

Nucleic acid vaccines

Technical field

The present invention relates to modular nanoparticle-based compositions based on nucleic acids, such as DNA and RNA, which are particularly useful in prophylaxis and/or treatment of diseases and disorders.

Background

Vaccines remain the most effective tools for preventing and controlling the spread of infectious diseases. Live-attenuated vaccines are highly immunogenic, inducing long-lived antibody responses even after a single immunization. In contrast, modern, subunit vaccines (i.e. based on a soluble protein antigen) show high safety, but reduced immunogenicity and fail to induce similar durable antibody responses in humans. Induction of a strong and long lasting immune response to pathogens as well as disease-associated antigens is thus very difficult to obtain with simple subunit vaccines.

The licensed Human papillomavirus (HPV) vaccines (Cervarix®, Gardasil®, and Gardasil 9®), however, make an important exception, since they seem to have comparable immunogenicity to live-attenuated vaccines and can induce highly potent, durable antibody responses in humans, even after a single dose (Schiller et al., 2018, Schiller et al., 2012, De Vincenzo et al., 2014). Importantly, this vaccine is formed by the self-assembly of the HPV major capsid protein into virus-like particles (VLPs), which is believed to be the cause of its high potency.

In fact, many studies have established a strong causal link between the high immunogenicity of VLPs and their structural similarities to native viruses, and several strategies have been pursued, exploiting VLPs as scaffolds for the presentation of heterologous antigens, including self-antigens. These studies have collectively shown that multivalent, repetitive antigen display can, in fact, significantly increase the immunogenicity of an antigen and even induce long-lasting immunity.

We have previously described the development of a modular VLP-based vaccine platform using a split-protein (Tag/Catcher) conjugation technology for attaching antigens in a unidirectional, multivalent and repetitive manner on the surface of VLPs

(WO 2016/112921). To date, this technology represents a highly powerful and versatile vaccine platform.

5 DNA- and/or RNA-based vaccines, in contrast to protein-based vaccines, possess major advantages, mainly due to their simplicity and low cost of production; omitting the steps of recombinant expression and purification of the vaccine antigen. In fact, usually clinical vaccine batches can already be generated shortly after a sequence encoding the antigen has become available. A further advantage is that the manufacturing process is cell-free and highly scalable. Additionally, the manufacturing of multiple
10 different vaccines at a facility requires minimal adaptation of the manufacturing processes to the specific vaccine formulations. Finally, *in vivo* expression of complex proteins that are difficult or impossible to generate recombinantly with current expression systems is possible with these vaccines.

15 However, so far DNA vaccines have been shown to induce only relatively weak immune responses against the antigen when tested in humans and non-human primates, limiting their commercial exploitation.

20 There is thus an urgent need for a vaccine that combines the high immunogenicity of multivalent particulate vaccines with the manufacturing advantages associated with nucleic acid based vaccines.

Summary

25 The present invention provides nucleic acid based vaccines, which upon delivery into eukaryotic cells during vaccination, are translated into self-assembling nanoparticles displaying the vaccine antigen in a unidirectional, repetitive and multivalent manner by exploiting a split-protein Tag/Catcher conjugation system.

30 The repetitive, multivalent antigen display increases the immunogenicity of the vaccine antigen, enabling induction of a strong antigen-specific immune response after vaccination. At the same time, the nucleic acid (DNA and/or mRNA) based vaccine technology holds major benefits in terms of manufacturing, as the up- and down-stream processes associated with recombinant production of the vaccine antigen can be omitted. Additionally, delivery of the vaccine antigen as a nucleic acid sequence allows

in vivo translation of the encoded proteins, which may be otherwise difficult or impossible to produce recombinantly.

Herein is provided a composition comprising:

- 5 i. a first polynucleotide encoding a protein fused to a first peptide tag; and
 ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

 wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

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Herein is also provided a composition as disclosed herein for use in the the prophylaxis and/or treatment of a disease in a subject in need thereof.

- 15 Herein is also provided a method of preventing or treating a disease in a subject in need thereof.

Herein is also provided an expression system comprising:

- i. a first polynucleotide encoding a protein fused to a first peptide tag; and
20 ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

 wherein upon expression of the first and second polynucleotides in a cell, the antigen and the protein are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

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Herein is also provided a cell expressing:

- i. a first polynucleotide encoding a protein fused to a first peptide tag, preferably as defined in any one of the preceding claims; and
30 ii. a second polynucleotide encoding an antigen fused to a second peptide tag, preferably as defined in any one of the preceding claims,

 wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

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Herein is also provided a host cell comprising an expression system as disclosed herein.

Herein is also provided a method of administering a composition for use in the prophylaxis and/or treatment of a disease in a subject in need thereof comprising the steps of

- i. obtaining at least one composition as disclosed herein, and
- ii. administering said composition to a subject at least once for prophylaxis and/or treatment of a disease as defined herein.

Herein is also provided a kit of parts comprising

- i. a composition or an expression system as defined herein, and
- ii. optionally, a medical instrument or other means for administering the composition, and
- iii. instructions for use.

Description of the drawings

Figure 1: Confocal laser scanning microscopy (CLSM) of C2C12 mouse cells 72 hours after co-transfection with SpyCatcher (SpyC)-eGFP and Spytagged (SpytT) HBcore.

The picture shows distinct particulate enhanced green fluorescent protein (eGFP) fluorescent signals with a peri-nuclear distribution (all panels).

Figure 2: Confocal laser scanning microscopy (CLSM) of C2C12 mouse cells 72 hours after co-transfected with SpyCatcher (SpyC)-eGFP and Spytagged (SpytT) AP205 coat protein. The picture shows an even green smear from the eGFP fluorescence throughout the cell (all panels). Scale bar shows 50 μ m.

Figure 3: Western blot (WB) probed with polyclonal anti-eGFP antibodies for detection of eGFP in pre (UC input) and post ultracentrifugation (UC) fractions (3-21 and 31) of sonicated C2C12 cell co-transfected with Spytagged (SpytT) AP205 coat protein and SpyCatcher (SpyC)-eGFP. The theoretical size of eGFP is 41kDa whereas the conjugation of eGFP and AP205 would be 59kDa. A band of approximately 59kDa is seen weakly in the eGFP and more strongly in the UC input sample, but not in any of the post UC fractionated samples i.e. there is no indication that AP205 particles have formed to which eGFP have bound.

Figure 4: Western blot (WB) probed with polyclonal anti-eGFP antibodies for detection of eGFP in pre (UC input) and post ultracentrifugation (UC) fractions (3-21 and 31) of sonicated C2C12 cell co-transfected with Spytagged (SpyC) HBcore antigen and SpyCatcher (SpyC)-eGFP. The theoretical size of eGFP is 41 kDa whereas the conjugation of eGFP and HBcore antigen is 64kDa. A band of approximately 64 kDa is seen in the UC-input sample as well as in post-UC fractions, indicating that particulate structures have formed from the conjugated SpyTHBc and SpyC eGFP proteins.

Figure 5: Vector map of pVAX1 (V26020, thermoFisher). CMV promoter: bases 137-724; T7 promoter/priming site: bases 664-683; multiple cloning site: bases 696-811; bovine growth hormone (BGH) reverse priming site: bases 823-840; BGH polyadenylation signal: bases 829-1053; kanamycin resistance gene: bases 1226-2020; pUC origin: bases 2320-2993.

Figure 6: Expression of particle-forming subunit proteins from plasmid DNA. Western blot (WB) images of particle-forming subunits after plasmid DNA transfection in HEK cells. HEK cells were transfected with plasmid DNA encoding for particle forming subunit. Cells and supernatant were harvested 6 days after transfection and ran on a WB. Relevant antibodies coupled to horseradish peroxidase (HRP) were used for detection of the particle forming subunit.

- A. Sample: sign3-SpyC-i301-ctag. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 35.7kDa.
- B. Sample: sign8-tandemHBc-SpyC. Primary Ab: aHBc (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 52.3kDa.
- C. Sample: sign8-tandemHBc-SpyT. Primary Ab: aHBc (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 23.12kDa.
- D. Sample: sign9-Ferritin-SpyC. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 32.6kDa.

These pictures show band of the expected size in cell and supernatant samples for all constructs, thus indicating expression and secretion of the particle-forming subunit proteins in HEK cells, after plasmid DNA transfection.

Figure 7: Expression of soluble proteins from plasmid DNA. Western blot images of soluble proteins after plasmid DNA transfection in HEK cells. HEK cells were transfected with plasmid DNA encoding for soluble protein. Cells and supernatant were

harvested 6 days after transfection and ran on a WB. Relevant antibodies coupled to HRP were used for detection of the soluble protein.

- A. Sample: sign8-SpyT-eGFP. Primary Ab: aGFP-HRP. Expected size: 30.8kDa.
- B. Sample: sign8-eGFP. Primary Ab: aGFP-HRP. Expected size: 29kDa.
- 5 C. Sample: sign8-SpyC-His. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 15.4kDa.
- D. Sample: sign7-Pfs25-SpyT-Ctag. Primary Ab: aCtag-biotin. Secondary Ab: strep-HRP. Expected size: 24.3kDa.
- E. Sample: sign8-SpyC-eGFP. Primary Ab: aGFP-HRP. Expected size: 41.7kDa.
- 10 These pictures show bands of the expected size in cell and supernatant for all constructs, thus indicating expression and secretion of soluble proteins in HEK cells, after plasmid DNA transfection.

Figure 8: Verification of the conjugation of eGFP to different particle-forming proteins.

- 15 Western blot (WB) images of coupled eGFP to particle forming proteins after plasmid DNA co-transfection in HEK cells. HEK cells were co-transfected with plasmid DNA encoding for eGFP+tag/catcher and plasmid DNA encoding for particle-forming proteins+corresponding tag/catcher. Cells and supernatant were harvested 6 days after co-transfection. Relevant antibodies coupled to HRP were used for detection of the coupled GFP to the particle forming subunit.
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- A. Sample: sign8-SpyT-eGFP and sign9-SpyC-Ferritin. Primary Ab: aGFP-HRP. Expected size: 62kDa.
- B. Sample: sign8-SpyC-eGFP and sign8-Hbc-SpyT. Primary Ab: aHbc (mouse serum). Secondary Ab: anti-mouse-HRP. Expected size: 64.8kDa.
- 25 C. Sample: sign8-tandemHbc-SpyC and sign8-SpyT-eGFP. Primary Ab: aGFP-HRP. Expected size: 83.1kDa.
- D. Sample: sign3-SpyC-i301-ctag and sign8-SpyT-eGFP. Primary Ab: aGFP-HRP. Expected size: 66.5kDa.
- E. Sample: sign9-SpyT-E2 and sign8-SpyC-eGFP. Primary Ab: aGFP-HRP. Expected size: 72.45kDa.
- 30 F. Sample: sign9-SpyT-LS and sign8-SpyC-eGFP. Primary Ab: aGFP-HRP. Expected size: 61.7kDa.

These pictures show bands of the expected size for coupled eGFP to different particle-forming subunit proteins in cells and supernatant for all constructs. This indicates that eGFP and the particles with the corresponding tag/catcher are able to couple *in vitro*, after co-transfection in HEK cells.

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- Figure 9:** Verification of the conjugation of SpyC to different particle-forming proteins. Western blot (WB) images of coupled SpyC to particle forming proteins after plasmid DNA co-transfection in HEK cells. HEK cells were co-transfected with plasmid DNA encoding for SpyC and plasmid DNA encoding for particle-forming proteins+corresponding tag. Cells and supernatant were harvested 6 days after co-transfection. Relevant antibodies coupled to HRP were used for detection of the coupled SpyC to the particle forming subunits.
- A. Sample: sign8-SpyC-His and sign9-SpyT-E2. Primary Ab: aHis-HRP. Expected size: 46.15kDa.
 - B. Sample: sign8-SpyC-His and sign9-LS-SpyT. Primary Ab: aHis-HRP. Expected size: 35.4kDa.
 - C. Sample: sign8-SpyC-His and sign8-Hbc-SpyT. Primary Ab: aHis-HRP. Expected size: 38.5kDa.
 - D. Sample: sign8-Norovirus-SpyT and sign8-SpyC-His. Primary Ab: aHis-HRP. Expected size: 78.16kDa.

These pictures show bands of the expected size for coupled SpyC to different particle-forming subunit proteins in cells and supernatant for all constructs. This indicates that SpyC and the particle with the corresponding tag/catcher are able to couple *in vitro*, after co-transfection in HEK cells.

- Figure 10:** Verification of the conjugation of Pfs25 to different particle-forming proteins. Western blot (WB) images of coupled Pfs25 to particle forming proteins after plasmid DNA co-transfection in HEK cells. HEK cells were co-transfected with plasmid DNA encoding for Pfs25-SpyT and plasmid DNA encoding for particle-forming proteins+corresponding catcher. Cells and supernatant were harvested 6 days after co-transfection. Relevant antibodies coupled to HRP were used for detection of the coupled Pfs25 to the particle forming subunit.

- A. Sample: sign9-SpyC-Ferritin and sign7-Pfs25-SpyT-Ctag. Primary Ab: aCtag-biotin. Secondary Ab: strep-HRP. Expected size: 56.9a.
- B. Sample: sign3-SpyC-i301-Ctag and sign7-Pfs25-SpyT-Ctag. Primary Ab: aCtag-biotin. Secondary Ab: strep-HRP. Expected size: 60kDa.

These pictures show bands of the expected size for coupled Pfs25 to different particle-forming subunit proteins in cell and supernatant for all constructs. This indicates that Pfs25 and the particle with the corresponding tag/catcher are able to couple *in vitro*, after co-transfection in HEK cells.

Figure 11: Verifying nanoparticle formation by ultracentrifugation (UC) and WB.

Western blot (WB) image of UC fractions from HEK cells transfected supernatant. HEK cells were transfected with plasmid DNA encoding for particle-forming subunit proteins. Supernatant was harvested 6 days after transfection and loaded on an optiprep density gradient. The gradient was spun down for 3h30, 47800RPM, 16°C, and fractionated into 12 fractions. Each fraction was run on a WB where relevant antibodies coupled to HRP were used for detection of the particle forming subunit. If any particles are formed, bands of the corresponding size will be expected in fractions 3-8.

- A. Sample: sign3-SpyC-i301-Ctag. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size 36kDa.
- B. Sample: sign8-tandemHBc-SpyCatcher. Primary Ab: aHBc (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size 52kDa.
- C. Sample: sign9-Ferritin-SpyC. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size 32.6kDa.

These pictures show particle formation in all constructs, as we can see bands of the expected size present in relevant fractions (3-8). Thus, this indicates that after transfection with plasmid DNA in HEK cells, there was not only expression and secretion of the particle subunit, but also formation of a particle *in vitro*.

Figure 12: Verifying coupled nanoparticle formation by UC and Western blot (WB).

WB image of UC fractions from HEK cells transfected supernatant. HEK cells were co-transfected with plasmid DNA encoding for soluble antigen+tag/catcher and plasmid DNA encoding for particle-forming proteins+corresponding tag/catcher. Supernatant was harvested 6 days after transfection and loaded on an optiprep density gradient. The gradient was spun down for 3h30, 47800RPM, 16°C, and fractionated into 12 fractions. Each fraction was run on a WB where relevant antibodies coupled to HRP were used for detection of the coupled antigen to the particle forming subunit. If any particles are formed, bands of the corresponding size will be expected in fractions 3-8.

- A. Sample: sign8-SpyT-eGFP + sign9-SpyC-Ferritin. Primary Ab: aGFP-HRP. Expected size 62kDa.
- B. Sample: sign8-SpyC-His + sign9-SpyT-E2. Primary Ab: aHis-HRP. Expected size 46.15kDa.
- C. Sample: sign8-SpyC-His + sign9-LS-SpyT. Primary Ab: aHis-HRP. Expected size 35.4kDa.

- D. Sample: sign9-SpyC-Ferritin + sign7-Pfs25-SpyT. Primary Ab: aCtag-biotin.
Secondary Ab: strep-HRP. Expected size 56.9kDa.
- E. Sample: sign3-SpyC-i301 + sign7-Pfs25-SpyT. Primary Ab: aCtag-biotin.
Secondary Ab: strep-HRP. Expected size 60kDa.
- 5 F. Sample: sign9-SpyT-E2 + sign8-SpyC-eGFP. Primary Ab: aGFP-HRP.
Expected size 72.45kDa.
- G. Sample: sign9-LS-SpyT + sign8-SpyC-eGFP. Primary Ab: aGFP-HRP.
Expected size 61.7kDa.

10 These pictures show coupled particle formation in all constructs, as we can see bands of the expected size present in relevant fractions (3-8). Thus, this indicates that after co-transfection with plasmid DNA in HEK cells, there was not only expression and secretion of the particle subunit and the soluble antigens, but also formation of a particle coupled to an antigen *in vitro*.

15 **Figure 13:** Visualisation of particle formation by transmission electronic microscopy (TEM). Electron microscopy images of UC purified supernatant from HEK cells transfected with plasmid DNA. HEK cells were transfected with plasmid DNA encoding for particle forming subunit or co-transfected with plasmid DNA encoding for soluble antigen+tag/catcher and plasmid DNA encoding for particle-forming
20 proteins+corresponding tag/catcher. Supernatant was harvested 6 days after transfection and loaded on an optiprep density gradient. The gradient was spun down for 3h30, 47800RPM, 16°C, and fractionated into 12 fractions. The fractions containing particle were pooled and dialysis into 1xPBS for TEM imaging.

- A. Sample: sign9-Ferritin-SpyC. Expected size: 12nm.
- 25 B. Sample: sign8-SpyC-His + sign9-LS-SpyT. Expected size: 12nm.
- C. Sample: sign9-SpyT-E2 + sign8-SpyC-His. Expected size: 12nm.
- D. Sample: sign8-SpyT-eGFP + sign9-SpyC-Ferritin. Expected size: 12nm
- E. Sample: sign3-SpyC-i301-Ctag + sign7-Pfs25-SpyT-Ctag. Expected size: 25nm.
- F. Sample: sign9-SpyC-Ferritin + sign7-Pfs25-SpyT-Ctag. Expected size: 12nm.

30 These pictures show particles of the expected size formed in the SN of transfected or co-transfected cells. Thus, we can further confirm that particles and coupled particles are able to be formed in the supernatant of transfected or co-transfected cells *in vitro* from plasmid DNA.

35 **Figure 14:** Verification that conjugation of the antigen to the nanoparticle-forming protein occurred intracellularly. Western blot (WB) images of coupled soluble antigen to

particle forming proteins after plasmid DNA co-transfection in HEK cells. HEK cells were co-transfected with plasmid DNA encoding for a soluble antigen+tag/catcher and plasmid DNA encoding for particle-forming proteins+corresponding tag/catcher. Cells and supernatant were harvested 6 days after co-transfection. Upon harvest, cells were resuspended in 1x SDS+DTT, to prevent further coupling. Cell samples in 1x SDS+DTT and supernatant were run on WB. Relevant antibodies coupled to HRP were used for detection of the coupled soluble antigen to the particle forming subunit. Thus if any coupling is seen on WB, it had to occur intracellularly before harvest.

- A. Sample: sign8-SpyC-His and sign9-LS-SpyT. Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 35.4kDa.
- B. Sample: sign8-SpyT-eGFP + sign9-SpyC-Ferritin (B1822+B1772). Primary Ab: aSpyC (mouse sera). Secondary Ab: anti-mouse-HRP. Expected size: 63.4kDa.
- C. Sample: sign3-SpyC-i301-ctag (B1774) and sign8-SpyT-eGFP (B1822). Primary Ab: aHis-HRP. Expected size: 66.5kDa.
- D. Sample: sign8-eGFP-SpyC (B2009) and sign9-LS-SpyT (B1929) Lane 1 (cell), 2 (SN) compared to sign8-eGFP (B2033) and sign9-LS-SpyT (B1929) Lane 3 (cell), 4 (SN). Primary Ab: aGFP-HRP. Expected: B2009+B1929 should be able to couple while B2033+B1929 should not (since there are not catcher on B2033).

These pictures show that particles and soluble antigens are able to couple intracellularly, and not only in the supernatant. Indeed, a band was visualised of the expected coupling size in cells harvested with SDS+DTT, thus indicating that the coupling occurred inside the cells in the in vitro culture.

Figure 15: Verification of the immunogenicity of the plasmid DNA vaccine encoding for particle forming subunit. ELISA titers of total IgG in mice after plasmid DNA immunization. Plasmid DNA encoding for particles and/or soluble antigen was used for vaccination in mice. Balb/c mice were immunized with 30ug of LS-SpyT (B1929) and 30ug of SpyC (B1928) (N=6), or 30ug of SpyC (B1928) (N=4), or 30ug of E2-SpyT (B1930) and 30ug of SpyC (B1928). DNA was formulated in PBS and injected in the right thigh muscle. Mice were immunized on day 0 and week 5, and blood was drawn on week 3 and 4 post prime and post boost. Serum was isolated from the blood and run on ELISA for detection of anti-SpyC IgG. For that purpose, 96-well plates (Nunc MaxiSorp) were coated SpyC in PBS. IgG against SpyC were detected with a HRP conjugated goat anti-mouse IgG (Life technologies, A16072). Plates were developed with TMB X-tra substrate (Kem-En-Tec, 4800A) and absorbance was measured at 450nm.

- A. ELISA titers against SpyC 3 weeks and 4 weeks post prime immunization in Balb/c mice
- B. ELISA titers against SpyC 3 weeks and 4 weeks post boost immunization in Balb/c mice

5 These ELISA titers show that mice receiving DNA encoding for the particles and DNA encoding for SpyC, have higher IgG titers against SpyC after a first immunization, compared to mice receiving only DNA encoding for SpyC. Additionally, this trend is even higher after a boost immunization. This is true for both groups of mice that have received SpyC in combination with either the LS particle or the E2 particle.

10 Detailed description of the invention

The present disclosure provides nucleic acid based vaccines, which upon delivery into eukaryotic cells during vaccination, are translated into self-assembling nanoparticles displaying the vaccine antigen in a unidirectional, repetitive and multivalent manner by exploiting a split-protein Tag/Catcher conjugation system.

15 The repetitive, multivalent antigen display increases the immunogenicity of the vaccine antigen, enabling induction of a strong antigen-specific immune response after vaccination. At the same time, the nucleic acid (DNA and/or mRNA) based vaccine technology holds major benefits in terms of manufacturing, as the up- and down-stream processes associated with recombinant production of the vaccine antigen can be omitted. In addition, delivery of the vaccine antigen as a nucleic acid sequence allows *in vivo* translation of the encoded proteins, which may be otherwise difficult or impossible to produce recombinantly.

25 The solution of the present invention represents a novel approach for making a versatile nucleic-acid based vaccine delivery platform capable of efficiently displaying antigen epitopes and of inducing long-term protective immunity.

Definitions

30 The term "isopeptide bond" as used herein, refers to an amide bond between a carboxyl group and an amino group at least one of which is not derived from a protein main chain or alternatively viewed is not part of the protein backbone. An isopeptide bond may form within a single protein or may occur between two peptides or a peptide and a protein. Thus, an isopeptide may form intramolecularly within a single

protein or intermolecularly i.e. between two peptide/protein molecules. Typically, an isopeptide bond may occur intramolecularly between two reactive amino acids: a lysine and an asparagine or aspartate. For the process to occur the two reactive amino acids need to be in close proximity in a hydrophobic environment often including aromatic residues. Finally, the autocatalytic process may be facilitated by a catalytic aspartate or glutamate residue, which do not themselves take part in the isopeptide bond.

