(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
30 April 2020 (30.04.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/084298 A1

(51) International Patent Classification:
C07K 16/28 (2006.01)

(21) International Application Number:
PCT/GB2019/053010

(22) International Filing Date:
22 October 2019 (22.10.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

1817188.4	22 October 2018 (22.10.2018)	GB
1904754.7	04 April 2019 (04.04.2019)	GB
1905032.7	09 April 2019 (09.04.2019)	GB

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *as to the identity of the inventor (Rule 4.17(i))*
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

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(54) Title: ANTIBODY LIBRARY AND METHOD

(57) Abstract: This disclosure relates to methods of generating antibody libraries, antibody libraries produced using such methods, and variant antibodies. Presently, methods of improving antibody binding (affinity maturation assays) require the screening of vast libraries of antibody variants (often $>10^{10}$) to identify a small fraction of variants with improved characteristics. The present invention involves taking the nucleotide sequence of the framework and complementarity determining region of a target antibody and identifying motifs which would be recognised by deamination somatic hypermutation enzymes. A small library of variants is then created which incorporate one or more of these mutations. It was found that a relatively high proportion of the variants have an increased affinity. The technique of the present invention was demonstrated on the trastuzumab and Cathepsin S antibodies, and the variants produced are also claimed.

Antibody Library and Method

Field of the Invention

The present invention relates to methods of generating antibody libraries, antibody libraries produced using such methods, and variant antibodies.

Background to the Invention

Antibody affinity is the measure of the strength of interaction between the antibody and protein that it specifically binds to, as a ratio of the association rate and dissociation rate. There are many reasons why optimisation of this ratio is desirable. Increased affinity may mean that an antibody therapeutic is more effective at a given dose, or it may mean that less of the drug is required per dose, and diagnostic tests could improve sensitivity. Reducing affinity may also be beneficial for some drugs that require tissue penetration and therefore a faster dissociation. It has also been shown that bispecific antibodies require intricate tuning of affinity of each binding site in order to maximise efficacy.

Existing affinity maturation platforms generally involve creating a large library of variants through random mutagenesis focussed within antibody complementarity determining regions (CDRs). This process has the disadvantage of screening a very large number of variants (often $>10^{10}$) to identify a small fraction of variants with improved characteristics.

Summary of the Invention

The present invention addresses many of the problems of the prior art. As described in the examples, the inventors have surprisingly shown that by limiting mutations to a nucleotide sequence encoding a given antibody sequence to sites which correspond to DNA motifs which are targeted by enzymes involved in somatic hypermutation, a library of variants of a given antibody sequence may be created, the library, when compared to those prepared by many existing technologies, being relatively small but comprising a relatively high proportion of variants having increased affinity, or aggregation, or melting point, or

expression level in CHO cells or combinations of the same. The inventors have exemplified the invention utilising two unrelated antibodies, an anti-CathepsinS antibody, Fsn0503h (Fusion Antibodies Ltd), and the anti-HER2 antibody trastuzumab, (Herceptin ®, Roche).

Accordingly a first aspect of the present invention provides a library of antibody molecules, wherein each antibody molecule is a variant of a reference antibody, wherein the amino acid sequence of each antibody molecule differs from the amino acid sequence of the reference antibody at one or more amino acid residues, wherein each of said amino acid residues are independently encoded from a DNA segment of the DNA sequence encoding the variant, wherein said DNA segment of the variant differs from that of the corresponding DNA sequence encoding the reference antibody by a point mutation in a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

A second aspect of the invention provides a method of generating/producing a library of variant antibody molecules, wherein said variant antibody molecules are variants of a reference antibody, said method comprising the steps:

a) providing a nucleotide sequence encoding the reference antibody,

b) in said nucleotide sequence, identifying one or more DNA motifs susceptible to deamination by a somatic hypermutation inducing enzyme;

c) for one or more of said DNA motifs, selecting at least one variant nucleotide residue to substitute for a residue of said DNA motif, wherein said substitution will result in a variant nucleotide sequence which encodes a variant antibody molecule having, relative to the reference antibody, a change in the amino acid sequence being encoded by said DNA motif; and

d) repeating steps (b) and (c);

such that a library containing a plurality of variants of said reference antibody is generated.

The reference antibody may be any antibody molecule of which variants are desired or required. In one embodiment said reference antibody is a humanised antibody molecule.

A third aspect of the invention provides a method of generating a variant antibody molecule, wherein said variant antibody molecule is a variant of a reference antibody, said method comprising the steps:

a) providing a nucleotide sequence encoding the reference antibody,

b) in said nucleotide sequence, identifying one or more DNA motifs susceptible to deamination by a somatic hypermutation inducing enzyme;

c) for one or more of said DNA motifs, selecting at least one variant nucleotide residue to substitute for a residue of said DNA motif, wherein said substitution will result in a variant nucleotide sequence which encoding a variant antibody molecule having, relative to the reference antibody, a change in the amino acid sequence being encoded by said DNA motif.

In one embodiment of the invention, said somatic hypermutation inducing enzyme is Activation-Induced Deaminase (AID).

In embodiments of the invention, said DNA motif is DGYW or WRCH, for example RGYW or WRCY, where D is adenine, guanine or thymine, R is adenine or guanine, G is guanine, C is cytosine, H is adenine or cytosine or thymine, W is adenine or thymine, and Y is cytosine or thymine.

Said DNA motif may be on either strand of the DNA. Where there are more than one of said DNA motifs, the motifs may overlap.

The inventors have shown that the antibody library of the invention or produced according to a method of the invention may be refined by further limiting the members of the library to those variants resulting from mutations at the DNA motif targeted by a somatic hypermutation inducing enzyme which do not result in either a STOP codon or indeed a other motifs in the variant antibody molecule which relative to the reference antibody would be considered to be potentially undesirable, for example in terms of stability or binding.

Accordingly, in certain embodiments of the invention, the DNA sequence of each variant does not comprise (or encode) a deamination site, isomerisation site, N-linked glycosylation site or oxidation site which originates from said point mutation in said DNA motif.

In some embodiments, one or more of said DNA motifs are in DNA sequences which encode CDRs of said antibody molecule. However, as described in the examples, the inventors have shown that using the method with some reference antibodies, the variants generated may not have any mutations in some CDRs compared to the corresponding CDRs of the reference antibody.

In one embodiment, the variants have no changes in one or more of its CDRs compared to the corresponding CDRs of the reference sequence.

In one such embodiment, the variants have no changes in Light chain CDR1 compared to the corresponding CDR of the reference sequence.

In another such embodiment, the variants have no changes in Light chain CDR2 compared to the corresponding CDR of the reference sequence.

In another such embodiment, the variants have no changes in Light chain CDR3 compared to the corresponding CDR of the reference sequence.

In another such embodiment, the variants have no changes in heavy chain CDR1 compared to the corresponding CDR of the reference sequence.

In another such embodiment, the variants have no changes in heavy chain CDR2 compared to the corresponding CDR of the reference sequence.

In another such embodiment, the variants have no changes in heavy chain CDR3 compared to the corresponding CDR of the parent sequence.

Moreover, as shown in the examples, for some variants of reference antibodies, many of the mutations characterising the variants may be in the framework regions of the variant antibody molecules.

Accordingly in particular embodiments of the library or method of the invention, one or more of said DNA motifs are in DNA sequences which encode framework regions of said

antibody molecule. In some such embodiments, all of said DNA motifs are in DNA sequences which encode framework regions of said antibody molecule.

In one embodiment of the invention, greater than 20%, for example greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80% or greater than 90% of the nucleotide residues of each variant antibody which differ from nucleotide residues in corresponding positions in the reference antibody are residues at a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

In some embodiments of the invention, the nucleotide sequence encoding each of said antibody molecules does not differ from that of the reference antibody at any residue other than residues of said DNA motif.

An antibody library of the invention and/or produced using the method of the invention may be further refined to enhance the proportion of variants of high affinity and/or stability.

An antibody library of the invention and/or produced using the method of the invention may be further refined to enhance the proportion of variants with differential aggregation, i.e. less able to aggregate to each other. An antibody library of the invention and/or produced using the method of the invention may be further refined to enhance the proportion of variants with particular melting point characteristics. An antibody library of the invention and/or produced using the method of the invention may be further refined to enhance the proportion of variants which show favourable or desired expression level in CHO cells. An antibody library of the invention and/or produced using the method of the invention may be further refined to enhance the proportion of variants with high affinity, stability, desired aggregation characteristics, desired melting point characteristics, desired expression level characteristics or combinations of the same.

Thus in the invention, the method may further comprise determining the affinity and/or stability of binding of said variant antibody molecule to the binding target of the reference antibody, melting point in relation to a reference antibody, aggregation in relation to a reference antibody, expression level in relation to a reference antibody or combinations of

the same. Accordingly in one embodiment of the method of the invention, said method further comprises screening said library of variants to determine binding to an epitope to which the reference antibody binds. Suitably, the method of the invention, may further comprises screening said library of variants to determine a melting point, expression level, aggregation level or stability of a variant with respect to the reference antibody. Those variants determined to bind to said epitope with an affinity and/or stability/ or have a melting characteristic or aggregation characteristic or expression characteristic less or greater than a predetermined value relative to the reference antibody may be used to generate an optimised library of variant antibody molecules. Said screening method may be via conventional in vitro techniques. Such techniques may include an affinity ELISA assay, a BIAcore assay, a kinetic method or an equilibrium/solution method. In an alternative, said screening may be via an *in silico* technique, using, for example computer implemented molecular docking software to model the binding of the variant to the epitope of the reference antibody.

As shown in the examples, using molecular docking software, variants can be ranked by predicted affinity and stability allowing the selection of a small library of variants for DNA synthesis and expression. The inventors have demonstrated that within such a small library a very high number of the variants have increased affinity compared to the number which would be expected to be identified in a library generated by existing technologies such as error-prone PCR and phage display.

Molecular docking software products are commercially available. Any suitable software or tool suitable for modelling antibody binding to an epitope may be used in the present invention. For example, suitable software includes Bioluminate software from Schrodinger but others are available.

Accordingly, in embodiments of the invention, the antibody library comprises greater than 1%, for example greater than 5%, 10%, 20%, 30% , 40% or 50% of variants having increased affinity to the epitope bound by the reference antibody compared to the affinity of the reference antibody to said epitope.

In one embodiment of the method of the invention, the method is a computer implemented method.

A fourth aspect of the invention provides a computer readable storage media comprising instructions to perform a method for generating a library of variant antibody molecules according to the second aspect of the invention.

In the methods of the invention, whether computer implemented or not, the method may further comprise the step of synthesising the variant antibody molecules.

In embodiments of the method of the invention, where the method comprised a computer implemented screening method by, for example, docking modelling software, the method of the invention may further comprise in vitro screening of said library of variants to determine binding to an epitope to which the reference antibody binds.

A fifth aspect of the present invention is a variant of a trastuzumab antibody, wherein said variant has (i) at least two amino acid changes in the light chain sequence when compared to the light chain amino acid sequence of a reference antibody, wherein said reference antibody is trastuzumab, (ii) at least two amino acid changes in the heavy chain sequence when compared to the heavy chain amino acid sequence of trastuzumab, or (iii) at least one amino acid change in the light chain sequence when compared to the light chain amino acid sequence of trastuzumab and at least one amino acid change in the heavy chain sequence when compared to the heavy chain amino acid sequence of trastuzumab; wherein each of said amino acid changes are at amino acid residues independently encoded from a DNA segment of the variant DNA sequence, wherein said DNA segment of the variant differs from that of the corresponding DNA sequence encoding the reference antibody by a point

mutation in a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

In one embodiment of the fifth aspect of the invention, said amino acid changes are selected from the group consisting of lc9N, lc9T, lc9I, lc9R, lc9K, lc25G, lc25V, lc25D, lc31N, lc31S, lc31I, lc32D, lc32G, lc32V, lc32T, lc32N, lc32S, lc32I, lc32P, lc32L, lc32F, lc33L, lc33I, lc34G, lc34V, lc34D, lc38E, lc38K, lc40A, lc40S, lc40T, lc43G, lc43V, lc43T, lc43N, lc43S, lc43I, lc43P, lc43L, lc43F, lc46V, lc46I, lc47V, lc51S, lc51P, lc51T, lc76R, lc76N, lc76T, lc76K, lc76I, lc79K, lc79E, lc80T, lc80S, lc80A, lc85S, lc85N, lc85I, lc89H, lc90E, lc90A, lc91N, lc91D, lc91Y, lc93S, lc93N, lc93I, lc94S, lc94N, lc94I, lc101D, lc102S, lc102N, hc2L, hc2I, hc3H, hc4M, hc4V, hc13K, hc13E, hc14A, hc14T, hc14S, hc16A, hc16V, hc16D, hc23E, hc23G, hc23V, hc23T, hc23K, hc23R, hc23I, hc23P, hc23L, hc23S, hc24D, hc24G, hc24V, hc24T, hc24N, hc24S, hc24I, hc24P, hc24L, hc24F, hc26A, hc26V, hc26D, hc28K, hc35N, hc35D, hc35Y, hc48L, hc48I, hc49G, hc49S, hc56A, hc56V, hc56D, hc58S, hc58N, hc58I, hc61G, hc61V, hc61D, hc79G, hc79V, hc79D, hc82E, hc82K, hc85R, hc88D, hc88T, hc88S, hc88P, hc88G, hc92G, hc92V, hc92D, hc103A, hc103V, hc103D, hc106D, hc106G, hc106V, hc106T, hc106N, hc106S, hc106I, hc106P, hc106L, hc106F, hc114S, hc114N, and hc114I.

In the context of the invention, mutations are identified using the above nomenclature, where lc=light chain, hc=heavy chain, the number refers to the amino acid residue of the chain and the capital letter is the one letter amino acid code for the amino acid mutation at said site. Thus, for example, in the amino acid changes listed above for the fourth aspect, lc9N, refers to an asparagine at position 9 of the variant trastuzumab light chain.

In an embodiment of the fifth aspect of the invention, said amino acid changes are selected from the group consisting of lc9T, lc9I, lc9R, lc9K, lc43F, lc47V, lc51P, lc51T, lc101D, hc2L,

hc3H, hc14S, hc16V, hc24P, hc26A, hc26V, hc48I, hc58S, hc61V, hc79V, hc85R, hc88G, hc92V, hc92D, hc103A, hc103V, hc106V, hc114S, hc114N, and hc114I.

In one embodiment of the fifth aspect, the variant light chain and heavy chain sequences do not differ from that of the reference antibody at any residue other than by the amino acid changes recited above in relation to the fifth aspect.

A sixth aspect of the invention provides a variant of a Cathepsin S antibody, wherein said variant has (i) at least two amino acid changes in the light chain sequence when compared to the light chain amino acid sequence of a reference antibody, wherein said reference antibody is Fsn503h, (ii) at least two amino acid changes in the heavy chain sequence when compared to the heavy chain amino acid sequence of Fsn503h, or (iii) at least one amino acid change in the light chain sequence when compared to the light chain amino acid sequence of Fsn503h and at least one amino acid change in the heavy chain sequence when compared to the heavy chain amino acid sequence of Fsn503h; wherein each of said amino acid changes are at amino acid residues independently encoded from a DNA segment of the variant DNA sequence, wherein said DNA segment of the variant differs from that of the corresponding DNA sequence encoding the reference antibody by a point mutation in a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

In one embodiment of the sixth aspect of the invention, said amino acid changes are selected from the group consisting of lc12A, lc12S, lc12T, lc19V, lc28R, lc32T, lc32I, lc45A, lc45S, lc45T, lc50H, lc51V, lc51F, lc51I, lc56L, lc56F, lc56I, lc58K, lc66S, lc69A, lc69V, lc81T, lc81I, lc81N, lc85P, lc85S, lc85T, lc90L, lc90F, lc96I, lc96S, lc96I, lc96N, lc108N, hc3H, hc4V, hc4M, hc10A, hc10V, hc14A, hc14S, hc24G, hc24V, hc30T, hc30I, hc31R, hc31T, hc37L, hc37F, hc40P, hc40S, hc52S, hc52I, hc53S, hc53I, hc84T, hc84I, hc92G, and hc92V.

In one embodiment of the sixth aspect of the invention, said amino acid changes are selected from the group consisting of group consisting of lc12A, lc12S, lc12T, lc19V, lc45S,

lc45T, lc50H, lc51V, lc56I, lc81I, lc96I, lc96S, lc96I, lc96N, lc108N, hc10A, hc10V, hc14S, hc30I, hc31R, hc37L, hc37F, hc40P, hc40S, hc52I, and hc92G.

In one embodiment of the sixth aspect of the invention, the variant light chain and heavy chain sequences do not differ from that of the reference antibody at any residue other than by the amino acid changes recited above in relation to the sixth aspect.

In one embodiment of the fifth aspect of the invention, said variant antibody molecule has the combination of amino acid mutations as shown for any of the antibodies listed in Tables 2 to 7. In another embodiment of the fourth aspect, said variant antibody molecule has the combination of amino acid mutations as shown for any of the antibodies listed in Table 8. In one such embodiment, said variant antibody has the combination of amino acid mutations as shown for any one of the antibodies listed in Table 8 and does not have any amino acid mutations relative to the Trastuzumab light chain and heavy chain sequences other than those shown for said antibody listed in Table 8.

In one embodiment of the fifth aspect of the invention, said variant antibody has the mutation lc43F.

In one embodiment of the fifth aspect of the invention, said variant antibody has the combination of amino acid mutations as shown for any of variant antibodies 19, 5 or 6 in Table 8. In one such embodiment, said variant does not have any amino acid mutations relative to the Trastuzumab light chain and heavy chain sequences other than those shown for said variant antibody in Table 8.

In one embodiment of the fifth aspect of the invention, the variant antibody is a variant of a trastuzumab antibody comprising relative to trastuzumab, the following amino acid changes: lc9K, lc43F, and hc106V. In one embodiment, the variant antibody is variant antibody 19 as listed in Table 8.

In another embodiment of the fifth aspect of the invention, the variant antibody is a variant of a trastuzumab antibody comprising relative to trastuzumab, the following amino acid

changes: lc9R, lc43F, and hc114S. In one embodiment, the variant antibody is variant antibody 5 as listed in Table 8.

In another embodiment of the fifth aspect of the invention, the variant antibody is a variant of a trastuzumab antibody comprising relative to trastuzumab, the following amino acid changes: lc9I, lc43F,lc101D, and hc79V. In one embodiment, the variant antibody is variant antibody 6 as listed in Table 8.

In one embodiment of the sixth aspect of the invention, said variant antibody molecule has the combination of amino acid mutations as shown for any of the antibodies listed in Table 1.

In certain embodiments of the fifth or sixth aspects of the invention, said variant light chain and heavy chain sequences comprise in total at least three, for example at least 4, at least 5 or at least 6 amino acid changes compared to the amino acid sequence of the reference antibody.

In certain embodiments of the fifth or sixth aspects of the invention, one or more of said amino acid changes are in framework regions of said variant antibody. In certain embodiments of the fifth or sixth aspects of the invention, all of said amino acid changes are in framework regions of said variant antibody.

In certain embodiments of the fifth or sixth aspects of the invention, one or more of said amino acid changes are in CDRs of said variant antibody.

In certain embodiments of the fifth or sixth aspects of the invention, the change of affinity of said variant antibody molecule relative to the reference antibody is greater than -2 and the change of stability of said variant antibody molecule relative to reference antibody is greater than -2. In certain embodiments of the fifth or sixth aspects of the invention, the change of affinity of said variant antibody molecule relative to the reference antibody is greater than -10, for example greater than – 15, for example, greater than -20, such as greater than -25.

In certain embodiments of the fifth or sixth aspects of the invention, the change of stability of said variant antibody molecule relative to the reference antibody is greater than -10, for example greater than – 30, for example, greater than -50, such as greater than -60. Suitably,

the aggregation characteristic, melting point characteristic or expression level may be at least 2 fold, three fold, 10 fold different to the reference antbody.

For the avoidance of doubt, the greater the negative affinity value, the greater the affinity. Thus an antibody with an affinity value of -10 is considered to have a greater affinity value of an antibody with an affinity value of -5. Likewise, the greater the negative stability value, the greater the stability. Thus an antibody with a stability value of -10 is considered to have a greater stability value of an antibody with a stability value of -5.

Affinity and stability may be assessed by any suitable method. In the examples, the inventors used the residue scanning affinity maturation tool as part of Schrodinger's Biologics tool Maestro. Values were relative to the parental antibody with the minimum value for improved affinity taken to be -2 kcal/mol for both affinity and stability.

Definitions

Unless otherwise defined, all technical and scientific terms used herein have the meaning commonly understood by a person who is skilled in the art in the field of the present invention.

Throughout the specification, unless the context demands otherwise, the terms "comprise" or "include", or variations such as "comprises" or "comprising", "includes" or "including" will be understood to imply the inclusion of a stated integer or group of integers, but not the exclusion of any other integer or group of integers.

As used herein, terms such as "a", "an" and "the" include singular and plural referents unless the context clearly demands otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well as two or more different active agents in combination, while references to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

The term"DNA segment" refers to a section of a DNA sequence. In the context of the present invention, the DNA segment may be a section of DNA residues, said section being part of a longer DNA sequence which encodes an antibody molecule. The DNA segment may consist of the DNA residues which form a DNA motif, said DNA motif being a sequence-specific binding site for a somatic hypermutation inducing enzyme

The term "consisting essentially of" as used herein means that the invention necessarily includes the listed items and is open to including unlisted items that do not materially affect the basic and novel properties of the invention.

As herein defined, an "epitope" refers to a plurality of amino acid residues which are capable of being recognised by, and bound to by, an antibody molecule. Epitopes are generally comprised of chemically active surface groups and have specific three-dimensional structural characteristics, as well as specific charge characteristics which contribute to the three-dimensional structure of the epitope.

Antibody molecules of or for use in the invention may bind to a non-contiguous epitope. A "non-contiguous epitope" is an epitope that is comprised of a series of amino acid residues that are non-linear in alignment, such that the residues are spaced or grouped in a non-continuous manner along the length of a polypeptide sequence.

The terms "peptide", "polypeptide" and "protein" are used herein interchangeably to describe a series of at least two amino acids covalently linked by peptide bonds or modified peptide bonds such as isosteres. No limitation is placed on the maximum number of amino acids which may comprise a peptide or protein. Furthermore, the term polypeptide extends to fragments, analogues and derivatives of a peptide, wherein said fragment, analogue or derivative retains the same biological functional activity as the peptide from which the fragment, derivative or analogue is derived.

The nomenclature used to describe the polypeptide constituents herein follows the conventional practice wherein the amino group (N) is presented to the left and the carboxyl group to the right of each amino acid residue.

Antibodies and Antibody Molecules

An "antibody" is an immunoglobulin, whether natural or partly or wholly synthetically produced. The term also covers any polypeptide, protein or peptide having a binding domain that is, or is homologous to, an antibody binding domain. These can be derived from natural sources, or they may be partly or wholly synthetically produced. Examples of antibodies are the immunoglobulin isotypes and their isotypic subclasses and fragments which comprise an antigen binding domain such as Fab, scFv, Fv, dAb or Fd, and a bi-specific antibody.

As antibodies can be modified in a number of ways, the term "antibody" and "antibody molecule" should be construed as covering any binding member or substance having a binding domain with the required specificity. An antibody molecule of and for use in the invention may be a monoclonal antibody, or a fragment, derivative, functional equivalent or homologue thereof. The term includes any polypeptide comprising an immunoglobulin binding domain, whether natural or wholly or partially synthetic. Chimeric molecules comprising an immunoglobulin binding domain, or equivalent, fused to another polypeptide are therefore included.

The constant region of the antibody may be of any suitable immunoglobulin subtype. In certain embodiments the subtype of the antibody may be of the class IgA, IgM, IgD and IgE where a human immunoglobulin molecule is used. Such an antibody may further belong to any subclass, e.g. IgG1, IgG2a, IgG2b, IgG3 and IgG4.

Fragments of a whole antibody can perform the function of antigen binding. Examples of such binding fragments are a Fab fragment comprising or consisting of the VL, VH, CL and CH1 antibody domains; an Fv fragment consisting of the VL and VH domains of a single antibody; a F(ab')2 fragment; a bivalent fragment comprising two linked Fab fragments; a single chain Fv molecule (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site; and a bi-specific antibody, which may be multivalent or multispecific fragments constructed by gene fusion.

In certain embodiments humanized antibodies may be used. A humanised antibody may be a modified antibody having the hypervariable region of non-human antibody and the constant region of a human antibody. Thus the binding member may comprise a human constant region. The variable region other than the hypervariable region may also be derived from the variable region of a human antibody and/or may also be derived from a non-human antibody. In other cases, the entire variable region may be derived from a non-human antibody and the antibody is said to be chimerised.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

An antibody may be selected from the group consisting of a human antibody, a humanised antibody, a chimeric antibody, a monoclonal antibody, a polyclonal antibody, a synthetic antibody, a camelid antibody, a shark antibody and an *in-vitro* antibody. In certain embodiments an antigen binding fragment may be used. The antigen binding fragment may be derived from any of the aforementioned antibodies. In certain embodiments the antigen binding fragment is selected from the group consisting of a Fab fragment, a scFv fragment, a Fv fragment and a dAb fragment. In certain embodiments the antibody comprises two complete heavy chains and two complete light chains, or an antigen binding fragment thereof. In certain embodiments the antibody is of the isotype IgG, IgA, IgE or IgM, or an antigen binding fragment thereof. In certain embodiments where the antibody is of the isotype IgG, the antibody may be of the subtype IgG1, IgG2 or IgG3, or an antigen binding fragment thereof. In certain embodiments the antibody is of the subtype IgG4, or an antigen binding fragment thereof.

Production of Antibodies

Antibodies may be provided by a number of techniques. For example, a combinatorial screening technique such as a phage display-based biopanning assay may be used in order to identify amino acid sequences which have binding specificity to an antigen. Such phage display biopanning techniques involve the use of phage display libraries, which are utilised in methods which identify suitable epitope binding ligands in a procedure which mimics immune selection, through the display of antibody binding fragments on the surface of filamentous bacteria. Phage with specific binding activity are selected. The selected phage can thereafter be used in the production of chimeric, CDR-grafted, humanised or human antibodies. Antibodies can be tested for their ability to bind to an antigen using methods known in the art.

Antibodies or antigen fragments for use in the present invention may also be generated wholly or partly by chemical synthesis. The antibodies can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available and are well known by the person skilled in the art. Further, they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry.

Another convenient way of producing antibodies or antibody fragments suitable for use in the present invention is to express nucleic acid encoding them by use of nucleic acid in an expression system.

Antibodies may be generated by mutagenesis of antibody genes to produce artificial repertoires of antibodies. This technique allows the preparation of antibody libraries. Artificial repertoires of immunoglobulins, such as artificial scFv repertoires, may be employed as an immunoglobulin source in order to identify binding molecules which have specificity for a specific epitope.

Methods for generating repertoires are well characterised in the art.

Any suitable means of generating antibody libraries may be used in conjunction with the present invention. Selection protocols for isolating desired members of large libraries are

known in the art, as typified by phage display techniques. Such systems, in which diverse peptide sequences are displayed on the surface of filamentous bacteriophage, have proven useful for creating libraries of antibody fragments (and the nucleotide sequences that encode them) for the *in-vitro* selection and amplification of specific antibody fragments that bind a target antigen. The nucleotide sequences encoding the VH and VL regions are linked to gene fragments which encode leader signals that direct them to the periplasmic space of *E. coli* and as a result the resultant antibody fragments are displayed on the surface of the bacteriophage, typically as fusions to bacteriophage coat proteins (for example pIII or pVIII). Alternatively, antibody fragments are displayed externally on lambda phage capsids (phage bodies). An advantage of phage-based display systems is that, because they are biological systems, selected library members can be amplified simply by growing the phage containing the selected library member in bacterial cells. Furthermore, since the nucleotide sequence that encodes the polypeptide library member is contained on a phage or phagemid vector, sequencing, expression and subsequent genetic manipulation is relatively straight forward.

Methods for the construction of bacteriophage antibody display libraries and lambda phage expression libraries are well known in the art.

A method for producing polypeptides may comprise culturing host cells transformed with a recombinant expression vector encoding a polypeptide under conditions that promote expression of the polypeptide, then recovering the expressed polypeptides from the culture. The person skilled in the art will recognise that the procedure for purifying the expressed polypeptides will vary according to such factors as the type of host cells employed, and whether the polypeptide is intracellular, membrane-bound or a soluble form that is secreted from the host cell.

Any suitable expression system may be employed. Vectors may include a DNA encoding a polypeptide or fragment of the invention, operably linked to suitable transcriptional or translational regulatory nucleotide sequences, such as those derived from a mammalian, avian, microbial, viral, bacterial, or insect gene. Nucleotide sequences are operably linked when the regulatory sequence functionally relates to the DNA sequence. Thus, a promoter nucleotide sequence is operably linked to a DNA sequence if the promoter nucleotide sequence controls the transcription of the DNA sequence. An origin of replication that

confers the ability to replicate in the desired host cells, and a selection gene by which transformants are identified, are generally incorporated into the expression vector.

In addition, a sequence encoding an appropriate signal peptide (native or heterologous) can be incorporated into expression vectors. A DNA sequence for a signal peptide (secretory leader) may be fused in frame to the nucleic acid sequence of the invention so that the DNA is initially transcribed, and the mRNA translated, into a fusion protein comprising the signal peptide. A signal peptide that is functional in the intended host cells promotes extracellular secretion of the polypeptide. The signal peptide is cleaved from the polypeptide during translation, but allows secretion of polypeptide from the cell.

Suitable host cells for expression of polypeptides include higher eukaryotic cells and yeast. Prokaryotic systems are also suitable.

Mammalian cells, and in particular CHO cells are particularly preferred for use as host cells. CHO cells are used widely in the production of proteins due to their ease of culture and transfectability. ExpiCHO-S cells are a suspension cell line that can grow to very high densities and allows for high expression of proteins. The plasmid DNA of interest can be transfected into ExpiCHO cells by complexing with ExpiFectamine (a cationic lipid-based transfection reagent) to allow the DNA to condense. This condensed DNA then enters the ExpiCHO cells by endocytosis and is expressed in the nucleus. The expressed proteins are present in the cell culture supernatant and harvested after an appropriate number of days have passed.

Nucleic acid

Nucleic acid for use in accordance with the present invention may comprise DNA or RNA and may be wholly or partially synthetic. In a preferred aspect, nucleic acid for use in the invention codes for antibodies or antibody fragments of the invention as defined above. The skilled person will be able to determine substitutions, deletions and/or additions to such nucleic acids which will still provide an antibody molecule of or for use in the present invention.

Nucleic acid sequences encoding antibodies or antibody fragments for use with the present invention can be readily prepared by the skilled person. These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) preparing cDNA sequences. DNA encoding antibody fragments may be generated and used in any suitable way known to those of skill in the art, including by taking encoding DNA, identifying suitable restriction enzyme recognition sites either side of the portion to be expressed, and cutting out said portion from the DNA. The portion may then be operably linked to a suitable promoter in a standard commercially available expression system. Another recombinant approach is to amplify the relevant portion of the DNA with suitable PCR primers. Modifications to the sequences can be made, e.g. using site directed mutagenesis, to lead to the expression of modified peptide or to take account of codon preferences in the host cells used to express the nucleic acid.

The nucleic acid may be comprised as constructs in the form of a plasmid, vector, transcription or expression cassette which comprises at least one nucleic acid as described above. The construct may be comprised within a recombinant host cell which comprises one or more constructs as above. Expression may conveniently be achieved by culturing under appropriate conditions recombinant host cells containing the nucleic acid. Following production by expression the antibody or antibody fragments may be isolated and/or purified using any suitable technique, then used as appropriate.

Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, mammalian cells, yeast, insect and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney cells, NS0 mouse myeloma cells. A common, preferred bacterial host is *E. coli*. The expression of antibodies and antibody fragments in prokaryotic cells such as *E. coli* is well established in the art. Expression in eukaryotic cells in culture is also available to those skilled in the art as an option for production of a binding member. General techniques for the production of antibodies are well known to the person skilled in the field.

In certain embodiments of the invention, recombinant nucleic acids comprising an insert

coding for a heavy chain variable domain and/or for a light chain variable domain of antibodies are provided. By definition such nucleic acids comprise coding single stranded nucleic acids, double stranded nucleic acids consisting of said coding nucleic acids and of complementary nucleic acids thereto, or these complementary (single stranded) nucleic acids themselves.

Furthermore, nucleic acids encoding a heavy chain variable domain and/or a light chain variable domain of antibodies can be enzymatically or chemically synthesised nucleic acids having the authentic sequence coding for a naturally-occurring heavy chain variable domain and/or for the light chain variable domain, or a mutant thereof.

Brief Description of the Figures

Embodiments of the present invention will now be described, by way of example only, with reference to the accompanying figures in which:

Figure 1 shows amino acid sequences of the Fsn0503h antibody light chain and heavy chain showing potential mutations above each chain (SEQ ID Nos 1 & 2). Doubly circled amino acids were not permitted in the library due to their uncommon usage at that position. Doubly circled asterisks (*)represent STOP codons that also were not permitted. Singly circled amino acid regions are CDRs.

Figure 2 is a schematic model showing predicted protein-protein interaction between Fsn0503h and Cathepsin S.

Figure 3 shows Table 1 which lists affinity and stability predictions of amino acid mutations in a combinatorial fashion for 1 to 6 mutations. Groups of mutations highlighted in blue represent variants with both improved stability and affinity where the change is greater than -2.

Figure 4 illustrates an ELISA comparison of each of the expressed variants with the parental Fsn0503h antibody.

Figure 5 shows affinity (KD) measurements of each variant alongside the parental Fsn0503h antibody, as measured by BLI with an Octet instrument (Pall).

Figure 6 illustrates an affinity on-off rate map of the variants of Fsn0503h. The parental antibody is shown as the hollow dot. The 4.45 nM line represents equal affinity to the parental antibody, and any variants with an increased affinity (<4.45 nM) are within the area to the left of the 4.45 nM line.

Figure 7 shows amino acid sequences of the trastuzumab antibody light chain (SEQ ID NO: 3) showing in bold the amino acid residue at each position of the wild type antibody light chain and, where applicable, in the boxes to the right of each residue, potential mutations. Amino acids shown with two lines were not permitted in the library due to their uncommon usage at that position. Asterisks (*) in two lines represent STOP codons that also were not permitted. Complementarity determining regions CDRs and framework regions (FR) are indicated.

Figure 8 shows amino acid sequences of the trastuzumab antibody heavy chain (SEQ ID NO: 4) showing in bold the amino acid residue at each position of the wild type antibody antibody chain and, where applicable, in the boxes to the right of each residue, potential mutations. Amino acids shown with two lines were not permitted in the library due to their uncommon usage at that position. Asterisks (*) in two lines represent STOP codons that also were not permitted. Complementarity determining regions CDRs and framework regions (FR) are indicated.

Figure 9 schematically illustrates how somatic hypermutations may be used to cause alterations to the DNA sequence of antibodies which results in amino acid changes in the protein structure of the antibody leading to enhanced affinity.

Figure 10 illustrates the fully resolved crystal structure of trastuzumab in complex with human HER2 which was used to perform residue scanning. Domain IV of the extracellular domain of HER2 was found to bind to trastuzumab.

Figure 11a shows Table 2 which lists trastuzumab variants having one mutation relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain. Δ Affinity is predicted free energy change (ΔΔG) in kcal/mol relative to trastuzumab affinity. Δ Stability is predicted free energy change (ΔΔG) relative to trastuzumab stability. Negative Δ Affinity and negative Δ Stability values are considered to represent improved affinity and improved stability relative to trastuzumab.

Figure 11b shows Table 3 which lists trastuzumab variants having two mutations relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain.

Figure 11c shows Table 4 which lists trastuzumab variants having three mutations relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain.

Figure 11d shows Table 5 which lists trastuzumab variants having four mutations relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain.

Figure 11e shows Table 6 which lists trastuzumab variants having five mutations relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain.

Figure 11f shows Table 7 which lists trastuzumab variants having six mutations relative to the reference trastuzumab antibody. In column 2, L indicates light chain, while H indicates heavy chain.

Figure 12 shows Table 8 which lists the 100 trastuzumab variants from tables 1 to 7 having both improved affinity and improved stability, ranked according to improved affinity.

Figure 13 schematically illustrates the top 5 trastuzumab variants scored based on affinity using the predicted free energy change (ΔΔG) in kcal/mol. In the first and third ranked variants there are a total of five mutations, while rank 2 and 4 have four mutations and finally rank 5 contains three amino acid mutations. Domain IV of HER2 where trastuzumab binds is coloured cyan, while the scFv is coloured green and CDRs coloured red. Mutations are highlighted in yellow.

Figure 14 illustrates sensorgrams from 20 samples showing report points used for ranking. Assay performed at 25°C.

Figure 15 shows a graph illustrating off-rate ranking of Trastuzumab variants binding to HER2 and shows stability_early vs stability_late for identification of stable binder (best binders circled in blue). In total, 89 samples were analyzed and ranked with respect to binding stability, however for clarity only 29 are shown. Trastuzumab is commercially available antibody material. Wild-Type is antibody having sequence of trastuzumab and transiently expressed in parallel with all Trastuzumab variants/mutants. Mutant is a Trastuzumab variant. Mutants 5, 6 and 19 are trastuzumab variants listed as numbers 5, 6 and 19 in Table 8.Assay performed at 25°C.

Figure 16. shows a graph illustrating off-rate ranking of Trastuzumab variants binding to HER2 and shows Stability_early vs stability_late for identification of stable binders from a small sub-set of the top binders shown in Figure 15 (those binders closest the 100% left line). In total, 14 Trastuzumab variants were analyzed and ranked with respect to binding stability. Trastuzumab is commercially available antibody. Wild-Type is material transiently expressed in parallel with all Trastuzumab variants/mutants. Mutant; is a Trastuzumab variant. Mutants 5, 6 and 19 are trastuzumab variants listed as numbers 5, 6 and 19 in Table 8. Assay performed at 37°C.

Figure 17 shows reference corrected BLI binding curves (black), monitored on a surface of non-covalently immobilised Trastuzumab (a), wild-type (b), mutant 5 (c) and (d) mutant 19 antibodies, for various HER2 concentrations (serial diluted two-fold; highest concentration shown on curve) at 37°C in running buffer. The apparent dissociation rate constant (k_d) and association rate (k_a) constants were determined by globally fitting (red) a simple 1:1 interaction model, A+B = AB, to the sensorgrams using the software supplied with the instrument. Global fitting results are summarised in table 9.

Figure 18 illustrates schematically the correlation between affinity prediction and measurement for trastuzumab variants.

Figure 19 shows blank corrected BLI binding curves (black), monitored on a surface of non-covalently immobilised HER2 at a range of concentrations of wild-type (a), mutant 5 (b) and (c) mutant 19 antibodies, at 37°C in running buffer. The apparent dissociation rate constant (k_d) and association rate (k_a) constants were determined by globally fitting (red) a simple 1:1 interaction model, A+B = AB, to the sensorgrams using the software supplied with the instrument. Global fitting results are summarised in table 10.

Figure 20 shows double-reference corrected BLI binding curves (black), monitored on a surface of non-covalently immobilised HER2 at coated at a range of concentrations,

presented to a concentration of wild-type (a), mutant 5 (b) and (c) mutant 19 antibodies, at 37°C in running buffer. The apparent dissociation rate constant (k_d) and association rate (k_a) constants were determined by globally fitting (red) a simple 1:1 interaction model, A+B = AB, to the sensorgrams using the software supplied with the instrument. Global fitting results are summarised in tables 11, 12 and 13.

Figure 21 displays the Expression yield of Fsn0503 variants expressed as mg of purified IgG by Octet immunoassay obtained per ml of transfected CHO supernatant. Wild-Type shown in orange.

Figure 22 displays the melting point determination values (TM1 and TM2) of Fsn0503 variants in degrees centrigrade. Wild-Type is in position 1.

Figure 23 Shows the measurement of proportion of monodisperse (non-aggregated) molecule of Fsn0503 variants by size-exclusion chromatography. Wild-Type shown in orange.

Examples

Materials and Methods

Library Design

An antibody library was generated for each of (i) a humanised anti-Cathepsin S antibody, Fsn0503h (Fusion Antibodies, Belfast) (Kwok et al. Molecular Cancer 2011, 10:147) and (ii) trastuzumab (Roche) by mimicking the natural somatic hypermutation in humans, searching for specific DNA sequence motifs RGYW in the 3' to 5' strand and WRCY in the 5' to 3' strand, recognised by the AID enzyme, responsible for making mutations within antibodies in the human body.

The DNA sequence has a point mutation introduced at the guanine in position 2 of the DNA sequence motif RGYW or the cytosine in position 3 of the DNA sequence motif WRCY, where the nucleotide is mutated to be any other nucleotide. The AID enzyme in humans introduces natural mutations at these positions. This can cause a possible change to the amino acid at a single point. All these possible mutations were identified throughout the antibody DNA sequence in both directions in the 3' to 5' and 5' to 3' strands to generate all the amino acids that would be possible naturally throughout the antibody sequence to form the initial library.

Each library's size was then curated by removing any recognised sequence liabilities, such as deamidation sites, isomerization sites, n-linked glycosylation sites and oxidation sites from the newly generated amino acid sequences.

