US 20210361764A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2021/0361764 A1**

FOTIN-MLECZEK et al. (43) **Pub. Date: Nov. 25, 2021**

(54) **POLYMERIC CARRIER CARGO COMPLEX FOR USE AS AN IMMUNOSTIMULATING AGENT OR AS AN ADJUVANT**

(71) Applicant: **CureVac AG**, Tubingen (DE)

(72) Inventors: **Mariola FOTIN-MLECZEK**, Sindelfingen (DE); **Regina HEIDENREICH**, Tübingen (DE)

(73) Assignee: **CureVac AG**, Tübingen (DE)

(21) Appl. No.: **17/393,362**

(22) Filed: **Aug. 3, 2021**

Related U.S. Application Data

(63) Continuation of application No. 16/445,134, filed on Jun. 18, 2019, now Pat. No. 11,110,166, which is a continuation of application No. 15/300,682, filed on Sep. 29, 2016, now Pat. No. 10,369,216, filed as application No. PCT/EP2015/000706 on Apr. 1, 2015.

(30) **Foreign Application Priority Data**

Apr. 1, 2014 (EP) PCT/EP2014/000869

Publication Classification

(51) **Int. Cl.**

A61K 39/385	(2006.01)
A61K 47/64	(2006.01)
A61K 39/12	(2006.01)
A61K 39/145	(2006.01)
A61K 39/155	(2006.01)
A61K 39/205	(2006.01)
C12N 7/00	(2006.01)
C12N 15/117	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 39/385*** (2013.01); ***A61K 47/6455*** (2017.08); ***A61K 39/12*** (2013.01); ***A61K 39/145*** (2013.01); *A61K 2039/55516* (2013.01); ***A61K 39/205*** (2013.01); ***C12N 7/00*** (2013.01); ***C12N 15/117*** (2013.01); ***A61K 39/155*** (2013.01)

(57) **ABSTRACT**

The present invention is directed to a polymeric carrier cargo complex, comprising as a cargo at least one nucleic acid molecule and as a preferably non-toxic and non-immunogenic polymeric carrier disulfide-crosslinked cationic components for use as an immunostimulating agent or as an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule, which encodes a protein or peptide. The inventive polymeric carrier cargo complex administered in combination with the second nucleic acid molecule allows for both efficient transfection of nucleic acids into cells in vivo and in vitro and/or for induction of an innate and/or adaptive immune response, preferably dependent on the nucleic acid to be transported as a cargo and on the second nucleic acid molecule. The present invention also provides pharmaceutical compositions, particularly vaccines, comprising the inventive polymeric carrier cargo complex and the second nucleic acid molecule, as well as the use of the inventive polymeric carrier cargo complex and the second nucleic acid molecule for transfecting a cell, a tissue or an organism, as a medicament, for therapeutic purposes as disclosed herein, and/or as an immunostimulating agent or adjuvant, e.g. for eliciting an immune response for the treatment or prophylaxis of diseases as mentioned herein. Finally, the invention relates to kits containing the inventive polymeric carrier cargo complex and the second nucleic acid molecule, the inventive pharmaceutical composition and/or the inventive vaccine or any of its components in one or more parts of the kit.

Specification includes a Sequence Listing.

R2564 (SEQ ID NO: 384)

GGGGCGCUGCCUACGGAGGUGGCAGCCAUCUCCUUCUCGGCAUCAAGCUUACCAUGAAGG
CCAUCCUGGUGGUCCUCCUGUACACCUUCGCCACCGCGAACGCCGACACGCUGUGCAUCG
GCUACCACGCCAACAACAGCACCGACACCGUGGACACCGUGCUCGAGAAGAACGUCACGG
UGACCCACUCCGUGAACCUGCUGGAGGACAAGCACAACGGGAAGCUCUGCAAGCUGCGGG
GCGUCGCCCCGCUGCACCUCGGGAAGUGCAACAUCGCCGGCUGGAUCCUGGGGAACCCGG
AGUGCGAGAGCCUGUCCACCGCGAGCUCCUGGAGCUACAUCGUGGAGACCUCCAGCUCCG
ACAACGGCACGUGCUACCCCGGCGACUUCAUCGACUACGAGGAGCUCCGCGAGCAGCUGA
GCUCCGUGAGCUCCUUCGAGCGGUUCGAGAUCUUCCCCAAGACCAGCUCCUGGCCCAACC
ACGACAGCAACAAGGGGGUCACCGCCGCCUGCCCGCACGCCGGCGCGAAGUCCUUCUACA
AGAACCUGAUCUGGCUCGUGAAGAAGGGGAACAGCUACCCCAAGCUGUCCAAGAGCUACA
UCAACGACAAGGGCAAGGAGGUGCUGGUCCUCUGGGGGAUCCACCACCCCAGCACCUCCG
CCGACCAGCAGAGCCUGUACCAGAACGCCGACGCCUACGUGUUCGUGGGCUCCAGCCGCU
ACUCCAAGAAGUUCAAGCCCGAGAUCGCCAUCCGGCCGAAGGUCCGCGACCAGGAGGGCC
GGAUGAACUACUACUGGACGCUGGUGGAGCCCGGGGACAAGAUCACCUUCGAGGCGACCG
GCAACCUCGUGGUCCCCCGCUACGCCUUCGCCAUGGAGCGGAACGCCGGGAGCGGCAUCA
UCAUCUCCGACACCCCCGUGCACGACUGCAACACGACCUGCCAGACCCCGAAGGGCGCCA
UCAACACCAGCCUGCCCUUCCAGAACAUCCACCCCAUCACGAUCGGGAAGUGCCCCAAGU
ACGUGAAGUCCACCAAGCUGCGCCUCGCGACCGGCCUGCGGAACGUCCCGAGCAUCCAGU
CCCGCGGGCUGUUCGGCGCCAUCGCCGGGUUCAUCGAGGGCGGCUGGACCGGGAUGGUGG
ACGGCUGGUACGGGUACCACCACCAGAACGAGCAGGGCAGCGGGUACGCCGCCGACCUCA
AGUCCACGCAGAACGCGAUCGACGAGAUCACCAACAAGGUGAACAGCGUCAUCGAGAAGA
UGAACACCCAGUUCACCGCCGUGGGCAAGGAGUUCAACCACCUGGAGAAGCGGAUCGAGA
ACCUGAACAAGAAGGUCGACGACGGCUUCCUCGACAUCUGGACGUACAACGCCGAGCUGC
UGGUGCUCCUGGAGAACGAGCGCACCCUGGACUACCACGACUCCAACGUGAAGAACCUCU
ACGAGAAGGUCCGGAGCCAGCUGAAGAACAACGCCAAGGAGAUCGGGAACGGCUGCUUCG
AGUUCUACCACAAGUGCGACAACACCUGCAUGGAGUCCGUGAAGAACGGGACCUACGACU
ACCCCAAGUACAGCGAGGAGGCCAAGCUGAACCGCGAGGAGAUCGACGGCGUGAAGCUCG
AGUCCACGCGGAUCUACCAGAUCCUGGCGAUCUACAGCACCGUCGCCAGCUCCCUGGUGC
UCGUGGUCAGCCUGGGGGCCAUCUCCUUCUGGAUGUGCAGCAACGGCUCCCUGCAGUGCC
GCAUCUGCAUCUGACCACUAGUGCAUCACAUUUAAAAGCAUCUCAGCCUACCAUGAGAAU
AAGAGAAAGAAAAUGAAGAUCAAUAGCUUAUUCAUCUCUUUUUCUUUUUCGUUGGUGUAA
AGCCAACACCCUGUCUAAAAAACAUAAAUUUCUUUAAUCAUUUUGCCUCUUUUCUCUGUG
CUUCAAUUAAUAAAAAAUGGAAAGAACCUAGAUCUAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUGCAUCCCCCCCCCCCCCC
CCCCCCCCCCCCCCCCAAAGGCUCUUUUCAGAGCCACCAGAAUU

Fig. 1

R2025 (SEQ ID NO: 385)
GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAGCCGGUAUUUU
UUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUAGUCUGCCUAUAAAGGUGCGG
AUCCACAGCUGAUGAAAGACUUGUGCGGUACGGUUAAUCUCCCCUUUUUUUUUUUUUUUU
UUUUUAGUAAAUGCGUCUACUGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAA
GUGCAUAUAGUAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU
CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCGGCUAUUGCAG
GAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUUCCGCUCACUAUGAUUAAGAACCAG
GUGGAGUGUCACUGCUCUCGAGGUCUCACGAGAGCGCUCGAUACAGUCCUUGGAAGAAUC
UUUUUUUUUUUUUUUUUUUUUUUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCU
GCUCUAG

Fig. 2

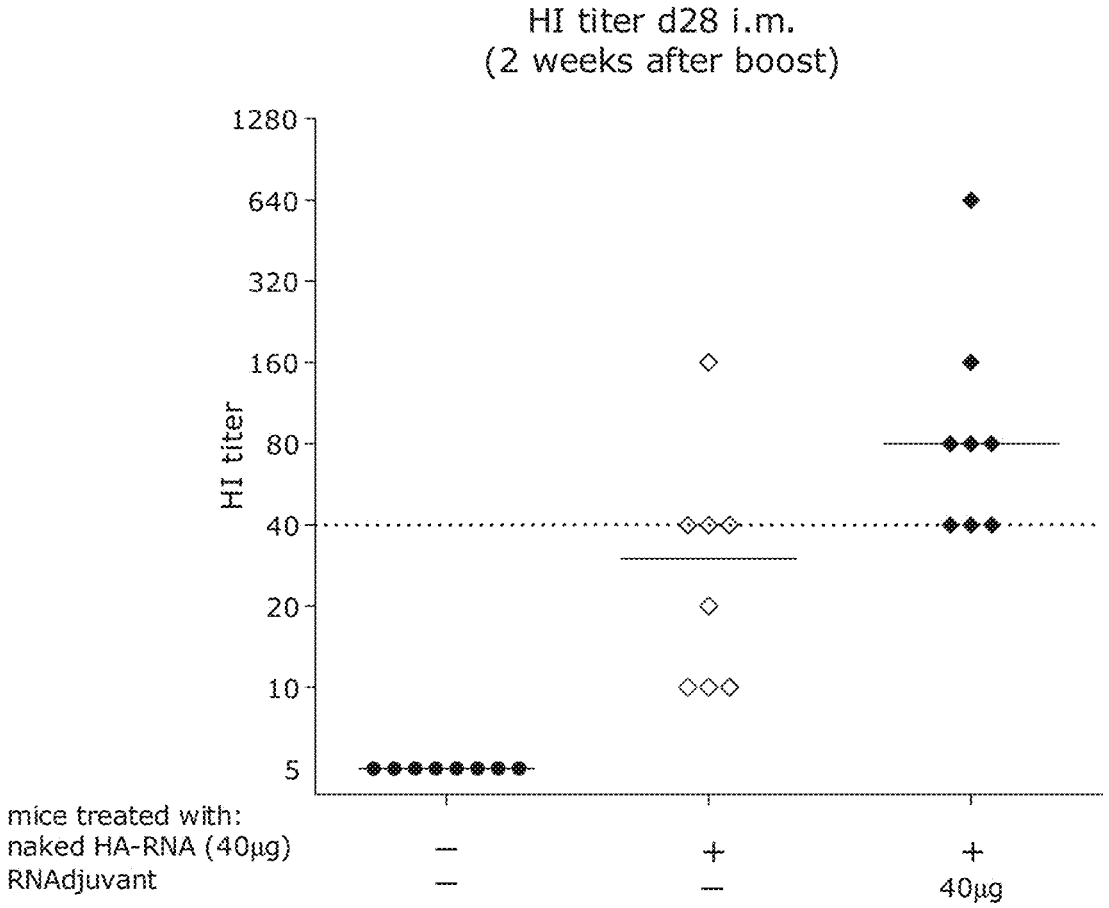

HI titer d28 i.m.
(2 weeks after boost)
HI titer
1280
640
320
160
80
40
20
10
5
mice treated with:
naked HA-RNA (40µg)
RNAdjuvant
–
–
+
–
+
40µg

Fig. 3

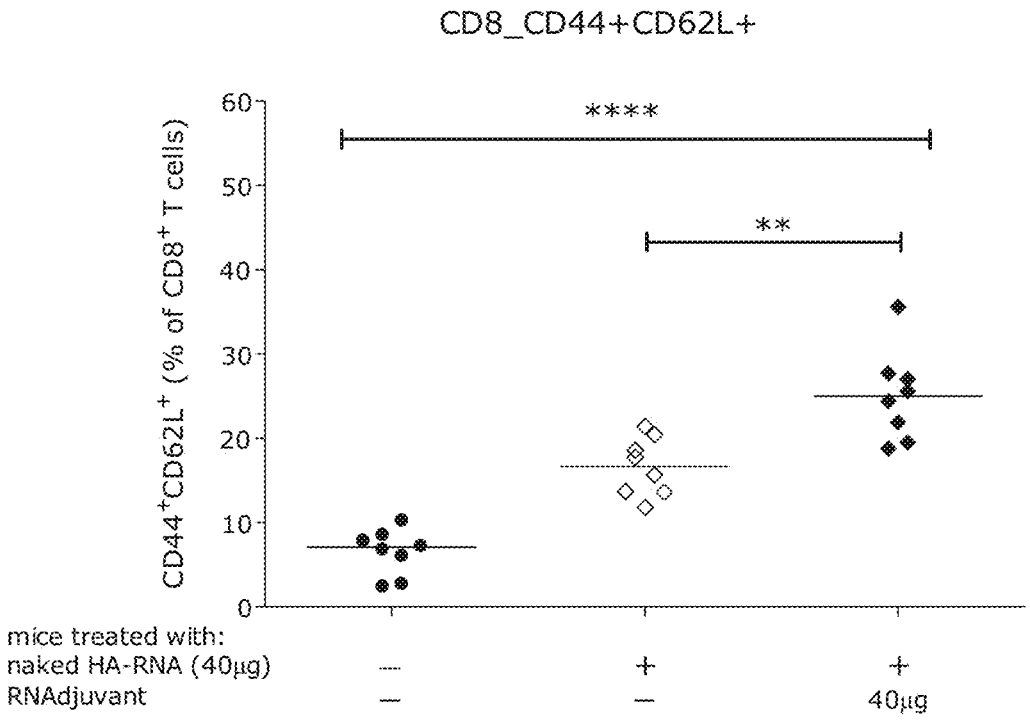

CD8_CD44+CD62L+
CD44+CD62L+ (% of CD8+ T cells)
60
50
40
30
20
10
0
****
**
mice treated with:
naked HA-RNA (40µg)
RNAdjuvant
—
—
+
—
+
40µg

Fig. 4

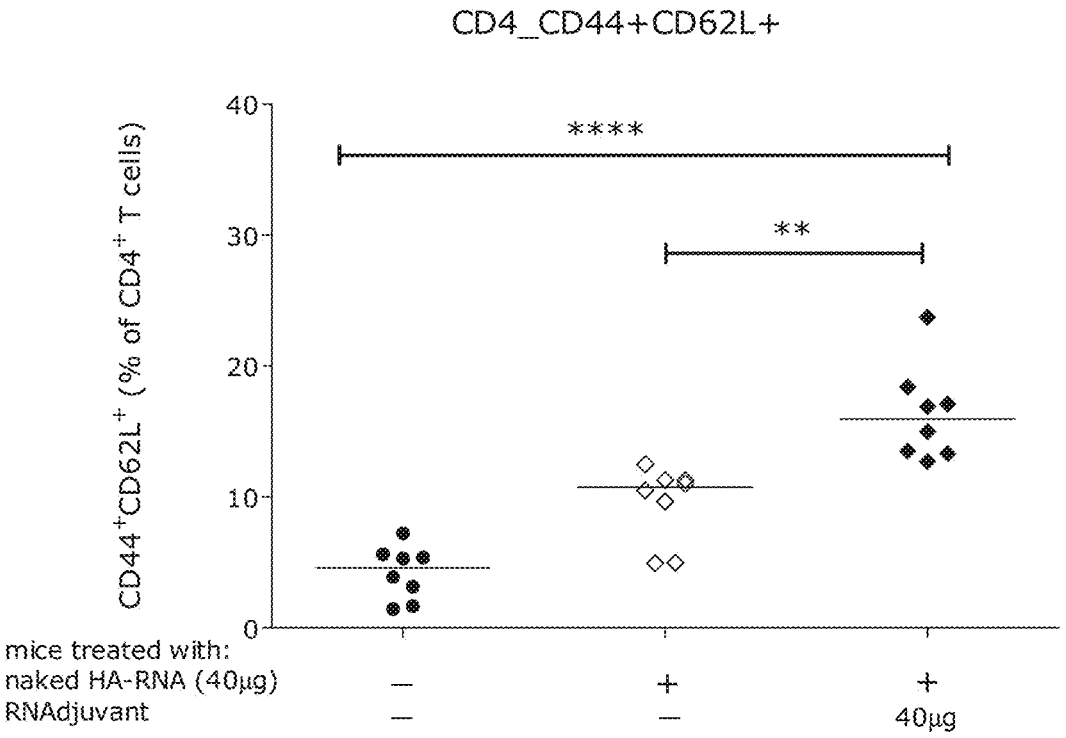

CD4_CD44+CD62L+
CD44+CD62L+ (% of CD4+ T cells)
40
30
20
10
0
****
**
mice treated with:
naked HA-RNA (40µg)
RNAdjuvant
–
–
+
–
+
40µg

Fig. 5

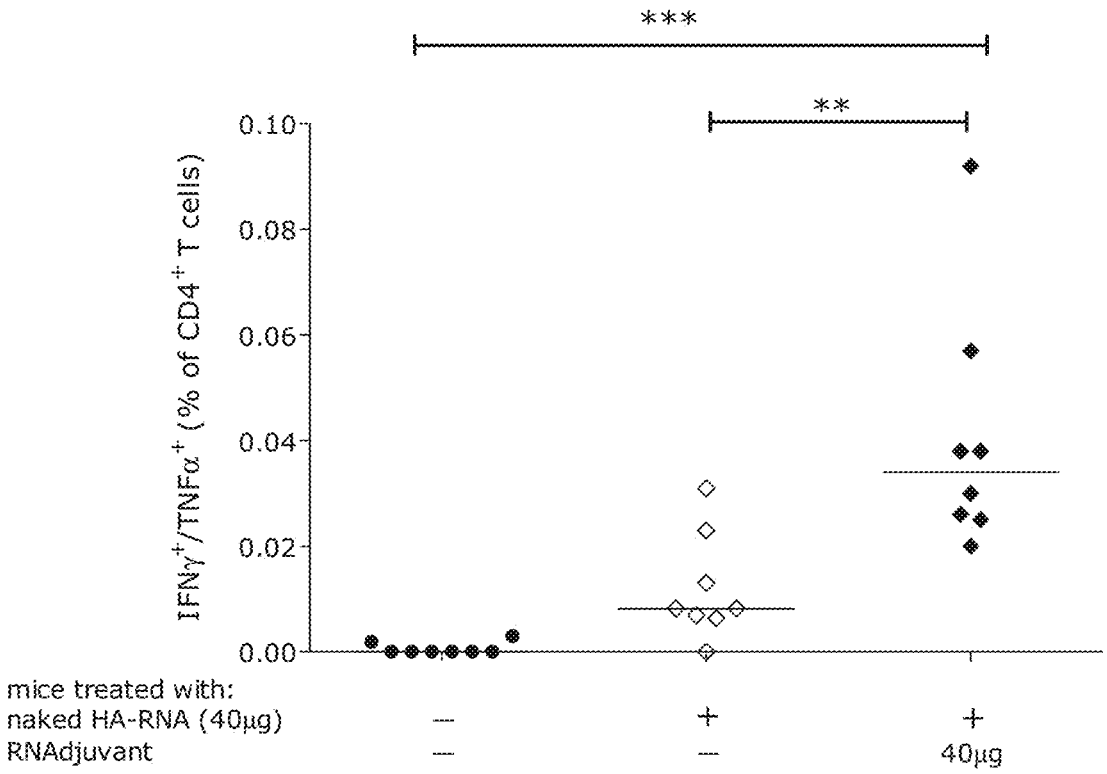

***
**
0.10
0.08
0.06
0.04
0.02
0.00
IFNγ+/TNFα+ (% of CD4+ T cells)
mice treated with:
naked HA-RNA (40µg)
RNAdjuvant
–
–
+
–
+
40µg

Fig. 6

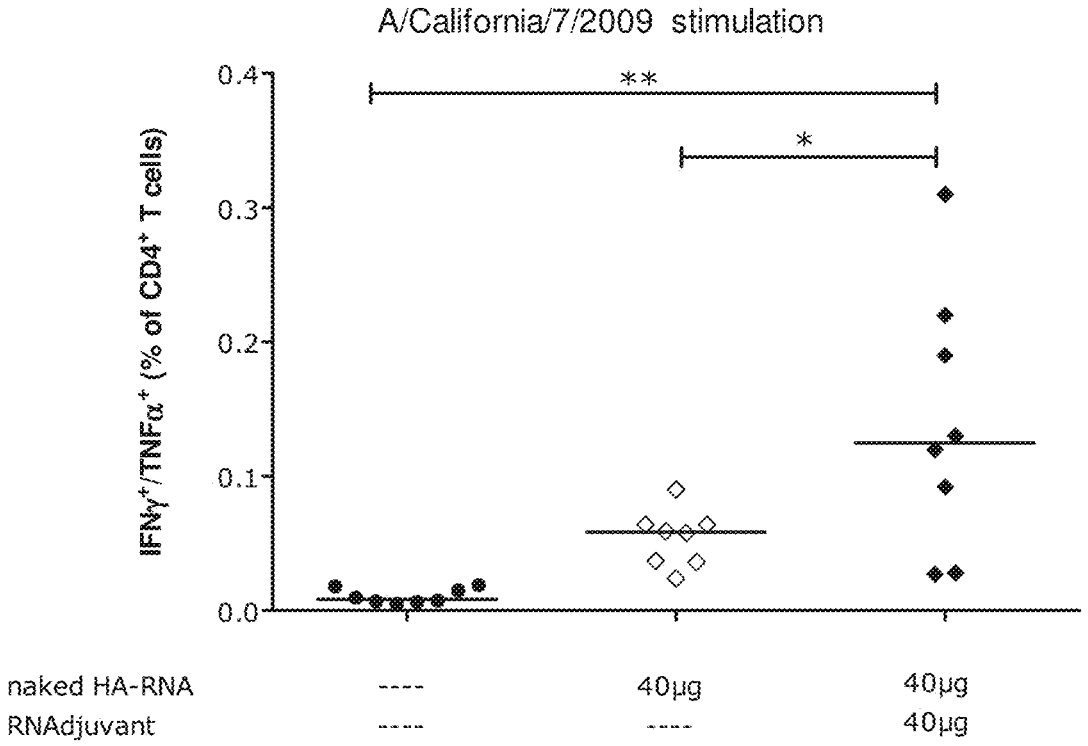

A/California/7/2009 stimulation
IFNγ+/TNFα+ (% of CD4+ T cells)
0.4
0.3
0.2
0.1
0.0
**
*
naked HA-RNA ---- 40µg 40µg
RNAdjuvant ---- ---- 40µg

Fig. 7

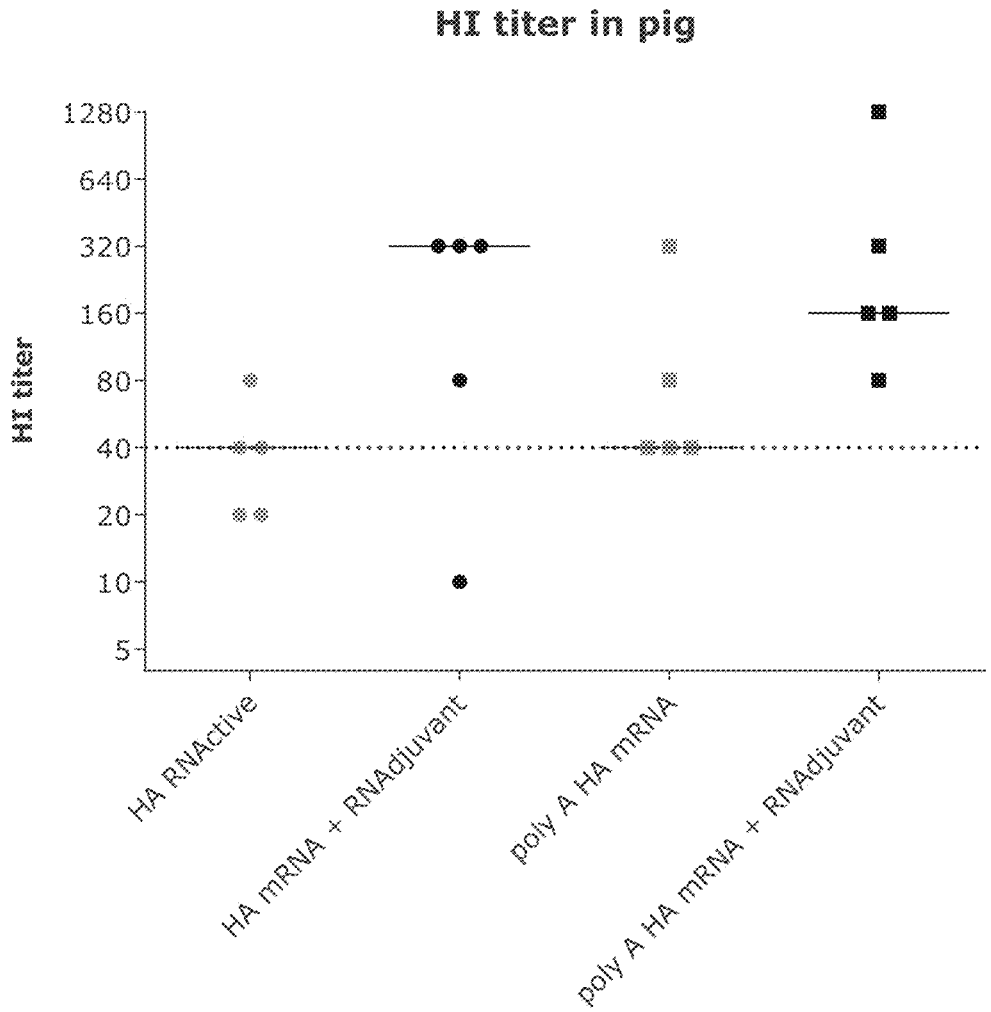

HI titer in pig
HI titer
1280
640
320
160
80
40
20
10
5
HA RNActive
HA mRNA + RNAdjuvant
poly A HA mRNA
poly A HA mRNA + RNAdjuvant

Fig. 8

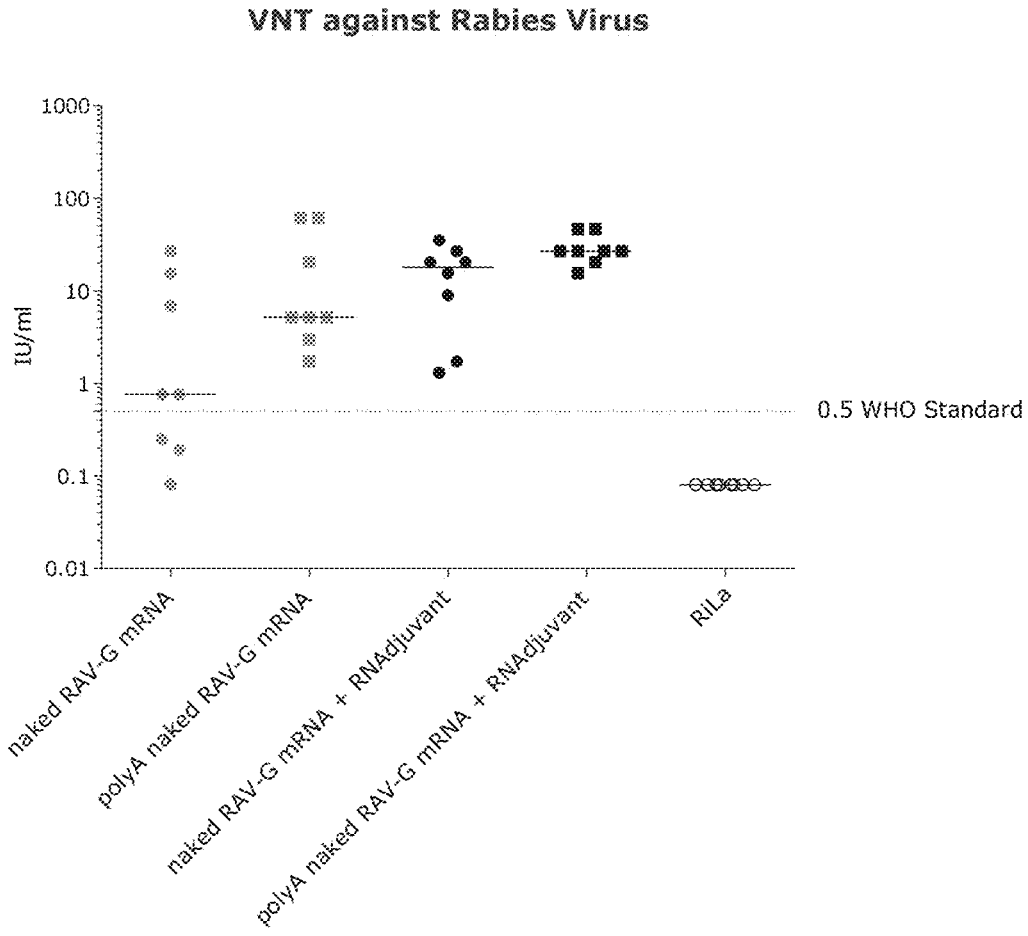

VNT against Rabies Virus
IU/ml
1000
100
10
1
0.1
0.01
0.5 WHO Standard
naked RAV-G mRNA
polyA naked RAV-G mRNA
naked RAV-G mRNA + RNAdjuvant
polyA naked RAV-G mRNA + RNAdjuvant
RiLa

Fig. 9

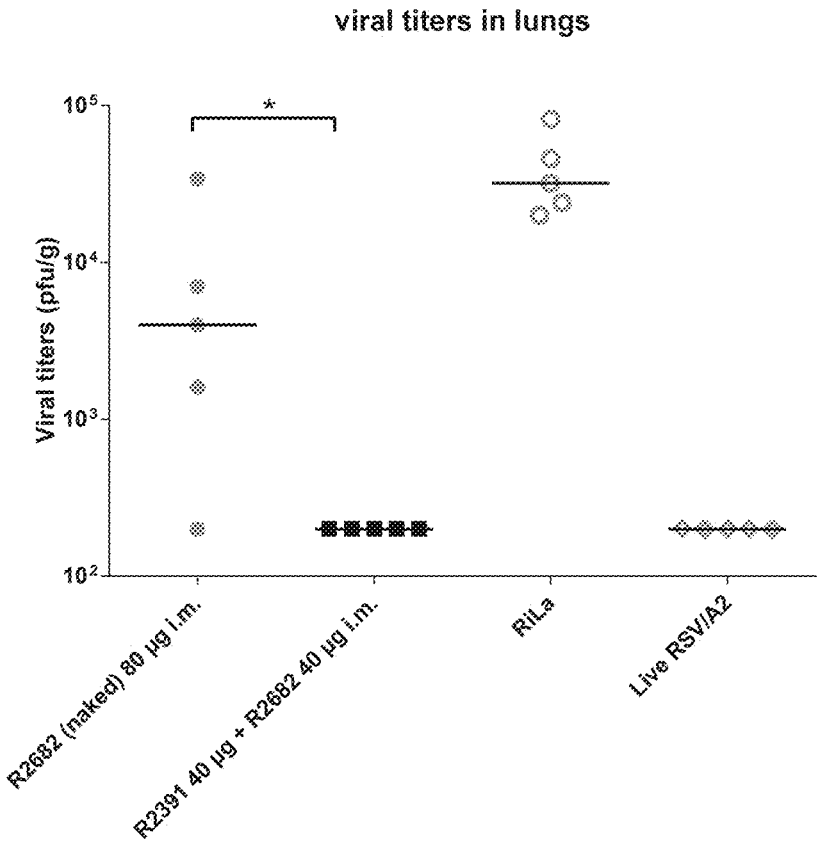

viral titers in lungs
Viral titers (pfu/g)
10^5
10^4
10^3
10^2
*
R2682 (naked) 80 µg i.m.
R2391 40 µg + R2682 40 µg i.m.
RiLa
Live RSV/A2

Fig. 10

R2506 (SEQ ID NO: 391)

GGGGCGCUGCCUACGGAGGUGGCAGCCAUCUCCUUCUCGGCAUCAAGCUUACCAUGGUGC
CCCAGGCCCUGCUCUUCGUCCCGCUGCUGGUGUUCCCCCUCUGCUUCGGCAAGUUCCCCA
UCUACACCAUCCCCGACAAGCUGGGGCCGUGGAGCCCCAUCGACAUCCACCACCUGUCCU
GCCCCAACAACCUCGUGGUCGAGGACGAGGGCUGCACCAACCUGAGCGGGUUCUCCUACA
UGGAGCUGAAGGUGGGCUACAUCAGCGCCAUCAAGAUGAACGGGUUCACGUGCACCGGCG
UGGUCACCGAGGCGGAGACCUACACGAACUUCGUGGGCUACGUGACCACCACCUUCAAGC
GGAAGCACUUCCGCCCCACGCCGGACGCCUGCCGGGCCGCCUACAACUGGAAGAUGGCCG
GGGACCCCCGCUACGAGGAGUCCCUCCACAACCCCUACCCCGACUACCACUGGCUGCGGA
CCGUCAAGACCACCAAGGAGAGCCUGGUGAUCAUCUCCCCGAGCGUGGCGGACCUCGACC
CCUACGACCGCUCCCUGCACAGCCGGGUCUUCCCCGGCGGGAACUGCUCCGGCGUGGCCG
UGAGCUCCACGUACUGCAGCACCAACCACGACUACACCAUCUGGAUGCCCGAGAACCCGC
GCCUGGGGAUGUCCUGCGACAUCUUCACCAACAGCCGGGGCAAGCGCGCCUCCAAGGGCA
GCGAGACGUGCGGGUUCGUCGACGAGCGGGGCCUCUACAAGUCCCUGAAGGGGGCCUGCA
AGCUGAAGCUCUGCGGCGUGCUGGGCCUGCGCCUCAUGGACGGGACCUGGGUGGCGAUGC
AGACCAGCAACGAGACCAAGUGGUGCCCCCCCGGCCAGCUGGUCAACCUGCACGACUUCC
GGAGCGACGAGAUCGAGCACCUCGUGGUGGAGGAGCUGGUCAAGAAGCGCGAGGAGUGCC
UGGACGCCCUCGAGUCCAUCAUGACGACCAAGAGCGUGUCCUUCCGGCGCCUGAGCCACC
UGCGGAAGCUCGUGCCCGGGUUCGGCAAGGCCUACACCAUCUUCAACAAGACCCUGAUGG
AGGCCGACGCCCACUACAAGUCCGUCCGCACGUGGAACGAGAUCAUCCCGAGCAAGGGGU
GCCUGCGGGUGGGCGGCCGCUGCCACCCCCACGUCAACGGGGUGUUCUUCAACGGCAUCA
UCCUCGGGCCCGACGGCAACGUGCUGAUCCCCGAGAUGCAGUCCAGCCUGCUCCAGCAGC
ACAUGGAGCUGCUGGUCUCCAGCGUGAUCCCGCUCAUGCACCCCCUGGCGGACCCCUCCA
CCGUGUUCAAGAACGGGGACGAGGCCGAGGACUUCGUCGAGGUGCACCUGCCCGACGUGC
ACGAGCGGAUCAGCGGCGUCGACCUCGGCCUGCCGAACUGGGGGGAAGUACGUGCUGCUCU
CCGCCGGCGCCCUGACCGCCCUGAUGCUGAUCAUCUUCCUCAUGACCUGCUGGCGCCGGG
UGAACCGGAGCGAGCCCACGCAGCACAACCUGCGCGGGACCGGCCGGGAGGUCUCCGUGA
CCCCGCAGAGCGGGAAGAUCAUCUCCAGCUGGGAGUCCUACAAGAGCGGCGGCGAGACCG
GGCUGUGAGGACUAGUGCAUCACAUUUAAAAGCAUCUCAGCCUACCAUGAGAAUAAGAGA
AAGAAAAUGAAGAUCAAUAGCUUAUUCAUCUCUUUUUCUUUUUCGUUGGUGUAAAGCCAA
CACCCUGUCUAAAAAACAUAAAUUUCUUUAAUCAUUUUGCCUCUUUUCUCUGUGCUUCAA
UUAAUAAAAAAUGGAAAGAACCUAGAUCUAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAUGCAUCCCCCCCCCCCCCCCCCCCCCC
CCCCCCCCCCAAAGGCUCUUUUCAGAGCCACCAGAAUU

Fig. 11

R2682 (SEQ ID NO: 392)

GGGGCGCUGCCUACGGAGGUGGCAGCCAUCUCCUUCUCGGCAUCAAGCUUACCAUGGAGC
UGCCCAUCCUCAAGGCCAACGCCAUCACCACCAUCCUGGCGGCCGUGACGUUCUGCUUCG
CCAGCUCCCAGAACAUCACCGAGGAGUUCUACCAGAGCACCUGCUCCGCCGUCAGCAAGG
GCUACCUGUCCGCCCUCCGGACCGGGUGGUACACGAGCGUGAUCACCAUCGAGCUGUCCA
ACAUCAAGGAGAACAAGUGCAACGGCACCGACGCGAAGGUGAAGCUGAUCAACCAGGAGC
UCGACAAGUACAAGAACGCCGUCACCGAGCUGCAGCUGCUCAUGCAGAGCACGACCGCCG
CCAACAACCGCGCGCGGCGCGAGCUGCCGCGGUUCAUGAACUACACCCUGAACAACACCA
AGAAGACGAACGUGACCCUCUCCAAGAAGCGCAAGCGGCGCUUCCUGGGGUUCCUGCUCG
GCGUGGGGAGCGCCAUCGCCUCCGGCAUCGCCGUCAGCAAGGUGCUGCACCUGGAGGGCG
AGGUGAACAAGAUCAAGUCCGCCCUCCUGAGCACCAACAAGGCGGUCGUGUCCCUGAGCA
ACGGGGUGUCCGUCCUCACCAGCAAGGUGCUGGACCUGAAGAACUACAUCGACAAGCAGC
UCCUGCCCAUCGUGAACAAGCAGUCCUGCCGGAUCAGCAACAUCGAGACGGUCAUCGAGU
UCCAGCAGAAGAACAACCGCCUGCUCGAGAUCACCCGGGAGUUCAGCGUGAACGCCGGCG
UGACCACCCCCGUCUCCACGUACAUGCUGACCAACAGCGAGCUGCUCUCCCUGAUCAACG
ACAUGCCCAUCACCAACGACCAGAAGAAGCUGAUGAGCAACAACGUGCAGAUCGUGCGCC
AGCAGUCCUACAGCAUCAUGUCCAUCAUCAAGGAGGAGGUCCUCGCCUACGUGGUGCAGC
UGCCGCUGUACGGGGUCAUCGACACCCCCUGCUGGAAGCUCCACACGAGCCCCCUGUGCA
CCACCAACACCAAGGAGGGCUCCAACAUCUGCCUGACGCGGACCGACCGCGGGUGGUACU
GCGACAACGCCGGCAGCGUGUCCUUCUUCCCCCCAGGCCGAGACCUGCAAGGUCCAGAGCA
ACCGGGUGUUCUGCGACACCAUGAACUCCCUCACGCUGCCGAGCGAGGUGAACCUGUGCA
ACGUCGACAUCUUCAACCCCAAGUACGACUGCAAGAUCAUGACCUCCAAGACCGACGUGA
GCUCCAGCGUGAUCACCUCCCUCGGCGCGAUCGUCAGCUGCUACGGGAAGACGAAGUGCA
CCGCCAGCAACAAGAACCGCGGCAUCAUCAAGACCUUCUCCAACGGGUGCGACUACGUGA
GCAACAAGGGCGUGGACACCGUCUCCGUGGGCAACACCCUGUACUACGUGAACAAGCAGG
AGGGGAAGAGCCUGUACGUCAAGGGCGAGCCCAUCAUCAACUUCUACGACCCCCUCGUGU
UCCCGUCCGACGAGUUCGACGCCAGCAUCUCCCAGGUGAACGAGAAGAUCAACCAGAGCC
UGGCCUUCAUCCGGAAGUCCGACGAGCUGCUGCACCACGUCAACGCCGGGAAGAGCACGA
CCAACAUCAUGAUCACCACCAUCAUCAUCGUGAUCAUCGUGAUCCUCCUGUCCCUGAUCG
CGGUCGGCCUCCUGCUGUACUGCAAGGCCCGCUGAGGACUAGUGCAUCACAUUUAAAAGC
AUCUCAGCCUACCAUGAGAAUAAGAGAAAGAAAAUGAAGAUCAAUAGCUUAUUCAUCUCU
UUUUCUUUUUCGUUGGUGUAAAGCCAACACCCUGUCUAAAAAACAUAAAUUUCUUUAAUC
AUUUUGCCUCUUUUCUCUGUGCUUCAAUUAAUAAAAAAUGGAAAGAACCUAGAUCUAAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
UGCAUCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCAAAGGCUCUUUUCAGAGCCACCAG
AAUU

Fig. 12

POLYMERIC CARRIER CARGO COMPLEX FOR USE AS AN IMMUNOSTIMULATING AGENT OR AS AN ADJUVANT

[0001] This application is a continuation of U.S. application Ser. No. 16/445,134, filed Jun. 18, 2019, which is a continuation of U.S. application Ser. No. 15/300,682, filed Sep. 29, 2016, now U.S. Pat. No. 10,369,216, which is a national phase application under 35 U.S.C. § 371 of International Application No. PCT/EP2015/000706, filed Apr. 1, 2015, which claims benefit of International Application No. PCT/EP2014/000869, filed Apr. 1, 2014, the entire contents of each of which are hereby incorporated by reference.

[0002] The sequence listing that is contained in the file named "CRVCP0157USC2.txt", which is 97 KB (as measured in Microsoft Windows®) and was created on Aug. 3, 2021, is filed herewith by electronic submission and is incorporated by reference herein.

[0003] The present invention is directed to a polymeric carrier cargo complex, comprising as a cargo at least one first nucleic acid molecule and as a preferably non-toxic and non-immunogenic polymeric carrier disulfide-crosslinked cationic components for use as an immunostimulating agent or as an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule, which encodes a protein or peptide. The inventive polymeric carrier cargo complex administered in combination with the second nucleic acid molecule allows for both efficient transfection of nucleic acids into cells in vivo and in vitro and/or for induction of an innate and/or adaptive immune response, preferably dependent on the nucleic acid to be transported as a cargo and on the second nucleic acid molecule. The present invention also provides pharmaceutical compositions, particularly vaccines, comprising the inventive polymeric carrier cargo complex and the second nucleic acid molecule, as well as the use of the inventive polymeric carrier cargo complex and the second nucleic acid molecule for transfecting a cell, a tissue or an organism, as a medicament, for therapeutic purposes as disclosed herein, and/or as an immunostimulating agent or adjuvant, e.g. for eliciting an immune response for the treatment or prophylaxis of diseases as mentioned herein. Finally, the invention relates to kits containing the inventive polymeric carrier cargo complex and the second nucleic acid molecule, the inventive pharmaceutical composition and/or the inventive vaccine or any of its components in one or more parts of the kit.

[0004] Many diseases today require administration of adjuvants to provide an innate immune response and, optionally, to support an adaptive immune response, particularly in the context of vaccinations. Some but not necessarily all of these diseases additionally or alternatively require administration of peptide-, protein-, and nucleic acid-based drugs, e.g. the transfection of nucleic acids into cells or tissues. These requirements usually represent different aspects in the treatment of such diseases and are typically difficult to address in one approach. As a consequence, the prior art usually handles such aspects via separate approaches.

[0005] In the above context, vaccination is generally believed to be one of the most effective and cost-efficient ways to prevent or treat diseases. Nevertheless, several problems in vaccine development have proved difficult to solve: Vaccines are often inefficient for the very young and the very old; many vaccines need to be given several times, and the protection they confer wanes over time, requiring booster administrations, and, for some diseases such as HIV, development of efficient vaccines is urgently needed. As generally accepted, many of these vaccines would be enabled or improved if they could elicit a stronger and more durable immune response.

[0006] Accordingly, the development of new efficient and safe adjuvants for vaccination purposes which support induction and maintenance of an adaptive immune response by initiating or boosting a parallel innate immune response represents a main challenging problem.

[0007] Adjuvants are usually defined as compounds that can increase and/or modulate the intrinsic immunogenicity of an antigen. To reduce negative side effects, new vaccines have a more defined composition that often leads to lower immunogenicity compared with previous whole-cell or virus-based vaccines. Adjuvants are therefore required to assist new vaccines to induce potent and persistent immune responses, with the additional benefit that less antigen and fewer injections are needed. Now it is clear that the adaptive immune response mainly depends on the level and specificity of the initial danger signals perceived by innate immune cells following infection or vaccination (Guy, B. (2007), Nat Rev Microbiol 5(7): 505-17.). In particular for new generation vaccine candidates, which will increasingly comprise highly purified recombinant proteins and, although very safe, are poorly immunogenic, efficient adjuvants will become increasingly necessary.

[0008] Unfortunately, only a few licensed adjuvants are available so far. Most prominent is Alum, which is known to be safe, but also represents a very weak adjuvant. Many further adjuvants have been developed, e.g. including the administration of pathogens, CpG-nucleotides, etc. Most of these new or "established" adjuvants, however, still do not satisfy the above requirements, since many new and emerging problems have to be considered and solved. These problems inter alia include new and re-emerging infectious diseases, repeated administrations, and threat of pandemic flu.

[0009] Furthermore, the new vaccine targets are usually more difficult to develop and—due to their specifically tailored immune responses—require more potent adjuvants to enable success. Moreover, there are still a significant number of important pathogens for which we do not even have effective vaccines at present. This represents a very challenging future target. To enable vaccine development against such targets, more potent adjuvants will be necessary. Such new adjuvants will need to offer advantages, including more heterologous antibody responses, covering pathogen diversity, induction of potent functional antibody responses, ensuring pathogen killing or neutralization and induction of more effective T cell responses, for direct and indirect pathogen killing, particularly the induction of cytotoxic T cells which are part of a Th1 immune response. In addition, adjuvants may be necessary to achieve more pragmatic effects, including antigen dose reduction and overcoming antigen competition in combination vaccines. Moreover, against the background of an aging population, which is increasingly susceptible to infectious diseases, new adjuvants will be necessary to overcome the natural deterioration of the immune response with age (O'Hagan, D. T. and E. De Gregorio (2009), Drug Discov Today 14(11-12): 541-51.).

[0010] The review of O'Hagan (2009; supra) summarizes some reasons for the urgent need of new effective adjuvants,

e.g. the requirement of a lower antigen dose in vaccines, the necessity to increase the breadth of an immune response and the heterologous activity, to enable complex combination vaccines, and to overcome antigenic competition, to overcome limited immune response in some groups of the population, such as the elderly, the young children, and infants, patients with chronic diseases and the immunocompromised, to increase effector T cell response and antibody titers, to induce protective responses more rapidly and also to extend the duration of response by enhancing memory B and T cell responses.

[0011] Summarizing the above, new efficient and safe immunostimulating agents or adjuvants are required, which are preferably efficient in inducing an innate immune response, particularly in inducing the anti-viral cytokine IFN-alpha; which are, preferably, also efficient in supporting an adaptive immune response; safe, i.e. not associated with any long-term effects; which are well tolerated; which are available via a simple synthetic pathway; which exhibit low cost storage conditions (particularly feasible lyophilisation); which require simple and inexpensive components; which are biodegradable; which are compatible with many different kinds of vaccine antigens; which are capable of co-delivery of antigen and immune potentiator, etc.

[0012] As already explained above, adjuvants or immunostimulating agents usually act via their capability to induce an innate immune response. The innate immune system forms the dominant system of host defense in most organisms and comprises barriers such as humoral and chemical barriers including, e.g., inflammation, the complement system and cellular barriers. The innate immune system is typically based on a small number of receptors, called pattern recognition receptors. They recognize conserved molecular patterns that distinguish foreign organisms, like viruses, bacteria, fungi and parasites, from cells of the host. Such pathogen-associated molecular patterns (PAMP) include viral nucleic acids, components of bacterial and fungal walls, flagellar proteins, and more. The first family of pattern recognition receptors (PAMP receptors) studied in detail was the Toll-like receptor (TLR) family. TLRs are transmembrane proteins which recognize ligands of the extracellular milieu or of the lumen of endosomes. Following ligand-binding they transduce the signal via cytoplasmic adaptor proteins which leads to triggering of a host-defence response and entailing production of antimicrobial peptides, proinflammatory chemokines and cytokines, antiviral cytokines, etc. (see e.g. Meylan, E., J. Tschopp, et al. (2006), Nature 442(7098): 39-44). Further relevant components of the immune system include e.g. the endosomal TLRs, cytoplasmic receptors, Type I interferons and cytoplasmic receptors.

[0013] Therefore, the immunostimulating agents or adjuvants are defined herein preferably as inducers of an innate immune response, which active pattern recognition receptors (PAMP receptors). Hereby, a cascade of signals is elicited, which e.g. may result in the release of cytokines (e.g. IFN-alpha) supporting the innate immune response. Accordingly, it is preferably a feature of an immunostimulating agent or adjuvant to bind to such receptors and activate such PAMP receptors. Ideally, such as an agent or adjuvant additionally supports the adaptive immune response by e.g. shifting the immune response such that the preferred class of Th cells is activated. Depending on the disease or disorder to be treated a shift to a Th1-based immune reponse may be preferred or, in other cases, a shift to a Th2 immune response may be preferred.

[0014] In the prior art there are some promising adjuvant candidates which fulfil at least some, but not all, of the above defined required characteristics.

[0015] As an example, among the above developed new adjuvants, some nucleic acids, like CpG DNA oligonucleotides or isRNA (immunostimulating RNA), turned out to be promising candidates for new immunostimulating agents or adjuvants as they allow the therapeutic or prophylactic induction of an innate immune response. Such nucleic acid based adjuvants usually have to be delivered effectively to the site of action to allow induction of an effective innate immune response without unnecessary loss of adjuvant activity and, in some cases, without the necessity to increase the administered volume above systemically tolerated levels.

[0016] One approach to solve this issue may be the transfection of cells which are part of the innate immune system (e.g. dendritic cells, plasmacytoid dendritic cells (pDCs)) with immunostimulatory nucleic acids, which are ligands of PAMP receptors, (e.g. Toll-like receptors (TLRs)), and thus may lead to immunostimulation by the nucleic acid ligand. Further approaches may be the direct transfection of nucleic acid based adjuvants. All of these approaches, however, are typically limited by inefficient delivery of the nucleic acid and consequently diminished adjuvant activity, in particular when administered locally.

[0017] However, one main disadvantage of such nucleic acid based adjuvant approaches until today is their limited ability to cross the plasma membrane of mammalian cells, resulting in poor cellular access and inadequate therapeutic efficacy. Until today this hurdle represents a major challenge for nucleic acid transfection based applications, e.g. biomedical developments and accordingly the commercial success of many biopharmaceuticals (see e.g. Foerg, C. & Merkle, H. P., *J Pharm Sci* 97, 144-62 (2008).

[0018] Transfection of nucleic acids or genes into cells or tissues has been investigated up to date in the context of in vitro transfection and in the context of gene therapeutic approaches. However, no adjuvants are available so far which are based on such gene delivery techniques which are efficient and safe, in particular no licensed adjuvants. This is presumably due to the complex requirements of adjuvants in general in combination with stability issues to be solved in the case of nucleic acid based adjuvants.

[0019] Nevertheless, transfection of nucleic acids or genes into cells or tissues for eliciting an innate and/or adaptive immune response appears to provide a promising approach to provide new adjuvants.

[0020] However, many of these approaches utilize transfection of nucleic acids or genes into cells or tissues without induction of an innate immune response. There are even some gene therapies, which have to strictly avoid induction of an innate immune response. Even in the rare cases, where vaccination is carried out to induce an adaptive antigen-specific immune response using administration of nucleic acids, e.g. in tumour vaccinations using DNA or mRNA encoded antigens, induction of an adaptive immune response is typically carried out as an active immunization against the encoded antigen but not as an accompanying adjuvant therapy and thus requires additional administration of a separate adjuvant to induce an innate immune response.

[0021] Even if a series of transfection methods are known in the art, transfer or insertion of nucleic acids or genes into an individual's cells still represents a major challenge today and is not yet solved satisfactorily. To address this complex issue a variety of methods were developed in the last decade. These include transfection by calcium phosphate, cationic lipids, cationic polymers, and liposomes. Further methods for transfection are electroporation and viral transduction.

[0022] However, as known to a skilled person, systems for transfer or insertion of nucleic acids or genes have to fulfil several requirements for in vivo applications which include efficient nucleic acid delivery into an individual's cells with high functionality, protection of the nucleic acid against ubiquitously occurring nucleases, release of the nucleic acid in the cell, no safety concerns, feasible manufacturing in a commercially acceptable form amenable to scale-up and storage stability under low cost conditions (e.g feasible lyophilisation). These requirements are to be added to the complex requirements of an adjuvant particularly if it is in the form of a nucleic acid as outlined above.

[0023] Some successful strategies for the transfer or insertion of nucleic acids or genes available today rely on the use of viral vectors, such as adenoviruses, adeno-associated viruses, retroviruses, and herpes viruses. Viral vectors are able to mediate gene transfer with high efficiency and the possibility of long-term gene expression. However, the acute immune response ("cytokine storm"), immunogenicity, and insertion mutagenesis observed in gene therapy clinical trials have raised serious safety concerns about some commonly used viral vectors.

[0024] Another solution to the problem of transfer or insertion of nucleic acids or genes may be found in the use of non-viral vectors. Although non-viral vectors are not as efficient as viral vectors, many non-viral vectors have been developed to provide a safer alternative. Methods of non-viral nucleic acid delivery have been explored using physical (carrier-free nucleic acid delivery) and chemical approaches (synthetic vector-based nucleic acid delivery). Physical approaches usually include needle injection, electroporation, gene gun, ultrasound, and hydrodynamic delivery, employ a physical force that permeates the cell membrane and facilitates intracellular gene transfer. The chemical approaches typically use synthetic or naturally occurring compounds (e.g. cationic lipids, cationic polymers, lipid-polymer hybrid systems) as carriers to deliver the nucleic acid into the cells. Although significant progress has been made in the basic science and applications of various nonviral nucleic acid delivery systems, the majority of non-viral approaches are still much less efficient than viral vectors, especially for in vivo gene delivery (see e.g. Gao, X., Kim, K. & Liu, D., *AAPS J* 9, E92-104 (2007)).

