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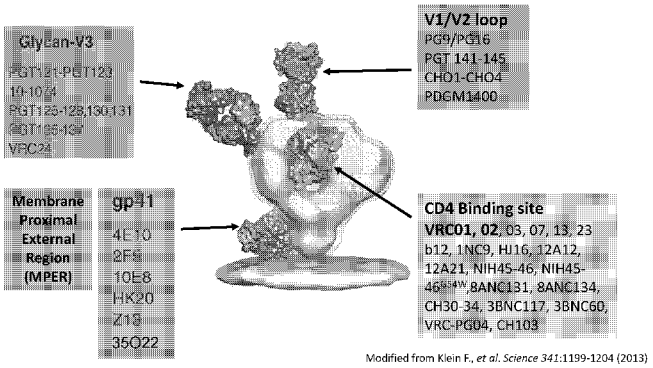
Fig. 1



Modified from Klein F., *et al. Science 341*:1199-1204 (2013)

(57) Abstract: This disclosure provides a multimeric human immunodeficiency virus (HIV) protein binding molecule, *e.g.*, an dimeric IgA or a pentameric or hexameric IgM binding molecule, comprising at least two bivalent binding units, or variants or fragments thereof, each comprising at least two antibody heavy chain constant regions or fragments thereof, wherein each heavy chain constant region or fragment thereof is associated with an HIV antigen binding domain. Also provided are compositions comprising the multimeric binding molecules, polynucleotides encoding the multimeric binding molecules, and methods to make and use the multimeric binding molecules.

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MULTI-VALENT HUMAN IMMUNODEFICIENCY VIRUS ANTIGEN BINDING MOLECULES AND USES THEREOF

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CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims benefit of U.S. Provisional Application Serial No. 62/149,460, filed on April 17, 2015, which is incorporated herein by reference in its entirety.

BACKGROUND

[0002] Human immunodeficiency virus (HIV) is a retrovirus of the *Lentivirus* family. HIV is a single stranded, positive sense enveloped RNA virus. HIV causes acquired immunodeficiency syndrome (AIDS) which leads to failure of the immune system due to destruction of T cells, macrophages and dendritic cells. HIV has a very high rate of genetic variability. Two types of HIV have been identified, including HIV-1 and HIV-2, both of which are transmitted by sexual contact or through blood and both cause AIDS. The two viruses differ in that HIV-1 is more prevalent, more virulent, and more easily transmitted than HIV-2. HIV-1 can be further divided into three groups based on sequence differences in the envelope (*env*) gene, group M, group N group O, and group P. Group M of HIV-1 is further divided into at least nine subtypes (or clades) based on difference in genomic sequence and geographic distribution (subtypes A, B, C, D, F, G, H, J and K). The structure of the HIV RNA genome includes nine genes: *gag*, *pol*, *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu*. Some HIV genomes include a tenth gene called *tev*, a fusion of *tat*, *env*, and *rev*. These genes encode the following proteins:

Viral Structural Proteins	
gag	proteolytically processed to yield matrix protein (p17, MA), capsid protein (p24, CA), nucleocapsid protein (p7, NC), spacer peptide 2 (SP2, p1) and P6 protein
pol	proteolytically processed to yield reverse transcriptase (RT), RNase H, integrase (IN) and HIV protease (PR)
env	gp160 envelope protein; processed proteolytically to yield gp120

Essential Regulatory Elements	
tat	RNA binding transcriptional activator protein
rev	Rev protein, which is a sequence-specific RNA binding regulator protein
Accessory Regulatory Proteins	
vpr	Vpr is lentivirus nucleocytoplasmic shuttling/transport regulatory protein
vif	Vif is a cell-specific regulatory phosphoprotein
nef	Nef is an N-terminal myristoylated membrane-associated regulatory phosphoprotein
vpu	Vpu is an HIV-1 specific integral membrane regulatory phosphoprotein
tev	*tev* is a *tat*/*env*/*rev* fusion gene yielding a fusion protein

[0003] The HIV envelope protein, gp160 is proteolytically cleaved by a host cell protease to yield gp120 and gp41. These two molecules form a cap and stem structure protruding from the viral envelope, also referred to as the spike. The cap is composed of three copies of gp120 and the stem is composed of three copies of gp41. The stem anchors the gp120/gp41 complex to the viral envelope. The envelope glycoprotein gp120 is expressed both on the surface of infected cells and the viral envelope of viral particles. The gp120 portion of the Env protein is responsible for binding to the CD4 receptor on target cells, such as helper T cells, enabling the virus to fuse to the host cell. Structure of the HIV spike glycoprotein is shown in **FIG. 1**.

[0004] The viral protein gp120 has been intensely focused upon in vaccine development research since it is the main point of contact and entry into host cells. However, the mechanism of HIV entry into cells includes masking of gp120 epitopes by covalently attached sugar moieties. It is only when the HIV virion is in close proximity to a host cell that the portion of gp120 that interacts with host cell receptors is unmasked. Likewise, glycoprotein gp41 is non-covalently associated with gp120 and becomes exposed only after gp120 binds to its target and undergoes a conformational change. The conformational change triggered by binding to the host cell allows gp41 to assist in the fusion of the virion to the host cell. Thus, gp41 has also received the attention of clinical research as a potential target for antiviral drugs. While gp120 and gp41 are highly immunogenic, the proteins can vary substantially between HIV types, groups, and/or clades. Moreover, the proteins frequently mutate to form antigenic variants and rapidly evolve to evade the host immune response.

[0005] Accordingly, the development of therapeutic monoclonal antibodies that can cross react with antigenic determinants on a large number of HIV types, groups, and clades is currently an area of intense investigation. The broadly neutralizing HIV antibodies, or bnAbs, are described in the

literature and have been collected at the web site "Broadly Neutralizing Antibodies Electronic Resource," www.bnaber.org (Eroshkin AM, *et al.*, *Nucleic Acids Res. 42(1)*:D1133-9 (2014)).

[0006] The epitopes of the bnAbs are found on the heterotrimer HIV envelope spike, composed of gp41 and gp120 and the surrounding glycan layer. A broad range of spike epitopes, both linear and conformational, have been identified, and four epitopes bound by bnAbs have been extensively characterized: the membrane proximal external region (MPER), the CD4 binding site, the variable region 1/variable region 2 (V1/V2) loop and the variable region 3 (V3) loop (Hepler NL. *et al. PLOS Comp. Biol. 10*(9):e1003842 (2014)).

[0007] MPER is located on the gp41 protein in the base of the HIV spike (Montero M. *et al. MMBR 72*(1):54-84 (2008)). BnAbs interacting with the CD4 binding site on gp120 can mimic CD4 binding. For example, the binding of bnAb VCR01 to the CD4 binding site causes a conformational shift that is thought to disable the virus receptor thereby neutralizing the HIV virus (Scheid JF. *et al.*, *Science 333*(6049):1633-7 (2011)). The V1/V2 loop on gp120 has been shown to be one of the most frequent epitopes for potent bnAbs (Moore P.L. *et al.* J. Virol. 69(9):5723-33 (2011)). The V3 loop on gp120 has been targeted by neutralizing antibodies in a quaternary-structure specific manner. The glycan shield can also contribute to the conformation of neutralizing HIV antibody epitopes and make direct contacts with bnAbs; conversely the glycans can inhibit antibody binding through steric hindrance.

[0008] HIV is thought to remain dormant in reservoir cells in various tissues throughout the body due to the immunoprivileged status of certain tissues, such as the central nervous system, the genitourinary tract, and lymphoid organs. (*See*, Iglesias-Ussel *et al.*, *AIDS Rev.*, 13:13-29, 2011). It has been postulated that the gastrointestinal tract (GIT) is a primary target for HIV infection, and a major cellular reservoir due to the abundance of macrophages located at mucosal sites in the GIT. (*See*, Brown *et al.*, *Clin. Vacc. Immunol.*, 21(11):1469-1473, 2014). However, latently infected memory T cells are the largest and best understood reservoir for HIV. Even when treated with Highly Active Antiretroviral Therapy (HAART), the T cell reservoir alone has a remarkably long half-life (Finzi, D., *et al.* 1999. Nature Med 5:512-7) resulting in rapid rebound and virus reemergence upon cessation of therapy. It is believed that infection of some CD4+ T cells can be followed by transition of the infected T cell into a quiescent state and ultimately formation of a memory CD4+ T cell which contains an integrated genomic copy of the viral genome (proviral DNA) which is not expressed until a later time when transcription is triggered. Others believe that

such reservoir cells are not be truly silent, but instead persistently or stochastically produce small amounts of virus. Memory T cells are ideal HIV reservoir cells since they are quiescent and do not undergo cell division, differentiation, or activation and their transcriptional machinery therefor exhibits only minimal activity. However, upon activation, memory T cells can produce large quantities of virus. Targeting of dormant HIV reservoirs is a subject of intensive study and represents the next major hurdle in managing and ultimately eliminating HIV infection. These HIV-infected reservoir cells are most often infected with mutated variants of the originally-infecting HIV virus. (*See*, Picker *et al*., *Nature*, 517:381-385, 2015).

[0009] Thus, chronically infected individuals, although not harboring detectable levels of expressed HIV nonetheless continue to carry HIV and the potential to succumb to HIV infection, or infect others, without further exposure to HIV. There is presently no publically available treatment known to clear chronic HIV infection. Available therapies merely halt further infection by precluding the virus from replicating. Various monoclonal antibodies and combination therapies have been investigated for the purpose of treating HIV, including chronic HIV infection, but none have been commercialized. Therefore, there remains a continuing need to develop new therapies targeting HIV, *e.g.*, reservoir cells harboring dormant HIV in chronic HIV infection; and/or to at least provide the public with a useful choice. Thus, there is a strong need for more potent treatments that are readily available and do not present cost barriers to clinical application and availability; and/or which at least provide the public with a useful choice.

SUMMARY

[009A] In a first aspect, the invention relates to a multimeric binding molecule comprising two or five bivalent binding units and a modified J-chain; wherein each binding unit comprises two antibody heavy chain constant regions, each associated with an antigen binding domain, wherein the antigen binding domains specifically bind to a human immunodeficiency virus (HIV) antigen expressed on the surface of HIV viral particles, on the surface of HIV-infected cells, or a combination thereof, wherein the modified J-chain comprises a J-chain or functional fragment or variant thereof and a heterologous polypeptide comprising a binding domain; and wherein the binding molecule can effect more potent antibody mediated, complement mediated, or T-cell mediated, killing of HIV infected cells than a

reference single binding unit molecule comprising the same antigen binding domains that specifically binds to an HIV antigen, wherein the reference single binding unit molecule is an IgG antibody.

[009B] In a second aspect, the invention relates to a composition comprising:

(a) a polynucleotide comprising a nucleic acid sequence that encodes a human IgM constant region or fragment thereof fused to the C-terminal end of a VH domain comprising:

the VH amino acid sequence SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99;

(b) a polynucleotide comprising a nucleic acid sequence encoding a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL comprising:

the VL amino acid sequence SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100; and

(c) a polynucleotide comprising a nucleic acid sequence encoding a modified J-chain or functional fragment or variant thereof comprising a heterologous polypeptide that comprises a binding domain.

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[009C] In a third aspect, the invention relates to the multimeric binding molecule according to the first aspect for use in preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity, wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG antibody comprising an identical HIV-binding antigen binding domain.

[009D] In a fourth aspect, the invention relates to a method of preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity in a subject in need thereof comprising administering an effective amount of the multimeric binding molecule according to the first aspect, wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG1 antibody comprising an identical HIV-binding antigen binding domain.

[009E] In a fifth aspect, the invention relates to use of the multimeric binding molecule according to the first aspect, in the manufacture of a medicament for preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity in a subject in need thereof, wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG1 antibody comprising an identical HIV-binding antigen binding domain.

BRIEF DESCRIPTION

[0010] Disclosed are various embodiments of multimeric binding molecules that possess specificity for binding one or more HIV antigens, *e.g.*, gp120/gp41 antigens.

[0011] This disclosure includes a multimeric binding molecule that includes at least two bivalent binding units, or variants or fragments thereof; where each binding unit includes at least two antibody heavy chain constant regions or fragments thereof, where each heavy chain constant region or fragment thereof is associated with an antigen binding domain, where at least one antigen binding domain specifically binds to a human immunodeficiency virus (HIV) antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof, and where the binding molecule is more potent in preventing, controlling or treating HIV infection than

a corresponding reference single binding unit molecule including the HIV antigen binding domain. The corresponding reference single binding unit molecule can be, *e.g.*, an IgG antibody.

[0012] In certain embodiments, the at least one antigen binding domain specifically binds to the HIV spike protein, *e.g.*, to an epitope on gp120, gp41, or a combination thereof. In certain embodiments the epitope is situated in the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, the carbohydrates associated with these regions, or a combination thereof.

[0013] In certain embodiments, the described binding molecule is multispecific, *e.g.*, bispecific, including at least two non-identical antigen binding domains. The two non-identical antigen binding domains can specifically bind, without limitation, to different epitopes of a common HIV antigen, to different HIV antigens, or to an HIV antigen and a heterologous antigen.

[0014] In certain embodiments, the described binding molecule is a dimeric binding molecule that includes two bivalent IgA binding units or fragments thereof and a J-chain or fragment thereof or variant thereof. According to these embodiments, each binding unit can include two IgA heavy chain constant regions or fragments thereof each associated with an antigen binding domain. In certain embodiments a dimeric binding molecule as described herein can further include an associated secretory component, or fragment or variant thereof. A dimeric binding molecule as described herein can include the Cα2 domain and/or the Cα3-tp domain if the IgA constant region, and can in some embodiments further include the Cα1 domain. In certain embodiments the IgA heavy chain constant region is a human IgA heavy chain constant region. An IgA-based dimeric binding molecule as described herein can include, in some embodiments, two IgA heavy chains each including a VH situated amino terminal to the IgA constant region or fragment thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region.

[0015] In certain embodiments the described binding molecule is a pentameric or a hexameric binding molecule including five or six bivalent IgM binding units, respectively, where each binding unit includes two IgA heavy chain constant regions or fragments thereof each associated with an antigen binding domain. In certain embodiments the IgM heavy chain constant regions or fragments thereof can each include a Cμ3 domain and a Cμ4-tp domain and can in some embodiments, further include a Cμ2 domain, a Cμ1 domain, or any combination thereof. Where the described binding molecule is pentameric, it can further include a J-chain, or fragment thereof,

or variant thereof. In certain embodiments, the IgM heavy chain constant region is a human IgM constant region.

[0016] In certain embodiments, the disclosure provides a bi-or multispecific dimeric or pentameric binding molecule that includes a J-chain. The J-chain can be a modified J-chain including, *e.g.*, a binding domain. In certain embodiments the modified J-chain is derived from a human J-chain, and can include the amino acid sequence 23 to 159 of SEQ ID NO: 2, or a functional fragment thereof. In certain embodiments the binding domain is a polypeptide sequence fused in frame with the J-chain or fragment thereof, either with or without a peptide linker. In certain embodiments the peptide linker can include at least 5 amino acids, but no more than 25 amino acids. In certain embodiments the peptide can consist of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, or SEQ ID NO: 104. In certain embodiments the modified J-chain can include the formula $X[L_n]J$ or $J[L_n]X$, where J includes a mature native J-chain or functional fragment thereof, X includes a heterologous binding domain, and $[L_n]$ is a linker sequence consisting of n amino acids, where n is a positive integer from 1 to 100, 1 to 50, or 1 to 25. In certain embodiments N is 5, 10, 15, or 20.

[0017] In certain embodiments the binding domain is situated at the C-terminus of the J-chain or fragment thereof. In certain embodiments the binding domain is situated at the N-terminus of the J-chain or fragment thereof. In certain embodiments the binding domain is inserted within the J-chain or fragment thereof. In certain embodiments the binding domain of the modified J-chain is an antibody or antigen binding fragment thereof, *e.g.*, an $F(ab')_2$, an $F(ab)_2$, an Fab', an Fab, an Fv, an scFv, or a single domain antibody, *e.g.*, a VHH. In certain embodiments, the binding domain of the modified J-chain binds to one or more effector cells, *e.g.*, T-cells, natural killer (NK) cells, macrophages and/or neutrophils. Where the effector cell is a T-cell, the binding domain can bind, *e.g.*, to CD3 or CD8 on the T-cell. Where the effector cell is an NK cells, the binding domain can bind, *e.g.*, to one or more of CD16, CD64, and/or NKG2D on the NK cell. Where the effector cell is a macrophage, the binding domain can bind to, *e.g.*, CD14 on the macrophage. Where the effector cell is a neutrophil, the binding domain can bind to, *e.g.*, CD16b and/or CD177 on the neutrophil. In certain embodiments, the heterologous polypeptide includes SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, or a combination thereof. In certain embodiments the modified J-chain includes SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or a combination thereof. In certain embodiments the modified J-chain can further include a signal peptide.

[0018] In certain embodiments, each binding unit of a binding molecule as described herein can include two heavy chains each including a VH situated amino terminal to the constant region or fragment thereof, and two immunoglobulin light chains each including a VL situated amino terminal to an immunoglobulin light chain constant region. In certain embodiments, at least one binding unit includes two antigen binding domains that specifically bind to an HIV antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof. In some embodiments the two heavy chains within the binding unit can be identical. In some embodiments the two light chains within the binding unit are identical. The two light chain constant regions can be, *e.g.*, human lambda constant regions or human kappa constant regions.

[0019] In certain embodiments the binding molecule as described herein can include at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or twelve antigen binding domains that specifically bind to an HIV antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof. In certain embodiments at least two, at least three, at least four, at least five, or at least six of the binding units are identical. In certain embodiments at least one antigen binding domain of the binding molecule as described herein can specifically bind to an HIV spike protein expressed or presented on the surface of HIV-infected reservoir cells, *e.g.*, a cell in which HIV antigens are expressed at a low level compared to HIV-infected cells.

[0020] In certain embodiments at least one antigen binding domain of the binding molecule as described herein includes an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), where the VH and VL can include the HCDR1, HCDR2, and HCDR3 regions, or HCDR1, HCDR2, and HCDR3 regions containing one or two single amino acid substitutions, and the LCDR1, LCDR2, and LCDR3 regions, or LCDR1, LCDR2, and LCDR3 containing one or two single amino acid substitutions, of the VH and VL amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ

ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 6, SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 81 and SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86, SEQ ID NO: 87 and SEQ ID NO: 88, SEQ ID NO: 89 and SEQ ID NO: 90, SEQ ID NO: 91 and SEQ ID NO: 92, SEQ ID NO: 93 and SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96, SEQ ID NO: 97 and SEQ ID NO: 98, or SEQ ID NO: 99 and SEQ ID NO: 100, respectively.

[0021] In certain embodiments, at least one antigen binding domain of the binding molecule as described herein includes an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), where the VH and VL include, respectively, amino acid sequences that are at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 6, SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 81 and

SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86, SEQ ID NO: 87 and SEQ ID NO: 88, SEQ ID NO: 89 and SEQ ID NO: 90, SEQ ID NO: 91 and SEQ ID NO: 92, SEQ ID NO: 93 and SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96, SEQ ID NO: 97 and SEQ ID NO: 98, or SEQ ID NO: 99 and SEQ ID NO: 100.

[0022] In certain embodiments the binding molecule as described herein is more potent in preventing, controlling or treating HIV infection, enhancing viral clearance, controlling HIV infectivity, and/or controlling HIV growth than a corresponding reference single binding unit molecule including the HIV-binding antigen binding domain. In certain embodiments the binding molecule can be more potent in neutralizing HIV, can bind to and neutralize more diverse HIV variants or clades, or a combination thereof, than a corresponding reference single binding unit molecule that includes the HIV-binding antigen binding domain. In certain embodiments the binding molecule can effect more potent antibody mediated, complement mediated, or cell mediated, *e.g.*, T-cell mediated, killing of HIV infected cells than a corresponding reference single binding unit molecule that includes the HIV-binding antigen binding domain. In certain embodiments the binding molecule can provide equivalent benefit at a lower dosage than a corresponding reference single binding unit molecule that includes the HIV-binding antigen binding domain.

[0023] The disclosure further includes an isolated IgM antibody or fragment thereof that includes a J-chain, or functional fragment or variant thereof, and five binding units, each including two heavy chains and two light chains, where each heavy chain or fragment thereof includes a human Mu constant region or fragment thereof, and the heavy chain variable region amino acid sequence SEQ ID NO: 7, and where each light chain includes a human kappa constant region and the light chain variable region amino acid sequence SEQ ID NO: 8; where the antibody or fragment thereof can assemble into a pentameric IgM antibody that can specifically bind to the CD4 binding site of the HIV spike glycoprotein. In certain embodiments the IgM antibody or fragment thereof can include the heavy chain amino acid sequence SEQ ID NO: 113 and the light chain amino acid sequence SEQ ID NO: 114. In certain embodiments the J-chain can include amino acids 23 to 159 of the amino acid sequence SEQ ID NO: 2 or a functional fragment thereof. In certain embodiments the J-chain or fragment thereof can be a modified J-chain that further includes a heterologous polypeptide that can be directly or indirectly fused to the J-chain. In certain embodiments the heterologous polypeptide can be fused to the J-chain or fragment thereof via a peptide linker, *e.g.*, a

peptide linker that includes at least 5 amino acids, but no more than 25 amino acids, *e.g.*, a peptide linker consisting of of SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, or SEQ ID NO: 104. In certain embodiments the heterologous polypeptide can be fused to the N-terminus of the J-chain or fragment thereof, the C-terminus of the J-chain or fragment thereof, or to both the N-terminus and C-terminus of the J-chain or fragment thereof. In certain embodiments the heterologous polypeptide can include a binding domain, *e.g.*, the heterologous polypeptide can be an antibody or antigen binding fragment thereof, *e.g.*, a scFv fragment, *e.g.*, a scFv fragment that can specifically bind to CD3. In certain embodiments the modified J-chain can include a heterologous polypeptide that includes the amino acid sequence SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, or SEQ ID NO: 111. In certain embodiments the modified J-chain can further include a signal peptide.

[0024] The disclosure further includes a composition that includes the binding molecule or the IgM antibody described herein.

[0025] In addition, the disclosure includes a polynucleotide that includes a nucleic acid sequence encoding a polypeptide subunit of the binding molecule as described herein, where the polypeptide subunit includes the IgM heavy chain constant region and at least the antibody VH portion of an antibody binding domain that specifically binds to an HIV spike protein antigen expressed on the surface of viral particles, on the surface if HIV-infected cells, or a combination thereof. In certain embodiments the polypeptide subunit can include a human IgM constant region or fragment thereof fused to the C-terminal end of a VH domain that includes: the HCDR1, HCDR2, and HCDR3 domains, or the HCDR1, HCDR2, and HCDR3 domains containing one or two single amino acid substitutions in one or more HCDRs, of the VH amino acid sequence SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99; or an amino acid sequence at least 80%, at least 85%, at least 90%, at

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least 95% or 100% identical to the SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99.

[0026] In addition, the disclosure includes a polynucleotide that includes a nucleic acid sequence encoding a polypeptide subunit of the binding molecule as described herein, where the polypeptide subunit includes the antibody VL portion of an antibody binding domain that specifically binds to an HIV spike protein antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof. In certain embodiments, the polypeptide subunit includes a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL that includes: the LCDR1, LCDR2, and LCDR3 domains, or the LCDR1, LCDR2, and LCDR3 domains containing one or two single amino acid substitutions in one or more LCDRs, of the VL amino acid sequence SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100; or an amino acid sequence at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID

NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100.

[0027] The disclosure further includes a composition that includes VH and VL-containing polynucleotide as described herein. In certain embodiments the polynucleotides are situated on the same vector. In certain embodiments the polynucleotides are situated on separate vectors. In certain embodiments the composition further includes a polynucleotide that includes a nucleic acid sequence encoding a J-chain, a modified J-chain, fragment thereof, or a variant thereof. In certain embodiments, the polynucleotides are situated on at least two separate vectors. In certain embodiments, the polynucleotides are situated on the same vector. The disclosure further includes the vector or vectors as described, a host cell that includes the described polynucleotide or the described polynucleotide composition, or the described vector or vectors. The disclosure further describes a method of producing the binding molecule described herein, where the method includes culturing the described host cell, and recovering the binding molecule.

[0028] The disclosure further describes a method of preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity, where the method includes contacting a mixture of HIV and HIV-susceptible cells with the binding molecule described herein; where the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding reference single binding unit molecule including the HIV-binding antigen binding domain. In certain embodiments, the binding molecule exhibits increased potency in neutralizing HIV in infected cells, as compared with the single binding unit molecule.

[0029] The disclosure further describes a method of treating an human immunodeficiency virus (HIV) infection in a patient, including administering to a patient infected with HIV the binding molecule as described herein; where the binding molecule is stronger, more potent in preventing, controlling or treating HIV infection or controlling HIV infectivity, or requires a lower binding molecule dose than a corresponding reference single binding unit molecule including the HIV-binding antigen binding domain. According to this method, the binding molecule can exhibit increased potency in (i) reducing the infectivity of an HIV virion, (ii) reducing the number of HIV-

infected cells, (iii) preventing HIV infection, (iv) enhancing viral clearance, (v) improving the signs and symptoms of HIV infection, or (vi) any combination thereof, as compared with the corresponding reference single binding unit molecule. In certain embodiments, the corresponding reference single binding unit molecule is an IgG antibody.

BRIEF DESCRIPTION OF THE FIGURES

[0030] **FIGURE 1**: Illustration of various antibody binding sites on the HIV spike glycoprotein (Modified from Modified from Klein F., *et al. Science 341*:1199-1204 (2013)).

[0031] **FIGURE 2A**: Expression and assembly of the HIV02 antibodies, as measured by non-reducing SDS native-PAGE.

[0032] **FIGURE 2B**: Assembly of HIV12, HIV32 and HIV72 IgM+J proteins. Proteins were electrophoresed in non-reducing SDS native-PAGE, transferred to membrane and probed with anti-J antibody. Lane 1, reference IgM+J; lane 2, HIV02; lane 3, HIV32; lane 4, HIV72.

[0033] **FIGURE 2C**: Assembly of HIV72 IgG, IgM+J and the IgM+V5J bispecific. Lane 1, Native markers, lane 2, HIV72 IgG; lane 3, HIV72 IgM; lane 4, HIV72 IgM+J; lane 5 HIV72 IgM+V5J. The left side of the figure shows Coomassie staining of the gel, and the right side shows an anti-J chain western blot.

[0034] **FIGURE 3A-F**: Effect of antigen coating concentration on the binding of HIV02 IgG and HIV02 IgM+J to gp120 by ELISA. **FIG. 3A**: 1 μg/ml antigen coating; **FIG. 3B**: 0.8 μg/ml antigen coating; **FIG. 3C**: 0.6 μg/ml antigen coating; **FIG. 3D**: 0.4 μg/ml antigen coating; **FIG. 3E**: 0.2 μg/ml antigen coating; **FIG. 3F**: 0.1 μg/ml antigen coating.

[0035] **FIGURE 4**: Comparison of HIV02 IgG and HIV02 IgM+J binding to gp120 by ELISA. The IgM+J molecule is 20x better than the IgG molecule at low antigen densities.

[0036] **FIGURE 5A-B**: HIV02 IgG (**FIG. 5A**) and IgM+J (**FIG. 5B**) binding to gp140-expressing CHO-PI cells.

[0037] **FIGURE 6**: Neutralization of HIV isolates from multiple clades by HIV02 IgM+J.

[0038] **FIGURE 7**: Antigen-dependent T-cell activation by HIV02 IgM+V10J.

[0039] **FIGURE 8**: PAGE analysis of HIV02+V10J HPLC-SEC column fractions.

[0040] **FIGURE 9**: Antigen-dependent T-cell activation by SEC-purified HIV02 IgM+V10J.

DETAILED DESCRIPTION

Definitions

[0041] The term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0042] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0042A] The term “comprising” as used in this specification and claims means “consisting at least in part of”. When interpreting statements in this specification, and claims which include the term “comprising”, it is to be understood that other features that are additional to the features prefaced by this term in each statement or claim may also be present. Related terms such as “comprise” and “comprised” are to be interpreted in similar manner.

[0043] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0044] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various embodiments or embodiments of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0045] As used herein, the term "non-naturally occurring" substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being "naturally-occurring," or that are, or might be at any time, determined or interpreted by a judge or an administrative or judicial body to be, "naturally-occurring."

[0046] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids are included within the definition of "polypeptide," and the term "polypeptide" can be used instead of, or interchangeably with any of these terms. The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, and derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide can be derived from a biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It can be generated in any manner, including by chemical synthesis.

[0047] A polypeptide as disclosed herein can be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides can have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides that do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a *protein* coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid, *e.g.*, a serine or an asparagine.

[0048] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated as disclosed herein, as are native or recombinant polypeptides that have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0049] As used herein, the term “non-naturally occurring” polypeptide, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the polypeptide that are well-understood by persons of ordinary skill in the art as being “naturally-occurring,” or that are, or might be at any time, determined or interpreted by a judge or an administrative or judicial body to be, “naturally-occurring.”

[0050] Other polypeptides disclosed herein are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" as disclosed herein include any polypeptides that retain at least some of the properties of the corresponding native antibody or polypeptide, for example, specifically binding to an antigen. Fragments of polypeptides include, for example, proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of, *e.g.*, a polypeptide include fragments as described above, and also polypeptides with altered amino acid sequences due to amino acid substitutions, deletions, or insertions. In certain embodiments, variants can be non-naturally occurring. Non-naturally occurring variants can be produced using art-known mutagenesis techniques. Variant polypeptides can comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives are polypeptides that have been altered so as to exhibit additional features not found on the original polypeptide. Examples include fusion proteins. Variant polypeptides can also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a polypeptide can also refer to a subject polypeptide having one or more amino acids chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides that contain one or more derivatives of the twenty standard amino acids. For example, 4-hydroxyproline can be substituted for proline; 5-hydroxylysine can be substituted for lysine; 3-methylhistidine can be substituted for histidine; homoserine can be substituted for serine; and ornithine can be substituted for lysine.

[0051] A "conservative amino acid substitution" is one in which one amino acid is replaced with another amino acid having a similar side chain. Families of amino acids having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. In certain embodiments, conservative substitutions in the sequences of the polypeptides and antibodies of the present disclosure do not abrogate the binding of the polypeptide or antibody containing the amino acid sequence, to the antigen to which the binding molecule binds. Methods of identifying nucleotide and amino acid conservative substitutions that do not eliminate antigen binding are well-known in the art (see, *e.g.*, Brummell *et al.*, *Biochem.* 32:1180-1 187 (1993); Kobayashi *et al.*, *Protein Eng.* 12(10):879-884 (1999); and Burks *et al.*, *Proc. Natl. Acad. Sci. USA* 94:.412-417 (1997)).

[0052] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA), cDNA, or plasmid DNA (pDNA). A polynucleotide can comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The terms "nucleic acid" or “nucleic acid sequence” refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide.

