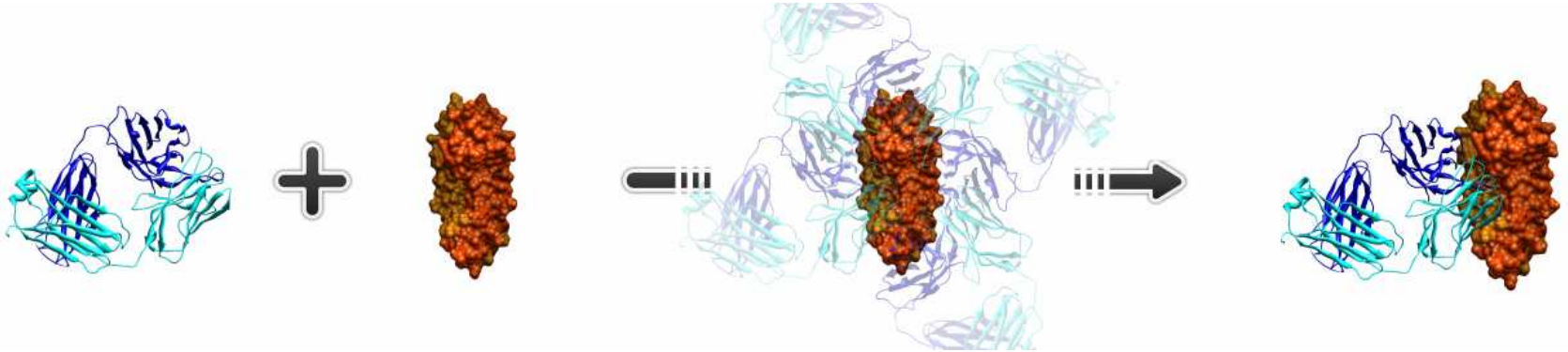
Rosetta Workshop

Aug 05, 2009

Modeling antibody-antigen complexes to improve biotherapeutics



Andrea Rossi

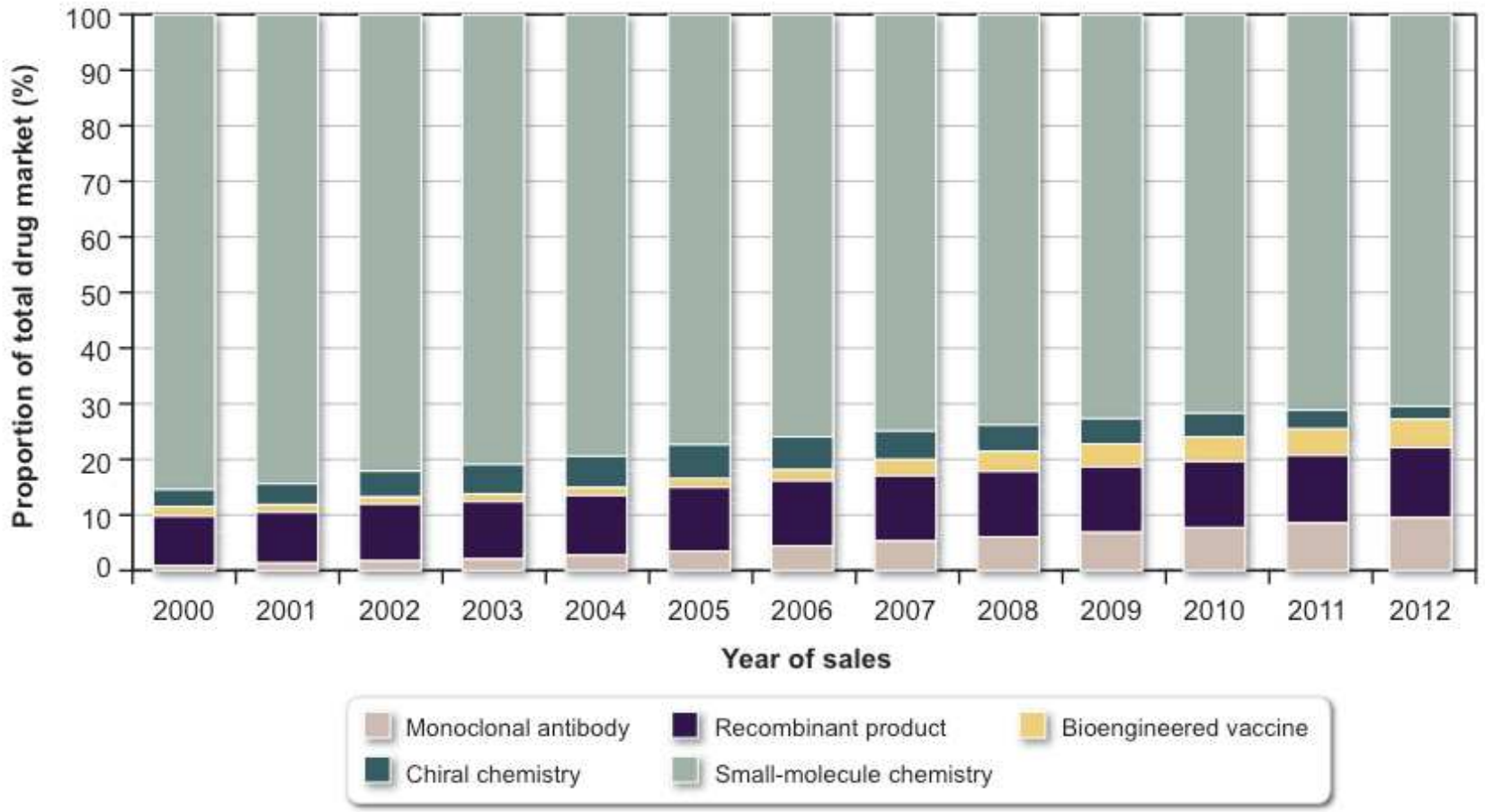
Overview

- Introduction
  - Rinat and the therapeutic antibody engineering field
  - Needs for epitope determination
  - Methods for epitope determination
- Protein-protein docking
  - The platform for protein-protein docking
  - An example success
  - Using the Amazon Cloud
  - Platform to model ab-ag complexes
- Assessing the platform
  - Ab modeling
  - Ab-ag benchmark
  - Preliminary results
- Need for experimental restraints
  - Collaboration with UCSF
  - Pepscan
  - Sidec
- Conclusion

Rinat/Pfizer

- Rinat span off from Genentech in 2001 to continue the neuroscience program
- Bought by Pfizer in May 2006 for two drugs:
  - anti-NGF for acute and chronic pain and
  - anti-amiloyd beta for Alzheimer's disease.
- Currently antibodies engineering hub for Pfizer with about 80 people (mostly scientists)
- Located in South San Francisco is part of a large biotech cluster

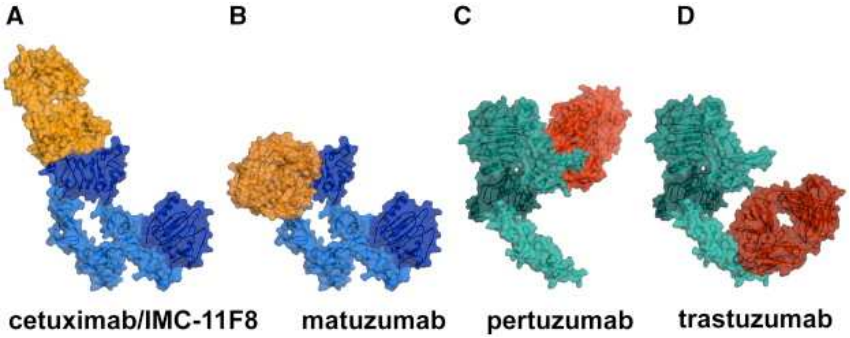
Therapeutical antibodies market

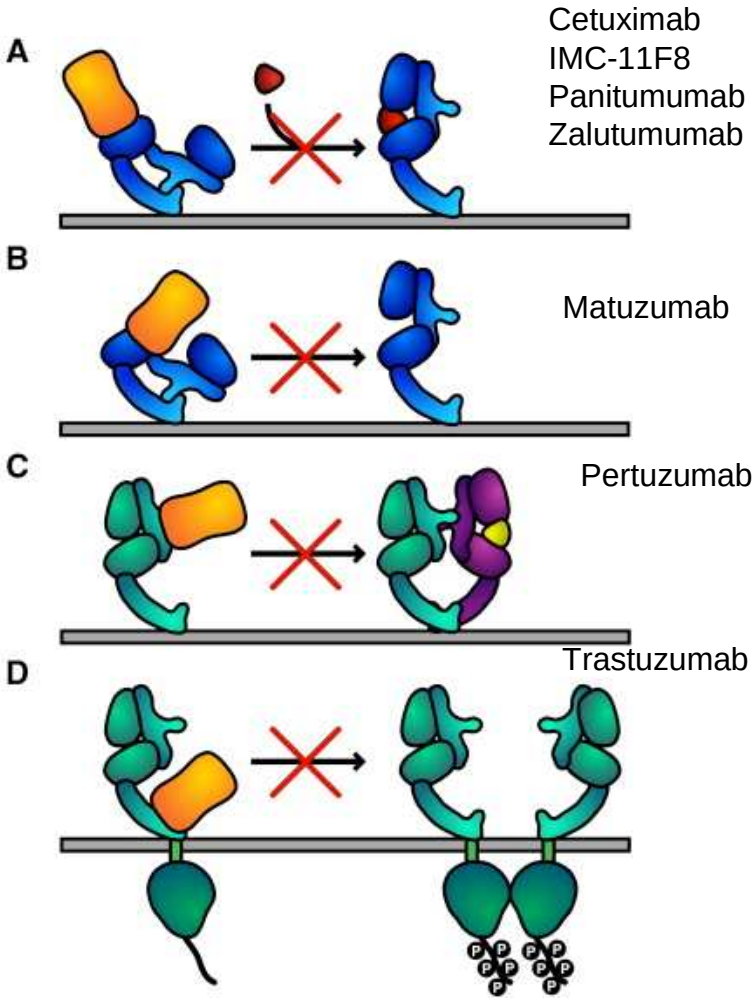


Approved therapeutical antibodies

Antibody ↓	Approval date ↑	Type ↓	Target ↓	Approved treatment(s) ↓	Structure
Muromonab-CD3	1986	murine	T cell CD3 Receptor	Transplant rejection	NA
Abciximab	1994	chimeric	inhibition of glycoprotein IIb/IIIa	Cardiovascular disease	NA
Daclizumab	1997	humanized	IL-2Rα receptor (CD25)	Transplant rejection	NA
Rituximab	1997	chimeric	CD20	Non-Hodgkin lymphoma	NA
Basiliximab	1998	chimeric	IL-2Rα receptor (CD25)	Transplant rejection	NA
Infliximab	1998	chimeric	inhibition of TNF-α signaling	Several autoimmune disorders	NA
Palivizumab	1998	humanized	an epitope of the RSV F protein	Respiratory Syncytial Virus	NA
Trastuzumab	1998	humanized	ErbB2	Breast cancer	2004; Genentech
Gemtuzumab	2000	humanized	CD33	Acute myelogenous leukemia (with calicheamicin)	NA
Alemtuzumab	2001	humanized	CD52	Chronic lymphocytic leukemia	NA
Adalimumab	2002	human	inhibition of TNF-α signaling	Several auto-immune disorders	NA
Efalizumab	2002	humanized	CD11a	Psoriasis	2009; Chinese Academy Sciences
Ibritumomab tiuxetan	2002	murine	CD20	Non-Hodgkin lymphoma (with yttrium-90 or indium-111)	NA
Tositumomab	2003	murine	CD20	Non-Hodgkin lymphoma	NA
Bevacizumab	2004	humanized	Vascular endothelial growth factor (VEGF)	Colorectal cancer	NA
Cetuximab	2004	chimeric	epidermal growth factor receptor	Colorectal cancer, Head and neck cancer	2005; Univ Pennsilvania
Omalizumab	2004	humanized	immunoglobulin E (IgE)	mainly allergy-related asthma	NA
Natalizumab	2006	humanized	alpha-4 (α4) integrin,	Multiple sclerosis and Crohn's disease	NA
Panitumumab	2006	human	epidermal growth factor receptor	Colorectal cancer	NA
Ranibizumab	2006	humanized	Vascular endothelial growth factor A (VEGF-A)	Macular degeneration	NA
Eculizumab	2007	humanized	Complement system protein C5	Paroxysmal nocturnal hemoglobinuria	NA
Certolizumab pegol	2008	humanized	inhibition of TNF-α signaling	Crohn's disease	NA

