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(54) Titre : CONCEPTION D'ANTICORPS A L'AIDE DE STRUCTURES CRISTALLINES D'ANTICORPS ANTI-LIPIDIQUES

(54) Title: ANTIBODY DESIGN USING ANTI-LIPID ANTIBODY CRYSTAL STRUCTURES

(57) Abrégé/Abstract:
The present invention provides crystalline forms of an anti-lipid antibody or fragment thereof, which may further comprise a lipid ligand of said antibody and/or salts, metals, or co-factors. Methods for making such crystals and co-crystals are provided. The lipid may be a bioactive lipid, including sphingolipids such as S1P. X-ray coordinates of such a crystal are provided, as are methods of using this information in antibody design or optimization. Methods for designing a humanized antibody to a lipid are provided. These methods may be performed in silico and may be intended to enhance binding affinity of an antibody to its original target lipid, and/or to alter binding specificity. Antibodies produced by these methods are also provided.

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(54) **Title**: ANTIBODY DESIGN USING ANTI-LIPID ANTIBODY CRYSTAL STRUCTURES

(57) **Abstract**: The present invention provides crystalline forms of an anti-lipid antibody or fragment thereof, which may further comprise a lipid ligand of said antibody and/or salts, metals, or co-factors. Methods for making such crystals and co-crystals are provided. The lipid may be a bioactive lipid, including sphingolipids such as S1P. X-ray coordinates of such a crystal are provided, as are methods of using this information in antibody design or optimization. Methods for designing a humanized antibody to a lipid are provided. These methods may be performed in silico and may be intended to enhance binding affinity of an antibody to its original target lipid, and/or to alter binding specificity. Antibodies produced by these methods are also provided.

WHAT IS CLAIMED IS:

1. A crystalline composition comprising an anti-lipid antibody or fragment thereof, wherein the lipid is a bioactive lipid, optionally a sphingolipid or a lysolipid, and wherein the anti-lipid antibody or fragment thereof optionally is a monoclonal anti-lipid antibody or fragment thereof, wherein the fragment optionally is a Fab fragment.

2. A crystalline composition according to claim 1 that further comprises a lipid ligand of the antibody.

3. A crystalline composition according to claim 1 that further comprises at least one co-factor, salt, or metal.

4. A crystalline composition according to claim 1 that comprises an anti-lipid antibody defined by a set of structure coordinates as presented in Table 10 or Table 11.

5. A crystalline composition according to claim 1 that comprises a co-crystal of an antibody Fab fragment and its bioactive lipid ligand.

6. A computer-readable storage medium comprising a data storage medium storing computer-readable data, wherein the data comprises structural coordinates of all or a selected portion of an anti-lipid antibody or fragment thereof derived from a crystalline composition according to claim 1, wherein the data optionally comprises all or a selected portion of the structural coordinates shown in Table 10 or Table 11.

7. Use of a crystalline composition according to claim 1 in:

a. determining the structure of the antibody;

b. determining the ligand-binding characteristics of the antibody or fragment thereof, or of a variant or fragment of variant of the antibody;

c. designing an antibody, or fragment thereof, specifically reactive with a lipid, optionally a bioactive lipid; and/or

d. optimizing or altering the affinity of a monoclonal antibody for a lipid,

8. A method of preparing a crystalline composition according to claim 2 comprising co-crystals comprising the antibody or antibody fragment and its lipid ligand, comprising:

a. providing an anti-lipid monoclonal antibody or fragment thereof, optionally a Fab fragment;

b. combining the antibody or fragment thereof with an excess of the lipid ligand under conditions in which antibody-ligand or antibody fragment-ligand complexes form; and

c. incubating the antibody-ligand or antibody fragment-ligand complexes under conditions in which antibody-ligand or antibody fragment-ligand co-crystals form, thereby preparing a co-crystal of antibody or antibody fragment and its lipid ligand.

9. A co-crystal of an antibody or antibody fragment and its lipid ligand prepared according to claim 8, wherein the lipid is optionally a bioactive lipid, optionally a sphingolipid or a lysolipid.

