Combining refoldability assessment and molecular dynamics simulations to select aggregation-resistant antibodies

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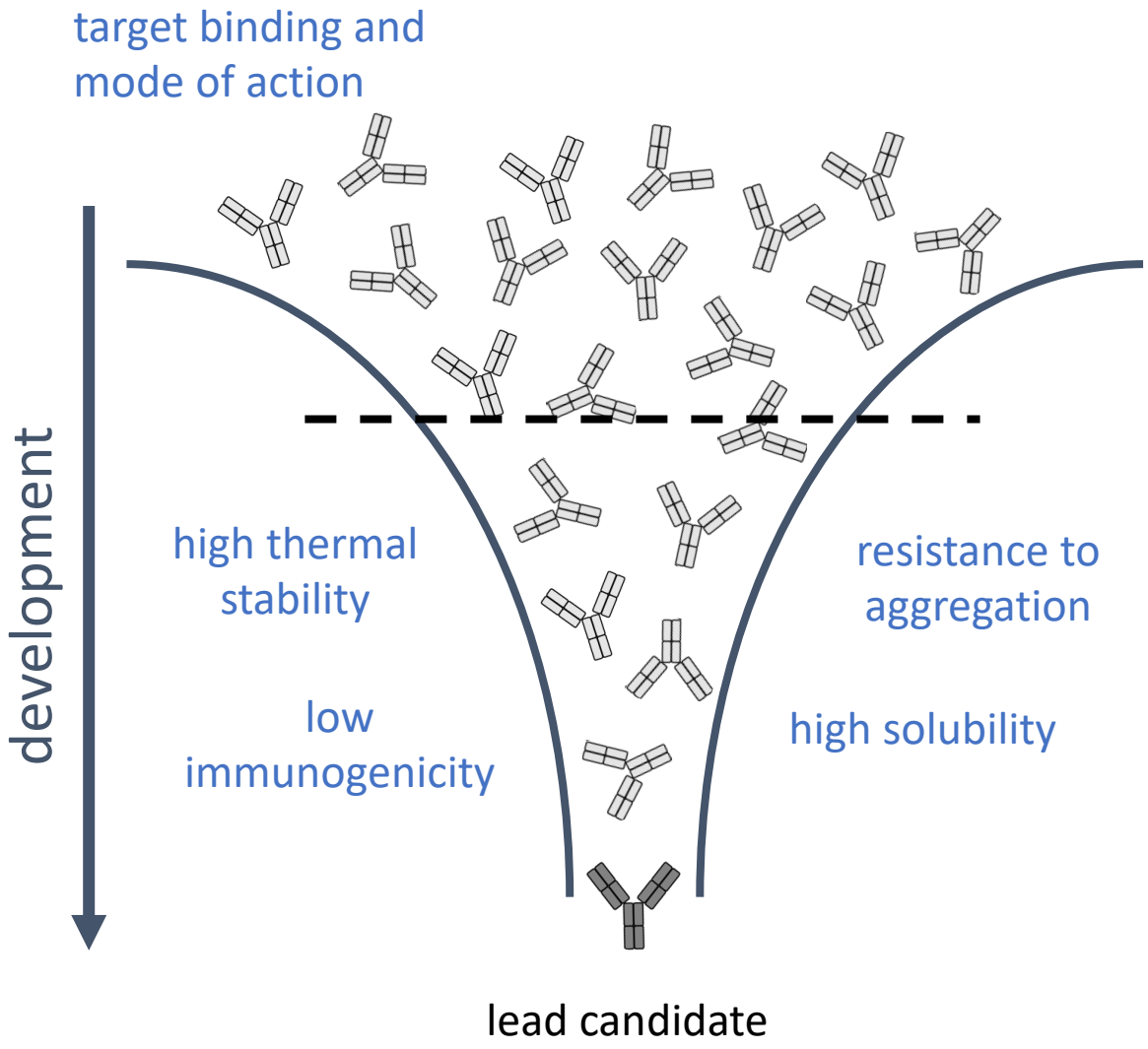
Antibody candidate selection



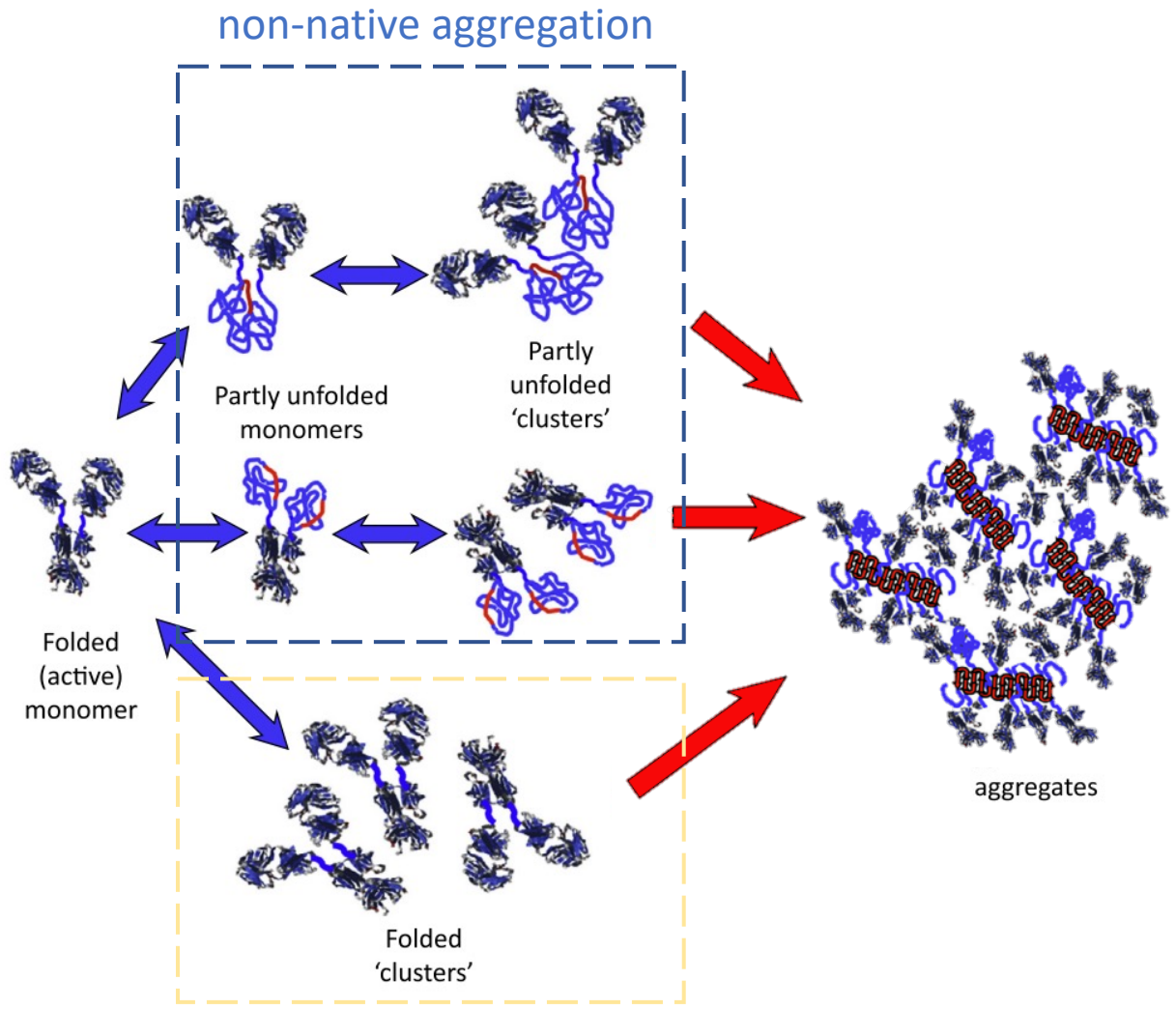
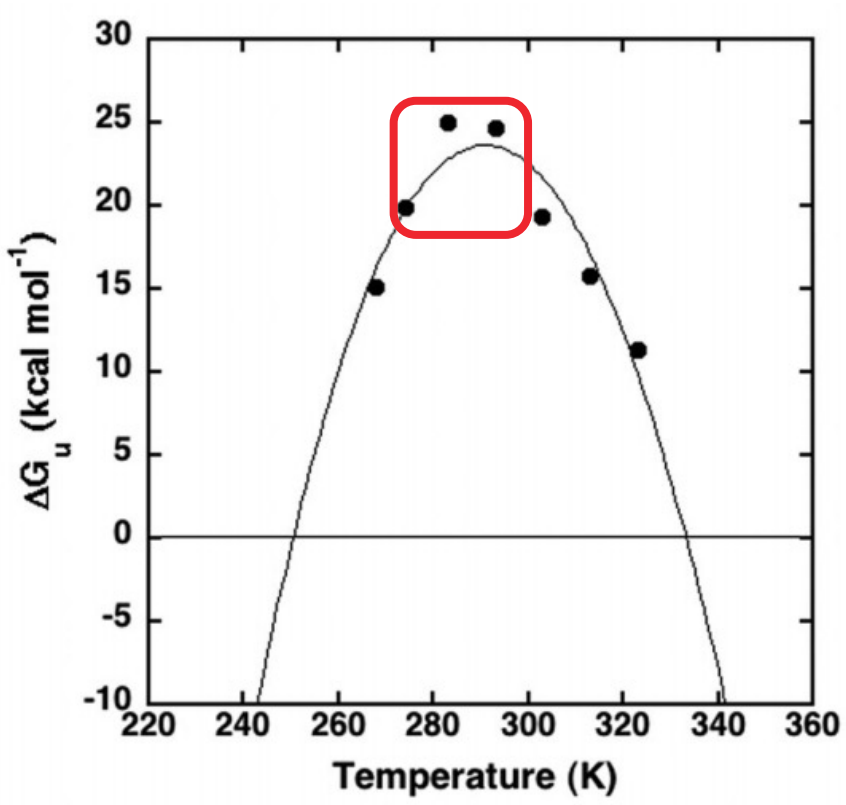


