University of Zurich[UZH]

WORLD adc FRANKFURT 2014

Select your lead candidate with confidence
improve preclinical predictability
Maximise clinical efficacy

17–20 FEBRUARY 2014 | Frankfurt, Germany

TROUBLESHOOTING AND ENGINEERING OF ANTIBODY CONSTRUCTS - PART I

Annemarie Honegger, PhD

Department of Biochemistry

University of Zürich

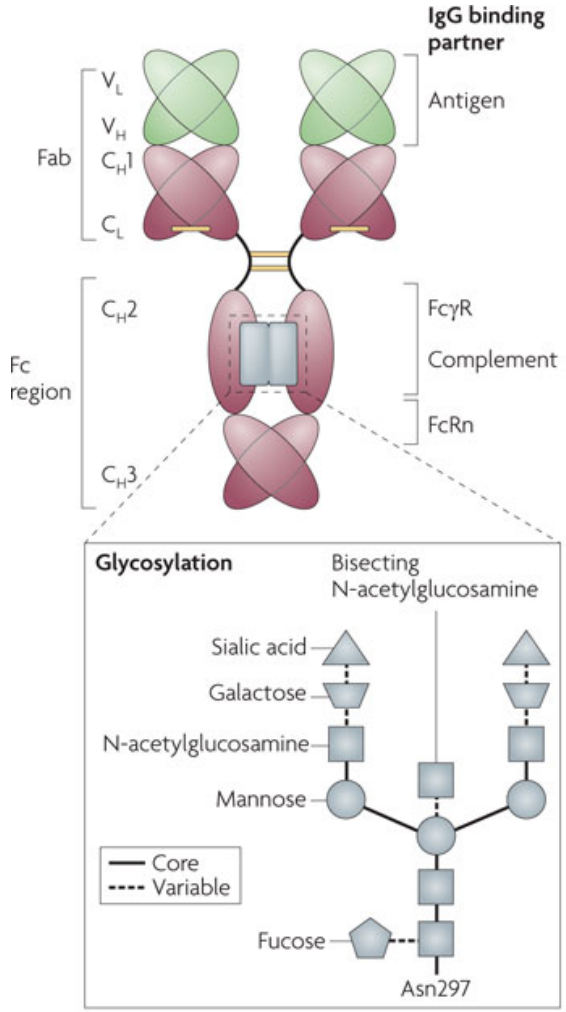
University of Zurich[UZH]

Outline

- Antibody fragments as building blocks for multivalent and multi-specific targeting constructs
- Source of antibody fragments: human antibodies and humanization of non-human antibodies
- Sequence variability and biophysical properties of human antibody variable domains
- Sequence and structural features that lead to unstable and aggregation-prone antibody domains
- Influence of domain interactions on the stability of antibody constructs
- Generalizable approaches to “repairing” poorly behaved antibody domains
- Discussion

University of Zurich[UZH]

Full-length IgG

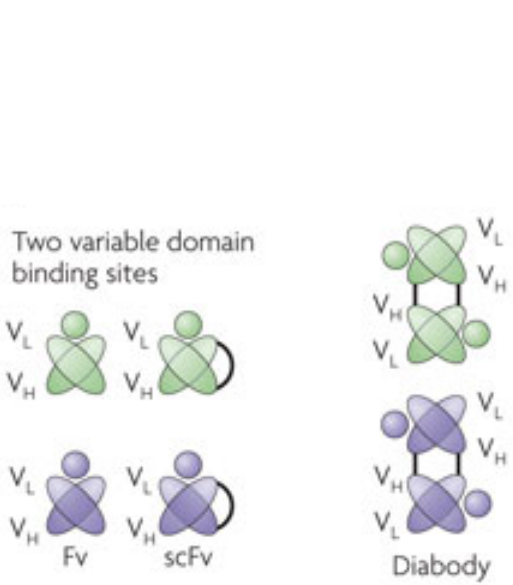


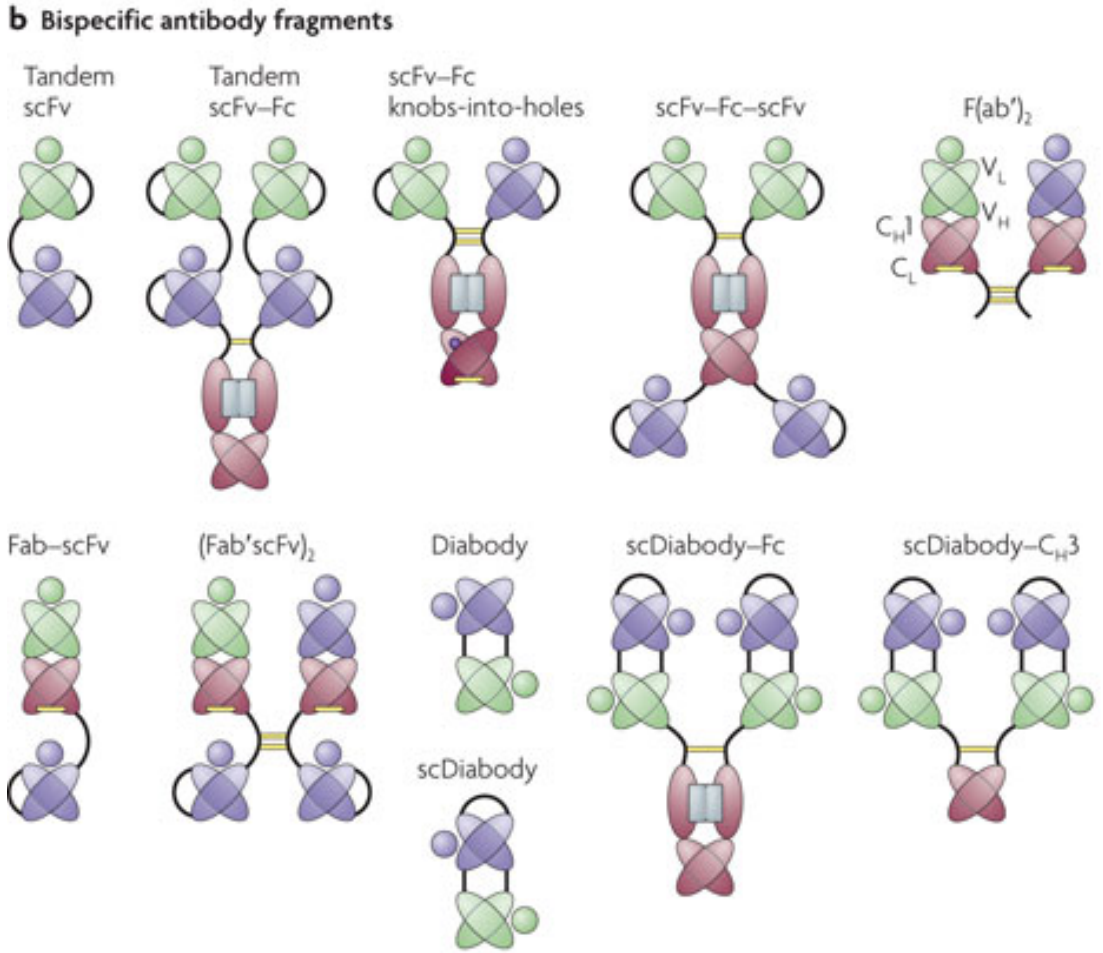
IgG binding partner	Protein strategies for modifying interactions	Potential impact of modifying interaction
Antigen	Mutate V domain sequences using display libraries and/or rationale design	Altered binding affinity or specificity
FcγR Complement	Mutate Fc sequence using display libraries and/or rationale design; select IgG isotype	↑ or ↓ ADCC ↑ or ↓ ADCP ↑ or ↓ CDC
FcRn	Mutate Fc sequence using display libraries and/or rationale design	↑ or ↓ half-life
	Antibody fragment lacking Fc	↓ Half-life, ↓ CDC, ↓ ADCC and ↓ ADCP
	Glycosylation strategies for modifying FcγR and complement interactions	
	Aglycosylation	↓ ADCC, ↓ ADCP and ↓ CDC
	Bisecting N-acetylglucosamine	↑ ADCC
	Non-fucosylation	↑ ADCC

Nature Reviews Immunology 10 (2010)301-316 "Therapeutic antibodies for autoimmunity and inflammation" Andrew C. Chan & Paul J.Carter

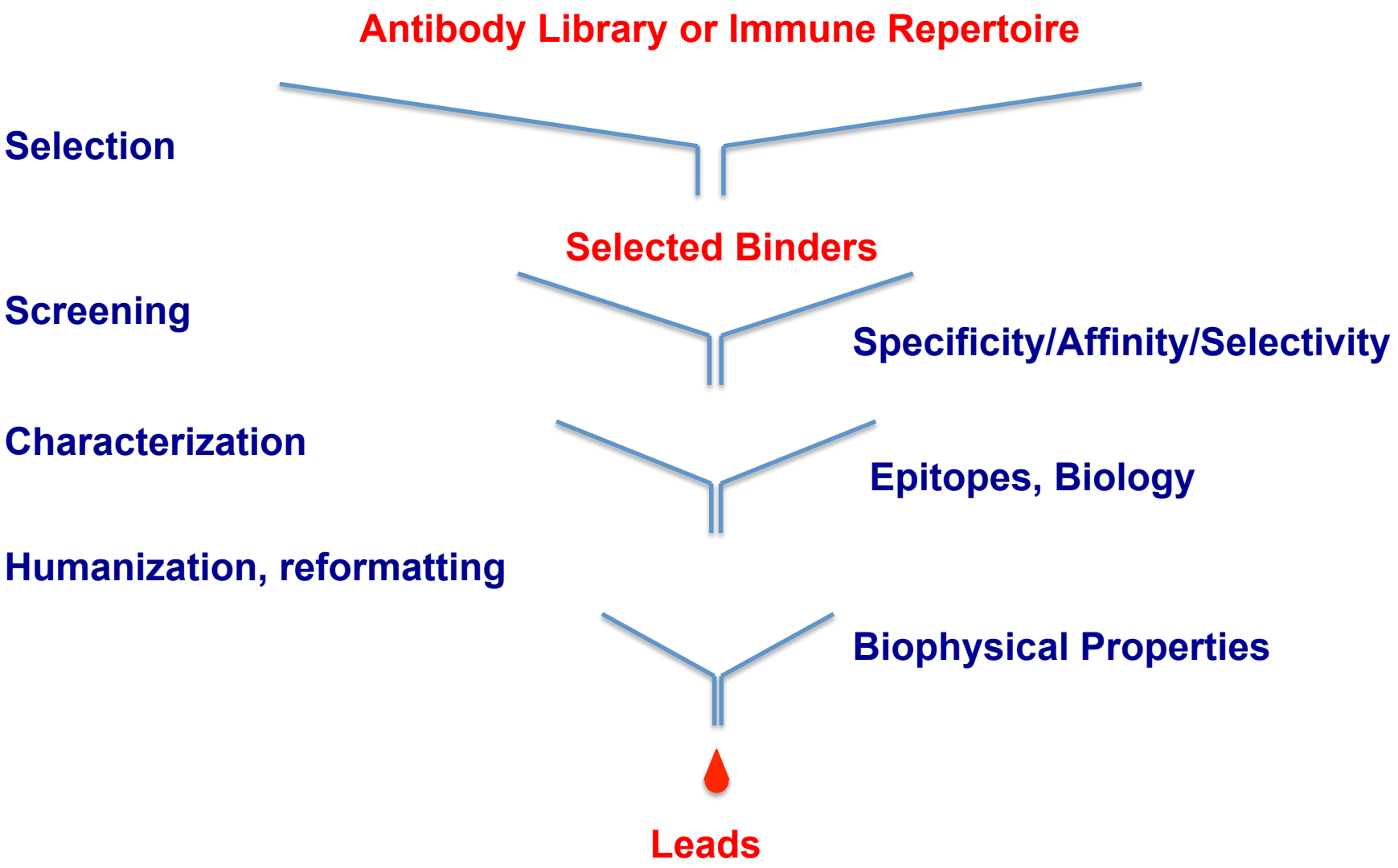
University of Zurich[UZH]

