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3DEXPERIENCE®

Accelerate Science-Led Innovation for Competitive Advantage

BIOVIA Discovery Studio

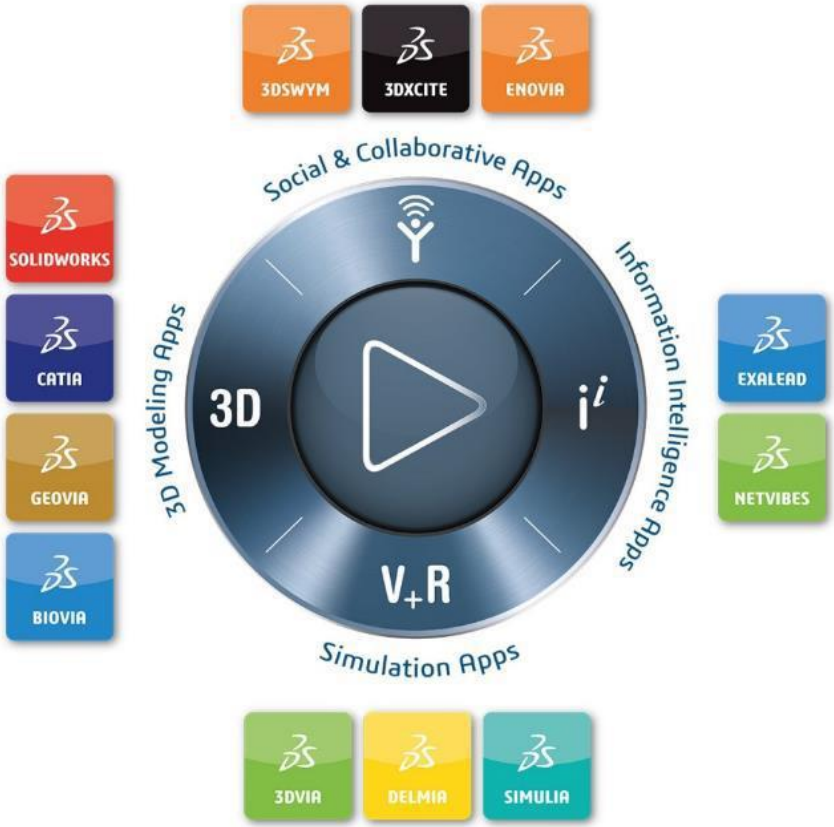
虛擬抗體設計課程

創源生技 分子視算中心

經理 陳冠文 (Gene)

創源生技 GGA

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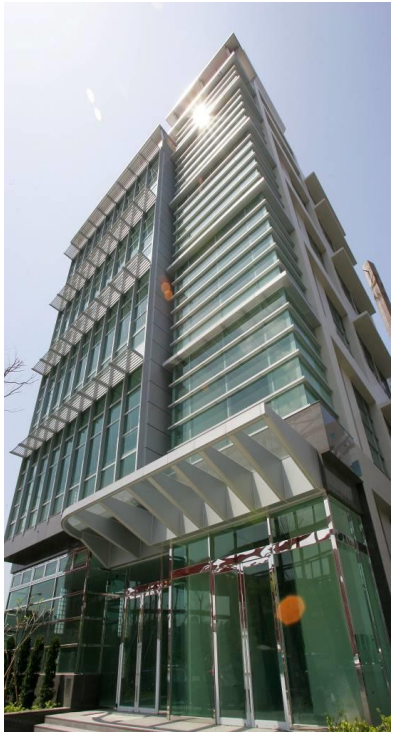
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GGA is part of the BIONET Group (訊聯生物科技)



CEO: Christopher Tsai, Ph.D. 蔡政憲 博士

Established: Nov. 2008

Main Product & Service Areas:

1. Genetic Testing
2. Molecular Diagnosis
3. **Scientific Informatics & Bio IT**

IPO Date: September 17, 2012

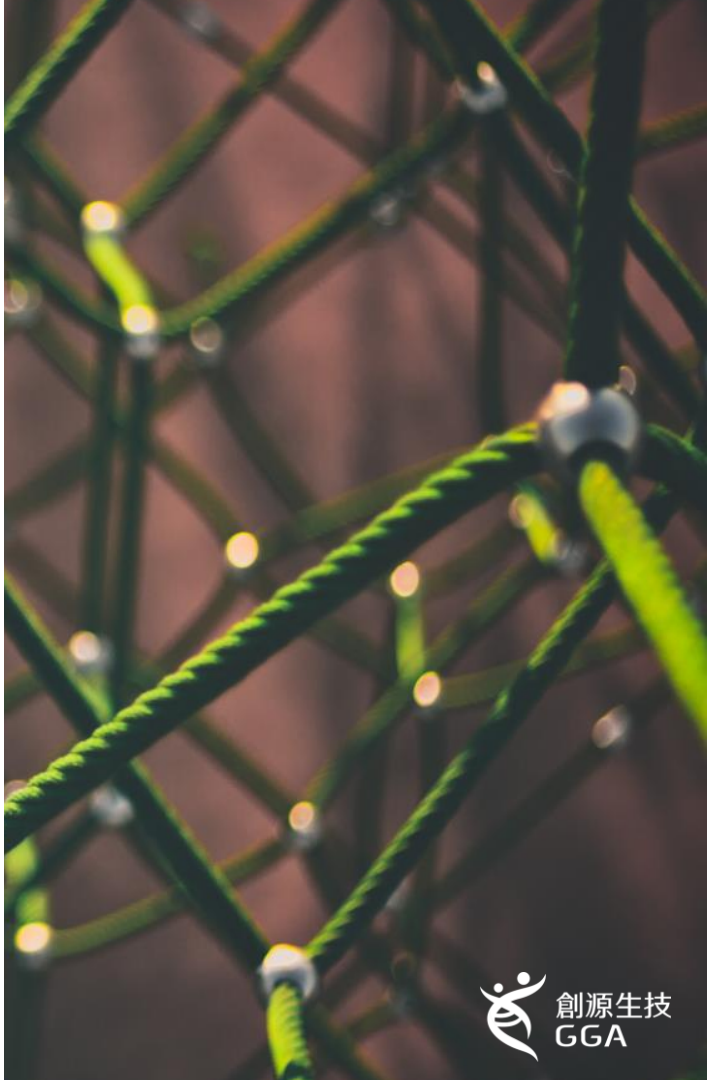
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Our Vision

用科學的知識與技術，提昇台灣產業科技創新能量。





Our Mission in 2019

- ✓ High throughput screening and machine learning for electrolyte additives
- ✓ Design optimization for industry 4.0
- ✓ 整合材料模擬與電子實驗記錄系統服務 (台灣前三大石化廠)
- ✓ 執行國家型基因體分析平台建置計劃
- ✓ 連結產學界計算模擬技術

創源生技
GGA



分子視算中心

Be Your Partner

我們是由物理、化工、生物、統計、資訊等超過十位不同領域的專家組成，具備多年產業經驗。

10+

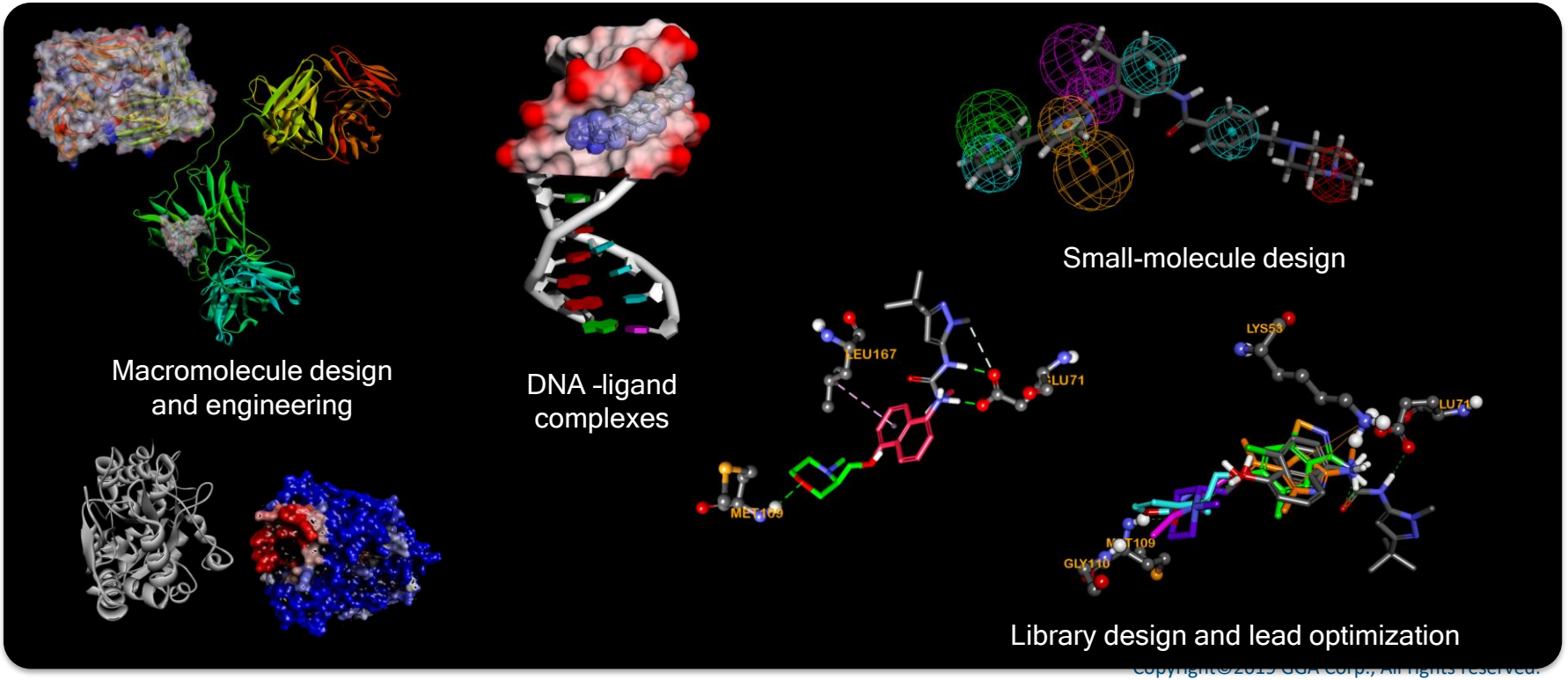
我們服務全台灣超過四十所大專院校，每年舉辦超過100場以上教育訓練課程。

100+

我們擁有包含化學、材料、生物領域，超過百萬筆資料數據，並有實際處理分析案例。

1M+

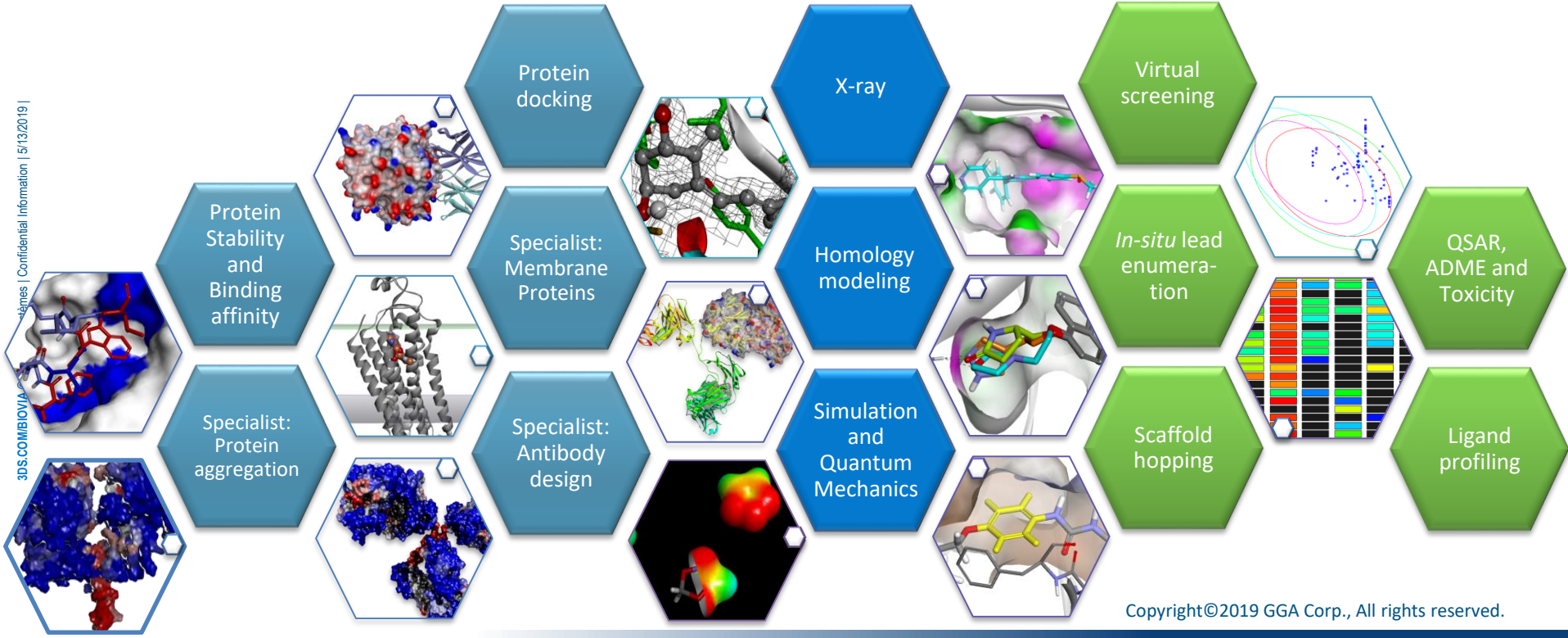
Discovery Studio



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Discovery Studio: A Comprehensive Portfolio



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Outline

- 抗體結構模擬應用簡介
- 抗體結構建模
- 特性優化(Affinity Saturation)
- 人類化預測(Humanization)

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Outline

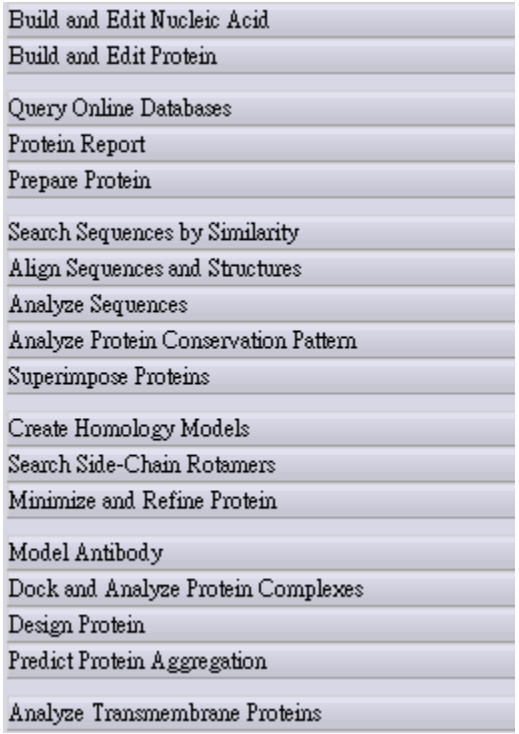
- 抗體結構模擬應用簡介
- 抗體結構建模
- 特性優化(Affinity Saturation)
- 人類化預測(Humanization)

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General introduction

- Tool sets in DS
  - Build and edit tool for Nucleic acid and Protein
  - Online search for sequences and structures
  - Prepare Protein
    - Structural information report
    - Standardize atom names
    - insert missing atoms in residues and remove alternate conformations
    - Loop rebuild and refine
    - Structure minimization
    - Calculate the pK and protonate the structure
  - Homology modeling
    - General modeling tools
    - Antibody modeling tools
  - Predict protein aggregation
  - Analyze transmembrane proteins



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Structural Information

- **Protein Data Bank – 117882 structures**
  - Macromolecules deposit by X-ray, NMR, etc techniques
  - www.rcsb.org
- **SCOP – 1195 folds**
  - Classification (Fold, Superfamily, Family)
  - http://scop.mrc-lmb.cam.ac.uk/scop/
  - June, 2009
  - SCOP2 - Structural Classification of Proteins 2
- **CATH – 173536 domain/2626 superfamily: 2011 Sep (CASH v3.5)**
  - Class(C), Architecture(A), Topology(T) and Homologous superfamily (H).
  - Fold information
  - http://www.cathdb.info/

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Difficulties for Solving Protein Structure

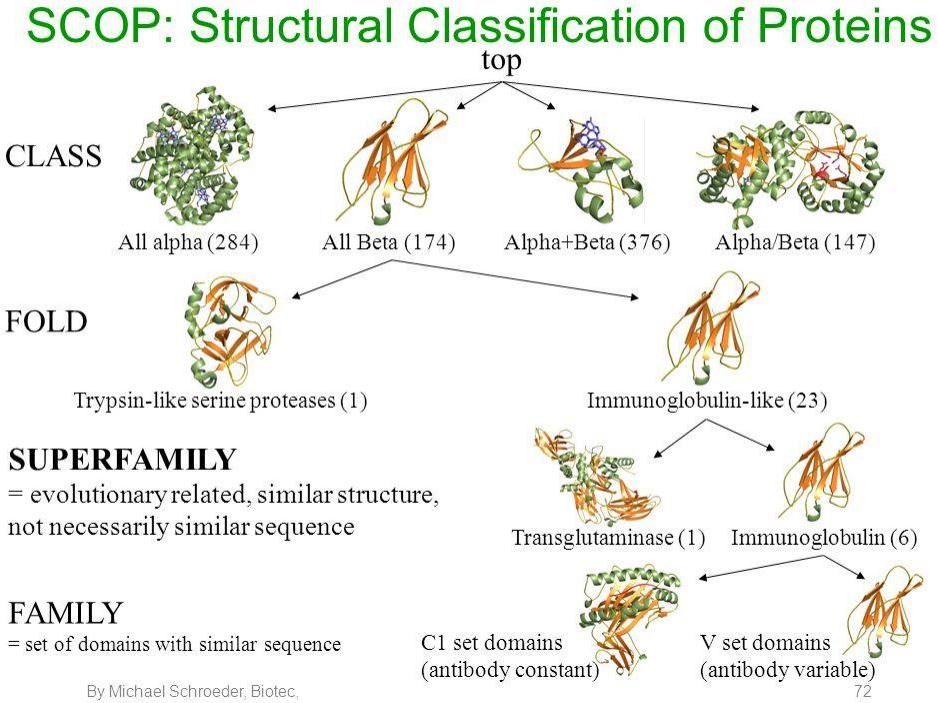
- Problem of X-ray
  - Sample preparation and protein purification
  - Crystallization
  - Diffraction
- Problem of NMR
  - Sample preparation and protein purification
  - Molecular weight limit
  - Signal labelling

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Structural Acquisition Issue

- Homology modeling
  - SCOP classification
  - Sequence similarity
  - Structural similarity
- Modeling materials
  - Aligned sequences
  - Templates

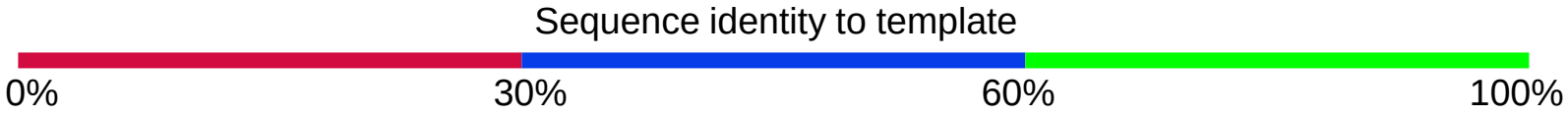


Evolutionarily related proteins have similar sequences and naturally occurring homologous proteins have similar protein structure.