Image adapted from CJ Roberts (2014) *Trends in Biotechnology*

Challenges to study the stability of partially unfolded states

mAb stability curve



$$dG = -RT\ln(K_{eq})$$

$$\text{folded} \underset{}{\overset{K_{eq}}{\rightleftharpoons}} \text{unfolded}$$

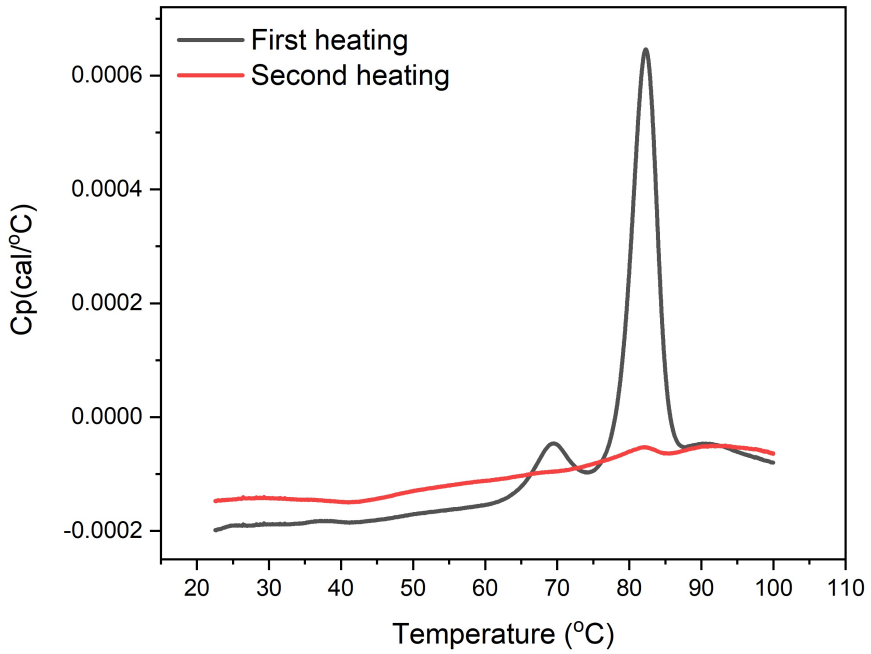
dG (kcal/mol)	% unfolded protein
0	50
2.7	1
5.5	0.01
9.6	0.00001
20	0.0000000000002

- the fraction of partially unfolded species is tiny at ambient conditions

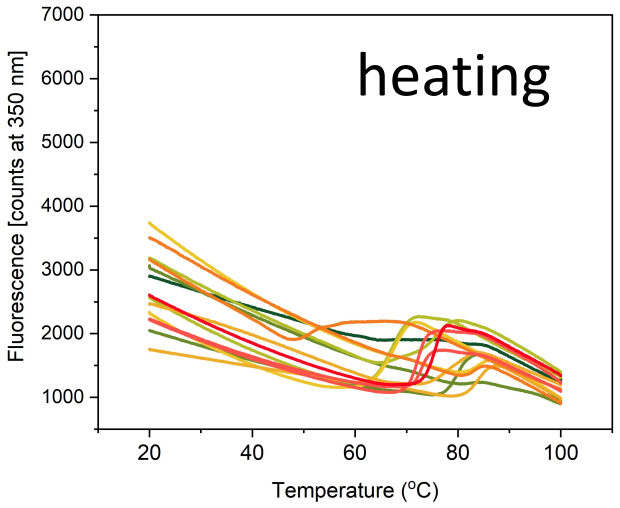
Lazar et al. *mAbs* (2010)

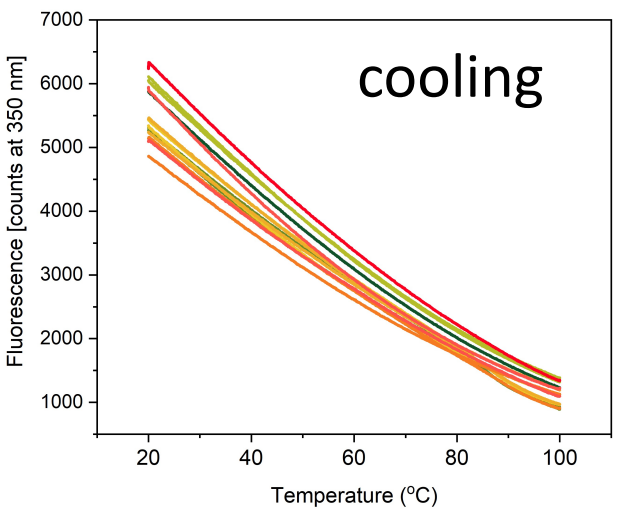
Traditional approach to test for refoldability after heating

