

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*

Ligand-Protein Interaction Sites: Ligplot+

LigPlot+: Multiple Ligand-Protein Interaction Diagnostics 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

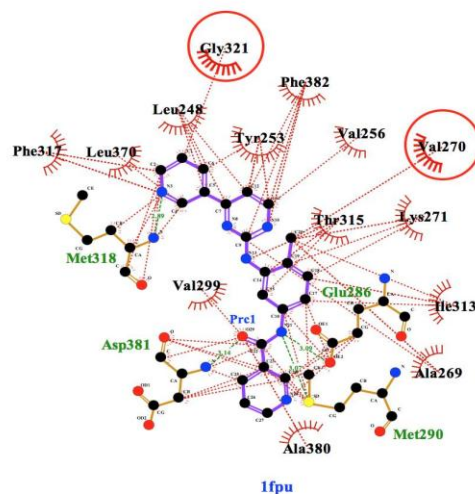
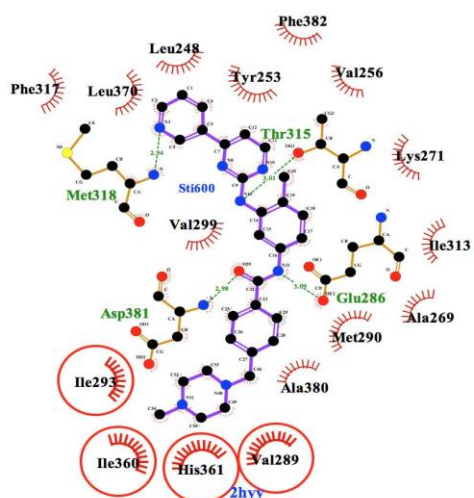
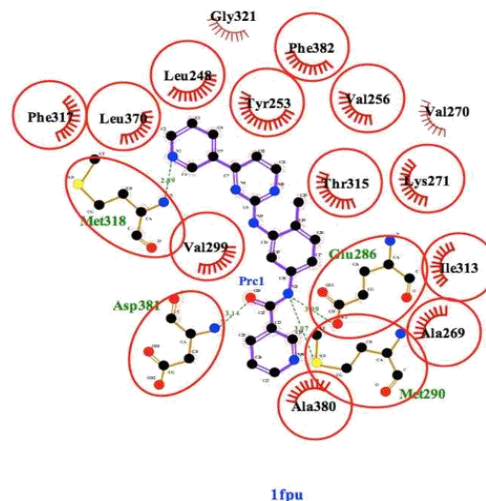
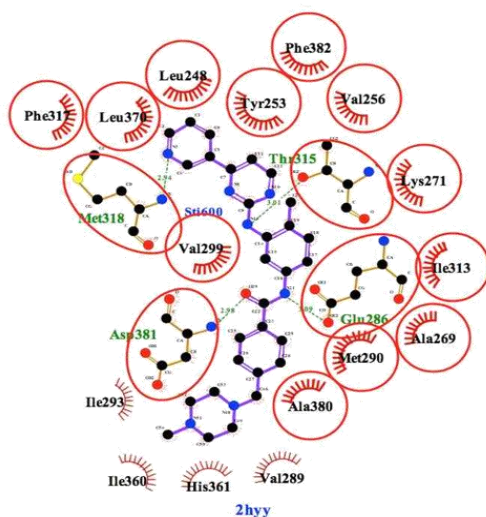
Enhancements over Ligplot include

- 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

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

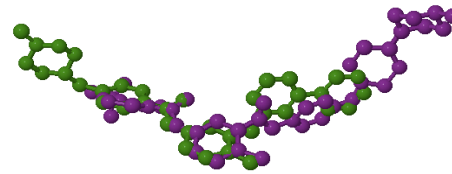
- **Conserved** interactions highlighted as circles.

Bottom Diagram

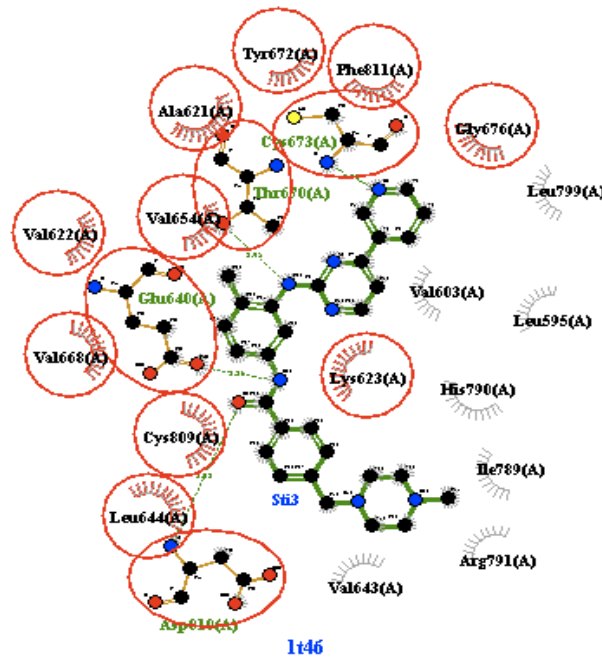
- **Unconserved** interactions highlighted
- Right-hand diagram also displays hydrophobic interactions as red lines

Ligplot+: Homologous proteins with different ligands

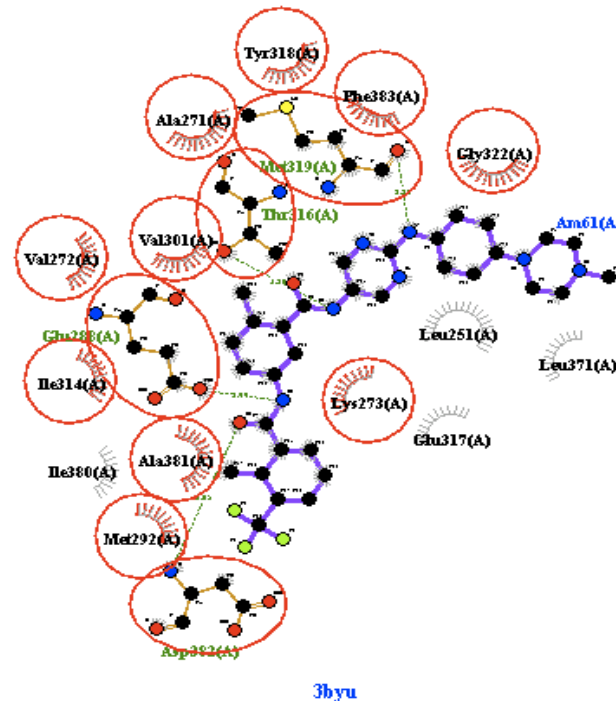
Ligplot conveys both ligand/protein similarity and 3D orientation.



