date. Other genetically encoded systems for enabling spontaneous amide bond formation include SnoopTag/ SnoopTagJr and SnoopCatcher; RrgATag/RrgATag2/DogTag and RrgACatcher, IsopepTag/ IsopepTag-N and Pilin-C or Pilin-N, PsCsTag and PsCsCatcher; and SnoopTagJr and DogTag (mediated by SnoopLigase), and variants of all these systems.

The present inventors have proven that use of large/multicomponent antigens in vaccine compositions is possible using genetically-encoded systems for enabling amide bond formation, which may improve the response to the large/multicomponent antigen. This is a surprising result.

SUMMARY

In one aspect of the present disclosure, there is provided a composition comprising a particle displaying an antigenic component, wherein said composition comprises:

i) an antigenic component comprising a first peptide tag, and

ii) a particulate moiety comprising a second peptide tag,

wherein the antigenic component and the moiety are linked via an isopeptide bond between said first and second peptide tags, and wherein the antigenic component is over 50 kDa.

In another aspect of the present disclosure, there is provided a vaccine comprising a composition disclosed herein for use in the prophylaxis and/or treatment of a disease.

In a further aspect of the present disclosure, there is provided a method of producing a composition disclosed herein, said method comprising:

- introducing a first nucleic acid which encodes a first genetic fusion of a first protein to a first peptide tag into a first host cell;
- incubating said first host cell under conditions for expressing said first genetic fusion;
- introducing a second nucleic acid which encodes a second genetic fusion of a second protein to a second peptide tag into a second host cell;
- incubating said second host cell under conditions for expressing said second genetic fusion;
- optionally purifying the expressed components;
- incubating the expressed components under conditions for formation of an isopeptide bond between the first peptide tag and the second peptide tag; and purifying the resultant composition

wherein the first protein comprises an antigenic component and the second protein comprises a moiety.

In another aspect of the present disclosure, there is provided a nucleic acid molecule for use in a method disclosed herein wherein the nucleic molecule encodes an amino sequence as set out in any of SEQ ID NOs: 27 to 41 .

In a further aspect of the present disclosure, there is provided a nucleic acid molecule for use in a method disclosed herein wherein the nucleic molecule encodes an amino sequence as set out in any of SEQ ID NOs: 50 to 58 .

In another aspect of the present disclosure, there is provided a vector comprising a nucleic acid molecule disclosed herein.

In a further aspect of the present disclosure, there is provided a host cell comprising a nucleic acid molecule disclosed herein, or a vector disclosed herein.

In one example of the present disclosure there is provided a composition comprising a particle displaying a protein component, wherein said composition comprises:

i) i) a protein component comprising a first peptide tag, and

ii) ii) a moiety comprising a second peptide tag,

wherein the protein component and the moiety are linked via an isopeptide bond between said first and second peptide tags, and wherein the protein component is over 50 kDa.

In another example of the present disclosure there is provided a composition comprising a particle displaying a protein component, wherein said composition comprises:

i) a protein component comprising a first peptide tag, and

ii) a moiety comprising a second peptide tag,

wherein the protein component and the moiety are linked via an isopeptide bond between said first and second peptide tags, and wherein the protein component is multimeric.

The protein component may have any function e.g. it may be an enzyme or have enzymatic properties. The protein component may be a full-length protein, or it may be a part, segment, domain or truncation of a full-length protein. The protein component may be an antigen or an immunogen. The protein component may also be called the antigenic component.

In another example of the present disclosure there is provided a composition comprising a particle displaying an antigenic component, wherein said composition comprises:

iii) an antigenic component comprising a first peptide tag, and

iv) a moiety comprising a second peptide tag,

wherein the antigenic component and the moiety are linked via an isopeptide bond between said first and second peptide tags, and wherein the antigenic component is over approximately 50 kDa.

In some examples of any example of the present disclosure the protein component or the antigenic component may be over 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 110 kDa, 120 kDa, 130

kDa, 140 kDa, 150 kDa, 160 kDa, 170 kDa, 180 kDa, 190 kDa or more, such as over 200kDa, over 300kDa or over 400kDa.

A multimer may comprise any number of subunits, which may or may not be covalently linked in the protein or antigenic component. The multimer may comprise 2-20 subunits, alternatively, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more subunits. Alternatively, the multimer may be a dimer, trimer, tetramer, pentamer, hexamer, septamer, octamer, nonamer or decamer. The multimer may be from any appropriate pathogen, but is preferably a viral multimer.

Non-limiting examples of large protein components, i.e. those over 50kDa or as described above, include the pentameric complex (PC) and gB glycoprotein from Human cytomegalovirus (HCMV), the G and F glycoproteins from RSV, the haemagglutinin (HA) and neuraminidase (NA) antigens from influenza A virus, Plasmodium *falciparum* Pfs230, P. falciparum CSP, Human HER2 receptor, PCSK9, VAR2CSA, P. falciparum RIPR, Varicella zoster virus (VZV) glycoprotein E, Rabies virus glycoprotein and the Epstein-Barr virus (EBV) gH/gL complex.

In some examples of the present disclosure, the protein component or the antigenic component may be a monomer or a multimer, for example a dimer, trimer, tetramer or pentamer. In some examples of the present disclosure, the protein component or the antigenic component may be a protein or peptide complex.

Non-limiting examples of multimeric antigenic components include the pentameric complex (PC) and gB trimer from Human cytomegalovirus (HCMV), the G and F glycoproteins from RSV, the haemagglutinin (HA) and neuraminidase (NA) antigens from influenza A virus, some of which are described herein. Other examples include components of disease agents such as viruses, bacteria, fungal pathogens, parasites or other disease vectors. Suitable multimeric antigenic components include, for example those derived from viruses such influenza (such as Influenza hemagglutinin (HA) (e.g. Flu trimer)), Respiratory syncytial virus (RSV) etc.