scFv as building blocks for multivalent constructs



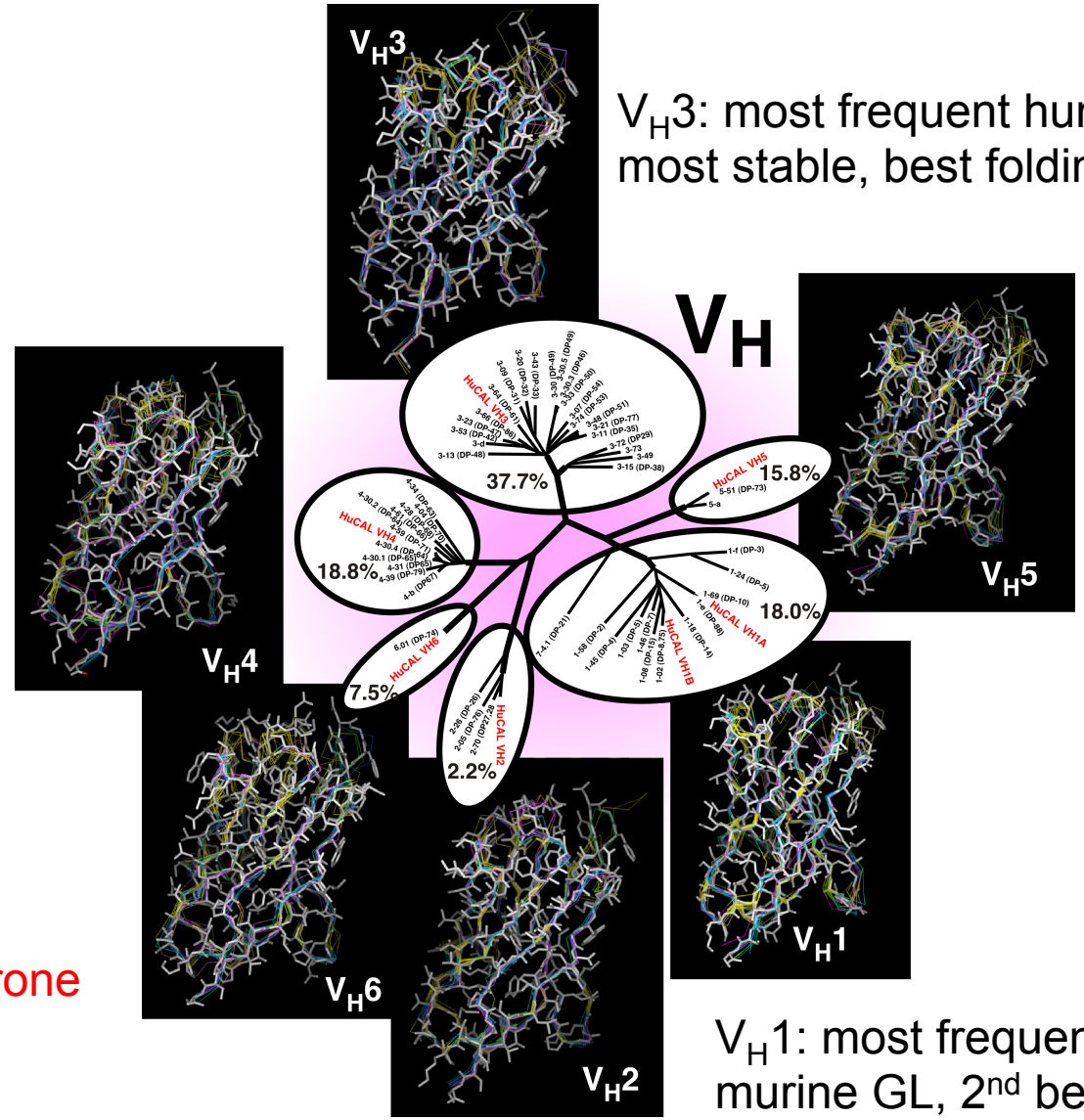


Nature Reviews Immunology 10 (2010) 301-316 "Therapeutic antibodies for autoimmunity and inflammation" Andrew C. Chan & Paul J.Carter


Antibody Library or Immune Repertoire
Selection
Selected Binders
Screening
Specificity/Affinity/Selectivity
Characterization
Epitopes, Biology
Humanization, reformatting
Biophysical Properties
Leads

University of Zurich[UZH]

Different V_H families differ widely in biophysical properties



V_H3: most frequent human GL
most stable, best folding properties

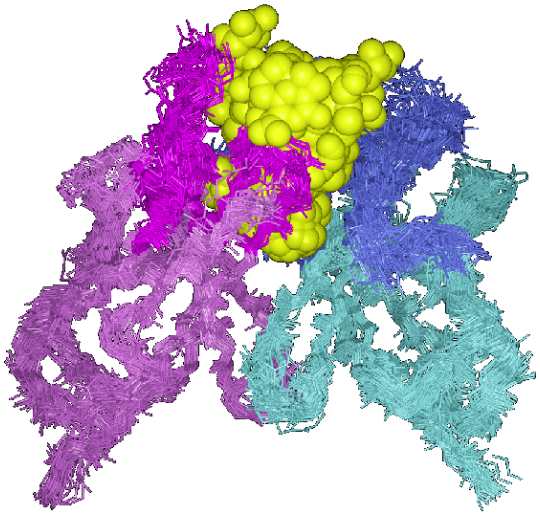
V_H2, V_H4, V_H6:
poor stability,
aggregation-prone

V_H1: most frequent
murine GL, 2nd best stability

University of Zurich[UZH]

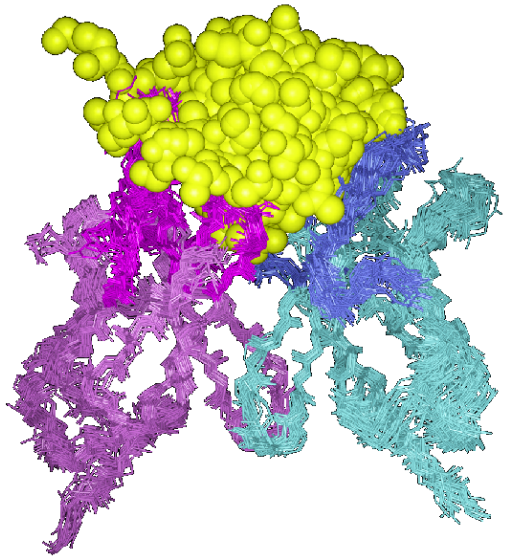
Why not just use the best framework?

Sequence features characteristic of particular germline families govern both stability and antigen binding characteristics



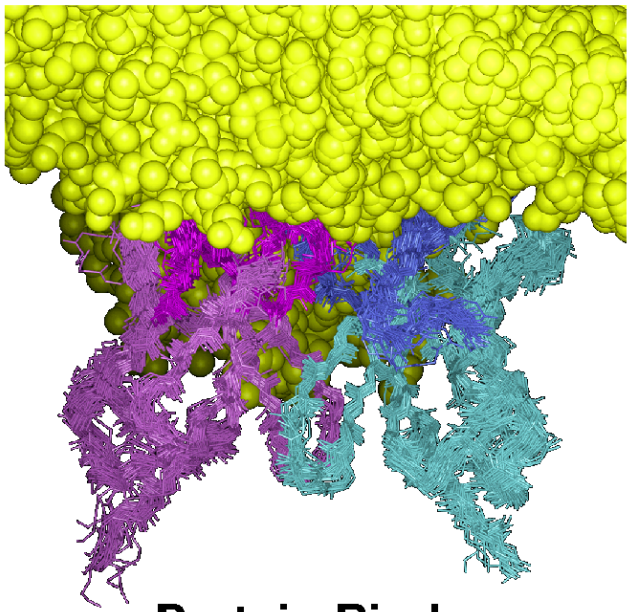
Hapten Binders

(52 structures)



Oligomer Binders

(30 structures)



Protein Binders

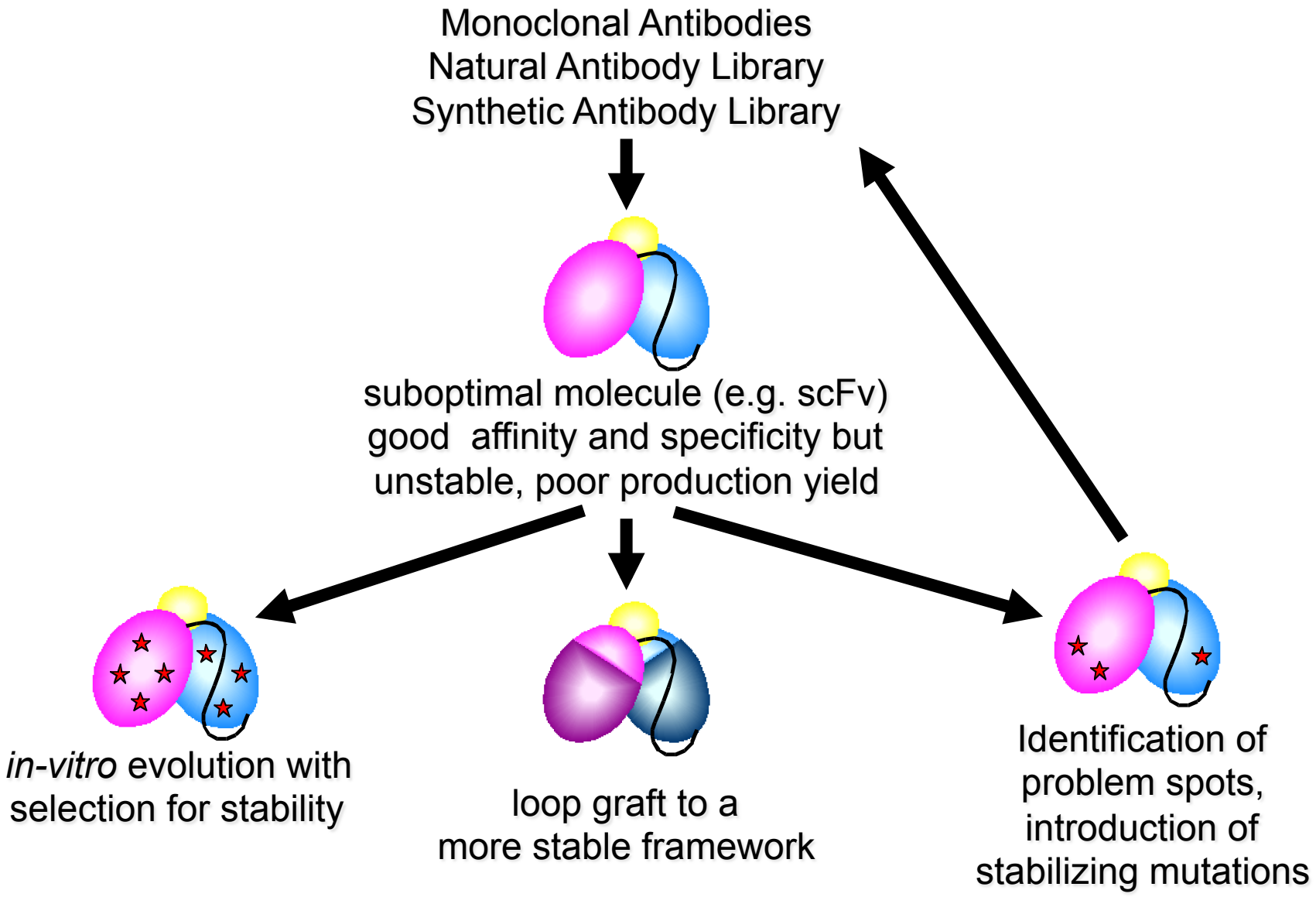
(45 structures)

Long CDR L1 and H2 loops
open CDR-H3 conformation

Short CDR L1 and H2 loops
closed CDR-H3 conformation

University of Zurich[UZH]

Strategies of Stabilization



University of Zurich[UZH]

Sources of antibody fragments

Non-human antibodies
need reengineering both for humanization and stabilization

Human repertoire libraries
no need for humanization, but may need stability engineering

Synthetic human antibody libraries
could be built based on stability-enhanced domain frameworks

University of Zurich[UZH]

Humanization

- Iteratively replace subsequences of the murine antibody variable domain by closest subsequence from the human repertoire, checking for T-cell epitopes

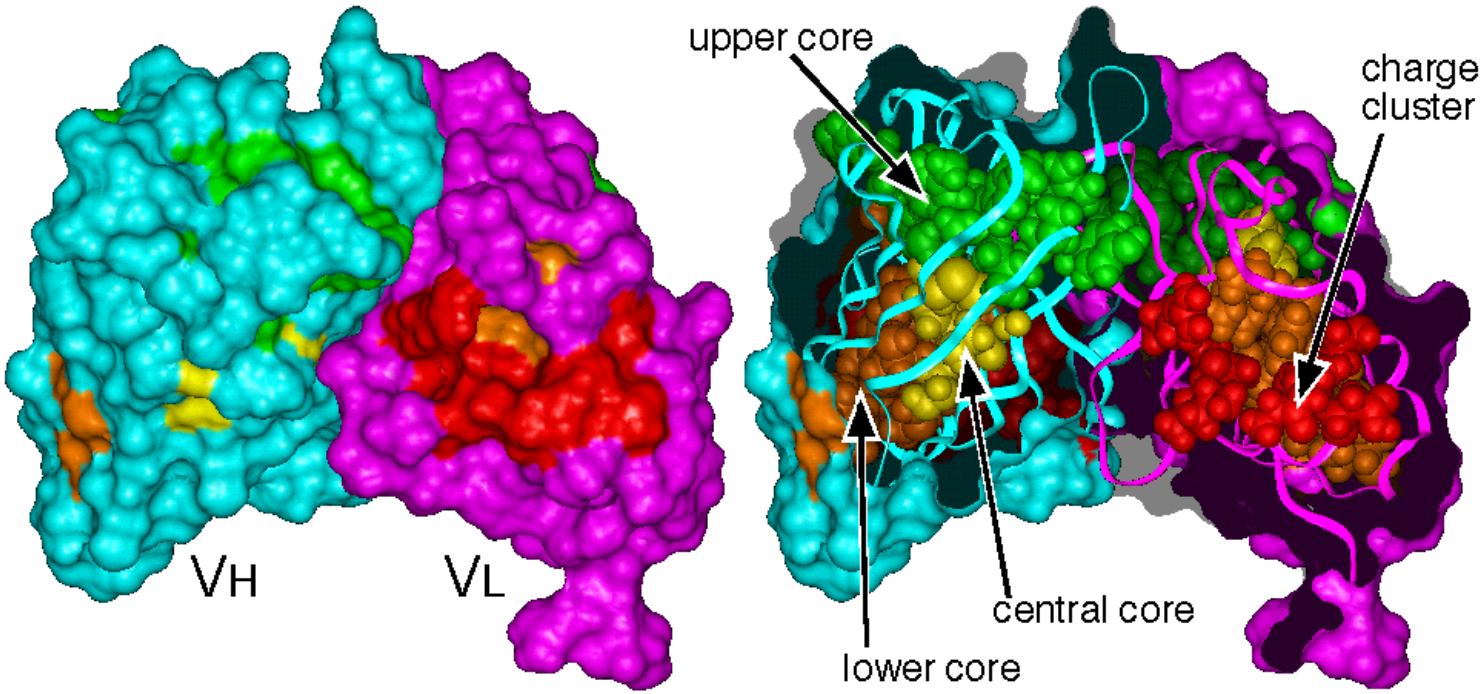
- Graft CDR regions to closest human framework