Molecular Docking

For each library, the antibody prediction tool in Maestro 11.7 (Schrödinger) was used to perform homology modelling, to generate an antibody model based on the amino acid sequences for the heavy and light variable chain regions. The antigen was imported from PDB.In thecase of Fsn0503h, the inventors imported the crystal structure of human Cathepsin S (Cat S) with a C25S mutation with a bound drug (PDB Code: 3MPE). In the case of the library for trastuzumab variants, the extracellular domain of human epidermal growth factor receptor 2 (HER2) (PDB Code: 1N8Z) was used.

The protein preparation wizard (Bioluminate, Schrodinger) was used to assign bond orders (using Chemical Component Dictionary (CCD) database), add hydrogens, create zero-order bonds to metals, create disulphide bonds, convert selenomethionines to methionines, fill in missing side chains and loops using Prime, and generating het states using Epik, for both the antibody and antigen models. The structures were further refined using ProtAssign (Bioluminate, Schrodinger) to define hydroxyl, asparagine, glutamine and histidine states. Waters with less than three bonds to non-waters were removed. Finally, the structures were minimized using the OPLS3e forcefield. (Bioluminate, Schrodinger).

Cathepsin S was docked to the surface of the antibody model using the protein-protein docking tool, Prime. Only the CDR regions of the antibody were considered for molecular docking. Non-CDR regions were masked. Using in vitro information about the epitope of Cat S, a suitable docked pose was selected based on rank and from inspection of shape complementarity and surface interactions (using the protein interaction analysis tool).

Likewise, for the trastuzumab variants, the extracellular domain of human epidermal growth factor receptor 2 (HER2) (PDB Code: 1N8Z) was docked to the surface of the antibody model using the protein-protein docking tool, Prime. Only the CDR regions of the antibody were considered for molecular docking. Non-CDR regions were masked. A suitable docked pose was selected based on rank and from our inspection of shape complementarity and surface interactions (using the protein interaction analysis tool).

Combinatorial Mutation Analysis

Residue scanning was performed on the docked pose of the antibody-antigen complex. Informed mutations were made to the antibody, avoiding highly conserved residues, to increase the affinity of the antibody for the antigen and to enhance stability.

Residue scanning was first done by generating models with a single amino acid variation from the original structure, and repeated (with the same mutations) for up to 6 simultaneous variations from the wild type Ab. The residue mutation tool calculated the stability and affinity of the mutants relative to the original wild type antibody-antigen complex.

The variants were then sorted by difference in affinity and difference in stability relative to the wild type. Results that scored below a threshold of -2 for difference in stability and difference in affinity were selected and ranked based on the combination of the two scores (prioritising difference in affinity).

The best variants of the Fsn503h antibody and of the trastuzumab antibody were synthesised and analysed in vitro.

Antibody Synthesis

Transient transfection:

Suspension adapted ExpiCHO cells were routinely cultivated at 4–6 x10^6 cells/ml at 130 rpm, 37°C, 8% CO_2, in ExpiCHO Expression Medium in 500ml vented Erlenmeyer flasks. For each of the Fsn0503h variants, 1µg/ml of DNA was diluted in 4% (v/v) OptiPRO SFM in a centrifuge tube. In a separate tube, 0.32% (v/v) ExpiFectamine was diluted in 3.7% OptiPRO SFM. The ExpiFectamine/OptiPRO mix was then added to the DNA/OptiPRO mix and incubated at room temperature for 3 minutes before adding to 25ml ExpiCHO cells at a final density of 6 x 10^6 cells/ml in 125ml vented Erlenmeyer flasks. Each transfected culture was cultivated at 37°C, 8% CO_2 and 130 rpm overnight. Twenty hours post transfection, cells were supplemented with 0.6% (v/v) ExpiCHO enhancer and 24% (v/v) ExpiCHO feed. Cultures were then transferred to incubators at 32°C, 5% CO_2 and 130 rpm. Cultures were harvested by centrifugation at 4000 rpm for 40 minutes at 18°C.

Purification:

Two-step Fsn0503h WT and variant antibody purifications were performed using a Tricorn 5/50 column (GE) packed with 1 ml of MabSelect^{TM} PrismA (GE) followed by a 10 ml (2x5ml) Hitrap Desalting (Desalt) column (GE). The MabSelect^{TM} PrismA affinity medium was chosen for its high mAb binding and specificity properties and its alkali tolerance for efficient Cleaning-in-place (CIP). All steps were performed at room temperature, using a flow-rate of 4 ml/min, unless otherwise stated. After loading (performed using the AKTA sample pump), the protein A column was washed (in reverse flow mode) with 10 column volumes (CV) of PBS followed by a one-step elution (in reverse flow mode) with 100 mM glycine, pH 3.0. The protein A eluate was collected in a 2 ml loop when the absorbance was above 120 mAU at 280 nm (AKTA equipped with a 10 mm flow cell) and injected immediately onto the pre-equilibrated Desalt column. The Desalt peak elution was collected in a 96-well-2 ml block at 2-8°C when the eluate had an absorbance above 100 mAU. To avoid any cross-contamination, the automated process also included a CIP of both the affinity and desalt columns. CIP was performed between each sample, for all contact pathways, using 0.2M NaOH (reverse flow mode was used for column cleaning).

The level of expression was determined as the total yield of material, following purification per ml of culture media.

Trastuzumab variants were synthesised using similar techniques.

Affinity Ranking

Enzyme-Linked Immunosorbent Assay:

MaxiSorp 96 well plates were coated with the sixty six 0503 variants at 1g/ml in PBS for 24hrs at 4°C. To obtain the EC50 results, the variants were serially diluted from 1000ng/ml to 1ng/ml with PBS and coated in duplicate. The standard curve was prepared with the parental 0503 antibody coated at 1µg/ml in PBS for 24hrs at 4°C. After 24hrs, the MaxiSorp plates were washed three times with PBS-T. 200µl of SuperBlock was added to each well, removed and replaced three times. 100µl of Cat S antigen at 200ng/ml was added to each well and left shaking at RT, 150rpm for 1hr 30mins. The plates were washed with PBS-T three times and dried. 100µl of anti-his-HRP at 5µg/ml was added to each well and left for 1hr 30mins shaking at 150rpm, RT. The plates were washed three time PBS-T, once with PBS and dried. 100µl of TMB was added to each well and incubated at 37 °C for 10mins, which was followed by 50µl of 1M HCL and the absorbance of the plates was measured at 450nm.

Affinity ranking using the Octet RED96 system (Fsn503h variants):

Affinity ranking assays were performed by first capturing IgG using anti-human Octet biosensors (ForteBio part no. 18-5060) followed by a baseline step of 2 minutes in HBS-EBT buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 1 mg/ml BSA, and 0.05% Tween-20, pH 7.4). The mAb capture biosensors were then submerged in wells containing 200 ng/ml of recombinant Cathepsin S antigen for 10 minutes (association step), followed by a 10 min dissociation step in running buffer. To allow for double reference correction, IgG-captured sensors were dipped into wells containing only buffer and blank sensors were also dipped into wells containing the antigen. This referencing provided a means of compensating for both the natural dissociation of the capture IgG and also non-specific binding of the antigen to the sensor surface. All steps were performed at 25°C in HBS-EBT buffer at a constant

flow-rate of 1000 rpm. New sensors were used for each sample. Dissociation rate constants (*k*off) were calculated using the ForteBio Data Analysis software. All consumables used were those recommended by ForteBio.

Antibody quantification using biolayer interferometry (the Octet RED96 system):

To measure IgG content, 200 µL aliquots of antibody standards (spanning 0.06 to 512 µg/ml) and IgG containing cell supernatants (diluted within the measurable range of calibration curve) were prepared in duplicate using 1x HBS-EBT buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA, 1 mg/ml BSA, and 0.05% Tween-20, pH 7.4) and placed in the wells of a 96-well black microtiter plate (Greiner Bio-One part no. 655209). All samples and standards were measured in duplicate using protein A Biosensors (Fortebio PN 18-5010). The plate was placed in the Octet and allowed to equilibrate to 25° C in the thermostatted chamber. The run was initiated by placing the sensors in the wells and measuring the change in layer thick-ness (in nanometers, nm) with time, all under computer control. Data were taken for each set of eight samples at a time (one plate column is measured simultaneously) for 180-600 sec at a flow rate of 400-1000 rpm (orbital flow). Data were processed automatically using the Octet User Software version 3.1. The measurement time and flow rate were altered according to the sensitivity required.

Affinity ranking using biolayer interferometry (the Octet RED96 system) (Trastuzumab variants):

Affinity ranking assays were performed by first capturing *IgG using anti-human Octet biosensors (ForteBio part no. 18-5060) followed by a baseline step of 2 minutes in HBS-P+ buffer (10 mM HEPES, 150 mM NaCl, 1 mg/ml BSA, and 0.05% Tween-20, pH 7.4). The mAb capture biosensors were then submerged in wells containing 5 nM of recombinant HER2 (Acro Biosystems; P/N. H5225) antigen for 15 minutes (association step), followed by a 20 min dissociation step in running buffer. To allow for double reference correction, IgG-captured sensors were dipped into wells containing only buffer and blank sensors were also dipped into wells containing the antigen. This referencing provided a means of compensating for both the natural dissociation of the capture IgG and also non-specific binding of the antigen to the

sensor surface. Steps were performed at either 25°C or 37°C in HBS-EBT buffer at a constant flow-rate of 1000 rpm. New sensors were used for each sample. Dissociation rate constants (*k*off) were calculated using the ForteBio Data Analysis software. All consumables used were those recommended by ForteBio.

*To allow for similar loading levels the quantified (as described above) IgG containing cell supernatants were diluted to an identical concentration.

K_D determination using biolayer interferometry (the Octet RED96 system):

Kinetic assays were performed by first capturing IgG using anti-human Fc Octet biosensors followed by two baseline steps of 2 minutes each in in HBS-P+ buffer running buffer. The mAb capture biosensors were then submerged in wells containing various concentrations of HER2 for 15 mins followed by 20 mins of dissociation time in running buffer. To allow for double reference correction, IgG-captured sensors were dipped into wells containing only buffer and blank sensors were also dipped into wells containing the antigen concentration series. This referencing providing a means of compensating for both the natural dissociation of the capture IgG and also non-specific binding of the antigen to the sensor surface. All steps were performed at 37°C in kinetics buffer at a constant flow-rate of 1000 rpm.

Melting Point Determination

Antibodies commonly display 2 measurable melting points in analysis, designated TM1 and TM2 as a result of thermal denaturing of different parts of the assembled molecule. These values were determined by Thermal Shift Assay. Solutions containing 5 µl of Sypro Orange ([diluted 1/200 in PBS, pH 7.4); Molecular Probes) and 45 µl of 0.3 mg ml^{-1} antibody were added to low profile PCR tubes (Bio-Rad; TLS0851). Tubes were sealed with optical ultra-clear sealing caps (Bio-Rad; TCS0803) and heated in an i-Cycler iQ5 real-time PCR detection system (Bio-Rad) from 20 to 90 °C in increments of 1 °C. Fluorescence changes in the wells of the plate were monitored simultaneously with a charge-coupled (CCD) camera. The wavelengths for excitation and emission were 485 and 575 nm, respectively. The temperature midpoint for the protein unfolding transition, T_m, was calculated using the Bio-Rad iQ5 software.

Determination of monodispersity

The level of monodispersity, defined as free individual molecules of immunoglobulin within an antibody preparation, of each variant was also shown to differ from the wild type molecule. This measurement is commonly used as an indicator of aggregation propensity for antibody molecules. Aggregation is the tendency of protein molecules to associate into multimeric complexes, diminishing the solubility and activity of an antibody preparation over time and is a key attribute contributing to the stability of an antibody molecule in solution.

This was determined on the purified antibody solutions using size-exclusion chromatography. All samples were diluted to a final concentration of 0.1 mg/ml using phosphate buffered saline. Highly purified samples of antibodies were loaded independently onto a Superdex 200 increase 10/300 GL gel-filtration column. 50 µl samples were injected and the column flow rate was maintained at 0.75 µl min^{-1}. Separations and equilibration steps were performed in phosphate buffered saline at 22°C. Protein peaks were monitored using absorbance at 280 & 214 nm and spectra were analysed using the Unicorn emulation software package (GE healthcare). Results are reported in V_r (ml) and relative peak area (%) of each peak.

Results

Example 1 _ Cathepsin S antibody variants

Library Design

The DNA sequences of the variable domains of the Fsn0503h antibody light and heavy light chains were analysed for motifs susceptible to mutation during somatic hypermutation, and the corresponding potential amino acid results of these mutation plotted above the parental sequence, as shown in Figure 1. Any undesirable amino acids or STOP codons generated as a result of the DNA mutations were identified.

It was a surprising discovery that there are more mutations within the framework regions than the CDRs, and in particular that CDR-H3 has no functional mutations.

Molecular Docking

The parental Fsn0503h antibody variable domains (Kwok et al. Molecular Cancer 2011, 10:147) were docked with the Cathepsin S protein using Schrödinger molecular docking software as described in the Methods. The outcome of the docking procedure is shown in Figure 2.

Combinatorial Mutagenesis

Within the docked structures amino acid residue mutations were introduced and the relative difference in both affinity and stability predicted. The mutations were introduced in increasing numbers until no further benefits to stability or affinity were predicted. The results of the combinatorial mutagenesis are shown in Figure 3. The inventors identified 66 variants that had a predicted improvement in both affinity and stability. The DNA coding for these variants was then synthesised and the antibodies expressed and purified as described in the Methods.

ELISA

Each of the purified variants was analysed by ELISA for binding to recombinant Cathepsin S protein. The results (Figure 4) show that 12 of the variants have a higher relative OD than the parental Fsn0503h, potentially indicating a higher affinity for the Cathepsin S target.

Affinity Ranking

In order to determine an accurate affinity comparison of each of the variants with the parental Fsn0503h antibody the interactions with Cathepsin S were measured by BLI using an Octet instrument as described in the Methods.

The results show that approximately 50% of the variants have improved affinity when measured against the average reading for the Fsn0503h antibody (Figure 6). The association (Kon) and dissociation (Kdis) rates are given in Figure 5.

Influence on Expression

The 66 variants expressed in CHO as described above also demonstrated a range of level of expression relative to the wild type. IgG level was determined by a quantitative human IgG immunoassay on the BLI Octet instrument following purification and expressed the total amount relative to the supernatant volume purified.

Modulation of melting point

The 66 variants also demonstrated a range of variation in stability characteristics associated with antibody molecules. This included changes in melting temperature profile at 2 melting points commonly observable in immunoglobulin molecules. The results of melting point determination are shown in Figure 22. It is noteable that some variants such as Mut 6 and Mut 49 appear to have lost their distinct 2-phase melting pattern, with only a single melting temperature being observable.

Modulation of monodispersity

This characteristic was analysed by analytical size exclusion chromatography of the 66 variants and show a range of values amongst the analysed variants as presented in Figure 23.

Example 2 – Trastuzumab variants

Library Design

A library of antibody variants was produced for trastuzumab based on naturally occurring somatic hypermutation in humans. Using the method described above somatic hypermutation was replicated, producing naturally occurring mutations in the DNA sequence of trastuzumab and subsequently translated to their respective amino acid mutations, as illustrated in Figure 9. These mutations were compiled into a library of

potential mutations at specific sites in both the complementary determining regions (CDRs) as well as in the framework regions (Figures 7 and 8), in which potential amino acid results of the method are detailed next to the parental sequence. Any undesirable amino acids or STOP codons generated as a result of the DNA mutations were identified.

The fully resolved crystal structure of trastuzumab in complex with the extracellular domain of human epidermal growth factor receptor 2 (HER2) (PDB Code: 1N8Z) is illustrated in figure 10. The library of trastuzumab mutations illustrated in Figures 7 and 8 was used for mutant scanning. Mutants were scanned consecutively whereby a single amino acid mutation was first analysed, followed by two mutations up until six total mutations in the heavy and light chains and subsequently variants were ranked based on affinity and stability. As with the Fsn503h library, the Schrödinger molecular docking software was used as described in the Methods.

Within the docked structures amino acid residue mutations were introduced and the relative difference in both affinity and stability predicted. The mutations were introduced in increasing numbers until no further benefits to stability or affinity were predicted. The results of the combinatorial mutagenesis are detailed in the tables of Figures 11 and 12. A total of 558 potential variants for trastuzumab were found to have both improved affinity and improved stability. The top five ranked affinities for variants are illustrated in figure 13. Among these variants, a total of 5 mutations across the heavy and light chains were found to be the most common. The variants were scored relative to the parental antibody affinity using the predicted free energy change (ΔΔG).

Off-rate ranking

A panel of 89 Trastuzuamb variants were screened for binding to the HER2 antigen using biolayer interferometry (see materials and methods for details). Sensorgrams from 20 samples with the report points indicated as colour bars are shown in **Figure 14**. **Figure 15** shows a scatterplot of the report points stability_early plotted against stability_late. The best binders (14 in total), with a high binding stability and slow dissociation, are highlighted with

a blue circle. The off-rate ranking experiments were repeated for these mutants at 37°C, to provide more biologically relevant data (**Figure 16**). Mutant 19 appeared to have enhanced stability compared to that of WT Trastuzumab and was taken forward for kinetic analysis.

KD determination

All samples were diluted in freshly prepared running buffer. Either Trastuzumab or a variant (Mutant) were immobilised onto the surface of a series of biosensors using the capture methods described (see materials and methods). HER2 was passed over the surface to generate a binding response. Binding data for the HER2 interactions were collected at 37°C on the biosensors. A dilution series of the HER2 antigen (5nM-0.078nM) was used in the association step, in order to fit results globally and get the best values for k_a, k_d, and K_D. The response data for the binding of antigen to the surface immobilised IgG were fitted to a 1:1 binding model, yielding the data traces (red – see **Figure 17**). The experiments were performed in duplicate and the average kinetic parameters are summarised in **Table 9**. For example, the data shows that Mutant 19 exhibits an apparent ~ 2-fold affinity increase compared with that of the wild-type control and commercial Trastuzumab. The increase in affinity is primarily due to the slower Kd, 6.04E-05 compared to 1.20E-04 for the wild-type antibody. Figure 18 schematically shows a comparison of actual versus predicted affinity for tratuzumab variants selected by in silico predictions of improved affinity and stability. Actual Octet affinity ranking correlates reasonably well for the top 20 predictions

Table 9: Kinetic parameters (fit to 1:1 interaction model) for Trastuzumab variants and HER2 interaction

Name	K_D (pM)	k_a ($m^{-1}s^{-1}$)	k_d (s^{-1})	R^2	X^2	Mean Rmax
Trastuzumab	192 ± 21	1.10E+06 ± 4.50E+04	2.12E-04 ± 3.15E-05	0.9960	0.2185	0.2112
HER2 WT	126 ± 6	1.01E+06 ± 1.85E+04	1.28E-04 ± 7.50E-06	0.9946	0.1283	0.1693
MUT 5	139 ± 5	1.05E+06 ± 5.00E+04	1.46E-04 ± 1.50E-06	0.9977	0.1395	0.2553
MUT 19	52 ± 16	1.12E+06 ± 1.30E+05	6.04E-05 ± 2.47E-05	0.9958	0.0984	0.1405

R^2 values indicate how well the fit and experimental data correlate and above 0.95 are considered a good fit; X^2 is the sum of the squared deviation should be generally below 3 X^2

is the measure of error between the experimental data and the fitted line. The smaller the X^2 indicates a better fit.

Monomeric KD determination

In order to further verify the affinity of the top variants and to understand the contribution of avidity to the measurement of affinity within the top 2 variants (MUT 5 and MUT 19), these molecules were prepared by enzymatic cleavage as Fragment Antigen Binding (Fab) fragments, consisting of the variable domain and CH1 constant domain of the heavy and light chain, in a monomeric form, with a single antigen binding domain per molecule.

An initial measurement was made after coating the probes with 2.5 µg/ml biotinylated Her2-recombinant Fc fusion protein. A range of concentrations of each of the 3 monomeric antibodies were assessed for binding to the Her2 surface. In this case double-referencing was not applied. The sensorgram data is shown in **Figure 19** and resulting kinetic calculations (on a 1:1 model) are listed in **Table 10**.

Table 10: Kinetic parameters (fit to 1:1 interaction model) for Trastuzumab variants as monomeric Fab and HER2 interaction at a HER2 coating concentration of 2.5 µg/ml

Antibody	Capture level (nm)	k_a ($m^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	K_D (pM)	K_D Steady State	R^2	X^2	Mean R_{max}
WT (4)	0.719	2.05E+06	6.17E-04	3.01E-10	300	6.40E-10	0.9513	0.141	0.059
Mut 5 (5)	0.853	1.14E+06	2.90E-04	2.56E-10	256	1.20E-10	0.9856	0.107	0.106
Mut 19 (3)	0.676	2.17E+06	1.13E-05	5.20E-12	5.2	1.00E-10	0.9848	0.0289	0.061

Sensors were then prepared by coating with 0.625, 1.25 or 2.5 µg/ml of biotinylated Her2-recombinant Fc fusion protein. The purified Fab fragments were then tested for their binding to these probes. The sensorgram traces are shown in **Figure 20** for these

measurements, with details shown in **Tables 11, 12 and 13**. This data was double-referenced to exclude any drift due to non-specific interactions at the probe surface, unlike the previous experiment.

Table 11: Kinetic parameters (fit to 1:1 interaction model) for Trastuzumab variants as monomeric Fab and HER2 interaction at a HER2 coating concentration of 2.5 µg/ml (double-referenced)

Sample	K_D	K_D Error	K_{ON}	K_{ON} Error	K_{OFF}	K_{OFF} Error	R^2	X^2	R_{MAX}
WT	2.86E-10	<1.0E-12	1.18E+06	5.34E-65	3.37E-04	<1.0E-07	0.9938	0.0012	0.0921
MUT 19	1.88E-10	2.74E+56	1.19E+06	3.24E+68	2.22E-04	3.24E+62	0.9959	0.0014	0.1157
MUT 5	3.55E-10	<1.0E-12	1.29E+06	2.94E-65	4.59E-04	<1.0E-07	0.9872	0.0035	0.1138

Table 12: Kinetic parameters (fit to 1:1 interaction model) for Trastuzumab variants as monomeric Fab and HER2 interaction at a HER2 coating concentration of 1.25 µg/ml (double-referenced)

Sample	K_D	K_D Error	K_{ON}	K_{ON} Error	K_{OFF}	K_{OFF} Error	R^2	X^2	R_{MAX}
WT	3.12E-10	<1.0E-12	1.10E+06	1.73E-65	3.43E-04	<1.0E-07	0.9908	0.0012	0.0717
MUT 19	1.68E-10	3.79E+56	1.03E+06	3.91E+68	1.73E-04	3.91E+62	0.9914	0.0021	0.0917
MUT 5	4.50E-10	<1.0E-12	1.26E+06	2.24E-65	5.67E-04	<1.0E-07	0.9883	0.0021	0.0904

Table 13: Kinetic parameters (fit to 1:1 interaction model) for Trastuzumab variants as monomeric Fab and HER2 interaction at a HER2 coating concentration of 0.625 µg/ml (double-referenced)

Sample	K_D	K_D Error	K_{ON}	K_{ON} Error	K_{OFF}	K_{OFF} Error	R^2	X^2	R_{MAX}
WT	3.15E-10	<1.0E-12	1.00E+06	1.79E-65	3.16E-04	<1.0E-07	0.9831	0.0013	0.0552
MUT 19	<1.0E-12	<1.0E-12	1.08E+06	1.94E-62	<1.0E-07	N/A	0.9894	0.0017	0.0698
MUT 5	3.40E-10	<1.0E-12	1.34E+06	2.10E-65	4.57E-04	<1.0E-07	0.9787	0.0018	0.0642

The affinity value appears to vary as a result of coating concentration so it is difficult to assign a definitive value for the increase in affinity for MUT 5 and MUT 19 compared to the WT Trastuzemab molecule from these monomeric analysis. Without wishing to be bound by theory, this is thought to be due to the problem of working at the limits of sensitivity of the FortéBio Octet Biosensor instrument.

Conclusion

Using the methods of the present invention, the inventors have successfully demonstrated that the affinity of an antibody for its target can be improved without the need to generate a very large physical library of antibody variants and subsequent selection/screening process. In addition, a pool of variants are generated which, upon expression, display a range of attributes of interest including affinity, expression level and physiochemical characteristics, which are all of interest to the potential developability of an antibody molecule.

Although the invention has been particularly shown and described with reference to particular examples, it will be understood by those skilled in the art that various changes in

the form and details may be made therein without departing from the scope of the present invention.

Claims

1. A variant of a reference antibody, wherein said variant has (i) at least two amino acid changes in the light chain sequence when compared to the light chain amino acid sequence of the reference antibody, (ii) at least two amino acid changes in the heavy chain sequence when compared to the heavy chain amino acid sequence of the reference antibody, or (iii) at least one amino acid change in the light chain sequence when compared to the light chain amino acid sequence of the reference antibody and at least one amino acid change in the heavy chain sequence when compared to the heavy chain amino acid sequence of the reference antibody; wherein each of said amino acid changes are at amino acid residues independently encoded from a DNA segment of the variant DNA sequence, wherein said DNA segment of the variant differs from that of the corresponding DNA sequence encoding the reference antibody by a point mutation in a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

2. The variant antibody according to claim 1 wherein said somatic hypermutation inducing enzyme is Activation-Induced Deaminase (AID) and said DNA motif is DGYW or WRCH, for example RGYW or WRCY, where D is adenine, guanine or thymine, R is adenine or guanine, G is guanine, C is cytosine, H is adenine or cytosine or thymine, W is adenine or thymine, and Y is any cytosine or thymine.

3. The variant antibody according to claim 1 or claim 2, wherein said reference antibody is trastuzumab.

4. The variant antibody according to claim 3, wherein said amino acid changes are selected from the group consisting of lc9N, lc9T, lc9I, lc9R, lc9K, lc25G,

lc25V, lc25D, lc31N, lc31S, lc31I, lc32D, lc32G, lc32V, lc32T, lc32N, lc32S, lc32I, lc32P, lc32L, lc32F, lc33L, lc33I, lc34G, lc34V, lc34D, lc38E, lc38K, lc40A, lc40S, lc40T, lc43G, lc43V, lc43T, lc43N, lc43S, lc43I, lc43P, lc43L, lc43F, lc46V, lc46I, lc47V, lc51S, lc51P, lc51T, lc76R, lc76N, lc76T, lc76K, lc76I, lc79K, lc79E, lc80T, lc80S, lc80A, lc85S, lc85N, lc85I, lc89H, lc90E, lc90A, lc91N, lc91D, lc91Y, lc93S, lc93N, lc93I, lc94S, lc94N, lc94I, lc101D, lc102S, lc102N, hc2L, hc2I, hc3H, hc4M, hc4V, hc13K, hc13E, hc14A, hc14T, hc14S, hc16A, hc16V, hc16D, hc23E, hc23G, hc23V, hc23T, hc23K, hc23R, hc23I, hc23P, hc23L, hc23S, hc24D, hc24G, hc24V, hc24T, hc24N, hc24S, hc24I, hc24P, hc24L, hc24F, hc26A, hc26V, hc26D, hc28K, hc35N, hc35D, hc35Y, hc48L, hc48I, hc49G, hc49S, hc56A, hc56V, hc56D, hc58S, hc58N, hc58I, hc61G, hc61V, hc61D, hc79G, hc79V, hc79D, hc82E, hc82K, hc85R, hc88D, hc88T, hc88S, hc88P, hc88G, hc92G, hc92V, hc92D, hc103A, hc103V, hc103D, hc106D, hc106G, hc106V, hc106T, hc106N, hc106S, hc106I, hc106P, hc106L, hc106F, hc114S, hc114N, and hc114I.

5. The variant antibody according to claim 4, wherein said amino acid changes are selected from the group consisting of lc9T, lc9I, lc9R, lc9K, lc43F, lc47V, lc51P, lc51T, lc101D, hc2L, hc3H, hc14S, hc16V, hc24P, hc26A, hc26V, hc48I, hc58S, hc61V, hc79V, hc85R, hc88G, hc92V, hc92D, hc103A, hc103V, hc106V, hc114S, hc114N, and hc114I.

6. The variant antibody according to any one of claims 3 to 5, wherein variant light chain and heavy chain sequences do not differ from that of the reference antibody at any residue other than by the amino acid changes recited in claim 4.

7. The variant antibody according to any one of claims 3 to 6, wherein said variant antibody molecule has the combination of amino acid mutations as

shown for variant number 19 of Table 8 or any of the other antibodies listed in Table 8.

8. The variant antibody according to any one of claims 3 to 6, wherein said variant antibody molecule has the combination of amino acid mutations as shown for any of the antibodies listed in Tables 2 to 7.

9. The variant antibody according to any one of claims 3 to 8, wherein the variant antibody is a variant of a trastuzumab antibody comprising relative to trastuzumab, the following amino acid changes: lc9K, lc43F, and hc106V.

10. The variant antibody according to claim 1 or claim 2, wherein said variant is a variant of a Cathepsin S antibody, wherein said, wherein said reference antibody is Fsn503h.

11. The variant antibody according to claim 10, wherein said amino acid changes are selected from the group consisting of lc12A, lc12S, lc12T, lc19V, lc28R, lc32T, lc32I, lc45A, lc45S, lc45T, lc50H, lc51V, lc51F, lc51I, lc56L, lc56F, lc56I, lc58K, lc66S, lc69A, lc69V, lc81T, lc81I, lc81N, lc85P, lc85S, lc85T, lc90L, lc90F, lc96I, lc96S, lc96I, lc96N, lc108N, hc3H, hc4V, hc4M, hc10A, hc10V, hc14A, hc14S, hc24G, hc24V, hc30T, hc30I, hc31R, hc31T, hc37L, hc37F, hc40P, hc40S, hc52S, hc52I, hc53S, hc53I, hc84T, hc84I, hc92G, and hc92V.

12. The variant antibody according to claim 11, wherein said amino acid changes are selected from the group consisting of lc12A, lc12S, lc12T, lc19V, lc45S, lc45T, lc50H, lc51V, lc56I, lc81I, lc96I, lc96S, lc96I, lc96N, lc108N, hc10A, hc10V, hc14S, hc30I, hc31R, hc37L, hc37F, hc40P, hc40S, hc52I, and hc92G..

13. The variant antibody according to any one of claims 10 to 12, wherein variant light chain and heavy chain sequences do not differ from that of the

reference antibody at any residue other than by the amino acid changes recited in claim 11.

14. The variant antibody according to any one of claims 10 to 13, wherein said variant antibody molecule has the combination of amino acid mutations as shown for any of the antibodies listed in Table 1.

15. The variant antibody according to any one of claims 1 to 14, wherein said variant light chain and heavy chain sequences comprise in total at least three, for example at least 4, at least 5 or at least 6 amino acid changes compared to the amino acid sequence of the reference antibody.

16. The variant antibody according to any one of claims 1 to 15 wherein one or more of said amino acid changes are in framework regions of said variant antibody.

17. The variant antibody according to any one of claims 1 to 16 wherein one or more of said amino acid changes are in CDRs of said variant antibody.

18. The variant antibody according to claims 16 wherein all of said amino acid changes are in framework regions of said variant antibody.

19. The variant antibody according to any one of claims 1 to 18, wherein the change of affinity of said variant antibody molecule relative to the reference antibody is greater than -2 and the change of stability of said variant antibody molecule relative to reference antibody is greater than -2.

20. A library of antibody molecules, wherein each antibody molecule is a variant of a reference antibody, wherein the amino acid sequence of each antibody molecule differs from the amino acid sequence of the reference antibody at

one or more amino acid residues, wherein each of said amino acid residues are independently encoded from a DNA segment of the variant DNA sequence, wherein said DNA segment of the variant differs from that of the corresponding DNA sequence encoding the reference antibody by a point mutation in a DNA motif susceptible to deamination by a somatic hypermutation inducing enzyme.

21. The library according to claim 20, wherein said somatic hypermutation inducing enzyme is Activation-Induced Deaminase (AID).

22. The library according to claim 21, wherein said DNA motif is DGYW or WRCH, for example RGYW or WRCY, where D is adenine, guanine or thymine, R is adenine or guanine, G is guanine, C is cytosine, H is adenine or cytosine or thymine, W is adenine or thymine, and Y is any cytosine or thymine.

23. The library according to any one of claims 20 to 22, wherein the nucleotide sequence encoding each of said antibody molecules does not differ from that of the reference antibody at any residue other than residues of said DNA motif.

24. The library according to any one of claims 20 to 23 wherein one or more of said DNA motifs are in DNA sequences which encode framework regions of said antibody molecule.

25. The library according to any one of claims 20 to 24 wherein one or more of said DNA motifs are in DNA sequences which encode CDRs of said antibody molecule.

26. The library according to claim 24, wherein all of said DNA motifs are in DNA sequences which encode framework regions of said antibody molecule.

27. The library according to any one of the claims 20 to 26 wherein the DNA sequence of each variant does not comprise (or encode) a deamination site, isomerisation site, N-linked glycosylation site or oxidation site which originates from said point mutation in said DNA motif.

28. A library of nucleotide sequences, wherein each member of the library encodes an antibody molecule of the library of antibody molecules according to any one of claims 20 to 27.

29. A method of generating/producing a library of variant antibody molecules, wherein said variant antibody molecules are variants of a reference antibody, said method comprising the steps:

a) providing a nucleotide sequence encoding the reference antibody,
b) in said nucleotide sequence, identifying one or more DNA motifs susceptible to deamination by a somatic hypermutation inducing enzyme;
c) for one or more of said DNA motifs, selecting at least one variant nucleotide residue to substitute for a residue of said DNA motif, wherein said substitution will result in a variant nucleotide sequence which encodes a variant antibody molecule having, relative to the reference antibody, a change in the amino acid sequence being encoded by said DNA motif; and
d) repeating steps (b) and (c);

such that a library containing a plurality of variants of said reference antibody is generated.

30. The method according to claim 29, wherein said method further comprises screening said library of variants to determine binding to an epitope to which the reference antibody binds.

31. The method according to claim 30, wherein those variants determined to bind to said epitope with an affinity and/or stability greater than a predetermined value relative to the reference antibody are used to generate an optimised library of variant antibody molecules.

32. The method according to any one of claims 29 to 31, wherein said method is a computer implemented method.

33. The method according to any one of claims 29 to 32, further comprising synthesising the variant antibody molecules.

34. The method according to any one of claims 29 to 33, wherein said library is a library of antibody molecules according to any one of claims 21 to 27.

35. The library according to any one of claims 20 to 27 or the method according to any one of claims 29 to 34, wherein the reference antibody is trastuzumab or the anti Cathepsin S antibody Fsn0503h.

Light Chain
SEQ ID NO: 1
D V V M T Q S P L S L P V T L G Q P A S I S C R S S Q S L V H S N G N T Y L H W
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
T D
S V
A G
N
I
T
C
D
V
A
S
K
L
I
F
V
H
T
S
I
L
A
T
S
N
P
K
V
S
N
R
F
S
T
F
G
Q
G
T
K
L
E
I
K
Y L Q K P G Q S P Q L L I Y K V S N R F S G V P D R F S G S G T D F T L K I
41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
*
N I T
I S F
T P L
I
F
L
N
I
S
P
*
W
N
S
T
Q
S
C
Y
Y
V
G
V
D
E
A
E
V
R
S
T
T
H
V
P
P
T
S 81 R 82 V 83 E 84 A 85 E 86 D 87 V 88 G 89 V 90 Y 91 Y 92 C 93 S 94 Q 95 T 96 T 97 H 98 V 99 P 100 P 101 T 102 F 103 G 104 P 105 Q 106 G 107 T 108 K 109 L 110 E 111 I 112 K

Figure 1A

Heavy Chain
SEQ ID NO: 2
E V Q L V E S G G G L V K P G G S L R L S C A A S G F A F S S Y D M S W V R Q A P G K G L E W V A Y I T T G G V N T Y Y A D T V K G R F T I S R D N A K N T L Y L Q M S S L K S E D T A V Y Y C A R H S Y P D F D Y W G Q G T T V T V S S
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115

Figure 1B

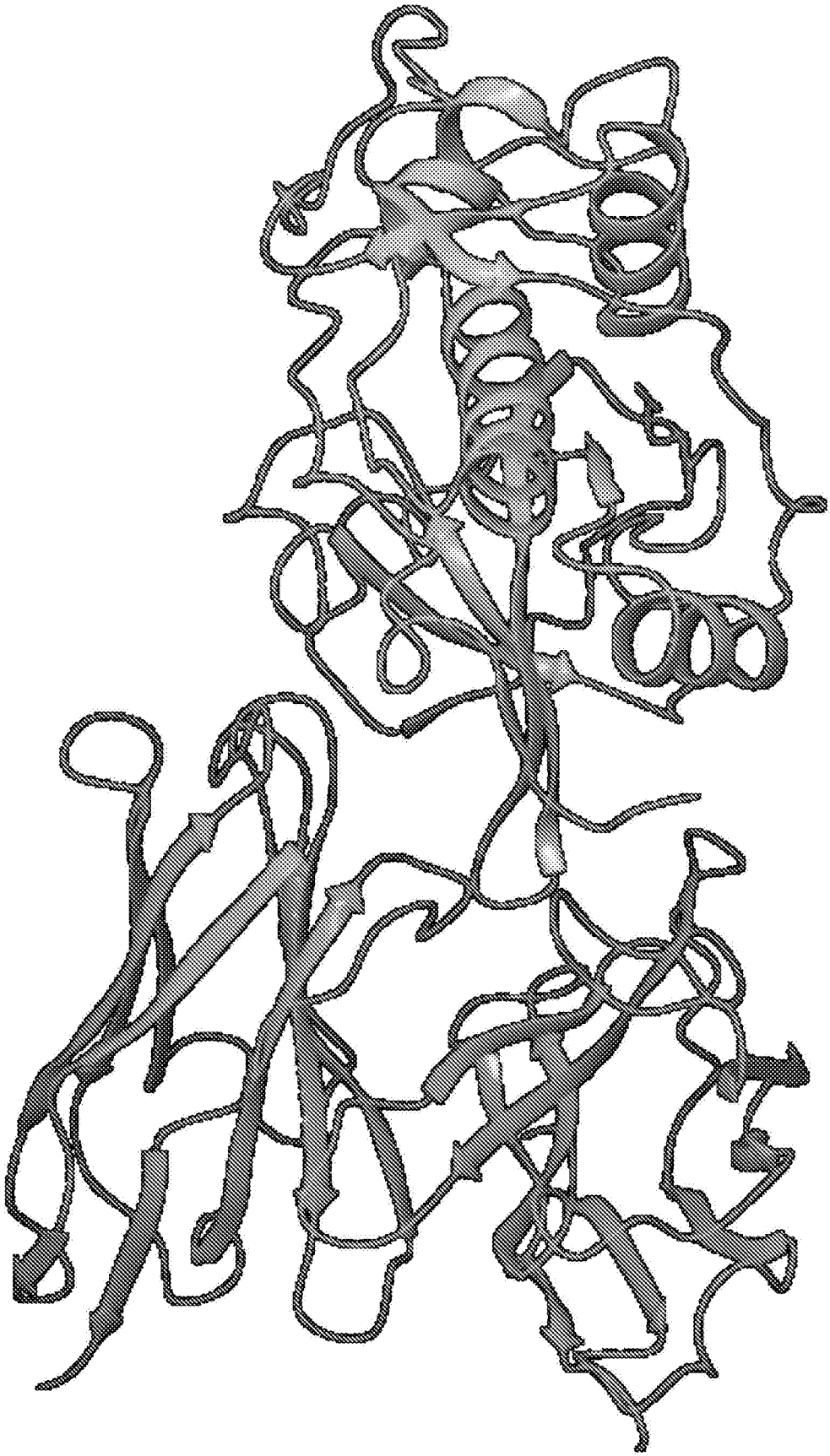


Figure 2

Figure 3

One Mutation					Two Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
H:31	SER	ARG	-12.49	-1	L:19	ALA	VAL	-10.68	4.62
					H:31	SER	ARG		
H:31	SER	ASN	-4.87	5.17					
H:31	SER	ILE	-4.46	-0.93					
L:96	THR	ILE	-3.16	4.48					
H:30	SER	ILE	-2.32	1.12					
H:31	SER	THR	-1.76	1.18					