In the case of intermolecular isopeptide bonds, the bond typically occurs between a lysine residue and an asparagine, aspartic acid, glutamine, or glutamic acid residue or the terminal carboxyl group of the protein or peptide chain or may occur between the alpha-amino terminus of the protein or peptide chain and an asparagine, aspartic acid, glutamine or glutamic acid. Each residue of the pair involved in the isopeptide bond is referred to herein as a reactive residue. Thus, an isopeptide bond may form between a lysine residue and an asparagine residue or between a lysine residue and an aspartic acid residue. Particularly, isopeptide bonds can occur between the side chain amine of lysine and carboxamide group of asparagine.

The term "open reading frame" as used herein refers to a nucleotide sequence comprising in a 5' to 3' direction 1) a translation initiation codon, 2) one or more codons coding for one or more gene products of interest, preferably one or more protein, and 3) a translation stop codon, whereby it is understood that 1), 2) and 3) are operably linked in frame. The open reading frame will thus consist of a multiple of 3 nucleotides (triplets).

The term "sequence variant" refers to a polypeptide and/or polynucleotide sequence with at least 70%, such as 75%, such as 80%, such as 85%, such as 90%, such as 95%, such as 96%, such as, 97%, such as 98%, such as 99%, such as 99,5%, such as 100% sequence identity to said polypeptide and/or polynucleotide sequence.

The term "antigenic variant" refers to a variant of the full length of a polypeptide or a fragment of said polypeptide, wherein the fragment comprises an epitope that is recognized by a cytotoxic T lymphocyte, helper T lymphocyte and/or B cell of the host. Said fragment may be more immunogenic and thus elicit a stronger and/or longer lasting immune response than the original polypeptide from which it is derived. Preferably, the immunogenic portion of the antigenic variant will comprise at least 30%,

preferably at least 50%, especially at least 75% and in particular at least 90% (e.g. 95% or 98%) of the amino acid sequence of the reference sequence. The immunogenic portion will preferably comprise all of the epitope regions of the reference sequence. The immunogenicity of said antigenic variant may be verified by any of the known methods in the art, such as the methods described in Wadhwa et al., 2015.

A first peptide tag and a second peptide tag (or binding partner) as discussed herein refer to a first and second peptide tag which bind to one another via an isopeptide bond, preferably a spontaneous isopeptide bond. Preferably the first peptide tag comprises one of the reactive residues involved in the isopeptide bond and the second peptide tag comprises the other reactive residue involved in that isopeptide bond.

The term "spontaneous" as used herein refers to a bond, in particular an isopeptide bond, which can form in a protein or between peptides or proteins (e.g. between the first peptide tag and the second peptide tag) without any other agent (e.g. an enzyme catalyst) being present and/or without chemical modification of the protein or peptide e.g. without native chemical ligation or chemical coupling. A spontaneous isopeptide bond may therefore form of its own accord in the absence of enzymes or other exogenous substances or without chemical modification. Particularly however, a spontaneous isopeptide or covalent bond may require the presence of a glutamic acid or an aspartic acid residue in one of the peptides/proteins involved in the bond to allow formation of the bond.

The term "virus-like particle" or "VLP" refers to one or several recombinantly expressed viral proteins such as viral capsid proteins, which spontaneously assemble into macromolecular particulate structures mimicking the morphology of a virus coat, but lacking infectious genetic material.

The term "particle" herein refers to a virus-like particle or to a nanoparticle, on the surface of which an antigen can be displayed as described herein. The surface may be an internal surface, i.e. facing towards the inner part of the particle, or an external surface, i.e. facing towards the surroundings of the particle.

The term "self-assembly" refers to a process in which a system of pre-existing components, under specific conditions, adopts a more organised structure through interactions between the components themselves. In the present context, self-

assembly refers to the intrinsic capacity of a protein, such as a viral protein, for example a capsid protein, and/or a phage protein to self-assemble into particles, in particular virus-like particles in the absence of other viral proteins, when subjected to specific conditions. "Self-assembly" does not preclude the possibility that cellular proteins, *e.g.* chaperones, participate in the process of intracellular VLP or nanoparticle assembly. The self-assembly process may be sensitive and fragile and may be influenced by factors such as, but not limited to, choice of expression host, choice of expression conditions, and conditions for maturing the virus-like particles. Virus capsid proteins may be able to form VLPs on their own, or in combination with several virus capsid proteins, these optionally all being identical.

The term "consistent orientation", as used herein, refers to the orientation of an antigen and its spatial orientation on the surface of a particle as disclosed herein, *i.e.* on an internal surface or on an external surface of the particle, preferably at least on the external surface. When linking an antigen fused to a second peptide tag to protein comprising a second peptide tag as disclosed herein, one molecule of antigen can only be linked to a single particle-forming protein at unique sites in both the antigen and the protein, thus creating a uniform and/or consistent presentation of said antigen with a consistent orientation. In contrast, for example, a streptavidin homo-tetramer may crosslink several proteins on the outer surface of a biotinylated VLP, thus creating an irregular and non-consistent orientation of said antigen. Besides, it is highly challenging to use streptavidin as a bridging molecule *e.g.* for conjugating biotinylated antigens onto biotinylated VLPs, since the multiple biotin binding sites will allow cross-linking and aggregation of the biotinylated VLPs.

The term "regularly spaced" as used herein, refers to antigens of the present invention which forms a pattern on a surface of a VLP or nanoparticle. Such pattern may be symmetric, circle-like, and/or bouquet like pattern of antigens.

The term "treatment" refers to the remediation of a health problem. Treatment may also be preventive and/or prophylactic or reduce the risk of the occurrence of a disease and/or infection. Treatment may also be curative or ameliorate a disease and/or infection.

The term “prophylaxis” refers to the reduction of risk of the occurrence of a disease and/or infection. Prophylaxis may also refer to the prevention of the occurrence of a disease and/or infection.

- 5 The term “loop” refers to a secondary structure of a polypeptide where the polypeptide chain reverses its overall direction and may also be referred to as a turn.

- 10 The term “vaccine cocktail” refers to a mixture of antigens administered together. A vaccine cocktail may be administered as a single dose or as several doses administered over a period of time. Time intervals may be, but are not limited to, administration within the same year, month, week, day, hour and/or minute. Co-vaccination and vaccine cocktail may be used interchangeably.

- 15 The term “self-antigens” refers to endogenous antigens that have been generated within previously normal cells as a result of abnormal cell metabolism.

Compositions

Herein is provided a composition comprising:

- 20 i. a first polynucleotide encoding a protein fused to a first peptide tag; and
 ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

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Said compositions are useful for prophylaxis and/or treatment of a disease or a disorder, such as those described herein below.

- 30 Administration of said composition comprising said first and second polynucleotides in a subject may induce a stronger immune response in said subject compared to administration of a composition comprising the polypeptides encoded by said first and second polynucleotides, wherein said first and second polypeptide have been linked via an isopeptide bond, or via an ester bond, between the first peptide tag and the second peptide tag prior to administration in the subject.

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The first peptide tag and the second peptide tag are selected from peptides having the intrinsic ability to form an isopeptide bond, or an ester bond, thereby binding to one another. The first polynucleotide encodes a protein which has the ability to form, preferably spontaneously, a particle, such as a nanoparticle or a virus-like particle (VLP). Upon expression in a cell, the first polynucleotide thus leads to the formation of particles formed by the protein fused to the first peptide tag, while the second polynucleotide leads to expression of a fusion protein comprising or consisting of an antigen fused to the second peptide tag. The assembled particles may closely resemble viruses or other pathogenic organisms that are recognized by the immune system, but are non-infectious because they contain no pathogenic genetic material. Due to the spontaneous formation of an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, particles are formed, which display the antigen on their surface, preferably on their external surface. Importantly, besides consistent antigen orientation, such nanoparticles and VLPs unexpectedly have unusually beneficial antigen display characteristics including consistent antigen orientation, high-density, and regular spacing. The obtained nanoparticles and VLPs can thus induce strong humoral responses and overcome B cell tolerance. The successful formation of such nanoparticles or VLPs may be assessed by relevant methods as known to the person skilled in the art, such as those disclosed in Example 2 and 3 of the present disclosure. Such nanoparticles or VLPs surprisingly show an increased efficiency of antigen coupling compared to chemical coupling methods, and allow antigen display with consistent antigen orientation, high-density, and regular spacing, for both large and small antigens.

The first and second polynucleotides may be DNA or RNA; preferably, the first polynucleotide and the second polynucleotides are both DNA, or both RNA.

The RNA constructs and/or the mRNA transcribed from the DNA constructs may be polycistronic. In some embodiments, the first and the second polynucleotides are encoded on the same ribonucleic acid molecule. In some embodiments, the first and the second polynucleotides lie within the same open reading frame, whereby only one promoter sequence is needed to transcribe both polynucleotides. In some embodiments, the first and the second polynucleotides lie within separate open reading frames and may thus be regulated by separate promoters.

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Proteins

In preferred embodiments, the protein is a particle-forming protein. For example, the protein may be a viral capsid protein or a viral envelope protein such as a glycoprotein. In some embodiments, the protein is from a mammalian virus, for example a human virus.

In some embodiments, the protein is a protein from a hepatitis virus such as hepatitis B or E, for example a core protein from hepatitis B virus. In some embodiments, the protein is a protein from a norovirus such as NoV. In some embodiments, the protein is a protein from a papilloma virus such as Human Papilloma Virus (HPV), preferably HPV16 or HPV18, such as HPV L1. In some embodiments, the protein is a protein from a polyomavirus such as polyomavirus vp1 (PyV). In some embodiments, the protein is a protein from a calicivirus such as feline calicivirus (FCV), preferably FCV VP1. In some embodiments, the protein is a protein from a circovirus such as a porcine circovirus (PCV), preferably PCV2 ORF2. In some embodiments, the protein is a protein from a nervous necrosis virus (NNV), such as NNV coat protein. In some embodiments, the protein is a protein from a parvovirus such as canine parvovirus (CVP), preferably CPV VP2, goose parvovirus (GPV) or porcine parvovirus (PPV), preferably structural proteins from GPV or PPV, or parvovirus B19. In some embodiments, the protein is a protein from a protoparvovirus such as an enteritis virus, for example mink enteritis virus (MEV), preferably MEV VP2, or duck plague virus (DPV), preferably a DPV structural protein.

The protein may be a protein from a plant virus, such as a cowpea virus, a tobacco virus, a tomato virus, a cucumber virus or a potato virus. In some embodiments, the plant virus is a mosaic virus, preferably Cowpea mosaic virus (CPMV). In some embodiments, the plant virus is a tobacco mosaic virus (TMV). In some embodiments, the plant virus is a tomato spotted wilt virus (TSWV). In some embodiments, the plant virus is a tomato yellow leaf curl virus (TYLCV). In some embodiments, the plant virus is a cucumber mosaic virus (CMV). In some embodiments, the plant virus is a potato virus Y (PVY).

In some embodiments, the protein is a bacteriophage protein, such as a protein from *Salmonella* virus P22, MS2, QBeta, PRR1, PP7, bacteriophage R17, bacteriophage fr, bacteriophage GA, bacteriophage SP, bacteriophage M11, bacteriophage MX1,

bacteriophage NL95, bacteriophage f2 or Cb5. Additional relevant bacteriophage proteins are described in Lieknina et al., 2019.

5 In some embodiments, the protein is a protein from a virus which is common in mammals, in particular in humans. Without being bound by theory, such viruses may be better suited for expression in mammalian cells, in particular human cells, than viruses commonly found in non-mammalian organisms. Thus, in some embodiments, the virus is a hepatitis virus, such as a hepatitis B or E virus. In some embodiments, the virus is a norovirus. In some embodiments, the virus is a papilloma virus, such as
10 Human Papilloma Virus (HPV), preferably HPV16 or HPV18. In some embodiments, the virus is a polyomavirus. In some embodiments, the virus is a parvovirus.

In some embodiments, the protein is a particle forming protein, such as ferritin, i301, replicase polyprotein 1a (pp1a) or a lumazine synthase. Other particle-forming proteins
15 are known in the art and may also be used.

First and second peptide tags

Peptide pairs, consisting of a first peptide tag and a second peptide tag which are capable of binding to one another via the (spontaneous) formation of an isopeptide bond, are known in the art, or can be designed or obtained by methods known in the
20 art, in particular as described in Zakeri et al., 2012, and in Zakeri et al., 2010.

The term "peptide tag" as used herein generally refers to a small peptide fragment which may be designed or derived directly from a protein which naturally forms an intramolecular isopeptide bond. Peptide tags may also be identified by using a known
25 binding partner, for example derived from a protein naturally forming an intramolecular isopeptide bond, to screen a peptide library. The candidate peptide tags may thus be from a library, e.g. a peptide library, which can be screened for candidate peptide tags. They may also be designed *in silico*.

30 A peptide pair as understood herein thus consists of two peptide tags which can interact via the spontaneous formation of an isopeptide bond, or via an ester bond. Generally, these are also called a "tag and catcher" system, where the longer of the two peptide tags is termed "catcher" while the shorter of the two peptide tags is termed

“tag”. For instance, the SpyTag/SpyCatcher system consists of a first peptide tag (SpyTag) and a second peptide tag (SpyCatcher).

5 In some embodiments, the peptide pair as understood herein consists of two peptide tags which can interact via the spontaneous formation of an ester bond. Useful peptide tags able to form such spontaneous ester bonds are further described in Young et al., 2017.

10 The “tag” may be between 5-50 amino acids in length e.g. from 10, 20, 30, 40 to 50 amino acids in length and may bind covalently via an isopeptide bond to a binding partner as defined herein. Thus, the “tag” may comprise one reactive residue involved in an isopeptide bond in the isopeptide protein used to design the binding partner (and the binding partner may comprise the other reactive residue involved in that bond), as described herein.

15 In some embodiments, the “tag” has a length between 7 and 47 amino acids, such as between 8 and 46 amino acids, such as between 9 and 45 amino acids, such as between 10 and 44 amino acids, such as between 11 and 43 amino acids, such as between 12 and 42 amino acids, such as between 13 and 41 amino acids, such as
20 between 14 and 40 amino acids, such as between 15 and 39 amino acids, such as between 16 and 38 amino acids, such as between 17 and 37 amino acids, such as between 18 and 36 amino acids, such as between 19 and 35 amino acids, such as between 20 and 34 amino acids, such as between 21 and 33 amino acids, such as between 22 and 32 amino acids, such as between 23 and 31 amino acids, such as
25 between 24 and 30 amino acids, such as between 25 and 29 amino acids, such as between 26 and 28 amino acids, such as 27 amino acids. In some embodiments, the “tag” has a length of 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45 or 46 amino acids.

30 In some embodiments, the “catcher” is at least 20 amino acids in length. Preferably, the “catcher” has a length of 5 amino acids or more, such as 10 amino acids or more, such as 15 amino acids or more, such as 20 amino acids or more, such as 25 amino acids, such as 30 amino acids, such as 35 amino acids, such as 40 amino acids, such as 45
35 amino acids, such as 50 amino acids, such as 60, 70, 80, 90, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325 or 350 amino acids or more. In preferred embodiments, the

“catcher” is at least 20 amino acids in length. In some embodiments, the “catcher” is between 75 to 125 amino acids in length

5 Preferably, the “catcher” has an amino acid sequence which consists of more amino acid residues than the “tag”.

In some embodiments, one of the first and second peptide tags is selected from the group consisting of a SpyTag (SEQ ID NO: 1), a SdyTag (SEQ ID NO: 2), a SnoopTag (SEQ ID NO: 3), a PhoTag (SEQ ID NO: 4), an EntTag (SEQ ID NO: 5), a KTag, a
10 BacTag (SEQ ID NO: 15), a Bac2Tag (SEQ ID NO: 16), a Bac3Tag (SEQ ID NO: 17), a Bac4Tag (SEQ ID NO: 18), a RumTrunkTag (SEQ ID NO: 13 or SEQ ID NO: 14), a Rum7Tag (SEQ ID NO: 12), a RumTag (SEQ ID NO: 6), a Rum2Tag (SEQ ID NO: 7), a Rum3Tag (SEQ ID NO: 8), a Rum4Tag (SEQ ID NO: 9), a Rum5Tag (SEQ ID NO: 10), a Rum6Tag (SEQ ID NO: 11) and a Bac5Tag (SEQ ID NO: 19). Nucleic acid
15 sequences encoding said tags are as follows: SpyTag (SEQ ID NO: 35), SdyTag (SEQ ID NO: 36), SnoopTag (SEQ ID NO: 37), PhoTag (SEQ ID NO: 38), EntTag (SEQ ID NO: 39), KTag, BacTag (SEQ ID NO: 49), Bac2Tag (SEQ ID NO: 50), Bac3Tag (SEQ ID NO: 51), Bac4Tag (SEQ ID NO: 52), RumTrunkTag (SEQ ID NO: 47 or SEQ ID NO: 48), Rum7Tag (SEQ ID NO: 46), RumTag (SEQ ID NO: 40), Rum2Tag (SEQ ID NO: 41), Rum3Tag (SEQ ID NO: 42), Rum4Tag (SEQ ID NO: 43), Rum5Tag (SEQ ID NO: 44), Rum6Tag (SEQ ID NO: 45) and Bac5Tag (SEQ ID NO: 46).

In some embodiments, the other of the first and second peptide tags is selected from the group consisting of a SpyCatcher (SEQ ID NO: 55), a SdyCatcher (SEQ ID NO: 56), a SnoopCatcher (SEQ ID NO: 57) and an esther-forming split-protein pair. An
25 example of an esther-forming split-protein pair is the fragment corresponding to amino acid residues 439-587 of cpe0147 (Uniprot B1R775) (SEQ ID NO: 34, DNA sequence: SEQ ID NO: 68) and the fragment corresponding to amino acid residues 565-587 of cpe0147 (Uniprot B1R775) (SEQ ID NO: 20; DNA sequence: SEQ ID NO: 54). Other
30 first or second peptide tags are presented in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, and SEQ ID NO: 33; the corresponding DNA sequences are SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, and SEQ ID NO: 67.

35

In some embodiments, the peptide pair comprises or consists of a SpyTag and a SpyCatcher. In some embodiments, the peptide pair comprises or consists of an SdyTag and an SdyCatcher. In some embodiments, the peptide pair comprises or consists of a SnoopTag and a SnoopCatcher.

5

In some embodiments, the peptide pair comprises or consists of truncated or modified versions of any of the above, i.e. further engineered peptide pairs, which however retain the ability to form an isopeptide bond.

10 A peptide tag may be altered, e.g. mutations or alterations may be introduced in any one or any two of the first or second peptide tag, i.e. in any one or any two of the tag and catcher. The peptide tag, i.e. the first, or the second peptide tag, should be able to covalently bind to a corresponding binding partner via an isopeptide bond, or via an ester bond, spontaneously. In this respect, each peptide tag preferably comprises one
15 of the reactive amino acid residues involved in the formation of an isopeptide bond in the isopeptide protein. Hence, each peptide tag comprises only one reactive residue from the isopeptide bond and does not comprise both reactive residues involved. Further, if the peptide tag is modified or mutated, the reactive residue in that fragment preferably remains unchanged. This means that when a homologue of a peptide tag is
20 used, the homologue preferably still contains the reactive residue which was originally present in the original peptide tag. In some embodiments, however, the reactive residue in that fragment is also changed if the peptide tag is modified or mutated.

25 Preferably, the reactive residue present in the tag is an asparagine or an aspartate residue, which can form an isopeptide bond with the reactive residue of the binding partner or modified binding partner (the catcher), as described above. Thus, one peptide tag contains one reactive residue while the other peptide tag contains the other reactive residue, and thus no single peptide tag contain both reactive residues.

30 In some embodiments, both reactive residues are involved in the formation of an isopeptide bond. In some embodiments, the reactive residue of the first peptide tag is different than the reactive residue of the second peptide tag. Preferably, the reactive residue present in the "catcher" is a lysine residue. In some embodiments, the reactive residue present in the "catcher" is an asparagine or an aspartate residue. Preferably,
35 the reactive residue present in the "tag" is an asparagine or an aspartate residue. In

some embodiments, the reactive residue present in the "tag" is a lysine residue. These residues together may form the isopeptide bond.

Without being bound by theory, a third residue may be involved in the formation of the isopeptide bond. While not directly participating in the bond, this third residue may mediate the formation of the bond. Typically, the third residue is a glutamate residue. The modified binding partner preferably comprises this third residue. In other words, the peptide tag, i.e. any of the first, second or third peptide tag, preferably does not comprise this third residue, which is instead present in the modified binding partner.

Consequently, as long as the peptide pair retains the ability to form an isopeptide bond, the peptide pair may comprise or consists of truncated or modified versions of any of the above mentioned peptide tags.

Thus, in some embodiments, one of the first and second peptide tags is selected from the group consisting of a SpyTag (SEQ ID NO: 1), a SdyTag (SEQ ID NO: 2), a SnoopTag (SEQ ID NO: 3), a PhoTag (SEQ ID NO: 4), an EntTag (SEQ ID NO: 5), a KTag, a BacTag (SEQ ID NO: 15), a Bac2Tag (SEQ ID NO: 16), a Bac3Tag (SEQ ID NO: 17), a Bac4Tag (SEQ ID NO: 18), a RumTrunkTag (SEQ ID NO: 13 or SEQ ID NO: 14), a Rum7Tag (SEQ ID NO: 12), a RumTag (SEQ ID NO: 6), a Rum2Tag (SEQ ID NO: 7), a Rum3Tag (SEQ ID NO: 8), a Rum4Tag (SEQ ID NO: 9), a Rum5Tag (SEQ ID NO: 10), a Rum6Tag (SEQ ID NO: 11), a Bac5Tag (SEQ ID NO: 19) and homologues thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto. Likewise, the nucleic acid sequence of one of the first and second peptide tags may be selected from the group consisting of a SpyTag (SEQ ID NO: 35), SdyTag (SEQ ID NO: 36), SnoopTag (SEQ ID NO: 37), PhoTag (SEQ ID NO: 38), EntTag (SEQ ID NO: 39), KTag, BacTag (SEQ ID NO: 49), Bac2Tag (SEQ ID NO: 50), Bac3Tag (SEQ ID NO: 51), Bac4Tag (SEQ ID NO: 52), RumTrunkTag (SEQ ID NO: 47 or SEQ ID NO: 48), Rum7Tag (SEQ ID NO: 46), RumTag (SEQ ID NO: 40), Rum2Tag (SEQ ID NO: 41), Rum3Tag (SEQ ID NO: 42), Rum4Tag (SEQ ID NO: 43), Rum5Tag (SEQ ID NO: 44), Rum6Tag (SEQ ID NO: 45), Bac5Tag (SEQ ID NO: 46) and variants thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such

as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%,
such as at least 91%, such as at least 92%, such as at least 93%, such as at least
94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at
least 98%, such as at least 99% sequence identity thereto.