Differential scanning calorimetry: consecutive heating scans



Nano differential scanning fluorimetry: heating and cooling scans

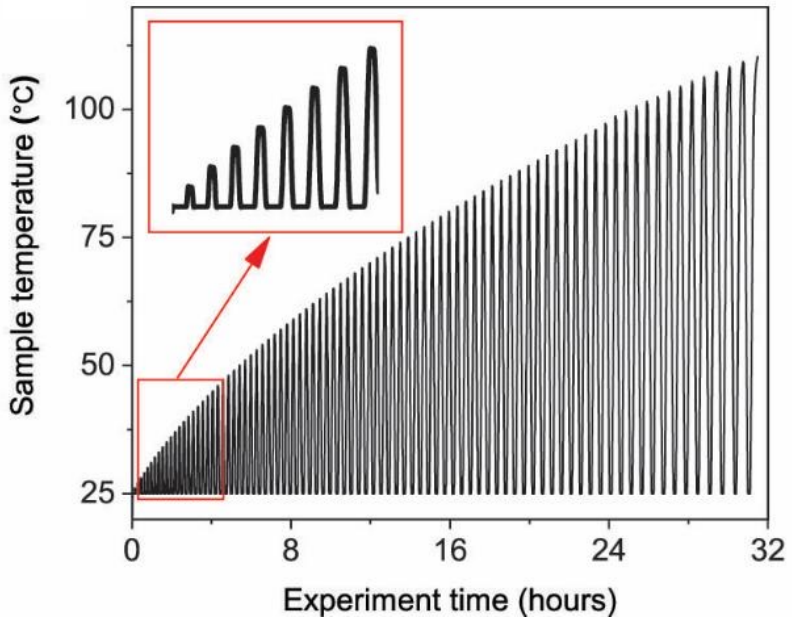




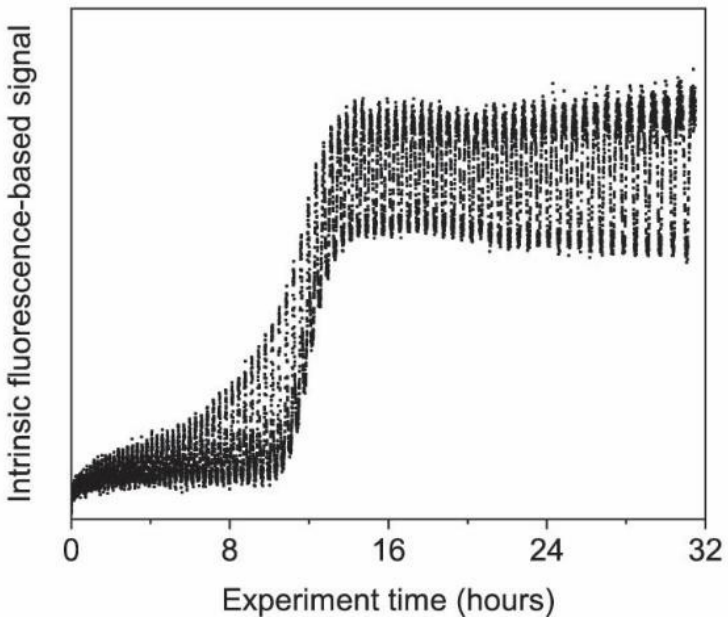
- Problem: overheating masks unfolding reversibility differences

Another approach – modulated scanning fluorimetry (MSF)

