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(54) Title: VIRAL DISK ASSEMBLIES AND METHODS OF USE THEREOF

(57) Abstract: The present disclosure provides variant viral coat proteins. The present disclosure provides conjugates comprising variant viral coat protein to which an agent is conjugated; the conjugates can self-assemble into disks which facilitate the delivery of the conjugated agent into cells. The present disclosure provides methods of use of the disks for delivering drugs or imaging agents.

VIRAL DISK ASSEMBLIES AND METHODS OF USE THEREOF

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/404,531, filed October 5, 2016, which application is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under Grant No. DE-AC02-05CH1123 awarded by the U.S. Department of Energy. The government has certain rights in the invention.

INTRODUCTION

[0003] Self-assembling protein nanomaterials derived from viruses have properties that make them useful for applications in drug delivery, disease imaging and diagnostics. These properties include uniform sizes and shapes, biodegradability, and multiple sets of functional handles for chemical manipulation. Intact virus nanoparticles have been functionalized for applications in drug delivery *in vivo*, typically through the covalent attachment of drugs or imaging agents onto the coat proteins. However, the injection of replication-competent viruses into subjects may limit their clinical appeal.

[0004] The development of spherical and rod-shaped virus nanoparticles has in both cases resulted in differential tumor accumulation, demonstrating the need to further expand the shape library of protein nanomaterials. However, expressing non-spherical virus-based protein nanomaterials without the genetic material that functions as a backbone to the assembly architecture can lead to significant challenges including polydiversity in size and shape, and change in assembly behavior in response to different conditions such as pH and ionic strength.

[0005] There is a need in the art for nanoparticles for drug delivery and as imaging agents.

SUMMARY

[0006] The present disclosure provides variant viral coat proteins. The present disclosure provides conjugates comprising variant viral coat protein to which an agent is conjugated; the conjugates can self-assemble into disks which facilitate the delivery of the conjugated agent into cells. The present disclosure provides methods of use of the disks for delivering drugs or imaging agents.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] **FIG. 1A-1I** provide amino acid sequences of wild-type tobacco mosaic virus (wtTMV) coat protein and variants such as RR-TMV.

[0008] **FIG. 2A-2E** depict the assembly of stable RR-TMV disks.

[0009] **FIG. 3** depicts representative sizes of assembled RR-TMV disks.

[0010] **FIG. 4A-4B** depict modification of RR-TMV with poly(ethylene glycol) (PEG) through either maleimide modification or oxidative coupling.

[0011] **FIG. 5A-5C** depict serum stability of RR-TMV.

[0012] **FIG. 6A-6E** depict RR-TMV conjugates generation and assembly into disks.

[0013] **FIG. 7A-7D** depict the delivery by RR-TMV of doxorubicin into glioblastoma cells.

[0014] **FIG. 8A-8D** depict serum stability of RR-TMV and RR-TMV modified with AF488.

[0015] **FIG. 9** depicts the modification of RR-$TMV_{AF488\ by}$ PEG.

[0016] **FIG. 10** depicts stability of RR-TMV in phosphate-buffered saline (PBS) after 5 days.

[0017] **FIG. 11A-11C** depict oxidation of DOX in RR-TMV_{DOX} by $NaIO_4$ but not $K_3Fe(CN)_6$.

DEFINITIONS

[0018] The term "linker" and "linker molecule" are used interchangeably herein to refer to a molecule including at least one covalent bond-forming reactive group as defined herein and at least one group that is either a member of a specific binding pair or a functional group capable of subsequent covalent bond formation.

[0019] The term "conjugated," as used in the context of conjugate comprising a variant TMV polypeptide described herein, refers to a covalent or non-covalent interaction between two entities, e.g., between a variant TMV polypeptide and a drug, between a variant TMV polypeptide and an imaging agent, etc.

[0020] The terms "polypeptide" and "protein", used interchangeably herein, refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and native leader sequences, with or without N-terminal methionine residues; immunologically tagged proteins; fusion proteins with detectable fusion partners, e.g., fusion proteins including as a fusion partner a fluorescent protein, β-galactosidase, luciferase, etc.; and the like.

[0021] The terms "nucleic acid" and "polynucleotide" are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. The terms encompass, e.g., DNA, RNA and modified forms thereof. Polynucleotides may have any three-dimensional structure, and may perform any function, known or unknown. Non-limiting examples of polynucleotides include a gene, a gene fragment, exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, control regions, isolated RNA of any sequence, nucleic acid probes, and primers. The nucleic acid molecule may be linear or circular.

[0022] A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST. See, e.g., Altschul *et al.* (1990), *J. Mol. Biol.* 215:403-10. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wisconsin, USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, California, USA. Of particular interest are alignment programs that permit gaps

in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970).

[0023] A "substitution" results from the replacement of one or more nucleotides or amino acids by different amino acids or nucleotides, respectively as compared to an amino acid sequence or nucleotide sequence of a polypeptide. If a substitution is conservative, the amino acid that is substituted into a polypeptide has similar structural or chemical properties (e.g., charge, polarity, hydrophobicity, and the like) to the amino acid that it is substituting. Conservative substitutions of naturally occurring amino acids usually result in a substitution of a first amino acid with second amino acid from the same group as the first amino acid, where exemplary amino acid groups are as follows: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; and (4) neutral non-polar amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. In some embodiments, polypeptide variants may have "non-conservative" changes, where the substituted amino acid differs in structural and/or chemical properties.

[0024] The terms "antibodies" and "immunoglobulin" include antibodies or immunoglobulins of any isotype, fragments of antibodies that retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies (scAb), single domain antibodies (dAb), single domain heavy chain antibodies, a single domain light chain antibodies, bi-specific antibodies, multi-specific antibodies, and fusion proteins comprising an antigen-binding (also referred to herein as antigen binding) portion of an antibody and a non-antibody protein. The antibodies can be detectably labeled, *e.g.*, with a radioisotope, an enzyme that generates a detectable product, a fluorescent protein, and the like. The antibodies can be further conjugated to other moieties, such as members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific binding pair), and the like. The antibodies can also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term are Fab', Fv, $F(ab')_2$, and or other

antibody fragments that retain specific binding to antigen, and monoclonal antibodies. As used herein, a monoclonal antibody is an antibody produced by a group of identical cells, all of which were produced from a single cell by repetitive cellular replication. That is, the clone of cells only produces a single antibody species. While a monoclonal antibody can be produced using hybridoma production technology, other production methods known to those skilled in the art can also be used (e.g., antibodies derived from antibody phage display libraries). An antibody can be monovalent or bivalent. An antibody can be an Ig monomer, which is a "Y-shaped" molecule that consists of four polypeptide chains: two heavy chains and two light chains connected by disulfide bonds.

[0025] "Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); domain antibodies (dAb; Holt et al. (2003) *Trends Biotechnol.* 21:484); single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

[0026] The term "nanobody" (Nb), as used herein, refers to the smallest antigen binding fragment or single variable domain (V_{HH}) derived from naturally occurring heavy chain antibody and is known to the person skilled in the art. They are derived from heavy chain only antibodies, seen in camelids (Hamers-Casterman et al., 1993; Desmyter et al., 1996). In the family of "camelids" immunoglobulins devoid of light polypeptide chains are found. "Camelids" comprise old world camelids (*Camelus bactrianus* and *Camelus dromedarius*) and new world camelids (for example, *Llama paccos*, *Llama glama*, *Llama guanicoe* and *Llama vicugna*). A single variable domain heavy chain antibody is referred to herein as a nanobody or a V_{HH} antibody.

[0027] "Fv" is the minimum antibody fragment that contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRS of each variable domain interact to define an antigen-binding site on the

surface of the V_H-V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0028] The "Fab" fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0029] "Single-chain Fv" or "sFv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).*

[0030] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H-V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

[0031] As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse affect attributable to the disease. "Treatment," as used herein, covers any treatment of a

disease in a mammal, particularly in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease or at risk of acquiring the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

[0032] The terms "subject," "individual," "host," and "patient" are used interchangeably herein, and refer to an animal amenable to therapy according to the methods of the disclosure or to which composition according to the present disclosure may be administered to achieve a desired effect. Animals include, e.g., humans and non-human mammals. Non-human mammals include, e.g., ungulates (e.g., cows, sheep, camels, pigs, horses, and goats); felines (e.g., cats); canines (e.g., dogs); non-human primates; rodents (e.g., rats; mice); lagomorphs (e.g., rabbits); and the like.

[0033] "Non-native", "non-endogenous", and "heterologous", in the context of a polypeptide, are used interchangeably herein to refer to a polypeptide having an amino acid sequence or, in the context of an expression system or a viral particle, present in an environment different to that found in nature.

[0034] As used herein, the terms "determining," "measuring," "assessing," and "assaying" are used interchangeably and include both quantitative and qualitative determinations.

[0035] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, 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 be limiting, since the scope of the present invention will be limited only by the appended claims.

[0036] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0037] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0038] It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a viral disk" includes a plurality of such viral disks and reference to "the variant TMV coat protein" includes reference to one or more variant TMV coat proteins and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

[0039] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[0040] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[0041] The present disclosure provides variant viral coat proteins. The present disclosure provides conjugates comprising variant viral coat protein to which an agent is conjugated; the conjugates can self-assemble into disks which facilitate the delivery of the conjugated agent into cells. The present disclosure provides methods of use of the disks for delivering drugs or imaging agents.

VARIANT TMV POLYPEPTIDES

[0042] The present disclosure provides a variant tobacco mosaic virus (TMV) coat protein, where the variant TMV coat protein includes self-assembles into viral disk assemblies that are more stable in maintaining disk architecture compared to the stability of viral disk assembly comprising a wild-type TMV coat protein.

[0043] Wild-type TMV coat protein comprises the amino acid sequence set forth in SEQ ID NO:1:

[0044] SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEV WKPSPQVTVRFPDSDFKVYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPTTA ETLDATRRVDDATVAIRSAINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:1).

[0045] In some cases, a variant TMV coat protein (also referred to herein as a "variant viral coat protein") comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; and comprises an amino acid substitution of K53. In some cases, the amino acid at position 53 is any amino acid (other than lysine) capable of forming a salt bridge. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg.

[0046] In some cases, a variant TMV coat protein (also referred to herein as a "variant viral coat protein") of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; and comprises substitution of amino acids K53 and K68. In some cases, the amino acid at positions 53 and 68 is any amino acid other than lysine. In some cases, the amino acid at

position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the substitutions are K53R and K68R. In some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; and comprises K53R and K68R substitutions. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:2 (as depicted in FIG. 1B); where the variant viral coat protein comprises R53 and R68, as shown in FIG. 1B.

[0047] In some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, and comprises substitution of amino acids K53, K68, and T104. In some cases, the amino acid at positions 53 and 68 is any amino acid other than lysine and the amino acid at position 104 is any amino acid other than threonine. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid 68 is Arg. In some cases, the amino acid at position 104 is Lys, His, or Arg. In some cases, the amino acid at position 104 is Lys. In some cases, the substitutions are K53R, K68R, and T104K. In some cases, the amino acid at position 123 is not substituted; i.e., the amino acid at position 123 is Ser. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in

SEQ ID NO:3 (as depicted in FIG. 1C); where the variant viral coat protein comprises R53, R68, K104, and S123, as shown in FIG. 1C.

[0048] In some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, and comprises substitution of amino acids K53, K68, and S123. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid 68 is Arg. In some cases, the amino acid at position 123 is other than Ser. In some cases, the amino acid at position 123 provides for maleimide modification. In some cases, the amino acid at position 123 is Cys. In some cases, a variant TMV coat protein does not include a substitution at T104; i.e., in some cases, the variant viral coat protein comprises substitution of amino acids K53, K68, and S123; and comprises a Thr at amino acid 104. In some cases, the variant viral coat protein comprises a K53R substitution, a K68R substitution, and a S123C substitution; and comprises a Thr at amino acid 104 For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:4 (as depicted in FIG. 1D); where the variant viral coat protein comprises R53, R68, T104, and C123, as shown in FIG. 1D.

[0049] In some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, and comprises substitution of amino acids K53, K68, T104, and S123. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His,

Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid 68 is Arg. In some cases, the amino acid at position 104 is Lys, His, or Arg. In some cases, the amino acid at position 104 is Lys. In some cases, the amino acid at position 123 is other than Ser. In some cases, the amino acid at position 123 provides for maleimide modification. In some cases, the amino acid at position 123 is Cys. In some cases, the substitutions are K53R, K68R, T104K, and S123C. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:5 (as depicted in FIG. 1E); where the variant viral coat protein comprises R53, R68, T104, and C123, as shown in FIG. 1E.

[0050] A variant viral coat protein of the present disclosure can include an N-terminal extension of from 1 to 10 amino acids, where such an N-terminal extension provides an additional site of attachment. For example, in some cases, a variant viral coat protein of the present disclosure can include an N-terminal extension of from 1 to 10 amino acids, where such an N-terminal extension provides an attachment site for a water-soluble polymer. In some cases, the N-terminal extension comprises, at its N-terminus, a proline. In some cases, the N-terminal extension comprises, at its N-terminus, a serine. In some cases, the N-terminal extension comprises, at its N-terminus, a threonine. In some cases, the N-terminal extension comprises, at its N-terminus, a lysine. In some cases, the N-terminal extension comprises, at its N-terminus, an arginine. In some cases, the N-terminal extension comprises, at its N-terminus, a histidine. In some cases, the N-terminal extension comprises amino acids Pro-Ala-Gly, and is 3 to 5 amino acids in length.

[0051] For example, in some cases, a variant TMV coat protein (also referred to herein as a "variant viral coat protein") of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; comprises substitution of amino acids K53 and K68, based on the amino acid

numbering of SEQ ID NO:1; and comprises an N-terminal extension of from 1 to 10 amino acids. In some cases, the amino acid at positions 53 and 68 is any amino acid other than lysine. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the substitutions are K53R and K68R. In some cases, the N-terminal extension comprises the amino acid sequence PAG, and has a length of 3 to 5 amino acids. For example in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1; comprises K53R and K68R substitutions based on the amino acid number of SEQ ID NO:1; and comprises an N-terminal extension of from 1 to 10 amino acids. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:6 (as depicted in FIG. 1F); where the variant viral coat protein comprises R53 and R68, based on the amino acid numbering of SEQ ID NO:1; and comprises the N-terminal extension PAG (Pro-Ala-Gly), as shown in FIG. 1F.

[0052] As another example, in some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, comprises substitution of amino acids K53, K68, and T104 based on the amino acid numbering of SEQ ID NO:1; and comprises an N-terminal extension of from 1 to 10 amino acids. In some cases, the amino acid at positions 53 and 68 is any amino acid other than lysine and the amino acid at position 104 is any amino acid other than threonine. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid

68 is Arg. In some cases, the amino acid at position 104 is Lys, His, or Arg. In some cases, the amino acid at position 104 is Lys. In some cases, the substitutions are K53R, K68R, and T104K. In some cases, the amino acid at position 123 is not substituted; i.e., the amino acid at position 123 is Ser. In some cases, the N-terminal extension comprises the amino acid sequence PAG, and has a length of 3 to 5 amino acids. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:7 (as depicted in FIG. 1G); where the variant viral coat protein comprises R53, R68, K104, and S123, based on the amino acid numbering of SEQ ID NO:1; and comprises the N-terminal extension PAG (Pro-Ala-Gly), as shown in FIG. 1G.

[0053] As another example, in some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, comprises substitution of amino acids K53, K68, and S123 based on the amino acid numbering of SEQ ID NO:1; and comprises an N-terminal extension of from 1 to 10 amino acids. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid 68 is Arg. In some cases, the amino acid at position 123 is other than Ser. In some cases, the amino acid at position 123 provides for maleimide modification. In some cases, the amino acid at position 123 is Cys. In some cases, a variant TMV coat protein does not include a substitution at T104; i.e., in some cases, the variant viral coat protein comprises substitution of amino acids K53, K68, and S123; and comprises a Thr at amino acid 104. In some cases, the variant viral coat protein comprises a K53R substitution, a K68R substitution, and a S123C substitution; and comprises a Thr at amino acid 104. In some cases, the N-terminal extension comprises the amino acid sequence PAG, and has a length of 3 to 5 amino acids. For example, in

some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:8 (as depicted in FIG. 1H); where the variant viral coat protein comprises R53, R68, T104, and C123, based on the amino acid numbering of SEQ ID NO:1; and comprises the N-terminal extension PAG (Pro-Ala-Gly), as shown in FIG. 1H.

