Claims

WHAT IS CLAIMED IS:

1. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising steps of:
    a. generating n-1 separate sets of mutant polypeptides, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
    b. assaying each set of mutant polypeptides for at least one predetermined property, characteristic or activity;
    c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
    d. creating a functional map.

2. The method of claim 1 wherein the functional map is used to identify one or more of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

3. The method of claim 2 wherein the functional map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

4. The method of claim 2 wherein the functional map is used to identify fully mutable sites compared to the template polypeptide.

5. The method of claim 2 wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

6. The method of claim 1 wherein X represents the 19 naturally occurring amino acid residues not present in a predetermined position of the template polypeptide.

7. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising steps of:
   a. generating a population of n-1 sets of mutant polypeptides, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
   b. assaying the population of mutant polypeptides for at least one predetermined property, characteristic or activity;
   c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
   d. creating a functional map.

8. The method of claim 7 wherein the functional map is used to identify one or more of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

9. The method of claim 8 wherein the functional map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

10. The method of claim 8 wherein the functional map is used to identify fully mutable sites compared to the template polypeptide.

11. The method of claim 8 wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

12. The method of claim 1 wherein X is 19.

13. The method of claim 6 or 12 wherein said generating step comprises:

i. subjecting a codon-containing polynucleotide encoding for said template polypeptide to polymerase-based amplification using a 64-fold degenerate oligonucleotide for each codon to be mutagenized, wherein each of said 64-fold degenerate oligonucleotides is comprised of a first homologous sequence and a degenerate N,N,N triplet sequence, so as to generate a set of progeny polynucleotides; and

ii. subjecting said set of progeny polynucleotides to clonal amplification such that polypeptides encoded by the progeny polynucleotides are expressed.

14. The method of claim 1 wherein n represents a subset or region of said template polypeptide.

15. A method of mapping a set of mutant antibodies formed from a template antibody having six complementary determining regions (CDRs), said CDRs together comprising n amino acid residues, the method comprising:

a. generating n separate sets of mutant antibodies, each set comprising mutant antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of mutant antibodies differs in the single predetermined position; and the number of different mutant antibodies generated is equivalent to n x X;

b. assaying each set for at least one predetermined property, characteristic or activity;
c. for each mutant antibodyidentifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a structural positional map.

16. The method of claim 15 wherein the structural map is used to identify one or more of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

17. The method of claim 16 wherein the structural map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

18. The method of claim 16 wherein the structural map is used to identify fully mutable sites compared to the template polypeptide.

19. The method of claim 16 wherein the structural map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

20. The method of claim 15 wherein said predetermined property, characteristic or property is at least one of binding affinity and immunogenicity.

21. A method of mapping a population of mutant antibodies formed from a template antibody having at least one complementary determining regions (CDRs), said CDRs together comprising n amino acid residues, the method comprising:
a. generating a population comprising n sets of mutant antibodies, each set comprising mutant antibodies having X number of different predetermined amino acid residues at a single predetermined position

of the CDR; wherein each set of mutant antibodies differs in the single predetermined position; and the number of different mutant antibodies generated is equivalent to n x X;

b. assaying the population for at least one predetermined property, characteristic or activity;

c. for each mutant antibody identifying any change in said property, characteristic or activity relative to the template polypeptide; and

d. creating a structural positional map.

22. The method of claim 21 wherein the structural map is used to identify one or more of the group consisting of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

23. The method of claim 16 wherein the structural map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

24. The method of claim 16 wherein the structural map is used to identify fully mutable sites compared to the template polypeptide.

25. The method of claim 16 wherein the structural map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

26. A functional positional map made by the method of claim 1, 7, 15 or 21.

27. A method of producing a set of mutant antibodies formed from a template antibody having m complementary determining regions (CDR), wherein m is an integer 1 to 6, each said CDR comprising n amino acid residues, the method comprising:

a. generating m x n separate sets of mutant antibodies, each set comprising mutant antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of mutant antibodies differs in the single predetermined position; and the number of different mutant antibodies generated is equivalent to m x n x X.