The protein component may be attached to the first peptide tag by genetic fusion, and expressed recombinantly in an appropriate cell. For components that include post-translational modifications such as glycosylation it may be preferable to express the recombinant protein in a eukaryotic or mammalian cell line.

In one example, the “moiety” is a component onto which a protein component or an antigenic component may be displayed e.g. made available to the immune system. In one example the moiety multimerises to form said particle. Suitably, such a moiety may be a virus, a bacteria, a multimerisation scaffold for vaccination or a protein component which multimerises to form a VLP (virus-like particle). Suitably, the moiety may be a component of a bacteriophage, tobacco mosaic virus particle, adeno-associated virus like particles (AAVLP), *E. coli* etc. In one

example, the moiety is itself a component of the virus, bacteria etc. such that the multimerisation (e.g. self-assembly) of the moiety forms the particle for displaying the protein component or the antigenic component. In one example the moiety may be a viral structural protein, for example a viral envelope or capsid protein or surface antigen. Examples of structural proteins include matrix M1 protein and viral envelope M2 protein from the influenza virus, HBsAg from Hepatitis B virus, *E. coli* bacteriophage AP205 viral coat protein (CP3), hemagglutinin-neuraminidase from a variety of viruses including Mumps and the like. Suitable viral structural proteins will be known to those skilled in the art. In other examples, the moiety may be a protein or peptide, such as a multimerisation domain such as IMX313, which forms nanoparticles, or a computationally derived particle such as MI3. In further examples the moiety may be a synthetic nanoparticle or a synthetic VLP, such as a gold, lipopeptide or poly(lactic-co-glycolic acid) (PLGA) nanoparticle. Other suitable moieties may include liposomes or outermembrane vesicles. Suitably a moiety comprising a second peptide tag is a moiety to which a second peptide tag is attached.

The use of a structural surface antigen from a virus may be preferred. The second peptide tag is therefore attached to the structural surface antigen, permitting the formation of a virus-like particle (VLP), to which the second peptide tag is attached, or to which is displaying the second peptide tag. VLPs are non-infectious self-assembling nanoparticles and their repetitive molecularly-defined architecture is attractive for engineering multivalency, notably for vaccination. VLPs have been produced from components of a wide variety of virus families including the Hepatitis B virus (including Hepatitis B small surface antigen (HBsAg)), Parvoviridae (e.g. adeno-associated virus), Retroviridae (e.g. HIV), Flaviviridae (e.g. Hepatitis C virus) and bacteriophages (e.g. Qβ, AP205). Any of these may be suitable for use as the moiety in the present present disclosure.

The second peptide tag may be attached to the moiety through genetic fusion. This genetic fusion may be at any appropriate point in the sequence, not simply limited to the termini. Those skilled in the art will appreciate that the fusion protein may be expressed recombinantly in an appropriate cell.

The second peptide tag may alternatively be displayed or attached to the moiety by means of chemical conjugation. This would require, for example, the presence of a reactive amine group in order to allow the conjugation to take place.

Accordingly, in one example, the moiety is a surface antigen of the hepatitis B virus (HBsAg). Suitably HBsAg has the amino acid sequence set out in SEQ ID NO: 41, as described herein (or functional equivalents thereof).

In one example of the present disclosure, the protein component or antigenic component is an immunogenic component of an HCMV pentamer. Suitably, an antigenic or immunogenic component is one which is capable of generating an immune response such as an antibody response against that component upon introduction into a subject such as a patient. Accordingly, an "immunogenic component of an HCMV pentamer", for example, is a component which is capable of generating an anti-HCMV antibody response in a subject. Suitably an immunogenic component comprises one or more of (at least one of) the HCMV pentamer subunit components selected from gH, gL, pUL128, pUL130 and pUL131 (also known as pUL131A). In some examples, the immunogenic component comprises one or more of those "pUL" or "UL" components. In other examples, the immunogenic component comprises one or more of those gH or gL components. In one example the immunogenic component comprises a combination of one or more "UL" components with one or more components selected from the gH or gL components. In another example of the present disclosure, the immunogenic component of an HCMV pentamer is the HCMV pentamer comprising all of the gH/gL/pUL128/pUL130/pUL131 subunits. Suitably the gH/gL/pUL128/pUL130/pUL131 subunits have amino acid sequences corresponding to those derived from any known HCMV strain (including both laboratory strains and/or clinical isolates), including Towne (GI:239909366), AD169 (GI:219879600), Toledo (GI:290564358) and Merlin (GI: 155573956), or functional equivalents thereof. By functional equivalents is meant amino acid sequences that share some homology and differ only in some amino acids but retain the functional property of being able to form an antigenic subunit or pentamer that provides protective antibodies. Suitable variants of the components gH/gL/pUL128/pUL130/pUL131A are described, for example, in WO2014/005959(see pages 4 to 10), hereby incorporated by reference. Advantageously, using HCMV pentamer subunits in a vaccine approach can provide immunogenic protection against infection from a wide range of HCMV viral strains, due to the high degree of homology between strains at the level of pentamer amino acid sequence.

In some examples, an antigenic component may correspond to a component of a disease agent or vector or a portion thereof. An antigenic component may, for example, lack a transmembrane domain for ease of manufacture. Suitably, in the HCMV pentamer, for example, the immunogenic component of an HCMV pentamer comprises a gH subunit with a truncated transmembrane domain (having been truncated by deletion of one or more amino acids from this region) such that the subunit is secreted into the cell supernatant, during protein production in host cells, for ease of purification.

