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(54) **Title**: ADENOVIRAL COAT PROTEIN DERIVED DELIVERY VEHICLES

(57) **Abstract**: The present invention relates to new adenoviral coat protein based delivery vehicles. They are based on a modified penton base protomers that assemble into VLPs. Exposed areas of the penton base proteins can be modified to allow the VLP to specifically bind to any target and/or to comprise any desired peptide epitope. Additional cargo, e.g. drugs, proteins, or nucleic acids, can reversibly or irreversibly attached to the VLP via engineered fibre protein fragments. The present invention relates to such engineered penton base protomers, engineered proteins comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer, VLPs comprising the engineered penton base protomers and optionally engineered proteins comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer, nucleic encoding the engineered proteins, the VLPs as well as methods of producing the proteins and VLPs.

ADENOVIRAL COAT PROTEIN DERIVED DELIVERY VEHICLES

TECHNICAL FIELD OF THE INVENTION

The present invention relates to new adenoviral coat protein based delivery vehicles. They are based on a modified penton base protomers that assemble into VLPs. Exposed areas of the penton base proteins can be modified to allow the VLP to specifically bind to any target and/or to comprise any desired peptide epitope. Additional cargo, e.g. drugs, polypeptides, proteins, or nucleic acids, can be reversibly or irreversibly attached to the VLP via engineered fibre protein fragments. The present invention relates to such engineered penton base protomers, engineered proteins comprising a fibre protein fragment capable of binding to the penton base protomer, VLPs comprising the engineered penton base protomers and optionally engineered proteins comprising a fibre protein fragment, nucleic encoding the engineered proteins, the VLPs as well as methods of producing the proteins and VLPs.

BACKGROUND OF THE INVENTION

Infectious diseases continue to plague and decimate populations world-wide. Among the means at our disposal to counter this threat, vaccination has proven to be exceptionally powerful. Small-pox has been eradicated, measles, polio and tetanus constrained from the world by vaccination. Nonetheless, severe threats continue to challenge human health, notably also from emergent viruses that have adapted and emerged as new diseases or pathogenic strains with attributes facilitating pathogenicity.

Recent such examples are the severe threat posed by Chikungunya and Zika, insect-born viruses that is transmitted to humans by the bite of a mosquito. Both viruses are rapidly spreading to Asia and Europe, by means of its mosquito host, causing considerable alarm. Chikungunya and Zika disease can potentially incur severe costs for affected communities and economies, and a potent vaccination strategy to counter this emerging threat would be highly desirable. However, powerful vaccines are utterly lacking to date.

Ideally, a vaccine will be safe, non-replicative, efficient, and tunable. Moreover, it will be produced easily at industrial scale. Recombinant Virus Like Particles (VLPs) can be such ideal vaccines and therefore hold enormous promise. In this proposal, we will create such a VLP vaccine. We will make use of an amazingly versatile bio-similar multiprotein platform called ADDomer (Adenovirus dodecahedron derived multimer). ADDomer will serve to create

vaccine candidates to combat infectious diseases caused by viruses (including but not limited to Chikungunya, Zika, others).

ADDomer is a synthetic scaffold derived from a virus-like particle (VLP) that in nature occurs during the human adenovirus serotype 3 (HAd3) replication cycle catalyzing internalization (Fender, P., *et al.* (2012) *J Virol 86*, 5380-5385. ADDomer is a designed bio-similar derived from this natural VLP, retaining the aptitude to autonomously self-assemble into a dodecahedron. ADDomer is uniquely suited to display multiple peptide and protein epitopes by means of fully flexible, solvent exposed loops. Engineering these loops does not destroy the global architecture of ADDomer particles. These loops offer convenient options to insert, by using methods from synthetic biology, multiple copies of highly immunogenic peptide epitopes, for example from viral pathogens. ADDomer is not limited to vaccine development against infectious diseases. A wide range of applications will potentially benefit from the ADDomer technology, including also cancer therapy. Moreover, ADDomer can not only display peptide epitopes. Proteins or protein domains can be likewise exposed by ADDomer, significantly broadening the scope of its application.

SUMMARY OF THE INVENTION

The present inventors have identified that certain regions in the penton base protomer are amenable to the introduction of heterologous peptide sequence without disrupting assembly of penton base protomers into penton subunits, which in turn can self-assemble into penton dodecamers forming virus like particles (VLPs) also referred to as ADDomers. The design is highly modular and enables rapid and flexible functionalization of extended loops for multipolypeptide display. The modularity is even further enhanced by using an adenovirus fibre protein fragment that specifically binds to the penton base protomer. The VLPs of the present invention are safe since they do not comprise genetic material. The penton base protomers can receive and display up to 180 foreign polypeptide motifs including antigens, neutralizing polypeptides, oncoepitope polypeptides, single chain antibodies, and nanobodies.

Accordingly, in a first aspect the present invention relates to an engineered polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), adenovirus fibre protein binding cleft and/or a N-terminal domain and comprises one or more of the following:

(i) at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop; and/or

(ii) one or more non-adenoviral polypeptides in the first, the second or both the first and the second RGD-loops and/or in the V loop; and/or

(iii) a non-adenoviral polypeptide at the N- and/or C-terminus of the penton base protomer; and/or

(iv) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer, wherein the N-terminus of the N-terminal domain within the penton base protomer is defined as follows:

X_1-G-R-N-S-I-R (SEQ ID NO: 44)

and the C-terminus of the N-terminal domain within the penton base protomer is defined as follows:

D-X_2-R-S-R-G (SEQ ID NO: 45),

wherein

X_1 is selected from the group consisting of G and E, and

X_2 is selected from the group consisting of D and E; and/or

(v) a drug or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or

(vi) at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer

and wherein the engineered polypeptide of the first aspect is preferably capable of assembling into VLPs.

In a second aspect the present invention relates to an engineered polypeptide comprising at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and:

(i) a non-adenoviral polypeptide; and/or

(ii) is covalently or non-covalently coupled to a drug or label.

In a third aspect the present invention relates to a nucleic acid encoding the engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or the engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment.

In a fourth aspect the present invention relates to an expression vector comprising the nucleic acid of the invention.

In a fifth aspect the present invention relates to a cloning vector encoding:

(i) a polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop and/or a binding site for adenovirus fibre protein adapted for introducing nucleic acids encoding non-adenoviral polypeptides into the nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop; or

(ii) polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment adapted for introducing nucleic acids encoding non-adenoviral polypeptides a the C- and/or N-terminus.

In a sixth aspect the present invention relates to a recombinant host cell comprising the expression vector of the invention or the cloning vector of the invention.

In a seventh aspect the present invention relates to a pentamer comprising five engineered polypeptides comprising adenovirus penton base protomer of the invention.

In an eight aspect the present invention relates to a virus-like particle (VLP) comprising 12 pentamers of the invention.

In a ninth aspect the present invention relates to a VLP comprising 12 pentamers each comprising five adenovirus penton base protomers and at least one engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment.

In a tenth aspect the present invention relates to a method for producing the engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or the engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment, comprising the steps of:

(a) providing a recombinant host cell of the invention;

(b) expressing the engineered polypeptide; and

(c) purifying the engineered polypeptide.

In an eleventh aspect the method for producing a VLP of the invention comprising the steps of the method of the tenth aspect of the invention and the further step of allowing the engineered polypeptides to assemble into a VLP.

In a twelfth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral polypeptides, comprising the steps of:

(a) providing a cloning vector of the invention;

(b) determining the amino acid sequence of disease or patient specific non-adenoviral polypeptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral polypeptides into nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop of the adenovirus penton base protomer, and/or at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an
5 adenovirus penton base protomer binding fibre protein fragment;

(d) expressing the engineered adenovirus penton base protomer in a host cell, optionally together with the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment; and

(e) purifying said VLP optionally comprising an adenovirus penton base protomer
10 binding fibre protein fragment, or said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment.

In a thirteenth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral polypeptides, comprising the steps of:

15 (a) providing a cloning vector of the invention;

(b) determining the amino acid sequence of disease or patient specific non-adenoviral polypeptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral polypeptides at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus
20 of the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment;

(d) expressing the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment in a host cell, optionally together with an adenovirus penton base protomer; and

25 (e1) purifying said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment and admixing with adenovirus penton base protomers or engineered adenovirus penton base protomers of the invention; or

(e2) purifying said VLP in case that the adenovirus penton base protomer was co-expressed.

30 In a fourteenth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral polypeptides, comprising the steps of:

(a) determining the amino acid sequence of disease or patient specific non-adenoviral polypeptides;

(b) synthetizing an engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment and at least one of said non-adenoviral polypeptides; and

(c) admixing said engineered polypeptide with adenovirus penton base protomers or engineered adenovirus penton base protomers of the invention with pentamers of the invention or with VLPs of the invention.

In a fifteenth aspect the present invention relates to a VLP producible by a method of producing a VLP of the invention.

In a sixteenth aspect the present invention relates to a pharmaceutical composition comprising the engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or the engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment, the nucleic acid encoding one or more of the engineered proteins of the invention, the expression vector of the invention or the VLP of the invention, and a pharmaceutically acceptable carrier and/or suitable excipient(s).

In a seventeenth aspect the present invention relates to an engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or the engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment, the nucleic acid encoding one or more of the engineered proteins of the invention, the expression vector of the invention or the VLP of the invention for treating and/or preventing an infectious disease, an immune disease or cancer.

FIGURES

Figure 1: Shows the synthetic self-assembling multimeric scaffolds of the invention also referred to as VLPs. 5 protomers form one penton subunit and 12 pentons spontaneously self-assemble into large superstructure which is alternatively termed a VLP or ADDomer.

Figure 2: Shows a side view of penton. The penton protomer contains two highly heteromorphic RGD-loops and one Variable loop (V loop) of unconserved, widely varied length and sequence. In this respect the present inventors determined them to be similar to antibody CDRs and suitable for introducing binding sites that allow the resulting VLP to bind to any desired target. It is suitable for display of multiple epitopes and can display up to 180 epitopes per VLP.

Figure 3: The penton base protomer comprises a region – a sticky patch - that interacts with adenovirus fibre protein. This sticky patch can bind with subnanomolar affinity to fragments of adenoviral fibre – also referred to as “STICKER”. It is preferred that these fibre

fragments are multimerized to increase binding affinity. The STICKER$_n$-tag (n is preferably 2 to 4) can be fused to the C- and/or N-terminus of a protein to be attached to the VLP or can be covalently or non-covalently coupled to any other cargo. The STICKER$_n$-tag, thus provides the ability to display on the surface of the VLP peptides, proteins, nucleic acids, liposomes and any 5 other cargo to be delivered by the VLP. An ADDomer has 12 sites for binding to a STICKER-tagged cargo. In one embodiment the sticky patch on the penton base protomer is modified to comprise a coupling residue, preferably a Cys and the STICKER$_n$-tag is also modified to comprise a Cys in a way that under non-reducing conditions the Cys in the sticky patch and the STICKER$_n$-tag can form a covalent bond, which will be severed under reducing conditions.

10 **Figure 4:** Shows diagrammatically the process of producing the ADDomers. The ADDomers are produced with high yield, are simple to purify and are exceptionally stable. The pACEBac-ADDOMER vector has three regions encoding the first and second RGD loop as well as the V loop that can easily be replaced with any desired peptide chain, e.g. a peptide conferring specific binding activities and/or an antigenic epitope.

15 **Figure 5:** The penton base protomer comprises a region that interacts with the neighboring protomer when assembling into dodecahedron, known as “strand swapping”. Mutation of relevant amino acids residues to cysteines can give rise to stabilization of the VLP super-structure by covalent disulfide bond formation, making the ADDomer thermostable. A schematic representing strand swapping residues mutated to cysteine is shown.

20 **Figure 6:** The sequences indicated are highly conserved throughout the species. The alignement shows wild-type penton base sequences from different natural serotypes. Sequences shown are : Subgroup C Ad2, accession number PO3276; subgroup B Ad3, S41389; subgroup B Ad7, AAR89958; subgroup B Ad11, AAP49205; subgroup F Ad41, AAF14179; subgroup A Ad12, P36716; subgroup D Ad17, NP_049379; subgroup E Ad25, NP_478405; and subgroup 25 D Ad37, CAC82544 were aligned. The V loop is highlighted with a dark grey box; the RGD-loops are highlighted with a light grey box. Those amino acid residues of the penton base protomer that bind to fibre are highlighted with an intermediated grey. They are all conserved amongst the serotypes.

Figures 7 and **8:** The structure of two preferred cloning vectors of the present invention 30 is shown. The nucleic acid sequences of the cloning vectors are respectively provided in SEQ ID NO: 61 and 62.

Figure 9: Plug&Play expression cassette and baculovirus transfer plasmid. The gene encoding ADDomer was designed in order to insert the “epitopes of interest” at three distinct loci (in dark grey) flanked by unique restriction sites. This cassette was inserted in

BamHI/HindIII of the pACEBac plasmid. BioBrick insertion using either ECoRI/RsrII, BssHII/SalI or SacI/XbaI of the epitope of interest can be easily done in construct pACEBac-ADDomer2.0.

Figure 10: ADDomer2.0 thermostability. Purified ADDomer was stored at different temperatures followed by electron microscopy. Storage at room temperature or 37°C resulted in full preservation of the particle. Dissociation in building blocks (pentamer) was observed only for temperature above 45°C. Thermal shift assay (TSA) confirmed stability up to 37°C, an onset of minor dissociation above 45°C, and a total denaturation only by incubation at 60°C.

Figure 11: Chikungunya epitope insertion and mode of epitope display. (a) The amino acids sequence incorporated in a display loop of ADDomer2.0 is shown on top (SEQ ID NO: 78). The major Chikungunya neutralizing epitope (highlighted in dark grey) was inserted. The N-terminus of the peptide contained extra amino acids encoding for a TEV cleavage site (highlighted in light grey). (b) The possibilities of epitope display are explained schematically. Expression results in ADDomer-TevCHIK, in which both ends of the peptide were linked to the ADDomer scaffold ('constrained epitopes'). Incubation with TEV protease released the N-terminus of the peptide in a 'nature-like' configuration ('relaxed epitopes'), while fully maintaining the integrity of the (now multiply nicked) ADDomer VLP. (c) Cleavage was monitored over time by SDS-PAGE analysis showing that intact ADDomer (around 60 kDa) is efficiently cleaved in two bands around 43 and 17kDa as expected (left). Despite cleavage, electron microscopy confirmed that ADDomer scaffold was not destroyed (right).

Figure 12: ELISA of CHIK epitope recognition by mouse sera. Three groups of eight mice were injected at w2 and w4 with 10 mg of either ADDomer scaffold only (no antigenic epitope in epitope presenting loops), ADDomer-TevCHIK^{exp} (exposed "nature-like" CHIK antigenic epitope in epitope presenting loops, free N-terminal end as in the live Chikungunya glycoprotein, C-terminal end covalently attached to scaffold) or ADDomer-TevCHIK^{constr} ("constrained" CHIK antigenic epitope in epitope presenting loop, N- and C-terminal ends attached to ADDomer scaffold. Sera were collected every two weeks and tested for CHIK antigenic epitope recognition (dilution 1/100). ADDomer-TevCHIK^{exp} with exposed, nature-like' epitope efficiently elicits response.

Figure 13: ADDomer with massively extended epitope presenting loop. A linear epitope encompassing 200 amino acids was inserted into the epitope presenting loops of the ADDomer scaffold and compared to ADDomer scaffold only (without insertion). SDS-PAGE gel evidences the insertion as reflected by the shift to higher molecular weight (left). Mass spectroscopy analysis confirmed the molecular weights (63,573Da for the ADDomer scaffold

without insertion; 81,179Da for the "extended" ADDomer comprising the extra 200 amino acid insertion in the epitope presenting loops.

Figure 14: Covalent coupling of "STICKER" peptide to ADDomer containing targeted cystein mutations. Wild-type ADDomer (wt) and ADDomer with one cysteine mutation (K363C, Q476C or A477C, respectively) were incubated with STICKER peptides (C20 (SEQ ID NO: 77) and C9 (SEQ ID NO: 75), respectively). SDS-PAGE analysis was performed under reducing (+bMeSH) and non-reducing (-bMeSH) conditions and transferred to a PVDF membrane. ADDomer (dark grey) and STICKER peptide (light grey) were visualized by binding of labelled antibody and Avidin binding, respectively, evidencing STICKER binding to cysteine-mutant ADDomer by specific disulfide bond formation (marked by white circle in lower panel)

DETAILED DESCRIPTION OF THE INVENTION

Before the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodology, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether *supra* or *infra*, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

Definitions

To practice the present invention, unless otherwise indicated, conventional methods of chemistry, biochemistry, and recombinant DNA techniques are employed which are explained in the literature in the field (cf., e.g., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. As used in this specification and the appended claims, the singular forms "a", "an", and "the" include plural referents, unless the content clearly dictates otherwise.

The terms "polynucleotide" and "nucleic acid" are used interchangeably herein and are understood as a polymeric or oligomeric macromolecule made from nucleotide monomers. Nucleotide monomers are composed of a nucleobase, a five-carbon sugar (such as but not limited to ribose or 2'-deoxyribose), and one to three phosphate groups. Typically, a polynucleotide is formed through phosphodiester bonds between the individual nucleotide monomers. In the context of the present invention referred to nucleic acid molecules include but are not limited to ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and mixtures thereof such as e.g. RNA-DNA hybrids. The nucleic acids, can e.g. be synthesized chemically, e.g. in accordance with the phosphotriester method (see, for example, Uhlmann, E. & Peyman, A. (1990) Chemical Reviews, 90, 543-584). "Aptamers" are nucleic acids which bind with high affinity to a polypeptide. Aptamers can be isolated by selection methods such as SELEmir146-a (see e.g. Jayasena (1999) Clin. Chem., 45, 1628-50; Klug and Famulok (1994) M. Mol. Biol. Rep., 20, 97-107; US 5,582,981) from a large pool of different single-stranded RNA molecules. Aptamers can also be synthesized and selected in their mirror-image form, for example as the L-ribonucleotide (Nolte et al. (1996) Nat. Biotechnol., 14, 1116-9; Klussmann et al. (1996) Nat. Biotechnol., 14, 1112-5). Forms which have been isolated in this way enjoy the advantage that they are not degraded by naturally occurring ribonucleases and, therefore, possess greater stability.

The terms "protein" and "polypeptide" are used interchangeably herein and refer to any peptide-bond-linked chain of amino acids, regardless of length or post-translational modification. Proteins usable in the present invention (including protein derivatives, protein variants, protein fragments, protein segments, protein epitopes and protein domains) can be

further modified by chemical modification. This means such a chemically modified polypeptide comprises other chemical groups than the 20 naturally occurring amino acids. Examples of such other chemical groups include without limitation glycosylated amino acids and phosphorylated amino acids. Chemical modifications of a polypeptide may provide advantageous properties as compared to the parent polypeptide, e.g. one or more of enhanced stability, increased biological half-life, or increased water solubility.

The term “penton base protein” or “penton base protomer” as used in the context of the present invention refers to an adenoviral protein that assembles into the so called “penton protein”. Each penton protein comprises five penton base proteins. The penton protein is one of three proteins forming the adenoviruses coat. The other proteins are hexon and fibre. The structure of an assembled adenovirus is shown in Fig. 1 upper left corner. Penton base proteins that are used in the present invention originate from adenovirus specific to any mammalian species. Preferably the adenovirus is a human or non-human great ape adenovirus, preferably Chimpanzee (Pan), Gorilla (Gorilla) and orangutans (Pongo), more preferably Bonobo (*Pan paniscus*) and common Chimpanzee (*Pan troglodytes*). It is understood by the skilled person that the penton base proteins of different adenovirus will vary in their amino acid sequence all such naturally occurring variants are encompassed by the term “penton base protein”. Additionally, the term encompasses artificial variants that comprise insertion, deletions and/or mutations of the naturally occurring penton base protein sequence. These mutations are in addition to the modifications of the N-terminal domain, V loop, first RGD, second RGD loop and/or sticky patch region described in more detail below. Any such artificial variants are comprised in as long as the artificially modified penton base protein assembles into penton subunits and 12 of these assemble into VLPs. Preferably, the artificial variants have at least 75%, more preferably at least 80%, more preferably at least 85%, more preferably at least 90%, more preferably at least 92%, more preferably 94%, more preferably 96%, and more preferably 98% sequence identity to a naturally occurring penton base protomer outside the N-terminal domain, the V loop, the first RGD, and the second RGD loop as defined below. Preferred penton base proteins are those indicated in SEQ ID NOs: 1 to 14. The penton base proteins as defined above are the basis for the engineered penton base proteins of the present invention. Thus, the engineered penton base proteins differ in sequence from naturally occurring penton base proteins by amino acid insertions, deletions and mutations as outlined in more detail below.

The phrase that the “engineered polypeptide is capable of assembling into VLPs” or “assembles into a VLP” as used interchangeably in the context of the present invention refers to the ability of five penton base protomers to self-assemble into a penton protein and

subsequently of twelve penton proteins to self-assemble into a small spherically shaped particle, i.e. a virus-like particle (VLP). The ability to assemble and to maintain the penton protein or preferably the VLP structure can be ascertained by methods known in the art and described herein, in particular by electron microscopy (EM). Preferred conditions at which the capability to assemble into VLPs is assessed is 20°C and physiologic buffer conditions. In a further preferred embodiment the term encompasses engineered polypeptides that not only assemble into VLPs but maintain the spherical shape at temperatures above 20°C, preferably at temperatures above 30°C, preferably at temperatures above 40°C, more preferably above 45°C and even more preferably above 50°C. The integrity of the spherical shape can be assessed by EM, preferably under physiological buffer conditions.

The term "first RGD-loop" as used in the context of the present invention refers to a polypeptide sequence of between 10 to 40 amino acids that is located N-terminally to the "RGD motif" comprised in the penton protomer (see Fig. 6). This polypeptide sequence is highly divergent between different adenoviruses. Accordingly, it cannot be defined by homology but can be defined by the sequence that is located N-terminally of its N-terminal end. Its C-terminal end within the penton protomer is determined by the RGD motif.

The term "second RGD-loop" as used in the context of the present invention refers to a polypeptide sequence of between 10 to 35 amino acids that is located C-terminally to the "RGD motif" comprised in the penton protomer (see Fig. 6). This polypeptide sequence is highly divergent between different adenoviruses. Accordingly, it cannot be defined by sequence homology. Its N-terminal end within the penton protomer is determined by the RGD motif. Its C-terminal end within the protomer can be defined by the sequence that is located C-terminally of its C-terminal end, which is conserved among different adenoviruses.

The term "RGD motif" as used in the context of the present invention refers to a three amino acid long polypeptide composed of arginine, glycine and aspartic acid. This motif was originally identified in fibronectin as mediating binding to integrins. The RGD-motif is also present in many other receptors and mediates both cell-substrate and cell-cell interactions. The RGD-motif in the penton protomers of the engineered polypeptides of the present invention may be intact or may be mutated in a way that the penton protomer does no longer bind to integrins.

The term "variable loop" as used in the context of the present invention corresponds to a sequence located between the beta sheet sheet b3 and the beta sheet b4 of the adenovirus penton base. Both the length and the aminoacids composition of this loop are are very variable amongst serotypes. The sequences corresponding to variable loops are highlighted in green in Figure 6.