28. The method of claim 27 wherein m is 6.

29. A method of producing a library of multi-site mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

a. generating n-1 separate sets of point mutation polypeptides, each set comprising point mutation polypeptides having X number of different predetermined single amino acid substitutions at a single predetermined site of the template polypeptide; wherein each set of point mutation polypeptides differs in the single predetermined site; and the number of different point mutation polypeptides generated is equivalent to [n-1] x X;
b. screening each set of point mutation polypeptides for at least one predetermined property, characteristic or activity;
c. identifying any change in said property, characteristic or activity relative to the template polypeptide for each point mutation polypeptide;
d. categorizing each amino acid point mutation as one of deactivating, non-deactivating or activating;
e. creating a functional map of such changes categorizing each site as one of non-mutable; partially mutable; or fully mutable;
f. utilizing the map to select non-deactivating amino acid point mutations within partially mutable sites that are adjacent to non-mutable sites; and
g. recombining permutations of two to twenty amino acid point mutations selected in step (f) simultaneously to generate a library of multi-site mutant polypeptides.

30. The method of claim 29 wherein the recombining step utilizes combinatorial protein synthesis.

31. The method of claim 29 wherein contact amino acid residues in the template polypeptide are categorized as non-mutable in the functional map.

32. The method of claim 29 wherein the template polypeptide is derived from a portion of an immunoglobulin molecule.

33. The method of claim 32 wherein the portion of the immunoglobulin molecule is selected from a heavy chain, light chain, variable domain, constant domain, hypervariable region, complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3).

34. A method of identifying a multi-site up-mutant polypeptide, the method comprising:
    a. generating a library of multi-site mutant polypeptides from a template polypeptide having n amino acid residues, said generating step comprising the steps of:
        i. generating n-1 separate sets of point mutation polypeptides, each set comprising point mutation polypeptides having X number of different predetermined single amino acid substitutions at a single predetermined site of the template polypeptide; wherein each set of point mutation polypeptides differs in the single predetermined site; and the number of different point mutation polypeptides generated is equivalent to [n-1] x X;
        ii. screening each set of point mutation polypeptides for at least one predetermined property, characteristic or activity;
        iii. identifying any change in said property, characteristic or activity relative to the template polypeptide for each point mutation polypeptide;

iv. categorizing each amino acid point mutation as one of deactivating, non-deactivating or activating;
v. creating a functional map of such changes categorizing each site as one of non-mutable; partially mutable; or fully mutable;
vi. utilizing the functional map to select non-deactivating amino acid point mutations within partially mutable sites that are adjacent to non-mutable sites; and
vii. recombining permutations of two to twenty amino acid point mutations selected in step (f) simultaneously to generate a library of multi-site mutant polypeptides;

b. screening the library of multi-site mutant polypeptides for at least one pre-determined property, characteristic or activity relative to the template polypeptide; and
c. identifying the multi-site up-mutant polypeptides as those with one or more improved properties, characteristics, or activities relative to the template polypeptide.

35. A method of selecting two or more sites in a template polypeptide for multi-site mutation, the method comprising:
a. obtaining a functional map of mutant polypeptides formed from a template polypeptide having n amino acid residues, said obtaining step comprising the steps of:
i. generating n-1 separate sets of mutant polypeptides, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
ii. assaying each set for at least one predetermined property, characteristic or activity;
iii. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
iv. creating a functional map of such changes identifying positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide;

b. utilizing the functional map to select partially mutable sites that are adjacent to non-mutable sites for multi-site mutation.

36. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

a. generating n-1 (n-2 in the case where the initial residue is methionine) separate mutant polypeptides, wherein each mutant polypeptide differs from the template polypeptide in that one amino acid is deleted at a single predetermined position;
b. assaying the population of mutant polypeptides for at least one predetermined property, characteristic or activity;
c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a functional map.

37. The method of claim 36 wherein the functional map is used to identify those deletions which result in one of the group consisting of (a) deletions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) deletions which result in a down-mutant compared to the template polypeptide; and (c) deletions which result in an up-mutant compared to the template polypeptide.