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Quality of Model Related to Quality of Template



- Assignment of function/protein fold
- Finding binding or active sites by 3D motif searching
- Success may require additional information

- Identify properties that do not occur in template (e.g. electrostatics, cavity volume)
- Could be used for protein engineering (e.g. stability)
- May help guide site-directed mutagenesis studies

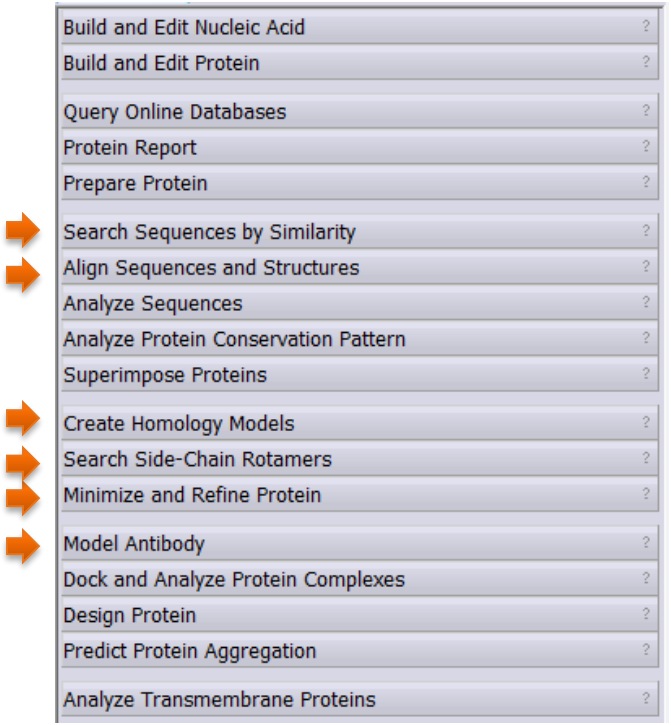
- Comparable to medium resolution X-ray/NMR structure
- May be useful for structure-based design
- Applicable for docking studies
- May help understand substrate or ligand specificity

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Homology Modeling in Discovery Studio

- Discovery Studio - Homology Modeling Function
- Macromolecules
  - Search for homologues
    - Search Sequence by Similarity
    - Model Antibody (AntiBody only)
  - Sequence Alignment of Hits
    - Align Sequences and Structures
  - Building the model
    - Create Homology Models
    - Model Antibody (AntiBody only)
  - Analysis of models
    - Create Homology Models
  - Refine of models
    - Minimize and Refine Protein
    - Search Side-Chain Rotamers
    - Model Antibody (AntiBody only)
    - Other…



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Top 25 Drug Products in the World

EvaluatePharma. World Preview 2018 Embracing the Patent Cliff

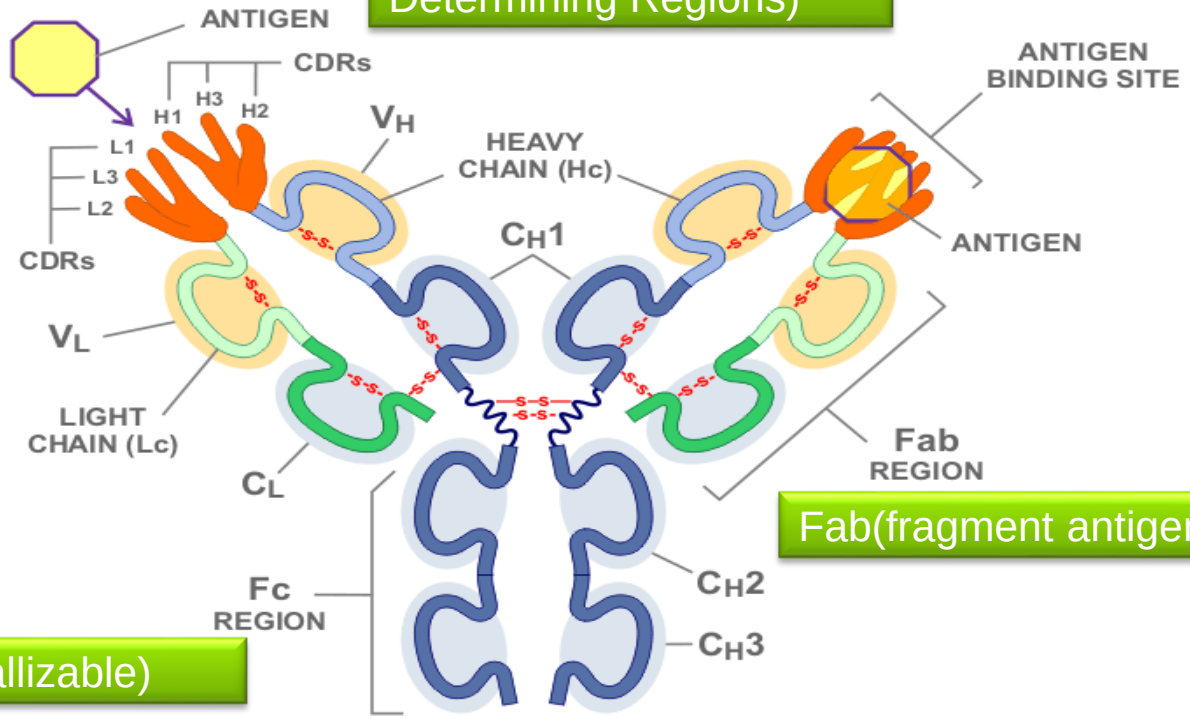
					WW Product Sales ($bn)				
	Product	Generic Name	Company	Pharmacological Class	2010	2011	% Growth	Age (Yrs)	Technology
1	Lipitor	atorvastatin calcium	Pfizer + Astellas + Almirall	Statin/ HMG CoA reductase inhibitor	11,967	10,840	-9%	15.3	Chiral chemistry
2	Plavix	clopidogrel bisulfate	BMS + Sanofi	Platelet aggregation inhibitor	9,148	9,654	6%	14.3	Small molecule
3	Humira	adalimumab	Abbott + Eisai	Anti-TNFa MAb6	6,742	8,236	22%	9.4	Antibody
4	Seretide/Advair	fluticasone; salmeterol	GSK + Almirall + Faes	Beta 2 adrenoreceptor agonist & corticosteroid	8,063	8,233	2%	13.7	Small molecule
5	Enbrel	etanercept	Amgen + Pfizer + Takeda	TNFa inhibitor	7,257	7,892	9%	13.6	Recombinant product
6	Remicade	infliximab	J&J + Merck + Mitsubishi Tanabe	Anti-TNFa Mab	6,520	7,187	10%	13.8	Antibody
7	Crestor	rosuvastatin calcium	AstraZeneca + Shionogi + Chiesi	Statin/ HMG CoA reductase inhibitor	6,106	7,161	17%	9.3	Small molecule
8	Rituxan	rituximab	Roche + Biogen Idec	Anti-CD20 Mab	6,113	6,790	11%	14.5	Antibody
9	Seroquel	quetiapine fumarate	AstraZeneca + Astellas	5-HT2 & dopamine D2 antagonist	5,611	6,180	10%	14.7	Small molecule
10	Avastin	bevacizumab	Roche	Anti-VEGF Mab	6,214	5,984	-4%	8.3	Antibody
11	Singulair	montelukast sodium	Merck + Kyorin + Sigma-Tau	Leukotriene D4 antagonist	5,410	5,967	10%	14.3	Small molecule
12	Herceptin	trastuzumab	Roche	Anti-HER2 Mab	5,221	5,940	14%	13.7	Antibody
13	Diovan	valsartan	Novartis + Ipsen + Recordati	Angiotensin II antagonist	6,134	5,737	-6%	15.7	Small molecule
14	Lantus	insulin glargine	Sanofi	Insulin	4,658	5,451	17%	11.9	Recombinant product
15	Abilify	aripiprazole	Otsuka + Bristol-Myers	5-HT1A & D2 partial agonist & 5-HT2 antagonist	4,593	5,216	14%	9.5	Small molecule
16	Januvia/Janumet	sitagliptin phosphate	Merck + Ono + Almirall						ule
17	Gleevec	imatinib mesylate	Novartis						ule
18	Zyprexa	olanzapine	Eli Lilly						ule
19	Nexium	esomeprazole magnesium	AstraZeneca + Daiichi Sankyo						stry
20	Spiriva	tiotropium bromide	Boehringer Ingelheim + Pfizer	Long-acting muscarinic antagonist	3,799	4,389	16%	10	Small molecule
21	Cymbalta	duloxetine hydrochloride	Eli Lilly + Shionogi						cule
22	Neulasta	pegfilgrastim	Amgen						roduct
23	Actos	pioglitazone hydrochloride	Takeda + Abbott						ule
24	Lyrica	pregabalin	Pfizer + Eisai						ule
25	Lucentis	ranibizumab	Novartis + Roche						

- **全球銷售前百大藥品比例排行**
  - 2011預計成長34%，2018預計成長49%
  - **2011→2018預計成長51% (142bn → 215bn)**

- **成功進入Phase II**臨床試驗階段 (2006 → 2010): 25%以上
  - 有機小分子藥物: 約10%
- 成功從前臨床試驗階段進入臨床試驗: 12%
  - 有機小分子藥物: 2%

Antibody Structure

CDRs(Complementary Determining Regions)



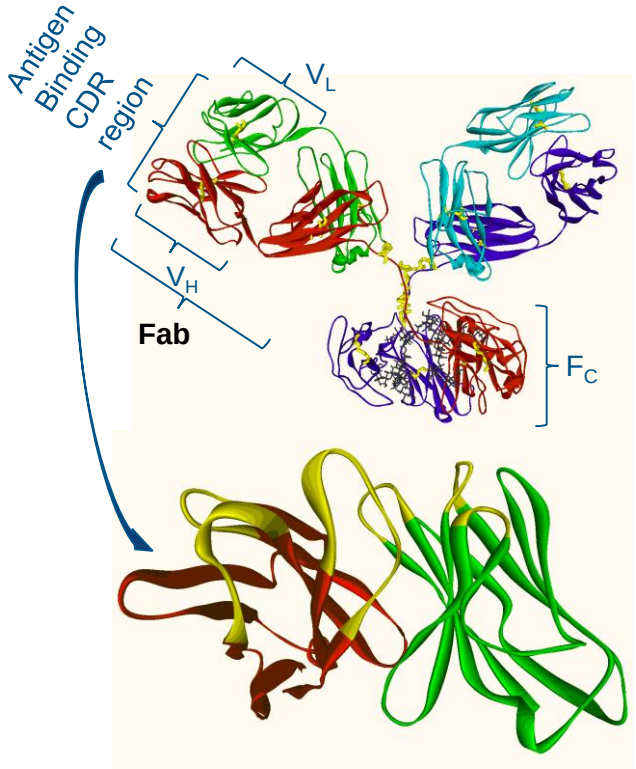
Fab(fragment antigen binding)

Fc(Fragment crystallizable)

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Antibody Modeling & Simulation



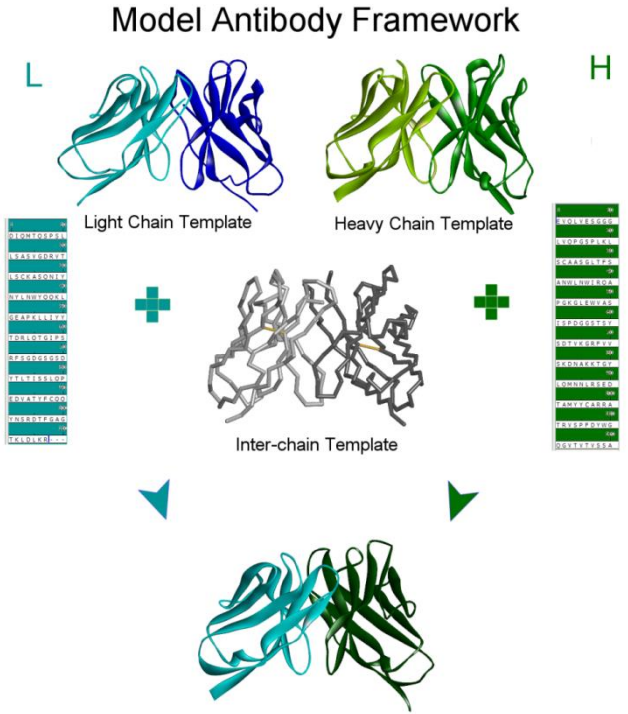
- Antibody Structure
  - Variable domain, light chain VL and heavy chain VH
  - Disulfide bridges within domain and linking domains
  - Each has 3 hypervariable (also called CDR) loops which together form the antigen binding site
- Model Antibody Framework
  - Full length antibody
  - Fab domain
  - Variable domains
- Model Antibody CDR loops
  - Template based approach
  - Ab initio approach
- Binding to Antigen
  - Protein – protein docking
  - Binding free energy

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Feature: Antibody Modeling Cascade

- Includes support for separate heavy and light chain templates (chimera reference)
- Input: light chain sequence, heavy chain sequence, heavy chain reference, heavy chain reference, optional inter-chain reference.
- Automated Steps:
  - Coordinate superimpositions of references
  - optionally based on separate inter-chain positioning reference
  - Removal of excess reference chains
- 3D Model building from target Light and Heavy chain sequences



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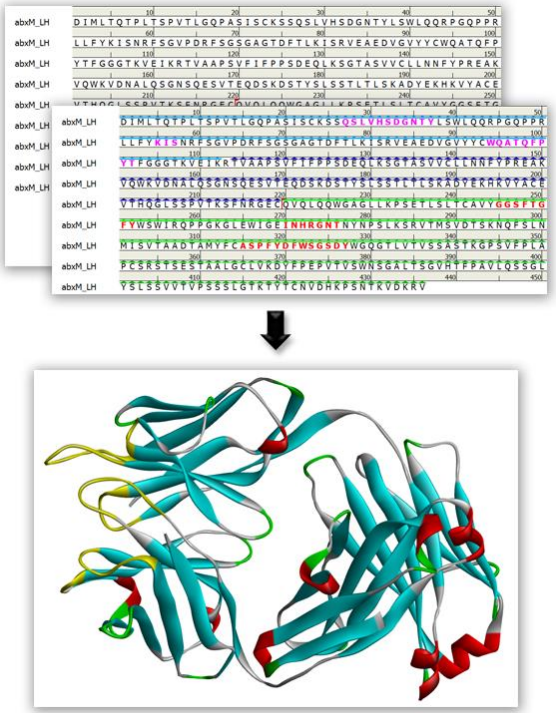
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Antibody modeling framework in DS

1. Identify Framework Templates
   - Find quality crystal structures with suitable framework region sequence similarity.
   - Results can optionally include complementarity-determining region (CDR) similarity and filter by organism.

2. Generate 3D model based on Framework Templates (Modeler)
   - Multiple Framework Templates can be used as input.

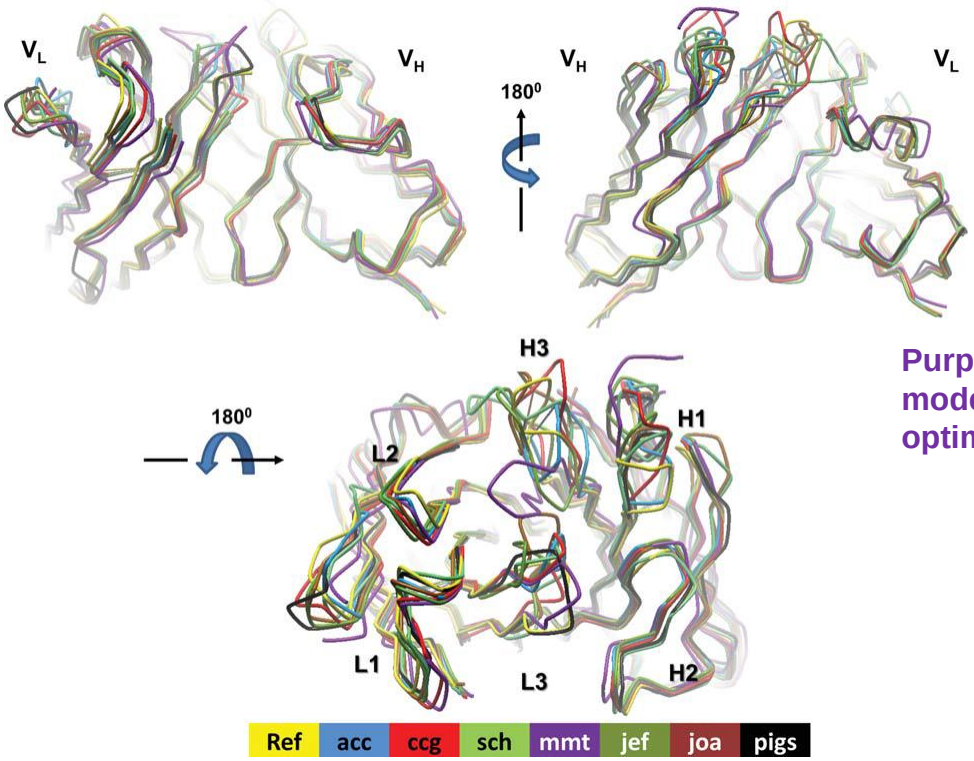
3. Refine Antibody Loops to predict CDR conformations
   - Refines the highly sequence variable CDR loops



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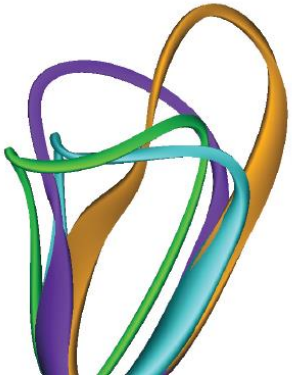
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Modeling result for the rabbit models.



