

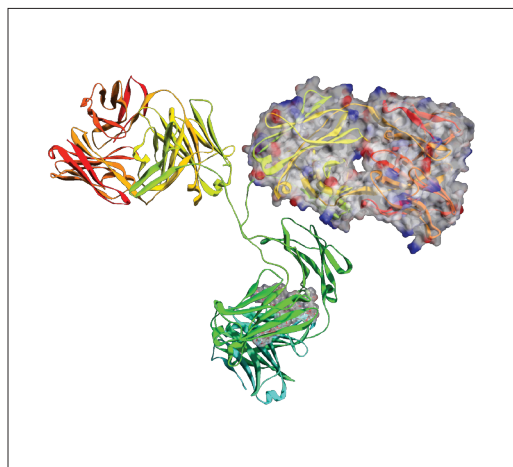
# ANTIBODY DEVELOPMENT WITH DISCOVERY STUDIO®

Antibodies have proven to be an effective treatment in a number of key diseases, including cancer, chronic inflammatory diseases, cardiovascular diseases, immune disorders, as well as infectious diseases<sup>1</sup>. Because of this potential, antibodies now represent one of the fastest growing classes of human therapeutic drugs<sup>2,3</sup>. With Discovery Studio, not only do you have the tools necessary to model antibody structures in an easy to use environment, but it also delivers the essential science to optimize their efficacy and pharmaceutical developability as therapeutic agents.

## INDUSTRY CHALLENGES TO DEVELOPING NOVEL THERAPEUTIC ANTIBODIES

Development of antibodies as drugs presents a number of specific design and development challenges, including:

- Modeling the antibody structure
  - $V_H$  and  $V_L$  Template structure identification
  - Full-length IgG1 structure models
  - Fab or Fv domain ( $V_{HL}$ ) Framework models
  - Accurately modeling CDR loops
  - Model refinements and simulations
- Optimizing affinity and specificity
  - Antigen epitope/Antibody paratope identification
  - Optimizing affinity and specificity of the antibody
- Optimizing stability
  - Thermal and pH dependent stability
  - Predicting protein aggregation propensity
- Humanization of antibodies
  - Grafting animal CDRs into human antibody frameworks
  - Predict stabilizing mutations to support the humanization process



**Figure 1:** Example structure of an intact full-length antibody [PDB: 1IGT]

With Discovery Studio, you have the science and technology available to tackle these design challenges.

## MODELING ANTIBODIES

Discovery Studio delivers a comprehensive portfolio of specialized tools for modeling antibody structures, including predicting the conformations of the CDR loops.

### 1) Annotate antibody domains and CDR loops

- Automatically identify the variable and constant domains of an antibody sequence or structure using HMM (Hidden Markov Model)
- For variable domains, report the CDR loops based on several commonly adopted definitions, including Chothia, Kabat, IMGT, and Honegger

### 2) Template identification

- Search PDB and curated PDB Antibody databases using Blast to identify optimal templates for each chain or domain
- Refine template selection, by specifying and filtering by particular species

### 3) Sequence alignment

- Quickly and accurately align model sequence with templates using multiple structure alignment and multiple sequence alignment algorithms
- Simultaneously, but independently perform alignments on either light or heavy chains

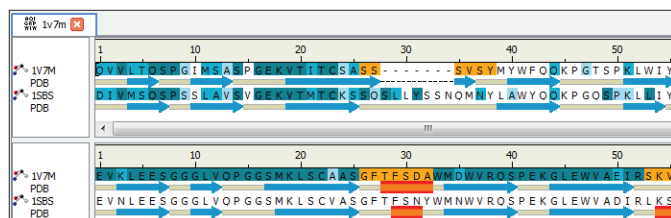


Figure 2: Example of light and heavy chain sequence alignments of antibody Fab domains [PDB: 1V7M and 1SBS]

### 4) Build homology models

Use the industry-standard MODELER<sup>4,5,6</sup> to build homology models of antibodies automatically in Discovery Studio:

- Antibody Framework modeling: Specify different templates for heavy chain and light chain respectively, allowing you to easily build a hybrid model. Use a framework template structure to determine the relative spatial orientation of the two chains

- Model Full-length Antibody: Build models from full-length sequences of Immunoglobulin gamma isotype (IgG), based on IgG1 and IgG2 template structures

### 5) Identification and refinement of CDR loops

- Automatically identify the CDR loops of an antibody structure using BLAST search against a database of known antibodies. Find the best templates for each loop region, and optionally, build models based on the loop templates
- Manual Loop Grafting: Copy the loop conformation from a template structure onto the target antibody model
- *Ab Initio* Loop Refinement: Use the LOOPER algorithm<sup>7</sup> to systematically search the main chain conformations that are then optimized and ranked using the CHARMM force field<sup>8,9</sup>

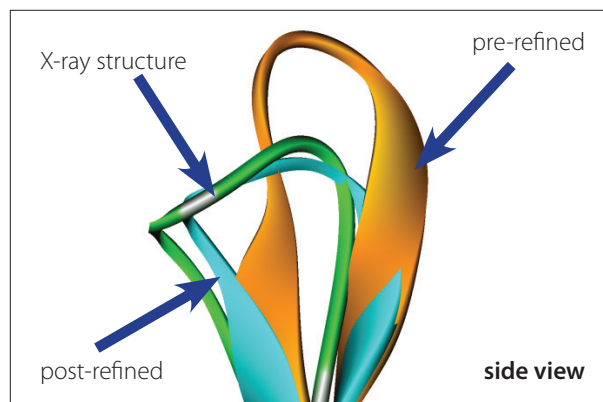


Figure 3: Loop refinement in antibody structure [PDB: 2AAB]

### 6) Model analysis

Discovery Studio provides tools and protocols to help you perform a detailed analysis of the quality of your antibody models.