5

In some embodiments, the other of the first and second peptide tags is selected from
the group consisting of a SpyCatcher (SEQ ID NO: 21), a SdyCatcher (SEQ ID NO:
22), a SnoopCatcher (SEQ ID NO: 23), an esther-forming split-protein pair (such as the
fragment corresponding to amino acid residues 439-587 of cpe0147 (Uniprot B1R775)
10 (SEQ ID NO: 34, DNA sequence: SEQ ID NO: 68) and the fragment corresponding to
amino acid residues 565-587 of cpe0147 (Uniprot B1R775) (SEQ ID NO: 20; DNA
sequence: SEQ ID NO: 54)), SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID
NO: 27, SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID
NO: 32, and SEQ ID NO: 33 and homologues thereof having at least 60% homology
15 thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at
least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as
at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such
as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%
homology thereto. Likewise, the nucleic acid sequence of the other of the first and
20 second peptide tags may be selected from the group consisting of the DNA sequence
of SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59,
SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64,
SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67 and variants thereof having at least
60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at
25 least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as
at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such
as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,
such as at least 99% sequence identity thereto.

30 In some embodiments, the peptide pair comprises or consists of a SpyTag (SEQ ID
NO: 1) or a homologue thereof having at least 60% homology thereto, such as at least
65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at
least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as
at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such
35 as at least 97%, such as at least 98%, such as at least 99% homology thereto, and a
SpyCatcher (SEQ ID NO: 21) or a homologue thereof having at least 60% homology

thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99%

5 homology thereto. Likewise, the nucleic acid sequence of the peptide pair may comprise or consist of a SpyTag (SEQ ID NO: 35) or a variant thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,

10 such as at least 99% sequence identity thereto, and a SpyCatcher (SEQ ID NO: 55) or a variant thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

15

In some embodiments, the peptide pair comprises or consists of an SdyTag (SEQ ID NO: 2) or a homologue thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto, and an SdyCatcher (SEQ ID NO: 22) or a homologue thereof having at least 60% homology

20 thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto. Likewise, the nucleic acid sequence of the peptide pair may

25 comprise or consist of an SdyTag (SEQ ID NO: 36) or a variant thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%,

30 such as at least 99% sequence identity thereto, and an SdyCatcher (SEQ ID NO: 56) or a variant thereof having at least 60% sequence identity thereto, such as at least

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65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

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In some embodiments, the peptide pair comprises or consists of a SnoopTag (SEQ ID NO: 3) or a homologue thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto, and a SnoopCatcher (SEQ ID NO: 23) or a homologue thereof having at least 60% homology thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto. Likewise, the nucleic acid sequence of the peptide pair may comprise or consist of a SnoopTag (SEQ ID NO: 37) or a variant thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto, and a SnoopCatcher (SEQ ID NO: 57) or a variant thereof having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity thereto.

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Fusion of the first peptide tag to the protein and fusion of the second peptide tag to the antigen

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Changing the position where the first peptide tag is fused to the protein may allow changing the orientation of the antigen on the particle. This may be performed to enable the best possible display of the most important epitopes of the antigen. The best possible orientation may be different from antigen to antigen.

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Epitopes of specific monoclonal antibodies may be mapped on the antigen structure, whereby it is possible to determine which epitopes are accessible after conjugation of the antigen to the particle. Specifically, one may measure binding between a specific monoclonal antibody and the complex of the antigen bound to the particle
5 (antigen:particle complex), such as by using ELISA or another affinity-measuring technique (e.g. Attana), and thereby determine the orientation of the antigen. Cryo-electron microscopy may also be used to determine the structure of the entire antigen:particle complex. If the antigen contains a functional binding epitope, binding-assays may be conducted to determine if the epitope is exposed or hidden in the final
10 antigen:particle complex.

Changing the position where the second peptide tag is fused to the antigen will allow changing the orientation of the antigen on the particle. This may be performed to enable the best possible display of the most important epitopes of the antigen. The
15 best possible orientation may be different from antigen to antigen.

In some embodiments, the first peptide tag is fused to the N-terminus of the protein. In other embodiments, the first peptide tag is fused to the C-terminus of the protein. In other embodiments, the first polynucleotide is inserted in-frame in the coding sequence
20 of the protein. The fusion protein may comprise a linker between the first peptide tag and the protein.

Similarly, in some embodiments, the second peptide tag is fused to the N-terminus of the antigen. In other embodiments, the second peptide tag is fused to the C-terminus of
25 the antigen. In other embodiments, the second polynucleotide is inserted in-frame in the coding sequence of the antigen. The fusion protein may comprise a linker between the second peptide tag and the antigen.

Diseases and medical indications

30 The present invention is a novel, generic, and easy-to-use-approach to conjugate various antigens to a nanoparticle or VLP directly in the cell in which the nanoparticle or VLP is to be expressed, i.e. *in vivo*. Depending on the antigen, the present compositions can be used for prophylaxis and/or treatment of a wide range of diseases. The diseases which the present invention may be used for prophylaxis
35 and/or treatment of include, but are not limited to, cancers, cardiovascular diseases,

allergic diseases, chronic diseases, neurologic diseases, and/or infectious diseases. Antigens are typically peptides, polypeptides or proteins or fragments thereof, i.e. they comprise or consist of an amino acid sequence.

5 In some embodiments, an antigen which is associated with at least one cancer disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions may be used for prophylaxis and/or treatment of the cancer and/or cancers which the antigen is associated with.

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In some embodiments, an antigen which is associated with at least one cardiovascular disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the cardiovascular disease and/or cardiovascular diseases which the antigen is associated with.

15

In some embodiments, an antigen which is associated with at least one allergic disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the allergic disease and/or allergic diseases which the antigen is associated with.

20

In some embodiments, an antigen which is associated with at least one infectious disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the infectious disease and/or infectious diseases which the antigen is associated with.

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30 In some embodiments, an antigen which is associated with at least one chronic disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the chronic disease and/or chronic diseases which the antigen is associated with.

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In some embodiments, an antigen which is associated with at least one neurologic disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the neurologic disease and/or neurologic diseases which the antigen is associated with.

In some embodiments, an antigen which is associated with at least one viral disease is linked to the protein, such as a particle-forming protein as described herein, via the interaction between the first peptide tag and second peptide tag. In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the viral disease which the antigen is associated with. In some embodiments, the viral disease is caused by a coronavirus such as SARS-CoV-2, malaria, tuberculosis, HIV or influenza.

A non-exhaustive list of antigens which may be used with the present invention is outlined in Table 1 and Table 2. In addition, table 1 shows examples of specific diseases the antigens are associated with as well as examples of patient groups which may be in need of prophylaxis and/or treatment using the present compositions.

Relevant antigens include: hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, neuraminidase, human melanoma protein gp100, human melanoma protein melan-A/MART1, HIV envelope protein, M2e, VAR2CSA, ICAM1, CSP, Dengue virus NS1, Dengue virus envelope protein, Chikungunya virus envelope protein, tyrosinase, HCV E2, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1, EGFRvIII, endoglin, ANGPTL-3, CSPG4, CTLA-4, HER2, IgE, IL-1 beta, IL-5, IL-13, IL-17, IL-22, IL-31, IL-33, TSLP, NGF and (IHNV) G-protein, a lymphotoxin such as lymphotoxin α or β , a lymphotoxin receptor, a receptor activator of nuclear factor κ B ligand, vascular endothelial growth factor VEGF, a VEGF receptor, IL-23 p19, ghrelin, CCL21, CXCL12, SDF-1, M-CSF, MCP-1, endoglin, GnRH, TRH, eotaxin, bradykinin, BLC, TNF- α , amyloid β peptide A, angiotensin, gastrin, progastrin, CETP, CCR5, C5a, CXCR4, Des-Arg-bradykinin, GnRH peptide, angiotensin peptide or TNF peptide.

In some embodiments, the antigen is an antigenic fragment or antigenic variant of hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, neuraminidase, human melanoma protein gp100, human melanoma protein melan-A/MART1, HIV envelope protein, M2e,

VAR2CSA, ICAM1, CSP, Dengue virus NS1, Dengue virus envelope protein, Chikungunya virus envelope protein, tyrosinase, HCV E2, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1, EGFRvIII, endoglin, ANGPTL-3, CSPG4, CTLA-4, HER2, IgE, IL-1 beta, IL-5, IL-13, IL-17, IL-22, IL-31, IL-33, TSLP, 5 NGF and (IHNV) G-protein, a lymphotoxin such as lymphotoxin α or β , a lymphotoxin receptor, a receptor activator of nuclear factor κ B ligand, vascular endothelial growth factor VEGF, a VEGF receptor, IL-23 p19, ghrelin, CCL21, CXCL12, SDF-1, M-CSF, MCP-1, endoglin, GnRH, TRH, eotaxin, bradykinin, BLC, TNF- α , amyloid β peptide A, angiotensin, gastrin, progastrin, CETP, CCR5, C5a, CXCR4, Des-Arg-bradykinin, 10 GnRH peptide, angiotensin peptide or TNF peptide. This antigenic fragment or antigenic variant may be better at inducing a stronger immune response than the corresponding antigen. Thus, in some embodiments, the protein sequence of the antigenic fragment or antigenic variant is a homologue of the corresponding antigen, having at least 60% homology thereto, such as at least 65%, such as at least 70%, 15 such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% homology thereto. In some embodiments, the antigenic fragment or antigenic variant is encoded by a polypeptide. Said polypeptide 20 may consist or comprise of a nucleic acid sequence variant of the corresponding natural antigen, the nucleic acid sequence variant having at least 60% sequence identity thereto, such as at least 65%, such as at least 70%, such as at least 75%, such as at least 80%, such as at least 85%, such as at least 90%, such as at least 91%, such as at least 92%, such as at least 93%, such as at least 94%, such as at least 25 95%, such as at least 96%, such as at least 97%, such as at least 98%, such as at least 99% sequence identity to the corresponding antigen.

It is desirable that the cell upon transfection and expression of the antigen and the particle-forming protein, each fused to a tag as described herein, can form self- 30 assembling particles displaying the antigen linked to the particle-forming protein. This may be assessed by relevant methods as known to the person skilled in the art, such as those disclosed in Example 2 and 3 of the present disclosure.

The compositions of the present invention may as well be used against other diseases 35 and/or use other antigens than the ones listed herein.

In an embodiment of the present invention, the medical indication is selected from the group consisting of a cardiovascular disease, an immune-inflammatory disease, a chronic disease, a neurologic disease, an infectious disease and cancer. In a particular embodiment, the medical indication is an immune-inflammatory disease. In another particular embodiment, the medical indication is a cardiovascular disease. In another embodiment the medical indication is a chronic disease. In another embodiment the medical indication is a neurologic disease. In another embodiment, the medical indication is an infectious disease. In another embodiment, the medical indication is cancer.

10

In another embodiment, the antigen is a polypeptide, peptide and/or an antigenic fragment of a polypeptide associated with an abnormal physiological response, such as a cardiovascular disease and/or an allergic reaction/disease. In a particular embodiment the abnormal physiological response is a cancer.

15

In a further embodiment the antigen is a protein, peptide and/or an antigenic fragment associated with a medical indication as disclosed herein.

Table 1. Non-exhaustive list of antigens or parts hereof that could be used in treatment of specific diseases/medical indications in various patient groups.

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Examples of antigens (non-exhaustive)	Examples of a specific disease (non-exhaustive)	Examples of patient group (non-exhaustive)
Her2/Neu (ERBB2)	Breast cancer	Females overexpressing Her2
Her2/Neu (ERBB2)	Gastric cancer	Males and females overexpressing Her2
Her2/Neu (ERBB2)	Ovarian cancer	Females overexpressing Her2
Her2/Neu (ERBB2)	Uterine serous carcinoma	Postmenopausal Females overexpressing Her2
Survivin	Cancer types overexpressing Survivin	Males and non-pregnant females overexpressing Survivin
PCSK9	Cardiovascular disease	Males and females with dyslipidemia
PCSK9	Cardiovascular disease	Males and females with atherosclerosis

PCSK9	Cardiovascular disease	Males and females with hypercholesterolemia
ANGPTL3	Cardiovascular disease	Males and females with dyslipidemia
ANGPTL3	Cardiovascular disease	Males and females with atherosclerosis
ANGPTL3	Cardiovascular disease	Males and females with hypercholesterolemia
Interleukin-5	Asthma	Males and females with eosinophilia
Interleukin-5	nasal polyposis	Males and females with eosinophilia
Interleukin-5	atopic dermatitis	Males and females with eosinophilia
Interleukin-5	eosinophilic esophagitis	Males and females with eosinophilia
Interleukin-5	Hypereosinophilic syndrome	Males and females with eosinophilia
Interleukin-5	Churg-Strauss syndrome	Males and females with eosinophilia
Ag85A	Tuberculosis	Males and females with tuberculosis
PfRH5	Malaria	Males and females with malaria
VAR2CSA	Malaria	Females with malaria
PfEMP1, CIDR1a	Malaria	Males and females with malaria
GLURP	Malaria	Males and females with malaria
MSP3	Malaria	Males and females with malaria
Pfs25	Malaria	Males and females with malaria
CSP	Malaria	Males and females with malaria
PfSEA-1	Malaria	Males and females with malaria
Hemagglutinin HA	Influenza	Males and females with influenza
Interleukin-17	Psoriasis	Males and females with Psoriasis
Interleukin-17	Multiple sclerosis	Males and females with multiple sclerosis
Interleukin-17	Rheumatoid arthritis	Males and females with rheumatoid arthritis
Interleukin-17	Inflammatory bowel diseases	Males and females with inflammatory bowel diseases
Interleukin-17	Asthma	Males and females with Asthma
IL-33	Asthma	Males and females with Asthma
IgE	Asthma	Males and females with Asthma

Gp160	HIV	Males and females with HIV
Gp120	HIV	Males and females with HIV
Gp40	HIV	Males and females with HIV
GD2	Cancer cell types expressing GD2 (e.g. melanomas, osteosarcoma and soft-tissue sarcomas)	Males and females with GD2 expressing tumors.
EGF-R	Cancer cell types expressing EGF-R (e.g. metastatic colorectal and head and neck cancer)	Males and females with EGF-R expressing tumors.
CEA	Cancer cell types expressing CEA (e.g. colon and rectal cancer or pancreatic, breast, ovary, or lung cancer.	Males and females with CEA expressing tumors.
CD52	Chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma or multiple sclerosis	Males and females with chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma or multiple sclerosis.
CD21	B-cell cancers	Males and females with B-cell cancers
human melanoma protein gp100	Cancer cell types expressing human melanoma protein gp 100 (e.g. Melanoma).	Males and females with melanoma.
human melanoma protein melan-A/MART-1	Cancer cell types expressing human melanoma protein melan-A/MART-1 (e.g. Melanoma)	Males and females with melanoma.
tyrosinase	Melanoma	Males and females with melanoma

NA17-A nt protein	Melanoma	Males and females with melanoma
MAGE-3 protein	melanoma, non-small cell lung cancer, hematologic malignancies.	Males and females with melanoma, non-small cell lung cancer or hematologic malignancies
p53 protein	Cancer cell types expressing p53	Males and females with tumors expressing p53
HPV 16 E7 protein	Cancers of the cervix, vulva, vagina, penis, oropharynx and anus.	HPV infected males and females
HPV L2	Cancers of the cervix, vulva, vagina, penis, oropharynx and anus.	HPV infected males and females
PD-L1	Cancer types PD-L1	Males and females with tumors expressing PD-L1
PD-L1	Cancer types PD1	Males and females with tumors expressing PD1
CTLA-4	Cancer types CTLA-4	Males and females with tumors expressing CTLA-4
hCG	Cancer cell types expressing hCG	Males and females with tumors expressing hCG
Fel d1	Cat allergy	Males and females allergic to cats
(IHN) G-protein	Infectious haematopoietic necrosis (IHN)	Salmon and trout infected with IHN

Table 2. Non-exhaustive list of diseases/medical indications and target antigen/organisms of the present VLP/nanoparticle vaccine.

5

Disease:	Target antigen/ Organism:
Cancer:	Her2/Neu (ERBB2) / <i>Homo Sapiens</i> Survivin (Baculoviral IAP repeat-containing protein 5) / <i>Homo Sapiens</i> GD2 / <i>Homo Sapiens</i>

	EGF-R / <i>Homo Sapiens</i>
	CEA / <i>Homo Sapiens</i>
	CD52 / <i>Homo Sapiens</i>
	human melanoma protein gp100 / <i>Homo Sapiens</i>
5	human melanoma protein melan-A/MART-1 / <i>Homo Sapiens</i>
	tyrosinase / <i>Homo Sapiens</i>
	NA17-A nt protein / <i>Homo Sapiens</i>
	MAGE-3 protein / <i>Homo Sapiens</i>
	p53 protein / <i>Homo Sapiens</i>
10	HPV 16 E7 protein / Human papillomavirus
	HPV L2 protein / Human papillomavirus
	PD1 / <i>Homo Sapiens</i>
	PD-L1 / <i>Homo Sapiens</i>
	CTLA-4 / <i>Homo Sapiens</i>
15	hCG / <i>Homo Sapiens</i>
	(IHNV) G-protein / Infectious haematopoietic necrosis virus
	Cardiovascular disease: PCSK9 (Proprotein convertase subtilisin/kexin type 9) /
	<i>Homo Sapiens</i>
20	
	Asthma / Allergies: IL-5 (Interleukin-5) / <i>Homo Sapiens</i>
	Fel d1 / <i>Felis catus</i>
	IL-17 / <i>Homo sapiens</i>
	IL-13 / <i>Homo sapiens</i>
25	IL-1B / <i>Homo sapiens</i>
	IgE / <i>Homo sapiens</i>
	Tuberculosis: Ag85A (Diacylglycerol cyltransferase/mycolyltransferase) /
	<i>Mycobacterium tuberculosis</i>
30	
	Malaria: Reticulocyte-binding protein homologue 5 (PfRH5) /
	<i>Plasmodium falciparum</i>
	VAR2CSA (domain, ID1-ID2a) / <i>Plasmodium falciparum</i>
	CIDR1a domain of PfEMP1, <i>Plasmodium falciparum</i>
35	Glutamate rich protein (GLURP) / <i>Plasmodium falciparum</i>

		Merozoite surface protein 3 (MSP3) / <i>Plasmodium falciparum</i>
		25 kDa ookinete surface antigen (Pfs25) / <i>Plasmodium falciparum</i>
5		Circumsporozoite protein (CSP) / <i>Plasmodium falciparum</i>
		Schizont egress antigen-1 (PfSEA-1) / <i>Plasmodium falciparum</i>
10	Multiple sclerosis	CD52 / Homo sapiens
	Contraception	hCG
	Influenza	HA
15	<u>Cancer and associated antigens</u>	
	In 2012 more than 14 million adults were diagnosed with cancer and there were more than 8 million deaths from cancer, globally. Consequently, there is a need for efficient cancer therapeutics.	
20	One characteristic of cancer cells is abnormal expression levels of genes and proteins. One example of a cancer associated gene is HER2, which is overexpressed in 20% of all breast cancers and is associated with increased metastatic potential and poor patient survival. Although cancer cells express cancer associated antigens in a way that immunologically distinguishes them from normal cells, most cancer associated	
25	antigens are only weakly immunogenic because most cancer associated antigens are "self" proteins which are generally tolerated by the host. The present compositions can be used to express, in a cell of a subject, particles displaying an antigen capable of activating the immune system to react against for example cancer associated antigens and overcome the immunological tolerance to such antigens. Different cancers are	
30	characterized by having different cancer associated antigens. Survivin is regarded to be overexpressed in most cancer cells and could also be used in the present invention. Therefore the present invention may be used in treatment/prophylaxis of most types of cancers that overexpress a tumor associated antigen.	

Thereby the present invention provides compositions capable of activating the immune system to react against for example cancer associated antigens and overcome immunological tolerance to such antigens. In an embodiment the present compositions can be used for prophylaxis and/or treatment of the cancer which the antigen is associated with.

In another embodiment the present invention is used in treatment/prophylaxis of any type of cancer which overexpresses an antigen. The type of cancer which the invention may be used against is determined by the choice of antigen.

It is known that oncoviruses can cause cancer. Therefore in an embodiment the vaccine of the present invention comprises an oncovirus associated antigen linked to a protein, preferably a particle-forming protein, via the isopeptide bond or ester bond.

In a further embodiment the present compositions can be used for prophylaxis and/or treatment of the cancer which the antigen is associated with.

In an embodiment the antigen is a protein or peptide or an antigenic fragment of a polypeptide associated with a cancer selected from the group comprising of adrenal cancer, anal cancer, bile duct cancer, bladder cancer, bone cancer, brain/CNS tumors in adults, brain/CNS tumors in children, breast cancer, breast cancer in men, cancer in adolescents, cancer in children, cancer in young adults, cancer of unknown primary (CUP), Castleman disease, cervical cancer, colon/rectum cancer, endometrial cancer, esophagus cancer, Ewing family of tumors, eye cancer, gallbladder cancer, gastrointestinal carcinoid tumors, gastrointestinal stromal tumor, gestational trophoblastic disease, Hodgkin disease, Kaposi sarcoma, kidney cancer, laryngeal and hypopharyngeal cancer, leukemia, acute lymphocytic in adults, leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, chronic myeloid leukemia, chronic myelomonocytic leukemia, leukemia in children, liver cancer, lung cancer, non-small cell lung cancer, small cell lung cancer, lung carcinoid tumor, lymphoma, lymphoma of the skin, malignant mesothelioma, multiple myeloma, myelodysplastic syndrome, nasal cavity and paranasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, non-Hodgkin lymphoma in children, oral cavity and oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, penile cancer, pituitary tumors, prostate cancer, retinoblastoma, rhabdomyosarcoma, salivary gland cancer, adult soft tissue cancer sarcoma, skin cancer, basal and squamous cell skin cancer,

melanoma skin cancer, Merkel cell skin cancer, small intestine cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, uterine sarcoma, vaginal cancer, vulvar cancer, Waldenstrom macroglobulinemia, and Wilms tumor.

- 5 In some embodiments the cancer is selected from the group consisting of breast cancer, gastric cancer, ovarian cancer, and uterine serous carcinoma.

10 Linking the Her2/Neu (ERBB2) and/or Survivin or an antigenic fragment hereof to the VLP or nanoparticle as described herein forms a VLP or nanoparticle which is capable of activating the immune system to react against for example cells with high Her2/Neu (ERBB2) and/or Survivin expression and overcome immunological tolerance. In an embodiment the Her2/Neu (ERBB2) and/or Survivin can be used for prophylaxis and/or treatment of the herein disclosed cancer disease and/or other cancer diseases. Using a similar reasoning other cancer disease associated antigens may be used against any
15 cancer disease. Such antigens may be chosen from the group consisting of interleukin-17, hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, human melanoma protein gp100, human melanoma protein melan-A/MART1, tyrosinase, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1 and (IHNV) G-protein.

- 20 In an embodiment the antigen of the present invention is Her2/Neu (ERBB2) and/or Survivin or an antigenic fragment hereof, wherein the antigen is associated with and directed against at least one of the herein disclosed types of cancers. In an embodiment the antigen of the present disclosure is interleukin-17, hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, human melanoma protein gp100, human melanoma
25 protein melan-A/MART1, tyrosinase, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1 and (IHNV) G-protein or an antigenic fragment thereof, wherein the antigen is associated with and directed against at least one of the herein disclosed types of cancers.

