that destabilization would reduce the protein's activity greater than the effects predicted by standard rules such as Q10, 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, there is provided 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.

[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 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.

[0006c] In a further aspect, there is provided 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 embodiments, the normal physiological condition is selected from one or more of temperature, pH, osmotic pressure, osmolality, oxidative stress and electrolyte concentration. In a particular embodiment, 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 embodiments, the conditionally active biologic protein is reversibly or irreversibly inactivated at the wild type normal physiological conditions. In one specific embodiment, 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 embodiment, the wild-type biologic protein is an enzyme, in certain embodiments, the wild-type biologic protein is selected from the group consisting of tissue plasminogen activator, streptokinase, urokinase, renin, and hyaluronidase.

[0008] In another embodiment, 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 embodiment, the biologic protein is an antibody.

[0010] In another embodiment, 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 embodiment, the inflammatory response mediator is selected from IL-6, IL-6 receptor, TNF-alpha, IL- 23 and IL- 12. In another embodiment, the first and second conditions are selected from conditions of pH, osmotic pressure, osmolality, oxidative stress and electrolyte concentration.

[0011] In another embodiment, 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.,