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(54) Title: MODIFIED VIRUS-LIKE PARTICLES OF BACTERIOPHAGE AP205

(57) Abstract: The present invention relates to a modified virus-like particle of RNA bacteriophage AP205 (AP205 VLP) comprising AP205 coat protein dimers to which antigenic polypeptides are fused at the N-terminus and/or at the C -terminus. The modified AP205 VLPs can be used as a platform, in particular for vaccine development, in generating immune responses against a variety of antigens.

MODIFIED VIRUS-LIKE PARTICLES OF BACTERIOPHAGE AP205

The present invention relates to a modified virus-like particle of RNA bacterioph AP205 (AP205 VLP) comprising AP205 coat protein dimers to which antigenic polypepti are fused at the N-terminus and/or at the C-terminus. The modified AP205 VLPs can be u as a platform, in particular for vaccine development, in generating immune responses again variety of antigens.

RELATED ART

Virus-like particles (VLPs) are shells of viruses, devoid of viral genome, morphologically and immunologically similar to the respective viruses. Therefore, VLPs be used to create efficient and safe vaccines against the respective viruses, the best-knc examples being Hepatitis B vaccine, composed of Hepatitis B virus S antigen VLPs vaccines against cervical cancer, which are composed of human papilloma virus VL Physically, VLPs are composed of multiple copies of viral coat protein, forming eit icosahedral or rod-like nanoparticles. Recombinant VLPs can be produced by overproduc the respective coat protein gene in bacterial, yeast or other expression systems. Class vaccines are normally composed of inactivated or attenuated pathogens, which still contain genetic information. In contrast, VLPs do not contain any information about the genome pathogens, therefore they are considered to be a safer alternative to classical vaccines. V based technology is not limited to creation of vaccines against the virus of VLP origin, but fact, VLPs can be used to generate immune responses against heterologous antigens (Frie KM et al., Curr Opin Virol. 2016, 18: 44–49; Aves KL et al. Viruses 2020, 12, 185). Amo them, VLPs of single stranded RNA bacteriophages like MS2 and Qβ have been usec construction of vaccines (Tars K, 2020 In: Witzany G. (eds) Biocommunication of Phag Springer, Cham.). Most frequently, two technologies are used – chemical coupling or gen fusion. Recombinant fusion proteins of foreign antigens and viral structural proteins of however, fail to assemble into VLPs due to folding, formation of insoluble products anc assembly problems of the obtained fusion protein. Moreover, genetic fusions are genera limited to small peptide antigens that do not inhibit the required particle assembly. By way example, for RNA bacteriophage MS2 VLPs, foreign peptides inserted within the virus c proteins are rarely compatible with protein folding and VLP assembly (Caldeira JC et al Nanobiotechnology. 2011; 9:22; Peabody DS, Arch Biochem Biophys. 1997; 347:85–

Plevka P et al., Protein Sci. 2009; 18:1653–1661; Peabody DS et al., J Mol Biol. 2008; 380:2 263; WO2008024427A2; US20090054246A1; O'Rourke et al., Current Opinion in Virol 2015, 11:76–82). Therefore, the success of genetic fusion is difficult to predict, let alone the successful identification of foreign antigen insertion sites in VLPs is still a major challe (Frietze KM et al., Curr Opin Virol. 2016, 18: 44–49; Aves KL et al. Viruses 2020, 12, 1 and references cited therein).

RNA-bacteriophage AP205 infects Acinetobacter bacteria and is very distantly rel to other RNA-bacteriophages such as MS2 or Qβ. Sequence alignment of its coat protein other RNA bacteriophage coat proteins revealed that only 5 amino acids are conser (Shishovs et al., J Mol Biol 2016, 428:4267–4279). The assembled AP205 VLPs have b described to be stable and suggested as vaccine platform and even found to tolerate fusion the N or the C terminus (WO04/007538, WO2006/032674, Tissot AC et al., PLoS One 5:e98 WO2016/112921).

Despite these achievements, there is still a need for a robust and versatile VLP platfo in particular for vaccine development, which is able to generate immune responses again variety of antigens, and in particular, to generate immune responses against desired antig and hereby irrespective and independent of the length of said antigens.

SUMMARY OF THE INVENTION

It has been surprisingly found that the modified virus-like particle of RNA bacterioph AP205 (AP205 VLP) of the present invention comprising AP205 coat protein dimers not c allows the fusion of antigens to the N or C-terminus of said AP205 coat protein dim irrespective of the size of the antigens, but, furthermore, the inventive modified AP205 V comprising AP205 coat protein dimers also allows fusion of antigens to both termini of AP205 coat protein dimers without affecting the ability to form VLPs. Thus, the pres invention provides a modified virus-like particle of RNA bacteriophage AP205 (AP205 V comprising AP205 coat protein dimers to which antigenic polypeptides are fused at the and/or at the C-terminus. The inventive modified AP205 VLPs can, thus, be used as a platfo in particular for vaccine development, in generating immune responses against a variety antigens and even against different antigens presented on the same VLP. In particular, provided AP205 coat protein dimers represents a tool to create recombinant VLPs with expo large antigens via genetic fusion of said antigens to the AP205 coat protein dimers and her at either the N-terminus and/or the C-terminus of said AP205 coat protein dimers. In additi

said inventive AP205 coat protein dimers, also named herein *coat protein tandem dime bacteriophage AP205 or tandem dimer of AP205*, allow genetic fusion with long anti sequences without compromising VLP integrity and stability.

In particular, the inventors have found that attempts to insert and fuse the 213 amino a long CspZ protein from *Borrelia burgdorferi* to the N- or the C- terminal part of the single c protein of AP205 failed and did not form VLPs but insoluble products in both cases schematically represented in Fig. 2. On the other hand, the resulting fusion proteins of AP205 coat protein dimers of the present invention with said CspZ protein from *Borr burgdorferi* lead to intact modified virus-like particles in both cases, i.e. either when said C antigen was fused at the N-terminus or when said CspZ antigen was fused to the C-terminu the AP205 coat protein dimer. Furthermore, and besides the bacterial antigen of CspZ pro from *Borrelia burgdorferi* having a length of 213 amino acids, the inventors further achie in providing modified AP205 VLPs in accordance with the present invention for antigen viral and mammalian origin, including ectodomain III (ED3) from E protein of Dengue fe virus serotype 1 having a length of 117 amino acids, the RBM domain of SARS-CoV-2 hav a length of 72 amino acids, and of canine interleukin-33 (cIL-33) with a length of 163 am acids. These modified VLPs are useful for vaccination against Lyme borreliosis, Dengue fe and canine atopic dermatitis, respectively. In summary, the present invention demonstrates VLPs of AP205 coat protein dimers in accordance with the present invention provid universal VLP platform, able to tolerate insertions of a wide variety of antigens, in partic of large antigens, by genetic fusion to either N- and/or C-terminus of said AP205 coat pro dimers.

Thus, in a first aspect, the present invention provides a modified virus-like particl RNA bacteriophage AP205 (AP205 VLP) comprising one or more fusion proteins, whe said fusion protein comprises, preferably consists of,

(i) an AP205 coat protein dimer, wherein said AP205 coat protein dimer compris first AP205 polypeptide and a second AP205 polypeptide, wherein said first AP polypeptide is fused at its C-terminus either directly or via an amino acid space the N-terminus of said second AP205 polypeptide, and wherein said first and s second AP205 polypeptide independently comprises

(a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or

(b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP (a), have a sequence identity of at least 90 %, preferably of at least 95%, fur

preferably of at least 98% and again more preferably of at least 99%; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is fused to the terminus and/or the C-terminus of said AP205 coat protein dimer either directly via an amino acid linker.

In a further aspect, the present invention provides the modified AP205 VLP of the pres invention for use as a medicament.

In again a further aspect, the present invention provides a pharmaceutical composit comprising (a) the AP205 VLP of the present invention, and (b) a pharmaceutically accepta carrier, diluent and/or excipient.

In a further aspect, the present invention provides the modified AP205 VLP or pharmaceutical composition of the present invention for use in a method of immunization animal or a human, comprising administering the modified AP205 VLP or the pharmaceut composition to said animal or human.

In another aspect, the present invention provides the modified AP205 VLP or pharmaceutical composition of the present invention for use in a method of treating preventing a disease or disorder in an animal or human, comprising administering the modif AP205 VLP or the pharmaceutical composition to said animal or human.

In another aspect, the present invention provides a modified virus-like particle of R bacteriophage AP205 (AP205 VLP) comprising one or more AP205 coat protein din wherein said AP205 coat protein dimer comprises a first AP205 polypeptide and a sec AP205 polypeptide, wherein said first AP205 polypeptide is fused at its C-terminus eit directly or via an amino acid spacer to the N-terminus of said second AP205 polypeptide, wherein said first and said second AP205 polypeptide independently comprises

(a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or

(b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP (a), have a sequence identity of at least 90 %, preferably of at least 95%, furt preferably of at least 98% and again more preferably of at least 99%.

Further aspects and embodiments of the present invention will be become apparent as description continues.

DESCRIPTION OF FIGURES

FIG. 1: AP205 tandem dimer coat protein. Two AP205 coat protein genes are fu together, resulting in production of tandem coat protein dimer, where C- terminus of monomer is covalently joined to the N-terminus of other monomer. The covalent dime depicted in rainbow colour from blue (N-terminus) to red (C-terminus). Electron microgr of AP205 tandem dimer VLPs is shown as well.

FIG. 2: Tolerance of VLPs of AP205 tandem dimer. Single AP205 coat protein fu with long antigen such as CspZ either at the N-terminus or at the C-terminus did not lea VLPs but to insoluble products, whereas the AP205 tandem dimer VLPs tolerate C- or terminal fusions of such long antigens.

FIG. 3A: Production and solubility of CspZ-AP205TD. Lane 1 - cell lysate, lane soluble fraction, lane 3 - insoluble fraction. Position of CspZ-AP205TD protein is indica with an arrow.

FIG. 3B: Production and solubility of AP205TD-CspZ. Lane 1 - cell lysate, lane soluble fraction, lane 3 - insoluble fraction. Position of AP205TD-CspZ protein is indica with an arrow.

FIG. 4A: Purification of CspZ-AP205TD by gel-filtration. Peak fractions are loaded SDS-PAGE gel. Position of CspZ-AP205TD protein is indicated with an arrow.

FIG. 4B: Purification of CspZ-AP205TD by gel-filtration. Peak fractions are loaded native agarose gel and stained both with ethidium bromide for the RNA content of VLPs (panel) and coomassie blue for the protein content (right panel).

FIG. 5: Electron micrograph of purified CspZ-AP205TD VLPs.

FIG. 6A: Purification of AP205TD-CspZ by gel-filtration. Peak fractions are loaded SDS-PAGE gel. Position of AP205TD-CspZ protein is indicated with an arrow.

FIG 6B: Peak fractions are loaded on native agarose gel and stained both with ethidi bromide for the RNA content of VLPs (left panel) and coomassie blue for the protein con (right panel).

FIG. 7: Electron micrograph of purified AP205TD-CspZ VLPs.

FIG. 8A: Production and solubility of ED3-AP205TD. Lane 1 - cell lysate, lane 2 - solu fraction, lane 3 - insoluble fraction. Position of ED3-AP205TD protein is indicated with arrow.

FIG. 8B: Refolding of ED3-AP205TD. Lane 1 - insoluble fraction (as in lane 3 of pa A), solubilized with 8 M urea, lane 2 - soluble fraction after refolding, lane 3 - insoluble fract after refolding. Position of ED3-AP205TD protein is indicated with an arrow.

FIG. 9A: Purification of ED3-AP205TD by gel-filtration. Peak fractions are loaded SDS-PAGE gel. Position of ED3-AP205TD protein is indicated with an arrow.

FIG. 9B: Purification of ED3-AP205TD by gel-filtration. Peak fractions are loaded native agarose gel and stained both with ethidium bromide for the RNA content of VLPs (panel) and coomassie blue for the protein content (right panel).

FIG. 10: Electron micrograph of purified ED3-AP205TD VLPs.

FIG. 11: Production and solubility of AP205TD-cIL33. Lane 1 - cell lysate, lane soluble fraction, lane 3 - insoluble fraction. Position of the ~47 kDa AP205TD-cIL33 prot is indicated with an arrow.

FIG. 12A: Purification of AP205TD-cIL33 by gel-filtration. Peak fractions are loaded SDS-PAGE gel. Position of AP205TD-cIL33 protein is indicated with an arrow.

FIG. 12B: Purification of AP205TD-cIL33 by gel-filtration. Peak fractions are loaded native agarose gel and stained both with ethidium bromide for the RNA content of VLPs (panel) and coomassie blue for the protein content (right panel).

FIG. 13: Electron micrograph of purified AP205TD-cIL33 VLPs.

FIG. 14: Immunization of mice with AP205TD-cIL33 VLPs to generate binding neutralizing antibodies. The upper panel shows IL33-binding IgG antibody titers measured ELISA and the lower panel shows IL33-neutralizing antibody titers determined by an *in v* cellular assay.

FIG. 15A: Schematic representation of AP205-RBM fusion construct.

FIG. 15B: 12% SDS-PAGE for AP205-RBM expression in *E.*coli showing soluble and insoluble (P) fractions.

FIG. 15C: 12% SDS-PAGE showing the purified AP205-RBM vaccine of ~37.4 kDa

FIG. 15D: Electron Microscopy of negatively stained AP205-RBM VLPs showing a s of ~30 nm, scale bar 200 nm

FIG. 16A: Vaccination with AP205-RBM vaccine induces high titer of RBD and Spi specific IgG Abs. RBD-specific IgG titer for the groups vaccinated with AP205 control AP205-RBM vaccine on days 14, 21, 35 and 49, measured by ELISA (OD50 in D: given reciprocal dilution values), three-fold serial serum dilution was used starting from 1:20.

FIG. 16B: Spike-specific IgG titer for the groups vaccinated with AP205 control AP205-RBM vaccine on days 14, 21, 35 and 49, measured by ELISA (OD50 in E: giver reciprocal dilution values), three-fold serial serum dilution was used starting from 1:20

FIG. 17A: The AP205-RBM vaccine-candidate induces antibodies neutralizing SA] CoV2. Neutralization titer of the induced antibodies using a SARS-CoV-2 pseudo-typed V virus assay.

FIG. 17B: Neutralization titer of the induced antibodies using CPE method and TCID50 of SARS-CoV-2/ABS/NL20 virus.

FIG. 18. Production and solubility of np12-AP205TD-cp12. M – protein lac (molecular weights as indicated), lane 1 – cell lysate before the induction with IPTG, lane cell lysate after the induction with IPTG, lane 3 – soluble fraction of the cell lysate, lane insoluble fraction. Position of np12-AP205TD-cp12 protein is indicated with an arrow.

FIG. 19A. Purification of np12-AP205TD-cp12 by gel-filtration. Analysis of p fractions on SDS-PAGE gel. Position of np12-AP205TD-cp12 protein is indicated with arrow.

FIG. 19B: Purification of np12-AP205TD-cp12 by gel-filtration. Analysis of p fractions on native agarose gel, stained with ethidium bromide for the RNA content of V] (left panel) and coomassie blue for the protein content (right panel).

FIG. 20A: Purification of np12-AP205TD-cp12 by ion exchange chromatograp Analysis of peak fractions on SDS-PAGE gel. Position of np12-AP205TD-cp12 protei indicated with an arrow.

FIG. 20B: Purification of np12-AP205TD-cp12 by ion exchange chromatograp Analysis of peak fractions on native agarose gel, stained with ethidium bromide for the R content of VLPs (left panel) and coomassie blue for the protein content (right panel).

FIG. 21: Electron micrograph of purified np12-AP205TD-cp12 VLPs.

DETAILED DESCRIPTION OF THE INVENTION

Unless defined otherwise, all technical and scientific terms used herein have the sa meanings as commonly understood by one of ordinary skill in the art to which this invent belongs. The herein described and disclosed embodiments, preferred embodiments and v preferred embodiments should apply to all aspects and other embodiments, prefe embodiments and very preferred embodiments irrespective of whether is specifically ag referred to or its repetition is avoided for the sake of conciseness. The articles “a” and “an”

used herein, refer to one or to more than one (i.e., to at least one) of the grammatical objec the article. The term "or", as used herein, should be understood to mean "and/or", unless context clearly indicates otherwise. As used herein, the terms "about" when referring to numerical value are intended to mean a value of ±10% of the stated value. In a prefer embodiment, said "about" when referring to any numerical value are intended to mean a va of ±5% of the stated value. In another preferred embodiment, said "about" when referrin any numerical value are intended to mean a value of ±3% of the stated value.

Virus-like particle (VLP): The term "virus-like particle (VLP)" as used herein, refer a non-replicative or non-infectious, preferably a non-replicative and non-infectious v particle, or refers to a non-replicative or non-infectious, preferably a non-replicative and n infectious structure resembling a virus particle, preferably a capsid of a virus. The term "n replicative", as used herein, refers to being incapable of replicating the genome comprised the VLP. The term "non-infectious", as used herein, refers to being incapable of entering host cell. A virus-like particle in accordance with the invention is non-replicative and n infectious since it lacks all or part of the viral genome or genome function. A virus-like part in accordance with the invention may contain nucleic acid distinct from their geno Recombinantly produced virus-like particles typically contain host cell derived RNA.

Modified virus-like particle of RNA bacteriophage AP205 (modified AP205 VLP): term "modified virus-like particle of RNA bacteriophage AP205 (modified AP205 VLP)" re to a virus-like particle comprising at least one, typically and preferably about 90, furt preferably exactly 90, AP205 coat protein dimers in accordance with the present inventi Typically and preferably, modified AP205 VLPs resemble the structure of the capsid of R bacteriophage AP205. Modified AP205 VLPs are non-replicative and/or non-infectious, lack at least the gene or genes encoding for the replication machinery of RNA bacterioph AP205, and typically also lack the gene or genes encoding the protein or proteins respons for viral attachment to or entry into the host. This definition includes also modified virus- particles in which the aforementioned gene or genes are still present but inactive. Preferal non-replicative and/or non-infectious modified virus-like particles are obtained by recombir gene technology and typically and preferably do not comprise the viral genome.

Polypeptide: The term "polypeptide" as used herein refers to a polymer composed amino acid monomers which are linearly linked by amide bonds (also known as peptide bon It indicates a molecular chain of amino acids and does not refer to a specific length of product. Thus, peptides, dipeptides, tripeptides, oligopeptides and proteins are included wi the definition of polypeptide. The term "polypeptide" as used herein should also refer, typic

and preferably to a polypeptide as defined before and encompassing modifications such as p translational modifications, including but not limited to glycosylations. In a prefe embodiment, said term “polypeptide” as used herein should refer to a polypeptide as defi before and not encompassing modifications such as post-translational modifications suc glycosylations. In particular, for said biologically active peptides, said modifications suc said glycosylations can occur even *in vivo* thereafter, for example, by bacteria.

AP205 polypeptide: The term “AP205 polypeptide” as used herein – be it for a “ AP205 polypeptide” or “second AP205 polypeptide” independently - refers to a polypep comprising or preferably consisting of: (i) an amino acid sequence of a coat protein of R bacteriophage AP205, or (ii) a mutated amino acid sequence, wherein said mutated amino sequence and said amino acid sequence of said coat protein of CMV, have a sequence iden of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again m preferably of at least 99%. Typically and preferably, the AP205 coat protein dimer compri a first AP205 polypeptide and a second AP205 polypeptide is capable of forming a virus- particle of AP205 upon expression by self-assembly.

Coat protein (CP) of RNA bacteriophage AP205: The term “coat protein (CP) of R bacteriophage AP205 (or, in short and interchangeably used, coat protein of AP205)”, as u herein, refers to a coat protein of the RNA bacteriophage AP205 which occurs in nature or amino acid sequence of said coat protein wherein the first methionine is cleaved. The seque of said coat proteins (CPs) of AP205 are described in and retrievable from the known datab such as Genbank, www.dpvweb.net, or www.ncbi.nlm.nih.gov/protein/. Specific and prefe examples CPs of AP205, and mutated amino acid sequences thereof, are described in Klov J., et al., 2002, J. Gen. Virol. 83:1523-33 and WO2006/032674, the disclosures of which explicitly incorporated herein by way of reference. In a preferred embodiment of the pre invention, said coat protein of AP205 consists of a length of about 131 amino acids, typic and preferably said coat protein of AP205 consists of a length of 128 to 133 amino acids. V preferred examples and embodiments of AP205 coat proteins are provided in SEQ ID NO and SEQ ID NO:26.

Recombinant polypeptide: In the context of the invention the term "recombinant” w used in the context of a polypeptide refers to a polypeptide which is obtained by a process w comprises at least one step of recombinant DNA technology. Typically and preferabl recombinant polypeptide is produced in a prokaryotic expression system. It is apparent for artisan that recombinantly produced polypeptides which are expressed in a prokary expression system such as *E. coli* may comprise an N-terminal methionine residue. The

terminal methionine residue is typically cleaved off the recombinant polypeptide in expression host during the maturation of the recombinant polypeptide. However, the cleav of the N-terminal methionine may be incomplete. Thus, a preparation of a recombir polypeptide may comprise a mixture of otherwise identical polypeptides with and withoui N-terminal methionine residue. Typically and preferably, a preparation of a recombir polypeptide comprises less than 10 %, more preferably less than 5 %, and still more prefera less than 1 % recombinant polypeptide with an N-terminal methionine residue.

Recombinant modified virus-like particle: In the context of the invention the t "recombinant modified virus-like particle" refers to a modified virus-like particle (VLP) wh is obtained by a process which comprises at least one step of recombinant DNA technology

Mutated amino acid sequence: The term "mutated amino acid sequence" refers to amino acid sequence which is obtained by introducing a defined set of mutations into an am acid sequence to be mutated. In the context of the invention, said amino acid sequence tc mutated typically and preferably is an amino acid sequence of a coat protein of AP205. Tl a mutated amino acid sequence differs from an amino acid sequence of a coat protein of AP in at least one amino acid residue, wherein said mutated amino acid sequence and said am acid sequence of a coat protein of AP205 have a sequence identity of at least 90 %. Typic and preferably said mutated amino acid sequence and said amino acid sequence of a coat pro of AP205 have a sequence identity of at least 91 %, 92 %, 93 % 94 %, 95 %, 96 %, 97 %, %, or 99 %. Preferably, said mutated amino acid sequence and said sequence of a coat pro of AP205 differ in at most 11, 10, 9, 8, 7, 6, 4, 3, 2, or 1 amino acid residues, wherein furt preferably said difference is selected from insertion, deletion and amino acid exchange ar combination thereof. Preferably, the mutated amino acid sequence differs from an amino a sequence of a coat protein of CMV in three, two or one amino acid, wherein preferably s difference is an amino acid exchange, deletion or addition, and a combination thereof.

Sequence identity: The sequence identity of two given amino acid sequences determined based on an alignment of both sequences. Algorithms for the determinatior sequence identity are available to the artisan. Preferably, the sequence identity of two am acid sequences is determined using publicly available computer homology programs sucl the "BLAST" program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) or the "CLUSTAL (http://www.genome.jp/tools/clustalw/), and hereby preferably by the "BLAST" progı provided on the NCBI homepage at http://blast.ncbi.nlm.nih.gov/Blast.cgi, using the def settings provided therein. Typical and preferred standard settings are: expect threshold: word size: 3; max matches in a query range: 0; matrix: BLOSUM62; gap costs: existence

extension 1; compositional adjustments: conditional compositional score matrix adjustmen

Amino acid exchange: The term amino acid exchange refers to the exchange of a gi amino acid residue in an amino acid sequence by any other amino acid residue having a differ chemical structure, preferably by another proteinogenic amino acid residue. Thus, in cont to insertion or deletion of an amino acid, the amino acid exchange does not change the t number of amino acids of said amino acid sequence. In case of an amino acid exchange wi the present invention and thus, also referred to as an amino acid substitution, conserva amino acid substitutions are preferred. Conservative amino acid substitutions, as understooc a skilled person in the art, include, and typically and preferably consist of isosteric substituti substitutions where the charged, polar, aromatic, aliphatic or hydrophobic nature of the am acid is maintained. Typical conservative substitutions are substitutions between amino ac within one of the following groups: Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr, C Lys, Arg; and Phe and Tyr.