30 Cardiovascular diseases and associated antigens

An estimated 17.3 million people died from cardiovascular diseases in 2008, representing 30% of all global deaths. Addressing risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity, high blood pressure, diabetes and raised lipids are important for prevention of cardiovascular diseases. However, the need for
35 preventive pharmaceutical measures is increasingly important. The present invention

may be used in treatment/prophylaxis of most types of cardiovascular diseases. The type of cardiovascular disease which the invention may be used against is decided by the choice of antigen.

5 In an embodiment of the invention the antigen is a protein or peptide or an antigenic fragment of a polypeptide associated with a disease selected from the group comprising a lipid disorder such as hyperlipidemia, type I, type II, type III, type IV, or type V hyperlipidemia, secondary hypertriglyceridemia, hypercholesterolemia, familial hypercholesterolemia, xanthomatosis, cholesterol acetyltransferase deficiency,
10 an atherosclerotic condition (e.g., atherosclerosis), a coronary artery disease, a cardiovascular disease.

In an embodiment of the invention the antigen is a protein or peptide or an antigenic fragment of a polypeptide associated with a cardiovascular disease. In a further
15 embodiment the cardiovascular disease is selected from the group consisting of dyslipidemia, atherosclerosis, and hypercholesterolemia.

One example of a polypeptide associated with a cardiovascular disease is PCSK9 which acts in cholesterol homeostasis. Blockage of PCSK9 has medical significance
20 and can lower the plasma and/or serum low-density lipoprotein cholesterol (LDL-C) levels. Reducing LDL-C reduces the risk of for example heart attacks.

Linking the PCSK9 antigen to the VLP or nanoparticle forms a PCSK9-VLP/nanoparticle based vaccine which is capable of activating the immune system to
25 produce antibodies that bind PCSK9 and either clear PCSK9 from the bloodstream or hinders binding of PCSK9 to the LDL receptor, thereby lowering the LDL-C levels and the risk of heart attacks. In an embodiment, the present compositions can be used for prophylaxis and/or treatment of the herein disclosed cardiovascular disease and/or other cardiovascular diseases. Using a similar reasoning other cardiovascular disease
30 associated antigens may be used against any cardiovascular disease.

In a preferred embodiment the antigen comprises PCSK9 or an antigenic fragment hereof, wherein the antigen is associated with and directed against at least one of the herein disclosed cardiovascular disease and/or other cardiovascular diseases.

35

Another example of a polypeptide associated with a cardiovascular disease is ANGPTL3 which acts in cholesterol homeostasis. Blockage of ANGPTL3 has medical significance and can lower the plasma and/or serum low-density lipoprotein cholesterol (LDL-C) levels. Reducing LDL-C reduces the risk of for example heart attacks.

5

Linking the ANGPTL3 antigen to the VLP or nanoparticle forms a ANGPTL3-VLP/nanoparticle based vaccine which is capable of activating the immune system to produce antibodies that bind ANGPTL3 and either clear ANGPTL3 from the bloodstream or hinders binding of ANGPTL3 to the LDL receptor, thereby lowering the LDL-C levels and the risk of heart attacks. In an embodiment, the present compositions can be used for prophylaxis and/or treatment of the herein disclosed cardiovascular disease and/or other cardiovascular diseases. Using a similar reasoning other cardiovascular disease associated antigens may be used against any cardiovascular disease.

10

15

In a preferred embodiment the antigen comprises ANGPTL3 or an antigenic fragment hereof, wherein the antigen is associated with and directed against at least one of the herein disclosed cardiovascular disease and/or other cardiovascular diseases.

20

Immune-inflammatory diseases and associated antigens

The prevalence of immune-inflammatory diseases worldwide is rising dramatically in both developed and developing countries. According to World Health Organization statistics, hundreds of millions of subjects in the world suffer from allergic rhinitis and it is estimated that 300 million have asthma markedly affecting the quality of life of these individuals and negatively impacting the socio-economic welfare of society.

25

Interleukin 5 (IL-5) has been shown to play an instrumental role in eosinophilic inflammation in various types of allergies, including severe eosinophilic asthma. Eosinophils are regulated in terms of their recruitment, activation, growth, differentiation and survival by IL-5 which, consequently, has identified this cytokine as a primary target for therapeutic interventions.

30

Linking an IL-5 antigen or a fragment hereof to the particle-forming protein of the present invention forms an IL-5-VLP/nanoparticle based vaccine which is capable of activating the immune system to react against IL-5. Consequently an IL-5-based composition described in the present invention may be used in the

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treatment/prophylaxis of eosinophilic asthma or other immune-inflammatory diseases. Other immune-inflammatory disease associated antigens (e.g. IgE or interleukin 17 or IL-17) may be used by the present invention using a similar reasoning. Consequently an IL-17-based vaccine described in the present invention may be used in the

5 treatment/prophylaxis of eosinophilic asthma or other immune-inflammatory diseases. The type of asthma or allergy or other immune-inflammatory disease which the invention may be used against is decided by the choice of antigen. In an embodiment the antigen is a protein or peptide or an antigenic fragment of a polypeptide associated with one or more asthma or immune-inflammatory diseases disclosed herein. In a

10 preferred embodiment the asthma or immune-inflammatory disease is selected from the group consisting of eosinophilic asthma, allergy, nasal polyposis, atopic dermatitis, eosinophilic esophagitis, hypereosinophilic syndrome, and Churg-Strauss syndrome.

In a preferred embodiment the antigen comprises IL-5, IL-17 or an antigenic fragment

15 hereof, wherein the antigen is associated with and directed against at least one of the herein disclosed asthma or allergy diseases and/or other immune-inflammatory diseases.

Infectious diseases and associated antigens

20 Tuberculosis and malaria are two major infectious diseases. In 2012, an estimated 207 million cases of malaria occurred which resulted in more than 500.000 deaths. Also in 2012, an estimated 8.6 million people developed tuberculosis and 1.3 million died from the disease. The current methods of treatment are insufficient and some have resulted in drug resistance. Consequently there is a need for new and efficient drugs for

25 treatment/prophylaxis of tuberculosis and malaria. Linking a malaria or tuberculosis associated-antigen or a fragment hereof to the VLP or nanoparticle of the present invention forms a VLP or nanoparticle based vaccine which is capable of activating the immune system to react against for example malaria or tuberculosis. Using a similar line of reasoning the present invention may be used in treatment/prophylaxis of most

30 infectious disease. The type of infectious disease which the invention may be used against is decided by the choice of antigen.

The year 2020 has been marked by the SARS-CoV-2/COVID-19 pandemic, resulting in millions of infected subjects worldwide, and this infectious disease is thus of great

35 interest. Influenza is another infectious disease of interest.

In an embodiment the antigen fused to the second peptide tag of the present invention is a protein or peptide or an antigenic fragment of a polypeptide associated with an infectious disease such as tuberculosis and/or malaria.

5

In one embodiment an antigen from *Plasmodium falciparum* is fused to the second peptide tag for use in treatment/prophylaxis of malaria.

10 In a further embodiment an antigen from *Mycobacterium tuberculosis* is fused to the second peptide tag for use in treatment/prophylaxis of tuberculosis.

15 In a further embodiment the antigen is selected from the group consisting of Ag85A from *Mycobacterium tuberculosis*, PfRH5 from *Plasmodium falciparum*, VAR2CSA (domain, ID1-ID2a) from *Plasmodium falciparum*, CIDR1a domain, of PfEMP1 from *Plasmodium falciparum*, GLURP from *Plasmodium falciparum*, MSP3 from *Plasmodium falciparum*, Pfs25 from *Plasmodium falciparum*, CSP from *Plasmodium falciparum*, and PfSEA-1 from *Plasmodium falciparum* or an antigenic fragment of the disclosed antigens. In another embodiment the antigen comprises a fusion construct between MSP3 and GLURP (GMZ2) from *Plasmodium falciparum*.

20

In a further embodiment the antigen is a hemagglutinin (HA) antigen from the influenza virus or an antigenic fragment thereof.

25 In another embodiment the antigen of the present invention comprises a protein, or an antigenic fragment hereof, from the pathogenic organism which causes the infectious disease.

30 In one embodiment, the antigen is a protein, peptide and/or an antigenic fragment of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). Thus, in some embodiments, the antigen is a protein, peptide and/or an antigenic fragment of a SARS-CoV-2 envelope protein. In some embodiments, the antigen is a protein, peptide and/or an antigenic fragment of a SARS-CoV-2 spike protein. In some embodiments, the antigen is a protein, peptide and/or an antigenic fragment of a SARS-CoV-2 nucleocapsid protein. In some embodiments, the antigen is a protein, peptide and/or an antigenic fragment of a SARS-CoV-2 envelope protein. In specific embodiments, the antigen is amino acids 319-591 of the SARS-CoV-2 spike protein RBD (GenBank

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accession number: QIA20044.1). Said antigen may be fused to a catcher or a tag, and may further comprise a C-tag purification tag.

Thus, in some embodiments, the composition as described herein comprises:

- 5 i. a first polynucleotide encoding a protein fused to a first peptide tag;
 and
- ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to a
 second peptide tag,

wherein the antigen and the protein upon expression in a cell are linked via an
10 isopeptide bond, or an ester bond, between the first peptide tag and the second
peptide tag, whereby i - ii form a particle displaying said SARS-CoV-2 antigen.

In some embodiments, the composition as described herein comprises:

- i. a first polynucleotide encoding a protein fused to a SpyCatcher; and
- 15 ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to a
 SpyTag.

In some embodiments, the composition as described herein comprises:

- i. a first polynucleotide encoding a protein fused to an SdyCatcher; and
- 20 ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to an
 SdyTag.

In some embodiments, the composition as described herein comprises:

- i. a first polynucleotide encoding a protein fused to a SnoopCatcher;
25 and
- ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to a
 SnoopTag.

In some embodiments, the composition as described herein comprises:

- 30 i. a first polynucleotide encoding a protein fused to a SpyTag; and
- ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to a
 SpyCatcher.

In some embodiments, the composition as described herein comprises:

- 35 i. a first polynucleotide encoding a protein fused to an SdyTag; and

- ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to an SdyCatcher.

In some embodiments, the composition as described herein comprises:

- 5 i. a first polynucleotide encoding a protein fused to a SnoopTag; and
- ii. a second polynucleotide encoding a SARS-CoV-2 antigen fused to a SnoopCatcher.

10 In one embodiment the antigen is a protein, peptide and/or an antigenic fragment of an influenza virus.

Antigens known to be difficult to express in heterologous expression systems

Some antigens are difficult to express in a heterologous expression system (for example mammalian antigens produced in *E. coli*). A number of antigens are also
15 present as multi-protein complexes, further complicating production and formulation. Protein degradation or aggregation during production and coupling can also cause issues for particle display, in particular for VLP display. This can result in antigens being insoluble and thus very complex or impossible to produce in vitro and used as vaccine for the VLP technology.

20 These antigens could thus benefit from being produced by DNA/mRNA *in vivo* and directly coupled to VLP *in vivo*. This would avoid the need to first produce and purify the protein/protein complex, and also avoids lengthy storage in potentially non-optimal buffers. Here are some examples of few antigens that could benefit from such a
25 technology:

Interleukins: In the context of allergy and asthma, levels of interleukins are involved in the severity of the disease. The particle technology, in particular the VLP technology, allows for a breach of immune tolerance and thus to control levels of interleukins, thus
30 alleviating some of the disease's symptoms. However, a number of interleukins are difficult to produce (particularly in *E. coli*, but also in a range of other expression systems) and often are insoluble. Among these, IL-13, IL-31 and IL-17A could benefit from the present mRNA/DNA technology.

Thus, in some embodiments, the antigen is IL-13. In some embodiments, the antigen is IL-31. In some embodiments, the antigen is IL-17A.

5 Similarly, PCSK9 is involved in cholesterol levels, thus research has been focusing on making a PCSK9 vaccine. In the SARS-CoV-2 pandemic, the mutation rate of the virus is quite high, thus, new vaccines might be needed in order to protect against the different variants. However, both of these antigens need to be produced in eukaryotic cells, making the production line costly and time consuming. Furthermore, PCSK9 has proven extremely difficult to couple stably to particles, including VLPs, with protein
10 aggregation and degradation issues causing severe delays in development and requiring novel PCSK9 designs for success. This antigen could thus benefit from mRNA/DNA delivery technology, in order to cut down on the production time and cost.

15 Thus, in some embodiments, the antigen is PCSK9.

Finally, HIV trimers and Flu stem trimers are also difficult proteins to couple to particles, such as VLPs, as they are quite large proteins, and have intrinsic stability issues for the artificially designed stem trimers. It is also known from literature that a number of HIV variant sequences has proven impossible to incorporate in stem-only HIV vaccine
20 designs due to instability issues (Zhang et al., 2021). In an *in vivo* system, where these antigens would be delivered as DNA/mRNA (where the coupling efficiency and pressure would be different while also removing the need to work with the unstable antigens for long periods before coupling to the particle), DNA/mRNA particle technology may be the solution.

25 Thus, in some embodiments, the antigen is an HIV trimer. In some embodiments, the antigen is an influenza virus trimer.

Expression systems

30 Herein is also provided an expression system comprising:

- i. a first polynucleotide encoding a protein fused to a first peptide tag; and
- ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

wherein upon expression of the first and second polynucleotides in a cell, the antigen
35 and the protein are linked via an isopeptide bond, or an ester bond, between the first

peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

5 The expression system may consist of or comprise a polycistronic RNA construct and/or a DNA construct, from which the transcribed mRNA is polycistronic. Thus, in some embodiments, the first and the second polynucleotides of the expression system are encoded on the same ribonucleic acid molecule. In some embodiments, the first and the second polynucleotides of the expression system lie within the same open reading frame, whereby only one promoter sequence is needed to transcribe both
10 polynucleotides. In some embodiments, the first and the second polynucleotides of the expression system lie within separate open reading frames and may thus be regulated by separate promoters.

15 The first peptide tag, the second peptide tag, the protein and/or the antigen may be as defined herein elsewhere.

20 The term "expression system" refers to a genetic construct designed to produce a protein and/or an RNA inside a cell. Thus, the expression system may comprise RNA and/or DNA, which is translated or transcribed to a protein or DNA, respectively, inside the cell.

25 The expression system may comprise the sequences necessary for gene expression in the cell. These may include a promoter, a translation initiation sequence such as a ribosomal binding site, a start codon, a termination codon, and a transcription termination sequence. There are differences in the enzymes responsible for protein synthesis between prokaryotes and eukaryotes, therefore the expression vectors must comprise elements for expression that are appropriate for the chosen host. For example, prokaryotic expression systems may comprise a Shine-Dalgarno sequence at the translation initiation site for the binding of ribosomes, while eukaryotic expression
30 systems may contain a Kozak consensus sequence.

35 The expression system may additionally comprise a marker, such as a selectable marker, i.e. a gene that confers a trait suitable for artificial selection, whereby cells comprising the expression system may be selected for, or a screenable marker, such as a reporter gene, i.e. a gene that allows for differentiation between cells comprising or not comprising the expression system, whereby cells comprising the expression

system may be identified. Examples of such markers include antibiotic resistance genes, auxotrophic markers and genes expressing detectable compounds, such as coloured and/or fluorescent compounds.

5 In some embodiments, the first polynucleotide and the second polynucleotide are both DNA polynucleotides. In some embodiments, the first polynucleotide and the second polynucleotide are both RNA polynucleotides. In some embodiments, the first polynucleotide or the second polynucleotide is a DNA polynucleotide and the other is an RNA polynucleotide.

10 The first polynucleotide and/or the second polynucleotide may be under the control of a promoter, such as an inducible promoter or a constitutive promoter. The first and/or the second polynucleotide may each be under the control of a first and/or second promoter, respectively, which may be identical or different. They may also be under the control of a single promoter.

15 The first and the second polynucleotides of the expression system may be comprised within the same molecule. The first and the second polynucleotides of the expression system may alternatively be comprised within different molecules, such as within two or more separate molecules.

20 The first and/or the second polynucleotide may further comprise a secretion or excretion signal to obtain a fusion protein comprising such a signal, whereby the protein fused to the first peptide tag and/or the antigen fused to the second peptide tag is secreted or excreted from the endoplasmic reticulum and optionally also from the cell.

25 The present expressions systems can be used for prophylaxis and/or treatment of a wide range of diseases as disclosed herein above.

30

Cells and host cells

The invention further relates to a cell, such as a host cell, comprising a polynucleotide and/or an expression system as disclosed herein. The polynucleotide and/or expression system may have a sequence that is codon-optimised. Codon optimisation methods are known in the art and allow optimised expression in a heterologous host

35

organism or cell. In an embodiment the cell may be selected from the group comprising bacteria, yeast, fungi, plant, mammalian and/or insect cells.

5 Methods for expressing a first polypeptide and/or a second polypeptide in a cell, such as a host cell, are known in the art. The first or second polypeptide may be heterologously expressed from corresponding polynucleotide sequences cloned into the genome of the cell or they may be comprised within a vector. For example, a first and/or second polynucleotide coding for the first and/or second polypeptide is cloned into the genome, and a first and/or second polynucleotide coding for the first and/or
10 second polypeptide is comprised within a vector transformed or transfected into the cell.

Expression of the first and second polypeptides in the cell may occur in a transient manner. When the polynucleotide encoding one of the polypeptides is cloned into the
15 genome, an inducible promoter may be cloned as well to control expression of the polypeptides. Such inducible promoters are known in the art. Alternatively, genes coding for suppressors of gene silencing may also be cloned into the genome or into a vector transfected within the cell.

20 Also provided herein is thus a cell expressing:
i. a first polynucleotide encoding a protein fused to a first peptide tag, preferably as defined in any one of the preceding claims; and
ii. a second polynucleotide encoding an antigen fused to a second peptide tag, preferably as defined in any one of the preceding claims;
25 wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.

30 In some embodiments, the cell is a bacterial cell. In some embodiments, the cell is a yeast cell. In some embodiments, the cell is a fungal cell. In some embodiments, the cell is a plant cell. In some embodiments, the cell is a mammalian cell, such as a human cell. In some embodiments, the cell is an insect cell.

35 In a particular embodiment the cell, such as the host cell, may be selected from the group comprising *Escherichia coli*, *Spodoptera frugiperda* (sf9), *Trichoplusia ni* (BTI-TN-5B1-4), *Pichia Pastoris*, *Saccharomyces cerevisiae*, *Hansenula polymorpha*,

Drosophila Schneider 2 (S2), *Lactococcus lactis*, Chinese hamster ovary (CHO), Human Embryonic Kidney 293, *Nicotiana tabacum* cv. Samsun NN and *Solanum tuberosum* cv. Solara. Thus in an embodiment, the cell is *Escherichia coli*. In another embodiment, the cell is *Spodoptera frugiperda*. In another embodiment, the cell is *Pichia Pastoris*. In another embodiment, the cell is *Saccharomyces cerevisiae*. In another embodiment, the cell is *Hansenula polymorpha*. In another embodiment, the cell is *Drosophila Schneider 2*. In another embodiment, the cell is *Lactococcus lactis*. In another embodiment, the cell is Chinese hamster ovary (CHO). In another embodiment, the cell is Human Embryonic Kidney 293. In another embodiment, the cell is *Trichoplusia ni* (BTI-TN-5B1-4). In another embodiment, the cell is *Nicotiana tabacum* cv. Samsun NN. In another embodiment, the cell is *Solanum tuberosum* cv. Solara.

In some embodiments, the cell, such as the host cell, expresses:

- i. a first polynucleotide encoding a protein fused to a first peptide tag; and
- ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen, and wherein the cell is selected from the group comprising bacteria, yeast, fungi, plant, mammalian and/or insect cells.

The present cells, such as host cells, can be used for prophylaxis and/or treatment of a wide range of diseases as disclosed herein above.

Examples

Example 1 – Materials and methods for Examples 2 and 3

Gene sequences of Spytagged Hepatitis B core antigen, Spytagged Acinobacter bacteriophage AP205 major coat protein and SpyCatcher-fused enhanced green fluorescent protein (eGFP) were codon optimized and synthesised by Geneart.

Sequences:

>pVax1_ SpyTag-AP205

AHIVMVDAYKPTKSGTAGGGSGSANKPMQPITSTANKIVWSDPTRLSTTFASLLRQ
 RVKVGIAELNNVSGQYVSVYKRPAPKPEGCADACVIMPNNENQSIRTVISGSAENLATL
 KAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTA* (SEQ ID NO: 85, DNA
 sequence in SEQ ID NO: 88)

5

>pVAX1_ HBc-SpyTag

MDIDPYKEFGASVELLSFLPSDFFPSIRDLLDTASALYREALESPEHVSPHHTALRQAIL
 CWGELMNLATWGSNLEDPASRELVSYNVNMGLKLRQILWFHISCLTFGRETVE
 YLVSFVWIRTPTAYRPPNAPILSTLPETTIVGGGGGSPGGGTPSPGGGGSQSPGG
 GGSQSGESQCGSAHIVMVDAYKPTK* (SEQ ID NO: 86, DNA sequence in SEQ ID
 NO: 89)

10

>pVAX1-SPYCEGFP

GAMVDTLSGLSSEQGQSGDMTIEEDSATHIKFSKRDEDGKELAGATMELRDSSGKTI
 STWISDGQVKDFYLYPGKYTFVETAAPDGYEVATAITFTVNEQGQVTVNGKATKGDA
 HIGGSGSMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTT
 GKLPVPWPTLVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNY
 KTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQKNGIKVNF
 KIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHVYSTQSALSKDPNEKRDHMLLEF
 VTAAGITLGMDELYK* (SEQ ID NO: 87, DNA sequence in SEQ ID NO: 90)

20

Modular combinatorial INFUSION shuffling and cloning of constructs into a plasmid
 vector suitable for DNA immunization (pVAX1 – see Figure 5) was performed.

25 Mouse derived C2C12 myoblast precursor cell line was transfected (reverse
 transfection, 2x24 well plates using Lipofectamine 2000 reagent) with:

- A) SPYCEGFP (n=3)
- B) SPYCEGFP and SpyTAP205 (n=3)
- C) SPYCEGFP and HBcSpyT (n=3)

30 ***Example 2 – Formation of distinct intracellular particles in transfected
 mammalian cells***

Co-transfection of eukaryotic cells with eGFP (genetically fused at N-terminus with
 SpyCatcher) and the Hepatitis B core antigen (genetically fused to SpyTag at C-
 terminus) resulted in the apparent expression of both components, which subsequently
 35 were able to bind (via interaction between SpyTag and SpyCatcher) to each other and

form particulate complexes displaying eGFP. Specifically, confocal laser scanning microscopy (CLSM) of mouse cells harvested 72 hours after co-transfected with SpyCatcher-eGFP and Spytagged HBcore, respectively, revealed a distinct perinuclear distribution of eGFP fluorescent signals, indicating particularization of eGFP (see Figure 1).

In contrast, transfection with SpyCatcher-eGFP and a Spytagged AP205 coat protein (previously shown to spontaneously form VLP upon expression in *E. coli*) resulted a diffuse/smeared eGFP fluorescent signal throughout the cell (see Figure 2).

Overall, these results indicate that both the SpyCatcher eGFP and HBcSpyT protein were successfully expressed and that the eGFP was able to bind (via spyTag/SpyCatcher) to particulate structures formed by the HBcSpyT capsid protein.

