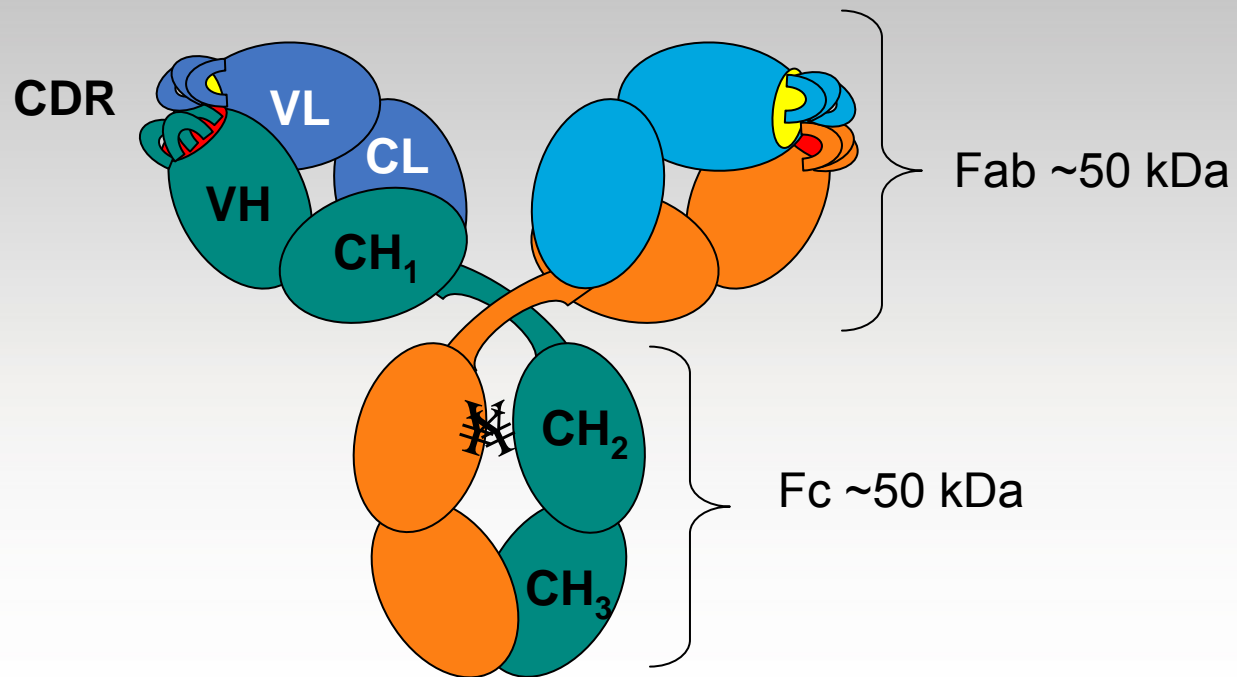


# Contribution of variable domains to the stability of humanized IgG1 monoclonal antibodies

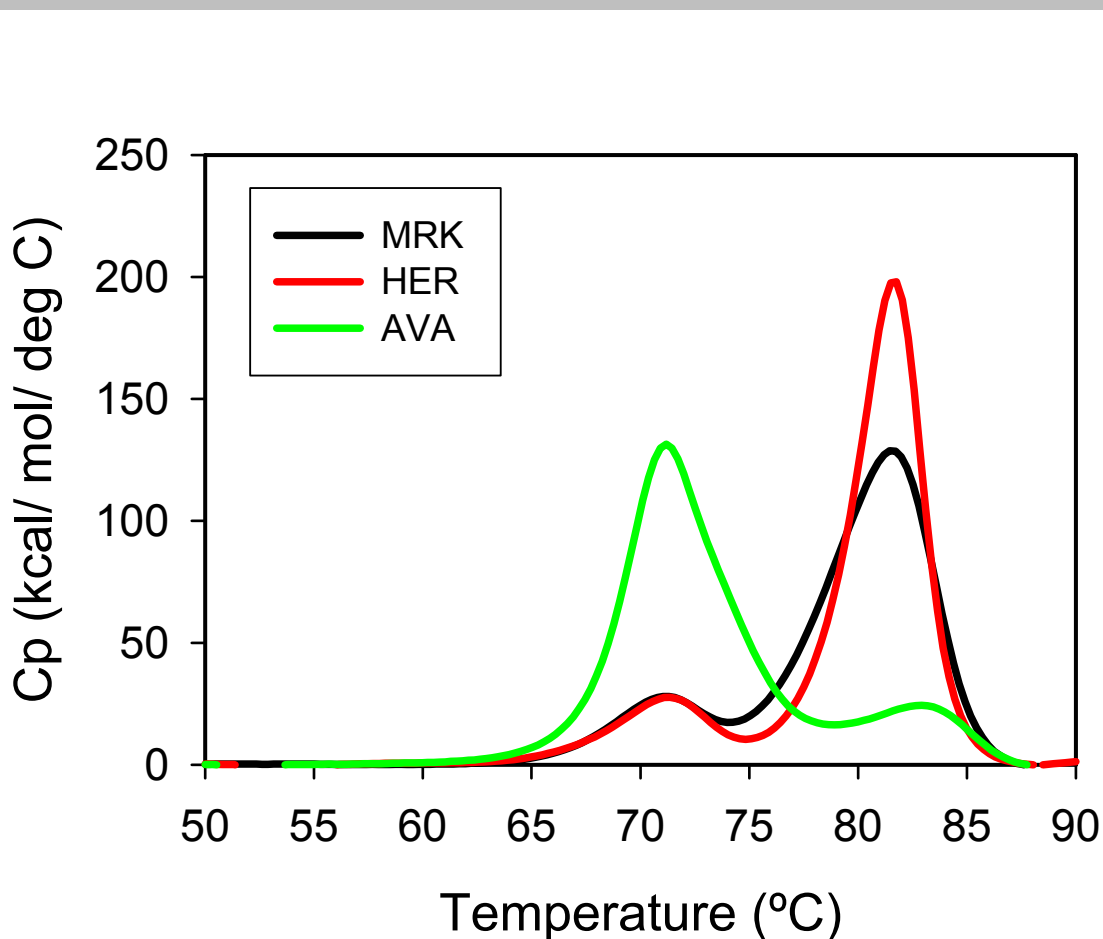
Presented by Roxana Ionescu