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
L:12	PRO	SER			L:12	PRO	SER		
H:31	SER	ARG	-9.72	0.93	L:96	THR	ILE	-16.92	-1.71
H:92	ALA	GLY			H:31	SER	ARG		
					H:52	THR	ILE		
L:12	PRO	THR							
H:31	SER	ARG	-9.72	-2.61	L:12	PRO	ALA		
H:92	ALA	GLY			L:96	THR	ILE	-15.33	-1.8
					H:31	SER	ARG		
L:12	PRO	ALA			H:52	THR	ILE		
H:31	SER	ARG	-9.72	0.84					
H:92	ALA	GLY			L:12	PRO	THR		
					L:96	THR	ILE	-15.33	-3.9
L:12	PRO	THR			H:31	SER	ARG		
H:31	SER	ASN	-4.6	2.33	H:52	THR	ILE		
H:92	ALA	GLY							
					L:12	PRO	SER		
L:12	PRO	SER			L:96	THR	ILE	-15.27	-12.76
H:31	SER	ASN	-4.48	4.5	H:30	SER	ILE		
H:92	ALA	GLY			H:31	SER	ARG		
L:12	PRO	ALA			L:12	PRO	THR		
H:31	SER	ASN	-4.48	4.41	L:96	THR	ILE	-15.26	-15
H:92	ALA	GLY			H:30	SER	ILE		
					H:31	SER	ARG		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
					L:12	PRO	SER		
L:12	PRO	SER			L:96	THR	ASN	-15.23	3.57
H:31	SER	ILE	-3.68	4.85	H:30	SER	ILE		
H:92	ALA	GLY			H:31	SER	ARG		
L:12	PRO	THR			L:12	PRO	ALA		
H:31	SER	ILE	-3.08	1.25	L:96	THR	ASN	-15.23	3.48
H:92	ALA	GLY			H:30	SER	ILE		
					H:31	SER	ARG		
L:12	PRO	ALA							
H:31	SER	ILE	-3.08	4.75	L:12	PRO	THR		
H:92	ALA	GLY			L:96	THR	ASN	-15.23	1.38
					H:30	SER	ILE		
					H:31	SER	ARG		
L:12	PRO	THR							
L:96	THR	ILE	-13.77	-8.03	L:12	PRO	ALA		
H:31	SER	ARG			L:96	THR	ASN	-14.18	3.42
					H:30	SER	THR		
L:12	PRO	SER			H:31	SER	ARG		
H:31	THR	ARG	-13.76	-6.2					
H:52	SER	ILE							
					L:12	PRO	THR		
					L:42	LEU	ILE	-14.04	5.11
L:12	PRO	THR			L:96	THR	ASN		
H:31	SER	ARG	-13.76	-8.43	H:31	SER	ARG		
H:52	THR	ILE							
					L:12	PRO	THR		
L:12	PRO	SER			L:96	THR	SER	-14	-4.06
L:96	THR	ASN	-13.67	2.78	H:31	SER	ARG		
H:31	SER	ARG			H:52	THR	ILE		
L:12	PRO	THR			L:12	PRO	ALA		
L:96	THR	ASN	-13.67	1.98	L:50	GLN	HID	-13.83	-6.97
H:31	SER	ARG			L:96	THR	ILE		
					H:31	SER	ARG		
L:12	PRO	ALA							
H:31	SER	ARG	-13.36	4.76	L:12	PRO	ALA		
H:52	THR	ILE			L:81	SER	ILE	-13.78	-12.11
					L:96	THR	ILE		
L:12	PRO	SER			H:31	SER	ARG		
H:30	SER	ILE	-12.93	-16.98					
H:31	SER	ARG			L:12	PRO	THR		
					H:31	SER	ARG	-13.76	-5.98
					H:37	VAL	LEU		
					H:52	THR	ILE		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
L:12	PRO	THR	-12.9	-16.3	L:12	PRO	ALA		
H:30	SER	ILE			H:31	SER	ARG	-13.76	-2.46
H:31	SER	ARG			H:37	VAL	LEU		
					H:52	THR	ILE		
L:12	PRO	ALA	-12.9	-14.14					
H:30	SER	ILE			L:12	PRO	SER		
H:31	SER	ARG			H:14	PRO	SER	-13.76	-3.52
					H:31	SER	ARG		
					H:52	THR	ILE		
					L:12	PRO	THR		
					H:14	PRO	SER	-13.75	-5.71
					H:31	SER	ARG		
					H:52	THR	ILE		
					L:12	PRO	SER		
					H:10	GLY	VAL	-13.73	-4.32
					H:31	SER	ARG		
					H:52	THR	ILE		
					L:12	PRO	SER		
					H:10	GLY	ALA	-13.73	1.16
					H:31	SER	ARG		
					H:52	THR	ILE		
					L:12	PRO	ALA		
					L:96	THR	ILE	-13.68	-12.9
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:85	ALA	SER	-13.67	2.35
					L:96	THR	ASN		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:96	THR	ASN	-13.67	-6.16
					H:31	SER	ARG		
					H:40	ALA	THR		
					L:12	PRO	SER		
					L:81	SER	THR	-13.67	3.31
					L:96	THR	ASN		
					H:31	SER	ARG		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
					L:12	PRO	THR		
					L:81	SER	THR	-13.67	1.13
					L:96	THR	ASN		
					H:31	SER	ARG		
					L:12	PRO	SER		
					L:96	THR	ASN	-13.62	2.18
					H:30	SER	ASN		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					L:96	THR	ASN	-13.62	2.1
					H:30	SER	ASN		
					H:31	SER	ARG		
					L:12	PRO	THR		
					H:31	SER	ARG	-13.59	-2.06
					H:52	VAL	ILE		
					H:83	THR	ILE		
					L:12	PRO	THR		
					L:45	PRO	SER	-13.51	5.12
					L:96	THR	ASN		
					H:31	SER	ARG		
					L:12	PRO	SER		
					L:96	THR	ASN	-13.49	1.81
					H:30	SER	THR		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:96	THR	ASN	-13.46	-0.37
					H:30	SER	THR		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:96	THR	ILE	-13.42	-1.36
					H:31	SER	ARG		
					H:92	ALA	GLY		
					L:12	PRO	SER		
					L:51	LEU	ILE	-13.41	-9.7
					H:30	SER	ILE		
					H:31	SER	ARG		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
					L:12	PRO	THR		
					L:51	LEU	ILE	-13.41	-13.29
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					L:51	LEU	ILE	-13.41	-9.79
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:27	GLN	ILE		
					H:31	SER	ARG	-13.26	-2.56
					H:37	VAL	ILE		
					H:52	THR	ILE		
					L:27	GLN	THR		
					H:31	SER	ARG	-13.22	3.55
					H:37	VAL	ILE		
					H:52	THR	ILE		
					L:12	PRO	THR		
					L:51	LEU	VAL	-13.06	1.69
					L:96	THR	ILE		
					H:31	SER	ARG		
					L:12	PRO	SER		
					L:50	GLN	HID	-13.01	-15.3
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					L:50	GLN	HID	-13	-15.39
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:50	GLN	HID	-12.97	-17.51
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:51	LEU	VAL	-12.95	-9.77
					H:30	SER	ILE		
					H:31	SER	ARG		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
					L:27	GLN	ARG		
					H:31	SER	ARG	-12.93	-2.78
					H:37	VAL	PHE		
					H:52	THR	ILE		
					L:12	PRO	SER		
					H:30	SER	ILE	-12.91	-17.22
					H:31	SER	ARG		
					H:40	ALA	SER		
					L:12	PRO	THR		
					H:30	SER	ILE	-12.91	-19.41
					H:31	SER	ARG		
					H:40	ALA	SER		
					L:12	PRO	THR		
					H:30	SER	ILE	-12.9	-24.43
					H:31	SER	ARG		
					H:40	ALA	THR		
					L:12	PRO	THR		
					L:96	THR	ASN	-12.82	-1.56
					H:30	SER	ASN		
					H:31	SER	ARG		
					L:12	PRO	SER		
					L:19	ALA	VAL	-12.8	-4.5
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					H:14	PRO	THR	-12.8	-12.04
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					H:14	PRO	SER	-12.79	-16.44
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	SER		
					H:14	PRO	SER	-12.79	-12.83
					H:30	SER	ILE		
					H:31	SER	ARG		

Figure 3 (Continued)

Three Mutations					Four Mutations				
Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:	Residue:	Original:	Mutation:	ΔAffinity:	ΔStability:
					L:12	PRO	THR		
					H:14	PRO	THR	-12.79	-14.14
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					H:14	PRO	SER	-12.79	-12.93
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	SER		
					H:14	PRO	SER	-12.79	-11.2
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:45	PRO	SER	-12.78	-13.38
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:45	PRO	THR	-12.78	-15.1
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	THR		
					L:108	LYS	ASN	-12.76	-15.47
					H:30	SER	ARG		
					H:31	SER	ASP		
					L:12	PRO	THR		
					L:56	VAL	ILE	-12.74	-15.73
					H:30	SER	ILE		
					H:31	SER	ARG		
					L:12	PRO	ALA		
					L:45	PRO	SER	-12.69	-7.41
					L:96	THR	ILE		
					H:31	SER	ARG		
					L:12	PRO	SER		
					L:108	LYS	ASN	-12.69	-15.77
					H:30	SER	ILE		
					H:31	SER	ARG		

Figure 3 (Continued)

Five Mutations						Six Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability		Residue	Original	Mutation	ΔAffinity	ΔStability
L:12	PRO	SER				L:12	PRO	SER		
H:30	SER	ASN				L:96	THR	ASN		
H:31	SER	ARG	-16.07	4.91		H:30	SER	ILE	-17.69	5.29
H:52	THR	ILE				H:31	SER	ARG		
H:92	ALA	GLY				H:40	ALA	THR		
						H:52	THR	ILE		
L:12	PRO	THR								
L:27	GLN	THR				L:12	PRO	THR		
H:31	SER	ARG	-13.9	2.53		L:96	THR	ASN		
H:52	THR	ILE				H:30	SER	ILE	-17.69	3.1
H:92	ALA	GLY				H:31	SER	ARG		
						H:40	ALA	THR		
L:12	PRO	SER				H:52	THR	ILE		
L:81	SER	THR								
H:31	SER	ARG	-13.8	2.46		L:12	PRO	ALA		
H:52	THR	ILE				L:51	LEU	VAL		
H:92	ALA	GLY				H:30	SER	ILE	-16.11	0.88
						H:31	SER	ARG		
L:12	PRO	SER				H:40	ALA	SER		
L:45	PRO	THR				H:52	THR	ILE		
H:31	SER	ARG	-13.8	4.36						
H:52	THR	ILE				L:23	PRO	THR		
H:92	ALA	GLY				L:51	LEU	VAL		
						H:30	SER	ILE	-16.1	-1.19
L:12	PRO	SER				H:31	SER	ARG		
L:96	THR	ILE				H:40	ALA	SER		
H:30	SER	ILE	-13.52	-6.31		H:52	THR	ILE		
H:31	SER	ARG								
H:92	ALA	GLY				L:12	PRO	SER		
						L:96	THR	ASN		
L:12	PRO	ALA				H:30	SER	ASN	-16.05	4.11
L:96	THR	ILE				H:31	SER	ARG		
H:30	SER	ILE	-13.52	-6.4		H:40	ALA	THR		
H:31	SER	ARG				H:52	THR	ILE		
H:92	ALA	GLY								
						L:12	PRO	ALA		
L:12	PRO	SER				H:10	GLY	ALA		
H:30	SER	ILE				H:30	SER	ILE	-15.37	-7.41
H:31	SER	ARG	-15.44	-8.88		H:31	SER	ARG		
H:40	ALA	SER				H:40	ALA	SER		
H:52	THR	ILE				H:52	THR	ILE		

Figure 3 (Continued)

Five Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability
L:12	PRO	THR		
H:30	SER	ILE		
H:31	SER	ARG	-15.28	-16.01
H:40	ALA	THR		
H:52	THR	ILE		
L:12	PRO	SER		
L:96	THR	ILE		
H:31	SER	ARG	-15.27	-10.16
H:40	ALA	THR		
H:52	THR	ILE		
L:12	PRO	SER		
H:30	SER	ILE		
H:31	SER	ARG	-15.26	-13.79
H:40	ALA	THR		
H:52	THR	ILE		
L:12	PRO	ALA		
H:30	SER	ILE		
H:31	SER	ARG	-15.25	-8.79
H:40	ALA	PRO		
H:52	THR	ILE		

Six Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability
L:12	PRO	SER		
H:30	SER	ILE		
H:31	SER	ARG	-15.36	2.08
H:40	ALA	SER		
H:52	THR	ILE		
H:83	MET	ILE		
L:12	PRO	THR		
H:30	SER	ILE		
H:31	SER	ARG	-15.36	-0.1
H:40	ALA	SER		
H:52	THR	ILE		
H:83	MET	ILE		
L:12	PRO	ALA		
H:10	GLY	ALA		
H:30	SER	ILE	-15.36	-12.36
H:31	SER	ARG		
H:40	ALA	THR		
H:52	THR	ILE		
L:12	PRO	SER		
H:10	GLY	ALA		
H:30	SER	ILE	-15.36	-7.31
H:31	SER	ARG		
H:40	ALA	SER		
H:52	THR	ILE		
L:12	PRO	SER		
H:10	GLY	VAL		
H:30	SER	ILE	-15.36	-7.14
H:31	SER	ARG		
H:40	ALA	SER		
H:52	THR	ILE		
L:12	PRO	SER		
L:45	PRO	ALA		
H:30	SER	ILE	-15.35	-5.15
H:31	SER	ARG		
H:40	ALA	SER		
H:52	THR	ILE		

Figure 3 (Continued)

Five Mutations						Six Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability		Residue	Original	Mutation	ΔAffinity	ΔStability
						L:12	PRO	SER		
						H:10	GLY	ALA		
						H:30	SER	ILE	-15.35	-12.26
						H:31	SER	ARG		
						H:40	ALA	THR		
						H:52	THR	ILE		
						L:12	PRO	ALA		
						H:30	SER	ILE		
						H:31	SER	ARG	-15.34	2.01
						H:40	ALA	SER		
						H:52	THR	ILE		
						H:83	MET	ILE		
						L:12	PRO	SER		
						H:30	SER	ILE		
						H:31	SER	ARG	-15.32	-2.58
						H:40	ALA	SER		
						H:52	THR	ILE		
						H:92	ALA	GLY		
						L:12	PRO	THR		
						L:27	GLN	ILE		
						H:30	SE5R	ILE	-15.31	-12.88
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		
						L:12	PRO	THR		
						L:27	GLN	ILE		
						H:30	SER	ILE	-15.31	4.6
						H:31	SER	ARG		
						H:40	ALA	PRO		
						H:52	THR	ILE		
						L:12	PRO	THR		
						L:27	GLN	ILE		
						H:30	SER	ILE	-15.3	-17.85
						H:31	SER	ARG		
						H:40	ALA	THR		
						H:52	THR	ILE		

Figure 3 (Continued)

Five Mutations						Six Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability		Residue	Original	Mutation	ΔAffinity	ΔStability
						L:12	PRO	SER		
						L:96	THR	ILE		
						H:10	GLY	VAL	-15.27	-3.35
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		
						L:12	PRO	SER		
						L:96	SER	ILE		
						H:10	THR	ILE	-15.26	-15.43
						H:31	SER	ARG		
						H:40	ALA	THR		
						H:52	THR	ILE		
						L:12	PRO	SER		
						L:96	THR	ILE		
						H:10	GLY	VAL	-15.26	-8.31
						H:31	SER	ARG		
						H:40	ALA	THR		
						H:52	THR	ILE		
						L:12	PRO	ALA		
						H:10	GLY	VAL		
						H:30	SER	ILE	-15.25	-7
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		
						L:12	PRO	THR		
						L:81	SER	THR		
						H:30	SER	ILE	-15.25	-10.28
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		
						L:12	PRO	THR		
						L:108	LYS	ASN		
						H:30	SER	ILE	-15.14	-11.18
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		

Figure 3 (Continued)

Five Mutations						Six Mutations				
Residue	Original	Mutation	ΔAffinity	ΔStability		Residue	Original	Mutation	ΔAffinity	ΔStability
						L:12	PRO	SER		
						L:51	LEU	VAL		
						H:30	SER	THR	-15.07	1.04
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		
						L:12	PRO	THR		
						L:51	LEU	VAL		
						H:30	SER	THR	-15.07	-1.15
						H:31	SER	ARG		
						H:40	ALA	SER		
						H:52	THR	ILE		

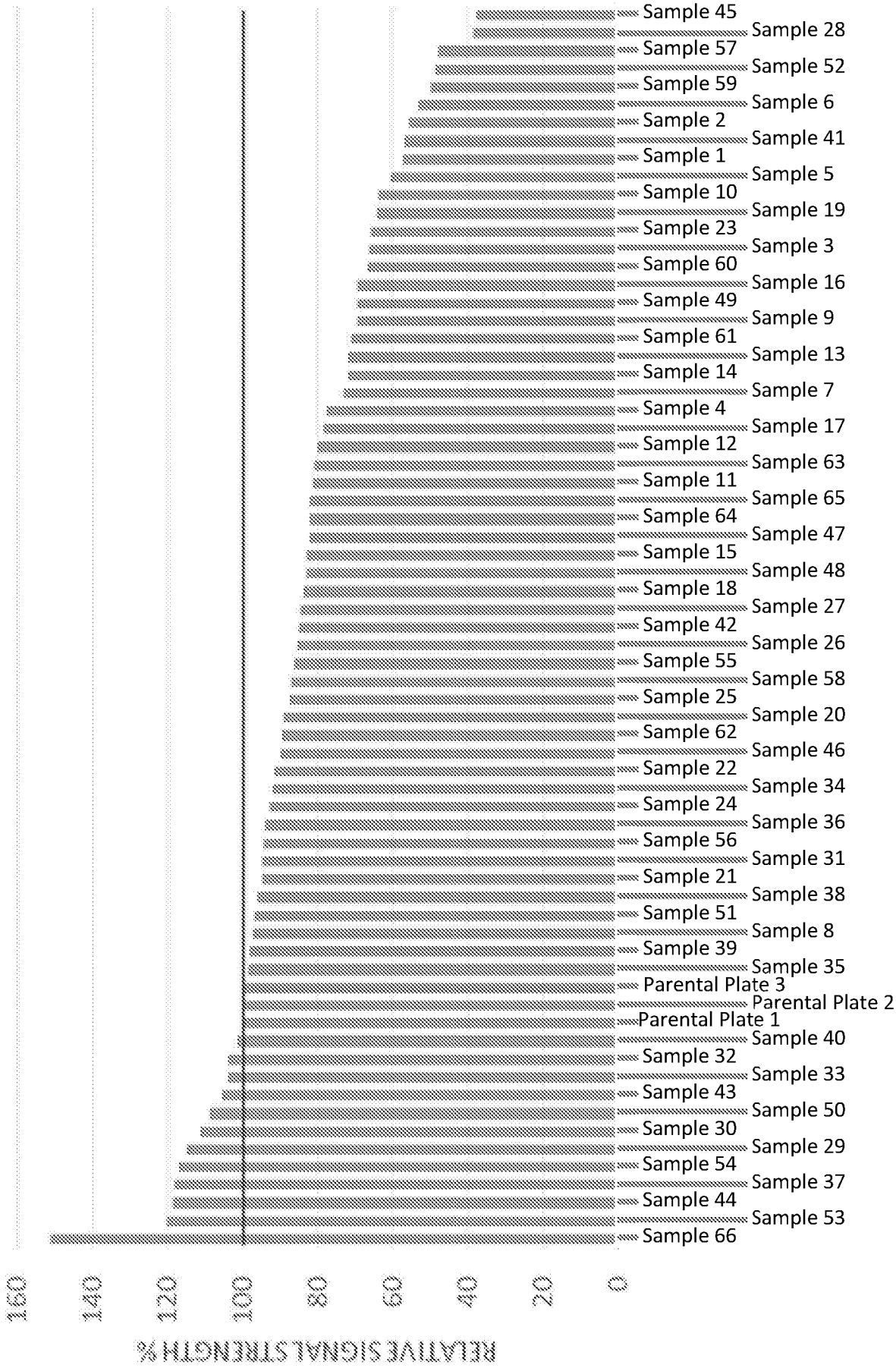

Sample 45
Sample 28
Sample 57
Sample 52
Sample 59
Sample 6
Sample 2
Sample 41
Sample 1
Sample 5
Sample 10
Sample 19
Sample 23
Sample 3
Sample 60
Sample 16
Sample 49
Sample 9
Sample 61
Sample 13
Sample 14
Sample 7
Sample 4
Sample 17
Sample 12
Sample 63
Sample 11
Sample 65
Sample 64
Sample 47
Sample 15
Sample 48
Sample 18
Sample 27
Sample 42
Sample 26
Sample 55
Sample 58
Sample 25
Sample 20
Sample 62
Sample 46
Sample 22
Sample 34
Sample 24
Sample 36
Sample 56
Sample 31
Sample 21
Sample 38
Sample 51
Sample 8
Sample 39
Sample 35
Parental Plate 3
Parental Plate 2
Parental Plate 1
Sample 40
Sample 32
Sample 33
Sample 43
Sample 50
Sample 30
Sample 29
Sample 54
Sample 37
Sample 44
Sample 53
Sample 66
160
140
120
100
80
60
40
20
0
RELATIVE SIGNAL STRENGTH %

Figure 4

Figure 5

	Sample ID	KD (M)	kon (1/Ms)	kdis (1/s)
Run 1	Fsn0503h	3.05E-09	3.96E+04	1.21E-04
	WT	*<1.0E-12*	4.51E+04	<1.0E-07
	Mut 1	3.63E-09	3.77E+04	1.37E-04
	Mut 2	*<1.0E-12*	2.02E+05	<1.0E-07
	Mut 3	*<1.0E-12*	8.72E+04	<1.0E-07
	Mut 4	*<1.0E-12*	2.96E+04	<1.0E-07
	Mut 5	**1.40E-09**	2.84E+04	3.99E-05
	Mut 6	1.75E-08	4.17E+04	7.31E-04
	Mut 7	4.88E-09	3.70E+04	1.80E-04
	Mut 8	7.69E-09	3.13E+04	2.41E-04
	Mut 9	5.66E-09	5.73E+04	2.11E-04
	Mut 10	8.01E-09	1.68E+04	1.34E-04
	Mut 11	*<1.0E-12*	4.28E+04	<1.0E-07
	Mut 12	8.42E-09	2.71E+04	2.28E-04
	Mut 13	7.31E-09	3.96E+04	2.89E-04
	Mut 14	7.28E-09	3.75E+04	2.73E-04
	Mut 15	6.04E-09	2.99E+04	1.80E-04
	Mut 16	6.24E-09	3.77E+04	2.35E-04
	Mut 17	3.10E-09	3.06E+04	1.13E-04
	Mut 18	5.03E-09	2.07E+04	1.04E-04
	Mut 19	*<1.0E-12*	1.78E+04	<1.0E-07

Sample ID	KD (M)	kon (1/Ms)	kdis (1/s)
Mut 20	**3.54E-10**	3.67E+04	1.30E-05
Mut 21	3.10E-09	3.16E+04	9.80E-05
Mut 22	4.55E-09	4.28E+04	1.95E-04
Mut 23	1.20E-08	2.53E+04	3.04E-04
Mut 24	1.48E-09	3.68E+04	5.44E-05
Mut 25	2.81E-09	3.83E+04	1.07E-04
Mut 26	*<1.0E-12*	1.17E+05	<1.0E-07
Mut 27	*<1.0E-12*	8.78E+04	<1.0E-07
Mut 29	4.78E-09	3.35E+04	1.60E-04
Mut 30	**2.80E-09**	3.80E+04	1.06E-04
Mut 31	5.33E-09	2.98E+04	1.59E-04
Mut 32	4.48E-09	4.27E+04	1.91E-04
Mut 33	3.13E-09	3.79E+04	1.18E-04
Mut 34	7.39E-09	3.99E+04	2.95E-04
Mut 35	6.82E-09	2.64E+04	1.80E-04
Mut 36	*<1.0E-12*	2.04E+05	<1.0E-07
Mut 37	5.53E-09	3.40E+04	1.88E-04
Mut 38	3.10E-09	4.34E+04	1.34E-04
Mut 39	3.92E-09	3.89E+04	1.52E-04
Mut 40	**3.03E-09**	3.81E+04	1.15E-04

	Sample ID	KD (M)	kon (1/Ms)	kdis (1/s)
Run 1	Mut 41	3.34E-09	2.72E+04	9.07E-05
	Mut 42	4.40E-09	4.11E+04	1.81E-04
	Mut 43	6.31E-09	1.93E+04	1.22E-04
	Mut 44	*<1.0E-12*	1.82E+05	<1.0E-07
	Mut 45	**1.56E-09**	1.74E+04	2.72E-05
	Mut 46	6.33E-09	1.84E+04	1.17E-04
	Mut 47	**2.55E-09**	1.22E+04	3.12E-05
	Mut 48	3.14E-09	2.09E+04	6.56E-05
	Mut 49	1.77E-08	2.38E+04	4.21E-04
	Mut 50	6.59E-09	1.96E+04	1.29E-04
	Mut 51	9.01E-09	3.73E+04	3.36E-04
	Mut 52	1.80E-08	2.21E+04	3.99E-04
	Fsn0503h	5.47E-09	2.62E+04	1.44E-04
	No hIgG	*<1.0E-12*	2.52E+05	<1.0E-07

	Sample ID	KD (M)	kon (1/Ms)	kdis (1/s)
Run 2	Fsn0503h	4.39E-09	4.28E+04	1.88E-04
	Mut 53	**4.04E-09**	3.83E+04	1.55E-04
	Mut 54	*<1.0E-12*	1.08E+05	<1.0E-07
	Mut 55	*<1.0E-12*	1.10E+05	<1.0E-07
	Mut 56	5.14E-09	4.02E+04	2.07E-04
	Mut 57	**4.49E-10**	3.79E+04	1.70E-05
	Mut 58	5.37E-09	1.69E+04	9.07E-05
	Mut 59	9.21E-09	1.87E+04	1.72E-04
	Mut 60	**2.89E-09**	2.33E+04	6.72E-05
	Mut 61	4.12E-08	3.15E+04	1.30E-03
	Mut 62	2.63E-08	1.16E+04	3.05E-04
	Mut 63	1.24E-08	1.27E+04	1.58E-04
	Mut 64	**2.78E-09**	3.57E+04	9.94E-05
	Mut 65	**2.63E-09**	1.50E+04	3.96E-05
	Mut 66	**2.31E-09**	3.44E+04	7.94E-05
	Fsn0503h	5.11E-09	4.73E-04	2.42E-04
	No hIgG	1.91E-08	1.81E+05	3.45E-03
	No hIgG	*<1.0E-12*	1.00E+05	<1.0E-07

Bold	= Affinity higher than best reading for Fsn0503h
Underline	= Fsn0503h control
Italic	= Affinity to be determined

Figure 6

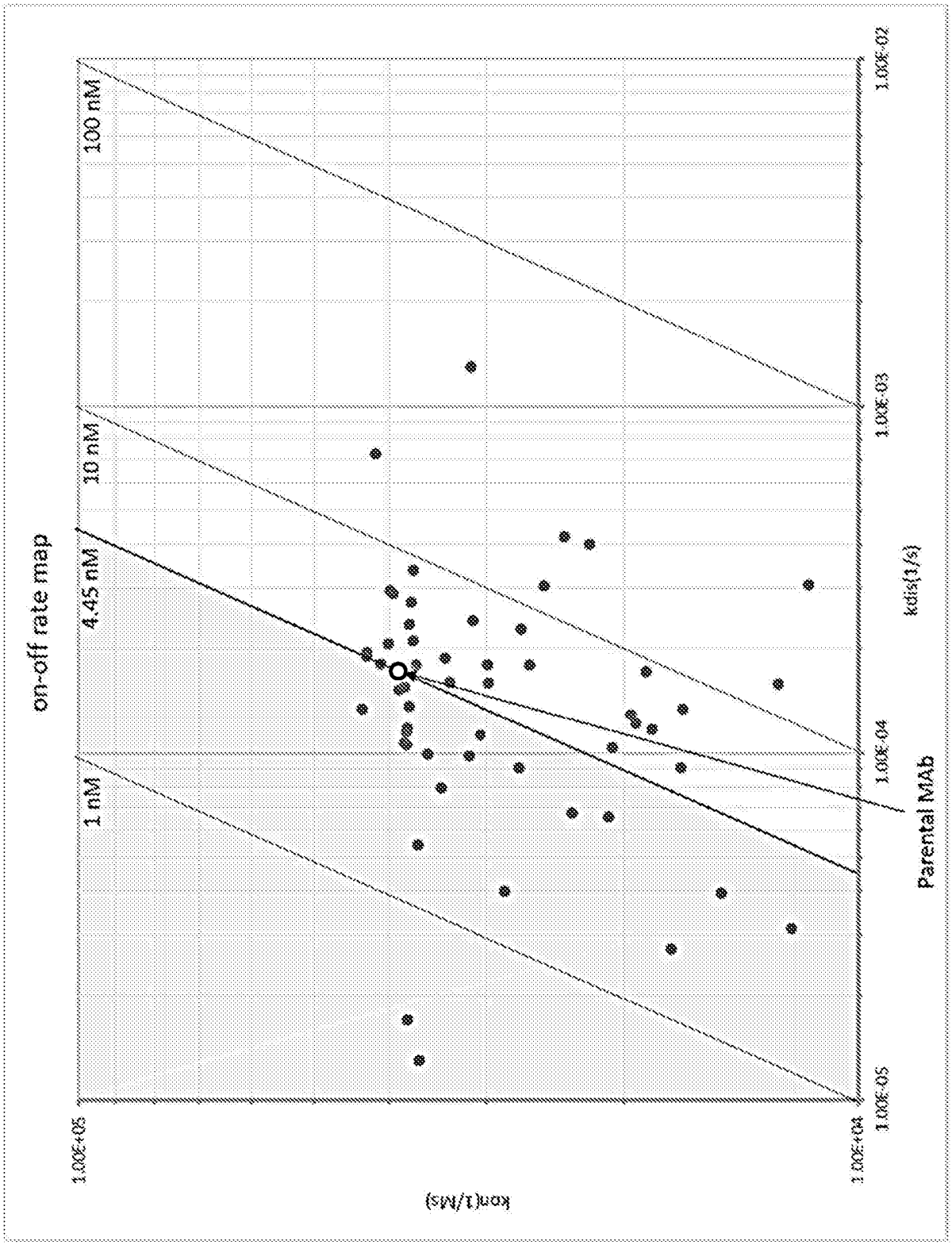

on-off rate map
100 nM
10 nM
4.45 nM
1 nM
1.00E+05
1.00E+04
1.00E-02
1.00E-03
1.00E-04
1.00E-05
kon(1/Ms)
koff(1/s)
Parental MAb

Figure 7

SEQ ID NO: 3

1		**D**										
2		**I**										
3		**Q**										
4		**M**										
5		**T**										
6		**Q**										
7		**S**										
8		**P**										
9		**S**	S	N	T	I	R	K				
10		**S**										
11		**L**										
12	**FR1**	**S**										
13		**A**										
14		**S**										
15		**V**										
16		**G**										
17		**D**										
18		**R**										
19		**V**										
20		**T**										
21		**I**										
22		**T**										
23		**C**	C	W	*							
24		**R**										
25		**A**	G	A	V	D						
26		**S**										
27		**Q**										
28		**D**										
29	**CDR1**	**V**										
30		**N**										
31		**T**	S	N	T	I						
32		**A**	A	D	G	V	T	N	S	I	P	L	F
33		**V**	L	I	V							
34		**A**	G	A	V	D						
35		**W**	C	W	STOP							
36		**Y**										
37		**Q**										
38		**Q**	E	STOP	Q	K						
39		**K**										
40		**P**	A	S	P	T						
41	**FR2**	**G**										
42		**K**										
43		**A**	A	D	G	V	T	N	S	I	P	L	F
44		**P**										
45		**K**										
46		**L**	V	L	I							
47		**L**	V	L	M							

Figure 7
(Continued)

48	FR2	I						
49		Y	Y	STOP				
50	CDR 2	S						
51		A	A	S	P	T		
52		S						
53		F						
54		L						
55		Y						
56		S						
57		G						
58		V						
59		P						
60		S						
61	FR3	R						
62		F						
63		S						
64		G						
65		S						
66		R						
67		S						
68		G						
69		T						
70		D						
71		F						
72		T						
73		L						
74		T						
75		I						
76		S	R	S	N	T	K	I
77		S						
78		L						
79		Q	K	E	Q	STOP		
80		P	T	S	P	A		
81		E						
82		D						
83		F						
84		A						
85		T	S	N	T	I		
86		Y						
87		Y	Y	STOP				
88		C						
89	CDR3	Q	Q	H				
90		Q	E	STOP	Q	A		
91		H	N	D	Y	H		
92		Y						
93		T	S	N	T	I		
94		T	S	N	T	I		

95		P				
96		P				
97	CDR3	T				
98	FR4	F				
99		G				
100		Q				
101		G	G	A	V	D
102		T	S	N	T	I
103		K				
104		V				
105		E				
106		I				
107		K				

Figure 7 (continued)

Figure 8

		SEQ ID NO: 4											
1		E											
2		V	V	L	I	F							
3		Q	Q	H									
4		L	M	V	L								
5		V											
6		E											
7		S											
8		G											
9		G											
10		G											
11		L											
12		V											
13		Q	K	E	Q	STOP							
14		P	P	A	T	S							
15	FR1	G											
16		G	G	A	V	D							
17		S											
18		L											
19		R											
20		L											
21		S											
22		C											
23		A	A	E	G	V	T	K	R	I	P	L	S
24		A	A	D	G	V	T	N	S	I	P	L	F
25		S											
26		G	G	A	V	D							
27		F											
28		N	K	N									
29		I											
30		K											
31		D											
32		T											
33	CDR1	Y											
34		I											
35		H	H	N	D	Y							
36		W											
37		V											
38		R											
39		Q											
40		A											
41		P											
42	FR2	G	G	A	V	D							
43		K											
44		G											
45		L											
46		E											
47		W											

Figure 8 (Continued)

48	FR2	V	F	L	V	I						
49		A	A	E	G	V	T	K	R	I	P	L	S
50	CDR2	R										
51		I										
52		Y										
53		P										
54		T										
55		N										
56		G	G	A	V	D						
57		Y										
58		T	S	N	T	I						
59		R										
60		Y										
61		A	G	A	V	D						
62		D										
63		S										
64		V										
65		K										
66		G										
67	FR3	R										
68		F										
69		T										
70		I										
71		S										
72		A										
73		D										
74		T										
75		S										
76		K										
77		N	K	N								
78		T										
79		A	G	A	V	D						
80		Y	Y	STOP								
81		L										
82		Q	E	K	Q	STOP						
83		M										
84		N	K	N								
85		S	R	S								
86		L										
87		R										
88		A	A	D	G	V	T	N	S	I	P	L	F
89		E										
90		D										
91		T										
92		A	G	A	V	D						
93		V										
94		Y										
95		Y										

96	FR3	C	F	S	Y	C						
97		S										
98		R										
99		W										
100	CDR3	G										
101		G										
102		D										
103		G	G	A	V	D						
104		F										
105		Y										
106		A	A	D	G	V	T	N	S	I	P	L	F
107		M										
108		D										
109		Y										
110	FR4	W										
111		G										
112		Q										
113		G										
114		T	S	N	T	I						
115		L										
116		V										
117		T										
118		V										
119		S										
120		S										

Figure 8 (Continued)

Figure 9

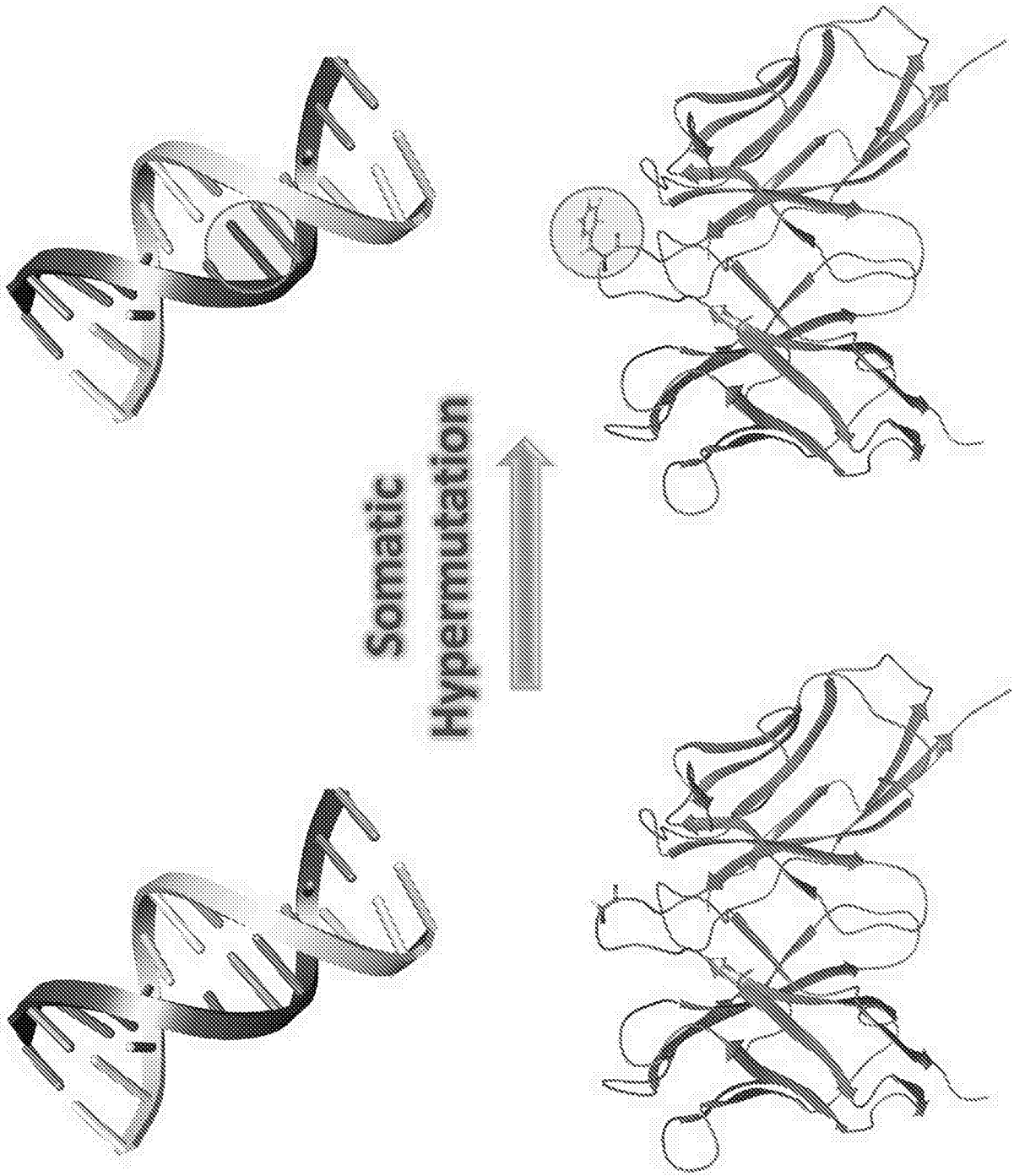


Figure 10

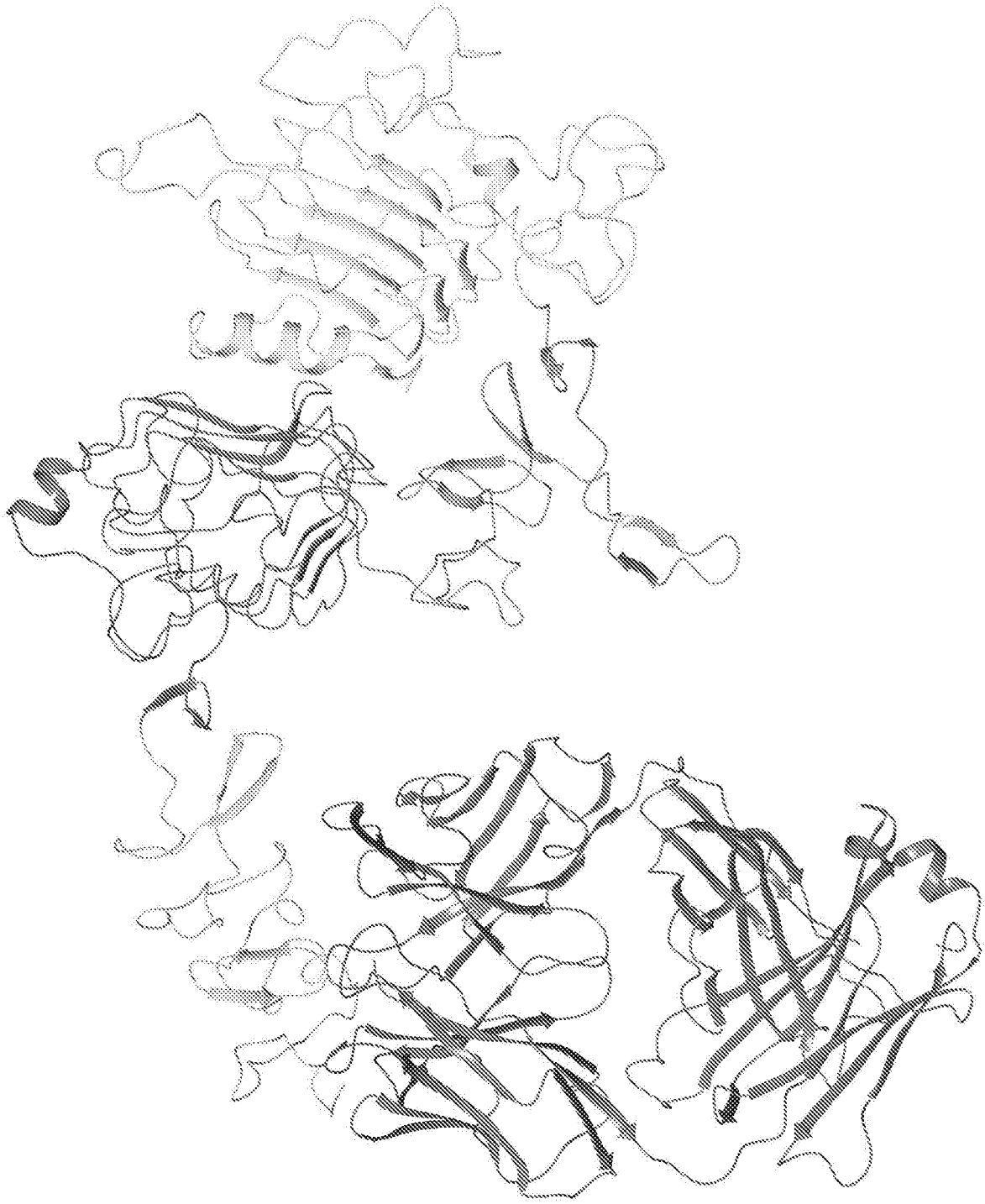


Figure 11A

1 Mutation

Variant Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
1	L:51	ALA	THR	**-4.33**	7.33
2	H:103	GLY	VAL	**-3.99**	19.62
3	L:31	THR	SER	**-3.56**	7.03
4	H:103	GLY	ALA	**-3.01**	1.12
5	L:25	ALA	VAL	-1.74	45.76
6	L:32	ALA	VAL	-1.42	37.08
7	L:93	THR	ILE	-0.91	***-6.9***
8	L:9	SER	LYS	-0.77	4.2
9	L:9	SER	ARG	-0.71	***-9.36***
10	L:93	THR	ASN	-0.49	6.78
11	L:9	SER	ILE	-0.34	***-5.4***
12	L:46	LEU	ILE	-0.28	11.43
13	H:85	SER	ARG	-0.25	***-10.35***
14	L:31	THR	ILE	-0.18	1.53
15	L:34	ALA	GLY	-0.17	5.6
16	H:114	THR	ILE	-0.17	***-9.08***
17	H:61	ALA	GLY	-0.16	4.56
18	L:51	ALA	PRO	-0.15	57.67
19	H:56	GLY	ALA	-0.15	0.43
20	H:79	ALA	GLY	-0.15	8.7
21	L:25	ALA	GLY	-0.11	8.48
22	L:46	LEU	VAL	-0.1	16.58
23	H:24	ALA	GLY	-0.1	6.2
24	H:79	ALA	VAL	-0.1	***-10.71***
25	H:114	THR	ASN	-0.07	-1.79
26	H:114	THR	SER	-0.06	0.82
27	L:47	LEU	VAL	-0.05	8.2
28	H:48	VAL	LEU	-0.05	10.23
29	L:101	GLY	VAL	-0.03	1.3
30	H:48	VAL	ILE	-0.02	0.64
31	L:43	ALA	GLY	-0.01	4.12
32	L:9	SER	THR	0	***-4.63***
33	L:51	ALA	SER	0	2.2
34	L:76	SER	THR	0	***-6.43***
35	L:80	PRO	THR	0	***-4.29***
36	L:80	PRO	SER	0	0.3
37	H:4	LEU	VAL	0	15.82
38	H:14	PRO	THR	0	1.28
39	H:14	PRO	ALA	0	6.03
40	H:14	PRO	SER	0	6.35
41	H:88	ALA	VAL	0	***-3.97***
42	H:88	ALA	GLY	0	2.56
43	L:40	PRO	THR	0.01	8.27
44	L:40	PRO	SER	0.01	6.92
45	L:80	PRO	ALA	0.01	2.89
46	L:101	GLY	ALA	0.01	3.6
47	H:16	GLY	ALA	0.01	1.65
48	L:40	PRO	ALA	0.02	6.79
49	L:43	ALA	PRO	0.03	***-2.02***
50	L:85	THR	SER	0.03	5.27
51	H:2	VAL	LEU	0.03	12.41
52	H:16	GLY	VAL	0.03	4.11
53	H:58	THR	SER	0.03	6.62
54	H:92	ALA	GLY	0.03	6.24
55	L:43	ALA	ASN	0.04	***-7.99***
56	H:2	VAL	ILE	0.04	***-2.26***
57	H:61	ALA	VAL	0.04	-1.73
58	L:102	THR	ILE	0.05	***-2.42***
59	L:43	ALA	PHE	0.06	0.82
60	H:3	GLN	HID	0.07	6.94
61	H:56	GLY	VAL	0.07	-1.4
62	L:76	SER	ILE	0.08	***-10.69***