Example 3 - Simultaneous expression and assembly of encoded proteins into particulate complexes in mammalian cells

To further investigate if the Spytagged coat proteins (AP205 and HBcore) could be expressed and form virus-like particles, and whether the SpyCatcher-eGFP protein could also bind (via spyTag / SpyCatcher) to such particles, we harvested the co-transfected C2C12 cells (48 hours after transfection), which were then sonicated for 45s (3 times, 30%) and fractionated by ultracentrifugation (UC) using an iodixanol ultracentrifugation density gradient column. The post-UC fractions (incl. controls) were run on SDS-PAGE and transferred to a nitrocellulose membrane, which was finally probed with polyclonal HRP conjugated anti-GFP antibodies.

This experiment indicated that co-transfection of SpyCatcher eGFP and SpyTagged AP205 may have led to expression and conjugation of the two proteins, as indicated by the appearance of a protein band in the UC input lane (/ pellet lane) having the approximate size of the theoretical size (i.e. 59 kDa) of the conjugate. However, there were not seen any protein bands of that size in any of the UC fractions (F3-F21), which could have contained particulate proteins (Figure 3).

However, a similar analysis of C2C12 cells co-transfected with SpyT-HBc and SpyCatcher-eGFP, shows a clear protein band of approx. 64 kDa (i.e. the theoretical size of a SpyTHBc:SpyCatcher-eGFP conjugate) both in the UC-input sample as well

as in post UC fractions (F3-21, F31 and F32) including in high-density fractions expected to contain particulate protein complexes (Figure 4). These results further indicate that co-transfection of Spytagged HBcore antigen and SpyCatcher-eGFP is able to simultaneously express and assemble into particulate complexes in a mammalian cell.

Example 4 – DNA sequences used in Example 5 to 10

The following nomenclature is used throughout examples 5 to 10 to refer to DNA sequences encoding the indicated amino acid SEQ ID NO:s.

Abbreviation	Description	Encodes SEQ ID NO:
Sign1	Secretion signal tag (from azurocidin)	79
Sign2	Secretion signal tag (from serum albumin)	80
Sign3	Secretion signal tag (from modified serum albumin)	81
Sign4	Secretion signal tag (from Ig kappa chain V III region)	82
Sign5	Secretion signal tag (from Modified Ig kappa chain V III region MOPC 63 like (mlgk C))	83
Sign6	Secretion signal tag (from Modified Ig kappa chain V III region VG (mlgk H))	84
Sign7	Secretion signal tag (from tissue plasminogen activator)	99
Sign8	Secretion signal tag (from chymotrypsinogen B)	100
Sign9	Secretion signal tag (from secreted embryonic alkaline phosphatase)	101
SpyC	SpyCatcher	21
i301	i301 particle-forming protein	72
ctag	C-terminal tag	91
Hbc	Hepatitis core protein	92
tandemHBc	Tandem hepatitis core protein construct	93
SpyT	SpyTag	1
Ferritin	Human ferritin (amino acids 5-174)	69
eGFP	Enhanced green fluorescent protein	94

His	His purification tag	95
Pfs25	Plasmodium falciparum antigen	96
E2	2-oxo acid dehydrogenase subunit E2	97
LS	Lumazine synthase	70
Norovirus	Norovirus capsid protein	98

Example 5 - Expression of soluble antigens and particle-forming proteins from plasmid DNA

Methods

5 DNA was cloned in pVAX1 vector (V26020, thermoFisher) and cloned in E.coli (One shot Top10, Invitrogen, C404006). Vectors were purified using a midiprep kit.

HEK293-Freestyle cells were transfected or co-transfected with 37.5ug in 30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies).

10

After incubation for 6days, cells and supernatant were harvested. Supernatant was frozen down at -20°C and represent the samples called supernatant (SN). Cells were pelleted by spinning down at 1200RPM, 5min, and resuspended in PBS with Complete™, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma, 11836170001). Cells
15 were sonicated at 30%, 45s, 3 times and spun down 20.000g, 10min, 4°C. The supernatant is frozen at -20°C and represent the samples called "cell".

Both cell and SN were run on denaturing SDS+DTT gels (15uL loaded), the SDS gel was then transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk
20 overnight. Proteins on the membrane were detected with a primary antibody (as specified for each gel) and if necessary a secondary antibody (as specified for each gel). Membrane was washed 3 times between each antibody and prior to development with TBS-T. The membrane was developed with ECL substrate according to manufacturer's instructions.

25 Results and conclusion

After DNA transfection in mammalian cells *in vitro*, expression and secretion (through a secretion signal) of particles and soluble antigens could be detected by WB of the cell and SN from transfected cells. The Western blots shows the appearance of a band at the expected size for the particle or soluble antigen.

This was shown for the following:

- Sign3-SpyC-i301-ctag
- 5 • Sign8-tandemHBc-SpyC
- Sign8-HBc-SpyT
- Sign9-Ferritin-SpyC
- Sign8-SpyT-eGFP
- Sign8-eGFP
- 10 • Sign8-SpyC-His
- Sign7-Pfs25-SpyT-Ctag
- Sign8-SpyC-eGFP

As seen in Figures 6 and 7, bands of the expected size in cell and supernatant
15 samples for all constructs were observed, thus indicating expression and secretion of
the particle-forming subunit proteins as well as soluble protein antigens in human
(HEK) cells, after plasmid DNA transfection.

Example 6 - Coupling of particles and proteins expressed from plasmid DNA

20 Methods

HEK293-Freestyle cells were co-transfected with 37,5ug (18.75ug of each vector) in
30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies).

Cells were co-transfected with a vector encoding for a particle and a vector encoding
25 for a protein with a compatible tag-catcher for coupling.

After incubation for 6 days, cells and supernatant were harvested. Supernatant was
frozen down at -20°C and represent the samples called supernatant (SN). Cells were
pelleted by spinning down at 1200RPM, 5 min, and resuspended in PBS with
30 Complete™, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma, 11836170001). Cells
were sonicated at 30%, 45s, 3 times and spun down 20 000g, 10min, 4°C. The
supernatant is frozen at -20°C and represent the samples called "cell".

Both cell and SN were run on denaturing SDS+DTT gels (15uL loaded), the SDS gel was then transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane were detected with a primary antibody (as specified for each gel) and if necessary a secondary antibody (as specified for each gel). Membrane was washed 3 times between each antibody and prior to development with TBS-T. The membrane was developed with ECL substrate according to the manufacturer's instructions.

Results and conclusion

After in vitro DNA co-transfection of mammalian (HEK293) cells, the isopeptide bond-mediated conjugation of individually expressed antigens and particle-forming proteins, respectively, could be verified by WB by detecting a band of the expected size of the conjugated antigen and particle-forming protein.

This was shown for the following:

- Sign8-SpyT-eGFP and sign9-SpyC-Ferritin
- Sign8-SpyC-eGFP and sign8-Hbc-SpyT
- Sign8-tandemHBc-SpyC and sign8-SpyT-eGFP
- Sign3-SpyC-i301-ctag and sign8-SpyT-eGFP
- Sign9-SpyT-E2 and sign8-SpyC-eGFP
- Sign9-SpyT-LS and sign8-SpyC-eGFP
- Sign8-SpyC-His and sign9-SpyT-E2
- Sign8-SpyC-His and sign9-LS-SpyT
- Sign8-SpyC-His and sign8-Hbc-SpyT
- Sign8-SpyC-Ferritin and sign7-Pfs25-SpyT
- Sign3-SpyC-i301-Ctag and sign7-Pfs25-SpyT
- Sign8-Norovirus-SpyT and sign8-SpyC-His

As seen in Figures 8, 9 and 10, bands of the expected size for coupled eGFP, SpyCatcher and Pfs25 (model antigens) to different particle-forming subunit proteins in cells and supernatant for all constructs were observed. This indicates that the antigens and the particles with the corresponding tag/catcher are able to couple *in vitro*, after co-transfection in human (HEK) cells.

Example 7 – Verification of nanoparticle formation by ultracentrifugation and Western blotting

Methods

After HEK cell transfection and harvest, supernatant was loaded onto an Optiprep step
5 gradient (23, 29 and 35%) followed by centrifuged for 3h 30min at 47800g, 16°C. The
gradient was then dripped into fractions (F1-F12) each fraction containing
approximately 250uL.

If a particle was formed it is expected that it will be found in the middle fractions (F3-
10 F8).

All fractions were run on denaturing SDS+DTT gels (15 µL loaded), the SDS gel was
then transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk
overnight. Proteins on the membrane were detected with a primary antibody (as
15 specified for each gel) and if necessary a secondary antibody (as specified for each
gel). The membrane was washed 3 times between each antibody and prior to
development with TBS-T. The membrane was developed with ECL substrate according
to the manufacturer's instructions.

Results and conclusion

20 After DNA co-transfection or transfection in mammalian cells in vitro, we could detect
potential particle formation and secretion. Particle formation can be expected when the
proteins are found within the fractions 3-8 from the density gradient. This was the case
for the following:

- 25 • Sign3-SpyC-i301-Ctag
- Sign8-tandemHBc-SpyCatcher
- Sign9-Ferritin-SpyC
- Sign8-SpyT-eGFP + sign9-SpyC-Ferritin
- Sign8-SpyC-His + sign9-SpyT-E2
- 30 • Sign8-SpyC-His + sign9-LS-SpyT
- Sign9-SpyC-Ferritin + sign7-Pfs25-SpyT
- Sign3-SpyC-i301 + sign7-Pfs25-SpyT
- Sign9-SpyT-E2 + sign8-SpyC-eGFP
- Sign9-LS-SpyT + sign8-SpyC-eGFP

35

As seen in Figures 11 and 12, particle formation as well as the formation of particles coupled to antigens were observed in all depicted constructs, as bands are seen of the expected sizes present in the relevant fractions (fractions 3-8). This thus indicates that after transfection with plasmid DNA in human (HEK) cells, there was not only expression and secretion of the particle subunits and the soluble antigens *in vivo*, but also formation of the particles and of the particles coupled to antigens *in vitro*.

Example 8 - Visualisation of particle formation by transmission electronic microscopy

After HEK cell transfection, harvest and purification by ultracentrifugation, samples were run on transmission electronic microscopy (TEM).

After DNA co-transfection or transfection in mammalian cells *in vitro*, secreted particle formation was visualized by TEM.

This was shown for the following:

- Sign9-Ferritin-SpyC
- Sign8-SpyC-His + sign9-LS-SpyT
- Sign9-SpyT-E2 + sign8-SpyC-His
- Sign8-SpyT-eGFP + sign9-SpyC-Ferritin
- Sign3-SpyC-i301-Ctag + sign7-Pfs25-SpyT-Ctag
- Sign9-SpyC-Ferritin + sign7-Pfs25-SpyT-Ctag

As can be seen from Figure 13, particles of the expected size formed in the supernatant of transfected or co-transfected cells. Thus, it is further confirmed that particles and coupled particles are able to form in the supernatant of transfected or co-transfected cells *in vitro* from plasmid DNA.

Example 9 - Verification of intracellular conjugation of the antigen to the nanoparticle-forming protein

Methods

DNA was cloned in pVAX1 (V26020, thermoFisher) vector and cloned in E.coli (One shot Top10, Invitrogen, C404006). Vectors were purified using a midiprep kit.

HEK293-Freestyle cells were transfected or co-transfected with 37.5ug in 30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies).

- 5 After incubation for 6days, cells and supernatant were harvested. Supernatant was frozen down at -20°C and represent the samples called supernatant (SN). Cells were pelleted by spinning down at 1200RPM, 5min, and resuspended in 1x SDS+DTT and frozen at -20°C and represent the samples called "cell".
- 10 Both cell and SN were run on denaturing SDS+DTT gels (15µL loaded), the SDS gel was then transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane were detected with a primary antibody (as specified for each gel) and if necessary a secondary antibody (as specified for each gel). Membrane was washed 3 times between each antibody and prior to development
- 15 with TBS-T. Membrane was developed with ECL substrate according to manufacturer's instructions.

Results and conclusion

- This technique allows for visualization of coupling between the particle and the soluble antigen in the cells. We have previously shown that some particles could couple with
- 20 some antigens, however we did not know if it was happening after secretion or already inside the cell. This experiment shows that coupling is happening intracellularly, as we observe the coupling band from the cell substrate harvested in SDS+DTT (these compounds prohibiting coupling after harvest).

- 25 This was shown for the following:

- Sign8-SpyC-His and sign9-LS-SpyT
- Sign8-SpyT-eGFP + sign9-SpyC-Ferritin
- Sign3-SpyC-i301-ctag and sign8-SpyT-eGFP
- 30 • Sign8-eGFP-SpyC and sign9-LS-SpyT

- As seen in Figure 14, particles and soluble antigens were able to couple intracellularly, and not only in the supernatant. Indeed, a band was visualised of the expected coupling size in cells harvested with SDS+DTT, thus indicating that the coupling occurred inside
- 35 the cells in the *in vitro* culture.

Example 10 - Plasmid DNA immunization in miceMethods

DNA encoding for particles and/or soluble antigen was used for vaccination in mice.

- 5 Balb/c mice were immunized with 30 µg of LS-SpyT and 30 µg of SpyC (N=6), or 30 µg of SpyC (N=4), or 30 µg of E2-SpyT and 30 µg of SpyC (N=6). DNA was formulated in PBS, and injected in the right thigh muscle.

- 10 Mice were immunized on day 0 and week 5, and blood was drawn on week 3 and 4 post prime and post boost. Serum was isolated from the blood and run on ELISA for detection of anti-SpyC IgG. For that purpose, 96-well plates (Nunc MaxiSorp) were coated overnight at 4°C with 0.1 µg/well SpyC in PBS. Plates were blocked for 1 hour at room temperature (RT) using 0.5% skimmed milk in PBS. Mouse serum was diluted 1:50 or 1:10 in blocking buffer, and added to the plate in a 2-fold dilution, followed by
- 15 incubation for 1 hour at RT. Plates were washed three times in PBS in between steps. In order to measure total serum IgG, Horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (Life technologies, A16072) was diluted 1:1000 in blocking buffer followed by 1 hour incubation at RT. Plates were developed with TMB X-tra substrate (Kem-En-Tec, 4800A) and absorbance was measured at 450 nm. Data were collected
- 20 on a BioSan HiPo MPP-96 microplate reader and analyzed using GraphPad Prism (San Diego, USA, version 8.4.3).

Results and conclusion

- 25 After DNA immunization in mice, it was seen that mice receiving DNA encoding the particles and DNA encoding for SpyC, have higher IgG titers against SpyC after only a first immunization, compared to mice receiving only the DNA encoding for the SpyC. Additionally, this trend is even higher after a boost immunization. This is true for both groups of mice that have received SpyC in combination with either the LS particle or the E2 particle.

30

This is shown in Figure 15, where mice receiving DNA encoding for the particles and DNA encoding for SpyC, have higher IgG titers against SpyC after a first immunization than mice receiving only DNA encoding for SpyC, as shown by ELISA.

Example 11 - Expression of soluble antigens and particle-forming proteins from mRNA

HEK293-Freestyle cells will be transfected or co-transfected with 37,5 µg in 30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies).

5

After incubation for 6 days, cells and supernatant will be harvested. Supernatant will be frozen down at -20°C and represent the samples called supernatant (SN). Cells will be pelleted by spinning down at 1200RPM, 5min, and resuspended in PBS with Complete™, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma, 11836170001). Cells will be sonicated at 30%, 45s, 3 times and spun down 20000g, 10min, 4°C. The supernatant is frozen at -20°C and represent the samples called "cell".

10

Both cell and SN will be run on denaturing SDS+DTT gels (15 µL loaded). The SDS gel will be transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane will be detected with a primary antibody and if necessary a secondary antibody. Membrane will be washed 3 times between each antibody and prior to development with TBS-T. Membranes will be developed with ECL substrate according to the manufacturer's instructions.

15

After mRNA transfection in mammalian cells *in vitro*, expression and secretion (through a secretion signal) of particles and soluble antigens will thus be detected by Western blotting similar to what was described in Example 5.

20

Bands of the expected size in cells and supernatant for all constructs (particle-forming subunit proteins or soluble protein antigens), will indicate expression and secretion of soluble proteins in human (HEK) cells, after mRNA transfection.

25

Example 12 - Verification of the conjugation of soluble antigen to different particle-forming proteins expressed from mRNA

HEK293-Freestyle cells will be co-transfected with 37,5 µg (18.75 µg of each RNA) in 30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies)

30

Cells will be co-transfected with a vector encoding for a particle and a vector encoding for a protein with a compatible tag-catcher for coupling.

35

After incubation for 6 days, cells and supernatant will be harvested. Supernatant will be frozen down at -20°C and represent the samples called supernatant (SN). Cells will be pelleted by spinning down at 1200RPM, 5min, and resuspended in PBS with Complete™, Mini, EDTA-free Protease Inhibitor Cocktail (Sigma, 11836170001). Cells
5 will be sonicated at 30%, 45s, 3 times and spun down 20000g, 10min, 4°C. The supernatant is frozen at -20°C and represent the samples called "cell".

Both cell and SN will be run on denaturing SDS+DTT gels (15 µL loaded). The SDS gel will be transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane will be detected with a primary antibody and if
10 necessary a secondary antibody. Membrane will be washed 3 times between each antibody and prior to development with TBS-T. Membranes will be developed with ECL substrate according to the manufacturer's instructions.

15 After mRNA co-transfection in mammalian cells *in vitro*, expression, secretion and coupling of particles to soluble antigens will be detected by WB, by detecting a band of the expected size of the particle and the antigen, similar to what was described in Example 6.

20 ***Example 13 – Verification of nanoparticle formation when expressed from transfected mRNA by ultracentrifugation and Western blotting***

After Hek cells transfection with mRNA and harvest, the supernatant will be loaded onto an Optiprep step gradient (23, 29 and 35%) followed by centrifuged for 3h 30min at 47800g, 16°C. The gradient will then be dripped into fractions (F1-F12), each
25 fraction containing approximately 250 µL.

If a particle is formed it is expected that it will be found in the middle fractions (F3-F8).

30 All fractions will be run on denaturing SDS+DTT gels (15 µL loaded). The SDS gel will be transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane will be detected with a primary antibody and if necessary a secondary antibody. Membranes will be washed 3 times between each antibody and prior to development with TBS-T. Membranes will be developed with ECL substrate according to the manufacturer's instructions.

35

After mRNA co-transfection or transfection in mammalian cells in vitro, potential particle formation and secretion will be detected, similar to what was described in Example 7. Particle formation can be expected when the proteins are found within fractions 3-8 from the density gradient.

5

Example 14 – Visualisation of particle formation by transmission electronic microscopy

As described in Example 8, secreted, assembled particles expressed from mRNA constructs in HEK cells will be detected by transmission electronic microscopy.

10

Example 15 – Verification of intracellular conjugation of the antigen to the nanoparticle-forming protein when expressed from mRNA

HEK293-Freestyle cells will be transfected or co-transfected with 37.5µg RNA in 30mL culture using FreeStyle™ MAX Reagent (16447100, Life Technologies)

15

After incubation for 6 days, cells and supernatant will be harvested. Supernatant will be frozen down at -20°C and represent the samples called supernatant (SN). Cells will be pelleted by spinning down at 1200RPM, 5min, and resuspended in 1x SDS+DTT and frozen down at -20°C and represent the samples called "cell".

20

Both cell and SN will be run on denaturing SDS+DTT gels (15 µL loaded). The SDS gel will be transferred on a Nitrocellulose membrane and blocked in TBS-T 5% milk overnight. Proteins on the membrane will be detected with a primary antibody and if necessary a secondary antibody. Membrane will be washed 3 times between each antibody and prior to development with TBS-T. Membranes will be developed with ECL substrate according to the manufacturer's instructions.

25

This will allow for visualization of coupling between the particle and the soluble antigen in the cells, similar to what was shown in Example 9. This will show that coupling occurs intracellularly when the constructs are expressed from mRNA.

30

Example 16 – mRNA immunization in mice

mRNA encoding particles and/or soluble antigens (the particle and antigen each fused to a separate peptide tag) will be used for vaccination in mice, similar to what was described in Example 10.

5

Balb/c mice will be immunized with either a combination of mRNA encoding the particle and mRNA encoding for soluble antigen, or with only mRNA encoding a soluble antigen. Mice will be immunized on day 0 and week 5, and blood will be drawn on week 3 and 4 post prime and post boost.

10

Serum will be isolated from the blood and run on ELISA for detection of antigen specific IgG. For that purpose, 96-well plates (Nunc MaxiSorp) will be coated overnight at 4°C with 0.1µg/well SpyC in PBS. Plates will be blocked for 1 hour at room temperature (RT) using 0.5% skimmed milk in PBS. Mouse serum will be diluted 1:50 in blocking buffer, and added to the plate in a 2-fold dilution, followed by incubation for 1 hour at RT. Plates will be washed three times in PBS in between steps. In order to measure total serum IgG, Horseradish peroxidase (HRP) conjugated goat anti-mouse IgG (Life technologies, A16072) will be diluted 1:1000 in blocking buffer followed by 1 hour incubation at RT. Plates will be developed with TMB X-tra substrate (Kem-En-Tec, 4800A) and absorbance measured at 450nm. Data will be collected on a BioSan HiPo MPP-96 microplate reader and analyzed using GraphPad Prism (San Diego, USA, version 8.4.3).

15

20

25

It is expected that after mRNA immunization in mice, the mice receiving RNA encoding the particles and soluble antigen (and thus forming coupled antigen-particle complexes) have higher IgG titers against the soluble antigen compared to mice receiving only the RNA encoding for the soluble antigen. Similar to the results shown in Example 10, this trend is expected to be even higher after a boost immunization.

