clinical trial screening disqualifies patients from enrollment, since NAbs severely attenuate therapeutic effectiveness and cause outcome variability.

[0004] To overcome AAV NAbs, several approaches have been employed: serial plasmapheresis, immune suppressive drugs, and alteration of the vector capsid to eliminate immune epitopes. Plasmapheresis is inefficient, requiring multiple rounds that remove 2-3 fold of the remaining NAbs each session and is only capable of addressing low titer of NAbs. Plasmapheresis is also time consuming and puts patients at risk for communication of nosocomial infections through simultaneous depletion of antibodies and repeated intravenous needle exposure. Steroids or pharmacological immune suppression to kill B-cells carries significant health risks and requires prolonged regimens to reduce NAbs by even 10-fold. AAV capsid engineering is an innovative method but ultimately inadequate against polyclonal anti-AAV sera, where a modest 10-fold increase in antibody escape is observed *in vivo*. Additionally, modification of the capsid structure to alter NAb recognition epitopes typically results in less potent vectors and defective vector production because those altered surface regions are multifunctional. None of the current approaches successfully overcome pre-existing anti-AAV NAbs above the typical thresholds set for clinical trial exclusion, or allow repeat administration of the same AAV vectors.

[0005] Mycoplasma protein M has been identified as capable of non-specifically binding antibodies and blocking their ability to bind antigen (US Patent No. 9,593,150; US Publication No, 2017/0320921).

[0006] The present invention overcomes shortcomings in the art by providing a vector independent protein based method to universally block Nabs and other methods and compositions based on antibody binding.

SUMMARY

[0007] The present disclosure is based, in part, on the development of a vector independent protein based method to universally block NAbs and demonstration that this method is effective against a broad range of pre-existing NAb concentrations. The disclosure pioneers the use of a unique mycoplasma-derived protein and its analogues, termed protein M, to enable successful gene delivery by preventing neutralization of heterologous agents (*e.g.*, AAV vectors) by NAbs upon administration of the heterologous agent to a subject. Protein M was shown to block mammalian IgG, IgM, and IgA antibody classes in a species and antigen independent manner by universally binding to conserved regions on the antibody light and heavy chains, causing structural interference with the antigen recognizing CDR

regions. Protein M binds to antibodies with nanomolar affinity, and prevents antigen-antibody union for a variety of different tested immunoglobulin/antigen pairs. The inventors discovered that protein M blocks antibody recognition of AAV and prevents antibody-mediated neutralization of AAV. It has been demonstrated that the level of AAV vector escape from NAbs is proportional to the NAb titer and volume, amount of protein M, and amount of AAV. Protein M mediated NAb escape has been validated *in vitro* and *in vivo* using human serum (IVIG) and serum from AAV immunized mice. It was demonstrated that protein M can be administered alone prior to AAV administration, or formulated with AAV for NAb evasion. The effectiveness of this approach depends on the interaction of protein M with immunoglobulin before AAV is neutralized. This approach can be used to overcome NAbs to multiple heterologous agents (*e.g.*, AAV vector serotypes) while maintaining the unique or beneficial properties of each agent for specific gene therapy applications.

[0008] The disclosure further relates to the use of protein M in other methods in which binding of antibodies is beneficial, including treatment of autoimmune disorders, treatment of disorders associated with excess antibodies, methods of isolating antibodies, and methods of performing immunoassays.

[0009] The disclosure further relates to modified protein M proteins having increased thermostability and/or other advantageous characteristics for carrying out the methods of the disclosure *in vivo* or at elevated temperatures.

[0009a] In one aspect, the disclosure relates to a modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase thermostability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof.

[0009b] In another aspect, the disclosure relates to a polynucleotide encoding the modified mycoplasma protein M or a functional fragment thereof of the disclosure.

[0009c] In another aspect, the disclosure relates to a vector comprising the polynucleotide of the disclosure.