In one example, the gH/gL/pUL128/pUL130/pUL131A subunits have the amino acid sequences set out in SEQ ID NOs: 28, 31, 35, 33, 36, respectively (or functional equivalents thereof)

(with or without the signal peptide indicated). By functional equivalents is meant amino acid sequences that share some homology and differ only in some amino acids but retain the functional property e.g. of being able to form an antigenic subunit or pentamer that provides protective antibodies. In some examples, a functional equivalent may share 70%, 80%, 90% homology, or more, with the relevant amino acid sequence. In another example, the gH/gL/pUL128/pUL130/pUL131A subunits are encoded by nucleic acid sequences such as those set out in SEQ ID NOs: 13, 16, 20, 18, 21, or codon optimised versions thereof (with or without the encoding sequence for the signal peptide). In some examples any one of the gH/gL/pUL128/pUL130/pUL131A subunits may have a signal peptide, for example that signal peptide present on the native protein for that strain, a functional equivalent of the signal peptide, or a signal peptide derived from a different strain of HCMV. In some examples any one of the gH/gL/pUL128/pUL130/pUL131A subunits may have a signal peptide derived from a heterologous protein. The choice of signal peptide may be determined in order to target the expressed protein to a particular cellular (or extracellular) location, or to confer other functionality. Following expression of the subunit(s), the signal peptide may be enzymatically cleaved (e.g. by a signal peptidase), either by native cellular machinery in the expression system used, or *in vitro*. In some examples, any one of the gH/gL/pUL128/pUL130/pUL131A subunits may be expressed without a signal peptide. In some examples, the native sequences, including introns, may be used where these may result in higher expression levels. Suitably, the native nucleic acid sequence for UL128 includes 2 introns. In another example, the nucleic acid sequence for UL131A includes one intron. In some examples, the introns may be removed. In some examples, the native sequences may be codon-optimised for the relevant expression system.

In one example of the present disclosure, the protein component or antigenic component is an immunogenic component of a RSV virus, such as the attachment glycoprotein (G protein) or fusion glycoprotein (F protein), both of which control the initial phase of infection. G is a highly glycosylated 90 kDa type II integral membrane protein, and can mediate viral attachment to the host cell membrane either through interaction with heparan sulphate on proteoglycans, and is a good candidate for a protein component.

The F protein is an integral membrane protein composed of three F_0 monomers that are processed during assembly into F_1 and F_2 subunits, which are covalently linked by two disulphide bonds. The F protein is highly conserved amongst RSV isolates from both A and B subgroups and the amino acid sequences show 90% or above identity. F is a 574 amino acid class I fusion protein consisting of a 50 kilodalton (kDa) carboxy-terminal F1 fragment and a 20 kDa amino-terminal F2 fragment; making it a trimer of heterodimers. It is distinguished by two furin cleavage sites that

liberate a 27 amino acid glycopeptide and expose the hydrophobic fusion peptide at the F1 amino terminus. There are two N-linked glycosylation sites in F2 and only one in F1. After removal of the 25 amino acid signal peptide and the 27 amino acid glycopeptide between F2 and F1, the remaining ectodomain of F consists of 472 amino acids. Only 25 amino acids in the F ectodomain differ between subtypes A and B.

In order to develop an antigenic composition from RSV-F protein, some studies have focussed upon making variants of the pre-fusion protein, which is a trimer. Variants have been produced by genetically fusing the two subunits of mature pre-F into a single chain. DS-Cav1 variants with F2 fused genetically to F1 and both fusion peptide and pep27 region deletions have been made. Differences in the linker between F2 and F1 subunits appeared to affect immunogenicity, and therefore variants may use a selection of different linkers. The native RSV-F protein sequence may be found at Accession number P03420.1.

Several versions of pre-fusion F proteins have been researched and developed, and consequently published. These pre-fusion trimers may all be suitable for use in the present present disclosure. The DS-Cav1-stabilized fusion glycoprotein is derived from the native protein. EP2222710, incorporated here by reference, also discloses recombinant RSV antigen comprising a soluble F protein polypeptide comprising an F2 domain and an F1 domain of an RSV-F protein polypeptide and a trimerisation domain. In Nat. Commun. 2015; 6: 8143, Krarup *et al*, a highly stable pre-fusion RSV-F protein is described, again incorporated by reference.

WO2014/160463, herein incorporated by reference, describes isolated recombinant RSV-F proteins that are stabilised in a pre-fusion conformation, as well as nucleic acid molecules encoding the recombinant RSV-F proteins.

WO2017/172890, herein incorporated by reference, describes substitution-modified pre-fusion RSV-F proteins, and nucleic acids coding therefor. Further description is given in Nat Struct Mol Biol. 2016 Sep; 23(9): 811–820, Iterative structure-based improvement of a respiratory syncytial virus fusion glycoprotein vaccine, M. Gordon Joyce, Baoshan Zhang, Li Ou, Man Chen, Gwo-Yu Chuang, Aliaksandr Druz, Wing-Pui Kong,Yen-Ting Lai, Emily J. Rundlet, Yaroslav Tsybovsky, Yongping Yang, Ivelin S. Georgiev, Miklos Guttman, Christopher R. Lees, Marie Pancera, Mallika Sastry, Cinque Soto, Guillaume B.E. Stewart-Jones, Paul V. Thomas, Joseph G. Van Galen, Ulrich Baxa, Kelly K. Lee, John R. Mascola, Barney S. Graham, and Peter D. Kwong, also incorporated herein by reference.

Exemplary nucleic acid sequences encoding recombinant F_2-F_1 ectodomain protomers linked to a T4 Fibritin trimerization domain are available as Accession Numbers: LP884611.1, LP884610.1, LP884609.1 and LP884608.1.

