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DEPARTMENT OF BIOINFORMATICS

CERTIFICATE

This is to certify that Ms. Prarthi Hrishit Kothari (Roll No: 115) of M. Sc. Bioinformatics (Part II) has satisfactorily completed the practical for Elective Paper: Immunoinformatics & Drug Designing (GNKPSBIEL1P503) for Semester III course prescribed by the University of Mumbai during the academic year 2024-2025.

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WEBLEM 1

Introduction to Sequence and Structure Database

INTRODUCTION:

UniProt provides access to a vast collection of protein sequences, including those that are experimentally determined and those that are computationally predicted. The database includes extensive information about the function of proteins, such as their role in biological processes, molecular functions, and involvement in various pathways. UniProt integrates data on the 3D structures of proteins, where available, often linking to related resources like the Protein Data Bank (PDB). It provides cross-references to other biological databases, such as genomic, enzyme, and pathway databases, enabling a broad spectrum of data connectivity. UniProt includes information on different protein isoforms and variants, which are important for understanding protein diversity and function. The database distinguishes between manually curated entries (UniProtKB/Swiss-Prot) and automatically annotated entries (UniProtKB/TrEMBL), providing users with information on the reliability and source of the data.

The UniProt databases support biological and biomedical research by providing a comprehensive collection of protein sequence data, along with functional information. UniProtKB combines expert-reviewed data (Swiss-Prot) with automated entries (TrEMBL). UniRef clusters sequences based on similarity, and UniParc stores all known sequences, including obsolete ones. UniProt links to 180 resources, ensuring data is findable, accessible, interoperable, and reusable (FAIR). Recognized for its data quality, UniProt received the ELIXIR Core Data Resource and CoreTrustSeal certifications. The database continually evolves, adding new sequences from projects like the Darwin Tree of Life, growing by over 65 million entries in two years.

UniProt is the central hub for the collection of functional information on proteins, with accurate, consistent and rich annotation. It consists of two sections:

- 1. Swiss-Prot (Reviewed)** – contains manually annotated records with data added by expert bio-curators giving information on protein function, structure, subcellular location and molecular interactions. Each entry in UniProt/Swiss-Prot represents a single, non-redundant gene from a specific organism and all proteins and peptides transcribed by that gene are described within the record.

- 2. TrEMBL (Unreviewed)** – contains computationally analysed records with additional information transferred from related well annotated records in UniProt/Swiss-Prot (automatic annotation). There may be several separate UniProt/TrEMBL entries describing the proteins derived from a specific gene.

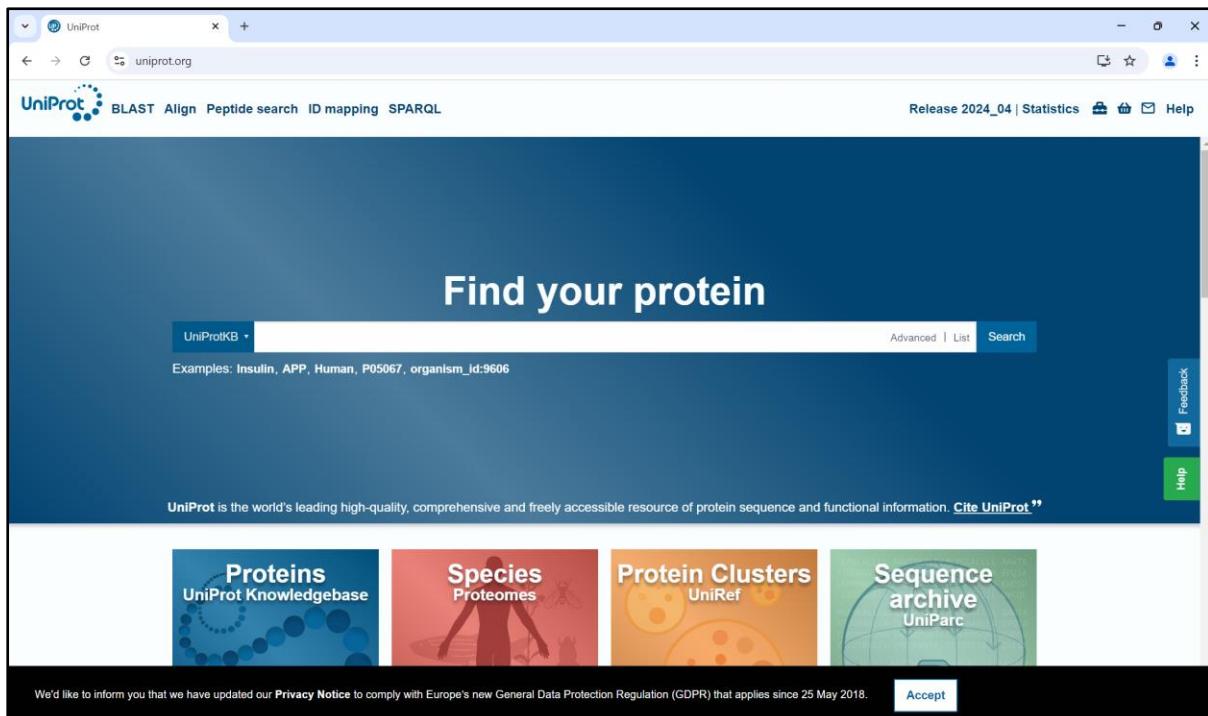


Fig 1: Homepage of UniProt database

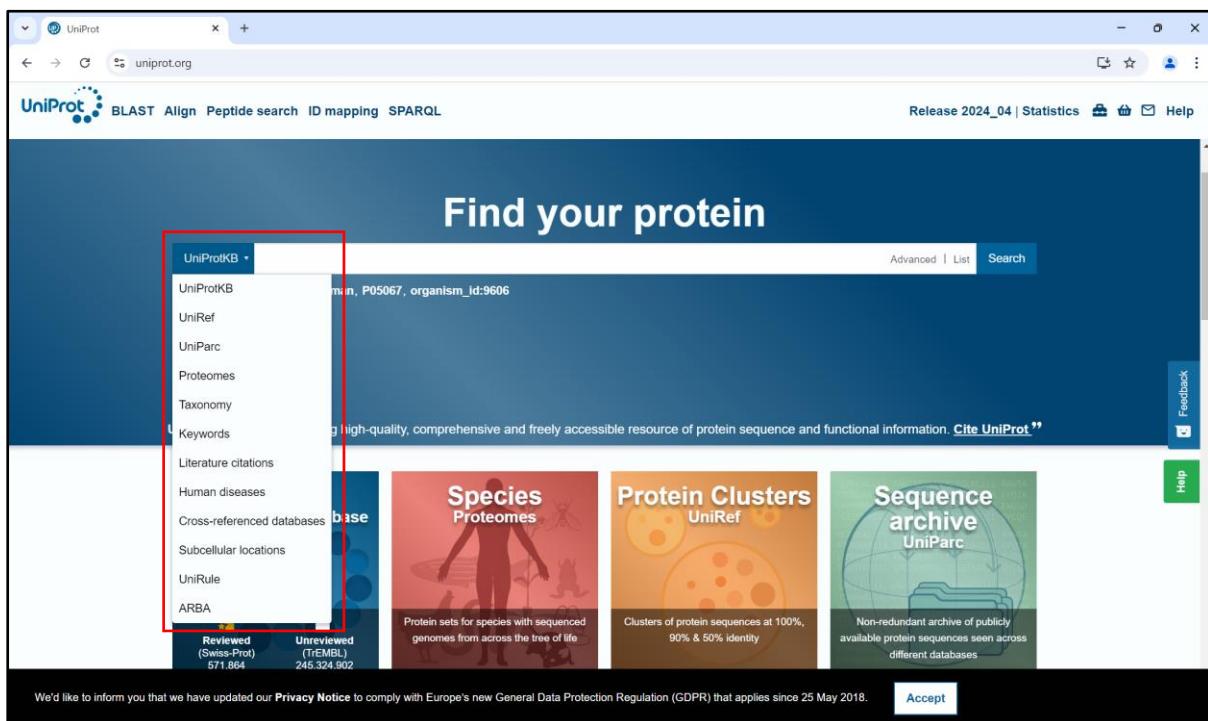


Fig 2: Search options in UniProt database

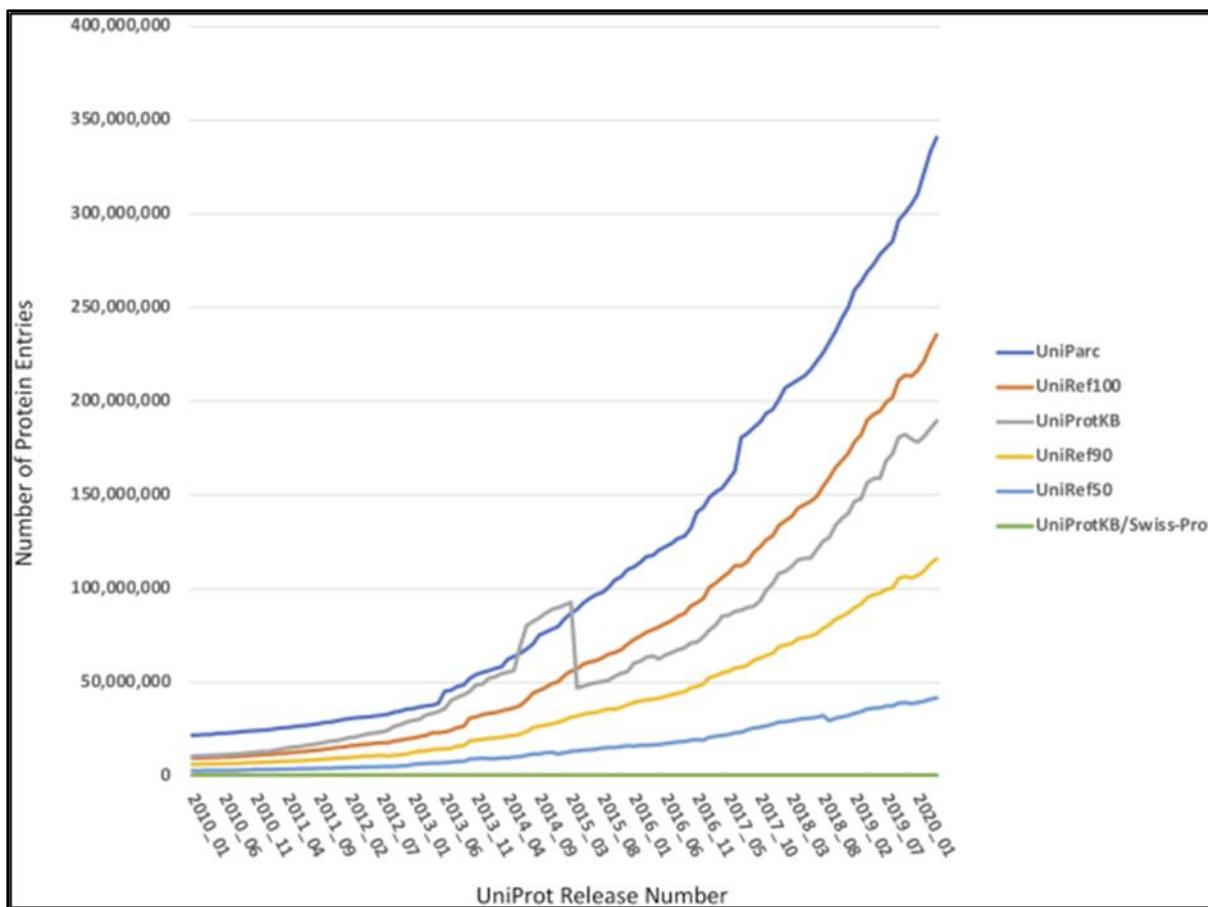


Fig 3: Growth in the number of entries in the UniProt database over the last decade

Applications

- Protein Function Prediction:** UniProt provides functional information about proteins, helping researchers predict the roles of unknown proteins based on sequence data.
- Drug Discovery:** By understanding protein structures and functions, researchers can identify potential drug targets and design therapies, especially in areas like cancer and infectious diseases.
- Genomics and Proteomics Research:** UniProt is essential for annotating genomes and identifying proteins in proteomics studies, aiding in the understanding of cellular functions.
- Evolutionary Studies:** Researchers use UniProt data to study protein evolution and compare protein sequences across species.
- Disease Research:** UniProt helps link genetic mutations to protein functions, assisting in the study of genetic disorders and personalized medicine.
- Metagenomics and Environmental Studies:** UniProt data is used to analyze microbial communities in environments, supporting research in biodiversity and ecosystem functioning.

Introduction to Structure Databases

INTRODUCTION:

Structural bioinformatics, a branch of bioinformatics, is related to the analysis and prediction of the three-dimensional structure of biological macromolecules such as proteins, RNA, and DNA. The main objective of structural bioinformatics is to create new methods for analyzing and manipulating biological macromolecular data to solve problems in biology and generate new insights.

Structural databases in bioinformatics are crucial resources that are modelled around experimentally determined protein structures, providing the biological community with access to valuable experimental data in a useful way. These databases aim to organize and annotate protein structures, and they often include three-dimensional coordinates, experimental information (such as unit cell dimensions and angles for x-ray crystallography determined structures), and sequence information. The primary attribute of a structure database is structural information, whereas sequence databases focus on sequence information and contain no structural information for the majority of entries. Protein structure databases are critical for many efforts in computational biology, such as structure-based drug design, and they are used to provide insights about the function of proteins.

Prominent examples of structural databases include the Protein Data Bank (PDB), which contains experimentally determined three-dimensional structures of biomolecules, the Nucleic Acid Data Base (NDB), which contains experimentally determined information about nucleic acids, the carbohydrate structure databases (CSDB) ,which providing a curated repository of structural, taxonomical, bibliographic, and NMR-spectroscopic data on natural carbohydrates and carbohydrate-related molecules from bacterial, fungal, and plant origins, the Reactome databases which provides information about metabolic pathways, the PDBSum databases provides a pictorial summary and detailed analyses of 3D macromolecular structures deposited in the Protein Data Bank, the PDBTM databases provides information about transmembrane proteins from the PDB, the CATH classifies protein domains based on their architecture, topology, and homology and the Structural Classification of Proteins (SCOP), which provides a comprehensive description of the structural and evolutionary relationships between structurally known proteins. These examples are introduced in detail below.

A. Protein Data Bank (PDB) Databases:

Protein data bank is an online structural library of biological macromolecules, which is the only worldwide repository of macromolecular structure. The PDB was organized in 1971 at Brookhaven National Laboratories (BNL) as a platform of crystal structures of biomolecules. Over the years, the data submitted to the PDB was modified and approaches to access the PDB have changed, as a result of advancements in technology.

In October 1998, Research Collaborator for Structural Bioinformatics (RCSB) has started to manage and maintain the activities of PDB. The major task of the RCSB is to generate such measures that allow the use and analysis of structural data. PDB stores 3D structural information of biological molecules mainly nucleic acid and proteins. The structural information of biomolecules is commonly acquired experimentally by NMR spectroscopy, X-ray crystallography, electron microscopy etc. Structural information of some chemical ligands and nucleotides are also available on PDB. PDB ID is a four-character identifier that is actually entitled as PDB entry. A Searching through PDB is done by a vast range of search engines ranges from PDB ID and keywords to structural features of proteins and other biomolecules.

There are two formats that PDB uses to keep structural data: The PDB file format and macromolecular crystallographic information file format (mmCIF). PDB file design is more commonly used in protein community as compared to mmCIF. PDB offers various molecular structural visualization softwares including RasMol, Jmol, PDB simple viewer, PDB protein workshop and RCSB-Kiosk. Structural confirmation of secondary structure is also provided by PDB. The PDB depository is run by an association, named the Worldwide Protein Data Bank (wwPDB) which guarantees that the information is freely accessible to the public. Structures for huge numbers of the proteins and nucleic acids required in the central procedures of life are available on PDB.

B. PDB file format:

The Protein Data Bank (PDB) file format is a standard for files containing atomic coordinates of biological macromolecules. The PDB file format consists of lines of information in a text file, with each line of information in the file called a Record. A PDB file generally contains several different types of records, arranged in a specific order to describe a structure. The most common record types include:

1. **ATOM:** atomic coordinate record containing the X, Y, Z orthogonal Å coordinates for atoms in standard residues (amino acids and nucleic acids).
2. **HETATM:** atomic coordinate record containing the X, Y, Z orthogonal Å coordinates for atoms in non-standard residues (ligands, cofactors, etc.).
3. **TER:** record indicating the end of a chain of residues.
4. **HEADER:** record containing general details about the molecules in the file, as well as the experiment(s) used to elucidate their structures.
5. **COMPND:** record containing information about the compound, including its name, synonyms, and other identifiers.
6. **REMARK:** record containing additional information about the structure, such as refinement details, experimental conditions, and other annotations.

The formats of these record types are given in the PDB file specification. The PDB file format is limited to 80 columns per line, with each line terminated by an end-of-line indicator. The columns in the PDB file format for the ATOM record type include the atom serial number, atom name, residue name, chain identifier, residue sequence number, and atomic coordinates. The HETATM record type is similar to the ATOM record type, but is used for non-standard residues. The TER record type indicates the end of a chain of residues. The HEADER, COMPND, and REMARK record types contain general information about the structure, such as the name of the molecule, the authors of the structure, and the method of structure determination.

C. Nucleic Acid Knowledgebase (NAKB) Databases:

The Nucleic Acid Database (NDB) played a pivotal role as the first comprehensive resource for three-dimensional (3D) structures of nucleic acids. Established in the 1990s at Rutgers University, NDB facilitated collaborative studies through a SQL-relational database, offering curated information from X-ray and nuclear magnetic resonance (NMR) experiments. Over its three-decade tenure, NDB evolved to become a valuable repository, collecting data from the Protein Data Bank (PDB) and the Cambridge Structural Database (CSD).

In response to the growing landscape of nucleic acid structures and emerging technologies like cryo electron microscopy (EM), the Nucleic Acid Knowledgebase (NAKB) emerged as the modern successor to NDB. Initiated in 2019 and officially launched in May 2023, NAKB aimed to preserve and enhance NDB's functionality while incorporating structures from diverse methods, providing comprehensive functional and structural annotations, and establishing links to broader nucleic acid-focused resources.

NAKB provides search, report, statistics, atlas and visualization pages for all nucleic-acid containing experimentally determined 3D structures held by NDB and by the Protein Data Bank (PDB), including all major methods: X-ray, NMR, and Electron Microscopy. For each structure, links are provided to external resources that annotate and analyze nucleic acid structures and their complexes.

The NAKB website (nakb.org), introduced in July 2022, offers efficient search tools, tabular reports, 2D and 3D structure visualizations, educational content, standards information, and a curated nucleic acid community web and software resource list. With a user-friendly interface and modern web architecture, NAKB ensures an enhanced experience for users, supporting accessibility on both large and small devices. The website undergoes weekly updates, maintaining its commitment to providing timely and relevant nucleic acid structural information. Notably, NDB was officially retired in July 2023, marking the seamless transition to the advanced capabilities of NAKB in serving the scientific community.

NOTE: NAKB replaces the Nucleic Acid Database (NDB) resource that will be retired in July 2023.

D. Carbohydrate Structure Database (CSDB)/ CCSD /Gly-Tou-Can Database:

The Carbohydrate Structure Database (CSDB) is a free curated database and service platform in glycoinformatics, launched in 2005 by a group of Russian scientists from N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences. The database aims to provide structural, bibliographic, taxonomic, NMR spectroscopic, and other information on glycan and glycoconjugate structures of prokaryotic, plant, and fungal origin. It serves as a platform for multiple glycoinformatic studies and web tools.

CSDB covers nearly all structures published up to the previous year in the scope of bacterial carbohydrates. Prokaryotic, plant, and fungal mean that a glycan was found in the organisms belonging to these taxonomic domains or was obtained by modification of those found in these organisms. Carbohydrate means a structure composed of any residues linked by glycosidic, ester, amidic, ketal, phospho- or sulpho-diester bonds in which at least one residue is a sugar or its derivative, except DNA/RNA.

The main source of data is retrospective literature analysis. About 20% of data were imported from CCSD (Carbbank, University of Georgia, Athens; structures published before 1996) with subsequent manual curation and approval. CSDB contains manually curated natural carbohydrate structures, taxonomy, bibliography, NMR, and other data from literature. Coverage is close to complete up to the year 2020 for bacterial and fungal carbohydrates. Users can search the database by IDs, bibliographic data and keywords, biological source, structural fragments, and NMR data. The substructure search supports graphic input, structure wizard, selection from the library, and query language (expert form).

E. REACTOME Databases:

Reactome stands as a cornerstone in the landscape of pathway databases, offering an open-source, open-access, and meticulously curated resource dedicated to human pathways and biological processes. Developed through the collaborative efforts of expert biologists and Reactome editorial staff, pathway annotations within this database undergo a rigorous peer-review process. Notably, Reactome's annotations are intricately cross-referenced with various authoritative sources, including protein and gene information from UniProt, NCBI EntrezGene, Ensembl, UCSC, and HapMap, as well as small molecule data from KEGG Compound and ChEBI. Primary research literature from PubMed and GO controlled vocabularies further enriches the annotations, ensuring a comprehensive and well-rounded knowledgebase.

The unique data model employed by Reactome broadens the traditional concept of a reaction, encompassing diverse biological events such as entity transformations, compartmental transport, interactions leading to complex formation, and classical biochemical reactions. This inclusive approach allows Reactome to capture a wide spectrum of biological processes spanning signaling, metabolism, transcriptional regulation, apoptosis, and synaptic transmission. The resulting dataset is presented in a single, internally consistent, and computationally navigable format, making Reactome an indispensable resource for basic research, genome analysis, pathway modeling, systems biology, and education.

In response to the rapid growth of knowledge in the field, Reactome has not only doubled in size over the past two years but has also introduced new tools for data aggregation and analysis. To support this continuous evolution, Reactome has undergone a redesign, encompassing both its web interface and data analysis software. This redesign reflects Reactome's commitment to staying at the forefront of pathway databases, providing an up-to-date and user-friendly platform for researchers.

F. PDBSum Databases:

In the early years of the Protein Data Bank (PDB), researchers faced challenges navigating experimentally determined protein structures due to text file storage, lack of a user-friendly interface, and laborious methods for identifying entries of interest. The growing repository necessitated innovative solutions to efficiently access and analyze structural information.

In response to these challenges, the advent of the World Wide Web (WWW) in the early 1990s ushered in a transformative era for protein structure analysis. Among the pioneering platforms that leveraged the emerging web technology was PDBsum, developed at University College London (UCL) in 1995. Designed to harness the capabilities of the WWW, PDBsum sought to streamline the exploration of structural information in the PDB by creating a visually-oriented catalog. This compendium aimed to provide a rich array of pictorial representations, including unique structural analyses not readily available elsewhere. Alongside PDBsum, other early servers such as PDDBrowse, the Swiss-3Dimage collection, and the IMB Jena Image Library of Biological Macromolecules emerged, each contributing distinct approaches to presenting and visualizing protein structures.

PDBsum's development persisted at UCL until its transfer to the European Bioinformatics Institute (EBI) in 2001, marking a pivotal moment in its evolution. Subsequent enhancements and additions have further refined the database, while concurrent advancements in other servers, particularly those operated by members of the worldwide Protein Data Bank (wwPDB) consortium, have collectively propelled the field of protein structure analysis into a new era of accessibility and functionality. This narrative encapsulates the dynamic evolution of databases like PDBsum, which, through strategic adaptation to technological advancements, continue to play pivotal roles in facilitating the exploration and understanding of protein structures on a global scale.

G. PDBTM Databases:

The Protein Data Bank (PDB) is a critical repository of biological macromolecular structures, yet the representation of transmembrane proteins within this vast resource is notably scarce, constituting less than 2% of entries, as highlighted by the PDBTM database. Established in 2004, the PDBTM database emerged to address the challenges associated with identifying and characterizing transmembrane protein structures within the PDB.

Transmembrane proteins, pivotal for cellular functions such as energy production, regulation, and metabolism, are also frequent targets for drug development, with approximately half of contemporary drugs impacting these proteins. Recognizing the importance of these proteins, the PDBTM database pioneered a methodology reliant solely on 3D coordinates to identify transmembrane segments, circumventing the limitations of existing annotations in PDB entries. Given the experimental intricacies in determining the orientation of transmembrane proteins relative to the lipid bilayer, the PDBTM database introduced the TMDET method to tackle this challenge. In the absence of solved atomic structures for the double lipid layer, theoretical methods, such as those employed by the PDBTM database, become indispensable for determining protein orientations.

Several other databases, each utilizing diverse theoretical algorithms, contribute to the understanding of transmembrane proteins. The OPM database offers a well-structured classification, emphasizing the protein-membrane relationship. The CGDB database employs sophisticated physics-based models derived from coarse-grained simulations, while Mpstruct stands out as a reliable resource for regularly updated membrane protein classifications.

In the landscape of transmembrane protein databases, PDBTM plays a distinctive role by systematically collecting and verifying the structures of transmembrane proteins from the PDB. This meticulous curation includes the correction of biologically active oligomer forms, definition of membrane orientation, and identification of transmembrane segments, re-entrant loops, and interfacial helices. Through these efforts, PDBTM significantly contributes to unraveling the complexities of transmembrane protein structures and their roles in cellular processes.

H. Class, Architecture, Topology, And Homologous Superfamily (CATH) Databases:

CLASS, ARCHITECTURE, TOPOLOGY, AND HOMOLOGOUS SUPERFAMILY (CATH) CATH, a database for hierarchical classification of protein domains was developed at University of London. The CATH database is a free, publicly available online resource that provides information on the evolutionary relationships of protein domains. It was created in the mid-1990s by Professor Christine Orengo and colleagues, and continues to be developed by the Orengo group at University College London.

At its core, CATH utilizes experimentally-determined protein three-dimensional structures sourced from the Protein Data Bank (PDB). These structures are meticulously dissected into their constituent polypeptide chains, and the identification of protein domains within these chains is a nuanced process involving a combination of automated methodologies and manual curation. The ensuing classification within the CATH structural hierarchy follows a multi-tiered approach.

The Class (C) level classification categorizes domains based on their secondary structure content, distinguishing between all-alpha, all-beta, a combination of alpha and beta, or domains with minimal secondary structure. Moving up the hierarchy, the Architecture (A) level considers the spatial arrangement of secondary structures in three-dimensional space. The Topology/fold (T) level focuses on the connectivity and arrangement of secondary structure elements. Finally, domains are assigned to the Homologous Superfamily (H) level when there is compelling evidence of evolutionary relatedness, indicating homology.

To supplement experimentally determined structures, CATH incorporates additional sequence data from Gene3D, a related resource. Gene3D provides information on domains lacking experimentally determined structures, aiding in the population of homologous super families, UniProtKB and Ensembl contribute to this process by having their protein sequences scanned against CATH Hidden Markov Models (HMMs), facilitating the prediction of domain sequence boundaries and the assignment to homologous super families.

This intricate classification process, combining automated tools and manual curation, results in a wealth of information that is freely accessible to the scientific community and beyond. Furthermore, the CATH database remains dynamic, receiving periodic updates to ensure that the latest advancements in protein domain classification are reflected, demonstrating its commitment to serving as a valuable resource for researchers and bioinformaticians alike.

I. SCOPe Databases (Structural Classification of Proteins – Extended):

The Structural Classification of Proteins (SCOPe) database, established 27 years ago as the successor to the classic SCOP, continues to be a cornerstone in the field of protein structure and evolution. Designed as a manually curated hierarchy of domains from known protein structures, SCOPe's primary objective is to unravel the structural and evolutionary relationships among proteins.

SCOPe maintains a dynamic knowledgebase that evolves with the influx of new protein structures from the Protein Data Bank (PDB). Its hierarchical organization encompasses Families, Superfamilies, Folds, and Classes, providing a comprehensive framework for understanding the relationships between related proteins at various structural and functional levels. Expert curation, particularly at the Superfamily level, integrates diverse information to discern common ancestry.

The database excels in uncovering ancient homologous relationships, utilizing structural evidence when sequence similarity is absent. SCOPe annotates these relationships, grouping homologous domains into Superfamilies or, when evidence is inconclusive, categorizing them under common Folds.

Beyond classification, SCOPe offers valuable resources for computational analyses. It provides sequences and PDB-style coordinate files for all domains, ensuring accessibility for researchers. Post-translationally modified amino acids are meticulously translated, and sequences are curated to eliminate errors.

In alignment with FAIR principles (Findable, Accessible, Interoperable, Reusable), SCOPe ensures data availability through versioned releases, enabling findability and traceability over time. Major stable releases, accompanied by periodic updates, reflect the commitment to maintaining a stable and accurate database. The monthly updates, synchronized with the PDB, reflect the dedication to staying current in the rapidly evolving field.

Since 2001, SCOPe has adhered to stable identifiers, ensuring consistency across releases. The database is designed for both machines and humans, supporting download in various formats and archived on Zenodo, an open-access data repository. The current SCOPe release, 2.08, stands as a testament to its growth, classifying 344,851 domains from 106,976 PDB entries. With each release, SCOPe continues to be a vital resource for researchers exploring the intricate world of protein structure and evolution.

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WEBLEM 1(A)
UniProt Database
(URL: <http://www.uniprot.org/>)

AIM:

To explore the UniProt database for further study of the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704)

INTRODUCTION:

UniProt Database

The UniProt database is a free resource for protein sequence and functional information. It contains over 60 million sequences, including over half a million that have been curated by experts. The database was originally created as a primary database for protein sequences and functional annotation based on experimental evidence. It now combines a network of sister databases that centralize all levels of annotation for protein sequences.

The UniProt databases are:

1. UniProt Knowledgebase (UniProtKB)
2. UniProt Reference Clusters (UniRef)
3. UniProt Archive (UniParc)

UniProt Database was created by combining Swiss-Prot, TrEMBL, and PIR. Many entries in the database are derived from genome sequencing projects. The Protein Data Bank (PDB) is the central archive of all experimentally determined protein structure data. The PDB was established in 1971 and is maintained by an international consortium known as the Worldwide Protein Data Bank (wwPDB).

Immunoglobulin lambda variable

Immunoglobulin lambda variable 2-14 (IGLV2-14) is a protein-coding gene in humans that plays a crucial role in the immune system. This gene is located in the human genome and is involved in the production of immunoglobulin light chains, which are essential components of antibodies.

IGLV2-14 encodes for the variable region of the lambda light chain of immunoglobulins, which is critical for antigen recognition. This function allows antibodies to bind specifically to antigens, facilitating the immune response against pathogens. The protein expressed by this gene is predicted to be involved in immune responses and is found in extracellular exosomes, suggesting its role in intercellular communication within the immune system.

IGLV2-14 has important paralogs, including IGLV2-18, which share similar functions and characteristics. The gene is part of a larger family of immunoglobulin genes that contribute to the diversity of antibodies produced by the immune system.

Understanding the function and expression of IGLV2-14 can provide insights into its role in various immune-related conditions and diseases, potentially aiding in the development of therapeutic strategies targeting immunological disorders.

METHODOLOGY:

1. Go to the UniProt database homepage and type the query ‘Immunoglobulin lambda variable’ into the search box.
2. Decide whether you choose to view your results as a table or cards.
3. Use several filters to look for the query, such as organism popularity, taxonomy, proteins having 3D structures, sequence length, etc.
4. Save data in the FASTA format.
5. Results can be sorted by functions, name, taxonomy, subcellular location, disease and variations, structure, family & domains, sequence, and related proteins when you click on a result.

OBSERVATIONS:

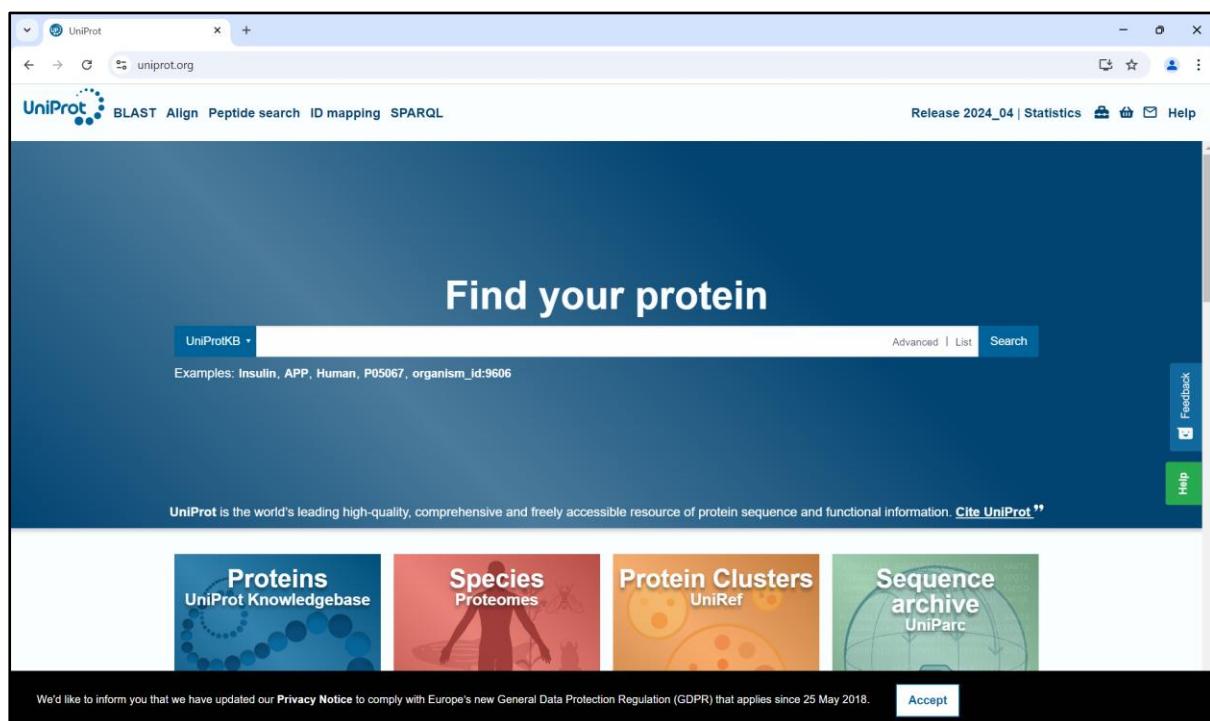


Fig 1: Homepage of the UniProt database

A drop – down list next to the search box allows you to specify the protein you want to look up, and the search box itself can be used to look up many proteins.

The screenshot shows the UniProtKB search results for the query 'immunoglobulin lambda variable'. The results are displayed in a table with columns: Entry, Entry Name, Protein Names, Gene Names, Organism, and Length. There are 15,847 results in total. The results are filtered to show 78 reviewed (Swiss-Prot) entries. One specific entry, P01704 (LV214_HUMAN), is highlighted with a red border.

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
P01703	LV140_HUMAN	Immunoglobulin lambda variable 1-40[...]	IGLV1-40	Homo sapiens (Human)	118 AA
P01705	LV223_HUMAN	Immunoglobulin lambda variable 2-23[...]	IGLV2-23	Homo sapiens (Human)	113 AA
P01699	LV144_HUMAN	Immunoglobulin lambda variable 1-44[...]	IGLV1-44	Homo sapiens (Human)	117 AA
P01700	LV147_HUMAN	Immunoglobulin lambda variable 1-47[...]	IGLV1-47	Homo sapiens (Human)	117 AA
P01709	LV208_HUMAN	Immunoglobulin lambda variable 2-8[...]	IGLV2-8	Homo sapiens (Human)	118 AA
P08048	LV321_HUMAN	Immunoglobulin lambda variable 3-21[...]	IGLV3-21	Homo sapiens (Human)	117 AA
P01704	LV214_HUMAN	Immunoglobulin lambda variable 2-14[...]	IGLV2-14	Homo sapiens (Human)	120 AA
A0M8Q6	IGLC7_HUMAN	Immunoglobulin lambda constant 7[...]	IGLC7	Homo sapiens (Human)	106 AA
P01701	LV151_HUMAN	Immunoglobulin lambda variable 1-51[...]	IGLV1-51	Homo sapiens (Human)	117 AA
P01721	LV657_HUMAN	Immunoglobulin lambda variable 6-57[...]	IGLV6-57	Homo sapiens (Human)	117 AA
P01706	LV211_HUMAN	Immunoglobulin lambda variable 2-11[...]	IGLV2-11	Homo sapiens (Human)	119 AA
P0CG04	IGLC1_HUMAN	Immunoglobulin lambda constant 1[...]	IGLC1	Homo sapiens (Human)	106 AA

We'd like to inform you that we have updated our [Privacy Notice](#) to comply with Europe's new General Data Protection Regulation (GDPR) that applies since 25 May 2018. [Accept](#)

Fig 2: Query ‘Immunoglobulin lambda variable’ searched
Reviewed (SwissProt) search: 78 hits
Total results obtained: 15,847 hits

The screenshot shows the detailed information for the UniProt ID P01704 · LV214_HUMAN. The page includes sections for Function, Names & Taxonomy, Subcellular Location, Disease & Variants, PTM/Processing, Expression, Interaction, Structure, Family & Domains, Sequence, and Similar Proteins. The 'Function' section describes the V region of the variable domain of immunoglobulin light chains. The 'Names & Taxonomy' section shows the protein name as Immunoglobulin lambda variable 2-14, gene name as IGLV2-14, and organism as Homo sapiens (Human). The 'Sequence' section shows the amino acid sequence of the protein.

We'd like to inform you that we have updated our [Privacy Notice](#) to comply with Europe's new General Data Protection Regulation (GDPR) that applies since 25 May 2018. [Accept](#)

Fig 3: Information for the query selected: ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704)

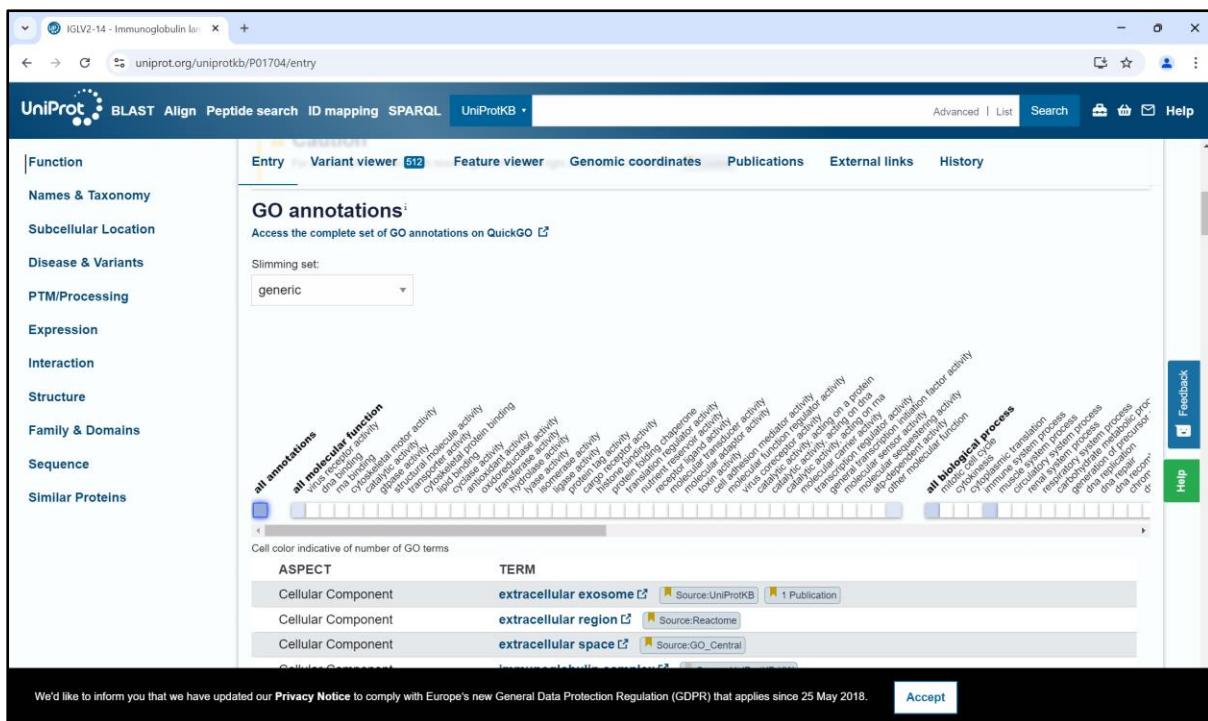


Fig 3.1: Number of annotations and all molecular functions of site

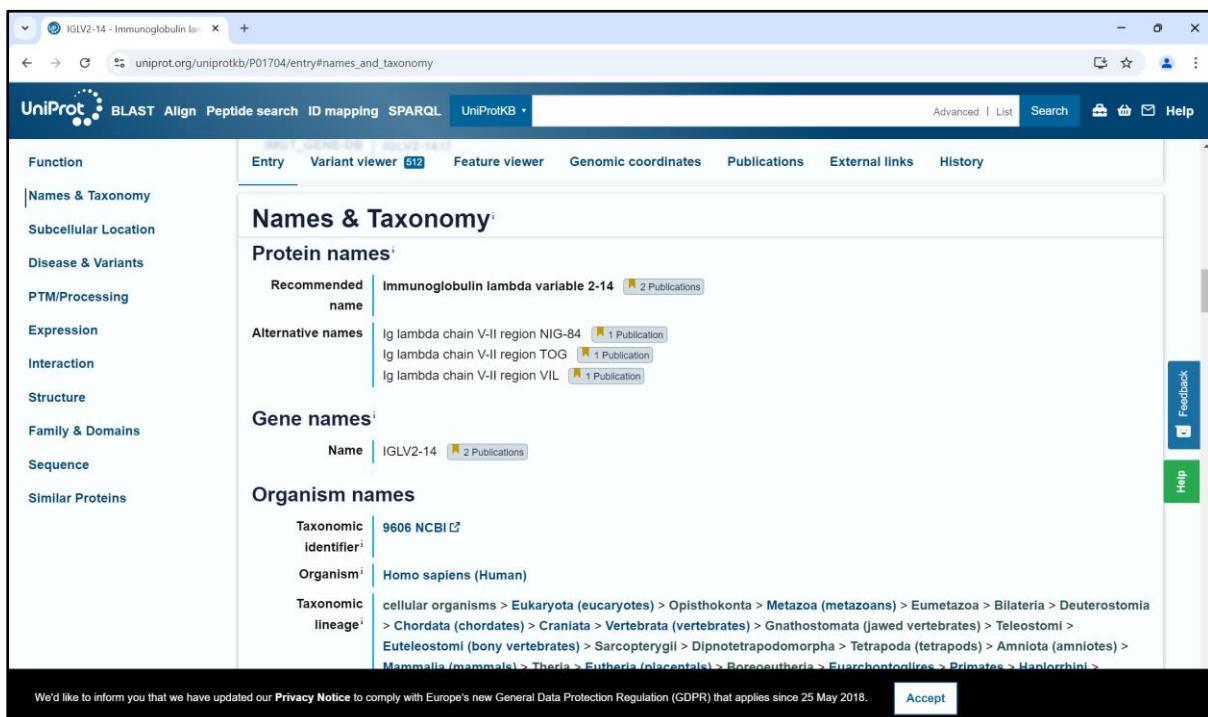


Fig 4: Names and Taxonomy of the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704)

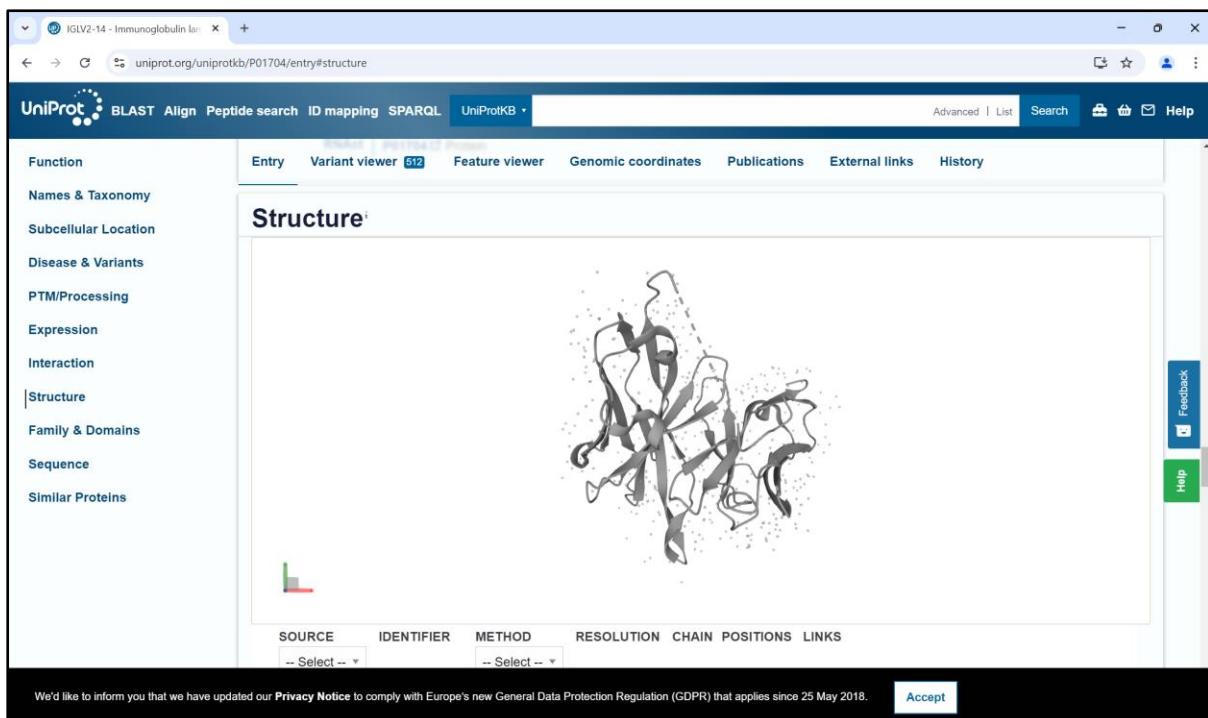


Fig 5: Structure of the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704)

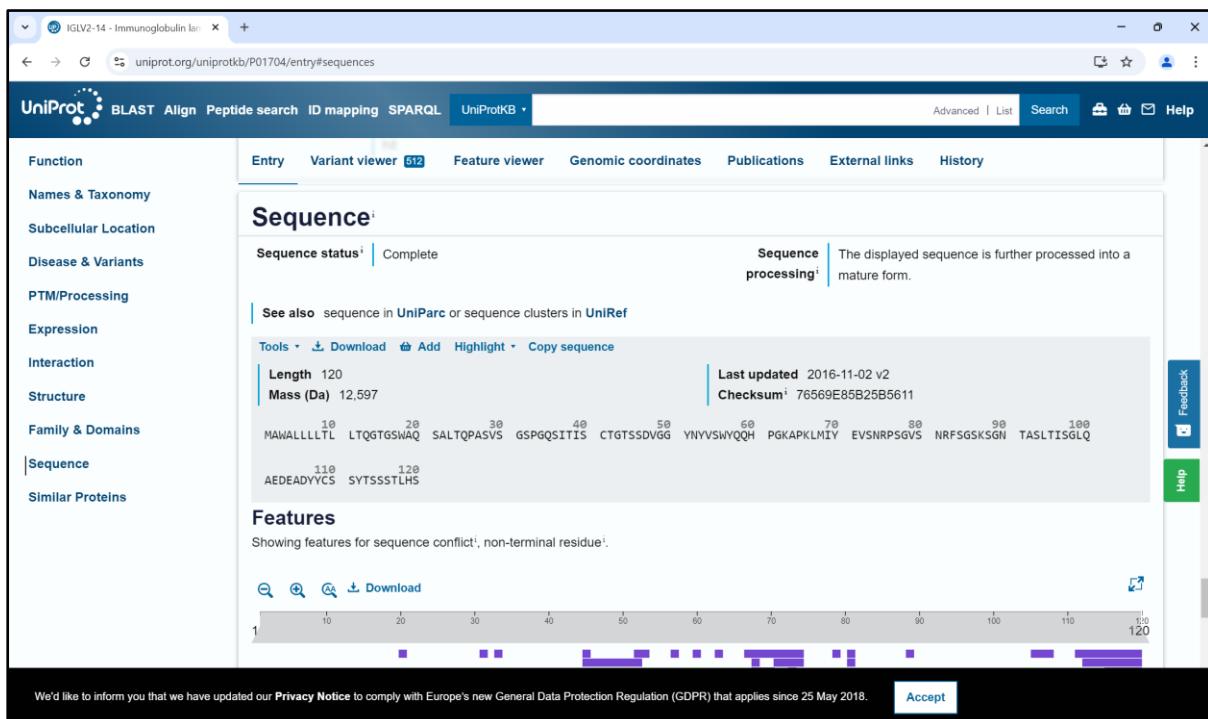


Fig 6: Sequence of the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704)

RESULTS:

The entry with the UniProt ID: P01704 in the 15,847 findings for the query ‘Immunoglobulin lambda variable 2-14’ is for the organism: *Homo sapiens* (Human) with 120 amino acids. Immunoglobulins, also known as antibodies, are specialized glycoproteins produced by B lymphocytes, featuring variable domains that undergo V-(D)-J rearrangement and somatic hypermutation, allowing them to bind with high specificity and affinity to antigens, playing a crucial role in both the recognition and effector phases of humoral immunity. This is shown using a function filter. Number of annotations and all molecular function of site along with name and taxonomy, structure and sequence of the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704) were observed.

CONCLUSION:

The UniProt, SwissProt and TrEMBL databases were explored for the query ‘Immunoglobulin lambda variable 2-14’ (UniProt ID: P01704) and related information was searched.

REFERENCES:

1. UniProt. (n.d.). <https://www.uniprot.org/>
 2. National Center for Biotechnology Information. (n.d.). IGLV2-14 immunoglobulin lambda variable 2-14 [Homo sapiens (human)]. U.S. Department of Health and Human Services. <https://www.ncbi.nlm.nih.gov/gene?Cmd=DetailsSearch&Db=gene&Term=28815>
 3. UniProt. (n.d.). <https://www.uniprot.org/uniprotkb/P01704/entry>
-

WEBLEM 1(B)
Protein Data Bank (PDB) Database
(URL: <https://www.rcsb.org/pdb/>)

AIM:

To study and explore the protein structure for the query ‘Catalytic Fab’ (PDB ID: 2GFB) using the Protein Data Bank (PDB) Database.

INTRODUCTION:

Protein Data Bank (PDB)

The Protein Data Bank (PDB) is a comprehensive database that houses three-dimensional structural data of biological macromolecules, including proteins and nucleic acids. Established in 1971, it is managed by the Worldwide Protein Data Bank (wwPDB), an international consortium responsible for overseeing the deposition, validation, curation, and open-access dissemination of 3D structural data.

The PDB is a vital resource for structural biology, particularly in fields like structural genomics, enabling scientists to study the 3D architecture of biological macromolecules. The archive contains atomic coordinates and other relevant information about proteins and key biological molecules, with the primary data being coordinate files that describe the atoms in each molecule and their spatial positions.

Noteworthy features of the PDB include its historical role as the first open-access digital platform for sharing protein structures, its importance in computational biology for applications such as structure-based drug design, and its continuous growth, reflecting the ongoing research in laboratories worldwide.

The PDB file format, a text-based format used to describe molecular structures, includes data on atomic coordinates, secondary structure assignments, and atomic connectivity. While the PDB format is a legacy system, the database now stores biological macromolecule data in the updated mmCIF format.

Catalytic Fab

The crystal structure of a catalytic Fab fragment are catalytic antibodies, often referred to as "abzymes", engineered to catalyze specific chemical reactions, mimicking natural enzymes. The esterase-like activity is attributed to specific amino acid residues in the combining site that facilitate substrate hydrolysis. The design of these antibodies often involves eliciting responses against transition state analogs, which guide the formation of catalytic sites tailored for specific reactions. The structure shows similarities with other catalytic antibodies, such as antibody 17E8, which also exhibits esterase activity. This suggests a conserved mechanism among different abzymes that utilize similar structural features for catalysis.

METHODOLOGY:

1. Open the homepage of the Protein Data Bank (PDB) Database.
2. Enter the query ‘Catalytic Fab’ and initiate the search.
3. After the retrieval of the query, observe the results. Apply specific refinements (filters) to narrow down the results based on the query.
4. Select a particular entry of interest [‘2GFB: CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY’] for further study in terms of its Structure Summary, 3D View, Annotations, Experiment, Sequence, Genome, and Versions.
5. To display and download the 3D structure of the protein, click on the ‘Display and Download’ option and select the desired format.