- Graft CDR regions to most stable framework

Going for the human sequence closest to the original one is least likely to cause problems with affinity loss,
but may result in stability problems

University of Zurich[UZH]

Domain Anatomy



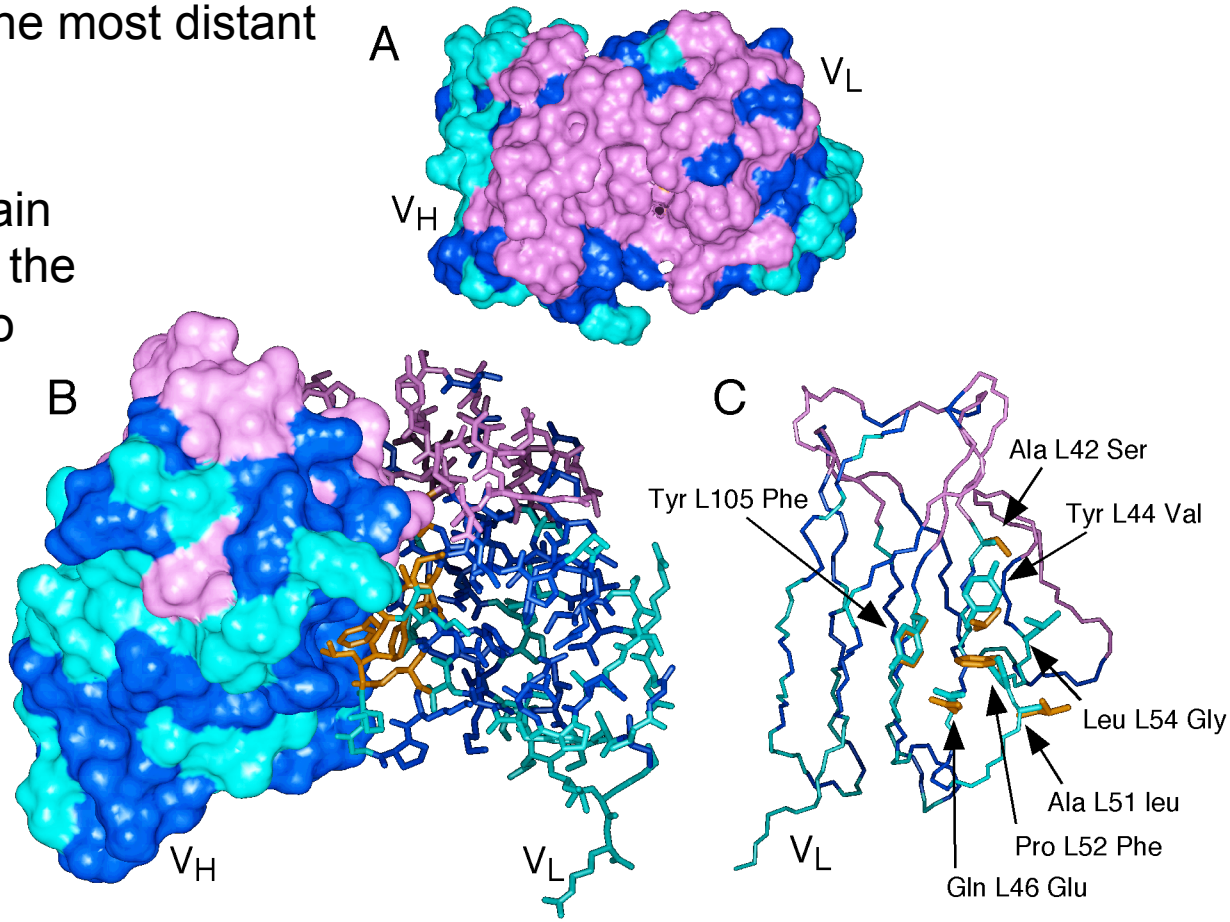
University of Zurich[UZH]

CDR-Graft

The most closely related human and murine antibody sequence are more similar than the sequences of two antibodies belonging to different germline sequences

However, grafts between the most distant Frameworks are possible

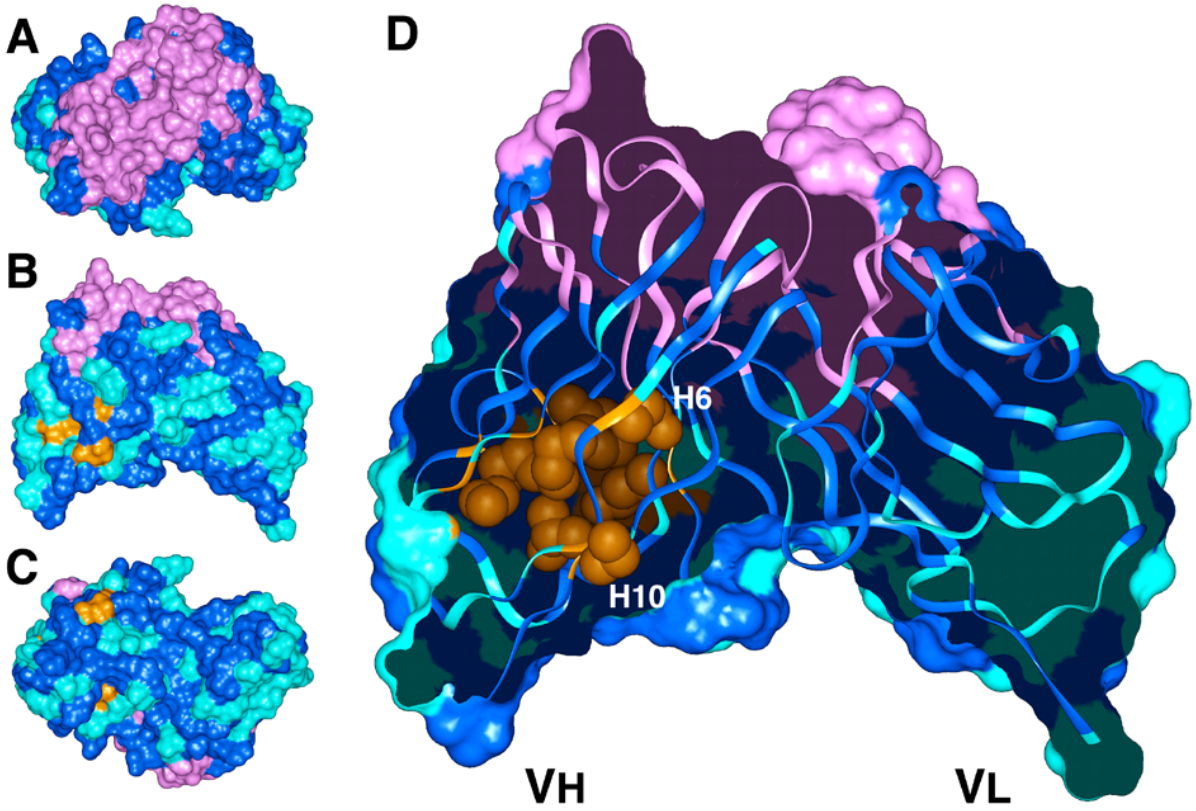
It may be necessary to retain destabilizing features from the original antibody in order to preserve binding



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

University of Zurich[UZH]

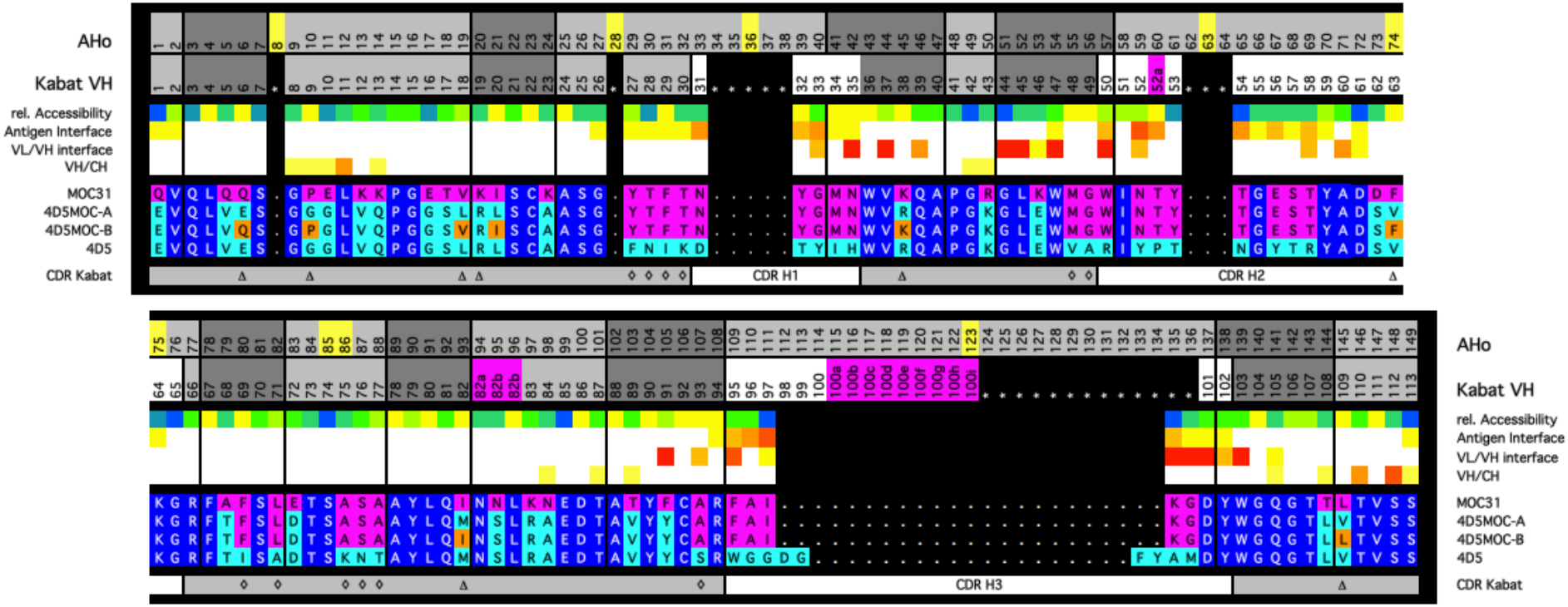
Mismatch in CDR-Grafts

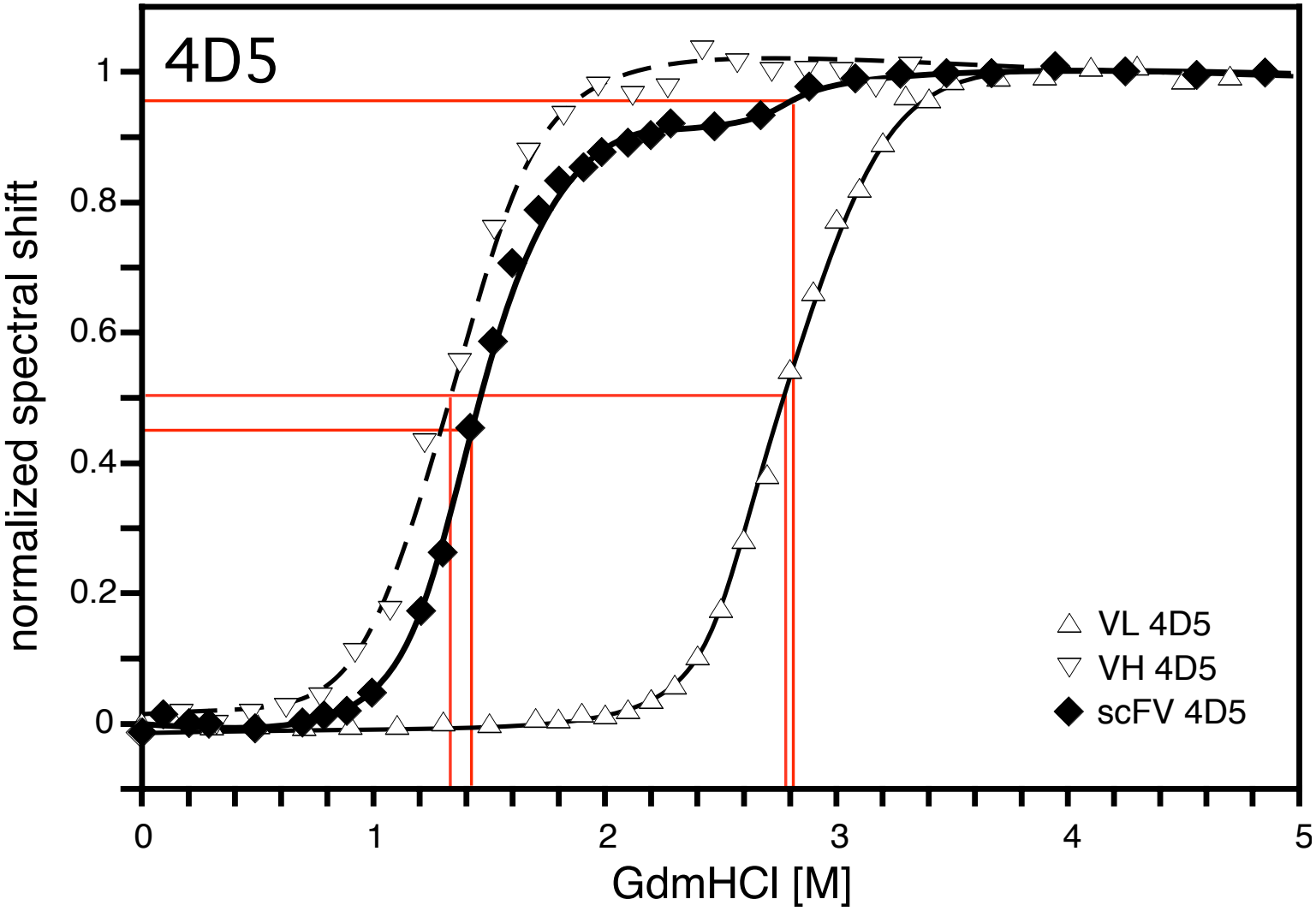


Although the huVH3 consensus domain is the most stable, grafts from VH1-like domains are less stable than expected

