STRUCTURE-BASED CONSTRUCTION OF HUMAN ANTIBODY LIBRARY

Methods and systems are provided for constructing recombinant antibody libraries based on three-dimensional structures of antibodies from various species including human. In one aspect, a library of antibodies with diverse sequences is efficiently constructed in silico to represent the structural repertoire of the vertebrate antibodies. Such a functionally representative library provides a structurally diverse and yet functionally more relevant source of antibody candidates which can then be screened for high affinity binding to a wide variety of target molecules, including but not limited to biomacromolecules such as protein, peptide, and nucleic acids, and small molecules.
STRUCTURE-BASED CONSTRUCTION OF
HUMAN ANTIBODY LIBRARY

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates generally to computer-aided design of human antibody sequence libraries, and, more particularly, relates to methods and systems for selecting and constructing fully human or human-derived antibody library based on three-dimensional structural frameworks of vertebrate antibody repertoire.

Description of Related Art

Antibodies are made by vertebrates in response to various internal and external stimuli (antigens). Synthesized exclusively by B cells, antibodies are produced in millions of forms, each with different amino acid sequence and a different binding site for antigen. Collectively called immunoglobulins (abbreviated as Ig), they are among the most abundant protein components in the blood, constituting about 20% of the total plasma protein by weight.


The mammalian immune system has evolved unique genetic mechanisms that enable it to generate an almost unlimited number of different light and heavy chains in a remarkably economical way by joining separate gene segments together before they are transcribed. For each type of Ig chain—κ light chains, λ light chains, and heavy chain—there is a separate pool of gene segments from which a single peptide chain is eventually synthesized. Each pool is on a different chromosome and usually contains a large number of gene segments.
encoding the V region of an Ig chain and a smaller number of gene
segments encoding the C region. During B cell development a complete
coding sequence for each of the two Ig chains to be synthesized is
assembled by site-specific genetic recombination, bringing together the
entire coding sequences for a V region and the coding sequence for a C
region. In addition, the V region of a light chain is encoded by a DNA
sequence assembled from two gene segments—a V gene segment and
short joining or J gene segment. The V region of a heavy chain is
encoded by a DNA sequence assembled from three gene segments—a V
gene segment, a J gene segment and a diversity or D segment.

The large number of inherited V, J and D gene segments
available for encoding Ig chains makes a substantial contribution on its
own to antibody diversity, but the combinatorial joining of these
segments greatly increases this contribution. Further, imprecise joining
of gene segments and somatic mutations introduced during the V-D-J
segment joining at the pre-B cell stage greatly increases the diversity of
the V regions.

After immunization against an antigen, a mammal goes through
a process known as affinity maturation to produce antibodies with
higher affinity toward the antigen. Such antigen-driven somatic
hypermutation fine-tunes antibody responses to a given antigen,
presumably due to the accumulation of point mutations specifically in
both heavy- and light-chain V region coding sequences and a selected
expansion of high-affinity antibody-bearing B cell clones.

Structurally, various functions of an antibody are confined to
discrete protein domains (regions). The sites that recognize and bind
antigen consist of three complementarity-determining regions (CDRs)
that lie within the variable \(V_{H} \) and \(V_{L} \) regions at the N-terminal ends of
the two H and two L chains. The constant domains are not involved
directly in binding the antibody to an antigen, but are involved in
various effector functions, such as participation of the antibody in
antibody-dependent cellular cytotoxicity.

The domains of natural light and heavy chains have the same
general structures, and each domain comprises four framework regions,
whose sequences are somewhat conserved, connected by three hyper-
variable or CDRs. The four framework regions largely adopt a β-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the β-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen binding site.

Generally all antibodies adopt a characteristic "immunoglobulin fold". Specifically, both the variable and constant domains of an antigen binding fragment (Fab, consisting of V_L and C_L of the light chain and V_H and C_H 1 of the heavy chain) consist of two twisted antiparallel β-sheets which form a β-sandwich structure. The constant regions have three- and four-stranded β-sheets arranged in a Greek key-like motif, while variable regions have a further two short β strands producing a five-stranded β-sheet.

The V_L and V_H domains interact via the five-stranded β sheets to form a nine-stranded β barrel of about 8.4 Å radius, with the strands at the domain interface inclined at approximately 50° to one another. The domain pairing brings the CDR loops into close proximity. The CDRs themselves form some 25% of the V_L/V_H domain interface.

The six CDRs, (CDR-L1, -L2 and -L3 for the light chain, and CDR-H1, -H2 and -H3 for the heavy chain), are supported on the β barrel framework, forming the antigen binding site. While their sequence is hypervariable in comparison with the rest of the immunoglobulin structure, some of the loops show a relatively high degree of both sequence and structural conservation. In particular, CDR-L2 and CDR-H1 are highly conserved in conformation.

Chothia and co-workers have shown that five of the six CDR loops (all except CDR-H3) adopt a discrete, limited number of main-chain conformations (termed canonical structures of the CDRs) by analysis of conserved key residues. Chothia and Lesk (1987) J. Mol. Biol. 196:901-917; Chothia et al. (1989) Nature (London) 342:877; and Chothia et al. (1998) J. Mol. Biol. 278:457-479. The adopted structure depends on both the CDR length and the identity of certain key amino acid residues, both in the CDR and in the contacting framework, involved in its packing. The canonical conformations were determined by specific packing, hydrogen bonding interactions, and stereochemical
constraints of only these key residues which serve as structural
determinants.

Various methods have been developed for modeling the three
dimensional structures of the antigen binding site of an antibody.
Other than x-ray crystallography, nuclear magnetic resonance (NMR)
spectroscopy has been used in combination with computer modeling
building to study the atomic details of antibody-ligand interactions.
used spin-labeled hapten to deduce the combining site of the MoPC 315
myeloma protein for dinitrophenyl. Similar analysis has also been done
26: 6958-6064) and on the anti-2-phenyloxazolone Fv fragments

Computer-implemented analysis and modeling of antibody
combining site (or antigen binding site) is based on homology analysis
comparing the target antibody sequence with those of antibodies with
known structures or structural motifs in existing data bases (e.g. the
Brookhaven Protein Data Bank). By using such homology modeling
methods approximate three-dimensional structure of the target
antibody is constructed. Early antibody modeling was based on the
conjecture that CDR loops with identical length and different sequence
Acad. Sci. USA 69: 960-964. A typical segment match algorithm is as
follows: given a loop sequence, the Protein Data Bank can be searched
for short, homologous backbone fragments (e.g. tripeptides) which are
then assembled and computationally refined into a new combining site
model.

More recently, the canonical loop concept has been incorporated
into computer-implemented structural modeling of antibody combining
site. In its most general form, the canonical structure concept assumes
that (1) sequence variation at other than canonical positions is
irrelevant for loop conformation, (2) canonical loop conformations are
essentially independent of loop-loop interactions, and (3) only a limited
number of canonical motifs exist and these are well represented in the
database of currently known antibody crystal structures. Based on this
concept, Chothia predicted all six CDR loop conformations in the lysozyme-binding antibody D1.3 and five canonical loop conformations in four other antibodies. Chothia (1989), supra. It is also possible to improve modeling of CDRs of antibody structures by combining the homology modeling with conformational search procedures. Martin, A.C.R. (1989) PNAS 86, 9268-72.

Besides modeling a specific antibody structure, efforts have been made in generating artificial (or synthetic) libraries of antibodies which are screened against one or more specific, target antigens. Various artificial sequences are generated by site-specific or random mutagenesis on the antibody sequence, especially into the CDR regions of the variable domains. For example, Iba et al. used computer-driven model building system to change the specificity of antibodies against steroid antigens by introducing mutations into the CDR regions. Iba et al. (1998) Protein Eng. 11:361-370. A phage-display library of Abs in which 16 residues of 17-α-hydroxyprogesterone (17-OHP) were mutated in three CDR regions of the heavy chain that appeared to form the steroid-binding pocket. The phage display library were screened against 17-OHP and cortisol that had been conjugated with bovine serum albumin. Many clones were isolated that had retained 17-OHP-binding ability as well as clones with the newly developed ability to bind cortisol in addition to 17-OHP.

Immunol. 22: 867; Marks et al. (1992) Biotechnology 10: 779; Marks et
30: 10832; Lerner et al. (1992) Science 258: 1313). Also see review by

Generally, a phage library is created by inserting a library of a
random oligonucleotide or a cDNA library encoding antibody fragment
such as \(V_L\) and \(V_H\) into gene 3 of M13 or fd phage. Each inserted gene is
expressed at the N-terminal of the gene 3 product, a minor coat protein
of the phage. As a result, peptide libraries that contain diverse peptides
can be constructed. The phage library is then affinity screened against
immobilized target molecule of interest, such as an antigen, and
specifically bound phages are recovered and amplified by infection into
Escherichia coli host cells. Typically, the target molecule of interest
such as a receptor (e.g., polypeptide, carbohydrate, glycoprotein, nucleic
acid) is immobilized by covalent linkage to a chromatography resin to
enrich for reactive phage by affinity chromatography) and/or labeled for
screen plaques or colony lifts. This procedure is called biopanning.

Finally, amplified phages can be sequenced for deduction of the specific
peptide sequences.

The sequences of the antibodies in these phage display libraries are
from natural sources. For example, cDNA of antibody gene pools have
been generated from immunized or naive human or rodents. Barbas
and Burton (1996) Trends Biotech. 14:230-234 (immunized donors); De
Haard et al. (1999) J. Biol. Chem. 274:18218-18230 (naive B-cell Ig
repertoires). The antibody cDNA library can be constructed by reverse
transcription of RNA encoding the gene pool from total RNA samples
isolated from B cells contained in peripheral blood supplied by human
or animal. First strand cDNA synthesis is usually performed using the
method of Marks et al. in which a set of heavy and light chain cDNA
primers are designed to anneal to the constant regions for priming the
synthesis of cDNA of heavy chain and light chains (both κ and λ)
21:985-991.

Synthetic or artificial libraries of antibody sequences were
constructed in vitro from human germline sequences. Griffiths et al.
(1994) EMBO J. 13:3245-3260. Highly diverse repertoires of heavy and
light chains were created entirely in vitro from a bank of human V gene
segments and then, by recombination of the repertoire in bacteria, an
even larger (close to 6.5x10^19) synthetic library of Fab fragments were
generated in bacteria and displayed on filamentous phage.

Highly diverse synthetic libraries of antibody sequences were also
constructed based on consensus sequences of each germline family of
human antibody repertoire. For example, a fully synthetic
combinatorial antibody library was constructed based on modular
consensus frameworks and CDR3 regions in heavy and light chains
296:57-86. Knappik et al. analysed the human antibody repertoire in
terms of structure, amino acid sequence diversity and germline usage.

Modular consensus framework sequences with seven V\textsubscript{H} and seven V\textsubscript{L}
were derived to cover 95% of variable germline families and optimized
for expression in E. coli. A consensus sequence was derived for each
highly used germline family and optimized for expression in E. coli.
Molecular modeling of their CDR loops of the consensus sequences
verified that all canonical classes were present. Diversity of the
antibody library was created by replacing the CDR3 regions of seven V\textsubscript{H}
and seven V\textsubscript{L} frameworks of the master genes by CDR3 library
cassette. A synthetic library of combinatorial antibody was generated
from mixed trinucleotides and biased towards natural human antibody
CDR3 sequences. This library was cloned into phagemid and expressed
as soluble proteins in the periplasm of E. coli.
SUMMARY OF THE INVENTION

The present invention provides a comprehensive methodology to map the functional space of proteins by exploiting the fundamental structure-sequence relationship within protein families. The methodology of the present invention provides for efficient in silico selection and construction of a library of antibodies with diverse sequences. By using the methodology libraries of antibodies can be constructed with diverse sequences in the CDR regions, and humanized frameworks of the variable regions having fully human, human-derived antibody, or antibody of human origin (collectively referred to herein as "human antibody") based on three-dimensional structures of antibodies generated by all species of vertebrates including human.

In one aspect of the invention, a method is provided for constructing a library of artificial antibodies in silico based on ensembles of 3D structures of existing antibodies of human origin, optional also including those of other vertebrate origins. By using the method, a master library of human antibody sequences can be selected to better represent all antibody structural repertoire in the vertebrate antibody repertoire that are functionally important for high affinity binding to antigens and eliciting antibody-dependent cellular responses. Such a functionally representative library provides a structurally diverse and yet functionally more relevant source of antibody candidates which can then be screened for binding to a wide variety of target molecules, including but not limited to biomacromolecules such as protein, peptide, and nucleic acids, and small molecules.

In one embodiment, the method comprises the steps of:
- clustering variable regions of a collection of antibodies having known 3D structures into at least two families of structural ensembles, each family of structural ensemble comprising at least two different antibody sequences but with substantially identical main chain conformations;
- selecting a representative structural template from each family of structural ensemble;
profiling a tester polypeptide sequence onto the representative structural template within each family of structural ensemble; and selecting the tester antibody sequence that is compatible to the structural constraints of the representative structural template.

According to the method, examples of the collection of antibodies include, but are not limited to, antibodies or immunoglobulins collected in a protein database such as the protein data bank of Brookhaven National Laboratory, genbank at the National Institute of Health, and Swiss-PROT protein sequence database.

The collection of antibodies having known 3D structures include any antibody having resolved X-ray crystal structure, NMR structure or a 3D structure based on structural modeling such as homology modeling.

The variable regions of a collection of antibodies may be the full length of the heavy chain or light chain variable region or a specific portion of the heavy chain or light chain variable region, such as a CDR (e.g., VH or VL CDR1, CDR2, and CDR3), a framework region (FR, e.g., VH or VL FR1, FR2, FR3, and FR4), and a combination thereof.

Also according to the method, the clustering step includes clustering the collection of antibodies such that the root mean square difference of the main chain conformations of antibody sequences in each family of the structural ensemble is preferably less than 4 Å, more preferably less than 3 Å, and most preferably less than 2 Å.

Optionally, the clustering step includes clustering the collection of antibodies such that the Z-score of the main chain conformations of antibody sequences in each family of the structural ensemble is preferably more than 2, more preferably more than 3, and most preferably more than 4.

The clustering step may be implemented by an algorithm selected from the group consisting of CE, Monte Carlo and 3D clustering algorithms.

Also according to the method, the profiling step includes reverse threading the tester polypeptide sequence onto the representative structural template within each family of structural ensemble.
Optionally, the profiling step is implemented by a multiple sequence alignment algorithm such as the profile HMM algorithm and PSI-BLAST (Position-Specific Iterated BLAST).

When the representative structural template is adopted by a CDR region, the profiling step includes profiling the tester polypeptide sequence that is a human or non-human antibody onto the representative structural template within each family of structural ensemble.

When the representative structural template is adopted by a FR region, the profiling step includes profiling the tester polypeptide sequence that is a human or non-human antibody, preferably a human germline antibody sequence, onto the representative structural template within each family of structural ensemble.

In another aspect of the invention, a method is provided for in silico selection of antibody sequences based on structural alignment with a target structural template. Diverse sequences which still retain the same functionally relevant structure as the target structural template can be selected by using reverse threading, the profile HMM algorithm and PSI-BLAST. By using the method, a library of diverse antibody sequences can be constructed and screened experimentally in vitro or in vivo for antibody mutants with improved or desired function(s).

In one embodiment, the method comprises the steps of:

- providing a target structural template of a variable region of one or more antibodies;
- profiling a tester polypeptide sequence onto the target structural template; and
- selecting the tester polypeptide sequence that is structurally compatible with the target structural template.

According to the method, the target structural template may be a 3D structure of a heavy chain or light chain variable region of an antibody (e.g., CDR, FR and a combination thereof), or a structural ensemble of heavy chain or light chain variable regions of at least two different antibodies.
Also according to the method, the profiling step includes reverse threading the tester polypeptide sequence onto the target structural template.

Optionally, the profiling step is implemented by a multiple sequence alignment algorithm such as the profile HMM algorithm and PSI-BLAST.

Also optionally, when the target structural template is adopted by a CDR region of the target antibody, the profiling step includes profiling a heavy chain or light chain variable region of the tester polypeptide sequence that is either a human antibody or a non-human antibody.

Also optionally, when the target structural template is adopted by a FR region of the target antibody, the profiling step includes profiling a heavy chain or light chain variable region of the tester polypeptide sequence that is a human antibody, preferably a human germline antibody, onto the target structural template.

According to any of the above method, the tester polypeptide sequence may be the sequence or a segment sequence of an expressed protein, preferably an antibody, more preferably a human antibody, and most preferably a human germline antibody sequence.

According to any of the above method, the selecting step includes selecting the tester polypeptide sequence by using an energy scoring function selected from the group consisting of electrostatic interactions, van der Waals interactions, electrostatic solvation energy, solvent-accessible surface solvation energy, and conformational entropy.

Optionally, the selecting step includes selecting the tester polypeptide sequence by using a scoring function incorporating a forcefield selected from the group consisting of the Amber forcefield, Charmm forcefield, the Discover cvff forcefields, the ECEPP forcefields, the GROMOS forcefields, the OPLS forcefields, the MMFF94 forcefield, the Tripose forcefield, the MM3 forcefield, the Dreiding forcefield, and UNRES forcefield, and other knowledge-based statistical forcefield (mean field) and structure-based thermodynamic potential functions.

In yet another aspect of the invention, a method is provided for in silico selection of antibody sequences based on homology alignment.
with a target sequence template. Remote homologues with diverse sequences but retaining the same functionally relevant structure can be selected by using profile hidden Markov Model (HMM) and PSI-BLAST. By using the method, a library of diverse antibody sequences can be constructed with a relatively smaller size than that constructed by complete randomization of the target sequence. This library can then be filtered using certain cutoff value based on, for example, the occurrence frequency of variants in each amino acid residue position, and screened experimentally in vitro or in vivo for antibody mutants with improved or desired function(s).

In one embodiment, the method comprises the steps of:

providing a target sequence of a heavy chain or light chain variable region of an antibody;

aligning the target sequence with a tester polypeptide sequence;

and

selecting the tester polypeptide sequence that has at least 15% sequence homology with the target sequence.

According to the method, the target sequence may be the full length of the heavy chain or light chain variable region, or a specific portion of the variable region, such as a CDR, a framework (FR) region and a combination thereof.

Also according to the method, the aligning step includes aligning the target sequence with the polypeptide segment of the tester protein by using a sequence alignment algorithm selected from the group consisting of BLAST, PSI-BLAST, profile HMM, and COBLATH.

Also according to the method, when the target sequence is a CDR region of the target antibody, the alignment step includes aligning any protein sequences that is of either human or non-human origin with the target sequence.

Also according to the method, when the target sequence is a CDR region of the target antibody, the tester polypeptide sequence is a heavy chain or light chain variable region of a human or non-human antibody.

Also according to the method, when the target sequence is a FR region of the target antibody, the tester polypeptide sequence is a heavy
chain or light chain variable region of a human antibody, preferably a human germline antibody sequence.

Also according to the method, the selecting step includes selecting the polypeptide segment of the tester protein that has preferably at least 25%, preferably at least 35%, and most preferably at least 45% sequence homology with the target sequence.

According to any of the above methods, the method further comprises:

introducing the DNA segment encoding the selected tester polypeptide into cells of a host organism;

expressing the DNA segment in the host cells such that a recombinant antibody containing the selected polypeptide or antibody sequence is produced in the cells of the host organism; and

selecting the recombinant antibody that binds to a target antigen with affinity higher than $10^5 \text{ M}^{-1}$.

The recombinant antibody may be a fully assembled antibody, a Fab fragment, an Fv fragment, or a single chain antibody.