38. The method of claim 37 wherein the functional map is used to identify deletions which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

39. The method of claim 37 wherein the functional map is used to identify deletions which result in a down-mutant compared to the template polypeptide.

40. The method of claim 37 wherein the functional map is used to identify deletions which result in an up-mutant compared to the template polypeptide.

41. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

a. generating n+[20 x (n-1)] separate mutant polypeptides, wherein each mutant polypeptide differs from the template polypeptide in that it has inserted after a specific position in the template one each of the 20 naturally occurring amino acids;
b. assaying the population for at least one predetermined property, characteristic or activity;
c. for each mutant polypeptide identifying any change in said property, characteristic or activity relative to the template polypeptide; and

d. creating a functional map.

42. The method of claim 36 wherein the functional map is used to identify those insertions which result in one of the group consisting of (a) insertions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) insertions which result in a down-mutant compared to the template polypeptide; and (c) insertions which result in an up-mutant compared to the template polypeptide.

43. The method of claim 37 wherein the functional map is used to identify insertions which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

44. The method of claim 37 wherein the functional map is used to identify insertions which result in a down-mutant compared to the template polypeptide.

45. The method of claim 37 wherein the functional map is used to identify insertions which result in an up-mutant compared to the template polypeptide.

46. A method of providing an optimized protein, the method comprising:

a. selecting a template polypeptide having n amino acid residues;
b. generating n-1 separate sets of mutant polypeptides from the template polypeptide, each set comprising mutant polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the number of different mutant polypeptides generated is equivalent to [n-1] x X;
c. assaying each mutant polypeptide for at least one predetermined property, characteristic or activity;
d. identifying any change in said property, characteristic or activity of the mutant polypeptide relative to the template polypeptide;

e. creating a functional map wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide;
f. combining two or more of each of the relevant positions and mutations identified from mutant polypeptides which result in a up-mutant compared to the template polypeptide to create a set of combinatorial polypeptides;
g. assaying each combinatorial polypeptide for at least one predetermined property, characteristic or activity; and
h. selecting a combinatorial polypeptide in which the predetermined property, characteristic or activity is optimized compared to up-mutant polypeptides.

47. The method of claims 1 or 7 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position in each member polypeptide in each set of mutant polypeptides.

48. The method of claims 15, 21 or 27 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position of the CDR in each mutant antibody in each set of mutant antibodies.

49. The method of claim 29 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of each of the intended predetermined single amino acid substitutions at the predetermined site in each point mutation polypeptide in each set of point mutation polypeptides.

50. The method of claim 34 wherein the generating step (a) further comprises confirming the sequence of each multi-site mutant polypeptide in the library by sequencing, or some other method.

51. The method of claim 36 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid deletion at the single predetermined position.

52. The method of claim 41 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid insertion at the specific position in the template.

53. A method of evolving a polypeptide encoding a functional protein, the method comprising:

a. generating and cloning at least 20 or more separate sets of mutant polypeptides, each set comprising mutant polypeptides having from 5 to 19 different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position;

b. confirming that each different mutant polypeptide was generated and cloned; and

c. assaying each set of mutant polypeptides for at least one predetermined property, characteristic or activity.

54. The method of claim 53, wherein in step (a), each set of mutant polypeptides comprises mutant polypeptides having from 10 to 19, from 15 to 19, or 19 different predetermined amino acid residues at a single predetermined position of the polypeptide.

55. The method of claim 53, further comprising:

d. performing bioinformatic analysis to identify positions of potential interest for further modification; and

(e) mutating positions identified in (d).

56. The method of claim 55 wherein bioinformatic analysis is generation of a functional map.

57. The method of claim 53 wherein the at least one predetermined property, characteristic or activity is modified expression

58. The method of claim 57 wherein the modified expression is improved expression.

59. A method of evolving a polypeptide encoding a functional protein and having n amino acid residues, the method comprising:

a. generating and cloning n-1 sets of mutant polypeptides, each set comprising mutant polypeptides having 19 different predetermined amino acid residues at a single predetermined position of the template polypeptide; wherein each set of mutant polypeptides differs in the single predetermined position; and the total number of different mutant polypeptides generated is equivalent to [n-1] x 19;

b. confirming that each different mutant polypeptide was generated and cloned; and

c. assaying each set of mutant polypeptide for at least one predetermined property, characteristic or activity;

60. The method of claim 59, the method further comprising:

(d) performing bioinformatic analysis to identify positions of potential interest for further modification; and

(e) mutating positions identified in (d).

