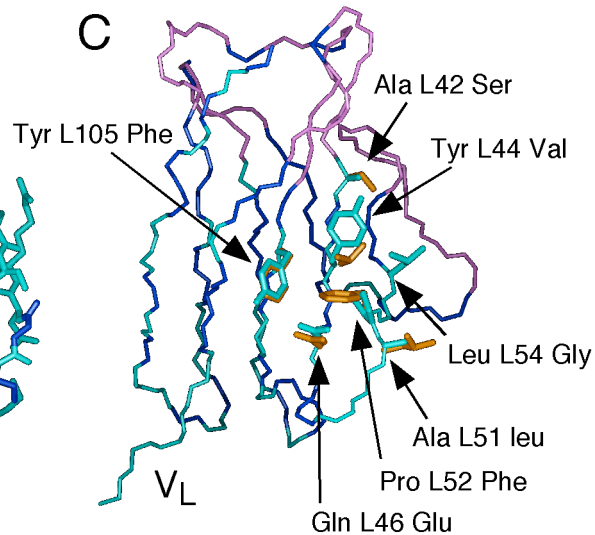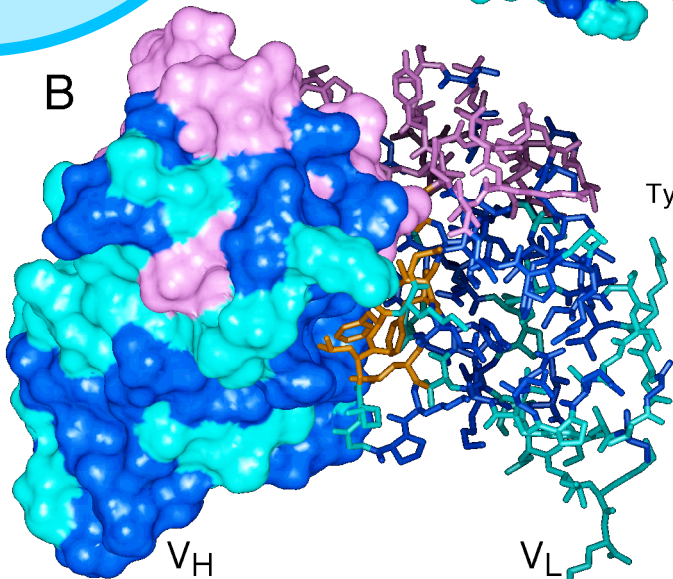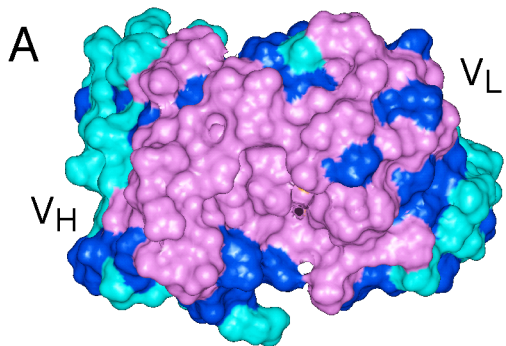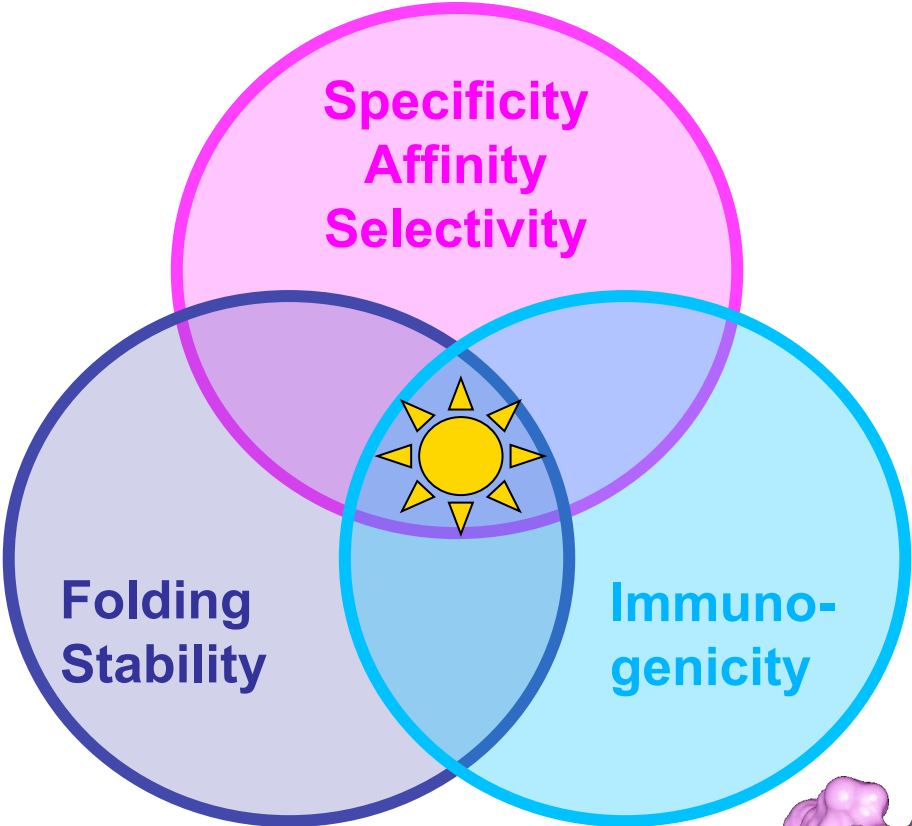**Recent applications of bioinformatics
to antibody engineering**

A second look at antibody humanization by CDR-Graft

Reasons why CDR grafts fail

Annemarie Honegger,
EMBL-EBI Industry Programme Workshop: Antibody Informatics 10-11 July 2012