Figure 11A (Continued)

63	L:93	THR	SER	0.08	2.35
64	L:102	THR	ASN	0.08	6.08
65	H:48	VAL	PHE	0.08	119.92
66	L:85	THR	ILE	0.09	***-5.15***
67	L:89	GLN	HID	0.09	13.16
68	H:24	ALA	PRO	0.09	32.5
69	H:4	LEU	MET	0.1	***-5.89***
70	L:34	ALA	VAL	0.11	9.25
71	H:26	GLY	ALA	0.11	***-7.27***
72	H:92	ALA	VAL	0.11	47.33
73	L:85	THR	ASN	0.14	3.85
74	H:106	ALA	GLY	0.15	7.17
75	L:102	THR	SER	0.16	0.32
76	L:76	SER	ASN	0.19	***-3.06***
77	H:26	GLY	VAL	0.21	***-7.3***
78	H:88	ALA	ASP	0.3	-0.16
79	H:58	THR	ASN	0.36	1.07
80	H:24	ALA	PHE	0.39	78.02
81	H:58	THR	ILE	0.49	2.2
82	H:24	ALA	ASN	0.5	17.06
83	H:26	GLY	ASP	0.53	***-7.85***
84	H:92	ALA	ASP	0.62	28.05
85	H:106	ALA	VAL	0.68	8.45
86	L:101	GLY	ASP	0.69	11.64
87	H:61	ALA	ASP	0.9	6.52
88	H:56	GLY	ASP	1.26	3.08
89	L:32	ALA	GLY	1.75	11.73
90	H:79	ALA	ASP	1.84	21.41
91	L:31	THR	ASN	1.91	0.6
92	L:25	ALA	ASP	2.84	86.7
93	H:106	ALA	ASP	3.94	28.94
94	L:34	ALA	ASP	4.24	36.04
95	L:32	ALA	ASP	11.37	16.31
96	H:103	GLY	ASP	12.53	16.85

Figure 11B

2 Mutations

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
1	L:9 L:43	SER ALA	LYS PHE	**-26.91**	66.79
2	L:9 L:43	SER ALA	THR PHE	**-26.08**	62.78
3	L:9 H:103	SER GLY	ARG VAL	**-24.17**	33.13
4	L:9 H:103	SER GLY	LYS VAL	**-23.96**	43.24
5	L:9 H:103	SER GLY	THR VAL	**-23.23**	35.33
6	L:9 H:103	SER GLY	ILE VAL	**-23.21**	35.04
7	L:9 H:103	SER GLY	LYS ALA	**-20.26**	1.22
8	L:9 H:103	SER GLY	ARG ALA	**-20.01**	**-11.42**
9	L:9 L:102	SER THR	THR ASN	**-18.24**	37.61
10	L:9 L:40	SER PRO	ARG ALA	**-18.91**	25.27
11	L:9 H:103	SER GLY	THR ALA	**-18.59**	**-5.37**
12	L:9 H:103	SER GLY	ILE ALA	**-18.57**	**-6.91**
13	L:9 L:32	SER ALA	THR VAL	**-18.32**	48.63
14	L:9 L:102	SER THR	THR ASN	**-18.24**	37.61
15	L:9 L:102	SER THR	ILE ASN	**-18.22**	37.32
16	L:9	SER	THR	**-18.2**	23.83
17	L:9	SER	ILE	**-18.19**	23.47
18	L:9 L:40	SER PRO	ILE ALA	**-18.18**	30.15
19	L:9 H:2	SER VAL	THR LEU	**-18.1**	27.69
20	L:9 L:93	SER THR	ARG ASN	**-17.86**	23.9
21	L:9 L:32	SER ALA	LYS VAL	**-17.51**	**-30.41**
22	L:9 L:32	SER ALA	ARG VAL	**-17.46**	**-42.8**
23	L:9 L:80	SER PRO	ARG ALA	**-16.83**	**-74.92**
24	L:9 L:51	SER ALA	ARG SER	**-16.81**	56.69
25	L:9 L:51	SER ALA	LYS PRO	**-16.7**	55.02
26	L:9 H:92	SER ALA	LYS VAL	**-16.64**	**-43.9**
27	L:9 H:14	SER PRO	LYS SER	**-16.6**	**-51.7**
28	L:9 H:79	SER ALA	ARG GLY	**-16.58**	**-60.45**
29	L:9 L:43	SER ALA	ARG ASN	**-16.58**	**-84.28**
30	L:9 L:25	SER ALA	ARG GLY	**-16.56**	69.73

Figure 11B (Continued)

31	L:9 L:93	SER THR	ARG SER	**-16.55**	**-69.14**
32	L:9 H:16	SER GLY	LYS ALA	**-16.55**	**-58.01**
33	L:9 L:93	SER THR	ARG ILE	**-16.55**	**-21.78**
34	L:9 H:58	SER THR	ILE SER	**-16.54**	**-63.79**
35	L:9 L:93	SER THR	THR ILE	**-16.53**	**-73.77**
36	L:9 L:102	SER THR	ARG ASN	**-16.53**	**-54.45**
37	L:9 H:48	SER VAL	LYS ILE	**-16.52**	**-54.76**
38	L:9 H:56	SER GLY	LYS ALA	**-16.51**	**-64.22**
39	L:9 L:47	SER LEU	LYS VAL	**-16.5**	**-51.96**
40	L:9 L:76	SER SER	ARG THR	**-16.5**	**-79.57**
41	L:9 L:101	SER GLY	ARG ALA	**-16.5**	**-71.42**
42	L:9 H:56	SER GLY	ILE ALA	**-16.49**	**-69.38**
43	L:9 H:2	SER VAL	ARG ILE	**-16.45**	**-75.03**
44	L:9 H:114	SER THR	ARG SER	**-16.44**	**-66.76**
45	L:9 H:14	SER PRO	ARG ALA	**-16.44**	**-71.28**
46	L:9 L:43	SER ALA	ARG PRO	**-16.43**	**-76.51**
47	L:9 H:61	SER ALA	LYS VAL	**-16.39**	**-60.92**
48	L:9 H:61	SER ALA	ARG VAL	**-16.38**	**-74.21**
49	L:9 L:43	SER ALA	ARG PHE	**-16.33**	60.52
50	L:9 H:58	SER THR	ARG ILE	**-16.32**	**-72.44**
51	L:9 L:31	SER THR	LYS SER	**-16.31**	**-54.16**
52	L:9 L:31	SER THR	ARG SER	**-16.29**	**-67.24**
53	L:9 H:88	SER ALA	LYS ASP	**-16.28**	**-61.14**
54	L:9 H:24	SER ALA	LYS ASN	**-16.25**	**-57.56**
55	L:9 H:58	SER THR	ARG SER	**-16.23**	1.68
56	L:9 L:46	SER LEU	ARG VAL	**-16.21**	12.27
57	L:9 H:24	SER ALA	ARG ASN	**-16.19**	**-69.82**
58	L:9 L:51	SER ALA	LYS THR	**-16.19**	**-9.02**
59	L:9 H:106	SER ALA	LYS VAL	**-16.16**	**-54.48**
60	L:9 H:114	SER THR	LYS ILE	**-16.16**	**-12.53**
61	L:9 L:93	SER THR	ILE ILE	**-16.16**	**-76.44**

Figure 11B (Continued)

62	L:9 H:88	SER ALA	THR VAL	**-16.14**	**-76.29**
63	L:9 L:51	SER ALA	ARG THR	**-16.12**	**-21.44**
64	L:9 L:101	SER GLY	THR ALA	**-16.12**	**-70.99**
65	L:9 L:34	SER ALA	ARG VAL	**-16.09**	6.58
66	L:9 L:51	SER ALA	THR PRO	**-16.09**	48.2
67	L:9 H:61	SER ALA	ARG GLY	**-16.08**	0.92
68	L:9 H:61	SER ALA	LYS GLY	**-16.06**	12.12
69	L:9 H:79	SER ALA	ARG VAL	**-16.06**	**-19.38**
70	L:9 L:85	SER THR	THR ILE	**-16.05**	**-78.22**
71	L:9 H:106	SER ALA	LYS GLY	**-16.05**	19.57
72	L:9 H:114	SER THR	LYS ASN	**-16.04**	**-5.16**
73	L:9 H:114	SER THR	ARG ASN	**-16.02**	**-17.55**
74	L:9 L:93	SER THR	LYS ILE	**-16.02**	**-13.85**
75	L:9 L:43	SER ALA	LYS GLY	**-16.01**	1.02
76	L:9 L:76	SER SER	LYS THR	**-16**	**-9.72**
77	L:9	SER	LYS	**-16**	**-3.24**
78	L:9 L:25	SER ALA	LYS VAL	**-16**	62.99
79	L:9 H:58	SER THR	LYS SER	**-15.99**	2.62
80	L:9 L:85	SER THR	LYS SER	**-15.98**	**-4.34**
81	L:9 L:85	SER THR	ARG SER	**-15.97**	**-16.83**
82	L:9 H:14	SER PRO	LYS THR	**-15.96**	**-3.27**
83	L:9 L:46	SER LEU	ILE ILE	**-15.96**	**-60.4**
84	L:9 L:80	SER PRO	ARG THR	**-15.96**	**-27.85**
85	L:9 L:43	SER ALA	ARG GLY	**-15.95**	**-11.52**
86	L:9 H:26	SER GLY	ARG VAL	**-15.94**	4.91
87	L:9 H:48	SER VAL	LYS LEU	**-15.93**	13.32
88	L:9 H:79	SER ALA	LYS VAL	**-15.93**	**-4.21**
89	L:9 H:48	SER VAL	ARG ILE	**-15.93**	**-17.62**
90	L:9 L:85	SER THR	ARG ILE	**-15.92**	**-8.89**
91	L:9 H:58	SER THR	THR SER	**-15.9**	**-61.71**
92	L:9 H:48	SER VAL	ARG PHE	**-15.89**	133.57
93	L:9 L:25	SER ALA	THR GLY	**-15.89**	**-54.93**

Figure 11B (Continued)

94	L:9 H:4	SER LEU	ARG MET	**-15.88**	**-4.6**
95	L:9 L:85	SER THR	LYS ILE	**-15.88**	**-9.08**
96	L:9 H:56	SER GLY	THR ALA	**-15.86**	**-67.29**
97	L:9 L:76	SER SER	ILE THR	**-15.86**	**-18.81**
98	L:9 L:93	SER THR	THR SER	**-15.86**	**-64.34**
99	L:9 H:14	SER PRO	THR SER	**-15.84**	**-58.59**
100	L:9 L:80	SER PRO	ILE SER	**-15.81**	**-69.43**
101	L:9 L:89	SER GLN	LYS HID	**-15.81**	3.09
102	L:9 H:48	SER VAL	THR LEU	**-15.81**	**-51.33**
103	L:9 H:16	SER GLY	THR ALA	**-15.79**	**-64.81**
104	L:9 L:43	SER ALA	THR PRO	**-15.79**	**-73.51**
105	L:9 H:3	SER GLN	LYS HID	**-15.78**	75.97
106	L:9 H:92	SER ALA	ILE VAL	**-15.78**	**-52.87**
107	L:9 L:80	SER PRO	THR ALA	**-15.78**	**-64.55**
108	L:9 L:93	SER THR	ILE SER	**-15.76**	**-63.25**
109	L:9 H:88	SER ALA	ILE VAL	**-15.76**	**-73.28**
110	L:9 L:43	SER ALA	ILE PRO	**-15.76**	**-75.35**
111	L:9 H:58	SER THR	LYS ILE	**-15.76**	-1.84
112	L:9 L:46	SER LEU	LYS VAL	**-15.75**	83.94
113	L:9 L:40	SER PRO	ILE SER	**-15.74**	**-62.95**
114	L:9 H:79	SER ALA	LYS GLY	**-15.72**	74.64
115	L:9 H:3	SER GLN	ARG HID	**-15.72**	64
116	L:9 L:25	SER ALA	ARG VAL	**-15.7**	42.01
117	L:9 L:25	SER ALA	LYS GLY	**-15.69**	75.85
118	L:9 H:4	SER LEU	LYS MET	**-15.68**	64.29
119	L:9 L:43	SER ALA	LYS ASN	**-15.67**	59.33
120	L:9 L:40	SER PRO	LYS THR	**-15.64**	75.96
121	L:9 H:24	SER ALA	LYS GLY	**-15.63**	73.96
122	L:9 H:56	SER GLY	ARG ALA	**-15.63**	63.88
123	L:9 L:46	SER LEU	ARG ILE	**-15.62**	69.38
124	L:9 H:24	SER ALA	ARG GLY	**-15.62**	61.59

Figure 11B (Continued)

125	L:9 H:14	SER PRO	ARG SER	**-15.61**	63.52
126	L:9 H:88	SER ALA	ARG GLY	**-15.61**	57.74
127	L:9 L:80	SER PRO	LYS SER	**-15.6**	67.39
128	L:9 H:56	SER GLY	LYS VAL	**-15.59**	**-4.12**
129	L:9 L:80	SER PRO	LYS THR	**-15.58**	**-6.83**
130	L:9 H:16	SER GLY	ARG ALA	**-15.58**	52.51
131	L:9 L:76	SER SER	ARG ILE	**-15.58**	44.51
132	L:9 L:102	SER THR	LYS ILE	**-15.58**	81.18
133	L:9 H:48	SER VAL	ARG LEU	**-15.58**	63.85
134	L:9 L:34	SER ALA	LYS GLY	**-15.58**	4.93
135	L:9 H:92	SER ALA	LYS GLY	**-15.57**	77.1
136	L:9 H:16	SER GLY	ARG VAL	**-15.57**	51.75
137	L:9 L:102	SER THR	ARG ILE	**-15.57**	68.15
138	L:9 L:34	SER ALA	ARG GLY	**-15.57**	**-7.48**
139	L:9 H:2	SER VAL	ARG LEU	**-15.56**	69.54
140	L:9 L:47	SER LEU	ARG VAL	**-15.56**	63.8
141	L:9 L:40	SER PRO	LYS SER	**-15.56**	77.58
142	L:9 L:80	SER PRO	ARG SER	**-15.56**	**-14.69**
143	L:9 H:26	SER GLY	LYS VAL	**-15.56**	68.81
144	L:9 H:85	SER SER	LYS ARG	**-15.55**	**-2.09**
145	L:9 L:76	SER SER	LYS ASN	**-15.55**	64.54
146	L:9 L:40	SER PRO	ARG SER	**-15.55**	65.26
147	L:9 L:89	SER GLN	ILE HID	**-15.55**	0.8
148	L:9 H:4	SER LEU	ARG VAL	**-15.55**	73.02
149	L:9 L:85	SER THR	LYS ASN	**-15.55**	73.89
150	L:9 H:16	SER GLY	LYS VAL	**-15.55**	**-5.53**
151	L:9 H:24	SER ALA	ARG PRO	**-15.54**	6.7
152	L:9 L:40	SER PRO	LYS ALA	**-15.53**	74.11
153	L:9 L:85	SER THR	ARG ASN	**-15.53**	**-11.09**

Figure 11C

3 Mutations

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
1	L:9 L:43 H:114	SER ALA THR	ARG PHE SER	-31.23	-36.46
2	L:9 L:43 H:114	SER ALA THR	THR PHE ASN	-30.47	-78.74
3	L:9 L:43 H:114	SER ALA THR	THR PHE ILE	-29.97	-76.82
4	L:9 L:43 H:106	SER ALA ALA	LYS PHE VAL	-28.37	-45.98
5	L:9 L:43 H:114	SER ALA THR	LYS PHE SER	-27.14	-21.57
6	L:9 L:43 H:114	SER ALA THR	THR PHE SER	-26.36	-37.27
7	L:9 L:43 H:106	SER ALA ALA	LYS PHE GLY	-25.73	-25.84
8	L:9 H:103 H:114	SER GLY THR	LYS ALA ASN	-24.74	-62.52
9	L:9 H:103 H:114	SER GLY THR	LYS ALA SER	-24.69	-56.48
10	L:9 H:103 H:106	SER GLY ALA	LYS ALA GLY	-24.39	-31.96
11	L:9 H:103 H:106	SER GLY ALA	ARG ALA VAL	-24.23	-44.64
12	L:9 H:103 H:114	SER GLY THR	ARG ALA SER	-23.91	-67.4
13	L:9 H:103 H:114	SER GLY THR	ARG ALA ILE	-23.83	-77.36
14	L:9 H:103 H:114	SER GLY THR	ARG ALA ASN	-23.61	-73.47
15	L:9 H:103 H:106	SER GLY ALA	ARG ALA GLY	-23.51	-43.94
16	L:9 H:103 H:106	SER GLY ALA	ILE ALA GLY	-23.44	-41.02
17	L:9 H:103 H:114	SER GLY THR	LYS ALA ILE	-23.39	-67.34
18	L:9 H:103 H:106	SER GLY ALA	LYS ALA VAL	-23.27	-35.88
19	L:9 H:103 H:106	SER GLY ALA	ILE ALA VAL	-23.14	-44.47
20	L:9 H:103 H:114	SER GLY THR	ILE ALA ASN	-23.13	-69.27

Figure 11C (Continued)

21	L:9 L:51 H:114	SER ALA THR	ARG THR SER	**-22.85**	**-32.94**
22	L:9 H:103 H:106	SER GLY ALA	THR ALA GLY	**-22.81**	**-38.94**
23	L:9 H:103 H:114	SER GLY THR	THR ALA ILE	**-22.68**	**-74.57**
24	L:9 H:103 H:114	SER GLY THR	ILE ALA ILE	**-22.54**	**-78.31**
25	L:9 H:103 H:114	SER GLY THR	THR ALA ASN	**-22.53**	**-67.13**
26	L:9 H:103 H:114	SER GLY THR	THR ALA SER	**-22.52**	**-64.56**
27	L:9 H:103 H:106	SER GLY ALA	THR ALA VAL	**-22.51**	**-42.54**
28	L:9 H:103 H:114	SER GLY THR	ILE ALA SER	**-22.49**	**-66.44**
29	L:9 H:114	SER THR	LYS ASN	**-22.18**	**-19.52**
30	L:9 H:24 H:114	SER ALA THR	ARG PRO ASN	**-22.16**	**-17.48**
31	L:9 L:76 H:114	SER SER THR	LYS ASN ASN	**-22.13**	**-35.83**
32	L:9 L:51 H:114	SER ALA THR	ARG THR ASN	**-22.09**	**-67.18**
33	L:9 H:92 H:114	SER ALA THR	ARG VAL SER	**-21.9**	**-21.63**
34	L:9 H:114	SER THR	ARG ASN	**-21.83**	**-42.75**
35	L:9 H:92 H:114	SER ALA THR	LYS ASP SER	**-21.81**	**-3.58**
36	L:9 L:46 H:114	SER LEU THR	ARG VAL ASN	**-21.75**	**-54.78**
37	L:9 H:2 H:114	SER VAL THR	LYS ILE ASN	**-21.74**	**-34.8**
38	L:9 L:47 H:114	SER LEU THR	ARG VAL ILE	**-21.68**	**-43.52**
39	L:9 H:24 H:114	SER ALA THR	LYS GLY ILE	**-21.64**	**-34.99**
40	L:9 L:51 H:114	SER ALA THR	LYS THR ASN	**-21.6**	**-59.8**
41	L:9 L:102 H:114	SER THR THR	LYS ASN ILE	**-21.58**	**-56.69**

Figure 11C (Continued)

42	L:9 L:32 H:114	SER ALA THR	LYS VAL ASN	**-21.57**	**-37.67**
43	L:9 L:34 H:114	SER ALA THR	LYS VAL SER	**-21.53**	**-18.63**
44	L:9 H:24 H:114	SER ALA THR	LYS GLY ASN	**-21.49**	**-27.52**
45	L:9 L:34 H:114	SER ALA THR	LYS VAL ASN	**-21.47**	**-9.63**
46	L:9 L:34 H:114	SER ALA THR	ARG VAL ILE	**-21.47**	**-46.2**
47	L:9 H:48 H:114	SER VAL THR	ARG PHE ILE	**-21.46**	**-18.79**
48	L:9 L:25 H:114	SER ALA THR	LYS GLY ILE	**-21.45**	**-32.92**
49	L:9 H:114	SER THR	LYS SER	**-21.43**	**-30.02**
50	L:9 H:24 H:114	SER ALA THR	LYS GLY SER	**-21.42**	**-23.34**
51	L:9 H:3 H:114	SER GLN THR	ARG HID ASN	**-21.42**	**-40.42**
52	L:9 L:34 H:114	SER ALA THR	ARG GLY ILE	**-21.36**	**-49.09**
53	L:9 L:43 H:114	SER ALA THR	LYS PRO ASN	**-21.34**	**-36.84**
54	L:9 H:114	SER THR	LYS ILE	**-21.33**	**-41.62**
55	L:9 H:85 H:114	SER SER THR	LYS ARG ILE	**-21.33**	**-33.57**
56	L:9 L:47 H:114	SER LEU THR	LYS VAL SER	**-21.33**	**-19.04**
57	L:9 L:47 H:114	SER LEU THR	ARG VAL ASN	**-21.32**	**-35.6**
58	L:9 L:80 H:114	SER PRO THR	ARG THR ASN	**-21.32**	**-50.76**
59	L:9 L:47 H:114	SER LEU THR	ARG VAL SER	**-21.31**	**-33.05**
60	L:9 L:46 H:114	SER LEU THR	LYS VAL ILE	**-21.31**	**-23.06**
61	L:9 L:40 H:114	SER PRO THR	LYS SER SER	**-21.31**	**-23.36**
62	L:9 L:47 H:114	SER LEU THR	LYS VAL ILE	**-21.31**	**-30.61**

Figure 11C (Continued)

63	L:9 H:4 H:114	SER LEU THR	LYS VAL SER	**-21.31**	**-16.24**
64	L:9 H:48 H:114	SER VAL THR	LYS PHE SER	**-21.31**	5.13
65	L:9 H:56 H:114	SER GLY THR	ARG ALA ILE	**-21.3**	**-78.22**
66	L:9 H:48 H:114	SER VAL THR	LYS PHE ILE	**-21.29**	5.19
67	L:9 L:102 H:114	SER THR THR	LYS ILE ILE	**-21.29**	**-14.8**
68	L:9 H:48 H:114	SER VAL THR	LYS LEU SER	**-21.29**	**-28.52**
69	L:9 H:92 H:114	SER ALA THR	LYS GLY SER	**-21.29**	**-17.89**
70	L:9 H:2 H:114	SER VAL THR	LYS ILE SER	**-21.28**	**-29.52**
71	L:9 H:48 H:114	SER VAL THR	ARG LEU SER	**-21.28**	**-40.34**
72	L:9 L:51 H:114	SER ALA THR	ILE SER SER	**-21.28**	**-43.45**
73	L:9 H:48 H:114	SER VAL THR	LYS LEU ILE	**-21.27**	**-40.08**
74	L:9 H:92 H:114	SER ALA THR	LYS GLY ILE	**-21.27**	**-29.49**
75	L:9 H:88 H:114	SER ALA THR	LYS GLY ASN	**-21.26**	**-16.12**
76	L:9 H:48 H:114	SER VAL THR	ARG PHE SER	**-21.24**	**-7.2**
77	L:9 H:114	SER THR	ARG SER	**-21.24**	**-39.89**
78	L:9 L:51 H:114	SER ALA THR	LYS THR ILE	**-21.23**	**-65.88**
79	L:9 H:114	SER THR	ARG ILE	**-21.22**	**-51.47**
80	L:9 H:92 H:114	SER ALA THR	ARG GLY SER	**-21.22**	**-30.22**
81	L:9 L:85 H:114	SER THR THR	ARG ASN ASN	**-21.22**	**-42.91**
82	L:9 H:2 H:114	SER VAL THR	ARG ILE SER	**-21.21**	**-41.86**
83	L:9 H:92 H:114	SER ALA THR	ARG GLY ILE	**-21.21**	**-41.81**

Figure 11C (Continued)

84	L:9 L:76 H:114	SER SER THR	LYS THR ASN	**-21.21**	**-25.66**
85	L:9 L:51 H:114	SER ALA THR	ARG PRO ILE	**-21.17**	-1.63
86	L:9 H:16 H:114	SER GLY THR	LYS ALA SER	**-21.16**	**-18.68**
87	L:9 H:3 H:114	SER GLN THR	LYS HID ILE	**-21.15**	**-34.4**
88	L:9 H:24 H:114	SER ALA THR	THR GLY ILE	**-21.14**	**-42.13**
89	L:9 L:47 H:114	SER LEU THR	LYS VAL ASN	**-21.12**	**-57.41**
90	L:9 L:51 H:114	SER ALA THR	LYS THR SER	**-21.11**	**-54.3**
91	L:9 H:3 H:114	SER GLN THR	LYS HID ASN	**-21.11**	**-27.04**
92	L:9 L:43 H:114	SER ALA THR	ARG PHE ASN	**-21.1**	**-75.76**
93	L:9 L:40 H:114	SER PRO THR	LYS SER ASN	**-21.09**	**-59.52**
94	L:9 L:25 H:114	SER ALA THR	LYS GLY SER	**-21.09**	**-22.45**
95	L:9 L:31 H:114	SER THR THR	LYS SER SER	**-21.09**	**-24.73**
96	L:9 H:79 H:114	SER ALA THR	LYS GLY SER	**-21.07**	**-23.51**
97	L:9 L:31 H:114	SER THR THR	ARG SER ILE	**-21.06**	**-51.18**
98	L:9 L:51 H:114	SER ALA THR	ARG THR ILE	**-21.06**	**-77.98**
99	L:9 H:16 H:114	SER GLY THR	LYS ALA ILE	**-21.06**	**-72.79**
100	L:9 L:102 H:114	SER THR THR	LYS SER ASN	**-21.05**	**-28.72**
101	L:9 L:51 H:114	SER ALA THR	ARG SER ASN	**-21.04**	**-89.85**
102	L:9 L:102 H:114	SER THR THR	ARG ILE ILE	**-21.04**	**-70.46**
103	L:9 L:25 H:114	SER ALA THR	LYS VAL ILE	**-21.03**	14.9

Figure 11C (Continued)

104	L:9 L:51 H:114	SER ALA THR	ARG SER SER	**-21.03**	**-87.23**
105	L:9 H:58 H:114	SER THR THR	LYS SER ILE	**-21.03**	**-38.04**
106	L:9 L:51 H:114	SER ALA THR	LYS SER ILE	**-21.02**	**-84.87**
107	L:9 L:34 H:114	SER ALA THR	LYS GLY SER	**-21.02**	**-21.01**
108	L:9 L:34 H:114	SER ALA THR	ARG GLY ASN	**-21.01**	**-41.09**
109	L:9 L:76 H:114	SER SER THR	ARG THR SER	**-21.01**	**-87.7**
110	L:9 L:102 H:114	SER THR THR	LYS ILE ASN	**-21**	**-20.09**
111	L:9 L:34 H:114	SER ALA THR	ARG GLY SER	**-21**	**-38.62**
112	L:9 H:61 H:114	SER ALA THR	LYS GLY ASN	**-21**	**-26.71**
113	L:9 L:40 H:114	SER PRO THR	LYS ALA SER	**-20.99**	**-21.92**
114	L:9 L:102 H:114	SER THR THR	LYS ILE SER	**-20.99**	**-17.55**
115	L:9 H:26 H:114	SER GLY THR	ARG VAL ILE	**-20.99**	**-54.75**
116	L:9 L:85 H:114	SER THR THR	LYS SER ILE	**-20.98**	**-37.13**
117	L:9 L:102 H:114	SER THR THR	ARG SER ASN	**-20.98**	**-40.92**
118	L:9 L:40 H:114	SER PRO THR	LYS ALA ILE	**-20.98**	**-33.49**
119	L:9 H:48 H:114	SER VAL THR	ARG PHE ASN	**-20.98**	**-15.82**
120	L:9 H:58 H:114	SER THR THR	ARG ILE ASN	**-20.98**	**-74.03**
121	L:9 L:80 H:114	SER PRO THR	LYS THR ASN	**-20.98**	**-37.74**
122	L:9 H:3 H:114	SER GLN THR	ARG HID SER	**-20.98**	**-35.12**
123	L:9 L:43 H:114	SER ALA THR	LYS PRO SER	**-20.98**	**-30.74**

Figure 11C (Continued)

124	L:9 L:51 H:114	SER ALA THR	ARG SER ILE	**-20.96**	**-97.11**
125	L:9 H:79 H:114	SER ALA THR	LYS GLY ASN	**-20.91**	**-63.43**
126	L:9 L:25 H:114	SER ALA THR	ARG VAL ASN	**-20.85**	**-62.63**
127	L:9 H:24 H:114	SER ALA THR	ARG GLY ASN	**-20.83**	**-71.62**
128	L:9 L:43 H:114	SER ALA THR	ARG PHE ILE	**-20.83**	**-81.93**
129	L:9 L:40 H:114	SER PRO THR	ARG ALA SER	**-20.83**	**-70.96**
130	L:9 L:51 H:114	SER ALA THR	ILE THR ASN	**-20.81**	**-68.13**
131	L:9 L:102 H:114	SER THR THR	ARG ASN ASN	**-20.77**	**-60.94**
132	L:9 L:76 H:114	SER SER THR	ARG THR ASN	**-20.76**	**-84.86**
133	L:9 L:43 H:114	SER ALA THR	ARG GLY ASN	**-20.76**	**-74.39**
134	L:9 H:24 H:114	SER ALA THR	ARG GLY ILE	**-20.76**	**-78.89**
135	L:9 L:76 H:114	SER SER THR	ARG ASN ASN	**-20.75**	**-83.79**
136	L:9 L:40 H:114	SER PRO THR	LYS SER ILE	**-20.75**	**-65.78**
137	L:9 H:79 H:114	SER ALA THR	LYS GLY ILE	**-20.74**	**-65.64**
138	L:9 H:14 H:114	SER PRO THR	ARG ALA ASN	**-20.74**	**-74.03**
139	L:9 L:51 H:114	SER ALA THR	LYS PRO ILE	**-20.73**	**-21.43**
140	L:9 L:80 H:114	SER PRO THR	ARG ALA SER	**-20.73**	**-72.9**
141	L:9 H:92 H:114	SER ALA THR	ARG GLY ASN	**-20.73**	**-65.47**
142	L:9 H:56 H:114	SER GLY THR	LYS VAL ILE	**-20.73**	**-71.64**
143	L:9 H:48 H:114	SER VAL THR	ARG LEU ASN	**-20.72**	**-76.27**

Figure 11C (Continued)

144	L:9 H:61 H:114	SER ALA THR	LYS VAL ILE	**-20.71**	**-74.97**
145	L:9 H:16 H:114	SER GLY THR	ARG ALA SER	**-20.71**	**-70.28**
146	L:9 H:4 H:114	SER LEU THR	ARG VAL ASN	**-20.7**	**-63.71**
147	L:9 H:16 H:114	SER GLY THR	ARG ALA ILE	**-20.7**	**-81.88**
148	L:9 H:56 H:114	SER GLY THR	ARG VAL ASN	**-20.68**	**-80.26**
149	L:9 H:56 H:114	SER GLY THR	LYS VAL ASN	**-20.68**	**-64.37**
150	L:9 H:106 H:114	SER ALA THR	LYS GLY ILE	**-20.68**	**-65.31**
151	L:9 H:106 H:114	SER ALA THR	LYS VAL ASN	**-20.67**	**-67.65**
152	L:9 L:80 H:114	SER PRO THR	ARG ALA ILE	**-20.67**	**-82.75**
153	L:9 H:61 H:114	SER ALA THR	LYS VAL ASN	**-20.67**	**-67.66**
154	L:9 L:25 H:114	SER ALA THR	ARG VAL SER	**-20.66**	**-57.1**
155	L:9 H:114	SER THR	THR ILE	**-20.66**	**-81.4**
156	L:9 H:114	SER THR	ILE SER	**-20.65**	**-68.93**
157	L:9 H:48 H:114	SER VAL THR	LYS ILE ILE	**-20.64**	**-79.52**
158	L:9 L:101 H:114	SER GLY THR	LYS ALA ILE	**-20.63**	**-73.89**
159	L:9 H:106 H:114	SER ALA THR	ARG GLY ILE	**-20.62**	**-77.61**
160	L:9 H:4 H:114	SER LEU THR	ARG VAL ILE	**-20.62**	**-70.14**
161	L:9 L:32 H:114	SER ALA THR	ARG VAL ASN	**-20.62**	**-49.41**
162	L:9 L:43 H:114	SER ALA THR	LYS PRO ILE	**-20.61**	**-74.45**
163	L:9 H:2 H:114	SER VAL THR	LYS LEU ILE	**-20.6**	**-55.85**
164	L:9 L:89 H:114	SER GLN THR	LYS HID ASN	**-20.59**	**-62.74**

Figure 11C (Continued)

165	L:9 L:40 H:114	SER PRO THR	ARG SER ILE	**-20.57**	**-75.46**
166	L:9 L:76 H:114	SER SER THR	ARG ASN SER	**-20.57**	**-81.18**
167	L:9 L:40 H:114	SER PRO THR	ARG THR ILE	**-20.57**	**-73.45**
168	L:9 H:58 H:114	SER THR THR	ARG ILE SER	**-20.55**	**-70.24**
169	L:9 H:24 H:114	SER ALA THR	LYS PRO ILE	**-20.55**	**-44.78**

Figure 11D

4 Mutations

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
1	L:9	SER	ARG	**-32.48**	**-47.75**
	L:43	ALA	PHE		
	H:26	GLY	ALA		
	H:58	THR	SER		
2	L:9	SER	ILE	**-31.91**	**-60.15**
	L:43	ALA	PHE		
	H:26	GLY	VAL		
	H:85	SER	ARG		
3	L:9	SER	ARG	**-31.54**	73.47
	L:43	ALA	PHE		
	H:24	ALA	PRO		
	H:61	ALA	VAL		
4	L:9	SER	THR	**-31.44**	61.87
	L:43	ALA	PHE		
	L:51	ALA	PRO		
	H:92	ALA	VAL		
5	L:9	SER	ILE	**-31.05**	**-53.04**
	L:43	ALA	PHE		
	L:101	GLY	ASP		
	H:79	ALA	VAL		
6	L:9	SER	ILE	**-31.02**	**-48.23**
	L:31	THR	SER		
	L:43	ALA	PHE		
	L:102	THR	SER		
7	L:9	SER	LYS	**-29.87**	**-5.55**
	L:43	ALA	PHE		
	L:51	ALA	SER		
	H:14	PRO	THR		
8	L:9	SER	ILE	**-29.74**	**-16.58**
	L:43	ALA	PHE		
	L:46	LEU	VAL		
	L:76	SER	ASN		
9	L:9	SER	ARG	**-29.36**	**-23.71**
	L:43	ALA	PHE		
	H:14	PRO	THR		
	H:88	ALA	VAL		
10	L:9	SER	THR	**-29.26**	**-4.8**
	L:43	ALA	PHE		
	L:101	GLY	ALA		
	H:79	ALA	GLY		
11	L:9	SER	ILE	**-28.95**	2.85
	L:43	ALA	PHE		
	L:102	THR	ASN		
	H:14	PRO	THR		
12	L:9	SER	ARG	**-28.55**	**-24.01**
	L:43	ALA	PHE		
	L:80	PRO	THR		
	H:61	ALA	ASP		
13	L:9	SER	ARG	**-28.1**	**-28.88**
	H:56	GLY	ALA		
	H:79	ALA	VAL		
	H:103	GLY	VAL		
14	L:9	SER	THR	**-26.36**	70.21
	H:92	ALA	VAL		
	H:103	GLY	VAL		
15	L:9	SER	ARG	**-26.27**	68.12
	H:92	ALA	VAL		
	H:103	GLY	VAL		

Figure 11D (Continued)

16	L:9 H:56 H:92 H:103	SER GLY ALA GLY	ARG VAL VAL VAL	**-26.03**	62.68
17	L:9 L:89 H:92 H:103	SER GLN ALA GLY	ARG HID VAL VAL	**-25.68**	86.35
18	L:9 L:89 H:92 H:103	SER GLN ALA GLY	ARG HID GLY VAL	**-25.67**	82.6
19	L:9 L:43 H:92 H:103	SER ALA ALA GLY	LYS ASN VAL VAL	**-25.48**	66.08
20	L:9 H:92 H:103	SER ALA GLY	LYS GLY VAL	**-25.41**	70.24
21	L:9 H:92 H:103	SER ALA GLY	LYS VAL VAL	**-25.41**	73.99
22	L:9 L:76 H:92 H:103	SER SER ALA GLY	LYS ILE GLY VAL	**-25.39**	65.53
23	L:9 L:40 H:92 H:103	SER PRO ALA GLY	LYS SER GLY VAL	**-25.39**	82.76
24	L:9 H:88 H:92 H:103	SER ALA ALA GLY	LYS GLY VAL VAL	**-25.38**	77.03
25	L:9 L:80 H:92 H:103	SER PRO ALA GLY	LYS THR VAL VAL	**-25.37**	69
26	L:9 H:56 H:92 H:103	SER GLY ALA GLY	LYS VAL VAL VAL	**-25.37**	72.36
27	L:9 H:56 H:92 H:103	SER GLY ALA GLY	LYS VAL GLY VAL	**-25.36**	68.57
28	L:9 L:85 H:92 H:103	SER THR ALA GLY	LYS ILE GLY VAL	**-25.33**	64.14
29	L:9 H:14 H:92 H:103	SER PRO ALA GLY	ARG THR GLY VAL	**-25.33**	57.8
30	L:9 L:85 H:92 H:103	SER THR ALA GLY	LYS ILE VAL VAL	**-25.33**	70.03
31	L:9 H:88 H:92 H:103	SER ALA ALA GLY	LYS GLY GLY VAL	**-25.32**	73.34