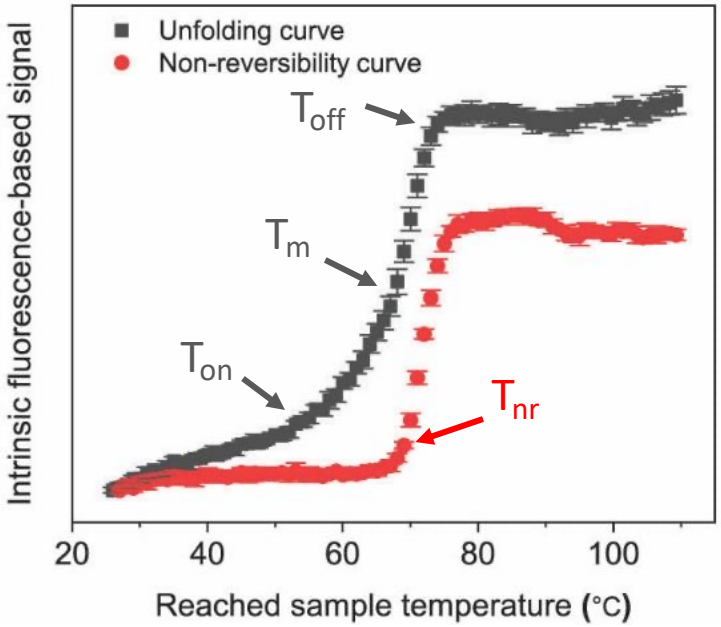
Incremental heating and cooling cycles



raw data



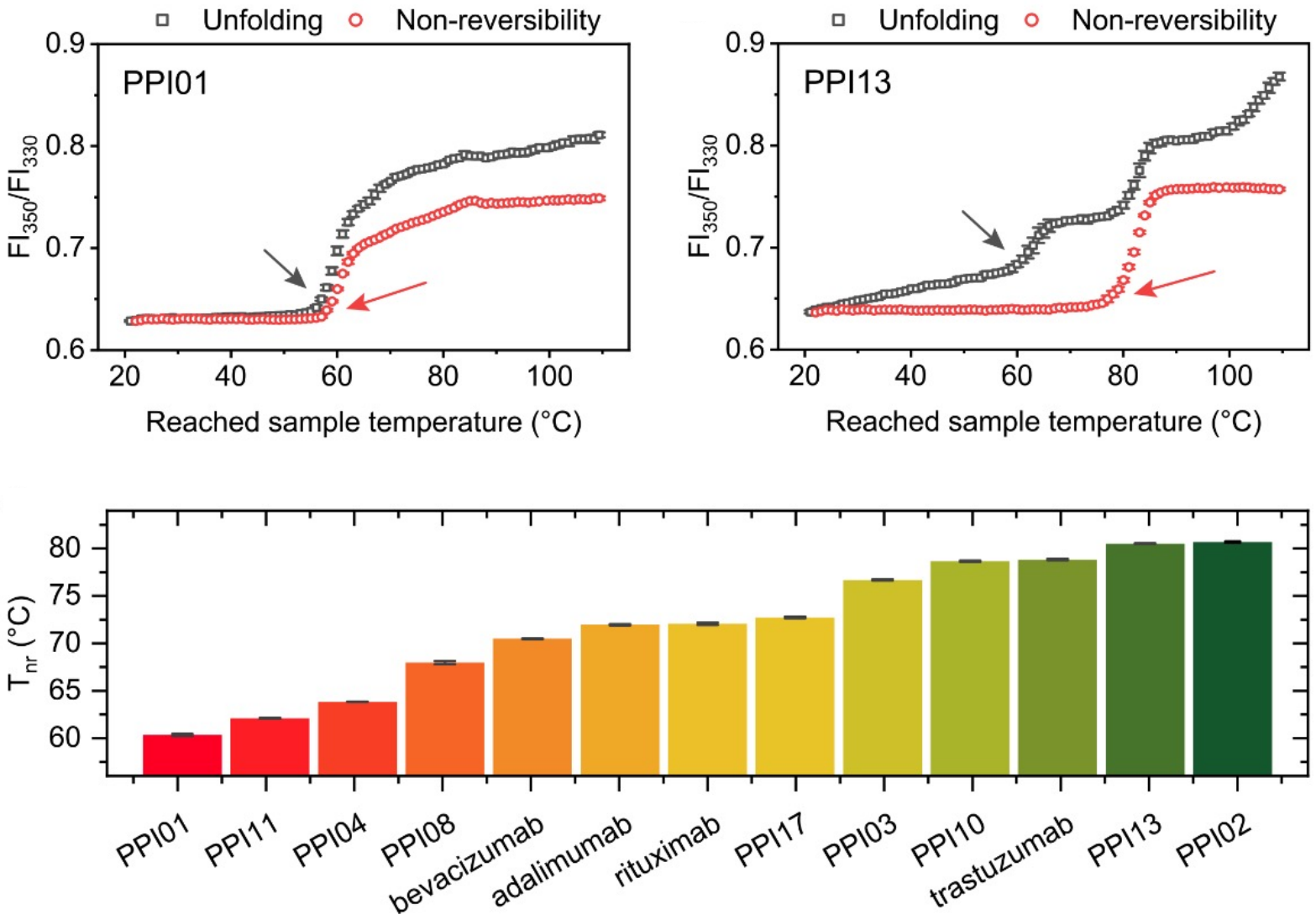
deconvoluted data



- distinct unfolding and non-reversibility curves are obtained

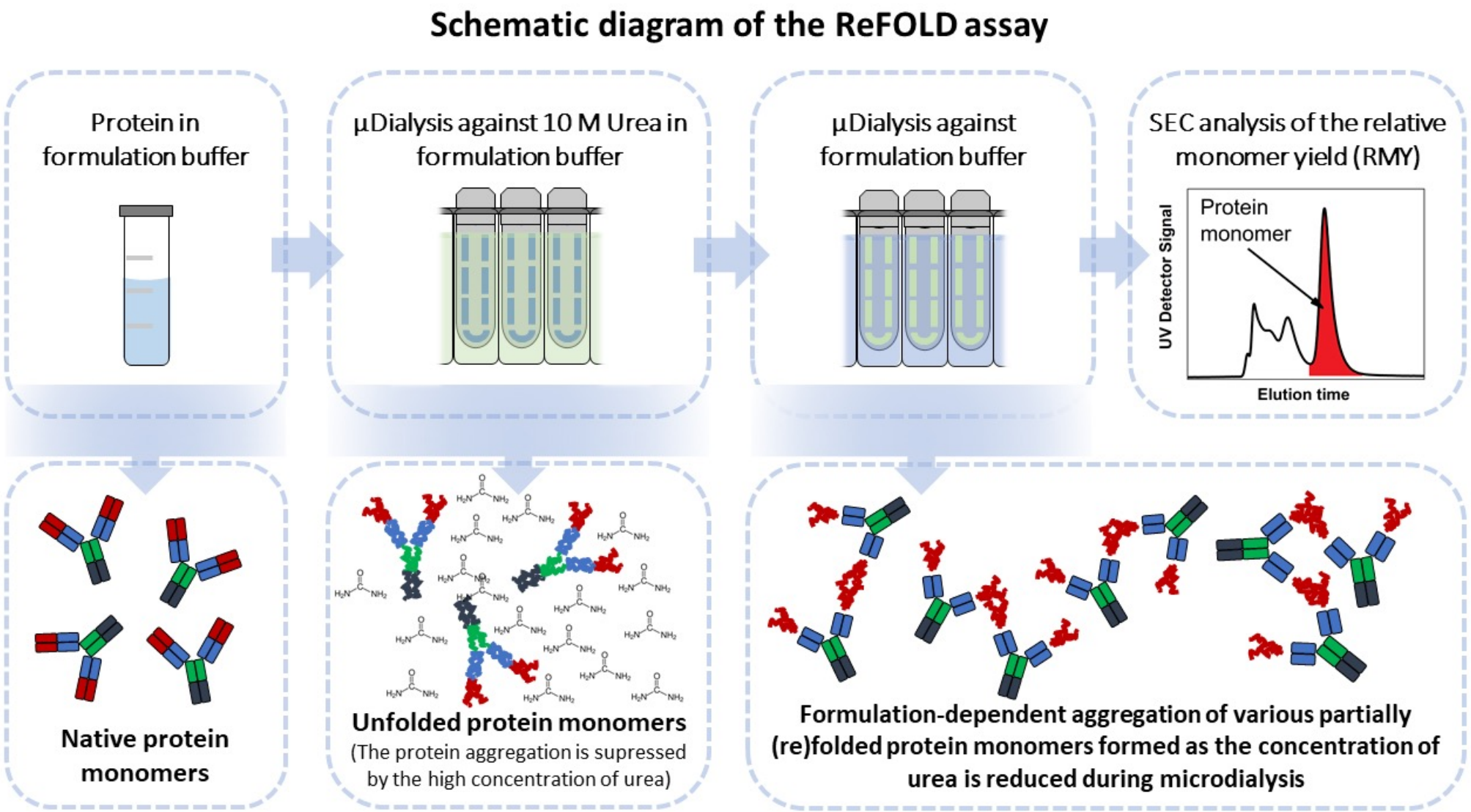
Svilenov et al. (2020)