[0025] Such transfection agents as defined above typically have been used successfully solely in in vitro reactions. For application of nucleic acids in vivo, however, further requirements have to be fulfilled. For example, complexes between nucleic acids and transfection agents have to be stable in physiological salt solutions with respect to aggregation. Furthermore, such complexes typically must not interact with parts of the complement system of the host and thus must not be immunogenic itself as the carrier itself shall not induce an adaptive immune response in the individual. Additionally, the complex shall protect the nucleic acid from early extracellular degradation by ubiquitously occurring nucleases.

[0026] In the art many transfection reagents are available, especially cationic lipids, which show excellent transfection activity in cell culture. However, most of these transfection reagents do not perform well in the presence of serum, and only a few are active in vivo. A dramatic change in size, surface charge, and lipid composition occurs when lipoplexes are exposed to the overwhelming amount of negatively charged and often amphipathic proteins and polysaccharides that are present in blood, mucus, epithelial lining fluid, or tissue matrix. Once administered in vivo, lipoplexes tend to interact with negatively charged blood components and form large aggregates that could be absorbed onto the surface of circulating red blood cells, trapped in a thick mucus layer, or embolized in microvasculatures, preventing them from reaching the intended target cells in the distal location. Some even undergo dissolution after they are introduced to the blood circulation (see e.g. Gao, X., Kim, K. & Liu, D., *AAPS J* 9, E92-104 (2007)).

[0027] One more promising approach utilizes cationic polymers. Cationic polymers turned out to be efficient in transfection of nucleic acids, as they can tightly complex and condense a negatively charged nucleic acid. Thus, a number of cationic polymers have been explored as carriers for in vitro and in vivo gene delivery. These include polyethylenimine (PEI), polyamidoamine and polypropylamine dendrimers, polyallylamine, cationic dextran, chitosan, cationic proteins and cationic peptides. Although most cationic polymers share the function of condensing DNA into small particles and facilitate cellular uptake via endocytosis through charge-charge interaction with anionic sites on cell surfaces, their transfection activity and toxicity differs dramatically.

[0028] Only in one approach in the art, the immunostimulatory effect of RNA complexed to short cationic peptides was demonstrated by Fotin-Mleczek et al. (WO 2009/030481). These formulations appear to efficiently induce the cytokine production in immunocompetent cells. Unfortunately Fotin-Mleczek et al. did not assess the induction of the preferable anti-viral cytokine IFN-α by these complexes. Additionally, these complexes turned out to be unstable during lyophilisation.

[0029] In the above context, cationic polymers exhibit better transfection efficiency with rising molecular weight. However, a rising molecular weight also leads to a rising toxicity of the cationic polymer. In this above context, high molecular weight PEI is perhaps the most active and most studied polymer for transfection of nucleic acids, in particular for gene delivery purposes. Unfortunately, it exhibits the same drawback due to its non-biodegradable nature and toxicity. Furthermore, even though polyplexes formed by high molecular weight polymers exhibit improved stability under physiological conditions, data have indicated that such polymers can hinder vector unpacking. To overcome this negative impact, Read et al. (see Read, M. L. et al., *J Gene Med.* 5, 232-245 (2003); and Read, M. L. et al., *Nucleic Acids Res* 33, e86 (2005)) developed a new type of synthetic vector based on a linear reducible polycation (RPC) prepared by oxidative polycondensation of the peptide Cys-Lysio-Cys. This peptide Cys-Lysio-Cys can be cleaved in the intracellular environment to facilitate release of nucleic acids. In this context, Read et al. (2003, supra) could show that polyplexes formed by these RPCs are destabilised by reducing conditions enabling efficient release of DNA and mRNA. However, examining the trans-

fection efficiency in vitro Read et al. (2003, supra) also observed that N/P (nitrogen to phosphor atoms) ratios of 2 were unsatisfying and higher N/P ratios were necessary to improve transfection efficiency. Additonally, Read et al. (2003, supra) observed that chloroquine or the cationic lipid DOTAP was additionally necessary to enhance transfection efficiency to adequate levels. As a consequence, Read et al. (2005, supra) included histidine residues into the RPCs which have a known endosomal buffering capacity and showed that such histidine-rich RPCs can be cleaved by the intracellular reducing environment. This approach enabled efficient cytoplasmic delivery of a broad range of nucleic acids, including plasmid DNA, mRNA and siRNA molecules without the requirement for the endosomolytic agent chloroquine.

[0030] Unfortunately, neither Read et al. (2003, supra) nor Read et al. (2005, supra) did assess as to whether RPCs can be directly used for in vivo applications. In their study in 2005, transfections were performed in the absence of serum to avoid masking the ability of histidine residues to enhance gene transfer that may have arisen from binding of serum proteins to polyplexes restricting cellular uptake. Preliminary experiments, however, indicated that the transfection properties of histidine-rich RPC polyplexes can be affected by the presence of serum proteins with a 50% decrease in GFP-positive cells observed in 10% FCS. For in vivo application Read et al. (2005, supra) proposed modifications with the hydrophilic polymer poly-[N-(2hydroxy-propyl) methacrylamide]. Unfortunately, they could not prevent aggregation of polyplexes and binding of polycationic complexes to serum proteins. Furthermore, strong cationic charged complexes are formed (positive zeta potential) when complexing the nucleic acid due to the large excess of cationic polymer, which is characterized by the high N/P ratio. Accordingly, such complexes are only of limited use in vivo due to their strong tendency of salt induced aggregation and interactions with serum contents. Additionally, these (positively charged) complexes may excite complement activation, when used for purposes of gene therapy. It has also turned out that these positively charged RPC based complexes showed poor translation of the nucleic acid cargo subsequent to local administration into the dermis.

[0031] In an approach similar to Read et al. McKenzie et al. (McKenzie, D. L., K. Y. Kwok, et al. (2000), J Biol Chem 275(14): 9970-7. and McKenzie, D. L., E. Smiley, et al. (2000), Bioconjug Chem 11(6): 901-9) developed crosslinking peptides as gene delivery agents by inserting multiple cysteines into short synthetic peptides. In their studies they examined the optimal complex formation with DNA and as a result they could show that an N/P ratio of at least 2 is necessary for fully formed peptide DNA condensates. Therefore only positively charged complexes appeared to show optimal DNA condensation. In contrast to these data they proposed the development of negatively charged complexes for in vivo gene delivery, since it was shown in previous studies that intravenous application of electropositive DNA condensates leads to rapid opsonisation and nonspecific biodistribution to lung and liver (Collard, W. T., Evers, D. L., McKenzie, D. L., and Rice, K. G. (2000), Carbohydr. Res. 323, 176-184). Therefore McKenzie et al. (2000; supra) proposed the derivatization of the carriers with polyethylene glycol and targeting ligands. To be noted, the approach of McKenzie et al. (2000, supra) is additionally subject of a patent (U.S. Pat. No. 6,770,740 B1), which particularly discloses the transfection of coding nucleic acids, antisense nucleic acids and ribozymes.

[0032] Thus, in vivo application of nucleic acids appears to be still one of the most challenging problems because plasma proteins with anionic charges may non-specifically bind to positively charged complexes and rapidly remove them e.g. via the reticulo-endothelial system. Opsonization and activation of the complement system by cationic complexes are additional physiological phenomena that can participate in lowering the efficacy of in vivo administered cationic complexes. This particularly applies to administration of nucleic acid-based drugs, e.g. the transfection of nucleic acids into cells or tissues, particularly if the expression of an encoded protein or peptide or transcription of an RNA of the transfected nucleic acid is intended. In particular, there continues to be a great need for a system that allows administration of nucleic acid-based drugs, particularly nucleic acid-based drugs comprising an adjuvant function, by a method, which warrants a high level of safety and efficacy and which can readily be applied in a variety of situations and without specific training.

[0033] Summarizing the above, the prior art does not provide feasible means or methods, which, on the one hand, allow to establish efficient and safe adjuvants for vaccination purposes, and which, on the other hand, are furthermore suited for in vivo delivery of nucleic acids, in particular for compacting and stabilizing a nucleic acid for the purposes of nucleic acid transfection in vivo without exhibiting the negative side effects as discussed above. More precisely, no means or methods are known in the prior art in the above context, which are, on the one hand, stable enough to carry a nucleic acid cargo to the target before they are metabolically cleaved, and which, on the other hand, can be cleared from the tissue before they can accumulate and reach toxic levels. In addition no means or method is known, which, additional to the above requirements, induces a desirable pattern of cytokines, particularly the anti viral cytokine IFN-α.

[0034] Accordingly, it is the object of the present invention to provide such means or methods, which address these problems.

[0035] The object underlying the present invention is solved by the subject matter of the present invention, preferably by the subject matter of the attached claims.

[0036] According to a first aspect, the object underlying the present invention is solved by a polymeric carrier cargo complex, comprising or consisting of

[0037] a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components and

[0038] b) as a cargo at least one first nucleic acid molecule,

preferably for use as a medicament, more preferably for use as an immunostimulating agent or adjuvant, e.g. in the treatment of a disease as defined herein, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule, which encodes a protein or a peptide, and wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly.

[0039] In a preferred embodiment, the invention relates to a polymeric carrier cargo complex, comprising:

[0040] a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and

[0041] b) as a cargo at least one first nucleic acid molecule,

[0042] for use as an immunostimulating agent or as an adjuvant,

wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, and wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly.

[0043] Alternatively, the problem is solved by a polymeric carrier cargo complex, comprising:

[0044] a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and

[0045] b) as a cargo at least one first nucleic acid molecule,

preferably for use as a medicament, more preferably for use as an immunostimulating agent or as an adjuvant, e.g. in the treatment of a disease as defined herein,

wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, and

wherein the second nucleic acid molecule is an RNA molecule, preferably an mRNA molecule.

[0046] In a preferred embodiment, the invention relates to a polymeric carrier cargo complex, comprising:

[0047] a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and

[0048] b) as a cargo at least one first nucleic acid molecule,

for use as an immunostimulating agent or as an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, wherein the second nucleic acid molecule is an RNA molecule, preferably an mRNA molecule.

[0049] As used herein, the term "first nucleic acid molecule" refers to a nucleic molecule, which is used as a cargo in the polymeric carrier cargo complex and is thus associated with the polymeric carrier. The term "second nucleic acid molecule", as used herein, typically refers to a nucleic acid, which is not part of the polymeric carrier cargo complex and which encodes a peptide or protein.

[0050] The term "immunostimulating agent" is typically understood not to include agents as e.g. antigens (of whatever chemical structure), which elicit an adaptive/cytotoxic immune response, e.g. a "humoral" or "cellular" immune response, in other words elicit immune reponses (and confer immunity by themselves) which are characterized by a specific response to structural properties of an antigen recognized to be foreign by immune competent cells. Rather "immunostimulating agent" is typically understood to mean agents/compounds/complexes which do not trigger any adaptive immune response by themselves, but which may exlusively enhance such an adaptive immune reponse in an unspecific way, by e.g. activating "PAMP" receptors and thereby triggering the release of cytokines which support the actual adaptive immune response. Accordingly, any immunostimulation by agents (e.g. antigens) which evoke an adaptive immune response by themselves (conferring immunity by themselves directly or indirectly) is typically disclaimed by the phrase "immunostimulating agent".

[0051] The term "adjuvant" is also understood not to comprise agents which confer immunity by themselves. Accordingly, adjuvants do not by themselves confer immunity, but assist the immune system in various ways to enhance the antigen-specific immune response by e.g. promoting presentation of an antigen to the immune system. Hereby, an adjuvant may preferably e.g. modulate the antigen-specific immune response by e.g. shifting the dominating Th2-based antigen specific response to a more Th1-based antigen specific response or vice versa. Accordingly, the terms "immunostimulating agent" and "adjuvant" in the context of the present invention are typically understood to mean agents, compounds or complexes which do not confer immunity by themselves, but exclusively support the immune reponse in an unspecific way (in contrast to an antigen-specific immune response) by effects, which modulate the antigen-specific (adaptive cellular and/or humoral immune response) by unspecific measures, e.g. cytokine expression/secretion, improved antigen presentation, shifting the nature of the arms of the immune response etc. Accordingly, any agents evoking by themselves immunity are typically disclaimed by the terms "adjuvant" or "immunostimulating agent".

[0052] The use of the polymeric carrier cargo complex in combination with a second nucleic acid molecule, preferably an RNA, allows provision of a more efficient and/or safer medicament. Advantageously, the polymeric carrier cargo complex is suited for in vivo delivery of nucleic acids, in particular for compacting and stabilizing a nucleic acid for the purposes of nucleic acid transfection, such as exhibiting one or more reduced negative side effects of high-molecular weight polymers as discussed above, such as poor biodegradability or high toxicity, agglomeration, low transfection activity in vivo, etc. The polymeric carrier cargo complex also provides for improved nucleic acid transfer in vivo, particularly via intradermal or intramuscular routes, including serum stability, salt stability, efficiency of uptake, reduced complement activation, nucleic acid release, etc. Such a polymeric carrier cargo complex furthermore may support induction and maintenance of an adaptive immune response by initiating or boosting a parallel innate immune response. It has been found that an improved adaptive immune response can further be obtained, in particular when the polymeric carrier cargo complex is administered in combination with a second nucleic acid molecule, preferably an RNA, encoding a protein or peptide, or when the polymeric carrier cargo complex is co-formulated in a pharmaceutical composition with a second nucleic acid molecule, preferably an RNA, encoding a protein or peptide, preferably an antigenic peptide or protein. It has proven as particularly beneficial in this respect to administer the pharmaceutical composition as defined herein or the polymeric carrier cargo complex in combination with the second nucleic acid molecule as defined herein via an intramuscular route. Additionally, the polymeric carrier cargo complex may exhibit improved storage stability, particularly during lyophilisation.

[0053] In particular, the polymeric carrier cargo complex as defined above enhances the immune response against a protein or peptide, which is encoded by a second nucleic acid molecule, preferably an RNA, more preferably an mRNA, that is administered in combination with the polymeric carrier cargo complex, preferably via an intramuscular route of administration.

[0054] The polymeric carrier cargo complex and/or the second nucleic acid molecule encoding a peptide or protein are preferably provided together with a pharmaceutically

acceptable carrier and/or vehicle. In the context of the present invention, a pharmaceutically acceptable carrier typically includes the liquid or non-liquid material, which is mixed with the polymeric carrier cargo complex and/or the second nucleic acid molecule. If the polymeric carrier cargo complex and/or the second nucleic acid molecule are provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or buffered aqueous solutions, e.g phosphate, citrate etc. buffered solutions. Ringer or Ringer-Lactate solution is particularly preferred as a liquid basis.

[0055] The phrase "administered in combination" as used herein refers to a situation, where the polymeric carrier cargo complex is administered to a subject before, concomittantly or after the administration of the second nucleic acid molecule encoding a protein or peptide to the same subject. Preferably, the time interval between the administration of the polymeric carrier cargo complex and the at least one second nucleic acid molecule, preferably an RNA, encoding a protein or peptide is less than about 48 hours, more preferably less than about 24 hours, 12 hours, 6 hours, 4 hours, 2 hours, 1 hour, most preferably less than about 30 minutes, 15 minutes or 5 minutes. In a particularly preferred embodiment, the phrase "administered in combination" refers to concomitant administration of the polymeric carrier cargo complex and the at least one second nucleic acid molecule, i.e. the simultaneous administration of both components or the administration of both components within a time frame that typically comprises less than 5 minutes. The phrase "administered in combination" does not only refer to a situation, where the pharmaceutical carrier cargo complex is in physical contact with the at least one second nucleic acid molecule or formulated together with said second nucleic acid molecule. The phrase "administered in combination" as used herein comprises also the separate administration of the polymeric carrier cargo complex and the second nucleic acid molecule (e.g. by two separate intramuscular injections), as long as the time interval between the two injections does not exceed the interval as defined above. Alternatively, the polymeric carrier cargo complex and the second nucleic acid molecule may be administered in combination by mixing the polymeric carrier cargo complex and the second nucleic acid molecule prior to administration and administering the mixture to a subject. When the polymeric carrier cargo complex is formulated together with the second nucleic acid molecule or when a pharmaceutical composition as defined herein is used, the polymeric carrier cargo complex and the second nucleic acid molecule may further, independently from each other, administered in combination via any of the administration routes as described herein.

[0056] According to a preferred embodiment, the second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex, is not comprised in the polymeric carrier cargo complex. More preferably, the second nucleic acid molecule is administered in combination with the polymeric carrier cargo complex as defined herein, without physically being a part or component of the polymeric carrier cargo complex. In particular, the second nucleic acid molecule is preferably not bound (e.g. covalently) to the polymeric carrier cargo complex. Further preferably, the at least one first nucleic acid molecule of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule, which is administered together with the polymeric carrier cargo complex, are not complexed by the same polymeric carrier.

[0057] In a further preferred embodiment, the present invention provides a polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly and wherein the polymeric carrier cargo complex and the second nucleic acid molecule are not administered together with a protein or peptide antigen selected from the group consisting of an antigen from a pathogen associated with infectious disease, an antigen associated with allergy or allergic disease, an antigen associated with autoimmune disease, an antigen associated with a cancer or tumour disease, or a fragment, variant and/or derivative of said protein or peptide antigen. More preferably, the present invention provides a polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly and wherein the polymeric carrier cargo complex and the second nucleic acid molecule are not administered together with a protein or peptide antigen.

[0058] The inventive polymeric carrier cargo complex as defined above comprises as one component a polymeric carrier formed by disulfide-crosslinked cationic components. The term "cationic component" typically refers to a charged molecule, which is positively charged (cation) at a pH value of about 1 to 9, preferably of a pH value of or below 9, of or below 8, of or below 7, most preferably at physiological pH values, e.g. about 7.3 to 7.4. Accordingly, a cationic peptide, protein or polymer according to the present invention is positively charged under physiological conditions, particularly under physiological pH value conditions of the cell in vivo. The term "cationic" may also refer to "oligocationic" or "polycationic" components. In the context of the present invention, the term "oligocationic" further refers to a compound, which preferably carries from two to five positive charges, i.e. which comprises from two to five cations, at a pH value of about 1 to 9, preferably of a pH value of or below 9, of or below 8, of or below 7, most preferably at physiological pH values, e.g. about 7.3 to 7.4. In this context, the term "polycationic" typically refers to a compound carrying at least six positive charges, i.e. comprising at least six cations, at a pH value of about 1 to 9, preferably of a pH value of or below 9, of or below 8, of or below 7, most preferably at physiological pH values, e.g. about 7.3 to 7.4.

[0059] Advantageously, in a cationic peptide or protein as used herein preferably at least 20% of the amino acid residues of said peptide or protein, more preferably at least 30% of the amino acid residues of said peptide or protein, even more preferably at least 40% of the amino acid residues of said peptide or protein, most preferably at least 50% of the amino acid residues of said protein or peptide are positively charged.

[0060] In this context the cationic components, which form the basis for the polymeric carrier of the inventive polymeric carrier cargo complex by disulfide-crosslinkage, are typically selected from any suitable cationic or polycationic peptide, protein or polymer suitable for this purpose,

particular any cationic or polycationic peptide, protein or polymer capable to complex a nucleic acid as defined according to the present invention, and thereby preferably condensing the nucleic acid. The cationic or polycationic peptide, protein or polymer, is preferably a linear molecule, however, branched cationic or polycationic peptides, proteins or polymers may also be used.

[0061] Each cationic or polycationic protein, peptide or polymer of the polymeric carrier contains at least one —SH moiety, most preferably at least one cysteine residue or any further chemical group exhibiting an —SH moiety, capable to form a disulfide linkage upon condensation with at least one further cationic or polycationic protein, peptide or polymer as cationic component of the polymeric carrier as mentioned herein.

[0062] Each cationic or polycationic protein, peptide or polymer or any further component of the polymeric carrier is preferably linked to its neighbouring component(s) (cationic proteins, peptides, polymers or other components) via disulfide-crosslinking. Preferably, the disulfide-crosslinking is a reversible disulfide bond (—S—S—) between at least one cationic or polycationic protein, peptide or polymer and at least one further cationic or polycationic protein, peptide or polymer or other component of the polymeric carrier. The disulfide-crosslinking is typically formed by condensation of —SH-moieties of the components of the polymeric carrier particularly of the cationic components. Such an —SH-moiety may be part of the structure of the cationic or polycationic protein, peptide or polymer or any further component of the polymeric carrier prior to disulfide-crosslinking or may be added prior to disulfide-crosslinking by a modification as defined below. In this context, the sulphurs adjacent to one component of the polymeric carrier, necessary for providing a disulfide bond, may be provided by the component itself, e.g. by a —SH moiety as defined herein or may be provided by modifying the component accordingly to exhibit a —SH moiety. These —SH-moieties are typically provided by each of the component, e.g. via a cysteine or any further (modified) amino acid or compound of the component, which carries a —SH moiety. In the case that the cationic component or any further component of the polymeric carrier is a peptide or protein it is preferred that the —SH moiety is provided by at least one cysteine residue. Alternatively, the component of the polymeric carrier may be modified accordingly with a —SH moiety, preferably via a chemical reaction with a compound carrying a —SH moiety, such that each of the components of the polymeric carrier carries at least one such —SH moiety. Such a compound carrying a —SH moiety may be e.g. an (additional) cysteine or any further (modified) amino acid or compound of the component of the polymeric carrier, which carries a —SH moiety. Such a compound may also be any non-amino compound or moiety, which contains or allows to introduce a —SH moiety into the component as defined herein. Such non-amino compounds may be attached to the component of the polymeric carrier according to the present invention via chemical reactions or binding of compounds, e.g. by binding of a 3-thio propionic acid or 2-iminothiolane (Traut's reagent), by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc.), by Michael addition (e.g maleinimide moieties, α,β unsatured carbonyls, etc.), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components. In some cases the —SH moiety may be masked by protecting groups during chemical attachment to the component. Such protecting groups are known in the art and may be removed after chemical coupling. In each case, the —SH moiety, e.g. of a cysteine or of any further (modified) amino acid or compound, may be present at the terminal ends or internally at any position of the component of the polymeric carrier. As defined herein, each of the components of the polymeric carrier typically exhibits at least one —SH-moiety, but may also contain two, three, four, five, or even more —SH-moieties. Additionally to binding of cationic components a —SH moiety may be used to attach further components of the polymeric carrier as defined herein, particularly an amino acid component, e.g. antigen epitopes, antigens, antibodies, cell penetrating peptides (e.g. TAT), ligands, etc.

[0063] As defined above, the polymeric carrier of the inventive polymeric carrier cargo molecule is formed by disulfide-crosslinked cationic (or polycationic) components.

[0064] According to one first alternative, at least one cationic (or polycationic) component of the polymeric carrier may be selected from cationic or polycationic peptides or proteins. Such cationic or polycationic peptides or proteins preferably exhibit a length of about 3 to 100 amino acids, preferably a length of about 3 to 50 amino acids, more preferably a length of about 3 to 25 amino acids, e.g. a length of about 3 to 10; 5 to 20; 5 to 15; 8 to 15, 16 or 17; 10 to 15, 16, 17, 18, 19, or 20; or 15 to 25 amino acids. Alternatively or additionally, such cationic or polycationic peptides or proteins may exhibit a molecular weight of about 0.1 kDa to about 100 kDa, including a molecular weight of about 0.5 kDa to about 100 kDa, preferably of about 10 kDa to about 50 kDa, even more preferably of about 10 kDa to about 30 kDa.

[0065] In the specific case that the cationic component of the polymeric carrier comprises a cationic or polycationic peptide or protein, the cationic properties of the cationic or polycationic peptide or protein or of the entire polymeric carrier, if the polymeric carrier is entirely composed of cationic or polycationic peptides or proteins, may be determined upon its content of cationic amino acids. Preferably, the content of cationic amino acids in the cationic or polycationic peptide or protein and/or the polymeric carrier is at least 10%, 20%, or 30%, preferably at least 40%, more preferably at least 50%, 60% or 70%, but also preferably at least 80%, 90%, or even 95%, 96%, 97%, 98%, 99% or 100%, most preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, or may be in the range of about 10% to 90%, more preferably in the range of about 15% to 75%, even more preferably in the range of about 20% to 50%, e.g. 20, 30, 40 or 50%, or in a range formed by any two of the afore mentioned values, provided, that the content of all amino acids, e.g. cationic, lipophilic, hydrophilic, aromatic and further amino acids, in the cationic or polycationic peptide or protein, or in the entire polymeric carrier, if the polymeric carrier is entirely composed of cationic or polycationic peptides or proteins, is 100%.

[0066] In this context, cationic amino acids are preferably the naturally occurring amino acids Arg (Arginine), Lys (Lysine), His (Histidine), and Orn (Ornithin). However, in a broader sense any non-natural amino acid carrying a cationic charge on its side chain may also be envisaged to carry out the invention. Preferably, however, are those cationic amino acids, the side chains of which are positively charged under physiological pH conditions. In a more preferred embodiment, these amino acids are Arg, Lys, and Orn.

[0067] Preferably, such cationic or polycationic peptides or proteins of the polymeric carrier, which comprise or are additionally modified to comprise at least one —SH moeity, are selected from, without being restricted thereto, cationic peptides or proteins such as protamine, nucleoline, spermine or spermidine, oligo- or poly-L-lysine (PLL), basic polypeptides, oligo or poly-arginine, cell penetrating peptides (CPPs), chimeric CPPs, such as Transportan, or MPG peptides, HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, members of the penetratin family, e.g. Penetratin, Antennapedia-derived peptides (particularly from *Drosophila* antennapedia), pAntp, pIsl, etc., antimicrobial-derived CPPs e.g. Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, MAP, KALA, PpTG20, Loligomere, FGF, Lactoferrin, histones, VP22 derived or analog peptides, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs), PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, Pep-1, L-oligomers, Calcitonin peptide(s), etc.

[0068] Alternatively or additionally, such cationic or polycationic peptides or proteins of the polymeric carrier, which comprise or are additionally modified to comprise at least one —SH moeity, are selected from, without being restricted thereto, following cationic peptides having the following sum formula (I):

$$\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\};$$

[0069] wherein l+m+n+o+x=3-100, and l, m, n or o independently of each other is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100 provided that the overall content of Arg (Arginine), Lys (Lysine), His (Histidine) and Orn (Ornithine) represents at least 10% of all amino acids of the oligopeptide; and Xaa is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and x is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, provided, that the overall content of Xaa does not exceed 90% of all amino acids of the oligopeptide. Any of amino acids Arg, Lys, His, Orn and Xaa may be positioned at any place of the peptide. In this context cationic peptides or proteins in the range of 7-30 amino acids are particular preferred. Even more preferred peptides of this formula are oligoarginines such as e.g. Arg_7, Arg_8, Arg_9, Arg_{12}, His_3Arg_9, Arg_9His_3, $His_3Arg_9His_3$, $His_6Arg_9His_6$, $His_3Arg_4His_3$, $His_6Arg_4His_6$, $TyrSer_2Arg_9Ser_2Tyr$, $(ArgLysHis)_4$, $Tyr(ArgLysHis)_2Arg$, etc.

[0070] According to a particular preferred embodiment, such cationic or polycationic peptides or proteins of the polymeric carrier having the empirical sum formula (I) as shown above, may, without being restricted thereto, comprise at least one of the following subgroup of formulae:

Arg_7, Arg_8, Arg_9, Arg_{10}, Arg_{11}, Arg_{12}, Arg_{13}, Arg_{14}, Arg_{15-30};

Lys_7, Lys_8, Lys_9, Lys_{10}, Lys_{11}, Lys_{12}, Lys_{13}, Lys_{14}, Lys_{15-30};

His_7, His_8, His_9, His_{10}, His_{11}, His_{12}, His_{13}, His_{14}, His_{15-30};

Orn_7, Orn_8, Orn_9, Orn_{10}, Orn_{11}, Orn_{12}, Orn_{13}, Orn_{14}, Orn_{15-30};

[0071] According to a further particularly preferred embodiment, cationic or polycationic peptides or proteins of the polymeric carrier, having the empirical sum formula (I) as shown above and which comprise or are additionally modified to comprise at least one —SH moeity, may be preferably selected from, without being restricted thereto, at least one of the following subgroup of formulae. The following formulae (as with empirical formula (I)) do not specify any amino acid order, but are intended to reflect empirical formulae by exclusively specifying the (number of) amino acids as components of the respective peptide. Accordingly, as an example, empirical formula $Arg_{(7-29)}Lys_1$ is intended to mean that peptides falling under this formula contain 7 to 19 Arg residues and 1 Lys residue of whatsoever order. If the peptides contain 7 Arg residues and 1 Lys residue, all variants having 7 Arg residues and 1 Lys residue are encompassed. The Lys residue may therefore be positioned anywhere in the e.g. 8 amino acid long sequence composed of 7 Arg and 1 Lys residues. The subgroup preferably comprises:

$Arg_{(4-29)}Lys_1$, $Arg_{(4-29)}His_1$, $Arg_{(4-29)}Orn_1$, $Lys_{(4-29)}His_1$, $Lys_{(4-29)}Orn_1$, $His_{(4-29)}Orn_1$, $Arg_{(3-28)}Lys_2$, $Arg_{(3-28)}His_2$, $Arg_{(3-28)}Orn_2$, $Lys_{(3-28)}His_2$, $Lys_{(3-28)}Orn_2$, $His_{(3-28)}Orn_2$, $Arg_{(2-27)}Lys_3$, $Arg_{(2-27)}His_3$, $Arg_{(2-27)}Orn_3$, $Lys_{(2-27)}His_3$, $Lys_{(2-27)}Orn_3$, $His_{(2-27)}Orn_3$, $Arg_{(1-26)}Lys_4$, $Arg_{(1-26)}His_4$, $Arg_{(1-26)}Orn_4$, $Lys_{(1-26)}His_4$, $Lys_{(1-26)}Orn_4$, $His_{(1-26)}Orn_4$, $Arg_{(3-28)}Lys_1His_1$, $Arg_{(3-28)}Lys_1Orn_1$, $Arg_{(3-28)}His_1Orn_1$, $Arg_1Lys_{(3-28)}His_1$, $Arg_1Lys_{(3-28)}Orn_1$, $Lys_{(3-28)}His_1Orn_1$, $Arg_1Lys_1His_{(3-28)}$, $Arg_1His_{(3-28)}Orn_1$, $Lys_1His_{(3-28)}Orn_1$;

$Arg_{(2-27)}Lys_2His_1$, $Arg_{(2-27)}Lys_1His_2$, $Arg_{(2-27)}Lys_2Orn_1$, $Arg_{(2-27)}Lys_1Orn_2$, $Arg_{(2-27)}His_2Orn_1$, $Arg_{(2-27)}His_1Orn_2$, $Arg_2Lys_{(2-27)}His_1$, $Arg_1Lys_{(2-27)}His_2$,

-continued

$Arg_2Lys_{(2-27)}Orn_1$, $Arg_1Lys_{(2-27)}Orn_2$,

$Lys_{(2-27)}His_{2Orn1}$, $Lys_{(2-27)}His_1Orn_2$,

$Arg_2Lys_1His_{(2-27)}$, $Arg_1Lys_2His_{(2-27)}$,

$Arg_2His_{(2-27)}Orn_1$, $Arg_1His_{(2-27)}Orn_2$,

$Lys_2His_{(2-27)}Orn_1$, $Lys_1His_{(2-27)}Orn_2$;

$Arg_{(1-26)}Lys_3His_1$, $Arg_{(1-26)}Lys_2His_2$,

$Arg_{(1-26)}Lys_1His_3$, $Arg_{(1-26)}Lys_3Orn_1$,

$Arg_{(1-26)}Lys_2Orn_2$, $Arg_{(1-26)}Lys_1Orn_3$,

$Arg_{(1-26)}His_3Orn_1$, $Arg_{(1-26)}His_2Orn_2$,

$Arg_{(1-26)}His_1Orn_3$, $Arg_3Lys_{(1-26)}His_1$,

$Arg_2Lys_{(1-26)}His_2$, $Arg_1Lys_{(1-26)}His_3$,

$Arg_3Lys_{(1-26)}Orn_1$, $Arg_2Lys_{(1-26)}Orn_2$,

$Arg_1Lys_{(1-26)}Orn_3$, $Lys_{(1-26)}His_3Orn_1$,

$Lys_{(1-26)}His_2Orn_2$, $Lys_{(1-26)}His_1Orn_3$,

$Arg_3Lys_1His_{(1-26)}$, $Arg_2Lys_2His_{(1-26)}$,

$Arg_1Lys_3His_{(1-26)}$, $Arg_3His_{(1-26)}Orn_1$,

$Arg_2His_{(1-26)}Orn_2$, $Arg_1His_{(1-26)}Orn_3$,

$Lys_3His_{(1-26)}Orn_1$, $Lys_2His_{(1-26)}Orn_2$,

$Lys_1His_{(1-26)}Orn_3$;

$Arg_{(2-27)}Lys_1His_1Orn_1$, $Arg_1Lys_{(2-27)}His_1Orn_1$,

$Arg_1Lys_1His_{(2-27)}Orn_1$, $Arg_1Lys_1His_1Orn_{(2-27)}$;

$Arg_{(1-26)}Lys_2His_1Orn_1$, $Arg_{(1-26)}Lys_1His_2Orn_1$,

$Arg_{(1-26)}Lys_1His_1Orn_2$, $Arg_2Lys_{(1-26)}His_1Orn_1$,

$Arg_1Lys_{(1-26)}His_2Orn_1$, $Arg_1Lys_{(1-26)}His_1Orn_2$,

$Arg_2LyS_1His_{(1-26)}Orn_1$, $Arg_1Lys_2His_{(1-26)}Orn_1$,

$Arg_1Lys_1His_{(1-26)}Orn_2$, $Arg_2Lys_1His_1Orn_{(1-26)}$,

$Arg_1Lys_2His_1Orn_{(1-26)}$, $Arg_1Lys_1His_2Orn_{(1-26)}$;

[0072] According to a further particular preferred embodiment, cationic or polycationic peptides or proteins of the polymeric carrier, having the empirical sum formula (I) as shown above and which comprise or are additionally modified to comprise at least one —SH moeity, may be, without being restricted thereto, selected from the subgroup consisting of generic formulas Arg_7 (also termed as R_7), Arg_9 (also termed R_9), Arg_{12} (also termed as R_{12}).

[0073] According to a one further particular preferred embodiment, the cationic or polycationic peptide or protein of the polymeric carrier, when defined according to formula $\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}$ (formula (I)) as shown above and which comprise or are additionally modified to comprise at least one —SH moeity, may be, without being restricted thereto, selected from subformula (Ia):

$$\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa')_x(Cys)_y\} \qquad \text{formula (Ia)}$$

wherein $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o$; and x are as defined herein, Xaa' is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His, Orn or Cys and y is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80 and 81-90, provided that the overall content of Arg (Arginine), Lys (Lysine), His (Histidine) and Orn (Ornithine) represents at least 10% of all amino acids of the oligopeptide.

[0074] This embodiment may apply to situations, wherein the cationic or polycationic peptide or protein of the polymeric carrier, e.g. when defined according to empirical formula $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$ (formula (I)) as shown above, comprises or has been modified with at least one cysteine as —SH moiety in the above meaning such that the cationic or polycationic peptide as cationic component carries at least one cysteine, which is capable to form a disulfide bond with other components of the polymeric carrier.

[0075] According to another particular preferred embodiment, the cationic or polycationic peptide or protein of the polymeric carrier, when defined according to formula $\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}$ (formula (I)) as shown above, may be, without being restricted thereto, selected from subformula (Ib):

$$Cys_1\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}Cys_2 \qquad \text{(formula (Ib))}$$

wherein empirical formula $\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}$ (formula (I)) is as defined herein and forms a core of an amino acid sequence according to (semiempirical) formula (I) and wherein Cys_1 and Cys_2 are Cysteines proximal to, or terminal to $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$. Exemplary examples may comprise any of the above sequences flanked by two Cys and following sequences:

(SEQ ID NOs: 1-14)

$Cys(Arg_7)Cys$, $Cys(Arg_8)Cys$, $Cys(Arg_9)Cys$,

$Cys(Arg_{10})Cys$, $Cys(Arg_{11})Cys$, $Cys(Arg_{12})Cys$,

$Cys(Arg_{13})Cys$, $Cys(Arg_{14})Cys$, $Cys(Arg_{15})Cys$,

$Cys(Arg_{16})Cys$, $Cys(Arg_{17})Cys$, $Cys(Arg_{18})Cys$,

$Cys(Arg_{19})Cys$, $Cys(Arg_{20})Cys$:

$CysArg_7Cys$

(SEQ ID NO. 1)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_8Cys$

(SEQ ID NO. 2)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_9Cys$:

(SEQ ID NO. 3)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{10}Cys$

(SEQ ID NO. 4)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{11}Cys$

(SEQ ID NO. 5)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{12}Cys$:

(SEQ ID NO. 6)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

-continued

$CysArg_{13}Cys$:

(SEQ ID NO. 7)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{14}Cys$:

(SEQ ID NO. 8)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{15}Cys$:

(SEQ ID NO. 9)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{16}Cys$:

(SEQ ID NO. 10)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{17}Cys$:

(SEQ ID NO. 11)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{18}Cys$:

(SEQ ID NO. 12)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{19}Cys$:

(SEQ ID NO. 13)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys

$CysArg_{20}Cys$:

(SEQ ID NO. 14)

Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg Cys

[0076] This embodiment may apply to situations, wherein the cationic or polycationic peptide or protein of the polymeric carrier, e.g. when defined according to empirical formula $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$ (formula (I)) as shown above, has been modified with at least two cysteines as —SH moieties in the above meaning such that the cationic or polycationic peptide of the inventive polymeric carrier cargo complex as cationic component carries at least two (terminal) cysteines, which are capable to form a disulfide bond with other components of the polymeric carrier.

[0077] In a preferred embodiment, the polymeric carrier cargo complex comprises a carrier, which comprises or consists of the peptide $CysArg_{12}Cys$ (SEQ ID NO: 6). Therein, the peptide having the sequence according to SEQ ID NO: 6 is preferably further modified by an amino acid component (AA) as defined herein.

[0078] According to a second alternative, at least one cationic (or polycationic) component of the polymeric carrier may be selected from e.g. any (non-peptidic) cationic or polycationic polymer suitable in this context, provided that this (non-peptidic) cationic or polycationic polymer exhibits or is modified to exhibit at least one —SH-moiety, which provide for a disulfide bond linking the cationic or polycationic polymer with another component of the polymeric carrier as defined herein. Thus, likewise as defined herein, the polymeric carrier may comprise the same or different cationic or polycationic polymers.

[0079] In the specific case that the cationic component of the polymeric carrier comprises a (non-peptidic) cationic or polycationic polymer the cationic properties of the (non-peptidic) cationic or polycationic polymer may be determined upon its content of cationic charges when compared to the overall charges of the components of the cationic polymer. Preferably, the content of cationic charges in the cationic polymer at a (physiological) pH as defined herein is at least 10%, 20%, or 30%, preferably at least 40%, more preferably at least 50%, 60% or 70%, but also preferably at least 80%, 90%, or even 95%, 96%, 97%, 98%, 99% or 100%, most preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, or may be in the range of about 10% to 90%, more preferably in the range of about 30% to 100%, even preferably in the range of about 50% to 100%, e.g. 50, 60, 70, 80%, 90% or 100%, or in a range formed by any two of the afore mentioned values, provided, that the content of all charges, e.g. positive and negative charges at a (physiological) pH as defined herein, in the entire cationic polymer is 100%.

[0080] Preferably, the (non-peptidic) cationic component of the polymeric carrier represents a cationic or polycationic polymer, typically exhibiting a molecular weight of about 0.1 or 0.5 kDa to about 100 kDa, preferably of about 1 kDa to about 75 kDa, more preferably of about 5 kDa to about 50 kDa, even more preferably of about 5 kDa to about 30 kDa, or a molecular weight of about 10 kDa to about 50 kDa, even more preferably of about 10 kDa to about 30 kDa. Additionally, the (non-peptidic) cationic or polycationic polymer typically exhibits at least one —SH-moiety, which is capable to form a disulfide linkage upon condensation with either other cationic components or other components of the polymeric carrier as defined herein.

[0081] In the above context, the (non-peptidic) cationic component of the polymeric carrier may be selected from acrylates, modified acrylates, such as pDMAEMA (poly (dimethylaminoethyl methylacrylate)), chitosanes, aziridines or 2-ethyl-2-oxazoline (forming oligo ethylenimines or modifed oligoethylenimines), polymers obtained by reaction of bisacrylates with amines forming oligo beta aminoesters or poly amido amines, or other polymers like polyesters, polycarbonates, etc. Each molecule of these (non-peptidic) cationic or polycationic polymers typically exhibits at least one —SH-moiety, wherein these at least one —SH-moiety may be introduced into the (non-peptidic) cationic or polycationic polymer by chemical modifications, e.g. using imonothiolan, 3-thio propionic acid or introduction of —SH-moieties containing amino acids, such as cysteine or any further (modified) amino acid. Such —SH-moieties are preferably as already defined above.

[0082] In the context of the polymeric carrier, the cationic components, which form basis for the polymeric carrier by disulfide-crosslinkage, may be the same or different from each other. It is also particularly preferred that the polymeric carrier of the present invention comprises mixtures of cationic peptides, proteins or polymers and optionally further components as defined herein, which are crosslinked by disulfide bonds as described herein.

[0083] In this context, the inventive polymeric carrier cargo complex due to its variable polymeric carrier advan-

tageously allows to combine desired properties of different (short) cationic or polycationic peptides, proteins or polymers or other components. The polymeric carrier, e.g., allows to efficiently compact nucleic acids for the purpose of efficient transfection of nucleic acids, for adjuvant therapy, for the purposes of gene therapy, for gene knock-down or others strategies without loss of activity, particularly exhibiting an efficient transfection of a nucleic acid into different cell lines in vitro but particularly transfection in vivo. The polymeric carrier and thus the inventive polymeric carrier cargo complex is furthermore not toxic to cells, provides for efficient release of its nucleic acid cargo, is stable during lyophilization and is applicable as immunostimulating agent or adjuvant. Preferably, the polymer carrier cargo complex may induce the anti-viral cytokine IFN-alpha.

[0084] In particular, the polymeric carrier formed by disulfide-linked cationic components allows considerably to vary its peptide or polymeric content and thus to modulate its biophysical/biochemical properties, particularly the cationic properties of the polymeric carrier, quite easily and fast, e.g. by incorporating as cationic components the same or different cationic peptide(s) or polymer(s) and optionally adding other components into the polymeric carrier. Even though consisting of quite small non-toxic monomer units the polymeric carrier forms a long cationic binding sequence providing a strong condensation of the nucleic acid cargo and complex stability. Under the reducing conditions of the cytosol (e.g. cytosolic GSH), the complex is rapidly degraded into its (cationic) components, which are further degraded (e.g. into oligopeptides). This supports the liberation of the nucleic acid cargo in the cytosol. Due to degradation into small oligopeptides or polymers in the cytosol, no toxicity is observed as known for high-molecular oligopeptides or polymers, e.g. from high-molecular polyarginine.

[0085] Accordingly, the polymeric carrier of the inventive polymeric carrier cargo complex may comprise different (short) cationic or polycationic peptides, proteins or polymers selected from cationic or polycationic peptides, proteins or (non-peptidic) polymers as defined above, optionally together with further components as defined herein.

[0086] Additionally, the polymeric carrier of the inventive polymeric carrier cargo complex as defined above, more preferably at least one of the different (short) cationic or polycationic peptides or (non-peptidic) polymers forming basis for the polymeric carrier via disulfide-crosslinking, may be, preferably prior to the disulfide-crosslinking, be modified with at least one further component. Alternatively, the polymeric carrier as such may be modified with at least one further component. It may also optionally comprise at least one further component, which typically forms the polymeric carrier disulfide together with the other the (short) cationic or polycationic peptides as defined above via disulfide crosslinking.

[0087] To allow modification of a cationic or polycationic peptide or a (non-peptidic) polymer as defined above, each of the components of the polymeric carrier may (preferably already prior to disulfide-crosslinking) also contain at least one further functional moiety, which allows attaching such further components as defined herein. Such functional moieties may be selected from functionalities which allow the attachment of further components, e.g. functionalities as defined herein, e.g. by amide formation (e.g. carboxylic acids, sulphonic acids, amines, etc.), by Michael addition (e.g maleinimide moieties, α,β unsatured carbonyls, etc.), by click chemistry (e.g. azides or alkines), by alkene/alkine methatesis (e.g. alkenes or alkines), imine or hydrozone formation (aldehydes or ketons, hydrazins, hydroxylamins, amines), complexation reactions (avidin, biotin, protein G) or components which allow S_n-type substitution reactions (e.g halogenalkans, thiols, alcohols, amines, hydrazines, hydrazides, sulphonic acid esters, oxyphosphonium salts) or other chemical moieties which can be utilized in the attachment of further components.

[0088] According to a particularly preferred embodiment, the further component, which may be contained in the polymeric carrier or which may be used to modify the different (short) cationic or polycationic peptides or (non-peptidic) polymers forming basis for the polymeric carrier of the inventive polymeric carrier cargo complex is an amino acid component (AA), which may e.g. modify the biophysical/biochemical properties of the polymeric carrier as defined herein. According to the present invention, the amino acid component (AA) comprises a number of amino acids preferably in a range of about 1 to 100, preferably in a range of about 1 to 50, more preferably selected from a number comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15-20, or may be selected from a range formed by any two of the afore mentioned values. In this context the amino acids of amino acid component (AA) can be chosen independently from each other. For example if in the polymeric carrier two or more (AA) components are present they can be the same or can be different from each other.

[0089] The amino acid component (AA) may contain or may be flanked (e.g. terminally) by a —SH containing moiety, which allows introducing this component (AA) via a disulfide bond into the polymeric carrier as defined herein. In the specific case that the —SH containing moiety represents a cysteine, the amino acid component (AA) may also be read as -Cys-(AA)-Cys- wherein Cys represents Cysteine and provides for the necessary —SH-moiety for a disulfide bond. The —SH containing moiety may be also introduced into amino acid component (AA) using any of modifications or reactions as shown above for the cationic component or any of its components.

[0090] Furthermore, the amino acid component (AA) may be provided with two —SH-moieties (or even more), e.g. in a form represented by formula HS-(AA)-SH to allow binding to two functionalities via disulfide bonds, e.g. if the amino acid component (AA) is used as a linker between two further components (e.g. as a linker between two cationic polymers). In this case, one —SH moiety is preferably protected in a first step using a protecting group as known in the art, leading to an amino acid component (AA) of formula HS-(AA)-S-protecting group. Then, the amino acid component (AA) may be bound to a further component of the polymeric carrier, to form a first disulfide bond via the non-protected —SH moiety. The protected —SH-moiety is then typically deprotected and bound to a further free —SH-moiety of a further component of the polymeric carrier to form a second disulfide bond.

[0091] Alternatively, the amino acid component (AA) may be provided with other functionalities as already described above for the other components of the polymeric carrier, which allow binding of the amino acid component (AA) to any of components of the polymeric carrier.

[0092] Thus, according to the present invention, the amino acid component (AA) may be bound to further components

of the polymeric carrier with or without using a disulfide linkage. Binding without using a disulfide linkage may be accomplished by any of the reactions described above, preferably by binding the amino acid component (AA) to the other component of the polymeric carrier using an amid-chemistry as defined herein. If desired or necessary, the other terminus of the amino acid component (AA), e.g. the N- or C-terminus, may be used to couple another component, e.g. a ligand L. For this purpose, the other terminus of the amino acid component (AA) preferably comprises or is modified to comprise a further functionality, e.g. an alkyn-species (see above), which may be used to add the other component via e.g. click-chemistry. If the ligand is bound via an acid-labile bond, the bond is preferably cleaved off in the endosome and the polymeric carrier presents amino acid component (AA) at its surface.

[0093] The amino acid component (AA) may occur as a further component of the polymeric carrier as defined above, e.g. as a linker between cationic components e.g. as a linker between one cationic peptide and a further cationic peptide, as a linker between one cationic polymer and a further cationic polymer, as a linker between one cationic peptide and a cationic polymer, all preferably as defined herein, or as an additional component of the polymeric carrier, e.g. by binding the amino acid component (AA) to the polymeric carrier or a component thereof, e.g. via side chains, SH-moieties or via further moieties as defined herein, wherein the amino acid component (AA) is preferably accordingly modified.

[0094] According to a further and particularly preferred alternative, the amino acid component (AA), may be used to modify the polymeric carrier, particularly the content of cationic components in the polymeric carrier as defined above.

[0095] In this context it is preferable, that the content of cationic components in the polymeric carrier is at least 10%, 20%, or 30%, preferably at least 40%, more preferably at least 50%, 60% or 70%, but also preferably at least 80%, 90%, or even 95%, 96%, 97%, 98%, 99% or 100%, most preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, or may be in the range of about 30% to 100%, more preferably in the range of about 50% to 100%, even preferably in the range of about 70% to 100%, e.g. 70, 80, 90 or 100%, or in a range formed by any two of the afore mentioned values, provided, that the content of all components in the polymeric carrier is 100%.

[0096] In the context of the present invention, the amino acid component (AA) may be selected from the following alternatives.

[0097] According to a first alternative, the amino acid component (AA) may be an aromatic amino acid component (AA). The incorporation of aromatic amino acids or sequences as amino aromatic acid component (AA) into the polymeric carrier of the present invention enables a different (second) binding of the polymeric carrier to the nucleic acid due to interactions of the aromatic amino acids with the bases of the nucleic acid cargo in contrast to the binding thereof by cationic charged sequences of the polymeric carrier molecule to the phosphate backbone. This interaction may occur e.g. by intercalations or by minor or major groove binding. This kind of interaction is not prone to decompaction by anionic complexing partners (e.g. Heparin, Hyaluronic acids) which are found mainly in the extracellular matrix in vivo and is also less susceptible to salt effects.

[0098] For this purpose, the amino acids in the aromatic amino acid component (AA) may be selected from either the same or different aromatic amino acids e.g. selected from Trp, Tyr, or Phe.

[0099] Alternatively, the amino acids (or the entire aromatic amino acid component (AA)) may be selected from following peptide combinations Trp-Tyr, Tyr-Trp, Trp-Trp, Tyr-Tyr, Trp-Tyr-Trp, Tyr-Trp-Tyr, Trp-Trp-Trp, Tyr-Tyr-Tyr, Trp-Tyr-Trp-Tyr, Tyr-Trp-Tyr-Trp, Trp-Trp-Trp-Trp, Phe-Tyr, Tyr-Phe, Phe-Phe, Phe-Tyr-Phe, Tyr-Phe-Tyr, Phe-Phe-Phe, Phe-Tyr-Phe-Tyr, Tyr-Phe-Tyr-Phe, Phe-Phe-Phe-Phe, Phe-Trp, Trp-Phe, Phe-Phe, Phe-Trp-Phe, Trp-Phe-Trp, Phe-Trp-Phe-Trp, Trp-Phe-Trp-Phe, or Tyr-Tyr-Tyr-Tyr, etc. (SEQ ID NOs: 15-42). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0100] Additionally, the aromatic amino acid component (AA) may contain or may be flanked by a —SH containing moiety, which allows introducing this component via a disulfide bond as a further part of the polymeric carrier as defined above, e.g. as a linker. Such a —SH containing moiety may be any moiety as defined herein suitable to couple one component as defined herein to a further component as defined herein. As an example, such a —SH containing moiety may be a cysteine. Then, e.g. the aromatic amino acid component (AA) may be selected from e.g. peptide combinations Cys-Tyr-Cys, Cys-Trp-Cys, Cys-Trp-Tyr-Cys, Cys-Tyr-Trp-Cys, Cys-Trp-Trp-Cys, Cys-Tyr-Tyr-Cys, Cys-Trp-Tyr-Trp-Cys, Cys-Tyr-Trp-Tyr-Cys, Cys-Trp-Trp-Trp-Cys, Cys-Tyr-Tyr-Tyr-Cys, Cys-Trp-Tyr-Trp-Tyr-Cys, Cys-Tyr-Trp-Tyr-Trp-Cys, Cys-Trp-Trp-Trp-Trp-Cys, Cys-Tyr-Tyr-Tyr-Tyr-Cys, Cys-Phe-Cys, Cys-Phe-Tyr-Cys, Cys-Tyr-Phe-Cys, Cys-Phe-Phe-Cys, Cys-Tyr-Tyr-Cys, Cys-Phe-Tyr-Phe-Cys, Cys-Tyr-Phe-Tyr-Cys, Cys-Phe-Phe-Phe-Cys, Cys-Tyr-Tyr-Tyr-Cys, Cys-Phe-Tyr-Phe-Tyr-Cys, Cys-Tyr-Phe-Tyr-Phe-Cys, or Cys-Phe-Phe-Phe-Phe-Cys, Cys-Phe-Trp-Cys, Cys-Trp-Phe-Cys, Cys-Phe-Phe-Cys, Cys-Phe-Trp-Phe-Cys, Cys-Trp-Phe-Trp-Cys, Cys-Phe-Trp-Phe-Trp-Cys, Cys-Trp-Phe-Trp-Phe-Cys, etc. Each Cys above may also be replaced by any modified peptide or chemical compound carrying a free —SH-moiety as defined herein. (SEQ ID NOs: 43-75) Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0101] Additionally, the aromatic amino acid component (AA) may contain or represent at least one proline, which may serve as a structure breaker of longer sequences of Trp, Tyr and Phe in the aromatic amino acid component (AA), preferably two, three or more prolines.

[0102] According to a second alternative, the amino acid component (AA) may be a hydrophilic (and preferably non charged polar) amino acid component (AA). The incorporation of hydrophilic (and preferably non charged polar) amino acids or sequences as amino hydrophilic (and preferably non charged polar) acid component (AA) into the polymeric carrier of the present invention enables a more flexible binding to the nucleic acid cargo. This leads to a more effective compaction of the nucleic acid cargo and hence to a better protection against nucleases and unwanted decompaction. It also allows provision of a (long) polymeric carrier which exhibits a reduced cationic charge over the

entire carrier and in this context to better adjusted binding properties, if desired or necessary.