The term "N-terminal domain" as used in the context of the present invention refers to a highly conserved region in the N-terminus of the penton base protomer. This part of the protein comprises the α1 and α2 helices, the ß1 and ß2 sheets as well as the B and C domain (see Figure 6). It is involved in the interaction between penton base protomers and, thus suitable for the
5 introduction of moieties, e.g. coupling residues that stabilize the interaction between the penton base protomers.

The term "adenovirus fibre protein binding cleft" as used in the context of the present invention refers to a fold of a penton base protomer forming the interaction surface with the adenovirus fibre protein. As can be seen in Fig. 6 the binding cleft is formed by several non-
10 contiguous stretches of polypeptide sequence which are conserved among different adenoviruses.

The term "target specific binding domain" as used throughout the specification refers to a polypeptide which facilitates specific binding to a target. The binding of such a target specific binding domain is considered specific to a given target if it binds with the highest affinity to the
15 respective target and only with lower affinity, e.g. at least 10-fold lower, preferably at least 100-fold lower affinity to other targets even to targets with a related amino acid sequence.

The term "target" as used in the present invention refers to a natural existing cellular or molecular structure towards which molecules have a certain binding affinity or to which molecules specifically bind. A target may comprise one or more epitopes. An antigen is a
20 preferred example of a target.

The term "antigen" as used in the context of the present invention to refer to any structure recognized by molecules of the immune response, e.g. antibodies, T cell receptors (TCRs) and the like. An antigen may be foreign or toxic to the body or may be a cellular protein that is associated with a particular disease. Antigens are recognized by highly variable antigen
25 receptors (B-cell receptor or T-cell receptor) of the adaptive immune system and may elicit a humoral or cellular immune response. Antigens that elicit such a response are also referred to as immunogen. A fraction of the proteins inside cells, irrespective of whether they are foreign or cellular, are processed into smaller peptides and presented to by the major histocompatibility complex (MHC). A cellular immune response is elicited, if the small peptide fragment is bound
30 by a T-cell receptor. Cell surface antigens can be selected from the group of cytokine receptors, integrins, cell adhesion molecules, cell type-specific cell surface antigen, tissue-specific cell surface antigen, cell surface-expressed tumor-associated antigen, cluster of differentiation antigens, or carbohydrates.

The term “specific binding” as used in the context of the present invention to mean that a binding moiety (e.g. an antibody) binds stronger to a target, such as an epitope, for which it is specific compared to the binding to another target if it binds to the first target with a dissociation constant (K_d) which is lower than the dissociation constant for the second target.
5 Targets can be recognized by their ligands which bind with a certain affinity to their targets and thus, the ligand binding to its respective target results in a biological effect. Preferably, the binding is both specific and occurs with a high affinity, preferably with K_d of less than 10^{-7}, 10^{-8}, 10^{-9}, 10^{-10} M or less. Such affinity is preferably measured at 37°C Suitable assays include surface plasmon resonance measurements (e.g. Biacore), quartz crystal microbalance
10 measurements (e.g. Attana), and competition assays.

The term “antibodies” as used in the context of the present invention are glycoproteins belonging to the immunoglobulin superfamily; the terms antibody and immunoglobulin are often used interchangeably. An antibody refers to a protein molecule produced by plasma cells and is used by the immune system to identify and neutralize foreign objects such as bacteria
15 and viruses. The antibody recognizes a unique part of the foreign target, its antigen.

The term “antibody fragment” as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. Examples of binding fragments encompassed within the term “antibody fragment” include a fragment antigen binding (Fab) fragment, a Fab’ fragment, a $F(ab')_2$ fragment, a heavy chain antibody, a single-domain
20 antibody (sdAb), a single-chain fragment variable (scFv), a fragment variable (Fv), a V_H domain, a V_L domain, a single domain antibody, a nanobody, an IgNAR (immunoglobulin new antigen receptor), a di-scFv, a bispecific T-cell engager (BITEs), a dual affinity re-targeting (DART) molecule, a triple body, a diabody, a single-chain diabody, an alternative scaffold protein, and a fusion protein thereof.

25 The term “diabody” as used within this specification refers to a fusion protein or a bivalent antibody which can bind different antigens. A diabody is composed of two single protein chains which comprise fragments of an antibody, namely variable fragments. Diabodies comprise a heavy chain variable domain (V_H) connected to a light-chain variable domain (V_L) on the same polypeptide chain (V_H-V_L, or V_L-V_H). By using a short peptide connecting the two
30 variable domains, the domains are forced to pair with the complementary domain of another chain and thus, create two antigen-binding sites. Diabodies can target the same (monospecific) or different antigens (bispecific).

The term “single domain antibody” as used in the context of the present invention refers to antibody fragments consisting of a single, monomeric variable domain of an antibody.

Simply, they only comprise the monomeric heavy chain variable regions of heavy chain antibodies produced by camelids or cartilaginous fish. Due to their different origins they are also referred to VHH or VNAR (variable new antigen receptor)-fragments. Alternatively, single-domain antibodies can be obtained by monomerization of variable domains of conventional mouse or human antibodies by the use of genetic engineering. They show a molecular mass of approximately 12-15 kDa and thus, are the smallest antibody fragments capable of antigen recognition. Further examples include nanobodies or nanoantibodies.

The term "antibody mimetic" as used within the context of the present specification refers to compounds which can specifically bind antigens, similar to an antibody, but are not structurally related to antibodies. Usually, antibody mimetics are artificial peptides or proteins with a molar mass of about 3 to 20 kDa which comprise one, two or more exposed domains specifically binding to an antigen. Examples include inter alia the LACI-D1 (lipoprotein-associated coagulation inhibitor); affilins, e.g. human-γ B crystalline or human ubiquitin; cystatin; Sac7D from *Sulfolobus acidocaldarius*; lipocalin and anticalins derived from lipocalins; DARPins (designed ankyrin repeat domains); SH3 domain of Fyn; Kunits domain of protease inhibitors; monobodies, e.g. the 10th type III domain of fibronectin; adnectins: knottins (cysteine knot miniproteins); atrimers; evibodies, e.g. CTLA4-based binders, affibodies, e.g. three-helix bundle from Z-domain of protein A from *Staphylococcus aureus*; Trans-bodies, e.g. human transferrin; tetranectins, e.g. monomeric or trimeric human C-type lectin domain; microbodies, e.g. trypsin-inhibitor-II; affilins; armadillo repeat proteins. Nucleic acids and small molecules are sometimes considered antibody mimetics as well (aptamers), but not artificial antibodies, antibody fragments and fusion proteins composed from these. Common advantages over antibodies are better solubility, tissue penetration, stability towards heat and enzymes, and comparatively low production costs.

As used herein, the term "K_d" (usually measured in "mol/L", sometimes abbreviated as "M") is intended to refer to the dissociation equilibrium constant of the particular interaction between a binding moiety (e.g. an antibody or fragment thereof) and a target molecule (e.g. an antigen or epitope thereof). Methods for determining K_d include, without limitation, ELISA and surface plasmon resonance assays.

The term an "epitope", also known as antigenic determinant, as used in the context of the present invention is the part of a macromolecule that is recognized by the immune system, specifically by antibodies, B cells, or T cells. As used herein, an "epitope" is the part of a macromolecule capable of binding to an antibody (e.g. an antibody or antigen-binding fragment thereof) as described herein. Epitopes usually consist of chemically active surface groupings of

molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes can be distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents.

5 As used herein, a “conformational epitope” refers to an epitope of a linear macromolecule (e.g. a polypeptide) that is formed by the three-dimensional structure of said macromolecule. In the context of the present application, a “conformational epitope” is a “discontinuous epitope”, i.e. the conformational epitope on the macromolecule (e.g. a polypeptide) which is formed from at least two separate regions in the primary sequence of the 10 macromolecule (e.g. the amino acid sequence of a polypeptide). In other words, an epitope is considered to be a “conformational epitope” in the context of the present invention, if the epitope consists of at least two separate regions in the primary sequence to which an antibody of the invention (or an antigen-binding fragment thereof) binds simultaneously, wherein these at least two separate regions are interrupted by one or more regions in the primary sequence to 15 which an antibody of the invention (or an antigen-binding fragment thereof) does not bind. Preferably, such a “conformational epitope” is present on a polypeptide, and the two separate regions in the primary sequence are two separate amino acid sequences to which an antibody of the invention (or an antigen-binding fragment thereof) binds, wherein these at least two separate amino acid sequences are interrupted by one more amino acid sequences in the primary 20 sequence to which an antibody of the invention (or an antigen-binding fragment thereof) does not bind. Preferably, the interrupting amino acid sequence is a contiguous amino acid sequence comprising two or more amino acids to which the antibody (or the antigen-binding fragment thereof) does not bind. The at least two separate amino acid sequences to which an antibody of the invention (or an antigen-binding fragment thereof) binds are not particularly limited with 25 regard to their length. Such a separate amino acid sequence may consists of only one amino acid as long as the total number of amino acids within said at least two separate amino acid sequences is sufficiently large to effect specific binding between the antibody (or the antigen-binding fragment thereof) and the conformational epitope.

The term “adenovirus fibre protein” as used in the context of the present invention refers 30 to an adenoviral protein that non-covalently binds to a penton protomer and aids in attachment of the adenovirus to the host cell.

The term "sequence identity" is used throughout the specification with regard to polypeptide and polynucleotide sequence comparisons. In case where two sequences are compared and the reference sequence is not specified in comparison to which the sequence

identity percentage is to be calculated, the sequence identity is to be calculated with reference to the longer of the two sequences to be compared, if not specifically indicated otherwise. If the reference sequence is indicated, the sequence identity is determined on the basis of the full length of the reference sequence indicated by SEQ ID, if not specifically indicated otherwise. For example, a polypeptide sequence consisting of 200 amino acids compared to a reference 300 amino acid long polypeptide sequence may exhibit a maximum percentage of sequence identity of 66.6% (200/300) while a sequence with a length of 150 amino acids may exhibit a maximum percentage of sequence identity of 50% (150/300). If 15 out of those 150 amino acids are different from the respective amino acids of the 300 amino acid long reference sequence, the level of sequence identity decreases to 45%. The similarity of nucleotide and amino acid sequences, i.e. the percentage of sequence identity, can be determined via sequence alignments. Such alignments can be carried out with several art-known algorithms, preferably with the mathematical algorithm of Karlin and Altschul (Karlin & Altschul (1993) Proc. Natl. Acad. Sci. USA 90: 5873-5877), with hmmalign (HMMER package, http://hmmer.wustl.edu/) or with the CLUSTAL algorithm (Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Nucleic Acids Res. 22, 4673-80) available e.g. on http://www.ebi.ac.uk/Tools/clustalw/ or on http://www.ebi.ac.uk/Tools/clustalw2/index.html or on http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html. Preferred parameters used are the default parameters as they are set on http://www.ebi.ac.uk/Tools/clustalw/ or http://www.ebi.ac.uk/Tools/clustalw2/index.html. The grade of sequence identity (sequence matching) may be calculated using e.g. BLAST, BLAT or BlastZ (or BlastX). A similar algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215: 403-410. BLAST polynucleotide searches are performed with the BLASTN program, score = 100, word length = 12. BLAST protein searches are performed with the BLASTP program, score = 50, word length = 3. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25: 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs are used. Sequence matching analysis may be supplemented by established homology mapping techniques like Shuffle-LAGAN (Brudno M., Bioinformatics 2003b, 19 Suppl 1:I54-I62) or Markov random fields. When percentages of sequence identity are referred to in the present application, these percentages are calculated in relation to the full length of the longer sequence, if not specifically indicated otherwise. "Hybridization" can also be used as a measure of sequence identity or homology between two nucleic acid sequences. A nucleic acid sequence encoding F, N, or M2-1, or a portion of any of these can be used as a

hybridization probe according to standard hybridization techniques. Hybridization conditions are known to those skilled in the art and can be found, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y., 6.3.1-6.3.6, 1991. "Moderate hybridization conditions" are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1X SSC, 0.1% SDS at 50°C. "Highly stringent conditions" are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

The term "coupling residue" as used in the context of the present invention refers to a natural or non-naturally occurring amino acid that has a side chain, capable of forming a covalent bond. Coupling residues can be inserted into a polypeptide of the present invention. If the coupling residue is a naturally occurring amino acid that is encoded by DNA the insertion of a coupling residue merely requires the modification of the DNA that is directing expression of the polypeptide of the invention, e.g. insertion of a codon that encodes such amino acid or mutation of an existing codon. Preferred examples of naturally occurring amino acids that are coupling residues within the meaning of this term are Asp, Glu, Lys and Cys. Cys is particularly preferred since it will form a disulfide bond with another Cys depending on the redox-status of the environment. In particular the latter allows the formation of a stable interconnection between two separate polypeptides.

The term "label" as used in the context of the present invention refers to any kind of compound being suitable for diagnostic purposes. Preferred compounds are selected from a fluorescent dye, a radioisotope and a contrast agent. A contrast agent is a dye or other substance that helps to show abnormal areas inside the body. In one embodiment the term label refers to a compound that comprises a chelating agent which forms a complex with divalent or trivalent metal cations. Preferred radioisotopes/fluorescence emitting isotopes are selected from the group consisting of alpha radiation emitting isotopes, gamma radiation emitting isotopes, Auger electron emitting isotopes, X-ray emitting isotopes, fluorescence emitting isotopes, such as ^{18}F, ^{51}Cr, ^{67}Ga, ^{68}Ga, ^{111}In, ^{99m}Tc, ^{140}La, ^{175}Yb, ^{153}Sm, ^{166}Ho,^{88}Y, ^{90}Y, ^{149}Pm, ^{177}Lu, ^{47}Sc, ^{142}Pr, ^{159}Gd, ^{212}Bi, ^{72}As, ^{72}Se, ^{97}Ru, ^{109}Pd, ^{105}Rh, $^{101m15}Rh$, ^{119}Sb, ^{128}Ba, ^{123}I, ^{124}I, ^{131}I, ^{197}Hg, ^{211}At, ^{169}Eu, ^{203}Pb, ^{212}Pb, ^{64}Cu, ^{67}Cu, ^{188}Re, ^{186}Re, ^{198}Au and ^{199}Ag. Preferred fluorescent dyes are selected from the following classes of dyes: Xanthens (e.g. Fluorescein), Acridines (e.g. Acridine Yellow), Oxazines (e.g. Oxazine 1), Cynines (e.g. Cy7 / Cy 3), Styryl dyes (e.g. Dye-28), Coumarines (e.g. Alexa Fluor 350), Porphines (e.g. Chlorophyll B), Metal-Ligand-Complexes (e.g. PtOEPK), Fluorescent proteins (e.g APC, R-Phycoerythrin), Nanocrystals (e.g QuantumDot 705), Perylenes (e.g. Lumogen Red F300) and Phtalocyanines (e.g.

IRDYE™700DX) as well as conjugates and combinations of these classes of dyes. Preferred contrast agents are selected from paramagnetic agents, e.g. Gd, Eu, W and Mn, preferably complexed with a chelating agent. Further options are supramagnetic iron (Fe) complexes and particles, compounds containing atoms of high atomic number, i.e. iodine for computer
5 tomography (CT), microbubbles and carriers such as liposomes that contain these contrast agents.

The term “drug” is to be understood in the context of the present invention in its broadest sense to refer to any compound that elicits a prophylactic, therapeutic or palliative effect in a patient. Preferably, it is a small molecule, e.g. with a molecular size of below 500 D.

10 A “linker” in the context of the present invention refers to any chemical moiety that is flexible and sterically separates two chemical moieties, e.g. an engineered polypeptide of the first aspect of the invention from a drug or label. Preferred linkers are moieties with have a length to width ratio of at least 10:1, preferably of at least 20:1, more preferably of at least 50:1. Preferably, linkers are linear molecules. It is preferred that the two moieties linked by a linker
15 are covalently or non-covalently, preferably covalently attached to the respective ends of the linker.

A "peptide linker" in the context of the present invention refers to an amino acid sequence, i.e. polypeptide, which sterically separates two parts within the engineered polypeptides of the present invention. Typically such linker consists of between 1 and 100,
20 preferably 3 to 50 more preferably 5 to 20 amino acids. Thus, such linkers have a minimum length of at least 1, 2, 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, or 30 amino acids, and a maximum length of at least 100, 95, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 40, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, or 15 amino acids or less. Peptide linkers may also provide flexibility among the two
25 parts that are linked together. Such flexibility is generally increased, if the amino acids are small. Accordingly, flexible peptide linkers comprise an increased content of small amino acids, in particular of glycins and/or alanines, and/or hydrophilic amino acids such as serines, threonines, asparagines and glutamines. Preferably, more than 20%, 30%, 40%, 50%, 60% or more of the amino acids of the peptide linker are small amino acids.

30 The terms "preparation" and "composition" are intended to include the formulation of the active compound, e.g. the VLPs of the present invention with a carrier and/or excipient.

"Pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

The term "carrier", as used herein, refers to a pharmacologically inactive substance such as but not limited to a diluent, excipient, surfactants, stabilizers, physiological buffer solutions or vehicles with which the therapeutically active ingredient is administered. Such pharmaceutical carriers can be liquid or solid. Liquid carrier include but are not limited to sterile liquids, such as saline solutions in water and oils, including but not limited to those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

Suitable pharmaceutical "excipients" include starch, glucose, lactose, sucrose, gelatine, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

"Surfactants" include anionic, cationic, and non-ionic surfactants such as but not limited to sodium deoxycholate, sodium dodecylsulfate, Triton X-100, and polysorbates such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65 and polysorbate 80.

"Stabilizers" include but are not limited to mannitol, sucrose, trehalose, albumin, as well as protease and/or nuclease antagonists.

"Physiological buffer solution" that may be used in the context of the present invention include but are not limited to sodium chloride solution, demineralized water, as well as suitable organic or inorganic buffer solutions such as but not limited to phosphate buffer, citrate buffer, tris buffer (tris(hydroxymethyl)aminomethane), HEPES buffer ([4 (2 hydroxyethyl)piperazino]ethanesulphonic acid) or MOPS buffer (3 morpholino-1 propanesulphonic acid). The choice of the respective buffer in general depends on the desired buffer molarity. Phosphate buffer are suitable, for example, for injection and infusion solutions.

The term "adjuvant" refers to agents that augment, stimulate, activate, potentiate, or modulate the immune response to the active ingredient of the composition at either the cellular or humoral level, e.g. immunologic adjuvants stimulate the response of the immune system to the actual antigen, but have no immunological effect themselves. Examples of such adjuvants include but are not limited to inorganic adjuvants (e.g. inorganic metal salts such as aluminium phosphate or aluminium hydroxide), organic adjuvants (e.g. saponins or squalene), oil-based adjuvants (e.g. Freund's complete adjuvant and Freund's incomplete adjuvant), cytokines (e.g. IL-1β, IL-2, IL-7, IL-12, IL-18, GM-CFS, and INF-γ) particulate adjuvants (e.g. immuno-

stimulatory complexes (ISCOMS), liposomes, or biodegradable microspheres), virosomes, bacterial adjuvants (e.g. monophosphoryl lipid A, or muramyl peptides), synthetic adjuvants (e.g. non-ionic block copolymers, muramyl peptide analogues, or synthetic lipid A), or synthetic polynucleotides adjuvants (e.g polyarginine or polylysine).

5 An "effective amount" or "therapeutically effective amount" is an amount of a therapeutic agent sufficient to achieve the intended purpose. The effective amount of a given therapeutic agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the therapeutic agent, and the purpose of the administration. The effective amount in each individual case may be determined 10 empirically by a skilled artisan according to established methods in the art.

EMBODIMENTS

The present invention provides inter alia the following advantages over the prior art: (i) an easily modified scaffold for antigen and/or target specific binding domain 15 insertion/presentation, which can be tailored to the need of a patient or easily adapted to the changing surface antigens of viruses, (ii) a stable composition that can be used for, e.g. vaccination even under adverse storage conditions, e.g. high heat, (iii) an extremely high density vehicle for presenting one or multiple antigens, (iv) the use of a fibre (STICKER) protein to add further antigens or other activities on the fly.

20 Accordingly, in a first aspect the present invention relates to an engineered polypeptide comprising, essentially consisting or consisting of an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), adenovirus fibre protein binding cleft and/or a N-terminal domain, and comprises one or more of the following:

25 (i) at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop; and/or

(ii) one or more non-adenoviral polypeptides in the first, the second or both the first and the second RGD-loops and/or in the V loop; and/or

(iii) a non-adenoviral polypeptide at the N- and/or C-terminus of the penton base 30 protomer; and/or

(iv) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer, wherein the N-terminus of the N-terminal domain within the penton base protomer is defined as follows:

35 X_1-G-R-N-S-I-R (SEQ ID NO: 44)

and the C-terminus of the N-terminal domain within the penton base protomer is defined as follows:

D-X_2-R-S-R-G (SEQ ID NO: 45),

wherein

5 X_1 is selected from the group consisting of G and E, preferably E, and

X_2 is selected from the group consisting of D and E, preferably E; and/or

(v) a drug, label and/or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or

10 (vi) at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer

and wherein the engineered polypeptide is preferably capable of assembling into VLPs.

If in one of above embodiments a residue or group of residues, e.g. a target specific binding domain, one or more non-adenoviral polypeptides or at least one heterologous coupling

15 residue, is indicated to be comprised in a certain region of the penton base protein this residue or group of residues may be inserted within the respectively indicated region of the penton base protein, i.e. may be an addition, or it may be inserted and additional at least one or all of the amino acids forming the respectively indicated first RGD-loop, second RGD-loops and/or in V loop may be deleted without affecting the capability to assemble into VLPs.

20 A preferred embodiment of the engineered polypeptide of the first aspect of the invention comprises, essentially consists or consists of an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), adenovirus fibre protein binding cleft and/or a N-terminal domain, and comprises one or more non-adenoviral polypeptides in the first, the second or both the first and the second RGD-

25 loops and/or in the V loop; and optionally further comprises one or more of the following:

(i) comprising at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop; and/or

(ii) a non-adenoviral polypeptide at the N- and/or C-terminus of the penton base protomer; and/or

30 (iii) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer, wherein the N-terminus of the N-terminal domain within the penton base protomer is defined as follows:

X_1-G-R-N-S-I-R (SEQ ID NO: 44)

and the C-terminus of the N-terminal domain within the penton base protomer is defined as follows:

D-X_2-R-S-R-G (SEQ ID NO: 45),

wherein

5 X_1 is selected from the group consisting of G and E, preferably E, and

X_2 is selected from the group consisting of D and E, preferably E; and/or

(iv) a drug, label and/or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or

10 (vi at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer

and wherein the engineered polypeptide is preferably capable of assembling into VLPs.

The at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop provides the penton base protomer and accordingly 15 the assembled VLP with the ability to specifically bind to a target structure, e.g. a cellular receptor on the surface of a cell. It is a surprising discovery of the present inventors that these parts of the penton base protomer can comprise a target specific binding domain of considerable length without disrupting penton or VLP formation. Additionally, the target specific binding domain comprised in these regions is free to interact with and bind to targets. The one or more 20 target specific binding domains may be inserted at any point in the respective loops, i.e. without removing any of the loop amino acids. Alternatively, all or part of the respective loop amino acids may be replaced by the amino acids of the target specific binding domain. The target specific binding domain may be flanked N- and/or C-terminally by peptide linkers.

