Overview

Knowledge based approaches driven by careful analysis

- Sequence and 3D structure based information
- Associated structure activity relationships
- Homology & other natural sequence variation

Improved research success

- Selectivity and specificity of compounds against targets
- Off-target candidate effects in the genome
- *Antibody humanness, anti-antibody response*

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Ligand-Protein Interaction Sites: Ligplot+

LigPlot+: Multiple Ligand–Protein Interaction Diagrams for Drug Discovery

Roman A. Laskowski*† and Mark B. Swindells‡
European Bioinformatics Institute, Wellcome Trust Genome Campus, Ebisu Ltd. United Kingdom

ACS Publications
MOST TRUSTED. MOST CITED. MOST READ.
Publications A-Z
JOURNAL OF CHEMICAL INFORMATION AND MODELING
J. Chem. Inf. Model. 2011, 51, 2778–2786

Enhancements over Ligplot include

- Allows multiple plots to be shown with binding sites oriented similarly
- User defined orientation
- Structurally conserved interactions automatically highlighted
- Distant homologue information can be applied
- Intuitive Java Interface

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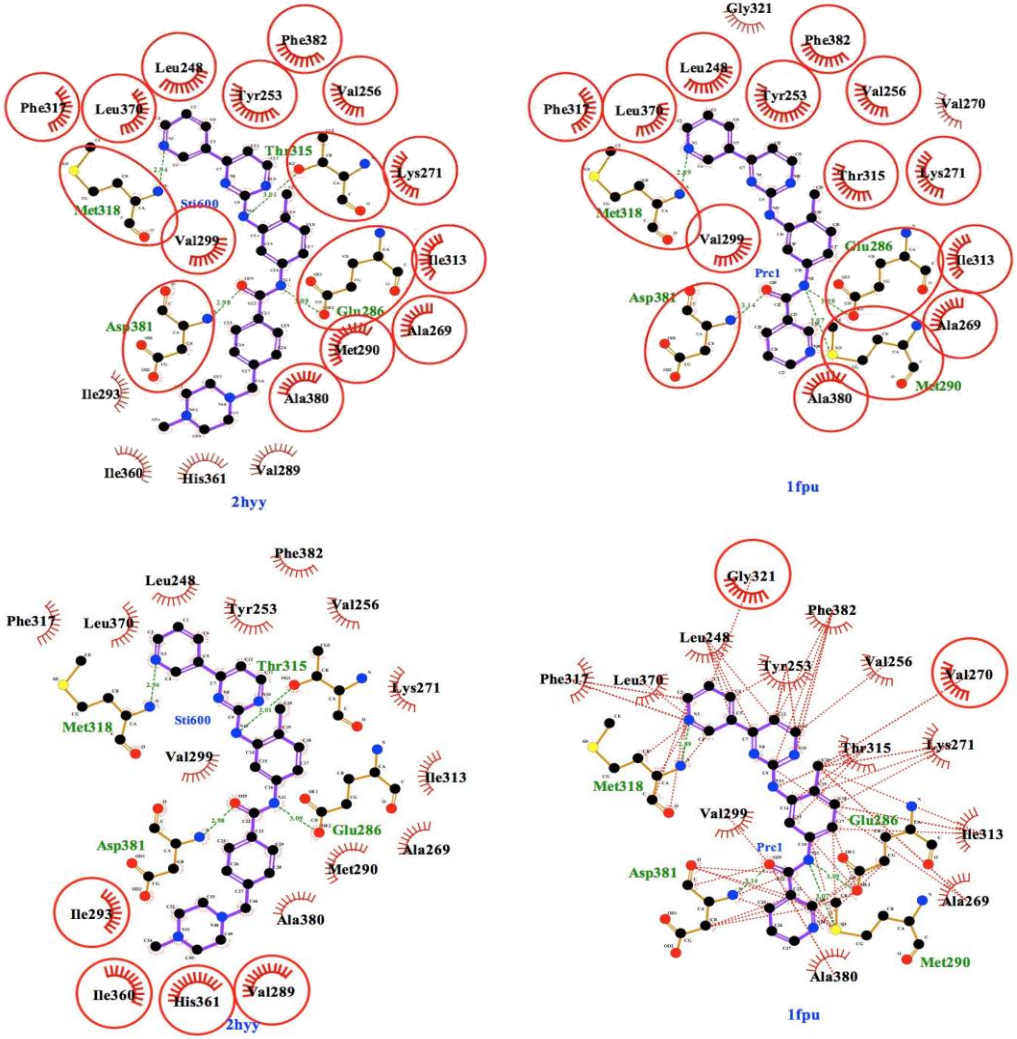
Ligplot+ Same protein different ligands

- c-abl bound to imatinib (PDB:2hyy) and compound PRC (PDB:1fpu)
- Tanimoto coefficient for ligands = 0.93

Ligplot automatically generates in same orientation.



Various display options

- Hydrogen Bonds shown in green
- Hydrophobic residues display as “eye lashes”

Top Diagram

- **Conserved** interactions highlighted as circles.

Bottom Diagram

- **Unconserved** interactions highlighted
- Right-hand diagram also displays hydrophobic interactions as red lines

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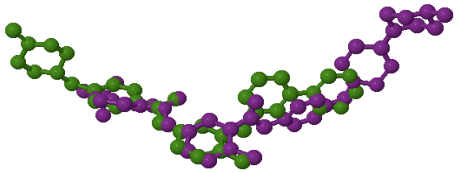
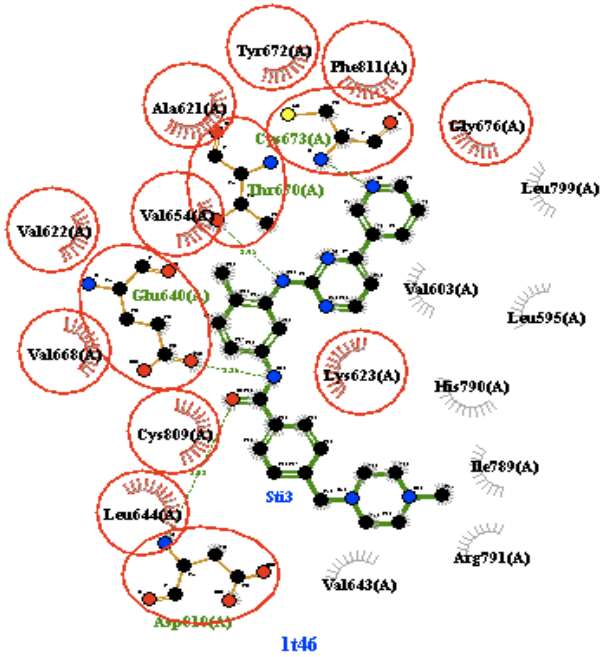
Ligplot+: Homologous proteins with different ligands

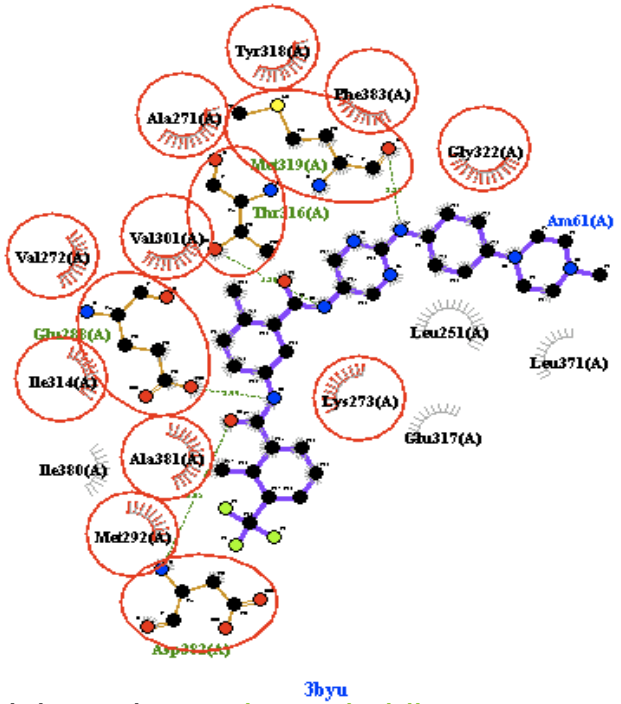
Ligplot conveys both ligand/protein similarity and 3D orientation.