[0054] As another example, in some cases, a variant TMV coat protein comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99% or 100% amino acid sequence identity to an amino acid sequence set forth in SEQ ID NO:1, comprises substitution of amino acids K53, K68, T104, and S123 based on the amino acid numbering of SEQ ID NO:1; and comprises an N-terminal extension of from 1 to 10 amino acids.. In some cases, the amino acid at position 53 and/or position 68 is any amino acid that can form a salt bridge. In some cases, the amino acid at position 53 and/or position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, the amino acid at position 53 and position 68 is Arg, Glu, Asp, His, Tyr, or Ser. In some cases, amino acid 53 is Arg. In some cases, amino acid 68 is Arg. In some cases, amino acid 53 is Arg; and amino acid 68 is Arg. In some cases, the amino acid at position 104 is Lys, His, or Arg. In some cases, the amino acid at position 104 is Lys. In some cases, the amino acid at position 123 is other than Ser. In some cases, the amino acid at position 123 provides for maleimide modification. In some cases, the amino acid at position 123 is Cys. In some cases, the substitutions are K53R, K68R, T104K, and S213C. In some cases, the N-terminal extension comprises the amino acid sequence PAG, and has a length of 3 to 5 amino acids. For example, in some cases, a variant viral coat protein of the present disclosure comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100%, amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:9 (as depicted in FIG. 1I); where the variant viral coat protein comprises R53, R68, T104, and C123, based on the amino acid numbering of SEQ ID NO:1; and comprises the N-terminal extension PAG (Pro-Ala-Gly), as shown in FIG. 1I.

[0055] A variant viral coat protein of the present disclosure can comprise a heterologous polypeptide, where the heterologous polypeptide is other than a TMV viral coat protein, i.e., the heterologous polypeptide comprises an amino acid sequence that is not present in a naturally-occurring TMV coat protein in nature. A variant viral coat protein of the present disclosure that comprises a heterologous polypeptide is a fusion protein comprising: a) a variant viral coat protein of the present disclosure; and b) a heterologous polypeptide. The heterologous polypeptide can also be referred to as a "fusion partner." In some cases, the heterologous polypeptide is inserted internally within the variant viral coat protein. In some cases, the heterologous polypeptide is appended to the N-terminus of the variant viral coat protein. In some cases, the heterologous polypeptide is appended to the C-terminus of the variant viral coat protein. The heterologous polypeptide can have a length of from 3 amino acids to about 50 amino acids; for example, the heterologous polypeptide can have a length of 3 amino acids (aa), 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, from 10 aa to 15 aa, from 15 aa to 20 aa, from 20 aa to 25 aa, from 25 aa to 30 aa, from 30 aa to 35 aa, from 35 aa to 40 aa, from 40 aa to 45 aa, or from 45 aa to 50 aa. Generally, the heterologous polypeptide does not substantially decrease stability of a disk assembly formed by the variant viral coat protein.

[0056] In some cases, the heterologous polypeptide provides for binding to the surface of a cell (e.g., a mammalian cell). For example, peptides with RGD (Arg-Gly-Asp), or DGR (Asp-Gly-Arg), or NGR (Asn-Gly-Arg) motifs, which bind to integrins or cell surface associated molecule, are suitable. For example, in some cases, the heterologous polypeptide comprises an Arg-Gly-Asp amino acid sequence.

[0057] In some cases, the heterologous polypeptide is a pro-apoptotic polypeptide. For example, a KLAK repeat peptide is suitable for use as a heterologous polypeptide (fusion partner). A KLAK peptide can comprise the following amino acid sequence: KLAKLAKKLAKLAK (SEQ ID NO:10).

[0058] In some cases, the heterologous polypeptide is an endosomal escape polypeptide. For example, a GALA peptide (an EALA repeat peptide) is suitable for use as a heterologous polypeptide (fusion partner). GALA peptides are pH responsive, and form alpha-helices at pH 4.5; these peptides then fuse with the endosome membrane and facilitate escape of a moiety (e.g., polypeptide; a disk) to which they are attached. See,

e.g., Li et al. (2004) *Adv. Drug Deliv. Rev.* 56(7) 967-985. As one example, a GALA peptide can comprise the following amino acid sequence: WEA ALA EAL AEA LAE HLA EAL AEA LEA LAA (SEQ ID NO:11); and can have a length of 30 amino acids. As another example, a KALA peptide is used as the heterologous polypeptide (fusion partner). A KALA peptide can comprise the following amino acid sequence: Trp-Glu-Ala-Lys-Leu-Ala-Lys-Ala-Leu-Ala-Lys-Ala-Leu-Ala-Lys-His-Leu-Ala-Lys-Ala-Leu-Ala-Lys-Ala-Leu-Lys-Ala-Cys-Glu-Ala (SEQ ID NO:12); and can have a length of 30 amino acids.

[0059] In some cases, a variant TMV coat protein of the present disclosure self-assembles into a disk. In some cases, a variant TMV coat protein of the present disclosure self-assembles into a double disk. In some cases, a variant TMV coat protein of the present disclosure self-assembles into nanoscale disk. In some cases, the nanoscale disks range in diameter from about 10 nm to about 25nm, e.g. from about 10.0 nm to about 10.5 nm, from about 10.5 nm to about 11.0, from about 11.0 nm to about 11.5 nm, from about 11.5 to about 12.0 nm, from about 12.0 nm to about 12.5, from about 12.5 nm to about 13.0 nm, from about 13.0 to about 13.5 nm, from about 13.5 nm to about 14.0 nm, from about 14.5 nm to about 15.0 nm, from about 15.0 nm to about 15.5 nm, from about 15.5 nm to about 16.0 nm, from about 16.0 nm to about 16.5 nm, from about 16.5 n to about 17.0 nm from about 17.0 nm to about 17.5 nm, from about 17.5 nm to about 18.0 nm, from about 18.0 nm to about 18.5 nm, from about 18.5 nm to about 19.0 nm, from about 19.0 nm to about 19.5 nm, from about 19.5 nm to about 20.0 nm, from about 20.0 nm to about 20.5 nm, from about 20.5 nm to about 21.0 nm, from about 21.0 nm to about 21.5 nm, from about 21.5 nm to about 22.0 nm, from about 22.0 nm to about 22.5 nm, from about 22.5 nm to about 23.0 nm, from about 23.0 nm to about 23.5 nm, from about 23.5 nm to about 24.0 nm, from about 24.0 nm to about 24.5 nm, from about 24.5 nm to about 25.0 nm.

[0060] In some cases, a variant TMV coat protein of the present disclosure self-assembles into double disk assemblies that maintain a double-disk architecture under varying conditions. In some cases, the double disk assemblies do not disassemble under various conditions. In some case, the double disk do not assemble into higher order assemblies, e.g. into assemblies comprising more than a double disk. In some cases, a variant TMV coat protein of the present disclosure self-assembles into stable disk assemblies in

solution at a pH of from 4 to 10, e.g., at pH 4.0 to 4.5, 4.5 to 5.0, 5.0 to 5.5, 5.5 to 6.0, 6.0 to 6.5, 6.5 to 7.0, 7.0 to 7.5, 7.5 to 8.0, 8.0 to 8.5, 8.5 to 9.0, or 9.0 to 9.5 at room temperature for a period of time of from about 10 minutes to about 6 months (e.g., from about 10 minutes to about 30 minutes, from about 30 minutes to 1 hour, from about 1 hour to about 6 hours, from about 6 hours to about 12 hours, from about 12 hours to about 1 day, from about 1 day to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, or from about 1 month to about 6 months). In some case, a variant viral coat protein of the present disclosure forms disk assemblies that remain stable for at least 5 days in solution at pH 7.5 at 37°C. In some cases, a variant viral coat protein of the present disclosure forms disk assemblies that remain stable (e.g., maintain a stable double disk architecture) at a temperature in a range of from about -80°C to about 40°C in solution at pH 7.5, for example at a temperature from about -80°C to about 0°C, from about 0°C to about 10°C, from about 4 °C to about 15 °C, from about 15 °C to about 25 °C, from about 10°C to about 25°C, from about 25 °C to about 37 °C, or from about 25°C to about 40°C. In some cases, a variant viral coat protein of the present disclosure forms disk assemblies that remain stable (e.g., maintain a stable double disk architecture) at a temperature in a range of from about -80°C to about 40°C in solution at pH 7.5, for example at a temperature from about -80°C to about 0°C, from about 0°C to about 10°C, from about 4 °C to about 15 °C, from about 15 °C to about 25 °C, from about 10°C to about 25°C, from about 25 °C to about 37 °C, or from about 25°C to about 40°C, for a period of time of from about 10 minutes to about 6 months (e.g., from about 10 minutes to about 30 minutes, from about 30 minutes to 1 hour, from about 1 hour to about 6 hours, from about 6 hours to about 12 hours, from about 12 hours to about 1 day, from about 1 day to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, or from about 1 month to about 6 months).

VIRAL COAT PROTEIN CONJUGATES

[0061] The present disclosure provides a viral coat protein conjugate comprising: a) a variant TMV coat protein of the present disclosure; and b) a drug or an imaging agent covalently or non-covalently linked to the variant TMV coat protein. In some cases, a viral coat protein conjugate of the present disclosure comprises a linker group that links the drug or imaging agent to the variant coat protein. A viral coat protein conjugate of the present

disclosure is also referred to herein as a "viral conjugate" or simply "a conjugate." The term "drug" is used interchangeably herein with "agent" and "active agent."

[0062] The active agent (e.g., drug) or imaging agent can be linked, directly or via a linker, to a cysteine present in the variant viral coat protein. In some cases, the active agent or imaging agent is linked, directly or via a linker, to C123 present in the variant viral coat protein (where the numbering is based on the amino acid numbering of SEQ ID NO:1). Where the drug or imaging agent is linked to the variant viral coat polypeptide via a linker, in some cases, the linker is an acid-labile linker. Where the drug or imaging agent is linked to the variant viral coat polypeptide via a linker, in some cases, the linker is a proteolytically cleavable linker. Where the drug or imaging agent is linked to the variant viral coat polypeptide via a linker, in some cases, the linker is a disulfide-containing linker that is cleavable by a reducing agent such as glutathione.

Active agents

[0063] Active agents suitable for linking (e.g., covalently or non-covalently linking, directly or via a linker) to a variant viral coat protein of the present disclosure include, but are not limited to, cancer chemotherapeutic agents; antibiotic agents; anti-parasite agents; anti-viral agents; anti-fungal agents; and anti-inflammatory agents.

Cancer chemotherapeutic agents

[0064] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) a cancer chemotherapeutic agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide.

[0065] Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

[0066] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin,

uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[0067] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

[0068] Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, *etc.*; podophyllotoxins, *e.g.* etoposide, teniposide, *etc.*; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, *etc.*; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, *etc.*; and the like.

[0069] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

[0070] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

[0071] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, *etc.*; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; *etc.*; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation, therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

[0072] Other chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, *etc.*; ureas, *e.g.* hydroxyurea; and hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc.. Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

[0073] "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).

[0074] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[0075] Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

Anti-inflammatory agents

[0076] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) an anti-inflammatory agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide. Suitable anti-inflammatory agents include, but are not limited to, naproxen sodium, diclofenac sodium, diclofenac potassium, celecoxib, sulindac, oxaprozin, diflunisal, etodolac, meloxicam, ibuprofen, ketoprofen, nabumetone, refecoxib, methotrexate, leflunomide, sulfasalazine, gold salts, RHo-D Immune Globulin, mycophenylate mofetil, cyclosporine, azathioprine, tacrolimus, basiliximab, daclizumab, salicylic acid, acetylsalicylic acid, methyl salicylate, diflunisal, salsalate, olsalazine, sulfasalazine, acetaminophen, indomethacin, sulindac, mefenamic acid, meclofenamate sodium, tolmetin, ketorolac, dichlofenac, flurbinprofen, oxaprozin, piroxicam, meloxicam, ampiroxicam, droxicam, pivoxicam, tenoxicam, phenylbutazone, oxyphenbutazone, antipyrine, aminopyrine, apazone, zileuton, aurothioglucose, gold sodium thiomalate, auranofin, methotrexate, colchicine, allopurinol, probenecid, sulfinpyrazone, benzbromarone, betamethasone, glucocorticoidspropionic acid derivatives, alminoprofen, benoxaprofen, bucloxic acid, carprofen, fenbufen, fenoprofen, fluprofen, flurbiprofen, ibuprofen, indoprofen, ketoprofen, miroprofen, naproxen, oxaprozin, pirprofen, pranoprofen, suprofen, tiaprofenic acid, tioxaprofen, indomethacin, acemetacin, alclofenac, clidanac, diclofenac, fenclofenac, fenclozic acid, fentiazac, furofenac, ibufenac, isoxepac, oxpinac, sulindac, tiopinac, tolmetin, zidometacin, zomepirac, flufenamic acid, meclofenamic acid, mefenamic acid, niflumic acid, olfenamic acid, diflunisal, ufenisal, isoxicam, piroxicam, sudoxicam, tenoxican, acetyl

salicylic acid, sulfasalazine, apazone, bezpiperylon, feprazone, mofebutazone, oxyphenbutazone, phenylbutazone, celecoxib and rofecoxib.

Antibiotics

[0077] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) an antibiotic linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide. Antibiotics include anti-bacterial agents and anti-mycobacterial agents.

[0078] Anti-bacterial and anti-mycobacterial agents are known in the art and include, e.g., beta-lactam antibiotics, tetracyclines, streptomycin, chloramphenicol, neomycin, gramicidin, bacitracin, sulfonamides, nitrofurazone, nalidixic acid, rifampicin, fluoroquinolones, isoniazid, pyrazinamide, vancomycin, methicillin etc.

[0079] Suitable anti-bacterial agents include, e.g., Aminoglycosides such as Amikacin, Apramycin, Arbekacin, Bambermycins, Butirosin, Dibekacin, Dihdrostreptomycin, Fortimicin(s), Gentamicin, Ispamicin, Kanamycin, Micronomicin, Neomycin, Neomycin Undecylenate, Netilmicin, Paromomycin, Ribostamycin, Sisomicin, Spectinomycin, Streptomycin, Streptonicozid and Tobramycin; Ansamycins such as Rifamide, Rifampin, Rifamycin and Rifaximin; β-lactams such as Carbapenems such as Imipenem; Cephalosporins such as Cefactor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefixime, Cefinenoxime, Cefodizime, Cefonicid, Cefoperazone, Ceforanide, Cefotaxime, Cefotiam, Cefpimizole, Cefpirimide, Cefpodoxime Proxetil, Cefroxadine, Cefsulodin, Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile Sodium, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin, Cephalothin, Cephapirin Sodium, Cephradine and Pivcefalexin; Cephamycins such as Cefbuperazone, Cefmetazole, Cefminox, Cefetan and Cefoxitin; Monobactams such as Aztreonam, Carumonam and Tigemonam; Oxacephems such as Flomoxef and Moxolactam; Penicillins such as Amidinocillin, Amdinocillin Pivoxil, Amoxicillin, Ampicillan, Apalcillin, Aspoxicillin, Azidocillan, Azlocillan, Bacampicillin, Benzylpenicillinic Acid, Benzylpenicillin Sodium, Carbenicillin, Carfecillin Sodium, Carindacillin, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenicillin Sodium, Epicillin, Fenbenicillin, Floxicillin, Hetacillin, Lenampicillin, Metampicillin, Methicillin Sodium, Mezlocillin, Nafcillin Sodium, Oxacillin, Penamecillin, Penethamate Hydriodide, Penicillin G Benethamine, Penicillin

G Benzathine, Penicillin G Benzhydrylamine, Penicillin G Calcium, Penicillin G Hydrabamine, Penicillin G Potassium, Penicillin G Procaine, Penicillen N, Penicillin O, Penicillin V, Penicillin V Benzathine, Penicillin V Hydrabamine, Penimepicycline, Phenethicillin Potassium, Piperacillin, Pivapicillin, Propicillin, Quinacillin, Sulbenicillin, Talampicillin, Temocillin and Ticarcillin; Lincosamides such as Clindamycin and Lincomycin; Macrolides such as Azithromycin, Carbomycin, Clarithromycin, Erythromycin, Erythromycin Acistrate, Erythromycin Estolate, Erythromycin Glucoheptonate, Erythromycin Lactobionate, Erythromycin Propionate, Erythromycin Stearate, Josamycin, Leucomycins, Midecamycins, Miokamycin, Oleandomycin, Primycin, Rokitamycin, Rosaramicin, Roxithromycin, Spiramycin and Troleandomycin; Polypeptides such as Amphomycin, Bacitracin, Capreomycin, Colistin, Enduracidin, Enviomycin, Fusafungine, Gramicidin(s), Gramicidin S, Mikamycin, Polymyxin, Polymyxin B-Methanesulfonic Acid, Pristinamycin, Ristocetin, Teicoplanin, Thiostrepton, Tuberactinomycin, Tyrocidine, Tyrothricin, Vancomycin, Viomycin, Viomycin Pantothenate, Virginiamycin and Zinc Bacitracin; Tetracyclines such as Apicycline, Chlortetracycline, Clomocycline, Demeclocycline, Doxycycline, Guamecycline, Lymecycline, Meclocycline, Methacycline, Minocycline, Oxytetracycline, Penimepicycline, Pipacycline, Rolitetracycline, Sancycline, Senociclin and Tetracycline; Cycloserine; Mupirocin; and Tuberin.

Antifungal agents

[0080] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) an anti-fungal agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide.

