polypeptides; and (c) collecting the expressed mutant polypeptides comprising an FcRn-binding domain from the host cell culture.

[10] There remains a need for conditionally active polypeptides having a higher activity and/or selectivity in particular environments and/or under particular conditions, and preferably also having an increased half-life in the plasma. The conditionally active polypeptides that also have an increased half-life will not only preferentially act at locations where aberrant conditions are present, such as a tumor microenvironment, but also provide extended action or an increase in overall activity because of their increased half-life. In addition, these conditionally active polypeptides will potentially cause less harmful side effects to normal tissues/organs where normal physiological conditions are present. The potential to reduce side effects allows more prolonged treatments with, or higher doses of, the conditionally active polypeptides leading to a higher efficacy.

[11] WO 2010/104821 and WO 2011/009058 disclose methods for evolving and screening for conditionally active proteins.

SUMMARY OF THE DISCLOSURE

[12] In one example, the disclosure relates to a method of producing a conditionally active polypeptide from a parent polypeptide, comprising steps of (i) evolving the parent polypeptide by introducing one or more mutations into the parent polypeptide to produce one or more mutant polypeptides that have a pI that is the same as or lower than a pI of the parent polypeptide, (ii) subjecting the one or more mutant polypeptides to a first assay under a normal physiological condition to measure the activity of the one or more mutant polypeptides under the normal physiological condition and a second assay under an aberrant condition to measure the activity of the one or more mutant polypeptides under the aberrant condition, wherein the normal physiological condition and the aberrant condition are the same condition but having different values, and (iii) selecting the conditionally active polypeptide from the one or more mutant polypeptides which exhibits an increased activity in the second assay compared to the same activity in the first assay.

[12a] In one aspect, the present disclosure refers to a method of producing a conditionally active polypeptide from a parent polypeptide, comprising steps of (i) evolving the parent polypeptide by introducing one or more mutations into the parent polypeptide to produce one or more mutant polypeptides that have a pI that is ~~the same as or~~ lower than a pI of the parent polypeptide, (ii) subjecting the one or more mutant polypeptides to a first assay under a normal physiological ~~condition selected from~~ pH~~, temperature, osmotic pressure, osmolality, oxidative stress, and electrolyte concentration~~ to measure the activity of the one or more mutant polypeptides under the normal physiological ~~condition~~ pH and a second assay under an aberrant ~~condition~~ pH to measure the activity of the one or more mutant polypeptides under the aberrant ~~condition~~pH, wherein the normal physiological ~~condition~~ pH and the aberrant ~~condition~~ pH are ~~the same condition but having~~ different ~~values~~, and (iii) selecting the conditionally active polypeptide from the one or more mutant polypeptides which exhibits an increased activity in the second assay compared to the same activity in the first assay, and has a pI lower than the pI of the parent polypeptide by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units.

[13] In the previous example, the conditionally active polypeptide may have a pI that is lower than the pI of the parent polypeptide.

[14] In any one of the previous examples, the conditionally active polypeptide may have a pI below 7.4, or a pI below 7.3, or a pI below 7.2, or a pI below 7.1 or a pI below 7.0.

[15] In any one of the previous examples, the one or more mutations may comprise at least one amino acid substitution of a residue of an amino acid for a residue of an amino acid in the

parent polypeptide that has a higher pI than a pI of the amino acid that is substituted into the parent polypeptide.

[16] In any one of the previous examples, the one or more mutations may comprise 2, 3, 4, 5, 6, 7, 8, 9, or 10 of said substitutions.

[17] In any one of the previous examples, the one or more mutations may comprise at least one insertion of a residue of an amino acid that has a lower pI than the pI of the parent polypeptide.

[18] In any one of the previous examples, the one or more mutations may comprise 2, 3, 4, or 5 of said insertions.

[19] In any one of the previous examples, the one or more mutations may comprise at least one deletion of a residue of an amino acid that has a higher pI than the pI of the parent polypeptide.

[20] In any one of the previous examples, the one or more mutations may comprise 2, 3, 4, or 5 of said deletions.