[0053] By an "isolated" nucleic acid or polynucleotide is intended any form of the nucleic acid or polynucleotide that is separated from its native environment. For example, gel-purified polynucleotide, or a recombinant polynucleotide encoding a polypeptide contained in a vector would be considered to be “isolated.” Also, a polynucleotide segment, *e.g.*, a PCR product, that has been engineered to have restriction sites for cloning is considered to be “isolated.” Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in a non-native solution such as a buffer or saline. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides, where the transcript is not one that would be found in nature. Isolated polynucleotides or nucleic acids further include such molecules produced synthetically. In

addition, polynucleotide or a nucleic acid can be or can include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0054] As used herein, a "non-naturally occurring" polynucleotide, or any grammatical variants thereof, is a conditional definition that explicitly excludes, but only excludes, those forms of the polynucleotide that are well-understood by persons of ordinary skill in the art as being "naturally-occurring," or that are, or that might be at any time, determined or interpreted by a judge or an administrative or judicial body to be, "naturally-occurring."

[0055] As used herein, a "coding region" is a portion of nucleic acid that consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it can be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector can contain a single coding region, or can comprise two or more coding regions, *e.g.*, a single vector can separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid can include heterologous coding regions, either fused or unfused to another coding region. Heterologous coding regions include without limitation, those encoding specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0056] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid that encodes a polypeptide normally can include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a

promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter can be a cell-specific promoter that directs substantial transcription of the DNA in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably associated with the polynucleotide to direct cell-specific transcription.

[0057] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions that function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0058] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0059] In other embodiments, a polynucleotide can be RNA, for example, in the form of messenger RNA (mRNA), transfer RNA, or ribosomal RNA.

[0060] Polynucleotide and nucleic acid coding regions can be associated with additional coding regions that encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide as disclosed herein. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence that is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells can have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains

the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, can be used. For example, the wild-type leader sequence can be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0061] Disclosed herein are certain binding molecules, or antigen binding fragments, variants, or derivatives thereof. Unless specifically referring to full-sized antibodies, the term "binding molecule" encompasses full-sized antibodies as well as antigen binding subunits, fragments, variants, analogs, or derivatives of such antibodies, *e.g.*, engineered antibody molecules or fragments that bind antigen in a manner similar to antibody molecules, but which use a different scaffold.

[0062] As used herein, the term “binding molecule” refers in its broadest sense to a molecule that specifically binds to a receptor, *e.g.*, an epitope or an antigenic determinant. As described further herein, a binding molecule can comprise one or more “antigen binding domains” described herein. A non-limiting example of a binding molecule is an antibody or fragment thereof that retains antigen-specific binding.

[0063] The terms “binding domain” and “antigen binding domain” are used interchangeably herein and refer to a region of a binding molecule that is necessary and sufficient to specifically bind to an epitope. For example, an “Fv,” *e.g.*, a variable heavy chain and variable light chain of an antibody, either as two separate polypeptide subunits or as a single chain, is considered to be a “binding domain.”

[0064] Other antigen binding domains include, without limitation, the variable heavy chain (VHH) of an antibody derived from a camelid species, or six immunoglobulin complementarity determining regions (CDRs) expressed in a fibronectin scaffold. A “binding molecule” as described herein can include one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve or more “antigen binding domains.”

[0065] The terms "antibody" and "immunoglobulin" can be used interchangeably herein. An antibody (or a fragment, variant, or derivative thereof as disclosed herein) includes at least the variable region of a heavy chain (for camelid species) or at least the variable regions of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988). Unless otherwise stated, the term “antibody” encompasses

anything ranging from a small antigen binding fragment of an antibody to a full sized antibody, *e.g.*, an IgG antibody that includes two complete heavy chains and two complete light chains, an IgA antibody that includes four complete heavy chains and four complete light chains and can include a J-chain and/or a secretory component, or an IgM antibody that includes ten or twelve complete heavy chains and ten or twelve complete light chains and can include a J-chain.

[0066] As will be discussed in more detail below, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4 or α1-α2)). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, IgA_2, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of this disclosure.

[0067] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class can be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. The basic structure of certain antibodies, *e.g.*, IgG antibodies, includes two heavy chain subunits and two light chain subunits covalently connected via disulfide bonds to form a "Y" structure, also referred to herein as an "H2L2" structure, or a "binding unit."

[0068] The term "binding unit" is used herein to refer to the portion of a binding molecule, *e.g.*, an antibody or antigen binding fragment thereof that corresponds to a standard immunoglobulin structure, *i.e.*, two heavy chains or fragments thereof and two light chains or fragments thereof, or two heavy chains or fragments thereof derived, *e.g.*, from a camelid or condricthoid antibody. In certain embodiments, *e.g.*, where the binding molecule is a bivalent, single binding unit IgG antibody or antigen binding fragment thereof, the terms "binding molecule" and "binding unit" are equivalent. In other embodiments, *e.g.*, where the binding molecule is an IgA dimer, an IgM pentamer, or an IgM hexamer, the binding molecule is "multimeric" and comprises two or more

"binding units." Two in the case of an IgA dimer, or five or six in the case of an IgM pentamer or hexamer, respectively. A binding unit need not include full-length antibody heavy and light chains, but will typically be bivalent, *i.e.*, will include two "antigen binding domains," as defined below. Certain binding molecules described in this disclosure are pentameric or hexameric, and include five or six bivalent binding units that include IgM constant regions or fragments thereof.

[0069] As used herein, a binding molecule comprising two or more binding units, *e.g.*, two, five, or six binding units, can be referred to as "multimeric." The term "multimeric" means possessing more than one unit. Thus, for example, a "multimeric binding molecule" will possess more than one binding unit. A multimeric binding molecule can possess two, three four, five or even six or more binding units. A "dimeric binding molecule" includes two binding units and is typically a dimeric IgA molecule that further comprises a J-chain. A "pentameric binding molecule" is typically a pentameric IgM binding molecule that further comprises a J-chain. A "hexameric binding molecule" is typically a hexameric IgM binding molecule. In contrast, a "single binding unit molecule" can be, *e.g.*, an IgG antibody.

[0070] The terms "wild-type (WT) J-chain," "native sequence J-chain" or "native J-chain" as used herein refer to a J-chain of native sequence IgM or IgA antibodies of any animal species, including mature human J-chain, the amino acid sequence of which is presented as SEQ ID NO: 2.

[0071] The term "modified J-chain" is used herein to refer to variants of a native J- chain polypeptide. A modified J-chain can be a full-length mature J-chain polypeptide or a functional fragment thereof, and can include, without limitation, amino acid insertions, deletions, substitutions, non-amino acid modifications such as glycosylation or lipidation. Moreover, a modified J-chain can include a heterologous moiety such as a binding moiety, either introduced into the J-chain amino acid sequence as a fusion protein, or attached or conjugated by other techniques, such as disulfide bonding, or chemical conjugation. A modified J-chain is typically functional, in that the modifications do not interfere with efficient polymerization of IgM or IgA and binding of such polymers to a target. Exemplary modified J-chains are described elsewhere herein and in PCT Publication No. WO 2015/153912, which is incorporated herein by reference in its entirety. The term "modified human J-chain" encompasses, without limitation, a native sequence human J-chain of the amino acid sequence of SEQ ID NO: 2 or functional fragment thereof modified as above, *e.g.*, by the introduction of a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an additional desired binding domain.

[0072] The terms "valency," "bivalent," "multivalent" and grammatical equivalents, refer to the number of antigen binding domains in given binding molecule or binding unit. For example, the terms "bivalent", "tetravalent", and "hexavalent" in reference to a given binding molecule, *e.g.*, an IgM antibody or fragment thereof, denote the presence of two antigen binding domains, four antigen binding domains, and six antigen binding domains, respectively. In a typical IgM-derived binding molecule, each binding unit is bivalent, whereas the binding molecule itself can have 10 or 12 valencies. A bivalent or multivalent binding molecule can be monospecific, *i.e.*, all of the antigen binding domains are the same, or can be bispecific or multispecific, *e.g.*, where two or more antigen binding domains are different, *e.g.*, bind to different epitopes on the same antigen, or bind to entirely different antigens.

[0073] The term "epitope" includes any molecular determinant capable of specific binding to an antibody. In certain embodiments, an epitope can include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, can have three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antibody.

[0074] "Multispecific binding molecules or antibodies" or "bispecific binding molecules or antibodies" refer to binding molecules, antibodies, or antigen binding fragments thereof that have the ability to specifically bind to two or more different epitopes on the same or different target(s). "Monospecific" refers to the ability to bind only one epitope.

[0075] The term "target" is used in the broadest sense to include substances that can be bound by a binding molecule. A target can be, *e.g.*, a polypeptide, a nucleic acid, a carbohydrate, a lipid, or other molecule. Moreover, a "target" can, for example, be a cell, an organ, or an organism that comprises an epitope bound that can be bound by a binding molecule.

[0076] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable regions (which can be called "variable domains" interchangeably herein) of both the variable light (VL) and variable heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (*e.g.*, CH1, CH2 or CH3) confer biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or

amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 (or CH4 in the case of IgM) and CL domains are at the carboxy-terminus of the heavy and light chain, respectively.

[0077] A "full length IgM antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable region (VH), an antibody constant heavy chain constant domain 1 (CM1 or Cμ1), an antibody heavy chain constant domain 2 (CM2 or Cμ2), an antibody heavy chain constant domain 3 (CM3 or Cμ3), and an antibody heavy chain constant domain 4 (CM4 or Cμ4), which can also include a tailpiece.

[0078] A "full length IgA antibody heavy chain" is a polypeptide that includes, in N-terminal to C-terminal direction, an antibody heavy chain variable region (V_H), an antibody constant heavy chain constant domain 1 (CA1 or Cα1), an antibody heavy chain constant domain 2 (CA2 or Cα2), an antibody heavy chain constant domain 3 (CA3 or Cα3), and a tailpiece and can be either an IgA1 or IgA2. The structure of monomeric, dimeric (J-chain-containing) and secretory IgA is described, *e.g.*, in Woof, JM and Russell, MW, *Mucosal Immunology 4*:590-597 (2011).

[0079] Both IgA and IgM possess an 18-amino acid extension in the C terminus called the "tail-piece" (tp). The IgM (μtp) and IgA (αtp) tail-pieces differ at seven amino acid positions. The IgM and IgA tail-piece is highly conserved among various animal species. The conserved penultimate cysteine residue in the IgA and IgM tail-pieces has been demonstrated to be involved in polymerization. Both tail-pieces contain an N-linked carbohydrate addition site, the presence of which is required for dimer formation in IgA and J-chain incorporation and pentamer formation in IgM. However, the structure and composition of the N-linked carbohydrates in the tail-pieces differ, suggesting differences in the accessibility of the glycans to processing by glycosyltransferases.

[0080] As indicated above, a variable region, *i.e.*, the "antigen binding domain," allows the binding molecule to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of a binding molecule, *e.g.*, an antibody combine to form the variable region that defines a three dimensional antigen binding site. More specifically, the antigen binding site is defined by three CDRs on each of the VH and VL chains. Certain antibodies form larger structures. For example, IgA can form a molecule that includes two H2L2 units and a J-chain, all covalently connected via disulfide bonds, and IgM can form a pentameric or hexameric molecule that includes five or six

H2L2 units and, in some embodiments, a J-chain covalently connected via disulfide bonds. In certain embodiments, polymeric IgA and IgM molecules can also contain a secretory component that can also be covalently connected via disulfide bonds. Further, it is known that both IgA and pentameric IgM bind to the polymeric immunoglobulin receptor (pIgR) and are secreted after binding. (*See*, Mostov K. E., *Ann. Rev. Immunol.*, 12:63-84, 1994, page 65).

[0081] The six "complementarity determining regions" or "CDRs" present in an antibody antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domain, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops that connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids that make up the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been defined in various different ways (*see*, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, *196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0082] In the case where there are two or more definitions of a term that is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described, for example, by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference. The Kabat and Chothia definitions include overlapping or subsets of amino acids when compared against each other. Nevertheless, application of either definition (or other definitions known to those of ordinary skill in the art) to refer to a

CDR of an antibody or variant thereof is intended to be within the scope of the term as defined and used herein, unless otherwise indicated. The appropriate amino acids that encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison. The exact amino acid numbers that encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine that amino acids comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1: CDR Definitions*

	Kabat	**Chothia**
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

*Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0083] Immunoglobulin variable domains can also be analyzed, *e.g.*, using the IMGT information system (www://imgt.cines.fr/) (IMGT®/V-Quest) to identify variable region segments, including CDRs. (*See, e.g.*, Brochet *et al.*, *Nucl. Acids Res.*, 36:W503-508, 2008).

[0084] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless use of the Kabat numbering system is explicitly noted, however, consecutive numbering is used for all amino acid sequences in this disclosure.

[0085] Binding molecules, *e.g.*, antibodies or antigen binding fragments, variants, or derivatives thereof include, but are not limited to, polyclonal, monoclonal, human, humanized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), single domain antibodies such as camelid VHH antibodies, fragments comprising either a VL or VH domain, fragments produced by a Fab expression library. ScFv molecules are known in the art and are

described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules encompassed by this disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. Also contemplated are immunoglobulin new antigen receptor (IgNAR) isotypes that are bivalent and comprise a single chain that includes an IgNAR variable domain (VNAR). (*See*, Walsh *et al.*, *Virology* 411:132-141, 2011).

[0086] By "specifically binds," it is generally meant that a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof binds to an epitope via its antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, a binding molecule is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain binding molecule binds to a certain epitope. For example, binding molecule "A" can be deemed to have a higher specificity for a given epitope than binding molecule "B," or binding molecule "A" can be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0087] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof disclosed herein can be said to bind a target antigen with an off rate (k(off)) of less than or equal to 5×10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5×10^{-3} sec^{-1}, 10^{-3} sec^{-1}, 5×10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5×10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5×10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5×10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0088] A binding molecule, *e.g.*, an antibody or antigen binding fragment, variant, or derivative disclosed herein can be said to bind a target antigen with an on rate (k(on)) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5×10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1}, 5×10^4 M^{-1} sec^{-1}, 10^5 M^{-1} sec^{-1}, 5×10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5×10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}.

[0089] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof is said to competitively inhibit binding of a reference antibody or antigen binding fragment to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody or antigen binding fragment to the epitope. Competitive inhibition can be determined by any method known in the art, for example, competition ELISA assays. A binding molecule can be said to competitively inhibit binding of the reference antibody or antigen binding

fragment to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0090] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with one or more antigen binding domains, *e.g.*, of an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of antigen binding domains and an antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual antigen binding domains in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity. An interaction between a between a bivalent monoclonal antibody with a receptor present at a high density on a cell surface would also be of high avidity.

[0091] Binding molecules or antigen binding fragments, variants or derivatives thereof as disclosed herein can also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of `a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, a binding molecule is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, can actually fit better than the original.

[0092] A binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can also be described or specified in terms of its binding affinity to an antigen. For example, a binding molecule can bind to an antigen with a dissociation constant or K_D no greater than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0093] Antibody fragments including single-chain antibodies or other antigen binding domains can exist alone or in combination with one or more of the following: hinge region, CH1, CH2, CH3, or CH4 domains, J-chain, or secretory component. Also included are antigen binding fragments that can include any combination of variable region(s) with one or more of a hinge region, CH1, CH2,

CH3, or CH4 domains, a J-chain, or a secretory component. Binding molecules, *e.g.*, antibodies, or antigen binding fragments thereof can be from any animal origin including birds and mammals. The antibodies can be human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region can be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and can in some instances express endogenous immunoglobulins and some not, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.*

[0094] As used herein, the term “heavy chain subunit” or “heavy chain domain” includes amino acid sequences derived from an immunoglobulin heavy chain, a binding molecule, *e.g.*, an antibody comprising a heavy chain subunit can include at least one of: a VH domain, a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, a CH4 domain, or a variant or fragment thereof. For example, a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH1 domain; CH1 domain, a hinge, and a CH2 domain; a CH1 domain and a CH3 domain; a CH1 domain, a hinge, and a CH3 domain; or a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain. In certain embodiments a binding molecule, *e.g.*, an antibody or fragment, variant, or derivative thereof can include, in addition to a VH domain, a CH3 domain and a CH4 domain; or a CH3 domain, a CH4 domain, and a J-chain. Further, a binding molecule for use in the disclosure can lack certain constant region portions, *e.g.*, all or part of a CH2 domain. It will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain subunit) can be modified such that they vary in amino acid sequence from the original immunoglobulin molecule.

[0095] The heavy chain subunits of a binding molecule, *e.g.*, an antibody or fragment thereof, can include domains derived from different immunoglobulin molecules. For example, a heavy chain subunit of a polypeptide can include a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain subunit can include a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain subunit can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0096] As used herein, the term "light chain subunit" or "light chain domain" includes amino acid sequences derived from an immunoglobulin light chain. The light chain subunit includes at least one of a VL or CL (*e.g.*, Cκ or Cλ) domain.

[0097] Binding molecules, *e.g.*, antibodies or antigen binding fragments, variants, or derivatives thereof can be described or specified in terms of the epitope(s) or portion(s) of an antigen that they recognize or specifically bind. The portion of a target antigen that specifically interacts with the antigen binding domain of an antibody is an "epitope," or an "antigenic determinant." A target antigen can comprise a single epitope or at least two epitopes, and can include any number of epitopes, depending on the size, conformation, and type of antigen.

[0100] As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH region" or "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain, extending, *e.g.*, from about amino acid 114 to about amino acid 223 of an IgG antibody using conventional numbering schemes (amino acids 114 to 223, Kabat numbering system; and amino acids 118-215, EU numbering system; see Kabat EA *et al.*, *op. cit*). The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of a typical IgG heavy chain molecule.

[0101] As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, *e.g.*, from about amino acid 244 to amino acid 360 of an IgG antibody using conventional numbering schemes (amino acids 244 to 360, Kabat numbering system; and amino acids 231-340, EU numbering system; see Kabat EA *et al.*, *op. cit*). The CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 amino acids. Certain immunoglobulin classes, *e.g.*, IgM, further include a CH4 region.

[0102] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain in IgG, IgA, and IgD heavy chains. This hinge region comprises approximately 25 amino acids and is flexible, thus allowing the two N-terminal antigen binding regions to move independently.

[0103] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In certain IgG molecules, the CH1 and CL regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0104] As used herein, the term "chimeric antibody" refers to an antibody in which the immunoreactive region or site is obtained or derived from a first species and the constant region (which can be intact, partial or modified) is obtained from a second species. In some embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0105] The terms "multispecific antibody, or "bispecific antibody" refer to an antibody that has antigen binding domains that are specific for two or more different epitopes within a single antibody molecule (or "binding unit"). Other binding molecules in addition to the canonical antibody structure can be constructed with two different binding specificities. Epitope binding by bispecific or multispecific antibodies can be simultaneous or sequential. Triomas and hybrid hybridomas are two examples of cell lines that can secrete bispecific antibodies. Bispecific antibodies can also be constructed by recombinant means. (Ströhlein and Heiss, *Future Oncol. 6*:1387-94 (2010); Mabry and Snavely, *IDrugs. 13*:543-9 (2010)). A bispecific antibody can also be a diabody. Thus, a bispecific binding molecule that is multimeric could potentially possess several different antigen binding domains, each with a different specificity. For instance, an IgM binding molecule would be considered multimeric, containing five or six binding units, and each binding unit possessing possibly two antigen binding domains. Such an IgM binding molecule could therefore have as many as two, three, four, five, six, seven, eight, nine, ten, eleven, or even twelve different specificities, since each antigen binding domain can bind a different, distinguishable epitope. In certain embodiments each binding unit is a monospecific H2L2 structure. Bispecific and multi-specific IgM and IgA binding molecules, including antibodies, are described, for example, in PCT Publication No. WO 2015/053887 and PCT Publication No. WO 2015/120474, the entire contents of

which are hereby expressly incorporated by reference. In certain embodiments a heterologous binding domain can be associated with a J-chain, as described in PCT Publication No. WO 2015/153912, and elsewhere herein.

[0106] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more amino acids in either the CDR or framework regions. In certain embodiments entire CDRs from an antibody of known specificity can be grafted into the framework regions of a heterologous antibody. Although alternate CDRs can be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, CDRs can also be derived from an antibody of different class, *e.g.*, from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity are grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." In certain embodiments not all of the CDRs are replaced with the complete CDRs from the donor variable region and yet the antigen binding capacity of the donor can still be transferred to the recipient variable domains. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0107] As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

[0108] As used herein, the terms "linked," "fused" or "fusion" or other grammatical equivalents can be used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the translational reading frame of the original

ORFs. Thus, a recombinant fusion protein is a single protein containing two or more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments can be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region can be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

[0109] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which amino acids that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

[0110] A portion of a polypeptide that is “amino-terminal” or “N-terminal” to another portion of a polypeptide is that portion that comes earlier in the sequential polypeptide chain. Similarly a portion of a polypeptide that is “carboxy-terminal” or “C-terminal” to another portion of a polypeptide is that portion that comes later in the sequential polypeptide chain. For example in a typical antibody, the variable domain is “N-terminal” to the constant region, and the constant region is “C-terminal” to the variable domain.

[0111] The term “expression” as used herein refers to a process by which a gene produces a biochemical, for example, a polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without limitation transcription of the gene into messenger RNA (mRNA), and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide that is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional

modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0112] Terms such as "treating" or "treatment" or "to treat" or "alleviating" or "to alleviate" refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or slow the progression of an existing diagnosed pathologic condition or disorder. Terms such as “prevent,” “prevention,” “avoid,” “deterrence” and the like refer to prophylactic or preventative measures that prevent the development of an undiagnosed targeted pathologic condition or disorder. Thus, “those in need of treatment” can include those already with the disorder; those prone to have the disorder; and those in whom the disorder is to be prevented.

[0113] By "subject" or "individual" or "animal" or "patient" or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sports, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, swine, cows, bears, and so on.

[0114] As used herein, phrases such as “a subject that would benefit from therapy” and “an animal in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of a binding molecule such as an antibody, comprising one or more antigen binding domains. Such binding molecules, *e.g.*, antibodies, can be used, *e.g.*, for a diagnostic procedures and/or for treatment or prevention of a disease.

Multimeric Binding Molecules

[0115] This disclosure includes a multimeric HIV binding molecule, *i.e.*, a binding molecule possessing at least two, *e.g.*, two, five, or six “binding units” as defined herein, where at least one antigen binding domain of the multimeric binding molecule can specifically bind to an HIV antigen, *e.g.*, an HIV protein, *e.g.*, gp120 and/or gp41. Exemplary epitopes on gp120 and/or gp41 that can be bound by a multimeric HIV binding molecule described herein, include, without limitation, the immunodominant region of gp41, the membrane proximal external region (MPER), the CD4 binding

site, the variable region 1/variable Region 2 (V1/V2) loop, the glycan-variable region 3 (V3) loop, and/or any carbohydrates associated with these regions. Exemplary multimeric binding molecules include, but are not limited to, an IgM binding molecule with five or six binding units (a pentameric or hexameric binding molecule), or an IgA binding molecule with two binding units.

[0116] A multimeric binding molecule as described herein can have improved binding characteristics or biological activity as compared to a binding molecule composed of a single binding unit, *e.g.*, a bivalent IgG antibody. In some embodiments, a multimeric binding molecule as described herein can more potently neutralize HIV, bind and neutralize more diverse HIV variants or clades, enhance viral clearance, improve tissue distribution (*e.g.*, to mucosal surfaces), and/or be more potent in preventing, controlling or treating HIV infection than a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains. In certain embodiments a multimeric binding molecule as described herein can be more potent in controlling HIV infectivity and growth as compared with a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains. In certain embodiments a multimeric binding molecule as described herein can be used to treat chronic infection, *e.g.*, by binding to and/or effecting antibody and/or cell-mediated killing of HIV infected cells, *e.g.*, reservoir cells that express extremely low levels of HIV antigens on their surface. In certain embodiments, the multimeric binding molecule can be more effective at activating and killing such HIV-infected cells or killing such cells after activation with an independent activating agent such as an effector cell. In certain embodiments, a multimeric binding molecule as described herein can describe equivalent benefit at a lower dosage than that of a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains. In certain embodiments administration of a multimeric binding molecule as described herein can allow for reduced or modified dosages of other retroviral therapies, *e.g.*, ART. See, *e.g.*, Example 7 below. The term "corresponding reference single binding unit molecule" refers to a binding molecule composed of a single binding unit, which has one or two HIV antigen binding domains similar or identical to one or more HIV antigen

binding domains of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody described herein.

[0117] In certain embodiments, a "corresponding reference single binding unit molecule" is an IgG antibody comprising two identical antigen binding domains, where those antigen binding domains are identical to those contained in at least one binding unit, or at least two, three, four, five, or six binding units, of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody described herein.

[0118] The term "improved binding characteristics" is a non-limiting term that can apply to any characteristic of the multimeric binding molecule that is improved or distinctive relative to a monomeric binding molecule. A multimeric binding molecule can, *e.g.*, neutralize HIV in infected cells, *e.g.*, cells in a human infected with HIV, and when administered to an individual in need thereof, can exhibit an activity that is empirically determined to be stronger, more potent, or require less binding molecule by mass or molar equivalents, such as, but not limited to (i) reducing the infectivity of an HIV virion, (ii) neutralizing more diverse HIV variants or clades, (iii) reducing the number of HIV-infected cells (including reservoir cells), (iv) preventing HIV infection, (v) enhancing viral clearance, and/or (vi) improving the signs and symptoms of HIV infection, as compared with a corresponding reference single binding unit molecule (*e.g.*, an IgG molecule) that possesses antigen binding domains similar or identical in sequence to those of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody described herein.

[0119] A corresponding reference single binding unit molecule as referred to above can be an IgG binding molecule. The reference IgG binding molecule can be of any isotype, such as IgG1, IgG2, IgG3, or IgG4, etc. The reference binding molecule is typically from the same animal. Thus if the multimeric binding molecule is human, the corresponding reference single binding unit molecule would typically also be human. Conversely, if the multimeric binding molecule is a rabbit binding molecule, the corresponding reference single binding unit molecule would also be a rabbit binding molecule.

IgM Binding Molecules

[0120] IgM is the first immunoglobulin produced by B cells in response to stimulation by antigen, and is present at around 1.5 mg/ml in serum with a half-life of 5 days. IgM is typically multimeric, *e.g.*, a pentameric or hexameric molecule. Thus, IgM molecules are "multimeric" binding molecules. Each of the five, or six, IgM binding units includes two light and two heavy chains. While IgG contains three heavy chain constant domains (CH1, CH2 and CH3), as explained above, the heavy (μ) chain of IgM additionally contains a fourth constant domain (CH4), that includes a C-terminal "tailpiece." The human IgM constant region typically comprises the amino acid sequence SEQ ID NO: 1. The human Cμ1 region ranges from about amino acid 5 to about amino acid 102 of SEQ ID NO: 1; the human Cμ2 region ranges from about amino acid 114 to about amino acid 205 of SEQ ID NO: 1, the human Cμ3 region ranges from about amino acid 224 to about amino acid 319 of SEQ ID NO: 1, the Cμ 4 region ranges from about amino acid 329 to about amino acid 430 of SEQ ID NO: 1, and the tailpiece ranges from about amino acid 431 to about amino acid 453 of SEQ ID NO: 1. The amino acid sequence of the human IgM constant region (SEQ ID NO: 1) is described below:

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSD
ISSTRGFPSVLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKE
KNVPLPVIAELPPKVSVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVS
WLREGKQVGSGVTTDQVQAEAKESGPTTYKVTSTLTIKESDWLGQSMFT
CRVDHRGLTFQQNASSMCVPDQDTAIRVFAIPPSFASIFLTKSTKLTCL
VTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSAVGEASICEDD
WNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQLNL
RESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRY
FAHSILTVSEEEWNTGETYTCVAHEALPNRVTERTVDKSTGKPTLYNVS
LVMSDTAGTCY

[0121] An IgM binding molecule can comprise five binding units (each an "IgM binding unit") that can form a complex with an additional small polypeptide chain (the J-chain) to form a pentameric IgM binding molecule. The human J-chain comprises the amino acid sequence SEQ ID NO: 2. As described elsewhere herein the J-chain can be a variant J-chain comprising, *e.g.*, a binding moiety such as an ScFv or a camelid antibody. Without the J-chain, IgM binding units typically assemble into a

hexameric IgM binding molecule. While not wishing to be bound by theory, the assembly of IgM binding units into a hexameric or pentameric binding molecule is thought to involve the Cμ3 and Cμ4 domains. Accordingly, a hexameric or pentameric IgM binding molecule included in this disclosure typically includes IgM constant regions that include at least the Cμ3 and Cμ4 domains. The amino acid sequence of the human J-chain (SEQ ID NO: 2) is described below:

MKNHLLFWGVLAVFIKAVHVKAQEDERIVLVDNKCKCARITSRIIRSSE
DPNEDIVERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTE
VELDNQIVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMV
ETALTPDACYPD

[0122] The signal peptide (amino acids 1 to 22 of SEQ ID NO: 2) is double underlined, the mature J-chain sequence is amino acids 23 to 159 of SEQ ID NO: 2.

[0123] An IgM heavy chain constant region can additionally include a Cμ2 domain or a fragment thereof, a Cμ1 domain or a fragment thereof, and/or other IgM heavy chain domains. In certain embodiments, a binding molecule as described herein can include a complete IgM heavy (μ) chain constant domain, *e.g.*, SEQ ID NO: 1, or a variant, derivative, or analog thereof.

Pentameric or Hexameric HIV Binding Molecules

[0124] This disclosure describes a pentameric or hexameric HIV binding molecule, a binding molecule that has five or six IgM-derived "binding units" as defined herein, which can specifically bind to an HIV antigen, *e.g.*, an HIV protein, *e.g.*, the HIV spike protein. In certain embodiments, each binding unit includes two IgM heavy chain constant regions or fragments thereof. In certain embodiments, the two IgM heavy chain constant regions are human heavy chain constant regions. In certain embodiments, the antigen binding domains in the IgM binding molecule are human in origin, or humanized, or a combination thereof. A pentameric or hexameric IgM binding molecule as described herein can have improved binding characteristics or biological activity as compared to a binding molecule composed of a single binding unit, *e.g.*, a bivalent IgG-derived antibody. In some embodiments, a pentameric or hexameric binding molecule described herein can, *e.g.*, through increased avidity or affinity, or enhanced effector functions, be more potent in targeting chronic

infections, *e.g.*, by binding to and/or effecting complement-mediated killing of HIV reservoir cells, as compared with a corresponding reference single binding unit molecule containing only two HIV-specific antigen binding domains.