The host organism includes any organism or its cell line that is capable of expressing transferred foreign genetic sequence, including but not limited to bacteria, yeast, plant, insect, and mammals.

The target antigen to be screened against includes small molecules and macromolecules such as proteins, peptides, nucleic acids and polycarbohydrates.

**BRIEF DESCRIPTION OF FIGURES**

**Figure 1** illustrates a flow chart of a computer-implemented process that can be used in the present invention to construct an antibody library in silico.

**Figure 2** shows $7 V_H$ and $7 V_L$ consensus sequences for $7 V_H$ and $7 V_L$ framework of HuCAL library in fasta format by Knappik et al., supra.

**Figure 3** shows the structures of the seven $V_H$ sequences superimposed on each other. The structures are aligned by superimposing the Ca
atoms using the CE with RMSD < 2Å and Z-score > 4.

**Figure 4** shows (A) the Ca trace of the superimposed structures of these 3 \( V_H \) sequences (1DHA in green, 1DHO in cyan, and 1DHW in yellow); (B) the superimposed structures with a ribbon representation of the \( \beta \)-sheets of the VH frameworks. As shown in both **Figures 4A** and 4B, the 3 \( V_H \) sequences (1DHA, 1DHO, and 1DHW) collapse into one structural family with RMSD <0.7 Å and Z-score >6 using 1DHA as standard, even though their sequence identity ranges widely from 72% to 87% relative to 1DHA.

**Figure 5** shows the structures of the seven \( V_L \) sequences retrieved from the PDB and superimposed on each other. The structures are aligned by using 1DGX as the reference structure with RMSD< 1.6Å and Z-score >6. The seven \( V_L \) sequences have a wide range of conformational variability, especially in the CDR regions highlighted (The structural flexibility at N- and C-termini are discarded here).

**Figure 6** shows the superpositioned 1DGX(green), 1DH4 (yellow), 1DH5 (color cyan) and 1DH6 (magenta) with similar conformation but varying length in the CDR regions. By using the CE algorithm, four \( V_L \) sequences (1DGX, 1DH4, 1DH5 and 1DH6) of the 7 consensus sequences families can be clustered into one structural family with RMSD < 0.6 Å and Z-score >6 and with sequence identity ranging from 67% to 80% using 1DGX as the structure reference. These four sequences also belong to the \( V_L \) kappa sequence family.

**Figure 7** shows three superimposed structures of 1DH7, 1DH8, and 1DH9 in lambda variable light chain, can be clustered into 1 structure family with RMSD < 1.5Å and Z-score > 6 using 1DGX as the reference.

**Figure 8** shows in (A) that CDR1 regions of the three lambda (\( \lambda \)) \( V_L \) sequences (1DH7, 1DH8 and 1DH9) adopt similar conformations with RMSD < 1Å. (B) that CDR1 regions of the 4 kappa (\( \kappa \)) \( V_L \) sequences (1DH4, 1DH6, 1DGX and 1DH5) adopt similar conformations with
RMSD < 0.6Å and gaps of 1-6 amino acids. (C) that CDR1 regions of the two kappa (κ) V_{L} sequences (1DH4 and 1DH6) adopt similar conformations with RMSD < 0.6Å and 1 amino acid gap in CDR1. Thus, structures of these two kappa V_{L} sequences are further clustered into one structural family according to the present invention. (D) that CDR1 regions of the two kappa (κ) V_{L} sequences (1DGX and 1DH5) adopt similar conformations with RMSD < 0.6Å and 1 amino acid gap in CDR1.

Figure 9 shows that clustering of the structures adopted by the seven consensus germline V_{L} sequences based on the structural families in the CDR1 region led to two to three distinct families of antibody structures: (1DH7, 1DH8 and 1DH9) for lambda variable light chains, (1DH4 and 1DH6), and/or (1DGX and 1DH5) for kappa variable light chains. The members within each family adopt similar conformations in their CDR1 regions with varying length in amino acids.

Figure 10 shows the PDB IDs of the consensus sequences of V_{H} and V_{L}, residues aligned, high score, P(N) sum, smallest probability, % identity with the query sequence, the germline family to which the identified germline sequence belongs.

Figure 11 shows the homology alignment for each of the selected human antibody germline sequences with the query sequence.

Figure 12A shows the flow chart for selecting the optimal remote homologous sequence(s) of structure-based multiple sequence alignment by using the profile Hidden Markov Model (HMM).

Figure 12B shows results generated by using the method diagramed in Figure 12A targeting V_{H} framework regions.

Figure 12C shows results generated by using the method diagramed in Figure 12A targeting kappa V_{L} CDR1.
**Figure 13** shows the top sequences from germline gene segments selected using the profile HMM method for various re-clustered structures.

**DEFINITION**

*Structural family:* a group of structures that are clustered into a family based on some empirically chosen cutoff values of the root mean square deviation (RMSD) (for example, their Ca atoms of the aligned residues) and statistical significance (Z-score). These values are empirically decided after an overall comparison among structures of interest. For example, for CE algorithms, the starting criteria used are RMSD < 2 Å and Z-score > 4.

*Structural ensemble:* It is well known that in the structural determination by NMR (nuclear magnetic resonance), the ensemble of structures rather than a single structure, with perhaps several members, all of which fit the NMR data and retain good stereochemistry, is deposited with the Protein Data Bank (PDB). Comparison between the models in this ensemble provides some information on how well the protein conformation was determined by the NMR constraints. In structural clustering, it is important to analyze the all members within a structural cluster to understand some consensus information about the distribution of all structural templates within a family and constraints on their sequences or sequence profiles within a structural family. It should be pointed out that all the sequences corresponding to NMR-determined ensemble structures have the same sequences (one protein with variable conformations). The structural ensemble here in the present invention refers to different proteins with variations in sequence and/or length but have similar main chain conformations.

*Ensemble average or representative structure:* if all members within a structural cluster has the same length of amino acids, the positions of atoms in main chain atoms of all structures are averaged, and the
average model is then adjusted to obey normal bond distances and angles ('restrained minimization'), similar to NMR-determined average structure. If all members within a structural cluster vary in the length of amino acids, a member which is representative of the average characteristics of all other members within the cluster will be chosen as the representative structure.

Canonical structures: the commonly occurring main-chain conformations of the hypervariable regions.

Structural repertoire: the collection of all structures populated by a class of proteins such as the modular structures and canonical structures observed for antibody framework and CDR regions.

Sequence repertoire: collection of sequences for a protein family.

Functional repertoire: a collection of all functions performed by proteins, such as the antibodies' diverse functional epitopes that are capable of binding to various antigens.

Germline gene segments: refers to the genes from the germline (the haploid gametes and those diploid cells from which they are formed). The germline DNA contain multiple gene segments that encode a single immunoglobulin heavy or light chain. These gene segments are carried in the germ cells but cannot be transcribed and translated into heavy and light chains until they are arranged into functional genes. During B-cell differentiation in the bone marrow, these gene segments are randomly shuffled by a dynamic genetic system capable of generating more than $10^8$ specificities. Most of these gene segments are published and collected by the germline database.

Rearranged immunoglobulin sequences: the functional immunoglobulin gene sequences in heavy and light chains that are generated by transcribing and translating the germline gene segments during B-cell differentiation and maturation process. Most of the
rearranged immunoglobulin sequences used here are from Kabat-Wu database.

**BLAST**: Basic Local Alignment Search Tool for pairwise sequence analysis. BLAST uses a heuristic algorithm with position-independent scoring parameters to detect similarity between two sequences.

**PSI-BLAST**: The Position-Specific Iterated BLAST, or PSI-BLAST program performs an iterative search in which sequences found in one round of searching are used to build a score model for the next round of searching. In PSI-BLAST the algorithm is not tied to a specific score matrix. Traditionally, it has been implemented using an AxA substitution matrix where A is the alphabet size. PSI-BLAST instead uses a QxA matrix, where Q is the length of the query sequence; at each position the cost of a letter depends on the position with respect to the query and the letter in the subject sequence. Two PSI-BLAST parameters have been adjusted: the pseudocount constant default has been changed from 10 to 7, and the E-value threshold for including matches in the PSI-BLAST model has been changed from 0.001 to 0.002.

**COBLATH**: A method that combines PSI-BLAST with Threading method for fold recognition and query-template alignment. It might be used to compare the compatibility between sequences and structural templates.

**Profile Hidden Markov Model (profile HMMs)**: statistical models of the primary structure consensus of a sequence family. They use position-specific scores for amino acids and for opening and extending an insertion and deletion to detect remote sequence homologues based on the statistical description of the consensus of a multiple sequence alignment. The multiple sequence alignments are given either by the multiple sequence alignment program such as ClustalW or structure-based multiple sequence alignment given by structural clustering.

**Threading**: a process of assigning the folding of the protein by
threading its sequence to a library of potential structural templates by using a scoring function that incorporates the sequence as well as the local parameters such as secondary structure and solvent exposure. The threading process starts from prediction of the secondary structure of the amino acid sequence and solvent accessibility for each residue of the query sequence. The resulting one-dimensional (1D) profile of the predicted structure is threaded into each member of a library of known 3D structures. The optimal threading for each sequence-structure pair is obtained using dynamic programming. The overall best sequence-structure pair constitutes the predicted 3D structure for the query sequence.

Reverse Threading: a process of searching for the optimal sequence(s) from sequence database by threading them onto a given target structure and/or structure cluster. Various scoring functions may be used to select for the optimal sequence(s) from the library comprising protein sequences with various lengths.

Reverse Engineering: the procedure to select and construct sequence or sequence libraries that are compatible to the structural constraints is called reverse engineering including but not limited to reverse threading.

Supervariable Region of Antibody: regions within antibody CDRs that show diverse structure, sequence and chain length variability compared to the other regions of CDRs or CDR ensembles which are relatively constant in structure, sequence and chain length. As exemplified in Figure 12C, the super-variability of a region of a specific CDR family can be exploited in CDR library construction.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a system and method for efficient in silico selection and construction of fully human and human-derived antibody libraries. The process is carried out computationally (i.e., in
in silico) in a high throughput manner by mining the ever-expanding databases of protein sequences of all organisms, especially human. The inventive methodology is developed by combining database mining of evolutionary sequences from nature with computational design of structurally relevant variants of the nature sequences.

In one aspect of the invention, the methodology is implemented by a computer system which computationally selects those human antibody sequences based on three-dimensional structural ensemble and/or ensemble average represented by a limited, discrete number of classes (or clusters) of antibody structures. By using the method, a master library of human antibody sequences can be constructed to better represent all antibody structures in the vertebrate antibody repertoire that are functionally important for high affinity binding to a large variety of antigens and eliciting antibody-dependent cellular responses.

In another aspect of the invention, the methodology is implemented by a computer system which computationally selects from the protein databases protein sequences, particularly antibody sequences, based on structural alignment with a target structural template. Diverse sequences which still retain the same functionally relevant structure as the target structural template can be selected by using reverse threading. By using the method, a library of diverse antibody sequences can be constructed and screened experimentally in vitro or in vivo for antibody mutants with improved or desired function(s).

In yet another aspect of the invention, the methodology is implemented by a computer system which computationally selects from the protein databases protein sequences, particularly antibody sequences, based on homology alignment with a target sequence template. Remote homologues with diverse sequences but retaining the same functionally relevant structure can be selected by using structure-based sequence alignment methods such as profile hidden Markov Model (HMM). By using the method, a library of diverse antibody sequences can be constructed with a relatively smaller size than that constructed by complete randomization of the target sequence. This
library can then be thoroughly screened experimentally in vitro or in vivo for antibody mutants with improved or desired function(s).

The inventive methodology can be used to design any protein with novel function or improved function over the target protein which serves as a lead in the process. In particular, mutant antibodies can be designed to include diverse sequences in the CDR regions, and to replace non-human sequences in the frameworks of the variable regions with human ones to reduce immunogenicity of the designed antibody when used as human therapeutics.

The library constructed by using the inventive methodology provides a structurally diverse and yet functionally more relevant source of antibody candidates for further screening for novel antibody with high affinity against a wide range of antigens and having no or minimum immunogenicity to human subject treated with antibody therapeutics.

1. **Principles of in silico selection and construction of a master library of functionally representative human antibody**

Antibody is a unique class of proteins which play profound roles in a vertebrate’s ability to defend itself against infection by neutralizing (or inactivating) viruses and bacterial toxins, and by recruiting the complement system and various types of white blood cells to kill extracellular microorganisms and larger parasites.

Like every protein of some biological significance, the biological functions of the proteins depend directly on the three-dimensional (3D) structure of the protein. The 3D structure or conformation determines the activity of an enzyme, how a receptor interacts with its ligand, and the affinity of the binding between the receptor and ligand. Thus, it would be biologically more relevant to screen a library of protein such as antibody based on the 3D structure a particular protein sequence adopts rather than the primary DNA or amino acid sequence of the protein.

In particular, as two of the most important handlers to map the functional space of proteins, the sequence and structure information of
antibodies have been accumulated for more than a few decades. Extensive analysis on their patterns have provided some of the most detailed understanding of fundamental process for molecular recognitions, which has a direct impact on the combinatorial technology in chemistry and biology.

So far, major efforts in mapping the functional diversity of antibodies have been focused on capturing the complexity in antibody sequence space by either simply increasing the size of antibody sequence pool (the so-called one-pot approach) or by generating large synthetic libraries in CDR regions. Only recently has systematic analysis of antibody sequence repertoire been utilized to design highly diverse consensus sequence library based on highly used human germline sequences as observed in the rearranged human antibody sequences. Knappik et al., supra. These consensus sequences were further analyzed to account for the canonical structures for the CDR regions.

In the present invention a distinctly novel approach is utilized to map the functional repertoire of antibody molecules. This approach is taken by exploiting the characteristics of antibody in sequence diversity and global structural conservation.

It is recognized that although a protein may have astronomical number of possible conformations (about 10^16 for a small protein of 100 residues (Dill (1985) Biochem. 24:1501-1509), all antibodies adopt a characteristic "immunoglobulin fold" globally. The natural antibody repertoire shows an amazing ability in recognizing a wide variety of molecules. To confer such diverse functions of binding ability to a vertebrate's antibodies, an extremely diverse sequence repertoire (about 10^19 possible combinations between the sequences of mouse heavy chain and light chains) is created by random genomic splicing of heavy and light chains with high variability in both sequence and length in their CDRs.

The structural repertoire to accommodate the much larger sequence repertoire is, however, surprisingly small. Only a limited number of canonical backbone conformations are found to account for structures adopted by the CDRs that are docked onto highly conserved
immunoglobin scaffold.

1) General approach

The general approach for constructing a structure-based human library is illustrated by a flow chart in Figure 1.

As illustrated in Figure 1, antibody structures and models in various protein structure databases such as the PDB are collected. The structural repertoire of these antibody molecules are mapped out in their three dimensional shape space. It is believed that conservation and variation in the shape space should make it possible to develop some general frameworks that remain constant across different species. On the other hand, variation in the shape space should make it possible to capture the functional diversity of antibody against a wide array of antigens in specific antibody regions.

Referring to Figure 1, the variable regions in shape space are clustered either separately (such as CDR3) or in combination (CDR1 & CDR2) into distinct families with or without certain conserved structural frameworks.

Still referring to Figure 1, these structural clusters, the ensemble average, and/or their corresponding sequence profiles are used to map out the corresponding sequence in human germline (or in a rearranged antibody sequence database) to find optimal sequences or sequence profiles within each family.

As diagramed in three boxes in the middle portion of Figure 1, at least three approaches can be taken to exploit the information generated by structure-based clustering of target antibody sequence(s). As described in the left box, one approach is to directly select sequences that fit onto the target structural template by using algorithms such as reverse threading, PSI-BLAST and profile HMM. For example, a library of recombinant antibodies can be generated by 1) selecting from a human antibody germline database sequence segments that fit onto a structural template of a target FR region of an antibody; 2) selecting from a protein database sequence segments that fit onto a structural template of a target CDR region of the antibody; and 3) combining the selected FR and CDR segments to build the library of
recombinant antibodies which are then synthesized and screened against a target antigen in vitro or in vivo.

As described in the right box in the middle section of Figure 1, another approach is to indirectly select antibody sequences using a target sequence or sequence profile built based on a structural template of a target antibody. For example, a library of recombinant antibodies can be generated by 1) aligning the target sequence or sequence profile with tester sequences from a protein database (e.g., human germline antibody sequence database or PDB) by using BLAST or multiple sequence alignment methods such as profile HMM; 2) selecting the tester sequences with homology to the target sequence (e.g., sequence homology of at least 15%); and optionally 3) evaluating the structural compatibility of the selected tester sequence with the structure template of the target sequence or sequence profile. This process can be carried out to construct a library of recombinant antibodies by targeting a particular region of an antibody such as a CDR, FR, and combination thereof. The selected tester sequences may be profiled based on variability in each amino acid residue and those variants with low occurrence frequency (e.g., 5 times out of 100 selected tester sequences) may be filtered and discarded. The rest of the selected tester sequences may be pooled and combined by a combinatorial combination of the amino acid variants in each residue position. The tester sequences selected by targeting the CDR region and the ones targeting the FR regions may also be combined; and the combined sequences may be filtered based on their structural compatibility with the target antibody. The library of recombinant antibodies can be synthesized and screened against a target antigen in vitro or in vivo.

As described in the middle box in the middle section of Figure 1, yet another approach is to select antibody sequences based on a target structural template combining methods described in the left and right boxes. For example, a library of recombinant antibodies can be generated by 1) aligning the sequence or sequence profile of the target structural template with tester sequences from a protein database (e.g., human germline antibody sequence database or PDB) and reverse threading the tester sequences onto the target structural template by
using a structure/sequence dual selection algorithm such as COBLATH; and 2) selecting the tester sequences with homology to the target sequence (e.g., sequence homology of at least 15%) and structurally compatible with the target structural template. This process can be carried out to construct a library of recombinant antibodies by targeting a particular region of an antibody such as a CDR, FR, and combination thereof. The selected tester sequences may be profiled based on variability in each amino acid residue and those variants with low occurrence frequency (e.g., 5 times out of 100 selected tester sequences) may be filtered and discarded. The rest of the selected tester sequences may be pooled and combined by a combinatorial combination of the amino acid variants in each residue position. The tester sequences selected by targeting the CDR region and the ones targeting the FR regions may also be combined; and the combined sequences may be filtered based on their structural compatibility with the target antibody. The library of recombinant antibodies can be synthesized and screened against a target antigen in vitro or in vivo.

There are several advantages associated with this approach of mapping the functional space of proteins using diversity libraries that are designed by sampling the diversity in shape space rather than in sequence space.

First, protein-protein interactions between ligand and receptor, antigen and antibody are conducted in well-defined conformation in space. Therefore, antibody libraries should be designed to map the 3-dimensional space populated by antibodies in order to target antigens with different shapes.

Second, compared to the larger sequence repertoire, structure repertoire of antibodies is limited to a small number of canonical structures in its main chain conformations in the CDR regions which are docked onto a common core structure for both the variable light and heavy chains. The simplicity in structure repertoire makes it easy to map the functional diversity based on variation in its 3-dimensional space and simple to cluster seemingly complicated sequence pools into distinct families for library construction.
Third, it is conceived that the conserved nature of the structural repertoire of immunoglobins across very different species (Barre et al. (1994) "Structural conservation of hypervariable regions in immunoglobins evolution" Nature Struct. Biol. 1:915-920) that clustering structure repertoire of antibodies from different species into distinct families is a viable approach to map its functional space. This approach is simple yet functionally more relevant for selecting and constructing the diversity libraries once it is applied to the sequence repertoire for a specific species. This is particularly important for constructing human antibody libraries for therapeutic application or for humanizing murine antibodies by using human-derived sequence repertoire for its counterparts. In contrast, sequence homology-based approaches would be less flexible and hard to transfer from species to species if sequence homology is relatively low.

