complex antigen mixtures, target expression can be low, and because certain display packages used to construct antibody libraries (*e.g.*, vaccinia virus antibody libraries) can bind to whole cells non-specifically. There remains a need for new methods to express and display target IMPs of interest in their native conformation at a sufficient concentration and with minimal competition from other cell proteins to allow for identification and selection of therapeutic antibodies and antibody-like molecules from display libraries.

SUMMARY

[0003] This disclosure provides compositions and methods for expressing and displaying isolated integral membrane proteins (IMPs) or fragments thereof in a native conformation for use in the screening, selecting, and identifying of antibodies or antibody-like molecules that bind to a target IMP of interest.

[0004] In one aspect, the present disclosure refers to an isolated polynucleotide comprising: (a) a first nucleic acid fragment that encodes an integral membrane protein (IMP) or fragment thereof, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and wherein a portion of the first nucleic acid fragment encoding at least one intra-membrane region is situated at the 5' or 3' end of the first nucleic acid fragment; and (b) a second nucleic acid fragment that encodes a vaccinia virus F13L protein or functional fragment thereof, wherein the second nucleic acid fragment is fused in frame to a portion of the first nucleic acid fragment that encodes an intra-membrane region of the IMP; wherein a poxvirus infected cell comprising the polynucleotide can express an IMP-F13L fusion protein as part of the outer envelope membrane of an extracellular enveloped virion (EEV). In certain examples, the disclosure provides an isolated polynucleotide that includes: a first nucleic acid fragment that encodes an integral membrane protein (IMP) or fragment thereof, where the IMP or fragment thereof includes at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and where a portion of the first nucleic acid fragment encoding at least one intra-membrane region is situated at the 5' or 3' end of the first nucleic acid fragment; and a second nucleic acid fragment that encodes a vaccinia virus F13L protein or functional fragment thereof, where the second nucleic acid fragment is fused in frame to a portion of the first nucleic acid fragment that encodes an intra-membrane region of the IMP.

According to these examples, a poxvirus infected cell containing the polynucleotide can express an IMP-F13L fusion protein as part of the outer envelope membrane of an extracellular enveloped virion (EEV). In certain examples, the F13L protein or functional fragment thereof can include the amino acid sequence SEQ ID NO: 1 or a functional fragment thereof. In certain examples the IMP is a multi-pass membrane protein comprising at least two, at least three, at least four, at least five, at least six or at least seven transmembrane domains. In certain examples the IMP is a multi-pass membrane protein listed in Table 1.

[0005] In certain examples the multi-pass IMP can have an odd number of transmembrane domains, the 5' end of the first nucleic acid fragment can encode an extra-membrane region, and the 3' end of the first nucleic acid fragment can encode an intra-membrane region fused to the 5' end of the second nucleic acid fragment. In certain examples the first nucleic acid

fragment of this type can encode, *e.g.*, a G-protein coupled receptor (GPCR). In certain examples the GPCR can be the human frizzled-4 protein (FZD4), or a fragment thereof, and the polynucleotide can encode a polypeptide that includes amino acids 20 to 892 of SEQ ID NO: 2. In certain examples the polypeptide can further include a signal peptide, e.g., amino acids 1 to 19 of SEQ ID NO: 2. In certain examples the GPCR can be a CXC chemokine receptor, e.g., CXCR4, or a fragment thereof, and the polynucleotide can encode a polypeptide that includes the amino acid sequence SEQ ID NO: 3.

[0006] In certain examples the multi-pass IMP can have an even number of transmembrane domains, and both the 5' and 3' ends of the first nucleic acid fragment can encode intra-membrane regions. In certain examples, the second nucleic acid fragment can be fused to 3' end of the first nucleic acid fragment. In certain examples the IMP can be, e.g., human CD20 protein, or a fragment thereof, and the polynucleotide can encode a polypeptide that includes the amino acid sequence SEQ ID NO: 4.

[0007] In certain examples, the first and second nucleic acid fragments of a polynucleotide provided herein can be directly fused. In certain examples the polynucleotide as provided herein can include a third nucleic acid fragment encoding a heterologous peptide, *e.g.*, a linker sequence, an amino acid tag or label, or a peptide or polypeptide sequence that facilitates purification, such as a histidine tag. In certain examples a polynucleotide as provided here can be operably associated with a poxvirus promoter, e.g., a p7.5, a T7, or H5 promoter.

[0008] The disclosure further provides an F13L fusion protein encoded by a polynucleotide as provided herein. In another aspect, the present disclosure further provides a poxvirus genome, *e.g.*, a vaccinia virus genome, that includes a polynucleotide as provided herein. In another aspect, the present disclosure further provides a recombinant vaccinia virus EEV that includes a poxvirus genome as provided herein.

