Hydrogen exchange mass spectrometry for higher-order structure determination in therapeutic protein discovery and development

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Department of Chemistry
Adams Institute for Bioanalytical Chemistry
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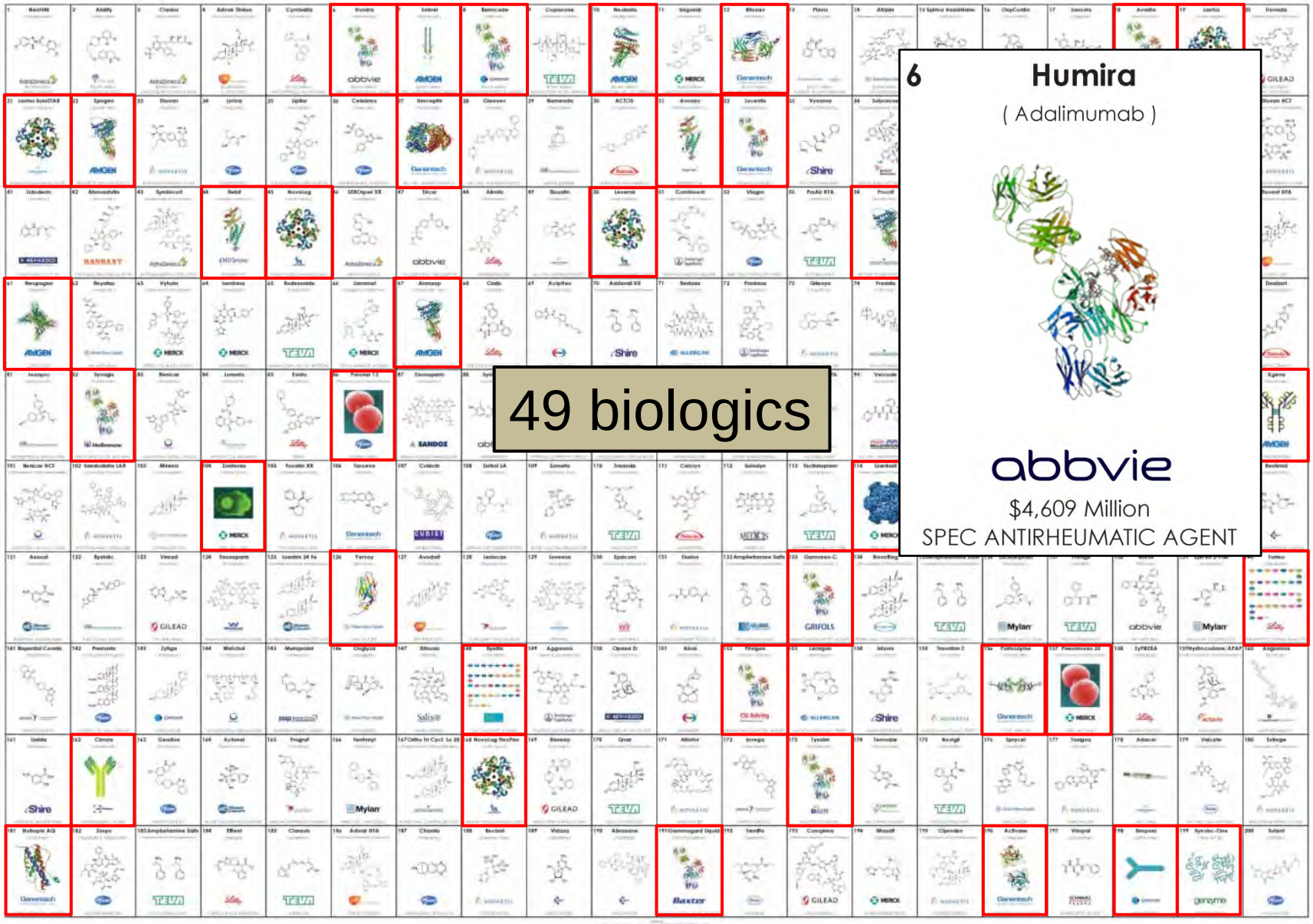
Citations and links for this work

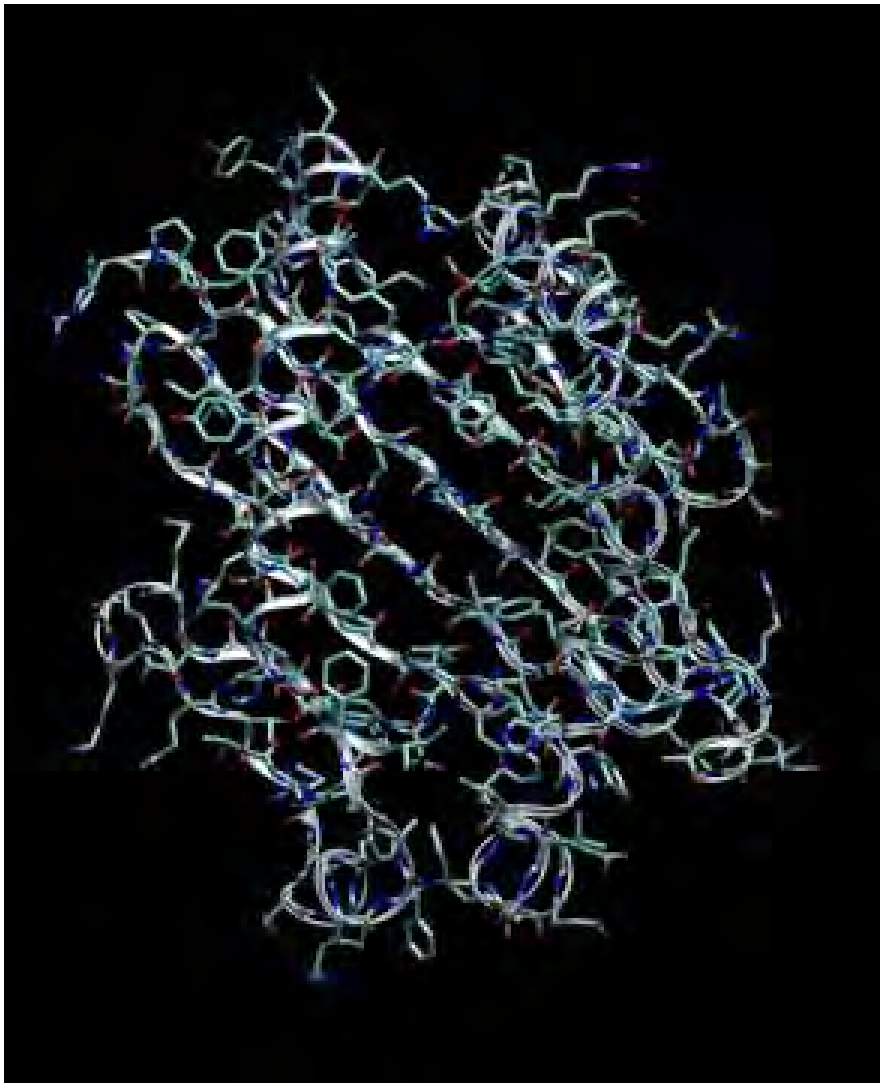
Arora, J; et al. Hydrogen exchange mass spectrometry reveals protein interfaces and distant dynamic coupling effects during the reversible self-association of an IgG1 monoclonal antibody. mAbs 2015, 7, 525-539.	http://www.tandfonline.com/doi/abs/10.1080/19420862.2015.1029217?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed#.VeRtuZdGwl8
Manikwar, P; et al. Correlating excipient effects on conformational and storage stability of an IgG1 monoclonal antibody with local dynamics as measured by hydrogen/deuterium-exchange mass spectrometry. J Pharm Sci 2013, 102, 2136-2151.	http://onlinelibrary.wiley.com/doi/10.1002/jps.23543/abstract;jsessionid=8285294C29C018B7BFA9F6CAD972C2D6.f03t03
Majumdar, R; et al. Effects of Salts from the Hofmeister Series on the Conformational Stability, Aggregation Propensity, and Local Flexibility of an IgG1 Monoclonal Antibody. Biochemistry 2013, 52, 3376–3389.	http://pubs.acs.org/doi/abs/10.1021/bi400232p
Majumdar, R; et al. Correlations between changes in conformational dynamics and physical stability in a mutant IgG1 mAb engineered for extended serum half-life. mAbs 2015, 7, 84-95.	http://www.tandfonline.com/doi/abs/10.4161/19420862.2014.985494?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed#.VeRt65dGwl8

Top 200 Pharmaceutical Products by US Retail Sales in 2012

Compiled and Produced by the Njardarson Group (The University of Arizona): Edon Vitaku, Elizabeth A. Ilardi, Jón T. Njarðarson



Higher order structure is essential for function

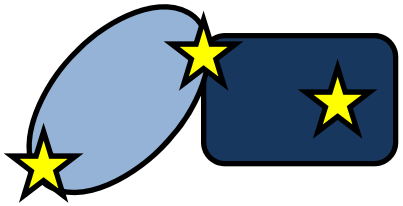


Major Histocompatibilty Complex (MHC) protein, http://www.youtube.com/watch?v=Y79Xl0LfYI4

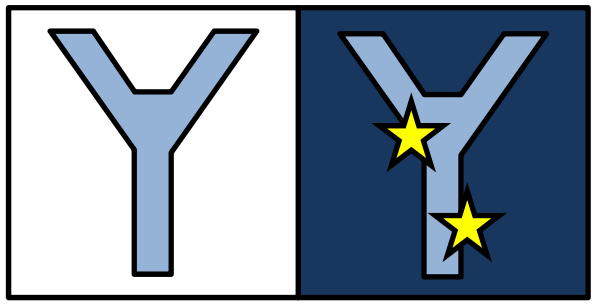
Applications of hydrogen exchange (HX)