[21] In any one of the previous examples, the one or more of the mutations may be located in a position exposed on the surface of the mutant polypeptide.

[22] In any one of the previous examples, the evolving step may comprise introduction of one or more additional mutations into the mutant polypeptide.

[23] Any one of the previous examples may further comprise a step before step (ii) for confirming that at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 98% of the mutant polypeptides have a pI that is the same as or lower than the pI of the parent polypeptide.

[24] The previous example may further comprise a step of discarding mutant polypeptides that have a pI greater than the pI of the parent polypeptide prior to step (ii).

[25] Any one of the previous examples may further comprise a step of measuring a pI of the conditionally active polypeptide.

[26] In any one of the previous examples, the pI of the conditionally active polypeptide may be lower than the pI of the parent polypeptide by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units.

[26a] In another aspect, the present disclosure refers to a conditionally active antibody or antibody fragment evolved ~~polypeptide derived~~ from a parent antibody or antibody fragment~~polypeptide~~ having a pI by introducing one or more mutations into the parent antibody or antibody fragment to produce one or more mutant antibodies or antibody fragments, said conditionally active antibody or antibody fragment ~~polypeptide~~ having:

a. a pI that is ~~the same as or~~ lower than the pI of the parent antibody or antibody fragment~~polypeptide, or lower than the pI of the parent polypeptide~~ by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units; and

b. a ratio of at least 1.3, or at least 1.5, or at least 1.7, or at least 2.0, or at least 3.0, or at least 4.0, or at least 6.0, or at least 8.0, or at least 10.0, or at least 20.0, or at least 40.0, or at least 60.0, or at least 100.0, of an activity in a second assay at an aberrant ~~condition~~ pH to the same activity in a first assay at a normal physiological ~~condition~~pH, and wherein the activity of the conditionally active antibody or antibody fragment~~polypeptide~~ is measured in the presence of at least one molecule or ion having a molecular weight of less than 900 a.m.u., or less than 500 a.m.u., less than 200 a.m.u., or less than 100 a.m.u.

[27] In any one of the previous examples, the parent polypeptide may be selected from antibodies, enzymes, hormones, growth factors, cytokines, regulatory proteins, functional peptides, biosimilars, immunomodulators, receptors, and ligands.

[51] In the previous example, the antibody may be an IgG antibody.

[52] In any one of the previous examples, the condition may be selected from pH, temperature, osmotic pressure, osmolality, oxidative stress, and electrolyte concentration.

[53] In any one of the previous examples, the normal physiological condition may be a pH in a range of from greater than 7.2 to less than 7.6, and the aberrant condition is a pH in a range of 5.5 to less than 7.2.

[54] In the previous example, the molecule or ion may have a pKa between the normal physiological pH and the aberrant pH.

[55] In the previous example, the pKa of the molecule or ion may be up to 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, or 1.0, 2.0 units away from the aberrant pH.

[56] In any one of the previous examples, the molecule or ion may be selected from histidine, histamine, hydrogenated adenosine diphosphate, hydrogenated adenosine triphosphate, citrate, bicarbonate, acetate, lactate, bisulfide, hydrogen sulfide, ammonium, dihydrogen phosphate and any combination thereof.

[57] In any one of the previous examples, the molecule or ion may be bicarbonate ion having a concentration in a range of from about 3 mM to about 200 mM, from about 5 mM to about 150 mM, from about 5 mM to about 100 mM, from about 10 mM to about 100 mM, from about 20 mM to about 100 mM, from about 25 mM to about 100 mM, from about 30 mM to about 100 mM, from about 35 mM to about 100 mM, from about 40 mM to about 100 mM, or from about 50 mM to about 100 mM.

[58] In any one of the previous examples, the molecule or ion may be bisulfide ion having a concentration in a range of from 1 mM to 100 mM, from 2nM to 500 nM, from 3 nM to 200 nM, from 5 nM to 100 nM.

[59] In any one of the previous example, the molecule or ion may be selected from sodium bicarbonate, potassium bicarbonate, sodium bisulfide, or potassium bisulfide.

