Assessing refoldability to select therapeutic proteins and formulations with lower aggregation propensity during storage

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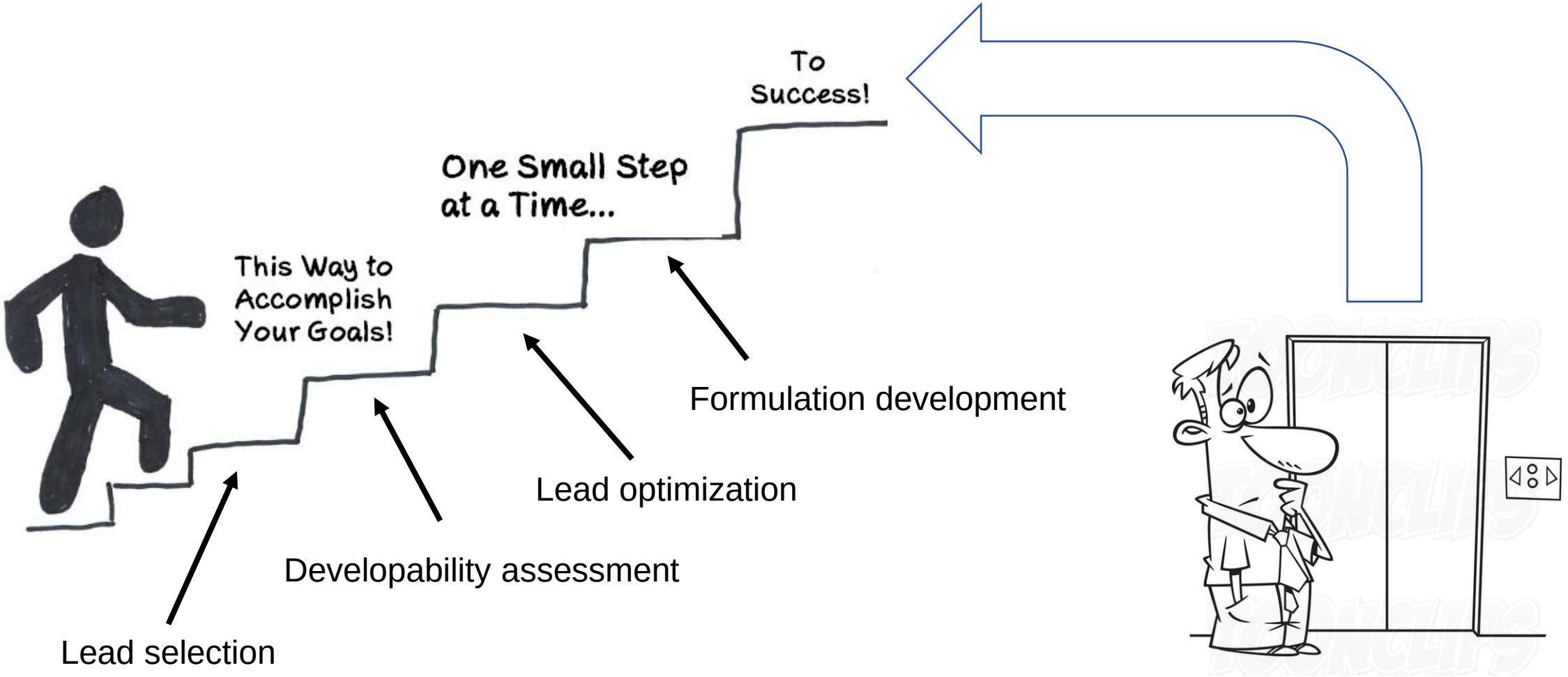
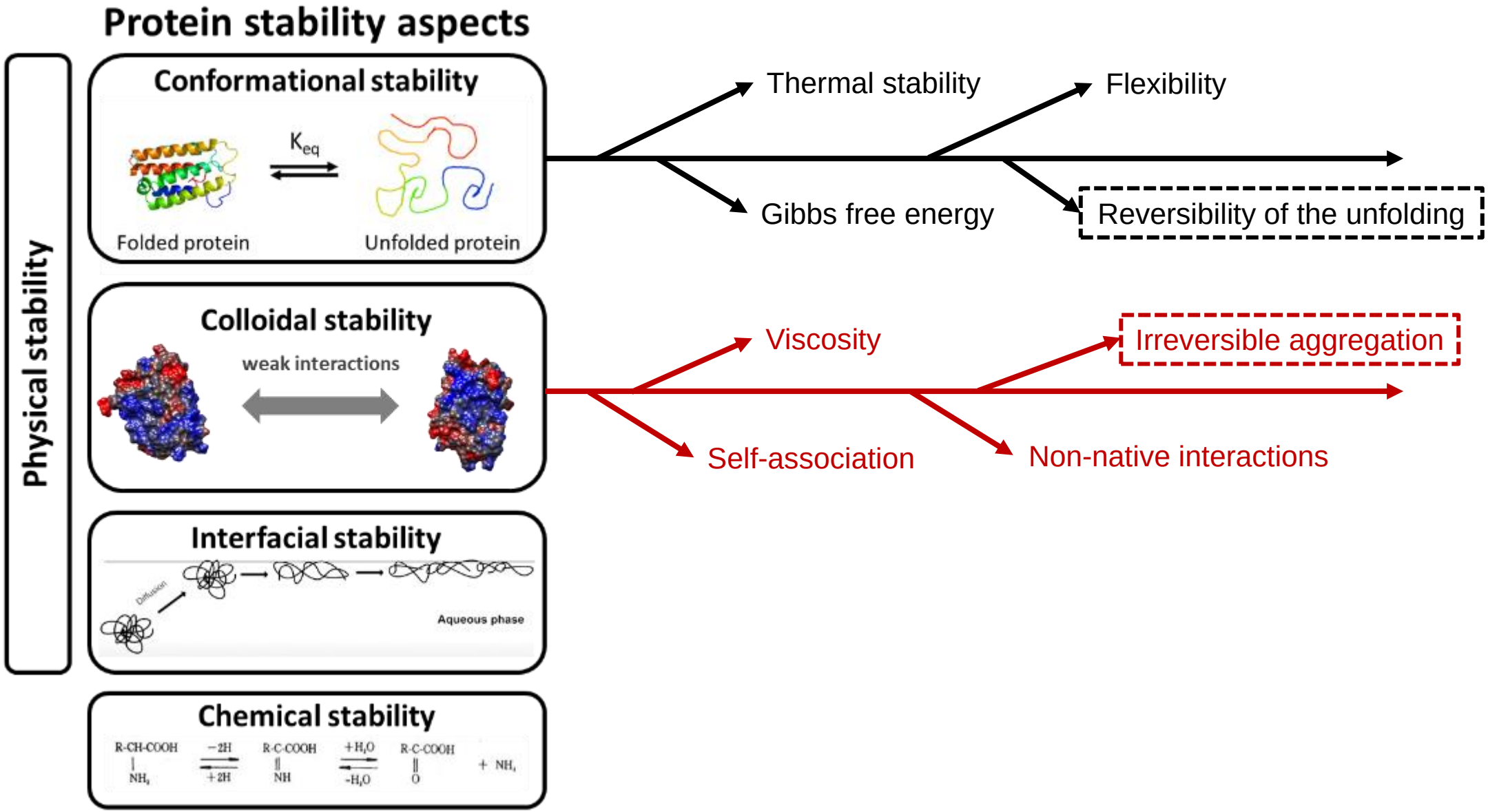
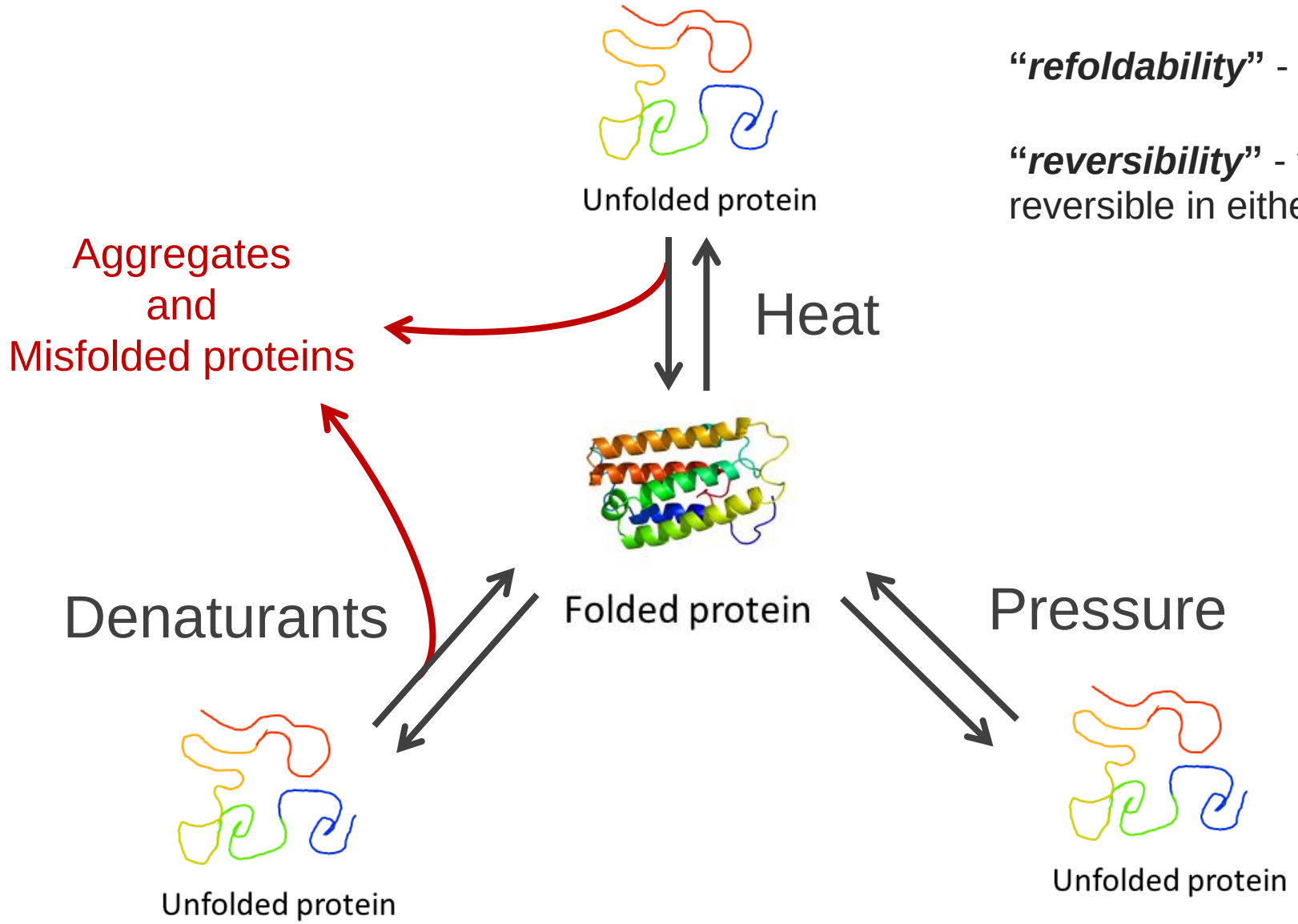
The staircase of antibody development