Moreover, the structure-based construction of sequence libraries makes it possible to apply various methods developed in structural biology to filter apparent complexity in sequence spaces based on structural or physical principles, in addition to the tools used in sequence analysis that are largely relied on the principles of evolution.

Accordingly, the present invention provides a method of constructing a master library of functionally representative antibody. This master library is formed by a repertoire of antibody sequences adopting distinct classes of structures that covers, ideally, almost all of the 3D structural ensembles and/or ensemble averages of all vertebrate antibodies.

According to the present invention, a master library of functionally representative antibody is represented by a library of antibody sequences adopting distinct classes of structures that covers, ideally, almost all families of the 3D structures of all vertebrate antibodies. Although a protein may have astronomical number of possible sequence combinations (about $10^{16}$ for a small protein of 100 residues (Dill (1985) Biochem. 24:1501-1509), all antibodies adopt a characteristic "immunoglobulin fold" globally. The natural antibody repertoire shows an amazing ability in recognizing a wide variety of molecules. To confer such diverse functions of binding ability to a
vertebrate's antibodies, an extremely diverse sequence repertoire (about $10^{12}$ possible combinations between the sequences of mouse heavy chain and light chains) is created by random genomic splicing of heavy and light chains with high variability in both sequence and length in their CDRs.

The structural repertoire to accommodate the much larger sequence repertoire is, however, surprisingly small. Only a limited number of canonical backbone conformations are found to account for structures adopted by the CDRs that are docked onto highly conserved immunoglobulin scaffold.

According to the present invention, it is believed that antibody achieves its functional diversity by decorating a diverse array of amino acids onto a finite number of CDR canonical structures. The present invention clusters antibodies with experimental or modeled structures into distinct families. By clustering the antibodies according to their 3D structures instead of using conventional methods of classification based on sequence homology, each family of the structure repertoire should better represent the population of antibodies with binding geometry complementary to the recognition sites of potential antigens, although the binding affinity could be further optimized by matching the shape and chemical nature of the specific amino acids. Therefore, the approach taken in the present invention tends to maximize the functional diversity of antibody in recognizing and binding to a wide array of antigens in silico and meanwhile to minimize the sequence space required for efficient screening in vitro or in vivo.

2) **Construction of antibody sequence library based on structural constraints**

Once structural families are identified, either the cluster containing multiple members, a representative member, or an ensemble average of the cluster if possible, can be used as structural constraints to either select for optimal sequences or to construct sequences for further constructing sequence libraries.

There are several ways to use these structural families from
sampling antibody structure databases as the constraints for constructing desired sequence libraries. The main chain conformations of 3D structures within a structural family or cluster are called structure ensembles or structure templates. The ensemble average is referred to the average structure of all members within a structure cluster or family when it is physically meaningful to take average of the main chain conformations. If it is not physically meaningful or possible to take average for all members within a structural cluster or family due to the difference in length, etc. a representative structure may be used to represent the "average structural properties" of all members within a structural family or cluster. The structure ensembles or templates, ensemble average, or representative structures described above are collectively referred to herein as the "structural templates".

The difference in using these terms in describing structural constraints depends on how much structural constraint within a cluster should be included in constructing sequence libraries. For structural constraints, the most stringent and reasonable approach should be to include all ensemble structures or templates within a cluster or family. The ensemble average if done properly, may be the simplest structural constraint and easy to compute. If taking ensemble average is not physically meaningful, the representative structure may be a compromise to replace constraints by structure cluster.

Once the structural constraints are identified, there are several ways to construct sequence libraries by applying structural constraints. The procedure to select and construct sequence or sequence libraries that are compatible to the structural constraints is called reverse engineering including but not limited to reverse threading. However, an important aspect of current invention is to restrict the sequence database for library construction to specific species and/or to even the specific population of the same species. For therapeutic purpose, the human immunoglobulin sequence database are preferably used to construct human-derived antibody libraries, especially in the frameworks of the variable regions. In the CDR regions, sequences with non-human origins may optionally be used to increase the diversity of these regions so as to increase the chance of finding antibodies with
novel or improved function(s). The methods in applying both the physical and evolutionary constraints to construct sequence libraries are described in detail below.

One method is to use the sequence that is compatible to the ensemble average structure or the representative member within a structure cluster to search for the optimal sequences from the germline sequence database. This will usually yield the sequence with the highest sequence identity to the query sequence using BLAST as demonstrated in Section 3 below (Figures 10 and 11).

The clustered structures within a structure family can give multiple sequence alignments based on 3D structures. These aligned sequences might come from different species; they may be close or remote sequence homologues. The multiple sequence alignment can be used, however, to build a profile Hidden Markov Model (HMM); and this HMM will then be used to search for the close and/or remote human homologues from human sequence database such as the human germline and/or rearranged sequence database as demonstrated in Section 3 below (Figures 12 and 13A-C).

A more direct way to search for sequences compatible to structural constraints is to thread amino acid sequences from human germline and/or rearranged sequence database onto structure templates of the structural cluster and to find out the optimal scoring sequences on their target structure templates. These sequences can be then used for constructing sequence libraries for the structure cluster. This procedure is called reverse threading because it tries to find the best sequences fitting to the target structure templates, which is the opposite of threading which tries to find the best structure template from a structure library for a given sequence.

Additionally, the top hits of the sequences found for a structure cluster or queried sequences may be profiled by threading multiple amino acids at each position in a combinatorial approach to select for the best "consensus sequence" compatible with the structural ensemble and/or ensemble average. This process of searching for consensus sequence is different from the consensus sequence from the method of using simple sequence average at each position described in Knappik,
et al, *supra*. The consensus sequence according to the present invention is created using the physically oriented reverse engineering approach using all possible combination of amino acids that are allowed at each position from the retrieved sequences but are optimized by scoring their compatibility with the structural constraints.

The human antibody sequences that are selected according to these criteria for the framework regions can serve as the sequence template for building a master framework for constructing the human antibody library of the present invention. These selected human sequences are then pooled together and included in the master framework. The same methods can be used to construct the sequence libraries for CDR regions if the structure templates for each canonical structure family of CDRs are used to construct the sequence libraries for these regions.

Once the master framework of human antibody is constructed, mutagenesis can be carried out to diversify specified region(s) in the master framework. For example, CDR regions, especially CDR3 of the heavy chain, of the master framework can be randomly mutagenized to mimic the natural process of antibody diversification. The mutagenized antibody sequence may be further selected *in silico* based their compatibility to the structural ensemble average. All of the antibody sequences selected in these processes are pooled to form a master library of human antibody which can be screened against a wide range of antigens *in vitro* or *in vivo*.

Since the selection and construction of the antibody library of the present invention is based on structural clustering, not simple sequence homology alignment, it is thus possible to further limit the number of antibody sequences in the library and yet not to sacrifice the functionally relevant sequences. For example, multiple human antibody sequences may be highly diverse in their sequences and yet adopt the same 3D structure when threaded onto the structural ensemble average.

Further, for those antibody sequences mutagenized randomly in the CDR3 region, not all structures of randomized CDR3 are compatible with the framework structural ensemble averages. Consequently, a
fewer number of CDR3 loops that are structurally diverse will be selected, and therefore a fewer number of human sequences selected. As a result, the sequence space of antibody to be screened is reduced in size without sacrificing diversity in antibody functionality.

By using the method, a master library of human antibody sequences is selected and constructed to better represent all antibody structures in the vertebrate antibody repertoire that are functionally important for high affinity binding to antigen and eliciting antibody-dependent cellular responses. Such a functionally representative library provides a structurally diverse, and yet functionally more relevant source of antibody candidates which can then be screened for binding to a wide variety of target molecules, including but not limited to biomacromolecules such as protein, peptide, and nucleic acids, and small molecules.

The method of present invention is an efficient way of constructing a digital library of antibody which represents most of the 3D structures of antibodies that are functionally relevant. Thus, the human antibody sequences selected from the reverse engineering process such as threading are finite and yet covers most of the functionally relevant structures of antibody in human antibody gene pool.

In contrast, the current methods of construction of antibody library in vitro involve isolation of cDNA libraries from immunized human antibody gene pool, naive B-cell Ig repertoire, or particular germline sequences. Barbas and Burton (1996), supra; De Haard et al. (1999), supra; and Griffiths et al (1994), supra. These libraries are very large and extremely diverse in terms of antibody sequences.

The conventional approach is to create a library of antibody as large, and as diverse as possible to mimic immunological response to antigen in vivo. Typically, these large libraries of antibody are displayed on phage surface and screened for antibodies with high affinity binding ability to a target molecule. Such a "fishing in a large pond" or "finding a needle in a huge hey stack" approach is based on the assumption that simple increase in the size of sequence repertoire should make it more likely to fish out the antibody that can bind to a target antigen with
high affinity.

There may be several problems associated with such a conventional approach. A simple increase in the size of sequence library may not necessarily correlate with an effective increase in functional diversity. Further, due to the physical limit on making an extremely large experimental library, it may be very difficult to construct a library with diversity over $10^{11}$ in vitro in the lab. The library that is actually screened experimentally probably presents only a fraction of the sequence repertoire at the theoretically predicted size. In addition, there is legitimate concern that with the difficulties and the under representation problems associated with handling and manipulation of an extremely large library in vitro, time and money may be lost in an effort trying to increase the size of the library and yet not increasing functional diversity significantly.

Another approach existing in the art is to design an artificial antibody library computationally and then construct a synthetic antibody library which is expressed in bacteria. Knappik et al., supra. The artificial antibody library was designed based the consensus sequence of each subgroup of the heavy chain and light chain sequences according to the germline families. The consensus was automatically weighted according to the frequency of usage. The most homologous rearranged sequences for each consensus sequence was identified by searching against the compilation of rearranged sequences, and all positions where the consensus differed from this nearest rearranged sequence were inspected. Furthermore, models for the seven $V_H$ and seven $V_L$ consensus sequences were built and analyzed according to their structural properties. A library of artificial antibodies were then constructed and expressed in E. coli. This library constructed can be used to screen for antibodies with high affinity binding to a target molecule.

However, there is a major problem concerning such an approach as far as therapeutic applications of the selected antibody are concerned. Although derived from human sequence pools, the consensus sequences found by using this approach, by definition, are not natural sequences. (1) Combination of sequences, albeit human
sequences, at various positions may give rise to new immunogenic epitopes, thus significantly limiting therapeutic applications of the selected antibodies to human, whereas the method described here can give either fully human sequences or human derived sequences or both. (2) Consensus sequence has its own serious limitation. Moreover, the definition of consensus sequence may be too arbitrary and such artificial sequences defined may not be representative of a natural, functional structure, although experimental test and structure analysis may eliminate some unfavorable amino acid combinations. (3)

Although the consensus sequences designed to cover mainly those human germline sequences that are highly used in rearranged human sequences, it might bias consensus sequence library toward a limited number of antigens exposed to human being so far, whereas sampling functional space by mapping structures of different species covers a wide range of functional epitopes of antibodies exposed to a wide array of antigens. This would be very important for designing antibody libraries to target novel antigens.

By contrast, the method of the present invention is based on structural constraints of antibodies directly or derived from natural sources. According to the present invention, a complete structural repertoire of all antibodies available including both human and other vertebrates can be analyzed for structural ensembles and/or ensemble averages within each representative 3D structural family. Based on this analysis, the structural models are clustered into distinct structure families, each of which includes one or more representative members. These structure families ideally should represent evenly the structure space which all antibodies, including those from humans and other animals, would adopt. Thus, by collecting and building structural models for each structural ensembles and/or their ensemble averages for these antibodies, a relatively comprehensive survey of functional repertoire of antibodies across the species may be achieved.

Further, the method of the present invention involves using selection of native human antibody sequence which fits the best onto the structural ensemble or ensemble average in each of the structural family. By selecting and pooling the native human sequences based on
the 3D structural templates in each family, a more focused human antibody library is created. The library may be smaller than the native antibody gene pool and yet representative of the functional repertoire of antibody in all vertebrates.

Moreover, the sequences of the antibody library constructed using the method of the present invention are closely related to human sequences. The antibody selected from this library against a target molecule should be more desirable than an artificial, non-human antibody for therapeutic applications and humanization of non-human antibodies. This approach can minimize the potential of creating new immunogenic epitopes associated with using synthetic antibody sequences derived from randomization of the consensus sequences.

In addition, the library generated according to the method of the present invention should encompass a broader spectrum of the basic function of an antibody: antigen recognition and neutralization. Since the family of the structural ensemble averages are clustered based on not only the structures adopted by known human sequences, but also structures collected from other vertebrates. In particular, monoclonal antibody produced by mice is a rich source of structures to be included in the process of clustering. Since these monoclonal antibodies are generated from immunization against a vast number of antigens, including these antibodies in the clustering process should tend to enlarge the functional repertoire, although a few special features specific to mice should be taken into account or avoided when applied to human. This approach may effectively avoid the problem associated with known human antibody sequences that are restricted to those isolated against a limited number of antigens.

2. **Process of clustering antibodies based on their structural ensemble or ensemble averages.**

According to the present invention, a master library of human antibody sequence can be constructed based on 3D structural clusters of antibodies from human and other vertebrates. The 3D structural ensembles and/or the ensemble averages serve as master frameworks
upon which human antibody sequences are mapped onto by threading etc and those best compatible are selected to form the master library of human antibody.

The structural ensemble or ensemble averages of antibody from various species may be modeled *in silico* by using various structural alignment methods for comparing antibodies with known 3D structures. By "known 3D structures" is meant x-ray crystal structures, NMR structures, and 3D structures of antibody modeled *in silico*. Currently, there are about 360 antibody 3D structures deposited in the Protein Data Bank (PDB) which include 306 X-ray structures, 17 NMR structures, and 32 modeled structures.


In an embodiment of the present invention, the antibody structural families are clustered by structural alignment using the CE algorithm. Compared to Monte Carlo and 3D clustering algorithms, the CE algorithm significantly reduces the search space and empirically establishes a reasonable target function. The CE target function assumes that alignment path is continuous when including gaps and there is an optimal match between the pair. Various protein properties can also be used with CE algorithm, for example, 1) structure superposition as rigid bodies; 2) inter-residue distance, 3) environmental properties (e.g., exposure, secondary structure); 4) conformational properties (e.g., bond angles, dihedral angles, and
orientation with respect to the protein center of mass).

As a proof of principle, 3D structures of a series of artificial antibody sequences were compared by using the CE algorithm and classified into a smaller number of clusters based on their 3D structural alignments. These artificial sequences tested are the consensus sequences of the subgroups of the heavy and light chain sequences according to the germline families. Knappik et al., supra. These sequences shown in Figure 2 consist of the following 7 VH and 7 VL consensus sequences:

\[
\begin{align*}
V_H & \quad V_L \\
1DHA [SEQ ID NO: 1] & 1DGX [SEQ ID NO: 8] \\
1DHO [SEQ ID NO: 2] & 1DH4 [SEQ ID NO: 9] \\
1DHQ [SEQ ID NO: 3] & 1DH5 [SEQ ID NO: 10] \\
1DHU [SEQ ID NO: 4] & 1DH6 [SEQ ID NO: 11] \\
1DHV [SEQ ID NO: 5] & 1DH7 [SEQ ID NO: 12] \\
1DHW [SEQ ID NO: 6] & 1DH8 [SEQ ID NO: 13] \\
1DHZ [SEQ ID NO: 7] & 1DH9 [SEQ ID NO: 14]
\end{align*}
\]

The seven \(V_H\) consensus sequences stored in the PDB, 1DHA, 1DHO, 1DHQ, 1DHU, 1DHV, 1DGW, and 1DHZ, correspond to VH1A, VH1B, VH2, VH3, VH4, and VH6, respectively, as described in Knappik et al., supra. The seven \(V_L\) consensus sequences stored in the PDB, 1DGX, 1DH4, 1DH5, 1DH6, 1DH7, 1DH8, and 1DH9, correspond to VL\(\alpha_1\), VL\(\alpha_2\), VL\(\alpha_3\), VL\(\kappa_4\), VL\(\lambda_1\), VL\(\lambda_2\), and VL\(\lambda_3\), respectively, as described in Knappik et al., supra.

The 3D structural models of these VH and VL consensus sequences built by Knappik et al. were retrieved from the PDB and compared by using the CE algorithm. It should be noticed that CDR3 of the heavy and light chains were the same for all frameworks in the modeled structures. The CE program compares pairs of protein structures of polypeptide chain or their segments based on the root mean square difference (RMSD), their statistical significance (Z-score), length difference, allowable gaps (given as a percentage of the total...
number of residues without a matching partner relative to the complete alignment) and sequence identity.

**Figure 3** shows the structures of the seven VH sequences superimposed on each other. The structures are aligned by superimposing the Ca atoms using the CE with RMSD < 2Å and Z-score > 4. As shown in **Figure 3**, the seven VH sequences have a range of conformational variability, especially in the CDR regions. According to Knappik et al., these seven structures cover all canonical classes of the CDRs of the VH structures.

However, by using the method of the present invention, a closer look into the seven structures reveals a striking conformational similarity between at least three of the seven VH sequences. By using the CE algorithm, five VH sequences (1DHA, 1DHO, 1DHW, 1DHZ, 1DHV) of the 7 consensus sequences families can be clustered into one structural family with RMSD < 1.5Å and Z-score > 4 and with sequence identity ranging from 48% to 87% using 1dha as standard. Further clustering of the 5 VH sequences (1DHA, 1DHO, 1DHW, 1DHZ, 1DHV) reveals that the 3 VH sequences (1DHA, 1DHO, and 1DHW) collapse into one structural family with RMSD <0.7 Å and Z-score >6 using 1DHA as standard, even though their sequence identity ranges widely from 72% to 87% relative to 1DHA.

**Figure 4A** shows the Ca trace of the superimposed structures of these 3 VH sequences (1DHA in green, 1DHO in cyan, and 1DHW in yellow). **Figure 4B** shows the superimposed structures with a ribbon representation of the β-sheets of the VH frameworks. As shown in both **Figures 4A and 4B**, these three structures have an almost perfect superposition (RMSD <0.7 Å) even in the CDR regions. According to the present invention, these three structures are clustered into one VH structure family based on the structural clustering criteria of the present invention. The rest of the 7 VH sequences: 1DHQ, 1DHU, 1DHV, and 1DHZ, have distinctly different structures and thus clustered into 4 distinct structural families with only one member within each family according to the present invention. Thus, by using the method of the present invention, the 7 consensus germlines VH sequences of human antibody designated by Knappik et al. can be
presented by 5 distinctly different structural families. The preferred criteria are RMSD < 1 Å for each structural family and Z-score > 6.

**Figure 5** shows the structures of the seven VL sequences retrieved from the PDB and superimposed on each other. The structures are aligned by using 1DGX as the reference structure with RMSD< 1.6Å and Z-score >6. As shown in **Figure 5**, the seven VL sequences have a wide range of conformational variability, especially in the CDR regions (The structural flexibility at N- and C-termini are discarded here). According to Knappik et al., these seven structures cover all canonical classes of the CDRs of the VL structures.

However, by using the method of the present invention, the seven VL sequences can be re-clustered into smaller number of families. By using the CE algorithm, four VL sequences (1DGX, 1DH4, 1DH5 and 1DH6) of the 7 consensus sequences families can be clustered into one structural family with RMSD < 0.6 Å and Z-score >6 and with sequence identity ranging from 67% to 80% using 1DGX as the structure reference. **Figure 6** shows the superimposed 1DGX(green),1DH4 (yellow), 1DH5 (color cyan) and 1DH6 (magenta) with similar conformation but varying length in the CDR regions. These four sequences also belong to the VL kappa sequence family.