Orange: H3-loop of homology model

Purple: H3-loop of homology model of 2aab after LOOPER optimization



Green: crystal structure for the H3-loop

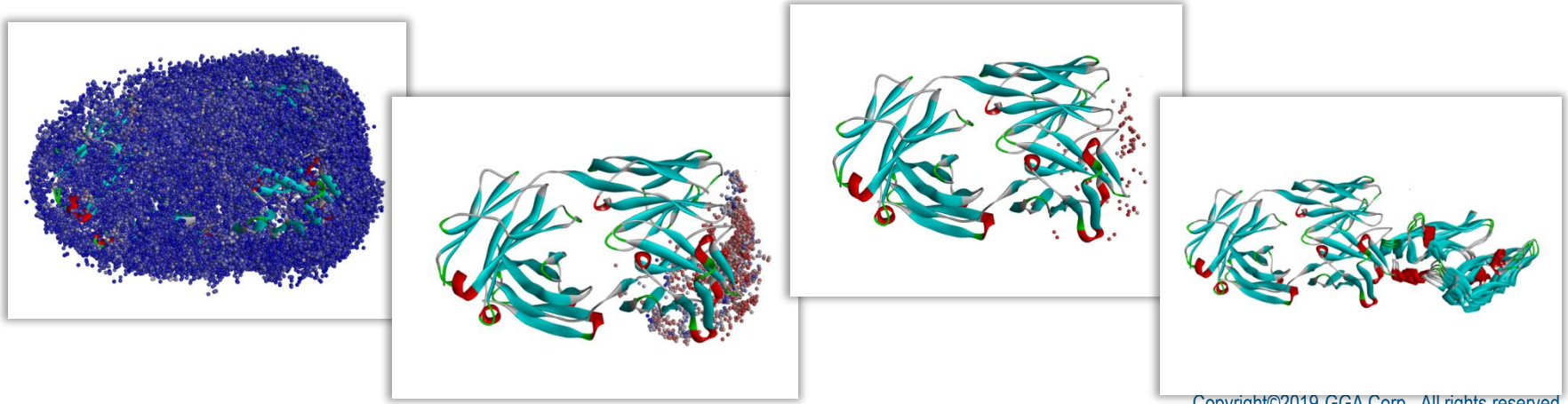
Cyan: H3-loop of homology model of after improved method refinement

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Protein-Protein Docking - ZDOCK

- ZDOCK uses a grid-based rigidbody docking search in six dimensions utilizing the Fast Fourier Transform (FFT) technique for efficiency
- The RDOCK protocol can be used subsequently for further refinement of the docked poses for the optimization of intermolecular interactions

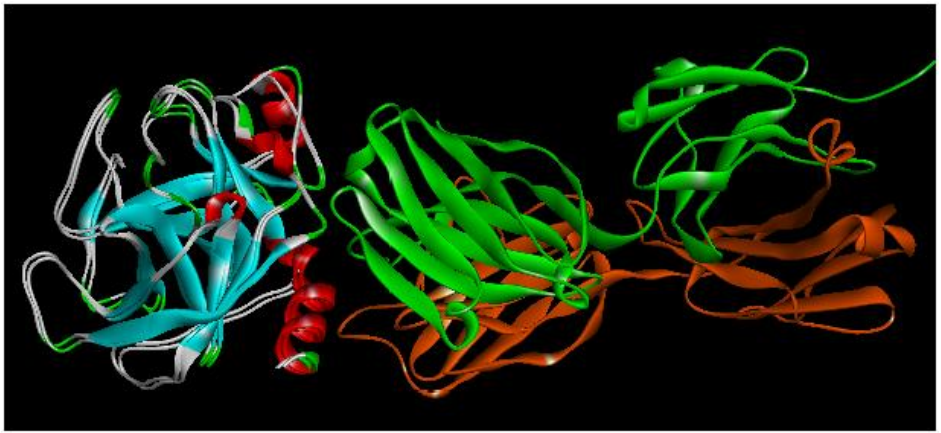


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Protein-Protein Docking - ZDOCK

- Developed in collaboration with Prof. Zhiping Weng at Boston University
  - http://zlab.bu.edu/zhiping/
- A fast, initial stage algorithm for unbound, rigid-body docking
- Docks complexes with minimum information of the binding site



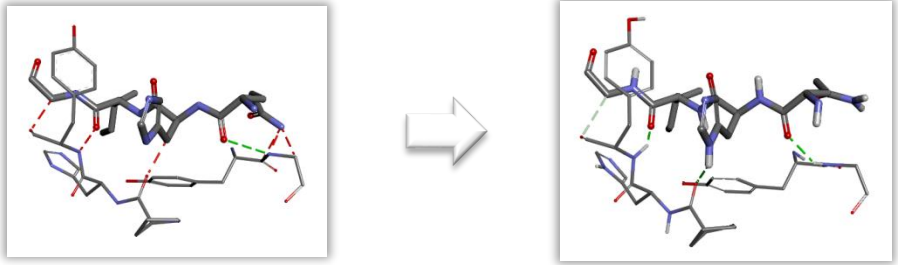
Protein ribbon diagram of the best docking prediction of the PSA antibody complex superimposed on the crystal structure from PDB 2ZCH. Antibody light chain (orange), heavy chain (green).

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Optimization of intermolecular interactions by RDOCK

- Post-processing refinement of docked complexes
- Uses a two-stage CHARMm-based energy minimization for refinement and scoring
  - Identifies near-native structures
- Re-ranks complexes with scoring function that includes desolvation and electrostatic energy
  - No side-chain or backbone conformational searches



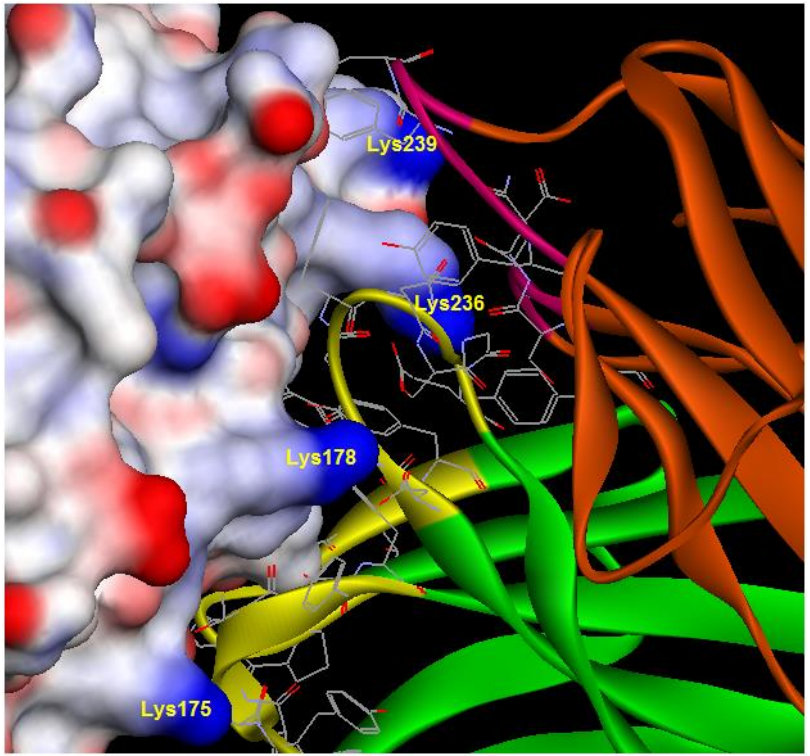
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Analyzing binding interface

- With the solvent surface displayed on PSA, it's easy to visualize the different areas where each antibody CDR loop residue interacts with PSA
- Four lysine residues Lys239, Lys236, Lys178, Lys175 involved in the binding interface.
- Additional interactions such as hydrophobic and favorable π can be displayed.





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Protein Aggregation Case Study – Avastin / VEGF

- Avastin from Genentech
  - For treatment of Cancer
  - Binds to Vascular Endothelial Growth Factor (VEGF) dimer
  - PDB code: 1BJ1
  - *In silico* affinity maturation mutagenesis comparing to experiment
  - Validate candidates with stability, electrostatics, and aggregation propensity



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Improve Avastin – Identify Binding Site Residues

- Select all residues on Avastin Fab domain within 5 Angstroms to VEGF
- Calculate mutation energies of the binding site residues
  - Using charmm-polar-H forcefield
  - Using pH-dependent electrostatics
  - Protein internal dielectic constant = 8

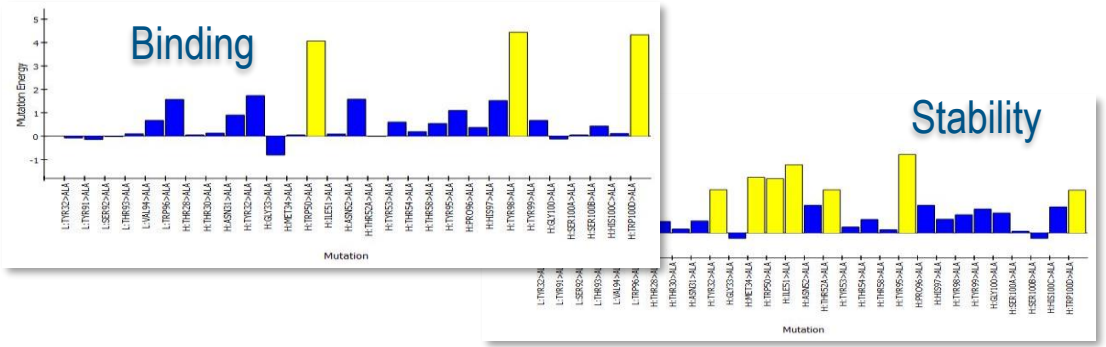


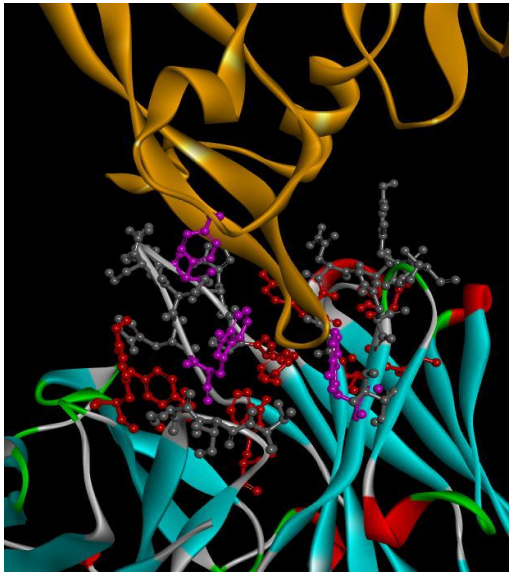
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Improve Avastin – Identify Hotspots

- Identify hotspots on Fab domain binding to VEGF
  - Run virtual alanine scanning of binding site residues to find binding and stability hotspots highlighted in yellow in the bar charts





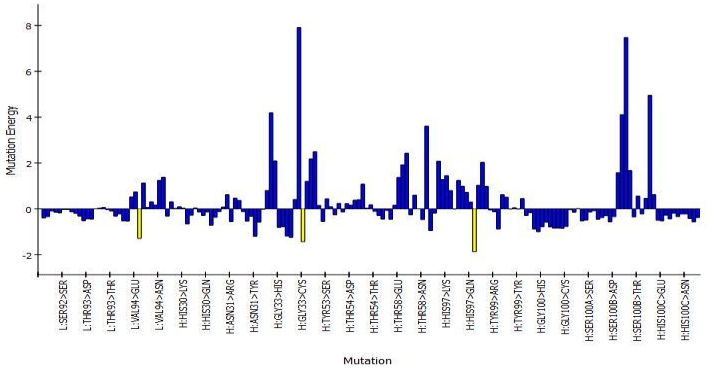
Red – stability hotspot
Magenta – binding hotspot
Grey – not hotspot

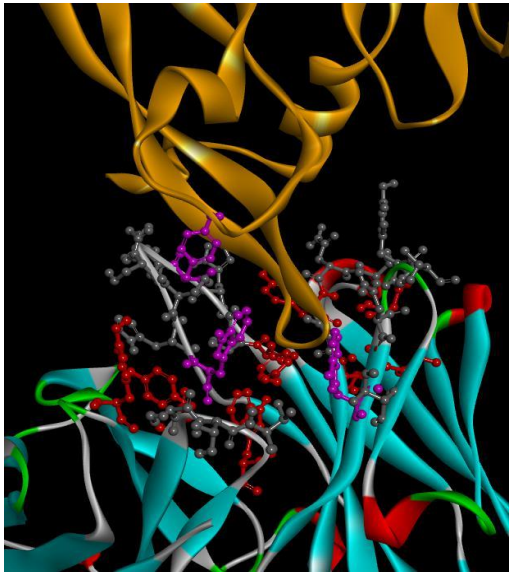
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Improve Avastin – Scanning Non-Hotspots

- For the non-hotspots, scanning each residue by 20 aa types
  - 165 mutations submitted to one job
  - **Completed in 1hr using 8 processors**
  - Identified the most stable mutations: L:VAL94>LYS, H:GLY33>CYS, H:HIS97>TYR (using Kabat numbering) highlighted in the bar chart





Red – stability hotspot
Magenta – binding hotspot
Grey – not hotspot

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Improve Avastin – Affinity Maturation

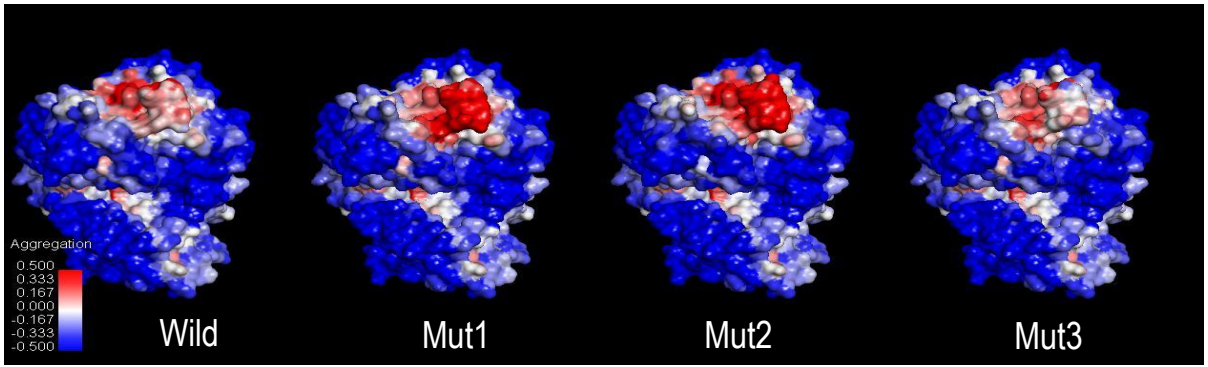
- Triple mutations L:VAL94>LYS+H:GLY33>CYS+H:HIS97>TYR
  - Predicted mutation energy = -4.34 kcal/mol
  - Most stabilizing single mutation H:HIS97>TYR agrees with Genentech affinity maturation experiment
- Also tested mutation published by Genentech:
  - Experimental 5 sites mutation
    - H:ILE34>MET+H:THR28>ASP+H:ASN31>HIS+H:HIS97>TYR+H:SER100a>THR
    - mutation energy = -2.8 kcal/mol
  - Calculated
    - H:THR28>ASP+H:ASN31>HIS+H:HIS97>TYR+H:SER100a>THR
    - pH = 9.6
    - Mutation energy = -1.48 kcal/mol

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Improving Avastin – Aggregation Propensity

- Mutants showed larger aggregation surface patches around CDR region:
  - Mut1 – Triple mutations L:VAL94>LYS+H:GLY33>CYS+H:HIS97>TYR
  - Mut2 – Genentech mutation H:ILE34>MET+H:THR28>ASP+H:ASN31>HIS+H:HIS97>TYR+H:SER100a>THR
- Introduce Mut3 – Add forth residue to Mut1 to offset the aggregation: H:TYR99>HIS, $\Delta\Delta G(mut)$ = -3.81 kcal/mol



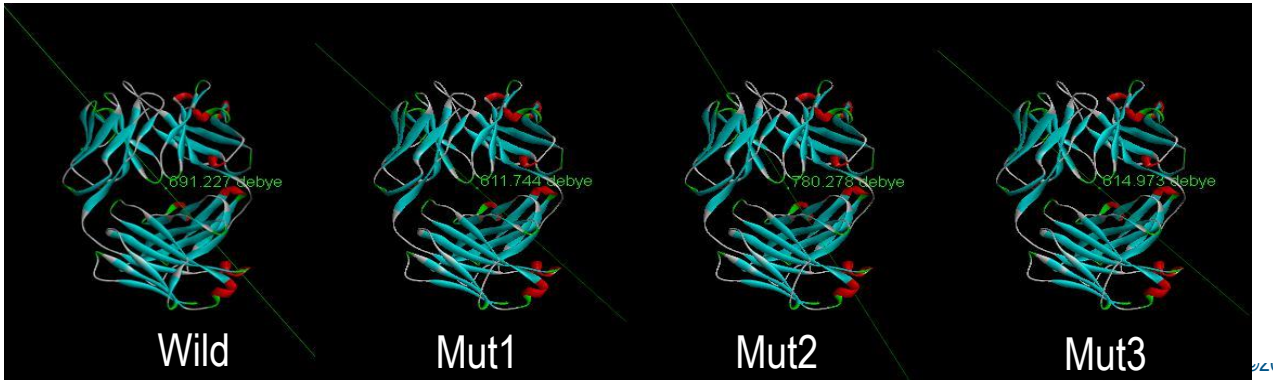
Red to blue – Increase likely hood to aggregate

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Improving Avastin – Electrostatics Property

- Calculate pK of ionizable residues and protonate protein at pH 7.4
- Mutants have similar isoelectric point (pI): 9.3-9.5
- Total charges mut2 = 4 and mut3 = 6
- Mutants have somewhat different dipole moments
  - Wild type (691) and Genentech Mutants (780) have larger dipole moment
  - DS mutants (612 and 615) have smaller dipole moments



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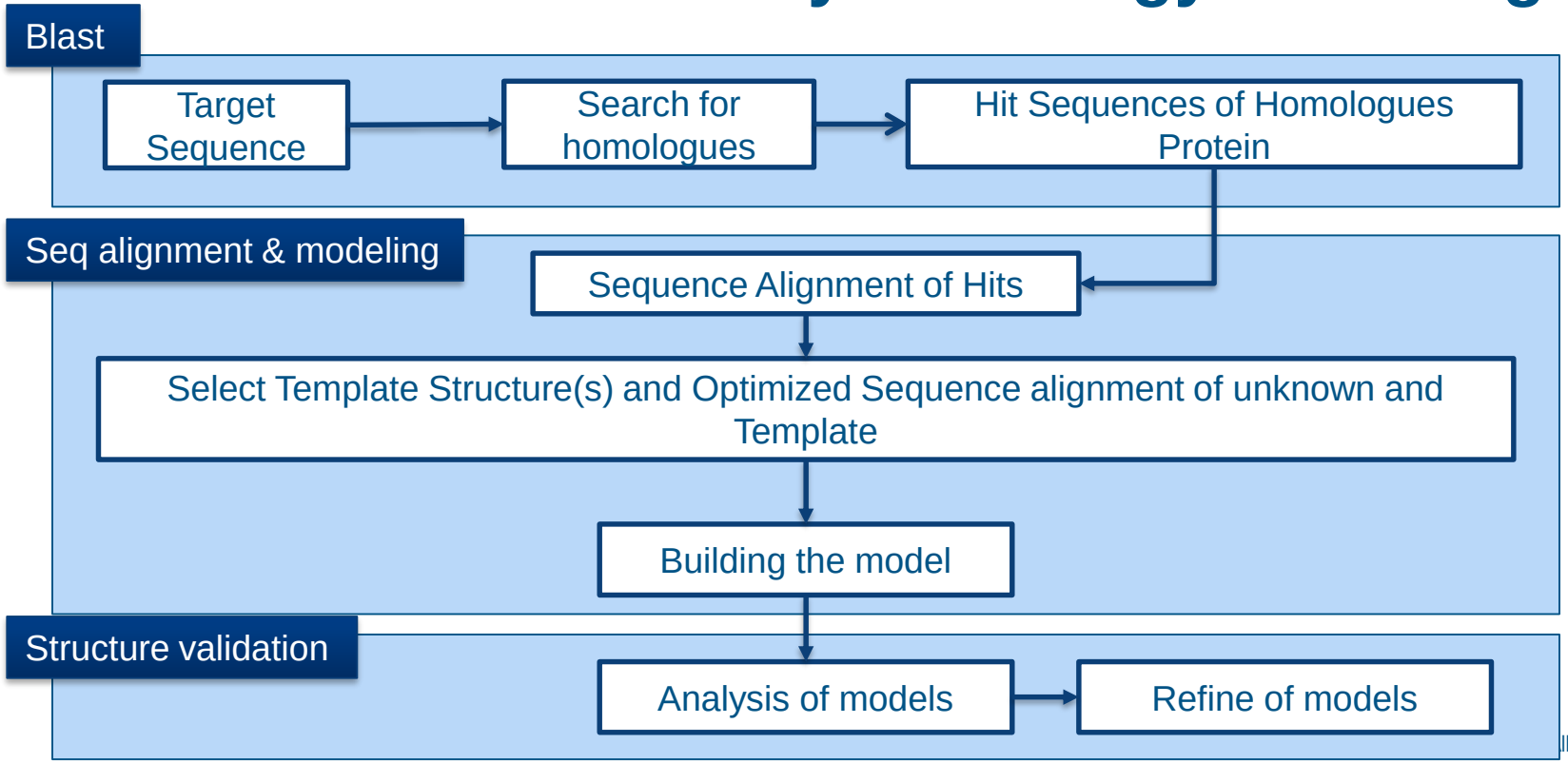
Obtaining Antibody Template Structures

- **Protein Data Bank**
  - URL http://www.rcsb.org/pdb
  - Run by the Research Collaboratory for Structural Bioinformatics (RCSB)
- **Abysis Databases**
  - www.bioinf.org.uk
  - Dr Andrew CR Martin's Group
- **IMGT – International ImMunoGeneTics information system®**
  - http://imgt.cines.fr/
- **Web Antibody Modeling**
  - http://antibody.bath.ac.uk/abmod.html
- **Prediction of ImmunoGlobulin Structure**
  - http://arianna.bio.uniroma1.it/pigs/

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served.