[60] In yet another example, the disclosure relates to a pharmaceutical composition comprising an effective amount of the conditionally active polypeptide and a pharmaceutically acceptable carrier.

[60a] In another aspect, the present disclosure refers to a pharmaceutical composition comprising an effective amount of the conditionally active antibody or antibody fragment~~polypeptide~~ as disclosed herein and a pharmaceutically acceptable carrier.

[61] In yet another example, the disclosure relates to a use of the conditionally active polypeptide for treatment of solid tumors, inflamed joints, or brain diseases or disorders.

[62] In yet another example, the disclosure relates to a method of treatment of solid tumors, inflamed joints, or brain diseases or disorders comprising a step of administering the conditionally active polypeptide to a patient in need of said treatment.

[62a] In another aspect, the present disclosure refers to the conditionally active antibody or antibody fragment~~polypeptide~~ as disclosed herein or the pharmaceutical composition as disclosed herein for use as a medicament.

[62b] In another aspect, the present disclosure refers to use of the conditionally active antibody or antibody fragment ~~polypeptide~~ as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating solid tumors, inflamed joints, or brain diseases or disorders.

WHAT IS CLAIMED IS:

1. A method of producing a conditionally active polypeptide from a parent polypeptide, comprising steps of:

(i) evolving the parent polypeptide by introducing one or more mutations into the parent polypeptide to produce one or more mutant polypeptides that have a pI that is ~~the same as or~~ lower than a pI of the parent polypeptide;

(ii) subjecting the one or more mutant polypeptides to a first assay under a normal physiological ~~condition selected from~~ pH~~, temperature, osmotic pressure, osmolality, oxidative stress, and electrolyte concentration~~ to measure the activity of the one or more mutant polypeptides under the normal physiological ~~condition~~ pH and a second assay under an aberrant ~~condition~~ pH to measure the activity of the one or more mutant polypeptides under the aberrant ~~condition~~pH, wherein the normal physiological ~~condition~~ pH and the aberrant ~~condition~~ pH are ~~the same condition but having~~ different ~~values~~; and

(iii) selecting the conditionally active polypeptide from the one or more mutant polypeptides which exhibits an increased activity in the second assay compared to the same activity in the first assay, and has a pI lower than the pI of the parent polypeptide by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units.

~~2. The method of claim 1, wherein the conditionally active polypeptide has a pI that is lower than the pI of the parent polypeptide.~~

~~3~~2. The method of ~~any one of claims 1-2~~claim 1, wherein the conditionally active polypeptide has a pI below 7.4, or a pI below 7.3, or a pI below 7.2, or a pI below 7.1 or a pI below 7.0.

4~~3~~. The method of any one of claims 1-~~3~~2, wherein the one or more mutations comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions of a residue of an amino acid for a residue of an amino acid in the parent polypeptide that has a higher pI than a pI of the amino acid that is substituted into the parent polypeptide.

5~~4~~. The method of any one of claims 1-4~~3~~, wherein the one or more mutations comprise 1, 2, 3, 4, or 5 insertions of a residue of an amino acid that has a lower pI than the pI of the parent

polypeptide, or lower than the pI of the parent polypeptide by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units.

~~6~~5. The method of any one of claims 1-~~5~~4, wherein the one or more mutations comprise 1, 2, 3, 4, or 5 deletions of a residue of an amino acid that has a higher pI than the pI of the parent polypeptide.

~~7~~6. The method of any one of claims 1-~~6~~5, wherein one or more of the mutations is located in a position exposed on the surface of the mutant polypeptide.

~~8~~7. The method of any one of claims 1-~~7~~6, further comprising a step before step (ii) for confirming that at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 95%, or at least 98% of the mutant polypeptides have a pI that is the same as or lower than the pI of the parent polypeptide and optionally, further comprising a step of discarding mutant polypeptides that have a pI greater than the pI of the parent polypeptide prior to step (ii).