[0125] A pentameric or hexameric HIV binding molecule as described herein can likewise possess distinctive characteristics as compared to univalent or multivalent binding molecules composed of synthetic or chimeric structures. For example, use of human IgM constant regions can afford reduced immunogenicity and thus increased safety relative to a binding molecule containing chimeric constant regions or synthetic structures. Moreover, an IgM-based binding molecule can consistently form hexameric or pentameric oligomers resulting in a more homogeneous expression product. Superior complement fixation can also be an advantageous effector function of IgM-based binding molecules.

[0126] The reference single binding unit referred to above can be an IgG binding unit. The reference IgG binding unit can be of any isotype, such as IgG1, IgG2, IgG3, or IgG4, etc. The reference binding unit is typically from the same animal. Thus if the multimeric binding molecule is human, the reference single binding unit would also be human, but not necessarily human. That is, the reference single binding unit can be a humanized antibody of the IgG type. Conversely, if the multimeric binding molecule is a rabbit binding molecule, the reference single binding unit would also be a rabbit binding unit. Further, if the multimeric binding molecule is comprised of one or more binding unit fragments, then the reference single binding unit would also be an equivalent single binding unit fragment. In other words, the reference single binding unit is otherwise identical in sequence and structure to the binding units contained in the multimeric binding molecule except that the reference single binding unit is an equivalent single binding unit.

[0127] In certain embodiments, the disclosure includes a pentameric or hexameric binding molecule comprising five or six binding units, respectively, where each binding unit includes two IgM heavy chain constant regions or fragments thereof. In certain embodiments, the two IgM heavy chain constant regions are human heavy chain constant regions. In some embodiments, the antigen binding domains in the IgM binding molecule are human in origin, or humanized, or a combination thereof.

[0128] Where the multimeric binding molecule described herein is pentameric, the binding molecule can further comprise a J-chain, or functional fragment thereof, or variant thereof. Where the pentameric IgM binding molecule contains a J-chain, the J-chain can be of the same species as the IgM binding molecule. That is, if the pentameric IgM binding molecule is human, the J-chain can also be human. In certain embodiments, the J-chain can be a modified J-chain comprising a heterologous moiety or one or more heterologous moieties, *e.g.*, a heterologous polypeptide sequence, *e.g.*, an additional desired binding domain introduced into the native sequence. In certain embodiments the additional binding domain specifically binds to CD3, *e.g.*, CD3ε, or CD16.

[0129] In certain embodiments each of the two IgM heavy chain constant regions in a binding unit is associated with an antigen binding domain, for example an Fv portion of an antibody, *e.g.*, a VH and a VL of a human or murine antibody. In certain embodiments, at least one antigen binding domain of a binding molecule as described herein is a cross-reactive HIV antigen binding domain, *e.g.*, an antigen binding domain that can bind to an HIV antigen from two or more HIV types, Groups, or clades. In certain embodiments, the antigen binding domain can bind to the HIV antigen from both HIV types (types 1 and 2). In certain embodiments, the binding molecule can bind to the HIV antigen from two or more Groups (M, N and O) of HIV-2. In certain embodiments, the antigen binding domain can specifically bind to the HIV antigen from two, three, four, or more HIV groups or clades. In certain embodiments, the binding molecule can bind to the HIV spike protein, *e.g.*, gp120 and/or gp41, of two or more HIV types, groups, or clades. Exemplary epitopes on gp120 and/or gp41 epitopes include, without limitation, gp41, *e.g.*, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions

[0130] In other embodiments, each antigen binding domain of a pentameric or hexameric HIV binding molecule as described herein can independently bind a different antigen or different epitope on the same antigen. Thus, a pentameric IgM binding molecule can bind as many as two, three, four, five, six, seven, eight, nine or even ten different antigens or epitopes, across different HIV groups, subtypes or clades. Likewise, a

hexameric IgM binding molecule can bind as many as two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve different antigens or epitopes, across different HIV groups, subtypes or clades.

[0131] In certain embodiments, a pentameric or hexameric HIV binding molecule as described herein comprises at least one antigen binding domain that binds to an epitope on the HIV spike protein, *e.g.*, gp120 and/or gp41, of two or more HIV types, groups, or clades. In other embodiments, two or more antigen binding domains of a pentameric or hexameric HIV binding molecule described herein, *e.g.*, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve antigen binding domains, can bind to two or more distinct HIV spike protein epitopes, *e.g.*, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions. In some embodiments, a pentameric or hexameric HIV binding molecule as described herein can comprise at least one antigen binding domain that specifically binds the HIV spike protein, and can comprise other antigen binding domains that specifically bind to other HIV proteins, *e.g.*, gag, pol, tat, rev, nef, vpr, vif, and/or vpu. Alternatively, all of the antigen binding domains can possess the same specificity, *e.g.*, for a specific epitope on the HIV spike protein. In one embodiment,all binding units of the pentameric or hexameric HIV binding molecule specifically bind to the HIV spike protein, for example, a pentameric or hexameric HIV binding molecule can comprise ten or twelve antigen binding domains that bind to the same spike protein epitope. In certain embodiments the ten or twelve antigen binding domains can be identical.

[0132] In certain embodiments, a pentameric or hexameric HIV binding molecule as described herein can bind to an HIV virion particle, and/or can bind to the surface of an HIV-infected cell. In certain embodiments, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve antigen binding domains of the pentameric or hexameric HIV binding molecule can specifically bind to an HIV virion particle, and/or can bind to the surface of an HIV-infected cell.

[0133] An HIV antigen or epitope bound by a pentameric or hexameric HIV binding molecule described herein can be any one or more of the HIV proteins, including the

HIV spike protein, *e.g.*, gp120 and/or gp41, *e.g.*, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions. Moreover, a pentameric or hexameric HIV binding molecule as described herein can be multispecific, including antigen binding domains that specifically bind to two or more antigens, *e.g.*, one or more HIV antigens or epitopes and one or more heterologous antigens or epitopes, or two or more HIV antigens or epitopes. For example in certain embodiments a multispecific hexameric or pentameric HIV binding molecule described herein can include five or six binding units each comprising two antigen binding domains, where at least two individual binding domains bind to different antigens or epitopes. In certain non-limiting embodiments one or more binding domains can bind to, *e.g.*, an epitope within the CD4 binding site of the spike protein, while one or more of the remaining binding domains can bind to, *e.g.*, the immunodominant region of gp41, another epitope within the CD4 binding site, an epitope within the MPER region, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions, or any other region of the spike protein (exemplary binding domains are described in Table 3), or an epitope of another HIV protein. In another embodiment,a multispecific IgM binding molecule can comprise binding domains that are specific for different subsets of or individual HIV groups or clades, thereby providing a binding molecule with activity over a broader range of HIV viruses. Methods of making bispecific and multi-specific IgM and IgA binding molecules, including antibodies, are described, for example, in PCT Publication No. WO 2015/053887 and PCT Publication No. WO 2015/120474, the entire contents of which are hereby expressly incorporated by reference. In certain embodiments a heterologous binding domain can be associated with a J-chain, as described in PCT Publication No. WO 2015/153912, and elsewhere herein.

IgA Binding Molecules

[0134] IgA plays a critical role in mucosal immunity, and comprises about 15% of total immunoglobulin produced. IgA is a monomeric or dimeric molecule. Dimeric IgA molecules are relatively smaller in size than IgM molecules, but can also possess

improved binding characteristics relative to a corresponding reference single binding unit molecule. Moreover, a dimeric IgA binding molecule can reach mucosal sites providing greater tissue distribution for the binding molecules described herein. Likewise, a dimeric IgA-derived binding molecule as described herein can possess binding characteristics or biological activity that can be distinguished from a binding molecule comprising five or six binding units, *e.g.*, a hexameric or pentameric IgM-derived binding molecule as described elsewhere herein. For example, a dimeric binding molecule would be smaller, and could, for example, achieve better tissue penetration. Dimeric IgA binding molecules can be manufactured by expression *in vitro* to include two IgA monomers and a J-chain. The dimeric J-chain-containing IgA molecules can then be administered to an individual where the IgA molecules that migrate to mucous membranes or mucosal tissue can bind to, and form a complex with, a membrane-bound secretory component (mSC, also referred to as the polymeric Ig receptor (pIgR)) produced by epithelial cells. The complex is translocated across epithelial cells and the mSC is cleaved, delivering sIgA to the mucosal surfaces. (See, Kaetzel *et al.*, *Proc. Natl. Acad. Sci. USA* 88(19):8796–8800, 1991). Therefore, delivery of IgA to the blood stream can provide targeting of mucosal tissues.

[0135] An IgA binding unit includes two light and two IgA heavy chains. IgA contains three heavy chain constant domains (Cα1, Cα2 and Cα3), and includes a C-terminal "tailpiece." Human IgA has two subtypes, IgA1 and IgA2. The mature human IgA1 constant region typically comprises the amino acid sequence SEQ ID NO: 3, described below:

ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVT
ARNFPPSQDASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVT
VPCPVPSTPPTPSPSTPPTPSPSCCHPRLSLHRPALEDLLLGSEANLTC
TLTGLRDASGVTFTWTPSSGKSAVQGPPERDLCGCYSVSSVLPGCAEPW
NHGKTFTCTAAYPESKTPLTATLSKSGNTFRPEVHLLPPPSEELALNEL
VTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAV
TSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVNVSV
VMAEVDGTCY

The human Cα1 region ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 3; the human Cα2 region ranges from about amino acid 125 to about amino acid 220 of SEQ ID NO:

3 the human Cα3 region ranges from about amino acid 228 to about amino acid 330 of SEQ ID NO: 3, and the tailpiece ranges from about amino acid 331 to about amino acid 352 of SEQ ID NO: 3.

[0136] The mature human IgA2 constant region typically comprises the amino acid sequence SEQ ID NO: 4, described below:

ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVT
ARNFPPSQDASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVT
VPCPVPPPPPCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGATF
TWTPSSGKSAVQGPPERDLCGCYSVSSVLPGCAQPWNHGETFTCTAAHP
ELKTPLTANITKSGNTFRPEVHLLPPPSEELALNELVTLTCLARGFSPK
DVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTSILRVAAEDWKK
GDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDGTCY

The human Cα1 region ranges from about amino acid 6 to about amino acid 98 of SEQ ID NO: 4; the human Cα2 region ranges from about amino acid 112 to about amino acid 207 of SEQ ID NO: 4, the human Cα3 region ranges from about amino acid 215 to about amino acid 317 of SEQ ID NO: 4, and the tailpiece ranges from about amino acid 318 to about amino acid 340 of SEQ ID NO: 4.

[0137] Two IgA binding units can form a complex with two additional polypeptide chains, the J-chain (SEQ ID NO: 2) and the secretory component (SEQ ID NO: 76) to form a secretory IgA (sIgA) antibody. The amino acid sequence of the mature secretory component (SEQ ID NO: 76) is described below:

KSPIFGPEEVNSVEGNSVSITCYYPPTSVNRHTRKYWCRQGARGGCITL
ISSEGYVSSKYAGRANLTNFPENGTFVVNIAQLSQDDSGRYKCGLGINS
RGLSFDVSLEVSQGPGLLNDTKVYTVDLGRTVTINCPFKTENAQKRKSL
YKQIGLYPVLVIDSSGYVNPNYTGRIRLDIQGTGQLLFSVVINQLRLSD
AGQYLCQAGDDSNSNKKNADLQVLKPEPELVYEDLRGSVTFHCALGPEV
ANVAKFLCRQSSGENCDVVVNTLGKRAPAFEGRILLNPQDKDGSFSVVI
TGLRKEDAGRYLCGAHSDGQLQEGSPIQAWQLFVNEESTIPRSPTVVKG
VAGGSVAVLCPYNRKESKSIKYWCLWEGAQNGRCPLLVDSEGWVKAQYE
GRLSLLEEPGNGTFTVILNQLTSRDAGFYWCLTNGDTLWRTTVEIKIIE
GEPNLKVPGNVTAVLGETLKVPCHFPCKFSSYEKYWCKWNNTGCQALPS
QDEGPSKAFVNCDENSRLVSLTLNLVTRADEGWYWCGVKQGHFYGETAA
VYVAVEERKAAGSRDVSLAKADAAPDEKVLDSGFREIENKAIQDPR

[0138] While not wishing to be bound by theory, the assembly of IgA binding units into a dimeric sIgA binding molecule is thought to involve the Cα3 and tailpiece domains. Accordingly, a dimeric sIgA binding molecule included in this disclosure typically

includes IgA constant regions that include at least the Cα3 and tailpiece domains. An IgA heavy chain constant region can additionally include a Cα2 domain or a fragment thereof, a Cα1 domain or a fragment thereof, and/or other IgA heavy chain domains. In certain embodiments, a binding molecule as described herein can include a complete IgA heavy (α) chain constant domain, *e.g.*, SEQ ID NO: 3 or SEQ ID NO: 4, or a variant, derivative, or analog thereof.

Dimeric HIV Binding Molecules

[0139] This disclosure includes a dimeric binding molecule comprising two or more IgA binding units as defined herein, where the dimeric HIV binding molecule comprises at least one antigen binding domain that can specifically bind to an HIV antigen, *e.g.*, an HIV protein, *e.g.*, the HIV spike protein. As explained above in the context of a pentameric or hexameric HIV binding molecule, a dimeric HIV binding molecule as described herein can possess improved binding characteristics or biological activity as compared to a binding molecule composed of corresponding reference single binding unit molecule, *e.g.*, a bivalent IgG antibody. For example, a dimeric HIV binding molecule can effect improved tissue penetration or tissue distribution, especially to mucosal surfaces. Thus, a dimeric IgA binding molecule processed for transport and secretion could target HIV-reservoir cells in the GIT.

[0140] The reference single binding unit referred to above can be an IgG binding unit. The reference IgG binding unit can be of any isotype, such as IgG1, IgG2, IgG3, or IgG4, etc. The reference binding unit is typically from the same animal. Thus if the multimeric binding molecule is human, the reference single binding unit would also be human, but not necessarily human. That is, the reference single binding unit can be a humanized antibody of the IgG type. Conversely, if the multimeric binding molecule is a rabbit binding molecule, the reference single binding unit would also be a rabbit binding unit. Further, if the multimeric binding molecule is comprised of one or more binding unit fragments, then the reference single binding unit would also be an equivalent single binding unit fragment. In other words, the reference single binding unit is otherwise identical in sequence and structure to the binding

units contained in the multimeric binding molecule except that the reference single binding unit is an equivalent single binding unit.

[0141] In certain embodiments, the disclosure includes a dimeric HIV binding molecule comprising two binding units, where each binding unit includes two IgA heavy chain constant regions or fragments thereof. In certain embodiments, the two IgA heavy chain constant regions are human heavy chain constant regions. The anti-HIV binding domain(s) can be, *e.g.*, human or humanized.

[0142] A dimeric HIV binding molecule as described herein can further comprise a J-chain, or fragment thereof, or variant thereof. A dimeric HIV binding molecule as described herein can further comprise a secretory component, or fragment thereof, or variant thereof.

[0143] An IgA heavy chain constant region can include one or more of a Cα1 domain, a Cα2 domain, and/or a Cα3 domain, provided that the constant region can serve a desired function in the binding molecule, *e.g.*, associate with second IgA constant region to form an antigen binding domain, or associate with another IgA binding unit to form a dimeric binding molecule. In certain embodiments the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each comprise a Cα3 domain or fragment thereof, a tailpiece (TP) or fragment thereof, or any combination of a Cα3 domain, a TP, or fragment thereof. In certain embodiments the two IgA heavy chain constant regions or fragments thereof within an individual binding unit each further comprise a Cα2 domain or fragment thereof, a Cα1 domain or fragment thereof, or a Cα1 domain or fragment thereof and a Cα2 domain or fragment thereof.

[0144] In certain embodiments each of the two IgA heavy chain constant regions in a given binding unit is associated with an antigen binding domain, for example an Fv portion of an antibody, *e.g.*, a VH and a VL of a human or murine antibody. In certain embodiments, at least one antigen binding domain of a binding molecule as described herein is a cross-reactive HIV antigen binding domain, *e.g.*, an antigen binding domain that can specifically bind to two, three, four, or more HIV types, subtypes or clades. In certain embodiments, the antigen binding domain can specifically bind to the HIV antigen from two, three, four, or more HIV groups or

clades. In certain embodiments, the binding molecule can bind to the HIV spike protein, *e.g.*, gp120 and/or gp41, of two or more HIV types, groups, or clades. Exemplary epitopes on gp120 and/or gp41 epitopes include, without limitation, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions.

[0145] In other embodiments, a dimeric HIV binding molecule of the present disclosure can comprise binding units wherein each binding unit can possess two antigen binding domains, each with a different and distinguishable specificity. Thus, a dimeric HIV binding molecule could possess as many as four different specificities.

[0146] In certain embodiments, the antigen binding domain can bind to the HIV antigen from both HIV types (types 1 and 2). In certain embodiments, the dimeric binding molecule can bind to the HIV antigen from two or more Groups (M, N and O) of HIV-2. In certain embodiments, the antigen binding domain can specifically bind to the HIV antigen from two, three, four, or more HIV groups or clades.

[0147] In other embodiments, each antigen binding domain of a dimeric HIV binding molecule as described herein can independently bind a different antigen or different epitope on the same antigen. Thus, a dimeric HIV binding molecule can bind as many as two, three, or four different antigens or epitopes, across different HIV groups, subtypes or clades.

[0148] In certain embodiments, a dimeric HIV binding molecule as described herein comprises at least one antigen binding domain that binds to an epitope on the HIV spike protein, *e.g.*, gp120 and/or gp41, of two or more HIV types, groups, or clades. In other embodiments, two or more antigen binding domains of a dimeric HIV binding molecule described herein, *e.g.*, two, three, or four antigen binding domains, can bind to two or more distinct HIV spike protein epitopes, *e.g.*, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions. In some embodiments, a dimeric HIV binding molecule as described herein can comprise at least one antigen binding domain that specifically binds to the HIV spike protein, and can comprise other antigen binding domains that specifically bind other HIV proteins, *e.g.*, gag, pol, tat, rev, nef, vpr, vif, and/or vpu. Alternatively, all of the

antigen binding domains can possess the same specificity, *e.g.*, for a specific epitope on the HIV spike protein. In one embodiment,all binding units of the dimeric HIV binding molecule specifically bind to the HIV spike protein; for example, a dimeric HIV binding molecule can comprise four antigen binding domains that bind to the same spike protein epitope. In certain embodiments the four antigen binding domains can be identical.

[0149] In certain embodiments, a dimeric HIV binding molecule as described herein can bind to an HIV virion particle, and/or can bind to the surface of an HIV-infected cell. In certain embodiments, at least two, at least three, or at least four antigen binding domains of the pentameric or hexameric HIV binding molecule can specifically bind to an HIV virion particle, and/or can bind to the surface of an HIV-infected cell.

[0150] An HIV antigen or epitope bound by a dimeric HIV binding molecule described herein can be any one or more of the HIV proteins, including the HIV spike protein, *e.g.*, gp120 and/or gp41, *e.g.*, the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, and/or any carbohydrates associated with these regions. Moreover, a dimeric HIV binding molecule as described herein can be multispecific, including antigen binding domains that specifically bind to two or more antigens, *e.g.*, one or more HIV antigens or epitopes and one or more heterologous antigens or epitopes, or two or more HIV antigens or epitopes. For example in certain embodiments a multispecific dimeric HIV binding molecule described herein can include two binding units each comprising two antigen binding domains, where at least two individual binding domains bind to different antigens or epitopes. In certain embodiments one or more binding domains can bind to, *e.g.*, an epitope within the CD4 binding site of the spike protein, while one or more of the remaining binding domains can bind to, *e.g.*, another epitope within the CD4 binding site, an epitope within the MPER region, any other region of the spike protein (exemplary binding domains are described in Table 3), or an epitope of another HIV protein. In another embodiment, a multispecific IgA binding molecule can comprise binding domains that are specific for different subsets of or individual HIV groups or clades, thereby providing a binding molecule with activity over a broader range of HIV viruses. Methods of making bispecific and multi-specific IgM and IgA binding

molecules, including antibodies, are described, for example, in PCT Publication No. WO 2015/053887 and PCT Publication No. WO 2015/120474, the entire contents of which are hereby expressly incorporated by reference. In certain embodiments a heterologous binding domain can be associated with a J-chain, as described in PCT Publication No. WO 2015/153912, and elsewhere herein.

Multispecific dimeric, pentameric, or hexameric HIV binding molecules

[0151] A multi-specific, *e.g.*, bispecific dimeric HIV binding molecule as described herein can be based on the dimeric form of an IgA antibody or a hexameric or pentameric form of an IgM antibody, in which two, five, or six pairs of heavy chain sequences can be present with or without associated light chain sequences. For example, a bispecific dimeric HIV binding molecule as described herein can be composed of two IgA (IgA1 or IgA2) binding units, or five or six IgM binding units, and can include a J-chain, *e.g.*, a modified J-chain as described elsewhere herein.

[0152] A multi-specific, *e.g.*, bispecific dimeric HIV binding molecule as described herein can include mono- and/or bispecific binding units as long as the molecule as a whole has at least two binding specificities, *e.g.*, at least two non-identical antigen binding domains, *e.g.*, different regions of the gp120/41 spike protein, spike protein epitopes and epitopes from other HIV antigens, or HIV antigens and heterologous antigens. In certain embodiments, one or more heterologous antigens can be situated on effector cells, *e.g.*, CD3 on T-cells or CD16 on NK cells. In certain embodiments the non-identical antigen binding domain can be part of a modified J-chain.

[0153] Thus, in one embodiment, a multi-specific, *e.g.*, bispecific dimeric binding molecule as described herein can include two monospecific binding units (AA, BB), each having bivalent binding specificity to a different binding target. In another embodiment, a multi-specific, *e.g.*, bispecific dimeric binding molecule as described herein can include two bispecific binding units, each binding unit binding to the same two binding targets (AB, AB) to form a bispecific dimeric binding molecule. In a further embodiment, one binding unit present in a multi-specific dimeric binding molecule as described herein is monospecific (AA) while the other binding units are bispecific (BC), resulting in a multispecific binding molecule with three (A, B, C)

binding specificities. In a further embodiment, each binding unit is bispecific, but one specificity is overlapping (*e.g.* AB, AC), resulting in a multispecific binding molecule with three (A, B, C) binding specificities. As discussed above for multispecific dimeric binding molecules, each of the five or six binding units can independently be monospecific or bispecific (*e.g.*, AA, BB, CC, etc.) or one or more binding units can be bispecific (*e.g.*, AB, AB, AC, CD, etc.). Thus, a multi-specific, *e.g.*, bispecific pentameric or hexameric binding molecule as described herein can include at least two independent antigen binding domains, and up to twelve different, independent antigen binding domains. Other combinations, *e.g.*, with four non-identical antigen binding domains (A, B, C, and D) can be readily made based on this disclosure. In another embodiment all of the IgM or IgA binding units can be monospecific (*e.g.*, AA) and the non-identical antigen binding domain can be part of a modified J-chain.

Modified J-chains

[0154] In certain embodiments, HIV binding molecules described herein can be multispecific, *e.g.*, bispecific, incorporating a modified J-chain. As described herein and in PCT Publication No. WO 2015/153912, a modified J-chain can comprise a heterologous moiety, *e.g.*, a heterologous polypeptide, *e.g.*, an additional desired binding domain, which can include, for example, a polypeptide binding domain capable of specifically binding to a target. The binding domain can be, for example, an antibody or antigen binding fragment thereof, an antibody-drug conjugate or antigen binding fragment thereof, or an antibody-like molecule. A polypeptide binding domain can be introduced into a J-chain by appropriately selecting the location and type of addition (*e.g.* direct or indirect fusion, chemical tethering, etc.).

[0155] In some embodiments, a modified J-chain can comprise a binding domain that can include without limitation a polypeptide capable of specifically binding to a target antigen. In certain embodiments, a binding domain associated with a modified J-chain can be an antibody or an antigen binding fragment thereof, including monospecific, bispecific, and multi-specific antibodies and antibody fragments. The antibody fragment can be, without limitation, a Fab fragment, a Fab' fragment, a

$F(ab')_2$ fragment, an scFv, $(scFv)_2$ fragment, single-chain antibody molecules, single domain antibodies, *e.g.*, camelid VHH antibodies, minibodies, or multispecific antibodies formed from antibody fragments. In certain embodiments, the antibody fragment is a scFv.

[0156] In other embodiments, the binding domain can be an antibody-like molecule, for example, a human domain antibody (dAb), Dual-Affinity Re-Targeting (DART) molecule, a diabody, a di-diabody, dual-variable domain antibody, a Stacked Variable Domain antibody, a Small Modular Immuno Pharmaceutical (SMIP), a Surrobody, a strand-exchange engineered domain (SEED)-body, or TandAb.

[0157] The binding domain can be introduced into the native J-chain sequence at any location that allows the binding of the binding domain to its binding target without interfering with the binding of the recipient IgM or IgA molecule to its binding target or binding targets or the ability of the J-chain to effectively incorporate into an IgA dimer or an IgM pentamer. In certain embodiments the binding domain can be inserted at or near the C-terminus, at or near the mature N-terminus (*i.e.*, amino acid number 23 of SEQ ID NO: 2 following cleavage of the signal peptide) or at an internal location that, based on the three-dimensional structure of the J-chain is accessible. In certain embodiments, the binding domain can be introduced into the native sequence J-chain without about 10 residues from the C-terminus or without about 10 amino acid residues from the mature N-terminus, of the human J-chain of SEQ ID NO: 2. In another embodiment,the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 2 in between cysteine residues 114 and 123 of SEQ ID NO: 2, or at an equivalent location of another native sequence J-chain. In a further embodiment,the binding domain can be introduced into a native sequence J-chain, such as a J-chain of SEQ ID NO: 2, at or near a glycosylation site. In certain embodiments, the binding domain can be introduced into the native sequence human J-chain of SEQ ID NO: 2 within about 10 amino acid residues from the C-terminus.

[0158] Introduction can be accomplished by direct or indirect fusion, *i.e.* by the combination of the J-chain and binding domain in one polypeptide chain by in-frame combination of their coding nucleotide sequences, with or without a peptide linker. The peptide

linker (indirect fusion), if used, can be about 1 to 50, or about 1 to 40, or about 1 to 30, or about 1 to 20, or about 1 to 10, or about 1 to 5, or about 10 to 20 amino acids in length, and can be present at one or both ends of the binding domain to be introduced into the J-chain sequence. In certain embodiments, the peptide linker can be about 1 to 100 amino acids long. In certain embodiments the peptide linker is 5, 10, 15, or 20 amino acids long.

[0159] In certain embodiments the mature modified J-chain comprises the formula $X[L_n]J$ or $J[L_n]X$, where J is a native J-chain or functional fragment thereof, *e.g.*, a native human J-chain (amino acids 23 to 159 of SEQ ID NO: 2), X is a binding domain, and $[L_n]$ is a linker sequence consisting of n amino acids, where n is a positive integer, *e.g.*, from 1 to 100, 1 to 50, or 1 to 25. In certain embodiments n=5, 10, 15, or 20. In certain embodiments L_n can consist of GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104). In certain embodiments, X can comprise an anti-CD3 binding domain, *e.g.*, an anti-CD3 ScFv. In certain embodiments X comprises or consists of SEQ ID NO: 105, SEQ ID NO: 106, or SEQ ID NO: 107.

[0160] It is also possible to introduce more than one heterologous polypeptide, *e.g.*, more than one binding domain, into a J-chain.

[0161] The modified J-chain can be produced by well-known techniques of recombinant DNA technology, by expressing a nucleic acid encoding the modified J-chain in a suitable prokaryotic or eukaryotic host organism.

[0162] The modified J-chain can be co-expressed with the heavy and light chains of the recipient IgM or IgA binding molecules as described elsewhere herein. The recipient binding molecule, prior to the modified J-chain incorporation can be monospecific, bispecific or multi-specific, *e.g.*, a monospecific, bispecific, or multispecific IgA or IgM antibody. Bispecific and multi-specific IgM and IgA binding molecules, including antibodies, are described, for example, in PCT Publication No. WO 2015/053887 and PCT Publication No. WO 2015/120474, the entire contents of which are hereby expressly incorporated by reference.

[0163] In certain embodiments, an anti-HIV IgM or IgA binding molecule as described herein can include a modified J-chain with binding specificity for an immune effector cell, such as a T-cell, NK-cell, a macrophage, or a neutrophil. In certain embodiments the effector cell is a T-cell and the binding target is CD3 (discussed below), or CD8. By activating and redirecting effector cells, *e.g.* effector T-cells (T-cell dependent killing or TDCC), or NK cells to infected cells expressing HIV antigens, *e.g.*, the HIV spike glycoprotein, on their surface, including reservoir cells, a bispecific anti-HIV IgM or IgA binding molecule comprising an effector cell-directed modified J-chain as described herein can produce an enhanced immune response against the target, the response comprising, *e.g.*, complement-mediated cytotoxicity, antibody dependent cellular cytotoxicity (ADCC), TDCC, and/or NK-cell mediated killing, thereby further increasing potency and efficacy. In certain embodiments, a bispecific anti-HIV IgM or IgA binding molecule as described herein comprising a modified J-chain can be used for the treatment of a disease or condition caused by, or exacerbated by infection with HIV, and/or can direct HIV neutralization, and/or clearance or killing of an HIV-infected cells, such as reservoir cells.

[0164] In the case of T-cells, cluster of differentiation 3 (CD3) is a multimeric protein complex, known historically as the T3 complex, and is composed of four distinct polypeptide chains (ε, γ, δ, ζ) that assemble and function as three pairs of dimers (εγ, εδ, ζζ). The CD3 complex serves as a T-cell co-receptor that associates non-covalently with the T-cell receptor (TCR). Components of this CD3 complex, especially CD3ε, can be targets for a modified J-chain of a bispecific IgM or IgA binding molecule described herein.

[0165] In certain embodiments, a bispecific anti-HIV x anti-CD3 IgM or IgA binding molecule binds to HIV-infected cells or HIV virus particles via the antibody binding domains, while the J-chain is modified to bind to CD3, *e.g.*, CD3ε.

[0166] In certain embodiments the anti- CD3 binding domain of a modified J-chain described herein is a scFv. The anti CD3 scFv can be fused at or near the N-terminus of the J-chain, or at or near the C-terminus of the J-chain either directly or indirectly via a synthetic linker introduced in between the scFv and the J-chain sequences, *e.g.*,

GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104). Suitable anti-CD3 binding domains for inclusion in a modified J-chain as described herein include, but are not limited to, ScFv antigen binding domains comprising the amino acid sequences SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, as shown in Table 2.