Figure 11D (Continued)

32	L:9 L:93 H:16 H:103	SER THR GLY GLY	LYS ILE VAL ALA	**-25.31**	**-52.18**
33	L:9 H:88 H:92 H:103	SER ALA ALA GLY	ARG GLY GLY VAL	**-25.31**	60.86
34	L:9 L:43 H:92 H:103	SER ALA ALA GLY	ARG ASN GLY VAL	**-25.3**	50.03
35	L:9 H:56 H:92 H:103	SER GLY ALA GLY	ARG VAL GLY VAL	**-25.3**	56.45
36	L:9 L:85 H:92 H:103	SER THR ALA GLY	ARG ILE GLY VAL	**-25.27**	51.96
37	L:9 H:4 H:92 H:103	SER LEU ALA GLY	LYS MET VAL VAL	**-25.26**	74.81
38	L:9 H:58 H:92 H:103	SER THR ALA GLY	LYS ILE VAL VAL	**-25.26**	75.35
39	L:9 L:85 H:92 H:103	SER THR ALA GLY	ARG ILE VAL VAL	**-25.24**	55.74
40	L:9 L:76 H:92 H:103	SER SER ALA GLY	LYS ASN VAL VAL	**-25.22**	70.92
41	L:9 H:24 H:92 H:103	SER ALA ALA GLY	LYS PHE VAL VAL	**-25.01**	95.86
42	L:9 H:24 H:92 H:103	SER ALA ALA GLY	LYS PHE GLY VAL	**-25**	92.16
43	L:9 H:24 H:92 H:103	SER ALA ALA GLY	ARG PHE GLY VAL	**-24.96**	78.74
44	L:9 H:2 H:92 H:103	SER VAL ALA GLY	LYS LEU VAL ALA	**-24.9**	74.08
45	L:9 H:2 H:103	SER VAL GLY	LYS LEU ALA	**-24.89**	59.87
46	L:9 H:2 H:92 H:103	SER VAL ALA GLY	LYS LEU GLY ALA	**-24.86**	69.88
47	L:9 H:3 H:56 H:103	SER GLN GLY GLY	ARG HID ALA VAL	**-24.85**	**-9.98**

Figure 11D (Continued)

48	L:9 H:103 H:114	SER GLY THR	ARG VAL ASN	**-24.84**	**-19.23**
49	L:9 H:2 H:92 H:103	SER VAL ALA GLY	ARG LEU VAL ALA	**-24.84**	62.12
50	L:9 H:79 H:103	SER ALA GLY	LYS VAL VAL	**-24.84**	**-18.78**
51	L:9 L:47 H:3 H:103	SER LEU GLN GLY	ARG VAL HID VAL	**-24.84**	-1.82
52	L:9 H:3 H:103	SER GLN GLY	ARG HID VAL	**-24.82**	**-10.4**
53	L:9 H:56 H:103	SER GLY GLY	ARG ALA VAL	**-24.81**	**-15.76**
54	L:9 H:4 H:79 H:103	SER LEU ALA GLY	LYS MET GLY VAL	**-24.81**	0.51
55	L:9 L:101 H:3 H:103	SER GLY GLN GLY	ARG ALA HID VAL	**-24.79**	**-12.68**
56	L:9 L:76 H:92 H:103	SER SER ALA GLY	LYS ILE ASP VAL	**-24.77**	78.59
57	L:9 H:88 H:92 H:103	SER ALA ALA GLY	LYS GLY ASP VAL	**-24.76**	89.79
58	L:9 H:79 H:103	SER ALA GLY	ARG VAL VAL	**-24.75**	**-29.31**
59	L:9 L:101 H:103	SER GLY GLY	ARG ALA VAL	**-24.75**	**-18.08**
60	L:9 L:47 H:92 H:103	SER LEU ALA GLY	LYS VAL ASP VAL	**-24.74**	111.08
61	L:9 L:43 H:92 H:103	SER ALA ALA GLY	THR ASN VAL VAL	**-24.71**	59.03
62	L:9 H:4 H:79 H:103	SER LEU ALA GLY	LYS MET VAL VAL	**-24.7**	**-19.87**
63	L:9 L:85 H:92 H:103	SER THR ALA GLY	LYS ILE ASP VAL	**-24.7**	83.23
64	L:9 L:43 H:92 H:103	SER ALA ALA GLY	THR ASN GLY VAL	**-24.7**	55.27

Figure 11D (Continued)

65	L:9 H:24 H:92 H:103	SER ALA ALA GLY	THR GLY VAL VAL	**-24.69**	76.82
66	L:9 H:79 H:85 H:103	SER ALA SER GLY	ARG GLY ARG ALA	**-24.68**	**-54.2**
67	L:9 L:85 H:92 H:103	SER THR ALA GLY	THR SER VAL VAL	**-24.67**	73.18
68	L:9 L:34 H:92 H:103	SER ALA ALA GLY	THR GLY VAL VAL	**-24.65**	77.6
69	L:9 L:85 H:92 H:103	SER THR ALA GLY	ARG ILE ASP VAL	**-24.64**	70.96
70	L:9 L:102 H:92 H:103	SER THR ALA GLY	THR ASN VAL VAL	**-24.64**	83.96
71	L:9 H:4 H:79 H:103	SER LEU ALA GLY	ARG MET VAL VAL	**-24.64**	**-30.61**
72	L:9 H:26 H:92 H:103	SER GLY ALA GLY	THR ALA VAL VAL	**-24.62**	72.5
73	L:9 H:14 H:92 H:103	SER PRO ALA GLY	THR ALA VAL VAL	**-24.61**	74.74
74	L:9 H:92 H:103 H:114	SER ALA GLY THR	THR VAL VAL ILE	**-24.61**	68.62
75	L:9 H:56 H:92 H:103	SER GLY ALA GLY	THR VAL VAL VAL	**-24.6**	65.31
76	L:9 H:85 H:103	SER SER GLY	ARG ARG ALA	**-24.6**	**-61.78**
77	L:9 H:14 H:92 H:103	SER PRO ALA GLY	THR ALA GLY VAL	**-24.6**	77.36
78	L:9 H:16 H:85 H:103	SER GLY SER GLY	LYS VAL ARG ALA	**-24.59**	**-52.03**
79	L:9 H:16 H:85 H:103	SER GLY SER GLY	ARG VAL ARG ALA	**-24.58**	**-64.84**
80	L:9 L:85 H:92 H:103	SER THR ALA GLY	ILE ILE GLY VAL	**-24.54**	55.22

Figure 11D (Continued)

81	L:9 H:3 H:85 H:103	SER GLN SER GLY	LYS HID ARG ALA	**-24.53**	**-56.52**
82	L:9 H:85 H:103	SER SER GLY	LYS ARG ALA	**-24.5**	**-62.14**
83	L:9 L:76 H:85 H:103	SER SER SER GLY	LYS ILE ARG ALA	**-24.48**	**-72.72**
84	L:9 L:93 H:26 H:103	SER THR GLY GLY	LYS ILE ALA ALA	**-24.48**	56.08
85	L:9 H:85 H:103 H:114	SER SER GLY THR	LYS ARG ALA SER	**-24.46**	**-53.1**
86	L:9 H:61 H:92 H:103	SER ALA ALA GLY	ARG ASP GLY VAL	**-24.44**	64.17
87	L:9 H:92 H:103 H:114	SER ALA GLY THR	THR VAL ALA ILE	**-24.42**	51.32
88	L:9 H:85 H:103 H:114	SER SER GLY THR	ARG ARG ALA SER	**-24.38**	**-60.85**
89	L:9 H:16 H:103	SER GLY GLY	LYS VAL ALA	**-24.37**	**-45.25**
90	L:9 H:24 H:92 H:103	SER ALA ALA GLY	ARG PHE ASP VAL	**-24.33**	110.82
91	L:9 H:85 H:103 H:106	SER SER GLY ALA	ARG ARG ALA GLY	**-24.32**	**-54.91**
92	L:9 L:93 H:26 H:103	SER THR GLY GLY	THR ILE ALA ALA	**-24.29**	48.96
93	L:9 H:92 H:103	SER ALA GLY	LYS ASP VAL	**-24.22**	18.85
94	L:9 L:80 H:92 H:103	SER PRO ALA GLY	LYS THR ASP VAL	**-24.22**	14.57
95	L:9 H:24 H:92 H:103	SER ALA ALA GLY	ILE PHE VAL VAL	**-24.21**	86.99
96	L:9 H:103 H:114	SER GLY THR	ARG ALA SER	**-24.19**	**-52.86**

Figure 11D (Continued)

97	L:9 H:58 H:85 H:103	SER THR SER GLY	ARG ILE ARG ALA	**-24.18**	**-61.95**
98	L:9 H:79 H:92 H:103	SER ALA ALA GLY	LYS VAL ASP VAL	**-24.17**	5.95
99	L:9 H:92 H:103	SER ALA GLY	THR VAL ALA	**-24.17**	52.92
100	L:9 H:24 H:85 H:103	SER ALA SER GLY	LYS ASN ARG ALA	**-24.16**	**-57.83**
101	L:9 H:79 H:92 H:103	SER ALA ALA GLY	THR VAL GLY VAL	**-24.15**	**-47.96**
102	L:9 L:93 H:26 H:103	SER THR GLY GLY	LYS ILE ASP ALA	**-24.14**	55.85
103	L:9 H:2 H:92 H:103	SER VAL ALA GLY	THR LEU VAL ALA	**-24.14**	67
104	L:9 H:3 H:56 H:103	SER GLN GLY GLY	ILE HID ALA VAL	**-24.12**	**-8.1**
105	L:9 L:25 H:92 H:103	SER ALA ALA GLY	ARG VAL GLY ALA	**-24.12**	34.13
106	L:9 H:24 H:85 H:103	SER ALA SER GLY	ARG ASN ARG ALA	**-24.09**	**-69.73**
107	L:9 H:92 H:103 H:106	SER ALA GLY ALA	THR VAL VAL VAL	**-24.07**	77.62
108	L:9 H:3 H:92 H:103	SER GLN ALA GLY	THR HID ASP VAL	**-24.06**	87.86
109	L:9 H:26 H:92 H:103	SER GLY ALA GLY	THR ALA ASP VAL	**-24.02**	84.67
110	L:9 H:92 H:103	SER ALA GLY	THR ASP VAL	**-24.02**	82.33
111	L:9 H:14 H:92 H:103	SER PRO ALA GLY	THR ALA ASP VAL	**-23.99**	87.75
112	L:9 L:76 H:92 H:103	SER SER ALA GLY	THR ILE ASP VAL	**-23.96**	71.75

Figure 11D (Continued)

113	L:9 L:85 H:92 H:103	SER THR ALA GLY	THR ILE ASP VAL	**-23.94**	76.39
114	L:9 L:85 H:92 H:103	SER THR ALA GLY	ILE ILE ASP VAL	**-23.91**	74.55
115	L:9 H:4 H:79 H:103	SER LEU ALA GLY	ILE MET VAL VAL	**-23.91**	**-28.76**
116	L:9 L:31 H:103 H:114	SER THR GLY THR	ARG ASN VAL ASN	**-23.89**	**-13.7**
117	L:9 H:3 H:14 H:103	SER GLN PRO GLY	LYS HID THR VAL	**-23.87**	78.54
118	L:9 H:14 H:103	SER PRO GLY	LYS THR VAL	**-23.83**	73
119	L:9 L:31 H:103	SER THR GLY	ARG ASN VAL	**-23.82**	**-11.93**
120	L:9 L:34 H:14 H:103	SER ALA PRO GLY	LYS VAL THR VAL	**-23.81**	90.99
121	L:9 L:31 L:101 H:103	SER THR GLY GLY	ARG ASN ALA VAL	**-23.81**	**-12.59**
122	L:9 H:3 H:85 H:103	SER GLN SER GLY	THR HID ARG ALA	**-23.76**	**-63.67**
123	L:9 L:46 H:26 H:103	SER LEU GLY GLY	THR ILE ALA ALA	**-23.74**	69
124	L:9 H:61 H:92 H:103	SER ALA ALA GLY	THR ASP VAL VAL	**-23.73**	76.6
125	L:9 H:26 H:103	SER GLY GLY	THR ALA ALA	**-23.72**	54.88
126	L:9 H:88 H:92 H:103	SER ALA ALA GLY	THR ASP ASP VAL	**-23.7**	86.85
127	L:9 L:93 H:26 H:103	SER THR GLY GLY	ILE ILE ALA ALA	**-23.69**	47.1
128	L:9 L:93 H:26 H:103	SER THR GLY GLY	THR ILE VAL ALA	**-23.68**	48.26

Figure 11D (Continued)

129	L:9 H:85 H:103 H:114	SER SER GLY THR	ILE ARG ALA SER	**-23.67**	**-62.25**
130	L:9 H:3 H:88 H:103	SER GLN ALA GLY	ARG HID VAL ALA	**-23.67**	**-52.39**
131	L:9 L:34 H:92 H:103	SER ALA ALA GLY	ARG VAL VAL ALA	**-23.65**	-1.63
132	L:9 L:31 H:103 H:106	SER THR GLY ALA	ARG ASN VAL VAL	**-23.62**	**-4.47**
133	L:9 L:101 H:16 H:103	SER GLY GLY GLY	LYS ASP ALA ALA	**-23.6**	**-40.42**
134	L:9 L:101 H:16 H:103	SER GLY GLY GLY	LYS ASP VAL ALA	**-23.6**	**-42.56**
135	L:9 H:3 H:92 H:103	SER GLN ALA GLY	ARG HID GLY ALA	**-23.56**	**-14.7**
136	L:9 H:26 H:92 H:103	SER GLY ALA GLY	ARG ALA VAL ALA	**-23.55**	**-14.96**
137	L:9 H:92 H:103	SER ALA GLY	ARG VAL ALA	**-23.55**	**-17.14**
138	L:9 L:93 H:92 H:103	SER THR ALA GLY	LYS ILE VAL ALA	**-23.53**	**-44.07**
139	L:9 H:92 H:103	SER ALA GLY	ARG GLY ALA	**-23.52**	**-20.47**
140	L:9 H:2 H:92 H:103	SER VAL ALA GLY	THR LEU ASP ALA	**-23.51**	80.07
141	L:9 L:93 H:2 H:103	SER THR VAL GLY	LYS ASN LEU ALA	**-23.51**	66.19
142	L:9 L:85 H:92 H:103	SER THR ALA GLY	ARG ILE VAL ALA	**-23.48**	**-23.63**
143	L:9 L:80 H:88 H:103	SER PRO ALA GLY	LYS ALA GLY ALA	**-23.47**	**-10.36**
144	L:9 H:79 H:92 H:103	SER ALA ALA GLY	ILE VAL ASP VAL	**-23.47**	**-26.24**

Figure 11D (Continued)

145	L:9 H:48 H:92 H:103	SER VAL ALA GLY	ARG LEU GLY ALA	**-23.46**	**-15.99**
146	L:9 H:79 H:92 H:103	SER ALA ALA GLY	THR VAL ASP VAL	**-23.41**	-0.55
147	L:9 H:58 H:92 H:103	SER THR ALA GLY	LYS ILE VAL ALA	**-23.38**	-0.82
148	L:9 L:76 H:92 H:103	SER SER ALA GLY	ARG ASN VAL ALA	**-23.37**	**-20.12**
149	L:9 L:93 H:26 H:103	SER THR GLY GLY	THR ILE ASP ALA	**-23.37**	48.84
150	L:9 H:3 H:56 H:103	SER GLN GLY GLY	ARG HID ASP VAL	**-23.35**	**-7.22**
151	L:9 H:58 H:92 H:103	SER THR ALA GLY	ARG ILE VAL ALA	**-23.33**	**-12.31**
152	L:9 H:56 H:92 H:103	SER GLY ALA GLY	THR ASP VAL VAL	**-23.3**	72.99
153	L:9 H:24 H:92 H:103	SER ALA ALA GLY	ARG ASN VAL ALA	**-23.22**	**-12.64**
154	L:9 L:31 L:101 H:103	SER THR GLY GLY	ILE ASN ALA VAL	**-23.08**	**-8.89**
155	L:9 H:61 H:92 H:103	SER ALA ALA GLY	ARG VAL ASP VAL	**-23.07**	**-14.2**
156	L:9 H:58 H:92 H:103	SER THR ALA GLY	LYS SER ASP ALA	**-23.05**	18.98
157	L:9 H:92 H:103	SER ALA GLY	LYS ASP ALA	**-22.98**	13.03
158	L:9 H:24 H:92 H:103	SER ALA ALA GLY	ARG ASN GLY ALA	**-22.97**	**-19.38**
159	L:9 H:92 H:103	SER ALA GLY	ARG GLY VAL	**-22.97**	**-29.82**
160	L:9 H:3 H:92 H:103	SER GLN ALA GLY	ARG HID ASP ALA	**-22.95**	19.39

Figure 11D (Continued)

161	L:9 H:92 H:103	SER ALA GLY	LYS VAL ALA	**-22.92**	**-38.05**
162	L:9 H:16 H:92 H:103	SER GLY ALA GLY	LYS ALA GLY VAL	**-22.92**	**-19.26**
163	L:9 H:92 H:103	SER ALA GLY	ARG ASP ALA	**-22.91**	13.64
164	L:9 H:92 H:103	SER ALA GLY	LYS GLY ALA	**-22.89**	**-43.34**
165	L:9 H:4 H:92 H:103	SER LEU ALA GLY	ARG VAL GLY VAL	**-22.86**	**-15.65**
166	L:9 H:16 H:92 H:103	SER GLY ALA GLY	ARG ALA GLY VAL	**-22.86**	**-31.55**
167	L:9 H:58 H:92 H:103	SER THR ALA GLY	ILE SER GLY ALA	**-22.85**	**-3.84**
168	L:9 H:58 H:92 H:103	SER THR ALA GLY	ILE SER VAL ALA	**-22.85**	**-5.21**
169	L:9 H:24 H:88 H:103	SER ALA ALA GLY	ARG GLY VAL ALA	**-22.84**	**-53.51**
170	L:9 L:76 H:92 H:103	SER SER ALA GLY	THR ASN VAL ALA	**-22.84**	**-14.9**
171	L:9 L:80 L:102 H:79	SER PRO THR ALA	LYS THR ILE VAL	**-22.83**	**-52.04**
172	L:9 H:24 H:85	SER ALA SER	ARG GLY ARG	**-22.82**	47.27
173	L:9 L:102 H:88 H:103	SER THR ALA GLY	ARG ASN GLY ALA	**-22.82**	**-41.36**
174	L:9 H:88 H:92 H:103	SER ALA ALA GLY	LYS GLY ASP ALA	**-22.82**	6.45
175	L:9 L:102 H:88 H:103	SER THR ALA GLY	ARG ASN VAL ALA	**-22.82**	**-47.74**
176	L:9 H:79 H:92 H:103	SER ALA ALA GLY	ILE VAL GLY VAL	**-22.82**	**-50.34**

Figure 11D (Continued)

177	L:9 H:2 H:48 H:103	SER VAL VAL GLY	ARG LEU ILE VAL	**-22.81**	**-32.27**
178	L:9 H:88 H:92 H:103	SER ALA ALA GLY	LYS GLY GLY ALA	**-22.8**	**-40.92**
179	L:9 H:88 H:103	SER ALA GLY	ARG GLY ALA	**-22.8**	**-54.17**
180	L:9 H:58 H:92 H:103	SER THR ALA GLY	LYS ILE ASP ALA	**-22.79**	14.4
181	L:9 H:48 H:88 H:103	SER VAL ALA GLY	ARG LEU VAL ALA	**-22.79**	**-63.37**
182	L:9 H:4 H:92 H:103	SER LEU ALA GLY	LYS VAL GLY ALA	**-22.79**	**-29.18**
183	L:9 H:88 H:92 H:103	SER ALA ALA GLY	ARG GLY GLY ALA	**-22.79**	**-53.46**
184	L:9 H:88 H:103	SER ALA GLY	ARG VAL ALA	**-22.78**	**-60.38**
185	L:9 H:85 H:88 H:103	SER SER ALA GLY	ARG ARG GLY ALA	**-22.78**	**-33.12**
186	L:9 L:34 H:2 H:103	SER ALA VAL GLY	ARG GLY ILE ALA	**-22.78**	**-49.05**
187	L:9 H:92 H:103	SER ALA GLY	ILE VAL ALA	**-22.77**	**-11.16**
188	L:9 L:34 H:2 H:103	SER ALA VAL GLY	ARG GLY LEU ALA	**-22.76**	**-35.07**
189	L:9 L:85 H:92 H:103	SER THR ALA GLY	ILE ILE GLY ALA	**-22.75**	**-27.49**
190	L:9 L:85 H:92 H:103	SER THR ALA GLY	ILE ILE VAL ALA	**-22.74**	**-20.7**
191	L:9 H:2 H:103	SER VAL GLY	ARG ILE ALA	**-22.73**	**-56.48**
192	L:9 L:25 H:92 H:103	SER ALA ALA GLY	LYS ASP VAL VAL	**-22.72**	109.94

Figure 11D (Continued)

193	L:9 H:56 H:79 H:103	SER GLY ALA GLY	LYS ALA VAL ALA	**-22.67**	**-21.71**
194	L:9 H:88 H:103	SER ALA GLY	LYS GLY ALA	**-22.63**	**-14.02**
195	L:9 H:103 H:114	SER GLY THR	LYS ALA SER	**-22.63**	**-6.9**
196	L:9 H:88 H:103 H:114	SER ALA GLY THR	LYS GLY ALA SER	**-22.63**	**-3.96**
197	L:9 L:80 H:26 H:103	SER PRO GLY GLY	LYS SER ALA ALA	**-22.62**	**-15.29**

Figure 11E

5 Mutations

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
	L:9	SER	ARG		
	L:43	ALA	PHE		
1	H:92	ALA	ASP	**-35.47**	21.7
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:43	ALA	PHE		
2	H:88	ALA	GLY	**-32.78**	**-75.08**
	H:103	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	ILE		
	L:43	ALA	PHE		
3	H:85	SER	ARG	**-31.99**	**-73.44**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:43	ALA	PHE		
4	H:16	GLY	VAL	**-30.43**	9.38
	H:85	SER	ARG		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:43	ALA	PHE		
5	H:2	VAL	LEU	**-29.51**	**-60.66**
	H:14	PRO	SER		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:43	ALA	PHE		
6	H:16	GLY	ALA	**-29.09**	**-81.47**
	H:58	THR	ASN		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:93	THR	ILE		
7	H:92	ALA	GLY	**-25.78**	**-12.61**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:102	THR	ILE		
8	H:92	ALA	GLY	**-25.44**	7.33
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	LYS		
	L:51	ALA	THR		
9	H:92	ALA	VAL	**-25.4**	7.37
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:48	VAL	PHE		
10	H:92	ALA	VAL	**-25.31**	114.9
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:25	ALA	VAL		
11	H:92	ALA	ASP	**-25.3**	81.52
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	H:48	VAL	PHE		
12	H:92	ALA	VAL	**-25.27**	108.05
	H:103	GLY	VAL		
	H:114	THR	ILE		

Figure 11E (Continued)

	L:9	SER	ARG		
	L:93	THR	ILE		
13	H:92	ALA	GLY	**-25.23**	**-13.15**
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	H:85	SER	ARG		
14	H:92	ALA	VAL	**-25.15**	**-23.8**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:80	PRO	THR		
15	H:92	ALA	GLY	**-25.09**	**-22.29**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	LYS		
	L:101	GLY	ALA		
16	H:92	ALA	GLY	**-25.07**	**-10.03**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:56	GLY	ALA		
17	H:79	ALA	VAL	**-25.03**	**-40.37**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:58	THR	SER		
18	H:92	ALA	VAL	**-25.01**	**-5.57**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:101	GLY	VAL		
19	H:92	ALA	VAL	**-24.99**	**-21.41**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	LYS		
	L:51	ALA	THR		
20	H:92	ALA	VAL	**-24.97**	11.01
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	L:80	PRO	ALA		
21	H:92	ALA	VAL	**-24.95**	1.03
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
22	H:92	ALA	VAL	**-24.94**	**-3.32**
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
23	H:92	ALA	VAL	**-24.93**	**-11.42**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:80	PRO	THR		
24	H:92	ALA	VAL	**-24.93**	**-14.81**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:101	GLY	VAL		
25	H:92	ALA	VAL	**-24.93**	**-14.01**
	H:103	GLY	VAL		
	H:114	THR	ASN		

Figure 11E (Continued)

26	L:9 L:101 H:92 H:103 H:114	SER GLY ALA GLY THR	ARG VAL VAL VAL SER	**-24.92**	**-11.41**
27	L:9 L:51 H:92 H:103 H:114	SER ALA ALA GLY THR	LYS THR GLY VAL SER	**-24.9**	9.21
28	L:9 H:48 H:92 H:103 H:114	SER VAL ALA GLY THR	ARG PHE VAL VAL SER	**-24.88**	118.53
29	L:9 H:92 H:103 H:114	SER ALA GLY THR	ARG VAL VAL ILE	**-24.87**	-0.53
30	L:9 H:61 H:103 H:114	SER ALA GLY THR	LYS GLY ALA ASN	**-24.86**	**-42.87**
31	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR VAL VAL SER	**-24.86**	4.94
32	L:9 H:56 H:92 H:103 H:114	SER GLY ALA GLY THR	ARG ALA GLY VAL SER	**-24.86**	**-5.83**
33	L:9 H:92 H:103 H:114	SER ALA GLY THR	ARG GLY VAL SER	**-24.86**	**-6.2**
34	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR GLY VAL SER	**-24.86**	**-10.58**
35	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR VAL VAL ILE	**-24.85**	**-6.72**
36	L:9 H:26 H:92 H:103 H:114	SER GLY ALA GLY THR	ARG ALA VAL VAL ILE	**-24.85**	2.01
37	L:9 L:40 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG ALA VAL VAL ILE	**-24.85**	4.58
38	L:9 H:92 H:103 H:114	SER ALA GLY THR	ARG GLY VAL ILE	**-24.84**	**-5.72**

Figure 11E (Continued)

	L:9	SER	ARG		
	L:51	ALA	PRO		
39	H:79	ALA	GLY	**-24.8**	3.96
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:2	VAL	ILE		
40	H:92	ALA	GLY	**-24.8**	**-8.78**
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	LYS		
	L:51	ALA	PRO		
41	H:61	ALA	GLY	**-24.77**	**-20.92**
	H:103	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	LYS		
	L:51	ALA	PRO		
42	H:79	ALA	VAL	**-24.76**	**-2.89**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:32	ALA	VAL		
43	H:92	ALA	GLY	**-24.75**	19.22
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	H:88	ALA	GLY		
44	H:92	ALA	VAL	**-24.72**	**-3.63**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:88	ALA	GLY		
45	H:92	ALA	GLY	**-24.71**	**-19.57**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:89	GLN	HID		
46	H:92	ALA	VAL	**-24.71**	**-6.52**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	LYS		
	L:51	ALA	SER		
47	H:26	GLY	ALA	**-24.7**	**-11.4**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:51	ALA	PRO		
48	H:79	ALA	VAL	**-24.7**	**-15.23**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	LYS		
	H:92	ALA	GLY		
49	H:103	GLY	VAL	**-24.7**	**-5.17**
	H:114	THR	SER		
	L:9	SER	LYS		
	H:24	ALA	PRO		
50	H:61	ALA	VAL	**-24.7**	**-44.49**
	H:103	GLY	ALA		
	H:114	THR	ILE		

Figure 11E (Continued)

51	L:9 H:88 H:92 H:103 H:114	SER ALA ALA GLY THR	LYS GLY VAL VAL ASN	**-24.68**	24.34
52	L:9 H:92 H:103 H:114	SER ALA GLY THR	LYS VAL VAL ASN	**-24.68**	21.38
53	L:9 H:48 H:92 H:103 H:114	SER VAL ALA GLY THR	LYS PHE GLY VAL SER	**-24.67**	75.26
54	L:9 L:101 H:92 H:103 H:114	SER GLY ALA GLY THR	LYS ALA GLY VAL SER	**-24.67**	**-6.39**
55	L:9 H:14 H:61 H:103 H:114	SER PRO ALA GLY THR	LYS ALA GLY ALA ASN	**-24.66**	**-36.74**
56	L:9 H:26 H:92 H:103 H:114	SER GLY ALA GLY THR	ARG VAL GLY VAL SER	**-24.66**	**-13.58**
57	L:9 H:79 H:92 H:103 H:114	SER ALA ALA GLY THR	LYS VAL VAL VAL ASN	**-24.66**	7.75
58	L:9 L:46 H:61 H:103 H:114	SER LEU ALA GLY THR	LYS ILE GLY ALA ASN	**-24.66**	**-42.3**
59	L:9 L:46 H:61 H:103 H:114	SER LEU ALA GLY THR	LYS ILE GLY ALA SER	**-24.65**	**-39.69**
60	L:9 L:51 H:26 H:103 H:114	SER ALA GLY GLY THR	ARG SER ALA VAL ASN	**-24.64**	**-23.69**
61	L:9 L:51 H:26 H:103 H:114	SER ALA GLY GLY THR	LYS SER ALA VAL ILE	**-24.62**	**-18.73**
62	L:9 L:101 H:92 H:103 H:114	SER GLY ALA GLY THR	ARG ALA GLY VAL ASN	**-24.62**	**-20.49**
63	L:9 L:102 H:103 H:114	SER THR GLY THR	ARG ASN VAL ASN	**-24.61**	**-9.85**

Figure 11E (Continued)

64	L:9 H:48 H:92 H:103 H:114	SER VAL ALA GLY THR	ARG PHE GLY VAL SER	**-24.61**	63.22
65	L:9 L:51 H:26 H:103 H:114	SER ALA GLY GLY THR	LYS SER VAL VAL ILE	**-24.6**	**-19.64**
66	L:9 L:102 H:26 H:103 H:114	SER THR GLY GLY THR	ARG ASN ALA VAL ASN	**-24.6**	**-7.84**
67	L:9 H:79 H:103 H:114	SER ALA GLY THR	ARG VAL VAL ASN	**-24.6**	**-39.78**
68	L:9 H:79 H:92 H:103 H:114	SER ALA ALA GLY THR	ARG VAL VAL VAL ASN	**-24.59**	**-3.99**
69	L:9 H:92 H:103 H:114	SER ALA GLY THR	ARG GLY VAL ASN	**-24.59**	**-17.28**
70	L:9 L:85 H:56 H:103 H:114	SER THR GLY GLY THR	ARG SER ALA VAL ILE	**-24.58**	**-27.72**
71	L:9 H:56 H:79 H:103 H:114	SER GLY ALA GLY THR	ARG ALA VAL VAL SER	**-24.58**	**-35.14**
72	L:9 L:43 H:92 H:103 H:114	SER ALA ALA GLY THR	ARG ASN VAL VAL ASN	**-24.57**	0.51
73	L:9 H:26 H:103 H:114	SER GLY GLY THR	ARG ALA VAL ASN	**-24.57**	**-22.13**
74	L:9 H:56 H:103 H:114	SER GLY GLY THR	ARG ALA VAL ILE	**-24.57**	**-34.36**
75	L:9 H:61 H:103 H:114	SER ALA GLY THR	LYS VAL ALA ASN	**-24.57**	**-50.23**
76	L:9 L:43 H:92 H:103 H:114	SER ALA ALA GLY THR	ARG PRO VAL VAL ASN	**-24.57**	6.46

Figure 11E (Continued)

	L:9	SER	ARG		
	H:56	GLY	ALA		
77	H:79	ALA	VAL	**-24.57**	**-46.74**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	LYS		
	H:56	GLY	VAL		
78	H:58	THR	ILE	**-24.56**	**-57.89**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	LYS		
	H:14	PRO	ALA		
79	H:61	ALA	VAL	**-24.56**	**-44.15**
	H:103	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:51	ALA	SER		
80	H:26	GLY	ALA	**-24.56**	**-31.07**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	ARG		
	H:48	VAL	ILE		
81	H:92	ALA	GLY	**-24.55**	**-24.23**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:48	VAL	ILE		
82	H:92	ALA	VAL	**-24.54**	**-7.63**
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:26	GLY	VAL		
83	H:56	GLY	ALA	**-24.54**	**-32.83**
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:80	PRO	ALA		
84	H:92	ALA	VAL	**-24.53**	4.65
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	H:14	PRO	SER		
85	H:92	ALA	VAL	**-24.53**	17.99
	H:103	GLY	VAL		
	H:114	THR	SER		
	L:9	SER	ARG		
	L:43	ALA	PHE		
86	H:92	ALA	GLY	**-24.52**	3.89
	H:103	GLY	VAL		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:14	PRO	SER		
87	H:92	ALA	VAL	**-24.52**	6.38
	H:103	GLY	VAL		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:43	ALA	PHE		
88	H:92	ALA	VAL	**-24.51**	9.25
	H:103	GLY	VAL		
	H:114	THR	ASN		

Figure 11E (Continued)

89	L:9 H:26 H:103 H:114	SER GLY GLY THR	ARG ALA VAL ILE	**-24.51**	**-32.32**
90	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR ASP VAL ILE	**-24.5**	7.11
91	L:9 H:2 H:92 H:103 H:114	SER VAL ALA GLY THR	ARG ILE VAL VAL ASN	**-24.49**	**-2.12**
92	L:9 H:26 H:103 H:114	SER GLY GLY THR	ARG VAL VAL ILE	**-24.49**	**-33.23**
93	L:9 L:93 H:92 H:103 H:114	SER THR ALA GLY THR	ILE ILE GLY VAL ILE	**-24.48**	**-11.16**
94	L:9 L:25 H:61 H:103 H:114	SER ALA ALA GLY THR	LYS VAL VAL ALA ASN	**-24.46**	**-7.7**
95	L:9 H:26 H:103 H:114	SER GLY GLY THR	ARG ALA ALA ASN	**-24.45**	**-57.56**
96	L:9 H:92 H:103 H:114	SER ALA GLY THR	ILE VAL VAL SER	**-24.41**	5.45
97	L:9 H:58 H:92 H:103 H:114	SER THR ALA GLY THR	THR SER VAL VAL ILE	**-24.36**	**-7.65**
98	L:9 H:24 H:61 H:103 H:114	SER ALA ALA GLY THR	LYS PRO VAL ALA ASN	**-24.34**	**-36.72**
99	L:9 H:56 H:58 H:103 H:114	SER GLY THR GLY THR	LYS VAL ILE ALA SER	**-24.34**	**-46.16**
100	L:9 L:102 H:103 H:106 H:114	SER THR GLY ALA THR	LYS ASN VAL VAL ASN	**-24.34**	10.69
101	L:9 H:24 H:61 H:103 H:114	SER ALA ALA GLY THR	LYS PRO VAL ALA SER	**-24.33**	**-34.04**

Figure 11E (Continued)

102	L:9 L:51 H:92 H:103 H:114	SER ALA ALA GLY THR	LYS THR ASP VAL SER	**-24.32**	26.27
103	L:9 L:102 H:103 H:106 H:114	SER THR GLY ALA THR	ARG ASN VAL VAL ASN	**-24.32**	**-2.3**
104	L:9 H:24 H:61 H:103 H:114	SER ALA ALA GLY THR	LYS PRO GLY ALA ILE	**-24.3**	**-36.39**
105	L:9 H:58 H:92 H:103 H:114	SER THR ALA GLY THR	THR SER VAL VAL ASN	**-24.3**	-0.25
106	L:9 H:24 H:92 H:103 H:114	SER ALA ALA GLY THR	THR GLY VAL VAL ASN	**-24.29**	-0.25
107	L:9 L:76 H:92 H:103 H:114	SER SER ALA GLY THR	ILE THR VAL ALA SER	**-24.29**	**-31.39**
108	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR ASP VAL SER	**-24.27**	18.76
109	L:9 H:24 H:61 H:103 H:114	SER ALA ALA GLY THR	ARG PRO VAL ALA ILE	**-24.25**	**-56.18**
110	L:9 L:102 H:92 H:103 H:114	SER THR ALA GLY THR	THR ASN VAL VAL ASN	**-24.25**	8.02
111	L:9 H:92 H:103 H:114	SER ALA GLY THR	THR VAL VAL ASN	**-24.23**	**-6.04**
112	L:9 L:51 H:92 H:103 H:114	SER ALA ALA GLY THR	THR THR VAL VAL ASN	**-24.22**	1.46
113	L:9 H:58 H:88 H:103 H:114	SER THR ALA GLY THR	LYS ILE GLY ALA ILE	**-24.22**	**-52.96**

Figure 11E (Continued)

114	L:9 H:58 H:103 H:114	SER THR GLY THR	LYS ILE ALA ILE	**-24.22**	**-55.9**
115	L:9 H:48 H:92 H:103 H:114	SER VAL ALA GLY THR	THR PHE VAL VAL ILE	**-24.19**	126.02
116	L:9 H:2 H:92 H:103 H:114	SER VAL ALA GLY THR	THR ILE VAL VAL ASN	**-24.19**	**-8.39**
117	L:9 L:43 H:92 H:103 H:114	SER ALA ALA GLY THR	THR ASN VAL VAL ASN	**-24.19**	**-13.87**
118	L:9 H:2 H:92 H:103 H:114	SER VAL ALA GLY THR	THR ILE GLY VAL ASN	**-24.18**	**-23.62**
119	L:9 H:24 H:92 H:103 H:114	SER ALA ALA GLY THR	ARG ASN VAL VAL SER	**-24.18**	6.15
120	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	THR THR VAL VAL SER	**-24.16**	8.3
121	L:9 H:79 H:92 H:103 H:114	SER ALA ALA GLY THR	ILE GLY GLY VAL SER	**-23.98**	0.84
122	L:9 H:92 H:103 H:114	SER ALA GLY THR	ILE GLY VAL SER	**-23.9**	**-6.97**
123	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ILE THR GLY VAL SER	**-23.89**	**-12**
124	L:9 H:79 H:88 H:103 H:114	SER ALA ALA GLY THR	THR GLY GLY ALA ILE	**-23.8**	**-32.54**
125	L:9 H:79 H:92 H:103 H:114	SER ALA ALA GLY THR	ILE GLY ASP VAL ASN	**-23.77**	25.36
126	L:9 H:79 H:88 H:103 H:114	SER ALA ALA GLY THR	THR GLY GLY ALA ASN	**-23.76**	**-25.29**

Figure 11E (Continued)

127	L:9	SER	THR	**-23.76**	**-14.8**
	L:25	ALA	VAL		
	H:61	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	ASN		
128	L:9	SER	LYS	**-23.72**	**-36.88**
	H:14	PRO	ALA		
	H:61	ALA	ASP		
	H:103	GLY	ALA		
	H:114	THR	ASN		
129	L:9	SER	THR	**-23.69**	**-29.09**
	L:76	SER	THR		
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	SER		
130	L:9	SER	THR	**-23.67**	**-19.85**
	H:88	ALA	GLY		
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	SER		
131	L:9	SER	THR	**-23.67**	**-22.8**
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	SER		
132	L:9	SER	ILE	**-23.67**	**-24.63**
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	SER		
133	L:9	SER	THR	**-23.65**	**-22.83**
	H:88	ALA	GLY		
	H:92	ALA	GLY		
	H:103	GLY	ALA		
	H:114	THR	ASN		
134	L:9	SER	ILE	**-23.64**	**-23.01**
	H:26	GLY	VAL		
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	SER		
135	L:9	SER	THR	**-23.64**	**-20.28**
	H:88	ALA	GLY		
	H:92	ALA	GLY		
	H:103	GLY	ALA		
	H:114	THR	SER		
136	L:9	SER	THR	**-23.61**	**-24.4**
	H:88	ALA	GLY		
	H:92	ALA	VAL		
	H:103	GLY	ALA		
	H:114	THR	ILE		
137	L:9	SER	ILE	**-23.61**	**-6.38**
	H:26	GLY	ASP		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
	H:114	THR	SER		
138	L:9	SER	THR	**-23.6**	**-34.47**
	H:14	PRO	THR		
	H:88	ALA	GLY		
	H:103	GLY	ALA		
	H:114	THR	ILE		
139	L:9	SER	THR	**-23.6**	**-40.38**
	H:88	ALA	GLY		
	H:103	GLY	ALA		
	H:114	THR	ILE		

Figure 11E (Continued)