University of Zurich[UZH]

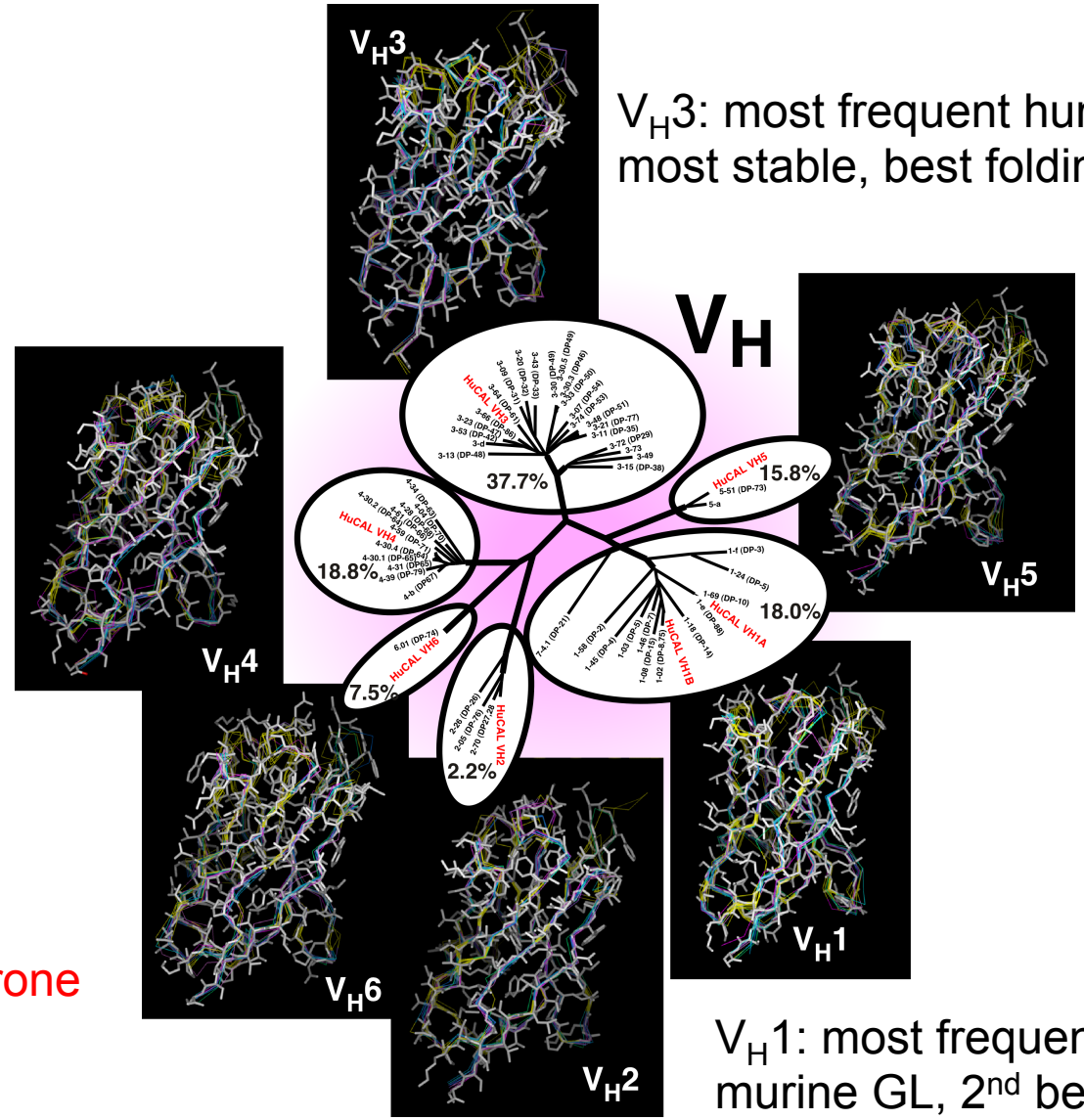
VH




4D5
normalized spectral shift
GdmHCl [M]
0
0.2
0.4
0.6
0.8
1
0
1
2
3
4
5
VL 4D5
VH 4D5
scFV 4D5

University of Zurich[UZH]

Different V_H families differ widely in biophysical properties



V_H3: most frequent human GL
most stable, best folding properties

V_H2, V_H4, V_H6:
poor stability,
aggregation-prone

V_H1: most frequent
murine GL, 2nd best stability

University of Zurich[UZH]

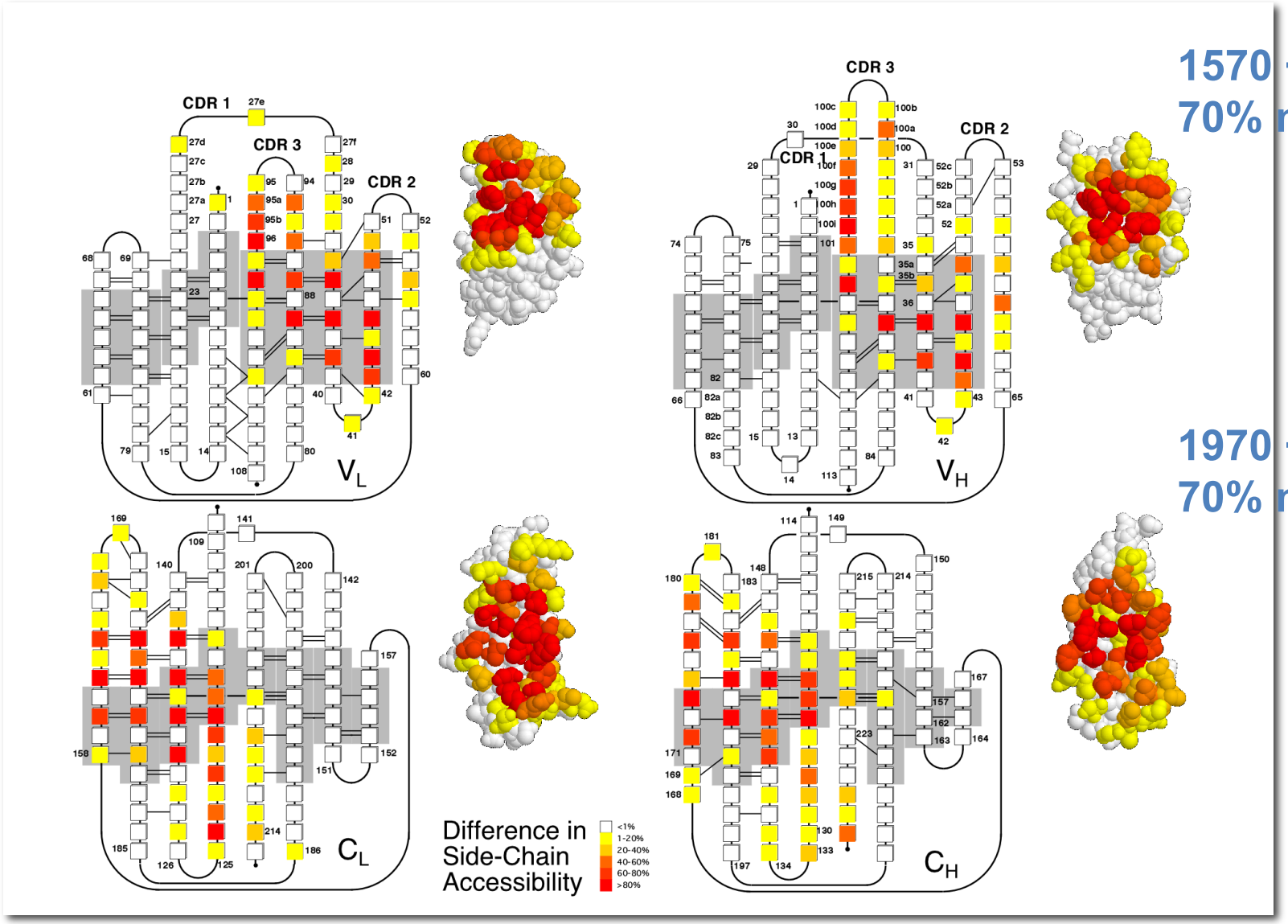
Stability of human consensus domains

Domain	yield mg/L_{OD10}	oligomeric state	T_m °C	$[GdmHCl]_{50}$ M	$\Delta G(H_2O)$ kJ/mol	m kJ L/mol^2
$hV_L\kappa1$	4.5	monomer	64	2.1	29	14
$hV_L\kappa2$	14.2	monomer	63	1.5	25	16
$hV_L\kappa3$	17.1	monomer	73	2.3	35	15
$hV_L\kappa4$	9.6	mono+dimer	58	1.5	*n.d.*	*n.d.*
$hV_L\lambda1$	0.3	monomer	64	2.1	24	11
$hV_L\lambda2$	1.9	monomer	50	1.0	16	16
$hV_L\lambda3$	0.8	mono+dimer	49	0.9	15	16

Domain	yield mg/L_{OD10}	oligomeric state	T_a °C	$[GdmHCl]_{50}$ M	$\Delta G(H_2O)$ kJ/mol	m kJ L/mol^2
hV_H1a	1.0	monomer	41	1.5	14	10
hV_H1b	1.2	monomer	51	2.1	26	13
hV_H2	refolded	*n.d.*	45	1.4	*n.d.*	*n.d.*
hV_H3	2.4	monomer	65	3.0	53	18
hV_H4	refolded	*n.d.*	44	2.3	*n.d.*	*n.d.*
hV_H5	refolded	monomer	44	2.2	17	7
hV_H6	refolded	*n.d*	39	1.2	*n.d.*	*n.d.*

University of Zurich[UZH]

V_L/V_H and C_L/C_H Interface



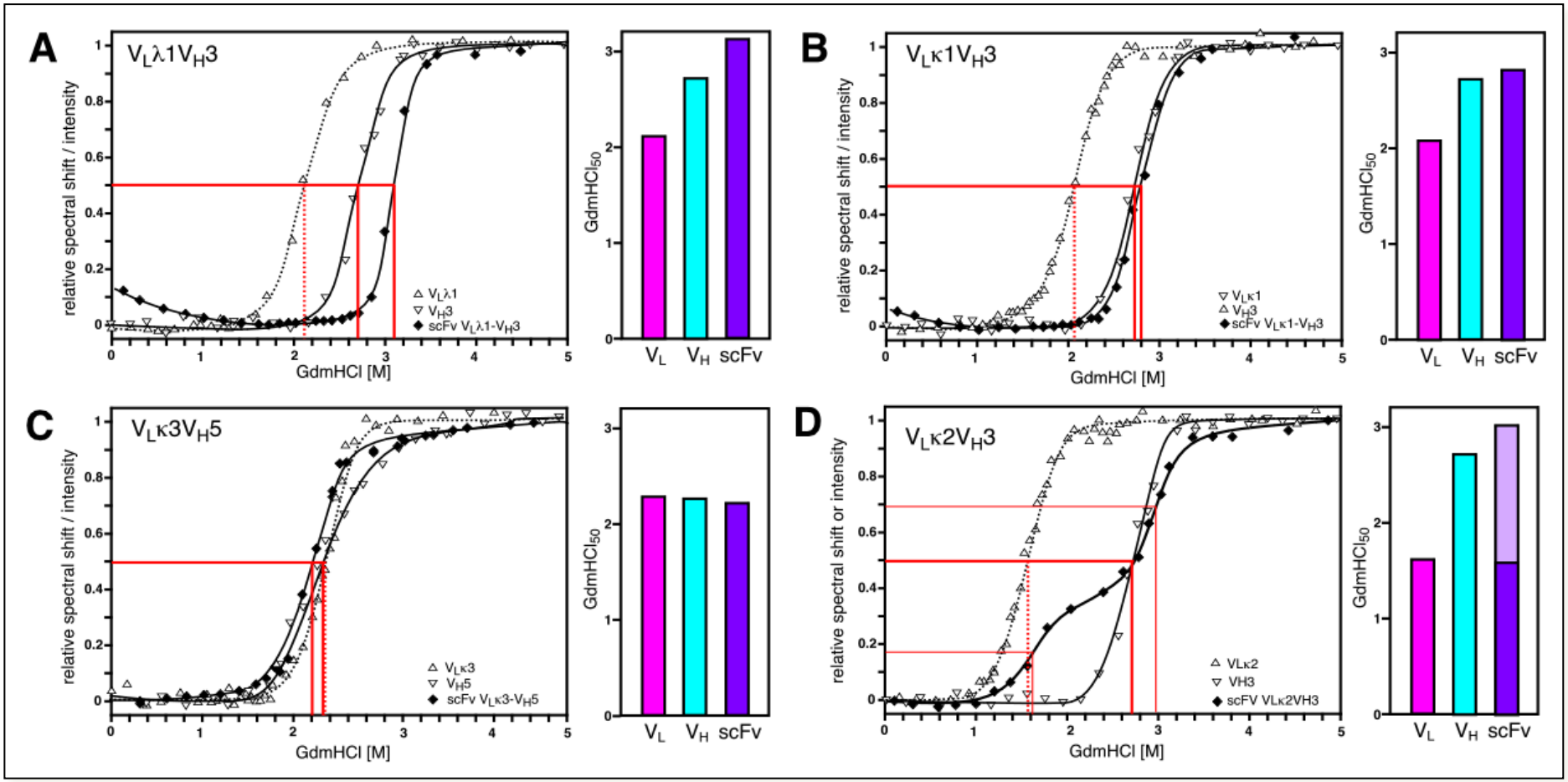
1570 +/- 160 Å^2
70% non-polar

1970 +/- 160 Å^2
70% non-polar

University of Zurich[UZH]