Tanimoto coefficient = 0.85
Protein Sequence Identity = 37%



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



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

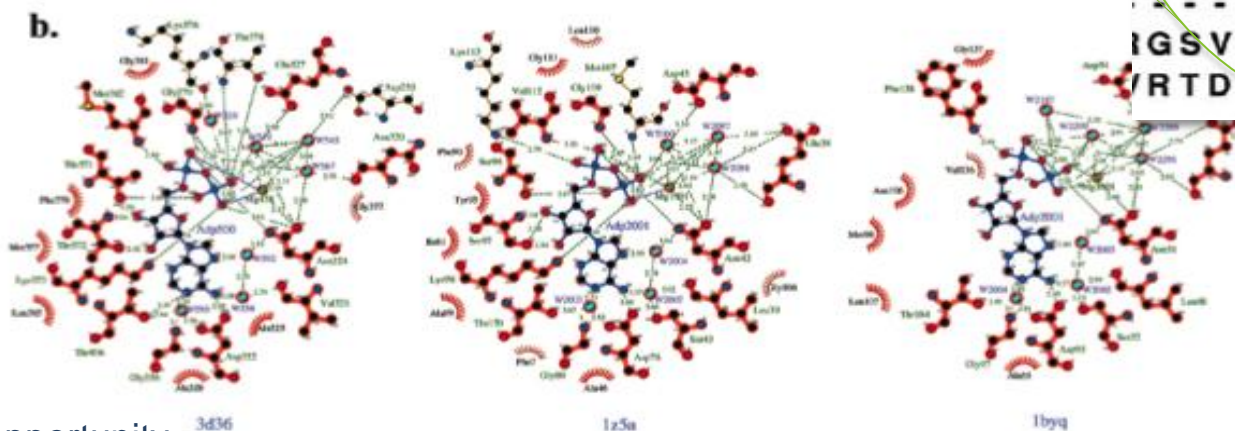
Ligplot+: Very Distant (<20% ID) Homologues

Journal of Chemical Information and Modeling

ARTICLE

Histidine Kinase
DNA Gyrase B
HSP-90

pdb 3d36	296VDIQA TLAPFSVIGEREFRQCLLNVMKNA	325	EKFRQCLLNVMKNA	325
pdb 1z5a	4KEKFTSLSPAEPFKRNPAPALYQTVRELIENS	43	RALYQTVRELIENS	43
pdb 1byq	11	PMEEEVETFAFQAEIAQLMGLINTFYS.....NK-EIFLRELISNS	52	IK-EIFLRELISNS	52
pdb 3d36	326	IEIMPN.....GGTLQVYVSI.....DNGRVLIRIATVY	356	IGRVLIRIADTGVG	356
pdb 1z5a	44	LDATDVHQI.....LPNLIKITIDLIDARQIYKVNVDNGIG	80	IQIYKVNVDNGIG	80
pdb 1byq	53	SDLKIRYETLTDP SKLDSGKELHINLIPNKQD.....RTLTI VDTGIG	97	---RTLTI VDTGIG	97
pdb 3d36	357	MTKEQLERLGEFYFTTKGVKG.....TGLMMVVYRIIES	391	TGLMMVVYRIIES	391
pdb 1z5a	81	IPPQEVNAPGRVLYSSKYVNRQTR.....GMYLVKAAVLYSQM	121	TGLGVKAAVLYSQM	121
pdb 1byq	98	MTKADLINLGTIAKSGTKAFMEALQAGADISMIQGFVGFYSAYLVA--	145	TGLGVFYSAYLVA--	145
pdb 3d36	392	-MNGTIRIESEIH.....KGT	407	---KGT	407
pdb 1z5a	122	HQDKPEIETSPVNSKRIYTFKLIKIDINKNEPIIVERGSVENTRGRFH	171	---IGSVENRGRFH	171
pdb 1byq	146	---EKYTVITKHNDDEQYAWESSAG-----GSFTVRTDTGPEMGRGK	185	---VRTDTGPEMGRGK	185
pdb 3d36	408	SIYPLAS.....	416	TGLGMMVVYRIIES	391
pdb 1z5a	172	VASIPGDWPKAKSRYYIKRTYIITPYAEFIKDPPEGNVTTYPRLT	219	TGLGVKAAVLYSQM	121
pdb 1byq	186	VILHKEDQTEYLEERRIKEIVKHSQFI-GYPITL FVE-----	223	TGLGVFYSAYLVA--	145
pdb 3d36	---	---	---	---	---
pdb 1z5a	220	NKI	222	---KGT	407
pdb 1byq	---	---	---	---IGSVENRGRFH	171
				---VRTDTGPEMGRGK	185



Research opportunity

Identify distant homologues.

