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WEBLEM 6
INTRODUCTION TO AG-AB INTERACTION DATABASE (AG-AB DB)

Antibodies are produced by vertebrates in response to antigens. Antigens are usually foreign molecules of invading pathogens. Antibodies are produced in billions of forms by B cells and are collectively referred to as immunoglobulins (abbreviated as Ig). The clonal selection theory states that all the antibodies produced by an individual B cell have the same antigen-binding site. Furthermore, every B cell producesa single species of antibody having a unique antigen-binding site.

An antibody molecule is a polymer of two light and two heavy chains. The two light chains are identical and are of a length of ~220 amino acids each. Similarly, the two heavy chains are identical with a typical length of ~440 amino acids each. The four chains are held together by various noncovalent and covalent (disulfi de) bonds. Every light chain has one variable and one constant region, whereas heavy chains have one variable and two to three constant regions. As a result, two identical antigen-binding sites are formed by the N-terminal variable regions of a pair of light and heavy chains. The tail (Fc) and hinge regions are however formed by the constant regions of two heavy chains. The antigen-binding site of an antibody is referred to as a “paratope”.

There are fi ve classes of antibodies such as IgA, IgD, IgE, IgG, and IgM, which are based on fi ve types of heavy chains such as α, δ, ε, γ, and μ. Each of these heavy chains is known to invoke a specific cascadeof reactions upon binding to an antigen. However, there are only two types of light chains (κ or λ) that pairwith one of the heavy chains. Therefore, the type of light chain does not seem to affect the properties of the antibody, other than its specifi city for the antigen.

Properties of Antigen–Antibody Interactions:
The binding of an antigen to an antibody is reversible, and both the molecules can exist independently. The antigen–antibody iinteractions are thus mediated by many relatively weak, non- covalent forces such as hydrogen bonds, hydrophobic interactions, van der Waals forces, and ionic interactions. Of all the forces, van der Waals forces are the weakest and can attract all kinds of molecules. Hydrogen or ion– dipole bonds are formed between oppositely charged atoms, whereas “hydrophobic” interactions are formed between atoms of nonpolar amino acids which do not form electric dipole. These weak forces are effective only when the antigen molecule is close enough to allow some of its atoms to fi t intocomplementary niches on the surface of the antibodies. The attractive forces exerted by ionic andhydrophobic bonds help the molecules to overcome hydration energies. This leads to the expulsion of water molecules and results in bringing the epitope and paratope closer. This spatial proximity facilitates van der Waals interactions. The overall strength of binding depends on goodness of fit between the epitopeand paratope and the total area of contact between them.

Antigen–antibody interactions are highly specifi c, and understand ing the molecular basis of the specifi city has been one of the goals of immunology. A large number of high-resolution X-ray struc tures of several antigens have been solved in the native (uncompleted) form as well as in complex with antibody, and the data are archived in Protein Data Bank (PDB). Analyses of these struc tures have helped in understanding characteristics of both epitopes (antibody-binding site on antigen) and paratopes (antigen- binding site of antibody), which are complementary to each other and are relational entities.

Ag-Ab Interaction Database (AgAbDb):
Antigen–Antibody Interaction Database (AgAbDb) is an immunoinformatics resource developed at the Bioinformatics Centre, University of Pune, and is available online at http://bioinfo.net.in/AgAbDb.html . Antigen–antibody interactions are a special class of protein-protein interactions that are characterized by high affi nity and strict specifi city of antibodies towards their antigens. Several co-crystal structures of antigen–antibody complexes have been solved and are available in the Protein Data Bank (PDB). AgAbDbis a derived knowledgebase developed with an objective to compile, curate, and analyze determinants of interactions between the respective antigen–antibody molecules. AgAbDb lists not only the residues of binding sites of antigens and antibodies, but also interacting residue pairs. It also helps in the identificationof interacting residues and buried residues that constitute antibody-binding sites of protein and peptide antigens. The Antigen–Antibody Interaction Finder (AAIF), a program developed in-house, is used to compile the molecular interactions, viz. van der Waals interactions, salt bridges, and hydrogen bonds.
A module for curating water- mediated interactions has also been developed. In addition, various residue level features, viz. accessible surface area, data on epitope segment, and secondary structural state of binding site residues, are also compiled. Apart from the PDB numbering, Wu–Kabat numbering and explicit Definitions of complementarity- determining regions are provided for residues of antibodies.