[0103] For this purpose, the amino acids in the hydrophilic (and preferably non charged polar) amino acid component (AA) may be selected from either the same or different hydrophilic (and preferably non charged polar) amino acids e.g. selected from Thr, Ser, Asn or Gln. Alternatively, the amino acids (or the entire hydrophilic (and preferably non charged polar) amino acid component (AA)) may be selected from following peptide combinations Ser-Thr, Thr-Ser, Ser-Ser, Thr-Thr, Ser-Thr-S er, Thr-Ser-Thr, Ser-Ser-Ser, Thr-Thr-Thr, Ser-Thr-Ser-Thr, Thr-Ser-Thr-Ser, Ser-Ser-Ser-Ser, Thr-Thr-Thr-Thr, Gln-Asn, Asn-Gln, Gln-Gln, Asn-Asn, Gln-Asn-Gln, Asn-Gln-Asn, Gln-Gln-Gln, Asn-Asn-Asn, Gln-Asn-Gln-Asn, Asn-Gln-Asn-Gln, Gln-Gln-Gln-Gln, Asn-Asn-Asn-Asn, Ser-Asn, Asn-Ser, Ser-Ser, Asn-Asn, Ser-Asn-Ser, Asn-Ser-Asn, Ser-Ser-Ser, Asn-Asn-Asn, Ser-Asn-Ser-Asn, Asn-Ser-Asn-Ser, Ser-Ser-Ser-Ser, or Asn-Asn-Asn-Asn, etc. (SEQ ID NOs: 76-111). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0104] Additionally, the hydrophilic (and preferably non-charged polar) amino acid component (AA) may contain or may be flanked by a —SH containing moiety, which allows introducing this component via a disulfide bond as a further part of generic formula (I) above, e.g. as a linker. Such a —SH containing moiety may be any moiety as defined herein suitable to couple one component as defined herein to a further component as defined herein. As an example, such a —SH containing moiety may be a cysteine. Then, e.g. the hydrophilic (and preferably non-charged polar) amino acid component (AA) may be selected from e.g. peptide combinations Cys-Thr-Cys, Cys-Ser-Cys, Cys-Ser-Thr-Cys, Cys-Thr-Ser-Cys, Cys-Ser-Ser-Cys, Cys-Thr-Thr-Cys, Cys-Ser-Thr-Ser-Cys, Cys-Thr-Ser-Thr-Cys, Cys-Ser-Ser-Ser-Cys, Cys-Thr-Thr-Thr-Cys, Cys-Ser-Thr-Ser-Thr-Cys, Cys-Thr-Ser-Thr-Ser-Cys, Cys-Ser-Ser-Ser-Ser-Cys, Cys-Thr-Thr-Thr-Thr-Cys, Cys-Asn-Cys, Cys-Gln-Cys, Cys-Gln-Asn-Cys, Cys-Asn-Gln-Cys, Cys-Gln-Gln-Cys, Cys-Asn-Asn-Cys, Cys-Gln-Asn-Gln-Cys, Cys-Asn-Gln-Asn-Cys, Cys-Gln-Gln-Gln-Cys, Cys-Asn-Asn-Asn-Cys, Cys-Gln-Asn-Gln-Asn-Cys, Cys-Asn-Gln-Asn-Gln-Cys, Cys-Gln-Gln-Gln-Gln-Cys, Cys-Asn-Asn-Asn-Asn-Cys, Cys-Asn-Cys, Cys-Ser-Cys, Cys-Ser-Asn-Cys, Cys-Asn-Ser-Cys, Cys-Ser-Ser-Cys, Cys-Asn-Asn-Cys, Cys-Ser-Asn-Ser-Cys, Cys-Asn-Ser-Asn-Cys, Cys-Ser-Ser-Ser-Cys, Cys-Asn-Asn-Asn-Cys, Cys-Ser-Asn-Ser-Asn-Cys, Cys-Asn-Ser-Asn-Ser-Cys, Cys-Ser-Ser-Ser-Ser-Cys, or Cys-Asn-Asn-Asn-Asn-Cys, etc. Each Cys above may also be replaced by any modified peptide or chemical compound carrying a free —SH-moiety as defined herein (SEQ ID NOs: 112-153). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0105] Additionally, the hydrophilic (and preferably non-charged polar) amino acid component (AA) may contain at least one proline, which may serve as a structure breaker of longer sequences of Ser, Thr and Asn in the hydrophilic (and preferably non charged polar) amino acid component (AA), preferably two, three or more prolines.

[0106] According to a third alternative, the amino acid component (AA) may be a lipohilic amino acid component (AA). The incorporation of lipohilic amino acids or sequences as amino lipohilic acid component (AA) into the polymeric carrier of the present invention enables a stronger compaction of the nucleic acid cargo and/or the polymeric carrier and its nucleic acid cargo when forming a complex. This is particularly due to interactions of one or more polymer strands of the polymeric carrier, particularly of lipophilic sections of lipohilic amino acid component (AA) and the nucleic acid cargo. This interaction will preferably add an additional stability to the complex between the polymeric carrier and its nucleic acid cargo. This stabilization may somehow be compared to a sort of non covalent crosslinking between different polymer strands. Especially in aqueous environment this interaction is typically strong and provides a significant effect.

[0107] For this purpose, the amino acids in the lipophilic amino acid component (AA) may be selected from either the same or different lipophilic amino acids e.g. selected from Leu, Val, Ile, Ala, Met. Alternatively, the amino acid AA (or the entire lipophilic amino acid component (AA)) may be selected from following peptide combinations Leu-Val, Val-Leu, Leu-Leu, Val-Val, Leu-Val-Leu, Val-Leu-Val, Leu-Leu-Leu, Val-Val-Val, Leu-Val-Leu-Val, Val-Leu-Val-Leu, Leu-Leu-Leu-Leu, Val-Val-Val-Val, Ile-Ala, Ala-Ile, Ile-Ile, Ala-Ala, Ile-Ala-Ile, Ala-Ile-Ala, Ile-Ile-Ile, Ala-Ala-Ala, Ile-Ala-Ile-Ala, Ala-Ile-Ala-Ile, Ile-Ile-Ile-Ile, Ala-Ala-Ala-Ala, Met-Ala, Ala-Met, Met-Met, Ala-Ala, Met-Ala-Met, Ala-Met-Ala, Met-Met-Met, Ala-Ala-Ala, Met-Ala-Met-Ala, Ala-Met-Ala-Met, or Met-Met-Met-Met etc. (SEQ ID NOs: 154-188). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0108] Additionally, the lipophilic amino acid component (AA) may contain or may be flanked by a —SH containing moiety, which allows introducing this component via a disulfide bond as a further part of the polymeric carrier above, e.g. as a linker. Such a —SH containing moiety may be any moiety as defined herein suitable to couple one component as defined herein to a further component as defined herein. As an example, such a —SH containing moiety may be a cysteine. Then, e.g. the lipophilic amino acid component (AA) may be selected from e.g. peptide combinations Cys-Val-Cys, Cys-Leu-Cys, Cys-Leu-Val-Cys, Cys-Val-Leu-Cys, Cys-Leu-Leu-Cys, Cys-Val-Val-Cys, Cys-Leu-Val-Leu-Cys, Cys-Val-Leu-Val-Cys, Cys-Leu-Leu-Leu-Cys, Cys-Val-Val-Val-Cys, Cys-Leu-Val-Leu-Val-Cys, Cys-Val-Leu-Val-Leu-Cys, Cys-Leu-Leu-Leu-Leu-Cys, Cys-Val-Val-Val-Val-Cys, Cys-Ala-Cys, Cys-Ile-Cys, Cys-Ile-Ala-Cys, Cys-Ala-Ile-Cys, Cys-Ile-Ile-Cys, Cys-Ala-Ala-Cys, Cys-Ile-Ala-Ile-Cys, Cys-Ala-Ile-Ala-Cys, Cys-Ile-Ile-Ile-Cys, Cys-Ala-Ala-Ala-Cys, Cys-Ile-Ala-Ile-Ala-Cys, Cys-Ala-Ile-Ala-Ile-Cys, Cys-Ile-Ile-Ile-Ile-Cys, or Cys-Ala-Ala-Ala-Ala-Cys, Cys-Met-Cys, Cys-Met-Ala-Cys, Cys-Ala-Met-Cys, Cys-Met-Met-Cys, Cys-Ala-Ala-Cys, Cys-Met-Ala-Met-Cys, Cys-Ala-Met-Ala-Cys, Cys-Met-Met-Met-Cys, Cys-Ala-Ala-Ala-Cys, Cys-Met-Ala-Met-Ala-Cys, Cys-Ala-Met-Ala-Met-Cys, Cys-Met-Met-Met-Met-Cys, or Cys-Ala-Ala-Ala-Ala-Cys, etc. Each Cys above may also be replaced by any modified peptide or chemical compound carrying a free —SH-moiety as defined herein. (SEQ ID NOs: 189-229). Such peptide

combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0109] Additionally, the lipophilic amino acid component (AA) may contain at least one proline, which may serve as a structure breaker of longer sequences of Leu, Val, Ile, Ala and Met in the lipophilic amino acid component (AA), preferably two, three or more prolines.

[0110] Finally, according to a fourth alternative, the amino acid component (AA) may be a weak basic amino acid component (AA). The incorporation of weak basic amino acids or sequences as weak basic amino acid component (AA) into the polymeric carrier of the present invention may serve as a proton sponge and facilitates endosomal escape (also called endosomal release) (proton sponge effect). Incorporation of such a weak basic amino acid component (AA) preferably enhances transfection efficiency.

[0111] For this purpose, the amino acids in the weak basic amino acid component (AA) may be selected from either the same or different weak amino acids e.g. selected from histidine or aspartate (aspartic acid). Alternatively, the weak basic amino acids (or the entire weak basic amino acid component (AA)) may be selected from following peptide combinations Asp-His, His-Asp, Asp-Asp, His-His, Asp-His-Asp, His-Asp-His, Asp-Asp-Asp, His-His-His, Asp-His-Asp-His, His-Asp-His-Asp, Asp-Asp-Asp-Asp, or His-His-His-His, etc. (SEQ ID NOs: 230-241). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0112] Additionally, the weak basic amino acid component (AA) may contain or may be flanked by a —SH containing moiety, which allows introducing this component via a disulfide bond as a further part of generic formula (I) above, e.g. as a linker. Such a —SH containing moiety may be any moiety as defined herein suitable to couple one component as defined herein to a further component as defined herein. As an example, such a —SH containing moiety may be a cysteine. Then, e.g. the weak basic amino acid component (AA) may be selected from e.g. peptide combinations Cys-His-Cys, Cys-Asp-Cys, Cys-Asp-His-Cys, Cys-His-Asp-Cys, Cys-Asp-Asp-Cys, Cys-His-His-Cys, Cys-Asp-His-Asp-Cys, Cys-His-Asp-His-Cys, Cys-Asp-Asp-Asp-Cys, Cys-His-His-His-Cys, Cys-Asp-His-Asp-His-Cys, Cys-His-Asp-His-Asp-Cys, Cys-Asp-Asp-Asp-Asp-Cys, or Cys-His-His-His-His-Cys, etc. Each Cys above may also be replaced by any modified peptide or chemical compound carrying a free —SH-moiety as defined herein (SEQ ID NOs: 242-255). Such peptide combinations may be repeated e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15 or even more times. These peptide combinations may also be combined with each other as suitable.

[0113] Additionally, the weak basic amino acid component (AA) may contain at least one proline, which may serve as a structure breaker of longer sequences of histidine or aspartate (aspartic acid) in the weak basic amino acid component (AA), preferably two, three or more prolines.

[0114] According to a fifth alternative, the amino acid component (AA) may be a signal peptide or signal sequence, a localization signal or sequence, a nuclear localization signal or sequence (NLS), an antibody, a cell penetrating peptide, (e.g. TAT), etc. Preferably such an amino acid component (AA) is bound to the polymeric carrier or to another component of the polymeric carrier via a (reversible) disulfide bond. In this context the signal peptide or signal sequence, a localization signal or sequence, a nuclear localization signal or sequence (NLS), an antibody, a cell penetrating peptide, (e.g. TAT), etc.; additionally comprises at least one —SH-moiety. In this context a signal peptide, a localization signal or sequence or a nuclear localization signal or sequence (NLS), may be used to direct the inventive polymeric carrier cargo complex to specific target cells (e.g. hepatocytes or antigen-presenting cells) and preferably allows a translocalization of the polymeric carrier to a specific target, e.g. into the cell, into the nucleus, into the endosomal compartment, sequences for the mitochondrial matrix, localisation sequences for the plasma membrane, localisation sequences for the Golgi apparatus, the nucleus, the cytoplasm and the cytoskeleton, etc. Such signal peptide, a localization signal or sequence or a nuclear localization signal may be used for the transport of any of the herein defined nucleic acids, preferably an RNA or a DNA, more preferably an shRNA or a pDNA, e.g. into the nucleus. Without being limited thereto, such a signal peptide, a localization signal or sequence or a nuclear localization signal may comprise, e.g., localisation sequences for the endoplasmic reticulum. Particular localization signals or sequences or a nuclear localization signals may include e.g. KDEL (SEQ ID NO: 256), DDEL (SEQ ID NO: 257), DEEL (SEQ ID NO: 258), QEDL (SEQ ID NO: 259), RDEL (SEQ ID NO: 260), and GQNLSTSN (SEQ ID NO: 261), nuclear localisation sequences, including PKKKRKV (SEQ ID NO: 262), PQKKIKS (SEQ ID NO: 263), QPKKP (SEQ ID NO: 264), RKKR (SEQ ID NO: 265), RKKRRQRRRAHQ (SEQ ID NO: 266), RQARRNRRRRWRERQR (SEQ ID NO: 267), MPLTRRRPAASQALAPPTP (SEQ ID NO: 268), GAALTILV (SEQ ID NO: 269), and GAALTLLG (SEQ ID NO: 270), localisation sequences for the endosomal compartment, including MDDQRDLISNNEQLP (SEQ ID NO: 271), localisation sequences for the mitochondrial matrix, including MLFNLRXXLNNAAFRHGHNFMVRNFRCGQPLX (SEQ ID NO: 272), localisation sequences for the plasma membrane: GCVCSSNP (SEQ ID NO: 273), GQTVTTPL (SEQ ID NO: 274), GQELSQHE (SEQ ID NO: 275), GNSPSYNP (SEQ ID NO: 276), GVSGSKGQ (SEQ ID NO: 277), GQTITTPL (SEQ ID NO: 278), GQTLTTPL (SEQ ID NO: 279), GQIFSRSA (SEQ ID NO: 280), GQIHGLSP (SEQ ID NO: 281), GARASVLS (SEQ ID NO: 282), and GCTLSAEE (SEQ ID NO: 283), localisation sequences for the endoplasmic reticulum and the nucleus, including GAQVSSQK (SEQ ID NO: 284), and GAQLSRNT (SEQ ID NO: 285), localisation sequences for the Golgi apparatus, the nucleus, the cytoplasm and the cytoskeleton, including GNAAAAKK (SEQ ID NO: 286), localisation sequences for the cytoplasm and cytoskeleton, including GNEASYPL (SEQ ID NO: 287), localisation sequences for the plasma membrane and cytoskeleton, including GSSKSKPK (SEQ ID NO: 288), etc. Examples of secretory signal peptide sequences as defined herein include, without being limited thereto, signal sequences of classical or non-classical MHC-molecules (e.g. signal sequences of MHC I and II molecules, e.g. of the MHC class I molecule HLA-A*0201), signal sequences of cytokines or immunoglobulins as defined herein, signal sequences of the invariant chain of immunoglobulins or antibodies as defined herein, signal sequences of Lamp1, Tapasin, Erp57, Calreticulin, Calnexin, and further membrane associated proteins or of proteins associated with the endoplasmic reticulum (ER) or

the endosomal-lysosomal compartment. Particularly preferably, signal sequences of MHC class I molecule HLA-A*0201 may be used according to the present invention. Such an additional component may be bound e.g. to a cationic polymer or to any other component of the polymeric carrier as defined herein. Preferably this signal peptide, localization signal or sequence or nuclear localization signal or sequence (NLS), is bound to the polymeric carrier or to another component of the polymeric carrier via a (reversible) disulfide bond. For this purpose the (AA) component additionally comprises at least one —SH moiety as defined herein. The binding to any of components of the polymeric carrier may also be accomplished using an acid-labile bond, preferably via a side chain of any of components of the polymeric carrier, which allows to detach or release the additional component at lower pH-values, e.g. at physiological pH-values as defined herein.

[0115] Additionally, according to another alternative, the amino acid component (AA) may be a functional peptide or protein, which may modulate the functionality of the polymeric carrier accordingly. Such functional peptides or proteins as the amino acid component (AA) preferably comprise any peptides or proteins as defined herein, e.g. as defined below as therapeutically active proteins. According to one alternative, such further functional peptides or proteins may comprise so called cell penetrating peptides (CPPs) or cationic peptides for transportation. Particularly preferred are CPPs, which induce a pH-mediated conformational change in the endosome and lead to an improved release of the polymeric carrier (in complex with a nucleic acid) from the endosome by insertion into the lipid layer of the liposome. These cell penetrating peptides (CPPs) or cationic peptides for transportation, may include, without being limited thereto protamine, nucleoline, spermine or spermidine, oligo- or poly-L-lysine (PLL), basic polypeptides, oligo or poly-arginine, cell penetrating peptides (CPPs), chimeric CPPs, such as Transportan, or MPG peptides, HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, members of the penetratin family, e.g. Penetratin, Antennapedia-derived peptides (particularly from *Drosophila* antennapedia), pAntp, pIsl, etc., antimicrobial-derived CPPs e.g. Buforin-2, Bac715-24, SynB, SynB(1), pVEC, hCT-derived peptides, SAP, MAP, KALA, PpTG20, Loligomere, FGF, Lactoferrin, histones, VP22 derived or analog peptides, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains (PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, Pep-1, L-oligomers, Calcitonin peptide(s), etc. Such an amino acid component (AA) may also be bound to any component of the polymeric carrier as defined herein. Preferably it is bound to the polymeric carrier or to another component of the polymeric carrier via a (reversible) disulfide bond. For the above purpose, the amino acid component (AA) preferably comprises at least one —SH moiety as defined herein. The binding to any of components of the polymeric carrier may also be accomplished using an SH-moiety or an acid-labile bond, preferably via a side chain of any of components of the polymeric carrier which allows to detach or release the additional component at lower pH-values, e.g. at physiological pH-values as defined herein.

[0116] According to a last alternative, the amino acid component (AA) may consist of any peptide or protein which can execute any favorable function in the cell. Particularly preferred are peptides or proteins selected from therapeutically active proteins or peptides, from antigens, e.g. tumour antigens, pathogenic antigens (animal antigens, viral antigens, protozoal antigens, bacterial antigens, allergic antigens), autoimmune antigens, or further antigens, from allergens, from antibodies, from immunostimulatory proteins or peptides, from antigen-specific T-cell receptors, or from any other protein or peptide suitable for a specific (therapeutic) application as defined below for coding nucleic acids. Particularly preferred are peptide epitopes from antigens as defined herein.

[0117] The polymeric carrier may comprise at least one of the above mentioned cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), wherein any of the above alternatives may be combined with each other, and may be formed by polymerizing same in a condensation polymerization reaction via their —SH-moieties.

[0118] According to another aspect, the polymeric carrier of the inventive polymeric carrier cargo complex or single components thereof, e.g. of the above mentioned cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), may be further modified with a ligand, preferably a carbohydrate, more preferably a sugar, even more preferably mannose. Preferably this ligand is bound to the polymeric carrier or to a component of the polymeric carrier via a (reversible) disulfide bond or via Michael addition. In the case that the ligand is bound by a disulfide bond the ligand additionally comprises at least one —SH-moiety. These ligands may be used to direct the inventive polymeric carrier cargo complex to specific target cells (e.g. hepatocytes or antigen-presenting cells). In this context mannose is particular preferred as ligand in the case that dendritic cells are the target especially for vaccination or adjuvant purposes.

[0119] According to a further embodiment of the invention, the inventive polymeric carrier cargo complex may comprise (AA) components as defined above which do not comprise —SH moieties. These (AA) components can be added before or during the complexation reaction of the at least one nucleic acid molecule. Thereby, the (AA) component(s) is/are (non-covalently) incorporated into the inventive polymeric carrier cargo complex without inclusion of the (AA) component(s) in the polymeric carrier itself by (covalent) polymerization.

[0120] According to one specific embodiment, the entire inventive polymeric carrier cargo complex may be formed by a polymerization or condensation (of at least one) of the above mentioned cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), via their —SH-moieties in a first step and complexing the first nucleic acid to such a polymeric carrier in a second step. The polymeric carrier may thus contain a number of at least one or even more of the same or different of the above defined cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), the number preferably determined by the above range.

[0121] According to one alternative specific embodiment, the inventive polymeric carrier cargo complex is formed by carrying out the polymerization or condensation of at least one of the above mentioned cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), via their —SH-moieties simultaneously to complexing the nucleic acid cargo to the (in situ prepared) polymeric carrier. Likewise, the polymeric carrier may thus also here contain

a number of at least one or even more of the same or different of the above defined cationic or polycationic peptides, proteins or polymers or further components, e.g. (AA), the number preferably determined by the above range.

[0122] The inventive polymeric carrier cargo complex additionally comprises as a cargo at least one first nucleic acid molecule. In the context of the present invention, such a first nucleic acid molecule may be any suitable nucleic acid, selected e.g. from any (single-stranded or double-stranded) DNA, preferably, without being limited thereto, e.g. genomic DNA, single-stranded DNA molecules, double-stranded DNA molecules, coding DNA, DNA primers, DNA probes, immunostimulatory DNA, a (short) DNA oligonucleotide ((short) oligodesoxyribonucleotides), viral DNA, or may be selected e.g. from any PNA (peptide nucleic acid) or may be selected e.g. from any (single-stranded or double-stranded) RNA, preferably, without being limited thereto, a (short) RNA oligonucleotide ((short) oligoribonucleotide), a coding RNA, a messenger RNA (mRNA), a viral RNA, replicons, an immunostimulatory RNA, a small interfering RNA (siRNA), an antisense RNA, a micro RNA, a small nuclear RNA (snRNA), a small-hairpin (sh) RNA or riboswitches, ribozymes or aptamers; etc. The nucleic acid molecule of the inventive polymeric carrier cargo complex may also be a ribosomal RNA (rRNA), a transfer RNA (tRNA), a messenger RNA (mRNA), or a viral RNA (vRNA). Preferably, the nucleic acid molecule of the inventive polymeric carrier cargo complex is an RNA. More preferably, the nucleic acid molecule of the inventive polymeric carrier cargo complex is a (linear) single-stranded RNA, even more preferably an mRNA or an immunostimulatory RNA. In the context of the present invention, an mRNA is typically an RNA, which is composed of several structural elements, e.g. an optional 5'-CAP structure, an optional 5'-UTR region, an upstream positioned ribosomal binding site followed by a coding region, an optional 3'-UTR region, which may be followed by a poly-A tail (and/or a poly-C-tail). An mRNA may occur as a mono-, di-, or even multicistronic RNA, i.e. a RNA which carries the coding sequences of one, two or more proteins or peptides. Such coding sequences in di-, or even multicistronic mRNA may be separated by at least one IRES sequence, e.g. as defined herein.

[0123] Furthermore, the nucleic acid of the inventive polymeric carrier cargo complex may be a single- or a double-stranded nucleic acid molecule or a partially double-stranded or partially single stranded nucleic acid, which are at least partially self complementary (both of these partially double-stranded or partially single stranded nucleic acid molecules are typically formed by a longer and a shorter single-stranded nucleic acid molecule or by two single stranded nucleic acid molecules, which are about equal in length, wherein one single-stranded nucleic acid molecule is in part complementary to the other single-stranded nucleic acid molecule and both thus form a double-stranded nucleic acid molecule in this region, i.e. a partially double-stranded or partially single stranded nucleic acid molecule. Preferably, the nucleic acid molecule may be a single-stranded nucleic acid molecule. Furthermore, the nucleic acid molecule may be a circular or linear nucleic acid molecule, preferably a linear nucleic acid molecule.

[0124] According to one alternative, the first nucleic acid molecule of the inventive polymeric carrier cargo complex may be a coding nucleic acid, e.g. a DNA or RNA. Moreover, the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule, which encodes a protein or a peptide.

[0125] According to one embodiment, the at least one first nucleic acid molecule and the at least one second nucleic acid molecule are both coding nucleic acid molecules. Preferably, the at least one first and the at least one second nucleic acid molecule each encode a different peptide or protein. In one embodiment, the first nucleic acid molecule has a sequence, which is distinct from the sequence of the second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex. Alternatively, the first nucleic acid molecule and the second nucleic acid molecule may comprise the same sequence or be identical.

[0126] In the case of the at least one first nucleic acid molecule and/or of the second nucleic acid molecule, such a coding DNA or RNA may be any DNA or RNA as defined herein. Preferably, such a coding DNA or RNA may be a single- or a double-stranded DNA or RNA, more preferably a single-stranded DNA or RNA, and/or a circular or linear DNA or RNA, more preferably a linear DNA or RNA. Furthermore such a coding DNA or RNA may be a genomic DNA, a viral RNA or DNA, a replicon, a plasmid DNA or an mRNA. Even more preferably, the coding DNA or RNA may be a (linear) single-stranded DNA or RNA. Most preferably, the nucleic acid molecule according to the present invention may be a linear single-stranded messenger RNA (mRNA). Such an mRNA may occur as a mono-, di-, or even multicistronic RNA, i.e. an RNA which carries the coding sequences of one, two or more proteins or peptides. Such coding sequences in di-, or even multicistronic mRNA may be separated by at least one IRES sequence, e.g. as defined herein.

[0127] In a preferred embodiment, the at least one second nucleic acid molecule encodes a therapeutically active protein or an antigen as defined herein. In a particularly preferred embodiment, the at least one second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex, encodes a peptide or a protein, which is capable of eliciting an immune response, preferably an adaptive immune response, after administration, especially intramuscular administration, to a host. Alternatively, the at least one second nucleic acid molecule encodes a therapeutically active peptide or protein.

Coding Nucleic Acids:

[0128] The at least one first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the at least one second nucleic acid molecule, which is administered together with the polymeric carrier cargo complex, may encode a protein or a peptide, which may be selected, without being restricted thereto, e.g. from therapeutically active proteins or peptides, including adjuvant proteins, from antigens, e.g. tumour antigens, pathogenic antigens (e.g. selected, from animal antigens, from viral antigens, from protozoal antigens, from bacterial antigens), allergenic antigens, autoimmune antigens, or further antigens, from allergens, from antibodies, from immunostimulatory proteins or peptides, from antigen-specific T-cell receptors, or from any other protein or peptide suitable for a specific (therapeutic) application, wherein the coding nucleic acid

may be transported into a cell, a tissue or an organism and the protein may be expressed subsequently in this cell, tissue or organism.

a) Therapeutically Active Proteins

[0129] In the context of the present invention, therapeutically active proteins or peptides may be encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Therapeutically active proteins are defined herein as proteins which have an effect on healing, prevent prophylactically or treat therapeutically a disease, preferably as defined herein, or are proteins of which an individual is in need of. These may be selected from any naturally or synthetically designed occurring recombinant or isolated protein known to a skilled person from the prior art. Without being restricted thereto therapeutically active proteins may comprise proteins, capable of stimulating or inhibiting the signal transduction in the cell, e.g. cytokines, lymphokines, monokines, growth factors, receptors, signal transduction molecules, transcription factors, etc; anticoagulants; antithrombins; antiallergic proteins; apoptotic factors or apoptosis related proteins, therapeutic active enzymes and any protein connected with any acquired disease or any hereditary disease.

[0130] A therapeutically active protein, which may be encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, may also be an adjuvant protein. In this context, an adjuvant protein is preferably to be understood as any protein, which is capable to elicit an innate immune response as defined herein. Preferably, such an innate immune response comprises activation of a pattern recognition receptor, such as e.g. a receptor selected from the Toll-like receptor (TLR) family, including e.g. a Toll like receptor selected from human TLR1 to TLR10 or from murine Toll like receptors TLR1 to TLR13. More preferably, the adjuvant protein is selected from human adjuvant proteins or from pathogenic adjuvant proteins, selected from the group consisting of, without being limited thereto, bacterial proteins, protozoan proteins, viral proteins, or fungal proteins, animal proteins, in particular from bacterial adjuvant proteins. In addition, nucleic acids encoding human proteins involved in adjuvant effects (e.g. ligands of pattern recognition receptors, pattern recognition receptors, proteins of the signal transduction pathways, transcription factors or cytokines) may be used as well.

b) Antigens

[0131] The first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may alternatively encode an antigen. According to the present invention, the term "antigen" refers to a substance which is recognized by the immune system and is capable of triggering an antigen-specific immune response, e.g. by formation of antibodies or antigen-specific T-cells as part of an adaptive immune response. In this context, the first step of an adaptive immune response is the activation of naïve antigen-specific T cells by antigen-presenting cells. This occurs in the lymphoid tissues and organs through which naïve T cells are constantly passing. The three cell types that can serve as antigen-presenting cells are dendritic cells, macrophages, and B cells. Each of these cells has a distinct function in eliciting immune responses. Tissue dendritic cells take up antigens by phagocytosis and macropinocytosis and are stimulated by infection to migrate to the local lymphoid tissue, where they differentiate into mature dendritic cells. Macrophages ingest particulate antigens such as bacteria and are induced by infectious agents to express MHC class II molecules. The unique ability of B cells to bind and internalize soluble protein antigens via their receptors may be important to induce T cells. By presenting the antigen on MHC molecules leads to activation of T cells which induces their proliferation and differentiation into armed effector T cells. The most important function of effector T cells is the killing of infected cells by $CD8^+$ cytotoxic T cells and the activation of macrophages by TH1 cells which together make up cell-mediated immunity, and the activation of B cells by both TH2 and TH1 cells to produce different classes of antibody, thus driving the humoral immune response. T cells recognize an antigen by their T cell receptors which does not recognize and bind antigen directly, but instead recognize short peptide fragments e.g. of pathogens' protein antigens, which are bound to MHC molecules on the surfaces of other cells.

[0132] T cells fall into two major classes that have different effector functions. The two classes are distinguished by the expression of the cell-surface proteins CD4 and CD8. These two types of T cells differ in the class of MHC molecule that they recognize. There are two classes of MHC molecules—MHC class I and MHC class II molecules—which differ in their structure and expression pattern on tissues of the body. $CD4^+$ T cells bind to a MHC class II molecule and $CD8^+$ T cells to a MHC class I molecule. MHC class I and MHC class II molecules have distinct distributions among cells that reflect the different effector functions of the T cells that recognize them. MHC class I molecules present peptides from pathogens, commonly viruses to $CD8^+$ T cells, which differentiate into cytotoxic T cells that are specialized to kill any cell that they specifically recognize. Almost all cells express MHC class I molecules, although the level of constitutive expression varies from one cell type to the next. But not only pathogenic peptides from viruses are presented by MHC class I molecules, also self-antigens like tumour antigens are presented by them. MHC class I molecules bind peptides from proteins degraded in the cytosol and transported in the endoplasmic reticulum. Thereby MHC class I molecules on the surface of cells infected with viruses or other cytosolic pathogens display peptides from these pathogen. The $CD8^+$ T cells that recognize MHC class I:peptide complexes are specialized to kill any cells displaying foreign peptides and so rid the body of cells infected with viruses and other cytosolic pathogens. The main function of $CD4^+$ T cells ($CD4^+$ helper T cells) that recognize MHC class II molecules is to activate other effector cells of the immune system. Thus MHC class II molecules are normally found on B lymphocytes, dendritic cells, and macrophages, cells that participate in immune responses, but not on other tissue cells. Macrophages, for example, are activated to kill the intravesicular pathogens they harbour, and B cells to secrete immunoglobulins against foreign molecules. MHC class II molecules are

prevented from binding to peptides in the endoplasmic reticulum and thus MHC class II molecules bind peptides from proteins which are degraded in endosomes. They can capture peptides from pathogens that have entered the vesicular system of macrophages, or from antigens internalized by immature dendritic cells or the immunoglobulin receptors of B cells. Pathogens that accumulate in large numbers inside macrophage and dendritic cell vesicles tend to stimulate the differentiation of TH1 cells, whereas extracellular antigens tend to stimulate the production of TH2 cells. TH1 cells activate the microbicidal properties of macrophages and induce B cells to make IgG antibodies that are very effective of opsonising extracellular pathogens for ingestion by phagocytic cells, whereas TH2 cells initiate the humoral response by activating naïve B cells to secrete IgM, and induce the production of weakly opsonising antibodies such as IgG1 and IgG3 (mouse) and IgG2 and IgG4 (human) as well as IgA and IgE (mouse and human).

[0133] In the context of the present invention, antigens as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex typically comprise any antigen, antigenic epitope or antigenic peptide, falling under the above definition, more preferably protein and peptide antigens, e.g. tumour antigens, allergenic antigens, auto-immune self-antigens, pathogenic antigens, etc. In particular antigens as encoded by the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be antigens generated outside the cell, more typically antigens not derived from the host organism (e.g. a human) itself (i.e. non-self antigens) but rather derived from host cells outside the host organism, e.g. viral antigens, bacterial antigens, fungal antigens, protozoological antigens, animal antigens, allergenic antigens, etc. Allergenic antigens (allergy antigens) are typically antigens, which cause an allergy in a human and may be derived from either a human or other sources. Additionally, antigens as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be furthermore antigens generated inside the cell, the tissue or the body. Such antigens include antigens derived from the host organism (e.g. a human) itself, e.g. tumour antigens, self-antigens or auto-antigens, such as auto-immune self-antigens, etc., but also (non-self) antigens as defined herein, which have been originally been derived from host cells outside the host organism, but which are fragmented or degraded inside the body, tissue or cell, e.g. by (protease) degradation, metabolism, etc.

[0134] One class of antigens as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises tumour antigens. "Tumour antigens" are preferably located on the surface of the (tumour) cell. Tumour antigens may also be selected from proteins, which are overexpressed in tumour cells compared to a normal cell. Furthermore, tumour antigens also include antigens expressed in cells which are (were) not themselves (or originally not themselves) degenerated but are associated with the supposed tumour. Antigens which are connected with tumour-supplying vessels or (re)formation thereof, in particular those antigens which are associated with neovascularization, e.g. growth factors, such as VEGF, bFGF etc., are also included herein. Antigens connected with a tumour furthermore include antigens from cells or tissues, typically embedding the tumour. Further, some substances (usually proteins or peptides) are expressed in patients suffering (knowingly or not-knowingly) from a cancer disease and they occur in increased concentrations in the body fluids of said patients. These substances are also referred to as "tumour antigens", however they are not antigens in the stringent meaning of an immune response inducing substance. The class of tumour antigens can be divided further into tumour-specific antigens (TSAs) and tumour-associated-antigens (TAAs). TSAs can only be presented by tumour cells and never by normal "healthy" cells. They typically result from a tumour specific mutation. TAAs, which are more common, are usually presented by both tumour and healthy cells. These antigens are recognized and the antigen-presenting cell can be destroyed by cytotoxic T cells. Additionally, tumour antigens can also occur on the surface of the tumour in the form of, e.g., a mutated receptor. In this case, they can be recognized by antibodies. Particular preferred tumour antigens are selected from the group consisting of 5T4, 707-AP, 9D7, AFP, AlbZIP HPG1, alpha-5-beta-1-integrin, alpha-5-beta-6-integrin, alpha-actinin-4/m, alpha-methylacyl-coenzyme A racemase, ART-4, ARTC1/m, B7H4, BAGE-1, BCL-2, bcr/abl, beta-catenin/m, BING-4, BRCA1/m, BRCA2/m, CA 15-3/CA 27-29, CA 19-9, CA72-4, CA125, calreticulin, CAMEL, CASP-8/m, cathepsin B, cathepsin L, CD19, CD20, CD22, CD25, CDE30, CD33, CD4, CD52, CD55, CD56, CD80, CDC27/m, CDK4/m, CDKN2A/m, CEA, CLCA2, CML28, CML66, COA-1/m, coactosin-like protein, collage XXIII, COX-2, CT-9/BRD6, Cten, cyclin B1, cyclin D1, cyp-B, CYPB1, DAM-10, DAM-6, DEK-CAN, EFTUD2/m, EGFR, ELF2/m, EMMPRIN, EpCam, EphA2, EphA3, ErbB3, ETV6-AML1, EZH2, FGF-5, FN, Frau-1, G250, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE7b, GAGE-8, GDEP, GnT-V, gp100, GPC3, GPNMB/m, HAGE, HAST-2, hepsin, Her2/neu, HERV-K-MEL, HLA-A*0201-R17I, HLA-A11/m, HLA-A2/m, HNE, homeobox NKX3.1, HOM-TES-14/SCP-1, HOM-TES-85, HPV-E6, HPV-E7, HSP70-2M, HST-2, hTERT, iCE, IGF-1R, IL-13Ra2, IL-2R, IL-5, immature laminin receptor, kallikrein-2, kallikrein-4, Ki67, KIAA0205, KIAA0205/m, KK-LC-1, K-Ras/m, LAGE-A1, LDLR-FUT, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A9, MAGE-A10, MAGE-A12, MAGE-B1, MAGE-B2, MAGE-B3, MAGE-B4, MAGE-B5, MAGE-B6, MAGE-B10, MAGE-B16, MAGE-B17, MAGE-C1, MAGE-C2, MAGE-C3, MAGE-D1, MAGE-D2, MAGE-D4, MAGE-E1, MAGE-E2, MAGE-F1, MAGE-H1, MAGEL2, mammaglobin A, MART-1/melan-A, MART-2, MART-2/m, matrix protein 22, MC1R, M-CSF, ME1/m, mesothelin, MG50/PXDN, MMP11, MN/CA IX-antigen, MRP-3, MUC-1, MUC-2, MUM-1/m, MUM-2/m, MUM-3/m, myosin class I/m, NA88-A, N-acetylglucosaminyltransferase-V, Neo-PAP, Neo-PAP/m, NFYC/m, NGEP, NMP22, NPM/ALK, N-Ras/m, NSE, NY-ESO-1, NY-ESO-B, OA1, OFA-iLRP, OGT, OGT/m, OS-9, OS-9/m, osteocalcin, osteopontin, p15, p190 minor bcr-abl, p53, p53/m, PAGE-4, PAI-1, PAI-2, PAP, PART-1, PATE, PDEF, Pim-1-Kinase, Pin-1,

Pml/PARalpha, POTE, PRAME, PRDX5/m, prostein, proteinase-3, PSA, PSCA, PSGR, PSM, PSMA, PTPRK/m, RAGE-1, RBAF600/m, RHAMM/CD168, RU1, RU2, S-100, SAGE, SART-1, SART-2, SART-3, SCC, SIRT2/m, Sp17, SSX-1, SSX-2/HOM-MEL-40, SSX-4, STAMP-1, STEAP, survivin, survivin-2B, SYT-SSX-1, SYT-SSX-2, TA-90, TAG-72, TARP, TEL-AML1, TGFbeta, TGFbetaRII, TGM-4, TPI/m, TRAG-3, TRG, TRP-1, TRP-2/6b, TRP/INT2, TRP-p8, tyrosinase, UPA, VEGF, VEGFR1, VEGFR-2/FLK-1, and WT1. Such tumour antigens preferably may be selected from the group consisting of MAGE-A1 (e.g. MAGE-A1 according to accession number M77481), MAGE-A2, MAGE-A3, MAGE-A6 (e.g. MAGE-A6 according to accession number NM_005363), MAGE-C1, MAGE-C2, melan-A (e.g. melan-A according to accession number NM_005511), GP100 (e.g. GP100 according to accession number M77348), tyrosinase (e.g. tyrosinase according to accession number NM_000372), surviving (e.g. survivin according to accession number AF077350), CEA (e.g. CEA according to accession number NM_004363), Her-2/neu (e.g. Her-2/neu according to accession number M11730), WT1 (e.g. WT1 according to accession number NM_000378), PRAME (e.g. PRAME according to accession number NM_006115), EGFRI (epidermal growth factor receptor 1) (e.g. EGFRI (epidermal growth factor receptor 1) according to accession number AF288738), MUC1, mucin-1 (e.g. mucin-1 according to accession number NM_002456), SEC61G (e.g. SEC61G according to accession number NM_014302), hTERT (e.g. hTERT accession number NM_198253), 5T4 (e.g. 5T4 according to accession number NM_006670), NY-Eso-1 (e.g. NY-Esol according to accession number NM_001327), TRP-2 (e.g. TRP-2 according to accession number NM_001922), STEAP, PCA, PSA, PSMA, etc.

[0135] According to another alternative, one further class of antigens as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises allergenic antigens. Such allergenic antigens may be selected from antigens derived from different sources, e.g. from animals, plants, fungi, bacteria, etc. Sources in this context include e.g. grasses, grass pollens, tree pollens, flower pollens, herb pollens, animals, dust mite, food, molds, animal venom (e.g. insect venom), drugs, or numerous environmental triggers, etc. Allergenic antigens typically belong to different classes of compounds, such as nucleic acids and their fragments, proteins or peptides and their fragments, carbohydrates, polysaccharides, sugars, lipids, phospholipids, etc. Of particular interest in the context of the present invention are antigens, which may be encoded by the first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, i.e. protein or peptide antigens and their fragments or epitopes, or nucleic acids and their fragments, particularly nucleic acids and their fragments, encoding such protein or peptide antigens and their fragments or epitopes.

[0136] According to another alternative, one further class of antigens as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises antigens from a pathogen associated with an infectious disease. Preferably, the pathogen is a viral, bacterial, fungal or protozoan pathogen. In this context particularly preferred are antigens from the pathogens *Acinetobacter baumannii, Anaplasma genus, Anaplasma phagocytophilum, Ancylostoma braziliense, Ancylostoma duodenale, Arcanobacterium haemolyticum, Ascaris lumbricoides, Aspergillus* genus, Astroviridae, *Babesia* genus, *Bacillus anthracis, Bacillus cereus, Bartonella henselae*, BK virus, *Blastocystis hominis, Blastomyces dermatitidis, Bordetella pertussis, Borrelia burgdorferi, Borrelia genus, Borrelia* spp, *Brucella* genus, *Brugia malayi, Bunyaviridae family, Burkholderia cepacia* and other *Burkholderia* species, *Burkholderia mallei, Burkholderia pseudomallei, Caliciviridae family, Campylobacter genus, Candida albicans, Candida* spp, *Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci*, CJD prion, *Clonorchis sinensis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium perfringens, Clostridium* spp, *Clostridium tetani, Coccidioides* spp, coronaviruses, *Corynebacterium diphtheriae, Coxiella burnetii*, Crimean-Congo hemorrhagic fever virus, *Cryptococcus neoformans, Cryptosporidium* genus, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), *Dientamoeba fragilis*, Ebolavirus (EBOV), *Echinococcus* genus, *Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia genus, Entamoeba histolytica, Enterococcus* genus, Enterovirus genus, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71), *Epidermophyton* spp, Epstein-Barr Virus (EBV), *Escherichia coli* O157:H7, 0111 and 0104:H4, *Fasciola hepatica* and *Fasciola gigantica*, FFI prion, Filarioidea superfamily, Flaviviruses, *Francisella tularensis, Fusobacterium genus, Geotrichum candidum, Giardia intestinalis, Gnathostoma* spp, GSS prion, Guanarito virus, *Haemophilus ducreyi, Haemophilus influenzae, Helicobacter pylori*, Henipavirus (Hendra virus Nipah virus), Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), *Histoplasma capsulatum*, HIV (Human immunodeficiency virus), *Hortaea werneckii*, Human bocavirus (HBoV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Human parainfluenza viruses (HPIV), Japanese encephalitis virus, JC virus, Junin virus, Kingella kingae, *Klebsiella granulomatis*, Kuru prion, Lassa virus, *Legionella pneumophila, Leishmania* genus, Leptospira genus, *Listeria monocytogenes*, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, *Malassezia* spp, Marburg virus, Measles virus, *Metagonimus* yokagawai, Microsporidia phylum, *Molluscum contagiosum* virus (MCV), Mumps virus, *Mycobacterium leprae* and *Mycobacterium lepromatosis, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Naegleria fowleri, Necator americanus, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Nocardia* spp, *Onchocerca volvulus, Orientia tsutsugamushi*, Orthomyxoviridae family, *Paracoccidioides brasiliensis, Paragonimus* spp, *Paragonimus westermani*, Parvovirus B19, *Pasteurella* genus, *Plasmodium* genus, *Pneumocystis jirovecii*, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhinovirus, rhinoviruses, *Rickettsia akari, Rickettsia genus, Rickettsia prowazekii, Rickettsia rickettsii, Rickettsia typhi*, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, *Salmonella* genus, *Sarcoptes scabiei*,

SARS coronavirus, *Schistosoma* genus, *Shigella* genus, Sin Nombre virus, Hantavirus, *Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Strongyloides stercoralis, Taenia genus, Taenia solium*, Tick-borne encephalitis virus (TBEV), *Toxocara canis* or *Toxocara cati, Toxoplasma gondii, Treponema pallidum, Trichinella spiralis, Trichomonas vaginalis, Trichophyton* spp, *Trichuris trichiura, Trypanosoma brucei, Trypanosoma cruzi, Ureaplasma urealyticum, Varicella zoster* virus (VZV), *Varicella zoster* virus (VZV), *Variola major* or *Variola minor*, vCJD prion, Venezuelan equine encephalitis virus, *Vibrio cholerae*, West Nile virus, Western equine encephalitis virus, *Wuchereria bancrofti*, Yellow fever virus, *Yersinia enterocolitica, Yersinia pestis*, and *Yersinia pseudotuberculosis*.

[0137] In specific embodiments according to the present invention, following antigens of pathogens associated with infectious disease are particularly preferred:

[0138] The Hemagglutinin (HA), the Neuraminidase (NA), the Nucleoprotein (NP), the M1 protein, the M2 protein, the NS1 protein, the NS2 protein (the NEP protein: nuclear export protein), the PA protein, the PB 1 protein (polymerase basic 1 protein), the PB 1-F2 protein and the PB2 protein in each case of Influenza virus;

[0139] the nucleoprotein (N), the phosphoprotein (P), the matrix protein (M), the glycoprotein (G), and the viral RNA polymerase (L), in each case of Rabies virus;

[0140] the Hepatitis B surface antigen (HBsAg), the Hepatitis B core antigen (HbcAg), the Hepatitis B virus DNA polymerase, the HBx protein, the preS2 middle surface protein, the large S protein, the virus protein VP1, the virus protein VP2, the virus protein VP3, and the virus protein VP4, in each case of Hepatitis B virus;

[0141] the E1 protein, the E2 protein, the E3 protein, the E4 protein, the E5 protein, the E6 protein, the E7 protein, the E8 protein, the L1 protein, and the L2 protein, in each case of human Papilloma virus (hPV);

[0142] the protective antigen (PA), the edema factor (EF), the lethal factor (LF), and the S-layer homology proteins (SLH), in each case of *Bacillus anthracis;*

[0143] the Fusion (F) protein, the nucleocapsid (N) protein, the phosphoprotein (P), the matrix (M) protein, the glycoprotein (G), the large protein (L; RNA polymerase), the non-structural protein 1 (NS1), the non-structural protein 2 (NS2), the small hydrophobic (SH) protein, the elongation factor M2-1, and the transcription regulation protein M2-2, in each case of respiratory syncytial virus (RSV);

[0144] the Glycoprotein L (UL1), the Uracil-DNA glycosylase UL2, the UL3 protein, the UL4 protein, the DNA replication protein UL5, the Portal protein UL6, the Virion maturation protein UL7, the DNA helicase ULB, the Replication origin-binding protein UL9, the Glycoprotein M (UL10), the UL11 protein, the Alkaline exonuclease UL12, the Serine-threonine protein kinase UL13, the Tegument protein UL14, the Terminase (UL15), the Tegument protein UL16, the UL17 protein, the Capsid protein VP23 (UL18), the Major capsid protein VP5 (UL19), the Membrane protein UL20, the Tegument protein UL21, the Glycoprotein H (UL22), the Thymidine Kinase UL23, the UL24 protein, the UL25 protein, the Capsid protein P40 (UL26, VP24, VP22A), the Glycoprotein B (UL27), the ICP18.5 protein (UL28), the Major DNA-binding protein ICP8 (UL29), the DNA polymerase UL30, the Nuclear matrix protein UL31, the Envelope glycoprotein UL32, the UL33 protein, the Inner nuclear membrane protein UL34, the Capsid protein VP26 (UL35), the Large tegument protein UL36, the Capsid assembly protein UL37, the VP19C protein (UL38), the Ribonucleotide reductase (Large subunit) UL39, the Ribonucleotide reductase (Small subunit) UL40, the Tegument protein/Virion host shutoff VHS protein (UL41), the DNA polymerase processivity factor UL42, the Membrane protein UL43, the Glycoprotein C (UL44), the Membrane protein UL45, the Tegument proteins VP11/12 (UL46), the Tegument protein VP13/14 (UL47), the Virion maturation protein VP16 (UL48, Alpha-TIF), the Envelope protein UL49, the dUTP diphosphatase UL50, the Tegument protein UL51, the DNA helicase/primase complex protein UL52, the Glycoprotein K (UL53), the Transcriptional regulation protein 1E63 (ICP27, UL54), the UL55 protein, the UL56 protein, the Viral replication protein ICP22 (1E68, US1), the US2 protein, the Serine/threonine-protein kinase US3, the Glycoprotein G (US4), the Glycoprotein J (US5), the Glycoprotein D (US6), the Glycoprotein I (US7), the Glycoprotein E (US8), the Tegument protein US9, the Capsid/Tegument protein US10, the Vmw21 protein (US11), the ICP47 protein (IE12, US12), the Major transcriptional activator ICP4 (1E175, RS1), the E3 ubiquitin ligase ICP0 (IE110), the Latency-related protein 1 (LRP1), the Latency-related protein 2 (LRP2), the Neurovirulence factor RL1 (ICP34.5), and the Latency-associated transcript (LAT), in each case of Herpes simplex virus (HSV);

[0145] the ESAT-6 protein, the ESX-1 protein, the CFP10 protein, the TB10.4 protein, the MPT63 protein, the MPT64 protein, the MPT83 protein, the MTB12 protein, the MTB8 protein, the AG85A protein, the AG85B protein, the Rpf-like proteins, the KATG protein, the PPE18 protein, the MTB32 protein, the MTB39 protein, the Crystallin, the HSP65 protein, the PST-S protein, and the HBHA protein, the 10 kDa filtrate antigen EsxB, the serine protease PepA, the fibronectin-binding protein D FbpD, the secreted protein MPT51, the periplasmic phosphate-binding lipoprotein PSTS1 (PBP-1), the periplasmic phosphate-binding lipoprotein PSTS3 (PBP-3, Phos-1), the PPE family protein PPE14, the PPE family protein PPE68, the protein MTB72F, the molecular chaperone DnaK, the cell surface lipoprotein MPT83, the lipoprotein P23, the Phosphate transport system permease protein PstA, the 14 kDa antigen, the fibronectin-binding protein C FbpC1, the Alanine dehydrogenase TB43, and the Glutamine synthetase 1, in each case of *Mycobacterium tuberculosis;*

[0146] the capsid protein C, the membrane protein M, the envelope protein E; the nonstructural protein NS1, the nonstructural protein NS2a, the nonstructural protein, the nonstructural protein NS2b, the nonstructural protein NS3, the nonstructural protein NS4a, the nonstructural protein NS4b, and the nonstructural protein NS5 in each case of Dengue virus;

[0147] the structural protein VP1, the structural protein VP2, the structural protein VP3, the structural protein

VP4, the structural protein VP6, the structural protein VP7, the nonstructural protein NSP1, the nonstructural protein NSP2, the nonstructural protein NSP3, the nonstructural protein NSP4, the nonstructural protein NSP5 and the nonstructural protein NSP6 in each case of Rotavirus;

[0148] the HIV p24 antigen, the HIV envelope proteins (Gp120, Gp41, Gp160), the polyprotein GAG, the negative factor protein Nef, the trans-activator of transcription Tat in each case of HIV (Human immunodeficiency virus); or

[0149] the glycoprotein GP, the nucleoprotein NP, the minor matrix protein VP24, the major matrix protein VP40, the transcription activator VP30, the polymerase cofactor VP35, the RNA polymerase L in each case of Ebolavirus (EBOV) or Marburg Virus.

[0150] Of particular interest in the context of the present invention are antigens, which may be encoded by the first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex or comprised in a pharmaceutical composition or vaccine, i.e. protein or peptide antigens and their fragments or epitopes, or nucleic acids and their fragments, particularly nucleic acids and their fragments, encoding such protein or peptide antigens and their fragments or epitopes.

c) Antibodies

[0151] According to a further alternative, the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may encode an antibody or an antibody fragment. According to the present invention, such an antibody may be selected from any antibody, e.g. any recombinantly produced or naturally occurring antibodies, known in the art, in particular antibodies suitable for therapeutic, diagnostic or scientific purposes, or antibodies which have been identified in relation to specific cancer diseases. Herein, the term "antibody" is used in its broadest sense and specifically covers monoclonal and polyclonal antibodies (including agonist, antagonist, and blocking or neutralizing antibodies) and antibody species with polyepitopic specificity. According to the invention, the term "antibody" typically comprises any antibody known in the art (e.g. IgM, IgD, IgG, IgA and IgE antibodies), such as naturally occurring antibodies, antibodies generated by immunization in a host organism, antibodies which were isolated and identified from naturally occurring antibodies or antibodies generated by immunization in a host organism and recombinantly produced by biomolecular methods known in the art, as well as chimeric antibodies, human antibodies, humanized antibodies, bispecific antibodies, intrabodies, i.e. antibodies expressed in cells and optionally localized in specific cell compartments, and fragments and variants of the aforementioned antibodies. In general, an antibody consists of a light chain and a heavy chain both having variable and constant domains. The light chain consists of an N-terminal variable domain, V_L, and a C-terminal constant domain, C_L. In contrast, the heavy chain of the IgG antibody, for example, is comprised of an N-terminal variable domain, V_H, and three constant domains, C_H1, C_H2 and C_H3.

[0152] In the context of the present invention, antibodies as encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may preferably comprise full-length antibodies, i.e. antibodies composed of the full heavy and full light chains, as described above. However, derivatives of antibodies such as antibody fragments, variants or adducts may also be encoded by the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Antibody fragments are preferably selected from Fab, Fab', $F(ab')_2$, Fc, Facb, pFc', Fd and Fv fragments of the aforementioned (full-length) antibodies. In general, antibody fragments are known in the art. For example, a Fab ("fragment, antigen binding") fragment is composed of one constant and one variable domain of each of the heavy and the light chain. The two variable domains bind the epitope on specific antigens. The two chains are connected via a disulfide linkage. A scFv ("single chain variable fragment") fragment, for example, typically consists of the variable domains of the light and heavy chains. The domains are linked by an artificial linkage, in general a polypeptide linkage such as a peptide composed of 15-25 glycine, proline and/or serine residues.

