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(71) Applicant: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA** [US/US]; 1111 Franklin Street, Twelfth Floor, Oakland, California 94607-5200 (US).

(72) Inventor; and

(71) Applicant: **MOROZ, Paul** [AU/AU]; c/o Philip Gehrig, Wrays, Level 20, 1 Castlereagh Street, Sydney, New South Wales 2000 (AU).

(72) Inventors: **YEATES, Todd**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US). **MILLER, Justin**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US). **CASTELLS-GRAELLS, Roger**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US). **ARBING, Mark Aubrey**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US). **MUNOZ, Aldo**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US). **NGUYEN, Brian Thuan**; c/o UCLA Technology Development Group, 10889 Wilshire Blvd., Suite 920, Los Angeles, California 90095-7191 (US).

(74) Agent: **SUNDBY, Suzannah K.**; Canady + Lortz LLP, 1050 30th Street, NW, Washington, District of Columbia 20007 (US).

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(54) **Title:** IMMUNOSORBENT NANOPARTICLES AND METHODS OF USING THEREOF

(57) **Abstract:** Disclosed herein are immunosorbent nanoparticles, devices, and methods for selective removal of a target protein such as beta-2 microglobulin (B2M) from a liquid such as blood.

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IMMUNOSORBENT NANOPARTICLES AND METHODS OF USING THEREOF

[0001] CROSS-REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Patent Application No. 63/508,514, filed June 16, 2023, which is herein incorporated by reference in its entirety.

[0003] REFERENCE TO A SEQUENCE LISTING SUBMITTED VIA EFS-WEB

[0004] The content of the XML file of the sequence listing named "20240611_034044_251WO1_ST26" which is 61,484 bytes in size was created on June 11, 2024 and electronically submitted via Patent Center herewith the application is incorporated herein by reference in its entirety.

[0005] ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

[0006] This invention was made with Government support under DE-FC02-02ER63421 awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

[0007] BACKGROUND OF THE INVENTION

[0008] 1. FIELD OF THE INVENTION

[0009] The field of the invention generally relates to filtration and separation media and hemodialysis.

[0010] 2. DESCRIPTION OF THE RELATED ART

[0011] Whole blood may be characterized as comprising three size classes of molecules: (i) small molecules having a size less than 1.5 nm such as water, electrolytes, glucose, urea, and small compounds, etc.; (ii) medium sized molecules having a mass of about 0.5 − 50 kDa or a size of about 1.5 − 4 nm (which are often referred to as "middle molecules"); and (iii) large molecules having a mass over 50 kDa or a size over 4 nm.

[0012] For patients afflicted by renal failure, hemodialysis is a lifesaving therapy wherein a hemodialyzer instrument fulfils the blood filtering function of healthy kidneys. However, the methods and devices used to filter blood are often ineffective at removing unwanted medium sized molecules, *e.g.*, uremic toxins. Additionally, prior art methods and devices lack of selectivity whereby unwanted medium sized molecules are removed from patient dialysate without removing or decreasing the amount of beneficial or necessary medium sized molecules such as albumin and immunoglobulins. Thus, prior art methods and devices for hemodialysis often lead to an unintended increase in the

concentration of medium sized molecules, such as beta-2 microglobulin (B2M), which has a mass of 11.8 kDa.

[0013] B2M is a member of the class of proteins known to transition from a functional folded state to a misfolded amyloid state. This transition occurs at elevated concentrations of the protein, those not normally found physiologically, but common in long-term dialysis patients. Patients with renal deficiency tend to exhibit highly elevated concentrations of circulating B2M, because 99% of B2M (which is produced naturally by the body at a high rate of 2-4 mg/kg/day on average) is cleared from circulation in the kidneys. Under renal failure, the high concentration of circulating B2M often results in amyloid deposition in a condition known as dialysis related amyloidosis (DRA). DRA is characterized by the formation of insoluble deposits of B2M throughout the body, resulting in pain and physiological dysfunction, with effective treatments mostly limited to pain management or surgical intervention. In patients with reduced renal function, who must undergo long-term dialysis, B2M can accumulate at concentrations more than 60 times higher than normal. This can lead to the aggregation of B2M into insoluble amyloid fibrils throughout the body, causing painful disorders of the bones and joints, including carpal tunnel syndrome and, in extreme cases, paraplegia. Thus, control B2M concentrations in subjects is of considerable medical importance.

[0014] Albumin is also a medium sized molecule (having a diameter of about 2.5 nm). Albumin, however, is an essential component in blood as it functions to maintain plasma oncotic pressure. Unfortunately, prior art methods and devices that remove B2M based on its size and/or mass from blood also remove necessary and beneficial proteins, such as albumin and immunoglobulins, from the blood.

[0015] Thus, a need exists for the selective removal of medium sized molecules of interest, such as B2M, from a fluid such as blood without removing other medium sized molecules such as albumin.

[0016] SUMMARY OF THE INVENTION

[0017] In some embodiments, the present invention is directed to an immunosorbent nanoparticle comprising or consisting of (a) a plurality of one or more subunit proteins which form a protein cage having an exterior surface, and (b) a binder against beta-2 microglobulin (B2M), said binder is recombinantly linked, directly or indirectly, to at least one of the one or more subunit proteins, wherein the binder is presented on the exterior surface. In some embodiments, the binder is (a) a nanobody comprising the following CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, or (b)

a DARPin comprising SEQ ID NO: 29 or SEQ ID NO: 30. In some embodiments, the binder is a nanobody having a sequence that (a) contains CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, and (b) at least 90% sequence identity to SEQ ID NO: 28. In some embodiments, the one or more subunit proteins are selected from the group consisting of: a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20; a subunit protein having 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23; and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24. In some embodiments, the immunosorbent nanoparticle comprises or consists of: a plurality of protein chains having SEQ ID NO: 42 and SEQ ID NO: 43; a plurality of protein chains having SEQ ID NO: 44 and SEQ ID NO: 45; a plurality of protein chains having SEQ ID NO: 46 and SEQ ID NO: 47; a plurality of protein chains having SEQ ID NO: 48 and SEQ ID NO: 49; or a plurality of protein chains having SEQ ID NO: 50 and SEQ ID NO: 51. In some embodiments, (1) the one or more subunit proteins are selected from the group consisting of a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24; and (2) the binder is selected from the group consisting of a nanobody comprising: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27; a nanobody comprising a sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 28, said sequence including SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27; a DARPin comprising SEQ ID NO: 29; and a DARPin comprising SEQ ID NO: 30. In some embodiments, the binder comprises or consists of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 33. In some embodiments, the immunosorbent nanoparticle is immobilized on solid substrate.

[0018] In some embodiments, the present invention is directed to an article comprising (a) a housing having at least one outlet, and (b) a plurality of immunosorbent nanoparticles, as described herein, contained within the housing. In some embodiments, the article comprising immunosorbent nanoparticles as described herein is in fluidic communication with a dialyzer.

[0019] In some embodiments, the present invention is directed to a kit comprising (1) a plurality of immunosorbent nanoparticles, as described herein, or (2) an article comprising a housing having at least one outlet and the plurality of immunosorbent nanoparticles.

[0020] In some embodiments, the present invention is directed to a hemodialysis device comprising (a) a dialyzer, and (b) an article comprising a housing having at least one outlet and a plurality of immunosorbent nanoparticles, as described herein. In some

embodiments, the article comprising immunosorbent nanoparticles as described herein is in fluidic communication with the dialyzer.

[0021] In some embodiments, the present invention is directed to a hemodialysis device comprising (a) a dialyzer, and (b) an article comprising a housing having at least one outlet, and (b) a plurality of self-assembling protein cages contained within the housing. In some embodiments, the plurality of self-assembling protein cages comprise one or more subunit proteins are selected from the group consisting of: a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to

SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22; a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23; and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24. In some embodiments, the article comprising immunosorbent nanoparticles as described herein is in fluidic communication with the dialyzer.

[0022] In some embodiments, the present invention is directed to a method of removing beta-2 microglobulin (B2M) from a liquid, which comprises (a) contacting the liquid with a plurality of immunosorbent nanoparticles, which are BACs as described herein, or flowing the liquid through an article or a hemodialysis device comprising the BACs, thereby binding the B2M to the plurality of BACs, and then (b) separating the liquid from the BACs having B2M bound thereto. The method according to claim 15, wherein the BACs having B2M bound thereto are removed using protein purification techniques in the art, *e.g.*, ion-exchange chromatography, immunoaffinity chromatography, size exclusion chromatography, or filtration.

[0023] Both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the invention as claimed. The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute part of this specification, illustrate several embodiments of the invention, and together with the description explain the principles of the invention.

[0024] DESCRIPTION OF THE DRAWINGS

[0025] This invention is further understood by reference to the drawings wherein:

[0026] FIG. 1: Assembled BACs bind to and coelute with B2M cargo. A: nbBAC-1-SL mixed with B2M elutes in size-exclusion chromatography (SEC) at a volume consistent with proper cage assembly (denoted with asterisk). SDS-PAGE analysis of the cage peak fraction indicates B2M co-elutes with BAC. B: nbBAC-2 mixed with B2M elutes in SEC at a volume consistent with proper cage assembly (denoted with asterisk). SDS-PAGE analysis of the cage peak fraction indicates B2M co-elutes with BAC.

[0027] FIG. 2: Purification and B2M binding of an improved B2M-binding nanoparticle, nbBAC-1-LL. The improved nbBAC-1-LL, mixed with B2M, elutes in SEC (left) at a volume consistent with proper cage assembly (denoted by an asterisk). SDS-PAGE analysis (right) of the cage peak fraction indicates B2M co-elutes with BAC.

[0028] FIG. 3: Binding of B2M by nbBAC-1-LL in a B2M retention assay. nbBAC-1-LL binds to soluble B2M and retains it in the supernatant of a concentrator column with a molecular weight cutoff of 100 kDa (right). When no nbBAC is added to the column, B2M flows through the membrane unimpeded (left). B2M has a MW of 13.8 kDa and nbBAC has a MW of 662 kDa. An SDS-PAGE gel shows the composition of the flow through in each experiment. B2M is only present in the gel run on the flow through of the experiment without nbBAC added, showing that B2M is fully retarded by the interaction between nbBAC-1-LL and B2M. This demonstrates retention of B2M – *i.e.*, removal of B2M from the flow-through—by the stable interaction between nbBAC-1-LL and B2M.

[0029] FIG. 4: Immunoblot analysis of flowthrough from the B2M retention assay. Serial dilutions of flow through from the size-filtration retention assay either with (top) or without (bottom) nbBAC-1-LL added to supernatant were analyzed for B2M via immunoblot. a positive control (1 μM B2M) is depicted in top right.

[0030] FIG. 5: Measurement of binding affinity of B2M to nbBAC-1-LL. Biolayer interferometry was used to estimate the equilibrium dissociation constant of nbBAC for B2M (64 nM ± 8 nM).

[0031] FIG. 6: Nanoparticle nbBAC-1-LL bound to a stationary matrix fully removes B2M from human serum. SDS-PAGE gels are analyzed by Western blotting with an anti-B2M antibody. A: Analysis of flow through of serum supplemented with B2M flowed over resin, either with nbBAC-1-LL bound to the column (lanes 5-7) or without (lanes 2-4). Lane 1 is 20X diluted serum supplemented with B2M. B: Analysis of resin-bound components from serum supplemented with B2M, eluted from the resin using 1M imidazole buffer, either with nbBAC-1-LL bound to the column (lanes 2-4) or without (lanes 5-7).

[0032] DETAILED DESCRIPTION OF THE INVENTION

[0033] Described herein are target adsorbent cages (TACs), which are nanoparticles comprising a protein cage as a scaffold that has on its surface multiple copies of a binder, *e.g.*, a nanobody or a DARPin, against a target molecule of interest. As the diameter of the exemplified tetrahedral protein cage itself is about 130 nm and the diameter of the

exemplified icosahedral protein cage is about 230 nm, the total diameter of a TAC having one or more copies of a target molecule of interest adsorbed thereon is at least 130 nm. Thus, as the experiments herein show, TACs may be used to remove small proteins and other molecules from a liquid phase via size-based separation methods in the art, (*e.g.*, size-based filtration) or adsorption chromatography (*e.g.*, polyvalent binding to a stationary matrix). As such, TACs may be used to remove abnormal or unwanted molecules from, *e.g.*, the blood of, subjects.