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30

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20

Sequence overview

Sequence ID NO:	Organism	Name	Sequence
Tags (amino acid)			
1	<i>Streptococcus pyogenes</i>	SpyTag	AHIVMVDAYKPTK
2	<i>Streptococcus dysgalactiae</i>	SdyTag	DPIVMIDNDKPIT
3	<i>Streptococcus pneumoniae</i>	SnoopTag	KLGDIEFIKVNK
4	<i>Streptococcus phocae</i>	PhoTag (LPXTG cell wall anchor domain-	LVTGTAHIVMVDNYKPIVETGD

		containing protein) (NCBI Reference Sequence: WP_082385550.1)	
5	<i>Enterococcus faecalis</i>	EntTag (hypothetical protein CUN42_14770, partial, GenBank PQC83400.1)	NTIVMVDKLKEVPPT
6	<i>Ruminococcus</i> sp. AF26-25AA	RumTag (Cna B-type domain-containing protein)	SENGNPLIVMVDDTTKVKIS
7	<i>Ruminococcus</i> sp. AF25-19	Rum2Tag (hypothetical protein DWY44_14000, partial)	GTPIVIMVDEAKPSLPD
8	<i>Ruminococcus</i> sp. Marseille-P6503	Rum3Tag (TonB-dependent receptor)	GNPLIVMIDEAEQKEIP
9	<i>Ruminococcus</i> sp.	Rum4Tag (hypothetical protein) (NCBI HCW12338.1)	AGGIIVMKDNTTKVSI
10	<i>Ruminococcus flavefaciens</i>	Rum5Tag (Cna B-type domain-containing protein) (NCBI	GNPIVTMIDDATLVKIS

		WP_051536060 .1)	
11	<i>Ruminococcus</i> <i>sp.</i>	Rum6Tag	GNSTITMVDDTTKVHIT
12	<i>Ruminococcus</i> <i>sp. AM43-6</i>	Rum7Tag (Cna B domain protein) (NCBI WP_118125159 .1)	GTPLIVMVDDTTKVEIS
13	Artificial sequence	Rumtrunk D9N	GNPLIVMVNDTTKVK
14	<i>Ruminococcus</i> <i>sp.</i>	Rumtrunk tag	GNPLIVMVDDTTKVK
15	<i>Bacillus cereus</i>	BacTag (choice- of-anchor A family protein) (NCBI WP_080470427 .1)	NEKVTGQFEIVKVDANDKTK
16	<i>Bacillus cereus</i>	Bac2Tag (hypothetical protein COD21_31890, partial) (NCBI PGT97799.1)	SKSLGQFEIVKVDAQDKTK
17	<i>Bacillus cereus</i>	Bac3Tag (choice-of- anchor A family protein) (NCBI WP_053565148 .1)	LGQFEIVKVDSQDKTK
18	<i>Bacillus cereus</i>	Bac4Tag (choice-of- anchor A family protein)	VTGQFEIVKVDAEDKTR

		(WP_088344882.1)	
19	<i>Bacillus cereus</i> VD022	Bac5Tag (hypothetical protein IC1_02949, partial, EJP89589.1)	EKVMGQFEIMKVDANDKTK
20	<i>Clostridium perfringens</i> B str. ATCC 3626	Cpe0147 (Uniprot: B1R775) aa 565-587 (esterbond forming TAG)	DTKQVVKHEDKNDKAQTLVVEKP
Catchers (amino acid)			
21	<i>Streptococcus pyogenes</i>	SpyCatcher	GAMVDTLSGLSSEQGQSGDMTIEEDS ATHIKFSKRDEGKELAGATMELRDS SGKTISTWISDGQVKDFYLYPGKYTF VETAAPDGYEVATAITFTVNEQGQVT VNGKATKGDHAI
22	<i>Streptococcus dysgalactiae</i>	SdyCatcher	IDTMSGLSGETGQSGNTTIEEDSTTH VKFSKRDSNGKELAGAMIELRNLSGQ TIQSWVSDGTVKDFYLMPGTYQFVET AAPEGYELAAPITFTIDEKGQIWDVS
23	<i>Streptococcus pneumoniae</i>	SnoopCatcher	SSGLVPRGSHMKPLRGAVFSLQKQHP DYPDIYGAIDQNGTYQNVRTGEDGKL TFKNLSDGKYRLFENSEPAGYKPVQN KPIVAFQIVNGEVRDVT SIVPQDIPA TYEFTNGKHYITNEPIPPK
24	<i>Actinomyces viscosus</i>	FimP domain 3	GSLSKYGKVILTKTGTDDLADKTKYN GAQFQVYECTKTASGATLRSDPSTQ TVDPLTIGGEKFTTAGQGTVEINYL RANDYVNGAKKDQLTDEDY YCLVETK APEGYNLQADPLPFRVLAEKAEKKA
25	<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Streptococcal ancillary pilin Domain 2	GSTTKVKLIKVDQDHNRLGEGVGFKL SVARDVSAAVPLIGEYRYSSSGQVG RTLYTDKNGEI FVTNLPLGNYRFKEV EPLAGYAVTTLDTDVQLVDHQLVT

26	<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Streptococcal ancillary pilin Domain 3	PRGNVDFMKVDGRNTNTSLQGAMFKVM KEESGHYTPVLQNGKEVVVTSKGDGR FRVEGLEYGTYLWELQAPTGYVQLT SPVSFTIGKDTRKELV
27	<i>Corynebacterium diphtheriae</i> NCTC 13129	Major Pilin SpaD Domain 3	VVTYHGKLVVKKDGKEAGKVLKGAE FELYQCTSAAVLGKGPLTVDGVKKWT TGDDGTFTIDGLHVTDFEDGKEAAPA TKKFLKETKAPAGYALPDPNVTEIE FTRAKISEKDKFEGDDEV
28	<i>Lactobacillus rhamnosus</i> GG	Pilin subunit (SpaA) domain 2	STNDTTTQNVVLTKYGFDDVTAIDR ATDQIWTGDGAKPLQGVDFTIYNVTA NYWASPKDYKGSFDSAPVAATGTTND KGQLTQALPIQSKDASGKTRAAVYLF HETNPRAGYNTSADFWLTLPAAAAAD GNVY
29	<i>Lactobacillus rhamnosus</i> GG	Pilin subunit (SpaA) domain 3	TTYERTFVKKDAETKEVLEGAGFKIS NSDGKFLKLTDKDQSVSIGEGFIDV LANNYRLTWVAESDATVFTSDKSGKF GLNGFADNTTYYTAVETNPVDGYDAA ANTDFKADNS
30	<i>Streptococcus agalactiae</i> A909	Surface protein Spb1 domain 3	GQITIKKIDGSTKASLQGAIFVLKNA TGQFLNFNDTNNVEWGTEANATEYTT GADGIITITGLKEGTYLVEKKAPLG YNLLDNSQKVILGDGATDTTNSDNLL VNP
31	<i>Streptococcus intermedius</i>	PsCsCatcher (LPXTG cell wall anchor domain-containing protein)	EQDVVFSKVNVAEEIAGAKIQLKDA QQQVHWSWTSKAGQSETVKLKAGTYT FHEASAPTGYLAVTDITFEVDVQGV TVKDANGNGVKAD
32	<i>Streptococcus pneumoniae</i>	RgA Catcher	KLGDIEFIKVNKNKKPLRGAVFSLQ KQHPDYPDIYGAIQNGTYQNVRTGE DGKLT FKNLSDGKYRLFENSEPAGYK PVQNKPIVAFQIVNGEVRDVT SIVPQ
33	<i>Corynebacterium diphtheriae</i> NCTC 13129	Major Pilin SpaD Domain 1	GSEKGSLLHKKKGAESEKRATGKE MDDVAGEPLNGVTFKITKLNFDLQNG DWAKFPKTAADAKGHETSTTKEVETS GNGTAVFDNLDLGIYLVEETKAPDGI VTGAPFIVSIPMVNEASDAWNYNVVA
34	<i>Clostridium perfringens</i> B str. ATCC 3626	Cpe0147 (Uniprot: B1R775) aa 439-587	NLPEVKDGTLRRTTVIADGVNGSSEKE ALVSFENSKDGVVDKDT INYEGLVAN QNYTLTGTLMHVKADGSLEEIATKTT NVTAGENGNGTWGLDFGNQKLQVGEK YVVFENAESVENLIDTDKDYNLDTKQ VVKHEDKNDKAQTLVVEKP

		(esterbond forming CATCHER)	
Tags (DNA)			
35	<i>Streptococcus pyogenes</i>	SpyTag DNA	GCTCACATCGTGATGGTGGACGCTTACAAGCCCACCAAG
36	<i>Streptococcus dysgalactiae</i>	SdyTag DNA	GACCCCATCGTGATGATCGACAACGA CAAGCCCATCACC
37	<i>Streptococcus pneumoniae</i>	SnoopTag DNA	AAACTGGGTGATATTGAATTTATTAA AGTTAATAAA
38	<i>Streptococcus phocae</i>	PhoTag DNA	CTGGTTACCGGCACCGCACATATTGT TATGGTTGATAACTATAAGCCGATCG TGGAAACCGGTGAT
39	<i>Enterococcus faecalis</i>	EntTag DNA	ACCGAAGTTAGCGGTAATACCATTGT GATGGTGGATAAACTGAAAGAAGTTC CGCCTACC
40	<i>Ruminococcus sp. AF26-25AA</i>	RumTag DNA	AGCGAAAACGGCAACCCGCTGATTGT GATGGTGGATGATACCACCAAAGTGA AAATTAGC
41	<i>Ruminococcus sp. AF25-19</i>	Rum2Tag DNA	GGCACCCCGATTGTGATTATGGTGGA TGAAGCGAAACCGAGCCTGCCGGAT
42	<i>Ruminococcus sp. Marseille-P6503</i>	Rum3Tag DNA	GGCAACCCGCTGATTGTGATGATTGA TGAAGCGGAACAGAAAGAAATTCG
43	<i>Ruminococcus sp.</i>	Rum4Tag DNA	GCGGGCGGCATTATTGTGATGAAAGA TAACACCACCAAAGTGAGCATTAGC
44	<i>Ruminococcus flavefaciens</i>	Rum5Tag DNA	GGCAACCCGATTGTGACCATGATTGA TGATGCGACCCTGGTGAAAATT
45	<i>Ruminococcus sp.</i>	Rum6Tag DNA	GGCAACAGCACCATTACCATGGTGGA TGATACCACCAAAGTGCATATTACC
46	<i>Ruminococcus sp. AM43-6</i>	Rum7Tag DNA	GGCACCCCGCTGATTGTGATGGTGGA TGATACCACCAAAGTGGAATTAGC
47	Artificial sequence	Rumtrunk D9N DNA	GGTAATCCGCTGATTGTGATGGTGAA TGATACCACCAAAGTGAAA
48	<i>Ruminococcus sp.</i>	Rumtrunk tag	GGCAACCCGCTGATTGTGATGGTGGA TGATACCACCAAAGTGAAA

49	<i>Bacillus cereus</i>	BacTag DNA	GGTCAGTTCGAAATTGTTAAAGTTGATGCAAACGATAAACTAAA
50	<i>Bacillus cereus</i>	Bac2Tag DNA	AGCAAAAGCCTGGGCCAGTTTGAAATGTGAAAGTGGATGCGCAGGATAAAACCAA
51	<i>Bacillus cereus</i>	Bac3Tag DNA	CTGGGCCAGTTTGAAATTGTTAAAGTTGATAGCCAGGATAAAACCAA
52	<i>Bacillus cereus</i>	Bac4Tag DNA	GTTACCGGTCAGTTTGAAATCGTTAAAGTTGATGCCGAAGATAAGACCCGT
53	<i>Bacillus cereus</i> VD022	Bac5Tag DNA	GAAAAAGTGTGATGGGCCAGTTTCGAAATCATGAAAGTTGATGCCAACGACAAGACCAA
54	<i>Clostridium perfringens</i> B str. ATCC 3626	cpe0147 (Uniprot: B1R775) aa 565-587 (esterbond forming TAG) DNA	GACACCAAGCAGGTGGTGAAGCACGAGGACAAGAACGACAAGGCCAGACCC TGGTGGTGGAGAAGCCC
Catchers (DNA)			
55	<i>Streptococcus pyogenes</i>	SpyCatcher DNA	GGTGCAATGGTTGATACCCTGAGCGGTCTGAGCAGCGAACAGGGTCAGAGCGGTGATATGACCATTGAAGAAGATAGCGCAACCCACATCAAATTCAGCAAACGTGATGAAGATGGTAAAGAACTGGCAGGCGCAACAATGGAAGTGCCTGATAGCAGCGGTAAAACCATTAGCACCTGGATAGTGATGGTTCAGGTGAAAGATTTTTATCTGTACCCTGGCAAATACACCTTTGTTGAAACCGCAGCACCGGATGGTTATGAAGTTGCAACCGCAATTACCTTACCGTTAATGAACAGGGCCAGGTTACGTGAATGGTAAAGCAACCAAGGTGATGCACATATT
56	<i>Streptococcus dysgalactiae</i>	SdyCatcher DNA	ATGGGTATTGATACCATGAGCGGTCTGAGCGGTGAAACCGGTCAGAGCGGTATACCACCATTTGAAGAGGATAGCACCACACATGTGAAATTCAGCAAACGCGATGCAAACGGCAAAGAACTGGCAGGCGCAATGATTGAACTGCGTAATCTGAGTGGTCAGACCATTCAGAGCTGGGTTAGTGATGGCACCGTTAAAGATTTTATCTGATGCCCTGGCACCTATCAGTTTGTTGAAACCGCAGCACCGGAAGGTTATGAGCTGGCAGCACCGATTACCTTTACCATTGATGAAAAAGGTCAGATTTGGGTTGATAGC

57	<i>Streptococcus pneumoniae</i>	SnoopCatcher DNA	AGCAGCGGCCTGGTGCCGCGCGGCAG CCATATGAAGCCGCTGCGTGGTGCCG TGTTTAGCCTGCAGAAACAGCATCCC GACTATCCCGATATCTATGGCGCGAT TGATCAGAATGGGACCTATCAAAATG TGCGTACCGGCGAAGATGGTAACTG ACCTTTAAGAATCTGAGCGATGGCAA ATATCGCCTGTTTGAAAAATAGCGAAC CCGCTGGCTATAAACCAGTGCAGAAT AAGCCGATTGTGGCGTTTCAGATTGT GAATGGCGAAGTGCCTGATGTGACCA GCATTGTGCCGACAGGATATTCGGCT ACATATGAATTTACCAACGGTAAACA TTATATCACCATGAACCGATACCGC CGAAA
58	<i>Actinomyces viscosus</i>	FimP domain 3 DNA	GGTAGCCTGAGCAAATATGGTAAAGT GATTCTGACCAAACCGGCACCGATG ATCTGGCAGATAAAACCAAATATAAC GGTGCACAGTTTCAGGTGTATGAATG TACCAAACAGCAAGCGGTGCAACCC TGCGTGATAGCGATCCGAGCACACAG ACCGTTGATCCGCTGACCATTGGTGG TGAAAAAACCTTTACCACCGCAGGTC AGGGCACCGTTGAAATTAATTATCTG CGTGCCAATGATTATGTGAACGGTGC AAAAAAAGATCAGCTGACCGATGAAG ATTATTACTGTCTGGTTGAAACCAAA GCACCGGAAGGTTATAATCTGCAGGC AGATCCGCTGCCGTTTCGTGTTCTGG CCGAAAAAGCAGAAAAAAAAGCC
59	<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Streptococcal ancillary pilin Domain 2 DNA	GGTAGCACCACCAAAGTGAACTGAT TAAAGTTGATCAGGATCACAATCGTC TGGAAGGTGTTGGTTTTAACTGGTT AGCGTTGCACGTGATGTTAGCGCAGC AGCAGTTCCGCTGATTGGTGAATATC GTTATAGCAGCAGCGTCAAGTTGGT CGTACCCTGTATACCGATAAAAAATGG CGAAATTTTCGTTACCAATCTGCCGC TGGGTAACATATCGTTTTAAAGAAGTT GAACCGCTGGCAGGTTATGCAGTTAC CACACTGGATACCGATGTTCACTGG TTGATCATCAGCTGGTGACC
60	<i>Streptococcus pneumoniae</i> serotype 4 (strain ATCC BAA-334/TIGR4)	Streptococcal ancillary pilin Domain 3 DNA	CCGCGTGGTAATGTTGATTTTATGAA AGTTGATGGTCGCACCAATACCAGCC TGCAGGGTGCAATGTTTAAAGTGATG AAAGAAGAAAGCGGTCACTATACACC GGTGCTGCAGAATGGTAAAGAAGTTG TTGTTACCAGCGGTAAAGATGGTCGT TTTCGTGTTGAAGGTCTGGAATATGG CACCTATTATCTGTGGGAACTGCAGG CACCGACCGTTATGTTCACTGACC AGTCCGGTTAGTTTTACCATTGGCAA AGATACCCGTAAAGAAGTGGTG

61	<i>Corynebacterium diphtheriae</i> NCTC 13129	Major Pilin SpaD Domain 3 DNA	GTTGTTACCTATCATGGTAAACTGAA AGTGGTGAAAAAAGACGGTAAAGAGG CAGGCAAAGTTCTGAAAAGTGCAGAA TTTGAACGTATCAGTGTACCAGCGC AGCAGTTTTAGGTAAAGGTCCGCTGA CCGTTGATGGTGTGAAAAATGGACC ACCGGTGATGATGGCACCTTTACCAT TGATGGTCTGCATGTTACCGATTTTG AAGATGGTAAAGAAGCCGCACCGGCA ACCAAAAAATTTCTGTCTGAAAGAAAC CAAAGCACCGGCAGGTTATGCACTGC CTGATCCGAATGTGACCGAAAATTGAA TTTACCGGTGCAAAAATCAGCGAGAA AGATAAATTTGAAGGCGACGATGAAG TGACC
62	<i>Lactobacillus rhamnosus</i> GG	Pilin subunit (SpaA) domain 2 DNA	AGCACCAATGATACCACCACACAGAA TGTTGTTCTGACCAAATATGGCTTCG ATAAAGATGTTACCGCAATTGATCGT GCAACCGATCAGATTTGGACCGGTGA TGGTGCAAAACCGCTGCAGGGTGTG ATTTTACCATTTATAACGTGACCGCC AATTATTTGGGCAAGCCCGAAAGATTA TAAAGGCAGCTTTGATAGCGCACCGG TTGCAGCCACCGGTACAACAAATGAT AAAGGCCAGCTGACCCAGGCACTGCC GATTCAGAGCAAAGATGCAAGCGGTA AAACCCGTGCAGCAGTTTACCTGTTT CACGAAACCAATCCGCGTGCAGGTTA TAATACCAGCGCAGATTTTGGCTGA CCCTGCCTGCAAAAGCAGCAGCAGAT GGTAATGTTTAT
63	<i>Lactobacillus rhamnosus</i> GG	Pilin subunit (SpaA) domain 3 DNA	ACCACCTATGAACGTACCTTTGTTAA AAAAGACGCCGAAACCAAAGAGTTC TGGAAGGCGCAGGCTTTAAAATCAGC AATAGTGATGGCAAATTCCTGAAACT GACCGATAAAGATGGTCAGAGCGTTA GCATTGGTGAAGGTTTTATTGATGTT CTGGCCAATAACTATCGTCTGACCTG GGTTGCAGAAAGTGATGCAACCGTTT TTACCAGCGATAAAAGCGGCAAATTT GGTCTGAATGGTTTTGCAGATAATAC CACCACCTATACCGCAGTTGAAACCA ATGTTCCGGATGGTTATGATGCAGCA GCAAACACCGATTTCAAAGCCGATAA TAGC
64	<i>Streptococcus agalactiae</i> A909	Surface protein Spb1 domain 3 DNA	GGTCAGATTACCATCAAAAAATCGA TGGTAGCACCAAAGCAAGCCTGCAGG GTGCAATTTTTGTTCTGAAAAATGCA ACCGGTGAGTTTCCTGAATTTTAACGA TACCAATAATGTTGAATGGGGCACCG AAGCAAATGCCACCGAATATAACCACC GGTGCAGATGGTATTATTACCATTAC CGGTCTGAAAGAAGGCACCTATTACC TGGTTGAAAAAAGCACCGCTGGGT

			TATAATCTGCTGGATAATTACAGAA AGTGATTTTAGGTGATGGTGCAACCG ATACCACCAATAGCGATAACCTGCTG GTTAATCCG
65	<i>Streptococcus intermedius</i>	PsCsCatcher (LPXTG cell wall anchor domain- containing protein) DNA	GAACAGGATGTTGTGTTTAGCAAAGT TAATGTTGCCGGTGAAGAAATTGCGG GTGCAAAAATCCAGCTGAAAGATGCA CAGGGTCAAGTTGTTTCATAGCTGGAC CAGCAAAGCAGGTGAGAGCGAAACCG TTAAACTGAAAGCAGGCACCTATACC TTTCATGAAGCAAGCGCACCGACCGG TTATCTGGCAGTTACCGATATTACCT TTGAAGTTGATGTTTCAGGGTAAAGTG ACCGTTAAAGATGCAAATGTTAATGG TGTGAAAGCCGAC
66	<i>Streptococcus pneumoniae</i>	RgA Catcher DNA	AAACTGGGTGATATTGAGTTCATCAA AGTGAACAAAAACGATAAAAAACCGC TGCGTGGTGCAGTTTTTAGCCTGCAG AAACAGCATCCGGATTACCCGATAT TTATGGTGCATTGATCAGAATGGCA CCTATCAGAATGTTTCGTACCGGTGAA GATGGTAAACTGACCTTTAAAAACCT GAGCGACGGTAAATATCGCCTGTTTG AAAATAGCGAACCAGGCGAGTTATAAA CCGGTTCAGAATAAACCGATTGTGGC CTTTCAGATTGTTAATGGTGAAGTTC GTGATGTGACCAGCATTTGTCCGCAG
67	<i>Corynebacteri um diphtheriae</i> NCTC 13129	Major Pilin SpaD Domain 1 DNA	GGTAGCGAACGTAAAGGTAGTCTGAC CCTGCATAAAAAGAAAGGTGCAGAAA GCGAAAAACGTGCAACCGGTAAAGAA ATGGATGATGTTGCCGGTGAACCGCT GAATGGTGTACCTTTAAAATCACCA AACTGAACCTCGATCTGCAGAATGGT GATTGGGCAAAATTTCCGAAAAACCGC AGCAGATGCAAAAGGTGATGAAACCA GCACCACCAAAGAAGTGGAACACGAGC GGTAATGGCACCGCAGTTTTTGATAA TCTGGATCTGGGTATTTACCTGGTGG AAGAAACCAAAGCACCGGATGGTATT GTTACAGGTGCACCGTTTATTGTTAG CATTCCGATGGTTAATGAAGCAAGTG ATGCCTGGAATTATAACGTGTTGCA
68	<i>Clostridium perfringens</i> B str. ATCC 3626	cpe0147(uniprot : B1R775) aa 439-587 (esterbond forming catcher) DNA	AACCTGCCCCGAGGTGAAGGACGGCAC CCTGAGGACCACCGTGATCGCCGACG GCGTGAACGGCAGCAGCGAGAAGGAG GCCCTGGTGAGCTTCGAGAACAGCAA GGACGGCGTGGACGTGAAGGACACCA TCAACTACGAGGGCCTGGTGGCCAAC CAGAACTACACCCTGACCGGCACCCT GATGCACGTGAAGGCCGACGGCAGCC TGGAGGAGATCGCCACCAAGACCACC AACGTGACCGCCGGCGAGAACGGCAA CGGCACCTGGGGCCTGGACTTCGGCA ACCAGAAGCTGCAGGTGGGCGAGAAG

