WHAT IS CLAIMED IS:

1. A method of preparing a conditionally active biologic protein, wherein the conditionally active biologic protein is a protein which exhibits a lower activity in an assay under a normal physiological condition compared to the activity of the conditionally active biologic protein in an assay under an aberrant condition, the method comprising a step of:

selecting the conditionally active biologic protein from a library of wild-type proteins wherein the conditionally active biologic protein exhibits a lower activity in the assay under the normal physiological condition compared to the activity of the conditionally active biologic protein in the assay under the aberrant condition.

2. The method of claim 1, wherein the selecting step comprises selecting the conditionally active biologic protein which exhibits both: (a) a decrease in the activity in the assay under the normal physiological condition compared to a reference protein, and (b) an increase in the activity in the assay under the aberrant condition compared to the reference protein, wherein the reference protein has substantially the same activity in both the assay under the normal physiological condition and the aberrant condition.

3. The method of any one of claims 1-2, wherein the wild-type proteins are wild-type antibodies.

4. The method of any one of claims 1-3, wherein the wild-type protein library is a collection of proteins encoded by cDNAs.

5. The method of any one of claims 1-4, wherein the wild-type protein library is a bacteriophage display library.

6. The method of any one of claims 1-5, wherein the wild-type protein library is a collection of recombinant proteins.

7. The method of any one of claims 1-6, wherein the selected conditionally active biologic protein is subjected to steps of:

i. evolving DNA which encodes the selected conditionally active biologic protein using one or more evolutionary techniques to create mutant DNAs;

ii. expressing the mutant DNAs to obtain mutant proteins; and

iii. selecting an improved conditionally active biologic protein from the mutant proteins which exhibits both (a) a decrease in activity in the assay under the normal physiological condition compared to the selected conditionally active biologic protein, and (b) an increase in activity in the assay under the aberrant condition compared to the selected conditionally active biologic protein.

8. The method of claim 7, wherein the improved conditionally active biologic protein has an increased ratio of the activity in the assay under the aberrant condition to the activity under the normal physiological condition, as compared to the selected conditionally active biologic protein employed in step (i).

9. The method of claim 7, wherein the improved conditionally active biologic protein has one or more of an improved binding affinity, expression and humanization, as compared to the selected conditionally active biologic protein employed in step (i).

10. A method of preparing a conditionally active biologic protein, wherein the conditionally active biologic protein is a protein which exhibits both: (a) a decrease in activity in an assay at a normal physiological condition compared to a wild-type biologic protein, and (b) an increase in activity in the assay under an aberrant condition compared to the wild-type biologic protein, the method comprising steps of:

i. selecting the wild-type biologic protein;

ii. evolving a DNA which encodes the wild-type biologic protein using one or more evolutionary techniques to create mutant DNAs;

iii. expressing the mutant DNAs to obtain mutant proteins at least one of which exhibits both (a) a decrease in activity in the assay at the normal physiological condition compared to the wild-type biologic protein, and (b) an increase in activity in the assay under the aberrant condition compared to the wild-type biologic protein; and

iv. selecting the conditionally active biologic protein from the mutant proteins that exhibits both (a) a decrease in activity in the assay at the normal physiological condition compared to the wild-type biologic protein, and (b) an increase in activity in the assay under the aberrant condition compared to the wild-type biologic protein,

wherein the assay under the normal physiological condition and the assay under the aberrant condition are performed in assay solutions containing at least one component selected from an inorganic compound, ion and an organic molecule.

11. The method of claim 10, wherein the at least one component has substantially same concentrations in the assay solutions for both the assay under the normal physiological condition and the assay under the aberrant condition.

12. The method of claim 10, wherein the at least one component is present in a bodily fluid of a mammal at a normal physiologic concentration.

13. The method of claim 12, wherein the at least one component has a concentration in the range of from about 5 % to about 500 % of the normal physiologic concentration, or from about 10 % to about 400 % of the normal physiologic concentration, or from about 30 % to about 300 % of the normal physiologic concentration, or from about 50 % to about 200 % of the normal physiologic concentration, or from about 75 % to about 150 % of the normal physiologic concentration.

14. The method of claim 10, wherein the inorganic compound is selected from boric acid, calcium chloride, calcium nitrate, di-ammonium phosphate, magnesium sulfate, mono-ammonium phosphate, mono-potassium phosphate, potassium chloride, potassium sulfate, copper sulfate, iron sulfate, manganese sulfate, zinc sulfate, magnesium sulfate, calcium nitrate, calcium chelate, copper chelate, iron chelate, iron chelate, manganese chelate, zinc chelate, ammonium molybdate, ammonium sulphate, calcium carbonate, magnesium phosphate, potassium bicarbonate, potassium nitrate, hydrochloric acid, carbon dioxide, sulfuric acid, phosphoric acid, carbonic acid, uric acid, hydrogen chloride, urea, phosphorus ion, sulfuric ion and chloride ion.

15. The method of claim 10, wherein the ion is selected from magnesium ion, sodium ion, potassium ion, ammonium ion, iron ion, zinc ion, and copper ion.

16. The method of any one of claims 14-15, where the component is selected from one or more of uric acid in concentration range of 2–7.0 mg/dL, calcium ion in a concentration range of 8.2–11.6 mg/dL, chloride ion in a concentration range of 355–381 mg/dL, iron ion in a concentration range of 0.028–0.210 mg/dL, potassium ion in a concentration range of 12.1–25.4 mg/dL, sodium ion in a concentration range of 300–330 mg/dL and carbonic acid in a concentration range of 15–30 mM.