[0034] Exemplified herein are TACs that comprise either nanobodies or DARPins against beta-2 microglobulin (B2M). As such, the exemplified TACs are referred to as "B2M adsorbent cages" (BACs). BACs comprising nanobodies as the binder are referred to as "nbBACs", and BACs comprising DARPins as the binder are referred to as "DARPBACs".

[0035] The BACs specifically bind B2M with nanomolar binding affinity (~64 nM). Because of the size of the protein cage having the nanobodies or DARPins thereon, the size of a B2M molecule is effectively increased by over 50-fold by having a BAC bound thereto. As such, B2M molecules complexed with BACs may be readily removed from a liquid phase (*e.g.*, plasma) using size-based separation methods in the art, (*e.g.*, size-based filtration) or adsorption chromatography (*e.g.*, polyvalent binding to a stationary matrix). Therefore, BACs may be employed in combination with hemodialysis to reduce the risk of and/or treat dialysis-related amyloidosis, existing methods for treating dialysis-related amyloidosis.

[0036] Self-assembling protein cages are known in the art. See, for example, US8969521, US9066870, US9630994, US10248758, US10501733, US20200397886, US20210163540, and WO2020/220044. Self-assembling protein cages comprise a plurality of polypeptide subunits that are held together via intermolecular forces to form a protein nanoparticle having a "shell", *i.e.*, an exterior surface. As used herein, a "protein cage" refers to a plurality of polypeptides ("subunits" or "oligomeric protein units") which collectively self-assemble to form a three-dimensional structure whereby some of the amino acids of a subunit are exterior residues that form part of the "shell", and the remainder of the amino acids of the subunit are interior residues which may form an interior cavity. The amino acid sequences of the subunits may be the same or different. Typically, protein cages are formed with (a) subunits having the same amino acid sequence, or (b) 2−3 different of subunits, *e.g.*, some subunits have a first amino acid sequence and some protein cage subunits have a second amino acid sequence. Each subunit of a protein cage typically occurs in multiple copies (*e.g.*, 12, 24, or 60 copies),

and at least one of the subunits has one or more amino acids at or near a terminal end that is exposed on the exterior surface of the protein cage. Generally, the subunits of the protein cages self-assemble into a symmetric geometric shapes that mimic the 3D shape of any one of the Platonic solids: tetrahedron, cube, octahedron, icosahedron, and dodecahedron.

[0037] The BACs exemplified herein are based on a tetrahedral protein cage known as "T33-51" (see Cannon (2020)) and an icosahedral protein cage known as "I53-50" (see Bale (2016)). As provided in the detailed examples, nbBAC-1 and DARPBAC-1 to -3 have a tetrahedral protein cage based on T33-51 and nbBAC-2 has a protein cage based on I53-50. Particularly, the sequences of the subunits of nbBAC-1 and DARPBAC-1 to -3 comprise SEQ ID NO: 1 ("subunit A") and SEQ ID NO: 2 ("subunit B", which is a truncation of SEQ ID NO: 6). In nbBAC-1, the binder is covalently linked to subunit B and subunit A comprises SEQ ID NO: 5. In DARPBAC-1 to -3, the binder is covalently linked to subunit A, *i.e.*, SEQ ID NO: 1 , which is a truncation of SEQ ID NO: 5. SEQ ID NO: 1 has about 90% sequence identity to SEQ ID NO: 5. The sequences of the subunits of nbBAC-2 comprise SEQ ID NO: 3 ("subunit A") and SEQ ID NO: 4 ("subunit B"). SEQ ID NO: 3 has 97.1% sequence identity to SEQ ID NO: 7 and SEQ ID NO: 4 has 95% sequence identity to SEQ ID NO: 8. In nbBAC-2, the binder is covalently linked to subunit B.

[0038] The protein cages based on T33-51 have 12 pairs of subunit chains A and B (*i.e.*, a stoichiometry of $A_{12}B_{12}$) and protein cages based on I53-50 have 60 pairs of subunit chains A and B (*i.e.*, a stoichiometry of $A_{60}B_{60}$). As the given binder is linked to either chain A or chain B, but not both, single molecules of nbBAC-1 and DARPBAC-1 to -3 have 12 copies and single molecules of nbBAC-2 have on their surface 60 copies of the given binder against B2M on their surfaces. The exemplified BACs comprise the given binder against B2M covalently linked to an amino acid residue of one of the subunit chains that is presented at the outer surface of the protein cage, which allows the given binder to interact with and bind B2M.

[0039] Any protein cage known in the art may be employed as the scaffold to which a binder against B2M is covalently linked thereto to give a BAC as contemplated herein. Suitable protein cages include: T33-51, I53-50, T33-31, T33-21, T33-28, I53-47, I53-40, I52-32, I32-28, ferritin, and sulfur oxygenase reductase, which are composed of subunits having the following amino acid sequences:

[0040] T33-51 protein cage (~130 nm dia.):

Subunit A:
mftrrgdqgetdlanrarvgkdspvvevqgtidelnsfigyalvLSRWDdiRNdlFRiqNdlfVl
gEdvsTgGKGRTVTmDmiiYlikRsvEmkAEigKIELfvvpGGSVEsaslhmaravsrrlerrik
aaselteinanvllyanmlsnilfmhalisnkRLNIPEKIWSIHRVSLE (SEQ ID NO: 5)

Subunit B:
mrittkvgdkgstrlfggeevwkddpiieangtldeltsfigeakhYVDEEmkGilEEiqNDiyK
imGeigsKGKIEGISeEriKWlaGliERyseMVNKLSFVLPGGTLesakldvcrtiarraerkva
tvlrefgigtlaaiylallsrllfllarvieIEKNKLKEVRSHHHHHH (SEQ ID NO: 6)

[0041] I53-50 protein cage (~230 nm dia.):

Subunit A:

MKMEElFkkhkiVavlransveeaiekavavfaggvhlieitftvpdadtvikalsvlkEkGaii
gagtvtsVeqcRkavEsGAEfivsphldEeisQfckEKGvfympgvmtptelvkamkLGHTilkl
fpgevvgpqfVkamkgpfpnvkfvptggvnldnvcewfkagvlavgvgsalvkgtpdevrekaka
fvekirgcte (SEQ ID NO: 7)

Subunit B:

MNQHSHKDYETVriavvrarwhAeivDacvSafEaamAdigGDRFAVDVFDVPGayeiplhartl
aeTGRygavlgtafvvnggiyrhefvasavidgmmnvqlstGvpvlsavltphryRDSdahtllf
lalfavkgmeaaracvEilAaREKIAAGSLE (SEQ ID NO: 8)

[0042] T33-31 protein cage (~115 nm dia.):

Subunit A:
MVrgirgaitveedtpaailaatiellLKmlEANGIQSYEElaaviftvtedltsafpaeaARLI
GMHRvpllsarevpvpgslprvirvlalwNTDTPQDRVRHVYLNEaVRlRPDLESAQLE (SEQ
ID NO: 9)

Subunit B:
MEevvlitvpsaLvavKiahAlveERLAAcvnivpgltsiyrEEGSVvsdhellllvktTTDafP
KlkervkElHPYEVpeivaLPIAEGnReylDwlREntgle (SEQ ID NO: 10)

[0043] T33-21 protein cage (~125 nm dia.):

Subunit A:
mrittkvgdkgstrlfggeevwkdspiieangtldeltsfigeaKHYVDEEmkGilEEiqNdiYK
imGeigskGKIEGISEEriaWllKlilRymEMVNLKSFVLPGGTLEsakldvcrtiarralrkvl
tvtrefgigaeaaaYllalsdllfllarvieIEKNKLKEVRS (SEQ ID NO: 11)

Subunit B:
mphlvieatanLRLETSPGEllEqanKalFasgqfGEAdiksrfVTLEAYRQGTAAVERaylhac
lsildgrdiatrtllgaslcavlaEAVAGGGEEGvqvsvevremerlsyakrvvarqrle (SEQ
ID NO: 12)

[0044] T33-28 protein cage (~125 nm dia.):

Subunit A:
mesvntsflspslvtirdfdngqfavlRIGRTGfpadkgdidlcldkmigvraaqiflgddtedg
fkgphirircVDIDDKHTYNAMvyvdlIVGTGASEveretaeeeaklalrvalqvdiaDEHSCVT
QFEMKLREELLSSDSFHpdkdEyyKDFL (SEQ ID NO: 13)

Subunit B:

MPviqtfvstpldhhkrlllaiiyrivtrvVLGKPEDLvmmtfhdstpmhffgsTDPvacvrvea
LGGYGPSepEkvtSivtAaiTavcgiVADrifvlyfSPLHCGWNGTNFLE (SEQ ID NO: 14)

[0045] I53-47 protein cage (~230 nm dia.):

Subunit A:

mpiftlntNIKATDVPSDflsltsrlvglilskpgsyvavhintDQQLSFGGSTNpaafgtlmsi
ggiepsknrdhsavlfdhlnaMLGIPKNRmyihfvnlngddvgwngttf (SEQ ID NO: 15)

Subunit B:

MNQHSHKDYETVriavvrarwhAdivDacvEafEiamAaigGDRFAVDVfdvpgayeiplhartl
aeTGRygavlgtafvvnggiyrhefvasavidgmmnvqlstGvpvlsavltphryrdsaehhrff
aahfavkgveaaraciEilAaREKIAAGSLE (SEQ ID NO: 16)

[0046] I53-40 protein cage (~240 nm dia.):

Subunit A:
mtkkvgivdttfarvdmasaailtlkmespnikiirktvpgikdlpvackklleeegcdivmalg
mpGKAEkdkvcaheaslglmlaqlmtnkhiievfvheDEAKDDaelkilaarraiehalnvyyll
fkpeyltrmagkglrqgfEDAGPARE (SEQ ID NO: 17)

Subunit B:
mstinNqlkalkvipviaidnaediiPlgKvlaEnglpaaeitfrssaavkaimllrsaqpemli
gagtilngvqalaakeagatfvvspgfnpntvracqiigidivpgvnnpstveaalemglttlkf
fpaeASGGISmvkSlvgpygdirlmptgGiTPSNIdNylAIPqvlacggtWMVdkKLvtNGEwdE
iaRltrEiveQVNPGSLE (SEQ ID NO: 18)

[0047] I52-32 protein cage (~260 nm dia.):

Subunit A:
MKYDGSKLrigilharwnleiiaalvagaikrlqeFGVKAENiiietvpgsfelpygsklfvekq
KRLGKPLDaiipigvlikgstmhfeyicdstthqlmklnfELGIpvifgvltcltdeqaearagl
iegkmhnhgedwgaaaveMATKFNLE (SEQ ID NO: 19)

Subunit B:
MGMKEkfvliithgdfgkgllsgaeviigkqenvhtvglnlgdniekvakevmriiiaklaedke
iiivvDlFGGspfNiaLemMKTFDVKvitginmPmlvEllTsinVYdtTEllEnisKigkDGIKV
IEKSSLKM (SEQ ID NO: 20)

[0048] I32-28 protein cage (~300 nm dia.):

Subunit A:
MGDDariaaigdvdelnsqigvllaeplpddvraalsAiqHdlfDlgGelCIPGHAAITEdhllr
lalwlvhyngqlppleefilpggargaalahvcrtvcrraersikalgaSEPLNIapaayvnlls
dllfvlarvlnrAaggadvlwdrtrah (SEQ ID NO: 21)

Subunit B:
MILSAEQSFTLRHPHGQAAaLAfvRepaAalAGVQRLRGLDSDGEqvwgellvrvpllgevdlpf
rseIVRTPQGAelrpLTLTGERAWVAVSGQATAAEGGEMAFAFQFQAHlatpeaegeggaafevm
vqaaagvtlllvamalpqglaAGLPPALE (SEQ ID NO: 22)