Getting a broad perspective on protein stability



Approaches to unfold proteins and study unfolding reversibility



"*refoldability*" - the ability to be refolded

"*reversibility*" - the quality of being reversible in either direction

Why bother about refoldability?

› Nat Biotechnol. 2004

Aggregation-resistant domain antibodies selected on phage by heat denaturation

Laurent Jespers, Oliver Schon, Kristoffer Famm, Greg Winter

› J Mol Biol. 2008

Thermodynamically stable aggregation-resistant antibody domains through directed evolution

Kristoffer Famm [1], Lars Hansen, Daniel Christ, Greg Winter

"This process appeared to select for domains with denatured states that resisted aggregation, but the domains only had low free energies of folding…"

Consecutive heating scans on an aggregation-resistant VH

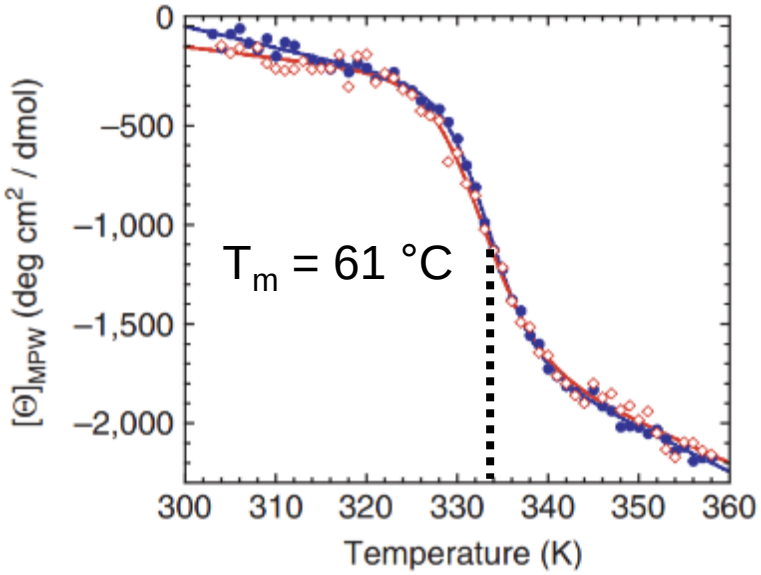
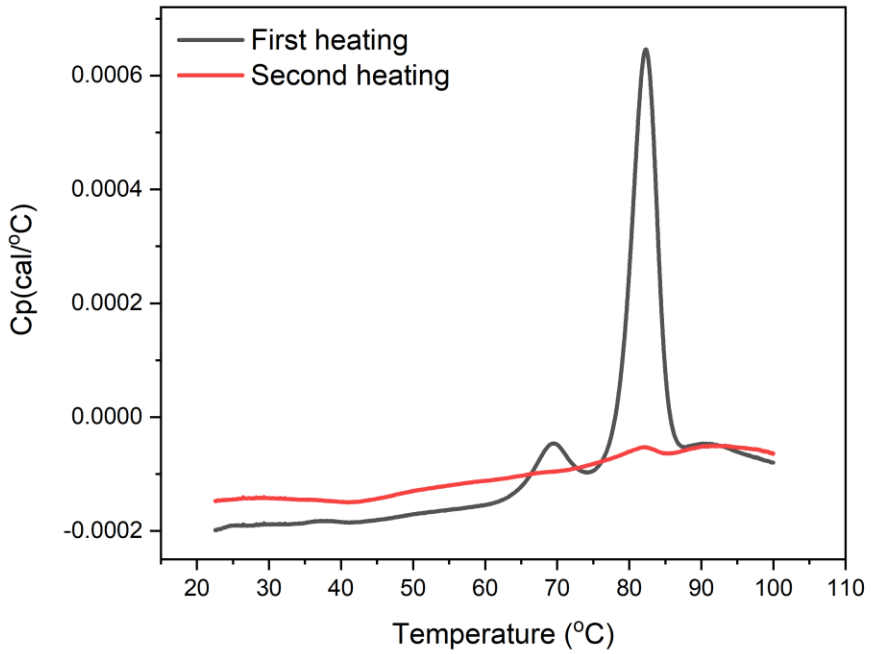


Image adapted from Jespers et al.

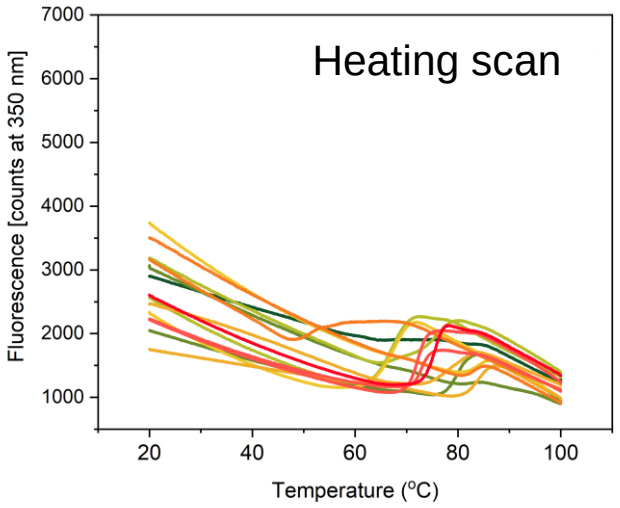
- Unfolding reversibility is related to low aggregation propensity of antibody domains
- However, many therapeutically relevant proteins do not unfold reversibly

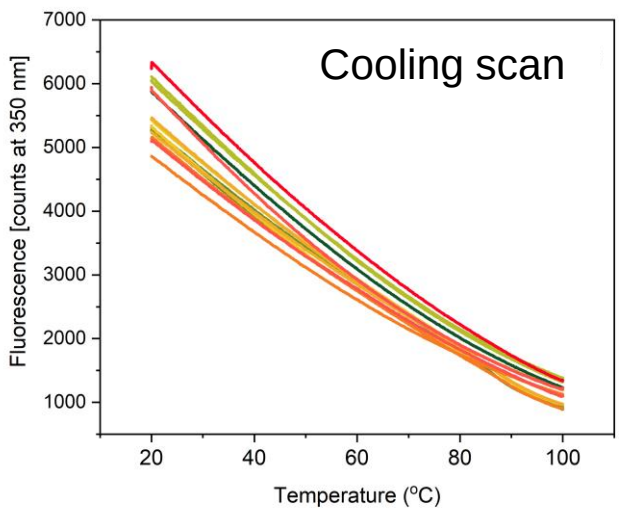
The traditional approach to test for reversibility after heating

Consecutive heating scans on a mAb



Heating and cooling scans on 14 different proteins

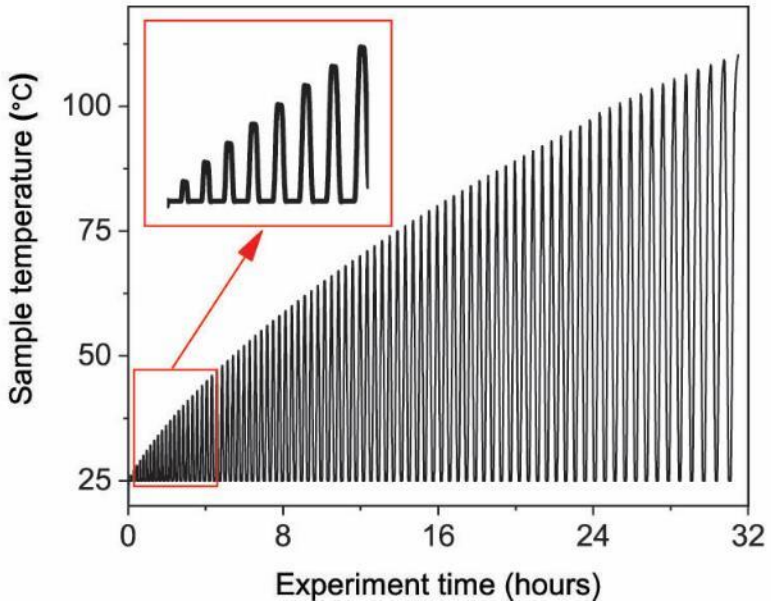




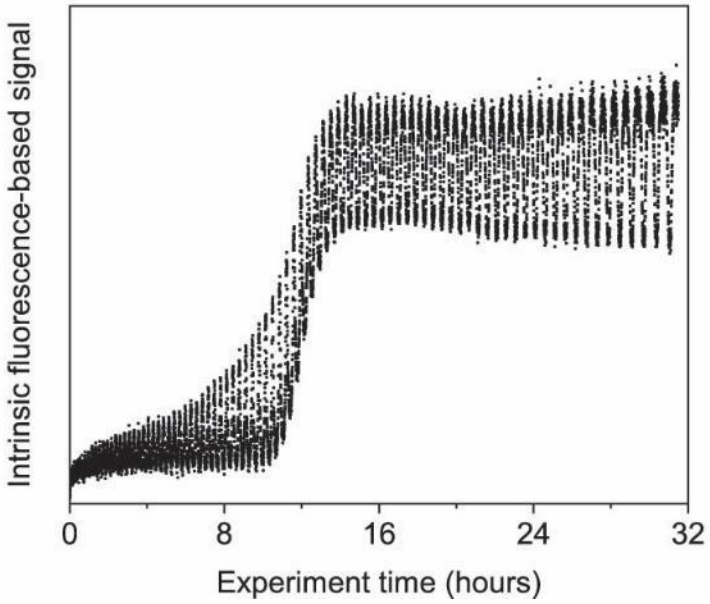
- Rapid aggregation at high temperatures → zero unfolding reversibility
- Overheating masks unfolding reversibility differences