Ligand binding

Protein interactions

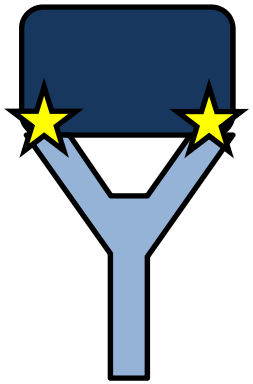


Formulations

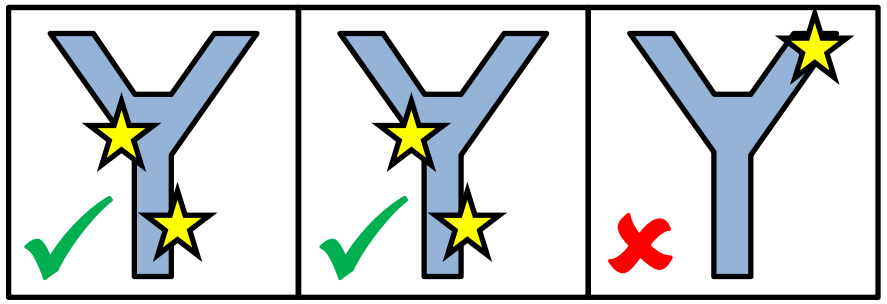


Disordered proteins

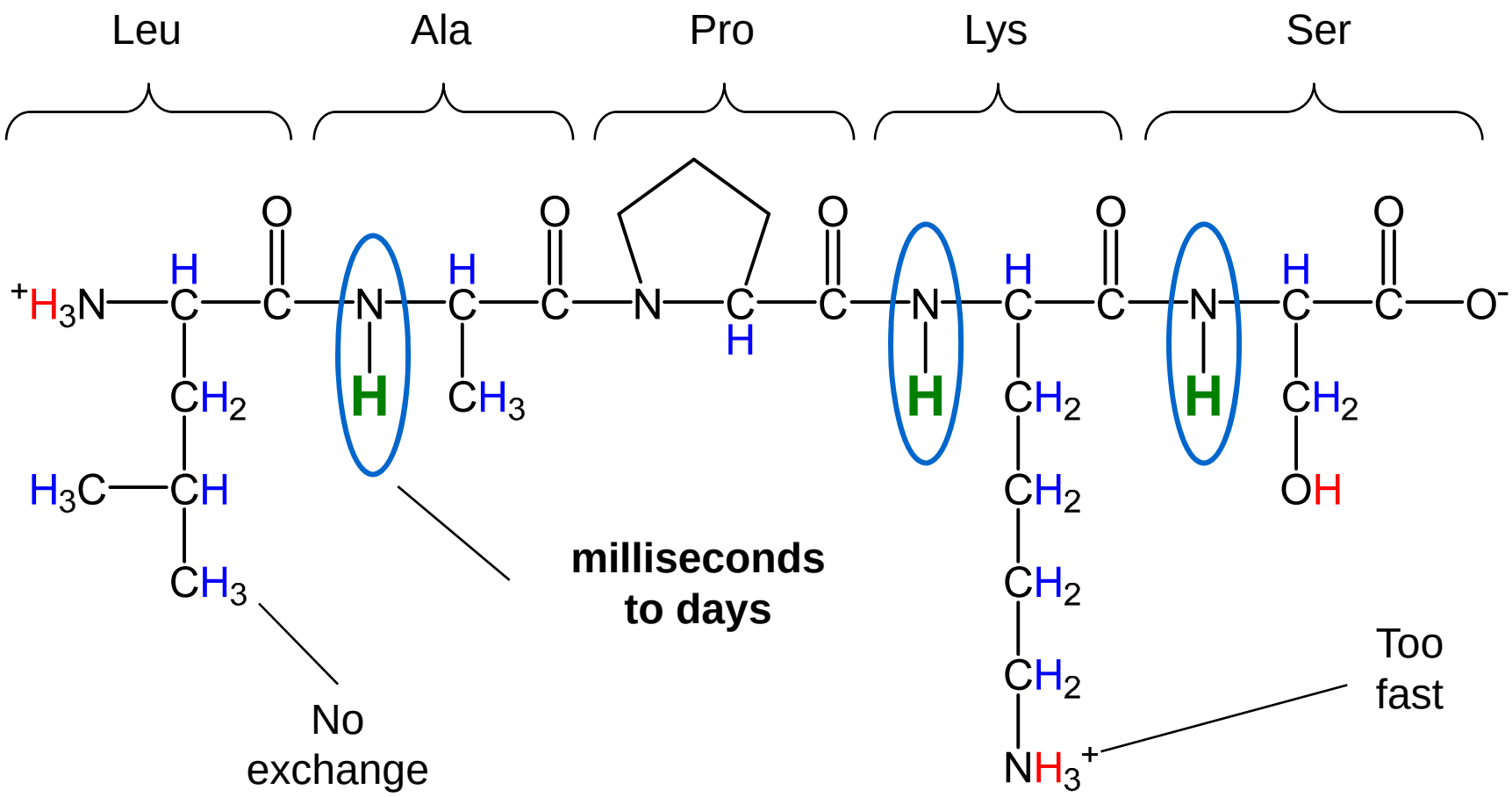
Epitope mapping



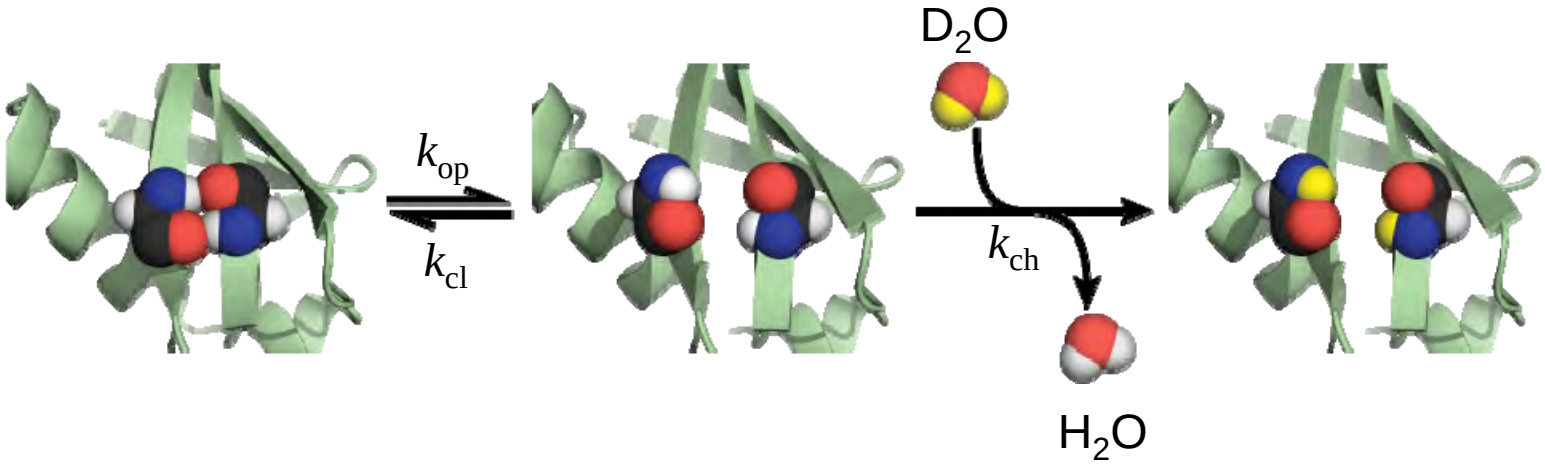
Comparability



Amide hydrogens serve as backbone sensors.



Hydrogen exchange reports on protein conformation and dynamics.

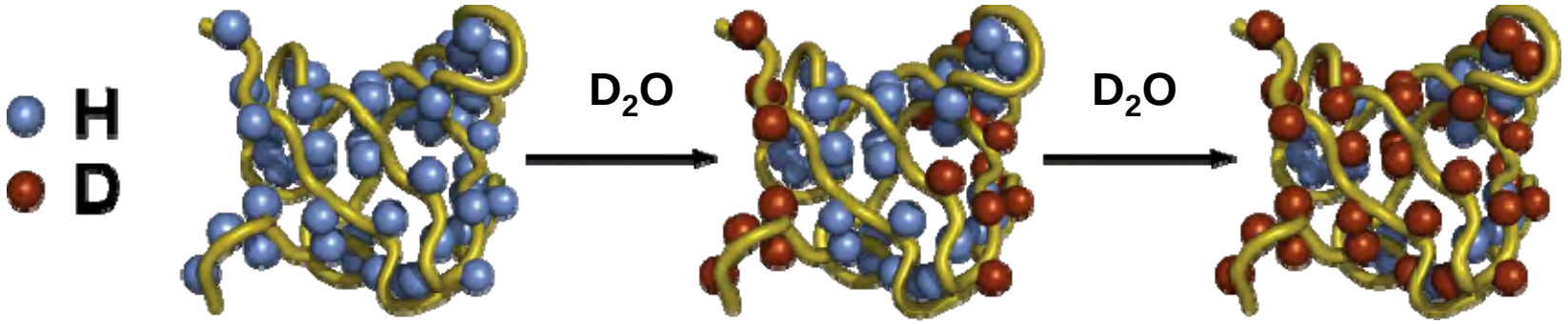


$$k_{obs} = \frac{k_{op}}{k_{cl}} \times k_{ch}$$

Conformation and dynamics

Chemical

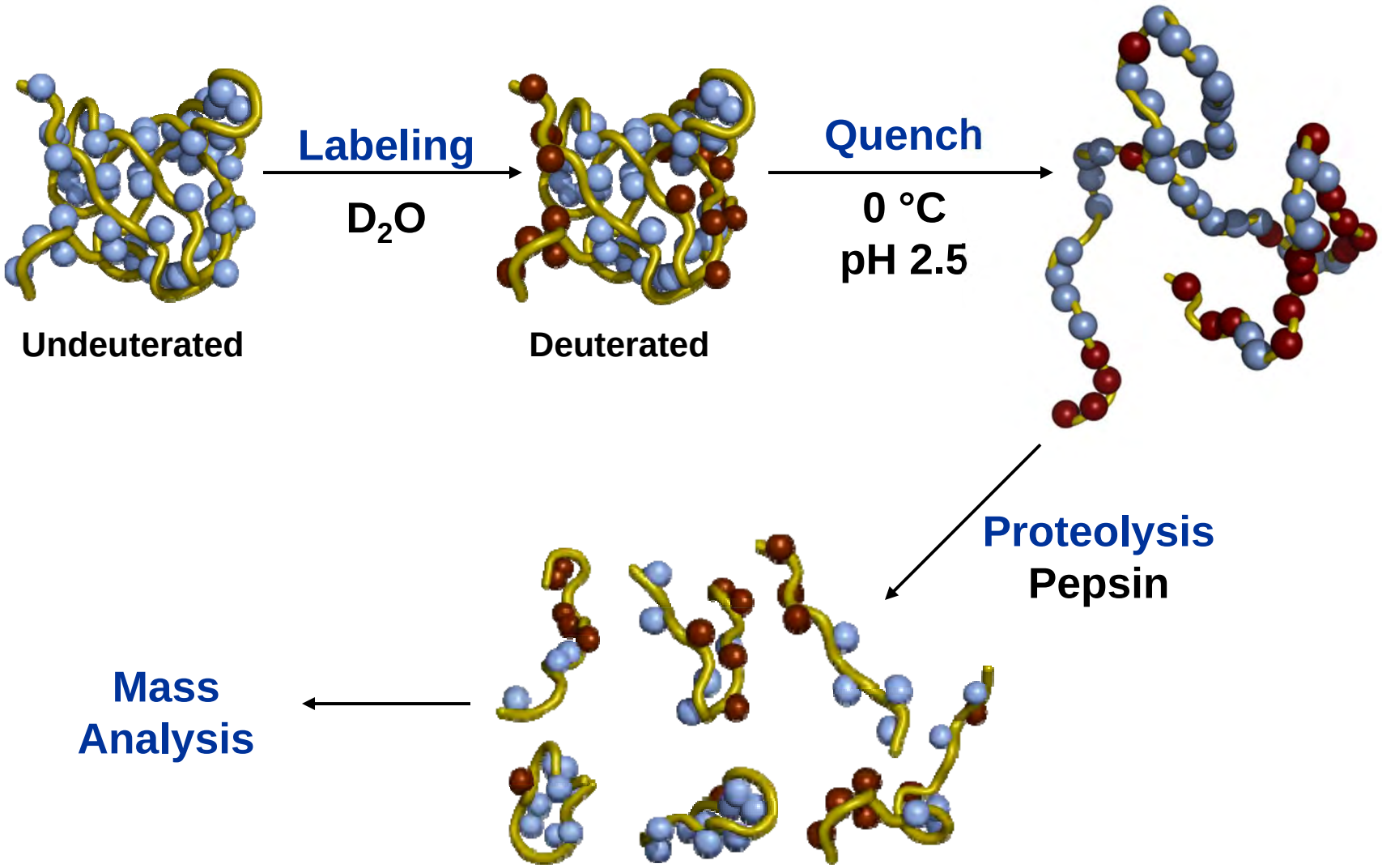
H/D exchange kinetics probes backbone dynamics.