Single chain fragments

V_H3 paired with any of the seven V_L fragments
$V_L\kappa3$ paired with any of the seven V_H fragments



University of Zurich[UZH]

scFv	yield mg/L_{OD10}	rel. yield %	% soluble	oligomeric state	[GdmHCl]$_{50}$ scFv V_L	[GdmHCl]$_{50}$ scFv V_H	[GdmHCl]$_{50}$ isol. domains V_L	[GdmHCl]$_{50}$ isol. domains V_H*
$V_L\kappa1$-V_H3	2.6	40	50	monomer	2.8		2.1	2.7
$V_L\kappa2$-V_H3	2.6	40	20	monomer	1.6	2.9	1.5	2.7
$V_L\kappa3$-V_H3	6.5	100	30	monomer	2.8		2.3	2.7
$V_L\kappa4$-V_H3	5.2	80	40	monomer	2.0	2.8	1.5	2.7
$V_L\lambda1$-V_H3	7.8	120	40	mono/dimer	3.0		2.1	2.7
$V_L\lambda2$-V_H3	5.9	90	10	mono/dimer	2.9		1.0	2.7
$V_L\lambda3$-V_H3	3.6	60	10	mono/dimer	2.8		0.9	2.7
$V_L\kappa3$-V_H1a	11.1	170	10	mono/dimer	2.8	1.8	2.3	1.2
$V_L\kappa3$-V_H1b	12.4	190	20	monomer	3.0	2.4	2.3	1.8
$V_L\kappa3$-V_H2	2.6	40	90	monomer	2.8	1.5	2.3	1.6
$V_L\kappa3$-V_H3	6.5	100	30	monomer	2.8		2.3	2.7
$V_L\kappa3$-V_H4	2.6	40	90	monomer	3.0	2.3	2.3	1.5
$V_L\kappa3$-V_H5	6.5	100	50	monomer	3.0	2.2	2.3	1.9
$V_L\kappa3$-V_H6	5.2	80	80	monomer	2.6	1.2	2.3	0.5

University of Zurich[UZH]

Role of interface stability

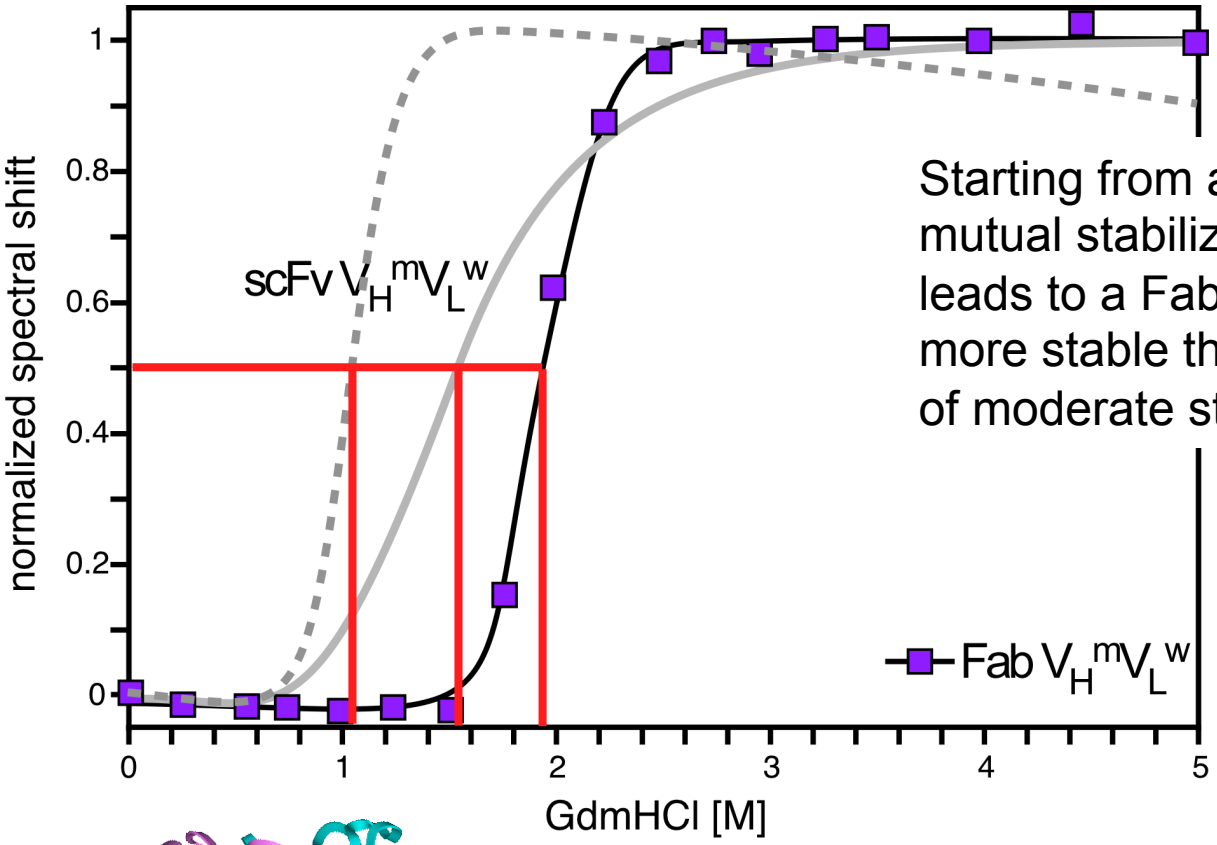
Changes in the fluorescence spectra in multistate unfolding of scFv showed no evidence of the two native domains dissociating.

Loss of the interface between VH and VL was always coupled to the unfolding of the weaker domain

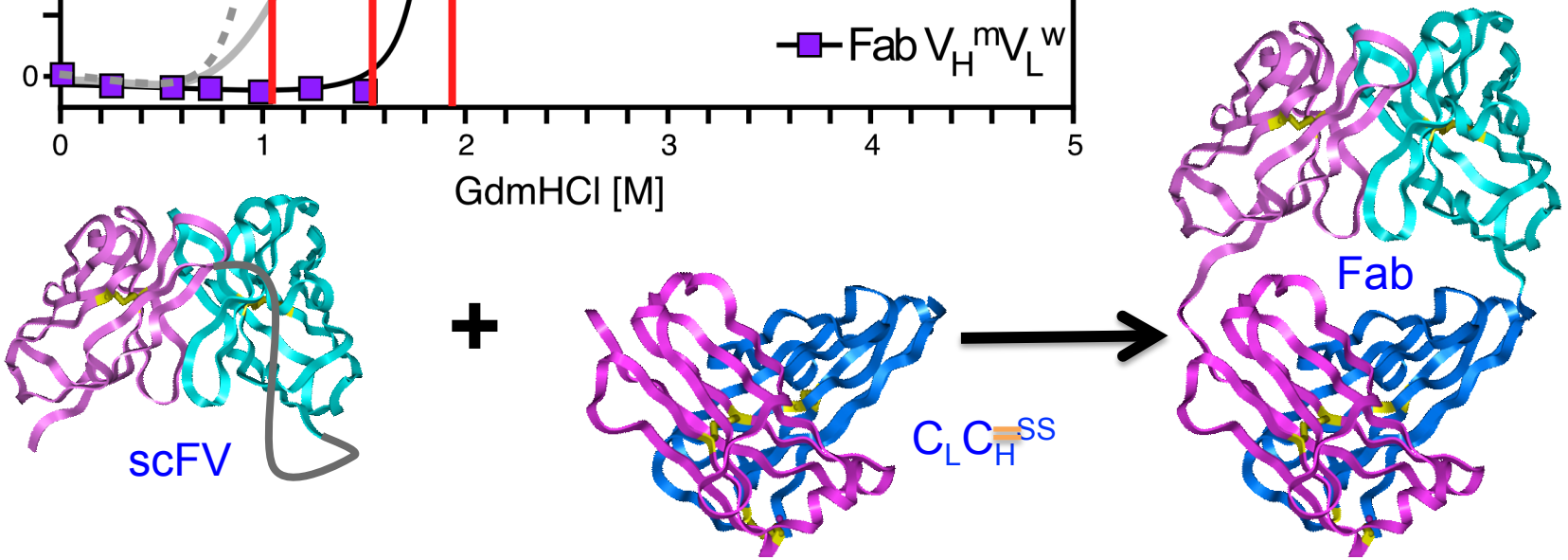
A weak interface can therefore be compensated by stabilizing the weaker of the two domains.

University of Zurich[UZH]

from scFv to Fab

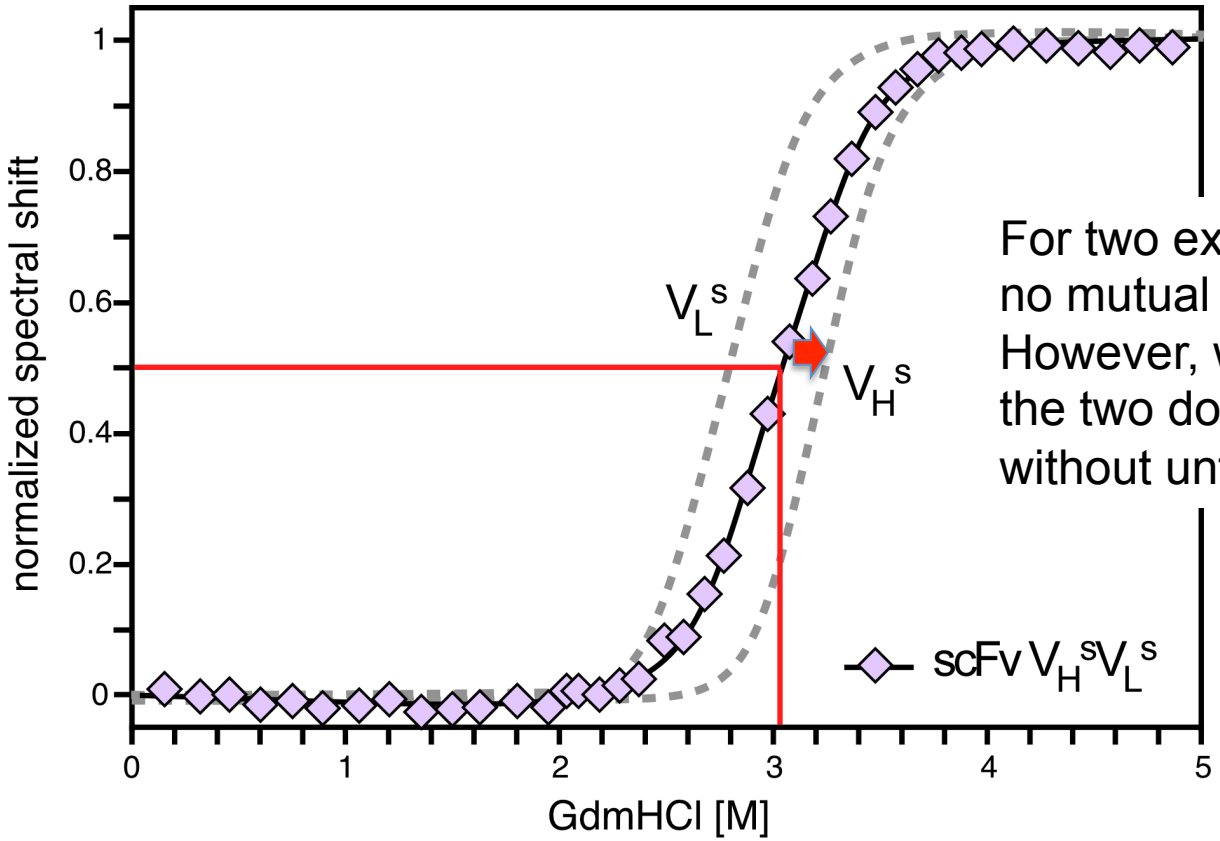


Starting from a weak scFV, mutual stabilization of Fv and $C_L C_H$ leads to a Fab fragment that is more stable than either, but is still of moderate stability