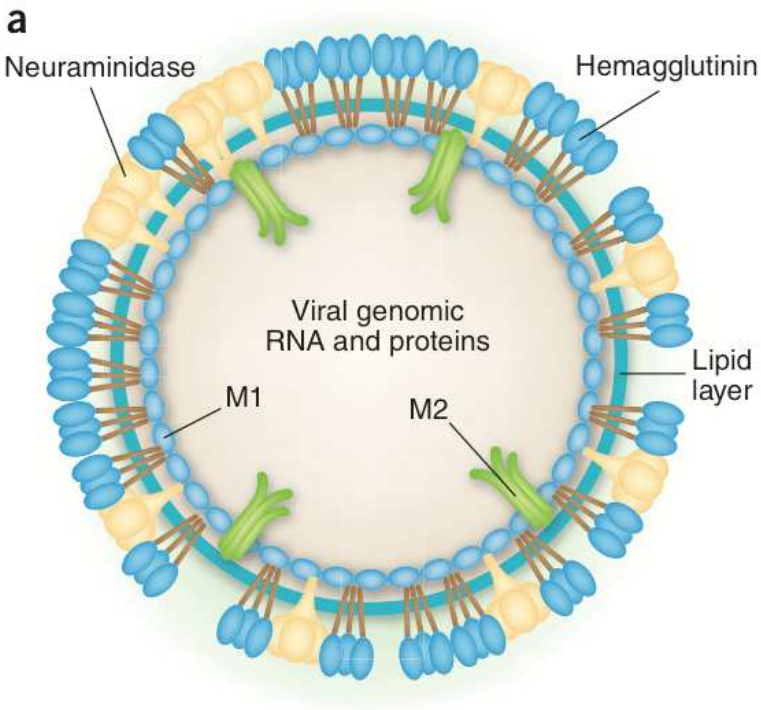
Mechanism of action: Antibodies against ErbB receptors

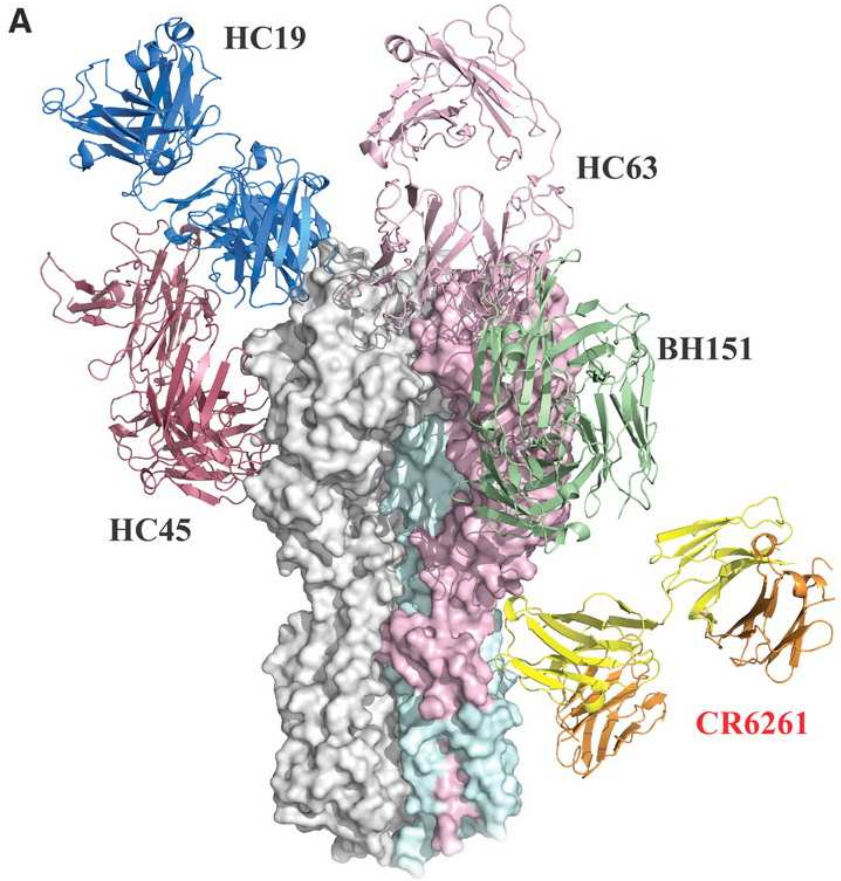




Schmitz KR, Ferguson KM., Exp Cell Res. 2009 315:659-70

Influenza virus:
An universal epitope





Ekiert DC et al., Antibody recognition of a highly conserved influenza virus epitope. Science. 2009 324:246-51

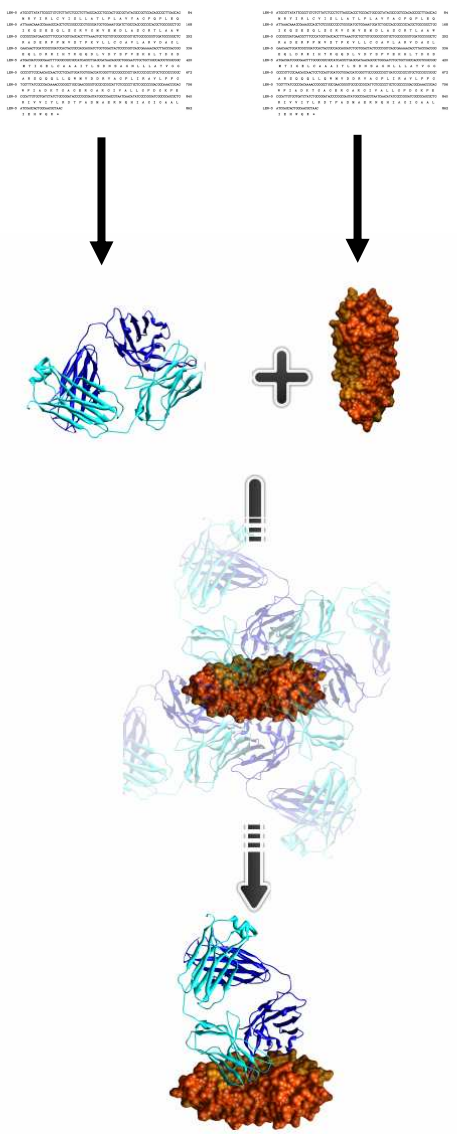
Sui J et al., Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. Nat Struct Mol Biol. 2009 16:265-73

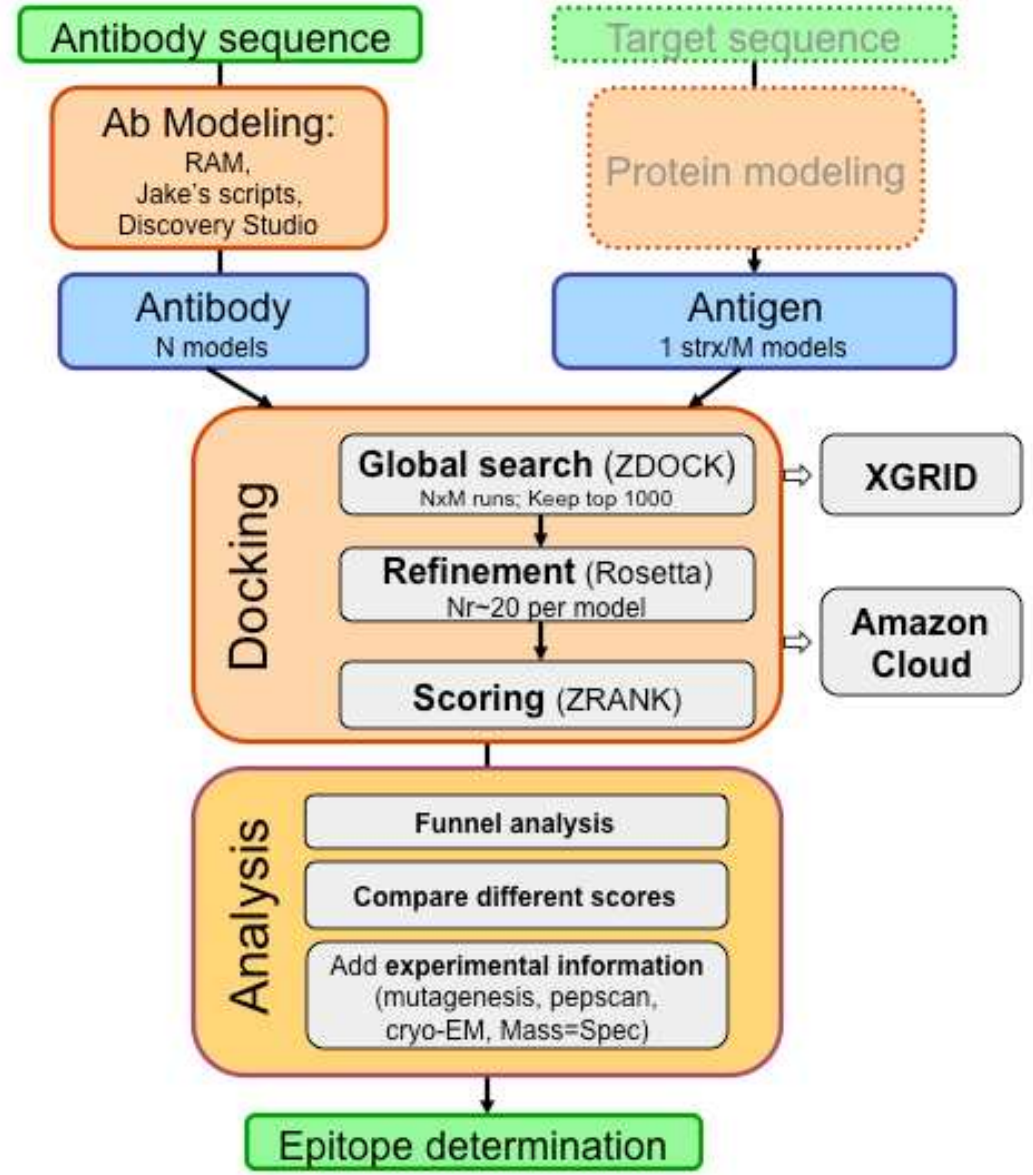
Epitope in mAb discovery

- Developing hypotheses for mechanism of action
- Strengthening intellectual property claims to efficacious molecules
- Aiding antibody selection for humanization
- Rational antibody design (humanization/optimization)
- Directing antibodies against a specific site
- In case of absence of function, study the coverage