If the penton base protomer comprises more than one target specific binding domain, it is 25 preferred that these are comprised in different loops of the penton base protomer, e.g. in the first and second RGD-loop, in the first RGD-loop and the V-loop, or the second RGD-loop and the V-loop. If the penton base protomer comprises more than one target specific binding domain it is also preferred that they bind to different targets, e.g. to a target on a first type of cell and to a different target on a second type of cell. Such dual or multiple specificities can be used to 30 bring together cells that do not normally or not frequently enough interact with each other. Examples of such cells are tumor cells and cells of the immune system, in particular cytotoxic T cells.

In an alternative embodiment (ii) that can be combined with one or more of the other alternative embodiments outlined above the first, the second or both the first and the second

RGD-loops and/or in the V loop comprise a non-adenoviral polypeptide. This embodiment is also based on the surprising observation that polypeptides inserted into one or more of these regions of the penton base protomer are sufficiently exposed to be recognized by cells of the immune system and, thus to elicit an immune response. The term “non-adenoviral” polypeptide refers to a polypeptide that has no sequence identity to any polypeptide present in an adenovirus, in particular in naturally occurring adenovirus penton base protomers over a length of at least 5 amino acids. Preferably, the non-adenoviral polypeptide has no sequence identity to any polypeptide present in adenovirus over a stretch of at least 10, preferably at least 15 amino acids. The one or more non-adenoviral polypeptides may be inserted in each case independently at any point in the respective loops, i.e. without removing any of the loop amino acids. Alternatively, all or part of the respective loop amino acids may be replaced by the amino acids of the target specific binding domain. The non-adenoviral polypeptide comprised in one or more of the loops may be flanked N- and/or C-terminally by peptide linkers. This may be preferred to increase exposure of the non-adenoviral polypeptide on the surface of the VLP. If at least one non-adenoviral polypeptide is inserted into each loop than each penton base protomer comprises at least three identical or different non-adenoviral polypeptides on its surface. Once assembled into VLPs at least 180 non-adenoviral polypeptides may be displayed at the surface of the VLPs of the present invention.

The present inventors have surprisingly found that long amino acid sequences of 50 or more, 100 or more, 150 or more, 200 or more, 250 or more or 300 or more amino acids may be introduced into the first, the second or both the first and the second RGD-loops, and/or in the V loop without disrupting the penton base protomers ability to assemble into penton proteins and subsequently into VLPs. Thus, in the embodiment indicated under (i) and/or (ii) amino acid sequences of above indicated length may be inserted (with or without deletion of some of the amino acids with the respective loops).

If the alternative embodiments indicated under (i) and (ii) are combined, it is further preferred that the non-adenoviral protein is inserted into a different loop than the target specific binding domain.

The present inventors have observed that polypeptides positioned at either N- and/or C-terminus of the penton base protomer do not interfere with penton and subsequently VLP assembly and are surface exposed in an assembled VLP. Thus, in a further alternative embodiment (iii) a non-adenoviral polypeptide may be linked with or without intervening peptide linker to the N- and/or C-terminus of the penton base protomer. Accordingly, if combined with the first and/or second embodiment a penton base protomer may comprise non-

adenoviral polypeptides in one or more of the loops, preferably all three loops and at the N-terminus, C-terminus or N- and C-terminus. It is preferred that this alternative embodiment is combined with at least one of the other alternative embodiments (i), (iii), (iv), (v) and/or (vi).

The observations of the present inventors relating to the possibility to insert heterologous 5 peptide sequence into the V-loop, the first RGD-loop and/or the second RGD-loop (with or without concomitant deletion of all or part of the respectively indicated loop) led to a further alternative embodiment (iv) that may be combined with one or more of the previously discussed alternative embodiments. In this embodiment at least one heterologous coupling residue is introduced in the first, the second, or both the first and the second RGD-loops and/or in the V 10 loop. By the insertion of one or more coupling residues it becomes possible to covalently couple further molecules to the loops. It is, for example envisioned that a VLP is first assembled from the engineered polypeptide of the first aspect comprising one or more coupling residue in one or more of the loops and that subsequently a polypeptide also comprising a coupling residue is coupled covalently to the VLP. Using this strategy it is possible to "decorate" the surface of the 15 VLP with polypeptides. Such VLPs may be used to elicit a humoral and/or cellular immune response against such polypeptides.

Furthermore, the present inventors have identified a region within the penton base protomer referred to as the "N-terminal domain of the penton base protomer". This domain is involved in the interaction between the penton base protomers within penton and also in the 20 interaction among pentons forming a VLP. The insertion of coupling residues into this region allows the formation of covalent bonds between two or more penton base protomers within the same or separate pentons. The formation of such covalent bonds stabilizes the penton as well as the assembled VLP. The N-terminal domain is highly conserved among different adenovirus species. It is, therefore possible to further delineate the N-terminal and C-terminal end of this 25 domain within the penton base protomer. Thus, it is preferred that one or more the coupling residues are comprised in the N-terminal domain. The coupling residue may replace an existing amino acid or may be inserted in addition to the amino acids forming the N-terminal domain. It is preferred that the one or more coupling residue replace a residue within the N-terminal domain. The N-terminus of the N-terminal domain within the penton base protomer is 30 preferably defined as follows:

X_1-G-R-N-S-I-R (SEQ ID NO: 44)

and the C-terminus of the N-terminal domain within the penton base protomer is preferably defined as follows:

D-X_2-R-S-R-G (SEQ ID NO: 45),

wherein

X_1 is selected from the group consisting of G and E, preferably E, and

X_2 is selected from the group consisting of D and E.

Accordingly, in this alternative embodiment (iv) one or more coupling residues are comprised within the amino acid sequence of the penton base protomer comprised in the engineered polypeptide of the present invention delimited by above N- and C-terminal region. It will be understood by the skilled person that it is also possible in this embodiment to replace one or more amino acid residues within SEQ ID NO: 44 or 45. The coupling residue may be positioned anywhere within the N-terminal domain in as long as it does not interfere with assembly of penton or VLP.

A preferred protomer amino acid sequence that can be modified according to alternatives (i) to (vi) of the first embodiment is SEQ ID NO: 64 (the encoding nucleotide sequence is indicated in SEQ ID NO: 63). It is preferred that the insertions of at least one target specific binding domain according to embodiment (i), and/or the insertion of one or more non-adenoviral peptides according to embodiment (ii), and/or the insertion of at least one heterologous coupling residue according to embodiment (iv), and/or the covalent or non-covalent coupling of the drug or polypeptide to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop according to embodiment (v) occurs in the first RGD-loop between amino acids 312 to 339 of SEQ ID NO: 64 and/or in the second RGD-loop between amino acids 343 to 367 of SEQ ID NO: 64 and/or in the V loop between amino acids 150 to 178 of SEQ ID NO: 64. Such insertion(s) may delete all or part of the respectively indicated amino acids belonging to the first and second RGD loop and the V loop.

Preferably, there has to be a PAIR of coupling residues, preferably mutations to cysteines to enable disulfide bond formation. The resulting stabilized VLP contains up to 120 disulfide bonds and is hyperstable at 37°C and even at 42°C, at least for several months. In a particularly preferred embodiment the coupling residues are located at amino acid position 51 and 54 with reference to SEQ ID NO: 64, i.e. a preferred penton base protomer amino acid sequence based on human Ad B3 or at analogous positions of a penton base protomer of another adenovirus, or at amino acid position 54 and 114 with reference to SEQ ID NO: 64 or at analogous positions of a penton base protomer of another adenovirus.

It has been further discovered that a coupling residue at amino acid position 53 (with reference to SEQ ID NO: 1) can form a covalent bond with a coupling residue at amino acid position 543 (with reference to SEQ ID NO: 64) or at analogous positions of a penton base

protomer of another adenovirus. The latter residue is outside the N-terminal domain. Thus, if a coupling residue is inserted at position 53 it is preferred that a second coupling residue is positioned at amino acid 541 with reference to SEQ ID NO: 64 or at analogous positions of a penton base protomer of another adenovirus. With reference to Fig. 6 and by including further penton base proteins in the alignment the skilled person can easily determine those residues in the respective penton base protomer that occupies an analogous position as amino acids. 51, 53, 54, 114 and 541 of SEQ ID NO: 64.

It is preferred in this embodiment of the engineered polypeptide of the present invention that the penton base protomer comprises the following sequences:

P-T-X_1-X_c-R-N-X_c-I-R (SEQ ID NO: 50);

P-T-X_1-G-R-X_c-S-I-R (SEQ ID NO: 51) and T-Q-T-I-N-X_{60}-Xc-X_{61} (SEQ ID NO: 52)

or

P-T-X_1-G-R-N-X_c-I-R (SEQ ID NO: 53) and

T-C-P-X_c-V-X_{62}-K-A-L-G (SEQ ID NO: 54)

wherein

X_1 is selected from the group consisting of G and E, preferably E

X_c in each case is a coupling residue, preferably C; D, E, and K, most preferably C;

X_{60} is selected from the group consisting of F, I, and L, preferably F and L, most preferably F,

X_{61} is selected from the group consisting of D and E, preferably E; and

X_{62} is selected from the group consisting of H and Y, preferably Y.

Particularly, preferred stabilized penton base protomers comprise or consist of the amino acid sequences according to SEQ ID NO: 65 to 67. It is further preferred that these amino acid sequences comprise one or more of the modifications according to alternative embodiments (i), (ii), (iii), (iv) in as far as this alternative embodiment is not relating to the N-terminal domain, (v) or (vi) of the first aspect of the invention described above.

It is preferred that the insertions of at least one target specific binding domain according to embodiment (i), and/or the insertion of one or more non-adenoviral peptides according to embodiment (ii), and/or the insertion of at least one heterologous coupling residue according to embodiment (iv), and/or the covalent or non-covalent coupling of the drug or polypeptide to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop according to embodiment (v) occurs in the first RGD-loop occurs between amino acids 312 to 339 of SEQ ID NO: 65 to 67 and/or that the insertion into the second RGD-loop occurs between amino acids 343 to 367 of SEQ ID NO: 65

to 67 and/or that the insertion into the V loop occurs between amino acids 150 to 178 of SEQ ID NO: 65 to 67. Such insertion(s) may delete all or part of the respectively indicated amino acids belonging to the first and second RGD loop and the V loop.

The thermal stabilization of penton and VLPs formed by the engineered proteins of the
5 present invention is desirable also in the context of any of the other alternative embodiments of the engineered protein of the present invention. Accordingly, the alternative embodiment mentioned under (iv) above in relation to the N-terminal domain is preferably combined with one or more alternative embodiments (i), (ii), (iii), (iv) in as far as this alternative embodiment is not relating to the N-terminal domain, (v) or (vi). It is also preferred that the alternative
10 embodiments mentioned under (iv) and (vi) are present in the engineered penton base protomer and are combined with one or more of (i), (ii), (iii), (iv) in as far as this alternative embodiment is not relating to the N-terminal domain, or (v).

In a further alternative embodiment (v) of the first aspect of the invention that may be combined with one or more of the other alternative embodiments of the first aspect of the
15 invention a drug, label and/or polypeptide is covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer. Again this embodiment is based on the observation that the coupling of moieties to these regions does not interfere with penton and VLP assembly and leads to decoration of the VLP with these moieties. In a preferred
20 embodiment the drug or label is attached to the penton base protomer through a linker, preferably a peptide linker, that is cleavable under physiologic condition, e.g. a protease thereby releasing the drug from the VLP at the site of action. In this preferred embodiment the linker, preferably peptide linker comprises an endopeptidase cleavage site.

In a preferred embodiment a fragment of adenoviral fibre is used to non-covalently attach
25 a moiety, e.g. a polypeptide, drug, or label etc. to the penton base protomer, the assembled penton and/or the assembled VLP. This interaction is mediated via the adenovirus fibre protein binding cleft of the penton base protomer that is present in the engineered polypeptide of the first aspect of the invention. In a preferred embodiment described below the fibre fragment comprises a heterologous coupling residue for covalent attachment of the fibre fragment to the
30 penton base protomer. Since a coupling residue requires a counter-part, i.e. a residue with which it can form a covalent bond, it is a further preferred alternative embodiment (vi) of the engineered protein of the first aspect of the invention that at least one heterologous coupling residue is comprised in the adenovirus fibre protein binding cleft of the penton base protomer. The coupling residue in the binding cleft and the fibre protein fragment are positioned in such

as to allow formation of a covalent bond once the fibre protein fragment is bound in the cleft of the penton base protomer.

Each penton base protomer interacts with one adenovirus fibre protein via the highly conserved region referred to herein as the "adenovirus fibre protein binding cleft of the penton base protomer". This interaction is used to indirectly attach a further moiety, preferably a polypeptide, a drug or label to the penton base protomer and to present upon assembly of 60 penton base protomers of the present invention up to 60 further moieties on the surface of the assembled VLP. Accordingly, in a second aspect the present invention relates to an engineered polypeptide comprising, essentially consisting or consisting of at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and:

(i) a non-adenoviral peptide, and/or

(ii) is covalently or non-covalently coupled to a drug or label.

The at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer comprised in the engineered polypeptide of the second aspect of the invention is throughout this specification also referred to as STICKER.

Surprisingly, a relatively small N-terminal fragment of adenovirus fibre protein was sufficient to specifically bind to a penton base protomer. The smaller the fibre fragment the bigger the moiety that can be attached to the penton base protomer. Furthermore, the reduction of the length of the adenoviral fibre fragment reduces the likelihood that a new immune response is elicited against adenovirus fibre and/or that fibre bound to VLPs are cleared by pre-existing anti-fibre antibody. It is, thus preferred that the fibre fragment has a length of 50 contiguous amino acids or less of N-terminal fibre sequence. It is more preferred that the length of the fragment is 40 amino acids or less, 35 amino acids or less, 30 amino acids or less, 25 amino acids or less or 20 amino acids or less. The minimal fibre amino acid sequence required for specific binding to the binding cleft of the penton base protomer is F-N-P-V-Y-P-Y. This minimal sequence is preferably flanked by other adenovirus fiber, preferably Ad3 amino acid sequence on both sides. This small fragment can be used for extending the versatility and/or the number or exposed epitope on the VLP surface. Alternatively, addition of the STICKER tag to any protein or epitope sequence enables their binding to the VLP surface. This is done by *in vitro* incubation of the STICKER containing protein with the VLP or by co-expression of both components in a baculovirus system.

It is preferred that the engineered polypeptide does not comprise any further fibre amino acid sequence contiguous with STICKER. More preferably the polypeptide of the second aspect of the invention does not comprise any other adenovirus proteins or polypeptides other than STICKER.

5 It has been surprisingly found that STICKER can be attached N- and/or C-terminally without interfering with its binding to a penton base protomer. Preferably, STICKER is attached to the N-terminus of a non-adenoviral polypeptide. This polypeptide can be any polypeptide for which it is desired to attach it to the surface of the VLPs of the present invention.The size of the polypeptide that is attached to STICKER is not particularly limited. It can be any size that
10 still allows specific binding to the fibre protein binding cleft of a penton base protomer. The engineered polypeptide may further comprise a peptide linker between the non-adenoviral polypeptide and STICKER. This may be required, if the non-adenoviral polypeptide has a size that prevents 60 of such polypeptides to bind via STICKER to the assembled VLP. A peptide linker may also be advantageous in situations in which the N- and/or C-terminus to which
15 STICKER is attached is buried within the polypeptide.

The majority of the human population has been exposed to human Ad5 and have memory B cells capable of mounting an immune response against human Ad5. Accordingly, if human Ad5 based protomers and/or fibre are comprised in the engineered proteins of the first and second aspect of the invention, the resulting VLPs are more likely to be cleared from the
20 circulation by a pre-existing immunity. Thus, in a preferred embodiment the adenoviral proteins comprised in the engineered polypeptide according to the first and/or second aspect of the invention is based on adenovirus penton and fibre proteins, respectively, from human or non-human great ape adenoviruses, preferably from Chimpanzee (Pan) adenoviruses, Gorilla (Gorilla) adenoviruses and orangutans (Pongo) adenoviruses, more preferably Bonobo (*Pan*
25 *paniscus*) and common Chimpanzee (*Pan troglodytes*).

It is particularly preferred that the engineered polypeptide comprising an adenovirus penton base protomer and the engineered polypeptide comprising at least one adenovirus penton base protomer binding fibre protein fragment are based on penton and fibre proteins, respectively, of adenovirus selected from the group consisting of hAd3, hAd4, hAd5, hAd7,
30 hAd11, hAd26, hAd35 and hAd49, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16, ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82, PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147 described in WO 2005/071093 and WO 2010/086189.

The engineered polypeptide of the first aspect of the invention is preferably based on the wild-type penton base protomer of SEQ ID NO: 1 to 14, i.e. SEQ ID NO: 1 to 14 reflects the sequence of the protein prior to modification according to alternative embodiments (i) to (vi) outlined above. It will be understood by the skilled person that the insertion of a target specific binding domain into the V-loop, first and/or second RGD loop will alter the sequence in that part of SEQ ID NO: 1 to 14. Similarly, the replacement of amino acids with coupling residues will also alter the amino acid sequence.

The modifications according to (i) and (ii) above require the modification of one or both of the RGD loops and/or the V loop. In a preferred embodiment of the engineered polypeptide of the invention the region to be modified are defined by consensus sequences common to the majority of adenoviruses. Thus, these consensus sequences are based on the alignment of several preferred penton base protomer amino acid sequences from adenoviral species and are, suitable to determine the N- and C-terminus respectively, of the part of the penton base protein to modify according to embodiment (i) or (ii) noted above. Preferably, the N-terminus of the first RGD-loop within the penton base protomer is defined as follows:

X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11} (SEQ ID NO: 15)

wherein

X_3 is selected from the group consisting of D, E and N, and is preferably D;

X_4 is selected from the group consisting of V, L, and I, and is preferably V;

X_5 is any amino acid, is preferably selected from the group consisting of A, D, E, K, S, and T, and is more preferably T;

X_6 is any amino acid, is preferably selected from the group consisting of A, D, E, and K, and is more preferably A;

X_7 is selected from the group consisting of F, Y, and W, and is preferably Y;

X_8 is selected from the group consisting of A, D, E, N, and Q, is preferably E or Q, and is more preferably E;

X_9 is any amino acid, preferably selected from the group consisting of A, D, E, N, and K, and is more preferably E;

X_{10} is selected from the group consisting of S or T, and is preferably S; and

X_{11} is any amino acid and constitutes the N-terminal amino acid of the first RGD loop;

and/or

the following sequence defines the C-terminus of the first RGD-loop and at the same time the N-terminus of the second RGD-loop within the penton base protomer:

X_{12}-X_{13}-X_{14}-X_{15}-X_{16} (SEQ ID NO: 16)

5

wherein

X_{12} is any amino acid and constitutes the C-terminal amino acid of the first RGD loop;

X_{13} is R;

X_{14} is G;

10 X_{15} is D; and

X_{16} is any amino acid and the N-terminal amino acid of the second RGD loop;

and/or

the following sequence constitutes the C-terminus of the second RGD-loop within the penton base protomer:

15

X_{15}-X_{16}-X_{17}-X_{18}-X_{19}-X_{20}-X_{21}-X_{22} (SEQ ID NO: 17);

wherein

X_{17} is any amino acid and constitutes the C-terminal amino acid of the second RGD loop;

X_{18} is selected from the group consisting of I, L and V, and is preferably I;

20 X_{19} is selected from the group consisting of D, E, K, N, Q, and V, is preferably Q or K, and is more preferably Q;

X_{20} is selected from the group consisting of C, G and P, and is preferably P;

X_{21} is selected from the group consisting of I, L and V, is preferably L or V and is more preferably L;

25 X_{22} is selected from the group consisting of D, E, S and T, is preferably E or T and is more preferably E;

X_{23} is selected from the group consisting of D, E, K, S and T, is preferably E, K or T, and is more preferably K; and

X_{24} is selected from the group consisting of D and E, and is preferably D.

30 Similarly, the following sequence defines the N-terminus of the V loop of a penton base protomer:

X_{25}-X_{26}-X_{27}-X_{28}-X_{29}-X_{30}-X_{31}-X_{32} (SEQ ID NO: 18).

wherein

X_{25} is selected from the group consisting of F, Y, and W, and is preferably F;

X_{26} is selected from the group consisting of H, K and R, and is preferably K;

X_{27} is selected from the group consisting of A, V, I, and L, and is preferably A;

X_{28} is selected from the group consisting of H, K, and R, and is preferably R;

5 X_{29} is selected from the group consisting of A, V, I, and L, and is preferably V;

X_{30} is selected from the group consisting of A, V, I, L and M, and is preferably M;

X_{31} is selected from the group consisting of A, V, I, and L, and is preferably V; and

X_{32} is any amino acid and constitutes the N-terminal amino acid of the V-loop;

and/or

10 the following sequence defines the C-terminus of the V loop

X_{33}-X_{34}-X_{35}-X_{36}-X_{37}-X_{38}-X_{39} (SEQ ID NO: 19)

wherein

15 X_{33} is any amino acid and constitutes the C-terminal amino acid of the V-loop;

X_{34} is selected from the group consisting of F, Y, and W, and is preferably Y;

X_{35} is selected from the group consisting of D, E, S and T, is preferably E or T and is more preferably E;

X_{36} is selected from the group consisting of F, Y, and W, and is preferably W;

20 X_{37} is selected from the group consisting of A, F, V, Y, and W, is preferably F or V and is more preferably F;

X_{38} is selected from the group consisting of D, E, S, and T, is preferably D, and E and is more preferably E; and

X_{39} is selected from the group consisting of F, Y, and W, and is preferably F.

25 X_{32} is the N-terminal amino acid and X_{33} is the C-terminal amino acid of the V loop. One or more or all amino acid of the V-loop may be replaced by the inserted target specific binding domain and/or the non-adenoviral polypeptide.

It has been set out above that the part of the penton base protomer specifically binding to STICKER is a non-contiguous epitope. Thus, preferably one or more of the following non-
30 contiguous peptides within the penton base protomer form the adenovirus fibre protein binding cleft (bold amino acids interact directly with fibre)

M-T-I-D-L-M-N-N-A-I-X_{40}-X_{41}-X_{42}-Y-**L**-X_{43}-X_{44}-G-**R**-Q-X_{45}-G-V-L-**E**-S (SEQ ID NO: 20);
W-**D**-P-X_{46}-T-X_{47}-**X_{48}**-**P**-G (SEQ ID NO: 46);

X_{49}-V-X_{50}-X_{51}-**Y**-X_{52}-X_{53} (SEQ ID NO: 47);
X_{54}-**X_{55}**-R-S-Y (SEQ II NO: 48); and/or
L-T-X_{56}-V-F-N-R-**F**-**P**-X_{57} (SEQ ID NO: 49)

wherein

5 X_{40} is selected from the group consisting of V, I, and L;
X_{41} is selected from the group consisting of E, and D;
X_{42} is selected from the group consisting of H, N and Q, preferably H, and N;
X_{43} is selected from the group consisting of K, E, R, Q, and A;
X_{44} is selected from the group consisting of V, L, and I, preferably V, and I;
10 X_{45} is selected from the group consisting of H, N and Q, preferably H, and N;
X_{46} is selected from the group consisting of V, I, L, E, or D, preferably V, and E;
X_{47} is selected from the group consisting of V, L, and I, preferably V, and I;
X_{48} is selected from the group consisting of M, T and S, preferably M and T;
X_{49} is selected from the group consisting of D, E, N, and Q, preferably D and N;
15 X_{50} is any amino acid, preferably selected from the group consisting of A, D, P, K and T;
X_{51} is selected from the group consisting of A, D, E, K and R, preferably A, E, and K;
X_{52} is selected from the group consisting of D, E, L, I, Q, and N, preferably, E, L, and Q;
X_{53} is selected from the group consisting of A, D, E, K, N, Q, and R, preferably A, E, N and K;
20 X_{54} is selected from the group consisting of K, R, S, and T, preferably K, S and T;
X_{55} is selected from the group consisting of A, D, E, G, K, N, Q, R, S, and T, preferably D, G, K, N, and S;
X_{56} is selected from the group consisting of H, K, and R, preferably H and R; and
X_{57} is selected from the group consisting of D and E.