The molecular interactions can be visualized using the program Jmol. AgAbDb can be used as a benchmark dataset to validate algorithms for prediction of B-cell epitopes. It can as well be used to improve accuracy of existing algorithms and to design new algorithms. AgAbDb can also be used to designmimotopes representing antigens as well as aid in designing processes leading to humanization of antibodies. A user-friendly web-enabled interface for AgAbDb (http:// 115.111.37.206:8080/agabdb2) has been designed and tested for all the web browsers. A “quick search box” is provided on all the web pages of the interface. The “quick search” supports the data base search using the PDB ID or the keywords. This in turn opens a page listing the search results. AgAbDb can be browsed by clicking on thePDB ID. The search results page also provides links to view the antigen–antibody interactions archived inAgAbDb using Jmol , to view the corresponding complex at the RCSB PDB site , and to download thefile from the RCSB PDB .

AgAbDb: Data Formats and Displays:
AgAbDb archives data of antigens, antibodies, and molecular interactions under eight categories, viz. Summary, IR: Epitope Paratope, IR: Epitope Segments, Binding Site: IR + BR, Atomic Level Interactions, Water-Mediated Interactions, View Interactions, and Statistics. The tables displaying interaction data under each of these eight categories can be exported as Excel fi les. The complex, binding site residues of antigen and antibody along with subsets of various interactions can be visualized using Jmol (http://www.jmol.org/). The snapshots of screens based on eight categories are shown in FiAgAbDb records for a complex of the antibody. NC10 Fv and neuraminidase from infl uenza virus ([39], PDB ID: 1A14) are shown. AgAbDb uses PDB ID as a unique identifi er to archive interaction data.
This section provides overall information of the complex, the antigen, and the antibody. Data are curated from the PDB and typically lists PDB ID, PubMed ID, resolution, release date, and citation information. The data on antibody includes name, class/ type, scientifi c and common names of the source, and the PDBchain identifiers for light and heavy chains. The data on antigen includes name, scientific and common names of the source, anantigen type (protein or peptide), and the PDB chain identifier.

Epitope–Paratope:
This section lists all the interacting residues of the binding sites. The residues of antibody (paratope) thatare interacting with the residues of antigen (epitope) are provided. For example, the numbers of interacting residues of paratope (NC10 Fv) and epitope (neuraminidase) are 12 and 17, respectively (PDB ID: 1A14). The paratope residues are listed with chain type (heavy or light chain), PDB numbering, and Kabat numbering. It is preferred to have both the numbering systems and their equivalence known as far as antibody numbering is concerned. The table also lists equivalence between the interacting residues of the antigen and antibody. This is one of the unique features of AgAbDb. It is very useful and facilitates interesting analyses as a residue may interact with one or more residues. The residues of both antigen or antibody having minimum and maximum contacts can be identifi ed. For example, Asn400 of the antigen interacts with two residues of CDR2 and one residue of CDR1 of heavy chain. Identifi cation of such impor tant residues or hot spots may have applications in mutation analysis, which is a prerequisite for designing

antigen scaffolds and/or peptide/subunit vaccines. Other immunoinformatics resources, viz. IEDB-3D and IMGT/3Dstructure-DB, do not provide the list of pairs of interacting residues in an explicit fashion. Generation of such a list using these resources calls for processing of the data through multiple steps. The "IR: Epitope-Paratope" table also lists secondary structural states of interacting residues of antigen, which are obtained from DSSP assignments [37]. Analysis pertaining to preference of secondarystructural states of antigens has always been the area of interest and has been used effectively in epitope prediction programs.

Binding Site: IR + BR:
This section lists all the residues of the respective binding sites of the antigens and antibodies. Separate tables for antigen and antibody molecules are generated. In addition to the interacting residues, several residues of epitope are buried under the footprint of an antibody. Such residues are a part of the binding site scaffold and may not directly interact with residues of CDRs and LDRs of an antibody. Similarly, CDR and LDR also have only a few interacting residues while the other residues forming the scaffold, though not interacting explicitly, are used to calculate the area of interface of antibody with antigen.

Atomic Level Interactions:
This section displays various non-covalent interactions between residues of the epitopes and paratopes. For example, NC10 antibody (PDB ID: 1A14) has about 107 non-covalent interactions of the types such as salt bridges (1), hydrogen bonds (7), short van der Waals interactions (2), and van der Waals interactions (97). These interactions are curated using the program AAIF.