Tanimoto coefficient = 0.85
Protein Sequence Identity = 37%



c-kit bound to imatinib (PDB:1t46)



lck bound to aminopyrimidine reverse amide (PDB:3byu)

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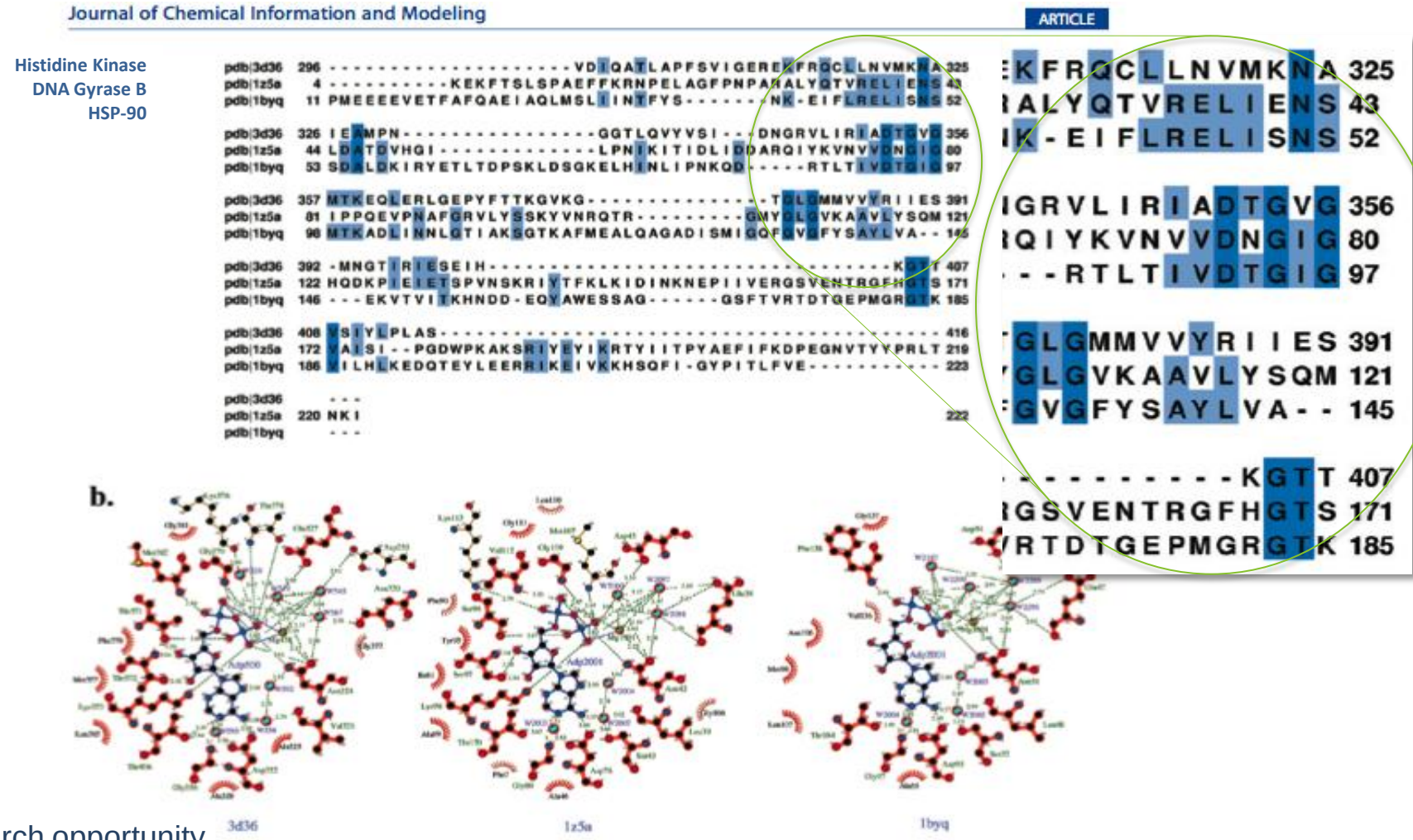
Ligplot+: Very Distant (<20% ID) Homologues



Research opportunity

Identify distant homologues.

Identify compounds in ChEMBL

Screen existing compounds or preferred fragments against your new distant homologue.

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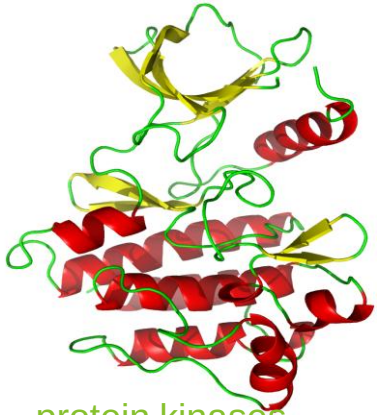
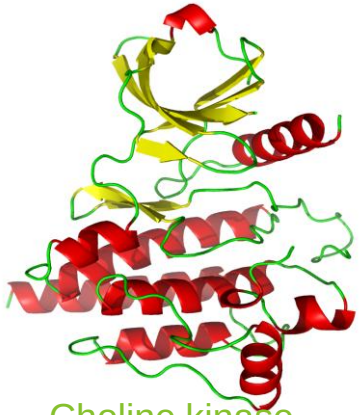
Exploring Distant Homologues

Protein Kinase Superfamily, other kinases, aminoglycoside phosphotransferase

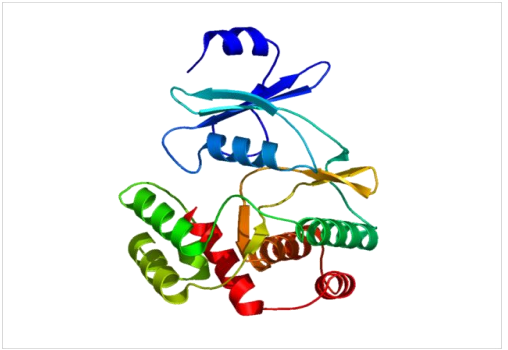
c-abl, syk and c-kit (below) are therapeutically relevant protein kinases