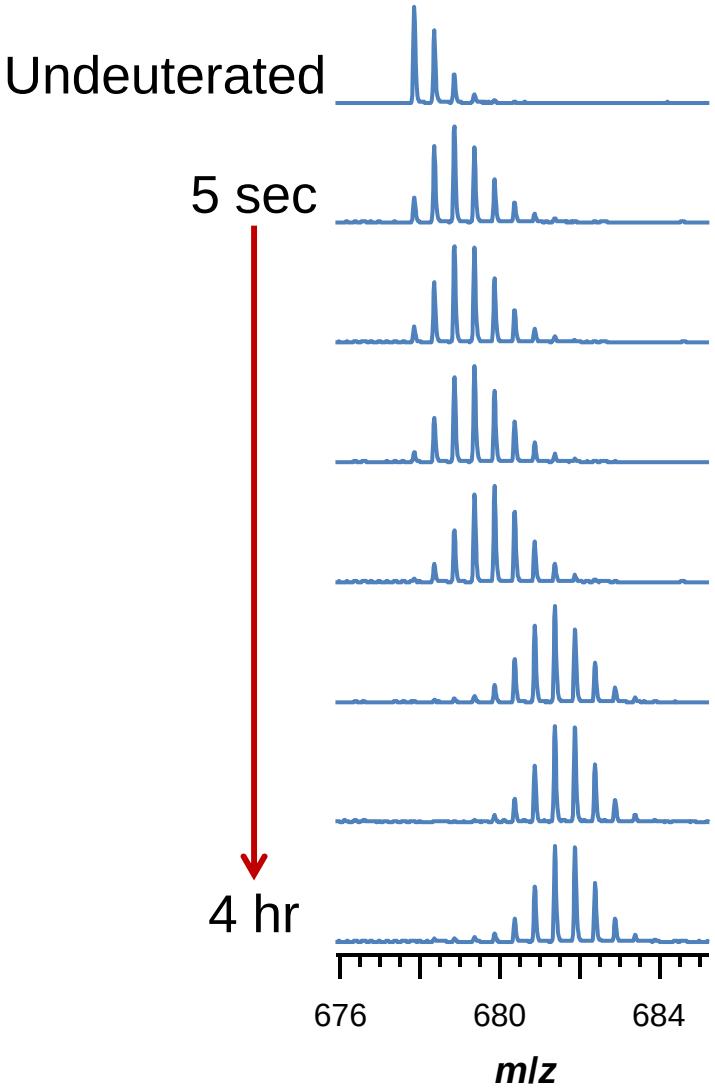
Flexible regions exchange rapidly

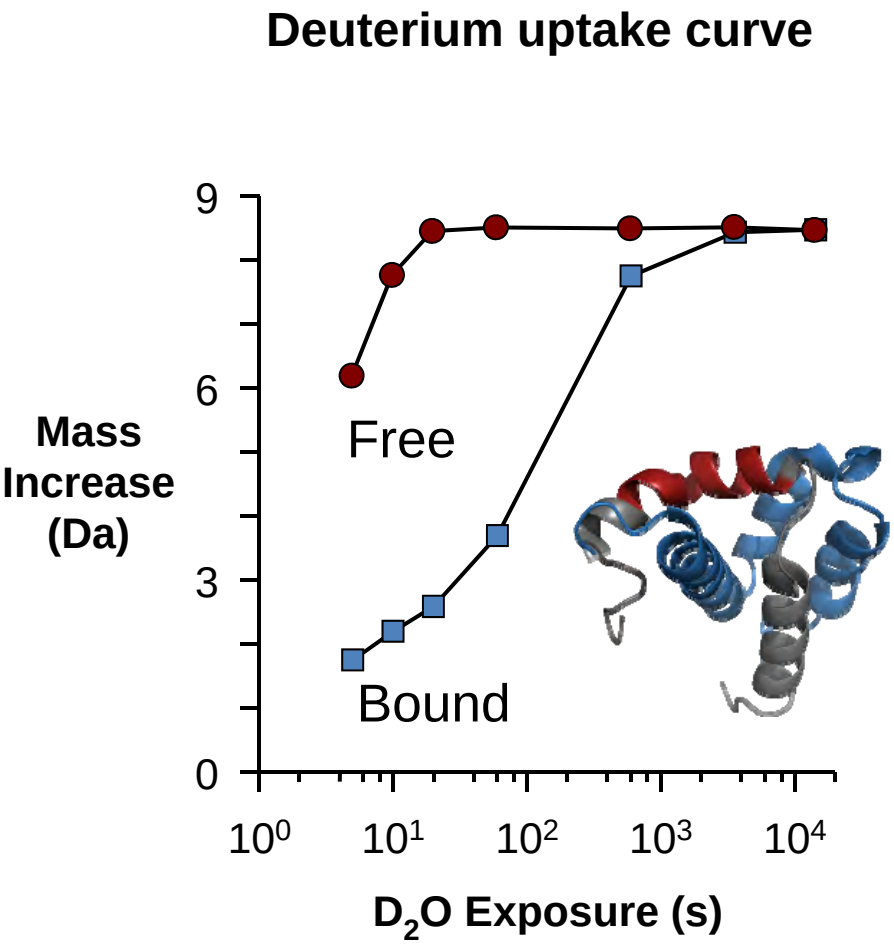
Rigid regions exchange slowly

MS approach uses quench and proteolysis.



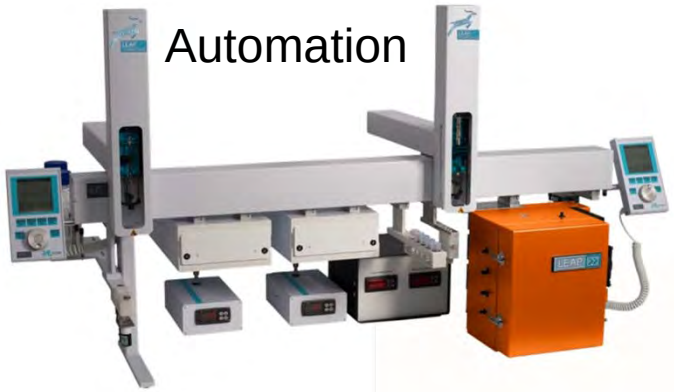
Peptides progressively gain mass.



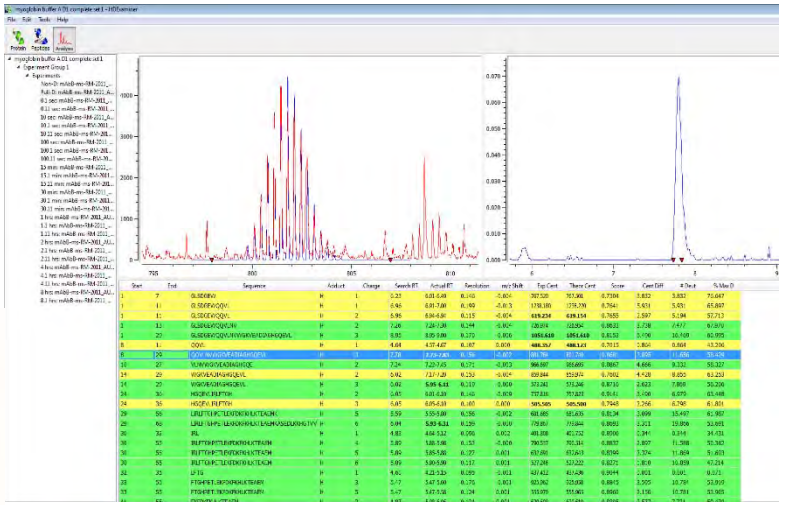


Efficient and robust platforms are now available

Automation



Informatics



HPLC

QTOF-MS

Year

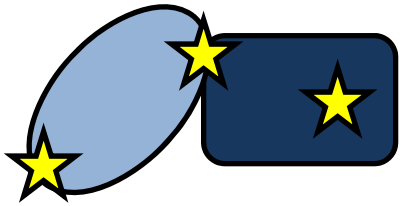
↓

Days

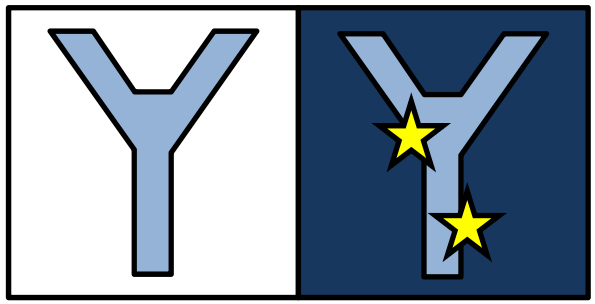
Applications of hydrogen exchange (HX)