CDR Grafts

A

V_L

V_H

B

V_H

V_L

C

Tyr L105 Phe

Ala L42 Ser

Tyr L44 Val

Leu L54 Gly

Ala L51 leu

Pro L52 Phe

Gln L46 Glu

V_L

J.Biol.Chem. 275, 2795-803 (2000)
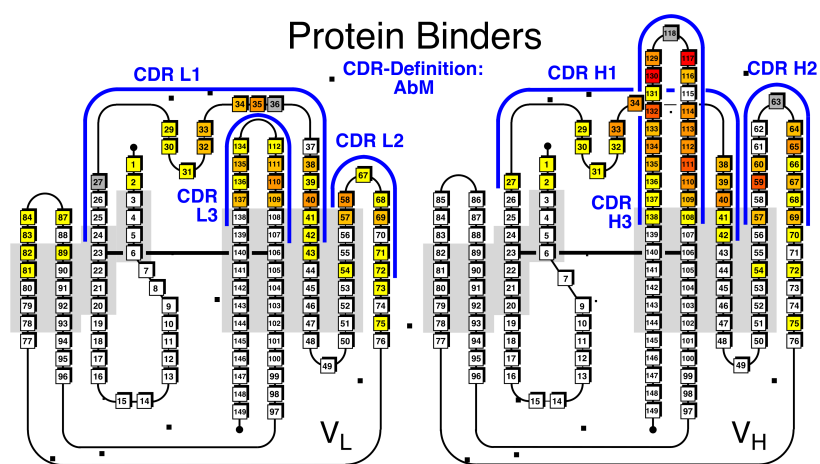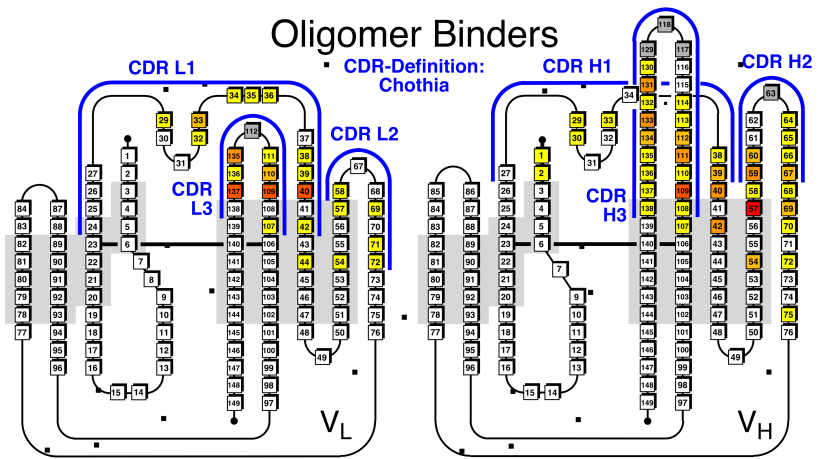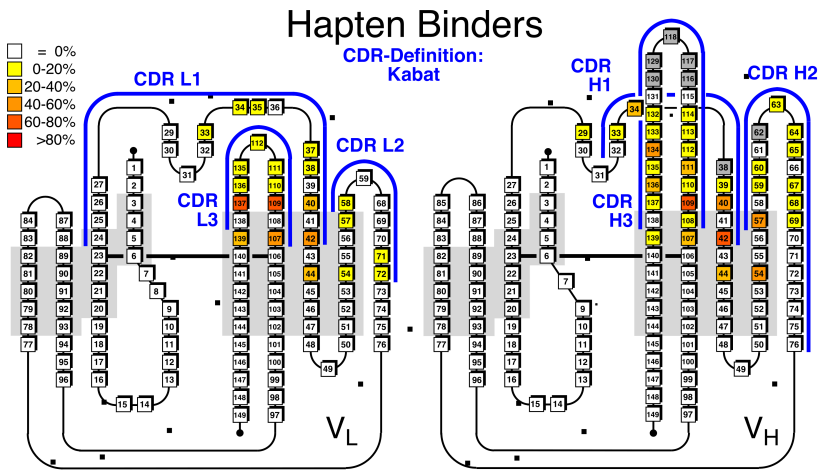
Specificity
Affinity
Selectivity

Folding
Stability

Immuno-
genicity

Upper core packing

upper core

charge cluster

lower core

central core

V_H V_L
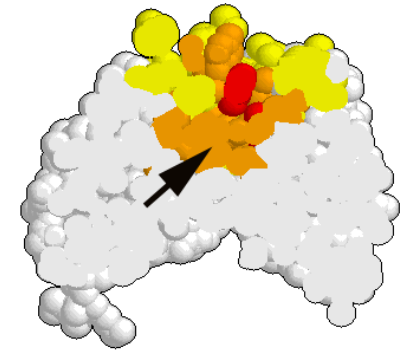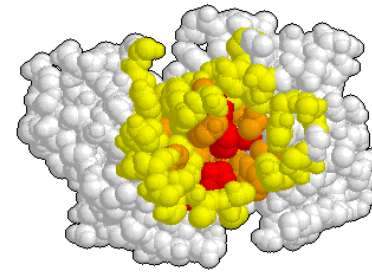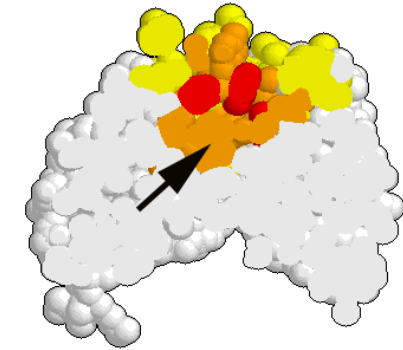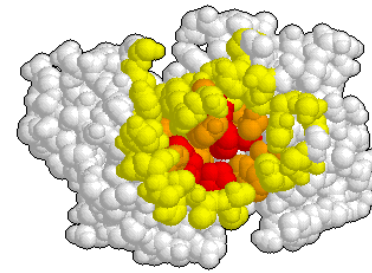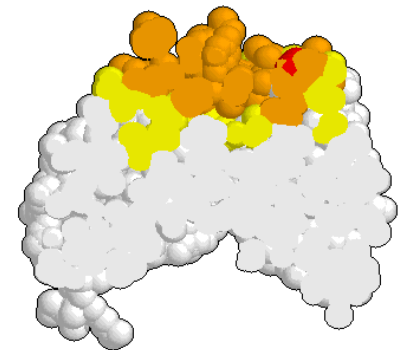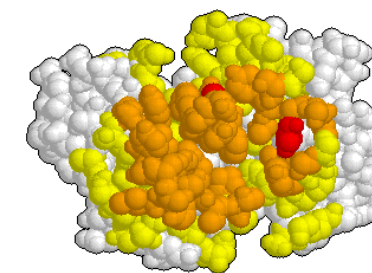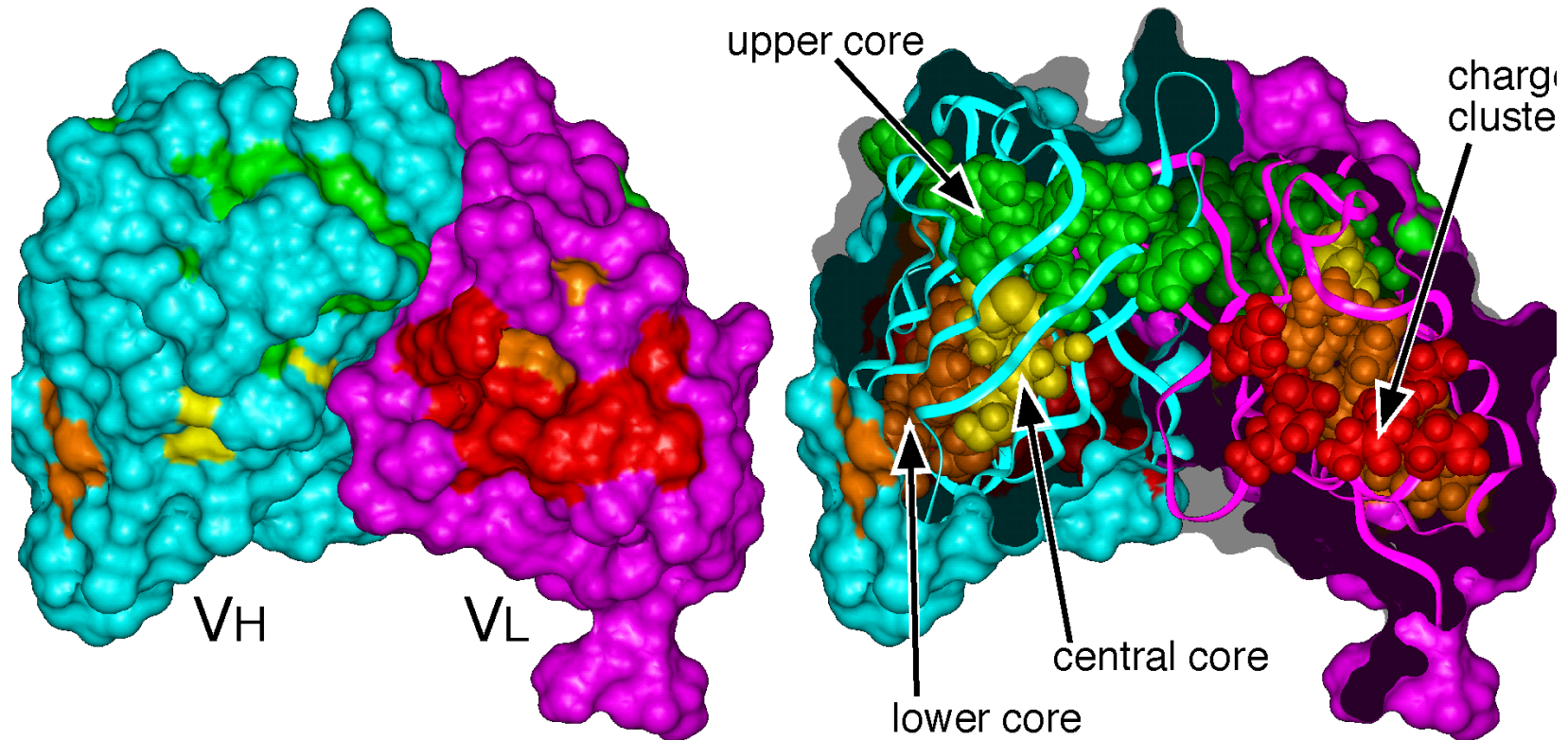
Nterm, outer loop