Protein docking

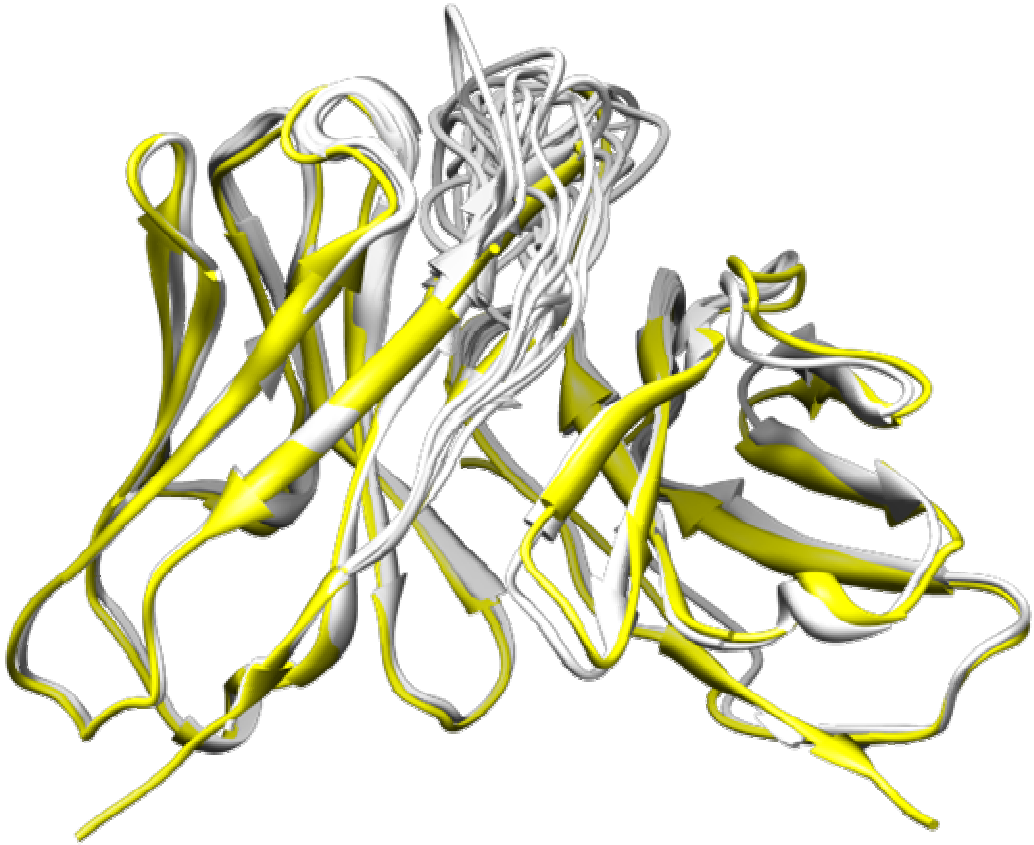
A protocol for docking



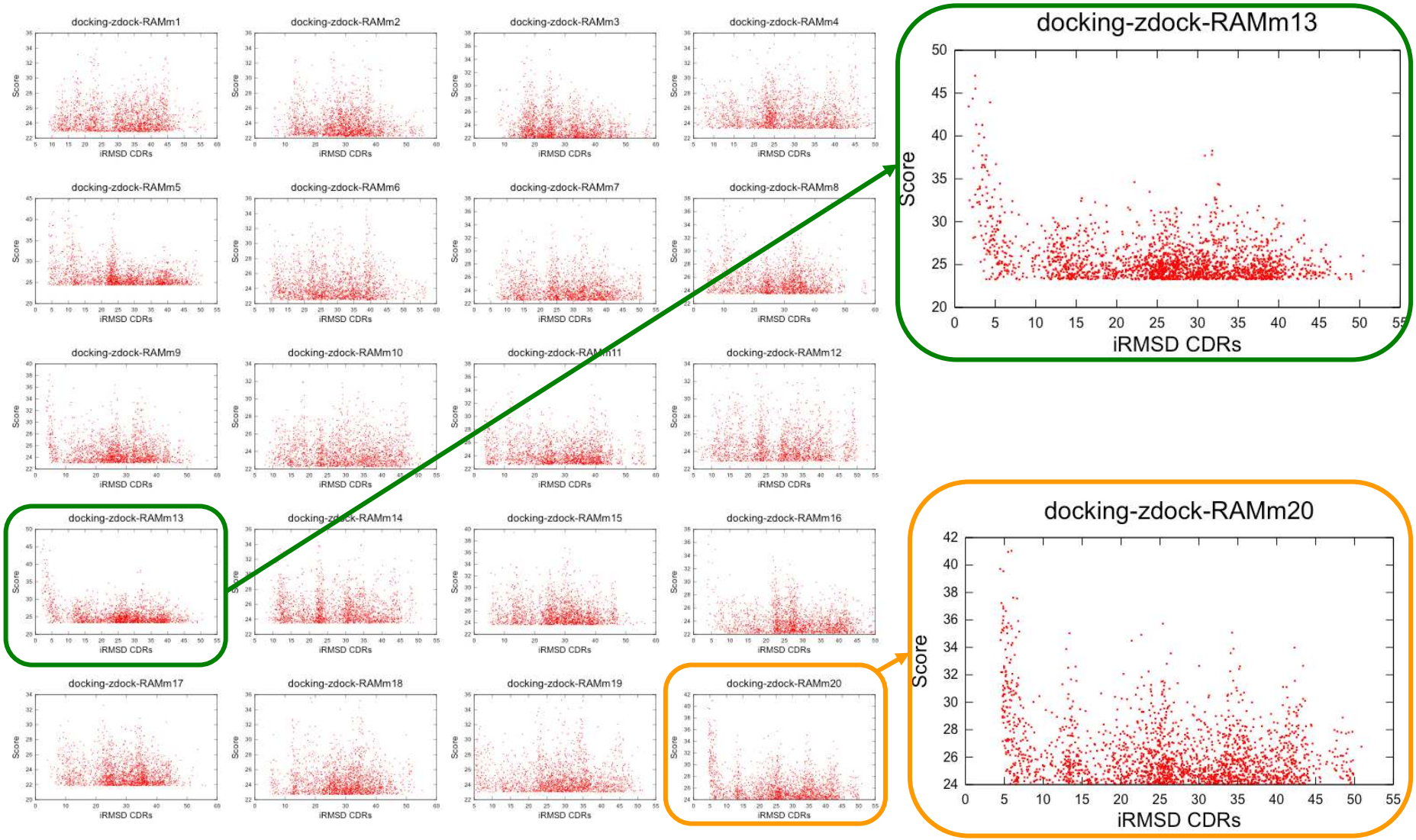


Antibody Modeling in Rosetta

- Automated generation of models
- Rosetta folding technology for H3 modeling



Docking multiple models



Clustering docking poses

	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C1
DOCK_1	7	8	8	2	1	3	1	1	1								
DOCK_2	1	3	5	3	4	3	4	1	1	5	1	3	2	1	1	1	
DOCK_3	2	2	5	13	5	2	1	1	1	1	1	1	1				
DOCK_4	9	3	3	2	3	2	1	2	2	1	1	1	1	1			
DOCK_5	11	7	6	8	4	2	9										
DOCK_6	2	2	8	3	3	2	1	1	2	2	1	1	1	2	1	1	
DOCK_7	13	2	2	5	1	1	3	1	4	2	1	1	1				
DOCK_8	2	4	10	3	4	2	4	4	1	1	1	1	1				
DOCK_9	9	7	1	2	3	3	10	1	2	1	1	2	1	1	1	1	
DOCK_10	5	3	1	7	6	2	1	1	1	1	1	1	1	1	1		
DOCK_11	7	2	2	8	7	2	4	2	3	1	1	1					
DOCK_12	4	2	4	2	4	1	5	3	2	4	1	1	1	1	1		
DOCK_13	14	15	2	3	1	1											
DOCK_14	2	3	6	3	7	2	2	1	1	2	1	1	1				
DOCK_15	4	3	3	11	1	1	1	1	1	1	1	2	2	1	2	1	1
DOCK_16	4	5	3	7	5	1	1	1	2	1							
DOCK_17	3	1	1	1	1	1	1	2	2	4	1	1	1	1	2	2	2
DOCK_18	1	7	4	2	2	2	4	2	5	1	1	1	2	1	1	1	1
DOCK_19	9	6	1	2	2	3	5	1	2	1	1	3	1	1	1	1	1
DOCK_20	21	12	2	2	2	1	1	1	1								

Microsoft Excel - Cluster13.htm

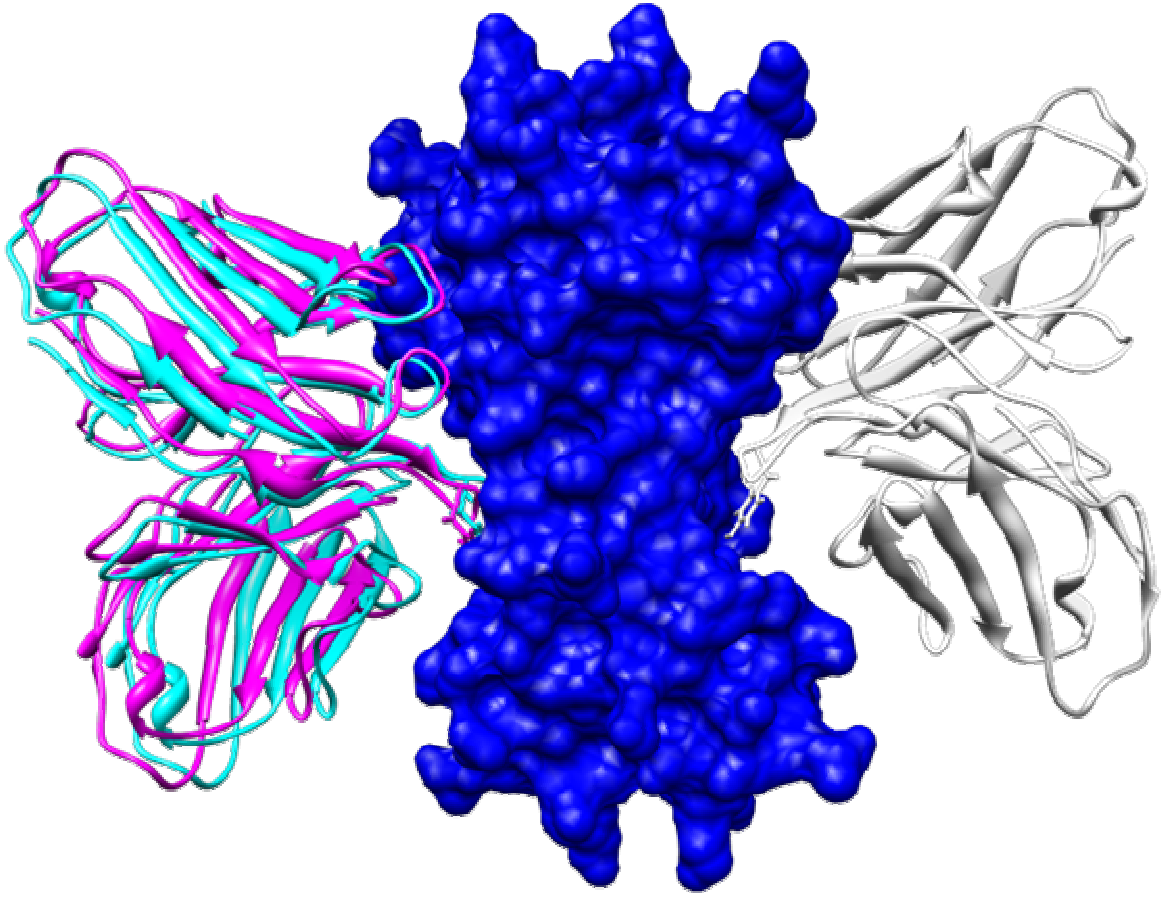
	A	B	C
1	Cluster #1	14 members	complex.1.pdb complex.5.pdb complex.6.pdb complex.7.pdb complex.8.pdb complex.15.pdb complex.18.pdb complex.20.pdb complex.23.pdb complex.24.pdb complex.25.pdb complex.33.pdb complex.34.pdb complex.35.pdb
2	Cluster #2	15 members	complex.2.pdb complex.3.pdb complex.4.pdb complex.9.pdb complex.10.pdb complex.12.pdb complex.14.pdb complex.17.pdb complex.19.pdb complex.21.pdb complex.22.pdb complex.26.pdb complex.28.pdb complex.31.pdb complex.32.pdb
3	Cluster #3	2 members	complex.11.pdb complex.29.pdb
4	Cluster #4	3 members	complex.13.pdb complex.16.pdb complex.30.pdb
5	Cluster #5	1 members	complex.27.pdb
6	Cluster #6	1 members	complex.36.pdb

Microsoft Excel - Cluster13.htm

	A	B	C
1	Cluster #1	21 members	complex.1.pdb complex.2.pdb complex.4.pdb complex.5.pdb complex.8.pdb complex.13.pdb complex.14.pdb complex.17.pdb complex.18.pdb complex.20.pdb complex.22.pdb complex.23.pdb complex.24.pdb complex.25.pdb complex.30.pdb complex.31.pdb complex.33.pdb complex.34.pdb complex.35.pdb complex.39.pdb complex.42.pdb
2	Cluster #2	12 members	complex.3.pdb complex.6.pdb complex.7.pdb complex.9.pdb complex.10.pdb complex.11.pdb complex.12.pdb complex.15.pdb complex.19.pdb complex.21.pdb complex.28.pdb complex.43.pdb
3	Cluster #3	2 members	complex.16.pdb complex.41.pdb
4	Cluster #4	2 members	complex.26.pdb complex.40.pdb
5	Cluster #5	2 members	complex.27.pdb complex.38.pdb
6	Cluster #6	1 members	complex.29.pdb
7	Cluster #7	1 members	complex.32.pdb
8	Cluster #8	1 members	complex.36.pdb
9	Cluster #9	1 members	complex.37.pdb