[0081] Suitable anti-fungal agents include, e.g., Polyenes such as Amphotericin-B (including various formulations of Amphotericin-B), Candicidin, Dermostatin, Filipin, Fungichromin, Hachimycin, Hamycin, Lucensomycin, Mepartricin, Natamycin, Nystatin, Pecilocin and Perimycin; and others such as Azaserine, Griseofulvin, Oligomycins, Neomycin Undecylenate, Pyrrolnitrin, Siccanin, Tubercidin and Viridin; Allylamines such as Naftifine and Terbinafine; Imidazoles such as Bifonazole, Butoconazole, Chlordantoin, Chlormidazole, Cloconazole, Clotrimazole, Econazole, Enilconazole, Fenticonazole, Isoconazole, Ketoconazole, Miconazole, Omoconazole,

Oxiconazole, Nitrate, Sulconazole and Tioconazole; Triazoles such as Fluconazole, Itraconazole and Terconazole; and other others such as Acrisorcin, Amorolfine, Biphenamine, Bromosalicylchloranilide, Buclosamide, Calcium Propionate, Chlophenesin, Ciclopirox, Cloxyquin, Coparaffinate, Diamthazole, Dihydrochloride, Exalamide, Flucytosine, Halethazole, Hexetidine, Loflucarban, Nifuratel, Potassium Iodide, Propionic Acid, Pyrithione, Salicylanilide, Sodium Propionate, Sulbentine, Tenonitrozole, Tolciclate, Tolindate, Tolnaftate, Tricetin, Ujothion, Undecylenic Acid and Zinc Propionate.

Anti-parasitic agents

[0082] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) an anti-parasitic agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide.

[0083] Anti-parasitic agents are known in the art and include, e.g., chloroquine, etc. For example, anti-malarial agents include, e.g., quinine, chloroquine, atovaquone, proguanil, primaquine, amodiaquine, mefloquine, piperaquine, artemisinin, methylene blue, pyrimethamine, sulfadoxine, artemether-lumefantrine, dapsone-chlorproguanil, artesunate, quinidine, clopidol, pyridine/pyridinol analogs, 4(1H)-quinolone analogs, dihydroartemisinin, a mixture of atovaquone and proguanil, an endoperoxide, and an acridone.

Imaging agents

[0084] As noted above, in some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) an imaging agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide. Examples of imaging agents include fluorescent compounds, radioactive isotopes, and MRI contrast agents. For example, in some embodiments, the imaging agent is a fluorescent molecule for fluorescent imaging. The detectable group can be any material having a detectable physical or chemical property. Such imaging agents have been well-developed in the field of fluorescent imaging, magnetic resonance imaging, positive emission tomography, and immunoassays and, in general, nearly any imaging agent useful in such methods can be conjugated to a variant viral coat polypeptide of the present disclosure. A suitable imaging agent is any compound detectable by

spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful imaging agents include magnetic beads (e.g. Dynabeads™) fluorescent dyes (e.g., fluorescein isothiocyanate, AlexaFluor555, Texas red, rhodamine, and the like), radiolabels (e.g., ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{121}I, ^{112}In, 99mTc), other imaging agents such as microbubbles (for ultrasound imaging), ^{18}F, ^{11}C, ^{15}O, (for Positron emission tomography), 99mTC, ^{111}In (for single photon emission tomography), and chelated lanthanides such as terbium, gadolinium, and europium (e.g., chelated gadolinium) or iron (for magnetic resonance imaging). Suitable imaging agents include cryptophane and cucurbituril molecules for use as 129Xe NMR/MRI contrast agents. See, e.g., US Patent Publication No. 2014/0004043. The choice of imaging agent depending on sensitivity required, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[0085] In some cases, the imaging agent is a magnetic resonance imaging agent. In the case of magnetic resonance imaging, the imaging agent can also be referred to as a contrast agent. Lanthanide elements are known to be useful as contrast agents. The lanthanide chemical elements comprises the fifteen metallic chemical elements with atomic numbers 57 through 71, and include lanthanum, cerium, praseodymium, neodymium, promethium, samarium, europium, gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium, and lutetium. Exemplary lanthanides include europium, gadolinium, and terbium. In order to more readily handle these rare earth metals, the lanthanides can be chelated. In some embodiments, the lanthanide selected for use as a contrast agent is gadolinium, or more specifically gadolinium (III).

Water-soluble polymers

[0086] In some cases, a viral protein conjugate of the present disclosure comprises, in addition to a drug or an imaging agent, a water-soluble polymer linked (covalently or non-covalently, directly or via a linker) to the variant viral coat protein. A "water-soluble polymer" refers to a polymer that is soluble in water, is substantially non-immunogenic, and can have an atomic molecular weight greater than about 1,000 Daltons. Attachment of a water-soluble polymer (e.g., PEG) to a variant viral coat polypeptide of the present disclosure (e.g., where the variant viral coat polypeptide is conjugated to an active (therapeutic) agent) can be desirable as such modification can increase therapeutic index by increasing serum half-life as a result of increased proteolytic stability and/or

decreased renal clearance. Additionally, attachment of one or more polymers (e.g., PEGylation) can reduce immunogenicity of protein pharmaceuticals.

[0087] In some cases, the water-soluble polymer has an effective hydrodynamic molecular weight of greater than about 10,000 Da, greater than about 20,000 to 500,000 Da, greater than about 40,000 Da to 300,000 Da, greater than about 50,000 Da to 70,000 Da, usually greater than about 60,000 Da. In some embodiments, the water-soluble polymer has an effective hydrodynamic molecular weight of from about 10 kDa to about 20 kDa, from about 20 kDa to about 25 kDa, from about 25 kDa to about 30 kDa, from about 30 kDa to about 50 kDa, or from about 50 kDa to about 100 kDa. By "effective hydrodynamic molecular weight" is intended the effective water-solvated size of a polymer chain as determined by aqueous-based size exclusion chromatography (SEC). When the water-soluble polymer contains polymer chains having polyalkylene oxide repeat units, such as ethylene oxide repeat units, each chain can have an atomic molecular weight of between about 200 Da and about 80,000 Da, or between about 1,500 Da and about 42,000 Da, or from about 2,000 to about 20,000 Da. Unless referred to specifically, molecular weight is intended to refer to atomic molecular weight. Linear, branched, and terminally charged water soluble polymers (e.g., PEG) are of particular interest.

[0088] Useful water-soluble polymers for conjugation to a variant viral coat protein of the present disclosure can have a wide range of molecular weights, and polymer subunits. These subunits may include a biological polymer, a synthetic polymer, or a combination thereof. Examples of such water-soluble polymers include: dextran and dextran derivatives, including dextran sulfate, P-amino cross linked dextrin, and carboxymethyl dextrin, cellulose and cellulose derivatives, including methylcellulose and carboxymethyl cellulose, starch and dextrines, and derivatives and hydroylactes of starch, polyalklyene glycol and derivatives thereof, including polyethylene glycol, methoxypolyethylene glycol, polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol, wherein said homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group, heparin and fragments of heparin, polyvinyl alcohol and polyvinyl ethyl ethers, polyvinylpyrrolidone, aspartamide, and polyoxyethylated polyols, with the dextran and dextran derivatives, dextrine and dextrine derivatives. It will be appreciated that various derivatives of the specifically recited water-soluble polymers are also contemplated.

[0089] Water-soluble polymers such as those described above are well known, particularly the polyalkylene oxide based polymers such as polyethylene glycol "PEG" (See. e.g., "Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications", J. M. Harris, Ed., Plenum Press, New York, N.Y. (1992); and "Poly(ethylene glycol) Chemistry and Biological Applications", J. M. Harris and S. Zalipsky, Eds., ACS (1997); and International Patent Applications: WO 90/13540, WO 92/00748, WO 92/16555, WO 94/04193,WO 94/14758, WO 94/17039, WO 94/18247, WO 94/28937, WO 95/11924, WO 96/00080, WO 96/23794, WO 98/07713, WO 98/41562, WO 98/48837, WO 99/30727, WO 99/32134, WO 99/33483, WO 99/53951, WO 01/26692, WO 95/13312, WO 96/21469, WO 97/03106, WO 99/45964, and U.S. Pat. Nos. 4,179,337; 5,075,046; 5,089,261; 5,100,992; 5,134,192; 5,166,309; 5,171,264; 5,213,891; 5,219,564; 5,275,838; 5,281,698; 5,298,643; 5,312,808; 5,321,095; 5,324,844; 5,349,001; 5,352,756; 5,405,877; 5,455,027; 5,446,090; 5,470,829; 5,478,805; 5,567,422; 5,605,976; 5,612,460; 5,614,549; 5,618,528; 5,672,662; 5,637,749; 5,643,575; 5,650,388; 5,681,567; 5,686,110; 5,730,990; 5,739,208; 5,756,593; 5,808,096; 5,824,778; 5,824,784; 5,840,900; 5,874,500; 5,880,131; 5,900,461; 5,902,588; 5,919,442; 5,919,455; 5,932,462; 5,965,119; 5,965,566; 5,985,263; 5,990,237; 6,011,042; 6,013,283; 6,077,939; 6,113,906; 6,127,355; 6,177,087; 6,180,095; 6,194,580; 6,214,966).

[0090] Exemplary polymers of interest include those containing a polyalkylene oxide, polyamide alkylene oxide, or derivatives thereof, including polyalkylene oxide and polyamide alkylene oxide comprising an ethylene oxide repeat unit of the formula -(CH2-CH2-O)-. Further exemplary polymers of interest include a polyamide having a molecular weight greater than about 1,000 Daltons of the formula -[C(O)-X-C(O)-NH-Y-NH]n- or -[NH-Y-NH-C(O)-X-C(O)]n-, where X and Y are divalent radicals that may be the same or different and may be branched or linear, and n is a discrete integer from 2-100, e.g., from 2 to 50 (e.g., n is 2, 3, 4, 5, from 5 to 10, from 10 to 15, from 15 to 20, from 20 to 25, from 25 to 30, from 30 to 35, from 35 to 40, from 40 to 45, or from 45 to 50), and where either or both of X and Y comprises a biocompatible, substantially non-antigenic water-soluble repeat unit that may be linear or branched. Further exemplary water-soluble repeat units comprise an ethylene oxide of the formula -(CH_2-CH_2-O)- or -(CH_2-CH_2-O)- . The number of such water-soluble repeat units can vary significantly,

with the usual number of such units being from 2 to 500, 2 to 400, 2 to 300, 2 to 200, 2 to 100, and most usually 2 to 50. An exemplary embodiment is one in which one or both of X and Y is selected from: -((CH_2) n1-(CH_2-CH_2-O)n2-(CH_2)- or -((CH_2)n1-(O-CH_2-CH_2)n2-(CH_2) n- 1-), where n1 is 1 to 6, 1 to 5, 1 to 4, e.g., from 1 to 3; where n2 is 2 to 50, 2 to 25, 2 to 15, 2 to 10, 2 to 8, e.g., from 2 to 5. A further exemplary embodiment is one in which X is -(CH_2-CH_2)-, and where Y is -(CH_2-(CH_2-CH_2-O)$_3$-CH_2-CH_2-CH_2)- or -(CH_2-CH_2-CH_2-(O-CH_2-CH_2)$_3$-CH_2)-.

[0091] The polymer can include one or more spacers or linkers. Exemplary spacers or linkers include linear or branched moieties comprising one or more repeat units employed in a water-soluble polymer, diamino and or diacid units, natural or unnatural amino acids or derivatives thereof, as well as aliphatic moieties, including alkyl, aryl, heteroalkyl, heteroaryl, alkoxy, and the like, which can contain, for example, up to 18 carbon atoms or even an additional polymer chain.

[0092] The polymer moiety, or one or more of the spacers or linkers of the polymer moiety when present, may include polymer chains or units that are biostable or biodegradable. For example, Polymers with repeat linkages have varying degrees of stability under physiological conditions depending on bond lability. Polymers with such bonds can be categorized by their relative rates of hydrolysis under physiological conditions based on known hydrolysis rates of low molecular weight analogs, e.g., from less stable to more stable, e.g., polyurethanes (-NH-C(O)-O) > polyorthoesters (-O-C((OR)(R'))-O-) > polyamides (-C(O)-NH-). Similarly, the linkage systems attaching a water-soluble polymer to a target molecule may be biostable or biodegradable, e.g., from less stable to more stable: carbonate (-O-C(O)-O-)>ester (-C(O)-O) > urethane (-NH-C(O)-O-) > orthoester (-O-C((OR)(R'))-O-) > amide (-C(O)-NH-). In general, it may be desirable to avoid use of sulfated polysaccharide, depending on the lability of the sulfate group. In addition, it may be less desirable to use polycarbonates and polyesters. These bonds are provided by way of example, and are not intended to limit the types of bonds employable in the polymer chains or linkage systems of the water-soluble polymers suitable for inclusion in a viral conjugate disclosed herein.

Antibodies

[0093] In some cases, an antibody is conjugated to a variant viral coat polypeptide of the present disclosure. For example, in some cases, a viral conjugate of the present

disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; b) an active agent or imaging agent linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide; and c) an antibody linked (covalently or non-covalently, directly or via a linker) to the variant viral coat polypeptide. In some cases, the antibody binds to a target cell, e.g., a cancer cell, etc.

[0094] The antibody can be any antigen-binding antibody-based polypeptide, a wide variety of which are known in the art. In some instances, the antibody is a single chain Fv (scFv). Other antibody based recognition domains (cAb VHH (camelid antibody variable domains) and humanized versions, IgNAR VH (shark antibody variable domains) and humanized versions, sdAb VH (single domain antibody variable domains) and "camelized" antibody variable domains are suitable for use. In some cases, the antibody is a nanobody.

[0095] In some cases, the antibody is specific for an epitope present in an antigen that is expressed by (synthesized by) a cancer cell, i.e., a cancer cell associated antigen. The cancer cell associated antigen can be an antigen associated with, e.g., a breast cancer cell, a B cell lymphoma, a pancreatic cancer, a Hodgkin lymphoma cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma, a lung cancer cell (e.g., a small cell lung cancer cell), a non-Hodgkin B-cell lymphoma (B-NHL) cell, an ovarian cancer cell, a prostate cancer cell, a mesothelioma cell, a lung cancer cell (e.g., a small cell lung cancer cell), a melanoma cell, a chronic lymphocytic leukemia cell, an acute lymphocytic leukemia cell, a neuroblastoma cell, a glioma, a glioblastoma, a medulloblastoma, a colorectal cancer cell, etc. A cancer cell associated antigen may also be expressed by a non-cancerous cell.

[0096] In some cases, the antibody is specific for an epitope present in a tissue-specific antigen. In some cases, the antigen-binding domain is specific for an epitope present in a disease-associated antigen.

[0097] Non-limiting examples of antigens to which an antibody can bind include, e.g., CD19, CD20, CD38, CD30, Her2/neu, ERBB2, CA125, MUC-1, prostate-specific membrane antigen (PSMA), CD44 surface adhesion molecule, mesothelin, carcinoembryonic antigen (CEA), epidermal growth factor receptor (EGFR), EGFRvIII, vascular endothelial growth factor receptor-2 (VEGFR2), high molecular weight-melanoma associated antigen (HMW-MAA), MAGE-A1, IL-13R-a2, GD2, and the like.

[0098] Non-limiting examples of antigens to which an antibody can bind include, e.g., Cadherins (CDH1-20), Integrins (alfa and beta isoforms), Ephrins, NCAMs, connexins, CD44, syndecan, CD47, DGalfa/beta, SV2, protocadherin, Fas, Dectin-1, CD7, CD40, Neuregulin, KIR, BTLA, Tim-2, Lag-3, CD19, CTLA4, CD28, TIGIT, and ICOS.

[0099] In some cases, the antibody is specific for a cell surface target, where non-limiting examples of cell surface targets include CD19, CD30, Her2, CD22, ENPP3, EGFR, CD20, CD52, CD 11a, and alpha-integrin.

[00100] Suitable antibodies include, but are not limited to, trastuzumab (Herceptin) , bevacizumab (Avastin™), cetuximab (Erbitux™), panitumumab (Vectibix™), Ipilimumab (Yervoy™), rituximab (Rituxan), alemtuzumab (Lemtrada™), Ofatumumab (Arzerra™), Oregovomab (OvaRex™), Lambrolizumab (MK-3475), pertuzumab (Perjeta™), ranibizumab (Lucentis™) etc.. Suitable antibodies include, but are not limited to, antibodies raised against tumor-associated antigens. Such antigens include, but are not limited to, CD20, CD30, CD33, CD52, EpCAM, CEA, gpA33, Mucins, TAG-72, CAIX, PSMA, Folate-binding protein, Gangliosides (e.g., GD2, GD3, GM2, etc.), Le ^{y} , VEGF, VEGFR, Integrin alpha-V-beta-3, Integrin alpha-5-beta-1, EGFR, ERBB2, ERBB3, MET, IGF1R, EPHA3, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, etc.

Chitin-binding domain

[00101] In some cases, a variant viral coat polypeptide of the present disclosure comprises a chitin binding domain linked to the variant viral coat polypeptide. Chitin binding domains are known in the art.

[00102] In some cases, a chitin-binding domain comprises an comprises an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to the following chitin binding domain amino acid sequence:

[0001] MTQSKVAPRH GTVSEPASRA HIYLAEWQSA GLESGKFFPE TQAGLADPSA PDDVRNDAXP TDGKIASAGQ DFAAELDKPG SDWQKHSVAA GQRLTITWSF HAPHKTRRWN YFLTREGWDP NAPLSRAQFE PDPIHQVQNS GQPYWSADDL LPENPTRHTI VLPQRQGYHV LLSVWEVADT AKGFYQVIDL NFTG (SEQ ID NO:13).