# Structural features of a monoclonal antibody

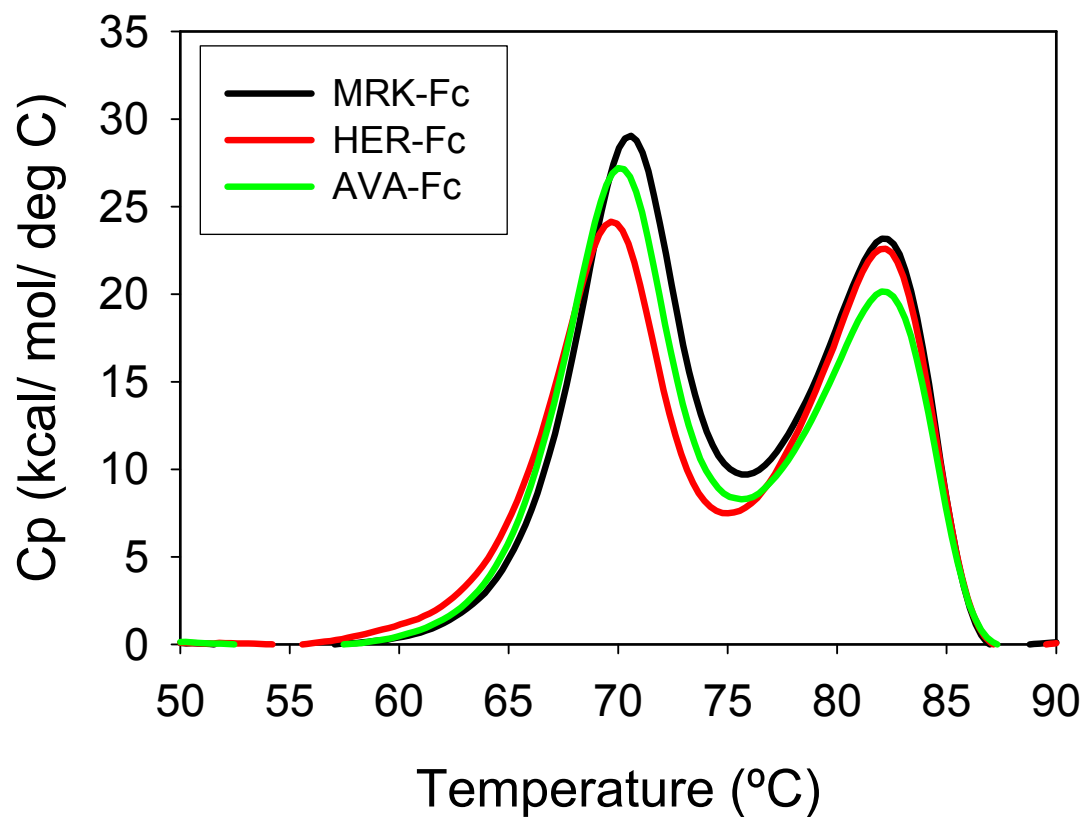


2 Fab: 1 Fc per intact mAB molecule

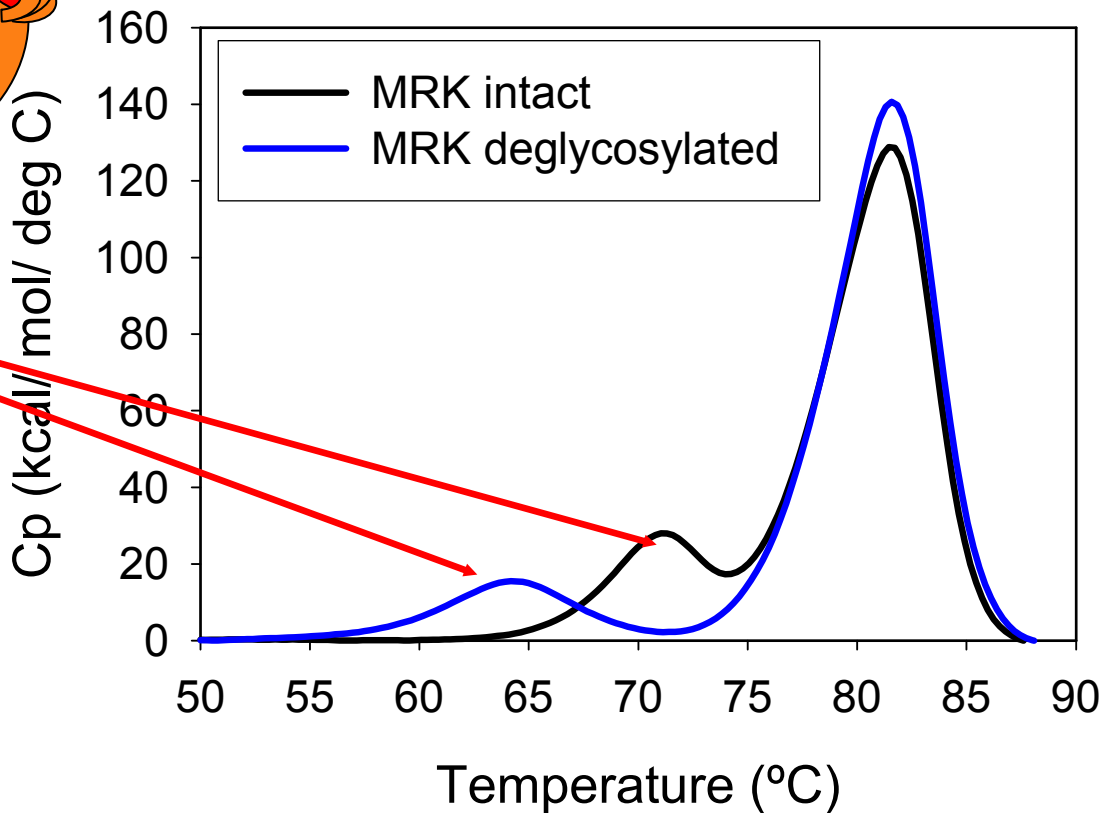
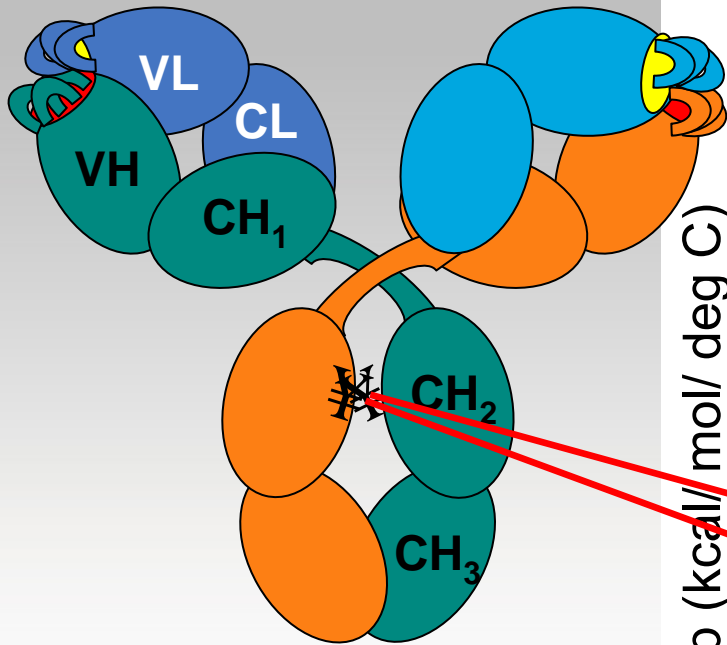
# Thermograms of three humanized IgG1 monoclonal antibodies