Structure Prediction by Homology Modeling



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Outline

- 抗體結構模擬應用簡介
- 抗體結構建模
- 特性優化(Affinity Saturation)
- 人類化預測(Humanization)

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創源生技
GGA

The Improved Workflow for Antibody Modeling in DS



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DASSAULT SYSTEMES | The 3DEXPERIENCE® Company

Training Materials

- Antibody Modeling
  - Mouse antibody 4D5
    - Light Chain
    - Heavy Chain
- Humanization
  - Trastuzumab
- Antigen Epitope Search
  - Targeting on Her2 extracellular domain

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Fab Antibody Modeling Workflow

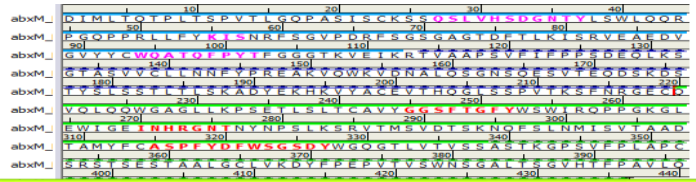
1.Structure Template Identification (Antibodies, Fv, Fab, IgG) Annotate Antibody Sequence Identify Framework Templates

2. Framework Model Generation
Model Antibody Framework from templates

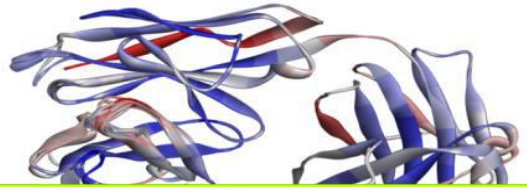
3. Analysis & Selection of Framework Model(s) Evaluate quality of templates

4. Loop Refinement (CDR) Model Antibody Loops

5. *De Novo* Loop Modeling (Optional)
Loop Refinement



Antibody Framework modeling:
1. Chimeric Method
2. Single Template Method



Refine loops refer to the similar template structure

Refine loops by *de novo* method

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Fab Antibody Modeling Workflow

1.Structure Template Identification (Antibodies, Fv, Fab, IgG) Annotate Antibody Sequence Identify Framework Templates

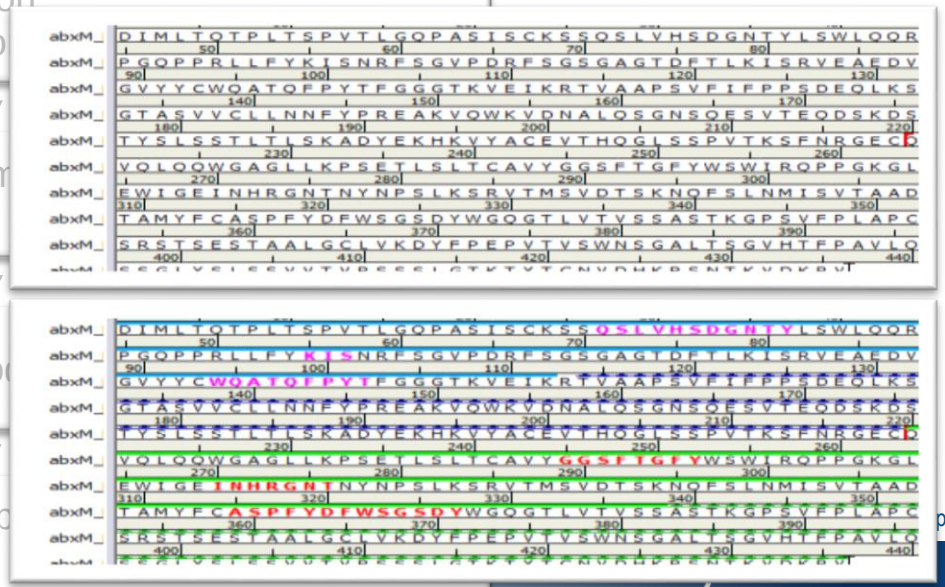
2. Framework Model Generation
Model Antibody Framework fro

3. Analysis & Selection of Fram
quality of templates

4. Loop Refinement (CDR) Mo

5. *De Novo* Loop Modeling (Op
Loop Refinement

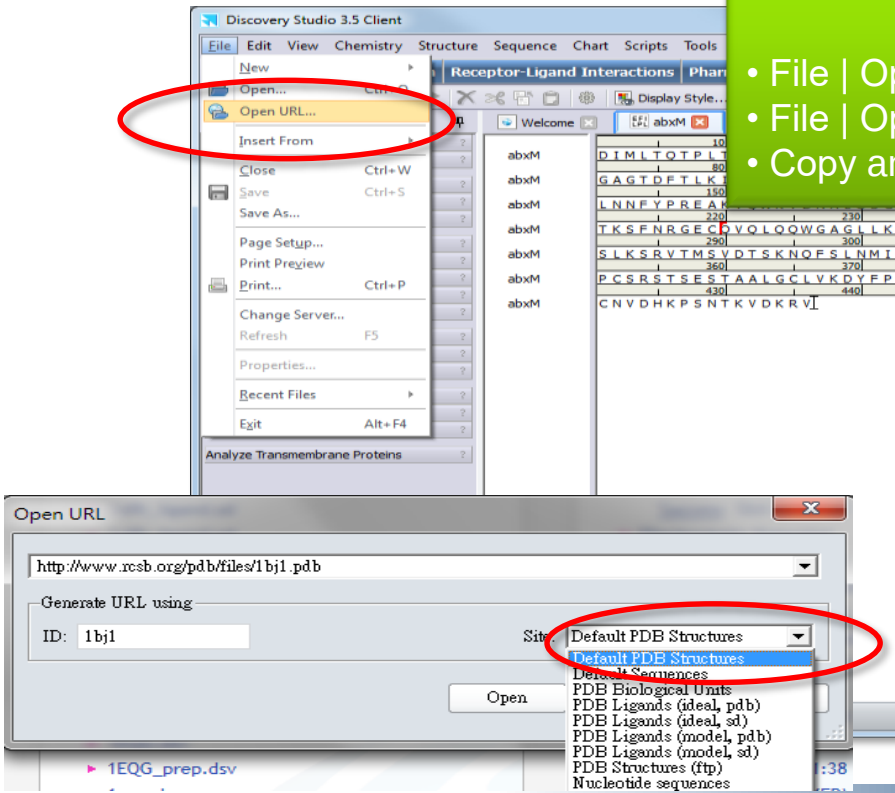
A.Load the Target Sequence
B.Annotate Antibody Sequence
C.Analyze Antibody Annotation
D.Identify Framework Templates



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1. Structure Template Identification



A. Load the Target Sequence

- File | Open URL…
- File | Open…
- Copy and paste

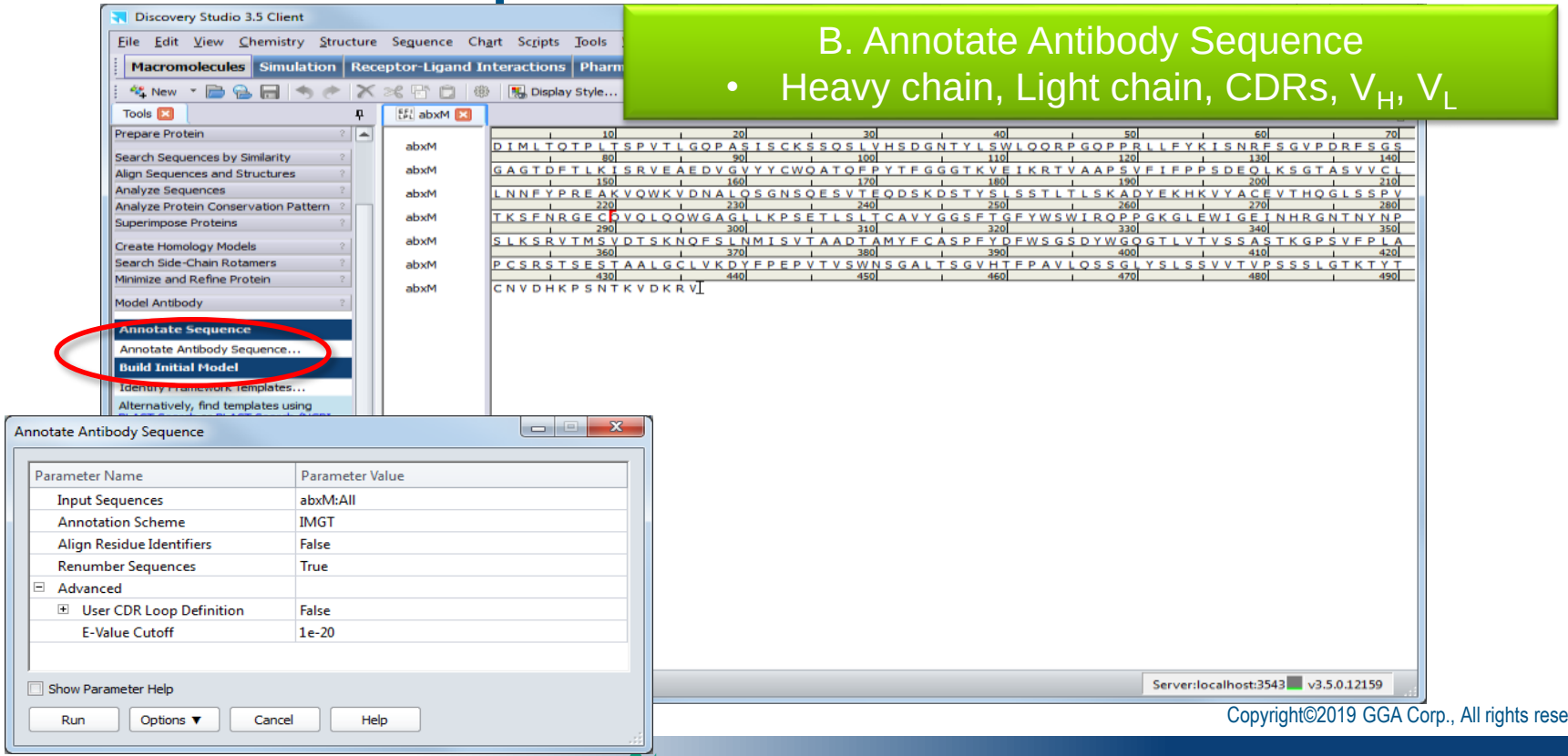
Supported sequence file formats:

-Bioinformatic Sequence Markup Language (.bsml) (default)
-Biosym format (.seq)
-Pearson (FASTA) format (.fasta)
-NBRF-PIR format (.pir)
-Swiss-Prot format (.sws)
-GenBank (.gb)
-ClustalW (.aln)
-Biosym Alignment (.align)
-Clustal (.aln)
-GDE (.gde)
-GCG format (.ssf and .rsf)
-GCG multiple sequence (.msf)

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1. Structure Template Identification



B. Annotate Antibody Sequence

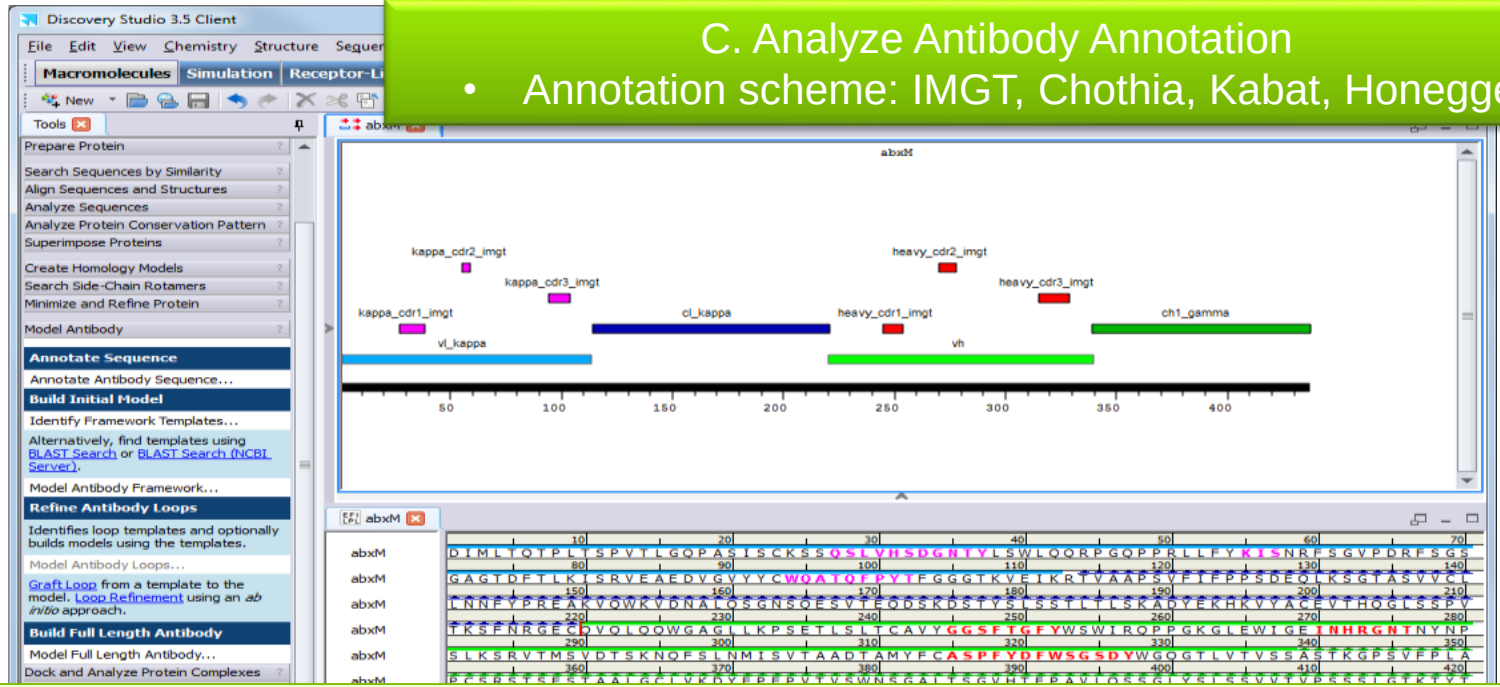
- Heavy chain, Light chain, CDRs, V_H, V_L

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1. Structure Template Identification

C. Analyze Antibody Annotation
- Annotation scheme: IMGT, Chothia, Kabat, Honegger



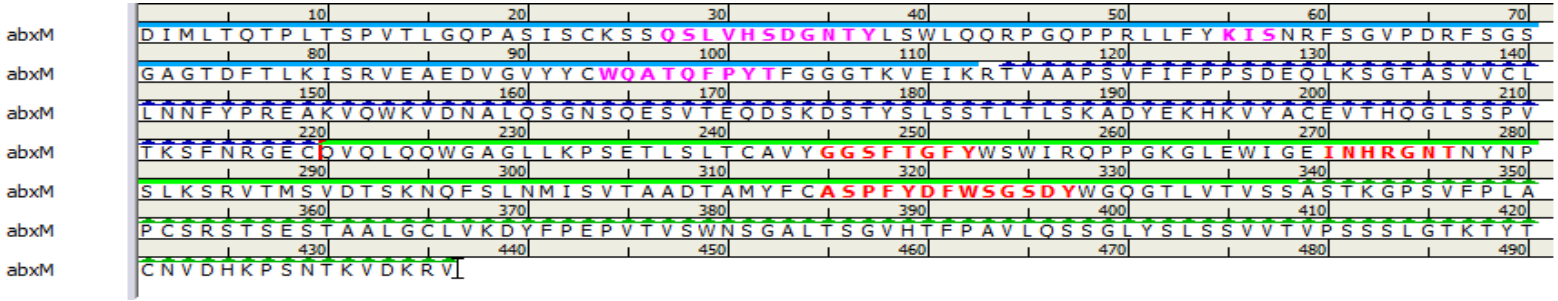
Users can customize the annotation on CDR by CDR loop definition parameters

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1. Structure Template Identification

Sequence Window annotation colors and patterns

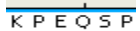
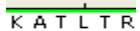
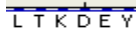
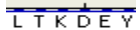
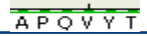
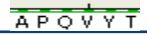
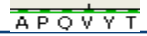
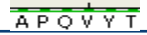
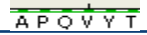
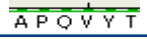
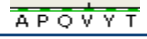
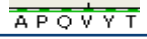
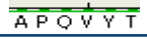
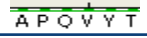


CDR Loops are highlighted
light chain variable domains - bold pink
heavy chain variable domains - bold red

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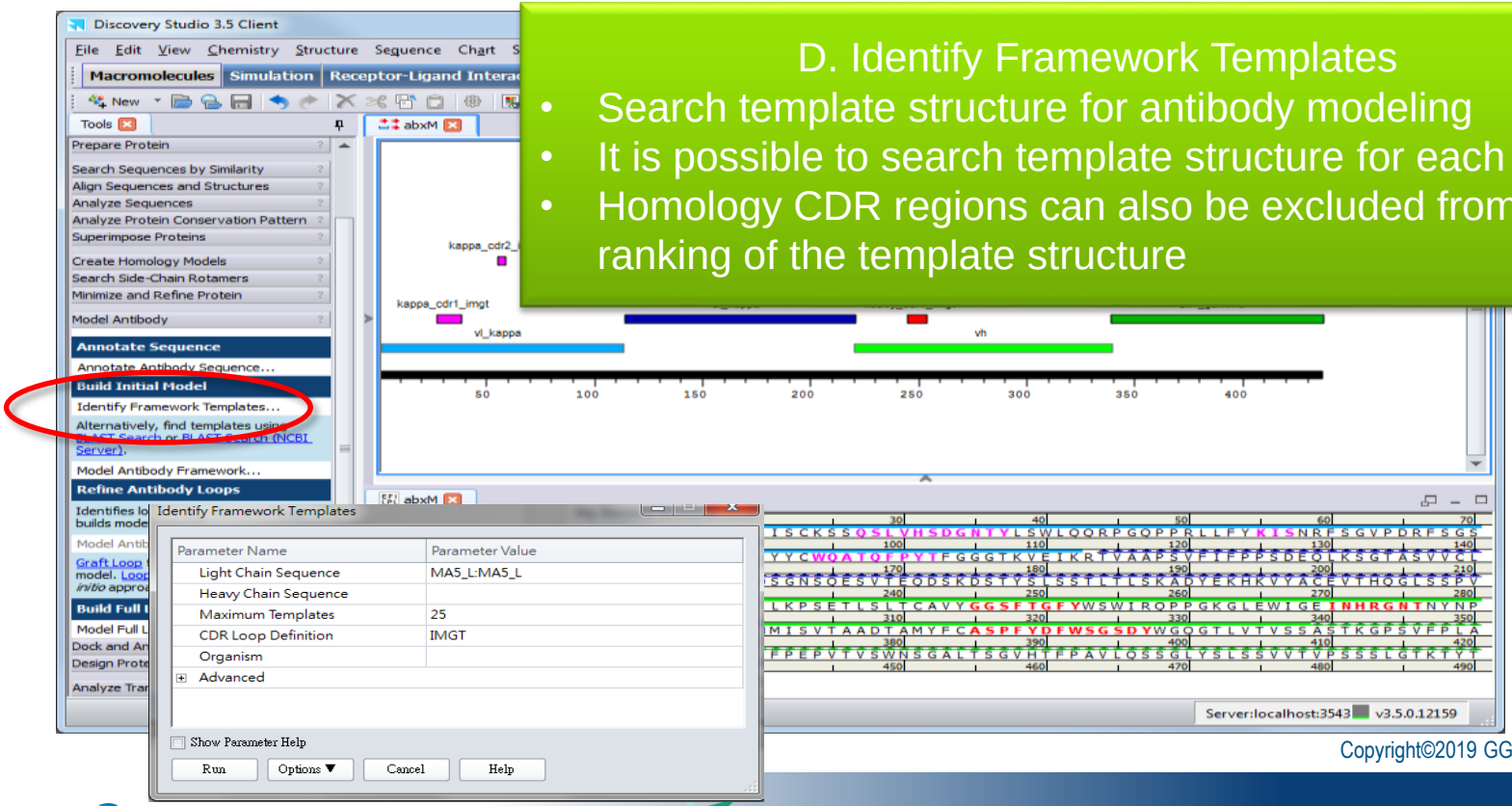
1. Structure Template Identification