[0002] In some cases, a chitin-binding domain comprises the following amino acid sequence: EGKGVEAVGDGR (SEQ ID NO:14); see, e.g., Khoushab et al. (2012) *Int. J. Biol. Macromolec.* 50:1267.

[0003] In some cases, a chitin-binding domain comprises the following amino acid sequence: VGECVRGRCPSGMCCSQFGYCGKGPKYCGR (SEQ ID NO:15); see, e.g., Broekaert et al. (1992) *Biochem.* 31:4308.

Linkers

[0004] As noted above, in some cases, a viral coat protein conjugate of the present disclosure comprises a linker group that links an active agent, an imaging agent, a polymer, or a polypeptide such as an antibody to the variant coat protein. These linkers groups include at least one specific binding moiety or a functional group capable of subsequent covalent bond formation. The linker molecule may include a single participating reactive group or a plurality of participating reactive groups. Where the linker molecules include a plurality of covalent bond-forming reactive groups, the number of covalent bond-forming reactive groups in the molecule may be 2, 3, 4, 5, 6, 7, 8, 9, 10 or more. The linker molecules may be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids or combinations thereof. The linker can be an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., Cancer Research 52:127-131 (1992); U.S. Pat. No.) may be used. Other linker molecules which can bind polypeptide may be used in light of this disclosure.

Proteolytically cleavable linkers

[0005] In some cases, a viral conjugate of the present disclosure comprises: a) a variant viral coat polypeptide of the present disclosure; and b) a drug or imaging agent covalently or non-covalently linked to the variant viral coat polypeptide, where the drug or imaging agent is linked to the variant viral coat polypeptide via a linker. In some cases, the linker is a proteolytically cleavable linker comprising a protease recognition sequence recognized by a protease. In some cases, the protease is selected from the group consisting of alanine carboxypeptidase, Armillaria mellea astacin, bacterial leucyl aminopeptidase, cancer procoagulant, cathepsin B, clostripain, cytosol alanyl aminopeptidase, elastase, endoproteinase Arg-C, enterokinase, gastricsin, gelatinase, Gly-X carboxypeptidase, glycyl endopeptidase, human rhinovirus 3C protease,

hypodermin C, IgA-specific serine endopeptidase, leucyl aminopeptidase, leucyl endopeptidase, lysC, lysosomal pro-X carboxypeptidase, lysyl aminopeptidase, methionyl aminopeptidase, myxobacter, nardilysin, pancreatic endopeptidase E, picornain 2A, picornain 3C, proendopeptidase, prolyl aminopeptidase, proprotein convertase I, proprotein convertase II, russellysin, saccharopepsin, semenogelase, T-plasminogen activator, thrombin, tissue kallikrein, tobacco etch virus (TEV), togavirin, tryptophanyl aminopeptidase, U-plasminogen activator, V8, venombin A, venombin AB, and Xaa-pro aminopeptidase. In some cases, the protease is an endogenous cellular protease present in a mammalian cell.

[0006] For example, the proteolytically cleavable linker can comprise a matrix metalloproteinase cleavage site, e.g., a cleavage site for a MMP selected from collagenase-1, -2, and -3 (MMP-1, -8, and -13), gelatinase A and B (MMP-2 and -9), stromelysin 1, 2, and 3 (MMP-3, -10, and -11), matrilysin (MMP-7), and membrane metalloproteinases (MT1-MMP and MT2-MMP). For example, the cleavage sequence of MMP-9 is Pro-X-X-Hy (wherein, X represents an arbitrary residue; Hy, a hydrophobic residue; SEQ ID NO:16), e.g., Pro-X-X-Hy-(Ser/Thr) SEQ ID NO:17, e.g., Pro-Leu/Gln-Gly-Met-Thr-Ser (SEQ ID NO:18) or Pro-Leu/Gln-Gly-Met-Thr (SEQ ID NO:19). Another example of a protease cleavage site is a plasminogen activator cleavage site, e.g., a uPA or a tissue plasminogen activator (tPA) cleavage site. Another example of a suitable protease cleavage site is a prolactin cleavage site. Specific examples of cleavage sequences of uPA and tPA include sequences comprising Val-Gly-Arg. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is a tobacco etch virus (TEV) protease cleavage site, e.g., ENLYTQS (SEQ ID NO:20), where the protease cleaves between the glutamine and the serine. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is an enterokinase cleavage site, e.g., DDDDK (SEQ ID NO:21) where cleavage occurs after the lysine residue. Another example of a protease cleavage site that can be included in a proteolytically cleavable linker is a thrombin cleavage site, e.g., LVPR (SEQ ID NO:22). Additional suitable linkers comprising protease cleavage sites include linkers comprising one or more of the following amino acid sequences: LEVLFQGP (SEQ ID NO:23), cleaved by PreScission protease (a fusion protein comprising human rhinovirus 3C protease and glutathione-S-transferase; Walker et al.

(1994) *Biotechnol.* 12:601); a thrombin cleavage site, e.g., CGLVPAGSGP (SEQ ID NO:24); SLLKSRMVPNFN (SEQ ID NO:25) or SLLIARRMPNFN (SEQ ID NO:26), cleaved by cathepsin B; SKLVQASASGVN (SEQ ID NO:27) or SSYLKASDAPDN (SEQ ID NO:28), cleaved by an Epstein-Barr virus protease; RPKPQQFFGLMN (SEQ ID NO:29) cleaved by MMP-3 (stromelysin); SLRPLALWRSFN (SEQ ID NO:30) cleaved by MMP-7 (matrilysin); SPQGIAGQRNFN (SEQ ID NO:31) cleaved by MMP-9; DVDERDVRGFASFL SEQ ID NO:32) cleaved by a thermolysin-like MMP; SLPLGLWAPNFN (SEQ ID NO:33) cleaved by matrix metalloproteinase 2(MMP-2); SLLIFRSWANFN (SEQ ID NO:34) cleaved by cathespin L; SGVVIATVIVIT (SEQ ID NO:35) cleaved by cathepsin D; SLGPQGIWGQFN (SEQ ID NO:36) cleaved by matrix metalloproteinase 1(MMP-1); KKSPGRVVGGSV (SEQ ID NO:37) cleaved by urokinase-type plasminogen activator; PQGLLGAPGILG (SEQ ID NO:38) cleaved by membrane type 1 matrix metalloproteinase (MT-MMP); HGPEGLRVGFYESDVMGRGHARLVHVEEPHT (SEQ ID NO:39) cleaved by stromelysin 3 (or MMP-11), thermolysin, fibroblast collagenase and stromelysin-1; GPQGLAGQRGIV (SEQ ID NO:40) cleaved by matrix metalloproteinase 13 (collagenase-3); GGSGQRGRKALE (SEQ ID NO:41) cleaved by tissue-type plasminogen activator(tPA); SLSALLSSDIFN (SEQ ID NO:42) cleaved by human prostate-specific antigen; SLPRFKIIGGFN (SEQ ID NO:43) cleaved by kallikrein (hK3); SLLGIAVPGNFN (SEQ ID NO:44) cleaved by neutrophil elastase; and FFKNIVTPRTPP (SEQ ID NO:45) cleaved by calpain (calcium activated neutral protease).

Conjugation methods

[0007] A variety of conjugation methods and chemistries can be used to conjugate a polypeptide to a moiety such as a drug, an imaging agent, an antibody, a non-peptide polymer, etc. Various zero-length, homo-bifunctional, and hetero-bifunctional crosslinking reagents can be used. Zero-length crosslinking reagents include direct conjugation of two intrinsic chemical groups with no introduction of extrinsic material. Agents that catalyze formation of a disulfide bond belong to this category. Another example is reagents that induce condensation of a carboxyl and a primary amino group to form an amide bond such as carbodiimides, ethylchloroformate, Woodward's reagent K (2-ethyl-5-phenylisoxazolium-3'-sulfonate), and carbonyldiimidazole. Homo- and hetero-

bifunctional reagents generally contain two identical or two non-identical sites, respectively, which may be reactive with amino, sulfhydryl, guanidino, indole, or nonspecific groups.

[0008] In some cases, a moiety for conjugation to a variant viral coat polypeptide of the present disclosure comprises an amino-reactive group for reacting with a primary amine group on the variant viral coat polypeptide, or on a linker. Suitable amino-reactive groups include, but are not limited to, N-hydroxysuccinimide (NHS) esters, imidoesters, isocyanates, acylhalides, arylazides, p-nitrophenyl esters, aldehydes, and sulfonyl chlorides.

[0009] In some cases, a moiety for conjugation to a variant viral coat polypeptide of the present disclosure comprises a sulfhydryl-reactive group, e.g., for reacting with a cysteine residue in the variant viral coat polypeptide. Suitable sulfhydryl-reactive groups include, but are not limited to, maleimides, alkyl halides, pyridyl disulfides, and thiophthalimides.

[0010] In other embodiments, carbodiimides soluble in both water and organic solvent, are used as carboxyl-reactive reagents. These compounds react with free carboxyl groups forming a pseudourea that can then couple to available amines, yielding an amide linkage.

[0011] As noted above, in some cases, a moiety for conjugation to a variant viral coat polypeptide of the present disclosure is conjugated to the variant viral coat polypeptide using a homobifunctional crosslinker.

[0012] In some cases, the homobifunctional crosslinker is reactive with primary amines. Homobifunctional crosslinkers that are reactive with primary amines include NHS esters, imidoesters, isothiocyanates, isocyanates, acylhalides, arylazides, p-nitrophenyl esters, aldehydes, and sulfonyl chlorides.

[0013] Non-limiting examples of homobifunctional NHS esters include disuccinimidyl glutarate (DSG), disuccinimidyl suberate (DSS), bis(sulfosuccinimidyl) suberate (BS), disuccinimidyl tartarate (DST), disulfosuccinimidyl tartarate (sulfo-DST), bis-2-(succinimidooxycarbonyloxy)ethylsulfone (BSOCOES), bis-2-(sulfosuccinimidooxycarbonyloxy)ethylsulfone (sulfo-BSOCOES), ethylene glycolbis(succinimidylsuccinate) (EGS), ethylene glycolbis(sulfosuccinimidylsuccinate) (sulfo-EGS), dithiobis(succinimidylpropionate (DSP), and dithiobis(sulfosuccinimidylpropionate(sulfo-DSP). Non-limiting examples of

homobifunctional imidoesters include dimethyl malonimidate (DMM), dimethyl succinimidate (DMSC), dimethyl adipimidate (DMA), dimethyl pimelimidate (DMP), dimethyl suberimidate (DMS), dimethyl-3,3'-oxydipropionimidate (DODP), dimethyl-3,3'-(methylenedioxy)dipropionimidate (DMDP), dimethyl-,3'-(dimethylenedioxy)dipropionimidate (DDDP), dimethyl-3,3'-(tetramethylenedioxy)dipropionimidate (DTDP), and dimethyl-3,3'-dithiobispropionimidate (DTBP).

[0014] Non-limiting examples of homobifunctional isothiocyanates include: p-phenylenediisothiocyanate (DITC), and 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene (DIDS). Non-limiting examples of homobifunctional isocyanates include xylene-diisocyanate, toluene-2,4-diisocyanate, toluene-2-isocyanate-4-isothiocyanate, 3-methoxydiphenylmethane-4,4'-diisocyanate, 2,2'-dicarboxy-4,4'-azophenyldiisocyanate, and hexamethylenediisocyanate. Non-limiting examples of homobifunctional arylhalides include 1,5-difluoro-2,4-dinitrobenzene (DFDNB), and 4,4'-difluoro-3,3'-dinitrophenyl-sulfone. Non-limiting examples of homobifunctional aliphatic aldehyde reagents include glyoxal, malondialdehyde, and glutaraldehyde. Non-limiting examples of homobifunctional acylating reagents include nitrophenyl esters of dicarboxylic acids. Non-limiting examples of homobifunctional aromatic sulfonyl chlorides include phenol-2,4-disulfonyl chloride, and α-naphthol-2,4-disulfonyl chloride. Non-limiting examples of additional amino-reactive homobifunctional reagents include erythritolbiscarbonate, which reacts with amines to give biscarbamates.

[0015] In some cases, the homobifunctional crosslinker is reactive with free sulfhydryl groups. Homobifunctional crosslinkers reactive with free sulfhydryl groups include, e.g., maleimides, pyridyl disulfides, and alkyl halides.

[0016] Non-limiting examples of homobifunctional maleimides include bismaleimidohexane (BMH), N,N'-(1,3-phenylene) bismaleimide, N,N'-(1,2-phenylene)bismaleimide, azophenyldimaleimide, and bis(N-maleimidomethyl)ether. Non-limiting examples of homobifunctional pyridyl disulfides include 1,4-di-3'-(2'-pyridyldithio)propionamidobutane (DPDPB). Non-limiting examples of homobifunctional alkyl halides include 2,2'-dicarboxy-4,4'-diiodoacetamidoazobenzene, α, α'-diiodo-p-xylenesulfonic acid, α, α'-dibromo-p-xylenesulfonic acid, N,N'-bis(b-

bromoethyl)benzylamine, N,N'-di(bromoacetyl)phenylhydrazine, and 1,2-di(bromoacetyl)amino-3-phenylpropane.

[0017] As noted above, in some cases, a moiety for conjugation to a variant viral coat polypeptide of the present disclosure is conjugated to a variant viral coat polypeptide of the present disclosure using a heterobifunctional reagent. Suitable heterobifunctional reagents include amino-reactive reagents comprising a pyridyl disulfide moiety; amino-reactive reagents comprising a maleimide moiety; amino-reactive reagents comprising an alkyl halide moiety; and amino-reactive reagents comprising an alkyl dihalide moiety.

[0018] Non-limiting examples of hetero-bifunctional reagents with a pyridyl disulfide moiety and an amino-reactive NHS ester include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), succinimidyl 6-3-(2-pyridyldithio)propionamidohexanoate (LC-SPDP), sulfosuccinimidyl 6-3-(2-pyridyldithio)propionamidohexanoate (sulfo-LCSPDP), 4-succinimidyloxycarbonyl-α-methyl-α-(2-pyridyldithio)toluene (SMPT), and sulfosuccinimidyl 6-α-methyl-α-(2-pyridyldithio)toluamidohexanoate (sulfo-LC-SMPT).

[0019] Non-limiting examples of heterobifunctional reagents comprising a maleimide moiety and an amino-reactive NHS ester include succinimidyl maleimidylacetate (AMAS), succinimidyl 3-maleimidylpropionate (BMPS), N-.gamma.-maleimidobutyryloxysuccinimide ester (GMBS)N-.gamma.-maleimidobutyryloxysulfosuccinimide ester (sulfo-GMBS) succinimidyl 6-maleimidylhexanoate (EMCS), succinimidyl 3-maleimidylbenzoate (SMB), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), and sulfosuccinimidyl 4-(p-maleimidophenyl)butyrate (sulfo-SMPB).

[0020] Non-limiting examples of heterobifunctional reagents comprising an alkyl halide moiety and an amino-reactive NHS ester include N-succinimidyl-(4-iodoacetyl)aminobenzoate (SIAB), sulfosuccinimidyl-(4-iodoacetyl)aminobenzoate (sulfo-SIAB), succinimidyl-6-(iodoacetyl)aminohexanoate (SIAX), succinimidyl-6-(6-((iodoacetyl)-amino)hexanoylamino)hexanoate (SIAXX), succinimidyl-6-(((4-(iodoacetyl)-

amino)methyl)-cyclohexane-1-carbonyl)aminohexanoate (SIACX), and succinimidyl-4((iodoacetyl)-amino)methylcyclohexane-1-carboxylate (SIAC).

[0021] A non-limiting example of a hetero-bifunctional reagent comprising an amino-reactive NHS ester and an alkyl dihalide moiety is N-hydroxysuccinimidyl 2,3-dibromopropionate (SDBP). A non-limiting example of a hetero-bifunctional reagent comprising an alkyl halide moiety and an amino-reactive p-nitrophenyl ester moiety include p-nitrophenyl iodoacetate (NPIA).

DELIVERY DISKS

[0022] The present disclosure provides delivery disks. A delivery disk of the present disclosure comprises a viral conjugate of the present disclosure, where the viral conjugate has self assembled into a disk. A disk of the present disclosure is also referred to herein as a "delivery nanoparticle."