~~9~~8. The method of any one of claims 1-~~8~~7, wherein the parent polypeptide is selected from antibodies, enzymes, hormones, growth factors, cytokines, regulatory proteins, functional peptides, biosimilars, immunomodulators, receptors, and ligands or the parent polypeptide is an antibody selected from a full-length antibody, a single chain antibody, an antibody fragment, a heavy chain, a light chain, an Fab, and Fc domain, or the parent polypeptide is a therapeutic antibody or a candidate antibody being developed for therapeutic use, or the parent polypeptide is an IgG antibody, optionally having the one or more mutations in a variable region of the IgG antibody or having the one or more mutations in a constant region of the IgG antibody or having the one or more mutations in one or more complementarity determining regions of the IgG antibody.

~~10~~9. The method of any one of claims 1-~~9~~8, wherein ~~the condition is pH and~~ the pH of the first assay is greater than 7.2 to less than 7.6 and the pH of the second assay is less than 7.2 or greater than 7.6.

~~11~~10. The method of any one of claims 1-~~10~~9, wherein a ratio of the activity of the conditionally active polypeptide in the second assay to the same activity in the first assay is at least 1.3, or 1.5, or

at least 1.7, or at least 2.0, or at least 3.0, or at least 4.0, or at least 6.0, or at least 8.0, or at least 10.0, or at least 20.0, or at least 40.0, or at least 60.0, or at least 100.0.

~~12~~11. The method of any one of claims 1-~~11~~10, wherein both the first assay and second assay are performed in the presence of a molecule or ion having a molecular weight of less than 900 a.m.u., less than 500 a.m.u., less than 200 a.m.u., or less than 100 a.m.u., wherein the molecule or ion is selected from histidine, histamine, hydrogenated adenosine diphosphate, hydrogenated adenosine triphosphate, citrate, bicarbonate, acetate, lactate, bisulfide, hydrogen sulfide, ammonium, dihydrogen phosphate and any combination thereof or the molecule or ion is bicarbonate ion having a concentration in a range of from ~~about~~ 3 mM to ~~about~~ 200 mM, from ~~about~~ 5 mM to ~~about~~ 150 mM, from ~~about~~ 5 mM to ~~about~~ 100 mM, from ~~about~~ 10 mM to ~~about~~ 100 mM, from ~~about~~ 20 mM to ~~about~~ 100 mM, from ~~about~~ 25 mM to ~~about~~ 100 mM, from ~~about~~ 30 mM to ~~about~~ 100 mM, from ~~about~~ 35 mM to ~~about~~ 100 mM, from ~~about~~ 40 mM to ~~about~~ 100 mM, or from ~~about~~ 50 mM to ~~about~~ 100 mM, or the molecule or ion is bisulfide ion having a concentration in a range of from 1 mM to 100 mM, from 2nM to 500 nM, from 3 nM to 200 nM, from 5 nM to 100 nM, or the molecule or ion is selected from sodium bicarbonate, potassium bicarbonate, sodium bisulfide, or potassium bisulfide.

~~13~~12. A conditionally active antibody or antibody fragment evolved~~polypeptide derived~~ from a parent antibody or antibody fragment~~polypeptide~~ having a pI by introducing one or more mutations into the parent antibody or antibody fragment to produce one or more mutant antibodies or antibody fragments, said conditionally active antibody or antibody fragment ~~polypeptide~~ having:

a. a pI that is ~~the same as or~~ lower than the pI of the parent antibody or antibody fragment~~polypeptide, or lower than the pI of the parent polypeptide~~ by at least 0.1, or at least 0.2, or at least 0.3, or at least 0.4, or at least 0.5, or at least 0.6, or at least 0.8, or at least 1.0, or at least 1.2, or at least 1.4, or at least 1.5, or at least 1.7, or at least 2.0, or at least 2.5, or at least 3.0, or at least 3.5, or at least 4.0, or at least 5.0 units; and

b. a ratio of at least 1.3, or at least 1.5, or at least 1.7, or at least 2.0, or at least 3.0, or at least 4.0, or at least 6.0, or at least 8.0, or at least 10.0, or at least 20.0, or at least 40.0, or at least 60.0, or at least 100.0, of an activity in a second assay at an aberrant ~~condition~~ pH to the same activity in a first assay at a normal physiological ~~condition~~pH, and

wherein the activity of the conditionally active antibody or antibody fragment~~polypeptide~~ is measured in the presence of at least one molecule or ion having a molecular weight of less than 900 a.m.u., or less than 500 a.m.u., less than 200 a.m.u., or less than 100 a.m.u.