[0009d] In another aspect, the disclosure relates to a transformed cell comprising the polynucleotide of the disclosure and/or the vector of the disclosure.

[0009e] In another aspect, the disclosure relates to a method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma protein M or a functional fragment thereof of the disclosure attached to a solid support, then

eluting the compound from the modified mycoplasma protein M or a functional fragment thereof.

[0009f] In another aspect, the disclosure relates to a method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof of the disclosure to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

[0009g] In another aspect, the disclosure relates to a kit comprising the modified mycoplasma protein M or a functional fragment thereof of the disclosure.

[0010] Thus, one example of the disclosure relates to a modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase or maintain thermostability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof.

[0011] An additional example of the disclosure relates to a polynucleotide encoding the modified mycoplasma protein M or a functional fragment thereof of the disclosure and a vector or transformed cell comprising the same.

[0012] Another example of the disclosure relates to a method of inhibiting neutralization of a heterologous agent by neutralizing antibodies upon administration of the heterologous agent to a subject, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby inhibiting neutralization of the heterologous agent.

[0013] A further example of the disclosure relates to a method of expressing a polypeptide or functional nucleic acid in a subject, comprising administering to the subject (a) a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective

amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0014] An additional example of the disclosure relates to a method of treating a disorder in a subject in need thereof, wherein the disorder is treatable by expressing a polypeptide or functional nucleic acid in the subject, comprising administering to the subject (a) a therapeutically effective amount of a nucleic acid delivery vector encoding the polypeptide or functional nucleic acid, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the disorder in the subject.

[0015] Another example of the disclosure relates to a method of editing a gene in a subject, comprising administering to the subject (a) a gene editing complex, and (b) an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby expressing the polypeptide or functional nucleic acid in the subject.

[0016] An additional example of the disclosure relates to a method of treating an autoimmune disease in a subject in need thereof, comprising administering to the subject an effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the autoimmune disease.

[0017] A further example of the disclosure relates to a method of treating a disorder associated with excess antibodies in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of mycoplasma protein M or a functional fragment or derivative thereof, thereby treating the disorder associated with excess antibodies.

[0018] Another example of the disclosure relates to a method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma protein M or a functional fragment thereof of the disclosure attached to a solid support, then eluting the compound from the modified mycoplasma protein M or a functional fragment thereof.

[0019] An additional example of the disclosure relates to a method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof of the disclosure to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

[0020] A further example of the disclosure relates to a kit comprising the modified mycoplasma protein M or a functional fragment thereof of the disclosure.

[0021] These and other examples of the disclosure are set forth in more detail in the description of the disclosure below.

What is claimed:

1. A modified mycoplasma protein M or a functional fragment thereof, having one or more amino acid mutations that increase thermostability of the mycoplasma protein M or a functional fragment thereof relative to wild-type mycoplasma protein M or a functional fragment thereof.

2. The modified mycoplasma protein M or a functional fragment thereof of claim 1, derived from protein M of *Mycoplasma genitalium* or *Mycoplasma pneumoniae*, optionally being a fragment from about residue 74 to about residue 479 of *M. genitalium* protein M (SEQ ID NO:3) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

3. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-2, wherein the one or more mutations are not at a residue within 5 Å of the antibody-binding site of protein M (residues 95, 99, 102, 103, 105, 106, 107, 109, 110, 114, 116, 117, 118, 119, 120, 144, 158, 160, 161, 162, 163, 177, 178, 179, 180, 181, 186, 187, 188, 191, 321, 338, 340, 341, 345, 381, 384, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 426, 427, 429, 436, 438, 439, 440, 441, 442, 444, 445, 446, 447, 448, 449, 452, 453, 455, 456, 457, 462, 466) or any of residues 469-479 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

4. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-3, wherein the one or more mutations are at residue 78, 81, 83, 84, 85, 89, 90, 91, 92, 93, 94, 96, 97, 100, 101, 108, 111, 112, 113, 122, 123, 125, 126, 127, 128, 130, 131, 133, 134, 136, 137, 139, 141, 142, 146, 147, 148, 149, 150, 153, 154, 155, 156, 164, 167, 170, 175, 176, 184, 185, 189, 192, 193, 196, 198, 201, 202, 204, 205, 206, 207, 209, 211, 215, 218, 220, 224, 225, 226, 227, 231, 232, 234, 235, 236, 237, 239, 241, 243, 244, 245, 246, 247, 249, 250, 252, 253, 254, 255, 256, 257, 258, 259, 264, 269, 270, 272, 274, 275, 276, 279, 282, 284, 286, 287, 288, 291, 297, 299, 300, 302, 303, 304, 305, 307, 308, 309, 310, 311, 313, 317, 318, 319, 320, 322, 326, 327, 329, 331, 332, 333, 335, 337, 342, 343, 347, 348, 351, 354, 355, 357, 358, 359, 360, 361, 362, 363, 367, 369, 370, 371, 372, 373, 374, 375, 378, 385, 399, 400, 401, 402, 405, 406, 407, 408, 409, 411, 413, 414, 417, 418, 419, 424, 428, 434, 435, 443, 450, 459, 460, 463,

464, 465, 468, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations is a mutation listed in Table 4 or any combination thereof, optionally wherein the one or more mutations are at residue 83, 90, 92, 94, 137, 142, 147, 150, 156, 184, 196, 198, 205, 211, 215, 225, 231, 232, 234, 235, 236, 237, 239, 243, 245, 250, 255, 256, 259, 264, 272, 274, 275, 276, 279, 282, 297, 300, 302, 310, 320, 326, 331, 332, 335, 342, 343, 347, 348, 355, 357, 361, 371, 374, 378, 385, 401, 402, 409, 413, 424, 460, 463, 464, 468, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations is a mutation listed in Table 5 or any combination thereof.

5. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-3, wherein the one or more mutations are at residue 150, 196, 198, 201, 205, 224, 232, 237, 274, 282, 342, 355, 373, 400, 402, 407, 409, 413, 135, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), wherein the one or more mutations are at residues selected from:

a) 237;

b) 232;

c) 282;

d) 150, 196, 198, 400, 402, 407, 409;

e) 413, 435;

f) 373, 400;

g) 402, 407, 409, 413;

h) 342;

i) 150, 196, 198, 232, 237, 282, 342, 373, 400, 402, 407, 409, 413, 435;

j) 274;

k) 150, 196, 198, 232, 237, 342, 400, 402, 407, 409;

l) 373, 413, 435;

m) 205;

n) 355;

o) 150, 196, 198, 342, 373, 400, 402, 407, 409;

p) 150, 196, 198, 232, 237, 342, 373, 400, 402, 407, 409;

q) 201, 224;

r) 150, 196, 198, 201, 224, 232, 237, 342, 400, 402, 407, 409;

s) 150, 196, 198, 232, 237, 342, 390, 400, 402, 407, 409, 444;

t) 150, 196, 198, 201, 205, 224, 232, 237, 274, 342, 355, 400, 402, 407, 409; or

u) 150, 196, 198, 232, 237, 342, 391, 400, 402, 407, 409

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations are selected from:

a) F237T;

b) S232Q;

c) Q282D;

d) S150E, S196R, S198P, V400I, N402I, K407P, S409V;

e) L413I, T435I;

f) V373I, V400I;

g) N402L, K407P, S409V, L413I;

h) A342V;

i) S150E, S196R, S198P, S232Q, F237T, Q282D, A342V, V373I, V400I, N402I, K407P, S409V, L413I, T435I;

j) N274D;

k) S150E, S196R, S198P, S232Q, F237T, A342V, V400I, N402I, K407P, S409V;

l) V373I, L413I, T435I;

m) A205P;