In some examples, a protein or antigenic component may correspond to a component of a disease agent or vector or a portion thereof. An antigenic component may, for example, lack a transmembrane domain for ease of manufacture. Suitably, in the RSV-F protein or a pre-fusion conformation thereof, for example, the immunogenic component of an F protein comprises a F_2-F_1 subunit with a truncated transmembrane domain (having been truncated by deletion of one or more amino acids from this region) such that the subunit is secreted into the cell supernatant, during protein production in host cells, for ease of purification. Therefore, the RSV-F protein lacks a functional TM domain. Alternatively, the genetic fusion with the first peptide tag may indeed prevent the F protein from residing in the membrane despite the presence of a functional transmembrane domain.

In one example, the pre-fusion stabilised subunits have the amino acid sequences set out in SEQ ID NO: 50 -58, respectively (or functional equivalents thereof). By functional equivalents is meant amino acid sequences that share some homology and differ only in some amino acids but retain the functional property e.g. of being able to form an antigenic subunit that provides protective antibodies. In some examples, a functional equivalent may share 70%, 80%, 90% homology, or more, with the relevant amino acid sequence. In one example, the pre-fusion stabilised RSV-F trimer may not include a heterologous trimerisation domain.

The protein component comprises a first peptide tag. This first peptide tag may be attached to the protein component by expressing a recombinant fusion protein. Those skilled in the art will be aware of techniques for the genetic fusion of peptide sequences in order to express the recombinant protein in suitable cell systems. For moieties that include post-translational modifications such as glycosylation it is preferable to express the recombinant protein in a eukaryotic or mammalian cell line.

Advantageously using a first peptide tag and a second peptide tag which form an isopeptide bond, such as the SpyTag-SpyCatcher system as described herein, allows “decoration” of the large and/or multimeric antigen, such as the HCMV pentamer, or immunogenic component thereof, onto the moiety which displays said large antigen, such as a VLP, in the correct formation and orientation such that the antigen is presented to the immune system in such a way as to be able to generate anti-antigen (e.g. anti-HCMV) antibodies which can provide a protective/neutralising/immunogenic

effect. Traditional approaches of vaccination that use a soluble antigen (even a large antigen such as a multimer/pentamer) may be less effective in producing a protective/neutralising/immunogenic effect. Advantageously, display of an antigen (e.g. a multimeric antigen) on a particle, such as a VLP or nanoparticle, results in the presentation of a geometric repetitive array of identical antigens that, in contrast to soluble antigens, are capable of robustly triggering an immune response. The larger size of VLPs or other suitable particles compared to 'free' antigens may also have a greater immunogenic effect. In addition, the orientation of display of a multimeric antigen, e.g. the HCMV pentamer, may be important to immunogenicity. The use of paired tags, such as the SpyTag-SpyCatcher system as described herein, to attach a multimeric antigen onto a particle permits the antigen to be attached to the particle in a particular advantageous orientation. For example in the case of HCMV, the gH/gL subunits may be less likely to have neutralising epitopes than the "UL" subunits. Thus, advantageously, the present disclosure permits the orientation of display of the HCMV pentamer on a particle to be determined by suitable positioning of the first peptide tag, such that, for example, the "UL" subunits are displayed towards the outside of the particle and therefore are more easily available to the immune system of an individual. Alternatively/additionally, the positioning of the first peptide tag on the antigen may be determined in order to produce a similar orientation of the antigen to that on the native virus, thereby presenting to the immune system a particle displaying an antigen in an orientation more likely to induce an immune response to an invading live virus.

In contrast, traditional approaches for presenting a protein onto a VLP may involve chemical linkage which has the disadvantages that such a chemical reaction may be more random, such that the correct (e.g. immunologically preferred) orientation of the antigen could not be obtained with certainty and may only represent a small proportion of the linkage reactions obtained. Moreover, the processes involved in a chemical conjugation may make it unlikely that the 3-D structure required for suitable antigen presentation could be maintained. Some disadvantages of traditional approaches are described, for example, in Brune et al. 2016; Scientific Reports**,** 6:19234, DOI: 10.1038/srep19234, Brune et al. Bioconjugate Chemistry, 2017, 28, 1544-1551, and Leneghan et al (2017) Scientific reports, 7:3811.

Similarly, genetic fusion of an antigen to a viral coat protein has proved challenging and time-consuming because of problems with misfolding and in determining expression conditions optimal for both of the two components. Moreover a genetic fusion would not be appropriate for expression of a large antigen or a multi-component antigen as effective expression in correct conformation would be too difficult to achieve.

In order to present the protein or antigenic component in such a way that it is immunogenic, the position of the first peptide tag needs to be carefully designed such that the native protein conformation is maintained, and optionally any post-translational modifications are retained, if appropriate. For some antigens, the retention of glycosylation does not affect the way the epitopes are presented, but for others either maintaining or removing them improves efficacy. For protein components that are transmembrane proteins, the transmembrane section of the protein component provides a good target for positioning the first peptide tag, since this sequence is not involved with the conformation of the protein component that is antigenic, and provides a role that will no longer be required in a vaccine, for example. If the protein component does not include a transmembrane protein, fusing the first peptide tag to a C- or N- terminus of the component or a subunit thereof (in the case of a multimer) may prove helpful, but the first peptide tag can also be included in any part of the sequence. Alternatively, it may be possible to locate the first peptide tag in a loop on the protein or antigenic component.

In order to present an immunogenic component, such as an immunogenic component of an HCMV pentamer, the position of the first peptide tag needs to be carefully designed such that the native protein conformation is maintained. For HCMV, in one example, attachment is via the gH subunit, suitably via the C-terminus of the gH subunit, or transmembrane domain (or portion thereof) of the gH subunit. In addition to maintaining the conformation of the pentamer (or component of the pentamer), this rational design also presents the target region of the pentamer towards the outside of the particle as discussed above. As used herein, the target region is the part of the protein known to raise antibodies with a neutralising effect, and may also be referred to as the immunogenic portion.