10. A method of designing an optimized antibody to a lipid comprising:

a. providing an amino acid sequence of at least one variable region of a heavy or light chain of a first humanized anti-lipid antibody, wherein the anti-lipid antibody is specific for a first lipid, optionally a sphingolipid or a lysolipid, and wherein optionally at least one complementarity-determining region within the variable region is identified;

b. replacing one or more amino acids within the at least one variable region with a different amino acid to yield a variant amino acid sequence, wherein the amino acid replacement(s) is(are) within a complementarity-determining region;

c. preparing a second humanized anti-lipid antibody containing the variant amino acid sequence, wherein the amino acid sequences of the first and second humanized anti-lipid antibodies differ only in the variant amino acid sequence;

d. determining one or more activity criteria of the second humanized antibody, optionally by molecular modeling, ELISA or surface plasmon resonance, wherein at least one of the activity criteria is optionally binding affinity for the first lipid, binding affinity for a second lipid, or specificity for the first lipid or specificity for a second lipid, wherein the first and second lipids are different lipid species; and

e. selecting an optimized antibody based on one or more of the activity criteria, wherein the optimized antibody is the second humanized antibody,

wherein the method is optionally performed in silico.

11. A method according to claim 10 further comprising use of three-dimensional structural information about the binding of the first antibody and the first lipid to select a location and/or identity of the amino acid replacement(s), optionally wherein the three-dimensional structural information is molecular modeling data or x-ray crystallography data.

12. An optimized antibody made according to claim 15.

13. A method according to claim 10 wherein the first humanized anti-lipid antibody is LT1009, optionally wherein the one or more amino acids replaced is/are selected from the group consisting of:

aspartic acid at positions 30, 31 and 32, glutamic acid at position 50, aspartic acid at position 92, leucine at position 94 and phenylalanine at position 96, all of the light chain; and threonine at position 33, histidine at position 35, alanine at position 50, serine at position 52, histidine at position 54, isoleucine at position 56, lysine at position 58, phenylalanine at position 97, tyrosine at position 98, threonine at position 100A or tryptophan at position 100C, all of the heavy chain.

14. An optimized antibody variant of LT1009 prepared according to claim 13, optionally wherein one or more amino acids within one or more of the variable regions of LT1009 is replaced with a different amino acid to yield a variant amino acid sequence, and wherein the one or more replaced amino acids is selected from the group consisting of: aspartic acid at positions 30, 31 and 32, glutamic acid at position 50, aspartic acid at position 92, leucine at position 94 and phenylalanine at position 96, all of the light chain; and threonine at position 33, histidine at position 35, alanine at position 50, serine at position 52, histidine at position 54, isoleucine at position 56, lysine at position 58, phenylalanine at position 97, tyrosine at position 98, glycine at position 99, serine at position 100, threonine at position 100A or tryptophan at position 100C, all of the heavy chain.

15. A method selected from the group consisting of:

a. a method of designing a consensus anti-lipid antibody specifically reactive with a target bioactive lipid, comprising:

(i) identifying at least a first CDR amino acid sequence from a first parent antibody species specifically reactive with a target bioactive lipid and at least a second CDR amino acid sequence from a second parent antibody species specifically reactive with the target bioactive lipid, wherein the first and second CDR amino acid sequences are both CDRH1, both CDRH2, both CDRH3, both CDRL1, both CDRL2 or both CDRL3 CDR amino acid sequences;

(ii) aligning the first CDR amino acid sequence and second CDR amino acid sequence to determine a consensus CDR amino acid sequence; and

(iii) engineering a nucleic acid sequence that encodes the consensus CDR amino acid sequence into a gene comprising a variable region of an antibody heavy or light chain, thereby designing a consensus anti-lipid antibody specifically reactive with a target bioactive lipid, and, optionally; and

(iv) producing an antibody or antibody fragment that binds the target bioactive lipid;

b. a method of designing an antibody variant or antibody fragment variant specifically reactive with a target bioactive lipid, comprising:

(i) providing a first structural representation comprising an initial representation of a target bioactive lipid in binding association with an antibody or antibody fragment specifically reactive with a source bioactive lipid, wherein the target bioactive lipid and the source bioactive lipid are the same or a different bioactive lipid species, wherein the initial representation comprises three-dimensional structural information for the antibody or antibody fragment optionally derived from molecular modeling data or x-ray crystallography data; and

(ii) substituting at least one amino acid residue represented in the first structural representation with a different amino acid residue in order to identify a second structural representation comprising a subsequent representation of the target bioactive lipid in modified binding association with the modified antibody or antibody fragment, thereby designing an antibody variant or antibody fragment variant that is specifically reactive with the target bioactive lipid, wherein the antibody variant or antibody fragment optionally has a modified binding association, optionally an improved binding association, for the bioactive lipid.

