that destabilization would reduce the protein's activity greater than the effects predicted by standard rules such as Ql0, therefore the ability to evolve proteins that work efficiently at lower temperature, for example, while being inactivated under their normal operating condition, creates an unexpected new class of proteins we refer to as conditionally active biologic proteins.

[0005] Throughout this application, various publications are referenced by author and date. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the disclosure described and claimed herein.

SUMMARY OF THE DISCLOSURE

[0006] The disclosure provides a method of preparing a conditionally active biologic protein, the method comprising: selecting a wild-type biologic protein; evolving the DNA which encodes the wild-type biologic protein using one or more evolutionary techniques to create a mutant DNA; expressing the mutant DNA to obtain a mutant protein; subjecting the mutant protein and the wild-type protein to an assay under a normal physiological condition and the assay under an aberrant condition; and selecting the conditionally active biologic protein from those mutant proteins which exhibit both (a) a decrease in activity in the assay at the normal physiological condition compared to the wild-type protein, and (b) an increase in activity in the assay under the aberrant condition compared to the wild-type protein.

[0006a] In one aspect, the present disclosure refers to 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 within a normal range at a site of administration of the conditionally active biologic protein, or at a tissue or organ at a site of action of the conditionally active biologic protein in a subject, compared to the activity of the conditionally active biologic protein in an assay under an aberrant condition that deviates from the normal range at the tissue or organ at the site of action of the conditionally active biologic protein in the subject, 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,

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 from100 μM-100 mM hydrogen sulfide and 25-100 mM bicarbonate.

[0006b] In another aspect, there is provided a method of preparing a conditionally active biologic protein, wherein the conditionally active biologic protein is a protein which exhibits a decrease in activity in an assay at a normal physiological condition within a normal range at a site of administration of the conditionally active biologic protein, or at a tissue or organ at a site of action of the conditionally active biologic protein in a subject compared to the same activity of the conditionally active biologic protein in an assay under an aberrant condition that deviates from the normal range at the tissue or organ at the site of action of the conditionally active biologic protein in the subject, the method comprising steps of:

i. selecting a parent polypeptide;

ii. evolving a DNA which encodes the parent polypeptide 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 a decrease in the activity in the assay at the normal physiological condition compared to in the same activity in the assay under the aberrant condition; and

iv. selecting the conditionally active biologic protein from the mutant proteins that exhibits a decrease in the activity in the assay at the normal physiological condition compared to the same activity in the assay under the aberrant condition,

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 at substantially the same concentration, said at least one component being selected from 100 μM-100 mM hydrogen sulfide and 20-100 mM bicarbonate.

[0006c] Further disclosed is 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.

[0006d] In various examples, the normal physiological condition is selected from one or more of temperature, pH, osmotic pressure, osmolality, oxidative stress and electrolyte concentration. In a particular example, the normal physiological condition is temperature;

wherein the conditionally active biologic protein is virtually inactive at the normal physiological temperature, but is active at an aberrant temperature less than the normal physiological temperature. In other examples, the conditionally active biologic protein is reversibly or irreversibly inactivated at the wild type normal physiological conditions. In one specific example, the protein is reversibly inactivated at the wild type normal physiological conditions. Alternatively, conditionally active biologic proteins are selected from those proteins which exhibit changes in activity, reversibly or irreversibly, in two or more different physiological conditions.

[0007] In one example, the wild-type biologic protein is an enzyme, in certain examples, the wild-type biologic protein is selected from the group consisting of tissue plasminogen activator, streptokinase, urokinase, renin, and hyaluronidase.

[0008] In another example, the wild-type biologic protein is selected from calcitonin gene-related peptide (CGRP), substance P (SP), neuropeptide Y (NPY), vasoactive intestinal peptide (VTP), vasopressin, and angiostatin.

[0009] In another example, the biologic protein is an antibody.

[0010] In another example, the disclosure provides a method of preparing a conditionally active biological response modifier, the method comprising: selecting an inflammatory response mediator; identifying a wild-type antibody to the mediator; evolving the wild-type antibody; screening differentially for mutants that exhibit decreased binding to the mediator relative to the wild-type antibody at a first condition, and exhibit increased binding affinity to the mediator at a second condition to identify up-mutants; and recombining the heavy chains and the light chains of the up-mutants to create recombined up-mutants; and screening the recombined up-mutants for mutants that exhibit decreased binding to the mediator relative to the wild-type antibody at the first condition, and show increased binding affinity to the mediator at the second condition to identify the conditionally active biological response modifier. In one example, the inflammatory response mediator is selected from IL-6, IL-6 receptor, TNF-alpha, IL- 23 and IL- 12. In another example, the first and second conditions are selected from conditions of pH, osmotic pressure, osmolality, oxidative stress and electrolyte concentration.

[0011] In another example, the disclosure provides a pharmaceutical composition comprising a conditionally active biologic protein, and a pharmaceutically acceptable carrier.

BRIEF DESCRIPTION OF THE DRAWING

[0012] FIG. 1 is a plot representing the conditionally active antibodies selected in Example 9 and their selectivity in pH 6.0 over pH 7.4.

DEFINITIONS

[0013] In order to facilitate understanding of the examples provided herein, certain frequently occurring methods and/or terms will be defined herein.

[0014] As used herein in connection with a measured quantity, the term "about" refers to the normal variation in that measured quantity that would be expected by the skilled artisan making the measurement and exercising a level of care commensurate with the objective of the measurement and the precision of the measuring equipment used. Unless otherwise indicated, "about" refers to a variation of +/- 10% of the value provided.

[0015] The term “activity” as used herein refers to any function that a protein can perform, including catalyzing reactions and binding to a partner. For enzymes, the activity may be an enzymatic activity of the enzyme. For antibodies, the activity may be a binding activity (i.e.,

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 within a normal range at a site of administration of the conditionally active biologic protein, or at a tissue or organ at a site of action of the conditionally active biologic protein in a subject, compared to the activity of the conditionally active biologic protein in an assay under an aberrant condition that deviates from the normal range at the tissue or organ at the site of action of the conditionally active biologic protein in the subject, 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,

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 100 μM-100 mM hydrogen sulfide and 25-100 mM bicarbonate.

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 which is the same type of protein as the wild-type proteins in the library, 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 assay under 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 a 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 a decrease in activity in an assay at a normal physiological condition within a normal range at a site of administration of the conditionally active biologic protein, or at a tissue or organ at a site of action of the conditionally active biologic protein in a subject compared to the same activity of the conditionally active biologic protein in an assay under an aberrant condition that deviates from the normal range at the tissue or organ at the site of action of the conditionally active biologic protein in the subject, the method comprising steps of:

i. selecting a parent polypeptide;

ii. evolving a DNA which encodes the parent polypeptide 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 a decrease in the activity in the assay at the normal physiological condition compared to in the same activity in the assay under the aberrant condition; and

iv. selecting the conditionally active biologic protein from the mutant proteins that exhibits a decrease in activity in the assay at the normal physiological condition compared to the same activity in the assay under the aberrant condition,

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 at substantially the same concentration, said at least one component being selected from 100 μM-100 mM hydrogen sulfide and 20-100 mM bicarbonate.

11. The method of claim 10, wherein the aberrant condition is a pH that is different from a normal physiological pH.