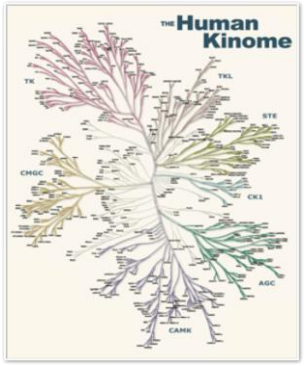
protein kinases

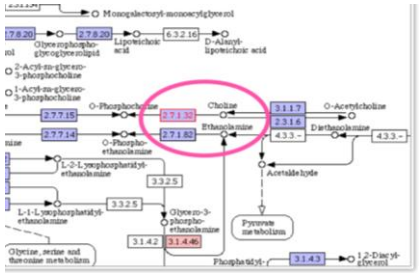


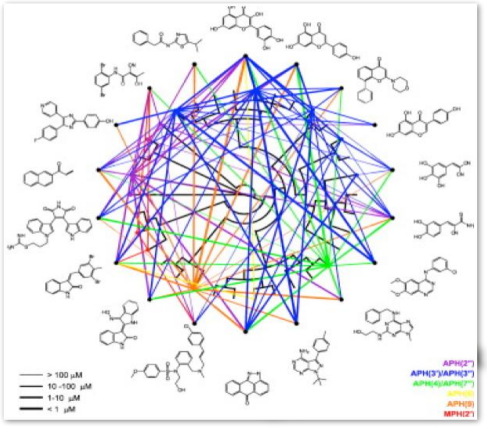
Choline kinase
Ethanolamine kinase



aminoglycoside
phosphotransferase







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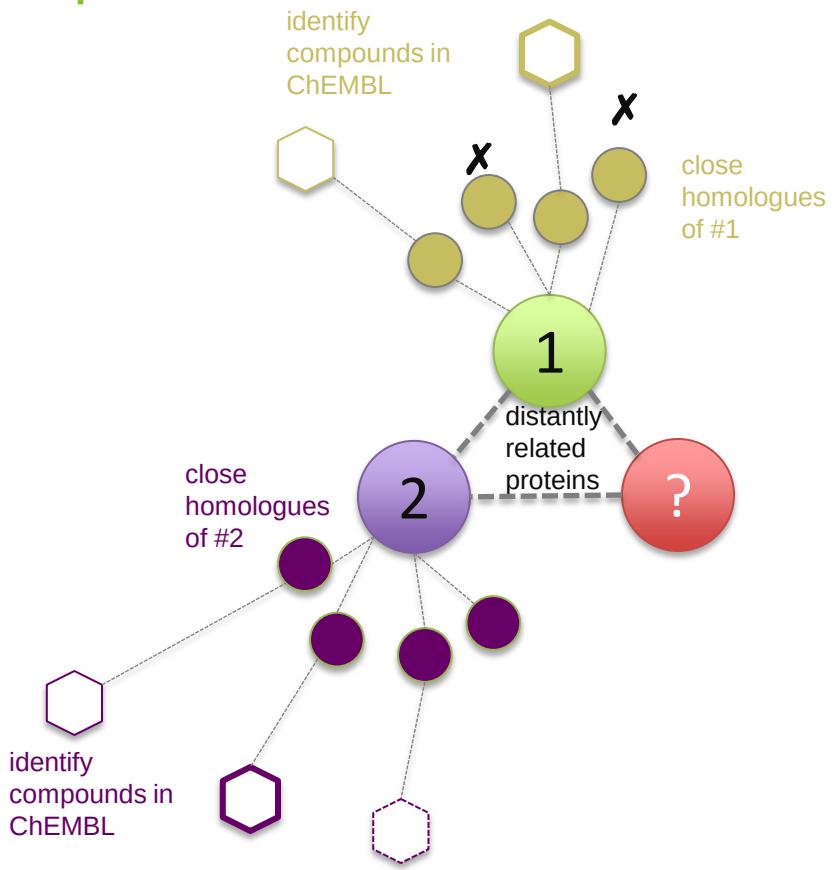
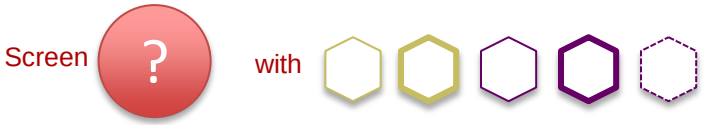
Knowledge-based screening

Step 1



Step 2



or generate knowledge based preferred fragments

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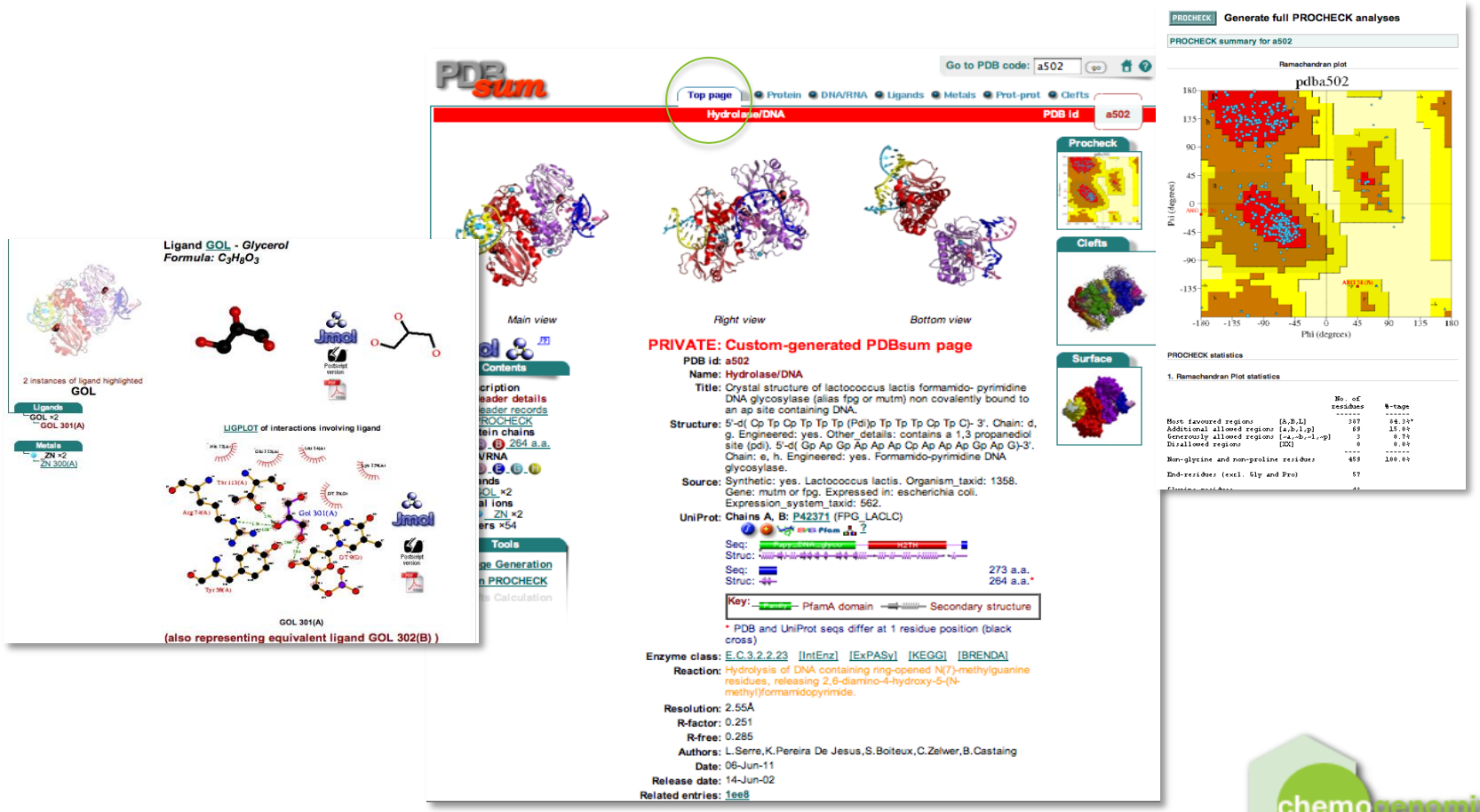
PDBsum*Proprietary* for an in-house Electronic Lab Notebook

- Registration, Storage, Dissemination of *in-house* protein structure complexes
- Built upon multiple integrated algorithms.