Identify compounds in ChEMBL

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



Exploring Distant Homologues

Protein Kinase Superfamily, other kinases, aminoglycoside phosphotransferase

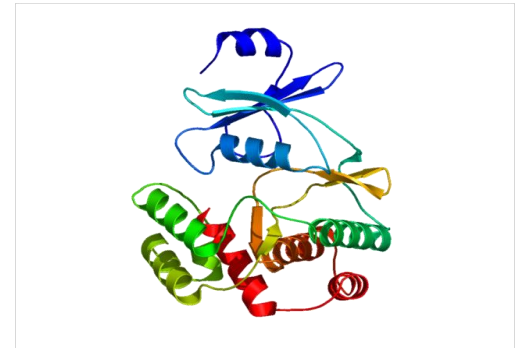
c-abl, syk and c-kit (below) are therapeutically relevant protein kinases



protein kinases

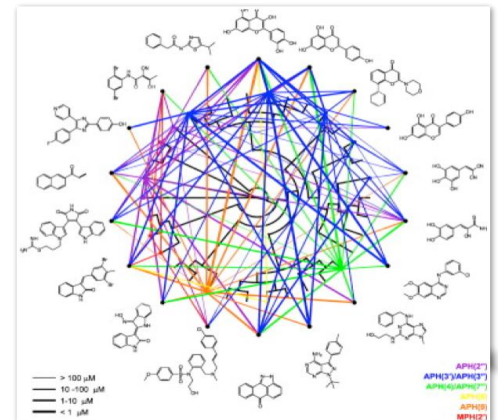
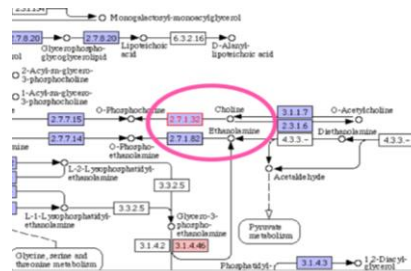
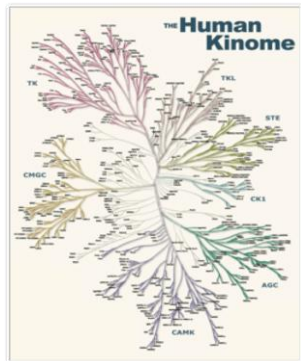


Choline kinase
Ethanolamine kinase



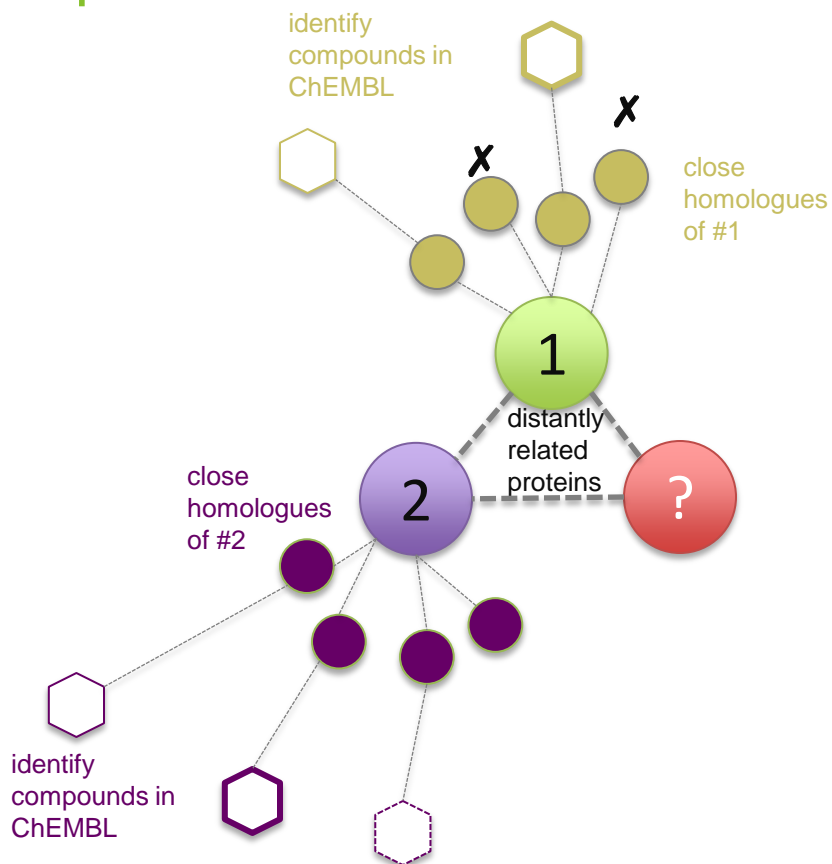
aminoglycoside
phosphotransferase

ebisu group

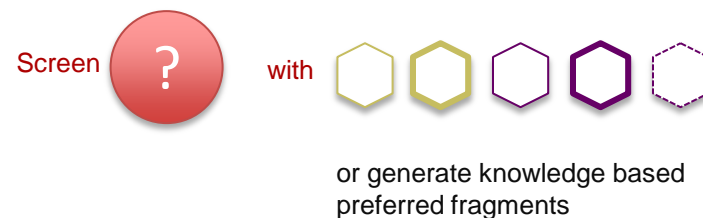


Knowledge-based screening

Step 1



Step 2



PDBsum^{Proprietary} for an in-house Electronic Lab Notebook

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

PRIVATE: Custom-generated PDBsum page

PDB id: a502
 Name: Hydrolase/DNA
 Title: Crystal structure of lactococcus lactis formamido-pyrimidine DNA glycosylase (alias fpg or mutm) non covalently bound to an ap site containing DNA.
 Structure: 5'-d(Cp Tp Cp Tp Tp Tp (Pd)j Tp Tp Tp Cp Tp C)-3'. Chain: d, g. Engineered: yes. Other_details: contains a 1,3 propanediol site (pd). 5'-d(Cp Ap Cp Ap Ap Ap Ap Cp Ap Ap Ap Cp Ap G)-3'. Chain: e, h. Engineered: yes. Formamido-pyrimidine DNA glycosylase.
 Source: Synthetic: yes. Lactococcus lactis. Organism_taxid: 1358. Gene: mutm or fpg. Expressed in: escherichia coli. Expression_system_taxid: 562.
 UniProt: Chains A, B: P42371 (FPG_LAACL)
 Seq: [Protein sequence bar chart]
 Struct: [Secondary structure bar chart] 273 a.a.
 Seq: [Protein sequence bar chart]
 Struct: [Secondary structure bar chart] 264 a.a.*
 Key: [Green bar] PfamA domain [Blue bar] Secondary structure
 * PDB and UniProt seqs differ at 1 residue position (black cross)