140	L:9 L:31 H:56 H:103 H:114	SER THR GLY GLY THR	ARG SER VAL ALA ILE	**-23.6**	**-64.89**
141	L:9 H:88 H:92 H:103 H:114	SER ALA ALA GLY THR	THR GLY GLY ALA ILE	**-23.58**	**-30.18**
142	L:9 H:26 H:92 H:103 H:114	SER GLY ALA GLY THR	ILE ASP GLY VAL SER	**-23.57**	**-6.67**
143	L:9 H:58 H:88 H:103 H:114	SER THR ALA GLY THR	ILE ILE GLY ALA ILE	**-23.43**	**-61.68**
144	L:9 L:43 H:92 H:103 H:114	SER ALA ALA GLY THR	ILE ASN ASP VAL ASN	**-23.41**	10.69
145	L:9 H:79 H:92 H:103 H:114	SER ALA ALA GLY THR	ILE GLY ASP VAL SER	**-23.38**	29.08
146	L:9 L:76 H:92 H:103 H:114	SER SER ALA GLY THR	ILE THR ASP VAL ASN	**-23.3**	12.29
147	L:9 H:92 H:103 H:114	SER ALA GLY THR	ILE ASP VAL ASN	**-23.3**	18.73
148	L:9 L:76 H:92 H:103 H:114	SER SER ALA GLY THR	ILE THR ASP VAL SER	**-23.29**	14.83
149	L:9 L:51 H:56 H:103 H:114	SER ALA GLY GLY THR	ARG PRO VAL ALA ILE	**-23.18**	**-34.67**
150	L:9 H:58 H:88 H:103 H:114	SER THR ALA GLY THR	THR ILE ASP ALA ILE	**-23.14**	**-59.75**
151	L:9 L:93 H:24 H:58 H:114	SER THR ALA THR THR	LYS ILE PRO ILE ILE	**-23.13**	**-36.78**

Figure 11E (Continued)

152	L:9 H:58 H:88 H:103 H:114	SER THR ALA GLY THR	ILE ILE ASP ALA ILE	**-23.11**	**-61.51**
153	L:9 H:88 H:92 H:103 H:114	SER ALA ALA GLY THR	THR GLY ASP ALA ASN	**-23.04**	5.79
154	L:9 H:2 H:58 H:103 H:114	SER VAL THR GLY THR	LYS ILE ASN ALA ASN	**-22.92**	**-63.41**
155	L:9 L:51 H:56 H:103 H:114	SER ALA GLY GLY THR	ARG PRO VAL ALA ASN	**-22.83**	**-26.68**
156	L:9 L:51 H:56 H:103 H:114	SER ALA GLY GLY THR	ARG PRO VAL ALA SER	**-22.82**	**-24.1**
157	L:9 L:102 H:24 H:103 H:114	SER THR ALA GLY THR	LYS ILE GLY ALA ILE	**-22.79**	**-62.42**
158	L:9 L:51 H:56 H:103 H:114	SER ALA GLY GLY THR	ARG PRO ALA ALA ILE	**-22.69**	**-31.89**
159	L:9 H:56 H:103 H:114	SER GLY GLY THR	ARG VAL ALA ILE	**-22.69**	**-73.38**
160	L:9 L:101 L:102 H:103 H:114	SER GLY THR GLY THR	ARG VAL ILE ALA SER	**-22.64**	**-64.67**
161	L:9 H:4 H:56 H:103 H:114	SER LEU GLY GLY THR	LYS VAL ALA ALA ILE	**-22.61**	**-47.9**
162	L:9 L:101 L:102 H:103 H:114	SER GLY THR GLY THR	ILE VAL ILE ALA SER	**-22.61**	**-65.92**
163	L:9 H:56 H:58 H:103 H:114	SER GLY THR GLY THR	LYS ALA ASN ALA ILE	**-22.6**	**-69.41**
164	L:9 H:56 H:103 H:114	SER GLY GLY THR	ARG ALA ALA ILE	**-22.58**	**-72.48**

Figure 11E (Continued)

	L:9	SER	LYS		
	L:102	THR	ILE		
165	H:24	ALA	GLY	**-22.57**	**-50.72**
	H:103	GLY	ALA		
	H:114	THR	SER		
	L:9	SER	LYS		
	H:56	GLY	ALA		
166	H:58	THR	ASN	**-22.56**	**-62**
	H:103	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	ARG		
	H:4	LEU	VAL		
167	H:56	GLY	ALA	**-22.54**	**-59.48**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	ARG		
	L:32	ALA	VAL		
168	L:93	THR	ASN	**-22.53**	**-53.88**
	L:101	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	ARG		
	L:31	THR	SER		
169	H:56	GLY	ALA	**-22.52**	**-65.69**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	LYS		
170	H:58	THR	ASN	**-22.48**	**-62.49**
	H:103	GLY	ALA		
	H:114	THR	ASN		
	L:9	SER	LYS		
	L:102	THR	ASN		
171	H:58	THR	ASN	**-22.4**	**-55.98**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	LYS		
172	H:58	THR	ASN	**-22.38**	**-70.02**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	ARG		
	H:24	ALA	PHE		
173	H:56	GLY	VAL	**-22.36**	24.42
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	LYS		
	L:102	THR	ILE		
174	H:24	ALA	PHE	**-22.29**	35.91
	H:103	GLY	ALA		
	H:114	THR	SER		
	L:9	SER	THR		
	H:4	LEU	VAL		
175	H:56	GLY	ALA	**-22.27**	**-55.67**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	LYS		
	L:25	ALA	GLY		
176	H:58	THR	ASN	**-22.1**	**-60.35**
	H:103	GLY	ALA		
	H:114	THR	ILE		
	L:9	SER	THR		
177	H:56	GLY	ALA	**-22.06**	**-58.43**
	H:103	GLY	ALA		
	H:114	THR	ASN		

Figure 11E (Continued)

178	L:9 L:34 L:93 H:88 H:114	SER ALA THR ALA THR	ARG GLY ASN GLY ILE	**-22.02**	**-61.28**
179	L:9 L:101 L:102 H:103 H:114	SER GLY THR GLY THR	THR VAL SER ALA SER	**-22.02**	**-57.14**
180	L:9 H:2 H:58 H:103 H:114	SER VAL THR GLY THR	LYS ILE ASN ALA SER	**-21.98**	**-59.74**
181	L:9 H:2 H:58 H:103 H:114	SER VAL THR GLY THR	LYS ILE ASN ALA ILE	**-21.97**	**-71.38**
182	L:9 L:101 L:102 H:103 H:114	SER GLY THR GLY THR	THR VAL ILE ALA SER	**-21.94**	**-58.27**
183	L:9 L:101 L:102 H:103 H:114	SER GLY THR GLY THR	THR VAL ASN ALA SER	**-21.94**	**-53.19**
184	L:9 H:79 H:88 H:114	SER ALA ALA THR	THR GLY GLY ASN	**-21.93**	**-22.74**
185	L:9 H:4 H:56 H:103 H:114	SER LEU GLY GLY THR	THR VAL ALA ALA ASN	**-21.91**	**-47.7**
186	L:9 H:4 H:56 H:103 H:114	SER LEU GLY GLY THR	THR VAL ALA ALA SER	**-21.9**	**-45.12**
187	L:9 H:56 H:103 H:114	SER GLY GLY THR	THR ALA ALA ILE	**-21.9**	**-68.29**
188	L:9 L:43 L:102 H:103 H:114	SER ALA THR GLY THR	THR GLY SER ALA ILE	**-21.88**	**-65.93**
189	L:9 L:31 H:56 H:103 H:114	SER THR GLY GLY THR	THR SER VAL ALA ILE	**-21.87**	**-63.44**
190	L:9 H:56 H:103 H:114	SER GLY GLY THR	THR ALA ALA SER	**-21.87**	**-56.66**

Figure 11E (Continued)

191	L:9 L:43 L:102 H:103 H:114	SER ALA THR GLY THR	THR GLY SER ALA ASN	**-21.85**	**-58.54**
192	L:9 H:26 H:61 H:103 H:114	SER GLY ALA GLY THR	THR ALA VAL ALA ASN	**-21.83**	**-66.04**
193	L:9 H:26 H:61 H:103 H:114	SER GLY ALA GLY THR	THR ALA VAL ALA SER	**-21.82**	**-63.47**
194	L:9 H:61 H:103 H:114	SER ALA GLY THR	THR VAL ALA ASN	**-21.82**	**-66.93**
195	L:9 L:102 H:24 H:103 H:114	SER THR ALA GLY THR	THR ILE GLY ALA SER	**-21.8**	**-58.27**
196	L:9 L:43 L:102 H:103 H:114	SER ALA THR GLY THR	THR GLY ASN ALA ASN	**-21.8**	**-53.97**
197	L:9 L:102 H:24 H:103 H:114	SER THR ALA GLY THR	THR ILE GLY ALA ILE	**-21.79**	**-69.89**
198	L:9 L:102 H:61 H:103 H:114	SER THR ALA GLY THR	THR SER VAL ALA ASN	**-21.79**	**-59.4**
199	L:9 H:2 H:58 H:103 H:114	SER VAL THR GLY THR	THR ILE ASN ALA ASN	**-21.78**	**-70.94**

Figure 11F

6 Mutations

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability
1	L:9	SER	LYS	-38.89	65.02
	L:25	ALA	GLY		
	H:24	ALA	ASN		
	H:56	GLY	ALA		
	H:92	ALA	VAL		
	H:106	ALA	VAL		
2	L:9	SER	LYS	-37.52	-1.22
	L:40	PRO	THR		
	L:43	ALA	PHE		
	H:16	GLY	VAL		
	H:85	SER	ARG		
	H:103	GLY	VAL		
3	L:9	SER	THR	-29.14	-29.16
	L:43	ALA	PHE		
	L:46	LEU	VAL		
	L:80	PRO	ALA		
	L:93	THR	SER		
	H:3	GLN	HID		
4	L:9	SER	ARG	-28.58	-15.8
	L:47	LEU	VAL		
	L:51	ALA	THR		
	H:48	VAL	ILE		
	H:58	THR	SER		
	H:103	GLY	VAL		
5	L:9	SER	LYS	-28.22	39.26
	L:40	PRO	ALA		
	L:43	ALA	ASN		
	L:46	LEU	VAL		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
6	L:9	SER	LYS	-28.22	39.26
	L:40	PRO	ALA		
	L:43	ALA	ASN		
	L:46	LEU	VAL		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
7	L:9	SER	LYS	-28.02	0.05
	L:51	ALA	THR		
	L:102	THR	SER		
	H:16	GLY	ALA		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
8	L:9	SER	LYS	-27.93	16.17
	L:89	GLN	HID		
	L:102	THR	SER		
	H:16	GLY	ALA		
	H:92	ALA	GLY		
	H:103	GLY	VAL		
9	L:9	SER	LYS	-27.92	20.59
	L:89	GLN	HID		
	L:102	THR	SER		
	H:16	GLY	ALA		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
10	L:9	SER	LYS	-27.91	1.39
	L:89	GLN	HID		
	H:16	GLY	ALA		
	H:48	VAL	LEU		
	H:92	ALA	GLY		
	H:103	GLY	VAL		

Figure 11F (Continued)

11	L:9 L:89 L:102 H:16 H:92 H:103	SER GLN THR GLY ALA GLY	LYS HID ILE ALA VAL VAL	**-27.87**	24.48
12	L:9 L:89 H:16 H:92 H:103	SER GLN GLY ALA GLY	LYS HID ALA GLY VAL	**-27.86**	6.53
13	L:9 L:43 L:76 H:92 H:103	SER ALA SER ALA GLY	ARG ASN ASN VAL VAL	**-27.69**	1.95
14	L:9 L:47 L:51 H:48 H:58 H:103	SER LEU ALA VAL THR GLY	ARG VAL PRO LEU SER VAL	**-27.69**	23.87
15	L:9 L:85 L:102 H:58 H:92 H:103	SER THR THR THR ALA GLY	ARG SER ILE SER GLY VAL	**-27.69**	**-3.14**
16	L:9 L:102 H:58 H:92 H:103	SER THR THR ALA GLY	ARG ILE SER GLY VAL	**-27.68**	**-10.06**
17	L:9 L:102 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	ARG ILE ILE SER GLY VAL	**-27.67**	**-17.6**
18	L:9 L:32 L:43 H:92 H:103	SER ALA ALA ALA GLY	ARG VAL ASN VAL VAL	**-27.65**	29.95
19	L:9 L:32 L:43 H:88 H:92 H:103	SER ALA ALA ALA ALA GLY	ARG VAL ASN VAL VAL VAL	**-27.65**	27.22
20	L:9 L:85 L:102 H:92 H:103	SER THR THR ALA GLY	ARG SER ILE GLY VAL	**-27.65**	**-8.58**
21	L:9 L:47 L:51 H:58 H:103	SER LEU ALA THR GLY	ARG VAL PRO SER VAL	**-27.65**	28.9

Figure 11F (Continued)

22	L:9 L:32 L:43 L:80 H:92 H:103	SER ALA ALA PRO ALA GLY	ARG VAL ASN THR VAL VAL	**-27.64**	24.67
23	L:9 L:40 L:85 L:102 H:92 H:103	SER PRO THR THR ALA GLY	ARG SER SER ILE GLY VAL	**-27.64**	-1.97
24	L:9 L:47 L:51 H:48 H:58 H:103	SER LEU ALA VAL THR GLY	ARG VAL PRO ILE SER VAL	**-27.63**	21.43
25	L:9 L:102 H:48 H:92 H:103	SER THR VAL ALA GLY	ARG ILE ILE GLY VAL	**-27.63**	**-23.04**
26	L:9 H:16 H:48 H:58 H:92 H:103	SER GLY VAL THR ALA GLY	LYS ALA LEU SER GLY VAL	**-27.62**	**-13.68**
27	L:9 L:102 H:16 H:92 H:103	SER THR GLY ALA GLY	LYS SER ALA VAL VAL	**-27.61**	-0.24
28	L:9 L:80 H:16 H:92 H:103	SER PRO GLY ALA GLY	ARG SER ALA VAL VAL	**-27.6**	12.84
29	L:9 L:31 L:32 H:85 H:103	SER THR ALA SER GLY	ARG ASN VAL ARG VAL	**-27.6**	8.08
30	L:9 L:40 L:102 H:92 H:103	SER PRO THR ALA GLY	ARG SER ILE GLY VAL	**-27.6**	**-3.99**
31	L:9 L:51 H:48 H:58 H:103	SER ALA VAL THR GLY	ARG PRO LEU SER VAL	**-27.59**	7.25
32	L:9 H:16 H:26 H:92 H:103	SER GLY GLY ALA GLY	ARG ALA ALA VAL VAL	**-27.59**	14.15

Figure 11F (Continued)

33	L:9 L:80 H:16 H:26 H:92 H:103	SER PRO GLY GLY ALA GLY	ARG SER ALA ALA VAL VAL	**-27.59**	12.87
34	L:9 L:102 H:48 H:61 H:92 H:103	SER THR VAL ALA ALA GLY	ARG ILE ILE VAL GLY VAL	**-27.59**	**-25.3**
35	L:9 L:51 H:48 H:58 H:92 H:103	SER ALA VAL THR ALA GLY	ARG PRO LEU SER GLY VAL	**-27.59**	9
36	L:9 H:16 H:48 H:92 H:103	SER GLY VAL ALA GLY	LYS ALA LEU GLY VAL	**-27.58**	**-19.13**
37	L:9 H:48 H:58 H:92 H:103	SER VAL THR ALA GLY	ARG LEU SER GLY VAL	**-27.58**	**-23.11**
38	L:9 L:40 L:43 L:46 H:92 H:103	SER PRO ALA LEU ALA GLY	LYS ALA ASN VAL ASP VAL	**-27.58**	52.88
39	L:9 L:43 L:101 H:92 H:103	SER ALA GLY ALA GLY	LYS PHE VAL GLY VAL	**-27.58**	**-15.08**
40	L:9 H:48 H:58 H:92 H:103	SER VAL THR ALA GLY	LYS ILE ASN VAL VAL	**-27.57**	**-19.84**
41	L:9 H:48 H:61 H:92 H:103	SER VAL ALA ALA GLY	ARG ILE VAL GLY VAL	**-27.57**	**-38.17**
42	L:9 L:25 L:43 L:76 H:92 H:103	SER ALA ALA SER ALA GLY	ARG VAL ASN ASN VAL VAL	**-27.57**	33.37
43	L:9 H:16 H:48 H:58 H:92 H:103	SER GLY VAL THR ALA GLY	ARG ALA LEU SER GLY VAL	**-27.57**	**-25.7**

Figure 11F (Continued)

44	L:9 L:80 H:48 H:58 H:92 H:103	SER PRO VAL THR ALA GLY	LYS ALA ILE ASN VAL VAL	**-27.56**	**-16.79**
45	L:9 L:40 L:85 L:102 H:92 H:103	SER PRO THR THR ALA GLY	ARG SER ILE ILE GLY VAL	**-27.55**	**-14.5**
46	L:9 L:32 H:16 H:26 H:92 H:103	SER ALA GLY GLY ALA GLY	ARG VAL ALA ALA VAL VAL	**-27.55**	38.96
47	L:9 L:32 L:80 H:92 H:103	SER ALA PRO ALA GLY	ARG VAL THR VAL VAL	**-27.55**	32.64
48	L:9 L:32 H:16 H:92 H:103	SER ALA GLY ALA GLY	ARG VAL ALA VAL VAL	**-27.54**	36.11
49	L:9 L:40 L:85 H:48 H:103	SER PRO THR VAL GLY	LYS ALA ILE PHE VAL	**-27.54**	-0.62
50	L:9 L:32 L:80 H:16 H:92 H:103	SER ALA PRO GLY ALA GLY	ARG VAL THR ALA VAL VAL	**-27.54**	30.8
51	L:9 L:43 L:102 H:92 H:103	SER ALA THR ALA GLY	ARG PHE ASN GLY VAL	**-27.52**	**-14.35**
52	L:9 L:40 L:43 L:46 H:92 H:103	SER PRO ALA LEU ALA GLY	ARG ALA ASN VAL ASP VAL	**-27.52**	40.44
53	L:9 L:80 H:48 H:58 H:92 H:103	SER PRO VAL THR ALA GLY	ARG ALA ILE ASN VAL VAL	**-27.51**	**-28.38**
54	L:9 L:43 L:101 H:4 H:92 H:103	SER ALA GLY LEU ALA GLY	LYS PHE VAL MET GLY VAL	**-27.48**	**-10.11**

Figure 11F (Continued)

55	L:9	SER	ARG	**-27.46**	26.87
	L:32	ALA	VAL		
	L:43	ALA	ASN		
	L:76	SER	ASN		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
56	L:9	SER	LYS	**-27.44**	**-5.02**
	L:80	PRO	SER		
	H:48	VAL	LEU		
	H:61	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	SER		
57	L:9	SER	LYS	**-27.44**	**-11.48**
	L:32	ALA	VAL		
	L:43	ALA	PRO		
	L:51	ALA	SER		
	H:92	ALA	GLY		
	H:103	GLY	VAL		
58	L:9	SER	ILE	**-27.43**	29.85
	L:40	PRO	ALA		
	L:43	ALA	ASN		
	L:46	LEU	VAL		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
59	L:9	SER	ARG	**-27.43**	**-7.56**
	L:102	THR	ASN		
	H:58	THR	ILE		
	H:92	ALA	GLY		
	H:103	GLY	VAL		
60	L:9	SER	LYS	**-27.42**	-1.61
	L:40	PRO	ALA		
	L:85	THR	ILE		
	H:4	LEU	MET		
	H:48	VAL	PHE		
	H:103	GLY	VAL		
61	L:9	SER	LYS	**-27.42**	**-6.93**
	L:32	ALA	VAL		
	L:43	ALA	PRO		
	L:51	ALA	SER		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
62	L:9	SER	ARG	**-27.42**	-0.96
	L:40	PRO	SER		
	L:102	THR	ASN		
	H:58	THR	ILE		
	H:92	ALA	GLY		
	H:103	GLY	VAL		
63	L:9	SER	ARG	**-27.42**	**-2.22**
	L:40	PRO	SER		
	L:102	THR	ILE		
	H:58	THR	ILE		
	H:92	ALA	GLY		
	H:103	GLY	VAL		
64	L:9	SER	ARG	**-27.41**	3.47
	L:40	PRO	SER		
	L:102	THR	ASN		
	H:58	THR	ILE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		

Figure 11F (Continued)

65	L:9 L:80 H:48 H:61 H:103	SER PRO VAL ALA GLY	LYS SER LEU GLY VAL	**-27.4**	**-14.41**
66	L:9 L:31 H:48 H:92 H:103	SER THR VAL ALA GLY	ARG SER ILE GLY VAL	**-27.39**	**-30.88**
67	L:9 H:48 H:61 H:103 H:114	SER VAL ALA GLY THR	LYS PHE GLY VAL SER	**-27.39**	11.61
68	L:9 L:80 H:16 H:24 H:92 H:103	SER PRO GLY ALA ALA GLY	ARG SER ALA ASN VAL VAL	**-27.39**	25.61
69	L:9 L:80 H:48 H:61 H:103 H:114	SER PRO VAL ALA GLY THR	LYS SER PHE GLY VAL SER	**-27.38**	10.35
70	L:9 L:34 L:40 H:48 H:103	SER ALA PRO VAL GLY	LYS VAL SER PHE VAL	**-27.38**	12.2
71	L:9 L:34 L:40 L:43 H:48 H:103	SER ALA PRO ALA VAL GLY	LYS VAL SER PRO PHE VAL	**-27.37**	8.42
72	L:9 L:31 H:48 H:61 H:92 H:103	SER THR VAL ALA ALA GLY	ARG SER ILE VAL GLY VAL	**-27.36**	**-33**
73	L:9 L:34 L:40 L:85 H:48 H:103	SER ALA PRO THR VAL GLY	LYS VAL SER ILE PHE VAL	**-27.36**	6.78
74	L:9 L:80 H:48 H:92 H:103	SER PRO VAL ALA GLY	LYS ALA ILE VAL VAL	**-27.36**	**-17.32**
75	L:9 L:43 L:102 H:58 H:92 H:103	SER ALA THR THR ALA GLY	ARG PHE ASN ILE GLY VAL	**-27.35**	**-12.66**

Figure 11F (Continued)

76	L:9 L:76 L:80 H:48 H:103	SER SER PRO VAL GLY	LYS THR SER LEU VAL	**-27.35**	**-23.57**
77	L:9 L:40 L:43 L:46 H:92 H:103	SER PRO ALA LEU ALA GLY	ARG ALA PHE VAL ASP VAL	**-27.34**	43.12
78	L:9 L:76 L:80 L:85 H:48 H:103	SER SER PRO THR VAL GLY	LYS THR SER ASN LEU VAL	**-27.33**	**-21.75**
79	L:9 L:40 L:43 H:92 H:103	SER PRO ALA ALA GLY	ILE ALA ASN VAL VAL	**-27.33**	13.27
80	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	LYS SER ILE ASN GLY VAL	**-27.33**	**-18.94**
81	L:9 L:34 L:40 L:43 H:48 H:103	SER ALA PRO ALA VAL GLY	LYS VAL SER PRO ILE VAL	**-27.33**	**-8.88**
82	L:9 L:34 L:40 L:43 H:48 H:103	SER ALA PRO ALA VAL GLY	LYS VAL ALA PRO ILE VAL	**-27.33**	**-8.83**
83	L:9 L:40 L:43 H:92 H:103	SER PRO ALA ALA GLY	LYS ALA PHE ASP VAL	**-27.33**	38.79
84	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	LYS SER ILE ASN VAL VAL	**-27.33**	**-14.67**
85	L:9 L:40 L:43 H:2 H:92 H:103	SER PRO ALA VAL ALA GLY	ILE ALA ASN ILE VAL VAL	**-27.31**	11.37
86	L:9 L:80 H:48 H:92 H:103	SER PRO VAL ALA GLY	ARG ALA ILE VAL VAL	**-27.31**	**-28.91**

Figure 11F (Continued)

87	L:9 L:34 L:40 L:85 H:48 H:103	SER ALA PRO THR VAL GLY	LYS VAL ALA ILE PHE VAL	**-27.3**	6.82
88	L:9 L:43 H:3 H:92 H:103	SER ALA GLN ALA GLY	ILE ASN HID VAL VAL	**-27.29**	11.32
89	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	ARG SER ILE ASN GLY VAL	**-27.28**	**-30.63**
90	L:9 L:40 L:43 H:3 H:92 H:103	SER PRO ALA GLN ALA GLY	ILE ALA ASN HID VAL VAL	**-27.28**	18.03
91	L:9 L:40 L:43 H:92 H:103	SER PRO ALA ALA GLY	ARG ALA PHE ASP VAL	**-27.27**	26.34
92	L:9 L:51 L:102 H:92 H:103	SER ALA THR ALA GLY	ILE THR SER GLY VAL	**-27.26**	**-10.74**
93	L:9 L:25 L:34 L:101 H:92 H:103	SER ALA ALA GLY ALA GLY	ILE GLY VAL ALA GLY VAL	**-27.26**	**-5.16**
94	L:9 L:51 L:102 H:92 H:103	SER ALA THR ALA GLY	ILE THR SER VAL VAL	**-27.25**	**-6.31**
95	L:9 L:51 L:102 H:16 H:92 H:103	SER ALA THR GLY ALA GLY	ILE THR SER ALA VAL VAL	**-27.24**	**-8.9**
96	L:9 L:40 L:102 H:26 H:92 H:103	SER PRO THR GLY ALA GLY	ARG SER ILE ASP GLY VAL	**-27.23**	**-3.17**
97	L:9 L:25 L:40 L:43 H:92 H:103	SER ALA PRO ALA ALA GLY	LYS VAL ALA PHE ASP VAL	**-27.21**	69.91

Figure 11F (Continued)

98	L:9	SER	ARG	**-27.17**	2.38
	L:46	LEU	ILE		
	H:61	ALA	GLY		
	H:79	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	ASN		
99	L:9	SER	THR	**-27.17**	**-14.19**
	L:43	ALA	ASN		
	L:47	LEU	VAL		
	L:51	ALA	THR		
	H:58	THR	SER		
	H:103	GLY	VAL		
100	L:9	SER	ARG	**-27.15**	57.48
	L:25	ALA	VAL		
	L:40	PRO	ALA		
	L:43	ALA	PHE		
	H:92	ALA	ASP		
	H:103	GLY	VAL		
101	L:9	SER	ARG	**-27.14**	**-20.43**
	L:43	ALA	ASN		
	L:46	LEU	ILE		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
	H:114	THR	ASN		
102	L:9	SER	ARG	**-27.13**	**-4.64**
	L:46	LEU	ILE		
	H:61	ALA	VAL		
	H:79	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	ASN		
103	L:9	SER	LYS	**-27.11**	**-8.25**
	L:43	ALA	ASN		
	L:46	LEU	ILE		
	L:80	PRO	SER		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
104	L:9	SER	ILE	**-27.11**	190.19
	L:80	PRO	SER		
	H:24	ALA	PHE		
	H:61	ALA	GLY		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
105	L:9	SER	LYS	**-27.09**	0.7
	L:80	PRO	SER		
	H:48	VAL	PHE		
	H:103	GLY	VAL		
	H:114	THR	ASN		
106	L:9	SER	ARG	**-27.09**	**-19.97**
	L:34	ALA	VAL		
	L:80	PRO	ALA		
	H:48	VAL	ILE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
107	L:9	SER	LYS	**-27.08**	7.67
	L:80	PRO	SER		
	H:48	VAL	PHE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
	H:114	THR	ASN		

Figure 11F (Continued)

108	L:9	SER	THR	**-27.07**	**-6.22**
	L:47	LEU	VAL		
	L:51	ALA	THR		
	H:58	THR	SER		
	H:103	GLY	VAL		
109	L:9	SER	ARG	**-27.06**	**-18.58**
	L:43	ALA	ASN		
	L:46	LEU	ILE		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
110	L:9	SER	ARG	**-27.06**	**-19.84**
	L:43	ALA	ASN		
	L:46	LEU	ILE		
	L:80	PRO	SER		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
111	L:9	SER	THR	**-27.05**	**-13.69**
	L:47	LEU	VAL		
	L:51	ALA	THR		
	H:48	VAL	ILE		
	H:58	THR	SER		
	H:103	GLY	VAL		
112	L:9	SER	ARG	**-27.04**	**-12.6**
	L:46	LEU	ILE		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
	H:114	THR	ASN		
113	L:9	SER	ARG	**-27.04**	-0.64
	L:25	ALA	GLY		
	L:80	PRO	SER		
	H:48	VAL	PHE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
114	L:9	SER	ARG	**-27.03**	**-5.96**
	L:46	LEU	ILE		
	H:61	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	ASN		
115	L:9	SER	LYS	**-27.03**	**-21.94**
	L:43	ALA	ASN		
	L:80	PRO	SER		
	H:61	ALA	VAL		
	H:103	GLY	VAL		
116	L:9	SER	ARG	**-27.03**	**-3.91**
	L:80	PRO	SER		
	H:48	VAL	PHE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
	H:114	THR	ASN		
117	L:9	SER	THR	**-27.02**	**-2.42**
	L:43	ALA	ASN		
	L:47	LEU	VAL		
	H:79	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	SER		
118	L:9	SER	ARG	**-27.01**	**-29.42**
	L:80	PRO	ALA		
	H:48	VAL	ILE		
	H:88	ALA	ASP		
	H:92	ALA	VAL		
	H:103	GLY	VAL		

Figure 11F (Continued)

119	L:9	SER	ARG	**-27.01**	**-15.68**
	L:25	ALA	GLY		
	L:80	PRO	SER		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
120	L:9	SER	ARG	**-27.01**	**-11.32**
	L:25	ALA	GLY		
	L:80	PRO	ALA		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
121	L:9	SER	ARG	**-27**	34.17
	L:80	PRO	SER		
	L:89	GLN	HID		
	H:24	ALA	ASN		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
122	L:9	SER	LYS	**-27**	19.68
	H:16	GLY	ALA		
	H:48	VAL	LEU		
	H:58	THR	SER		
	H:92	ALA	ASP		
	H:103	GLY	VAL		
123	L:9	SER	THR	**-26.99**	**-11.87**
	L:43	ALA	ASN		
	L:47	LEU	VAL		
	H:79	ALA	GLY		
	H:103	GLY	VAL		
124	L:9	SER	ARG	**-26.98**	**-6.88**
	L:46	LEU	ILE		
	L:101	GLY	VAL		
	H:61	ALA	GLY		
	H:103	GLY	VAL		
	H:114	THR	ASN		
125	L:9	SER	ARG	**-26.98**	**-9.2**
	L:25	ALA	GLY		
	L:80	PRO	ALA		
	L:93	THR	SER		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
126	L:9	SER	THR	**-26.97**	**-4.88**
	L:43	ALA	ASN		
	L:47	LEU	VAL		
	H:58	THR	SER		
	H:103	GLY	VAL		
	H:114	THR	SER		
127	L:9	SER	THR	**-26.97**	**-19.34**
	L:43	ALA	ASN		
	L:47	LEU	VAL		
	H:48	VAL	ILE		
	H:79	ALA	GLY		
	H:103	GLY	VAL		
128	L:9	SER	ARG	**-26.97**	**-9.61**
	L:80	PRO	SER		
	H:48	VAL	PHE		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
129	L:9	SER	LYS	**-26.97**	**-9.64**
	L:43	ALA	GLY		
	L:80	PRO	SER		
	H:61	ALA	VAL		
	H:103	GLY	VAL		

Figure 11F (Continued)

130	L:9 L:51 L:76 L:80 L:101 H:103	SER ALA SER PRO GLY GLY	LYS SER THR SER ASP VAL	**-26.96**	**-19.77**
131	L:9 L:25 L:43 L:76 H:92 H:103	SER ALA ALA SER ALA GLY	ARG VAL ASN ASN ASP VAL	**-26.96**	46.25
132	L:9 L:43 H:3 H:103 H:106	SER ALA GLN GLY ALA	ILE ASN HID VAL VAL	**-26.94**	11.01
133	L:9 L:43 H:3 H:24 H:103 H:106	SER ALA GLN ALA GLY ALA	ILE ASN HID PHE VAL VAL	**-26.94**	7.36
134	L:9 L:43 L:47 H:103 H:114	SER ALA LEU GLY THR	THR ASN VAL VAL SER	**-26.94**	**-10.37**
135	L:9 L:25 L:43 L:76 H:92 H:103	SER ALA ALA SER ALA GLY	ARG VAL PRO ASN ASP VAL	**-26.85**	50.46
136	L:9 L:25 L:43 H:92 H:103	SER ALA ALA ALA GLY	ARG VAL PRO ASP VAL	**-26.84**	53.4
137	L:9 L:40 L:43 L:46 H:92 H:103	SER PRO ALA LEU ALA GLY	THR ALA ASN VAL ASP VAL	**-26.81**	45.8
138	L:9 L:43 H:3 H:92 H:103 H:106	SER ALA GLN ALA GLY ALA	ILE ASN HID VAL VAL VAL	**-26.79**	18.85
139	L:9 L:80 H:24 H:61 H:92 H:103	SER PRO ALA ALA ALA GLY	THR SER ASN GLY VAL VAL	**-26.77**	39.56

Figure 11F (Continued)

140	L:9	SER	ILE	**-26.74**	37.47
	L:80	PRO	SER		
	H:24	ALA	ASN		
	H:61	ALA	GLY		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
141	L:9	SER	ILE	**-26.71**	117.37
	L:43	ALA	ASN		
	H:3	GLN	HID		
	H:48	VAL	PHE		
	H:103	GLY	VAL		
	H:106	ALA	VAL		
142	L:9	SER	THR	**-26.7**	33.42
	L:80	PRO	SER		
	H:24	ALA	ASN		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
143	L:9	SER	ARG	**-26.69**	5.9
	L:51	ALA	SER		
	L:85	THR	ILE		
	H:79	ALA	GLY		
	H:103	GLY	ALA		
144	L:9	SER	ARG	**-26.51**	42.72
	L:102	THR	ASN		
	H:2	VAL	ILE		
	H:48	VAL	LEU		
	H:103	GLY	VAL		
	H:114	THR	ASN		
145	L:9	SER	ARG	**-26.48**	33.67
	L:31	THR	ASN		
	L:80	PRO	SER		
	H:24	ALA	ASN		
	H:92	ALA	VAL		
	H:103	GLY	VAL		
146	L:9	SER	ARG	**-26.29**	**-2.22**
	L:43	ALA	ASN		
	L:51	ALA	SER		
	L:85	THR	ILE		
	H:79	ALA	GLY		
	H:103	GLY	ALA		
147	L:9	SER	ARG	**-26.25**	22.64
	L:46	LEU	VAL		
	L:51	ALA	SER		
	L:85	THR	ILE		
	H:79	ALA	GLY		
	H:103	GLY	ALA		
148	L:9	SER	LYS	**-26.22**	69.47
	L:25	ALA	VAL		
	L:43	ALA	PHE		
	L:93	THR	ASN		
	H:92	ALA	ASP		
	H:103	GLY	VAL		
149	L:9	SER	LYS	**-26.19**	26.7
	L:51	ALA	SER		
	L:85	THR	ILE		
	H:14	PRO	SER		
	H:79	ALA	GLY		
	H:103	GLY	ALA		

Figure 11F (Continued)

150	L:9 L:51 L:85 H:14 H:79 H:103	SER ALA THR PRO ALA GLY	ARG SER ILE SER GLY ALA	**-26.18**	14.17
151	L:9 L:43 L:85 H:14 H:103 H:106	SER ALA THR PRO GLY ALA	LYS ASN ILE SER VAL GLY	**-26.08**	48.55
152	L:9 L:43 L:85 H:103 H:106	SER ALA THR GLY ALA	LYS ASN ILE VAL GLY	**-26.08**	40.27
153	L:9 L:101 H:88 H:92 H:103 H:114	SER GLY ALA ALA GLY THR	LYS ALA VAL VAL VAL ASN	**-26**	9.4
154	L:9 L:43 L:85 H:24 H:103 H:106	SER ALA THR ALA GLY ALA	LYS ASN ILE PRO VAL GLY	**-25.98**	63.87
155	L:9 L:51 L:85 H:79 H:103	SER ALA THR ALA GLY	ARG THR ILE GLY ALA	**-25.96**	15.38
156	L:9 L:43 L:93 H:92 H:103	SER ALA THR ALA GLY	LYS PHE ASN ASP VAL	**-25.92**	38.36
157	L:9 H:2 H:48 H:92 H:103 H:114	SER VAL VAL ALA GLY THR	ARG ILE LEU VAL VAL ASN	**-25.92**	36.49
158	L:9 H:2 H:48 H:103 H:114	SER VAL VAL GLY THR	ARG ILE LEU VAL ASN	**-25.91**	26.05
159	L:9 L:40 L:43 L:93 H:92 H:103	SER PRO ALA THR ALA GLY	LYS ALA PHE ASN ASP VAL	**-25.9**	45.1
160	L:9 L:43 L:85 H:24 H:103 H:106	SER ALA THR ALA GLY ALA	LYS ASN ILE PRO ALA GLY	**-25.9**	49.78

Figure 11F (Continued)

161	L:9 L:25 L:43 L:93 H:92 H:103	SER ALA ALA THR ALA GLY	LYS VAL PRO ASN ASP VAL	**-25.86**	71.07
162	L:9 H:2 H:48 H:92 H:103	SER VAL VAL ALA GLY	ARG ILE LEU VAL VAL	**-25.85**	37.97
163	L:9 L:80 H:2 H:48 H:92 H:103	SER PRO VAL VAL ALA GLY	ARG SER ILE LEU VAL VAL	**-25.85**	36.69
164	L:9 L:25 L:43 L:93 H:92 H:103	SER ALA ALA THR ALA GLY	ARG VAL GLY ASN ASP VAL	**-25.84**	66.82
165	L:9 L:85 H:14 H:79 H:103	SER THR PRO ALA GLY	LYS ILE SER GLY ALA	**-25.84**	32.49
166	L:9 L:43 L:85 H:14 H:103 H:106	SER ALA THR PRO GLY ALA	LYS ASN ILE SER ALA GLY	**-25.83**	32.37
167	L:9 L:85 H:14 H:79 H:103 H:106	SER THR PRO ALA GLY ALA	LYS ILE SER GLY ALA GLY	**-25.81**	40.8
168	L:9 L:25 L:43 L:93 H:92 H:103	SER ALA ALA THR ALA GLY	ARG VAL PRO ASN ASP VAL	**-25.8**	58.69
169	L:9 L:85 H:24 H:92 H:103	SER THR ALA ALA GLY	LYS ILE PRO GLY ALA	**-25.78**	59.84
170	L:9 L:85 H:24 H:103 H:106	SER THR ALA GLY ALA	LYS ILE PRO ALA GLY	**-25.78**	57.94
171	L:9 L:85 H:24 H:92 H:103 H:106	SER THR ALA ALA GLY ALA	LYS ILE PRO GLY ALA GLY	**-25.76**	68.1

Figure 11F (Continued)

172	L:9 L:85 H:14 H:103 H:106	SER THR PRO GLY ALA	LYS ILE SER ALA GLY	**-25.73**	32.89
173	L:9 L:85 H:14 H:103 H:106 H:114	SER THR PRO GLY ALA THR	LYS ILE SER ALA GLY SER	**-25.72**	42.98
174	L:9 L:40 L:85 H:14 H:103 H:106	SER PRO THR PRO GLY ALA	LYS SER ILE SER ALA GLY	**-25.71**	39.62
175	L:9 L:76 L:85 H:14 H:103 H:106	SER SER THR PRO GLY ALA	LYS ILE ILE SER ALA GLY	**-25.68**	22.3
176	L:9 L:85 H:24 H:58 H:92 H:103	SER THR ALA THR ALA GLY	LYS ILE PRO ASN GLY ALA	**-25.6**	61.71
177	L:9 L:46 H:26 H:88 H:103 H:106	SER LEU GLY ALA GLY ALA	LYS ILE ASP GLY VAL GLY	**-25.59**	22.05
178	L:9 L:46 L:85 H:24 H:58 H:103	SER LEU THR ALA THR GLY	LYS ILE ILE ASN ASN ALA	**-25.57**	46.88
179	L:9 H:24 H:48 H:92 H:103	SER ALA VAL ALA GLY	ARG ASN LEU VAL VAL	**-25.56**	41.78
180	L:9 L:46 L:85 H:24 H:58 H:103	SER LEU THR ALA THR GLY	ARG ILE ILE ASN ASN ALA	**-25.56**	34.36
181	L:9 L:85 H:24 H:58 H:103	SER THR ALA THR GLY	LYS ILE ASN ASN ALA	**-25.55**	32.65
182	L:9 L:85 H:24 H:58 H:92 H:103	SER THR ALA THR ALA GLY	LYS ILE ASN ASN GLY ALA	**-25.55**	39.26

Figure 11F (Continued)