[0009] In another aspect, the present disclosure further provides a method of producing a recombinant vaccinia virus EEV as provided herein where the method includes infecting a host cell permissive for vaccinia virus infectivity with a vaccinia virus comprising a poxvirus genome as provided herein, and recovering EEV released from the host cell.

[0010] In another aspect, the present disclosure refers to a method to display an integral membrane protein (IMP) or fragment thereof in a native conformation comprising: (a) infecting host cells permissive for poxvirus infectivity with a recombinant poxvirus that

expresses the IMP or fragment thereof as a fusion protein with poxvirus EEV-specific protein or membrane-associated fragment thereof, wherein EEV produced by the infected host cell comprise the IMP fusion protein as part of the EEV outer envelope membrane; (b) recovering EEV released from the host cell, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and wherein the IMP or fragment thereof displays on the surface of the EEV in a native conformation. The disclosure further provides a method to display an integral membrane protein (IMP) or fragment thereof in a native conformation where the method includes infecting host cells permissive for poxvirus infectivity with a recombinant poxvirus that expresses an IMP or fragment thereof as a fusion protein with poxvirus EEV-specific protein or membrane-associated fragment thereof, where EEV produced by the infected host cell comprise the

IMP fusion protein as part of the EEV outer envelope membrane and recovering EEV released from the host cell. In certain examples the IMP or fragment thereof displays on the surface of the EEV in a native conformation. In certain examples the EEV-specific protein can be the vaccinia virus A33R protein, A34R protein, A56R protein, B5R protein, A36R protein, F13L protein, any membrane-associated fragment thereof, or any combination thereof.

[0011] In certain examples the EEV-specific protein is F13L (SEQ ID NO: 1) or a functional fragment thereof. In certain examples the IMP is a multi-pass membrane protein that includes at least two, at least three, at least four, at least five, at least six or at least seven transmembrane domains. In certain examples the IMP can be a G-protein coupled receptor (GPCR), e.g., human FZD4 or CXCR4 as described above, that includes seven transmembrane domains, and the F13L protein can be fused to the C-terminus of the IMP. In certain examples the IMP or fragment thereof can have an even number of transmembrane domains, e.g., human CD20 as described above, where both the N-terminus and the C-terminus of the IMP or fragment thereof are intra-membrane, and the F13L can be fused to the N-terminus or the C-terminus of the IMP.

[0012] In certain examples the membrane-associated EEV specific protein fragment can include or consist of the stalk, transmembrane, and intra-membrane domains of the vaccinia virus A56R protein, e.g., amino acids 108 to 314 of SEQ ID NO: 5. In certain examples IMP portion of the A56R fusion protein can include the extracellular domain of human FZD4, e.g., the fusion protein can include amino acids 20 to 370 of SEQ ID NO: 6, the extracellular domain of human ErbB2 (Her2), e.g., the fusion protein can include amino acids 20 to 855 of SEQ ID NO: 7, or the extracellular domain of human CD100 (Semaphorin 4D), e.g., the fusion protein can include amino acids 20 to 935 of SEQ ID NO: 8.

[0013] In certain examples the membrane-associated EEV specific protein fragment can include or consist of the transmembrane and intra-membrane domains, or the stalk, transmembrane, and intra-membrane domains of the vaccinia virus B5R protein, e.g., amino acids 276 to 317 of SEQ ID NO: 9 or amino acids 238 to 317 of SEQ ID NO: 9, respectively. In certain examples the IMP portion of the B5R fusion protein can include the extracellular domain of human FZD4, e.g., the fusion protein can includes amino acids 20 to 243 of SEQ ID NO: 10 or amino acids 20 to 281 of SEQ ID NO: 11.