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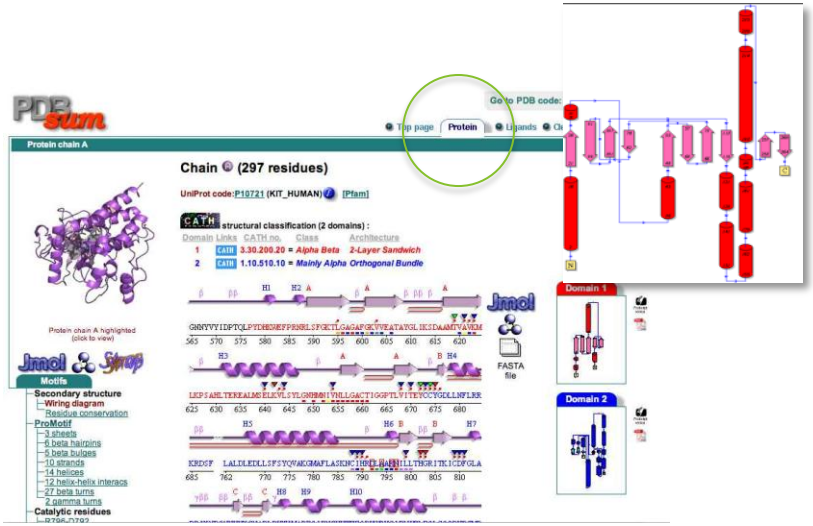
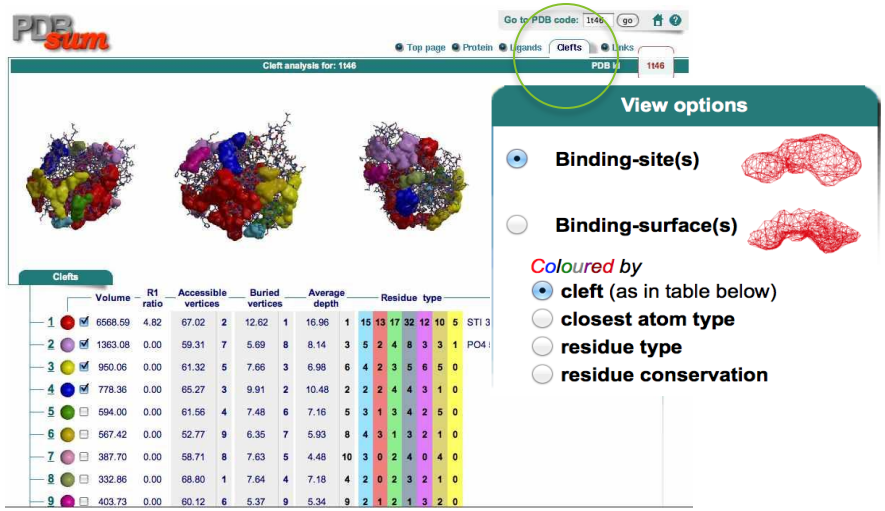
PDBsum*Proprietary*

After installing PDBsum*Proprietary* system at your site, load your proprietary PDB formatted structures, together with appropriate Uniprot reference code for each protein structure.

PDBsum will then automatically generate all necessary pages.



Secondary structure and topology overview page



Cleft overview page generated using surfnet

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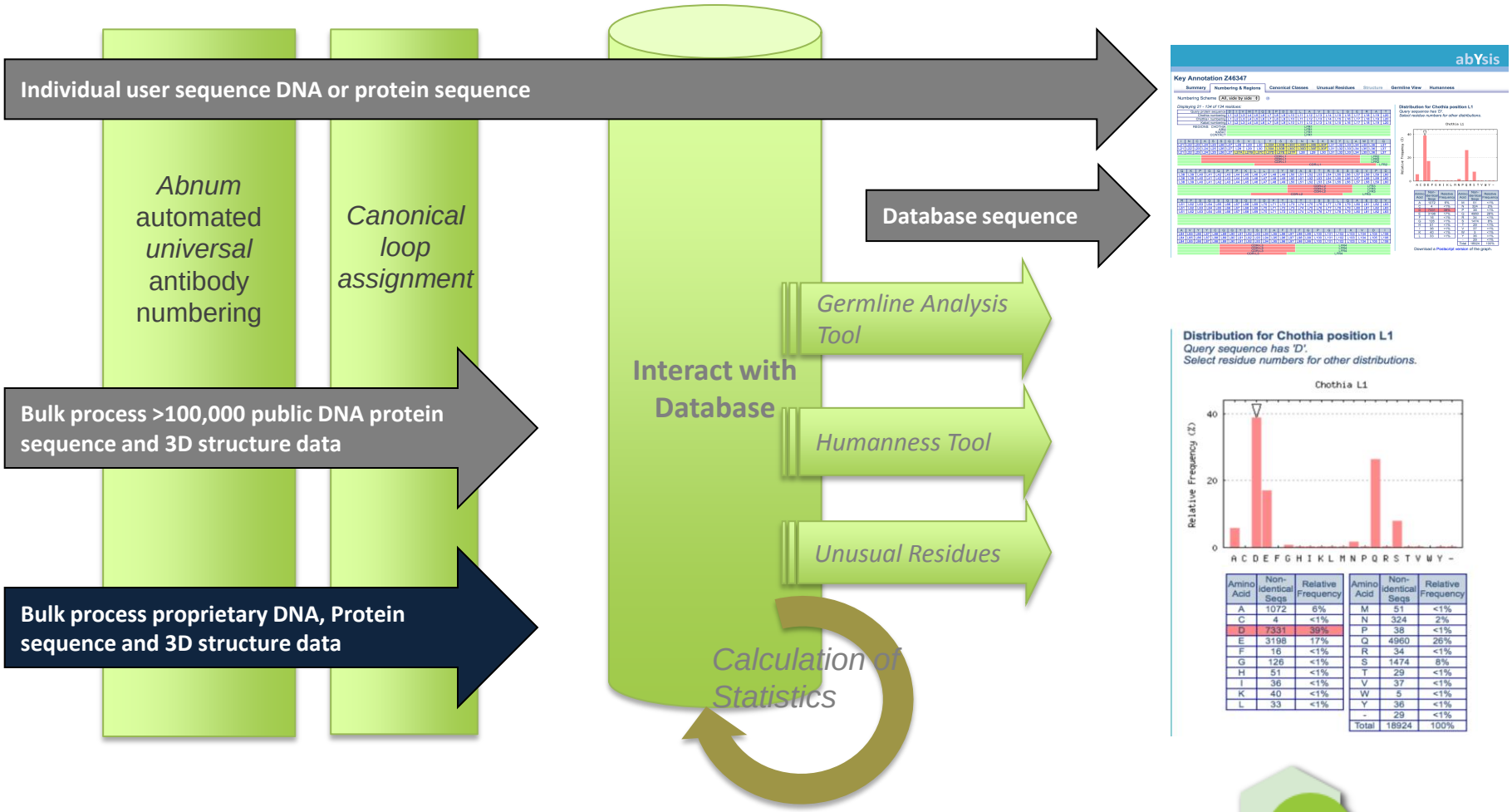
Antibody Drug Discovery

New Tools for Biotherapeutics



Amino Acid	Non-identical Seqs	Relative Frequency
A	1072	6%
C	4	<1%
D	7331	39%
E	3198	17%
F	16	<1%
G	126	<1%
H	51	<1%
I	36	<1%
K	40	<1%
L	33	<1%