Antigenic polypeptide: As used herein, the term "antigenic polypeptide" refers t molecule capable of being bound by an antibody or a T-cell receptor (TCR) if presented MHC molecules. An antigenic polypeptide is additionally capable of being recognized by immune system and/or being capable of inducing a humoral immune response and/or cell immune response leading to the activation of B- and/or T- lymphocytes. An antige polypeptide can have one or more epitopes (B- and T-epitopes). Antigenic polypeptides as u herein may also be mixtures of several individual antigenic polypeptides. The inventive fus proteins which are forming the inventive modified AP205 VLPs comprise the antige polypeptide. In case, the inventive fusion proteins which are forming the inventive modi AP205 VLPs comprise an antigenic polypeptide fused to the N-terminus of said AP205 c protein dimer and an antigenic polypeptide fused to the C-terminus of said AP205 coat pro dimer in accordance with the present invention, the terms “first antigenic polypeptide” “second antigenic polypeptide” are also used herein, preferably to distinguish the antige polypeptide fused to the N-terminus of said AP205 coat protein dimer and the antige polypeptide fused to the C-terminus of said AP205 coat protein dimer. However, all antige polypeptides mentioned herein as preferred antigenic polypeptides are understood to equ read on first antigenic polypeptides and/or second antigenic polypeptides irrespective whe it is explicitly stated as such. For the sake of conciseness such repetitions have been omitte

Epitope: The term epitope refers to continuous or discontinuous portions of an antige polypeptide, wherein said portions can be specifically bound by an antibody or by a T- receptor within the context of an MHC molecule. With respect to antibodies, specific bind

excludes non-specific binding but does not necessarily exclude cross-reactivity. An epit typically comprise 5-20 amino acids in a spatial conformation which is unique to the antige site.

Receptor binding domain: The term “protein domain” and “receptor binding domain' used herein, refers to parts of proteins that either occur alone or together with partner dom on the same protein chain. Most domains correspond to tertiary structure elements and are a to fold independently. All domains exhibit evolutionary conservation, and many either perf specific functions or contribute in a specific way to the function of their proteins (Forslund et al, Methods Mol Biol. (2019) 1910:469-504). Viral structural proteins, such as Coronav S proteins, can contain several functional domains, which are necessary for the cell infect process. One such domain in Coronavirus S protein is the receptor binding domain (RI which binds to corresponding cell receptor.

Receptor binding motif: The term “receptor binding motif (RBM)”, as used herein, part of receptor binding domain and represent a linear amino acid sequence and/or a structure located on outer surface of the virus and making direct contact with target receptors (Sobhy H, Proteomes (2016) 4(1): 3). For Coronaviruses, the amino acids sequen of RBMs have low homology due to different target cellular receptors. For SARS-CoV2, amino acids of RBM make direct contacts with human ACE2 receptor (Lan et al., Structur the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor, Nature, 2C 581, 215-220).

Adjuvant: The term "adjuvant" as used herein refers to non-specific stimulators of immune response or substances that allow generation of a depot in the host which w combined with the vaccine and pharmaceutical composition, respectively, of the pre invention may provide for an even more enhanced immune response. Preferred adjuvants complete and incomplete Freund's adjuvant, aluminum containing adjuvant, prefera aluminum hydroxide, and modified muramyldipeptide. Further preferred adjuvants are min gels such as aluminum hydroxide, surface active substances such as lyso lecithin, pluro polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, human adjuvants such as BCG (bacille Calmette Guerin) and Corynebacterium parvum. S adjuvants are also well known in the art. Further adjuvants that can be administered with compositions of the invention include, but are not limited to, Monophosphoryl li immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts (Alum), MF- OM- 174, OM- 197, OM-294, and Virosomal adjuvant technology. The adjuvants may a comprise mixtures of these substances. Virus-like particles have been generally described a

adjuvant. However, the term "adjuvant", as used within the context of this application, refer an adjuvant not being the inventive modified virus-like particle. Rather "adjuvant" relates tc additional, distinct component of the inventive compositions, vaccines or pharmaceut compositions.

Amino acid linker: The term “amino acid linker” as used herein, refers to a lin consisting exclusively of amino acid residues. The amino acid residues of the amino acid lin are composed of naturally occurring amino acids or unnatural amino acids known in the art, L or all-D or mixtures thereof. The amino acid residues of the amino acid linker are prefera naturally occurring amino acids, all-L or all-D or mixtures thereof.

Amino acid spacer: The term “amino acid spacer” as used herein, refers to a link consisting exclusively of amino acid residues linking the first AP205 polypeptide with second AP205 polypeptide of the present invention. The amino acid residues of the amino a spacer are composed of naturally occurring amino acids or unnatural amino acids known in art, all-L or all-D or mixtures thereof. The amino acid residues of the amino acid spacer preferably naturally occurring amino acids, all-L or all-D or mixtures thereof.

GS-linker: The term “GS-linker”, as used herein refers to a linker solely consisting glycine and serine amino acid residues. The GS-linker in accordance with the present invent comprise at least one glycine and at least one serine residue. Typically and preferably, the (linker in accordance with the present invention has a length of at most 50 amino acids, typically and further preferably, the GS-linker in accordance with the present invention ha length of at most 30 amino acids, further preferably of at most 15 amino acids.

Animal: The term “animal”, as used herein and being the subject in need of treatmen prevention with the inventive modified AP205 VLPs, may be an animal (e.g., a non hur animal), a vertebrate animal, a mammal, a rodent (e.g., a guinea pig, a hamster), a canine (e a dog), a feline (e.g., a cat), a porcine (e.g., a pig), an equine (e.g., a horse), a primate, (human. In the context of this invention, it is particularly envisaged that animals are to be trea which are economically, agronomically or scientifically important. Scientifically impor organisms include, but are not limited to, mice, rats, and rabbits. Non-limiting examples agronomically important animals are sheep, cattle and pigs, while, for example, cats, dogs horses may be considered as economically important animals. Preferably, the subject i mammal; more preferably, the subject is a human or a non-human mammal (such as, e.g dog, a cat, a horse, a sheep, cattle, or a pig).

Effective amount: As used herein, the term “effective amount” refers to an amc necessary or sufficient to realize a desired biologic effect. An effective amount of

composition, or alternatively the pharmaceutical composition, would be the amount achieves this selected result, and such an amount could be determined as a matter of routine a person skilled in the art. The effective amount can vary depending on the partic composition being administered and the size of the subject. One of ordinary skill in the art empirically determine the effective amount of a particular composition of the present invent without necessitating undue experimentation.

Therapeutically effective amount: As used herein, the term "therapeutically effec amount" refers to an amount that (i) treats the particular disease, condition, or disorder, attenuates, ameliorates, or eliminates one or more symptoms of the particular dise condition, or disorder, or (iii) prevents or delays the onset of one or more symptoms of particular disease, condition, or disorder described herein. For example, in case of a cancer, therapeutically effective amount may reduce the number of cancer cells; reduce the tumor s inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into periph organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to sc extent, tumor growth; and/or relieve to some extent one or more of the symptoms associa with the cancer.

Treatment: As used herein, the terms "treatment", "treat", "treated" or "treating" refe prophylaxis and/or therapy. In one embodiment, the terms "treatment", "treat", "treated" "treating" refer to a therapeutic treatment. In another embodiment, the terms "treatme "treat", "treated" or "treating" refer to a prophylactic treatment

In a first aspect, the present invention provides a modified virus-like particle of R bacteriophage AP205 (AP205 VLP) comprising one or more fusion proteins, wherein s fusion protein comprises, preferably consists of,

(i) an AP205 coat protein dimer, wherein said AP205 coat protein dimer compris first AP205 polypeptide and a second AP205 polypeptide, wherein said first AP polypeptide is fused at its C-terminus either directly or via an amino acid space the N-terminus of said second AP205 polypeptide, and wherein said first and s second AP205 polypeptide independently comprises
(a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or
(b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP (a), have a sequence identity of at least 90 %, preferably of at least 95%, furt preferably of at least 98% and again more preferably of at least 99%; and

(ii) an antigenic polypeptide, wherein said antigenic polypeptide is fused to the terminus and/or the C-terminus of said AP205 coat protein dimer either directl via an amino acid linker.

In another aspect, the present invention provides a modified virus-like particle of R bacteriophage AP205 (AP205 VLP) comprising one or more fusion proteins, wherein s fusion protein comprises, preferably consists of,

(i) an AP205 coat protein dimer, wherein said AP205 coat protein dimer compris first AP205 polypeptide and a second AP205 polypeptide, wherein said first AP polypeptide is fused at its C-terminus either directly or via an amino acid space the N-terminus of said second AP205 polypeptide, and wherein said first and s second AP205 polypeptide independently comprises

(a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or

(b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP (a), have a sequence identity of at least 90 %, preferably of at least 95%, furt preferably of at least 98% and again more preferably of at least 99%; and

(ii) (1) an antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer;

(2) an antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer; or

(3) a first antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer and a second antigenic polypeptide fu directly or via an amino acid linker to the C-terminus of said AP205 coat pro dimer.

In another aspect and preferred embodiment, the present invention provides a modi virus-like particle of RNA bacteriophage AP205 (AP205 VLP) comprising one or more fus proteins, wherein said fusion protein comprises, preferably consists of,

(i) an AP205 coat protein dimer, wherein said AP205 coat protein di comprises a first AP205 polypeptide and a second AP205 polypept wherein said first AP205 polypeptide is fused at its C-terminus ei directly or via an amino acid spacer to the N-terminus of said second AP polypeptide, and wherein said first and said second AP205 polypep independently comprises

(a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or

(b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP: (a), have a sequence identity of at least 90 %, preferably of at least 95%, furt preferably of at least 98% and again more preferably of at least 99%, and wher preferably said AP205 coat protein dimer comprises, preferably consists of, amino sequence of SEQ ID NO: 7 or an amino acid sequence having a seque identity of at least 90 %, preferably of at least 95%, further preferably of at l(98% and again more preferably of at least 99% with said SEQ ID NO: 7; and

(ii) (1) an antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer, wherein said antigenic polypeptide a length of at least 60 amino acids and at most 300 amino acids, and wher preferably said antigenic polypeptide has a length of at least 70 amino acids an most 300 amino acids;

(2) an antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer, wherein said antigenic polypeptide a length of at least 60 amino acids and at most 300 amino acids, and wher preferably said antigenic polypeptide has a length of at least 70 amino acids an most 300 amino acids; or

(3) a first antigenic polypeptide fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer and a second antigenic polypeptide fu directly or via an amino acid linker to the C-terminus of said AP205 coat pro dimer, wherein said first antigenic polypeptide and said second antige polypeptide independently has a length of at least 5 amino acids and at most amino acids, and wherein preferably said first antigenic polypeptide and s second antigenic polypeptide independently has a length of at least 5 amino ac and at most 60 amino acids, again further preferably, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length c least 5 amino acids and at most 50 amino acids;

and wherein preferably said modified AP205 VLP consists of said fusion proteins.

In a preferred embodiment, said first and said second AP205 polypeptide independer comprises (a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or (l mutated amino acid sequence, wherein said mutated amino acid sequence (b) and said am acid sequence of a coat protein of RNA bacteriophage AP205 (a), have a sequence identit least 95%. In a preferred embodiment, said first and said second AP205 polypep

independently comprises (a) an amino acid sequence of a coat protein of RNA bacterioph AP205, or (b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of a coat protein of RNA bacteriophage AP205 (a), hav sequence identity at least 98%. In a preferred embodiment, said first and said second AP polypeptide independently comprises (a) an amino acid sequence of a coat protein of R bacteriophage AP205, or (b) a mutated amino acid sequence, wherein said mutated amino a sequence (b) and said amino acid sequence of a coat protein of RNA bacteriophage AP205 have a sequence identity at least 99%.

In a preferred embodiment, said first and said second AP205 polypeptide independe comprises (a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or (mutated amino acid sequence, wherein said mutated amino acid sequence (b) and said am acid sequence of said coat protein of RNA bacteriophage AP205 (a) differ in at most 11, 1C 8, 7, 6, 4, 3, 2, or 1 amino acid residues, wherein said differences are independently sele from insertion, deletion, amino acid exchange and a combination thereof. In a prefe embodiment, the mutated amino acid sequence differs from an amino acid sequence of a c protein of RNA bacteriophage AP205 in 1, 2, 3, 4 or 5 amino acids, said differences independently selected from insertion, deletion, amino acid exchange and a combinat thereof. In a preferred embodiment, the mutated amino acid sequence differs from an am acid sequence of a coat protein of RNA bacteriophage AP205 in 1, 2 or 3 amino acids, s differences are independently selected from insertion, deletion, amino acid exchange an combination thereof. In a preferred embodiment, the mutated amino acid sequence differs fr an amino acid sequence of a coat protein of RNA bacteriophage AP205 in 3 amino acids, s differences are independently selected from insertion, deletion, amino acid exchange an combination thereof. In a preferred embodiment, the mutated amino acid sequence differs fr an amino acid sequence of a coat protein of RNA bacteriophage AP205 in 2 amino acids, s differences are independently selected from insertion, deletion, amino acid exchange an combination thereof. In a preferred embodiment, the mutated amino acid sequence differs fr an amino acid sequence of a coat protein of RNA bacteriophage AP205 in 1 amino acid, s difference is selected from insertion, deletion, and amino acid exchange.

In a preferred embodiment, said first and said second AP205 polypeptide independe comprises (a) an amino acid sequence of a coat protein of RNA bacteriophage AP205.

In a preferred embodiment, said first and said second AP205 polypeptide independe comprises, preferably consists of, an amino acid sequence selected from any of the SEQ NO: 25 to 33 and an amino sequence having a sequence identity of at least 90%, preferabl

at least 95%, further preferably of at least 98%, and again further preferably of at least 9
with any of the SEQ ID NO: 25 to 33. In a preferred embodiment, said first and said sec
AP205 polypeptide independently comprises, preferably consists of, an amino acid seque
selected from any of the SEQ ID NO: 25 to 33. In a preferred embodiment, said first AP
polypeptide comprises, preferably consists of, an amino acid sequence selected from any of
SEQ ID NO: 25, 26, 28, 29, 31 and 32. In a preferred embodiment, said first AP205 polypep
comprises, preferably consists of, an amino acid sequence selected from any of the SEQ
NO: 25, 28 and 31. In a very preferred embodiment, said first AP205 polypeptide compri
preferably consists of, the amino acid sequence of SEQ ID NO: 25. In a preferred embodim
said second AP205 polypeptide comprises, preferably consists of, an amino acid seque
selected from any of the SEQ ID NO: 26, 27, 29, 30, 32 and 33. In a preferred embodim
said second AP205 polypeptide comprises, preferably consists of, an amino acid seque
selected from any of the SEQ ID NO: 26 and 27. In a very preferred embodiment, said sec
AP205 polypeptide comprises, preferably consists of, the amino acid sequence of SEQ ID N
26. In a very preferred embodiment, said second AP205 polypeptide comprises, prefera
consists of, the amino acid sequence of SEQ ID NO: 27.

In a preferred embodiment, said first and said second AP205 polypeptide independe
comprises (a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or (
mutated amino acid sequence, wherein said mutated amino acid sequence (b) and said am
acid sequence of a coat protein of RNA bacteriophage AP205 (a), have a sequence identit
least 90%, wherein said coat protein of RNA bacteriophage AP205 comprises, prefera
consists of, the amino acid sequence of SEQ ID NO: 26. In a preferred embodiment, said
and said second AP205 polypeptide independently comprises (a) an amino acid sequence
coat protein of RNA bacteriophage AP205, or (b) a mutated amino acid sequence, wherein
mutated amino acid sequence (b) and said amino acid sequence of a coat protein of R
bacteriophage AP205 (a), have a sequence identity at least 95%, wherein said coat protei
RNA bacteriophage AP205 comprises, preferably consists of, the amino acid sequence of S
ID NO: 26. In a preferred embodiment, said first and said second AP205 polypep
independently comprises (a) an amino acid sequence of a coat protein of RNA bacterioph
AP205, or (b) a mutated amino acid sequence, wherein said mutated amino acid sequence
and said amino acid sequence of a coat protein of RNA bacteriophage AP205 (a), hav
sequence identity at least 98%, wherein said coat protein of RNA bacteriophage AP
comprises, preferably consists of, the amino acid sequence of SEQ ID NO: 26.

In a preferred embodiment, said first AP205 polypeptide is fused at its C-terminus dire

to the N-terminus of said second AP205 polypeptide.

In another preferred embodiment, said first AP205 polypeptide is fused at its C-termi via an amino acid spacer to the N-terminus of said second AP205 polypeptide. In anot preferred embodiment, said amino acid spacer is selected from the group consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5;
(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ v r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1; and wherein preferably said glycine-sei linker has a length of at most 15, further preferably of at most 10, amino acids; and
(c.) an amino acid sequence comprising at least one Gly, at least one Ser, and at least amino acid selected from Thr, Ala, Lys, Asp and Glu, wherein said amino acid seque has a length of at most 15 amino acids.

In another preferred embodiment, said amino acid spacer is selected from the gr consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and
(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ v r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1; and wherein preferably said glycine-sei linker has a length of at most 15, further preferably of at most 10, amino acids.

In another preferred embodiment, said amino acid spacer is selected from the gr consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and
(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ v r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1;

wherein said amino acid spacer has a length of at most 15 amino acids, wherein preferably s amino acid spacer has a length of at most 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 amino acid

In another preferred embodiment, said amino acid spacer has a length of at most 10 am acids. In another preferred embodiment, said amino acid spacer has a length of at most 5 am acids. In another preferred embodiment, said amino acid spacer is a glycine-serine linker ((linker) comprising at least one glycine and at least one serine, and has a length of at most amino acids. In another preferred embodiment, said amino acid spacer is a glycine-serine lin (GS-linker) comprising at least one glycine and at least one serine, and has a length of at m 5 amino acids. In another preferred embodiment, said amino acid spacer is the di-amino a

glycine-serine linker GS. In another preferred embodiment, said first AP205 polypeptid fused at its C-terminus via an amino acid spacer to the N-terminus of said second AP polypeptide, wherein said amino acid spacer is a glycine-serine linker, wherein said amino a spacer has a length of at most 5 amino acids, and wherein preferably said amino acid space the di-amino acid glycine-serine linker GS.

In a preferred embodiment, said amino acid spacer is selected from the group consist of (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ wherein r, s, t, u a

(i.) r=0, s=1, w=1, t=1, and u=0; (ii.) r=0, s=1, w=1, t=2, and u=0; (iii.) r=0, s=2, w t=1, and u=0; (iv.) r=0, s=3, w=1, t=1, and u=0; (v.) r=0, s=4, w=1, t=1, and u=0; (r=1, s=1, w=0, t=1, u=0; (vii.) r=1, s=2, w=0, t=1, u=0.

In a preferred embodiment, said amino acid spacer is a glycine-serine linker (GS-linl comprising at least one glycine and at least one serine, wherein said GS linker has the am acid sequence of GS, corresponding to the amino acid sequence $(GS)_r(G_sS_w)_t(GS)_u$ wherein r s=1, w=1, t=1, and u=0.

In another preferred embodiment, said amino acid spacer is selected from the gr consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and

(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ v r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1;

wherein said amino acid spacer has a length of at most 15 amino acids.

In a preferred embodiment, said antigenic polypeptide is fused directly to the N-termi or the C-terminus of said AP205 coat protein dimer. In a preferred embodiment, said antige polypeptide is fused directly to the N-terminus of said AP205 coat protein dimer. In a prefer embodiment, said antigenic polypeptide is fused directly to the C-terminus of said AP205 c protein dimer.

In a preferred embodiment, an antigenic polypeptide is fused directly or via an amino a linker to the N-terminus of said AP205 coat protein dimer; or an antigenic polypeptide is fu directly or via an amino acid linker to the C-terminus of said AP205 coat protein dimer; (first antigenic polypeptide is fused directly or via an amino acid linker to the N-terminus of s AP205 coat protein dimer and a second antigenic polypeptide is fused directly or via an am acid linker to the C-terminus of said AP205 coat protein dimer.

In a preferred embodiment, a first antigenic polypeptide is fused directly or via an am acid linker to the N-terminus of said AP205 coat protein dimer and a second antige polypeptide is fused directly or via an amino acid linker to the C-terminus of said AP205 c protein dimer. In a preferred embodiment, a first antigenic polypeptide is fused directly to N-terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fused an amino acid linker to the C-terminus of said AP205 coat protein dimer. In a prefe embodiment, a first antigenic polypeptide is fused via an amino acid linker to the N-termi of said AP205 coat protein dimer and a second antigenic polypeptide is fused directly to the terminus of said AP205 coat protein dimer. In a preferred embodiment, a first antige polypeptide is fused directly to the N-terminus of said AP205 coat protein dimer and a sec antigenic polypeptide is fused directly to the C-terminus of said AP205 coat protein dimer a preferred embodiment, a first antigenic polypeptide is fused via an amino acid linker to N-terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fused an amino acid linker to the C-terminus of said AP205 coat protein dimer.

In a preferred embodiment, said first antigenic polypeptide is fused to the N-terminu said AP205 coat protein dimer with the opposite orientation as is said second antige polypeptide fused to the C-terminus of said AP205 coat protein dimer. By way of example, s first antigenic polypeptide is fused with its C-terminus directly or via an amino acid linke the N-terminus of said AP205 coat protein dimer, wherein said second antigenic polypeptid fused with its N-terminus directly or via an amino acid linker to the C-terminus of said AP coat protein dimer.

In a preferred embodiment, said first antigenic polypeptide and said second antige polypeptide is the same antigenic polypeptide, wherein typically and preferably said f antigenic polypeptide and said second antigenic polypeptide comprises, preferably consists the same amino acid sequence. In a preferred embodiment, said first antigenic polypeptide fu directly or via an amino acid linker to the N-terminus of said AP205 coat protein dimer said second antigenic polypeptide fused directly or via an amino acid linker to the C-termi of said AP205 coat protein dimer, wherein said first antigenic polypeptide and said sec antigenic polypeptide is the same antigenic polypeptide, and typically and prefera comprises, preferably consists of, the same amino acid sequence, and wherein said f antigenic polypeptide is fused directly or via an amino acid linker to the N-terminus of s AP205 coat protein dimer with the opposite orientation as is said second antigenic polypep fused directly or via an amino acid linker to the C-terminus of said AP205 coat protein din By way of example, said first antigenic polypeptide is fused with its C-terminus directly or

an amino acid linker to the N-terminus of said AP205 coat protein dimer, wherein said sec antigenic polypeptide is fused with its N-terminus directly or via an amino acid linker to the terminus of said AP205 coat protein dimer.

In a preferred embodiment, said first antigenic polypeptide and said second antige polypeptide are different antigenic polypeptides, and typically and preferably compr preferably consist of, different amino acid sequences. In a preferred embodiment, said f antigenic polypeptide fused directly or via an amino acid linker to the N-terminus of said AP coat protein dimer and said second antigenic polypeptide fused directly or via an amino a linker to the C-terminus of said AP205 coat protein dimer, wherein said first antige polypeptide and said second antigenic polypeptide are antigenic polypeptides, and typically preferably comprise, preferably consist of, the different amino acid sequences, wherein s first antigenic polypeptide and said second antigenic polypeptide comprise different epito of the same pathogenic or pathologic antigenic polypeptide target.