[0153] In the present context it is preferable that the different chains of the antibody or antibody fragment are encoded by a multicistronic nucleic acid molecule. Alternatively, the different strains of the antibody or antibody fragment are encoded by several monocistronic nucleic acid(s) (sequences).

siRNA:

[0154] According to a further alternative, the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex may be in the form of dsRNA, preferably siRNA. A dsRNA, or a siRNA, is of interest particularly in connection with the phenomenon of RNA interference. The in vitro technique of RNA interference (RNAi) is based on double-stranded RNA molecules (dsRNA), which trigger the sequence-specific suppression of gene expression (Zamore (2001) Nat. Struct. Biol. 9: 746-750; Sharp (2001) Genes Dev. 5:485-490: Hannon (2002) Nature 41: 244-251). In the transfection of mammalian cells with long dsRNA, the activation of protein kinase R and RnaseL brings about unspecific effects, such as, for example, an interferon response (Stark et al. (1998) Annu. Rev. Biochem. 67: 227-264; He and Katze (2002) Viral Immunol. 15: 95-119). These unspecific effects are avoided when shorter, for example 21- to 23-mer, so-called siRNA (small interfering RNA), is used, because unspecific effects are not triggered by siRNA that is shorter than 30 bp (Elbashir et al. (2001) Nature 411: 494-498).

[0155] The nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex may thus be a double-stranded RNA (dsRNA) having a length of from 17 to 29, preferably from 19 to 25, and preferably is at least 90%, more preferably 95% and especially 100% (of the nucleotides of a dsRNA) complementary to a section of the nucleic acid molecule of a (therapeutically relevant) protein or antigen described (as active ingredient) hereinbefore or of any further protein as described herein, either a coding or a non-coding section, preferably a coding section. Such a (section of the) nucleic acid molecule may be termed herein a "target sequence" and may be any nucleic acid molecule as defined herein, preferably a genomic DNA, a cDNA, a

RNA, e.g. an mRNA, etc. 90% complementary means that with a length of a dsRNA described herein of, for example, 20 nucleotides, the dsRNA contains not more than 2 nucleotides showing no complementarity with the corresponding section of the target sequence. The sequence of the double-stranded RNA used according to the invention is, however, preferably wholly complementary in its general structure with a section of the target sequence. In this context the nucleic acid molecule of the inventive polymeric carrier cargo complex may be a dsRNA having the general structure 5'-(N_{17-29})-3', preferably having the general structure 5'-(N_{19-25})-3', more preferably having the general structure 5'-(N_{19-24})-3', or yet more preferably having the general structure 5'-(N_{21-23})-3', wherein for each general structure each N is a (preferably different) nucleotide of a section of the target sequence, preferably being selected from a continuous number of 17 to 29 nucleotides of a section of the target sequence, and being present in the general structure 5'-(N_{17-29})-3' in their natural order. In principle, all the sections having a length of from 17 to 29, preferably from 19 to 25, base pairs that occur in the target sequence can serve for preparation of a dsRNA as defined herein. Equally, dsRNAs used as nucleic acid molecule of the inventive polymeric carrier cargo complex can also be directed against nucleotide sequences of a (therapeutically relevant) protein or antigen described (as active ingredient) hereinbefore that do not lie in the coding region, in particular in the 5' non-coding region of the target sequence, for example, therefore, against non-coding regions of the target sequence having a regulatory function. The target sequence of the dsRNA used as nucleic acid molecule of the inventive polymeric carrier cargo complex can therefore lie in the translated and untranslated region of the target sequence and/or in the region of the control elements of a protein or antigen described hereinbefore. The target sequence for a dsRNA used as the nucleic acid molecule of the inventive polymeric carrier cargo complex can also lie in the overlapping region of untranslated and translated sequence; in particular, the target sequence can comprise at least one nucleotide upstream of the start triplet of the coding region, e.g. of a genomic DNA, a cDNA, a RNA, or an mRNA, etc.

Immunostimulatory Nucleic Acids:

a) Immunostimulatory CpG Nucleic Acids:

[0156] According to another alternative, the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex may be in the form of a(n) (immunostimulatory) CpG nucleic acid, in particular CpG-RNA or CpG-DNA, which preferably induces an innate immune response. A CpG-RNA or CpG-DNA used according to the invention can be a single-stranded CpG-DNA (ss CpG-DNA), a double-stranded CpG-DNA (dsDNA), a single-stranded CpG-RNA (ss CpG-RNA) or a double-stranded CpG-RNA (ds CpG-RNA). The CpG nucleic acid used according to the invention is preferably in the form of CpG-RNA, more preferably in the form of single-stranded CpG-RNA (ss CpG-RNA). Also preferably, such CpG nucleic acids have a length as described above. Preferably the CpG motifs are unmethylated.

b) Immunostimulatory RNA (isRNA):

[0157] Likewise, according to a further alternative, the (immunostimulatory) nucleic acid molecule of the inventive polymeric carrier cargo complex may be in the form of an immunostimulatory RNA (isRNA), which preferably elicits an innate immune response. Such an immunostimulatory RNA may be any (double-stranded or single-stranded) RNA, e.g. a coding RNA, as defined herein. In a preferred embodiment, the immunostimulatory RNA is a non-coding RNA. Preferably, the immunostimulatory RNA may be a single-stranded, a double-stranded or a partially double-stranded RNA, more preferably a single-stranded RNA, and/or a circular or linear RNA, more preferably a linear RNA. More preferably, the immunostimulatory RNA may be a (linear) single-stranded RNA. Even more preferably, the immunostimulatory RNA may be a (long) (linear) single-stranded) non-coding RNA. In this context it is particular preferred that the isRNA carries a triphosphate at its 5'-end which is the case for in vitro transcribed RNA. An immunostimulatory RNA may also occur as a short RNA oligonucleotide as defined herein.

[0158] An immunostimulatory RNA as used herein may furthermore be selected from any class of RNA molecules, found in nature or being prepared synthetically, and which can induce an innate immune response and may support an adaptive immune response induced by an antigen. In this context, an immune response may occur in various ways. A substantial factor for a suitable (adaptive) immune response is the stimulation of different T-cell sub-populations. T-lymphocytes are typically divided into two sub-populations, the T-helper 1 (Th1) cells and the T-helper 2 (Th2) cells, with which the immune system is capable of destroying intracellular (Th1) and extracellular (Th2) pathogens (e.g. antigens). The two Th cell populations differ in the pattern of the effector proteins (cytokines) produced by them. Thus, Th1 cells assist the cellular immune response by activation of macrophages and cytotoxic T-cells. Th2 cells, on the other hand, promote the humoral immune response by stimulation of B-cells for conversion into plasma cells and by formation of antibodies (e.g. against antigens). The Th1/Th2 ratio is therefore of great importance in the induction and maintenance of an adaptive immune response. In connection with the present invention, the Th1/Th2 ratio of the (adaptive) immune response is preferably shifted in the direction towards the cellular response (Th1 response) and a cellular immune response is thereby induced. According to one example, the innate immune system which may support an adaptive immune response, may be activated by ligands of Toll-like receptors (TLRs). TLRs are a family of highly conserved pattern recognition receptor (PRR) polypeptides that recognize pathogen-associated molecular patterns (PAMPs) and play a critical role in innate immunity in mammals. Currently at least thirteen family members, designated TLR1-TLR13 (Toll-like receptors: TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13), have been identified. Furthermore, a number of specific TLR ligands have been identified. It was e.g. found that unmethylated bacterial DNA and synthetic analogs thereof (CpG DNA) are ligands for TLR9 (Hemmi H et al. (2000) Nature 408:740-5; Bauer S et al. (2001) Proc Natl Acad Sci USA 98, 9237-42). Furthermore, it has been reported that ligands for certain TLRs include certain nucleic acid molecules and that certain types of RNA are immunostimulatory in a sequence-independent or sequence-dependent manner, wherein these various immunostimulatory RNAs may e.g. stimulate TLR3, TLR7, or TLR8, or intracellular receptors such as RIG-I, MDA-5, etc. E.g. Lipford et al. determined certain G,U-containing oli-

goribonucleotides as immunostimulatory by acting via TLR7 and TLR8 (see WO 03/086280). The immunostimulatory G,U-containing oligoribonucleotides described by Lipford et al. were believed to be derivable from RNA sources including ribosomal RNA, transfer RNA, messenger RNA, and viral RNA.

[0159] The immunostimulatory RNA (isRNA) used as the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex may thus comprise any RNA sequence, which enhances an immune response in a host. Preferably, the isRNA used as the first nucleic acid molecule of the polymeric carrier cargo complex enhances the immune response, which is preferably an adaptive immune response elicited by a peptide or protein encoded by the second nucleic acid molecule, preferably an mRNA, that is administered to the host in combination with the polymeric carrier cargo complex. The isRNA used as the first nucleic acid molecule of the polymeric carrier cargo complex may thus comprise any RNA sequence known to be immunostimulatory, including, without being limited thereto, RNA sequences representing and/or encoding ligands of TLRs, preferably selected from human family members TLR1-TLR10 or murine family members TLR1-TLR13, more preferably selected from (human) family members TLR1-TLR10, even more preferably from TLR7 and TLR8, ligands for intracellular receptors for RNA (such as RIG-I or MDA-5, etc.) (see e.g. Meylan, E., Tschopp, J. (2006). Toll-like receptors and RNA helicases: two parallel ways to trigger antiviral responses. Mol. Cell 22, 561-569), or any other immunostimulatory RNA sequence. Furthermore, (classes of) immunostimulatory RNA molecules, used as the nucleic acid molecule of the inventive polymeric carrier cargo complex may include any other RNA capable of eliciting an innate immune response. Without being limited thereto, such an immunostimulatory RNA may include ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), and viral RNA (vRNA). Such an immunostimulatory RNA may comprise a length of 1000 to 5000, of 500 to 5000, of 5 to 5000, or of 5 to 1000, 5 to 500, 5 to 250, of 5 to 100, of 5 to 50 or of 5 to 30 nucleotides.

[0160] According to a particularly preferred embodiment, such immunostimulatory nucleic acid sequences is preferably RNA preferably consisting of or comprising a nucleic acid of formula (II) or (III):

$$G_lX_mG_n, \quad \text{(formula (II))}$$

wherein:

[0161] G is guanosine, uracil or an analogue of guanosine or uracil;

[0162] X is guanosine, uracil, adenosine, thymidine, cytosine or an analogue of the above-mentioned nucleotides;

[0163] l is an integer from 1 to 40,

[0164] wherein

[0165] when l=1 G is guanosine or an analogue thereof,

[0166] when l>1 at least 50% of the nucleotides are guanosine or an analogue thereof;

[0167] m is an integer and is at least 3;

[0168] wherein

[0169] when m=3 X is uracil or an analogue thereof,

[0170] when m>3 at least 3 successive uracils or analogues of uracil occur;

[0171] n is an integer from 1 to 40,

[0172] wherein

[0173] when n=1 G is guanosine or an analogue thereof,

[0174] when n>1 at least 50% of the nucleotides are guanosine or an analogue thereof.

$$C_lX_mC_n, \quad \text{(formula (III))}$$

wherein:

[0175] C is cytosine, uracil or an analogue of cytosine or uracil;

[0176] X is guanosine, uracil, adenosine, thymidine, cytosine or an analogue of the above-mentioned nucleotides;

[0177] l is an integer from 1 to 40,

[0178] wherein

[0179] when l=1 C is cytosine or an analogue thereof,

[0180] when l>1 at least 50% of the nucleotides are cytosine or an analogue thereof;

[0181] m is an integer and is at least 3;

[0182] wherein

[0183] when m=3 X is uracil or an analogue thereof,

[0184] when m>3 at least 3 successive uracils or analogues of uracil occur;

[0185] n is an integer from 1 to 40,

[0186] wherein

[0187] when n=1 C is cytosine or an analogue thereof,

[0188] when n>1 at least 50% of the nucleotides are cytosine or an analogue thereof.

[0189] The nucleic acids of formula (II) or (III), which may be used the nucleic acid cargo of the inventive polymeric carrier cargo complex may be relatively short nucleic acid molecules with a typical length of approximately from 5 to 100 (but may also be longer than 100 nucleotides for specific embodiments, e.g. up to 200 nucleotides), from 5 to 90 or from 5 to 80 nucleotides, preferably a length of approximately from 5 to 70, more preferably a length of approximately from 8 to 60 and, more preferably a length of approximately from 15 to 60 nucleotides, more preferably from 20 to 60, most preferably from 30 to 60 nucleotides. If the nucleic acid of the inventive nucleic acid cargo complex has a maximum length of e.g. 100 nucleotides, m will typically be <=98. The number of nucleotides G in the nucleic acid of formula (II) is determined by l or n. l and n, independently of one another, are each an integer from 1 to 40, wherein when l or n=1 G is guanosine or an analogue thereof, and when l or n>1 at least 50% of the nucleotides are guanosine or an analogue thereof. For example, without implying any limitation, when l or n=4 G_l or G_n can be, for example, a GUGU, GGUU, UGUG, UUGG, GUUG, GGGU, GGUG, GUGG, UGGG or GGGG, etc.; when l or n=5 G_l or G_n can be, for example, a GGGUU, GGUGU, GUGGU, UGGGU, UGGUG, UGUGG, UUGGG, GUGUG, GGGGU, GGGUG, GGUGG, GUGGG, UGGGG, or GGGGG, etc.; etc. A nucleotide adjacent to X_m in the nucleic acid of formula (II) according to the invention is preferably not a uracil. Similarly, the number of nucleotides C in the nucleic acid of formula (III) according to the invention is determined by l or n. l and n, independently of one another, are each an integer from 1 to 40, wherein when l or n=1 C is cytosine or an analogue thereof, and when l or n>1 at least 50% of the nucleotides are cytosine or an analogue thereof. For example, without implying any limitation, when l or n=4, C_l or C_n can be, for example, a CUCU, CCUU, UCUC, UUCC, CUUC, CCCU, CCUC, CUCC, UCCC or CCCC, etc.; when l or n=5 C_l or C_n can be, for example, a CCCUU, CCUCU, CUCCU, UCCCU, UCCUC, UCUCC, UUCCC, CUCUC, CCCCU, CCCUC, CCUCC, CUCCC, UCCCC, or CCCCC, etc.; etc. A nucleotide adja-

cent to X_m in the nucleic acid of formula (III) according to the invention is preferably not a uracil. Preferably, for formula (II), when l or n>1, at least 60%, 70%, 80%, 90% or even 100% of the nucleotides are guanosine or an analogue thereof, as defined above. The remaining nucleotides to 100% (when guanosine constitutes less than 100% of the nucleotides) in the flanking sequences G_l and/or G_n are uracil or an analogue thereof, as defined hereinbefore. Also preferably, 1 and n, independently of one another, are each an integer from 2 to 30, more preferably an integer from 2 to 20 and yet more preferably an integer from 2 to 15. The lower limit of l or n can be varied if necessary and is at least 1, preferably at least 2, more preferably at least 3, 4, 5, 6, 7, 8, 9 or 10. This definition applies correspondingly to formula (III).

[0190] According to a particularly preferred embodiment, a nucleic acid according to any of formulas (II) or (III) above, which may be used as nucleic acid of the inventive polymeric carrier cargo complex, may be selected from a sequence consisting or comprising any of the following sequences:

(SEQ ID NO: 289)
GGUUUUUUUUUUUUUUUGGG;

(SEQ ID NO: 290)
GGGGGUUUUUUUUUUGGGGG;

(SEQ ID NO: 291)
GGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGG;

(SEQ ID NO: 292)
GUGUGUGUGUGUUUUUUUUUUUUUUUUUGUGUGUGUGUGU;

(SEQ ID NO: 293)
GGUUGGUUGGUUUUUUUUUUUUUUUUUUGGUUGGUUGGUU;

(SEQ ID NO: 294)
GGGGGGGGGUUUGGGGGGGG;

(SEQ ID NO: 295)
GGGGGGGGUUUUGGGGGGGG;

(SEQ ID NO: 296)
GGGGGGGUUUUUUGGGGGGG;

(SEQ ID NO: 297)
GGGGGGGUUUUUUUGGGGGG;

(SEQ ID NO: 298)
GGGGGGUUUUUUUUGGGGGG;

(SEQ ID NO: 299)
GGGGGGUUUUUUUUUGGGGG;

(SEQ ID NO: 300)
GGGGGGUUUUUUUUUUGGGG;

(SEQ ID NO: 301)
GGGGGUUUUUUUUUUUGGGG;

(SEQ ID NO: 302)
GGGGGUUUUUUUUUUUUGGG;

(SEQ ID NO: 303)
GGGGUUUUUUUUUUUUUGGG;

(SEQ ID NO: 304)
GGGGUUUUUUUUUUUUUUGG;

(SEQ ID NO: 305)
GGUUUUUUUUUUUUUUUUGG;

-continued

(SEQ ID NO: 306)
GUUUUUUUUUUUUUUUUUUG;

(SEQ ID NO: 307)
GGGGGGGGGGUUUGGGGGGGG;

(SEQ ID NO: 308)
GGGGGGGGGUUUUGGGGGGGG;

(SEQ ID NO: 309)
GGGGGGGGUUUUUUGGGGGGG;

(SEQ ID NO: 310)
GGGGGGGGUUUUUUUGGGGGG;

(SEQ ID NO: 311)
GGGGGGGUUUUUUUUGGGGGG;

(SEQ ID NO: 312)
GGGGGGGUUUUUUUUUGGGGG;

(SEQ ID NO: 313)
GGGGGGGUUUUUUUUUUGGGG;

(SEQ ID NO: 314)
GGGGGGUUUUUUUUUUUGGGG;

(SEQ ID NO: 315)
GGGGGGUUUUUUUUUUUUGGG;

(SEQ ID NO: 316)
GGGGGUUUUUUUUUUUUUGGG;

(SEQ ID NO: 317)
GGGGGUUUUUUUUUUUUUUGG;

(SEQ ID NO: 318)
GGGUUUUUUUUUUUUUUUUGG;

(SEQ ID NO: 319)
GGUUUUUUUUUUUUUUUUUUGG;

(SEQ ID NO: 320)
GGGGGGGGGGGUUUGGGGGGGGG;

(SEQ ID NO: 321)
GGGGGGGGGGUUUUGGGGGGGGG;

(SEQ ID NO: 322)
GGGGGGGGGUUUUUUGGGGGGGG;

(SEQ ID NO: 323)
GGGGGGGGGUUUUUUUGGGGGGG;

(SEQ ID NO: 324)
GGGGGGGGUUUUUUUUGGGGGGG;

(SEQ ID NO: 325)
GGGGGGGGUUUUUUUUUGGGGGG;

(SEQ ID NO: 326)
GGGGGGGGUUUUUUUUUUGGGGG;

(SEQ ID NO: 327)
GGGGGGGUUUUUUUUUUUGGGGG;

(SEQ ID NO: 328)
GGGGGGGUUUUUUUUUUUUGGGG;

(SEQ ID NO: 329)
GGGGGGUUUUUUUUUUUUUGGGG;

(SEQ ID NO: 330)
GGGGGGUUUUUUUUUUUUUUGGG;

(SEQ ID NO: 331)
GGGGUUUUUUUUUUUUUUUUGGG;

-continued

(SEQ ID NO: 332)
GGGUUUUUUUUUUUUUUUUUUUGGG;

(SEQ ID NO: 333)
GUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUG;

(SEQ ID NO: 334)
GGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGG;

(SEQ ID NO: 335)
GGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGG;

(SEQ ID NO: 336)
GGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGG;

(SEQ ID NO: 337)
GGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGG;

(SEQ ID NO: 338)
GGGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGGG;

(SEQ ID NO: 339)
GGGGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGGGG;

(SEQ ID NO: 340)
GGGGGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGGGGG;

(SEQ ID NO: 341)
GGGGGGGGGUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUUGGGGGGGGG;

(SEQ ID NO: 342)
GGUUUGG;

(SEQ ID NO: 343)
GGUUUUGG;

(SEQ ID NO: 344)
GGUUUUUGG;

(SEQ ID NO: 345)
GGUUUUUUGG;

(SEQ ID NO: 346)
GGUUUUUUUGG;

(SEQ ID NO: 347)
GGUUUUUUUUGG;

(SEQ ID NO: 348)
GGUUUUUUUUUGG;

(SEQ ID NO: 349)
GGUUUUUUUUUUGG;

(SEQ ID NO: 350)
GGUUUUUUUUUUUGG;

(SEQ ID NO: 351)
GGUUUUUUUUUUUUGG;

(SEQ ID NO: 352)
GGUUUUUUUUUUUUUGG;

(SEQ ID NO: 353)
GGUUUUUUUUUUUUUUGG;

(SEQ ID NO: 354)
GGUUUUUUUUUUUUUUUGG;

(SEQ ID NO: 355)
GGGUUUGGG;

(SEQ ID NO: 356)
GGGUUUUGGG;

(SEQ ID NO: 357)
GGGUUUUUGGG;

-continued

(SEQ ID NO: 358)
GGGUUUUUUGGG;

(SEQ ID NO: 359)
GGGUUUUUUUGGG;

(SEQ ID NO: 360)
GGGUUUUUUUUGGG;

(SEQ ID NO: 361)
GGGUUUUUUUUUGGG;

(SEQ ID NO: 362)
GGGUUUUUUUUUUGGG;

(SEQ ID NO: 363)
GGGUUUUUUUUUUUGGG;

(SEQ ID NO: 364)
GGGUUUUUUUUUUUUGGG;

(SEQ ID NO: 365)
GGGUUUUUUUUUUUUUGGG;

(SEQ ID NO: 366)
GGGUUUUUUUUUUUUUUUUGGGUUUUUUUUUUUUUUUUGGGUUUUUUUUUUUU

UUUUGGG;

(SEQ ID NO: 367)
GGGUUUUUUUUUUUUUUUUGGGGGGUUUUUUUUUUUUUUUUGGG;

(SEQ ID NO: 368)
GGGUUUGGGUUUGGGUUUGGGUUUGGGUUUGGGUUUGGGUUUGGGUUUGG

G;

(short GU-rich, SEQ ID NO: 369)
GGUUUUUUUUUUUUUUUUGGG
or

(SEQ ID NO: 370)
CCCUUUUUUUUUUUUUUUUCCCUUUUUUUUUUUUUUUUCCCUUUUUUUUUU

UUUUCCC

(SEQ ID NO: 371)
CCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCCCUUUCC

C

(SEQ ID NO: 372)
CCCUUUUUUUUUUUUUUUUCCCCCCUUUUUUUUUUUUUUUCCC

or from a sequence having at least 60%, 70%, 80%, 90%, or even 95% sequence identity with any of these sequences

[0191] According to a further particularly preferred embodiment, such immunostimulatory nucleic acid sequences particularly isRNA consist of or comprise a nucleic acid of formula (IV) or (V):

$$(N_uG_lX_mG_nN_v)_a, \quad \text{(formula (IV))}$$

wherein:

[0192] G is guanosine (guanine), uridine (uracil) or an analogue of guanosine (guanine) or uridine (uracil), preferably guanosine (guanine) or an analogue thereof;

[0193] X is guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine), or an analogue of these nucleotides (nucleosides), preferably uridine (uracil) or an analogue thereof;

[0194] N is a nucleic acid sequence having a length of about 4 to 50, preferably of about 4 to 40, more preferably of about 4 to 30 or 4 to 20 nucleic acids, each N

independently being selected from guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of these nucleotides (nucleosides);

[0195] a is an integer from 1 to 20, preferably from 1 to 15, most preferably from 1 to 10;

[0196] l is an integer from 1 to 40,

[0197] wherein when l=1, G is guanosine (guanine) or an analogue thereof,

[0198] when l>1, at least 50% of these nucleotides (nucleosides) are guanosine (guanine) or an analogue

[0199] thereof;

[0200] m is an integer and is at least 3;

[0201] wherein when m=3, X is uridine (uracil) or an analogue thereof, and

[0202] when m>3, at least 3 successive uridines (uracils) or analogues of uridine (uracil) occur;

[0203] n is an integer from 1 to 40,

[0204] wherein when n=1, G is guanosine (guanine) or an analogue thereof,

[0205] when n>1, at least 50% of these nucleotides (nucleosides) are guanosine (guanine) or an analogue

[0206] thereof;

[0207] u, v may be independently from each other an integer from 0 to 50,

[0208] preferably wherein when u=0, v≥1, or

[0209] when v=0, u≥1;

wherein the nucleic acid molecule of formula (IV) has a length of at least 50 nucleotides, preferably of at least 100 nucleotides, more preferably of at least 150 nucleotides, even more preferably of at least 200 nucleotides and most preferably of at least 250 nucleotides.

$$(N_uC_lX_mC_nN_v)_a, \qquad \text{(formula (V))}$$

wherein:

[0210] C is cytidine (cytosine), uridine (uracil) or an analogue of cytidine (cytosine) or uridine (uracil), preferably cytidine (cytosine) or an analogue thereof;

[0211] X is guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of the above-mentioned nucleotides (nucleosides), preferably uridine (uracil) or an analogue thereof;

[0212] N is each a nucleic acid sequence having independent from each other a length of about 4 to 50, preferably of about 4 to 40, more preferably of about 4 to 30 or 4 to 20 nucleic acids, each N independently being selected from guanosine (guanine), uridine (uracil), adenosine (adenine), thymidine (thymine), cytidine (cytosine) or an analogue of these nucleotides (nucleosides);

[0213] a is an integer from 1 to 20, preferably from 1 to 15, most preferably from 1 to 10;

[0214] l is an integer from 1 to 40,

[0215] wherein when l=1, C is cytidine (cytosine) or an analogue thereof,

[0216] when l>1, at least 50% of these nucleotides (nucleosides) are cytidine (cytosine) or an analogue

[0217] thereof;

[0218] m is an integer and is at least 3;

[0219] wherein when m=3, X is uridine (uracil) or an analogue thereof,

[0220] when m>3, at least 3 successive uridines (uracils) or analogues of uridine (uracil) occur;

[0221] n is an integer from 1 to 40,

[0222] wherein when n=1, C is cytidine (cytosine) or an analogue thereof,

[0223] when n>1, at least 50% of these nucleotides (nucleosides) are cytidine (cytosine) or an analogue

[0224] thereof.

[0225] u, v may be independently from each other an integer from 0 to 50,

[0226] preferably wherein when u=0, v≥1, or

[0227] when v=0, u≥1;

wherein the nucleic acid molecule of formula (V) according to the invention has a length of at least 50 nucleotides, preferably of at least 100 nucleotides, more preferably of at least 150 nucleotides, even more preferably of at least 200 nucleotides and most preferably of at least 250 nucleotides.

[0228] For formula (V), any of the definitions given above for elements N (i.e. N_u and N_v) and X (X_m), particularly the core structure as defined above, as well as for integers a, l, m, n, u and v, similarly apply to elements of formula (V) correspondingly, wherein in formula (V) the core structure is defined by $C_lX_mC_n$. The definition of bordering elements N_u and N_v is identical to the definitions given above for N_u and N_v.

[0229] According to a very particularly preferred embodiment, the inventive nucleic acid molecule according to formula (IV) may be selected from e.g. any of the following sequences:

(SEQ ID NO: 373)
UAGCGAAGCUCUUGGACCUAGGUUUUUUUUUUUUUUUGGGUGCGUUCCUA

GAAGUACACG

(SEQ ID NO: 374)
UAGCGAAGCUCUUGGACCUAGGUUUUUUUUUUUUUUUGGGUGCGUUCCUA

GAAGUACACGAUCGCUUCGAGAACCUGGAUCCAAAAAAAAAAAAAAAACCC

ACGCAAGGAUCUUCAUGUGC

(SEQ ID NO: 375)
GGGAGAAAGCUCAAGCUUGGAGCAAUGCCCGCACAUUGAGGAAACCGAGU

UGCAUAUCUCAGAGUAUUGGCCCCCGUGUAGGUUAUUCUUGACAGACAGU

GGAGCUUAUUCACUCCCAGGAUCCGAGUCGCAUACUACGGUACUGGUGAC

AGACCUAGGUCGUCAGUUGACCAGUCCGCCACUAGACGUGAGUCCGUCAA

AGCAGUUAGAUGUUACACUCUAUUAGAUC

(SEQ ID NO: 376)
GGGAGAAAGCUCAAGCUUGGAGCAAUGCCCGCACAUUGAGGAAACCGAGU

UGCAUAUCUCAGAGUAUUGGCCCCCGUGUAGGUUAUUCUUGACAGACAGU

GGAGCUUAUUCACUCCCAGGAUCCGAGUCGCAUACUACGGUACUGGUGAC

AGACCUAGGUCGUCAGUUGACCAGUCCGCCACUAGACGUGAGUCCGUCAA

AGCAGUUAGAUGUUACACUCUAUUAGAUCUCGGAUUACAGCUGGAAGGAG

CAGGAGUAGUGUUCUUGCUCUAAGUACCGAGUGUGCCCAAUACCCGAUCA

GCUUAUUAACGAACGGCUCCUCCUCUUAGACUGCAGCGUAAGUGCGGAAU

CUGGGGAUCAAAUUACUGACUGCCUGGAUUACCCUCGGACAUAUAACCUU

GUAGCACGCUGUUGCUGUAUAGGUGACCAACGCCCACUCGAGUAGACCAG

-continued

CUCUCUUAGUCCGGACAAUGAUAGGAGGCGCGGUCAAUCUACUUCUGGCU

AGUUAAGAAUAGGCUGCACCGACCUCUAUAAGUAGCGUGUCCUCUAG

(SEQ ID NO: 377)

GGGAGAAAGCUCAAGCUUGGAGCAAUGCCCGCACAUUGAGGAAACCGAGU

UGCAUAUCUCAGAGUAUUGGCCCCCGUGUAGGUUAUUCUUGACAGACAGU

GGAGCUUAUUCACUCCCAGGAUCCGAGUCGCAUACUACGGUACUGGUGAC

AGACCUAGGUCGUCAGUUGACCAGUCCGCCACUAGACGUGAGUCCGUCAA

AGCAGUUAGAUGUUACACUCUAUUAGAUCUCGGAUUACAGCUGGAAGGAG

CAGGAGUAGUGUUCUUGCUCUAAGUACCGAGUGUGCCCAAUACCCGAUCA

GCUUAUUAACGAACGGCUCCUCCUCUUAGACUGCAGCGUAAGUGCGGAAU

CUGGGGAUCAAAUUACUGACUGCCUGGAUUACCCUCGGACAUAUAACCUU

GUAGCACGCUGUUGCUGUAUAGGUGACCAACGCCCACUCGAGUAGACCAG

CUCUCUUAGUCCGGACAAUGAUAGGAGGCGCGGUCAAUCUACUUCUGGCU

AGUUAAGAAUAGGCUGCACCGACCUCUAUAAGUAGCGUGUCCUCUAGAGC

UACGCAGGUUCGCAAUAAAAGCGUUGAUUAGUGUGCAUAGAACAGACCUC

UUAUUCGGUGAAACGCCAGAAUGCUAAAUUCCAAUAACUCUUCCCAAAAC

GCGUACGGCCGAAGACGCGCGCUUAUCUUGUGUACGUUCUCGCACAUGGA

AGAAUCAGCGGGCAUGGUGGUAGGGCAAUAGGGGAGCUGGGUAGCAGCGA

AAAAGGGCCCCUGCGCACGUAGCUUCGCUGUUCGUCUGAAACAACCCGGC

AUCCGUUGUAGCGAUCCCGUUAUCAGUGUUAUUCUUGUGCGCACUAAGAU

UCAUGGUGUAGUCGACAAUAACAGCGUCUUGGCAGAUUCUGGUCACGUGC

CCUAUGCCCGGGCUUGUGCCUCUCAGGUGCACAGCGAUACUUAAAGCCUU

CAAGGUACUCGACGUGGGUACCGAUUCGUGACACUUCCUAAGAUUAUUCC

ACUGUGUUAGCCCCGCACCGCCGACCUAAACUGGUCCAAUGUAUACGCAU

UCGCUGAGCGGAUCGAUAAUAAAAGCUUGAAUU;

(SEQ ID NO: 378)

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAG

CCGGUAUUUUUUUUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUA

GUCUGCCUAUAAAGGUGCGGAUCCACAGCUGAUGAAAGACUUGUGCGGUA

CGGUUAAUCUCCCCUUUUUUUUUUUUUUUUUUUUUUUAGUAAAUGCGUCUAC

UGAAUCCAGCGAUGAUGCUGGCCCAGAUC

(SEQ ID NO: 379)

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAG

CCGGUAUUUUUUUUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUA

GUCUGCCUAUAAAGGUGCGGAUCCACAGCUGAUGAAAGACUUGUGCGGUA

CGGUUAAUCUCCCCUUUUUUUUUUUUUUUUUUUUUUUAGUAAAUGCGUCUAC

UGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAAGUGCAUAUAG

UAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU

CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCG

GCUAUUGCAGGAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUCCGC

UCACUAUGAUUAAGAACCAGGUGGAGUGUCACUGCUCUCGAGGUCUCACG

AGAGCGCUCGAUACAGUCCUUGGAAGAAUCUUUUUUUUUUUUUUUUUUU

UUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCUGCUCUA

(R2025/R2391 SEQ ID NO: 385)

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAG

CCGGUAUUUUUUUUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUA

GUCUGCCUAUAAAGGUGCGGAUCCACAGCUGAUGAAAGACUUGUGCGGUA

CGGUUAAUCUCCCCUUUUUUUUUUUUUUUUUUUUUUUAGUAAAUGCGUCUAC

UGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAAGUGCAUAUAG

UAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU

CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCG

GCUAUUGCAGGAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUCCGC

UCACUAUGAUUAAGAACCAGGUGGAGUGUCACUGCUCUCGAGGUCUCACG

AGAGCGCUCGAUACAGUCCUUGGAAGAAUCUUUUUUUUUUUUUUUUUUU

UUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCUGCUCUAG

(SEQ ID NO: 380)

GGGAGAAAGCUCAAGCUUAUCCAAGUAGGCUGGUCACCUGUACAACGUAG

CCGGUAUUUUUUUUUUUUUUUUUUUUUUUUGACCGUCUCAAGGUCCAAGUUA

GUCUGCCUAUAAAGGUGCGGAUCCACAGCUGAUGAAAGACUUGUGCGGUA

CGGUUAAUCUCCCCUUUUUUUUUUUUUUUUUUUUUUUAGUAAAUGCGUCUAC

UGAAUCCAGCGAUGAUGCUGGCCCAGAUCUUCGACCACAAGUGCAUAUAG

UAGUCAUCGAGGGUCGCCUUUUUUUUUUUUUUUUUUUUUUUGGCCCAGUU

CUGAGACUUCGCUAGAGACUACAGUUACAGCUGCAGUAGUAACCACUGCG

GCUAUUGCAGGAAAUCCCGUUCAGGUUUUUUUUUUUUUUUUUUUUUCCGC

UCACUAUGAUUAAGAACCAGGUGGAGUGUCACUGCUCUCGAGGUCUCACG

AGAGCGCUCGAUACAGUCCUUGGAAGAAUCUUUUUUUUUUUUUUUUUUU

UUGUGCGACGAUCACAGAGAACUUCUAUUCAUGCAGGUCUGCUCUAGAAC

GAACUGACCUGACGCCUGAACUUAUGAGCGUGCGUAUUUUUUUUUUUUUU

UUUUUUUUUCCUCCCAACAAAUGUCGAUCAAUAGCUGGGCUGUUGGAGAC

GCGUCAGCAAAUGCCGUGGCUCCAUAGGACGUGUAGACUUCUAUUUUUUU

UUUUUUUUUUUUUUCCCGGGACCACAAAUAAUAUUCUUGCUUGGUUGGGC

GCAAGGGCCCCGUAUCAGGUCAUAAACGGGUACAUGUUGCACAGGCUCCU

UUUUUUUUUUUUUUUUUUUUUUUCGCUGAGUUAUUCCGGUCUCAAAAGACG

GCAGACGUCAGUCGACAACACGGUCUAAAGCAGUGCUACAAUCUGCCGUG

UUCGUGUUUUUUUUUUUUUUUUUUUUGUGAACCUACACGGCGUGCACUGU

AGUUCGCAAUUCAUAGGGUACCGGCUCAGAGUUAUGCCUUGGUUGAAAAC

UGCCCAGCAUACUUUUUUUUUUUUUUUUUUUCAUAUUCCCAUGCUAAGC

AAGGGAUGCCGCGAGUCAUGUUAAGCUUGAAUU

[0230] According to another very particularly preferred embodiment, the nucleic acid molecule according to formula (V) may be selected from e.g. any of the following sequences:

(SEQ ID NO: 381)
UAGCGAAGCUCUUGGACCUACCUUUUUUUUUUUUUUCCCUGCGUUCCUAG

AAGUACACG;

(SEQ ID NO: 382)
UAGCGAAGCUCUUGGACCUACCUUUUUUUUUUUUUUCCCUGCGUUCCUA

GAAGUACACGAUCGCUUCGAGAACCUGGAUGGAAAAAAAAAAAAAAAGGG

ACGCAAGGAUCUUCAUGUGC;

or

[0231] In a further preferred embodiment the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may also occur in the form of a modified nucleic acid.

[0232] In this context, the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be provided as a "stabilized nucleic acid", preferably as a stabilized RNA or DNA, more preferably as a RNA that is essentially resistant to in vivo degradation (e.g. by an exo- or endo-nuclease).

[0233] Preferably, the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may contain backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present invention is a modification in which phosphates of the backbone of the nucleotides contained in the nucleic acid molecule of the inventive polymeric carrier cargo complex are chemically modified. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of the first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Furthermore, a base modification in connection with the present invention is a chemical modification of the base moiety of the nucleotides of the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Such a modification preferably increases the stability of the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, more preferably an RNA molecule, and/or the expression of a protein encoded by the first and/or the second nucleic acid molecule. Several nucleic acid modifications are known in the art, which can be applied to a nucleic acid molecule in the context of the present invention.

Chemical Modifications:

[0234] The term "nucleic acid modification" as used herein may refer to chemical modifications comprising backbone modifications as well as sugar modifications or base modifications.

[0235] In this context, the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may contain nucleotide analogues/modifications, e.g. backbone modifications, sugar modifications or base modifications. A backbone modification in connection with the present invention is a modification, in which phosphates of the backbone of the nucleotides contained in a nucleic acid molecule, preferably an RNA molecule as defined herein, are chemically modified. A sugar modification in connection with the present invention is a chemical modification of the sugar of the nucleotides of a nucleic acid molecule as defined herein. Furthermore, a base modification in connection with the present invention is a chemical modification of the base moiety of the nucleotides of the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. In this context, nucleotide analogues or modifications are preferably selected from nucleotide analogues, which are applicable for transcription and/or translation.

Sugar Modifications:

[0236] The modified nucleosides and nucleotides, which may be incorporated into the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, can be modified in the sugar moiety. For example, the 2' hydroxyl group (OH) can be modified or replaced with a number of different "oxy" or "deoxy" substituents. Examples of "oxy"-2' hydroxyl group modifications include, but are not limited to, alkoxy or aryloxy (—OR, e.g., R=H, alkyl, cycloalkyl, aryl, aralkyl, heteroaryl or sugar); polyethyleneglycols (PEG), —$O(CH_2CH_2O)_nCH_2CH_2OR$; "locked" nucleic acids (LNA) in which the 2' hydroxyl is connected, e.g., by a methylene bridge, to the 4' carbon of the same ribose sugar; and amino groups (—O-amino, wherein the amino group, e.g., NRR, can be alkylamino, dialkylamino, heterocyclyl, arylamino, diarylamino, heteroarylamino, or diheteroaryl amino, ethylene diamine, polyamino) or aminoalkoxy.

[0237] "Deoxy" modifications include hydrogen, amino (e.g. NH_2; alkylamino, dialkylamino, heterocyclyl, arylamino, diaryl amino, heteroaryl amino, diheteroaryl amino, or amino acid); or the amino group can be attached to the sugar through a linker, wherein the linker comprises one or more of the atoms C, N, and O.

[0238] The sugar group can also contain one or more carbons that possess the opposite stereochemical configuration than that of the corresponding carbon in ribose. Thus, nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex can include nucleotides containing, for instance, arabinose as the sugar.

Backbone Modifications:

[0239] The phosphate backbone may further be modified in the modified nucleosides and nucleotides, which may be incorporated into the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. The phosphate groups of the backbone can be modified by replacing one or more of

the oxygen atoms with a different substituent. Further, the modified nucleosides and nucleotides can include the full replacement of an unmodified phosphate moiety with a modified phosphate as described herein. Examples of modified phosphate groups include, but are not limited to, phosphorothioate, phosphoroselenates, borano phosphates, borano phosphate esters, hydrogen phosphonates, phosphoroamidates, alkyl or aryl phosphonates and phosphotriesters. Phosphorodithioates have both non-linking oxygens replaced by sulfur. The phosphate linker can also be modified by the replacement of a linking oxygen with nitrogen (bridged phosphoroamidates), sulfur (bridged phosphorothioates) and carbon (bridged methylene-phosphonates).

Base Modifications:

[0240] The modified nucleosides and nucleotides, which may be incorporated into the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, can further be modified in the nucleobase moiety. Examples of nucleobases found in nucleic acid molecules include, but are not limited to, adenine, guanine, cytosine and uracil. For example, the nucleosides and nucleotides described herein can be chemically modified on the major groove face. In some embodiments, the major groove chemical modifications can include an amino group, a thiol group, an alkyl group, or a halo group.

[0241] In particularly preferred embodiments of the present invention, the nucleotide analogues/modifications are selected from base modifications, which are preferably selected from 2-amino-6-chloropurineriboside-5'-triphosphate, 2-Aminopurine-riboside-5'-triphosphate; 2-aminoadenosine-5'-triphosphate, 2'-Amino-2'-deoxycytidine-triphosphate, 2-thiocytidine-5'-triphosphate, 2-thiouridine-5'-triphosphate, 2'-Fluorothymidine-5'-triphosphate, 2'-O-Methyl inosine-5'-triphosphate 4-thiouridine-5'-triphosphate, 5-aminoallylcytidine-5'-triphosphate, 5-aminoallyluridine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, 5-bromouridine-5'-triphosphate, 5-Bromo-2'-deoxycytidine-5'-triphosphate, 5-Bromo-2'-deoxyuridine-5'-triphosphate, 5-iodocytidine-5'-triphosphate, 5-Iodo-2'-deoxycytidine-5'-triphosphate, 5-iodouridine-5'-triphosphate, 5-Iodo-2'-deoxyuridine-5'-triphosphate, 5-methylcytidine-5'-triphosphate, 5-methyluridine-5'-triphosphate, 5-Propynyl-2'-deoxycytidine-5'-triphosphate, 5-Propynyl-2'-deoxyuridine-5'-triphosphate, 6-azacytidine-5'-triphosphate, 6-azauridine-5'-triphosphate, 6-chloropurineriboside-5'-triphosphate, 7-deazaadenosine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 8-azaadenosine-5'-triphosphate, 8-azidoadenosine-5'-triphosphate, benzimidazole-riboside-5'-triphosphate, N1-methyladenosine-5'-triphosphate, N1-methylguanosine-5'-triphosphate, N6-methyladenosine-5'-triphosphate, O6-methylguanosine-5'-triphosphate, pseudouridine-5'-triphosphate, or puromycin-5'-triphosphate, xanthosine-5'-triphosphate. Particular preference is given to nucleotides for base modifications selected from the group of base-modified nucleotides consisting of 5-methylcytidine-5'-triphosphate, 7-deazaguanosine-5'-triphosphate, 5-bromocytidine-5'-triphosphate, and pseudouridine-5'-triphosphate.

[0242] In some embodiments, modified nucleosides include pyridine-4-one ribonucleoside, 5-aza-uridine, 2-thio-5-aza-uridine, 2-thiouridine, 4-thio-pseudouridine, 2-thio-pseudouridine, 5-hydroxyuridine, 3-methyluridine, 5-carboxymethyl-uridine, 1-carboxymethyl-pseudouridine, 5-propynyl-uridine, 1-propynyl-pseudouridine, 5-taurinomethyluridine, 1-taurinomethyl-pseudouridine, 5-taurinomethyl-2-thio-uridine, 1-taurinomethyl-4-thio-uridine, 5-methyl-uridine, 1-methyl-pseudouridine, 4-thio-1-methyl-pseudouridine, 2-thio-1-methyl-pseudouridine, 1-methyl-1-deaza-pseudouridine, 2-thio-1-methyl-1-deaza-pseudouridine, dihydrouridine, dihydropseudouridine, 2-thio-dihydrouridine, 2-thio-dihydropseudouridine, 2-methoxyuridine, 2-methoxy-4-thio-uridine, 4-methoxy-pseudouridine, and 4-methoxy-2-thio-pseudouridine.

[0243] In some embodiments, modified nucleosides include 5-aza-cytidine, pseudoisocytidine, 3-methyl-cytidine, N4-acetylcytidine, 5-formylcytidine, N4-methylcytidine, 5-hydroxymethylcytidine, 1-methyl-pseudoisocytidine, pyrrolo-cytidine, pyrrolo-pseudoisocytidine, 2-thio-cytidine, 2-thio-5-methyl-cytidine, 4-thio-pseudoisocytidine, 4-thio-1-methyl-pseudoisocytidine, 4-thio-1-methyl-1-deaza-pseudoisocytidine, 1-methyl-1-deaza-pseudoisocytidine, zebularine, 5-aza-zebularine, 5-methyl-zebularine, 5-aza-2-thio-zebularine, 2-thio-zebularine, 2-methoxy-cytidine, 2-methoxy-5-methyl-cytidine, 4-methoxy-pseudoisocytidine, and 4-methoxy-1-methyl-pseudoisocytidine.

[0244] In other embodiments, modified nucleosides include 2-aminopurine, 2, 6-diaminopurine, 7-deaza-adenine, 7-deaza-8-aza-adenine, 7-deaza-2-aminopurine, 7-deaza-8-aza-2-aminopurine, 7-deaza-2,6-diaminopurine, 7-deaza-8-aza-2,6-diaminopurine, 1-methyladenosine, N6-methyladenosine, N6-isopentenyladenosine, N6-(cis-hydroxyisopentenyl) adenosine, 2-methylthio-N6-(cis-hydroxyisopentenyl) adenosine, N6-glycinylcarbamoyladenosine, N6-threonylcarbamoyladenosine, 2-methylthio-N6-threonyl carbamoyladenosine, N6,N6-dimethyladenosine, 7-methyladenine, 2-methylthio-adenine, and 2-methoxy-adenine.

[0245] In other embodiments, modified nucleosides include inosine, 1-methyl-inosine, wyosine, wybutosine, 7-deaza-guanosine, 7-deaza-8-aza-guanosine, 6-thio-guanosine, 6-thio-7-deaza-guanosine, 6-thio-7-deaza-8-aza-guanosine, 7-methyl-guanosine, 6-thio-7-methyl-guanosine, 7-methylinosine, 6-methoxy-guanosine, 1-methylguanosine, N2-methylguanosine, N2,N2-dimethylguanosine, 8-oxo-guanosine, 7-methyl-8-oxo-guanosine, 1-methyl-6-thio-guanosine, N2-methyl-6-thio-guanosine, and N2,N2-dimethyl-6-thio-guanosine.

[0246] In some embodiments, the nucleotide can be modified on the major groove face and can include replacing hydrogen on C-5 of uracil with a methyl group or a halo group.

[0247] In specific embodiments, a modified nucleoside is 5'-O-(1-Thiophosphate)-Adenosine, 5'-O-(1-Thiophosphate)-Cytidine, 5'-O-(1-Thiophosphate)-Guanosine, 5'-0-(1-Thiophosphate)-Uridine or 5'-O-(1-Thiophosphate)-Pseudouridine.

[0248] In further specific embodiments, the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may comprise nucleoside modifications selected from 6-aza-cytidine, 2-thio-cytidine, a-thio-cytidine, Pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodouridine, N1-methyl-pseudouridine, 5,6-dihydrouridine, a-thio-uridine, 4-thio-

uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, 5-methyl-uridine, Pyrrolo-cytidine, inosine, a-thio-guanosine, 6-methyl-guanosine, 5-methyl-cytidine, 8-oxo-guanosine, 7-deaza-guanosine, N1-methyl-adenosine, 2-amino-6-Chloro-purine, N6-methyl-2-amino-purine, Pseudo-iso-cytidine, 6-Chloro-purine, N6-methyl-adenosine, a-thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine.

Modification of the 5'-End of a Modified RNA Molecule:

[0249] According to another preferred embodiment of the invention, the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be an RNA molecule, preferably a modified RNA molecule as defined herein, which is modified by the addition of a so-called "5' CAP" structure.

[0250] A 5'-cap is an entity, typically a modified nucleotide entity, which generally "caps" the 5'-end of a mature mRNA. A 5'-cap may typically be formed by a modified nucleotide, particularly by a derivative of a guanine nucleotide. Preferably, the 5'-cap is linked to the 5'-terminus via a 5'-5'-triphosphate linkage. A 5'-cap may be methylated, e.g. m7GpppN, wherein N is the terminal 5' nucleotide of the nucleic acid carrying the 5'-cap, typically the 5'-end of an RNA. m7GpppN is the 5'-CAP structure which naturally occurs in mRNA transcribed by polymerase II and is therefore not considered as modification comprised in a modified RNA in this context. According to a preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may thus comprise a m7GpppN as 5'-CAP, and preferably comprise, in addition, at least one further modification as defined herein.

[0251] Further examples of 5'cap structures include glyceryl, inverted deoxy abasic residue (moiety), 4',5' methylene nucleotide, 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide, 1,5-anhydrohexitol nucleotide, L-nucleotides, alpha-nucleotide, modified base nucleotide, threo-pentofuranosyl nucleotide, acyclic 3',4'-seco nucleotide, acyclic 3,4-dihydroxybutyl nucleotide, acyclic 3,5 dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety, 3'-3'-inverted abasic moiety, 3'-2'-inverted nucleotide moiety, 3'-2'-inverted abasic moiety, 1,4-butanediol phosphate, 3'-phosphoramidate, hexylphosphate, aminohexyl phosphate, 3'-phosphate, 3'phosphorothioate, phosphorodithioate, or bridging or non-bridging methylphosphonate moiety. These modified 5'-CAP structures are regarded as at least one modification in this context.

[0252] Particularly preferred modified 5'-CAP structures are CAP1 (methylation of the ribose of the adjacent nucleotide of m7G), CAP2 (methylation of the ribose of the 2nd nucleotide downstream of the m7G), CAP3 (methylation of the ribose of the 3rd nucleotide downstream of the m7G), CAP4 (methylation of the ribose of the 4th nucleotide downstream of the m7G), ARCA (anti-reverse CAP analogue, modified ARCA (e.g. phosphothioate modified ARCA), inosine, N1-methyl-guanosine, 2'-fluoro-guanosine, 7-deaza-guanosine, 8-oxo-guanosine, 2-amino-guanosine, LNA-guanosine, and 2-azido-guanosine.

[0253] According to a further embodiment, the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex can contain a lipid modification. Such a lipid-modified nucleic acid typically comprises a nucleic acid as defined herein. Such a lipid-modified first nucleic acid molecule of the inventive polymeric carrier cargo complex or a lipid-modified second nucleic acid molecule administered in combination with the polymeric carrier cargo complex typically further comprises at least one linker covalently linked with that nucleic acid molecule, and at least one lipid covalently linked with the respective linker. Alternatively, the lipid-modified nucleic acid molecule comprises at least one nucleic acid molecule as defined herein and at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid molecule. According to a third alternative, the lipid-modified nucleic acid molecule comprises a nucleic acid molecule as defined herein, at least one linker covalently linked with that nucleic acid molecule, and at least one lipid covalently linked with the respective linker, and also at least one (bifunctional) lipid covalently linked (without a linker) with that nucleic acid molecule.

[0254] The first nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may likewise be stabilized in order to prevent degradation of the nucleic acid molecule by various approaches, particularly, when RNA or mRNA is used as a nucleic acid molecule for the inventive purpose. It is known in the art that instability and (fast) degradation of RNA in general may represent a serious problem in the application of RNA based compositions. This instability of RNA is typically due to RNA-degrading enzymes, "RNAases" (ribonucleases), wherein contamination with such ribonucleases may sometimes completely degrade RNA in solution. Accordingly, the natural degradation of RNA in the cytoplasm of cells is very finely regulated and RNase contaminations may be generally removed by special treatment prior to use of said compositions, in particular with diethyl pyrocarbonate (DEPC). A number of mechanisms of natural degradation are known in this connection in the prior art, which may be utilized as well. E.g., the terminal structure is typically of critical importance particularly for an mRNA. As an example, at the 5' end of naturally occurring mRNAs there is usually a so-called "cap structure" (a modified guanosine nucleotide), and at the 3' end is typically a sequence of up to 200 adenosine nucleotides (the so-called poly-A tail).

[0255] According to another embodiment, the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be modified, and thus stabilized, especially if the nucleic acid molecule is in the form of a coding nucleic acid e.g. an mRNA, by modifying the G/C content of the nucleic acid molecule, particularly an mRNA, preferably of the coding region thereof.

[0256] In a particularly preferred embodiment of the present invention, the G/C content of the coding region of the first nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or of the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid molecule is in the form of an mRNA, is modified, particularly increased, compared to the G/C content of the coding region of its particular wild type coding sequence,

i.e. the unmodified mRNA. The encoded amino acid sequence of the nucleic acid sequence is preferably not modified compared to the coded amino acid sequence of the particular wild type mRNA.

[0257] The modification of the G/C-content of the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid molecule is in the form of an mRNA or codes for an mRNA, is based on the fact that the sequence of any mRNA region to be translated is important for efficient translation of that mRNA. Thus, the composition and the sequence of various nucleotides are important. In particular, sequences having an increased G (guanosine)/C (cytosine) content are more stable than sequences having an increased A (adenosine)/U (uracil) content. According to the invention, the codons of the coding sequence or mRNA are therefore varied compared to its wild type coding sequence or mRNA, while retaining the translated amino acid sequence, such that they include an increased amount of G/C nucleotides. In respect to the fact that several codons code for one and the same amino acid (so-called degeneration of the genetic code), the most favourable codons for the stability can be determined (so-called alternative codon usage).

[0258] Preferably, the G/C content of the coding region of the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, is increased by at least 7%, more preferably by at least 15%, particularly preferably by at least 20%, compared to the G/C content of the coded region of the wild type mRNA. According to a specific aspect at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, more preferably at least 70%, even more preferably at least 80% and most preferably at least 90%, 95% or even 100% of the substitutable codons in the region coding for a protein or peptide as defined herein or its fragment or variant thereof or the whole sequence of the wild type mRNA sequence or coding sequence are substituted, thereby increasing the G/C content of said sequence.