Amino Acid	Non-identical Seqs	Relative Frequency
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S	1474	8%
T	29	<1%
V	37	<1%
W	5	<1%
Y	36	<1%
-	29	<1%
Total	18924	100%

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Comprehensive numbering, assignments & distributions

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abYsis

Key Annotation Z46347

Summary | Numbering & Regions | Canonical Classes | Unusual Residues | Structure | Germline View | Humanness

Numbering Scheme: All, side by side

Displaying 21 - 134 of 134 residues:

Query protein sequence	D	I	V	M	T	Q	S	P	D	S	L	A	V	S	L	G	E	R	A	T
Chothia numbering	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
Chothia+ numbering	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
Kabat numbering	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18	L19	L20
REGIONS: CHOTHIA	LFR1																			
ABM	LFR1																			
KABAT	LFR1																			
CONTACT	LFR1																			

I	N	C	K	S	S	Q	S	V	L	Y	S	S	N	N	K	N	Y	L	A	W	Y	Q
L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L30A	L30B	L30C	L30D	L30E	L30F	L31	L32	L33	L34	L35	L36	L37
L21	L22	L23	L24	L25	L26	L27	L28	L29	L30	L30A	L30B	L30C	L30D	L30E	L30F	L31	L32	L33	L34	L35	L36	L37
L21	L22	L23	L24	L25	L26	L27	L27A	L27B	L27C	L27D	L27E	L27F	L28	L29	L30	L31	L32	L33	L34	L35	L36	L37
				CDR-L1																LFR2		
				CDR-L1																LFR2		
				CDR-L1																LFR2		
										CDR-L1											LFR2	

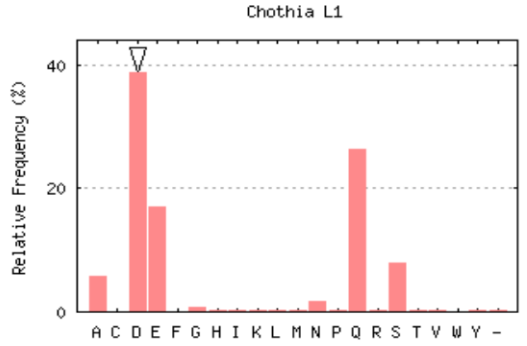
Q	K	P	G	Q	P	P	K	L	L	I	Y	W	A	S	T	R	E	S	G	V	P	D
L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60
L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60
L38	L39	L40	L41	L42	L43	L44	L45	L46	L47	L48	L49	L50	L51	L52	L53	L54	L55	L56	L57	L58	L59	L60
												CDR-L2								LFR3		
												CDR-L2								LFR3		
												CDR-L2								LFR3		
										CDR-L2										LFR3		

R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	A	E	D	V
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83

A	V	Y	Y	C	Q	Q	Y	Y	S	T	A	F	T	F	G	P	G	T	K	V	D	I
L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102	L103	L104	L105	L106
L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102	L103	L104	L105	L106
L84	L85	L86	L87	L88	L89	L90	L91	L92	L93	L94	L95	L96	L97	L98	L99	L100	L101	L102	L103	L104	L105	L106
					CDR-L3											LFR4						
					CDR-L3											LFR4						
					CDR-L3											LFR4						
					CDR-L3											LFR4						

Distribution for Chothia position L1

Query sequence has 'D'.
Select residue numbers for other distributions.



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T	29	<1%
V	37	<1%
W	5	<1%
Y	36	<1%
-	29	<1%
Total	18924	100%

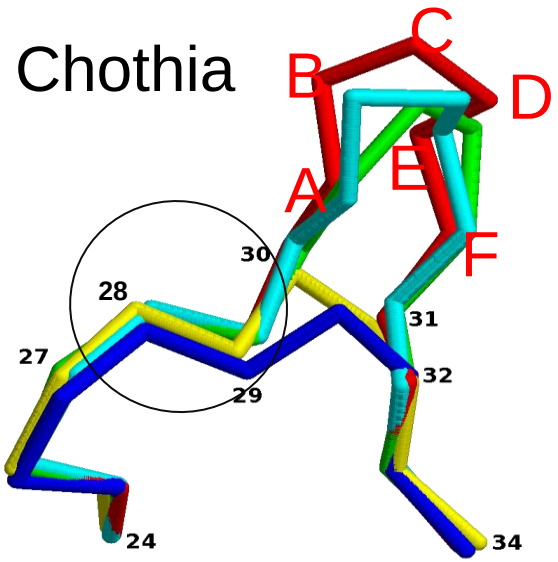
Download a Postscript version of the graph.

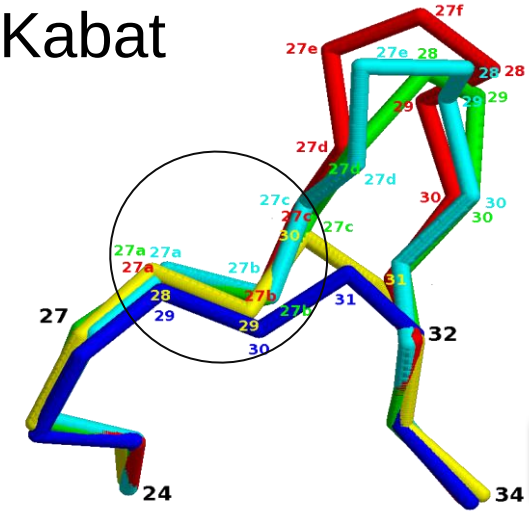
Numbering systems are important

Antigen binding regions vary in critical ways.

Numbering needs to take structural and functional variation into account

K	**S**	**S**	**Q**	**S**	**V**	**L**	**Y**	**S**	**S**	**N**	**N**	**K**	**N**	**Y**	**L**	**A**	17
L24	L25	L26	L27	L28	L29	L30	L30A	L30B	L30C	L30D	L30E	L30F	L31	L32	L33	L34	Chothia
L24	L25	L26	L27	L27A	L27B	L27C	L27D	L27E	L27F	L28	L29	L30	L31	L32	L33	L34	Kabat
R	**S**	**S**	**Q**	**S**	**L**	**V**	**H**	**T**	**N**	**G**	**N**		**T**	**Y**	**L**	**H**	16
L24	L25	L26	L27	L28	L29	L30	L30A	L30B	L30C	L30D	L30E		L31	L32	L33	L34	Chothia
L24	L25	L26	L27	L27A	L27B	L27C	L27D	L27E	L28	L29	L30		L31	L32	L33	L34	Kabat
T	**G**	**T**	**S**	**S**	**V**	**V**	**G**	**G**	**Y**				**N**	**Y**	**V**	**S**	14
L24	L25	L26	L27	L28	L29	L30	L30A	L30B	L30C				L31	L32	L33	L34	Chothia
L24	L25	L26	L27	L27A	L27B	L27C	L28	L29	L30				L31	L32	L33	L34	Kabat





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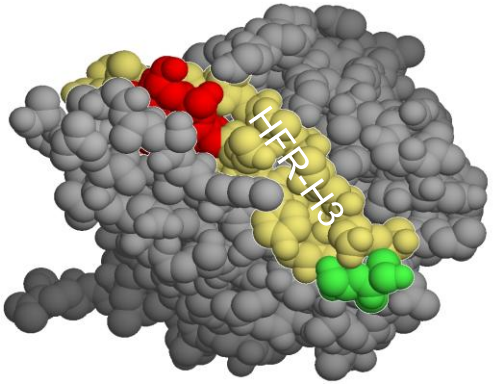
Frameworks can vary too