[0049] Human H chain Ferritin protein cage (~125 nm dia.):

mttastsqvrqnyhqDseAainRqinLelYasyvylsmsyyfdrddvalknfakyflhqsheere
haeklmklqnqrggRIFLQDIQKPDCDDWEsglNameCalHlekNvnQsllElhkLatDKndphl
cdfiethylneqvkaikelgdhvTnlRKMGApESglaeylfdkhtlgdsdnes (SEQ ID NO:
23)

[0050] Sulfur Oxygenase Reductase (SOR) protein cage (~150 nm dia.):

MPKPYvainmaelknepktfemfasvgpkvcmvtaRHPGfvgfqnhiqigilpfgnryggakmd
mtkesstvrvlqytfwKDWKDheEmhRqnwsylfrlcyscasqmiwgpwepIYEIIYAnmpin
temtdftavvgkkfaEGKPldipvisqpygkrvvafaehsvipgKeKqfeDaiVrtlEmlkkap
gflgamvlkeigvsgigsmqfgakgfhqvlenpgslepdpNnvMYSvpEakntpqqyivhvewa
ntdalmfgmgrvllypElrqVhdEvldtlvygpyirilnpmmegtfwrEYLNENAWRHPQFGG
(SEQ ID NO: 24)

[0051] In the above sequences, the capital letters represent "exterior residues", *i.e.*, amino acid residues that are exposed on the exterior surface of the protein cages. As such, a given binder against B2M is preferably covalently linked to any one of the exterior residues of one of the subunit chains of a protein cage above to result in a BAC as contemplated herein. In some embodiments, the given binder is recombinantly fused to one of the exterior residues of one of the subunit chains of a protein cage. In some embodiments, the given binder is covalently attached to one of the exterior residues of one of the subunit chains of a protein cage using chemical protein synthesis techniques in the art. In some embodiments, the exterior residue to which the binder is covalently linked to is one of the last exterior residues at or near the C-terminus of the subunit chain. In some embodiments, a subunit chain may be truncated to remove 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 residues at the N-terminus and/or the C-terminus and the binder is covalently linked to the N- or C-terminus of the truncated subunit chain. In some embodiments, a subunit chain may be truncated to remove any interior residues at the C-terminus and the binder is covalently linked to the C-terminus of the truncated subunit chain. In some embodiments, a subunit chain may be truncated to remove any interior residues at the N-terminus and the binder is covalently linked to the N-terminus of the truncated subunit chain. In some embodiments, a subunit chain is modified by adding to its N- or C-terminus one or more amino acid residues, which will be presented on the exterior of the protein cage, and the binder is covalently linked to the one or more amino acid residues that were added.

[0052] As exemplified herein, the binder of the nbBAC constructs is a nanobody having the following CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27. Thus, in some embodiments, the binder is a nanobody comprising the following CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27. In some embodiments, the nanobody sequence comprises CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, and at least 90% sequence identity to SEQ ID NO: 28.

[0053] As exemplified herein, the binder of the DARPBAC constructs is a DARPin. The sequences of the exemplified DARPins comprise at least about 80% sequence identity to each other and have the following consensus sequence:
K-K-X1-L-D-A-A-S-A-G-X2-D-D-X3-V-X4-X5-L-X6-A-X7-G-A-D-V-N-A-S-X8-X9-X10-G-X11-T-P-L-H-X12-A-A-X13-X14-G-H-L-E-I-V-X15-V-L-L-X16-X17-G-A-D-X18-N-A-S-D-X19-Y-G-W-T-P-L-H-X20-A-A-X21-X22-G-H-L-E-I-V-X23-X24-L-L-X25-X26-G-A-D-V-N-A-Q-D-K-F-G-K-T-P-F-D-L-A-I-D-N-G-N-E-D-I-A-E-V-L-Q-

K-A-A (SEQ ID NO: 29). Therefore, in some embodiments, the binder is a DARPin comprising SEQ ID NO: 29, wherein each X is independently any amino acid. In some embodiments, the binder is a DARPin comprising SEQ ID NO: 30, wherein X1 is Q or L; X2 is Y or Q; X3 is Q or E; X4 is A or R; X5 is A or I; X6 is L or M; X7 is K or N; X8 is D or N; X9 is W or N; X10 is N or W; X11 is W or Y; X12 is S or A; X13 is Q or Y; X14 is W, R or D; X15 is E or D; X16 is K or A; X17 is R, N or Y; X18 is I or V; X19 is W or Y; X20 is V or S; X21 is T or R; X22 is W, N or Q; X23 is E or D; X24 is L or V; X25 is R or A; and X26 is W or H.

[0054] The BACs exemplified herein indicate that subunit proteins forming a protein cage of BAC (excluding the binder and any linkers and tags) need only have at least about 90% sequence identity to the parental sequences, *i.e.*, the sequences of the subunits of the known protein cages from which the BACs are based. Therefore, in some embodiments, the sequences of subunits forming the protein cage of a BAC are at least 90% identical to the parental sequences of the protein cages described herein. Thus, where the BAC is based on the T33-51 protein cage, then the sequences of the subunits forming the protein cage of the BAC have at least 90% sequence identity to SEQ ID NO: 5 and at least 90% sequence identity to SEQ ID NO: 6. As another example, where the BAC is based on the I53-50 protein cage, then the sequences of the subunits forming the protein cage of the BAC have at least 90% sequence identity to SEQ ID NO: 7 and at least 90% sequence identity to SEQ ID NO: 8. In some embodiments, BACs comprise:
(1) a protein cage formed of:

- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6;

- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22;
- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23;

- a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24; and

(2) a binder selected from the group consisting of

- a nanobody comprising: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27;
- a nanobody comprising a sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 28, said sequence including SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27;
- a DARPin comprising SEQ ID NO: 29; and
- a DARPin comprising SEQ ID NO: 30.

[0055] *BAC Design*

[0056] The subunit proteins of the BACs exemplified herein were over-expressed in *E. coli* cells. The BACs self-assemble and to therefore appear in their assembled forms upon expression and purification. The BACs were purified by affinity chromatography. For binding tests, the BACs were mixed with purified B2M protein and assessed for binding using size exclusion chromatography (SEC). In the event of a stable interaction between the BAC and B2M, co-elution of proteins would be expected. If no interaction occurs, B2M would be expected to elute much later due to its significantly smaller size relative to the BAC assembly. nbBAC-1-SL and nbBAC-2 co-eluted with B2M. *See* FIG. 1. However, nbBAC-1-SL displayed limited stability in its assembled state, precipitating out of solution over time, and exhibited a propensity to disassemble into smaller subunits; and nbBAC-2 displayed sub-stoichiometric binding to B2M when visualized on SDS-PAGE.

[0057] Therefore, a BAC comprising a longer 12-residue $(GS)_6$ linker, *i.e.*, nbBAC-1-LL, was designed and tested. nbBAC-1-LL exhibited better stability and a higher yield of protein eluting at the expected elution volume compared nbBAC-1-SL. *See* FIG. 2. nbBAC-2 exhibited a limited capacity for B2M binding compared to the nbBAC-1 constructs. As such, BACs based on T33-51 protein cages are preferred over BACs based on I53-50 protein cages.

[0058] *B2M Removal*

[0059] To characterize the ability of the BACs to bind and sequester B2M, size-based retention assays utilizing a centrifugal filter with a molecular weight cutoff of 100 kDa, which falls between the size of B2M and the BACs, were employed. When centrifuged, a stable interaction between B2M and BAC should prevent B2M from passing through

the filter, whereas B2M alone should pass through the filter unimpeded. *See* FIG. 3. That is, adding the BAC to a solution of B2M should prevent any protein material from passing through the size filter, and this was confirmed experimentally.

[0060] Using a BSA assay, the protein concentration was measured in the flow through of both experiments, testing 100 nM B2M plus or minus addition of nbBAC-1-LL at 1 μM. The resulting protein concentration in the flow through was determined to be at least 5 times lower when nbBAC-1-LL was added (above the centrifugal filter) compared to when it was absent. The BAC effectively sequesters the B2M by forming a complex therewith, which complex is unable to pass through the filter membrane. Owing to the detection limit of the BSA assay, the precise depletion factor for removal of B2M in this size-based experiment is likely considerably higher than the limiting value of 5 noted above. Indeed, B2M was undetectable by immunoblot in the flow-through fraction; chemiluminescent signal is only visible on the blot containing flowthrough from the experiment without nbBAC-1-LL added to the B2M. *See* FIG. 4.

[0061] Biolayer interferometry was used to determine the affinity of the binding interaction between nbBAC-1-LL and B2M. The equilibrium dissociation constant was determined to be about 64 nM. *See* FIG. 5. Whether the BAC remained stable under conditions relevant for dialysis applications was determined. nbBAC-1-LL was incubated at 37°C in human serum for 4 hours and then assayed for its oligomeric state. After pulling down nbBAC-1-LL based on its poly-histidine tag using Ni-NTA resin, and then eluting with imidazole, SEC analysis indicates that the major species had a size that corresponded to that of the assembled protein cage thereby indicating that BACs are structurally stable under hemodialysis conditions.

[0062] To evaluate the capacity of BACs as a binding matrix for unwanted proteins associated with hemodialysis applications, nbBAC-1-LL was immobilized on a metal affinity (His-trap) column and then human serum supplemented with 50 ug/ml B2M was flowed therethrough and the amount of B2M in the flow-through (after resulting serum had passed through the column) was assayed. The amount of B2M in the column eluate after washing off the BAC from the column using imidazole was also assayed. Western blot was used to analyze samples for B2M and B2M was observed in the BAC elution fraction only. No detectable amounts of B2M were present in the flow-through. *See* FIG. 6. These results indicate that BACs as described herein are capable of removing B2M from serum and blood and can therefore be used to effectively remove B2M in the in the serum and blood of subjects, who may have elevated levels of B2M as a result of hemodialysis.

[0063] As such, in some embodiments, the BACs may be used to remove B2M from the blood or serum of subjects. In some embodiments, BACs are immobilized on a solid substrate, *e.g.*, beads or other solid phase media, and B2M is removed from blood or plasma by contacting the blood or plasma with the BACs immobilized on the solid substrate and then separating the blood or plasma from the solid substrate. For example, (a) BACs may be immobilized on the stationary matrix of a purification column, then the B2M in blood or plasma is removed by flowing the blood or plasma through the purification column; or (b) BACs may be immobilized on magnetic beads, then the B2M in blood or plasma is removed by contacting the blood or plasma with the magnetic beads and then the magnetic beads having B2M bound thereto are removed from the blood or plasma via magnetic fields.

[0064] In some embodiments, size-exclusion methods in the art may be used to remove B2M from blood or plasma. For example, BACs may be added to the blood or plasma and then the BACs and BACs having B2M complexed therewith are separated from the blood or plasma using, *e.g.*, membrane filtration or size exclusion chromatography. For example, if the protein cage of the BAC, excluding any bound B2M has a diameter of 130 nm, then the pore size of the membrane used for membrane filtration should be < 130 nm. Similarly, if the protein cage of the BAC has a diameter of 230 nm, then the pore size of the filtration membrane should be < 230 nm. Likewise, other forms of size-based exclusion media should have a channel size or pore size smaller than the diameter of the protein cage of the given BAC being employed. Because suitable protein cages have a protein size that is typically between 500 kDa and 2 MDa in size, in some embodiments, the size exclusion media employed has a channel or pore size that prevents proteins of 500 kDa or larger from passing therethrough. In some embodiments, the pore size of the membrane or size-based media may be considerably smaller, while retaining the property that proteins of 500 kDa are prevented from passing through. The use of BACs to remove B2M from blood or plasma may be employed in combination with existing hemodialysis methods in the art. In some embodiments, BACs may be used to remove B2M concurrently with hemodialysis, *e.g.*, at the same time a subject's blood is passed through the dialyzer of a hemodialysis machine. In these embodiments, the BACs are added to the blood or plasma to be dialyzed, *i.e.*, added upstream of the dialyzer of a hemodialysis machine. The dialyzer will then remove the BACs and any B2M complexed thereto at the same time the dialyzer removes other unwanted molecules from the subject's blood or plasma. In some embodiments, BACs are used to remove B2M in a step that is "in series" with the step where the blood or

plasma is passed through the dialyzer. For example, the blood or plasma is contacted with the BACs and the BACs and any B2M complexed thereto are removed before the blood or plasma is passed through the dialyzer. As another example, after blood or plasma has passed through the dialyzer, the dialyzed blood or plasma is contacted with the BACs and then the BACs and any B2M complexed thereto are removed therefrom before the blood or plasma is returned to the subject's body. In some embodiments, BACs are used to remove B2M from a subject's blood or plasma in a procedure that is independent of any hemodialysis procedure that the subject may have undergone.