In a preferred embodiment, a first antigenic polypeptide is fused directly to the terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fused dire to the C-terminus of said AP205 coat protein dimer. In a preferred embodiment, said f antigenic polypeptide is fused to the N-terminus of said AP205 coat protein dimer with opposite orientation as is said second antigenic polypeptide fused to the C-terminus of s AP205 coat protein dimer. By way of example, said first antigenic polypeptide is fused with C-terminus directly to the N-terminus of said AP205 coat protein dimer, wherein said sec antigenic polypeptide is fused with its N-terminus directly to the C-terminus of said AP205 c protein dimer. In a preferred embodiment, said first antigenic polypeptide and said sec antigenic polypeptide is the same antigenic polypeptide, wherein typically and preferably s first antigenic polypeptide and said second antigenic polypeptide comprises, preferably cons of, the same amino acid sequence. In a preferred embodiment, said first antigenic polypept fused directly to the N-terminus of said AP205 coat protein dimer and said second antige polypeptide fused directly to the C-terminus of said AP205 coat protein dimer, wherein s first antigenic polypeptide and said second antigenic polypeptide is the same antige polypeptide, and typically and preferably comprises, preferably consists of, the same am acid sequence, and wherein said first antigenic polypeptide is fused directly to the N-termi of said AP205 coat protein dimer with the opposite orientation as is said second antige polypeptide fused directly to the C-terminus of said AP205 coat protein dimer. By way example, said first antigenic polypeptide is fused with its C-terminus directly to the N-termi of said AP205 coat protein dimer, wherein said second antigenic polypeptide is fused with

N-terminus directly to the C-terminus of said AP205 coat protein dimer.

In a preferred embodiment, said first antigenic polypeptide and said second antige polypeptide are different antigenic polypeptides, and typically and preferably compr preferably consist of, different amino acid sequences. In a preferred embodiment, said f antigenic polypeptide fused directly to the N-terminus of said AP205 coat protein dimer said second antigenic polypeptide fused directly to the C-terminus of said AP205 coat pro dimer, wherein said first antigenic polypeptide and said second antigenic polypeptide antigenic polypeptides, and typically and preferably comprise, preferably consist of, different amino acid sequences, wherein said first antigenic polypeptide and said sec antigenic polypeptide comprise different epitopes of the same pathogenic antigenic polypep target.

In another preferred embodiment, said antigenic polypeptide is fused via an amino a linker to the N-terminus and/or the C-terminus of said AP205 coat protein dimer. In anot preferred embodiment, said antigenic polypeptide is fused via an amino acid linker to the terminus of said AP205 coat protein dimer. In another preferred embodiment, said antige polypeptide is fused via an amino acid linker to the C-terminus of said AP205 coat prot dimer.

In a preferred embodiment, a first antigenic polypeptide is fused via an amino acid lin to the N-terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fu via an amino acid linker to the C-terminus of said AP205 coat protein dimer. In a prefer embodiment, said first antigenic polypeptide is fused to the N-terminus of said AP205 c protein dimer with the opposite orientation as is said second antigenic polypeptide fused to C-terminus of said AP205 coat protein dimer. By way of example, said first antige polypeptide is fused with its C-terminus via an amino acid linker to the N-terminus of s AP205 coat protein dimer, wherein said second antigenic polypeptide is fused with its terminus via an amino acid linker to the C-terminus of said AP205 coat protein dimer. I preferred embodiment, said first antigenic polypeptide and said second antigenic polypept is the same antigenic polypeptide, wherein typically and preferably said first antige polypeptide and said second antigenic polypeptide comprises, preferably consists of, the sa amino acid sequence. In a preferred embodiment, said first antigenic polypeptide fused via amino acid linker to the N-terminus of said AP205 coat protein dimer and said second antige polypeptide fused via an amino acid linker to the C-terminus of said AP205 coat protein din wherein said first antigenic polypeptide and said second antigenic polypeptide is the sa antigenic polypeptide, and typically and preferably comprises, preferably consists of, the sa

amino acid sequence, and wherein said first antigenic polypeptide is fused via an amino a
spacer to the N-terminus of said AP205 coat protein dimer with the opposite orientation a
said second antigenic polypeptide fused via an amino acid linker to the C-terminus of s
AP205 coat protein dimer. By way of example, said first antigenic polypeptide is fused wit
C-terminus via an amino acid linker to the N-terminus of said AP205 coat protein din
wherein said second antigenic polypeptide is fused with its N-terminus via an amino acid lir
to the C-terminus of said AP205 coat protein dimer.

In a preferred embodiment, said first antigenic polypeptide and said second antige
polypeptide are different antigenic polypeptides, and typically and preferably compr
preferably consist of, different amino acid sequences. In a preferred embodiment, said f
antigenic polypeptide fused via an amino acid linker to the N-terminus of said AP205 c
protein dimer and said second antigenic polypeptide fused via an amino acid linker to the
terminus of said AP205 coat protein dimer, wherein said first antigenic polypeptide and s
second antigenic polypeptide are antigenic polypeptides, and typically and preferably compr
preferably consist of, the different amino acid sequences, wherein said first antige
polypeptide and said second antigenic polypeptide comprise different epitopes of the sa
pathogenic antigenic polypeptide target.

In another preferred embodiment, said amino acid linker is selected from the gr
consisting of:

(a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and
(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein preferably said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ v r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1; and wherein preferably said glycine-se linker has a length of at most 15, further preferably of at most 10, amino acids;
(c.) an amino acid linker comprising at least one Gly, at least one Ser, and at least one am acid selected from Thr, Ala, Lys, Asp and Glu, wherein said amino acid sequence ha length of at most 15 amino acids.

In a preferred embodiment, said amino acid linker has a length of at most 10 amino ac
In a preferred embodiment, said amino acid linker has a length of at most 8 amino acids. I
preferred embodiment, said amino acid linker has a length of at most 5 amino acids. I
preferred embodiment, said amino acid linker has a length of 4 amino acids. In a prefe
embodiment, said amino acid linker has a length of 3 amino acids. In a preferred embodim
said amino acid linker has a length of 2 amino acids. In another preferred embodiment, s
amino acid linker has a length of 1 amino acid.

In a preferred embodiment, said amino acid linker is selected from the group consist of (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ with r=0 or 1, s= w=0 or 1; t=1-3 and u=0 or 1; and wherein preferably said glycine-serine linker has a lengtl at most 15, further preferably of at most 10, amino acids.

In a preferred embodiment, said amino acid linker is selected from the group consist of (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein said GS linker has has a length of at most 15 amino acids.

In a preferred embodiment, said amino acid linker is selected from the group consist of (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein said GS linker has has a length of at most 10 amino acids. In a preferred embodim said amino acid linker is selected from the group consisting of (a.) a polyglycine linker (G of a length of n=2-5; and (b.) a glycine-serine linker (GS-linker) comprising at least one glyc and at least one serine, wherein said GS linker has has a length of at most 8 amino acids. preferred embodiment, said amino acid linker is selected from the group consisting of (a polyglycine linker $(Gly)_n$ of a length of n=2-5; and (b.) a glycine-serine linker (GS-linl comprising at least one glycine and at least one serine, wherein said GS linker has has a len of at most 5 amino acids.

In a preferred embodiment, said amino acid linker is selected from the group consist of (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n=2-5; and a glycine-serine linker (GS-linker) comprising at least one glycine and at least one ser wherein said GS linker has an amino acid sequence of $(GS)_r(G_sS_w)_t(GS)_u$ wherein r, s, t, u a

(i.) r=0, s=1, w=1, t=1, and u=0; (ii.) r=0, s=1, w=1, t=2, and u=0; (iii.) r=0, s=2, w t=1, and u=0; (iv.) r=0, s=3, w=1, t=1, and u=0; (v.) r=0, s=4, w=1, t=1, and u=0; (r=1, s=1, w=0, t=1, u=0; (vii.) r=1, s=2, w=0, t=1, u=0.

In a preferred embodiment, said amino acid linker is a glycine-serine linker (GS-linl comprising at least one glycine and at least one serine, wherein said GS linker has the am acid sequence of GS, corresponding to the amino acid sequence $(GS)_r(G_sS_w)_t(GS)_u$ wherein r s=1, w=1, t=1, and u=0.

In a preferred embodiment, said antigenic polypeptide is fused to the N-terminus anc the C-terminus of said AP205 coat protein dimer either directly or via an amino acid linl

wherein said linker consists of at most five amino acids, preferably of at most four amino ac In a preferred embodiment, said antigenic polypeptide is fused to the N-terminus and/or the terminus of said AP205 coat protein dimer either directly or via an amino acid linker, wher said linker consists of at most four amino acids, preferably of at most two amino acids. I preferred embodiment, said antigenic polypeptide is fused to the N-terminus and/or the terminus of said AP205 coat protein dimer either directly or via an amino acid linker, wher said linker consists of two amino acids, wherein preferably said two amino acid linker is the amino acid glycine-serine linker GS.

In a preferred embodiment, a first antigenic polypeptide is fused to the N-terminus of s AP205 coat protein dimer either directly or via an amino acid linker and a second antige polypeptide is fused to the C-terminus of said AP205 coat protein dimer either directly or an amino acid linker, wherein said linker consists of at most four amino acids, preferably c most two amino acids. In a preferred embodiment, a first antigenic polypeptide is fused to N-terminus of said AP205 coat protein dimer either directly or via an amino acid linker ar second antigenic polypeptide is fused to the C-terminus of said AP205 coat protein dimer eit directly or via an amino acid linker, wherein said linker consists of two amino acids, wher preferably said two amino acid linker is the di-amino acid glycine-serine linker GS. I preferred embodiment, a first antigenic polypeptide is fused to the N-terminus of said AP coat protein dimer directly and a second antigenic polypeptide is fused to the C-terminus said AP205 coat protein dimer directly.

In a preferred embodiment, said antigenic polypeptide is fused to the N-terminus of s AP205 coat protein dimer either directly or via an amino acid linker, wherein said lin consists of at most five amino acids, preferably of at most four amino acids. In a prefer embodiment, said antigenic polypeptide is fused to the N-terminus of said AP205 coat pro dimer either directly or via an amino acid linker, wherein said linker consists of at most f amino acids, preferably of at most two amino acids. In a preferred embodiment, said antige polypeptide is fused to the N-terminus of said AP205 coat protein dimer either directly or an amino acid linker, wherein said linker consists of two amino acids, wherein preferably s two amino acid linker is the di-amino acid glycine-serine linker GS.

In a preferred embodiment, said antigenic polypeptide is fused to the C-terminus of s AP205 coat protein dimer either directly or via an amino acid linker, wherein said lin consists of at most five amino acids, preferably of at most four amino acids. In a prefer embodiment, said antigenic polypeptide is fused to the C-terminus of said AP205 coat pro dimer either directly or via an amino acid linker, wherein said linker consists of at most f

amino acids, preferably of at most two amino acids. In a preferred embodiment, said antige polypeptide is fused to the C-terminus of said AP205 coat protein dimer either directly or an amino acid linker, wherein said linker consists of two amino acids, wherein preferably s two amino acid linker is the di-amino acid glycine-serine linker GS.

In a preferred embodiment, said antigenic polypeptide is fused to the N-terminus of s AP205 coat protein dimer via an amino acid linker, wherein said linker consists of at most f amino acids, preferably of at most four amino acids. In a preferred embodiment, said antige polypeptide is fused to the N-terminus of said AP205 coat protein dimer via an amino a linker, wherein said linker consists of at most four amino acids, preferably of at most two am acids. In a preferred embodiment, said antigenic polypeptide is fused to the N-terminus of s AP205 coat protein dimer via an amino acid linker, wherein said linker consists of two am acids, wherein preferably said two amino acid linker is the di-amino acid glycine-serine lin GS.

In a preferred embodiment, said antigenic polypeptide is fused to the C-terminus of s AP205 coat protein dimer via an amino acid linker, wherein said linker consists of at most f amino acids, preferably of at most four amino acids. In a preferred embodiment, said antige polypeptide is fused to the C-terminus of said AP205 coat protein dimer via an amino a linker, wherein said linker consists of at most four amino acids, preferably of at most two am acids. In a preferred embodiment, said antigenic polypeptide is fused to the C-terminus of s AP205 coat protein dimer via an amino acid linker, wherein said linker consists of two am acids, wherein preferably said two amino acid linker is the di-amino acid glycine-serine lin GS.

In a preferred embodiment, said AP205 coat protein dimer comprises, preferably cons of, the amino sequence of SEQ ID NO:111 or an amino acid sequence having a seque identity of at least 90 %, preferably of at least 95%, further preferably of at least 98% and ag more preferably of at least 99% with said SEQ ID NO:111. In a preferred embodiment, s AP205 coat protein dimer comprises, preferably consists of, the amino sequence of SEQ NO:111 or an amino acid sequence having a sequence identity of at least 95% with said S ID NO:111. In a preferred embodiment, said AP205 coat protein dimer comprises, prefera consists of, the amino sequence of SEQ ID NO:111 or an amino acid sequence havin sequence identity of at least 98% with said SEQ ID NO:111. In a preferred embodiment, s AP205 coat protein dimer comprises, preferably consists of, the amino sequence of SEQ NO:111 or an amino acid sequence having a sequence identity of at least 99% with said S ID NO:111. In a preferred embodiment, said AP205 coat protein dimer comprises, prefera

consists of, the amino sequence of SEQ ID NO:111. In a preferred embodiment, said AP: coat protein dimer consists of the amino sequence of SEQ ID NO:111 or an amino ε sequence having a sequence identity of at least 95% with said SEQ ID NO:111. In a prefeı embodiment, said AP205 coat protein dimer consists of the amino sequence of SEQ ID NO: or an amino acid sequence having a sequence identity of at least 98% with said SEQ ID NO:1 In a preferred embodiment, said AP205 coat protein dimer consists of the amino sequence SEQ ID NO:111 or an amino acid sequence having a sequence identity of at least 99% v said SEQ ID NO:111. In a preferred embodiment, said AP205 coat protein dimer consists the amino sequence of SEQ ID NO:111.

In a preferred embodiment, said AP205 coat protein dimer comprises, preferably cons of, the amino sequence of SEQ ID NO: 7 or an amino acid sequence having a sequence iden of at least 90 %, preferably of at least 95%, further preferably of at least 98% and again m preferably of at least 99% with said SEQ ID NO: 7. In a preferred embodiment, said AP: coat protein dimer comprises, preferably consists of, the amino sequence of SEQ ID NO: ' an amino acid sequence having a sequence identity of at least 95% with said SEQ ID NO: 7 a preferred embodiment, said AP205 coat protein dimer comprises, preferably consists of, amino sequence of SEQ ID NO: 7 or an amino acid sequence having a sequence identity c least 98% with said SEQ ID NO: 7. In a preferred embodiment, said AP205 coat protein diı comprises, preferably consists of, the amino sequence of SEQ ID NO: 7 or an amino ε sequence having a sequence identity of at least 99% with said SEQ ID NO: 7. In a prefeı embodiment, said AP205 coat protein dimer comprises, preferably consists of, the am sequence of SEQ ID NO: 7. In a preferred embodiment, said AP205 coat protein dimer cons of the amino sequence of SEQ ID NO: 7 or an amino acid sequence having a sequence iden of at least 95% with said SEQ ID NO: 7. In a preferred embodiment, said AP205 coat pro dimer consists of the amino sequence of SEQ ID NO: 7 or an amino acid sequence havin sequence identity of at least 98% with said SEQ ID NO: 7. In a preferred embodiment, ş AP205 coat protein dimer consists of the amino sequence of SEQ ID NO: 7 or an amino ε sequence having a sequence identity of at least 99% with said SEQ ID NO: 7. In a prefeı embodiment, said AP205 coat protein dimer consists of the amino sequence of SEQ ID NO

In a preferred embodiment, said modified AP205 VLP consists of said fusion prote Thus, in a preferred embodiment, said modified AP205 VLP consists of about 90, prefera exactly 90, AP205 coat protein dimers to which about 90, preferably exactly 90, antige polypeptide are fused to in accordance with the present invention. Thus, the present invent provides a platform with higher antigen density as compared to, for example, mosaic V

platforms consisting of both native and genetically modified VLP subunits (Pokorski, JK e ChemBioChem 2011, 12, 2441–2447; Lino CA et al., J Nanobiotechnol (2017) 15:13; A KL et al. Viruses 2020, 12, 185).

In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids. In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids and at most 300 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids and at most 300 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids and at most 300 amino ac

In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids. In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids and at most 250 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids and at most 250 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids and at most 250 amino ac

In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids. In a preferred embodiment, said antigenic polypeptide has a length of at least 50 am acids and at most 220 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids. In a preferred embodiment, said antigenic polypeptide a length of at least 60 amino acids and at most 220 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids. In a preferred embodim said antigenic polypeptide has a length of at least 70 amino acids and at most 240 amino ac In a preferred embodiment, said antigenic polypeptide has a length of at least 70 amino ac and at most 230 amino acids. In a preferred embodiment, said antigenic polypeptide ha length of at least 70 amino acids and at most 220 amino acids. In a preferred embodiment, s antigenic polypeptide has a length of at least 70, 75, 80, 85, 90, 95 or 100 amino acids an most 250 amino acids. In a preferred embodiment, said antigenic polypeptide has a length c least 70 amino acids and at most 250, 240, 235, 230, 225 or 220 amino acids. In a prefe embodiment, said antigenic polypeptide has a length of at least 80 amino acids. In a prefe embodiment, said antigenic polypeptide has a length of at least 90 amino acids. In a prefe embodiment, said antigenic polypeptide has a length of at least 100 amino acids.

In another preferred embodiment, a first antigenic polypeptide is fused directly or via

amino acid linker to the N-terminus of said AP205 coat protein dimer and a second antige polypeptide is fused directly or via an amino acid linker to the C-terminus of said AP205 c protein dimer, wherein said first antigenic polypeptide and said second antigenic polypep independently has a length of at least 5, 7, 8, 10 or 12 amino acids. In a preferred embodim wherein said first antigenic polypeptide and said second antigenic polypeptide independe has a length of at least 5, 7, 8, 10 or 12 amino acids and at most 250, 200, or 150 amino ac In a preferred embodiment, wherein said first antigenic polypeptide and said second antige polypeptide independently has a length of at least 5, 7, 8, 10 or 12 amino acids and at most amino acids. In a preferred embodiment, wherein said first antigenic polypeptide and s second antigenic polypeptide independently has a length of at least 5, 7, 8, 10 or 12 amino a and at most 80, 70, 60, 50 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 80 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 70 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 60 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 50 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 40 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 35 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 30 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 25 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 20 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 80 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 70 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas

amino acids and at most 60 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 50 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 40 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 35 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 30 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 25 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 20 amino acids.In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 80 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 70 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 60 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 50 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 40 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 35 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 30 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 25 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at leas amino acids and at most 20 amino acids. In a preferred embodiment, wherein said first antige polypeptide and said second antigenic polypeptide independently has a length of at least 5 8, 10 or 12 amino acids and at most 50, 40, 35, 30, 25 or 20 amino acids.

In a preferred embodiment, said antigenic polypeptide is a polypeptide derived from

group consisting of: (a) allergens; (b) viruses; (c) bacteria; (d) parasites; (e) tumors; (f) s molecules; (g) hormones; (h) growth factors; (i) cytokines; (j) chemokines; and (k) biologic: active peptides. In a preferred embodiment, said antigenic polypeptide is of bacterial, vira mammalian origin. In a preferred embodiment, said antigenic polypeptide is an allerge polypeptide derived from a viral pathogen, a polypeptide derived from a bacterial pathoge tumor antigen, a self antigen, a polypeptide derived from a hormone, a polypeptide deri from a growth factor, a cytokine or a chemokine. In another preferred embodiment, s antigenic polypeptide is an allergen, a self antigen, a tumor antigen, or a polypeptide c pathogen. In a preferred embodiment, said antigenic polypeptide is an allergen, a polypep derived from a viral pathogen, a polypeptide derived from a bacterial pathogen, a self antig a cytokine or a chemokine. In a preferred embodiment, said antigenic polypeptide is an allerg In a preferred embodiment, said antigenic polypeptide is of viral origin. In a prefe embodiment, said antigenic polypeptide is a polypeptide derived from a virus. In a prefe embodiment, said antigenic polypeptide is of bacterial origin. In a preferred embodiment, s antigenic polypeptide is a polypeptide derived from a bacteria. In a preferred embodiment, s antigenic polypeptide is a polypeptide derived from a parasite. In a preferred embodiment, s antigenic polypeptide is a tumor antigen. In a preferred embodiment, said antigenic polypep is a self antigen. In a preferred embodiment, said antigenic polypeptide is a polypeptide deri from a parasite. In a preferred embodiment, said antigenic polypeptide is a hormone. I preferred embodiment, said antigenic polypeptide is a growth factor. In a prefe embodiment, said antigenic polypeptide is cytokine. In a preferred embodiment, said antige polypeptide is chemokine. In a preferred embodiment, said antigenic polypeptide is biologic: active peptide.

In a further preferred embodiment said antigenic polypeptide is an allergen, wherein s allergen is derived from the group consisting of: (a) pollen extract; (b) dust extract; (c) c mite extract; (d) fungal extract; (e) mammalian epidermal extract; (f) feather extract; (g) in: extract; (h) food extract; (i) hair extract; (j) saliva extract; and (k) serum extract. In a furt preferred embodiment said antigenic polypeptide is an allergen, wherein said allerge selected from the group consisting of: (a) trees; (b) grasses; (c) house dust; (d) house dust m (e) aspergillus; (f) animal hair; (g) animal feather; (h) bee venom; (i) animal products; (j) p products; (k) animal dander and (l) peanut allergens.

In a further preferred embodiment said antigenic polypeptide is a recombir polypeptide derived from an allergen selected from the group consisting of: (a) bee ven phospholipase A2; (b) ragweed pollen Amb a 1; (c) birch pollen Bet v I; (d) white faced ho

venom 5 DoI m V; (e) house dust mite Der p 1; (f) house dust mite Der f 2; (g) house dust n Der p 2; (h) dust mite Lep d; (i) fungus allergen Alt a 1; (j) fungus allergen Asp f 1; (k) fun allergen Asp f 16; (l) peanut allergens (m) cat allergen Fel d1; (n) Canine allergens Can f1, (f2 (o) peanut-derived allergens; or (p) Japanese cedar allergen Cry J2.

In a further preferred embodiment said antigenic polypeptide is a recombinant allerg wherein said allergen is selected from the group consisting of: (a) bee venom phospholip A2; (b) ragweed pollen Amb a 1; (c) birch pollen Bet v I; (d) white faced hornet venom 5 l m V; (e) house dust mite Der p 1; (f) house dust mite Der f 2; (g) house dust mite Der p 2; dust mite Lep d; (i) fungus allergen Alt a 1; (j) fungus allergen Asp f 1; (k) fungus allergen . f 16; (l) peanut allergens (m) cat allergen Fel d1; (n) Canine allergens Can f1, Can f2 (o) pea derived allergens; or (p) Japanese cedar allergen Cry J2.

In a further preferred embodiment, said antigenic polypeptide is an allergen derived fi Japanese Cedar Cry J 2. Preferably, said antigenic polypeptide is derived from Japanese Ce Cry J 2 of SEQ ID NO:34. Preferably, said antigenic polypeptide is derived from Japan Cedar Cry J 2 and comprises the amino acid sequence of SEQ ID NO:34.

In a further preferred embodiment, said antigenic polypeptide is an allergen derived fi ragweed pollen Amb a1. Preferably, said antigenic polypeptide is derived from ragweed pol Amb a 1 of SEQ ID NO:35. Preferably, said antigenic polypeptide is derived from ragw pollen Amb a1 and comprises the amino acid sequence of SEQ ID NO:35.

In a further preferred embodiment said antigenic polypeptide is a tumor antigen, whe said tumor antigen is selected from the group consisting of: (a) a polypeptide of breast car cells; (b) a polypeptide of kidney cancer cells; (c) a polypeptide of prostate cancer cells; ((polypeptide of skin cancer cells; (e) a polypeptide of brain cancer cells; and (f) a polypep of leukemia cells.