In order to present an immunogenic component, such as an immunogenic component of an RSV pre-fusion F protein, the position of the first peptide tag needs to be carefully designed such that the native protein conformation is maintained. For RSV-F pre-fusion protein, in one example, attachment suitably via the C-terminus of the F pre-fusion , via the 3’ end of the nucleic acid encoding the same. In addition to maintaining the conformation of the pre-fusion F protein (or component thereof), this rational design also presents the most neutralising epitopes of the pre-fusion F protein towards the outside of the particle as discussed above. The same considerations will apply for any other variation of the F protein. Inclusion of the first peptide tag at the C-terminus has been demonstrated to work by the present inventors, leaving an immunogenic protein component to fold correctly.

In one example the first and second peptide tags are part of a peptide tag/binding partner pair capable of forming an isopeptide bond. This isopeptide bond may be spontaneous, i.e.

without assistance, or require assistance, i.e. from a ligase or other helper. Suitably, the first and second peptide tag are a SpyTag/SpyCatcher pair. Suitably, the first and second peptide tag are selected from the list comprising SpyTag/SpyCatcher, SnoopTag/ SnoopTagJr and SnoopCatcher; RrgATag/RrgATag2/DogTag and RrgACatcher, IsopepTag/ IsopepTag-N and Pilin-C or Pilin-N, PsCsTag and PsCsCatcher; and SnoopTagJr and DogTag (mediated by SnoopLigase), and variants, derivatives and modifications of all these systems.

Suitably, the first peptide tag is the peptide tag from a peptide tag/binding partner pair, such as SpyTag, and the second peptide tag is the binding partner, such as SpyCatcher. In another example, the first peptide tag is the binding partner, such as SpyCatcher, and the second peptide tag is the peptide tag component from the peptide tag/binding partner pair, such as SpyTag.

Suitably, the first peptide tag is the peptide tag from a peptide tag/binding partner pair, such as SnoopTag, and the second peptide tag is the binding partner, such as SnoopCatcher. In another example, the first peptide tag is the binding partner, such as SnoopCatcher, and the second peptide tag is the peptide tag component from the peptide tag/binding partner pair, such as SnoopTag. Thus, it can be seen that the first peptide tag can be either the “tag” or “catcher”; with the second peptide tag being the partner for this pair, the “catcher” or the “tag”, respectively. Suitable peptide tag/binding partner pairs are described in detail in WO2011/09877, WO2016/193746, WO2018/18951 and WO2018/197854, herein incorporated by reference.

In one example, the protein or antigenic component is attached to any one of SpyTag, SnoopTag, RrgATag, RrgATag2, DogTag, IsopepTag, IsopepTag-N, PsCsTag and SnoopTagJr as a first peptide tag.

The first peptide tag may be attached via a linker, if required, which may be rigid or flexible. Those skilled in the art will appreciate which linker would be appropriate.

In another example, the moiety is attached to any one of SpyCatcher, SnoopCatcher, RrgACatcher, Pilin-C, Pilin-N, PsCsCatcher and DogTag (mediated by SnoopLigase) as a second peptide tag.

The moiety may be any suitable moiety, as discussed previously, including synthetic multimerisation platforms.

The second peptide tag may be attached to any suitable position in the moiety, which does not affect its ability to fold and form an appropriate conformation. Genetic fusion may be preferred. It may be preferable to include the second peptide tag at the C- or N- terminus of the moiety but the second peptide tag can also be included in any part of the sequence. Alternatively, it may be possible to locate the second peptide tag in a loop on the moiety. For example, genetically fused SpyCatcher to the N-terminus of the viral coat protein (CP3) of the RNA bacteriophage AP205 is

described in Brune *et al*, Scientific Reports volume 6, Article number: 19234 (2016). Alternative fusions using self-assembling synthetic proteins as multimerisation platforms are discussed in Bruun *et al*, ACS Nano, 2018, 12 (9), pp 8855–8866. The second peptide tag may alternatively be attached via chemical conjugation.

The second peptide tag may be attached via a linker, if required, which may be rigid or flexible. Those skilled in the art will appreciate which linker would be appropriate.

In one example, the antigenic component, such as the HCMV pentamer or immunogenic component thereof, is attached to SpyTag. A suitable SpyTag has the amino acid sequence set out in SEQ ID NO: 30.

The SpyTag may be attached via a linker. Suitable linkers include the linker having the amino acid sequence set out in SEQ ID NO: 29.

In another example, the moiety is attached to a SpyCatcher binding partner (second peptide tag). The moiety may suitably be HBsAg. A suitable SpyCatcher has the amino acid sequence set out in SEQ ID NO: 38. In one example, SpyCatcher is attached via a linker. The linker may be a rigid linker or a flexible linker, suitably wherein the linker has the amino acid sequence set out in SEQ ID NO: 39.

In another example, the protein composition or antigenic composition in accordance with any example or example of the present disclosure further comprises another, preferably different, protein comprising a first peptide tag.

In another example, the composition in accordance with any example of the present disclosure further comprises another, preferably different, antigen comprising a first peptide tag, such as another HCMV antigen. Suitably the other HCMV antigen is glycoprotein B. Suitably glycoprotein B sequences are described, for example, in WO2014/005959, see SEQ ID NOs: 21, 22, 23 or 36. In one example, the composition comprises particles (e.g. VLPs) displaying both the HCMV pentamer and the other HCMV antigen.