Ligand binding

Protein interactions

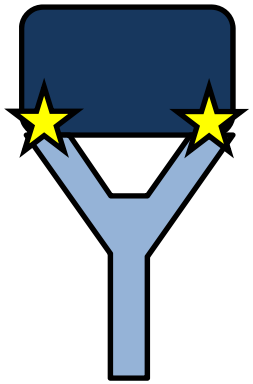


Formulations

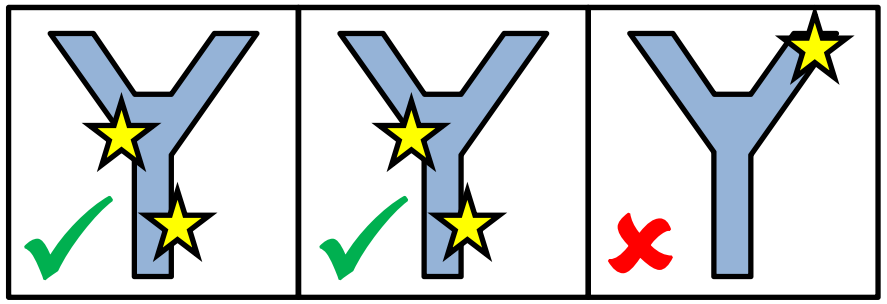


Disordered proteins

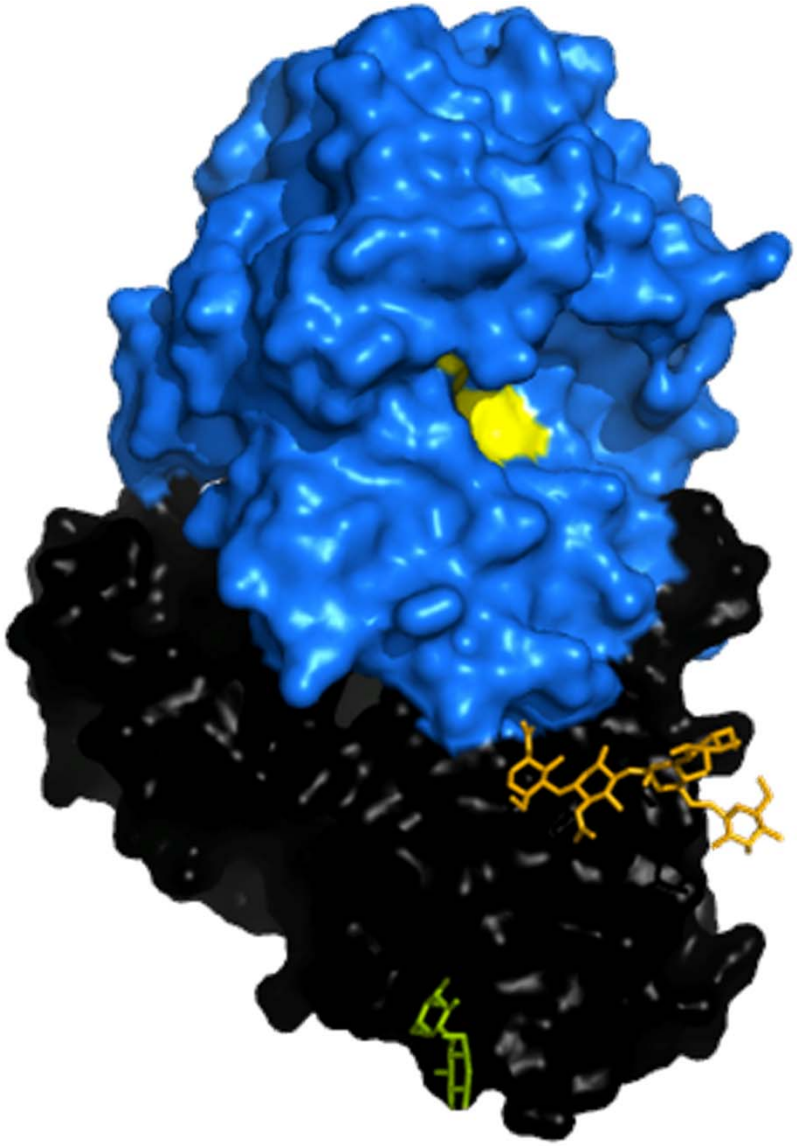
Epitope mapping



Comparability

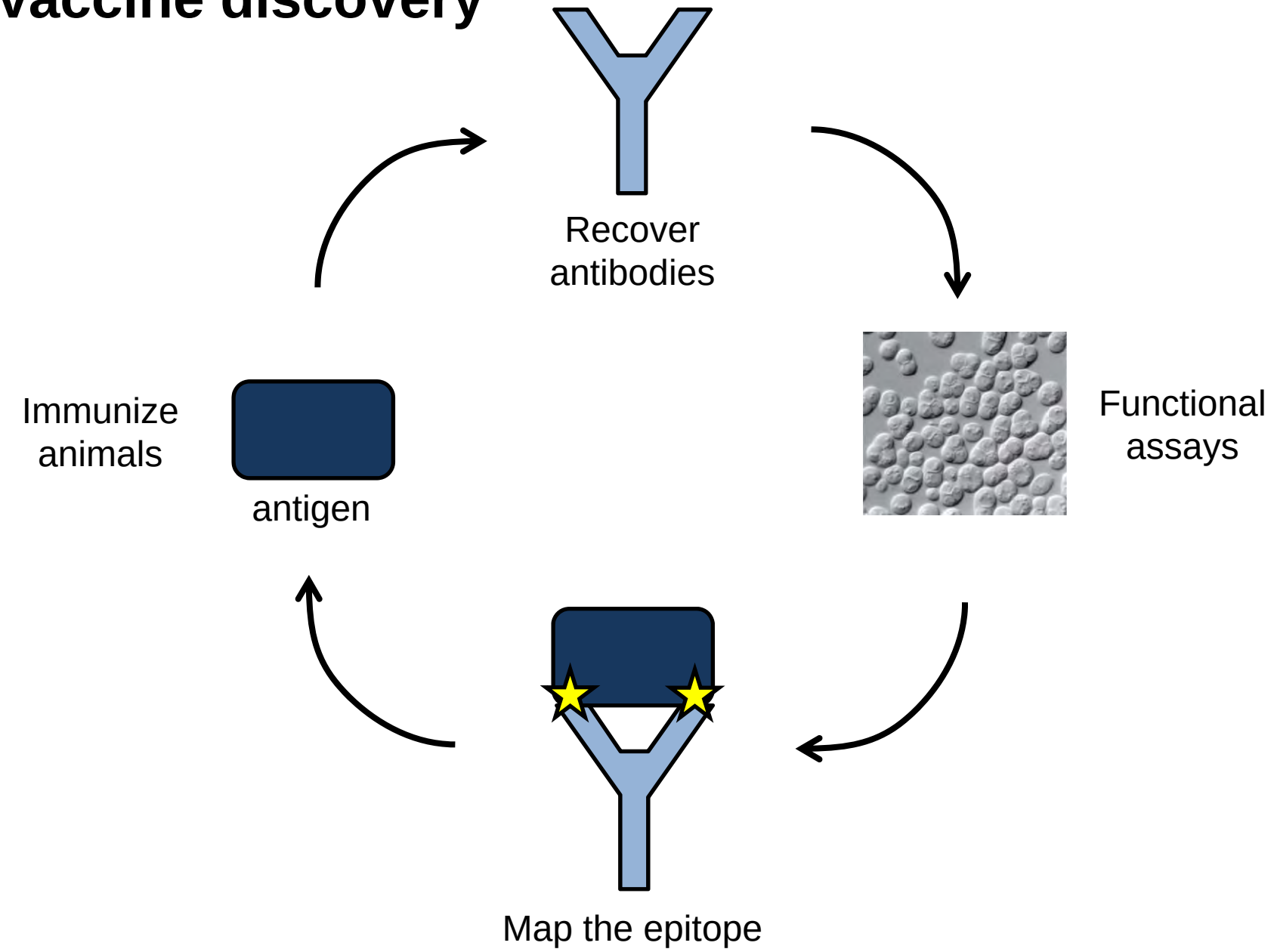


Ricin as a vaccine target

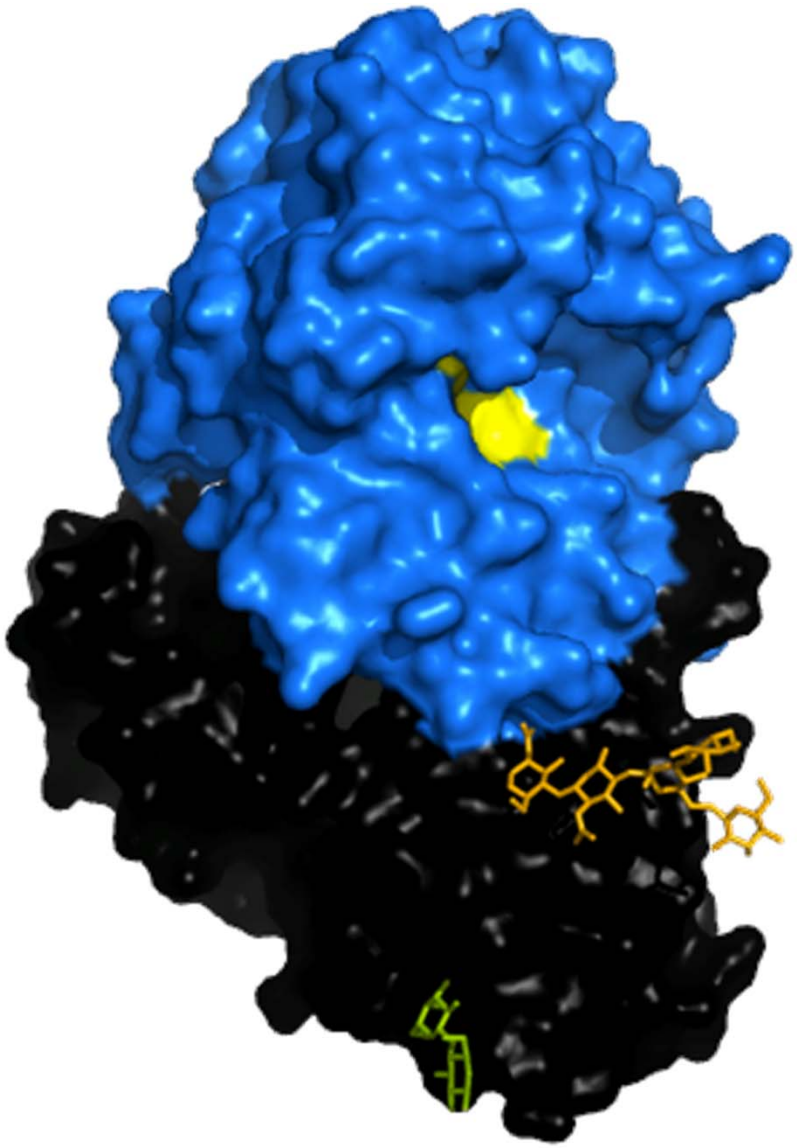


- Extremely toxic
  (~1 µg/kg inhaled)
- Multiple organ failure
- Lethal within 72 hrs
- Potential terror agent

