

AMENDED CLAIMS

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What is claimed is:

1. A genetically modified non-human animal comprising in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L segment sequences,

wherein the human V_L and J_L segment sequences of the single rearranged human immunoglobulin light chain variable region gene sequence comprise at least one histidine codon that is not encoded by corresponding human germline V_L and J_L gene sequences.
2. The animal of claim 1, wherein the single rearranged human immunoglobulin variable region sequence is operably linked to an immunoglobulin light chain constant region gene sequence.
3. The animal of claim 2, wherein the immunoglobulin light chain constant region gene sequence is a non-human immunoglobulin light chain constant region gene sequence.
4. The animal of claim 3, wherein the non-human immunoglobulin light chain constant region gene sequence is an endogenous non-human immunoglobulin light chain constant region gene sequence.
5. The animal of claim 1, further comprising in its germline an immunoglobulin heavy chain locus that comprises an unrearranged immunoglobulin heavy chain variable region gene sequence comprising human V_H, D_H, and J_H segments operably linked to an immunoglobulin heavy chain constant region gene sequence.
6. The animal of claim 5, wherein the immunoglobulin heavy chain constant region gene sequence is a non-human immunoglobulin heavy chain constant region gene sequence.
7. The animal of claim 6, wherein the non-human heavy chain constant region gene sequence is an endogenous non-human immunoglobulin constant region gene sequence.

8. The animal of claim 1, wherein the animal lacks a functional unrearranged immunoglobulin light chain variable region.
9. The animal of claim 1, wherein the immunoglobulin light chain locus is at an endogenous non-human immunoglobulin light chain locus.
10. The animal of claim 5, wherein the immunoglobulin heavy chain locus is at an endogenous non-human immunoglobulin heavy chain locus.
11. The animal of claim 1, wherein the at least one histidine codon is in the nucleotide sequence encoding a complementary determining region (CDR) 3.
12. The animal of claim 11, wherein the at least one histidine codon is one, two, three, or four CDR3 codon(s).
13. The animal of claim 1, wherein the single rearranged human immunoglobulin light chain variable region is derived from a human V κ 1-39 or V κ 3-20 gene segment.
14. The animal of claim 13, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 or V κ 3-20/J κ 1 gene sequence.
15. The animal of claim 14, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 gene sequence, and wherein the V κ 1-39/J κ 5 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof.

16. The animal of claim 14, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 3-20/J κ 1 gene sequence, and wherein the V κ 3-20/J κ 1 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.
17. The animal of claim 1, wherein the animal comprises a population of B cells in response to an antigen of interest that is enriched for antibodies that exhibit a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold.
18. The animal of claim 17, wherein the decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH is about 30 fold or more.
19. The animal of claim 1, wherein the animal is a rodent.
20. The rodent of claim 19, wherein the rodent is a rat or a mouse.
21. The rodent of claim 20, wherein the rodent is a mouse.
22. The animal of claim 1, wherein the animal expresses an antibody comprising a human immunoglobulin light chain variable domain comprising at least one histidine residue at an amino acid position encoded by the at least one histidine codon in the immunoglobulin light chain variable region gene sequence.
23. A genetically modified mouse comprising in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V $_L$ and J $_L$ segment sequences,

wherein the human V_L and J_L segment sequences of the single rearranged human immunoglobulin light chain variable region sequence comprise at least one histidine codon that is not encoded by corresponding human germline V_L and J_L gene sequences, and

wherein the mouse lacks a functional unrearranged immunoglobulin light chain variable region.

24. The mouse of claim 23, wherein the single rearranged immunoglobulin light chain variable region gene sequence is operably linked to an immunoglobulin light chain constant region gene sequence.

25. The mouse of claim 24, wherein the immunoglobulin light chain constant region gene sequence is selected from a rat or a mouse immunoglobulin light chain constant region gene sequence.

26. The mouse of claim 23, further comprising in its germline an immunoglobulin heavy chain locus that comprises an unrearranged immunoglobulin heavy chain variable region sequence comprising human V_H , D_H , and J_H segments operably linked to an immunoglobulin heavy chain constant region gene sequence.