[0023] In some cases, a delivery disk of the present disclosure ranges in diameter from about 10 nm to about 25nm, e.g. from about 10.0 nm to about 10.5 nm, from about 10.5 nm to about 11.0, from about 11.0 nm to about 11.5 nm, from about 11.5 to about 12.0 nm, from about 12.0 nm to about 12.5, from about 12.5 nm to about 13.0 nm, from about 13.0 to about 13.5 nm, from about 13.5 nm to about 14.0 nm, from about 14.5 nm to about 15.0 nm, from about 15.0 nm to about 15.5 nm, from about 15.5 nm to about 16.0 nm, from about 16.0 nm to about 16.5 nm, from about 16.5 n to about 17.0 nm from about 17.0 nm to about 17.5 nm, from about 17.5 nm to about 18.0 nm, from about 18.0 nm to about 18.5 nm, from about 18.5 nm to about 19.0 nm, from about 19.0 nm to about 19.5 nm, from about 19.5 nm to about 20.0 nm, from about 20.0 nm to about 20.5 nm, from about 20.5 nm to about 21.0 nm, from about 21.0 nm to about 21.5 nm, from about 21.5 nm to about 22.0 nm, from about 22.0 nm to about 22.5 nm, from about 22.5 nm to about 23.0 nm, from about 23.0 nm to about 23.5 nm, from about 23.5 nm to about 24.0 nm, from about 24.0 nm to about 24.5 nm, from about 24.5 nm to about 25.0 nm.

[0024] In some cases, a population of delivery disks has an "average" diameter, where the average diameter of a population of delivery disks of the present disclosure ranges in diameter from about 10 nm to about 25nm, e.g. from about 10.0 nm to about 10.5 nm, from about 10.5 nm to about 11.0, from about 11.0 nm to about 11.5 nm, from about 11.5 to about 12.0 nm, from about 12.0 nm to about 12.5, from about 12.5 nm to about

13.0 nm, from about 13.0 to about 13.5 nm, from about 13.5 nm to about 14.0 nm, from about 14.5 nm to about 15.0 nm, from about 15.0 nm to about 15.5 nm, from about 15.5 nm to about 16.0 nm, from about 16.0 nm to about 16.5 nm, from about 16.5 n to about 17.0 nm from about 17.0 nm to about 17.5 nm, from about 17.5 nm to about 18.0 nm, from about 18.0 nm to about 18.5 nm, from about 18.5 nm to about 19.0 nm, from about 19.0 nm to about 19.5 nm, from about 19.5 nm to about 20.0 nm, from about 20.0 nm to about 20.5 nm, from about 20.5 nm to about 21.0 nm, from about 21.0 nm to about 21.5 nm, from about 21.5 nm to about 22.0 nm, from about 22.0 nm to about 22.5 nm, from about 22.5 nm to about 23.0 nm, from about 23.0 nm to about 23.5 nm, from about 23.5 nm to about 24.0 nm, from about 24.0 nm to about 24.5 nm, from about 24.5 nm to about 25.0 nm.

[0025] In some cases, a delivery disk of the present disclosure is stable in a liquid solution at a pH of from 4 to 10, e.g., at pH 4.0 to 4.5, 4.5 to 5.0, 5.0 to 5.5, 5.5 to 6.0, 6.0 to 6.5, 6.5 to 7.0, 7.0 to 7.5, 7.5 to 8.0, 8.0 to 8.5, 8.5 to 9.0, or 9.0 to 9.5 at room temperature for a period of time of from about 10 minutes to about 6 months (e.g., from about 10 minutes to about 30 minutes, from about 30 minutes to 1 hour, from about 1 hour to about 6 hours, from about 6 hours to about 12 hours, from about 12 hours to about 1 day, from about 1 day to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, or from about 1 month to about 6 months In some cases, a delivery disk of the present disclosure is stable for at least 5 days in solution at pH 7.5 at 37°C.

[0026] In some cases, a delivery disk of the present disclosure is stable (e.g., maintains a stable double disk architecture) at a temperature in a range of from about -80°C to about 40°C in solution at pH 7.5, for example at a temperature from about -80°C to about 0°C, from about 0°C to about 10°C, from about 4 °C to about 15 °C, from about 15 °C to about 25 °C, from about 10°C to about 25°C, from about 25 °C to about 37 °C, or from about 25°C to about 40°C. In some cases, a delivery disk of the present disclosure is stable (e.g., maintain a stable double disk architecture) at a temperature in a range of from about -80°C to about 40°C in solution at pH 7.5, for example at a temperature from about -80°C to about 0°C, from about 0°C to about 10°C, from about 4 °C to about 15 °C, from about 15 °C to about 25 °C, from about 10°C to about 25°C, from about 25 °C to about 37 °C, or from about 25°C to about 40°C, for a period of time of from about 10 minutes to about 6 months (e.g., from about 10 minutes to about 30 minutes, from about 30 minutes to 1

hour, from about 1 hour to about 6 hours, from about 6 hours to about 12 hours, from about 12 hours to about 1 day, from about 1 day to about 1 week, from about 1 week to about 2 weeks, from about 2 weeks to about 1 month, or from about 1 month to about 6 months).

PHARMACEUTICAL COMPOSITIONS

[0027] The present disclosure provides a pharmaceutical composition comprising: a) a delivery disk, as described above; and b) a pharmaceutically acceptable carrier, diluent, excipient, or buffer. In some embodiments, the pharmaceutically acceptable carrier, diluent, excipient, or buffer is suitable for use in a human.

[0028] Such excipients, carriers, diluents, and buffers include any pharmaceutical agent that can be administered without undue toxicity. Pharmaceutically acceptable excipients include, but are not limited to, liquids such as water, saline, glycerol and ethanol. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such vehicles. A wide variety of pharmaceutically acceptable excipients are known in the art and need not be discussed in detail herein. Pharmaceutically acceptable excipients have been amply described in a variety of publications, including, for example, A. Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th edition, Lippincott, Williams, & Wilkins; Pharmaceutical Dosage Forms and Drug Delivery Systems (1999) H.C. Ansel et al., eds., 7th ed., Lippincott, Williams, & Wilkins; and Handbook of Pharmaceutical Excipients (2000) A.H. Kibbe et al., eds., 3rd ed. Amer. Pharmaceutical Assoc.

METHODS OF DELIVERING AN ACTIVE AGENT

[0029] The present disclosure provides a method of delivering a drug to an individual, the method comprising administering to the individual a delivery disk of the present disclosure, where the delivery disk comprise an active agent linked to a variant viral coat polypeptide of the present disclosure. In some cases, delivering a drug can provide treatment of a disease.

[0030] In one embodiment, the present disclosure provides a method of treating cancer, the method comprising administering to an individual in need thereof an effective amount of

a delivery disk of the present disclosure, where the delivery disk comprises a cancer chemotherapeutic agent linked to a variant viral coat polypeptide of the present disclosure.

[0031] In one embodiment, the present disclosure provides a method of treating an inflammatory disease or disorder, the method comprising administering to an individual in need thereof an effective amount of a delivery disk of the present disclosure, where the delivery disk comprises an anti-inflammatory agent linked to a variant viral coat polypeptide of the present disclosure.

[0032] A delivery disk of the present disclosure can be administered via intravenous, intraarterial, intramuscular, intratumoral, peritumoral, intradermal, subcutaneous, or intraperitoneal administration. A delivery disk assembly of the present disclosure can be administered via convection-enhanced delivery.

[0033] A "therapeutically effective amount" of a delivery disk of the present disclosure includes a relatively broad range that can be determined through experimentation and/or clinical trials. Effective dosages can be readily established by one of ordinary skill in the art through routine trials establishing dose response curves.

Cancer treatment

[0034] Where a delivery disk of the present disclosure comprises a cancer chemotherapeutic agent, a method of the present disclosure can treat a wide variety of cancers, including carcinomas, sarcomas, leukemias, and lymphomas.

[0035] Carcinomas that can be treated using a subject method include, but are not limited to, esophageal carcinoma, hepatocellular carcinoma, basal cell carcinoma (a form of skin cancer), squamous cell carcinoma (various tissues), bladder carcinoma, including transitional cell carcinoma (a malignant neoplasm of the bladder), bronchogenic carcinoma, colon carcinoma, colorectal carcinoma, gastric carcinoma, lung carcinoma, including small cell carcinoma and non-small cell carcinoma of the lung, adrenocortical carcinoma, thyroid carcinoma, pancreatic carcinoma, breast carcinoma, ovarian carcinoma, prostate carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, renal cell carcinoma, ductal carcinoma in situ or bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical

carcinoma, uterine carcinoma, testicular carcinoma, osteogenic carcinoma, epithelial carcinoma, and nasopharyngeal carcinoma, etc.

[0036] Sarcomas that can be treated using a subject method include, but are not limited to, fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, chordoma, osteogenic sarcoma, osteosarcoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's sarcoma, leiomyosarcoma, rhabdomyosarcoma, and other soft tissue sarcomas.

[0037] Other solid tumors that can be treated using a subject method include, but are not limited to, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

[0038] Leukemias that can be treated using a subject method include, but are not limited to, a) chronic myeloproliferative syndromes (neoplastic disorders of multipotential hematopoietic stem cells); b) acute myelogenous leukemias (neoplastic transformation of a multipotential hematopoietic stem cell or a hematopoietic cell of restricted lineage potential; c) chronic lymphocytic leukemias (CLL; clonal proliferation of immunologically immature and functionally incompetent small lymphocytes), including B-cell CLL, T-cell CLL prolymphocytic leukemia, and hairy cell leukemia; and d) acute lymphoblastic leukemias (characterized by accumulation of lymphoblasts). Lymphomas that can be treated using a subject method include, but are not limited to, B-cell lymphomas (e.g., Burkitt's lymphoma); Hodgkin's lymphoma; and the like.

[0039] Brain cancers that can be treated using subject method include, but are not limited to glioblastoma multiforme, oligodendroglioma, astrocytoma, oligogastrocytoma, ependymoma, medulloblastoma, and meningioma.

Combination therapies

[0040] In some cases, a delivery disk of the present disclosure is administered as an adjuvant therapy to a standard cancer therapy. Standard cancer therapies include surgery (e.g., surgical removal of cancerous tissue), radiation therapy, bone marrow transplantation, and certain combinations of the foregoing.

IMAGING METHODS

[0041] The present disclosure provides a method of delivering an imaging agent to an individual, the method comprising administering to the individual a delivery disk of the

present disclosure, where the delivery disk comprises an imaging agent linked to a variant viral coat polypeptide of the present disclosure. Once the delivery disk is administered to an individual, imaging can be carried out using any suitable method and instrumentation appropriate to the imaging agent. Suitable imaging methods include, but are not limited to, MRI, PET, and the like.

[0042] A delivery disk of the present disclosure can be administered via intravenous, intraarterial, intramuscular, intratumoral, peritumoral, intradermal, subcutaneous, or intraperitoneal administration. A delivery disk assembly of the present disclosure can be administered via convection-enhanced delivery.

Examples of Non-Limiting Aspects of the Disclosure

[0043] Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure numbered 1-40 are provided below. As will be apparent to those of skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below:

[0044] Aspect 1. A variant viral coat protein comprising an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 98%, or at least 99%, amino acid sequence identity to SEQ ID NO:1, and comprising a K53R substitution and a K68R substitution.

[0045] Aspect 2. The variant viral coat protein of aspect 1, wherein the protein comprises a non-naturally occurring cysteine.

[0046] Aspect 3. The variant viral coat protein of aspect 2, wherein the non-naturally occurring cysteine is at amino acid 123 of the amino acid sequence set forth in SEQ ID NO:1.

[0047] Aspect 4. The variant viral coat protein of any one of aspects 1-3, comprising an N-terminal extension of from 1 to 10 amino acids, wherein the N-terminal amino acid provides an attachment site.

[0048] Aspect 5. The variant viral coat protein of aspect 4, wherein the N-terminal extension comprises Pro-Ala-Ser.

[0049] Aspect 6. The variant viral coat protein of any one of aspects 1-5, comprising a heterologous polypeptide that provides for: a) binding to a cell surface; b) pro-apoptotic

activity; or c) endosomal escape, wherein the heterologous polypeptide is fused at the N-terminus of the variant viral coat protein, at the C-terminus of the variant viral coat protein, or at an internal site within the variant viral coat protein.

[0050] Aspect 7. The variant viral coat protein of aspect 6, wherein the heterologous polypeptide comprises the amino acid sequence Arg-Gly-Asp, wherein the heterologous polypeptide comprises a GALA peptide, wherein the heterologous polypeptide comprises a KALA wherein the heterologous polypeptide comprises, or wherein the heterologous polypeptide comprises a KALK peptide.

[0051] Aspect 8. A viral coat protein conjugate comprising: a) the variant viral coat protein of any one of aspects 1-7; and b) a drug or an imaging agent covalently linked to the variant viral coat protein.

[0052] Aspect 9. The viral coat protein conjugate of aspect 8, wherein the conjugate self-assembles into a disk.

[0053] Aspect 10. The viral protein conjugate of aspect 8, wherein the drug is a cancer chemotherapeutic agent.

[0054] Aspect 11. The viral protein conjugate of any one of aspects 8-10, wherein the drug is an anti-inflammatory agent, an anti-bacterial agent, an anti-fungal agent, or an anti-parasitic agent.

[0055] Aspect 12. The viral protein conjugate of aspect 8, wherein the imaging agent is selected from an agent that detectable by optical imaging, near-infrared imaging, nuclear magnetic resonance imaging, magnetic resonance imaging, x-ray imaging, computed tomography imaging, K-edge imaging, ultrasound imaging, photoacoustic imaging, acoustic optical imaging, microwave imaging, or nuclear imaging.

[0056] Aspect 13. The viral protein conjugate of aspect 12, wherein the imaging agent is a radioisotope, an MRI contrast agent, or a PET imaging agent.

[0057] Aspect 14. The viral coat protein conjugate of any one of aspects 8-13, comprising a stabilization agent covalently linked to the variant viral coat protein.

[0058] Aspect 15. The viral coat protein conjugate of aspect 14, wherein the stabilization agent is a polyalkylene glycol.

[0059] Aspect 16. The viral coat protein conjugate of aspect 15, wherein the polyalkylene glycol is polyethylene glycol (PEG).

[0060] Aspect 17. The viral coat protein conjugate of any one of aspects 8-16, comprising an antibody linked to the conjugate, wherein the antibody is specific for an antigen on a target cell.

[0061] Aspect 18. A composition comprising:

[0062] a) the viral protein conjugate of any one of aspects 8-17; and

[0063] b) a pharmaceutically acceptable carrier.

[0064] Aspect 19. A delivery disk comprising a plurality of the viral protein conjugate of any one of aspects 8-17.

[0065] Aspect 20. The delivery disk of aspect 19, wherein the diameter of the disk is from about 10 nm to about 25 nm.

[0066] Aspect 21. A composition comprising: a) a delivery disk of aspect 19 or aspect 20; and b) a pharmaceutically acceptable carrier.

[0067] Aspect 22. A method of delivering a drug to an individual, the method comprising administering the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises the drug linked to the variant viral coat polypeptide.

[0068] Aspect 23. The method of aspect 22, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

[0069] Aspect 24. A method for treating a disease or condition in an individual, the method comprising administering to the individual an effective amount of the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises a drug linked to the variant viral coat polypeptide.

[0070] Aspect 25. The method of any one of aspects 22-24, wherein the individual is a human.

[0071] Aspect 26. The method of aspect 24 or aspect 25, wherein the disease is cancer, inflammation, or an infection.

[0072] Aspect 27. A method of delivering an imaging agent to an individual, the method comprising administering the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises an imaging agent linked to the variant viral coat polypeptide.

[0073] Aspect 28. The method of aspect 27, wherein the imaging agent is a diagnostic agent, an MRI contrast agent, or a PET imaging agent.

[0074] Aspect 29. A method for treating cancer in an individual, the method comprising administering to the individual an effective amount of the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises a cancer chemotherapeutic agent linked to the variant viral coat polypeptide.

[0075] Aspect 30. The method of aspect 29, wherein the individual is a human.

[0076] Aspect 31. The method of aspect 29 or aspect 30, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

[0077] Aspect 32. A method for treating inflammation in an individual, the method comprising administering to the individual an effective amount of the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises an anti-inflammatory agent linked to the variant viral coat polypeptide.

[0078] Aspect 33. The method of aspect 32, wherein the individual is a human.

[0079] Aspect 34. The method of aspect 32 or aspect 33, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

[0080] Aspect 35. A method for treating an infection in an individual, the method comprising administering to the individual an effective amount of the composition of aspect 21 to the individual, wherein the viral protein conjugate comprises an active agent suitable for treating the infection linked to the variant viral coat polypeptide.

[0081] Aspect 36. The method of aspect 35, wherein the individual is a human.

[0082] Aspect 37. The method of aspect 35 or aspect 36, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

[0083] Aspect 38. The method of any one of aspects 35-37, wherein the infection is a bacterial infection, and the active agent is an antibiotic.

[0084] Aspect 39. The method of any one of aspects 35-37, wherein the infection is a fungal infection, and the active agent is an antimycotic agent.

[0085] Aspect 40. The method of any one of aspects 35-37, wherein the infection is a parasitic infection, and the active agent is an anti-parasitic agent.

EXAMPLES

[0086] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

[0087] Described below is a viral derived delivery disk assembly, term RR-TMV disk, which can effectively deliver drug or imaging agents into cells in culture. RR-TMV disks includes variant TMV coat proteins that are conjugated with a drug or an imaging agent and may also include a serum stabilizing agent. RR-TMV disks can be internalized by cells via endocytosis. After endocytosis, the drug or imaging agent can be released into the cytoplasm.

MATERIALS AND METHODS

[0088] The following materials and methods were used in the Examples, below.