25 In a preferred embodiment of the engineered polypeptide of the invention of each other the amino acid sequence of X_3 to X_{10} is independently selected from the group consisting of DVTAYEES (SEQ ID NO: 21), DVDAYENS (SEQ ID NO: 22), DVAEYEKS (SEQ ID NO: 23), DVEAYEKS (SEQ ID NO: 24), DVDAYEKS (SEQ ID NO: 25), DVSKYEAS (SEQ ID NO: 26), NVKAYEDS (SEQ ID NO: 27), DVKKYENS (SEQ ID NO: 28), DVDAYQAS (SEQ 30 ID NO: 29), and DVDAYQAS (SEQ ID NO: 30), the amino acid sequence of X_{18} to X_{24} is selected from the group consisting of IQPLEKD (SEQ ID NO: 31), IQPVEKD (SEQ ID NO: 32), IKPLEKD (SEQ ID NO: 33), IVPLTKD (SEQ ID NO: 34), IEPVETD (SEQ ID NO: 35) and IKPLTED (SEQ ID NO: 36), the amino acid sequence of X_{25} to X_{31} is selected from the group consisting of FKARVMV (SEQ ID NO: 37), FRAKLMV (SEQ ID NO: 38), and

FRAKVMV (SEQ ID NO: 39), the amino acid sequence of X_{33} to X_{39} is selected from the group consisting of YEWFEF (SEQ ID NO: 40), YEWVEF (SEQ ID NO: 41), and YEWAEF (SEQ ID NO: 42).

It has been surprisingly found that large heterologous polypeptides can be inserted and or replace the first and/or second RGD loop without disrupting assembly of penton and subsequently the VLPs of the invention.

In a preferred embodiment of the engineered polypeptide of the invention each of the target specific binding domain of the first RGD loop independently of each other has a length of between 5 to 300 amino acids, preferably between 6 to 200 amino acids; the target specific binding domain of the second RGD loop has a length of between 5 to 300 amino acids, preferably between 10 to 200 amino acid; and/or the target specific binding domain in the V loop has a length of between 5 to 300 amino acids, preferably between 10 to 200 amino acid.

In one alternative the target bound by the target specific binding domain is a moiety present on the surface of a cell or in the extracellular matrix. The specificity of the target specific binding domain is chosen, if the VLPs are targeted to a specific cell type in order to deliver its payload, e.g. drug or label. In an alternative preferred embodiment of the engineered polypeptide of the invention the at least one target specific binding domain is capable of specifically binding to an immunogenic peptide, pathogen neutralizing peptide, viral peptide, bacterial peptide, immune-modulating peptide, cancer peptide, to the surface of a cell, preferably a cellular receptor, a low molecular weight tag, preferably biotin or chitin. This provides an alternative and rapid way of binding various peptides to the surface of the VLPs.

In a preferred embodiment of the engineered polypeptide of the first or second aspect of the invention the non-adenoviral polypeptide or the polypeptide inserted or attached is selected from the group consisting of immunogenic peptides, pathogen neutralizing peptides, viral peptides, bacterial peptides, immune-modulating peptides, and cancer peptides. Particularly preferred are viral peptides of Dengue HAKKQDVVVLGSQEGAM (SEQ ID NO: 55), Chikungunya STKDNFNVYKATRPYLAH (SEQ ID NO: 56) and of Zika virus STKDNFNVYKATRPLAH (SEQ ID NO: 57). Examples of the engineered polypeptide of the second aspect of the invention comprising STICKER and a Chikungunya peptide are AKRARLSTSFNPVPYEDESSTKDNFNVYKATRPYLAH (SEQ ID NO: 58), AKRARLSTSFNPVPYEDE**C**SSTKDNFNVYKATRPYLAH (SEQ ID NO: 59) and AKRARLST**C**FNPVPYEDESSTKDNFNVYKATRPYLAH (SEQ ID NO: 60). The latter two examples comprise a coupling residue, i.e. Cys to form a covalent bond to a corresponding coupling residue in the binding cleft for fibre of the penton base protomer.

Preferred examples of target specific binding domains are antibodies, single chain antibodies, antibody fragments, nanobodies, light or heavy chains, variable light or variable heavy chains, diabodies, or antibody mimetics. Preferred antibody fragments comprise a fragment antigen binding (Fab) fragment, a Fab' fragment, a $F(ab')_2$ fragment, a heavy chain 5 antibody, a single-domain antibody (sdAb), a single-chain fragment variable (scFv), a fragment variable (Fv), a V_H domain, a V_L domain, a single domain antibody, a nanobody, an IgNAR (immunoglobulin new antigen receptor), a di-scFv, a bispecific T-cell engager (BITEs), a dual affinity re-targeting (DART) molecule, a triple body, a diabody, a single-chain diabody, an alternative scaffold protein, and a fusion protein thereof.

10 If a non-adenoviral peptide or the peptide is inserted into the first RGD loop they preferably have a length of between 5 to 60 amino acids, preferably of between 6 to 45 amino acids. If the non-adenoviral peptide or the peptide is inserted into the second RGD loop they can have a length of between 5 to 50 amino acids, preferably between 10 to 36 amino acids. If the non-adenoviral peptide or the peptide is inserted into the second V loop they can have a 15 length of between 5 to 30 amino acids, preferably between 10 to 21 amino acids.

In a preferred embodiment of the engineered polypeptide of the first or second aspect invention the non-adenoviral polypeptide or the polypeptide comprises a protease cleavage site, preferably a sequence specific endopeptidase cleavage site, more preferably a TEV cleavage site. A preferred example of such a TEV cleavage site is ENLYFQG (SEQ ID NO: 60). Such a 20 cleavage site allows cleavage of the polypeptide of the first aspect of the invention once assembled into penton or VLP. Some antigens require exposure of the free N- and/or C-terminus to elicit an immune response. Thus, if penton proteins or VLPs have been assembled treatment of those with a protease will expose the N- and/or C-terminal sequence of such antigens, if the cleavage site is positioned at the N- and/or C-terminal end of such a non-25 adenoviral polypeptide. Quite surprisingly the present inventors have found such cleavage does not disrupt the assembled penton or VLP. This is extremely useful in situations in which the a strong antigen specific immune response requires the exposure of free N- and/or C-terminii of the antigen. Alternatively, the cleavage sit can be comprised in an engineered polypeptide of the first and/or second aspect of the invention to facilitate purification of the engineered 30 polypeptide, e.g. it can be placed at the N- or C-terminus of the respective engineered polypeptide separating the penton or fibre comprising part of the engineered polypeptide from an affinity tag, e.g. biotin, chitin binding protein, Myc-taq. Such an affinity tag allows immobilization of the engineered polypeptide on a suitable affinity matrix and release of purified engineered polypeptide from the matrix.

In a preferred embodiment of the engineered polypeptide of the first or second aspect of the invention the coupling residue is selected from the group comprising Lys, Cys, Asp, and Glu, preferably Cys.

In a preferred embodiment of the engineered polypeptide of the first or second aspect of 5 the invention the drug is selected from the group of chemotherapeutic drug, antipathogenic drug, immune modulating drug, and anti-inflammatory drug.

In a preferred embodiment of the engineered polypeptide of the second aspect of the invention the fibre protein fragment comprises, consists essentially of or consists of:

X_{58}-F-N-P-V-Y-P-Y-X_{59} (SEQ ID NO: 43)

10 wherein

X_{58} is selected from the group consisting of S, D and T, preferably S or D, and is more preferably S; and

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E.

15 Preferably, the fibre protein fragment has a length between 9 to 20 contiguous amino acids of fibre.

It is further preferred that the engineered polypeptide of the second aspect of the invention comprises 2, 3, 4, 5, 6, 7, or 8 repeats of the fragment of the fibre protein. The multimerization increases binding affinity. It has been observed by the present inventors that 2 or 3, preferably 20 consecutive repeats are suitable to mediate binding to the fibre binding cleft on the penton base protomer with subnanomolar affinities. Preferably, the multimers are arranged in a head-to-tail orientation.

As has been set out above, it is preferred according to alternative embodiment (vi) that one or more coupling residues are comprised in the binding cleft of the penton base protomer. 25 To facilitate formation of covalent bonds between these one or more coupling residues in the penton base protomer and coupling residues comprised in the engineered polypeptide of the second aspect of the invention. In a preferred embodiment of the engineered polypeptide of the second aspect of the invention the at least one coupling residue is inserted into and/or positioned at the N- and/or C-terminus of the fibre protein fragment, preferably inserted into and/or 30 positioned at the N- and/or C-terminus of SEQ ID NO: 43 or attached to an amino acid of the fibre protein fragment. As has been set out above coupling residues have to form covalent bonds with corresponding coupling residues. Once two polypeptides interact it is preferred that the coupling residue of the one polypeptide is sterically close to the coupling residue in the other polypeptide.

In a preferred embodiment of alternative embodiment (vi) the coupling residue is positioned in the penton base protomer comprised in the engineered polypeptide of the first aspect of the invention at amino acid position 476 and/or 477 (with reference to the amino acid sequence of SEQ ID NO: 64) or at an analogous amino acid position of a penton base protomer
5 of another adenovirus. The analogous position in another adenovirus penton base protomer can be determined by aligning the sequence according to SEQ ID NO: 64 with other adenoviral penton base protomer sequences with a standard alignment tool, like, e.g. CLUSTAL. The skilled person can easily determine the amino acid that occupies an analogous position to amino acids 476 or 477 in another adenovirus penton protein. It is preferred that the engineered protein
10 according to the first aspect of the invention and according to alternative embodiment (iv) comprises the sequence

KSFX_{64}N$X_{c1}$$X_{c2}$AVY (SEQ ID NO: 68)

wherein

15 X_{64} is selected from the group consisting of Y and F, preferably Y;

X_{c1} is selected from the group consisting of D, E and a coupling residue, preferably Cys;

X_{c2} is selected from the group consisting of L, Q and a coupling residue, preferably Cys;

and wherein at least one, preferably both X_{c1} and X_{c2} are a coupling residue, preferably Cys.

If the coupling residue in the penton base protomer is positioned at either amino acid
20 position 476 and/or 477 or at an analogous amino acid position of a penton base protomer of another adenovirus than it is preferred that a corresponding coupling reside of the engineered polypeptide of the second aspect of the invention comprises a coupling residue at the C-terminus of the STICKER part of the polypeptide. It is preferred that the coupling residue is located in STICKER as shown in below sequence:

25

X_{58}-F-N-P-V-Y-P-Y-X_{59}-$(X_{63})_n$-X_c (SEQ ID NO: 69)

wherein

X_{58} is selected from the group consisting of S, D and T, preferably S or D, and is more preferably S; and

30 X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E;

X_{63} is in each case independently any amino acid, preferably those naturally occurring in fibre proteins at this or these positions;

X_c is a coupling residue, preferably C, D, E, and K, most preferably C; and

n is an integer between 0 to 10, i.e. 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, preferably between 1 and 5, more preferably 2.

Thus, the engineered protein of the second aspect of the invention comprises in a preferred embodiment a STICKER polypeptide according to SEQ ID NO: 69. A particularly preferred
5 STICKER polypeptide that may be comprised in the engineered protein of the second aspect of the invention is

A-K-R-A-R-L-S-T-X_{58}-F-N-P-V-Y-P-Y-X_{59}-D-E-X_c (SEQ ID NO: 76)

wherein

10 X_{58} is selected from the group consisting of S, D and T, preferably S or D, and is more preferably S;

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E; and

X_c is a coupling residue, preferably C, D, E, and K, most preferably C.

15 In a particularly preferred embodiment the STICKER polypeptide comprises the coupling residue Cys at position 20 and consists of the following amino acid sequence:
A-K-R-A-R-L-S-T-S-F-N-P-V-Y-P-Y-E-D-E-C (SEQ ID NO: 77).

In an alternative preferred embodiment of alternative embodiment (vi) the coupling residue is positioned in the penton base protomer comprised in the engineered polypeptide of
20 the first aspect of the invention at Lys376 of the penton base protomer according to SEQ ID NO: 64 or an analogous position of a penton base protomer of another adenovirus. The engineered protein of the first aspect preferably comprises the following sequence:

X_c-X_{65}-R-S-Y-N (SEQ ID NO: 73)

25 wherein

X_c is a coupling residue, preferably C, D, E, and K, most preferably C; and

X_{65} is any amino acid, is preferably selected from the group consisting of D, E, G, K, N, or S, more preferably S or N.

If the coupling residue is comprised at this position it is preferred that the engineered
30 polypeptide of the second aspect of the present invention comprises a corresponding coupling residue as indicated in below amino acid sequence:

X_c-F-N-P-V-Y-P-Y-X_{59} (SEQ ID NO: 70)

wherein

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E; and/or

X_c is a coupling residue preferably C, D, E, and K, most preferably C.

Accordingly, the engineered protein of the second aspect of the invention comprises in a
5 preferred embodiment a STICKER polypeptide according to SEQ ID NO: 70. A particularly preferred STICKER polypeptide that may be comprised in the engineered protein of the second aspect of the invention is

A-K-R-A-R-L-S-T-X_c-F-N-P-V-Y-P-Y-X_{59}-D-E-S (SEQ ID NO: 74)

10 wherein

X_c is a coupling residue, preferably C, D, E, and K, most preferably C; and

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E.

In a particularly preferred embodiment the STICKER polypeptide comprises the coupling
15 residue Cys at position 9 and consists of the following amino acid sequence:
A-K-R-A-R-L-S-T-C-F-N-P-V-Y-P-Y-E-D-E-S (SEQ ID NO: 75).

In some embodiments of the engineered polypeptide of the first aspect of the invention it is desired that the RGD motif positioned between the first and second RGD loop is intact to facilitate binding of the penton base protomer, penton or the VLP to certain cellular and
20 extracellular structures present in a patient. Alternatively, if such targeting is not desired in a particular application of the engineered polypeptide of the first aspect of the invention, the RGD motif may be mutated in such that it loses its ability to bind to integrin.

In a third aspect the present invention relates to a nucleic acid encoding the engineered polypeptide of the first aspect of the invention and/or the engineered polypeptide of the second
25 aspect of the invention.

In a fourth aspect the present invention relates to an expression vector comprising the nucleic acid of the invention. Expression vectors comprise plasmids as well as viral vectors and contain a coding sequence encoding the engineered protein of the first and/or second aspect of the invention and appropriate DNA sequences necessary for the expression of the operably
30 linked coding sequence in a particular host organism (e.g., bacteria, yeast, plant, insect, or mammal) or in *in vitro* expression systems. Cloning vectors are generally used to engineer and amplify a certain desired DNA fragment and may lack functional sequences needed for expression of the desired DNA fragments.

It has been realized in the art that immunization against diseases that rapidly change their antigenic epitopes, e.g. influenza, or that are characterized by a patient specific epitope mixture require rapid adaption or individualization of the vaccine. The VLPs of the present invention can be rapidly adapted to present the respectively desired antigens. Accordingly, the present invention relates in a fifth aspect to a cloning vector amenable to rapid insertion of nucleic acid segments into the first and/or second RGD-loop or V-loop, which encode one or more desired antigen. The cloning vector of this aspect of the invention comprises:

(i) a polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop and/or a binding site for adenovirus fibre protein adapted for introducing nucleic acids encoding non-adenoviral peptides into the nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop; or

(ii) polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment adapted for introducing nucleic acids encoding non-adenoviral peptides a the C- and/or N-terminus.

In a preferred embodiment cloning vector of the present invention the adaptation comprises one or more restriction enzyme sites, preferably BamHI, KpnI, KasI, NarI, SfdI, EcoRI and RsrII, PfoI, BssHII, SalI, SacI, XbaI, BstEII, and HindIII. The nucleic acid sequence of preferred examples of such a cloning vector is provided in SEQ ID NO: 61 and 62. The structure of these vectors termed pACEBac-ADDOmer1.0 and pACEBac-ADDOmer2.0 is provided in Figures 7 and 8. The sequence of the cloning vector pACEBac-ADDOmer2.0 comprising the preferred Chikungunya virus antigenic epitope is provided in SEQ ID NO: 71 and 72.

In a sixth aspect the present invention relates to a recombinant host cell comprising the expression vector of the present invention or the cloning vector of the present invention. The expression vector of the present invention or the cloning vector of the present invention may be found inside the host cell (i) freely dispersed as such, or (ii) integrated into the host cell genome or mitochondrial DNA. The recombinant host cell is used for expression of the engineered polypeptides of the invention. The term "recombinant host cell" includes the progeny of the original cell which has been transformed, transfected, or infected with the polynucleotide or the recombinant vector of the invention. A recombinant host cell may be a bacterial cell such as an *E. coli* cell, a yeast cell such as *Saccharomyces cerevisiae* or *Pichia pastoris*, a plant cell, an insect cell such as SF9 or Hi5 cells, or a mammalian cell. Preferred examples of mammalian

cells are Chinese hamster ovary (CHO) cells, green African monkey kidney (COS) cells, human embryonic kidney (HEK293) cells, HELA cells, and the like.

The engineered polypeptides of the first aspect of the present invention are capable in spite of their modification according to alternative embodiments (i) to (vi) in comparison to the respective wild-type penton base protomers to assembe into a penton subunit, i.e. to form a pentamer. Whether a given engineered protein of the first aspect of the invention assembles into pentamers can be easily assessed by methods well known to the skilled person that comprise non-denaturing polyacrylamide gel electrophoresis, size exclusion chromatography, mass spectroscopy or the like. Thus, in a seventh aspect the present invention relates to a pentamer comprising five engineered polypeptides comprising adenovirus penton base protomer of the invention. This pentamer may additionally comprise between 1 and engineered polypeptides of the second aspect of the present invention.

The pentamers assembled from the engineered polypeptides of the first aspect of the present invention are further capable of assembly into a VLP. Accordingly, in an eight aspect the present invention relates to a virus-like particle (VLP) comprising 12 pentamers of the present invention. The VLP is stable and the most suitable composition for administration to a patient. Preferably, the VLP is assembled and/or stored under non-reducing conditions to allow the formation of covalent bonds between coupling residues in the penton base protomers.

In a preferred embodiment the VLP of the present invention further comprises at least one engineered polypeptide of the second aspect of the invention, preferably up to 60 engineered polypeptide of the second aspect of the invention. In the latter embodiment all fibre protein binding clefts of the penton base proteins of the engineered polypeptide of the first aspect of the invention are occupied by engineered proteins of the second aspect of the invention. It is preferred that the VLPs of the present invention comprise an engineered protein of the first aspect of the invention comprising the modification according to alternative (vi), preferably combined with a modification according to alternative (i), (ii), (iii), (iv), and/or (v) and an engineered protein of the second aspect of the invention comprising at least one coupling residue, preferably the at least one coupling residue in inserted into and/or positioned at the N- and/or C-terminus of the fibre protein fragment, preferably inserted into and/or positioned at the N- and/or C-terminus of SEQ ID NO: 43 or attached to an amino acid of the fibre protein fragment. Preferably, the engineered protein of the first aspect of the invention comprises KSFX_{64}N$X_{c1}X_{c2}$AVY (SEQ ID NO: 68), wherein X_{64}, X_{c1}, and X_{c2} have the meaning outlined above and the engineered protein of the second aspect of the invention comprises X_{58}-F-N-P-V-Y-P-Y-X_{59}-$(X_{63})_n$-X_c (SEQ ID NO: 69), wherein X_{58}, X_{59}, X_{63}, X_c, and n have the

meaning outlined above. In an alternative preferred embodiment the engineered protein of the first aspect of the invention comprises the following sequence X_c-X_{65}-R-S-Y-N (SEQ ID NO: 73), wherein X_c and X_{65} have the meaning outlined above and the engineered protein of the second aspect of the invention comprises X_c-F-N-P-V-Y-P-Y-X_{59} (SEQ ID NO: 70), wherein
5 X_c and X_{59} have the meaning outlined above.

Preferably, the VLPs which comprise coupling residues to covalently couple the engineered polypeptide of the first aspect of the invention to the engineered polypeptide second present invention is assembled and/or stored under non-reducing conditions to allow the formation of covalent bonds between coupling residues in the penton base protomers and the
10 STICKER polypeptide comprised in the engineered polypeptide of the second aspect of the invention.

It is also envisioned that VLPs consisting of or comprising wild-type penton base protomers are used to provide a vehicle and that these are decorated with different non-adenoviral polypeptides by using the engineered polypeptide of the second aspect of the
15 invention. Due to the short length that the engineered polypeptide of the second aspect of the invention has in preferred embodiments, it can be synthesized, e.g. by solid state chemistry within less than a day. Thus, in a ninth aspect the present invention relates to a VLP comprising 12 pentamers each comprising five adenovirus penton base protomers and at least one engineered polypeptide of the second aspect of the invention. It is preferred that all fibre protein
20 binding clefts of the penton base protomers are occupied and, thus that these VLPs comprise 60 engineered polypeptides of the second aspect of the invention.

In a tenth aspect the present invention relates to a method for producing an engineered polypeptide of the first or second aspect of the present invention, comprising the steps of:

(a) providing a recombinant host cell of the present invention;
25 (b) expressing the engineered polypeptide; and
(c) purifying the engineered polypeptide.

In an eleventh aspect the method for producing a VLP of the invention comprising the steps of the method of the tenth aspect of the invention and the further step of allowing the engineered polypeptides to assemble into a VLP.

30 The method of the tenth aspect of the present invention further comprising the step of incubating the VLP with a protease, preferably a sequence specific endopeptidase cleavage site, more preferably TEV.

In a twelfth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:

(a) providing a cloning vector of the invention;

5 (b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral peptides into nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop of the adenovirus penton base protomer, and/or at nucleic acid position preceding or subsequent 10 to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment;

(d) expressing the engineered adenovirus penton base protomer in a host cell, optionally together with the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment; and

15 (e) purifying said VLP optionally comprising an adenovirus penton base protomer binding fibre protein fragment, or said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment.

In a thirteenth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral peptides, comprising the 20 steps of:

(a) providing a cloning vector of the invention;

(b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral peptides at 25 nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment;

(d) expressing the engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment in a host cell, optionally together with an adenovirus 30 penton base protomer; and

(e1) purifying said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment and admixing with adenovirus penton base protomers or engineered adenovirus penton base protomers of the invention; or

(e2) purifying said VLP in case that the adenovirus penton base protomer was co-expressed.