Enzyme class: E.C.3.2.2.23 [IntEnz] [Expasy] [KEGG] [BRENDA]
 Reaction: Hydrolysis of DNA containing ring-opened N(7)-methylguanine residues, releasing 2,6-diamino-4-hydroxy-5-(N-methyl)formamidopyrimidine.
 Resolution: 2.55Å
 R-factor: 0.251
 R-free: 0.285
 Authors: L.Serre, K.Pereira De Jesus, S.Boiteux, C.Zelwer, B.Castaing
 Date: 06-Jun-11
 Release date: 14-Jun-02
 Related entries: 1ee8

Ligand GOL - Glycerol
 Formula: C₃H₈O₃

2 instances of ligand highlighted
 GOL
 GOL *2
 GOL 301(A)
 Metals
 ZN *2
 ZN 300(A)

LIGPLOT of interactions involving ligand

GOL 301(A)
 (also representing equivalent ligand GOL 302(B))

PROCHECK summary for a502

Ramachandran plot
 pdba502

PROCHECK statistics

1. Ramachandran Plot statistics

	No. of residues	%-age
Most favoured regions	387	84.34
Additional allowed regions	69	15.66
Generously allowed regions	2	0.55
Disallowed regions	0	0.00
Non-glycine and non-proline residues	469	100.00
Dnd-residues (excl. Gly and Pro)	57	
Clashes (excl. H-bonds)	41	

PDBsumProprietary

After installing PDBsumProprietary 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.

Chain A (297 residues)
UniProt code: P19721 (KIT_HUMAN) [Pfam]

CATH structural classification (2 domains):
 Domain Links: CATH no. Class Architecture
 1 **CATH** 3.30.200.20 = Alpha Beta 2-Layer Sandwich
 2 **CATH** 1.10.510.10 = Mainly Alpha Orthogonal Bundle

Secondary structure
 -Wiring diagram
 -Residue conservation
 -ProfMod
 -11 sheets
 -1 beta hairpins
 -5 beta bulges
 -14 helices
 -10 strands
 -12 beta-helix interacs
 -2 alpha bulbs
 -2 alpha turns
 -Catalytic residues
 -Lipids

Secondary structure and topology overview page

Cleft analysis for: 1t46

View options

- Binding-site(s)
- Binding-surface(s)

Coloured by

- cleft (as in table below)
- closest atom type
- residue type
- residue conservation

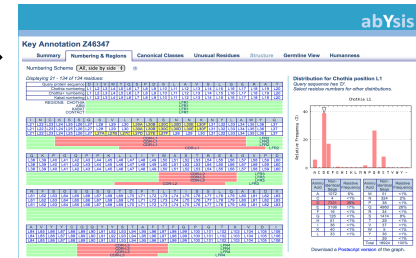
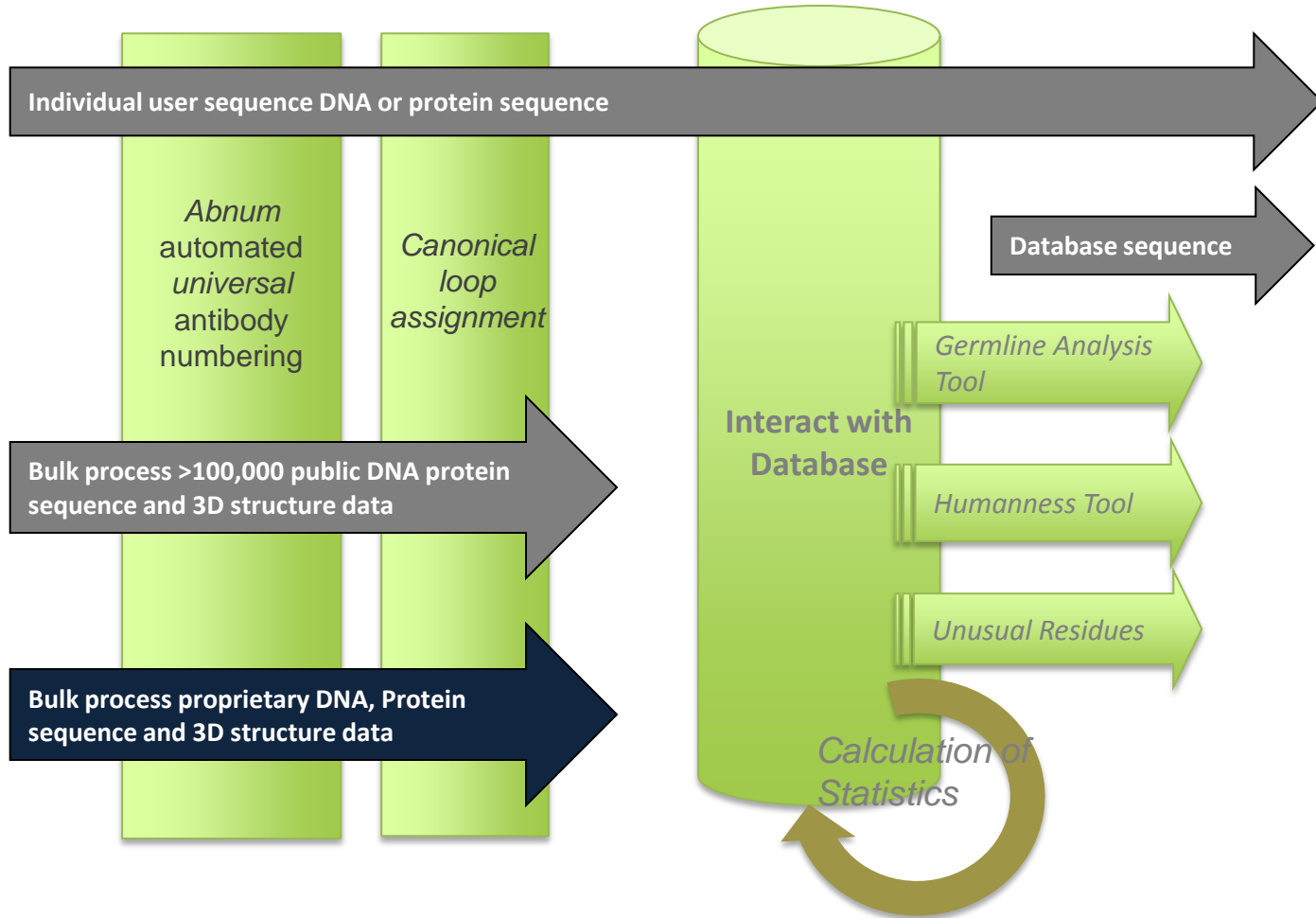
Clefts	Volume	R1 ratio	Accessible vertices	Buried vertices	Average depth	Residue type
1	6568.69	4.82	67.02	12.62	16.98	15 13 17 32 12 10 5 STI 3
2	1363.08	0.00	59.31	7	5.69	8 14 3 5 2 4 8 3 3 1 PO4
3	950.06	0.00	61.32	5	7.66	3 6.98 6 4 2 3 5 6 5 0
4	778.36	0.00	65.27	3	9.91	2 10.48 2 2 2 4 4 3 1 0
5	594.00	0.00	61.56	4	7.48	6 7.16 5 3 1 3 4 2 5 0
6	567.42	0.00	52.77	9	6.35	7 5.93 8 4 3 1 3 2 1 0
7	387.70	0.00	58.71	8	7.63	5 4.48 10 3 0 2 4 0 4 0
8	332.86	0.00	68.80	1	7.64	4 2 0 2 3 2 1 0
9	403.73	0.00	60.12	6	5.37	9 2 1 2 1 3 2 0