[0089] All solvents and reagents, including doxorucibin-HCl, were purchased from commercial suppliers and used without further purification. DOX-EMCH was prepared according to literature procedures. Thin layer chromatography (TLC) was performed on silica gel 60 F_{254} (E. Merck) and visualized under a UV lamp at 254 nm. A C18 column was used for analytical and semi-preparative reverse phase high performance liquid chromatography (RP-HPLC) on an Agilent 1100 Series Capillary LC. Runs were eluted with H_2O/MeCN (0.1 % v/v TFA) and monitored using a UV-Vis detector. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker Avance 400 with working frequencies of 400

MHz for ^{1}H NMR, and 100 MHz for ^{13}C NMR, respectively. UV-Vis spectra were obtained using a NanoDrop (Thermo Scientific).

Protein expression and purification

[0090] The starting point for the RR-TMV protein was a gene for the coat protein of the TMV U1 strain optimized for the codon usage of *E. coli* (Genscript, Piscataway, NJ). Site-directed mutagenesis was performed using QuikChange mutagenesis (Stratagene, Santa Clara, CA). For N-terminal oxidative coupling, the N terminus of the RR-TMV was extended from SYS to PAGSYS using the primers:

sense: 5' – GAAGGAGATATACATATGCCTGCCGGCAGCTATAGCATTACC – 3' (SEQ ID NO:46)

antisense: 5' – TGCTATAGCTGCCGGCAGGCATATGTATATCTCCTTCTTAAG – 3' (SEQ ID NO:47)

[0091] The RR-TMV coat protein was expressed to contain K53R, K68R, T104K, S123C, and PAGSYS N-terminal mutations. BL21 DE3 RIL Codon+ cells were transformed with the vector described above, and cultured in Terrific Broth with 100 μg/L ampicillin at 37 °C. When cultures reached optical densities of 0.6 to 0.8, IPTG was added to a final concentration of 30 μM. Cultures were grown 24 h at 30 °C, harvested by centrifugation, and stored at -80 °C. Cells (from a 1 L expression batch) were thawed, resuspended in 20 mL of 20 mM TEA pH 8, and lysed by sonicating with a 2 s on, 4 s off cycle for a total of 30 min using a standard disruptor horn at 90% amplitude (Fischer Scientific). The resulting lysate was cleared by ultracentrifugation for 30 min at 45,000 rpm using a Beckman 45 Ti rotor in an Optima L-80 XP (Beckman Coulter). The clarified lysate was decanted, warmed to room temperature, and stirred while adding a saturated solution of ammonium sulfate dropwise to a final concentration of 50% (v/v). After 5 min, the white ppt that formed was pelleted by ultracentrifugation (30 min at 45,000 rpm in a Beckman 45 Ti rotor), and resuspended in 20 mM TEA pH 7.2. The resulting protein solution was next loaded onto a diethylaminoethanol (DEAE) Sepharose column and eluted with a 0 – 300 mM NaCl gradient. Purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography (HPLC). This preparation provided pure RR-TMV in yields up to 100 mg/L culture.

Size Exclusion Chromatography (SEC)

[0092] Analytical size exclusion was performed on an Agilent 1100 series HPLC equipped with a PolySep-GFC-P 5000 column (Phenomenex, Torrance, CA), at a flow rate of 1 mL/min.

Transmission Electron Microscopy (TEM)

[0093] Samples were prepared for TEM analysis by applying an analyte solution (approximately 0.2 mg/mL in RR-TMV protein) to carbon-coated copper grids for 2 min, followed by triple rinsing with dd-H_2O. The grids were then exposed to a 1.6% aqueous solution of uranyl acetate for 2 min as a negative stain. Images were obtained at the Berkeley Electron Microscope Lab using a FEI Tecnai 12 transmission electron microscope with 120 kV accelerating voltage.

Dynamic light scattering (DLS) and zeta potential studies

[0094] DLS and zeta potential measurements were obtained using a Malvern Instruments Zetasizer Nano ZS. Data plots and standard deviations were calculated from an average of three measurements, each of which consisted of 10 runs of 45 s each. Measurement data are presented as a number distribution.

[0095] The zeta potentials of derivatives of modified RR-TMV conjugated to PEG were measured to determine the effects of charge shielding by the PEG.

Table 1

Samples	Zeta Potential (mV) ± S.D.
Unmodified RR-TMV	-19.5 $\pm$ 1.25
S213C-PEG modified RR-TMV	-6.67 $\pm$ 0.73
N-term-PEG modified RR-TMV	-7.46 $\pm$ 0.86

Mass spectrometry

[0096] Protein bioconjugates were analyzed using an Agilent 1200 series liquid chromatograph (Agilent Technologies, USA) that was connected in-line with an Agilent 6224 Time-of Flight (TOF) LC/MS system equipped with a Turbospray ion source.

Gel Analyses

[0097] For protein analysis, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on a Mini-Protean apparatus (Bio-Rad, Hercules, CA), using a 10-20% precast linear gradient polyacrylamide gel (Bio-Rad). All protein

electrophoresis samples were heated for 5-10 min at 95 °C in the presence of 1,4-dithiothreitol (DTT) to ensure reduction of disulfide bonds. Gels were run for 75-90 min at 120 V to separate the bands. Commercially available markers (Bio-Rad) were applied to at least one lane of each gel for assignment of apparent molecular masses. Visualization of protein bands was accomplished by staining with Coomassie Brilliant Blue R-250 (Bio-Rad). ImageJ was used to determine the level of modification by optical densitometry.

General procedure for Alexa Fluor 488 conjugation to RR-TMV

[0098] To a solution of RR-TMV (PAGSYS N-terminus, S123C, T104K, 100 μM) in 10 mM pH 7 NaPhos buffer, 1.5 equiv of AF488 (22.6 μL of 1 mM stock solution) was added. The solution was incubated for 1 h, protected from light at room temperature. The solution was then spin concentrated 3-5 times into 10 mM pH 7 NaPhos buffer.

General procedure for aminophenol-PEG$_{5k}$ conjugation to RR-TMV

[0099] To a solution of RR-TMV (PAGSYS N-terminus, S123C, T104K, 100 μM) in 20 mM pH 7.5 NaPhos buffer, 2 equiv of *N*-ethyl maleimide (0.8 μL of a 100 mM stock solution) was added. The reaction was incubated for 1 h at room temperature. An aliquot of the mixture was analyzed by ESI-TOF MS to confirm the formation of the RR-TMV$_{NEM}$ complex. After 1 h of reaction time, 5 equiv of aminophenol-PEG$_{5k}$ (133 μL of 3 mM stock solution) was added. Following this, 50 equiv of $K_3Fe(CN)_6$ (80 μL of a 3 mM stock solution) was added. After 30 min of incubation at room temperature, the solution was spin concentrated 3-5 times into 10 mM pH 7 NaPhos buffer with a 30k molecular weight cutoff (MWCO) spin concentrator (Millipore).

General procedure for maleimide-PEG$_{5k}$ conjugation to RR-TMV

[00100] To a solution of RR-TMV (PAGSYS N-terminus, S123C, T104K, 100μM) in 20 mM NaPhos buffer pH 7.5, 10 equiv of maleimide-PEG$_{5k}$ (8 μL of 50 mM stock solution) was added. After 2 h of incubation at room temperature, the solution was spin concentrated 3-5 times into 10 mM NaPhos buffer pH 7 with a 30k MWCO spin concentrator.

General procedure for doxorubicin and aminophenol-PEG$_{5k}$ conjugation to RR-TMV

[00101] To a solution of RR-TMV (PAGSYS N-terminus, S123C, T104K, 100 μM) in 20 mM pH 7.5 NaPhos buffer, 1 equiv of DOX-EMCH (2 μL of a 100 mM stock solution

in DMSO) was added. The solution was incubated for 1 h, protected from light at room temperature. An aliquot of the mixture was analyzed by ESI-TOF MS to confirm the formation of the RR-TMV_{DOX} complex. Then, 5 equiv of PEG_{5k}-aminophenol (400 µL of a 2.5 mM stock) was added to the solution. Following this, 50 equiv of $K_3Fe(CN)_6$, (200 µL of a 50 mM stock solution) was added. After 30 min of incubation at room temperature, the solution was spin concentrated 3-5 times into PBS with a 30k MWCO spin concentrator.

Cell culture

[00102] U87MG human glioblastoma cells were obtained from the UC Berkeley Cell Culture Facility. Cells were cultured in DMEM containing phenol red (ATCC, Manassas, VA) or DMEM without phenol red (Thermo, Waltham, MA) with 10% fetal bovine serum (Omega Scientific, Tarzana, CA) and 1% penicillin/streptomycin (Thermo) at 37 °C and 5% CO_2.

MTS cell viability assay

[00103] A 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS) cell viability assay was purchased from Promega and used according to the manufacturer's instructions. U87MG cells were cultured in high glucose DMEM containing 10% fetal bovine serum and penicillin and streptomycin. Cells were plated in 96-well microtiter plates at 5000 cells per well and incubated overnight to allow adhesion. After overnight incubation, the media was removed and exchanged with DOX, RR-TMV, or RR-$TMV_{DOX\text{-}PEG}$ stock solutions prepared in cell media at the appropriate concentration. Cell viability was measured after 72 h using an MTS cell viability assay and was used according to the supplier's instructions. The cell media was replaced after 72 h with a stock of 20% MTS solution in cell media. The plate was incubated for 1–3 h, and the absorbance was read using a microplate reader (490 nm). Cell viability was calculated as an absorbance percent relative to the untreated cell control. Cells were treated in triplicate.

Cell uptake studies

[00104] U87MG cells were seeded in a 96 well plate as 200 µL solutions of 50,000 cells/mL. The cells were allowed to grow and adhere to the plate for 48 h in DMEM media with 10% FBS and 1% P/S at 37 °C and 5% CO_2. The media was removed and replaced with 200 µL DMEM without phenol red containing RR-$TMV_{DOX\text{-}PEG}$

conjugates at a final concentration of 1 μM DOX (3 μM RR-TMV coat protein) or 2 μM unmodified RR-TMV coat protein. The cells were incubated with the agents for an additional 48 h. Incucyte Zoom Live-Cell Analysis System (EssenBio, Ann Arbor, MI) was used to collect images every hour post incubation. Phase and green fluorescence images at 20x magnification were collected, capitalizing on the intrinsic fluorescence of doxorubicin. The images were processed using Incucyte Zoom proprietary software v.2016A, and the Top-Hat background subtraction algorithm (radius 10 μm, threshold 0.5% of green calibration dye signal, GCU) was used to define the boundaries of the cells (green objects). The mean green object fluorescence intensities of 4 images taken in each well were plotted against the incubation time.

Example 1: TMV coat proteins variant self-assemble into disk

[00105] The gene for the coat protein of the TMV U1 stain was mutated using site-directed mutagenesis to express two replace two native lysines at positions K53 and K68 with two non-native arginines. In addition, a non-native lysine was introduced at position T104. This variant, also referred herein as "RR-TMV" was then expressed in *E.coli* and purified by anion exchange chromatography to provide high yields of pure protein. Representative cryogenic transmission electron microscopy images as in FIG 2. demonstrate that RR-TMV variants assemble into disks and short stacks of disks in NaPhos buffer at pH 7.

Example 2: TMV coat protein variant have enhanced disk self-assembly stability under varying conditions

[00106] Size exclusion chromatography (HPLC-SEC) was used to assess the stability of RR-TMV variants disk assemblies under varying pH conditions. FIG 3 shows that under all conditions tested, only SEC elution times of 8-9 min were observed, corresponding to the known double disk assembly of TMV. TEA and borate buffers (both at pH 8) were also evaluated, as they have been previously shown to promote disassembly of TMV disks into monomers; however, disks were predominantly observed by SEC for both buffers. FIG 3B shows that disks were predominantly observed by SEC for both buffers. Transmission electron microscopy (TEM) was conducted on RR-TMV in both phosphate buffer and 50 mM NaOAc pH 5.5, as NaOAc has been reported to drive rod assembly for TMV proteins. FIGs 3C and 3D show that under both conditions, only RR-TMV disks and short stacks of disks were observed.

Example 3: TMV coat protein self-assemble into nanoscale disk assemblies

[00107] Dynamic light scattering was used to assess the size of the disk formed RR-TMV variant assembly. FIG 4 shows that the RR-TMV disks have a diameter of 18.2 nm, closely matching the known diameter of TMV assemblies.

Example 4: TMV coat protein variants assemble in C_2 oriented disks

[00108] Experiments were conducted to determine whether the RR-TMV variant assembles into double disks in which both disks face the same direction or into C_2-symmetric arrangement in which common faces are in contact. Collected data including TEM are consistent with a C_2 arrangement for RR-TMV variant disk assemblies.

Example 5: TMV coat protein variant conjugation with PEG is possible at two distinct conjugation sites

[00109] Experiments were conducted to determine how effectively the RR-TMV disk assemblies could be PEGylated with PEG_{5k}. Conjugation of nanomaterial developed for drug delivery to polyethylene glycol (PEG) polymers is commonly used to reduce immunogenicity and increase serum stability. RR-TMV coat protein variants were expressed to contain two orthogonal handles for bioconjugation: cysteine S123C for maleimide conjugation and an N-terminal proline for an oxidative coupling. FIG 5A demonstrates a schematic of the strategy for PEG conjugation at S123C or at an N-terminal proline for an oxidative coupling. FIG 5B shows that PEG_{5k} was conjugated to either site at varying equivalents to achieve modifications ranging from 14 to 40%.

Example 6: TMV coat protein variants form disk assemblies stabilized in serum for up to five days

[00110] Experiments were conducted to monitor the serum stability of RR-TMV and PEGylated RR-TMV variant disks by HPLC-SEC. RR-TMV disks were labeled with Alexa Fluor 488 maleimide in order to detect at a wavelength that is not effected by background absorbance from serum proteins, and incubated in 10% fetal bovine serum (FBS) solution at 37 °C. FIG 6C shows that RR-TMV can be modified by AF488 maleimide. To determine if any serum interactions could be minimized with the RR-TMV, $RR\text{-}TMV_{AF488}$ was PEGylated with aminophenol-PEG_{5k} (FIG 6A) and incubated in 10% FBS solution for 5 d. FIG 6B shows that the SEC trace at 8.5 min remained the dominant observed peak after 5 d of incubation; however, new peaks at 9.3 and 9.9 min corresponding to thiol exchange between small molecules in serum and the AF488-

maleimide (AF488-SM). FIG 6E shows that LC/MS analysis of the 8.5 min peak revealed RR-TMV and RR-TMV_{AF488} while only BSA and a small peak corresponding to BSA_{AF488} were observed in the 9.3 min peak by LC/MS. FIG 6D shows that AF488 – maleimide alone elutes at about 10min. PEGylated-RR-TMV_{AF488} also demonstrated the emergence of 9.3 and 9.9 min peaks, corresponding to the AF488 thiol exchange products, but no peak at 7.5 min was observed even after 5 d of incubation. FIG 7 shows that no disassembly was observed for RR-TMV that was incubated in PBS at 37 °C for 5 days.

Example 7: TMV coat protein variants-drug conjugates formation

[00111] Experiments were conducted to determine whether the chemotherapy drug Doxorubicin (DOX) could be conjugated to the RR-TMV coat protein variant using maleimide chemistry. DOX was condensed onto the hydrazide-maleimide linker EMCH, and was conjugated via maleimide chemistry to the RR-TMV disks at the S123C position. EMCH allows for acid-sensitive release of the DOX upon endocytosis of the RR-TMV disks by cancer cells. FIG 8A depicts a schematic of the strategy for modifying RR-TMV with DOX at S123 and PEGylation at N-terminal proline. FIG 8B and 8C show that incubation with 1 equiv of DOX-EMCH afforded ~40% modification as observed by ESI-TOF MS and UV-vis spectroscopy. FIG 8D shows that Conjugation of RR-TMV_{DOX} with aminophenol-PEG_{5k} in the presence of the oxidant $K_3Fe(CN)_6$ resulted in 30% PEGylation. FIG 8E demonstrates by SEC traces of RR-$TMV_{DOX\text{-}PEG}$ conjugates that the disk assembly state was maintained after conjugation.

Example 8: Cell viability when incubated with TMV coat protein variants-DOX conjugate disk assemblies

[00112] U87MG glioblastoma cells (5000 cells per well) were incubated with RR-$TMV_{DOX\text{-}PEG}$ conjugates at varying concentrations, and cell viability was monitored via an MTS assay. . FIG 9A shows that after 72 h of incubation, significant cell death was observed for both DOX-treated and RR-$TMV_{DOX\text{-}PEG}$-treated cells when compared to cell controls. Cells incubated with RR-TMV alone displayed 100.9 ± 0.2% viability at TMV concentrations equal to the highest concentration of RR-$TMV_{DOX\text{-}PEG}$ samples. The difference in cell viability curves of DOX alone and RR-$TMV_{DOX\text{-}PEG}$ is most likely due to the slow release profile of the hydrazone linkage between the DOX and EMCH.