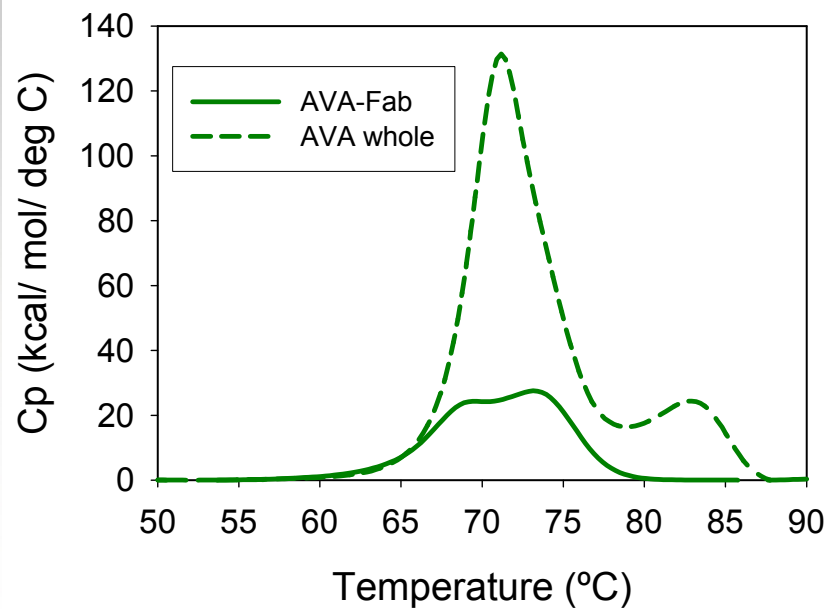
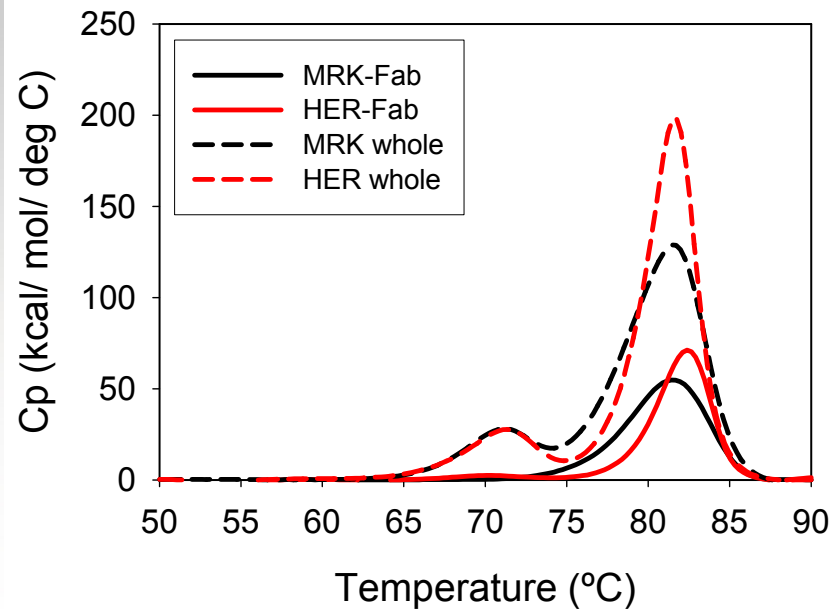
# Temperature-induced unfolding of Fc fragments