Domain Name	Domain Type	Sequence Window	Annotation Window
VL_Kappa	variable light chain, kappa		
VL_Lambda	variable light chain, lambda		
VH	variable heavy chain		
CL_Kappa	constant light chain, kappa		
CL_Lambda	constant light chain, lambda		
CH1_ALPHA	constant heavy chain CH1, alpha		
CH1_GAMMA	constant heavy chain CH1, gamma		
CH1_MU	constant heavy chain CH1, mu		
CH2_ALPHA	constant heavy chain CH2, alpha		
CH2_EPSILON	constant heavy chain CH2, epsilon		
CH2_GAMMA	constant heavy chain CH2, gamma		
CH3_ALPHA	constant heavy chain CH3, alpha		
CH3_EPSILON	constant heavy chain CH3, epsilon		
CH3_GAMMA	constant heavy chain CH3, gamma		
CH4_EPSILON	constant heavy chain CH4, epsilon		

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1. Structure Template Identification



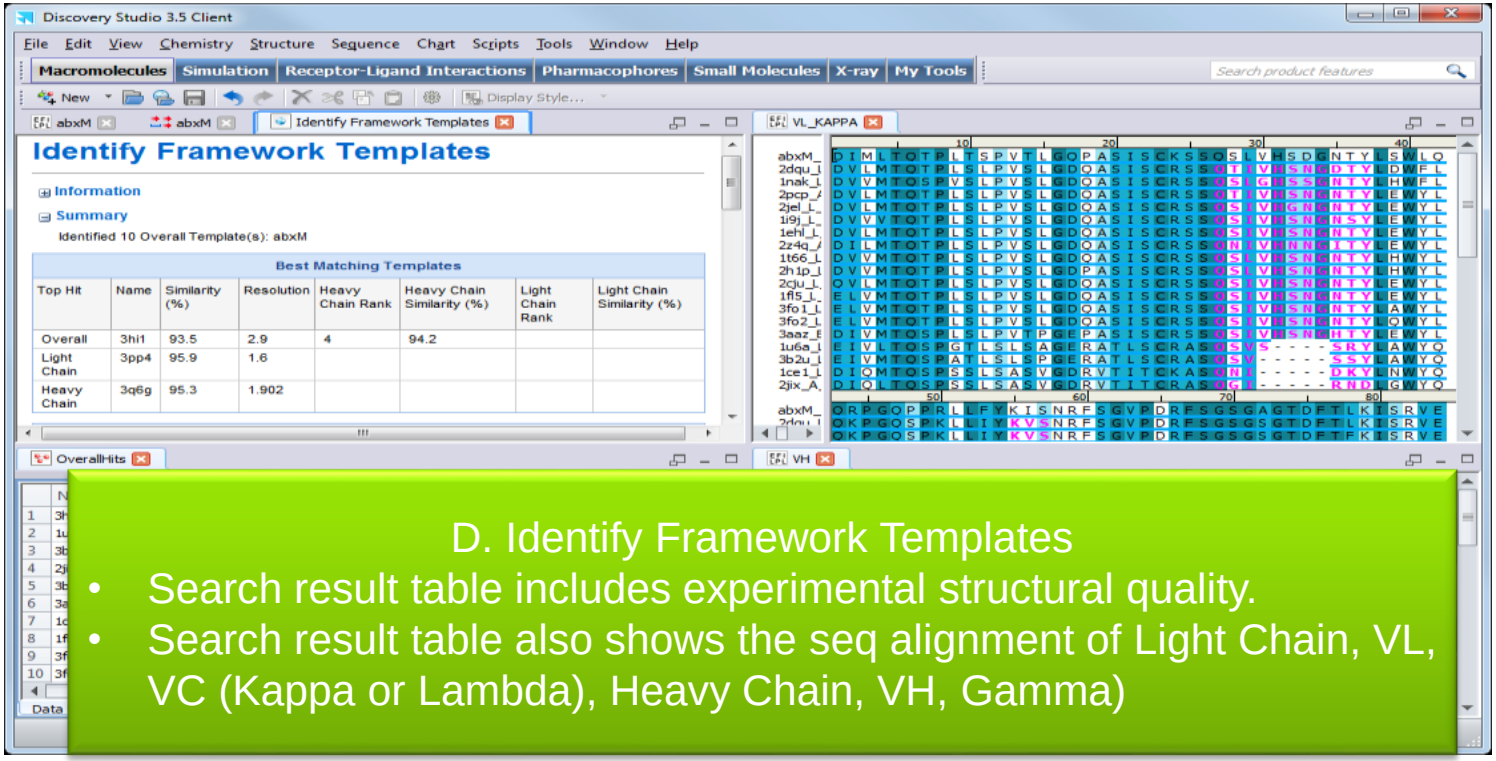
D. Identify Framework Templates

- Search template structure for antibody modeling
- It is possible to search template structure for each chain
- Homology CDR regions can also be excluded from the ranking of the template structure

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1. Structure Template Identification



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1. Structure Template Identification

- Template includes…
  - Heavy chain
  - Light chain
  - Interface
- How to choose a good template?
  - Homology (identity and similarity)
  - Experimental structure quality
  - Alignment results

GIGO - Garbage in, garbage out

- If you use bad template, the bad model structure obtained.
- Determine template structure is very important!

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erved.

Fab Antibody Modeling Workflow

1.Structure Template Identification (Antibodies, Fv, Fab, IgG) Annotate Antibody Sequence Identify Framework Templates

2. Framework Model Generation
Model Antibody Framework from templates

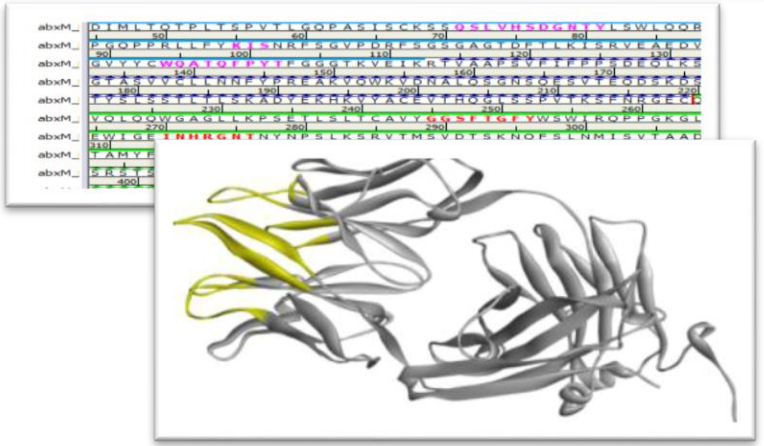
3. Analysis & Selection of Framework Model(s) Evaluate quality of templates

4. Loop Refinement (CDR) Model Antibody Loops

5. *De Novo* Loop Modeling (Optional)
Loop Refinement

Chimeric Method

Use H, L chain templates respectively



Single Template Method Homology modeling workflow

A. Align Sequence to Templates
B. Build Homology Models

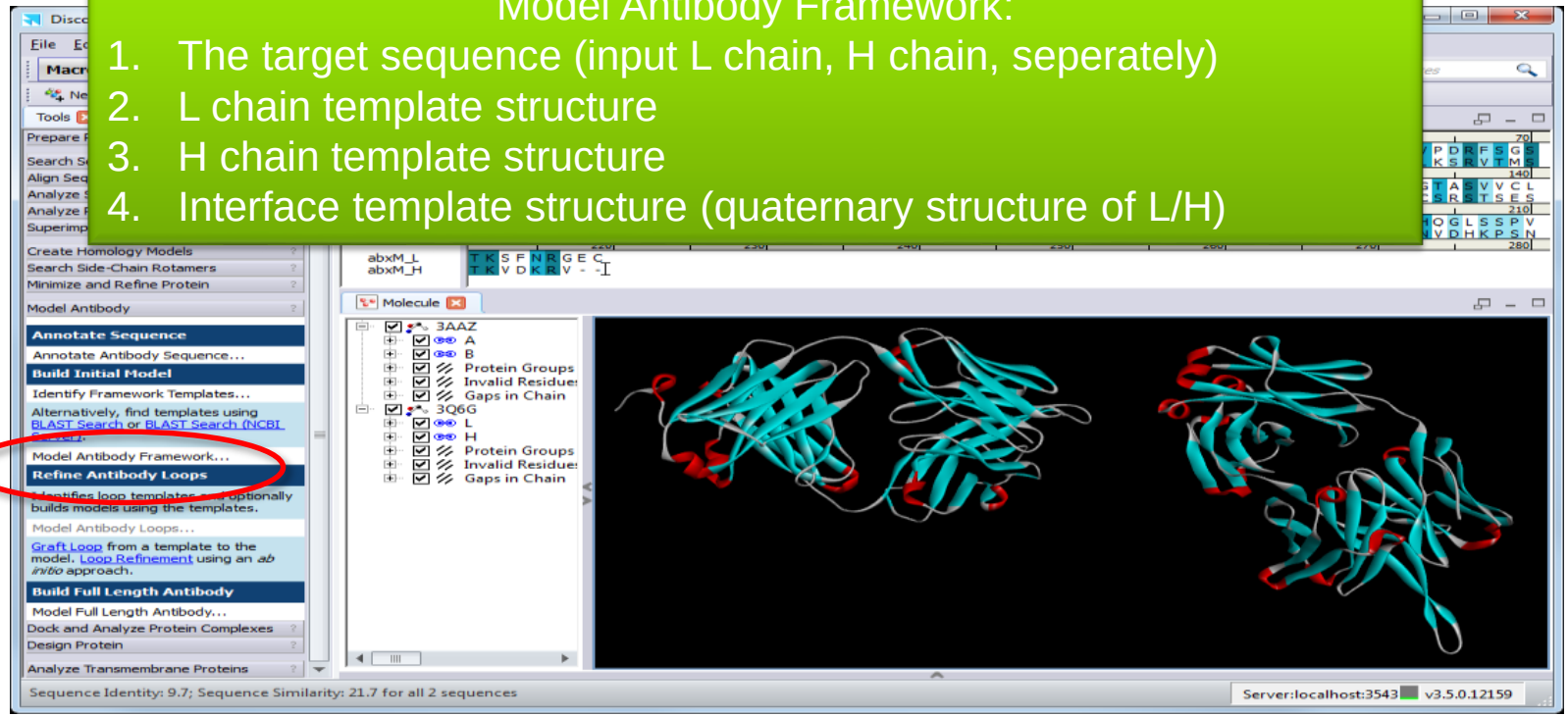
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ved.

2. Framework Model Generation

Model Antibody Framework:

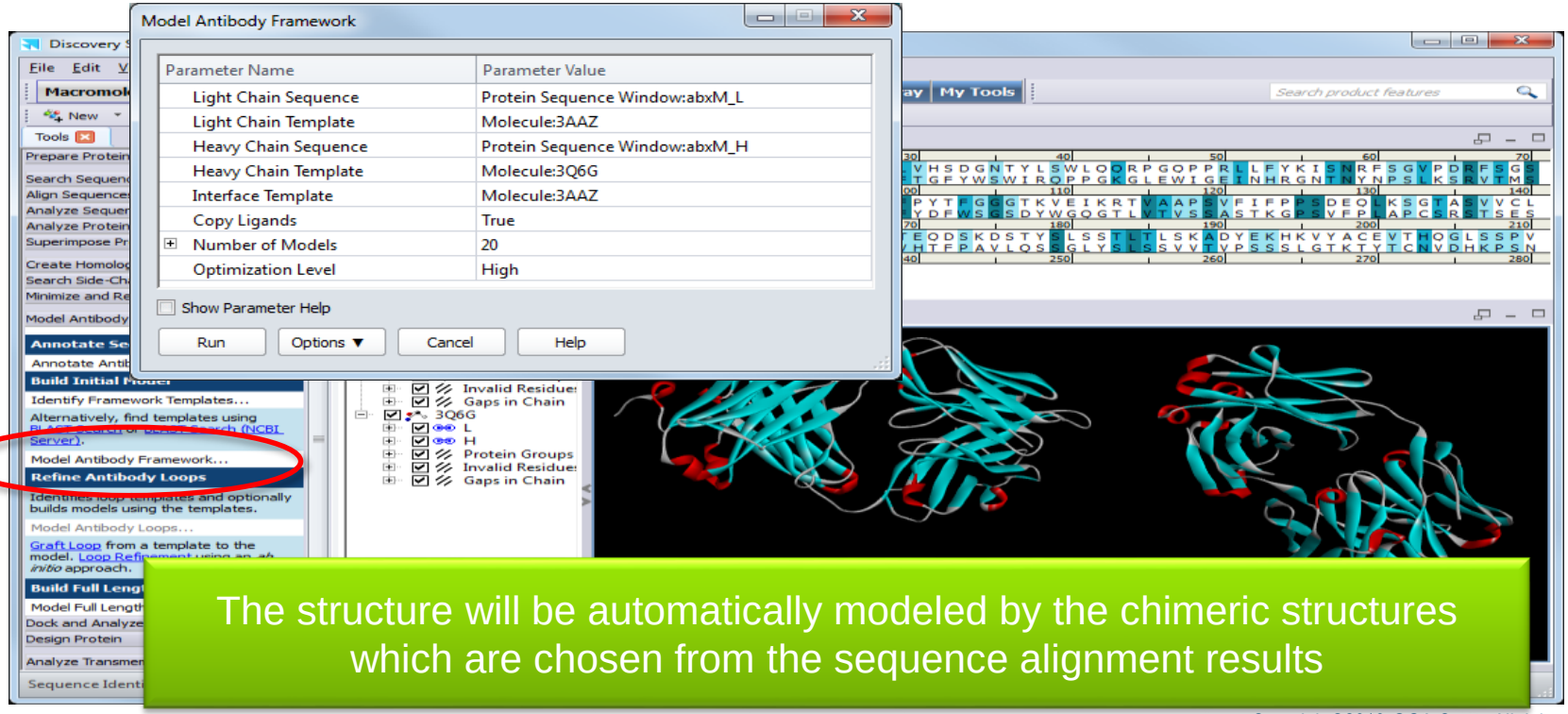
1. The target sequence (input L chain, H chain, seperately)
2. L chain template structure
3. H chain template structure
4. Interface template structure (quaternary structure of L/H)



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2. Framework Model Generation

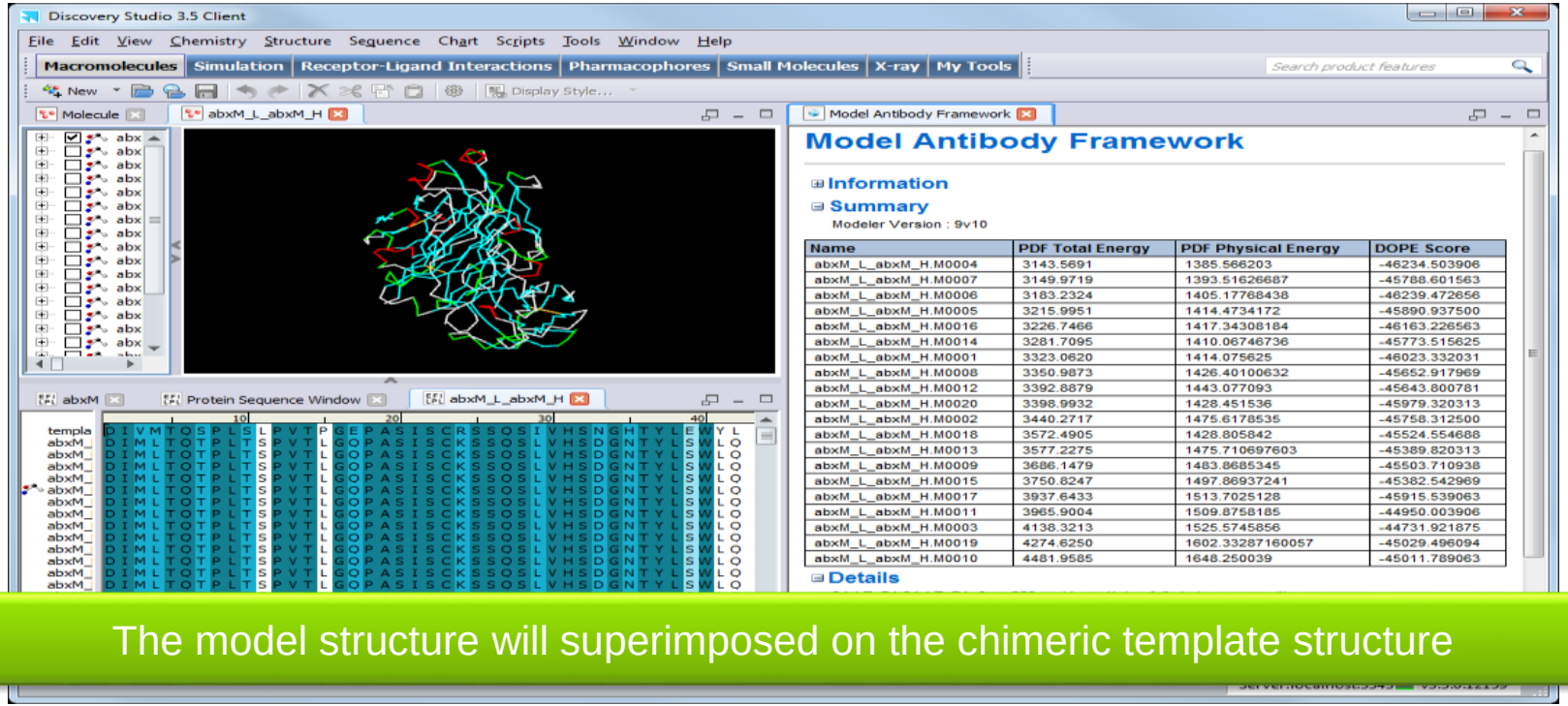


The structure will be automatically modeled by the chimeric structures which are chosen from the sequence alignment results

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2. Framework Model Generation



The model structure will superimposed on the chimeric template structure

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Fab Antibody Modeling Workflow

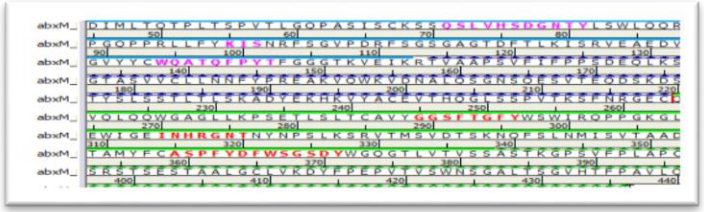
1.Structure Template Identification (Antibodies, Fv, Fab, IgG) Annotate Antibody Sequence Identify Framework Templates

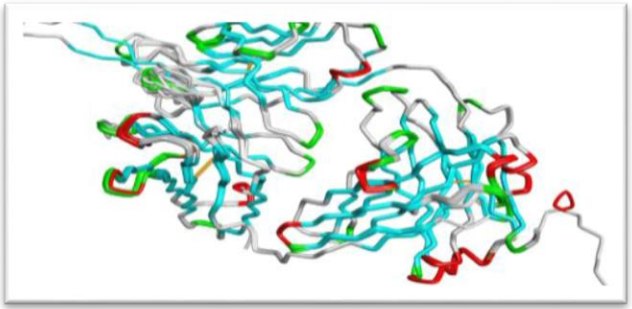
2. Framework Model Generation
Model Antibody Framework from templates