[0259] In this context, it is particularly preferable to increase the G/C content of the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, to the maximum (i.e. 100% of the substitutable codons), in particular in the region coding for a protein, compared to the wild type sequence.

[0260] According to the invention, a further preferred modification of the nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, is based on the finding that the translation efficiency is also determined by a different frequency in the occurrence of tRNAs in cells. Thus, if so-called "rare codons" are present in the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, to an increased extent, the corresponding modified nucleic acid molecule is translated to a significantly poorer degree than in the case where codons coding for relatively "frequent" tRNAs are present.

[0261] Especially if the modified nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex is in the form of an mRNA or codes for an mRNA, the coding region of the modified nucleic acid is preferably modified compared to the corresponding region of the wild type mRNA or coding sequence such that at least one codon of the wild type sequence which codes for a tRNA which is relatively rare in the cell is exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and carries the same amino acid as the relatively rare tRNA. By this modification, the sequences of the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, is modified such that codons for which frequently occurring tRNAs are available are inserted. In other words, according to the invention, by this modification all codons of the wild type sequence which code for a tRNA which is relatively rare in the cell can in each case be exchanged for a codon which codes for a tRNA which is relatively frequent in the cell and which, in each case, carries the same amino acid as the relatively rare tRNA.

[0262] Which tRNAs occur relatively frequently in the cell and which, in contrast, occur relatively rarely is known to a person skilled in the art; cf. e.g. Akashi, Curr. Opin. Genet. Dev. 2001, 11(6): 660-666. The codons which use for the particular amino acid the tRNA which occurs the most frequently, e.g. the Gly codon, which uses the tRNA which occurs the most frequently in the (human) cell, are particularly preferred.

[0263] According to the invention, it is particularly preferable to link the sequential G/C content which is increased, in particular maximized, in the modified nucleic acid molecule of the herein defined inventive polymeric carrier cargo complex and/or the modified second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA, with the "frequent" codons without modifying the amino acid sequence of the protein encoded by the coding region of the nucleic acid molecule. This preferred aspect allows provision of a particularly efficiently translated and stabilized (modified) nucleic acid molecule, especially if the nucleic acid is in the form of an mRNA or codes for an mRNA.

[0264] According to a further preferred embodiment of the invention, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of a coding nucleic acid molecule, preferably has at least one 5' and/or 3' stabilizing sequence. These stabilizing sequences in the 5' and/or 3' untranslated regions have the effect of increasing the half-life of the nucleic acid in the cytosol. These stabilizing sequences can have 100% sequence identity to naturally occurring sequences which occur in viruses, bacteria and eukaryotes,

but can also be partly or completely synthetic. The untranslated sequences (UTR) of the (alpha-)globin gene, e.g. from *Homo sapiens* or *Xenopus laevis* may be mentioned as an example of stabilizing sequences which can be used in the present invention for a stabilized nucleic acid. Another example of a stabilizing sequence has the general formula (C/U)CCAN$_x$CCC(U/A)Py$_x$UC(C/U)CC (SEQ ID NO: 383), which is contained in the 3'UTR of the very stable RNA which codes for (alpha-)globin, type(I)-collagen, 15-lipoxygenase or for tyrosine hydroxylase (cf. Holcik et al., Proc. Natl. Acad. Sci. USA 1997, 94: 2410 to 2414). Such stabilizing sequences can of course be used individually or in combination with one another and also in combination with other stabilizing sequences known to a person skilled in the art.

[0265] In one embodiment of the invention, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may be an RNA molecule, which is preferably modified as defined herein, more preferably an mRNA molecule, wherein the mRNA molecule comprises at least one selected from the group consisting of a 5'-UTR, a 3'-UTR, a poly(A) sequence, a poly(C) sequence and a histone stem-loop sequence. In a particularly preferred embodiment, the second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex is an mRNA molecule, preferably an mRNA molecule comprising at least one modification as defined herein, wherein the mRNA preferably comprises at least one selected from the group consisting of a 5'-UTR, a 3'-UTR, a poly(A) sequence, a poly(C) sequence and a histone stem-loop sequence.

[0266] In a preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises a 5'-UTR and/or a 3'-UTR.

[0267] In the context of the present invention, a 3'-UTR is typically the part of an mRNA, which is located between the protein coding region (i.e. the open reading frame) and the 3'-terminus of the mRNA. A 3'-UTR of an mRNA is not translated into an amino acid sequence. The 3'-UTR sequence is generally encoded by the gene, which is transcribed into the respective mRNA during the gene expression process. In the context of the present invention, a 3'-UTR corresponds to the sequence of a mature mRNA which is located 3' to the stop codon of the protein coding region, preferably immediately 3' to the stop codon of the protein coding region, and which extends to the 5'-side of the 3'-terminus of the mRNA or of the poly(A) sequence, preferably to the nucleotide immediately 5' to the poly(A) sequence. The term "corresponds to" means that the 3'-UTR sequence may be an RNA sequence, such as in the mRNA sequence used for defining the 3'-UTR sequence, or a DNA sequence which corresponds to such RNA sequence. In the context of the present invention, the term "a 3'-UTR of a gene", such as "a 3'-UTR of an albumin gene", is the sequence, which corresponds to the 3'-UTR of the mature mRNA derived from this gene, i.e. the mRNA obtained by transcription of the gene and maturation of the pre-mature mRNA. The term "3'-UTR of a gene" encompasses the DNA sequence and the RNA sequence of the 3'-UTR. Preferably, the 3'-UTR used according to the present invention is heterologous to the coding region of the mRNA sequence. Even if 3'-UTR's derived from naturally occurring genes are preferred, also synthetically engineered UTR's may be used in the context of the present invention.

[0268] As used herein, the term '5'-UTR' typically refers to a particular section of messenger RNA (mRNA). It is located 5' of the open reading frame of the mRNA. Typically, the 5'-UTR starts with the transcriptional start site and ends one nucleotide before the start codon of the open reading frame. The 5'-UTR may comprise elements for controlling gene expression, also called regulatory elements. Such regulatory elements may be, for example, ribosomal binding sites or a 5'-Terminal Oligopyrimidine Tract. The 5'-UTR may be posttranscriptionally modified, for example by addition of a 5'-CAP. In the context of the present invention, a 5'-UTR corresponds to the sequence of a mature mRNA, which is located between the 5'-CAP and the start codon. Preferably, the 5'-UTR corresponds to the sequence, which extends from a nucleotide located 3' to the 5'-CAP, preferably from the nucleotide located immediately 3' to the 5'-CAP, to a nucleotide located 5' to the start codon of the protein coding region, preferably to the nucleotide located immediately 5' to the start codon of the protein coding region. The nucleotide located immediately 3' to the 5'-CAP of a mature mRNA typically corresponds to the transcriptional start site. The term "corresponds to" means that the 5'-UTR sequence may be an RNA sequence, such as in the mRNA sequence used for defining the 5'-UTR sequence, or a DNA sequence, which corresponds to such RNA sequence. In the context of the present invention, the term "a 5'-UTR of a gene", such as "a 5'-UTR of a TOP gene", is the sequence, which corresponds to the 5'-UTR of the mature mRNA derived from this gene, i.e. the mRNA obtained by transcription of the gene and maturation of the pre-mature mRNA. The term "5'-UTR of a gene" encompasses the DNA sequence and the RNA sequence of the 5'-UTR. Preferably, the 5'-UTR used according to the present invention is heterologous to the coding region of the mRNA sequence. Even if 5'-UTR's derived from naturally occurring genes are preferred, also synthetically engineered UTR's may be used in the context of the present invention.

[0269] In a particularly preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises at least one 5'-untranslated region (5'-UTR). More preferably, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises a 5'-UTR, which comprises or consists of a nucleic acid sequence which is derived from a 5'-UTR of a TOP gene, or which is derived from a fragment, homolog or variant of the 5'-UTR of a TOP gene.

[0270] The 5'terminal oligopyrimidine tract (TOP) is typically a stretch of pyrimidine nucleotides located at the 5' terminal region of a nucleic acid molecule, such as the 5' terminal region of certain mRNA molecules or the 5' terminal region of a functional entity, e.g. the transcribed region, of certain genes. The sequence starts with a cytidine, which usually corresponds to the transcriptional start site, and is followed by a stretch of usually about 3 to 30 pyrimidine nucleotides. For example, the TOP may comprise 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,

24, 25, 26, 27, 28, 29, 30 or even more nucleotides. The pyrimidine stretch and thus the 5' TOP ends one nucleotide 5' to the first purine nucleotide located downstream of the TOP. Messenger RNA that contains a 5'terminal oligopyrimidine tract is often referred to as TOP mRNA. Accordingly, genes that provide such messenger RNAs are referred to as TOP genes. TOP sequences have, for example, been found in genes and mRNAs encoding peptide elongation factors and ribosomal proteins.

[0271] In the context of the present invention, a TOP motif is typically a nucleic acid sequence, which corresponds to a 5'TOP as defined above. Thus, a TOP motif in the context of the present invention is preferably a stretch of pyrimidine nucleotides having a length of 3-30 nucleotides. Preferably, the TOP-motif consists of at least 3 pyrimidine nucleotides, preferably at least 4 pyrimidine nucleotides, preferably at least 5 pyrimidine nucleotides, more preferably at least 6 nucleotides, more preferably at least 7 nucleotides, most preferably at least 8 pyrimidine nucleotides, wherein the stretch of pyrimidine nucleotides preferably starts at its 5' end with a cytosine nucleotide. In TOP genes and TOP mRNAs, the TOP-motif preferably starts at its 5'end with the transcriptional start site and ends one nucleotide 5' to the first purine residue in said gene or mRNA. A TOP motif in the sense of the present invention is preferably located at the 5'end of a sequence, which represents a 5'-UTR or at the 5'end of a sequence, which codes for a 5'-UTR. Thus, preferably, a stretch of 3 or more pyrimidine nucleotides is called "TOP motif" in the sense of the present invention if this stretch is located at the 5'end of a respective sequence, such as the inventive mRNA, the 5'-UTR of the inventive mRNA, or the nucleic acid sequence, which is derived from the 5'-UTR of a TOP gene as described herein. In other words, a stretch of 3 or more pyrimidine nucleotides, which is not located at the 5'-end of a 5'-UTR but anywhere within a 5'-UTR is preferably not referred to as "TOP motif".

[0272] In this context, a TOP gene is typically characterised by the presence of a 5' terminal oligopyrimidine tract. Furthermore, most TOP genes are characterized by a growth-associated translational regulation. However, also TOP genes with a tissue specific translational regulation are known. As defined above, the 5'-UTR of a TOP gene corresponds to the sequence of a 5'-UTR of a mature mRNA derived from a TOP gene, which preferably extends from the nucleotide located 3' to the 5'-CAP to the nucleotide located 5' to the start codon. A 5'-UTR of a TOP gene typically does not comprise any start codons, preferably no upstream AUGs (uAUGs) or upstream open reading frames (uORFs). Therein, upstream AUGs and upstream open reading frames are typically understood to be AUGs and open reading frames that occur 5' of the start codon (AUG) of the open reading frame that should be translated. The 5'-UTRs of TOP genes are generally rather short. The lengths of 5'-UTRs of TOP genes may vary between 20 nucleotides up to 500 nucleotides, and are typically less than about 200 nucleotides, preferably less than about 150 nucleotides, more preferably less than about 100 nucleotides. Exemplary 5'-UTRs of TOP genes in the sense of the present invention are the nucleic acid sequences extending from the nucleotide at position 5 to the nucleotide located immediately 5' to the start codon (e.g. the ATG) in the sequences according to SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the international patent application WO2013/143700 or homologs or variants thereof, whose disclosure is incorporated herewith by reference. In this context a particularly preferred fragment of a 5'-UTR of a TOP gene is a 5'-UTR of a TOP gene lacking the 5'TOP motif. The term '5'-UTR of a TOP gene' preferably refers to the 5'-UTR of a naturally occurring TOP gene.

[0273] In a specific embodiment, the 5'-UTR does not comprise a TOP-motif or a 5'TOP, as defined herein.

[0274] In some embodiments, the nucleic acid sequence of the 5'-UTR, which is derived from a 5'-UTR of a TOP gene terminates at its 3'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 upstream of the start codon (e.g. A(U/T)G) of the gene or mRNA it is derived from. Thus, the 5'-UTR does not comprise any part of the protein coding region. Thus, preferably, the only protein coding part of the inventive mRNA sequence is provided by the coding region.

[0275] The nucleic acid sequence, which is derived from a 5'-UTR of a TOP gene, is preferably derived from a eukaryotic TOP gene, preferably a plant or animal TOP gene, more preferably a chordate TOP gene, even more preferably a vertebrate TOP gene, most preferably a mammalian TOP gene, such as a human TOP gene.

[0276] For example, the 5'-UTR preferably comprises or consists of a nucleic acid sequence, which is derived from a nucleic acid sequence selected from the group consisting of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, whose disclosure is incorporated herein by reference, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from a variant thereof, or preferably from a corresponding RNA sequence. The term "homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700" refers to sequences of other species than *Homo sapiens*, which are homologous to the sequences according to SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700.

[0277] In a preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from a nucleic acid sequence extending from nucleotide position 5 (i.e. the nucleotide that is located at position 5 in the sequence) to the nucleotide position immediately 5' to the start codon (located at the 3' end of the sequences), e.g. the nucleotide position immediately 5' to the ATG sequence, of a nucleic acid sequence selected from SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700 from a variant thereof, or a corresponding RNA sequence. It is particularly preferred that the 5' UTR is derived from a nucleic acid sequence extending from the nucleotide position immediately 3' to the 5'TOP to the nucleotide position immediately 5' to the start codon (located at the 3' end of the sequences), e.g. the nucleotide position immediately 5' to the ATG sequence, of a nucleic acid sequence selected from SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from the homologs of SEQ ID Nos. 1-1363, SEQ ID NO. 1395, SEQ ID NO. 1421 and SEQ ID NO. 1422 of the patent application WO2013/143700, from a variant thereof, or a corresponding RNA sequence.

[0278] In a particularly preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 5'-UTR of a ribosomal protein gene, preferably from a 5'-UTR of a TOP gene encoding a ribosomal protein or from a variant of a 5'-UTR of a TOP gene encoding a ribosomal protein. For example, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 5'-UTR of a nucleic acid sequence according to any of SEQ ID NOs: 67, 170, 193, 244, 259, 554, 650, 675, 700, 721, 913, 1016, 1063, 1120, 1138, and 1284-1360 of the patent application WO2013/143700, a corresponding RNA sequence, a homolog thereof, or a variant thereof as described herein, preferably lacking the 5'TOP motif. As described above, the sequence extending from position 5 to the nucleotide immediately 5' to the ATG (which is located at the 3'end of the sequences) corresponds to the 5'-UTR of said sequences.

[0279] Preferably, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 5'-UTR of a TOP gene encoding a ribosomal Large protein (RPL) or from a homolog or variant of a 5'-UTR of a TOP gene encoding a ribosomal Large protein (RPL). For example, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 5'-UTR of a nucleic acid sequence according to any of SEQ ID NOs: 67, 259, 1284-1318, 1344, 1346, 1348-1354, 1357, 1358, 1421 and 1422 of the patent application WO2013/143700, a corresponding RNA sequence, a homolog thereof, or a variant thereof as described herein, preferably lacking the 5'TOP motif.

[0280] In a particularly preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from the 5'-UTR of a ribosomal protein Large 32 gene, preferably from a vertebrate ribosomal protein Large 32 (L32) gene, more preferably from a mammalian ribosomal protein Large 32 (L32) gene, most preferably from a human ribosomal protein Large 32 (L32) gene, or from a variant of the 5'-UTR of a ribosomal protein Large 32 gene, preferably from a vertebrate ribosomal protein Large 32 (L32) gene, more preferably from a mammalian ribosomal protein Large 32 (L32) gene, most preferably from a human ribosomal protein Large 32 (L32) gene, wherein preferably the 5'-UTR does not comprise the 5'TOP of said gene.

[0281] A preferred sequence for a 5'-UTR element corresponds to SEQ ID NO. 1368 of the patent application WO2013/143700 and reads as follows:

Nucleotide sequence for 5'-UTR element (SEQ ID NO. 386)

GGCGCTGCCTACGGAGGTGGCAGCCATCTCCTTCTCGGCATC

[0282] Accordingly, in a particularly preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 1368 of the patent application WO2013/143700 (5'-UTR of human ribosomal protein Large 32 lacking the 5' terminal oligopyrimidine tract, SEQ ID NO. 32) or preferably to a corresponding RNA sequence, or wherein the at least one 5'-UTR comprises or consists of a fragment of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 31 or more preferably to a corresponding RNA sequence, wherein, preferably, the fragment is as described above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR. Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

[0283] In some embodiments, the inventive mRNA sequence comprises a 5'-UTR, which comprises or consists of a nucleic acid sequence, which is derived from the 5'-UTR of a vertebrate TOP gene, such as a mammalian, e.g. a human TOP gene, selected from RPSA, RPS2, RPS3, RPS3A, RPS4, RPS5, RPS6, RPS7, RPS8, RPS9, RPS10, RPS11, RPS12, RPS13, RPS14, RPS15, RPS15A, RPS16, RPS17, RPS18, RPS19, RPS20, RPS21, RPS23, RPS24, RPS25, RPS26, RPS27, RPS27A, RPS28, RPS29, RPS30, RPL3, RPL4, RPL5, RPL6, RPL7, RPL7A, RPL8, RPL9, RPL10, RPL10A, RPL11, RPL12, RPL13, RPL13A, RPL14, RPL15, RPL17, RPL18, RPL18A, RPL19, RPL21, RPL22, RPL23, RPL23A, RPL24, RPL26, RPL27, RPL27A, RPL28, RPL29, RPL30, RPL31, RPL32, RPL34, RPL35, RPL35A, RPL36, RPL36A, RPL37, RPL37A, RPL38, RPL39, RPL40, RPL41, RPLP0, RPLP1, RPLP2, RPLP3, RPLP0, RPLP1, RPLP2, EEF1A1, EEF1B2, EEF1D, EEF1G, EEF2, EIF3E, EIF3F, EIF3H, EIF2S3, EIF3C, EIF3K, EIF3EIP, EIF4A2, PABPC1, HNRNPA1, TPT1, TUBB1, UBA52, NPM1, ATP5G2, GNB2L1, NME2, UQCRB, or from a homolog or variant thereof, wherein preferably the 5'-UTR does not comprise a TOP-motif or the 5'TOP of said genes, and wherein optionally the 5'-UTR starts at its 5'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 downstream of the 5'terminal oligopyrimidine tract (TOP) and wherein further optionally the 5'-UTR, which is derived from a 5'-UTR of a TOP gene, terminates at its 3'-end with a nucleotide located at position 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 upstream of the start codon (A(U/T)G) of the gene it is derived from.

[0284] In further particularly preferred embodiments, the 5'-UTR comprises or consists of a nucleic acid sequence, which is derived from the 5'-UTR of a ribosomal protein Large 32 gene (RPL32), a ribosomal protein Large 35 gene (RPL35), a ribosomal protein Large 21 gene (RPL21), an ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, an hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), an androgen-induced 1 gene (AIG1), cytochrome c oxidase subunit VIc gene (COX6C), or a N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, preferably from a vertebrate ribosomal protein Large 32 gene (RPL32), a vertebrate ribosomal protein Large 35 gene (RPL35), a vertebrate ribosomal protein Large 21 gene (RPL21), a vertebrate ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a vertebrate hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a vertebrate androgen-induced 1 gene (AIG1), a vertebrate cytochrome c

oxidase subunit VIc gene (COX6C), or a vertebrate N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, more preferably from a mammalian ribosomal protein Large 32 gene (RPL32), a ribosomal protein Large 35 gene (RPL35), a ribosomal protein Large 21 gene (RPL21), a mammalian ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a mammalian hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a mammalian androgen-induced 1 gene (AIG1), a mammalian cyto-chrome c oxidase subunit VIc gene (COX6C), or a mammalian N-acylsphingosine ami-dohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, most preferably from a human ribosomal protein Large 32 gene (RPL32), a human ribosomal protein Large 35 gene (RPL35), a human ribosomal protein Large 21 gene (RPL21), a human ATP syn-thase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle (ATP5A1) gene, a human hydroxysteroid (17-beta) dehydrogenase 4 gene (HSD17B4), a human androgen-induced 1 gene (AIG1), a human cytochrome c oxidase subunit VIc gene (COX6C), or a human N-acylsphingosine amidohydrolase (acid ceramidase) 1 gene (ASAH1) or from a variant thereof, wherein preferably the 5'-UTR does not comprise the 5'TOP of said gene.

[0285] Accordingly, in a particularly preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 1368, or SEQ ID NOs 1412-1420 of the patent application WO2013/143700, or a corresponding RNA sequence, or wherein the at least one 5'-UTR comprises or consists of a fragment of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 1368, or SEQ ID NOs 1412-1420 of the patent application WO2013/143700, wherein, preferably, the fragment is as described above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR. Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

[0286] Accordingly, in a particularly preferred embodiment, the 5'-UTR comprises or consists of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according SEQ ID NO. 1414 of the patent application WO2013/143700 (5'-UTR of ATP5A1 lacking the 5' terminal oligopyrimidine tract) or preferably to a corresponding RNA sequence, or wherein the at least one 5'-UTR comprises or consists of a fragment of a nucleic acid sequence, which has an identity of at least about 40%, preferably of at least about 50%, preferably of at least about 60%, preferably of at least about 70%, more preferably of at least about 80%, more preferably of at least about 90%, even more preferably of at least about 95%, even more preferably of at least about 99% to the nucleic acid sequence according to SEQ ID NO. 1414 of the patent application WO2013/143700 or more preferably to a corresponding RNA sequence, wherein, preferably, the fragment is as described above, i.e. being a continuous stretch of nucleotides representing at least 20% etc. of the full-length 5'-UTR. Preferably, the fragment exhibits a length of at least about 20 nucleotides or more, preferably of at least about 30 nucleotides or more, more preferably of at least about 40 nucleotides or more. Preferably, the fragment is a functional fragment as described herein.

[0287] In a further preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, preferably an mRNA, comprises at least one 3'-UTR.

[0288] More preferably, the mRNA comprises or consists of a nucleic acid sequence derived from the 3'-UTR of a chordate gene, preferably a vertebrate gene, more preferably a mammalian gene, most preferably a human gene, or from a variant of the 3'-UTR of a chordate gene, preferably a vertebrate gene, more preferably a mammalian gene, most preferably a human gene.

[0289] Preferably, the inventive mRNA sequence comprises a 3'-UTR, which may be derivable from a gene that relates to an mRNA with an enhanced half-life (that provides a stable mRNA), for example a 3'-UTR as defined and described below.

[0290] In a particularly preferred embodiment, the 3'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 3'-UTR of a gene selected from the group consisting of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, and a collagen alpha gene, such as a collagen alpha 1(I) gene, or from a variant of a 3'-UTR of a gene selected from the group consisting of an albumin gene, an α-globin gene, a (3-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, and a collagen alpha gene, such as a collagen alpha 1(I) gene according to SEQ ID NO. 1369-1390 of the patent application WO2013/143700 whose disclosure is incorporated herein by reference. In a particularly preferred embodiment, the 3'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 3'-UTR of an albumin gene, preferably a vertebrate albumin gene, more preferably a mammalian albumin gene, most preferably a human albumin gene according SEQ ID No: 1369 of the patent application WO2013/143700. The mRNA sequence may comprise or consist of a nucleic acid sequence, which is derived from the 3'-UTR of the human albumin gene according to GenBank Accession number NM_000477.5, or from a fragment or variant thereof.

[0291] In this context, it is particularly preferred that the mRNA comprises a 3'-UTR comprising a corresponding RNA sequence derived from the nucleic acid sequences according to SEQ ID NO. 1369-1390 of the patent application WO2013/143700 or a fragment, homolog or variant thereof.

[0292] Most preferably the 3'-UTR comprises the nucleic acid sequence derived from a fragment of the human albumin gene according to SEQ ID No: 1376 of the patent application WO2013/143700, in the following referred to as SEQ ID NO. 33.

Nucleotide sequence of 3'-UTR element of human albumin gene

(SEQ ID NO. 387)

CATCACATTTAAAAGCATCTCAGCCTACCATGAGAATAAGAGAAAGAAAA

TGAAGATCAATAGCTTATTCATCTCTTTTTCTTTTTCGTTGGTGTAAAGC

CAACACCCTGTCTAAAAAACATAAATTTCTTTAATCATTTTGCCTCTTTT

CTCTGTGCTTCAATTAATAAAAAATGGAAAGAACCT

[0293] In another particularly preferred embodiment, the 3'-UTR comprises or consists of a nucleic acid sequence, which is derived from a 3'-UTR of an α-globin gene, preferably a vertebrate α- or β-globin gene, more preferably a mammalian α- or β-globin gene, most preferably a human α- or β-globin gene according to SEQ ID NO. 1370 of the patent application WO2013/143700 (3'-UTR of *Homo sapiens* hemoglobin, alpha 1 (HBA1)), or according to SEQ ID NO. 1371 of the patent application WO2013/143700 (3'-UTR of *Homo sapiens* hemoglobin, alpha 2 (HBA2)), or according to SEQ ID NO. 1372 of the patent application WO2013/143700 (3'-UTR of *Homo sapiens* hemoglobin, beta (HBB)).

[0294] For example, the 3'-UTR may comprise or consist of the center, a-complex-binding portion of the 3'-UTR of an α-globin gene, such as of a human α-globin gene, preferably according to SEQ ID NO. 388 (corresponding to SEQ ID NO. 1393 of the patent application WO2013/143700).

Nucleotide sequence of 3'UTR element of an α-globin gene

(SEQ ID NO. 388)

GCCCGATGGGCCTCCCAACGGGCCCTCCTCCCCTCCTTGCACCG

[0295] In this context it is particularly preferred that the 3'-UTR of the inventive mRNA comprises or consists of a corresponding RNA sequence of the nucleic acid sequence according to the above or a homolog, a fragment or variant thereof.

[0296] The term 'a nucleic acid sequence, which is derived from the 3'-UTR of a [. . .] gene' preferably refers to a nucleic acid sequence, which is based on the 3'-UTR sequence of a [. . .] gene or on a part thereof, such as on the 3'-UTR of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, or a collagen alpha gene, such as a collagen alpha 1(I) gene, preferably of an albumin gene or on a part thereof. This term includes sequences corresponding to the entire 3'-UTR sequence, i.e. the full length 3'-UTR sequence of a gene, and sequences corresponding to a fragment of the 3'-UTR sequence of a gene, such as an albumin gene, α-globin gene, β-globin gene, tyrosine hydroxylase gene, lipoxygenase gene, or collagen alpha gene, such as a collagen alpha 1(I) gene, preferably of an albumin gene.

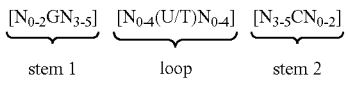
[0297] The term 'a nucleic acid sequence, which is derived from a variant of the 3'-UTR of a [. . .] gene' preferably refers to a nucleic acid sequence, which is based on a variant of the 3'-UTR sequence of a gene, such as on a variant of the 3'-UTR of an albumin gene, an α-globin gene, a β-globin gene, a tyrosine hydroxylase gene, a lipoxygenase gene, or a collagen alpha gene, such as a collagen alpha 1(I) gene, or on a part thereof as described above. This term includes sequences corresponding to the entire sequence of the variant of the 3'-UTR of a gene, i.e. the full length variant 3'-UTR sequence of a gene, and sequences corresponding to a fragment of the variant 3'-UTR sequence of a gene. A fragment in this context preferably consists of a continuous stretch of nucleotides corresponding to a continuous stretch of nucleotides in the full-length variant 3'-UTR, which represents at least 20%, preferably at least 30%, more preferably at least 40%, more preferably at least 50%, even more preferably at least 60%, even more preferably at least 70%, even more preferably at least 80%, and most preferably at least 90% of the full-length variant 3'-UTR. Such a fragment of a variant, in the sense of the present invention, is preferably a functional fragment of a variant as described herein.

[0298] Preferably, the at least one 5'-UTR and the at least one 3'-UTR act synergistically to increase protein production from the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex as described above.

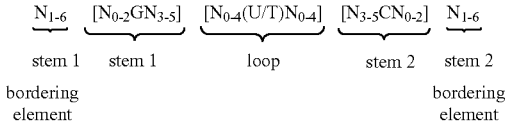
[0299] In a particularly preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises a histone stem-loop sequence/structure. Such histone stem-loop sequences are preferably selected from histone stem-loop sequences as disclosed in WO 2012/019780, whose disclosure is incorporated herewith by reference.

[0300] A histone stem-loop sequence, suitable to be used within the present invention, is preferably selected from at least one of the following formulae (VI) or (VII):

Formula (VI) (Stem-Loop Sequence without Stem Bordering Elements):



Formula (VII) (Stem-Loop Sequence with Stem Bordering Elements):



wherein:

[0301] stem1 or stem2 bordering elements N_{1-6} is a consecutive sequence of 1 to 6, preferably of 2 to 6, more preferably of 2 to 5, even more preferably of 3 to 5, most preferably of 4 to 5 or 5 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C, or a nucleotide analogue thereof;

[0302] stem1 [N_{0-2} GN_{3-5}] is reverse complementary or partially reverse complementary with element stem2, and is a consecutive sequence between of 5 to 7 nucleotides;

[0303] wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

[0304] wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof, and

[0305] wherein G is guanosine or an analogue thereof, and may be optionally replaced by a cytidine or an analogue thereof, provided that its complementary nucleotide cytidine in stem2 is replaced by guanosine;

[0306] loop sequence [N_{0-4} $(U/T)N_{0-4}$] is located between elements stem1 and stem2, and is a consecutive sequence of 3 to 5 nucleotides, more preferably of 4 nucleotides;

[0307] wherein each N_{0-4} is independent from another a consecutive sequence of 0 to 4, preferably of 1 to 3, more preferably of 1 to 2 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

[0308] wherein U/T represents uridine, or optionally thymidine;

[0309] stem2 [N_{3-5} CN_{0-2}] is reverse complementary or partially reverse complementary with element stem1, and is a consecutive sequence between of 5 to 7 nucleotides;

[0310] wherein N_{3-5} is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

[0311] wherein N_{0-2} is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G or C or a nucleotide analogue thereof; and

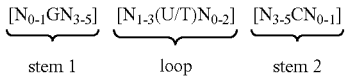
[0312] wherein C is cytidine or an analogue thereof, and may be optionally replaced by a guanosine or an analogue thereof provided that its complementary nucleoside guanosine in stem1 is replaced by cytidine;

wherein

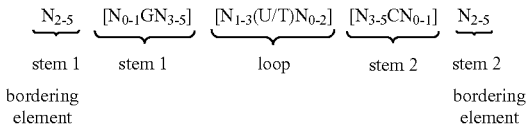
stem1 and stem2 are capable of base pairing with each other forming a reverse complementary sequence, wherein base pairing may occur between stem1 and stem2, e.g. by Watson-Crick base pairing of nucleotides A and U/T or G and C or by non-Watson-Crick base pairing e.g. wobble base pairing, reverse Watson-Crick base pairing, Hoogsteen base pairing, reverse Hoogsteen base pairing or are capable of base pairing with each other forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2, on the basis that one ore more bases in one stem do not have a complementary base in the reverse complementary sequence of the other stem.

[0313] According to a further preferred embodiment of the first inventive aspect, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may comprise at least one histone stem-loop sequence according to at least one of the following specific formulae (VIa) or (VIIa):

Formula (VIa) (Stem-Loop Sequence without Stem Bordering Elements):



Formula (VIIa) (Stem-Loop Sequence with Stem Bordering Elements):

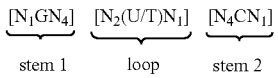


wherein:

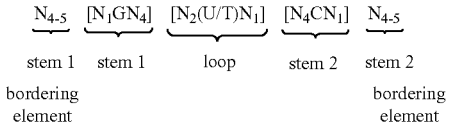
[0314] N, C, G, T and U are as defined above.

[0315] According to a further more particularly preferred embodiment of the first aspect, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex may comprise at least one histone stem-loop sequence according to at least one of the following specific formulae (VIb) or (VIIb):

Formula (VIb) (Stem-Loop Sequence without Stem Bordering Elements):



Formula (VIIb) (Stem-Loop Sequence with Stem Bordering Elements):



wherein:

[0316] N, C, G, T and U are as defined above.

[0317] A particular preferred histone stem-loop sequence is the nucleic acid sequence according to SEQ ID NO. 389.

Histone stern-loop nucleotide sequence (SEQ ID NO. 389)

CAAAGGCTCTTTTCAGAGCCACCA

[0318] More preferably the stem-loop sequence is the corresponding RNA sequence of the nucleic acid sequence according to SEQ ID NO. 390.

Histone stem-loop RNA sequence (SEQ ID NO. 390)

CAAAGGCUCUUUUCAGAGCCACCA

[0319] In a particularly preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex comprises a nucleic acid sequence derived from a 5'-TOP-UTR, a GC-optimized coding sequence, a nucleic acid sequence derived from the 3'-UTR of an albumin gene, a poly(A)-sequence, a poly(C)-sequence, and a histone stem loop. It is particularly preferred that the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex is an mRNA molecule, which comprises a nucleic acid sequence derived from a 5'-TOP-UTR, a GC-optimized coding sequence, a nucleic acid sequence derived from the 3'-UTR of an albumin gene, a poly(A)-sequence, a poly(C)-sequence, and a histone stem loop, wherein each of these features is preferably as defined herein.

[0320] Nevertheless, substitutions, additions or eliminations of bases are preferably carried out with the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA, using a DNA matrix for preparation of the nucleic acid molecule by techniques of the well known site directed mutagenesis or with an oligonucleotide ligation strategy (see e.g. Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 3rd ed., Cold Spring Harbor, N.Y., 2001). In such a process, for preparation of the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA, a corresponding DNA molecule may be transcribed in vitro. This DNA matrix preferably comprises a suitable promoter, e.g. a T7 or SP6 promoter, for in vitro transcription, which is followed by the desired nucleotide sequence for the nucleic acid molecule, e.g. mRNA, to be prepared and a termination signal for in vitro transcription. The DNA molecule, which forms the matrix of the at least one RNA of interest, may be prepared by fermentative proliferation and subsequent isolation as part of a plasmid which can be replicated in bacteria. Plasmids which may be mentioned as suitable for the present invention are e.g. the plasmids pT7 Ts (GenBank accession number U26404; Lai et al., Development 1995, 121: 2349 to 2360), pGEM® series, e.g. pGEM®-1 (GenBank accession number X65300; from Promega) and pSP64 (GenBank accession number X65327); cf. also Mezei and Storts, Purification of PCR Products, in: Griffin and Griffin (ed.), PCR Technology: Current Innovation, CRC Press, Boca Raton, Fla., 2001.

[0321] Nucleic acid molecules used according to the invention as defined herein may be prepared using any method known in the art, including synthetic methods such as e.g. solid phase synthesis, as well as in vitro methods, such as in vitro transcription reactions.

[0322] According to another particularly preferred embodiment, the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of a coding nucleic acid molecule may additionally or alternatively encode a secretory signal peptide. Such signal peptides are sequences, which typically exhibit a length of about 15 to 30 amino acids and are preferably located at the N-terminus of the encoded peptide, without being limited thereto. Signal peptides as defined herein preferably allow the transport of the protein or peptide as encoded by the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, especially if the nucleic acid is in the form of an mRNA, into a defined cellular compartment, preferably the cell surface, the endoplasmic reticulum (ER) or the endosomal-lysosomal compartment. Examples of secretory signal peptide sequences as defined herein include, without being limited thereto, signal sequences of classical or non-classical MHC-molecules (e.g. signal sequences of MHC I and II molecules, e.g. of the MHC class I molecule HLA-A*0201), signal sequences of cytokines or immunoglobulins as defined herein, signal sequences of the invariant chain of immunoglobulins or antibodies as defined herein, signal sequences of Lamp1, Tapasin, Erp57, Calreticulin, Calnexin, and further membrane associated proteins or of proteins associated with the endoplasmic reticulum (ER) or the endosomal-lysosomal compartment. Particularly preferably, signal sequences of MHC class I molecule HLA-A*0201 may be used according to the present invention.

[0323] Any of the above modifications may be applied to the nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein, or to the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex and/or to any nucleic acid as used in the context of the present invention and may be, if suitable or necessary, be combined with each other in any combination, provided, these combinations of modifications do not interfere with each other in the respective nucleic acid. A person skilled in the art will be able to take his choice accordingly.

[0324] The nucleic acid molecule of the inventive polymeric carrier cargo complex as defined herein and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex as well as proteins or peptides as encoded by these nucleic acid molecules may comprise fragments or variants of those sequences. Such fragments or variants may typically comprise a sequence having a sequence identity with one of the above mentioned nucleic acids, or with one of the proteins or peptides or sequences, if encoded by the at least one nucleic acid molecule, of at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, preferably at least 70%, more preferably at least 80%, equally more preferably at least 85%, even more preferably at least 90% and most preferably at least 95% or even 97%, 98% or 99%, to the entire wild type sequence, either on nucleic acid level or on amino acid level.

[0325] "Fragments" of proteins or peptides in the context of the present invention (encoded by a nucleic acid as defined herein) may comprise a sequence of a protein or peptide as defined herein, which is, with regard to its amino acid sequence (or its encoded nucleic acid molecule), N-terminally, C-terminally and/or intrasequentially truncated compared to the amino acid sequence of the original (native)

protein (or its encoded nucleic acid molecule). Such truncation may thus occur either on the amino acid level or correspondingly on the nucleic acid level. A sequence identity with respect to such a fragment as defined herein may therefore preferably refer to the entire protein or peptide as defined herein or to the entire (coding) nucleic acid molecule of such a protein or peptide. Likewise, "fragments" of nucleic acids in the context of the present invention may comprise a sequence of a nucleic acid as defined herein, which is, with regard to its nucleic acid molecule 5'-, 3'- and/or intrasequentially truncated compared to the nucleic acid molecule of the original (native) nucleic acid molecule. A sequence identity with respect to such a fragment as defined herein may therefore preferably refer to the entire nucleic acid as defined herein.

[0326] Fragments of proteins or peptides in the context of the present invention (e.g. as encoded by the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex) may furthermore comprise a sequence of a protein or peptide as defined herein, which has a length of about 6 to about 20 or even more amino acids, e.g. fragments as processed and presented by MHC class I molecules, preferably having a length of about 8 to about 10 amino acids, e.g. 8, 9, or 10, (or even 6, 7, 11, or 12 amino acids), or fragments as processed and presented by MHC class II molecules, preferably having a length of about 13 or more amino acids, e.g. 13, 14, 15, 16, 17, 18, 19, 20 or even more amino acids, wherein these fragments may be selected from any part of the amino acid sequence. These fragments are typically recognized by T-cells in form of a complex consisting of the peptide fragment and an MHC molecule, i.e. the fragments are typically not recognized in their native form.

[0327] Fragments of proteins or peptides as defined herein (e.g. as encoded by the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or by the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex) may also comprise epitopes of those proteins or peptides. Epitopes (also called "antigen determinants") in the context of the present invention are typically fragments located on the outer surface of (native) proteins or peptides as defined herein, preferably having 5 to 15 amino acids, more preferably having 5 to 12 amino acids, even more preferably having 6 to 9 amino acids, which may be recognized by antibodies or B-cell receptors, i.e. in their native form. Such epitopes of proteins or peptides may furthermore be selected from any of the herein mentioned variants of such proteins or peptides. In this context antigenic determinants can be conformational or discontinous epitopes which are composed of segments of the proteins or peptides as defined herein that are discontinuous in the amino acid sequence of the proteins or peptides as defined herein but are brought together in the three-dimensional structure or continuous or linear epitopes which are composed of a single polypeptide chain.

[0328] "Variants" of proteins or peptides as defined in the context of the present invention (e.g. as encoded by a nucleic acid as defined herein) may be encoded by the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex. Thereby, a protein or peptide may be generated, having an amino acid sequence which differs from the original sequence in one or more mutation(s), such as one or more substituted, inserted and/or deleted amino acid(s). Preferably, these fragments and/or variants have the same biological function or specific activity compared to the full-length native protein, e.g. its specific antigenic property.

[0329] "Variants" of proteins or peptides as defined in the context of the present invention (e.g. as encoded by a nucleic acid as defined herein) may comprise conservative amino acid substitution(s) compared to their native, i.e. non-mutated physiological, sequence. Those amino acid sequences as well as their encoding nucleotide sequences in particular fall under the term variants as defined herein. Substitutions in which amino acids, which originate from the same class, are exchanged for one another are called conservative substitutions. In particular, these are amino acids having aliphatic side chains, positively or negatively charged side chains, aromatic groups in the side chains or amino acids, the side chains of which can enter into hydrogen bridges, e.g. side chains, which have a hydroxyl function. This means that e.g. an amino acid having a polar side chain is replaced by another amino acid having a likewise polar side chain, or, for example, an amino acid characterized by a hydrophobic side chain is substituted by another amino acid having a likewise hydrophobic side chain (e.g. serine (threonine) by threonine (serine) or leucine (isoleucine) by isoleucine (leucine)). Insertions and substitutions are possible, in particular, at those sequence positions, which cause no modification to the three-dimensional structure or do not affect the binding region. Modifications to a three-dimensional structure by insertion(s) or deletion(s) can easily be determined e.g. using CD spectra (circular dichroism spectra) (Urry, 1985, Absorption, Circular Dichroism and ORD of Polypeptides, in: Modern Physical Methods in Biochemistry, Neuberger et al. (ed.), Elsevier, Amsterdam).

[0330] Furthermore, variants of proteins or peptides as defined herein, which may be encoded by the nucleic acid molecule of the inventive polymeric carrier cargo complex and/or the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex, may also comprise those sequences, wherein nucleotides of the nucleic acid are exchanged according to the degeneration of the genetic code, without leading to an alteration of the respective amino acid sequence of the protein or peptide, i.e. the amino acid sequence or at least part thereof may not differ from the original sequence in one or more mutation(s) within the above meaning.

[0331] In order to determine the percentage, to which two sequences are identical, e.g. nucleic acid sequences or amino acid sequences as defined herein, preferably the amino acid sequences encoded by a nucleic acid sequence of the polymeric carrier as defined herein or the amino acid sequences themselves, the sequences can be aligned in order to be subsequently compared to one another. Therefore, e.g. a position of a first sequence may be compared with the corresponding position of the second sequence. If a position in the first sequence is occupied by the same component as is the case at a position in the second sequence, the two sequences are identical at this position. If this is not the case, the sequences differ at this position. If insertions occur in the second sequence in comparison to the first sequence, gaps can be inserted into the first sequence to allow a further alignment. If deletions occur in the second sequence in comparison to the first sequence, gaps can be inserted into the second sequence to allow a further alignment. The

percentage to which two sequences are identical is then a function of the number of identical positions divided by the total number of positions including those positions, which are only occupied in one sequence. The percentage to which two sequences are identical can be determined using a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used is the algorithm of Karlin et al. (1993), PNAS USA, 90:5873-5877 or Altschul et al. (1997), Nucleic Acids Res., 25:3389-3402. Such an algorithm is integrated in the BLAST program. Sequences, which are identical to the sequences of the present invention to a certain extent, can be identified by this program.

[0332] In the inventive polymeric carrier cargo complex, the cationic component of the polymeric carrier as defined herein and the nucleic acid cargo are typically provided in a molar ratio of about 1 to 10000, preferably in a molar ratio of about 5 to 5000, more preferably in a molar ratio of about 10 to 2500, even more preferably in a molar ratio of about 25 to 2000, and most preferably in a molar ratio of about 25 to 1000 of polymeric carrier to nucleic acid.

[0333] Furthermore, in the inventive polymeric carrier cargo complex, the cationic component of the polymeric carrier as defined herein and the nucleic acid cargo are preferably provided in an N/P-ratio of at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1, 1.5 or 2. Preferably, the N/P-ratio lies within a range of about 0.1, 0.3, 0.4, 0.5, 0.75, 1.0, 1.5 or 2 to 20, preferably in a range of about 0.2 (0.5 or 0.75 or 1.0) to 12, and even more preferably in an N/P-ratio of about 0.4 (0.75 or 1.0) to 10. Most preferably, the N/P ratio lies in a ratio between 0.1 and 0.9. In this context, the N/P ratio is a measure of the ionic charge of the cationic (side chain) component of the polymeric carrier or of the polymeric carrier as such. In particular, if the cationic properties of the cationic component are generated by nitrogens (of the amino acid side chains), the N/P ratio expresses the ratio of basic nitrogen atoms to phosphate residues in the nucleotide backbone, considering that (side chain) nitrogen atoms in the cationic component of the polymeric carrier contribute to positive charges and phosphate of the phosphate backbone of the nucleic acid contribute to the negative charge. A formula is given in the Examples. The N/P-ratio is defined as the nitrogen/phosphate ratio (N/P-ratio) of the entire inventive polymeric carrier cargo complex. This is typically illustrative for the content/amount of cationic components, in the polymeric carrier and characteristic for the content/amount of nucleic acids bound or complexed in the inventive polymeric carrier cargo complex. It may be calculated on the basis that, for example, 1 μg RNA typically contains about 3 nmol phosphate residues, provided that RNA exhibits a statistical distribution of bases. Additionally, 1 nmol peptide typically contains about x nmol nitrogen residues, dependent on the molecular weight and the number of its (cationic) amino acids.

[0334] In this context it is preferable that in the inventive polymeric carrier cargo complex, the cationic component of the polymeric carrier as defined herein and the nucleic acid cargo are provided in an N/P-ratio of at least about 1 or, preferably, of a range of about 1 to 20 for in vitro transfection purposes.

[0335] If the expression of an encoded protein or the transcription of an encoded nucleic acid e.g. an mRNA or siRNA of the nucleic acid cargo is intended for therapeutical purposes (in vivo application) an N/P ratio of at least 0.1 (0.2, 0.3, 0.4, 0.5, 0.6), preferably of a range of about 0.1 (0.2, 0.3, 0.4., 0.5, or 0.6) to 1.5 is preferred. Even more preferred is an N/P ratio range of 0.2 to 0.9 or an N/P ratio range of 0.5 to 0.9. In the case that the inventive polymeric carrier cargo complex is used for (in vivo) immunostimulation e.g. as an immunostimulating agent or adjuvant (for the purpose to induce an innate immune response), an N/P ratio of about 0.1 to 20 is preferred, more particular an N/P ratio of 0.1 to 5 or 0.1 to 1.5.

[0336] In the specific case that the induction of IFN-α is intended using the inventive polymeric cargo complex as an (in vivo) immunostimulating agent or adjuvant an N/P ratio of at least 0.1 (0.2, 0.3, 0.4, 0.5, or 0.6) or an N/P ratio range of 0.1 to 1 is preferred or more preferred is an N/P ratio range of 0.2 to 0.9 or an N/P ratio range of 0.5 to 0.9. Otherwise if the induction of TNFα is intended using the inventive polymeric cargo complex as an (in vivo) immunostimulating agent or adjuvant an N/P ratio of 1 to 20 is particularly preferred.

[0337] The N/P ratio significantly influences the surface charge of the resulting inventive polymeric carrier cargo complex. Thus it is preferable that the resulting inventive polymeric carrier cargo complex is positively charged for in vitro transfection purposes and negatively or neutrally charged for in vivo transfection purposes, especially if the expression of an encoded protein or the transcription of an encoded nucleic acid of the nucleic acid cargo is intended. The surface charge of the resulting inventive polymeric carrier cargo complex can be indicated as Zetapotential which may be measured by Doppler electrophoresis method using a Zetasizer Nano (Malvern Instruments, Malvern, UK).

[0338] The molar ratio of the nucleic acid molecule used as a cargo in the polymeric carrier cargo complex ("first nucleic acid molecule") to the second nucleic acid molecule administered in combination with the polymeric carrier cargo complex is preferably in the range from 0.01 to 100, more preferably in the range from 0.1 to 10, even more preferably in the range from 0.5 to 2, most preferably about 1.

[0339] The second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex, is typically used in non-packaged form, i.e. the second nucleic acid molecule, preferably an RNA, in the context of the present invention is preferably not packaged in particles. Preferably, the second nucleic acid molecule, which is administered in combination with the polymeric carrier cargo complex, is not packaged in a virus particle, an inactivated virus particle or a virus-like particle. "Non-packaged" in this context refers to a nucleic acid molecule, which may be a naked nucleic acid molecule or a nucleic acid molecule, which is complexed by another compound, preferably a cationic compound. In one embodiment, the second nucleic acid molecule, preferably an RNA, is complexed by another compound, thus forming another polymeric complex distinct from the polymeric carrier cargo complex as defined herein. Accordingly, the second nucleic acid molecule may be in the form of a complex, wherein the complex comprising the second nucleic acid molecule is distinct from the polymeric carrier cargo complex, in particular with respect to the nucleic acid sequence of the respective first nucleic acid molecules and/or with respect to the compound, by which the first nucleic acid molecule, respectively, is complexed.

[0340] Accordingly, in a preferred embodiment, the second nucleic acid molecule, preferably an RNA, encoding a peptide or a protein, is administered in the form of a naked nucleic acid.

[0341] Alternatively, the second nucleic acid molecule, preferably an RNA, is complexed by a cationic or polycationic compound. According to one embodiment, the cationic or polycationic compound is selected from protamine, nucleoline, spermine or spermidine, poly-L-lysine (PLL), basic polypeptides, poly-arginine, cell penetrating peptides (CPPs), chimeric CPPs, including Transportan, or MPG peptides, HIV-binding peptides, Tat, HIV-1 Tat (HIV), Tat-derived peptides, oligoarginines, members of the penetratin family, including Penetratin, Antennapedia-derived peptides (from *Drosophila* antennapedia), pAntp, pIsl, antimicrobial-derived CPPs including Buforin-2, Bac715-24, SynB, SynB (1), pVEC, hCT-derived peptides, SAP, MAP, KALA, PpTG20, Proline-rich peptides, L-oligomers, Arginine-rich peptides, Calcitonin-peptides, FGF, Lactoferrin, poly-L-Lysine, poly-Arginine, histones, VP22 derived or analog peptides, HSV, VP22 (Herpes simplex), MAP, KALA or protein transduction domains including PTDs, PpT620, prolin-rich peptides, arginine-rich peptides, lysine-rich peptides, Pep-1, and Calcitonin peptide(s), or from proteins or peptides having the following total formula: $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$, wherein l+m+n+o+x=8-15, and l, m, n or o independently of each other may be any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15, provided that the overall content of Arg, Lys, His and Orn represents at least 50% of all amino acids of the oligopeptide; and Xaa may be any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and x may be any number selected from 0, 1, 2, 3, 4, 5, 6, 7, or 8 provided, that the overall content of Xaa does not exceed 50% of all amino acids of the oligopeptide,

or from oligoarginines including Arg_7, Arg_8, Arg_9, Arg_7, H_3R_9, R_9H3, $H_3R_9H_3$, $YSSR_9SSY$, $(RKH)_4$, $Y(RKH)_2R$,

or from cationic polysaccharides, including chitosan, polybrene, cationic polymers, polyethyleneimine (PEI), cationic lipids, DOTMA: [1-(2,3-sioleyloxy)propyl]-N,N,N-trimethylammonium chloride, DMRIE, di-C14-amidine, DOTIM, SAINT, DC-Chol, BGTC, CTAP, DOPC, DODAP, DOPE (Dioleyl phosphatidylethanol-amine), DOSPA, DODAB, DOIC, DMEPC, DOGS (Dioctadecylamidoglicyl-spermin), DIMRI: Dimyristo-oxypropyl dimethyl hydroxyethyl ammonium bromide, DOTAP: dioleoyloxy-3-(trimethylammonio)propane, DC-6-14: O,O-ditetradecanoyl-N-(a-trimethylammonioacetyl)diethanolamine chloride, CLIP1: rac-[(2,3-dioctadecyloxypropyl)(2-hydroxyethyl)]-dimethylammonium chloride, CLIP6: rac-[2(2,3-dihexadecyloxypropyl-oxymethyloxy)ethyl]trimethylammonium, CLIPS: rac-[2(2,3-dihexadecyloxypropyl-oxysuccinyloxy)ethyl]-trimethylammonium, oligofectamine, or from cationic or polycationic polymers, including modified polyaminoacids, including as (3-aminoacid-polymers or reversed polyamides, modified polyethylenes, including PVP (poly(N-ethyl-4-vinylpyridinium bromide)), modified acrylates, including pDMAEMA (poly(dimethylaminoethyl methylacrylate)), modified Amidoamines including pAMAM (poly (amidoamine)), modified polybetaaminoester (PBAE), including diamine end modified 1,4 butanediol diacrylate-co-5-amino-1-pentanol polymers, dendrimers, including polypropylamine dendrimers or pAMAM based dendrimers, polyimine(s), including PEI: poly(ethyleneimine), poly(propyleneimine), polyallylamine, sugar backbone based polymers, including cyclodextrin based polymers, dextran based polymers, Chitosan, silan backbone based polymers, including PMOXA-PDMS copolymers, block polymers consisting of a combination of one or more cationic blocks (selected of a cationic polymer as defined above) and of one or more hydrophilic- or hydrophobic blocks (including polyethyleneglycol).

[0342] Preferably, the second nucleic acid molecule is administered in combination with the polymeric carrier cargo complex, without being comprised in the polymeric carrier cargo complex. In particular, the second nucleic acid molecule is administered in combination with the polymeric carrier cargo complex as defined herein, without physically being a part or component of the polymeric carrier cargo complex. Preferably, the second nucleic acid molecule is not bound (e.g. covalently) to the polymeric carrier cargo complex. Further preferably, the at least one first nucleic acid molecule of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule, which is administered together with the polymeric carrier cargo complex, are not complexed by the same polymeric carrier.

[0343] In a further aspect the present invention also provides a method of preparing the inventive polymeric carrier cargo complex as defined herein comprising following steps:

[0344] a) providing at least one cationic protein or peptide as defined herein and/or at least one cationic or polycationic polymer and optionally at least one amino acid component (AA) as defined herein, each comprising at least one —SH moiety,

[0345] b) providing at least one first nucleic acid molecule as defined herein, preferably in the above mentioned ratios,

[0346] c) mixing the components provided in steps a) and b), preferably in a basic or neutral milieu as defined herein, preferably in the presence of oxygen or a further starter as defined herein, preferably at a pH, at a temperature and at time as defined herein, and thereby condensing and thus polymerizing the cationic components provided in step a) with each other via disulfide bonds (in a condensation polymerization or polycondensation) to obtain the polymeric carrier and complexing the nucleic acid molecule provided in step b) with the cationic components provided in step a)

[0347] d) optionally purifying the inventive polymeric carrier cargo complex obtained according to step c), preferably using a method as defined herein;

[0348] e) optionally lyophilization of the inventive polymeric carrier cargo complex obtained according to step c) or d).