[0065] As provided herein, nbBAC-1-LL complexes with B2M to produce an approximately 825 kDa assembly with a capacity to bind 12 copies of B2M on its exterior. As evidenced herein, BACs have the ability to remove B2M in a size-based separation, *i.e.*, by employing a semi-permeable membrane filter that allows smaller proteins (*e.g.*, albumin) to pass. While the experiments herein attached BACs to a nickelated substrate based on polyhistidine tails, other methods in the art may be used to immobilize the BACs on a given substrate. For example, the BACs may be biotinylated and immobilized on a substrate via binding to streptavidin functional groups on the surface of the substrate. Like other immunosorbents, the substrate comprising BACs having B2M bound thereto may be reused by eluting the BACs, sterilizing the substrate, and then immobilizing fresh, unused BACs thereto.

[0066] As exemplified herein, removal of B2M with BACs was achieved with only one passage of the serum over the solid phase medium, which is a striking improvement over existing size-based filtration methods that require multiple, *e.g.*, up to 20, passages over filtration media. Additionally, a single passage using BACs resulted in a significantly greater reduction in B2M concentrations as compared to prior art methods. As such, BACs are significantly advantageous over prior art methods and materials and may be used to significantly lower B2M concentrations in subjects and thereby inhibit, reduce, and/or treat dialysis-related amyloidosis in subjects.

[0067] These experiments indicate that TACs (including BACs) may be used to selectively remove a target molecule of interest, which has a mass of about 50 kDa or less and/or or a diameter of 4 nm or less, from a liquid (*e.g.*, plasma) using, *e.g.*, size-based separation media and adsorption chromatography.

[0068] Sized Based Separations: In some embodiments, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 115 nm. In embodiments where the protein cage is T33-51, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a

given liquid is about 130 nm. In embodiments where the protein cage is I53-50, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 230 nm. In embodiments where the protein cage is T33-31, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 115 nm. In embodiments where the protein cage is T33-21, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 125 nm. In embodiments where the protein cage is T33-28, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 125 nm. In embodiments where the protein cage is I53-47, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 230 nm. In embodiments where the protein cage is I53-40, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 240 nm. In embodiments where the protein cage is I52-32, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 260 nm. In embodiments where the protein cage is I32-28, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 300 nm. In embodiments where the protein cage is Human H chain Ferritin, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 125 nm. In embodiments where the protein cage is SOR, the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 150 nm. In some embodiments, the TAC comprises or consists of a plurality of protein chains having SEQ ID NO: 42 and SEQ ID NO: 43 and the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 130 nm. In some embodiments, the TAC comprises or consists of a plurality of protein chains having SEQ ID NO: 44 and SEQ ID NO: 45 and the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 230 nm. In some embodiments, the TAC comprises or consists of a plurality of protein chains having SEQ ID NO: 46 and SEQ ID NO: 47 and the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 130 nm. In some embodiments, the TAC comprises or consists of a plurality of protein chains having SEQ ID NO: 48 and SEQ ID NO: 49 and the diameter of the pores of the size

exclusion media used to separate the bound and unbound TACs from a given liquid is about 130 nm. In some embodiments, the TAC comprises or consists of a plurality of protein chains having SEQ ID NO: 50 and SEQ ID NO: 51 and the diameter of the pores of the size exclusion media used to separate the bound and unbound TACs from a given liquid is about 130 nm.

[0069] Adsorption Chromatography: In some embodiments, the TACs are immobilized on a solid substrate and the target molecule of interest is removed from a liquid by contacting the liquid with the TACs immobilized on the solid substrate. Exemplary solid substrate materials to which TACs may be immobilized include cellulose based materials (*e.g.*, cellulose, cellulose acetate, and the like), polystyrene, nylon, polyethersulfone (PES), polypropelene, polyvinylidene fluoride (PVDF), polyamide, and biologically inert materials known in the art.

[0070] The proteins, *i.e.*, subunits of TACs (including BACs) described herein may be made using methods in the art including chemical synthesis, biosynthesis or *in vitro* synthesis using recombinant DNA methods, and solid phase synthesis. *See*, *e.g.*, Kelly & Winkler (1990) Genetic Engineering Principles and Methods, vol. 12, J.K. Setlow ed., Plenum Press, NY, pp. 1-19; Merrifield (1964) J Amer Chem Soc 85:2149; Houghten (1985) PNAS USA 82:5131-5135; and Stewart & Young (1984) Solid Phase Peptide Synthesis, 2 ed. Pierce, Rockford, IL, which are herein incorporated by reference. Protein purification techniques in the art such as reverse phase high-performance liquid chromatography (HPLC), ion-exchange or immunoaffinity chromatography, filtration or size exclusion, or electrophoresis may be used to separate or remove TACs and complexes therewith from fluids and other molecules. *See*, *e.g.*, Olsnes and Pihl (1973) Biochem 12(16):3121-3126; and Scopes (1982) Protein Purification, Springer-Verlag, NY, which are herein incorporated by reference. Polynucleotides that encode the TACs and BACs as described herein are also contemplated herein. In some embodiments, the proteins and polynucleotides are isolated.

[0071] As used herein, an "isolated" compound refers to a compound that is isolated from its native environment. For example, an isolated polynucleotide is a one which does not have the bases normally flanking the 5' end and/or the 3' end of the polynucleotide as it is found in nature. As another example, an isolated polypeptide is a one which does not have its native amino acids, which correspond to the full-length polypeptide, flanking the N-terminus, C-terminus, or both. In some embodiments, isolated polynucleotides and polypeptides are made "by the hand of man", *e.g.*, using synthetic and/or recombinant techniques.

[0072] As used herein, a compound (*e.g.*, a binder as described herein) "specifically binds" a given target (*e.g.*, B2M) if it reacts or associates more frequently, more rapidly, with greater duration, and/or with greater binding affinity with the given target than it does with a given alternative, and/or indiscriminate binding that gives rise to non-specific binding and/or background binding. As used herein, "non-specific binding" and "background binding" refer to an interaction that is not dependent on the presence of a specific structure (*e.g.*, a given epitope).

[0073] As used herein, "binding affinity" refers to the propensity of a compound to associate with (or alternatively dissociate from) a given target and may be expressed in terms of its dissociation constant, Kd. Binding affinity can be determined using methods in the art, such as equilibrium dialysis, equilibrium binding, gel filtration, immunoassays, surface plasmon resonance, and spectroscopy using experimental conditions that exemplify the conditions under which the compound and the given target may come into contact and/or interact. Dissociation constants may be used determine the binding affinity of a compound for a given target relative to a specified alternative. Alternatively, methods in the art, *e.g.*, immunoassays, *in vivo* or *in vitro* assays for functional activity, *etc.*, may be used to determine the binding affinity of the compound for the given target relative to the specified alternative.

[0074] As used herein, the term "sample" is used in its broadest sense and includes specimens and cultures obtained from any source, as well as biological samples and environmental samples. Biological samples may be obtained from animals (including humans) and encompass fluids, solids, tissues, and gases. Biological samples include blood products, such as plasma, serum, and the like. a biological sample can be obtained from a subject using methods in the art. a sample to be analyzed using one or more methods described herein can be either an initial unprocessed sample taken from a subject or a subsequently processed, *e.g.*, partially purified, diluted, concentrated, fluidized, pretreated with a reagent (*e.g.*, protease inhibitor, anti-coagulant, *etc.*), and the like. In some embodiments, the sample is a blood sample. In some embodiments, the blood sample is a whole blood sample, a serum sample, or a plasma sample. In some embodiments, the sample may be processed, *e.g.*, condensed, diluted, partially purified, and the like. In some embodiments, the sample is pretreated with a reagent, *e.g.*, a protease inhibitor.

[0075] TACs may comprise a detectable label attached thereto. For example, BACs may comprise a detectable label so that they may be detected, measured, visualized, etc., in, *e.g.*, a hemodialysis system. As used herein, a "detectable label" is a compound or

composition that produces or can be induced to produce a signal that is detectable by, *e.g.*, visual, spectroscopic, photochemical, biochemical, immunochemical, or chemical means. a detectable label can be attached directly or indirectly by way of a linker (*e.g.*, an amino acid linker or a chemical moiety). Examples of detectable labels include radioactive and non-radioactive isotopes (*e.g.*, 125I, 18F, 13C, *etc.*), enzymes (*e.g.*, β-galactosidase, peroxidase, *etc.*) and fragments thereof, enzyme substrates, enzyme inhibitors, coenzymes, catalysts, fluorophores (*e.g.*, rhodamine, fluorescein isothiocyanate, *etc.*), dyes, chemiluminescers and luminescers (*e.g.*, dioxetanes, luciferin, *etc.*), and sensitizers.

[0076] *Kits and Devices*

[0077] In some embodiments, the present invention provides kits for removing a given protein of interest, *e.g.*, B2M, from a fluid sample, *e.g.*, blood, from a subject. In some embodiments, the kits comprise BACs as described herein packaged together with (a) reagents for immobilizing the BACs on a solid substrate, or (b) a device for delivering or contacting the BACs with the fluid sample to be treated. In some embodiments, the device is a cassette, *i.e.*, a housing, that contains the BACs packaged therein. In some embodiments, the cassette comprises at least one connector whereby the cassette can be removably attached to a hemodialysis machine and placed in fluidic communication with the blood flow pathway of the hemodialysis machine. In some embodiments, the cassette has an inlet connector and an outlet connector. The connectors may be luer connectors, quick connect couplings, dialysis connectors, and the like, which are known in the art. In some embodiments, the cassette is connected in line with the venous blood line of a hemodialysis machine upstream of the dialyzer of the hemodialysis machine. In some embodiments, the cassette, via its outlet connector, is connected directly to and upstream of the dialyzer of a hemodialysis machine. In some embodiments, the cassette is connected in line with the arterial blood line of a hemodialysis machine downstream of the dialyzer of the hemodialysis machine. In some embodiments, the cassette, via its inlet connector, is connected directly to and downstream of the dialyzer of a hemodialysis machine. In some embodiments, the BACs in a cassette are immobilized on a solid substrate, *e.g.*, solid phase media, contained within the cassette. In some embodiments, the cassette is a reservoir from which BACs are dispensed therefrom into the venous blood upstream of the dialyzer of a hemodialysis machine.

[0078] In some embodiments, the kits include a carrier, package, or container that may be compartmentalized to receive one or more containers, such as vials, tubes, and the

like. In some embodiments, the kits optionally include an identifying description or label or instructions relating to its use. In some embodiments, the kits include information prescribed by a governmental agency that regulates the manufacture, use, or sale of compounds and compositions as contemplated herein.

[0079] The following examples are intended to illustrate but not to limit the invention.

[0080] EXAMPLES

[0081] *BACs Sequences*

[0082] nbBAC-1-SL:

[0083] Subunit A

MFTRRGDQGETDLANRARVGKDSPVVEVQGTIDELNSFIGYALVLSRWDDIRNDLFRIQNDLFVL
GEDVSTGGKGRTVTMDMIIYLIKRSVEMKAEIGKIELFVVPGGSVESASLHMARAVSRRLERRIK
AASELTEINANVLLYANMLSNILFMHALISNKRLNIPEKIWSIHRVSLE (SEQ ID NO: 40)

[0084] Subunit B

MRITTKVGDKGSTRLFGGEEVWKDDPIIEANGTLDELTSFIGEAKHYVDEEMKGILEEIQNDIYK
IMGEIGSKGKIEGISEERIKWLAGLIERYSEMVNKLSFVLPGGTLESAKLDVCRTIARRAERKVA
TVLREFGIGTLAAIYLALLSRLLFLLARVIEIEKNKLKEVRS**GGS**QVQLQESGGGSVQAGGSLRL
SCAAS**GYTDSRY**CMAWFRQAPGKEREWVAR**INSGRDIT**YYADSVKGRFTFSQDNAKNTVYLQMDS
LEPEDTATYYCAT**DIPLRCRDIVAKGGDGFRY**WGQGTQVTVSS*HHHHHH* (SEQ ID NO: 41)

The binder (nanobody) sequence (underlined) is SEQ ID NO: 28, the CDR sequences (bold underlined) are SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively, the linker sequence is GGS, *i.e.*, SEQ ID NO: 34, and the tag is SEQ ID NO: 38.