In one example, the composition is an immunogenic composition or vaccine composition. Preferably said immunogenic or vaccine composition is one which is capable of inducing an immune response, such as an antibody response, upon administration to an individual. Suitably the immune response may be a protective immune response. A suitable immunogenic composition may further comprise additional components including adjuvants, immunostimulants and/or pharmaceutically acceptable excipients.

Suitable adjuvants, for example, may be based on aluminium, peptides, squalene, liposomes, oil-in-water emulsions and saponin, and may include Alhydrogel®, MF59, AS01, MatrixM, muramyl

dipepide and Quil A. Water-in-oil adjuvants are also suitable. Squalene-Oil-in-water emulsions, such as Addavax™, are suitable.

Accordingly, in another example of the present disclosure, there is provided an immunogenic or vaccine composition comprising a composition in accordance with the present disclosure. Suitably, a vaccine composition comprises a vaccine dose which is an amount of composition in accordance with the present disclosure which provides an immunogenic, preferably immunoprotective effect from an infective agent/vector, such as a neutralising effect from HCMV infection. Suitably, a vaccine composition comprises a vaccine dose which is an amount of composition in accordance with the present disclosure which provides a neutralising effect from an infective agent/vector, such as a neutralising effect from RSV infection. Antibodies, preferably neutralising antibodies generated to an immunogenic composition may be detected and measured by methods familiar to those skilled in the art, including standardised ELISA assays or microneutralisation assays, as described herein, for example.

In another example there is provided a VLP comprising:

i) a moiety comprising a first peptide tag

ii) a protein comprising a second peptide tag

wherein said first peptide tag and said second peptide tag form an isopeptide bond. In some examples, the moiety is HBsAg. However, any suitable moiety may be used, as described previously.

Suitably, the first peptide tag is the peptide tag from a peptide tag/binding partner pair, such as SpyTag, and the second peptide tag is the binding partner, such as SpyCatcher. In another example, the first peptide tag is the binding partner, such as SpyCatcher, and the second peptide tag is the peptide tag from the peptide tag/binding partner pair, such as SpyTag. Other suitable peptide tag/binding partner pairs are described herein and will be known to those skilled in the art. Suitably, the first and second peptide tag are selected from the list comprising SpyTag/SpyCatcher, SnoopTag/ SnoopTagJr and SnoopCatcher; RrgATag/RrgATag2/DogTag and RrgACatcher, IsopepTag/ IsopepTag-N and Pilin-C or Pilin-N, PsCsTag and PsCsCatcher; and SnoopTagJr and DogTag (mediated by SnoopLigase), and variants, derivatives and modifications of all these systems.

Suitably the protein comprising the second peptide tag is a protein or peptide complex which is greater than 50 kDa. The protein comprising the second peptide tag may be a protein or peptide complex which is greater than 50 kDa, 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 110 kDa, 120 kDa, 130 kDa, 140 kDa, 150 kDa or 160 kDa, 170 kDa, 180 kDa, 190 kDa or more, such as over 200 kDa, over 300 kDa or over 400 kDa.

In one example, the protein comprising the second peptide tag is a multimeric protein. In one example, the protein comprising the second peptide tag is an antigen, preferably a multimeric antigen. Suitably, the multimeric antigen may be HCMV pentamer as described herein. Suitably, the protein may be an RSV-F protein or derivative thereof (such as the pre-fusion F protein). In one example, the protein comprising a second peptide tag is an immunogenic component of HCMV pentamer. The HCMV pentamer (gH/gL/pUL128/pUL130/pUL131) as described herein and including suitable linkers and tags has a molecular weight of over 160 kDa. Other suitable large or multimeric proteins or antigens include antigens from other infectious agents including viruses such as influenza virus, RSV and so forth.

Advantageously, using HBsAg as a carrier (VLP) in this way would also be likely to generate an anti-HepB boost, alternatively described as a an anti-Hepatitis B virus (HBV) response

In another example there is provided a VLP comprising:

i) a protein comprising a first peptide tag

ii) a moiety comprising a second peptide tag

wherein said first peptide tag and said second peptide tag form an isopeptide bond. In some examples, the moiety is HBsAg. However, any suitable moiety may be used, as described previously.

Suitably, the first peptide tag is the peptide tag from a peptide tag/binding partner pair, such as SpyTag, and the second peptide tag is the binding partner, such as a SpyCatcher. In another example, the first peptide tag is the binding partner, such as a SpyCatcher, and the second peptide tag is the peptide tag from the peptide tag/binding partner pair, such as SpyTag. Other suitable peptide tag/binding partner pairs are described herein and will be known to those skilled in the art. Suitably, the first and second peptide tag are selected from the list comprising SpyTag/SpyCatcher, SnoopTag/ SnoopTagJr and SnoopCatcher; RrgATag/RrgATag2/DogTag and RrgACatcher, IsopepTag/ IsopepTag-N and Pilin-C or Pilin-N, PsCsTag and PsCsCatcher; and SnoopTagJr and DogTag (mediated by SnoopLigase), and variants, derivatives and modifications of all these systems.

Suitably the protein comprising the first peptide tag is a protein or peptide complex which is greater than 50 kDa. The protein comprising the first peptide tag may be a protein or peptide complex which is greater than 50 kDa, 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 110 kDa, 120 kDa, 130 kDa, 140 kDa, 150 kDa or 160 kDa or more, notably 200 kDa, 300 kDa or even 400 kDa or more. In one example, the protein comprising the first peptide tag is a multimeric protein. In one example, the protein comprising the second peptide tag is an antigen, preferably a multimeric antigen. Suitably, the multimeric antigen may be HCMV pentamer as described herein. Suitably, the

protein may be an RSV-F protein or derivative thereof (such as the pre-fusion F protein). In one example, the protein comprising a first peptide tag is an immunogenic component of HCMV pentamer. The HCMV pentamer (gH/gL/pUL128/pUL130/pUL131A) as described herein and including suitable linkers and tags has a molecular weight of over 160 kDa. Other suitable large or multimeric proteins or antigens include antigens from other infectious agents including viruses such as influenza virus, RSV and so forth.