[0349] The method of preparing the inventive polymeric carrier cargo complex as defined herein comprises a multistep condensation polymerization or polycondensation reaction via —SH moieties of the educts e.g. cationic peptides or polymers as defined herein and optionally further amino acid components (AA) in step c). The condensation polymerization or polycondensation reaction which occurs simultaneously to the complexation or electrostatic binding of the nucleic acid molecule preferably leads to the inventive polymeric carrier cargo complex wherein the polymeric carrier is a condensation polymer, wherein the single components are linked by disulfide bonds.

[0350] As defined herein in a step a) of the inventive method of preparing the inventive polymeric carrier cargo complex, at least one cationic or polycationic protein or peptide as defined herein and/or at least one cationic or polycationic polymer as defined herein are provided, preferably in the ratios indicated above. These components are mixed in step c) with the nucleic acid molecule provided in step b), preferably in a basic or neutral milieu as defined herein, preferably in the presence of oxygen or a further starter as defined herein, preferably at a pH, and at a temperature and at a time as defined herein, and thereby condensing and thus polymerizing these components with each other via disulfide bonds (in a condensation polymerization or polycondensation) to obtain a polymeric carrier complexed to the at least one first nucleic acid molecule as defined herein.

[0351] According to an alternative, in step a) of the inventive method of preparing the inventive polymeric carrier cargo complex at least one cationic or polycationic protein or peptide and/or at least one cationic or polycationic polymer are provided as defined herein, and optionally at least one amino acid component (AA), are provided in step a) as defined herein, and are used for a condensation polymerization or polycondensation and complexation reaction prior to adding the nucleic acid of step b) but using the same polymerization conditions outlined for step c). The polymerized polymeric carrier and the nucleic acid of step b) are then mixed in step c). Preferably, the components are all provided in the ratios indicated above and mixed, preferably in a basic or neutral milieu as defined herein, preferably in the presence of oxygen or a further starter as defined herein, preferably at a pH, at a temperature and at time as defined herein. Upon mixing and starting the reaction, the components are condensed and thus polymerized with each other via disulfide bonds (in a condensation polymerization or polycondensation) to obtain a polymeric carrier complexed to the nucleic acid molecule as defined herein.

[0352] In both of the above alternatives, different polymeric carriers, particularly different peptides and/or different polymers, and may be selected in the condensation polymerization as indicated above. In this context, the selection of different component(s) of the polymeric carrier is typically dependent upon the desired properties of the final polymeric carrier and the desired cationic strength of the final polymeric carrier. Accordingly, the content of cationic components, may furthermore be "diluted" or modified in the above alternative of step a) e.g. by introducing an amino acid component (AA) as defined herein, preferably in the above defined ratios.

[0353] Thereby, a modified polymeric carrier may be obtained, wherein the cationic character of the unmodified polymeric carrier typically remains in the limitations as defined herein. The properties of the final polymeric carrier may thus be adjusted as desired with properties of components (AA) by inserting amino acid component (AA) as defined herein in steps a).

[0354] In step c), the at least one cationic or polycationic protein or peptide as defined herein and/or at least one cationic or polycationic polymer as defined herein, and optionally at least one amino acid component (AA) and the at least one first nucleic acid as defined herein, are preferably contained in a basic or neutral milieu in the step a) of the inventive method of preparing the inventive polymeric carrier cargo complex. Such a basic or neutral milieu typically exhibits a pH range of about 5 to about 10, preferably a pH range of about 6 to about 9, more preferably a pH range of about 7 to about 8, e.g. about 6.5, 7, 7.5, 8, 8.5, or 9 or any range selected from any two of these or the aforementioned values.

[0355] Furthermore, the temperature of the solution in step c) is preferably in a range of about 5° C. to about 60° C., more preferably in a range of about 15° C. to about 40° C., even more preferably in a range of about 20° C. to about 30° C., and most preferably in a range of about 20° C. to about 25° C., e.g. about 25° C.

[0356] In step c) of the inventive method of preparing the inventive polymeric carrier cargo complex as defined herein buffers may be used as suitable. Preferred buffers may comprise, but are not limited to carbonate buffers, borate buffers, Bicine buffer, CHES buffer, CAPS buffer, Ethanolamine containing buffers, HEPES, MOPS buffer, Phosphate buffer, PIPES buffer, Tris buffer, Tricine buffer, TAPS buffer, and/or TES buffer as buffering agents. Particularly preferred is a carbonate buffer.

[0357] Upon mixing the components, preferably in the presence of oxygen, preferably in the presence of a basic or neutral mileu as defined herein, the condensation polymerization or polycondensation reaction and the complexation of the at least one nucleic acid molecule is started. For this purpose, the mixture in step c) is preferably exposed to oxygen or may be started using a further starter, e.g. a catalytic amount of an oxidizing agent, e.g. DMSO, etc. Upon start of the condensation polymerization or polycondensation reaction of the at least one cationic or polycationic protein or peptide and/or at least one cationic or polycationic polymer and optionally at least one amino acid component (AA) as defined herein, are condensed and thus polymerized with each other via disulfide bonds (condensation polymerization or polycondensation). In this reaction step a) preferably linear polymers are created using monomers with at least one reactive —SH moiety, i.e. at least one cationic or polycationic protein or peptide and/or at least one cationic or polycationic polymer and optionally at least one amino acid component (AA) as defined herein, each component exhibiting at least one free —SH-moieties as defined herein, e.g. at their terminal ends. However, components with more than one, preferably two free —SH-moieties may be used, which may lead to branched polymers. Simultaneously to the polymerization reaction the cationic polymers bind to the at least one nucleic acid molecule and thereby complexing it.

[0358] According to one alternative, the inventive polymeric carrier cargo complex additionally may be modified with a component (AA) as defined herein.

[0359] According to a first example, a component (AA) (e.g. a ligand) is attached to the cationic component prior to providing the cationic component in step a) via any functionality as defined herein, e.g. a —SH moiety. This component (AA) or (e.g. a ligand) is preferably attached to the cationic component at one terminus of these components. If the attachment is carried out via —SH bonds, the cationic components are preferably provided with two (or even more) —SH-moieties. The component (AA) or (e.g. a ligand) preferably carries only one —SH moiety. In this case, one —SH moiety of the cationic component is preferably protected in a first step using a protecting group as known in the art. Then, the cationic component may be bound to a component L to form a first disulfide bond via the

non-protected —SH moiety. The protected —SH-moiety of the cationic component is then typically deprotected for further reactions.

[0360] Alternatively, the above mentioned component (AA) or (e.g. a ligand) may be used in step c) to be coupled with the cationic components provided in step a) above, e.g. via disulfide bonds without blocking the free —SH moieties. But in this context all methods known to a skilled person or defined herein may be used to attach the component (AA) to the cationic component or to the polymeric carrier.

[0361] Alternatively, a component (AA) or (e.g. a ligand) can be bound to the inventive polymeric carrier cargo complex after step c) via any functionality as defined herein, e.g. a —SH moiety. In this context it is preferable that the component (AA) (e.g. a ligand) is bound via free —SH moieties of the polymeric carrier components.

[0362] According to step c) of the inventive method of preparing the inventive polymeric carrier cargo complex as defined herein, at least one nucleic acid molecule as defined herein is mixed with the cationic components provided in step b), preferably in the above mentioned ratios. Typically, in the inventive polymeric carrier cargo complex, the cationic components as defined herein, and the at least one nucleic acid molecule are provided in a molar ratio of about 5 to 10000, preferably in a molar ratio of about 5 to 5000, more preferably in a molar ratio of about 10 to 2500, even more preferably in a molar ratio of about 10 to 1000 cationic polymer to nucleic acid. The N/P ratios are preferably as indicated above. In this context it is particularly preferred that the N/P ratios are selected thereby avoiding agglomeration and toxicity in vivo.

[0363] In a specific embodiment, (AA) components as defined above which do not comprise —SH moieties can be added in step c) which are thereby incorporated into the inventive polymeric carrier cargo complex without polymerization by (terminal) —SH moieties. Thereby these (AA) components is/are typically not covalently linked and included non-covalently in the inventive complex as a further component.

[0364] According to a further step d) of the inventive method of preparing the inventive polymeric carrier cargo complex as defined herein, the inventive polymeric carrier cargo complex obtained according to step c) is optionally purified. Purification may occur by using chromatographic methods, such as HPLC, FPLC, GPS, dialysis, etc.

[0365] According to a further step e) of the inventive method of preparing the inventive polymeric carrier cargo complex as defined herein, the inventive polymeric carrier cargo complex obtained according to step c) or d) is optionally lyophilized. For this purpose any suitable cryoprotectant or lyoprotectant may be added to the inventive polymeric carrier cargo complex obtained in step c) or d).

[0366] The inventive method of preparing the inventive polymeric carrier cargo complex as defined herein is particularly suitable to adapt the chemical properties of the desired inventive polymeric carrier cargo complex due to specific selection of its components of the polymeric carrier thereby avoiding agglomeration and toxicity in vivo.

[0367] According to a further aspect the present invention also provides a method for transfecting a cell, a tissue or an organism, thereby applying or administering the inventive polymeric carrier cargo complex in combination with at least one second nucleic acid molecule, particularly for therapeutic purposes. In this context, typically after preparing the inventive polymeric carrier cargo complex as described above, the inventive polymeric carrier cargo complex is administered to a cell, a tissue or an organism, in combination with the at least one second nucleic acid encoding a protein or peptide as described herein, or as a pharmaceutical composition or vaccine as described herein, more preferably using any of the administration modes as described herein. The method for transfecting a cell may be carried out in vitro, in vivo or ex vivo.

[0368] Likewise, according to another aspect, the present invention also relates to the use of the inventive polymeric carrier cargo complex administered in combination with at least one second nucleic acid molecule, particularly for therapeutic purposes, for transfecting a cell, a tissue or an organism, thereby applying or administering the inventive polymeric carrier cargo complex as described above to a cell, a tissue or an organism in combination with the at least one second nucleic acid molecule encoding a protein or peptide as described herein, preferably in non-packaged form or as a pharmaceutical composition or vaccine as described herein, more preferably using any of the administration modes as described herein. The administration may be carried out in vitro, in vivo or ex vivo.

[0369] The polymeric carrier cargo complex in combination with the second nucleic acid molecule, preferably an RNA, as described herein, may also be used as a medicament. The polymeric carrier cargo complex in combination with the second nucleic acid molecule, preferably an RNA, as described herein, is preferably used in the treatment or prophylaxis of a disease selected from a tumour or a cancer disease, an infectious disease, an autoimmune disease or an allergy or for use as an immunostimulating agent or adjuvant in the treatment or prophylaxis of a disease selected from a tumour or a cancer disease, a cardiovascular disease, an infectious disease, an autoimmune disease or an allergy. The polymeric carrier cargo complex in combination with the second nucleic acid molecule, preferably an RNA, as described herein, may be administered, preferably in a safe and effective amount as defined herein, orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraarticular, intranodal, intrasynovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques. Preferably, the polymeric carrier cargo complex and the second nucleic acid molecule encoding a protein or peptide are administered intramuscularly.

[0370] The polymeric carrier cargo complex in combination with the second nucleic acid molecule, preferably an RNA, as described herein, may be administered by parenteral injection, more preferably by subcutaneous, intravenous, intramuscular, intraarticular, intranodal, intrasynovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or via infusion techniques. Particularly preferred is intradermal and intramuscular injection. In one particularly preferred embodiment, the polymeric carrier cargo complex in combination with the second nucleic acid molecule is administered intramuscularly.

[0371] Methods for intramuscular administration are known in the art. Typically, a liquid is injected into a skeletal

muscle (such as M. gluteus, M. deltoideus or M. vastus lateralis) using, for example, a syringe or a needle-free injection system, such as a jet injection system.

[0372] In a preferred embodiment, the polymeric carrier cargo complex administered in combination with the second nucleic acid molecule as defined herein is used as a medicament, immunostimulating agent or adjuvant, wherein the only two active ingredients are the polymeric carrier cargo complex and the second nucleic acid molecule. An "active ingredient", in this context, may be any compound having a therapeutic effect, particularly capable of eliciting an immune response or of stimulating/modulating an immune response.

[0373] In a further aspect, the present invention also provides a pharmaceutical composition, comprising the inventive polymeric carrier cargo complex, which is formulated together with at least one second nucleic acid molecule encoding a protein or peptide, wherein the second nucleic acid molecule is preferably an RNA molecule.

[0374] In one embodiment, the invention provides a pharmaceutical composition comprising:

[0375] (A) a polymeric carrier cargo complex, comprising:

[0376] a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and

[0377] b) as a cargo at least one first nucleic acid molecule, and

[0378] (B) at least one additional pharmaceutically active component, preferably a second nucleic acid molecule, wherein the at least one second nucleic acid molecule is an RNA molecule encoding a protein or a peptide.

[0379] The pharmaceutical composition optionally comprises a pharmaceutically acceptable carrier and/or vehicle.

[0380] As a first ingredient (component (A)), the inventive pharmaceutical composition comprises the inventive polymeric carrier cargo complex formed by the nucleic acid cargo and the polymeric carrier as defined herein (and, optionally, (AA) component(s)).

[0381] As a second ingredient (component (B)), the inventive pharmaceutical composition may comprise at least one additional pharmaceutically active component. A pharmaceutically active component in this connection is a compound that has a therapeutic effect to heal, ameliorate or prevent a particular indication, preferably cancer diseases, autoimmune disease, allergies or infectious diseases. Such compounds include, without implying any limitation, peptides or proteins, preferably as defined herein, nucleic acids, preferably as defined herein, (therapeutically active) low molecular weight organic or inorganic compounds (molecular weight less than 5000, preferably less than 1000), sugars, antigens or antibodies, preferably as defined herein, therapeutic agents already known in the prior art, antigenic cells, antigenic cellular fragments, cellular fractions; cell wall components (e.g. polysaccharides), modified, attenuated or de-activated (e.g. chemically or by irradiation) pathogens (virus, bacteria etc.), adjuvants, preferably as defined herein, etc.

[0382] In a preferred embodiment, the second ingredient (component (B)) is a second nucleic acid molecule as defined herein, preferably an RNA, more preferably an mRNA, encoding a protein or peptide, wherein the protein or peptide has a therapeutic effect to heal, ameliorate or prevent a particular indication, preferably cancer diseases, autoimmune disease, allergies or infectious diseases. In this context it is particularly preferred, that the encoded peptides or proteins are antigenic peptides or proteins.

[0383] In particularly preferred embodiments, the pharmaceutical composition comprises as a second ingredient (component (B)) an RNA, preferably an mRNA, preferably as defined herein with respect to the inventive polymeric carrier cargo complex for use as an immunostimulating agent or as an adjuvant. In particular, any one of the features or any combination of features described herein with regard to the inventive polymeric carrier cargo complex for use as an immunostimulating agent or as an adjuvant may likewise be applied to the RNA comprised in the inventive pharmaceutical composition as a second ingredient (component (B)). In particular, the RNA comprised in the inventive pharmaceutical composition as a second ingredient (component (B)) may comprise at least one selected from the group consisting of a 5'-UTR, a 3'-UTR, a poly(A) sequence, a poly(C) sequence and a histone stem-loop sequence, wherein each of these features is preferably as defined herein.

[0384] In a particularly preferred embodiment, the RNA comprised in the inventive pharmaceutical composition as a second ingredient (component (B)) comprises a nucleic acid sequence derived from a 5'-TOP-UTR, a GC-optimized coding sequence, a nucleic acid sequence derived from the 3'-UTR of an albumin gene, a poly(A)-sequence, a poly(C)-sequence, and a histone stem loop, wherein each of these features is preferably as defined herein.

[0385] Preferably, component (B) is not covalently linked, in particular not by a disulfide bond, with component (A). Thus, component (B) is preferably not covalently linked, such as by a disulfide bond, to the polymeric carrier and/or the at least one nucleic acid molecule of the polymeric carrier cargo complex. Preferably, the at least one second nucleic acid molecule is not covalently linked to the polymeric carrier cargo complex, in particular not to the polymeric carrier of the polymeric carrier cargo complex. For example, preferably, the at least one second nucleic acid molecule, is not covalently linked to the polymeric carrier cargo complex, such as to the polymeric carrier, by a disulfide bond. However, in an embodiment, wherein component (A) and component (B) are linked via disulfide bonds, such linkage is preferably not realized via a crosslinker, such as via a 3,6-Dioxa-1,8-octanedithiol (DODT) crosslinker. Furthermore, in an embodiment, wherein component (A) and component (B) are linked via disulfide bonds, component (B) is preferably not ovalbumine or a fragment of ovalbumine. Moreover, in a preferred embodiment, components (A) and (B) do not form a micelle structure together, in particular, the polymeric carrier preferably does not form a micelle structure.

[0386] Furthermore, the inventive pharmaceutical composition may comprise a pharmaceutically acceptable carrier and/or vehicle. In the context of the present invention, a pharmaceutically acceptable carrier typically includes the liquid or non-liquid basis of the inventive pharmaceutical composition. If the inventive pharmaceutical composition is provided in liquid form, the carrier will typically be pyrogen-free water; isotonic saline or buffered (aqueous) solutions, e.g phosphate, citrate etc. buffered solutions. The injection buffer may be hypertonic, isotonic or hypotonic with reference to the specific reference medium, i.e. the buffer may have a higher, identical or lower salt content with reference to the specific reference medium, wherein prefer-

ably such concentrations of the afore mentioned salts may be used, which do not lead to damage of cells due to osmosis or other concentration effects. Reference media are e.g. liquids occurring in "in vivo" methods, such as blood, lymph, cytosolic liquids, or other body liquids, or e.g. liquids, which may be used as reference media in "in vitro" methods, such as common buffers or liquids. Such common buffers or liquids are known to a skilled person. Ringer or Ringer-Lactate solution is particularly preferred as a liquid basis.

[0387] However, one or more compatible solid or liquid fillers or diluents or encapsulating compounds may be used as well for the inventive pharmaceutical composition, which are suitable for administration to a patient to be treated. The term "compatible" as used here means that these constituents of the inventive pharmaceutical composition are capable of being mixed with the inventive polymeric carrier cargo complex and the at least one additional pharmaceutically active component as defined herein in such a manner that no interaction occurs which would substantially reduce the pharmaceutical effectiveness of the inventive pharmaceutical composition under typical use conditions. Pharmaceutically acceptable carriers, fillers and diluents must, of course, have sufficiently high purity and sufficiently low toxicity to make them suitable for administration to a person to be treated. Some examples of compounds which can be used as pharmaceutically acceptable carriers, fillers or constituents thereof are sugars, such as, for example, lactose, glucose and sucrose; starches, such as, for example, corn starch or potato starch; cellulose and its derivatives, such as, for example, sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; tallow; solid glidants, such as, for example, stearic acid, magnesium stearate; calcium sulfate; vegetable oils, such as, for example, groundnut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil from theobroma;

[0388] polyols, such as, for example, polypropylene glycol, glycerol, sorbitol, mannitol and polyethylene glycol; alginic acid.

[0389] In certain embodiments of the invention, the polymeric carrier cargo complex comprised in the inventive pharmaceutical composition is used as an adjuvant. For example, it is used as an adjuvant, and/or has adjuvant properties, as may be readily determined by the person of ordinary skill using routine methodologies, and including methodologies as described herein.

[0390] As a first ingredient (component (A)) the inventive pharmaceutical composition includes (e.g. as an adjuvant) at least one polymeric carrier cargo complex, comprising

[0391] a) (as a carrier) a polymeric carrier comprising disulfide-crosslinked cationic components, preferably formed by disulfide-crosslinked cationic components, and

[0392] b) (as a cargo) at least one (first) nucleic acid molecule.

[0393] According to a specific embodiment, the inventive pharmaceutical composition may comprise an (additional) adjuvant. In this context, an adjuvant may be understood as any compound, which is suitable to initiate or increase an immune response of the innate immune system, i.e. a nonspecific immune response. With other words, when administered, the inventive pharmaceutical composition typically elicits an innate immune response due to the adjuvant, optionally contained therein. Such an adjuvant may be selected from any adjuvant known to a skilled person and suitable for the present case, i.e. supporting the induction of an innate immune response in a mammal.

[0394] In particular, such an adjuvant may be selected from any adjuvant known to a skilled person and suitable for the present case, i.e. supporting the induction of an innate immune response in a mammal and/or suitable for depot and delivery of the components of the inventive pharmaceutical composition. Preferred as adjuvants suitable for depot and delivery are cationic or polycationic compounds as defined above. Likewise, the adjuvant may be selected from the group consisting of, without being limited thereto, cationic or polycationic compounds as defined above, from chitosan, TDM, MDP, muramyl dipeptide, pluronics, alum solution, aluminium hydroxide, ADJUMER™ (polyphosphazene); aluminium phosphate gel; glucans from algae; algammulin; aluminium hydroxide gel (alum); highly protein-adsorbing aluminium hydroxide gel; low viscosity aluminium hydroxide gel; AF or SPT (emulsion of squalane (5%), Tween 80 (0.2%), Pluronic L121 (1.25%), phosphate-buffered saline, pH 7.4); AVRIDINE™ (propanediamine); BAY R1005™ ((N-(2-deoxy-2-L-leucylamino-b-D-glucopyranosyl)-N-octadecyl-dodecanoyl-amide hydroacetate); CALCITRIOL™ (1-alpha,25-dihydroxy-vitamin D3); calcium phosphate gel; CAP™ (calcium phosphate nanoparticles); cholera holotoxin, cholera-toxin-A1-protein-A-D-fragment fusion protein, sub-unit B of the cholera toxin; CRL 1005 (block copolymer P1205); cytokine-containing liposomes; DDA (dimethyldioctadecylammonium bromide); DHEA (dehydroepiandrosterone); DMPC (dimyristoylphosphatidylcholine); DMPG (dimyristoylphosphatidylglycerol); DOC/alum complex (deoxycholic acid sodium salt); Freund's complete adjuvant; Freund's incomplete adjuvant; gamma inulin; Gerbu adjuvant (mixture of: i)N-acetylglucosaminyl-(P1-4)-N-acetylmuramyl-L-alanyl-D35 glutamine (GMDP), ii) dimethyldioctadecylammonium chloride (DDA), iii) zinc-L-proline salt complex (ZnPro-8); GM-CSF); GMDP (N-acetylglucosaminyl-(b1-4)-N-acetylmuramyl-L47 alanyl-D-isoglutamine); imiquimod (1-(2-methypropyl)-1H-imidazo [4,5-c]quinoline-4-amine); ImmTher™ (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Ala-glycerol dipalmitate); DRVs (immunoliposomes prepared from dehydration-rehydration vesicles); interferon gamma; interleukin-1beta; interleukin-2; interleukin-7; interleukin-12; ISCOMS™; ISCOPREP 7.0.3.™; liposomes; LOXORIBINE™ (7-allyl-8-oxoguanosine); LT 5 oral adjuvant (*E. coli* labile enterotoxin-protoxin); microspheres and microparticles of any composition; MF59™; (squalenewater emulsion); MONTANIDE ISA 51™ (purified incomplete Freund's adjuvant); MONTANIDE ISA720™ (metabolisable oil adjuvant); MPL™ (3-Q-desacyl-4'-monophosphoryl lipid A); MTP-PE and MTP-PE liposomes ((N-acetyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1,2-dipalmitoyl-sn-glycero-3-(hydroxyphosphoryloxy))-ethylamide, monosodium salt); MURAMETIDE™ (Nac-Mur-L-Ala-D-Gln-OCH3); MURAPALMITINE™ and DMURAPALMITINE™ (Nac-Mur-L-Thr-D-isoGln-sn-glyceroldipalmitoyl); NAGO (neuraminidase-galactose oxidase); nanospheres or nanoparticles of any composition; NISVs (non-ionic surfactant vesicles); PLEURAN™ ((3-glucan); PLGA, PGA and PLA (homo- and co-polymers of lactic acid and glycolic acid; micro spheres/nano spheres); PLURONIC L121™; PMMA (polymethylmethacrylate); PODDS™ (proteinoid microspheres); polyethylene carbam-

ate derivatives; poly-rA: poly-rU (polyadenylic acid-polyuridylic acid complex); polysorbate 80 (Tween 80); protein cochleates (Avanti Polar Lipids, Inc., Alabaster, Ala.); STIMULON™ (QS-21); Quil-A (Quil-A saponin); 5-28463 (4-amino-otec-dimethyl-2-ethoxymethyl-1H-imidazo [4,5-c]quinoline-1-ethanol); SAF-1™ ("Syntex adjuvant formulation"); Sendai proteoliposomes and Sendaicontaining lipid matrices; Span-85 (sorbitan trioleate); Specol (emulsion of Marcol 52, Span 85 and Tween 85); squalene or Robane® (2,6,10,15,19,23-hexamethyltetracosan and 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexane); stearyltyrosine (octadecyltyrosine hydrochloride); Theramid® (N-acetylglucosaminyl-N-acetylmuramyl-L-Ala-D-isoGlu-L-Aladipalmitoxypropylamide); Theronyl-MDP (Termurtide™ or [thr 1]-MDP; N-acetylmuramyl-Lthreonyl-D-isoglutamine); Ty particles (Ty-VLPs or virus-like particles); Walter-Reed liposomes (liposomes containing lipid A adsorbed on aluminium hydroxide), and lipopeptides, including Pam3Cys, in particular aluminium salts, such as Adju-phos, Alhydrogel, Rehydragel; emulsions, including CFA, SAF, IFA, MF59, Provax, TiterMax, Montanide, Vaxfectin; copolymers, including Optivax (CRL1005), L121, Poloaxmer4010), etc.; liposomes, including Stealth, cochleates, including BIORAL; plant derived adjuvants, including QS21, Quil A, Iscomatrix, ISCOM; adjuvants suitable for costimulation including Tomatine, biopolymers, including PLG, PMM, Inulin, microbe derived adjuvants, including Romurtide, DETOX, MPL, CWS, Mannose, CpG nucleic acid sequences, CpG7909, ligands of human TLR 1-10, ligands of murine TLR 1-13, ISS-1018, 35 IC31, Imidazoquinolines, Ampligen, Ribi529, IMOxine, IRIVs, VLPs, cholera toxin, heat-labile toxin, Pam3Cys, Flagellin, GPI anchor, LNFPIII/Lewis X, antimicrobial peptides, UC-1V150, RSV fusion protein, cdiGMP; and adjuvants suitable as antagonists including CGRP neuropeptide.

[0395] The inventive pharmaceutical composition may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The term parenteral as used herein includes subcutaneous, intravenous, intramuscular, intraarticular, intranodal, intrasynovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or infusion techniques.

[0396] Preferably, the inventive pharmaceutical composition may be administered by parenteral injection, more preferably by subcutaneous, intravenous, intramuscular, intraarticular, intranodal, intrasynovial, intrasternal, intrathecal, intrahepatic, intralesional, intracranial, transdermal, intradermal, intrapulmonal, intraperitoneal, intracardial, intraarterial, and sublingual injection or via infusion techniques. Particularly preferred is intradermal and intramuscular injection. In one particularly preferred embodiment, the pharmaceutical composition is administered intramuscularly.

[0397] Methods for intramuscular administration are known in the art. Typically, a liquid is injected into a skeletal muscle (such as M. gluteus, M. deltoideus or M. vastus lateralis) using, for example, a syringe or a needle-free injection system, such as a jet injection system.

[0398] Sterile injectable forms of the inventive pharmaceutical compositions may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil may be employed including synthetic mono- or di-glycerides. Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents or bioavailability enhancers which are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation of the inventive pharmaceutical composition.

[0399] The inventive pharmaceutical composition as defined herein may also be administered orally in any orally acceptable dosage form including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredients, i.e. the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule, are combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added.

[0400] The inventive pharmaceutical composition may also be administered topically, especially when the target of treatment includes areas or organs readily accessible by topical application, e.g. including diseases of the skin or of any other accessible epithelial tissue. Suitable topical formulations are readily prepared for each of these areas or organs. For topical applications, the inventive pharmaceutical composition may be formulated in a suitable ointment, containing the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule suspended or dissolved in one or more carriers. Carriers for topical administration include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the inventive pharmaceutical composition can be formulated in a suitable lotion or cream. In the context of the present invention, suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

[0401] The inventive pharmaceutical composition typically comprises a "safe and effective amount" of the components of the inventive pharmaceutical composition, particularly of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule as defined herein. As used herein, a "safe and effective amount" means

an amount of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule as such that is sufficient to significantly induce a positive modification of a disease or disorder as defined herein. At the same time, however, a "safe and effective amount" is small enough to avoid serious side-effects and to permit a sensible relationship between advantage and risk. The determination of these limits typically lies within the scope of sensible medical judgment. A "safe and effective amount" of the components of the inventive pharmaceutical composition, particularly of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule as defined herein, will furthermore vary in connection with the particular condition to be treated and also with the age and physical condition of the patient to be treated, the body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the activity of the inventive polymeric carrier cargo complex and the at least one second nucleic acid molecule, the severity of the condition, the duration of the treatment, the nature of the accompanying therapy, of the particular pharmaceutically acceptable carrier used, and similar factors, within the knowledge and experience of the accompanying doctor. The inventive pharmaceutical composition may be used for human and also for veterinary medical purposes, preferably for human medical purposes, as a pharmaceutical composition in general or as a vaccine, immunostimulating agent or adjuvant.

[0402] According to a particular preferred aspect, the inventive pharmaceutical composition (or the inventive polymeric carrier cargo complex) may be provided or used as an immunostimulating agent. In this context, the inventive pharmaceutical composition is preferably as defined above. More preferably, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the pharmaceutical composition, is typically an immunostimulatory nucleic acid as defined herein, e.g. a CpG-DNA or an immunostimulatory RNA (isRNA). Alternatively or additionally, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the pharmaceutical composition, is a coding nucleic acid as defined herein, preferably a cDNA or an mRNA, more preferably encoding an adjuvant protein preferably as defined herein.

[0403] In a specific embodiment in this context, it is preferred that an adjuvant protein is a component of the inventive polymeric carrier cargo complex and, preferably, of the polymeric carrier.

[0404] According to an even more preferred embodiment, the inventive pharmaceutical composition (or the inventive polymeric carrier cargo complex) may be provided or used as an adjuvant. In this context, the adjuvant is preferably defined as the inventive pharmaceutical composition above. More preferably, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the adjuvant, is typically an immunostimulatory nucleic acid as defined herein, e.g. a CpG-DNA or an immunostimulatory RNA (isRNA). Alternatively or additionally, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the adjuvant, is a coding nucleic acid as defined herein, preferably a cDNA or an mRNA, more preferably encoding an adjuvant protein, preferably as defined herein. The inventive polymeric carrier cargo complex, preferably contained in the adjuvant, typically initiates an innate immune response in the patient to be treated. Such an adjuvant may be utilized in any accompanying therapy, with any known vaccine or any further (known) therapeutic agent, preferably prior to, concurrent with or subsequent to administration of the main therapy, prior to, concurrent with or subsequent to administration of a further (known) vaccine or a (known) further therapeutic agent.

[0405] The polymeric carrier cargo complex, which is administered in combination with a second nucleic acid molecule as described herein, or the inventive pharmaceutical composition as defined herein provided or used as an adjuvant is preferably capable of triggering a non-antigen-specific, (innate) immune reaction (as provided by the innate immune system), preferably in an immunostimulating manner. An immune reaction can generally be brought about in various ways. An important factor for a suitable immune response is the stimulation of different T-cell sub-populations. T-lymphocytes typically differentiate into two subpopulations, the T-helper 1 (Th1) cells and the T-helper 2 (Th2) cells, with which the immune system is capable of destroying intracellular (Th1) and extracellular (Th2) pathogens (e.g. antigens). The two Th cell populations differ in the pattern of effector proteins (cytokines) produced by them. Thus, Th1 cells assist the cellular immune response by activation of macrophages and cytotoxic T-cells. Th2 cells, on the other hand, promote the humoral immune response by stimulation of B-cells for conversion into plasma cells and by formation of antibodies (e.g. against antigens). The Th1/Th2 ratio is therefore of great importance in the immune response. In connection with the present invention, the Th1/Th2 ratio of the immune response is preferably displaced by the immune-stimulating agent, namely the inventive polymeric carrier cargo complex in the direction towards the cellular response, that is to say the Th1 response, and a predominantly cellular immune response is thereby induced. As defined above, the inventive polymeric carrier cargo complex exerts by itself an unspecific innate immune response, which allows the inventive polymeric carrier cargo complex be used as such (without adding another pharmaceutically active component) as an immunostimulating agent. If administered together with another pharmaceutically active component, preferably a specifically immunogenic component, preferably an antigen and more preferably the at least one second nucleic acid molecule encoding an antigenic peptide or protein, the nucleic acid of the polymeric carrier cargo complex serves as an adjuvant supporting the specific adaptive immune response elicited by the other pharmaceutically active component e.g. an antigen.

[0406] In a preferred embodiment, the pharmaceutical composition contains as the only pharmaceutically active ingredients the polymeric carrier cargo complex and the second nucleic acid molecule as defined herein. An "active ingredient", in this context, may be any compound having a therapeutic effect, capable of eliciting an immune response or of stimulating/modulating an immune response, such as, for instance, a nucleic acid encoding a peptide antigen or a protein antigen.

Determination of the Immunostimulatory or Adjuvant Capacity of an Inventive Compound or an Inventive Complex:

[0407] For the determination of the immunostimulatory capacity of an inventive compound or an inventive complex several methods are known in the art and may be used. E.g., in vitro methods are advantageous to screen for compounds as to their capacity to induce cytokines, which are (exclu-

sively or at least typically) part of the innate immune system and thereby (as an additional arm of the immune system) typically improve the induction of an antigen-specific immune response caused by an antigen. For this purpose, e.g. PBMCs may be isolated from blood samples and stimulated with the particular compound or complex. After incubation, secretion of the desired cytokines (e.g. as a reaction of an activation of the PAMP receptors) being typically part of the innate immune system (and not of the antigen-specific immune system) is determined by ELISA. These selected cytokines may be used in the art as determinants of the induction of an innate immune response in the body. In this context, the secretion of TNF-alpha and IFN-alpha is preferably measured to determine the unspecific (innate immune response) evoked by a compound or complex. Especially, IFN-alpha plays an important role in the induction of an unspecific immune response after viral infection. Accordingly, it is particularly preferred that the the immunostimulatory compound or complex, which shall be identified by the screening assay, induces the secretion of e.g. IFN-alpha. Such a compound or complex may then be applied e.g. for the use as an immunostimulating agent in vaccination therapies.

[0408] IFN-alpha is part of the family of type I interferons. Type I interferons (IFN) are pleiotropic cytokines that are essential for supporting anti-viral immune responses. They induce apoptosis of virus-infected cells and cellular resistance to viral infection, in addition to activating natural killer (NK) and T cells. Type I interferons have effects on a large set of cytokines and chemokines that i.a. influence immunocyte maturation, homing, effector functions and apoptosis. Typically, a major role of IFN-α/β is the induction of a priming state affecting the production and regulation of other mediators, including cytokines. For example, IFN-α/β signaling upregulates IFN-γ production by dendritic cells (DCs) and T cells and thereby favours the induction and maintenance of Th1 cells. Shifting of an immune response in direction of a Th1 immune response may become important, once protein or peptide vaccines are used, because these vaccines usually induce a Th2-based immune response which consequently prevents the induction of cytotoxic T cells.

[0409] Therefore, it is preferred that a compound or complex to be used as an adjuvant may preferably have the property of shifting an antigen-specific immune response caused by a vaccine to a Th1-based immune response. The direction of an immune response induced by a vaccine is usually measured by determination of the induction of several subtypes of antigen-specific antibodies and the induction of antigen-specific cytotoxic CD8+ T cells. In this context, the subtype antibody IgG1 represents the induction of a Th2-based immune response and the induction of the subtype antibody IgG2a and the induction of cytotoxic T cells represent the induction of a Th1-based immune response. The induction of antigen-specific antibodies is determined by measurement of the antibody titer in the blood of the vaccinee by ELISA. The induction of antigen-specific cytotoxic T cells is determined by measurement of IFN-gamma secretion in splenocytes after stimulation with antigen-specific peptides by ELISPOT. In this context, the induction of IFN-gamma secretion proves that antigen-specific cytotoxic T cells are present in the spleen which can specifically attack cells which present epitopes of the antigen on MHC I molecules on their surface.

[0410] Thus, for the determination of beneficial properties of an adjuvant in vivo vaccinations are performed. Therewith, it is possible to find out, if the adjuvant or immunostimulatory compound or complex improves an antigen-specific immune response caused by the vaccine and, furthermore, if it can shift an antigen-specific immune response in the desired direction to display adjuvant properties. Particularly, in the induction of an anti-tumoral immune response the induction of a Th1-shifted immune response, especially the induction of cytotoxic T cells plays a major role, because the induction of antigen-specific cytotoxic T cells represents an indispensable prerequisite for the successful combat of a tumour.

[0411] Accordingly, the methods to screen for compounds or complexes which actually exhibit properties as immunostimulating agents and/or adjuvants are well known in the art and may readily be applied e.g. by ELISA tests measuring the immune response elicited by the tested compounds/complexes.

[0412] According to another particularly preferred aspect, the inventive pharmaceutical composition (or the inventive polymeric carrier cargo complex, which is administered in combination with a second nucleic acid molecule as defined herein) may be provided or used as a vaccine.

[0413] In this context, the vaccine is preferably defined as an adjuvant or as an inventive pharmaceutical composition as disclosed above. More preferably, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in such a vaccine, may be any nucleic acid as defined above, preferably an immunostimulatory nucleic acid as defined herein, e.g. a CpG-DNA or an immunostimulatory RNA (isRNA). Alternatively or additionally, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the vaccine, is a coding nucleic acid as defined herein, preferably a cDNA or an mRNA, more preferably encoding an adjuvant protein, preferably as defined herein. Alternatively or additionally, the nucleic acid of the inventive polymeric carrier cargo complex, preferably contained in the vaccine, is a coding nucleic acid as defined herein, preferably a cDNA or an mRNA, more preferably encoding an antigen, preferably as defined herein. Furthermore, the vaccine comprises a second nucleic acid molecule, preferably an RNA, encoding a protein or peptide as defined herein. In addition, the inventive vaccine may contain an antigen, preferably as defined above, as a protein or peptide, or antigenic cells, antigenic cellular fragments, cellular fractions; cell wall components (e.g. polysaccharides), modified, attenuated or de-activated (e.g. chemically or by irradiation) pathogens (virus, bacteria etc.).

[0414] As described above, the present invention provides a polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant, wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly and wherein the polymeric carrier cargo complex and the second nucleic acid molecule are preferably not administered together with a protein or peptide antigen selected from the group consisting of an antigen from a pathogen associated with infectious disease, an antigen associated with allergy or allergic disease, an antigen associated with autoimmune disease, an antigen associated with a cancer or tumour disease, or a fragment, variant and/or

derivative of said protein or peptide antigen. According to a preferred embodiment, the inventive pharmaceutical composition or the inventive vaccine do likewise not comprise a protein or peptide antigen selected from the group consisting of an antigen from a pathogen associated with infectious disease, an antigen associated with allergy or allergic disease, an antigen associated with autoimmune disease, an antigen associated with a cancer or tumour disease, or a fragment, variant and/or derivative of said protein or peptide antigen. More preferably, the inventive pharmaceutical composition or the inventive vaccine does not comprise a protein or peptide antigen.

[0415] According to a first embodiment such an inventive vaccine supports or elicits an innate immune response of the immune system of a patient to be treated, preferably due to an immunostimulatory capacity of the inventive polymeric carrier cargo complex.

[0416] According to a second embodiment, the inventive vaccine may further elicit an adaptive immune response, preferably due to the protein or peptide encoded by the second nucleic acid molecule as defined herein, which is suitable to elicit an adaptive immune response. Alternatively, an additional antigen can be provided in form of a peptide, a protein or an epitope of said antigen.

[0417] The antigen may also be a component of the inventive polymeric carrier, e.g. as a (AA) component, as defined herein.

[0418] The inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant may also comprise a pharmaceutically acceptable carrier, adjuvant, and/or vehicle as defined herein for the inventive pharmaceutical composition. In the specific context of the inventive vaccine, the choice of a pharmaceutically acceptable carrier is determined in principle by the manner in which the inventive vaccine is administered. The inventive vaccine can be administered, for example, systemically or locally. Routes for systemic administration in general include, for example, transdermal, oral, parenteral routes, including subcutaneous, intravenous, intramuscular, intraarterial, intradermal and intraperitoneal injections and/or intranasal administration routes.

[0419] Intramuscular administration, e.g. via needle injection or needle-free injection (e.g. jet injection), is particularly preferred.

[0420] Routes for local administration in general include, for example, topical administration routes but also intradermal, transdermal, subcutaneous, or intramuscular injections or intralesional, intracranial, intrapulmonal, intracardial, and sublingual injections. More preferably, vaccines may be administered by an intradermal, subcutaneous, or intramuscular route, most preferably by intramuscular route. Inventive vaccines are therefore preferably formulated in liquid (or sometimes in solid) form. The suitable amount of the inventive vaccine to be administered can be determined by routine experiments with animal models. Such models include, without implying any limitation, rabbit, sheep, mouse, rat, dog and non-human primate models. Preferred unit dose forms for injection include sterile solutions of water, physiological saline or mixtures thereof. The pH of such solutions should be adjusted to about 7.4. Suitable carriers for injection include hydrogels, devices for controlled or delayed release, polylactic acid and collagen matrices. Suitable pharmaceutically acceptable carriers for topical application include those, which are suitable for use in lotions, creams, gels and the like. If the inventive vaccine is to be administered orally, tablets, capsules and the like are the preferred unit dose form. The pharmaceutically acceptable carriers for the preparation of unit dose forms which can be used for oral administration are well known in the prior art. The choice thereof will depend on secondary considerations such as taste, costs and storability, which are not critical for the purposes of the present invention, and can be made without difficulty by a person skilled in the art.

[0421] The inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant can additionally contain one or more auxiliary substances in order to increase its immunogenicity or immunostimulatory capacity, if desired. A synergistic action of the inventive polymeric carrier cargo complex and the second nucleic acid molecule as defined herein and of an auxiliary substance, which may be optionally contained in the inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant as defined herein, is preferably achieved thereby. Depending on the various types of auxiliary substances, various mechanisms can come into consideration in this respect. For example, compounds that permit the maturation of dendritic cells (DCs), for example lipopolysaccharides, TNF-alpha or CD40 ligand, form a first class of suitable auxiliary substances. In general, it is possible to use as auxiliary substance any agent that influences the immune system in the manner of a "danger signal" (LPS, GP96, etc.) or cytokines, such as GM-CFS, which allow an immune response to be enhanced and/or influenced in a targeted manner. Particularly preferred auxiliary substances are cytokines, such as monokines, lymphokines, interleukins or chemokines, that further promote the innate immune response, such as IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, IL-21, IL-22, IL-23, IL-24, IL-25, IL-26, IL-27, IL-28, IL-29, IL-30, IL-31, IL-32, IL-33, INF-alpha, IFN-beta, INF-gamma, GM-CSF, G-CSF, M-CSF, LT-beta or TNF-alpha, growth factors, such as hGH.

[0422] Further additives which may be included in the inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant are emulsifiers, such as, for example, Tween®; wetting agents, such as, for example, sodium lauryl sulfate; colouring agents; taste-imparting agents, pharmaceutical carriers; tablet-forming agents; stabilizers; antioxidants; preservatives.

[0423] The inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant can also additionally contain any further compound, which is known to be immunostimulating due to its binding affinity (as ligands) to human Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, or due to its binding affinity (as ligands) to murine Toll-like receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11, TLR12 or TLR13.

[0424] The inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant can also additionally or alternatively contain an immunostimulatory RNA, i.e. an RNA derived from an immunostimulatory RNA, which triggers or increases an (innate) immune response. Preferably, such an immunostimulatory RNA may be in general be as defined hereinbefore.

[0425] Another class of compounds, which may be added to an inventive vaccine, pharmaceutical composition, immunostimulating agent or adjuvant in this context, may be CpG

nucleic acids, in particular CpG-RNA or CpG-DNA. A CpG-RNA or CpG-DNA can be a single-stranded CpG-DNA (ss CpG-DNA), a double-stranded CpG-DNA (dsDNA), a single-stranded CpG-RNA (ss CpG-RNA) or a double-stranded CpG-RNA (ds CpG-RNA). The CpG nucleic acid is preferably in the form of CpG-RNA, more preferably in the form of single-stranded CpG-RNA (ss CpG-RNA). The CpG nucleic acid preferably contains at least one or more (mitogenic) cytosine/guanine dinucleotide sequence(s) (CpG motif(s)). According to a first preferred alternative, at least one CpG motif contained in these sequences, that is to say the C (cytosine) and the G (guanine) of the CpG motif, is unmethylated. All further cytosines or guanines optionally contained in these sequences can be either methylated or unmethylated. According to a further preferred alternative, however, the C (cytosine) and the G (guanine) of the CpG motif can also be present in methylated form.

[0426] The present invention furthermore provides several applications and uses of the inventive polymeric carrier cargo complex, which is administered in combination with a second nucleic acid molecule encoding a protein or peptide, as defined herein, the inventive pharmaceutical composition, the inventive immunostimulating agent or adjuvant and the inventive vaccine comprising same or of kits comprising same.

[0427] According to one specific embodiment, the present invention is directed to the first medical use of the inventive polymeric carrier cargo complex in combination with a second nucleic acid molecule as defined herein as a medicament, preferably as an immunostimulating agent, adjuvant or vaccine.

[0428] According to another embodiment, the present invention is directed to the second medical use of the inventive polymeric carrier cargo complex administered in combination with at least one second nucleic acid molecule as defined herein, for the treatment of diseases as defined herein, preferably to the use of the inventive polymeric carrier cargo complex in combination with a second nucleic acid molecule as defined herein, of a pharmaceutical composition, vaccine, immunostimulating agent, adjuvant or vaccine comprising same or of kits comprising same for the preparation of a medicament for the prophylaxis, treatment and/or amelioration of various diseases as defined herein, particularly prophylaxis, treatment and/or amelioration of such diseases as defined herein. Preferably, the pharmaceutical composition, an immunostimulating agent, an adjuvant or a vaccine is used or administered to a patient in need thereof for this purpose.

[0429] Preferably, diseases as mentioned herein are selected from cancer or tumour diseases, infectious diseases, preferably (viral, bacterial or protozoan) infectious diseases, autoimmune diseases, allergies or allergic diseases, monogenetic diseases, i.e. (hereditary) diseases, or genetic diseases in general, diseases which have a genetic inherited background and which are typically caused by a single gene defect and are inherited according to Mendel's laws, cardiovascular diseases, neuronal diseases or any disease which can be influenced by the present invention.

[0430] Such diseases include cancer or tumor diseases, preferably selected from melanomas, malignant melanomas, colon carcinomas, lymphomas, sarcomas, blastomas, renal carcinomas, gastrointestinal tumors, gliomas, prostate tumors, bladder cancer, rectal tumors, stomach cancer, oesophageal cancer, pancreatic cancer, liver cancer, mammary carcinomas (=breast cancer), uterine cancer, cervical cancer, acute myeloid leukaemia (AML), acute lymphoid leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), hepatomas, various virus-induced tumors such as, for example, papilloma virus-induced carcinomas (e.g. cervical carcinoma=cervical cancer), adenocarcinomas, herpes virus-induced tumors (e.g. Burkitt's lymphoma, EBV-induced B-cell lymphoma), hepatitis B-induced tumors (hepatocell carcinomas), HTLV-1- and HTLV-2-induced lymphomas, acoustic neuroma, lung carcinomas (=lung cancer=bronchial carcinoma), small-cell lung carcinomas, pharyngeal cancer, anal carcinoma, glioblastoma, rectal carcinoma, astrocytoma, brain tumors, retinoblastoma, basalioma, brain metastases, medulloblastomas, vaginal cancer, pancreatic cancer, testicular cancer, Hodgkin's syndrome, meningiomas, Schneeberger disease, hypophysis tumor, Mycosis fungoides, carcinoids, neurinoma, spinalioma, Burkitt's lymphoma, laryngeal cancer, renal cancer, thymoma, corpus carcinoma, bone cancer, non-Hodgkin's lymphomas, urethral cancer, CUP syndrome, head/neck tumors, oligodendroglioma, vulval cancer, intestinal cancer, colon carcinoma, oesophageal carcinoma (=oesophageal cancer), wart involvement, tumors of the small intestine, craniopharyngeomas, ovarian carcinoma, genital tumors, ovarian cancer (=ovarian carcinoma), pancreatic carcinoma (=pancreatic cancer), endometrial carcinoma, liver metastases, penile cancer, tongue cancer, gall bladder cancer, leukaemia, plasmocytoma, lid tumor, prostate cancer (=prostate tumors), etc.

[0431] According to one further specific embodiment, diseases as defined herein comprise infectious diseases, preferably (viral, bacterial or protozoological) infectious diseases. Such infectious diseases, are preferably caused by a viral, bacterial, fungal or protozoan pathogen, preferably selected from the pathogens *Acinetobacter baumannii, Anaplasma genus, Anaplasma phagocytophilum, Ancylostoma braziliense, Ancylostoma duodenale, Arcanobacterium haemolyticum, Ascaris lumbricoides, Aspergillus* genus, Astroviridae, *Babesia* genus, *Bacillus anthracis, Bacillus cereus, Bartonella henselae*, BK virus, *Blastocystis hominis, Blastomyces dermatitidis, Bordetella pertussis, Borrelia burgdorferi, Borrelia genus, Borrelia* spp, *Brucella* genus, *Brugia malayi, Bunyaviridae family, Burkholderia cepacia* and other *Burkholderia* species, *Burkholderia mallei, Burkholderia pseudomallei, Caliciviridae family, Campylobacter genus, Candida albicans, Candida* spp, *Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci*, CJD prion, *Clonorchis sinensis, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium perfringens, Clostridium* spp, *Clostridium tetani, Coccidioides* spp, coronaviruses, *Corynebacterium diphtheriae, Coxiella burnetii*, Crimean-Congo hemorrhagic fever virus, *Cryptococcus neoformans, Cryptosporidium* genus, Cytomegalovirus, Dengue viruses (DEN-1, DEN-2, DEN-3 and DEN-4), *Dientamoeba fragilis*, Ebolavirus (EBOV), *Echinococcus* genus, *Ehrlichia chaffeensis, Ehrlichia ewingii, Ehrlichia genus, Entamoeba histolytica, Enterococcus* genus, Enterovirus genus, Enteroviruses, mainly Coxsackie A virus and Enterovirus 71 (EV71), *Epidermophyton* spp, Epstein-Barr Virus (EBV), *Escherichia coli* O157:H7, 0111 and 0104:H4, *Fasciola hepatica* and *Fasciola gigantica*, FFI prion, Filarioidea superfamily, Flaviviruses, *Francisella tularensis, Fusobac-*

terium genus, *Geotrichum candidum, Giardia intestinalis, Gnathostoma* spp, GSS prion, Guanarito virus, *Haemophilus ducreyi, Haemophilus influenzae, Helicobacter pylori*, Henipavirus (Hendra virus Nipah virus), Hepatitis A Virus, Hepatitis B Virus, Hepatitis C Virus, Hepatitis D Virus, Hepatitis E Virus, Herpes simplex virus 1 and 2 (HSV-1 and HSV-2), *Histoplasma capsulatum*, HIV (Human immunodeficiency virus), *Hortaea werneckii*, Human bocavirus (HBoV), Human herpesvirus 6 (HHV-6) and Human herpesvirus 7 (HHV-7), Human metapneumovirus (hMPV), Human papillomavirus (HPV), Human parainfluenza viruses (HPIV), Japanese encephalitis virus, JC virus, Junin virus, Kingella kingae, *Klebsiella granulomatis*, Kuru prion, Lassa virus, *Legionella pneumophila, Leishmania* genus, Leptospira genus, *Listeria monocytogenes*, Lymphocytic choriomeningitis virus (LCMV), Machupo virus, *Malassezia* spp, Marburg virus, Measles virus, *Metagonimus* yokagawai, Microsporidia phylum, *Molluscum contagiosum* virus (MCV), Mumps virus, *Mycobacterium leprae* and *Mycobacterium* lepromatosis, *Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Naegleria fowleri, Necator americanus, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Nocardia* spp, *Onchocerca volvulus*, Orientia tsutsugamushi, Orthomyxoviridae family, *Paracoccidioides brasiliensis, Paragonimus* spp, *Paragonimus westermani*, Parvovirus B19, *Pasteurella* genus, *Plasmodium* genus, *Pneumocystis jirovecii*, Poliovirus, Rabies virus, Respiratory syncytial virus (RSV), Rhinovirus, rhinoviruses, *Rickettsia akari, Rickettsia genus, Rickettsia prowazekii, Rickettsia rickettsii, Rickettsia typhi*, Rift Valley fever virus, Rotavirus, Rubella virus, Sabia virus, *Salmonella* genus, *Sarcoptes scabiei*, SARS coronavirus, *Schistosoma* genus, *Shigella* genus, Sin Nombre virus, Hantavirus, *Sporothrix schenckii, Staphylococcus genus, Staphylococcus genus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Strongyloides stercoralis, Taenia genus, Taenia solium*, Tick-borne encephalitis virus (TBEV), *Toxocara canis* or *Toxocara cati, Toxoplasma gondii, Treponema pallidum, Trichinella spiralis, Trichomonas vaginalis, Trichophyton* spp, *Trichuris trichiura, Trypanosoma brucei, Trypanosoma cruzi, Ureaplasma urealyticum, Varicella zoster* virus (VZV), *Varicella zoster* virus (VZV), *Variola major* or *Variola minor*, vCJD prion, Venezuelan equine encephalitis virus, *Vibrio cholerae*, West Nile virus, Western equine encephalitis virus, *Wuchereria bancrofti*, Yellow fever virus, *Yersinia enterocolitica, Yersinia pestis*, and *Yersinia pseudotuberculosis*. In this context, an infectious disease, preferably a viral, bacterial or protozoan infectious diseases, is typically selected from influenza, malaria, SARS, yellow fever, AIDS, Lyme borreliosis, Leishmaniasis, anthrax, meningitis, viral infectious diseases such as AIDS, Condyloma acuminata, hollow warts, Dengue fever, three-day fever, Ebola virus, cold, early summer meningoencephalitis (FSME), flu, shingles, hepatitis, herpes simplex type I, herpes simplex type II, Herpes zoster, influenza, Japanese encephalitis, Lassa fever, Marburg virus, measles, foot-and-mouth disease, mononucleosis, mumps, Norwalk virus infection, Pfeiffer's glandular fever, smallpox, polio (childhood lameness), pseudo-croup, fifth disease, rabies, warts, West Nile fever, chickenpox, cytomegalic virus (CMV), bacterial infectious diseases such as miscarriage (prostate inflammation), anthrax, appendicitis, borreliosis, botulism, *Camphylobacter, Chlamydia trachomatis* (inflammation of the urethra, conjunctivitis), cholera, diphtheria, donavanosis, epiglottitis, typhus fever, gas gangrene, gonorrhoea, rabbit fever, *Heliobacter pylori*, whooping cough, climatic bubo, osteomyelitis, Legionnaire's disease, leprosy, listeriosis, pneumonia, meningitis, bacterial meningitis, anthrax, otitis media, *Mycoplasma hominis*, neonatal sepsis (Chorioamnionitis), noma, paratyphus, plague, Reiter's syndrome, Rocky Mountain spotted fever, *Salmonella paratyphus, Salmonella typhus*, scarlet fever, syphilis, tetanus, tripper, tsutsugamushi disease, tuberculosis, typhus, vaginitis (colpitis), soft chancre, and infectious diseases caused by parasites, protozoa or fungi, such as amoebiasis, bilharziosis, Chagas disease, *Echinococcus*, fish tapeworm, fish poisoning (Ciguatera), fox tapeworm, athlete's foot, canine tapeworm, candidosis, yeast fungus spots, scabies, cutaneous Leishmaniosis, lambliasis (giardiasis), lice, malaria, microscopy, onchocercosis (river blindness), fungal diseases, bovine tapeworm, schistosomiasis, porcine tapeworm, toxoplasmosis, trichomoniasis, trypanosomiasis (sleeping sickness), visceral Leishmaniosis, nappy/diaper dermatitis or miniature tapeworm.