Another approach – use incremental heating and cooling cycles

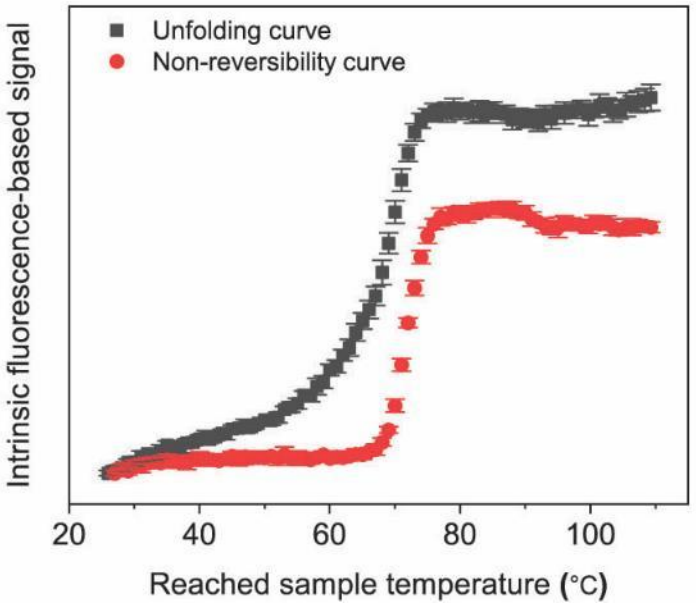
Heating program



Raw data



Deconvoluted data

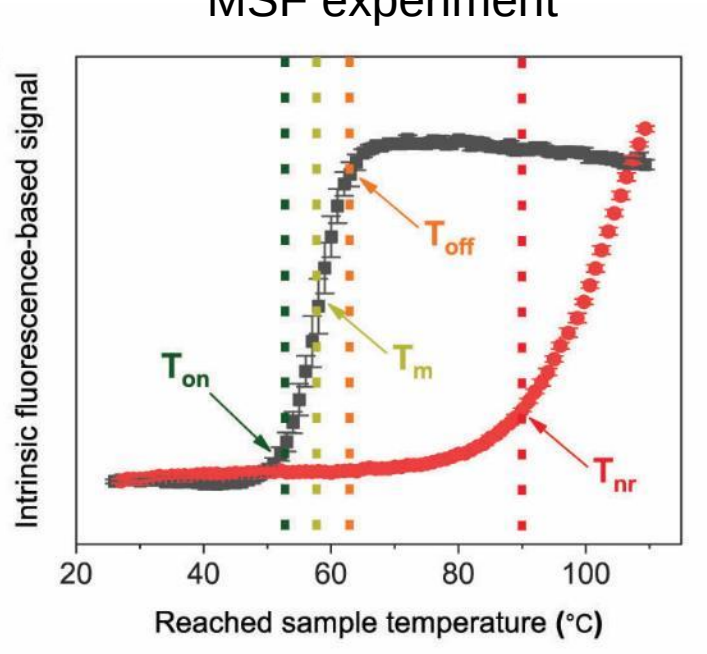


- We call it modulated scanning fluorimetry (MSF)
- The MSF analyser software from Klaus Richter deconvolutes the raw data
- Distinct unfolding and non-reversibility curves are obtained

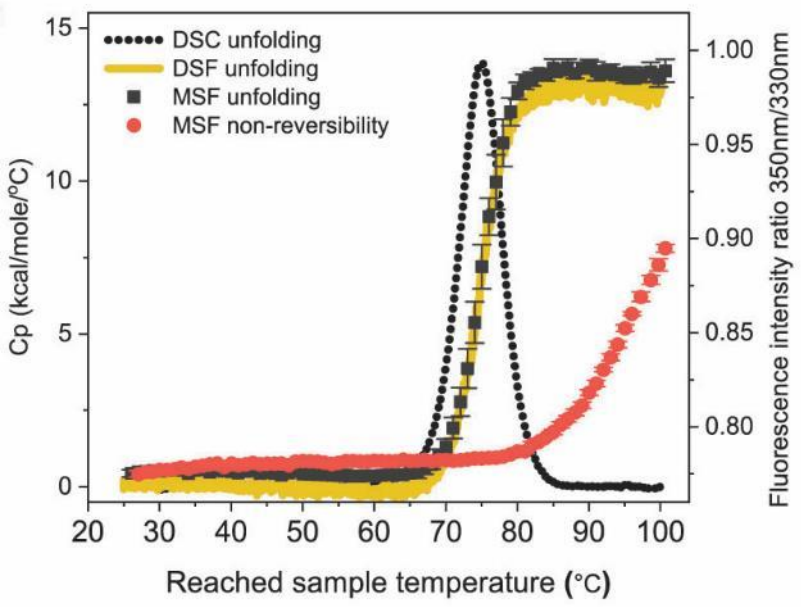
Svilenov et al. (2020)