Cleft overview page generated using surfnet

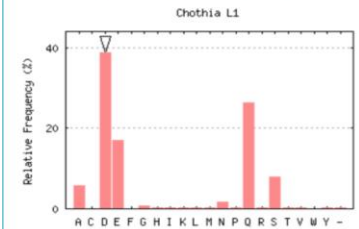
Antibody Drug Discovery

New Tools for Biotherapeutics

ebisu group



Distribution for Chothia position L1
Query sequence has 'D'.
Select residue numbers for other distributions.



Amino Acid	Non-identical Segs	Relative Frequency	Amino Acid	Non-identical Segs	Relative Frequency
A	1072	6%	M	51	<1%
C	4	<1%	N	324	2%
D	2331	36%	P	33	<1%
E	3198	17%	Q	4960	26%
F	16	<1%	R	34	<1%
G	126	<1%	S	1474	8%
H	51	<1%	T	29	<1%
I	36	<1%	V	37	<1%
K	40	<1%	W	5	<1%
L	33	<1%	Y	36	<1%
-			-	29	<1%
Total	18924	100%			



Comprehensive numbering, assignments & distributions

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	

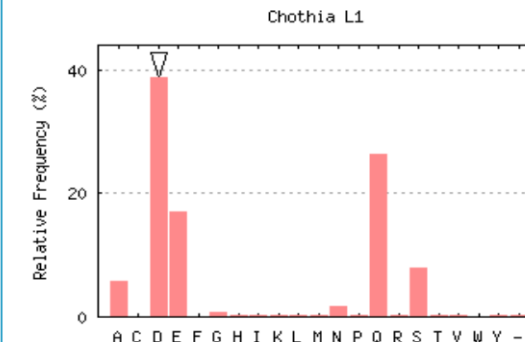
R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S	S	L	Q	A	E	D	V
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	L79	L80	L81	L82	L83
L61	L62	L63	L64	L65	L66	L67	L68	L69	L70	L71	L72	L73	L74	L75	L76	L77	L78	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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L	33	<1%	Y	36	<1%
			-	29	<1%
			Total	18924	100%

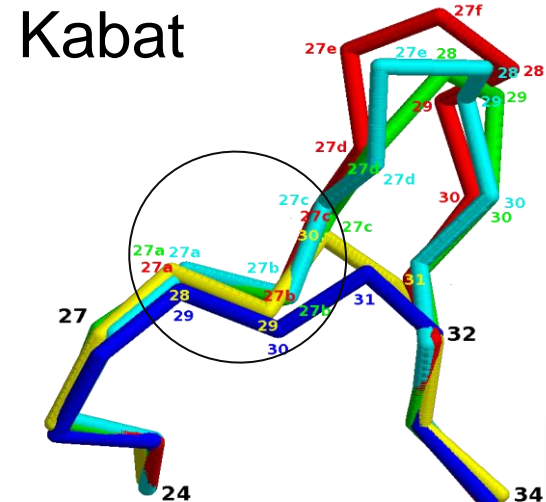
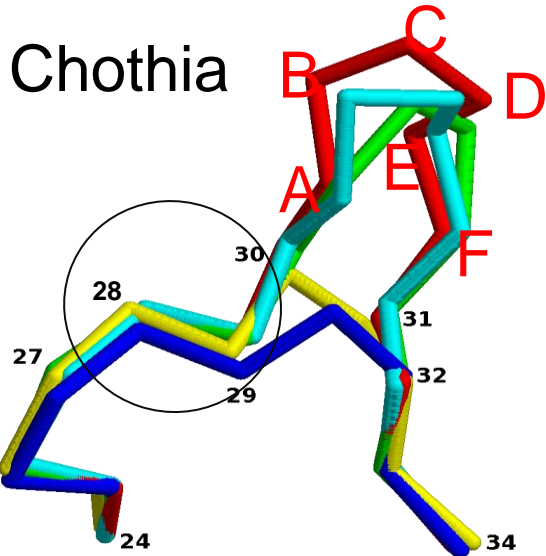
Download a [Postscript version](#) of the graph.

