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 selecting and optionally further evolving a wild-type biologic protein with conditional activity, wherein the wild-type biologic protein with conditional activity 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 wild-type biologic protein, or at a tissue or organ at a site of action of the wild-type biologic protein in a subject, compared to the same activity of the wild-type 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 wild-type biologic protein in the subject, the method comprising:

selecting the wild-type biologic protein with conditional activity from a library of wild-type proteins wherein the selected wild-type biologic protein exhibits the lower activity in the assay under the normal physiological condition compared to the

same activity of the wild-type biologic protein with conditional 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 from100 μM-100 mM hydrogen sulfide and 25-100 mM bicarbonate; and

wherein the normal physiological condition and the aberrant condition for the assays are the same condition, said condition being selected from temperature, pH, osmotic pressure, osmolality, oxidative stress, electrolyte concentration, and a combination thereof.

**[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 the same activity of the mutant proteins in the assay under the aberrant condition; and

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

said condition being selected from temperature, pH, osmotic pressure, osmolality, oxidative stress, electrolyte concentration, and a combination thereof.

**[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;

## CLAIMS

1. A method of selecting and optionally further evolving a wild-type biologic protein with conditional activity, wherein the wild-type biologic protein with conditional activity 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 wild-type biologic protein, or at a tissue or organ at a site of action of the wild-type biologic protein in a subject, compared to the same activity of the wild-type 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 wild-type biologic protein in the subject, the method comprising:

selecting the wild-type biologic protein with conditional activity from a library of wild-type proteins wherein the selected wild-type biologic protein exhibits the lower activity in the assay under the normal physiological condition compared to the same activity of the wild-type biologic protein with conditional 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 25-100 mM bicarbonate; and

wherein the normal physiological condition and the aberrant condition for the assays are the same condition, said condition being selected from temperature, pH, osmotic pressure, osmolality, oxidative stress, electrolyte concentration, and a combination thereof.

2. The method of claim 1, wherein the selecting step comprises selecting the wild-type biologic protein with conditional activity 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 wild-type biologic protein with conditional activity is subjected to steps of:

i. evolving a DNA which encodes the selected wild-type biologic protein with conditional activity using one or more evolutionary techniques to create mutant DNAs;

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

iii. selecting a 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 wild-type biologic protein with conditional activity employed in step (i), and (b) an increase in activity in the assay under the aberrant condition compared to the wild-type biologic protein with conditional activity employed in step (i).

8. The method of claim 7, wherein the 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 wild-type biologic protein with conditional activity employed in step (i).

9. The method of claim 7, wherein the conditionally active biologic protein has one or more of an improved binding affinity, expression and humanization, as compared to the wild-type biologic protein with conditional activity 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 the same activity of the mutant proteins in the assay under the aberrant condition; and

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

said condition being selected from temperature, pH, osmotic pressure, osmolality, oxidative stress, electrolyte concentration, and a combination thereof.

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