Further clustering of the 4 VL sequences (1DGX, 1DH4, 1DH5 and 1DH6) reveals that the 2 VL sequences (1DH4 and 1DH6) collapse into a structural family with RMSD <0.6 Å and Z-score > 6 with length of CDR1 loop closer to each other, using 1DGX as the reference, while two VL sequences (1DGX and 1DH5) can be clustered into another structural family (data not shown).

**Figure 7** shows three superimposed structures of 1DH7, 1DH8, and 1DH9 in lamda variable light chain, can be clustered into 1 structure family with RMSD < 1.5Å and Z-score > 6 using 1DGX as the reference according to the present invention.

Thus, by using the method of the present invention, the 7 consensus germlines VL sequences of human antibody designated by Knappik et al. can be represented by 2 to 3 distinctly different structural families. Combined with the clustering of the 7 consensus germlines VH sequences into a 5 structural families, the total structural
family for human antibody germline can be represented by $5 \times (2 \text{ to } 3) = 10$ to 15 distinct families, a much reduced structural repertoire than the germline sequence repertoire of Knappik et al.: $7 \times 7 = 49$.

The structures of the consensus germline VH and VL sequences can also be clustered based on the conformation ensemble adopted by a specific region of the VH or VL, such as a particular CDR region.

**Figure 8A** shows that CDR1 regions of the three lambda (\(\lambda\)) VL sequences (1DH7, 1DH8 and 1DH9) adopt similar conformations with RMSD < 1Å. Thus, structures of these three lambda VL sequences are clustered into one structural family according to the present invention.

**Figure 8B** shows that CDR1 regions of the 4 kappa (\(\kappa\)) VL sequences (1DH4, 1DH6, 1DGX and 1DH5) adopt similar conformations with RMSD < 0.6Å and gaps of 1-6 amino acids. Thus, structures of these four kappa VL sequences are clustered into one structural family according to the present invention.

**Figure 8C** shows that CDR1 regions of the two kappa (\(\kappa\)) VL sequences (1DH4 and 1DH6) adopt similar conformations with RMSD < 0.6Å and 1 amino acid gap in CDR1. Thus, structures of these two kappa VL sequences are further clustered into one structural family according to the present invention.

**Figure 8D** shows that CDR1 regions of the two kappa (\(\kappa\)) VL sequences (1DGX and 1DH5) adopt similar conformations with RMSD < 0.6Å and 1 amino acid gap in CDR1. Thus, structures of these two kappa VL sequences are further clustered into one structural family according to the present invention.

As a result of such clustering with a focus on a specific region of the VH or VL, regions, the number of antibody structure families might be clustered differently. **Figure 9** shows that clustering of the structures adopted by the seven consensus germline VL sequences based on the structural families in the CDR1 region led to two to three distinct families of antibody structures: (1DH7, 1DH8 and 1DH9), (1DH4 and 1DH6), and/or (1DGX and 1DH5). As shown in **Figure 9**, within each family, the members adopt similar conformations in its CDR1 regions with varying length in amino acids.

Thus, by further clustering of antibody structures based on a
more focused region of the global structure, i.e., CDR1, the seven consensus germline VL sequences of human antibody designated by Knappik et al. can also be represented by 2 to 3 distinctly different structural families. Combined with the clustering of the 7 consensus germlines VH sequences into 5 structural families, the total structural families for human antibody framework sequences can be represented by 5x(2 to 3) = 10 to 15 distinct families, a much more reduced structural repertoire than the consensus framework sequence repertoire of Knappik et al.: 7x7=49.

As illustrated by the above example, the method of the present invention enables one to reduce the size of the antibody sequence library by clustering them according to their 3D structural families. Since the structure of a protein or antibody determines its function in the biological system, the structural ensemble or ensemble average in each structure family of the present invention should represent the population of diverse antibody sequences sharing similar functions, e.g. in antigen recognition and affinity binding.

The above-described method of clustering structures of consensus human germline antibody sequences only serves as an example to illustrate the principal of the invention. It should be noted that such a clustering method is not limited to these structures. In a broader application, structures of both human and non-human vertebrate antibodies can be combined in a pool and clustered based their structural ensemble or ensemble averages or representative structure. This approach presumably reduces the risk of the biased library consisting only of structures of human antibodies generated by limited exposure to various antigens. By combining and clustering structures from both human and non-human vertebrate antibodies, this structural ensemble or ensemble average determined should better represent the functional epitope of the antibody family. In addition, compared to the approach based on consensus antibody sequences, the structural ensemble or ensemble average generated by using the methods of the present invention is based on some well-established structural principle instead of the ill-defined consensus sequences.

The following lists the principles followed in clustering
structures:

a). Align structures based on the RMSD for C alpha carbon atoms in the backbone and Z-score and gaps in the length of amino acids.
b). Clustering structures into the same family progressively based on smaller RMSD values and smaller gaps in amino acids.
c). Clustering structure using globe or important motifs.

It is believed that because structural repertoire is better way to represent functional repertoire, starting from structure should provide an important and more rational basis for library construction. The antibody-antigen interaction occurs on the 3D structure space rather than 1D sequence space. The structure change in CDRs should be better represented in 3D space. Using the structure as the criteria without details into the exact interaction between Ag and Ab should be make it possible to score for human sequence better compatible with the representative structure motif or ensemble.

3. Selection of Antibody Sequences that Fit onto the Targeted Structural Ensemble or Ensemble Average

Once the structures of the antibodies are clustered using methods described above, either the structure ensemble within a cluster or its ensemble average or its representative member can serve as the target structural scaffold in the search for those human antibody sequences that adopt the same or similar 3D structure. For example, an ensemble average of the structures of a target antibody can be used as a structural template in the search in a protein database for antibody sequences with diverse sequences and yet retaining the same functionally relevant structure.

In a preferred embodiment, the human antibody sequences are selected from the human immunoglobulin germline sequences. The germline sequences have been clustered into different sequence families including the V-genes, D-genes and J-genes. The rearranged immunoglobulin sequences are collected in the Kabat-Wu sequence databases (Johnson & Wu, Kabat Database and its applications: future
directions (2001) 29, 205-206). These human immunoglobulin sequences are retrieved from the Kabat-Wu sequence databases and stored in the human immunoglobulin (or antibody) sequence data of the present invention (Figure 1).

According to the present invention, a variety of methods can be used to search for those human antibody sequences that adopt the same or similar structure as the target structural scaffold. The following are examples of the methods that may be used for achieving this purpose.

1) Reverse threading

The conventional threading of protein sequence is used to predict the 3D structure scaffold of a protein. Typically, it is a process of assigning the folding of the protein by threading its sequence to a library of potential structural templates by using a scoring function that incorporates the sequence as well as the local parameters such as secondary structure and solvent exposure. Bowie et al. (1991) Science 253:164-170; Rost et al. (1997) 270:471-480; Xu and Xu (2000) Proteins: Structure, Function, and Genetics 40:343-354; and Panchenko et al. (2000) J. Mol. Biol. 296:1319-1331. For example, Rost et al. supra the threading process starts from prediction of the secondary structure of the amino acid sequence and solvent accessibility for each residue of the query sequence. The resulting one-dimensional (1D) profile of the predicted structure is threaded into each member of a library of known 3D structures. The optimal threading for each sequence-structure pair is obtained using dynamic programming. The overall best sequence-structure pair constitutes the predicted 3D structure for the query sequence.

In contrast, the reverse threading of the present invention is a process of finding the optimal sequence within a library of sequences to fit onto a target structure. Various scoring functions may be used to select for the optimal sequence(s) from the library comprising antibody sequences with various lengths. In a preferred embodiment, the scoring function is capable of discriminating the following interactions among different sequences with different lengths: (a) The interactions between
the side chains and backbone template as well as between side chains; and (b) the gap penalties for sequences with varying lengths in CDR1, CDR2 and CD3 regions.

For example, amino acid sequences from a human germline immunoglobulin database can be threaded onto the 3D structure of the target structural template (or scaffold) and to search for the sequences with optimal acceptable scores.

2) Matching the target structure with the optimal sequence composition of multiple aligned sequence family

For this method, the optimal sequence that will fit onto the target structure is selected by matching the target structure with the optimal sequence composition of multiple aligned sequence family. The top hitting sequences found from human antibody sequence database can be optimized at each position with all possible composition to yield the best sequence composition that fits a target structure based on the scoring of the interactions between side chains and backbone and side chain and side chain.

3) Selecting the optimal sequence by homology alignment with the sequence of the target structure

Another method of selecting human antibody sequence that will fit onto the structural scaffold of each member of the structural family is through homologous alignment with the amino acid sequence of the representative structure within a family. Such a method of structure-based sequence alignment can be practiced by the following procedure.

The target structure may be a member of the structural family clustered by using the method described in Section 1. This target structure serves as a structural scaffold with which a library of human antibody sequences are matched. The matching process is performed through homologous alignment of the library of human antibody sequences with the amino acid sequence of the target structure (the sequence template). This method is a process of indirect structure-based sequence query, instead of directly searching for sequences that can be thread onto the structural scaffold in a reverse threading
process described in Section 1) above. Through homologous alignment
with the sequence template of the target structure, optimal human
antibody sequences will be efficiently selected based on simple sequence
alignment method such as BLAST.

The following is an example of selecting optimal human antibody
sequence(s) by using the indirect structure-based sequence alignment
according to the present invention.

This example demonstrates that fully human antibody sequences
with extremely high sequence homology (100% sequence identity) could
be found by matching the library of human antibody sequences against
the sequence template of the target structure, i.e., the query sequence.
It can be reasonably assumed that the antibody sequence having the
highest sequence identity with the query sequence should adopt the
same or a very similar structure as that of the query sequence. This
sequence(s) is included in the library of selected human antibody to
represent the same scaffold as the target structure. For each member
of the structural family, human antibody sequences can be selected to
match the sequence of the structural ensemble or ensemble average
(there is only one member within each family). The selected human
antibody sequences are combined to form a library relatively small in
sequence space and yet functionally diverse.

In this example, the library of human antibody germline
sequences (HuCal sequences) serves as the library of human antibody
sequences (Figure 1). The HuCal sequences in fasta format as shown
in Figure 2 were divided into variable light chains and variable heavy
chains. These sequences were then used to compare with human
germline sequences using Blast (Basic Local Alignment Search Tool).

The amino acid sequences of the consensus human germline
sequences that are clustered by using the method described in Section
1 serve as the query sequences. Each of the query sequences and the
human germline sequences were aligned and ranked in decreasing
identity. Figure 10 shows the PDB IDs of the query sequence, name of
the retrieved germline gene segment, sequence id no, residues aligned,
high score, P(N) sum, smallest probability, % identity with the query
sequence, the germline family to which the identified germline sequence
belongs to (vhaagp-f1.aa stands for the f1 subfamily of VH chain; 
vkallaa-f1 stands for f1 subfamily of VL kappa chain; vlallaa-f1.aa 
stands for f1 subfamily of VL lamda chain).

**Figure 11** shows the homology alignment for each of the selected 
human antibody germline sequences with the query sequence.

As shown in **Figures 10** and **11**, human antibody germline 
sequences with up to 100% homology with the query sequence can be 
found from the library of human antibody germline sequences. For 
example, 1DHA, 1DHW and 1DHV have the identical sequence as the 
ergmline sequence segment, while close germline homologues can be 
found for other sequences corresponding to the target structural 
models. These are trivial cases because there is only one query 
sequence for each structural template.

4) **Selecting the optimal remote homologous sequence(s) of 
structure-based multiple sequence alignment by using profile Hidden 
Markov Model.**

Given one clustered structure family, how to search for optimal 
sequence(s) that match with their aligned multiple sequence profile 
corresponding to their structure alignment? The flow chart in **Figure 
12A** illustrates an indirect approach to search for remote homologues 
consistent with multiple sequence alignment from clustered structures. 
The clustered structures within a structure family can give multiple 
sequence alignment based on their 3D structures. These aligned 
sequences might come from different species; they may be close or 
remote sequence homologues. The multiple sequence alignment can be 
used, however, to build a profile Hidden Markov Model (HMM); and this 
HMM can then be used to select the close and/or remote human 
homologues from human sequence database such as the human 
germline and/or rearranged sequence database.

**Figure 12B** shows the result generated by using the method 
diagramed in **Figure 12A** based on a sequence profile of a structure 
cluster of the FR regions of 3 V_H sequences. The structure cluster of 
the framework regions of 3 V_H sequences, 1dha, 1dho and 1dhw, is
shown in Figure 4A. Sequences of the FR regions of these 3 $V_H$ in the structure cluster were obtained by removing CDR1-3 from $V_H$, which are designated as FR123 (Figure 12B). FR123 sequences were used to build HMM and search human germline antibody sequences or humanized sequences. Fifty-two human germline antibody sequences (i.e., hits for FR123) were found. Variants in each position of the amino acid residues were profiled. Variants that occur less than 5 times in the position were filtered (i.e., cutoff value = 5) and discarded. The rest of the variants were combined combinatorially to produce a library of recombinant FR sequences. The hits for FR123 and/or the recombinant FR sequences can be scored based on their structurally compatibility with the structure cluster of the framework regions.

Figure 12C shows the result generated by using the method diagramed in Figure 12A based on a sequence profile of a structure cluster of the CDR1 of 4 kappa $V_L$ sequences. The structure cluster of the 4 kappa $V_L$ sequences, 1dgx, 1dh5, 1dh4, and 1dh6, is shown in Figure 8B. Sequences of CDR1 of these 4 kappa $V_L$ sequences in the structure cluster were used to build HMM and search Kabat database. The regions in these 4 kappa $V_L$ sequences showing a greater variability, i.e., the supervariable regions, are highlighted in red (Figure 12C). Numerous hits were found with diverse sequences and variable lengths. The hits were grouped according to their lengths. The group having the same length as one of the 4 kappa $V_L$ CDR1 sequences were compared and profiled based on variability in each amino acid residue. Such a variant profile was built for each of the 4 kappa $V_L$ CDR1 sequences, 1dgx, 1dh5, 1dh4, and 1dh6. To demonstrate, hits with lengths different than these 4 target sequences were also selected by using the inventive method, three artificial sequences, 1dh5a, 1dh5b, and 1dh5c, were constructed by inserting more residues into the supervariable region of 1dh5 and used as references to group these hits. As shown in the right portion of Figure 12C, hits with lengths different from the 4 "real" target sequences, 1dgx, 1dh5, 1dh4, and 1dh6, were also be found, variant profiles of which were shown underneath each of the 3 artificial sequences.

The variant profiles shown in Figure 12C reveal that there is a
much higher variability in the supervariable region than the rest of the CDR1. The amino acid residues in the supervariable region may make a greater contribution to the specific and high affinity binding of the antibody to its antigen. This region can be specifically targeted to generate a more focused library of recombinant antibodies for structural and functional screening in silico, in vitro or in vivo.

As also shown in Figure 12C, the CDR1 variants with less than 5% of occurrence frequency were filtered and discarded. The rest of the variants were combined combinatorially to produce a library of recombinant CDR1 sequences. The hits for CDR1 and/or the recombinant CDR1 sequences can be scored based on their structurally compatibility with the structure cluster of the 4 kappa V\(_L\) CDR1 sequences.

For the reclustered structure family in the Hucal model of Knappik et al., three of the V\(_H\) structures are re-clustered into one family based on the structure criteria (superimposition and gaps), these three sequences should be used as the profiled sequences to build their HMM and then search the corresponding human germline sequence that is closest to all of them. Figure 13 A-C show the results of the search using this method. The identified human germline sequences (labeled as “Top Hits”) can then be used to represent the corresponding structure in our diversity library for the target structures.

As shown in Figure 13A, the seven VHs of the Hucal models are clustered into 5 structure families: (1DGA, 1DHO, 1DHW), 1DHQ, 1DHU, 1DV, and 1DHZ. The seven VLs of the Hucal models are clustered into 3 structure families: (1DGX, 1DHS) and (1DH4, 1DH6) for Kappa VL, and (1DH7, 1DH8, 1DH9) for Lamda VL. Figure 13B shows the alignment of the amino acid sequences based on the structures of the members within each structure family.

Figure 13 C lists the top hits of human germline antibodies identified by using the profile HMM method (HMMER2.1.1). The HMM has been calibrated; and E-values are empirical estimates. The top hits to the query sequence profile shows some important features which make it necessary to capture in order to make a comprehensive library for the clustered structure family of 1DHA, 1DHW and 1DHO. It is
noted that 1DHW belongs to a different family of VH (f5 see Figure 10) where 1DHA and 1DHO belong to the same family of VH (f1 in Figure 10) based on the sequence homology classification. It is also apparent that comparison between hits and query sequence profile show that in some regions the sequence are highly conservative whereas in other regions sequence variability is large. The constant region should be good part for making master framework whereas the highly variable regions are some position for making sequence library.

It should be noted that the order of the top hits depends sensitively on the multiple sequence alignment derived from structure-based alignment. This demonstrates that the structure information is important for selecting the hit sequences. As shown in Figure 13C, some of the top hits are nontrivial from those obtained by BLAST.

5). Matching the library of structural template with the library of sequence pools

A powerful approach to compare the target structural template library with the sequence database is to match them in both directions. Using threading to find the optimal template for each sequence among the sequence database and then using reverse threading to match each template structure to sequence in the sequence database. The convergence of the both direction should give a reliable sequence to construct the sequence libraries for the desired target structures. This method can be also used in combination with other sequence searching method such as COBLATH that combines PSI-BLAST with Threading method.

4. Examples of Structural Computational Engines

Many programs are available for modeling structures or structural ensembles of antibodies. For example, a molecular mechanics software may be employed for these purposes, examples of which include, but are not limited to CONGEN, SCWRL, UHBD, and GENPOL.
CONGEN (CONformation GENerator) is a program performing conformational searches on segments of proteins (R. E. Brucoleri (1993) Molecular Simulations 10, 151-174 (1993); R. E. Brucoleri, E. Haber, J. Novotny, (1988) Nature 335, 564-568 (1988); R. Brucoleri, M. Karplus. (1987) Biopolymers 26, 137-168. It is most suited to problems where one needs to construct underdetermined loops or segments in a known structure, i.e. homology modeling. The program is a modification of CHARMM version 16, and has most of the capabilities of that version of CHARMM (Brooks BR, Brucoleri BE, Olafson BD, States DJ, Swaminathan S, Karplus M. (1983) J. Comput. Chem. 4, 187-217). The energy functions of the total energy include bonds, angles, torsional angels, improper term, vdw and electrostatic interactions with distance dependent dielectric constant using Amber94 forcefield in CONGEN. It provides a simple yet fast way to scan sequence library for their compatibility with their template structure with decent correlation with the more refined scoring energy functions.

The CONGEN program is a modeling stratagem based on the theory that the lowest energy conformation should be close or correspond to the naturally occurring one. Brucoleri and Karplus (1987) Biopolymers 26:137-168; and Brucoleri and Novotny (1992) Immunomethods 96-106. Given an accurate Gibbs function and a short loop sequence, all of the stereochemically acceptable structures of the loop can be generated and their energies calculated. The one with the lower energy is selected.

The program can be used to perform both conformational searches and structural evaluation using standard scoring function. The program can calculate other properties of the molecules such as the solvent accessible surface area and conformational entropies, given steric constraints. Each one of these properties in combination with other properties described below can be used to score the digital libraries.