CDR-Donors: Rabbit Antibodies

- Germline sequence alignment retrieved from IMGT
- ~1000 rabbit V_H, ~500 $V\kappa$ and ~30 $V\lambda$ retrieved from NCBI
- Less sequence variability than human and murine antibodies,
- frameworks $huV\kappa 1$ and $huV_H 3$-like
- Several features found that are not seen in human and murine variable domains
- Only two rabbit antibody structures found in the pdb:
  3NL4 (1.54Å res.) is annotated as such,
  2X7L isn't.
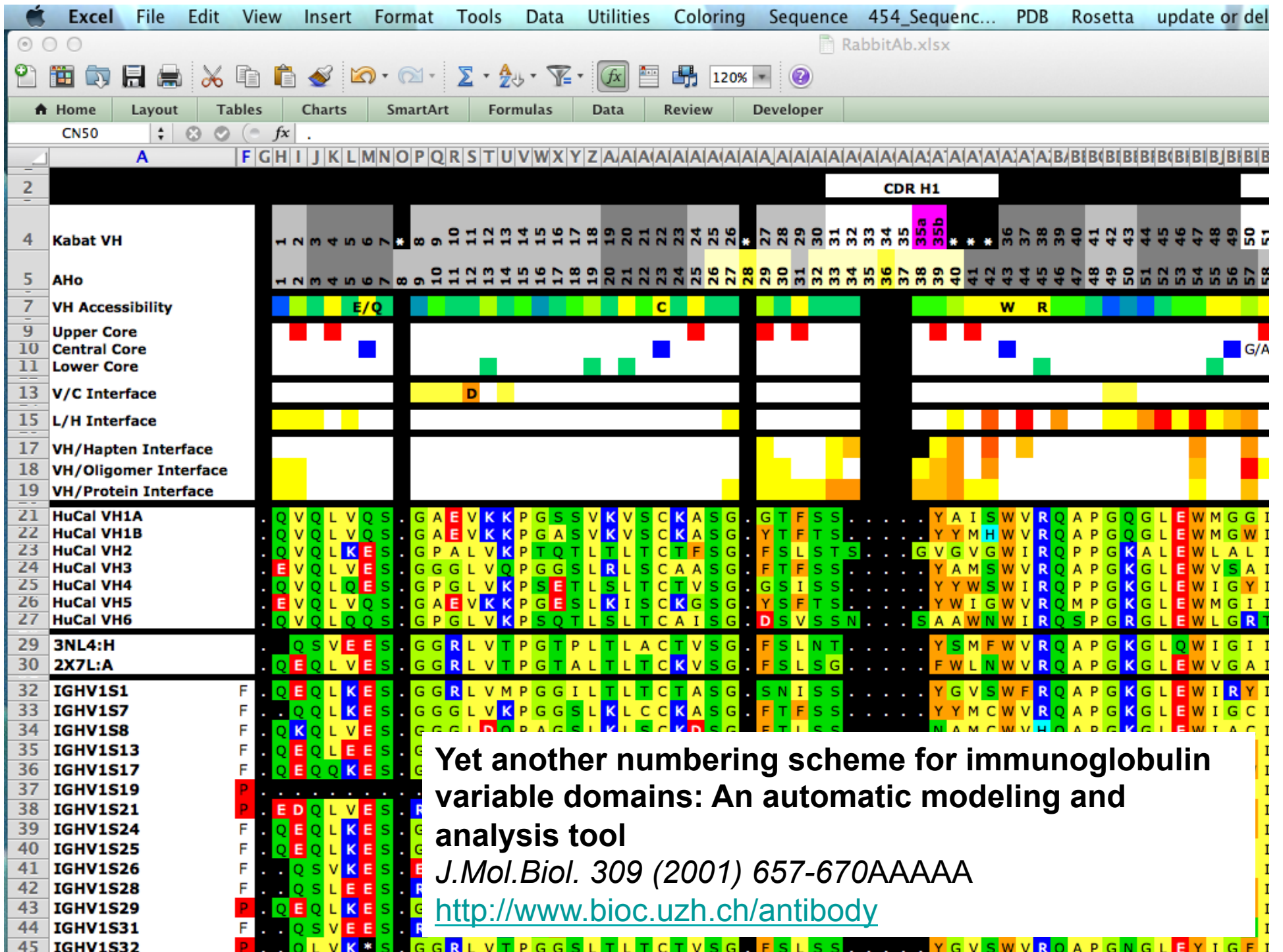
Yet another numbering scheme for immunoglobulin variable domains: An automatic modeling and analysis tool
J.Mol.Biol. 309 (2001) 657-670AAAAA
http://www.bioc.uzh.ch/antibody

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Murine and human antibody repertoires are quite similar – the rabbit repertoire is different:

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- There is less length variability in CDR-L1 of rabbit Vκ domains than in human and murine kappa domainsCDR L3 in rabbit Vκ lack Gln L108 (L90) and *cis*-Pro L136 (L?), which in human and murine Vκ domains produce the typical Ω-loop conformation. This produces a lambda-like CDR-L3 which might increase the flexibility of the V_L/V_H interface.