Table 2: Exemplary CD3 Heterologous Binding Domains

SEQ ID NO	Source	Binds to	Sequence
105	Kung, P.C., *et al.* (1979) Science, 206, 347–349	CD3ε	QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRP GQGLEWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQL SSLTSEDSAVYYCARYYDDHYSLDYWGQGTTLTVSSGGGGSG GGGSGGGGSQIVLTQSPAIMSASPGEKVTMTCSASSSVSYMN WYQQKSGTSPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISG MEAEDAATYYCQQWSSNPFTFGSGTKLEIK
106	U.S. Patent No. 5,834,597	CD3ε	QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAP GQGLEWMGYINPRSGYTHYNQKLKDKATLTADKSASTAYME LSSLRSEDTAVYYCARSAYYDYDGFAYWGQGTLVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCSASSSVSY MNWYQQKPGKAPKRLIYDTSKLASGVPSRFSGSGSGTDFTLTI SSLQPEDFATYYCQQWSSNPPTFGGGTKLEIK
107	Beverley, P. C. & Callard, R. E. (1981) Eur. J. Immunol. 11, 329–334	CD3ε	EVQLVESGGGLVQPGGSLRLSCAASGYSFTGYTMNWVRQAPG KGLEWVALINPYKGVTTYADSVKGRFTISVDKSKNTAYLQMN SLRAEDTAVYYCARSGYYGDSDWYFDVWGQGTLVTVSSGGG GSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQDIRN YLNWYQQKPGKAPKLLIYYTSRLESGVPSRFSGSGSGTDYTLTI SSLQPEDFATYYCQQGNTLPWTFGQGTKVEIK
112	GenBank: ABQ52435.1	CD16	EVQLVESGGELVQAGGSLRLSCAASGLTFSSYNMGWFRRAPG KEREFVASITWSGRDTFYADSVKGRFTISRDNAKNTVYLQMSS LKPEDTAVYYCAANPWPVAAPRSGTYWGQGTQVTVSS

[0167] In certain embodiments the modified J-chain comprises the mature scFv amino acid sequence of SEQ ID NO: 105 fused to the N-terminus of the human J-chain through a linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or

GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as OL_nJ, where n=5, 10, 15, or 20. OL_nJ can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments the mature modified J-chain comprises a scFv of SEQ ID NO: 105 fused to the C-terminus of the human J-chain through an amino acid linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as JL_nO, where n=5, 10, 15, or 20. JL_nO can further include a signal peptide, *e.g.*, amino acids 1 to 22 of SEQ ID NO: 2, to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments, other signal peptides can be used. Selection and inclusion of suitable signal peptides to facilitate expression, secretion, and incorporation of a modified J-chain into an anti-HIV IgM or IgA binding molecule as described herein is well within the capabilities of a person of ordinary skill in the art.

[0168] In certain embodiments the modified J-chain comprises the mature scFv amino acid sequence SEQ ID NO: 106 fused to the N-terminus of the human J-chain through a linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as VL_nJ, where n=5, 10, 15, or 20. VL_nJ can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments the mature modified J-chain comprises a scFv of SEQ ID NO: 106 fused to the C-terminus of the human J-chain through an amino acid linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as JL_nV, where n=5, 10, 15, or 20. JL_nV can further include a signal peptide, *e.g.*, amino acids 1 to 22 of SEQ ID NO: 2, to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments, other signal peptides can be used. Selection and inclusion of suitable signal peptides to

facilitate expression, secretion, and incorporation of a modified J-chain into an anti-HIV IgM or IgA binding molecule as described herein is well within the capabilities of a person of ordinary skill in the art. Exemplary modified J chains include, without limitation V5J (SEQ ID NO: 108), V10J (SEQ ID NO: 109), V15J (SEQ ID NO: 110), or V20J (SEQ ID NO: 111). For each sequence, the mature anti-CD3 ScFv sequence is single underlined, and the mature human J-chain sequence is shown in italics. In certain embodiments, the mature modified J chain can SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or a combination thereof.

V5J

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMG
YINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCAR
SAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGG
SQEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRE
NISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSA
TETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD (SEQ ID NO: 108).

V10J

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMG
YINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCAR
SAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGG
SGGGGS*QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNIRIIVP*
LNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTATQSNIC
DEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYPD
(SEQ ID NO: 109).

J-chain sequence for V15J

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMG
YINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCAR
SAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGG
SGGGGSGGGGS*QEDERIVLVDNKCKCARITSRIIRSSEDPNEDIVERNI*
RIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQIVTAT
QSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTPDACYP
D (SEQ ID NO: 110).

J-chain sequence for V20J

QVQLVQSGAEVKKPGASVKVSCKASGYTFISYTMHWVRQAPGQGLEWMG
YINPRSGYTHYNQKLKDKATLTADKSASTAYMELSSLRSEDTAVYYCAR
SAYYDYDGFAYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLS
ASVGDRVTITCSASSSVSYMNWYQQKPGKAPKRLIYDTSKLASGVPSRF
SGSGSGTDFTLTISSLQPEDFATYYCQQWSSNPPTFGGGTKLEIKGGGG
SGGGGSGGGGSGGGGS*QEDERIVLVDNKCKCARITSRIIRSSEDPNEDI*
VERNIRIIVPLNNRENISDPTSPLRTRFVYHLSDLCKKCDPTEVELDNQ
IVTATQSNICDEDSATETCYTYDRNKCYTAVVPLVYGGETKMVETALTP
DACYPD (SEQ ID NO: 111).

[0169] In certain embodiments the modified J-chain comprises the mature scFv amino acid sequence SEQ ID NO: 107 fused to the N-terminus of the human J-chain through a linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as UL_nJ, where n=5, 10, 15, or 20. UL_nJ can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments the mature modified J-chain comprises a scFv of a SEQ ID NO: 107 fused to the C-terminus of the human J-chain through an amino acid linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as JL_nU, where n=5, 10, 15, or 20. JL_nU can further include a signal peptide, *e.g.*, amino acids 1 to 22 of SEQ ID NO: 2, to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments, other signal peptides can be used. Selection and inclusion of suitable signal peptides to facilitate expression, secretion, and incorporation of a modified J-chain into an anti-HIV IgM or IgA binding molecule as described herein is well within the capabilities of a person of ordinary skill in the art.

[0170] A person of ordinary skill in the art would readily be able to make additional modified J-chains according to the description described herein.

[0171] In certain embodiments such as those noted above, a modified J-chain associated with a dimeric or pentameric HIV binding molecule as described herein can

comprise an antigen binding domain that binds to an effector cell, *e.g.* a T cell, an NK cell, or a macrophage. In certain embodiments the effector cell is a T cell, *e.g.*, a cytotoxic T cell, expressing CD3, CD8, or a combination thereof. According to this embodiment,the J-chain can be modified by covalent attachment of a CD3 (CD3ε) binding domain or a CD8 binding domain. In this configuration, a dimeric or pentameric binding molecule as described herein provides binding specificity to a target HIV antigen, *e.g.*, an HIV spike protein, while the T-cell tethering of the J-chain through CD3 binding or CD8 binding delivers cytotoxic potency. The CD3 binding domain or CD8 binding domain covalently attached to a J-chain, or a variant thereof, can, for example be a single-chain Fv (scFv) of an anti-CD3 antibody, or a naturally occurring heavy chain only antibody, *e.g.* a camelid (camels, llamas, alpacas) or single-chain antibody of cartilaginous fish (sharks, rays), a scaffold, *e.g.* fibronectin (*e.g.* fibronectin III) with CD3 binding specificity.

[0172] In another embodiment,the effector cell is a Natural killer (NK) cell. NK cells are important components of the innate immunity and play a key role in host defense by virtue of their ability to release cytokines and to mediate cytolytic activity against tumor cells and virus-infected cells. NK cell antigens include, without limitation, CD16, CD32a, CD56, CD57, CD64, CD117 (or c-kit), adhesion molecules including lymphocyte-associated molecule-2 (LFA-2 or CD2), LFA-3 (CD58), and LFA-1 (CD11a/CD18). According to this embodiment,the J-chain can be modified, *e.g.*, by covalent attachment of a CD16 binding domain. In this configuration, a dimeric or pentameric binding molecule as described herein provides binding specificity to a target HIV antigen, *e.g.*, an HIV spike protein, while the NK-cell tethering of the J-chain delivers cytotoxic potency. The CD16 binding domain covalently attached to a J-chain, or a variant thereof can, for example be a single-chain Fv (scFv) of an anti-CD16 antibody, or a naturally occurring heavy chain only antibody (VHH), *e.g.* a camelid (camels, llamas, alpacas) or single-chain antibody of cartilaginous fish (sharks, rays), a scaffold, *e.g.* fibronectin (*e.g.* fibronectin III) with CD16 binding specificity.

[0173] In certain embodiments the modified J-chain comprises the mature VHH amino acid sequence SEQ ID NO: 112 (Table 2) fused to the N-terminus of the human J-chain

through a linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as CD16L_nJ, where n=5, 10, 15, or 20. CD16L_nJ can further include a signal peptide to facilitate transport and assembly into an IgM or IgA binding molecules. An exemplary modified J-chain according to this embodiment can comprise the amino acid sequence SEQ ID NO: 125, described in Example 1. In certain embodiments the mature modified J-chain comprises a VHH of SEQ ID NO: 112 fused to the C-terminus of the human J-chain through an amino acid linker, *e.g.*, GGGGS (L_5, SEQ ID NO: 101), GGGGSGGGGS (L_{10}, SEQ ID NO: 102), GGGGSGGGGSGGGGS (L_{15}, SEQ ID NO: 103), or GGGGSGGGGSGGGGSGGGGS (L_{20}, SEQ ID NO: 104), a modified J-chain referred to herein as JL_nCD16, where n=5, 10, 15, or 20. JL_nCD16 can further include a signal peptide, *e.g.*, amino acids 1 to 22 of SEQ ID NO: 2, to facilitate transport and assembly into an IgM or IgA binding molecules. In certain embodiments, other signal peptides can be used. Selection and inclusion of suitable signal peptides to facilitate expression, secretion, and incorporation of a modified J-chain into an anti-HIV IgM or IgA binding molecule as described herein is well within the capabilities of a person of ordinary skill in the art.

[0174] In another embodiment, the effector cell is a macrophage. According to this embodiment,the J-chain can be modified, *e.g.*, by covalent attachment of a CD14 binding domain. In this configuration, a dimeric or pentameric binding molecule as described herein provides binding specificity to a target HIV antigen, *e.g.*, an HIV spike protein, while the macrophage tethering of the J-chain delivers cytotoxic potency. The CD14 binding domain covalently attached to a J-chain, or a variant thereof can, for example be a single-chain Fv (scFv) of an anti-CD14 antibody, or a naturally occurring heavy chain only antibody, *e.g.* a camelid (camels, llamas, alpacas) or single-chain antibody of cartilaginous fish (sharks, rays), a scaffold, *e.g.* fibronectin (*e.g.* fibronectin III) with CD14 binding specificity.

[0175] In another embodiment,the effector cell is a neutrophil. According to this embodiment,the J-chain can be modified, *e.g.*, by covalent attachment of a CD16b or

CD177 binding domain. In this configuration, a dimeric or pentameric binding molecule as described herein provides binding specificity to a target HIV antigen, *e.g.*, an HIV spike protein, while the neutrophil tethering of the J-chain delivers cytotoxic potency. The CD16b or CD177 binding domain covalently attached to a J-chain, or a variant thereof can, for example be a single-chain Fv (scFv) of an anti-CD16b or CD177 antibody, or a naturally occurring heavy chain only antibody, *e.g.* a camelid (camels, llamas, alpacas) or single-chain antibody of cartilaginous fish (sharks, rays), a scaffold, *e.g.* fibronectin (*e.g.* fibronectin III) with CD16b or CD177 binding specificity.

Engineered HIV Antigen Binding Domains

[0176] In certain embodiments an HIV antigen binding domain as described herein can include as many as six immunoglobulin complementarity determining regions HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3, wherein at least one, at least two, at least three, at least four, at least five, or at least six CDRs are related to, or in some embodiments identical, or identical except for one, two, three, four, five, six, seven, eight, nine, or ten single amino acid substitutions, to the corresponding CDRs of the HIV mAbs set forth in Table 3, below.

[0177] Methods for genetically engineering cloned variable regions into immunoglobulin domains, and expressing and purifying such constructs are published and within the capability of one of skill in the art. (*See*, for instance, Wu *et al.*, *MAbs*, 1(4):339–47, 2009, and Wu *et al.*, *Nat. Biotechnol.*, 25:1290–7, 2007).

[0178] Exemplary peptide sequences encoding mature VH and VL domains that combine to form antibodies specific for HIV epitopes are set forth in Table 3. These sequences, or variants, fragments, or derivatives thereof, can then be engineered into a standard pentameric or hexameric IgM structure, as explained above, or an IgA structure.

Table 3: Exemplary Monoclonal Antibodies that Bind to HIV

Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
Wu *et al.*, *Science*, 329(5993):856-861, 2010; WO 2011/038290; USPN 8,637,036	CD4bs	5	QVQLVQSGGQMKKPGESMRISCRAS GYEFIDCTLNWIRLAPGKRPEWMGW LKPRGGAVNYARPLQGRVTMTRDVY SDTAFLELRSLTVDDTAVYFCTRGKN CDYNWDFEHWGRGTPVIVSS	6	EIVLTQSPGTLSLSPGETAIISCRTS QYGSLAWYQQRPGQAPRLVIYSG STRAAGIPDRFSGSRWGPDYNLTI SNLESGDFGVYYCQQYEFFGQGT KVQVDIKR
	CD4bs	7	QVQLVQSGGQMKKPGESMRISCQAS GYEFIDCTLNWVRLAPGRRPEWMGW LKPRGGAVNYARPLQGRVTMTRDVY SDTAFLELRSLTADDTAVYYCTRGKN CDYNWDFEHWGRGTPVTVSS	8	EIVLTQSPGTLSLSPGETAIISCRTS QYGSLAWYQQRPGQAPRLVIYSG STRAAGIPDRFSGSRWGPDYNLTI RNLESGDFGLYYCQQYEFFGQGT KVQVDIKR
	CD4bs	9	QVQLVQSGAVIKTPGSSVKISCRASGY NFRDYSIHWVRLIPDKGFEWIGWIKPL WGAVSYARQLQGRVSMTRQLSQDPD DPDWGVAYMEFSGLTPADTAEYFCV RRGSCDYCGDFPWQYWGQGTVVVV SS	10	EIVLTQSPGILSLSPGETATLFCKA SQGGNAMTWYQKRRGQVPRLLI YDTSRRASGVPDRFVGSGSGTDF FLTINKLDREDFAVYYCQQFEFFG LGSELEVHR
Wu *et al.*, *Science*, 333(6049):1593-1602, 2011	CD4bs	11	QVQLVQSGSGVKKPGASVRVSCWTS EDIFERTELIHWVRQAPGQGLEWIGW VKTVTGAVNFGSPDFRQRVSLTRDRD LFTAHMDIRGLTQGDTATYFCARQKF YTGGQGWYFDLWGRGTLIVVSS	12	EIVLTQSPGTLSLSPGETASLSCTA ASYGHMTWYQKKPGQPPKLLIFA TSKRASGIPDRFSGSQFGKQYTLT ITRMEPEDFARYYCQQLEFFGQG TRLEIRR
	CD4bs	13	QVQLVQSGSGVKKPGASVRVSCWTS EDIFERTELIHWVRQAPGQGLEWIGW VKTVTGAVNFGSPNFRHRVSLTRDRD LFTAHMDIRGLTQGDTATYFCARQKF ERGGQGWYFDLWGRGTLIVVSS	14	EIVLTQSPGTLSLSPGETASLSCTA ASYGHMTWYQKKPGQPPKLLIFA TSKRASGIPDRFSGSQFGKQYTLT ITRMEPEDFAGYYCQQVEFFGQG TRLEIR
	CD4bs	15	QVQLVQSGAAVRKPGASVTVSCKFA EDDDYSPYWVNPAPEHFIHFLRQAPG	16	DIQMTQSPSSLSASLGDRVTITCQ ASRGIGKDLNWYQQKAGKAPKL

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
			QQLEWLAWMNPTNGAVNYAWYLN GRVTATRDRSMTTAFLEVKSLRSDDT AVYYCARAQKRGRSEWAYAHWGQG TPVVVSS		LVSDASTLEGGVPSRFSGSGFHQ NFSLTISSLQAEDVATYFCQQYET FGQGTKVDIK
	CD4bs	17	QVQLVQSGAAVRKPGASVTVSCKFA EDDDYSPHWVNPAPEHYIHFLRQAPG QQLEWLAWMNPTNGAVNYAWQLH GRLTATRDGSMTTAFLEVRSLRSDDT AVYYCARAQKRGRSEWAYAHWGQG TPVAVSS	18	DIQMTQSPSSLSASLGDRVTITCQ ASRGIGKDLNWYQQKPGKAPKL LVSDASILEGGVPSRFSGSGFHQN FSLTISSLQPEDVATYFCQQYETF GQGTKVDIK
	CD4bs	19	QVQLVQSGAAVRKPGASVTVSCKFA EDDDFSPHWVNPAPEHYIHFLRQAPG QQLEWLAWMKPTNGAVNYAWQLQ GRVTVTRDRSQTTAFLEVKNLRSDDT AVYYCARAQKRGRSEWAYAHWGQG TPVVISA	20	DIQMTQSPSSLSASLGDRVTITCQ ASRGIGKDLNWYQQKPGRAPKL LVSDASILEGGVPTRFSGSGFHQN FSLTISSLQAEDVATYFCQQYETF GQGTKVDIK
	CD4bs	21	QVQLVQSGAAVRKPGASISVSCKFAD ADDYSPHWMNPAPEHYIHFLRQAPG QQLEWLAWMNPTNGAVNYAWYLN GRVTATRDRSMTTAFLEVRSLRSDDT AVYYCARAQKRARSEWAYAHWGQG TPVVVSS	22	DIQMTQSPSSLSASLGDRVTITCQ ASRGIGKDLNWYQQKRGRAPRL LVSDASVLEGGVPSRFSGSGFHQ NFSLTISTLQPEDVATYFCQQYET FGQGTKVDIK
	CD4bs	23	QVQLVQSGAAVRKPGASVTVSCKFA EDDDWSPHWVNPAPEHYIHFLRQAP GQQLEWLAWMNPTNGAVNYAWQL NGRLTATRDTSMTTAFLEVKSLRSDD TAVYYCARAQKRGRSEWAYAHWGQ GTPVVVSS	24	DIQMTQSPSSLSASLGDRVTITCQ ASRGIGKDLNWYQQKAGKAPKL LVSDASILEGGVPSRFSGSGFHQN FSLTISSLQPEDVATYFCQQYETF GQGTKVDIK
Lehman *et al.*, *Science*, 326(5950):285-289, 2009	V1/V2	25	QRLVESGGGVVQPGSSLRLSCAASGF DFSRQGMHWVRQAPGQGLEWVAFIK YDGSEKYHADSVWGRLSISRDNSKDT LYLQMNSLRVEDTATYFCVREAGGP	26	QSALTQPASVSGSPGQSITISCNGT SNDVGGYESVSWYQQHPGKAPK VVIYDVSKRPSGVSNRFSGSKSG NTASLTISGLQAEDEGDYYCKSL

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
			DYRNGYNYYDFYDGYYNYHYMDV WGKGTTVTVSS		TSTRRRVFGTGTKLTVL
	V1/V2	27	QEQLVESGGGVVQPGGSLRLSCLASG FTFHKYGMHWVRQAPGKGLEWVALI SDDGMRKYHSDSMWGRVTISRDNSK NTLYLQFSSLKVEDTAMFFCAREAGG PIWHDDVKYYDFNDGYYNYHYMDV WGKGTTVTVSS	28	QSALTQPASVSGSPGQTITISCNG TSSDVGGFDSVSWYQQSPGKAPK VMVFDVSHRPSGISNRFSGSKSG NTASLTISGLHIEDEGDYFCSSLT DRSHRIFGGGTKVTVL
Scheid *et al.*, *Science*, 333(6049):1633 -1637, 2011	CD4bs	29	QVQLLQSGAAVTKPGASVRVSCEASG YNIRDYFIHWWRQAPGQGLQWVGWI NPKTGQPNNPRQFQGRVSLTRHASW DFDTFSFYMDLKALRSDDTAVYFCAR QRSDYWDFDVWGSGTQVTVSSASTK GP	30	DIQMTQSPSSLSASVGDTVTITCQ ANGYLNWYQQRRGKAPKLLIYD GSKLERGVPSRFSGRRWGQEYNL TINNLQPEDIATYFCQVYEFVVPG TRLDLKRTVAAP
	CD4bs	31	QVRLSQSGGQMKKPGESMRLSCRAS GYEFLNCPINWIRLAPGRRPEWMGWL KPRGGAVNYARKFQGRVTMTRDVYS DTAFLELRSLTSDDTAVYFCTRGKYC TARDYYNWDFEHWGRGAPVTVSSAS TKGPSV	32	EIVLTQSPATLSLSPGETAIISCRTS QSGSLAWYQQRPGQAPRLVIYSG STRAAGIPDRFSGSRWGADYNLSI SNLESGDFGVYYCQQYEFFGQGT KVQVDIKRTVAAP
	Other	33	QIHLVQSGTEVKKPGSSVTVSCKAYG VNTFGLYAVNWVRQAPGQSLEYIGQI WRWKSSASHHFRGRVLISAVDLTGSS PPISSLEIKNLTSDDTAVYFCTTTSTYD KWSGLHHDGVMAFSSWGQGTLISVS AASTKGPSVF	34	DIQMTQSPSTLSASIGDTVRISCRA SQSITGNWVAWYQQRPGKAPRL LIYRGAALLGGVPSRFSGSAAGT DFTLTIGNLQAEDFGTFYCQQYD TYPGTFGQGTKVEVKRTVAAPSV FIFPPSDEQLKSGT
	CD4bs	35	QVHLSQSGAAVTKPGASVRVSCEASG YKISDHFIHWWRQAPGQGLQWVGWI NPKTGQPNNPRQFQGRVSLTRQASW DFDTYSFYMDLKAVRSDDTAIYFCAR QRSDFWDFDVWGSGTQVTVSSASTK GPSX	36	DIQMTQSPSSLSARVGDTVTITCQ ANGYLNWYQQRRGKAPKLLIYD GSKLERGVPARFSGRRWGQEYN LTINNLQPEDVATYFCQVYEFIVP GTRLDLKRTVAA

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
	CD4bs	37	SQHLVQSGTQVKKPGASVRVSCQAS GYTFTNYILHWWRQAPGQGLEWMG LIKPVFGAVNYARQFQGRIQLTRDIYR EIAFLDLSGLRSDDTAVYYCARDESG DDLKWHLHPWGQGTQVIVSPASTKG P	38	DIQMTQSPSSLSASVGDRVTINCQ AGQGIGSSLNWYQKKPGRAPKLL VHGASNLQRGVPSRFSGSGFHTT FTLTISSLQPDDVATYFCAVFQWF GPGTKVDIKRT
Walker *et al.*, *Nature*, 477(7365):466-70, 2011	V3glycan	39	QMQLQESGPGLVKPSETLSLTCSVSG ASISDSYWSWIRRSPGKGLEWIGYVH KSGDTNYSPSLKSRVNLSLDTSKNQV SLSLVAATAADSGKYYCARTLHGRRI YGIVAFNEWFTYFYMDVWGNGTQVT VSS	40	SDISVAPGETARISCGEKSLGSRA VQWYQHRAGQAPSLIIYNNQDRP SGIPERFSGSPDSPFGTTATLTITS VEAGDEADYYCHIWDSRVPTKW VFGGGTTLTVL
	V3glycan	41	QVHLQESGPGLVKPSETLSLTCNVSG TLVRDNYWSWIRQPLGKQPEWIGYV HDSGDTNYNPSLKSRVHLSLDKSKNL VSLRLTGVTAADSAIYYCATTKHGRR IYGVVAFKEWFTYFYMDVWGKGTSV TVSS	42	TFVSVAPGQTARITCGEESLGSRS VIWYQQRPGQAPSLIIYNNNDRPS GIPDRFSGSPGSTFGTTATLTITSV EAGDEADYYCHIWDSRRPTNWV FGEGTTLIVL
	V3glycan	43	QLHLQESGPGLVKPPETLSLTCSVSGA SINDAYWSWIRQSPGKRPEWVGYVH HSGDTNYNPSLKRRVTFSLDTAKNEV SLKLVDLTAADSATYFCARALHGKRI YGIVALGELFTYFYMDVWGKGTAVT VSS	44	SSMSVSPGETAKISCGKESIGSRA VQWYQQKPGQPPSLIIYNNQDRP AGVPERFSASPDFRPGTTATLTIT NVDAEDEADYYCHIYDARGGTN WVFDRGTTLTVL
	V3glycan	45	QSQLQESGPRLVEASETLSLTCNVSGE STGACTYFWGWVRQAPGKGLEWIGS LSHCQSFWGSGWTFHNPSLKSRLTISL DTPKNQVFLKLTSLTAADTATYYCAR FDGEVLVYNHWPKPAWVDLWGRGIP VTVTVSS	46	QSALTQPPSASGSPGQSITISCNGT ATNFVSWYQQFPDKAPKLIIFGV DKRPPGVPDRFSGSRSGTTASLTV SRLQTDDEAVYYCGSLVGNWDV IFGGGTTLTVL
	V3glycan	47	QPQLQESGPGLVEASETLSLTCTVSGD STAACDYFWGWVRQPPGKGLEWIGG	48	QSALTQPPSASGSPGQSISISCTGT SNRFVSWYQQHPGKAPKLVIYGV

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
			LSHCAGYYNTGWTYHNPSLKSRLTIS LDTPKNQVFLKLNSVTAADTAIYYCA RFDGEVLVYHDWPKPAWVDLWGRG TLVTVTVSS		NKRPSGVPDRFSGSKSGNTASLT VSGLQTDDEAVYYCSSLVGNWD VIFGGGTKLTVL
	V3glycan	49	QPQLQESGPGLVEASETLSLTCTVSGD STGRCNYFWGWVRQPPGKGLEWIGS LSHCRSYYNTDWTYHNPSLKSRLTISL DTPKNQVFLRLTSVTAADTATYYCAR FGGEVLVYRDWPKPAWVDLWGRGT LVTVSS	50	QSALTQPPSASGSPGQSITISCTGT SNNFVSWYQQYPGKAPKLVIYEV NKRPSGVPDRFSGSKSGSTASLTV SGLQADDEGVYYCSSLVGNWDV IFGGGTKLTVL
	V3glycan	51	QPQLQESGPTLVEASETLSLTCAVSGD STAACNSFWGWVRQPPGKGLEWVGS LSHCASYWNRGWTYHNPSLKSRLTL ALDTPKNLVFLKLNSVTAADTATYYC ARFGGEVLRYTDWPKPAWVDLWGR GTLVTVSS	52	QSALTQPPSASGSPGQSITISCTGT SNNFVSWYQQHAGKAPKLVIYD VNKRPSGVPDRFSGSKSGNTASL TVSGLQTDDEAVYYCGSLVGNW DVIFGGGTKLTVL
	V3glycan	53	QVQLQESGPGLVKPAETLSLTCSVSG ESINTGHYYWGWVRQVPGKGLEWIG HIHYTTAVLHNPSLKSRLTIKIYTLRN QITLRLSNVTAADTAVYHCVRSGGDI LYYYEWQKPHWFSPWGPGIHVTVSS	54	QSALTQPPSASGSLGQSVTISCNG TSSDIGGWNFVSWYQQFPGRAPR LIIFEVNKRPSGVPGRFSGSKSGNS ASLTVSGLQSDDEGQYFCSSLFG RWDVVFGGGTKLTVL
	V3glycan	55	QVQLQESGPGLVKPSETLSLTCTVSG DSINTGHHYWGWVRQVPGKGPEWIA HIHYNTAVLHNPALKSRVTISIFTLKN LITLSLSNVTAADTAVYFCVRSGGDIL YYIEWQKPHWFYPWGPGILVTVSS	56	QSALTQPPSASGSLGQSLTISCSGT GSDIGSWNFVSWYQQFPGRAPNL IIFEVNRRRSGVPDRFSGSKSGNT ASLTVSGLRSEDEAEYFCSSLSGR WDIVFGGGTKVTVL
	V3glycan	57	QLQMQESGPGLVKPSETLSLSCTVSG DSIRGGEWGDKDYHWGWVRHSAGK GLEWIGSIHWRGTTHYKESLRRRVSM SIDTSRNWFSLRLASVTAADTAVYFC ARHRHHDVFMLVPIAGWFDVWGPGV QVTVSS	58	EIVMTQSPDTLSVSPGETVTLSCR ASQNINKNLAWYQYKPGQSPRL VIFETYSKIAAFPARFVASGSGTEF TLTINNMQSEDVAVYYCQQYEE WPRTFGQGTKVDIK

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
	V1/V2	59	QVQLVQSGPEVKKPGSSVKVSCKASG NTFSKYDVHWVRQATGQGLEWVGW MSHEGDKTESAQRFKGRVTFTRDTSA STAYMELRGLTSDDTAIYYCTRGSKH RLRDYVLYDDYGLINYQEWNDYLEF LDVWGHGTAVTVSS	60	DTVVTQSPLSLPVTPGEAASMSCS STQSLRHSNGANYLAWYQHKPG QSPRLLIRLGSQRASGVPDRFSGS GSGTHFTLKISRVEAEDAAIYYC MQGLNRPWTFGKGTKLEIK
	V1/V2	61	QVQLEQSGAEVKKPGSSVKVSCKASG NTFSKYDVHWVRQATGQGLEWVGW MSHEGDKTESAQRFKGRVTFTRDTSA STAYMELRGLTSDDTAIYYCTRGSKH RLRDYVLYDDYGLINYQEWNDYLEF LDVWGHGTAVTVSS	62	DTVVTQSPLSLPVTPGEAASMSC TSTQSLRHSNGANYLAWYQHKP GQSPRLLIRLGSQRASGVPDRFSG SGSGTHFTLKISRVEPEDAAIYYC MQGLNRPWTFGKGTKLEIK
	V1/V2	63	QVQLVQSGAEVKKPGSSVKVSCKAS GNTFRKYDVHWVRQATGQGLEWVG WMSHEGDKTESAQRFKGRVSFTRDN SASTAYIELRGLTSDDTAIYYCTGGSK HRLRDYVLYDDYGLINQQEWNDYLE FLDVWGHGTAVTVSS	64	DTVVTQSPLSLSVTPGEAASMSC TSTQSLRHSNGANYLAWYQHKP GQSPRLLIRLGSQRASGVPDRFSG SGSGTHFTLKISRVEADDAAIYYC MQGLNRPWTFGKGTKLEIK
	V1/V2	65	QVQLVQSGAEVKKPGSSVKVSCKAS GNSFSNHDVHWVRQATGQGLEWMG WMSHEGDKTGLAQKFQGRVTITRDS GASTVYMELRGLTADDTAIYYCLTGS KHRLRDYFLYNEYGPNYEEWGDYLA TLDVWGHGTAVTVSS	66	EVVITQSPLFLPVTPGEAASLSCK CSHSLQHSTGANYLAWYLQRPG QTPRLLIHLATHRASGVPDRFSGS GSGTDFTLKISRVESDDVGTYYC MQGLHSPWTFGQGTKVEIK
	V1/V2	67	QVQLVQSGPEVKKPGSSVKVSCKASG NTFSKYDVHWVRQATGQGLEWVGW ISHERDKTESAQRFKGRVTFTRDTSAT TAYMELRGLTSDDTAIYYCTRGSKHR LRDYVLYDDYGLINYQEWNDYLEFL DVWGHGTAVTVSS	68	DTVVTQSPLSLPVTPGEAASMSCS STQSLRHSNGANYLAWYQHKPG QSPRLLIRLGSQRASGVPDRFSGS GSGTHFTLKISRVEAEDAAIYYC MQGLNRPWTFGKGTKLEIK
Zhu *et al.*, *J. Virol.*,	MPER	69	QVQLVQSGAEVKKPGESLKISCKVSG YNFASEWIGWVRQMPGKGLEWMGII	70	DIQLTQSPSSLSASLGDKVTITCR ASQHIKKYLNWYQQKPGKAPKL