OBSERVATIONS:

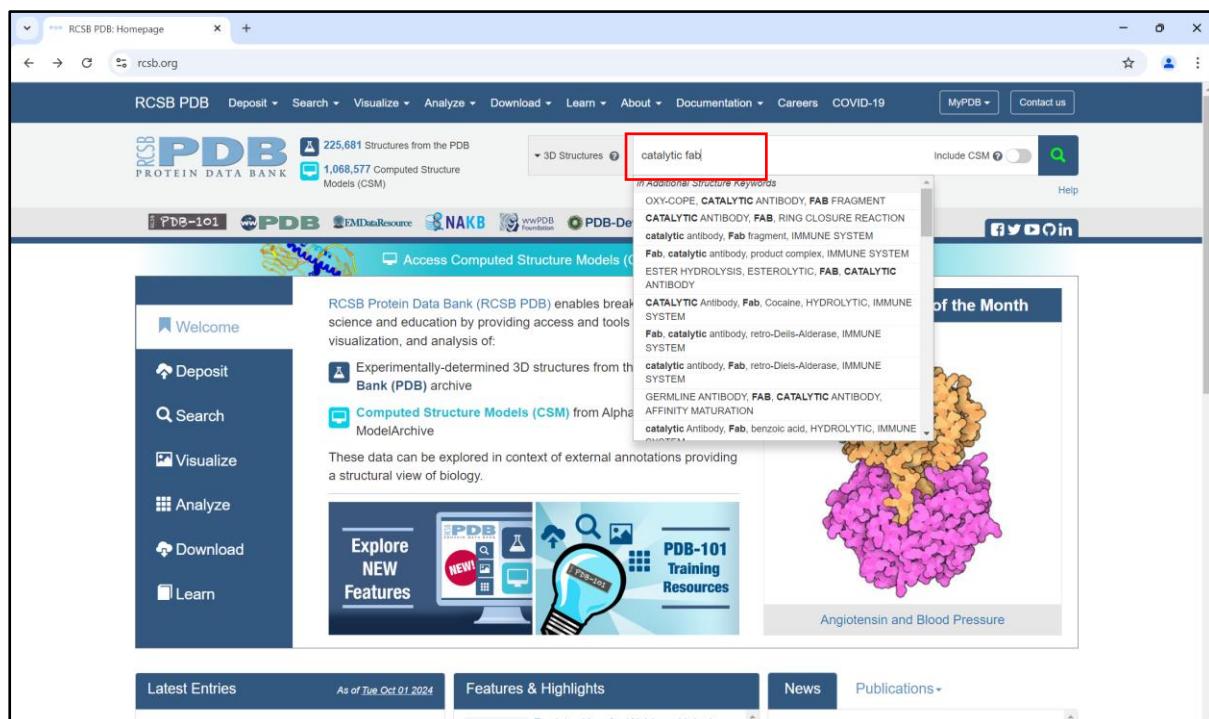


Fig 1: Homepage of the Protein Data Bank (PDB) Database

Search Summary This query matches 153,949 Structures.

Refinements

Structure Determination Methodology

- experimental (153,949)

Scientific Name of Source Organism

- Homo sapiens (52,890)
- Mus musculus (6,153)
- Escherichia coli (5,734)
- synthetic construct (4,869)
- Escherichia coli K-12 (3,547)
- Severe acute respiratory syndrome coronavirus 2 (3,520)
- Bos taurus (3,128)
- Saccharomyces cerevisiae (2,633)
- Rattus norvegicus (2,609)
- Saccharomyces cerevisiae S288C (2,133)
- More...

Taxonomy

- Eukaryota (89,045)
- Bacteria (52,079)
- Riboviria (10,772)
- other sequences (4,915)
- Archaea (4,556)
- Duplodoniviria (2,155)
- Eukaryota (eukaryotes) (841)
- Vardinaviria (457)
- unclassified sequences (274)

1EAP
CRYSTAL STRUCTURE OF A CATALYTIC ANTIBODY WITH A SERINE PROTEASE ACTIVE SITE
Zhou, G.W., Guo, J., Huang, W., Scanlan, T.S., Fletterick, R.J.
(1994) Science 265: 1059-1064

5XQW
Catalytic antibody 7B9
Ito, N., Fujii, I., Tsumuraya, T.
(2018) Bioorg Med Chem 26: 1412-1417

Fig 2: Number of hits obtained for the basic search of the query ‘Catalytic Fab’

Scientific Name of Source Organism

- Homo sapiens (52,890)
- Mus musculus (6,153)
- Escherichia coli (5,734)
- synthetic construct (4,869)
- Escherichia coli K-12 (3,547)
- Severe acute respiratory syndrome coronavirus 2 (3,520)
- Bos taurus (3,128)
- Saccharomyces cerevisiae (2,633)
- Rattus norvegicus (2,609)
- Saccharomyces cerevisiae S288C (2,133)
- [More...](#)

Experimental Method

- X-RAY DIFFRACTION (134,700)
- ELECTRON MICROSCOPY (14,533)
- SOLUTION NMR (4,460)
- NEUTRON DIFFRACTION (171)
- ELECTRON CRYSTALLOGRAPHY (128)
- SOLID-STATE NMR (52)
- SOLUTION SCATTERING (34)
- POWDER DIFFRACTION (18)
- EPR (5)
- THEORETICAL MODEL (3)
- [More...](#)

Fig 3: List of refinements (filters) applied

Search Summary This query matches 4,716 Structures.

Refinements

- Structure Determination Methodology
 - experimental (4,716)
- Scientific Name of Source Organism
 - Mus musculus (4,716)
 - Homo sapiens (898)
 - synthetic construct (159)
 - Gallus gallus (82)
 - Rattus norvegicus (60)
 - Streptomyces lividans (57)
 - Escherichia coli (37)
 - Bos taurus (36)
 - Oryctolagus cuniculus (34)
 - Cricetulus griseus (32)
 - More...
- Taxonomy
 - Eukaryota (4,716)
 - Bacteria (195)
 - Riboviria (165)
 - other sequences (161)
 - Eukaryota (eukaryotes) (87)
 - Riboviria (RNA viruses and viroids) (22)
 - Duplodnaviria (15)
 - Monodnaviria (15)
 - Archaea (9)

1EAP
CRYSTAL STRUCTURE OF A CATALYTIC ANTIBODY WITH A SERINE PROTEASE ACTIVE SITE
Zhou, G.W., Guo, J., Huang, W., Scanlan, T.S., Fletterick, R.J.
(1994) Science 265: 1059-1064

Released 1994-12-20
Method X-RAY DIFFRACTION 2.4 Å
Organisms Mus musculus
Macromolecule IGG2B-KAPPA 17E8 FAB (HEAVY CHAIN) (protein)
IGG2B-KAPPA 17E8 FAB (LIGHT CHAIN) (protein)
Unique Ligands HEP

5XQW
Catalytic antibody 7B9
Ito, N., Fujii, I., Tsumuraya, T.
(2018) Bioorg Med Chem 26: 1412-1417

Released 2018-04-18
Method X-RAY DIFFRACTION 2.2 Å
Organisms Mus musculus
Macromolecule Fab fragment of catalytic antibody 7B9, heavy chain (protein)
Fab fragment of catalytic antibody 7B9, light chain (protein)
Unique Ligands 8EU

Fig 4: Results obtained after applying refinements (filters)

Structure Summary Structure Annotations Experiment Sequence Genome Versions

2GFB
CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY
PDB DOI: <https://doi.org/10.2210/pdb2GFB/pdb>

Classification: IMMUNOGLOBULIN
Organism(s): Mus musculus
Mutation(s): No

Deposited: 1994-07-07 **Released:** 1994-09-30
Deposition Author(s): Golinielli-Pimpneau, B., Knossow, M.

Experimental Data Snapshot
Method: X-RAY DIFFRACTION
Resolution: 3.00 Å
R-Value Work: 0.213
R-Value Observed: 0.213

wwPDB Validation
Metric Classscore Percentile Ranks Value
Ramachandran outliers 17 3.7%
Sidechain outliers 9.8%
Percentile relative to all X-ray structures
Percentile relative to X-ray structures of similar resolution

Fig 5: Entry with the PDB ID: 2GFB opened that displays the ‘Structure Summary’

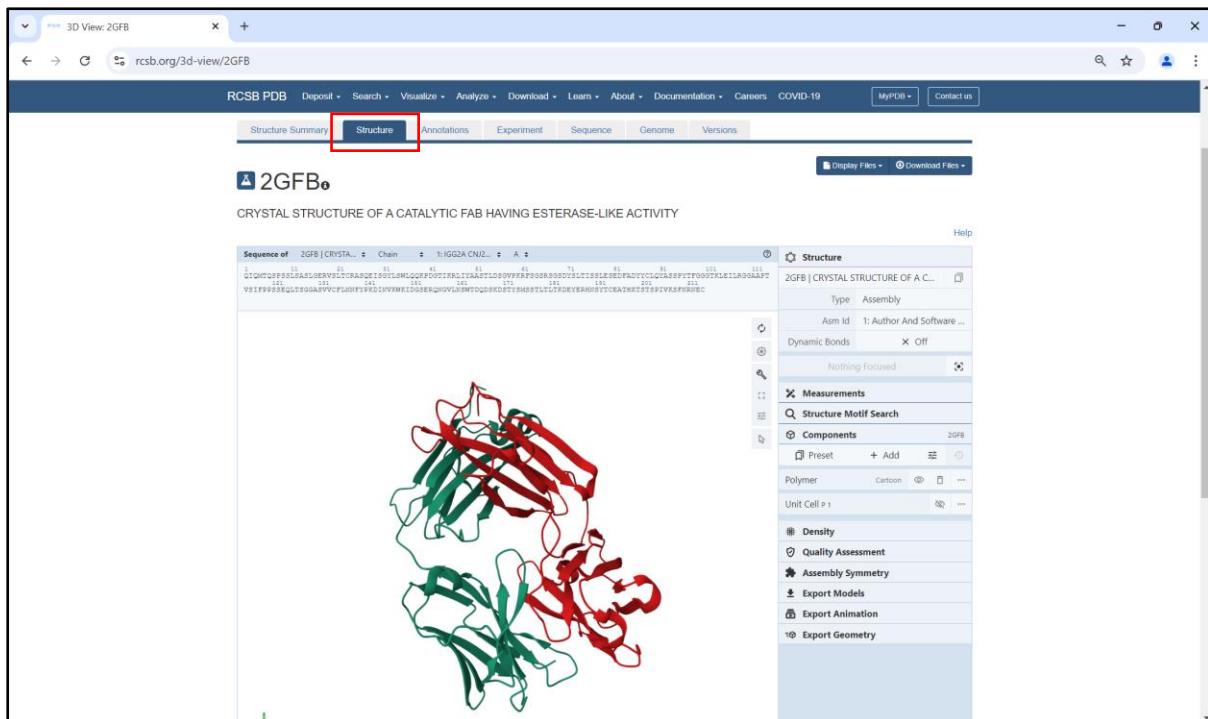


Fig 6: View of the ‘Structure’ of the ‘Catalytic Fab’ (PDB ID: 2GFB)

Chains	Domain Info	Class	Fold	Superfamily	Family	Domain	Species	Provenance
A	d2gfb1	All beta proteins	Immunoglobulin -like beta-sandwich	Immunoglobulin	V set domains (antibody variable domain-like)	Immunoglobulin light chain kappa variable domain, VL-kappa	(Mus musculus) [TaxId: 10090]	SCOPe (2.08)
A	d2gfb2	All beta proteins	Immunoglobulin -like beta-sandwich	Immunoglobulin	C1 set domains (antibody constant domain-like)	Immunoglobulin light chain kappa constant domain, CL-kappa	(Mus musculus) [TaxId: 10090]	SCOPe (2.08)
C	d2gfb1	All beta proteins	Immunoglobulin -like beta-sandwich	Immunoglobulin	V set domains (antibody variable)	Immunoglobulin light chain kappa variable	(Mus musculus) [TaxId: 10090]	SCOPe (2.08)

Fig 7: View of the ‘Annotations’ section

Experiment

2GFB
CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY

X-RAY DIFFRACTION

Crystallization

Crystal Properties	
Matthews coefficient	Solvent content
2.4	48.74

Crystal Data

Unit Cell		Symmetry	
Length (Å)	Angle (°)	Space Group	P 1
a = 198.7	α = 71.9		
b = 68.06	β = 112.2		
c = 83.66	γ = 119.6		

Refinement

Statistics						
Structure	Cut-off	Number	Number	Percent	Mean	

Fig 8: View of the ‘Experiment’ section



Fig 9: View of the ‘Sequence’ section

The screenshot shows the RCSB PDB website for entry 2GFB. The 'Genome' tab is highlighted with a red box. The page title is '2GFB' and the subtitle is 'CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY'. Below the title, it says 'No genome alignments are available'. The bottom of the page contains navigation links for 'About', 'Help', 'RCSB PDB is hosted by', 'RCSB PDB is a member of the', and 'RCSB Partners'.

Fig 10: View of the ‘Genome’ section

The screenshot shows the RCSB PDB website for entry 2GFB in the 'Versions' section. The 'Versions' tab is highlighted with a red box. The page title is '2GFB' and the subtitle is 'CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY'. It includes a note about PDB versioning and a table of version history. The table has columns for Version Number, Version Date, Version Type/Reason, Version Change, and Revised CIF Category. The table shows four versions: 1.0 (Initial release), 1.1 (Version format compliance), 1.2 (Version format compliance), and 1.3 (Data collection, Database references, Other). The Revised CIF Category for version 1.3 is 'chem_comp_atom, chem_comp_bond, database_2, pdbx_database_status, struct_ref_seq_dif'. There is a 'Download' button next to the table. The bottom of the page contains navigation links for 'About', 'Help', 'RCSB PDB is hosted by', 'RCSB PDB is a member of the', and 'RCSB Partners'.

Fig 11: View of the ‘Versions’ section

2GFB
CRYSTAL STRUCTURE OF A CATALYTIC FAB

PDB DOI: <https://doi.org/10.2210/pdb2GFB/pdb>

Classification: IMMUNOGLOBULIN
Organism(s): *Mus musculus*
Mutation(s): No

Deposited: 1994-07-07 Released: 1994-09-30
Deposition Author(s): Golinelli-Pimpaneau, B., Knossow, M.

Experimental Data Snapshot

Metric	Percentile Ranks	Value
Clashscore	17	3.7%
Ramachandran outliers	9.8%	Better
Sidechain outliers	9.8%	Better

This is version 1.3 of the entry. See complete history.

Fig 12: Display options for the ‘Catalytic Fab’ (PDB ID: 2GFB)

2GFB
CRYSTAL STRUCTURE OF A CATALYTIC FAB

PDB DOI: <https://doi.org/10.2210/pdb2GFB/pdb>

Classification: IMMUNOGLOBULIN
Organism(s): *Mus musculus*
Mutation(s): No

Deposited: 1994-07-07 Released: 1994-09-30
Deposition Author(s): Golinelli-Pimpaneau, B., Knossow, M.

Experimental Data Snapshot

wwPDB Validation

Metric	Percentile Ranks	Value
Clashscore	17	3.7%
Ramachandran outliers	9.8%	Better
Sidechain outliers	9.8%	Better

This is version 1.3 of the entry. See complete history.

Literature

Crystal structure of a catalytic antibody Fab with a chymotrypsin-like activity. Golinelli-Pimpaneau, B., Gicquel, B., Rizkallah, T., Knossow, M. *J Mol Biol* 1995, 257, 101-112.

Fig 13: Download options for the ‘Catalytic Fab’ (PDB ID: 2GFB)

```

2gfb.pdb
File Edit View
HEADER IMMUNOGLOBULIN 07-JUL-94 2GFB
TITLE CRYSTAL STRUCTURE OF A CATALYTIC FAB HAVING ESTERASE-LIKE ACTIVITY
COMPID MOL_ID: 1
COMPID 2 MOLECULE: IgG2A CN3206 FAB (LIGHT CHAIN);
COMPID 3 CHAIN: A, C, E, G, I, K, M, O;
COMPID 4 MOL_ID: 2;
COMPID 5 MOLECULE: IgG2A CN3206 FAB (HEAVY CHAIN);
COMPID 6 CHAIN: B, D, F, H, J, L, N, P
SOURCE MOL_ID: 1;
SOURCE 2 ORGANISM_SCIENTIFIC: MUS MUSCULUS;
SOURCE 3 ORGANISM_COMMON: HOUSE MOUSE;
SOURCE 4 ORGANISM_TAXID: 10090;
SOURCE 5 MOL_ID: 2;
SOURCE 6 ORGANISM_SCIENTIFIC: MUS MUSCULUS;
SOURCE 7 ORGANISM_COMMON: HOUSE MOUSE;
SOURCE 8 ORGANISM_TAXID: 10090
KEYWDS IMMUNOGLOBULIN
EXPDTA X-RAY DIFFRACTION
AUTHOR B.GOLINELLI-PIMPANEAU,M.KNOSSOW
REVDAT 4 05-JUN-24 2GFB 1 SEQADV
REVDAT 3 24-FEB-09 2GFB 1 VERSN
REVDAT 2 01-APR-03 2GFB 1 JRNL
REVDAT 1 30-SEP-94 2GFB 0
JRNL AUTH B.GOLINELLI-PIMPANEAU,B.GIGANT,T.BIZZARD,J.NAVAZA,
JRNL AUTH 2 P.SALUDJIAN,R.ZEMEL,D.S.TAWFIK,Z.ESHHAR,B.S.GREEN,M.KNOSSOW
JRNL TITL CRYSTAL STRUCTURE OF A CATALYTIC ANTIBODY FAB WITH
JRNL TITL 2 ESTERASE-LIKE ACTIVITY.
JRNL REF STRUCTURE V. 2 175 1994
JRNL REF ISSN 0969-2126
JRNL PMID 8089632
JRNL DOI 10.1016/S0969-2126(00)00019-8
REMARK 1
REMARK 1 REFERENCE 1
REMARK 1 AUTH R.ZEMEL,D.G.SCHINDLER,D.S.TAWFIK,Z.ESHHAR,B.S.GREEN
REMARK 1 TITL DIFFERENCES IN THE BIOCHEMICAL PROPERTIES OF ESTEROLYTIC
REMARK 1 TITL 2 ANTIBODIES CORRELATE WITH STRUCTURAL DIVERSITY
REMARK 1 REF MOL IMMUNOL. V. 31 127 1994
REMARK 1 REFN ISSN 0161-5890
REMARK 2
REMARK 2 RESOLUTION. 3.00 ANGSTROMS.
REMARK 3
REMARK 3 REFINEMENT.
REMARK 3 PROGRAM : X-PLOR
REMARK 3 AUTHORS : BRUNGER
REMARK 3
REMARK 3 DATA USED IN REFINEMENT.
Ln 1 Col 1 22,63,707 characters

```

Fig 14: View of the sequence in PDB file format (Header)

RESULTS:

The Protein Data Bank (PDB) database was examined to investigate protein structures using the query ‘Catalytic Fab’ with the PDB ID: 2GFB. A total of 153,949 protein structure entries were initially obtained through a basic search. The results have been categorized into different sections, including Structure Summary, 3D View, Annotations, Experiment, Sequence, Genome and Versions. The entry can be displayed and downloaded in the desired format for further analysis.

CONCLUSION:

The Protein Data Bank (PDB) stands as an essential and foundational resource in structural biology and bioinformatics. It serves as a repository for experimentally determined three-dimensional structures of biological macromolecules, including proteins, nucleic acids, and complex assemblies. Key features and contributions of the PDB include Comprehensive Repository, Global Collaboration, Structural Insights, etc. Thus, the Protein Data Bank remains an indispensable resource for structural biologists, researchers, educators, and clinicians worldwide. Its wealth of structural information plays a pivotal role in advancing scientific knowledge, aiding in various research endeavours, and paving the way for innovations in biomedicine and biotechnology.

REFERENCES:

1. Berman, H. M. (2000, January 1). The Protein Data Bank. Nucleic Acids Research, 28(1), 235–242. <https://doi.org/10.1093/nar/28.1.235>
 2. Bernstein, F. C., Koetzle, T. F., Williams, G. J., Meyer, E. F., Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T., & Tasumi, M. (1977). The Protein Data Bank: A computer-based archival file for macromolecular structures. Journal of Molecular Biology, 112(3), 535-542. [https://doi.org/10.1016/s0022-2836\(77\)80200-3](https://doi.org/10.1016/s0022-2836(77)80200-3)
 3. Charbonnier, J. B., Carpenter, E., Gigant, B., Golinelli-Pimpaneau, B., Eshhar, Z., Green, B. S., & Knossow, M. (1995). Crystal structure of the complex of a catalytic antibody Fab fragment with a transition state analog: structural similarities in esterase-like catalytic antibodies. Proceedings of the National Academy of Sciences of the United States of America, 92(25), 11721–11725. <https://doi.org/10.1073/pnas.92.25.11721>
-

WEBLEM 2

Structural Antibody Database (SAbDab)

(URL: <https://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabdab>)

AIM:

To study the antibody structure information using SAbDab Database.

INTRODUCTION:

Antibodies form the foundations of the vertebrate immune response. These proteins form complexes with potentially pathogenic molecules called antigens and inhibit their function or recruit other components of the immunological machinery to destroy them. In addition to the biological importance of antibodies, their ability to be raised against an almost limitless number of molecules has made them useful laboratory tools and increasingly useful as therapeutic agents in humans. This biopharmaceutical application has motivated the desire to understand how binding, stability and immunogenic properties of the antibody are determined and how they can be modified.

Computational analyses and tools are increasingly being employed to aid the antibody engineering process. Many of these tools now use only the antibody data, as opposed to general protein data, because this has been shown to increase performance. The publicly available structural data for most types of proteins are too sparse to merit protein-specific prediction methods. However, since the first antibody structure was deposited in 1976, the number of antibody structures in the protein data bank (PDB) has grown, and it now represents approximately 1.75% of the total 91939 entries.

Several databases that handle antibody data currently exist (7–13). Of these, most are sequence-based or are antibody discovery tools. The most recent, DIGIT, provides sequence information for immunoglobulins and has the advantage over earlier sequence databases [Kabat, IMGT, Vbase2] of providing heavy and light chain sequence pairings. However, it does not incorporate structural data. Antigen DB and IEDB-3D do include structural data. However, both focus on collecting epitope data and do not include unbound antibody structures. In comparison, both IMGT and the Abysis portal provide the ability to inspect and download individual bound and unbound antibody structures. Neither allow for the generation of bespoke datasets nor for the download of an ensemble of curated structural data.

To address this problem, we have developed a Structural Antibody Database (SAbDab), a database devoted to automatically collecting, curating and presenting antibody structural data in a consistent manner for both bulk analysis and individual inspection. SAbDab updates on a weekly basis and provides users with a range of methods to select sets of structures. For example, users can select by species, experimental details (e.g. method, resolution and r-factor), similarity to a given antibody sequence, amino acid composition at certain positions and antibody–antigen affinity. Entries can also be selected using structural annotations including, for example, the canonical form of the complementarity determining regions (CDR), orientation between the antibody variable domains and the presence of constant domains in the structure. Structures can be inspected individually or downloaded en masse either as the

original file from the PDB or as a structure that has been annotated using the Chothia numbering scheme. In all cases, a tab-separated file detailing heavy and light chain pairing, antibody–antigen pairing and all other annotations is generated.

Structural antibody database is an online resource containing all the publicly available antibody structures annotated and presented in a consistent fashion. The data are annotated with several properties including experimental information, gene details, correct heavy and light chain pairings, antigen details and, where available, antibody-antigen binding affinity. The user can select structures, according to these attributes as well as structural properties such as complementarity determining region loop conformation and variable domain orientation. Individual structures, datasets and the complete database can be downloaded.

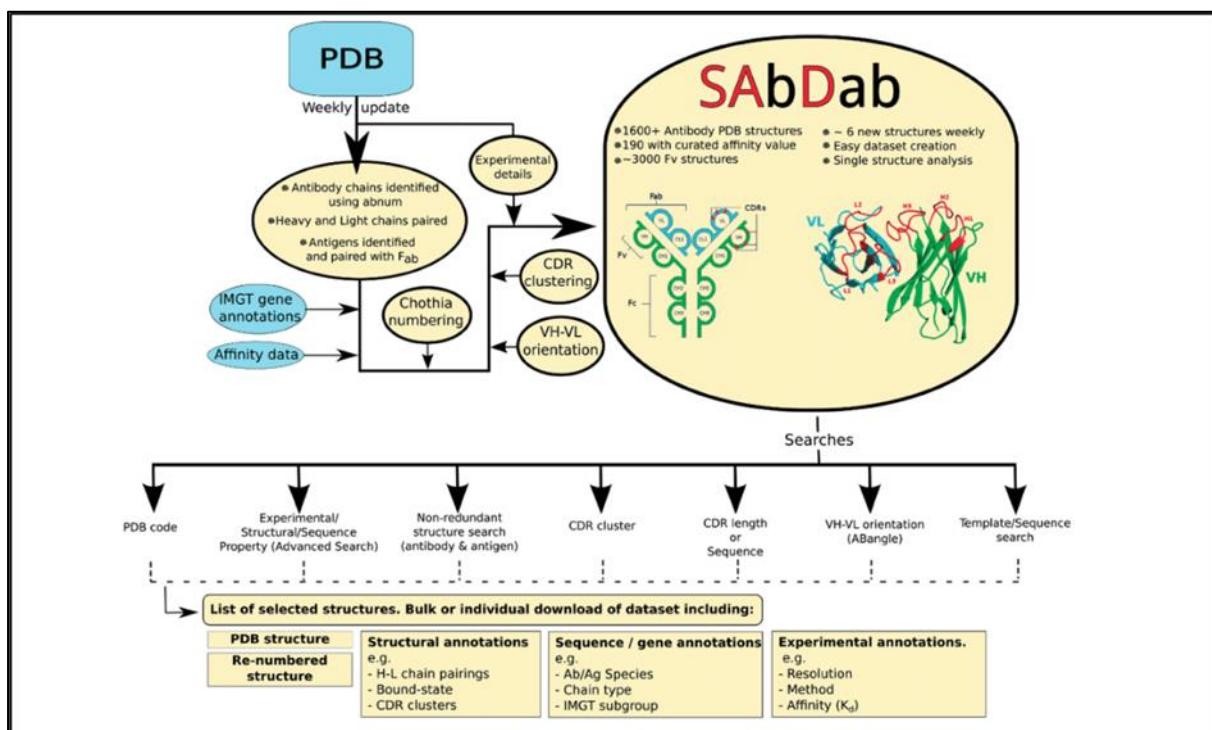


Fig 1: Workflow of SAbDab database

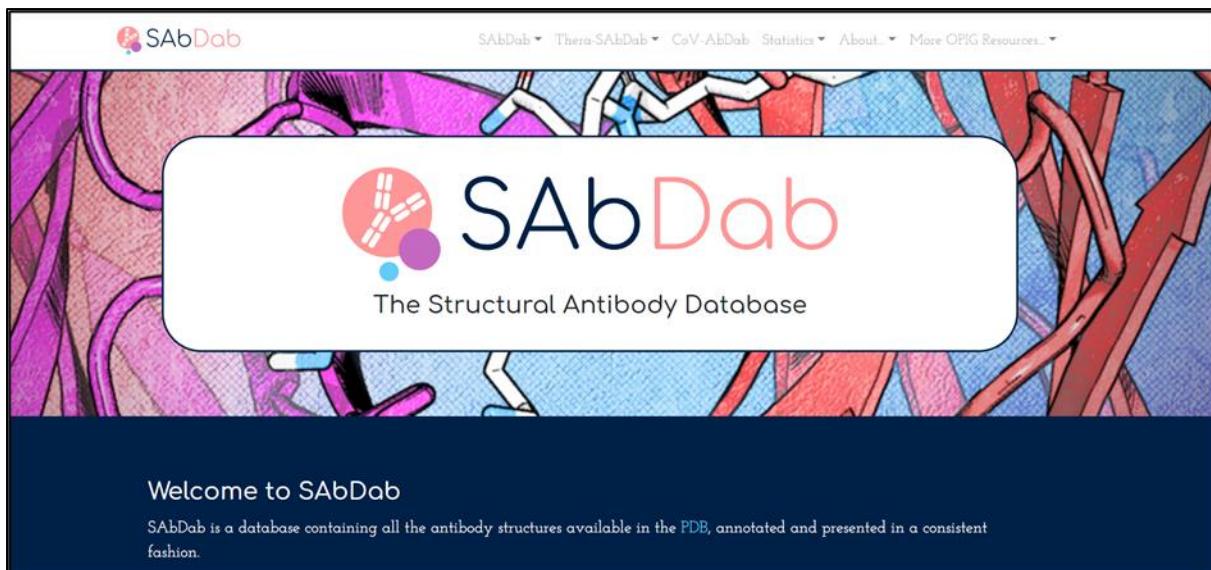


Fig 2: Homepage of SAbDab Database

The screenshot shows the "About Thera-SAbDab" page. The top navigation bar is identical to Fig 2. The main title "About Thera-SAbDab" is centered on a dark blue banner against a background of antibody structures. On the left, there is a sidebar with three items: "About Thera-SAbDab" (which is highlighted in blue), "Example Queries", and "Contact". The main content area starts with a section titled "About Thera-SAbDab" which describes the database as a collection of immunotherapeutic variable domain sequences and their structural representatives from SAbDab. It mentions weekly updates and sequence matching. Below this, another section discusses updates based on the WHO International Non-proprietary Name (INN) list, mentioning AdisInsight and the Thera-SAbDab search page.

Fig 3: Homepage of Thera – SAbDab

The [Oxford Protein Informatics Group](#) (Dept. of Statistics, University of Oxford) is collaborating in efforts to understand the immune response to SARS-CoV2 infection and vaccination. As part of our investigations, we are releasing and maintaining this public database to [document all published/patented antibodies and nanobodies able to bind to coronaviruses, including SARS-CoV2, SARS-CoV1, and MERS-CoV](#).

Explanations and a preliminary analysis of the database contents can be found in our [Applications Note](#) in Bioinformatics. Please consider citing it if you are making use of our database in your research. [BibTex Reference](#).

Fig 4: Homepage of CoV – SAbDab

Method	No. of Structures
X-Ray Diffraction	1032
Electron Microscopy	685

Fig 5: SAbDab – nano Statistics

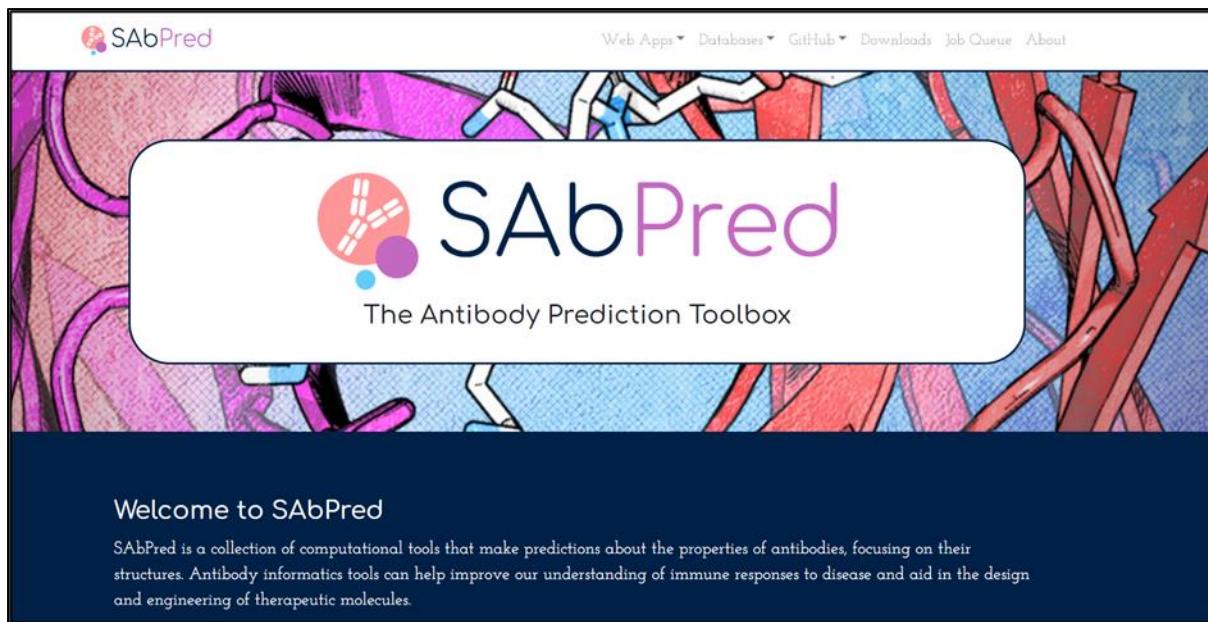


Fig 6: The Antibody Prediction Toolkit

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1. Dunbar, J., Krawczyk, K., Leem, J., Baker, T., Fuchs, A., Georges, G., Shi, J., & Deane, C. M. (2014). SAbDab: the structural antibody database. Nucleic acids research, 42(Database issue), D1140–D1146. <https://doi.org/10.1093/nar/gkt1043>
2. Dunbar, J., Krawczyk, K., Leem, J., Baker, T., Fuchs, A., Georges, G., Shi, J., & Deane, C. (2013). SAbDab: the structural antibody database. <https://www.semanticscholar.org/paper/SAbDab%3A-the-structural-antibody> database-Dunbar-Krawczyk/fefea2b9ed93a0c3163432c52a67cf34efa868f7

WEBLEM 2(A)

The Structural Antibody Database (SAbDab)

(URL: <https://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabdab>)

AIM:

To study the Antibody structure for the query 'DRD1_LSD complex' (PDB ID: 8JXR) using the Structural Antibody Database (SAbDab).

INTRODUCTION:

SAbDab Database

The Structural Antibody Database (SAbDab) is a comprehensive online resource dedicated to the collection and curation of antibody structures. It provides researchers with access to all publicly available antibody structures, which are annotated and presented in a standardized format. This database is particularly valuable for those working in the fields of antibody structure prediction, docking, and therapeutic design.

Key Features of SAbDab

1. **Extensive Data Collection:** SAbDab includes a significant number of antibody structures, with around 7,184 variable domain structures recorded from 3,663 entries in the Protein Data Bank (PDB) as of August 2019.
2. **Detailed Annotations:** Each structure in the database is annotated with various properties, such as experimental details, gene information, heavy and light chain pairings, antigen details, and where available, antibody-antigen binding affinities. This comprehensive annotation allows users to filter and select structures based on specific criteria, including experimental methods and structural properties.
3. **User-Friendly Tools:** The database features several tools for users, such as:
 - a. **ABangle Tool:** This tool allows users to characterize the orientation between the antibody's variable domains (VH and VL) and visualize conformational changes.
 - b. **CDR Search and Clustering:** Users can select hyper-variable loops based on their length and type, facilitating the study of antibody variability.
 - c. **Template Search:** Users can submit antibody sequences to find structural templates suitable for homology modeling.
4. **Regular Updates:** SAbDab is updated weekly, ensuring that it reflects the latest entries from the PDB and includes new sequence data as it becomes available. This continuous updating process enhances the database's relevance for ongoing research.
5. **Accessibility:** The database is freely available for public use, encouraging collaboration and innovation in antibody research. Users can download individual structures or entire datasets for further analysis.

DRD1_LSD complex

The nanobody-bound DRD1_LSD complex has been structurally characterized using cryo-electron microscopy (cryo-EM), revealing significant insights into the interaction between the dopamine D1 receptor (DRD1) and the psychedelic compound LSD, as well as the role of a β-arrestin-mimicking nanobody.

The complex consists of the dopamine D1 receptor (DRD1) bound to LSD and a nanobody (NBA3) that mimics β-arrestin. This setup is crucial for studying the receptor's signaling pathways, particularly in the context of G protein coupling versus β-arrestin coupling. LSD exhibits a unique binding mode within the DRD1 receptor, with its ergoline moiety oriented toward transmembrane helix 4 (TM4). This orientation is pivotal for understanding how LSD influences receptor activity and signaling dynamics. The findings from this structural analysis provide a foundational understanding of the dynamics of G protein-coupled receptors (GPCRs) and their relevance to signal transduction, particularly in the context of psychoactive substances like LSD.

METHODOLOGY:

1. Open the Protein Data Bank (PDB) website. (URL: <https://www.rcsb.org/>) to obtain the PDB ID of the structure (query).
2. Open the Structural Antibody Database (SAbDab). (URL: <https://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabdab>) that contains structural information on antibodies and antibody-antigen complexes.
3. Select the ‘Structure Search’ option from the SAbDab portal.
4. Select the ‘Search for a specific PDB entry’ option to search for antibody structures by their PDB ID.
5. Enter the PDB ID of the query (PDB ID: 8JXR), obtained from the Protein Data Bank (PDB) and select the entry from the results obtained to view detailed information about the antibody structure.
6. Study the Structure Details, Structure Visualization, Fv regions. Structures can further be downloaded using the links provided in the ‘Download’ section.

OBSERVATIONS:

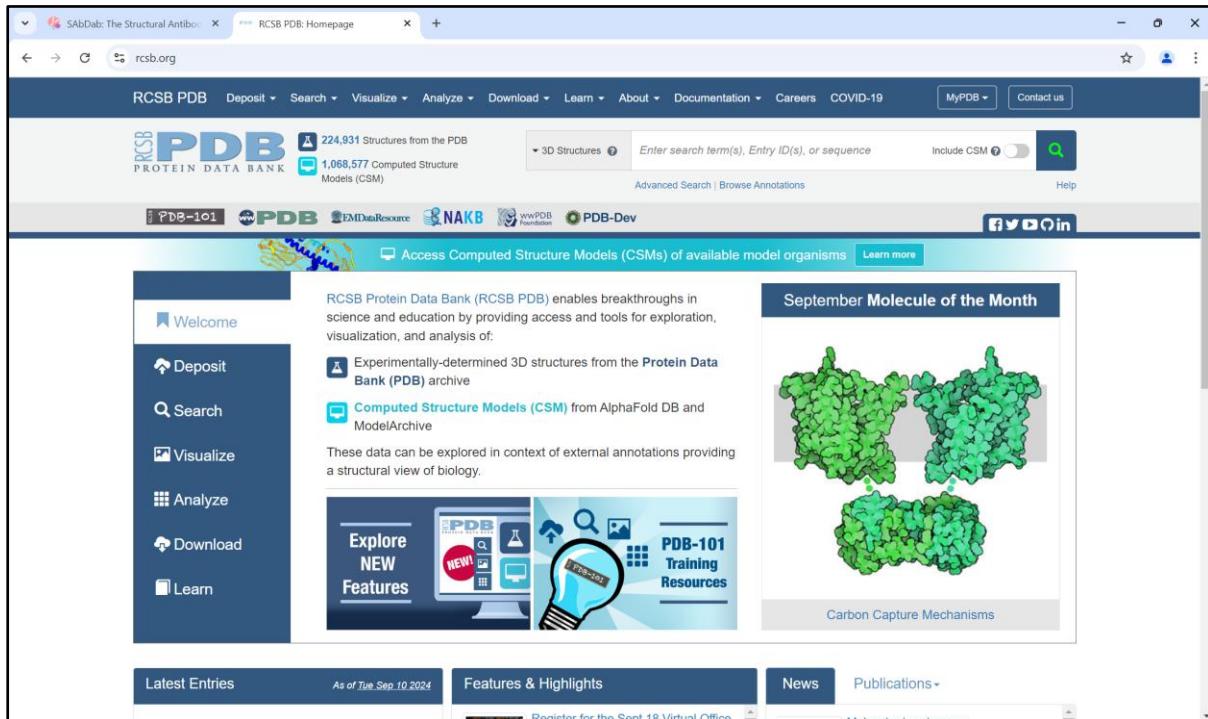


Fig 1: Homepage of the Protein Data Bank (PDB) database

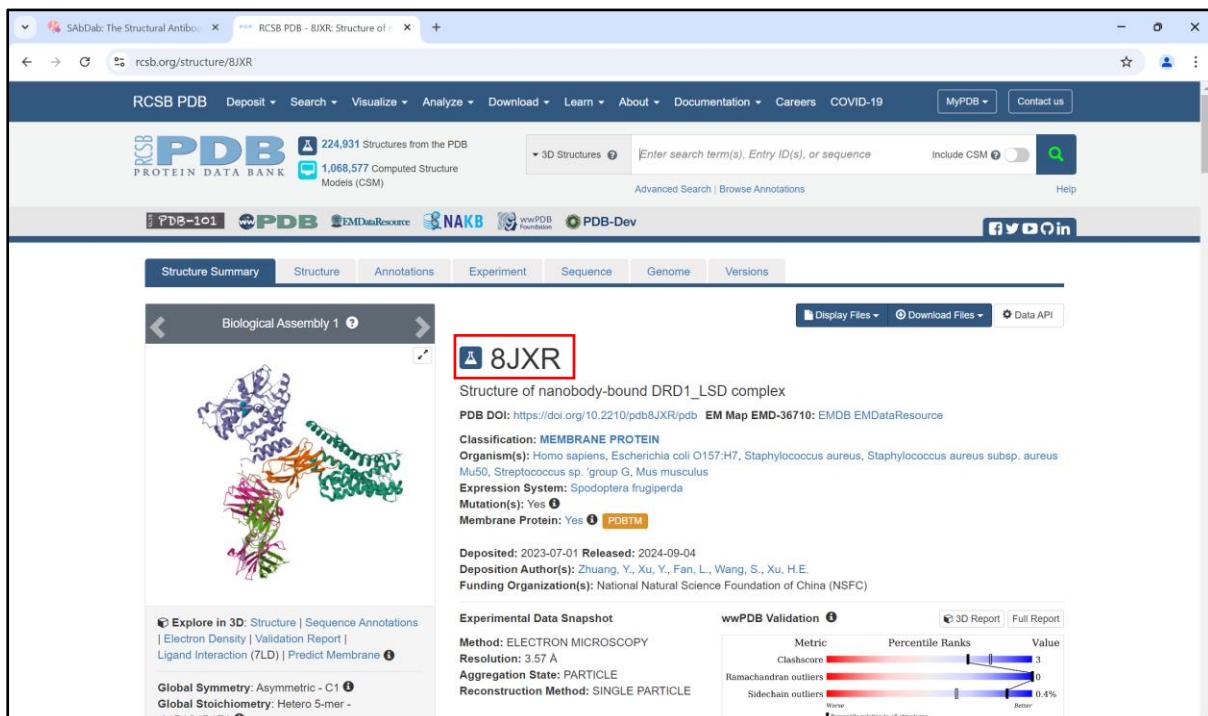


Fig 2: Retrieving the query 'DRD1_LSD complex' (PDB ID: 8JXR) from the PDB database

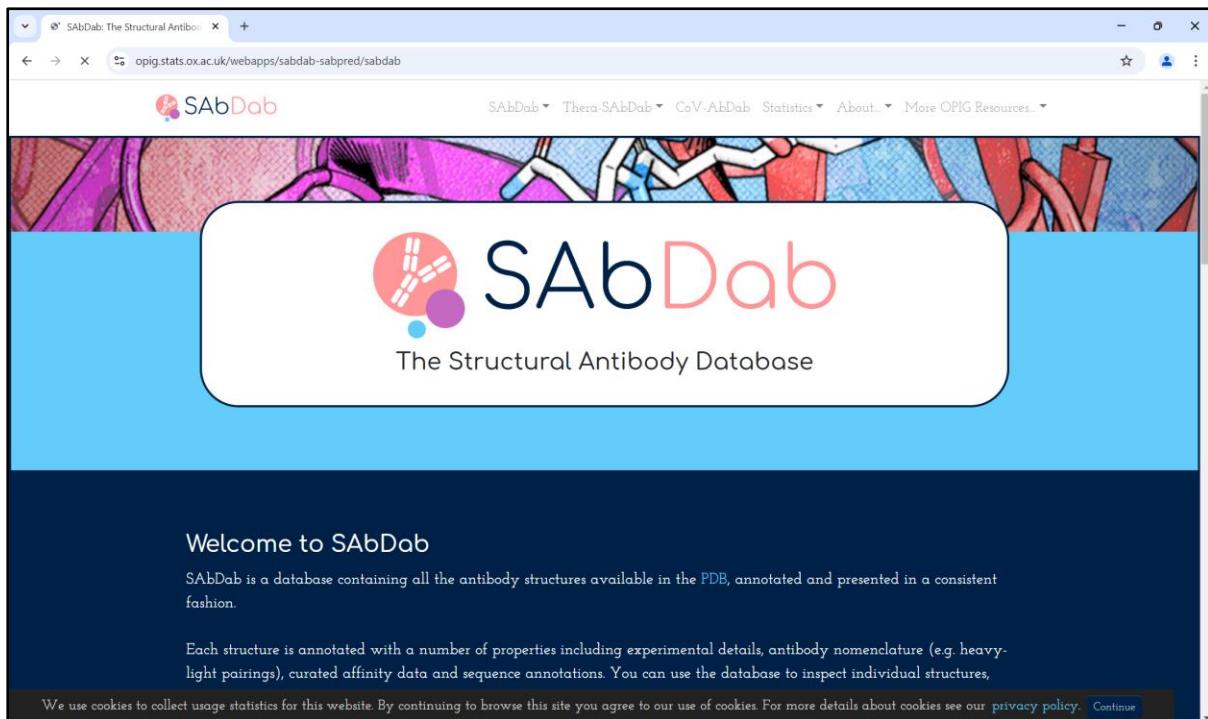


Fig 3: Homepage of the Structural Antibody Database (SAbDab)

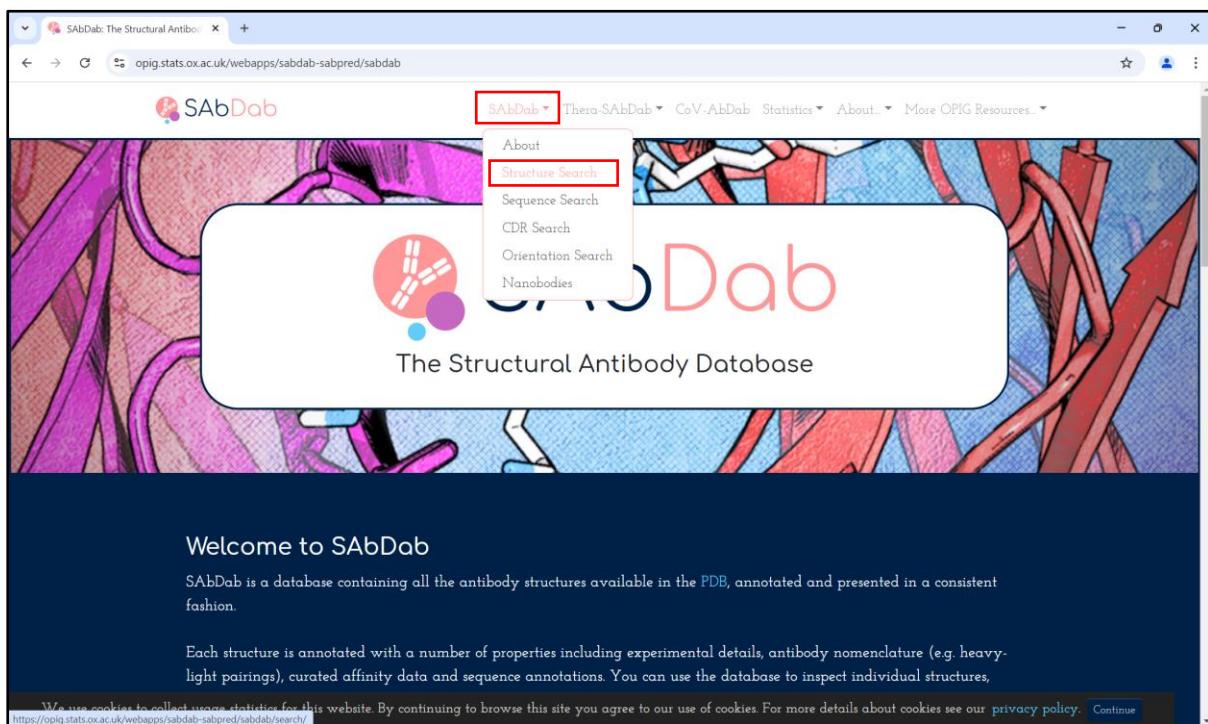


Fig 4: Selecting the ‘Structure Search’ option in the SAbDab portal

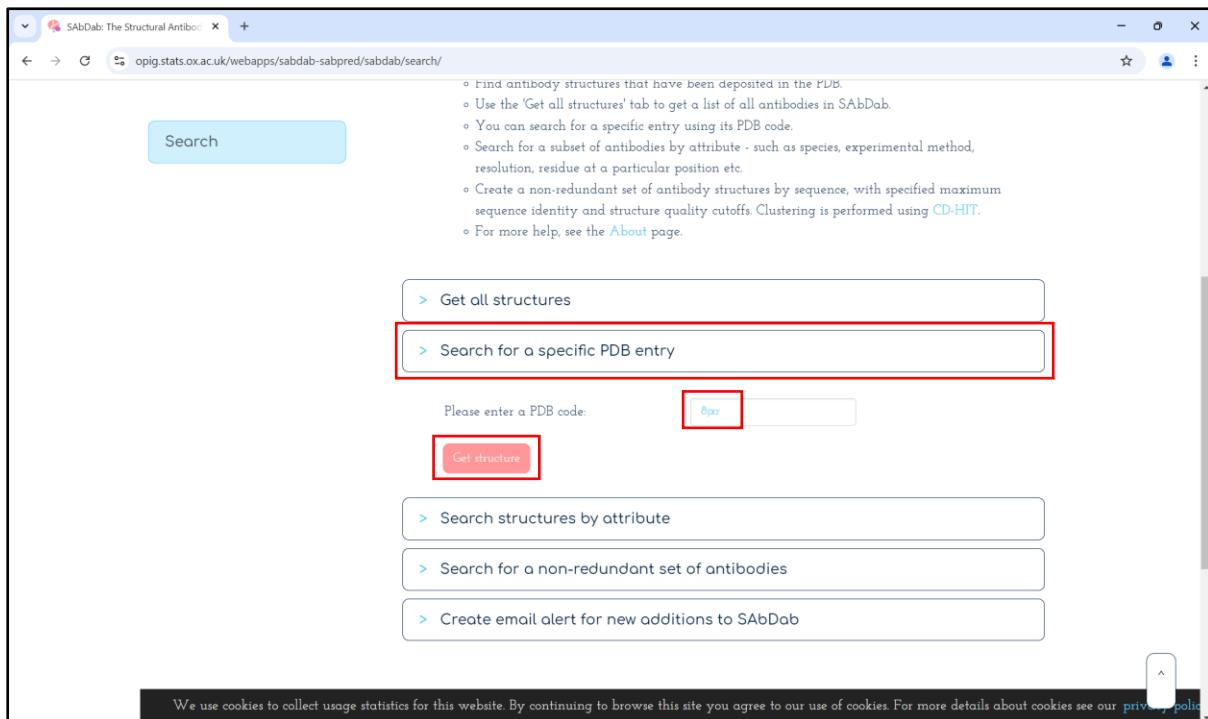


Fig 5: Selecting the ‘Search for a specific PDB entry’ option and searching for the PDB code: ‘8jxr’

The screenshot shows the search results page for the PDB ID 8jxr. The main heading is "Search Structures". On the left, there is a sidebar with links: "View results" (highlighted with a blue box), "Downloads", and "Search".

The main content area displays the search results:

1 structure(s) fit your criteria. Click on the PDB code to view the structure.

PDB	Species	Method	Resolution	Chain Pairings	Antigens	Downloads
8jxr	MUS MUSCUS; HOMO SAPIENS	ELECTRON MICROSCOPY	3.57 Å	Fv no. 1: VH: H VL: L Fv no. 2: VH: B	protein	<input type="radio"/> Structure (as PDB) <input type="radio"/> Structure (Chothia) <input type="radio"/> Structure (IMGT) <input type="radio"/> Summary File

At the bottom, there is a cookie consent message: "We use cookies to collect usage statistics for this website. By continuing to browse this site you agree to our use of cookies. For more details about cookies see our [privacy policy](#)".

Fig 6: Search Results obtained for searching the PDB ID: 8JXR

The screenshot shows the SAbDab Structure Viewer interface for PDB entry 8jxr. The main title "Structure Viewer: 8jxr" is displayed prominently at the top. On the left, a vertical navigation menu includes "Details" (which is selected and highlighted in blue), "Visualisation", "Fvs", "Downloads", and "PDB ^". The "Structure details" section contains the following information:

PDB	8jxr
Species	MUS MUSCULUS; HOMO SAPIENS
Method	ELECTRON MICROSCOPY
Resolution	3.57 Å
Number of Fvs	2
In complex	True

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Fig 7: Results obtained for the particular entry

This screenshot is identical to Fig 7, but the "Details" tab in the navigation menu is highlighted with a red box, indicating it is the active selection.

Fig 8: Results obtained: ‘Structure Details’

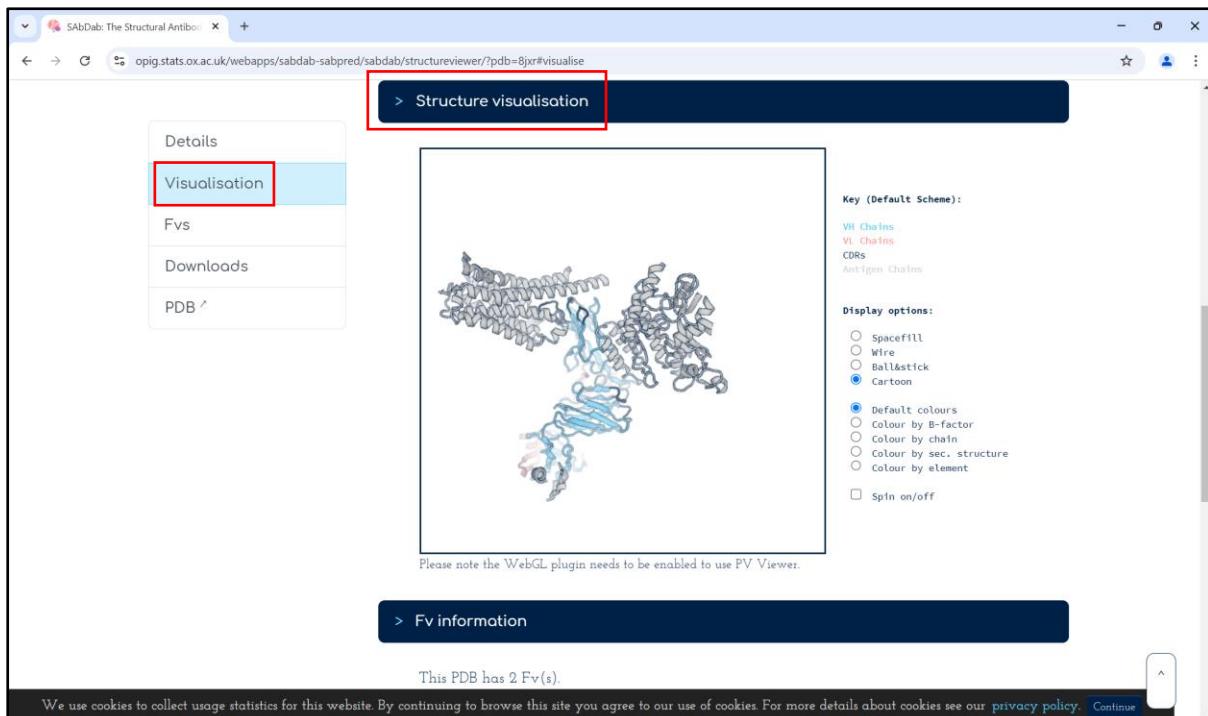


Fig 9: Results obtained: ‘Visualization’

The screenshot shows the SabDab structure viewer interface. The URL is opig.stats.ox.ac.uk/webapps/sabdab-sabpred/sabdab/structureviewer/?pdb=8jxr#chains. A red box highlights the 'Fvs' button in the sidebar. Another red box highlights the 'Fv information' button at the top. The main area shows 'This PDB has 2 Fv(s)'. Below it, a section for the first Fv (H/L) is shown with a red box highlighting the 'Fv Details' header. The details include Heavy chain (H), Light chain (L), Heavy subgroup (IGHV5), Light subgroup (IGKV8), Species (MUS MUSCULUS), In complex? (True), scFv? (False), and Has constant domain? (True). At the bottom, there is a 'Numbered Sequences (chothia)' table for the Heavy chain:

Heavy chain	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
D	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	K	S	

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Fig 10: Results obtained: ‘Fv Information’ [Header information for the 1st Fv (H/L)]

The screenshot shows the SABDab structure viewer interface. On the left, a sidebar menu includes 'Details', 'Visualisation', 'Fvs' (selected), 'Downloads', and 'PDB'. The main panel has a header 'Has constant domain? True'. Below this, there are two tables of amino acid sequences:

- Heavy chain:** Rows 21 to 37. A red box highlights the sequence from 26 to 37: S G F T F S N F.
- Light chain:** Rows 23 to 37. A red box highlights the sequence from 24 to 37: C K S T Q S I L Y N S N Q K T Y L.