In a further preferred embodiment said antigenic polypeptide is a tumor antigen selec from the group consisting of: (a) Her2; (b) ganglioside GD2; (c) EGF-R; (d) carcino embryc antigen (CEA); (e) CD52; (f) CD21; (g) human melanoma gp100; (h) human melanc melanA/MART-1; (i) Human melanoma melanA/MART-1 analogue; (j) tyrosinase; (k) NA A nt; (1) MAGE3; (m) p53 protein; and (n) antigenic fragments of any of the tumor antigen (a) to (m).

In a further preferred embodiment said antigenic polypeptide is a polypeptide selec from the group consisting of: (a) IgE, (b) IL-6 (c) receptor activator of nuclear factor kB lig (RANKL); (d) vascular endothelial growth factor (VEGF); (e) vascular endothelial gro factor receptor (VEGF-R); hepatocyte growth factor (HGF) (f) interleukin-1α; (g) interleul

1 β; (h) interleukin-5; (i) interleukin-8; (j) interleukin-13; (k) interleukin-15; (1) interleukin (IL- 17); (m) IL-23; (n) Ghrelin; (o) angiotensin; (p) chemokine (C-C motif) (CCL21); chemokine (C-X motif) (CXCL 12); (r) stromal cell derived factor 1 (SDF-I); (s) macroph colony stimulating factor (M-CSF); (t) monocyte chemotactic protein 1 (MCP-I); (u) endog (v) resistin; (w) gonadotropin releasing hormone (GnRH); (x) growth hormone releas (GHRH); (y) lutenizing hormone releasing hormone (LHRH); (z) thyreotropin releas hormone (TRH); (aa) macrophage migration inhibitory factor (MIF); (bb) glucose-depenc insulinotropic peptide (GIP); (cc) eotaxin; (dd) bradykinin; (ee) Des-Arg bradykinin; (ff) lymphocyte chemoattractant (BLC); (gg) macrophage colony stimulating factor M-CSF; (tumor necrosis factor α (TNFα); (ii) amyloid beta peptide (Aβl-42); (jj) amyloid beta pep (Aβ3-6); (kk) human IgE; (ii) CCR5 extracellular domain; (mm) CXCR4 extracellular dom (nn) Gastrin; (oo) CETP; (pp) C5a; (qq) epidermal growth factor receptor (EGF-R); (rr) CG (ss) α-synuclein; (tt) calcitonin gene-related peptide (CGRP) (uu) Amylin; (vv) myosta (ww) interleukin-4; (xx) thymic stromal lymphopoietin; (yy) interleukin-33; (zz) interleul 25; (aaa) interleukin-13 or (bbb) a fragment of any one of the polypeptides (a) to (aaa); (ccc) an antigenic mutant or fragment of any one of the polypeptides (a) to (aaa).

In a further preferred embodiment said antigenic polypeptide is a self antigen, whe said self antigen is a polypeptide selected from the group consisting of: (a) IgE, (b) IL-6 receptor activator of nuclear factor kB ligand (RANKL); (d) vascular endothelial growth fa (VEGF); (e) vascular endothelial growth factor receptor (VEGF-R); hepatocyte growth fa (HGF) (f) interleukin-1 α; (g) interleukin-1 β; (h) interleukin-5; (i) interleukin-8; (j) i leukin-13; (k) interleukin-15; (1) interleukin-17 (IL- 17); (m) IL-23; (n) Ghrelin; angiotensin; (p) chemokine (C-C motif) (CCL21); (q) chemokine (C-X motif) (CXCL 12) stromal cell derived factor 1 (SDF-I); (s) macrophage colony stimulating factor (M-CSF) monocyte chemotactic protein 1 (MCP-I); (u) endoglin; (v) resistin; (w) gonadotropin releas hormon (GnRH); (x) growth hormon releasing (GHRH); (y) lutenizing hormon releas hormon (LHRH); (z) thyreotropin releasing hormon (TRH); (aa) macrophage migra inhibitory factor (MIF); (bb) glucose-dependent insulinotropic peptide (GIP); (cc) eotaxin; (bradykinin; (ee) Des-Arg bradykinin; (ff) B-lymphocyte chemoattractant (BLC); (macrophage colony stimulating factor M-CSF; (hh) tumor necrosis factor α (TNFα); amyloid beta peptide (Aβ1-42); (jj) amyloid beta peptide (Aβ3-6); (kk) human IgE; (ii) CC extracellular domain; (mm) CXCR4 extracellular domain; (nn) Gastrin; (oo) CETP; (pp) C (qq) epidermal growth factor receptor (EGF-R); (rr) CGRP; (ss) α-synuclein; (tt) calcitc

gene-related peptide (CGRP) (uu) Amylin; (vv) myostatin; (ww) interleukin-4; (xx) thy
stromal lymphopoietin; (yy) interleukin-33; (zz) interleukin-25; (aaa) interleukin-13 or (b
a fragment of any one of the polypeptides (a) to (aaa); and (ccc) an antigenic mutant or fragm
of any one of the polypeptides (a) to (aaa).

In a preferred embodiment, said antigenic polypeptide is interleukin 17 (IL-
preferably human IL-17. Interleukin 17 is a T cell-derived cytokine that induces the releas
pro-inflammatory mediators in a wide range of cell types. Aberrant Th17 responses
overexpression of IL-17 have been implicated in a number of autoimmune disorders includ
rheumatoid arthritis psoriasis, ankylosing spondylitis, and multiple sclerosis. Molec
blocking IL-17 such as IL-17-specific monoclonal antibodies have proved to be effectiv
ameliorating disease in animal models. Moreover, active immunization targeting IL-17
recently been suggested using virus-like particles conjugated with recombinant IL-17 (R
TA, et al., Eur J Immunol (2006) 36: 1-11). Immunization with IL-17-VLP induced high le
of anti-IL-17 antibodies thereby overcoming natural tolerance, even in the absence of ad
adjuvant. Mice immunized with IL-17-VLP had lower incidence of disease, slower progress
to disease and reduced scores of disease severity in both collagen-induced arthritis
experimental autoimmune encephalomyelitis.

Thus, in a preferred embodiment, said antigenic polypeptide comprises, or prefera
consists of SEQ ID NO:36. Furthermore, the inventive modified AP205 VLPs are used i
method of treating an inflammatory disease, preferably a chronic inflammatory disease in
animal or human. Preferably, said inflammatory disease is selected from RA, MS, Psoria
asthma, Crohns, Colitis, COPD, diabetes, neurodermatitis (allergic dermatitis), again prefera
wherein said inflammatory disease is MS, and wherein further preferably said antige
polypeptide comprises, or preferably consists of SEQ ID NO:82.

In another preferred embodiment, said antigenic polypeptide is IL-5, preferably hum
canine, feline or horse IL-5. In another preferred embodiment, said antigenic polypeptid
human IL-5. In again a further preferred embodiment, said antigenic polypeptide comprises
preferably consists of SEQ ID NO:37. Furthermore, the inventive modified AP205 V
comprising antigenic polypeptides of IL-5 are used in a method of treating an inflammat
disease, preferably a chronic inflammatory disease in an animal or human. Preferably, s
inflammatory disease is selected from RA, MS, Psoriasis, asthma, Crohns, Colitis, CO
diabetes, neurodermatitis (allergic dermatitis), eosinophilic granulomatosis, feline atopic s
syndrome and insect bite hypersensitivity. In another preferred embodiment, said antige
polypeptide comprises, or preferably consists of SEQ ID NO:37.

In another preferred embodiment, said antigenic polypeptide is canine IL-5. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:38 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:38. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:38. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:38.

In another preferred embodiment, said antigenic polypeptide is feline IL-5. In a prefer embodiment, said antigenic polypeptide comprises, or preferably consists of, SEQ ID NO: SEQ ID NO:40, SEQ ID:41 or an amino acid sequence having a sequence identity of at le 90%, preferably of at least 92 %, further preferably of at least 95%, and again further prefera of at least 98% amino acid sequence identity with SEQ ID NO:39, SEQ ID NO:40, SEQ ID In a further preferred embodiment, said antigenic polypeptide comprises SEQ ID NO:39, S ID NO:40 or SEQ ID:41. In a further preferred embodiment, said antigenic polypeptide cons of SEQ ID NO:39, SEQ ID NO:40 or SEQ ID:41. In a further preferred embodiment, s antigenic polypeptide comprises, or preferably consists of, SEQ ID NO:39 or an amino a sequence having a sequence identity of at least 90%, preferably of at least 92 %, furt preferably of at least 95%, and again further preferably of at least 98% amino acid seque identity with SEQ ID NO:39.

In another preferred embodiment, said antigenic polypeptide is equine IL-5. In a prefer embodiment, said antigenic polypeptide comprises, or preferably consists of, SEQ ID NO or an amino acid sequence having a sequence identity of at least 90%, preferably of at least %, further preferably of at least 95%, and again further preferably of at least 98% amino a sequence identity with SEQ ID NO:42.

In another preferred embodiment, said antigenic polypeptide is IL-4, preferably hur Il-4. In again a further preferred embodiment, said antigenic polypeptide comprises, preferably consists of SEQ ID NO:43. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:43.

In another preferred embodiment, said antigenic polypeptide is canine IL-4. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:44 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:44. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:44. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:44.

In another preferred embodiment, said antigenic polypeptide is feline IL-4. In agai

further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:45 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:45. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:45. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:45. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:45.

In another preferred embodiment, said antigenic polypeptide is equine IL-4.

In another preferred embodiment, said antigenic polypeptide is IL-13, preferably hur IL-13. Furthermore, the inventive modified AP205 VLPs comprising antigenic polypeptide IL-13 are used in a method of treating an inflammatory disease, preferably an alle inflammation, allergic lung disease, asthma or atopic dermatitis. In again a further prefe embodiment, said antigenic polypeptide comprises, or preferably consists of SEQ ID NO In another preferred embodiment, said antigenic polypeptide consists of SEQ ID NO:46.

In another preferred embodiment, said antigenic polypeptide is canine IL-13. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:47 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:47. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:47. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:47.

In another preferred embodiment, said antigenic polypeptide is feline IL-13. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:48 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:48. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:48. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:48.

In another preferred embodiment, said antigenic polypeptide is equine IL-13. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:49 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:49. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:49. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:49.

In a further preferred embodiment, said antigenic polypeptide is TNFα. Furthermore, inventive modified AP205 VLPs comprising antigenic polypeptides of TNFα are used i

method of treating an inflammatory disease, preferably multisystem inflammatory disea rheumatoid arthritis, inflammatory bowel disease, Crohn’s disease, ulcerative colitis, psoria psoriatic arthritis, juvenile idiopathic arthritis or ankylosing spondylitis. In another prefe embodiment, said antigenic polypeptide is IL-1α, preferably human IL-1α. In again a furt preferred embodiment, said antigenic polypeptide comprises, or preferably consists of SEQ NO:50. In another preferred embodiment, said antigenic polypeptide consists of SEQ NO:50.

In another preferred embodiment, said antigenic polypeptide is canine IL-1α. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:51 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:51. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:51. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:51.

In another preferred embodiment, said antigenic polypeptide is feline IL-1α. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:52 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:52. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:52. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:52.

In another preferred embodiment, said antigenic polypeptide is equine IL-1α. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:53 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:53. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:53. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:53.

In another very preferred embodiment, said antigenic polypeptide is IL-33, prefera human IL-33. Furthermore, the inventive modified AP205 VLPs comprising antige polypeptides of IL-33 are used in a method of treating an inflammatory disease, prefera atopic dermatitis, asthma, a cardiovascular disease, a musculoskeletal disease, inflammat bowel disease, or an allergy such as food allergy, or cancer or Alzheimer disease. In agai further very preferred embodiment, said antigenic polypeptide comprises, or preferably cons of SEQ ID NO:54. In another preferred embodiment, said antigenic polypeptide consist SEQ ID NO:54.

Canine atopic dermatitis is a form of inflammation of skin, causing itch, which prom dogs to scratch extensively and eventually, loose their fur around scratched places. Sev interleukins appear to be involved in the driving of itching, including canine interleukins c 31 and cIL-33. It has been shown previously that vaccination of dogs with the inven modified VLPs, decorated with cIL-31 raises autoantibodies and reduces itching. Sim effects are plausible when the inventive modified VLPs decorated with cIL-33 are usec evidenced by the data of Example 3.

Thus, in another very preferred embodiment, said antigenic polypeptide is canine IL In again a further very preferred embodiment, said antigenic polypeptide comprises, preferably consists of any one of SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:112 or SEQ NO:113, or an amino acid sequence having a sequence identity of at least 90%, preferably c least 95%, with any one of SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:112 or SEQ NO:113. Preferably, said antigenic polypeptide comprises, or preferably consists of any on SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:112 or SEQ ID NO:113. In another prefe embodiment, said antigenic polypeptide consists any one of SEQ ID NO:55, SEQ ID NO SEQ ID NO:112 or SEQ ID NO:113. In again a further very preferred embodiment, s antigenic polypeptide comprises, or preferably consists of SEQ ID NO:56 or an amino a sequence having a sequence identity of at least 90%, preferably of at least 95%, with SEQ NO:56. Preferably, said antigenic polypeptide comprises, or preferably consists of SEQ NO:56. In another preferred embodiment, said antigenic polypeptide consists of SEQ NO:56. In again a further very preferred embodiment, said antigenic polypeptide comprises preferably consists of SEQ ID NO:113 or an amino acid sequence having a sequence iden of at least 90%, preferably of at least 95%, with SEQ ID NO:113. Preferably, said antige polypeptide comprises, or preferably consists of SEQ ID NO:113. In another prefe embodiment, said antigenic polypeptide consists of SEQ ID NO:113.

In another very preferred embodiment, said antigenic polypeptide is feline IL-33. In ag a further very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:57 or an amino acid sequence having a sequence identity of at l 90%, preferably of at least 95%, with SEQ ID NO:57. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:57. In another preferred embodiment, s antigenic polypeptide consists of SEQ ID NO:57.

In another very preferred embodiment, said antigenic polypeptide is equine IL-33 again a further very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:58 or an amino acid sequence having a sequence identity of at l

90%, preferably of at least 95%, with SEQ ID NO:58. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:58. In another preferred embodiment, s antigenic polypeptide consists of SEQ ID NO:58.

In another preferred embodiment, said antigenic polypeptide is IL-25, preferably hur IL-25. In again a further preferred embodiment, said antigenic polypeptide comprises, preferably consists of SEQ ID NO:59. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:59.

In another preferred embodiment, said antigenic polypeptide is canine IL-25. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:60 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:60. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:60. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:60.

In another preferred embodiment, said antigenic polypeptide is feline IL-25. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:61 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:61. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:61. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:61.

In another preferred embodiment, said antigenic polypeptide is equine IL-25. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:62 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:62. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:62. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:62.

In a further preferred embodiment, said antigenic polypeptide is IL-1β, preferably hur IL-1β. Furthermore, the inventive modified AP205 VLPs comprising antigenic polypepti of IL-1β are used in a method of treating an inflammatory disease, preferably multisys inflammatory diseases associated with inflammasome dysregulation including osteoarthr juvenile idiopathic arthritis, Familial Mediterranean Fever, cryopyrin associated peric syndrome, Muckle-Wells Syndrome, hyperimmunoglobulin D syndrome, Stills disease, gc arthritis, rheumatoid arthritis, chronic obstructive pulmonary disease and coronary art disease. In again a further preferred embodiment, said antigenic polypeptide comprises,

preferably consists of SEQ ID NO:63.

In a further preferred embodiment, said antigenic polypeptide is canine IL-1β. I preferred embodiment, said antigenic polypeptide comprises, or preferably consists of, SEQ NO:64, SEQ ID NO:65, SEQ ID NO:66 or an amino acid sequence having a sequence iden of at least 90%, preferably of at least 92 %, further preferably of at least 95%, and again furt preferably of at least 98% amino acid sequence identity with SEQ ID NO:64, SEQ ID NO: SEQ ID NO:66. In a further preferred embodiment, said antigenic polypeptide comprises S ID NO:64, SEQ ID NO:65, SEQ ID NO:66. In a further preferred embodiment, said antige polypeptide consists of SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66. In a further prefe embodiment, said antigenic polypeptide comprises, or preferably consists of, SEQ ID NO or an amino acid sequence having a sequence identity of at least 90%, preferably of at least %, further preferably of at least 95%, and again further preferably of at least 98% amino a sequence identity with SEQ ID NO:64.

In a further preferred embodiment, said antigenic polypeptide is feline IL-1β. In aga further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:67.

In a further preferred embodiment, said antigenic polypeptide is IL-12/23, prefera human IL-12/23. In a further preferred embodiment, said antigenic polypeptide is canine 12/23. In a further preferred embodiment, said antigenic polypeptide is feline IL-12/23. I further preferred embodiment, said antigenic polypeptide is equine IL-12/23.

In another preferred embodiment, said antigenic polypeptide is IL-31, preferably hur IL-31. Furthermore, the inventive modified AP205 VLPs comprising antigenic polypeptide IL-31 are used in a method of treating an inflammatory disease, preferably atopic dermat bullous pemphigoid, chronic urticaria or asthma. In again a further preferred embodiment, s antigenic polypeptide comprises, or preferably consists of SEQ ID NO:68. In another prefe embodiment, said antigenic polypeptide consists of SEQ ID NO:68.

In another preferred embodiment, said antigenic polypeptide is canine IL-31. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist SEQ ID NO:69 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:69. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:69. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:69.

In another preferred embodiment, said antigenic polypeptide is feline IL-31. In agai

further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:70 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:70. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:70. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:70.

In another preferred embodiment, said antigenic polypeptide is equine IL-31. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:71 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:71. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:71. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:71.

In another preferred embodiment, said antigenic polypeptide is thymic stroı lymphopoietin (TLSP), preferably human thymic stromal lymphopoietin (TLSP). In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:72. In another preferred embodiment, said antigenic polypeptide consists of S ID NO:72.

In another preferred embodiment, said antigenic polypeptide is canine TLSP. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:73 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:73. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:73. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:73.

In another preferred embodiment, said antigenic polypeptide is feline TLSP. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:74 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:74. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:74. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:74.

In another preferred embodiment, said antigenic polypeptide is equine TLSP. In agai further preferred embodiment, said antigenic polypeptide comprises, or preferably consist: SEQ ID NO:75 or an amino acid sequence having a sequence identity of at least 90%, prefera of at least 95%, with SEQ ID NO:75. Preferably, said antigenic polypeptide comprises preferably consists of SEQ ID NO:75. In another preferred embodiment, said antige polypeptide consists of SEQ ID NO:75.

In again a further preferred embodiment, said antigenic polypeptide is IgE or a peptid domain comprised in IgE.

In again a further preferred embodiment, said antigenic polypeptide is a peptide deri the N-terminus from Aβ-1-42 (SEQ ID NO:76), in particular a fragment of Aβ-1-42 (SEQ NO: 76) of at most 7 consecutive amino acids in length, preferably a fragment of Aβ-1-42 (S ID NO: 76) of at most 6 consecutive amino acids in length. Thus, in a further prefe embodiment, said antigenic polypeptide is selected from Aβ-1-6 (SEQ ID NO:77), Aβ- (SEQ ID NO:78), Aβ-3-6 (SEQ ID NO:79), Aβ-1-5 (SEQ ID NO:80), Aβ-2-6 (SEQ ID NO:8 or Aβ-3-7 (SEQ ID NO:82).

In another preferred embodiment, said antigenic polypeptide is α-synuclein or a pep derived from α-synuclein, and wherein preferably said peptide consists of 6 to 14 amino ac and wherein further preferably said antigenic polypeptide is a peptide derived from α-synuc selected from any one of SEQ D NO:83, SEQ ID NO:84, SEQ ID NO:85 and SEQ ID NO Further preferred peptides derived from α-synuclein are disclosed in WO 2011/020133, wh is incorporated herein by way of reference.

Alpha-synuclein (α-Syn), a small protein with multiple physiological and patholog functions, is one of the dominant proteins found in Lewy Bodies, a pathological hallmark Lewy body disorders, including Parkinson's disease (PD). More recently, α-Syn has been fo in body fluids, including blood and cerebrospinal fluid, and is likely produced by b peripheral tissues and the central nervous system. Exchange of α-Syn between the brain peripheral tissues could have important pathophysiologic and therapeutic implications (Ga SJ et al., PLoS ONE (2013) 8(8): e71634). The evidence implicating alpha-synuclein (a-s in the pathogenesis of Parkinson's Disease (PD) is overwhelming.

Thus, in a further preferred embodiment, said antigenic polypeptide is selected from one of the sequences selected from SEQ D NO:83, SEQ ID NO:84, SEQ ID NO:85 and S ID NO:86. In a further preferred embodiment, said antigenic polypeptide is SEQ D NO:83 a further preferred embodiment, said antigenic polypeptide is SEQ D NO:84. In a fur preferred embodiment, said antigenic polypeptide is SEQ D NO:85. In a further prefe embodiment, said antigenic polypeptide is SEQ D NO:86.

In another preferred embodiment, a first antigenic polypeptide is fused directly or via amino acid linker to the N-terminus of said AP205 coat protein dimer and a second antige polypeptide is fused directly or via an amino acid linker to the C-terminus of said AP205 c protein dimer, wherein first antigenic polypeptide and said second antigenic polypeptid

independently selected from any one of the SEQ ID NO:77 to SEQ ID NO:86.

In again a further preferred embodiment, said antigenic polypeptide is Amylin.

In a further preferred embodiment, said antigenic polypeptide is derived from Afri Swine Fever (ASF) protein. In a preferred embodiment, said antigenic polypeptide compri preferably is, SEQ ID NO:102. In another preferred embodiment, a first antigenic polypep is fused directly or via an amino acid linker to the N-terminus of said AP205 coat protein di and a second antigenic polypeptide is fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer, wherein first antigenic polypeptide and said sec antigenic polypeptide is derived from African Swine Fever (ASF) protein. In another prefe embodiment, a first antigenic polypeptide is fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fused dire or via an amino acid linker to the C-terminus of said AP205 coat protein dimer, wherein f antigenic polypeptide and said second antigenic polypeptide is SEQ ID NO:102. This modi AP205 VLP comprising antigenic polypeptides derived from African Swine Fever (A protein can be used to address African Swine Fever infections.

In a further preferred embodiment, said antigenic polypeptide is Gonadotropin Releas Hormone (GnRH). In one preferred embodiment, the antigenic polypeptide is GnRH c fragment thereof. Such fragments useful in the production of modified AP205 VLPs vaccines in accordance with the present invention are disclosed in WO2006/027300, whic incorporated herein by reference in its entirety. In a preferred embodiment, said antige polypeptide comprises, preferably is, SEQ ID NO:114 or SEQ ID NO:115. In another prefe embodiment, a first antigenic polypeptide is fused directly or via an amino acid linker to the terminus of said AP205 coat protein dimer and a second antigenic polypeptide is fused dire or via an amino acid linker to the C-terminus of said AP205 coat protein dimer, wherein f antigenic polypeptide and said second antigenic polypeptide is GnRH or a fragment thereof another preferred embodiment, a first antigenic polypeptide is fused directly or via an am acid linker to the N-terminus of said AP205 coat protein dimer and a second antige polypeptide is fused directly or via an amino acid linker to the C-terminus of said AP205 c protein dimer, wherein first antigenic polypeptide and said second antigenic polypeptid independently selected from SEQ ID NO:114 or SEQ ID NO:115. In a further prefe embodiment, the N-terminal glutamic acid of SEQ ID NO:114 is a pyroglutamic acid (pGl pE).

This modified AP205 VLP comprising antigenic polypeptides derived from GnRH be used to address boar taint, fertility and behavior management. Thus, this modified AP

VLP comprising antigenic polypeptides derived from GnRH can be administered to a mamr such as pig to prevent the boar taint in the meat. This modified AP205 VLP comprising Gn can be administered to an animal, such as dog, cat, sheep, cattle, horse to control their behavi and/or to reduce their reproductivity. This modified 205 VLP comprising GnRH can administered to human having gonadal steroid hormone dependent cancers. Moreover, modified 205 VLP comprising GnRH can be administered to an animal or human to lo steroid hormone, preferably testosterone, levels in an animal or human.