61. The method of claim 60 wherein the bioinformatic analysis is generation of a functional map.

62. The method of claim 59 wherein the at least one predetermined property, characteristic or activity is modified expression.

63. The method of claim 62 wherein the modified expression is improved expression.

64. A method of evolving a polynucleotide encoding a functional protein, the method comprising:

a. incorporating N,N,N at a predetermined codon position in the polynucleotide in order to generate one or more predetermined codon mutants within a set of predetermined mutant polynucleotides and cloning each predetermined mutant polynucleotide;

b. confirming that each predetermined mutant polynucleotide in the set was generated and cloned;

c. producing a set of predetermined mutant polypeptides from set of predetermined mutant polynucleotides; and

d. assaying each predetermined mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

65. The method of claim 64 wherein the at least one predetermined property, characteristic or activity is modified expression.

66. The method of claim 65 wherein the modified expression is improved expression.

67. The method of claim 64 wherein the confirming step comprises sequencing of each mutant polynucleotide.

68. A method of evolving a polypeptide from a functional protein, the method comprising:

a. incorporating N,N,N at every codon position in a polynucleotide encoding the functional protein in order to generate a set of predetermined mutant polynucleotides containing every possible codon sequence and cloning each predetermined mutant polynucleotide;

b. confirming by sequencing, or some other method, that each predetermined mutant polynucleotide in the set was generated and cloned;

c. producing a set of predetermined mutant polypeptides from the set of predetermined mutant polynucleotides; and

d. assaying each mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

69. A method of evolving a polypeptide from a functional protein, the method comprising:

a. incorporating N,N,N at a codon position in a template polynucleotide encoding the functional protein in order to generate one or more predetermined codons within the set of mutant polynucleotides and cloning and expressing each mutant polynucleotides to produce a set of predetermined mutant polypeptides;

b. confirming by sequencing or some other method that greater than 75%, 50%, 25%, or 1% of the predetermined mutant polypeptides in the set were generated, cloned and expressed; and

c. assaying each predetermined mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

70. A method of evolving a polypeptide from a functional protein, the method comprising:

a. incorporating N,N,N at a codon position in a template polynucleotide encoding the functional protein in order to generate one or more predetermined codons within the set of mutant polynucleotides and cloning and expressing each mutant polynucleotide to produce a set of predetermined mutant polypeptides;

b. confirming by sequencing, or some other method, that any number of the predetermined mutant polypeptides in the set were generated, cloned and expressed; and

c. assaying each predetermined mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

Claims

What is claimed is:

1. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:
    a. generating n-1 separate sets of mutant polypeptides, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;
    b. assaying each set for at least one predetermined property, characteristic or activity;
    c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
    d. creating a functional map.
2. The method of claim 1 wherein the functional map is used to identify one or more of the group consisting of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.
3. The method of claim 2 wherein the functional map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.
4. The method of claim 2 wherein the functional map is used to identify fully mutable sites compared to the template polypeptide.
5. The method of claim 2 wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.
6. The method of claim 1 wherein X represents the 19 naturally occurring amino acid residues not present in a given position of the template polypeptide.
7. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

a. generating a population of n-1 sets of mutant polypeptides, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;
b. assaying the population for at least one predetermined property, characteristic or activity;
c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a functional map.

8. The method of claim 7 wherein the functional map is used to identify one or more of the group consisting of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

9. The method of claim 8 wherein the functional map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

10. The method of claim 8 wherein the functional map is used to identify fully mutable sites compared to the template polypeptide.

11. The method of claim 8 wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

12. The method of claim 1 wherein X represents the 19 naturally occurring amino acid residues not present in a given position of the template polypeptide.