MSF to study antibody candidates



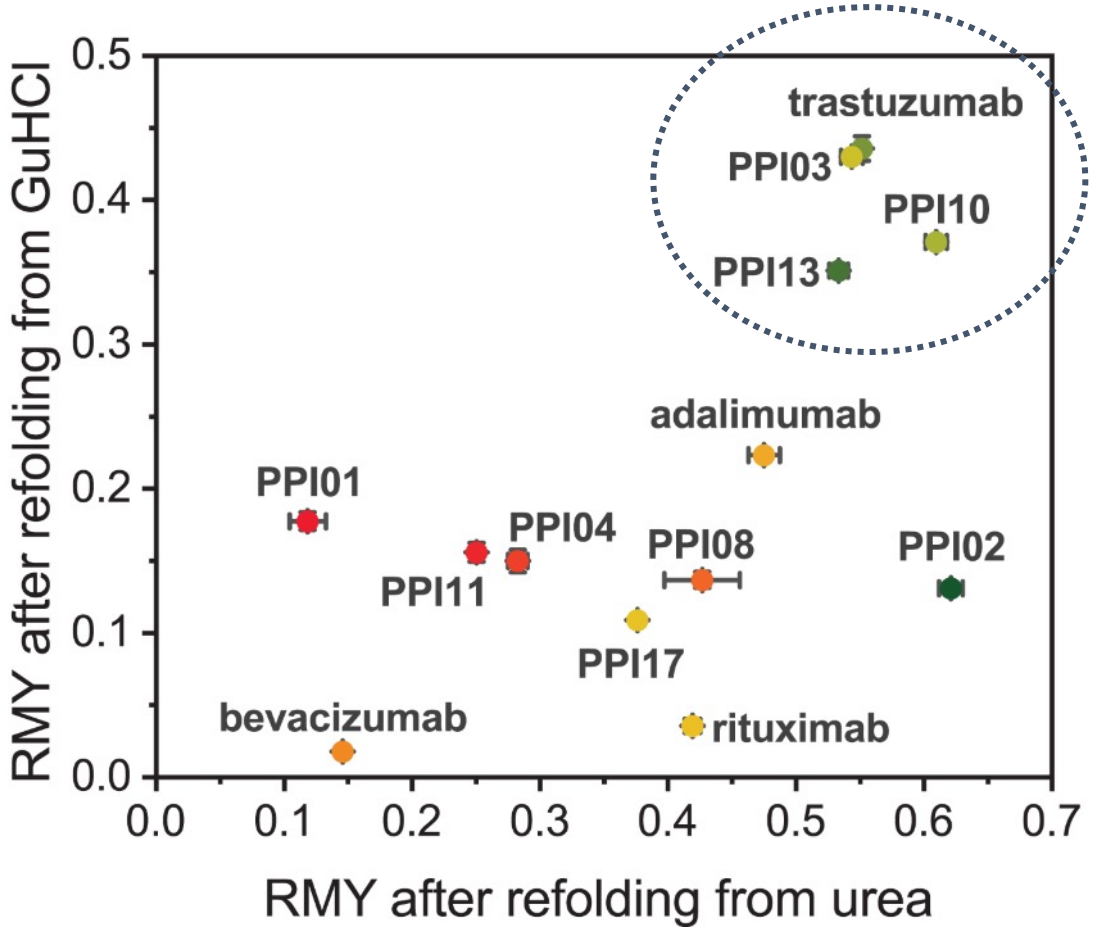
Berner et al. (2021)

Refoldability after unfolding with chemical denaturants



Svilenov and Winter (2019)

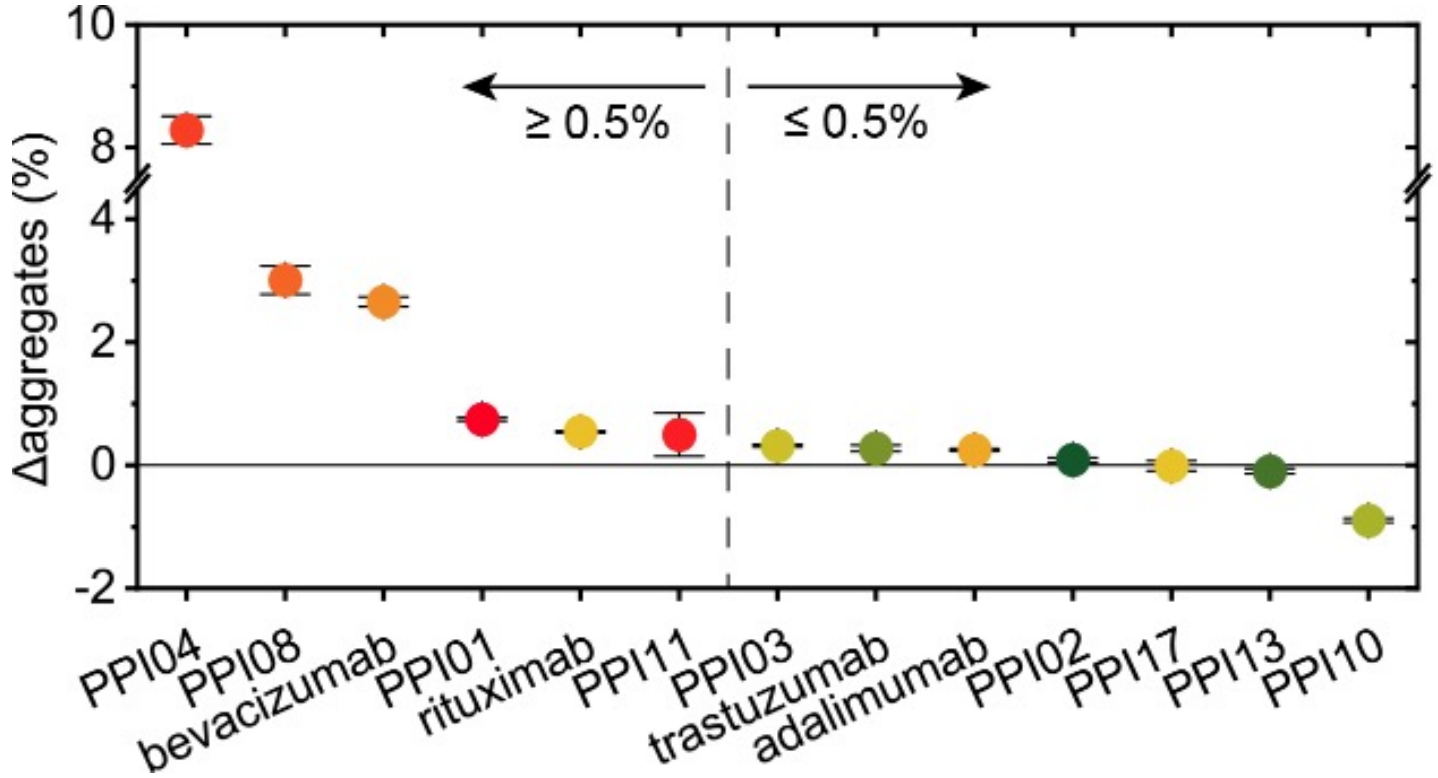
RMY after unfolding with chemical denaturants



- some antibodies exhibit high RMY after refolding from either urea or GuHCl

Berner et al. (2021)