[0085] nbBAC-1-LL:

[0086] Subunit A

MFTRRGDQGETDLANRARVGKDSPVVEVQGTIDELNSFIGYALVLSRWDDIRNDLFRIQNDLFVL
GEDVSTGGKGRTVTMDMIIYLIKRSVEMKAEIGKIELFVVPGGSVESASLHMARAVSRRLERRIK
AASELTEINANVLLYANMLSNILFMHALISNKRLNIPEKIWSIHRVSLE (SEQ ID NO: 42)

[0087] Subunit B

MRITTKVGDKGSTRLFGGEEVWKDDPIIEANGTLDELTSFIGEAKHYVDEEMKGILEEIQNDIYK
IMGEIGSKGKIEGISEERIKWLAGLIERYSEMVNKLSFVLPGGTLESAKLDVCRTIARRAERKVA
TVLREFGIGTLAAIYLALLSRLLFLLARVIEIEKNKLKEVRS**GGSGGGSGGGGS**QVQLQESGGGS
VQAGGSLRLSCAAS**GYTDSRY**CMAWFRQAPGKEREWVAR**INSGRDIT**YYADSVKGRFTFSQDNAK
NTVYLQMDSLEPEDTATYYCAT**DIPLRCRDIVAKGGDGFRY**WGQGTQVTVSS*HHHHHH* (SEQ ID NO: 43)

The binder (nanobody) sequence (underlined) is SEQ ID NO: 28, the CDR sequences (bold underlined) are SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively, the linker sequence is SEQ ID NO: 35, and the tag is SEQ ID NO: 38.

[0088] nbBAC-2:

[0089] Subunit A

MKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAII
GAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHDILKL
FPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCKWFKAGVLAVGVGKALVKGKPDEVREKAKK
FVKKIRGCTE (SEQ ID NO: 44)

[0090] Subunit B

MNQHSHKDHETVRIAVVRARWHAEIVDACVSAFEAAMRDIGGDRFAVDVFDVPGAYEIPLHARTL
AETGRYGAVLGTAFVVNGGIYRHEFVASAVINGMMNVQLNTGVPVLSAVLTPHNYDKSKAHTLLF
LALFAVKGMEAARACVEILAAREKIAAGS**GGSGGSGGS**QVQLQESGGGSVQAGGSLRLSCAAS**GY**
TDSRYCMAWFRQAPGKEREWVAR**INSGRDIT**YYADSVKGRFTFSQDNAKNTVYLQMDSLEPEDTA
TYYCAT**DIPLRCRDIVAKGGDGFRY**WGQGTQVTVSS*AHHSEDPHHHHHH* (SEQ ID NO: 45)

The binder (nanobody) sequence (underlined) is SEQ ID NO: 28, the CDR sequences (bold underlined) are SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, respectively, the linker sequence is SEQ ID NO: 36, and the tag is SEQ ID NO: 39.

[0091] DARPBAC-1:

[0092] Subunit A

MFTRRGDQGETDLANRARVGKDSPVVEVQGTIDELNSFIGYALVLSRWDDIRNDLFRIQNDLFVL
GEDVSTGGKGRTVTMDMIIYLIKRSVEMKAEIGKIELFVVPGGSVESASLHMARAVSRRLERRIK
AASELTEINANVLLYANMLSNILFMHALISNKR**KEELD**KKQLDAASAGYDDQVAALLAKGADVNA
SDWNGWTPLHSAAQWGHLEIVEVLLKRGADINASDWYGWTPLHVAATWGHLEIVELLLRWGADVN
AQDKFGKTPFDLAIDNGNEDIAEVLQKAA (SEQ ID NO: 46)

The binder (DARPin) sequence (underlined) is SEQ ID NO: 31, and the linker sequence is SEQ ID NO: 37.

[0093] Subunit B

MRITTKVGDKGSTRLFGGEEVWKDDPIIEANGTLDELTSFIGEAKHYVDEEMKGILEEIQNDIYK
IMGEIGSKGKIEGISEERIKWLAGLIERYSEMVNKLSFVLPGGTLESAKLDVCRTIARRAERKVA
TVLREFGIGTLAAIYLALLSRLLFLLARVIEIEKNKLKEVRS*HHHHHH* (SEQ ID NO: 47)

The tag (italicized) is SEQ ID NO: 38.

[0094] DARPBAC-2:

[0095] Subunit A

MFTRRGDQGETDLANRARVGKDSPVVEVQGTIDELNSFIGYALVLSRWDDIRNDLFRIQNDLFVL
GEDVSTGGKGRTVTMDMIIYLIKRSVEMKAEIGKIELFVVPGGSVESASLHMARAVSRRLERRIK
AASELTEINANVLLYANMLSNILFMHALISNKR**KEELD**KKLLDAASAGQDDEVRILMANGADVNA
SDNWGYTPLHAAAYRGHLEIVDVLLANGADVNASDYYGWTPLHVAARNGHLEIVDVLLAHGADVN
AQDKFGKTPFDLAIDNGNEDIAEVLQKAAKLN (SEQ ID NO: 48)

The binder (DARPin) sequence (underlined) is SEQ ID NO: 32, and the linker sequence is SEQ ID NO: 37.

[0096] Subunit B

MRITTKVGDKGSTRLFGGEEVWKDDPIIEANGTLDELTSFIGEAKHYVDEEMKGILEEIQNDIYK
IMGEIGSKGKIEGISEERIKWLAGLIERYSEMVNKLSFVLPGGTLESAKLDVCRTIARRAERKVA
TVLREFGIGTLAAIYLALLSRLLFLLARVIEIEKNKLKEVRS*HHHHHH* (SEQ ID NO: 49)

The tag (italicized) is SEQ ID NO: 38.

[0097] DARPBAC-3:

[0098] Subunit A

MFTRRGDQGETDLANRARVGKDSPVVEVQGTIDELNSFIGYALVLSRWDDIRNDLFRIQNDLFVL
GEDVSTGGKGRTVTMDMIIYLIKRSVEMKAEIGKIELFVVPGGSVESASLHMARAVSRRLERRIK
AASELTEINANVLLYANMLSNILFMHALISNKR**KEELD**KKLLDAASAGQDDEVRILMANGADVNA
SNWWGYTPLHAAAQDGHLEIVDVLLAYGADVNASDWYGWTPLHSAATQGHLEIVDVLLAHGADVN
AQDKFGKTPFDLAIDNGNEDIAEVLQKAAKLN (SEQ ID NO: 50)

The binder (DARPin) sequence (underlined) is SEQ ID NO: 33, and the linker sequence is SEQ ID NO: 37.

[0099] Subunit B

MRITTKVGDKGSTRLFGGEEVWKDDPIIEANGTLDELTSFIGEAKHYVDEEMKGILEEIQNDIYK
IMGEIGSKGKIEGISEERIKWLAGLIERYSEMVNKLSFVLPGGTLESAKLDVCRTIARRAERKVA
TVLREFGIGTLAAIYLALLSRLLFLLARVIEIEKNKLKEVRS*HHHHHH* (SEQ ID NO: 51)

The tag (italicized) is SEQ ID NO: 38.

[0100] *Cloning and Expression*

[0101] DNA encoding the BAC subunits were ordered from Twist Biosciences and cloned using Gibson assembly into the bacterial pET22b expression vector. Expression of the BACs were performed by cloning sequence-verified BAC-encoding expression vectors into Shuffle T7 Express lysY cells (New England Biolabs) to allow for proper folding of disulfide bond-containing nanobodies. Cells were grown in autoinduction media (see Studier (2005)) at 25°C for 48 hours. Cells were harvested at 4000 x g and stored at −20°C until purification.

[0102] *BAC Purification*

[0103] Frozen pelleted cells were solubilized in lysis buffer containing 50 mM TRIS pH 8.0, 250 mM NaCl, and protease inhibitor tablets (Pierce). Cells were lysed using a C3 Emulsiflex with 4 passages through the instrument. Lysate was clarified by centrifugation at 18000 x g for 35 minutes after which the supernatant fraction was passed over a gravity-flow column containing Ni-NTA functionalized agarose beads (Thermo Fisher). The column was washed with lysis buffer containing imidazole of sequential concentrations: 50 mM, 75 mM, and 100 mM. BACs were eluted from the column using lysis buffer supplemented with 500 mM imidazole. BAC proteins were then buffer-exchanged using dialysis into assay buffer containing: 50 mM TRIS, 150 mM NaCl, and 0.02% Tween-20. SEC was performed to isolate correctly assembled nanoparticles using a Superose-6 column (Cytiva Life Sciences). The identity of fractions containing purified BACs were verified using SDS-PAGE.

[0104] *Purification of B2M*

[0105] Avi and 6xHis- tagged B2M was expressed and purified using methods in the art. B2M was then enzymatically biotinylated with the addition of BirA enzyme using methods in the art.

[0106] *Binding Affinity Measurements*

[0107] Binding affinities were determined using an Octet RED96 BLI system (Sartorius). Biosensors functionalized with streptavidin were used to bind biotinylated B2M at a concentration of 2.5 µg/ml and tested for binding with soluble BACs. Buffer used for experiments was 50 mM TRIS pH 8.0, 150 mM NaCl, 0.02% Tween-20.

[0108] *Immunoblots*

[0109] 3 µl aliquots of flow through samples from spin columns were applied to nitrocellulose paper, allowed to dry then repeated at the same location once. Established methods for western blotting were then used develop and image the blot. Anti-biotin antibodies were used to recognize biotinylated B2M (Invitrogen). For western blots, Mini-Protean Any-KD SDS-PAGE gels (Bio-Rad) were used to separate proteins, and then transferred to nitrocellulose membrane (GE-Healthcare) using a Trans-Blot SD system (Bio-Rad). The membrane was rocked in blocking buffer: TBST with 5% milk powder for 1 hour at room temperature with rocking. Primary anti-B2M antibodies were purchased from AbClonal (A1562) and used at a dilution of 1:1000 in blocking buffer. Membrane was allowed to incubate with primary antibody for 1 hour at room temperature. The membrane was washed thrice in TBST, then incubated with anti-rabbit goat secondary antibody with HRP conjugate for an additional 1 hour at room temperature. The membrane was washed of excess secondary antibody thrice with TBST, then incubated with Clarity Western ECL substrate (Bio-rad) and imaged on an Azure imager (Azure Biosystems).

[0110] *Size-Excluded Flow Through Experiment*

[0111] 500 nM B2M with and without 1 µM BAC was added to the supernatant of a centrifuge Amicon Ultra 100 kDa cutoff microcentrifuge concentrator, and then spun for 2.5 minutes at 4000 x g. Flow through and supernatant were collected for further experimentation.

[0112] *Serum Challenge Assay*

[0113] SEC-purified BAC was added to human serum and incubated at 37°C for 4 hours. Serum mixture was then flowed over a column containing HisPur Ni-NTA resin (Thermo Fisher Scientific), and washed and eluted with buffers identical to those used in IMAC purification. Elution fraction was analyzed by SDS-PAGE and concentrated and injected into a Superose 6 gel filtration column (Cytiva Life Sciences) for oligomeric state determination.

[0114] *B2M Removal Experiment*

[0115] 1 ml His-Trap columns (Cytiva life sciences) were used as a stationary matrix to immobilize BACs. Following incubation with SEC-purified BACs, human serum supplemented with 50 µg/ml of B2M was flowed over the column and the flow-through was collected. Column was washed with binding buffer (50 mM Tris pH 8.0, 200 mM NaCl, 20 mM Imidazole), and then eluted with elution buffer containing 50 mM Tris pH 8.0, 200 mM NaCl, 500 mM Imidazole. These fractions were analyzed by Western blot.