16. A method according to claim 15 performed in silico.

17. An antibody or antibody fragment, optionally a humanized antibody or fragment thereof, produced according to claim 15(a).

18. A method according to claim 15(b) wherein the target bioactive lipid is (i) the same as the source bioactive lipid or (ii) different than the source bioactive lipid, wherein in either case the target bioactive lipid optionally is S1P.

19. A method according to claim 15(b) further comprising engineering one or more nucleotide sequences that encode the antibody variant or antibody fragment variant that binds the target bioactive lipid, and optionally further comprising producing the antibody variant or antibody fragment variant.

20. An antibody or antibody fragment specifically reactive with a target bioactive lipid, or a composition comprising such an antibody or antibody fragment, wherein in either case the antibody or antibody fragment is produced in accordance with claim 19.

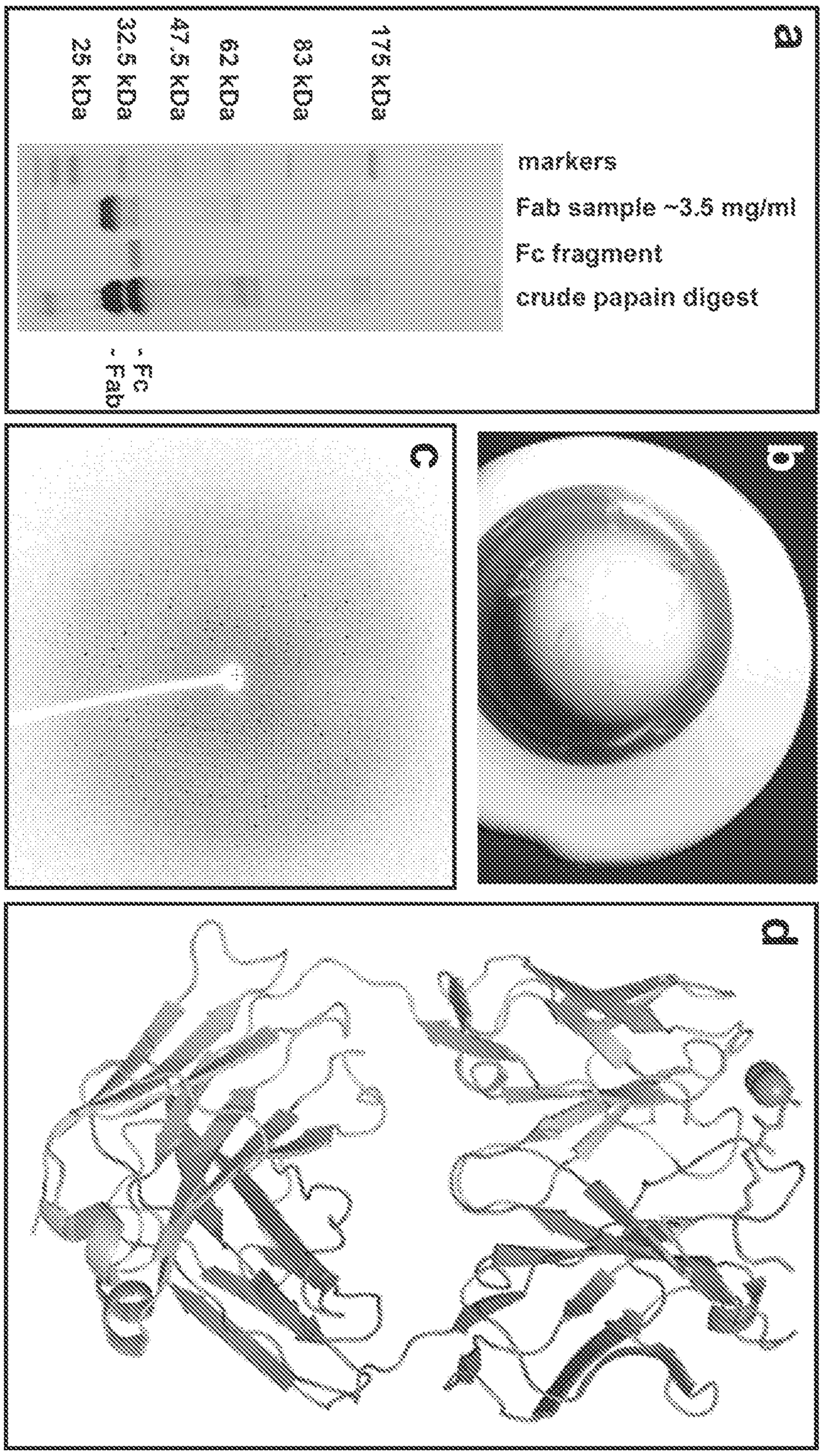
a
175 kDa
83 kDa
62 kDa
47.5 kDa
32.5 kDa
25 kDa
markers
Fab sample ~3.5 mg/ml
Fc fragment
crude papain digest
Fab
Fc
b
c
d