Vaccine discovery

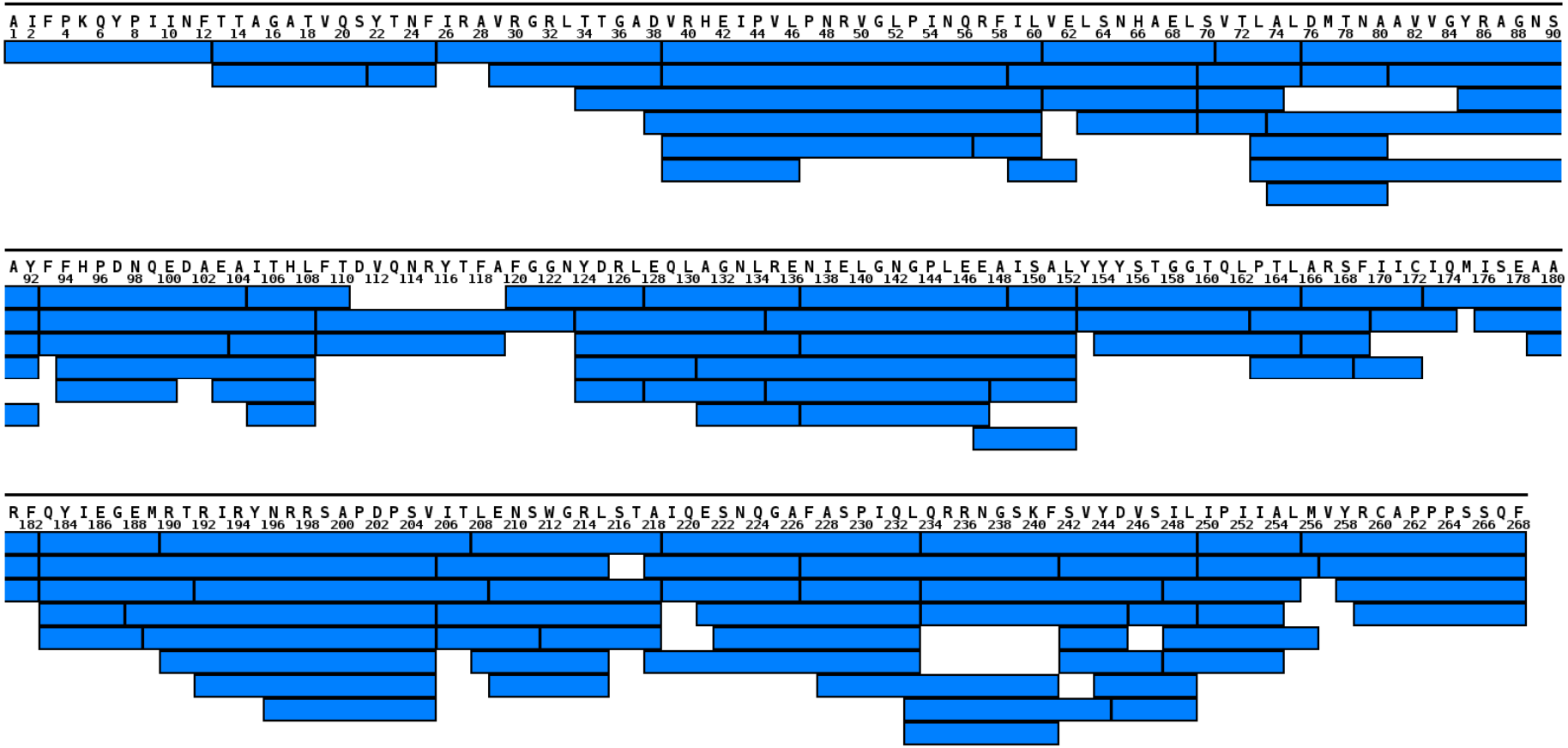


Eliciting *neutralizing* antibodies

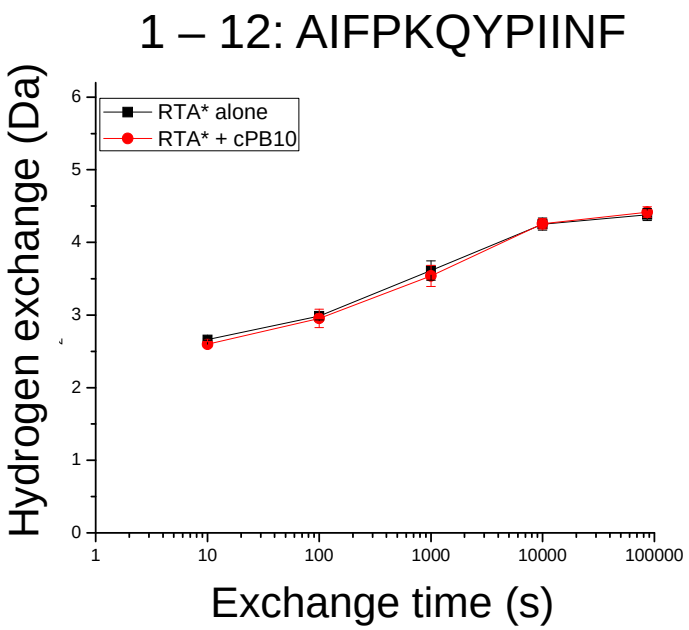


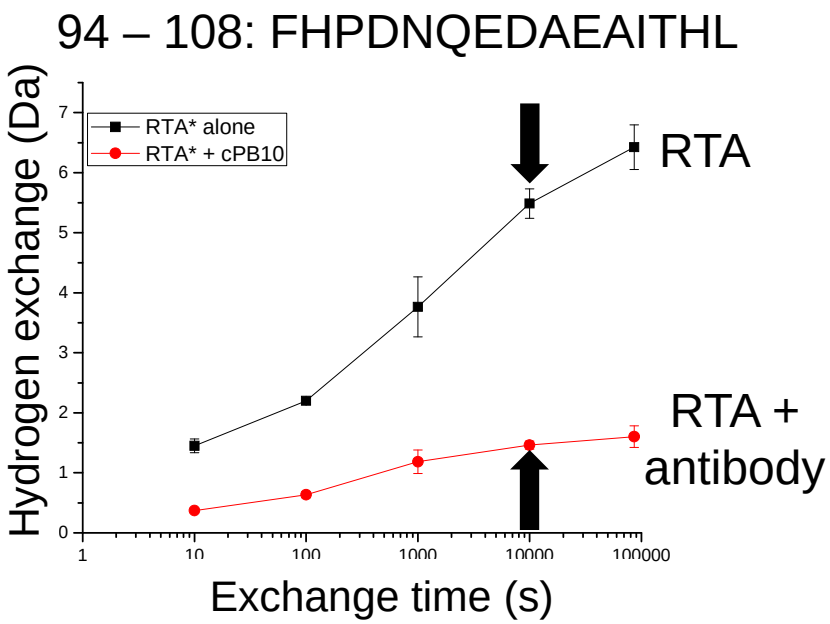
- Location?
- Mechanism?
- Rational design?