PDB ID	Chain																									

Kabat VL kappa		70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	
AHo		88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	
VL Side Chain Accessibility		D	F									E	D					Y	Y	C				

Upper Core

Central Core

Lower Core

VC Interface

LH Interface

VL/Haptens

VL/Oligomers

VL/Proteins

HuCal Vk1		D	F	T	L	T	I	S	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	.	.	
HuCal Vk2		D	F	T	L	K	I	S	R	V	E	A	E	D	V	G	V	Y	Y	C	Q	.	.	
HuCal Vk3		D	F	T	L	T	I	S	S	L	E	P	E	D	F	A	V	Y	Y	C	Q	.	.	
HuCal Vk4		D	F	T	L	T	I	S	S	L	Q	A	E	D	V	A	V	Y	Y	C	Q	.	.	

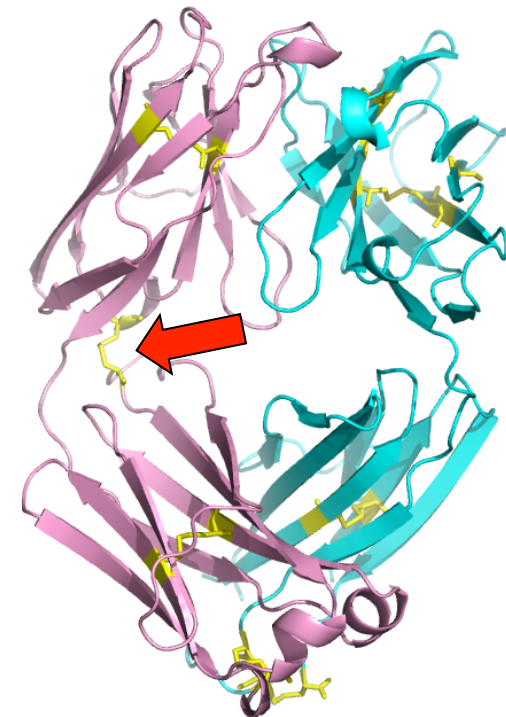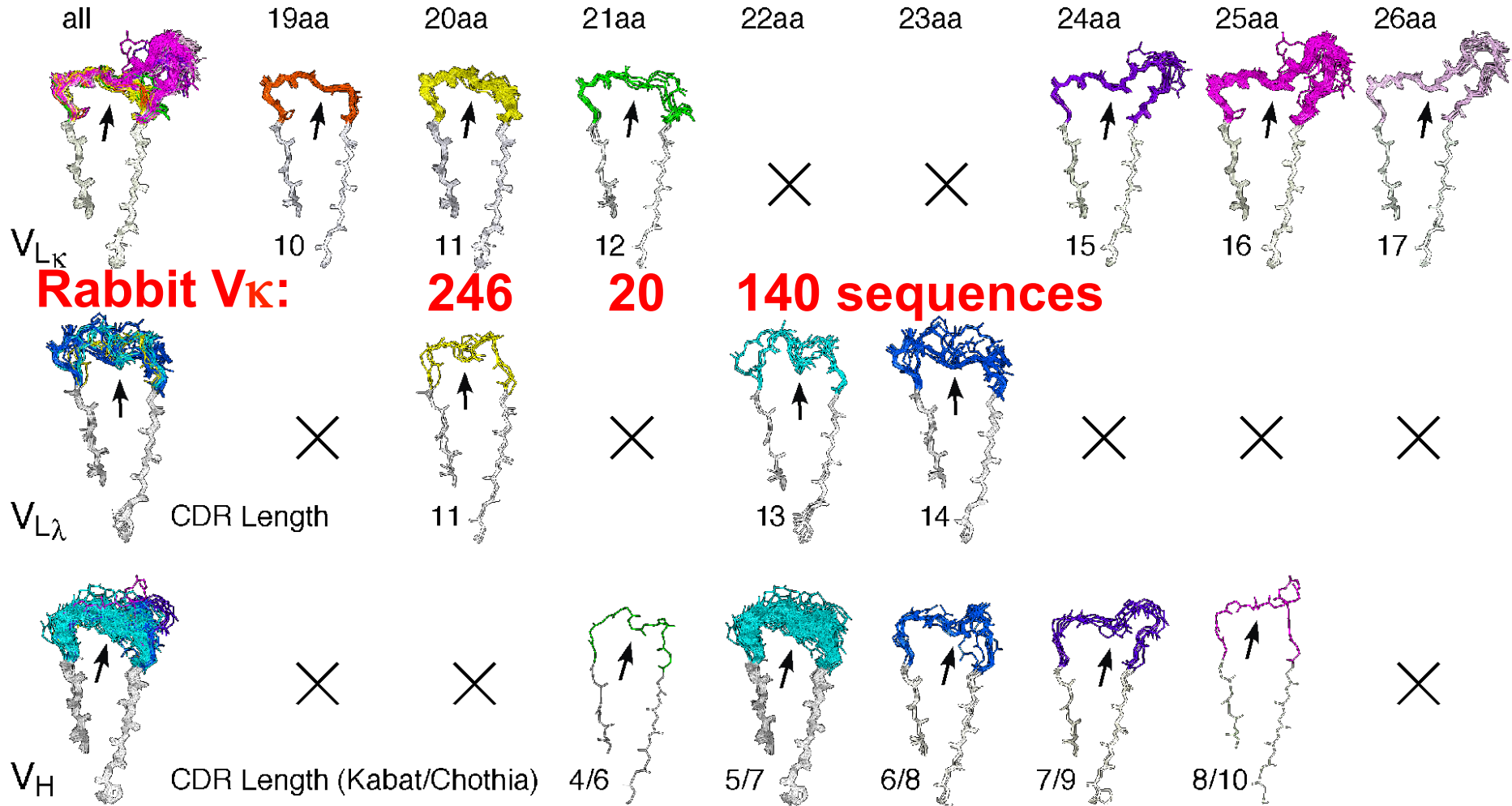| IGKV1S1 | IGKV1S1*0 | Q | F | T | L | T | I | S | G | V | E | C | D | D | A | A | T | Y | Y | C | A | G | Y | K | S | Y | S | K | * | * | Y |
IGKV1S2	IGKV1S2*0	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	G	S	Y	Y	S	S	G	W	Y
IGKV1S3	IGKV1S3*0	E	Y	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	H	G	Y	I	Y	S	S	G	D
IGKV1S4	IGKV1S4*0	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	G	G	Y	Y	N	S	G	W	Y
IGKV1S5	IGKV1S5*0	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	A	G	Y	Y	Y	S	G	S	G	T	D
IGKV1S6	IGKV1S6*0	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	C	T	Y	S	S	S	T	G
IGKV1S7	IGKV1S7*0	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	C	T	Y	S	S	S	T	G
IGKV1S8	IGKV1S8*0	Q	F	T	L	T	I	N	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	C	T	Y	S	S	S	T	G
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IGKV1S10	IGKV1S10*	Q	F	T	L	T	I	S	D	L	E	C	A	D	A	A	T	Y	Y	C	Q	S	N	Y	Y	S	S	S	N	G
IGKV1S11	IGKV1S11*	Q	F	T	L	T	I	N	G	V	Q	C	D	D	A	A	T	Y	Y	C	A	A	R	Y	S	G	N	I	Y
IGKV1S12	IGKV1S12*	Q	F	T	L	T	I	S	G	V	Q	C	D	D	A	A	T	Y	Y	C	Q	G	A	Y	S	G	N	I	Y
IGKV1S13	IGKV1S13*	Q	F	T	L	T	I	S	G	A	Q	C	D	D	A	A	T	Y	Y	C	A	G	Y	K	N	Y	S	N	D	D