[0116] REFERENCES

[0117] The following references are herein incorporated by reference in their entirety with the exception that, should the scope and meaning of a term conflict with a definition explicitly set forth herein, the definition explicitly set forth herein controls:

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[0118] All scientific and technical terms used in this application have meanings commonly used in the art unless otherwise specified.

[0119] As used herein, the terms “subject”, “patient”, and “individual” are used interchangeably to refer to humans and non-human animals. The terms “non-human animal” and “animal” refer to all non-human vertebrates, *e.g.*, non-human mammals and non-mammals, such as non-human primates, horses, sheep, dogs, cows, pigs, chickens, and other veterinary subjects and test animals. In some embodiments, the subject is a mammal. In some embodiments, the subject is a human.

[0120] As used herein, the term “diagnosing” refers to the physical and active step of informing, *i.e.*, communicating verbally or by writing (on, *e.g.*, paper or electronic media), another party, *e.g.*, a patient, of the diagnosis. Similarly, “providing a prognosis” refers to the physical and active step of informing, *i.e.*, communicating verbally or by writing (on, *e.g.*, paper or electronic media), another party, *e.g.*, a patient, of the prognosis.

[0121] The use of the singular can include the plural unless specifically stated otherwise. As used in the specification and the appended claims, the singular forms "a", "an", and "the" can include plural referents unless the context clearly dictates otherwise.

[0122] As used herein, "and/or" means "and" or "or". For example, "A and/or B" means "A, B, or both A and B" and "A, B, C, and/or D" means "A, B, C, D, or a combination thereof" and said "A, B, C, D, or a combination thereof" means any subset of A, B, C, and D, for example, a single member subset (*e.g.*, A or B or C or D), a two-member subset (*e.g.*, A and B; A and C; *etc.*), or a three-member subset (*e.g.*, A, B, and C; or A, B, and D; *etc.*), or all four members (*e.g.*, A, B, C, and D).

[0123] As used herein, the phrase "one or more of", *e.g.*, "one or more of A, B, and/or C" means "one or more of A", "one or more of B", "one or more of C", "one or more of A and one or more of B", "one or more of B and one or more of C", "one or more of A and one or more of C" and "one or more of A, one or more of B, and one or more of C".

[0124] The phrase "comprises or consists of A" is used as a tool to avoid excess page and translation fees and means that in some embodiments the given thing at issue: comprises A or consists of A. For example, the sentence "In some embodiments, the composition comprises or consists of A" is to be interpreted as if written as the following two separate sentences: "In some embodiments, the composition comprises A. In some embodiments, the composition consists of A."

[0125] Similarly, a sentence reciting a string of alternates is to be interpreted as if a string of sentences were provided such that each given alternate was provided in a sentence by itself. For example, the sentence "In some embodiments, the composition comprises A, B, or C" is to be interpreted as if written as the following three separate sentences: "In some embodiments, the composition comprises A. In some embodiments, the composition comprises B. In some embodiments, the composition comprises C." As another example, the sentence "In some embodiments, the composition comprises at least A, B, or C" is to be interpreted as if written as the following three separate sentences: "In some embodiments, the composition comprises at least A. In some embodiments, the composition comprises at least B. In some embodiments, the composition comprises at least C."

[0126] As used herein, the terms "protein", "polypeptide" and "peptide" are used interchangeably to refer to two or more amino acids linked together. Groups or strings of amino acid abbreviations are used to represent peptides. Except when specifically indicated, peptides are indicated with the N-terminus on the left and the sequence is written from the N-terminus to the C-terminus. Except when specifically indicated,

peptides are indicated with the N-terminus on the left and the sequences are written from the N-terminus to the C-terminus. Similarly, except when specifically indicated, nucleic acid sequences are indicated with the 5' end on the left and the sequences are written from 5' to 3'.

[0127] As used herein, a given percentage of "sequence identity" refers to the percentage of nucleotides or amino acid residues that are the same between sequences, when compared and optimally aligned for maximum correspondence over a given comparison window, as measured by visual inspection or by a sequence comparison algorithm in the art, such as the BLAST algorithm, which is described in Altschul *et al.*, (1990) J Mol Biol 215:403-410. Software for performing BLAST (*e.g.*, BLASTP and BLASTN) analyses is publicly available through the National Center for Biotechnology Information (ncbi.nlm.nih.gov). The comparison window can exist over a given portion, *e.g.*, a functional domain, or an arbitrarily selection a given number of contiguous nucleotides or amino acid residues of one or both sequences. Alternatively, the comparison window can exist over the full length of the sequences being compared. For purposes herein, where a given comparison window (*e.g.*, over 80% of the given sequence) is not provided, the recited sequence identity is over 100% of the given sequence. Additionally, for the percentages of sequence identity of the proteins provided herein, the percentages are determined using BLASTP 2.8.0+, scoring matrix BLOSUM62, and the default parameters available at blast.ncbi.nlm.nih.gov/Blast.cgi. *See also* Altschul, *et al.*, (1997) Nucleic Acids Res 25:3389-3402; and Altschul, *et al.*, (2005) FEBS J 272:5101-5109.

[0128] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, Adv Appl Math 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J Mol Biol 48:443 (1970), by the search for similarity method of Pearson & Lipman, PNAS USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection.

[0129] To the extent necessary to understand or complete the disclosure of the present invention, all publications, patents, and patent applications mentioned herein are expressly incorporated by reference therein to the same extent as though each were individually so incorporated.

[0130] Having thus described exemplary embodiments of the present invention, it should be noted by those skilled in the art that the within disclosures are exemplary only and

that various other alternatives, adaptations, and modifications may be made within the scope of the present invention. Accordingly, the present invention is not limited to the specific embodiments as illustrated herein, but is only limited by the following claims.

What is claimed is:

1. An immunosorbent nanoparticle comprising or consisting of (a) a plurality of one or more subunit proteins which form a protein cage having an exterior surface, and (b) a binder against beta-2 microglobulin (B2M), said binder is recombinantly linked, directly or indirectly, to at least one of the one or more subunit proteins, wherein the binder is presented on the exterior surface.

2. The immunosorbent nanoparticle according to claim 1, wherein the binder is (a) a nanobody comprising the following CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, or (b) a DARPin comprising SEQ ID NO: 29 or SEQ ID NO: 30.

3. The immunosorbent nanoparticle according to claim 1, wherein the binder is a nanobody having a sequence that (a) contains CDR sequences: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27, and (b) at least 90% sequence identity to SEQ ID NO: 28.

4. The immunosorbent nanoparticle according to any one of claims 1 – 3, wherein the one or more subunit proteins are selected from the group consisting of:

a) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2;

b) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4;

c) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6;

d) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8;

e) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10;

f) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12;

g) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14;

h) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16;

i) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18;

j) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20;

k) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22;

l) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23; and

m) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24.

5. The immunosorbent nanoparticle according to any one of claims 1 – 3, wherein the immunosorbent nanoparticle comprises or consists of:

a) a plurality of protein chains having SEQ ID NO: 42 and SEQ ID NO: 43;

b) a plurality of protein chains having SEQ ID NO: 44 and SEQ ID NO: 45;

c) a plurality of protein chains having SEQ ID NO: 46 and SEQ ID NO: 47;

d) a plurality of protein chains having SEQ ID NO: 48 and SEQ ID NO: 49; or

e) a plurality of protein chains having SEQ ID NO: 50 and SEQ ID NO: 51.

6. The immunosorbent nanoparticle according to claim 1, wherein

(1) the one or more subunit proteins are selected from the group consisting of

a) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2;

b) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4;

c) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6;

d) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8;

e) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10;

f) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12;

g) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14;

h) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16;

i) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18;

j) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20;

k) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22;

l) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23;

m) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24; and

(2) the binder is selected from the group consisting of

a) a nanobody comprising: SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27;

b) a nanobody comprising a sequence having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 28, said sequence including SEQ ID NO: 25, SEQ ID NO: 26, and SEQ ID NO: 27;

c) a DARPin comprising SEQ ID NO: 29; and

d) a DARPin comprising SEQ ID NO: 30.

7. The immunosorbent nanoparticle according to any one of claims 1 – 6, wherein the binder comprises or consists of SEQ ID NO: 28, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO: 32, or SEQ ID NO: 33.

8. The immunosorbent nanoparticle according to any one of claims 1 – 7, wherein the immunosorbent nanoparticle is immobilized on solid substrate.

9. An article comprising (a) a housing having at least one outlet, and (b) a plurality of immunosorbent nanoparticles according to any one of claims 1 – 8 contained within the housing.

10. A kit comprising a plurality of immunosorbent nanoparticles according to any one of claims 1 – 8 or the article according to claim 9.

11. A hemodialysis device comprising (a) a dialyzer, and (b) the article according to claim 9.

12. A hemodialysis device comprising (a) a dialyzer, and (b) an article comprising a housing having at least one outlet, and (b) a plurality of self-assembling protein cages contained within the housing.

13. The hemodialysis device according to claim 12, wherein the plurality of self-assembling protein cages comprise one or more subunit proteins selected from the group consisting of:

a) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 1, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 2;

b) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 3, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 4;

c) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 5, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 6;

d) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 8;

e) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 9, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 10;

f) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 11, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 12;

g) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 13, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 14;

h) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 15, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 16;

i) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 17, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 18;

j) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 19, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 20;

k) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 21, and a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 22;

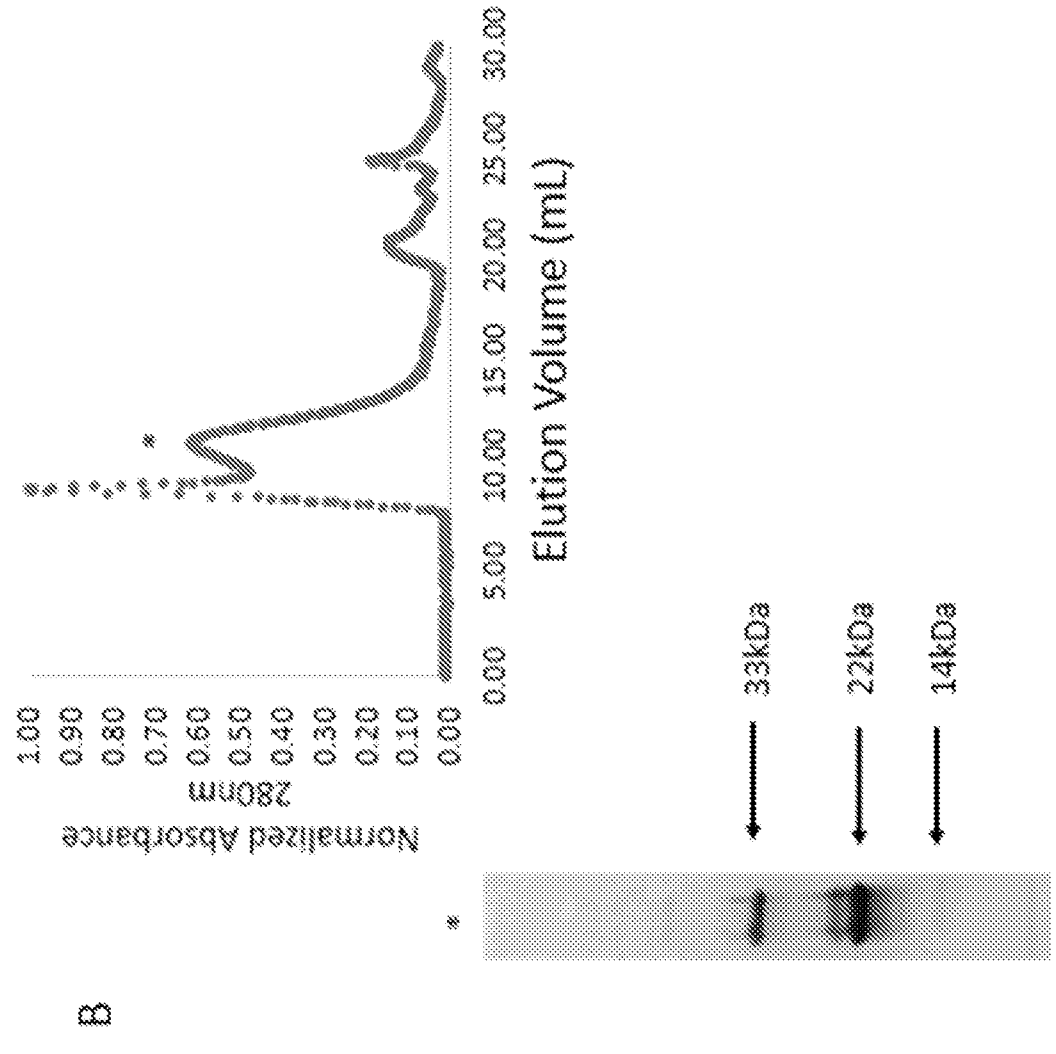
l) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 23; and

m) a subunit protein having 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 24.