Figure 1

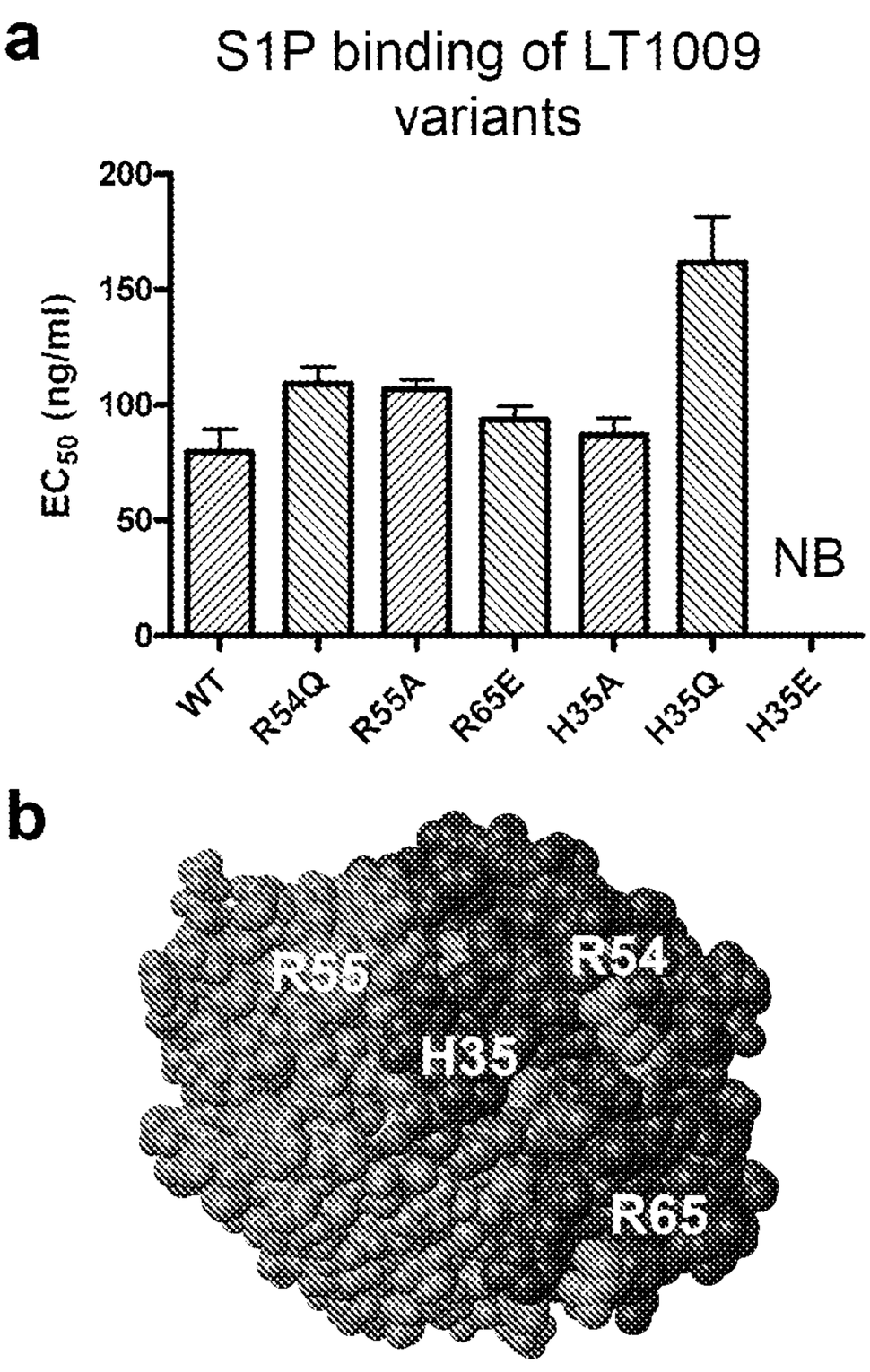
a
S1P binding of LT1009 variants
200
150
100
50
0
EC50 (ng/ml)
NB
WT
R54Q
R55A
R65E
H35A
H35Q
H35E
b
R55
R54
H35
R65