Information obtained with modulated scanning fluorimetry

Parameters derived from the MSF experiment



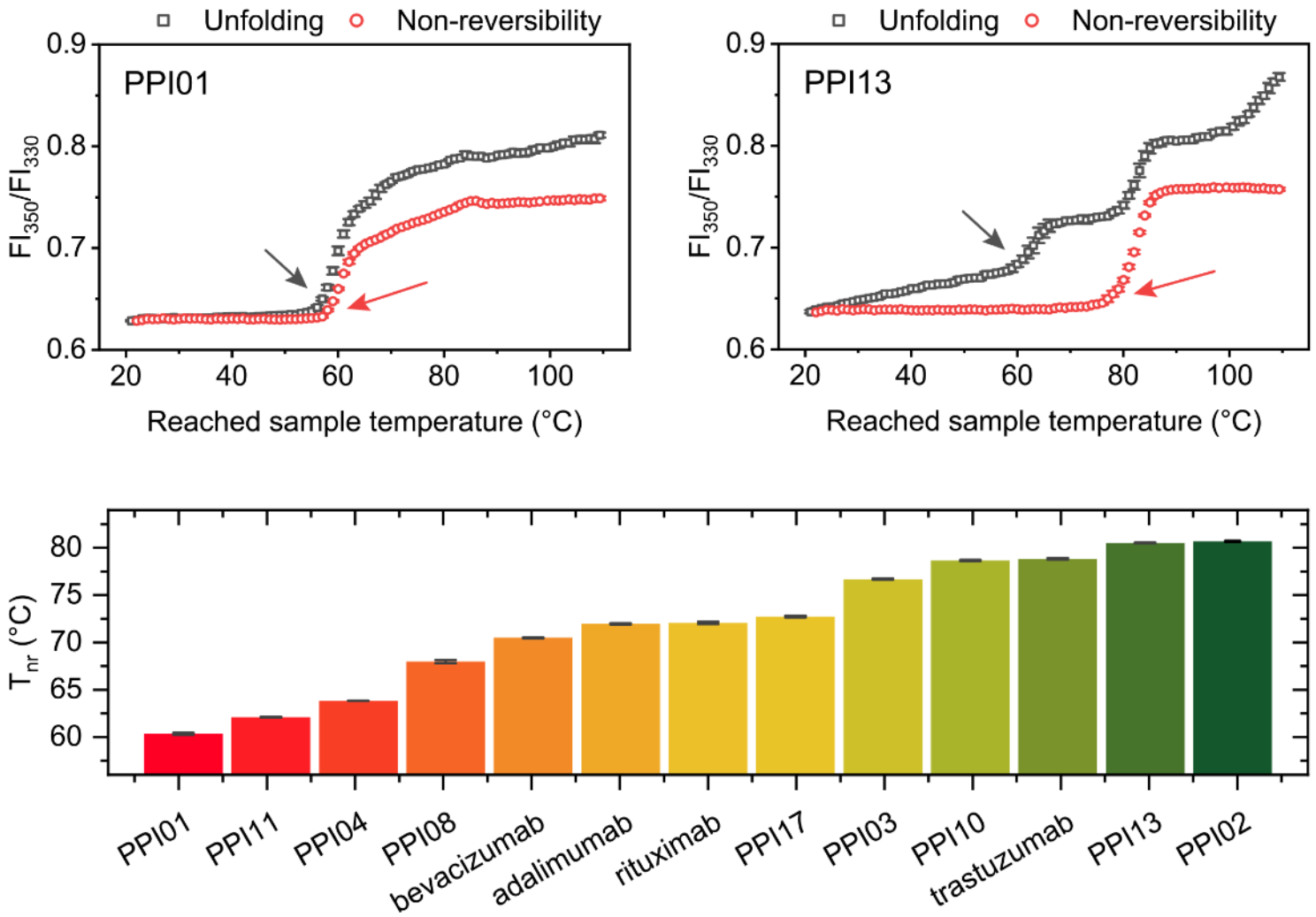
Overlay of thermal denaturation data from different methods



- Non-reversibility onset temperature (T_{nr}) can be obtained with MSF
- The non-reversibility curve provides orthogonal data to DSC and DSF
- Applicable to various proteins and concentrations

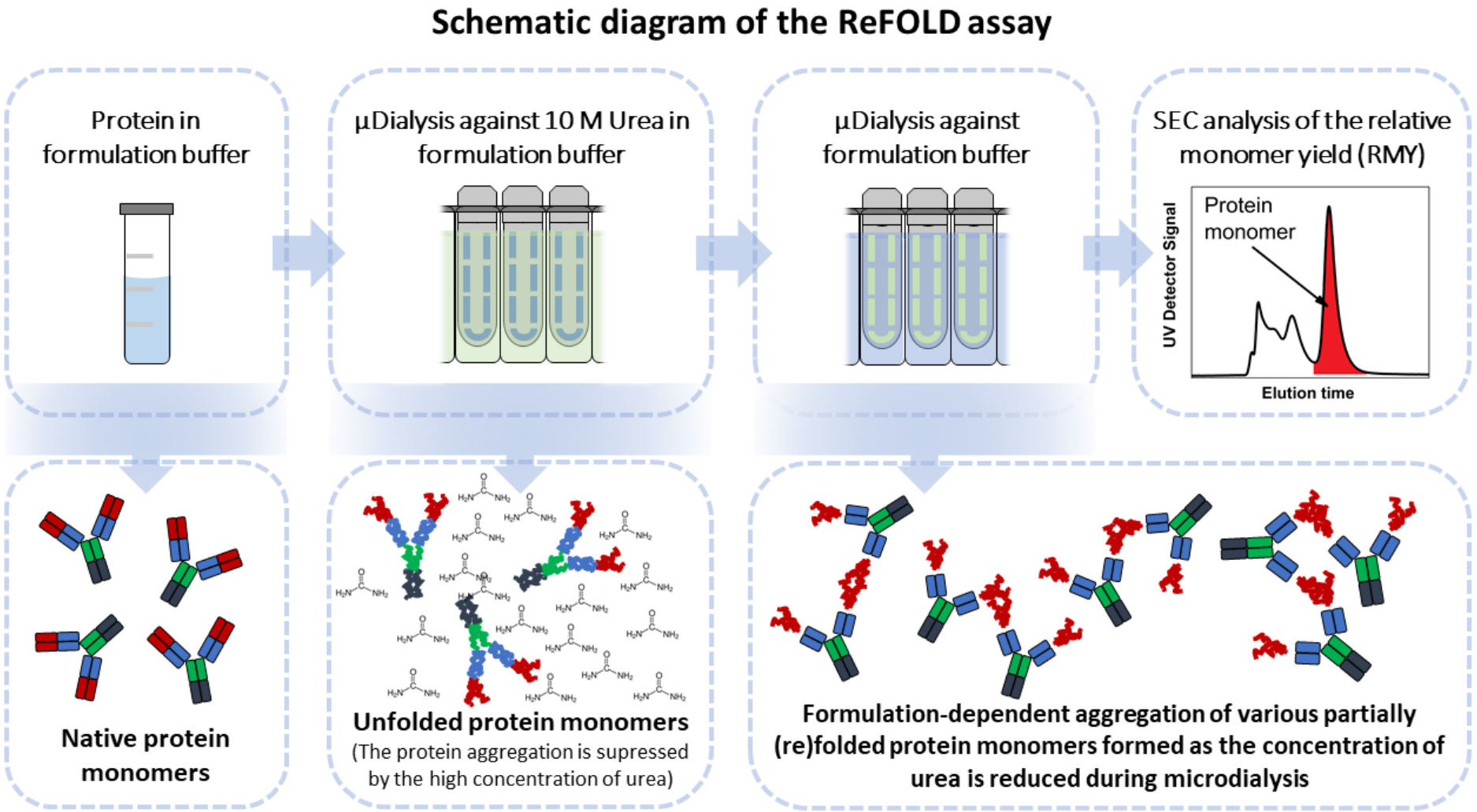
Svilenov et al. (2020)