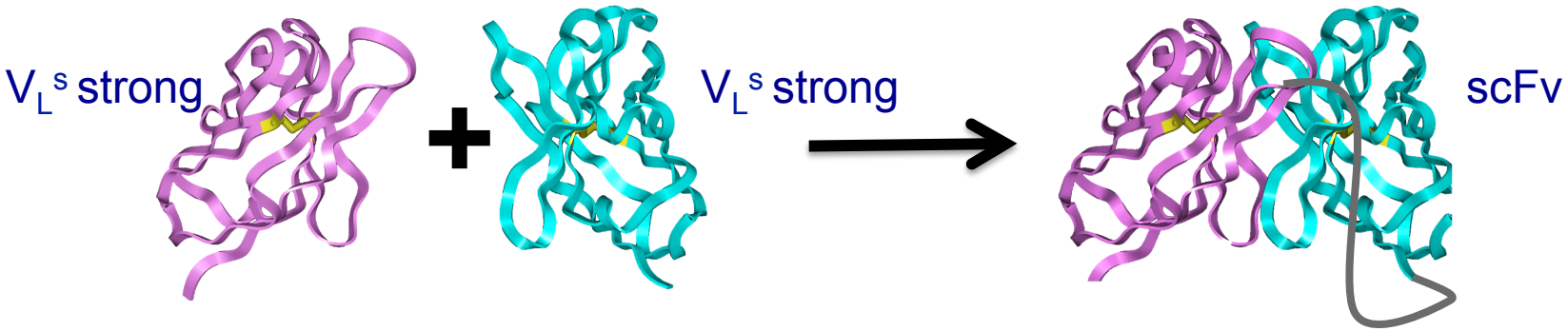


University of Zurich[UZH]

Mutual stabilization of V_L and V_H in scFV

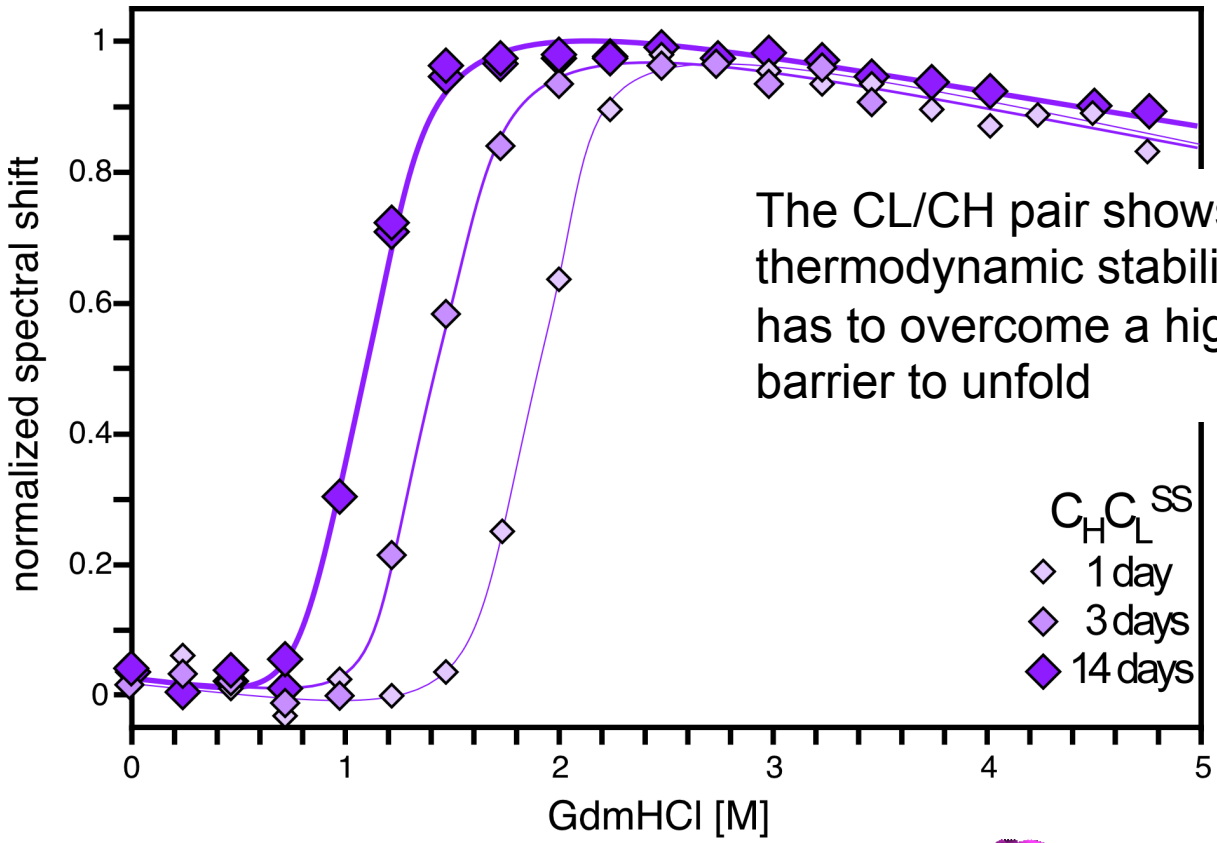


For two extremly stable domains, no mutual stabilization is observed. However, we found no evidence of the two domains dissociating without unfolding

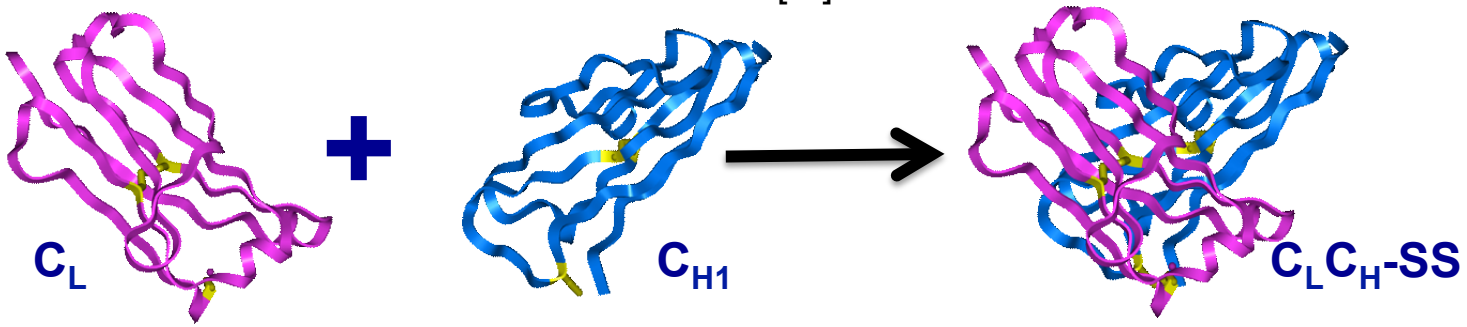


University of Zurich[UZH]

Mutual Stabilization of C_L and C_H

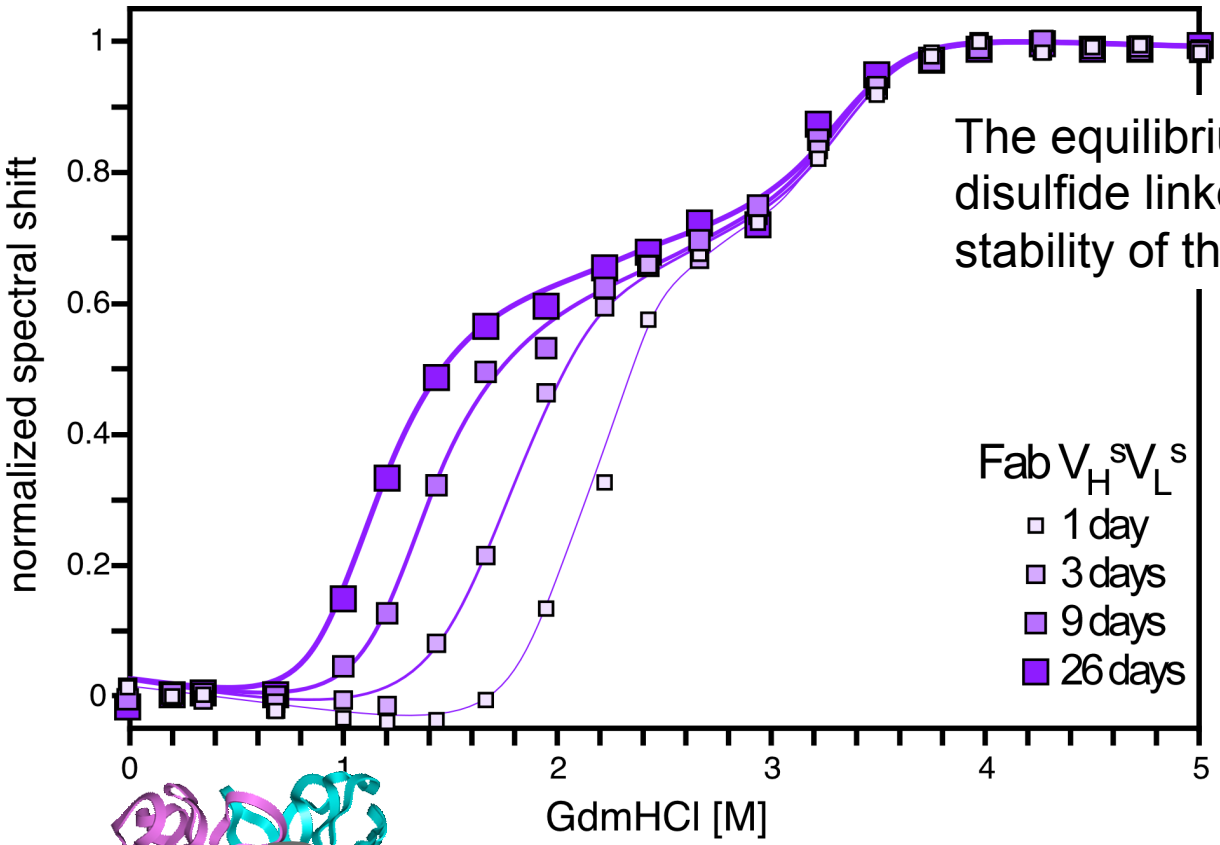


The CL/CH pair shows poor thermodynamic stability, but has to overcome a high activation barrier to unfold

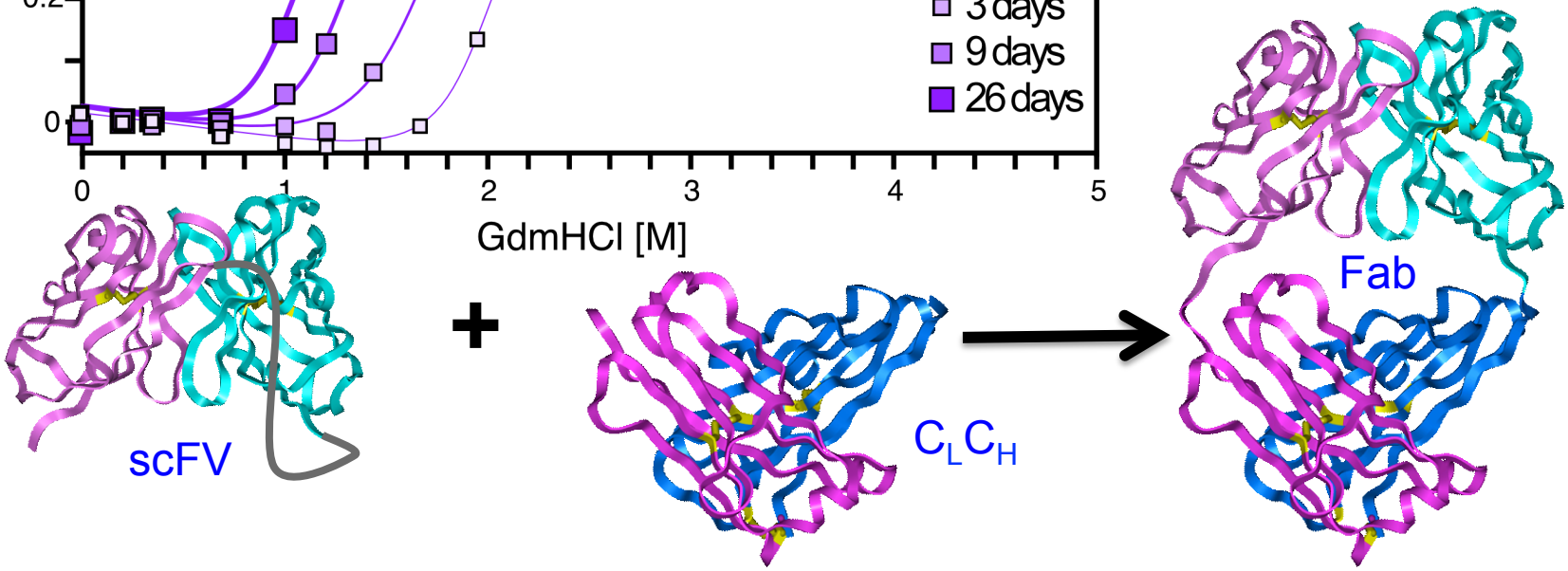


University of Zurich[UZH]