3. Analysis & Selection of Framework Model(s) Evaluate quality of templates

4. Loop Refinement (CDR) Model Antibody Loops

5. *De Novo* Loop Modeling (Optional)
Loop Refinement





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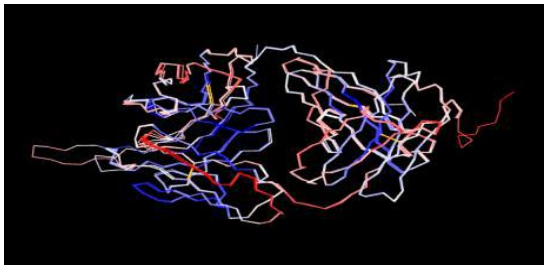
3. Analysis & Selection of Framework Models

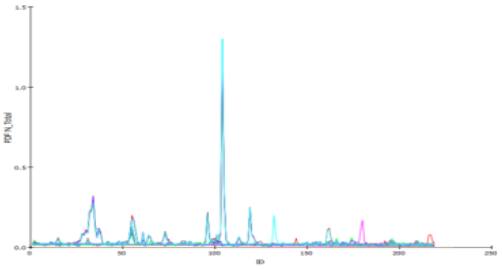
Select good model structure for optimization of the CDR regions

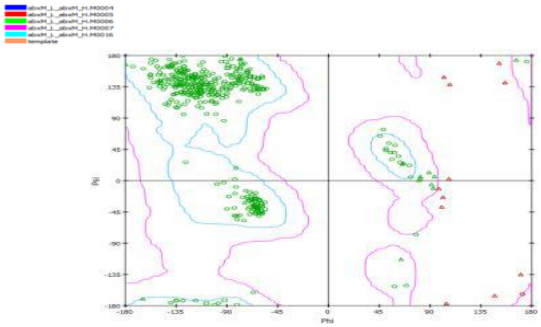
You can validate model quality by…

1. PDF energy
2. Verify protein
3. Ramachandron plot

for overall structure or heavy chain, light chain







Name	Verify Score	Verify Expected High Score	Verify Expected Low Score
abxM_L_abxM_H.M0004	202.17	198.992	89.5463
abxM_L_abxM_H.M0007	212.22	198.992	89.5463
abxM_L_abxM_H.M0006	213.25	198.992	89.5463
abxM_L_abxM_H.M0005	213.36	198.992	89.5463
abxM_L_abxM_H.M0016	213.86	198.992	89.5463
template	225.15	211.416	95.1373

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Fab Antibody Modeling Workflow

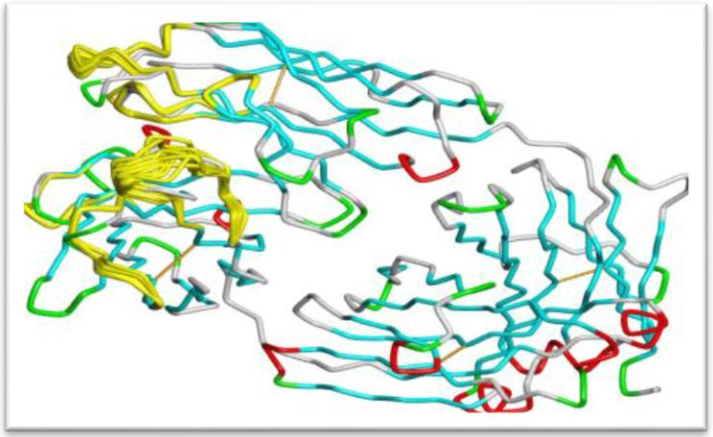
1.Structure Template Identification (Antibodies, Fv, Fab, IgG) Annotate Antibody Sequence Identify Framework Templates

2. Framework Model Generation
Model Antibody Framework from templates

3. Analysis & Selection of Framework Model(s) Evaluate quality of templates

4. Loop Refinement (CDR) Model Antibody Loops

5. *De Novo* Loop Modeling (Optional)
Loop Refinement

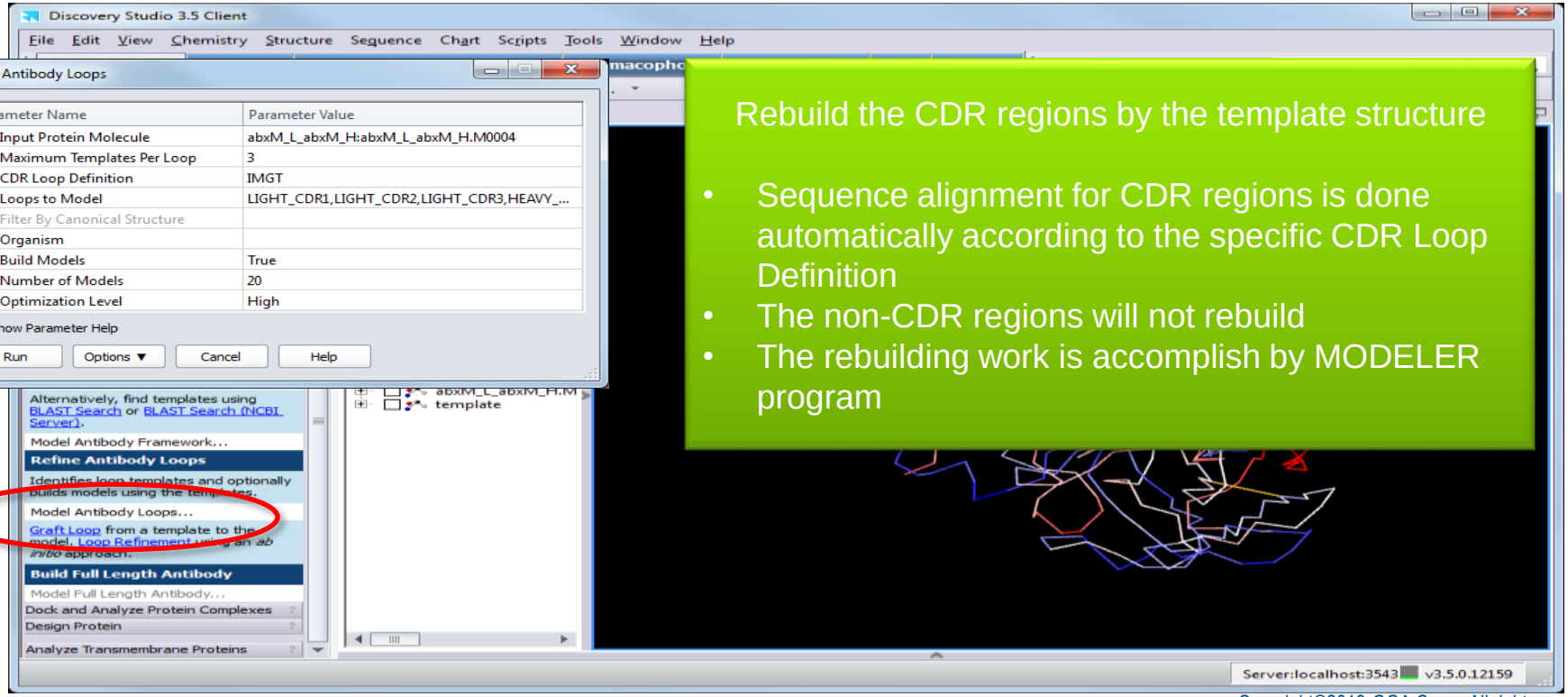


Accuracy of CDR regions determine the success or failure of antibody modeling

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4. Loop Refinement (CDR)



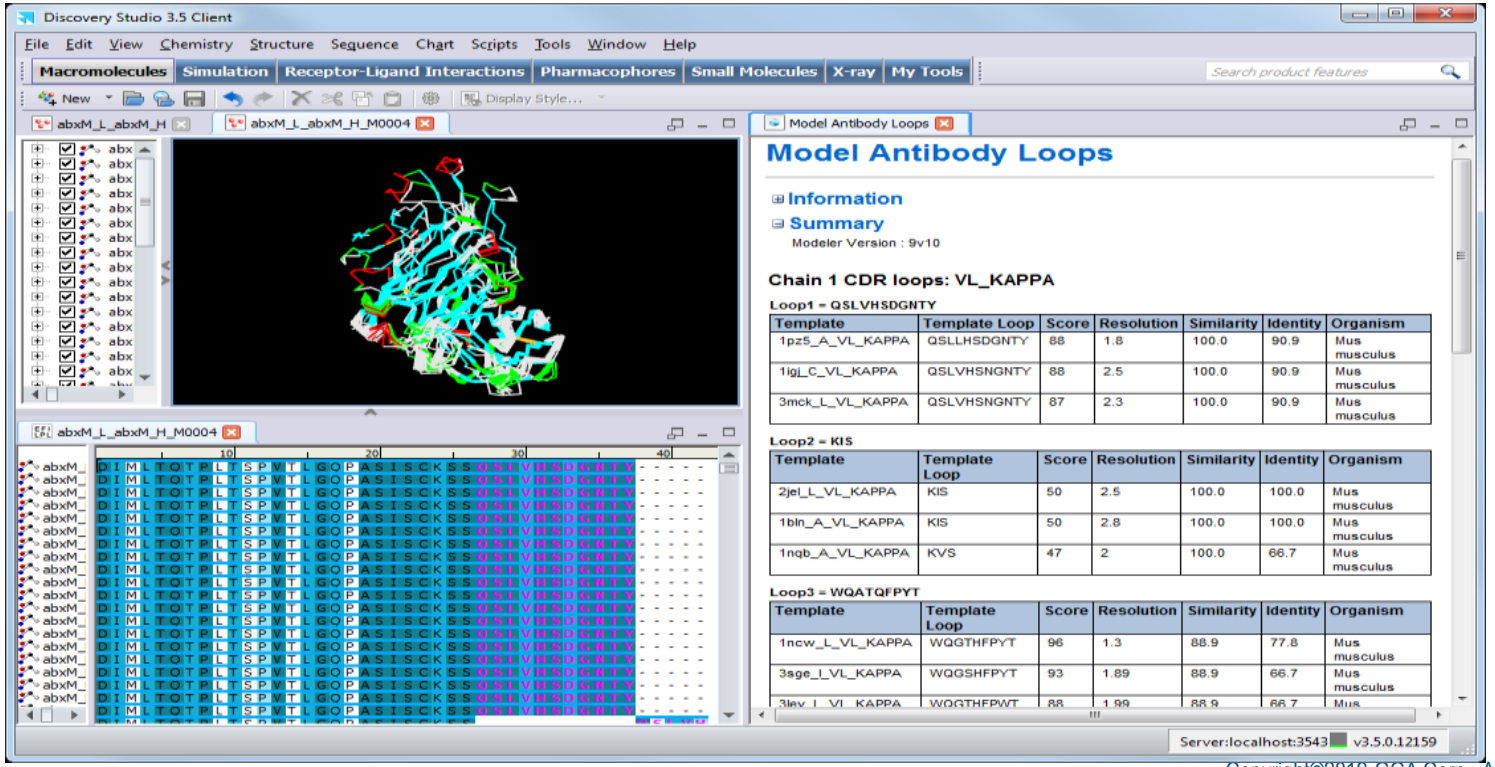
Rebuild the CDR regions by the template structure

- Sequence alignment for CDR regions is done automatically according to the specific CDR Loop Definition
- The non-CDR regions will not rebuild
- The rebuilding work is accomplish by MODELER program

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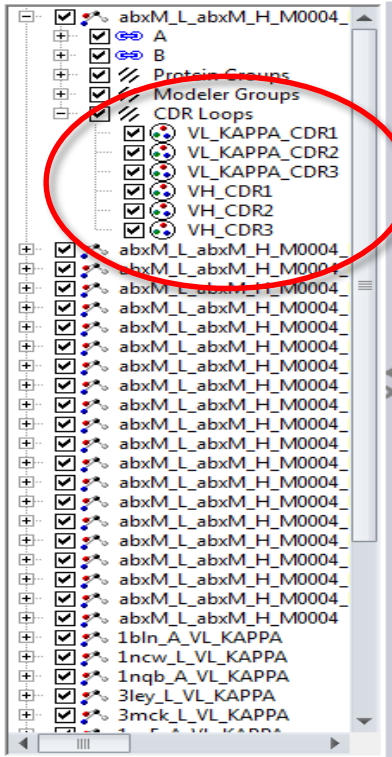
4. Loop Refinement (CDR)

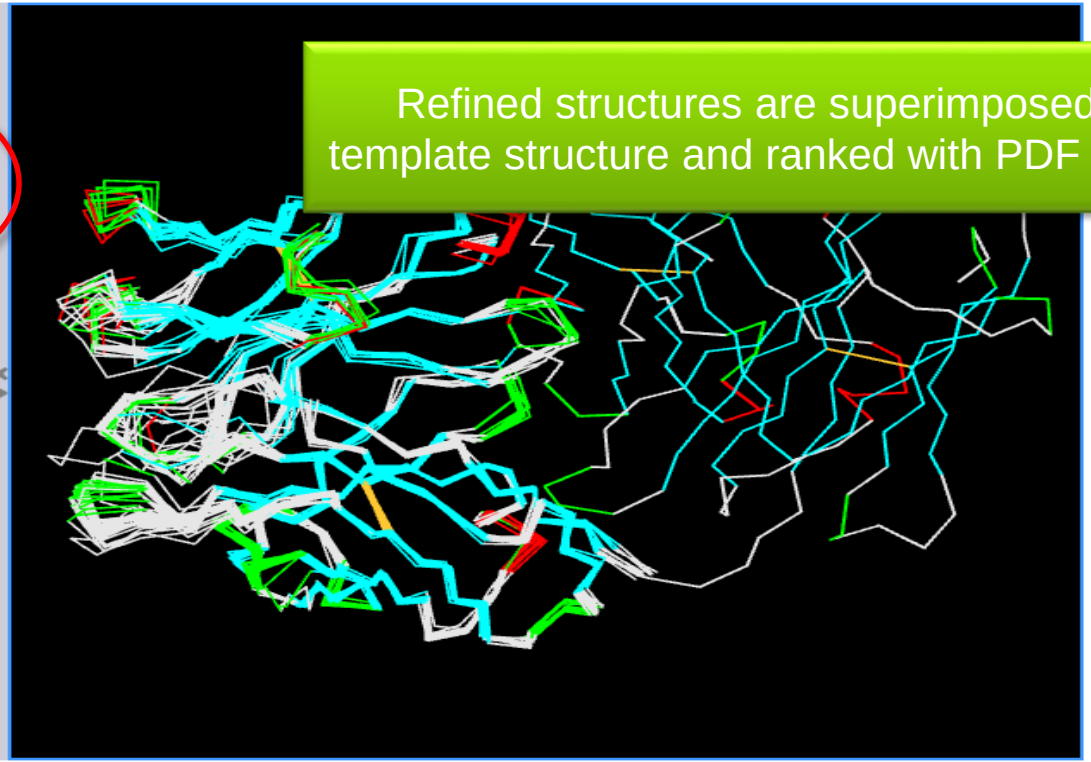


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4. Loop Refinement (CDR)





Refined structures are superimposed on template structure and ranked with PDF energy

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4. Loop Refinement (CDR)

- How to determine the “better” loop structure
  - PDF, Verify Score, Ramachandran Plot
  - Compare with template structures
  - Compare with canonical structures
- If the better one is not available…
  - Find other templates for antibody loop modeling
  - Chose single template for antibody loop modeling
  - Use *de novo* method

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Fab Antibody Modeling Workflow

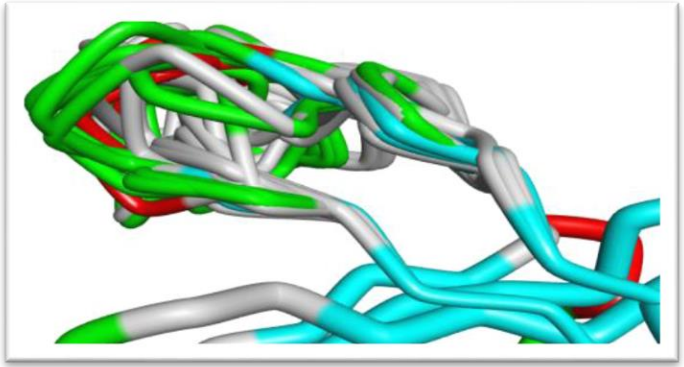
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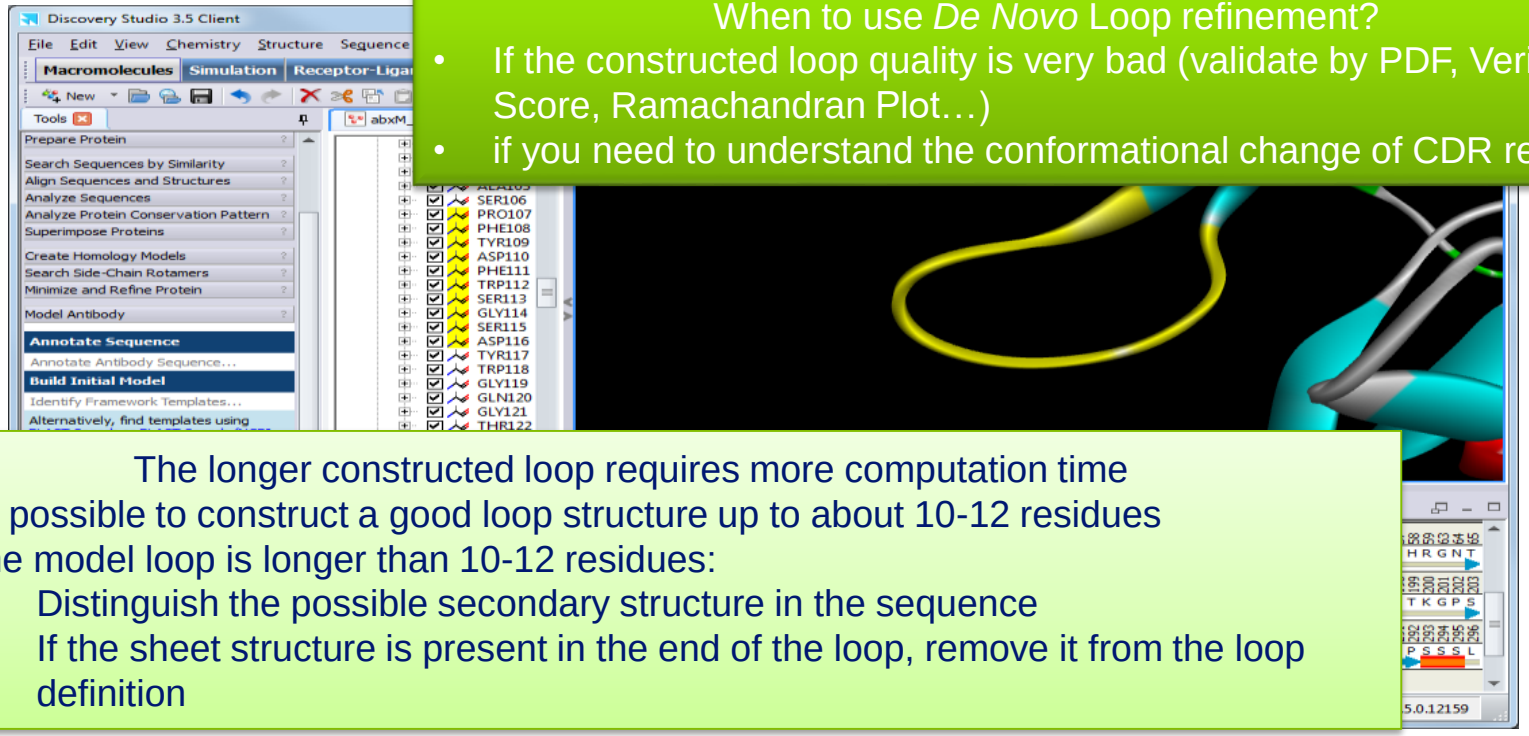
5. *De Novo* Loop Modeling (Optional)
Loop Refinement



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5. De Novo Loop Modeling (Optional)



When to use *De Novo* Loop refinement?