Aggregates formed during storage at 40 °C

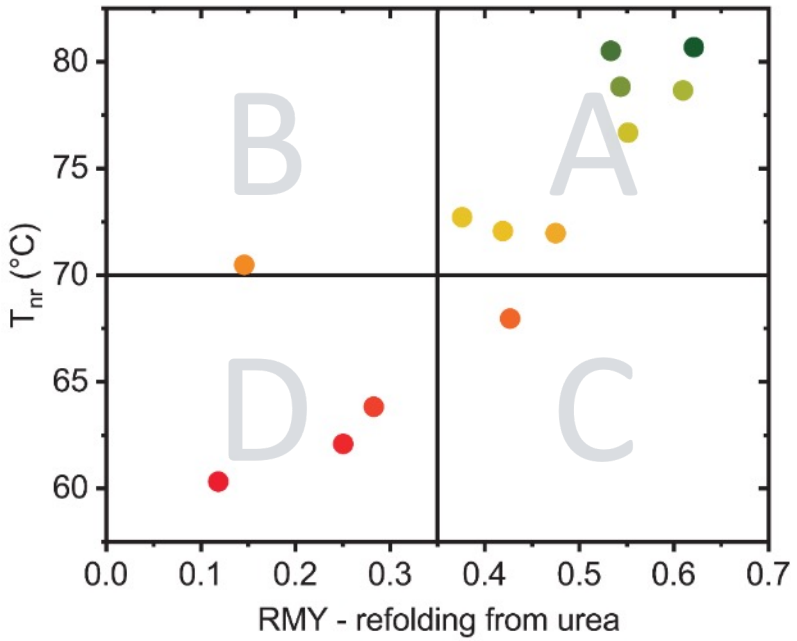


- the antibodies exhibit different aggregation during storage at 40 °C

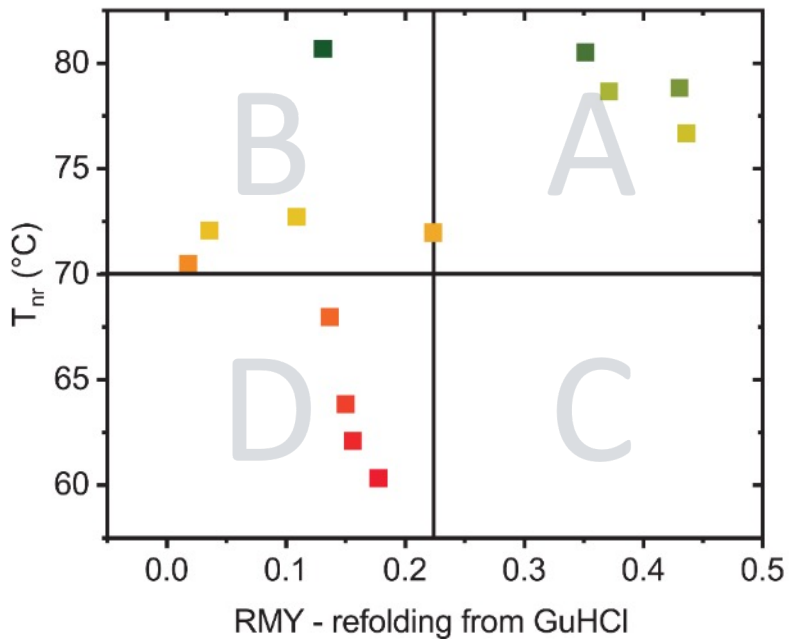
Berner et al. (2021)

Classifying proteins and formulations based on MSF and ReFOLD

experiment with urea



experiment with GuHCl

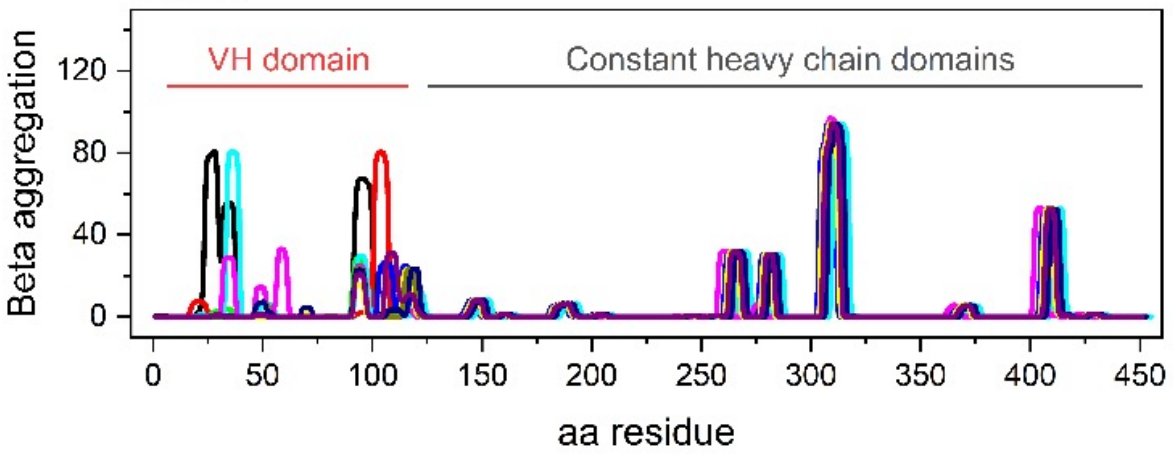


- aggregation-resistant antibodies cluster in Group A; antibodies that aggregated during storage cluster in Group D

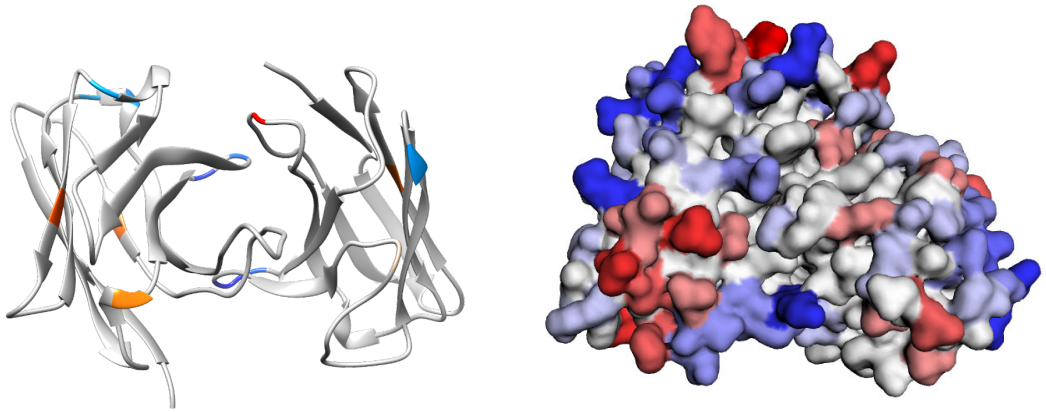
Berner et al. (2021)

Can we explain refoldability with certain molecular features?

from primary sequence
(e.g. TANGO, AggreScan)



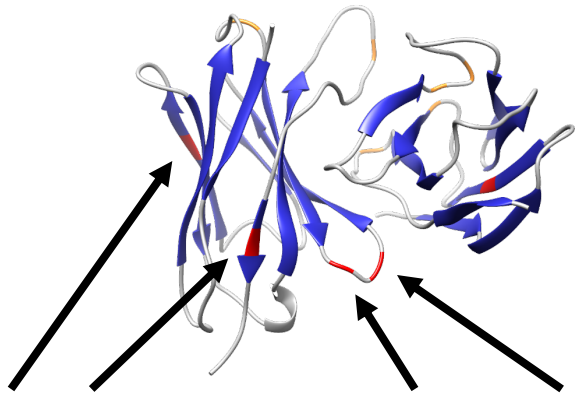
from native structure
(e.g. CamSol, AggreScan 3D, SAP)