- H3 Framework insertions occur in many antibodies
- Our own “Chothia+” system also considers Framework

R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E
H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H82A	H82B	H82C	H83	H84	H85
H66	H67	H68	H69	H70	H71	H72	H72A	H72B	H72C	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85
H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H82A	H82B	H82C	H83	H84	H85
											HFR3											

HFR-H3



With our approach framework insertions are also put in appropriate structural locations

H82A, H82B H82C ✗ Chothia, Kabat

H72A, H72B H72C ✓ Martin (Chothia+)

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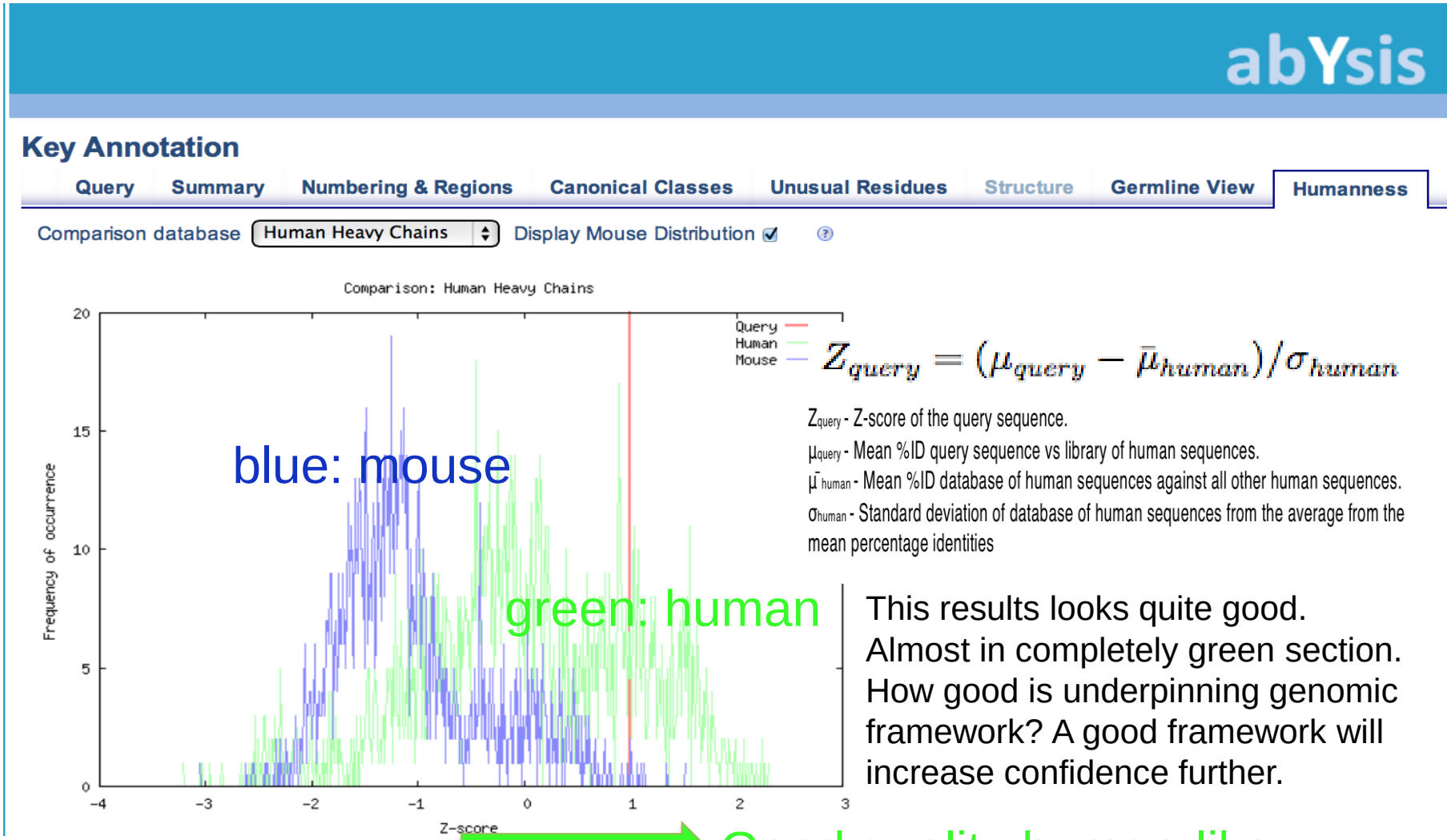
1. Humanness of Herceptin



This results looks quite good. Almost in completely green section. How good is underpinning genomic framework? A good framework will increase confidence further.

Good quality human-like

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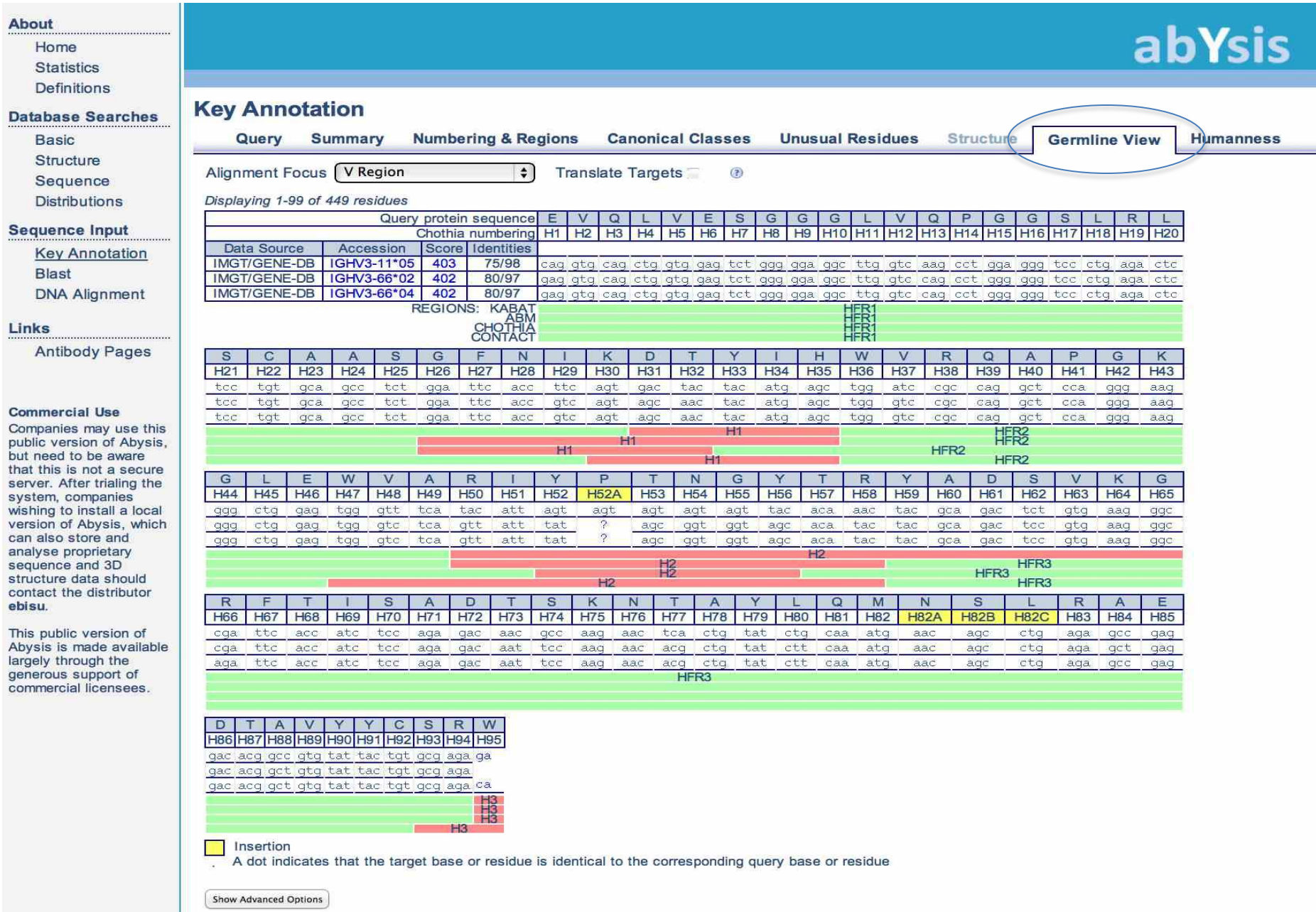
Germline Origin of herceptin