Figure 2

CA 02745436 2011-06-01

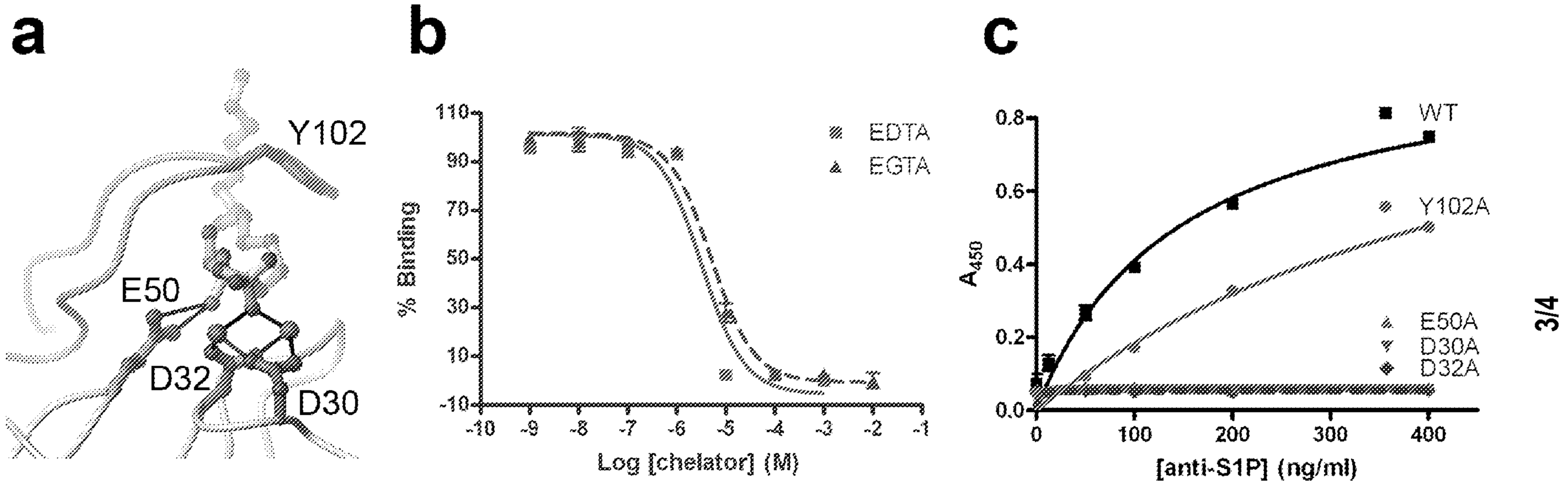

Figure 3

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