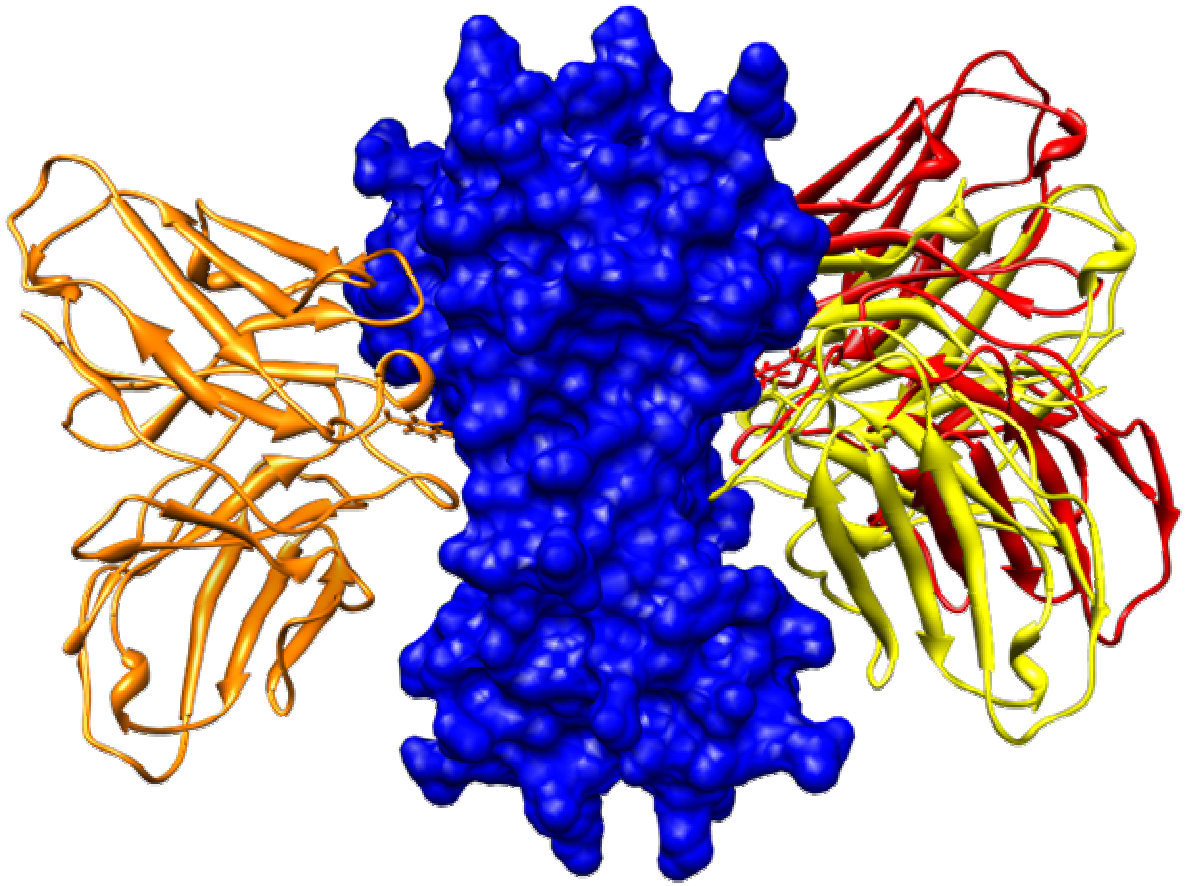
Docking model RAMm13

First top three complexes

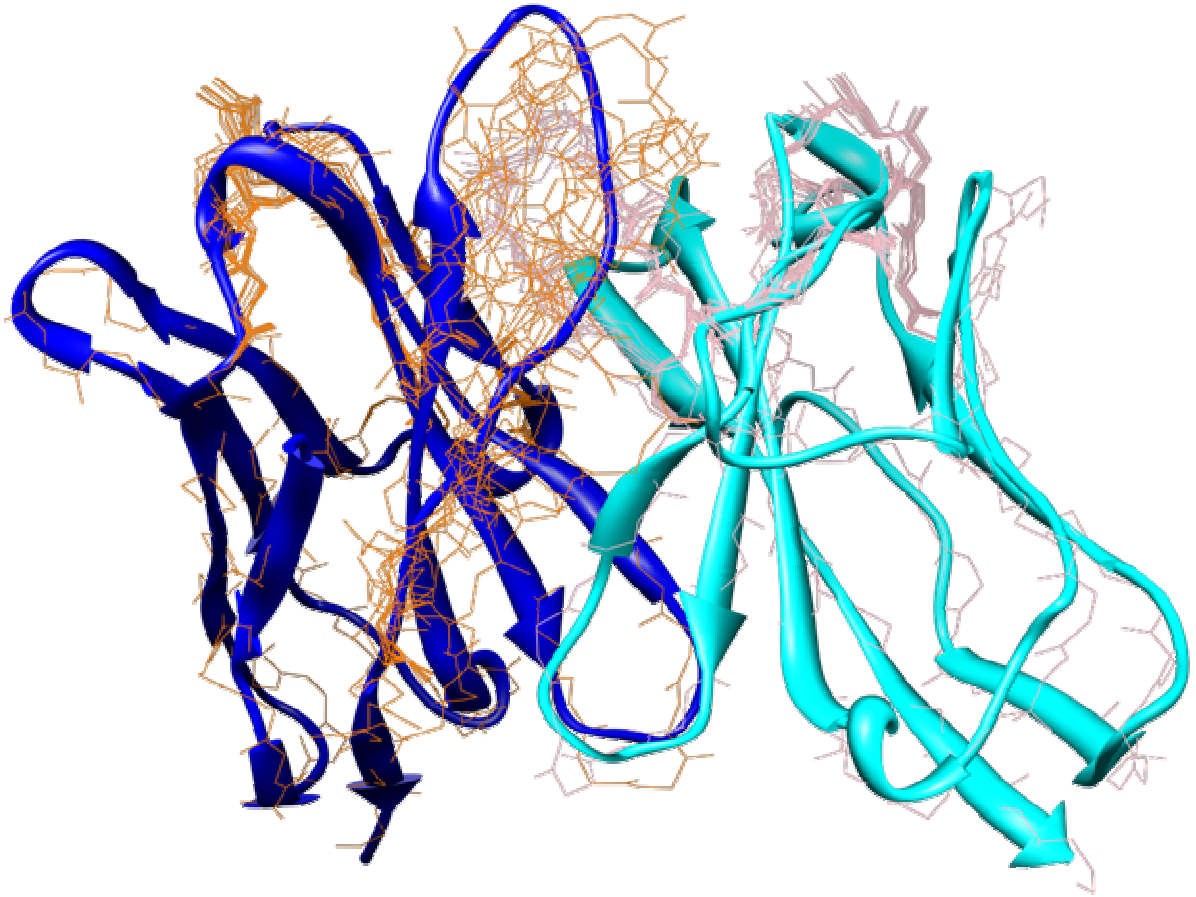


Docking model RAMm20

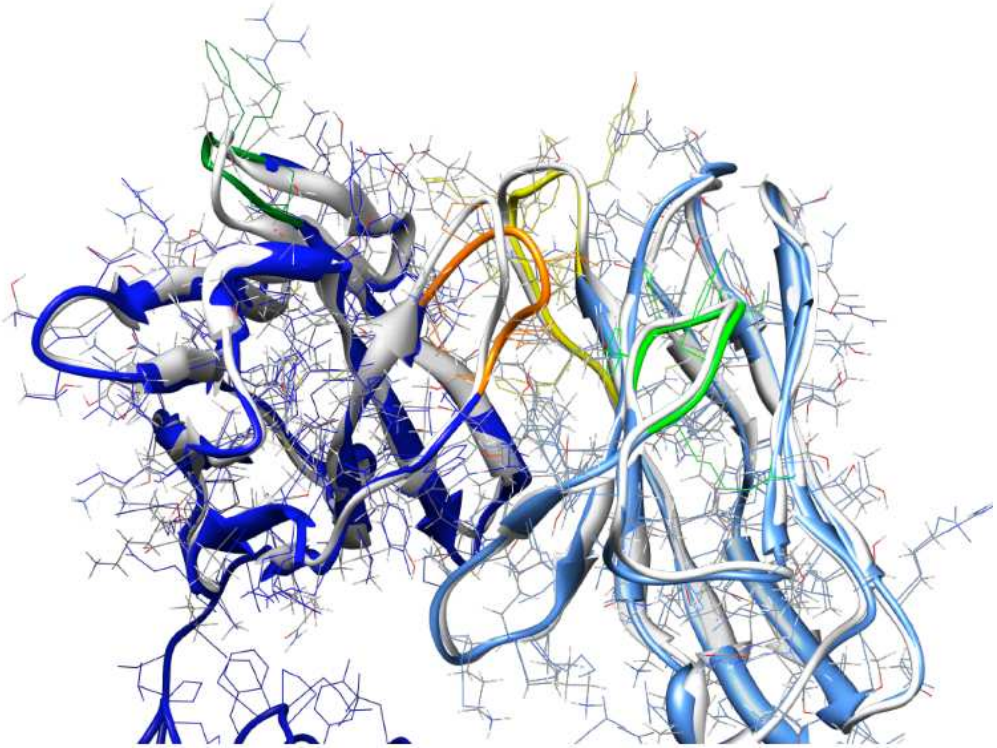
First top three complexes



Amgen-mab1: Modeling doesn't work

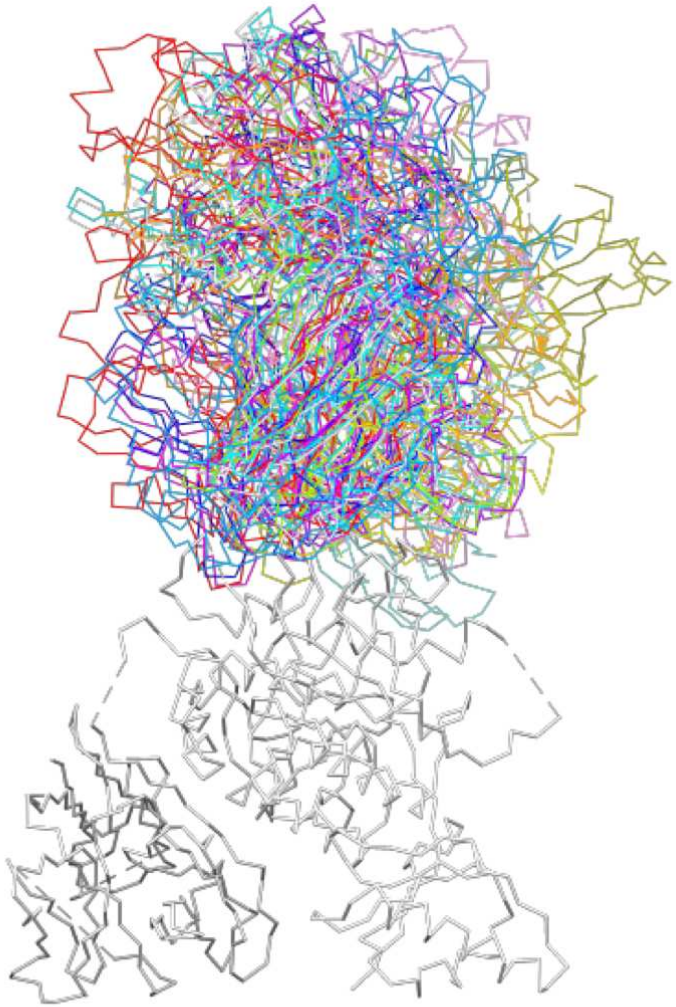


Modeling Amgen-mab2: A successful example



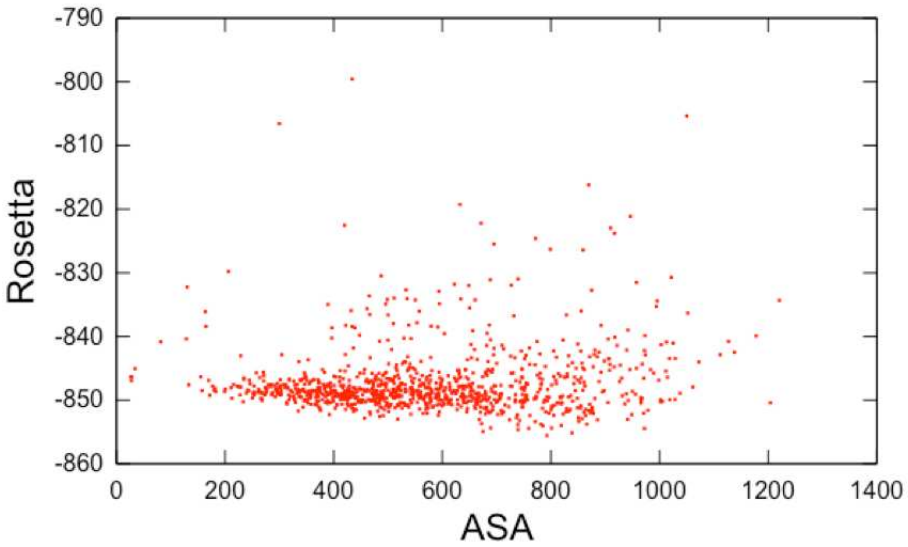
- RAM top model is very close to bound Xtal structure
- Are these deviations enough for docking to fail?