			TACGTGGTGTTCGAGAACGCCGAGAG CGTGGAGAACCTGATCGACACCGACA AGGACTACAACCTGGACACCAAGCAG GTGGTGAAGCACGAGGACAAGAACGA CAAGGCCAGACCCTGGTGGTGGAGA AGCCC
Particle-forming proteins (amino acid)			
69	<i>Homo sapiens</i>	Ferritin, aa 5-174	RMLKALNDQLNRELYSAYLYFAMAAY FEDLGLEGFANWMKAQAEIEIGHALR FYNYIYDRNGRVELDEIPKPPKEWES PLKAFAEAYEHEKFISKSTYELAALA EEEKDYSTRAFLEWFINEQVEEEASV KKILDKLKFAKDSPQILFMLDKELSA RAPKLPGLLMQGGGE
70	<i>Homo sapiens</i>	Lumazine synthase	MQIYEGKLTAEGLRFGIVASRFNHAL VDRLVEGAIDCIVRHGGREEDITLVR VPGSWEIPVAAGELARKEDIDAVIAI GVLIRGATPHFDYIASEVSKGLANLS LELRKPITFGVITADTLEQAIERAGT KHGKNGWEAALSAIEMANLFKSLR
71	<i>Homo sapiens</i>	NSP10	PANSTVLSFCAFAVDPKAYKDYLAS GGQPITNCVKMLCTHTGTGQAITVTP EANMDQESFGGASCCLYCRCHIDHPN PKGFCDLKGKYVQIPTTCANDPVGFT LRNTVCTVCGMWKGYGCS
72	<i>Homo sapiens</i>	I301	MKMEELFKKHKIVAVLRANSVEEAKK KALAVFLGGVHLIEITFTVPDADTVI KELSFLKEMGAIIGAGTVTSVEQARK AVESGAEFIVSPHLDEEISQFAKEKG VFYMPGVMTPTLVKAMKLGHTILKL FPGEVVGPFVKAMKGFPPNVKFVPT GGVNLNDVCEWFKAGVLAVGVGSALV KGTPVEVAEKAKAFVEKIRGCTE
73	<i>Thermotoga maritima</i>	5KP9	MGARASGSKSGSGSDSGSKMEELFKK HKIVAVLRANSVEEAKKALAVFLGG VHLIEITFTVPDADTVIKELSFLKEM GAIIGAGTVTSVEQCRKAVESGAEFI VSPHLDEEISQFCKEKGVFYMPGVMT PTLVKAMKLGHTILKLFPGEVVGPFQ FVKAMKGFPPNVKFVPTGGVNLNDVC EWFKAGVLAVGVGSALVKGTPVEVAE KAKAFVEKIRGCTEQKLISEEDLQSR PEPTAPPEESFRSGVETTTTPQKQEP IDKELYPLTSLRSLFGNDPSSQ
Particle-forming proteins (DNA)			
74	<i>Homo sapiens</i>	Ferritin DNA sequence covering aa 5-174	AGAATGCTGAAGGCCCTGAACGACCA GCTGAACAGAGAGCTGTACTCCGCT ACCTGTACTTCGCTATGGCCGCTTAC TTCGAGGACCTGGGCCTTGAGGGATT

			CGCCAACTGGATGAAGGCTCAGGCCG AGGAAGAGATCGGCCACGCTCTGAGA TTCTACAAC TACATCTACGACAGAAA CGGCCGCGTGGAAGTGGACGAGATCC CCAAGCCTCCTAAAGAGTGGGAGAGC CCTCTGAAGGCTTTCGAGGCTGCTTA CGAGCACGAGAAGTTCATCAGCAAGA GCATCTACGAGCTGGCCGCTCTGGCC GAAGAGGAAAAGGACTACTCTACCAG AGCCTTCCTGGAATGGTTCATCAACG AACAGGTGGAAGAGGAAGCCAGCGTC AAGAAGATCCTGGACAAGCTGAAGTT CGCCAAGGACAGCCCTCAGATCCTGT TCATGCTGGACAAAGAGCTGAGCGCC AGGGCTCCTAAACTGCCTGGACTGCT TATGCAAGGCGGCCGAA
75	<i>Homo sapiens</i>	Lumazine synthase DNA	ATGCAGATCTACGAGGGCAAGCTGAC CGCCGAGGGCCTGAGGTTCCGGCATCG TGGCCAGCAGGTTCAACCACGCCCTG GTGGACAGGCTGGTGGAGGGCGCCAT CGACTGCATCGTGAGGCACGGCGGCA GGGAGGAGGACATCACCCTGGTGAGG GTGCCCCGGCAGCTGGGAGATCCCCGT GGCCGCCGGCGAGCTGGCCAGGAAGG AGGACATCGACGCCGTGATCGCCATC GGCGTGCTGATCAGGGGCGCCACCCC CCACTTCGACTACATCGCCAGCGAGG TGAGCAAGGGCCTGGCCAACCTGAGC CTGGAGCTGAGGAAGCCCATCACCTT CGGCGTGATCACCGCCGACACCCTGG AGCAGGCCATCGAGAGGGCCGGCACC AAGCACGGCAACAAGGGCTGGGAGGC CGCCCTGAGCGCCATCGAGATGGCCA ACCTGTTCAAGAGCCTGAGG
76	<i>Homo sapiens</i>	NSP10 DNA	CCCGCCAACAGCACCGTGCTGAGCTT CTGCGCCTTCGCCGTGGACCCCGCCA AGGCCTACAAGGACTACCTGGCCAGC GGCGGCCAGCCATCACCAACTGCGT GAAGATGCTGTGCACCCACACCGGCA CCGGCCAGGCCATCACCGTGACCCCC GAGGCCAACATGGACCAGGAGAGCTT CGGCGGCCCGAGCTGCTGCCTGTACT GCAGGTGCCACATCGACCACCCCAAC CCCAAGGGCTTCTGCGACCTGAAGGG CAAGTACGTGCAGATCCCCACCACCT GCGCCAACGACCCCGTGGGCTTCAAC CTGAGGAACACCGTGTGCACCGTGTG CGGCATGTGGAAGGGCTACGGCTGCA GC
77	<i>Homo sapiens</i>	I301 DNA sequence (optimized for human expression)	ATGAAGATGGAGGAGCTGTTCAAGAA GCACAAGATCGTGGCCGTGCTGAGGG CCAACAGCGTGGAGGAGGCCAAGAAG AAGGCCCTGGCCGTGTTCTGGGCGG CGTGCACCTGATCGAGATCACCTTCA

			CCGTGCCCGACGCCGACACCGTGATC AAGGAGCTGAGCTTCCTGAAGGAGAT GGGCGCCATCATCGGCGCCGCCACCG TGACCAGCGTGGAGCAGGCCAGGAAG GCCGTGGAGAGCGGCGCCGAGTTCAT CGTGAGCCCCCACCTGGACGAGGAGA TCAGCCAGTTCGCCAAGGAGAAGGGC GTGTTCTACATGCCC GGCGTGATGAC CCCCACCGAGCTGGTGAAGGCCATGA AGCTGGGCCACACCATCCTGAAGCTG TTCCCCGGCGAGGTGGTGGGCCCCCA GTTCGTGAAGGCCATGAAGGGCCCCCT TCCCCAACGTGAAGTTCGTGCCCACC GGCGGCGTGAACCTGGACAACGTGTG CGAGTGGTTCAAGGCCGGCGTGCTGG CCGTGGGCGTGGGCAGCGCCCTGGTG AAGGGCACCCCCGTGGAGGTGGCCGA GAAGGCCAAGGCCTTCGTGGAGAAGA TCAGGGGCTGCACCGAG
78	<i>Thermotoga maritima</i>	5KP9 DNA	ATGGGGCGCCAGGGCCAGCGGCAGCAA GAGCGGCAGCGGCAGCGACAGCGGCA GCAAGATGGAGGAGCTGTTCAAGAAG CACAAGATCGTGGCCGTGCTGAGGGC CAACAGCGTGGAGGAGGCCAAGAAGA AGGCCCTGGCCGTGTTCTGGGCGGC GTGCACCTGATCGAGATCACCTTCAC CGTGCCCGACGCCGACACCGTGATCA AGGAGCTGAGCTTCCTGAAGGAGATG GGCGCCATCATCGGCGCCGGCACCGT GACCAGCGTGGAGCAGTGCAGGAAGG CCGTGGAGAGCGGCGCCGAGTTCATC GTGAGCCCCACCTGGACGAGGAGAT CAGCCAGTTCCTGCAAGGAGAAGGGCG TGTTCACATGCCC GGCGTGATGACC CCCACCGAGCTGGTGAAGGCCATGAA GCTGGGCCACACCATCCTGAAGCTGT TCCCCGGCGAGGTGGTGGGCCCCCAG TTCGTGAAGGCCATGAAGGGCCCCCTT CCCCCAACGTGAAGTTCGTGCCCACCG GCGGCGTGAACCTGGACAACGTGTGC GAGTGGTTCAAGGCCGGCGTGCTGGC CGTGGGCGTGGGCAGCGCCCTGGTGA AGGGCACCCCCGTGGAGGTGGCCGAG AAGGCCAAGGCCTTCGTGGAGAAGAT CAGGGGCTGCACCGAGCAGAAGCTGA TCAGCGAGGAGGACCTGCAGAGCAGG CCCGAGCCCACCGCCCCCCCCGAGGA GAGCTTCAGGAGCGGCGTGGAGACCA CCACCCCCCCCCAGAAGCAGGAGCCC ATCGACAAGGAGCTGTACCCCTGAC CAGCCTGAGGAGCCTGTTCCGGCAACG ACCCCAGCAGCCAG
Signal peptides (amino acid)			

79	<i>Homo sapiens</i>	SP of azurocidin	MTRLTVLALLAGLLASSRA
80	<i>Homo sapiens</i>	Serum albumin	MKWVTFISLLFLFSSAYS
81	<i>Homo sapiens</i>	Modified serum albumin	MKWVTFISLLFLFSSSSRA
82	<i>Cricetulus griseus</i>	Ig kappa chain V III region	MGSAALLLWVLLLWVPGSNG
83	<i>Cricetulus griseus</i>	Modified Ig kappa chain V III region MOPC 63 like (mlgk C)	MGSAALLLWVLLLWVPSSRA
84	<i>Homo sapiens</i>	Modified Ig kappa chain V III region VG (mlgk H)	MEAPAQLLFLLLLWLPSSRA
Others			
85	Artificial	pVax1_SpyTag-AP205 (amino acid)	AHIVMVDAYKPTKSGTAGGGSGSAN KPMQPITSTANKIIVSDPTRLSTTFS ASLLRQRVKVGIAELNNVSGQYVSVY KRPAPKPEGCADACVIMPNENQSIRT VISGSAENLATLKAETHKRNVDTL FASGNAGLGFLDPTAAIVSSDTTA
86	Artificial	pVAX1_HBc-SpyTag (amino acid)	MDIDPYKEFGASVELLSFLPSDFFPS IRDLLDTASALYREALESPHEVSPHH TALRQAILCWGELMNLATWVGSNLED PASRELVSIVNVNMGLKLRQILWFH ISCLTFGRETVLEYLVSFGVWIRTPT AYRPPNAPILSTLPETTVVGGGGGSP GGGTPSPGGGGSQSPGGGGSQSGESQ CGSAHIVMVDAYKPTK
87	Artificial	pVAX1-SPYCEGFP (amino acid)	GAMVDTLGSLSEQQSGDMTIEEDS ATHIKFSKRDEGKELAGATMELRDS SGKTISTWISDGQVKDFLYPGKYTF VETAAPDGYEVATAITFTVNEQGQVT VNGKATKGDHIGGSGSMVSKGEELF TGVVPILVELDGDVNGHKFSVSgege GDATYGKLTCLKFICTTGKLPVPWPTL VTTLTYGVQCFSRYPDHMKQHDFFKS AMPEGYVQERTIFFKDDGNYKTRAEV KFEGDTLVNRIELKGIDFKEDGNILG HKLEYNYNSHNVYIMADKQKNGIKVN FKIRHNIEDGSVQLADHYQQNTPIGD GPVLLPDNHYLSTQSALSKDPNEKRD HMLLEFVTAAGITLGMDELYK
88	Artificial	pVax1_SpyTag-AP205 (DNA)	GCTCACATCGTGATGGTGGACGCCTA CAAGCCACAAAAGGCTCTGGAACAG CTGGCGGCGGATCTGGCTCTGCCAAC AAACCTATGCAGCCCATCACCAGCAC CGCCAACAAGATCGTTTGGAGCGACC

			CCACCAGACTGAGCACCACATTCAGC GCTAGCCTGCTGAGACAGCGCGTGAA AGTGGGAATCGCCGAGCTGAACAATG TGTCCGGCCAGTACGTGTCCGTGTAC AAGAGGCCTGCTCCTAAGCCTGAGGG CTGTGCCGATGCCGTGTGTGATCATGC CCAACGAGAACCAGAGCATCAGAACC GTGATCAGCGGCAGCGCCGAGAACCT GGCTACACTGAAGGCTGAGTGGGAGA CACACAAGAGAAACGTGGACACCCTG TTCGCCTCTGGCAACGCTGGACTGGG CTTCTGGATCCTACAGCCGCTATCG TGCTAGCGACACCACAGCCTGA
89	Artificial	pVAX1_ HBC-SpyTag (DNA)	ATGGACATCGACCCCTACAAAGAATT TGGCGCCAGCGTGGAACGTCTGAGCT TCCTGCCTAGCGACTTCTTCCCTTCC ATCCGGGACCTGCTGGATACAGCCAG CGCACTGTATAGAGAGGCCCTGGAAT CTCCCGAGCACGTGTACCTCACCAC ACAGCTCTGAGACAGGCCATCCTGTG TTGGGGCGAGCTGATGAACCTGGCCA CATGGGTGCGAAGCAACCTGGAAGAT CCCGCCAGCAGAGAACTGGTGGTGTC CTACGTGAACGTGAACATGGGCCTGA AGCTGAGACAGATCCTGTGGTTCCAC ATCAGCTGCCTGACCTTCGGCAGAGA AACCGTGCTGGAATACCTGGTGTCTT TCGGCGTGTGGATCAGAACCCCTACC GCCTACAGACCTCCTAACGCTCCCAT CCTGAGCACCCCTGCCTGAGACAACAG TTGTTGGCGGAGGCGGAGGATCTCCT GGCGGAGGAACACCTTCTCCAGGTGG TGGTGGATCTCAAAGTCTTGGTGGTG GCGGTTCTCAGAGCGGCGAATCTCAG TGTGGCTCTGCCCACATCGTGATGGT GGACGCCCTACAAGCCCACCAATGA
90	Artificial	pVAX1-SPYCEGFP (DNA)	GAGCCATGGTGGATACACTGTCTGGA CTGTCTAGCGAGCAGGGCCAGAGCGG CGACATGACAATCGAGGAAGATAGCG CCACACACATCAAGTTCAGCAAGCGC GACGAGGACGGCAAAGAACTGGCTGG CGCTACCATGGAACCTGAGAGACAGCA GCGGCAAGACCATCAGCACCTGGATC TCTGACGGCCAAGTGAAGGACTTCTA TCTGTACCCCGGCAAGTACACCTTCG TGGAACAGCCGCTCCTGACGGATAC GAGGTGGCCACAGCCATCACCTTCAC CGTGAACGAGCAGGGACAAGTGACAG TGAACGGCAAGGCCACAAAGGGCGAC GCTCACATTGGCGGCTCTGGCTCCAT GGTGTCCAAGGGCGAAGAACTGTTCA CCGGCGTGGTGGCCATTCTGGTGGAA CTGGATGGGGATGTGAACGGCCACAA GTTCTCCGTGTCTGGCGAAGGCGAAG

			GGGATGCCACATACGGCAAGCTGACC CTGAAGTTCATCTGCACCACCGGAAA GCTGCCCCGTGCCTTGGCCTACACTGG TCACCACACTGACATACGGCGTGACG TGCTTCAGCAGATACCCCGACCATAT GAAGCAGCACGACTTCTTCAAGAGCG CCATGCCTGAGGGCTACGTGCAAGAG AGAACCATCTTCTTTAAGGACGACGG CAACTACAAGACCAGGGCCGAAGTGA AGTTCGAGGGCGACACCCCTGGTCAAC AGAATCGAGCTGAAGGGCATCGACTT CAAAGAGGATGGCAACATCTGGGCC ACAAGCTCGAGTACAACTACAACAGC CACAACGTGTACATCATGGCCGACAA GCAGAAAAACGGCATCAAAGTGAAC TCAAGATCCGGCACAACATCGAGGAC GGAAGCGTGCAGCTGGCCGATCACTA CCAGCAGAACACACCTATCGGCGACG GACCTGTGCTGCTGCCTGATAACCAC TACCTGAGCACACAGAGCGCCCTGAG CAAGGACCCTAACGAGAAGAGGGGACC ACATGGTGTGCTGGAATTTGTGACC GCCGCTGGCATCACACTCGGCATGGA CGAGCTGTACAAATGA
91	Artificial	C-tag	EPEA
92	Hepatitis B virus	Hepatitis core protein	MDIDPYKEFGASVELLSFLPSDFFPS IRDLLDTASALYREALESPHVSPPH TALRQAILCWGELMNLATWVGSNLED PASRELVSYSVNVNMGKLRQILWFH ISCLTFGRETVLEYLVSFGVWIRTPT AYRPPNAPILSTLPETTVVGGGGGSP GGGTPSPGGGGSQSPGGGGSQSGESQ C
93	Artificial	Tandem hepatitis core protein	MDIDPYKEFGATVELLSFLPSDFFPS VRDLLDTASALYREALESPHVSPPH TALRQAILCWGELMTLATWVGNNLED PASRDLVVNYVNTNMGLKIRQLLWFH ISCLTFGRETVLEYLVSFGVWIRTPT AYRPPNAPILSTLPETTVVGGSGGSG GSGSGGSMIDIDPYKEFGATVELLSF LPSDFFPSVRDLLDTASALYREALESPHVSPPH TALRQAILCWGELMTLATWVGNNLED
94	Artificial	eGFP	MVSKGEELFTGVVPILVELDGDVNGH KFSVSGEGEGDATYGKLTLLKFICTTG KLPVPWPTLVTTLTYGVCFSRYPDH MKQHDFFKSAMPEGYVQERTIFFKDD GNYKTRAEVKFEGDTLVNRIELKGID FKEDGNILGHKLEYNNSHNVYIMAD KQKNGIKVNFKIRHNIEDGSVQLADH YQQNTPIGDGPVLLPDNHYLSTQSAL SKDPNEKRDHMLLEFVTAAGITLGM DELYK

95	Artificial	His purification tag	HHHHHH
96	<i>Plasmodium falciparum</i>	Plasmodium falciparum antigen	KVTVDTVCKRGFLIQMSGHLECKCEN DLVLVNEETCEEKVLKDEKTVNKPC GDFSKCIKIDGNPVSACKCNLGYDM VNNVCIPNECKQVTCGNGKCILDTSN PVKTGVCSCNIGKVPNVQDQNKCSKD GETKCSLKCLKEQETCKAVDGIYKCD CKDGFIIIDQESSICT
97	<i>Natronobacterium gregoryi</i>	2-oxo acid dehydrogenase subunit E2	KAAAEKAAPAAAKPATTEGEFPETR EKMSGIRRAIAKAMVHSKHTAPHVTL MDEADVTKLVAHRKKFKAIAAEKGIK LTFLPYVVKALVSALREYPVLNTSID DETEEIIQKHYYNIGIAADTDRLGLLV PVIKHADRKPIFALAQEINELAEEKAR DGKLTPEMKGASCTITNIGSAGGQW FTPVINHPEVAILGIGRIAEKPIVRD GEIVAAPMLALSLSFDHRMIDGATAQ KALNHIKRLSDPELLLMEA
98	Norovirus	Norovirus capsid protein	KMASSDANPSDGSAAANLVPEVNNEVM ALEPVVGAIAAPVAGQQNVIDPWIR NNFVQAPGGFTVSPRNAPGEILWSA PLGPDLPYLSHLARMYNGYAGGFV QVILAGNAFTAGKVIFAAVPPNFPTE GLSPSQVTMFPHIVDVRQLEPVLIP LPDVRNNFYHYNQSNPTIKLIAMLY TPLRANNAGDDVFTVSCRVLTRPSD FDFIFLVPPTVESRTKPFVSVPVLTVE EMTNSRFPIPLEKLFTGPSSAFVVQP QNGRCTTDGVLLGTTQLSPVNICTFR GDVTHITGSRNYTMNLASQNWNDYDP TEEIPAPLGTPDFVGKIQGVLTQTTR TDGSTRGHKATVYTGSAFAPKLGRV QFETDTRDFEANQNTKFTPVGVIQD GGTTHRNEPQQWVLPSSYSGRNTHNVH LAPAVAPTFFGEQLLFFRSTMPGCSG YPNMDLDCLLPQEWVQYFYQEAPPAQ SDVALLRFVNPDTGRVLFECKLHKSG YVTVAHGTGQHDLVIPPNGYFRFDSWV NQFYTLAPMGNGTGRRRAV
99	<i>Homo sapiens</i>	Sign7	MKRGLCCVLLLCGAVFVSPSQEIHAR FERRA
100	<i>Homo sapiens</i>	Sign8	MAFLWLLSCWALLGTTFG
101	<i>Homo sapiens</i>	Sign9	MLLLLLLLGLRLQLSLG

Items

1. A composition for use in the prophylaxis and/or treatment of a disease wherein the composition comprises:
 - i. a first polynucleotide encoding a protein fused to a first peptide tag; and
 - 5 ii. a second polynucleotide encoding an antigen fused to a second peptide tag,wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.
- 10 2. The composition for the use according to item 1, wherein the protein is a particle-forming protein, such as a viral capsid protein or a viral envelope protein such as a glycoprotein.
- 15 3. The composition for the use according to item 2, wherein the protein is a protein from a hepatitis virus such as hepatitis B or E, for example a core protein from hepatitis B virus; a protein from a novovirus such as NoV; a protein from a papilloma virus such as Human Papilloma Virus (HPV), preferably HPV16 or HPV18, such as HPV L1; a protein from a polyomavirus such as polyomavirus vp1
- 20 (PyV); a protein from a calicivirus such as feline calicivirus (FCV), preferably FCV VP1; a protein from a circovirus such as a porcine circovirus (PCV), preferably PCV2 ORF2; a protein from a nervous necrosis virus (NNV), such as NNV coat protein; a protein from a parvovirus such as canine parvovirus (CVP), preferably CPV VP2, goose parvovirus (GPV) or porcine parvovirus (PPV), preferably
- 25 structural proteins from GPV or PPV, or parvovirus B19; a protein from a protoparvovirus such as an enteritis virus, for example mink enteritis virus (MEV), preferably MEV VP2, or duck plague virus (DPV), preferably a DPV structural protein.
- 30 4. The composition for the use according to any one of the preceding items, wherein the protein is a bacteriophage protein, such as a protein from *Salmonella* virus P22, from MS2, from QBeta, PRR1, PP7, bacteriophage R17, bacteriophage fr, bacteriophage GA, bacteriophage SP, bacteriophage M11, bacteriophage MX1, bacteriophage NL95, bacteriophage f2 or Cb5.

