AAV9 AFFINITY AGENTS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/852,717, filed on May 24, 2019 and U.S. Provisional Application No. 62/949,878, filed on December 18, 2019, the contents of each of which are hereby incorporated by reference herein in their entirety.

BACKGROUND

[0002] The purity of biologically produced therapeutics is tightly scrutinized and regulated by authorities to ensure safety and efficacy. Thus, there remains a need for means to efficiently purify of biologically produced therapeutics to a high degree of purity.

SUMMARY

[0003] To support the clinical efforts for therapeutic proteins, compositions and methods to efficiently purify proteins from recombinant sources are needed. Affinity purification is a means to isolate and/or achieve desired purity of a protein in few steps, or a single step. However, the development of affinity agents (e.g., comprising an affinity ligand) can be a resource intensive and time consuming task and hence affinity agents exist for very few proteins. In the absence of an affinity agent, purification typically involves inefficient processes, such as a multi-column process.

[0004] Recombinant adeno-associated viruses (rAAV) are one of the most investigated viral vectors for the delivery of gene therapies in humans. AAV serotypes exhibit various cellular tropisms and interactions with cell receptors to allow entry into the cells and delivery of genetic cargo into the nucleus for expression. AAV9 is of particular interest to biopharmaceutical developers due to its reported ability to cross the blood brain barrier, which is of particular importance to potentially address an array of central nervous system (CNS) disorders. In addition to its application for CNS disorders, and due to a broad tropism towards liver, skeletal muscle and lung tissues, AAV9 is also being used to address non-CNS disorders.

CLAIMS

1. An affinity agent comprising a ligand, wherein the ligand comprises a binding motif having an amino acid sequence of:

(a) any one of SEQ ID NOs: 1 - 5; or

(b) an amino acid sequence that differs from any one of SEQ ID NOs: 1 - 5 by no more than two substitutions, additions, or deletions.

2. An affinity agent comprising a ligand, wherein the ligand comprises a polypeptide having an amino acid sequence of:

(a) any one of SEQ ID NOs: 6 - 47, or

(b) an amino acid sequence that differs from any one of SEQ ID NOs: 6 - 47 by no more than two substitutions, additions, or deletions.

3. The affinity agent of claim 2, wherein the ligand comprises a binding motif having an amino acid sequence of:

(a) any one of SEQ ID NOs: 21, 23, 28, 32, 46, or 47; or

(b) an amino acid sequence that differs from any one of SEQ ID NOs: 21, 23, 28, 32, 46, or 47 by no more than two substitutions, additions, or deletions.

4. The affinity agent of any one of claims 1-3, wherein the N-terminus of the ligand is acetylated.

5. The affinity agent of any one of claims 1-4, wherein the ligand further comprises a C-terminal lysine.

6. The affinity agent of claim 5, wherein the C-terminal lysine of the ligand is amidated.

7. The affinity agent of any one of claims 1-6, wherein the ligand further comprises at least one polyethylene glycol (PEG).

8. The affinity agent of claim 7, wherein the at least one polyethylene glycol is $PEG_{(3)}$.

9. The affinity agent of any one of claims 1-8, wherein the ligand is attached to a solid surface.

10. The affinity agent of claim 9, wherein the solid surface is a column, resin, matrix, bead, test tube, microtiter dish, solid particle, microchip, membrane, or biological surface to which the ligand may be attached.

11. The affinity agent of claim 10, wherein the solid surface is a resin or bead.

12. The affinity agent of claim 10, wherein the solid surface is a membrane.

13. The affinity agent of claim 10, wherein the solid surface is a monolith.

14. The affinity agent of any one of claims 9-13, wherein the ligand is conjugated to the solid surface via a linker.

15. The affinity agent of claim 14, wherein the linker comprises PEG and/or lysine.

16. The affinity agent of claim 15, wherein the PEG linker has a molecular weight of from about 100 to 5000 kDa.

17. The affinity agent of claim 16, wherein the PEG linker has a molecular weight of from about 100 to 500 kDa.

18. An affinity agent comprising a ligand according to any one of claims 1-17, wherein the ligand comprises a cyclic polypeptide.

19. An affinity agent of any one of claims 1-18 that is used for purification of an adeno-associated virus.

20. A method of making an affinity agent comprising:

conjugating a ligand according to any of claims 1-19 to a solid surface.

21. A method of affinity purification of AAV comprising:

(a) contacting AAV, produced by cell culture, with a resin comprising an affinity agent of any one of claims 1-19 under conditions favorable to form a complex between the affinity agent and AAV;

(b) washing away non-binding materials; and

(c) eluting the AAV with an elution buffer.

22. The method of claim 21, further comprising:

(d) exposing the resin to 0.5M NaOH for 30 minutes; and

(e) repeating steps a-c;

wherein the yield of AAV is at least 60%.