From scFV to non-disulfide linked Fab

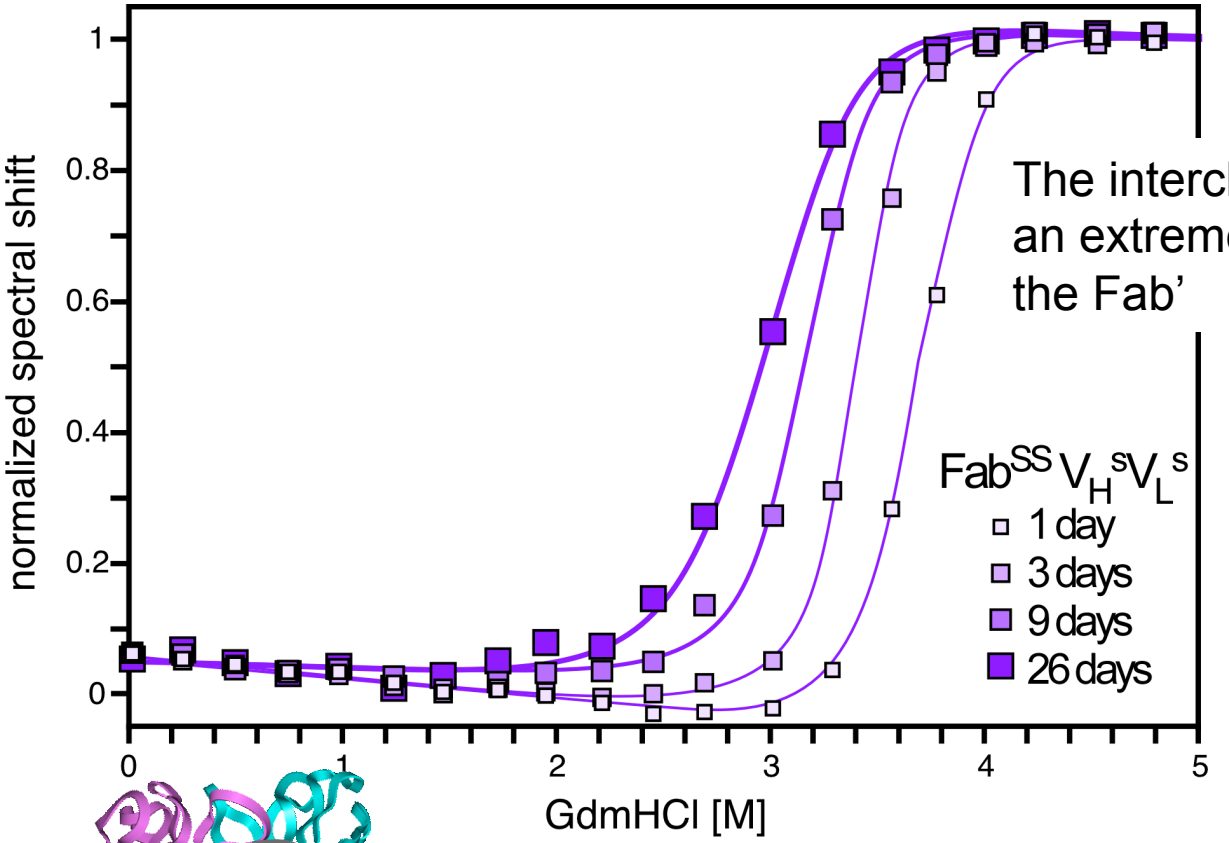


The equilibrium stability of a non-disulfide linked Fab is limited by the stability of the C_L/C_H pair

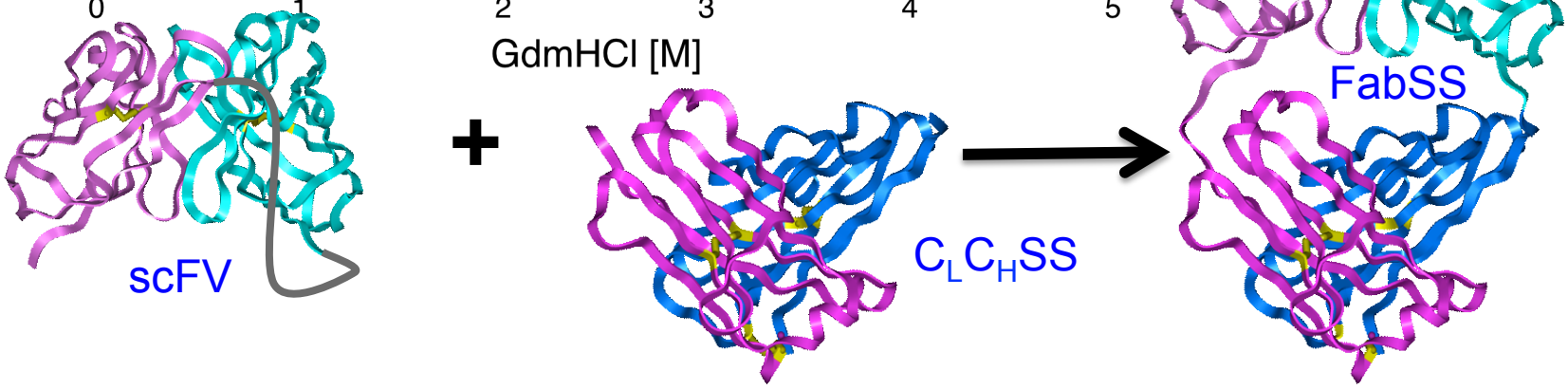


University of Zurich[UZH]

Influence of the inter-chain disulfide bond



The interchain disulfide bond has an extreme effect on the stability of the Fab'



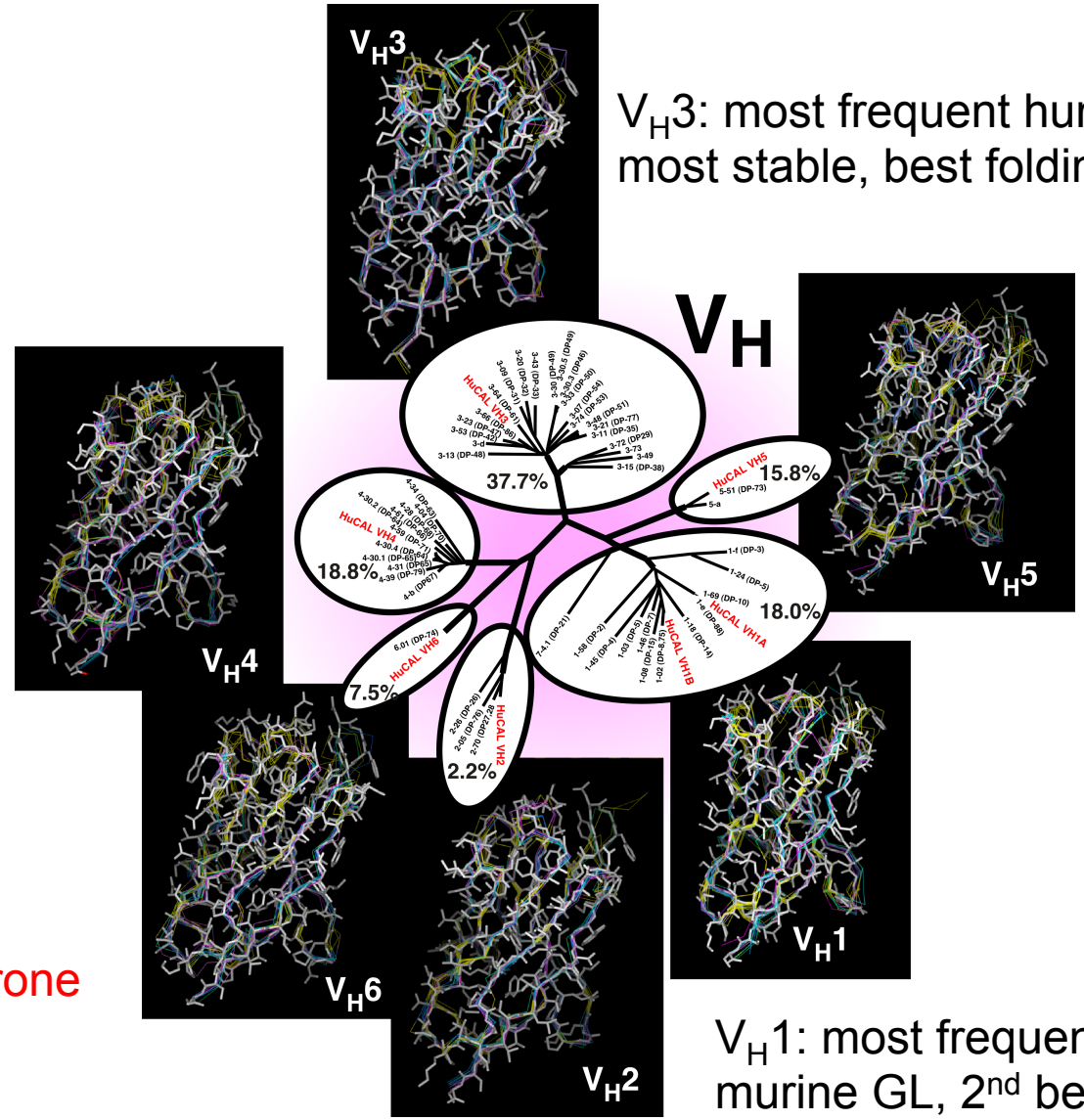
University of Zurich[UZH]

Summary

- The extent of mutual stabilization of V_L and V_H depends on the individual sequences due to the strong contribution of CDR-3s to the domain interface, and is mainly relevant for weak domains.
- There is no stabilization between V_L and C_L in the isolated light chain, nor between V_H and C_H in the Fd fragment.
- The $C_L C_H$ heterodimer dissociates in the absence of an interdomain disulfide bond.
- $[GdmHCl]_{50}$ of $C_L C_H^{SS}$ equals $[GdmHCl]_{50}$ of the isolated C_L domain.
- Kinetic stabilization of the disulfide linked $C_L C_H^{SS}$ heterodimer.
- Above a $[GdmHCl]_{50}$ of the scFv of 1.5 - 2 M, the stability of the constant domains becomes limiting for the stability of the non-disulfide-linked Fab
- In the disulfide-linked Fab', even strong variable domains profit from the kinetic stabilization of the $C_L C_H^{SS}$ heterodimer, while the $C_L C_H^{SS}$ is significantly stabilized by its interaction with the $V_L V_H$ heterodimer.

University of Zurich[UZH]

Different V_H families differ widely in biophysical properties



V_H3: most frequent human GL
most stable, best folding properties

V_H2, V_H4, V_H6:
poor stability,
aggregation-prone

V_H1: most frequent
murine GL, 2nd best stability

How many mutations are needed to “repair” the weak human germline fragments?

University of Zurich[UZH]

Features that lead to unstable and aggregation-prone antibody domains

Conserved hydrogen bonding interactions

core hydrogen bonding network (Glu/Gln 6, Thr 143, Tyr 104)

Hydrophobic core packing

steric clashes and cavities destabilize the domain,
mutations to hydrophilic residues are destabilizing

Hydropathic contrast between core and surface

hydrophobic surface residue can decrease folding efficiency

Conserved charge interactions

buried charge cluster (Arg 77, Asp 100, Glu 99, Arg/Gln 45, Glu/Arg 53)

Conserved unusual main-chain torsion angles

positions which enforce a positive Φ torsion angle, conserved Gly

Conserved Pro positions

cis-Pro L8 and and L136 of $V_L\kappa$, conserved trans-Pro in various positions

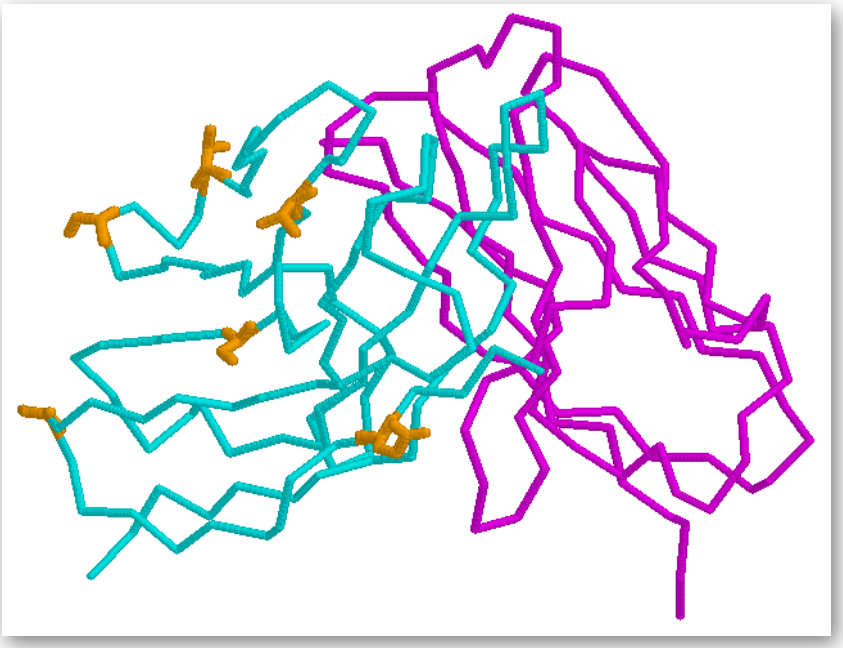
Secondary structure propensity and torsional preference

University of Zurich[UZH]

Two different scFv: 2C2 (V_κ3-V_H6) and 6B3 (V_λ3-V_H6):

	yield	stability
Gln H5 Val (secondary structure propensity)	+	+
Ser H16 Gly (pos. Φ, conformational strain)	+	+
Thr H58 Ile (hydrophobic packing, to V_H consensus)	0	+
Ser H76 Gly (pos. Φ, conformational strain)	+	+
Ser H90 Tyr (semiexposed hydrophobic, to V_H cons.)	+	0
Val H72 Asp (exposed hydrophobic residue)	+	0