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CDR 1 in murine/human antibodies

Segment length (residues 20-47):

all	19aa	20aa	21aa	22aa	23aa	24aa	25aa	26aa

$V_{L\kappa}$

10   11   12   ×   ×   15   16   17

Rabbit Vκ: **246**   **20**   **140 sequences**

$V_{L\lambda}$ CDR Length   11   13   14

V_H CDR Length (Kabat/Chothia)   4/6   5/7   6/8   7/9   8/10
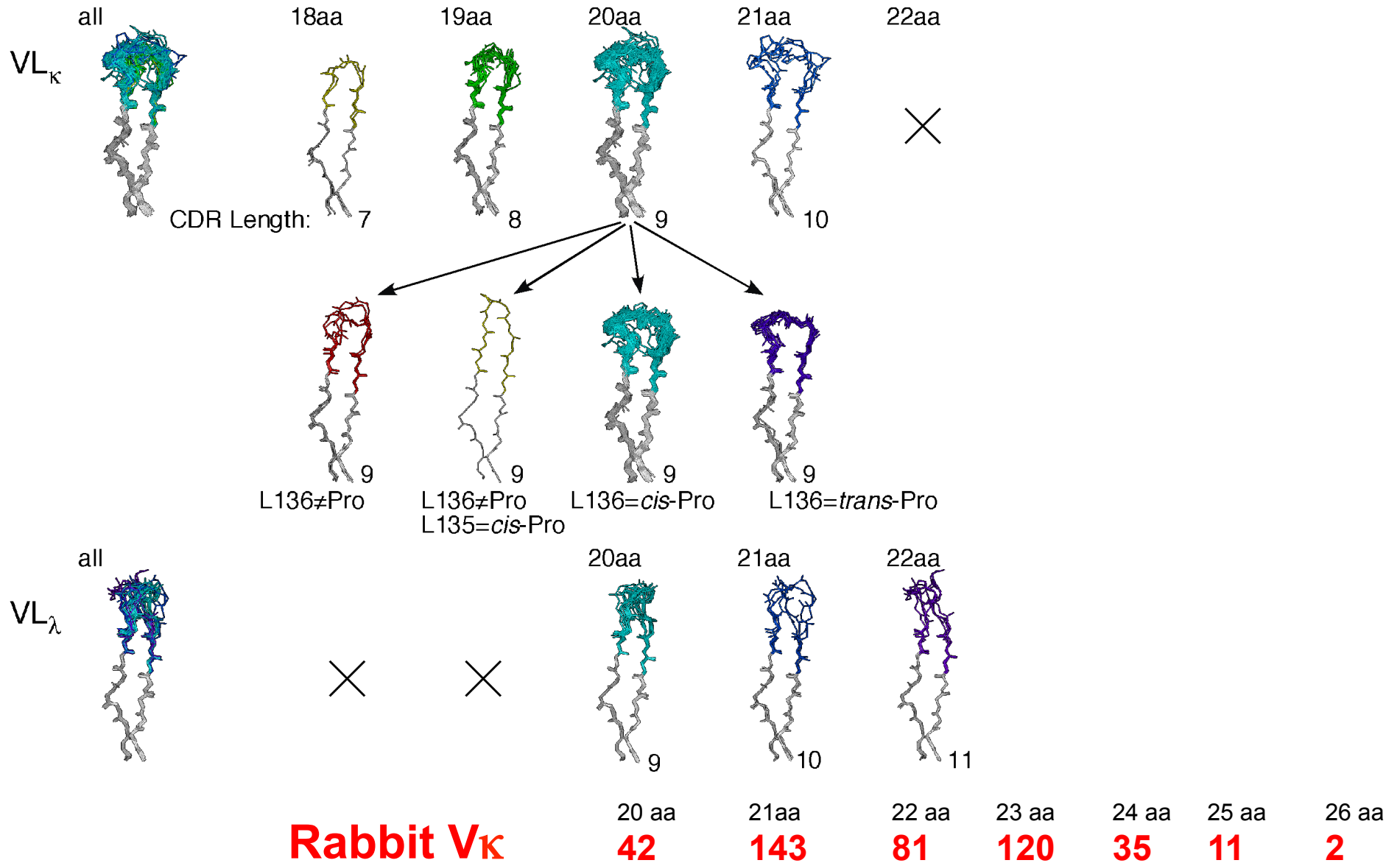
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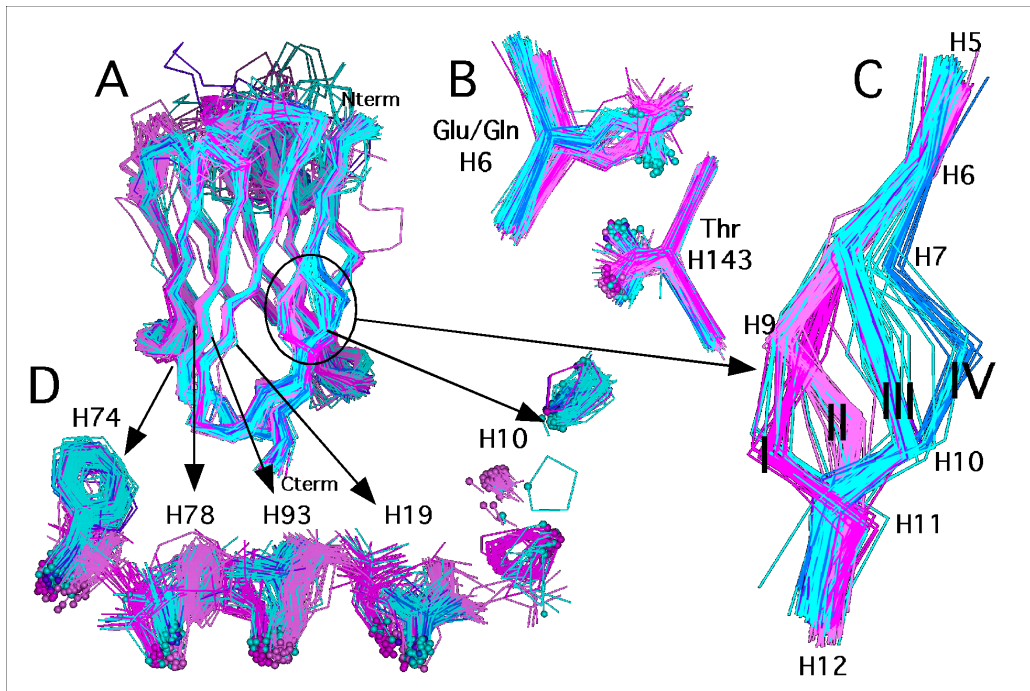
CDR L3 in murine/human light chains