# Impact of deglycosylation on the stability of a monoclonal antibody

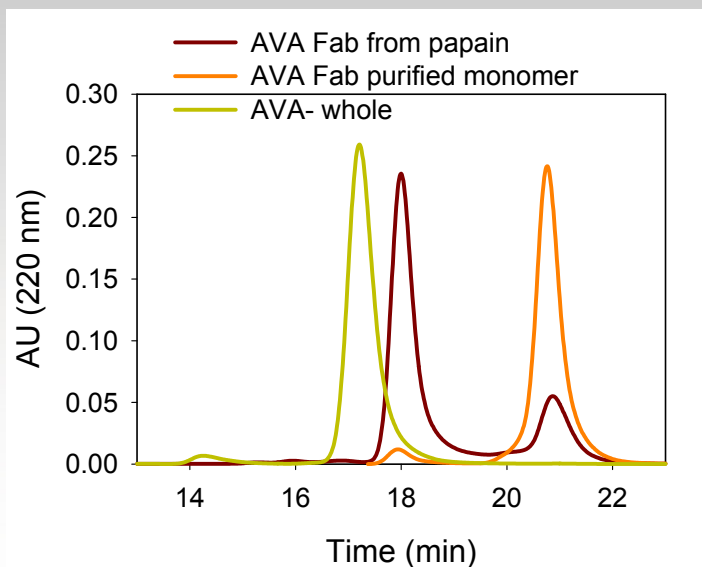


# Temperature-induced unfolding of Fab fragments

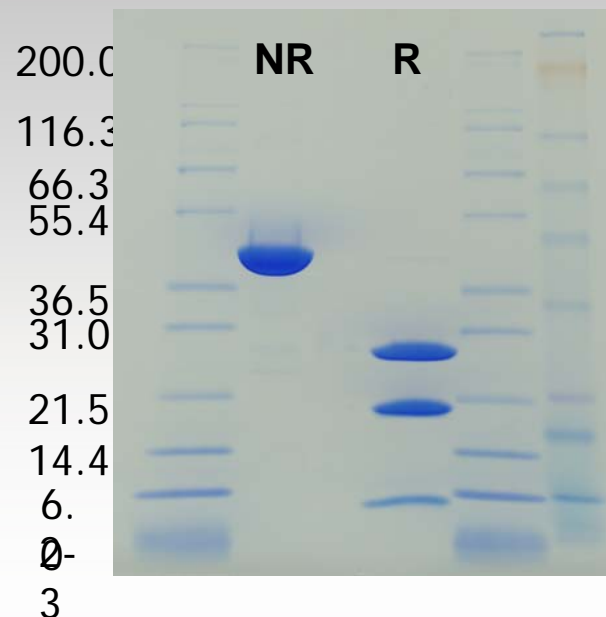


# Characterization of Ava Fab fragment resulted from papain digestion

## SEC-HPLC on Ava-Fab



## SDS-PAGE on Ava-Fab



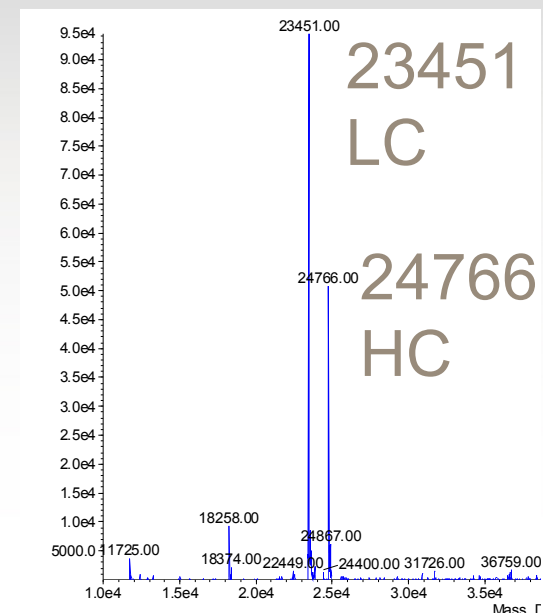
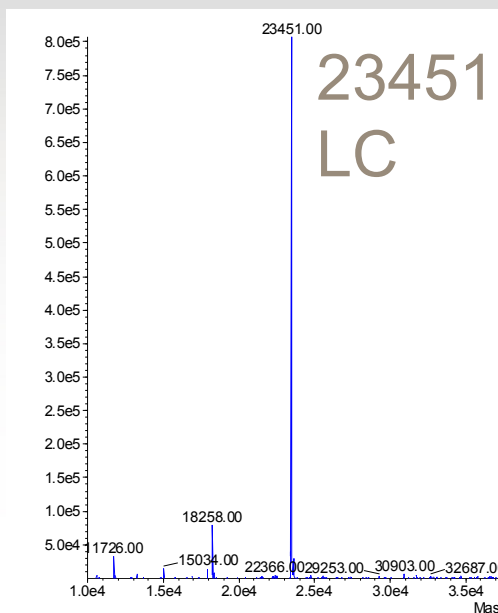
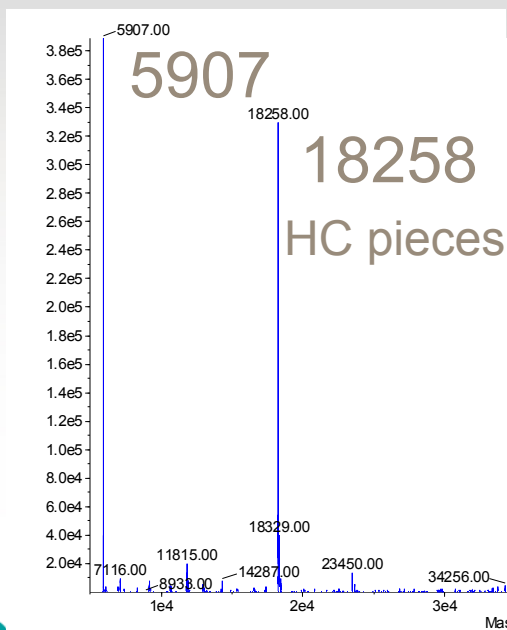
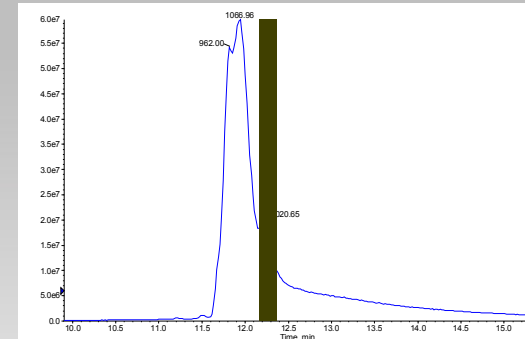
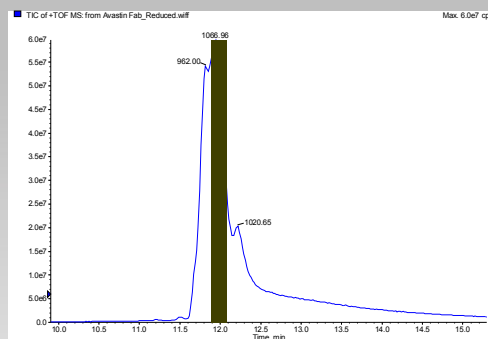
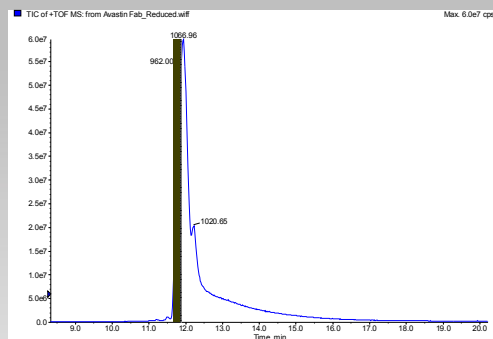
$Ava + Ava \rightleftharpoons (Ava)_2$   $K_D = 3.5 \times 10^{-4} M$  Moore et al. Biochemistry (1999) 38, 13960-7

$Fab-Ava^* + Fab-Ava^* \rightleftharpoons (Fab-Ava^*)_2$   $K_D \approx 3.5 \times 10^{-4} M$