The defined canonical structures are available for five of the CDRs (V_{L} CDR1, 2, and 3, and V_{H} CDR1 and 2) except for V_{H} CDR3. V_{H} CDR3 is known to show large variation in its length and conformations, although progress has been made in modeling its conformation with
increasing number of antibody structures becoming available in the PDB (protein data bank) database. CONGEN may be used to generate conformations of a loop region (e.g., \( V_H \) CDR3) if no canonical structure is available, to replace the side chains of the template sequence with the corresponding side chain rotamers of the target amino acids. Third, the model will be further optimized by energy minimization or molecular dynamic simulation or other protocols to relieve the steric clash etc in the structure model.

SCWRL is a side chain placing program that can be used to generate side chain rotamers and combinations of rotamers using the backbone dependent rotamer library (Dunbrack RL Jr, Karplus M (1993) J Mol Biol 230:543-574). SCWRL is a program for adding sidechains to a protein backbone based on the backbone-dependent rotamer library (Bower, MJ, Cohen FE, Dunbrack RL (1997) J Mol Biol 267, 1268-1282). The library provides lists of chi1-chi2-chi3-chi4 values and their relative probabilities for residues at given phi-psi values. The program can further explore these conformations to minimize sidechain-backbone clashes and sidechain-sidechain clashes. Once the steric clash is minimized, the side chains and the backbone of the substituted segment can be energy minimized to relieve local strain using CONGEN (Brunoculli and Karplus (1987) Biopolymers 26:137-168). Each structure is scored using a custom energy function that measures the relative stability of the sequence in the lead structural template.

Several automatic programs that are developed specifically for building antibody structures may be used for structural modeling of antibody in the present invention. The ABGEN program is an automated antibody structure generation algorithm for obtaining structural models of antibody fragments. Mandal et al. (1996) Nature Biotech. 14:323-328. ABGEN utilizes a homology based scaffolding technique and includes the use of invariant and strictly conserved residues, structural motifs of known Fab, canonical features of hypervariable loops, torsional constraints for residue replacements and key inter-residue interactions. Specifically, the ABGEN algorithm consists of two principal modules, ABalign and ABbuild. ABalign is the
program that provides the alignment of the antibody V-region sequence whose structure is desired with all the V-region sequences of antibodies whose structures are known and computes scores for the fitting. The highest scoring library sequence is considered to be the best fit to the test sequence. ABBuild then uses this best fit model output by Abalign to generate the three-dimensional structure and provides Cartesian coordinates for the desired antibody sequence.

WAM (Whitelegg NRJ and Rees, AR (2000) Protein Engineering 13, 819-824) is an improved version of ABM which is uses a combined algorithm by (Martin, ACR, Cheetham, JC, and Rees AR (1989) PNAS 86, 9268-9272) Rees etc.- to model the CDR conformations using the canonical conformations of CDRs loops from x-ray PDB database and loop conformations generated using CONGEN (see reference by Rees 1995 (Ab antibody engineering)). In short, the modular nature of antibody structure make it possible to model its structure using a combination of protein homology modeling and structure predictions.

In a preferred embodiment, the following procedure will be used to model antibody structure. Because antibody is one of the most conserved proteins in both sequence and structure, homology models of antibodies are relatively straightforward, except for certain CDR loops that are not yet determined within existing canonical structures or with insertion or deletions. These loop structures can be, however, modeled using a combined algorithms that combines homology modeling with conformational search (for example, CONGEN can be used for such purpose).

The defined canonical structures for five of the CDRs (L1,2,3 and VH1,2) except for H3 (i.e., VH CDR3) are used. VH H3 is known to show large variation in its length and conformations, although progress has been made in modeling its conformation with increasing number of antibody structures become available in the (protein data bank) PDB database using protein structure prediction methods, including threading and comparative modeling, which aligns the sequence of unknown structure with at least one known structure based on the similarity spanning modeled sequence. The de novo or ab initio methods also show increasing promising to predict the structure from
sequence alone. The unknown loop conformations can be sampled using CONGEN if no canonical structure is available (Brucoleri RE, Haber E, Novotny J (1988) Nature 355, 564-568). Alternatively, ab initio methods including but not limited to Rosetta ab initio method can be used to predict antibody CDR structures (Bonneau R, Tsai J, Ruczinski I, Chivian D, Rohl C, Strauss CE, Baker D (2001) Proteins Suppl 5, 119-126) without relying on similarity at the fold level between the modeled sequence and any of the known structures. The more accurate method that uses the state-of-the-art explicit solvent molecular dynamics and implicit solvent free energy calculations can be used to refine and select for native-like structures from models generated from either CONGEN or Rossetta ab initio method (Lee MR, Tsai J, Baker D, Kollman PA (2001) J Mol Biol 313, 417-430). The interactions between CDRs are first scored using the principles that determine the structure of β-sheet barrels in proteins.

5. Scoring functions for evaluating structural compatibility of tester sequence and structural template

In the implementation of the inventive methods described above, thermodynamic computational analysis can be used for evaluating structural compatibility of a tester sequence with a target structural template. The structural evaluation is based on an empirical and parameterized scoring function and is intended to reduce the number of subsequent in vitro screenings necessary. The scoring function consists of three energy terms: nonpolar solvation, sidechain entropy, and electrostatic energy (Sharp KA. (1998) Proteins 33, 39-48; Novotny J, Brucoleri RE, Davis M, Sharp KA (1997) J Mol Biol 268, 401-411).

For energy functions, there are many that can be used to score compatibility of sequences with template structure or structure ensemble. The scoring function is composed of several terms including contribution from electrostatic and van der Waals interactions, $\Delta G_{MM}$ calculated using molecular mechanic forcefield, contribution from solvation including electrostatic solvation and solvent-accessible surface, $\Delta G_{sol}$, and contribution from the conformational entropy.
A simple fast way for computational screening is to calculate structural stability of a sequence using the total or combination of energy terms from molecular mechanic forcefield such as Amber94 implemented in CONGEN.

\[ \Delta E_{total} = E_{vdw} + E_{bond} + E_{angle} + E_{electrostatics} + E_{solvation} \]

or alternatively, the binding free energy is calculated as

\[ \Delta G_b = \Delta G_{MM} + \Delta G_{sol} (Ag-Ab) - \Delta G_{sol} (Ag) - \Delta G_{sol} (Ab) - T\Delta S \]

where:

\[ \Delta G_{MM} = \Delta G_{ele} + \Delta G_{vdw} \] (1)
\[ \Delta G_{sol} = \Delta G_{ele-sol} + \Delta G_{ASA} \] (2)

The \( \Delta G_{ele} \) and \( \Delta G_{vdw} \) electrostatic and van der Waals interaction energy are calculated using Amber94 parameters implemented in CONGEN for \( \Delta G_{MM} \), whereas the \( \Delta G_{ele-sol} \) is electrostatic solvation energy required to move a heterogeneously distributed charges from the gas phase into an aqueous phase. This is calculated by solving the Poisson-Boltzmann equation for the electrostatic potential for the reference and mutant structures. \( \Delta G_{ASA} \), the nonpolar energy is the energetic cost of moving nonpolar solute groups into polar solvent, resulting in reorganization of the solvent molecules. This has been shown to correlate linearly with the solvent accessible surface area of the molecule (Sitkoff D, Sharp, KA, Honig B (1994) J Phys Chem 98, 1978-1988).

The change in sidechain entropy is a measure of the effect on the local sidechain conformational space particularly at the binding interface. This is calculated from the ratio of the number of allowed sidechain conformations in the reference and mutant structures, in the bound and unbound states. For general scoring purposes, the independent sidechain approximation is applied to the mutated sidechains in order to reduce computational time resulting from sampling the huge conformational space for individual side chains in various structural context.
6. **Energy Functions**

Many energy functions can be used to score the compatibility between sequences and structures. There are four kinds of energy functions can be used: (1) empirical physical chemistry-based forcefields based on simple model compounds such as standard molecular mechanic forcefields discussed below; (2) knowledge-based statistical forcefields extracted from protein structures, the so-called potential of mean force (PMF) or the threading score derived from the structure-based sequence profiling (3) parameterized forcefield by fitting the forcefield parameters using experimental model system; (4) combinations of one or several terms from (1) to (3) with various weighting factor for each term.

The following well-tested physical-chemistry forcefields can be used or incorporated into the scoring functions. For example, amber 94 forcefield was used in Congen to score the sequence-structure compatibility in the examples below. The forcefields include but are not limited to the following forcefields which are widely used for those skilled at the art. Amber 94 (Cornell, WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW and Kollman PA. JACS (1995) 117, 5179-5197 (1995); Charmm forcefields (Brooks, B.R., Bruccoleri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., Karplus, M. (1983) J. Comp. Chem. 4, 187-217.; MacKerell, A D ; Bashford, D; Bellott, M; Dunbrack, R L; Eva seck, J D; Field, M J; Fischer, S; Gao, J; Guo, H; Ha, S; JosephMcCarthy, D; Kuczyn, L; Kuczera, K; Lau, F T K; Mattos, C; Michnick, S; Ngo, T; Nguyen, D T; Pro hom, B; Reiher, W E; Roux, B; Schlenkrich, M; Smith, J C; Stote, R; Straub, J; W tanabe, M; WiorkiewiczKuczera, J; Yin, D; Karplus, M (1998) J. Phys. Chem., B 102, 3586-3617). The Discover cvff forcefields (Dauber-Osguthorpe, P.; Roberts, V. A.; Osguthorpe, D. J.; Wolff, J.; Genest, M.; Hagler, A. T. (1988) Proteins: Structure, Function and Genetics, 4, 31-47.) The ECEPP forcefields (Momany, F. A., McGuire, R. F., Burgess, A. W., & Scheraga, H. A., (1975) J. Phys. Chem. 79, 2361-2381.; Nemethy, G., Pottle, M. S., & Scheraga, H. A.,


A combination of various terms from molecular mechanic forcefields plus some specific components has been used in most protein design programs. In a preferred embodiment, the forcefield is composed of one or several some terms such as the van der Waals,
hydrogen bonding and electrostatic interactions from the standard molecular mechanics forcefields such as Amber, Charmm, OPLS, cvff, ECEPP, plus one or several terms that are believed to control the stability of proteins.

7. **Examples of forcefields for protein design**

It is understood that as a general solution to protein design problem, the energy surface describing the interactions among all elements of the system are sampled as a function of its atomic coordinates over all available sequences and their conformational space. Such a procedure may be implemented in following steps: i) providing a target scaffold with the backbone structure, e.g., a X-ray crystal structure retrieved from protein databank (PDB) or a structural model built by modeling; ii) building side chain models of amino acid variants onto a selected backbone by using a rotamer library derived from a protein structure database; iii) assigning forcefield parameters such as charge, radii, etc. to each atom to construct the target function; and iv) searching the energy surface of the target function using deterministic and/or stochastic algorithms to find optimal solution or solutions ranked in their scores.

Each individual protein design method is distinguished mainly from each other in terms of the forcefield and sampling algorithm. However, scoring functions and sampling algorithms in these protein design methods may optionally be used for a structure-based evaluation of the sequences from the hit and/or hit variant library.

For example, as an important interaction for scoring the correct packing interactions inside the core of proteins, van der Waals (vdw) interaction was used to design the protein core sequences by testing allowed rotamer sequences in enumeration (Ponder JW, Richards FM (1987) J Mol Biol 193, 775-791. A group of sequences can be selected under a potential function using simulated evolution with stochastic algorithm; the ranking order of the energies of selected sequences for residues in the hydrophobic cores of proteins correlates well with their biological activities (Hellinga HW, Richards FM (1994) PNAS 91, 5803-5807).
Similar approaches were also used to design proteins using stochastic algorithm (Desjarlais J, Handel T, (1995) Protein Science 4, 2006-2018; Kono H, Doi J (1994) Proteins, 19, 244-255). Effect of potential function on the designed sequences of a target scaffold has been evaluated by including van der Waals, electrostatics, and surface-dependent semiempirical environmental free energy or combinations of terms in an automatic protein design method that keeps the composition of amino acid sequence unchanged. It was shown that each additional term of the energy function increases progressively the performance of the designed sequences with vdw for packing, electrostatics for folding specificity and environmental solvation term for burial of the hydrophobic residues and for exposure of the hydrophilic residue (Koehl P, Levitt M (1999) J Mol Biol 293, 1161-1181).


Forcefields have been also optimized specifically for protein design purpose. The energy function is decomposed into pairwise functional forms that combine molecular mechanic energy terms with specific solvation term is used for residues at the core, boundary and surface positions; dead end elimination algorithm is used to sip through huge number of combinatorial rotameric sequences (Dahiyat BI, Mayo...
SL (1996) Protein Science 5, 895-903). The stringency of force fields and rigid inverse folding protocol with fix backbone used in protein design has inevitably resulted a significant rate of false negative: rejection of many sequences that might be acceptable if soft energy function or flexible backbone is allowed. Moreover, the energy function used for protein design is so different from forcefields such as Amber or Charmm that are widely used and tested for studying protein folding or stability (Gordon DB, Marshall SA, Mayo SL (1999) Curr Opin Stru Biol 9, 509-513). Cautions should be excised to compare the sequences designed using specific protocol with others from alternative methods because a direct comparison among them may not be possible due to the false negative issues involved in protein design protocols.

The inventor believes that although a high false negative rate in protein design is not a problem for designing proteins with no restriction, this will pose serious problem for designing proteins for pharmaceutical application because only small restrictive region is allowed to have altered sequences to improve protein functions such as the CDRs in antibodies and a few positions in the framework regions. Therefore, it is accuracy rather than the speed of computational screening that matters the most for functional improvement in order to identify those few mutants in the targeted region.

These methods can be used to generate structure ensembles by molecular dynamics calculations or computational methods of proteins in the native or unfolded states which provide more accurate methods to score sequence and its variants based on the ensemble averages of the energy functions (Kollman PA, Massova I, Reyes C, Kuhn B, Huo SH, Chong LT, Lee M, Lee TS, Duan Y, Wang W, Donini O, Cieplak P, Srinivasan P, Case DA, and Cheatham TE (2000) Acc. Chem Res. 33, 889-897). The ensemble averages calculated from ensemble structures show better correlation with corresponding data from experimental measurement.

In a particular embodiment, standard terms from MM terms have been combined with the solvation terms including electrostatic solvation and solvent-accessible solvation term calculated with continuous solvent model for electrostatic solvation; these MM-PBSA or MM-GBSA
method, together with contribution from the conformational entropy including backbone and side chains, have shown good correlation between experimental and calculated values in the free energy change (Wang W, Kollman P (2001) JMB). Compared to other scoring functions used in protein and drug design, MM-PBSA or MM-GBSA is better physical model for scoring and would handle various problems on an uniform basis, although it is computational expensive because multiple trajectories from molecular dynamic simulation in explicit water is required to calculate the ensemble averages for the system.
CLAIMS

What is claimed is:

1. A method for constructing a library of recombinant antibodies, comprising the steps of:
   clustering variable regions of a collection of antibodies having known 3D structures into at least two families of structural ensembles, each family of structural ensemble comprising at least two different antibody sequences but with substantially identical main chain conformations;
   selecting a representative structural template from each family of structural ensemble;
   profiling a tester polypeptide sequence onto the representative structural template within each family of structural ensemble; and
   selecting the tester antibody sequence that is compatible to the structural constraints of the representative structural template.

2. The method of claim 1, wherein the collection of antibodies include antibodies or immunoglobulins collected in a protein database.

3. The method of claim 2, wherein the protein database is selected from the group consisting of the protein data bank of Brookhaven National Laboratory, genbank at the National Institute of Health, and Swiss-PROT protein sequence database.

4. The method of claim 1, wherein the collection of antibodies having known 3D structures include antibodies having resolved X-ray crystal structures, NMR structures or 3D structures based on structural modeling.

5. The method of claim 1, wherein the variable regions of the collection of antibodies are the full length heavy chain or light chain variable regions or specific portions of the heavy chain or light chain
variable region selected from the group consisting of CDR, FR, and a combination thereof.

6. The method of claim 5, wherein the CDR is CDR1, CDR2, or CDR3 of an antibody.

7. The method of claim 5, wherein the FR is FR1, FR2, FR3, or FR4 of an antibody.

8. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the root mean square difference of the main chain conformations of antibody sequences in each family of the structural ensemble is less than 4 Å.

9. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the root mean square difference of the main chain conformations of antibody sequences in each family of the structural ensemble is less than 3 Å.

10. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the root mean square difference of the main chain conformations of antibody sequences in each family of the structural ensemble is less than 2 Å.

11. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the root mean square difference of the main chain conformations of antibody sequences in each family of the structural ensemble is between about 0.1-4.0 Å.

12. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the Z-score of the main chain conformations of antibody sequences in each family of the structural ensemble is more than 2.
13. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the Z-score of the main chain conformations of antibody sequences in each family of the structural ensemble is more than 3.

14. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the Z-score of the main chain conformations of antibody sequences in each family of the structural ensemble is more than 4.

15. The method of claim 1, wherein the clustering step includes clustering the collection of antibodies such that the Z-score of the main chain conformations of antibody sequences in each family of the structural ensemble is between about 2-8.

16. The method of claim 1, wherein the clustering step is implemented by an algorithm selected from the group consisting of CE, Monte Carlo and 3D clustering algorithms.

17. The method of claim 1, wherein the profiling step includes reverse threading the tester polypeptide sequence onto the representative structural template within each family of structural ensemble.

18. The method of claim 1, wherein the profiling step is implemented by a multiple sequence alignment algorithm.

19. The method of claim 18, wherein the multiple sequence alignment algorithm is profile HMM algorithm or PSI-BLAST.

20. The method of claim 1, wherein the representative structural template is adopted by a CDR region, and the profiling step includes profiling the tester polypeptide sequence that is a variable region of a human or non-human antibody onto the representative structural template within each family of structural ensemble.
21. The method of claim 1, wherein the representative structural template is adopted by a FR region, and the profiling step includes profiling the tester polypeptide sequence that is a variable region of a human antibody onto the representative structural template within each family of structural ensemble.

22. The method of claim 21, wherein the tester polypeptide sequence is a variable region of human germline antibody sequence.

23. The method of claim 1, wherein the tester polypeptide sequence is the sequence or a segment sequence of an expressed protein.

24. The method of claim 1, wherein the tester polypeptide sequence is a region of an antibody.

25. The method of claim 24, wherein the antibody is a human antibody.

26. The method of claim 1, wherein the tester polypeptide sequence is a region of a human germline antibody sequence.

27. The method of claim 1, wherein the selecting step includes selecting the tester polypeptide sequence by using an energy scoring function selected from the group consisting of electrostatic interactions, van der Waals interactions, electrostatic solvation energy, solvent-accessible surface solvation energy, and conformational entropy.

28. The method of claim 1, wherein the selecting step includes selecting the tester polypeptide sequence by using a scoring function incorporating a forcefield selected from the group consisting of the Amber forcefield, Charmm forcefield, the Discover cvff forcefields, the ECEPP forcefields, the GROMOS forcefields, the OPLS forcefields, the MMFF94 forcefield, the Tripose forcefield, the MM3 forcefield, the Dreiding forcefield, and UNRES forcefield, and other knowledge-based
statistical forcefield (mean field) and structure-based thermodynamic potential functions.

29. The method of claim 1, further comprising the steps of:
building an amino acid positional variant profile of the selected
tester polypeptide sequences;
filtering out the variants with occurrence frequency lower than 3;
and
combining the variants remained to produce a combinatorial
library of antibody sequences.

30. The method of claim 29, wherein the filtering step includes
filtering out the variants with occurrence frequency lower than 5.

31. The method of claim 1, further comprising the following:
introducing the DNA segment encoding the selected tester
polypeptide into cells of a host organism;
expressing the DNA segment in the host cells such that a
recombinant antibody containing the selected polypeptide sequence is
produced in the cells of the host organism; and
selecting the recombinant antibody that binds to a target antigen
with affinity higher than $10^6 \, M^{-1}$.