Segment length (residues 102-144):

CDR-Donors: Rabbit Antibodies
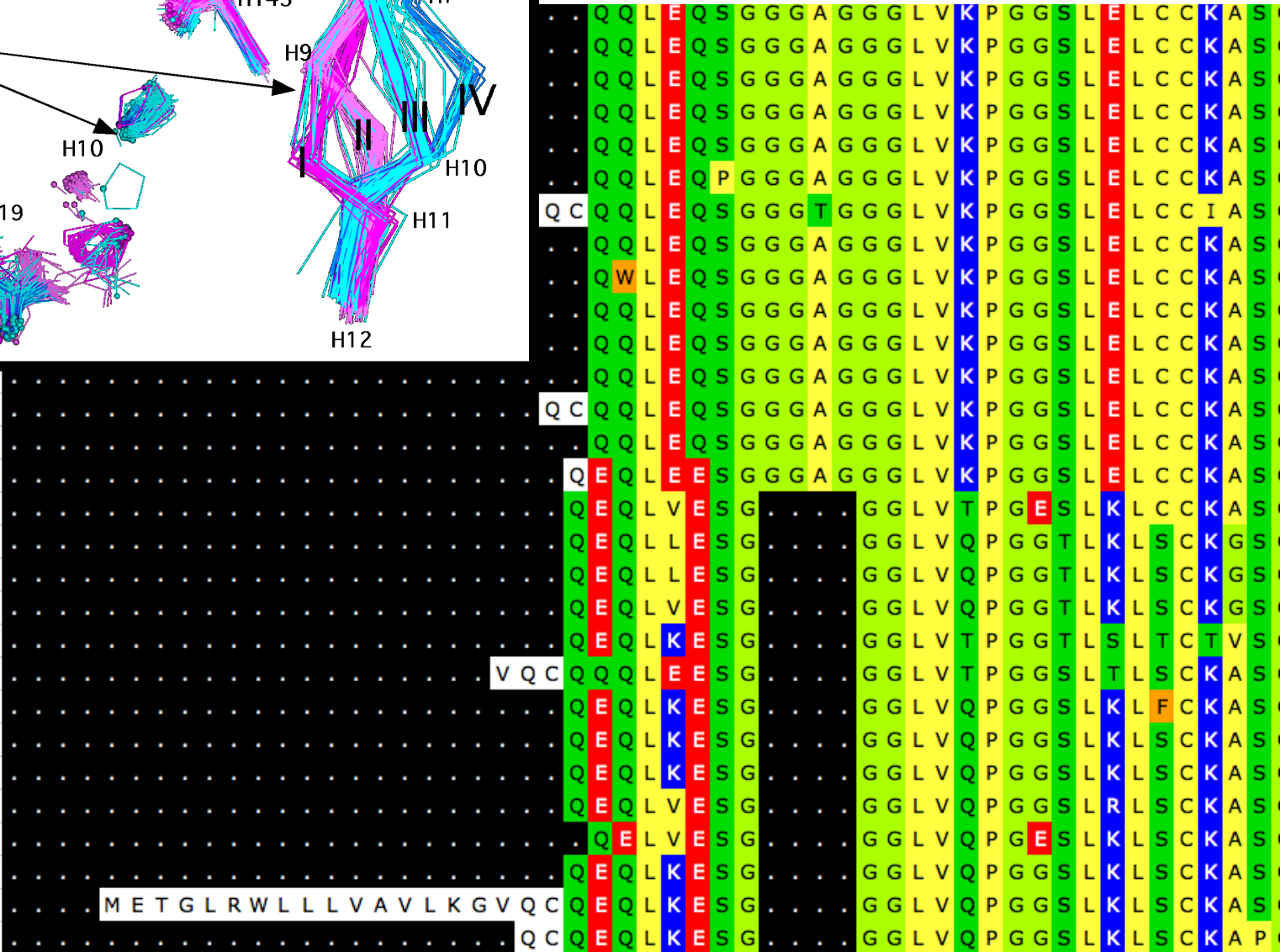
VH-Domains

- A'-strand (N-terminus) is frequently shortened by one residue

- Upper core residue H2 is hydrophilic

- Some V_H domains have a flexible insertion (4 or 5 residues) in the kink between strands A' and A''.

- These V_H domains have additional Cys residues in positions H22 (H21) and H90 (H79) that can form a disulfide bond connecting strand B to strand F.

- Others have additional Cys in positions H42 (H?) and H57 (H50), allowing a disulfide bond that connects strands C and D.

- Some combine both additional disulfide bonds

- Rabbit V_H domains have a highly conserved additional Trp at the base of CDR H2