In a preferred embodiment, said antigenic polypeptide is angiotensin I or a pep derived from angiotensin I. In another preferred embodiment, said antigenic polypeptid angiotensin II or a peptide derived from angiotensin II.

Modified AP205 VLP comprising angiotensin derived antigenic polypeptides are us for the treatment of diseases or disorders associated with the renin-activated angiotensin syst and in particular for the treatment of diseases selected from the group consisting of hypertens and high blood pressure, stroke, infarction, congestive heart failure, kidney failure, prefera cat chronic kidney disease, and retinal hemorrhage. Such angiotensin derived antige polypeptides are disclosed in WO03031466, which is incorporated herein by reference in entirety. In a preferred embodiment, said antigenic polypeptide comprises, preferably is, S ID NO:116, SEQ ID NO:117 or SEQ ID NO:118.

In another preferred embodiment, a first antigenic polypeptide is fused directly or via amino acid linker to the N-terminus of said AP205 coat protein dimer and a second antige polypeptide is fused directly or via an amino acid linker to the C-terminus of said AP205 c protein dimer, wherein first antigenic polypeptide and said second antigenic polypeptide is angiotensin derived antigenic polypeptide. In another preferred embodiment, a first antige polypeptide is fused directly or via an amino acid linker to the N-terminus of said AP205 c protein dimer and a second antigenic polypeptide is fused directly or via an amino acid lin to the C-terminus of said AP205 coat protein dimer, wherein first antigenic polypeptide said second antigenic polypeptide is independently selected from SEQ ID NO:116, SEQ NO:117 or SEQ ID NO:118.

In a further preferred embodiment, said antigenic polypeptide is eotaxin.

In another preferred embodiment, said antigenic polypeptide is myostatin, preferably c myostatin. In again a further preferred embodiment, said antigenic polypeptide comprises preferably consists of SEQ ID NO:87 or an amino acid sequence having a sequence identity at least 90%, preferably of at least 95%, with SEQ ID NO:87. Preferably, said antige polypeptide comprises, or preferably consists of SEQ ID NO:87. In another prefer

embodiment, said antigenic polypeptide consists of SEQ ID NO:87.

In a further preferred embodiment said antigenic polypeptide is a polypeptide c parasite, wherein preferably said pathogen is selected from the group consisting of: Toxoplasma spp.; (b) Plasmodium falciparum; (c) Plasmodium vivax; (d) Plasmodium ov (e) Plasmodium malariae; (f) Leishmania; (g) Schistosoma and (h) Nematodes. Preferably, s antigenic polypeptide is derived from Plasmodium falciparum or Plasmodium Vivax (SEQ NO: 88).

In a further preferred embodiment, said antigenic polypeptide is a polypeptide c bacterium, wherein preferably said bacterium is selected from the group consisting of: Chlamydia (b) Streptococcus; (c) Pneumococcus; (d) Staphylococcus; (e) Salmonella; Mycobacteria; (g) Clostridia (h) Vibrio (i) Yersinia (k) Meningococcus (l) Borrelia.

Lyme disease is the most prevalent tick-born disease in Europe and North America, v about 400,000 registered cases annually. Disease may have different complications – pai joints, neurological disorders, symptoms like multiple sclerosis and arthritis. Although disease can be cured with antibiotics, symptoms may persist for years even after antibi treatment. Currently, no vaccine against Lyme disease is available in the market. In 19 SmithKline Beecham Biologicals (now part of GlaxoSmithKline) developed LYMErix a Lyme vaccine, but it was removed from the market due to complaints about side-effects multiple lawsuit cases. Therefore, at a global scale there is a need for a new, efficient and s anti-Lyme vaccine. *Borrelia* genus bacteria, which cause Lyme disease, have many diffe proteins, located on their surface, creating an immune response against which may kill pathogen. This approach was used in the Lymerix vaccine, which consisted of outer surf protein OspA. Since then, several other surface proteins of *Borrelia burgdorferi* have been t as vaccine candidates, but none of them have reached the market so far. Borrelia spe produce a number of surface proteins, which help to evade the destruction of bacteria by complement system of the host. So-called CRASPs (complement regulator-acquiring prote are able bind complement regulator factor H (CFH) and CFH-like protein-1 (CFHL-1), wh both inhibit complement activation and formation of membrane attack complex. CspZ is on CRASPs, being able to bind both CFH and CFHL-1. Therefore, anti-CspZ antibodies wo not only mark the surface of bacteria for attack of the immune system, but also reduce the abi of bacteria to avoid the complement.

Thus, in another preferred embodiment, said antigenic polypeptide is CspZ protein fr *Borrelia burgdorferi*. In a very preferred embodiment, said antigenic polypeptide comprises preferably consists of SEQ ID NO:89 or an amino acid sequence having a sequence identity

at least 90%, preferably of at least 95%, with SEQ ID NO:89. Preferably, said antige polypeptide comprises, or preferably consists of SEQ ID NO:89. In another very prefe embodiment, said antigenic polypeptide consists of SEQ ID NO:89.

In a very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:90 or an amino acid sequence having a sequence identity of at l 90%, preferably of at least 95%, with SEQ ID NO:90. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:90. In another very preferred embodim said antigenic polypeptide consists of SEQ ID NO:90.

As a consequence, the inventive modified virus-like particle of RNA bacterioph AP205 comprising CspZ protein as the antigenic polypeptide can be useful as vaccine protecting from Lyme borreliosis.

In a further preferred embodiment said antigenic polypeptide is a viral antigen, whe preferably said viral antigen is a polypeptide derived from a virus selected from the gr consisting of: (a) Retrovirus, preferably HIV; (b) Influenza virus, preferably influenza A extracellular domain or HA or HA globular domain; (c) a polypeptide of Hepatitis B vi preferably preSl; (d) Hepatitis C virus; (e) HPV, preferably HPV16E7; (f) RSV; Coronavirus, preferably SARS-CoV-1, SARS-CoV-2, MERS, further preferably SARS-C 2; (h) Flavivirus, preferably Dengue virus, Zika Virus, West Nile Virus and Hand Foot Mouth Disease Virus, and further preferably ectodomain III (ED3) from E protein of Den fever virus serotype 1; (i) Alphavirus, preferably Chikungunya; (k) Herpesvirus, prefera CMV; (l) Rotavirus. In a further preferred embodiment, said antigenic polypeptide is derived from RSV.

In a further very preferred embodiment, said antigenic polypeptide is the derived f Dengue virus. Dengue fever is a vector-borne tropical disease, caused by Dengue fever vi Each year about 390 million cases occur worldwide. Symptoms include fever, heada vomiting, pain in joints and muscle and characteristic skin rash. In rare cases illness progre to Dengue haemorrhagic fever, which is a life threatening condition, causing around 40, deaths worldwide annually. The first and only dengue vaccine that successfully compl clinical development has been withdrawn from the market in many countries due to sa concerns. Therefore, there still is a need for a safe dengue vaccine.

Envelope (E) protein is found on the surface of mature dengue virus particles and i composed of three ectodomains EDI, EDII, EDIII (ED3) and a transmembrane region. It been shown previously that ED3 alone results in production of high levels of EDIII-spec neutralizing antibodies. Therefore, ED3 could be used in fusion with the tandem dimers lead

to the inventive modified VLPs as an efficient vaccine.

Thus, in another preferred embodiment, said antigenic polypeptide is derived, prefera is, from ectodomain III (ED3) from E protein of Dengue fever virus. In another prefe embodiment, said antigenic polypeptide is derived from ectodomain III (ED3) from E pro of Dengue fever virus serotype 1. Thus, in another preferred embodiment, said antige polypeptide is ectodomain III (ED3) from E protein of Dengue fever virus serotype 1. In a v preferred embodiment, said antigenic polypeptide comprises, or preferably consists of SEQ NO:91 or an amino acid sequence having a sequence identity of at least 90%, preferably o least 95%, with SEQ ID NO:91. Preferably, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:91. In another very preferred embodiment, said antigenic polypep consists of SEQ ID NO:91.

In a very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:92 or an amino acid sequence having a sequence identity of at l 90%, preferably of at least 95%, with SEQ ID NO:92. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:92. In another very preferred embodim said antigenic polypeptide consists of SEQ ID NO:92.

In a further preferred embodiment, said antigenic polypeptide comprises, or prefera consists of, position 9 to 99, position 9 to 109 or position 9 to 112 of SEQ ID NO:92 o amino acid sequence having a sequence identity of at least 90%, preferably of at least 9 with SEQ ID NO:92. Preferably, said antigenic polypeptide comprises, or preferably cons of, position 9 to 99, position 9 to 109 or position 9 to 112 of SEQ ID NO:92. In another v preferred embodiment, said antigenic polypeptide consists of position 9 to 99, position 9 to or position 9 to 112 of SEQ ID NO:92.

In a preferred embodiment, said antigenic polypeptide is the extracellular domai Influenza A virus M2 protein, or an antigenic fragment thereof. In a preferred embodiment s antigenic polypeptide comprises or preferably consists of the extracellular domain of Influenza A virus M2 protein, wherein preferably said extracellular domain of the Influenz virus M2 protein is SEQ ID NO:93. In another preferred embodiment, said antige polypeptide is the globular domain of Influenza virus. In another preferred embodiment, s antigenic polypeptide comprises the protease cleavage site of HA Influenza virus.

In a preferred embodiment, said antigenic polypeptide is a receptor binding dom (RBD) of a coronavirus (CoV), or a fragment thereof. In another preferred embodiment, s antigenic polypeptide is the receptor binding domain (RBD), preferably the receptor bind motif (RBM), of a spike (S) protein of a human coronavirus (HCoV), or a fragment ther

wherein said HCoV is selected from SARS-CoV-2, SARS-CoV, MERS-CoV, HCoV-22 HCoV-NL63, HCoV-OC43 and HCoV-HKU1, preferably from SARS-CoV-2, SARS-CoV MERS-CoV, and again further preferably from SARS-CoV-2.

In a very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of the amino acid sequence selected from SEQ ID NO:94, SEQ ID NO:95, SEQ NO:96, SEQ ID NO:97, and an amino acid sequence having a sequence identity of at least %, preferably of at least 90%, further preferably of at least 95% with any of SEQ ID NO: SEQ ID NO:95, SEQ ID NO:96 and SEQ ID NO:97.

In a very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:94 or an amino acid sequence having a sequence identity of at l 90%, preferably of at least 95%, with SEQ ID NO:94. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:94. In another very preferred embodim said antigenic polypeptide consists of SEQ ID NO:94.

In a very preferred embodiment, said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:95 or an amino acid sequence having a sequence identity of at l 90%, preferably of at least 95%, with SEQ ID NO:95. Preferably, said antigenic polypep comprises, or preferably consists of SEQ ID NO:95. In another very preferred embodim said antigenic polypeptide consists of SEQ ID NO:95.

In a further very preferred embodiment, said fusion protein is selected from the gr consisting of SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO:21, SEQ ID NO:24, SEQ NO:101, SEQ ID NO:106 and SEQ ID NO:110.

In a further aspect the invention provides the modified AP205 virus-like particle of invention for use as a medicament.

In a further aspect the invention provides a vaccine comprising or alternatively consist of the modified AP205 virus-like particle of the invention. Encompassed are vaccines whe said modified AP205 VLPs comprise any one of the technical features disclosed herein, eit alone or in any possible combination. In one embodiment the vaccine further comprises adjuvant. In a further embodiment the vaccine is devoid of an adjuvant. In a prefe embodiment said vaccine comprises an effective amount of the composition of the inventic

In a further aspect, the invention relates to a pharmaceutical composition comprising: a modified AP205 VLP of the invention or a vaccine of the invention; and (b pharmaceutically acceptable carrier, diluent and/or excipient. Said diluent includes ste aqueous (e.g., physiological saline) or non-aqueous solutions and suspensions. Examples non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as o

oil, and injectable organic esters such as ethyl oleate. Carriers or occlusive dressings can used to increase skin permeability and enhance antigen absorption. Pharmaceut compositions of the invention may be in a form which contain salts, buffers, adjuvants, or ot substances which are desirable for improving the efficacy of the conjugate. Examples materials suitable for use in preparation of pharmaceutical compositions are provided numerous sources including Remington's Pharmaceutical Sciences (Osol, A, ed., M Publishing Co., (1990)). In one embodiment said pharmaceutical composition comprises effective amount of the vaccine of the invention.

A further aspect of the invention is a method of immunization an animal or a hur comprising administering a modified AP205 VLP of the invention, a vaccine of the inventi or a pharmaceutical composition of the invention to said animal or human. In a prefer embodiment said method comprises administering a modified AP205 VLP of the inventio said animal or human. A further aspect of the invention is a method of immunization an ani or a human comprising administering a modified AP205 VLP of the invention to said ani or human. A further aspect of the invention is a method of immunization an animal compris administering a modified AP205 VLP of the invention to said animal. A further aspect of invention is a method of immunization a human comprising administering a modified AP VLP of the invention to said human.

A further aspect of the invention is the use of the modified AP205 VLP of the inventi the vaccine of the invention, or the pharmaceutical composition of the invention in manufacture of a medicament for the treatment or prevention of a disease or a disorder in animal or in a human. A further aspect of the invention is an use of the modified AP205 V of the invention in the manufacture of a medicament for the treatment or prevention of a dise or a disorder in an animal. A further aspect of the invention is an use of the modified AP VLP of the invention in the manufacture of a medicament for the treatment or prevention (disease or a disorder in a human.

A further aspect of the invention is a method of treating or preventing a disease (disorder in an animal said method comprising administering a modified AP205 VLP of invention, a vaccine of the invention, or a pharmaceutical composition of the invention to s animal, wherein preferably said animal can be a human. In a further preferred embodiment s modified AP205 VLP, said vaccine, or said pharmaceutical composition is administered to s animal subcutaneously, intravenously, intradermally, intranasally, orally, intranodal transdermally.

A further aspect of the invention is a method of treating or preventing a disease (

disorder in an animal said method comprising administering a modified AP205 VLP of invention to said animal, wherein preferably said animal can be a human. In a further prefei embodiment said modified AP205 VLP is administered to said animal subcutaneou intravenously, intradermally, intranasally, orally, intranodal or transdermally. A further ası of the invention is a method of treating or preventing a disease or a disorder in a human ı method comprising administering a modified AP205 VLP of the invention to said human. further preferred embodiment said modified AP205 VLP is administered to said hur subcutaneously, intravenously, intradermally, intranasally, orally, intranodal or transderma

In a further very preferred embodiment, said disease or disorder is selected from the gr consisting of an allergy, a cancer, an autoimmune disease, an inflammatory disease, infectious disease.

In a further very preferred embodiment, said disease or disorder is selected from the gr consisting of RA, MS, Psoriasis, asthma, Crohns, Colitis, COPD, diabetes, neuroderma (allergic dermatitis), Alzheimer`s disease, Parkinson`s disease, influenza A virus infecti Dengue virus infection, corona virus infection, preferably SARS-CoV2 infection, Afri Swine Fever Virus infection, Lyme disease preferably Lyme borreliosis, malaria, R infection.

In a further very preferred embodiment, said disease or disorder is an inflammat disease. In a further very preferred embodiment, said disease or disorder is an inflammat disease selected from RA, MS, Psoriasis, asthma, Crohns, Colitis, COPD, diabe neurodermatitis (allergic dermatitis).

In a further very preferred embodiment, said disease or disorder is an infectious dise In a further very preferred embodiment, said disease or disorder is an infectious disease selec from influenza A virus infection, Dengue virus infection, African Swine Fever Virus infecti SARS-CoV2 infection, malaria, RSV infection. In a further very preferred embodiment, ı disease or disorder is a Dengue virus infection, a corona virus infection, preferably a SA CoV2 infection, Lyme disease, preferably Lyme borreliosis and atopic dermatitis prefera canine atopic dermatitis.

EXAMPLES

EXAMPLE 1

Cloning, expression and purification of recombinant AP205 tandem dimer VLPs wi CspZ in N- or in C- terminal ends

Cloning of the AP205 tandem dimer

AP205 tandem dimer was cloned in pET-Duet1 vector (Novagen) in two steps. In the f step PCR fragment containing AP205 gene with Nco I and BamH I restriction sites for clon in pET-Duet1 was generated with upstream primer: APncof 5`-tacaccatggcaaataagccaatg (SEQ ID NO:1) and downstream primer: APbamr 5`-tacattaggatccagcagtagtatcagacgata (SEQ ID NO:2) and template plasmid, containing AP205 coat protein gene sequence (NC Reference Sequence: NC_002700.2; SEQ ID NO:3).The PCR product was digested with N and BamHI and cloned in the same restriction sites into pET-Duet1. In the second step P fragment containing AP205 gene with BamH I and Pst I restriction sites was generated v upstream primer: APbamf 5`-tacaggatccgcaaataagccaatgcaacc-3` (SEQ ID NO:4) downstream primer APnher 5`-tacactgcagttagctagcagtagtatcagacgatac-3` (SEQ ID NO:5) template plasmid, containing AP205 coat protein gene sequence (NCBI Reference Sequer NC_002700.2; SEQ ID NO:3). The PCR product was digested with BamHI and Pst I and clo in the same restriction sites into the plasmid, obtained in the first step. As a result, express plasmid pET-Duet1-AP205TD (SEQ ID NO:6) was obtained, encoding AP205 coat pro tandem dimer AP205TD (SEQ ID NO:7). The AP205TD sequence contains two AP205 c protein genes, separated by a two amino acid glycine-serine (GS) linker and possessing an e serine residue at the C-terminus. GS linker was added to allow some flexibility between b coat protein halves and C-terminal serine is a consequence of engineered NheI restriction to allow insertions of foreign sequences.

Cloning of the CspZ gene in the N-terminus of AP205 tandem dimer

PCR fragment, containing CspZ gene sequence of Borrelia burgdorferi B31 strain (NC Reference Sequence: NC_001853.1; SEQ ID NO:8) and NcoI restriction sites in both ends generated with upstream primer CspZf 5`-tacaccatggcaagaaatattaatgagcttaaaatt-3` (SEQ NO:9), downstream primer CspZr 5`-cataccatggctaataaagtttgcttaatagctttat-3` (SEQ ID NO: and template plasmid, containing CspZ gene of Borrelia burgdorferi B31 strain (NC Reference Sequence: NC_001853.1 SEQ ID NO:8). The PCR product was cleaved with N and cloned in the same restriction site of plasmid pET-Duet1-AP205TD (SEQ ID NO:6) this way we obtained the expression plasmid pET-Duet1-CspZ-AP205TD (SEQ ID NO: encoding fusion protein CspZ-AP205TD (SEQ ID NO:12).

Cloning of the CspZ gene in the C-terminus of AP205 tandem dimer

PCR fragment, containing CspZ gene sequence and NheI restriction sites in both e was generated with upstream primer: CspZf2 5`-tacagctagcagaaatattaatgagcttaaatt-3`(SEQ

NO:13) and downstream primer CspZr2 5`-catagctagctaataaagtttgcttaatagctttat-3` (SEQ NO:14) and template plasmid, containing CspZ gene of Borrelia burgdorferi B31 strain (N(Reference Sequence: NC_001853.1 SEQ ID NO:8). The PCR product was cleaved with N and cloned in the same restriction site of plasmid pET-Duet1-AP205TD (SEQ ID NO:6) this way we obtained the expression plasmid pET-Duet1-AP205TD-CspZ (SEQ ID NO: encoding fusion protein AP205TD-CspZ (SEQ ID NO:16).

Production of Recombinant CspZ-AP205TD and AP205TD-CspZ fusion proteins

E. coli cells BL21(DE3) were transformed with plasmid pET-Duet1-AP205TD-Csp pET-Duet1-CspZ-AP205TD. 5 ml of LB liquid medium with 20 µg/ml ampicillin w inoculated with a single colony and incubated at 37 °C for 16-24 h without shaking. prepared inoculum was diluted 1:100 in 100-300 ml of LB medium, containing 20 µg ampicillin and incubated at 37°C overnight without shaking. The resulting second inocul was diluted 1:50 in 2xTY medium and incubated with shaking at 37°C to an OD 600 of (1.0. Then the expression was induced with 0.2 mM (final concentration) IPTG. Incubation continued on the rotary shaker at 20°C for 18 – 20 h. Cells were harvested by centrifugat and frozen at -20°C. The presence of produced recombinant proteins was verified by S PAGE analysis (Fig 3A and Fig. 3B).

Purification of recombinant CspZ-AP205TD protein VLPs

Frozen cells were thawed and resuspended in a lysis buffer (4ml of buffer per 1g cells). The mixture was sonicated at 24 kHz and 4°C for 10min, with on/off intervals of 0.5 After sonification, urea was added to a final concentration of 1M and lysate incubated on for 30min. The lysate was then centrifuged for 30minutes at 10000g. The pooled superna was loaded on a Sepharose 4FF column and proteins were eluted with PBS (Akta Prime P GE Healthcare). The fractions were analyzed by 15% PAGE-SDS and native agarose gel (agarose in TAE buffer, stained with 0.05% ethidium bromide). Fractions with visible V bands in agarose gel were pooled and concentrated with 15 ml 100kDa cutoff Ami concentration filters (Millipore). Concentrated VLPs were loaded on Superose6 gel filtrat column and eluted with PBS (Akta Prime Plus, GE Healthcare). The fractions were analy by 15% PAGE-SDS and native agarose gel and VLP-containing fractions, containing puri CspZ-AP205TD protein VLPs pooled. See Fig. 4a and Fig. 4B. For electron microscopy, protein samples were adsorbed on carbon–Formvar-coated copper grids and negatively stai with 1 % uranyl acetate aqueous solution. The grids were examined with a JEM-1230 elect microscope (JEOL Ltd., Tokyo, Japan) at 100 kV. The presence of VLPs was confirmed

electron microscopy (Fig 5). The outcome was about 4.5 – 5.0 mg of purified protein per gı of wet cells.

Purification of recombinant AP205TD-CspZ protein VLPs

Frozen cells were thawed and resuspended in a lysis buffer (4ml of buffer per 1g cells). The mixture was sonicated at 24 kHz and 4°C for 10min, with on/off intervals of 0.5s After sonication, urea was added to a final concentration of 1M and lysate incubated on ice 30min. The lysate was then centrifuged for 30minutes at 10000g. Clarified cell lysate precipitated for one hour with 20% saturated ammonium sulfate. The precipitate was collec by centrifugation and dissolved in a lysis buffer. Clarified supernatant was loaded o Sepharose 4FF column and proteins were eluted with PBS (Akta Prime Plus, GE Healthca The fractions were analyzed by 15% PAGE-SDS and native agarose gel (see Fig. 6A and] 6B). Fractions with visible VLP bands in agarose gel were pooled and concentrated with 15 100 kDa cutoff Amicon concentration filters (Millipore). The presence of VLPs was confirr by electron microscopy (Fig. 7). The outcome was about 0.6 mg of purified protein per gı of wet cells.

Solutions and buffers for Example 1:

Lysis buffer: 50mM Tris-HCl pH 8.0, 150mM NaCl, 0.1% tritonX100, 1mM PMSF.

PBS: 137mM NaCl, 2.7mM KCl, 10mM Na_2HPO_4, 1.8mM KH_2PO_4.

Thus, CspZ antigen was inserted both in C- and N-terminal parts of AP205TD and in b cases soluble VLPs were formed. We also attempted to insert CspZ antigen in N- and terminal parts of single (non-tandem) coat protein of AP205 but failed to obtain VLPs in b cases (Fig 3).

EXAMPLE 2

Cloning, expression and purification of recombinant AP205 tandem dimer VLPs wi dengue virus serotype 1 E protein ectodomain EDIII in N-terminal end

Cloning of the ED3 in N-terminal end of AP205 tandem dimer.