Example 9: Cellular delivery of DOX by RR-$TMV_{DOX-PEG}$ disk assemblies

[00113] Experiments were conducted to determine whether RR-$TMV_{DOX-PEG}$ conjugates could deliver DOX into cells *in vitro*. FIG 9B shows the cellular uptake of RR-$TMV_{DOX-PEG}$ monitored by fluorescence microscopy, taking advantage of the fluorescent properties of DOX. Cellular internalization was observed after 48 h of incubation, with fluorescence observed throughout the cytoplasm and in the nuclei, where the DOX can take effect. Uptake kinetics were monitored via time lapsed live cell fluorescent microscopy. FIG 9C shows that significant uptake was observed starting after 12 h of incubation.

Example 10: Cellular delivery of DOX by RR-$TMV_{DOX-PEG}$ disk assemblies

[00114] Experiments were conducted to assess two different oxidants for the oxidative conjugation of PEG at the N-terminal proline of a RR-TMV variant-DOX conjugate. $NaIO_4$, can cause the oxidation of DOX and can lead to further degradation. A milder oxidant, $K_3Fe(CN)_6$, was used to avoid any decomposition and loss of functionality of DOX. FIG 10C shows that treatment of RR-TMV_{DOX} with $NaIO_4$ led to a mass loss of 129 Da, corresponding to the cleavage of the sugar moiety). However, no such changes were observed upon treatment with $K_3Fe(CN)_6$ (FIG 10B).

[00115] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

CLAIMS

What is claimed is:

1. A variant viral coat protein comprising an amino acid sequence having at least 85% amino acid sequence identity to the amino acid sequence set forth in SEQ ID NO:1, and comprising a K53R substitution and a K68R substitution.

2. The variant viral coat protein of claim 1, comprising a non-naturally occurring cysteine.

3. The variant viral coat protein of claim 2, wherein the non-naturally occurring cysteine is at amino acid 123 of the amino acid sequence set forth in SEQ ID NO:1.

4. The variant viral coat protein of any one of claims 1-3, comprising an N-terminal extension of from 1 to 10 amino acids, wherein the N-terminal amino acid provides an attachment site.

5. The variant viral coat protein of claim 4, wherein the N-terminal extension comprises Pro-Ala-Ser.

6. The variant viral coat protein of any one of claims 1-5, comprising a heterologous polypeptide that provides for binding to a cell surface, that provides pro-apoptotic activity, or provides for escape from an endosome.

7. The variant viral coat protein of claim 6, wherein the heterologous polypeptide comprises the amino acid sequence Arg-Gly-Asp, a GALA peptide, a KALA peptide, or a KALK peptide

8. A viral coat protein conjugate comprising:

a) the variant viral coat protein of any one of claims 1-7; and

b) a drug or an imaging agent covalently linked to the variant viral coat protein.

9. The viral coat protein conjugate of claim 8, wherein the conjugate self-assembles into a disk.

10. The viral protein conjugate of claim 8, wherein the drug is a cancer chemotherapeutic agent.

11. The viral protein conjugate of any one of claims 8-10, wherein the drug is an anti-inflammatory agent, an anti-bacterial agent, an anti-fungal agent, or an anti-parasitic agent.

12. The viral protein conjugate of claim 8, wherein the imaging agent is selected from an agent that detectable by optical imaging, near-infrared imaging, nuclear magnetic resonance imaging, magnetic resonance imaging, x-ray imaging, computed tomography imaging, K-edge imaging, ultrasound imaging, photoacoustic imaging, acoustic optical imaging, microwave imaging, or nuclear imaging.

13. The viral protein conjugate of claim 12, wherein the imaging agent is a radioisotope, an MRI contrast agent, or a PET imaging agent.

14. The viral coat protein conjugate of any one of claims 8-13, comprising a stabilization agent covalently linked to the variant viral coat protein.

15. The viral coat protein conjugate of claim 14, wherein the stabilization agent is a polyalkylene glycol.

16. The viral coat protein conjugate of claim 15, wherein the polyalkylene glycol is polyethylene glycol (PEG).

17. The viral coat protein conjugate of any one of claims 8-16, comprising an antibody linked to the conjugate, wherein the antibody is specific for an antigen on a target cell.

18. A composition comprising:

a) the viral protein conjugate of any one of claims 8-17; and

b) a pharmaceutically acceptable carrier.

19. A delivery disk comprising a plurality of the viral protein conjugate of any one of claims 8-17.

20. The delivery disk of claim 19, wherein the diameter of the disk is from about 10 nm to about 25 nm.

21. A composition comprising:

a) a delivery disk of claim 19 or 20; and

b) a pharmaceutically acceptable carrier.

22. A method of delivering a drug to an individual, the method comprising administering the composition of claim 21 to the individual, wherein the viral protein conjugate comprises the drug linked to the variant viral coat polypeptide.

23. The method of claim 22, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

24. A method for treating a disease or condition in an individual, the method comprising administering to the individual an effective amount of the composition of claim 21 to the individual, wherein the viral protein conjugate comprises a drug linked to the variant viral coat polypeptide.

25. The method of any one of claims 22-24, wherein the individual is a human.

26. The method of claim 24 or 25, wherein the disease is cancer, inflammation, or an infection.

27. A method of delivering an imaging agent to an individual, the method comprising administering the composition of claim 21 to the individual, wherein the viral protein conjugate comprises an imaging agent linked to the variant viral coat polypeptide.

28. The method of claim 27, wherein the imaging agent is a diagnostic agent.

29. A method for treating cancer in an individual, the method comprising administering to the individual an effective amount of the composition of claim 21 to the individual, wherein the viral protein conjugate comprises a cancer chemotherapeutic agent linked to the variant viral coat polypeptide.

30. The method of claim 29, wherein the individual is a human.

31. The method of claim 29 or 30, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

32. A method for treating inflammation in an individual, the method comprising administering to the individual an effective amount of the composition of claim 21 to the individual, wherein the viral protein conjugate comprises an anti-inflammatory agent linked to the variant viral coat polypeptide.

33. The method of claim 32, wherein the individual is a human.

34. The method of claim 32 or 33, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

35. A method for treating an infection in an individual, the method comprising administering to the individual an effective amount of the composition of claim 21 to the individual, wherein the viral protein conjugate comprises an active agent suitable for treating the infection linked to the variant viral coat polypeptide.

36. The method of claim 35, wherein the individual is a human.

37. The method of claim 35 or 36, wherein said administering is intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal administration, or wherein said administering comprises convection-enhanced delivery.

38. The method of any one of claims 35-37, wherein the infection is a bacterial infection, and the active agent is an antibiotic.

39. The method of any one of claims 35-37, wherein the infection is a fungal infection, and the active agent is an antimycotic agent.

40. The method of any one of claims 35-37, wherein the infection is a parasitic infection, and the active agent is an anti-parasitic agent.

FIG. 1A

SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**K**PSPQVT

VRFPDSDF**K**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**T**AETLDATRRVDDATV

AIR**S**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:1)

FIG. 1B

SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**T**AETLDATRRVDDATV

AIR**S**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:2)

FIG. 1C

SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**K**AETLDATRRVDDATV

AIR**S**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:3)

FIG. 1D

SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**T**AETLDATRRVDDATV

AIR**C**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:4)

FIG. 1E

SYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**K**AETLDATRRVDDATV

AIR**C**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:5)

FIG. 1F

PAGSYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**T**AETLDATRRVDDATV

AIR**S**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:6)

FIG. 1G

PAGSYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**K**AETLDATRRVDDATV

AIR**S**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:7)

FIG. 1H

PAGSYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**T**AETLDATRRVDDATV

AIR**C**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:8)

FIG. 1I

PAGSYSITTPSQFVFLSSAWADPIELINLCTNALGNQFQTQQARTVVQRQFSEVW**R**PSPQVT

VRFPDSDF**R**VYRYNAVLDPLVTALLGAFDTRNRIIEVENQANPT**K**AETLDATRRVDDATV

AIR**C**AINNLIVELIRGTGSYNRSSFESSSGLVWTSGPAT (SEQ ID NO:9)

Figure 2- Assembly characterization of RR-TMV disks

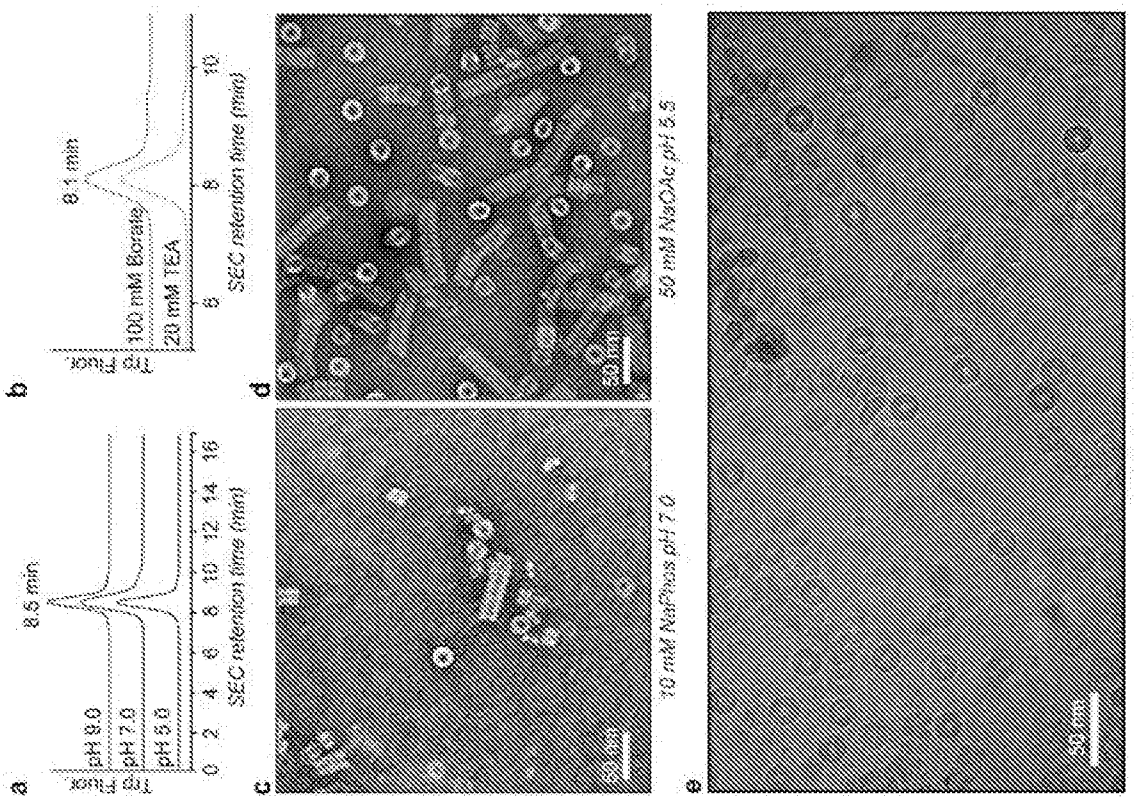


Figure 3- size of RR-TMV disks

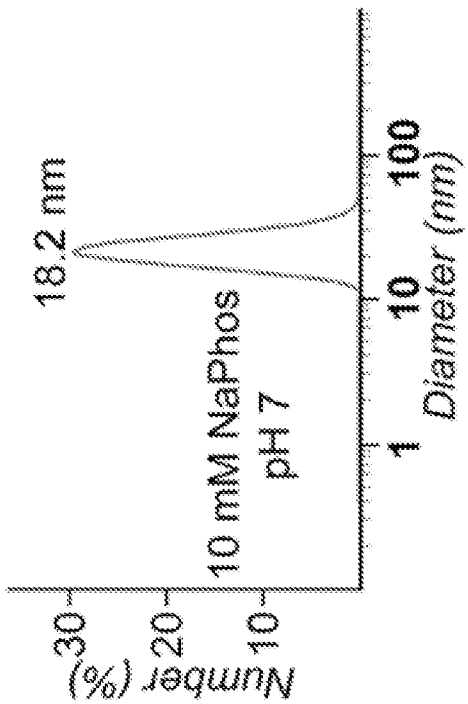


Figure 4

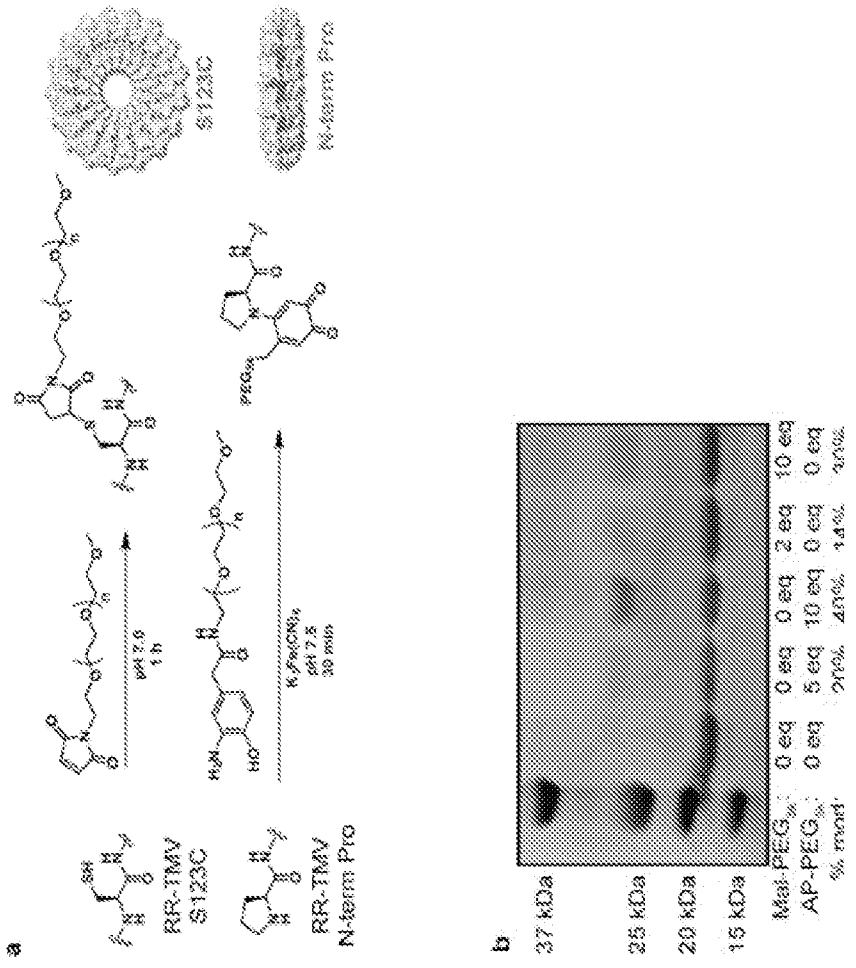

a
RR-TMV S123C
RR-TMV N-term Pro
pH 7.5 1 h
K3Fe(CN)6 pH 7.5 30 min
S123C
N-term Pro
b
37 kDa
25 kDa
20 kDa
15 kDa
Mal-PEG5k: 0 eq 0 eq 0 eq 2 eq 10 eq
AP-PEG5k: 0 eq 5 eq 10 eq 0 eq 0 eq
% mod: 20% 40% 14% 40%