RTA peptic peptide map gives 100% coverage



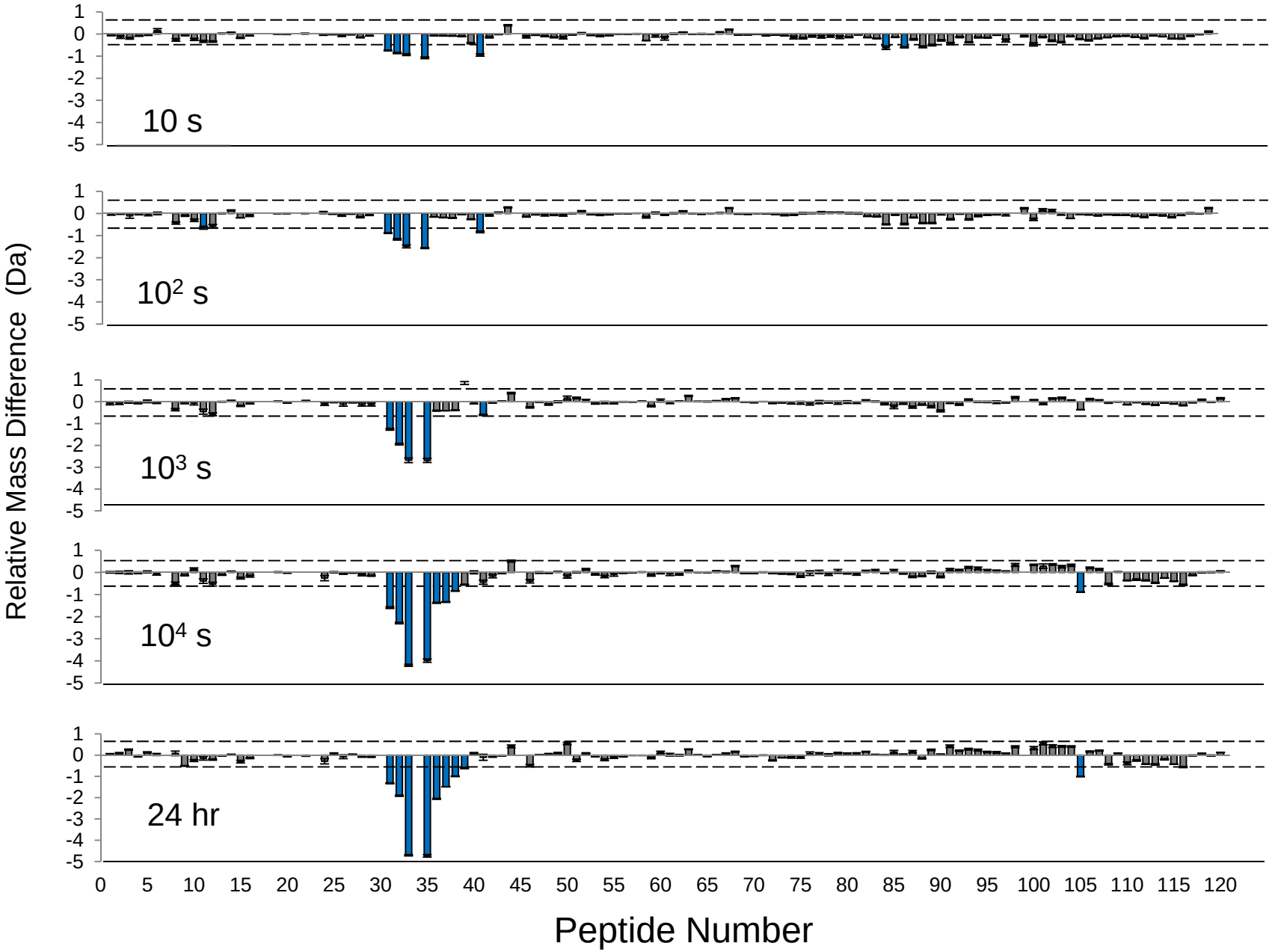
Epitope mapping with hydrogen exchange



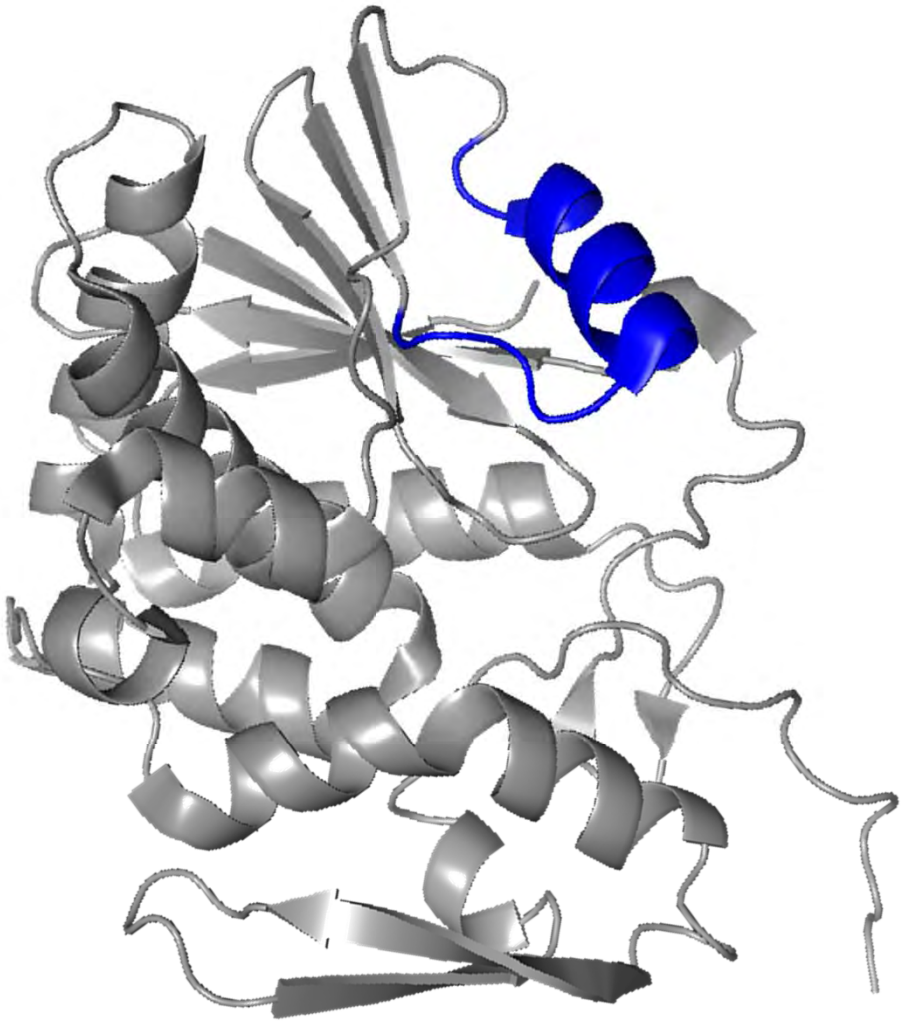


$$\Delta m = m_{\text{bound}} - m_{\text{free}}$$

Epitope mapping by HX protection

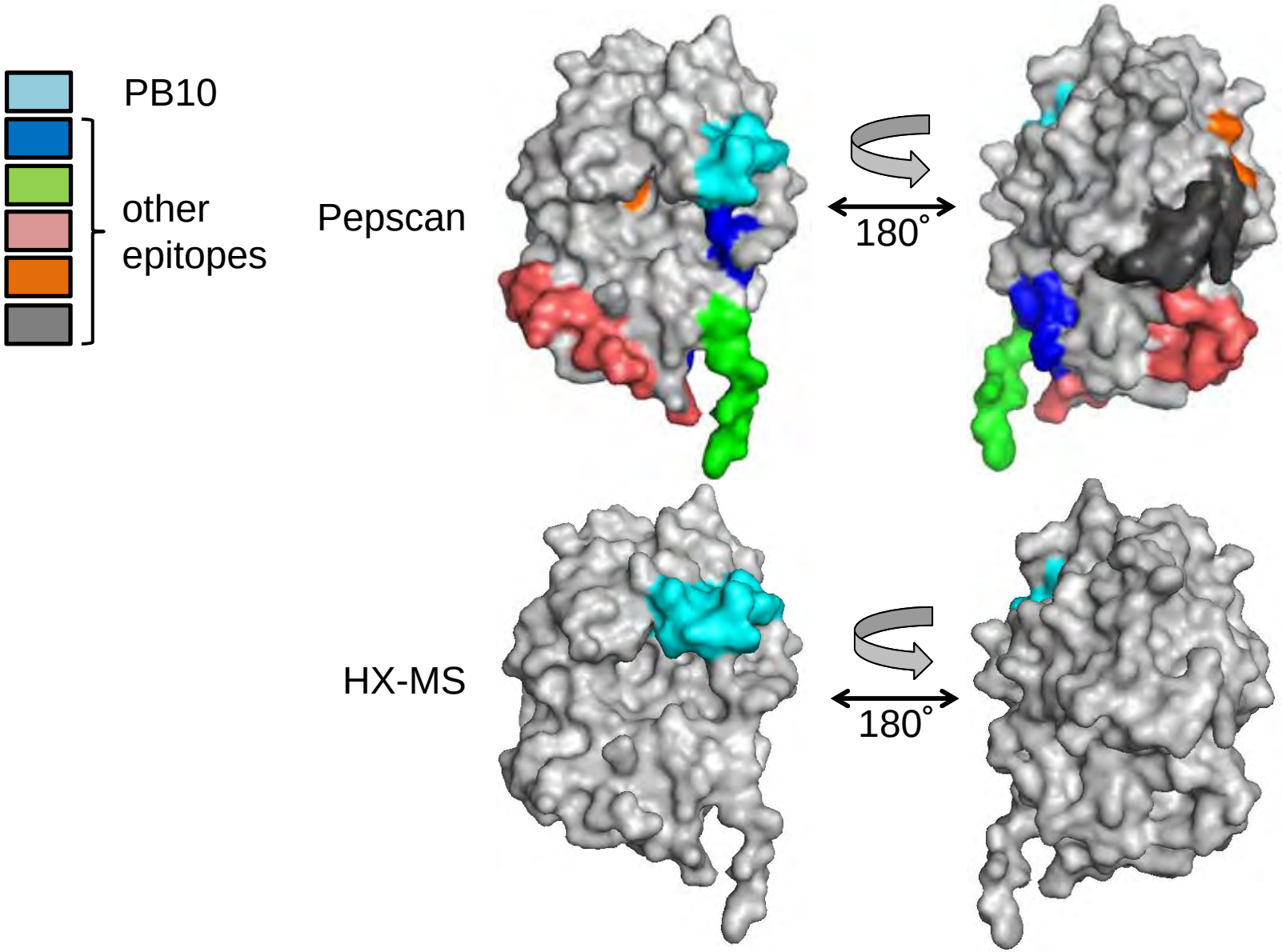


Location of PB10 epitope by HX-MS



PDB:3SRT

HX-MS agrees with Pepscan



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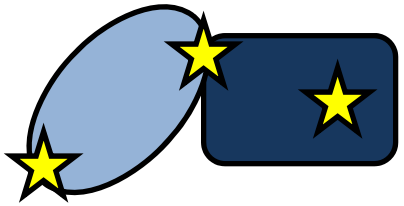
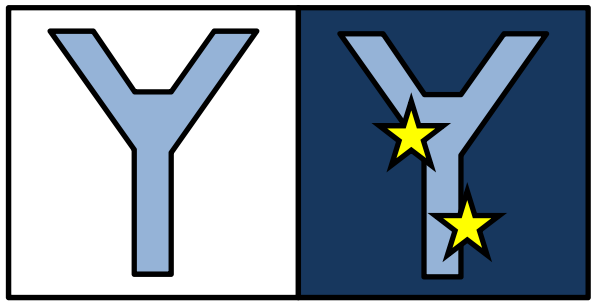
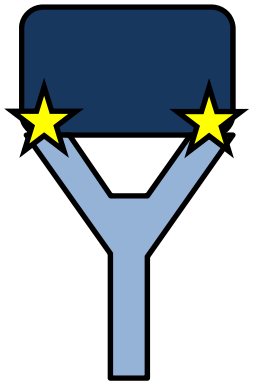