In a fourteenth aspect the present invention relates to a method for producing a VLP of the invention comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:

(a) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(b) synthesizing an engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment and at least one of said non-adenoviral peptides; and

(c) admixing said engineered polypeptide with adenovirus penton base protomers or engineered adenovirus penton base protomers of the invention with pentamers of the invention or with VLPs of the invention.

In a fifteenth aspect the present invention relates to a VLP producible by a method of producing a VLP of the invention.

In a sixteenth aspect the present invention relates to a pharmaceutical composition comprising the engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment of the invention, the nucleic acid encoding one or more of the engineered proteins of the invention, the expression vector of the invention or the VLP of the invention, and a pharmaceutically acceptable carrier and/or suitable excipient(s). Preferably, such composition is a pharmaceutical composition. In preferred embodiments the pharmaceutical composition further comprises a pharmaceutically acceptable carrier and/or excipient and optionally one or more additional active substances. Preferably, the composition of the fifth aspect contains a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier and/or excipient so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

The pharmaceutical compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The pharmaceutical composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides.

For preparing pharmaceutical compositions of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form compositions include powders, tablets, pills, capsules, lozenges, cachets, suppositories, and dispersible granules. A solid excipient can be one or more substances, which may also act as diluents, flavouring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material. In powders,

the excipient is preferably a finely divided solid, which is in a mixture with the finely divided inhibitor of the present invention. In tablets, the active ingredient is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired. Suitable excipients are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogeneous mixture is then poured into convenient sized moulds, allowed to cool, and thereby to solidify. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

Liquid form compositions include solutions, suspensions, and emulsions, for example, water, saline solutions, aqueous dextrose, glycerol solutions or water/propylene glycol solutions. For parenteral injections (e.g. intravenous, intraarterial, intraosseous infusion, intramuscular, subcutaneous, intraperitoneal, intradermal, and intrathecal injections), liquid preparations can be formulated in solution in, e.g. aqueous polyethylene glycol solution. A saline solution is a preferred carrier when the pharmaceutical composition is administered intravenously.

Preferably, the pharmaceutical composition is in unit dosage form. In such form the composition may be subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged composition, the package containing discrete quantities of the composition, such as packaged tablets, capsules, and powders in vials or ampoules. Also, the unit dosage form can be a capsule, an injection vial, a tablet, a cachet, or a lozenge itself, or it can be the appropriate number of any of these in packaged form.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

Furthermore, such pharmaceutical composition may also comprise other pharmacologically active substance such as but not limited to adjuvants and/or additional active ingredients. Adjuvants in the context of the present invention include but are not limited to inorganic adjuvants, organic adjuvants, oil-based adjuvants, cytokines, particulate adjuvants, virosomes, bacterial adjuvants, synthetic adjuvants, or synthetic polynucleotides adjuvants.

In a seventeenth aspect the present invention relates to an engineered polypeptide comprising an adenovirus penton base protomer of the invention and/or engineered polypeptide of the invention comprising an adenovirus penton base protomer binding fibre protein fragment, the nucleic acid encoding one or more of the engineered proteins of the invention, the expression vector of the invention or the VLP of the invention for treating and/or preventing an infectious disease, an immune disease or cancer.

EXAMPLES

ADDomers were designed and produced in very high yields (tens of grams per liter expression culture). A generic three-step protocol was established to purify ADDomer to homogeneity (see below). In a proof-of-concept project, it was experimentally established that highly immunogenic Chikungunya epitopes can be inserted into the functionalized loops of ADDomer, without perturbing particle formation or decreasing yield noticeably. ADDomers containing Chikungunya epitopes were purified to homogeneity and cell-based and animal studies were initiated to establish their potency as vaccine candidates. The ADDomers were validated with a range of techniques including electron microscopy, evidencing homogenously structured, discrete multimers (dodecahedra). Cysteine-disulfide chemistry was implemented to even further increase the already remarkable thermostability of ADDomer (elimination of cold chain requirement). Moreover, ADDomers were preparaed which contain not only peptide epitopes but also protein domains and entire proteins including high-affiny binders (nanobodies DARPins, antibody fragments) and are establishing efficient protocols to manufacture these in large scale. Triggered by the recent emergence of Zika virus, ADDomer-based Zika vaccine candidates were designed, and also ADDomers to potentially combat more than one disease simultaneously (combo vaccine). Cell-based and animal experiments to validate these are performed.

In the following, experiments and protocols are described to produce and validate ADDomer VLPs and ADDomer VLP vaccines.

1. ADDomer design

The atomic structure of naturally occurring dodecamer species (e.g. derived from Adenovirus Ad3 serotype) has been determined by X-ray crystallography (Szolajska E *et al.*, PLoS One. 2012;7(9):e46075 and Zubieta C *et al.*, Mol. Cell 2005: 17(1):121-35). Careful inspection of the atomic structures revealed the presence of extended loop structures. More precisely, one variable loop (denominated V-loop) and two regions in the so-called RGD-loop of the wild-type dodecahedron were indentified as potential sites of functionalization. Comparison of a number of dodecahedron protomers revealed a wide variability of the V-loop and the two RGD-loop regions throughout the species, both in length and in sequence composition, underscoring their potential. Using this information, we designed de novo a DNA sequence encoding for a synthetic designer dodecahedron promoter. BioBrick design (Shetty et al. J. Biol. End. 2008) was applied by introducing DNA sequences representing endo-nuclease cleavage sites, to facilitate designed variations (and even randomization) of the amino acids

representing the V-loop and the two RGD-loop regions (RGDloop2, RGDloop2). The protomer design was optimized iteratively until a protomer was identified that would give rise to recombinant dodecahedra (ADDomer) characterized by complete BioBrick design of the above described loop regions, while maintaining the high solubility and structure integrity of wild-
5 type human Ad3 serotype dodecahedron.

2. Engineering hyperstable ADDomer

ADDomer is already remarkably thermostable and can be stored at 37°C for protracted times, indicative of not requiring a cold-chain in remote areas with poor infrastructure.
10 Inspection of the crystal coordinates of native Ad3 particles revealed a so-called 'strand-swapping' region, where segments of the protomers extended to the vicinity of the adjacent protomers resulting in juxtaposition of amino acids that were within a distance that could allow covalent bond formation. We genetically substituted these amino acids in ADDomer with cysteines, such that two cysteines each coming from distinct protomers were within the distance
15 required for disulfide bond formation.

3. MultiBac-based ADDomer expression

Next, ADDomers were expressed by using the MultiBac system. The gene encoding for ADDomer was synthesized from scratch (SEQ ID NO: 63 and the encoded ADDomer is
20 provided in SEQ ID NO: 64) and inserted by classical cloning methods (restriction/ligation) into pACEBac, a transfer plasmid of the MultiBac system. MultiBac was developed by one of the inventors (Berger) specifically for the production of complex biologics such as ADDomer. Composite MultiBac virus containing the ADDomer gene was prepared (see Figures 7 and 8) and insect cell cultures infected following previously described protocols (Berger I et al. J Vis
25 Exp. (2013) (77):e50159 and Fitzgerald DJ *et al.* Nat Methods. (2006) 3(12):1021). ADDomer protein-containing cell pellets were prepared by centrifugation as described. Cell pellets were stored at -80 degrees. Expression of ADDomer with peptide or protein epitopes inserted, and also expression of hyperstable ADDomer, all gave rise to comparable, very high yields and homogeneously structured dodecahedral particles.

30

3. Neutralizing epitope

Example ADDomer-CHIKADDomer-based VLP vaccine candidate presenting multiple copies of the major neutralizing Chikungunya immune epitope HAKKQDVVVLGSQEGAM

(SEQ ID NO: 55) were constructed. The major neutralizing immune epitope is part of a Chikungunya envelope protein, and is a linear peptide epitope located at the extreme N-terminus (Kam YW *et al.* EMBO Mol Med. (2012); 4(4):330-4). In an overwhelming majority of patient sera, antibodies are found which react with this linear peptide epitope. ADDomer
5 provides the means to present linear epitopes either in a constrained fashion (bot N- and C-termini covalently linked to ADDomer scaffold), or in an uncontrained fashion (N-termini liberated by cleavage with a specific protease) or a combination of constrained or unconstrained epitopes, all the while preserving structural integrity of the ADDomer scaffold. Preferred arrangements used were the following:
10 AKRARLSTSFNPVPYEDESSTKDNFNVYKATRPYLAH (SEQ ID NO: 58), AKRARLSTSFNPVPYEDE**C**SSTKDNFNVYKATRPYLAH (SEQ ID NO: 59) and AKRARLST**C**FNPVPYEDESSTKDNFNVYKATRPYLAH (SEQ ID NO: 60). Nature-like presentation of the Chikungunya major neutralizing epitope was achieved by TEV protease-mediated cleavage of ADDomer presenting multiple copies of the epitope, each containing a
15 specific TEV cleavage site preceding the neutralizing epitope sequence, preserving thye native N-terminus. Similar approaches can be used for any epitope or peptide or protein domain displayed by ADDomer.

4. Purification of ADDomer and variants

20 *Spodoptera frugiperda* Sf21 insect cells pellets were lysed by freeze-thawing. Lysate was cleared by centrifugation following standard protocols for insect cells (Berger I *et al.* J Vis Exp. (2013) (77):e50159 and Fitzgerald DJ *et al.* Nat Methods. 2006 3(12):1021). Cleared supernatant was loaded on a 15 to 40% sucrose gradient and centrifuged utilizing a Beckman SW41 rotor overnight. Fractions of 1.1mL were collected from the top of the gradient and
25 loaded on denaturing SDS polyacrylamide gels (SDS-PAGE) for analyzing protein content and identifying fractions containing ADDomers for pooling Size exclusion chromatography (SEC) and/or ion-exchange (IEX) was then carried out after dialyzing away the sucrose.

5. Validation of ADDomer by electron microscopy

30 Purified ADDomer and ADDomer variants were visualized by negative-stain electron microscopy (EM) to assess their assembly status and their structural integrity. Standard mica-carbon preparation was utilized with ADDomer at approximately 0.1 mg/ml concentration prior to deposition on the support material. Samples were stained using 1% (wt/vol) sodium

silicotungstate (pH 7.0) and visualized on a JEOL electron microscope at 100 kV. Images were recorded and analysed was carried out using software supplied by Gatan.

For thermostability experiments, ADDomers were stored either frozen, at 4°C, at room temperature (RT) or at 37°C for one week. Electron microscopy showed that storage at RT or at 37°C resulted in correctly auto-assembled particles demonstrating their thermostability. Incubation of ADDomer (SEQ ID NO: 64) for 2H at 45°C resulted in reversible particle disassembling which reassemble when turned back at RT. This reversible dissociation was also observed by Thermal Shift Assay (Fig 10, see arrow) but irreversible dissociation was seen only for temperature above 50°C.

6. Design of animal (murine) experiments to assess ADDomer immunogenicity

For murine immune analysis of ADDomer-CHIK Chikungunya ADDomer VLP vaccine candidates, six weeks old BALB/c female mice were used. Four groups of eight mice were designated per immunization with ADDomer species (e.g, in case of Chikungunya VLP vaccine candidate: i) ADDomer, ii) ADDomer CHIK unconstrained epitope, iii) ADDomer CHIK constrained epitope, iv) isolated CHIK major neutralizing peptide epitope cross-linked to KLH as positive control). Each animal was injected with 10 µg of ADDomer and ADDomer variants at 2 week intervals. IgA, IgM, total IgG, IgG1 IgG2a and anti-CHIK antibodies were titered from mouse sera by ELISA. Immune analysis of other ADDomer VLP vaccine candidates were designed in an analogous fashion. Two kind of epitope display at the ADDomer surface were tested (Constrained and Relaxed, see below: point 7). A time dependent response was observed (Week 0 to 6). The superior potential of the relaxed form of the epitope over the constrained form to trigger an anti-Chik epitope response was shown (Fig 12).

7. Exposition of the epitope of interest at the ADDomer surface

The addition of a TEV cleavage site upstream the epitope of interest enables its display under two different configurations: constrained or relaxed. Upon purification, the epitopes are naturally constrained in the ADDomer loops. By addition of the TEV (Tobacco Etch Virus) protease (1/100 w:w) for 2H at RT the epitope can be relaxed and displayed in a linear form at the scaffold surface. The cleavage efficiency is easily monitored by SDS-PAGE. Of note, the overall ADDomer scaffold is not affected by this cleavage (Fig.11).

8. Extension of the epitope insertion capacity in ADDomer

The capacity of ADDomer to carry large epitope sequence was assessed. To this end, a 200 amino acid in length artificial epitope was inserted in ADDomer (named extended ADDomer). This resulted in correctly auto-assembled ADDomer. The insertion was confirmed by both SDS-PAGE analysis and mass spectroscopy as shown in Figure 13.

5

9. Covalent linkage of epitope at the ADDomer surface

To widen the ADDomer potential, a system enabling the addition of extra epitopes at the ADDomer was developed. Single cysteines were inserted at specific locationd of the ADDomer sequence (either K363C or Q476C or A477C). K363C was designed to form
10 covalent disulfide bridge with a fibre protein fragment of 20 amino acids length (peptide C20 (SEQ ID NO: 77) derived from SEQ ID NO: 59) while Q476C or A477C were designed to do the same with another fibre protein fragment (peptide C9 (SEQ ID NO: 75) derived from SEQ ID NO 60). The covalent interaction of the peptides with their corresponding Cys modified ADDomer was checked by incubating the particle with their corresponding peptide under
15 oxidative condition (i.e. absence of beta-mercaptoethanol) or reducing condition (addition of beta-mercaptoethanol). Complexes were run on SDS-PAGE and ADDomer was detected by a specific antibody and a secondary antibody labelled with Cy3 while the biotinylated peptide was detected by Alexa488 labeled avidin. When the right Cys-ADDomer/peptide was used, the presence of peptide was detected at the ADDomer band size (circle in Fig 14). This interaction
20 was specific of the disulfide bridge created between the Cys-ADDomer and the peptide since the interaction was prevented under reducing conditions.

The present invention relates to the following aspects:

25 1. An engineered polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), an adenovirus fibre protein binding cleft and/or a N-terminal domain, and comprises one or more of the following:
   (i) at least one target specific binding domain in the first, the second or both the first and
30 the second RGD-loops, and/or in the V loop; and/or
   (ii) one or more non-adenoviral peptides in the first, the second or both the first and the second RGD-loops and/or in the V loop; and/or
   (iii) a non-adenoviral peptide at the N- and/or C-terminus of the penton base protomer; and/or

(iv) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer, wherein the N-terminus of the N-terminal domain within the penton base protomer is defined as follows:

5 X_1-G-R-N-S-I-R (SEQ ID NO: 44)

and the C-terminus of the N-terminal domain within the penton base protomer is defined as follows:

D-X_2-R-S-R-G (SEQ ID NO: 45),

wherein

10 X_1 is selected from the group consisting of G and E, and

X_2 is selected from the group consisting of D and E; and/or

(v) a drug, label or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or

15 (vi) at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer

and wherein the engineered polypeptide is preferably capable of assembling into VLPs.

2. An engineered polypeptide comprising at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base
20 protomer and:

(i) a non-adenoviral peptide and/or

(ii) is covalently or non-covalently coupled to a drug or label.

3. The engineered polypeptide according to item 1 or 2, wherein the adenovirus is a human or non-human great ape adenovirus, preferably Chimpanzee (Pan), Gorilla (Gorilla) and
25 orangutans (Pongo), more preferably Bonobo (*Pan paniscus*) and common Chimpanzee (*Pan troglodytes*).

4. The engineered polypeptide according to item 3, wherein the adenovirus is selected from the group consisting of hAd3, hAd4, hAd5, hAd7, hAd11, hAd26, hAd35 and hAd49, ChAd3, ChAd4, ChAd5, ChAd6, ChAd7, ChAd8, ChAd9, ChAd10, ChAd11, ChAd16,
30 ChAd17, ChAd19, ChAd20, ChAd22, ChAd24, ChAd26, ChAd30, ChAd31, ChAd37, ChAd38, ChAd44, ChAd63 and ChAd82, PanAd1, PanAd2, PanAd3, ChAd55, ChAd73, ChAd83, ChAd146, and ChAd147.

5. The engineered polypeptide according to item 1 or 3 or 4, wherein the sequence of the wild-type penton base protomer on which the engineered protein is based is selected from the group consisting of SEQ ID NO: 1 to 14.
6. The engineered polypeptide according to item 1 or 3 to 5, wherein the following sequence defines the N-terminus of the first RGD-loop within the penton base protomer:

X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11} (SEQ ID NO: 15)

wherein

X_3 is selected from the group consisting of D, E and N, and is preferably D;

X_4 is selected from the group consisting of V, L, and I, and is preferably V;

X_5 is any amino acid, is preferably selected from the group consisting of A, D, E, K, S, and T, and is more preferably T;

X_6 is any amino acid, is preferably selected from the group consisting of A, D, E, and K, and is more preferably A;

X_7 is selected from the group consisting of F, Y, and W, and is preferably Y;

X_8 is selected from the group consisting of A, D, E, N, and Q, is preferably E or Q, and is more preferably E;

X_9 is any amino acid, preferably selected from the group consisting of A, D, E, N, and K, and is more preferably E;

X_{10} is selected from the group consisting of S or T, and is preferably S; and

X_{11} is any amino acid and constitutes the N-terminal amino acid of the first RGD loop;

and/or

the following sequence defines the C-terminus of the first RGD-loop and the N-terminus of the second RGD-loop within the penton base protomer:

X_{12}-X_{13}-X_{14}-X_{15}-X_{16} (SEQ ID NO: 16)

wherein

X_{12} is any amino acid and constitutes the C-terminal amino acid of the first RGD loop;

X_{13} is R;

X_{14} is G;

X_{15} is D; and

X_{16} is any amino acid and constitutes the N-terminal amino acid of the second RGD loop;

and/or

5 the following sequence defines the C-terminus of the second RGD-loop within the penton base protomer:

X_{17}-X_{18}-X_{19}-X_{20}-X_{21}-X_{22}-X_{23}-X_{24} (SEQ ID NO: 17);

wherein

10 X_{17} is any amino acid and constitutes the C-terminal amino acid of the second RGD loop;

X_{18} is selected from the group consisting of I, L and V, and is preferably I;

X_{19} is selected from the group consisting of D, E, K, N, Q, and V, is preferably Q or K, and is more preferably Q;

15 X_{20} is selected from the group consisting of C, G and P, and is preferably P;

X_{21} is selected from the group consisting of I, L and V, is preferably L or V and is more preferably L;

X_{22} is selected from the group consisting of D, E, S and T, is preferably E or T and is more preferably E;

20 X_{23} is selected from the group consisting of D, E, K, S and T, is preferably E, K or T, and is more preferably K; and

X_{24} is selected from the group consisting of D and E, and is preferably D;

and/or

25 the following sequence defines the N-terminus of the V loop:

X_{25}-X_{26}-X_{27}-X_{28}-X_{29}-X_{30}-X_{31}-X_{32} (SEQ ID NO: 18).

wherein

X_{25} is selected from the group consisting of F, Y, and W, and is preferably F;

30 X_{26} is selected from the group consisting of H, K and R, and is preferably K;

X_{27} is selected from the group consisting of A, V, I, and L, and is preferably A;

X_{28} is selected from the group consisting of H, K, and R, and is preferably R;

X_{29} is selected from the group consisting of A, V, I, and L, and is preferably V;

X_{30} is selected from the group consisting of A, V, I, L and M, and is preferably M;

X_{31} is selected from the group consisting of A, V, I, and L, and is preferably V; and
X_{32} is any amino acid and constitutes the N-terminal amino acid of the V loop;
and/or

5 the following sequence defines the C-terminus of the V loop

X_{33}-X_{34}-X_{35}-X_{36}-X_{37}-X_{38}-X_{39} (SEQ ID NO: 19)

wherein
10 X_{33} is any amino acid and constitutes the C-terminal amino acid of the V loop;
X_{34} is selected from the group consisting of F, Y, and W, and is preferably Y;
X_{35} is selected from the group consisting of D, E, S and T, is preferably E or T and is more preferably E;
X_{36} is selected from the group consisting of F, Y, and W, and is preferably W;
15 X_{37} is selected from the group consisting of A, F, V, Y, and W, is preferably F or V and is more preferably F;
X_{38} is selected from the group consisting of D, E, S and T, is preferably D or E and is more preferably E; and
X_{39} is selected from the group consisting of F, Y, and W, and is preferably F;
20

and/or one or more of the following non-continuous peptides within the penton base protomer form the adenovirus fibre protein binding cleft (bold amino acids interact directly with fibre)

25 M-T-I-D-L-M-N-N-A-I-X_{40}-X_{41}-X_{42}-Y-**L**-X_{43}-X_{44}-G-**R**-Q-X_{45}-G-V-L-**E**-S (SEQ ID NO: 20);
W-**D**-P-X_{46}-T-X_{47}-**X_{48}**-**P**-G (SEQ ID NO: 46);
X_{49}-V-X_{50}-X_{51}-**Y**-X_{52}-X_{53} (SEQ ID NO: 47);
X_{54}-**X_{55}**-R-S-Y (SEQ II NO: 48); and/or
30 L-T-X_{56}-V-F-N-R-**F**-**P**-X_{57} (SEQ ID NO: 49)

wherein
X_{40} is selected from the group consisting of V, I, and L;
X_{41} is selected from the group consisting of E, and D;
X_{42} is selected from the group consisting of H, N and Q, preferably H, and N;

X_{43} is selected from the group consisting of K, E, R, Q, and A;
X_{44} is selected from the group consisting of V, L, and I, preferably V, and I;
X_{45} is selected from the group consisting of H, N and Q, preferably H, and N;
X_{46} is selected from the group consisting of V, I, L, E, or D, preferably V, and E;
5 X_{47} is selected from the group consisting of V, L, and I, preferably V, and I;
X_{48} is selected from the group consisting of M, T and S, preferably M and T;
X_{49} is selected from the group consisting of D, E, N, and Q, preferably D and N;
X_{50} is any amino acid, preferably selected from the group consisting of A, D, P, K and T;
X_{51} is selected from the group consisting of A, D, E, K and R, preferably A, E, and K;
10 X_{52} is selected from the group consisting of D, E, L, I, Q, and N, preferably, E, L, and Q;
X_{53} is selected from the group consisting of A, D, E, K, N, Q, and R, preferably A, E, N and K;
X_{54} is selected from the group consisting of K, R, S, and T, preferably K, S and T;
X_{55} is selected from the group consisting of A, D, E, G, K, N, Q, R, S, and T, preferably
15 D, G, K, N, and S;
X_{56} is selected from the group consisting of H, K, and R, preferably H and R; and
X_{57} is selected from the group consisting of D and E.