14. The hemodialysis device according to any one of claims 11 − 13, wherein the article is in fluidic communication with the dialyzer.

15. A method of removing beta-2 microglobulin (B2M) from a liquid, which comprises (a) contacting the liquid with a plurality of immunosorbent nanoparticles according to any one of claims 1 − 8 or flowing the liquid through the article according to claim 9 or the hemodialysis device according to claim 11 or 14, thereby binding the B2M to the plurality of immunosorbent nanoparticles, and then (b) separating the liquid from the immunosorbent nanoparticles having B2M bound thereto.

16. The method according to claim 15, wherein the immunosorbent nanoparticles having B2M bound thereto are removed using protein purification techniques in the art, *e.g.*, ion-exchange chromatography, immunoaffinity chromatography, size exclusion chromatography, or filtration.


A
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0.6
0.4
0.2
0
0.00
10.00
20.00
30.00
Elution Volume (mL)
*
*
34kDa
21kDa
14kDa
B
Normalized Absorbance
280nm
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00
5.00
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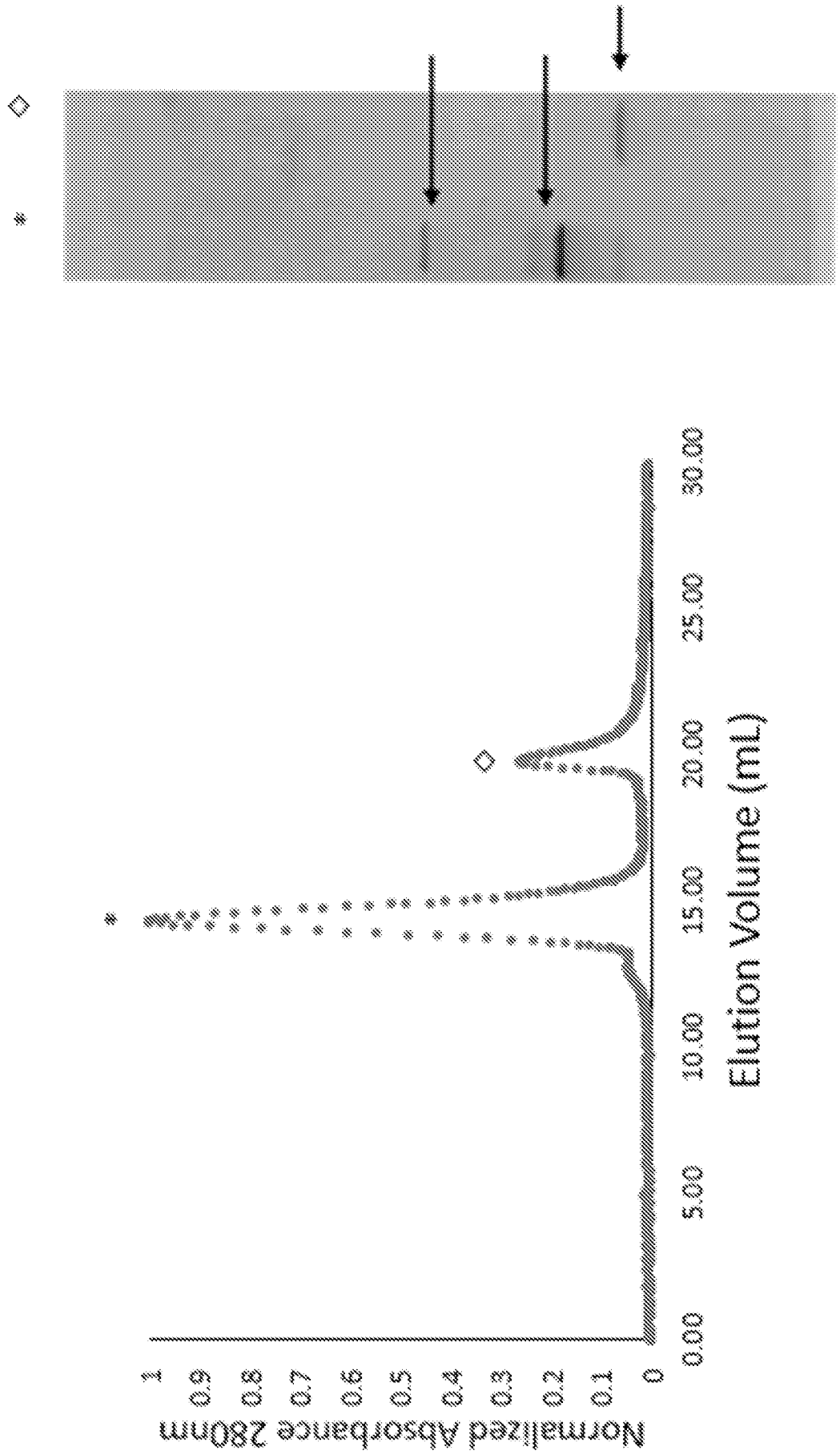
25.00
30.00
Elution Volume (mL)
*
*
33kDa
22kDa
14kDa

FIG. 1


Normalized Absorbance 280nm
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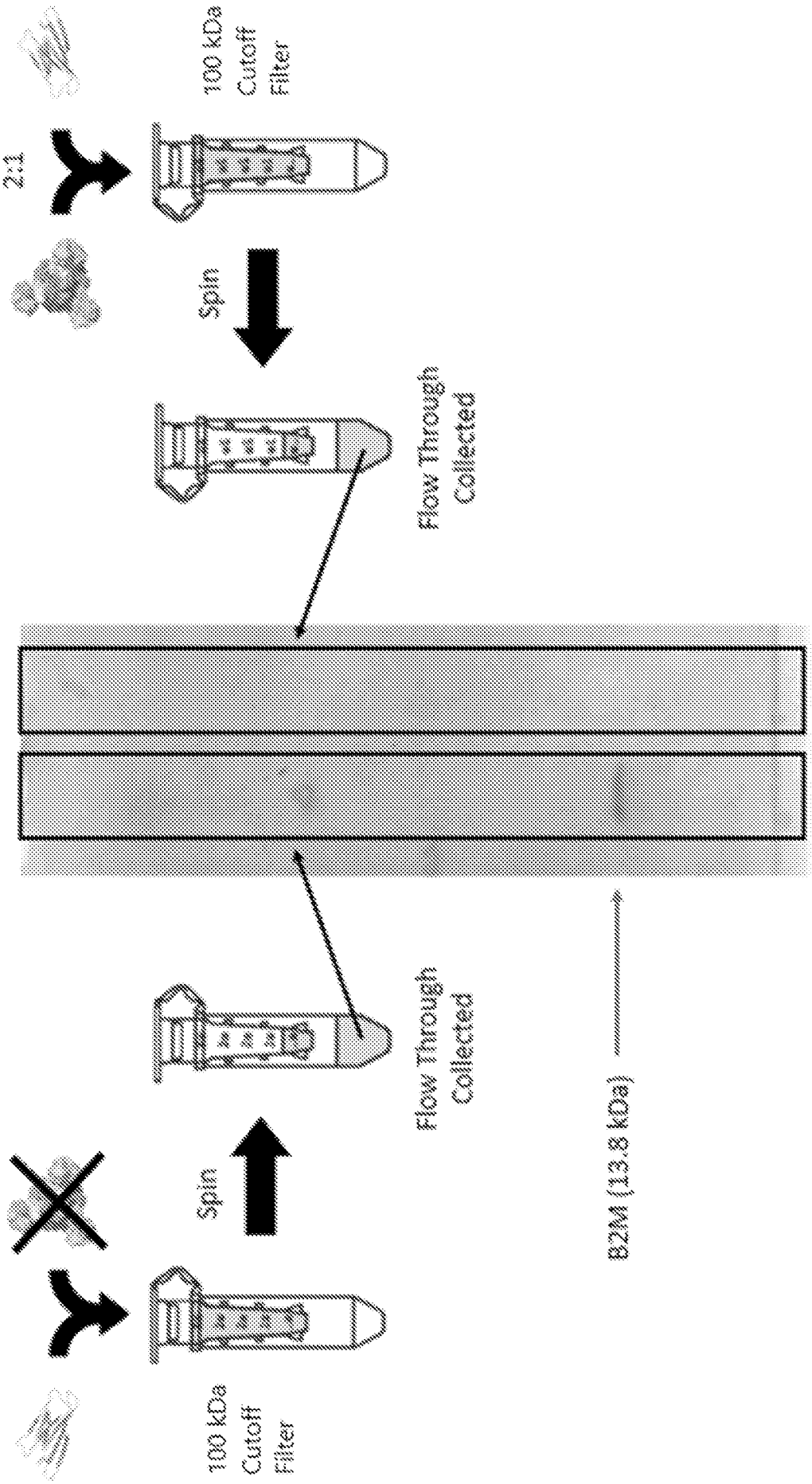
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0.00
5.00
10.00
15.00
20.00
25.00
30.00
Elution Volume (mL)

FIG. 2


100 kDa
Cutoff
Filter
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Spin
Flow Through
Collected
B2M (13.8 kDa)
Flow Through
Collected
Spin
100 kDa
Cutoff
Filter