Formulations

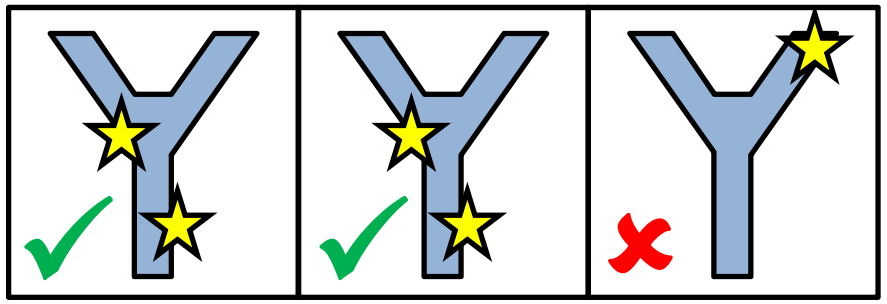


Disordered proteins

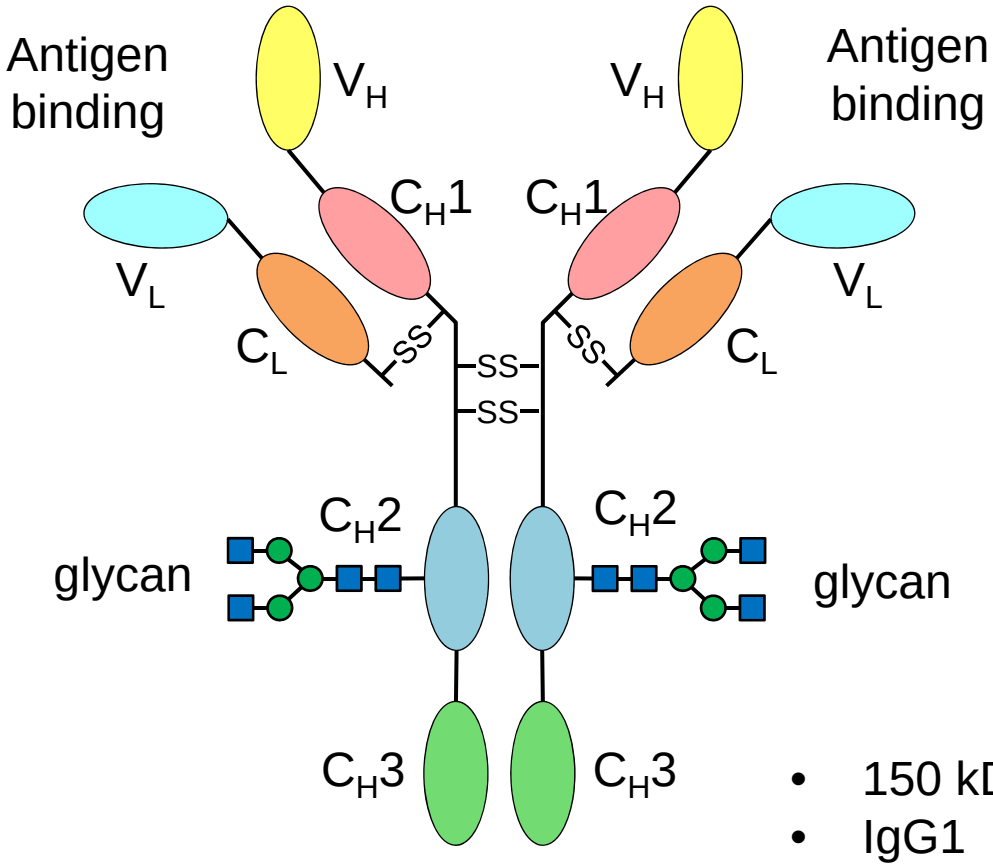
Epitope mapping



Comparability



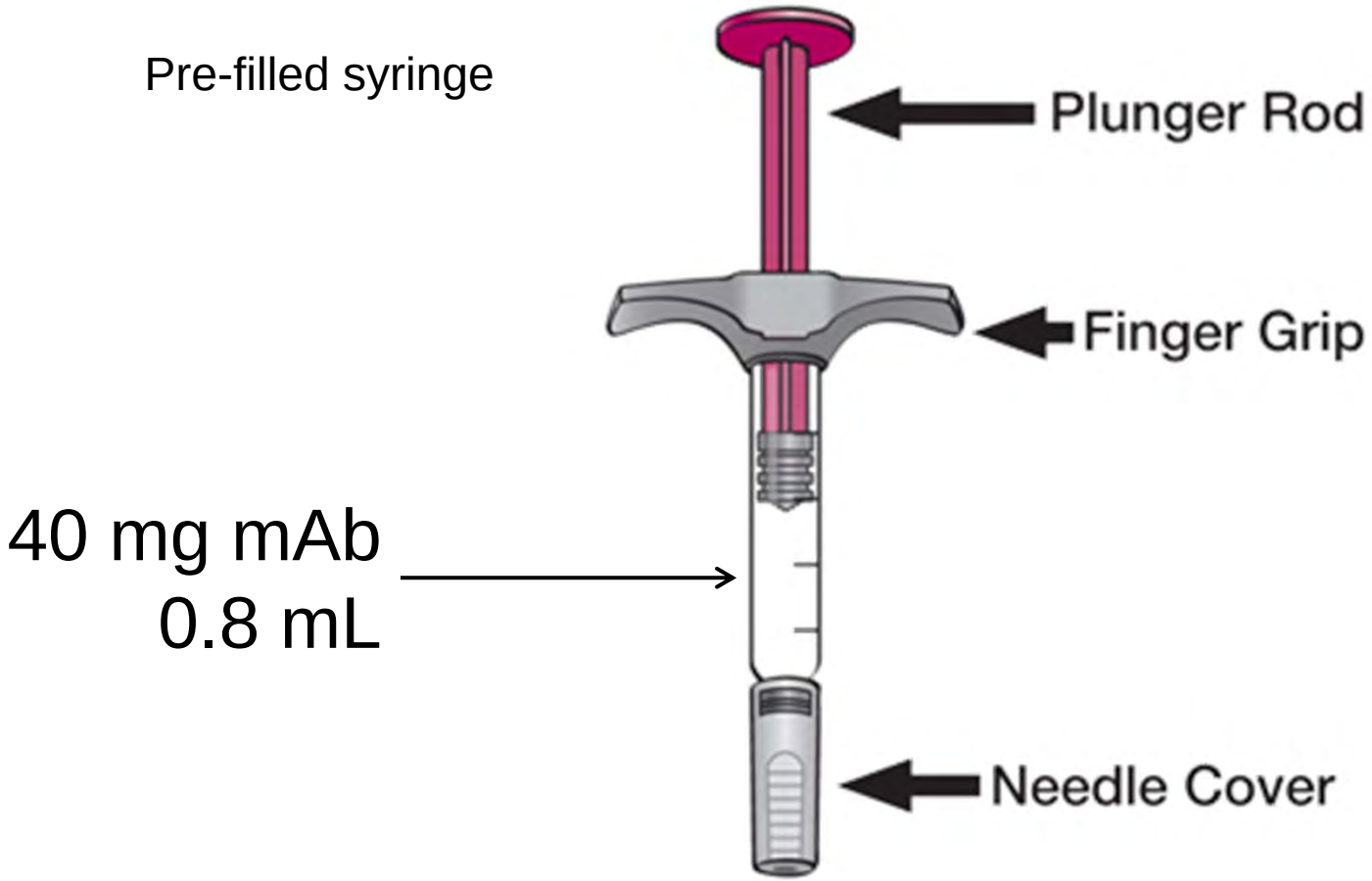
mAbs are the Cadillacs of biotherapeutics.



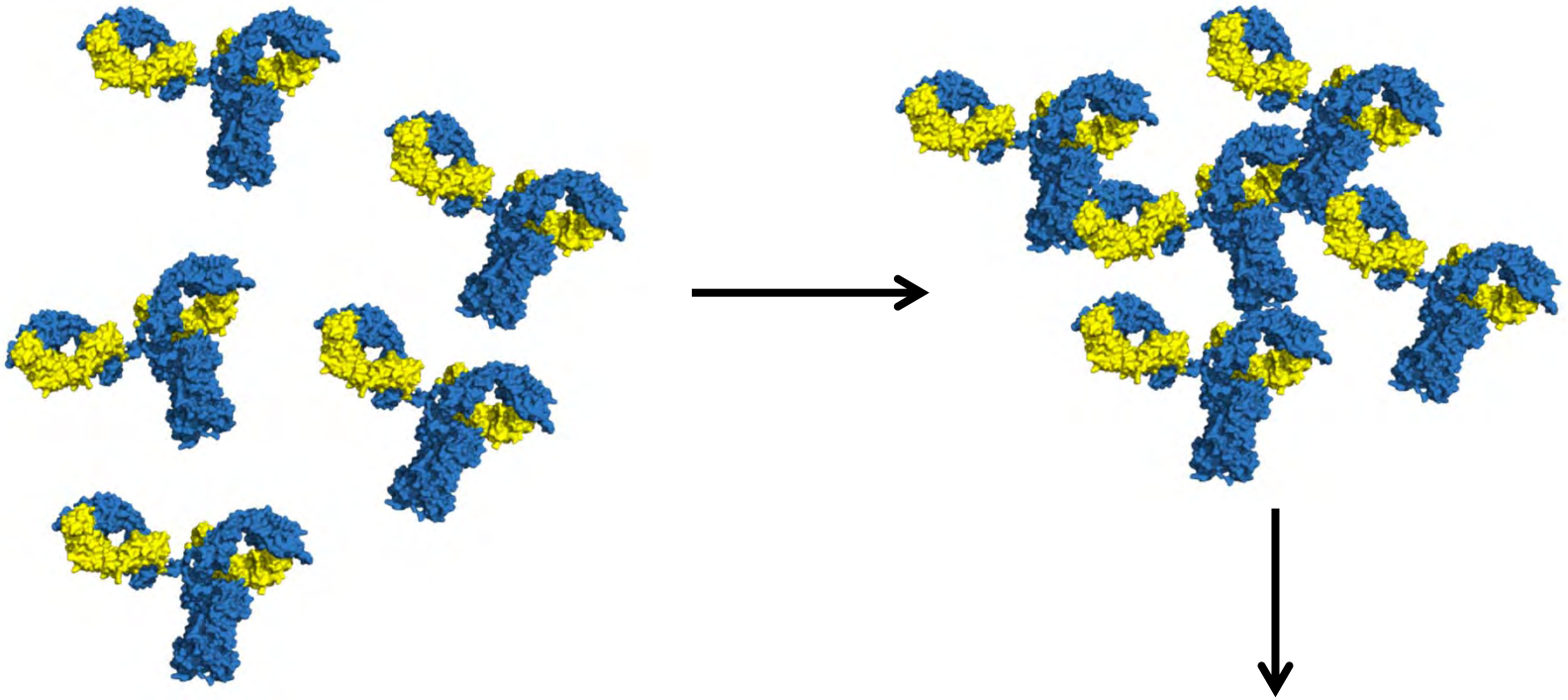
- 150 kDa
- IgG1
- Glycosylated
- 12 disulfide bonds
- 50 mg/mL, pH 6