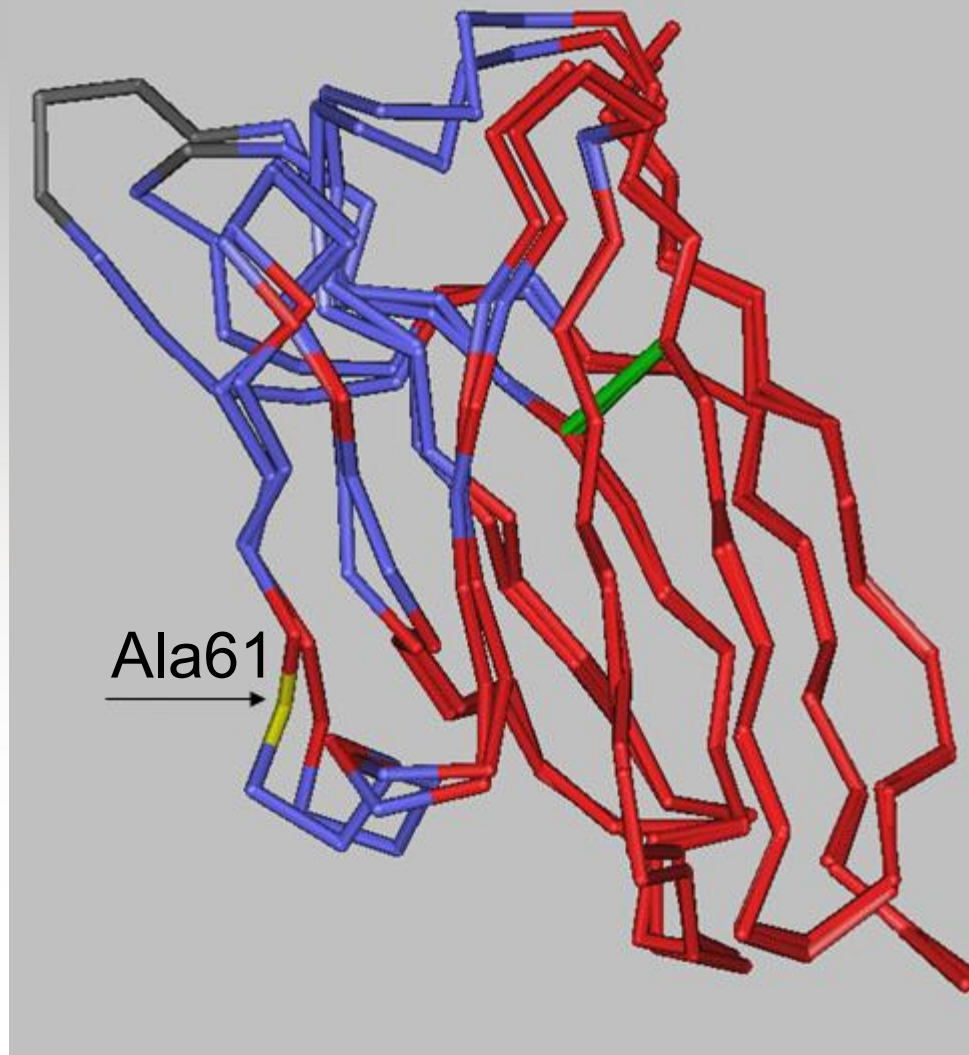
$Fab-Ava + Fab-Ava \rightleftharpoons (Fab-Ava)_2$   $K_D \sim 3.5 \times 10^{-4} M$