27. The mouse of claim 26, wherein the immunoglobulin heavy chain constant region gene sequence is a rat or a mouse heavy chain constant region gene sequence.

28. The mouse of claim 23, wherein the immunoglobulin light chain locus is at an endogenous mouse immunoglobulin light chain locus.

29. The mouse of claim 26, wherein the immunoglobulin heavy chain locus is at an endogenous mouse immunoglobulin heavy chain locus.

30. The mouse of claim 23, wherein the at least one histidine codon is in the nucleotide sequence encoding a complementary determining region (CDR) 3.
31. The mouse of claim 30, wherein the at least one histidine codon is one, two, three, or four CDR3 codon(s).
32. The mouse of claim 23, wherein the single rearranged human immunoglobulin light chain variable region is derived from a human V κ 1-39 or V κ 3-20 gene segment.
33. The mouse of claim 32, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 or V κ 3-20/J κ 1 gene sequence.
34. The mouse of claim 33, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 gene sequence, and wherein the V κ 1-39/J κ 5 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof.
35. The mouse of claim 34, wherein the V κ 1-39/J κ 5 gene sequence comprises a replacement of non-histidine codons with histidine codons designed to express histidines at positions 105, 106, 108, and 111.
36. The mouse of claim 34, wherein the V κ 1-39/J κ 5 gene sequence comprises a replacement of non-histidine codons with histidine codons designed to express histidines at positions 106, 108 and 111.
37. The mouse of claim 33, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 3-20/J κ 1 gene sequence, and wherein the V κ 3-20/J κ 1 gene sequence comprises replacement of at least one non-histidine codon with a

histidine codon designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof.

38. The mouse of claim 37, wherein the V κ 3-20/J κ 1 gene sequence comprises a replacement of non-histidine codons with histidine codons designed to express histidines at positions 105, 106, 107, and 109.

39. The mouse of claim 37, wherein the V κ 3-20/J κ 1 gene sequence comprises a replacement of non-histidine codons with histidine codons designed to express histidines at positions 105, 106 and 109.

40. The mouse of claim 23, wherein the mouse expresses a population of antigen-specific antibodies in response to an antigen of interest wherein all antibodies comprise:

immunoglobulin light chain variable domains derived from the same single rearranged human light chain variable region gene sequence comprising human V_L and J_L segment sequences that comprise at least one histidine codon that is not encoded by corresponding human germline V_L and J_L gene sequences, and

immunoglobulin heavy chains comprising human heavy chain variable domains derived from a repertoire of human heavy chain V, D, and J segments.

41. A method of generating an antibody that exhibits pH-dependent binding to an antigen of interest comprising:

generating a mouse of claim 23;

immunizing the mouse with an antigen of interest; and

selecting an antibody that binds to the antigen of interest with a desired affinity at a neutral pH while displaying reduced binding to the antigen of interest at an acidic pH.

42. The method of claim 41, wherein the antibody exhibits a dissociative half-life ($t_{1/2}$) at acidic pH and 37°C of about 2 minutes or less.

43. A method of generating an antibody that exhibits pH-dependent binding to an antigen of interest comprising:

generating in a non-human animal comprising in its germline an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence comprising human V_L and J_L segment sequences a first antibody that binds to an antigen of interest with a desired affinity,

modifying the human V_L and J_L segment sequences of the single rearranged human immunoglobulin light chain variable region gene sequence to comprise histidine codon that is not encoded by corresponding human germline V_L and J_L segment sequences,

expressing an immunoglobulin heavy chain of the first antibody and the modified immunoglobulin light chain in a cell, and

selecting a second antibody expressed in the cell that retains a desired affinity for the antigen of interest at neutral pH and displays reduced binding to the antigen of interest at an acidic pH.

44. The method of claim 43, wherein the first antibody generated in the non-human animal comprises an immunoglobulin heavy chain sequence derived from a repertoire of human V_H , D_H , and J_H segments.

45. The method of claim 43, wherein the single rearranged human immunoglobulin light chain variable region sequence is selected from $V_{\kappa}1-39/J_{\kappa}5$ and $V_{\kappa}3-20/J_{\kappa}1$ gene sequence.