- If the constructed loop quality is very bad (validate by PDF, Verify Score, Ramachandran Plot…)
- if you need to understand the conformational change of CDR regions

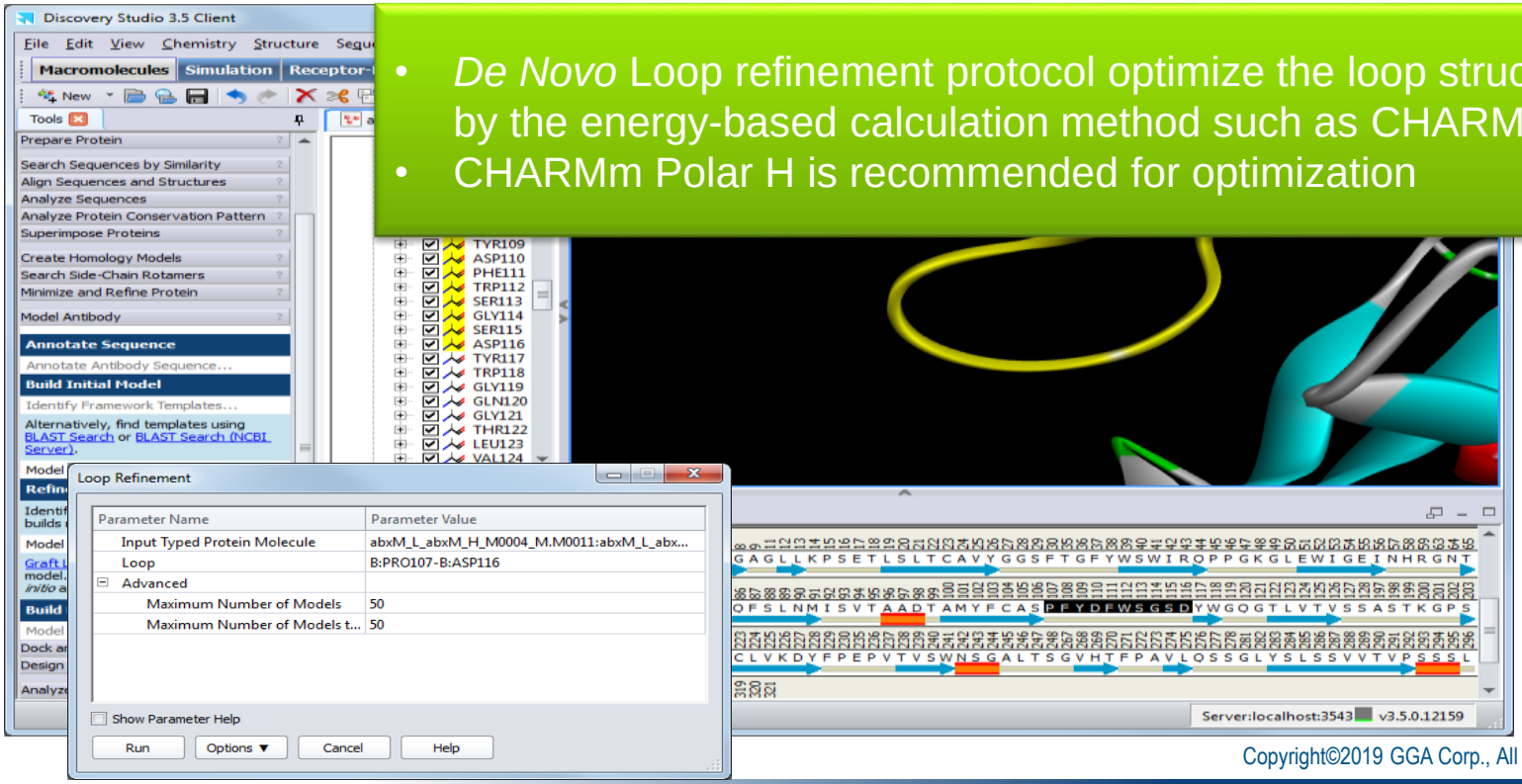
The longer constructed loop requires more computation time

- It is possible to construct a good loop structure up to about 10-12 residues
- If the model loop is longer than 10-12 residues:
  - Distinguish the possible secondary structure in the sequence
  - If the sheet structure is present in the end of the loop, remove it from the loop definition

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5. De Novo Loop Modeling (Optional)



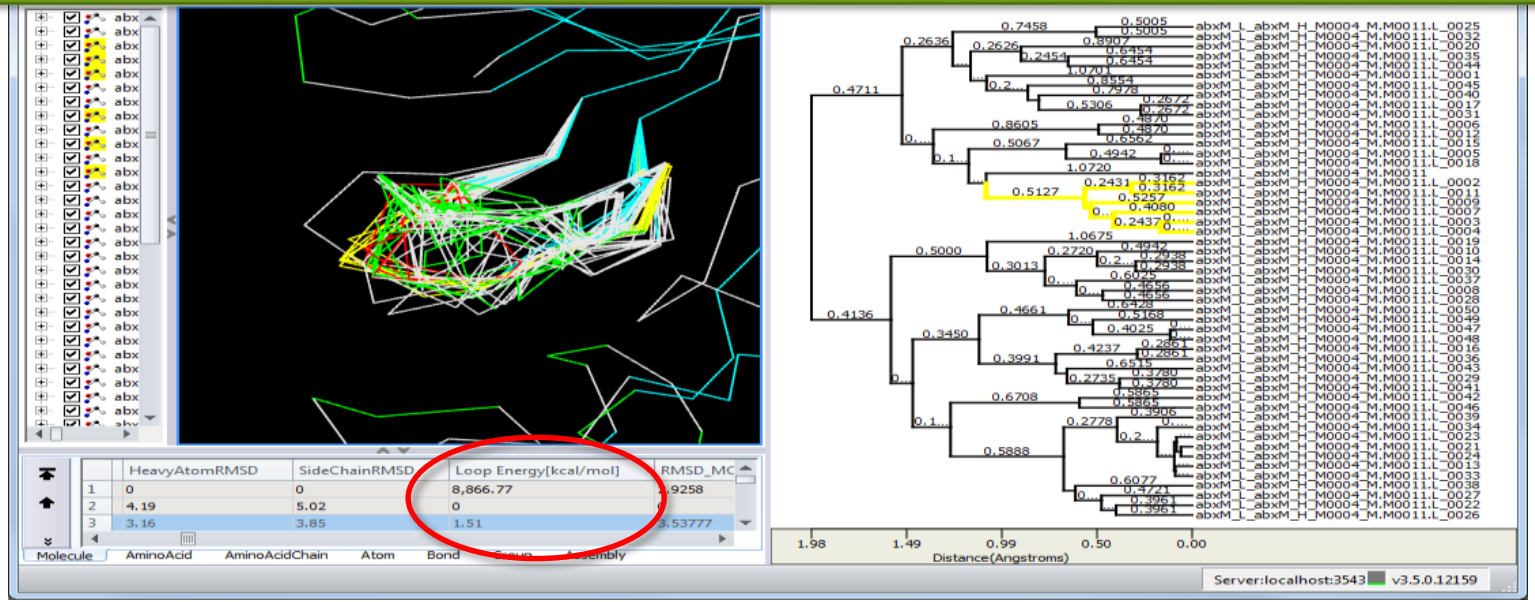
- *De Novo* Loop refinement protocol optimize the loop structure by the energy-based calculation method such as CHARMm
- CHARMm Polar H is recommended for optimization

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5. De Novo Loop Modeling (Optional)

- All of the loop refined models are ranked with energy
- The clustering result (RMSD of backbone Ca atom) is displayed in the dendrogram



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Fab Antibody Modeling Workflow

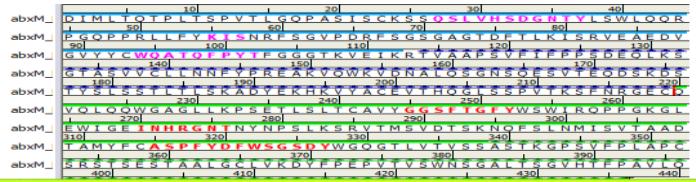
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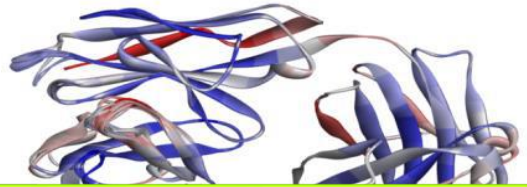
3. Analysis & Selection of Framework Model(s) Evaluate quality of templates

4. Loop Refinement (CDR) Model Antibody Loops

5. *De Novo* Loop Modeling (Optional)
Loop Refinement



Antibody Framework modeling:
1. Chimeric Method
2. Single Template Method



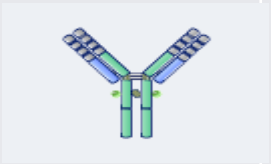
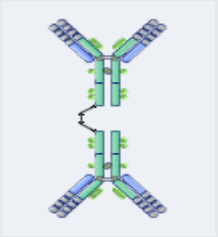
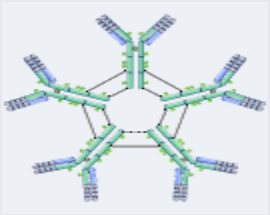
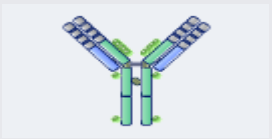
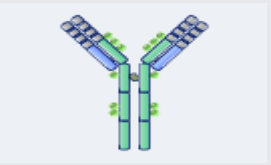
Refine loops refer to the similar template structure

Refine loops by *de novo* method

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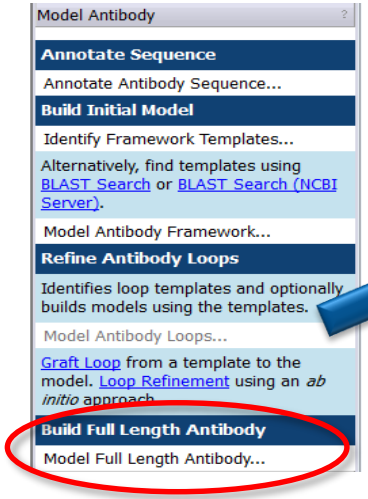
Antibody Types

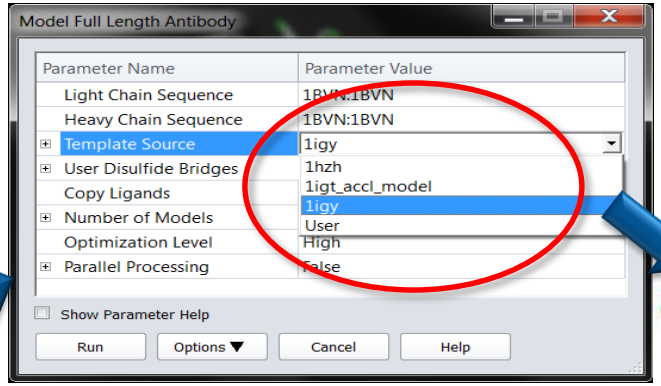
	IgG	IgA	IgM	IgD	IgE
% of serum antibodies	80	13	6	1	0.002
Average serum half-life (days)	23	6	5	3	2.5
Notes	• 初次免疫的二次抗體 • 長期免疫	• 存在於血液、黏膜中(唾液、眼淚、母乳中) • 保護呼吸器官與腸胃道	• 初次免疫的一次抗體	• B細胞膜表面上的結合受體	• 發炎和過敏反應因子 • 嗜鹼性球和肥大細胞表面的過敏原
Structure	Monomer 	Dimer, Monomer 	Pentamer 	Monomer 	Monomer 

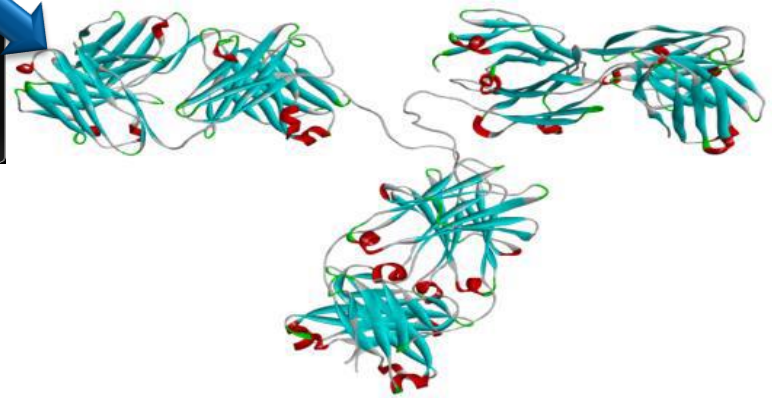
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Model Full Length Antibody

In Discovery Studio, full length antibody modeling with Fv and Fab is possible



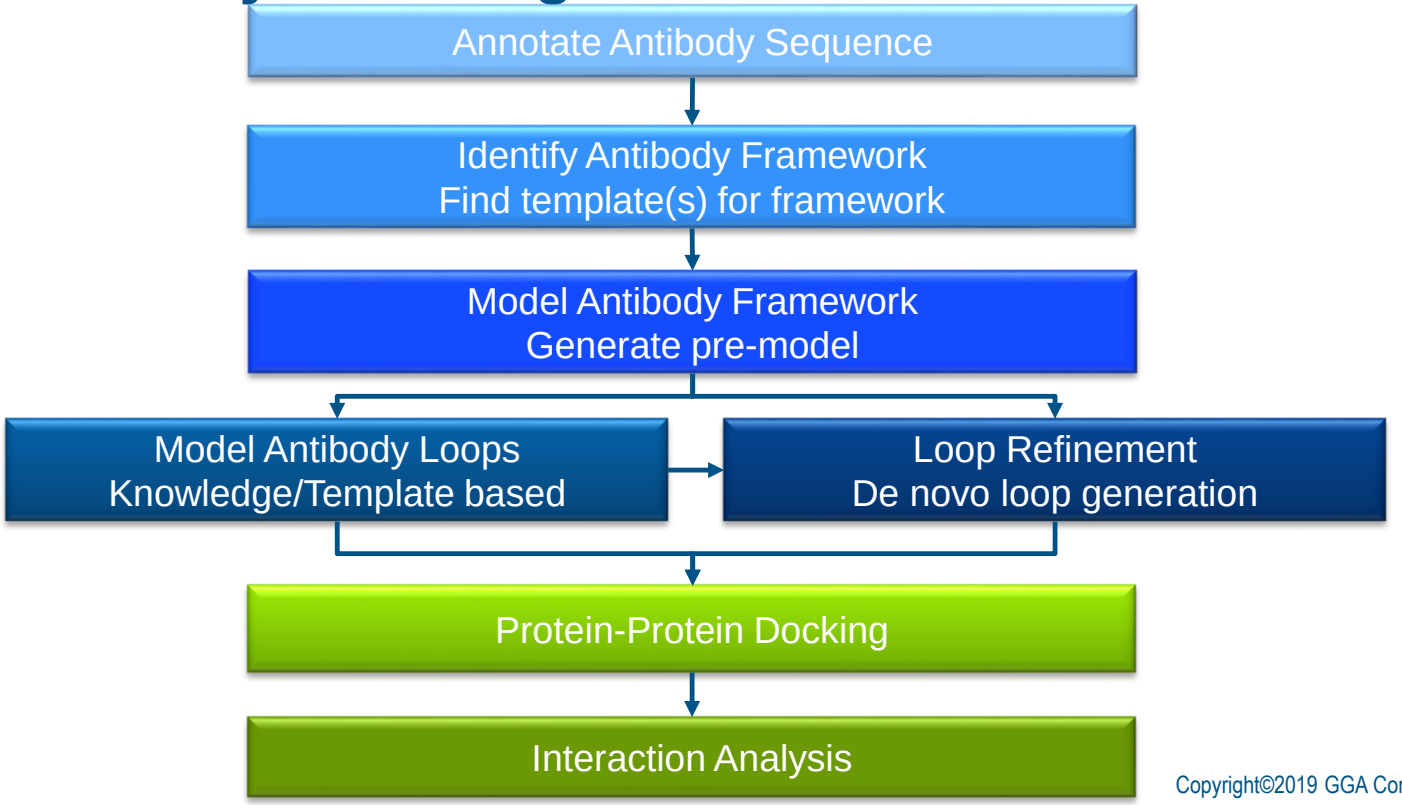




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Fab Antibody Modeling Workflow

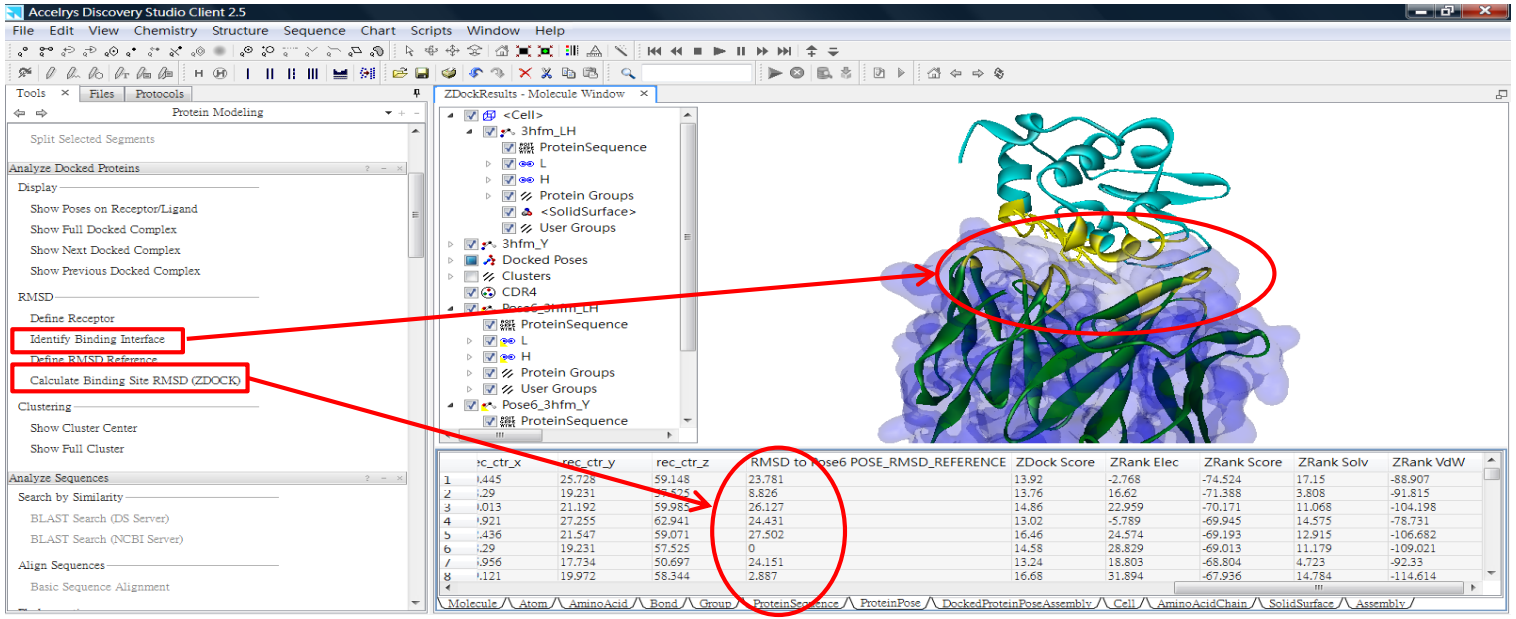


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Antibody Docking

- Define binding site residues (yellow region)
- Calculate RMSD (overall or binding site residues only)