Statistics:
This section provides a residue-wise summary of various inter actions. Separate tables are provided forthe antigen (epitope) and antibody (paratope), which list the residues that contribute mamaximallyo the antigen–antibody interactions. This section provides a summary of interactions for every residue and includes data on the total number of interactions, which is a sum of the total number of hydrogen bonds, van der Waals interactions, and salt bridges. The table also lists the total number of residues (from the partner mol ecule) with which a given residue is interacting. This section also helps to quickly enlistwhich of the 20 amino acids are parts of the paratope and epitope. For example, NC10 antibody CDRs have only 7 (S, T, N, F, L, D, Y) amino acids whereas the near aminidase epitope has 11 (S, K, T, N, G,A, D, I Y, P, W) amino acids as characterized in the complex 1A14 .

AgAbDb also helps in analyzing how every CDR partici pates in binding to the epitope. This utility is provided under the "Search" option on the main menu bar. Three CDRs on light chain are termed as LDR 1–3. There are three LDRs (light chain) and three CDRs (heavy chain). Since the PDB numbering may or may not be in accordance with the position of a given residue in sequence and/or Kabat scheme of numbering, AgAbDb provides equivalence between PDB and Kabat numbering. "CDR statistics" for NC10 antibody (PDB ID: 1A14) reveals that two of the six CDRs such as LDR2 and CDR1 do not participate in the antigen binding at all. The LDR1, LDR3, CDR2, and CDR3, respectively, have 2, 4, 3, and 3 residues interacting with various residues of the antigen. Of the 107 total interactions, 25, 34, 27, and 21 interactions are contributed by LDR1, LDR3, CDR2, and CDR3, respectively. Thus, AgAbDb can be used to perform various queries and to study the multiple aspects of antigen–antibody interactions.