Below the sequences is a section titled 'Antigen Details' containing the following information:

- Antigen chains: C
- Antigen type: protein
- Antigen name: maltose/maltodextrin-binding periplasmic protein, immunoglobulin g-binding protein a, immunoglobulin g-binding protein g
- Antigen species: ESCHERICHIA COLI O157:AUREUS, STAPHYLOCOCCUS AUREUS SUBSP. AUREUS MUS6, STREPTOCOCCUS SP. 'GROUP G'

At the bottom, a cookie consent banner reads: 'We use cookies to collect usage statistics for this website. By continuing to browse this site you agree to our use of cookies. For more details about cookies see our [privacy policy](#). [Continue](#)'.

Fig 10a: Marked positions for the heavy chain and light chain for the 1st Fv (H/L) through the chothia numbering system

This screenshot shows the same SABDab interface as Fig 10a, but the 'Antigen Details' section is highlighted with a red box. The information is identical to Fig 10a.

Below the antigen details is a section titled 'Antigen sequence' containing a long sequence of amino acids:

```

MGSSHHHHHHSSGLVPRGSHMKIEEGKLVIWINGDKGYNGLAEVG
KFKEKOTGIKTVHEPKLEKTFPVAAATGGDPDIFWAIDRFGG
YAQSGLLAEITPDKAFQOKLYPFTWDARYNGKLAYPIAVEALS
LIYNKOLLNPWPKTWEETPALDKELKAKGKSALMNLNQEPPYTMW
LIAADGGYAFKYENGKYOIKODVGNAGAKAGLTFLVLDLKHKHM
NADTDYSAEAFNKGETAMTITNGPWNNSNIDTSKVNYGTVLPT
FKGQPSPKFVGVLSAGINAASPILAKEFLENYLDEGLEAVN
KOKPLGAVALKSVEELAKOPRTIAATNEAQKGEMLPNIPQMSAF
WYAVRTAVINAAASGRQTVDQALAFQQLIMPNTTEQRNGFSQL
KDDPSVSKELIAEAKLNHEQAPKGSSGGSGSDQGSASFYELNM
PNLNEAQNGFIQSLKQDPSQSTNVLGAEAKLNESQAGGGSSGGG
GGSATVTTKLVINGKTLGEETTAKVDAETAEKAFKQYANDGV
GWTYDDATKTFVTVEGSG

```

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Fig 10b: Antigen details for the 1st Fv (H/L)

The screenshot shows the SabDab structure viewer interface for PDB ID 8jxr#chains. The left sidebar has tabs for Details, Visualisation, Fvs (selected), Downloads, and PDB. The main content area is divided into two sections: 'CDR Sequences (chothia definition)' and 'Orientation Angles (from Abangle)'. Both sections are highlighted with red boxes.

CDR Sequences (chothia definition)

CDRH1	GFTFSNF
CDRH2	SSGSTT
CDRH3	RPLYDGDYGYPMDY
CDRL1	KSTQSILYNSNQKTYLA
CDRL2	WASTRAS
CDRL3	HQYLSAWT

Orientation Angles (from Abangle)

HL	-62.43°
HC1	73.79°
HC2	118.97°
LC1	120.44°
LC2	80.93°
dc	16.6 Å

> B/-

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Fig 10c: CDR Sequences (chothia definition) and Orientation angles for the 1st Fv (H/L)

The screenshot shows the SabDab structure viewer interface for PDB ID 8jxr#chains. The left sidebar has tabs for Details, Visualisation, Fvs (selected), Downloads, and PDB. The main content area is divided into three sections: 'Fv Details', 'Numbered Sequences (chothia)', and 'Antigen Details'. The 'Fv Details' section is highlighted with a red box.

Fv Details

Heavy chain	B
Light chain	
Heavy subgroup	IGHV1
Light subgroup	NA
Species	HOMO SAPIENS
In complex?	True
scFv?	False
Has constant domain?	False

Numbered Sequences (chothia)

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Q	V	Q	L	Q	E	S	G	G	G	L	V	Q	A	G	G	S

Antigen Details

Antigen chains	A
Antigen type	protein

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Fig 11: Results obtained: ‘Fv Information’ [Header information for the 2nd Fv (B/-)]

The screenshot shows the SabDab structure viewer interface. On the left, a sidebar menu includes 'Details', 'Visualisation', 'Fvs' (selected), 'Downloads', and 'PDB'. The main panel displays 'Fv Details' for chain B, including Heavy chain (B), Light chain (IGHV1), Heavy subgroup (NA), Light subgroup (NA), Species (HOMO SAPIENS), In complex? (True), scFv? (False), and Has constant domain? (False). Below this is a section titled 'Numbered Sequences (chothia)' which shows the amino acid sequence of the Heavy chain from position 21 to 37. The sequence is: S C A A S G S I F A L N I M G W Y. The positions are numbered 21 through 37 above the sequence. At the bottom of the main panel, there is a 'Antigen Details' section for chain A, listing Antigen chains (A), Antigen type (protein), Antigen name (d(1a) dopamine receptor), and Antigen species (HOMO SAPIENS). A cookie consent banner at the bottom states: 'We use cookies to collect usage statistics for this website. By continuing to browse this site you agree to our use of cookies. For more details about cookies see our [privacy policy](#). [Continue](#)'.

Fig 11a: Marked positions for the heavy chain for the 2nd Fv (B/-) through the chothia numbering system

This screenshot shows the same SabDab interface as Fig 11a, but with a different focus. The 'Antigen Details' section is highlighted with a red box. It contains the same information as Fig 11a: Antigen chains (A), Antigen type (protein), Antigen name (d(1a) dopamine receptor), and Antigen species (HOMO SAPIENS). Below this is the 'Antigen sequence' section, which displays a long sequence of amino acids: GTGLVVERDFSVRILTACFLSLLILSTLLGNTLVAACAVIRFRHLR SKVTFVFSISLAVSDLLVALVNPKAVALAEIAQGMPPGSFCNIMW AFDIMCSTASIWNLVISVDRYWAISSPPRYERMRKTPKAFAFLIS VAWTLSVLISFIPVQLSHHKAKPTPSDGNATSLAEIDNCSSL SRTYAISSSVISIYIPVAIMIVITYTRIVYRAQKQRRIAALERAA VHAKNCQTTTNGNOKPVECSQPESSFKMSFRKTKLKTLSVINGV FVCWMLPFIFINCLIPCGSGETQFCFIDSNTDFVFWFOWANSA LNPIIYAFNADFRKAFSTLLGCYRLCPATNAlEVS. The 'CDR Sequences (chothia definition)' section is also highlighted with a red box and lists CDRH1 (GSIFALN), CDRH2 (HSGGT), and CDRH3 (KDFGAIVADRDY). A 'Downloads' button is visible at the bottom left, and a cookie consent banner at the bottom right.

Fig 11b: Antigen Details and CDR Sequences (chothia definition) for the 2nd Fv (B/-)

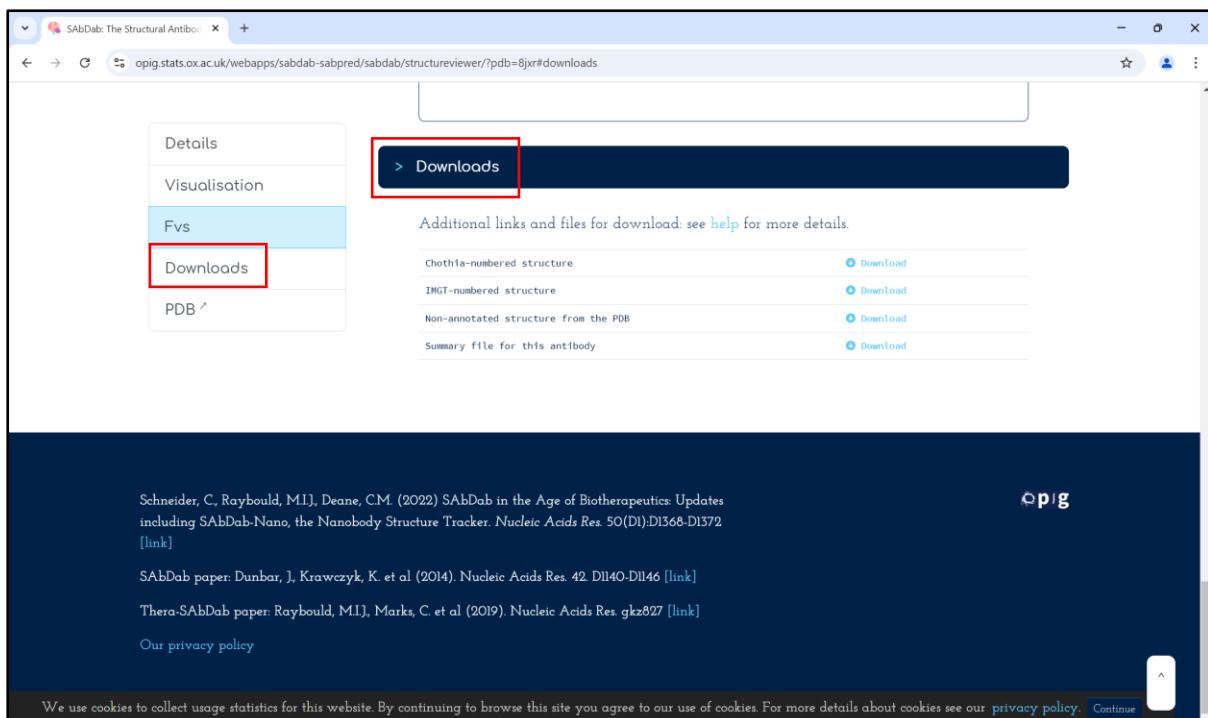


Fig 12: Links for downloading the structure under the ‘Downloads’ section

RESULTS:

The query ‘DRD1_LSD complex’ (PDB ID: 8JXR) was searched and studied using the Structural Antibody Database (SAbDab). Following information was studied for the selected entry:

1. Structure Details:

Name	Structure of Nanobody-Bound Drd1_Lsd complex
PDB	8jxr
Species	MUS MUSCULUS; HOMO SAPIENS
Method	ELECTRON MICROSCOPY
Resolution	3.57Å
Number of Fvs	2
In complex	True
Light chain type	Kappa,NA
Has constant region	True

2. Structure Visualization:

The structure can be observed in terms of VH Chains, VL Chains and CDRs in the form of various display options (example: Wire) and colours.

3. Fvs:

Following information was studied for each of the Fvs:

Fv	Header Information		Numbered Sequences (chothia)
H/L	Heavy chain	H	Heavy chain: 26 – 32, 52 – 56, 95 – 102 Light chain: 24 – 34, 50 – 56, 89 – 97
	Light chain	L	
	Heavy subgroup	IGHV5	
	Light subgroup	IGKV8	
	Species	MUS MUSCULUS	
	In complex?	True	
	scFv?	False	
	Has constant domain?	True	
B/-	Heavy chain	B	Heavy chain: 26 – 32, 52 – 56, 95 – 102
	Light chain		
	Heavy subgroup	IGHV1	
	Light subgroup	NA	
	Species	HOMO SAPIENS	
	In complex?	True	
	scFv?	False	
	Has constant domain?	False	

Further information was studied about antigen details, CDR sequences (chothia definition) and orientation angles for each of the Fvs.

4. Downloads:

Various links have been provided for the downloading:

- a. Chothia-numbered structure
- b. IMGT-numbered structure
- c. Non-annotated structure from the PDB
- d. Summary file for this antibody

CONCLUSION:

The Antibody structure for the query ‘DRD1_LSD complex’ (PDB ID: 8JXR) was studied using the Structural Antibody Database (SAbDab). The SAbDab entry page provides extensive details about the antibody structure, including:

1. **Structure details:** resolution, R-factors, experimental method, etc.
2. **Visualization tools:** to view the antibody structure
3. Information on the **antibody variable (Fv) regions**
4. Options to **download** the structure coordinates in various formats

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 3. wwPDB.org. (n.d.). wwPDB: 8JXR. https://www.wwpdb.org/pdb?id=pdb_00008jxr
-

WEBLEM: 3

AntiBodies Chemically Defined Database (ABCD)

(URL: <https://web.expasy.org/abcd/>)

AIM:

To study antibody sequence using ABCD database.

INTRODUCTION:

The ABCD (for AntiBodies Chemically Defined) database is a repository of sequenced antibodies, integrating curated information about the antibody and its antigen with cross-links to standardized databases of chemical and protein entities. It is freely available to the academic community, accessible through the ExPASy server (<https://web.expasy.org/abcd/>). The ABCD database aims at helping to improve reproducibility in academic research by providing a unique, unambiguous identifier associated to each antibody sequence. It also rapidly determines whether a sequenced antibody is available for a given antigen.

The ABCD (AntiBodies Chemically Defined) database is a manually curated repository of sequenced antibodies, developed by the Geneva Antibody Facility at the University of Geneva, in collaboration with the CALIPHO and Swiss-Prot groups at SIB Swiss Institute of Bioinformatics. The ABCD database is part of a broader project, aiming to promote the widespread use of recombinant antibodies by academic researchers and, ultimately, the replacement of animal-produced antibodies. This concerted effort also includes the Geneva Antibody Facility (for discovering and producing antibodies) and the scientific journal Antibody Reports (publishing technical articles on antibody characterization).

ABCD is a huge collection of AD-related data of molecular markers. The web interface contains information concerning the proteins, genes, transcription factors, SNPs, miRNAs, mitochondrial genes, and expressed genes implicated in AD pathogenesis. In addition to the molecular-level data, the database has information for animal models, medicinal candidates and pathways involved in the AD and some image data for AD patients.

The ABCD (AntiBodies Chemically Defined) Database

The ABCD (AntiBodies Chemically Defined) database is a manually curated depository of **sequenced antibodies**, developed by the [Geneva Antibody Facility](#) at the University of Geneva, in collaboration with the [CALIPHO](#) and [Swiss-Prot](#) groups at [SIB Swiss Institute of Bioinformatics](#).

Search by antibody name, species or target ([UniProt](#) or [ChEBI](#) ID)

Example searches: 9E10 , P07766 , 37926 , Escherichia coli , Protein tag , Nanobody

The ABCD database is part of a broader project, with the mission of promoting the widespread use of **recombinant antibodies** by academic researchers and, ultimately, the replacement of animal-produced antibodies. This concerted effort also includes the [Geneva Antibody Facility](#) (for discovery and production of antibodies) and the scientific journal [Antibody Reports](#) (publishing technical articles on antibody characterization).

Release information: Version 14.0 (January 2024)
26'410 sequenced antibodies, against 4'292 different targets
If you'd like to cite the ABCD database: Lima WC, Gasteiger E, Marcatili P, Duek P, Bairoch A, Cosson P. The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Res.* 2020, 48:D261-D264. doi: 10.1093/nar/gkz714

Fig 1: Homepage of ABCD Database

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WEBLEM 3(A)

AntiBodies Chemically Defined Database (ABCD)

(URL: <https://web.expasy.org/abcd/>)

AIM:

To study 'Insulin' antibody sequence using ABCD Database.

INTRODUCTION:

ABCD Database

Antibodies are one of the most widespread tools used in biological sciences. However, they are currently deemed one of the major culprits in the reproducibility crisis plaguing bio-medical research. Problems include batch-to-batch variability, poorly characterized and/or non-validated antibodies that sometimes do not recognize the presumptive target, or recognize more than one target, lack of explicitly described procedures adapted to each antibody, decreasing scrutiny of results by scientists and misleading antibody nomenclature. The 2 million antibodies available on the market might represent as few as 250,000 actual clones.

The ABCD (for AntiBodies Chemically Defined) database is a repository of sequenced antibodies, integrating curated information about the antibody and its antigen with cross-links to standardized databases of chemical and protein entities. It is freely available to the academic community, accessible through the ExPASy server (<https://web.expasy.org/abcd/>). The ABCD database aims at helping to improve reproducibility in academic research by providing a unique, unambiguous identifier associated to each antibody sequence. It also allows to determine rapidly if a sequenced antibody is available for a given antigen.

Insulin

Insulin, a crucial hormone for glucose metabolism, can elicit an immune response in some individuals, leading to the production of antibodies against it. These antibodies can significantly impact insulin's effectiveness and the management of diabetes.

Types of Insulin Antibodies

1. **Insulin Autoantibodies (IAA):** These antibodies develop spontaneously in some individuals, particularly children, before they are exposed to exogenous insulin. They are often associated with the onset of Type 1 diabetes.
2. **Insulin Antibodies (IA):** These are typically produced in response to exogenous insulin therapy. They can be of various immunoglobulin classes, with IgG being the most prevalent. Other classes include IgM, IgA, and occasionally IgE, which is linked to allergic reactions.

Insulin antibodies can bind to insulin molecules, potentially forming complexes that reduce the availability of free insulin in circulation. This can lead to increased insulin resistance and variability in blood glucose levels. High levels of insulin antibodies may diminish the action

of insulin, causing issues such as hyperglycemia or hypoglycemia. In some cases, these antibodies can even act as agonists at the insulin receptor, leading to unexpected hypoglycemic episodes. The presence of these antibodies is not always clinically significant; however, they can complicate diabetes management by necessitating adjustments in therapy or insulin type. The interaction between insulin and its antibodies is a complex aspect of diabetes management that requires careful monitoring and understanding. While not all individuals on insulin therapy will develop significant antibody responses, those who do may face challenges that necessitate tailored therapeutic approaches.

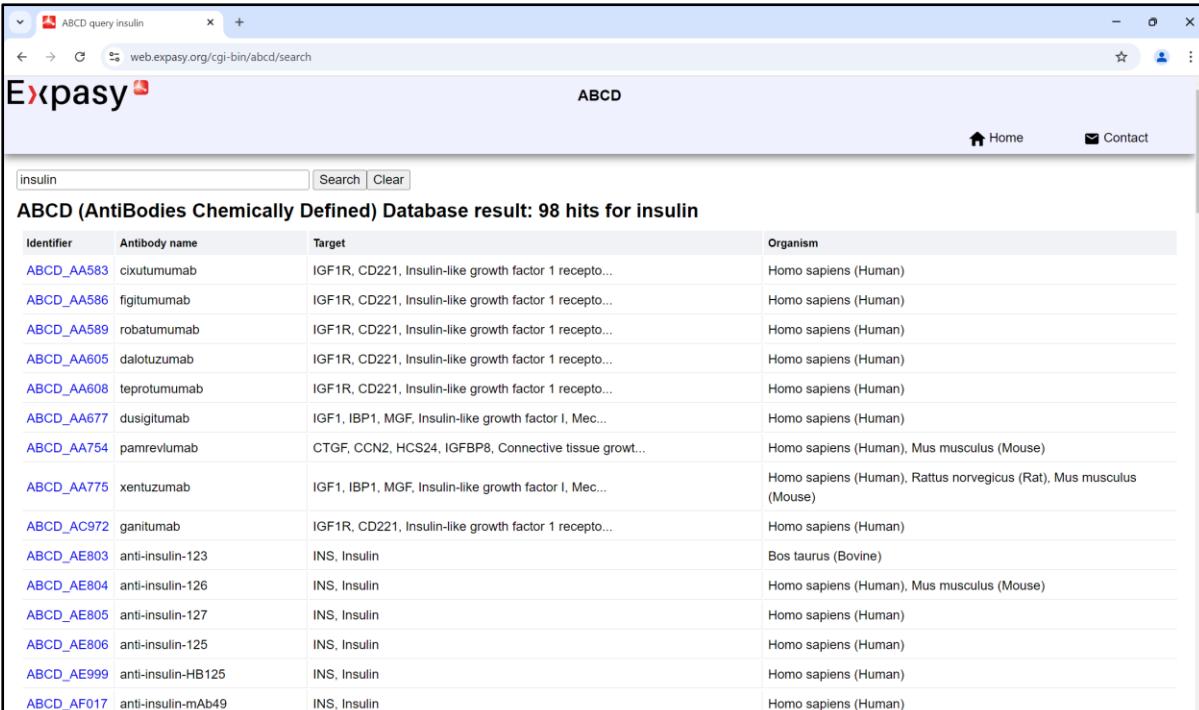
METHODOLOGY:

1. Open the home page of ABCD Database (URL: <https://web.expasy.org/abcd/>)
2. Search for query ‘Insulin’.
3. Open one entry (ID: ABCD_AC463) from the obtained entries.
4. Interpret the results.

OBSERVATIONS:

The screenshot shows the ABCD (AntiBodies Chemically Defined) Database homepage. At the top, there is a search bar with placeholder text "Search by antibody name, species or target (UniProt or ChEBI ID)" and buttons for "Search" and "Clear". Below the search bar, there is a section titled "Example searches: 9E10 , P07766 , 37926 , Escherichia coli , Protein tag , Nanobody". To the right of the search bar, there is a large circular logo for the "GENEVA ANTIBODY FACILITY". From this central circle, four arrows point to four smaller circles representing different processes: "Discovery" (with a hand icon), "Production" (with a factory icon), "Database" (with a database icon), and "Hybridoma Sequencing" (with a DNA helix icon). The main content area contains text about the database being a manually curated depository of sequenced antibodies, developed by the Geneva Antibody Facility at the University of Geneva, in collaboration with the CALIPHO and Swiss-Prot groups at SIB Swiss Institute of Bioinformatics. It also mentions that the database is part of a broader project to promote the widespread use of recombinant antibodies and includes the Geneva Antibody Facility (for discovery and production of antibodies) and the scientific journal *Antibody Reports* (publishing technical articles on antibody characterization). Release information is provided, stating Version 15.0 (September 2024) with 28'088 sequenced antibodies against 4'259 different targets. There is also a citation for the database in a scientific paper: Lima WC, Gasteiger E, Marcattili P, Duek P, Baiocco A, Cosson P. The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Res.* 2020, 48:D261-D264. doi: 10.1093/nar/gkz714. Navigation links include "About us", "Frequently asked questions (FAQ)", "Submit a new Antibody", and "Antibodies to Protein tags and Subcellular markers".

Fig 1: Homepage of ABCD database



ExPasy ABCD

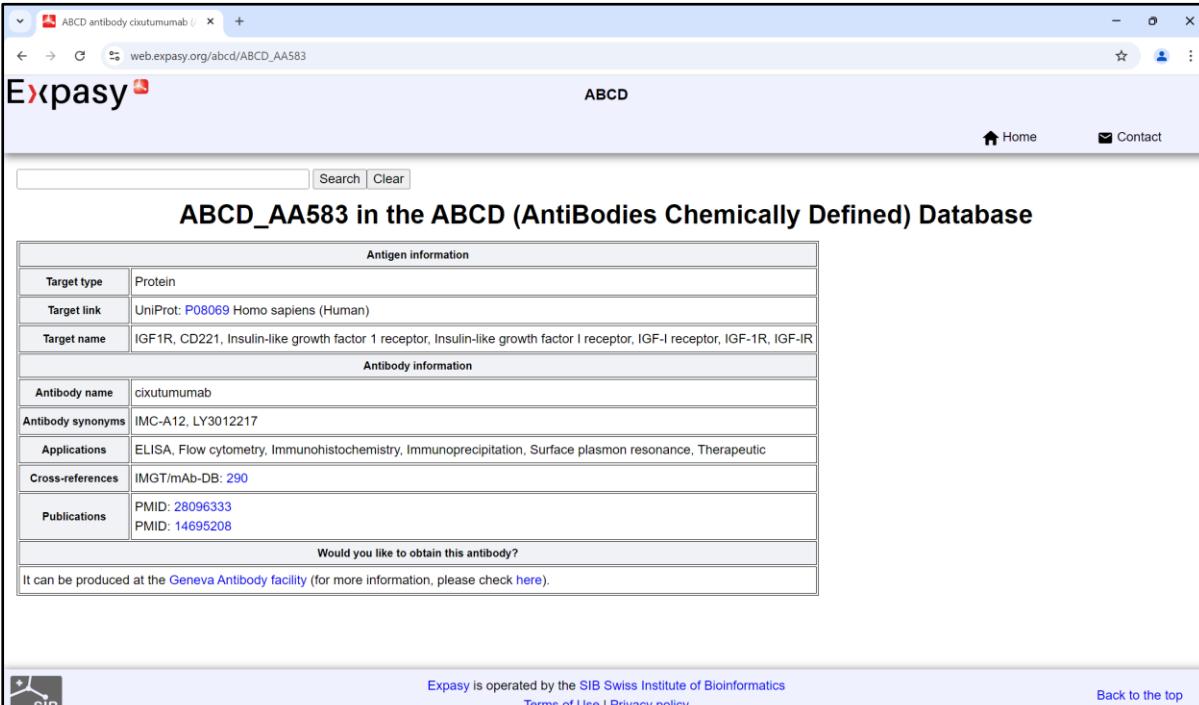
Home Contact

insulin Search Clear

ABCD (AntiBodies Chemically Defined) Database result: 98 hits for insulin

Identifier	Antibody name	Target	Organism
ABCD_AA583	cixutumumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AA586	figitumumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AA589	robatumumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AA605	dalotuzumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AA608	tepotumumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AA677	dusigatumab	IGF1, IBP1, MGF, Insulin-like growth factor I, Mec...	Homo sapiens (Human)
ABCD_AA754	pamrevlumab	CTGF, CCN2, HCS24, IGFBP8, Connective tissue growth factor, Insulin-like growth factor I, Mec...	Homo sapiens (Human), Mus musculus (Mouse)
ABCD_AA775	xentuzumab	IGF1, IBP1, MGF, Insulin-like growth factor I, Mec...	Homo sapiens (Human), Rattus norvegicus (Rat), Mus musculus (Mouse)
ABCD_AC972	ganitumab	IGF1R, CD221, Insulin-like growth factor 1 receptor, IGF-I receptor, IGF-1R, IGF-IR	Homo sapiens (Human)
ABCD_AE803	anti-insulin-123	INS, Insulin	Bos taurus (Bovine)
ABCD_AE804	anti-insulin-126	INS, Insulin	Homo sapiens (Human), Mus musculus (Mouse)
ABCD_AE805	anti-insulin-127	INS, Insulin	Homo sapiens (Human)
ABCD_AE806	anti-insulin-125	INS, Insulin	Homo sapiens (Human)
ABCD_AE999	anti-insulin-HB125	INS, Insulin	Homo sapiens (Human)
ABCD_AF017	anti-insulin-mAb49	INS, Insulin	Homo sapiens (Human)

Fig 2: Result page for the query ‘Insulin’



ExPasy ABCD

Home Contact

Search Clear

ABCD_AA583 in the ABCD (AntiBodies Chemically Defined) Database

Antigen information	
Target type	Protein
Target link	UniProt: P08069 Homo sapiens (Human)
Target name	IGF1R, CD221, Insulin-like growth factor 1 receptor, Insulin-like growth factor I receptor, IGF-I receptor, IGF-1R, IGF-IR
Antibody information	
Antibody name	cixutumumab
Antibody synonyms	IMC-A12, LY3012217
Applications	ELISA, Flow cytometry, Immunohistochemistry, Immunoprecipitation, Surface plasmon resonance, Therapeutic
Cross-references	IMGT/mAb-DB: 290
Publications	PMID: 28096333 PMID: 14695208
Would you like to obtain this antibody?	
It can be produced at the Geneva Antibody facility (for more information, please check here).	

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Back to the top

Fig 3: Information obtained after selecting the entry with the identifier: ABCD_AA583

RESULTS:

ABCD Database was explored to study the antigen-antibody information for query ‘Insulin’. The query was searched and 98 hits were obtained. Out of which one entry was selected and studied that contained information about target type, target link (UniProt: P08069), antibody name, cross references, etc.

CONCLUSION:

ABCD Database was explored for query ‘Insulin’ for antigen and antibody information. ABCD Database represents a vital resource for organizations seeking to optimize data management and analysis. Its combination of usability, analytical capabilities, and security makes it an invaluable tool for driving informed decisions and enhancing operational efficiency. As organizations increasingly rely on data-driven strategies, the ABCD Database is well-positioned to support their needs effectively.

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1. Lima, W. C., Gasteiger, E., Marcatili, P., Duek, P., Bairoch, A., & Cosson, P. (2019). The ABCD database: a repository for chemically defined antibodies. *Nucleic Acids Research*, 48(D1), D261–D264. <https://doi.org/10.1093/nar/gkz714>.
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WEBLEM 4

Antibody Numbering using KabatMan and Chothia Database and AbRSA Numbering tool as Demo

AIM:

To use KabatMan database and AbRSA numbering tool as demo.

INTRODUCTION:

An important prerequisite for antibody humanization requires standardized numbering methods to define precisely complementary determining regions (CDR), frameworks and residues from the light and heavy chains that affect the binding affinity and/or specificity of the antibody-antigen interaction. The recently generated deep-sequencing data and the increasing number of solved three-dimensional structures of antibodies from human and non-human origins have led to the emergence of numerous databases. However, these different databases use different numbering conventions and CDR definitions. In addition, the large fluctuation of the variable chain lengths, especially in CDR3 of heavy chains (CDRH3), hardly complicates the comparison and analysis of antibody sequences and the identification of the antigen binding residues. This review compares and discusses the different numbering schemes and “CDR” definition that were established up to date. Furthermore, it summarizes concepts and strategies used for numbering residues of antibodies and CDR residues identification. Finally, it discusses the importance of specific sets of residues in the binding affinity and/or specificity of immunoglobulins.

Antibody engineering methods require precise identification of the residues that have an impact on the interaction or affinity of the antibody for its target antigen. CDR-grafting aims to decrease the immunogenicity of non-human antibodies by engineering the variable regions directed against the target antigen. This method requires an accurate identification of the CDRs and therefore an adequate alignment of antibody sequences from human and non-human species. Moreover, it has been shown that residues from the framework regions might also exert a strong impact on the antibody affinity. Thus, the precise identification of corresponding positions in human and animal immunoglobulin chains is essential. However, the use of different amino acid numbering schemes currently available in the literature is confusing and might lead to aberrant identification of framework and CDR residues. Therefore, it is of crucial importance to understand the different numbering schemes and, consequently, being able to compare them.

KabatMan database

To enter KabatMan database (<http://www.bioinf.org.uk/abs/simkab.html>). The purpose of maintaining the Kabat Database of aligned sequences of proteins of immunological interest, it provides useful correlations between structure and function for this special group of proteins from their nucleotide and amino acid sequences to their tertiary structures. The Kabat Database was initially started in 1970 to determine the combining site of antibodies based on the available amino acid sequences at that time. Bence Jones proteins, mostly from human, were aligned, using the now-known Kabat numbering system, and a quantitative measure, variability, was calculated for every position. It has been used extensively by immunologists to derive useful structural and functional information from the primary sequences of these proteins. The Kabat Database may be accessed for searching, sequence retrieval and analysis by a few different methods: electronic mail, WWW and ftp.

AbRSA tool

To enter AbRSA tool enter (<http://cao.labshare.cn/AbRSA/index.html>). Antibody sequence numbering and complementarity determining region (CDR) delimitation have wide applications in antibody engineering. They are generally obtained by mapping query sequences to the retrospective patterns. However, due to the enormous diversity of antibody sequences, novel patterns are often generated in antibody affinity maturation. They may not be recognized by the traditional methods. Antibody Region-Specific Alignment (AbRSA) integrates the biological insight of antibody region-specific feature with dynamic programming to improve the robustness of antibody numbering. Benchmarks show AbRSA is a powerful method in numbering unusual antibodies and distinguishing between antibody and non-antibody sequences.

Workflow

The pipeline of AbRSA web service is shown in the following Figure. The input could be either the protein sequence or structure. Multiple protein sequences are supported if the sequences are in FASTA format. The program judges whether it is a heavy chain, light chain or neither by comparing the sequence identities with consensus sequences. After all the possible heavy or light chains are found out, the program will output the numbering results and the location of FRs and CDRs in the sequences. If the input is a protein structure (PDB format), the web page will generate its interactive 3D visualization powered by 3Dmol JavaScript library. CDRs will be highlighted in colours. The 3D view can be rotated, translated, and re-sized by dragging, scrolling the mouse. We believe this feature could help to understand where and how antibody binding with antigen.

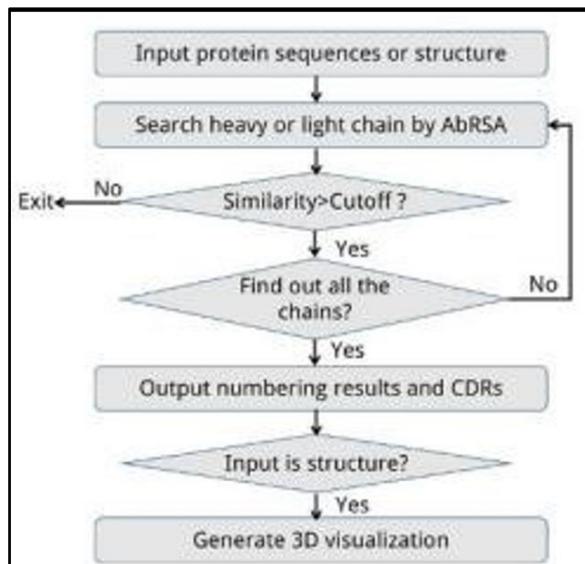


Fig 1: Workflow of AbRSA tool

OBSERVATIONS:

KabatMan Database



Fig 2: Main page to enter KabatMan Database

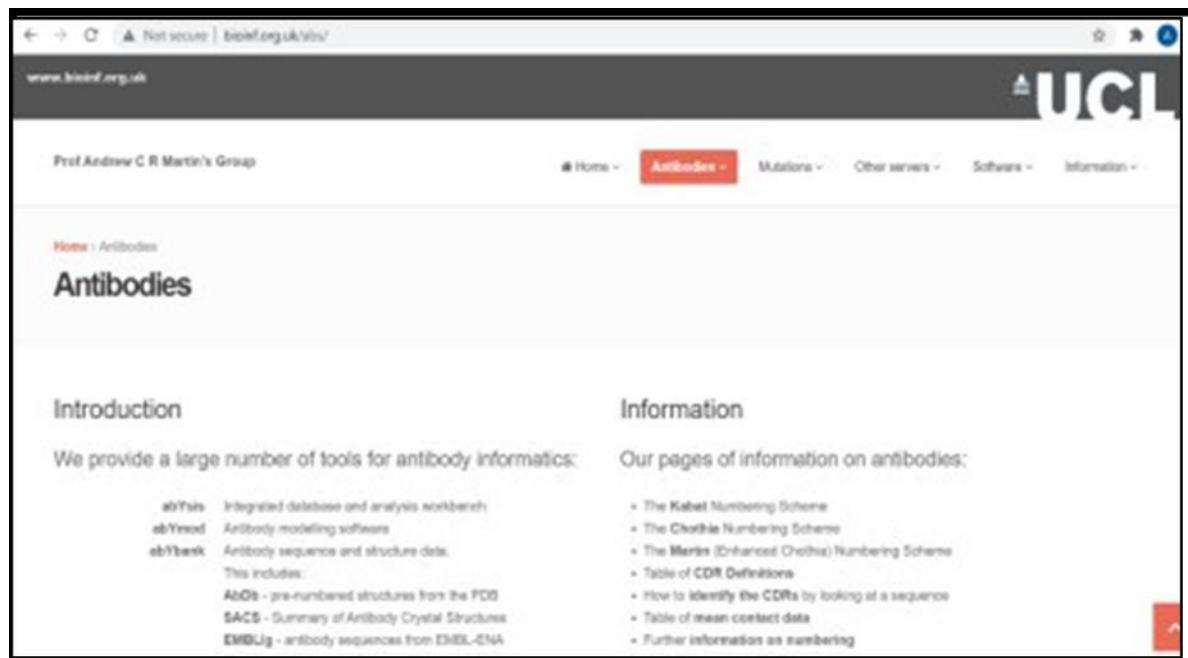


Fig 3: Antibodies page which gives the list of all available tools

A screenshot of the 'Antibodies' page from the abYsis website, focusing on the KabatMan database section. The page title 'We provide a large number of tools for antibody informatics:' is followed by a list of tools: abYsis, abYmod, abYbank, Humanness (G), and PAPS. Below this, a note states 'The following are included within abYsis, but also available as standalone tools:'. A table lists these tools: KabatMan (Query the Kabat sequence data), AbCheck (Test a sequence against the Kabat data for unusual residues), Chothia canonicals (Identify canonical classes for CDRs from your sequence), Human subgroups (Assign the human subgroup for your sequence), and Humanness (H) (Assess humanness against expressed sequences in Kabat). To the right, the 'Information' column is expanded to include 'Information and links to accompany my book chapter Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duszel, S. and Kortenmann, R., Springer-Verlag, Heidelberg). [Information] [Purchase]'. It also notes that the famous Kabat book is now available online as a scanned copy via Google Books: 'Elin A. Kabat, Tai Te Wu, Carl Fosher, Harold M. Perry, Kay S. Gottschman (1991) Sequences of Proteins of Immunological Interest.'

Fig 4: Antibodies page which shows KabatMan database

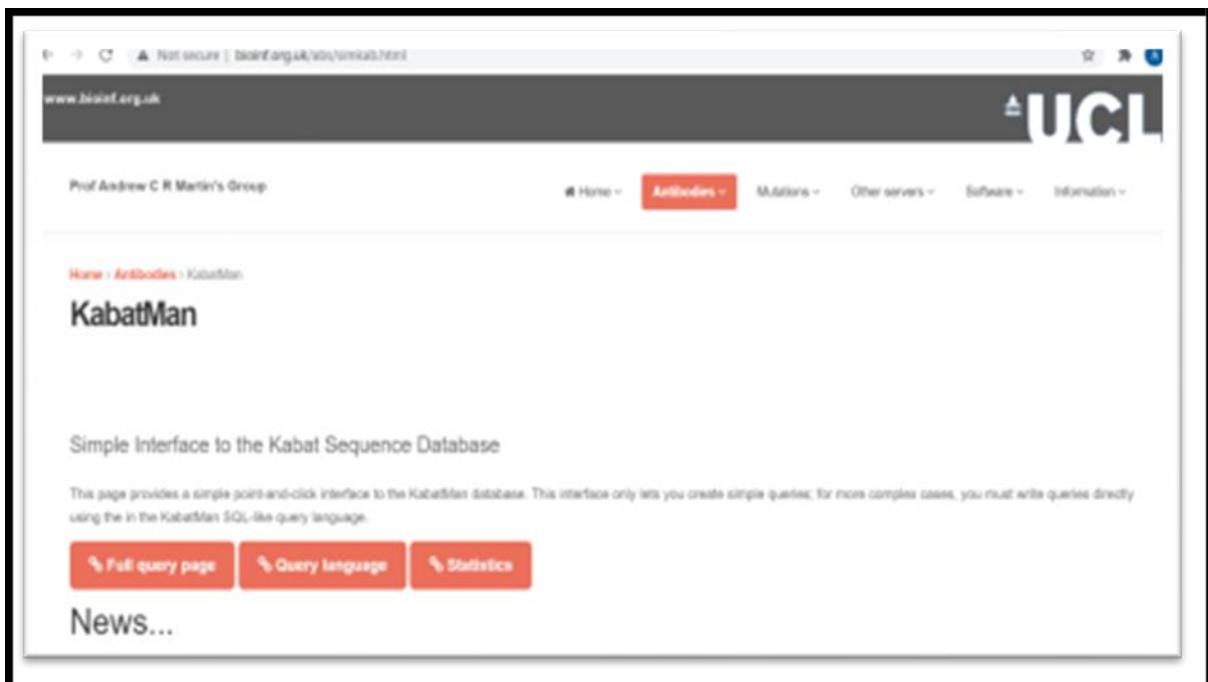


Fig 5: Homepage of KabatMan database

A screenshot of the KabatMan database showing four numbered examples of SQL queries. 1. Find all complete antibodies where the antigen is known with loop lengths:

```
SELECT name, antigen, length(l1), length(l2), length(l3),
       length(l4), length(l5), length(l6)
  WHERE antigen ne '' complete eq true AND
```

2. Get the sequences of all complete mouse antibodies which bind to lysozyme, display the results in PIR format:

```
SELECT pir
  WHERE source includes mouse
        antigen includes lysozyme AND
        complete eq true AND
```

3. Find all antibodies with 11 residue CDR-L1s and a proline at the sixth position:

```
SELECT name, ll
  WHERE len(ll) eq 11 res(L2B) eq P AND
```

4. Find all complete antibodies with the sequence Ser-Ala-Ser-Ser-Ser in the light chain:

Note that there must be no spaces in the sequence

```
SELECT name, light
  WHERE complete = 1
        light includes SASSS AND
```

Fig 6: Example queries available in KabatMan database

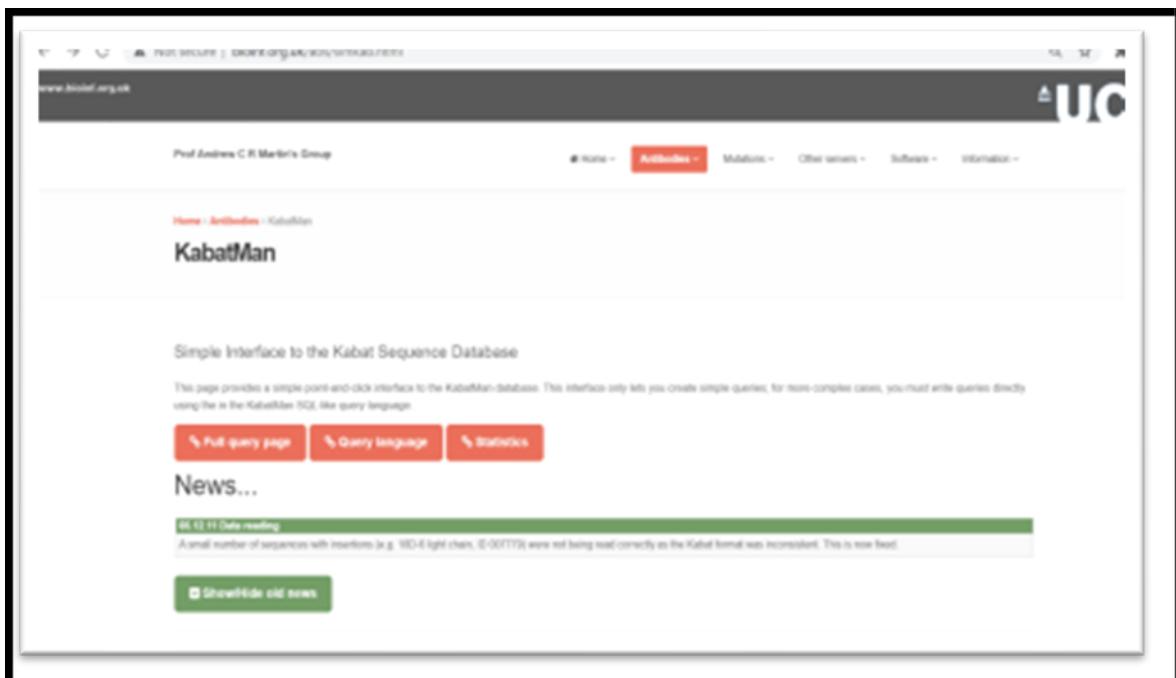


Fig 7: Click on ‘full query’ on KabatMan homepage



Fig 8: Search bar to enter the sequence taken from the PDB database

The screenshot shows a web browser window with the URL <http://blinlong.uk/lib/kabatman.cgi>. The title bar says "KabatMan Query Results". The content area displays the following text:

KabatMan V2.26

Copyright (c) 1994-2009, Dr. Andrew C.R. Martin / University College London / University of Reading.
This program is copyright. Any copying without the permission of the
author is prohibited.

The query was:

```
SELECT name, li  
WHERE len(li) eq 11 AND res(L29) eq P AND
```

Results were:

```
SL_ S6ALPQWVAF  
IS-27'CL_ S604LPQWVAF  
TCR8'CL_ S60ALPQWVAF  
CV-65'CL_ S60ALPQWVAF  
H88'CL_ S60ALPQWVAF  
RI-387'CL_ S60ALPQWVAF  
RI-112'CL_ S60ALPQWVAF  
TCR8'CL_ S60ALPQWVAF  
DR_ S60ALPQWVAF  
WP_ S60ALPQWVAF  
BB'CL_ S60ALPQWVAF  
RR_ S60ALPQWVAF  
AP_ S60VLPQWVAF  
G HER1'CL_ S60ALPQWVAF  
wigg1158'CL_ S60ALPQWVAF
```

Fig 9: Result page for the query

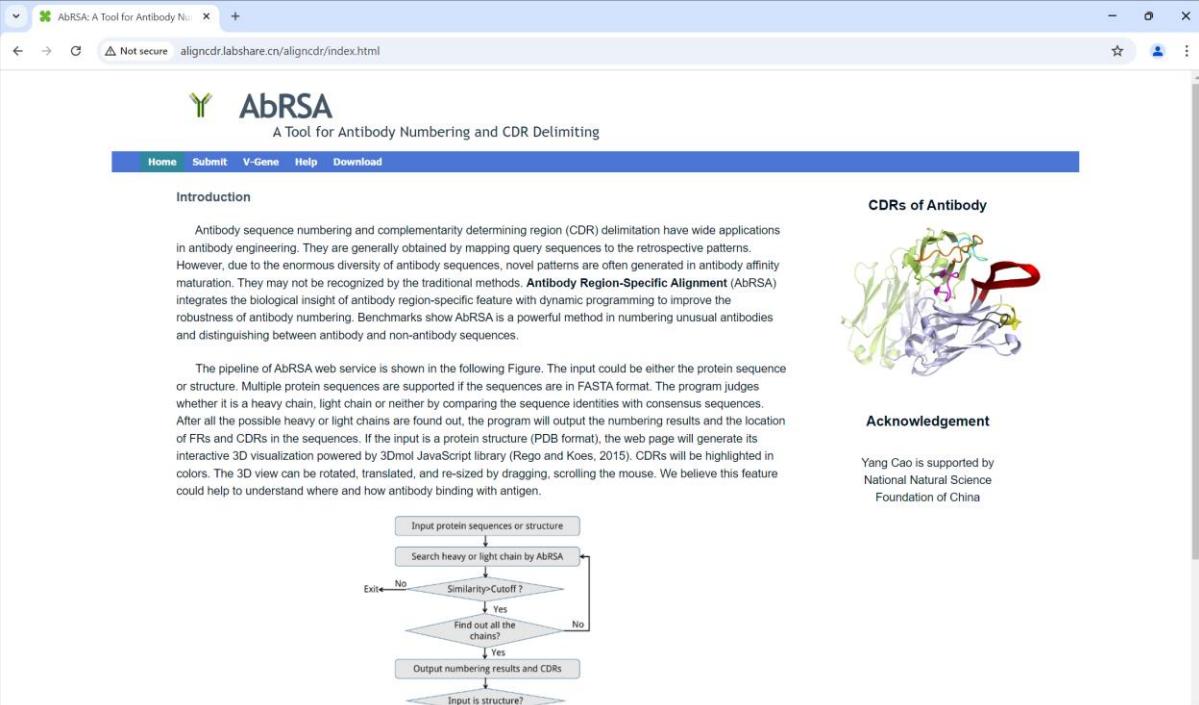
The screenshot shows a web browser window with the URL <http://blinlong.uk/lib/kabatman.cgi>. The title bar says "Not secure". The content area displays the following text:

```
C5yN1A'CL_ S60ALPQWVAF  
C5yN4G'CL_ S60ALPQWVAF  
C5yN8Z'CL_ S60ALPQWVAF  
C5yN5T'CL_ S60ALPQWVAF  
SPILL4E'CL_ S60ALPQWVAF  
SPILL4F'CL_ S60ALPQWVAF  
SPILL5F'CL_ S60ALPQWVAF  
SPILL8I'CL_ S60ALPQWVAF  
SPILL5Y'CL_ S60ALPQWVAF  
SPILL5Y'CL_ S60ALPQWVAF  
SPILL5Y'CL_ S60ALPQWVAF  
SPILL11'CL_ S60ALPQWVAF  
SPILL24'CL_ S60ALPQWVAF  
SPILL27'CL_ S60ALPQWVAF  
SPILL31'CL_ S60ALPQWVAF  
TPILL11'CL_ S60ALPQWVAF  
TPILL12'CL_ S60ALPQWVAF  
TPILL13'CL_ S60ALPQWVAF  
TPILL15'CL_ S60ALPQWVAF  
TPILL16'CL_ S60ALPQWVAF  
TPILL18'CL_ S60ALPQWVAF  
TPILL19'CL_ S60ALPQWVAF  
TPILL20'CL_ S60ALPQWVAF  
TPILL21'CL_ S60ALPQWVAF  
TPILL29'CL_ S60ALPQWVAF  
TPILL30'CL_ S60ALPQWVAF  
TPILL30'CL_ S60ALPQWVAF  
TPILL31'CL_ S60ALPQWVAF  
TPILL35'CL_ S60ALPQWVAF  
TPILL39'CL_ S60ALPQWVAF  
TPILL72'CL_ S60ALPQWVAF  
TPILL73'CL_ S60ALPQWVAF  
TPILL76'CL_ S60ALPQWVAF  
TPILL87'CL_ S60ALPQWVAF
```

| Number of Hits = 100 (Dataset created Wed Jul 22 09:58:03 2009)

Fig 10: Result page showing the number of hits for the entered query

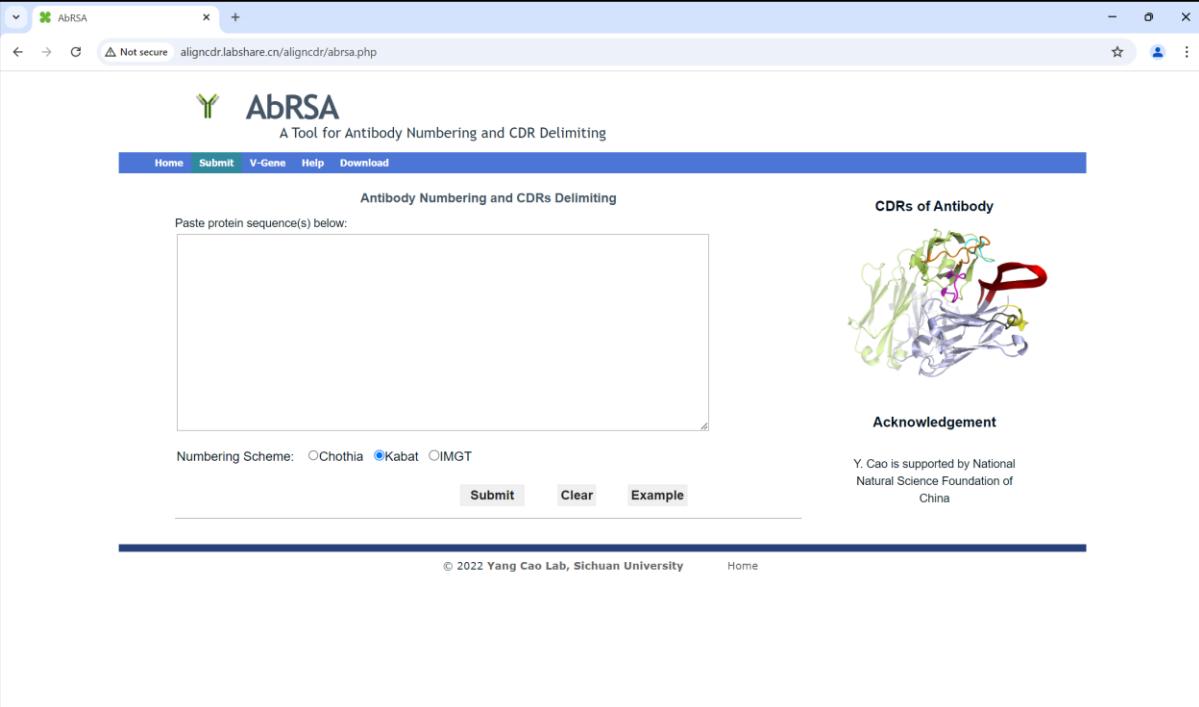
AbRSA Tool



The screenshot shows the AbRSA homepage with a navigation bar at the top: Home, Submit, V-Gene, Help, Download. Below the navigation bar is a section titled "Introduction" which contains text about antibody sequence numbering and CDR delimitation. To the right of the introduction is a 3D molecular model of an antibody structure with colored regions representing CDRs. Below the model is a section titled "Acknowledgement" which credits Yang Cao's support from the National Natural Science Foundation of China. At the bottom of the page is a flowchart illustrating the tool's process:

```
graph TD; A[Input protein sequences or structure] --> B[Search heavy or light chain by AbRSA]; B --> C{Similarity-Cutoff?}; C -- No --> D[Find out all the chains?]; C -- Yes --> E[Output numbering results and CDRs]; D -- Yes --> E; D -- No --> F[Input is structure?]; F --> G[Input protein sequences or structure]
```

Fig 11: Homepage of AbRSA tool



The screenshot shows the "Submit" page of the AbRSA tool. The page title is "AbRSA: A Tool for Antibody Numbering and CDR Delimiting". It features a "Paste protein sequence(s) below:" text input field. To the right of the input field is a 3D antibody structure visualization and an "Acknowledgement" section. At the bottom of the page are buttons for "Submit", "Clear", and "Example". The footer includes copyright information: "© 2022 Yang Cao Lab, Sichuan University" and "Home".