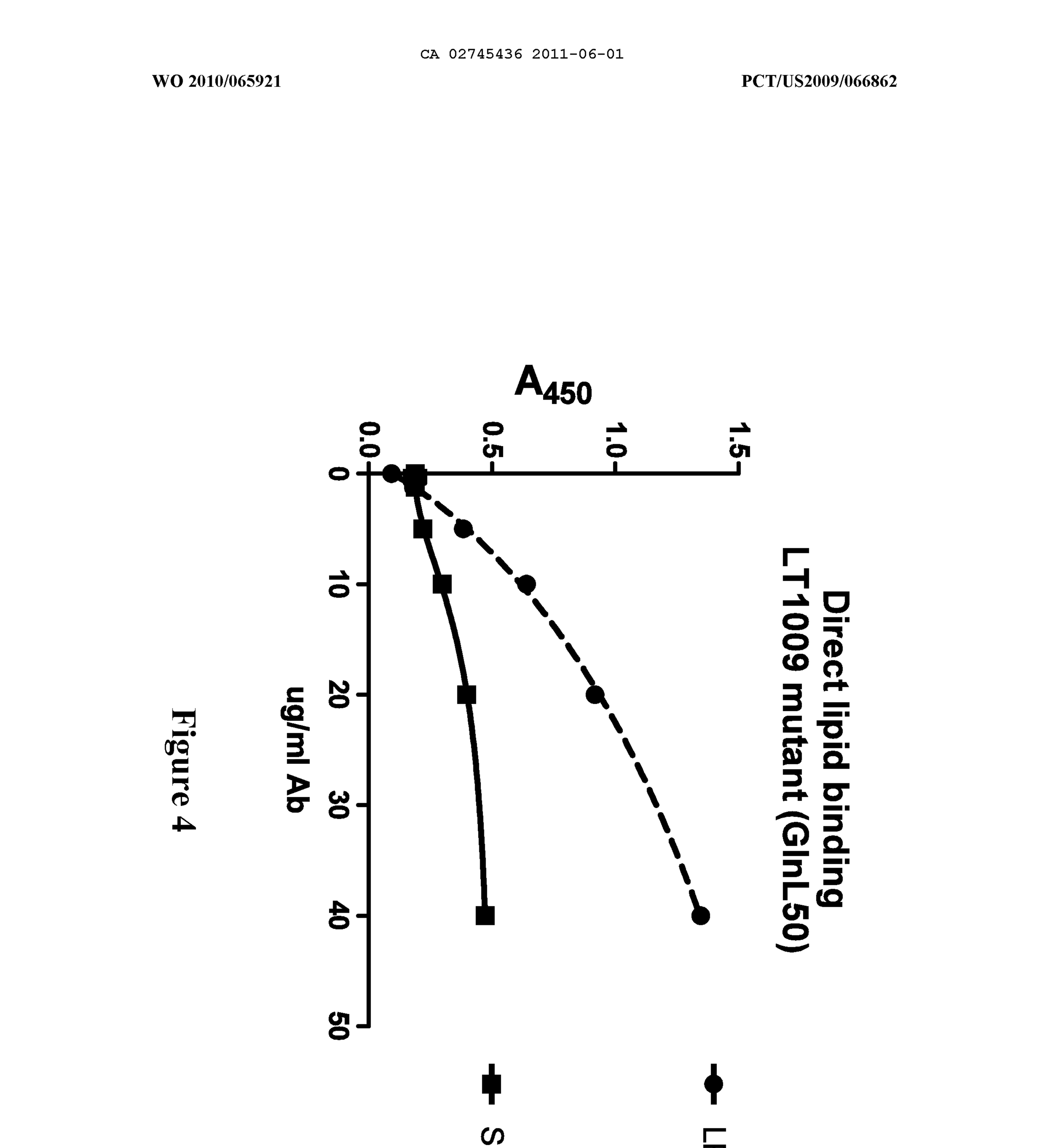
A450
0.0
0.5
1.0
1.5
0
10
20
30
40
50
ug/ml Ab
Direct lipid binding
LT1009 mutant (GlnL50)
S1P
LPA

Figure 4