

CLAIMS

We claim:

1. An antibody-peptide fusion protein, comprising:
 - an amyloid-reactive peptide; and
 - an antibody capable of inducing phagocytosis and serving as an opsonin, wherein the antibody comprises a heavy chain comprising a heavy chain variable region (VH) and a light chain comprising a light chain variable region (VL), wherein the amyloid-reactive peptide and the antibody are linked at the N-terminal end or the C-terminal end of the heavy chain or the light chain,
 - wherein the amyloid-reactive peptide is linked to the antibody via a spacer comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23-24, 27, 83-86.
2. The antibody-peptide fusion protein of claim 1, wherein the light chain comprises a light chain constant region, and the heavy chain comprises a heavy chain constant region.
3. The antibody-peptide fusion protein of claim 1 or 2, wherein the amyloid-reactive peptide and the antibody are linked at the C-terminal end of the light chain.
4. The antibody-peptide fusion protein of claim 1 or 2, wherein the spacer is selected from the group consisting of SEQ ID NO:83 and SEQ ID NO:86.
5. The antibody-peptide fusion protein of any one of claims 1-4, wherein the amyloid-reactive peptide comprises an amino acid sequence having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 100% sequence identity to any one of the amino acid sequences set forth as SEQ ID NOs: 1-13.
6. The antibody-peptide fusion protein of any one of claims 1-5, wherein the antibody-peptide fusion protein comprises two heavy chains and two light chains and wherein each light chain is linked at the C-terminal end to the amyloid-reactive peptide.
7. The antibody-peptide fusion protein of any one of claims 1-6, wherein the antibody is a chimeric antibody or humanized antibody.
8. The antibody-peptide fusion protein of any one of claims 1-7, wherein the antibody binds to human amyloid fibrils.

9. The antibody-peptide fusion protein of any one of claims 1-8, wherein
- a) the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NOs: 64-70, a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22, and the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:18, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19;
 - b) the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO: 20; a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22, and the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NOs: 71-81; and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19; or
 - c) the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NOs: 64-70, a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22, and the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NOs: 71-81; and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19.
10. The antibody-peptide fusion protein of any one of claims 1-8, wherein the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO:64, a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22, and the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:73, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19.
11. The antibody-peptide fusion protein of any one of claims 1-8, wherein:
- a) the VL comprises an amino acid sequence set forth in SEQ ID NO:34, and the VH comprises an amino acid sequence set forth in SEQ ID NO:48;

- b) the VL comprises an amino acid sequence set forth in SEQ ID NO:35, and the VH comprises an amino acid sequence set forth in SEQ ID NO:51;
 - c) the VL comprises an amino acid sequence set forth in SEQ ID NO:36, and the VH comprises an amino acid sequence set forth in SEQ ID NO:55;
 - d) the VL comprises an amino acid sequence set forth in SEQ ID NO:35, and the VH comprises an amino acid sequence set forth in SEQ ID NO:52;
 - e) the VL comprises an amino acid sequence set forth in SEQ ID NO:35, and the VH comprises an amino acid sequence set forth in SEQ ID NO:50; or
 - f) the VL comprises an amino acid sequence set forth in SEQ ID NO:35, the VH comprises an amino acid sequence set forth in SEQ ID NO:49.
12. The antibody-peptide fusion protein of any one of claims 1-11, wherein the VL comprises an amino acid sequence set forth in SEQ ID NO:36, and the VH comprises an amino acid sequence set forth in SEQ ID NO:55.
13. The antibody-peptide fusion protein of any one of claims 1-12, wherein the antibody is a full-length antibody.
14. The antibody-peptide fusion protein of claim 13, wherein the Fc region is of an IgG1 isotype.
15. An antibody-peptide fusion protein, comprising:
- an antibody that binds to amyloid fibrils comprising a first polypeptide and a second polypeptide each comprising a light chain of the antibody, and a third and a fourth polypeptide each comprising a heavy chain of the antibody, and
 - an amyloid-reactive peptide that is linked to the N-terminus or the C-terminus of the light chain or the heavy chain, wherein
- a) the first polypeptide and second polypeptide comprise the amino acid set forth in SEQ ID NO:87, and the third and fourth polypeptide comprise the amino acid sequence set forth in SEQ ID NO:91;
 - b) the first polypeptide and second polypeptide comprise the amino acid set forth in SEQ ID NO:88, and the third and fourth polypeptide comprise the amino acid sequence set forth in SEQ ID NO:92;