13. The method of claim 6 or 12 wherein said generating step comprises:

i. subjecting a codon-containing polynucleotide encoding for said template polypeptide to polymerase-based amplification using a 64-fold degenerate oligonucleotide for each codon to be mutagenized, wherein each of said 64-fold

degenerate oligonucleotides is comprised of a first homologous sequence and a degenerate N,N,N triplet sequence, so as to generate a set of progeny polynucleotides; and

ii. subjecting said set of progeny polynucleotides to clonal amplification such that polypeptides encoded by the progeny polynucleotides are expressed.

14. The method of claim 1 wherein n represents a subset or region of said template polypeptide.

15. A method of mapping a set of mutant antibodies formed from a template antibody having six complementary determining regions (CDRs), said CDRs together comprising n amino acid residues, the method comprising:

a. generating n separate sets of mutant antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to n x X;

b. assaying each set for at least one predetermined property, characteristic or activity;

c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and

d. creating a structural positional map.

16. The method of claim 15 wherein the structural map is used to identify one or more of the group consisting of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

17. The method of claim 16 wherein the structural map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

18. The method of claim 16 wherein the structural map is used to identify fully mutable sites compared to the template polypeptide.

19. The method of claim 16 wherein the structural map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

20. The method of claim 15 wherein said predetermined property, characteristic or property is at least one of binding affinity and immunogenicity.

21. A method of mapping a population of mutant antibodies formed from a template antibody having at least one complementary determining regions (CDRs), said CDRs together comprising n amino acid residues, the method comprising:
    a. generating a population comprising n sets of mutant antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to n x X;
    b. assaying the population for at least one predetermined property, characteristic or activity;
    c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
    d. creating a structural positional map.

22. The method of claim 21 wherein the structural map is used to identify one or more of the group consisting of (a) positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) fully mutable sites compared to the template polypeptide; and (c) positions and mutations which result in an up-mutant compared to the template polypeptide.

23. The method of claim 16 wherein the structural map is used to identify positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

24. The method of claim 16 wherein the structural map is used to identify fully mutable sites compared to the template polypeptide.

25. The method of claim 16 wherein the structural map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide.

26. A functional positional map made by the method of claim 1, 7, 15 or 21.

27. A method of producing a set of mutant antibodies formed from a template antibody having m complementary determining regions (CDR), wherein m is an integer 1 to 6, each said CDR comprising n amino acid residues, the method comprising:

    a. generating m x n separate sets of mutant antibodies, each set comprising member antibodies having X number of different predetermined amino acid residues at a single predetermined position of the CDR; wherein each set of antibodies differs in the single predetermined position; and the number of different member antibodies generated is equivalent to m x n x X.

28. The method of claim 27 wherein m is 6.

29. A method of producing a library of multi-site mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

    a. generating n-1 separate sets of point mutation polypeptides, each set comprising member point mutation polypeptides having X number of different predetermined single amino acid substitutions at a single predetermined site of the polypeptide; wherein each set of polypeptides differs in the single predetermined site; and the number of different member point mutation polypeptides generated is equivalent to [n-1] x X;
    b. screening each set of point mutation polypeptides for at least one predetermined property, characteristic or activity;
    c. identifying any change in said property, characteristic or activity relative to the template polypeptide for each member;
    d. categorizing each amino acid point mutation as one of deactivating, non-deactivating or activating;
    e. creating a functional map of such changes categorizing each site as one of non-mutable; partially mutable; or fully mutable;
    f. utilizing the map to select non-deactivating amino acid point mutations within partially mutable sites that are adjacent to non-mutable sites; and

g. recombining permutations of two to twenty amino acid point mutations selected in step (f) simultaneously to generate a library of multi-site mutant polypeptides.

30. The method of claim 29 wherein the recombining step utilizes combinatorial protein synthesis.

31. The method of claim 29 wherein contact amino acid residues in the template polypeptide are categorized as non-mutable in the functional map.