- different APRs mostly in the variable antibody domains

Solubility of the native variable domains

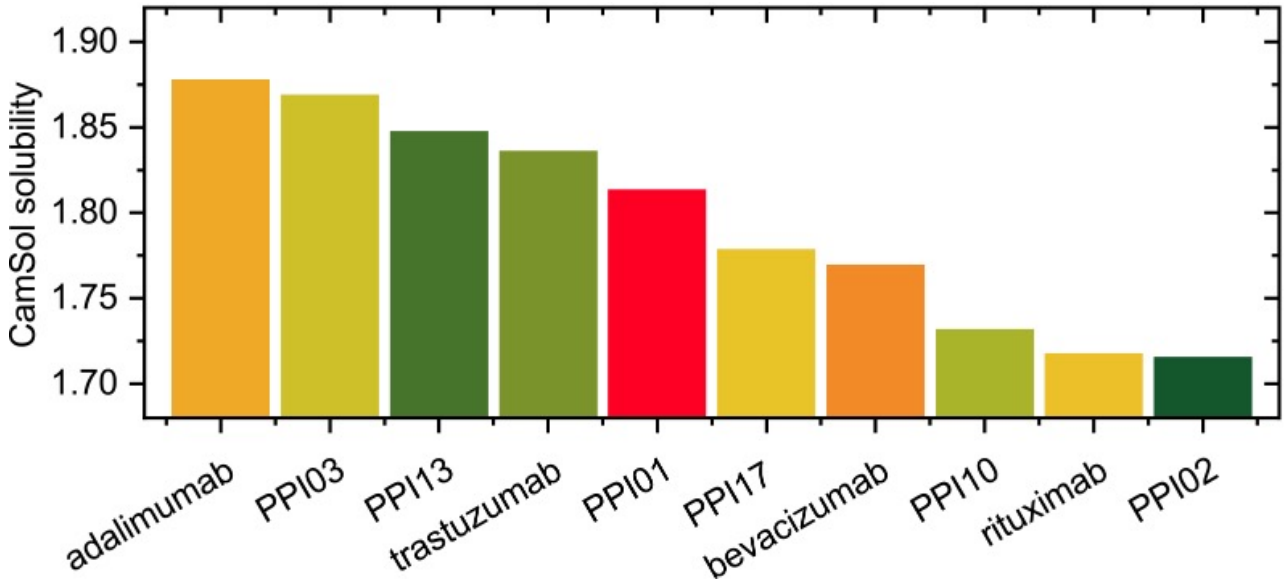
bevacizumab Fv



buried APRs

exposed APRs

CamSol ranking based on native variable domains

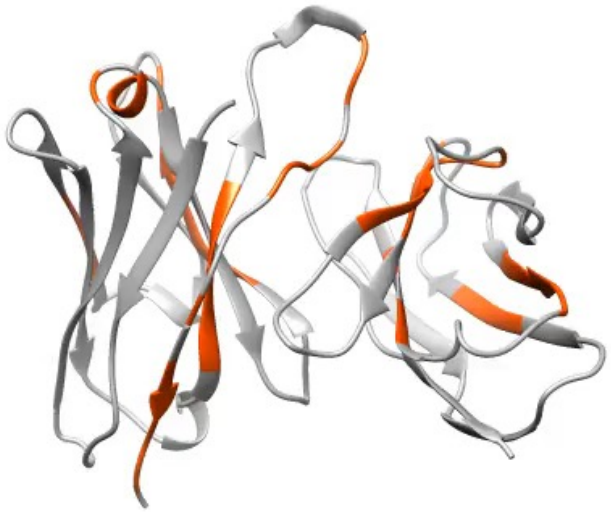


- APRs are often buried in the variable antibody domains

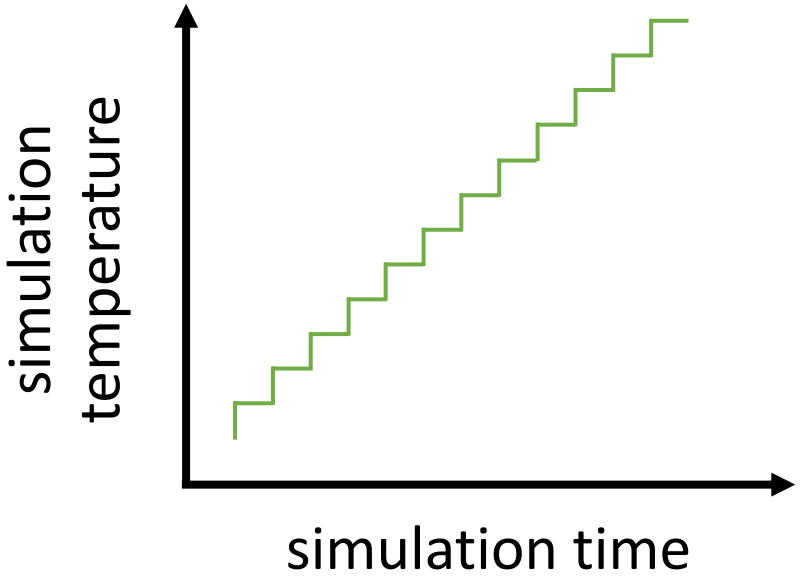
Molecular dynamics (MD) simulations to induce partial unfolding