7. The engineered polypeptide according to item 6, wherein independently of each other the amino acid sequence of X_3 to X_{10} is selected from the group consisting of DVTAYEES
20 (SEQ ID NO: 21), DVDAYENS (SEQ ID NO: 22), DVAEYEKS (SEQ ID NO: 23), DVEAYEKS (SEQ ID NO: 24), DVDAYEKS (SEQ ID NO: 25), DVSKYEAS (SEQ ID NO: 26), NVKAYEDS (SEQ ID NO: 27), DVKKYENS (SEQ ID NO: 28), DVDAYQAS (SEQ ID NO: 29), and DVDAYQAS (SEQ ID NO: 30), the amino acid sequence of X_{18} to X_{24} is selected from the group consisting of IQPLEKD (SEQ ID NO: 31), IQPVEKD
25 (SEQ ID NO: 32), IKPLEKD (SEQ ID NO: 33), IVPLTKD (SEQ ID NO: 34), IEPVETD (SEQ ID NO: 35) and IKPLTED (SEQ ID NO: 36), the amino acid sequence of X_{25} to X_{31} is selected from the group consisting of FKARVMV (SEQ ID NO: 37), FRAKLMV (SEQ ID NO: 38), and FRAKVMV (SEQ ID NO: 39), the amino acid sequence of X_{33} to X_{39} is selected from the group consisting of YEWFEF (SEQ ID NO: 40), YEWVEF (SEQ
30 ID NO: 41), and YEWAEF (SEQ ID NO: 42).

8. The engineered polypeptide according to any of items 1 or 3 to 7, wherein independently of each other the target specific binding domain of the first RGD loop has a length of between 5 to 300 amino acids, preferably between 6 to 200 amino acids; the target specific binding domain of the second RGD loop has a length of between 5 to 300 amino acids,

preferably between 10 to 200 amino acid; and/or the target specific binding domain in the V loop has a length of between 5 to 300 amino acids, preferably between 10 to 200 amino acid.

9. The engineered polypeptide according to any of items 1 or 3 to 8, wherein at least one of the target specific binding domain is capable of specifically binding to an immunogenic peptide, pathogen neutralizing peptide, viral peptide, bacterial peptide, immune-modulating peptide, cancer peptide, to the surface of a cell, preferably a cellular receptor, a low molecular weight tag, preferably biotin or chitin.
10. The engineered polypeptide according to any of items 1 to 9, wherein the non-adenoviral polypeptide or the polypeptide is selected from the group consisting of immunogenic peptides, pathogen neutralizing peptides, viral peptides, bacterial peptides, immune-modulating peptides, and cancer peptides.
11. The engineered polypeptide according to any of item 10, wherein the non-adenoviral peptide or the peptide comprises a protease cleavage site, preferably a sequence specific endopeptidase cleavage site, more preferably tobacco etch virus NIa protease (TEV).
12. The engineered polypeptide according to any of items 1 to 11, wherein the coupling residue is selected from the group comprising Lys, Cys, Asp, and Glu, preferably Cys.
13. The engineered polypeptide according to any of items 1 to 12, wherein the drug is selected from the group chemotherapeutic drug, antipathogenic drug, immune modulating drug, and anti-inflammatory drug.
14. The engineered polypeptide according to item 2, wherein the fibre protein fragment comprises:

X_{58}-F-N-P-V-Y-P-Y-X_{59} (SEQ ID NO: 43)

wherein

X_{58} is selected from the group consisting of S, D and T, preferably S or D, and is more preferably S; and

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is more preferably E.

15. The engineered polypeptide according to item 14, wherein the fibre protein fragment has a length between 9 to 20 amino acids.
16. The engineered polypeptide according to item 2 and 14 or 15, wherein at least one coupling residue in inserted into and/or positioned at the N- and/or C-terminus of the fibre

protein fragment, preferably inserted into and/or positioned at the N- and/or C-terminus of SEQ ID NO: 43 or attached to an amino acid of the fibre protein fragment.

17. A nucleic acid encoding the polypeptide according to any of items 1 to 16.
18. An expression vector comprising the nucleic acid of item 17.
19. A cloning vector encoding:
    (i) a polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop and/or a binding site for adenovirus fibre protein adapted for introducing nucleic acids encoding non-adenoviral peptides into the nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop; or
    (ii) polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer adapted for introducing nucleic acids encoding non-adenoviral peptides a the C- and/or N-terminus.
20. The cloning vector of item 19, wherein the adaptation comprises one or more restriction enzyme sites, preferably BamHI, KpnI, KasI, NarI, SfdI, EcoRI and RsrII, PfoI, BssHII, SalI, SacI, XbaI, BstEII, and HindIII.
21. A recombinant host cell comprising the expression vector of item 18 or the cloning vector of item 19 or 20.
22. A pentamer comprising five engineered polypeptides comprising adenovirus penton base protomer according items 1, 3 to 13.
23. A virus-like particle (VLP) comprising 12 pentamers according to item 22.
24. The VLP according to item 23, which further comprises at least one engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer according to items 2 to 4 and 10 to 16.
25. The VLP according to item 24, which further comprises at least one mutation in Cys in the amino acids residues such as G51C, S555C, G53C, Y64C, S54C, D114C.
26. A VLP comprising 12 pentamers each comprising five adenovirus penton base protomers and at least one engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer according to items 2 to 4 and 10 to 16.
27. A method for producing an engineered polypeptide according to any of items 1 to 16, comprising the steps of:

(a) providing a recombinant host cell of item 21;
(b) expressing the engineered polypeptide; and
(c) purifying the engineered polypeptide.

28. A method for producing a VLP according to any of items 23 to 26 comprising the steps of the method of item 27 and the further step of allowing the engineered polypeptides to assemble into a VLP.
29. The method of item 28 further comprising the step of incubating the VLP with a protease, preferably a sequence specific endopeptidase cleavage site, more preferably TEV.
30. A method for producing a VLP according to any of items 23 to 26 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:
    (a) providing a cloning vector of item 19 and/or 20;
    (b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;
    (c) inserting nucleic acids encoding at least one of said non-adenoviral peptides into nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop of the adenovirus penton base protomer, and/or at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer;
    (d) expressing the engineered adenovirus penton base protomer in a host cell, optionally together with the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer; and
    (e) purifying said VLP optionally comprising an adenovirus penton base protomer binding fibre protein fragment, or said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment.
31. A method for producing a VLP according to any of items 23 to 26 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:
    (a) providing a cloning vector of item 20;
    (b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;
    (c) inserting nucleic acids encoding at least one of said non-adenoviral peptides at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or

C-terminus of the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer;

(d) expressing the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer in a host cell, optionally together with an adenovirus penton base protomer; and

(e1) purifying said engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and admixing with adenovirus penton base protomers or engineered adenovirus penton base protomers of any of items 1 or 3 to 13; or

(e2) purifying said VLP in case that the adenovirus penton base protomer was co-expressed.

32. A method for producing a VLP according to any of items 23 to 26 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:

(a) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(b) synthesizing an engineered polypeptide according to any of items 2 to 4 and 10 to 16 comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and at least one of said non-adenoviral peptides; and

(c) admixing said engineered polypeptide with adenovirus penton base protomers or engineered adenovirus penton base protomers of any of items 1 or 3 to 10 with pentamers according to item 18 of with VLPs according to item 19.

33. A VLP producible by a method of any of items 28 to 32.

34. A pharmaceutical composition comprising the engineered polypeptides according to any of items 1 to 16, the nucleic acid of item 17, the expression vector of item 18 or the VLP of any of items 23 to 26 or 33, and a pharmaceutically acceptable carrier and/or suitable excipient(s).

35. An engineered polypeptide according to any of items 1 to 16, the nucleic acid of item 17, the expression vector of item 18 or the VLP of any of items 23 to 26 or 33 for treating and/or preventing an infectious disease, an immune disease or cancer.

CLAIMS

1. An engineered polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), an adenovirus fibre protein binding cleft and/or a N-terminal domain, and comprises one or more non-adenoviral peptides in the first, the second or both the first and the second RGD-loops and/or in the V loop; and optionally one or more of the following
   (i) at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop; and/or
   (ii) a non-adenoviral peptide at the N- and/or C-terminus of the penton base protomer; and/or
   (iii) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer, wherein the N-terminus of the N-terminal domain within the penton base protomer is defined as follows:

   X_1-G-R-N-S-I-R (SEQ ID NO: 44),

   and the C-terminus of the N-terminal domain within the penton base protomer is defined as follows:

   D-X_2-R-S-R-G (SEQ ID NO: 45),

   wherein
   X_1 is selected from the group consisting of G and E, and
   X_2 is selected from the group consisting of D and E; and/or
   (iv) a drug, label or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or
   (v) at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer,

   and wherein the engineered polypeptide is capable of assembling into VLPs.

2. An engineered polypeptide comprising at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and:
   (i) a non-adenoviral peptide and/or
   (ii) is covalently or non-covalently coupled to a drug or label.

3. The engineered polypeptide according to claim 1, wherein the following sequence defines the N-terminus of the first RGD-loop within the penton base protomer:

X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11} (SEQ ID NO: 15)

5

wherein

X_3 is selected from the group consisting of D, E and N, and is preferably D;

X_4 is selected from the group consisting of V, L, and I, and is preferably V;

X_5 is any amino acid, is preferably selected from the group consisting of A, D, E, K,

10 S, and T, and is more preferably T;

X_6 is any amino acid, is preferably selected from the group consisting of A, D, E, and K, and is more preferably A;

X_7 is selected from the group consisting of F, Y, and W, and is preferably Y;

X_8 is selected from the group consisting of A, D, E, N, and Q, is preferably E or Q, and

15 is more preferably E;

X_9 is any amino acid, preferably selected from the group consisting of A, D, E, N, and K, and is more preferably E;

X_{10} is selected from the group consisting of S or T, and is preferably S; and

X_{11} is any amino acid and constitutes the N-terminal amino acid of the first RGD loop;

20 and/or

the following sequence defines the C-terminus of the first RGD-loop and the N-terminus of the second RGD-loop within the penton base protomer:

25 X_{12}-X_{13}-X_{14}-X_{15}-X_{16} (SEQ ID NO: 16)

wherein

X_{12} is any amino acid and constitutes the C-terminal amino acid of the first RGD loop;

X_{13} is R;

30 X_{14} is G;

X_{15} is D; and

X_{16} is any amino acid and constitutes the N-terminal amino acid of the second RGD loop;

and/or

the following sequence defines the C-terminus of the second RGD-loop within the penton base protomer:

5 X_{17}-X_{18}-X_{19}-X_{20}-X_{21}-X_{22}-X_{23}-X_{24} (SEQ ID NO: 17);

wherein

X_{17} is any amino acid and constitutes the C-terminal amino acid of the second RGD loop;

X_{18} is selected from the group consisting of I, L and V, and is preferably I;

10 X_{19} is selected from the group consisting of D, E, K, N, Q, and V, is preferably Q or K, and is more preferably Q;

X_{20} is selected from the group consisting of C, G and P, and is preferably P;

X_{21} is selected from the group consisting of I, L and V, is preferably L or V and is more preferably L;

15 X_{22} is selected from the group consisting of D, E, S and T, is preferably E or T and is more preferably E;

X_{23} is selected from the group consisting of D, E, K, S and T, is preferably E, K or T, and is more preferably K; and

X_{24} is selected from the group consisting of D and E, and is preferably D;

20 and/or

the following sequence defines the N-terminus of the V loop:

X_{25}-X_{26}-X_{27}-X_{28}-X_{29}-X_{30}-X_{31}-X_{32} (SEQ ID NO: 18).

25 wherein

X_{25} is selected from the group consisting of F, Y, and W, and is preferably F;

X_{26} is selected from the group consisting of H, K and R, and is preferably K;

X_{27} is selected from the group consisting of A, V, I, and L, and is preferably A;

X_{28} is selected from the group consisting of H, K, and R, and is preferably R;

30 X_{29} is selected from the group consisting of A, V, I, and L, and is preferably V;

X_{30} is selected from the group consisting of A, V, I, L and M, and is preferably M;

X_{31} is selected from the group consisting of A, V, I, and L, and is preferably V; and

X_{32} is any amino acid and constitutes the N-terminal amino acid of the V loop;

and/or

the following sequence defines the C-terminus of the V loop

X_{33}-X_{34}-X_{35}-X_{36}-X_{37}-X_{38}-X_{39} (SEQ ID NO: 19)

5

wherein

X_{33} is any amino acid and constitutes the C-terminal amino acid of the V loop;

X_{34} is selected from the group consisting of F, Y, and W, and is preferably Y;

X_{35} is selected from the group consisting of D, E, S and T, is preferably E or T and is
10 more preferably E;

X_{36} is selected from the group consisting of F, Y, and W, and is preferably W;

X_{37} is selected from the group consisting of A, F, V, Y, and W, is preferably F or V and is more preferably F;

X_{38} is selected from the group consisting of D, E, S and T, is preferably D or E and is
15 more preferably E; and

X_{39} is selected from the group consisting of F, Y, and W, and is preferably F;

and/or one or more of the following non-continuous peptides within the penton base protomer form the adenovirus fibre protein binding cleft (bold amino acids interact
20 directly with fibre)

M-T-I-D-L-M-N-N-A-I-X_{40}-X_{41}-X_{42}-Y-**L**-X_{43}-X_{44}-G-**R**-Q-X_{45}-G-V-L-**E**-S
(SEQ ID NO: 20);

W-**D**-P-X_{46}-T-X_{47}-**X_{48}**-**P**-G (SEQ ID NO: 46);

25 X_{49}-V-X_{50}-X_{51}-**Y**-X_{52}-X_{53} (SEQ ID NO: 47);

X_{54}-**X_{55}**-R-S-Y (SEQ II NO: 48); and/or

L-T-X_{56}-V-F-N-R-**F**-**P**-X_{57} (SEQ ID NO: 49)

wherein

X_{40} is selected from the group consisting of V, I, and L;

30 X_{41} is selected from the group consisting of E, and D;

X_{42} is selected from the group consisting of H, N and Q, preferably H, and N;

X_{43} is selected from the group consisting of K, E, R, Q, and A;

X_{44} is selected from the group consisting of V, L, and I, preferably V, and I;

X_{45} is selected from the group consisting of H, N and Q, preferably H, and N;

X_{46} is selected from the group consisting of V, I, L, E, or D, preferably V, and E;
X_{47} is selected from the group consisting of V, L, and I, preferably V, and I;
X_{48} is selected from the group consisting of M, T and S, preferably M and T;
X_{49} is selected from the group consisting of D, E, N, and Q, preferably D and N;
5 X_{50} is any amino acid, preferably selected from the group consisting of A, D, P, K and T;
X_{51} is selected from the group consisting of A, D, E, K and R, preferably A, E, and K;
X_{52} is selected from the group consisting of D, E, L, I, Q, and N, preferably, E, L, and Q;
X_{53} is selected from the group consisting of A, D, E, K, N, Q, and R, preferably A, E, N and K;
10 X_{54} is selected from the group consisting of K, R, S, and T, preferably K, S and T;
X_{55} is selected from the group consisting of A, D, E, G, K, N, Q, R, S, and T, preferably D, G, K, N, and S;
X_{56} is selected from the group consisting of H, K, and R, preferably H and R; and
X_{57} is selected from the group consisting of D and E.

15

4. The engineered polypeptide according to claim 2, wherein the fibre protein fragment comprises:

X_{58}-F-N-P-V-Y-P-Y-X_{59} (SEQ ID NO: 43)

20

wherein

X_{58} is selected from the group consisting of S, D and T, preferably S or D, and is more preferably S; and

X_{59} is selected from the group consisting of E, D and G, is preferably E or D, and is
25 more preferably E.

5. A nucleic acid encoding the polypeptide according to any of claims 1 to 4.

6. An expression vector comprising the nucleic acid of claim 5 or a cloning vector
30 encoding:

(i) a polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop and/or a binding site for adenovirus fibre protein adapted for introducing nucleic

acids encoding non-adenoviral peptides into the nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop; or

(ii) polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base
5 protomer adapted for introducing nucleic acids encoding non-adenoviral peptides a the C- and/or N-terminus.

7. A recombinant host cell comprising the expression vector of claim or the cloning vector of claim 6.

10

8. A pentamer comprising five engineered polypeptides comprising adenovirus penton base protomer according claims 1, or 3.

9. A virus-like particle (VLP) comprising 12 pentamers according to claim 6.

15

10. A VLP comprising 12 pentamers each comprising five adenovirus penton base protomers and at least one engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer according to claims 2 or 4.

20

11. A method for producing an engineered polypeptide according to any of claims 1 to 4, comprising the steps of:
(a) providing a recombinant host cell of claim 7;
(b) expressing the engineered polypeptide; and
25 (c) purifying the engineered polypeptide.

12. A method for producing a VLP according to claim 9 or 10 comprising the steps of the method of claim 11 and the further step of allowing the engineered polypeptides to assemble into a VLP.

30

13. A method for producing a VLP according to claim 9 or 10 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:
(a) providing a cloning vector of claim 6;

(b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral peptides into nucleic acids encoding the first RGD-loop, the second RGD-loop and/or the variable loop of the adenovirus penton base protomer, and/or at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer;

(d) expressing the engineered adenovirus penton base protomer in a host cell, optionally together with the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer; and

(e) purifying said VLP optionally comprising an adenovirus penton base protomer binding fibre protein fragment, or said engineered polypeptide comprising an adenovirus penton base protomer binding fibre protein fragment.

14. A method for producing a VLP according to claim 9 or 10 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:

(a) providing a cloning vector of claim 6;

(b) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

(c) inserting nucleic acids encoding at least one of said non-adenoviral peptides at nucleic acid position preceding or subsequent to nucleic acids encoding the N- or C-terminus of the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer;

(d) expressing the engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer in a host cell, optionally together with an adenovirus penton base protomer; and

(e1) purifying said engineered polypeptide comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft

of a penton base protomer and admixing with adenovirus penton base protomers or engineered adenovirus penton base protomers of claims 1 or 3; or

(e2) purifying said VLP in case that the adenovirus penton base protomer was co-expressed.

5

15. A method for producing a VLP according to claim 9 or 10 comprising disease and/or patient specific non-adenoviral peptides, comprising the steps of:

(a) determining the amino acid sequence of disease or patient specific non-adenoviral peptides;

10 (b) synthesizing an engineered polypeptide according to claim 2 or 4 comprising an adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and at least one of said non-adenoviral peptides; and

(c) admixing said engineered polypeptide with adenovirus penton base protomers or

15 engineered adenovirus penton base protomers according to claim 1 or 3 with pentamers according to claim 8 or with VLPs according to claim 9 or 10.

16. A VLP producible by a method of any of claims 28 to 32.

20 17. A pharmaceutical composition comprising the engineered polypeptides according to any of claims 1 to 4, the nucleic acid of claim 5, the expression vector of claim 6 or the VLP according to any of claims 7, 8 or 14, and a pharmaceutically acceptable carrier and/or suitable excipient(s).

25 18. An engineered polypeptide according to any of claims 1 to 4, the nucleic acid of claim 5, the expression vector of claim 6 or the VLP according to any of claims 7, 8 or 14 for treating and/or preventing an infectious disease, an immune disease or cancer.