32. The method of claim 29 wherein the template polypeptide is derived from a portion of an immunoglobulin molecule.

33. The method of claim 32 wherein the portion of the immunoglobulin molecule is selected from a heavy chain, light chain, variable domain, constant domain, hypervariable region, complementarity determining region 1 (CDR1), complementarity determining region 2 (CDR2), and complementarity determining region 3 (CDR3).

34. A method of identifying a multi-site up-mutant polypeptide, the method comprising:

a. generating a library of multi-site mutant polypeptides;

b. screening the library of multi-site mutant polypeptides for at least one pre-determined property, characteristic or activity relative to the template polypeptide; and

c. identifying the multi-site up-mutant polypeptides as those with one or more improved properties, characteristics, or activities relative to the template polypeptide.

35. The method of claim 34 wherein the generating step comprises a method of producing a library of multi-site mutant polypeptides formed from a template polypeptide having n amino acid residues, the method of producing comprising:

a. generating n-1 separate sets of point mutation polypeptides, each set comprising member point mutation polypeptides having X number of different predetermined single amino acid substitutions at a single predetermined site of the polypeptide; wherein each set of polypeptides differs

in the single predetermined site; and the number of different member point mutation polypeptides generated is equivalent to [n-1] x X;

b. screening each set of point mutation polypeptides for at least one predetermined property, characteristic or activity;
c. identifying any change in said property, characteristic or activity relative to the template polypeptide for each member;
d. categorizing each amino acid point mutation as one of deactivating, non-deactivating or activating;
e. creating a functional map of such changes categorizing each site as one of non-mutable; partially mutable; or fully mutable;
f. utilizing the map to select non-deactivating amino acid point mutations within partially mutable sites that are adjacent to non-mutable sites; and
g. recombining permutations of two to twenty amino acid point mutations selected in step (f) simultaneously to generate a library of multi-site mutant polypeptides.

36. A method of selecting two or more sites in a template polypeptide for multi-site mutation, the method comprising:

a. obtaining a functional map of mutant polypeptides formed from a template polypeptide;
b. utilizing the map to select partially mutable sites that are adjacent to non-mutable sites for multi-site mutation.

37. The method of claim 36 wherein the obtaining step comprises a method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method of mapping comprising:

a. generating n-1 separate sets of mutant polypeptides, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;

b. assaying each set for at least one predetermined property, characteristic or activity;
c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a functional map of such changes identifying positions and mutations which do not affect the activity of the mutant polypeptide compared to the template polypeptide.

38. A method of providing a deimmunized variant polypeptide by masking immunogenicity with glycosylation while maintaining function as compared to the template polypeptide, the method comprising:
a. preparing a library of novel glycosylated variant polypeptides;
b. assaying the library for at least one predetermined property, characteristic or activity relative to the template polypeptide;
c. testing the immunogenicity of the variant polypeptides *in vitro* by T cell proliferation assay, compared to the immunogenicity of the template polypeptide in the same assay; and
d. selecting a variant polypeptide with activity commensurate with the template polypeptide and with reduced immunogenicity relative to the template polypeptide.

39. The method of claim 38 wherein the preparing step comprises a method of generating a novel glycosylated variant polypeptide library compared to a template polypeptide, the method generating comprising;
a. utilizing a functional map to identify fully mutable sites as target positions;
b. generating a codon variant library by insertion of a codon which codes for either Asn, Ser or Thr at one or more of the target positions;
c. utilizing the codon variant library to prepare the novel glycosylated variant polypeptide library in any expression system which has the ability to glycosylate.

40. A method to improve the expression of an immunoglobulin molecule encoded by a variant nucleic acid in a production host cell line compared to the expression of the

template immunoglobulin molecule encoded by the wild-type nucleic acid, the method comprising:

a. generating an Fc codon variant library by use of sets of forward and reverse degenerate primers comprising codons which allow generation of all silent mutations at each target position;
b. annealing corresponding forward and reverse primers to a template;
c. extending to full length products;
d. transforming full length extension products into *E. coli*;
e. sequencing selected *E. coli* colonies to confirm mutants;
f. utilizing confirmed mutants to prepare miniprep plasmid DNA;
g. transfecting miniprep plasmid DNA into mammalian cells; and
h. screening for improved expression.

41. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:

a. generating n-1 (n-2 in the case where the initial residue is methionine) separate mutant polypeptides, wherein each polypeptide differs from the template polypeptide in that one amino acid is deleted at a single predetermined position;
b. assaying the population for at least one predetermined property, characteristic or activity;
c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
d. creating a functional map.

42. The method of claim 41 wherein the functional map is used to identify those deletions which result in one of the group consisting of (a) deletions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) deletions which result in a down-mutant compared to the template polypeptide; and (c) deletions which result in an up-mutant compared to the template polypeptide.

43. The method of claim 42 wherein the functional map is used to identify deletions which do not affect the activity of the mutant polypeptide compared to the template polypeptide.
44. The method of claim 42 wherein the functional map is used to identify deletions which result in a down-mutant compared to the template polypeptide.
45. The method of claim 42 wherein the functional map is used to identify deletions which result in an up-mutant compared to the template polypeptide.

46. A method of mapping mutant polypeptides formed from a template polypeptide having n amino acid residues, the method comprising:
    a. generating n+[20 x (n-1)] separate mutant polypeptides, wherein each polypeptide differs from the template polypeptide in that it has inserted after a specific position in the template one each of the 20 naturally occurring amino acids;
    b. assaying the population for at least one predetermined property, characteristic or activity;
    c. for each member identifying any change in said property, characteristic or activity relative to the template polypeptide; and
    d. creating a functional map.

47. The method of claim 41 wherein the functional map is used to identify those insertions which result in one of the group consisting of (a) insertions which do not affect the activity of the mutant polypeptide compared to the template polypeptide; (b) insertions which result in a down-mutant compared to the template polypeptide; and (c) insertions which result in an up-mutant compared to the template polypeptide.
48. The method of claim 42 wherein the functional map is used to identify insertions which do not affect the activity of the mutant polypeptide compared to the template polypeptide.
49. The method of claim 42 wherein the functional map is used to identify insertions which result in a down-mutant compared to the template polypeptide.
50. The method of claim 42 wherein the functional map is used to identify insertions which result in an up-mutant compared to the template polypeptide.
51. A method of providing an optimized protein, the method comprising:

a. selecting a template polypeptide having n amino acid residues;
b. generating n-1 separate sets of mutant polypeptides from the template polypeptide, each set comprising member polypeptides having X number of different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the number of different member polypeptides generated is equivalent to [n-1] x X;
c. assaying each member polypeptide for at least one predetermined property, characteristic or activity;
d. identifying any change in said property, characteristic or activity of the member polypeptide relative to the template polypeptide;
e. creating a functional map wherein the functional map is used to identify positions and mutations which result in an up-mutant compared to the template polypeptide;
f. combining two or more of each of the relevant positions and mutations identified from member polypeptides which result in a up-mutant compared to the template polypeptide to create a set of combinatorial polypeptides;
g. assaying each combinatorial polypeptide for at least one predetermined property, characteristic or activity; and
h. selecting a combinatorial polypeptide in which the predetermined property, characteristic or activity is optimized compared to up-mutant member polypeptides.

52. The method of claims 1, 7 or 37 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position in each member polypeptide in each set of mutant polypeptides.

53. The method of claims 15, 21 or 27 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of the intended predetermined amino acid residue at the single predetermined position of the CDR in each member antibody in each set of mutant antibodies.

54. The method of claim 29 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the presence of each of the intended predetermined single amino acid substitutions at the predetermined site in each member point mutation polypeptide in each set of point mutation polypeptides.

55. The method of claim 34 wherein the generating step (a) further comprises confirming the sequence of each multi-site mutant polypeptide in the library by sequencing, or some other method.

56. The method of claim 38 wherein the generating step (a) further comprises confirming the sequence of each glycosylated variant polypeptide in the library by sequencing, or some other method.

57. The method of claim 41 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid deletion at the single predetermined position.

58. The method of claim 46 wherein the generating step (a) further comprises confirming by sequencing, or some other method, the intended amino acid insertion at the specific position in the template.