[0432] According to another specific embodiment, diseases as defined herein comprise autoimmune diseases as defined in the following. Autoimmune diseases can be broadly divided into systemic and organ-specific or localised autoimmune disorders, depending on the principal clinicopathologic features of each disease. Autoimmune diseases may be divided into the categories of systemic syndromes, including systemic lupus erythematosus (SLE), Sjögren's syndrome, Scleroderma, Rheumatoid Arthritis and polymyositis or local syndromes which may be endocrinologic (type I diabetes (Diabetes mellitus Type 1), Hashimoto's thyroiditis, Addison's disease etc.), dermatologic (pemphigus vulgaris), haematologic (autoimmune haemolytic anaemia), neural (multiple sclerosis) or can involve virtually any circumscribed mass of body tissue. The autoimmune diseases to be treated may be selected from the group consisting of type I autoimmune diseases or type II autoimmune diseases or type III autoimmune diseases or type IV autoimmune diseases, such as, for example, multiple sclerosis (MS), rheumatoid arthritis, diabetes, type I diabetes (Diabetes mellitus Type 1), chronic polyarthritis, Basedow's disease, autoimmune forms of chronic hepatitis, colitis ulcerosa, type I allergy diseases, type II allergy diseases, type III allergy diseases, type IV allergy diseases, fibromyalgia, hair loss, Bechterew's disease, Crohn's disease, Myasthenia gravis, neurodermitis, Polymyalgia rheumatica, progressive systemic sclerosis (PSS), Reiter's syndrome, rheumatic arthritis, psoriasis, vasculitis, etc, or type II diabetes. While the exact mode as to why the immune system induces an immune reaction against autoantigens has not been elucidated so far, there are several findings with regard to the etiology. Accordingly, the autoreaction may be due to a T-Cell bypass. A normal immune system requires the activation of B-cells by T-cells before the former can produce antibodies in large quantities. This requirement of a T-cell can be by-passed in rare instances, such as infection by organisms producing super-antigens, which are capable of initiating polyclonal activation of B-cells, or even of T-cells, by directly binding to the β-subunit of T-cell receptors in a nonspecific fashion. Another explanation deduces autoimmune diseases from a Molecular Mimicry. An exogenous antigen may share structural similarities with certain host antigens; thus, any antibody produced against this

antigen (which mimics the self-antigens) can also, in theory, bind to the host antigens and amplify the immune response. The most striking form of molecular mimicry is observed in Group A beta-haemolytic streptococci, which shares antigens with human myocardium, and is responsible for the cardiac manifestations of rheumatic fever.

[0433] Additionally, according to one further specific embodiment, diseases as defined herein comprise allergies or allergic diseases, i.e. diseases related to allergies. Allergy is a condition that typically involves an abnormal, acquired immunological hypersensitivity to certain foreign antigens or allergens, such as the allergy antigens as defined herein. Such allergy antigens or allergens may be selected from allergy antigens as defined herein antigens derived from different sources, e.g. from animals, plants, fungi, bacteria, etc. Allergens in this context include e.g. grasses, pollens, molds, drugs, or numerous environmental triggers, etc. Allergies normally result in a local or systemic inflammatory response to these antigens or allergens and lead to immunity in the body against these allergens. Without being bound to theory, several different disease mechanisms are supposed to be involved in the development of allergies. According to a classification scheme by P. Gell and R. Coombs the word "allergy" was restricted to type I hypersensitivities, which are caused by the classical IgE mechanism. Type I hypersensitivity is characterised by excessive activation of mast cells and basophils by IgE, resulting in a systemic inflammatory response that can result in symptoms as benign as a runny nose, to life-threatening anaphylactic shock and death. Well known types of allergies include, without being limited thereto, allergic asthma (leading to swelling of the nasal mucosa), allergic conjunctivitis (leading to redness and itching of the conjunctiva), allergic rhinitis ("hay fever"), anaphylaxis, angiodema, atopic dermatitis (eczema), urticaria (hives), eosinophilia, respiratory, allergies to insect stings, skin allergies (leading to and including various rashes, such as eczema, hives (urticaria) and (contact) dermatitis), food allergies, allergies to medicine, etc. Treatment of such allergic disorders or diseases may occur preferably by desensitizing the immune reaction which triggers a specific immune response. Such a desensitizing may be carried out by administering an effective amount of the allergen or allergic antigen encoded by the nucleic acid as defined herein, preferably, when formulated as a pharmaceutical composition, to induce a slight immune reaction. The amount of the allergen or allergic antigen may then be raised step by step in subsequent administrations until the immune system of the patient to be treated tolerates a specific amount of allergen or allergic antigen.

[0434] In a further aspect, the inventive polymeric carrier cargo complex which is administered in combination with a second nucleic acid molecule may be used for the preparation of a pharmaceutical composition, an immunostimulating agent, an adjuvant or a vaccine.

[0435] The inventive pharmaceutical composition, immunostimulating agent, adjuvant or vaccine may furthermore be used for the prophylaxis or treatment of a disease or a disorder as defined herein.

[0436] According to a final aspect, the present invention also provides kits, particularly kits of parts, comprising as components alone or in combination with further ingredients at least one inventive polymeric carrier cargo complex which is administered in combination with a second nucleic acid molecule as defined herein, at least one pharmaceutical composition, immunostimulating agent, adjuvant or vaccine comprising same and/or kits comprising same, and optionally technical instructions with information on the administration and dosage of the polymeric carrier molecule, the nucleic acid, the inventive polymeric carrier complex, and/or the inventive pharmaceutical composition. Such kits, preferably kits of parts, may be applied, e.g., for any of the above mentioned applications or uses. Such kits, when occurring as a kit of parts, may further contain each component of the inventive pharmaceutical composition, immunostimulating agent, adjuvant or vaccine in a different part of the kit. Preferably, at least one component is present in lyophilized form.

[0437] The inventive kit may thus comprise the pharmaceutical composition and/or the vaccine as described herein, and optionally a liquid vehicle for solubilising and optionally technical instructions with information on the administration and dosage of the active composition and/or the vaccine. In a preferred embodiment, the kit comprises a Ringer-lactate solution.

[0438] In the present invention, if not otherwise indicated, different features of alternatives and embodiments may be combined with each other, where suitable. Furthermore, the term "comprising" shall not be construed as meaning "consisting of", if not specifically mentioned. However, in the context of the present invention, term "comprising" may be substituted with the term "consisting of", where suitable.

FIGURES

[0439] The figures shown in the following are merely illustrative and shall describe the present invention in a further way. These figures shall not be construed to limit the present invention thereto.

[0440] FIG. **1**: G/C-enriched mRNA sequence R2564 coding for the hemagglutinin (HA) protein of influenza A virus (A/Netherlands/602/2009(H1N1)), corresponding to SEQ ID NO: 384.

[0441] FIG. **2**: RNA sequence of the non-coding immunostimulatory RNA R2025, corresponding to SEQ ID NO: 385.

[0442] FIG. **3**: FIG. **3** shows that intramuscular vaccination with a combination of HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, prepared according to Example 1) induces higher titers of antibodies against HA protein compared to vaccination with HA-mRNA (R2564) alone.

[0443] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 14 either with 40 μg HA-mRNA (R2564, SEQ ID NO: 384, "naked HA-RNA") alone or with 40 μg HA-mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of functional humoral responses was analysed on day 28 by determining the hemagglutination inhibition (HI) antibody titer, which is generally used as a surrogate marker of immune protection against influenza virus infection. A HI titer of 1:40 or greater is typically considered to confer protection. The experiment was performed as described in Example 2.

[0444] As can be seen in FIG. **3**, all mice vaccinated with the co-formulation developed HI-titers 1:40. In contrast, only 50% of mice vaccinated with HA-mRNA alone showed HI-titers 1:40.

[0445] Each dot represents an individual animal and the horizontal lines represent median values.

[0446] FIG. **4**: FIG. **4** shows that intramuscular vaccination with a combination of HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, prepared according to Example 1) leads to a significant increase in the number of central memory CD8+ cells.

[0447] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 14 with either 40 μg HA mRNA (R2564, SEQ ID NO: 384, "naked HA-RNA") alone or with 40 μg HA-mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of memory T cell responses in the bone marrow was analysed 7 weeks after boost vaccination. The experiment was performed as described in Example 2.

[0448] As can be seen in FIG. **4**, vaccination with the co-formulation led to a significant increase in the number of central memory CD8+ T cells compared to mice vaccinated with HA-mRNA alone.

[0449] Each dot represents an individual animal and the horizontal lines represent median values. Statistical assessment was performed with the unpaired t-test (**: p=0.0022; ****: p<0.0001).

[0450] FIG. **5**: FIG. **5** shows that intramuscular vaccination with a combination of HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, prepared according to Example 1) leads to significant increase in the number of central memory CD4+ cells.

[0451] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 14 with either 40 μg HA mRNA (R2564, SEQ ID NO: 384, "naked HA-RNA") alone or with 40 μg HA-mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of memory T cell responses in the bone marrow was analysed 7 weeks after boost vaccination. The experiment was performed as described in Example 2.

[0452] As can be seen in FIG. **5**, vaccination with the co-formulation led to a significant increase in the number of central memory CD4+ T cells compared to mice vaccinated with HA-mRNA alone.

[0453] Each dot represents an individual animal and the horizontal lines represent median values. Statistical assessment was performed with the unpaired t-test (**: p=0.0010; ****: p<0.0001).

[0454] FIG. **6**: FIG. **6** shows that the intramuscular vaccination with a combination of HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, prepared according to Example 1) leads to significant increase in the number of multifunctional CD4+ T cells.

[0455] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 14 with either 40 μg HA-mRNA (R2564, SEQ ID NO: 384, "naked HA-RNA") alone or 40 μg HA-mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of IFNγ/TNFα double-positive multifunctional CD4+ T cells in the spleen was analysed 7 weeks after boost vaccination by intracellular cytokine staining as described in Example 2.

[0456] As can be seen in FIG. **6**, vaccination with the co-formulation led to a significant increase in the number of multifunctional CD4+ T cells compared to mice vaccinated with HA-mRNA alone.

[0457] Each dot represents an individual animal and the horizontal lines represent median values. Statistical assessment was performed with the unpaired t-test (***: p=0.0003; **: p<0.007).

[0458] FIG. **7**: FIG. **7** shows that the intramuscular vaccination with a combination of the HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, prepared according to Example 1) leads to significant increase in the number of effector CD4+ T cells.

[0459] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 14 either with 40 μg HA-mRNA alone (R2564, SEQ ID NO: 384, "naked HA-RNA") or with 40 μg HA-mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of IFNγ/TNFα double-positive multifunctional CD4+ T cells in the spleen was analysed 7 days after boost vaccination by intracellular cytokine staining as described in Example 3.

[0460] As can be seen in FIG. **7**, vaccination with the co-formulation led to a significant increase in the number of multifunctional CD4+ T cells compared to mice vaccinated with HA-mRNA alone.

[0461] Each dot represents an individual animal and the horizontal lines represent median values. Statistical assessment was performed with the unpaired t-test (*: p=0.0286; **: p=0.0022).

[0462] FIG. **8**: shows that intramuscular vaccination of domestic pigs with a combination of HA-mRNA (R2564, SEQ ID NO: 384) and the polymeric carrier cargo complex (R2391, RNAdjuvant; prepared according to Example 1) induces higher titers of antibodies against HA protein compared to vaccination with HA-mRNA vaccine (R2630 RNActive®) alone. This effect is also detectable with enzymatically polyadenylated mRNA (R2564 pA).

[0463] Domestic pigs (n=5 per group) were vaccinated intramuscularly on days 1 and 29 either with 200 μg HA-mRNA vaccine (R2630 RNActive®) or R2564 pA (SEQ ID NO: 384, "naked polyadenylated HA-RNA") alone or a co-formulation of R2564 or R2564 pA and 200 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Pre-immune sera served as negative controls. Induction of functional humoral responses was analysed on day 57 by determining the hemagglutination inhibition (HI) antibody titer, which is generally used as a surrogate marker of immune protection against influenza virus infection. A HI titer of 1:40 or greater is typically considered to confer protection. The experiment was performed as described in Example 4.

[0464] As can be seen in FIG. **8**, the co-formulation with RNAdjuvant increased the functional antibodies compared to an mRNA vaccine (RNActive®) or compared to naked polyadenylated mRNA.

[0465] Each dot represents an individual animal, the horizontal lines represent median values.

[0466] FIG. **9**: shows that intramuscular vaccination of mice with a combination of RAV-G mRNA (R2506, SEQ ID NO: 391, and R3344) and the polymeric carrier cargo complex (R2391, RNAdjuvant; prepared according to Example 1) induces higher virus neutralization titers compared to vaccination with RAV-G-mRNA alone.

[0467] Balb/c mice (n=8 per group) were vaccinated intramuscularly on days 0 and 21 either with 20 μg RAV-G mRNA alone (R2506, SEQ ID NO: 391, "naked RAV-G RNA"; or R3344; enzymatically polyadenylated naked

RAV-G mRNA) or with 20 μg RAV-G mRNA co-formulated with 40 μg of the polymeric carrier cargo complex (R2391, "RNAdjuvant"). Buffer treated mice served as negative controls. Induction of virus neutralization titers was analysed 7 days after boost vaccination as described in Example 5. According to WHO guidelines, virus neutralization titers of ≤0.5 IU/ml are regarded as protective titers.

[0468] As can be seen in FIG. **9**, vaccination with the co-formulation led to increased functional antibody titers.

[0469] Each dot represents an individual animal, the horizontal lines represent median values.

[0470] FIG. **10**: shows that intramuscular vaccination of cotton rats with RSV-F mRNA (R2682; HRSV(Long-VR26)-Fdel554-574 mutant, SEQ ID NO: 392) in combination with the polymeric carrier cargo complex (R2391, "RNAdjuvant") significantly reduce lung titers in cotton rats challenged with RSV virus compared to vaccination with RSV-F mRNA alone.

[0471] The experiment was performed as described in Example 6.

[0472] FIG. **11**: G/C-enriched mRNA sequence R2506 (SEQ ID NO: 391) encoding the RAV-G protein.

[0473] FIG. **12**: G/C-enriched mRNA sequence R2682 (SEQ ID NO: 392) encoding the RSV-F protein (HRSV (Long-VR26)Fdel554-574).

EXAMPLES

[0474] The following examples are intended to illustrate the invention further. They are not intended to limit the subject matter of the invention thereto.

Example 1: Preparation of the RNA

[0475] 1. Preparation of DNA and mRNA Constructs

[0476] For the present example, a DNA sequence encoding the hemagglutinin (HA) protein of influenza A virus (A/Netherlands/602/2009(H1N1)) was prepared and used for subsequent in vitro transcription reactions.

[0477] According to a first preparation, the DNA sequence coding for the above mentioned mRNA was prepared. The construct R2564 (SEQ ID NO: 384) was prepared by introducing a 5'-TOP-UTR derived from the ribosomal protein 32L, modifying the wild type coding sequence by introducing a GC-optimized sequence for stabilization, followed by a stabilizing sequence derived from the albumin-3'-UTR, a stretch of 64 adenosines (poly(A)-sequence), a stretch of 30 cytosines (poly(C)-sequence), and a histone stem loop. In SEQ ID NO: 384 (see FIG. **1**) the sequence of the corresponding mRNA is shown.

[0478] For further examples, DNA sequences encoding the RAV-G protein of Rabies Virus and the RSV F protein (HRSV(Long-VR26)Fdel554-574) were prepared as already exemplified for mRNA coding for the hemagglutinin (HA) protein of influenza A virus (A/Netherlands/602/2009 (H1N1)) and used for subsequent in vitro transcription reactions. The corresponding mRNA sequences (SEQ ID NOs 391 and 392) are shown in FIGS. **11** and **12**.

2. Preparation of DNA and Non-Coding Immunostimulatory RNA Constructs

[0479] For the present example a DNA sequence encoding the non-coding immunostimulatory RNA (isRNA) R2025 was prepared and used for subsequent in vitro transcription reactions.

[0480] According to a first preparation, the DNA sequence coding for the above mentioned RNA was prepared. In SEQ ID NO: 385 (see FIG. **2**) the sequence of the corresponding RNA is shown.

TABLE 1

RNA constructs

RNA	Description	Figure	SEQ ID NO.
R2564 R2630	Influenza HA encoding mRNA	1	SEQ ID NO. 384
R2025 R2391	Non-coding immunostimulatory RNA	2	SEQ ID NO. 385
R2506	RAV-G encoding mRNA	11	SEQ ID NO. 391
R2682	RSV-F encoding mRNA (HRSV(Long-VR26)Fdel554-574)		SEQ ID NO. 392

3. In Vitro Transcription

[0481] The respective DNA plasmids prepared according to section 1 above were transcribed in vitro using T7 polymerase. The in vitro transcription of influenza HA encoding R2564, RAV-G encoding R2506 and R3344 or RSV F encoding R2682, respectively, was performed in the presence of a CAP analog (m^7GpppG). The isRNA R2025 was prepared without CAP analog. Subsequently the RNA was purified using PureMessenger® (CureVac, Tubingen, Germany; WO2008/077592A1).

Enzymatic Adenylation

[0482] RNA was reacted with *E. coli* poly(A) polymerase (Cellscript) using 1 mM ATP at 37° C. for 30 or 60 min. Immediately afterwards, RNA was purified by precipitation with lithium chloride (incubation for 1 h at −20° C.). The pellet was then washed with cold 75% ethanol and was finally resolved in water. RNA was run on a gel to assess RNA extension.

4. Preparation of the (Adjuvant) Polymeric Cargo Complex (RNAdjuvant®)

[0483] Cationic peptide as cationic component of the polymeric carrier:

CR12C: (SEQ ID NO: 6)
Cys-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Arg-Cys (Cys-Arg_{12}-Cys)

Synthesis of Polymeric Carrier Cargo Complexes:

[0484] An RNA molecule having the RNA sequence R2025 as defined in section 2 above was mixed with the cationic $CR_{12}C$ peptide component as defined above. The specified amount of the RNA was mixed with the respective cationic component in mass ratios as indicated, thereby forming a complex. If polymerizing cationic components were used according to the present invention, polymerization of the cationic components took place simultaneously to complexation of the nucleic acid cargo. Afterwards, the resulting solution was adjusted with water to a final volume of 50 μl and incubated for 30 minutes at room temperature. Further details are described in WO2012013326.

[0485] The mass ratio of peptide:RNA was 1:3,7. The polymeric carrier cargo complex is formed by the disulfide-crosslinked cationic peptide $CR_{12}C$ as carrier and the immunostimulatory R2025 as nucleic acid cargo. This polymeric carrier cargo complex R2025/$CR_{12}C$ (designated R2391) was used as adjuvant in the following examples. It is also referred to as 'RNAdjuvant®'.

5. Preparation of the Vaccine

[0486] The naked mRNAs R2564, R2506, R3344, and R2682 were administered in Ringer's Lactate solution. The lyophilyzed polymeric carrier cargo complex R2391 was dissolved in Ringer's Lactate solution to a final concentration of 1 □μg/μl. The co-formulation of naked mRNA R2564, R2506, R3344, or R2682 and R2391 was generated by mixing both components directly before administration.

[0487] For protamine-complexation, the mRNA R2564 was complexed with protamine in a mass ratio of 2:1. After incubation the same amount of naked mRNA R2564 was added to the nanoparticles. This vaccine formulation is referred to as R2630 RNActive®.

Example 2: Induction of a Humoral and Cellular Immune Response Against Hemagglutinin of Influenza Virus after Intramuscular Vaccination of Mice

Immunization:

[0488] On day zero, BALB/c mice were intramuscularly (i.m.) injected into both M. tibialis with the influenza HA-encoding mRNA (R2564) alone or in combination the polymeric carrier cargo complex (R2391) as shown in Table 2. Therein, the indicated amount in μg refers to the mass of the nucleic acid molecule per se, i.e. in the case of group 3, for instance, where the polymeric carrier cargo complex R2391 is used, animals received a polymeric carrier cargo complex (R2391), which comprised 20 μg of RNA. Mice injected with Ringer Lactate (RiLa) buffer served as controls. All animals received boost injections on day 14. Blood samples were collected on day 28 for the determination serum anti-HA antibody titers in the hemagglutination inhibition assay. Spleens and bone marrow were collected on day 45.

TABLE 2

Animal groups							
Group	Strain sex	No. mice	Route volume	RiLa buffer	HA RNA R2564	Polymeric carrier cargo complex R2391	Vaccination schedule
1	BALB/c Female	8	i.m. 2 × 30 μl	2 × 30 μl	—	—	d0: prime, d14: boost
2	BALB/c Female	8	i.m. 2 × 30 μl	—	2 × 20 μg	—	d0: prime, d14: boost
3	BALB/c Female	8	i.m. 2 × 30 μl	—	2 × 20 μg	2 × 20 μg	d0: prime, d14: boost

Protocols

Hemagglutination Inhibition Assay

[0489] For hemagglutination inhibition (HI) assay mouse sera were heat inactivated (56° C., 30 min), incubated with kaolin, and pre-adsorbed to chicken red blood cells (CRBC) (both Labor Dr. Merck & Kollegen, Ochsenhausen, Germany). For the HI assay, 50 μl of 2-fold dilutions of pre-treated sera were incubated for 45 minutes with 4 hemagglutination units (HAU) of inactivated A/California/5 7/2009 (NIBSC, Potters Bar, UK) and 50 μl 0.5% CRBC were added.

Isolation of Bone Marrow and Memory Cell Analyses

[0490] Femurs and tibias were removed and both ends of the bone were cut with scissors. The marrow was flushed with RPMI-1640 (Lonza, Verviers, Belgium) using a 5m1 syringe (Norm-Ject, Tuttlingen, Germany) with 23G needle (Braun Medical, Emmenbrücke, Germany). Cluster cells were dissociated by vigorous pipetting. Red blood cells were removed using an RBC lysis buffer. Cells were counted and plated on the 96-well V bottom plate (3×10^6 cells/well) for FACS staining. Cells were first incubated for 15 minutes at 4° C. with an anti-CD16/CD32 antibody (eBioscience, Frankfurt, Germany) to block unspecific binding followed by staining with PE-labelled HA-specific pentamer (H-2Kd IYSTVASSL, Proimmune, Oxford, UK) according to the manufacturer's instructions. Next, the cells were incubated with the following antibodies: CD44-FITC (1:200), Ly6C-PerCP-Cy5.5 (1:400), Thy1.2-APC (1:500), CD62L-PE-Cy7 (1:900), CD8α-APC-Cy7 (1:100) (BioLegend, Fell, Germany) and CD4-BD Horizon V450 (1:900) (BD Biosciences). After 30 minutes incubation at 4° C. cells were washed and stained with live/dead cell marker (AmCyan Aqua dye, Invitrogen, Life Technologies, Darmstadt, Germany) following by washing and FACS analyses using Fortessa or Canto II flow cytometer (Beckton Dickinson, Heidelberg, Germany). Flow cytometry data were analysed using FlowJo software (Tree Star, Inc., Ashland, Oreg., USA).

Intracellular Cytokine Staining

[0491] Splenocytes from vaccinated and control mice were isolated according to a standard protocol. Briefly,

isolated spleens were grinded through a cell strainer and washed in PBS/1% FBS followed by red blood cell lysis. After an extensive washing step with PBS/1% FBS splenocytes were seeded into 96-well plates (2×10^6 cells/well). Cells were stimulated with either Influenza Antigen A/California/7/2009 (5 μg/ml Health Protection Agency GB) or HAI (LYEKVKSQL) and HA2 (IYSTVASSL) peptides (5 μg/ml each, EMC Microcollections) and 2.5 μg/ml of an anti-CD28 antibody (BD Biosciences, Heidelberg, Germany) for 6 hours at 37° C. in the presence of the mixture of GolgiPlug™/GolgiStop™ (Protein transport inhibitors containing Brefeldin A and Monensin, respectively; BD Biosciences). Cells incubated with medium or DMSO were used as controls, respectively. After stimulation cells were washed and stained for intracellular cytokines using the Cytofix/Cytoperm reagent (BD Biosciences Frankfurt, Germany) according to the manufacturer's instructions. The following antibodies were used for staining: CD8-PECy7 (1:200), CD3-FITC (1:200), IL2-PerCP-Cy5.5 (1:100), TNFα-PE (1:100), IFNγ-APC (1:100) (eBioscience), CD4-BD Horizon V450 (1:200) (BD Biosciences) and incubated with Fey-block diluted 1:100. Aqua Dye was used to distinguish live/dead cells (Invitrogen, Life Technologies, Darmstadt, Germany). Cells were collected using a Canto II flow cytometer (Beckton Dickinson, Heidelberg, Germany). Flow cytometry data were analysed using FlowJo software (Tree Star, Inc., Ashland, Oreg., USA).

Results

[0492] FIG. **3** shows that the intramuscular vaccination with a combination of the HA-mRNA (R2564) and the polymeric carrier cargo complex (R2391) induces higher antibody titers against the HA protein compared to vaccination with the HA-mRNA (R2564) alone.

[0493] FIG. **4** shows that the intramuscular vaccination with a combination of the HA-mRNA (R2564) and the polymeric carrier cargo complex (R2391) leads to significant increase in the number of central memory CD8+ cells.

[0494] FIG. **5** shows that the intramuscular vaccination with a combination of the HA-mRNA (R2564) and the polymeric carrier cargo complex (R2391) leads to significant increase in the number of central memory CD4+ cells.

[0495] FIG. **6** shows that the intramuscular vaccination with a combination of the HA-mRNA (R2564) and the polymeric carrier cargo complex (R2391) leads to significant increase in the number of multifunctional CD4+ T cells.

Example 3: Induction of a Humoral and Cellular Immune Response Against Hemagglutinin of Influenza Virus after Intramuscular Vaccination of Mice

Immunization

[0496] On day zero, BALB/c mice were intramuscularly (i.m.) injected into both M. tibialis with the influenza HA-encoding mRNA (R2564) alone or in combination the polymeric carrier cargo complex (RNA R2391) as shown in Table 3. Therein, the indicated amounts refer to the amount of RNA per se (see also Example 2 above). Mice injected with Ringer Lactate (RiLa) buffer served as controls. All animals received boost injections on day 14. Blood samples were collected on days 14 and 21 for the determination of serum anti-HA antibody titers in the hemagglutination inhibition (HAI) assay as in example 2. Spleens were harvested on day 21, splenocytes were isolated and T cells were analysed by intracellular cytokine staining as described in example 2.

TABLE 3

Animal groups							
Group	Strain sex	No. mice	Route volume	RiLa buffer	HA RNA R2564	Polymeric carrier cargo complex R2391	Vaccination schedule (day)
1	BALB/c Female	8	i.m. 2 × 25 μl	2 × 25 μl	—	—	d0: prime, d14: boost
2	BALB/c Female	8	i.m. 2 × 25 μl	—	2 × 20 μg	—	d0: prime, d14: boost
3	BALB/c Female	8	i.m. 2 × 25 μl	—	2 × 20 μg	2 × 20 μg	d0: prime, d14: boost

Results

[0497] As can be seen in FIG. **7**, the intramuscular vaccination with 40 μg HA-mRNA (R2564) combined with 40 μg of polymeric carrier cargo complex (R2391) induced elevated numbers of IFNγ/TNFα double-positive multifunctional $CD4^+$ T cells as determined by intracellular cytokine staining after stimulation with Influenza Antigen A/California/7/2009 compared to vaccination with 40 μg of HA-mRNA (R2564) alone.

Example 4: Induction of a Humoral Immune Response Against Hemagglutinin of Influenza Virus H1N1 after Intramuscular Vaccination of Pigs

[0498] Domestic pigs were screened for swine influenza using the hemagglutinin inhibition assay at the breeding facility. Only seronegative pigs were introduced into the study.

Animal Groups and Treatment:

[0499]

Group	Animals	No.	Left leg i.m.	Vaccination schedule (day)
1	Female domestic pig, Hungarian large white	5	200 μg R2630 RNActive ®	d1: prime, d29: boost
2	Female domestic pig, Hungarian large white	5	200 μg R2564 + 200 μg R2391	d1: prime, d29: boost
3	Female domestic pig, Hungarian large white	5	200 μg R2564pA	d1: prime, d29: boost
4	Female domestic pig, Hungarian large white	5	200 μg R2564pA + 200 μg R2391	d1: prime, d29: boost

[0500] The RNA formulations prepared according to Example 1 were injected intramuscularly into the left hind leg. The treatment days were study day 1 and 29. Blood samples were taken on day −7, day 29, day 43 and day 57.

Hemagglutination Inhibition Assay:

[0501] For the hemagglutination inhibition (HI) assay, pig sera were treated with RDE (II) "SEIKEN" (WAK-Chemie Medical GmbH, Steinbach/Ts, Germany) o/n at 37° C., heat inactivated (56° C., 60 min), incubated with kaolin (Labor Dr. Merck & Kollegen, Ochsenhausen, Germany), and pre-adsorbed to chicken red blood cells (CRBC) (Lohmann Tierzucht, Cuxhaven, Germany). For the

[0502] HI assay, 50 μl of 2-fold dilutions of pre-treated sera were incubated for 45 minutes with 4 hemagglutination units (HAU) of inactivated A/California/5 7/2009 (NIBSC, Potters Bar, UK) and 50 μl 0.5% CRBC were added.

Results

[0503] As can be seen in FIG. **8**, the intramuscular vaccination with 200 μg of HA-mRNA (R2564) combined with 200 μg of polymeric carrier cargo complex (R2391) induced elevated neutralizing antibody titers against the HA protein compared to vaccination with the HA-mRNA vaccine (R2630 RNActive®) without the polymeric carrier cargo complex (R2391). Enzymatic polyadenylation increased the neutralizing antibody titers induced by HA-encoded mRNA (R2564 pA), but also in this case the addition of the polymeric carrier cargo complex (R2391) further increased the neutralizing antibody titers against the HA protein.

Example 5: Induction of Virus Neutralization Titers Against Rabies Virus after Intramuscular Vaccination of Mice

[0504] Balb/c mice were vaccinated 2 times (d0 and d21) with 20 μg RAV-G mRNA (R2506) or enzymatically polyadenylated RAV-G mRNA (R3344) alone or in combination with 40 μg RNAdjuvant prepared according to Example 1 into both M. tibialis. Therefore, 8 animals (group A) were vaccinated i.m. with R2506 (naked RAV-G mRNA), 8 animals (group B) were vaccinated i.m. with R3344 (enzymatically polyadenylated R2506—naked RNA), 8 animals (group C) were vaccinated i.m. with R2506 (naked RAV-G mRNA) in combination with RNAdjuvant and 8 animals (group D) were vaccinated i.m. with R3344 (enzymatically polyadenylated naked RAV-G mRNA) in combination with RNAdjuvant®. 8 mice injected with Ringer-Lactate solution (RiLa) served as negative controls. Blood was collected 28 days after prime (7 days after boost). Serum was analyzed for virus neutralization titers (VNT).

Animal Groups and Treatment

[0505]

group	n	mice	RNA	RNAdjuvant	vaccination
A	8	Balb/c	20 μg R2506	—	d0, d21
B	8	Balb/c	20 μg R3344	—	d0, d21
C	8	Balb/c	20 μg R2506	40 μg R2391	d0, d21
D	8	Balb/c	20 μg R3344	40 μg R2391	d0, d21
E	8	Balb/c	RiLa	—	d0, d21

Virus Neutralization Test

[0506] The virus neutralizing antibody response (specific B-cell immune response) was detected by using a virus neutralisation assay. The result of that assay is referred to as virus neutralization titer (VNT). According to WHO standards, an antibody titer is considered protective if the respective VNT is at least 0.5 IU/ml. Therefore, blood samples were taken from vaccinated mice on day 28 and sera were prepared. These sera were used in fluorescent antibody virus neutralisation (FAVN) test using the cell culture adapted challenge virus strain (CVS) of rabies virus as recommended by the OIE (World Organisation for Animal Health) and first described in Cliquet F., Aubert M. & Sagne L. (1998); *J. Immunol. Methods,* 212, 79-87. Shortly, heat inactivated sera are tested in microplates as quadruplicates in serial two-fold dilutions for their potential to neutralize 100 $TCID_{50}$ (tissue culture infectious doses 50%) of CVS in a volume of 50 μl. Therefore, sera dilutions were incubated with virus for 1 hour at 37° C. (in humid incubator with 5% CO_2) and subsequently trypsinized BHK-21 cells were added (4×10^5 cells/ml; 50 μl per well After an incubation period of 48 hours in humid incubator at 37° C. and 5% CO_2, cells were fixed in 80% aceton at room temperature for 30 minutes. Infection of cells was analysed using FITC anti-rabies conjugate (30 minutes at 37° C.). Plates were washed twice using PBS and excess of PBS was removed. Cell cultures are scored positive or negative for the presence of rabies virus. For each well, the presence or absence of fluorescent cells is evaluated. Wells with no detectable fluorescent cell are scored negative. Negative scored sera treated wells represent neutralization of rabies virus. Each FAVN tests includes WHO or OIE standard serum (positive reference serum) that serves as reference for standardisation of the assay. Neutralization activity of test sera was calculated with reference to the standard serum provided by the WHO and displayed as International Units/ml (IU/ml).

Results

[0507] As can be seen in FIG. **9**, the intramuscular vaccination with 20 μg of naked RAV-G mRNA (R2506) or enzymatically polyadenylated naked RAV-G mRNA (R3344) combined with 40 μg of polymeric carrier cargo complex (R2391; RNAdjuvant) induced elevated virus neutralization titers compared to vaccination with the RAV-G mRNAs alone.

Example 6: Reduction of RSV Virus Titers in the Lung after Vaccination with mRNA Encoding RSV F Protein

Groups and Treatment:

[0508]

Group	Strain/ sex	Nr.	Treatment RNA/mouse	Route, Volume	Immunisation schedule	chal-lenge
A	Cotton rats/female	5	R2682 80 μg	i.m. 1 × 100 μl	d0, d14	d49
B	Cotton rats/female	5	R2391 40 μg + R2682 40 μg	i.m. 1 × 100 μl	d0, d14	d49
C	Cotton rats/female	5	RiLa	i.d. 2 × 50 μl	d0, d14	d49
D	Cotton rats/female	5	Live RSV/A2		d0	d49
E	Cotton rats/female	5	untreated	—	—	d49

[0509] Cotton rats represent an established and widely accepted animal model for RSV that is routinely used for the development of RSV vaccines. Cotton rats respond to formalin-inactivated RSV virus vaccine preparations with enhanced lung pathology. This allows the evaluation of the safety of a vaccination in terms of enhanced disease phenomenon.

[0510] In order to assess the effect of the RSV-F encoding mRNA (R2682), the mRNA was administered intramuscularly on day 0 and 14 either alone or in combination with the polymeric cargo complex (R2391; RNAdjuvant) as shown above. An additional group was immunized intramuscularly (i.m.) with live RSV/A2 (Sigmovir) (10^5 plaque forming units, pfu) to compare their immunogenicity to mRNA vaccines. After immunization, the cotton rats were challenged by intranasal (i.n.) infection with RSV/A2 virus (105 PFU in 100 μl; Sigmovir). On day 54 the lung was harvested en bloc for viral titration.

Results:

[0511] As shown in FIG. **10**, intramuscular vaccination with 40 μg of naked RSV-F mRNA (R2682) combined with 40 μg of polymeric carrier cargo complex (R2391; RNAdjuvant) led to significantly reduced viral titers in the lung compared to vaccination with the RSV-F mRNA alone.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 392

<210> SEQ ID NO 1
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 1

Cys Arg Arg Arg Arg Arg Arg Arg Cys
1 5

<210> SEQ ID NO 2
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 2

Cys Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10

<210> SEQ ID NO 3
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

-continued

<400> SEQUENCE: 3

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10

<210> SEQ ID NO 4
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 4

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10

<210> SEQ ID NO 5
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 5

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10

<210> SEQ ID NO 6
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 6

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10

<210> SEQ ID NO 7
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 7

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10 15

<210> SEQ ID NO 8
<211> LENGTH: 16
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 8

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Cys
1 5 10 15

<210> SEQ ID NO 9
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 9

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Cys

<210> SEQ ID NO 10
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 10

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Arg Cys

<210> SEQ ID NO 11
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 11

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Arg Arg Cys

<210> SEQ ID NO 12
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or

-continued

polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 12

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Arg Arg Arg Cys
20

<210> SEQ ID NO 13
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 13

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Arg Arg Arg Arg Cys
20

<210> SEQ ID NO 14
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Sequence: cationic or
polycationic peptide of formula (Ib): Cys

(Orn)o;(Xaa)x
Cys

<400> SEQUENCE: 14

Cys Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1 5 10 15

Arg Arg Arg Arg Arg Cys
20

<210> SEQ ID NO 15
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 15

Trp Tyr
1

<210> SEQ ID NO 16
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 16

Tyr Trp

-continued

1

<210> SEQ ID NO 17
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 17

Trp Trp
1

<210> SEQ ID NO 18
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 18

Tyr Tyr
1

<210> SEQ ID NO 19
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 19

Trp Tyr Trp
1

<210> SEQ ID NO 20
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 20

Tyr Trp Tyr
1

<210> SEQ ID NO 21
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 21

Trp Trp Trp
1

<210> SEQ ID NO 22
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 22

-continued

Tyr Tyr Tyr
1

<210> SEQ ID NO 23
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 23

Trp Tyr Trp Tyr
1

<210> SEQ ID NO 24
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 24

Tyr Trp Tyr Trp
1

<210> SEQ ID NO 25
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 25

Trp Trp Trp Trp
1

<210> SEQ ID NO 26
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 26

Phe Tyr
1

<210> SEQ ID NO 27
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 27

Tyr Phe
1

<210> SEQ ID NO 28
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

-continued

<400> SEQUENCE: 28

Phe Phe
1

<210> SEQ ID NO 29
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 29

Phe Tyr Phe
1

<210> SEQ ID NO 30
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 30

Tyr Phe Tyr
1

<210> SEQ ID NO 31
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 31

Phe Phe Phe
1

<210> SEQ ID NO 32
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 32

Phe Tyr Phe Tyr
1

<210> SEQ ID NO 33
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 33

Tyr Phe Tyr Phe
1

<210> SEQ ID NO 34
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 34

Phe Phe Phe Phe
1

<210> SEQ ID NO 35
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 35

Phe Trp
1

<210> SEQ ID NO 36
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 36

Trp Phe
1

<210> SEQ ID NO 37
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 37

Phe Phe
1

<210> SEQ ID NO 38
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 38

Phe Trp Phe
1

<210> SEQ ID NO 39
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 39

Trp Phe Trp
1

<210> SEQ ID NO 40
<211> LENGTH: 4

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 40

Phe Trp Phe Trp
1

<210> SEQ ID NO 41
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 41

Trp Phe Trp Phe
1

<210> SEQ ID NO 42
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 42

Tyr Tyr Tyr Tyr
1

<210> SEQ ID NO 43
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 43

Cys Tyr Cys
1

<210> SEQ ID NO 44
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 44

Cys Trp Cys
1

<210> SEQ ID NO 45
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 45

Cys Trp Tyr Cys
1

-continued

<210> SEQ ID NO 46
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 46

Cys Tyr Trp Cys
1

<210> SEQ ID NO 47
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 47

Cys Trp Trp Cys
1

<210> SEQ ID NO 48
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 48

Cys Tyr Tyr Cys
1

<210> SEQ ID NO 49
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 49

Cys Trp Tyr Trp Cys
1 5

<210> SEQ ID NO 50
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 50

Cys Tyr Trp Tyr Cys
1 5

<210> SEQ ID NO 51
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 51

Cys Trp Trp Trp Cys
1 5

-continued

<210> SEQ ID NO 52
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 52

Cys Tyr Tyr Tyr Cys
1 5

<210> SEQ ID NO 53
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 53

Cys Trp Tyr Trp Tyr Cys
1 5

<210> SEQ ID NO 54
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 54

Cys Tyr Trp Tyr Trp Cys
1 5

<210> SEQ ID NO 55
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 55

Cys Trp Trp Trp Trp Cys
1 5

<210> SEQ ID NO 56
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 56

Cys Tyr Tyr Tyr Tyr Cys
1 5

<210> SEQ ID NO 57
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 57

-continued

Cys Phe Cys
1

<210> SEQ ID NO 58
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 58

Cys Phe Tyr Cys
1

<210> SEQ ID NO 59
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 59

Cys Tyr Phe Cys
1

<210> SEQ ID NO 60
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 60

Cys Phe Phe Cys
1

<210> SEQ ID NO 61
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 61

Cys Tyr Tyr Cys
1

<210> SEQ ID NO 62
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 62

Cys Phe Tyr Phe Cys
1 5

<210> SEQ ID NO 63
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

-continued

<400> SEQUENCE: 63

Cys Tyr Phe Tyr Cys
1 5

<210> SEQ ID NO 64
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 64

Cys Phe Phe Phe Cys
1 5

<210> SEQ ID NO 65
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 65

Cys Tyr Tyr Tyr Cys
1 5

<210> SEQ ID NO 66
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 66

Cys Phe Tyr Phe Tyr Cys
1 5

<210> SEQ ID NO 67
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 67

Cys Tyr Phe Tyr Phe
1 5

<210> SEQ ID NO 68
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 68

Cys Phe Phe Phe Phe Cys
1 5

<210> SEQ ID NO 69
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 69

Cys Phe Trp Cys
1

<210> SEQ ID NO 70
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 70

Cys Trp Phe Cys
1

<210> SEQ ID NO 71
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 71

Cys Phe Phe Cys
1

<210> SEQ ID NO 72
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 72

Cys Phe Trp Phe Cys
1 5

<210> SEQ ID NO 73
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 73

Cys Trp Phe Trp Cys
1 5

<210> SEQ ID NO 74
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 74

Cys Phe Trp Phe Trp Cys
1 5

<210> SEQ ID NO 75
<211> LENGTH: 6
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary aromatic amino acid component (AA)

<400> SEQUENCE: 75

Cys Trp Phe Trp Phe Cys
1 5

<210> SEQ ID NO 76
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 76

Ser Thr
1

<210> SEQ ID NO 77
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 77

Thr Ser
1

<210> SEQ ID NO 78
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 78

Ser Ser
1

<210> SEQ ID NO 79
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 79

Thr Thr
1

<210> SEQ ID NO 80
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 80

-continued

Ser Thr Ser
1

<210> SEQ ID NO 81
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 81

Thr Ser Thr
1

<210> SEQ ID NO 82
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 82

Ser Ser Ser
1

<210> SEQ ID NO 83
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 83

Thr Thr Thr
1

<210> SEQ ID NO 84
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 84

Ser Thr Ser Thr
1

<210> SEQ ID NO 85
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 85

Thr Ser Thr Ser
1

<210> SEQ ID NO 86
<211> LENGTH: 4

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 86

Ser Ser Ser Ser
1

<210> SEQ ID NO 87
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 87

Thr Thr Thr Thr
1

<210> SEQ ID NO 88
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 88

Gln Asn
1

<210> SEQ ID NO 89
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 89

Asn Gln
1

<210> SEQ ID NO 90
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 90

Gln Gln
1

<210> SEQ ID NO 91
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

-continued

<400> SEQUENCE: 91

Asn Asn
1

<210> SEQ ID NO 92
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 92

Gln Asn Gln
1

<210> SEQ ID NO 93
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 93

Asn Gln Asn
1

<210> SEQ ID NO 94
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 94

Gln Gln Gln
1

<210> SEQ ID NO 95
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 95

Asn Asn Asn
1

<210> SEQ ID NO 96
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 96

Gln Asn Gln Asn
1

-continued

<210> SEQ ID NO 97
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 97

Asn Gln Asn Gln
1

<210> SEQ ID NO 98
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 98

Gln Gln Gln Gln
1

<210> SEQ ID NO 99
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 99

Asn Asn Asn Asn
1

<210> SEQ ID NO 100
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 100

Ser Asn
1

<210> SEQ ID NO 101
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 101

Asn Ser
1

<210> SEQ ID NO 102
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non

-continued

charged polar) amino acid component (AA)

<400> SEQUENCE: 102

Ser Ser
1

<210> SEQ ID NO 103
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 103

Asn Asn
1

<210> SEQ ID NO 104
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 104

Ser Asn Ser
1

<210> SEQ ID NO 105
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 105

Asn Ser Asn
1

<210> SEQ ID NO 106
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 106

Ser Ser Ser
1

<210> SEQ ID NO 107
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 107

Asn Asn Asn
1

-continued

<210> SEQ ID NO 108
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 108

Ser Asn Ser Asn
1

<210> SEQ ID NO 109
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 109

Asn Ser Asn Ser
1

<210> SEQ ID NO 110
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 110

Ser Ser Ser Ser
1

<210> SEQ ID NO 111
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 111

Asn Asn Asn Asn
1

<210> SEQ ID NO 112
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 112

Cys Thr Cys
1

<210> SEQ ID NO 113
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 113

Cys Ser Cys
1

<210> SEQ ID NO 114
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 114

Cys Ser Thr Cys
1

<210> SEQ ID NO 115
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 115

Cys Thr Ser Cys
1

<210> SEQ ID NO 116
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 116

Cys Ser Ser Cys
1

<210> SEQ ID NO 117
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 117

Cys Thr Thr Cys
1

<210> SEQ ID NO 118
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 118

-continued

Cys Ser Thr Ser Cys
1 5

<210> SEQ ID NO 119
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 119

Cys Thr Ser Thr Cys
1 5

<210> SEQ ID NO 120
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 120

Cys Ser Ser Ser Cys
1 5

<210> SEQ ID NO 121
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 121

Cys Thr Thr Thr Cys
1 5

<210> SEQ ID NO 122
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 122

Cys Ser Thr Ser Thr Cys
1 5

<210> SEQ ID NO 123
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 123

Cys Thr Ser Thr Ser Cys
1 5

<210> SEQ ID NO 124
<211> LENGTH: 6

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 124

Cys Ser Ser Ser Ser Cys
1 5

<210> SEQ ID NO 125
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 125

Cys Thr Thr Thr Thr Cys
1 5

<210> SEQ ID NO 126
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 126

Cys Asn Cys
1

<210> SEQ ID NO 127
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 127

Gln Cys Gln
1

<210> SEQ ID NO 128
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 128

Cys Gln Asn Cys
1

<210> SEQ ID NO 129
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

-continued

<400> SEQUENCE: 129

Cys Asn Gln Cys
1

<210> SEQ ID NO 130
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 130

Cys Gln Gln Cys
1

<210> SEQ ID NO 131
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 131

Cys Asn Asn Cys
1

<210> SEQ ID NO 132
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 132

Cys Gln Asn Gln Cys
1 5

<210> SEQ ID NO 133
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 133

Cys Asn Gln Asn Cys
1 5

<210> SEQ ID NO 134
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 134

Cys Gln Gln Gln Cys
1 5

-continued

<210> SEQ ID NO 135
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 135

Cys Asn Asn Asn Cys
1 5

<210> SEQ ID NO 136
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 136

Cys Gln Asn Gln Asn Cys
1 5

<210> SEQ ID NO 137
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 137

Cys Asn Gln Asn Gln Cys
1 5

<210> SEQ ID NO 138
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 138

Cys Gln Gln Gln Gln Cys
1 5

<210> SEQ ID NO 139
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 139

Cys Asn Asn Asn Asn Cys
1 5

<210> SEQ ID NO 140
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non

-continued

charged polar) amino acid component (AA)

<400> SEQUENCE: 140

Cys Asn Cys
1

<210> SEQ ID NO 141
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 141

Cys Ser Cys
1

<210> SEQ ID NO 142
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 142

Cys Ser Asn Cys
1

<210> SEQ ID NO 143
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 143

Cys Asn Ser Cys
1

<210> SEQ ID NO 144
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 144

Cys Ser Ser Cys
1

<210> SEQ ID NO 145
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 145

Cys Asn Asn Cys
1

-continued

<210> SEQ ID NO 146
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 146

Cys Ser Asn Ser Cys
1 5

<210> SEQ ID NO 147
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 147

Cys Asn Ser Asn Cys
1 5

<210> SEQ ID NO 148
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 148

Cys Ser Ser Ser Cys
1 5

<210> SEQ ID NO 149
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 149

Cys Asn Asn Asn Cys
1 5

<210> SEQ ID NO 150
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 150

Cys Ser Asn Ser Asn Cys
1 5

<210> SEQ ID NO 151
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 151

Cys Asn Ser Asn Ser Cys
1 5

<210> SEQ ID NO 152
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 152

Cys Ser Ser Ser Ser Cys
1 5

<210> SEQ ID NO 153
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary hydrophilic (and preferably non charged polar) amino acid component (AA)

<400> SEQUENCE: 153

Cys Asn Asn Asn Asn Cys
1 5

<210> SEQ ID NO 154
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 154

Leu Val
1

<210> SEQ ID NO 155
<211> LENGTH: 1
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 155

Leu
1

<210> SEQ ID NO 156
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 156

Leu Leu
1

-continued

<210> SEQ ID NO 157

<400> SEQUENCE: 157

000

<210> SEQ ID NO 158
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 158

Leu Val Leu
1

<210> SEQ ID NO 159
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 159

Val Leu Val
1

<210> SEQ ID NO 160
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 160

Leu Leu Leu
1

<210> SEQ ID NO 161
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 161

Val Val Val
1

<210> SEQ ID NO 162
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 162

Leu Val Leu Val
1

<210> SEQ ID NO 163
<211> LENGTH: 4
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 163

Val Leu Val Leu
1

<210> SEQ ID NO 164
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 164

Leu Leu Leu Leu
1

<210> SEQ ID NO 165
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 165

Val Val Val Val
1

<210> SEQ ID NO 166
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 166

Ile Ala
1

<210> SEQ ID NO 167
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 167

Ala Ile
1

<210> SEQ ID NO 168
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 168

Ile Ile
1

<210> SEQ ID NO 169

-continued

<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 169

Ala Ala
1

<210> SEQ ID NO 170
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 170

Ile Ala Ile
1

<210> SEQ ID NO 171
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 171

Ala Ile Ala
1

<210> SEQ ID NO 172
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 172

Ile Ile Ile
1

<210> SEQ ID NO 173
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 173

Ala Ala Ala
1

<210> SEQ ID NO 174
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 174

Ile Ala Ile Ala
1

-continued

<210> SEQ ID NO 175
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 175

Ala Ile Ala Ile
1

<210> SEQ ID NO 176
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 176

Ile Ile Ile Ile
1

<210> SEQ ID NO 177
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 177

Ala Ala Ala Ala
1

<210> SEQ ID NO 178
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 178

Met Ala
1

<210> SEQ ID NO 179
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 179

Ala Met
1

<210> SEQ ID NO 180
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 180

Met Met

-continued

1

<210> SEQ ID NO 181
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 181

Ala Ala
1

<210> SEQ ID NO 182
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 182

Met Ala Met
1

<210> SEQ ID NO 183
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 183

Ala Met Ala
1

<210> SEQ ID NO 184
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 184

Met Met Met
1

<210> SEQ ID NO 185
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 185

Ala Ala Ala
1

<210> SEQ ID NO 186
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 186

-continued

Met Ala Met Ala
1

<210> SEQ ID NO 187
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 187

Ala Met Ala Met
1

<210> SEQ ID NO 188
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 188

Met Met Met Met
1

<210> SEQ ID NO 189
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 189

Cys Val Cys
1

<210> SEQ ID NO 190
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 190

Cys Leu Cys
1

<210> SEQ ID NO 191
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 191

Cys Leu Val Cys
1

<210> SEQ ID NO 192
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

-continued

<400> SEQUENCE: 192

Cys Val Leu Cys
1

<210> SEQ ID NO 193
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 193

Cys Leu Leu Cys
1

<210> SEQ ID NO 194
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 194

Cys Val Val Cys
1

<210> SEQ ID NO 195
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 195

Cys Leu Val Leu Cys
1 5

<210> SEQ ID NO 196
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 196

Cys Val Leu Val Cys
1 5

<210> SEQ ID NO 197
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 197

Cys Leu Leu Leu Cys
1 5

<210> SEQ ID NO 198
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence

-continued

<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 198

Cys Val Val Val Cys
1 5

<210> SEQ ID NO 199
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 199

Cys Leu Val Leu Val Cys
1 5

<210> SEQ ID NO 200
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 200

Cys Val Leu Val Leu Cys
1 5

<210> SEQ ID NO 201
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 201

Cys Leu Leu Leu Leu Cys
1 5

<210> SEQ ID NO 202
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 202

Cys Val Val Val Val Cys
1 5

<210> SEQ ID NO 203
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 203

Cys Ala Cys
1

<210> SEQ ID NO 204
<211> LENGTH: 3

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 204

Cys Ile Cys
1

<210> SEQ ID NO 205
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 205

Cys Ile Ala Cys
1

<210> SEQ ID NO 206
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 206

Cys Ala Ile Cys
1

<210> SEQ ID NO 207
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 207

Cys Ile Ile Cys
1

<210> SEQ ID NO 208
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 208

Cys Ala Ala Cys
1

<210> SEQ ID NO 209
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 209

Cys Ile Ala Ile Cys
1   5

-continued

<210> SEQ ID NO 210
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 210

Cys Ala Ile Ala Cys
1 5

<210> SEQ ID NO 211
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 211

Cys Ile Ile Ile Cys
1 5

<210> SEQ ID NO 212
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 212

Cys Ala Ala Ala Cys
1 5

<210> SEQ ID NO 213
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 213

Cys Ile Ala Ile Ala Cys
1 5

<210> SEQ ID NO 214
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 214

Cys Ala Ile Ala Ile Cys
1 5

<210> SEQ ID NO 215
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 215

Cys Ile Ile Ile Ile Cys
1 5

-continued

<210> SEQ ID NO 216
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 216

Cys Ala Ala Ala Ala Cys
1 5

<210> SEQ ID NO 217
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 217

Cys Met Cys
1

<210> SEQ ID NO 218
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 218

Cys Met Ala Cys
1

<210> SEQ ID NO 219
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 219

Cys Ala Met Cys
1

<210> SEQ ID NO 220
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 220

Cys Met Met Cys
1

<210> SEQ ID NO 221
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 221

-continued

Cys Ala Ala Cys
1

<210> SEQ ID NO 222
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 222

Cys Met Ala Met Cys
1 5

<210> SEQ ID NO 223
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 223

Cys Ala Met Ala Cys
1 5

<210> SEQ ID NO 224
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 224

Cys Met Met Met Cys
1 5

<210> SEQ ID NO 225
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 225

Cys Ala Ala Ala Cys
1 5

<210> SEQ ID NO 226
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 226

Cys Met Ala Met Ala Cys
1 5

<210> SEQ ID NO 227
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

-continued

<400> SEQUENCE: 227

Cys Ala Met Ala Met Cys
1 5

<210> SEQ ID NO 228
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 228

Cys Met Met Met Met Cys
1 5

<210> SEQ ID NO 229
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary lipophilic amino acid component (AA)