Fig 12: Paste the FASTA sequence taken from the PDB database

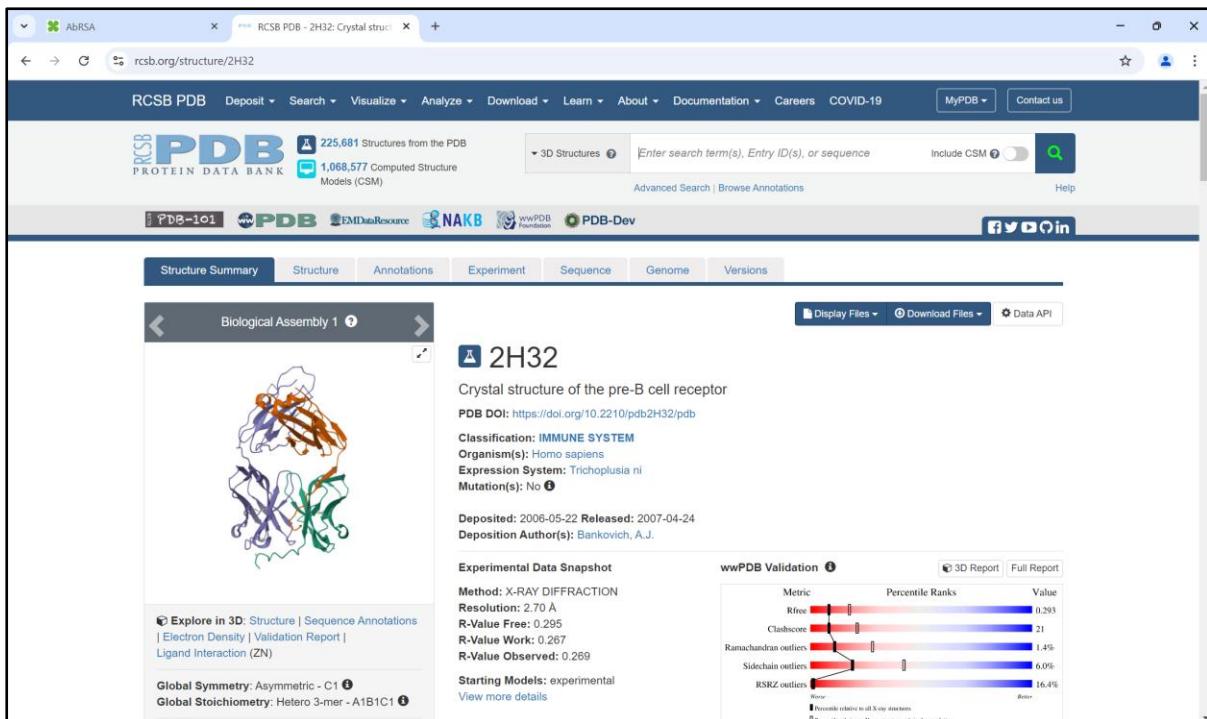


Fig 13: Result page of the PDB database

```
>2H32_1|Chain A|Immunoglobulin iota chain [Homo sapiens (9606)
QPVHQPPAMMSALGTTIIRLCTCLRNHDIDGV/SVYVQQRPGRHPFPRFLRFSQSKSQQGPVPPRFSGSKDVARNRGYLISISELQPDEEAMYYCAGARSSEKEEREREEEEEPETAARTRPV
>2H32_2|Chain B|Immunoglobulin omega chain [Homo sapiens (9606)
SVTHVFSGSTQLTVLSQPKATPSVTLFPPSSEEQANKATLVCLMNDFYPGILT
>2H32_3|Chain C[auth H]|Immunoglobulin heavy chain [Homo sapiens (9606)
EVOLVQSGAEVKPGESLKISKCGSGYSFTSYWIGWVRQMPGKLEWMGIIYPG
DSDTRYSPSFQGVQTISADKSISTAYLQWSLKLASDTAMYYCARHYYYYGMDV
WGOGTTTVSSWSASAPTLFPLVSCENSPTSSVAVGCLAQDFLPDSITFSWK
YKNNSDISSTRGFPsvLRGGKYAATSQVLLPSKDVMQGTDEHVCKVQHPNGNK
CKVQPHNGNKEKNVPLP
```

Fig 14: FASTA sequence retrieved from the PDB database

AbRSA
A Tool for Antibody Numbering and CDR Delimiting

Antibody Numbering and CDRs Delimiting

Paste protein sequence(s) below:

```
>2H32_2|chain B|Immunoglobulin omega chain [Homo sapiens (9606)
SVTHVFSGSTQLTVLSQPKATPSVTLFPPSSEEQANKATLVCLMNDFYPGILT
>2H32_3|chain C[auth H]|Immunoglobulin heavy chain [Homo sapiens (9606)
EVOLVQSGAEVKPGESLKISKCGSGYSFTSYWIGWVRQMPGKLEWMGIIYPG
DSDTRYSPSFQGVQTISADKSISTAYLQWSLKLASDTAMYYCARHYYYYGMDV
WGOGTTTVSSWSASAPTLFPLVSCENSPTSSVAVGCLAQDFLPDSITFSWK
YKNNSDISSTRGFPsvLRGGKYAATSQVLLPSKDVMQGTDEHVCKVQHPNGNK
EKNVPLP
```

CDRs of Antibody

Acknowledgement

Y. Cao is supported by National Natural Science Foundation of China

Fig 15: FASTA sequence pasted in AbRSA tool page

AbRSA

A Tool for Antibody Numbering and CDR Delimiting

Home Submit V-Gene Help Download

AbRSA Result (Kabat)

#Warning: No Antibody Variable Domain Sequence was Detected for 2H32_2|CHAIN B|IMMUNOGLOBULIN OMEGA CHAIN|HOMO SAPIENS (9606)

Summary of CDRs

Name	Type	CDR1	CDR2	CDR3
2H32_3 CHAIN C[AUTH H] IMMUNOGLOBULIN HEAVY CHAIN HOMO SAPIENS (9606)	VH	SYWIG	IIPPGDSDTRYSPSFQG	HHYYYYGMDV
2H32_1 CHAIN A IMMUNOGLOBULIN IOTA CHAIN HOMO SAPIENS (9606)	VL	TLRNDHDIGVSVY	YFSQSDKSQGP	AMGARSSEKEEREREWEWEE

Download Numbering Results: NumberingFile

Variable Domain

>2H32_1|CHAIN A|IMMUNOGLOBULIN IOTA CHAIN|HOMO SAPIENS (9606)

```
1 QPVLHQPPAMSSALGTTIRLCTLRNDHDIGVSVYWYQQRPGHPPRFLLRYFSQSDKS 60
61 GPQVPFRSGSKDVARNRGVLISSELQPEDEAMYCAMGARSSEKEEREREWEEMPTA 120
121 ARTRVP
```

>2H32_2|CHAIN B|IMMUNOGLOBULIN OMEGA CHAIN|HOMO SAPIENS (9606)

>2H32_3|CHAIN C[AUTH H]|IMMUNOGLOBULIN HEAVY CHAIN|HOMO SAPIENS (9606)

```
1 EVLVQSGAEVKPGESLKIISCKGSYFTSYWIGWVRQMPGKLEWMGIITYPGDSDTRY 60
```

CDRs of Antibody

Acknowledgement

Y. Cao is supported by National Natural Science Foundation of China

Fig 16: Result page of AbRSA tool which shows the summary of CDRs

AbRSA

Acknowledgement

Y. Cao is supported by National Natural Science Foundation of China

Variable Domain

>2H32_1|CHAIN A|IMMUNOGLOBULIN IOTA CHAIN|HOMO SAPIENS (9606)

```
1 QPVLHQPPAMSSALGTTIRLCTLRNDHDIGVSVYWYQQRPGHPPRFLLRYFSQSDKS 60
61 GPQVPFRSGSKDVARNRGVLISSELQPEDEAMYCAMGARSSEKEEREREWEEMPTA 120
121 ARTRVP
```

>2H32_2|CHAIN B|IMMUNOGLOBULIN OMEGA CHAIN|HOMO SAPIENS (9606)

>2H32_3|CHAIN C[AUTH H]|IMMUNOGLOBULIN HEAVY CHAIN|HOMO SAPIENS (9606)

```
1 EVLVQSGAEVKPGESLKIISCKGSYFTSYWIGWVRQMPGKLEWMGIITYPGDSDTRY 60
61 SPFGQGVTISADKSISTAYIQLWSSLKASDTAMYCARHYYYYYGMDVWQGTTTVSSW 120
121 SASAPTLFPLVSCENSPTSDTSSVAVGCLAQDFLPDSITFSWKYKHNSDISSTRGFFSPLR 180
181 GGKYAATSVQVLLPSKDVMQGTDEHVVCVKVQHPNGNKEKNVPLF
```

- CDRs are highlighted in colors (CDR1, CDR2, CDR3).
- The gray letters indicate the non-variable-domain region.
- The underlined black letters indicate variable domain of heavy chain while the other black letters indicate variable domain of light chain.

Numbering

2H32_1|CHAIN Light Chain

Q	P	V	L	H	Q	P	P	A	M	S	S	A	L	G	T	T	I	R	L
1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21

Fig 17: Results of Variable domains

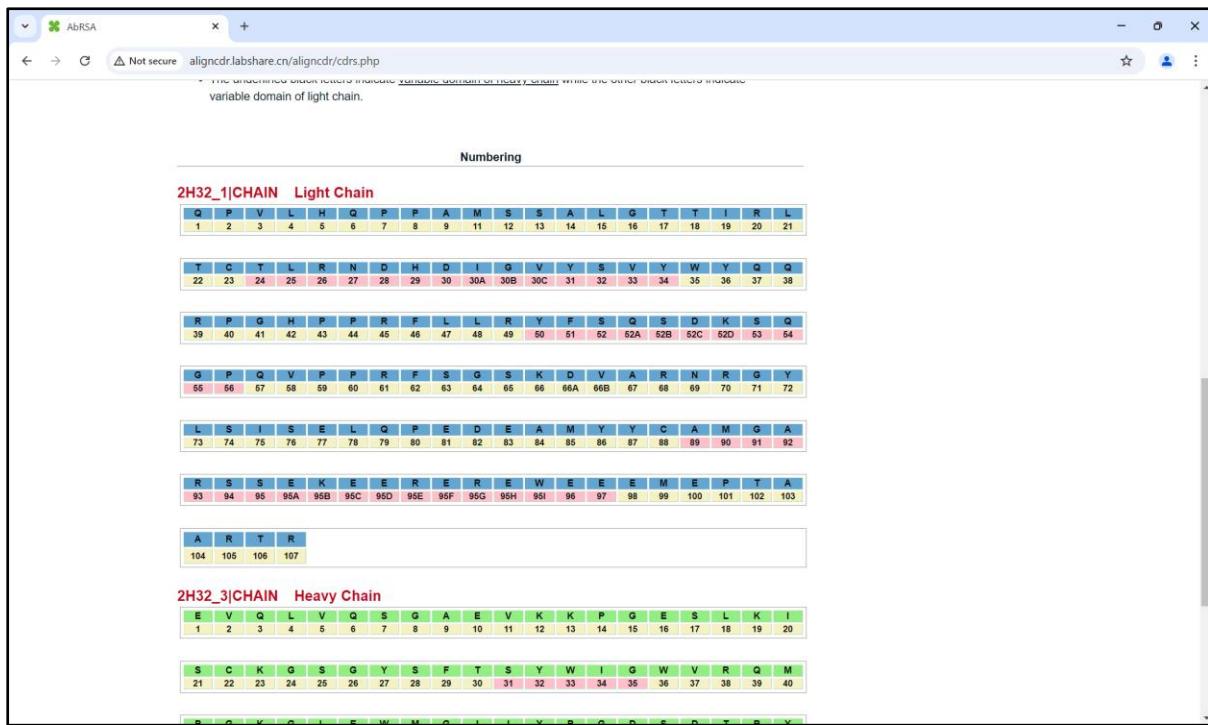


Fig 18: Light chains of the query sequence entered



Fig 19: Heavy chain for the sequence entered

CONCLUSION:

KabatMan database and AbRSA tool was used for antibody numbering.

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WEBLEM 5

Introduction to STCRDab Database

(URL: <https://opig.stats.ox.ac.uk/webapps/stcrdab-stcrpred/>)

INTRODUCTION:

The Single-chain T-cell Receptor Database (STCRDab) is a specialized repository designed to support the study and development of single-chain T-cell receptors (scTCRs), which are key players in the immune response. T-cell receptors (TCRs) are essential in recognizing antigenic peptides presented by Major Histocompatibility Complex (MHC) molecules, allowing T-cells to identify and attack infected or abnormal cells. Single-chain T-cell receptors, which are engineered forms of natural TCRs, are increasingly used in research and immunotherapy, particularly in personalized cancer treatments and autoimmune disease research. The STCRDab provides a comprehensive collection of sequence, structure, and functional data on these receptors, thus facilitating their analysis, modification, and application in various biological and clinical contexts.

T-cell receptors are surface proteins found on T-lymphocytes, specialized white blood cells that play a critical role in the adaptive immune system. TCRs enable T-cells to detect and bind to specific antigens, such as viral or tumor-derived peptides, that are presented on the surface of infected or abnormal cells via MHC molecules. Once the TCR recognizes an antigen, the T-cell is activated and triggers an immune response to eliminate the threat.

TCRs are typically composed of two chains: α (alpha) and β (beta) in the majority of T-cells, or γ (gamma) and δ (delta) in a smaller subset. These chains combine to form a unique antigen-binding site, providing the ability to recognize a vast array of foreign peptides. TCRs do not directly bind free-floating antigens, unlike antibodies. Instead, they recognize antigenic peptides that are presented by MHC molecules on the surface of cells.

This specific interaction between TCRs, MHC molecules, and peptides is at the heart of T-cell-mediated immunity, which is crucial for identifying and eliminating virus-infected, malignant, or abnormal cells.

Single-Chain T-Cell Receptors (scTCRs)

Single-chain T-cell receptors (scTCRs) are engineered constructs that combine the antigen-binding domains of TCRs into a single polypeptide chain. These constructs simplify the natural heterodimeric structure of TCRs into a single chain, retaining the binding specificity while making them more stable and easier to produce for therapeutic purposes. scTCRs have become highly valuable in immunotherapy, particularly in CAR-T cell therapies (Chimeric Antigen Receptor T-cells), where engineered TCRs are modified to recognize specific tumor antigens, enabling precision targeting of cancer cells.

The primary advantage of scTCRs lies in their ability to be custom-designed for particular antigens, making them powerful tools for personalized medicine. They can be used in treating cancers, viral infections, and autoimmune diseases by enabling highly targeted immune responses.

The STCRDab database was created to provide a centralized platform for the accumulation, sharing, and analysis of single-chain T-cell receptor data. Its goal is to advance the understanding of TCRs and their therapeutic applications by providing a comprehensive resource for researchers engaged in T-cell biology, immunotherapy, and vaccine development.

STCRDab serves as a multi-functional database offering several key features to facilitate research:

- 1. TCR Sequences and Annotations:** The database contains a vast array of TCR sequences, focusing on both natural and engineered single-chain TCRs. Each sequence entry is accompanied by relevant annotations, such as the antigen it binds to, the MHC class involved, and structural features.
- 2. Structural Data:** For many TCRs and scTCRs, structural data is available, allowing researchers to explore the three-dimensional conformation of these receptors. The structure-function relationship is critical for designing scTCRs with enhanced binding properties or specificity, making this feature of great importance to those involved in rational drug design or antigen-targeted therapy.
- 3. Functional Information:** In addition to sequence and structural data, STCRDab includes functional characterizations of TCRs, such as antigen specificity, binding affinity, and T-cell activation strength. This data helps researchers understand how different TCRs function in diverse immunological contexts, which is crucial for developing therapies targeting specific diseases like cancer or autoimmune disorders.
- 4. Mutational Analysis:** STCRDab offers information on mutated TCRs and their effects on binding affinity, antigen specificity, and MHC interaction. This feature supports the engineering of optimized scTCRs for use in experimental models or therapeutic applications.
- 5. Cross-Species Data:** The database includes TCR data from various species, allowing researchers to perform comparative studies that might offer insights into evolutionary conservation and diversity of T-cell receptors. This cross-species comparison is particularly valuable for preclinical studies where animal models are used to test TCR-based therapies.
- 6. MHC-TCR-Peptide Complex Information:** Given the critical role of MHC in TCR-antigen recognition, STCRDab provides information on MHC-bound peptides and their interactions with TCRs. This helps in understanding the specificity of TCRs for certain MHC alleles and the potential for cross-reactivity with other peptides.
- 7. Search and Analysis Tools:** The database offers a suite of bioinformatics tools that allow users to search, compare, and analyze TCR data. This includes sequence alignment, structural modeling, and antigenic epitope prediction, which are crucial for researchers looking to design novel TCRs for therapeutic use.

STCRPred

STCRPred is a bioinformatics tool used for modeling the structure of T-cell receptors (TCRs). It includes several computational tools designed to predict and analyze TCR structures, which are essential for understanding immune responses and designing therapeutic TCR-related proteins.

One of its main functions is the **sequence-based prediction** of complementarity-determining regions (CDRs), which are crucial for TCR recognition of antigens. STCRPred uses a tool called SCALOP-TCR to predict the canonical form of CDRs based on sequence data. The tool assigns sequences to clusters by analyzing the structural information available from databases like the Protein Data Bank (PDB).



Fig 1: Homepage of STCRDab

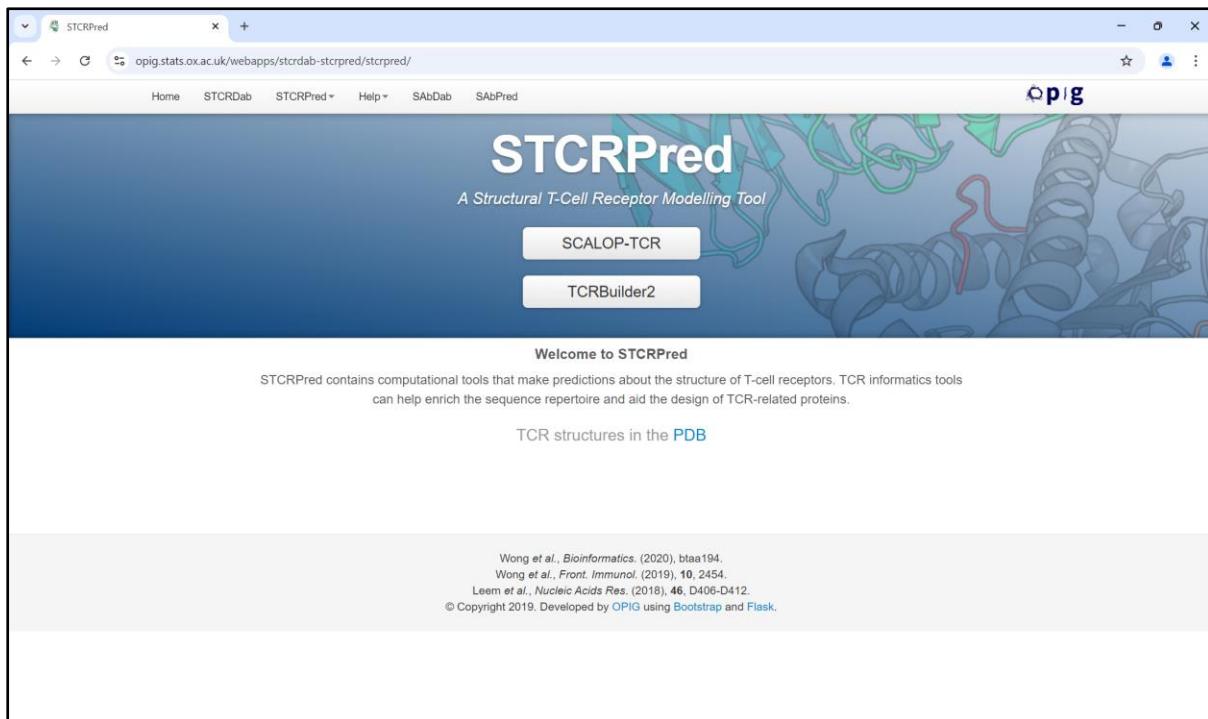


Fig 2: Homepage of STCRPred

REFERENCES:

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2. Morris, G. P., & Allen, P. M. (2012). How the TCR balances sensitivity and specificity for the recognition of self and pathogens. *Nature Immunology*, 13(2), 121–128. <https://doi.org/10.1038/ni.2190>
3. Smith, J. A., & June, C. H. (2019). CAR T cell therapies: The road to universal T cells. *Nature Reviews Drug Discovery*, 18(7), 481–493. <https://doi.org/10.1038/s41573-019-0025-2>

WEBLEM 5(A)

Structural T-cell Receptor Database (STCRDab)

(URL: <https://opig.stats.ox.ac.uk/webapps/stcrdab-stcrpred/>)

AIM:

To retrieve CDR position in query '7SG1' using STCRDab database.

INTRODUCTION:

The STCRDab Database is an essential online resource designed to assist researchers working on single-chain T-cell receptors (scTCRs), which play a pivotal role in the immune system's ability to recognize and respond to antigens. scTCRs are engineered constructs that simplify the natural T-cell receptor, allowing for greater stability and targeted therapeutic use. This database offers a rich collection of information on TCR sequences, structural data, and functional characterizations, facilitating in-depth studies into how these receptors interact with antigens presented by MHC molecules.

The database serves as a valuable tool for advancing research in areas such as cancer immunotherapy, where engineered TCRs are used to precisely target tumor antigens, and in the study of infectious diseases, where TCRs recognize pathogen-derived peptides. Additionally, STCRDab provides insights into autoimmune conditions by analyzing how TCRs interact with self-antigens. Key features of STCRDab include a robust set of tools for sequence alignment, structural modeling, and mutational analysis, as well as comprehensive data on TCR-antigen specificity and MHC interactions. The database supports cross-species comparisons, helping researchers understand evolutionary aspects of TCR diversity and function. By centralizing TCR-related data, STCRDab accelerates discoveries in immunology, fosters the development of novel therapies, and plays a critical role in the growing field of personalized medicine.

METHODOLOGY:

1. Visit the homepage of the STCRDab Database by accessing the URL: <https://opig.stats.ox.ac.uk/webapps/stcrdab-stcrpred/>.
2. Retrieve the antibody PDB ID: 7SG1 from the PDB Database.
3. Input the retrieved PDB ID (7SG1) into the PDB search option in STCRDab.
4. Examine each section of the results displayed.
5. Provide an interpretation of the observed results.

OBSERVATIONS:



Fig 1: Homepage of STCRDab database

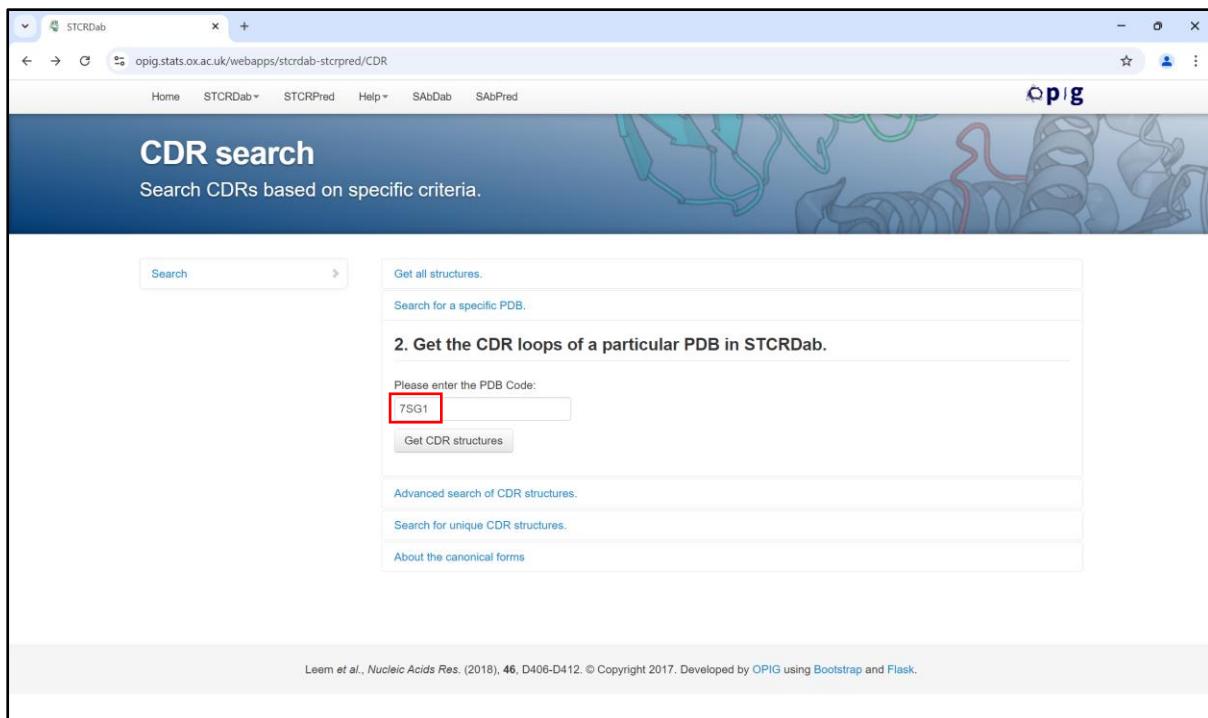


Fig 2: Enter the PDB ID: 7SG1 in CDR Search of STCRDab

CDR search
Search CDRs based on specific criteria.

PDB	Species	Method	Resolution (Å)	View CDR loop Structure	View TCR Structure	Downloads
7sg1	human	X-RAY DIFFRACTION	3.1	TCR JR: CDR1: NIATNDY CDR2: GYTK CDR3: LVQGLARDMR CDRB1: SOVTM CDRB2: ANQGSEA CDRB3: SVALGSDTGELF TCR ED: CDR1: NIATNDY CDR2: GYTK CDR3: LVQGLARDMR CDRB1: SOVTM CDRB2: ANQGSEA CDRB3: SVALGSDTGELF	View Structure	<ul style="list-style-type: none"> IMGT-numbered Structure Summary file

1 structure(s) fit your criteria.

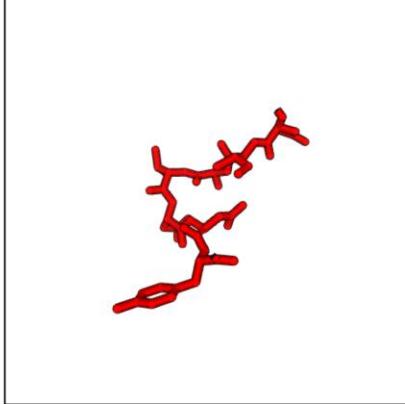
Download results

- Download the [summary file](#) for this search. See [help](#) for more details on file formats.
- Download an archived [zip file](#) for this search. See [help](#) for more details on file formats.

Fig 3: Result page of PDB ID: 7SG1

Details for 7sg1 CDRA1
Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (Default Scheme):
[IMGT-defined CDR loop](#)

Display options:
[Cartoon model](#)
[Spacefill model](#)
[Wire model](#)
[Ball&stick model](#)
[Default colouring](#)
[Color by atom](#)
[Color by B-factor](#)
[Color by element](#)
[Spin: on off](#)

Show anchor residues (?):

[Get anchors](#)

Fig 4: CDRA1 loop structure summary of 7SG1

The screenshot shows a web browser window titled "STCRPred". The URL in the address bar is "opig.stats.ox.ac.uk/webapps/stcrdab-stcrpred/CDRViewer?pdb=7sg1&loop=CDRA1&chain=I". The page content includes:

- Basic parent structure information**: A table with the following data:

Item	Info
PDB	7sg1
Organism	HOMO SAPIENS
Method	X-RAY DIFFRACTION
Resolution	3.1 Å
- CDR loop information**: A table showing the IMGT Numbered sequence for the CDR loop of chain I:

A27	A28	A29	A30	A36	A37	A38
N	I	A	T	N	D	Y
- Available downloads**: A section listing available download links.

At the bottom of the page, there is a copyright notice: "Wong et al., Bioinformatics. (2020), btaa194. Wong et al., Front. Immunol. (2019), 10, 2454. Leem et al., Nucleic Acids Res. (2018), 46, D406-D412. © Copyright 2019. Developed by OPiG using Bootstrap and Flask."

Fig 4.1: CDRA1 details of 7SG1 (TCR JI)

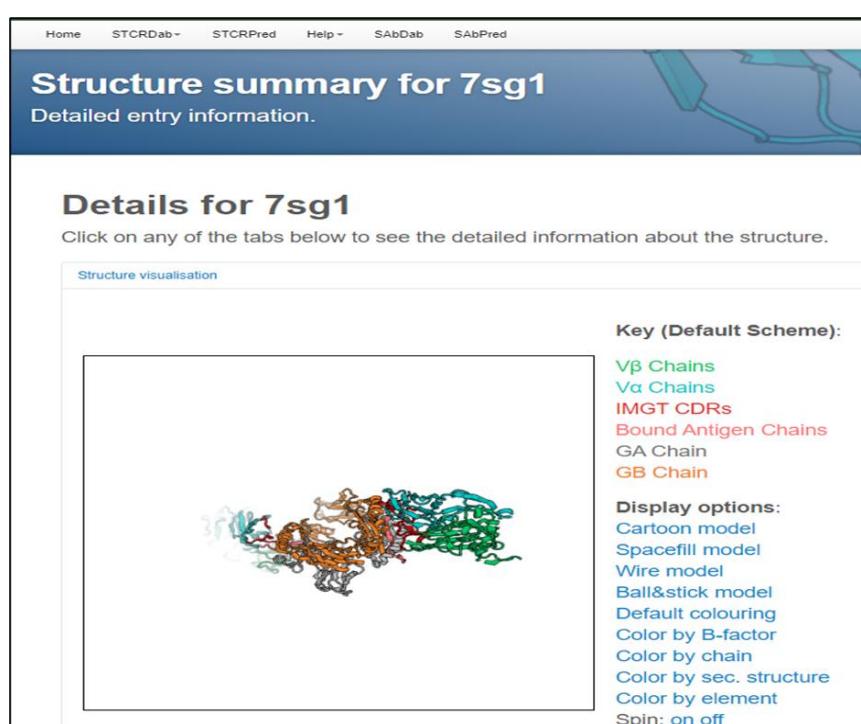
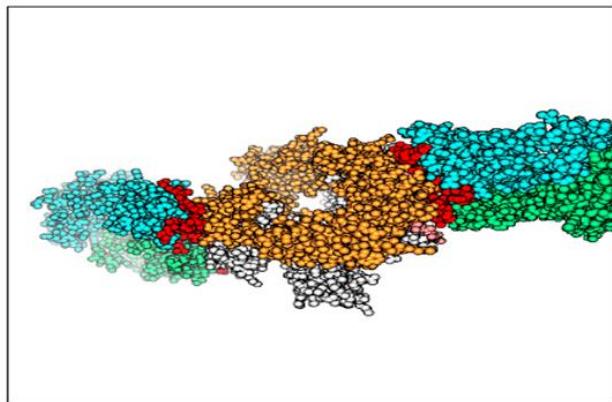


Fig 5: Structure summary for 7SG1

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (Default Scheme):

V β Chains
V α Chains
IMGT CDRs
Bound Antigen Chains
GA Chain
GB Chain

Display options:

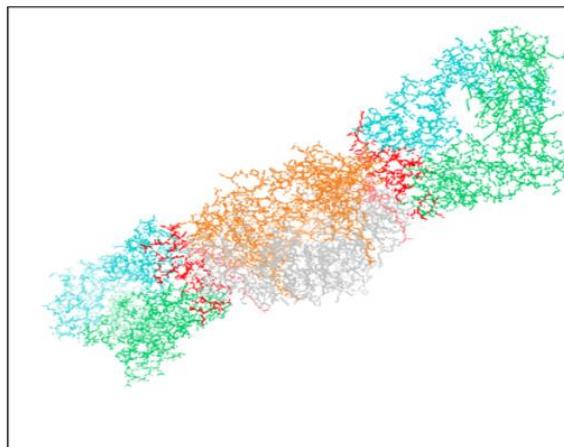
Cartoon model
Spacefill model
Wire model
Ball&stick model
Default colouring
Color by B-factor
Color by chain
Color by sec. structure
Color by element
Spin: on off

Fig 5.1: Visualization of structure in spacefill model

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (Default Scheme):

V β Chains
V α Chains
IMGT CDRs
Bound Antigen Chains
GA Chain
GB Chain

Display options:

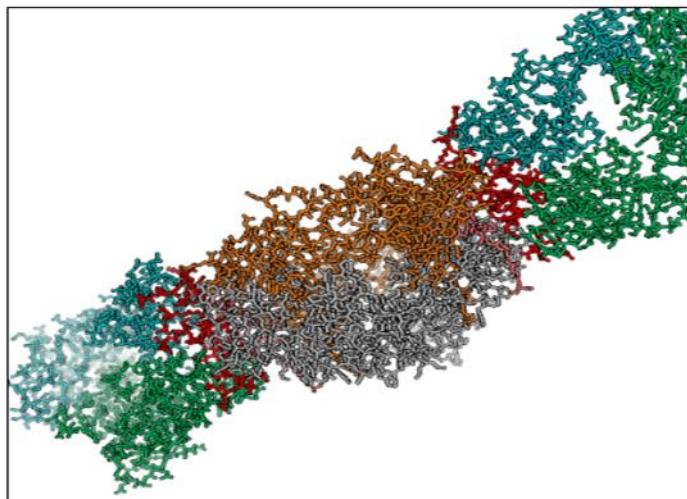
Cartoon model
Spacefill model
Wire model
Ball&stick model
Default colouring
Color by B-factor
Color by chain
Color by sec. structure
Color by element
Spin: on off

Fig 5.2: Visualization of structure in wire model

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (Default Scheme):

V β Chains
V α Chains
IMGT CDRs
Bound Antigen Chains
GA Chain
GB Chain

Display options:

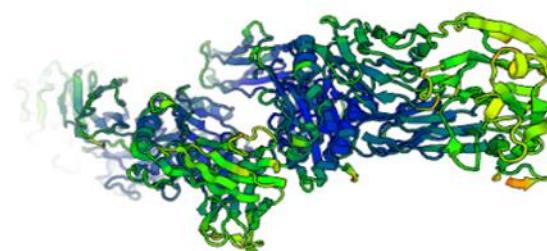
Cartoon model
Spacefill model
Wire model
Ball&stick model
Default colouring
Color by B-factor
Color by chain
Color by sec. structure
Color by element
Spin: on off

Fig 5.3: Visualization of structure in Ball and stick model

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (B-factor):

Each atom is coloured by its B-factor.

Display options:

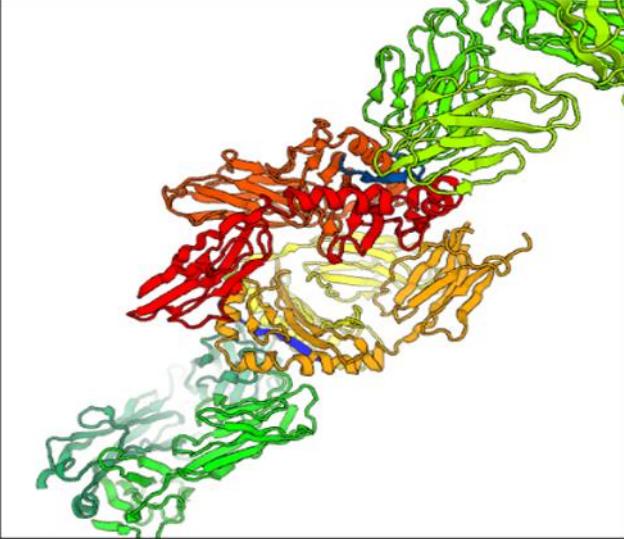
Cartoon model
Spacefill model
Wire model
Ball&stick model
Default colouring
Color by B-factor
Color by chain
Color by sec. structure
Color by element
Spin: on off

Fig 5.4: Visualization of structure in color by B – factor

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation



Key (B-factor):
Each atom is coloured by its B-factor.

Display options:
[Cartoon model](#)
[Spacefill model](#)
[Wire model](#)
[Ball&stick model](#)
[Default colouring](#)
[Color by B-factor](#)
[Color by chain](#)
[Color by sec. structure](#)
[Color by element](#)
Spin: [on](#) [off](#)

Fig 5.5: Visualization of structure in color by chain

STCRPred

opig.stats.ox.ac.uk/webapps/stcrdab-stcprpred/CDRViewer?pdb=7sg1&loop=CDRA1&chain=l

Home STCRDab STCPRpred Help SAbDab SAbPred

opig

Details for 7sg1 CDRA1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation

Basic parent structure information

Xpa5 TCR in complex with HLA-Dq2-alpha1

Item	Info
PDB	7sg1
Organism	HOMO SAPIENS
Method	X-RAY DIFFRACTION
Resolution	3.1 Å

CDR loop information

Available downloads

Fig 6: Structure information details for 7SG1

Details for 7sg1

Click on any of the tabs below to see the detailed information about the structure.

Structure visualisation

Structure information

Paired chains information

This PDB has 2 TCR(s).

E / D

TCR Details:

Item	Info
VB chain	E
VA chain	D
VB IMGT details	TRBV29/TRBJ2
VA IMGT details	TRA4/TRAJ43
Species	human

Antigen Details:

Item	Info
Antigen Chain	C
Antigen Type	Peptide
Antigen Organism	HOMO SAPIENS
Antigen Sequence	LQPFPQPELPYGSGGG
Antigen Length	16

MHC details:

Item	Info
MHC Chain	A B
MHC Type	MH2
MHC Species	human
MHC IMGT details	HLA-DQA1*0501 / HLA-DQB1*0201

Fig 7: Pair chain information of E / D TCR showing TCR, Antigen details and MHC details

CDR Sequences:

Loop	Sequence	Predicted canonical form	CDR Length
CDRB3	SVALGSDTGELF	None	12
CDRB2	ANQGSEA	None	7
CDRB1	SQVTM	None	5
CDRA3	LVGGLARDMR	None	10
CDRA2	GYTKK	None	5
CDRA1	NIATNDY	None	7

Orientation and docking angles:

Angle	Value
BC2	108.08°
BC1	69.88°
BA	-51.96°
AC2	88.28°
AC1	133.28°
dc	18.06Å
Docking angle	37.20°

TCRs with similar orientations:

TCR PDB	BC2	BC1	BA	AC2	AC1	dc	TRangle Distance
7sg2_ED	106.67°	69.23°	-53.38°	88.19°	132.97°	18.09Å	2.1
3l0e_DC	104.85°	71.10°	-52.97°	88.52°	133.53°	17.82Å	3.6
3o6f_HG	104.58°	69.75°	-54.07°	87.97°	132.76°	17.81Å	4.1
8sgb_DC	104.44°	69.27°	-53.20°	89.63°	135.21°	18.20Å	4.6
3poy_JI	107.68°	69.73°	-56.20°	90.17°	132.20°	18.07Å	4.8

Fig 7.1: Pair chain information of J / I TCR showing CDR sequences, orientation and docking angles and TCRs with similar orientations

RESULTS:

The CDR search for the query PDB ID: 7SG1 was conducted in the STCRDab database to identify the CDR position for drug design purposes. STCRDab is a database of T-cell receptor (TCR) structures. The results section showed one structure, along with its species name, method, and resolution. One of the CDR loop structures, 'CDRA1,' was visualized, and both its parent structure information and CDR loop details were explored. Following this, the TCR structure of 7SG1 was visualized using different display options. The paired chain information of the 7SG1 structure revealed two TCRs, named E/D and J/I, displaying details such as TCR, antigen, MHC information, numbered sequences, CDR sequences, orientation and docking angles, and TCRs with similar orientations.

CONCLUSION:

The retrieval of CDR positions in query '7SG1' using the STCRDab database provided valuable structural insights into the antigen-binding mechanisms of T-cell receptors. This information was crucial for understanding the CDR loops, which played an essential role in drug design and therapeutic development.

REFERENCES:

1. Davis, M. M., & Bjorkman, P. J. (1988). T-cell antigen receptor genes and T-cell recognition. *Nature*, 334(6181), 395–402. <https://doi.org/10.1038/334395a0>
 2. Garcia, K. C., Adams, J. J., Feng, D., & Ely, L. K. (2009). The molecular basis of TCR germline bias for MHC is surprisingly simple. *Nature Immunology*, 10(2), 143–147. <https://doi.org/10.1038/ni.1698>
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WEBLEM 6

Introduction to Yvis Database

(URL: <http://bioinfo.icb.ufmg.br/yvis/>)

INTRODUCTION:

Yvis Database is a modern, versatile data management platform that addresses the growing needs of today's data-centric environments. It supports a wide range of data types, including relational, non-relational, and semi-structured formats, making it ideal for applications ranging from IoT data streams and big data analytics to cloud-native solutions. One of its key strengths lies in its high performance and scalability, enabling it to handle large-scale, high-volume transactions with ease. Its distributed architecture ensures efficient processing, while resources are dynamically adjusted to maintain peak performance.

Security is a core focus for Yvis, with robust encryption, role-based access control (RBAC), and compliance with major standards such as GDPR, HIPAA, and SOC2. This ensures that sensitive data remains secure at all times. Yvis also integrates real-time analytics and machine learning, allowing users to process and analyze data as it's ingested and apply predictive analytics or anomaly detection without exporting data to separate systems.

The platform is designed for flexibility, supporting seamless integration with various data sources, including traditional SQL databases, cloud services, and data lakes. Continuous data ingestion from APIs and streaming services like Kafka is also easily managed. Users benefit from both an intuitive graphical interface and comprehensive API support, enabling efficient data management regardless of technical proficiency. Yvis Database is a powerful solution that combines speed, security, and flexibility for the most demanding data-driven applications.

Antibodies or immunoglobulins are vertebrate immune system proteins that are produced by B cells and can bind to antigens with high specificity and affinity. For this reason, antibodies are an important tool in diagnosis, therapy and experimental biology. To elucidate the antibody characteristics, large numbers of antibody structures and sequences have been generated in the last years. The number of antibodies or antibody fragment structures deposited in Protein Data Bank (PDB) has increased exponentially, leading to the development of databases of antibody structures. Moreover, many antibody sequences have been obtained by high-throughput sequencing of the B-cell receptor repertoire. This extraordinary and still increasing number of antibody structures and sequences demands integrative data organization and tools for their analysis, comparison and visualization. One of the major bottlenecks in this field is the concomitant visualization of a large amount of antibody data. AbYsis and IMGT/3Dstructure-DB allow antibody visualization, but only a limited number of sequences can be analysed at a time. Indeed, abYsis presents a classical multiple sequence alignment (MSA) that displays a limited number of sequences and positions each time. IMGT/3Dstructure-DB display only one antibody sequence using the IMGT/Collier des Perles representation that allows sequence

analysis related to the antibody structure. To fill this gap, we developed the antibody high-density alignment visualization and analysis (Yvis) platform that includes:

1. an updated weekly and curated antibody structure database (Yvis database)
2. integrated antibody analysis resources, such as an antibody high-density alignment visualization called Collier de Diamants, and multiple filter options to analyse data from user files or from the Yvis database.

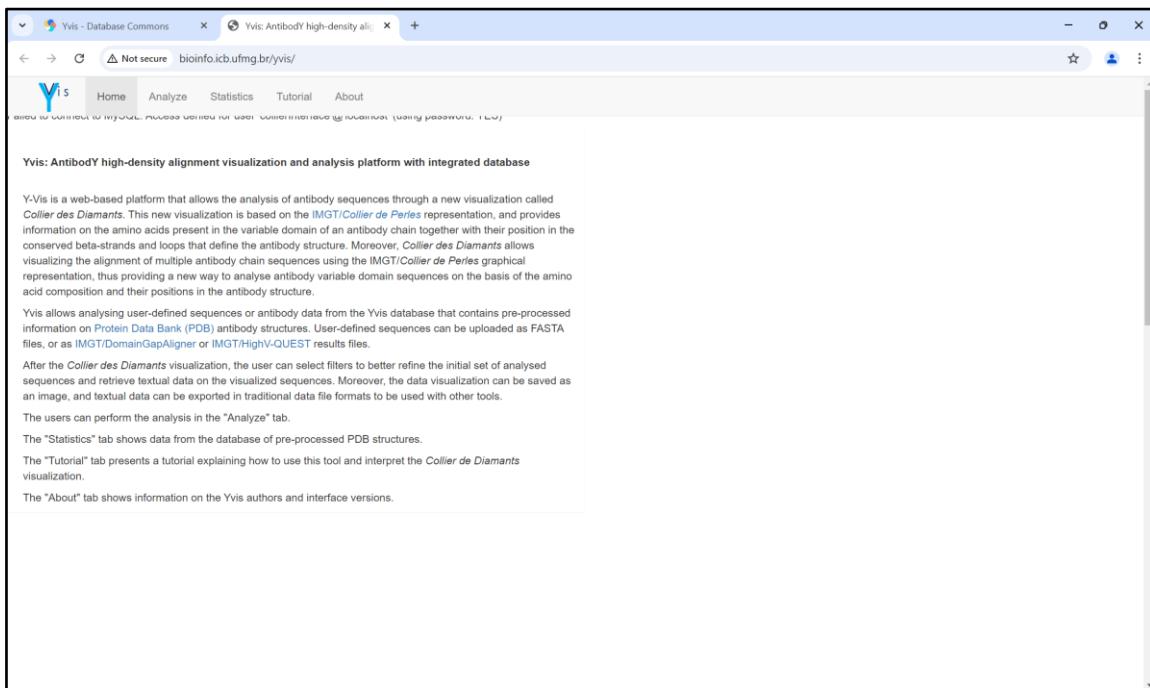


Fig 1: Homepage of Yvis database

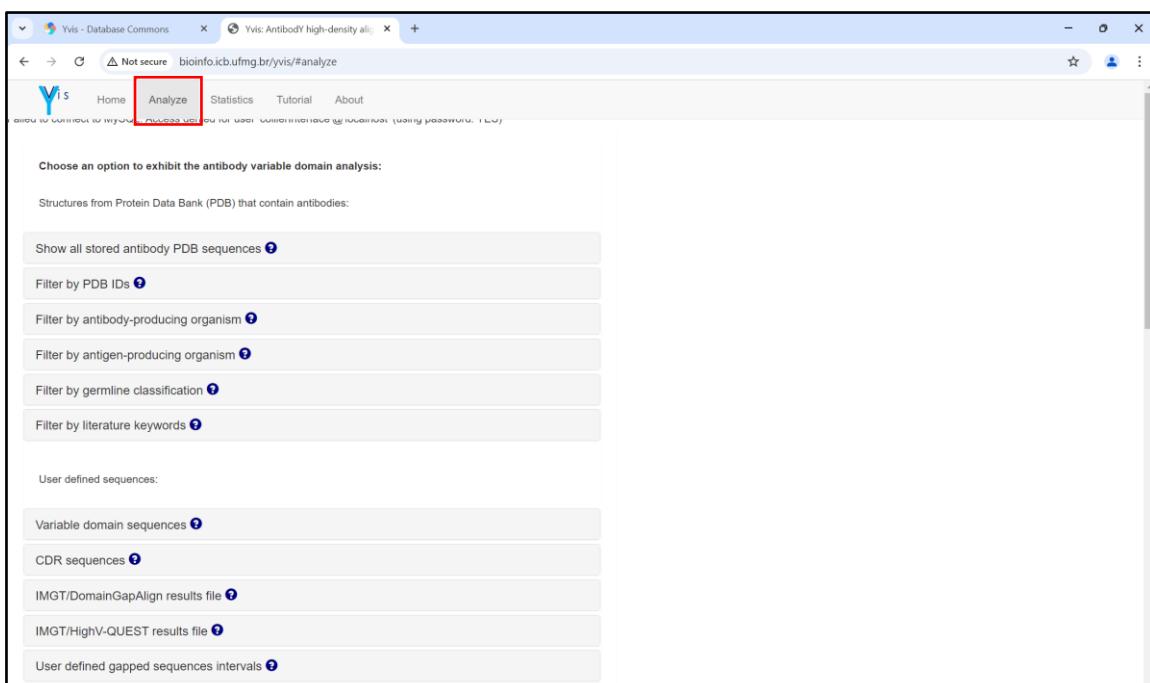


Fig 2: Analysis options in Yvis database

1. Structure from Protein Data Bank (PDB) that contain antibodies:

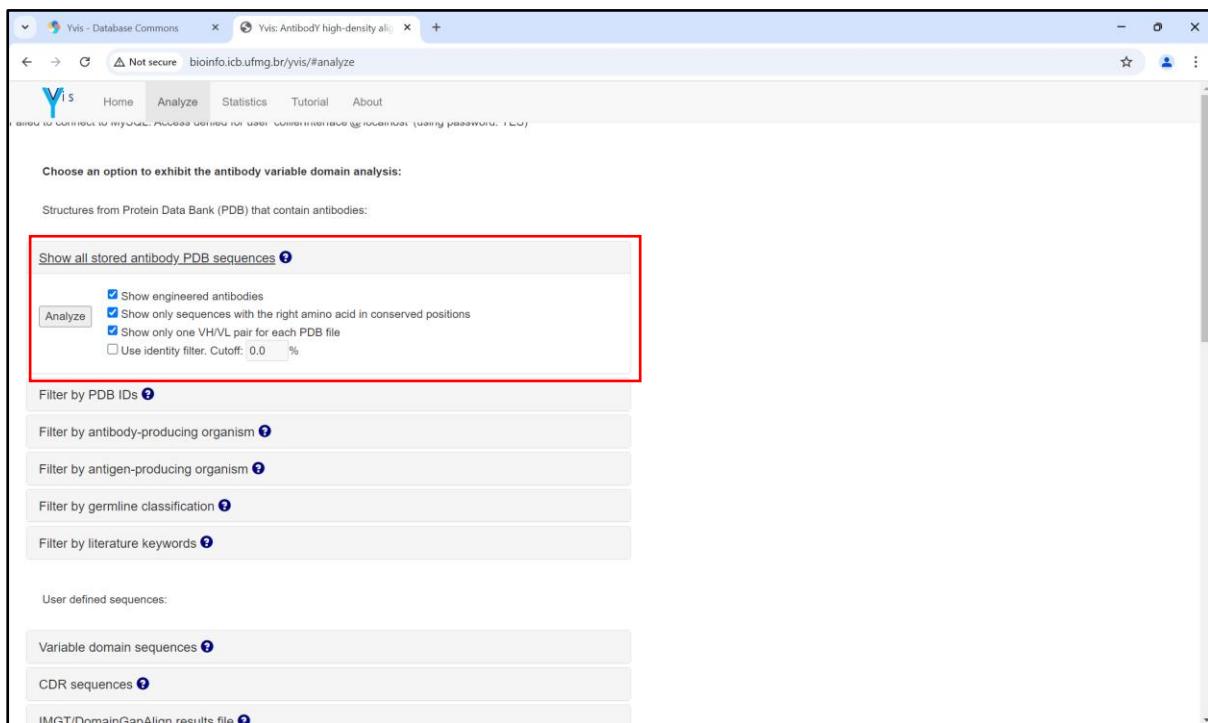


Fig 3: Show all stored antibody PDB structures

Select this option to show information on all antibody sequences from PDB and stored in Yvis database.

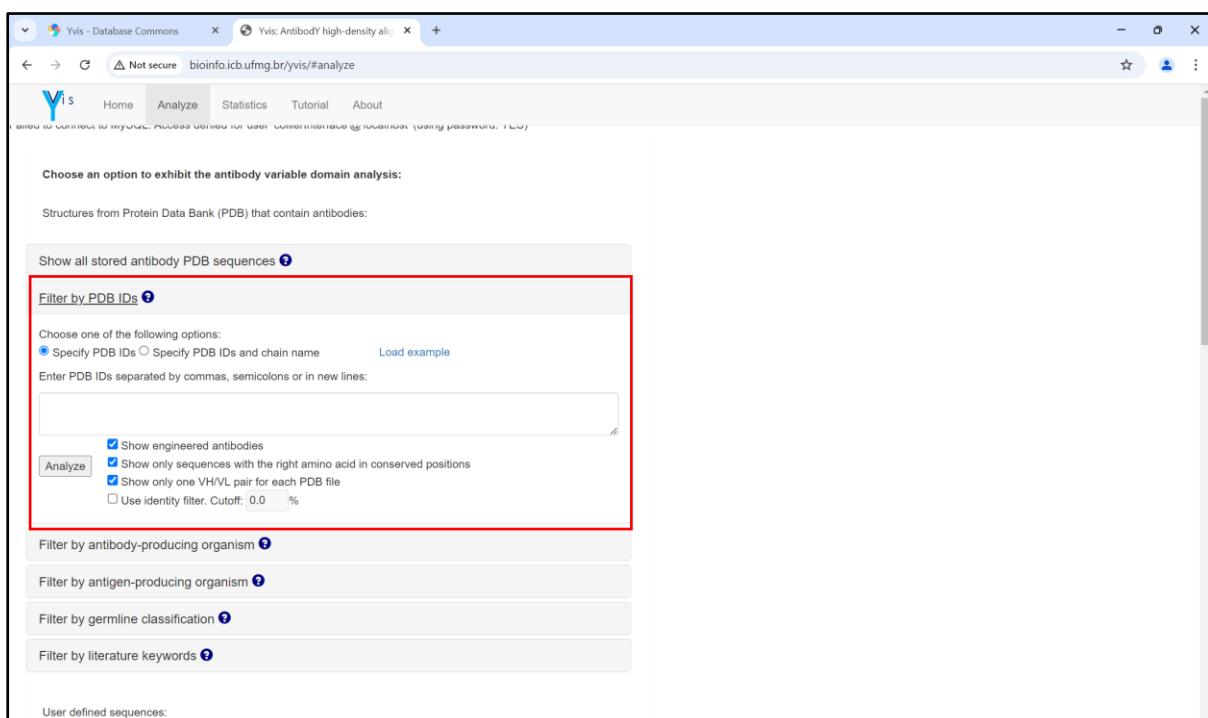


Fig 4: Filter by PDB IDs

Select this option to show only chains from structures of a user – defined list of PDB identifiers, with or without chain specifications.