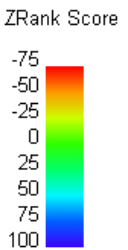


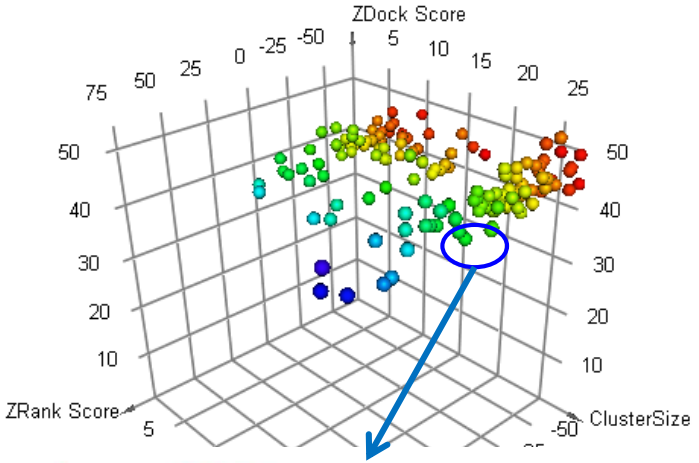
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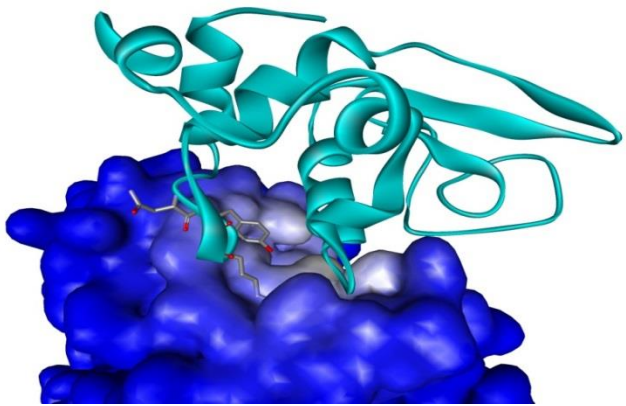
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Plots of the Data

- Data in the Data Table can be plotted
  - May help in better visualization of the data
- Look for
  - High ZRank scores
  - High ZDock scores
  - Large clusters
- 2D point plot of ClusterSize vs. ZRank Score
- 3D point plot of ClusterSize, ZDock Score, and ZRank Score







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Outline

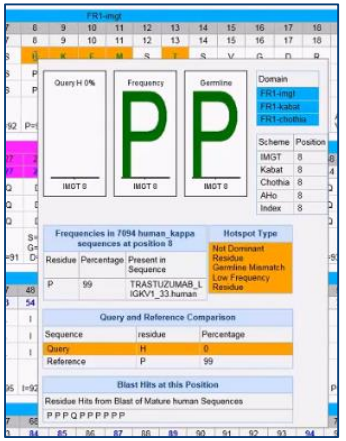
- 抗體結構模擬應用簡介
- 抗體結構建模
- 特性優化(Affinity Saturation)
- 人類化預測(Humanization)

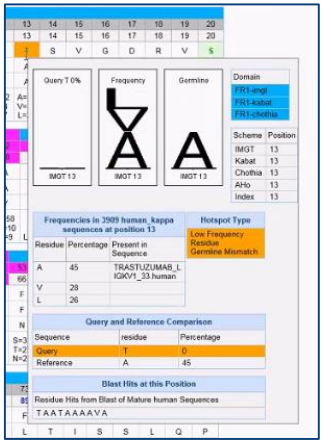
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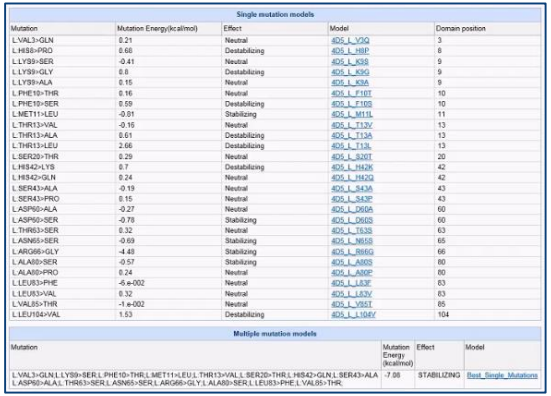
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Antibody Humanization Workflow

- Search query Light and Heavy chain antibody sequences for humanizing mutations
  - Search against reference germline databases
  - Optionally, compare to a reference sequence
- Highlight residue humanization hotspots
  - Sites where residue occurs in less than 5% frequency
    and do not match germlines
  - See humanizing residues *via* ‘logo plots’
  - Optionally, exclude CDR and Vernier regions
- Predict most stabilizing humanized mutations
  - Either supply a pre-built antibody structure model
  - Or, use the Antibody Modeling Cascade to automatically build a 3D model



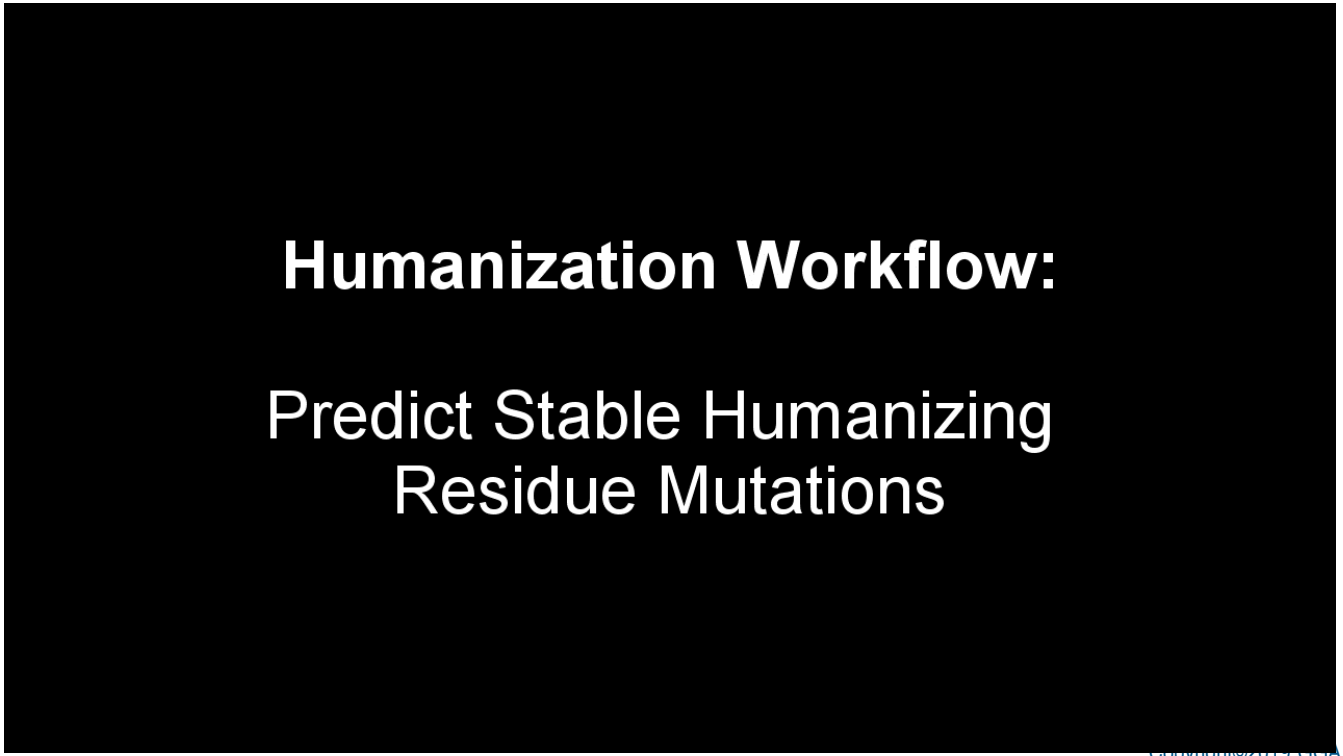




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Antibody Humanization Workflow



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Humanizing an antibody Fv sequence

- Case – Trastuzumab (Herceptin)
- Humanizing murine monoclonal antib
- Region: VH and VL



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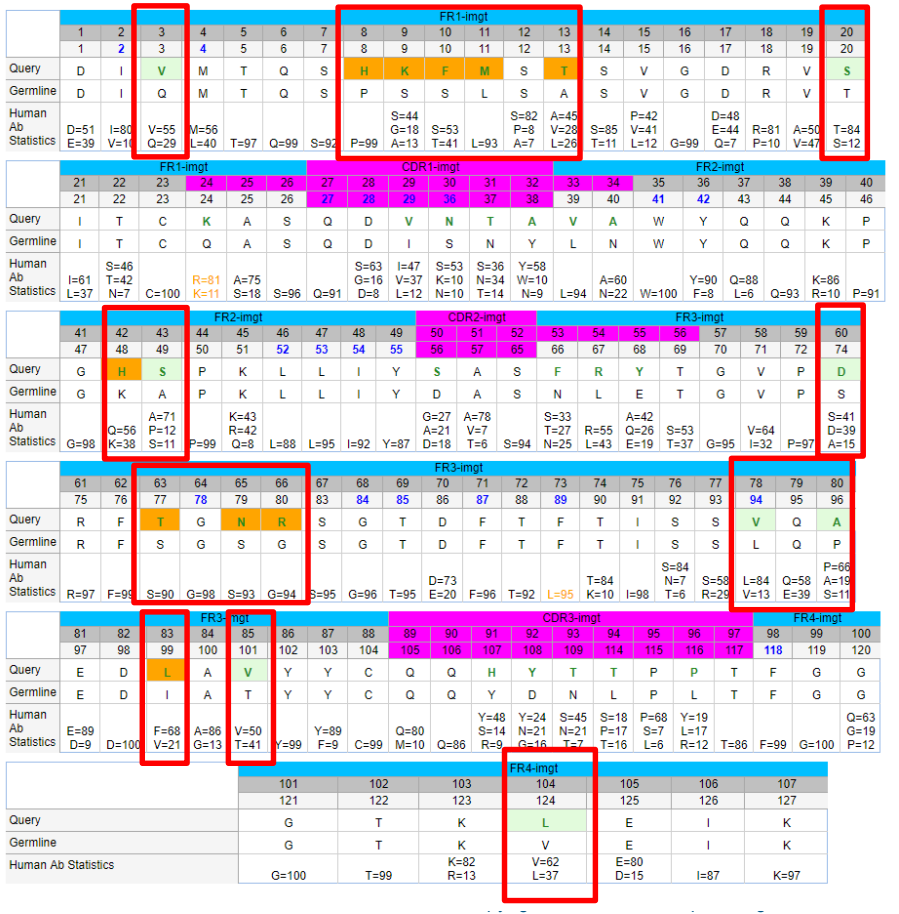
Example

- VL_Kappa

Residue Analysis report for 4D5 VL_KAPPA

Legend for Table	
Display Style	Description
Red Background	Heavy chain CDR region
Bright Green Background	Heavy chain framework region
Magenta Background	Light chain CDR region
Mid Blue Background	Light chain framework region
Dark Gray Background	Kabat scheme residue number
Light Gray Background	IMGT scheme residue number
Bold Blue IMGT position	Vernier zone residue
Orange Residue Background	Frequency less than 5% and not in CDR region
Light Green Residue Background	Query residue does not match germline and is not in the CDR region
Bold Dark Green Residue Label	Query residue does not match germline
Orange Frequency Labels	Neither query or germline matches dominant (frequency >75%) residue type

Legend for Tooltip	
Display Style	Description
Red Logo	Acidic Residue
Blue Logo	Basic Residue
Purple Logo	Uncharged Hydrophilic Residue
Black Logo	Hydrophobic Residue
Green Logo	Neutral Residue
Orange Background	Hotspot
Red Background	Heavy CDR residue
Bright Green Background	Heavy framework residue
Magenta Background	Light CDR residue
Mid Blue Background	Light framework residue

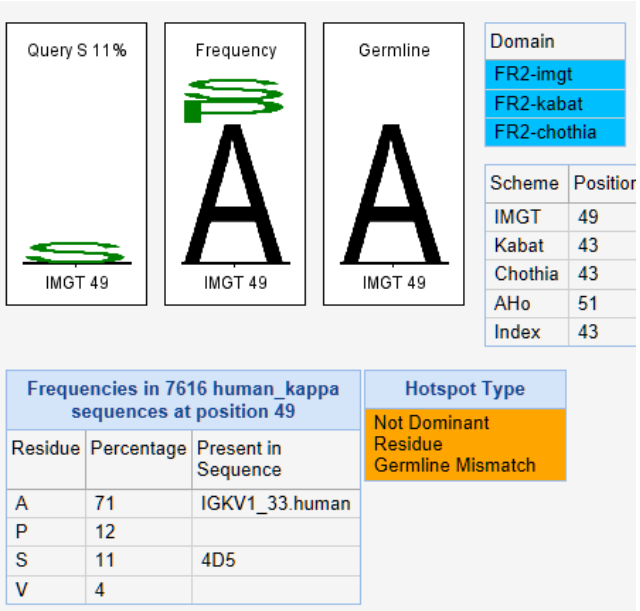


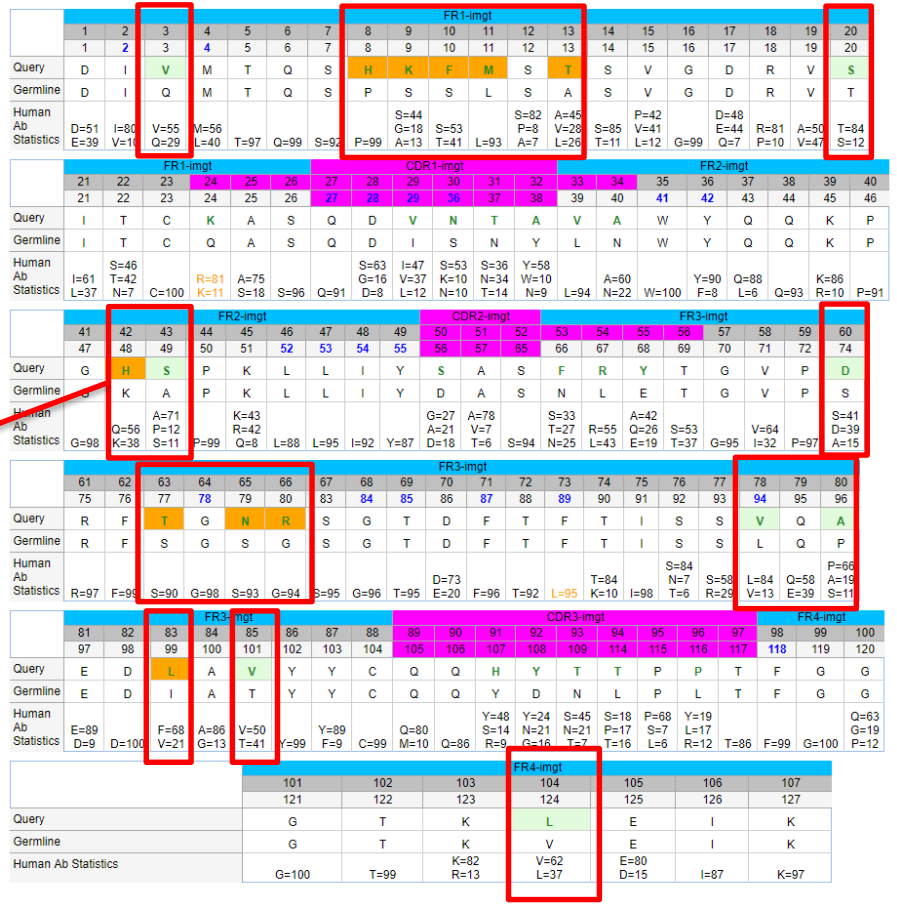
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Example

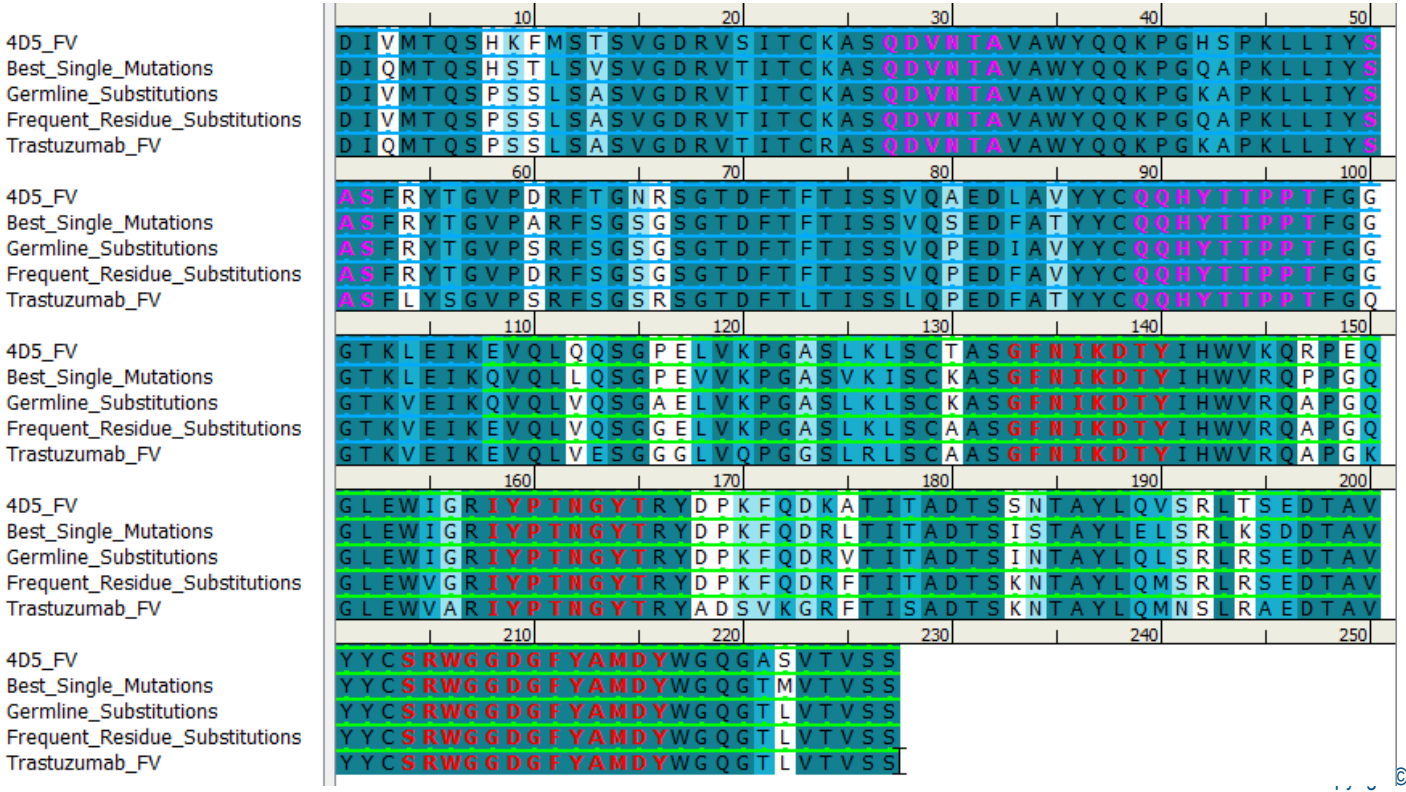
- VL_Kappa





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Result



Total Variants:59
Total Hits: 37
Best Model Hits: 11
Frequent Hit: 31

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Q&A

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