~~14~~13. The conditionally active antibody or antibody fragment~~polypeptide~~ of claim ~~13~~12, wherein the conditionally active antibody or antibody fragment~~polypeptide~~ is ~~selected from antibodies, enzymes, hormones, growth factors, cytokines, regulatory proteins, functional peptides, biosimilars, immunomodulators, receptors, and ligands or the conditionally active polypeptide is an antibody~~ selected from a full-length antibody, a single chain antibody, an antibody fragment, a heavy chain, a light chain, an Fab, and Fc domain ~~or~~ and ~~the conditionally active polypeptide is~~ an IgG antibody.

~~15~~14. The conditionally active antibody or antibody fragment~~polypeptide~~ of any one of claims ~~13~~12-~~14~~13, wherein ~~the condition is selected from pH, temperature, osmotic pressure, osmolality, oxidative stress, and electrolyte concentration or~~ the normal physiological ~~condition~~ pH is a pH in a range of from greater than 7.2 to less than 7.6, and the aberrant ~~condition~~ pH is a pH in a range of 5.5 to less than 7.2 and, optionally,
the molecule or ion has a pKa between the normal physiological pH and the aberrant pH and the pKa of the molecule or ion is up to 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8, or 1.0, 2.0 units away from the aberrant pH.

~~16~~15. The conditionally active antibody or antibody fragment~~polypeptide~~ of any one of claims ~~13~~12-~~15~~14, wherein the molecule or ion is selected from histidine, histamine, hydrogenated adenosine diphosphate, hydrogenated adenosine triphosphate, citrate, bicarbonate, acetate, lactate, bisulfide, hydrogen sulfide, ammonium, dihydrogen phosphate and any combination thereof or the molecule or ion is bicarbonate ion having a concentration in a range of from ~~about~~ 3 mM to ~~about~~ 200 mM, from ~~about~~ 5 mM to ~~about~~ 150 mM, from ~~about~~ 5 mM to ~~about~~ 100 mM, from ~~about~~ 10 mM to ~~about~~ 100 mM, from ~~about~~ 20 mM to ~~about~~ 100 mM, from ~~about~~ 25 mM to ~~about~~ 100 mM, from ~~about~~ 30 mM to ~~about~~ 100 mM, from ~~about~~ 35 mM to ~~about~~ 100 mM, from ~~about~~ 40 mM to ~~about~~ 100 mM, or from ~~about~~ 50 mM to ~~about~~ 100 mM or the molecule or ion is bisulfide ion having a concentration in a range of from 1 mM to 100 mM, from 2nM to 500 nM, from 3 nM to 200 nM, from 5 nM to 100 nM or the molecule or ion is selected from sodium bicarbonate, potassium bicarbonate, sodium bisulfide, or potassium bisulfide.

~~17~~16. A pharmaceutical composition comprising an effective amount of the conditionally active antibody or antibody fragment~~polypeptide~~ of any one of claims ~~13~~12-~~16~~15 and a pharmaceutically acceptable carrier.

~~18~~17. The conditionally active antibody or antibody fragment~~polypeptide~~ of any one of claims ~~13~~12-~~16~~ 15 or the pharmaceutical composition of claim ~~17~~ 16 for use as a medicament.

~~19~~18. Use of the conditionally active antibody or antibody fragment~~polypeptide~~ of any one of claims ~~13~~12-~~16~~ 15 or the pharmaceutical composition of claim ~~17~~ 16 in the manufacture of a medicament for treating solid tumors, inflamed joints, or brain diseases or disorders.