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
85(21):11401-11408, 2011			YPGDSDTKYSPSFQGQVIISADKSINTAYLQWSSLKASDTAIYYCARQNHYGSGSYFYRTAYYYAMDVWGQGTTVTVSS		LIYGALNLQSGVPSRFSGRGSGTDFTLTISSLQPEDFATYYCQQSYSTPFTFGPGTKVDIKR
Liao *et al.*, *Nature*, 496(7446):469-476, 2013	CD4bs	71	SETLSLTCTVSGGSMGGTYWSWLRLSPGKGLEWIGYIFHTGETNYSPSLKGRVSISVDTSEDQFSLRLRSVTAADTAVYFCASLPRGQLVNAYFRNWGRGSLVSVTA	72	SYELTQPPSVSVSPGQTATITCSGASTNVCWYQVKPGQSPEVVIFENYKRPSGIPDRFSGSKSGSTATLTIRGTQAIDEADYYCQVWDSFSTFVFGSGTQVTVL
Huang *et al.*, *Nature*, 491(7424):406-412, 2012; WO 2013/070776	MPER	73	EVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNLRMEDSGLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSS	74	SYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFGGGTKLTVLX
WO 2013/086533	CD4bs	75	QVRLSQSGGQMKKPGDSMRISCRASGYEFINCPINWIRLAPGKRPEWMGWMKPRGGAVSYARQLQGRVTMTRDMYSETAFLELRSLTSDDTAVYFCTRGKYCTARDYYNWDFEHWGQGTPVTVSS	6	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEFFGQGTKVQVDIKR
WO 2013/142324	CD4bs/bl9	77	QVQLVQPGTAMKSLGSSLTITCRVSGDDLGSFHFGTYFMIWVRQAPGQGLEYMGGILPSTKTPTYAHKFRGRVSISAPGVPPVLSLALTNLTYDDTATYFCARERGRHFEPKNRDNLEGKFFDLWGRGTFVRVSP	78	QSALTQPASVSGSPGQSINISCAGRSDRVSWYQQRPNGVPKLLMFDVYRRPSGVSDRFSGSHSGDTAFLTISGLQTEDEADYYCTSHPYAFGAGTKVNVL
Diskin *et al.*, *J. Exp. Med.*, 210(6):1235-1249, 2013	CD4bs	79	XVRLSQSGGQMKKPGESMRLSCRASGYEFLNCPINWIRLAPGRRPEWMGWLKPRWGAVNYARKFQGRVTMTRDVYSDTAFLELRSLTSDDTAVYFCTRGKYCTARDYYNWDFEHWGRGAPVTVSS	80	EIVLTQSPATLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGADYNLSISNLESGDFGVYYCQQYEFFGQGTKVQVDIKR
Mouquet *et al.*,	V3 glycan	81	QVQLQESGPGLVKPSETLSVTCSVSG	82	SYVRPLSVALGETARISCGRQAL

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
PNAS USA, 109:E3268-E3277, 2012			DSMNNYYWTWIRQSPGKGLEWIGYIS DRESATYNPSLNSRVVISRDTSKNQLS LKLNSVTPADTAVYYCATARRGQRIY GVVSFGEFFYYYSMDVWGKGTTVTV SS		GSRAVQWYQHRPGQAPILLIYNN QDRPSGIPERFSGTPDINFGTRATL TISGVEAGDEADYYCHMWDSRS GFSWSFGGATRLTVLG
Huang, J. *et al. Nature* 515, 138–142 (2014)	face of contiguous areas of gp41 and gp120	83	QGQLVQSGAELKKPGASVKISCKTSG YRFNFYHINWIRQTAGRGPEWMGWIS PYSGDKNLAPAFQDRVIMTTDTEVPV TSFTSTGAAYMEIRNLKFDDTGTYFC AKGLLRDGSSTWLPYLWGQGTLLTV SS	84	QSVLTQSASVSGSLGQSVTISCTG PNSVCCSHKSISWYQWPPGRAPT LIIYEDNERAPGISPRFSGYKSYW SAYLTISDLRPEDETTYYCCSYTH NSGCVFGTGTKVSVL
Bonsignori M, *et al.*, J Virol. 85(19):9998-10009 2011.	V1/V2	85	EVQLVESGANVVRPGGSLRLSCKASG FIFENFGFSWVRQAPGKGLQWVAGL NWNGGDTRYADSVKGRFRMSRDNSR NFVYLDMDKVGVDDTAFYYCARGT DYTIDDAGIHYQGSGTFWYFDLWGR GTLVSVSS	86	EIVLAQSPGTLSLSPGERATLSCR ASHNVHPKYFAWYQQKPGQSPR LLIYGGSTRAAGIPGKFSGSGSGT DFTLTISRVDPEDFAVYYCQQYG GSPYTFGQGTKVEIK
	V1/V2	87	EVQLVESGGSVVRPGGSLRLSCRASG FIFENYGLTWVRQVPGKGLHWVSGM NWNGGDTRYADSVRGRFSMSRDNSN NIAYLQMNNLRVEDTALYYCARGTD YTIDDQGRFYQGSGTFWYFDFWGRG TLVTVSS	88	EIVLTQSPATLSVSPGERATLSCR ASQNVHPRYFAWYQQKRGQSPR LLIHSGSTRAAGIADRFSGGGSGM HFTLTITRVEPEDFAVYFCQQYG GSPYTFGQGTRVELR
	V1/V2	89	EVQLVESGGGVVRPGGSLRLSCAASG FIFENYGLTWVRQVPGKGLHWVSGM NWNGGDTRYADSVRGRFSMSRDNSN NIAYLQMKNLRVDDTALYYCARGTD YTIDDQGIFYKGSGTFWYFDLWGRGT LVTVSS	90	EIVLTQSPATLSLSPGERATLSCR ASQSVHPKYFAWYQQKPGQSPR LLIYSGSTRAAGIADRFSGGGSGI HFTLTITRVEPEDFAVYFCQQYG GSPYTFGQGTKVELR
	V1/V2	91	EVQLVESGGGLIRPGGSLRLSCKGSGF IFENFGFGWVRQGPGKGLEWVSGTN WNGGDSRYGDSVKGRFTISRDNSNNF	92	EIVLTQSPDTLSLSPGERATLSCR ASQSVHSRYFAWYQHKPGQPPRL LIYGGSTRATGIPNRFSAGGSGTQ

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Reference	Binding Site	VH SEQ ID NO	VH Sequence	VL SEQ ID NO	VL Sequence
			VYLQMNSLRPEDTAIYYCARGTDYTI DDQGIRYQGSGTFWYFDVWGRGTLV TVSS		FTLTVNRLEAEDFAVYYCQQYGR SPYTFGQGTKVEIR
Sok, D. *et al.*, *Proc. Natl. Acad. Sci. USA* 111:17624-17629 (2014)		93	QVHLTQSGPEVRKPGTSVKVSCKAPG NTLKTYDLHWVRSVPGQGLQWMGW ISHEGDKKVIVERFKAKVTIDWDRST NTAYLQLSGLTSGDTAVYYCAKGSK HRLRDYALYDDDGALNWAVDVDYL SNLEFWGQGTAVTVSS	94	DFVLTQSPHSLSVTPGESASISCKS SHSLIHGDRNNYLAWYVQKPGRS PQLLIYLASSRASGVPDRFSGSGS DKDFTLKISRVETEDVGTYYCMQ GRESPWTFGQGTKVDIK
Buchacher, A., *et al.*, AIDS Res. Hum. Retroviruses 10:359–369 (1994); WO 2011/035205	V3/glycan	95	EVQLVESGGGLVKAGGSLILSCGVSN FRISAHTMNWVRRVPGGGLEWVASIS TSSTYRDYADAVKGRFTVSRDDLEDF VYLQMHKMRVEDTAIYYCARKGSDR LSDNDPFDAWGPGTVVTVSP	96	DIQMTQSPSTLSASVGDTITITCRA SQSIETWLAWYQQKPGKAPKLLI YKASTLKTGVPSRFSGSGSGTEFT LTISGLQFDDFATYHCQHYAGYS ATFGQGTRVEIK
Pincus SH, *et al*. J Immunol 170: 2236–2241(2003)	The immuno-dominant region of gp41	97	QVQLVQSGGGVFKPGGSLRLSCEASG FTFTEYYMTWVRQAPGKGLEWLAYI SKNGEYSKYSPSSNGRFTISRDNAKNS VFLQLDRLSADDTAVYYCARADGLT YFSELLQYIFDLWGQGARVTVSS	98	DIVMTQSPDSLAVSPGERATIHCK SSQTLLYSSNNRHSIAWYQQRPG QPPKLLLYWASMRLSGVPDRFSG SGSGTDFTLTINNLQAEDVAIYYC HQYSSHPPTFGHGTRVEIK
Moore, J.P., and J. Sodroski J. Virol 70:1863-1872 (1996); WO 2006/044410	CD4bs	99	QVQLQESGPGLVKPSQTLSLSCTVSG GSSSSGAHYWSWIRQYPGKGLEWIGY IHYSGNTYYNPSLKSRITISQHTSENQF SLKLNSVTVADTAVYYCARGTRLRTL RNAFDIWGQGTMVTVSS	100	QSVLTQPPSASGSPGQSVTISCTG TSSDVGGYNYVSWYQHHPGKAP KLIISEVNNRPSGVPDRFSGSKSG NTASLTVSGLQAEDEAEYYCSSY TDIHNFVFGGGTKLTVLR

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[0179] In certain embodiments the HIV antigen binding domain of a dimeric, hexameric, or pentameric binding molecule as described herein comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein the VH region, the VL region, or both the VH and VL regions are related to the corresponding VH and VL of HIV monoclonal antibodies disclosed in the references set forth in Table 3, above. In certain embodiments, the binding molecules described herein exhibit greater potency than an IgG antibody comprising the VH and VL of antibodies listed in Table 3.

[0180] In certain embodiments the VH can comprise an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one or more of the amino acid sequences of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99.

[0181] In certain embodiments the VL can comprise an amino acid sequence at least at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one or more of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74,

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SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100.

[0182] In certain embodiments the VH and VL amino acid sequences can comprise amino acid sequences at least at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 6, SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 81 and SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86, SEQ ID NO: 87 and SEQ ID NO: 88, SEQ ID NO: 89 and SEQ ID NO: 90, SEQ ID NO: 91 and SEQ ID NO: 92, SEQ ID NO: 93 and SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96, SEQ ID NO: 97 and SEQ ID NO: 98, or SEQ ID NO: 99 and SEQ ID NO: 100, respectively.

[0183] In certain embodiments the HIV antigen binding domain of a dimeric, hexameric, or pentameric binding molecule as described herein comprises the HCDR1, HCDR2, and HCDR3 regions, or HCDR1, HCDR2, and HCDR3 regions containing one or

two single amino acid substitutions, and the LCDR1, LCDR2, and LCDR3 regions, or LCDR1, LCDR2, and LCDR3 containing one or two single amino acid substitutions, of the VH and VL amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 6, SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 81 and SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86, SEQ ID NO: 87 and SEQ ID NO: 88, SEQ ID NO: 89 and SEQ ID NO: 90, SEQ ID NO: 91 and SEQ ID NO: 92, SEQ ID NO: 93 and SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96, SEQ ID NO: 97 and SEQ ID NO: 98, or SEQ ID NO: 99 and SEQ ID NO: 100.

[0184] In certain embodiments a hexameric or pentameric IgM antibody designated herein as HIV02M is described comprising an IgM heavy chain comprising the amino acid sequence SEQ ID NO: 114 and a kappa light chain comprising the amino acid sequence SEQ ID NO: 115. In certain embodiments a hexameric or pentameric IgM antibody designated herein as HIV12M is described comprising an IgM heavy chain comprising the amino acid sequence SEQ ID NO: 117 and a kappa light chain

comprising the amino acid sequence SEQ ID NO: 118. In certain embodiments a hexameric or pentameric IgM antibody designated herein as HIV32M is described comprising an IgM heavy chain comprising the amino acid sequence SEQ ID NO: 120 and a kappa light chain comprising the amino acid sequence SEQ ID NO: 121. In certain embodiments a hexameric or pentameric IgM antibody designated herein as HIV72M is described comprising an IgM heavy chain comprising the amino acid sequence SEQ ID NO: 123 and a kappa light chain comprising the amino acid sequence SEQ ID NO: 124. Where the IgM antibody HIV02M, HIV12M, HIV32M, or HIV72M is pentameric, it can further include a J-chain or functional fragment thereof, *e.g.*, a wild-type human J-chain comprising amino acids 23 to 159 of SEQ ID NO: 2, or a modified J-chain as described elsewhere herein, *e.g.*, a J-chain comprising the formula X[L_n]J or J[L_n]X, where J is a native J-chain or functional fragment thereof, *e.g.*, a native human J-chain (amino acids 23 to 159 of SEQ ID NO: 2), X is a binding domain, *e.g.*, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, or SEQ ID NO: 112, and [L_n] is a linker sequence consisting of n amino acids, where n is a positive integer, *e.g.*, from 1 to 100, 1 to 50, or 1 to 25. In certain embodiments n=5, 10, 15, or 20, *e.g.*, SEQ ID NO: 101, SEQ ID NO: 102, SEQ ID NO: 103, or SEQ ID NO: 104. In certain embodiments the modified J-chain can comprise or consist of V5J (SEQ ID NO: 108), V10J (SEQ ID NO: 109), V15J (SEQ ID NO: 110), V20J (SEQ ID NO: 111), C15J (SEQ ID NO: 125), or any combination thereof.

[0185] In certain embodiments an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M, either without a J-chain or further comprising a wild-type or modified J-chain as described herein can exhibit greater potency than a single binding unit antibody, *e.g.*, an IgG antibody comprising the corresponding VH and VL of an antibody listed in Table 3. For example, an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can more potently neutralize HIV, bind and neutralize more diverse HIV variants or clades, enhance viral clearance, and/or be more potent in preventing, controlling or treating HIV infection than a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains.

Moreover, an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can be more potent in controlling HIV infectivity and growth as compared with a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains. In addition, an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can be used to treat chronic infection, *e.g.*, by binding to and/or effecting antibody and/or cell-mediated killing of HIV infected cells, *e.g.*, reservoir cells that express extremely low levels of HIV antigens on their surface. In a further example, an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can be more effective at activating and killing such HIV-infected cells or killing such cells after activation with an independent activating agent such as an effector cell, *e.g.*, a T-cell. In a further example, an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can describe equivalent benefit at a lower dosage than that of a corresponding reference single binding unit molecule comprising only two HIV antigen binding domains. In certain embodiments, administration of an IgM antibody as described herein, *e.g.*, an IgM antibody comprising HIV02M, HIV12M, HIV32M, or HIV72M can allow for reduced or modified dosages of other anti-retroviral therapies, such as ART (see, *e.g.*, Example 7 below).

[0186] While a variety of different dimeric, hexameric, and pentameric binding molecules can be contemplated by a person of ordinary skill in the art based on this disclosure, and as such are included in this disclosure, in certain embodiments, a binding molecule as described above is described in which each binding unit comprises two IgM heavy chains each comprising a VH situated amino terminal to the IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region. In certain embodiments, a binding molecule as described above is included in which each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA constant region or fragment thereof, and two

immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region.

[0187] Moreover in certain embodiments, at least one binding unit of the binding molecule, or two, three, four, five, or six binding units of the binding molecule, each comprise two of the HIV antigen binding domains as described above. In certain embodiments the two HIV antigen binding domains in the one binding unit of the binding molecule, or two, three, four, five, or six binding units of the binding molecule, can be different from each other, or they can be similar or identical.

[0188] In certain embodiments, the two IgA heavy chains within one binding unit of the binding molecule, or two binding units of the binding molecule, are identical. In certain embodiments, the two IgM heavy chains within one binding unit of the binding molecule, or two, three, four, five, or six binding units of the binding molecule, are identical.

[0189] In certain embodiments, the two light chains within one binding unit of the binding molecule, or two, three, four, five, or six binding units of the binding molecule, are identical. In certain embodiments, two identical light chains within at least one binding unit, or within two, three, four, five, or six binding units of the binding molecule are kappa light chains, *e.g.*, human kappa light chains, or lambda light chains, *e.g.*, human lambda light chains.

[0190] In certain embodiments at least one, two, three, four, five, or six binding units of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody described by this disclosure comprises or each comprise two identical IgA or IgM heavy chains, and two identical light chains. According to this embodiment,the HIV antigen binding domains in the one binding unit of the binding molecule, or two, three, four, five, or six binding units of the binding molecule, can be identical. Further according to this embodiment,a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein can comprise at least one, two, three, four, five, six, seven, eight, nine, ten, eleven, or twelve copies of an HIV antigen binding domain as described above. In certain embodiments at least two, at least three, at least four, at least five, or at least six of the binding units can be identical and, in certain embodiments the binding units can comprise identical

antigen binding domains, *e.g.*, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve HIV antigen binding domains can be identical.

[0191] In certain embodiments, a dimeric, pentameric, or hexameric HIV binding molecule as described herein can possess advantageous structural and/or functional properties, or "improved binding characteristics," as compared to other binding molecules, such as a corresponding reference single binding unit molecule comprising the same antigen binding domain. For example, the dimeric, pentameric, or hexameric HIV binding molecule can possess improved activity or potency in a biological assay, either *in vitro* or *in vivo*, relative to a corresponding reference single binding unit molecule, *e.g.*, an IgG1 binding molecule comprising the same VH and VL region sequences as are present in the multimeric binding molecule, as described above. Biological assays include, but are not limited to, Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) assays, T-cell dependent Cellular Cytotoxicity (TDCC) assays, Complement-Dependent Cytotoxicity (CDC) assays, Cell-To-Cell Spread (CTCS) assays, viral transcytosis assays, complement-dependent virolysis assays, virus neutralization assays, cell attachment assays, viral egress assays, immunohistochemical assays, direct cytotoxicity assays, complement-mediated cytotoxicity assays, etc. Suitable HIV glycoprotein-expressing cells for performing such assays include, but are not limited to .g., CHO-gp120, CHO-gp140, Jurkat-522 F/Y cells, or mammalian cells expressing membrane anchored trimeric forms of gp140, *e.g.*, strain JR-FL (Go *et al.* 2015 *J Virol 89*:8245-8257). In certain embodiments a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein can direct HIV neutralization, and/or clearance or killing of an HIV-infected cells, such as reservoir cells, at higher potency than an equivalent amount of a monospecific, bivalent IgG1 antibody or fragment thereof that specifically binds to the same HIV epitope as the HIV antigen binding domain.

[0192] By "potency" or "binding characteristics" refers to the ability of a binding molecule to achieve a given biological result. For example, potency can be referred to as the amount of a given binding molecule necessary to achieve a given biological result (EC100 or IC100) or the amount of a given binding molecule necessary to achieve

50% of a desired biological result (EC50 or IC50). Biological results can include, for example, binding to recombinant gp120 or gp41, binding to gp120/41 expressing cells, binding to chronically and/or latently infected cell lines, neutralization of HIV viruses or HIV pseudo-typed viruses, neutralization of more diverse HIV viruses or HIV pseudo-typed viruses, killing of latent HIV-infected cells, reduction of HIV virus or HIV infected cells in therapeutic animal models, or prolonged absence of HIV virus after cessation of ART.

Polynucleotides, Vectors, and Host Cells

[0193] The disclosure further includes a polynucleotide, *e.g.*, an isolated, recombinant, and/or non-naturally-occurring polynucleotide, comprising a nucleic acid sequence that encodes a polypeptide subunit of the dimeric, hexameric, or pentameric binding molecule as described above. By "polypeptide subunit" is meant a portion of a binding molecule, binding unit, or antigen binding domain that can be independently translated. Examples include, without limitation, an antibody variable domain, *e.g.*, a VH or a VL, a J-chain, a secretory component, a single chain Fv, an antibody heavy chain, an antibody light chain, an antibody heavy chain constant region, an antibody light chain constant region, and/or any fragment, variant, or derivative thereof.

[0194] In certain embodiments, the polypeptide subunit can comprise an IgM or an IgA heavy chain constant region or fragment thereof, and VH portion of an HIV antigen binding domain. In certain embodiments the polynucleotide can encode a polypeptide subunit comprising a human IgM or IgA constant region or fragment thereof fused to the C-terminal end of a VH, where the VH comprises the HCDR1, HCDR2, and HCDR3 regions, or the HCDR1, HCDR2, and HCDR3 regions containing one or two single amino acid substitutions of a VH comprising the amino acid sequence SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59,

SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99.

[0195] In certain embodiments, the polypeptide subunit can comprise an antibody VL portion of an HIV antigen binding domain as described above. In certain embodiments the polypeptide subunit can comprise a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL, where the VL comprises LCDR1, LCDR2, and LCDR3 regions, or the LCDR1, LCDR2, and LCDR3 regions containing one or two single amino acid substitutions of a VL comprising the amino acid sequence SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100.

[0196] In certain embodiments the polynucleotide can encode a polypeptide subunit comprising a human IgM or IgA constant region or fragment thereof fused to the C-terminal end of a VH, where the VH comprises an amino acid sequence at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one or more of the amino acid sequences of SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO:

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43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99.

[0197] In certain embodiments the polynucleotide can encode a polypeptide subunit comprising a human light chain constant region or fragment thereof fused to the C-terminal end of a VL, where the VL comprises an amino acid sequence at least at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or 100% identical to any one or more of SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100.

[0198] Thus, to form the antigen binding domains, the variable regions of antibodies that specifically bind to an HIV antigen, *e.g.*, that specifically bind to an epitope on the HIV spike protein, can be inserted into expression vector templates for IgM and/or IgA structures, thereby creating multimeric binding molecules having at least two bivalent binding units. In brief, nucleic acid sequences encoding the heavy and light chain variable domain sequences can be synthesized or amplified from existing molecules, and inserted into vectors in the proper orientation and in frame such that upon expression, the vector will yield a full length heavy or light chain. Vectors useful for these purposes are known in the art. Such vectors can also comprise enhancer and other sequences needed to achieve expression of the desired chains.

Multiple vectors or single vectors can be used. These vectors are transfected into host cells and then the chains are expressed and purified. Upon expression the chains form fully functional multimeric binding molecules, as has been reported in the literature. The fully assembled multimeric binding molecules can then be purified by standard methods. The expression and purification processes can be performed at commercial scale, if needed.

[0199] The disclosure further includes a composition comprising two or more polynucleotides, where the two or more polynucleotides collectively can encode a dimeric, hexameric, or pentameric binding molecule as described above. In certain embodiments the composition can include a polynucleotide encoding an IgM and/or IgA heavy chain or fragment thereof, *e.g.*, a human IgM heavy chain as described above where the IgM and/or IgA heavy chain comprises at least the VH of an HIV antigen binding domain, and a polynucleotide encoding a light chain or fragment thereof, *e.g.*, a human kappa or lambda light chain that comprises at least the VL of an HIV antigen binding domain. A polynucleotide composition as described can further include a polynucleotide encoding a J-chain, *e.g.*, a human J-chain, or a fragment, variant, or derivative thereof. In certain embodiments the polynucleotides making up a composition as described herein can be situated on two, three, or more separate vectors, *e.g.*, expression vectors. Such vectors are included in the disclosure. In certain embodiments two or more of the polynucleotides making up a composition as described herein can be situated on a single vector, *e.g.*, an expression vector. Such a vector is included in the disclosure.

[0200] The disclosure further includes a host cell, *e.g.*, a prokaryotic or eukaryotic host cell, comprising a polynucleotide or two or more polynucleotides encoding a dimeric, pentameric, or hexameric HIV binding molecule as described herein, or any subunit thereof, a polynucleotide composition as described herein, or a vector or two, three, or more vectors that collectively encode a dimeric, pentameric, or hexameric HIV binding molecule as described herein, or any subunit thereof. In certain embodiments a host cellincluded in the disclosure can express a dimeric, pentameric, or hexameric HIV binding molecule as included in this disclosure, or a subunit thereof.

[0201] In a related embodiment,the disclosure includes a method of producing a dimeric, pentameric, or hexameric HIV binding molecule as included in this disclosure, where the method comprises culturing a host cell as described above, and recovering the binding molecule.

Methods of Use

[0202] This disclosure includes improved methods for preventing, controlling, or treating HIV infection, and/or methods for neutralizing HIV infectivity, *e.g.*, across two or more types, groups, or clades, using a dimeric IgA-based HIV binding molecule, or pentameric or hexameric IgM-based HIV binding molecule. The methods described below can utilize multimeric binding molecules comprising HIV antigen binding domains including without limitation, the antibodies and corresponding VH and VL sequences disclosed in the references set forth in Table 3, or variants, derivatives, or analogs thereof, where the dimeric, pentameric, or hexameric HIV binding molecule can provide improved virus neutralization and/or clearance potency as compared to a corresponding reference single binding unit molecule, fragment, variant, derivative, or analog, as disclosed and explained above. Exemplary corresponding single binding unit molecules are described in the references presented in Table 3. Based on this disclosure, construction of a dimeric IgA binding molecule, or pentameric or hexameric IgM binding molecule comprising any HIV-specific antigen binding domain of interest is well within the capabilities of a person of ordinary skill in the art. The improved binding characteristics of such compositions can, for example, allow a reduced dose to be used, or can result in more effective neutralization of viruses resistant to neutralization by the original antibody, as explained above. By "resistant" is meant any degree of reduced activity of an HIV antibody, on HIV infectivity, replication, release, etc.

[0203] In certain embodiments, this disclosure includes a method for directing improved neutralization of HIV, or killing of HIV infected cells, where the method includes contacting an HIV, or an HIV-infected cell with a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein, where the binding molecule can direct virus neutralization, or killing of HIV reservoir cells, at a higher

potency than an equivalent amount of a corresponding reference single binding unit molecule, *e.g.*, a monospecific, bivalent IgG antibody or fragment thereof. In certain embodiments a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein can direct virus neutralization of two or more HIV types, subtypes or clades at higher potency than an equivalent amount of a corresponding reference single binding unit molecule, where the corresponding reference single binding unit molecule is, or comprises similar or identical VH and VL regions as at least one binding unit of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein.

[0204] In certain embodiments, this disclosure includes a method for testing the ability of a given binding molecule to bind to and effect killing of HIV reservoir cells. The method includes providing cells, *e.g.*, cells from chronically infected HIV patients, or a series of recombinant cell lines that express an HIV antigen, *e.g.*, an HIV protein, *e.g.*, to an epitope on the HIV spike protein, *e.g.*, gp120 and/or gp41 at a series of predetermined levels from high copy number down to low, or even a single copy number. The cells can then be contacted with a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein under conditions that would allow antibody-dependent, T-cell dependent, or complement-dependent killing of the cells, and recovering those binding molecules that can effect killing of the cells expressing the lowest copy numbers of the HIV antigen. In certain embodiments the dimeric, pentameric, or hexameric HIV binding molecule can direct killing of cells expressing a lower copy number of the HIV antigen than cells killed by an equivalent amount of a corresponding reference single binding unit molecule, *e.g.*, a monospecific, bivalent IgG antibody or fragment thereof.

[0205] For instance, methods include screening of various binding molecules whose affinities and/or avidities for enveloped HIV viral particles of a different type, group, or clade, have not been determined. The present methods can be employed to identify more broadly neutralizing binding molecules that bind to the surface of HIV viral particles, on the surface if HIV-infected cells, such as reservoir cells, or a combination thereof. In this manner, additional binding molecules useful in the methods of the present disclosure can be identified and utilized, as disclosed herein.

[0206] This screening method can be accomplished by contacting a test binding molecule known to specifically bind to an HIV or HIV-infected cell of a first type, group, or clade with an HIV or HIV-infected cell of a second type, group, or clade, and measuring the affinity and/or avidity of the test binding molecule for binding to the second HIV or infected cell. The dimeric, pentameric, or hexameric HIV binding molecule can thus be tested for cross-reactivity that might not have been evident for a single binding unit molecule having the same antigen binding domains.

[0207] The cells in such methods can be any cell capable of being infected by HIV, such as a human cell.

[0208] In certain embodiments, this disclosure includes a method for directing more broadly cross-reacting neutralization of HIV, or killing of HIV reservoir cells, where the method includes contacting an HIV virion, or an HIV-infected cell with a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein, where the virus is of a different type, group, or clade than that typically bound by the one or more antigen binding domains of the binding molecule, where the binding molecule can direct virus neutralization, or killing of HIV reservoir cells of the different type, group, or clade, at a higher potency than an equivalent amount of a corresponding reference single binding unit molecule, *e.g.*, a monospecific, bivalent IgG antibody or fragment thereof. In certain embodiments a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein can direct virus neutralization of more HIV types, subtypes or clades than an equivalent amount of a corresponding reference single binding unit molecule, where the corresponding reference single binding unit molecule is, or comprises similar or identical VH and VL regions as at least one binding unit of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein.

[0209] For instance, methods include screening of various binding molecules whose affinities and/or avidities for enveloped HIV viral particles of a different type, group, or clade, have not been determined. The present methods can be employed to identify more broadly neutralizing binding molecules that bind to the surface of HIV viral particles, on the surface if HIV-infected cells, such as reservoir cells, or a

combination thereof. In this manner, additional binding molecules useful in the methods of the present disclosure can be identified and utilized, as disclosed herein.

[0210] This screening method can be accomplished by contacting a test binding molecule known to specifically bind to an HIV or HIV-infected cell of a first type, group, or clade with an HIV or HIV-infected cell of a second type, group, or clade, and measuring the affinity and/or avidity of the test binding molecule for binding to the second HIV or infected cell. The dimeric, pentameric, or hexameric HIV binding molecule can thus be tested for cross-reactivity that might not have been evident for a single binding unit molecule having the same antigen binding domains.

[0211] The cells in such methods can be any cell capable of being infected by HIV, such as a human cell.