[0014] In another aspect, the present disclosure refers to a fusion protein comprising: (a) amino acids 20 to 892 of SEQ ID NO: 2; (b) SEQ ID NO: 3; or (c) SEQ ID NO: 4; wherein the fusion protein, when expressed by a recombinant poxvirus, appears on the surface of a poxvirus extracellular enveloped virion (EEV) in a native conformation. The disclosure further provides a fusion protein comprising: amino acids 20 to 892 of SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 4; amino acids 20 to 370 of SEQ ID NO: 6; amino acids 20 to 855 of SEQ ID NO: 7; amino acids 20 to 935 of SEQ ID NO: 8; amino acids 20 to 243 of SEQ ID NO: 10; amino acids 20 to 281 of SEQ ID NO: 11, amino acids 20 to 506 of SEQ ID NO: 16, or amino acids 20 to 235 of SEQ ID NO: 17. A fusion protein as provided, when expressed by a recombinant poxvirus, e.g., a vaccinia virus, can appear on the surface of a poxvirus extracellular enveloped virion (EEV) in a native conformation. A recombinant poxvirus EEV comprising the fusion protein is also provided. The disclosure further provides a recombinant poxvirus EEV that includes a heterologous IMP or fragment thereof fused to a poxvirus EEV-specific protein or membrane-associated fragment thereof, where the fusion protein is situated in the EEV outer envelope membrane, and where the IMP or fragment thereof displays on the surface of the EEV in its native conformation. In another aspect, the present disclosure refers to a recombinant poxvirus EEV comprising the fusion protein as disclosed herein. In another example, the present disclosure refers to a recombinant poxvirus EEV comprising a heterologous IMP or fragment thereof fused to a poxvirus EEV-specific protein or membrane-associated fragment thereof, wherein the fusion protein is situated in the EEV outer envelope membrane, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region and wherein the IMP or fragment thereof displays on the surface of the EEV in its native conformation. In certain examples the recombinant poxvirus EEV is a vaccinia virus EEV.

[0015] In another aspect, the present disclosure refers to a method to select antibodies that bind to a multi-pass membrane protein comprising: (a) attaching the recombinant EEV of any one of claims 16, or 23 to 25 to a solid support; (b) providing an antibody display library, wherein the library comprises display packages displaying a plurality of antigen binding domains; (c) contacting the display library with the EEV such that display packages displaying antigen binding domains that specifically binds to the IMP expressed on the EEV can bind thereto; (d) removing unbound display packages; and (e) recovering display

packages that display an antigen binding domain specific for the IMP expressed on the EEV; wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region. The disclosure further provides a method to select antibodies that bind to a multi-pass membrane protein where the method includes attaching recombinant EEV as provided herein to a solid support; providing an antibody display library, where the library comprises display packages displaying a plurality of antigen binding domains; contacting the display library with the EEV such that display packages displaying antigen binding domains that specifically binds to the IMP expressed on the EEV can bind thereto; removing unbound display packages; and recovering display packages that display an antigen binding domain specific for the IMP expressed on the EEV. In certain examples of this method the recombinant EEV are inactivated prior to attachment to the solid support, e.g., by incubation with Psoralen (Trioxsalen, 4'-aminomethyl-, hydrochloride) in the presence of UV irradiation. In certain examples of this method the recombinant EEV are attached to the solid surface via reaction with tosyl groups attached to the surface. In certain examples the solid surface can be tosyl-activated magnetic beads. In certain examples of this method the recombinant EEV are biotinylated and attached to a streptavidin coated solid surface, e.g., streptavidin-coated magnetic beads. In another aspect, the present disclosure refers to an IMP-EEV fusion protein, which comprises (a) an integral membrane protein (IMP) or fragment thereof, which comprises at least one extra-membrane region, at least one transmembrane domain, and at least one intra-membrane region; and (b) a vaccinia virus extracellular enveloped virion (EEV) protein or membrane-associates fragment thereof.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0016] **FIGURE 1A-C**: Diagrammatic depiction of integral membrane proteins (IMPs) or fragment thereof fused to vaccinia virus extracellular enveloped virion (EEV)-specific

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising:

(a) a first nucleic acid fragment that encodes an integral membrane protein (IMP) or fragment thereof, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and wherein a portion of the first nucleic acid fragment encoding at least one intra-membrane region is situated at the 5' or 3' end of the first nucleic acid fragment; and

(b) a second nucleic acid fragment that encodes a vaccinia virus F13L protein or functional fragment thereof, wherein the second nucleic acid fragment is fused in frame to a portion of the first nucleic acid fragment that encodes an intra-membrane region of the IMP;

wherein a poxvirus infected cell comprising the polynucleotide can express an IMP-F13L fusion protein as part of the outer envelope membrane of an extracellular enveloped virion (EEV).

2. The polynucleotide of claim 1, wherein the F13L protein or functional fragment thereof comprises the amino acid sequence SEQ ID NO: 1 or a functional fragment thereof.

3. The polynucleotide of claim 1 or claim 2, wherein the IMP is a multi-pass membrane protein comprising at least two, at least three, at least four, at least five, at least six or at least seven transmembrane domains.

4. The polynucleotide of claim 3, wherein the IMP has an odd number of transmembrane domains, wherein the 5' end of the first nucleic acid fragment encodes an extra-membrane region, wherein the 3' end of the first nucleic acid fragment encodes an intra-membrane region, and wherein the 5' end of the second polynucleotide is fused to the 3' end of the first nucleic acid fragment.