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



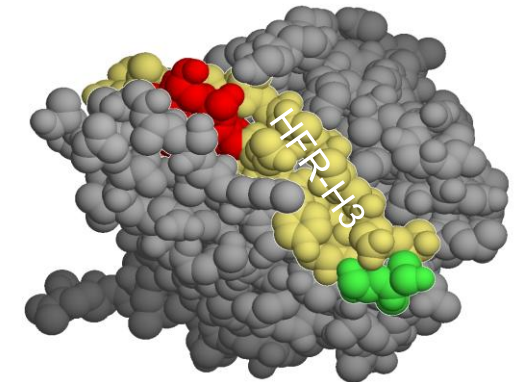
Frameworks can vary too

- 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

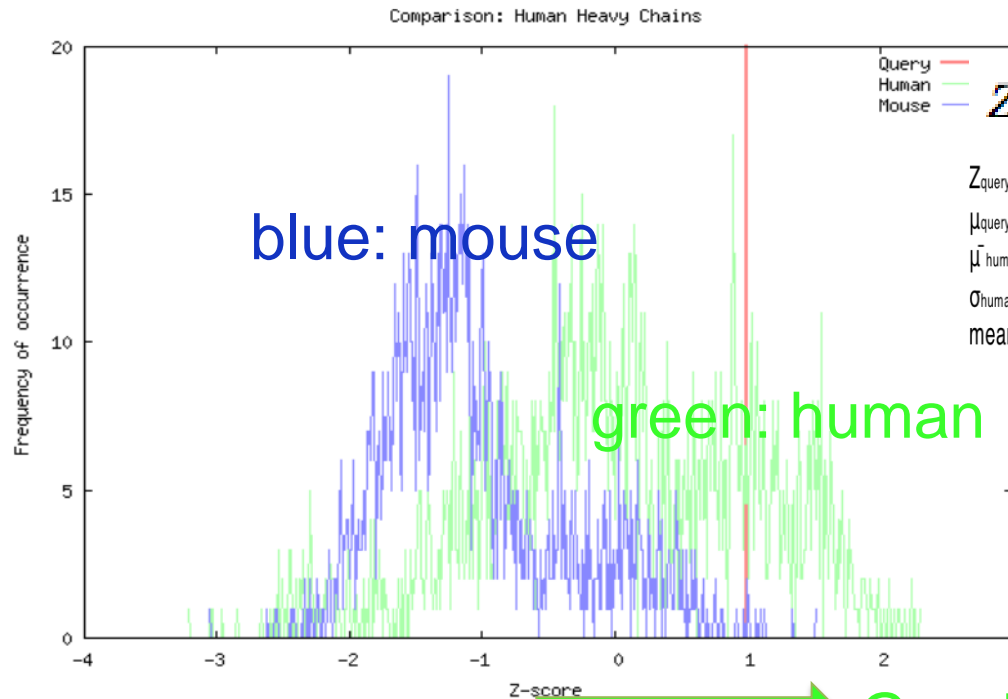
H72A, H72B H72C ✅ Martin (Chothia+)

1. Humanness of Herceptin

Key Annotation

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

Comparison database **Human Heavy Chains** Display Mouse Distribution



blue: mouse

green: human

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

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

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

ebisu group



Germline Origin of herceptin

About

- Home
- Statistics
- Definitions

Database Searches

- Basic
- Structure
- Sequence
- Distributions

Sequence Input

- Key Annotation
- Blast
- DNA Alignment

Links

- Antibody Pages

Commercial Use

Companies may use this public version of Abysis, but need to be aware that this is not a secure server. After trialing the system, companies wishing to install a local version of Abysis, which can also store and analyse proprietary sequence and 3D structure data should contact the distributor **ebisu**.

This public version of Abysis is made available largely through the generous support of commercial licensees.

Key Annotation

- Query
- Summary
- Numbering & Regions
- Canonical Classes
- Unusual Residues
- Structure
- Germline View**
- Humanness

Alignment Focus Translate Targets

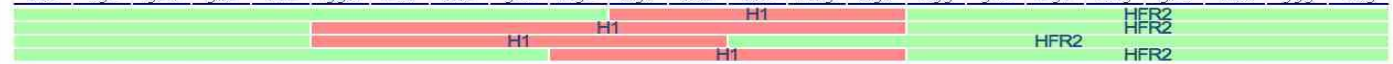
Displaying 1-99 of 449 residues

Query protein sequence				E	V	Q	L	V	E	S	G	G	G	L	V	Q	P	G	G	S	L	R	L
Chothia numbering				H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20
Data Source	Accession	Score	Identities																				
IMGT/GENE-DB	IGHV3-11*05	403	75/98	cag	gtg	caq	ctg	gtg	gaq	tct	ggg	gga	ggc	ttg	gtc	aag	cct	gga	ggg	tcc	ctg	aga	ctc
IMGT/GENE-DB	IGHV3-66*02	402	80/97	gag	gtg	caq	ctg	gtg	gaq	tct	ggg	gga	ggc	ttg	gtc	caq	cct	ggg	ggg	tcc	ctg	aga	ctc
IMGT/GENE-DB	IGHV3-66*04	402	80/97	gag	gtg	caq	ctg	gtg	gaq	tct	ggg	gga	ggc	ttg	gtc	caq	cct	ggg	ggg	tcc	ctg	aga	ctc

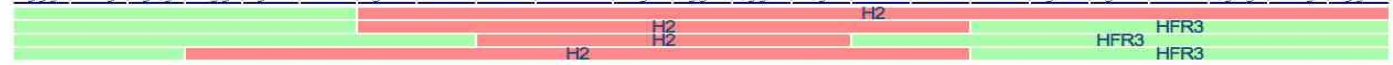
REGIONS: KABAT
ABM
CHOITHA
CONTACT

HFR1
HFR1
HFR1

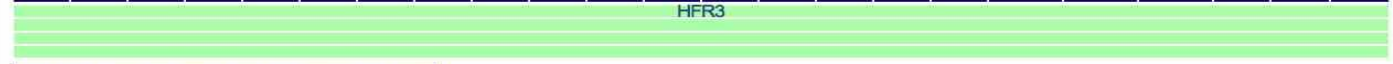
S	C	A	A	S	G	F	N	I	K	D	T	Y	I	H	W	V	R	Q	A	P	G	K
H21	H22	H23	H24	H25	H26	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40	H41	H42	H43
tcc	tgt	gca	gcc	tct	gga	ttc	acc	ttc	agt	gac	tac	tac	atg	agc	tgg	atc	cgc	caq	gct	cca	ggg	aag
tcc	tgt	gca	gcc	tct	gga	ttc	acc	gtc	agt	agc	aac	tac	atg	agc	tgg	gtc	cgc	caq	gct	cca	ggg	aag
tcc	tgt	gca	gcc	tct	gga	ttc	acc	gtc	agt	agc	aac	tac	atg	agc	tgg	gtc	cgc	caq	gct	cca	ggg	aag