# LC-MS of reduced Ava-Fab: expected MW HC 24766 Da; LC 23451 Da

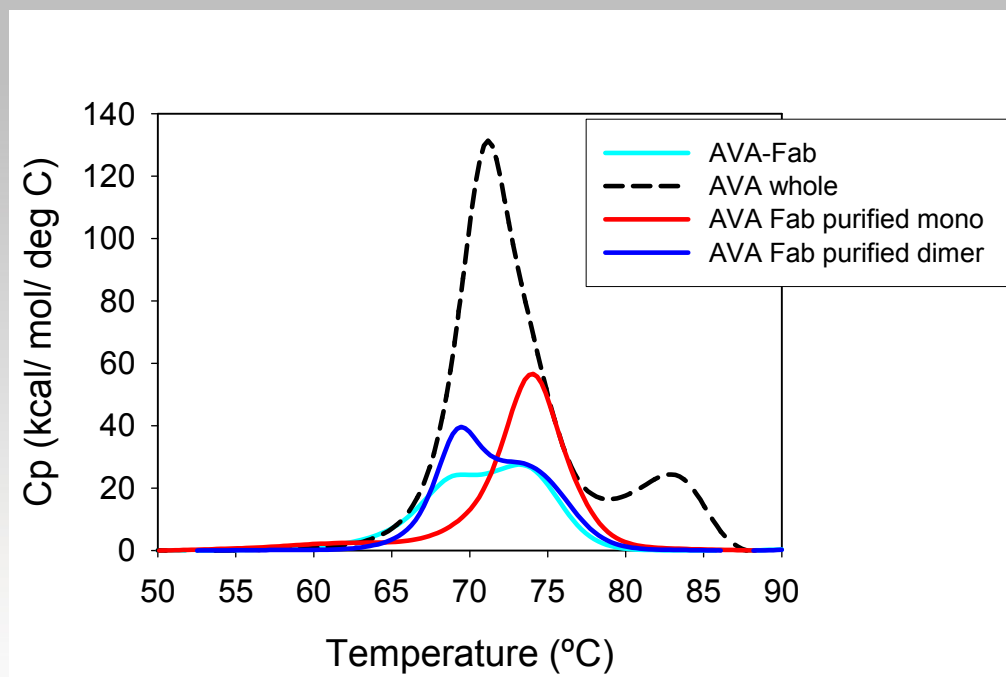
TIC



# Overlay of Her and Ava heavy chain variable domains: additional clipping site of papain in Ava Fab

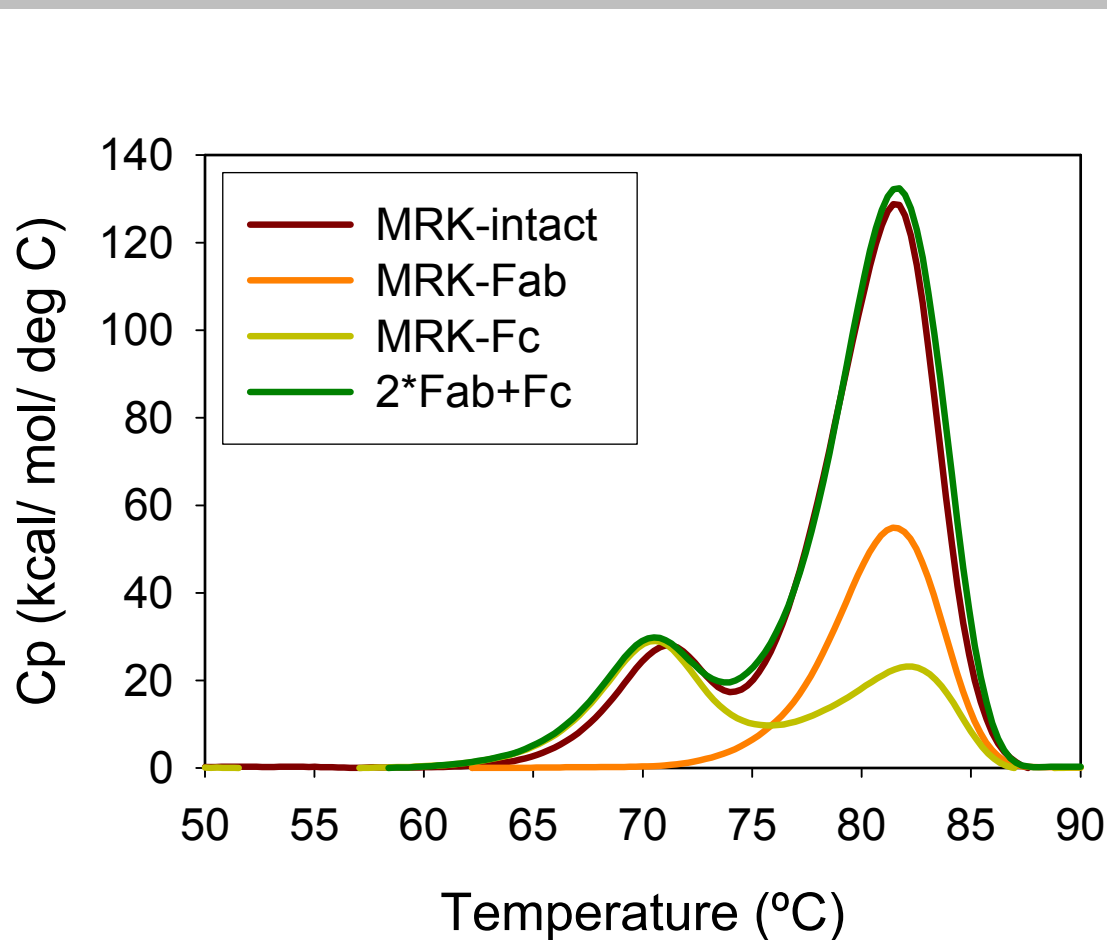


# Thermograms of purified fractions of Ava Fab

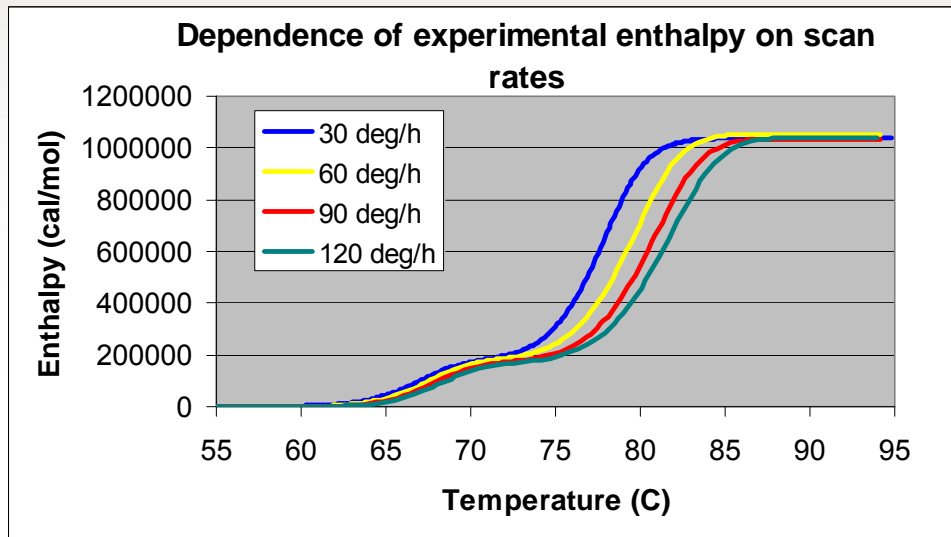
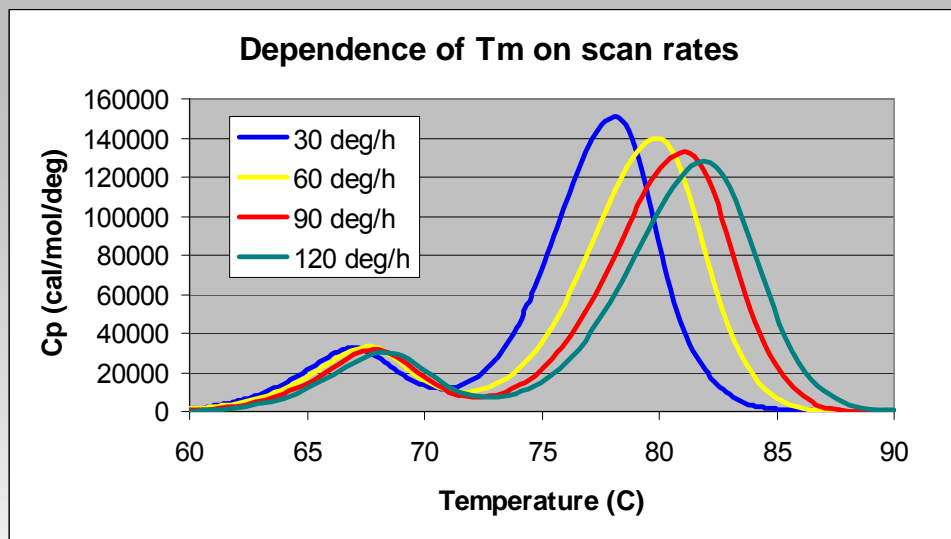


By SEC, the sample composition after purification is:  
“AVA Fab purified monomer”: 95% monomer, 5% dimer  
“AVA Fab purified dimer”: 12% monomer, 88% dimer  
“Monomer” Ava-Fab intact  
“Dimer” (Ava-Fab\*)<sub>2</sub> clipped

# For Mrk mAB, Fc and Fab unfold independently



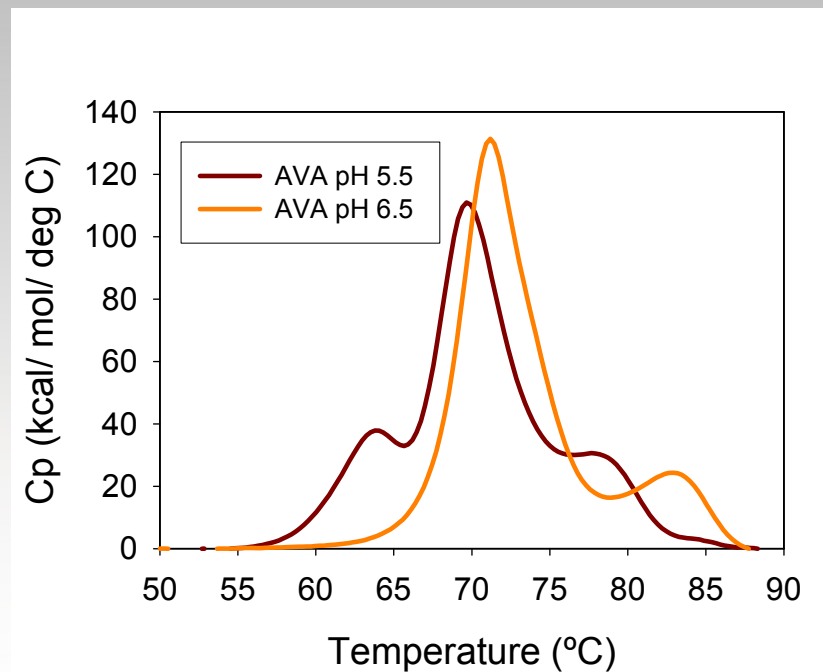
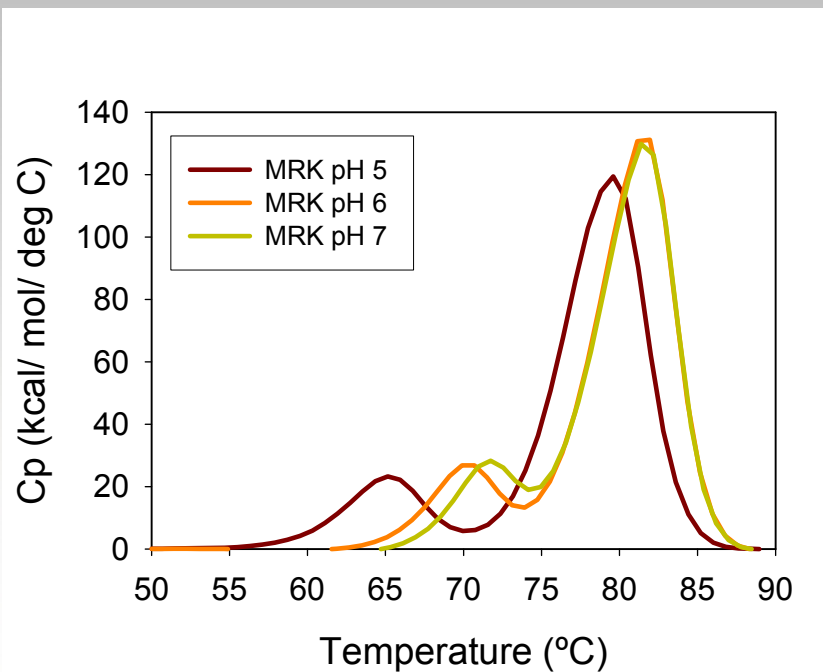
# Dependence of melting temperatures and experimental enthalpy of unfolding on scan rate