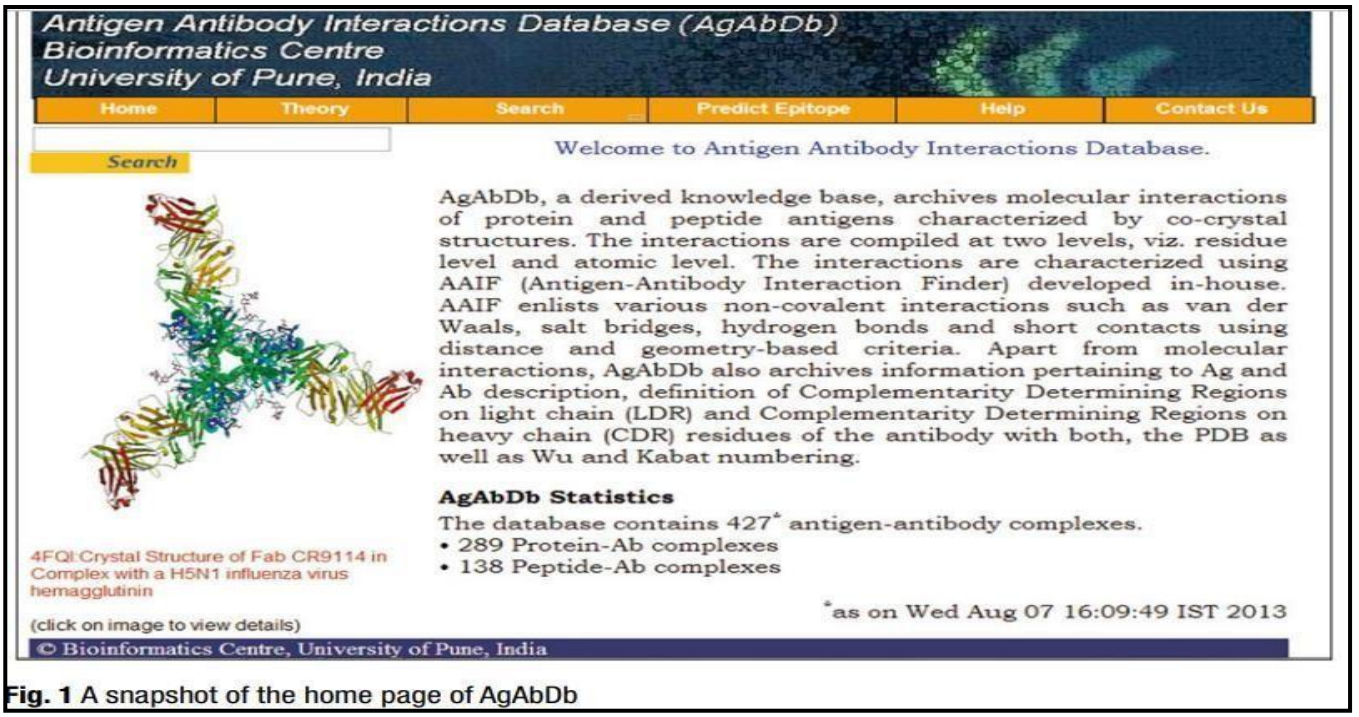


Fig. 1 A snapshot of the home page of AgAbDb

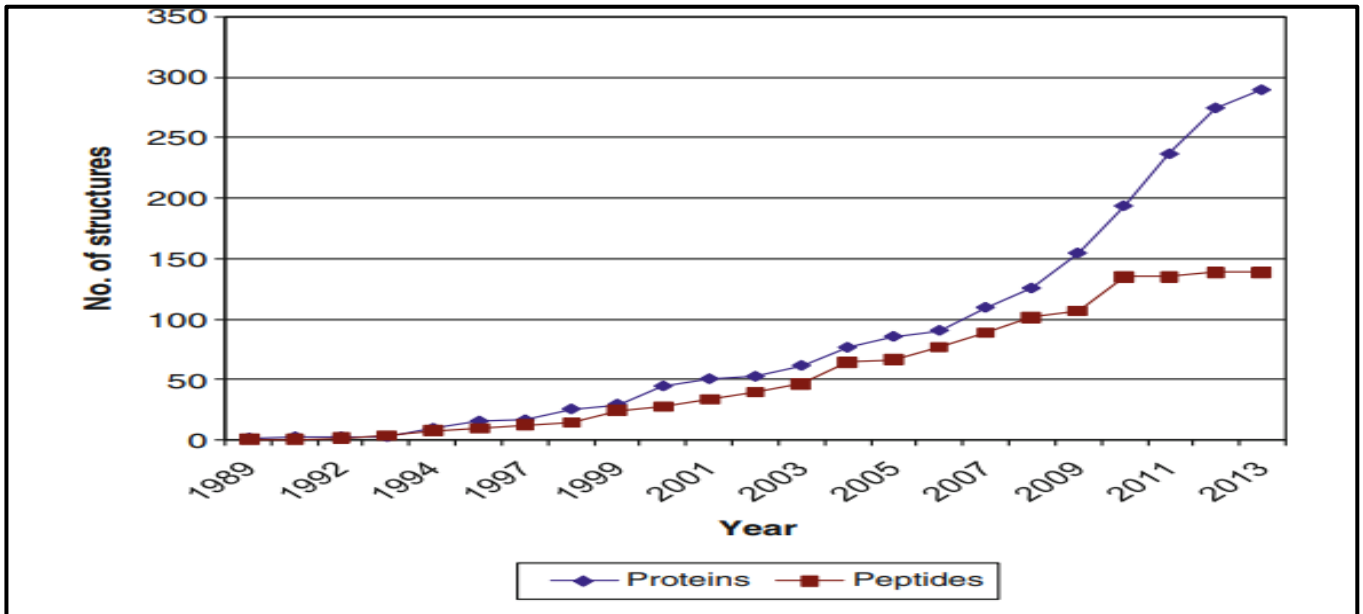
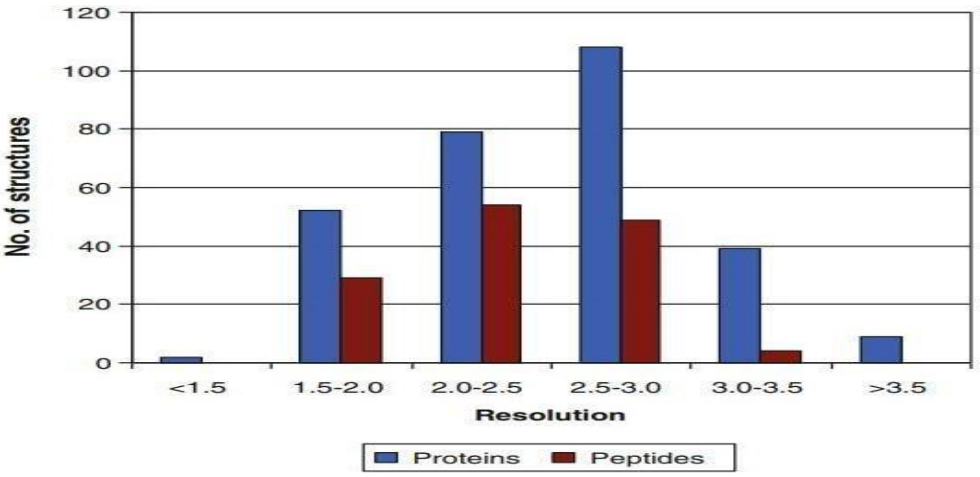