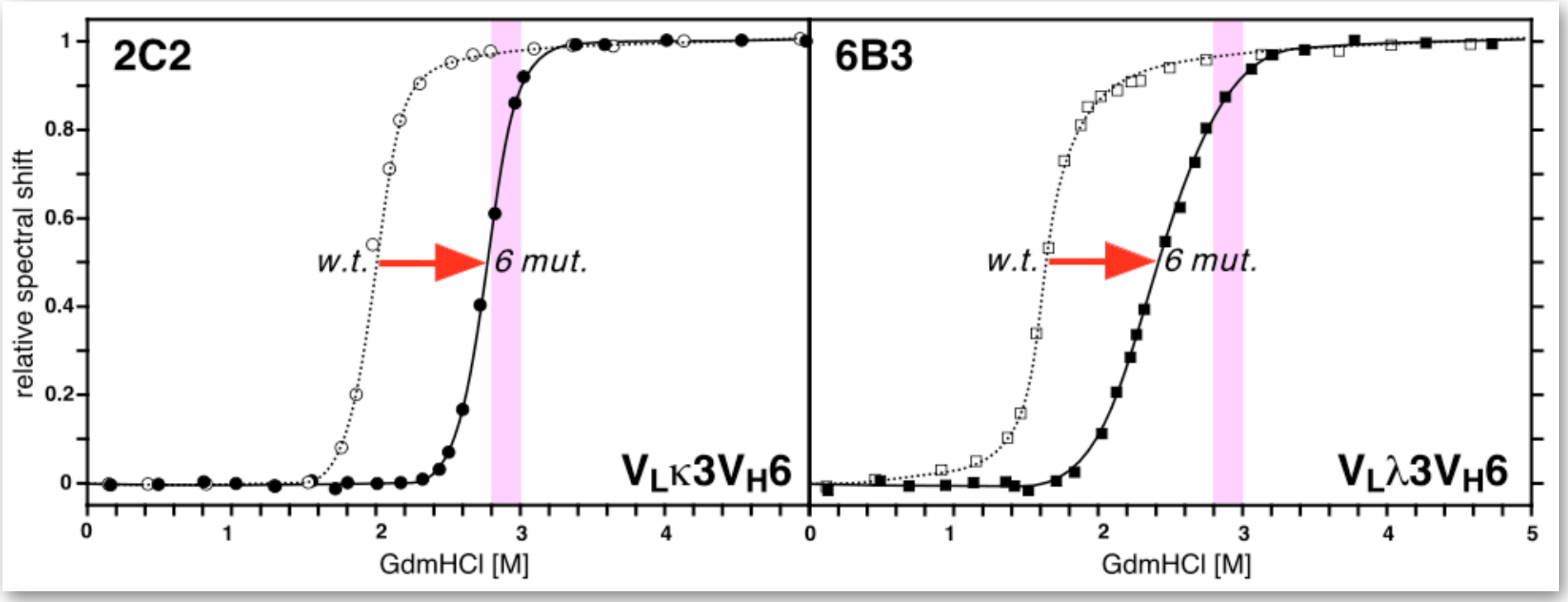
$[GdmHCl]_{50}$ shifted from 2.0 to 2.8 M
and from 0.7 to 2.5 M *
Total stabilisation by 21 and 25 kJ/mol
from 51 to 72 kJ/mol
and from 42 to 67 kJ/mol *
Total increase in yield 4.3 and 4.2-fold,
from 1.2 mg/L to 5 mg/L
and from 0.4 mg/L to 1.7 mg/L

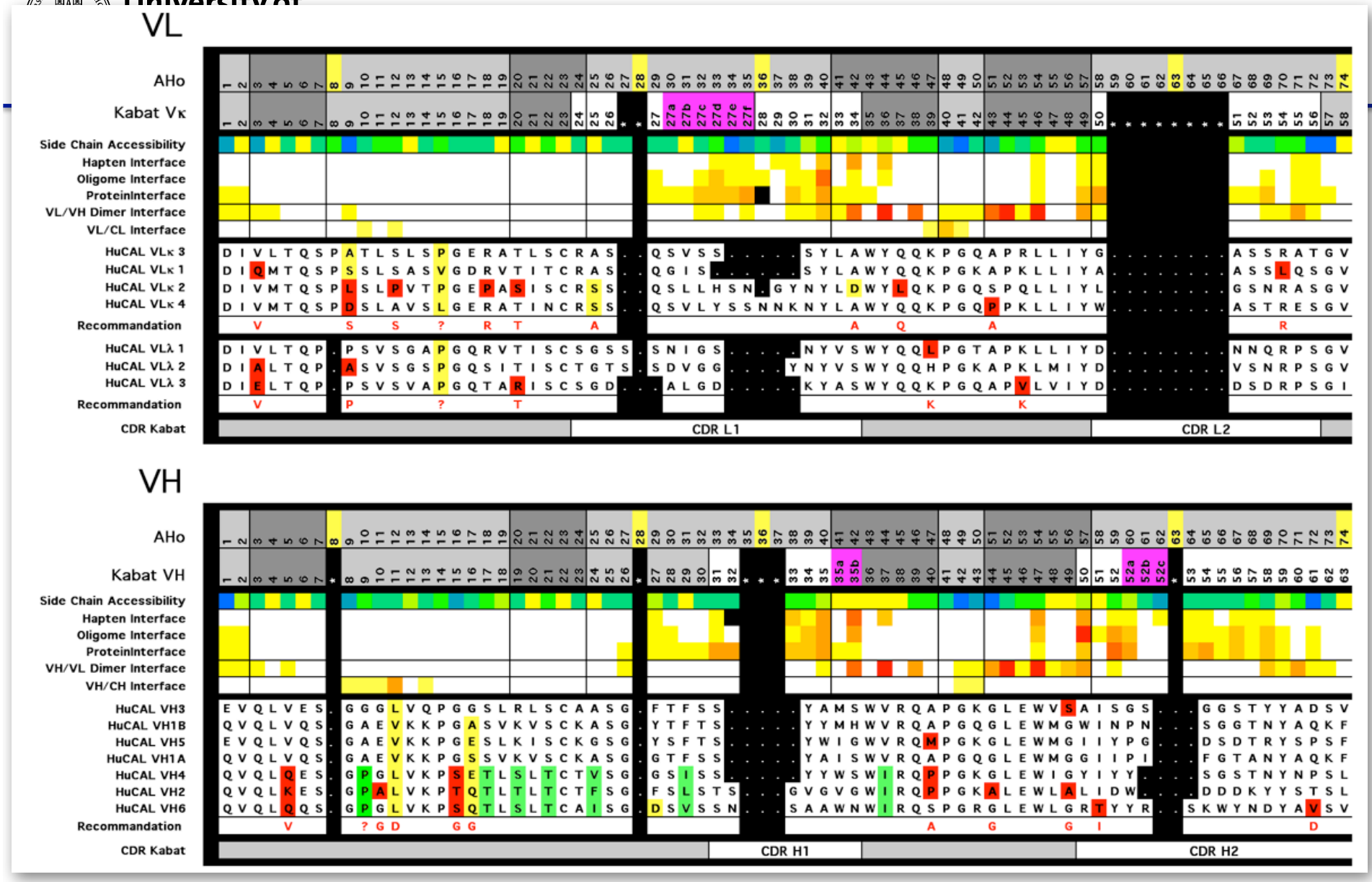


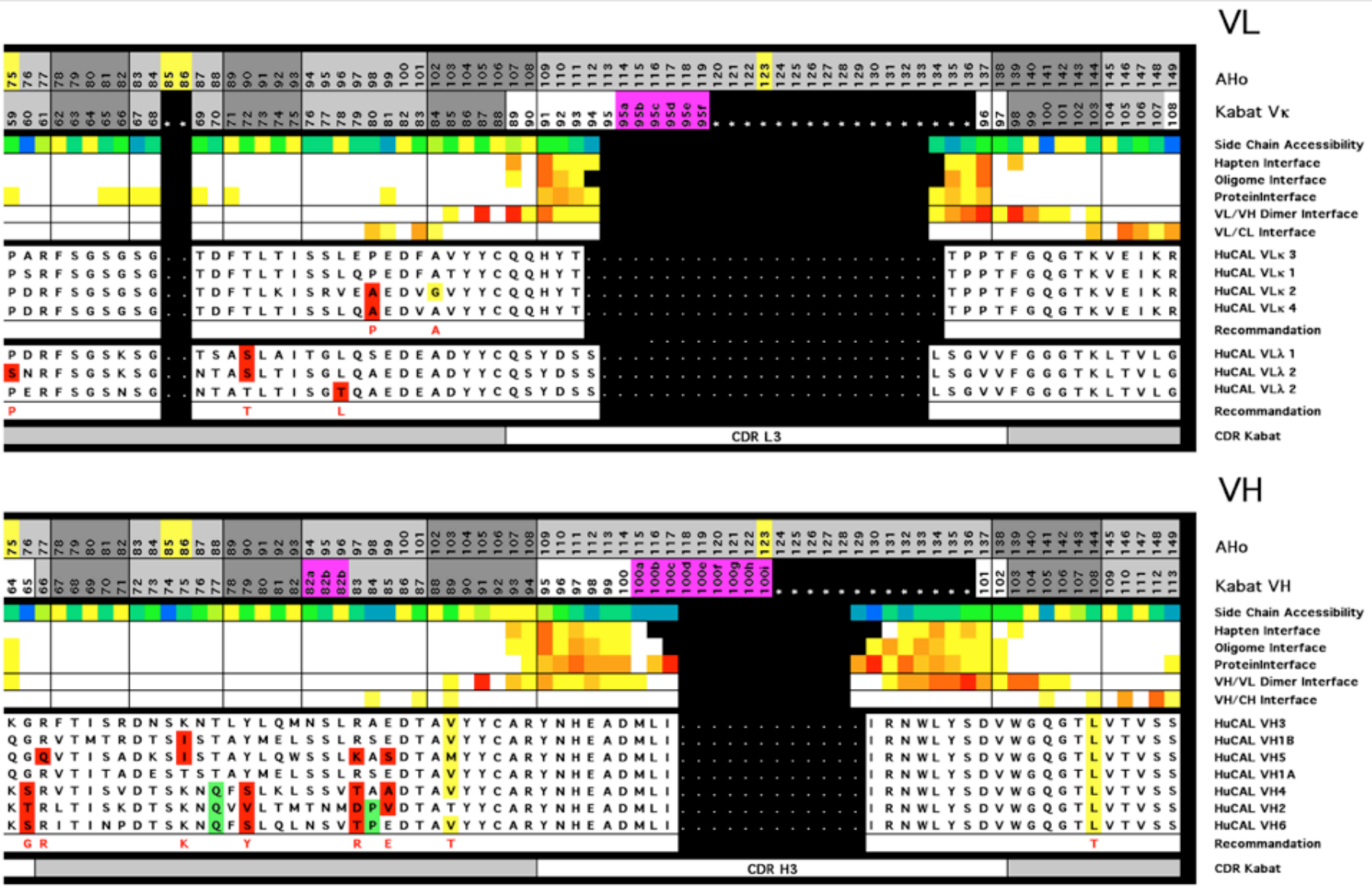
University of Zurich[UZH]

Improving the huVH6 consensus framework

Six mutations were needed, five of them common to huV$_H$2, huV$_H$4 and huV$_H$6:
Three mutations improved both stability and yield
Two improved the folding yield, but had no measurable effect on thermodynamic stability
One significantly improved stability without affecting the folding yield




VL
AHo
Kabat Vκ
Side Chain Accessibility
Hapten Interface
Oligome Interface
ProteinInterface
VL/VH Dimer Interface
VL/CL Interface
HuCAL VLκ 3
HuCAL VLκ 1
HuCAL VLκ 2
HuCAL VLκ 4
Recommandation
HuCAL VLλ 1
HuCAL VLλ 2
HuCAL VLλ 3
Recommandation
CDR Kabat
CDR L1
CDR L2
VH
AHo
Kabat VH
Side Chain Accessibility
Hapten Interface
Oligome Interface
ProteinInterface
VH/VL Dimer Interface
VH/CH Interface
HuCAL VH3
HuCAL VH1B
HuCAL VH5
HuCAL VH1A
HuCAL VH4
HuCAL VH2
HuCAL VH6
Recommandation
CDR Kabat
CDR H1
CDR H2


VL
AHo
Kabat Vκ
Side Chain Accessibility
Hapten Interface
Oligome Interface
ProteinInterface
VL/VH Dimer Interface
VL/CL Interface
HuCAL VLκ 3
HuCAL VLκ 1
HuCAL VLκ 2
HuCAL VLκ 4
Recommandation
HuCAL VLλ 1
HuCAL VLλ 2
HuCAL VLλ 2
Recommandation
CDR Kabat
CDR L3
VH
AHo
Kabat VH
Side Chain Accessibility
Hapten Interface
Oligome Interface
ProteinInterface
VH/VL Dimer Interface
VH/CH Interface
HuCAL VH3
HuCAL VH1B
HuCAL VH5
HuCAL VH1A
HuCAL VH4
HuCAL VH2
HuCAL VH6
Recommandation
CDR Kabat
CDR H3

University of Zurich[UZH]

Does variable domain stability matter for a whole IgG expressed in mammalian cells?