5. The composition for the use according to any one of the preceding items, wherein the first and/or second peptide tag is derived from the splitting of a protein containing an intramolecular isopeptide bond, identified from a library by using a known binding partner, or designed *in silico*, to obtain complementary binding partners each containing a reactive amino acid involved in isopeptide bond formation, preferably wherein the first or second peptide tag comprises a tag selected from the group consisting of a SpyTag (SEQ ID NO: 1), a SdyTag (SEQ ID NO: 2), a SnoopTag (SEQ ID NO: 3), a PhoTag (SEQ ID NO: 4), an EntTag (SEQ ID NO: 5), a KTag, a BacTag (SEQ ID NO: 15), a Bac2Tag (SEQ ID NO: 16), a Bac3Tag (SEQ ID NO: 17), a Bac4Tag (SEQ ID NO: 18), a RumTrunkTag (SEQ ID NO: 13 or SEQ ID NO: 14), a Rum7Tag (SEQ ID NO: 12), a RumTag (SEQ ID NO: 6), a Rum2Tag (SEQ ID NO: 7), a Rum3Tag (SEQ ID NO: 8), a Rum4Tag (SEQ ID NO: 9), a Rum5Tag (SEQ ID NO: 10), a Rum6Tag (SEQ ID NO: 11) and a Bac5Tag (SEQ ID NO: 19), and/or preferably wherein the first or second peptide tag comprises a tag selected from the group consisting of a SpyCatcher (SEQ ID NO: 21), a SdyCatcher (SEQ ID NO: 22), a SnoopCatcher (SEQ ID NO: 23) and an ester-forming split-protein pair.
6. The composition for the use according to any one of the preceding items, wherein the disease is cancer, a lipid disorder, a cardiovascular disease, an immune-inflammatory disease, a chronic disease, a neurological disease, an allergic reaction/disease and/or an infectious disease, such as an infectious disease selected from the group consisting of malaria, tuberculosis, and a disease caused by a virus, such as a coronavirus, for example SARS-CoV-2, malaria, tuberculosis, HIV, HCV, Dengue fever, Chikungunya, Yellow fever, HBV or influenza.
7. The composition for the use according to any one of the preceding items, wherein said antigen is a protein, peptide and/or an antigenic fragment from the group consisting of cancer-specific polypeptides, polypeptides associated with cardiovascular diseases, polypeptides associated with asthma, polypeptides associated with nasal polyposis, polypeptides associated with atopic dermatitis, polypeptides associated with eosinophilic esophagitis, polypeptides associated with hypereosinophilic syndrome, polypeptides associated with Churg-Strauss syndrome and polypeptides associated with pathogenic organisms, preferably wherein the antigen is a protein, peptide and/or an antigenic fragment of Severe

acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

8. The composition for the use according to any one of the preceding items, wherein the antigen is selected from the group consisting of: hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, neuraminidase, human melanoma protein gp100, human melanoma protein melan-A/MART1, HIV envelope protein, M2e, VAR2CSA, ICAM1, CSP, Dengue virus NS1, Dengue virus envelope protein, Chikungunya virus envelope protein, tyrosinase, HCV E2, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1, EGRFvIII, endoglin, ANGPTL-3, CSPG4, CTLA-4, HER2, IgE, IL-1 beta, IL-5, IL-13, IL-17, IL-22, IL-31, IL-33, TSLP, NGF, (IHNV) G-protein, a lymphotoxin such as lymphotoxin α or β , a lymphotoxin receptor, a receptor activator of nuclear factor κ B ligand, vascular endothelial growth factor VEGF, a VEGF receptor, IL-23 p19, ghrelin, CCL21, CXCL12, SDF-1, M-CSF, MCP-1, endoglin, GnRH, TRH, eotaxin, bradykinin, BLC, TNF- α , amyloid β peptide A, angiotensin, gastrin, progastrin, CETP, CCR5, C5a, CXCR4, Des-Arg-bradykinin, GnRH peptide, angiotensin peptide, TNF peptide, or an antigenic fragment or antigenic variant thereof, preferably wherein the antigen is selected from the group consisting of a SARS-CoV-2 envelope protein, a SARS-CoV-2 spike protein, a SARS-CoV-2 nucleocapsid protein and a SARS-CoV-2 envelope protein.

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9. A composition according to any one of the preceding items.

10. An expression system comprising:

- i. a first polynucleotide encoding a protein fused to a first peptide tag; and
ii. a second polynucleotide encoding an antigen fused to a second peptide tag,

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wherein upon expression of the first and second polynucleotides in a cell, the antigen and the protein are linked via an isopeptide bond between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen, optionally wherein the first peptide tag, the second peptide tag, the protein and/or the antigen are as defined in any one of items 1 to 8.

30

11. A cell expressing:

- i. a first polynucleotide encoding a protein fused to a first peptide tag, preferably as defined in any one of the preceding items; and

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- ii. a second polynucleotide encoding an antigen fused to a second peptide tag, preferably as defined in any one of the preceding items;
wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond between the first peptide tag and the second peptide tag, whereby
5 i - ii form a particle displaying said antigen, optionally wherein the cell comprises the expression system according to item 10.
12. A host cell, wherein the host cell comprises an expression system according to item
10.
13. The composition for the use according to any one of items 1 to 8, the expression
system according to item 10, and/or the cell or host cell according to any one of
items 11 to 12, for use in a method for inducing an immune response in a subject,
the method comprising the step of administering said composition, polynucleotide
15 or expression system to said subject at least once.
14. A method of administering a composition for use in the prophylaxis and/or
treatment of a disease in a subject in need thereof comprising the steps of
20 i. obtaining at least one composition as defined in any one of items 1 to 9;
and
ii. administering said composition to a subject at least once for prophylaxis
and/or treatment of a disease as defined in any one of the preceding
items.
- 25 15. A kit of parts comprising
i. a composition as defined in any one of items 1 to 9 or an expression
system according to item 10; and
ii. optionally, a medical instrument or other means for administering the
composition; and
30 iii. instructions for use.

Claims

1. A composition for use in the prophylaxis and/or treatment of a disease wherein the composition comprises:
 - i. a first polynucleotide encoding a protein fused to a first peptide tag; and
 - 5 ii. a second polynucleotide encoding an antigen fused to a second peptide tag,wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.
- 10 2. The composition for the use according to claim 1, wherein the particle is a virus-like particle.
3. The composition for the use according to any one of the preceding claims, wherein
15 the particle is a nanoparticle.
4. The composition for the use according to any one of the preceding claims, wherein the first and/or second polynucleotide is DNA.
- 20 5. The composition for the use according to any one of the preceding claims, wherein the first and/or second polynucleotide is RNA.
6. The composition for the use according to claim 5, wherein the RNA is formulated in a lipid particle formulation.
- 25 7. The composition for the use according to any one of the preceding claims, wherein the protein is a particle-forming protein.
8. The composition for the use according to any one of the preceding claims, wherein
30 the protein is a viral capsid protein or a viral envelope protein such as a glycoprotein.
9. The composition for the use according to any one of the preceding claims, wherein
35 the protein is a viral capsid protein or a viral envelope protein from a mammalian virus such as a human virus.

10. The composition for the use according to claim 9, wherein the protein is a protein from a hepatitis virus such as hepatitis B or E, for example a core protein from hepatitis B virus; a protein from a norovirus such as NoV; a protein from a papilloma virus such as Human Papilloma Virus (HPV), preferably HPV16 or HPV18, such as HPV L1; a protein from a polyomavirus such as polyomavirus vp1 (PyV); a protein from a calicivirus such as feline calicivirus (FCV), preferably FCV VP1; a protein from a circovirus such as a porcine circovirus (PCV), preferably PCV2 ORF2; a protein from a nervous necrosis virus (NNV), such as NNV coat protein; a protein from a parvovirus such as canine parvovirus (CVP), preferably CPV VP2, goose parvovirus (GPV) or porcine parvovirus (PPV), preferably structural proteins from GPV or PPV, or parvovirus B19; a protein from a protoparvovirus such as an enteritis virus, for example mink enteritis virus (MEV), preferably MEV VP2, or duck plague virus (DPV), preferably a DPV structural protein.
11. The composition for the use according to any one of the preceding claims, wherein the protein is a protein from a plant virus, such as a cowpea virus, a tobacco virus, a tomato virus, a cucumber virus or a potato virus.
12. The composition for the use according to claim 11, wherein the plant virus is a mosaic virus, preferably Cowpea mosaic virus (CPMV); tobacco mosaic virus (TMV); tomato spotted wilt virus (TSWV); tomato yellow leaf curl virus (TYLCV); cucumber mosaic virus (CMV); or from potato virus Y (PVY).
13. The composition for the use according to any one of the preceding claims, wherein the protein is a bacteriophage protein, such as a protein from *Salmonella* virus P22, from MS2, from QBeta, PRR1, PP7, bacteriophage R17, bacteriophage fr, bacteriophage GA, bacteriophage SP, bacteriophage M11, bacteriophage MX1, bacteriophage NL95, bacteriophage f2 or Cb5.
14. The composition for the use according to any one of the preceding claims, wherein the protein is a particle forming protein, such as ferritin as set forth in SEQ ID NO: 69, i301 as set forth in SEQ ID NO: 72, replicase polyprotein 1a (pp1a), a lumazine synthase as set forth in SEQ ID NO: 70, Hbc as set forth in SEQ ID NO: 92, tandemHBc as set forth in SEQ ID NO: 93, the 2-oxo acid dehydrogenase subunit

E2 as set forth in SEQ ID NO: 97, or the norovirus capsid protein as set forth in SEQ ID NO: 98.

15. The composition for the use according to any one of the preceding claims, wherein
5 the first or second peptide tag comprises a tag selected from the group consisting of a SpyTag as set forth in SEQ ID NO: 1, a SdyTag as set forth in SEQ ID NO: 2, a SnoopTag as set forth in SEQ ID NO: 3, a PhoTag as set forth in SEQ ID NO: 4, an EntTag as set forth in SEQ ID NO: 5, a KTag, a BacTag as set forth in SEQ ID NO: 15, a Bac2Tag as set forth in SEQ ID NO: 16, a Bac3Tag as set forth in SEQ ID
10 NO: 17, a Bac4Tag as set forth in SEQ ID NO: 18, a RumTrunk D9N tag as set forth in SEQ ID NO: 13 or SEQ ID NO: 14, a Rum7Tag as set forth in SEQ ID NO: 12, a RumTag as set forth in SEQ ID NO: 6, a Rum2Tag as set forth in SEQ ID NO: 7, a Rum3Tag as set forth in SEQ ID NO: 8, a Rum4Tag as set forth in SEQ ID NO: 9, a Rum5Tag as set forth in SEQ ID NO: 10, a Rum6Tag as set forth in SEQ ID
15 NO: 11 and a Bac5Tag as set forth in SEQ ID NO: 19.
16. The composition for the use according to any one of the preceding claims, wherein the first or second peptide tag comprises a tag selected from the group consisting of a SpyCatcher as set forth in SEQ ID NO: 21, a SdyCatcher as set forth in SEQ
20 ID NO: 22, a SnoopCatcher as set forth in SEQ ID NO: 23 and an ester-forming split-protein pair.
17. The composition for the use according to any one of the preceding claims, wherein the composition further comprises a SpyLigase, whereby the two peptide tags may
25 be locked together.
18. The composition for the use according to any one of the preceding claims, wherein one of the first and second peptide tags is an SdyTag and the other of the first and second peptide tags is an SdyCatcher.
30
19. The composition for the use according to any one of the preceding claims, wherein one of the first and second peptide tags is a SpyTag, such as the SpyTag as set forth in SEQ ID NO: 1 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, and the other of the first and
35 second peptide tags is a SpyCatcher, such as the SpyCatcher as set forth in SEQ

ID NO: 21 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto.

- 5 20. The composition for the use according to any one of the preceding claims, wherein one of the first and second peptide tags is a SnoopTag and the other of the first and second peptide tags is a SnoopCatcher.
- 10 21. The composition for the use according to any one of the preceding claims, wherein one or more first peptide tags are fused to the N-terminal end, to the C-terminal end of the protein and/or inserted in-frame into the coding sequence of the protein, optionally by a linker.
- 15 22. The composition for the use according to any one of the preceding claims, wherein one or more second peptide tags are fused to the N-terminal end, to the C-terminal end of the antigen and/or inserted in-frame into the coding sequence of the antigen, optionally by a linker.
- 20 23. The composition for the use according to any one of the preceding claims, wherein the disease is cancer, a lipid disorder, a cardiovascular disease, an immune-inflammatory disease, a chronic disease, a neurological disease, an infectious disease and/or an allergic reaction/disease.
- 25 24. The composition for the use according to claim 23, wherein the cancer is selected from the group consisting of breast cancer, gastric cancer, ovarian cancer, glioblastoma, bone cancer, skin cancer and uterine serous carcinoma.
- 30 25. The composition for the use according to any one of claims 23 to 24, wherein the lipid disorder is selected from the group consisting of a lipid disorder such as hyperlipidemia, type I, type II, type III, type IV, or type V hyperlipidemia, secondary hypertriglyceridemia, hypercholesterolemia, familial hypercholesterolemia, xanthomatosis and cholesterol acetyltransferase deficiency.
- 35 26. The composition for the use according to any one of claims 23 to 25, wherein the cardiovascular disease is selected from the group consisting of dyslipidemia, atherosclerosis, a coronary artery disease and/or hypercholesterolemia.

27. The composition for the use according to any one of claims 23 to 26, wherein the chronic or immune-inflammatory disease is selected from the group consisting of eosinophilic asthma, allergy, nasal polyposis, atopic dermatitis, eosinophilic esophagitis, hypereosinophilic syndrome, contact allergy and Churg-Strauss syndrome.
28. The composition for the use according to any one of claims 23 to 27, wherein the neurological disease is Alzheimer's disease.
29. The composition for the use according to any one of claims 23 to 28, wherein the infectious disease is selected from the group consisting of malaria, tuberculosis, and a disease caused by a virus, such as a coronavirus, for example SARS-CoV-2, malaria, tuberculosis, HIV, HCV, Dengue fever, Chikungunya, Yellow fever, HBV or influenza.
30. The composition for the use according to any one of the preceding claims, wherein the antigen is a polypeptide, peptide and/or an antigenic fragment of a polypeptide associated with or specific for an abnormal physiological response.
31. The composition for the use according to claim 30, wherein the abnormal physiological response is an autoimmune disease, an allergic reaction and/or a cancer.
32. The composition for the use according to any one of the preceding claims, wherein said antigen is a protein, peptide and/or an antigenic fragment from the group consisting of cancer-specific polypeptides, polypeptides associated with cardiovascular diseases, polypeptides associated with asthma, polypeptides associated with nasal polyposis, polypeptides associated with atopic dermatitis, polypeptides associated with eosinophilic esophagitis, polypeptides associated with hypereosinophilic syndrome, polypeptides associated with Churg-Strauss syndrome and polypeptides associated with pathogenic organisms.
33. The composition for the use according to any one of the preceding claims, wherein said antigen is a protein, peptide and/or an antigenic fragment of Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

34. The composition for the use according to any one of the preceding claims, wherein the antigen is selected from the group consisting of: hemagglutinin, GD2, EGF-R, CEA, CD52, CD21, neuraminidase, human melanoma protein gp100, human melanoma protein melan-A/MART1, HIV envelope protein, M2e, VAR2CSA, ICAM1, CSP, Dengue virus NS1, Dengue virus envelope protein, Chikungunya virus envelope protein, tyrosinase, HCV E2, NA17-A nt, MAGE-3, HPV 16 E7, HPV L2, PD1, PD-L1, CTLA-4, p53, hCG, Fel d1, EGRFvIII, endoglin, ANGPTL-3, CSPG4, CTLA-4, HER2, IgE, IL-1 beta, IL-5, IL-13, IL-17, IL-22, IL-31, IL-33, TSLP, NGF, (IHNV) G-protein, a lymphotoxin such as lymphotoxin α or β , a lymphotoxin receptor, a receptor activator of nuclear factor κ B ligand, vascular endothelial growth factor VEGF, a VEGF receptor, IL-23 p19, ghrelin, CCL21, CXCL12, SDF-1, M-CSF, MCP-1, endoglin, GnRH, TRH, eotaxin, bradykinin, BLC, TNF- α , amyloid β peptide A, angiotensin, gastrin, progastrin, CETP, CCR5, C5a, CXCR4, Des-Arg-bradykinin, GnRH peptide, angiotensin peptide, TNF peptide, or an antigenic fragment or antigenic variant thereof.
35. The composition for the use according to any one of the preceding claims, wherein the antigen is selected from the group consisting of a SARS-CoV-2 envelope protein, a SARS-CoV-2 spike protein, a SARS-CoV-2 nucleocapsid protein and a SARS-CoV-2 envelope protein.
36. The composition for the use according to any one of the preceding claims, wherein the antigen is selected from the group consisting of IL-13, IL-31, IL-17A, PCSK9, an HIV trimer and an influenza virus trimer.
37. The composition for the use according to any one of the preceding claims, wherein the antigen is selected from the group consisting of eGFP as set forth in SEQ ID NO: 94 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, Pfs25 as set forth in SEQ ID NO: 96 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, and the His purification tag as set forth in SEQ ID NO: 95 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto.
38. The composition for the use according to any one of the preceding claims for use in treatment of an infectious disease, wherein the antigen is a protein or a peptide

specific to the pathogenic organism which causes the infectious disease, or a fragment thereof.

39. The composition for the use according to any one of the preceding claims, wherein
5 the antigen is capable of eliciting an immune reaction in an animal, such as a mammal, such as a *Homo sapiens*, cow, pig, horse, sheep, goat, llama, mouse, rat, monkey, and/or a bird, such as a chicken and/or a fish, such as a salmon.

40. The composition for the use according to any one of the preceding claims, wherein
10 the antigen further comprises a polyhistidine tag.

41. A composition as defined in any one of the preceding claims, wherein the protein is selected from the group consisting of i301 as set forth in SEQ ID NO: 72 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90%
15 sequence identity thereto, Hbc as set forth in SEQ ID NO: 92 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, tandemHBc as set forth in SEQ ID NO: 93 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, human ferritin as set forth in SEQ ID NO: 69 or a homologue thereof
20 having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, the 2-oxo acid dehydrogenase subunit E2 as set forth in SEQ ID NO: 97 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, lumazine synthase as set forth in SEQ ID NO: 70 or a homologue thereof having at least 70%, such as at least 80%, such as at least
25 90% sequence identity thereto, and the norovirus capsid protein as set forth in SEQ ID NO: 98 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto.

42. The composition according to claim 41, wherein the first and the second peptide
30 tags are selected from the group consisting of SpyTag as set forth in SEQ ID NO: 1 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, and SpyCatcher as set forth in SEQ ID NO: 21 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto.

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43. The composition according to any one of claims 41 to 42, wherein the antigen is selected from the group consisting of eGFP as set forth in SEQ ID NO: 94 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, Pfs25 as set forth in SEQ ID NO: 96 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto, and the His purification tag as set forth in SEQ ID NO: 95 or a homologue thereof having at least 70%, such as at least 80%, such as at least 90% sequence identity thereto.
44. An expression system comprising:
- i. a first polynucleotide encoding a protein fused to a first peptide tag; and
 - ii. a second polynucleotide encoding an antigen fused to a second peptide tag,
- wherein upon expression of the first and second polynucleotides in a cell, the antigen and the protein are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.
45. The expression system according to claim 44, wherein the first peptide tag, the second peptide tag, the protein and/or the antigen are as defined in any one of claims 1 to 40.
46. The expression system according to any one of claims 44, wherein the first polynucleotide and the second polynucleotide are both DNA polynucleotides or RNA polynucleotides.
47. The expression system according to any one of claims 44 to 46, wherein the first and second polynucleotides are comprised within one vector such as a viral vector or a plasmid, or wherein the first and second polynucleotides are comprised within two vectors such as two viral vectors; two plasmids; or one viral vector and one plasmid.
48. The expression system according to claim 47, wherein the viral vector is an adenoviral vector, such as a modified adenoviral vector, e.g. the replication-deficient simian adenovirus vector ChAdOx1, or a modified vaccinia Ankara (MVA) vector.

49. The expression system according to any one of claims 44 to 47, wherein the expression system comprises or consists of a plasmid.
50. The expression system according to claim 49, wherein said plasmid is pVAX1.
51. The expression system according to any one of claims 44 to 50, wherein the expression system comprises or consists of an mRNA.
52. The expression system according to any one of claims 44 to 51, wherein the first polynucleotide comprises or consists of a first polynucleotide encoding a protein comprising a first peptide tag, and the second polynucleotide comprises or consists of a second polynucleotide encoding an antigen comprising a second peptide tag.
53. The expression system according to any one of claims 44 to 52, wherein the first polynucleotide and the second polynucleotide are comprised within the same nucleic acid molecule, or within two different nucleic acid molecules.
54. The expression system according to any one of claims 44 to 53, further comprising a first promoter upstream of the first polynucleotide.
55. The expression system according to any one of claims 44 to 54, further comprising a second promoter upstream of the second polynucleotide.
56. The expression system according to any one of claims 44 to 55, wherein the first promoter is a constitutive promoter and the second promoter is an inducible promoter, such as a vitamin D-inducible promoter.
57. The expression system according to any one of claims 44 to 55, wherein the first and the second promoters are constitutive promoters.
58. The expression system according to any one of claims 44 to 57, wherein the first polynucleotide further comprises a secretion or excretion signal, whereby the protein fused to the first peptide tag is secreted or excreted from the endoplasmic reticulum of the cell.

59. The expression system according to any one of claims 44 to 58, wherein the second polynucleotide further comprises a secretion or excretion signal, whereby the antigen fused to the second peptide tag is secreted or excreted from the endoplasmic reticulum of the cell.
- 5
60. The expression system according to any one of claims 44 to 59, wherein the secretion or excretion signal comprises or consists of SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83 or SEQ ID NO: 84.
- 10
61. A cell expressing:
- i. a first polynucleotide encoding a protein fused to a first peptide tag, preferably as defined in any one of the preceding claims; and
 - ii. a second polynucleotide encoding an antigen fused to a second peptide tag, preferably as defined in any one of the preceding claims;
- 15
- wherein the antigen and the protein upon expression in a cell are linked via an isopeptide bond, or an ester bond, between the first peptide tag and the second peptide tag, whereby i - ii form a particle displaying said antigen.
- 20
62. The cell according to claim 61, comprising the expression system according to any one of claims 44 to 60.
63. A host cell, wherein the host cell comprises an expression system according to any one of claims 44 to 60.
- 25
64. The host cell according to claim 63, wherein the host cell is selected from the group consisting of a bacterial cell, a yeast cell, a fungal cell, a plant cell, a mammalian cell and an insect cell.
- 30
65. The composition for the use according to any one of claims 1 to 40, the expression system according to any one of claims 44 to 60, and/or the cell or host cell according to any one of claims 61 to 64, for use in a method for inducing an immune response in a subject, the method comprising the step of administering said composition, polynucleotide or expression system to said subject at least once.
- 35

66. A method of administering a composition for use in the prophylaxis and/or treatment of a disease in a subject in need thereof comprising the step of
- 5 i. administering at least one composition as defined in any one of claims 1 to 40 to a subject at least once for prophylaxis and/or treatment of a disease as defined in any one of the preceding claims.
67. The method of administering a composition for the use according to claim 66, wherein the composition is boosted by administration in a form or body part different from the previous administration.
- 10 68. The method of administering a composition for the use according to any one of claims 66 to 67, wherein the composition is administered to the area most likely to be the receptacle of a given disease.
- 15 69. The method of administering a composition for the use according to any one of claims 66 to 68, wherein the subject is an animal, such as a mammal, such as a cow, pig, horse, sheep, goat, llama, mouse, rat, monkey, most preferably such as a human being; or a bird, such as a chicken or a fish, such as a salmon.
- 20 70. The method of administering a composition for the use according to any one of claims 66 to 69, wherein the composition is administered in combination with any other vaccine.
- 25 71. The method of administering a composition for the use according to any one of claims 66 to 70, wherein the composition forms a part of a vaccine cocktail.
72. A kit of parts comprising
- 30 i. a composition as defined in any one of the preceding claims or an expression system according to any one of claims 44 to 60, and
- ii. optionally, a medical instrument or other means for administering the composition, and
- iii. instructions for use.
73. The kit of parts according to claim 72, comprising a second active ingredient.
- 35