46. The method of claim 45, wherein the single rearranged human immunoglobulin light chain variable region sequence is $V_{\kappa}1-39/J_{\kappa}5$, and the modification comprises a substitution of at least one non-histidine codon with a histidine codon in the CDR3 codon at a position selected from 105, 106, 108, 111, and a combination thereof.

47. The method of claim 45, wherein the single rearranged human immunoglobulin light chain variable region sequence is $V_{\kappa}3-20/J_{\kappa}1$, and the modification comprises a substitution of

at least one non-histidine codon with a histidine codon in the CDR3 codon at a position selected from 105, 106, 107, 109, and a combination thereof.

48. The method of claim 43, wherein the antibody displays a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold.

49. The method of claim 43, wherein the antibody exhibits a dissociative half-life ($t_{1/2}$) at acidic pH and 37°C of about 2 minutes or less.

50. The method of claim 43, wherein the non-human animal is a mouse.

51. A method of making a non-human animal that comprises a genetically modified immunoglobulin light chain locus in its germline, the method comprising:

modifying a genome of a non-human animal to delete or render non-functional endogenous immunoglobulin light chain V and J segments in an immunoglobulin light chain locus, and

placing in the genome a single rearranged human light chain variable region gene sequence comprising human V_L and J_L segment sequences comprising at least one histidine codon that is not encoded by corresponding human germline V_L and J_L segment sequences.

52. The method of claim 51, wherein the method results in a genetically modified non-human animal that comprises a population of B cells enriched for antibodies exhibiting pH-dependent binding to an antigen of interest.

53. The method of claim 51, wherein the animal is a rodent.

54. The method of claim 53, wherein the animal is a mouse or a rat.
55. The method of claim 51, wherein the single rearranged human immunoglobulin light chain variable region sequence is derived from a human V κ 1-39/J κ 5 or V κ 3-20/J κ 1 gene sequence, and the at least one histidine codon is a CDR3 codon.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A genetically modified non-human animal comprising in its germline genome an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence derived from a rearranged V κ 1-39/J κ or V κ 3-20/J κ sequence, and wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine in one, two, three, or four CDR3 codon(s).
2. The animal of claim 1, wherein the single rearranged human immunoglobulin light chain variable region sequence is operably linked to an immunoglobulin light chain constant region gene sequence.
3. The animal of claim 2, wherein the immunoglobulin light chain constant region gene sequence is a non-human immunoglobulin light chain constant region gene sequence.
4. The animal of claim 3, wherein the non-human immunoglobulin light chain constant region gene sequence is an endogenous non-human immunoglobulin light chain constant region gene sequence.
5. The animal of any one of claims 1 to 4, further comprising in its germline genome an immunoglobulin heavy chain locus that comprises an unrearranged immunoglobulin heavy chain variable region gene sequence comprising human V_H, D_H, and J_H segments operably linked to an immunoglobulin heavy chain constant region gene sequence, such as a non-human immunoglobulin heavy chain constant region gene sequence, that is optionally an endogenous non-human immunoglobulin constant region gene sequence, and wherein the immunoglobulin heavy chain locus is optionally at an endogenous non-human immunoglobulin heavy chain locus.
6. The animal of any one of claims 1 to 5, wherein the animal lacks a functional unrearranged immunoglobulin kappa light chain variable region.
7. The animal of any one of claims 1 to 6, wherein the immunoglobulin kappa light chain locus is at an endogenous non-human immunoglobulin light chain locus.