PCR fragment, containing ED3 gene sequence and Nco I restriction sites in both e was generated with upstream primer: ED3f 5`-gatataccatggataaactgaccctgaaag-3`(SEQ NO:17) and downstream primer ED3r 5`-atttgccatggcaccgctgcccattttgccaat-3` (SEQ ID NO: and template plasmid, containing ED3 gene of Dengue serotype 1 (SEQ ID NO:19). The P product was cleaved with NcoI and cloned in the same restriction site of plasmid pET-Du AP205TD (SEQ ID NO:6). In this way we obtained the expression plasmid pET-Duet1-E]

AP205TD (SEQ ID NO:20), encoding fusion protein ED3-AP205TD (SEQ ID NO:21).

Production of Recombinant ED3-AP205TD VLPs

E. coli cells BL21(DE3) were transformed with plasmid pET-Duet1-ED3-AP205TI ml of LB liquid medium with 20μg/ml ampicillin were inoculated with a single colony incubated at 37°C for 16-24h without shaking. The prepared inoculum was diluted 1:10(100-300ml of LB medium, containing 20μg/ml of ampicillin and incubated at 37°C overni without shaking. The resulting second inoculum was diluted 1:50 in 2xTY medium incubated with shaking at 37°C to an OD 600 of 0.8–1.0. Then the expression was induced v 0.2mM IPTG (final concentration). Incubation was continued on the rotary shaker at 20°C 18 – 20h. Cells were harvested by centrifugation and frozen at -20°C. The presence of produ recombinant proteins was verified by SDS-PAGE analysis (Fig 8A).

Purification of recombinant ED3-AP205TD VLPs

Frozen cells were thawed and resuspended in a lysis buffer (10ml buffer per 1g wet cel The mixture was sonicated at 24 kHz for10 min at +4°C, with on/off intervals of 0.5sec. (lysate was centrifuged for 30min at 10000g at 4°C and supernatant discarded. The pellet ' washed 3 times in a lysis buffer (same volume as taken for cell lysis). A single step of wash was performed in the same way as cell lysis – pellet was resuspended in the lysis buf sonicated, centrifuged and supernatant discarded. The pellet was further resuspended in solubilization buffer and incubated for 16h at 4°C on end-over-end rotator (30rpm). N suspension was centrifuged for 30min at 10000g at 4°C. The obtained supernatant was furt dialyzed against 100 volumes of RB I buffer for 24h at 4°C, subsequently – 100 volumes of II buffer for 24-36h at 4°C, and finally against 100 volumes of PBS at 4°C. The dialy supernatant was further centrifuged for 30min, at 10000g at 4°C and pellet discarded. ' refolding efficiency was assessed by SDS-PAGE electrophoresis (Fig. 8B). Supernatant ' loaded on a Sepharose 4FF column and proteins were eluted with PBS (Akta Prime Plus, Healthcare). The fractions were analyzed by 15% PAGE-SDS (Fig. 9A) and native agarose (1% agarose in TAE buffer, stained with 0.05% ethidium bromide) (Fig. 9B). Fractions v visible VLP bands in agarose gel were pooled and concentrated with 15ml 100 kDa cu Amicon concentration filters (Millipore). The presence of VLPs was confirmed by elect microscopy (Fig. 10). The outcome was about 2 mg of purified protein per gram of wet cel

Solutions and buffers for Example 2:

Lysis buffer: PBS with 0.1% Triton X100

IB solubilization buffer: 8 M urea, 50 mM Tris-HCl pH 8.0, 150 mM NaCl

RB I buffer: 2 M urea, 100 mM PB pH 8.0, 0.5 M arginine, 5 mM reduced glutathion 0.5 mM oxidized glutathione

RB II buffer: 100 mM PB pH 8.0, 0.5 M arginine, 5 mM glutathione reduced, 0.5 mM glutathione oxidized.

Thus, the generation of fusion proteins with ED3 was successfully achieved regardles ED3 placement at the N- or C- terminus of AP205TD (only N-terminal fusion describe detail). The expression yielded first insoluble products, which formed soluble modified AP VLPs after re-folding.

EXAMPLE 3

Cloning, expression and purification of recombinant AP205 tandem dimer VLPs wi canine IL-33 in C-terminal end and immunization of mice to generate binding and neutralizing antibodies

Cloning of the cIL33 in C-terminal end of AP205 tandem dimer.

cIL33 codon-optimized gene (SEQ ID NO:22) was purchased from BioCat (Gen Biosystems, Inc) in the form of a plasmid. The IL33 was cut from the plasmid with restrict endonucleases *Nhe I* and *Pst I* and the resulting DNA fragment, containing the cIL33 g sequence cloned into the same restriction sites of plasmid pET-Duet1-AP205TD (SEQ NO:6). In this way we obtained the expression plasmid pET-Duet1-AP205TD-cIL33 (SEQ NO:23), encoding fusion protein AP205TD-cIL33 (SEQ ID NO:24).

Production of Recombinant AP205TD-cIL33 VLPs

E. coli cells BL21(DE3) were transformed with plasmid pET-Duet1-AP205TD-cIL3 ml of LB liquid medium with 20μg/ml ampicillin were inoculated with a single colony incubated at 37°C for 16-24 h without shaking. The prepared inoculum was diluted 1:10 100-300ml of LB medium, containing 20μg/ml of ampicillin and incubated at 37°C overni without shaking. The resulting second inoculum was diluted 1:50 in 2xTY medium incubated with shaking at 37°C to an OD 600 of 0.8–1.0. Then the expression was induced v 0.2mM IPTG (final concentration). Incubation was continued on a rotary shaker at 20°C fo – 20h. Cells were harvested by centrifugation and frozen at -20°C. The presence of indu recombinant protein of ~ 47 kDa was verified by SDS-PAGE analysis (Fig. 11).

Purification of recombinant AP205TD-cIL33 VLPs

Frozen cells were thawed and resuspended in a 50mM Tris-HCl pH 8.0, 150mM Na

0.1% tritonX100, 1mM PMSF (4ml of buffer per 1g wet cells). The mixture was sonicate 24kHz and 4°C for10 min, with on/off intervals of 0.5sec. After sonification the lysate centrifuged for 30 minutes at 10000g and the pellet discarded. Ammonium sulphate was ad to supernatant to 40% saturation, and the solution centrifuged for 30 minutes at 10000g and supernatant discarded. The pellet was dissolved in PBS and loaded onto a Sepharose column. Proteins were eluted with PBS (Akta Prime Plus, GE Healthcare) and fracti analyzed by 15% PAGE-SDS and native agarose gel (1% agarose in TAE buffer, stained 0.05% ethidium bromide) (Fig. 12). Fractions with visible VLP bands in agarose gel pooled and concentrated using a 100 kDa cutoff Amicon concentration filters (Millipore). presence of VLPs was confirmed by electron microscopy (Fig. 13). The yield was 4.5 – 5.0 of purified protein per gram of wet cells.

Thus, fusion of cIL33 to C-terminus of AP205TD was demonstrated to result in solu 47 kDa fusion proteins (comprising AP205TD and cIL-33) which assembled into integral 30 nm modified AP205 VLPs in accordance with the present invention and composed of ab 90 x tandem dimers of AP205 each displaying 1 x cIL33.

Immunization of mice to generate binding and neutralizing antibodies

Groups of five female Balb/c mice were injected via the intra-peritoneal route with ul of AP205TD-cIL33 (30 ug/dose) VLPs formulated with 15 ug Quil-A® adjuvant (Brenn Biosector) in phosphate buffered saline or with phosphate buffered saline alone. After 14 d the mice were injected again with the same formulations. Mice were bled on days 0 (immune), day 14, 28, 42, 56, and 98. Sera were analyzed for IL-33-specific binding antibodies by ELISA and for neutralizing antibodies using a bioassay.

For the ELISA, NUNC plates were coated with canine IL-33 (recombinantly produce *E. coli*) in PBS with a concentration of 1 µg/ml overnight at 4°C. The plates were blocked Superblock (Invitrogen). A serial dilution of the sera was performed in order to calculate OD Values. OD50 describes the reciprocal of the dilution, which reaches half of the maximal value. Antibodies of the subtype gamma (IgG) specific for cIL-33 were detected with an a mouse IgG antibody directly labeled to horseradish dish peroxidase (HRPO) purchased fr Jackson. The conversion of o-phenylenediamine dihydrochloride (OPD) by the HRPO measured as color reaction at 450 nm, which was stopped by adding 5% sulfuric acid (H2S after 7 minutes incubation.

For the neutralization assay, HEK-Blue IL-33 cells from InvivoGen (hkb-hil33) w used. In this assay IL33 signaling leads to the activation of NF-kB and AP-1 pathways, wh

result in the production of a secreted alkaline phosphatase reporter which can be measure(the cell supernatant. The cell culture and the neutralization setup were performed accordin; the manufacturer's instructions with the exceptions that on day one of the assay the volume well was 100 ul and on day two, 40 ul of HEK-Blue IL-33 cell supernatant was added to ul QUANTI-Blue solution per well. Serial diluted mice sera were incubated in the presenc(5ng/ mL canine IL-33 (Sino Biological 700005-DNAE) before addition to HEK-Blue IL cells.

For mice receiving the AP205TD-cIL33 vaccine, canine IL-33 specific IgG antibo(were detected in sera collected on day 14; after a single immunization (Fig. 14, upper pan Titers were substantially boosted by a second immunization administered on day 14. Peak ti were measured in day 28 sera and declined slowly over the course of the 98-day experim Neutralizing antibodies were detected in day 28 sera of 4 of 5 mice and in day 98 sera in mice (Fig. 14, lower panel). The group receiving PBS had no detectable canine IL-33 bind or neutralizing antibodies.

Thus, the data show the AP205TD-cIL33 VLP vaccine was capable of induc antibodies that were able to both bind and neutralize canine IL-33.

EXAMPLE 4

Cloning, expression and purification of recombinant AP205 tandem dimer VLPs wi the RBM domain of SARS-CoV-2 in C-terminal end and immunization of mice to generate binding and neutralizing antibodies

The AP205 coat protein dimer of the present invention can be utilized efficiently generating a modified AP205 VLP in accordance with the present invention, and thus f(fusion vaccine against SARS-CoV-2. The exemplified prepared fusion protein described in example is abbreviated for the sake of ease and named AP205-RBM (FIG. 15A).

Cloning of the RBM domain of SARS-CoV-2 at the C-terminal end of AP205 tanc dimer

DNA encoding the Receptor Binding Motif (RBM) corresponding to residues 437- of SARS-CoV-2 Spike protein (GenBank accession number QIA98606.1, SEQ ID NO:95) amplified by PCR with Q5® High-Fidelity Master Mix (New England Biolabs, Ipswich, U using a codon optimized pUCIDT-SARS-CoV-2-RBD plasmid (SEQ ID NO:98) as temp and fused at the C-terminus of AP205TD between the *Bmt* I and *Hind* III sites in the pETD 1-AP205 dimer plasmid (SEQ ID NO:6) with a C-terminal 6xHis-tag. For PCR amplificat

the following primers were used: F: 5'-tctgatactactgctagcggatccaacagcaacaacc-3' (SEQ NO:99) and R: 5'-attatgcggccgcaagctttagtgatggtgatggtgatgactagtatacggctgatag-3' (SEQ NO:100). The corresponding PCR fragment was analyzed in 1.2% agarose gel and purified v *GeneJet Gel Extraction* kit (Thermo Scientific, USA). The PCR product and plasmid pETDı 1-AP205 were digested with enzymes *Bmt* I and *Hind* III (Thermo Fischer Scientific, Walth Massachusetts) and ligated, resulting in plasmid pETDuet-1-AP205-RBM (SEQ ID NO:1(*E.coli* XL1-Blue host cells were used for cloning and plasmid amplification. After sequenci plasmid were transformed into T7 Express Competent *E. coli* C2566 (High Efficiency) (N England Biolabs, Ipswich, USA).

Expression of AP205-RBM

E. coli C2566 were grown in LB medium containing Ampicillin (100 µg/ml) on a rot shaker (200 rpm) at 37 °C to an OD600 of 0.4 – 0.8. Following addition of 0.1 mM Isopro β-D-thiogalactopyranoside the expression phase was performed at 16 °C for 16 h. The biom was collected by low-speed centrifugation and frozen at -70 °C. After thawing on ice, cells w suspended in 20 mM Tris-HCl pH 8.0 100 mM NaCl, 2mM EDTA, 1 mM PMSF, 5 % glyce and 0. 1% Triton X-100 and disrupted by ultrasonication. Insoluble and soluble proteins w separated by centrifugation.SDS-PAGE using a 12% gel showed the 37.4 kDa construct expressed in an insoluble form (FIG. 15B).

Protein refolding and purification

Cell pellets were, the pellets were resuspended in lysis buffer (above) by sonication centrifuged for 20 min, 10,000 g at 4 °C. The process was repeated for 4x. The pellet contain inclusion bodies was solubilized in 8 M urea, 20 mM Tris-HCl and 100 mM NaCl for 16 l 4°C on a rotating wheel. Following centrifugation for 20 min at 10,000 g, the supernatant collected and sequentially dialyzed against 4M urea, 20mM Tris-HCl, 0.5 M Arginine, 5 r reduced Glutathione and 0.5 mM L- oxidized Glutathione for 24 h at 4 °C, then 2M urea, 20r Tris-HCl, 0.5 M Arginine, 5 mM reduced Glutathione and 0.5 mM L- oxidized Glutathic for 24 h at 4°C and finally 20mM Tris-HCl, 0.5 M Arginine, 5 mM reduced Glutathione 0.5 mM L- oxidized Glutathione for 36 h at 4°C. After centrifugation for 20 min at10,000 4 °C, the soluble refolded fusion protein which reassembles into VLPswas purified HisTrap™ (GE Healthcare, Germany) and analyzed on 12% SDS-PAGE and elect microscopy (FIG. 15C and FIG 15D respectively). For EM 2µl of purified AP205-RBM pro (1mg/ml) suspension for negative staining was adsorbed on glow discharged and carbon coa copper grids (Plano, Wetzlar; Germany) for 1 min at RT. After washing 3 x with pure wa grids were stained with 2% uranyl acetate solution (Electron Microscopy Science, Hatfi

USA) for 30 seconds. Excess fluid was removed and samples examined at 80kV wit transmission electron microscope (Tecnai Spirit, FEI, Hillsboro, USA) equipped with a dig camera (Veleta, Olympus, Münster, Germany)

In summary, expression of pETDuet-1-AP205-RBM resulted in large amounts insoluble aggregates of AP205-RBM which could be easily denatured and refolded into solu 37.4 kDa fusion proteins in accordance with the present invention which assembled into inte 25-30 nm modified AP205 VLPs composed of typically and preferably 90 x tandem dimer AP205 each displaying 1 x RBM domain. (Figure. 15B and 15C).

Immune response to AP205-RBM VLP vaccine.

Vaccination regimen. Wild type Balb/c female mice were vaccinated subcutaneou (s.c.) with 100µg AP205-RBM VLPs or AP205 VLPs in 100µl PBS on day 0 and 28 and s collected on days 0, 14, 21, 35 and 49. Sera from immunized mice were used to measure antibodies capable of binding RBD and Spike proteins of SARS-CoV-2 (ELISA) neutralizing antibodies (Pseudotype virus neutralization assay).

Enzyme-linked immunosorbent assay (ELISA). ELISA plates were coated overnight v 0.1µg/ml and 1.0 µg/ml of S protein RBD or full spike protein (Sinobiological, Beijing, Chi respectively. Plates were washed with PBS-0.01% Tween and blocked using 100µl PBS-Ca 0.15% for 2h in RT. For titration purposes, sera, initially diluted 1/20 then serially diluted were added (100 ul per well) to the wells.. Plates were incubated for 1h at RT. After wash with PBS-0.01%Tween, goat anti-mouse IgG conjugated to Horseradish Peroxidase (H (Jackson ImmunoResearch, West Grove, Pennsylvania) was added 1/2000 and incubated 1h at RT. Plates were developed and OD 450 reading was performed.

Neutralization assay. Vesicular stomatitis pseudotyped virus production has b described elsewhere (Whitt M. A., 2010, J. Virol. Meth. 169 365-374) and used a modification to incorporate SARS-CoV-2_Spike and the $TCID_{50}$ was tested on HEK293T cells transiently expressing ACE2 and transmembrane protease serine subtype 2 (TMPRSS

Neutralization assays were undertaken using $100xTCID_{50}$ per well of 96-well plate. virus was incubated for 1 hour at 37°C (5% CO_2) along with the heat-inactivated serum, wh was diluted over a range of 1:20-1:500. After which, $2x10^4$ HEK293T/17 cells transie expressing ACE2 and TMPRSS2 were added to each well and the plate left to incubate (37 5% CO_2) for a further 48 hours. The media was then discarded and the level of reporter g activity assessed using a 50:50 mix of non-supplemented media:BrightGlo and a read i GloMax Discover (Promega).

CPE-based assay: the capacity of the induced antibodies in neutralizing wild-t

SARS-CoV-2 (SARS-CoV-2/ABS/NL20) was also performed. Serum samples were h inactivated for 30min at 56°C. Two-fold serial dilutions were prepared starting at 1:20 u 1:160. 100 TCID50 of the virus was added to each well and incubated for 37°C for 1h. mixture has been added on a monolayer of Vero cells and incubated again for 37°C for 4 d Four days later the cells were inspected for cytopathic effect (CPE). The titer was expresse the highest dilution that fully inhibits formation of CPE. Data were analyzed and presente mean ± SEM using GraphPad PRISM 8. P-values $^{**}P < 0.01$; $^{*}P < 0.05$.

Results of the assessment of the immune response to AP205-RBM VLP. Immuniza of naïve mice with AP205-RBM VLP vaccine resulted in an increase in RBD-specific (F 16A) and Spike protein (FIG. 16B) specific IgG antibody titers 14 days after priming w were further increased following injection on day 28. No RBD-specific antibodies w detected in the mice vaccinated with AP205 as a control. These data show the IgG antibo induced by the AP205-RBM VLPs in accordance with the present invention are capabl recognizing the larger eukaryotically expressed RBD domain from which the RBM was deri and the still larger spike protein which contains the RBD within a trimeric native struct Moreover, the induced antibodies were able to neutralize a pseudotype vesicular stomatitis v (17A) as well as SARS-CoV-2/ABS/NL20 (FIG. 17A and FIG. 17B) demonstrating that AP205-RBM VLP is an effective vaccine candidate.

EXAMPLE 5

Cloning, expression and purification of recombinant AP205 tandem dimer VLPs wi African Swine Fever Virus (ASFV) p12 protein surface exposed peptide in both N- a C- terminal end

Cloning of the ASFV p12 in N- and C- terminal ends of AP205 tandem dimer.

In the first step, ASFV p12 protein surface exposed peptide (further – p12) gene cloned into the N-terminal end of AP205 tandem dimer. Since the inserted peptide relatively short, only 12 residues (SEQ ID NO:102), p12 gene was purchased from Meta International AG (Germany) as two single stranded oligonucleotides. One oligonucleo (SEQ ID NO:103) encoded p12 gene in forward orientation and the other one (SEQ ID NO:1 – in reverse orientation. The oligonucleotides were complementary to each other and had N recognition sites at both ends. Both oligonucleotides were annealed into a single dsD fragment by mixing them together in equimolar amounts (30 mM each) and incubating at t melting temperature (88°C) for 1 min. The obtained dsDNA fragment containing p12 g

sequence was cleaved with NcoI and cloned in the same restriction site of plasmid pET-Du AP205TD. As a result, expression plasmid pET-Duet1-np12-AP205TD (SEQ ID NO:1 encoding fusion protein np12-AP205TD (SEQ ID NO:106) was prepared. The np12-AP205 sequence contains the AP205TD sequence (SEQ ID NO:7) and possessing an extra alar residue at the N-terminus of the AP205TD sequence as a consequence of the cloning strate

Further, we used the obtained pET-Duet1-np12-AP205TD (SEQ ID NO:105) to in p12 sequence into the C-terminal end of AP205 tandem dimer. For this, another two sir stranded oligonucleotides encoding p12 were ordered from Metabion International (Germany). This time forward (SEQ ID NO:107) and reverse (SEQ ID NO:1 oligonucleotides contained NheI and PstI recognition sites. Double stranded DNA fragm encoding p12 protein was obtained as described above, except that the melting temperature adjusted to 90°C. Then DNA fragment was cleaved with NheI and PstI and cloned into the sa restriction sites of plasmid pET-Duet1-np12-AP205TD (SEQ ID NO:105). This way obtained a new expression plasmid pET-Duet1-np12-AP205TD-cp12 (SEQ ID NO:1 encoding fusion protein np12-AP205TD-cp12 (SEQ ID NO:110). The presence of sequence at both AP205 tandem dimer terminal ends was verified by Sanger sequencing.

Production of recombinant np12-AP205TD-cp12 VLPs

E. coli cells BL21(DE3) were transformed with plasmid pET-Duet1-np12-AP205 cp12. A single colony was put into 30 ml of LB liquid medium with 50 μg/ml of ampicillin incubated at 37°C for 16-24 h without shaking. The prepared inoculum was diluted 1:1 2xTY medium and incubated with shaking at 37°C to an OD 600 of 0.8–1.0. Then expression of the fusion protein was induced with 0.5 mM IPTG (final concentrati Incubation was continued on the rotary shaker at 20°C for 18 – 20h. Cells were harvested centrifugation and frozen at -20°C. The presence of produced recombinant proteins was veri by SDS-PAGE analysis (Fig. 18).

Purification of recombinant np12-AP205TD-cp12 VLPs

Frozen cells were thawed and resuspended in a lysis buffer (4ml of buffer per 1 g cells). The mixture was sonicated at 24 kHz and 4°C for 10, with on/off intervals of 0.5 After sonification the lysate was centrifuged for 30 minutes at 10 000g and pellet discarc Ammonium sulphate was added to the supernatant to 40% saturation and incubated overni at +4 °C. The mixture was centrifuged for 30 minutes at 10 000g and the supernatant discarc The pellet was dissolved in the extraction buffer, centrifuged as described before and obtai supernatant loaded on a Sepharose 4FF (30 ml) column. Proteins were eluted with TBS (A Prime Plus, GE Healthcare). The fractions were analyzed by 15% PAGE-SDS and na

agarose gel (1% agarose in TAE buffer, stained with 0.05% ethidium bromide) (Fig. 19A, F 19B). Fractions with visible VLP bands in agarose gel were pooled for further ion excha chromatography. The fractions were loaded on a DEAE Fractogel M (5 ml) column. Bo proteins were eluted with 20 column volumes of linear gradient of 0-1M NaCl in TBS. ' fractions were analyzed by 15% PAGE-SDS and native agarose gel (FIG. 20A, FIG. 2C Fractions containing purified VLPs were dialysed against PBS buffer and concentrated witl ml 100 kDa cutoff Amicon concentration filters (Millipore). The presence of VLPs ' confirmed by electron microscopy (FIG. 21). The outcome was about 0.6-0.8 mg of puri VLP per gram of wet cells.

Solutions and buffers for Example 5:

Lysis buffer: 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.1% tritonX100, 0.5 Urea, 1 mM PMSF

Extraction buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.5 M Urea, 0.1 Tween20, 1 mM PMSF

TBS buffer: 20 mM Tris-HCl pH 8.0, 150 mM NaCl

PBS buffer: 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO

Thus, the generation of fusion proteins with antigenic polypeptides derived from Afri Swine Fever Virus (ASFV), and in particular from ASFV p12 protein surface exposed pep in both, N- and C-terminal end, of AP205TD was successfully achieved.