Advantageously, using HBsAg as a carrier (VLP) in this way would also be likely to generate an anti-HBV boost.

In another example of the present disclosure, there is provided an HCMV pentamer linked to a SpyTag, as described herein.

In accordance with another example of the present disclosure there is provided a method of producing a composition or VLP in accordance with the present disclosure, said method comprising:

- introducing a first nucleic acid which encodes a first genetic fusion of a first protein to a first peptide tag into a first host cell;
- incubating said first host cell under conditions for expressing said first genetic fusion; optionally purifying the expressed components;
- introducing a second nucleic acid which encodes a second genetic fusion of a second protein to a second peptide tag into a second host cell;
- incubating said second host cell under conditions for expressing said second genetic fusion; optionally purifying the expressed components;
- incubating the expressed components under conditions for formation of an isopeptide bond between the first peptide tag and the second peptide tag; optionally purifying the resultant composition.

Suitably, the expressed components are incubated together in order for the isopeptide bond to form. The formation of the isopeptide bond may require co-incubation with a ligase or similar.

Suitably, the method of producing a composition or VLP in accordance with the present disclosure may be for producing a composition comprising an antigenic component displayed on a VLP.

In some examples, where the “immunogenic component of the HCMV pentamer” comprises the entire HCMV pentamer, recombinant production of the components of the HCMV pentamer requires each subunit to be expressed in the right stoichiometry for the pentamer to be formed, as well as to fold correctly for assembly. In these examples, complexes of just parts of the required pentamer (e.g. gH/gL dimers and tetramers, or tetramers lacking any one of the five subunits) need to be excluded from the final product. Advantageously, the present present disclosure

overcomes the problems that would otherwise be associated by expressing all of the vaccine components in one system (i.e. HBsAg and 5 subunits of HCMV pentamer) by providing a simple approach of making the components separately and then conjugating them. Accordingly, in one example, a purification tag is incorporated onto UL130 (Hofmann et al, DOI 10.1002/bit 25670). Similar principles would be applicable to other immunogenic components.

In some examples, where the "immunogenic component of the RSV-F protein" comprises the entire F protein or a derivative thereof, recombinant production of the components of the F protein or derivative thereof requires it to fold correctly for assembly, with derivatives including the pre-fusion F protein trimer.

Suitably, the method is for producing a composition comprising an HCMV pentamer displayed on an HBsAg VLP. Suitably, the method is for producing a composition comprising an RSV-F pre-fusion F protein trimer displayed on an HBsAg VLP.

In another example of the present disclosure, there is provided a vaccine for use in the prophylaxis and/or treatment of a disease. Suitably, said vaccine comprises a composition or VLP in accordance with any example of the present disclosure. In one example, the disease is HCMV infection. In another example there is provided a prophylactic method of treatment for HCMV. Suitably, the vaccine is for use in humans. Suitably the vaccine is for use in adult humans, for example women of reproductive age or pregnant women. In another example, the present disclosure provides a method of inducing an immunogenic response, for example a protective immune response, for HCMV in an individual wherein the method comprises administering a composition in accordance with any example of the present disclosure.

In another example of the present disclosure there is provided a composition in accordance with any example of the present disclosure for use as a medicament.

In a further example of the present disclosure there is provided a composition in accordance with any example of the present disclosure for use as a vaccine, preferably a vaccine for use in prophylaxis and/or treatment of HCMV infection. A composition for use as a medicament or a vaccine in accordance with the present disclosure may be administered to human adults, for example women of reproductive age or pregnant women.

In another example, the present disclosure provides nucleic acid molecules for use in a method in accordance with the present disclosure. In one example, a nucleic acid molecule in accordance with the present disclosure comprises a nucleic acid sequence encoding an amino acid sequence as set out in any of SEQ ID NOs: 27 to 41. In one example, a nucleic acid molecule in accordance with the present disclosure comprises a nucleic acid sequence as set out in any of SEQ ID NOs: 12 to 26 or 42 to 46.

In another example, the present disclosure provides a plurality of nucleic acid molecules comprising those nucleic acid molecules encoding an amino acid sequence as set out in SEQ ID NOs: 27 to 41. In one example, the nucleic acid molecules of the present disclosure include those having a sequence as set out in any of SEQ ID NOS: 12 to 26 or 42 to 46.

In another example, the present disclosure provides nucleic acid molecules for use in a method in accordance with the present disclosure. In one example, a nucleic acid molecule in accordance with the present disclosure comprises a nucleic acid sequence encoding an amino acid sequence as set out in any of SEQ ID NOs: 50 to 58. In one example, a nucleic acid molecule in accordance with the present disclosure comprises a nucleic acid sequence as set out in any of SEQ ID NOs: 47 to 55.

In another example, the present disclosure provides a plurality of nucleic acid molecules comprising those nucleic acid molecules encoding an amino acid sequence as set out in SEQ ID NOs: 50 to 58. In one example, the nucleic acid molecules of the present disclosure include those having a sequence as set out in any of SEQ ID NOS: 47 to 55.

In another example, the present disclosure provides a vector comprising a nucleic acid molecule or a plurality of nucleic acid molecules in accordance with the present disclosure. Suitably a vector is an expression vector for expressing the amino acid sequence of any component of a composition in accordance with the present disclosure.

