

A complete modeling workflow for antibody humanization projects

- Build chimeric models of antibodies and obtain highly optimized homology models

- Refine CDR loop regions

- Accurately model antibody-target complexes

- Sequence analysis

- Homology modeling

- Mutational analysis

- Model Refinement

- Model Characterization

- Exposed vs. buried residues

- Electrostatics

- Advanced and intuitive visualization

Antibody Modeling Solutions in Discovery Studio 2.5

Antibodies prove to be an effective tool in preventing and treating infectious diseases due to their high affinity for target antigens. Therapeutic antibodies even have the ability to treat cancer, arthritis, cardiovascular diseases, as well as immune disorders¹. Furthermore, antibodies are commonly used in diagnostic tests such as the PCA test for prostate cancer, as well as laboratory tasks such as purification, signal detection, and catalysis. Discovery Studio not only contains the tools necessary to construct modeling framework from antibodies, but also enables structure based prediction of antibodies by physical properties with the goal of uncovering novel antibody designs.

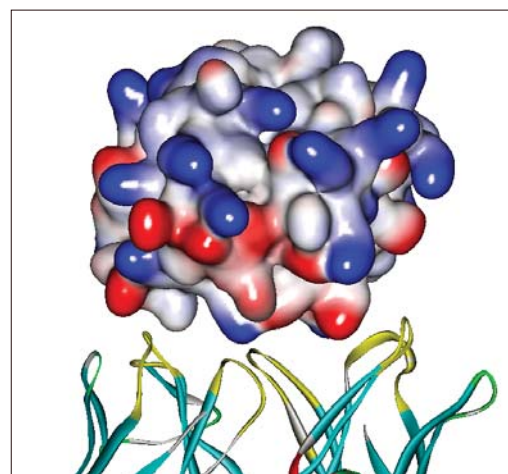
Challenges and Solutions

Antibodies have a high degree of global structural similarity, however many regions, such as the antigen binding region comprised of the CDR's, do not share such high degree of homology. Furthermore, the flexibility of the CDR loops is difficult to model and find an appropriate template for. There are many templates available, but very few many share high homology domains.

In order to address these challenges, two main approaches can be taken. The first method is the template based approach in which loops are grafted into a conserved core. The second approach is to use de novo methods to reconstruct the loop and propose high quality loop conformations. Through using both approaches, it is possible to provide clues as to what the optimal loop conformation is.

The Discovery Studio Research Environment

Homology modeling and antibody structure refinement is all part of the Discovery Studio research environment. Discovery Studio is built on Pipeline Pilot platform, allowing a powerful integration with multiple software applications that will guide you through template identification, sequence alignments, homology modeling, identification and refinement of CDR loops, as well as all subsequent analysis.



Features

A full suite of protein modeling tools is available to help in the homology models of antibodies. With these capabilities, Discovery Studio quickly guides you through the steps necessary to build the antibody framework.

Template Identification

- BLAST search against PDB_nr95 database: capable of identifying the best templates for individual chain or domain
- BLAST search against PDB_Antibody: capable of identifying the best templates for multi-domains, especially for building the domain interface
- Filter search results by specific organism

Antibody Modeling Solutions

Sequence Alignment

- Quickly and accurately align model sequence with templates using multiple structure alignment and multiple sequence alignment algorithms

Homology Model Building

- Customizable Build Homology Models protocol
- MODELER^{2,3} performs comparative protein structure modeling through the satisfaction of spatial restraints
- Specify and link disulfide bridges
- Copy ligands from the template to the model

Identification and Refinement of CDR Loops

- Template based or *Ab initio* based modeling
- For template based modeling
 - Automatically identify the loop type and loop region from the input structure
 - Customizable protocol allows customer to change the loop definition
 - Identifies the best template(s) to use for each loop region
 - Keep the core structures fixed while modifying loop region
- For *Ab initio* based modeling using LOOPER⁴ algorithm
 - Systematically searches for backbone conformation
 - Minimizes and ranks conformations using CHARMM⁵ energy

Side Chain Refinement

- Systematically search for side-chain conformation using CHARMM simulations⁶
- Selects the best conformation based on CHARMM energy

Analysis of Models

Discovery Studio provides tools and protocols to assess the quality of your models. With a wide array of diverse tools you can be sure you are receiving a detailed analysis of your discoveries.