<400> SEQUENCE: 229

Cys Ala Ala Ala Ala Cys
1 5

<210> SEQ ID NO 230
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 230

Asp His
1

<210> SEQ ID NO 231
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 231

His Asp
1

<210> SEQ ID NO 232
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 232

Asp Asp
1

<210> SEQ ID NO 233
<211> LENGTH: 2
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 233

His His
1

<210> SEQ ID NO 234
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 234

Asp His Asp
1

<210> SEQ ID NO 235
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 235

His Asp His
1

<210> SEQ ID NO 236
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 236

Asp Asp Asp
1

<210> SEQ ID NO 237
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 237

His His His
1

<210> SEQ ID NO 238
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 238

Asp His Asp His
1

<210> SEQ ID NO 239
<211> LENGTH: 4
<212> TYPE: PRT

-continued

<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 239

His Asp His Asp
1

<210> SEQ ID NO 240
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 240

Asp Asp Asp Asp
1

<210> SEQ ID NO 241
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 241

His His His His
1

<210> SEQ ID NO 242
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 242

Cys His Cys
1

<210> SEQ ID NO 243
<211> LENGTH: 3
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 243

Cys Asp Cys
1

<210> SEQ ID NO 244
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 244

Cys Asp His Cys
1

<210> SEQ ID NO 245

-continued

<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 245

Cys His Asp Cys
1

<210> SEQ ID NO 246
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 246

Cys Asp Asp Cys
1

<210> SEQ ID NO 247
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 247

Cys His His Cys
1

<210> SEQ ID NO 248
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 248

Cys Asp His Asp Cys
1 5

<210> SEQ ID NO 249
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 249

Cys His Asp His Cys
1 5

<210> SEQ ID NO 250
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 250

Cys Asp Asp Asp Cys
1 5

-continued

<210> SEQ ID NO 251
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 251

Cys His His His Cys
1 5

<210> SEQ ID NO 252
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 252

Cys Asp His Asp His Cys
1 5

<210> SEQ ID NO 253
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 253

Cys His Asp His Asp Cys
1 5

<210> SEQ ID NO 254
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 254

Cys Asp Asp Asp Asp Cys
1 5

<210> SEQ ID NO 255
<211> LENGTH: 6
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Exemplary weak basic amino acid component (AA)

<400> SEQUENCE: 255

Cys His His His His Cys
1 5

<210> SEQ ID NO 256
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide,
localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 256

-continued

Lys Asp Glu Leu
1

<210> SEQ ID NO 257
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 257

Asp Asp Glu Leu
1

<210> SEQ ID NO 258
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 258

Asp Glu Glu Leu
1

<210> SEQ ID NO 259
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 259

Gln Glu Asp Leu
1

<210> SEQ ID NO 260
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 260

Arg Asp Glu Leu
1

<210> SEQ ID NO 261
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 261

Gly Gln Asn Leu Ser Thr Ser Asn
1   5

<210> SEQ ID NO 262
<211> LENGTH: 7

-continued

<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 262

Pro Lys Lys Lys Arg Lys Val
1 5

<210> SEQ ID NO 263
<211> LENGTH: 7
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 263

Pro Gln Lys Lys Ile Lys Ser
1 5

<210> SEQ ID NO 264
<211> LENGTH: 5
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 264

Gln Pro Lys Lys Pro
1 5

<210> SEQ ID NO 265
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 265

Arg Lys Lys Arg
1

<210> SEQ ID NO 266
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 266

Arg Lys Lys Arg Arg Gln Arg Arg Arg Ala His Gln
1 5 10

<210> SEQ ID NO 267
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

-continued

<400> SEQUENCE: 267

Arg Gln Ala Arg Arg Asn Arg Arg Arg Arg Trp Arg Glu Arg Gln Arg
1 5 10 15

<210> SEQ ID NO 268
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 268

Met Pro Leu Thr Arg Arg Arg Pro Ala Ala Ser Gln Ala Leu Ala Pro
1 5 10 15

Pro Thr Pro

<210> SEQ ID NO 269
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 269

Gly Ala Ala Leu Thr Ile Leu Val
1 5

<210> SEQ ID NO 270
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 270

Gly Ala Ala Leu Thr Leu Leu Gly
1 5

<210> SEQ ID NO 271
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 271

Met Asp Asp Gln Arg Asp Leu Ile Ser Asn Asn Glu Gln Leu Pro
1 5 10 15

<210> SEQ ID NO 272
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (7)..(8)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid
<220> FEATURE:

-continued

<221> NAME/KEY: misc_feature
<222> LOCATION: (32)..(32)
<223> OTHER INFORMATION: Xaa can be any naturally occurring amino acid

<400> SEQUENCE: 272

Met Leu Phe Asn Leu Arg Xaa Xaa Leu Asn Asn Ala Ala Phe Arg His
1 5 10 15

Gly His Asn Phe Met Val Arg Asn Phe Arg Cys Gly Gln Pro Leu Xaa
20 25 30

<210> SEQ ID NO 273
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 273

Gly Cys Val Cys Ser Ser Asn Pro
1 5

<210> SEQ ID NO 274
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 274

Gly Gln Thr Val Thr Thr Pro Leu
1 5

<210> SEQ ID NO 275
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 275

Gly Gln Glu Leu Ser Gln His Glu
1 5

<210> SEQ ID NO 276
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 276

Gly Asn Ser Pro Ser Tyr Asn Pro
1 5

<210> SEQ ID NO 277
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

-continued

<400> SEQUENCE: 277

Gly Val Ser Gly Ser Lys Gly Gln
1 5

<210> SEQ ID NO 278
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 278

Gly Gln Thr Ile Thr Thr Pro Leu
1 5

<210> SEQ ID NO 279
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 279

Gly Gln Thr Leu Thr Thr Pro Leu
1 5

<210> SEQ ID NO 280
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 280

Gly Gln Ile Phe Ser Arg Ser Ala
1 5

<210> SEQ ID NO 281
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 281

Gly Gln Ile His Gly Leu Ser Pro
1 5

<210> SEQ ID NO 282
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 282

Gly Ala Arg Ala Ser Val Leu Ser
1 5

-continued

<210> SEQ ID NO 283
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 283

Gly Cys Thr Leu Ser Ala Glu Glu
1 5

<210> SEQ ID NO 284
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 284

Gly Ala Gln Val Ser Ser Gln Lys
1 5

<210> SEQ ID NO 285
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 285

Gly Ala Gln Leu Ser Arg Asn Thr
1 5

<210> SEQ ID NO 286
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 286

Gly Asn Ala Ala Ala Ala Lys Lys
1 5

<210> SEQ ID NO 287
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 287

Gly Asn Glu Ala Ser Tyr Pro Leu
1 5

<210> SEQ ID NO 288
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: Description of sequence: signal peptide, localization signal or sequence or nuclear localization signal

<400> SEQUENCE: 288

Gly Ser Ser Lys Ser Lys Pro Lys
1 5

<210> SEQ ID NO 289
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 289

gguuuuuuuu uuuuuuuggg 20

<210> SEQ ID NO 290
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 290

ggggguuuuu uuuuuggggg 20

<210> SEQ ID NO 291
<211> LENGTH: 40
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 291

ggggguuuuu uuuuuuuuuu uuuuuuuuuu uuuuuggggg 40

<210> SEQ ID NO 292
<211> LENGTH: 39
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 292

gugugugugu guuuuuuuuu uuuuuuugug ugugugugu 39

<210> SEQ ID NO 293
<211> LENGTH: 39
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 293

gguugguugg uuuuuuuuuu uuuuuuuggu ugguugguu 39

<210> SEQ ID NO 294
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

-continued

<400> SEQUENCE: 294

ggggggggggu uuggggggggg 20

<210> SEQ ID NO 295
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 295

ggggggggguu uuggggggggg 20

<210> SEQ ID NO 296
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 296

ggggggguuu uuuggggggg 20

<210> SEQ ID NO 297
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 297

ggggggguuu uuuuggggggg 20

<210> SEQ ID NO 298
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 298

ggggggguuuu uuuuggggggg 20

<210> SEQ ID NO 299
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 299

ggggggguuuu uuuuuggggg 20

<210> SEQ ID NO 300
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 300

ggggggguuuu uuuuuuggggg 20

-continued

<210> SEQ ID NO 301
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 301

ggggguuuuu uuuuuugggg 20

<210> SEQ ID NO 302
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 302

ggggguuuuu uuuuuuggg 20

<210> SEQ ID NO 303
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 303

gggguuuuuu uuuuuuggg 20

<210> SEQ ID NO 304
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 304

gggguuuuuu uuuuuuugg 20

<210> SEQ ID NO 305
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 305

gguuuuuuuu uuuuuuugg 20

<210> SEQ ID NO 306
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 306

guuuuuuuuu uuuuuuuug 20

<210> SEQ ID NO 307
<211> LENGTH: 22
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 307

ggggggggggg uuuggggggg gg 22

<210> SEQ ID NO 308
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 308

ggggggggggu uuuggggggg gg 22

<210> SEQ ID NO 309
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 309

ggggggggguu uuuuggggggg gg 22

<210> SEQ ID NO 310
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 310

ggggggggguu uuuuuggggg gg 22

<210> SEQ ID NO 311
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 311

ggggggguuu uuuuuggggg gg 22

<210> SEQ ID NO 312
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 312

ggggggguuu uuuuuuggggg gg 22

<210> SEQ ID NO 313
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

-continued

<400> SEQUENCE: 313

ggggggguuu uuuuuuuggg gg 22

<210> SEQ ID NO 314
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 314

ggggggguuu uuuuuuuggg gg 22

<210> SEQ ID NO 315
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 315

ggggggguuu uuuuuuuugg gg 22

<210> SEQ ID NO 316
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 316

gggggguuuuu uuuuuuuugg gg 22

<210> SEQ ID NO 317
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 317

gggggguuuuu uuuuuuuuug gg 22

<210> SEQ ID NO 318
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 318

gggguuuuuuu uuuuuuuuug gg 22

<210> SEQ ID NO 319
<211> LENGTH: 22
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 319

gguuuuuuuu uuuuuuuuuu gg 22

-continued

<210> SEQ ID NO 320
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 320

gggggggggg guuuggggggg gggg 24

<210> SEQ ID NO 321
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 321

gggggggggg uuuuggggggg gggg 24

<210> SEQ ID NO 322
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 322

ggggggggggu uuuuuggggg gggg 24

<210> SEQ ID NO 323
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 323

ggggggggggu uuuuuuggggg gggg 24

<210> SEQ ID NO 324
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 324

ggggggggguu uuuuuuggggg gggg 24

<210> SEQ ID NO 325
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 325

ggggggggguu uuuuuuuggg gggg 24

<210> SEQ ID NO 326
<211> LENGTH: 24
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 326

ggggggggguu uuuuuuuugg gggg 24

<210> SEQ ID NO 327
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 327

ggggggguuu uuuuuuuugg gggg 24

<210> SEQ ID NO 328
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 328

ggggggguuu uuuuuuuuug gggg 24

<210> SEQ ID NO 329
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 329

gggggguuuu uuuuuuuuug gggg 24

<210> SEQ ID NO 330
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 330

gggggguuuu uuuuuuuuuu gggg 24

<210> SEQ ID NO 331
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 331

gggguuuuuu uuuuuuuuuu gggg 24

<210> SEQ ID NO 332
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

-continued

<400> SEQUENCE: 332

ggguuuuuuu uuuuuuuuuu uggg 24

<210> SEQ ID NO 333
<211> LENGTH: 32
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 333

guuuuuuuuu uuuuuuuuuu uuuuuuuuuu ug 32

<210> SEQ ID NO 334
<211> LENGTH: 34
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 334

gguuuuuuuu uuuuuuuuuu uuuuuuuuuu uugg 34

<210> SEQ ID NO 335
<211> LENGTH: 36
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 335

ggguuuuuuu uuuuuuuuuu uuuuuuuuuu uuuggg 36

<210> SEQ ID NO 336
<211> LENGTH: 37
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 336

gggguuuuuu uuuuuuuuuu uuuuuuuuuu uuuuggg 37

<210> SEQ ID NO 337
<211> LENGTH: 39
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 337

gggggguuuu uuuuuuuuuu uuuuuuuuuu uuuuugggg 39

<210> SEQ ID NO 338
<211> LENGTH: 41
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 338

gggggguuuu uuuuuuuuuu uuuuuuuuuu uuuuugggg g 41

-continued

<210> SEQ ID NO 339
<211> LENGTH: 43
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 339

ggggggguuu uuuuuuuuuu uuuuuuuuuu uuuuuuuggg ggg 43

<210> SEQ ID NO 340
<211> LENGTH: 45
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 340

ggggggggguu uuuuuuuuuu uuuuuuuuuu uuuuuuuugg ggggg 45

<210> SEQ ID NO 341
<211> LENGTH: 47
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 341

ggggggggggu uuuuuuuuuu uuuuuuuuuu uuuuuuuuug ggggggg 47

<210> SEQ ID NO 342
<211> LENGTH: 7
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 342

gguuugg 7

<210> SEQ ID NO 343
<211> LENGTH: 8
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 343

gguuuugg 8

<210> SEQ ID NO 344
<211> LENGTH: 9
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 344

gguuuuugg 9

<210> SEQ ID NO 345
<211> LENGTH: 10
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 345

gguuuuuugg 10

<210> SEQ ID NO 346
<211> LENGTH: 11
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 346

gguuuuuuug g 11

<210> SEQ ID NO 347
<211> LENGTH: 12
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 347

gguuuuuuuu gg 12

<210> SEQ ID NO 348
<211> LENGTH: 13
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 348

gguuuuuuuu ugg 13

<210> SEQ ID NO 349
<211> LENGTH: 14
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 349

gguuuuuuuu uugg 14

<210> SEQ ID NO 350
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 350

gguuuuuuuu uuugg 15

<210> SEQ ID NO 351
<211> LENGTH: 16
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

-continued

<400> SEQUENCE: 351

gguuuuuuuu uuuugg 16

<210> SEQ ID NO 352
<211> LENGTH: 17
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 352

gguuuuuuuu uuuuugg 17

<210> SEQ ID NO 353
<211> LENGTH: 18
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 353

gguuuuuuuu uuuuuugg 18

<210> SEQ ID NO 354
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 354

gguuuuuuuu uuuuuuugg 19

<210> SEQ ID NO 355
<211> LENGTH: 9
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 355

ggguuuggg 9

<210> SEQ ID NO 356
<211> LENGTH: 10
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 356

ggguuuuggg 10

<210> SEQ ID NO 357
<211> LENGTH: 11
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 357

ggguuuuugg g 11

-continued

<210> SEQ ID NO 358
<211> LENGTH: 12
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 358

ggguuuuuug gg 12

<210> SEQ ID NO 359
<211> LENGTH: 13
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 359

ggguuuuuuu ggg 13

<210> SEQ ID NO 360
<211> LENGTH: 14
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 360

ggguuuuuuu uggg 14

<210> SEQ ID NO 361
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 361

ggguuuuuuu uuggg 15

<210> SEQ ID NO 362
<211> LENGTH: 16
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 362

ggguuuuuuu uuuggg 16

<210> SEQ ID NO 363
<211> LENGTH: 17
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 363

ggguuuuuuu uuuuggg 17

<210> SEQ ID NO 364
<211> LENGTH: 18
<212> TYPE: RNA

-continued

<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 364

ggguuuuuuu uuuuuggg 18

<210> SEQ ID NO 365
<211> LENGTH: 19
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 365

ggguuuuuuu uuuuuuggg 19

<210> SEQ ID NO 366
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 366

ggguuuuuuu uuuuuuuugg guuuuuuuuu uuuuuugggu uuuuuuuuuu uuuuggg 57

<210> SEQ ID NO 367
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II)

<400> SEQUENCE: 367

ggguuuuuuu uuuuuuuugg gggguuuuuu uuuuuuuuug gg 42

<210> SEQ ID NO 368
<211> LENGTH: 51
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (III)

<400> SEQUENCE: 368

ggguuugggu uuggguuugg guuuggguuu ggguuugggu uuggguuugg g 51

<210> SEQ ID NO 369
<211> LENGTH: 20
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (II) - Short GU rich

<400> SEQUENCE: 369

gguuuuuuuu uuuuuuuggg 20

<210> SEQ ID NO 370
<211> LENGTH: 57
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:

-continued

<223> OTHER INFORMATION: nucleic acid sequence according to formula (III)

<400> SEQUENCE: 370

cccuuuuuuu uuuuuuuucc cuuuuuuuuu uuuuuucccu uuuuuuuuuu uuuuccc 57

<210> SEQ ID NO 371
<211> LENGTH: 51
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (III)

<400> SEQUENCE: 371

cccuuucccu uucccuuucc cuuucccuuu cccuuucccu uucccuuucc c 51

<210> SEQ ID NO 372
<211> LENGTH: 42
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (III)

<400> SEQUENCE: 372

cccuuuuuuu uuuuuuuucc ccccuuuuuu uuuuuuuuuc cc 42

<210> SEQ ID NO 373
<211> LENGTH: 60
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 373

uagcgaagcu cuuggaccua gguuuuuuuu uuuuuuuggg ugcguuccua gaaguacacg 60

<210> SEQ ID NO 374
<211> LENGTH: 120
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 374

uagcgaagcu cuuggaccua gguuuuuuuu uuuuuuuggg ugcguuccua gaaguacacg 60

aucgcuucga gaaccuggau ccaaaaaaaa aaaaaaaccc acgcaaggau cuucaugugc 120

<210> SEQ ID NO 375
<211> LENGTH: 229
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 375

gggagaaagc ucaagcuugg agcaaugccc gcacauugag gaaaccgagu ugcauaucuc 60

agaguauugg cccccgugua gguuauucuu gacagacagu ggagcuuauu cacucccagg 120

auccgagucg cauacuacgg uacuggugac agaccuaggu cgucaguuga ccaguccgcc 180

acuagacgug aguccgucaa agcaguuaga uguuacacuc uauuagauc 229

-continued

<210> SEQ ID NO 376
<211> LENGTH: 547
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 376

gggagaaagc ucaagcuugg agcaaugccc gcacauugag gaaaccgagu ugcauaucuc 60

agaguauugg cccccgugua gguuauucuu gacagacagu ggagcuuauu cacucccagg 120

auccgagucg cauacuacgg uacuggugac agaccuaggu cgucaguuga ccaguccgcc 180

acuagacgug aguccgucaa agcaguuaga uguuacacuc uauuagaucu cggauuacag 240

cuggaaggag caggaguagu guucuugcuc uaaguaccga gugugcccaa uacccgauca 300

gcuuauuaac gaacggcucc uccucuuaga cugcagcgua agugcggaau cuggggauca 360

aauuacugac ugccuggauu acccucggac auauaaccuu guagcacgcu guugcuguau 420

aggugaccaa cgcccacucg aguagaccag cucucuuagu ccggacaaug auaggaggcg 480

cggucaaucu acuucuggcu aguuaagaau aggcugcacc gaccucuaua aguagcgugu 540

ccucuag 547

<210> SEQ ID NO 377
<211> LENGTH: 1083
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 377

gggagaaagc ucaagcuugg agcaaugccc gcacauugag gaaaccgagu ugcauaucuc 60

agaguauugg cccccgugua gguuauucuu gacagacagu ggagcuuauu cacucccagg 120

auccgagucg cauacuacgg uacuggugac agaccuaggu cgucaguuga ccaguccgcc 180

acuagacgug aguccgucaa agcaguuaga uguuacacuc uauuagaucu cggauuacag 240

cuggaaggag caggaguagu guucuugcuc uaaguaccga gugugcccaa uacccgauca 300

gcuuauuaac gaacggcucc uccucuuaga cugcagcgua agugcggaau cuggggauca 360

aauuacugac ugccuggauu acccucggac auauaaccuu guagcacgcu guugcuguau 420

aggugaccaa cgcccacucg aguagaccag cucucuuagu ccggacaaug auaggaggcg 480

cggucaaucu acuucuggcu aguuaagaau aggcugcacc gaccucuaua aguagcgugu 540

ccucuagagc uacgcagguu cgcaauaaaa gcguugauua gugugcauag aacagaccuc 600

uuauucggug aaacgccaga augcuaaauu ccaauaacuc uucccaaaac gcguacggcc 660

gaagacgcgc gcuuaucuug uguacguucu cgcacaugga agaaucagcg ggcauggugg 720

uagggcaaua ggggagcugg guagcagcga aaaagggccc cugcgcacgu agcuucgcug 780

uucgucugaa acaacccggc auccguugua gcgaucccgu uaucaguguu auucuugugc 840

gcacuaagau ucauggugua gucgacaaua acagcgucuu ggcagauucu ggucacgugc 900

ccuaugcccg ggcuugugcc ucucaggugc acagcgauac uuaaagccuu caagguacuc 960

gacgugggua ccgauucgug acacuuccua agauuauucc acuguguuag ccccgcaccg 1020

ccgaccuaaa cugguccaau guauacgcau ucgcugagcg gaucgauaau aaaagcuuga 1080

-continued

auu 1083

<210> SEQ ID NO 378
<211> LENGTH: 229
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 378

gggagaaagc ucaagcuuau ccaaguaggc uggucaccug uacaacguag ccgguauuuu 60

uuuuuuuuuu uuuuuuuuga ccgucucaag guccaaguua gucugccuau aaaggugcgg 120

auccacagcu gaugaaagac uugugcggua cgguuaaucu ccccuuuuuu uuuuuuuuuu 180

uuuuuaguaa augcgucuac ugaauccagc gaugaugcug gcccagauc 229

<210> SEQ ID NO 379
<211> LENGTH: 546
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 379

gggagaaagc ucaagcuuau ccaaguaggc uggucaccug uacaacguag ccgguauuuu 60

uuuuuuuuuu uuuuuuuuga ccgucucaag guccaaguua gucugccuau aaaggugcgg 120

auccacagcu gaugaaagac uugugcggua cgguuaaucu ccccuuuuuu uuuuuuuuuu 180

uuuuuaguaa augcgucuac ugaauccagc gaugaugcug gcccagaucu ucgaccacaa 240

gugcauauag uagucaucga gggucgccuu uuuuuuuuuu uuuuuuuuuu uggcccaguu 300

cugagacuuc gcuagagacu acaguuacag cugcaguagu aaccacugcg gcuauugcag 360

gaaaucccgu ucagguuuuu uuuuuuuuuu uuuuuuccgc ucacuaugau uaagaaccag 420

guggaguguc acugcucucg aggucucacg agagcgcucg auacaguccu uggaagaauc 480

uuuuuuuuuu uuuuuuuuuu uugugcgacg aucacagaga acuucuauuc augcaggucu 540

gcucua 546

<210> SEQ ID NO 380
<211> LENGTH: 1083
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (IV)

<400> SEQUENCE: 380

gggagaaagc ucaagcuuau ccaaguaggc uggucaccug uacaacguag ccgguauuuu 60

uuuuuuuuuu uuuuuuuuga ccgucucaag guccaaguua gucugccuau aaaggugcgg 120

auccacagcu gaugaaagac uugugcggua cgguuaaucu ccccuuuuuu uuuuuuuuuu 180

uuuuuaguaa augcgucuac ugaauccagc gaugaugcug gcccagaucu ucgaccacaa 240

gugcauauag uagucaucga gggucgccuu uuuuuuuuuu uuuuuuuuuu uggcccaguu 300

cugagacuuc gcuagagacu acaguuacag cugcaguagu aaccacugcg gcuauugcag 360

gaaaucccgu ucagguuuuu uuuuuuuuuu uuuuuuccgc ucacuaugau uaagaaccag 420

guggaguguc acugcucucg aggucucacg agagcgcucg auacaguccu uggaagaauc 480

-continued

uuuuuuuuuu uuuuuuuuuu uugugcgacg aucacagaga acuucuauuc augcaggucu 540

gcucuagaac gaacugaccu gacgccugaa cuuaugagcg ugcguauuuu uuuuuuuuuu 600

uuuuuuuuuc cucccaacaa augucgauca auagcugggc uguuggagac gcgucagcaa 660

augccguggc uccauaggac guguagacuu cuauuuuuuu uuuuuuuuuu uuuucccggg 720

accacaaaua auauucuugc uugguugggc gcaagggccc cguaucaggu cauaaacggg 780

uacauguugc acaggcuccu uuuuuuuuuu uuuuuuuuuu uucgcugagu uauuccgguc 840

ucaaaagacg gcagacguca gucgacaaca cggucuaaag cagugcuaca aucugccgug 900

uucguguuuu uuuuuuuuuu uuuuuuguga accuacacgg cgugcacugu aguucgcaau 960

ucauagggua ccggcucaga guuaugccuu gguugaaaac ugcccagcau acuuuuuuuu 1020

uuuuuuuuuu uucauauucc caugcuaagc aagggaugcc gcgagucaug uuaagcuuga 1080

auu 1083

<210> SEQ ID NO 381
<211> LENGTH: 59
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (V)

<400> SEQUENCE: 381

uagcgaagcu cuuggaccua ccuuuuuuuu uuuuuucccu gcguuccuag aaguacacg 59

<210> SEQ ID NO 382
<211> LENGTH: 120
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleic acid sequence according to formula (V)

<400> SEQUENCE: 382

uagcgaagcu cuuggaccua ccuuuuuuuu uuuuuuuccc ugcguuccua gaaguacacg 60

aucgcuucga gaaccuggau ggaaaaaaaa aaaaaaaggg acgcaaggau cuucaugugc 120

<210> SEQ ID NO 383
<211> LENGTH: 15
<212> TYPE: RNA
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Description of sequence: generic stabilizing sequence of the formula (C/U)CCANxCCC(U/A)PyxUC(C/U)CC
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: /replace="cytosine" /replace="uracile"
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1)
<223> OTHER INFORMATION: nucleic acid = cytosine or uracil
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: Nx = a, g, c or u or any other nucleic acid
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: /replace="cytosine" /replace="uracile" /replace="guanosine" /replace="adenosine", or any other nucleic acid
<220> FEATURE:

-continued

<221> NAME/KEY: repeat_unit
<222> LOCATION: (5)..(5)
<223> OTHER INFORMATION: x = any number
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: nucleic acid = uracil or adenosine
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (9)..(9)
<223> OTHER INFORMATION: /replace="uracile" /replace="adonosine"
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: Py = pyrimidine
<220> FEATURE:
<221> NAME/KEY: repeat_unit
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: x = any number
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (10)..(10)
<223> OTHER INFORMATION: /replace="pyrimidine"
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: nucleic acid = cytosine or uracil
<220> FEATURE:
<221> NAME/KEY: variation
<222> LOCATION: (13)..(13)
<223> OTHER INFORMATION: /replace="cytosine" /replace="uracile"

<400> SEQUENCE: 383

nccancccnn ucncc 15

<210> SEQ ID NO 384
<211> LENGTH: 2083
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R2564 - exemplary second nucleic acid molecule

<400> SEQUENCE: 384

ggggcgcugc cuacggaggu ggcagccauc uccuucucgg caucaagcuu accaugaagg 60

ccauccuggu gguccuccug uacaccuucg ccaccgcgaa cgccgacacg cugugcaucg 120

gcuaccacgc caacaacagc accgacaccg uggacaccgu gcucgagaag aacgucacgg 180

ugacccacuc cgugaaccug cuggaggaca agcacaacgg gaagcucugc aagcugcggg 240

gcgucgcccc gcugcaccuc gggaagugca acaucgccgg cuggauccug gggaacccgg 300

agugcgagag ccuguccacc gcgagcuccu ggagcuacau cguggagacc uccagcuccg 360

acaacggcac gugcuacccc ggcgacuuca ucgacuacga ggagcuccgc gagcagcuga 420

gcuccgugag cuccuucgag cgguucgaga ucuuccccaa gaccagcucc uggcccaacc 480

acgacagcaa caaggggguc accgccgccu gcccgcacgc cggcgcgaag uccuucuaca 540

agaaccugau cuggcucgug aagaagggga acagcuaccc caagcugucc aagagcuaca 600

ucaacgacaa gggcaaggag gugcuggucc ucugggggau ccaccacccc agcaccuccg 660

ccgaccagca gagccuguac cagaacgccg acgccuacgu guucgugggc uccagccgcu 720

acuccaagaa guucaagccc gagaucgcca uccggccgaa gguccgcgac caggagggcc 780

ggaugaacua cuacuggacg cugguggagc ccggggacaa gaucaccuuc gaggcgaccg 840

gcaaccucgu gguccccgc uacgccuucg ccauggagcg gaacgccggg agcggcauca 900

-continued

ucaucuccga caccccgug cacgacugca acacgaccug ccagaccccg aagggcgcca 960

ucaacaccag ccugcccuuc cagaacaucc accccaucac gaucgggaag ugccccaagu 1020

acgugaaguc caccaagcug cgccucgcga ccggccugcg gaacgucccg agcauccagu 1080

cccgcgggcu guucggcgcc aucgccgggu ucaucgaggg cggcuggacc gggauggugg 1140

acggcuggua cggguaccac caccagaacg agcagggcag cggguacgcc gccgaccuca 1200

aguccacgca gaacgcgauc gacgagauca ccaacaaggu gaacagcguc aucgagaaga 1260

ugaacaccca guucaccgcc gugggcaagg aguucaacca ccuggagaag cggaucgaga 1320

accugaacaa gaaggucgac gacggcuucc ucgacaucug gacguacaac gccgagcugc 1380

uggugcuccu ggagaacgag cgcacccugg acuaccacga cuccaacgug aagaaccucu 1440

acgagaaggu ccggagccag cugaagaaca acgccaagga gaucgggaac ggcugcuucg 1500

aguucuacca caagugcgac aacaccugca uggaguccgu gaagaacggg accuacgacu 1560

accccaagua cagcgaggag gccaagcuga accgcgagga gaucgacggc gugaagcucg 1620

aguccacgcg gaucuaccag auccuggcga ucuacagcac cgucgccagc ucccuggugc 1680

ucguggucag ccugggggcc aucuccuucu ggaugugcag caacggcucc cugcagugcc 1740

gcaucugcau cugaccacua gugcaucaca uuuaaaagca ucucagccua ccaugagaau 1800

aagagaaaga aaaugaagau caauagcuua uucaucucuu uuucuuuuuc guugguguaa 1860

agccaacacc cugucuaaaa aacauaaauu ucuuuaauca uuuugccucu uuucucugug 1920

cuucaauuaa uaaaaaaugg aaagaaccua gaucuaaaaa aaaaaaaaaa aaaaaaaaaa 1980

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaau gcaucccccc ccccccccc 2040

ccccccccc ccccaaagg cucuuuucag agccaccaga auu 2083

<210> SEQ ID NO 385
<211> LENGTH: 547
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R2025 - exemplary nucleic acid cargo

<400> SEQUENCE: 385

gggagaaagc ucaagcuuau ccaaguaggc uggucaccug uacaacguag ccgguauuuu 60

uuuuuuuuuu uuuuuuuuga ccgucucaag guccaaguua gucugccuau aaaggugcgg 120

auccacagcu gaugaaagac uugugcggua cgguuaaucu ccccuuuuuu uuuuuuuuuu 180

uuuuuaguaa augcgucuac ugaauccagc gaugaugcug gcccagaucu ucgaccacaa 240

gugcauauag uagucaucga gggucgccuu uuuuuuuuuu uuuuuuuuuu uggcccaguu 300

cugagacuuc gcuagagacu acaguuacag cugcaguagu aaccacugcg gcuauugcag 360

gaaaucccgu ucagguuuuu uuuuuuuuuu uuuuuuccgc ucacuaugau uaagaaccag 420

guggaguguc acugcucucg aggucucacg agagcgcucg auacaguccu uggaagaauc 480

uuuuuuuuuu uuuuuuuuuu uugugcgacg aucacagaga acuucuauuc augcaggucu 540

gcucuag 547

<210> SEQ ID NO 386
<211> LENGTH: 42
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

-continued

<223> OTHER INFORMATION: sequence corresponding to preferred 5'-UTR sequence

<400> SEQUENCE: 386

ggcgctgcct acggaggtgg cagccatctc cttctcggca tc 42

<210> SEQ ID NO 387
<211> LENGTH: 186
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sequence corresponding to 3'-UTR element derived from human albumin gene

<400> SEQUENCE: 387

catcacattt aaaagcatct cagcctacca tgagaataag agaaagaaaa tgaagatcaa 60

tagcttattc atctcttttt ctttttcgtt ggtgtaaagc caacaccctg tctaaaaaac 120

ataaatttct ttaatcattt tgcctctttt ctctgtgctt caattaataa aaaatggaaa 180

gaacct 186

<210> SEQ ID NO 388
<211> LENGTH: 44
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sequence corresponding to 3'-UTR element derived from an alpha globin gene

<400> SEQUENCE: 388

gcccgatggg cctcccaacg ggccctcctc ccctccttgc accg 44

<210> SEQ ID NO 389
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: sequence corresponding to preferred histon stem-loop sequence

<400> SEQUENCE: 389

caaaggctct tttcagagcc acca 24

<210> SEQ ID NO 390
<211> LENGTH: 24
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: preferred histone stem-loop sequence

<400> SEQUENCE: 390

caaaggcucu uuucagagcc acca 24

<210> SEQ ID NO 391
<211> LENGTH: 1957
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R2506 RAV-G encoding mRNA

<400> SEQUENCE: 391

ggggcgcugc cuacggaggu ggcagccauc uccuucucgg caucaagcuu accauggugc 60

-continued

cccaggcccu gcucuucguc ccgcugcugg uguucccccu cugcuucggc aaguucccca 120

ucuacaccau ccccgacaag cuggggccgu ggagccccau cgacauccac caccuguccu 180

gccccaacaa ccucgugguc gaggacgagg gcugcaccaa ccugagcggg uucuccuaca 240

uggagcugaa ggugggcuac aucagcgcca ucaagaugaa cggguucacg ugcaccggcg 300

uggucaccga ggcggagacc uacacgaacu ucgugggcua cgugaccacc accuucaagc 360

ggaagcacuu ccgccccacg ccggacgccu gccgggccgc cuacaacugg aagauggccg 420

gggacccccg cuacgaggag ucccuccaca accccuaccc cgacuaccac uggcugcgga 480

ccgucaagac caccaaggag agccugguga ucaucucccc gagcguggcg gaccucgacc 540

ccuacgaccg cucccugcac agccgggucu uccccggcgg gaacugcucc ggcguggccg 600

ugagcuccac guacugcagc accaaccacg acuacaccau cuggaugccc gagaacccgc 660

gccuggggau guccugcgac aucuucacca acagccgggg caagcgcgcc uccaagggca 720

gcgagacgug cgggguucguc gacgagcggg gccucuacaa gucccugaag ggggccugca 780

agcugaagcu cugcggcgug cugggccugc gccucaugga cgggaccugg guggcgaugc 840

agaccagcaa cgagaccaag uggugccccc ccggccagcu ggucaaccug cacgacuucc 900

ggagcgacga gaucgagcac cucguggugg aggagcuggu caagaagcgc gaggagugcc 960

uggacgcccu cgaguccauc augacgacca agagcguguc cuuccggcgc cugagccacc 1020

ugcggaagcu cgugcccggg uucggcaagg ccuacaccau cuucaacaag acccugaugg 1080

aggccgacgc ccacuacaag uccguccgca cguggaacga gaucaucccg agcaaggggu 1140

gccugcgggu gggcggccgc ugccacccce acgucaacgg gguguucuuc aacggcauca 1200

uccucgggcc cgacggcaac gugcugaucc ccgagaugca guccagccug cuccagcagc 1260

acauggagcu gcuggucucc agcgugaucc cgcucaugca cccccuggcg gaccccucca 1320

ccguguucaa gaacggggac gaggccgagg acuucgucga ggugcaccug cccgacgugc 1380

acgagcggau cagcggcguc gaccucggcc ugccgaacug ggggaaguac gugcugcucu 1440

ccgccggcgc ccugaccgcc cugaugcuga ucaucuuccu caugaccugc uggcgccggg 1500

ugaaccggag cgagcccacg cagcacaacc ugcgcgggac cggccgggag gucuccguga 1560

ccccgcagag cgggaagauc aucuccagcu gggaguccua caagagcggc ggcgagaccg 1620

ggcugugagg acuagugcau cacauuuaaa agcaucucag ccuaccauga gaauaagaga 1680

aagaaaauga agaucaauag cuuauucauc ucuuuuucuu uuucguuggu guaaagccaa 1740

cacccugucu aaaaaacaua aauuucuuua aucauuuugc cucuuuucuc ugugcuucaa 1800

uuaauaaaaa auggaaagaa ccuagaucua aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1860

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaugcaucc cccccccccc cccccccccc 1920

ccccccccca aaggcucuuu ucagagccac cagaauu 1957

<210> SEQ ID NO 392
<211> LENGTH: 2044
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: R2682 RSV-F encoding mRNA (HRSV(Long-VR26)
Fdel554-574)

<400> SEQUENCE: 392

ggggcgcugc cuacggaggu ggcagccauc uccuucucgg caucaagcuu accauggagc 60

-continued

ugcccauccu caaggccaac gccaucacca ccauccuggc ggccgugacg uucugcuucg 120

ccagcuccca gaacaucacc gaggaguucu accagagcac cugcuccgcc gucagcaagg 180

gcuaccuguc cgcccuccgg accggguggu acacgagcgu gaucaccauc gagcugucca 240

acaucaagga gaacaagugc aacggcaccg acgcgaaggu gaagcugauc aaccaggagc 300

ucgacaagua caagaacgcc gucaccgagc ugcagcugcu caugcagagc acgaccgccg 360

ccaacaaccg cgcgcggcgc gagcugccgc gguucaugaa cuacacccug aacaacacca 420

agaagacgaa cgugacccuc uccaagaagc gcaagcggcg cuuccugggg uuccugcucg 480

gcguggggag cgccaucgcc uccggcaucg ccgucagcaa ggugcugcac cuggagggcg 540

aggugaacaa gaucaagucc gcccuccuga gcaccaacaa ggcggucgug ucccugagca 600

acggguguc cguccucacc agcaaggugc uggaccugaa gaacuacauc gacaagcagc 660

uccugcccau cgugaacaag caguccugcc ggaucagcaa caucgagacg gucaucgagu 720

uccagcagaa gaacaaccgc cugcucgaga ucacccggga guucagcgug aacgccggcg 780

ugaccacccc cgucuccacg uacaugcuga ccaacagcga gcugcucucc cugaucaacg 840

acaugcccau caccaacgac cagaagaagc ugaugagcaa caacgugcag aucgugcgcc 900

agcaguccua cagcaucaug uccaucauca aggaggaggu ccucgccuac guggugcagc 960

ugccgcugua cggggucauc gacacccccu gcuggaagcu ccacacgagc ccccugugca 1020

ccaccaacac caaggagggc uccaacaucu gccugacgcg gaccgaccgc gggugguacu 1080

gcgacaacgc cggcagcgug uccuucuucc cccaggccga gaccugcaag guccagagca 1140

accggguguu cugcgacacc augaacuccc ucacgcugcc gagcgaggug aaccugugca 1200

acgucgacau cuucaacccc aaguacgacu gcaagaucau gaccuccaag accgacguga 1260

gcuccagcgu gaucaccucc cucggcgcga ucgucagcug cuacgggaag acgaagugca 1320

ccgccagcaa caagaaccgc ggcaucauca agaccuucuc caacgggugc gacuacguga 1380

gcaacaaggg cguggacacc gucuccgugg gcaacacccu guacuacgug aacaagcagg 1440

aggggaagag ccuguacguc aagggcgagc ccaucaucaa cuucuacgac ccccucgugu 1500

ucccguccga cgaguucgac gccagcaucu cccaggugaa cgagaagauc aaccagagcc 1560

uggccuucau ccggaagucc gacgagcugc ugcaccacgu caacgccggg aagagcacga 1620

ccaacaucau gaucaccacc aucaucaucg ugaucaucgu gauccuccug ucccugaucg 1680

cggucggccu ccugcuguac ugcaaggccc gcugaggacu agugcaucac auuuaaaagc 1740

aucucagccu accaugagaa uaagagaaag aaaaugaaga ucaauagcuu auucaucucu 1800

uuuucuuuuu cguuggugua aagccaacac ccugucuaaa aaacauaaau uucuuuaauc 1860

auuuugccuc uuuucucugu gcuucaauua auaaaaaaug gaaagaaccu agaucuaaaa 1920

aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 1980

ugcaucccccc ccccccccccc ccccccccccc ccccccaaag gcucuuuuca gagccaccag 2040

aauu 2044

1. A polymeric carrier cargo complex, comprising:
a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and
b) as a cargo at least one first nucleic acid molecule,
for use as an immunostimulating agent or as an adjuvant,
wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, and
wherein the polymeric carrier cargo complex and the second nucleic acid molecule are administered intramuscularly.

2. A polymeric carrier cargo complex, comprising:
a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and
b) as a cargo at least one first nucleic acid molecule,
for use as an immunostimulating agent or as an adjuvant,
wherein the polymeric carrier cargo complex is administered in combination with at least one second nucleic acid molecule encoding a protein or a peptide, wherein the second nucleic acid molecule is an RNA molecule.

3. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim 1, wherein the second nucleic acid molecule is an RNA molecule.

4. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 3, wherein the second nucleic acid molecule is either naked or complexed with a cationic component.

5. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 4, wherein the second nucleic acid molecule is not packaged in a particle, such as a virus particle, an inactivated virus particle or a virus-like particle.

6. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 4, wherein the second nucleic acid molecule is not comprised in the polymeric carrier cargo complex.

7. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 6, wherein the polymeric carrier cargo complex and the second nucleic acid molecule are not administered together with a protein or peptide antigen selected from the group consisting of an antigen from a pathogen associated with infectious disease, an antigen associated with allergy or allergic disease, an antigen associated with autoimmune disease, an antigen associated with a cancer or tumour disease, or a fragment, variant and/or derivative of said protein or peptide antigen.

8. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 6, wherein the polymeric carrier cargo complex and the second nucleic acid molecule are not administered together with a protein or peptide antigen.

9. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 8, wherein the second nucleic acid molecule is an mRNA molecule.

10. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim 9, wherein the mRNA molecule comprises at least one selected from the group consisting of a 5'-UTR, a 3'-UTR, a poly(A) sequence, a poly(C) sequence and a histone stem-loop sequence.

11. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim 10, wherein the 3'-UTR comprises a nucleic acid sequence derived from the 3'-UTR of an albumin gene.

12. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim 10 or 11, wherein the 3'-UTR comprises the nucleic acid sequence corresponding to SEQ ID NO. 388.

13. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 10 to 12, wherein the histone stem-loop sequence comprises a nucleic acid sequence corresponding to SEQ ID NO. 389.

14. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 10 to 13, wherein the 5'-UTR comprises a nucleic acid sequence derived from a 5'-UTR of a TOP gene.

15. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 10 to 14, wherein the 5'-UTR comprises a nucleic acid sequence derived from a ribosomal protein gene.

16. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 10 to 15, wherein the 5'-UTR comprises a nucleic acid sequence derived from ribosomal protein 32L gene.

17. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 9 to 16, wherein the mRNA molecule comprises a nucleic acid sequence derived from a 5'-TOP-UTR, a GC-optimized coding sequence, a nucleic acid sequence derived from the 3'-UTR of an albumin gene, a poly(A)-sequence, a poly(C)-sequence, and a histone stem loop.

18. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 17, wherein the at least one first nucleic acid molecule is an RNA molecule.

19. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 18, wherein the at least one first nucleic acid molecule is an immunostimulatory nucleic acid, preferably a non-coding immunostimulatory nucleic acid.

20. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 19, wherein the nitrogen/phosphate (N/P) ratio of the cationic components to the at least one first nucleic acid molecule is in the range of 0.1-20, or in the range of 0.1-5, or in the range of 0.1-1.

21. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims 1 to 20, wherein the polymeric carrier comprises functional peptides or proteins additionally to the cationic components.

22. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim 21, wherein the functional peptides or proteins are peptide or protein antigens or antigen epitopes.

23. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims **1** to **22**, wherein the polymeric carrier additionally comprises a ligand.

24. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim **23**, wherein the ligand is mannose.

25. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims **1** to **24**, wherein the cationic components are cationic peptides, preferably selected from oligocationic and polycationic peptides.

26. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim **25**, wherein the cationic peptides are selected from peptides according to formula (I)

$$(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x,$$

wherein

l+m+n+o+x=3-100, and

l, m, n or o=independently of each other is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100, provided that the overall content of Arg, Lys, His and Orn represents at least 10% of all amino acids of the cationic peptide; and Xaa is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and

x=any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, provided, that the overall content of Xaa does not exceed 90% of all amino acids of the cationic peptide,

or are selected from peptides according to subformula (Ia)

$$\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa')_x(Cys)_y\}$$

or from peptides according to subformula (Ib)

$$Cys_1\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}Cys_2$$

wherein $(Arg)_l$; $(Lys)_m$; $(His)_n$; $(Orn)_o$; and x are as defined above; Xaa' is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His, Orn; or Cys and y is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, and 81-90, provided that the overall content of Arg (Arginine), Lys (Lysine), His (Histidine) and Orn (Ornithine) represents at least 10% of all amino acids of the oligopeptide and wherein Cys_1 and Cys_2 are Cysteines proximal to, or terminal to $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$.

27. The polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim **25** or **26**, wherein the disulfide-bonds are formed by cysteine residues contained in the cationic peptides.

28. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to claim **27**, wherein the cysteine residue is located proximal to, preferably at the terminal ends of the cationic peptides.

29. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims **1** to **28**, wherein the cationic component comprises the peptide $CysArg_{12}Cys$.

30. A polymeric carrier cargo complex for use as an immunostimulating agent or an adjuvant according to any one of claims **1** to **29** comprising:

a. as a carrier a polymeric carrier formed by disulfide-crosslinked cationic polymers, and

b. as a cargo at least one nucleic acid (molecule),

in the treatment or prophylaxis of a disease selected from a tumour or a cancer disease, an infectious disease, an autoimmune disease or an allergy.

31. A pharmaceutical composition comprising:

(A) a polymeric carrier cargo complex, comprising:

a) as a carrier a polymeric carrier formed by disulfide-crosslinked cationic components, and

b) as a cargo at least one first nucleic acid molecule, and

(B) at least one second nucleic acid molecule, wherein the at least one second nucleic acid molecule is an RNA molecule encoding a protein or a peptide.

32. The pharmaceutical composition according to claim **31**, wherein the at least one second nucleic acid molecule encodes a protein or peptide antigen that is selected from the group consisting of an antigen from a pathogen associated with infectious disease; an antigen associated with allergy; an antigen associated with autoimmune disease; and an antigen associated with cancer or tumour disease;

or a fragment, variant and/or derivative of said antigen.

33. The pharmaceutical composition according to claim **31** or **32**, wherein component (B) is not covalently linked to (A).

34. The pharmaceutical composition according to any one of claims **31** to **33**, which does not comprise a protein or peptide antigen selected from the group consisting of an antigen from a pathogen associated with infectious disease, an antigen associated with allergy or allergic disease, an antigen associated with autoimmune disease, an antigen associated with a cancer or tumour disease, or a fragment, variant and/or derivative of said protein or peptide antigen.

35. The pharmaceutical composition according to any one of claims **31** to **33**, which does not comprise a protein or peptide antigen.

36. The pharmaceutical composition according to any one of claims **31** to **35**, wherein the second nucleic acid molecule is either naked or complexed with a cationic component.

37. The pharmaceutical composition according to any one of claims **31** to **36**, wherein the second nucleic acid molecule is not packaged in a particle, such as a virus particle, an inactivated virus particle or a virus-like particle.

38. The pharmaceutical composition according to any one of claims **31** to **37**, wherein the antigen is derived from a pathogen, preferably a viral, bacterial, fungal or protozoan pathogen, preferably selected from the list consisting of: Rabies virus, Hepatitis B virus, human Papilloma virus (hPV), *Bacillus anthracis*, Respiratory syncytial virus (RSV), Herpes simplex virus (HSV), Dengue virus, Rotavirus, Influenza virus and *Mycobacterium tuberculosis*.

39. The pharmaceutical composition according to any one of claims **31** to **37**, wherein the antigen is associated with allergy or allergic disease and is derived from a source selected from the list consisting of: grass pollen, tree pollen, flower pollen, herb pollen, dust mite, mold, animals, food, and insect venom.

40. The pharmaceutical composition according to any one of claims **31** to **37**, wherein the antigen is associated with a cancer or tumour disease and is selected from the list

consisting of: p53, CA125, EGFR, Her2/neu, hTERT, PAP, MAGE-A1, MAGE-A3, MAGE-C1, MAGE-C2, Mesothelin, MUC-1, NY-ESO-1, GP100, MART-1, Tyrosinase, PSA, PSCA, PSMA, VEGF, VEGFR1, VEGFR2, Ras, CEA, Survivin, 5T4, STEAP and WT1.

41. The pharmaceutical composition according to any one of claims **31** to **40**, wherein the polymeric carrier cargo complex is for use as an immunostimulating agent or as an adjuvant.

42. The pharmaceutical composition according to any one of claims **31** to **41**, wherein the at least one first nucleic acid molecule is an RNA.

43. The pharmaceutical composition according to any one of claims **31** to **42**, wherein the at least one first nucleic acid molecule is an immunostimulatory nucleic acid.

44. The pharmaceutical composition according to any one of claims **31** to **43**, wherein the nitrogen/phosphate (N/P) ratio of the cationic components to the at least one first nucleic acid molecule is in the range of 0.1-20, or in the range of 0.1-5, or in the range of 0.1-1.

45. The pharmaceutical composition according to any one of claims **31** to **44**, wherein the polymeric carrier additionally comprises a ligand.

46. The pharmaceutical composition according to claim **45**, wherein the ligand is mannose.

47. The pharmaceutical composition according to any one of claims **31** to **46**, wherein the cationic components are cationic peptides.

48. The pharmaceutical composition according to any one of claims **31** to **47**, wherein the cationic peptides are selected from peptides according to formula (I)

$$(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x,$$

wherein

l+m+n+o+x=3-100, and

l, m, n or o=independently of each other is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90 and 91-100, provided that the overall content of Arg, Lys, His and Orn represents at least 10% of all amino acids of the cationic peptide; and Xaa is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His or Orn; and

x=any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, 81-90, provided, that the overall content of Xaa does not exceed 90% of all amino acids of the cationic peptide,

or are selected from peptides according to subformula (Ia)

$$\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa')_x(Cys)_y\}$$

or from peptides according to subformula (Ib)

$$Cys_1\{(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x\}Cys_2$$

wherein $(Arg)_l$; $(Lys)_m$; $(His)_n$; $(Orn)_o$; and x are as defined above; Xaa' is any amino acid selected from native (=naturally occurring) or non-native amino acids except of Arg, Lys, His, Orn; or Cys and y is any number selected from 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21-30, 31-40, 41-50, 51-60, 61-70, 71-80, and 81-90, provided that the overall content of Arg (Arginine), Lys (Lysine), His (Histidine) and Orn (Ornithine) represents at least 10% of all amino acids of the oligopeptide and wherein Cys_1 and Cys_2 are Cysteines proximal to, or terminal to $(Arg)_l;(Lys)_m;(His)_n;(Orn)_o;(Xaa)_x$.

49. The pharmaceutical composition according to claim **47** or **48**, wherein the disulfide-bonds are formed by cysteine residues contained in the cationic peptides.

50. The pharmaceutical composition according to claim **49**, wherein the cysteine residue is located proximal to, preferably at the terminal ends of the cationic peptides.

51. The pharmaceutical composition according to claim **49** or **50**, wherein the cationic component comprises the peptide $CysArg_{12}Cys$.

52. A vaccine, comprising a pharmaceutical composition according to any one of claims **31** to **51**.

53. The vaccine according to claim **52**, wherein the pharmaceutical composition according to any of claims **31** to **51** elicits an adaptive immune response.

54. The vaccine according to claim **52** or **53**, wherein the polymeric carrier cargo complex is used as an immunostimulating agent or adjuvant.

55. The vaccine according to any one of claims **52** to **54**, wherein the vaccine further comprises a pharmaceutically acceptable carrier.

56. A kit, preferably a kit of parts, comprising the pharmaceutical composition according to any one of claims **31** to **51**, and/or the vaccine according to any one of claims **52** to **55**, and optionally a liquid vehicle for solubilising and optionally technical instructions with information on the administration and dosage of the active composition and/or the vaccine.

57. The kit according to claim **56**, wherein the at least one second nucleic acid molecule, which encodes a protein or peptide, is provided in lyophilized form as a separate part.

58. The kit according to claim **56** or **57**, wherein the kit contains as a part Ringer-Lactate solution.

59. The pharmaceutical composition according to any one of claims **31** to **51** or the kit according to any one of claims **56** to **58**, for use in the treatment or prophylaxis of an infectious disease; an allergy; an autoimmune disease; or a cancer or tumour disease.

* * * * *