32. The method of claim 31, wherein the recombinant antibody is a
fully assembled antibody, a Fab fragment, an Fv fragment, or a single
chain antibody.

33. The method of claim 31, wherein the host organism is selected
from the group consisting of bacteria, yeast, plant, insect, and mammal.

34. The method of claim 31, wherein the target antigen is a small
molecule, proteins, peptide, nucleic acid or polycarbohydrate.

35. A method of constructing a library of recombinant antibodies
based on
a target structural template, comprising the steps of:

  providing a target structural template of a variable region of one or more antibodies;
  
  profiling a tester polypeptide sequence onto the target structural template; and
  
  selecting the tester polypeptide sequence that is structurally compatible with the target structural template.

36. The method of claim 35, wherein the target structural template is a 3D structure of a heavy chain or light chain variable region of an antibody.

37. The method of claim 36, wherein the heavy chain or light chain variable region of an antibody is a CDR, a FR or a combination thereof.

38. The method of claim 35, wherein the target structural template is a 3D structural ensemble of heavy chain or light chain variable regions of at least two different antibodies.

39. The method of claim 38, wherein the heavy chain or light chain variable regions are CDRs, FRs or combinations thereof.

40. The method of claim 35, wherein the profiling step includes reverse threading the tester polypeptide sequence onto the target structural template.

41. The method of claim 35, wherein the profiling step is implemented by a multiple sequence alignment algorithm.

42. The method of claim 41, wherein the multiple sequence alignment algorithm is profile HMM algorithm or PSI-BLAST.

43. The method of claim 35, wherein the target structural template is adopted by a CDR region, and the profiling step includes profiling the tester polypeptide sequence that is a variable region of a human or non-
human antibody onto the representative structural template within each family of structural ensemble.

44. The method of claim 35, wherein the target structural template is adopted by a FR region, and the profiling step includes profiling the tester polypeptide sequence that is a variable region of a human antibody onto the representative structural template within each family of structural ensemble.

45. The method of claim 44, wherein the tester polypeptide sequence is a variable region of human germline antibody sequence.

46. The method of claim 35, wherein the tester polypeptide sequence is the sequence or a segment sequence of an expressed protein.

47. The method of claim 35, wherein the tester polypeptide sequence is a region of an antibody.

48. The method of claim 35, wherein the antibody is a human antibody.

49. The method of claim 35, wherein the tester polypeptide sequence is a region of a human germline antibody sequence.

50. The method of claim 35, wherein the selecting step includes selecting the tester polypeptide sequence by using an energy scoring function selected from the group consisting of electrostatic interactions, van der Waals interactions, electrostatic solvation energy, solvent-accessible surface solvation energy, and conformational entropy.

51. The method of claim 35, wherein the selecting step includes selecting the tester polypeptide sequence by using a scoring function incorporating a forcefield selected from the group consisting of the Amber forcefield, Charmm forcefield, the Discover cvff forcefields, the ECEPP forcefields, the GROMOS forcefields, the OPLS forcefields, the
MMFF94 forcefield, the Tripose forcefield, the MM3 forcefield, the
Dreiding forcefield, and UNRES forcefield, and other knowledge-based
statistical forcefield (mean field) and structure-based thermodynamic
potential functions.

52. The method of claim 35, further comprising the steps of:
building an amino acid positional variant profile of the selected
tester polypeptide sequences;
filtering out the variants with occurrence frequency lower than 3;
and
combining the variants remained to produce a combinatorial
library of antibody sequences.

53. The method of claim 52, wherein the filtering step includes
filtering out the variants with occurrence frequency lower than 5.

54. The method of claim 35, further comprising the following:
introducing the DNA segment encoding the selected tester
polypeptide into cells of a host organism;
expressing the DNA segment in the host cells such that a
recombinant antibody containing the selected polypeptide sequence is
produced in the cells of the host organism; and
selecting the recombinant antibody that binds to a target antigen
with affinity higher than $10^6$ M$^{-1}$.

55. The method of claim 54, wherein the recombinant antibody is a
fully assembled antibody, a Fab fragment, an Fv fragment, or a single
chain antibody.

56. The method of claim 54, wherein the host organism is selected
from the group consisting of bacteria, yeast, plant, insect, and mammal.

57. The method of claim 54, wherein the target antigen is a small
molecule, proteins, peptide, nucleic acid or polycarbohydrate.
58. A method for constructing a library of recombinant antibodies, comprising the steps of:
   providing a target sequence of a heavy chain or light chain variable region of a target antibody;
   aligning the target sequence with a tester polypeptide sequence;
   and
   selecting the tester polypeptide sequence that has at least 15% sequence homology with the target sequence.

59. The method of claim 58, wherein the target sequence is the full length heavy chain or light chain variable region of the target antibody or specific portions of the variable regions of the target antibody selected from the group consisting of CDR, FR, and a combination thereof.

60. The method of claim 59, wherein the CDR is CDR1, CDR2, or CDR3 of the target antibody.

61. The method of claim 59, wherein the FR is FR1, FR2, FR3, or FR4 of the target antibody.

62. The method of claim 58, wherein the aligning step includes aligning the target sequence with the polypeptide segment of the tester polypeptide sequence by using a sequence alignment algorithm.

63. The method of claim 62, wherein the sequence alignment algorithm is selected from the group consisting of BLAST, PSI-BLAST, profile HMM, and COBLATH.

64. The method of claim 58, wherein the target sequence is a CDR region of the target antibody, and the alignment step includes aligning the tester polypeptide sequence that is the sequence or segment sequence of an expressed protein with the target sequence.
65. The method of claim 58, wherein the target sequence is a FR region of the target antibody, and the alignment step includes aligning the tester polypeptide sequence that is the sequence or segment sequence of a human antibody protein with the target sequence.

66. The method of claim 58, wherein the selecting step includes selecting the tester polypeptide sequence that has at least 25% sequence homology with the target sequence.

67. The method of claim 58, wherein the selecting step includes selecting the tester polypeptide sequence that has at least 35% sequence homology with the target sequence.

68. The method of claim 58, wherein the selecting step includes selecting the tester polypeptide sequence that has at least 35% sequence homology with the target sequence.

69. The method of claim 58, further comprising the steps of:
   building an amino acid positional variant profile of the selected tester polypeptide sequences;
   filtering out the variants with occurrence frequency lower than 3;
   and
   combining the variants remained to produce a combinatorial library of antibody sequences.

70. The method of claim 58, wherein the filtering step includes filtering out the variants with occurrence frequency lower than 5.

71. The method of claim 58, further comprising the following:
   introducing the DNA segment encoding the selected tester polypeptide into cells of a host organism;
   expressing the DNA segment in the host cells such that a recombinant antibody containing the selected polypeptide sequence is produced in the cells of the host organism; and
selecting the recombinant antibody that binds to a target antigen with affinity higher than $10^6 \text{ M}^{-1}$.

72. The method of claim 51, wherein the recombinant antibody is a fully assembled antibody, a Fab fragment, an Fv fragment, or a single chain antibody.
FIGURE 2

> 1DGH: L IMMUNOGLOBULIN VL DOMAIN
DIQMTQSPSLSAVDRVTITCRASQIGSSYLYAWYQQPKKAPKLKLIAASLLQLGSVPS
RFSGGSQSDTFLTISSLQPDFATYQQHYTPTPFTGQGKVHEIRK

> 1DH4: L IMMUNOGLOBULIN VKAPPA DOMAIN
DIVMTQSPSLPVTPGPASISCRSSQSSLHNSQNYLDNYLQKPGQSPQLLLIYLGSNRA
SGVPDRFSGSGSTDTLKLISVEAEVDGYYQCQHYTPTPFTGQGKVHEIRK

> 1DH5: L IMMUNOGLOBULIN VL KAPPA DOMAIN
DIVLTTPOPLSLPGERTLSRACRSSQVSSSYLYAWYQQPKQAPRLLILYGASSRATGVP
ARFGSGSSGTDFLTESSLIPEDFQVVYYQCQHYTPTPFTGQGKVHEIRK

> 1DH6: L IMMUNOGLOBULIN VL KAPPA DOMAIN
DIVMTQSPSDLQSLGERATINCRSSQVLYSNKNQNYLYAWYQQPKQPPKLYIYAWSRT
EGVPDRFSGSGSTDFLTESSLQAEAVYYQCQHYTPTPFTGQGKVHEIRK

> 1DH7: L IMMUNOGLOBULIN VL LAMBDA DOMAIN
QSALTQPSVSQPQGQTSITCGTSGSTQVGGNYVSVYYYQHPKPKLIMYDNVSNRPQGV
SNRFSGSKSGTASLTALSGSTGQAEDDYFQHYTTPEPFGGKTTLVTLG

> 1DH8: L IMMUNOGLOBULIN VL LAMBDA DOMAIN
QSALTQPSVSQPQGQTSITCGTSGSTQVGGNYVSVYYYQHPKPKLIMYDNVSNRPQGV
SNRFSGSKSGTASLTALSGSTGQAEDDYFQHYTTPEPFGGKTTLVTLG

> 1DH9: L IMMUNOGLOBULIN VL LAMBDA DOMAIN
SYELTQPSVSQPQAPQTARISCGSDLGDKASWSYQQPKQAPAVLVYDDSRPFGSIFER
FSGSNQGNTALTSTLIGSTGQAEDDYFQHYTTPEPFGGKTTLVTLG

> 1DHA: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHB: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHC: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHD: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHE: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHF: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHI: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

> 1DHJ: H IMMUNOGLOBULIN HEAVY CHAIN VARIABLE DOMAIN
QVQLVQIGKPSQVPSSKSVKSCSASGTFTSSAYAIWVRQAPGQGLEWMDGWIIIFGDANY
AQKPGQRTTITADESTSTAYMELSDRSEDGATYCARWGGDFGYAMDYWGQGTLVTVSS

SUBSTITUTE SHEET (RULE 26)
Structural clustering of seven variable heavy chains

Figure 3

Nterm
1dga: green
1dho: cyan
1dhw: yellow
1dhz: magenta
1dhv: grey
1dhq: blue
1dhu: red

Cterm
Three VH (1dha, 1dho, 1dhw) are clustered into one structure family

Figure 4A

1dga: green; 1dho: cyan; 1dhw: yellow
Structural clustering of seven variable light chains

Figure 5

1dgx: green
1dh4: yellow
1dh5: cyan
1dh6: magenta
1dh7: red
1dh8: grey
1dh9: blue
Structural clustering of 4 VL kappa (1dgx, 1dh4, 1dh5, 1dh6)

N-term

CDR3

1dgx: green
1dh4: yellow
1dh5: cyan
1dh6: magenta

CDR1

CDR2

C-term

Figure 6
Structural clustering of 3 VL lambda (1dh7, 1dh8, 1dh9)

Figure 7

CDR1

CDR2

CDR3

N-term

C-term

1dh7: red
1dh8: grey
1dh9: blue
Clustering CDR1 of 3 VL lamda

1DH8 : L TGTSS-DVGGYN-YVSW
1DH9 : L SGDAL-G---DK-YASW
1DH7 : L SGSSS-NIG-SN-YVSW

Figure 8A
Clustering CDR1 of 4 kappa VL

Figure 8B

1DGX:L  RASQGI S------ SYLAW
1DH5:L  RASQSV-SS------ SYLAW
1DH4:L  RSSQSL-LHSNGY- NYLDW
1DH6:L  RSSQSV-LYSSNNK NYLAW
Clustering CDR1 of 2 kappa VL

1DH4:L  RSSQSL-LHS NGY- NYLDW
1DH6:L  RSSQSV-LYS SNNK NYLAW
Clustering CDR1 of 2 kappa VL

Figure 8D
Clustering CDR1 of 7 VL chains
FIGURE 10

SELECTED FULLY HUMAN ANTIBODY SEQUENCES WITH EXTREMELY HIGH HOMOLOGY TO
THE SEQUENCES OF THE TARGET STRUCTURES

<table>
<thead>
<tr>
<th>PDB_ID</th>
<th>Germline Name</th>
<th>SEQ ID NO</th>
<th>Aligned Residue</th>
<th>High Score</th>
<th>P(N) smallest Sum probability</th>
<th>Identity%</th>
<th>Germline Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>1DHA</td>
<td>VHGL1.8</td>
<td>15</td>
<td>120:98</td>
<td>502</td>
<td>7.9e-52</td>
<td>100</td>
<td>vhaagrp-f1.aa</td>
</tr>
<tr>
<td>1DHW</td>
<td>DP-73/V5-51...+</td>
<td>16</td>
<td>120:98</td>
<td>523</td>
<td>8.3e-55</td>
<td>100</td>
<td>vhaagrp-f5.aa</td>
</tr>
<tr>
<td>1DHO</td>
<td>DP-75/V1-2...+</td>
<td>17</td>
<td>120:98</td>
<td>498</td>
<td>2.1e-51</td>
<td>94</td>
<td>vhaagrp-f1.aa</td>
</tr>
<tr>
<td>1DHQ</td>
<td>S12-9/DP-27...+</td>
<td>18</td>
<td>121:100</td>
<td>494</td>
<td>1.9e-51</td>
<td>94</td>
<td>vhaagrp-f2.aa</td>
</tr>
<tr>
<td>1DHU</td>
<td>DP-47/V3-23...+</td>
<td>19</td>
<td>120:98</td>
<td>500</td>
<td>3.4e-51</td>
<td>97</td>
<td>vhaagrp-f3.aa</td>
</tr>
<tr>
<td>1DHV</td>
<td>DP-71/3d197d...+</td>
<td>20</td>
<td>119:97</td>
<td>510</td>
<td>1.3e-52</td>
<td>100</td>
<td>vhaagrp-f4.aa</td>
</tr>
<tr>
<td>1DHZ</td>
<td>DP-74/VH-VI...+</td>
<td>21</td>
<td>123:101</td>
<td>530</td>
<td>1.4e-56</td>
<td>99</td>
<td>vhaagrp-f6.aa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>V\textsubscript{L} Kappa Chains:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1DGX</td>
<td>DPK9/012...+</td>
<td>22</td>
<td>108:95</td>
<td>466</td>
<td>4.0e-48</td>
<td>95</td>
<td>vkallaa-f1.aa</td>
</tr>
<tr>
<td>1DH5</td>
<td>13K18</td>
<td>23</td>
<td>109:74</td>
<td>367</td>
<td>6.2e-38</td>
<td>97</td>
<td>vkallaa-f3.aa</td>
</tr>
<tr>
<td>1DH4</td>
<td>DPK15/A19...+</td>
<td>24</td>
<td>113:100</td>
<td>502</td>
<td>4.5e-52</td>
<td>96</td>
<td>vkallaa-f2.aa</td>
</tr>
<tr>
<td>1DH6</td>
<td>DPK24/VkIVKlob...+</td>
<td>25</td>
<td>114:101</td>
<td>519</td>
<td>2.0e-55</td>
<td>97</td>
<td>vkallaa-f4.aa</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>V\textsubscript{L} Lamda Chains:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1DH7</td>
<td>VI-17+</td>
<td>26</td>
<td>109:98</td>
<td>436</td>
<td>2.1e-45</td>
<td>93</td>
<td>vlallaa-f1.aa</td>
</tr>
<tr>
<td>1DH8</td>
<td>2b2.400B5+</td>
<td>27</td>
<td>110:99</td>
<td>448</td>
<td>1.6e-46</td>
<td>95</td>
<td>vlallaa-f2.aa</td>
</tr>
<tr>
<td>1DH9</td>
<td>3r.9C5/DPL23...+</td>
<td>28</td>
<td>107:95</td>
<td>421</td>
<td>2.0e-43</td>
<td>89</td>
<td>vlallaa-f3.aa</td>
</tr>
</tbody>
</table>
**FIGURE 11**

**Sequence Alignment between the Sequence of the Target Structure and the Selected Human Antibody Germline Sequence**

**IDHA> VHGL1.8**

Length = 98

Score = 502 (176.7 bits), Expect = 7.9e-52, P = 7.9e-52

Identities = 98/98 (100%), Positives = 98/98 (100%)

**Query:**
1 QVQLVQSGAEVKPKGSSVKSCASKATGFSSYAIWSWRQAPGQGLEWGMGIIPIGTANY 60
2 QVQLVQSGAEVKPKGSSVKSCASKATGFSSYAIWSWRQAPGQGLEWGMGIIPIGTANY

**Sbjct:**
1 QVQLVQSGAEVKPKGSSVKSCASKATGFSSYAIWSWRQAPGQGLEWGMGIIPIGTANY 60
2 QVQLVQSGAEVKPKGSSVKSCASKATGFSSYAIWSWRQAPGQGLEWGMGIIPIGTANY

**Query:**
61 AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR 98
2 AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR

**Sbjct:**
61 AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR 98
2 AQKFQGRVTITADESTSTAYMELSSLRSEDTAVYYCAR

**IDHW>DP-73/V5-51...+**

Length = 98

Score = 523 (184.1 bits), Expect = 8.3e-55, P = 8.3e-55

Identities = 98/98 (100%), Positives = 98/98 (100%)

**Query:**
1 EVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY 60
2 EVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY

**Sbjct:**
1 EVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY 60
2 EVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY

**Query:**
61 SPFQPGVTISADKSIAYLQWSSLKASDTAMYYCAR 98
2 SPFQPGVTISADKSIAYLQWSSLKASDTAMYYCAR

**Sbjct:**
61 SPFQPGVTISADKSIAYLQWSSLKASDTAMYYCAR 98
2 SPFQPGVTISADKSIAYLQWSSLKASDTAMYYCAR

**IDHO>DP-75/VI-2...+**

Length = 98

Score = 498 (175.3 bits), Expect = 2.1e-51, P = 2.1e-51

Identities = 93/98 (94%), Positives = 94/98 (95%)

**Query:**
1 QVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY 60
2 QVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY

**Sbjct:**
1 QVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY 60
2 QVQLVQSGAEVKPKGSLSKISCKGSIGFSSYNIGGWVRQMPKGRLEWMGSIMIPGDSTRY

**Query:**
61 AQKFQGRVTMTDRDSSSTAYMELSSLRSEDTAVYYCAR 98
2 AQKFQGRVTMTDRDSTAYMELS LRS+DTAVYYCAR

**Sbjct:**
61 AQKFQGRVTMTDRDSSSTAYMELSSLRSEDTAVYYCAR 98
2 AQKFQGRVTMTDRDSTAYMELS LRS+DTAVYYCAR
FIGURE 11- continued

1DHQ>S12-9/DP-27...+ .
Length = 100

Score = 494 (173.9 bits), Expect = 1.9e-51, P = 1.9e-51
Identities = 94/99 (94%), Positives = 96/99 (96%)

Query:  1 QVQLKESGPAVLKPQTLTLCTFTSFGLSTSGVGVGVRQPPGKALEWLALIDWDDDKY 60
         QV L+ESGPAVLKPQTLTLCTFTSFGLSTSG V WIQPPGKALEWLALIDWDDDKY
Sbjct:  1 QVTLRESGPAVLKPQTLTLCTFTSFGLSTSGMCVSWIRQPPGKALEWLALIDWDDDKY 60

Query:  61 YSTSLKRTLITSKDNQVVLTMNMDPVDTATYYCAR 99
         YSTSLKRTLITSKDNQVVLTMNMDPVDTATYYCAR
Sbjct:  61 YSTSLKRTLITSKDNQVVLTMNMDPVDTATYYCAR 99

1DHU>DP-47/V3-23...+ .
Length = 98

Score = 500 (176.0 bits), Expect = 3.4e-51, P = 3.4e-51
Identities = 96/98 (97%), Positives = 98/98 (100%)