Fig. 2 The growth of co-crystal structures of protein- and peptide–antibody complexes in AgAbDb



Resolution graph for 427 complexes

Fig. 3 Distribution of co-crystal structures of antigen–antibody complexes based on resolution

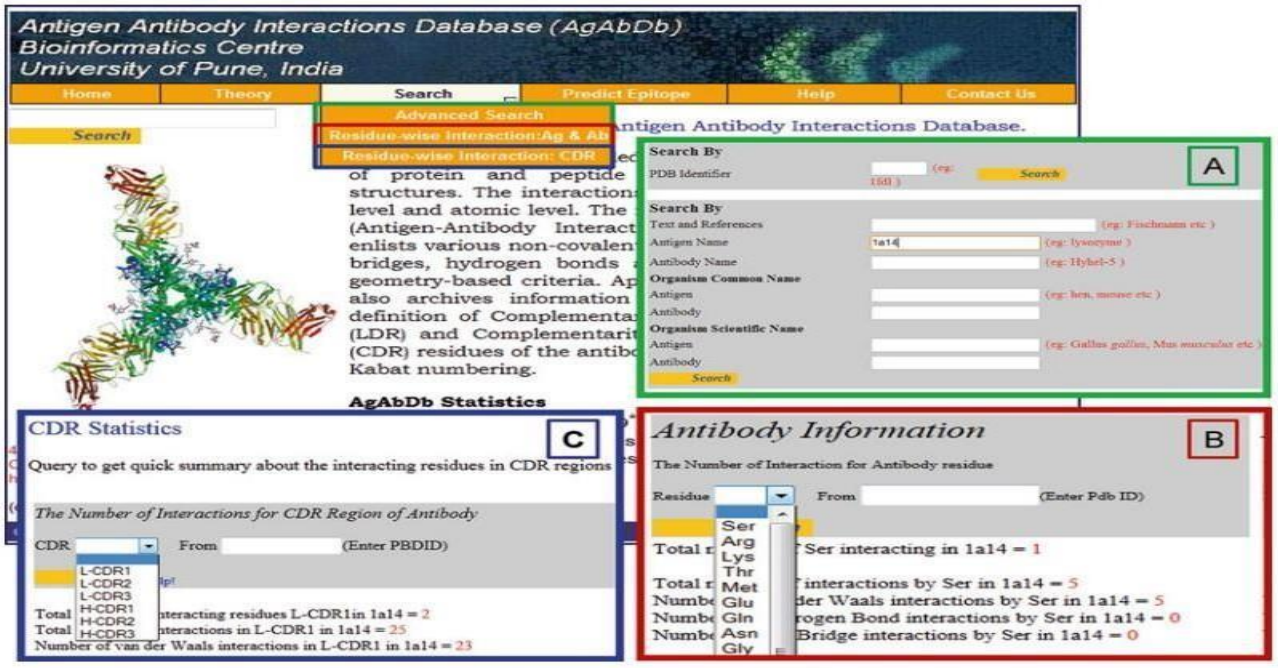


Fig. 4 Snapshots of various search strategies in AgAbDb

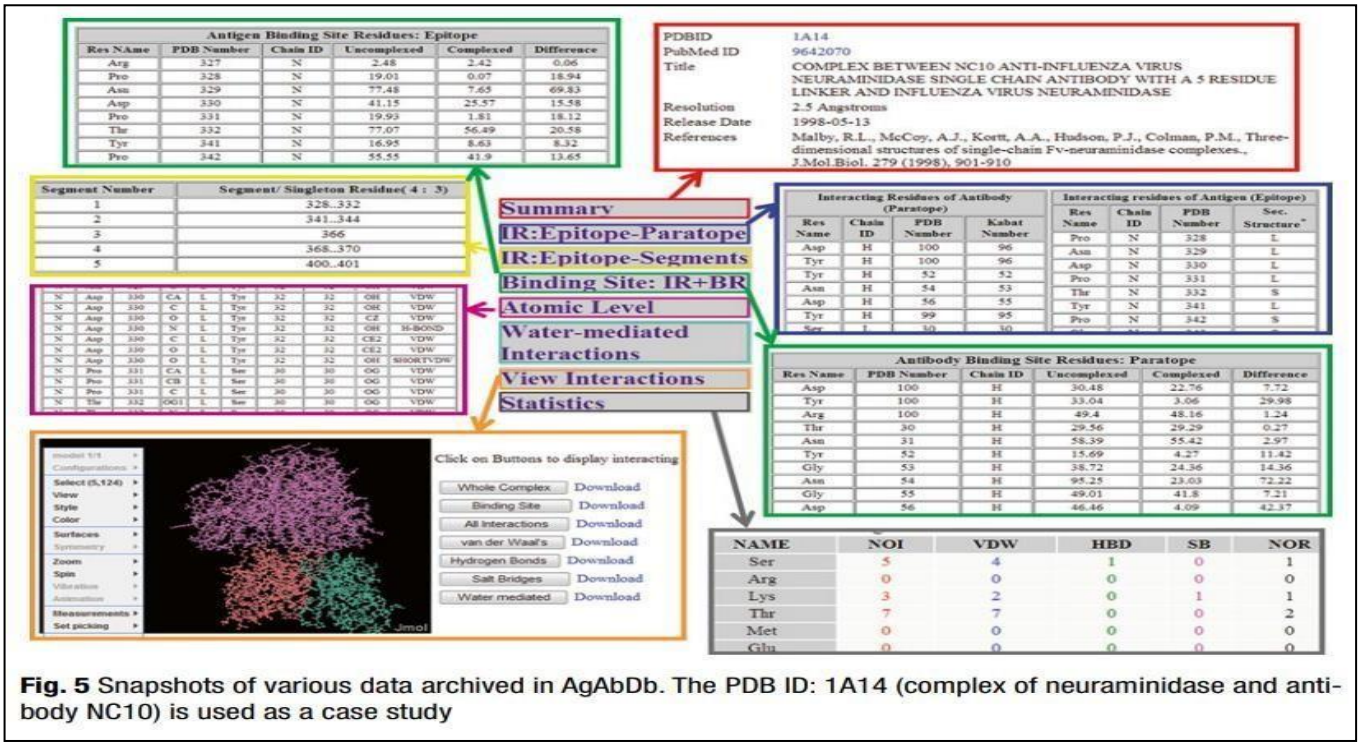


Fig. 5 Snapshots of various data archived in AgAbDb. The PDB ID: 1A14 (complex of neuraminidase and antibody NC10) is used as a case study

RESULT:

Various methods and search analysis were studied while exploring the AG-Ab database. The analysis methods for various Ag-Ab interactions studies in AG-Ab database were

Advanced search: This analysis section gives us a complete summary and information about various interactions such as Atomic, water-mediated, Binding sites, IR- Epitope, Paratope and IR- Epitopesegments. Residue- wise interaction; Ag &Ab: This analysis section gives us a complete information about the Antigen and Antibody Interaction along with the Number of interactions for antibody/antigen residue, Number of van der Waals interactions by residue in the query PDB ID, Number of Hydrogen Bond interactions by residue in the query PDB ID, Number of salt bridge interactions by residue in the query PDB ID in a tabular format. Residue- Wise interaction; CDR: This analysis section gives us a complex information about the number of interactions for CDR regions of Antibody along with a tabular format which contains information about the Antigen and Antibody with respect to information such as the Res name, Chain ID, PDB Number and Kabat Number respectively.

CONCLUSION:

AgAbDb can be used as a benchmark dataset to validate algorithms for prediction of B-cell epitopes. It can as well be used to improve accuracy of existing algorithms and to design new algorithms. AgAbDb can also be used to design mimotopes representing antigens as well as aid in designing processes leading to humanization of antibodies. The Database is currently unavailable as it is under maintenance.

REFERENCE:

Kulkarni-Kale, U., Raskar-Renuse, S., Natekar-Kalantre, G., & Saxena, S. A. (2014). Antigen- Antibody Interaction Database (AgAbDb): a compendium of antigen-antibody interactions. Methods in molecular biology (Clifton, N.J.), 1184, 149–164. https://doi.org/10.1007/978-1-4939-1115-8_8