- 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/reports 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 more

## FORCEFIELD-BASED PROTEIN MODELING

Perform a range of model refinements including amino acid side chain optimization, Minimization and Molecular Dynamics.

### 1) Side-chain refinement

- Systematically optimize amino acid side-chain conformation using CHARMM simulations<sup>10</sup>

### 2) Predict protein ionization and residue pKs

Using a novel in-house computational method<sup>11</sup>, quickly and accurately calculate protein ionization using a Generalized-Born solvent model in CHARMM<sup>12,13,14</sup>, incorporating an iterative mobile clustering approach<sup>15</sup> to the equilibria of proton binding.

- Predicts the pK and titration curves for each of the titratable amino acid residues
- Calculate the total protein charge as a function of pH and predict the isoelectric point (pI)
- Calculates the electrostatic contribution to the free energy of folding as a function of pH
- Protonates the residues at a given pH according to the predicted pK

### 3) Perform simulations on antibody structures

- Perform implicit solvent-based Molecular Dynamics (MD) simulations using CHARMM
- Alternatively, connect to NAMD<sup>16</sup> and perform simulations\*, with explicit solvation on a full antibody structure

## OPTIMIZING AFFINITY AND SELECTIVITY

Discovery Studio includes a suite of tools to help researchers identify the epitope binding site on antigens and also to predict the binding affinity with the Fab domain of antibodies:

### 1) Antigen-Antibody Docking

- Use ZDOCK<sup>17,18</sup> to comprehensively and effectively search protein-protein interaction patterns and output possible docking poses
- Increase the accuracy of docked poses using the ZRANK scoring function<sup>19</sup>

### 2) Predict binding affinity

- Perform amino acid scanning mutagenesis on a set of selected amino acid residues to evaluate the effect of single-point mutations on the binding affinity of molecular partners

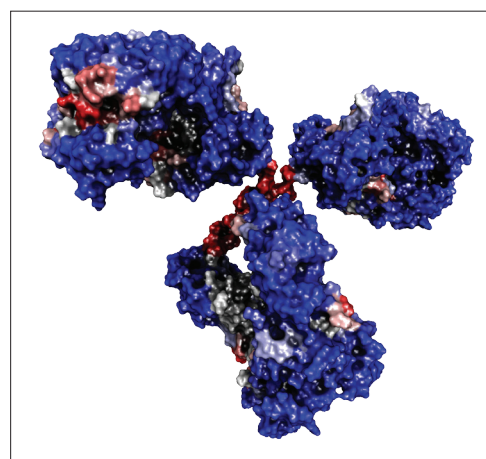
## OPTIMIZING STABILITY

### 1) Optimize protein stability

- Calculate the effect of a single-point mutation on protein stability at a given temperature
- Predict the most stabilizing two- or three-site mutations in a protein structure, among a list of specified mutations

### 2) Spatial aggregation propensity algorithm

- Use the experimentally validated spatial aggregation propensity algorithm, licensed from the Massachusetts Institute of Technology and developed by Prof. Trout<sup>20,21,22</sup>
- Identify the size and location of regions on antibodies prone to aggregation
  - Predict mutations leading to improved stability
  - Additionally, predict other protein-protein recognition sites, including the paratope, Fc receptor, protein A, and protein G binding regions<sup>23</sup>



**Figure 4:** Example protein aggregation map for IgG1, showing predicted sites of aggregation in red. [PDB: 1IGT]

\*NAMD is available from the University of Illinois at Urbana-Champaign. <http://www.ks.uiuc.edu/Research/namd/>

## HUMANIZATION OF ANTIBODIES

Improve effective dosage of antibodies in the human immune system by reducing immunogenicity of monoclonal antibodies from xenogenic sources:

- 1) Use the framework homology modeling tools to graft rodent CDR's into human framework structures
- 2) Alternatively, use the 'Calculate Mutation Energy (Stability)' tool and 'Calculate Mutation Energy (Stability)' tool together, to guide and prioritize the selection of humanizing residue mutations

To learn more about Discovery Studio, go to [accelrys.com/discovery-studio](http://accelrys.com/discovery-studio)

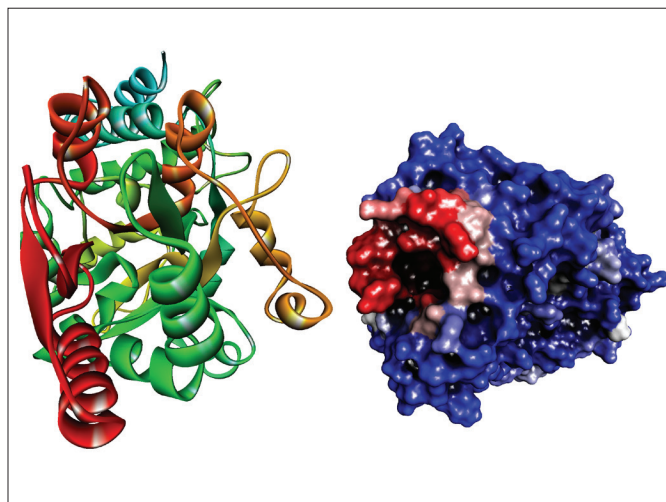


Figure 5: Binding site 'hot-spot' on a Fab domain of an antibody and its antigen [PDB: 3PGF]

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