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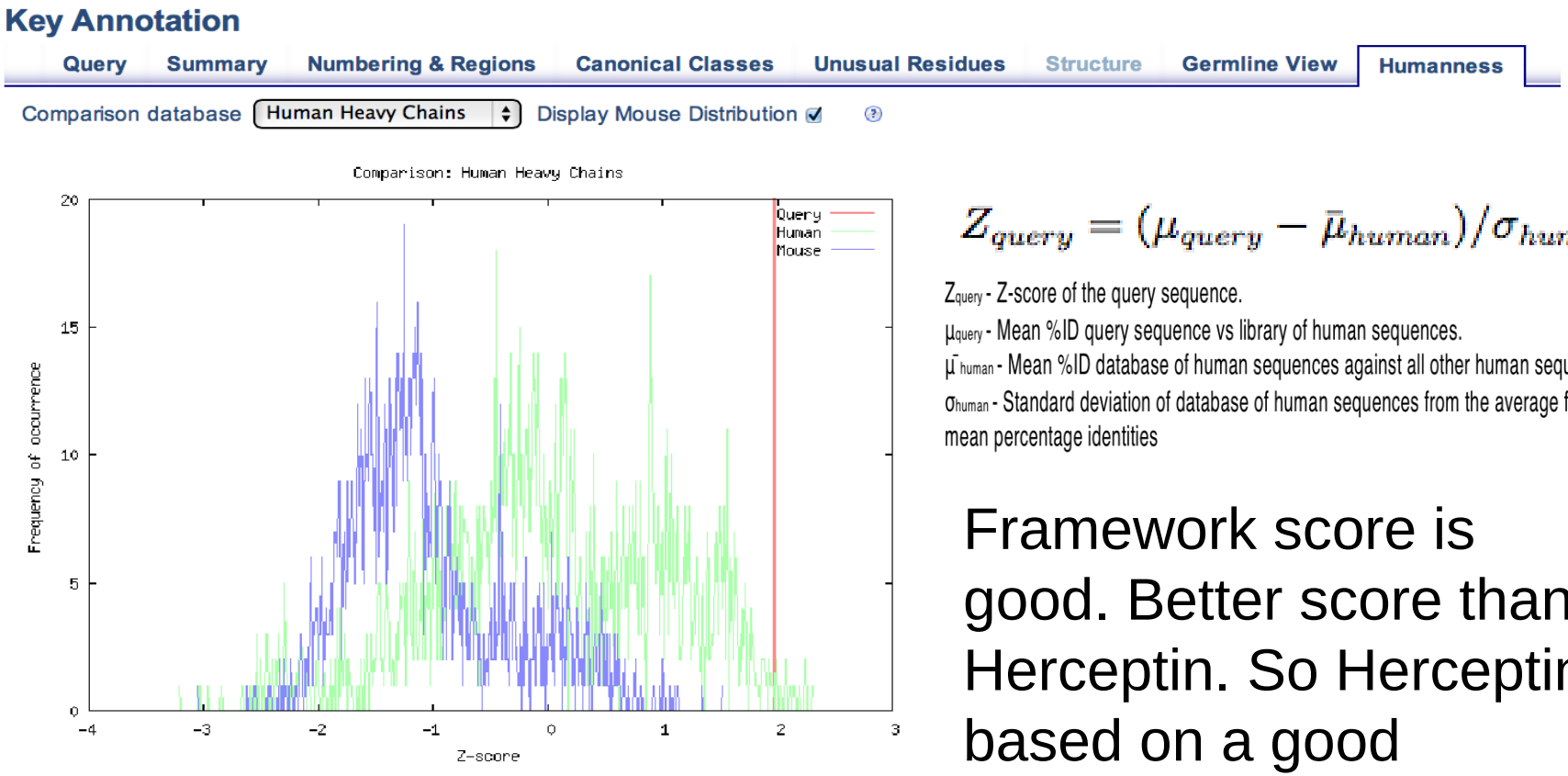
Humanness of Herceptin genomic framework



$$Z_{query} = (\mu_{query} - \bar{\mu}_{human})/\sigma_{human}$$

Z_{query} - Z-score of the query sequence.

μ_{query} - Mean %ID query sequence vs library of human sequences.

$\bar{\mu}_{human}$ - Mean %ID database of human sequences against all other human sequences.

σ_{human} - Standard deviation of database of human sequences from the average from the mean percentage identities

Framework score is good. Better score than Herceptin. So Herceptin based on a good framework.

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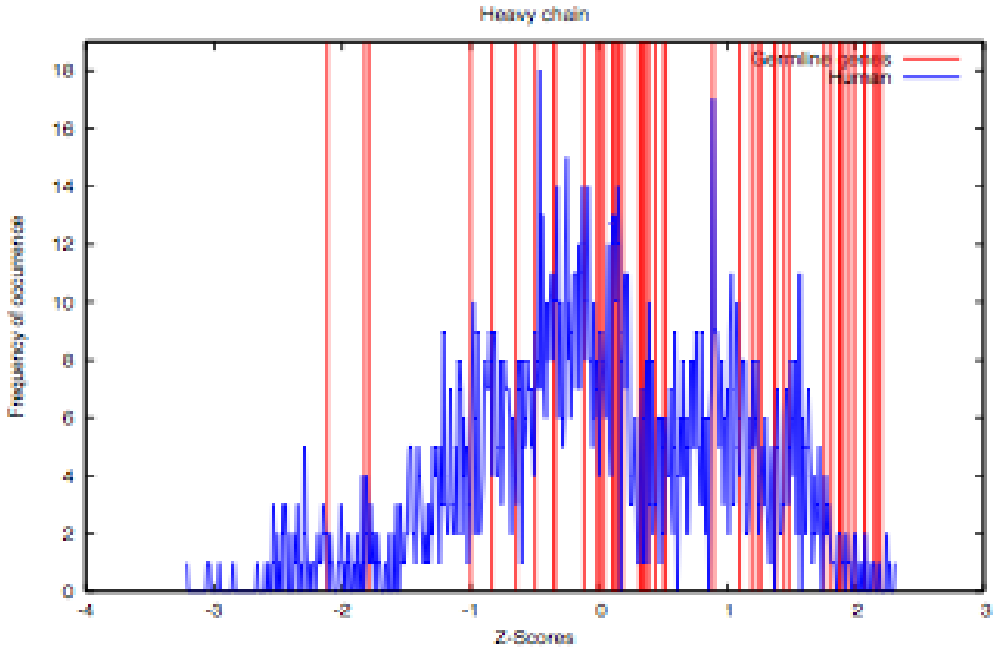
Variation in genomic frameworks

Investigative work has been implemented in Abysis to reflect how similar an antibody is to the mature human repertoire.