[0212] Dimeric, pentameric, or hexameric HIV binding molecules for use in the methods described herein can possess advantageous structural or functional properties compared to other binding molecules. For example, a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody for use in the methods described herein can possess improved binding characteristics in a biological assay, as described above, either *in vitro* or *in vivo*, than a corresponding reference single binding unit molecule, *e.g.*, IgG or a variant, analog, or derivative thereof, as also described above. Biological assays include, but are not limited to, T-cell Dependent Cell-mediated Cytotoxicity assays (TDCC), Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) assays, Complement-Dependent Cytotoxicity (CDC) assays, Cell-To-Cell Spread (CTCS) assays, complement-dependent virolysis assays, virus neutralization assays, cell attachment assays, viral egress assays, immunohistochemical assays, or direct cytotoxicity assays.

Pharmaceutical Compositions and Administration Methods

[0213] Methods of preparing and administering a multimeric, *e.g.*, a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody as described herein to a subject in need thereof are well known to or are readily determined by those skilled in the art in view of this disclosure. The route of administration of a multimeric binding molecule can be, for example, oral, parenteral, by inhalation or topical. The

term parenteral as used herein includes, *e.g.*, intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, rectal, or vaginal administration. While these forms of administration are contemplated as suitable forms, another example of a form for administration would be a solution for injection, in particular for intravenous or intraarterial injection or drip. A suitable pharmaceutical composition can comprise a buffer (*e.g.* acetate, phosphate or citrate buffer), a surfactant (*e.g.* polysorbate), and in some embodiments a stabilizer agent (*e.g.* human albumin).

[0214] A dimeric, pentameric, or hexameric HIV binding molecule as described herein can be administered in a pharmaceutically effective amount for the *in vivo* treatment of diseases or disorders in which it is desirable to clear, remove or otherwise eliminate an HIV infection in a subject infected with HIV. In this regard, it will be appreciated that the disclosed multimeric binding molecules can be formulated so as to facilitate administration and promote stability of the active agent. Pharmaceutical compositions accordingly can comprise a pharmaceutically acceptable, non-toxic, sterile carrier such as physiological saline, non-toxic buffers, preservatives and the like. A pharmaceutically effective amount of a dimeric, pentameric, or hexameric HIV binding molecule as described herein means an amount sufficient to achieve effective binding to a target and to achieve a therapeutic benefit. Suitable formulations are described in Remington's Pharmaceutical Sciences (Mack Publishing Co.) 16th ed. (1980).

[0215] Certain pharmaceutical compositions described herein can be orally administered in an acceptable dosage form including, *e.g.*, capsules, tablets, aqueous suspensions or solutions. Certain pharmaceutical compositions also can be administered by nasal aerosol or inhalation. Such compositions can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other conventional solubilizing or dispersing agents.

[0216] The amount of a dimeric, pentameric, or hexameric HIV binding molecule that can be combined with carrier materials to produce a single dosage form will vary depending, *e.g.*, upon the subject treated and the particular mode of administration. The composition can be administered as a single dose, multiple doses or over an

established period of time in an infusion. Dosage regimens also can be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response).

[0217] In keeping with the scope of the present disclosure, a dimeric, pentameric, or hexameric HIV binding molecule as described herein can be administered to a subject in need of therapy in an amount sufficient to produce a therapeutic effect. A multimeric binding molecule as described herein can be administered to the subject in a conventional dosage form prepared by combining the antibody or antigen binding fragment, variant, or derivative thereof of the disclosure with a conventional pharmaceutically acceptable carrier or diluent according to known techniques. The form and character of the pharmaceutically acceptable carrier or diluent can be dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables.

[0218] By "therapeutically effective dose or amount" or "effective amount" is intended an amount of a dimeric, pentameric, or hexameric HIV binding molecule, that when administered brings about a positive therapeutic response with respect to treatment of a patient with a disease or condition to be treated.

[0219] Therapeutically effective doses of the compositions disclosed herein, for treatment of HIV infection is desired, can vary depending upon many different factors, including means of administration, target site, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. In certain embodiments, the subject or patient is a human, but non-human mammals including transgenic mammals can also be treated. Treatment dosages can be titrated using routine methods known to those of skill in the art to optimize safety and efficacy.

[0220] The amount of a dimeric, pentameric, or hexameric HIV binding molecule, *e.g.*, an IgM antibody to be administered is readily determined by one of ordinary skill in the art without undue experimentation given this disclosure. Factors influencing the mode of administration and the respective amount of a multimeric binding molecule include, but are not limited to, the severity of the disease, the history of the disease, and the age, height, weight, health, and physical condition of the individual undergoing therapy. Similarly, the amount of a dimeric, pentameric, or hexameric

HIV binding molecule to be administered will be dependent upon the mode of administration and whether the subject will undergo a single dose or multiple doses of this agent.

[0221] This disclosure also includes the use of a dimeric, pentameric, or hexameric HIV binding molecule in the manufacture of a medicament for treating, preventing, or managing a disease or disorder caused by HIV infection.

[0222] This disclosure employs, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are explained fully in the literature. (See, for example, Sambrook *et al.*, ed. (1989) Molecular Cloning A Laboratory Manual (2nd ed.; Cold Spring Harbor Laboratory Press); Sambrook *et al.*, ed. (1992) Molecular Cloning: A Laboratory Manual, (Cold Springs Harbor Laboratory, NY); D. N. Glover ed., (1985) DNA Cloning, Volumes I and II; Gait, ed. (1984) Oligonucleotide Synthesis; Mullis *et al.* U.S. Pat. No. 4,683,195; Hames and Higgins, eds. (1984) Nucleic Acid Hybridization; Hames and Higgins, eds. (1984) Transcription And Translation; Freshney (1987) Culture Of Animal Cells (Alan R. Liss, Inc.); Immobilized Cells And Enzymes (IRL Press) (1986); Perbal (1984) A Practical Guide To Molecular Cloning; the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Miller and Calos eds. (1987) Gene Transfer Vectors For Mammalian Cells, (Cold Spring Harbor Laboratory); Wu *et al.*, eds., Methods In Enzymology, Vols. 154 and 155; Mayer and Walker, eds. (1987) Immunochemical Methods In Cell And Molecular Biology (Academic Press, London); Weir and Blackwell, eds., (1986) Handbook Of Experimental Immunology, Volumes I-IV; Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1986); and in Ausubel *et al.* (1989) Current Protocols in Molecular Biology (John Wiley and Sons, Baltimore, Md.).

[0223] General principles of antibody engineering are set forth in Borrebaeck, ed. (1995) Antibody Engineering (2nd ed.; Oxford Univ. Press). General principles of protein engineering are set forth in Rickwood *et al.*, eds. (1995) Protein Engineering, A Practical Approach (IRL Press at Oxford Univ. Press, Oxford, Eng.). General

principles of antibodies and antibody-hapten binding are set forth in: Nisonoff (1984) Molecular Immunology (2nd ed.; Sinauer Associates, Sunderland, Mass.); and Steward (1984) Antibodies, Their Structure and Function (Chapman and Hall, New York, N.Y.). Additionally, standard methods in immunology known in the art and not specifically described can be followed as in Current Protocols in Immunology, John Wiley & Sons, New York; Stites *et al.*, eds. (1994) Basic and Clinical Immunology (8th ed; Appleton & Lange, Norwalk, Conn.) and Mishell and Shiigi (eds) (1980) Selected Methods in Cellular Immunology (W.H. Freeman and Co., NY).

[0224] Standard reference works setting forth general principles of immunology include Current Protocols in Immunology, John Wiley & Sons, New York; Klein (1982) J., Immunology: The Science of Self-Nonself Discrimination (John Wiley & Sons, NY); Kennett *et al.*, eds. (1980) Monoclonal Antibodies, Hybridoma: A New Dimension in Biological Analyses (Plenum Press, NY); Campbell (1984) "Monoclonal Antibody Technology" in Laboratory Techniques in Biochemistry and Molecular Biology, ed. Burden *et al.*, (Elsevier, Amsterdam); Goldsby *et al.*, eds. (2000) Kuby Immunology (4th ed.; H. Freeman & Co.); Roitt *et al.* (2001) Immunology (6th ed.; London: Mosby); Abbas *et al.* (2005) Cellular and Molecular Immunology (5th ed.; Elsevier Health Sciences Division); Kontermann and Dubel (2001) Antibody Engineering (Springer Verlag); Sambrook and Russell (2001) Molecular Cloning: A Laboratory Manual (Cold Spring Harbor Press); Lewin (2003) Genes VIII (Prentice Hall, 2003); Harlow and Lane (1988) Antibodies: A Laboratory Manual (Cold Spring Harbor Press); Dieffenbach and Dveksler (2003) PCR Primer (Cold Spring Harbor Press).

[0225] All of the references cited above, as well as all references cited herein, are incorporated herein by reference in their entireties. The following examples are offered by way of illustration and not by way of limitation.

[0225A] In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that

such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

[0225B] Certain statements that appear herein are broader than what appears in the statements of the invention. These statements are provided in the interests of providing the reader with a better understanding of the invention and its practice. The reader is directed to the accompanying claim set which defines the scope of the invention.

EXAMPLES

Example 1: Construction and assembly of engineered anti-HIV binding molecules

[0226] VH and VL regions of various HIV antibodies described herein can be cloned into IgG and IgM backgrounds by standard methods through a commercial contractor. The mature proteins presented below can be expressed with a signal peptide to promote secretion.

[0227] HIV02: The VH and VL of a human antibody specific for the CD4 binding site on gp120 described in U.S. Patent No. 8,637,036, presented herein as SEQ ID NO: 7 and SEQ ID NO: 8, respectively, were cloned into appropriate vectors to encode the human IgG and IgM heavy chains comprising the amino acid sequences SEQ ID NO: 113 and SEQ ID NO: 114, respectively, and the kappa light chain comprising SEQ ID NO: 115. The vectors were transfected in to HEK293 cells (with, where appropriate, a vector encoding a human wild-type or modified J-chain as described below) and expression was permitted, producing the IgG molecule HIV02 IgG (HIV02G), the IgM molecule HIV02 IgM (HIV02M), the IgM+J (HIV02M+J), or the IgM+J containing a modified J-chain.

[0228] SEQ ID NO: 113: HIV02 Gamma 1 heavy chain

QVQLVQSGGQMKKPGESMRISCQASGYEFIDCTLNWVRLAPGRRPEWMG
WLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTADDTAVYYCTR
GKNCDYNWDFEHWGRGTPVTVSSASTKGPSVFPLAPSSKSTSGGTAALG
CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS
LGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK
PREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA

KGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPG

[0229] SEQ ID NO: 114: HIV02 Mu heavy chain

QVQLVQSGGQMKKPGESMRISCQASGYEFIDCTLNWVRLAPGRRPEWMG
WLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTADDTAVYYCTR
GKNCDYNWDFEHWGRGTPVTVSSGSASAPTLFPLVSCENSPSDTSSVAV
GCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLLPS
KDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDGFF
GNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKESGP
TTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDTAI
RVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHT
NISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISR
PKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQRG
QPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVAHE
ALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[0230] SEQ ID NO: 115 HIV02 Kappa light chain

EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSG
STRAAGIPDRFSGSRWGPDYNLTIRNLESGDFGLYYCQQYEFFGQGTKV
QVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD
NALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

[0231] HIV12: The VH and VL of a human antibody specific for the V3/glycan region on gp120 described in Buchacher, A., I., *AIDS Res. Hum. Retroviruses 10*:359–369 (1994) and in WO 2011/035205, presented herein as SEQ ID NO: 95 and SEQ ID NO: 96, respectively, were cloned into appropriate vectors to encode the human IgG and IgM heavy chains comprising the amino acid sequences SEQ ID NO: 116 and SEQ ID NO: 117, respectively, and the kappa light chain comprising SEQ ID NO: 118. The vectors were transfected in to HEK293 cells (with, where appropriate, a vector encoding a human wild-type or modified J-chain as described below) and expression was permitted, producing the IgG molecule HIV12 IgG (HIV12G), the IgM molecule HIV12 IgM (HIV12M), the IgM+J (HIV12M+J), or the IgM+J containing a modified J-chain.

[0232] SEQ ID NO: 116: HIV12 Gamma 1 heavy chain

EVQLVESGGGLVKAGGSLILSCGVSNFRISAHTMNWVRRVPGGGLEWVA
SISTSSTYRDYADAVKGRFTVSRDDLEDFVYLQMHKMRVEDTAIYYCAR
KGSDRLSDNDPFDAWGPGTVVTVSPASTKGPSVFPLAPSSKSTSGGTAA

LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPG

[0233] SEQ ID NO: 117: HIV12 Mu heavy chain

EVQLVESGGGLVKAGGSLILSCGVSNFRISAHTMNWVRRVPGGGLEWVA
SISTSSTYRDYADAVKGRFTVSRDDLEDFVYLQMHKMRVEDTAIYYCAR
KGSDRLSDNDPFDAWGPGTVVTVSPASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPG

[0234] SEQ ID NO: 118: HIV12 Light chain

DIQMTQSPSTLSASVGDTITITCRASQSIETWLAWYQQKPGKAPKLLIY
KASTLKTGVPSRFSGSGSGTEFTLTISGLQFDDFATYHCQHYAGYSATF
GQGTRVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ
WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC

[0235] HIV32: The VH and VL of a human antibody specific for the CD4 binding site on gp120 described in Moore, J.P., and J. Sodroski J. Virol 70:1863-1872 (1996) and in WO 2006/044410, presented herein as SEQ ID NO: 99 and SEQ ID NO: 100, respectively, were cloned into appropriate vectors to encode the human IgG and IgM heavy chains comprising the amino acid sequences SEQ ID NO: 119 and SEQ ID NO: 120, respectively, and the kappa light chain comprising SEQ ID NO: 121. The vectors were transfected in to HEK293 cells (with, where appropriate, a vector encoding a human wild-type or modified J-chain as described below) and expression was permitted, producing the IgG molecule HIV32 IgG (HIV32G), the IgM molecule HIV32 IgM (HIV32M), the IgM+J (HIV32M+J), or the IgM+J containing a modified J-chain.

[0236] SEQ ID NO: 119: HIV32 Gamma 1 heavy chain

QVQLQESGPGLVKPSQTLSLSCTVSGGSSSSGAHYWSWIRQYPGKGLEW
IGYIHYSGNTYYNPSLKSRITISQHTSENQFSLKLNSVTVADTAVYYCA
RGTRLRTLRNAFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGTAA
LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNH
YTQKSLSLSPG

[0237] SEQ ID NO: 120: HIV32 Mu heavy chain

QVQLQESGPGLVKPSQTLSLSCTVSGGSSSSGAHYWSWIRQYPGKGLEW
IGYIHYSGNTYYNPSLKSRITISQHTSENQFSLKLNSVTVADTAVYYCA
RGTRLRTLRNAFDIWGQGTMVTVSSGSASAPTLFPLVSCENSPSDTSSV
AVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQVLL
PSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPPRDG
FFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAKES
GPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPDQDT
AIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKT
HTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLKQTI
SRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQWMQ
RGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTCVVA
HEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[0238] SEQ ID NO: 121: HIV32 Kappa light chain

QSVLTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWYQHHPGKAPKLI
ISEVNNRPSGVPDRFSGSKSGNTASLTVSGLQAEDEAEYYCSSYTDIHN
FVFGGGTKLTVLRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA
KVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

[0239] HIV72: The VH and VL of a human antibody specific for the immunodominant region of gp41, described in Pincus SH, *et al.*, *J Immunol 170*: 2236–2241(2003), presented herein as SEQ ID NO: 97 and SEQ ID NO: 98, respectively, were cloned into appropriate vectors to encode the human IgG and IgM heavy chains comprising the amino acid sequences SEQ ID NO: 122 and SEQ ID NO: 123, respectively, and the kappa light chain comprising SEQ ID NO: 124. The vectors were transfected in to HEK293 cells (with, where appropriate, a vector encoding a human wild-type or modified J-chain as described below) and expression was permitted, producing the

IgG molecule HIV72 IgG (HIV72G), the IgM molecule HIV72 IgM (HIV72M), the IgM+J (HIV72M+J), or the IgM+J containing a modified J-chain.

[0240] SEQ ID NO: 122: HIV72 Gamma-1 heavy chain

QVQLVQSGGGVFKPGGSLRLSCEASGFTFTEYYMTWVRQAPGKGLEWLA
YISKNGEYSKYSPSSNGRFTISRDNAKNSVFLQLDRLSADDTAVYYCAR
ADGLTYFSELLQYIFDLWGQGARVTVSSASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH
NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK
TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL
HNHYTQKSLSLSPG

[0241] SEQ ID NO: 123: HIV72 Mu heavy chain

QVQLVQSGGGVFKPGGSLRLSCEASGFTFTEYYMTWVRQAPGKGLEWLA
YISKNGEYSKYSPSSNGRFTISRDNAKNSVFLQLDRLSADDTAVYYCAR
ADGLTYFSELLQYIFDLWGQGARVTVSSGSASAPTLFPLVSCENSPSDT
SSVAVGCLAQDFLPDSITFSWKYKNNSDISSTRGFPSVLRGGKYAATSQ
VLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKVSVFVPP
RDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEA
KESGPTTYKVTSTLTIKESDWLSQSMFTCRVDHRGLTFQQNASSMCVPD
QDTAIRVFAIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEA
VKTHTNISESHPNATFSAVGEASICEDDWNSGERFTCTVTHTDLPSPLK
QTISRPKGVALHRPDVYLLPPAREQLNLRESATITCLVTGFSPADVFVQ
WMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSILTVSEEEWNTGETYTC
VVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[0242] SEQ ID NO: 124 HIV72 Kappa Light chain

DIVMTQSPDSLAVSPGERATIHCKSSQTLLYSSNNRHSIAWYQQRPGQP
PKLLLYWASMRLSGVPDRFSGSGSGTDFTLTINNLQAEDVAIYYCHQYS
SHPPTFGHGTRVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHK
VYACEVTHQGLSSPVTKSFNRGEC

[0243] Wild type and modified J-Chains: Modified J-chains, V5J (SEQ ID NO: 108) and V10J (SEQ ID NO: 109), comprising an anti-CD3 ScFv corresponding to visilizumab fused to the N-terminus of the mature human J-chain via a 5-amino acid linker (GGGGS, SEQ ID NO: 101) or a 10-amino acid linker (GGGGSGGGGS, SEQ ID NO: 102), respectively were constructed by standard methods. See, *e.g.*, PCT Publication No. WO 2015/153912.

[0244] The mature expressed construct has a molecular weight of about 45 kD and can bind to soluble epsilon chain of CD3 (Sino Biological), or T-cells (data not shown).

[0245] The DNA constructs corresponding to the various anti-HIV heavy and light chains as well as those corresponding to either the wild-type (wt) J-chain or a modified J-chain (*e.g.*, V5J or V10J) sequences were co-transfected into HEK293 cells, and proteins were expressed and purified according to standard methods. HEK293 cells transfected with IgG or IgM+J versions of HIV02, HIV12, HIV32 and HIV72 antibodies produced sufficient protein to allow purification by standard methods.

[0246] Non-Reducing SDS-Native-PAGE. Protein samples were loaded into a NativePAGE 3-12% Bis-Tris gel (Novex). Tris-Acetate SDS Running Buffer (Novex) was added and the gel was run at 40V for 15 min and then at 90V for 2 hours. The gel was then fixed in 40% methanol, 10% acetic acid for 10 minutes, stained using a Colloidal Blue Staining Kit (Novex) for at least 3 hours and subsequently de-stained in water.

[0247] Western Blot Detection. After was complete, the gel was removed from the XCell SureLock Mini-Cell and transferred to a PVDF membrane at 30 volts for 1 hour (refer to Life Technologies' manual). The PVDF membrane was then blocked with 20 ml 3% BSA in PBST at 25 ºC for 1 hour.

[0248] For anti-J-chain Western blots, anti-human J chain antibody (SP105, Thermo Fisher) was added at a 1:500 dilution in 3% bovine serum albumin (BSA) in phosphate-buffered saline, 0.05% TWEEN™ 20 (PBST) overnight at 4 ºC. After washing with PBST four times at room temperature, horseradish peroxidase (HRP)-conjugated goat anti rabbit IgG (Jackson Immunology) was added at 1:5,000 dilution in 3% BSA in PBST and was incubated for 1 hour at room temperature. The membrane was washed with PBST 4 times at room temperature and was developed by addition of 10 ml of HRP chemiluminescent substrate (Thermo Fisher) for 10 minutes before exposing the blot to film. Anti-J-chain antibody only reacts with IgM which is co-expressed with either unmodified J-chain or modified J-chain.

[0249] Antibody expression and assembly: Antibodies present in cell supernatants were recovered by affinity chromatography using CaptureSelectM (BAC, ThermoFisher catalog 2890.05) for IgM antibodies or Protein A for IgG antibodies per the manufacturer's instructions. The purified proteins were evaluated as outlined below.

[0250] Expression and assembly of the HIV02 antibodies, as assessed by non-reducing SDS native-PAGE is shown in **FIG. 2A**. HIV02G expressed well and efficiently assembled into an IgG antibody. HIV02M expressed without a J-chain produced a mixture of assembled high molecular weight IgM and lower molecular weight antibody forms. When HIV02M was expressed with a wild-type J-chain (HIV02M+J), most of the material produced ran as a fully assembled IgM antibody. HIV02M also properly assembled into a high molecular weight bispecific IgM antibody when expressed with a modified J-chain targeting CD3 (*e.g.*, HIV02M+V10J; see FIG. 9).

[0251] Expression and assembly of HIV12M+J, HIV32M+J, and HIV72M+J is shown in **FIG. 2B**. All three IgM+J anti-HIV proteins properly assembled into high molecular weight IgM antibodies, as evidenced by Western analysis demonstrating the presence of J-chain in each of the proteins.

[0252] Expression and assembly of HIV72G, HIV72M, HIV72M+J and HIV72M+V5J is shown in **FIG. 2C**. The heavy and light chains of HIV72G expressed well and properly assembled into and IgG antibody (lane 2). The mu and light chains of HIV72M, expressed without a J-chain, were also produced well and mostly assembled into a high molecular weight IgM antibody (lane 3). Co-expression of the HIV72 mu and light chains with wild type J-chain or a modified J-chain targeting CD3 (V5J) resulted in efficient assembly of monospecific (HIV72M+J; lane 4) or bispecific (HIV72M+V5J; lane 5) IgM antibodies, respectively, as evidenced by Coomassie staining of the gel (left) or by an anti-J-chain Western of the electrophoresed proteins.

[0253] Other modified J-chains: Alternatively, modified J-chains can be constructed that allow binding to the CD16 antigen on natural killer cells (NK cells). For example, a modified J-chain can be constructed that expresses a camelid VHH binding domain specific for CD16 (*e.g.*, SEQ ID NO: 112). The binding domain is linked to a J-chain using a flexible amino acid linker (*e.g.*, 15 amino acids) to produce anti-CD16 camelid domain linked to the J chain (C15J). The bispecific IgM is expressed and purified as described above and assembly is confirmed by analyzing on non-reducing

SDS-Native-PAGE gels. Further, incorporation of the modified J-chain (C15J, SEQ ID NO: 125) into the pentameric IgM is confirmed using the Western blot method.

[0254] SEQ ID NO: 125

EVQLVESGGELVQAGGSLRLSCAASGLTFSSYNMGWFRRAPGKEREFVA
SITWSGRDTFYADSVKGRFTISRDNAKNTVYLQMSSLKPEDTAVYYCAA
NPWPVAAPRSGTYWGQGTQVTVSSGGGGSGGGGSGGGGSQEDERIVLVD
NKCKCARITSRIIRSSEDPNEDIVERNIRIIVPLNNRENISDPTSPLRT
RFVYHLSDLCKKCDPTEVELDNQIVTATQSNICDEDSATETCYTYDRNK
CYTAVVPLVYGGETKMVETALTPDACYPD

[0255] As described above, an expression vector containing DNA corresponding to this sequence is synthesized and transfected into HEK293 cells along with the heavy and light chains for an anti-HIV gp120/41 IgM (SEQ ID NOs 114 and 115) to produce protein which is then purified using the camelid antibody affinity matrix specific for IgM. J-chains fused to the new anti-CD16 binding domain with the 15 amino acid linker are able to incorporate into the IgM and the pentameric form of bi-specific IgM with the corresponding J-chain is distinguishable from the hexameric form without a J-chain.

Example 2: Binding of HIV specific IgM binding molecules to HIV antigens and antigen-expressing cells

[0256] HIV02G and HIV02M+J were evaluated for binding to HIV gp120 by an ELISA assay. The assays were carried out by the following method. Wells of 96-well microtiter plates (polystyrene, MaxiSorp ELISA plates, Nunc) were coated with gp120 antigen (strain HXB2, Immune Tech, cat #IT-001-0022p-PBS) at 1.0 μg/mL (high-density antigen) or dilutions down to 0.1 μg/mL (low density antigen) in 100 μL coating buffer (100 mM bicarbonate, pH 9.5) overnight at 4 °C. The plates were then washed with 0.05% PBS-Tween and blocked with 2% BSA-PBS. After blocking, 100 μL of the serial diluted samples (purified protein or cell culture supernatant) were added to the wells and incubated at room temperature for 1 hour. The plates were then washed and incubated with HRP conjugated mouse anti-human kappa antibody (Southern Biotech, 9230-05. 1:6000 diluted in 2% BSA-PBS) for 1 hour. After 5 final washes using 0.05% PBS-Tween, 100 μL TMB substrate (BD

Biosciences, 555214) was added to each well and incubated in the dark for 15 min. The reaction was then stopped by adding 50 µL of 2N H2SO4 or 2N HCl per well. A450 data was then collected on a microplate reader and analyzed with Prism software (GraphPad) using a 4-parameter logistic model.

[0257] The results for binding to gp120 HBX2 are shown in **FIG. 3A-F.** At a typical high-antigen coating of 1 µg/mL (**FIG. 3A**), HIV02M+J exhibited more effective binding than the IgG version (HIV02G), exhibiting an EC50 of 2,300 ng/mL vs 320 ng/mL, respectively, a 7-fold difference by weight. On a molar basis, HIV02M+J bound gp120 42-fold better than HIV02G at this coating concentration.

[0258] When the gp120 coating concentration was successively decreased from 1.0 µg/mL to 0.8, 0.6, 0.4, 0.2 and 0.1 µg/mL (**FIG. 3B** to **FIG. 3F**), thereby reducing the antigen density on the plate, HIV02M+J continued to show strong binding whereas HIV02G binding decreased rapidly. At the lowest antigen density (**FIG. 3F**, 0.1 µg/mL coat), HIV02G binding was barely detectable.

[0259] Since binding of HIV02G to gp120 fell off rapidly as the coating concentration was decreased, EC10 values were interpolated from the binding curves to allow a binding comparison of antigen binding at all coating concentrations. The results of this analysis are shown in **FIG. 4**. As the gp120 coating concentration in the ELISA decreased from 1.0 µg/mL to 0.1 µg/mL, the binding of IgM+J relative to that of the IgG increased 5-fold to 20-fold on a weight basis. On a molar basis, the IgM+J bound gp120 120-fold better than the IgG when the antigen was coated at the lowest concentration tested (0.1 µg/mL).

[0260] HIV02G and HIV02M+J were further evaluated for binding to cells transfected to express gp120 on their cell surface, by the following method.

[0261] CHO cells engineered to express HBX2 gp140 (gp120 + the extracellular portion of gp41; CHO-gp140) or gp160 (full-length protein; CHO-gp160) on their surface (Weiss *et al.*, 1993 *J Virol 67*:7060-7066) were obtained from the NIH AIDS Reagent Program (catalog # 2284 & 2239) and cultured per their instructions. Cells were passaged in T75 flask at 3x10^6 cells per flask in 20 ml of media – cells were replenished with fresh media containing 10 mM of Sodium Butyrate (Sigma, cat#B5887) 15 to 18 hrs prior to use. On the day of staining, the cells were dislodged

with 10 mL of cell dissociation buffer (Gibco, cat#131510). After aspirating off the media the cells were rinsed with PBS without Calcium & Magnesium. Ten mL of cell dissociation buffer was added to all the flasks and incubated for 20~30 min at 37 °C. The cells were then dislodged by tapping the flask to create a single cell suspension. An equal volume of the media was added to the flask to neutralize the cell dissociation buffer and live cell counts were determined using trypan blue exclusion on a cell counter (BioRad TC20). Cells at 1.5x10^4/well in 60 µL of FACS 2% FBS buffer (BD Pharmingen, cat# 554656) were added to "V" bottom 96 well plates. Antibodies and isotype controls were diluted in FACS 2% FBS buffer and 50 µL were added to the respective wells such that the final concentration was 30 µg/mL. The plates were then incubated for 30 min at 4°C. After washing the cells with 150 µL of FACS 2% FBS buffer, the plates were centrifuged (Sorvall Legend XIR centrifuge) at 1200 rpm for 5 min and supernatants were gently aspirated without disturbing the cell pellets. Antibody binding was detected by incubating the treated cells with AlexaFluor 647-labeled anti human kappa light chain (BioLegend, cat#316514) at 4 °C for 30 min. The wash step was repeated as above and the cells were resuspended in 60 µL of FACS 2% FBS containing 1:100 7_AAD (BD Pharm, cat#68981E). At least 1,000 events were acquired for each sample on a FACSCALIBUR™ (Becton Dickinson) and data analysis was done in FLOWJO™ (FlowJo LLC).

[0262] The binding of HIV02 antibodies to CHO-gp140 cells is shown in **FIG. 5A** and **FIG. 5B**. HIV02G binding to CHO-gp140 cells was relatively low when analyzed by FACS (**FIG. 5A**). However, binding of the IgM version HIV02M+J was readily detected and much stronger (**FIG. 5B**).

[0263] Similarly, the binding of anti-HIV antibodies to cells chronically or latently infected with HIV can be examined. Chronically infected cell lines such as CEM cells (CEM-IIIb) (Popovic, M., *et al.*, 1984. *Science 224*:497-500) constitutively express HIV gp120/41 envelope glycoprotein on the cell surface whereas the latently infected cell lines ACH2 (Folks TM, *et al.*, *Proc Natl Acad Sci USA 86*:2365-2368, 1989.), Jl.l (Perez VL, *et al.*, *J Immunol 147*:3145-3148, 1991) and OM10 (Butera *et al.*, 1991 *J Virol. 65*(9):4645–4653) cell lines are activated with the cytokine tumor necrosis

alpha to express HIV gp-120/41 envelope glycoprotein on the cell surface. Serial dilutions of the monoclonal or bispecific IgM antibodies as described above as well as appropriate controls, *e.g.*, IgG antibodies carrying equivalent anti-HIV binding domains, are incubated with the respective cell lines, washed to remove unbound antibody, then mixed with a fluorochrome-labeled secondary antibody which is specific for the isotype of antibody to be detected *e.g.*, IgG, IgM, or the kappa or lambda light chain of an antibody. After incubation the binding of monoclonal or bispecific antibodies is analyzed by flow cytometry on a FACSCALIBUR™ (Becton Dickson).