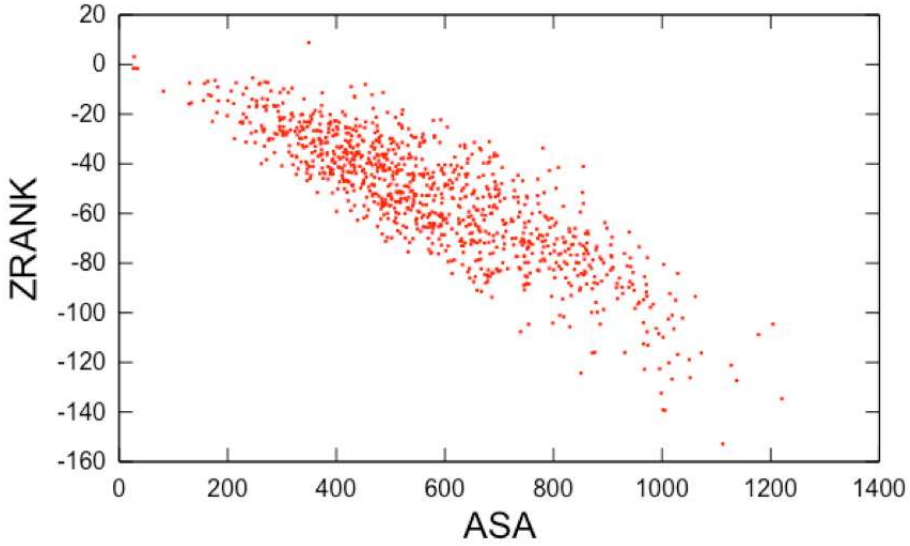
PCSK9-Docking mAb2 (Perturbation)

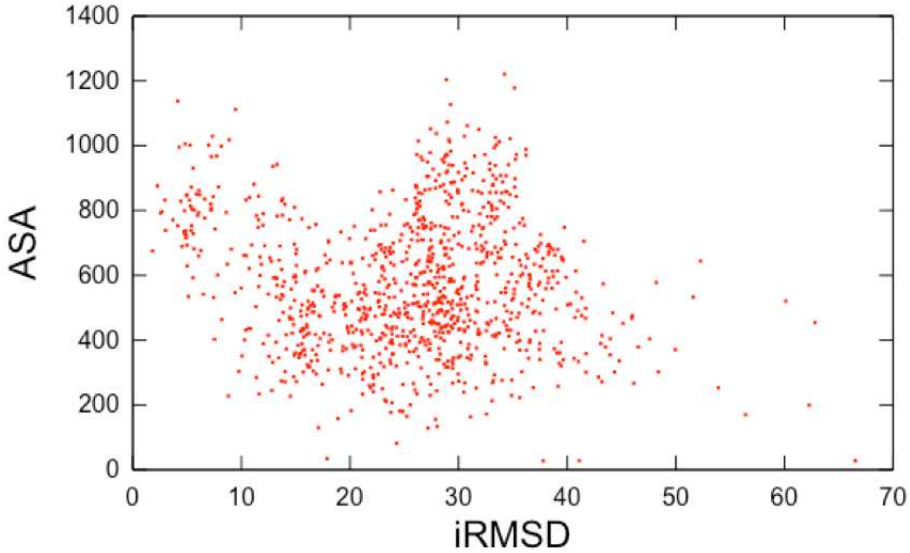


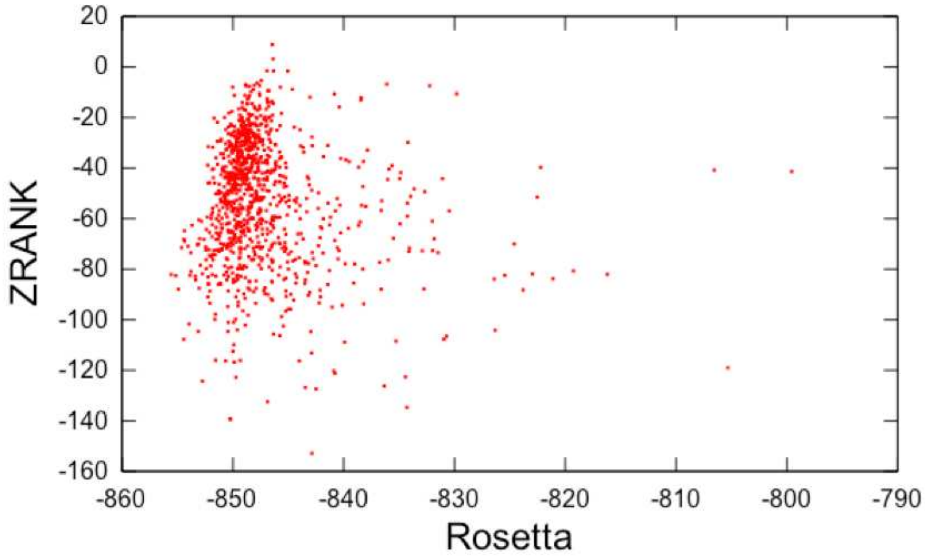
- Perturbation studies allow detailed exploration of scoring function landscape close to the native
- It allows to decompose the scoring function from the optimization protocol

Rosetta Score vs ZRANK









The dataset

Bound		Unbound					
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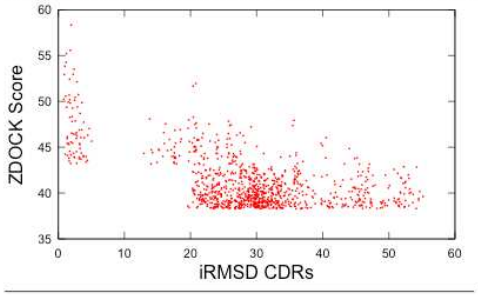
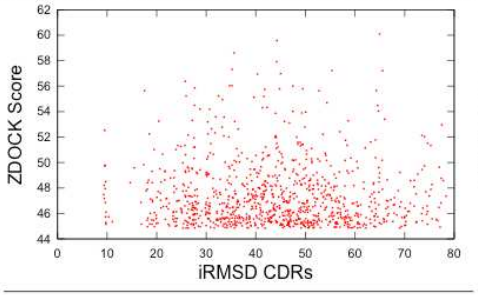
Pdb Id Complex	Cat	Pdb1	Desc 1	Pdb2	Desc 2	RMSD	ASA
1AHW_AB:C	A	1FGN_LH	Fab 5g9	1TFH_A	Tissue factor	0.69	1899
1BGX_HL:T	A	1AY1_HL	Fab	1CMW_A	Taq polymerase	1.48	5814
1BJ1_HL:VW	AB	1BJ1_HL	Fab	2VPF_GH	vEGF	0.5	1731
1BVK_DE:F	A	1BVL_BA	Fv Hulys11	3LZT_	HEW lysozyme	1.24	1321
1DQJ_AB:C	A	1DQQ_CD	Fab Hyhel63	3LZT_	HEW lysozyme	0.75	1765
1E6J_HL:P	A	1E6O_HL	Fab	1A43_	HIV-1 capsid protein p24	1.05	1245
1FSK_BC:A	AB	1FSK_BC	Fab	1BV1_	Birch pollen antigen Bet V1	0.45	1623
1I9R_HL:ABC	AB	1I9R_HL	Fab	1ALY_ABC	Cd40 ligand	1.3	1498
1IQD_AB:C	AB	1IQD_AB	Fab	1D7P_M	Factor VIII domain C2	0.48	1976
1JPS_HL:T	A	1JPT_HL	Fab D3H44	1TFH_B	Tissue factor	0.51	1852
1K4C_AB:C	AB	1K4C_AB	Fab	1JVM_ABCD	Potassium Channel Kcsa	0.53	1601
1MLC_AB:E	A	1MLB_AB	Fab44.1	3LZT_	HEW lysozyme	0.6	1392
1NCA_HL:N	AB	1NCA_HL	Fab	7NN9_	Flu virus neuraminidase N9	0.24	1953
1NSN_HL:S	AB	1NSN_HL	Fab N10	1KDC_	Staphylococcal nuclease	0.35	1776
1QFW_HL:AB	AB	1QFW_HL	Fv	1HRP_AB	Human chorionic gonadotropin	1.31	1580
1QFW_IM:AB	AB	1QFW_IM	Fv	1HRP_AB	Human chorionic gonadotropin	0.73	1637
1VFB_AB:C	A	1VFA_AB	Fv D1.3	8LYZ_	HEW lysozyme	1.02	1383
1WEJ_HL:F	A	1QBL_HL	Fab E8	1HRC_	Cytochrome C	0.31	1177
2FD6_HL:U	A	2FAT_HL	Plasminogen receptor Ab	1YWH_A	Plasminogen activator receptor	1.07	1139
2HMI_CD:AB	AB	2HMI_CD	Fab 28	1S6P_AB	HIV1 reverse transcriptase	2.26	1234
2JEL_HL:P	AB	2JEL_HL	Fab Jel42	1POH_	HPr	0.17	1501
2VIS_AB:C	A	1GIG_LH	Fab	2VIU_ACE	Flu virus hemagglutinin	0.8	1296

1AHW

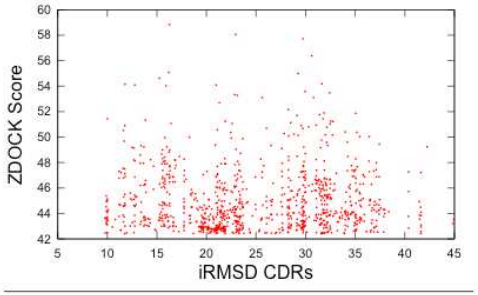
1AHW b



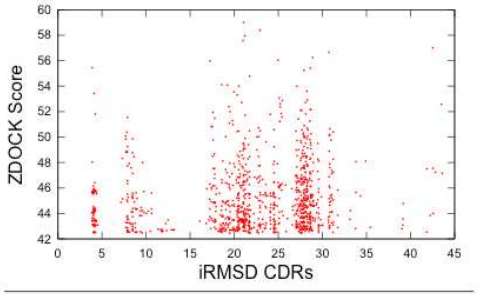
1AHW u



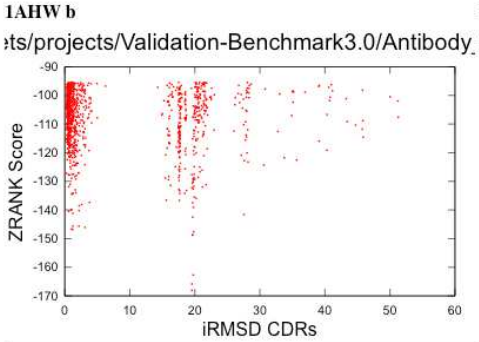
1AHW mb



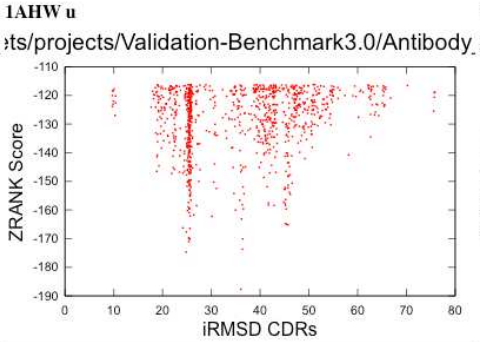
1AHW mu



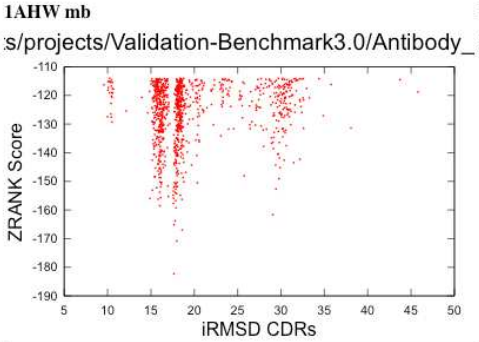
1AHW b



1AHW u



1AHW mb



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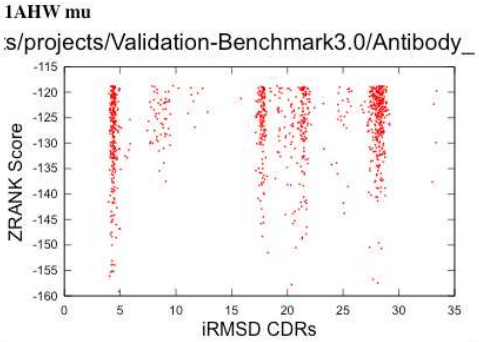