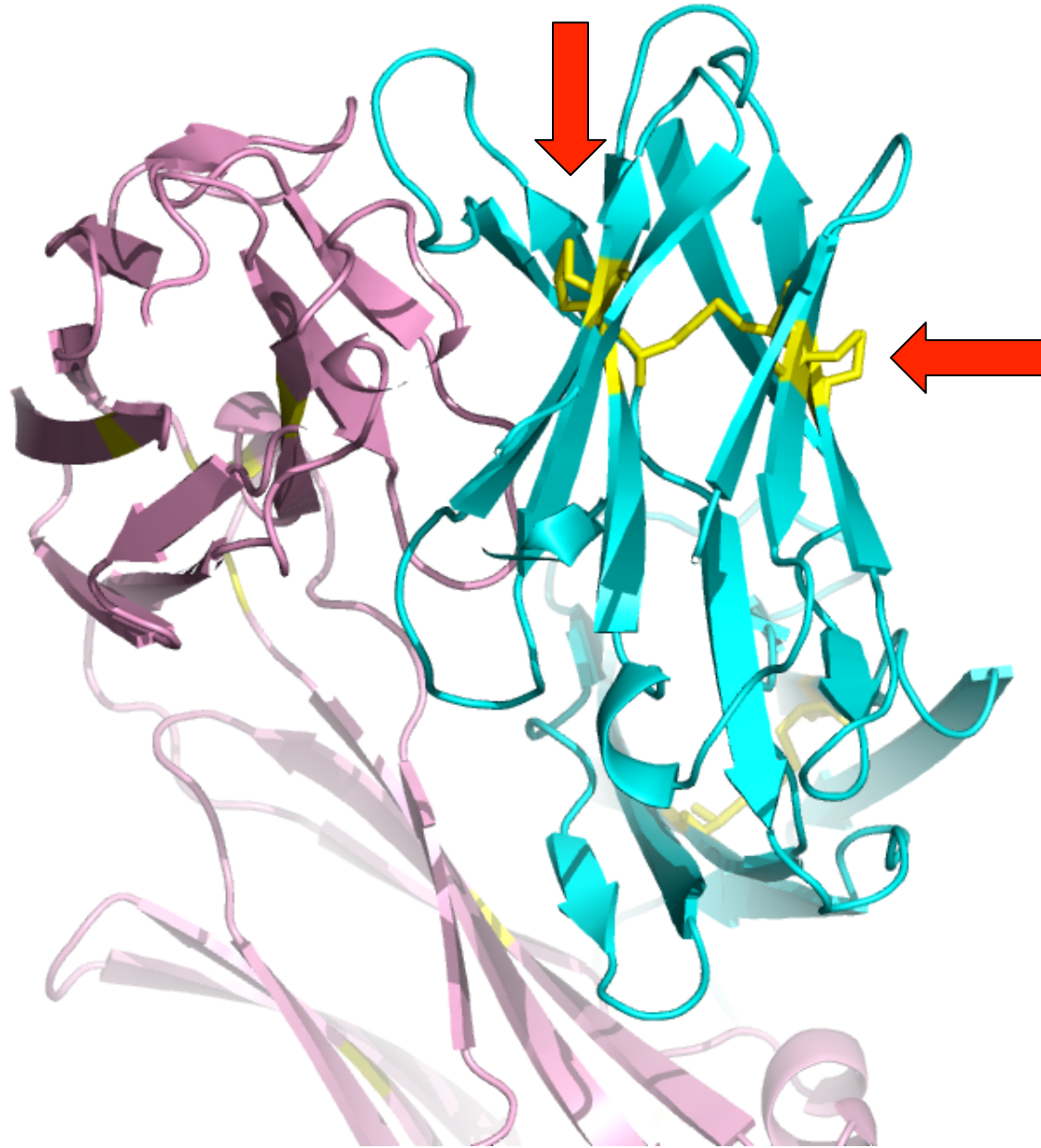- Rabbit V_H domains show length variability in the outer loop

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>gi	9799096	IGHV1S7	80.2	
>gi	2920459	IGHV1S7	80.8	
>gi	165196	IGHV1S7	84.0	
>gi	2920467	IGHV1S7	86.6	
>gi	9799316	IGHV1S7	81.1	
>gi	9799304	IGHV1S7	81.1	
>gi	9799249	IGHV1S7	82.1	
>gi	2920496	IGHV1S7	74.2	
>gi	2407786	IGHV1S7	75.0	
>gi	2920469	IGHV1S7	86.6	
>gi	2920490	IGHV1S7	85.6	
>gi	3930276	IGHV1S7	80.4	
>gi	27373563	IGHV1S7	74.2	
>gi	2920498	IGHV1S7	70.1	
>gi	2920471	IGHV1S7	83.5	
>gi	165250	IGHV1S7	73.0	METGLRWLLLVAVLKGVQC
>gi	9799143	IGHV1S7	84.7	

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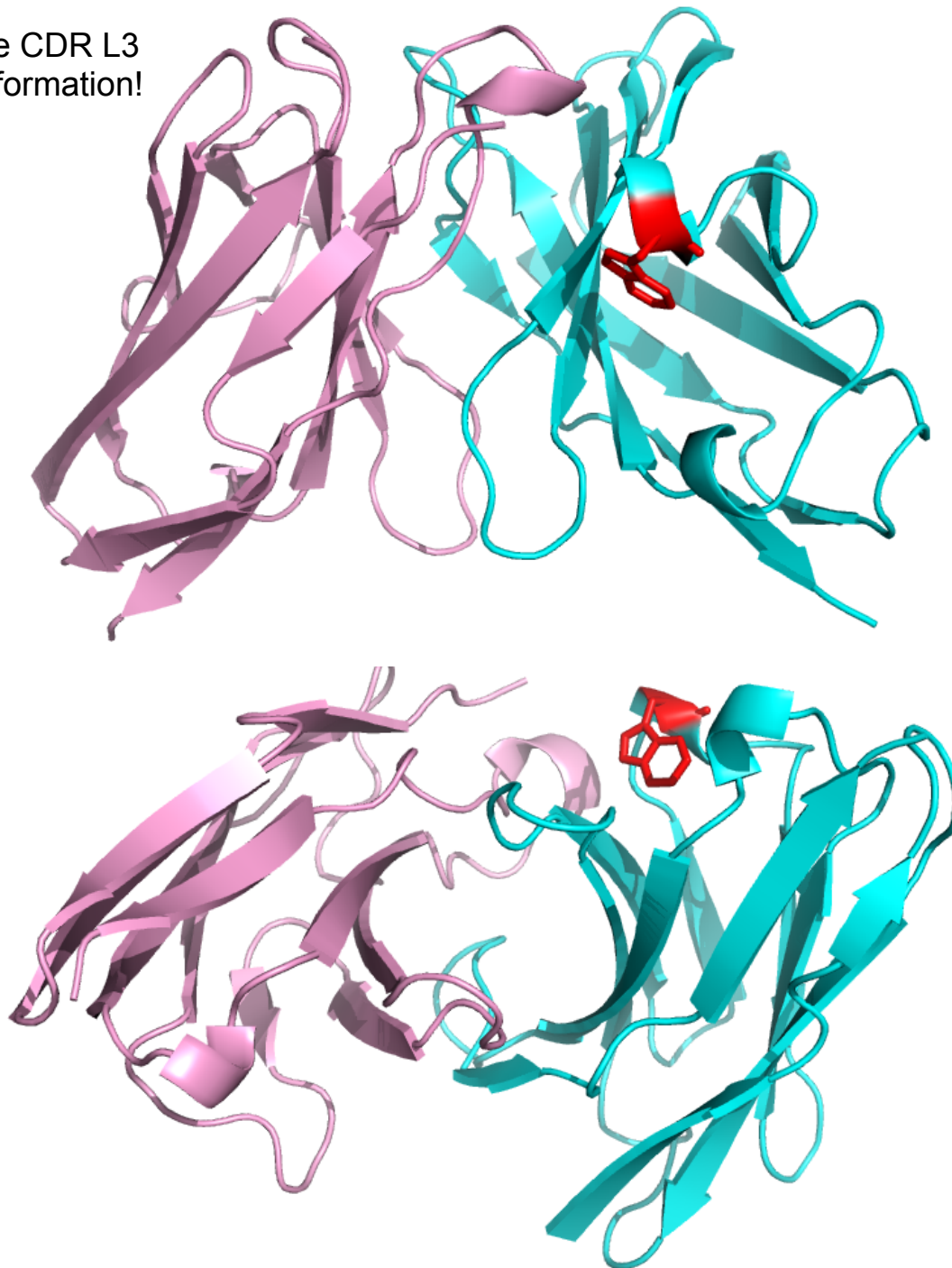- Rabbit V_H domains show length variability in the outer loop