G	L	E	W	V	A	R	I	Y	P	T	N	G	Y	T	R	Y	A	D	S	V	K	G
H44	H45	H46	H47	H48	H49	H50	H51	H52	H52A	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65
ggg	ctg	gag	tgg	ggt	tca	tac	att	agt	agt	agt	agt	tac	aca	aac	tac	gca	gac	tct	gtg	aag	ggc	
ggg	ctg	gag	tgg	gtc	tca	ggt	att	tat	?	agc	ggt	ggt	agc	aca	tac	tac	gca	gac	tcc	gtg	aag	ggc
ggg	ctg	gag	tgg	gtc	tca	ggt	att	tat	?	agc	ggt	ggt	agc	aca	tac	tac	gca	gac	tcc	gtg	aag	ggc

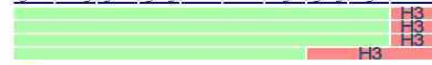


R	F	T	I	S	A	D	T	S	K	N	T	A	Y	L	Q	M	N	S	L	R	A	E
H66	H67	H68	H69	H70	H71	H72	H73	H74	H75	H76	H77	H78	H79	H80	H81	H82	H82A	H82B	H82C	H83	H84	H85
cga	ttc	acc	atc	tcc	aga	gac	aac	gcc	aag	aac	tca	ctg	tat	ctg	caa	atg	aac	agc	ctg	aga	gcc	gag
cga	ttc	acc	atc	tcc	aga	gac	aat	tcc	aag	aac	acg	ctg	tat	ctt	caa	atg	aac	agc	ctg	aga	gct	gag
aga	ttc	acc	atc	tcc	aga	gac	aat	tcc	aag	aac	acg	ctg	tat	ctt	caa	atg	aac	agc	ctg	aga	gcc	gag



D	T	A	V	Y	Y	C	S	R	W
H86	H87	H88	H89	H90	H91	H92	H93	H94	H95

gac acg gcc gtg tat tac tgt gcg aga ga
gac acg gct gtg tat tac tgt gcg aga
gac acg gct gtg tat tac tgt gcg aga ca



Insertion

A dot indicates that the target base or residue is identical to the corresponding query base or residue

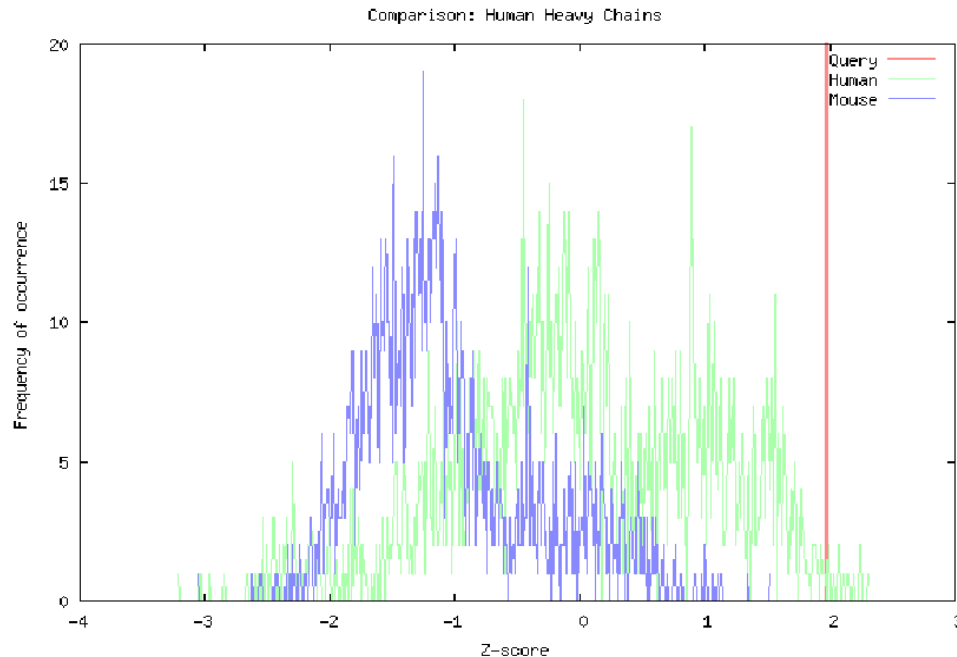
Show Advanced Options

Humanness of Herceptin genomic framework

Key Annotation

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

Comparison database Human Heavy Chains Display Mouse Distribution [?](#)



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

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.