Analysis

B=Bound,
U=Unbound,
MU=Model Bound,
MB=Model Unbound

rossia07@chime:/tools/datasets/projects/Validation-Benchmark3.0/Monitor

File Edit View Window Help

```
rossia07@chime:/tools/datasets/projects/Validation-Benchmark3.0/Monitor> ./stat-project.pl
1AHW <B>  1.9  0.9  0.8  30  50  51  64 100  <U> 65.0 25.8  9.5   0   0   1   4  75  <MB> 16.3 11.8 10.0   0   0   0  33 100  <MU> 21.1  3.9  3.9   0   3   9  24 100
1BVK <B> 22.9 21.4 14.6   0   0   0  17 100  <U> 17.5 16.2  9.7   0   0   1  35 100  <MB> 24.1 11.3  0.0   2   2   2  20 100  <MU> 27.6 23.1  0.0   1   1   1   2 100
1FSK <B>  0.9  0.9  0.9  52  67  67  74 100  <U>  2.0  1.9  1.3  23  37  37  38  93  <MB> 28.5  6.6  6.5   0   0  19  35 100  <MU>  7.6  7.0  6.7   0   0  29  58 100
1JPS <B>  0.8  0.8  0.6  19  19  20  36 100  <U> 38.8 31.8 25.9   0   0   0   0  75  <MB> 26.5 24.9 23.2   0   0   0   0  99  <MU> 27.6  8.8  8.8   0   0   2  49 100
1NCA <B>  1.3  1.3  1.2  27  29  29  37 100  <U> 60.4 19.8  3.8   0   5   5  10  60  <MB> 39.1 12.2  9.3   0   0   1  17 100  <MU> 17.1 12.1 12.1   0   0   0  28 100
1VFB <B> 32.0 12.3 12.1   0   0   0  18 100  <U> 31.0 17.2  9.4   0   0   2  32 100  <MB> 14.7 13.9 10.5   0   0   0  39 100  <MU> 29.7 19.9 11.3   0   0   0  12 100
2HMI <B> 23.1 19.3 19.3   0   0   0   4  63  <U> 97.5 58.6 54.5   0   0   0   0   0  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
2VIS <B> 29.5 25.7  1.0   1   1   6  34 100  <U> 42.1 42.1 42.1   0   0   0   0 100  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1BGX <B>  1.5  0.8  0.8  12  21  23  29  86  <U> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MB> 59.2 53.2 43.9   0   0   0   0  13  <MU> 18.6 18.6 11.2   0   0   0  11  99
1DQJ <B>  1.0  1.0  1.0  24  30  30  63 100  <U> 42.0 26.1 16.5   0   0   0   4  67  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1I9R <B> 53.3 25.2 17.6   0   0   0   4  78  <U> 70.8 56.5 35.1   0   0   0   0  20  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1K4C <B>  1.2  1.0  1.0  47  52  52  62 100  <U> 34.2 31.2 31.2   0   0   0   0 100  <MB> 33.5 32.0 31.1   0   0   0   0 100  <MU> 31.3 16.8 10.1   0   0   0  14  99
1NSN <B> 18.1 15.8  0.9   5   8   8  44 100  <U> 56.3 26.2 14.0   0   0   0   2  77  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1WEJ <B>  1.0  0.9  0.9  15  19  20  31 100  <U> 56.4 26.9  4.4   0   1   1   5  74  <MB> 21.4 16.8  6.0   0   0   7  24 100  <MU> 25.4 11.6  8.3   0   0   7  32 100
2JEL <B>  5.3  5.3  0.9   8  17  28  34 100  <U>  0.0  0.0  0.0   1   2   2  15  95  <MB> 18.9  7.9  0.0   1   1   6  81 100  <MU> 19.5  6.5  6.5   0   0   1  78 100
1BJ1 <B>  1.3  0.8  0.8  44  45  45  45  55  <U> 31.9 25.5  5.3   0   0   1   4  88  <MB> 16.8 13.5  9.6   0   0   2  64 100  <MU> 19.1  9.6  9.6   0   0   1  66 100
1E6J <B> 54.6 21.5  1.0   6   6   6   7  57  <U> 50.8 49.5 27.2   0   0   0   0  11  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1IQD <B>  0.9  0.8  0.8  38  47  47  55 100  <U> 38.3 31.1 23.7   0   0   0   0  81  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1MLC <B>  1.3  0.9  0.9  28  30  30  46 100  <U> 53.1 22.3  1.7   2   5   5  10  87  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
1QFW <B> 53.0  1.3  0.8   9   9   9  18  86  <U> 37.0 18.1  6.1   0   0   1   8 100  <MB> 43.4 32.6 19.4   0   0   0   2  91  <MU> 40.5 32.8 13.7   0   0   0   8  90
2FD6 <B> 22.7  7.6  0.9   5   7  39  48 100  <U> 47.1 34.2 24.3   0   0   0   0  83  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
2QFW <B> 16.5  1.3  1.2   8   9   9  33 100  <U> 19.7 14.9 14.3   0   0   0  33  99  <MB> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1  <MU> -1.0 -1.0 -1.0  -1  -1  -1  -1  -1
rossia07@chime:/tools/datasets/projects/Validation-Benchmark3.0/Monitor>
```

Best RMSD in top (1,10,100) predictions

Number of models in top 100 predictions with an RMSD less than (2.5,5,10,50,100)

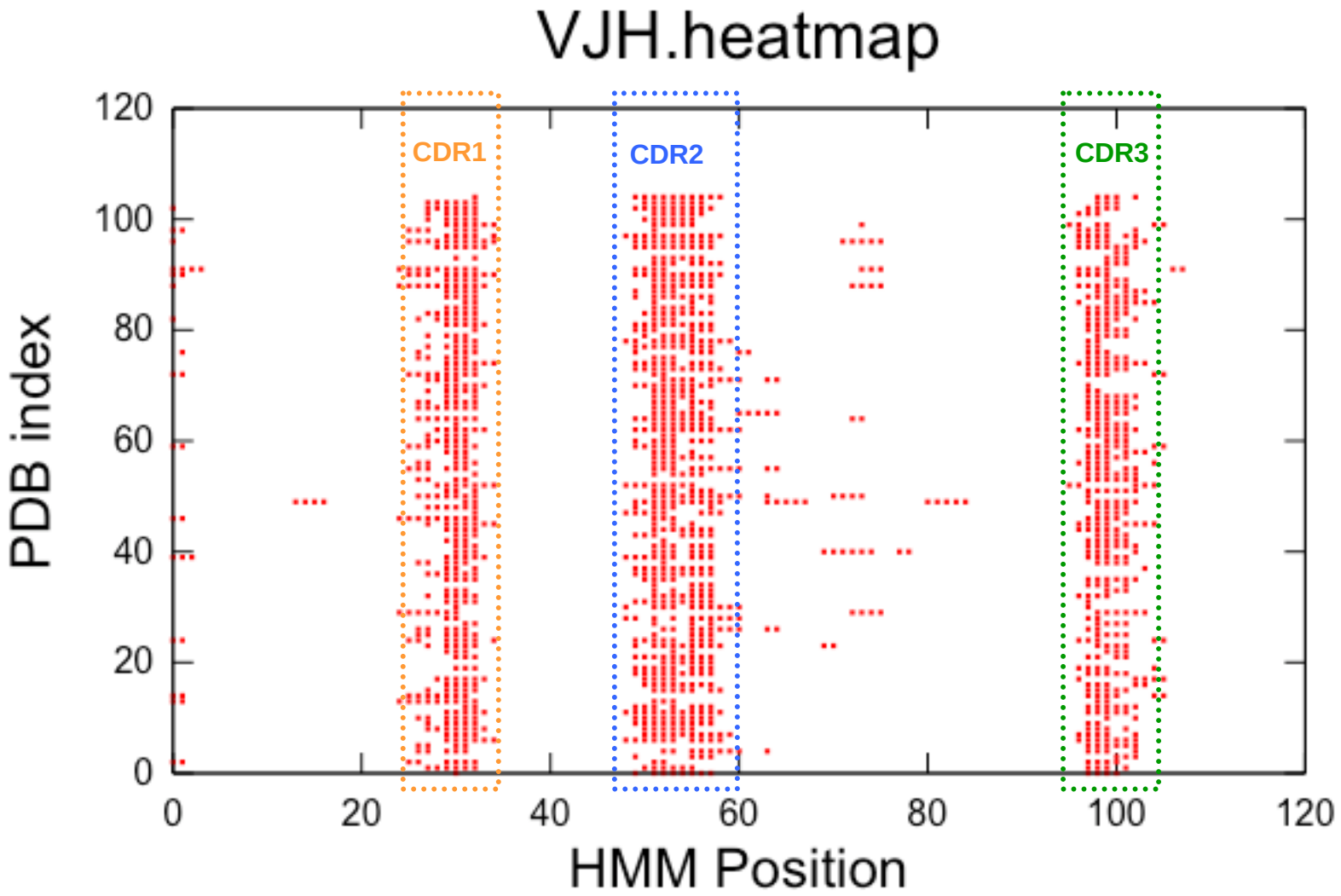
COMPLEX	b_zdock	b_refine	u_zdock	u_refine	mb_zdock	mb_refine	mu_zdock	mu_refine
1AHW	51	45	1	0	0	0	9	42
1BVK	0	0	1	0	2	0	1	0
1FSK	67	38	37	22	19	17	29	28
1JPS	20	45	0	0	0	0	2	2
1NCA	29	70	5	2	1	0	0	1
1VFB	0	0	2	0	0	0	0	0
2HMI	0	-1	0	-1	-1	-1	-1	-1
2VIS	6	-1	0	-1	-1	-1	-1	-1
1BGX	23	-1	-1	-1	0	0	0	0
1DQJ	30	-1	0	-1	-1	-1	-1	-1
1I9R	0	0	0	0	-1	-1	-1	-1
1K4C	52	65	0	0	0	0	0	0
1NSN	8	6	0	0	-1	-1	-1	-1
1WEJ	20	24	1	0	7	6	7	2
2JEL	28	-1	2	0	6	1	1	0
1BJ1	45	48	1	-1	2	0	1	0
1E6J	6	-1	0	-1	-1	-1	-1	-1
1IQD	47	63	0	0	-1	-1	-1	-1
1MLC	30	55	5	0	-1	-1	-1	-1
1QFW	9	-1	1	-1	0	0	0	0
2FD6	39	57	0	0	-1	-1	-1	-1
2QFW	9	-1	0	0	-1	-1	-1	-1