- c) the first polypeptide and second polypeptide comprise the amino acid set forth in SEQ ID NO:89, and the third and fourth polypeptide comprise the amino acid sequence set forth in SEQ ID NO:91; or
 - d) the first polypeptide and second polypeptide comprise the amino acid set forth in SEQ ID NO:90, and the third and fourth polypeptide comprise the amino acid sequence set forth in SEQ ID NO:91.
16. An antibody-peptide fusion protein, comprising:
- an amyloid-reactive peptide comprising the amino acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:2; and
 - an antibody that binds to a human amyloid fibrils wherein the antibody comprises a variable heavy chain (VH) and a variable light chain (VL) wherein the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:73, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19, and the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO:64, a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22; wherein the amyloid-reactive peptide and antibody are linked at the C-terminal end of the light chain,
- wherein the amyloid-reactive peptide is linked to the antibody via a spacer comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 23-24, 27, 83-86.
17. An antibody-peptide fusion protein, comprising:
- an amyloid-reactive peptide comprising the amino acid sequence set forth in SEQ ID NO:2; and
 - an antibody that binds to human amyloid fibrils wherein the antibody comprises a variable heavy chain (VH) and a variable light chain (VL) wherein the VH comprises a CDR-H1 comprising the amino acid sequence set forth in SEQ ID NO:17, a CDR-H2 comprising the amino acid sequence set forth in SEQ ID NO:73, and a CDR-H3 comprising the amino acid sequence set forth in SEQ ID NO:19, and the VL comprises a CDR-L1 comprising the amino acid sequence set forth in SEQ ID NO:64, a CDR-L2 comprising the amino acid sequence set forth in SEQ ID NO:21, and a CDR-L3 comprising the amino acid sequence set forth in SEQ ID NO:22; wherein the amyloid-reactive peptide and antibody are linked at the C-terminal end of the light chain,

wherein the amyloid-reactive peptide is linked to the antibody via a spacer comprising the amino acid sequence set forth in SEQ ID NO:83.

18. The antibody-peptide fusion protein of any one of claims 1-17, wherein the antibody-peptide fusion protein exhibits an EC₅₀ less than 1.5 nM for an amyloid substrate.

19. The antibody-peptide fusion protein of any one of claims 1-18, wherein the antibody-peptide fusion protein is conjugated to a detectable label wherein the detectable label comprises a fluorescent label or a radiolabel.

20. The antibody-peptide fusion protein of claim 19, wherein the radiolabel is I-123, I-124, F-18, ZR-89, or Tc-99m.

21. The antibody-peptide fusion protein of any one of claims 1-20, wherein the antibody-peptide fusion protein exhibits one or more *in vivo* features selected from among improved biodistribution, pan amyloid reactivity, and enhanced phagocytosis compared to a reference IgG antibody.

22. The antibody-peptide fusion protein of any one of claims 1-21, wherein the antibody-peptide fusion protein binds to rV λ 6W μ 1, A β , A β (1-40), IAAP, AL κ 4, A μ 1, ATTR, α -synuclein, or Tau 441 fibrils.

23. An antibody-peptide fusion protein, comprising an antibody that binds to human amyloid fibrils comprising i) a heavy chain and ii) a light chain with an amyloid-reactive peptide fused to the C-terminus of the light chain via a linker, wherein the heavy chain comprises the amino acid sequence of SEQ ID NO:91, and the light chain with the amyloid-reactive peptide fused to the C terminus of the light chain via the linker comprises the amino acid sequence of SEQ ID NO:89.

24. A composition comprising
an antibody-peptide fusion protein, comprising:

i) an amyloid-reactive peptide; and

ii) an antibody that is capable of inducing phagocytosis and serving as an opsonin, wherein the antibody comprises a heavy chain comprising a heavy chain variable region (VH) and a light chain comprising a light chain variable region (VL), wherein the

amyloid-reactive peptide and antibody are linked at the N-terminal end or the C-terminal end of the heavy chain or the light chain, wherein the amyloid-reactive peptide is linked to the antibody via a spacer, or without a spacer; and

wherein at least 90% of the antibody-peptide fusion protein is intact.

25. The composition of claim 24, wherein the intact antibody-peptide fusion protein comprises the antibody-peptide fusion protein of any one of claims 1-23.

26. The composition of claim 24, wherein the composition comprises no more than 10% of a cleavage product, wherein the cleavage product comprises a heavy chain lacking one or more amino acid residues from the N-terminus or C-terminus compared to the amino acid sequence set forth by SEQ ID NO:89 or a light chain lacking one or more amino acid residues from the N-terminus or C-terminus compared to the amino acid sequence set forth in SEQ ID NO:91.

27. The composition of any one of claims 24-26, wherein antibody-peptide fusion protein exhibits an EC₅₀ binding affinity for one or more amyloid substrate, wherein the EC₅₀ binding affinity is less than 1.5 nM.