Fig. 1

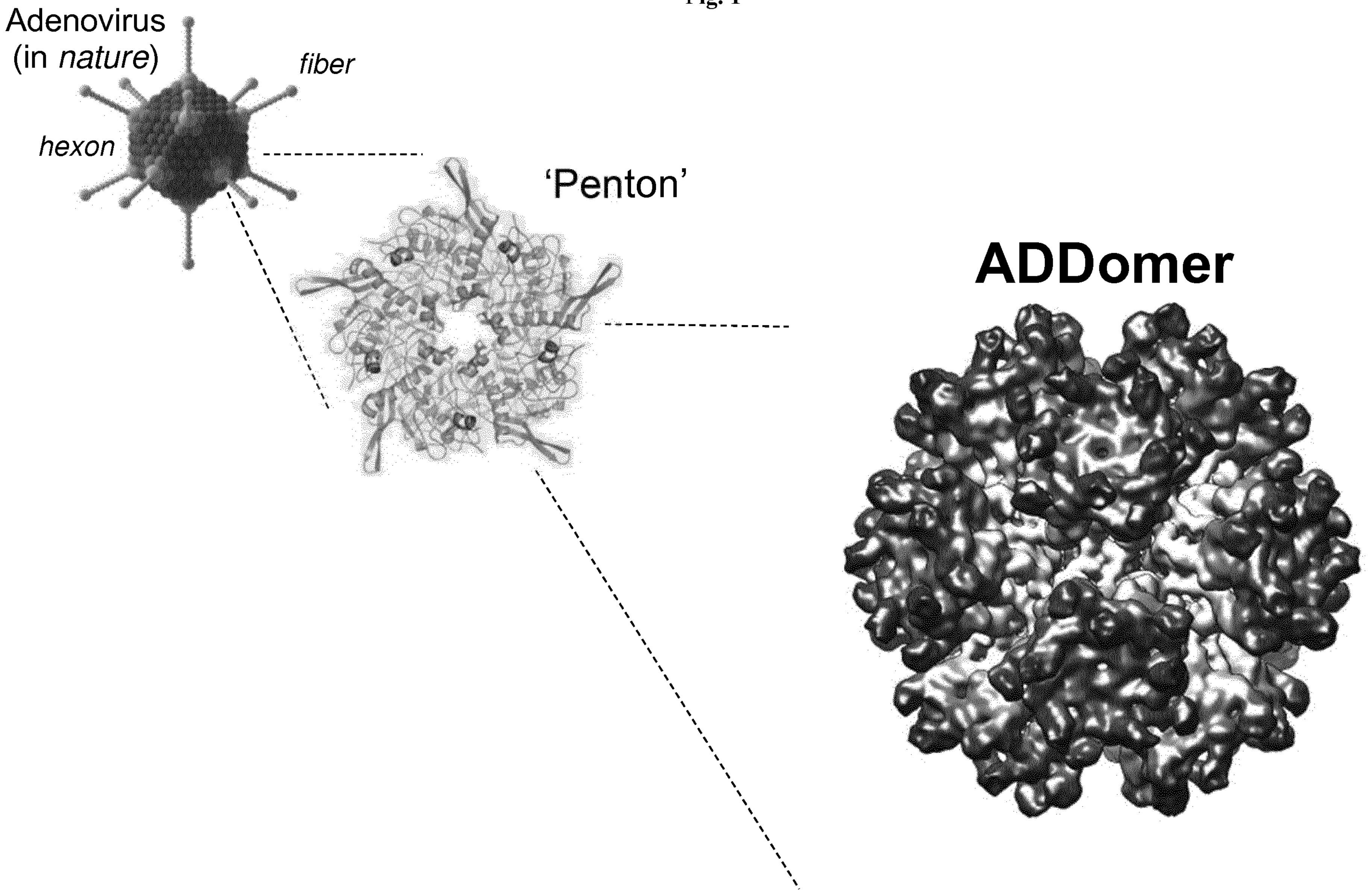


Fig. 2

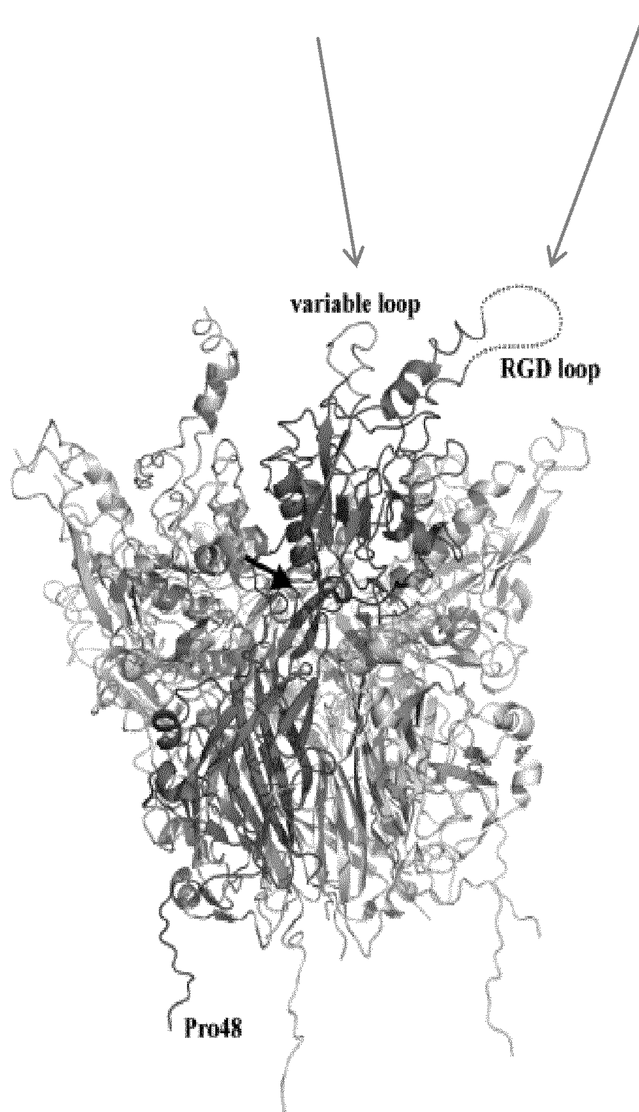


Fig. 3

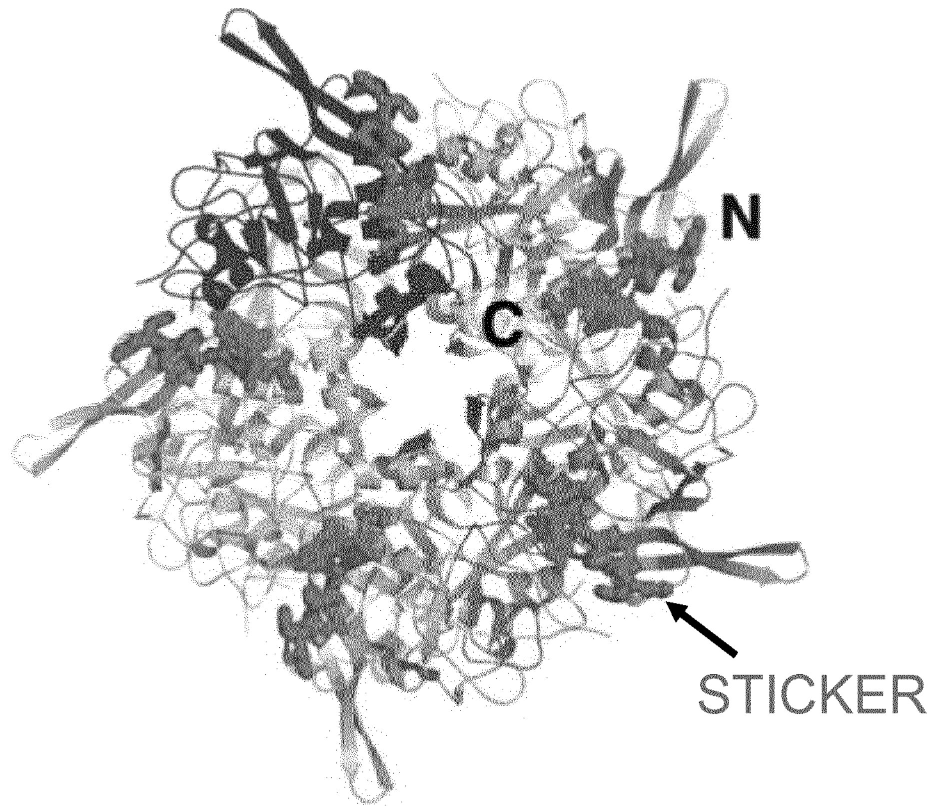
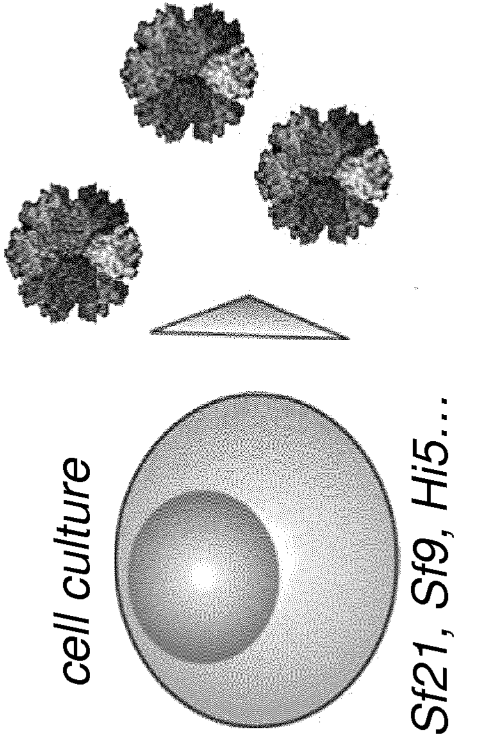
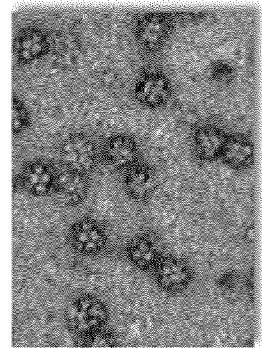