Number of top 100 structures below 10.0A from native

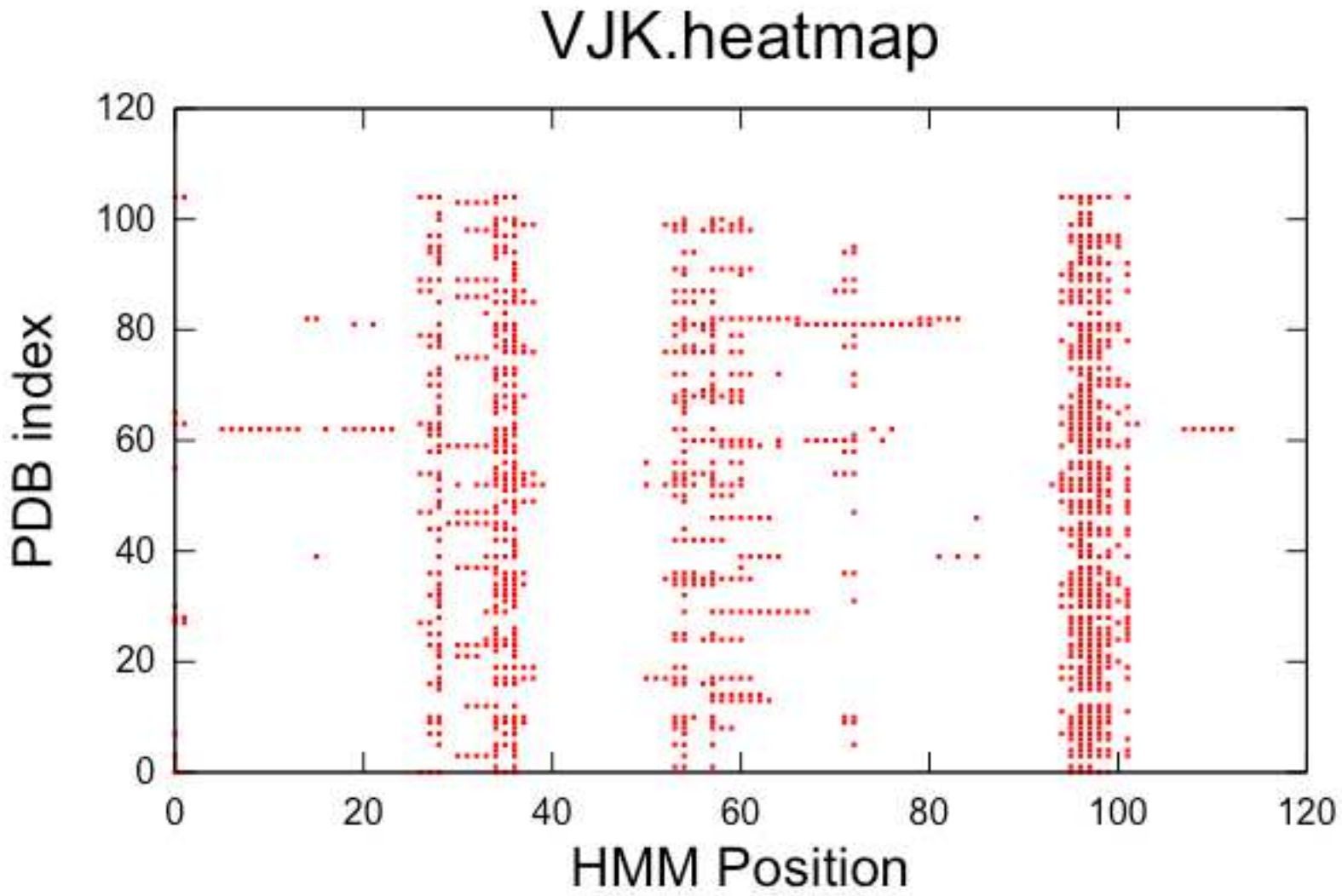
COMPLEX	b_zdock	b_refine	u_zdock	u_refine	mb_zdock	mb_refine	mu_zdock	mu_refine
1AHW	0.9	1.0	25.8	24.8	11.8	16.0	3.9	4.1
1BVK	21.4	20.2	16.2	19.7	11.3	25.1	23.1	23.4
1FSK	0.9	21.5	1.9	2.0	6.6	7.8	7.0	7.7
1JPS	0.8	0.6	31.8	28.9	24.9	25.8	8.8	14.1
1NCA	1.3	0.9	19.8	20.9	12.2	13.8	12.1	17.9
1VFB	12.3	23.2	17.2	20.0	13.9	18.2	19.9	24.3
2HMI	19.3	-1.0	58.6	-1.0	-1.0	-1.0	-1.0	-1.0
2VIS	25.7	-1.0	42.1	-1.0	-1.0	-1.0	-1.0	-1.0
1BGX	0.8	-1.0	-1.0	-1.0	53.2	46.5	18.6	18.3
1DQJ	1.0	-1.0	26.1	-1.0	-1.0	-1.0	-1.0	-1.0
1I9R	25.2	24.4	56.5	51.5	-1.0	-1.0	-1.0	-1.0
1K4C	1.0	31.2	31.2	32.6	32.0	33.7	16.8	24.4
1NSN	15.8	28.5	26.2	36.6	-1.0	-1.0	-1.0	-1.0
1WEJ	0.9	29.3	26.9	18.4	16.8	6.1	11.6	15.7
2JEL	5.3	-1.0	0.0	34.2	7.9	14.7	6.5	17.6
1BJ1	0.8	0.6	25.5	-1.0	13.5	14.0	9.6	14.7
1E6J	21.5	-1.0	49.5	-1.0	-1.0	-1.0	-1.0	-1.0
1IQD	0.8	0.8	31.1	28.6	-1.0	-1.0	-1.0	-1.0
1MLC	0.9	0.9	22.3	26.6	-1.0	-1.0	-1.0	-1.0
1QFW	1.3	-1.0	18.1	-1.0	32.6	20.7	32.8	16.7
2FD6	7.6	6.8	34.2	34.5	-1.0	-1.0	-1.0	-1.0
2QFW	1.3	-1.0	14.9	28.3	-1.0	-1.0	-1.0	-1.0

Min RMSD from native among top 10 conformations

Blocking VH


VJH.heatmap
CDR1
CDR2
CDR3
PDB index
HMM Position
0
20
40
60
80
100
120

Blocking VL



Resources

Name	Location	Software	CPUs	Disk	Pros	Cons
Clinical Grid	Groton	LSF	256?	Local	Available unless clinical trials	Difficult to install software; difficult to mount external hard drives
CamBlade (IBM)	Rinat+Cambridge	LSF	~20+40	Mount (local/remote)	Easy to install; available	Remote master is slow in writing on disk; remote slaves are extremely slow because limits in I/O.
Apple Grid	Rinat	XGRID	72 (powerful)	Mount (local)	Fast machines; lots of diskpace; easy to install;	Need to recompile most software;
Amazon Cloud	Distributed	SQS,S3,EC2,SimpleDB	~500	Distributed	Pay what you use – you built your cluster on the fly; virtual machines allows easy installation of virtually any software	Different paradigm - requires additional training (Bioteam); Not designed for scientific computing; Requires set of scripts to access the infromation

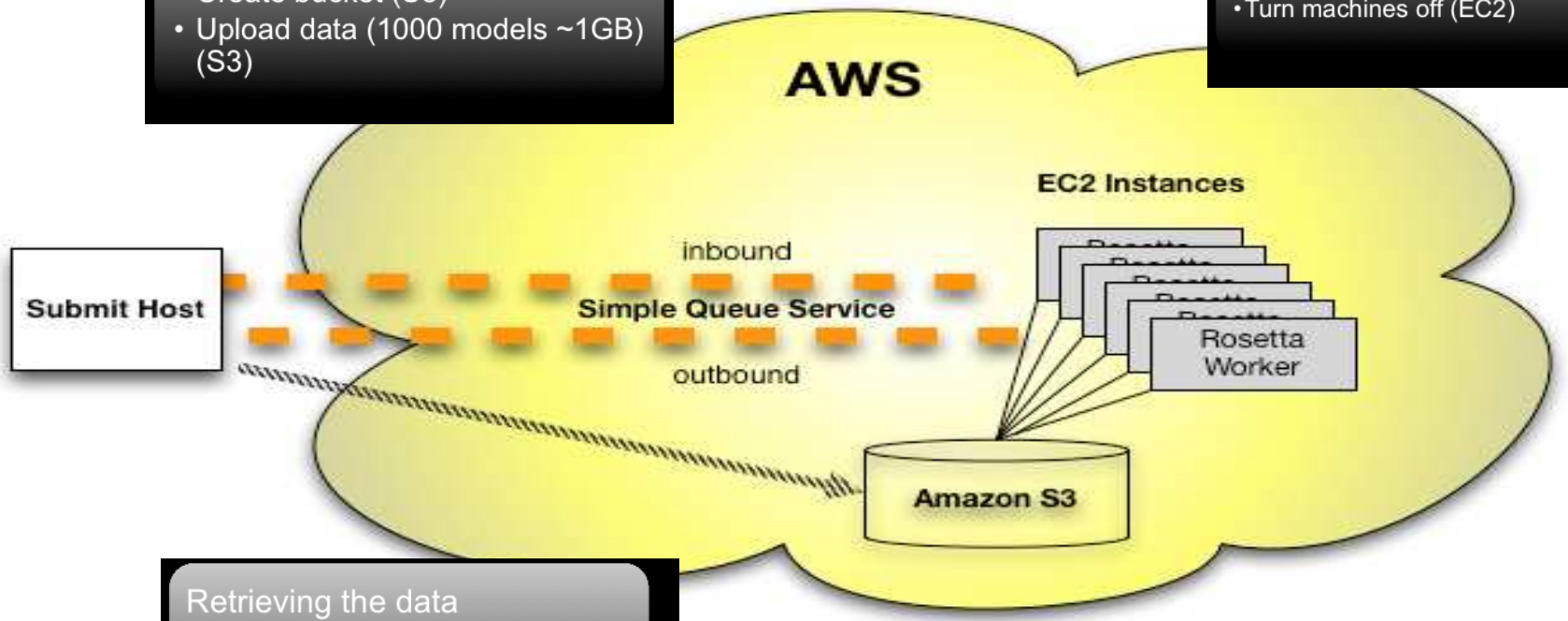
Amazon Cloud (Refinement)

Setting the system up

- Submit jobs (1000 jobs) (SQS)
- Create bucket (S3)
- Upload data (1000 models ~1GB) (S3)

Generating the data (20x models ~ 20GB)

- Turn virtual machines on (EC2)
- Monitor number of jobs and machines (EC2,S3,SQS)
- Turn machines off (EC2)



Retrieving the data

- Download zrank files (S3)
- Determine top 1000 pdb files
- Download top 1000 pdb files (S3)
- Clear queue (SQS)
- Clear bucket (S3)

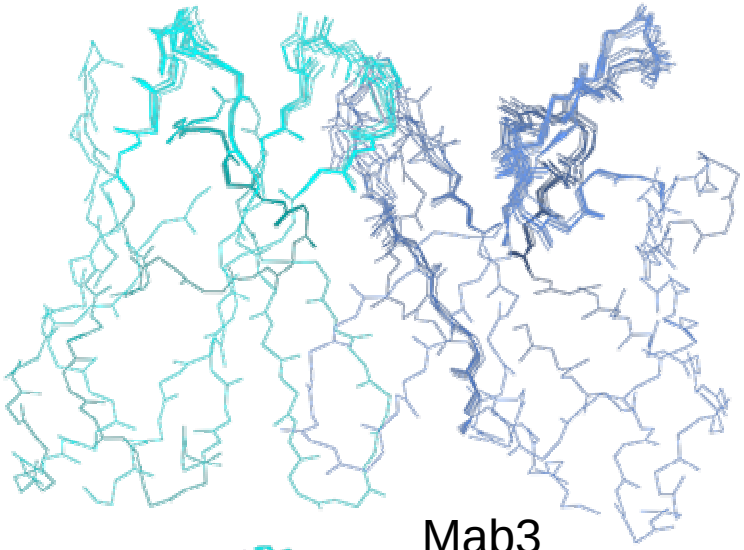
Partial conclusions

- Ab modeling + global docking is in general not a viable choice

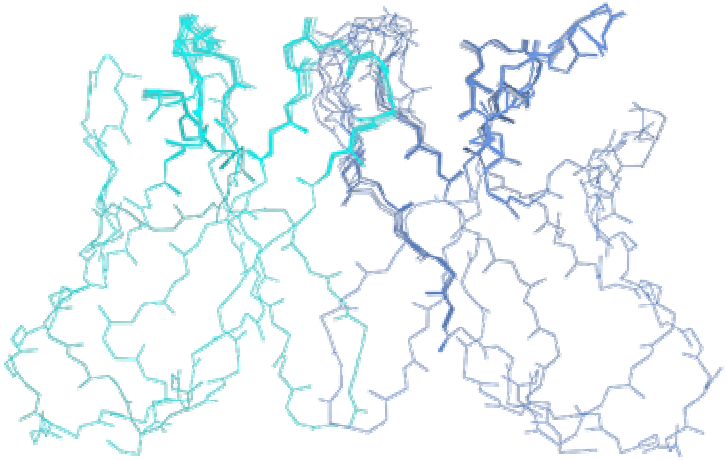
- Continuous improvement in the software RosettaAntibody, EnsembleDock, SnugDock
- We can leverage from biacore studies (in particular epitope binning)
- Experimental information