Query:  1 EVQLVESGGGLVQPGGLSRLSCAASGFTSFNYAMSWMVRQAPGKGLENVSAISGSGSTY 60
         EVQ+ESGGGLVQPGGLSRLSCAASGFTSFNYAMSWMVRQAPGKGLENVSAISGSGSTY
Sbjct:  1 EVQLLESGGGLVQPGGLSRLSCAASGFTSFNYAMSWMVRQAPGKGLENVSAISGSGSTY 60

Query:  61 ADSVKGRFTISRDNSKNTVQLMQNSLRAEDTAVYCYCAR 98
         ADSVKGRFTISRDNSKNTVQLMQNSLRAEDTAVYCYCA
Sbjct:  61 ADSVKGRFTISRDNSKNTVQLMQNSLRAEDTAVYCAK 98

1DHV>DP-71/3d197d...+ .
Length = 97

Score = 510 (179.5 bits), Expect = 1.3e-52, P = 1.3e-52
Identities = 97/97 (100%), Positives = 97/97 (100%)

Query:  1 QVQLQESGPGVLKPSLETSLCTTVSGSISYYWSIRQPPGKLGWGYIYYSGSTNNY 60
         QVQLQESGPGVLKPSLETSLCTTVSGSISYYWSIRQPPGKLGWGYIYYSGSTNNY
Sbjct:  1 QVQLQESGPGVLKPSLETSLCTTVSGSISYYWSIRQPPGKLGWGYIYYSGSTNNY 60

Query:  61 PSLKSRVTISVDTSDKNQFSLKLSVTAADTAVYCYCAR 97
         PSLKSRVTISVDTSDKNQFSLKLSVTAADTAVYCYCAR
Sbjct:  61 PSLKSRVTISVDTSDKNQFSLKLSVTAADTAVYCYCAR 97
FIGURE 11- continued

1DHZ>DP-74/VH-VI...+  .
Length = 101

Score = 530 (186.6 bits), Expect = 1.4e-56, P = 1.4e-56
Identities = 100/101 (99%), Positives = 100/101 (99%)

Query:  1 QVQLQQSGGGLVPKPSQTLSLCAISGDSSVNSAAWNRQRPSGRGELWRLGRTYRKSRYW 60
         QVQLQQSGGGLVPKPSQTLSLCAISGDSSVNSAAWNRQRPSGRGELWRLGRTYRKSRYW
Sbjct:  1 QVQLQQSGGGLVPKPSQTLSLCAISGDSSVNSAAWNRQRPSGRGELWRLGRTYRKSRYW 60

Query:  61 NDYAVSVKRSRTIPNTDSKQNFSLSNLQNSVTPEDAVYCAR 101
         NDYAVSVKRSRTIPNTDSKQNFSLSNLQNSVTPEDAVYCAR 101
Sbjct:  61 NDYAVSVKRSRTIPNTDSKQNFSLSNLQNSVTPEDAVYCAR 101

1DGX>DPK9/O12...+  ..
Length = 95

Score = 466 (164.0 bits), Expect = 4.0e-48, P = 4.0e-48
Identities = 91/95 (95%), Positives = 92/95 (96%)

Query:  1 DIQMTQSPSSLSASVGRDVTITCRASQGISSYLAWSYQKPGKAPKLLYAAASSLQSGVPS 60
         DIQMTQSPSSLSASVGRDVTITCRASQGISSYLAWSYQKPGKAPKLLYAAASSLQSGVPS
Sbjct:  1 DIQMTQSPSSLSASVGRDVTITCRASQGISSYLAWSYQKPGKAPKLLYAAASSLQSGVPS 60

Query:  61 RFSGSGSTFTDLTISLQPEDFAYYCQHYTTP 95
         RFSGSGSTFTDLTISLQPEDFAYYCQHYTTP
Sbjct:  61 RFSGSGSTFTDLTISLQPEDFAYYCQHYTTP 95

1DHS>13K18  ..
Length = 74

Score = 367 (129.2 bits), Expect = 6.2e-38, P = 6.2e-38
Identities = 72/74 (97%), Positives = 73/74 (98%)

Query:  8 PATLSSLSPGERATLSCRASQSVSSSAYQKPGQAPRLLYIAGSSRTAVPQPARFSGSG 67
         PATLSSLSPGERATLSCRASQSVSSSAYQKPGQAPRLLYIAGSSRTAVPQPARFSGSG
Sbjct:  1 PATLSSLSPGERATLSCRASQSVSSSAYQKPGQAPRLLYIAGSSRTAVPQPARFSGSG 60

Query:  68 SGDTFTLTISLEP 81
         SGDTFTLTISLEP
Sbjct:  61 SGDTFTLTISLEP 74

SUBSTITUTE SHEET (RULE 26)
FIGURE 11- continued

1DH4/DPK15/A19...+ ..
Length = 100

Score = 502 (176.7 bits), Expect = 4.5e-52, P = 4.5e-52
Identities = 96/100 (96%), Positives = 96/100 (96%)

Query: 1 DIVMTQSPSLPSPRPGETEAPSPSASCRRSSQQQLSSHNLNGINLYLDWYLQPQGPLPQPLLLIYLSNRA 60
        DIVMTQSPSLPSPRPGETEAPSPSASCRRSSQQQLSSHNLNGINLYLDWYLQPQGPLPQPLLLIYLSNRA
Sbjct: 1 DIVMTQSPSLPSPRPGETEAPSPSASCRRSSQQQLSSHNLNGINLYLDWYLQPQGPLPQPLLLIYLSNRA 60

Query: 61 SGVPDRFSGSGGTDTFLKISRVEAEVAVYVYQQHYTTTP 100
        SGVPDRFSGSGGTDTFLKISRVEAEVAVYVYQCMQALQTP 100
Sbjct: 61 SGVPDRFSGSGGTDTFLKISRVEAEVAVYVYQCMQALQTP 100

1DH6/DPK24/VkIVKlob...+ ..
Length = 101

Score = 519 (182.7 bits), Expect = 2.0e-55, P = 2.0e-55
Identities = 98/101 (97%), Positives = 101/101 (100%)

Query: 1 DIVMTQSPDSLAVSLGERATINCRRSSQSVLYSSNNKNYKLYQKPGQPKLILYVSTR 60
        DIVMTQSPDSLAVSLGERATINCRRSSQSVLYSSNNKNYKLYQKPGQPKLILYVSTR
Sbjct: 1 DIVMTQSPDSLAVSLGERATINCRRSSQSVLYSSNNKNYKLYQKPGQPKLILYVSTR 60

Query: 61 ESVPDRFSGSGGTDTFLTLISSQLAQEDVAVYVYQQHYTTTP 101
        ESVPDRFSGSGGTDTFLTLISSQLAQEDVAVYVYQQHYTTTP+Y+TP
Sbjct: 61 ESVPDRFSGSGGTDTFLTLISSQLAQEDVAVYVYQQHYTTTP 101

1DH7>V1-17+ ..
Length = 98

Score = 436 (153.5 bits), Expect = 2.1e-45, P = 2.1e-45
Identities = 83/89 (93%), Positives = 85/89 (95%)

Query: 1 QSVLTQPPSVSAGAGQVRITISCQSFSQSNIGHYVSWQQLFTAPKLLIYDNNQRPAGVP 60
        QSVLTQPPSVSAGAGQVRITISCQSFSQSNIGHYVSWQQLFTAPKLLIYDNNQRPAGVP
Sbjct: 1 QSVLTQPPSVSAGAGQVRITISCQSFSQSNIGHYVSWQQLFTAPKLLIYDNNQRPAGVP 60

Query: 61 DRFSGSKSGTASLAIQLQSEDAYYCY 89
        DRFSGSKSGTASLAIQLQSEDAYYCY+GL+SEDAADYCY
Sbjct: 61 DRFSGSKSGTASLAIQLQSEDAYYCY 89

SUBSTITUTE SHEET (RULE 26)
FIGURE 11- continued

1DH8>2b2.400B5+ ..
Length = 99

Score = 448 (157.7 bits), Expect = 1.6e-46, P = 1.6e-46
Identities = 86/90 (95%), Positives = 87/90 (96%)

Query: 1 QSAIQTQASVSQSIPSSITSTGTSSDTVGGYNVSSWYQHGPQPKLPQIYDVSNRPSGVC60
       QSAIQTQASVSQSIPSSITSTGTSSDVGYNSWYQHGPQPKLPQIY+VS RPSGVC60
Sbjct: 1 QSAIQTQASVSQSIPSSITSTGTSSDVGSYNLVSWYQHGPQPKLPQIYEVSKRPSGVC60

Query: 61 SNRFSGSKSGNATASLTISGLQAEDEADYYCV90
       SNRFSGSKSGNATASLTISGLQAEDEADYYCV90
Sbjct: 61 SNRFSGSKSGNATASLTISGLQAEDEADYYCV90

1DH9>3r.9C5/DPL23...+ ..
Length = 95

Score = 421 (148.2 bits), Expect = 2.0e-43, P = 2.0e-43
Identities = 79/88 (89%), Positives = 82/88 (93%)

Query: 1 SYELIQPSVSVPAGQTARISCSGDLGDKYASHWYQKPQAPVLYDSDRPSGIPER60
       SYELIQPSVSVPAGQTARISCSGDLGDKYASHWYQKPQAPVLYDSDRPSGIPER60
Sbjct: 1 SYELIQPSVSVPAGQTARISCSGDLGDKYASHWYQKPQAPVLYDSDRPSGIPER60

Query: 61 FSGSNSTALTSTGQAEDEADYYCQ88
       FSGSNSTALTSTGQAEDEADYYCQ88
Sbjct: 61 FSGSNSTALTSTGQAEDEADYYCQ88

SUBSTITUTE SHEET (RULE 26)
Selecting sequence(s) using profile Hidden Markov Model derived from a structure family

Figure 12A

A clustered Structure family

A structure-based multiple sequence alignment

Build the HMM model and calibrate the model
Search the germline and rearranged sequence database

The top hits are ranked, analyzed, pooled
Sequence profile of VH framework region for one structurally clustered family of (1dha, 1dho, 1dhw)

Figure 12B
Sequence/variant profile of CDR1 of 4 kappa VL

Structure-based multiple sequence alignment

1DGX:L  RASQGIS------SYLAW
1DH5:L  RASQSVSS------SYLAW
1DH4:L  RASSQLHNSGY-NYLDW
1DH6:L  RASSQVLSSNKNNYLAW

Score hit and/or combinatorial sequences for structural compatibility
Build HMM and search Kabat database
Hit sequences and variant profile

5% cutoff of occurrence frequency
FIGURE 13A
Structure-Based Clustering of the Hucal Library

a) The structure-based clustering of the VH chains
(1DGA, 1DHO, 1DHW), 1DHQ, 1DHU, 1DHV, 1DHZ

b) The structure-based clustering of 7 VL chains
----------Kappa---------- -----Lamda-------
((1DGX, 1DH5), (1DH4, 1DH6)) (1DH7, 1DH8, 1DH9)
FIGURE 13B
Structure-Based Multiple Sequence Alignment for the Clustered Structure Family Established by Using CE Algorithm

a) For 1DGA, 1DHO and 1DHW of 3 VH

1DHA:H QVQLVQSGAEVKPGSPSVKVSCKASGVTFFS---YISWVRQAPGQGLEWMIQIYP-IFGT  
1DHO:H QVQLVQSGAEVKPGSPSVKVSCKASGVTFFS---YISWVRQAPGQGLEWMIQIYP-NSG  
1DHW:H EVQLVQSGAEVKPGSPSVKVSCKASGVTFFS---YISWVRQAPGQGLEWMIQIYP-GDS  

1DHA:H AYWAQQFQGRVITADESTSTAYMELSLSRSEDTAVYCARWGGDGFRAMDYNWQQTLTVYSS  
1DHO:H NYAQQFQGRVITADESTSTAYMELSLSRSEDTAVYCARWGGDGFRAMDYNWQQTLTVYSS  
1DHW:H TRYSRFSFQGVVTISADVSITAYLQWSSLLKASDTAMYCARWGGDGFRAMDYNWQQTLTVYSS  

b) For 1DGX and 1DH5 of 2 kappa VL

1DGX:L DIQMTQSPSSLSASVGDRVTITCRASQG---S-----SYLAWYQQKPGKPKLLI  
1DH5:L DIQMTQSPSSLSASVGDRVTITCRASQG---S-----SYLAWYQQKPGKPKLLI  

1DGX:L YASSLQGQPSRGSRLGEDFTLTISSLQPEDFATYYCQKYTVKPSVFPLPQGDKVEIK  
1DH5:L YGASSRATGVPSRGSRLGEDFTLTISSLQPEDFATYYCQKYTVKPSVFPLPQGDKVEIK  

c) For 1DH4 and 1DH6 of 2 kappa VL

1DH4:L DIQMTQSPSSLSASVGDRVTITCRASQG---S-----SYLAWYQQKPGKPKLLI  
1DH6:L DIQMTQSPSSLSASVGDRVTITCRASQG---S-----SYLAWYQQKPGKPKLLI  

1DH4:L YLGSNRAQGVPRFRGSGSGSTSLFRTKISERVEAEQTVQYCEQHYTTPFTGKVEIK  
1DH6:L YQASTRSGQVFPRFRGSGSGSTSLFRTKISERVEAEQTVQYCEQHYTTPFTGKVEIK  

d) For 1DH7, 1DH8 and 1DH9 of 3 lambda VL

1DH7:L QSYLTVQPS-VSGAPQGRVTISCGSNGS---SLYVENQYOQQLQAPKLIYDNNQPRSG  
1DH8:L QSYLTVQPS-VSGAPQGRVTISCGSNGS---SLYVENQYOQQLQAPKLIYDNNQPRSG  
1DH9:L SYELTVQPS-VSGAPQGRVTISCGSNGS---SLYVENQYOQQLQAPKLIYDNNQPRSG  

1DH7:L VPDRFGSKGTSASLAIATGLQSEDAEYYCQHLYTTPPVTGKTLTVLG  
1DH8:L VSMRFSGSKSNTASLIGSLQAEDAEDYCYQHLYTTPPVTGKTLTVLG  
1DH9:L IPEFSGNSGNTATLSGTQEAEDAEDYCYQHLYTTPPVTGKTLTVLG
# FIGURE 13C

Top Hits of Human Germline Antibody Sequences that Adopt the Target Structure or Scaffold of the reclustered Hucal family by Using the Profile HMM Method

1) For the clustered structure family for 1DHA, 1DHO and 1DHW of VH, the top hits are listed below:

The sequences (1DHA:H, 1DHO:H and 1DHW:H) used to build the HMM are aligned with its top hits from germline sequences using HMMER 2.1.1

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Description</th>
<th>Score</th>
<th>E-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP-73/V5-51...+</td>
<td></td>
<td>232.9</td>
<td>4.5e-68</td>
<td>1</td>
</tr>
<tr>
<td>VHVCW/COS-24+</td>
<td></td>
<td>232.9</td>
<td>4.5e-68</td>
<td>1</td>
</tr>
<tr>
<td>DP-88/hv1051K...+</td>
<td></td>
<td>232.1</td>
<td>8.1e-68</td>
<td>1</td>
</tr>
<tr>
<td>DP-10/hv1051...+</td>
<td></td>
<td>231.4</td>
<td>1.3e-67</td>
<td>1</td>
</tr>
<tr>
<td>2M27/11M27...</td>
<td></td>
<td>231.4</td>
<td>1.3e-67</td>
<td>1</td>
</tr>
<tr>
<td>VHGL1.8</td>
<td></td>
<td>231.4</td>
<td>1.3e-67</td>
<td>1</td>
</tr>
<tr>
<td>VHJ8</td>
<td></td>
<td>229.8</td>
<td>3.9e-67</td>
<td>1</td>
</tr>
<tr>
<td>VHGLL.2</td>
<td></td>
<td>229.8</td>
<td>3.9e-67</td>
<td>1</td>
</tr>
<tr>
<td>RR_VH1.2</td>
<td></td>
<td>229.4</td>
<td>8e-67</td>
<td>1</td>
</tr>
<tr>
<td>6M27</td>
<td></td>
<td>229.3</td>
<td>5.6e-67</td>
<td>1</td>
</tr>
<tr>
<td>VH251Shen+</td>
<td></td>
<td>229.1</td>
<td>6.2e-67</td>
<td>1</td>
</tr>
<tr>
<td>DP-75/VI-2...+</td>
<td></td>
<td>229.0</td>
<td>6.8e-67</td>
<td>1</td>
</tr>
</tbody>
</table>

The top hits to the profile HMM are aligned to the original structure-based sequence alignment are shown below.

1DHA:H    QVQLVQSGAEVKPGGSSVKVSCKASGGTFTSSYAIISRVRQPAGGQGLEWMGG
1DHO:H    QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
1DHW:H    EVQLVQSGAEVKPGKSLKISCKGSGYFTSFWQVRQAPAGGQGLEWGG

DP-73/V5-51...+    EVQLVQSGAEVKPGGSLKISCKGSGYFTSFWQVRQAPAGGQGLEWGG
VHVCW/COS-24+     EVQLVQSGAEVKPGGSLKISCKGSGYFTSFWQVRQAPAGGQGLEWGG
DP-88/hv1051K...+ QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
DP-10/hv1051...+  QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
2M27/11M27...     QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
VHGL1.8         QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
VHJ8            QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
VHGLL.2         QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
6M27            QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG
VH251Shen+      QVQLVQSGAEVKPGGSSVKVSCKASGTQFTSSYAIISRVRQAPAGGQGLEWGG

1DHA:H    IIIPITDTANYAQKFQQIVFTITATDESTSTAYMESSLRLSDETDAYYCAR
1DHO:H    INPNSGTTNYAQKFQGTVMTRDKSSSTAYMESSLRLSDETDAYYCAR
1DHW:H    IYPGDSDTYRSFSPQFQVTISADKSIYATLAYQWSSKLASKADTMYCAR

DP-73/V5-51...+    IYPGDSDTYRSFSPQFQVTISADKSIYATLAYQWSSKLASKADTMYCAR
VHVCW/COS-24+     IYPGDSDTYRSFSPQFQVTISADKSIYATLAYQWSSKLASKADTMYCAR
DP-88/hv1051K...+ QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR
DP-10/hv1051...+  QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR
2M27/11M27...     QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR
VHGL1.8         QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR
VHJ8            QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR
VHGLL.2         QVQIFITDANYAQKFQGVRFTITATDESTSTAYMESSLRLSDETDAYYCAR

SUBSTITUTE SHEET (RULE 26)
2) For 1DGX and 1DH5

Scores for complete sequences (score includes all domains):

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Description</th>
<th>Score</th>
<th>E-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPK9/O12+</td>
<td></td>
<td>228.8</td>
<td>1.5e-67</td>
<td>1</td>
</tr>
<tr>
<td>DPK2/125+</td>
<td></td>
<td>222.1</td>
<td>2.7e-65</td>
<td>1</td>
</tr>
<tr>
<td>V268</td>
<td></td>
<td>213.3</td>
<td>3e-65</td>
<td>1</td>
</tr>
<tr>
<td>V1b+</td>
<td></td>
<td>221.2</td>
<td>1.4e-64</td>
<td>1</td>
</tr>
<tr>
<td>L10a+</td>
<td></td>
<td>218.2</td>
<td>2.3e-64</td>
<td>1</td>
</tr>
<tr>
<td>DPK5/Vb+</td>
<td></td>
<td>218.2</td>
<td>2.3e-64</td>
<td>1</td>
</tr>
<tr>
<td>DPK6/Vb'</td>
<td></td>
<td>218.2</td>
<td>2.3e-64</td>
<td>1</td>
</tr>
</tbody>
</table>