Blue: Mean % ID of each human antibody to all others in the set as Z score.

Red: Human VH germline antibodies



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σ_{human} - Standard deviation of database of human sequences from the average from the mean percentage identities

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2. Clinical antibody hu3S193

Targets LewisY antigen in epithelial cancers

Anti-antibody response

JOURNAL OF CLINICAL ONCOLOGY
Official Journal of the American Society of Clinical Oncology

Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition).
Vol 22, No 14S (July 15 Supplement), 2004: 2567

Abstract

Phase I trial of hu3S193 in patients with advanced epithelial cancers which express the Lewis-y antigen

A. M. Scott, N. Tebbutt, F.-T. Lee, T. Cavicchiolo, Z. Liu, A. Poon, M. W. Brechbiel, E. Stockert, E. W. Hoffman and L. J. Old

Ludwig Institute for Cancer Research, Heidelberg, Victoria, Australia; National Institutes of Health, Bethesda, MD; Ludwig Institute for Cancer Research, New York, NY

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Background: The Lewis-y (Le^y) antigen is a blood group related antigen that is expressed in a high proportion of epithelial cancers. We have generated a humanised antibody (hu3S193) against Le^y, which has potent immune effector function, and efficacy in murine tumour models (Scott et al, Cancer Res 60: 3254–3261, 2000). **Methods:** An open label dose escalation Phase I trial of hu3S193 in patients (pts) with advanced Le^y positive epithelial cancers has been conducted. Inclusion criteria included +ve Le^y expression in tumour assessed prior to study entry. Pts received 4 infusions of hu3S193 at weekly intervals, with four dose levels (5, 10, 20 and 40 mg/m^2). The first infusion of hu3S193 was trace labelled with ^{111}In to evaluate targeting. Biodistribution, pharmacokinetics, and immune response were evaluated in all patients. **Results:** A total of 12 pts (6M:6F; age range 42–76 yrs; 5 breast, 7 colorectal cancer) have been accrued into the study, completing the 5 (3 pts), 10 (6 pts) and 20 (3 pts) mg/m^2 dose levels. No infusion related AEs were observed. There was one episode of

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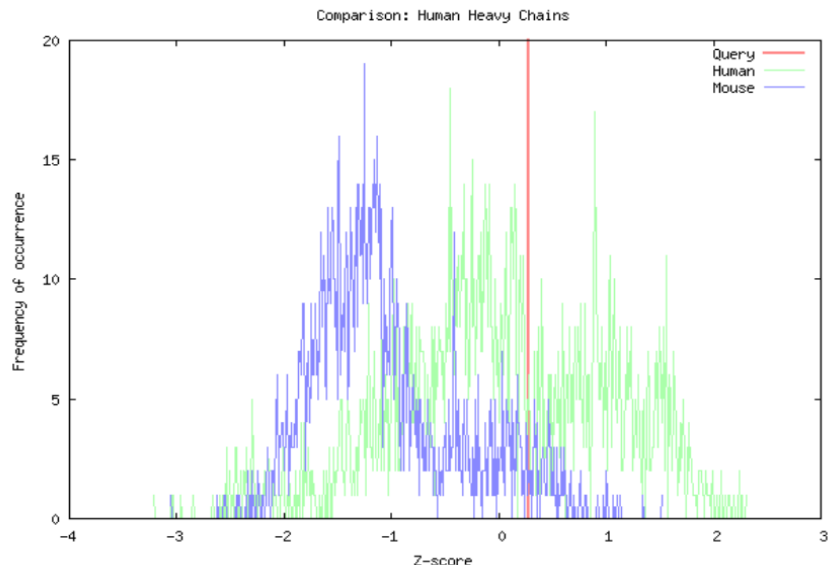
Humanness scores for hu3S193
Heavy and Light Chains significantly worse than Herceptin

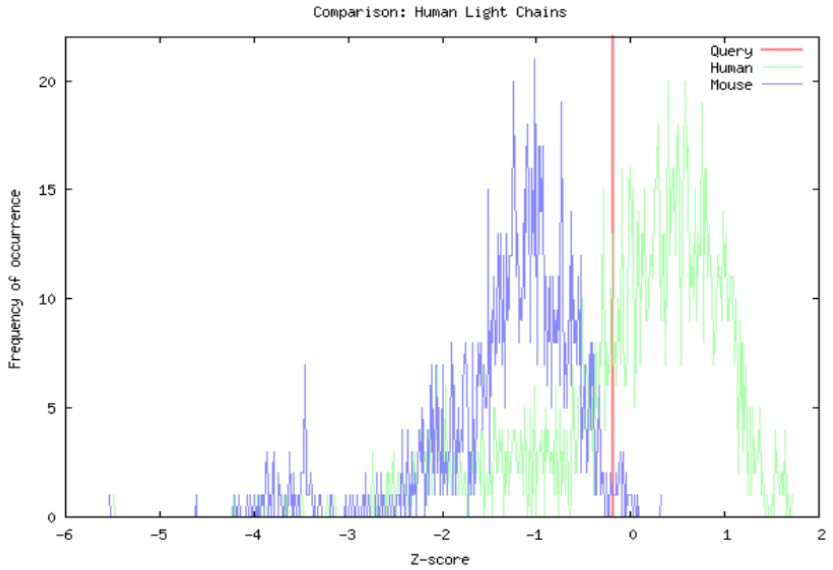
Heavy

Comparison database: Human Heavy Chains Display Mouse Distribution



Light

Comparison database: Human Light Chains Display Mouse Distribution



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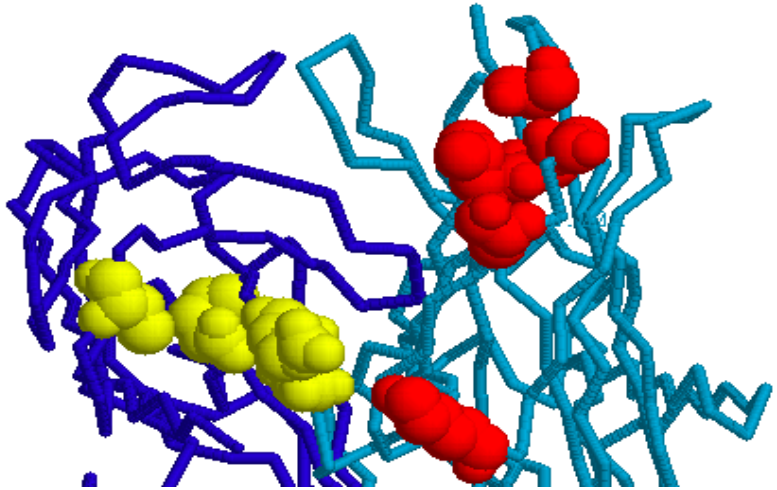
3. Humira (adalimumab)

How ‘perfect’ is it?

3-year follow-up study* in RA patients indicates higher anti-antibody response.

- Up to 28% developing anti-antibodies
- Presence of antibodies linked to failure of treatment and higher RA score
- Only 4% of those with anti-adalimumab antibodies had sustained remission
- 34% of antibody negative set has sustained remission

Analysis of Humira sequence identifies unusual residues



Residues cluster in 3D

30:L, 90:L, 93:L, 94:L Yellow

30:H, 52:H, 53:H, 56:H, 64:H Red

* Bartelds et al., JAMA.2011;305(14):1460-1468

^superposed on 1yqv.

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