Example 3: HIV virus neutralization by HIV specific IgM binding molecules

[0264] *In vitro* HIV virus neutralization assays can be conducted using a variety of standard techniques, such as those described by Richman *et al.*, *PNAS USA, 100*(7): 4144-4149, 2003.

[0265] In this assay, anti-HIV antibodies were examined for activity and potency by using HIV pseudo-virus capable of a single round of replication. One or more HIV pseudo-viruses are produced by co-transfection of HEK293 cells with a sub-genomic plasmid, pHIV-1lucΔu3, that incorporates a firefly luciferase indicator gene and a second plasmid, pCXAS that expresses the HIV-1 envelope proteins of interest. Following transfection, pseudo-viruses were harvested and incubated for 1 hour at 37 °C with serial dilutions of the antibodies to be tested. U87 cells that express CD4 plus the CCR5 and CXCR4 co-receptors were then inoculated with the virus-antibody dilutions in the absence of added cations. Virus infectivity was determined 72 hour post-inoculation by measuring the amount of luciferase activity expressed in infected cells. Neutralizing activity is described as the percent inhibition of viral replication (luciferase activity) at each antibody dilution compared with an antibody-negative control: % inhibition = {1 − [luciferase + Ab/luciferase − Ab]} × 100.

[0266] The results of a virus neutralization assay are shown in **FIG. 6**. HIV02M+J was tested against a panel of viruses that included clades A, B, C, D, F, G, AE, AG, CRF07-BC and CRF08-BC. HIV02M+J successfully neutralized all the viruses

tested, exhibiting 75% to 100% neutralization at 10 μg/mL, indicating that HIV02M+J binds to all the different gp120 proteins expressed by these clinically relevant HIV clades. Irrelevant isotype control IgM+J antibodies did not neutralized any of the pseudo-viruses tested (data not shown).

Example 4: T-Cell activation by HIV specific IgM binding molecules

[0267] To demonstrate whether a bispecific HIVxCD3 antibody can activate T cells upon binding to antigen-positive target cells, the following assay was performed. Engineered Jurkat T cells (Promega CS176403) and CHO-gp140 cells expressing gp120/gp41 were cultured in RPMI (Invitrogen) supplemented with 10% Fetal Bovine Serum (Invitrogen). Serial dilutions of HIV02M, HIV02M+J, and HIV02M+V10J were incubated with the antigen-expressing cells in 20 μL in a white 384 well assay plate for 2h at 37 ºC with 5% CO_2. The engineered Jurkat cells (25000) were then added to the mixture to a final volume of 40 μL. The mixture was incubated for 5h at 37 ºC with 5% CO_2. The cell mixtures were then mixed with 20 μL lysis buffer containing luciferin (Promega, Cell Titer Glo) to allow measurement of luciferase reporter activity. Light output was measured by EnVision plate reader.

[0268] The results of an experiment examining the gp120-specific HIV02M, HIV02M+J, and HIV02M+V10J antibodies is shown in **FIG. 7**. Whereas the monospecific HIV02M and HIV02M+J antibodies were without activity, the gp120xCD3 bispecific HIV02M+V10J antibody caused T-cell activation in a dose-dependent fashion.

[0269] PAGE analysis indicated that this preparation of HIV02M+V10J contained a mixture of unassembled and fully assembled IgM proteins (**FIG. 8**, lanes 2 & 8). The preparation was subsequently further purified by size-exclusion chromatography utilizing a Waters 2695 HPLC system equipped with a TOSOH G4000 SWXL chromatography column. The column was equilibrated with degassed mobile phase (0.1 M Sodium Phosphate, 0.1 M Sodium Sulfate pH 6.7) for 45 minutes at a flow rate of 1 ml/min and a temperature of 25 ºC. Samples were filtered and 100 μl was injected at a flow rate of 1 ml/min for a run time of 15 minutes. The absorbance was

monitored at 280 nm using Waters EMPOWER™ software suite, which was also used to generate and analyze the chromatograms. One mL fractions were collected using a Waters Fraction Collector II. Collected fractions were subsequently concentrated and buffer exchanged into 20 mM citric acid, 150 mM sodium chloride, pH 6.0 using Spin X UF-6 Concentrators (Corning, Ref# 431486).

[0270] Analysis of the HIV02M+V10J antibody is shown in **FIG. 8**. Based on non-reduced SDS-Native-PAGE, size-exclusion HPLC separated the preparation into purified high molecular weight properly assembled IgM HIV02M+V10J bispecific (fractions 3 and 4) and unassembled, lower molecular weight material (fractions 5 and 6).

[0271] The antigen-dependent T-cell activation induced by the highly purified HIV02M+V10J is shown in **FIG. 9**. In this assay, the purified HIV02M+V10J antibody was more potent than the HIV02M+V10J starting material as evidenced by the higher activation signal.

Example 5: Complement-dependent cytotoxicity of HIV specific IgM binding molecules

[0272] Antibodies of the IgM phenotype are particularly well-suited to use the efficient engagement of complement protein C1q to affect complement dependent cytotoxicity (CDC) activity on target cells. To measure CDC, recombinant cells expressing gp120 on their surface are used (*e.g.*, CHO-gp120, CHO-gp140, Jurkat-522 F/Y cells, or mammalian cells expressing membrane anchored trimeric forms of gp140, *e.g.*, strain JR-FL (Go *et al.* 2015 *J Virol 89*:8245-8257)). The target cells are washed and resuspended in CDC assay medium (RPMI 1640, 10% heat-inactivated FBS) at a density of 1.0 x 10^6 cells/mL and 10 µL/well is added to a Nunc 384-well tissue culture-treated white polystyrene plate. Serial 3-fold dilutions of test antibodies including, *e.g.*, a pentameric or hexameric HIV binding molecule, *e.g.*, an IgM antibody such as HIV02M, HIV02M+J, or HIV02M+V10J and appropriate controls, are prepared in assay medium, 10 µL/well is added to the assay plate, and the plate is incubated for 2 hr at 37 °C in a 5% CO_2 incubator to allow opsonization to occur. Normal human serum complement (Quidel) is diluted to 30% in assay medium, and 10 µL/well is added to the assay plate. The plate is incubated for 4 hr at

37 °C in a 5% CO_2 incubator. Cell Titer-Glo reagent (Promega) is thawed for use and 15 µL/well is added to the assay plate. The plate is gently mixed for 2 min on a plate shaker to lyse the cells and then for another 10 min at room temperature before measuring luminescence on an EnVision plate reader (Perkin-Elmer). After subtracting background signal, percent viability is plotted against antibody concentration and EC50 values are determined using GraphPad Prism.

[0273] Alternatively, chronically HIV-infected cell lines (*e.g.*, CEM-IIIb) or latently infected cell lines (*e.g.*, ACH2, Jl.l, OM10) can be used. The CEM-IIIb cells constitutively express the gp120/41 HIV envelope glycoprotein on their cell surface, whereas the latent cell lines are activated to express the gp120/41 HIV envelope glycoprotein that is indicative of HIV latency. Cells are seeded at 50,000 cells per well in a 96-well plate, serially diluted monoclonal or bispecific antibodies such as those described herein are added, and then human serum complement (Quidel cat. #A113) is added to a final concentration of 10 percent of normal serum. The reaction mixture is incubated at 37 °C for 4 hours. Cell Titer Glo reagent (Promega cat. #G7572) is added at a volume equal to the volume of culture medium present in each well. The plate is shaken for 2 minutes, incubated for 10 minutes at room temperature, and luminescence is then measured on a luminometer.

Example 6: T-cell directed killing of HIV antigen-expressing cells by HIV specific IgM binding molecules

[0274] Bispecific HIVxCD3 antibodies such as, but not limited to HIV02M+V10J, are tested for the induction of T-cell dependent cell cytotoxicity (TDCC) using HIV+ cells as targets. The target HIV+ cells used are cells recombinantly expressing gp120/gp41 on their surface (*e.g.*, CHO-gp140, CHO-gp160, Jurkat-522 F/Y, or mammalian cells expressing membrane anchored trimeric forms of gp140, *e.g.*, strain JR-FL (Go *et al.* 2015 *J Virol 89*:8245-8257)), chronically infected CEM cells (*e.g.*, CEM-IIIb) or latently infected cell lines (*e.g.*, ACH2, Jl.l, OM10). Bispecific HIVxCD3 antibodies are serially diluted and mixed with the target cells and PBMCs or purified/enriched CD8+ T cells from normal donors as effector cells at various target:effector cell ratios. After 24-72 hours the amount of cell lysis or killing is

analyzed by the addition of Cell titer glo (Promega) and luminescence is then measured on a luminometer.

[0275] Alternately, TDCC co-culture experiments can be conducted using CD8+ T-cell acute lymphoblastic leukemia (TALL) cells. gp120/pg41-expressing cells (about 6 x 10^3 cells), *e.g.*, CHO-gp140, CHO-gp160, Jurkat-522 F/Y, or mammalian cells expressing membrane anchored trimeric forms of gp140, *e.g.*, strain JR-FL (Go *et al.* 2015 *J Virol 89*:8245-8257)), chronically infected CEM cells (*e.g.*, CEM-IIIb) or latently infected cell lines (*e.g.*, ACH2, J1.1, OM10), are co-cultured with 3 x 10^4 TALL cells (ATCC CRL-11386) in the presence of different concentrations of test compounds, *e.g.*, HIVxCD3 bispecific antibodies, in 45 µL total volume of RPMI 1640 media supplemented with 10% heat-inactivated FBS per well on a 384-well black tissue culture plate. After 24 hours of incubation at 37 °C in a 5% CO_2 incubator, 15 µL of CytoTox-ONE substrate reagent (Promega, G7891) is added to each well to measure the level of LDH released from dead cells. The plates are shaken briefly to mix the reagents, and then incubated at room temperature for 90 min before measuring fluorescence signal (485 nm for excitation and 615 nm for emission) on an EnVision plate reader (Perkin-Elmer). The data is then analyzed with Prism software (GraphPad) to determine the EC50.

Example 7: Treatment of HIV-infected animals with HIV IgM binding molecules

[0276] Effective control of HIV-1 infection in humans is achieved using combinations of antiretroviral therapy (ART) drugs (Bartlett *et al.*, *AIDS 15*(11): 1369–1377). However, when ART is stopped HIV virus re-appears or "rebounds" after a short period of time indicating a small number of latently infected cells still remain in the ART-treated individual. This latent "reservoir" is the single biggest obstacle to HIV-infected individuals being cured of their infection. Therefore, since ART effectively reduces HIV infection to below detectable levels there is considerable interest in targeting and killing the reservoir of latently infected cells which can result in the HIV-infected individual being cured of their infection.

[0277] A model of HIV latency has been described in humanized BLT mice (Denton *et al.*, *J Virol.* 2012 Jan; *86*(1):630-4 (2012)) which has been used by Horowitz *et al.*, (*Proc. Natl. Acad. Sci.* USA. 2013 *110*(41):16538-43 and Klein *et al.*, *Nature 492*(7427): 118–122 (2012)) to analyze the combination of ART plus immunotherapy to determine the possibility of suppressing or eliminating the rebound of virus after ART is stopped as a promising approach toward developing a cure for HIV infection. Humanized mice are screened at 8 weeks of age for reconstitution of human lymphocytes as described by Klein *et al. Nature 492*(7427): 118–122 (2012. Mice with measurable human lymphocytes are injected intra-peritoneally with infectious HIV-1 YU2 virus and screened for viremia at 2 to 3 weeks post-infection by quantitative reverse-transcriptase PCR. Individual tablets of tenofovir disproxil-fumarate (TDF; Gilead Sciences), emtricitabine (FTC; Gilead Sciences), raltegravir (RAL; Merck), and efavirenz (EFV; Bristol-Myers Squibb) are crushed into a fine powder form using a mortar and pestle and suspended in PBS. ART preparations are aliquotted into 200-µL doses in sterile Eppendorf tubes and are administered daily by oral gavage at 2.5, 1.5, 1.2, and 2.5 mg per mouse for TDF, FTC, RAL, and EFV, respectively, based on effective doses reported by Denton *et al.* (2012). Four (4) groups of humanized YU2-infected mice will begin receiving ART by oral lavage daily on day 0 for 5 days and then groups 2, 3, and 4 will begin receiving HIV02G, HIV02M, and HIV02M+V10J, respectively, at 20 mg/kg on day 5. Groups 2, 3, and 4 continue to receive antibody therapy twice a week from day 5 to day 42. On day 21, ART is terminated in all groups. HIV plasma viral load, cell-associated HIV DNA and RNA are determined at various time points through the duration of the study to day 63 to determine the kinetics of virus rebound from latency. Similarly, other humanized mouse models can be used (see Xhang & Su 2012 *Cell. & Mol. Immunol. 9*,237-244).

[0278] Alternately, *in vivo* efficacy studies can be conducted in non-human primates. In one such model of chronic infection (Barouch *et al.* 2013 *Nature* 503, 224–228), specific pathogen-free rhesus monkeys (*Macaca mulatta*) that do not express the class I alleles Mamu-A*01, Mamu-B*08, and Mamu-B*17 associated with spontaneous virologic control are used. Groups are balanced for susceptible and resistant TRIM5α

alleles. Groups of 4 to 5 monkeys are randomly allocated to balance baseline viral loads. Animals are infected by the intrarectal route with rhesus-derived SHIV-SF162P3 challenge stock for 9 months prior to antibody administration. Dimeric, pentameric, or hexameric HIV binding molecules, *e.g.*, IgM antibodies such as HIV02M, HIV02M+J, or HIV02M+V10J, and appropriate controls, are administered to monkeys once or twice by the intravenous route at doses up to 10 mg/kg, and the monkeys are bled up to three times per week for assessment of viral loads. Alternatively, infected monkeys are treated with or without anti-retroviral therapy (*e.g.*, ART) in addition to the dimeric, pentameric, or hexameric HIV binding molecules. Once the anti-retroviral therapy has stopped, viral rebound is quantified. Similarly, infected monkeys can be treated with anti-retroviral therapy and, once stopped, then treated with the dimeric, pentameric, or hexameric HIV binding molecules and viral rebound is quantified.

***

[0279] The breadth and scope of the present disclosure should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims and their equivalents.

WHAT IS CLAIMED IS:

1. A multimeric binding molecule comprising two or five bivalent binding units and a modified J-chain; wherein each binding unit comprises two antibody heavy chain constant regions, each associated with an antigen binding domain, wherein the antigen binding domains specifically bind to a human immunodeficiency virus (HIV) antigen expressed on the surface of HIV viral particles, on the surface of HIV-infected cells, or a combination thereof, wherein the modified J-chain comprises a J-chain or functional fragment or variant thereof and a heterologous polypeptide comprising a binding domain; and wherein the binding molecule can effect more potent antibody mediated, complement mediated, or T-cell mediated, killing of HIV infected cells than a reference single binding unit molecule comprising the same antigen binding domains that specifically binds to an HIV antigen, wherein the reference single binding unit molecule is an IgG antibody.

2. The binding molecule of claim 1, which is a dimeric binding molecule comprising two bivalent IgA binding units, wherein each binding unit comprises two IgA heavy chains each comprising a VH situated amino terminal to the IgA heavy chain constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region, and wherein the IgA heavy chain constant regions or fragments thereof each comprise a Cα2 domain or a Cα3-tail piece (tp) domain.

3. The binding molecule of claim 1, which is a pentameric binding molecule comprising five bivalent IgM binding units, wherein each binding unit comprises two heavy chains each comprising a VH situated amino terminal to an IgM constant region or fragment thereof, and two immunoglobulin light chains each comprising a VL situated amino terminal to an immunoglobulin light chain constant region, and wherein the IgM heavy chain constant regions or fragments thereof each comprise a Cμ3 domain and a Cμ4-tp domain, and optionally further comprise a Cμ2 domain, a Cμ1 domain, or any combination thereof.

4. The binding molecule of any one of claims 1 to 3, wherein the modified J-chain comprises the amino acids 23 to 159 of the amino acid sequence SEQ ID NO: 2 or a functional fragment or variant thereof.

5. The binding molecule of any one of claims 1 to 4, wherein the heterologous polypeptide is directly or indirectly fused to the J-chain or functional fragment or variant thereof, and wherein the heterologous polypeptide is fused to the N-terminus of the J-chain or functional fragment or variant thereof, the C-terminus of the J-chain or functional fragment or variant thereof, or to both the N-terminus and C-terminus of the J-chain or functional fragment or variant thereof.

6. The binding molecule of claim 5, wherein the heterologous polypeptide comprises an antibody or antigen binding fragment thereof that binds to an effector cell.

7. The binding molecule of claim 6, wherein the antigen-binding fragment comprises a single-chain Fv (scFv) fragment.

8. The binding molecule of claim 7, wherein the scFv specifically binds to the T-cell antigen CD3 or the NK cell antigen CD16.

9. The binding molecule of any one of claims 1 to 8, wherein the at least one antigen binding domain specifically binds to an epitope on gp120, gp41, or a combination thereof, wherein the epitope is situated in the immunodominant region of gp41, the MPER, the CD4 binding site, the V1/V2 loop, the V3 loop, carbohydrates associated with any of these regions, or a combination thereof, and wherein at least one binding unit comprises two antigen binding domains that specifically bind to an HIV antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof, and wherein the two heavy chains within the at least one binding unit are identical, and wherein the two light chains within the at least one binding unit are identical.

10. The binding molecule of claim 9, comprising at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, or at least twelve antigen binding domains that specifically bind to an HIV antigen expressed on the surface of viral particles, on the surface of HIV-infected cells, or a combination thereof.

11. The binding molecule of any one of claims 1 to 10, wherein the at least one antigen binding domain comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL), wherein the VH and VL comprise the amino acid sequences of SEQ ID NO: 5 and SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, SEQ ID NO: 9 and SEQ ID NO: 10, SEQ ID NO: 11 and SEQ ID NO: 12, SEQ ID NO: 13 and SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16, SEQ ID NO: 17 and SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, SEQ ID NO: 21 and

SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26, SEQ ID NO: 27 and SEQ ID NO: 28, SEQ ID NO: 29 and SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 32, SEQ ID NO: 33 and SEQ ID NO: 34, SEQ ID NO: 35 and SEQ ID NO: 36, SEQ ID NO: 37 and SEQ ID NO: 38, SEQ ID NO: 39 and SEQ ID NO: 40, SEQ ID NO: 41 and SEQ ID NO: 42, SEQ ID NO: 43 and SEQ ID NO: 44, SEQ ID NO: 45 and SEQ ID NO: 46, SEQ ID NO: 47 and SEQ ID NO: 48, SEQ ID NO: 49 and SEQ ID NO: 50, SEQ ID NO: 51 and SEQ ID NO: 52, SEQ ID NO: 53 and SEQ ID NO: 54, SEQ ID NO: 55 and SEQ ID NO: 56, SEQ ID NO: 57 and SEQ ID NO: 58, SEQ ID NO: 59 and SEQ ID NO: 60, SEQ ID NO: 61 and SEQ ID NO: 62, SEQ ID NO: 63 and SEQ ID NO: 64, SEQ ID NO: 65 and SEQ ID NO: 66, SEQ ID NO: 67 and SEQ ID NO: 68, SEQ ID NO: 69 and SEQ ID NO: 70, SEQ ID NO: 71 and SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74, SEQ ID NO: 75 and SEQ ID NO: 6, SEQ ID NO: 77 and SEQ ID NO: 78, SEQ ID NO: 79 and SEQ ID NO: 80, SEQ ID NO: 81 and SEQ ID NO: 82, SEQ ID NO: 83 and SEQ ID NO: 84, SEQ ID NO: 85 and SEQ ID NO: 86, SEQ ID NO: 87 and SEQ ID NO: 88, SEQ ID NO: 89 and SEQ ID NO: 90, SEQ ID NO: 91 and SEQ ID NO: 92, SEQ ID NO: 93 and SEQ ID NO: 94, SEQ ID NO: 95 and SEQ ID NO: 96, SEQ ID NO: 97 and SEQ ID NO: 98, or SEQ ID NO: 99 and SEQ ID NO: 100, respectively.

12. A composition comprising:

(a) a polynucleotide comprising a nucleic acid sequence that encodes a human IgM constant region or fragment thereof fused to the C-terminal end of a VH domain comprising:

the VH amino acid sequence SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, or SEQ ID NO: 99;

(b) a polynucleotide comprising a nucleic acid sequence encoding a human antibody light chain constant region or fragment thereof fused to the C-terminal end of a VL comprising:

the VL amino acid sequence SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 56, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 6, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, or SEQ ID NO: 100;and

(c) a polynucleotide comprising a nucleic acid sequence encoding a modified J-chain or functional fragment or variant thereof comprising a heterologous polypeptide that comprises a binding domain.

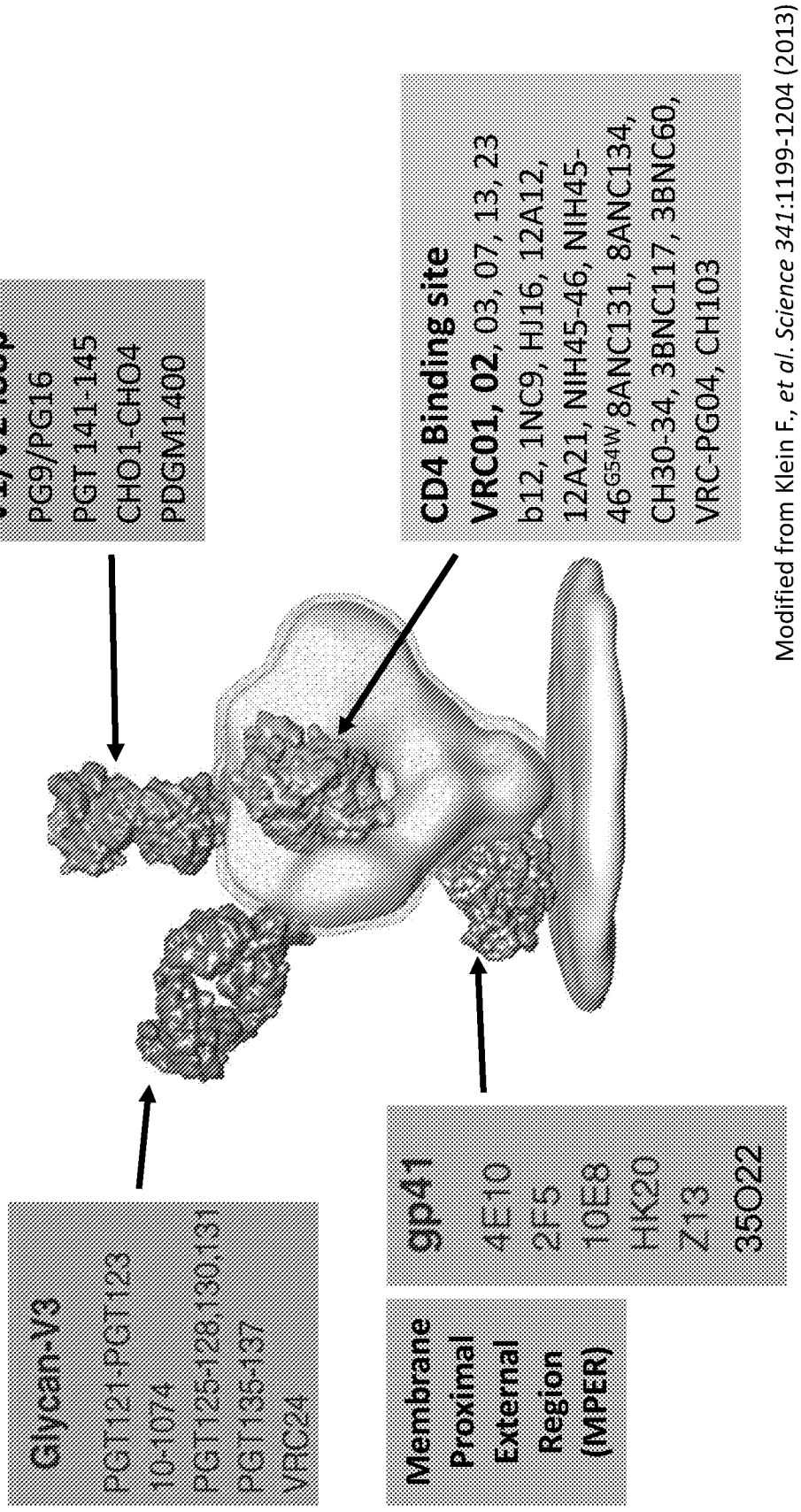
13. The multimeric binding molecule of any one of claims 1 to 11 for use in preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity,

wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG antibody comprising an identical HIV-binding antigen binding domain.

14. A method of preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity in a subject in need thereof comprising administering an effective amount of multimeric binding molecule of any one of claims 1 to 11, wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG1 antibody comprising an identical HIV-binding antigen binding domain.

15. Use of the multimeric binding molecule of any one of claims 1 to 11 in the manufacture of a medicament for preventing, controlling, or treating HIV infection, or controlling human immunodeficiency virus (HIV) infectivity in a subject in need thereof, wherein the binding molecule is more potent in preventing, controlling or treating HIV infection, or in controlling HIV infectivity, than a corresponding IgG1 antibody comprising an identical HIV-binding antigen binding domain.

Fig. 1



Modified from Klein F., *et al. Science 341*:1199-1204 (2013)

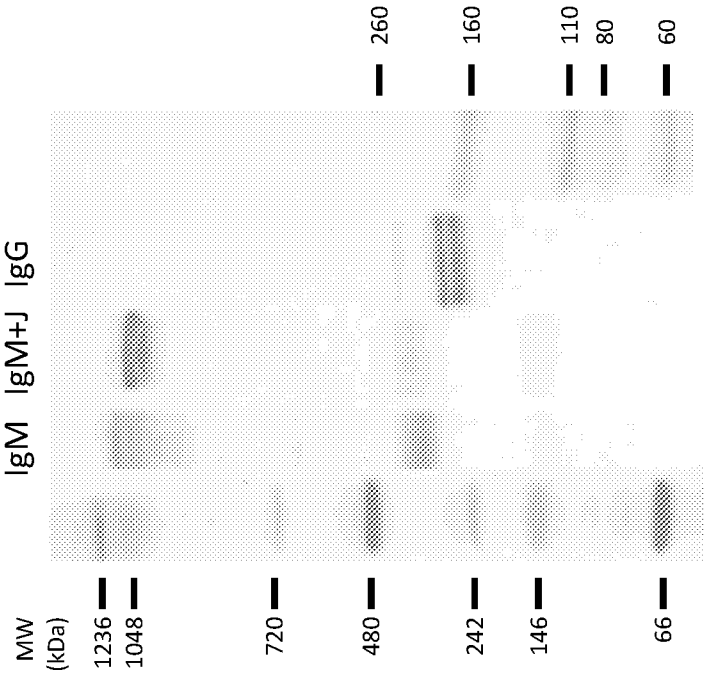

IgM
IgM+J
IgG
MW
(kDa)
1236
1048
720
480
242
146
66
260
160
110
80
60

Fig. 2A

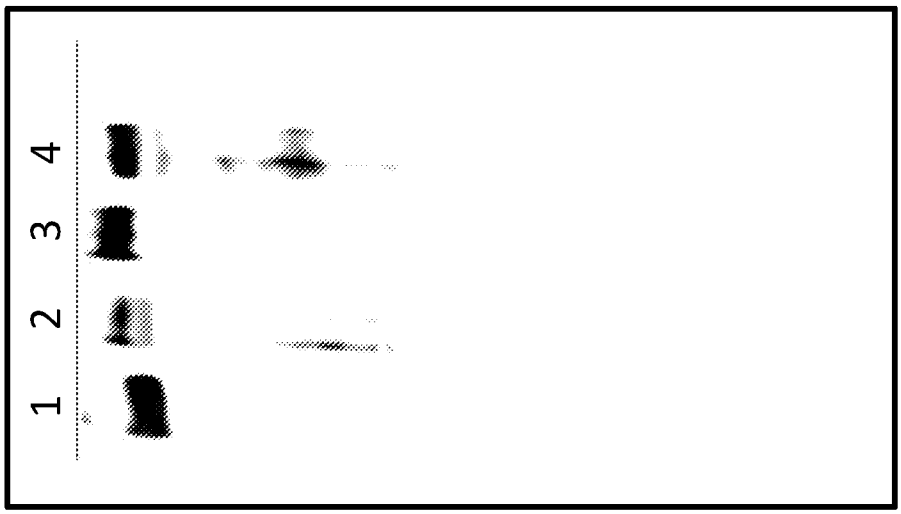


Fig. 2B

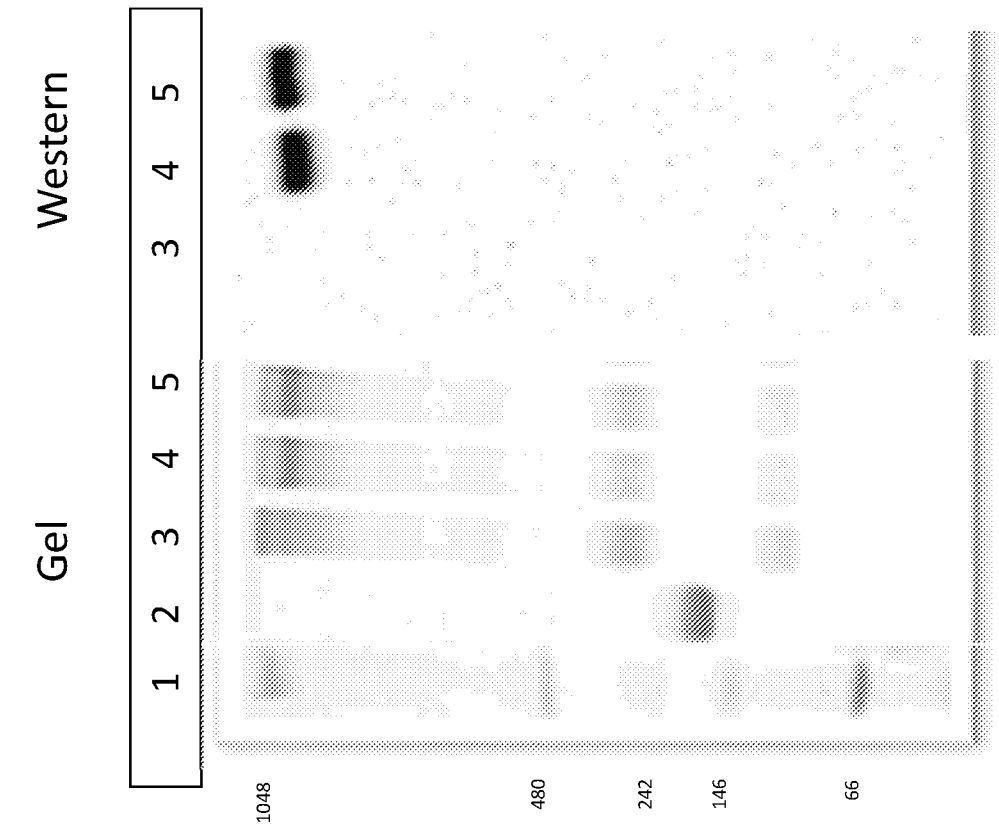


Fig. 2C

FIG. 3A

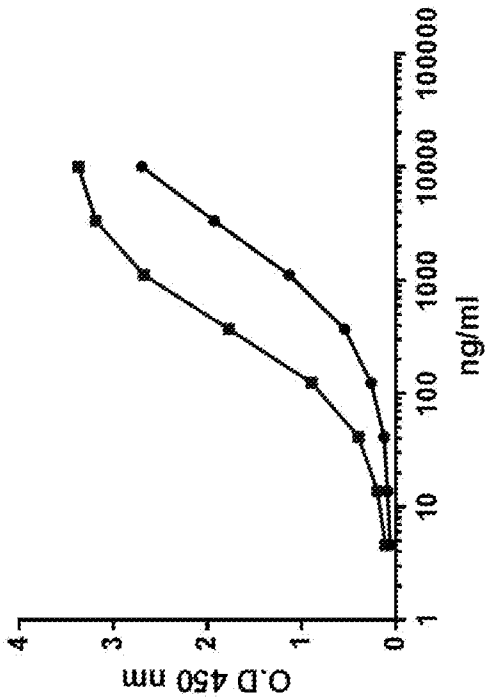

@1ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml

FIG. 3B

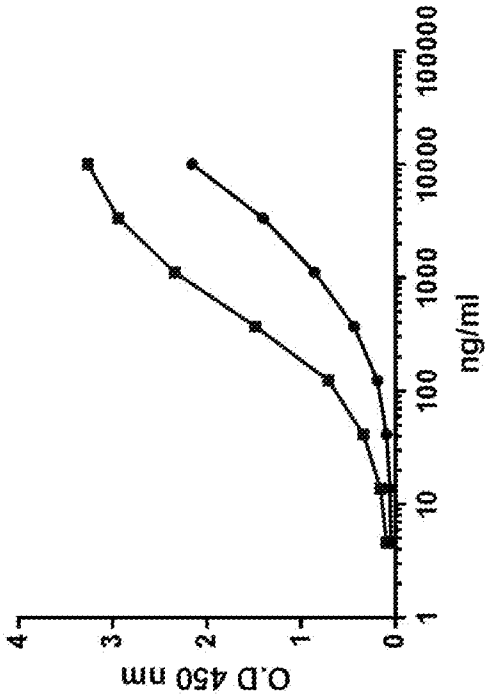

@0.8 ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml
HIV02 IgG
HIV02 IgM+J