5. The polynucleotide of claim 4, wherein the IMP comprises a G-protein coupled receptor (GPCR).

6. The polynucleotide of claim 5, wherein the IMP is the human frizzled-4 protein (FZD4), or a fragment thereof.

7. The polynucleotide of claim 6, which encodes a polypeptide comprising amino acids 20 to 892 of SEQ ID NO: 2.

8. The polynucleotide of claim 5, wherein the IMP is a CXC chemokine receptor.

9. The polynucleotide of claim 8, wherein the CXC chemokine receptor is CXCR4, or a fragment thereof.

10. The polynucleotide of claim 3, wherein the IMP has an even number of transmembrane domains, and wherein both the 5' and 3' ends of the first nucleic acid fragment encode intra-membrane regions and wherein the second nucleic acid fragment is fused to 3' end of the first nucleic acid fragment.

11. The polynucleotide of claim 10, wherein the IMP is human CD20 protein, or a fragment thereof.

12. The polynucleotide of any one of claims 1 to11, which is operably associated with a poxvirus promoter.

13. The F13L fusion protein encoded by the polynucleotide of any one of claims 1 to 12.

14. A poxvirus genome comprising the polynucleotide of any one of claims 1 to 13.

15. The poxvirus genome of claim 14, which is a vaccinia virus genome.

16. A recombinant vaccinia virus EEV comprising the poxvirus genome of claim 15.

17. A method of producing the recombinant vaccinia virus EEV of claim 16, comprising:

(a) infecting a host cell permissive for vaccinia virus infectivity with a vaccinia virus comprising the poxvirus genome of claim 14, and

(b) recovering EEV released from the host cell.

18. A method to display an integral membrane protein (IMP) or fragment thereof in a native conformation comprising:

(a) infecting host cells permissive for poxvirus infectivity with a recombinant poxvirus that expresses the IMP or fragment thereof as a fusion protein with

poxvirus EEV-specific protein or membrane-associated fragment thereof, wherein EEV produced by the infected host cell comprise the IMP fusion protein as part of the EEV outer envelope membrane;

(b) recovering EEV released from the host cell

wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region, and wherein the IMP or fragment thereof displays on the surface of the EEV in a native conformation.

19. The method of claim 18, wherein the EEV-specific protein is the vaccinia virus A33R protein, A34R protein, A56R protein, B5R protein, A36R protein, F13L protein, any membrane-associated fragment thereof, or any combination thereof.

20. The method of claim 19, wherein the EEV-specific protein is F13L (SEQ ID NO: 1) or a functional fragment thereof.

21. The method of claim 20, wherein the IMP is a multi-pass membrane protein comprising at least two, at least three, at least four, at least five, at least six or at least seven transmembrane domains.

22. A fusion protein comprising:

(a) amino acids 20 to 892 of SEQ ID NO: 2;

(b) SEQ ID NO: 3; or

(c) SEQ ID NO: 4;

wherein the fusion protein, when expressed by a recombinant poxvirus, appears on the surface of a poxvirus extracellular enveloped virion (EEV) in a native conformation.

23. A recombinant poxvirus EEV comprising the fusion protein of claim 22.

24. A recombinant poxvirus EEV comprising a heterologous IMP or fragment thereof fused to a poxvirus EEV-specific protein or membrane-associated fragment thereof, wherein the fusion protein is situated in the EEV outer envelope membrane, wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at

least one intra-membrane region and wherein the IMP or fragment thereof displays on the surface of the EEV in its native conformation.

25. The recombinant poxvirus EEV of claim 23 or claim 24, wherein the poxvirus EEV is a vaccinia virus EEV.

26. A method to select antibodies that bind to a multi-pass membrane protein comprising:

(a) attaching the recombinant EEV of any one of claims16, or 23 to 2525 to a solid support;

(b) providing an antibody display library, wherein the library comprises display packages displaying a plurality of antigen binding domains;

(c) contacting the display library with the EEV such that display packages displaying antigen binding domains that specifically binds to the IMP expressed on the EEV can bind thereto;

(d) removing unbound display packages; and

(e) recovering display packages that display an antigen binding domain specific for the IMP expressed on the EEV; wherein the IMP or fragment thereof comprises at least one extra-membrane region, at least one transmembrane domain and at least one intra-membrane region.

27. An IMP-EEV fusion protein, which comprises (a) an integral membrane protein (IMP) or fragment thereof, which comprises at least one extra-membrane region, at least one transmembrane domain, and at least one intra-membrane region; and (b) a vaccinia virus extracellular enveloped virion (EEV) protein or membrane-associates fragment thereof.