59. A method of evolving a polypeptide encoding a functional protein, the method comprising:

a. generating and cloning at least 20 or more separate sets of mutant polypeptides, each set comprising member polypeptides having from 5 to 19 different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position;

b. confirming that each different mutant polypeptide was generated and cloned; and

c. assaying each set for at least one predetermined property, characteristic or activity.

60. The method of claim 59, wherein in step (a), each set of mutant polypeptides comprises member polypeptides having from 10 to 19, from 15 to 19, or 19 different predetermined amino acid residues at a single predetermined position of the polypeptide.

61. The method of claim 59, further comprising:

d. performing bioinformatic analysis to identify positions of potential interest for further modification; and

(e) mutating positions identified in (d).

62. The method of claim 61 wherein bioinformatic analysis is generation of a functional map.

63. The method of claim 59 wherein the at least one predetermined property, characteristic or activity is modified expression

64. The method of claim 63 wherein the modified expression is improved expression.

65. A method of evolving a polypeptide encoding a functional protein and having n amino acid residues, the method comprising:

a. generating and cloning n-1 sets of mutant polypeptides, each set comprising member polypeptides having 19 different predetermined amino acid residues at a single predetermined position of the polypeptide; wherein each set of polypeptides differs in the single predetermined position; and the total number of different member polypeptides generated is equivalent to [n-1] x 19;

b. confirming that each different mutant polypeptide was generated and cloned; and

c. assaying each set for at least one predetermined property, characteristic or activity;

66. The method of claim 65, the method further comprising:

(d) performing bioinformatic analysis to identify positions of potential interest for further modification; and

(e) mutating positions identified in (d).

67. The method of claim 66 wherein the bioinformatic analysis is generation of a functional map.

68. The method of claim 65 wherein the at least one predetermined property, characteristic or activity is modified expression.

69. The method of claim 68 wherein the modified expression is improved expression.

70. A method of evolving a polynucleotide encoding a functional protein, the method comprising:

a. incorporating N,N,N at a predetermined codon position in the polynucleotide in order to generate one or more predetermined codon mutants within a set of predetermined mutant polynucleotides and cloning each predetermined mutant polynucleotide;

b. confirming that each predetermined mutant polynucleotide in the set was generated and cloned;

c. producing a set of predetermined polypeptides from set of predetermined polynucleotides; and

d. assaying each predetermined polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

71. The method of claim 70 wherein the at least one predetermined property, characteristic or activity is modified expression.

72. The method of claim 71 wherein the modified expression is improved expression.

73. The method of claim 70 wherein the confirming step comprises sequencing of each mutant polynucleotide.

74. A method of evolving a polypeptide from a functional protein, the method comprising:

a. incorporating N,N,N at every codon position in a polynucleotide encoding the functional protein in order to generate a set of predetermined mutant polynucleotides containing every possible codon sequence and cloning each predetermined mutant polynucleotide;

b. confirming by sequencing, or some other method, that each predetermined mutant polynucleotide in the set was generated and cloned;
c. producing a set of predetermined polypeptides from the set of predetermined polynucleotides; and
d. assaying each mutant polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

75. A method of evolving a polypeptide from a functional protein, the method comprising:
a. incorporating N,N,N at a codon position in a template polynucleotide encoding the functional protein in order to generate one or more predetermined codons within the set of mutant polynucleotides and cloning and expressing each mutant polynucleotides to produce a set of predetermined polypeptides;
b. confirming by sequencing or some other method that greater than 75%, 50%, 25%, or 1% of the predetermined polypeptides in the set were generated, cloned and expressed; and
c. assaying each predetermined polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.

76. A method of evolving a polypeptide from a functional protein, the method comprising:
a. incorporating N,N,N at a codon position in a template polynucleotide encoding the functional protein in order to generate one or more predetermined codons within the set of mutant polynucleotides and cloning and expressing each mutant polynucleotide to produce a set of predetermined polypeptides;
b. confirming by sequencing, or some other method, that any number of the predetermined polypeptides in the set were generated, cloned and expressed; and
c. assaying each predetermined polypeptide for at least one predetermined property, characteristic or activity compared to the functional protein.