Docking multiple antibodies

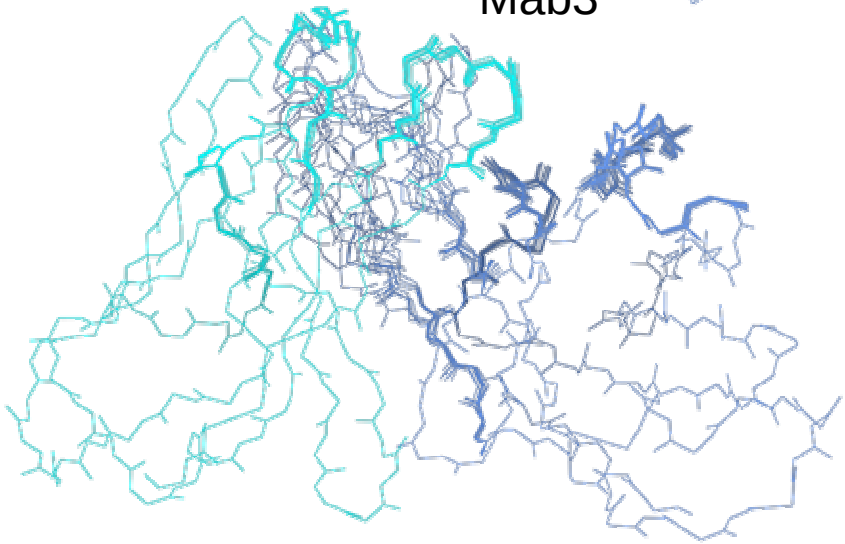
Mab1



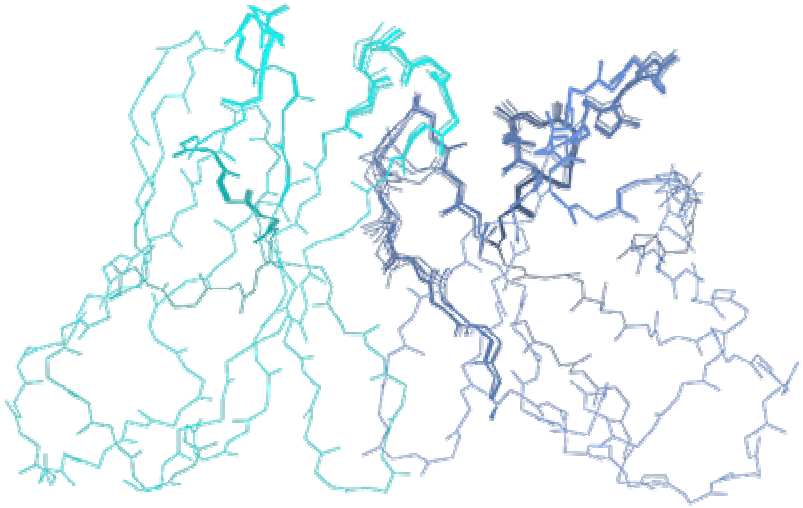
Mab2



Mab3

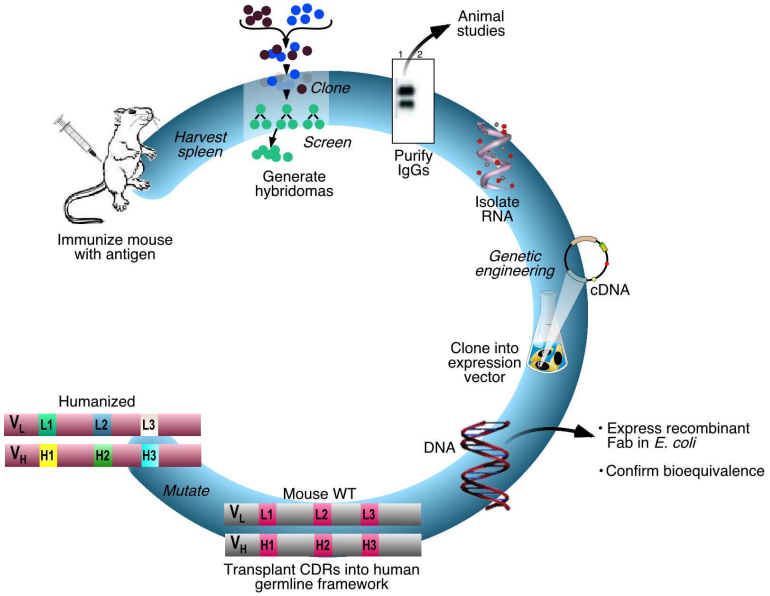


Mab4

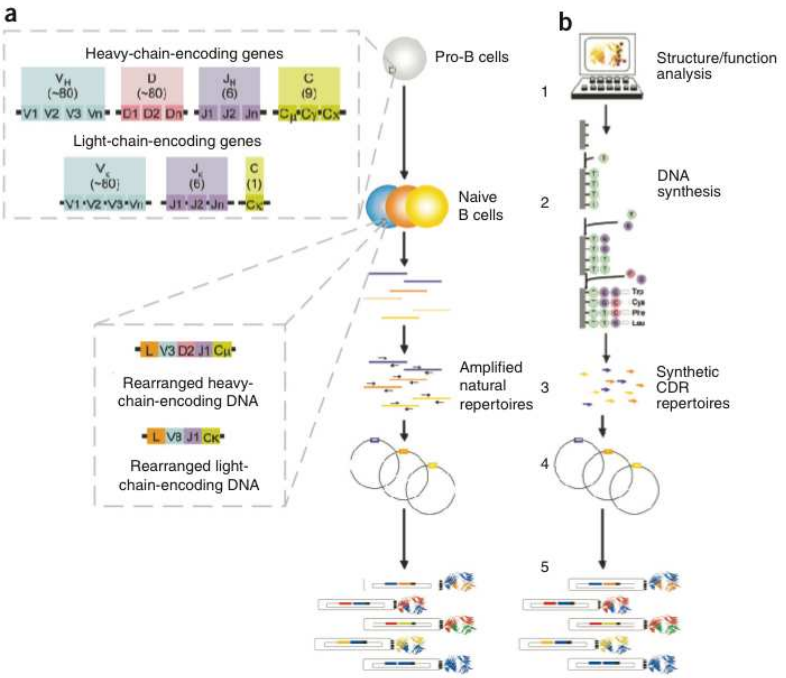


How do antibodies get “discovered”?

Hybridoma technology



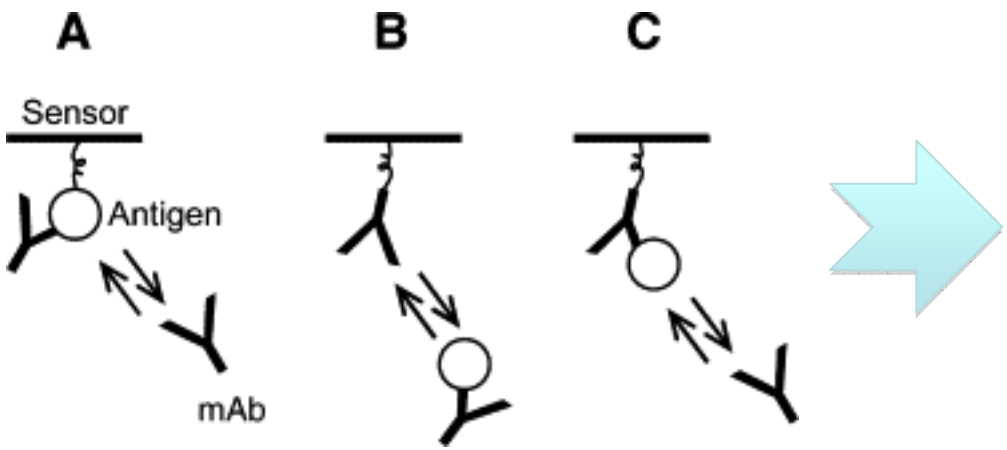
Display libraries
Synthetic libraries
Human libraries
Naïve libraries



Methods for epitope determination

Method	Time	Res.	Unc.	M. Abs	Pros	Cons
Peptides	~1 m	Low	Low	Yes	Fast and reliable	Linear epitopes only
Mass-spec (i.e., H/D exchange, chem. mod., e.g. ExSAR)	~3-6months	Low	Medium	Yes/No	Reasonably fast; potentiality for improvement	Requires data interpretation
Conf restrained peptides (e.g., Pepscan)	~2 ms	Low	High	Yes	Extends previous method - Potentially works for any type of binding site	High degree of uncertainty
Mutagenesis	~3months	Low	Medium	Yes	General approach Provides detailed binding information	Time consuming; Need data interpretation
X-Ray	6-12months	Highest	None	No	The gold standard	Requires crystallization; Present a static image of the complex
SAXS	1month	M/H	Medium	Yes	Doesn't require crystallization; can process multiple abs	Doesn't work for flexible molecules; requires data interpretation
NMR	3-12mt	High	Low	Yes/No		
Electron Microscopy	3-9months	Lowest	Low	No	No need to crystallize; single particles; capture flexibility of the complex	Expensive; needs further development
Protein-protein docking	1week	Highest	High	Yes	Superfast and high resolution if enough computer power available; provides additional information of the interaction; allows processing multiple abs	Success depends on correctly modeling individual components; force field not accurate enough for certain interactions (water)

Binning mabs by SPR

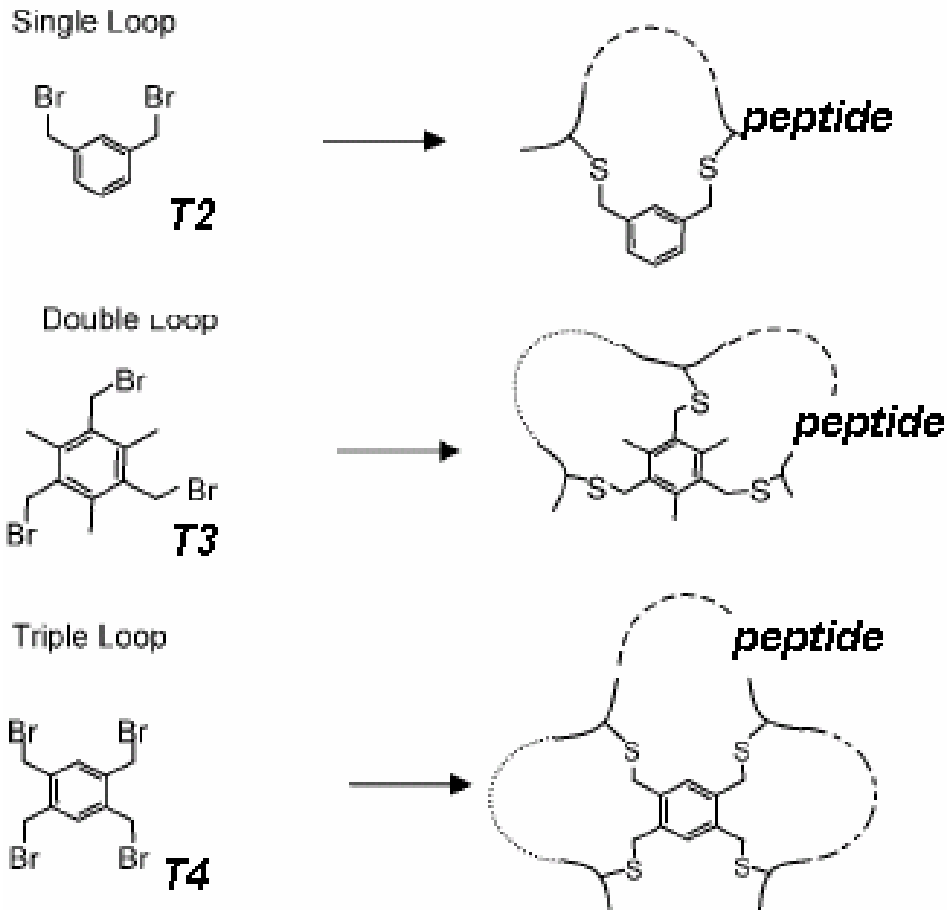


Saturating mAb	Competing mAb				
	2	3	4	5	6
1	Y	Y	Y	N	N
2	Y	Y	Y	Y	Y
3	Y	Y	Y	N	N
4	Y	Y	Y	Y	Y
5	Y	N	Y	Y	Y
6	Y	N	Y	Y	Y
7	Y	Y	Y	Y	Y

Peptide scanning: beyond linear epitopes

Basis of the CLIPS technology

spacially defined peptides to mimic complex protein structures.



CLIPS, Chemically LInked Peptides on Scaffolds.

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Pavel Strop

Rosetta Community

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David Baker