Additional S-S bridges in V$_H$

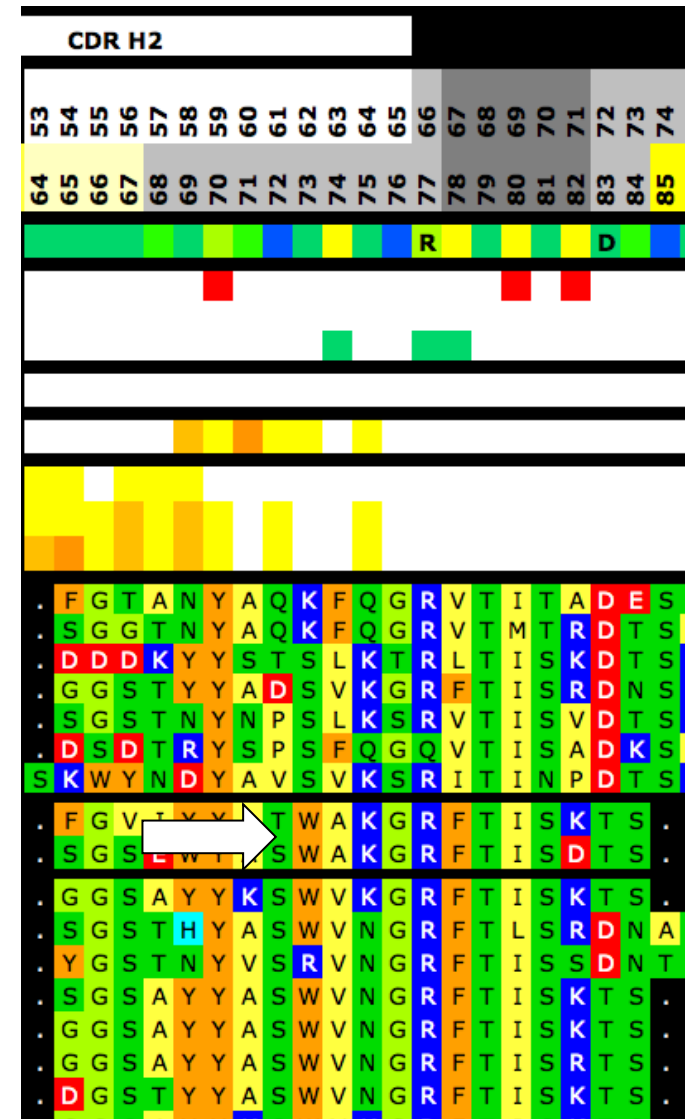# CDR-Donors: Rabbit Antibodies

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Note CDR L3 conformation!

Trp H73(H62)

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Outer Loops
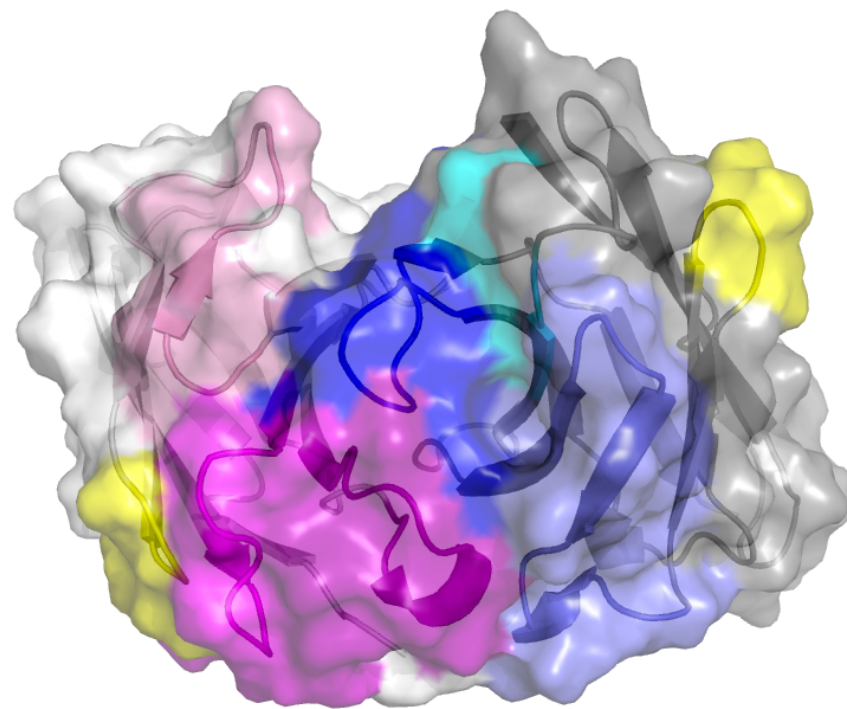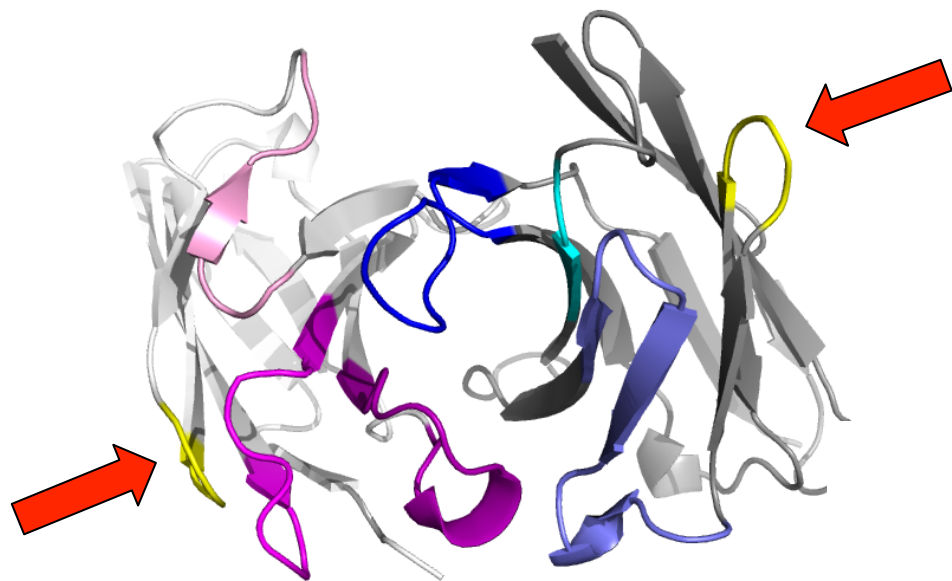
Why did those CDR Grafts fail?

Go to Graft Designer