FIG. 3

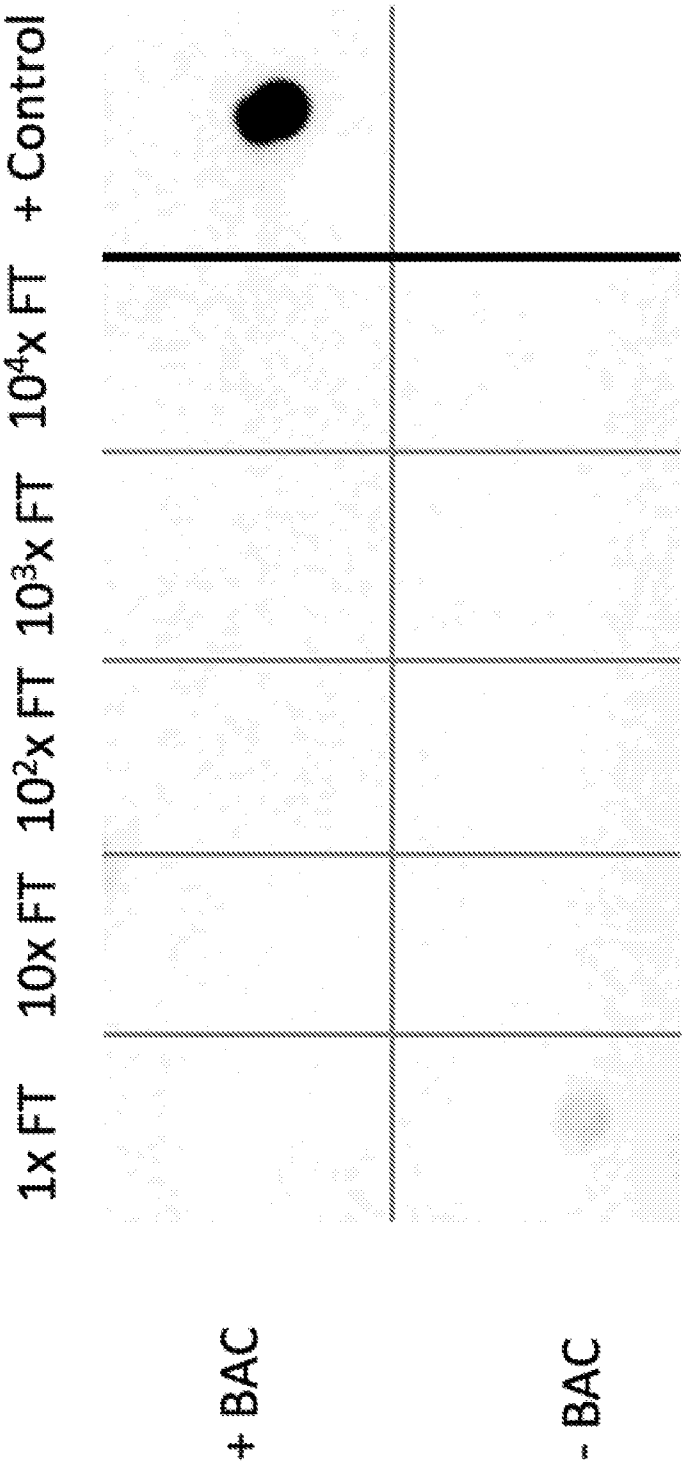


FIG. 4

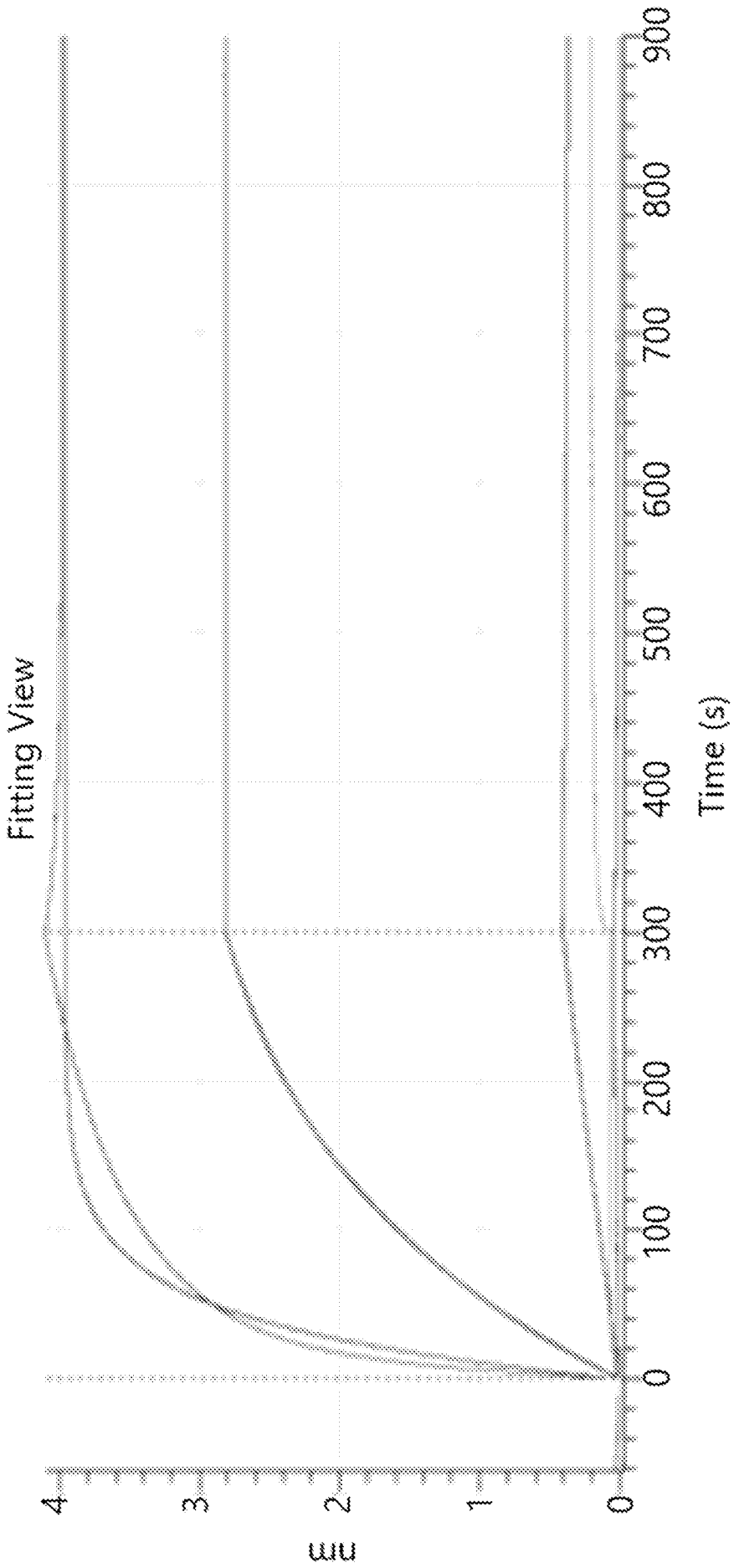
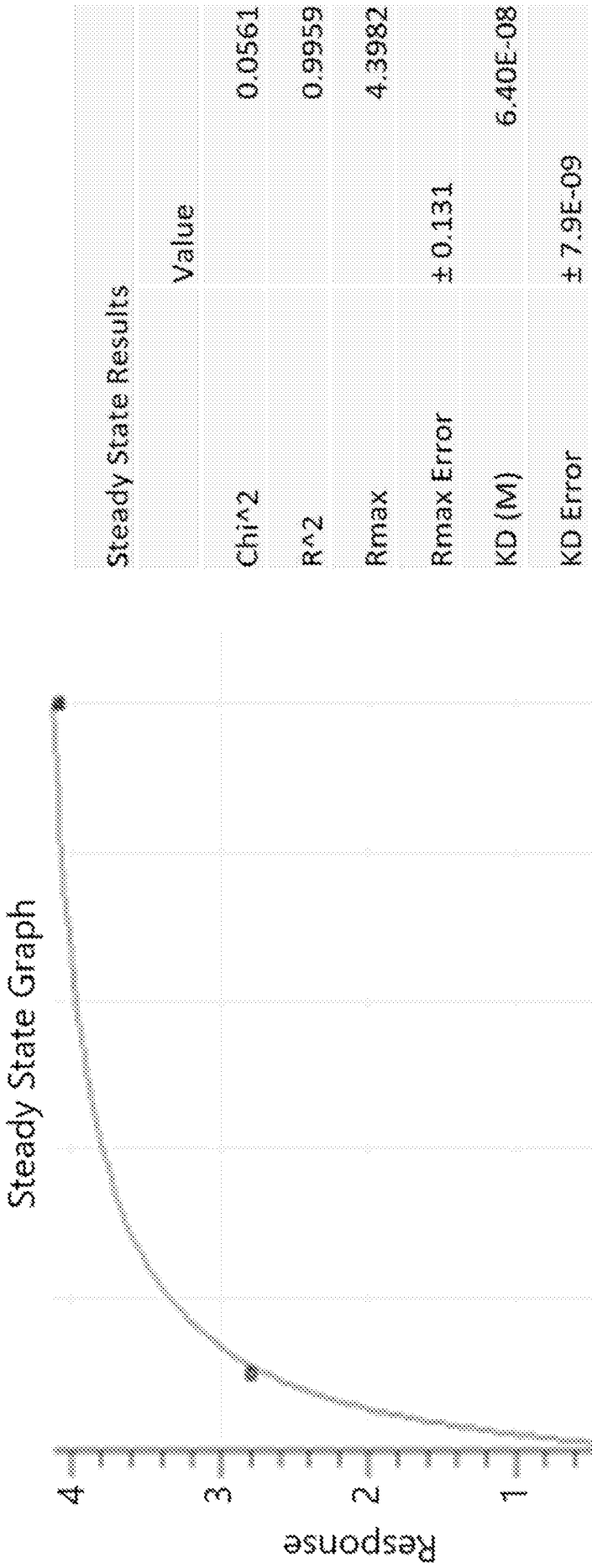

Fitting View
nm
0
1
2
3
4
0
100
200
300
400
500
600
700
800
900
Time (s)

FIG. 5


Steady State Results
Value
Chi^2
0.0561
R^2
0.9959
Rmax
4.3982
Rmax Error
± 0.131
KD (M)
6.40E-08
KD Error
± 7.9E-09
Steady State Graph
Response
Conc. (nM)
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1
2
3
4
0
200
400
600
800
1000

FIG. 5 cont.

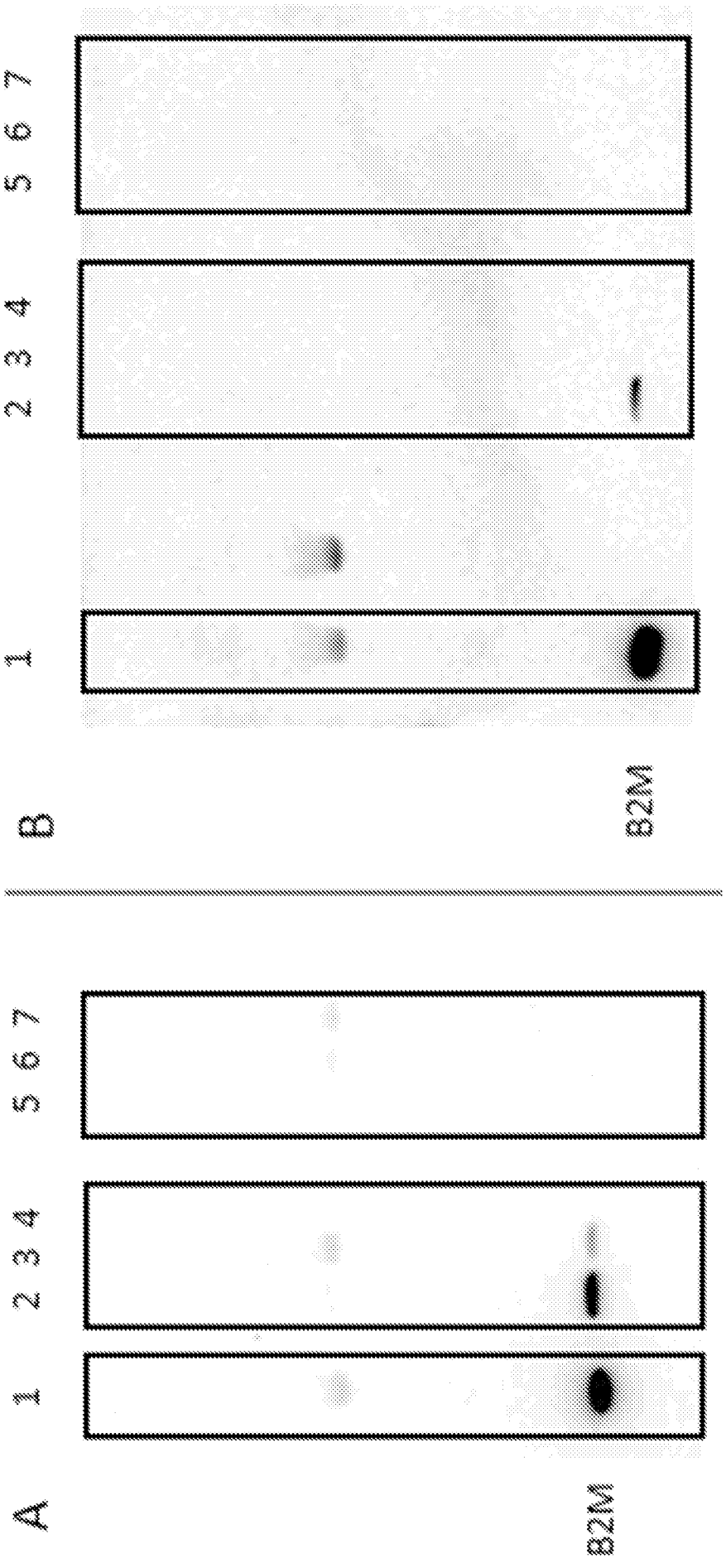

FIG. 6