In another example, the present disclosure provides host cells for expressing the components of a composition in accordance with the present disclosure. Suitable host cells may be those for transient or stable expression of those components. Methods and host cells for expressing CMV proteins are described, for example, in WO2014/005959 and WO2016/067239, both incorporated by reference. In some examples, the components may be glycosylated.

In another example of the present disclosure, there is provided a kit comprising a composition in accordance with the present disclosure for use in a prime-boost vaccination regime. Suitably said kit may comprise a prime composition comprising a first immunogenic composition in accordance with the present disclosure and a boost composition comprising a second immunogenic composition in accordance with the present disclosure. Alternatively, the kit may be provided to provide a single or multiple dose vaccination regime, i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses. Accordingly, in another example the present disclosure provides a dosage regime comprising doses applied at approximately 3 week intervals.

FIGURES

Figure 1. SDS-PAGE and Western blot analysis of purified pentamer-SpyTag under non-reducing and reducing conditions. Lane 1: ColorPlus Prestained Broad Range Protein Ladder, sizes indicated in kDa; Lane 2: non-reduced sample; Lane 3: reduced sample. A) SDS-PAGE and Coomassie staining

Claims

1. A composition comprising a particle displaying an antigenic component, wherein said composition comprises:

i) an antigenic component comprising a first peptide tag, and

ii) a particulate moiety comprising a second peptide tag,

wherein the antigenic component and the moiety are linked via an isopeptide bond between said first and second peptide tags, and wherein the antigenic component is over 50 kDa.

2. A composition as claimed in claim 1 wherein the antigenic component is over 60 kDa, 70 kDa, 80 kDa, 90 kDa, 100 kDa, 110 kDa, 120 kDa, 130 kDa, 140 kDa, 150 kDa , 160 kDa, 170 kDa, 180 kDa, 190 kDa, 200 kDa, 300k Da or 400 kDa.

3. A composition as claimed in claim 1 or claim 2 wherein the antigenic component is a monomer or multimer, optionally wherein the multimer is a trimer, tetramer, pentamer, hexamer, septamer, octamer, nonamer or decamer.

4. A composition as claimed in any preceding claim wherein the moiety is a virus, bacteria, multimerisation scaffold for vaccination, a protein component which multimerises to form a virus-like particle (VLP), a viral structural protein, a multimerisation domain which forms nanoparticles, a synthetic nanoparticle or a synthetic VLP.

5. A composition as claimed in any preceding claim wherein the moiety is an HBsAg particle.

6. A composition as claimed in any preceding claim wherein the first and second peptide tag are selected from any one of a SpyTag and SpyCatcher pair; a SnoopTag or SnoopTagJr and SnoopCatcher pair; a RrgATag, RrgATag2 or DogTag and RrgACatcher pair, an IsopepTag Pilin-C pair; an IsopepTag-N and Pilin-N pair; a PsCsTag and PsCsCatcher pair; and SnoopTagJr and DogTag pair mediated by SnoopLigase; or variants, derivatives or modifications thereof, optionally wherein the first and second peptide tags are a SpyTag/SpyCatcher pair.

7. A composition as claimed in any preceding claim wherein the antigenic component comprises an immunogenic component of an HCMV pentamer, wherein the immunogenic component of an HCMV pentamer comprises one or more of the gH, gL, pUL128, pUL130 or pUL131 subunits..

8. A composition as claimed in claim 7 wherein the immunogenic component of an HCMV pentamer comprises each of the gH, gL, pUL128, pUL130 or pUL131 subunits.

9. A composition as claimed in claim 7 or claim 8 wherein the immunogenic component of an HCMV pentamer comprises a gH subunit with a truncated transmembrane domain.

10. A composition as claimed in any of claims 7 to claim 9, wherein the first peptide tag is attached to the gH subunit.

11. A composition as claimed in any one of claim 1 to claim 6 wherein the antigenic component comprises an immunogenic component of an RSV-F protein, wherein the immunogenic component of an RSV-F protein is a pre-fusion F protein.

12. A composition as claimed in claim 11 wherein said RSV-F protein wherein the first peptide tag is attached to the C-terminus of the pre-fusion F protein.

13. A composition as claimed in any preceding claim wherein the first peptide tag is a SpyTag.

14. A composition as claimed in any preceding claim wherein the moiety is HBsAg, and optionally wherein the second peptide tag is a SpyCatcher.

15. A composition as claimed in any preceding claim wherein the composition is an immunogenic or vaccine composition.

16. A vaccine comprising a composition as claimed in any of claims 1 to 15 for use in the prophylaxis and/or treatment of a disease.

17. A method of producing a composition in accordance with any of claims 1 to 16, said method comprising:

- introducing a first nucleic acid which encodes a first genetic fusion of a first protein to a first peptide tag into a first host cell;
- incubating said first host cell under conditions for expressing said first genetic fusion;
- introducing a second nucleic acid which encodes a second genetic fusion of a second protein to a second peptide tag into a second host cell;
- incubating said second host cell under conditions for expressing said second genetic fusion;
- optionally purifying the expressed components;
- incubating the expressed components under conditions for formation of an isopeptide bond between the first peptide tag and the second peptide tag; and purifying the resultant composition

wherein the first protein comprises an antigenic component and the second protein comprises a moiety.

18. A nucleic acid molecule for use in a method as claimed in claim 17 wherein the nucleic molecule encodes an amino sequence as set out in any of SEQ ID NOs: 27 to 41 .

19. A nucleic acid molecule for use in a method as claimed in claim 17 wherein the nucleic molecule encodes an amino sequence as set out in any of SEQ ID NOs: 50 to 58 .

20. A vector comprising a nucleic acid molecule as claimed in claim 18 or 19.

21. A host cell comprising a nucleic acid molecule as claimed in claim 18 or 19, or a vector as claimed in claim 20.