FIG. 3C

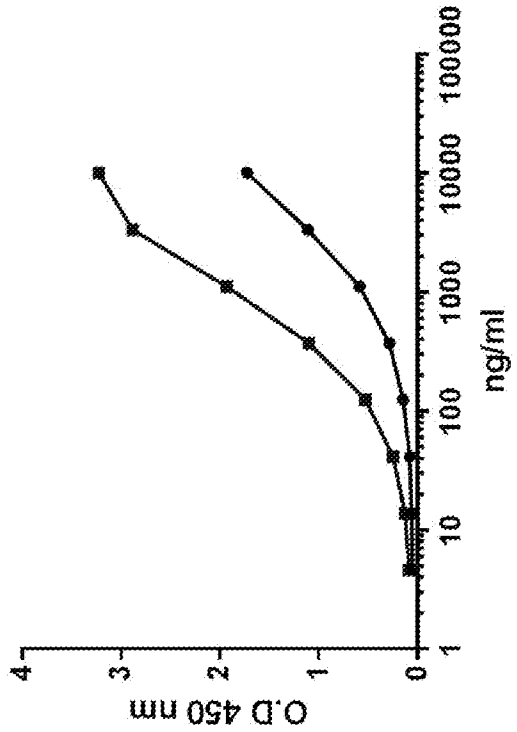
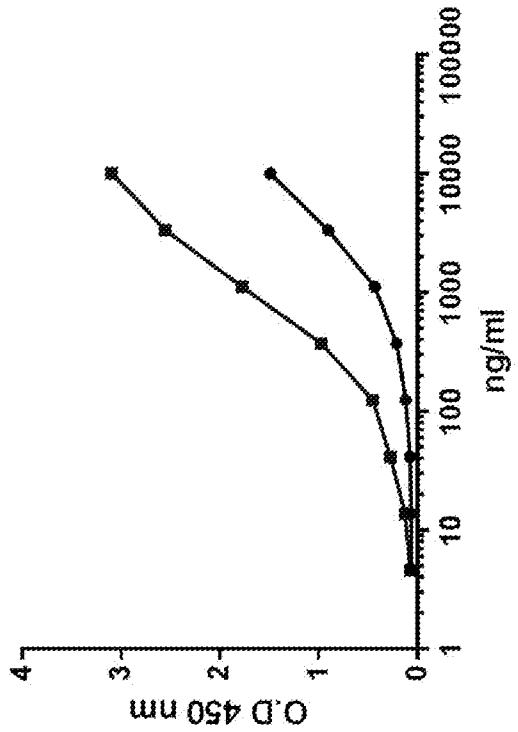

@0.6 ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml

FIG. 3D


@0.4 ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml

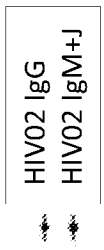

HIV02 IgG
HIV02 IgM+J

FIG. 3E

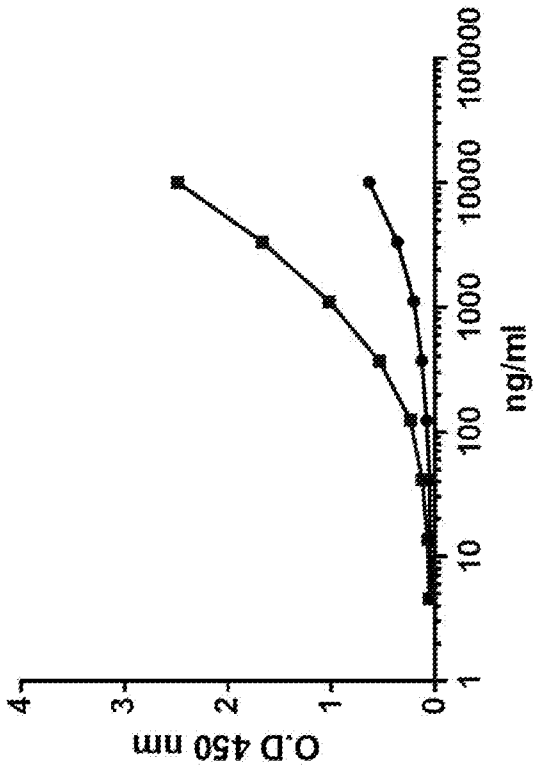
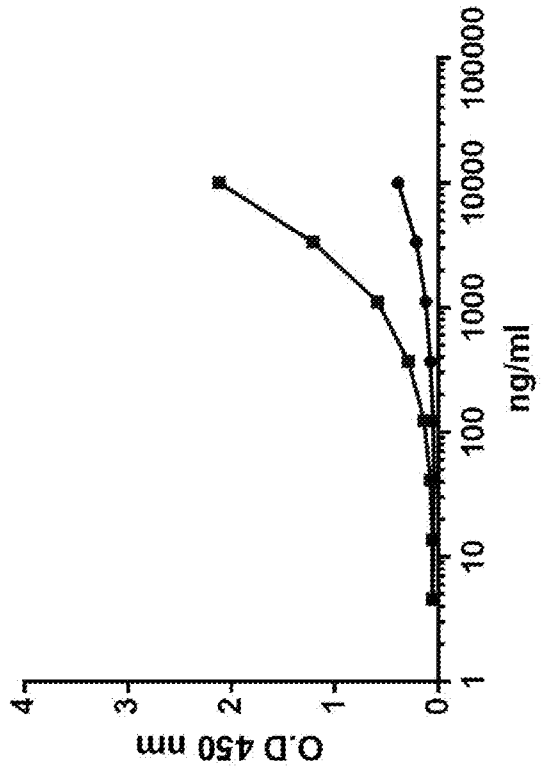
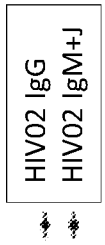

@0.2 ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml

FIG. 3F


@0.1 ug/ml coating
O.D 450 nm
0
1
2
3
4
1
10
100
1000
10000
100000
ng/ml


HIV02 IgG
HIV02 IgM+J

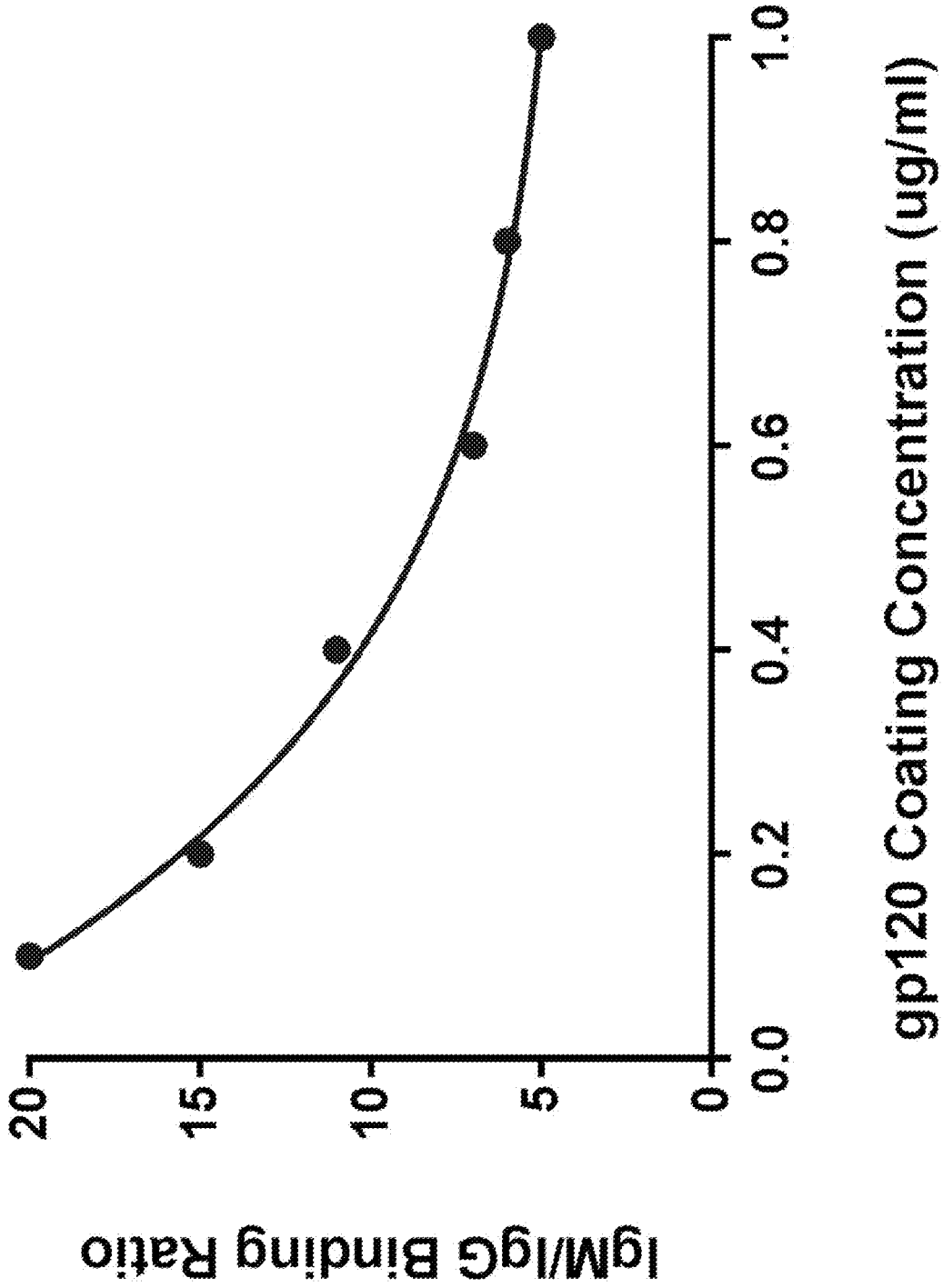

IgM/IgG Binding Ratio
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
gp120 Coating Concentration (ug/ml)

Fig. 4

Fig. 5A

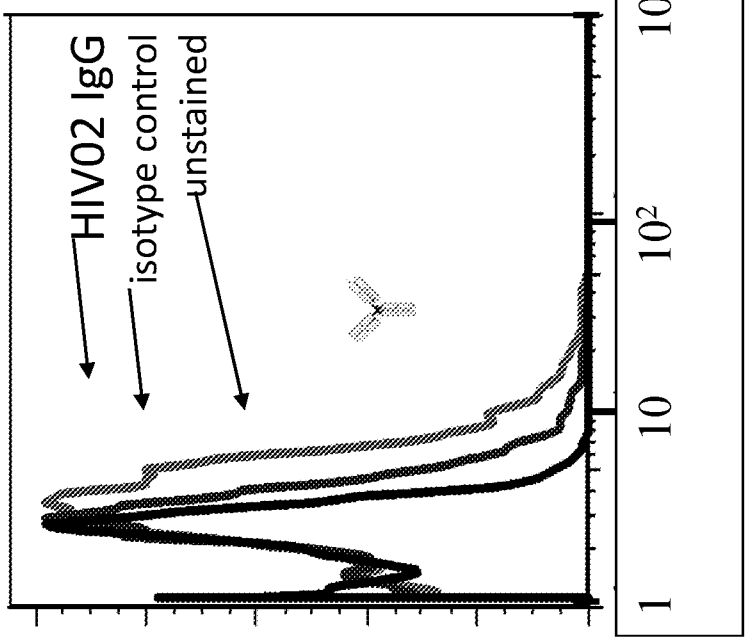
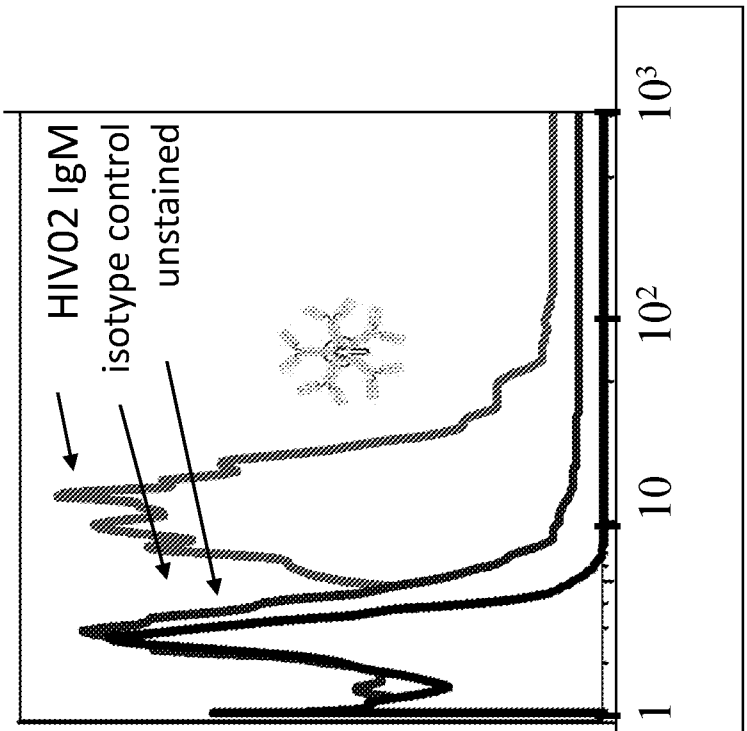

HIV02 IgG
isotype control
unstained
1
10
10^2
10^3

Fig. 5B


HIV02 IgM
isotype control
unstained
1
10
10^2
10^3

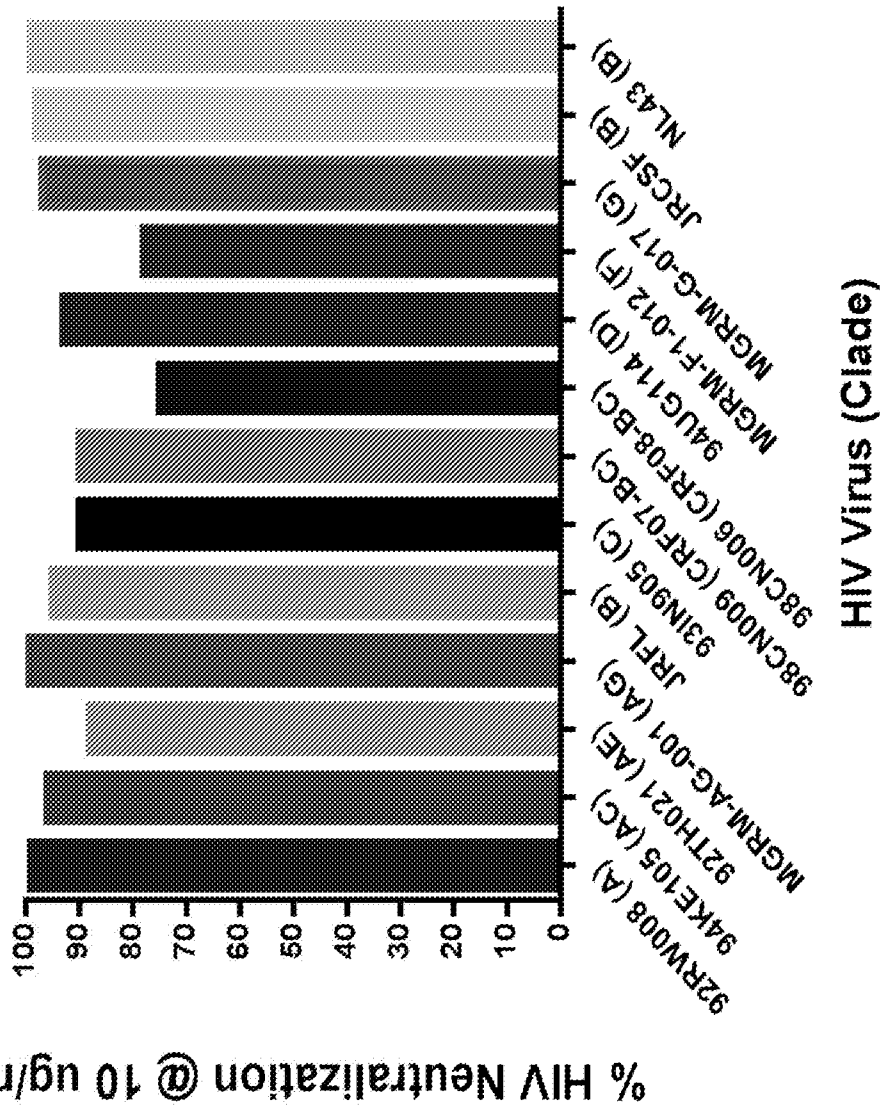

% HIV Neutralization @ 10 ug/mL
100
90
80
70
60
50
40
30
20
10
0
92RW008 (A)
94KE105 (AC)
92TH021 (AE)
MGRM-AG-001 (AG)
JRFL (B)
93IN905 (C)
98CN009 (CRF07-BC)
98CN006 (CRF08-BC)
94UG114 (D)
MGRM-F1-012 (F)
MGRM-G-017 (G)
JRCSF (B)
NL43 (B)
HIV Virus (Clade)

Fig. 6

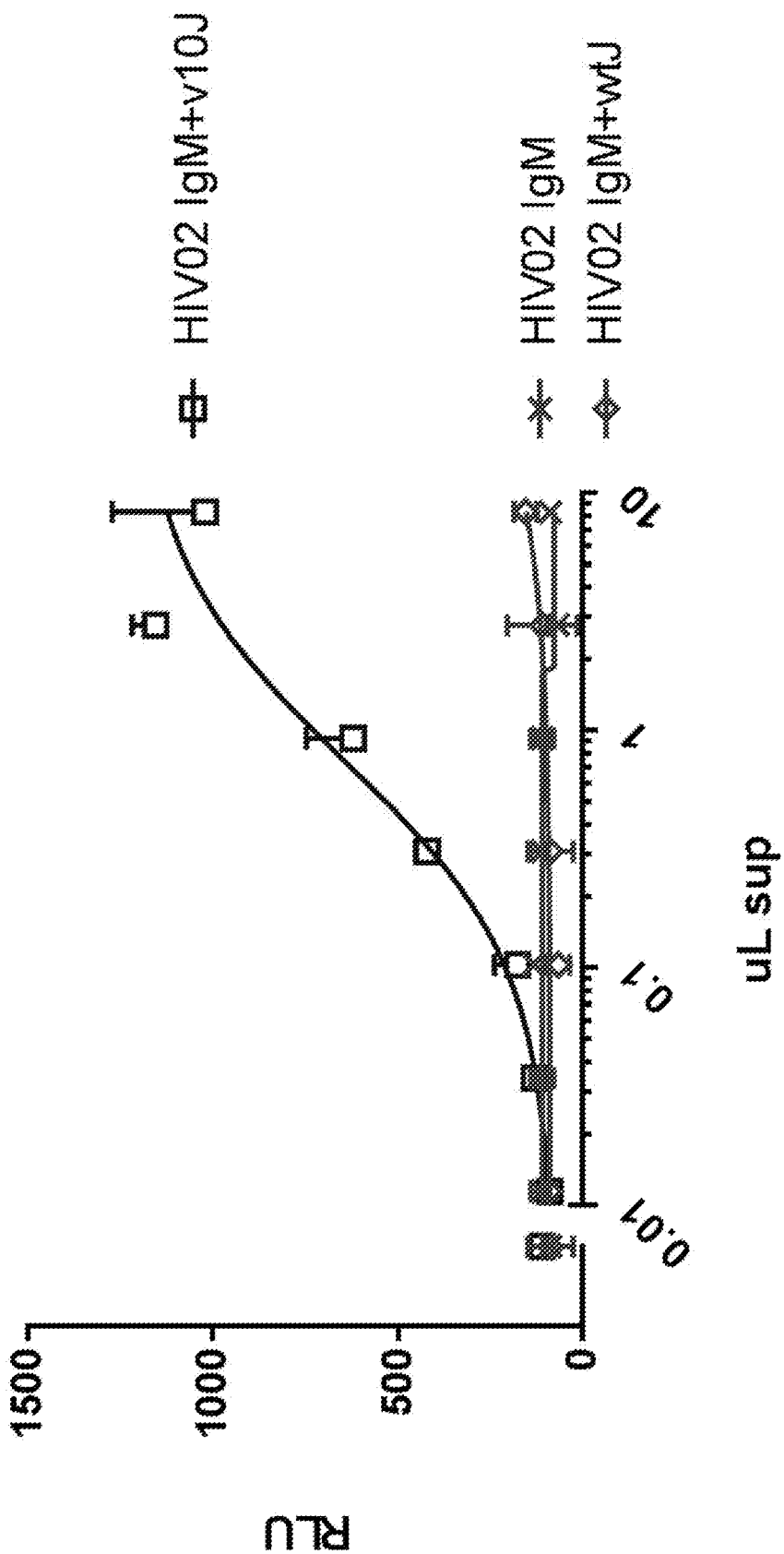
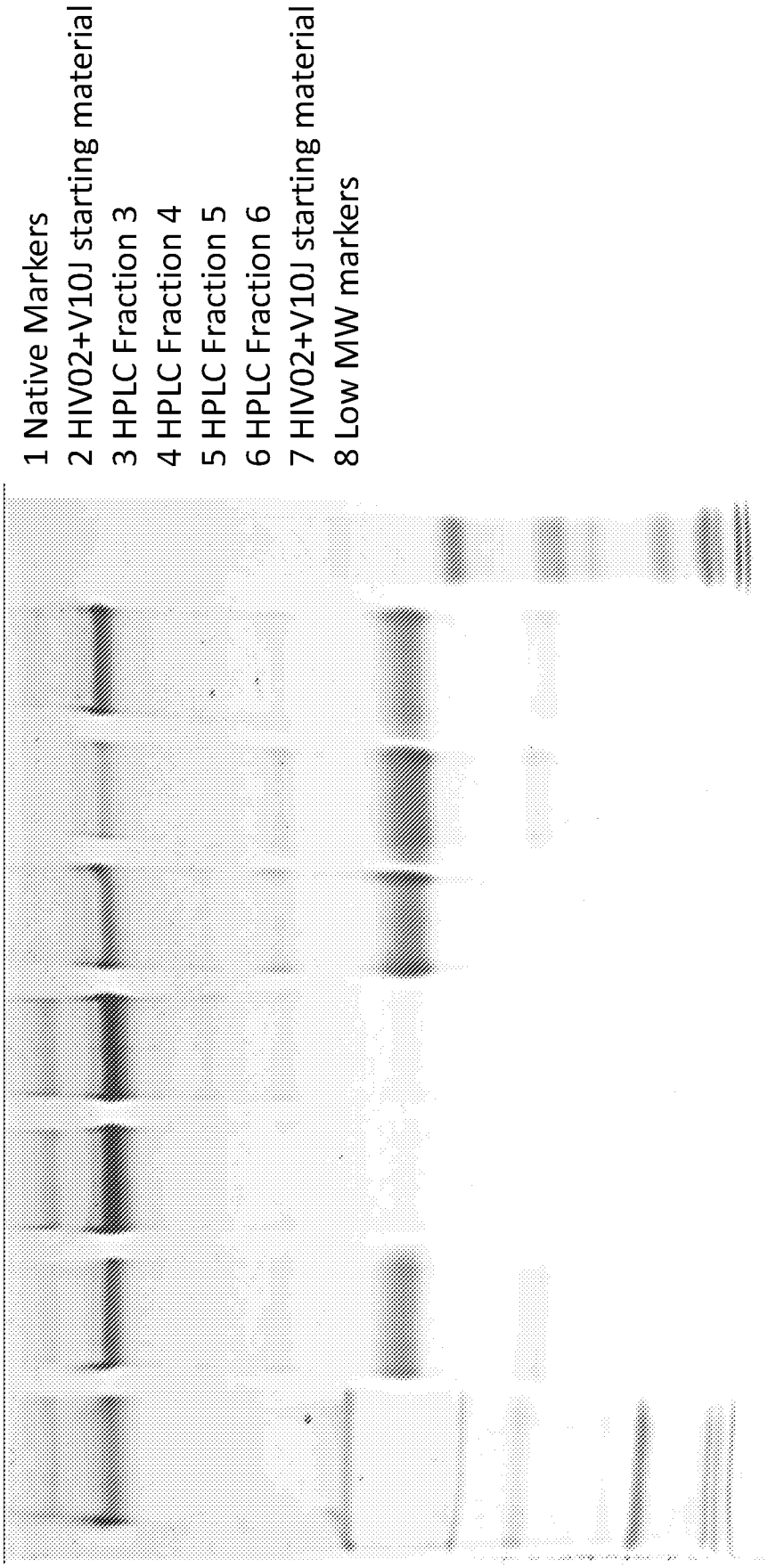

HIV02 IgM+v10J
HIV02 IgM
HIV02 IgM+wtJ
RLU
1500
1000
500
0
10
1
0.1
0.01
uL sup

Fig. 7

Fig. 8


1 Native Markers
2 HIV02+V10J starting material
3 HPLC Fraction 3
4 HPLC Fraction 4
5 HPLC Fraction 5
6 HPLC Fraction 6
7 HIV02+V10J starting material
8 Low MW markers
1
2
3
4
5
6
7
8

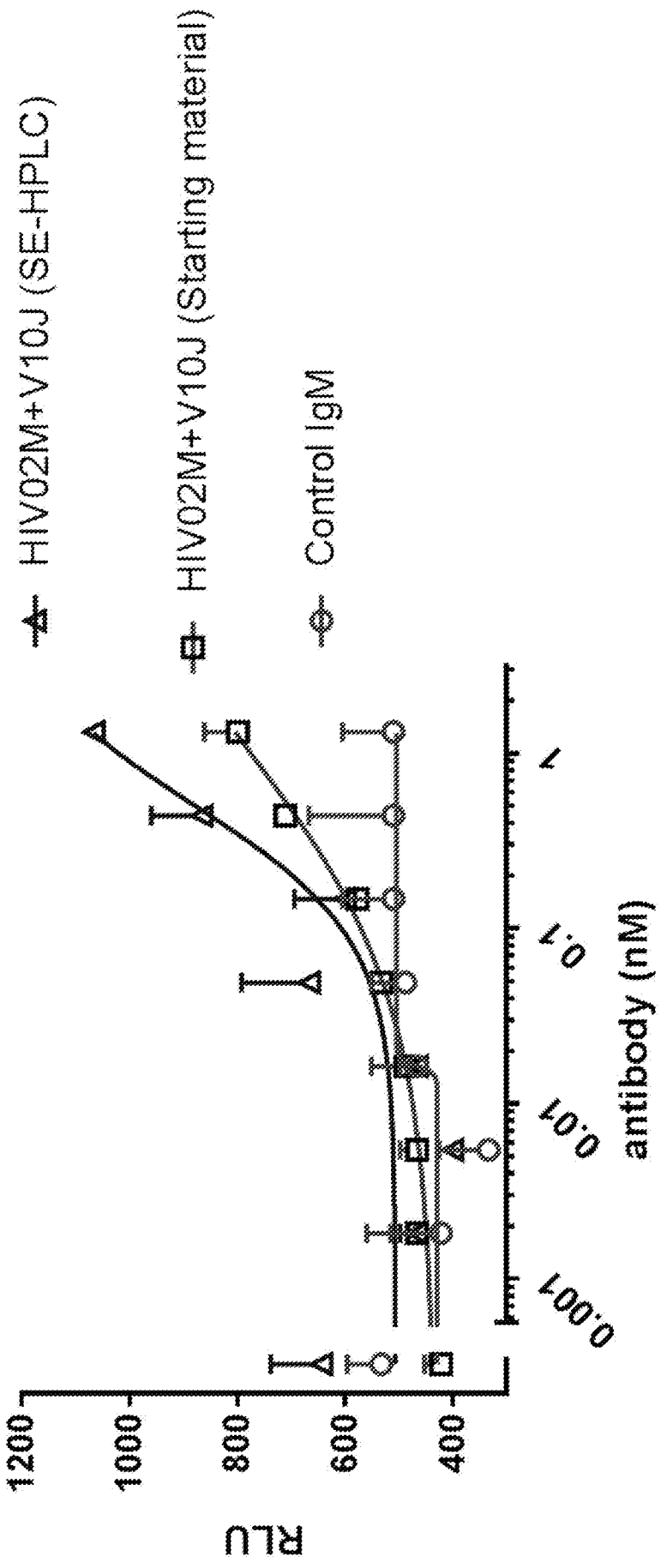

HIV02M+V10J (SE-HPLC)
HIV02M+V10J (Starting material)
Control IgM
RLU
1200
1000
800
600
400
1
0.1
0.01
0.001
antibody (nM)

Fig. 9