- Verify Protein (Profiles 3D) calculates the likelihood of each residue to be found in its specific local 3D environment. Expected Verify Scores create indicators of the quality of the model.
- Verify Protein (MODELER) scores the model conformation using a statistical potential
- Ramachandran plots to verify the distribution of phi and psi angles of amino acids
- Capabilities to look at main-chain angular conformation, side-chain deviations from a known rotamer library, and other structural parameters

Applications

Humanization

- Improve activation of antibodies in the human immune system by reducing immunogenicity of monoclonal antibodies from xenogeneic sources
- Graft rodent CDR's into human framework
- Designing of humanized antibody to adjust physical characteristics and improve binding affinity

Protein – Protein Docking

- Rigid docking using ZDOCK⁷ for an efficient systematic search in rotational and translational space
- Use an RMSD matrix to find the largest number of neighbors for clustering
- RDOCK for refining and re-ranking with electrostatic and ACE desolvation energy
- ZRANK to rank poses based on empirical energy function

Integration

Because Discovery Studio is built upon the Pipeline Pilot platform, it enables technology and data integration from disparate sources.

Pipeline Pilot

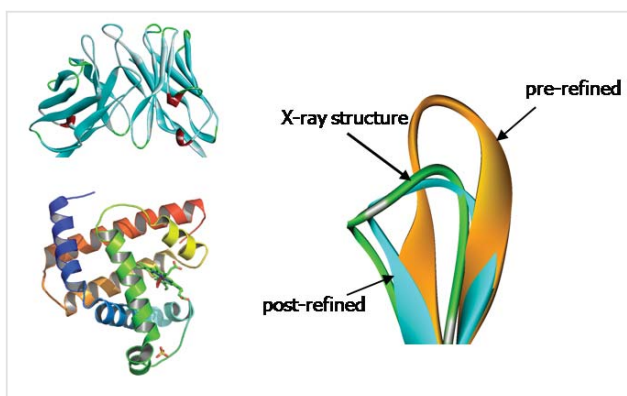
- Industry standard for data pipelining methods
- Customizable protocols Flexibility to create new workflows
- Automation of complex processes
- Client/Server architecture coupled with close integration to the DS Client

PDB Databank

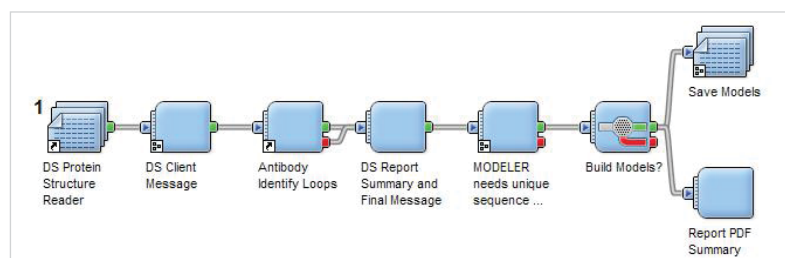
- Mine PDB databank via protocol with specific query
- Search using protocols specifically for antibodies, X-ray applications, fragment based design, structure based design, etc.
- A standard means of information exchange in macromolecular structure determination

BLAST

- Industry standard for searching sequence alignments
- Side chain or full structure based searching capabilities
- Filtering by individual organisms



Loop refinement in antibody structures



A customizable workflow for antibody loop modeling

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4. Spassov, V.Z., Flook, P.K., and Yan, L., *Protein Engineering, Design & Selection*, **2008**, 21, 91-100
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