28. The composition of any one of claims 24-27, further comprising a pharmaceutically acceptable carrier.

29. A polynucleotide encoding the antibody-peptide fusion protein of any one of claims 1-23.

30. A vector comprising the polynucleotide of claim 29.

31. A host cell comprising the vector of claim 30.

32. The host cell of claim 31, wherein the host cell is a mammalian cell, optionally a Chinese hamster ovary (CHO) cell.

33. A method of producing an antibody-peptide fusion protein comprising
a) culturing a host cell comprising a vector encoding an antibody-peptide fusion protein under perfusion cell culture conditions suitable for expression of the antibody-peptide fusion protein; and

b) recovering the antibody-peptide fusion protein about every 12-36 hours;

wherein the antibody-peptide fusion protein comprises

- i) an amyloid-reactive peptide; and
- ii) an antibody that is capable of inducing phagocytosis and serving as an opsonin, wherein the antibody comprises a heavy chain comprising a heavy chain variable region (VH) and a light chain comprising a light chain variable region (VL), wherein the amyloid-reactive peptide and antibody are linked at the C-terminal end of the light chain, wherein the amyloid-reactive peptide is linked to the antibody via a spacer, or without a spacer.

34. The method of claim 33, further comprising applying the antibody-peptide fusion recovered in step b) to a cation exchange chromatography column and eluting the antibody-peptide fusion protein from the cation exchange chromatography column.

35. The method of claim 34, wherein the antibody-peptide fusion protein is eluted separately from a truncated antibody-peptide fusion protein.

36. The method of any one of claims 33-35, wherein the antibody-peptide fusion protein comprises the antibody-peptide fusion protein of any one of claims 1-23.

37. The method of any one of claims 33-36, wherein the host cell is a CHO cell.

38. The method of any of claims 33-37, further comprising determining the purity of the antibody-peptide fusion protein, wherein the purity of the antibody-peptide fusion protein is determined using one or more analytical methods comprising sodium dodecyl sulfate capillary electrophoresis (CE-SDS), liquid chromatography (LC), mass spectrometry (MS), or a combination thereof.

39. The method of any one of claims 33-38, wherein the antibody-peptide fusion protein is purified to at least 90% intact antibody-peptide fusion protein.

40. An antibody-peptide fusion protein produced by the method of any of claims 33-39.

41. Use of the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28 in the manufacture of a medicament for treating a subject having an amyloid related disorder comprising an amyloid deposit.

42. The use of claim 41, wherein the amyloid related disorder is systematic or localized amyloidosis.
43. The use of claim 41 or 42, wherein the amyloid related disorder is selected from the group consisting of AL, AH, A β 2M, ATTR, transthyretin, AA, AApoAI, AApoAII, AGel, ALys, ALect2, AFib, ACys, ACal, AMed, AIAPP, APro, AIns, APrP, Parkinson's disease, Alzheimer's disease, or A β amyloidosis.
44. The use of any one of claims 41-43, wherein the amyloid deposit is opsonized by the antibody-peptide fusion protein.
45. The use of any one of claims 41-44, wherein treating the subject with the antibody-peptide fusion protein causes phagocytosis of the amyloid deposit.
46. Use of an amyloid-peptide fusion protein in the manufacture of a medicament for treating a subject having an amyloid-based disease or suspected of having an amyloid-based disease, wherein
- a) to determine whether the subject has an amyloid deposit:
 - i) the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28 is to be administered to the subject, wherein the antibody-peptide fusion protein comprises a detectable label, and
 - ii) whether a signal associated with the detectable label can be detected from the subject is to be determined; and
 - b) if the signal is detected, an amyloidosis treatment is to be administered to the subject.
47. The use of claim 46, wherein, if a signal is not detected, the subject is to be monitored for a later development of an amyloid deposit.
48. The method of claim 46 or 47, wherein the intensity of the signal is to be determined and the signal is compared to a threshold value, above which the subject is determined to possess an amyloid deposit.
49. The use of any one of claims 46-48, wherein the antibody-peptide fusion protein is detected by SPECT/CT imaging, PET/CT imaging, gamma scintigraphy, or optical imaging.

50. The use of any of claims 46-49, wherein the amyloidosis treatment comprises administering the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28 to the subject.
51. A method of identifying an amyloid deposit in a subject, wherein the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28 is to be administered to the subject, wherein the antibody-peptide fusion protein comprises a detectable label, and a signal from the antibody peptide fusion protein is detected.
52. A method of monitoring amyloid clearance in a subject wherein the amyloid substrate in the subject is to be contacted with the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28, wherein the antibody-peptide fusion protein comprises a detectable label, and wherein the peptide of the antibody-peptide fusion protein has binding affinity for an amyloid substrate; and
a signal from the detectable label is determined, thereby detecting the amyloid clearance.
53. The use or method of any one of claims 41-52, wherein the subject is a human.
54. A kit comprising the antibody-peptide fusion protein of any one of claims 1-23 and 40 or the composition of any one of claims 24-28, for use in the use of any of claims 41-50 and method of any one of claims 50-52.