You can specify a list of PDB IDs by selecting the ‘Specify PDB IDs’ option and inserting in the textbox the PDB IDs separated by commas, semicolons, or by putting each ID in a new line. In this case, Yvis will show the chains stored in the Yvis database that are part of the indicated structures.

If you want to restrict the analysis to specific chains, you should select the ‘Specify PDB IDs and chain name’ option and insert in the textbox a list of chains separated by commas, semicolons, or in new lines. Each chain must be specified by the PDB ID followed by a colon and the chain name.

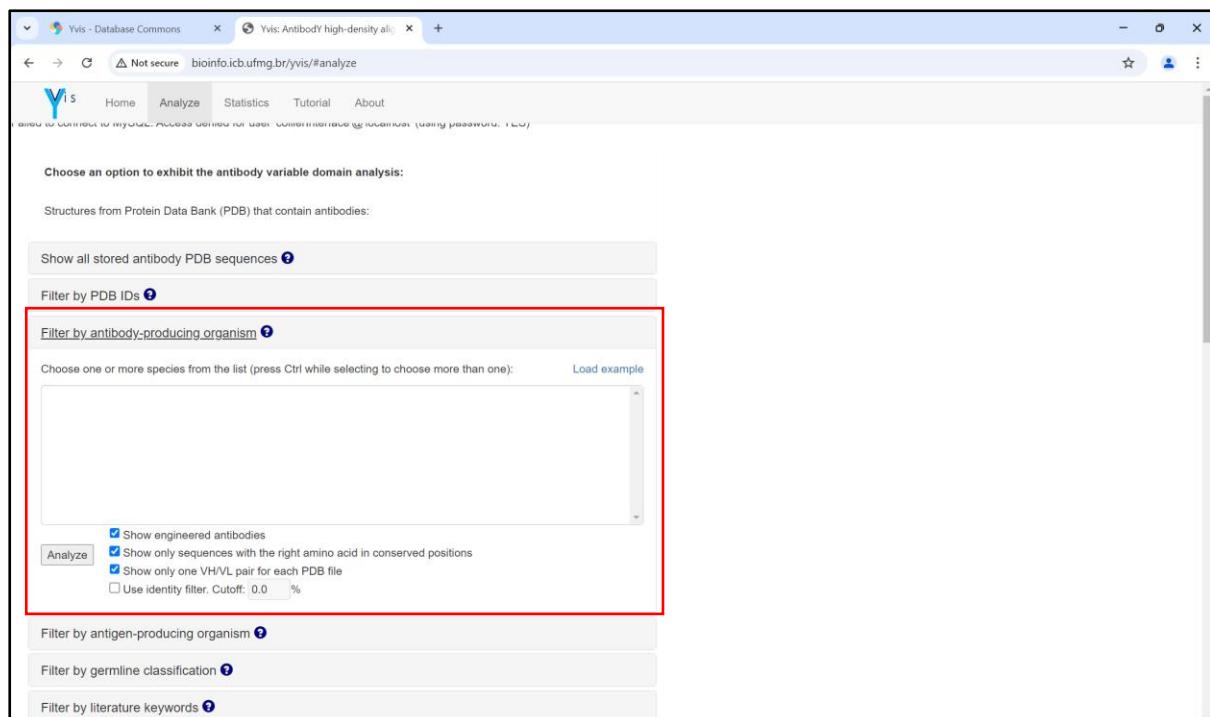


Fig 5: Filter by antibody producing organism

Select this option to show only chains of antibodies produced by specific organisms. Choose one or more species from the list of all antibody-producing organisms stored in the database, standardized by species, based on the [UniProt Taxonomy Database](#).

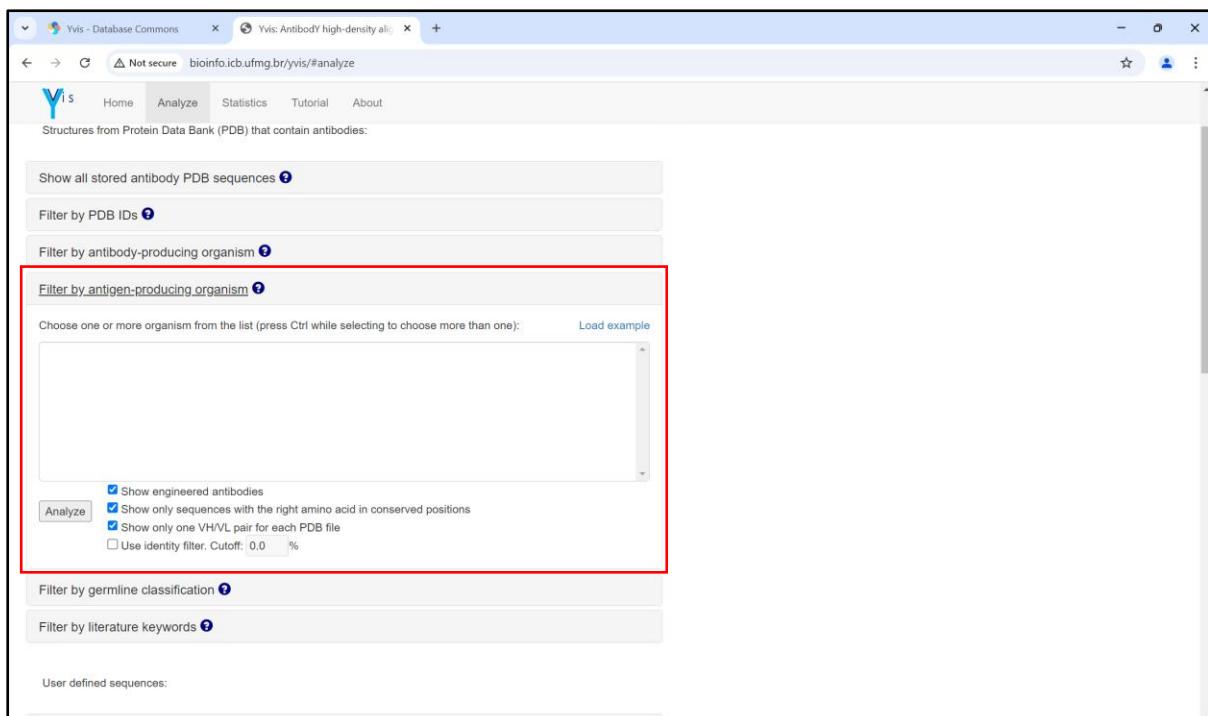


Fig 6: Filter by antigen producing organism

Select this option to show only chains from antibody structures that presents an antibody-antigen complex, or choose the "None" option to show only chains of antibodies that are not in complex with antigens. The list presents non-protein antigens type (carbohydrate, hapten, and nucleic acid) and, for proteins or peptide antigens, the antigen-producing organisms stored in the database, standardized by species, based on the UniProt Taxonomy Database. Choose one or more items from this list.

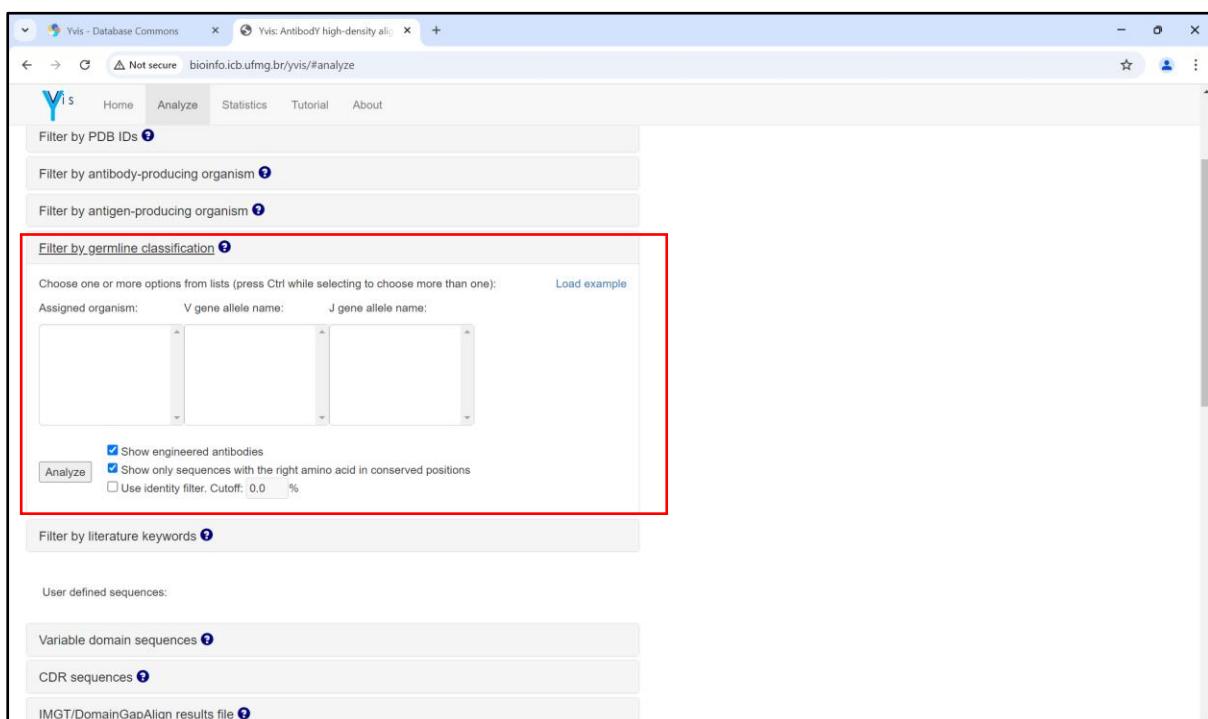


Fig 7: Filter by germline classification

Select this option to show only chains assigned to specific germline alleles by IMGT/DomainGapAlign. You can restrict the assigned species and V or J alleles by choosing one or more options from the lists. If you do not want to restrict the analysis, select all options.

The screenshot shows a web browser window for the Yvis platform. The URL is bioinfo.icb.ufmg.br/yvis/#analyze. The page has a navigation bar with links for Home, Analyze, Statistics, Tutorial, and About. A red box highlights the 'Filter by literature keywords' section. This section contains input fields for Title keywords, Summary keywords, Authors, Publication/Year, and Article identifier (DOI, PMID or PMCID). Below these fields is a button labeled 'Analyze'. Underneath the 'Analyze' button are four checkboxes: 'Show engineered antibodies' (checked), 'Show only sequences with the right amino acid in conserved positions' (checked), 'Show only one VH/VL pair for each PDB file' (checked), and 'Use identity filter. Cutoff: 0.0 %' (unchecked).

Fig 8: Filter by literature keywords

Select this option to show only antibody chains from PDB structures filtered on the basis of literature information. You can specify paper title or summary, authors' names, publication year, or article identifier (DOI, PMID or PMCID). These fields accept multiple keywords and can be defined with Boolean operators (AND, OR and NOT).

2. User – defined sequences:

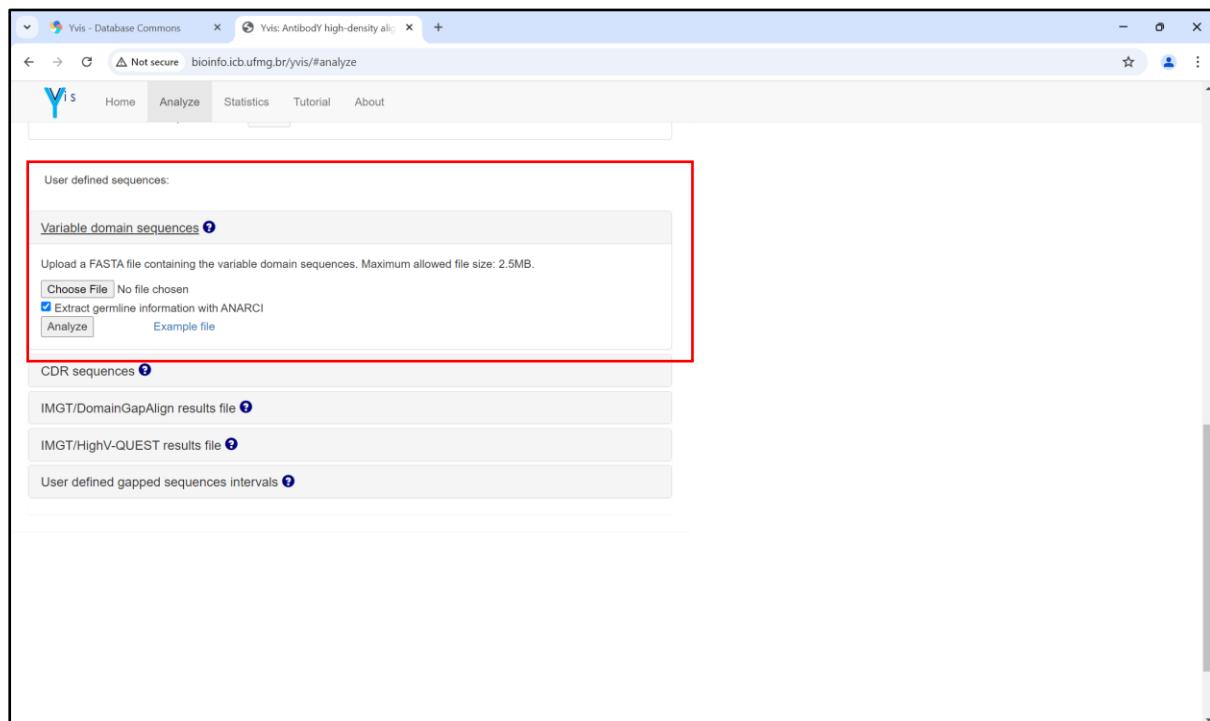


Fig 9: Variable domain sequences

Select this option to insert a FASTA file that contains amino acid sequences of variable domains of antibody chains. The Yvis server uses ANARCI to gap sequences.

In sequence name and comments line (line starting with ">"), the user can insert the following information, separated by "|": PDB/identification source, chain identification, chain type (it is overwritten if ANARCI finds a different type), antibody-producing organism, engineered antibody information (engineered or not), antigen-producing organism, antigen molecule description, assigned germline species, V gene, percentage of V gene identity, J gene, and percentage of J gene identity. These are suggested information and could be used in analysis filters; however, only the sequence identification is mandatory (and will be used in the PDB identification field). Optionally, you can select the option “Extract germline information with ANARCI” to obtain germline information from ANARCI instead of getting them from the user’s file.

As the time of execution of this analysis is in function of the number of uploaded/chosen sequences, in the case of large files this time will be long (the user’s browser might present a slow script dialog). In the case of a huge number of sequences, users are invited first to submit them to IMGT/DomainGapAlign and then upload the results page into Yvis, using the “IMGT/DomainGapAligner results file” input option.

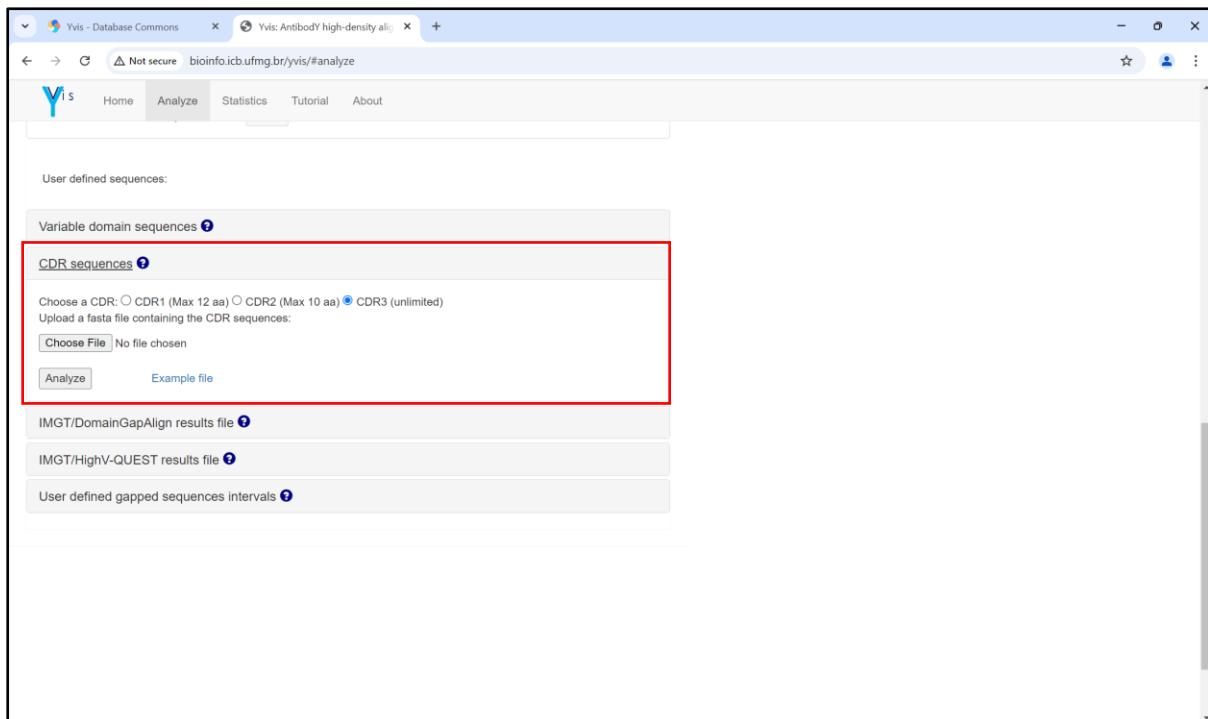


Fig 10: CDR sequences

Select this option to insert a FASTA file containing complementarity-determining region (CDR) amino acid sequences. Choose the type of CDR sequences (CDR1, CDR2, or CDR3; heavy and light chain are treated in the same way). The sequence length must be at most equal to the number of amino acids indicated in each CDR. The Yvis platform will gap sequences according to the chosen CDR. In sequence name and comments line (line starting with ">"), the user can insert the following information, separated by "|": PDB/identification source, chain identification, chain type (H or L, otherwise the information will be ignored), antibody-producing organism, engineered antibody information (engineered or not), antigen-producing organism, antigen molecule description, assigned germline species, V gene, percentage of V gene identity, J gene, and percentage of J gene identity. These are suggested data and could be used in analysis filters; however, only the sequence identification is mandatory (and will be used in the PDB identification field).

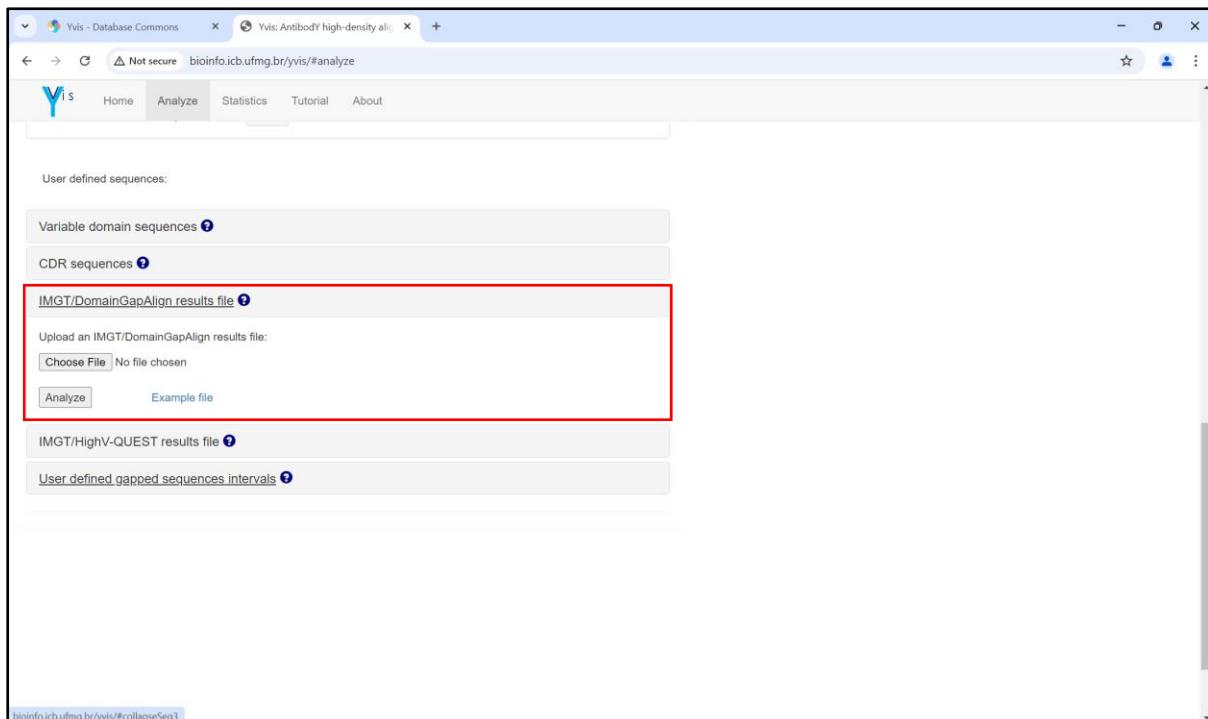


Fig 11: IMGT / DomainGapAlign results file

Select this option to insert an IMGT/DomainGapAlign results file. IMGT/ Domain Gap Align allows aligning amino acid sequences, gapping uploaded sequences, and indicating the closest germline V and J genes. When uploading sequences into IMGT/DomainGapAlign, it is recommended to choose 1 as input in the "Displayed alignments" option, because all displayed alignment sequences will be analysed by Yvis, even if there are multiple alignments of the same sequence. After submitting sequences to IMGT/DomainGapAlign, save the webpage that presents the results in your computer (HTML file: .htm or .html extension). Then submit this file to Yvis.

Yvis will process the submitted file on the user's web browser, extracting the chain identification, chain type, and antibody numbering and germline information. As IMGT/DomainGapAlign ignores the additional information passed on the sequence headers from the FASTA file, some information will be missing in the data table (e.g., engineered, antigen and antibody species, and molecule description).

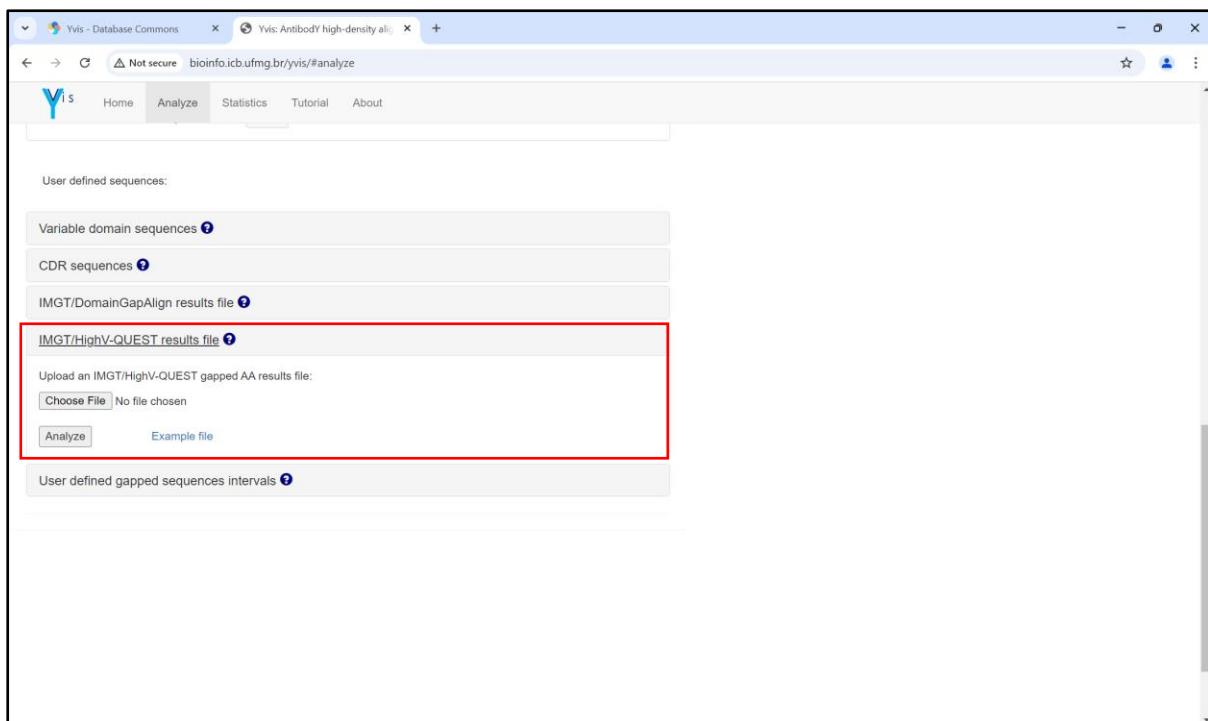


Fig 12: IMGT / HighV – QUEST results file

Select this option to insert an IMGT/HighV-QUEST results file. IMGT/HighV-QUEST analyses next-generation sequencing (NGS) data on antigen receptors. Users must submit a FASTA file containing the nucleotide sequences to IMGT/HighV-QUEST. This tool will generate a set of files that can be downloaded as a compressed file. After decompressing the file, submit the gapped amino acid file, identified as “4_IMGT-gapped-AA-sequences.txt” to Yvis. This file has a header row followed by several antibody chain rows. Each row has the following fields, as described in IMGT/V-QUEST Documentation, separated by tabs: Yvis will present the Collier de Diamants visualization of sequences that are marked as productive in “V-DOMAIN Functionality” and do not have ambiguous amino acids.

As the time of execution of this analysis is in function of the number of inputted sequences, users should be patient in the case of large files, even if their browser presents a slow script dialog.

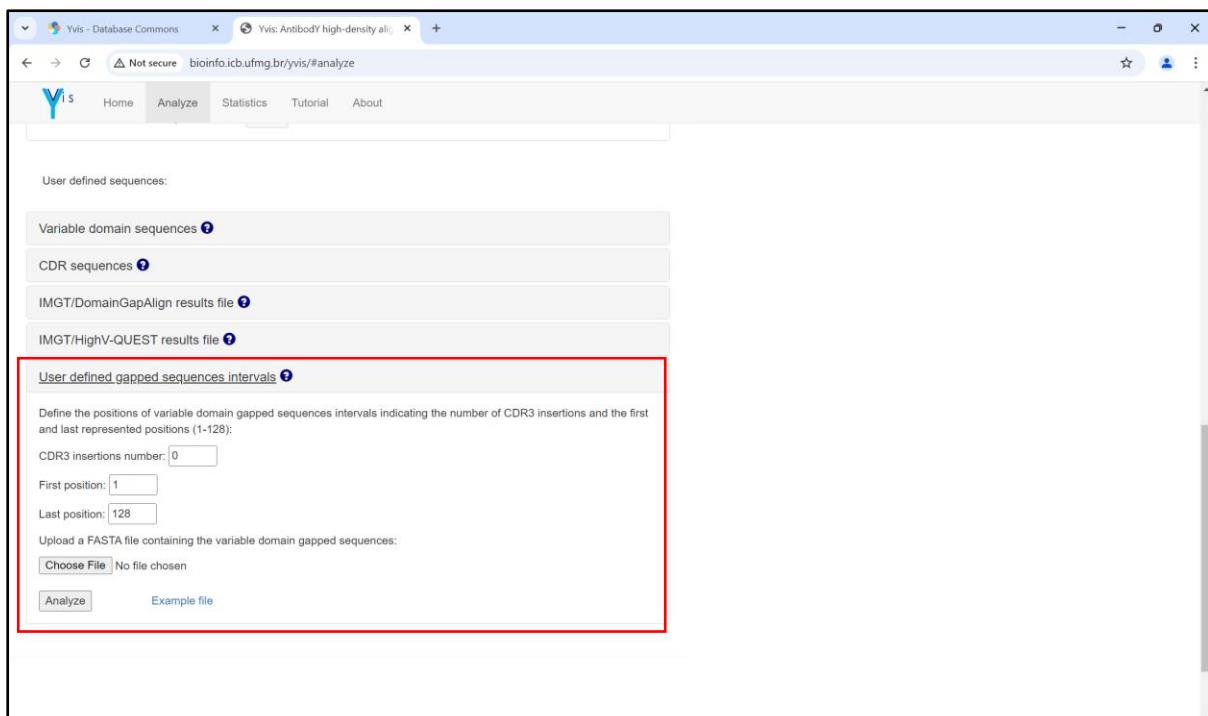


Fig 13: User – defined gapped sequences intervals

Select this option to upload a FASTA file containing gapped amino acid sequences of variable domains of antibodies chains. As Yvis will not change the sequences, they must be aligned in the submitted file. If the uploaded sequences have CDR3 insertions, the user must indicate the number of insertions in the corresponding field. It is also possible to insert a sequence of only one part of the variable domain. In this case, the first and last positions in the corresponding fields must be changed.

In sequence name and comments line (line starting with ">"), the user can insert the following information, separated by "|": PDB/source identification, chain identification, chain type (H or L, otherwise the information will be ignored), antibody-producing organism, engineered antibody information (engineered or not), antigen-producing organism, antigen molecule description, assigned germline species, V gene, percentage of V gene identity, J gene, and percentage of J gene identity. These are suggested information and could be used in analysis filters. However, only the sequence identification is mandatory (and will be used in the PDB identification field).

REFERENCES:

1. Yvis: AntibodY high-density alignment visualization and analysis platform with integrated database. (n.d.). <http://bioinfo.icb.ufmg.br/yvis/>
2. Carvalho, M., Molina, F., & Felicori, L. L. (2019). Yvis: antibody high-density alignment visualization and analysis platform with an integrated database. Nucleic Acids Research. <https://doi.org/10.1093/nar/gkz387>
3. YVIS - Database Commons. (n.d.). <https://ngdc.cncb.ac.cn/databasecommons/database/id/6818>

WEBLEM 6(A)

Yvis: AntibodY high-density alignment visualization and analysis platform with integrated database
(URL: <http://bioinfo.icb.ufmg.br/yvis/>)

AIM:

To study the variable and constant domain along with the Topology Diagram using Yvis Platform.

INTRODUCTION:

Yvis Database

AntibodY high-density alignment visualization and analysis platform with integrated database. Yvis is a web-based platform designed for the analysis of antibody sequences through a novel visualization method called Collier des Diamants, an adaptation of the IMGT/Collier de Perles representation. This platform enables users to examine amino acids in the variable domains of antibody chains, aligned with their structural positions in conserved beta-strands and loops. Yvis facilitates the analysis of multiple antibody chain sequences using this graphical representation, offering insights into their composition and structural arrangement. Users can upload sequences or use pre-processed data from the Yvis database, and the platform supports exportable visual and textual data for further analysis.

- The users can perform the analysis in the "Analyze" tab.
- The "Statistics" tab shows data from the database of pre-processed PDB structures.
- The "Tutorial" tab presents a tutorial explaining how to use this tool and interpret the Collier de Diamants visualization.
- The "About" tab shows information on the Yvis authors and interface versions.

Zika virus (ZIKV):

A number of emerging and re-emerging infections have taken a heavy toll on the public health around the globe. ZIKV was first identified, almost 70 years ago, in rhesus monkeys during a yellow fever surveillance in the Zika Forest in Uganda and was initially reported in humans in 1952. ZIKV is one of the re-emerging arboviruses (arthropod borne) which is transmitted by Aedes mosquito. It is a single-stranded RNA virus belonging to the genus Flavivirus of the Flaviviridae family and has been related to the other Flaviviruses including yellow fever virus, dengue virus (DENV), chikungunya virus, and West Nile virus. ZIKV virus belongs to two phylogenetic types: Asian and African. ZIKV in Africa is maintained in a life cycle (sylvatic transmission) that mainly includes monkeys and apes with humans as occasional hosts, but on the other hand, the Asian lineage of ZIKV includes humans as the main host. In the majority of people, infection by the Zika virus is mild and self-limiting. Diseases caused by Zika virus are predominately arboviral and transmitted by the bite of female *Aedes aegypti* and *Aedes albopictus* mosquitoes. Besides a mosquito bite, the virus can also be transmitted sexually.

METHODOLOGY:

1. Open a web browser and navigate to the homepage of the YVis Database.
2. Select 'Analyze' Option from the available menu on the Homepage.
3. Select Input Options -Select User-defined sequences, followed by choosing the 'CDR sequences' option.
(To proceed with the analysis, download a sample FASTA file containing complementarity-determining region (CDR) sequences.)
4. Click on 'Example file' and download the file containing 68 CDR3 sequences of anti-Zika virus antibody heavy chains isolated from four infected donors.
5. Click on the 'Choose File' button and select the downloaded FASTA file containing the CDR sequences.
6. Choose the 'CDR3' option to analyze the complementarity-determining region 3 (CDR3).
7. Click on the 'Analyze' button to initiate the analysis of the antibody variable domain sequences.

OBSERVATIONS:

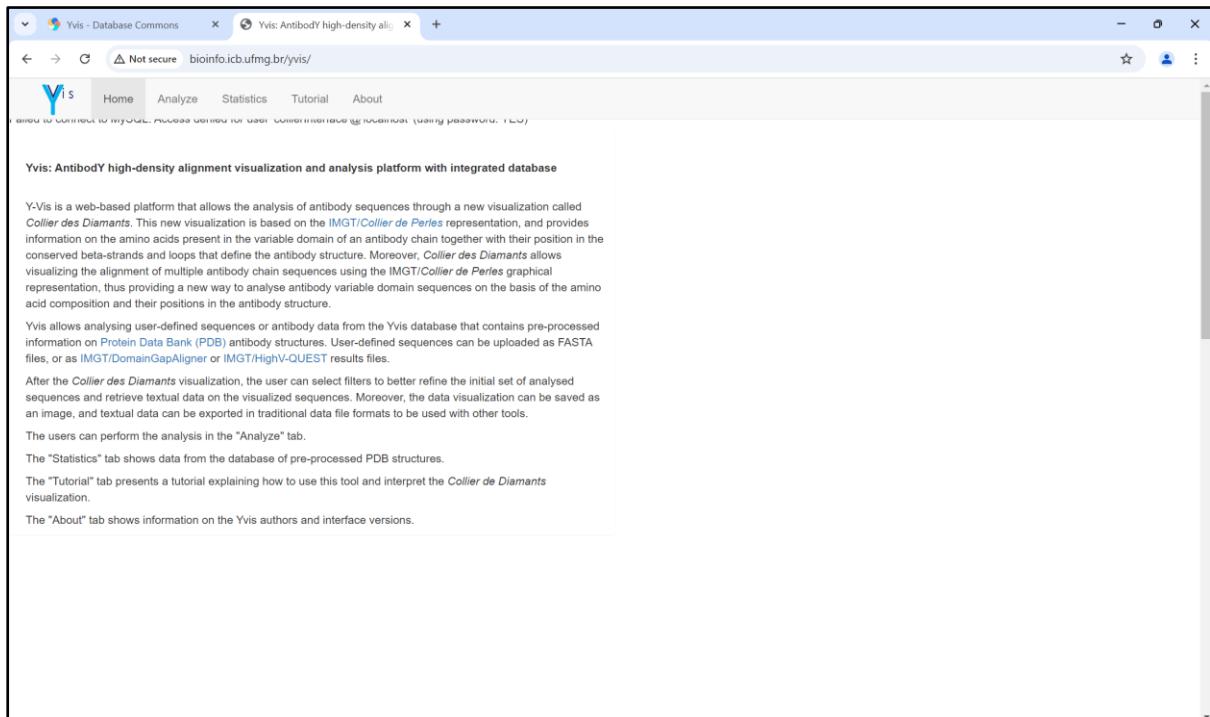


Fig 1: Homepage of Yvis: Antibody high – density alignment visualization and analysis platform with Integrated databases

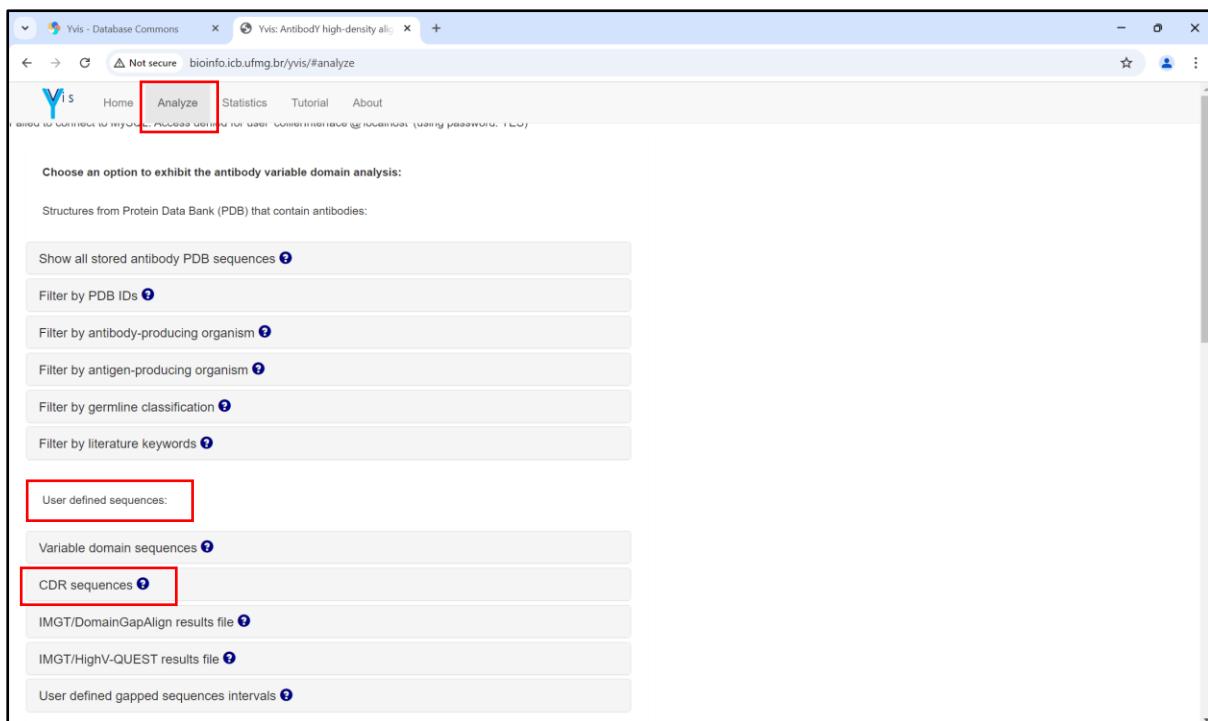


Fig 2: Selecting the ‘Analyze’ option in the Yvis portal and selecting the ‘CDR Sequences’ option

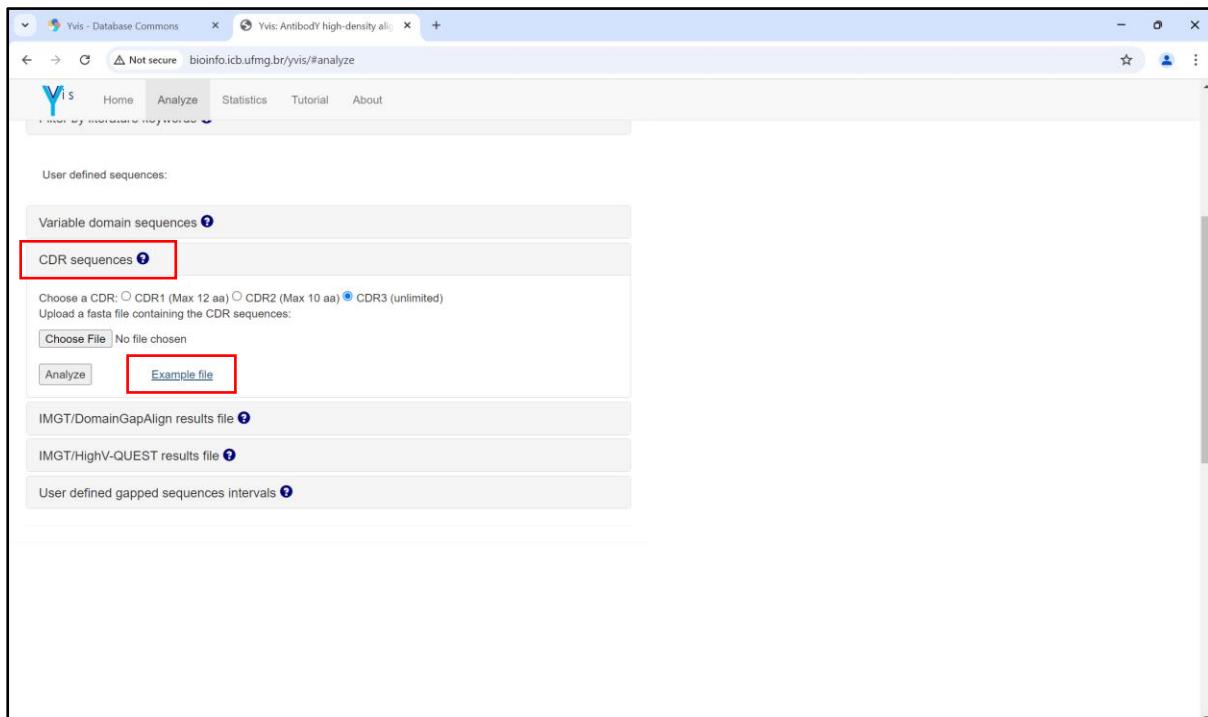


Fig 3: Selecting the ‘Example file’

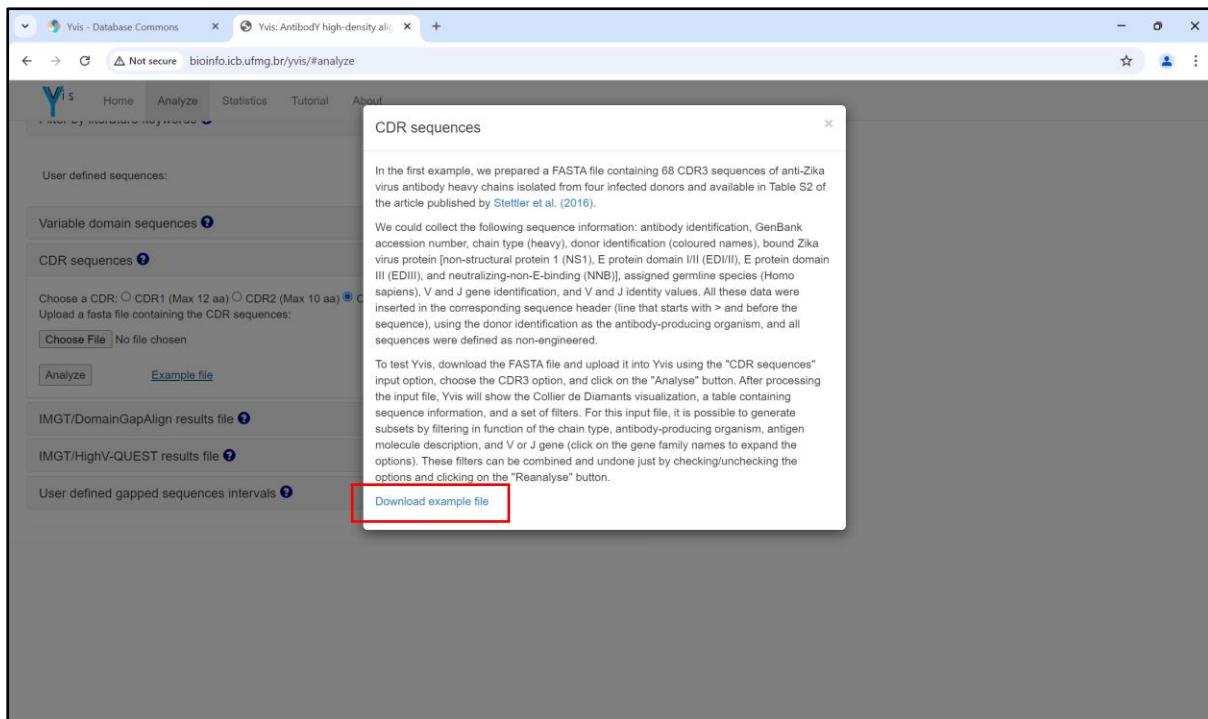


Fig 4: Click on ‘Download Example File’ option

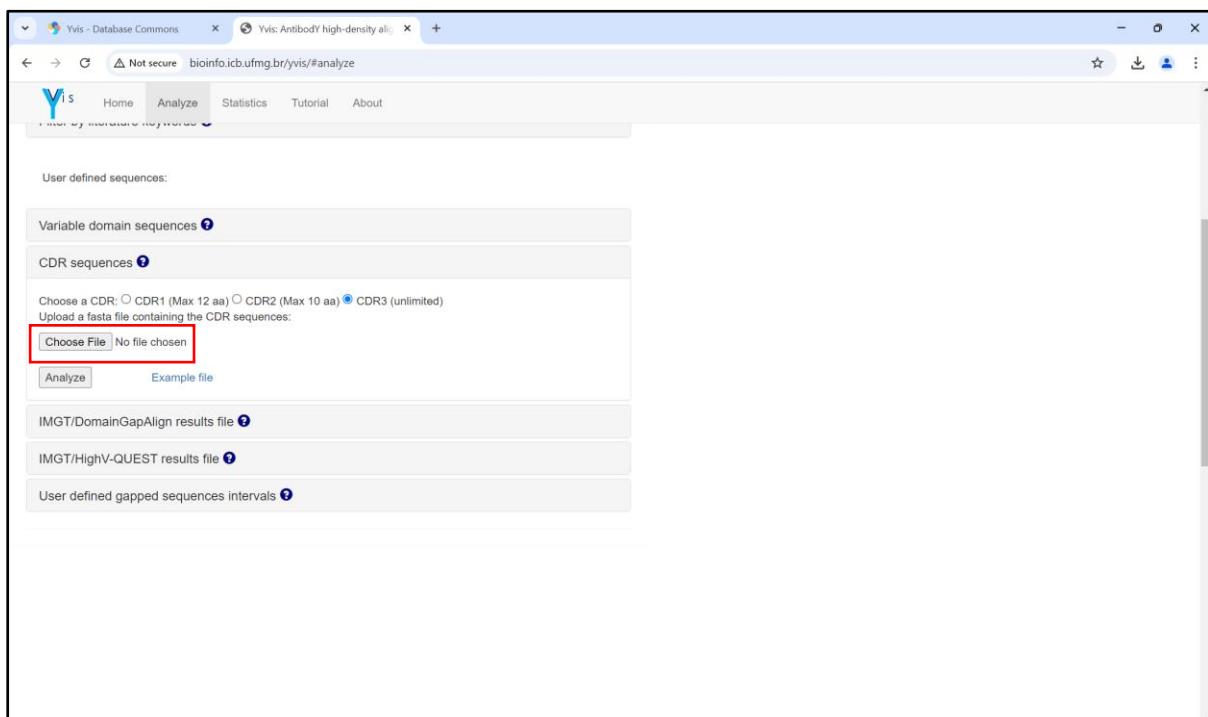


Fig 5: Click on ‘Choose File’ option

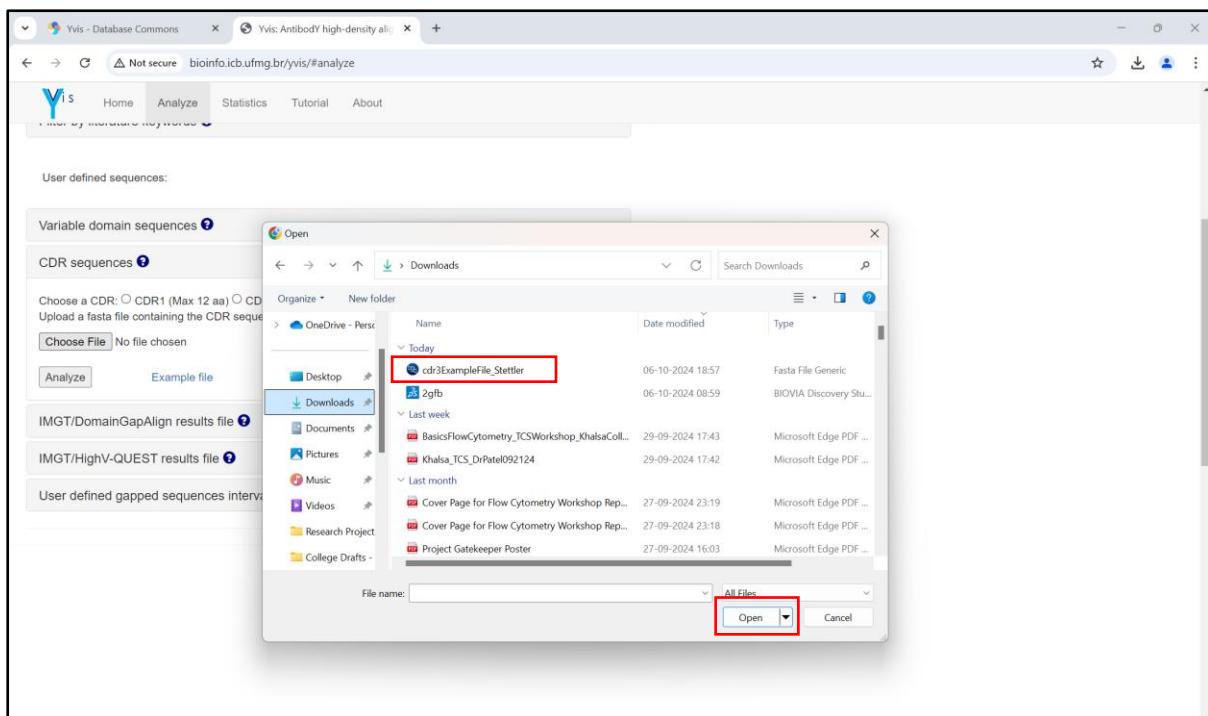


Fig 6: Select the FASTA file downloaded and click on ‘Open’

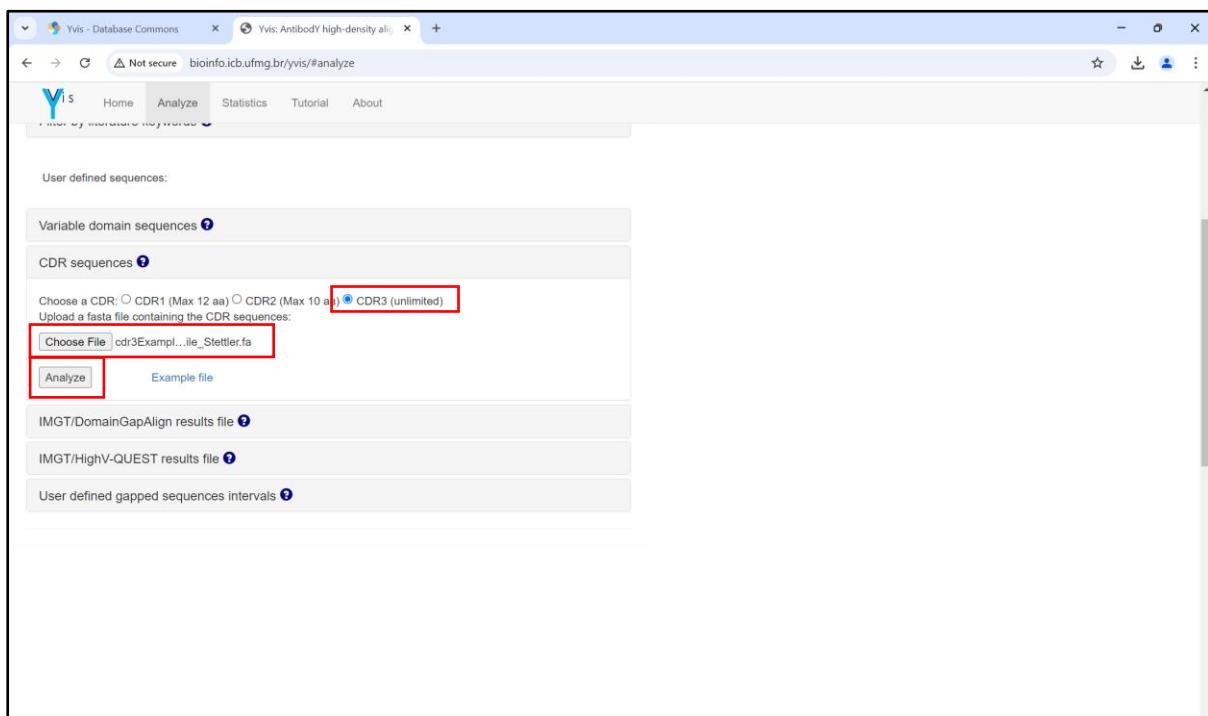


Fig 7: Choose the option ‘CDR3 (Unlimited)’

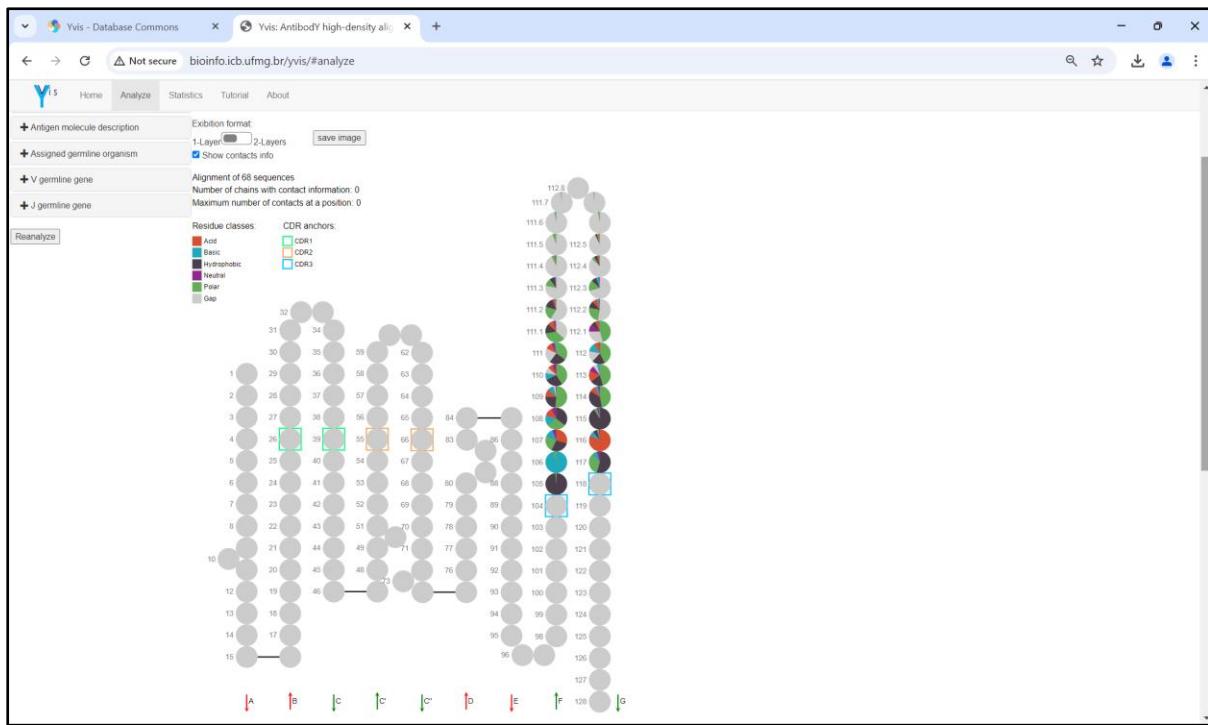


Fig 8: Result page showing user – defined antibody sequences obtained from cdr3ExampleFile_Stettler.fa file

a. IMGT / *Collier de Perles* (Pearl Necklace) visualization in one layers

Squares indicate the CDR anchors, one position before the CDR start regions and one after the CDR end regions (i.e., green for CDR1, orange for CDR2, and blue for CDR3)

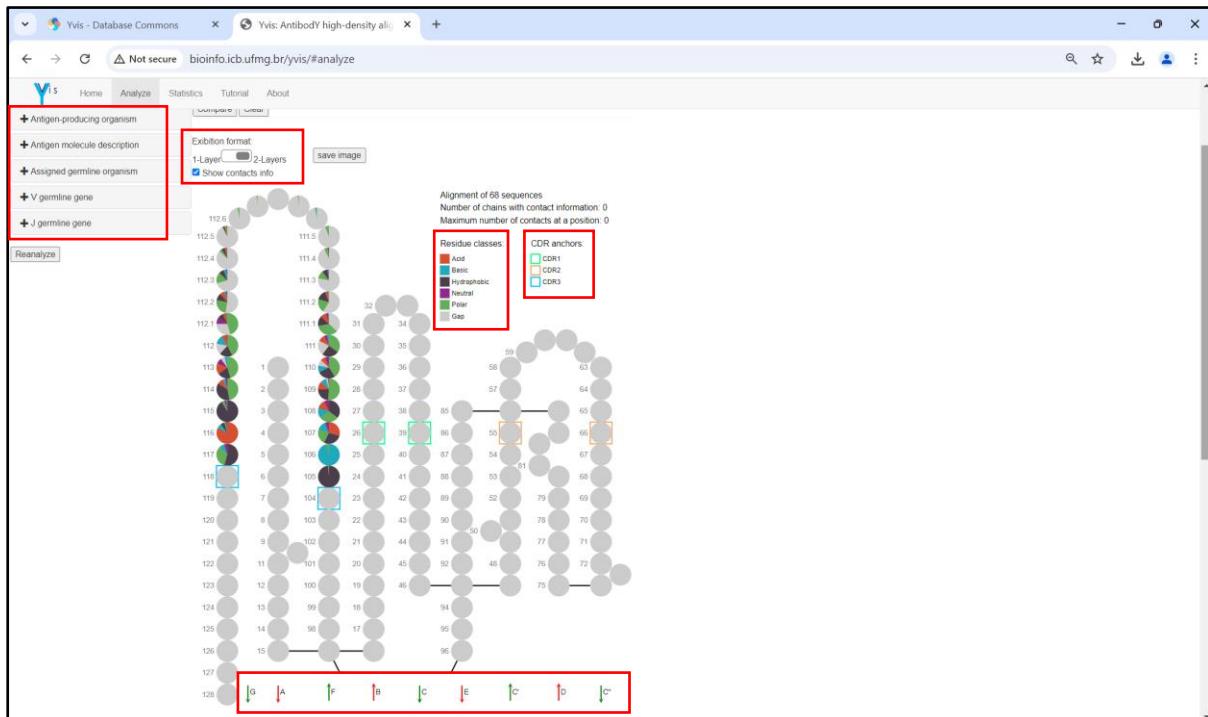


Fig 8.1: b. IMGT / *Collier de Perles* visualization in two layers

The “pie slice” (sector) represents the number of sequences with an amino acid of a specific class (defined by a colour) in that position. Green represents polar amino acids (G, S, T, Y, C,

Q, N), blue represents basic (K, R, H), red represents acidic (D, E), and black represents hydrophobic amino acids (A, V, L, I, P, W, F, M). In Yvis, gaps are in grey.