Using MSF to study and rank antibody candidates



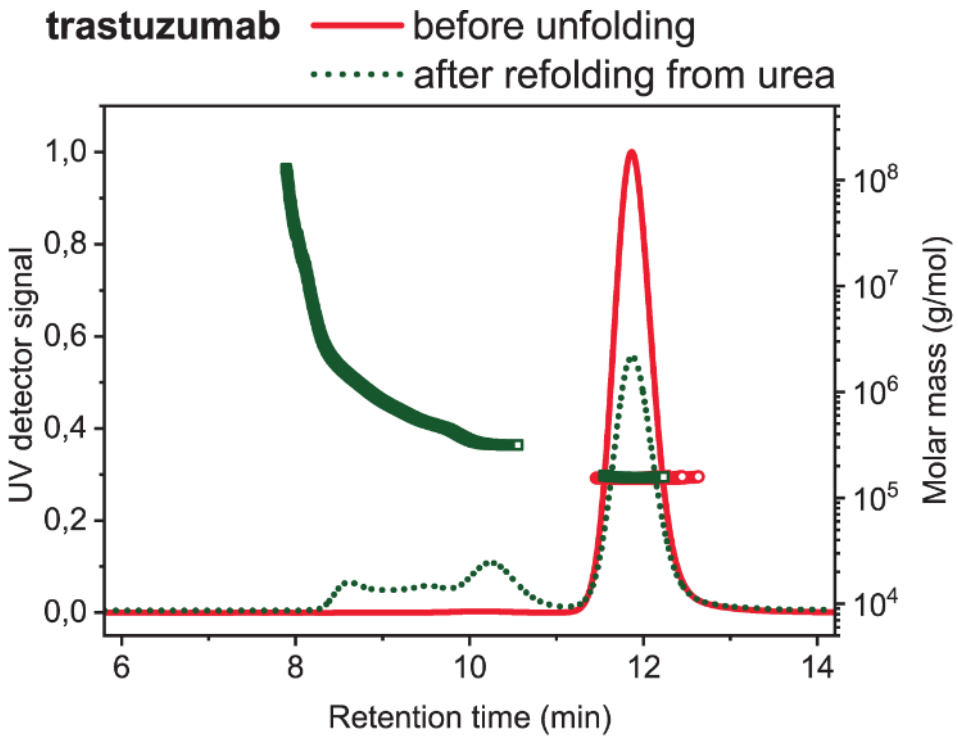
- Unique unfolding and non-reversibility traces are obtained for different antibodies
- Very different non-reversibility onset temperature (T_{nr}) of the candidates

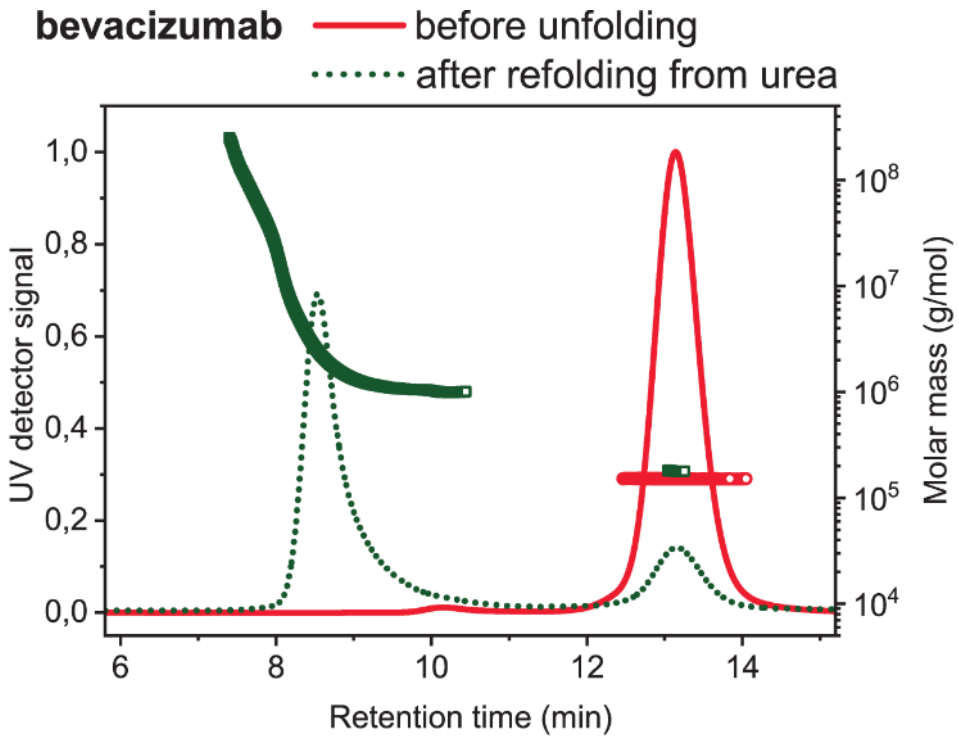
Studying refoldability after unfolding with chemical denaturants



Svilenov and Winter (2019)

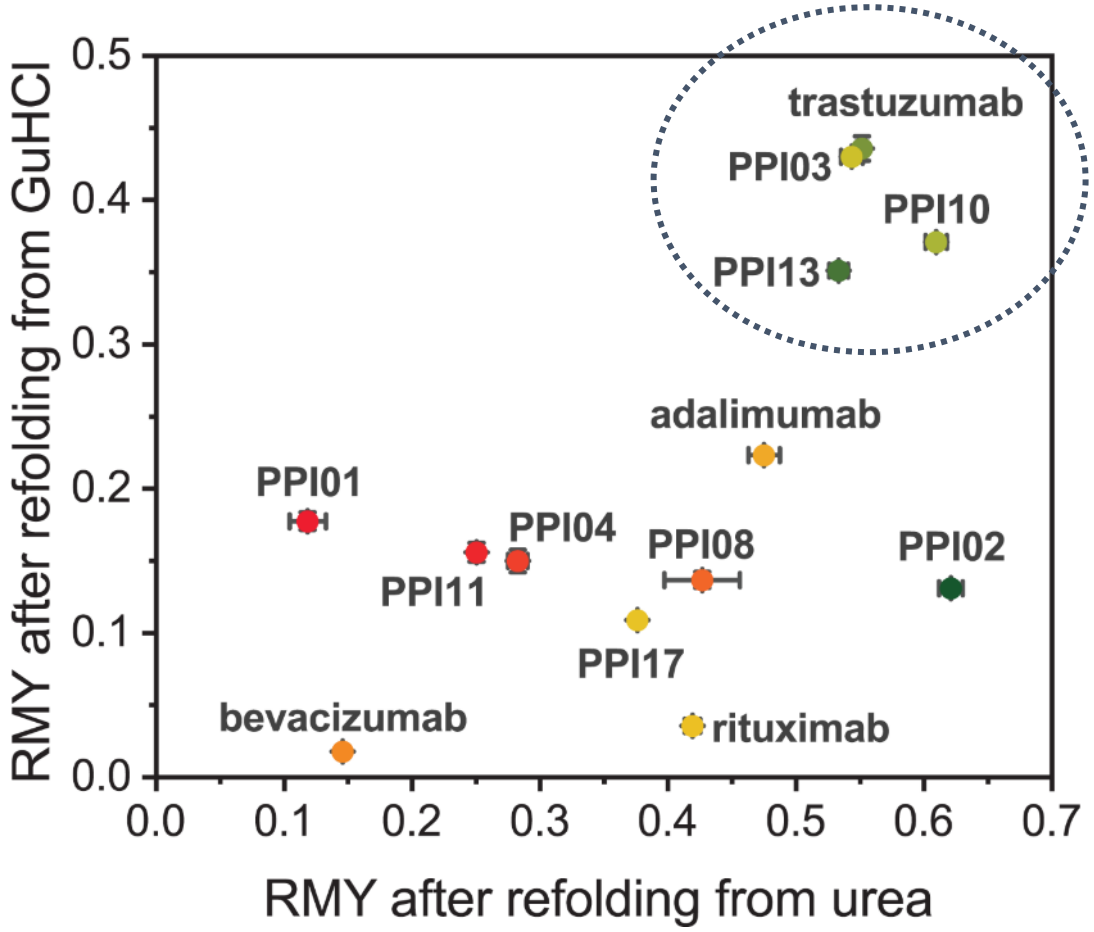
SEC-MALS analysis on native and refolded mAbs