CLAIMS

1. A modified virus-like particle of RNA bacteriophage AP205 (AP205 VLP) comprisi one or more fusion proteins, wherein said fusion protein comprises, preferably consis of,
   (i) an AP205 coat protein dimer, wherein said AP205 coat protein di comprises a first AP205 polypeptide and a second AP205 polypept wherein said first AP205 polypeptide is fused at its C-terminus ei directly or via an amino acid spacer to the N-terminus of said second AP polypeptide, and wherein said first and said second AP205 polypep independently comprises
      (a) an amino acid sequence of a coat protein of RNA bacterioph AP205, or
      (b) a mutated amino acid sequence, wherein said mutated amino a sequence (b) and said amino acid sequence of said coat protein of R bacteriophage AP205 (a), have a sequence identity of at least 90 preferably of at least 95%, further preferably of at least 98% and ag more preferably of at least 99%; and
   (ii) an antigenic polypeptide, wherein said antigenic polypeptide is fused to N-terminus and/or the C-terminus of said AP205 coat protein dimer ei directly or via an amino acid linker.

2. The modified AP205 VLP of claim 1, said first and said second AP205 polypep independently comprises (a) an amino acid sequence of a coat protein of R bacteriophage AP205, or (b) a mutated amino acid sequence, wherein said mutated am acid sequence and said amino acid sequence of a coat protein of RNA bacterioph AP205 have a sequence identity at least 90%, wherein said coat protein of R bacteriophage AP205 comprises, preferably consists of, the amino acid sequence of S ID NO: 26.

3. The modified AP205 VLP of claim 1 or claim 2, wherein said amino acid spacer a length of at most 15 amino acids and is selected from the group consisting of:
   (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n

5; and

(b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at le one serine, wherein preferably said GS linker has an amino acid sequenc $(GS)_r(G_sS_w)_t(GS)_u$ with r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1.

4. The modified AP205 VLP of any one of the preceding claims, wherein said amino a linker is selected from the group consisting of:
   (a.) a polyglycine linker $(Gly)_n$ of a length of n=2-10, preferably a length of n 5; and
   (b.) a glycine-serine linker (GS-linker) comprising at least one glycine and at le one serine, wherein preferably said GS linker has an amino acid sequenc $(GS)_r(G_sS_w)_t(GS)_u$ with r=0 or 1, s=1-5, w=0 or 1; t=1-3 and u=0 or 1; wherein preferably said glycine-serine linker has a length of at most 15 am acids;
   (c.) an amino acid linker comprising at least one Gly, at least one Ser, and at le one amino acid selected from Thr, Ala, Lys, Asp and Glu, wherein said am acid sequence has a length of at most 15 amino acids.

5. The modified AP205 VLP of any one of the preceding claims, wherein said AP205 c protein dimer comprises, preferably consists of, the amino sequence of SEQ ID NO: ' an amino acid sequence having a sequence identity of at least 90 %, preferably of at le 95%, further preferably of at least 98% and again more preferably of at least 99% v said SEQ ID NO: 7.

6. The modified AP205 VLP of any one of the preceding claims, wherein an antige polypeptide is fused directly or via an amino acid linker to the N-terminus of said AP coat protein dimer and an antigenic polypeptide is fused directly or via an amino a linker to the C-terminus of said AP205 coat protein dimer.

7. The modified AP205 VLP of any one of the preceding claims, wherein said modi AP205 VLP consists of said fusion proteins.

8. The modified AP205 VLP of any one of the preceding claims, wherein said antige polypeptide is a polypeptide derived from the group consisting of: (a) allergens;

viruses; (c) bacteria; (d) parasites; (e) tumors; (f) self-molecules; (g) hormones; growth factors; (i) cytokines; and (j) chemokines.

9. The composition of any one of the preceding claims, wherein said antigenic polypep is selected from
   (a) IL- 17, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, SEQ ID NO:36;
   (b) IL-5, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:37 to SEQ ID NO:42;
   (c) IL-4, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:43 to SEQ ID NO:45;
   (d) IL-13, and wherein preferably said antigenic polypeptide comprises, or prefera consists of SEQ ID NO:49;
   (e) IL-1α, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:50 to SEQ ID NO:53;
   (f) IL-33, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:54 to SEQ ID NO:58;
   (g) IL-25, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:59 to SEQ ID NO:62;
   (h) IL-1β, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:63 to SEQ ID NO:67;
   (i) IL-12/23;
   (j) TNF-α;
   (k) IL-31, wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:68 to SEQ ID NO:71;
   (l) thymic stromal lymphopoietin (TLSP), wherein preferably said antige polypeptide comprises, or preferably consists of, any one the SEQ ID NO:7 SEQ ID NO:75;
   (m) the dog allergen Can f1 or Can f2;
   (n) myostatin, and wherein preferably said antigenic polypeptide comprises, preferably consists of, SEQ ID NO:87;
   (o) an antigenic polypeptide derived from Plasmodium falciparum or Plasmodi Vivax, and wherein preferably said antigenic polypeptide comprises, or prefera

consists of, SEQ ID NO:88;

(p) CspZ protein from Borrelia burgdorferi, and wherein preferably said antige polypeptide comprises, or preferably consists of, SEQ ID NO:89 or SEQ ID NO:
(q) an antigenic polypeptide derived from RSV;
(r) Dengue viral antigenic polypeptide, wherein preferably said Dengue viral antige polypeptide comprises, or preferably consists of, SEQ ID NO:91 or SEQ ID NO:
(s) an antigenic polypeptide derived from a receptor binding domain (RBD) c receptor binding motif (RBM) of a coronavirus (CoV), preferably of SARS-C 2, and wherein preferably said antigenic polypeptide comprises, or prefera consists of, any one the SEQ ID NO:94 to SEQ ID NO:97;
(t) an antigenic polypeptide derived from African Swine Fever;
(u) calcitonin gene-related peptide (CGRP);
(v) Amylin; and
(w) GnRH.

10. The modified AP205 VLP of any one of the preceding claims, wherein said fusion pro is selected from the group consisting of SEQ ID NO:12, SEQ ID NO:16, SEQ ID NO SEQ ID NO:24, SEQ ID NO:101, SEQ ID NO:106 and SEQ ID NO:110.

11. A pharmaceutical composition comprising:
    (a) the AP205 VLP of any one of claims 1 to 10; and
    (b) a pharmaceutically acceptable carrier, diluent and/or excipient.

12. The modified AP205 VLP of any one of claims 1 to 10, or the pharmaceut composition of claim 11 for use in a method of immunization an animal or a hur comprising administering the modified AP205 VLP of any one of claims 1 to 10, or pharmaceutical composition of claim 11 to said animal or human.

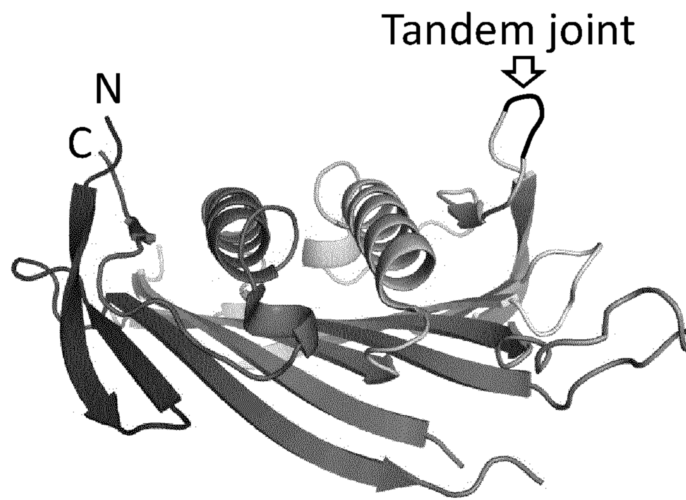
13. The modified AP205 VLP of any one of claims 1 to 10, or the pharmaceut composition of claim 11 for use in a method for the treatment of a disease or disorde an animal or human, wherein preferably said disease or disorder is selected from the gr consisting of autoimmune disease, an inflammatory disease, an infectious disease c cancer.

14. The modified AP205 VLP or the pharmaceutical composition for use of claim 13, whei
    a) said modified AP205 VLP is the modified AP205 VLP of any one of the claims 9 to 9(l), and wherein said disease or disorder is an inflammatory disease in an ani or human, and wherein preferably said inflammatory disease is selected from I MS, Psoriasis, ankylosing spondylitis, asthma, Crohns, Colitis, COPD, diabe neurodermatitis (allergic dermatitis);
    b) said modified AP205 VLP is the modified AP205 VLP of claim 9(m), and whei said disease or disorder is an allergy in a dog;
    c) said modified AP205 VLP is the modified AP205 VLP of claim 9(0) for prevent or treating malaria;
    d) said modified AP205 VLP is the modified AP205 VLP of claim 9(p), and whei said disease or disorder is Lyme borreliosis;
    e) said modified AP205 VLP is the modified AP205 VLP of claim 9(q) to 9(t), wherein said disease or disorder is an infectious disease in an animal or human;
    f) said modified AP205 VLP is the modified AP205 VLP of claim 9(u), and whei said disease or disorder is migraine in an animal or human;
    g) said modified AP205 VLP is the modified AP205 VLP of claim 9(v), and whei said disease, disorder or condition is type II diabetes in an animal or human; or
    h) said modified AP205 VLP is the modified AP205 VLP of claims 9(w), and whei said disease or disorder is to lower testosterone levels in an animal or human.

15. A modified virus-like particle of RNA bacteriophage AP205 (AP205 VLP) compris one or more AP205 coat protein dimer, wherein said AP205 coat protein dimer compri a first AP205 polypeptide and a second AP205 polypeptide, wherein said first AP polypeptide is fused at its C-terminus either directly or via an amino acid spacer to the terminus of said second AP205 polypeptide, and wherein said first and said second AP polypeptide independently comprises

    (a) an amino acid sequence of a coat protein of RNA bacteriophage AP205, or

    (b) a mutated amino acid sequence, wherein said mutated amino acid sequence and said amino acid sequence of said coat protein of RNA bacteriophage AP (a), have a sequence identity of at least 90 %, preferably of at least 95%, furt preferably of at least 98% and again more preferably of at least 99%.



AP205 CP	AP205 CP

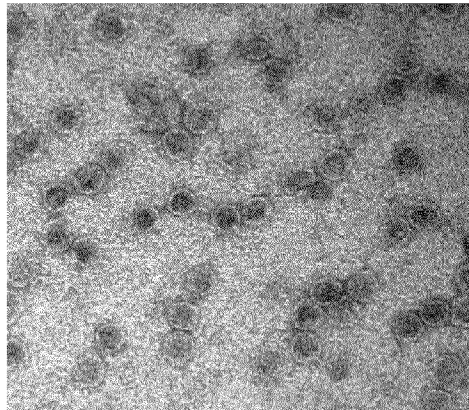
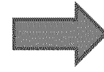
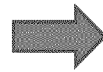
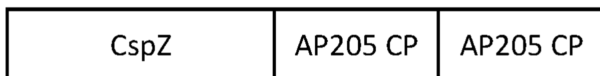
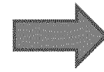
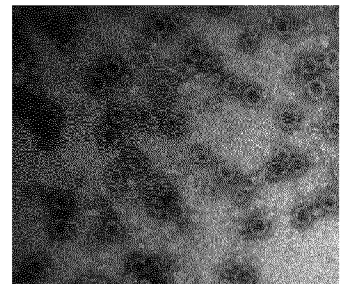


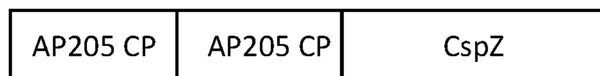
FIG. 1


No VLPs, product unsoluble


No VLPs, product unsoluble





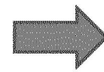
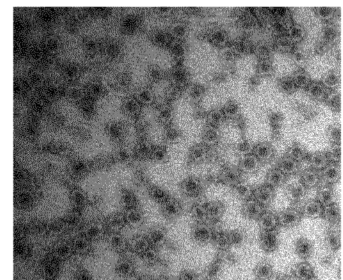