183	L:9 H:3 H:26 H:48 H:103	SER GLN GLY VAL GLY	LYS HID ALA ILE ALA	**-25.5**	66.26
184	L:9 H:24 H:48 H:79 H:92 H:103	SER ALA VAL ALA ALA GLY	ARG ASN LEU VAL VAL VAL	**-25.5**	29.66
185	L:9 L:46 L:85 H:24 H:103	SER LEU THR ALA GLY	ARG ILE ASN ASN ALA	**-25.48**	45.43
186	L:9 L:46 L:85 H:24 H:58 H:103	SER LEU THR ALA THR GLY	ARG ILE ASN ASN ASN ALA	**-25.47**	47.52
187	L:9 H:24 H:26 H:48 H:103	SER ALA GLY VAL GLY	LYS PRO ALA PHE ALA	**-25.44**	144.15
188	L:9 H:3 H:24 H:26 H:48 H:103	SER GLN ALA GLY VAL GLY	LYS HID PRO ALA ILE ALA	**-25.4**	91.26
189	L:9 H:3 H:24 H:26 H:48 H:103	SER GLN ALA GLY VAL GLY	LYS HID PRO ALA PHE ALA	**-25.4**	149.66
190	L:9 L:25 H:24 H:92 H:103 H:106	SER ALA ALA ALA GLY ALA	ARG GLY ASN VAL ALA VAL	**-25.39**	49.6
191	L:9 L:25 H:24 H:103 H:106	SER ALA ALA GLY ALA	ARG GLY ASN ALA VAL	**-25.37**	42.92
192	L:9 L:25 L:46 H:24 H:103 H:106	SER ALA LEU ALA GLY ALA	ARG GLY ILE ASN ALA VAL	**-25.36**	56.72
193	L:9 L:51 L:102 H:2 H:103 H:114	SER ALA THR VAL GLY THR	THR PRO ASN LEU VAL ASN	**-25.32**	82.73

Figure 11F (Continued)

194	L:9 L:46 H:24 H:103 H:106	SER LEU ALA GLY ALA	ARG ILE ASN ALA VAL	**-25.31**	46.14
195	L:9 H:2 H:24 H:48 H:92 H:103	SER VAL ALA VAL ALA GLY	ARG ILE ASN LEU VAL VAL	**-25.28**	40.29
196	L:9 L:43 L:101 H:92 H:103	SER ALA GLY ALA GLY	ARG PHE VAL VAL VAL	**-25.24**	**-4.24**
197	L:9 L:102 H:2 H:48 H:103 H:114	SER THR VAL VAL GLY THR	THR ASN ILE LEU VAL ASN	**-25.22**	45.28
198	L:9 L:46 L:85 H:24 H:58 H:103	SER LEU THR ALA THR GLY	ARG ILE ASN ASN ILE ALA	**-25.21**	45.46
199	L:9 L:102 H:2 H:103 H:114	SER THR VAL GLY THR	THR ASN LEU VAL ASN	**-25.19**	47.81
200	L:9 L:102 H:2 H:48 H:103 H:114	SER THR VAL VAL GLY THR	THR ASN LEU LEU VAL ASN	**-25.17**	59.94

Figure 12

Number	Residue	Original	Mutated	Δ Affinity	Δ Stability	# Mutations
1	L:9	SER	ARG	-32.78	-75.08	5
	L:43	ALA	PHE			
	H:88	ALA	GLY			
	H:103	GLY	ALA			
	H:114	THR	ASN			
2	L:9	SER	ARG	-32.48	-47.75	4
	L:43	ALA	PHE			
	H:26	GLY	ALA			
	H:58	THR	SER			
3	L:9	SER	ILE	-31.99	-73.44	5
	L:43	ALA	PHE			
	H:85	SER	ARG			
	H:103	GLY	ALA			
	H:114	THR	ILE			
4	L:9	SER	ILE	-31.91	-60.15	4
	L:43	ALA	PHE			
	H:26	GLY	VAL			
	H:85	SER	ARG			
5	L:9	SER	ARG	-31.23	-36.46	3
	L:43	ALA	PHE			
	H:114	THR	SER			
6	L:9	SER	ILE	-31.05	-53.04	4
	L:43	ALA	PHE			
	L:101	GLY	ASP			
	H:79	ALA	VAL			
7	L:9	SER	ILE	-31.02	-48.23	4
	L:31	THR	SER			
	L:43	ALA	PHE			
	L:102	THR	SER			
8	L:9	SER	THR	-30.47	-78.74	3
	L:43	ALA	PHE			
	H:114	THR	ASN			
9	L:9	SER	THR	-29.97	-76.82	3
	L:43	ALA	PHE			
	H:114	THR	ILE			
10	L:9	SER	LYS	-29.87	-5.55	4
	L:43	ALA	PHE			
	L:51	ALA	SER			
	H:14	PRO	THR			
11	L:9	SER	ILE	-29.74	-16.58	4
	L:43	ALA	PHE			
	L:46	LEU	VAL			
	L:76	SER	ASN			
12	L:9	SER	ARG	-29.51	-60.66	5
	L:43	ALA	PHE			
	H:2	VAL	LEU			
	H:14	PRO	SER			
	H:114	THR	ILE			
13	L:9	SER	ARG	-29.36	-23.71	4
	L:43	ALA	PHE			
	H:14	PRO	THR			
	H:88	ALA	VAL			
14	L:9	SER	THR	-29.26	-4.8	4
	L:43	ALA	PHE			
	L:101	GLY	ALA			
	H:79	ALA	GLY			

Figure 12 (Continued)

15	L:9 L:43 L:46 L:80 L:93 H:3	SER ALA LEU PRO THR GLN	THR PHE VAL ALA SER HID	**-29.14**	**-29.16**	6
16	L:9 L:43 H:16 H:58 H:114	SER ALA GLY THR THR	ARG PHE ALA ASN ILE	**-29.09**	**-81.47**	5
17	L:9 L:47 L:51 H:48 H:58 H:103	SER LEU ALA VAL THR GLY	ARG VAL THR ILE SER VAL	**-28.58**	**-15.8**	6
18	L:9 L:43 L:80 H:61	SER ALA PRO ALA	ARG PHE THR ASP	**-28.55**	**-24.01**	4
19	L:9 L:43 H:106	SER ALA ALA	LYS PHE VAL	**-28.37**	**-45.98**	3
20	L:9 H:56 H:79 H:103	SER GLY ALA GLY	ARG ALA VAL VAL	**-28.1**	**-28.88**	4
21	L:9 L:85 L:102 H:58 H:92 H:103	SER THR THR THR ALA GLY	ARG SER ILE SER GLY VAL	**-27.69**	**-3.14**	6
22	L:9 L:102 H:58 H:92 H:103	SER THR THR ALA GLY	ARG ILE SER GLY VAL	**-27.68**	**-10.06**	5
23	L:9 L:102 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	ARG ILE ILE SER GLY VAL	**-27.67**	**-17.6**	6
24	L:9 L:85 L:102 H:92 H:103	SER THR THR ALA GLY	ARG SER ILE GLY VAL	**-27.65**	**-8.58**	5
25	L:9 L:102 H:48 H:92 H:103	SER THR VAL ALA GLY	ARG ILE ILE GLY VAL	**-27.63**	**-23.04**	5
26	L:9 H:16 H:48 H:58 H:92 H:103	SER GLY VAL THR ALA GLY	LYS ALA LEU SER GLY VAL	**-27.62**	**-13.68**	6

Figure 12 (Continued)

27	L:9 L:40 L:102 H:92 H:103	SER PRO THR ALA GLY	ARG SER ILE GLY VAL	**-27.6**	**-3.99**	5
28	L:9 L:102 H:48 H:61 H:92 H:103	SER THR VAL ALA ALA GLY	ARG ILE ILE VAL GLY VAL	**-27.59**	**-25.3**	6
29	L:9 H:16 H:48 H:92 H:103	SER GLY VAL ALA GLY	LYS ALA LEU GLY VAL	**-27.58**	**-19.13**	5
30	L:9 H:48 H:58 H:92 H:103	SER VAL THR ALA GLY	ARG LEU SER GLY VAL	**-27.58**	**-23.11**	5
31	L:9 L:43 L:101 H:92 H:103	SER ALA GLY ALA GLY	LYS PHE VAL GLY VAL	**-27.58**	**-15.08**	5
32	L:9 H:48 H:58 H:92 H:103	SER VAL THR ALA GLY	LYS ILE ASN VAL VAL	**-27.57**	**-19.84**	5
33	L:9 H:48 H:61 H:92 H:103	SER VAL ALA ALA GLY	ARG ILE VAL GLY VAL	**-27.57**	**-38.17**	5
34	L:9 H:16 H:48 H:58 H:92 H:103	SER GLY VAL THR ALA GLY	ARG ALA LEU SER GLY VAL	**-27.57**	**-25.7**	6
35	L:9 L:80 H:48 H:58 H:92 H:103	SER PRO VAL THR ALA GLY	LYS ALA ILE ASN VAL VAL	**-27.56**	**-16.79**	6
36	L:9 L:40 L:85 L:102 H:92 H:103	SER PRO THR THR ALA GLY	ARG SER ILE ILE GLY VAL	**-27.55**	**-14.5**	6
37	L:9 L:43 L:102 H:92 H:103	SER ALA THR ALA GLY	ARG PHE ASN GLY VAL	**-27.52**	**-14.35**	5

Figure 12 (Continued)

38	L:9 L:80 H:48 H:58 H:92 H:103	SER PRO VAL THR ALA GLY	ARG ALA ILE ASN VAL VAL	**-27.51**	**-28.38**	6
39	L:9 L:43 L:101 H:4 H:92 H:103	SER ALA GLY LEU ALA GLY	LYS PHE VAL MET GLY VAL	**-27.48**	**-10.11**	6
40	L:9 L:80 H:48 H:61 H:103 H:114	SER PRO VAL ALA GLY THR	LYS SER LEU GLY VAL SER	**-27.44**	**-5.02**	6
41	L:9 L:32 L:43 L:51 H:92 H:103	SER ALA ALA ALA ALA GLY	LYS VAL PRO SER GLY VAL	**-27.44**	**-11.48**	6
42	L:9 L:102 H:58 H:92 H:103	SER THR THR ALA GLY	ARG ASN ILE GLY VAL	**-27.43**	**-7.56**	5
43	L:9 L:32 L:43 L:51 H:92 H:103	SER ALA ALA ALA ALA GLY	LYS VAL PRO SER VAL VAL	**-27.42**	**-6.93**	6
44	L:9 L:40 L:102 H:58 H:92 H:103	SER PRO THR THR ALA GLY	ARG SER ILE ILE GLY VAL	**-27.42**	**-2.22**	6
45	L:9 L:80 H:48 H:61 H:103	SER PRO VAL ALA GLY	LYS SER LEU GLY VAL	**-27.4**	**-14.41**	5
46	L:9 L:31 H:48 H:92 H:103	SER THR VAL ALA GLY	ARG SER ILE GLY VAL	**-27.39**	**-30.88**	5
47	L:9 L:80 H:48 H:92 H:103	SER PRO VAL ALA GLY	LYS ALA ILE VAL VAL	**-27.36**	**-17.32**	5
48	L:9 L:31 H:48 H:61 H:92 H:103	SER THR VAL ALA ALA GLY	ARG SER ILE VAL GLY VAL	**-27.36**	**-33**	6

Figure 12 (Continued)

49	L:9 L:76 L:80 H:48 H:103	SER SER PRO VAL GLY	LYS THR SER LEU VAL	**-27.35**	**-23.57**	5
50	L:9 L:43 L:102 H:58 H:92 H:103	SER ALA THR THR ALA GLY	ARG PHE ASN ILE GLY VAL	**-27.35**	**-12.66**	6
51	L:9 L:76 L:80 L:85 H:48 H:103	SER SER PRO THR VAL GLY	LYS THR SER ASN LEU VAL	**-27.33**	**-21.75**	6
52	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	LYS SER ILE ASN GLY VAL	**-27.33**	**-18.94**	6
53	L:9 L:34 L:40 L:43 H:48 H:103	SER ALA PRO ALA VAL GLY	LYS VAL SER PRO ILE VAL	**-27.33**	**-8.88**	6
54	L:9 L:34 L:40 L:43 H:48 H:103	SER ALA PRO ALA VAL GLY	LYS VAL ALA PRO ILE VAL	**-27.33**	**-8.83**	6
55	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	LYS SER ILE ASN VAL VAL	**-27.33**	**-14.67**	6
56	L:9 L:80 H:48 H:92 H:103	SER PRO VAL ALA GLY	ARG ALA ILE VAL VAL	**-27.31**	**-28.91**	5
57	L:9 L:31 H:48 H:58 H:92 H:103	SER THR VAL THR ALA GLY	ARG SER ILE ASN GLY VAL	**-27.28**	**-30.63**	6
58	L:9 L:51 L:102 H:92 H:103	SER ALA THR ALA GLY	ILE THR SER GLY VAL	**-27.26**	**-10.74**	5
59	L:9 L:25 L:34 L:101 H:92 H:103	SER ALA ALA GLY ALA GLY	ILE GLY VAL ALA GLY VAL	**-27.26**	**-5.16**	6

Figure 12 (Continued)

60	L:9 L:51 L:102 H:92 H:103	SER ALA THR ALA GLY	ILE THR SER VAL VAL	**-27.25**	**-6.31**	5
61	L:9 L:51 L:102 H:16 H:92 H:103	SER ALA THR GLY ALA GLY	ILE THR SER ALA VAL VAL	**-27.24**	**-8.9**	6
62	L:9 L:40 L:102 H:26 H:92 H:103	SER PRO THR GLY ALA GLY	ARG SER ILE ASP GLY VAL	**-27.23**	**-3.17**	6
63	L:9 L:43 L:47 L:51 H:58 H:103	SER ALA LEU ALA THR GLY	THR ASN VAL THR SER VAL	**-27.17**	**-14.19**	6
64	L:9 L:43 H:114	SER ALA THR	LYS PHE SER	**-27.14**	**-21.57**	3
65	L:9 L:43 L:46 H:61 H:103 H:114	SER ALA LEU ALA GLY THR	ARG ASN ILE VAL VAL ASN	**-27.14**	**-20.43**	6
66	L:9 L:46 H:61 H:79 H:103 H:114	SER LEU ALA ALA GLY THR	ARG ILE VAL GLY VAL ASN	**-27.13**	**-4.64**	6
67	L:9 L:43 L:46 L:80 H:61 H:103	SER ALA LEU PRO ALA GLY	LYS ASN ILE SER VAL VAL	**-27.11**	**-8.25**	6
68	L:9 L:34 L:80 H:48 H:92 H:103	SER ALA PRO VAL ALA GLY	ARG VAL ALA ILE VAL VAL	**-27.09**	**-19.97**	6
69	L:9 L:47 L:51 H:58 H:103	SER LEU ALA THR GLY	THR VAL THR SER VAL	**-27.07**	**-6.22**	5
70	L:9 L:43 L:46 H:61 H:103	SER ALA LEU ALA GLY	ARG ASN ILE VAL VAL	**-27.06**	**-18.58**	5

Figure 12 (Continued)

71	L:9 L:43 L:46 L:80 H:61 H:103	SER ALA LEU PRO ALA GLY	ARG ASN ILE SER VAL VAL	**-27.06**	**-19.84**	6
72	L:9 L:47 L:51 H:48 H:58 H:103	SER LEU ALA VAL THR GLY	THR VAL THR ILE SER VAL	**-27.05**	**-13.69**	6
73	L:9 L:46 H:61 H:103 H:114	SER LEU ALA GLY THR	ARG ILE VAL VAL ASN	**-27.04**	**-12.6**	5
74	L:9 L:46 H:61 H:103 H:114	SER LEU ALA GLY THR	ARG ILE GLY VAL ASN	**-27.03**	**-5.96**	5
75	L:9 L:43 L:80 H:61 H:103	SER ALA PRO ALA GLY	LYS ASN SER VAL VAL	**-27.03**	**-21.94**	5
76	L:9 L:80 H:48 H:92 H:103 H:114	SER PRO VAL ALA GLY THR	ARG SER PHE VAL VAL ASN	**-27.03**	**-3.91**	6
77	L:9 L:43 L:47 H:79 H:103 H:114	SER ALA LEU ALA GLY THR	THR ASN VAL GLY VAL SER	**-27.02**	**-2.42**	6
78	L:9 L:25 L:80 H:92 H:103	SER ALA PRO ALA GLY	ARG GLY SER VAL VAL	**-27.01**	**-15.68**	5
79	L:9 L:25 L:80 H:92 H:103	SER ALA PRO ALA GLY	ARG GLY ALA VAL VAL	**-27.01**	**-11.32**	5
80	L:9 L:80 H:48 H:88 H:92 H:103	SER PRO VAL ALA ALA GLY	ARG ALA ILE ASP VAL VAL	**-27.01**	**-29.42**	6
81	L:9 L:43 L:47 H:79 H:103	SER ALA LEU ALA GLY	THR ASN VAL GLY VAL	**-26.99**	**-11.87**	5

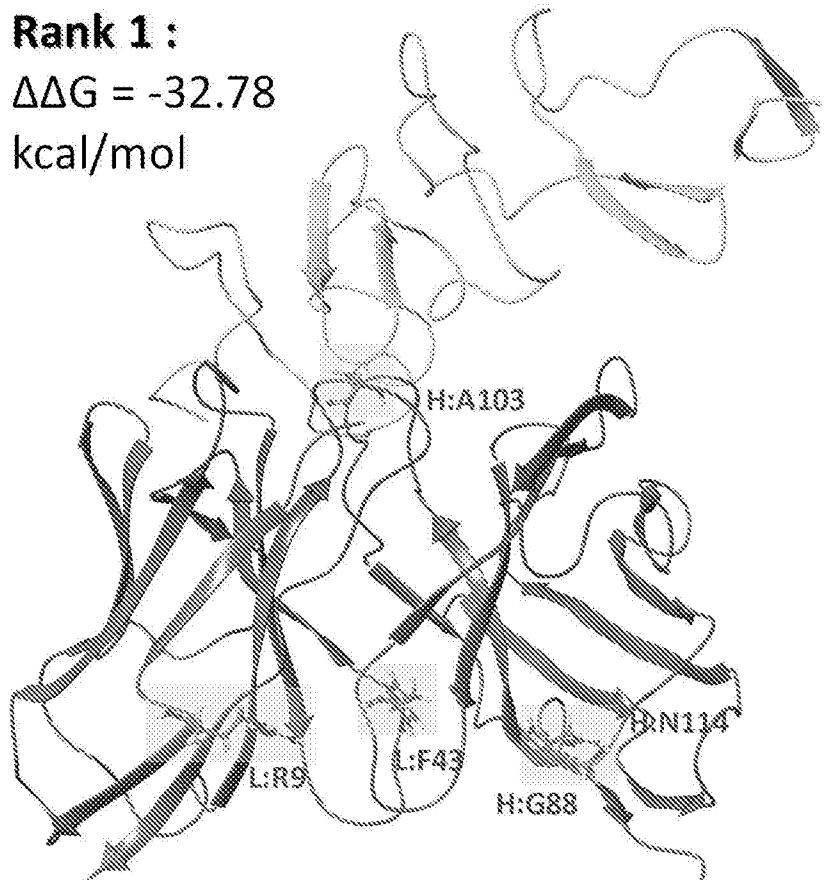
Figure 12 (Continued)

82	L:9 L:46 L:101 H:61 H:103 H:114	SER LEU GLY ALA GLY THR	ARG ILE VAL GLY VAL ASN	**-26.98**	**-6.88**	6
83	L:9 L:25 L:80 L:93 H:92 H:103	SER ALA PRO THR ALA GLY	ARG GLY ALA SER VAL VAL	**-26.98**	**-9.2**	6
84	L:9 L:80 H:48 H:92 H:103	SER PRO VAL ALA GLY	ARG SER PHE VAL VAL	**-26.97**	**-9.61**	5
85	L:9 L:43 L:80 H:61 H:103	SER ALA PRO ALA GLY	LYS GLY SER VAL VAL	**-26.97**	**-9.64**	5
86	L:9 L:43 L:47 H:58 H:103 H:114	SER ALA LEU THR GLY THR	THR ASN VAL SER VAL SER	**-26.97**	**-4.88**	6
87	L:9 L:43 L:47 H:48 H:79 H:103	SER ALA LEU VAL ALA GLY	THR ASN VAL ILE GLY VAL	**-26.97**	**-19.34**	6
88	L:9 L:51 L:76 L:80 L:101 H:103	SER ALA SER PRO GLY GLY	LYS SER THR SER ASP VAL	**-26.96**	**-19.77**	6
89	L:9 L:43 L:47 H:103 H:114	SER ALA LEU GLY THR	THR ASN VAL VAL SER	**-26.94**	**-10.37**	5
90	L:9 L:43 H:114	SER ALA THR	THR PHE SER	**-26.36**	**-37.27**	3
91	L:9 L:43 L:51 L:85 H:79 H:103	SER ALA ALA THR ALA GLY	ARG ASN SER ILE GLY ALA	**-26.29**	**-2.22**	6
92	L:9 L:93 H:92 H:103 H:114	SER THR ALA GLY THR	ARG ILE GLY VAL ILE	**-25.78**	**-12.61**	5
93	L:9 L:43 H:106	SER ALA ALA	LYS PHE GLY	**-25.73**	**-25.84**	3

Figure 12 (Continued)

94	L:9 L:93 H:16 H:103	SER THR GLY GLY	LYS ILE VAL ALA	**-25.31**	**-52.18**	4
95	L:9 L:43 L:101 H:92 H:103	SER ALA GLY ALA GLY	ARG PHE VAL VAL VAL	**-25.24**	**-4.24**	5
96	L:9 L:93 H:92 H:103 H:114	SER THR ALA GLY THR	ARG ILE GLY VAL SER	**-25.23**	**-13.15**	5
97	L:9 H:85 H:92 H:103 H:114	SER SER ALA GLY THR	ARG ARG VAL VAL ASN	**-25.15**	**-23.8**	5
98	L:9 L:80 H:92 H:103 H:114	SER PRO ALA GLY THR	ARG THR GLY VAL ILE	**-25.09**	**-22.29**	5
99	L:9 L:101 H:92 H:103 H:114	SER GLY ALA GLY THR	LYS ALA GLY VAL ASN	**-25.07**	**-10.03**	5
100	L:9 H:56 H:79 H:103 H:114	SER GLY ALA GLY THR	ARG ALA VAL VAL ASN	**-25.03**	**-40.37**	5

Figure 13A


Rank 1 :
ΔΔG = -32.78
kcal/mol
H:A103
H:N114
L:R9
L:F43
H:G88

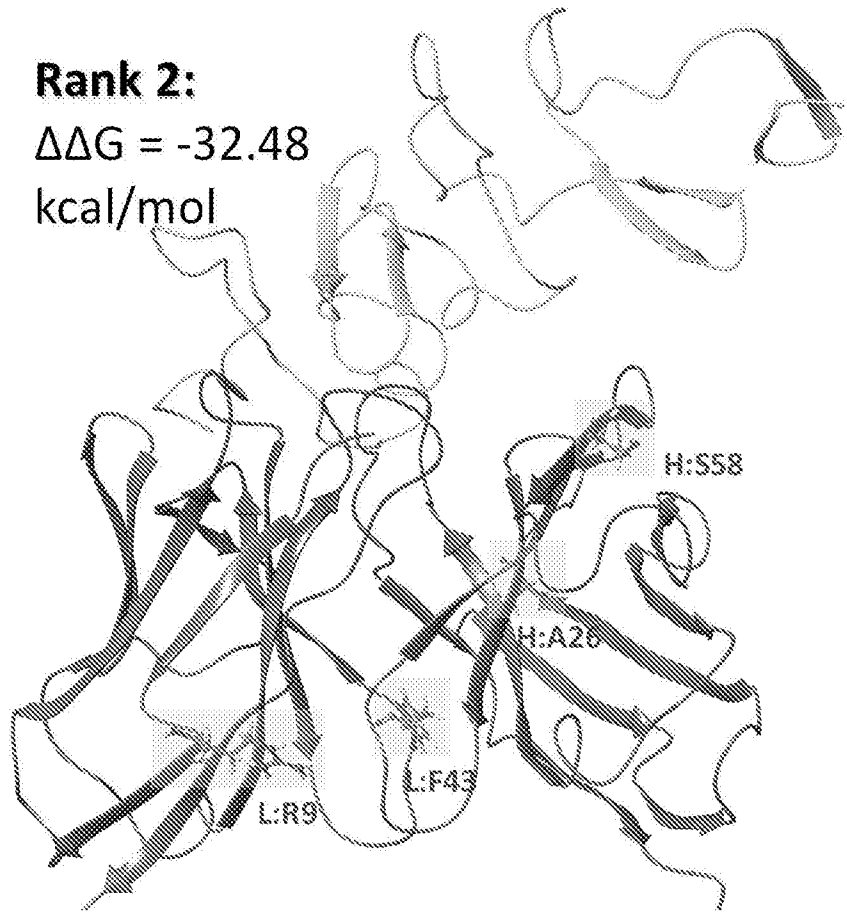
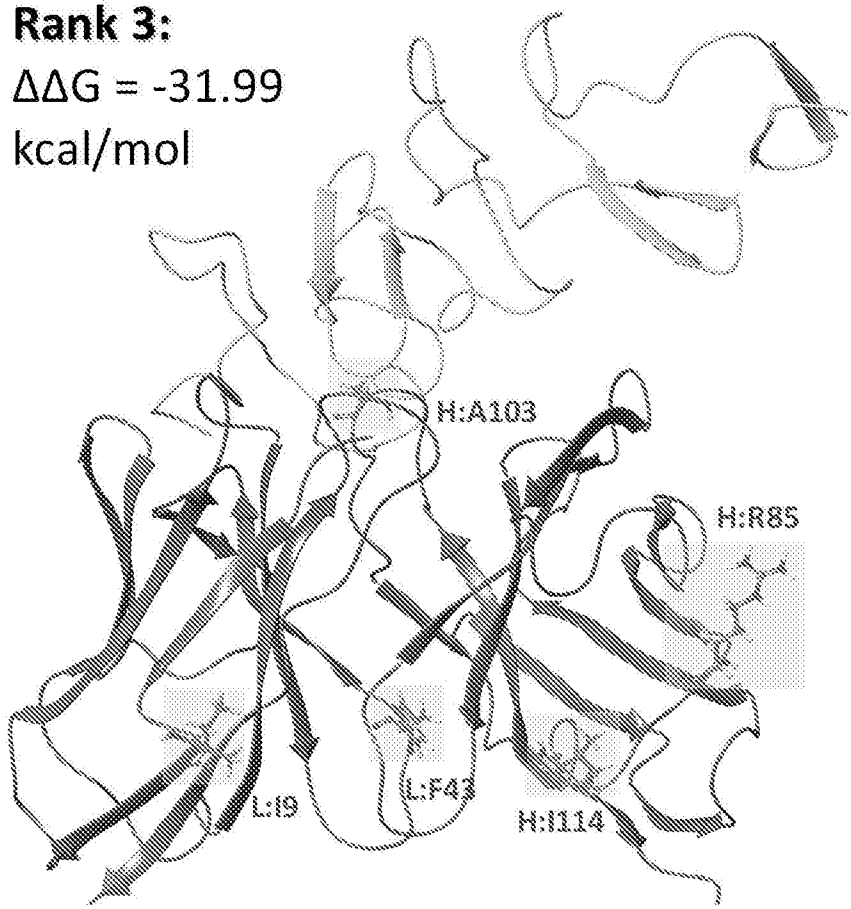

Rank 2:
ΔΔG = -32.48
kcal/mol
H:S58
H:A26
L:F43
L:R9

Figure 13B

Rank 3:

ΔΔG = -31.99 kcal/mol



Rank 4:

ΔΔG = -31.91 kcal/mol

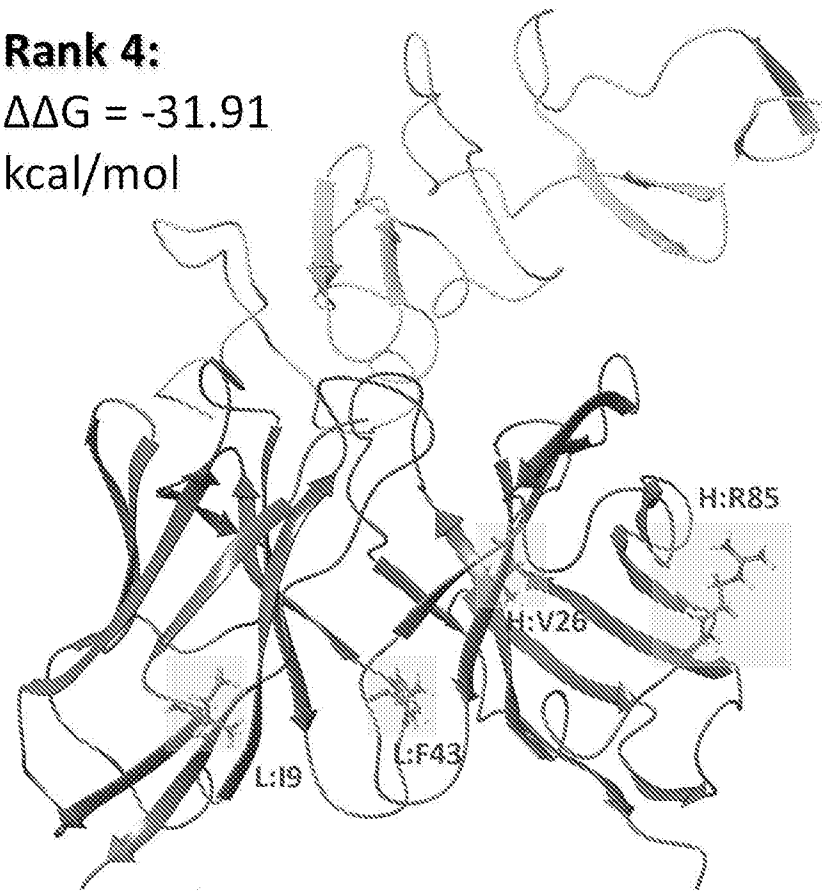
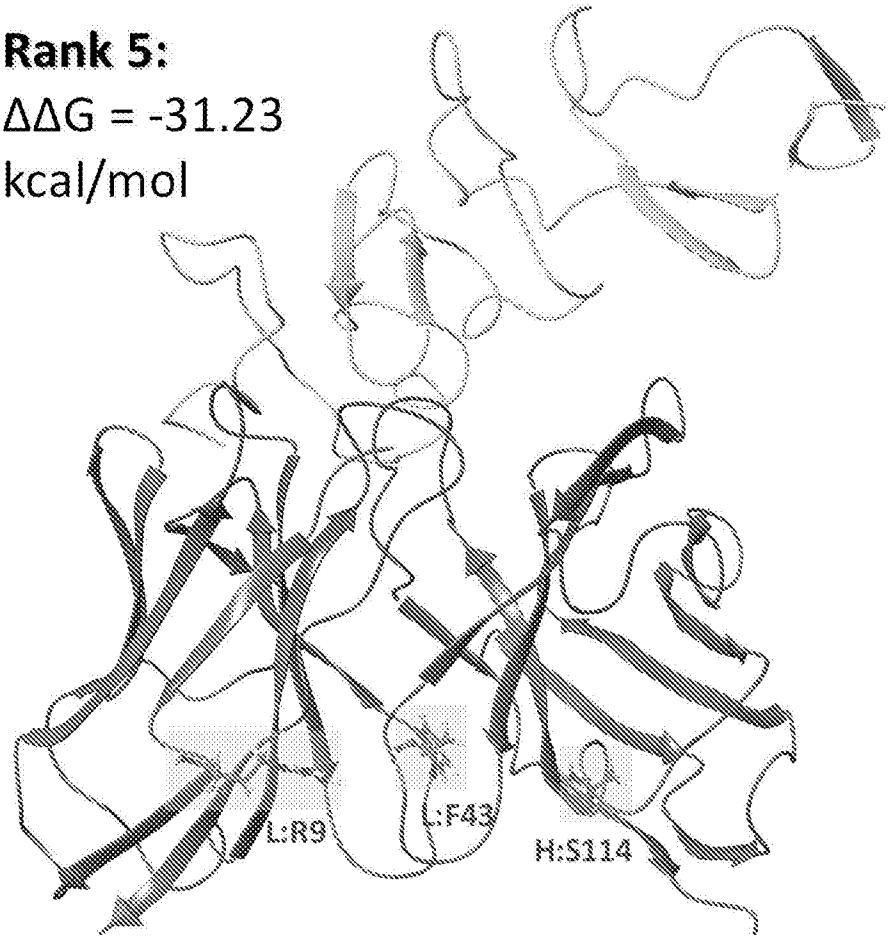


Figure 13C



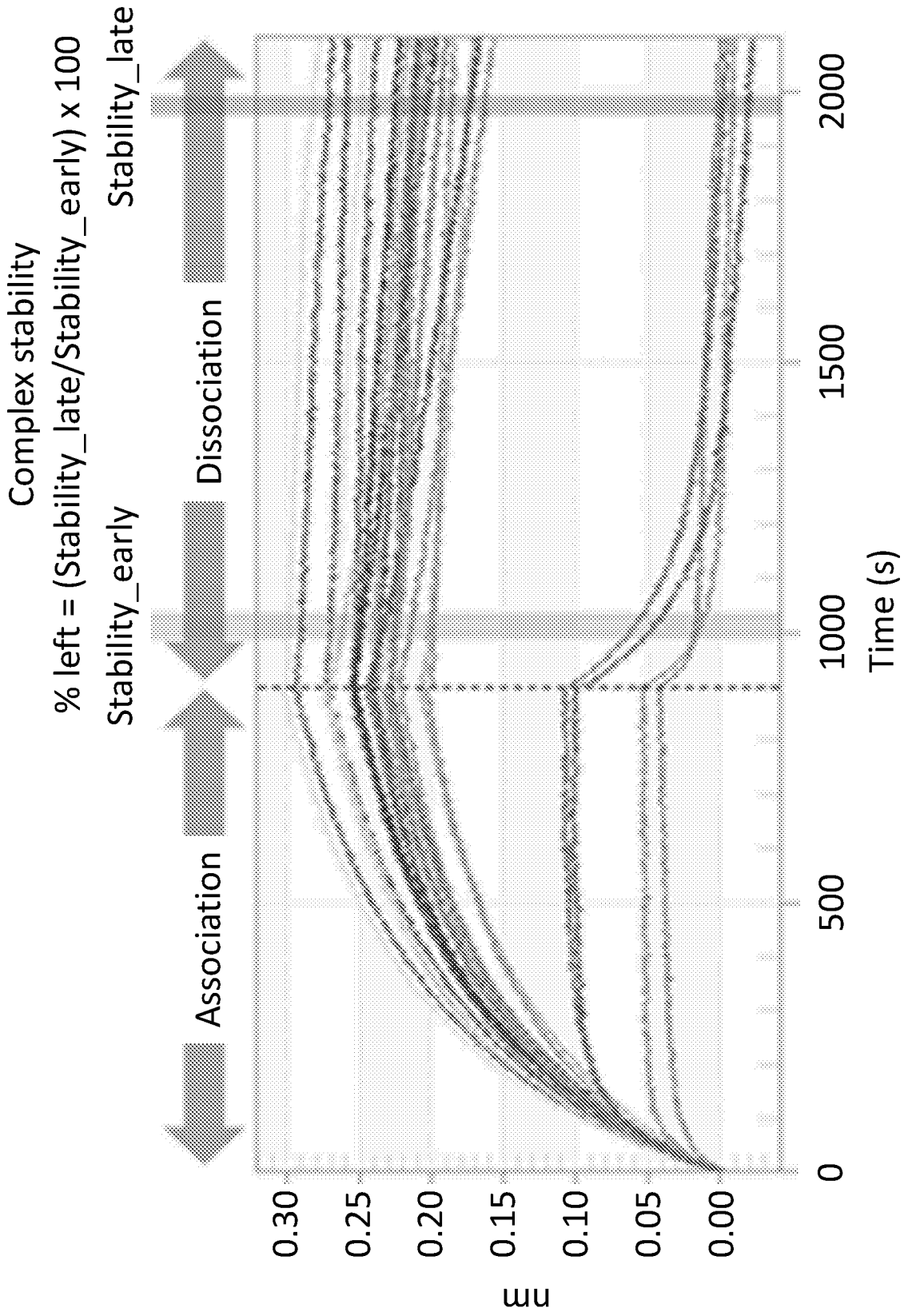

Complex stability
% left = (Stability_late/Stability_early) x 100
Stability_early
Association
Dissociation
Stability_late
nm
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
500
1000
1500
2000
Time (s)

Figure 14

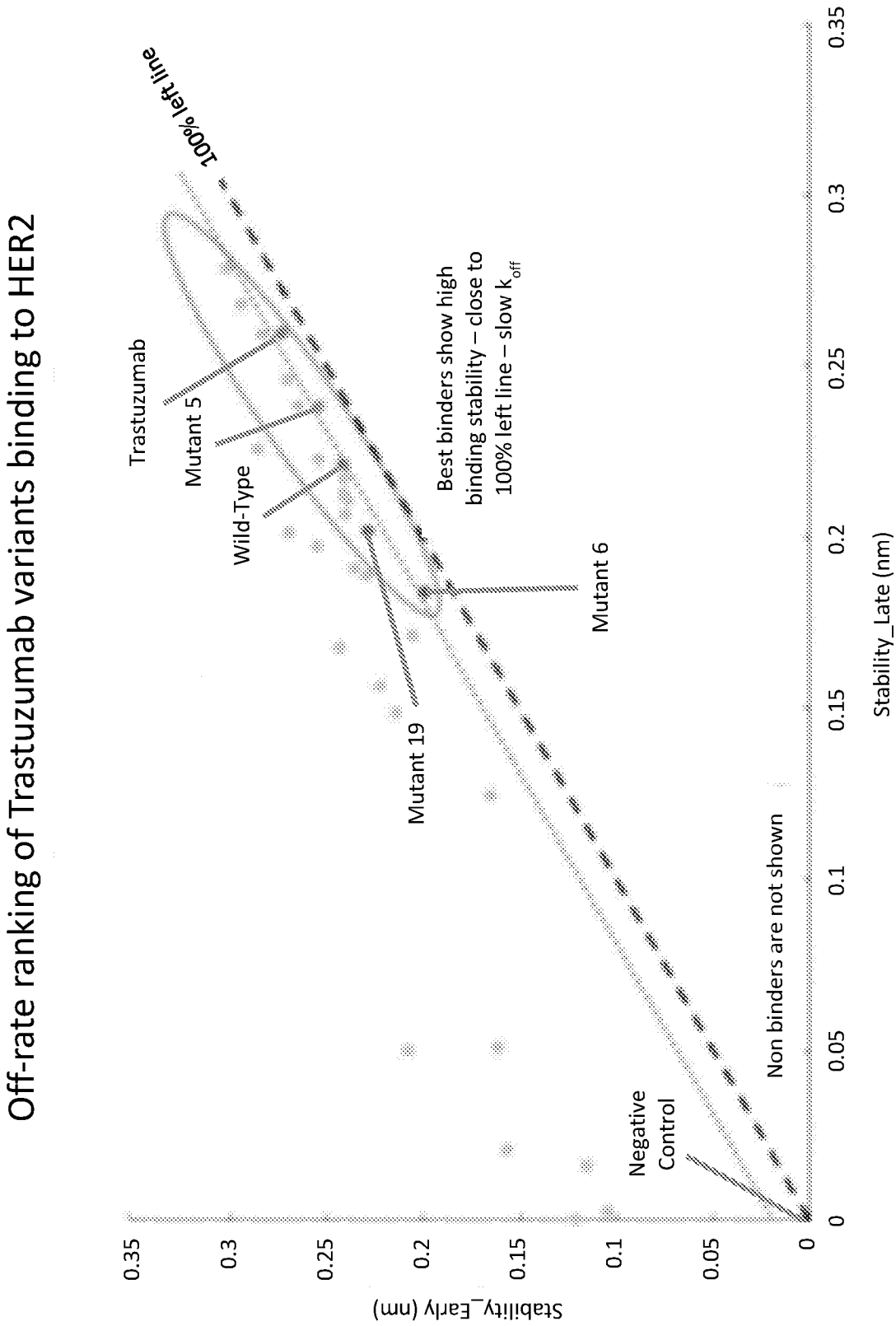

Off-rate ranking of Trastuzumab variants binding to HER2
100% left line
Trastuzumab
Mutant 5
Wild-Type
Best binders show high binding stability – close to 100% left line – slow k_{off}
Mutant 6
Mutant 19
Negative Control
Non binders are not shown
Stability_Early (nm)
Stability_Late (nm)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35