The strands of the variable domain are identified by letters (A-G) and arrows at the bottom of *Collier de Perles* visualization.

Filter option, is to analyse a subset of the initial dataset.

PDB Id	Chain Id	Antibody Chain Type	Antibody Species	Engineered Antibody	Antigen Organism	Antigen Molecule Description	Gapped Sequence	CDR highlights:	Putative contact highlights:
ZKA10	KX496835	Heavy	Blue	No	Zika virus	NS1
ZKA117	KX496861	Heavy	Blue	No	Zika virus	EDI/II
ZKA134	KX496852	Heavy	Blue	No	Zika virus	EDIII
ZKA160	KX496843	Heavy	Blue	No	Zika virus	NNB
ZKA172	KX496833	Heavy	Blue	No	Zika virus	NNB
ZKA174	KX496850	Heavy	Blue	No	Zika virus	NNB
ZKA18	KX496830	Heavy	Blue	No	Zika virus	NS1
ZKA185	KX496858	Heavy	Blue	No	Zika virus	NNB
ZKA189	KX496825	Heavy	Blue	No	Zika virus	NNB
ZKA190	KX496868	Heavy	Blue	No	Zika virus	EDIII

Fig 9: Data table – Yvis presents a table with information on each analyzed sequence. Click on the PDB ID to compare with the multiple sequence alignment presented in the *Collier de Perles*

A table containing the information available for all chains presented in the multiple sequence alignment. Information table contains the following fields: PDB/source identification, chain identification, chain type (heavy or light), antibody-producing organism, engineered antibody information (if the chain was marked as engineered), antigen-producing organism, antigen molecule description, gapped and ungapped chain sequences, assigned germline species, V gene, percentage of V gene identity, J gene, and percentage of J gene identity. At the gapped sequence column, the CDR positions are highlighted (green for CDR1, orange for CDR2, and blue for CDR3) as well as the putative contacts (salmon).

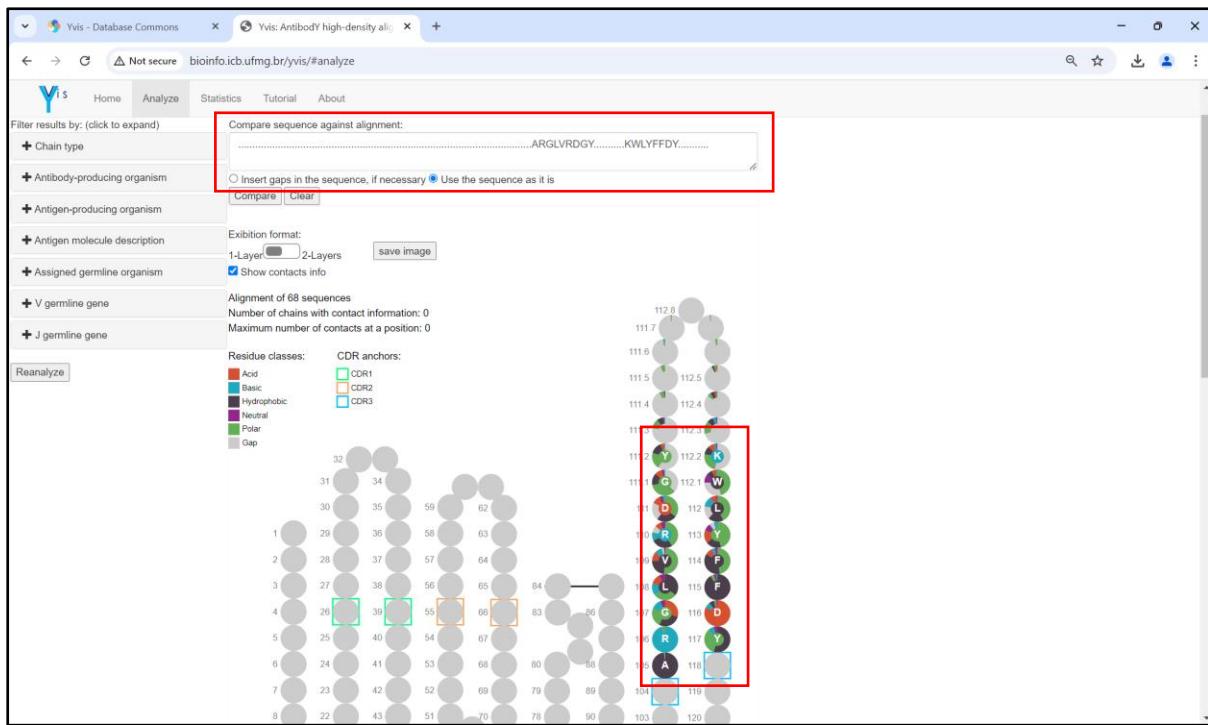


Fig 10: Result page for comparison of the selected sequence with the multiple sequence alignment

Centre of each pie chart that represents a position, a small circle with the inputted sequence amino acid corresponding to that position.

RESULTS:

Yvis platform were explored and the Collier de perles visualization presents multiple sequence alignments using pie charts, where each sector represents amino acid classes by color. Conserved positions show dominant sectors, while variable ones display multiple sectors. It highlights key structural regions like CDRs: CDR1 corresponds to positions 26-39, CDR2 corresponds to positions 55-66, and CDR3 corresponds to position 104-118, allowing visualization of residues involved in antigen binding.

The IMGT/Collier de Perles, presented in one or two layers were observed. The two-layers version presents the variable domain strands in a position closer to the 3D structure, while the one-layer version has a representation closer to the variable domain sequence.

The result for the comparing the multiple sequence alignment with using the sequence as it is by selecting PDB Id from the data table containing the information available for all chains presented in the multiple sequence alignment, were observed.

CONCLUSIONS:

The Yvis platform was explored and a detailed study of both the variable and constant regions, along with the topology diagram, offering a comprehensive analysis of the antibody's structure and function were studied. The Yvis platform, used for high-density antibody alignment and analysis, effectively assessed the similarity between the query and amino acid sequences, aiding in determining the relevance of associated antigens. By applying relevant filters, the platform enabled an in-depth investigation into sequence alignments.

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1. Wolford RW, Schaefer TJ. Zika Virus. [Updated 2023 Aug 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK430981/>
 2. Rawal, G., Yadav, S., & Kumar, R. (2016). Zika virus: An overview. Journal of family medicine and primary care, 5(3), 523–527. <https://doi.org/10.4103/2249-4863.197256>
 3. Masmejan, S., Musso, D., Vouga, M., Pomar, L., Dashraath, P., Stojanov, M., Panchaud, A., & Baud, D. (2020). Zika Virus. Pathogens (Basel, Switzerland), 9(11), 898. <https://doi.org/10.3390/pathogens9110898>
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WEBLEM 7 AgAbDb Database

AIM:

Introduction to Ag-Ab Interaction Database (AgAbDb)

INTRODUCTION:

The function of antibodies (Abs) involves specific binding to antigens (Ags) and activation of other components of the immune system to fight pathogens. The six hypervariable loops within the variable domains of Abs, commonly termed complementarity determining regions (CDRs), are widely assumed to be responsible for Ag recognition, while the constant domains are believed to mediate effector activation. Recent studies and analyses of the growing number of available Ab structures, indicate that this clear functional separation between the two regions may be an oversimplification. Some positions within the CDRs have been shown to never participate in Ag binding and some off CDRs residues often contribute critically to the interaction with the Ag. Moreover, there is now growing evidence for non-local and even allosteric effects in Ab-Ag interaction in which Ag binding affects the constant region and vice versa. The CDRs have different approaches for their identification and their relationship to the Ag interface. We also review what is currently known about the contribution of non- CDRs regions to Ag recognition, namely the framework regions (FRs) and the constant domains. The suggested mechanisms by which these regions contribute to Ag binding are discussed. Beyond improving the understanding of immunity, characterization of the functional role of different parts of the Ab molecule may help in Ab engineering, design of CDR-derived peptides, and epitope prediction.

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 produces a single species of antibody having a unique antigen-binding site.

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.htm>. Antigen–antibody interactions are a special class of protein– protein interactions that are characterized by high affinity and strict specificity 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). AgAbDb is a derived knowledge base 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 identification of 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 design mimotopes representing antigens as well as aid in designing processes leading to humanization of antibodies.

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2. Qiu, Tianyi, et al. “Proteochemometric Modeling of the Antigen-Antibody Interaction: New Fingerprints for Antigen, Antibody and Epitope-Paratope Interaction.” PLOS ONE, vol. 10, no. 4, 22 Apr. 2015, p. e0122416, 10.1371/journal.pone.0122416.

NOTE: The portal is not working for AgAbDb database.

WEBLEM 8
Immune Epitope Database (IEDB)
(URL: <https://www.iedb.org/homev3.php>)

AIM:

Introduction to IEDB Database for the prediction of the Cytotoxic and Helper T cell epitopes (MHC Class 1 epitopes and MHC Class 2 epitopes)

INTRODUCTION:

The Immune Epitope Database (IEDB) is a comprehensive and freely accessible resource that provides detailed information on immune epitopes, which are crucial for understanding adaptive immune responses. Established in 2003 and continually updated, the IEDB serves as a vital tool for researchers studying various aspects of immunology, including vaccine development, allergy research, and autoimmune diseases.

Key Features of IEDB

1. Extensive Data Repository:

- The IEDB contains information on over 2.2 million epitopes related to infectious diseases, allergies, autoimmunity, and transplantation.
- It includes curated data from more than 20,000 published manuscripts and covers both T cell and B cell epitopes across multiple species, including humans and non-human primates.

2. User-Friendly Interface:

- The database features a web portal (www.iedb.org) that allows users to easily search and access epitope data. This includes tools for predicting and analysing B cell and T cell epitopes.
- Users can download data in various formats, including Microsoft Excel, XML, or MySQL.

3. Curation Process:

- Data is meticulously curated from scientific literature and submissions by researchers. The IEDB employs rigorous automated validation processes to ensure data accuracy and relevance. The curation process has evolved to reflect the increasing complexity of immune epitope data, accommodating advancements in scientific techniques and standards.

4. Analysis Resources:

- The IEDB Analysis Resource (IEDB-AR) is a companion site offering computational tools for epitope prediction and analysis. These tools include epitope clustering, sequence conservancy analysis, and predictions of T cell receptor (TCR) and B cell

receptor (BCR) structures. New tools are regularly added to enhance functionality, such as those for predicting naturally processed ligands for MHC class I and II.

5. Accessibility:

- The IEDB is funded by the National Institute of Allergy and Infectious Diseases (NIAID) and is available to the public without any cost. Its continuous updates ensure that it remains a relevant resource for ongoing research in immunology.

T Cell Epitopes

T cell epitopes are specific peptide fragments derived from proteins (antigens) that are recognized by T cells, a crucial component of the adaptive immune system. These epitopes are presented on the surface of antigen-presenting cells (APCs) bound to major histocompatibility complex (MHC) molecules, allowing T cells to initiate an immune response.

Types of T Cell Epitopes

1. CD8 T Cell Epitopes:

- Recognized by CD8+ T cells and presented by MHC class I molecules.
- These epitopes typically originate from intracellular proteins, including viral or mutated proteins, allowing CD8+ T cells to identify and destroy infected or cancerous cells.

2. CD4 T Cell Epitopes:

- Recognized by CD4+ T cells and presented by MHC class II molecules.
- These epitopes are usually derived from extracellular proteins and play a vital role in orchestrating the immune response by helping other immune cells.

Mechanism of Recognition

The recognition of T cell epitopes involves several key steps:

- 1. Antigen Processing:** Proteins are broken down into smaller peptides within the APC.
- 2. Peptide-MHC Binding:** The processed peptides bind to MHC molecules, which then transport these complexes to the cell surface.
- 3. T Cell Activation:** The T cell receptor (TCR) on T cells recognizes the peptide-MHC complex, leading to T cell activation and proliferation.

Importance of T Cell Epitope Prediction

Identifying T cell epitopes is essential for various applications, including:

- 1. Vaccine Development:** Understanding which epitopes can elicit a strong immune response aid in designing effective vaccines.
- 2. Immunotherapy:** Predicting neoepitopes (cancer-specific peptides) can enhance personalized cancer treatment strategies.
- 3. Disease Understanding:** Epitope mapping helps in elucidating mechanisms of autoimmune diseases and allergies, where inappropriate immune responses occur.

T cell prediction

1. T Cell Epitopes - MHC Binding Prediction:

- Peptide binding to MHC class I molecules**

This tool will take in an amino acid sequence, or set of sequences and determine each subsequence's ability to bind to a specific MHC class I molecule.

- Peptide binding to MHC class II molecules**

This tool employs different methods to predict MHC Class II epitopes, including a consensus approach which combines NN-align, SMM-align and Combinatorial library methods.

- TepiTool**

The Tepitool provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps.

2. T Cell Epitopes - Processing Prediction:

These tools predict epitope candidates based upon the processing of peptides in the cell.

- Proteasomal cleavage/TAP transport/MHC class I combined predictor**

This tool combines predictors of proteasomal processing, TAP transport, and MHC binding to produce an overall score for each peptide's intrinsic potential of being a T cell epitope.

- Neural network-based prediction of proteasomal cleavage sites (NetChop) and T cell epitopes (NetCTL and NetCTLpan)**

NetChop is a predictor of proteasomal processing based upon a neural network. NetCTL and NetCTLpan are predictors of T cell epitopes along a protein sequence. It also employs a neural network architecture.

- MHC-NP: Prediction of peptides naturally processed by the MHC**

MHC-NP employs data obtained from MHC elution experiments in order to assess the probability that a given peptide is naturally processed and binds to a given MHC molecule.

- MHCII-NP**

This tool utilizes MHC II ligand elution data to predict naturally processed MHC II ligands by scanning the given peptide sequences

3. T Cell Epitopes - Immunogenicity Prediction:

These tools make predictions about the relative ability of a peptide/MHC complex to elicit an immune response.

- T cell class I pMHC immunogenicity predictor**

This tool uses amino acid properties as well as their position within the peptide to predict the immunogenicity of a class I peptide MHC (pMHC) complex.

- Deimmunization**

The deimmunization tool is attempt to identify immunodominant regions in a given therapeutically important protein, and suggest amino-acid substitutions that create non-immunogenic versions of the proteins.

- **CD4 T cell immunogenicity prediction**

The server is developed to predict the allele independent CD4 T cell immunogenicity at population level. User can predict the T cell immunogenicity using 7-allele method, immunogenicity method and combined method (IEDB recommended). The combined method predicts the final score that combines the predictions from 7-allele method and immunogenicity method.

- **AXEL-F (Antigen Expression based Epitope Likelihood-Function)**

AXEL-F incorporates antigen abundance estimates with MHC binding predictions to enhance epitope predictions.

TCR Analysis

TCRMatch compares input CDR3b sequences against curated CDR3b sequences in the IEDB to find matches that are predicted to share epitope specificity. Matches are determined by sequence similarity, which is scored using a comprehensive k-mer comparison.

Structure Tools

1. LYRA (Lymphocyte Receptor Automated Modelling):

The LYRA server predicts structures for either T-Cell Receptors (TCR) or B-Cell Receptors (BCR) using homology modelling. Framework templates are selected based on BLOSUM score, and complementary determining regions (CDR) are then selected if needed based on a canonical structure model and grafted onto the framework templates.

2. SCEptRe: Structural Complexes of Epitope Receptor

SCEptRe provides weekly updated, non-redundant, user customized benchmark datasets with information on the immune receptor features for receptor-specific epitope predictions.

3. Docktope

DockTopo is a web-based tool, based on D1-EM-D2 approach, intended to allow the pMHC-I modelling. This tool has been developed by Gustavo Fioravanti Vieira's group and has been deployed to the IEDB-AR servers with minimal modification by the IEDB team.

B Cell Epitopes

B cell epitopes are the specific regions of an antigen that are recognized by B cell receptors or secreted antibodies. These epitopes can be classified into two main categories based on their structure:

1. Linear (Continuous) Epitopes

- Consist of contiguous amino acid residues in the primary sequence of the antigen.
- Represent about 10% of all identified epitopes.
- Can be recognized by antibodies out of the remaining protein context and can replace the whole protein for antibody production.

2. Conformational (Discontinuous) Epitopes

- Include amino acid residues that are not sequential in the primary structure but are close in space due to the three-dimensional folding of the antigen.
- Make up the majority (about 90%) of B cell epitopes.
- The minimal amino acid sequence required for proper folding may range from 20 to 400 residues.

Importance of B Cell Epitope Mapping

Identifying B cell epitopes is crucial for various applications:

- 1. Development of epitope-based vaccines:** Epitopes can be used to replace the entire antigen for antibody production.
- 2. Design of therapeutic antibodies:** Knowledge of epitopes aids in developing antibody-based therapeutics.
- 3. Improvement of immunodiagnostic tools:** Epitopes can be used in serodiagnostic assays for disease detection.

B Cell Epitope Prediction

1. Prediction of linear epitopes from protein sequence

A collection of methods to predict linear B cell epitopes based on sequence characteristics of the antigen using amino acid scales and HMMs.

2. Discotope - Prediction of epitopes from protein structure

This method incorporates solvent-accessible surface area calculations, as well as contact distances into its prediction of B cell epitope potential along the length of a protein sequence.

3. ElliPro - Epitope prediction based upon structural protrusion

This method predicts epitopes based upon solvent-accessibility and flexibility.

4. Methods for modelling and docking of antibody and protein 3D structures

This page provides information on available methods for modelling and docking of antibody and protein 3D structures.

Structure Tools

1. LYRA (Lymphocyte Receptor Automated Modelling):

The LYRA server predicts structures for either T-Cell Receptors (TCR) or B-Cell Receptors (BCR) using homology modelling.

2. SCEptRe: Structural Complexes of Epitope Receptor

SCEptRe provides weekly updated, non-redundant, user customized benchmark datasets with information on the immune receptor features for receptor-specific epitope predictions. This tool extracts weekly updated 3D complexes of antibody-antigen, TCR-pMHC and MHC-ligand from the Immune Epitope Database (IEDB) and clusters them based on antigens, receptors and epitopes to generate benchmark datasets.

DiscoTope in IEDB

DiscoTope is a specialized tool within the Immune Epitope Database (IEDB) designed for the prediction of B cell epitopes based on the three-dimensional (3D) structures of proteins. This tool is crucial for researchers aiming to identify potential epitopes that can elicit an immune response, particularly in the context of vaccine development and therapeutic antibody design.

Key Features of DiscoTope

1. Structure-Based Prediction:

- DiscoTope employs a structure-based approach to predict discontinuous (conformational) B cell epitopes. It utilizes 3D structural data to assess surface accessibility and calculate contact numbers, which are essential for determining how well an epitope can be recognized by antibodies.

2. Improved Prediction Algorithms:

- The latest version, DiscoTope-3.0, features significant advancements over previous iterations. It incorporates inverse folding structure representations and utilizes a positive-unlabelled learning strategy, enabling it to predict epitopes from both solved and predicted protein structures. This enhances its applicability across various datasets and reduces dependency on experimentally solved structures.

3. High Performance:

- DiscoTope-3.0 has demonstrated improved predictive performance compared to earlier methods, achieving high accuracy in identifying both linear and conformational epitopes across multiple independent datasets. This is particularly beneficial for large-scale predictions involving numerous proteins.

4. Accessibility:

- The tool is accessible through the IEDB Analysis Resource, allowing users to input PDB (Protein Data Bank) IDs or upload their own PDB files for analysis. Users can select different versions of DiscoTope for their predictions, ensuring flexibility based on their specific research needs.

5. Integration with Other Databases:

- DiscoTope interfaces with databases such as RCSB PDB and AlphaFold DB, facilitating large-scale predictions across a vast catalog of proteins. This integration allows researchers to leverage structural data from multiple sources for more comprehensive epitope mapping.
- DiscoTope is a tool used for predicting discontinuous epitopes from protein 3D structures. The transition from version 1.1 to version 2.0 introduced several significant changes in methodology and performance.

Algorithm Enhancements

1. Proximity Scoring Function

- Version 1.1 utilizes a non-weighted proximity scoring function that evaluates the full-sphere neighbour count to determine the likelihood of nearby epitopes.
- Version 2.0 introduces a modified, weighted proximity scoring function that focuses on an upper half-sphere neighbour count. This adjustment improves prediction accuracy by concentrating on residues that are more likely to be exposed on the protein surface.

2. Surface Exposure Measurement

- Version 1.1 measures surface exposure based on a neighbour count within a 10 Å radius.
- Version 2.0 expands this radius to 14 Å and limits the measurement to the upper half-sphere, providing a more precise evaluation of residues that are accessible for antibody binding.

The screenshot shows the IEDB homepage with a search interface. On the left, there's a 'Welcome' section with a brief introduction to the database. Below it are sections for 'Upcoming Events & News' (Virtual User Workshop Nov 5-7, 2024, Festival of Biologics Apr 23-25, 2025, AACR 2025 Apr 25-30, 2025, Immunology 2025 May 3-7, 2025) and 'Summary Metrics' (Peptidic Epitopes 1,620,437, Non-Peptidic Epitopes 3,188, T Cell Assays 540,375, B Cell Assays 1,410,336, MHC Ligand Assays 4,881,465, Epitope Source Organisms 4,545, Restricting MHC Alleles 1,011, References 25,211). The main search area has tabs for 'Epitope', 'Assay', 'Epitope Source', 'MHC Restriction', 'Host', and 'Disease'. The 'Assay' tab is selected, showing options for T Cell, B Cell, and MHC Ligand. The 'Epitope' tab shows a chemical structure of a peptide. The right side features sections for 'T Cell Epitope Prediction', 'B Cell Epitope Prediction', and 'Epitope Analysis Tools'. A 'CORE TEAM' logo is at the bottom.

Fig 1: Homepage of the IEDB database

The screenshot shows the IEDB Analysis Resource page. At the top, there's a navigation bar with links for Overview, T Cell Tools, B Cell Tools, Analysis Tools, Tools-API, Usage, Download, Datasets, Contribute Tools, and References. The main content area is titled 'Epitope Prediction and Analysis Tools'. It includes sections for 'T Cell Epitope Prediction Tools' (MHC class I & II binding predictions, peptide processing, immunogenicity), 'B Cell Epitope Prediction Tools' (predict regions of proteins likely to be recognized as epitopes), and 'Analysis Tools' (for detailed analysis of known epitope sequence or group of sequences). To the right, there's a 'IEDB-AR News' box with a link to the 'Next-generation Tools site available!' and a 'IEDB-AR Release Notes' box with links to release notes for version 2.28 (26 Apr 2024), 2024-05-08, NetMHCIpan 4.2 and NetMHCIpan 4.3 incorporated into mhci binding web, API, and standalone tools; [Bugfix] Axel-F support..., version 2.27 (25 May 2023), and version 2.26 (24 Feb 2022). The footer contains copyright information and a note about being supported by the National Institute of Allergy and Infectious Diseases.

Fig 2: Overview of the IEDB database

The screenshot shows the IEDB Analysis Resource homepage. At the top, there are tabs for Home, Specialized Searches, Analysis Resource (which is selected), Help, and More IEDB. A banner at the top right encourages users to "Check out our new IEDB updates! (1) Learn how to customize your database exports and (2) try our new analysis tools site for all your analysis and prediction needs." Below the banner, the "START YOUR SEARCH HERE" section contains several search fields:

- Epitope**: Options include Any, Linear peptide, Non-peptidic, Discontinuous, and Non-peptidic. A search field for "Exact M" with the value "SIINFEKL" is shown.
- Assay**: Options include T Cell, B Cell, and MHC Ligand. A search field for "Ex: neutralization" with the value "Find" is shown.
- MHC Restriction**: Options include Any, Class I, Class II, Non-classical, and Non-classical. A search field for "Ex: HLA-A*02:01" with the value "Find" is shown.
- Epitope Source**: Options include Organism (e.g., influenza, peanut) and Antigen (e.g., core, capsid, myo).
- Host**: Options include Any, Human, Mouse, and Non-human primate. A search field for "Ex: dog, camel" with the value "Find" is shown.
- Disease**: Options include Any, Infectious, Allergic, Autoimmune, and Non-classical. A search field for "Ex: asthma" with the value "Find" is shown.

Below these search fields are "Reset" and "Search" buttons. To the right, the "Epitope Analysis Resource" section is visible, containing links for T Cell Epitope Prediction, B Cell Epitope Prediction, and Epitope Analysis Tools. The "T Cell Epitope Prediction" section includes options for MHC I Binding, MHC II Binding, MHC I Processing (Proteasome, TAP), and MHC I Immunogenicity. The "B Cell Epitope Prediction" section includes options for Antigen Sequence Properties and Predict discontinuous B cell epitopes using Discotope and ElliPro. The "Epitope Analysis Tools" section includes Population Coverage, Conservation Across Antigens, and Clusters with Similar Sequences.

Fig 3: Different resources available in the IEDB database

The screenshot shows the "T Cell Tools" page under the "IEDB Analysis Resource" header. The navigation bar includes Overview, T Cell Tools (selected), B Cell Tools, Analysis Tools, Tools-API, Usage, Download, Datasets, Contribute Tools, and References. The main content area is titled "T Cell Epitope Prediction Tools".

T Cell Epitopes - MHC Binding Prediction: Describes tools for predicting IC50 values for peptides binding to specific MHC molecules. It includes sections for Peptide binding to MHC class I molecules (using TepTool) and Peptide binding to MHC class II molecules (using TepTool).

T Cell Epitopes - Processing Prediction: Describes tools for predicting epitope candidates based upon the processing of peptides in the cell. It includes sections for Proteasomal cleavage/TAP transport/MHC class I combined predictor (using NetChop), Neural network based prediction of proteasomal cleavage sites (NetChop) and T cell epitopes (NetCTL and NetCTLpan), and MHC-NP: Prediction of peptides naturally processed by the MHC (using MHCII-NP).

T Cell Epitopes - Immunogenicity Prediction: Describes tools for predicting naturally processed MHC II ligands. It includes a section for MHCII-NP, which uses MHC II ligand elution data to predict naturally processed MHC II ligands by scanning given peptide sequences.

Fig 4: T cell Prediction tool

The screenshot shows the 'B Cell Epitope Prediction Tools' section of the IEDB Analysis Resource. It includes sections for 'Prediction of linear epitopes from protein sequence', 'Discotope - Prediction of epitopes from protein structure', 'EliPro - Epitope prediction based upon structural protrusion', and 'Methods for modeling and docking of antibody and protein 3D structures'. A note indicates that the page provides information on available methods for modeling and docking of antibody and protein 3D structures. Below these are sections for 'Structure Tools': 'LYRA (Lymphocyte Receptor Automated Modelling)' and 'SCEnRe: Structural Complexes of Epitope Receptor'. A note states that LYRA provides weekly updated, non-redundant, user-customized benchmark datasets with information on the immune receptor features for receptor-specific epitope predictions. A note also indicates that tools under AR Labs are experimental and not quite ready for production yet.

Fig 5: B cell Prediction tool

The screenshot shows the 'Analysis Tools' section of the IEDB Analysis Resource. It includes sections for 'Population Coverage', 'Epitope Conservancy Analysis', 'Epitope Cluster Analysis', 'Computational Methods for Mapping Mimotopes to Protein Antigens', 'RATE (Restrictor Analysis Tool for Epitopes)', 'ImmunoBrowser', 'PepSysSco', and 'PepX (Peptide Expression Annotation)'. A note indicates that the tools below are intended for the detailed analysis of a known epitope sequence or group of sequences.

Fig 6: Analysis tools

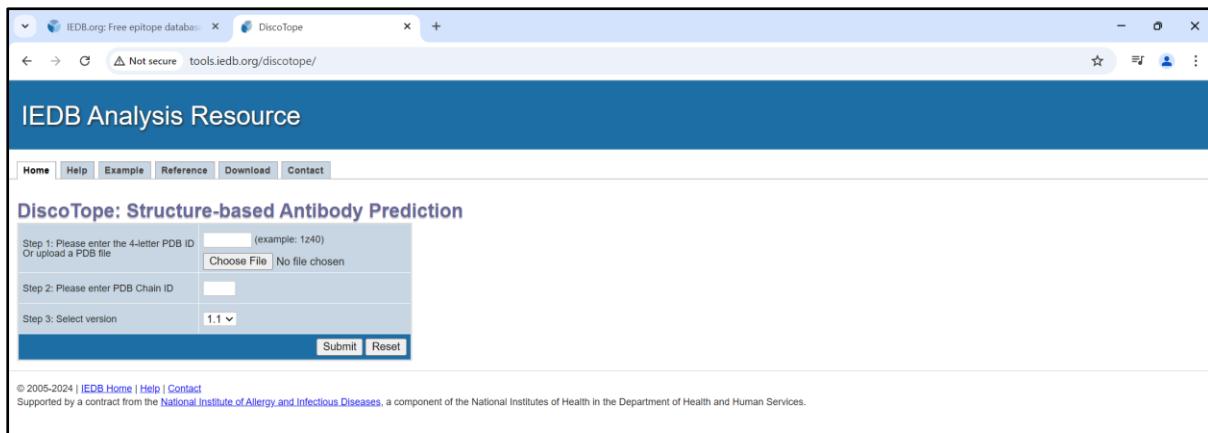


Fig 7: Homepage of DiscoTope program

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WEBLEM: 8(A)
Immune Epitope Database (IEDB)
(URL: <https://www.iedb.org/>)

AIM:

To predict B-Cell epitope for query ‘maltodextrin – binding protein’ (PDB ID: 1IUD) using Discotope Server 1.1 from IEDB Database.

INTRODUCTION:

The Immune Epitope Database (IEDB) is a free, publicly accessible resource established in 2004 that catalogs experimental data on antibody and T cell epitopes. It serves as a comprehensive platform for researchers to access curated epitope data from scientific literature, currently housing over 1.6 million experiments related to various fields, including infectious diseases, allergies, autoimmunity, and transplantation. The IEDB allows users to easily search for epitope information and integrates data from multiple external resources, enhancing usability and accessibility. The IEDB also hosts epitope prediction and analysis tools, and has a companion site, [CEDAR](#) (funded by [NCI](#)), which houses cancer epitopes.

IEDB Analysis Resource (IEDB-AR) and DiscoTope

Accompanying the IEDB is the IEDB Analysis Resource (IEDB-AR), which provides computational tools for predicting and analyzing B and T cell epitopes. Among its various tools is DiscoTope, specifically designed to predict B cell epitopes based on structural data. DiscoTope utilizes amino acid statistics, surface accessibility, and spatial information to identify potential epitopes on the surface of antigens. DiscoTope is a method for predicting discontinuous epitopes from 3D structures of proteins in PDB format. This tool has become invaluable in antibody engineering and vaccine design, allowing researchers to pinpoint B cell epitopes that can be targeted by antibodies. The IEDB-AR continues to evolve, offering enhanced features and improved performance for epitope prediction and analysis.

Maltodextrin – binding protein

The maltodextrin-binding protein (MBP) with PDB ID 1IUD is a recombinant form of the protein from *Escherichia coli*, characterized by an insertion/deletion mutation that includes a B-cell epitope from the PRES2 region of the hepatitis B virus.

Structural Overview

- **Type:** Insertion/Deletion mutant
- **Function:** MBP serves as a high-affinity binding component in the active transport system for maltooligosaccharides, which are crucial for bacterial nutrient uptake.
- **Significance:** The insertion of the hepatitis B virus epitope may have implications for immunological studies and vaccine development.

Key Features

- **Crystal Structure:** The 3D structure reveals how the inserted epitope interacts with the protein, potentially affecting its binding properties and overall conformation.
- **Research Applications:** Understanding this structure can help elucidate mechanisms of protein interactions and transport processes in bacteria, as well as contribute to vaccine design strategies.

The study of MBP has been pivotal in understanding oligosaccharide binding and transport mechanisms. The crystal structure has been referenced in various studies exploring the dynamics of ligand binding and the effects of mutations on protein function.

METHODOLOGY:

1. Open the Protein Data Bank (PDB) website. (URL: <https://www.rcsb.org/>) to obtain the PDB ID of the structure (query).
2. Open the Immune Epitope Database and Tools (IEDB) (<https://www.iedb.org/>) that contains experimental data on antibody and T-cell epitope, also host epitope prediction.
3. Select the ‘DiscoTope’ option under B Cell Epitope prediction from Epitope Analysis Resource section.
4. Enter the 4-letter PDB ID or upload a PDB file for the query (PDB ID: 1IUD), Chain ID for protein chain of interest and select the DiscoTope version 1.1.
5. Click on Submit.
6. Analyse the results in Chart view, Table view and 3D View.
7. The prediction can be saved in .csv extension.

OBSERVATIONS:

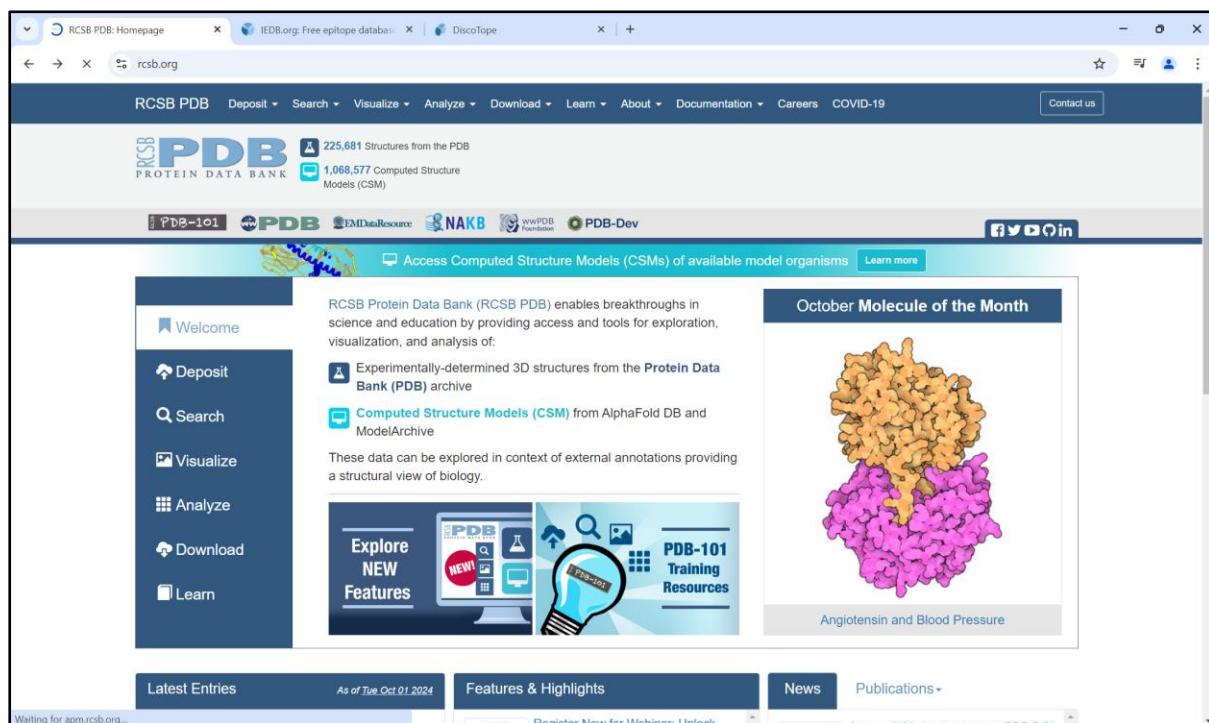


Fig 1: Homepage of the Protein Data Bank (PDB) database

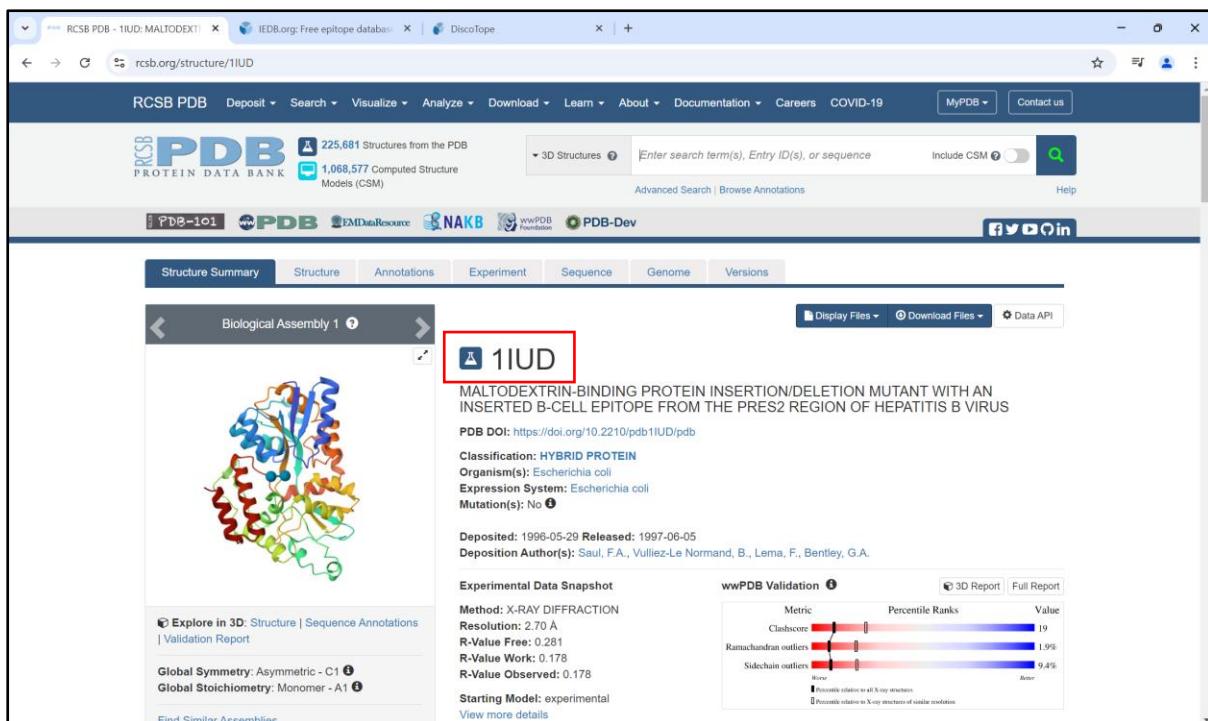


Fig 2: Retrieving the query ‘maltodextrin – binding protein’ (PDB ID: 1IUD) from the PDB database

Fig 3: Homepage of the Immune Epitope Database (IEDB)

The screenshot shows the IEDB.org homepage with a red box highlighting the 'Epitope Analysis Resource' section. Within this section, the 'T Cell Epitope Prediction' and 'B Cell Epitope Prediction' boxes are visible. The 'DiscoTope' option under 'B Cell Epitope Prediction' is highlighted with a red box.

Fig 4: Selecting the ‘Discotope’ option in the Epitope Analysis Resource section

The screenshot shows the DiscoTope prediction program homepage. It features a form with three steps: Step 1 (PDB ID: 1IUD), Step 2 (PDB Chain ID: A), and Step 3 (Version: 1.1). The 'Submit' button is highlighted with a red box.

Fig 5: Homepage of the DiscoTope program for prediction

This screenshot is identical to Fig 5, but it highlights specific input fields with red boxes: the PDB ID field containing '1IUD', the PDB Chain ID field containing 'A', and the 'Submit' button at the bottom.

Fig 6: Searching for the PDB code: 1IUD, Chain ID: A ; Select the version 1.1 and click on ‘Submit’

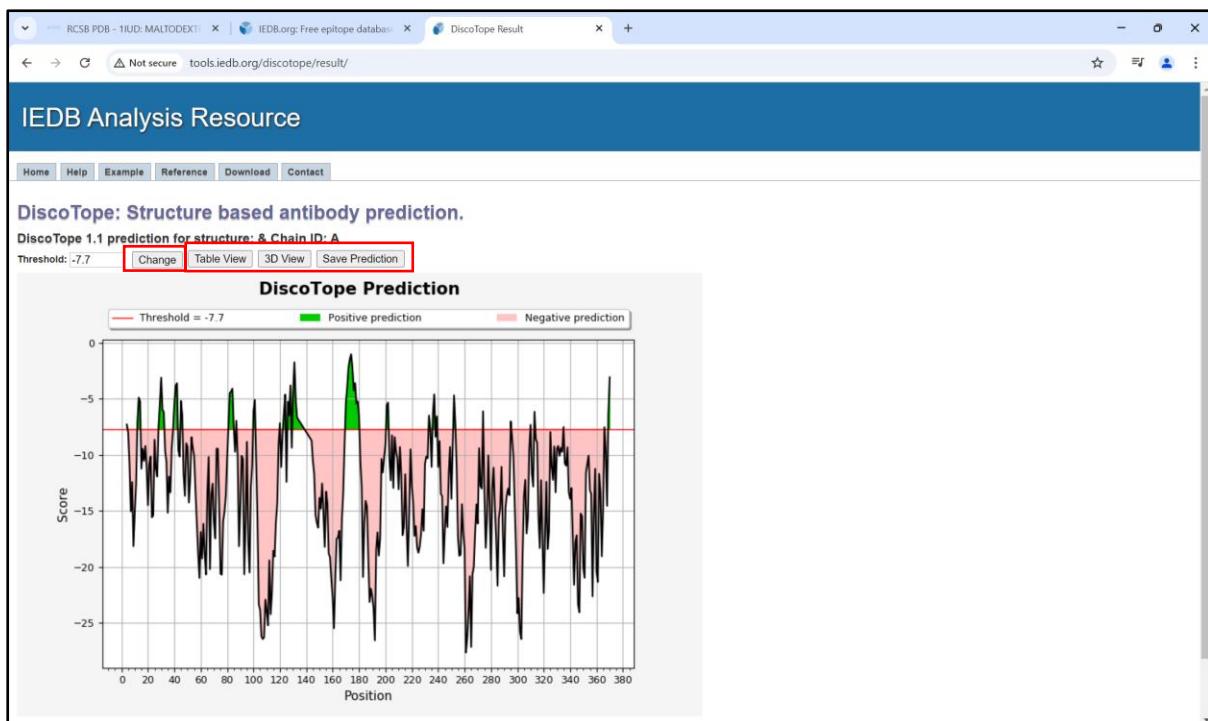


Fig 7: Search results obtained can be analyzed in Chart View, Table View and 3D View

Description: To change the threshold value, enter a different threshold and click on 'Change'. The default value for version 1.1 is -7.7 and version 2.0 is -3.7, which corresponds to a specificity of 75%. Higher values correspond to higher specificity. A specificity of 0.75 means that 25% of the nonepitope residues were predicted as part of epitopes. A sensitivity of 0.47 means that 47% of the epitope residues were predicted as part of epitopes.

In the chart, predictions above the threshold (red line) are positive predictions (displayed in green) and predictions below the threshold are negative prediction (displayed in orange).

The screenshot shows a web browser window with the title "IEDB Analysis Resource" and the sub-page "DiscoTope - Result". The URL is tools.iedb.org/discotope/table/. The page displays a table of results for "DiscoTope 1.1 prediction for structure: & Chain ID: A". It states that positive predictions are displayed in green. There are three tabs at the top: "Chart View", "3D View", and "Save Prediction". The "3D View" tab is currently selected.

Chain ID	Residue ID	Residue Name	Contact Number	Propensity Score	Discotope Score
A	174	GLY	7	2.487	-1.013
A	173	ASN	8	2.542	-1.458
A	370	LYS	8	0.932	-3.068
A	30	ASP	9	1.401	-3.099
A	82	ASP	9	0.028	-4.472
A	83	LYS	9	0.167	-4.333
A	84	ALA	9	0.415	-4.085
A	101	GLY	9	-0.599	-5.099
A	172	GLU	10	2.805	-2.195
A	237	THR	10	0.402	-4.598
A	274	GLU	10	-1.103	-6.103
A	4	GLU	11	-1.78	-7.28
A	42	LYS	11	1.897	-3.603
A	100	ASN	11	-0.586	-6.086
A	175	LYS	11	3.253	-2.247
A	211	SER	11	-3.789	-9.289
A	310	GLU	11	-1.814	-7.314
A	313	LYS	11	-0.645	-6.145
A	5	GLY	12	-2.013	-8.013
A	29	LYS	12	0.56	-5.44
A	41	ASP	12	2.203	-3.797
A	45	GLU	12	0.829	-5.171
A	53	THR	12	-2.408	-8.408
A	81	PRO	12	-1.422	-7.422
A	201	ASN	12	0.431	-5.569
A	239	LYS	12	-0.55	-6.55
A	31	THR	13	0.540	-5.951

Fig 8: Results obtained in Table View

Description:

- **Chain ID:** The chain id of the protein chain used in prediction (specified by the user)
- **Residue ID:** PDB Residue id
- **Residue Name:** Name of the residue
- **Contact Number:** The residue contact number is the number of C α atoms in the antigen within a distance of 10 Å of the residue's C α atom. A low contact number correlates with localization of the residue close to the surface or in protruding regions of the antigen's structures.
- **Propensity Score:** This score tells you about the probability/tendency of being part of an epitope for that particular residue. The propensity is reflected in amino acid epitope log-odds ratios, which were calculated on a set of 75 antigens. The propensity score is calculated by sequentially averaging epitope log-odds ratios within a window of 9 residues. Then the scores are summed up based on the proximity in the 3D structure of the antigen. For any given residue, the sequentially averaged log-odds scores from all residues within 10Å are summed to give the propensity score.
- **Discotope Score:** This score is calculated by combining the contact numbers with propensity score. DiscoTope score above the threshold value indicates positive predictions and that below the threshold value indicates negative predictions. Positive predictions are displayed in green. Click on header to sort column.