17. The method of claim 10, wherein the organic molecule is an amino acid selected from Histidine, Alanine, Isoleucine, Arginine, Leucine, Asparagine, Lysine, Aspartic acid, Methionine, Cysteine,

Phenylalanine, Glutamic acid, Threonine, Glutamine, Tryptophan, Glycine, Valine, Pyrrolysine, Proline, Selenocysteine, Serine, and Tyrosine.

18. The method of claim 10, wherein the organic molecule is an organic acid selected from citric acid, α-ketoglutaric acid, succinic acid, malic acid, fumaric acid, acetoacetic acid, β-hydroxybutyric acid, lactic acid, pyruvic acid, α-ketonic acid, acetic acid and volatile fatty acids.

19. The method of claim 10, wherein the organic molecule is a sugar selected from glucose, pentose, hexose, xylose, ribose, mannose, galactose, lactose, GlcNAcβ1-3Gal, Galα1-4Gal, Manα1-2Man, GalNAcβ1-3Gal and O-, N-, C-, and S-glycosides.

20. The method of claim 10, wherein the ion is selected from magnesium ion, sulfate ion, bisulfate ion, carbonate ion, bicarbonate ion, nitrate ion, nitrite ion, phosphate ion, hydrogen phosphate ion, dihydrogen phosphate ion, persulfate ion, monopersulfate ion, borate ion, and ammonium ion.

21. The method of claim 20, wherein the ion has a concentration in the range of from about 5 % to about 500 % of the normal physiologic concentration, or from about 10 % to about 400 % of the normal physiologic concentration, or from about 30 % to about 300 % of the normal physiologic concentration, or from about 50 % to about 200 % of the normal physiologic concentration, or from about 75 % to about 150 % of the normal physiologic concentration.

22. The method of claim 20, wherein the aberrant condition is a pH that is different from the normal physiological pH.

23. The method of claim 22, wherein the ion has a pKa that is within about 1 pH unit from the pH of the aberrant condition, or about 0.8 pH unit from the pH of the aberrant condition, or about 0.6 pH unit from the pH of the aberrant condition, or about 0.5 pH unit from the pH of the aberrant condition, or about 0.4 pH unit from the pH of the aberrant condition, or about 0.3 pH unit from the pH of the aberrant condition, or about 0.2 pH unit from the pH of the aberrant condition.

24. A method of preparing a conditionally active biologic protein, wherein the conditionally active biologic protein is a protein which exhibits both: (a) a decrease in activity in an assay at a normal physiological condition compared to a wild-type biologic protein, and (b) an increase in activity in

the assay under an aberrant condition compared to the wild-type biologic protein, the method comprising steps of:

i. selecting a fragment of a wild-type protein, a mutant protein or a fragment of a mutant protein with a desired property;

ii. evolving a DNA which encodes the protein or protein fragment selected in step (i) using one or more evolutionary techniques to create mutant DNAs;

iii. expressing the mutant DNAs to obtain mutant proteins at least one of which exhibits both (a) a decrease in activity in the assay at the normal physiological condition compared to the reference protein, and (b) an increase in activity in the assay under the aberrant condition compared to the reference protein; and

iv. selecting a mutant protein exhibits both (a) a decrease in activity in the assay at the normal physiological condition compared to the reference protein, and (b) an increase in activity in the assay under the aberrant condition compared to the reference protein.

25. The method of claim 24, wherein the protein selected in step (i) is a fragment of a wild-type protein.

26. The method of any one of claims 24-25, wherein the protein selected in step (i) is generated using a mutagenesis technique.

27. The method of any one of claims 24-26, wherein the protein selected in step (i) is an antibody or an antibody fragment.

28. The method of any one of claims 24-27, wherein the desired property is selected from binding affinity, expression level and humanization.

29. The method of any one of claims 24-28, further comprising a step of evolving the conditionally active biologic protein selected in step (iv) to produce an improved conditionally active biologic protein with at least one improved property.

30. The method of claim 29, wherein the at least one improved property is an increased ratio of the activity in the assay under the aberrant condition to the activity under the normal physiological condition, as compared to the conditionally active biologic protein selected in step (iv).

31. The method of any one of claims 24-28, further comprising a step of conjugating the conditionally active protein selected in step (iv) to an agent selected from cytokines, interleukins, enzymes, hormones, growth factors, cytotoxic agents, chemotherapy drugs, radioactive particles and diagnostic agents.

32. The method of any one of claims 29-30, further comprising a step of conjugating the improved conditionally active protein to an agent selected from cytokines, interleukins, enzymes, hormones, growth factors, cytotoxic agents, chemotherapy drugs, radioactive particles and diagnostic agents.

33. The method of any one of claims 24-28 and 31, wherein the protein is an antibody and further comprising a step of engineering the conditionally active antibody selected in step (iv) to be a bi-specific antibody.

34. The method of any one of claims 29-30 and 32, wherein the protein is an antibody and further comprising a step of engineering the improved conditionally active antibody to be a bi-specific antibody.

35. The method of any one of claims 33-34, wherein the engineering step engineers the bi-specific antibody to bind to both an immune effector cell surface antigen and a different target antigen.

36. The method of claim 35, wherein the immune effector cell is selected from a natural killer cell, a macrophage, a lymphokine-activated killer cell and a T-cell.

37. The method of claim 35, wherein the immune effector cell is a T-cell and the immune effector cell surface antigen is CD3.

38. The method of any one of claims 35-37, wherein the target antigen is an antigen specific to a diseased cell or diseased tissue.

39. The method of claim 38, wherein the diseased tissue is tumor tissue.

40. The method of any one of claims 24-28, further comprising the step of engineering the conditionally active biologic protein selected in step (iv) to include at least one of a masking moiety and a cleavable moiety.

41. The method of claim 40, wherein one or both of the masking moiety and the cleavable moiety is adapted to be cleaved from the conditionally active biologic protein when proximate to a diseased cell or diseased tissue.