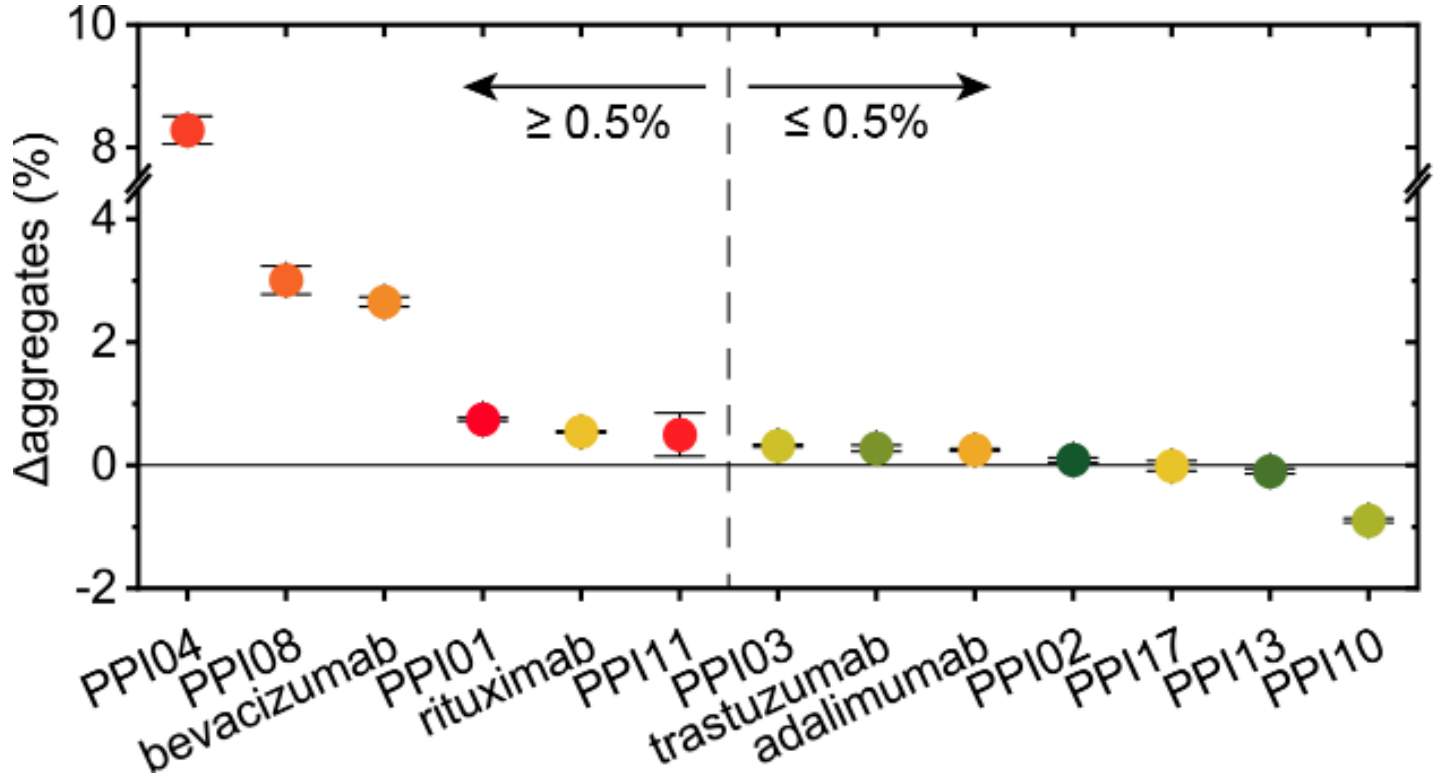
- Antibodies have different relative monomer yield after refolding from denaturants
- Aggregate distribution after refolding is protein-specific