Fig. 4





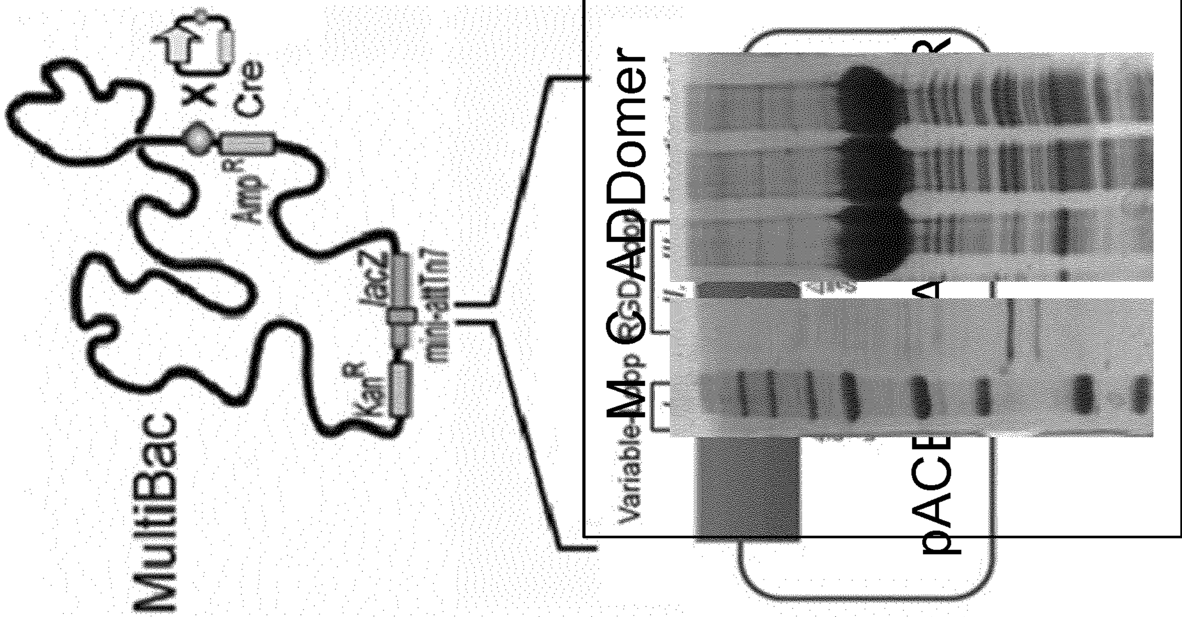


Fig. 5

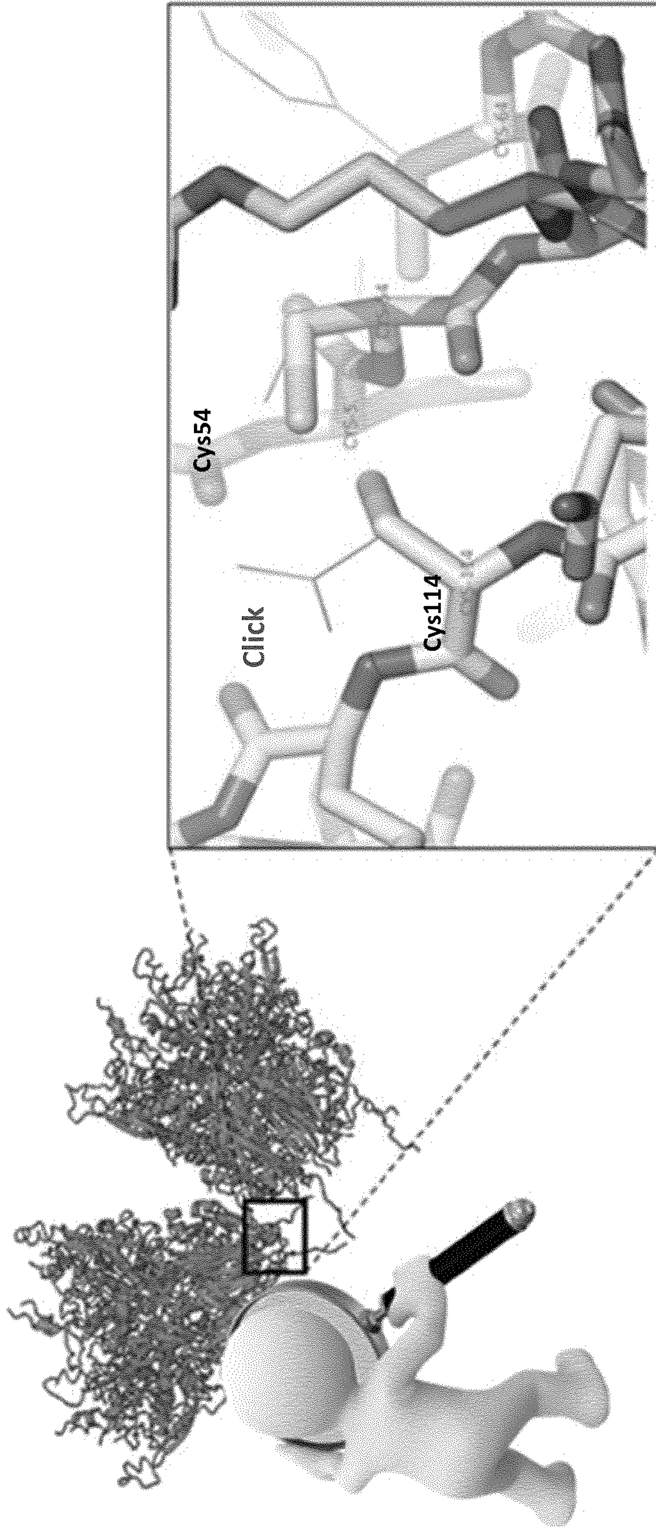


Fig. 6

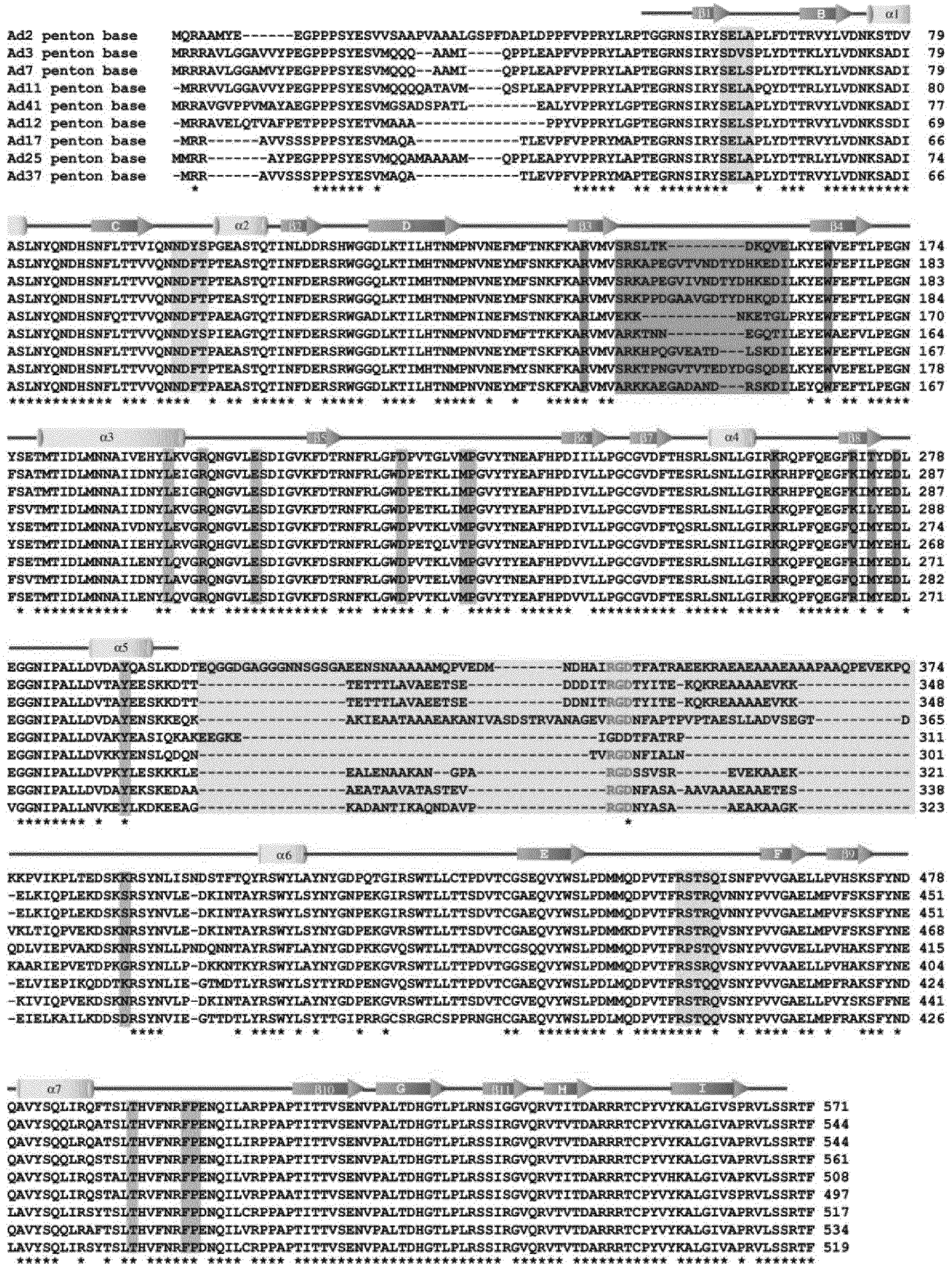


Fig. 7

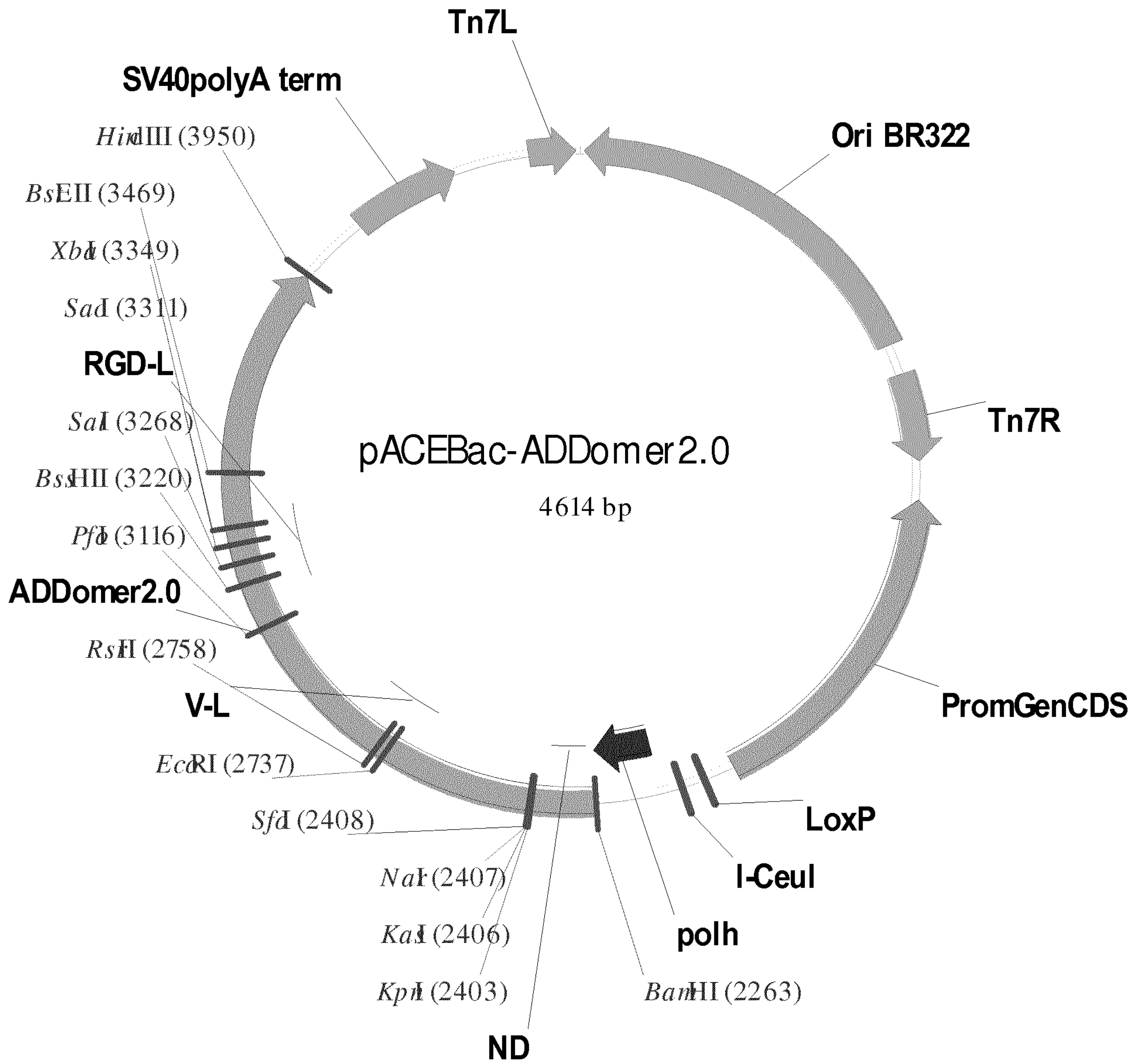


Fig. 8

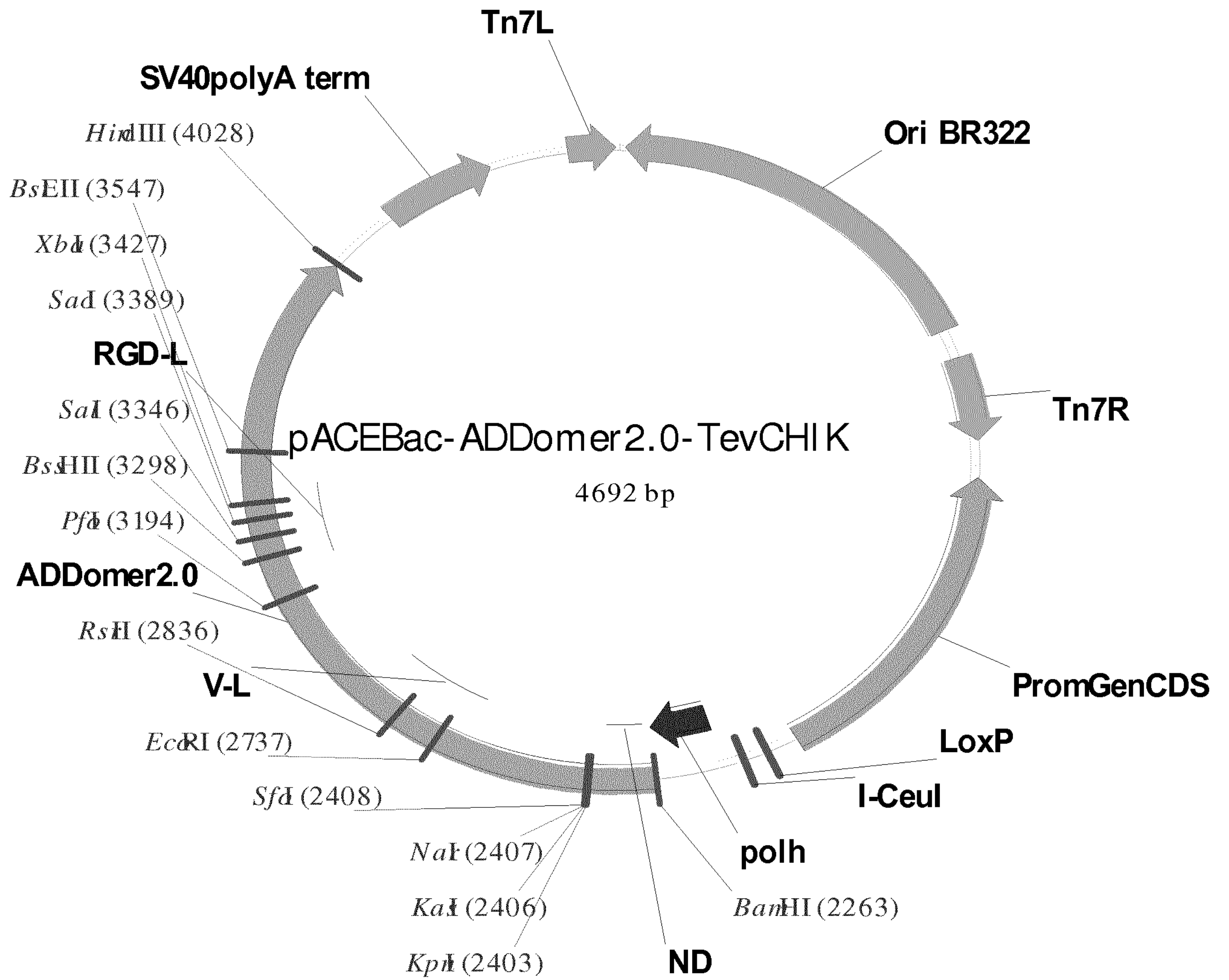


Fig. 9

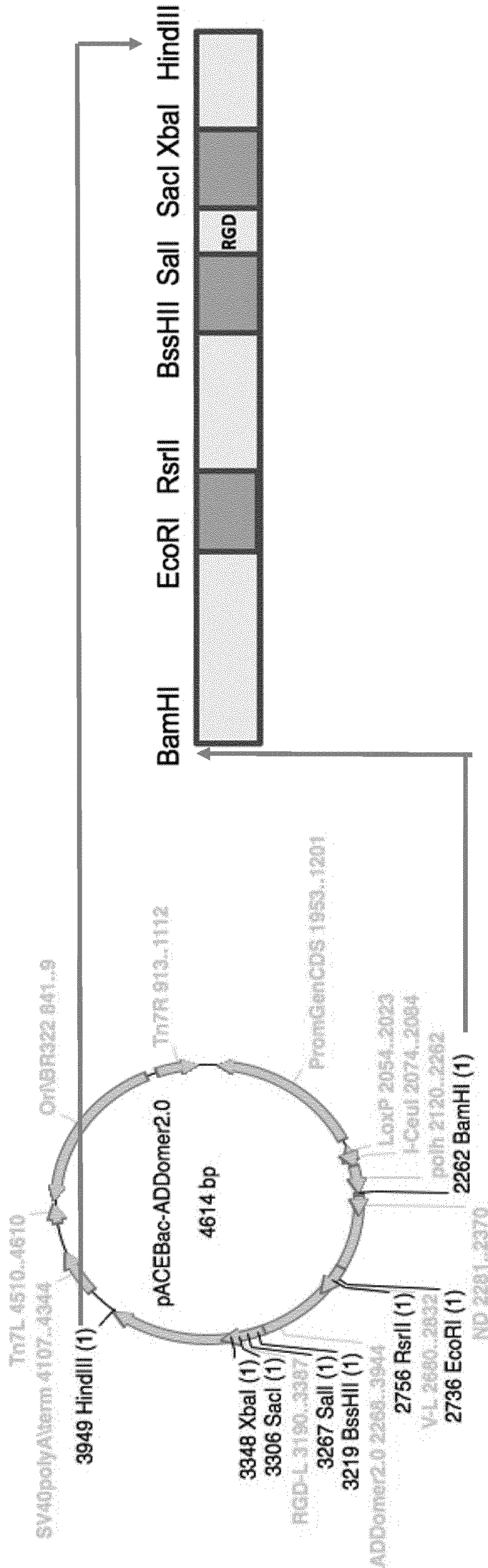


Fig. 10

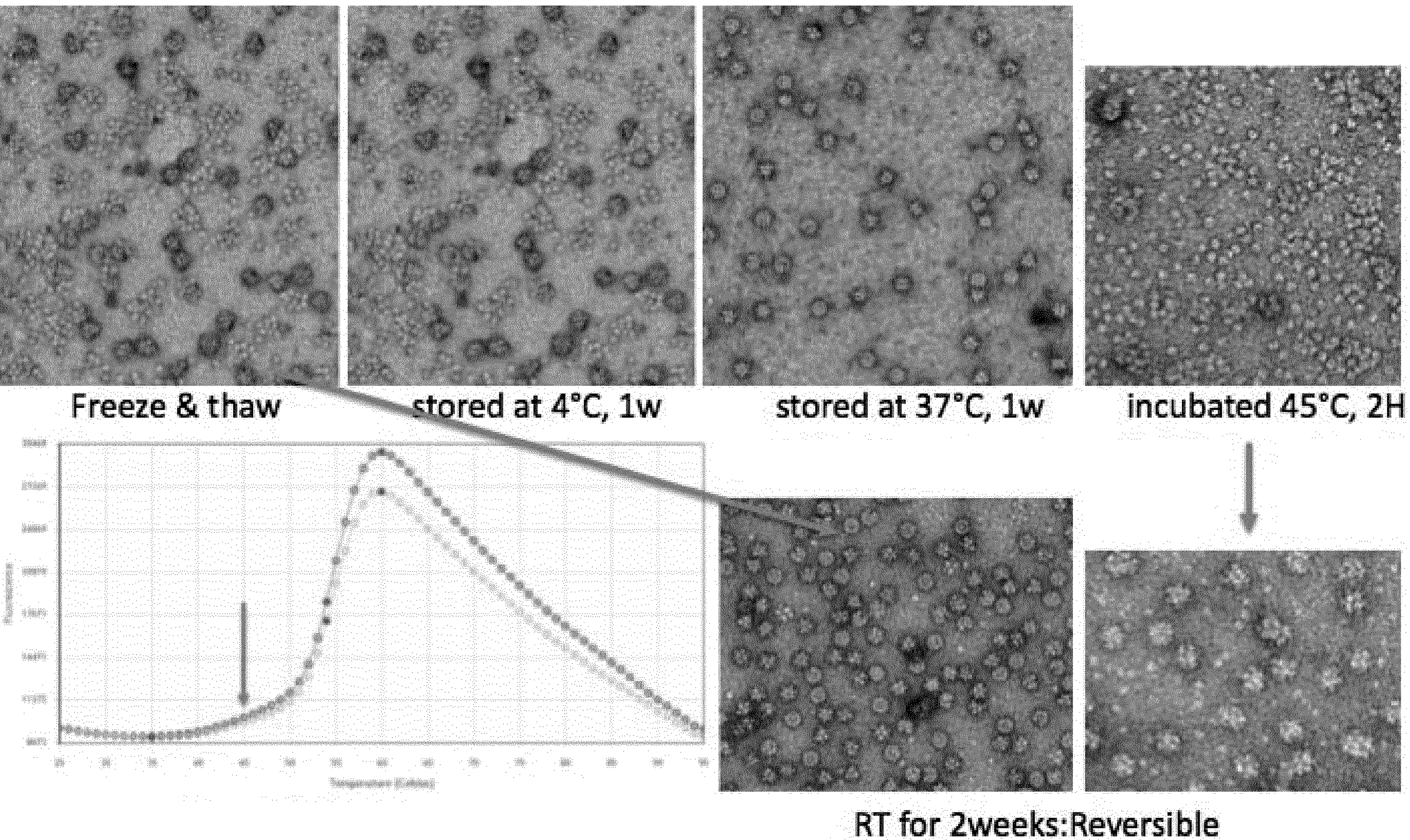
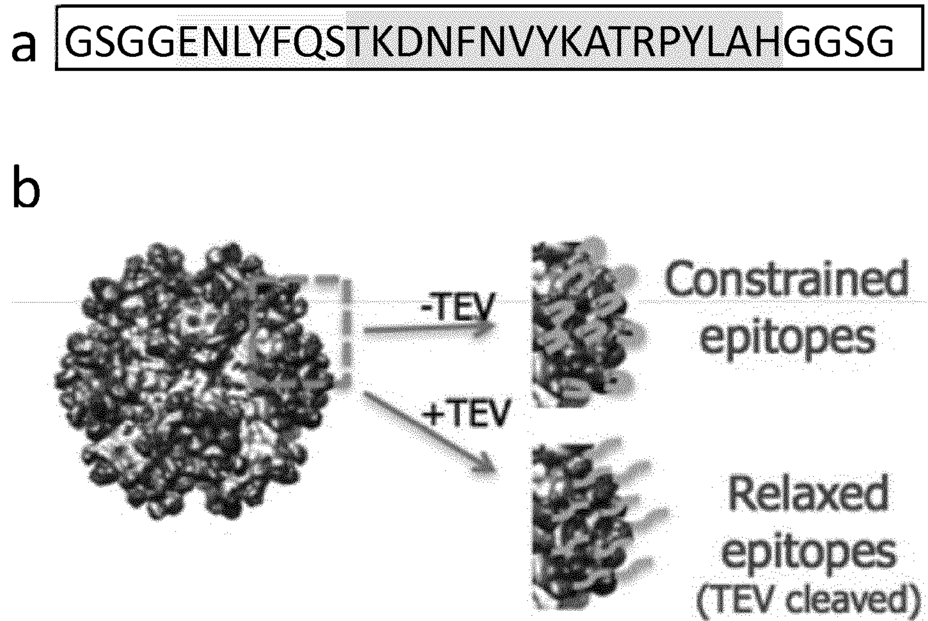


Fig. 11

a GSGGENLYFQSTKDNFNVYKATRPYLAHGGSG

Major CHIK neutralizing epitope configuration in ADDomer-TevCHIK

b



c

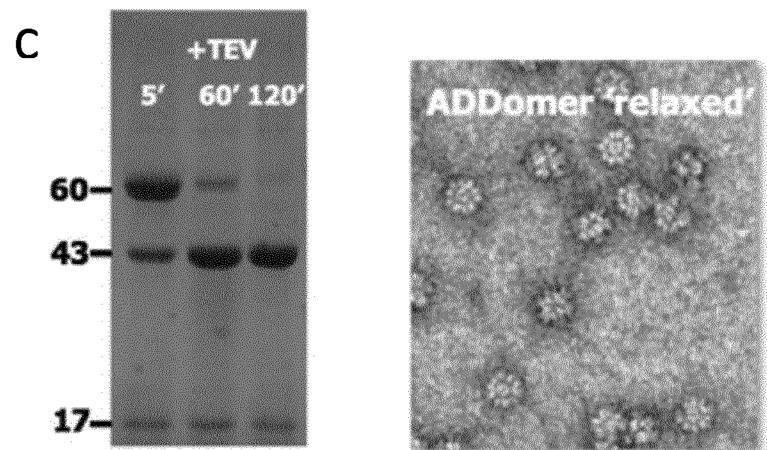


Fig. 12

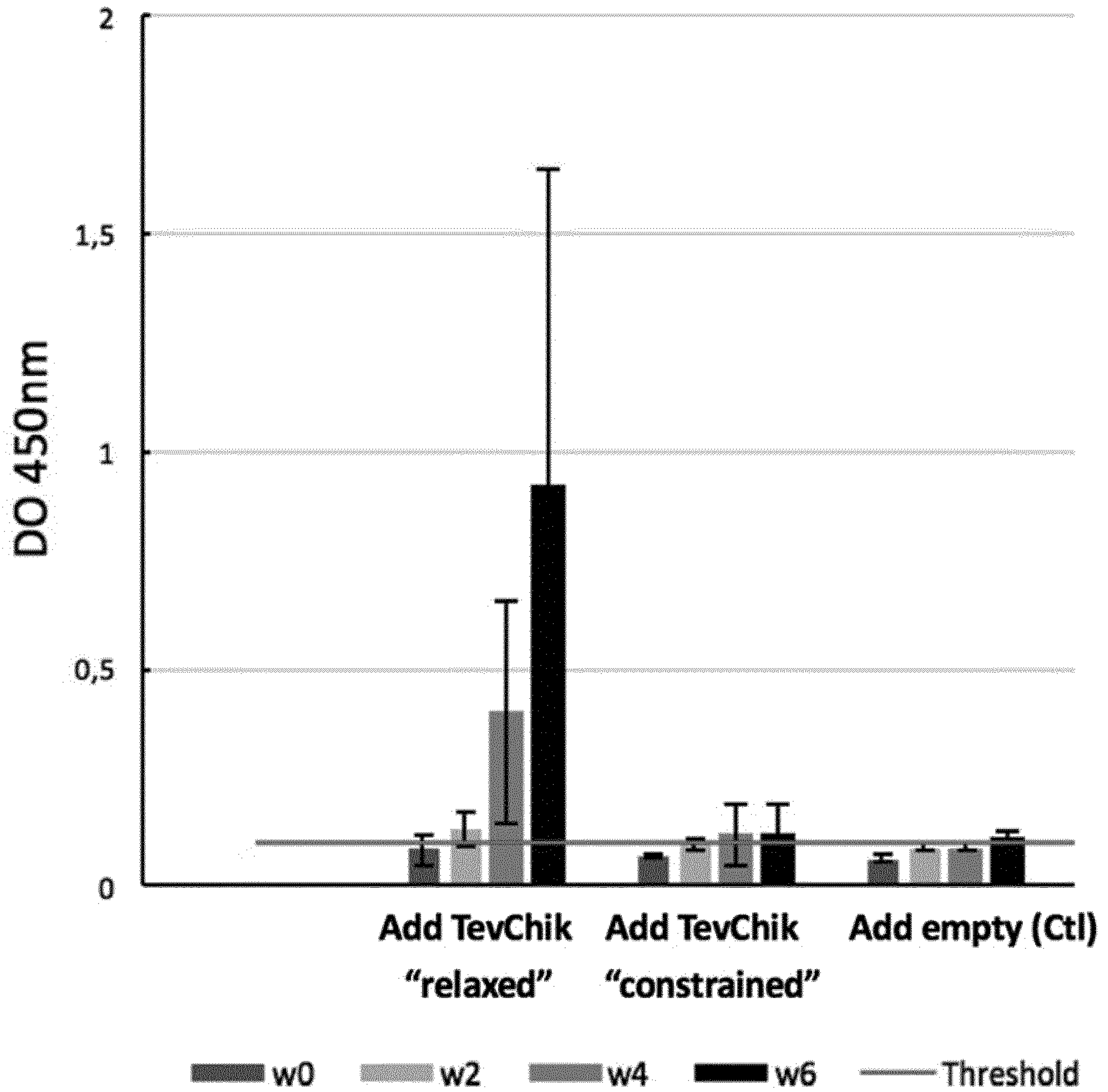
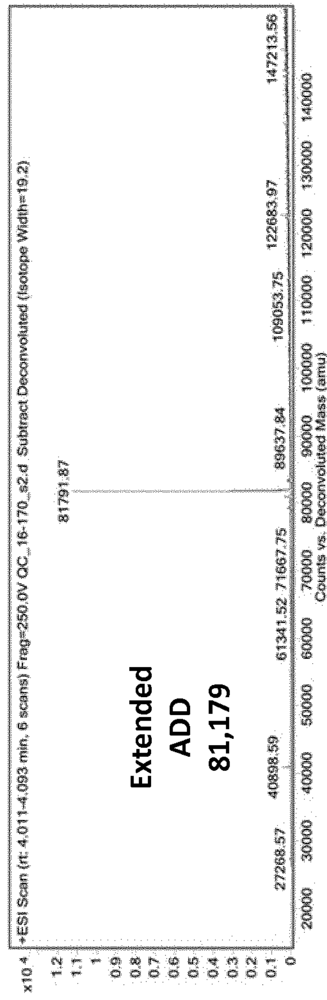
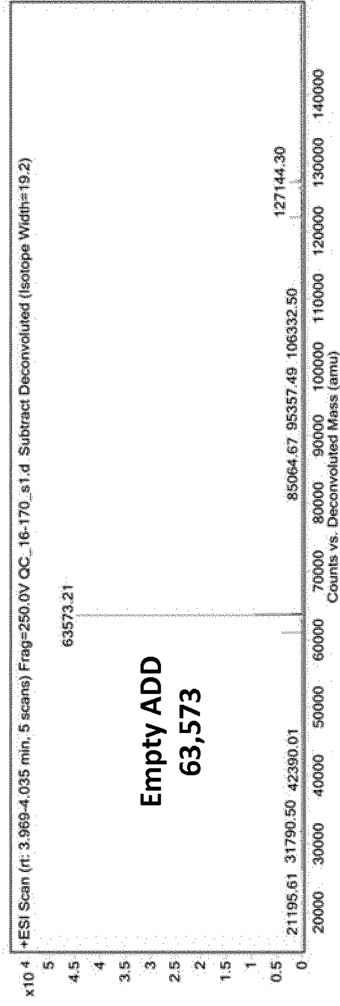


Fig. 13





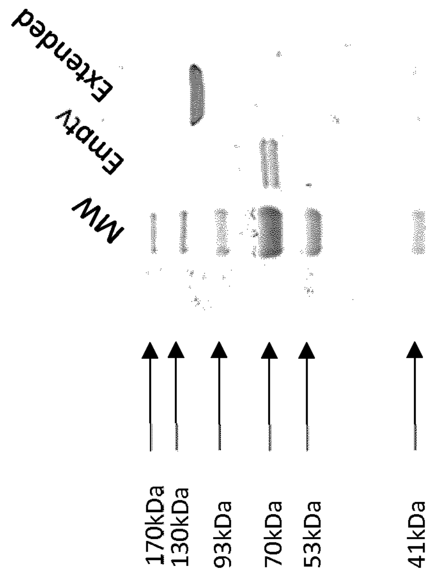
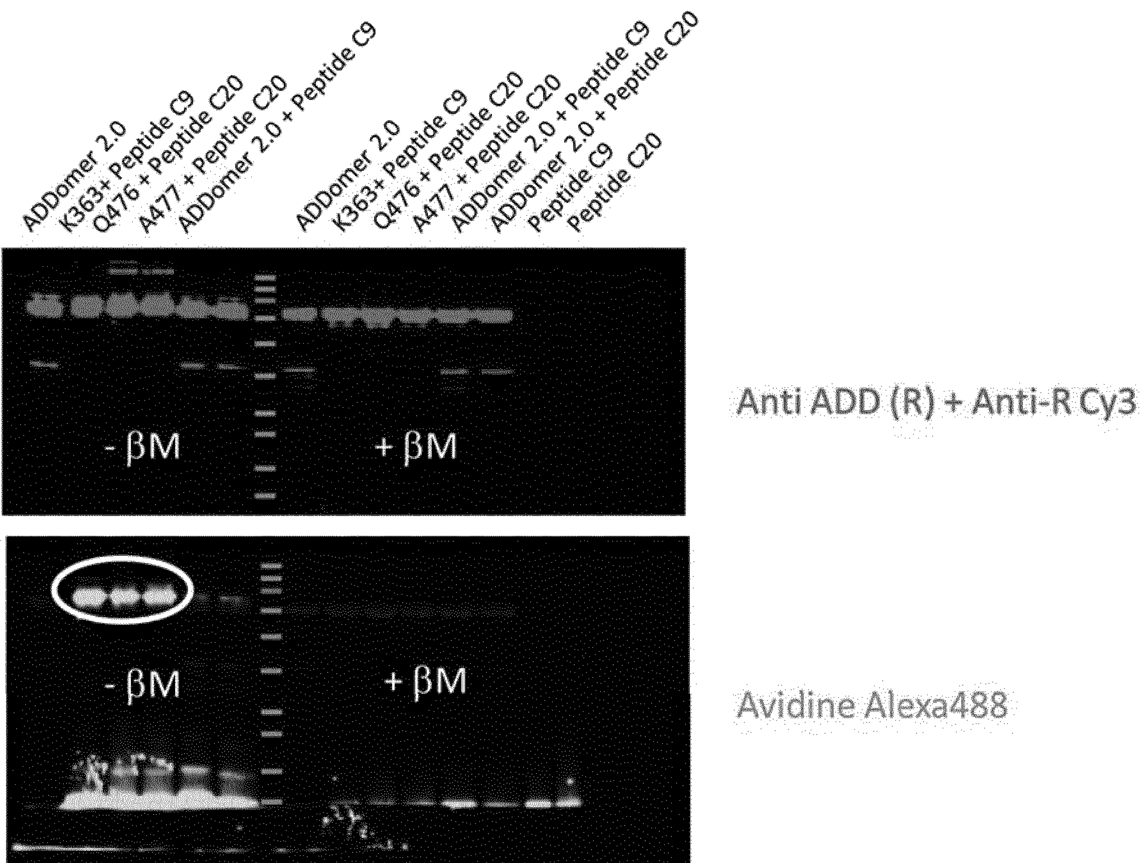


Fig. 14



AKRARLSTCFNPVYPYEDES (peptide C9)

AKRARLSTSFNPVYPYEDEC (Peptide C20)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/057747

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86 C07K14/005 C12N7/00 A61K39/12
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2010/151159 A2 (INST BIOCHEMII I BIOFIZYKI [PL]; SZOLAJSKA EWA [PL]; CHROBOCZEK JADWIG) 29 December 2010 (2010-12-29) claims 6,8 -----	1,3,5-9, 11,12, 16-18 13-15
A	CHLOE ZUBIETA ET AL: "The Structure of the Human Adenovirus 2 Penton", MOLECULAR CELL., vol. 17, no. 1, 2005, pages 121-135, XP055279729, US ISSN: 1097-2765, DOI: 10.1016/j.molcel.2004.11.041 figures 1, 3A ----- -/--	1,3,5-9, 11-18

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 June 2017	07/08/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Deleu, Laurent

2

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/027860 A2 (GENVEC INC [US]; US NAVY NAVAL RES LAB [US]; BRUDER JOSEPH T [US]; KOV) 8 March 2007 (2007-03-08) page 20, paragraph 52 -----	1,5-7,11
X	WICKHAM T J ET AL: "TARGETING OF ADENOVIRUS PENTON BASE TO NEW RECEPTORS THROUGH REPLACEMENT OF ITS RGD MOTIF WITH OTHER RECEPTOR-SPECIFIC PEPTIDE MOTIFS", GENE THERAPY, NATURE PUBLISHING GROUP, GB, vol. 2, no. 10, December 1995 (1995-12), pages 750-756, XP000565559, ISSN: 0969-7128 table 1 -----	1,5-7,11
Y	MATTHEW M. GUBIN ET AL: "Tumor neoantigens: building a framework for personalized cancer immunotherapy", JOURNAL OF CLINICAL INVESTIGATION, vol. 125, no. 9, 10 August 2015 (2015-08-10), pages 3413-3421, XP055280211, US ISSN: 0021-9738, DOI: 10.1172/JCI80008 page 3418 -----	13-15
Y	WO 01/92549 A2 (GENVEC INC [US]; WICKHAM THOMAS J [US]; KOVESDI IMRE [US]; ROELVINK PE) 6 December 2001 (2001-12-06) paragraph [0018] -----	13-15
A	WO 97/18317 A1 (COMMISSARIAT ENERGIE ATOMIQUE [FR]; CENTRE NAT RECH SCIENT [FR]; CHROB) 22 May 1997 (1997-05-22) example 3 -----	14,15
A	WO 2010/117287 A2 (INST BIO CHEMII I BIOFIZKI PAN [PL]; SZOLAJSKA EWA [PL]; CHROBOCZEK JA) 14 October 2010 (2010-10-14) claim 16; figure 1; example IV -----	1
A	WO 97/20575 A1 (UNIV ALABAMA RES FOUND [US]) 12 June 1997 (1997-06-12) figure 2 -----	14,15
X,P	WO 2016/118433 A1 (SHAYAKHMETOV DMITRY M [US]; DI PAOLO NELSON C [US]) 28 July 2016 (2016-07-28) claims 1-4 -----	1,5-7,11

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2017/057747

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1, 3, 8, 9(completely); 5-7, 11-18(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1, 3, 8, 9(completely); 5-7, 11-18(partially)

An engineered polypeptide comprising an adenovirus penton base protomer, wherein said penton base protomer comprises a first RGD-loop, a second RGD-loop, a variable loop (V loop), an adenovirus fibre protein binding cleft and/or a N-terminal domain, and comprises one or more non-adenoviral peptides in the first, the second or both the first and the second RGD-loops and/or in the V loop; and optionally comprises one or more of the following:(i) at least one target specific binding domain in the first, the second or both the first and the second RGD-loops, and/or in the V loop; and/or (ii) a non-adenoviral peptide at the N- and/or C-terminus of the penton base protomer; and/or (iii) at least one heterologous coupling residue in the first, the second, or both the first and the second RGD-loops, in the V loop and/or in the N-terminal domain of the penton base protomer; and/or (iv) a drug, label or polypeptide covalently or non-covalently coupled to one or more amino acids of the first, the second or both the first and the second RGD-loops and/or one or more amino acids of the V loop of the penton base protomer; and/or (v) at least one heterologous coupling residue in the adenovirus fibre protein binding cleft of the penton base protomer and wherein the engineered polypeptide is capable of assembling into VLPs. VLPs comprising said protomer, methods to produce said VLPs and said VLPs for their use as a medicament.

---

2. claims: 2, 4, 10(completely); 5-7, 11-18(partially)

An engineered polypeptide comprising at least one adenovirus fibre protein N-terminal fragment specifically binding to an adenovirus fibre protein binding cleft of a penton base protomer and:(i) a non-adenoviral peptide and/or (ii) is covalently or non-covalently coupled to a drug or label.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/057747

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010151159 A2	29-12-2010	EP 2461827 A2	13-06-2012
		PL 222497 B1	31-08-2016
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