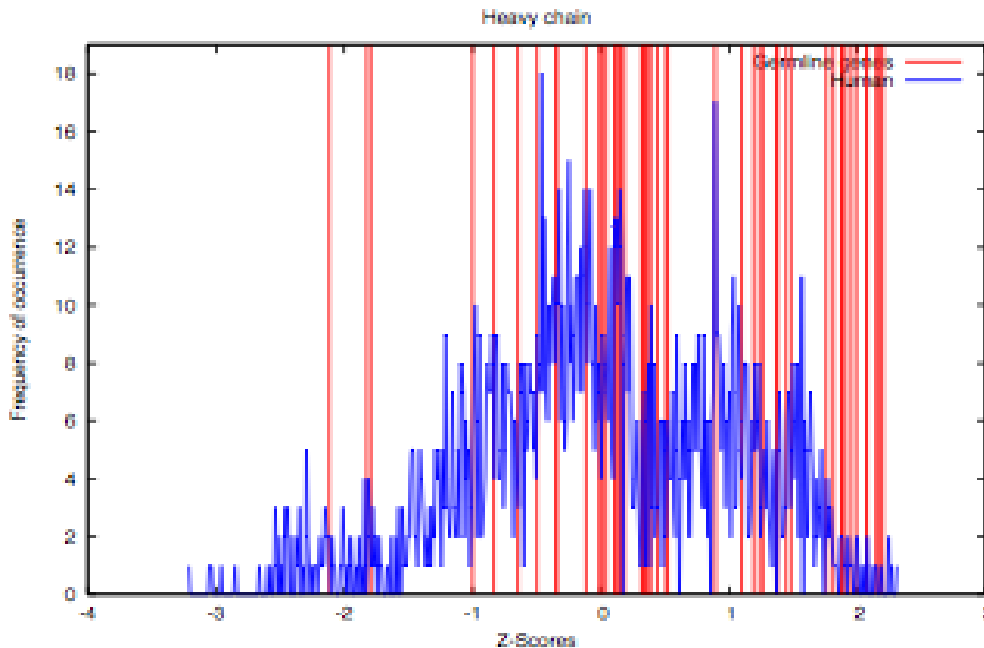


Variation in genomic frameworks

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



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

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.

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2. Clinical antibody hu3S193

Targets LewisY antigen in epithelial cancers

Anti-antibody response

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Journal of Clinical Oncology, 2004 ASCO Annual Meeting Proceedings (Post-Meeting Edition).

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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

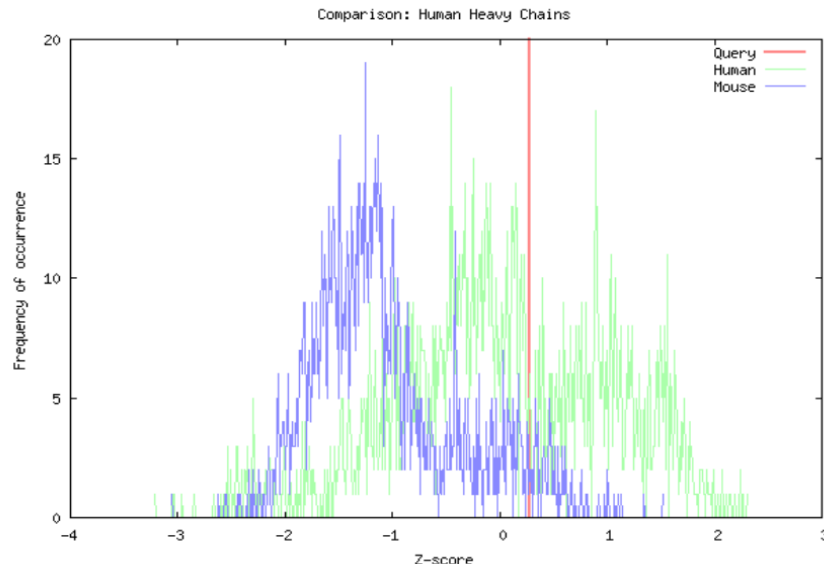
2567

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/m²). The first infusion of hu3S193 was trace labelled with ¹¹¹In 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/m² dose levels. No infusion related AEs were observed. There was one episode of

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

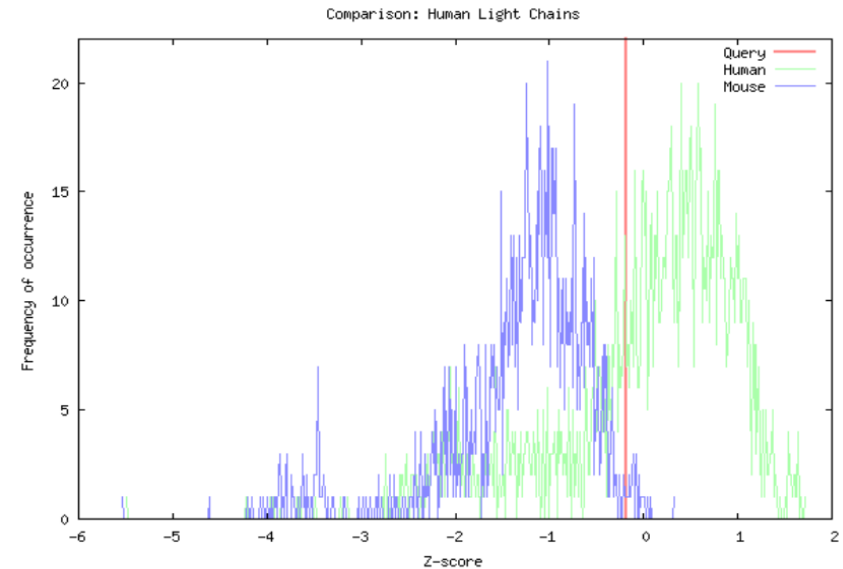
Heavy

Comparison database **Human Heavy Chains** Display Mouse Distribution [?](#)



Light

Comparison database **Human Light Chains** Display Mouse Distribution [?](#)



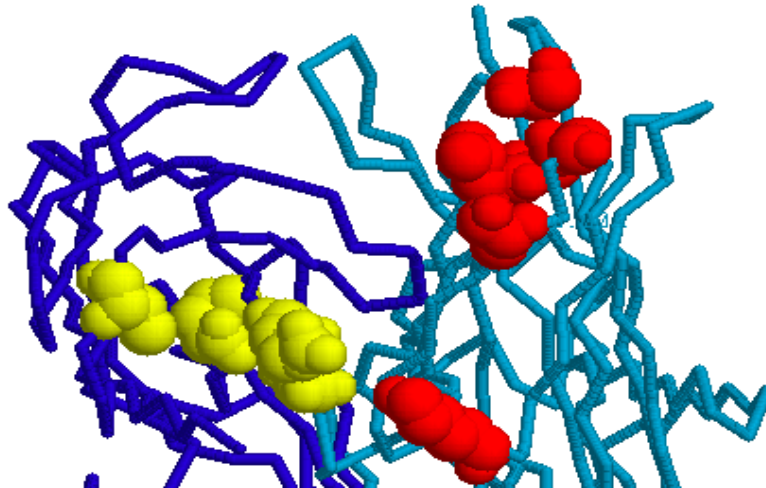
3. Humira (adalimumab)

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.