Chain ID	Residue ID	Residue Name	Contact Number	Propensity Score	DiscoTope Score	View
A	4	GLU	11	-1.78	-7.28	CPK
A	12	ASN	19	2.418	-7.082	CPK
A	13	GLY	15	2.621	-4.879	CPK
A	14	ASP	14	1.769	-5.211	CPK
A	28	GLU	15	-0.002	-7.502	CPK
A	29	LYS	12	0.56	-5.44	CPK
A	30	ASP	9	1.401	-3.999	CPK
A	31	THR	13	0.549	-5.951	CPK
A	32	GLY	13	0.317	-6.183	CPK
A	40	PRO	14	1.555	-5.445	CPK
A	41	ASP	12	2.203	-3.797	CPK
A	42	LYS	11	1.897	-3.603	CPK
A	45	GLU	12	0.829	-5.171	CPK
A	46	LYS	14	0.514	-6.486	CPK
A	81	PRO	12	-1.422	-7.422	CPK
A	82	ASP	9	0.028	-4.472	CPK
A	83	LYS	9	0.167	-4.333	CPK
A	84	ALA	9	0.415	-4.085	CPK
A	85	PHE	13	-0.922	-7.422	CPK
A	87	ASP	13	-0.447	-6.947	CPK

Fig 9: Results obtained in 3D Viewer

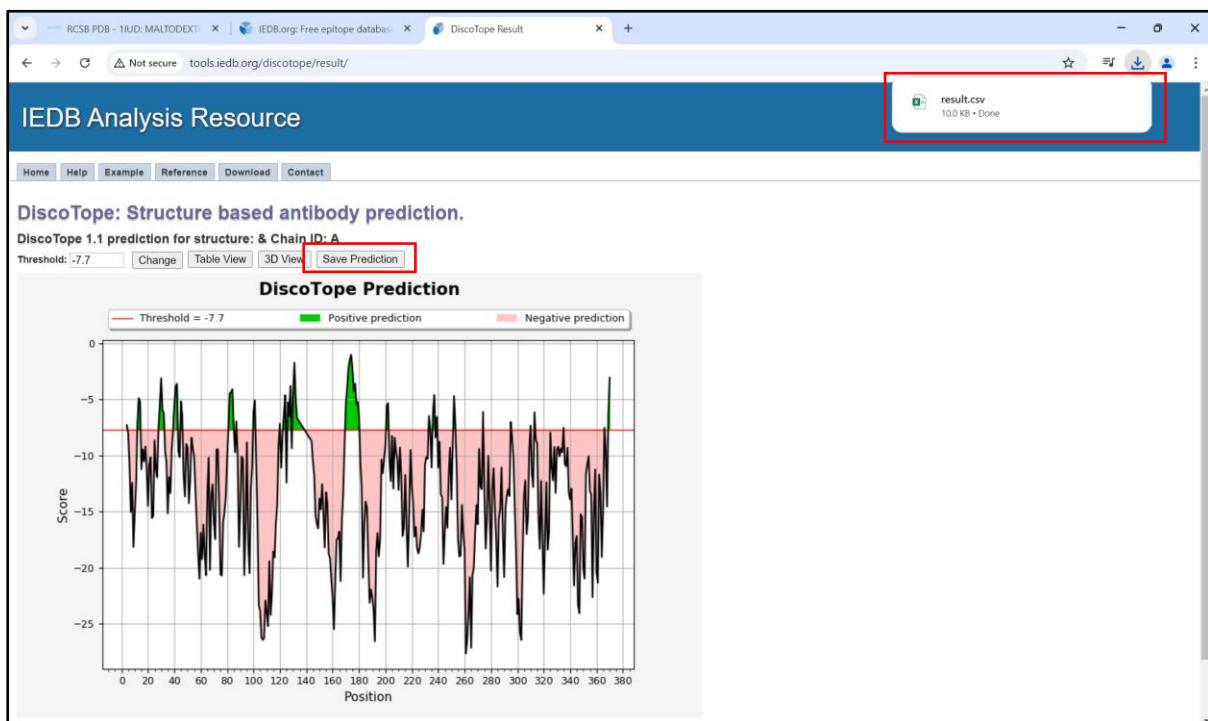


Fig 10: Save the prediction in .csv file

RESULTS:

The DiscoTope analysis of the ‘maltodextrin – binding protein’ (PDB ID: 1IUD) provided several critical insights regarding potential B cell epitopes:

1. **Chart View:** The predictions indicated that several residues had DiscoTope scores above the threshold value i.e., -7.7, which are considered positive predictions, displayed in green. This score suggests a high likelihood of these residues being part of an epitope, indicating their potential accessibility for antibody binding.
2. **Table View:** The detailed results included the following key metrics:
 - **Chain ID:** The chain ID for the analyzed protein, which is Chain A.
 - **Residue ID:** The specific identifier for the glycine residue (GLY).
 - **Contact Number:** The contact number for this glycine residue is 7, indicating that there are seven C α atoms from other residues within a distance of 10 Å of this glycine's C α atom. A low contact number suggests that this residue is well-exposed on the surface of the protein and accessible for antibody binding. In this context, a contact number of 7 indicates reasonable accessibility, although it may not be as optimal as residues with lower contact numbers. Generally, low contact numbers (e.g., 1 or 2) are preferred for predicting epitopes, as they correlate with increased exposure on the protein surface.
 - **Propensity Score:** The propensity score for this glycine is 2.487, reflecting a strong likelihood that this residue is part of an epitope based on its amino acid characteristics and statistical analysis from known antigens.
 - **DiscoTope Score:** The DiscoTope score for this residue is -1.013. This negative score indicates a lower probability of this residue being involved in immune recognition, despite its contact number and favorable propensity score.
3. **3D View:** The spatial visualization allowed for examination of predicted epitopes on the IgG4 structure, facilitating insights into their interactions with antibodies.

CONCLUSION:

The analysis highlighted a glycine residue with a contact number of 7, indicating reasonable accessibility, a high propensity score of 2.487 suggesting it has a strong likelihood of being part of an epitope, but a DiscoTope score of -1.013 indicating that it may not be as strongly favored for immune recognition compared to other residues with higher positive scores.

Additionally, the findings underscore the importance of utilizing tools like DiscoTope within the Immune Epitope Database (IEDB) to predict B cell epitopes effectively. The IEDB serves as a comprehensive resource for researchers, offering curated data and analytical tools that enhance our understanding of immune responses and facilitate vaccine development and therapeutic design. Understanding the range of contact scores is crucial; while lower scores indicate better surface exposure and accessibility for antibody binding, higher scores can still suggest reasonable accessibility depending on the context. Overall, these insights contribute significantly to advancing research in immunology and related fields by providing a clearer picture of how structural features influence epitope prediction and antibody interactions.

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-

WEBLEM 9

PaDELPy

(URL: <https://github.com/ecrl/padelpy>)

AIM:

Introduction to molecular Descriptors and PADEL Descriptor software.

INTRODUCTION:

PaDELPy is a powerful and versatile Python package that serves as a wrapper for PaDEL-Descriptor, a widely-used software in the field of cheminformatics and computational chemistry. This package bridges the gap between the Java-based PaDEL-Descriptor and the Python ecosystem, enabling researchers and data scientists to seamlessly integrate molecular descriptor calculations into their Python workflows. By providing a convenient Python interface to PaDEL-Descriptor, PaDELPy facilitates the calculation of a wide array of molecular descriptors and fingerprints, which are essential for various cheminformatics applications.

The primary purpose of PaDELPy is to simplify the process of calculating molecular descriptors and fingerprints, making these crucial tools more accessible to researchers working in Python environments. It offers a comprehensive set of features that make it an invaluable asset in computational chemistry. PaDELPy can compute a vast array of molecular descriptors, including 1D, 2D, and 3D descriptors. These encompass constitutional descriptors, which provide basic information about the molecule's composition; topological descriptors, which capture the connectivity and shape of molecules; geometrical descriptors, which describe the three-dimensional structure of molecules; electronic descriptors, which relate to the distribution of charge in molecules; and hybrid descriptors, which combine multiple types of molecular information.

In addition to descriptor calculation, PaDELPy supports the generation of various types of molecular fingerprints, such as MACCS keys, PubChem fingerprints, and substructure fingerprints. These fingerprints are crucial for tasks like similarity searching and machine learning applications in drug discovery. The package accepts multiple input formats, including SMILES strings, SDF files, and MOL2 files, providing flexibility in handling different molecular representations. This input flexibility allows researchers to work with their preferred molecular formats without the need for additional conversion steps.

One of the key strengths of PaDELPy is its customization options. Users can tailor their calculations by selecting specific descriptors or fingerprints and adjusting calculation parameters to suit their research needs. This level of control allows researchers to focus on the molecular features most relevant to their studies, potentially improving the efficiency and relevance of their analyses. To use PaDELPy, researchers must have both Python and Java installed on their system. The package can be easily installed using pip, the Python package

installer, with a simple command: "pip install padel-py". This straightforward installation process makes PaDELPy readily accessible to researchers and developers working in Python environments. Once installed, using PaDELPy in a Python script is straightforward. For example, to calculate descriptors for a molecule represented as a SMILES string, one would first import the PaDELDescriptor class from padel_py, initialize it, and then use the calculate_descriptors method with the SMILES string as input.

Molecular Descriptors

Molecular descriptors are numerical values that describe the chemical structure of molecules. They can represent various properties such as molecular weight, atom counts, functional groups, and 3D spatial information. These descriptors are essential for quantitative structure-activity relationship (QSAR) modeling, virtual screening, and other cheminformatics applications. Here are the main types of molecular descriptors up to 6D:

1. 0D Descriptors (Constitutional Descriptors):

- These descriptors are simple counts of atoms, bonds, or molecular fragments without considering the molecule's connectivity or spatial arrangement.
- **Examples:** Molecular weight, number of atoms, number of bonds, and atom type counts.

2. 1D Descriptors (Structural Descriptors):

- 1D descriptors represent the sequence of atoms or specific chemical groups in a molecule without taking molecular topology into account.
- **Examples:** Molecular formulas, number of specific functional groups (e.g., hydroxyl groups, halogens).

3. 2D Descriptors (Topological Descriptors):

- These descriptors represent the connectivity or topology of a molecule's structure in two dimensions. They are derived from the molecular graph, where atoms are represented as nodes and bonds as edges.
- **Examples:**
 - a. Topological indices like Wiener index, Balaban index.
 - b. Atom connectivity indices (degree of atoms, valence values).
 - c. Fragment-based descriptors (counts of specific substructures or functional groups).

4. 3D Descriptors (Geometric/Spatial Descriptors):

- 3D descriptors represent the three-dimensional arrangement of atoms in space, capturing molecular shape, size, and volume. These are crucial for understanding stereochemistry and interactions in docking studies.
- **Examples:**
 - a. Molecular surface area (van der Waals or solvent-accessible surface).
 - b. Molecular volume.
 - c. Dipole moment.
 - d. Shape indices (e.g., radius of gyration).

5. 4D Descriptors (Molecular Dynamics Descriptors):

- 4D descriptors incorporate time-dependent information to represent the dynamic behavior of molecules in different environments (e.g., solution, gas phase). These descriptors are generated from molecular dynamics (MD) simulations and capture conformational changes over time.
- **Examples:**
 - a. Time-averaged molecular properties (e.g., average distances between atoms).
 - b. Conformational flexibility measures.

6. 5D Descriptors (Quantum Descriptors):

- 5D descriptors account for quantum mechanical properties and interactions, often used in quantum chemistry. These descriptors take into account the electronic structure of molecules and how they change under different conditions.
- **Examples:**
 - a. Electron density distribution.
 - b. Molecular orbitals (HOMO, LUMO).
 - c. Quantum mechanical energy levels.

7. 6D Descriptors (Pharmacophore Descriptors):

- 6D descriptors are used to represent the pharmacophoric properties of molecules. A pharmacophore is a set of features (like hydrogen bond donors, acceptors, hydrophobic regions) that are responsible for the biological activity of a molecule. These descriptors aim to capture the spatial arrangement and dynamic nature of pharmacophoric features in a molecule.
- **Examples:**
 - a. Pharmacophoric patterns based on 3D alignments of molecular features.
 - b. Dynamic pharmacophore models (time-dependent movements of pharmacophoric features).

Significance of Molecular Descriptors

1. **Drug Design and Discovery:** Aid in predicting biological activity, toxicity, and pharmacokinetic properties for identifying potential drug candidates.
2. **Structure-Activity Relationship (SAR):** Help correlate molecular structures with biological activity, enabling QSAR modeling to understand structure-function relationships.
3. **Predictive Modeling:** Serve as inputs for machine learning models to predict chemical properties like toxicity, solubility, and binding affinity.
4. **Chemical Property Analysis:** Provide insights into molecular properties like hydrophobicity, polarity, molecular weight, etc., essential for understanding molecular interactions.

Key Molecular Descriptors and Their Symbols:

Descriptor Type	Symbol	Definition
Molecular Weight	MW	The sum of the atomic weights of all atoms in a molecule, indicating its size.
Log P (Partition Coefficient)	Log P	A measure of lipophilicity, indicating how a compound partitions between water and octanol.
Topological Polar Surface Area (TPSA)	TPSA	The surface area of polar atoms in a molecule, affecting solubility and permeability.
Molecular Volume	V _m	The 3D space occupied by a molecule, often computed from van der Waals radii.
Molecular Surface Area	A _s	The total surface area of a molecule, which can impact interactions with biological targets.
Hydrogen Bond Donor Count	HBD	The number of hydrogen bond donors in a molecule, influencing solubility and interaction.
Hydrogen Bond Acceptor Count	HBA	The number of hydrogen bond acceptors in a molecule, affecting its interaction properties.
Rotatable Bonds	RB	The number of rotatable bonds in a molecule, indicating flexibility and conformational change.
Log D (Distribution Coefficient)	Log D	A measure of the distribution of a compound between two phases, considering pH and ionization.
Molecular Shape Index	-	Describes the overall shape of a molecule, which can influence biological activity.
Dipole Moment	μ	A vector quantity that represents the polarity of a molecule, indicating charge distribution.
Polarizability	α	The ability of a molecule to have its electron cloud distorted by an external electric field.
Solvent Accessible Surface Area (SASA)	SASA	The surface area of a molecule that is accessible to solvent, relevant for solubility studies.
Constitutional Index	CI	A measure that considers the connectivity of atoms in a molecule, providing insight into stability.
3D-Radius of Gyration	R _g	A measure of the distribution of atoms around the molecule's center of mass, indicating compactness.

PaDEL-Descriptor

PaDEL-Descriptor is a software for calculating molecular descriptors and fingerprints. The software currently calculates 797 descriptors (663 1D, 2D descriptors, and 134 3D descriptors) and 10 types of fingerprints. These descriptors and fingerprints are calculated mainly using The Chemistry Development Kit. Some additional descriptors and fingerprints were added, which include atom type electrotopological state descriptors, McGowan volume, molecular linear free energy relation descriptors, ring counts, count of chemical substructures identified by Laggner, and binary fingerprints and count of chemical substructures identified by Klekota and Roth. Although a large number of descriptors can be calculated using various descriptor calculation software, considering the information available in a PubChem fingerprint, only PubChem

fingerprints of the compounds were used to train the model. PaDELPy, a Python wrapper for PaDEL-Descriptor software, was used for calculating the PubChem fingerprints.

INSTALLATION:

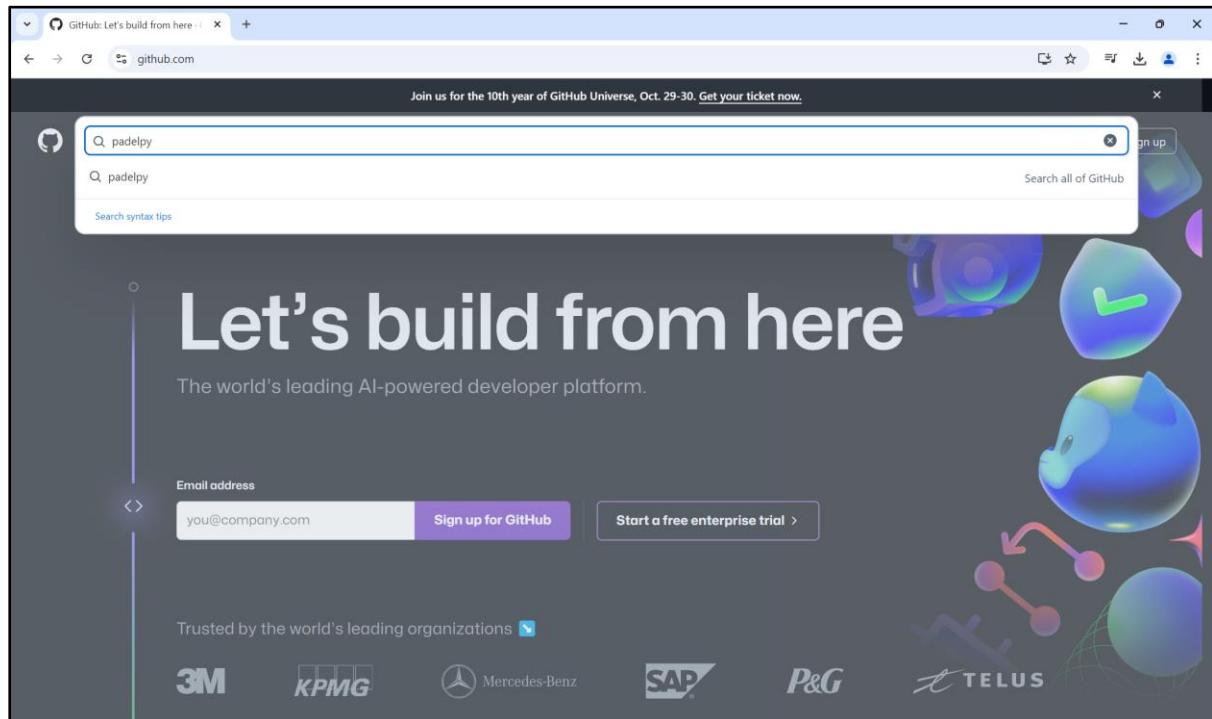


Fig 1: Open GitHub and search for ‘PaDelPy’

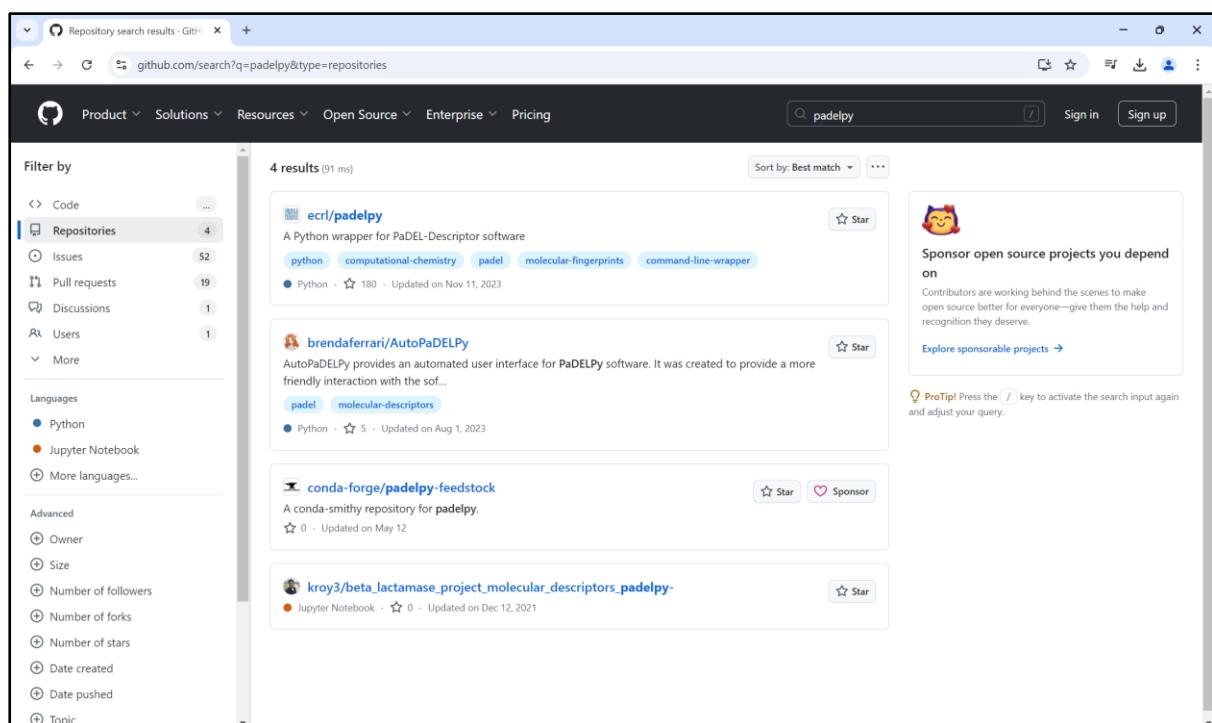


Fig 2: Open ‘ecrl/padelpy’

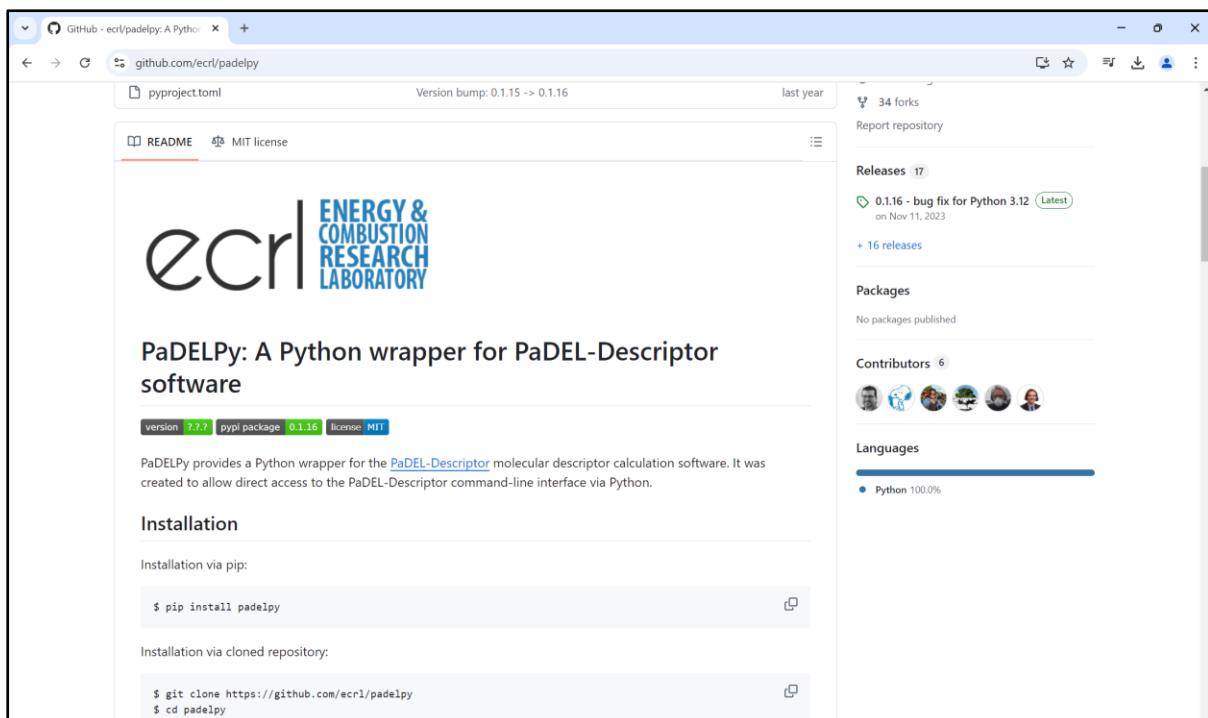


Fig 3: Python wrapper for PaDEL – Descriptor Software

A screenshot of a Windows Command Prompt window titled 'Administrator: Command Prompt'. The user runs the command '\$ pip3 install padelpy', which triggers a pip3 installation process. The output shows the download of the package from GitHub, the extraction of files, and the successful installation of version 0.1.16. The command prompt then returns to the user's directory.

Fig 4: Installing PaDELpy via Command Prompt

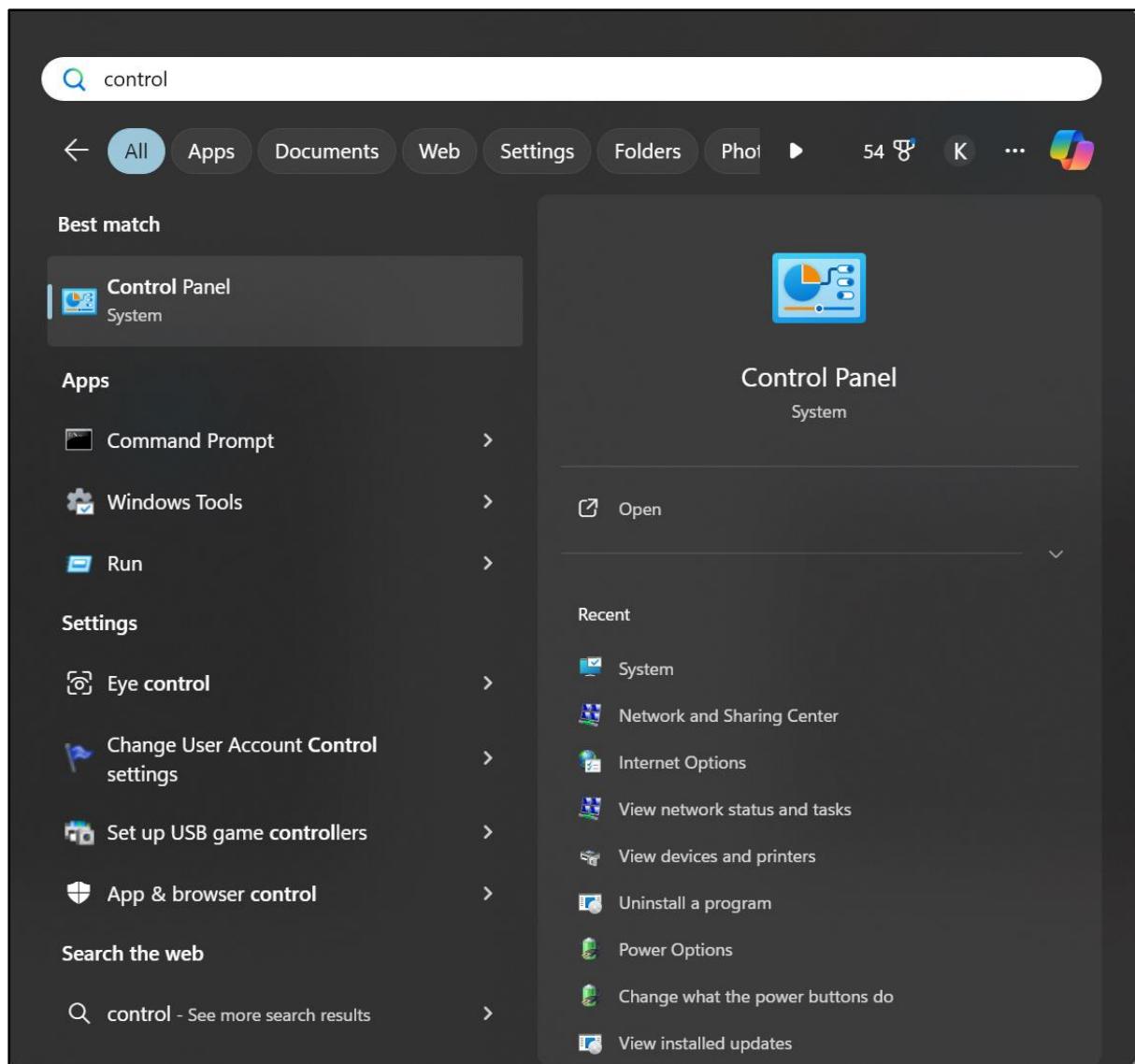


Fig 5: Open Control Panel

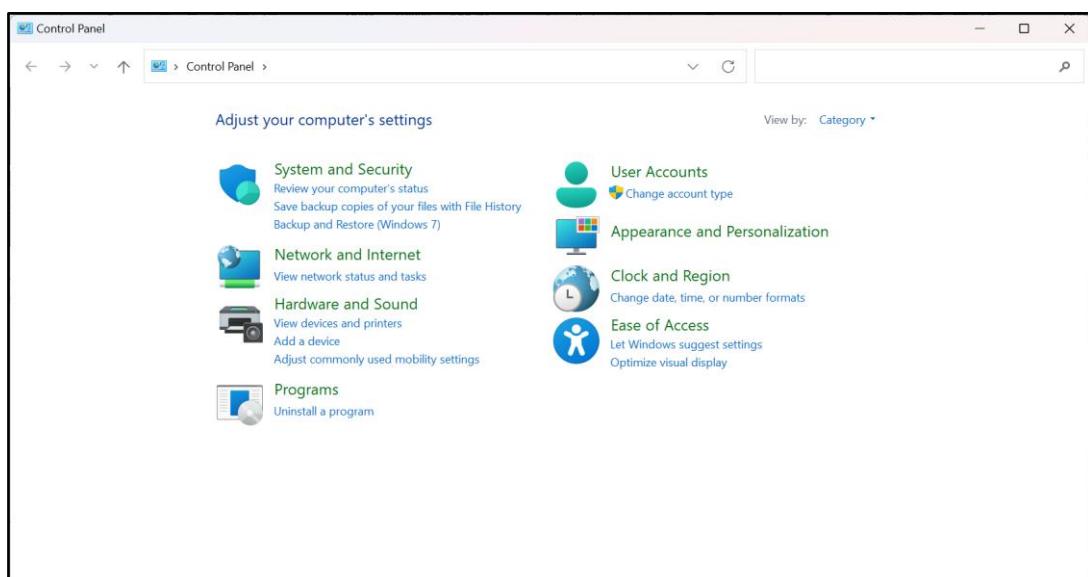


Fig 6: Select 'System and Security'

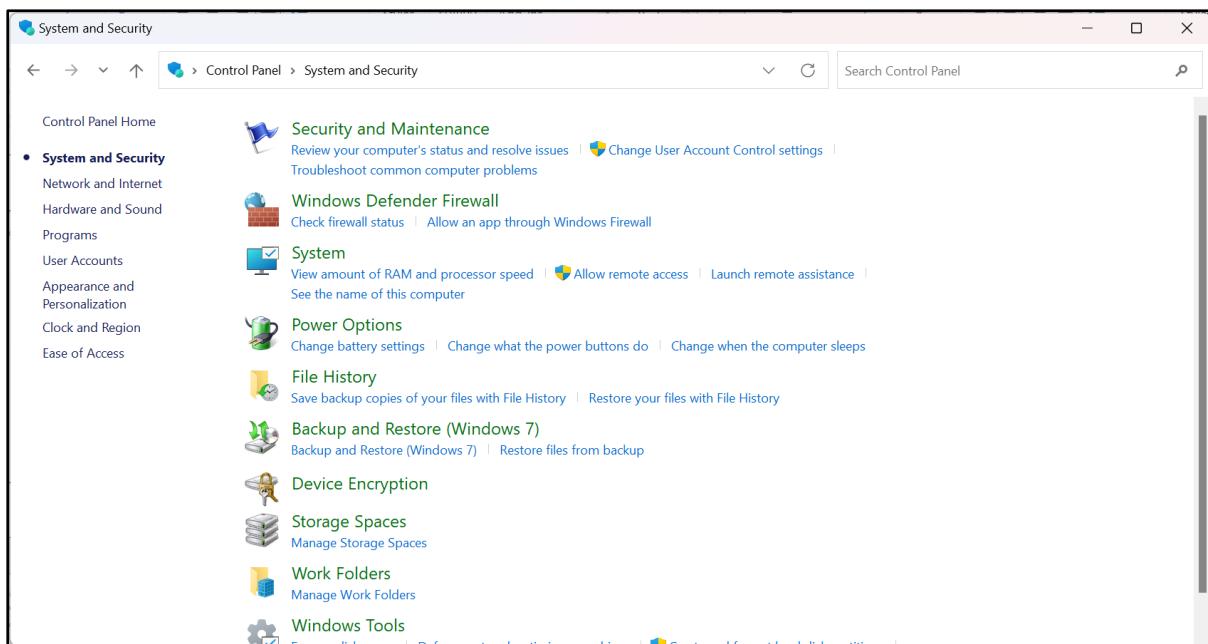


Fig 7: Select ‘System’

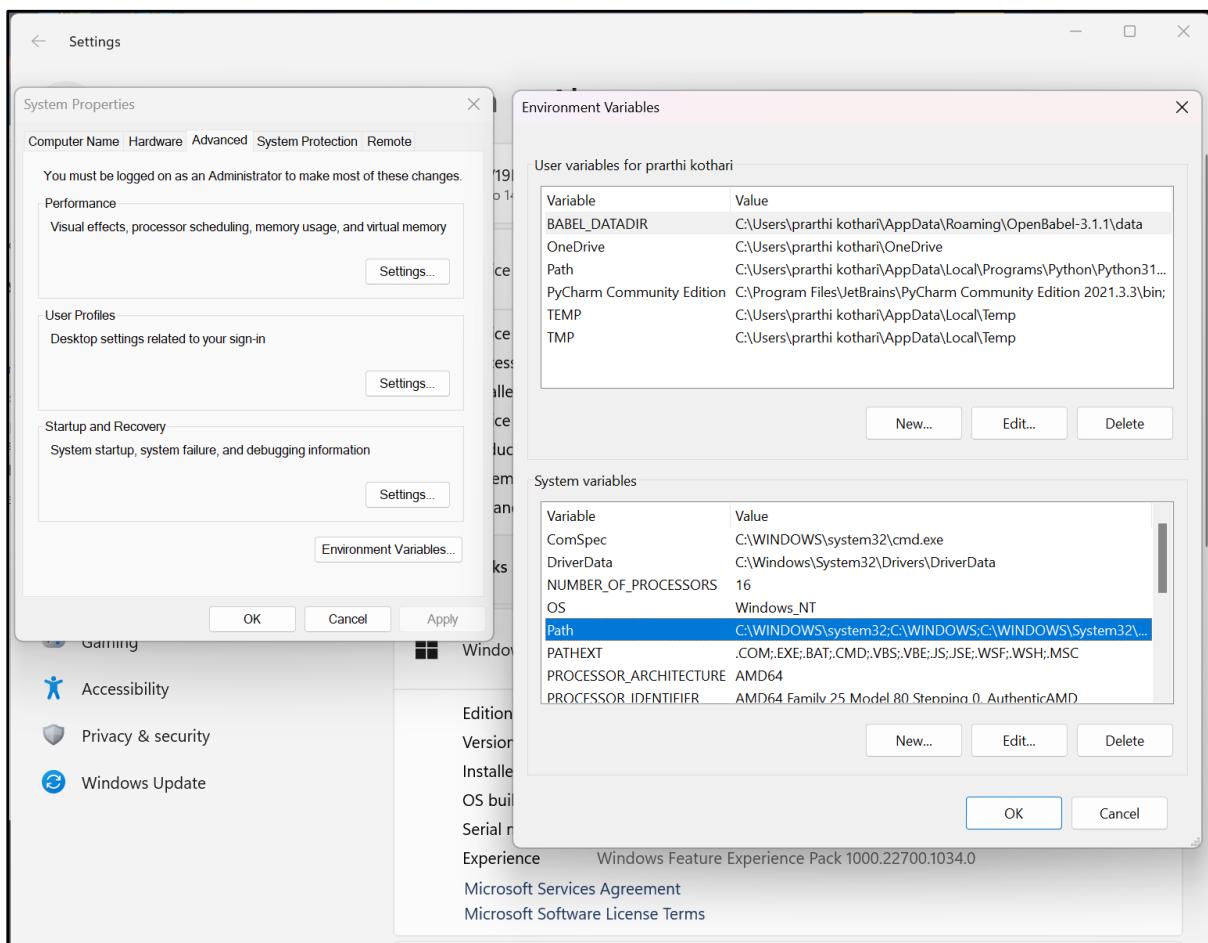


Fig 8: Click on ‘Environment variables’ and then under ‘System variables’, click on ‘path’

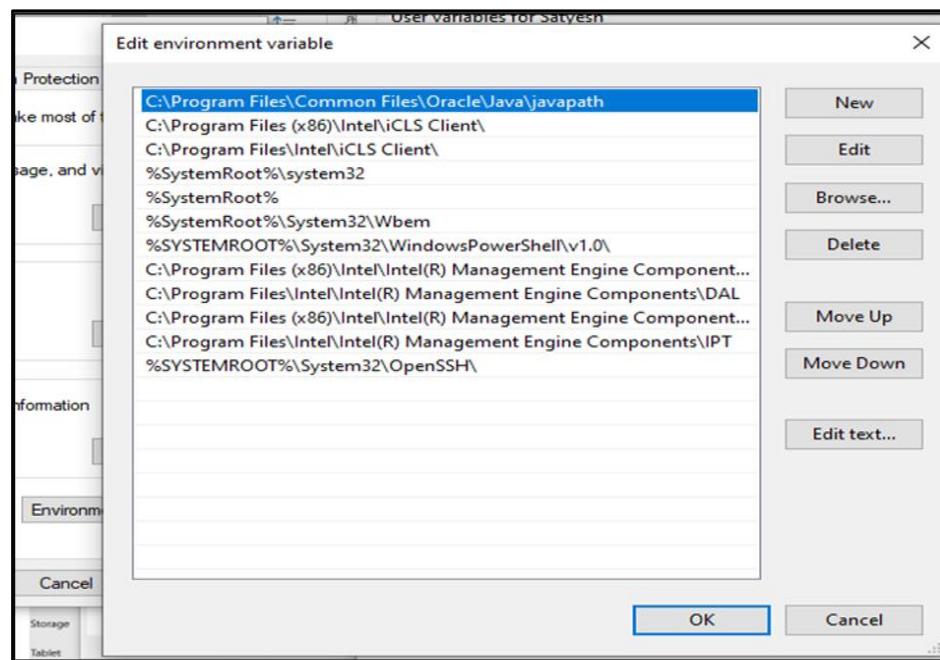


Fig 9: Add ‘Python’ and ‘JDK’ files path over here and click ‘OK’

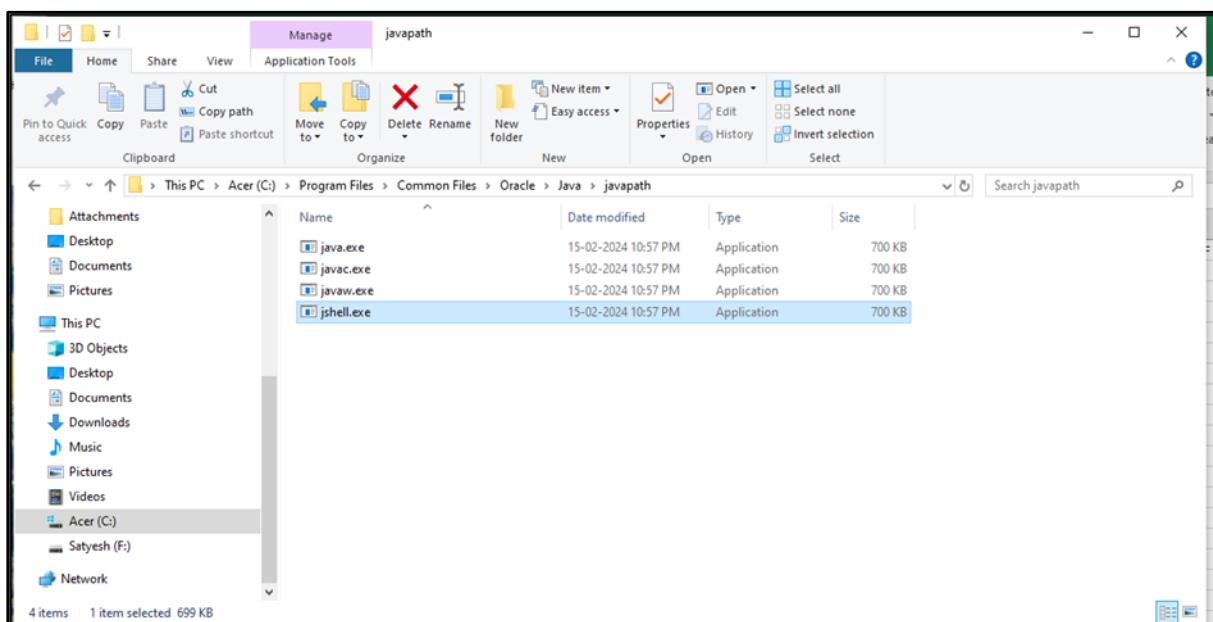


Fig 10: PaDELPy installed successfully in the assigned path

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WEBLEM 9(A)

PaDELPy: A Python wrapper for PaDEL-Descriptor software

(URL: <https://github.com/ecrl/padelpy>)

AIM:

To study ID, 2D & 3D descriptors for ‘Donepezil’ (PubChem CID: 3152) using PaDELPy Software.

INTRODUCTION:

PaDELPy

PaDELPy simplifies the process of calculating molecular descriptors and fingerprints by providing a Python interface for the PaDEL-Descriptor software. PaDEL-Descriptor, written in Java, generates molecular descriptors that are used to describe molecular properties in various computational chemistry and cheminformatics applications. It helps correlate molecular structures with biological activities, which is essential for building machine learning models in scientific research, particularly in drug discovery, toxicology, and other biological studies.

PaDELPy eliminates the need for manual handling of the Java-based PaDEL-Descriptor. This means users no longer have to install and execute the Java `jar` file separately. Instead, the library automates the process, allowing users to calculate molecular fingerprints directly in Python. This reduces the complexity of installation and streamlines the workflow for researchers and data scientists working on chemical data and machine learning model creation.

Donepezil

Donepezil, known by its brand name Aricept, is a piperidine derivative and a centrally acting, reversible acetylcholinesterase inhibitor primarily used for treating dementia associated with Alzheimer's disease. Approved by the FDA in 1996, it works by inhibiting the enzyme acetylcholinesterase, which breaks down acetylcholine, thereby increasing its availability at synapses and enhancing cholinergic transmission. Although donepezil does not alter the progression of Alzheimer's disease, it can improve cognitive function and behavioral symptoms in patients with mild to severe dementia. The drug is available in various forms, including oral tablets and a transdermal delivery system, making it accessible for patients with swallowing difficulties. Common side effects include nausea, diarrhea, and insomnia, while serious effects may involve abnormal heart rhythms and seizures.

METHODOLOGY:

1. Search for your query in the PubChem database.
2. Retrieve canonical SMILES of the best match.
3. Open PaDELPy in GitHub and copy the code for “SMILES to Descriptors/Fingerprints”.
4. Using Python IDLE:
 - a. Install PaDELPy via pip: pip install padelpy
 - b. Paste the code copied and change the name of the query and input the SMILES.
 - c. Run the code and interpret the results in an excel file containing the data for the descriptor.
5. Using Google Colab:
 - a. Install PaDELPy via pip: pip install padelpy
 - b. Paste the code copied and change the name of the query and input the SMILES.
 - c. Run the code and interpret the results in table containing the data for the descriptor.

CODE:

```
from padelpy import from_smiles

# calculate molecular descriptors for propane
descriptors =
from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC')

# in addition to descriptors, calculate PubChem fingerprints
desc_fp =
from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC',
fingerprints=True)

# only calculate fingerprints
fingerprints =
from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC',
fingerprints=True, descriptors=False)

# setting the number of threads, this uses one cpu thread to compute
descriptors =
from_smiles(['COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC'],
threads = 1)

# save descriptors to a CSV file
_ = from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC',
output_csv='descriptors.csv')
```

OBSERVATIONS:

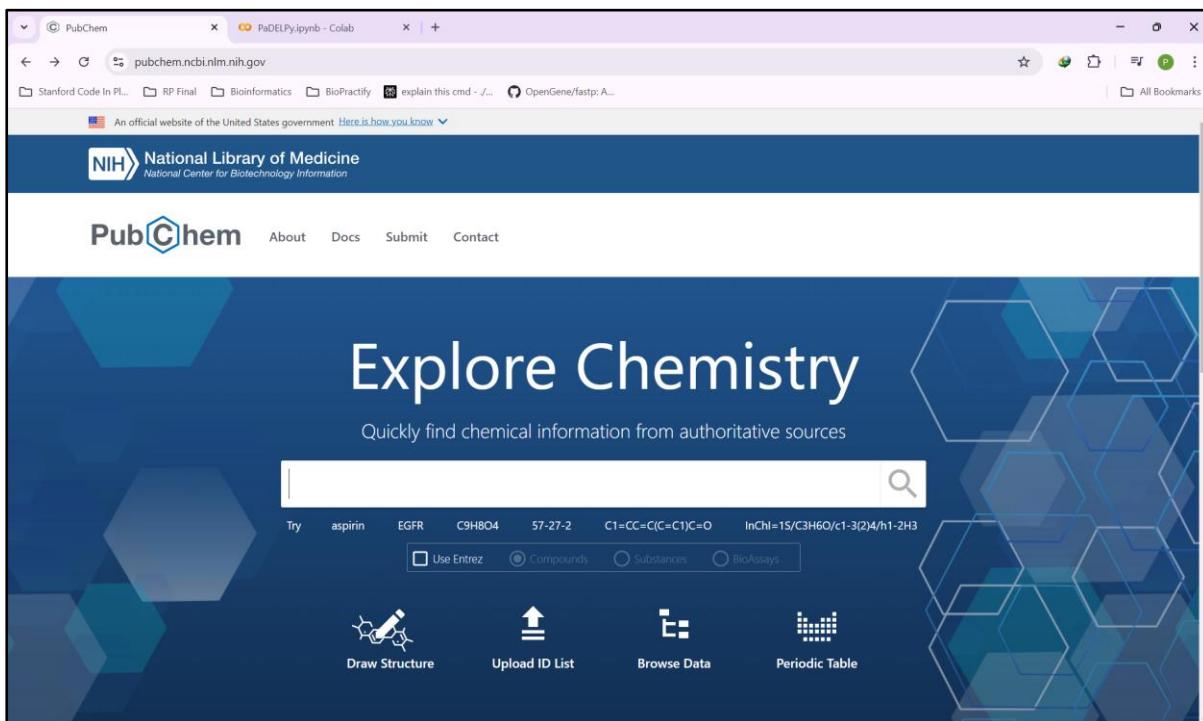


Fig 1: Homepage of PubChem database

A screenshot of a web browser showing the search results for 'donepezil' on the PubChem database. The search bar at the top contains 'donepezil'. Below the search bar, a 'SEARCH FOR' section shows the query 'donepezil'. The main content area is titled 'BEST MATCH' and displays the following information for PubChem CID 3152:

donepezil; 120014-06-4; 2-((1-Benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydro-1H-inden-1-one; Aricept; Donaz; donepezilo; donepezilum; Domepezil; ...

Compound CID: 3152
MF: $C_{22}H_{29}NO_4$ MW: 379.5g/mol
IUPAC Name: 2-((1-benzylpiperidin-4-yl)methyl)-5,6-dimethoxy-2,3-dihydroinden-1-one
Isomeric SMILES: COC1=C(C=C(C=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=C(C=C4)OC
InChIKey: ADEBPSSDOVVL-UHFFFAOYSA-N
InChI: InChI=1S/C24H29NO3/c1-27-22-14-19-13-20(24(26)21(19)15-23(22)28-2)/2-12-8-10-25(11-9-17)16-18-6-4-3-5-7-18/h3-7,14-15,17,20H,8-13,16H2,1-2H3
Create Date: 2005-03-25

Below this summary, there are links for 'Summary', 'Similar Structures Search', 'Related Records', and 'PubMed (MeSH Keyword)'.

Fig 2: Best Match for the query ‘Donepezil’ (PubChem CID: 3152)

PubChem Donepezil (Compound)

2.1.3 InChIKey

ADEBPSSDYVVL-DUHFFAOYSA-N
Computed by InChI 1.0.6 (PubChem release 2021.10.14)

2.1.4 SMILES

COC1=C(C=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC
Computed by OEChem 2.3.0 (PubChem release 2021.10.14)

2.2 Molecular Formula

C₂₄H₂₉NO₃
Computed by PubChem 2.2 (PubChem release 2021.10.14)

2.3 Other Identifiers

2.3.1 CAS

120014-06-4
CAS Common Chemistry; ChemIDplus; DrugBank; EPA DSSTox; European Chemicals Agency (ECHA); FDA Global Substance Registration...

CONTENTS

- 1 Structures
- 2 Names and Identifiers
- 3 Chemical and Physical Properties
- 4 Spectral Information
- 5 Related Records
- 6 Chemical Vendors
- 7 Drug and Medication Information
- 8 Pharmacology and Biochemistry
- 9 Use and Manufacturing
- 10 Identification
- 11 Safety and Hazards
- 12 Toxicity
- 13 Associated Disorders and Diseases
- 14 Literature
- 15 Patents
- 16 Interactions and Pathways
- 17 Biological Test Results
- 18 Classification
- 19 Information Sources

Fig 3: SMILES format of the query ‘Donepezil’ (PubChem CID: 3152)

SMILES to Descriptors/Fingerprints

The "from_smiles" function accepts a SMILES string or list of SMILES strings as an argument, and returns a Python dictionary with descriptor/fingerprint names/values as keys/values respectively - if multiple SMILES strings are supplied, "from_smiles" returns a list of dictionaries.

```
from padelpy import from_smiles

# calculate molecular descriptors for propane
descriptors = from_smiles('CCC')

# calculate molecular descriptors for propane and butane
descriptors = from_smiles(['CCC', 'CCCC'])

# in addition to descriptors, calculate PubChem fingerprints
desc_fp = from_smiles('CCC', fingerprints=True)

# only calculate fingerprints
fingerprints = from_smiles('CCC', fingerprints=True, descriptors=False)

# setting the number of threads, this uses one cpu thread to compute descriptors
descriptors = from_smiles(['CCC', 'CCCC'], threads = 1)

# save descriptors to a CSV file
_ = from_smiles('CCC', output_csv='descriptors.csv')
```

MDL MolFile to Descriptors/Fingerprints

The "from_mdl" function accepts a filepath (to an MDL MolFile) as an argument, and returns a list. Each list element is a dictionary with descriptors/fingerprints corresponding to each supplied molecule (indexed as they appear in the MolFile).

Fig 4: SMILES to Descriptors Code on PaDELPy GitHub

```
[1] pip install padelpy
Collecting padelpy
  Downloading padelpy-0.1.14-py3-none-any.whl (7.7 kB)
  Downloading padelpy-0.1.14-py2.py3-none-any.whl (20.9 kB)
Installing collected packages: padelpy
Successfully installed padelpy-0.1.14
```

Fig 5: Installing padelpy using pip in Google Colab

```
from padelpy import from_smiles

# calculate molecular descriptors for propane
descriptors = from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC')

# in addition to descriptors, calculate PubChem fingerprints
desc_fp = from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC', fingerprints=True)

# only calculate fingerprints
fingerprints = from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC', fingerprints=True, descriptors=False)

# setting the number of threads, this uses one cpu thread to compute descriptors
descriptors = from_smiles(['COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC'], threads = 1)

# save descriptors to a CSV file
_= from_smiles('COC1=C(C=C2C(=C1)CC(C2=O)CC3CCN(CC3)CC4=CC=CC=C4)OC', output_csv='descriptors.csv')
```

Fig 6: Code for SMILES to Descriptor / Fingerprints

Name	nAacid	ALogP	ALogP2	AMR	apol	naAromAtom	nAromBond	nAtom	nHeavyAtom	nH	nB	nC	nN	nO	nS	nI
AUTOGEN_20241006183439070876715089683	0	0.363800000000000084	0.13235044000000062	115.78649999999999	65.08299699999995	12	12	57	28	29	0	24	1	3	0	0

Fig 7: Output in .csv format

RESULTS:

Using PaDELPy, a Python wrapper for the PaDEL-Descriptor, molecular descriptors for the compound ‘Donepezil’ (PubChem CID: 3152) were calculated. These descriptors provide key numerical values representing the chemical and structural properties of the molecule. For example:

1. **AlogP:** Represents the lipophilicity (hydrophobicity) of the molecule.
2. **nAtom:** The total number of atoms in the molecule.
3. **nHeavyAtom:** The number of non-hydrogen atoms (heavy atoms) in the molecule.
4. **nH:** The number of hydrogen atoms.
5. **nC:** The number of carbon atoms.

These descriptors, along with many others, help quantify the chemical characteristics of molecules, which is crucial for predicting molecular behaviour in biological systems. This information is particularly useful in computational tasks such as molecular docking simulations, where the binding affinities and interactions between ligands (such as drugs) and biological targets (like proteins) are predicted.

CONCLUSION:

PaDEL-Descriptor, an open-source and multithreaded molecular descriptor calculation software, provides a powerful and efficient tool for extracting molecular descriptors. Its ability to handle large datasets and compute a wide range of molecular descriptors quickly makes it a valuable tool in cheminformatics, drug discovery, and computational biology. With its cross-platform compatibility and ease of use, PaDELPy enhances productivity in scientific research by facilitating the integration of molecular descriptor calculations into machine learning models and other data analysis workflows.

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WEBLEM 10

Web – Based Tools For Vaccine Designing

AIM:

To understand various web-based tools for vaccine designing.

INTRODUCTION:

Immunization is a cornerstone of public health policy and is demonstrably highly cost effective when used to protect child health. Although it could be argued that immunology has not thus far contributed much to vaccine development, in that most of the vaccines we use today were developed and tested empirically, it is clear that there are major challenges ahead to develop new vaccines for difficult-to target pathogens, for which we urgently need a better understanding of protective immunity. Moreover, recognition of the huge potential and challenges for vaccines to control disease outbreaks and protect the older population, together with the availability of an array of new technologies, make it the perfect time for immunologists to be involved in designing the next generation of powerful immunogens. This Review provides an introductory overview of vaccines, immunization and related issues and thereby aims to inform a broad scientific audience about the underlying immunological concepts.

Vaccines

A vaccine is a biological product that can be used to safely induce an immune response that confers protection against infection and/or disease on subsequent exposure to a pathogen. To achieve this, the vaccine must contain antigens that are either derived from the pathogen or produced synthetically to represent components of the pathogen. The essential component of most vaccines is one or more protein antigens that induce immune responses that provide protection. However, polysaccharide antigens can also induce protective immune responses and are the basis of vaccines that have been developed to prevent several bacterial infections, such as pneumonia and meningitis caused by *Streptococcus pneumoniae*, since the late 1980s. Protection conferred by a vaccine is measured in clinical trials that relate immune responses to the vaccine antigen to clinical end points. Vaccines are generally classified as live or non-live (sometimes loosely referred to as ‘inactivated’) to distinguish those vaccines that contain attenuated replicating strains of the relevant pathogenic organism from those that contain only components of a pathogen or killed whole organisms. In addition to the ‘traditional’ live and non-live vaccines, several other platforms have been developed over the past few decades, including viral vectors, nucleic acid-based RNA and DNA vaccines, and virus like particles.

History

Epidemics of smallpox swept across Europe in the seventeenth and eighteenth centuries, accounting for as much as 29% of the death rate of children in London¹³⁷. Initial efforts to control the disease led to the practice of variolation, which was introduced to England by Lady Mary Wortley Montagu in 1722, having been used in the Far East since the mid 1500s. In variolation, material from the scabs of smallpox lesions was scratched into the skin in an attempt to provide protection against the disease. Variolation did seem to induce protection, reducing the attack rate during epidemics, but sadly some of those who were variolated developed the disease and sometimes even died. It was in this context that Edward Jenner wrote an Inquiry into the Causes and Effects of the Variole Vaccine in 1798. His demonstration, undertaken by scratching material from cowpox lesions taken from the hands of a milkmaid, Sarah Nelms, into the skin of an 8 year-old boy, James Phipps, who he subsequently challenged with smallpox, provided early evidence that vaccination could work.