simulation with bevacizumab Fv

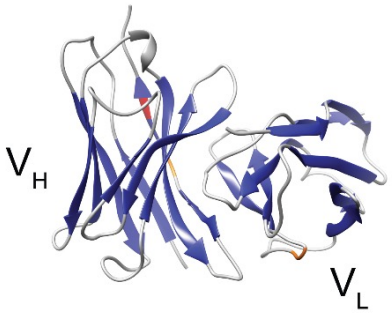
V_H



V_L

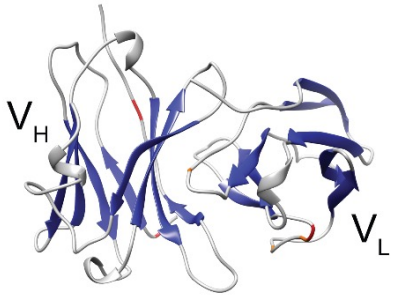


MD simulations combined with CamSol

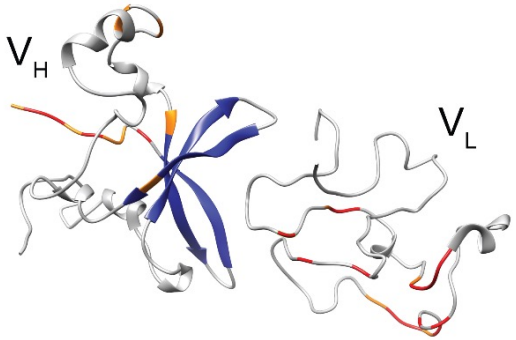


300 K

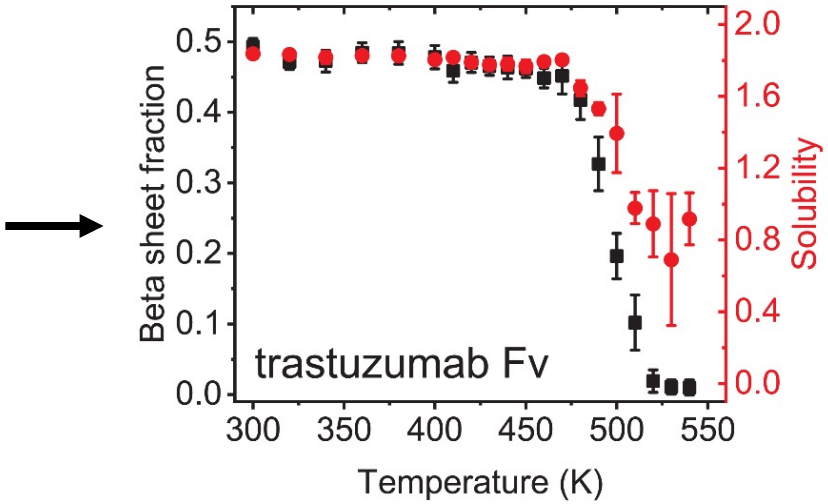
trastuzumab Fv



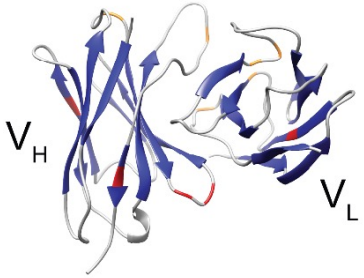
460 K



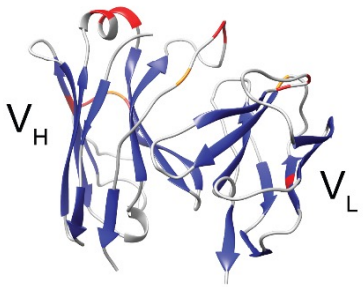
500 K



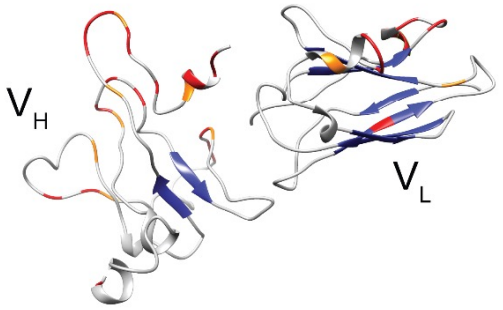
bevacizumab Fv



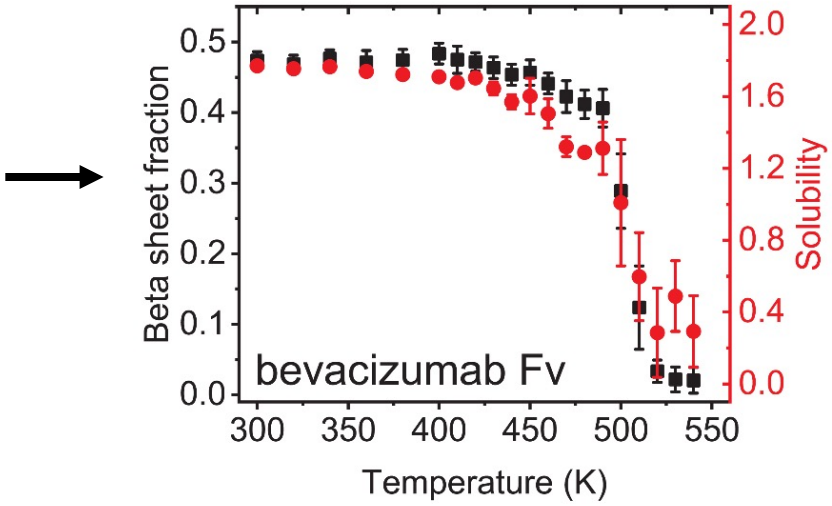
300 K



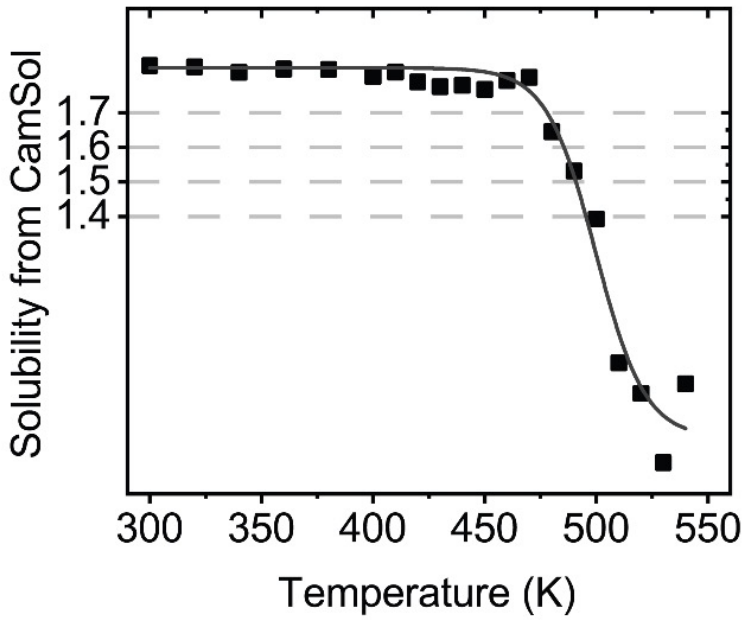
460 K

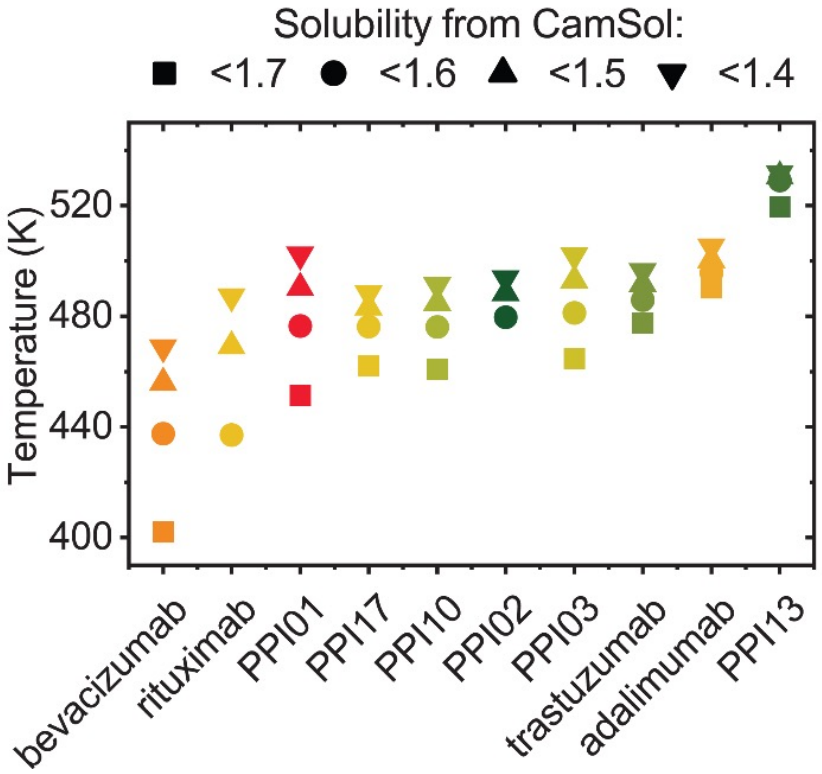


500 K



Ranking antibodies with temperature-ramped MD simulations





- antibody Fvs have different behaviour in temperature-ramped MD

Summary

- MSF and ReFOLD to study protein refoldability
  - MSF indicates what temperatures cause non-reversible structural changes
  - ReFOLD determines the fraction of protein that remains monomeric after refolding from denaturants
- MD simulations with partially unfolded antibodies can complement MSF and ReFOLD
- an aggregation-resistant antibody has two features:
  - high temperature of non-reversibility onset
  - high relative monomer yield after refolding from chemical denaturants

Acknowledgements

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