Figure 5A

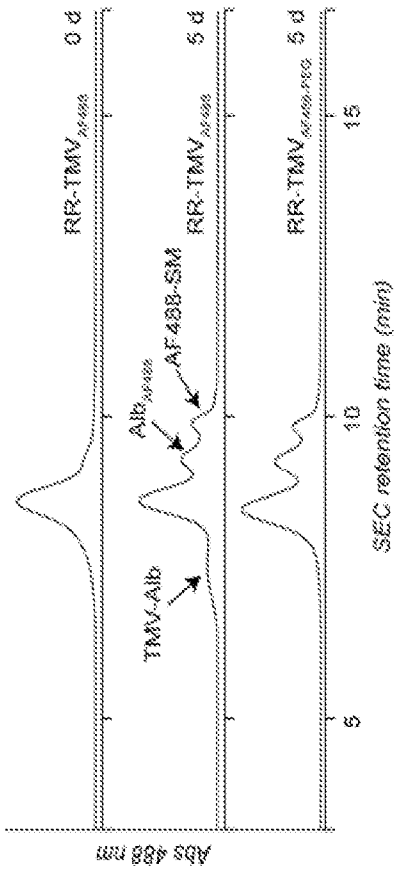


Figure 5B

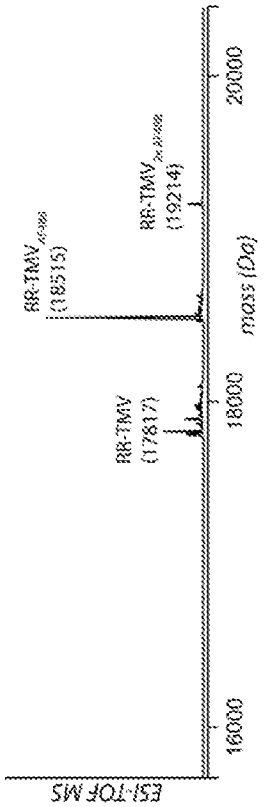


Figure 5C

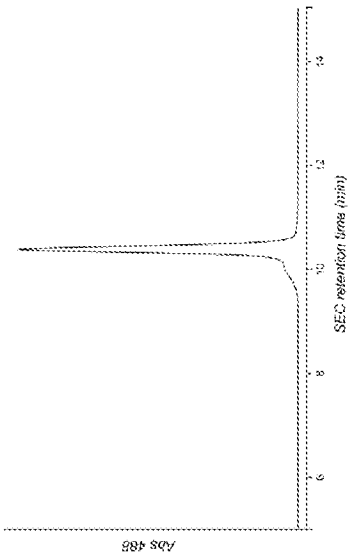


Figure 6 – Characterization of RR-TMV$_{DOX-PEG}$ conjugates

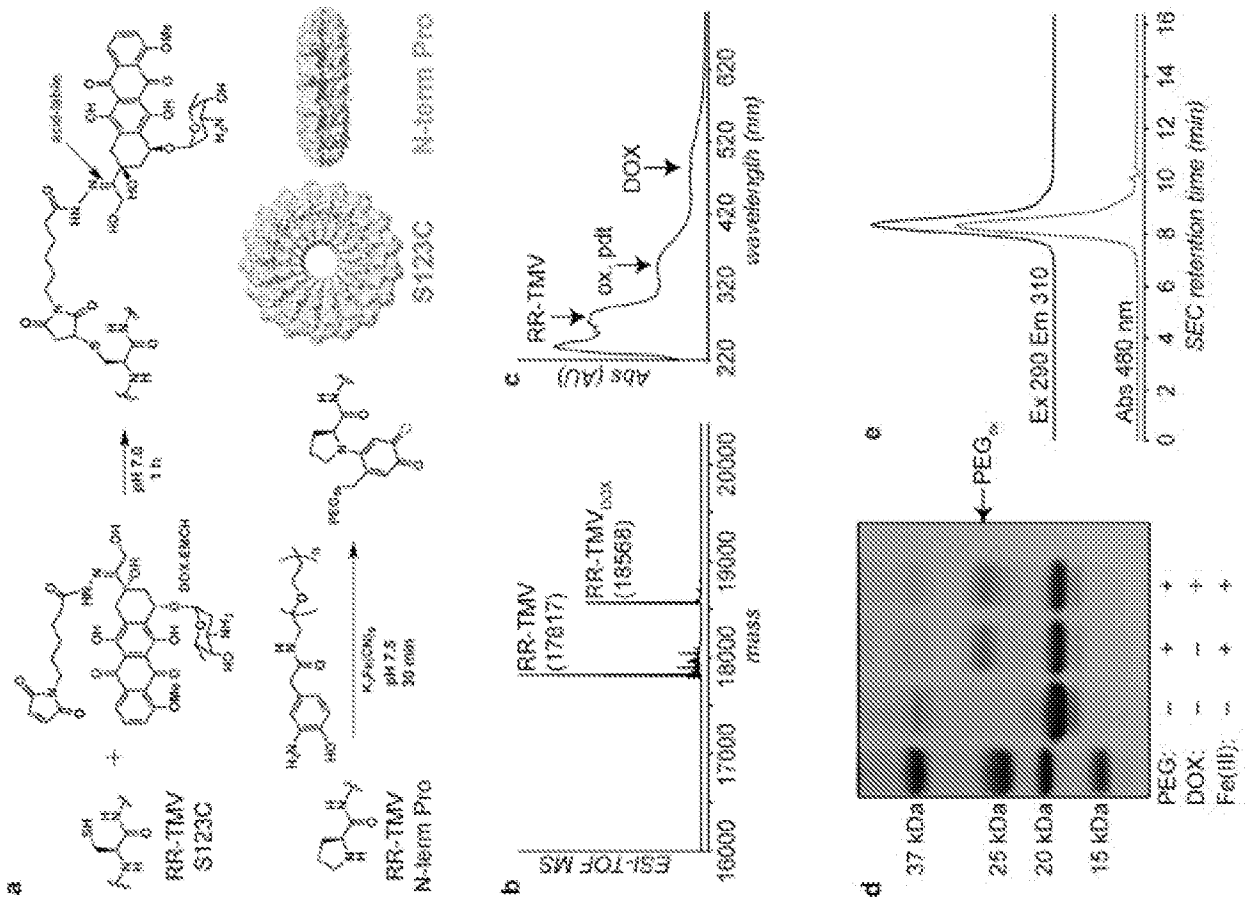


Figure 7A

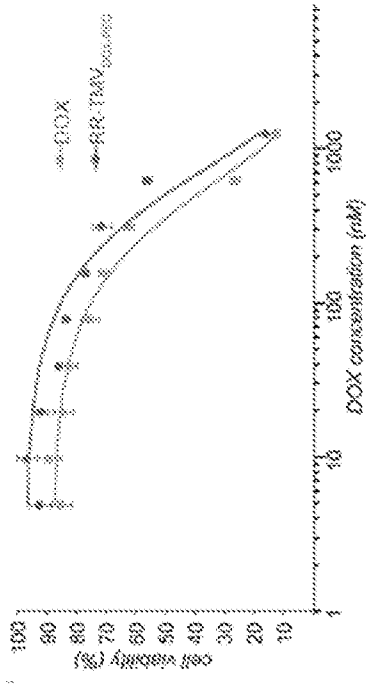


Figure 7B

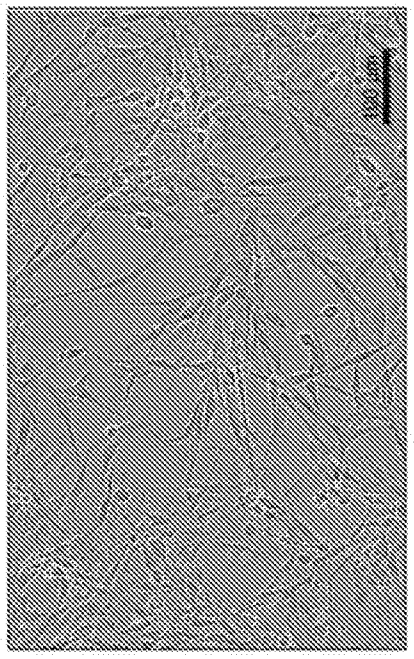


Figure 7C

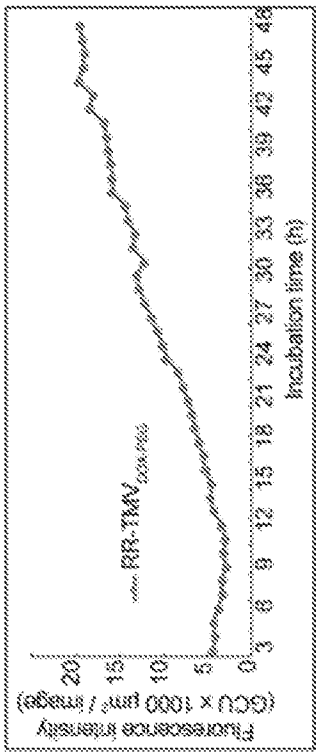


Figure 7D

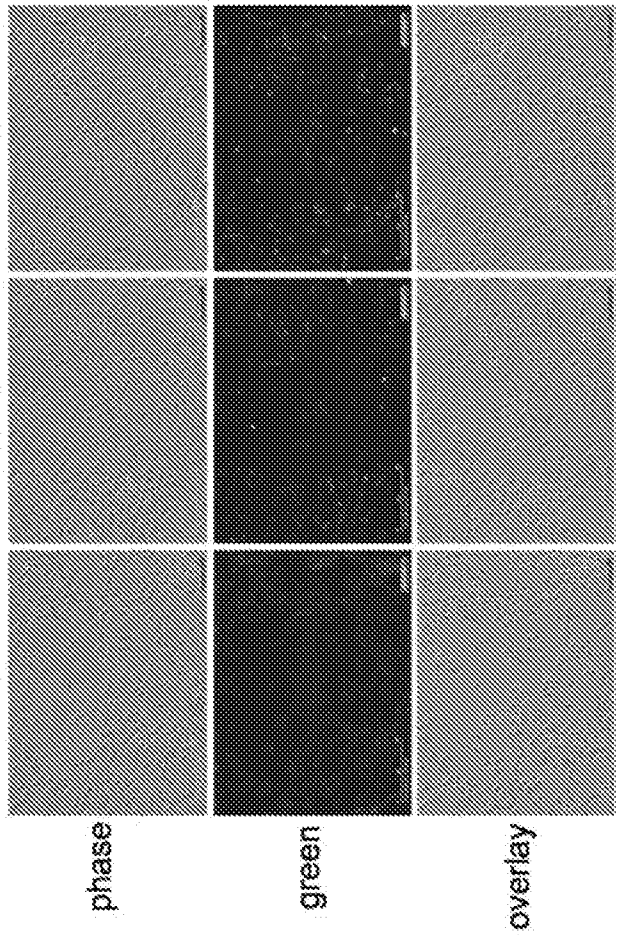


Figure 8A

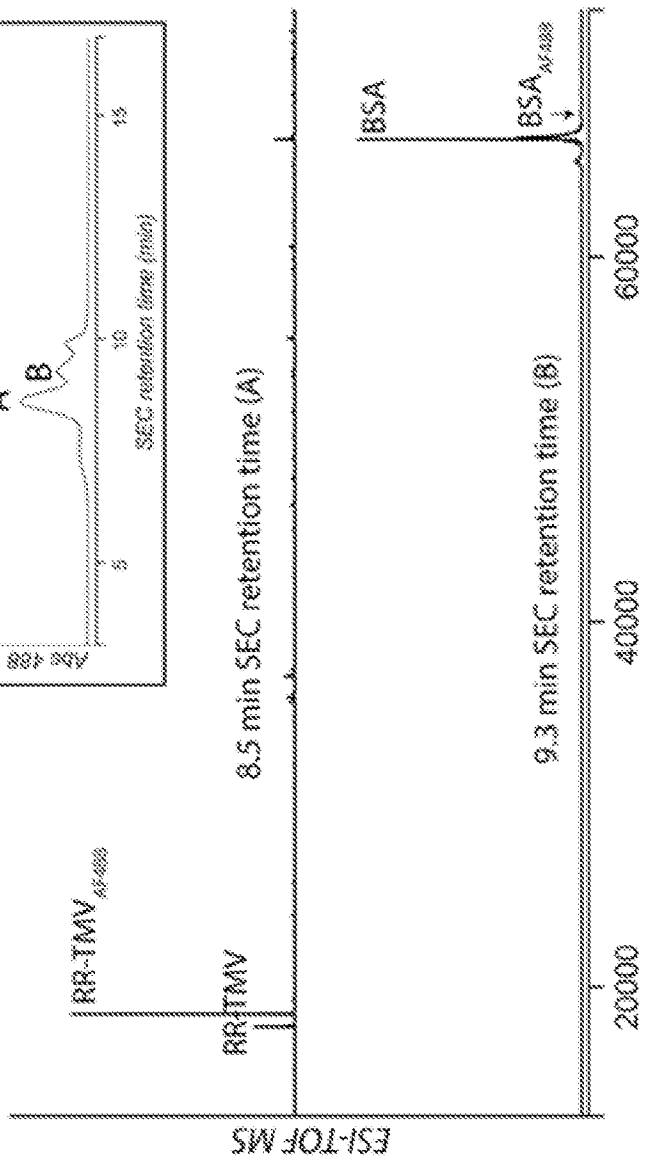


Figure 8B

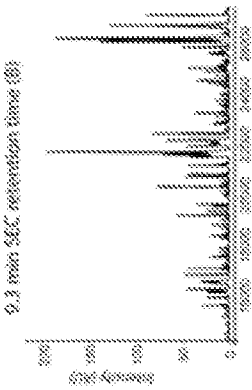


Figure 8C

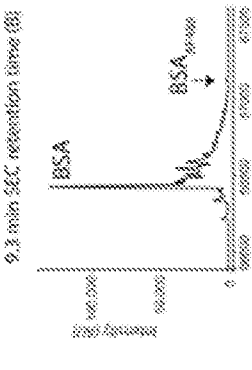


Figure 8D

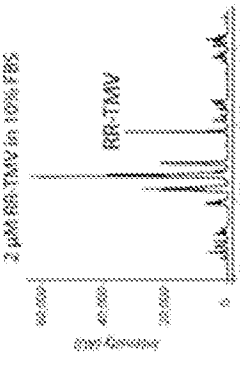


Figure 9

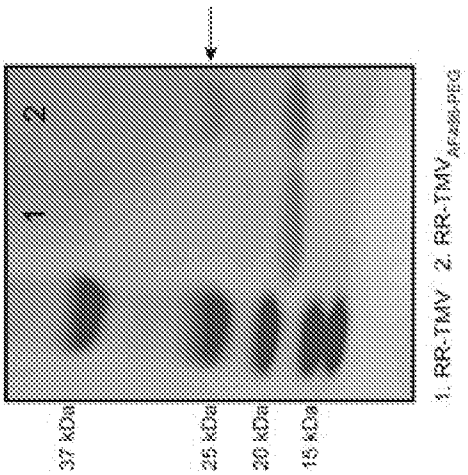


Figure 10- Stability of RR-TMV disks in PBS

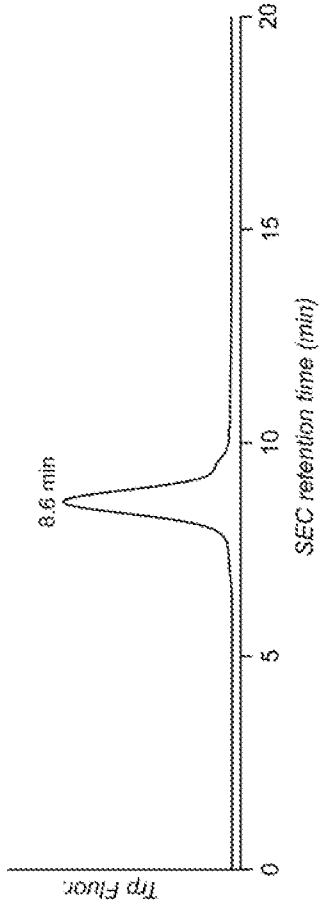


Figure 11A

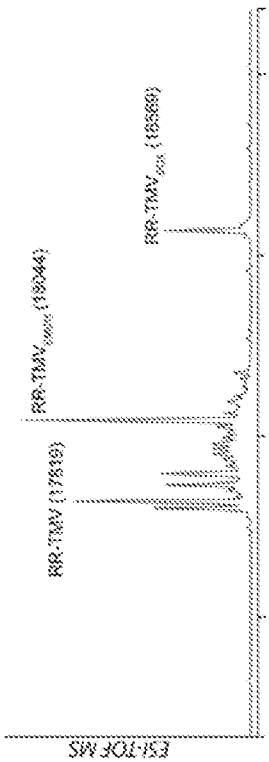


Figure 11B

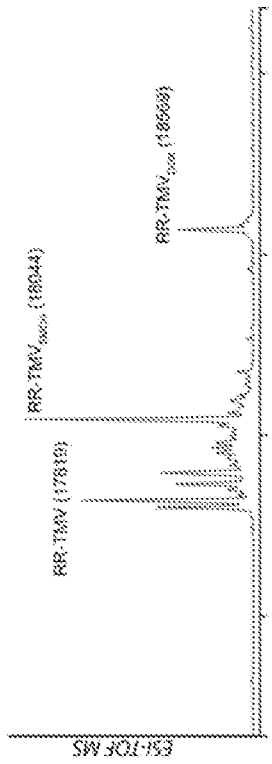
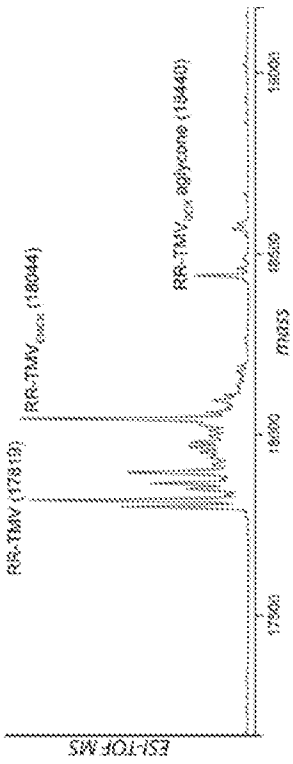


Figure 11C



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/54762

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07K 14/005, C07K 14/08, C07K 19/00 (2017.01)
CPC - C07K 14/005, C07K 2319/00, C12N 2795/10022

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)
See Search History Document

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	DEDEO, Development of Scaffolds for Light Harvesting and Photocatalysis from the Coat Protein of Tobacco Mosaic Virus. PhD Dissertation, University of California, Berkeley, 1 January 2012 [online]. [Retrieved on 17 December 2017]. Retrieved from the Internet: <URL: https://escholarship.org/uc/item/4fz7j4vf> Especially p 40, para 2; p 61, para 2-3; p 62, para 1, 8	1-4 ----- 5
Y	US 2005/0226885 A1 (SOEGAARD et al.) 13 October 2005 (13.10.2005) para [0083], [0086]	5

☐ Further documents are listed in the continuation of Box C. ☐ 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
18 December 2017	18 JAN 2018
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 17/54762

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.: 6-40
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:

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 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.:

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.