Jenner's contribution to medicine was thus not the technique of inoculation but his startling observation that milkmaids who had had mild cowpox infections did not contract smallpox, and the serendipitous assumption that material from cowpox lesions might immunize against smallpox. Furthermore, Jenner brilliantly predicted that vaccination could lead to the eradication of smallpox; in 1980, the World Health Assembly declared the world free of naturally occurring smallpox. Almost 100 years after Jenner, the work of Louis Pasteur on rabies vaccine in the 1880s heralded the beginning of a frenetic period of development of new vaccines, so that by the middle of the twentieth century, vaccines for many different diseases (such as diphtheria, pertussis and typhoid) had been developed as inactivated pathogen products or toxoid vaccines. However, it was the coordination of immunization as a major public health tool from the 1950s onwards that led to the introduction of comprehensive vaccine programmes and their remarkable impact on child health that we enjoy today. In 1974, the World Health Organization launched the Expanded Programme on Immunization and a goal was set in 1977 to reach every child in the world with vaccines for diphtheria, pertussis, tetanus, poliomyelitis, measles and tuberculosis by 1990. Unfortunately, that goal has still not been reached; although global coverage of 3 doses of the diphtheria–tetanus–pertussis vaccine has risen to more than 85%, there are still more than 19 million children who did not receive basic vaccinations in 2019.

MATERIALS AND METHODS:

Vaccines induce antibodies:

The adaptive immune response is mediated by B cells that produce antibodies (humoral immunity) and by T cells (cellular immunity). All vaccines in routine use, except BCG which is believed to induce T cell responses that prevent severe disease and innate immune responses and are thought to mainly confer protection through the induction of antibodies. There is considerable supportive evidence that various types of functional antibody are important in vaccine induced protection, and this evidence comes from three main sources: immunodeficiency states, studies of passive protection and immunological data.

Vaccines need T cell help:

The role of T cells in protection is poorly characterized, except for their role in providing help for B cell development and antibody production in lymph nodes. From studies of individuals with inherited or acquired immunodeficiency, it is clear that whereas antibody deficiency increases susceptibility to acquisition of infection, T cell deficiency results in failure to control a pathogen after infection. For example, T cell deficiency results in uncontrolled and fatal varicella zoster virus infection, whereas individuals with antibody deficiency readily develop infection but recover in the same way as immunocompetent individuals. The relative suppression of T cell responses that occurs at the end of pregnancy increases the severity of infection with influenza and varicella zoster viruses. Studies show that sterilizing immunity against carriage of *S. pneumoniae* in mice can be achieved by the transfer of T cells from donor mice exposed to *S. pneumoniae*, which indicates that further investigation of T cell-mediated immunity is warranted to better understand the nature of T cell responses that could be harnessed to improve protective immunity. Although somewhat simplistic, the evidence therefore indicates that antibodies have the major role in prevention of infection (supported by TH cells), whereas cytotoxic T cells are required to control and clear established infection.

Epitope-based vaccines:

Epitopes are of particular interest to both clinical and basic biomedical researchers as they hold huge potential for vaccine design, disease prevention, diagnosis, and treatment. Using rDNA technologies, we can isolate specific epitopes which can replace the whole pathogen in a vaccine. However, within the diversity of epitopes in a pathogen, it is important to notice that not all of the epitopes, even those that seem to be dominant, are equal in their ability to elicit antibody production. The proteins that contain many epitopes recognized by the common MHC alleles are known as promiscuous binders. The human leukocyte antigen (HLA) supertype refers to a set of HLA alleles with overlapping peptide binding specificities. The alleles in the given HLA supertype often represent the same epitope, which refers to the region on the surface of an antigen capable of eliciting an immune response for T cell recognition. On the other hand, elicitation of humoral responses relies on the recognition of linear epitopes and conformational epitopes. The latter constitute a challenge for chimeric vaccine design as they must retain their native conformation to be functional. Therefore, knowledge on the whole antigen structure is necessary to aid in the rational design of vaccines targeting conformational B cell epitopes.

Bioinformatics tools to prediction of potential T cell binding-epitopes:

The first step on applying bioinformatics to vaccine development consists of discriminating epitopes that are potentially immune-protective from epitopes that are not. Since T-cell epitopes are bound in a linear form to MHCs, the interface between ligands and T-cells can be modeled with accuracy. It is currently well known that epitopes link together into the binding groove of MHC Class I and Class II molecules through interactions between their R group side chains and pockets located on the floor of the MHC. Based on this knowledge, a large number of T-cell epitope- mapping algorithms have been established and used to develop tools to rapidly identify putative T-cell epitopes. MHC-I binding predictors are currently very efficient and have wide allelic coverage, a prediction accuracy in the range of 90–95% positive predictive value has been estimated.

Among the numerous servers for MHC-I alleles is RANKPEP, which predicts peptide binders to MHC-I and MHC-II molecules from protein sequences or sequence alignments using Position Specific Scoring Matrices (PSSMs). In addition, it predicts those MHC-I ligands whose C-terminal end is likely to be the result of proteasomal cleavage. This is a friendly platform which offers the widest allelic coverage to MHC-I and MHC-II alleles for humans and mice. To search epitopes sequences for MHC-I ligands using PSSMs, a dynamic algorithm written in Python is used; which scores all protein segments with the length of the PSSM width and sorts them accordingly. Scoring starts at the beginning of each sequence and the PSSM is slid over the sequence one residue at a time until reaching the end of the sequence. Furthermore, to narrow down the potential binders from the list of ranked peptides, a binding threshold is defined as the score value that includes 90% of the peptides within the PSSM. This binding threshold is built into each matrix, delineating the range of putative binders among the top scoring peptides.

Bioinformatics tools for predicting potential B cell binding epitopes:

B cell epitopes are recognized by B cell receptors or antibodies in their native structure. Continuous B cell epitope prediction is very similar to T cell epitope prediction, which has mainly been based on the amino acid properties such as hydrophilicity, charge, exposed surface area and secondary structure. Discontinuous B cell epitope prediction requires 3D structure of the antigen. Some specific resources to predict continuous or discontinuous B-cell epitopes are available on the Web. To predict linear B-cell epitopes, the Bcepred tool is based on physicochemical properties such as hydrophilicity, flexibility, polarity, and exposed surface on a non-redundant dataset. The dataset consists of 1029 B-cell epitopes obtained from the Bcipep database and an equal number of non-epitopes obtained randomly from the Swiss-Prot database. The prediction accuracy for models based on these properties varies from 52.92% to 57.53%.

The ABCpred server, which is based on neural networks, has an estimated accuracy of 65.93%. Another server called BepiPred predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method. The servers mentioned above are easy to use and properly organized. Among the tools used to predict discontinuous B cell epitopes we can mention DiscoTope, which uses the three dimensional structure of proteins to determine the surface accessibility and a novel epitope propensity amino acid score. The final scores are calculated by combining the propensity scores of residues in spatial proximity and the contact numbers. This server also predicts epitopes in complexes of multiple chains. This tool along with BEpro (formerly known as PEPITO) and SEPPA (Spatial Epitope Prediction of Protein Antigens) requires a 3-D structure as input, specifically, in PDB format. Using SEPPA, each residue in the query protein will be given a score according to information from its neighborhood residues. The higher score corresponds to the higher probability of the residue to be involved in an epitope. One of the most complete tools in this field is ElliPro. This server predicts linear and discontinuous epitopes based on a protein antigen's 3D structure. ElliPro associates each predicted epitope with a score, defined as a PI (Protrusion Index) value. Compared with databases mentioned earlier, in ElliPro the input is a protein sequence. A 3-D structure will be predicted for the input protein sequence by homology

modeling based on user selected structural template. Afterwards, linear and discontinuous epitopes will be computed based on the predicted protein structure. All of these integrative tools represent an opportunity for the development of new vaccines, in special those that aim at the elicitation of humoral responses.

Bioinformatics strategies for emergent peptide-based vaccines against hypervariable viruses:

Historically, most of the known successful vaccines have been developed empirically. However the emergence of highly sophisticated viruses, such as HIV and influenza characterized by having a high degree of genetic and antigenic diversity, has impeded the development of effective, broad-coverage vaccines using traditional methods. The rapid emergence of these viral pathogens underscores the need for improved and accelerated processes to develop and produce vaccines, a need that can be addressed by the methods described above allowing a rapid, *in silico*-based approach to formulate vaccine candidates. This section briefly discusses some approaches developed for the case of the human immunodeficiency (HIV) and influenza viruses as examples on how successful candidate vaccine design can be achieved in the case of hypervariable viruses using bioinformatic tools.

PERSPECTIVES:

Bioinformatics tools have enabled the capability of selecting potential epitopes without running the risks involved in cultivating the pathogen of interest. This kind of methodology represents a huge advantage over conventional vaccinology techniques, including faster outputs and lower costs. The application of omics technologies to this field has also revolutionized the way in which potential vaccine candidates can be identified. Proteomics and transcriptomics have been used as complementary approaches to genomics and are often more useful in identifying surface proteins during host pathogen interaction. Despite that numerous epitope prediction methods are available, developing a systematic assessment of different methods on standard benchmark datasets is still a need.

Launching a Critical Assessment of Techniques for Epitope Prediction will indeed benefit the field. It has been proposed that computational methods will be used to perform blinded *de novo* epitope prediction from query proteins previously screened experimentally. Comparison of different methods is yet a complex task due to many aspects including the following: (i) inadequate documentation of datasets and prediction methods, (ii) unavailability of the benchmark dataset used to evaluate the methods, (iii) unavailability of the code that implements the method, (iv) the lack of a unified output format, which complicates the process of combining the results of several servers in order to obtain consensus predictions. Therefore it is necessary to develop standardized data representations; this will enable the evaluation of different prediction methods on a standardized benchmark datasets in order to compare the methods and develop meta-servers combining the predictions of multiple prediction tools.

CONCLUSIONS AND FUTURE DIRECTIONS:

Immunization protects populations from diseases that previously claimed the lives of millions of individuals each year, mostly children. Under the United Nations Convention on the Rights of the Child, every child has the right to the best possible health, and by extrapolation a right to be vaccinated. Despite the outstanding success of vaccination in protecting the health of our children, there are important knowledge gaps and challenges to be addressed. An incomplete understanding of immune mechanisms of protection and the lack of solutions to overcome antigenic variability have hampered the design of effective vaccines against major diseases such as HIV/AIDS and TB. Huge efforts have resulted in the licensing of a partially effective vaccine against malaria, but more effective vaccines will be needed to defeat this disease. Moreover, it is becoming clear that variation in host response is an important factor to take into account.

New technologies and analytical methods will aid the delineation of the complex immune mechanisms involved, and this knowledge will be important to design effective vaccines for the future. Apart from the scientific challenges, sociopolitical barriers stand in the way of safe and effective vaccination for all. Access to vaccines is one of the greatest obstacles, and improving infrastructure, continuing education and enhancing community engagement will be essential to improve this, and novel delivery platforms that eliminate the need for a cold chain could have great implications. There is a growing subset of the population who are skeptical about vaccination and this requires a response from the scientific community to provide transparency about the existing knowledge gaps and strategies to overcome these.

Constructive collaboration between scientists and between scientific institutions, governments and industry will be imperative to move forwards. The COVID-19 pandemic has indeed shown that, in the case of an emergency, many parties with different incentives can come together to ensure that vaccines are being developed at unprecedented speed but has also highlighted some of the challenges of national and commercial interests. As immunologists, we have a responsibility to create an environment where immunization is normal, the science is accessible and robust, and access to vaccination is a right and expectation.

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WEBLEM 11
Introduction To Tepi Tool
(URL: <http://tools.iedb.org/tepitool/>)

AIM:

Introduction to IEDB Database for prediction of cytotoxic and helper T cell epitopes (MHC Class I epitopes and MHC Class II epitopes).

INTRODUCTION:

TepiTool is a powerful online platform developed as part of the Immune Epitope Database (IEDB) to facilitate the prediction of T-cell epitope candidates through the analysis of peptide binding to Major Histocompatibility Complex (MHC) class I and class II molecules. Accurate prediction of peptide-MHC binding is critical for understanding T-cell responses, which play a central role in the immune system's ability to recognize and respond to pathogens, cancer cells, and other antigens. This capability is particularly relevant for applications in vaccine design, immunotherapy, and diagnostics. TepiTool was created to address the growing need for accessible, accurate, and easy-to-use tools in epitope prediction, helping immunologists and researchers identify peptides that can potentially elicit immune responses. MHC molecules present peptides to T cells, activating an immune response when the peptide fits well in the MHC molecule's binding groove. MHC class I molecules bind shorter peptides (8-11 amino acids) and are recognized by CD8+ T cells, while MHC class II molecules bind longer peptides (12-20 amino acids) and are recognized by CD4+ T cells. Predicting which peptides will successfully bind to these MHC molecules is a vital step in identifying epitopes—regions of antigens that are recognized by T cells. This information is crucial in designing vaccines and therapies that target specific immune responses.

TepiTool simplifies this complex task by integrating state-of-the-art computational algorithms for MHC binding prediction, ensuring that researchers can efficiently identify candidate epitopes for further experimental validation. A key advantage of TepiTool is its user-friendly, step-by-step interface, which allows users to easily input amino acid sequences and specify parameters such as the species of interest, MHC class, and peptide length. This is particularly helpful for researchers unfamiliar with computational prediction tools, as it guides them through the entire process, ensuring accurate results without requiring extensive technical expertise. Furthermore, TepiTool supports predictions for hundreds of MHC alleles across multiple species, including humans and common model organisms like mice and pigs, making it highly versatile for a wide range of immunological studies. In addition to its simplicity and accessibility, TepiTool is equipped with some of the most advanced MHC binding prediction algorithms, including artificial neural networks and machine learning techniques. These algorithms have been refined to provide accurate and reliable predictions of peptide-MHC interactions, ensuring that researchers can quickly and effectively identify the most promising epitopes for further investigation. TepiTool also allows users to customize input parameters, such as MHC allele selection and peptide binding thresholds, offering flexibility to meet the

specific needs of various research projects. TepiTool has become an essential resource for immunology research, offering applications in vaccine development, cancer immunotherapy, autoimmune disease studies, and diagnostics. By facilitating the prediction of T-cell epitopes, TepiTool enables researchers to accelerate the discovery of novel immunotherapies and vaccines that can precisely target disease mechanisms. Its combination of cutting-edge algorithms and an intuitive interface ensures that it remains a key tool for both experienced researchers and those new to the field of epitope prediction.

The screenshot shows the IEDB.org homepage with a red box highlighting the 'Epitope Analysis Resource' section. This section includes 'T Cell Epitope Prediction' (Scan an antigen sequence for amino acid patterns indicative of: MHC I Binding, MHC II Binding, MHC I Processing (Proteasome,TAP), MHC II Immunogenicity), 'B Cell Epitope Prediction' (Predict linear B cell epitopes using: Antigen Sequence Properties, Predict discontinuous B cell epitopes using antigen structure via: Discotope, ElliPro), and 'Epitope Analysis Tools' (Analyze epitope sets of: Population Coverage, Conservation Across Antigens, Clusters with Similar Sequences).

Fig 1: Homepage of IEDB database and select ‘Epitope Analysis’ resource

The screenshot shows the 'T Cell Epitope Prediction Tools' section of the IEDB Analysis Resource. It includes tools for 'T Cell Epitopes - MHC Binding Prediction', 'T Cell Epitopes - Processing Prediction', and 'T Cell Epitopes - Immunogenicity Prediction'. The 'TepiTool' section is highlighted with a red box. The 'TepiTool' description states: 'The "TepiTool" provides prediction of peptides binding to MHC class I and class II molecules. Tool is designed as a wizard with 6 steps as described below. Each field (except sequences and alleles) is filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per your specific needs. You can go back to previous steps to change your selection before submission of the job. Once you submit the job (at the end of step-6), you will not be able to make any more changes and will have to start the prediction all over again with updated input parameters.'

Fig 2: Selection of Tepi tool from T Cell Epitope Prediction Tools of IEDB Analysis Resource

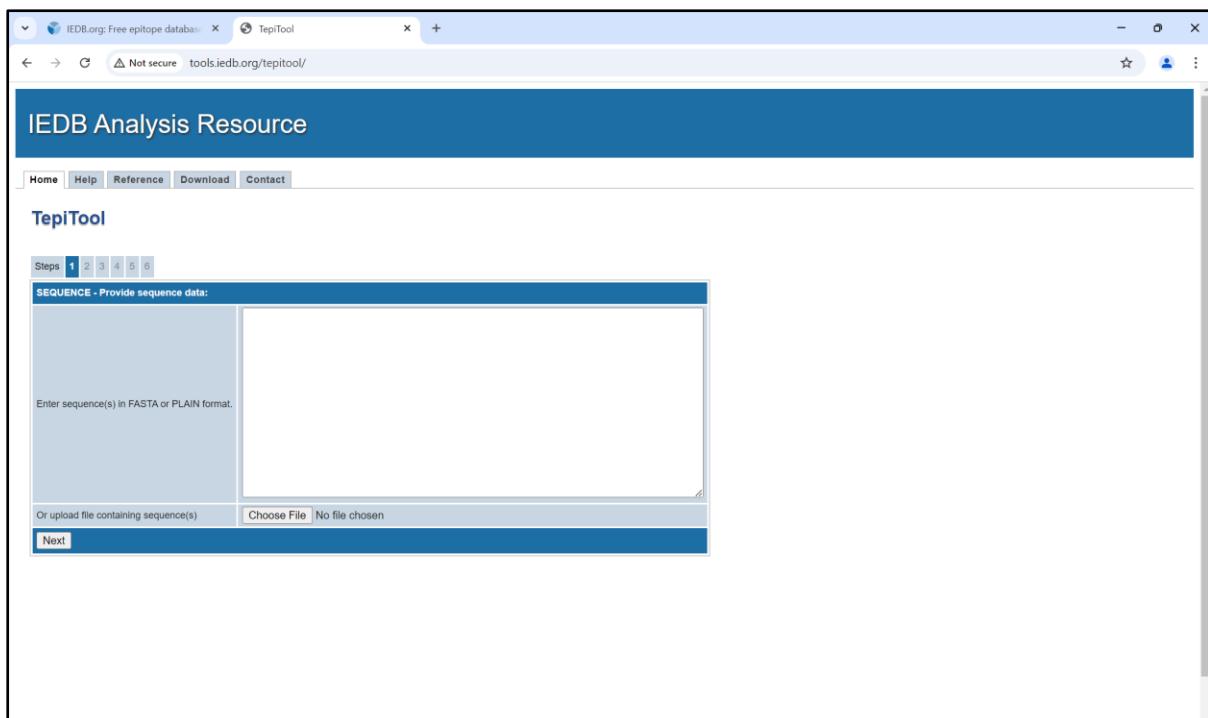


Fig 3: Homepage of TepiTool

Basic Protocol:

Computational Prediction of Peptides Binding to MHC Class I AND Class II Molecules
This protocol explains prediction of T cell epitope candidates from a given set of amino acid sequences, based on predicted peptide binding to MHC class I and class II molecules, using the online computational MHC binding prediction tool called TepiTool. The tool is designed as a wizard where the user is led through a series of well-defined steps to complete the task. Each step is a client-side web form that takes user input data that is in turn processed at the server-side when the user submits the entire form. All fields except sequences and alleles are filled with default recommended settings for prediction and selection of optimum peptides. The input parameters can be adjusted as per the user's specific needs, and the user can go back to previous steps to change the selection before final submission of the job. The TepiTool has six steps as described below.

Protocol steps to be followed:

1. Provide sequence data
2. Select the host species and MHC allele class
3. Select the alleles for binding prediction
4. Select peptides to be included in prediction
5. Select preferred methods for binding prediction and peptide selection and cutoff values
6. Review selections, enter job details and submit data

Step 1: Provide sequence data

Users input protein sequences in single-letter amino acid code, either by direct entry or by uploading a FASTA file. This step is foundational as it determines the specific proteins to be analyzed for potential T cell epitopes, setting the stage for all subsequent predictions.

Step 2: Select Host Species and MHC Allele Class

Users choose the species (e.g., human, mouse) and MHC class (I or II) relevant to their research. This selection is crucial because it dictates which MHC alleles will be considered in binding predictions, impacting the relevance of the results to the specific biological context.

Step 3: Select Alleles for Binding Prediction

Users specify which MHC alleles from the chosen species will be analyzed. Tailoring the analysis to specific alleles allows for more precise predictions that are relevant to the target population or experimental model.

Step 4: Select Peptides to Include in Prediction

Users can define peptide lengths and whether to include duplicates in their analysis. This step enables users to control the dataset's size and composition, which can significantly influence the quality and interpretability of the prediction results.

Step 5: Select Preferred Methods for Binding Prediction

Users choose algorithms for predicting binding affinities and set parameters like cutoff values for peptide selection. Customizing prediction methods allows users to optimize results based on their specific research needs or hypotheses, enhancing the accuracy of epitope identification.

Step 6: Review Selections and Submit Data

Users review all inputs, enter job details, and submit data for processing. This final step confirms that all parameters are correct before running predictions, ensuring that users have control over their analysis and can avoid errors before submission.

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WEBLEM 11(A)
Tepi Tool
(URL:<http://tools.iedb.org/tepitool/>)

AIM:

To Predict MHC Class I and Class II Molecules for the query ‘Chemokine’ (Accession ID: O00590) using TepiTool.

INTRODUCTION:

Tepi Tool

T-cell epitope prediction plays a crucial role in a variety of applications, including vaccine discovery, diagnostic development, and mitigating immune responses against therapeutic proteins. Despite ongoing advancements in MHC binding prediction tools, their widespread adoption among immunologists has been slow. This is primarily due to the lack of intuitive interfaces and clear guidance regarding key aspects such as allele selection, peptide lengths, and appropriate cutoff values. Current tools often provide minimal advice on these important factors, leaving users without the necessary insights to optimize their predictions.

To address these challenges, TepiTool—a newly developed online resource available through the Immune Epitope Database (IEDB)—offers a user-friendly interface and integrates top-performing MHC binding prediction algorithms. Designed to simplify the prediction process, TepiTool supports multiple species, including humans, chimpanzees, bovines, gorillas, macaques, mice, and pigs, making it a versatile tool for researchers. With step-by-step instructions and built-in recommendations, TepiTool streamlines the identification of optimal T-cell epitope candidates. Freely accessible at TepiTool, this tool enables immunologists and researchers to efficiently predict and analyze T-cell epitopes, enhancing applications in immunotherapy, vaccine development, and immune response modulation.

Chemokine

Chemokines are vital signaling molecules in the immune system that guide T cell migration and regulate their differentiation, playing a key role in immune responses. They attract naïve and effector T cells to sites of infection by creating gradients, while also influencing the behavior of antigen-presenting cells (APCs). T cell epitopes, short peptide fragments presented by major histocompatibility complex (MHC) molecules, are crucial for T cell activation. The interaction between T cell receptors (TCRs) and these peptide-MHC complexes is essential for mounting effective immune responses. Additionally, understanding the relationship between chemokines and T cell epitopes is important for developing immunotherapies, particularly in cancer treatment, where targeting specific neoepitopes can enhance T cell responses against tumors.

METHODOLOGY:

1. Access the UniProt database and search for the query ‘Chemokine’.
2. Locate the epitope of interest and copy its FASTA sequence.
3. Navigate to the TepiTool server and paste the copied FASTA sequence of the query ‘Chemokine’ (Accession ID: O00590). Provide the sequence data.
4. Select the host species and MHC allele class.
5. Choose the alleles for prediction.
6. Select the peptides to be included in the prediction.
7. Select for preferred methods for binding prediction, peptide selection strategy, and cutoff values.
8. Review your selections, enter job details, and submit the data.

OBSERVATIONS:

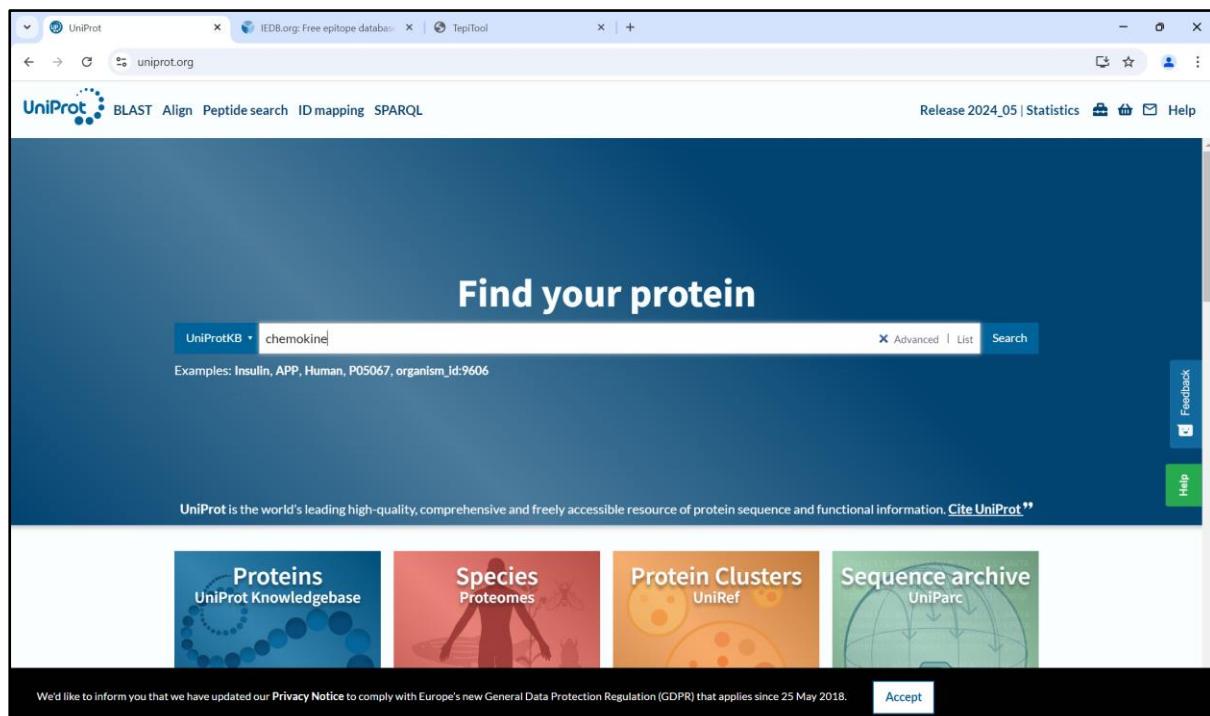


Fig 1: Homepage of UniProt database with the entered query ‘Chemokine’

UniProtKB 147,988 results or search "chemokine" as a Gene Ontology, Protein Name, Protein family, or Gene Name

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
O00590	ACKR2_HUMAN	Atypical chemokine receptor 2	ACKR2, CCBP2, CCR10, CMKBR9, D6	Homo sapiens (Human)	384 AA
O15444	CCL25_HUMAN	C-C motif chemokine 25 [...]	CCL25, SCYA25, TECK	Homo sapiens (Human)	150 AA
Q9Y4X3	CCL27_HUMAN	C-C motif chemokine 27 [...]	CCL27, ILC, SCYA27	Homo sapiens (Human)	112 AA
O00421	CCRL2_HUMAN	C-C chemokine receptor-like 2	CCRL2, CCR11, CCR6, CKRX, CRAM, HCR [...]	Homo sapiens (Human)	344 AA
O08707	ACKR2_MOUSE	Atypical chemokine receptor 2	Ackr2, Ccbp2, D6	Mus musculus (Mouse)	378 AA
Q92583	CCL17_HUMAN	C-C motif chemokine 17 [...]	CCL17, SCYA17, TARC	Homo sapiens (Human)	94 AA
O35903	CCL25_MOUSE	C-C motif chemokine 25 [...]	Ccl25, Scya25, Teck	Mus musculus (Mouse)	144 AA
O15467	CCL16_HUMAN	C-C motif chemokine 16 [...]	CCL16, ILINCK, NCC4, SCYA16	Homo sapiens (Human)	120 AA

Fig 2: Selected the entry with the Accession ID: O00590

Fig 3: Copy the FASTA sequence of the entry

Fig 4: Homepage of the TepiTool database

The screenshot shows a web browser window with the URL tools.iedb.org/tepitool/. The title bar says "ACKR2 - Atypical chemokine receptor 2". The main content area is titled "IEDB Analysis Resource" and "TepiTool". A progress bar at the top indicates "Steps 1 2 3 4 5 6". Below it, a section titled "SEQUENCE - Provide sequence data:" contains a text input field with a long FASTA sequence for ACKR2_HUMAN. The sequence starts with >sp|000590|ACKR2_HUMAN Atypical chemokine receptor 2 OS=Homo sapiens OX=9606 GN=ACKR2 PE=1 SV=2. Below the input field, it says "Enter sequence(s) in FASTA or PLAIN format:" and "Or upload file containing sequence(s)". A "Choose File" button is present, showing "No file chosen". A note "FASTA format detected." is displayed below the sequence input. At the bottom of the form are "Next" and "Back" buttons.

Fig 5: Enter the FASTA sequence

MHC Class I

The screenshot shows the same browser window as Fig 5, now at Step 2. The title bar still says "ACKR2 - Atypical chemokine receptor 2". The main content area is titled "IEDB Analysis Resource" and "TepiTool". The progress bar shows "Steps 1 2 3 4 5 6", with step 2 highlighted. A section titled "SPECIES & ALLELE CLASS - Select the host species and MHC allele class:" contains dropdown menus for "Host species" (set to "Human") and "Allele class" (set to "Class I"). To the right, a box titled "Current selections:" shows "No. of sequences: 1". At the bottom are "Start Over", "Back", and "Next" buttons.

Fig 6: Select the host species and MHC Allele Class I

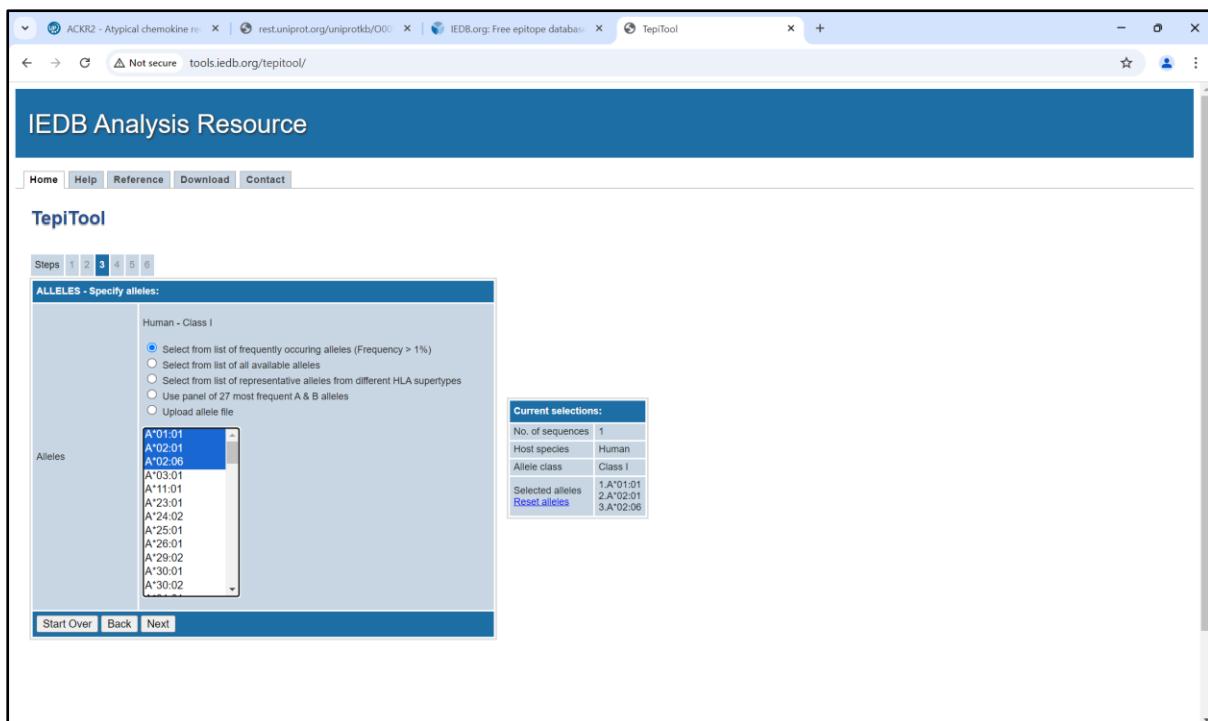


Fig 7: Select the 3 alleles for prediction

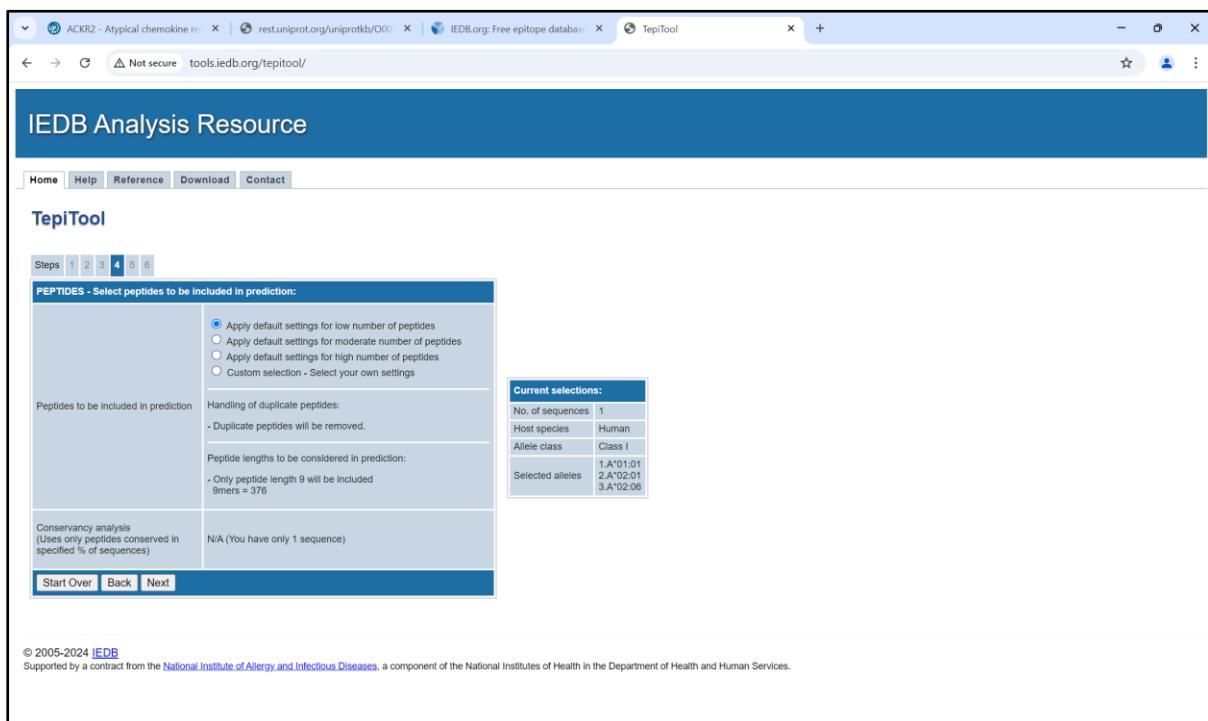


Fig 8: Choose peptides to be included in prediction

The screenshot shows the IEDB Analysis Resource TepiTool interface. At the top, there are tabs for ACKR2 - Atypical chemokine receptor, rest.uniprot.org/uniprotkb/O00..., IEDB.org: Free epitope database, and TepiTool. Below the tabs, the URL is tools.iedb.org/tepi tool/. The main header is "IEDB Analysis Resource" and "TepiTool". A navigation bar at the top has links for Home, Help, Reference, Download, and Contact. Below the navigation bar, a "Steps" menu shows Step 5 selected. The main content area is titled "METHOD - Select prediction & peptide selection methods and cutoff values:". It contains two dropdown menus: "Prediction method to use" set to "IEDB recommended" and "Selection of predicted peptides" set to "Select peptides based on predicted percentile rank" with a value of 1. To the right, a "Current selections" table provides detailed information about the analysis parameters:

Current selections:	
No. of sequences	1
Host species	Human
Allele class	Class I
Selected alleles	1.A*01:01 2.A*02:01 3.A*02:06
Duplicate peptides	Removed
Peptide lengths selected	9mers
No. of peptides included (Not considering conservancy analysis)	376
Conservancy analysis	Peptides conserved in at least % sequences

At the bottom of the page, there are "Start Over", "Back", and "Next" buttons. A copyright notice at the bottom left reads: © 2005-2024 IEDB. Supported by a contract from the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health in the Department of Health and Human Services.

Fig 9: Select prediction method, peptide selection strategy and cutoff values

The screenshot shows the IEDB Analysis Resource TepiTool interface. The steps menu now shows Step 6 selected. The main content area is titled "REVIEW: Review selections, enter job details & submit data:". It contains two sections: "Summary:" and "Job details:". The "Summary:" section lists the same parameters as in Fig 9, including the number of sequences (1), host species (Human), allele class (Class I), selected alleles (1.A*01:01, 2.A*02:01, 3.A*02:06), and peptide length (9mers). The "Job details:" section includes fields for "Job name (optional)" and "Email (optional - will notify when job is finished)". At the bottom, there are "Start Over", "Back", and "Submit" buttons. A note at the bottom states: "(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)"

Fig 10: Review summary, enter job details and submit data

The screenshot shows a web browser window with four tabs open. The active tab is 'TepiTool results' from tools.iedb.org/tepi tool/. The page title is 'IEDB Analysis Resource' and the sub-section is 'TepiTool'. Below this, it says 'Prediction results - concise (Download table)'. A table is displayed with the following columns: Seq #, Peptide start, Peptide end, Peptide, Percentile rank, and Allele. The data in the table is as follows:

Seq #	Peptide start	Peptide end	Peptide	Percentile rank	Allele
1	27	35	YLDDEVAFNL	0.01	HLA-A*02:06
1	16	24	DSENNSSFYY	0.01	HLA-A*01:01
1	27	35	YLDDEVAFNL	0.01	HLA-A*02:01
1	219	227	FLLFLLAAV	0.07	HLA-A*02:01
1	163	171	LLATIVWAV	0.08	HLA-A*02:01
1	139	147	SLDRYLEIV	0.09	HLA-A*02:01
1	219	227	FLLFLLAAV	0.11	HLA-A*02:06
1	175	183	VSIIDINIFV	0.12	HLA-A*02:06
1	89	97	AISNILLFLV	0.12	HLA-A*02:06
1	322	330	YLKAFLAAV	0.12	HLA-A*02:01
1	60	68	GLSGNLILL	0.12	HLA-A*02:01
1	163	171	LLATIVWAV	0.14	HLA-A*02:06
1	121	129	STLYTINFY	0.15	HLA-A*01:01
1	334	342	HLAIGTQAQ	0.17	HLA-A*02:01
1	322	330	YLKAFLAAV	0.18	HLA-A*02:06
1	139	147	SLDRYLEIV	0.18	HLA-A*02:06
1	260	268	FVLWFFYNL	0.18	HLA-A*02:06
1	215	223	NELGFLFLF	0.18	HLA-A*02:01
1	89	97	AISNILLFLV	0.18	HLA-A*02:01
1	334	342	HLAIGTQAQ	0.19	HLA-A*02:06
1	135	143	ISCMNSLDKY	0.2	HLA-A*01:01
1	314	322	FSSHFRFQY	0.21	HLA-A*01:01
1	118	126	EDVSTLYTIZ	0.21	HLA-A*02:01
1	115	123	FVCHMWWTTI	0.21	HLA-A*02:01

Fig 11: Predicted result for MHC Class I

- Number (Seq #):** Indicates the order of the predicted peptides Sequence.
- Peptide Start:** The position in the chemokine sequence where the predicted peptide begins.
- Peptide End:** The position in the chemokine sequence where the predicted peptide ends.
- Peptide:** The amino acid sequence of the predicted peptide.
- Percentile Rank:** Represents the predicted binding affinity of the peptide to a specific MHC Class I allele. A lower percentile rank indicates a stronger binding affinity and therefore a higher likelihood of the peptide functioning as a T-cell epitope.
- Allele:** Refers to the specific MHC Class I molecules (HLA alleles) that the peptide is predicted to bind.

The screenshot shows the TepiTool results page. At the top, there are tabs for 'ACKR2 - Atypical chemokine receptor 2' and 'rest.uniprot.org/uniprotkb/O00...'. The main content area has a header 'Download results details:' with a link to 'Complete results' and 'Prediction results of all peptides'. Below this is 'Citation information' with a note about including predictions in manuscripts and a link to 'References'. The 'Input sequences:' section shows a table with one row: Seq # 1, Seq title sp|O00590|ACKR2_HUMAN Atypical chemokine receptor 2 OS=Homo sapiens OX=9606 GN=ACKR2 PE=1 SV=2, and Sequence MAATASPOPLATEDADSENSSFYYYDYLDEVAFMLCRKDAVVSFGKVFLPVFSLIVLGLSGNLLLMVLLRYVPRRRMVEIYLLNLAINSLFLVTPW. The 'Other input parameters:' section contains a table of settings: No. of sequences (1), Host species (Human), Allele class (Class I), Alleles (A*01:01, A*02:01, A*02:06), Duplicate peptides (Removed), Peptide lengths selected (9mers), Peptide overlap (N/A), Conservancy analysis (No), Prediction method (iEDB recommended), Peptide selection criterion (Predicted percentile rank), Cutoff for peptide selection criterion (1), Job name (empty), and Email (empty). At the bottom, a copyright notice for iEDB (2005-2024) and a funding statement from the National Institute of Allergy and Infectious Diseases are displayed.

Fig 12: Input sequences and other parameters

MHC Class II

The screenshot shows the TepiTool interface at Step 2 of 6. The title bar says 'IEDB Analysis Resource' and 'TepiTool'. The navigation bar includes 'Home', 'Help', 'Reference', 'Download', and 'Contact'. The main content area is titled 'SPECIES & ALLELE CLASS - Select the host species and MHC allele class:'. It features two dropdown menus: 'Host species' set to 'Human' and 'Allele class' set to 'Class II'. To the right, a 'Current selections:' box shows 'No. of sequences: 1'. At the bottom are buttons for 'Start Over', 'Back', and 'Next'.

Fig 13: Select the host species and MHC Allele Class II

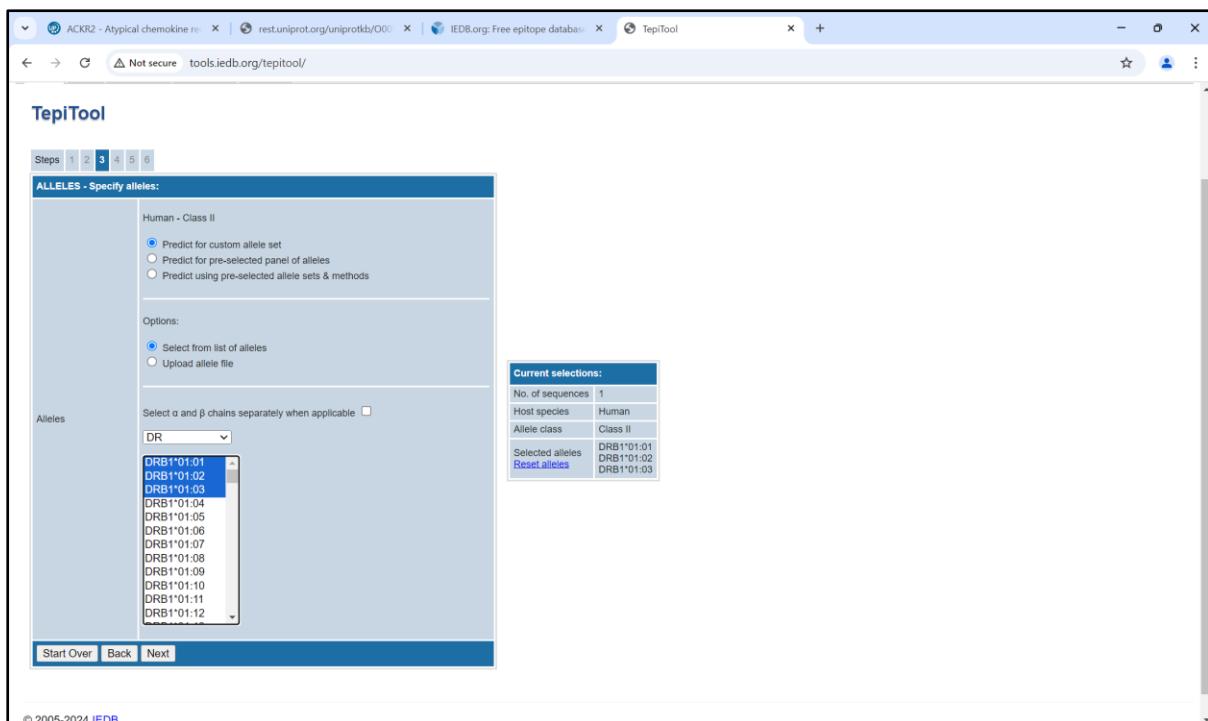


Fig 14: Select the allele for prediction

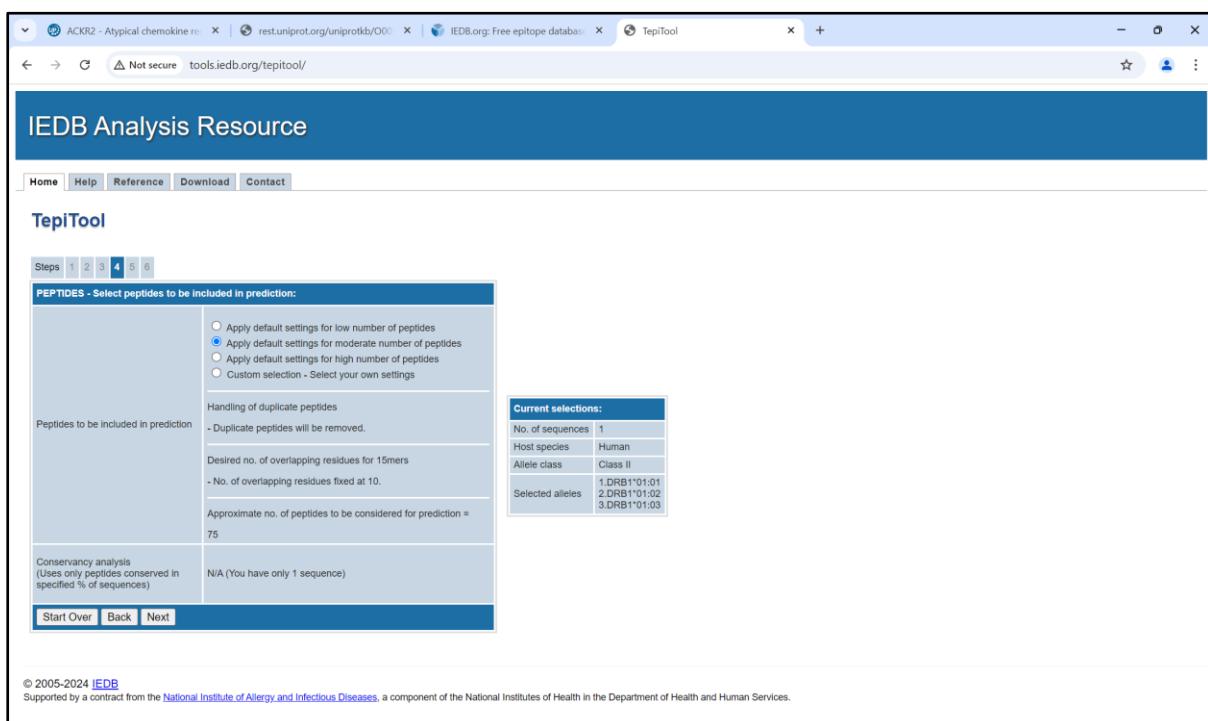


Fig 15: Choose peptides to be included in prediction

The screenshot shows the IEDB Analysis Resource TepiTool interface at Step 5. The main panel displays a form titled "METHOD - Select prediction & peptide selection methods and cutoff values:". It includes fields for "Prediction method to use" (set to "IEDB recommended") and "Selection of predicted peptides" (set to "Select peptides based on predicted percentile rank" with a cutoff of "10"). Below the form are "Start Over", "Back", and "Next" buttons. To the right is a "Current selections" table:

Current selections:	
No. of sequences	1
Host species	Human
Allele class	Class II
Alleles selected	1.DRB1*01:01 2.DRB1*01:02 3.DRB1*01:03
Duplicate peptides	Removed
Peptide overlap	10 AA residues
Approx no. of peptides included (Not considering conservancy analysis)	75
Conservancy analysis	Peptides conserved in at least % sequences

At the bottom left, a copyright notice reads: © 2005-2024 IEDB. Supported by a contract from the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health in the Department of Health and Human Services.

Fig 16: Select the prediction method, peptide selection strategy and cutoff values

The screenshot shows the IEDB Analysis Resource TepiTool interface at Step 6. The main panel displays a "REVIEW: Review selections, enter job details & submit data:" section. It contains two tabs: "Summary" and "Job details". The "Summary" tab shows the same information as Fig 16. The "Job details" tab includes fields for "Job name (optional)" and "Email (optional - will notify when job is finished)". Below these are "Start Over", "Back", and "Submit" buttons. A note at the bottom states: "(Please note that you will not be able to make any more changes once submitted. You will have to start again if you want to do so.)"

Fig 17: Review summary, enter job details and submit data

ACKR2 - Atypical chemokine receptor 2 OS=Homo sapiens OX=9606 GN=ACKR2 PE=1 SV=2

rest.uniprot.org/uniprotkb/O00

IEDB.org: Free epitope database

TepiTool results

Not secure tools.iedb.org/tepi tool/

Iedb Analysis Resource

Home Help Reference Download Contact

TepiTool

Prediction results - concise (Download table)

Seq #	Peptide start	Peptide end	Peptide sequence	Percentile rank	Allele
1	324	338	KAFIAAVLQWHLAPG	2.5	HLA-DRB1*01:01
1	245	259	QGRALKIAAAALVVAF	2.7	HLA-DRB1*01:01
1	288	302	QLDLYAQLQVTEISIAF	5.5	HLA-DRB1*01:01
1	24	38	YIDYDLEVAFLNLCKK	5.6	HLA-DRB1*01:01
1	140	154	LCKYLVLIWHAQPYHR	6.1	HLA-DRB1*01:01
1	165	179	ATIVWAVASLAVSIPD	7.4	HLA-DRB1*01:01
1	329	343	AVLWHWILAPLQTQAS	7.6	HLA-DRB1*01:01
1	177	191	IEFDNVEVQVTHENFEG	8.2	HLA-DRB1*01:01
1	324	338	KAFIAAVLQWHLAPG	2.1	HLA-DRB1*01:02
1	245	259	QGRALKIAAAALVVAF	2.8	HLA-DRB1*01:02
1	140	154	LCKYLVLIWHAQPYHR	3.1	HLA-DRB1*01:02
1	177	191	IEFDNVEVQVTHENFEG	7.5	HLA-DRB1*01:02
1	234	248	OCVLVRLRPAQQORA	8.4	HLA-DRB1*01:02
1	140	154	LCKYLVLIWHAQPYHR	3.9	HLA-DRB1*01:03
1	308	322	SPILYAFFFSSHRFRFQV	4.4	HLA-DRB1*01:03
1	245	259	QGRALKIAAAALVVAF	5.0	HLA-DRB1*01:03
1	324	338	KAFIAAVLQWHLAPG	5.3	HLA-DRB1*01:03
1	148	162	HAQTYHRLTRAKSL	5.7	HLA-DRB1*01:03
1	177	191	IEFDNVEVQVTHENFEG	7.5	HLA-DRB1*01:03

Download results details:

Non-redundant results: Prediction results with redundant peptides within each sequence removed - Includes positives and negatives

Complete results: Prediction results of all peptides

Fig 18: Predicted result for MHC Class II

ACKR2 - Atypical chemokine receptor 2 OS=Homo sapiens OX=9606 GN=ACKR2 PE=1 SV=2

rest.uniprot.org/uniprotkb/O00

IEDB.org: Free epitope database

TepiTool results

Not secure tools.iedb.org/tepi tool/

Iedb Analysis Resource

Download results details:

Non-redundant results: Prediction results with redundant peptides within each sequence removed - Includes positives and negatives

Complete results: Prediction results of all peptides

Citation information:

If you use these predictions in a manuscript, please include the following in the method section:
For complete list of references please click here: [References](#)

Input sequences:

Seq #	Seq title	Sequence
1	sp O00590 ACKR2_HUMAN Atypical chemokine receptor 2 OS=Homo sapiens OX=9606 GN=ACKR2 PE=1 SV=2	MAATASPOPPLATEDADSENSSFFYYDDYLDEVAFMLCRKDAVVSFGKVFLPVFYSLIVLGLSGNLLLMVLLRYVPRRRMVIEYLLNLAINSLFLVTLPPV

Other input parameters:

Input summary:	
No. of sequences	1
Host species	Human
Allele class	Class II
Alleles	DRB1*01:01 DRB1*01:02 DRB1*01:03
Duplicate peptides	Removed
Peptide lengths selected	15mers (Only one length for class II)
Peptide overlap	10 AA residues
Conservancy analysis	No
Prediction method	IEDB recommended
Peptide selection criterion	Predicted percentile rank
Cutoff for peptide selection criterion	10
Job name	
Email	

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Supported by a contract from the National Institute of Allergy and Infectious Diseases, a component of the National Institutes of Health in the Department of Health and Human Services.

Fig 19: Input sequences and other parameters

RESULTS:

The prediction results for MHC Class I and II molecules for the query ‘Chemokine’ (Accession ID: O00590) were obtained using IEDB's TepiTool. The predicted result in concise table shows the percentile rank. The low percentile ranks (less than 1) indicate these peptides are strong candidates for further experimental validation as potential MHC Class I and II epitopes for Chemokine. Best percentile rank 0.01 for the allele peptides are predicted to bind well to the corresponding MHC Class I molecules and for MHC Class II the percentile rank is 2.5.

CONCLUSION:

TepiTooL was used to perform T cell epitope predictions on the IEDB database. It identified potential epitopes for both Class I and Class II MHC molecules. TepiTooL is a tool designed to predict peptide sequences that can bind to MHC molecules, aiding in the identification of T cell epitopes crucial for immune responses. It provides a ranking based on binding affinity, helping prioritize peptides for further research.

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