8. The animal of any one of claims 1 to 7, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 or V κ 3-20/J κ 1 gene sequence.
9. The animal of any one of claims 1 to 8, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 1-39/J κ 5 gene sequence, wherein the V κ 1-39/J κ 5 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 108, 111, and a combination thereof, and wherein the positions are according to IMGT numbering.
10. The animal of any one of claims 1 to 9, wherein the single rearranged human immunoglobulin light chain variable region is derived from a rearranged V κ 3-20/J κ 1 gene sequence, wherein the V κ 3-20/J κ 1 gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine at a position selected from 105, 106, 107, 109, and a combination thereof, and wherein the positions are according to IMGT numbering.
11. The animal of any one of claims 1 to 10, wherein the animal comprises a population of B cells in response to an antigen of interest that is enriched for antibodies that exhibit a decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH of at least about 2-fold, at least about 3-fold, at least about 4-fold, at least about 5-fold, at least about 10-fold, at least about 15-fold, at least about 20-fold, at least about 25-fold, or at least about 30-fold.
12. The animal of claim 11 wherein the decrease in dissociative half-life ($t_{1/2}$) at an acidic pH as compared to neutral pH is about 30 fold or more.
13. The animal of any one of claims 1 to 12, wherein the animal is a rodent.
14. The rodent of claim 13, wherein the rodent is a rat or a mouse.
15. The rodent of claim 14, wherein the rodent is a mouse.
16. The animal of any one of claims 1 to 15, wherein the animal expresses an antibody comprising a human immunoglobulin light chain variable domain with a substitution of at least one non-histidine residue with a histidine at an amino acid position encoded by the at least one

codon substituted in the single rearranged human immunoglobulin light chain variable region gene sequence.

17. A method of making the non-human animal of any one of claims 1 to 16, wherein the method comprises replacing an immunoglobulin light chain variable region gene sequence in the animal with a single rearranged human immunoglobulin light chain variable region gene derived from a rearranged V_κ1-39/J_κ or V_κ3-20/J_κ sequence, wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine in one, two, three, or four CDR3 codon(s).

18. A method of generating an antibody that exhibits pH-dependent binding to an antigen of interest comprising:

immunizing the non-human animal of any one of claims 1 to 16 or the non-human animal made by the method of claim 17 with an antigen of interest; and

obtaining a B cell expressing an antibody that binds to the antigen of interest with a desired affinity at a neutral pH while displaying reduced binding to the antigen of interest at an acidic pH and optionally (a) using the B cell to make a hybridoma or (b) isolating DNA encoding the antibody from the B cell or the hybridoma made from the B cell and expressing the DNA in a host cell.

19. The method of claim 17, wherein the antibody exhibits a dissociative half-life ($t_{1/2}$) at acidic pH and 37°C of about 2 minutes or less.

20. The method of claim 18 or 19, wherein the method is for making a bispecific pH dependent antibody.

21. The method of claim 20, wherein the method comprises:

(a) identifying a B cell that encodes a first heavy chain variable domain that binds a first epitope of interest and expresses an antibody that exhibits pH dependent antigen binding and isolating a first nucleic acid encoding the heavy chain variable region; and

(b) identifying a B cell that encodes a second heavy chain variable domain that binds a second epitope of interest and expresses an antibody that exhibits pH dependent

antigen binding and isolating a second nucleic acid encoding the second heavy chain variable region;

(c) isolating a third nucleic acid sequence that encodes a light chain variable domain; and

(d) expressing the first, second and third nucleic acid sequences to form the bispecific antibody.

22. A non-human cell isolated from the non-human animal of any one of claims 1 to 16 or isolated from a non-human animal made by the method of claim 17, wherein the cell comprises an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence derived from a rearranged V κ 1-39/J κ or V κ 3-20/J κ sequence, and wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine in one, two, three, or four CDR3 codon(s).

23. The non-human cell of claim 22 wherein the cell is a B cell.

24. A hybridoma made from a B cell isolated from the mouse of any one of claims 1 to 16 or a non-human animal made by the method of claim 17, wherein the hybridoma comprises an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene derived from a rearranged V κ 1-39/J κ or V κ 3-20/J κ sequence, and wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine in one, two, three, or four CDR3 codon(s).

25. An ES cell comprising an immunoglobulin light chain locus that comprises a single rearranged human immunoglobulin light chain variable region gene sequence derived from a rearranged V1-39/J or V3-20/J sequence, and wherein the single rearranged human immunoglobulin light chain variable region gene sequence comprises a replacement of at least one non-histidine codon with a histidine codon designed to express a histidine in one, two, three, or four CDR3 codon(s).