n) T355D;

o) T355P;

p) S150E, S196R, S198P, A342V, V373I, V400I, N402I, K407P, S409V;

q) 150, 196, 198, 232, 237, 342, 373, 400, 402, 407, 409;

r) S201C, A224C;

s) S150E, S196R, S198P, S201C, A224C, S232Q, F237T, A342V, V400I, N402I, K407P, S409V;

t) S150E, S196R, S198P, S232Q, F237T, A342V, F390E, V400I, N402I, K407P, S409V Y444K;

u) S150E, S196R, S198P, S201C, A205P, A224C, S232Q, F237T, N274D, A342V, T355P, V400I, N402I, K407P, S409V; or
v) S150E, S196R, S198P, S232Q, F237T, A342V, A391P, V400I, N402I, K407P, S409V
of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

6. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-3, wherein the one or more mutations are at residues 155, 203, 243, 248, and 358 of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations are A155E, K203R, H243T, V248Q, and A358V of *M. pneumoniae* protein M (SEQ ID NO:23).

7. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-3, wherein the one or more mutations are at residues selected from:
a) 468;
b) 150;
c) 147;
d) 272;
e) 355;
f) 276,277,279;
g) 300;
h) 378;
i) 156;
j) 232;
k) 245;
l) 276;
m) 225; or
n) 310
of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations are selected from:
a) R468Q;
b) S150E;

c) H147F;

d) S272G;

e) T355G;

f) S276E,Q277L,N279R;

g) N300Q;

h) N378Y;

i) S156K;

j) S232L;

k) A245Q;

l) S276D;

m) K225P; or

n) V310E

of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23).

8. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-7, wherein the modified mycoplasma protein M or a functional fragment thereof is further modified to remove one or more glycosylation sites or to add one or more glycosylation sites.

9. The modified mycoplasma protein M or a functional fragment thereof of claim 1, further comprising one or more mutations that enhance the affinity of the modified mycoplasma protein M or a functional fragment thereof to antibodies, optionally wherein the one or more mutations are at residue 95, 102, 103, 106, 107, 114, 116, 160, 161, 162, 163, 181, 186, 321, 381, 384, 389, 390, 391, 396, 397, 426, 429, 436, 438, 439, 441, 442, 447, 448, 449, 452, 453, 455, 456, 462, or 466, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations is a mutation listed in Table 6 or any combination thereof, optionally wherein the one or more mutations are at residue 95, 103, 116, 186, 321, 389, 429, 442, or 466, or any combination thereof of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M.*

pneumoniae protein M (SEQ ID NO:23), optionally wherein the one or more mutations is a mutation listed in Table 7 or any combination thereof.

10. The modified mycoplasma protein M or a functional fragment thereof of claim 1, further comprising one or more mutations that reduce the affinity of the modified mycoplasma protein M or a functional fragment thereof to antibodies, optionally wherein the one or more mutations are at residue 390 and/or 444 of *M. genitalium* protein M (SEQ ID NO:1) or the equivalent residues of *M. pneumoniae* protein M (SEQ ID NO:23), optionally wherein the one or more mutations is 390E and/or Y444K.

11. The modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-10, further comprising a secretion peptide at the N-terminus.

12. A polynucleotide encoding the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-11.

13. A vector comprising the polynucleotide of claim 12.

14. A transformed cell comprising the polynucleotide of claim 12 and/or the vector of claim 13.

16. A method of isolating a compound comprising an antibody light chain variable region and/or heavy chain variable region from a sample, the method comprising contacting the compound with the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-11 attached to a solid support, then eluting the compound from the modified mycoplasma protein M or a functional fragment thereof.

17. A method of performing an immunoassay, the method comprising using the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-11 to bind a compound comprising an antibody light chain variable region and/or heavy chain variable region.

18. A kit comprising the modified mycoplasma protein M or a functional fragment thereof of any one of claims 1-11.