# Experimental enthalpy: key for assignment of thermal transition

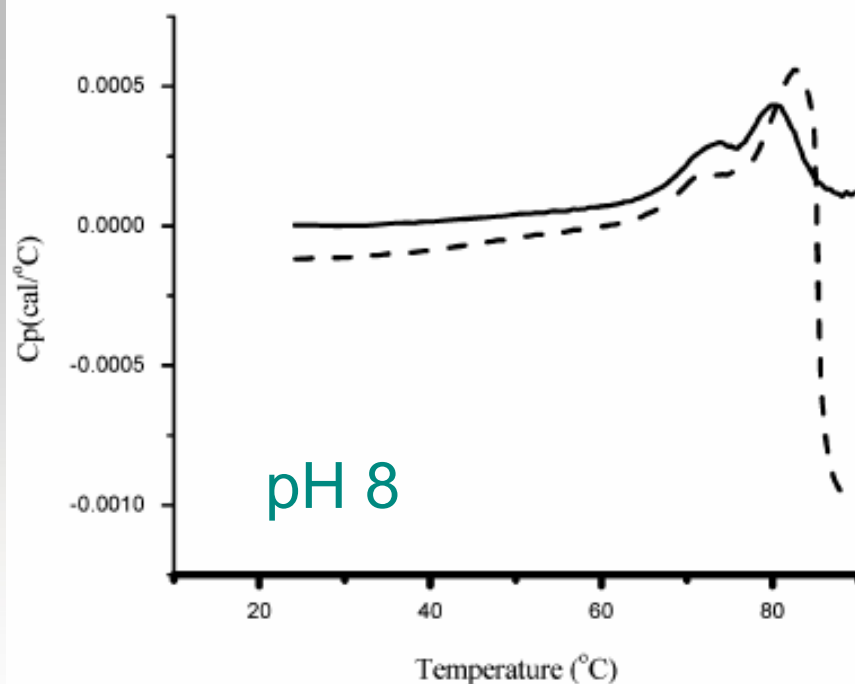
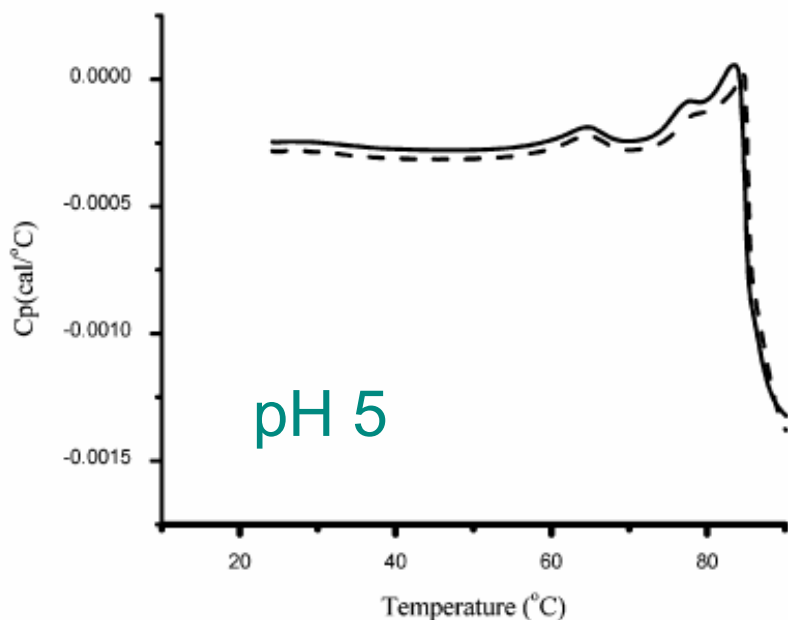
Protein	First transition		Second transition		Total Enthalpy (kcal/mol)	
	T <sub>m</sub> (°C)	Enthalpy (kcal/mol)	T <sub>m</sub> (°C)	Enthalpy (kcal/mol)		
Mrk	Intact	71.0	157	81.5	786	943
	Fab			81.4	341	341
	Fc	70.6	192	82.0	158	350
Her	Intact	71.4	163	81.8	835	998
	Fab			82.4	318	318
	Fc	69.7	170	82.2	155	325
Ava	Intact	71.2	800	82.9	138	938
	Fab	74.1	316			316
	Fc	70.0	185	82.0	138	323

# Impact of pH on the stability of IgG1 monoclonal antibodies



# A post-transition baseline is a pre-requisite for correct assignment of transitions

*Biochemistry, Vol. 46, No. 6, 2007 1539*



Aditya A. Wakankar,<sup>\*,‡</sup> Ronald T. Borchardt,<sup>§</sup> Charles Eigenbrot,<sup>||</sup> Steven Shia,<sup>||</sup> Y. John Wang,<sup>‡</sup>  
Steve J. Shire,<sup>‡</sup> and Jun L. Liu<sup>‡</sup>

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# Non-cooperative unfolding of Fab fragment can produce multiple peaks in the DSC profile