3) For 1DH4 and 1DH6, the top hits from human germline database

Scores for complete sequences (score includes all domains):

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Description</th>
<th>Score</th>
<th>E-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPK24/Vk</td>
<td></td>
<td>251.0</td>
<td>3.1e-74</td>
<td>1</td>
</tr>
<tr>
<td>DPK15/A19</td>
<td></td>
<td>236.0</td>
<td>9.8e-70</td>
<td>1</td>
</tr>
<tr>
<td>DPK13/O11</td>
<td></td>
<td>205.9</td>
<td>1.2e-61</td>
<td>1</td>
</tr>
<tr>
<td>DPK3/V21</td>
<td></td>
<td>201.5</td>
<td>2.5e-59</td>
<td>1</td>
</tr>
<tr>
<td>DPK12/A2+</td>
<td></td>
<td>199.1</td>
<td>1.3e-58</td>
<td>1</td>
</tr>
<tr>
<td>A2b/A2c+</td>
<td></td>
<td>198.7</td>
<td>1.7e-58</td>
<td>1</td>
</tr>
<tr>
<td>DPK27/A29</td>
<td></td>
<td>196.4</td>
<td>7.8e-58</td>
<td>1</td>
</tr>
<tr>
<td>A1b+</td>
<td></td>
<td>196.5</td>
<td>9.5e-58</td>
<td>1</td>
</tr>
<tr>
<td>DPK18/A17+</td>
<td></td>
<td>196.0</td>
<td>1.1e-57</td>
<td>1</td>
</tr>
<tr>
<td>A13+</td>
<td></td>
<td>194.8</td>
<td>2.6e-57</td>
<td>1</td>
</tr>
<tr>
<td>DPK19/A1+</td>
<td></td>
<td>190.7</td>
<td>4.3e-56</td>
<td>1</td>
</tr>
<tr>
<td>DPK28/A18+</td>
<td></td>
<td>189.8</td>
<td>8.3e-56</td>
<td>1</td>
</tr>
<tr>
<td>DPK16/A21+</td>
<td></td>
<td>188.1</td>
<td>2.7e-55</td>
<td>1</td>
</tr>
</tbody>
</table>

4) For 1DH7, 1DH8 and 1DH9, the top hits from human germline database

Scores for complete sequences (score includes all domains):

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Description</th>
<th>Score</th>
<th>E-value</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPL12+</td>
<td></td>
<td>174.8</td>
<td>5.7e-51</td>
<td>1</td>
</tr>
<tr>
<td>2e.2.1/V1-3+</td>
<td></td>
<td>174.8</td>
<td>5.7e-51</td>
<td>1</td>
</tr>
<tr>
<td>2a.2.272A12/DPL11+</td>
<td></td>
<td>174.3</td>
<td>8e-51</td>
<td>1</td>
</tr>
<tr>
<td>2c.118D9/V1-2+</td>
<td></td>
<td>171.6</td>
<td>5.1e-50</td>
<td>1</td>
</tr>
<tr>
<td>VL2.1(1GLV281)+</td>
<td></td>
<td>170.2</td>
<td>1.4e-49</td>
<td>1</td>
</tr>
<tr>
<td>2h2.400B5+</td>
<td></td>
<td>169.9</td>
<td>1.7e-49</td>
<td>1</td>
</tr>
<tr>
<td>1v2046</td>
<td></td>
<td>169.7</td>
<td>1.9e-49</td>
<td>1</td>
</tr>
<tr>
<td>2d.29D11/DPL13+</td>
<td></td>
<td>169.2</td>
<td>2.7e-49</td>
<td>1</td>
</tr>
<tr>
<td>1v216.21</td>
<td></td>
<td>169.1</td>
<td>2.9e-49</td>
<td>1</td>
</tr>
<tr>
<td>DPL10/V1-7+</td>
<td></td>
<td>167.3</td>
<td>9e-49</td>
<td>1</td>
</tr>
</tbody>
</table>
Abmaxis.706.ST25.txt
SEQUENCE LISTING

<110> Abmaxis, Inc.
       Luo, Peizhi

<120> STRUCTURE-BASED CONSTRUCTION OF HUMAN ANTIBODY LIBRARY

<130> 26050-706

<150> US 60/284,407
<151> 2001-04-17

<160> 28

<170> PatentIn version 3.1

<210> 1
<211> 120
<212> PRT
<213> Artificial Sequence

<220>
<223> Human consensus antibody heavy chain variable region
<400> 1

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1       5        10        15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Tyr
20      25        30

Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35      40        45

Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50      55        60

Page 1
Abmaxis.706.Str25.txt

Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120

<210> 2

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Human consensus antibody heavy chain variable region

<400> 2

Gln Val Gln Leu Val Gln Ser Gly Ala Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Glu Trp Met
35 40 45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50 55 60

Gln Gly Arg Val Thr Met Thr Arg Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ser
115 120
Abmaxis.706.ST25.txt

<210> 3
<211> 120
<212> PRT
<213> Artificial Sequence

<220>

<223> Human consensus antibody heavy chain variable region

<400> 3

Gln Val Gln Leu Lys Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1      5
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20     25 30
Gly Val Gly Val Gly Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35     40 45
Trp Leu Ala Leu Ile Asp Trp Asp Asp Asp Lys Tyr Tyr Ser Thr Ser
50     55 60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65     70 75  80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85     90 95
Cys Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly
100    105 110
Gln Gly Thr Leu Val Thr Val Ser
115    120

<210> 4
<211> 120
<212> PRT
<213> Artificial Sequence

<220>

<223> Human consensus antibody heavy chain variable region
<400>  4
Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly 1
     5     10     15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr 20
     25    30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val 35
     40    45
Ser Ala Ile Ser Gly Ser Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Val 50
     55    60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr 65
     70    75    80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys 85
     90    95
Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln 100
     105   110
Gly Thr Leu Val Thr Val Ser Ser 115
     120

<210>  5
<211> 119
<212> PRT
<213> Artificial Sequence

<220>
<223> Human consensus antibody heavy chain variable region
<400>  5
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu 1
     5     10     15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Ser Tyr 20
     25    30
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile 35
     40    45

Page 4
Abmaxis.706.ST25.txt

Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50 55 60

Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65 70 75 80

Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85 90 95

Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Leu Val Thr Val Ser Ser
115

<210> 6

<211> 120

<212> PRT

<213> Artificial Sequence

<220>

<223> Human consensus antibody heavy chain variable region

<400> 6

Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1 5 10 15

Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Tyr
20 25 30

Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35 40 45

Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50 55 60

Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65 70 75 80

Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys
85 90 95

Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr Trp Gly Gln
100 105 110
Abmaxis.706.ST25.txt

Gly Thr Leu Val Thr Val Ser Ser

<210> 7
<211> 123
<212> PRT
<213> Artificial Sequence

<220>

<223> Human consensus antibody heavy chain variable region
<400> 7

Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1     5       10       15

Thr'Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn
20   25       30

Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Gly Arg Gly Leu Glu
35   40       45

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
50   55       60

Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
65   70       75       80

Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
85   90

Tyr Tyr Cys Ala Arg Trp Gly Gly Asp Gly Phe Tyr Ala Met Asp Tyr
100  105      110

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser

<210> 8
<211> 108
<212> PRT
<213> Artificial Sequence
Abmaxis.706.ST25.txt

<220>
<223> Human consensus antibody light chain variable region
<400> 8
Asp Ile Glu Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1  5 10 15

Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Ser Tyr
20 25 30

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

Tyr Ala Ala Ser Ser Leu Glu Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80

Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro
85 90 95

Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100 105

<210> 9
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> Human consensus antibody light chain variable region
<400> 9
Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1  5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro
Abmaxis.706.str25.txt

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65
Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Gln Gln His
85
Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100
Arg

<210> 10
<211> 109
<212> PRT
<213> Artificial Sequence

<220>
<223> Human consensus antibody light chain variable region
<400> 10
Asp Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1
Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
5
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
20
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Ser
25
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
35
Ile Tyr Gln Gly Ala Ser Ser Arg Ala Thr Gln Val Pro Ala Arg Phe Ser
50
Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gln Val Pro Ala Arg Phe Ser
55
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
65
Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu
70
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro
85
Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg
100
105
Human consensus antibody light chain variable region

Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1  5  10  15
Glu Arg Ala Thr Ile Asn Cys Arg Ser Ser Gln Ser Val Leu Tyr Ser
20 25 30
Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45
Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85 90 95
His Tyr Thr Thr Pro Pro Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
100 105 110
Lys Arg
Abmaxis.706.ST25.txt

Gln Ser Val Leu Thr Gln Pro Pro Ser Val Ser Gly Ala Pro Gly Gln
1 5 10 15

Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Ser Asn Ile Gly Ser Asn
20 25 30

Tyr Val Ser Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35 40 45

Ile Tyr Asp Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
50 55 60

Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Leu Gln
65 70 75 80

Ser Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro
85 90 95

Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
100 105

<210> 13

<211> 110

<212> PRT

<213> Artificial Sequence

<220>

<223> Human consensus antibody light chain variable region

<400> 13

Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
1 5 10 15

Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Gly Tyr
20 25 30

Asn Tyr Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
35 40 45

Met Ile Tyr Asp Val Ser Asn Arg Pro Ser Gly Val Ser Asn Arg Phe
50 55 60

Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
65 70 75 80

Page 10
Abmaxis.706.ST25.txt

Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Gln His Tyr Thr Thr
  85     90

Pro Pro Val Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105 110

<210> 14
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> Human consensus antibody light chain variable region
<400> 14

Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ala Pro Gly Gln
  1  5  10  15

Thr Ala Arg Ile Ser Cys Ser Gly Asp Ala Leu Gly Asp Lys Tyr Ala
  20  25  30

Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Val Leu Val Ile Tyr
  35  40  45

Asp Asp Ser Asp Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
  50  55  60

Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Glu
  65  70  75  80

Asp Glu Ala Asp Tyr Tyr Cys Gln Gln His Tyr Thr Thr Pro Pro Val
  85  90  95

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly
 100 105

<210> 15
<211> 98
<212> PRT
<213> Homo sapiens
<400> 15
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser
1  5  10  15
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser Ser Ser Tyr
20  25  30
Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35  40  45
Gly Gly Ile Ile Pro Ile Phe Gly Thr Ala Asn Tyr Ala Gln Lys Phe
50  55  60
Gln Gly Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Ser Thr Ala Tyr
65  70  75  80
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85  90  95
Ala Arg

<210> 16
<211> 98
<212> PRT
<213> Homo sapiens

<400> 16
Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Glu
1  5  10  15
Ser Leu Lys Ile Ser Cys Lys Gly Ser Gly Tyr Ser Phe Thr Ser Ser Tyr
20  25  30
Trp Ile Gly Trp Val Arg Gln Met Pro Gly Lys Gly Leu Glu Trp Met
35  40  45
Gly Ile Ile Tyr Pro Gly Asp Ser Asp Thr Arg Tyr Ser Pro Ser Phe
50  55  60
Gln Gly Gln Val Thr Ile Ser Ala Asp Lys Ser Ile Ser Thr Ala Tyr
65  70  75  80

Page 12
Leu Gln Trp Ser Ser Leu Lys Ala Ser Asp Thr Ala Met Tyr Tyr Cys

85

90

95

Ala Arg

<210>  17
<211>  98
<212> PRT
<213> Homo sapiens

<400>  17
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala
1      5     10     15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Gly Tyr
20    25     30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
35    40     45

Gly Trp Ile Asn Pro Asn Ser Gly Gly Thr Asn Tyr Ala Gln Lys Phe
50    55     60

Gln Gly Arg Val Thr Met Thr Arg Asp Thr Ser Ile Ser Thr Ala Tyr
65    70     75     80

Met Glu Leu Ser Arg Leu Arg Ser Asp Asp Thr Ala Val Tyr Tyr Cys
85    90     95

Ala Arg

<210>  18
<211>  99
<212> PRT
<213> Homo sapiens

<400>  18
Gln Val Thr Leu Arg Glu Ser Gly Pro Ala Leu Val Lys Pro Thr Gln
1      5     10     15
Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
20 25 30
Gly Met Cys Val Ser Trp Ile Arg Gln Pro Pro Gly Lys Ala Leu Glu
35 40 45
Trp Leu Ala Leu Ile Asp Trp Asp Asp Lys Tyr Tyr Ser Thr Ser
50 55 60
Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
65 70 75 80
Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
85 90 95
Cys Ala Arg

<210> 19
<211> 98
<212> PRT
<213> Homo sapiens

<400> 19
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
20 25 30
Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
35 40 45
Ser Ala Ile Ser Gly Ser Gly Ser Thr Tyr Tyr Ala Asp Ser Ser Val
50 55 60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95
Ala Lys
Abmaxis.706.ST25.txt

<210> 20
<211> 97
<212> PRT
<213> Homo sapiens

<400> 20
Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu
1      5      10   15
Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Tyr
20
25
Tyr Trp Ser Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Ile
35
40
45
Gly Tyr Ile Tyr Tyr Ser Gly Ser Thr Asn Tyr Asn Pro Ser Leu Lys
50
55
60
Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser Leu
65
70
75
80
Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Val Tyr Tyr Cys Ala
85
90
95
Arg

<210> 21
<211> 101
<212> PRT
<213> Homo sapiens

<400> 21
Gln Val Gln Leu Gln Gln Ser Gly Pro Gly Leu Val Lys Pro Ser Gln
1      5      10   15
Thr Leu Ser Leu Thr Cys Ala Ile Ser Gly Asp Ser Val Ser Ser Asn
20
25
30
Ser Ala Ala Trp Asn Trp Ile Arg Gln Ser Pro Ser Arg Gly Leu Glu

Trp Leu Gly Arg Thr Tyr Tyr Arg Ser Lys Trp Tyr Asn Asp Tyr Ala
Val Ser Val Lys Ser Arg Ile Thr Ile Asn Pro Asp Thr Ser Lys Asn
Gln Phe Ser Leu Gln Leu Asn Ser Val Thr Pro Glu Asp Thr Ala Val
Tyr Tyr Cys Ala Arg

<210> 22
<211> 95
<212> PRT
<213> Homo sapiens

<400> 22
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
1 5 10 15
Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr
20 25 30
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
35 40 45
Tyr Ala Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65 70 75 80
Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro
85 90 95

<210> 23
<211> 74
<212> PRT
<213> Homo sapiens
<table>
<thead>
<tr>
<th>Protein Sequence</th>
<th>Abmaxis.706.ST25.txt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pro Ala Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Arg Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala Trp Tyr Gln Gln</strong></td>
<td>20</td>
</tr>
<tr>
<td><strong>Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ala Ser Ser Arg</strong></td>
<td>35</td>
</tr>
<tr>
<td><strong>Ala Thr Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp</strong></td>
<td>50</td>
</tr>
<tr>
<td><strong>Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro</strong></td>
<td>65</td>
</tr>
<tr>
<td><strong>&lt;210&gt; 24</strong></td>
<td></td>
</tr>
<tr>
<td><strong>&lt;211&gt; 100</strong></td>
<td></td>
</tr>
<tr>
<td><strong>&lt;212&gt; PRT</strong></td>
<td></td>
</tr>
<tr>
<td><strong>&lt;213&gt; Homo sapiens</strong></td>
<td></td>
</tr>
<tr>
<td><strong>&lt;400&gt; 24</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly</strong></td>
<td>1</td>
</tr>
<tr>
<td><strong>Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser</strong></td>
<td>20</td>
</tr>
<tr>
<td><strong>Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser</strong></td>
<td>35</td>
</tr>
<tr>
<td><strong>Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro</strong></td>
<td>50</td>
</tr>
<tr>
<td><strong>Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile</strong></td>
<td>65</td>
</tr>
<tr>
<td><strong>Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala</strong></td>
<td>85</td>
</tr>
<tr>
<td><strong>Leu Gln Thr Pro</strong></td>
<td>100</td>
</tr>
</tbody>
</table>
Abmaxis.706.ST25.txt

<210> 25
<211> 101
<212> PRT
<213> Homo sapiens

<400> 25
Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu Ala Val Ser Leu Gly
1  5  10  15
Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln Ser Val Leu Tyr Ser
20  25  30
Ser Asn Asn Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35  40  45
Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50  55  60
Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65  70  75  80
Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val Tyr Tyr Cys Gln Gln
85  90  95
Tyr Tyr Ser Thr Pro
100

<210> 26
<211> 89
<212> PRT
<213> Homo sapiens

<400> 26
Gln Ser Val Leu Thr Gln Pro Pro Pro Ser Ala Ser Gly Thr Pro Gly Gln
1  5  10  15
Arg Val Thr Ile Ser Cys Ser Gly Ser Ser Asn Ile Gly Ser Asn
20  25  30
Tyr Val Tyr Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu
35  40  45
Abmaxis.706.ST25.txt

Ile Tyr Ser Asn Asn Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser
 50
Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Ser Gly Leu Arg
 65
Ser Glu Asp Glu Ala Asp Tyr Tyr Cys
 85

<210> 27
<211> 90
<212> PRT
<213> Homo sapiens

<400> 27
Gln Ser Ala Leu Thr Gln Pro Ala Ser Val Ser Gly Ser Pro Gly Gln
 1 5 10 15
Ser Ile Thr Ile Ser Cys Thr Gly Thr Ser Ser Asp Val Gly Ser Tyr
 20 25 30
Asn Leu Val Ser Trp Tyr Gln Gln His Pro Gly Lys Ala Pro Lys Leu
 35 40 45
Met Ile Tyr Glu Val Ser Lys Arg Pro Ser Gly Val Ser Asn Arg Phe
 50 55 60
Ser Gly Ser Lys Ser Gly Asn Thr Ala Ser Leu Thr Ile Ser Gly Leu
 65 70 75 80
Gln Ala Glu Asp Glu Ala Asp Tyr Tyr Cys
 85 90

<210> 28
<211> 88
<212> PRT
<213> Homo sapiens

<400> 28
Ser Tyr Glu Leu Thr Gln Pro Pro Ser Val Ser Val Ser Pro Gly Gln
Thr Ala Ser Ile Thr Cys Ser Gly Asp Lys Leu Gly Asp Lys Tyr Ala
Cys Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Val Leu Val Ile Tyr
Gln Asp Ser Lys Arg Pro Ser Gly Ile Pro Glu Arg Phe Ser Gly Ser
Asn Ser Gly Asn Thr Ala Thr Leu Thr Ile Ser Gly Thr Gln Ala Met
Asp Glu Ala Asp Tyr Tyr Cys Gln
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(7) : G01N 31/00; G06F 19/00
 US CL. : 702/27

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
 U.S. : 702/27

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>KNAPP Et al. Fully Synthetic Human Combinatorial Antibody Libraries (HuCAL) Based on Modular Consensus Frameworks and CDRs Randomized with Triaminoethanes</td>
<td>1-72</td>
</tr>
<tr>
<td>X</td>
<td>IBA et al. Changes in the specificity of antibodies against steroid antigens by introduction of mutations into complementarity-determining regions of the VH domain. Protein Engineering. Issued May 1998, Volume 11, Number 5, pages 361-370, see entire document.</td>
<td>1-6, 35-39, and 58-60</td>
</tr>
</tbody>
</table>

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

Date of the actual completion of the international search

Date of mailing of the international search report
27 AUG 2002

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703)305-3230

Authorized officer
Channing S. Mahalan
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet) (July 1998)
Continuation of B. FIELDS SEARCHED Item 3:
US PATENT FULL, MEDLINE, BIOSIS, BIOTECHDS, CAPLUS, EMBASE
search terms: antibody, combinatorial, library, phage display, immunoglobulin, CDR, framework, computer, 3D, three dimensional, database, protein, crystal structure