RMY after unfolding with chemical denaturants to rank antibodies



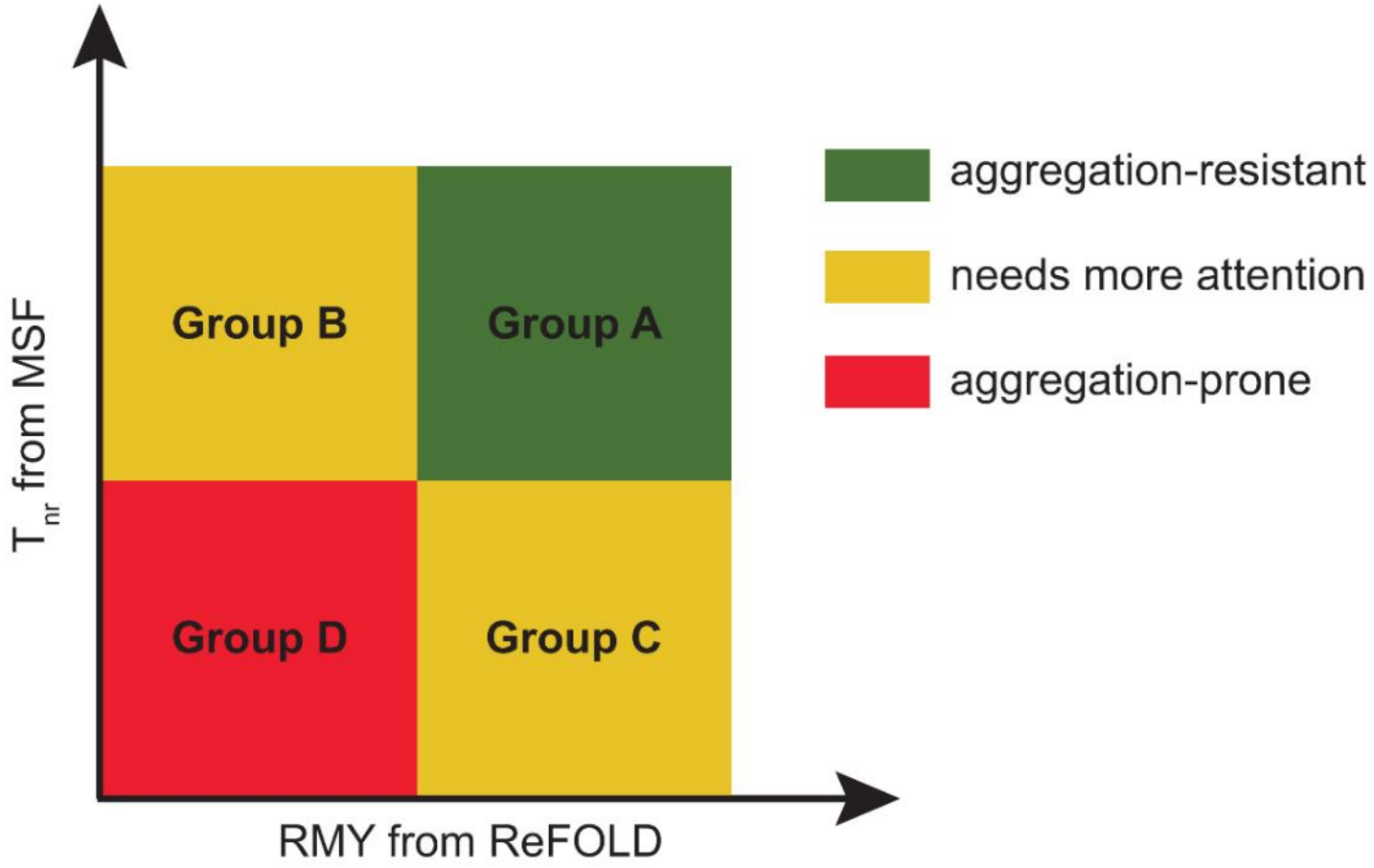
- Some antibodies exhibit high RMY after refolding from either urea or GuHCl

Aggregates formed by the antibodies during storage at 40 °C



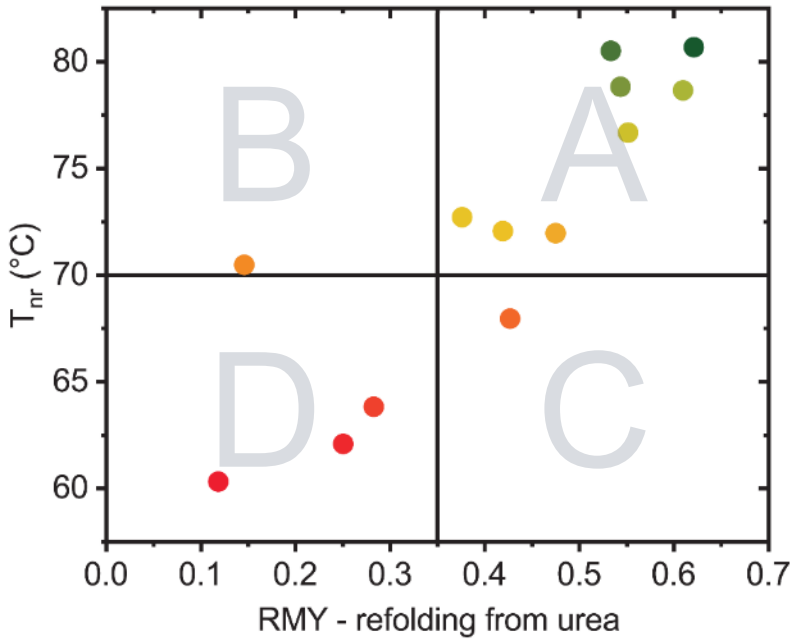
- The increase in aggregates was analyzed with size-exclusion chromatography
- The antibodies exhibit very different aggregation during storage for 3 months at 40 °C
- Only PPI11 aggregated at 4 °C (ca. 1.5% aggregates after 12 months)

Classifying proteins and formulations based on MSF and ReFOLD

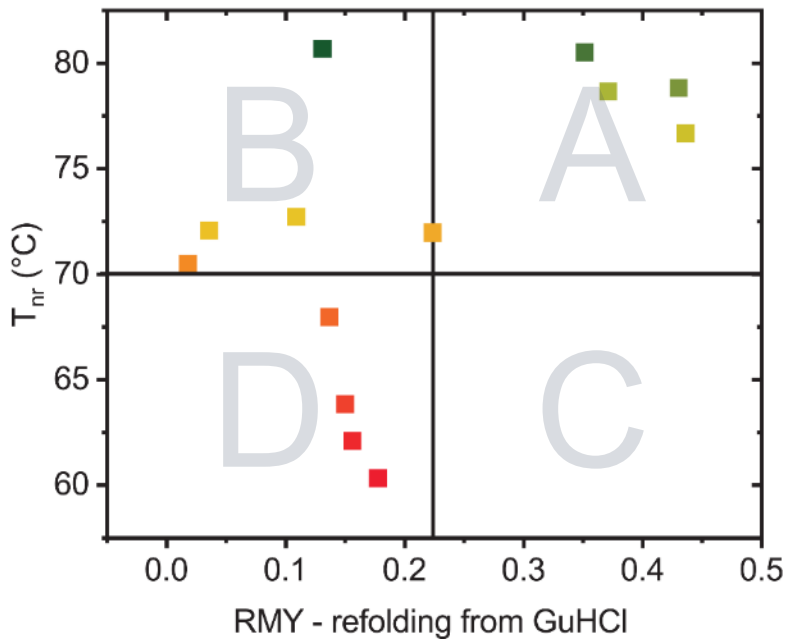


Classifying proteins and formulations based on MSF and ReFOLD

Experiment with urea



Experiment with GuHCl



- Aggregation-resistant antibodies cluster in Group A
- Antibodies that aggregated during storage cluster in Group D

Wrap up and take-home messages

- **Two approaches to study protein refoldability were presented**
- **MSF indicates what temperatures cause non-reversible structural changes**
- **ReFOLD gives you the fraction of protein that remains monomeric after refolding from denaturants**
- **The two approaches are complementary**
- **Ideally an aggregation-resistant antibody will have two properties:**
  1. **High temperature of non-reversibility onset**
  2. **High relative monomer yield after refolding from chemical denaturants**

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