**“A broad range of Fab stabilities within a host of therapeutic IgGs “**

**Ellen Garber and Stephen J. Demarest**

**BBRC (2007) 355, 751-757**

# Why is it important to have a correct understanding of the thermal transitions of an monoclonal antibody

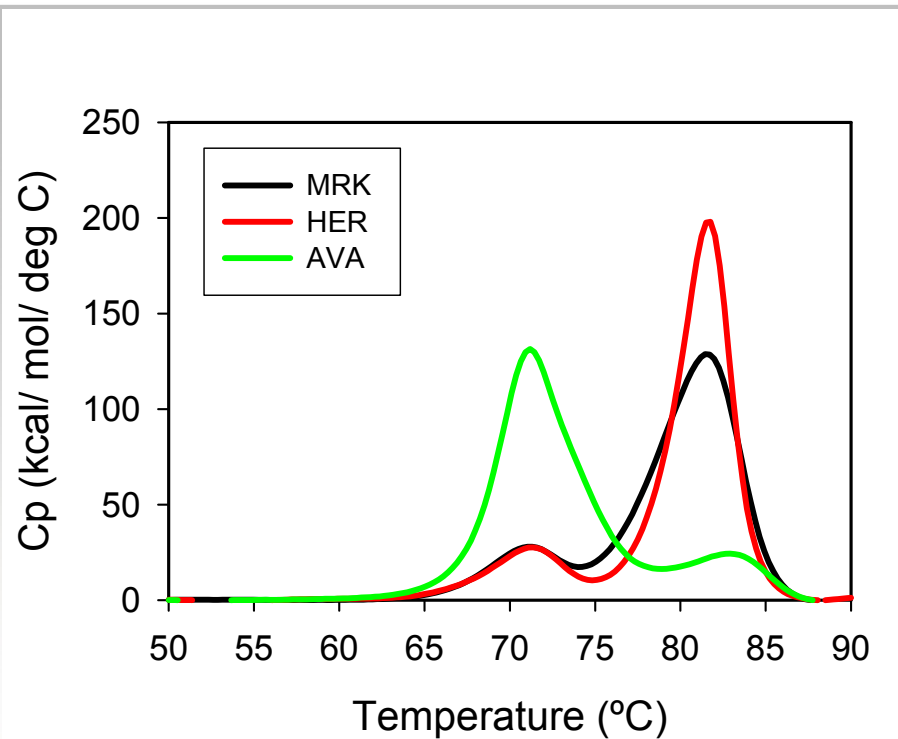
## **Support process development**

- Participate in clone selection
- Demonstrate batch-to-batch consistency
- Improve production yield (alleviate aggregation issues)

## **Support formulation development**

- Excipient screening
- Correlation with long-term stability (?)

# Formulation of three humanized IgG1 monoclonal antibodies



Her: lyophilized formulation (shelf life 3y)

Ava: liquid formulation at 25 mg/mL protein (shelf life 2y)

Mrk: liquid formulation at 20 mg/mL (shelf life  $\geq 2y$ )

# Sequence alignment of Her and Ava monoclonal antibodies

```

1                               50
1N8Z_HC EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR
1BJ1_HC EVQLVESGGG LVQPGGSLRL SCAASGYTFT NYGMNWVRQA PGKGLEWVGW

51                               100
1N8Z_HC IYPTNGYT..RYADSVKGR FTISADTSKN TAYLQMNSLR AEDTAVYYCS
1BJ1_HC I...NTYTGE PTYAADFRR FTFSLDTSKS TAYLQMNSLR AEDTAVYYCA

101                               150
1N8Z_HC RWG...GDGF YAMDYWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA
1BJ1_HC KYPHYYGSSH WYFDVWGQGT LVTVSSASTK GPSVFPLAPS SKSTSGGTAA

1                               50
1N8Z_LC DIQMTQSPSS LSASVGRDRT ITCRASQDVN TAVAWYQOKP GKAPKLLIYS
1BJ1_LC DIQMTQSPSS LSASVGRDRT ITCASQDIS NYLWYQOKP GKAPKLLIYF

51                               100
1N8Z_LC ASFLYSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ HYTTPPTFGQ
1BJ1_LC TSSLHSGVPS RFSGSRSGTD FTLTISSLQP EDFATYYCQQ YSTVPWTFGQ

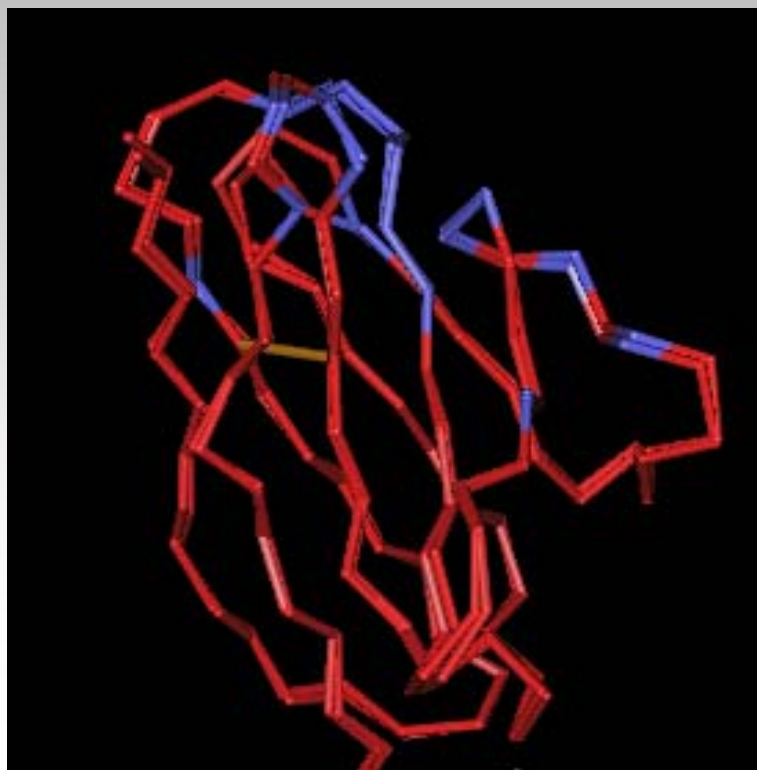
101                               150
1N8Z_LC GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNMFY PREAKVQWKV
1BJ1_LC GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNMFY PREAKVQWKV

```

“Hot spots”  
 N30 -LC Her  
 D102-HC Her  
 N55 -HC Her

Harris et al. J. Chromat. 2001

# VAST alignment of Herceptin (1n8z.pdb) and Avastin (1bj1.pdb)



Light chain- variable domains



Heavy chain- variable domains

# Acknowledgement

**Josef Vlasak**

**Colleen Price**

**Henryk Mach**

**Marc Kirchmeier**