FIG. 2

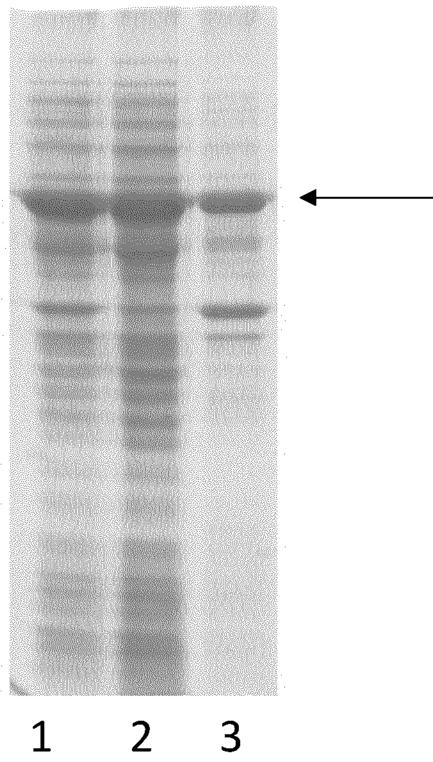


FIG. 3A

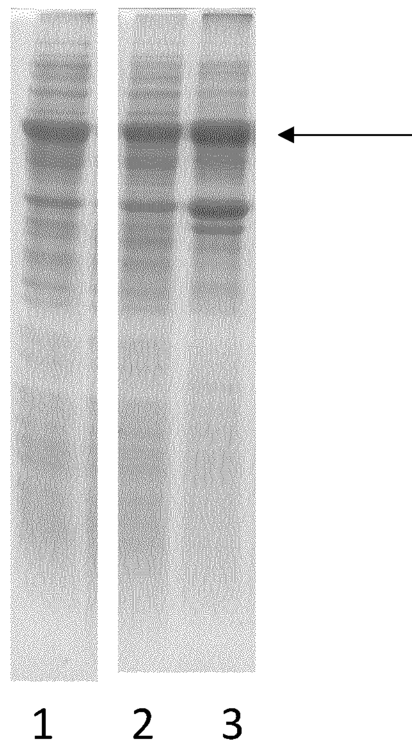


FIG. 3B

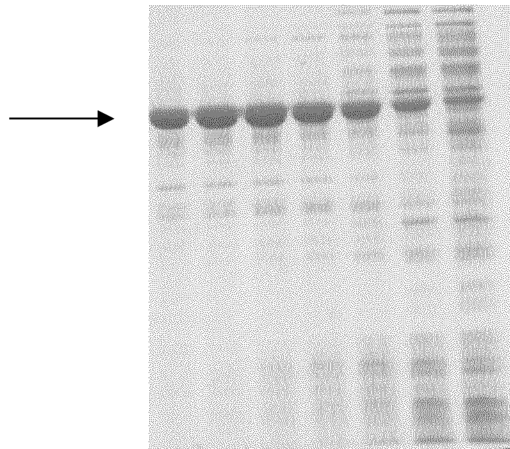


FIG. 4A

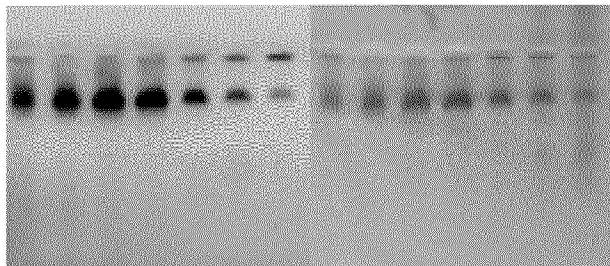


FIG. 4B

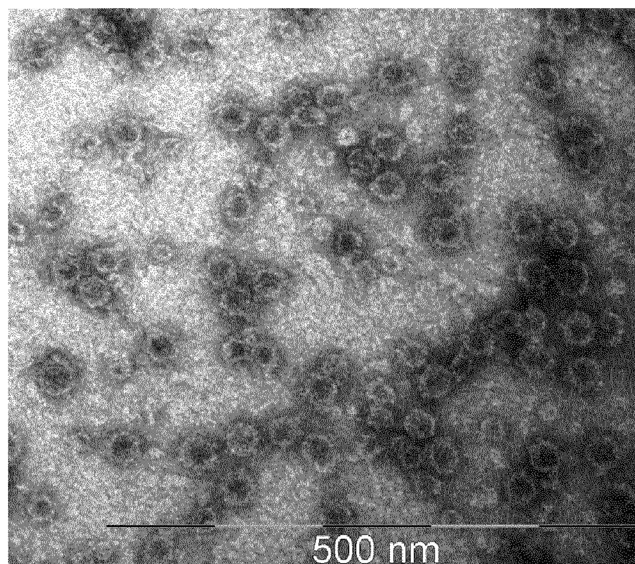


FIG. 5

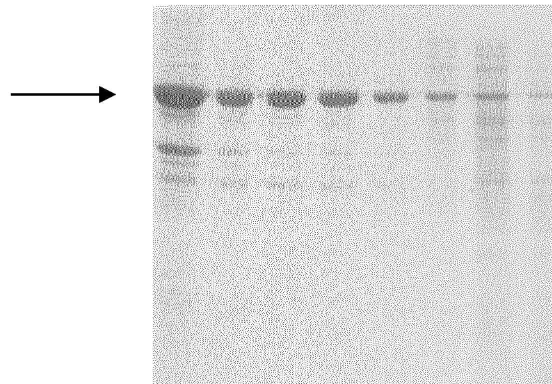


FIG. 6A

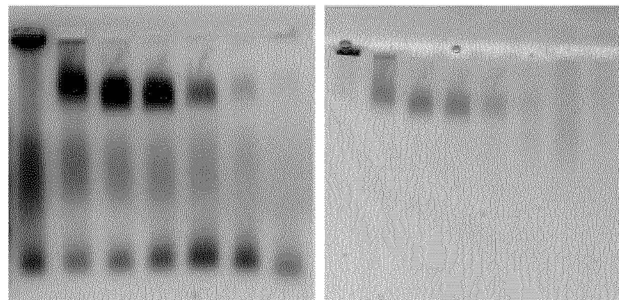


FIG. 6B

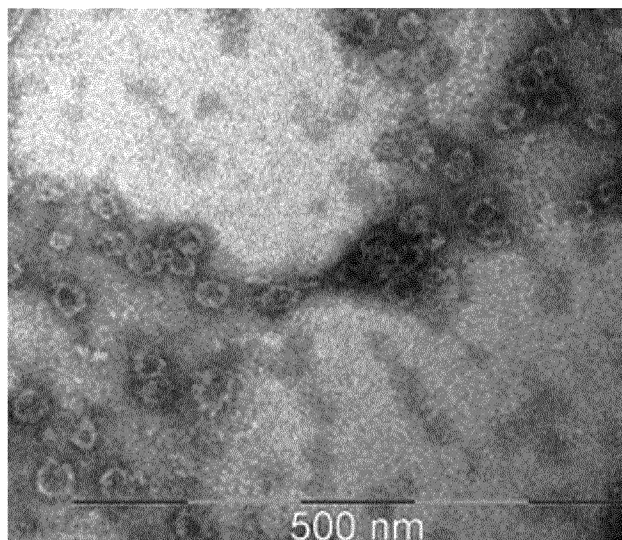


FIG. 7

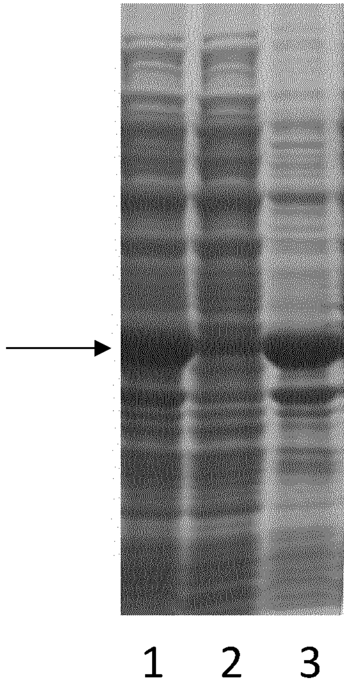


FIG. 8A

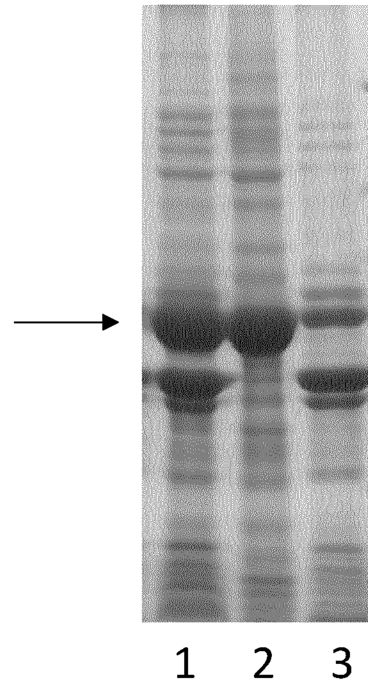


FIG. 8B

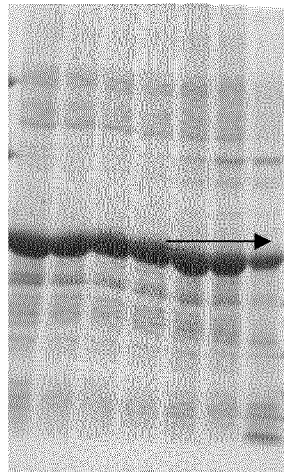


FIG. 9A

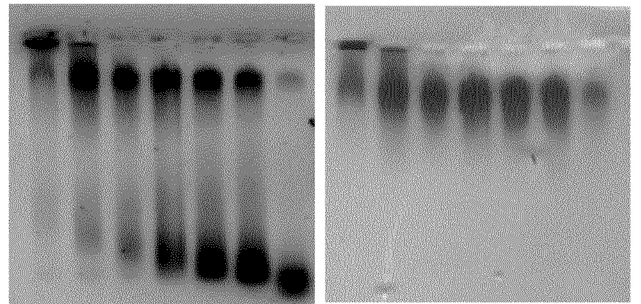


FIG. 9B

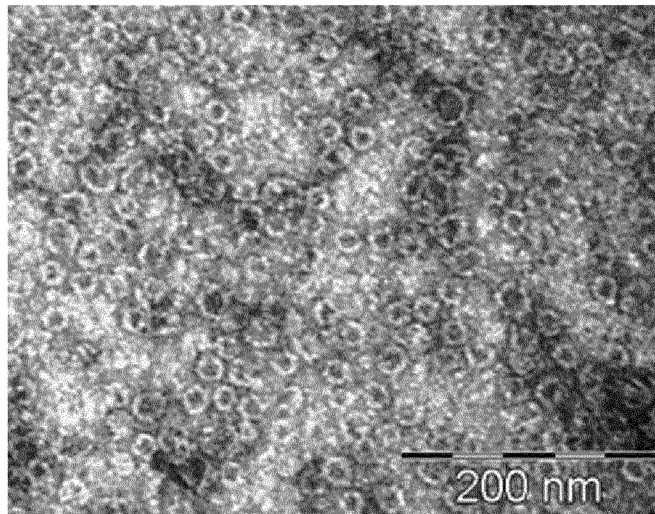


FIG. 10

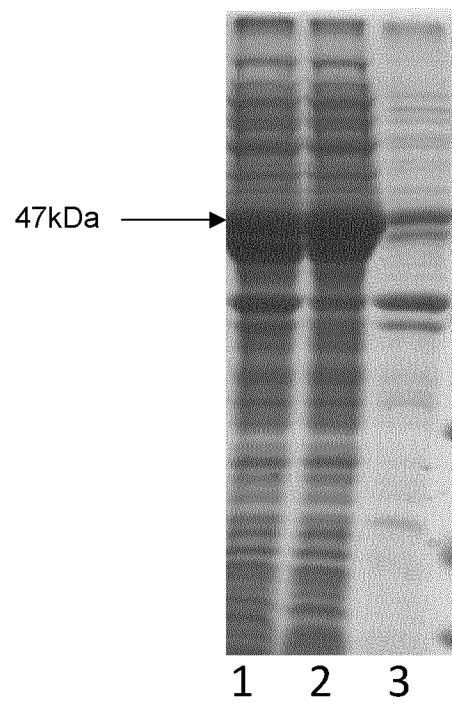


FIG. 11

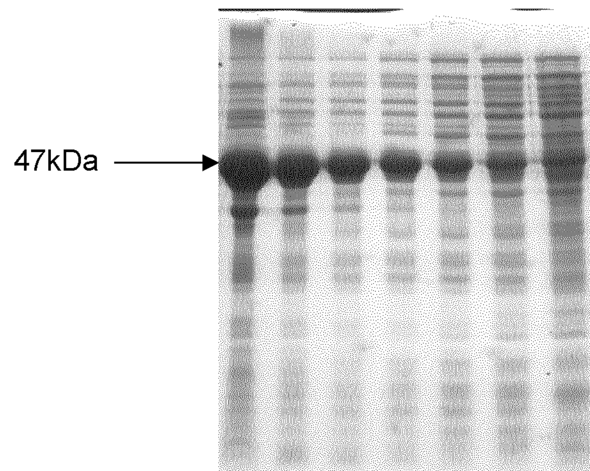


FIG. 12A

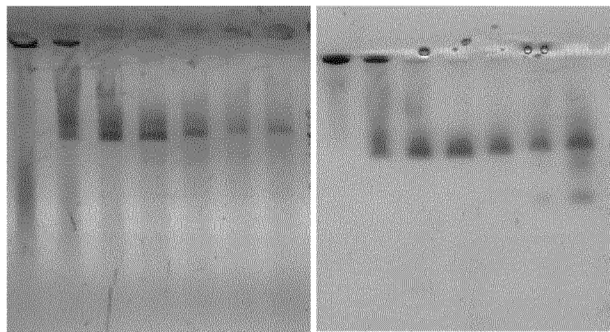


FIG. 12B

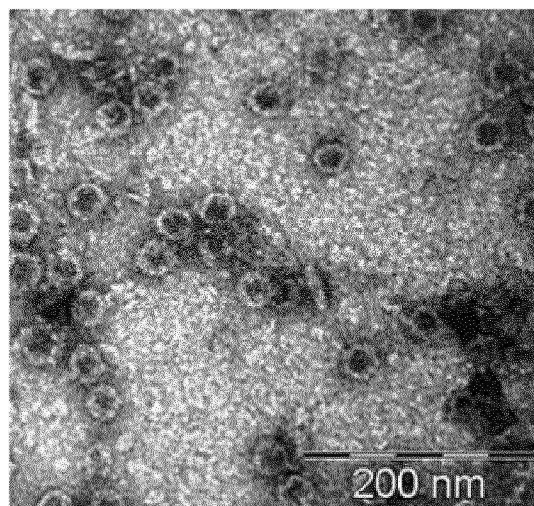


FIG. 13

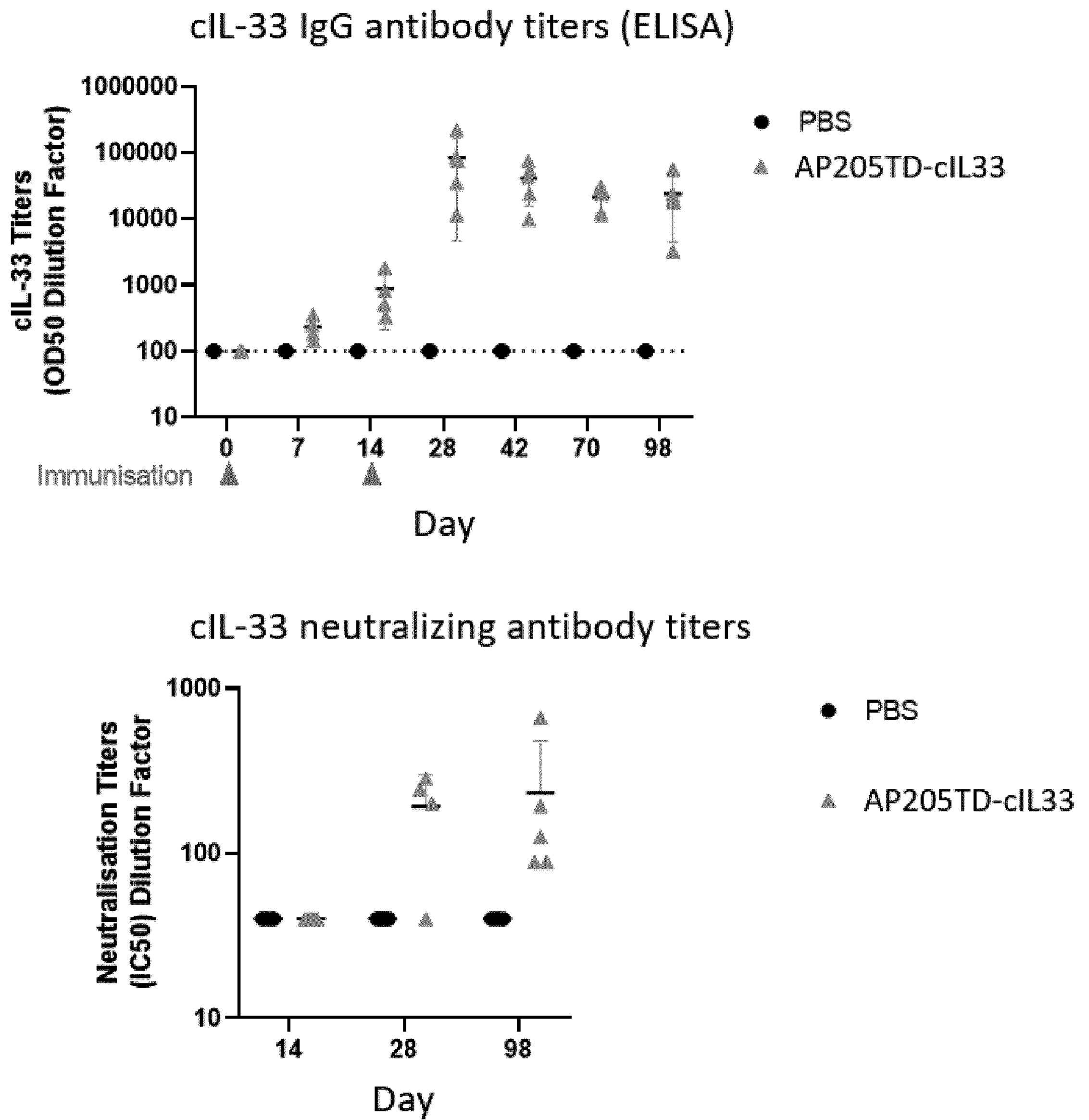


FIG. 14

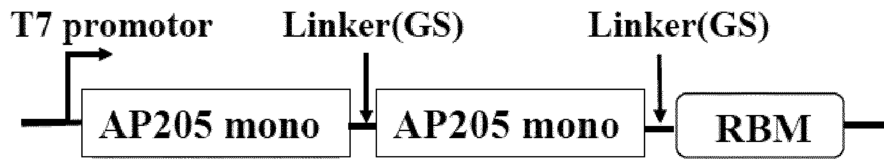


FIG. 15A

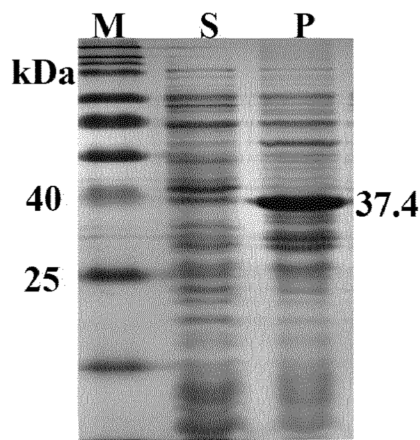


FIG. 15B

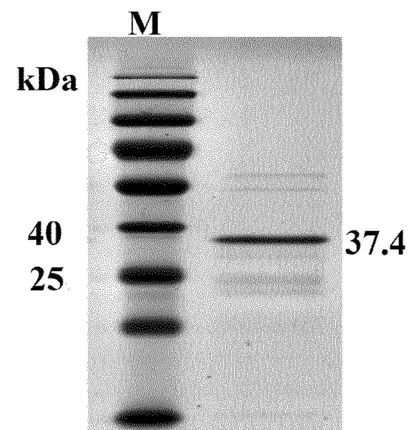


FIG. 15C

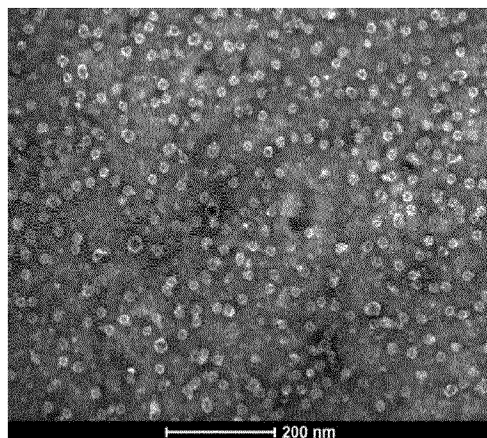


FIG. 15D

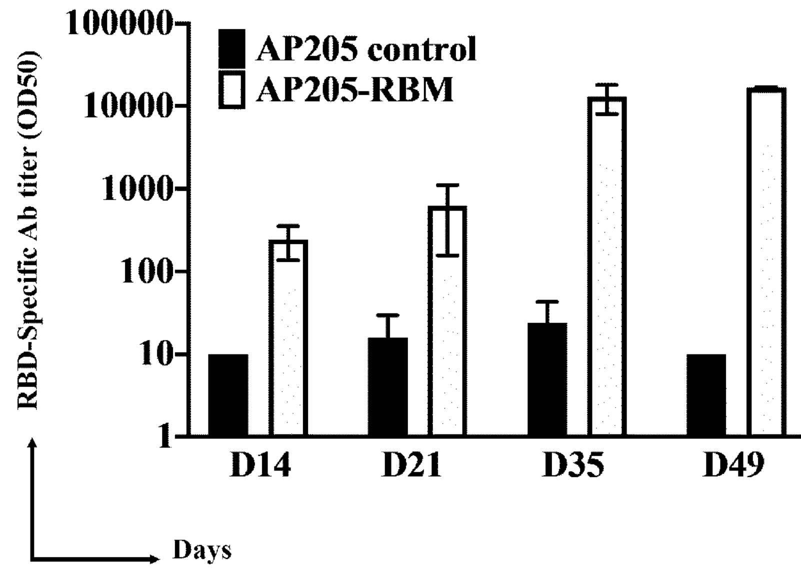


FIG. 16A

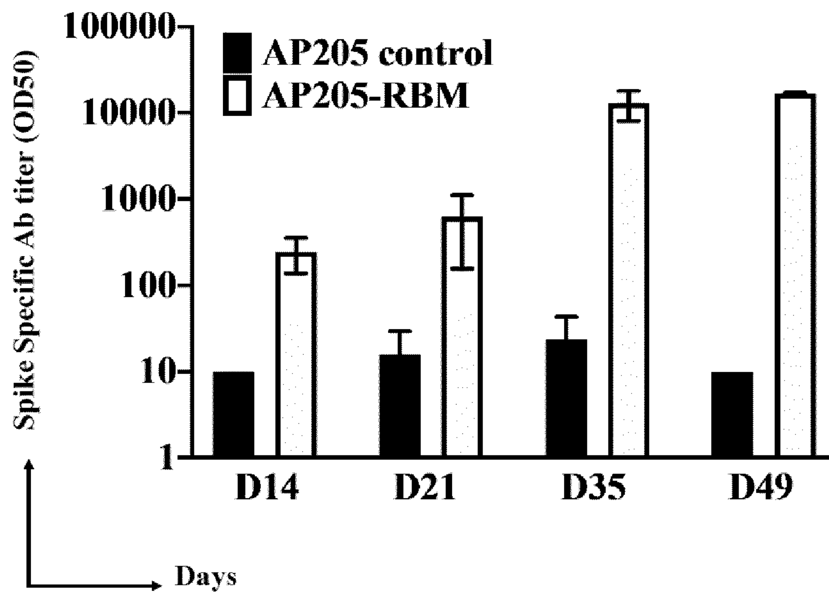


FIG. 16B

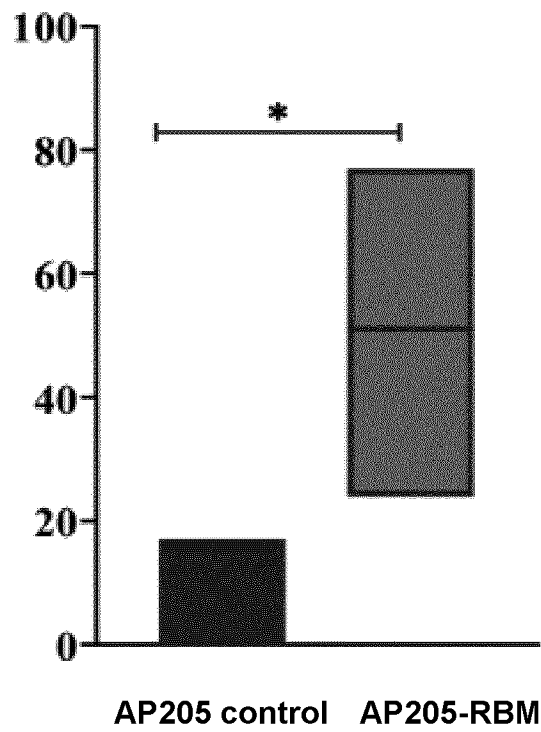


FIG. 17A

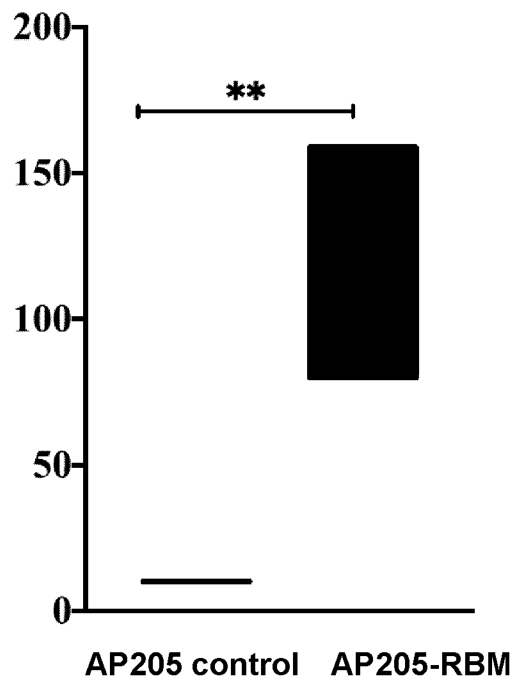


FIG. 17B

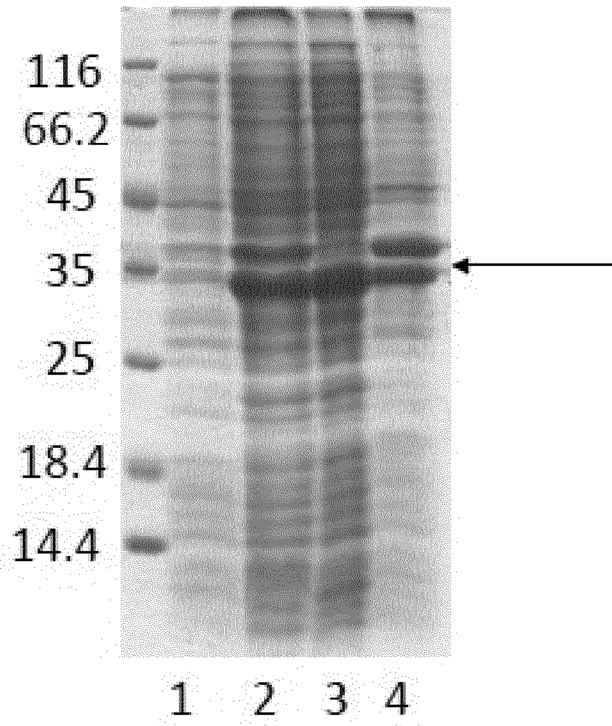


FIG. 18

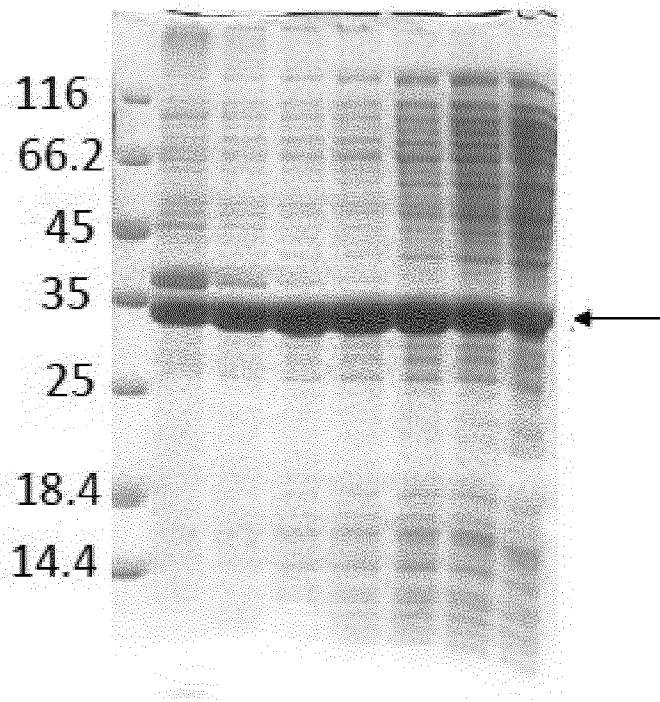


FIG. 19A

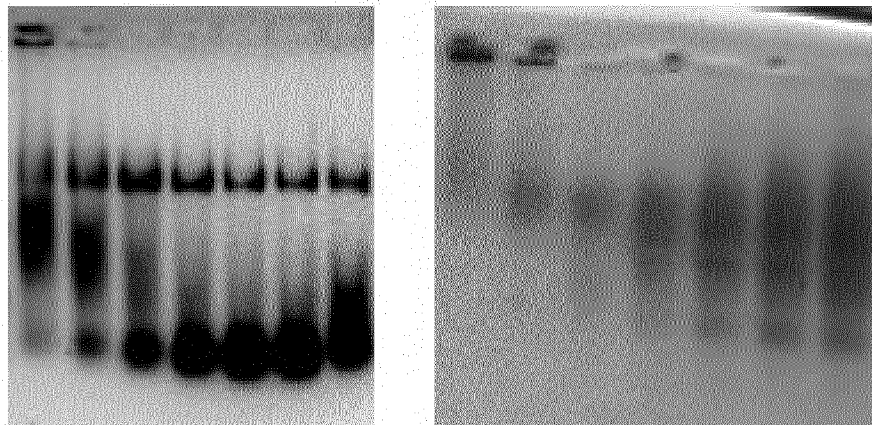


FIG. 19B

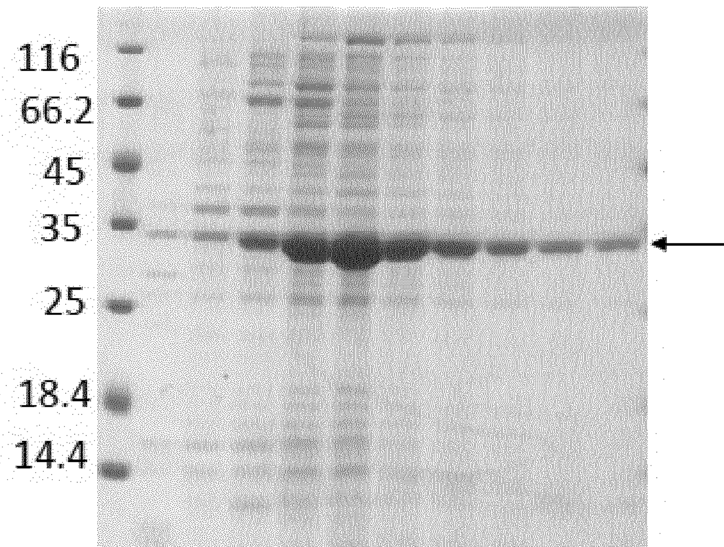


FIG. 20A

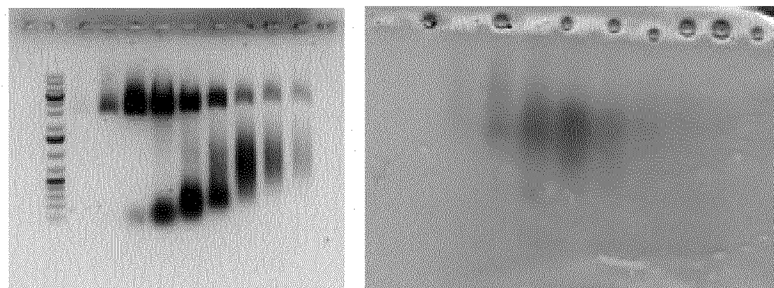


FIG. 20B

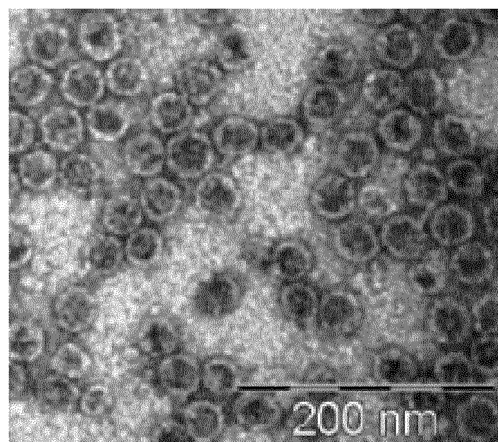


FIG. 21

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2022/059646

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/10 C07K16/12 C07K16/24 C07K16/08 A61P31/04
A61P31/12 A61P31/14 A61P31/20
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

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

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

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2009/080823 A2 (CYTOS BIOTECHNOLOGY AG [CH]; BACHMANN MARTIN [CH] ET AL.) 2 July 2009 (2009-07-02) paragraph [0106] - paragraph [0107]; examples 1,2; sequence 41	1-15
X	WO 2019/086548 A1 (VIB VZW [BE]; UNIV BRUSSEL VRIJE [BE]) 9 May 2019 (2019-05-09) figure 40; example 15; sequence 171	1,5-7, 11,15

-/--

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

[x] See patent family annex.

* Special categories of cited documents :

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

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"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
23 June 2022	01/07/2022

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Authorized officer

Scheffzyk, Irmgard

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KLOVINS J ET AL: "Nucleotide sequence of a ssRNA phage from Acinetobacter: Kinship to coliphages", JOURNAL OF GENERAL VIROLOGY,, vol. 83, no. 6, 1 June 2002 (2002-06-01), pages 1523-1533, XP002260479, ISSN: 0022-1317 the whole document -----	1-15
X	WO 2016/112921 A1 (UNIV COPENHAGEN [DK] ET AL.) 21 July 2016 (2016-07-21) cited in the application claims 1,22; sequence 58 -----	1-15
A	SPOHN GUNTHER ET AL: "A VLP-based vaccine targeting domain III of the West Nile virus E protein protects from lethal infection in mice", VIROLOGY JOURNAL, BIOMED CENTRAL, LONDON, GB, vol. 7, no. 1, 6 July 2010 (2010-07-06), page 146, XP021080076, ISSN: 1743-422X, DOI: 10.1186/1743-422X-7-146 the whole document -----	1-15
A	SHISHOVS MIHAILS ET AL: "Structure of AP205 Coat Protein Reveals Circular Permutation in ssRNA Bacteriophages", JOURNAL OF MOLECULAR BIOLOGY, ACADEMIC PRESS, UNITED KINGDOM, vol. 428, no. 21, 31 August 2016 (2016-08-31), pages 4267-4279, XP029762985, ISSN: 0022-2836, DOI: 10.1016/J.JMB.2016.08.025 cited in the application the whole document -----	1-15

International application No.

PCT/EP2022/059646

INTERNATIONAL SEARCH REPORT

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2022/059646

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		US 2020115420 A1	16-04-2020
		WO 2016112921 A1	21-07-2016