Figure 15

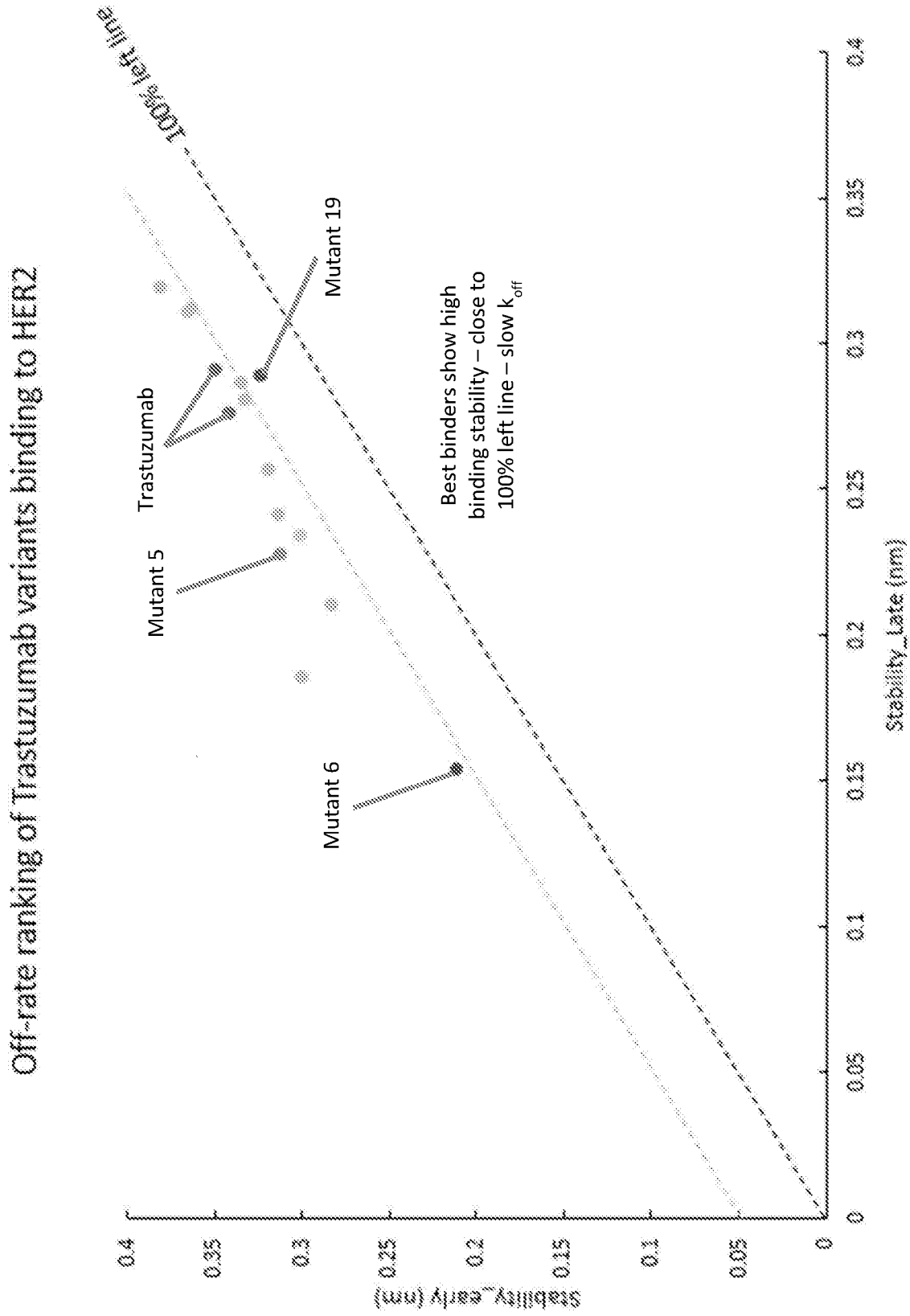

Off-rate ranking of Trastuzumab variants binding to HER2
100% left line
Trastuzumab
Mutant 5
Mutant 6
Mutant 19
Best binders show high binding stability – close to 100% left line – slow k_{off}
Stability_Late (nm)
Stability_early (nm)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4

Figure 16

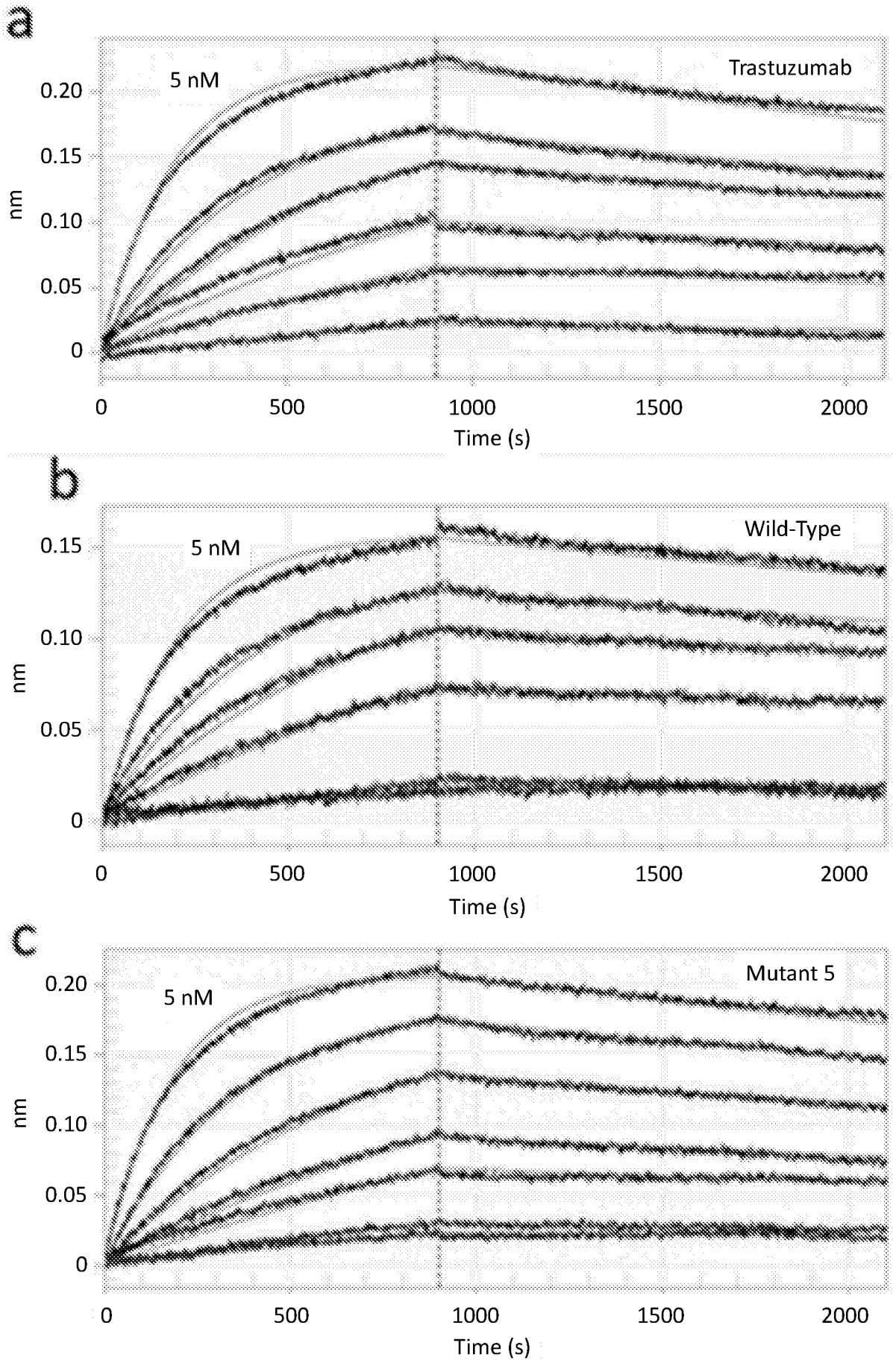

a
5 nM
Trastuzumab
0.20
0.15
0.10
0.05
0
nm
0
500
1000
1500
2000
Time (s)
b
5 nM
Wild-Type
0.15
0.10
0.05
0
nm
0
500
1000
1500
2000
Time (s)
c
5 nM
Mutant 5
0.20
0.15
0.10
0.05
0
nm
0
500
1000
1500
2000
Time (s)

Figure 17

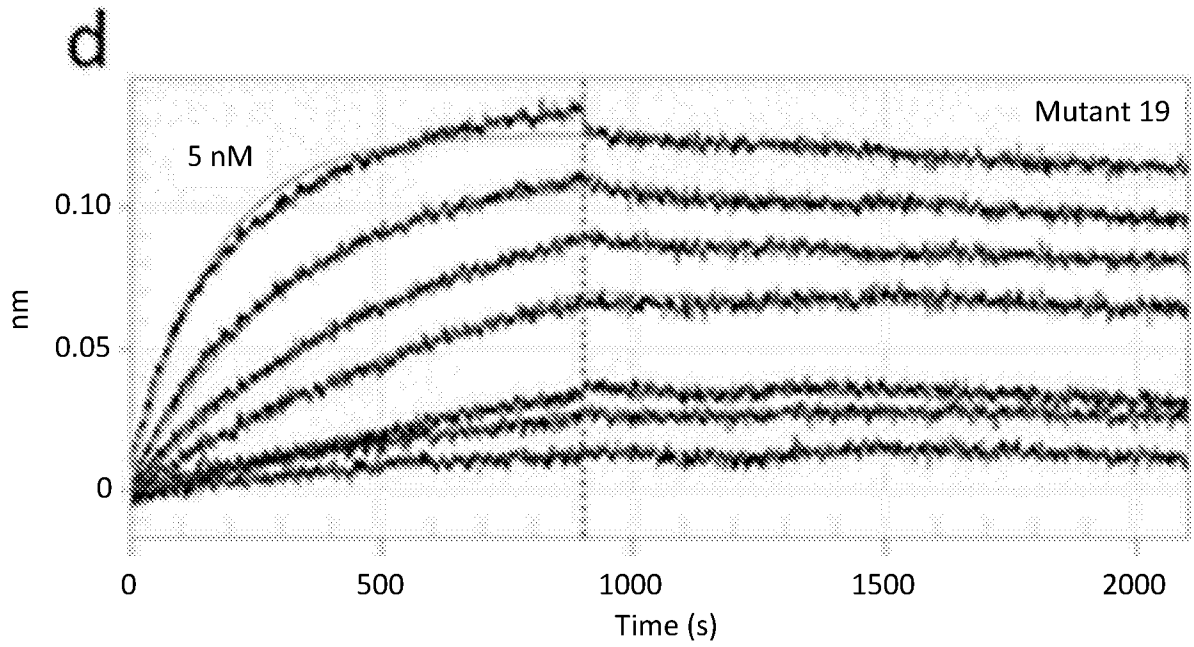


Figure 17 (continued)

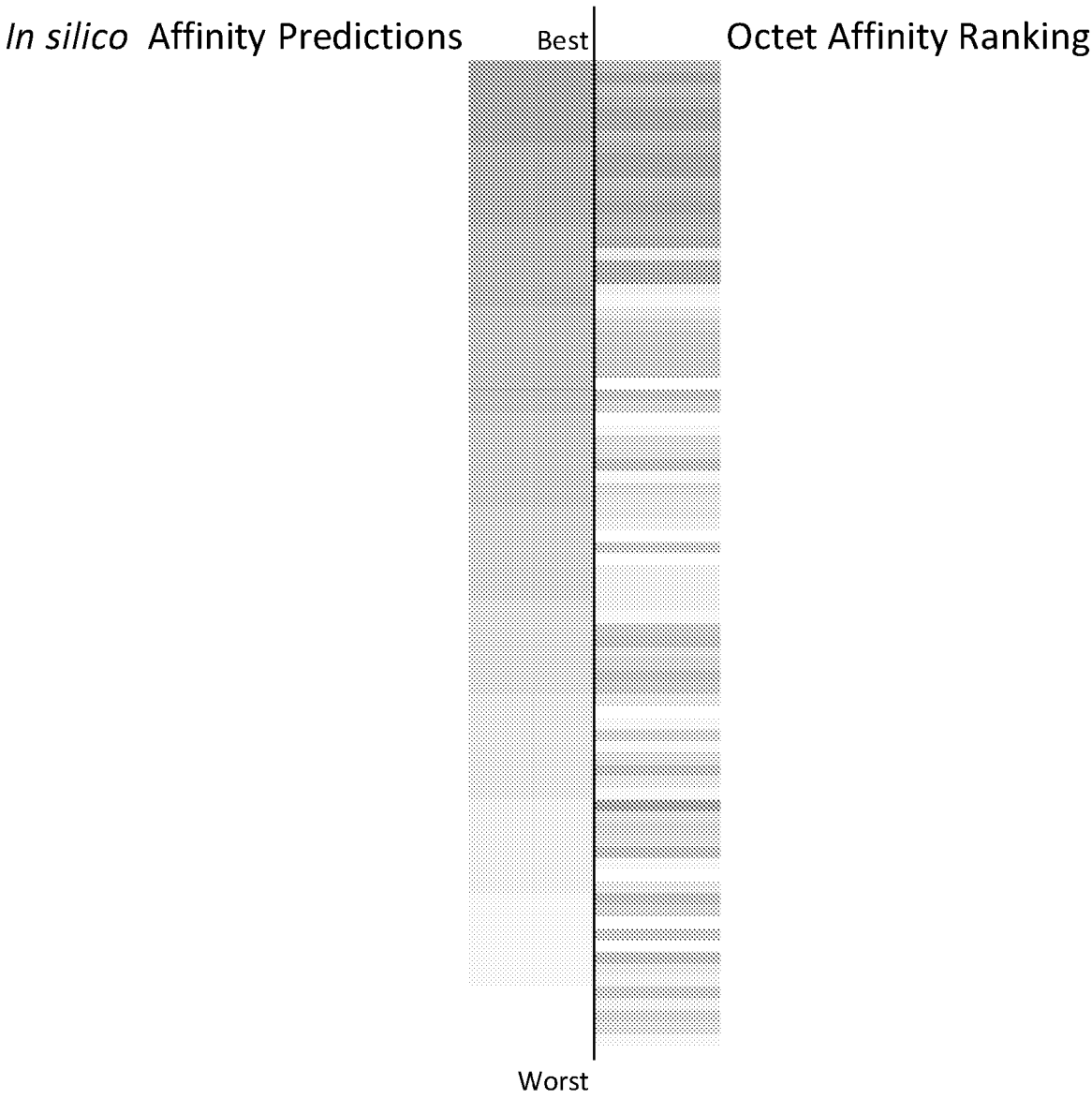

In silico Affinity Predictions
Best
Octet Affinity Ranking
Worst

Figure 18

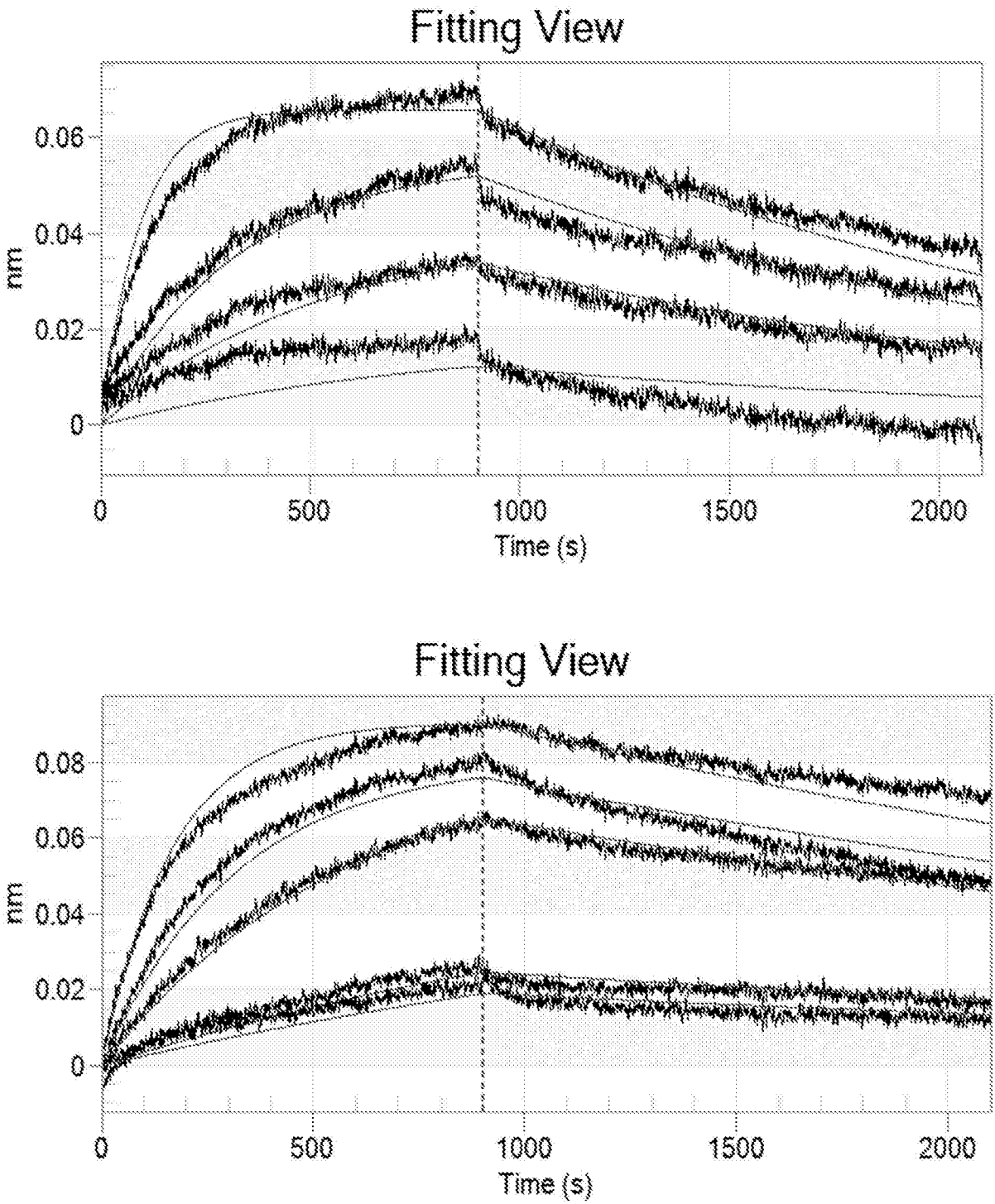

Fitting View
nm
0.06
0.04
0.02
0
0
500
1000
1500
2000
Time (s)
Fitting View
nm
0.08
0.06
0.04
0.02
0
0
500
1000
1500
2000
Time (s)

Figure 19

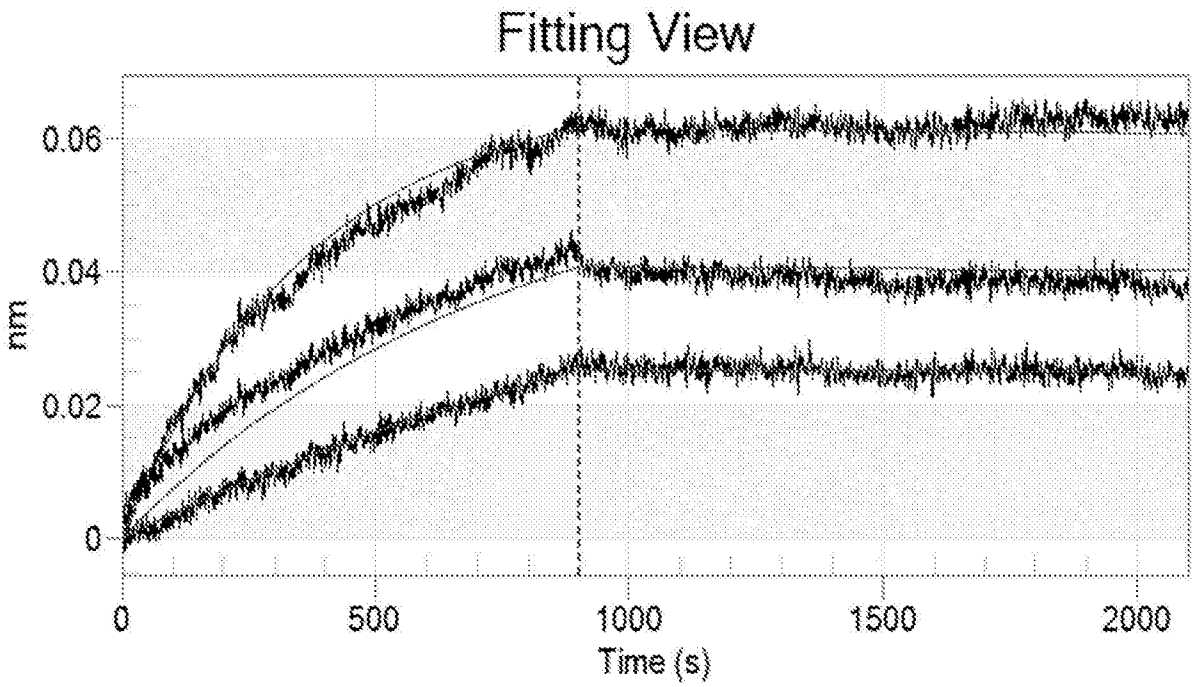


Figure 19 (continued)

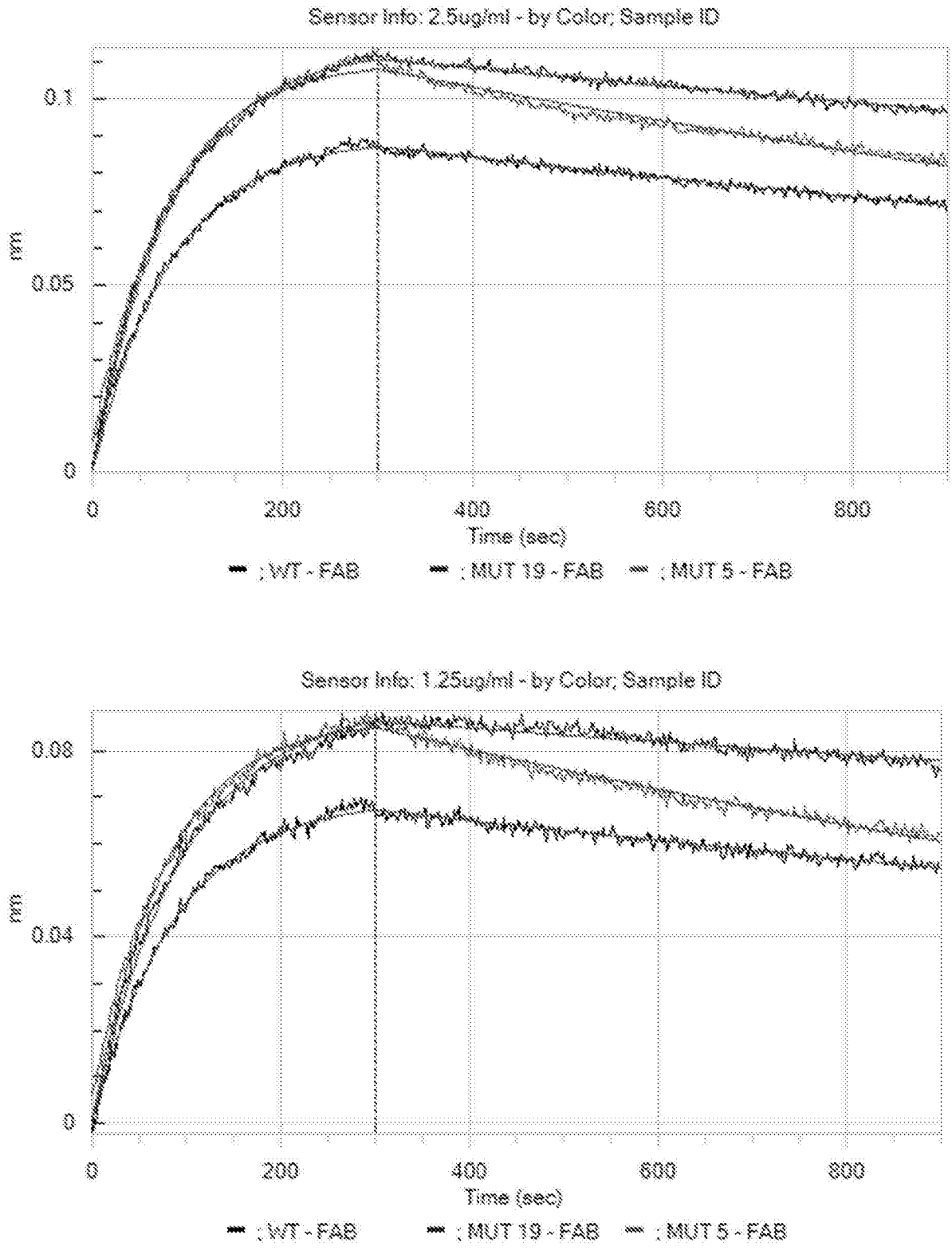

Sensor Info: 2.5ug/ml - by Color; Sample ID
nm
0.1
0.05
0
0
200
400
600
800
Time (sec)
; WT - FAB
; MUT 19 - FAB
; MUT 5 - FAB
Sensor Info: 1.25ug/ml - by Color; Sample ID
nm
0.08
0.04
0
0
200
400
600
800
Time (sec)
; WT - FAB
; MUT 19 - FAB
; MUT 5 - FAB

Figure 20

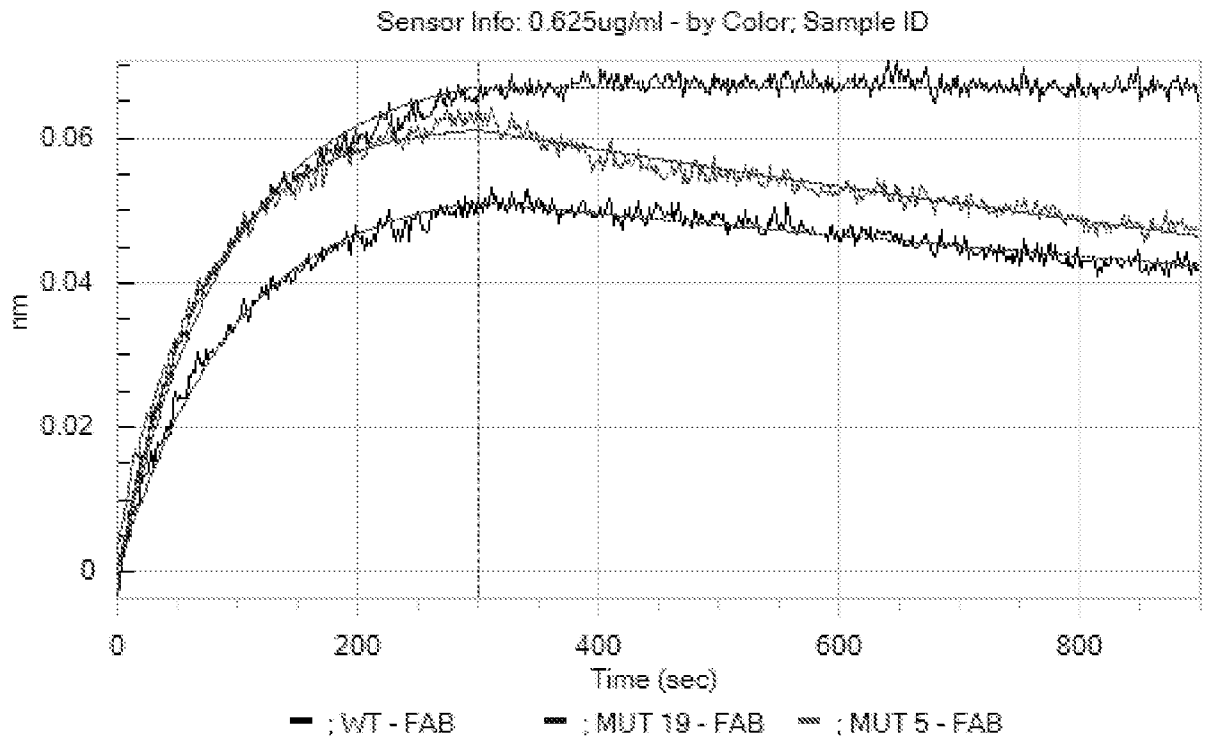


Figure 20 (continued)

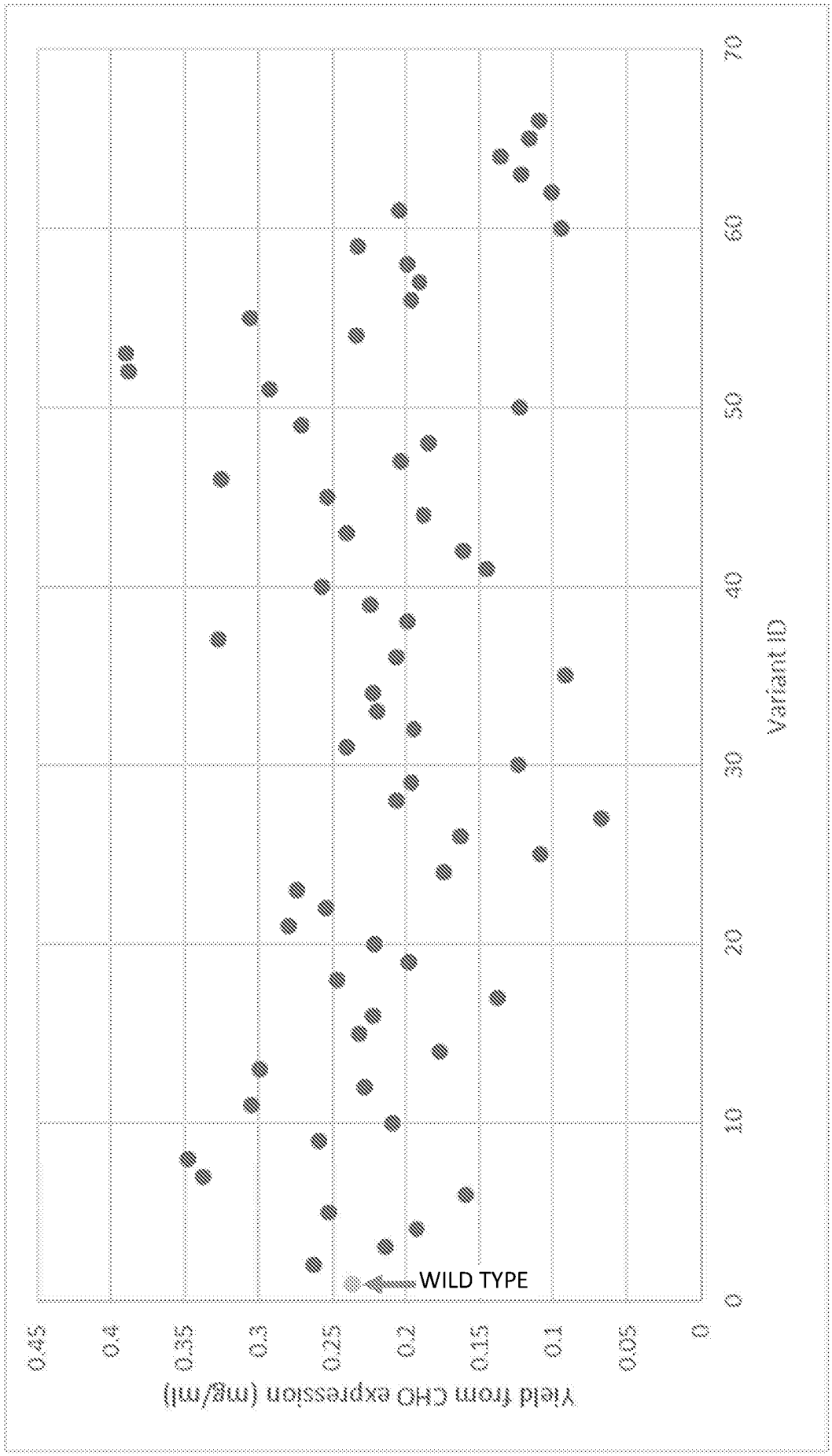

Yield from CHO expression (mg/ml)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
WILD TYPE
0
10
20
30
40
50
60
70
Variant ID

Figure 21

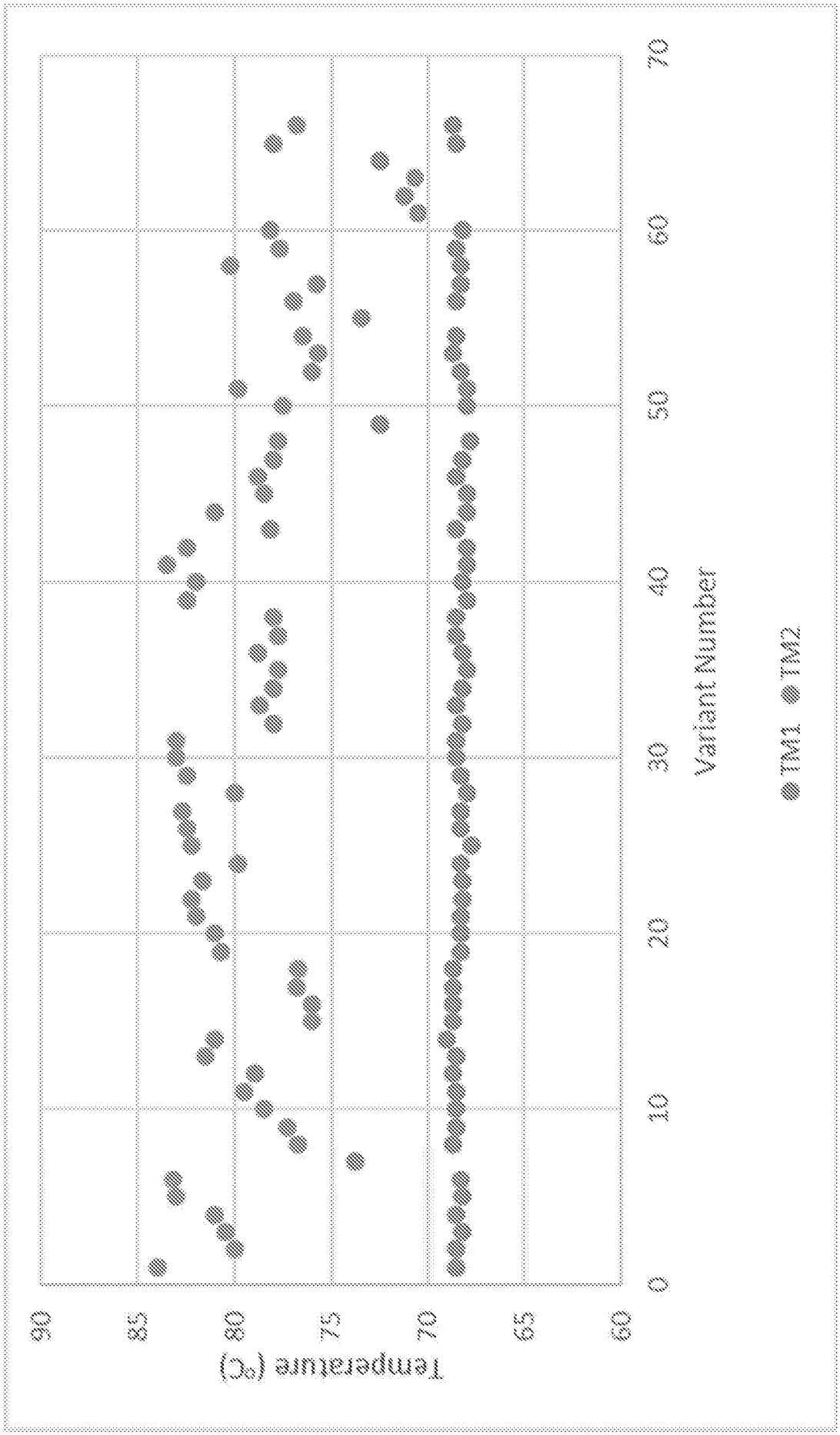

Temperature (°C)
90
85
80
75
70
65
60
0
10
20
30
40
50
60
70
Variant Number
TM1
TM2

Figure 22

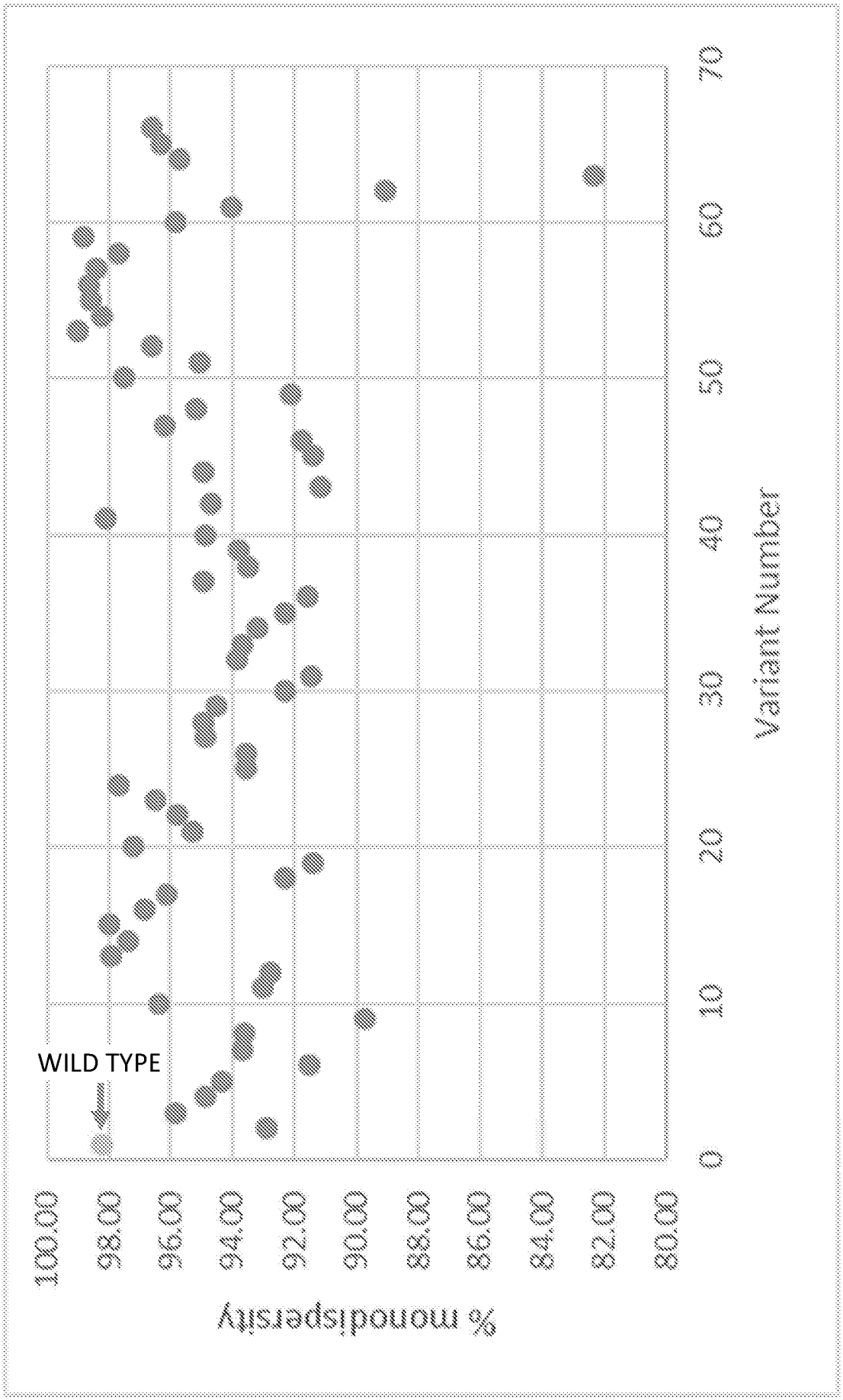

WILD TYPE
% monodispersity
100.00
98.00
96.00
94.00
92.00
90.00
88.00
86.00
84.00
82.00
80.00
Variant Number
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30
40
50
60
70

Figure 23

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Asn Gly Asn Thr Tyr Leu His Xaa Tyr Leu Gln Lys Xaa Gly Gln Ser
        35   40   45

Pro Xaa Xaa Leu Ile Tyr Lys Xaa Ser Xaa Arg Phe Ser Gly Val Pro
    50   55   60

Asp Xaa Phe Ser Xaa Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65   70   75   80

Xaa Arg Val Glu Xaa Glu Asp Val Gly Xaa Tyr Tyr Xaa Ser Gln Xaa
                85   90   95

Thr His Val Pro Pro Thr Phe Gly Gln Gly Thr Xaa Leu Glu Ile Lys
            100   105   110

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Asp Met Ser Trp Xaa Arg Gln Xaa Pro Gly Lys Gly Leu Glu Trp Val
        35   40   45

Ala Tyr Ile Xaa Xaa Gly Gly Val Asn Thr Tyr Tyr Pro Asp Thr Val
    50   55   60

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Xaa Lys Asn Thr Leu Tyr
65   70   75   80

Leu Gln Met Xaa Ser Leu Lys Ser Glu Asp Thr Xaa Val Tyr Tyr Cys
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Ala Arg His Ser Tyr Phe Asp Tyr Trp Gly Gln Xaa Thr Thr Val Thr
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Xaa Xaa Xaa Tyr Gln Xaa Lys Xaa Gly Lys Xaa Pro Lys Xaa Xaa Ile
        35                  40                  45


Tyr Ser Xaa Ser Phe Leu Tyr Ser Gly Val Pro Ser Arg Phe Ser Gly
    50                  55                  60


Ser Arg Ser Gly Thr Asp Phe Thr Leu Thr Ile Xaa Ser Leu Xaa Xaa
65                  70                  75                  80


Glu Asp Phe Ala Xaa Tyr Tyr Cys Xaa Xaa Xaa Tyr Xaa Xaa Pro Pro
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Ser Leu Arg Leu Ser Cys Xaa Xaa Ser Xaa Phe Xaa Ile Lys Asp Thr
            20   25   30

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Tyr Ile Xaa Trp Val Arg Gln Ala Pro Xaa Lys Gly Leu Glu Trp Xaa
        35                  40                  45

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    50                  55                  60

Lys Gly Arg Phe Thr Ile Ser Ala Asp Thr Ser Lys Xaa Thr Xaa Tyr
65                  70                  75                  80

Leu Xaa Met Xaa Xaa Leu Arg Xaa Glu Asp Thr Xaa Val Tyr Tyr Xaa
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Ser Arg Trp Gly Gly Asp Xaa Phe Tyr Xaa Met Asp Tyr Trp Gly Gln
            100                 105                 110

Gly Xaa Leu Val Thr Val Ser Ser
        115                 120
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