Large dose, small volume

Pre-filled syringe



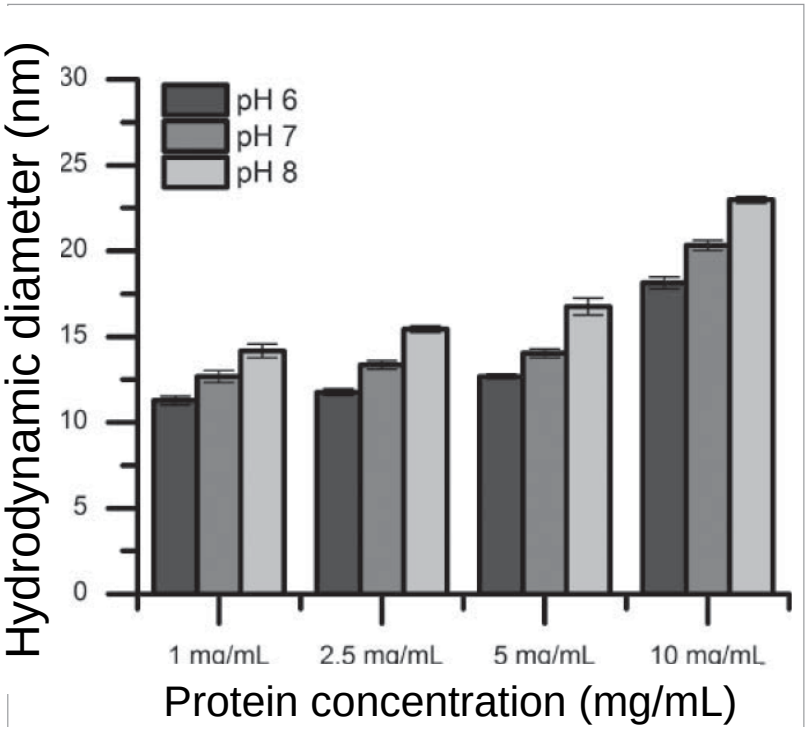
Reversible self-association

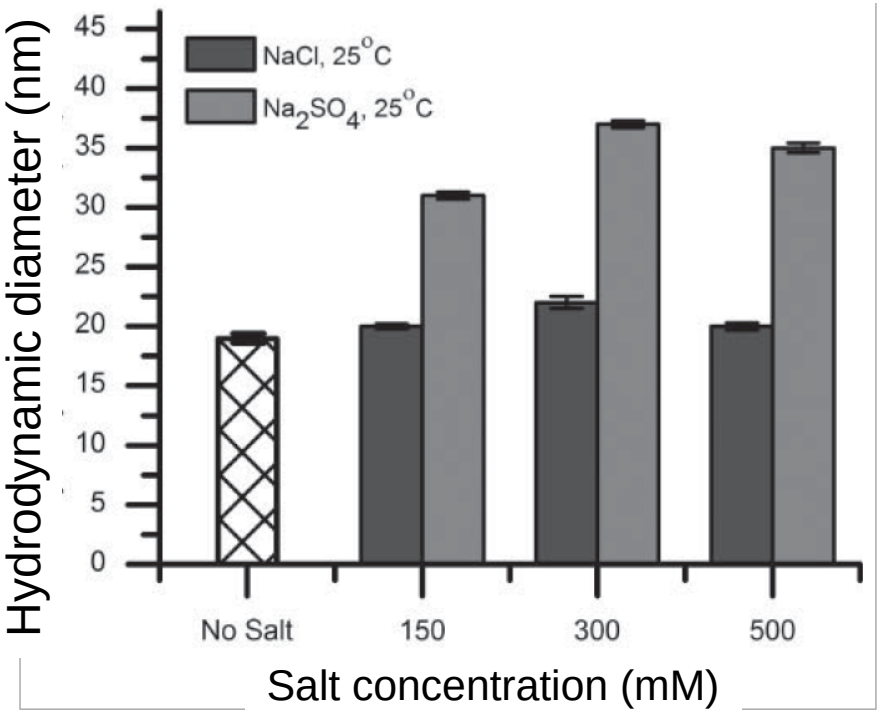


40 mg in 0.8 mL

Viscosity
Loss of efficacy
Immunogenicity

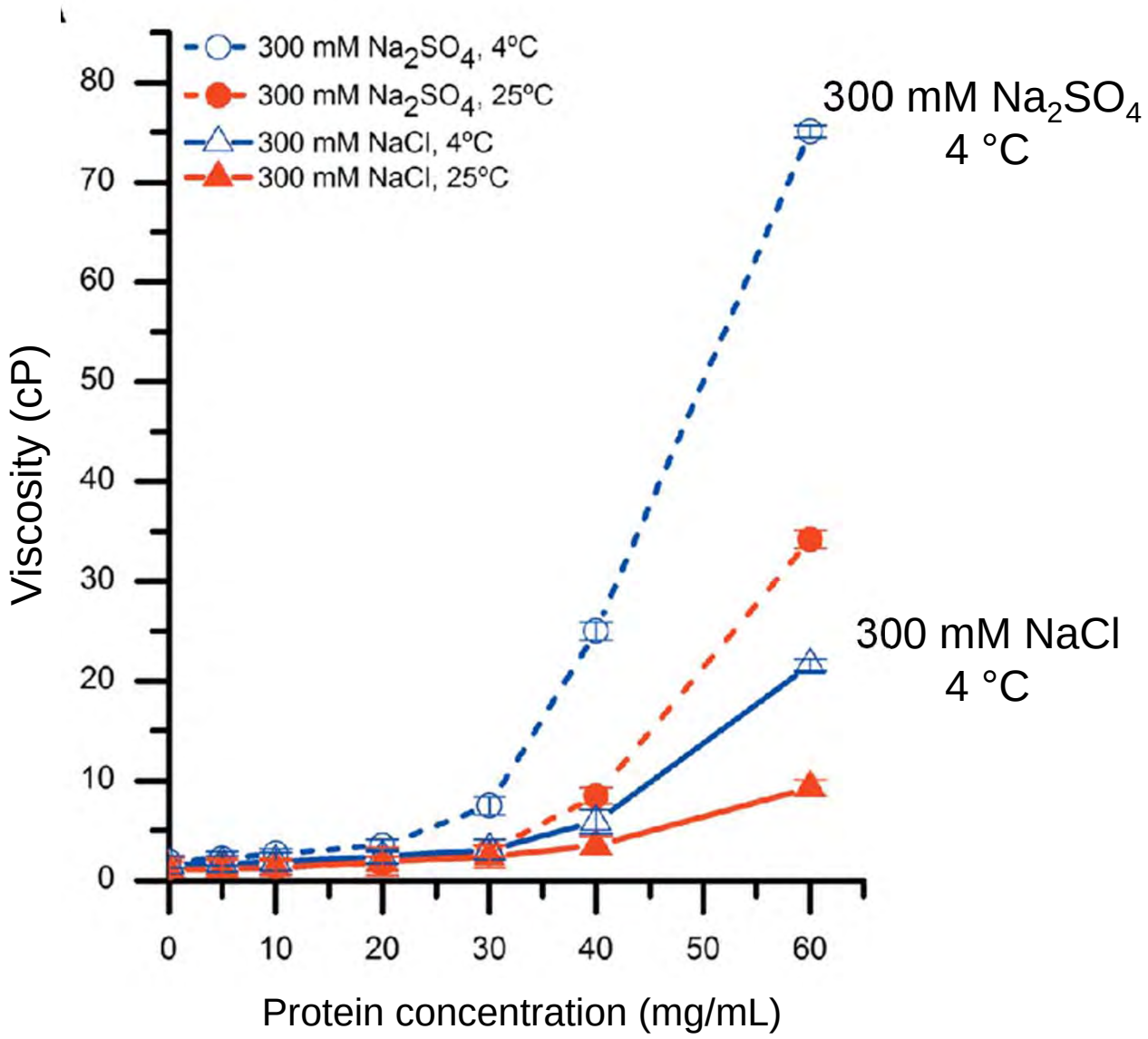
mAb C undergoes reversible self-association



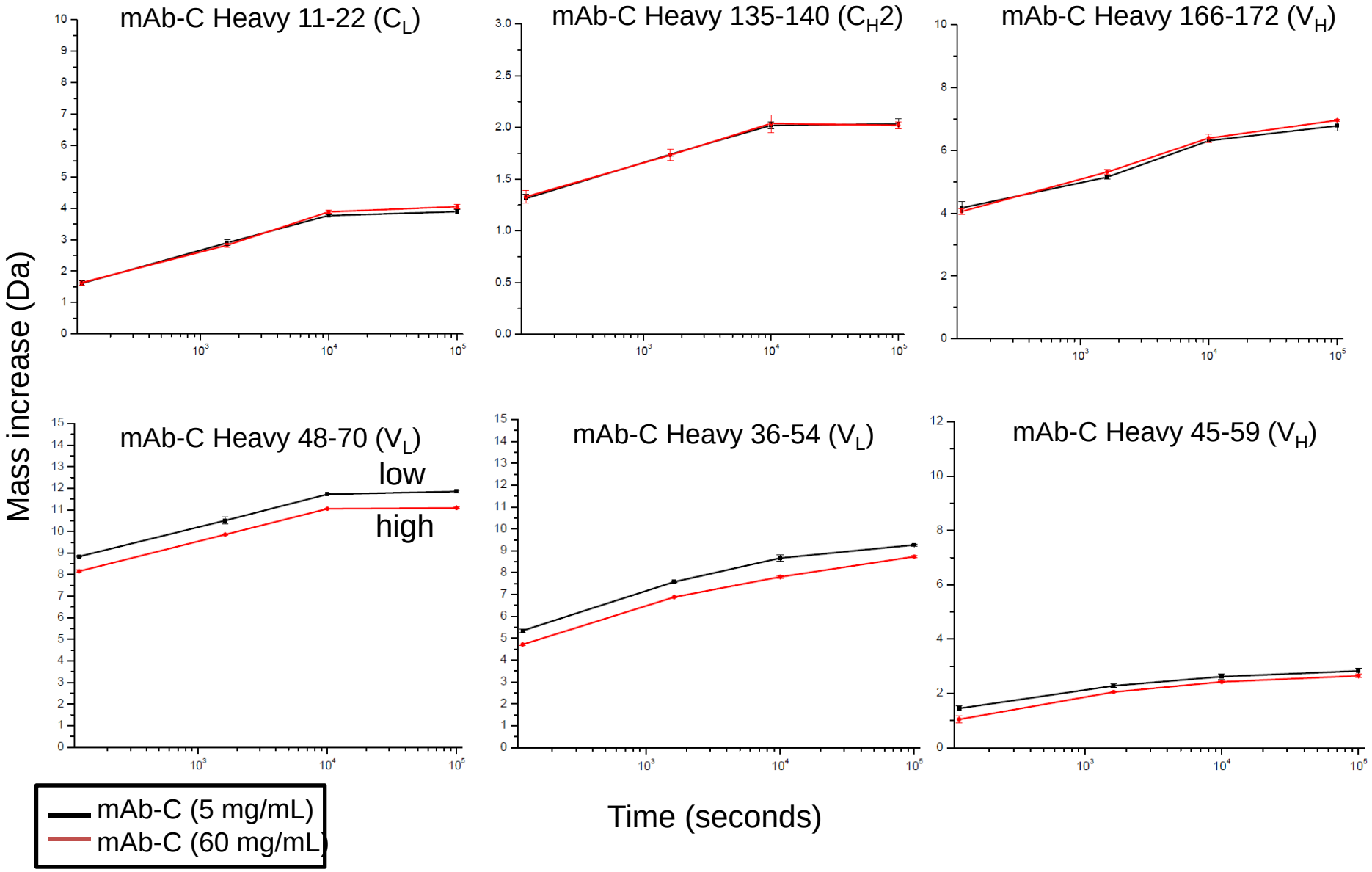


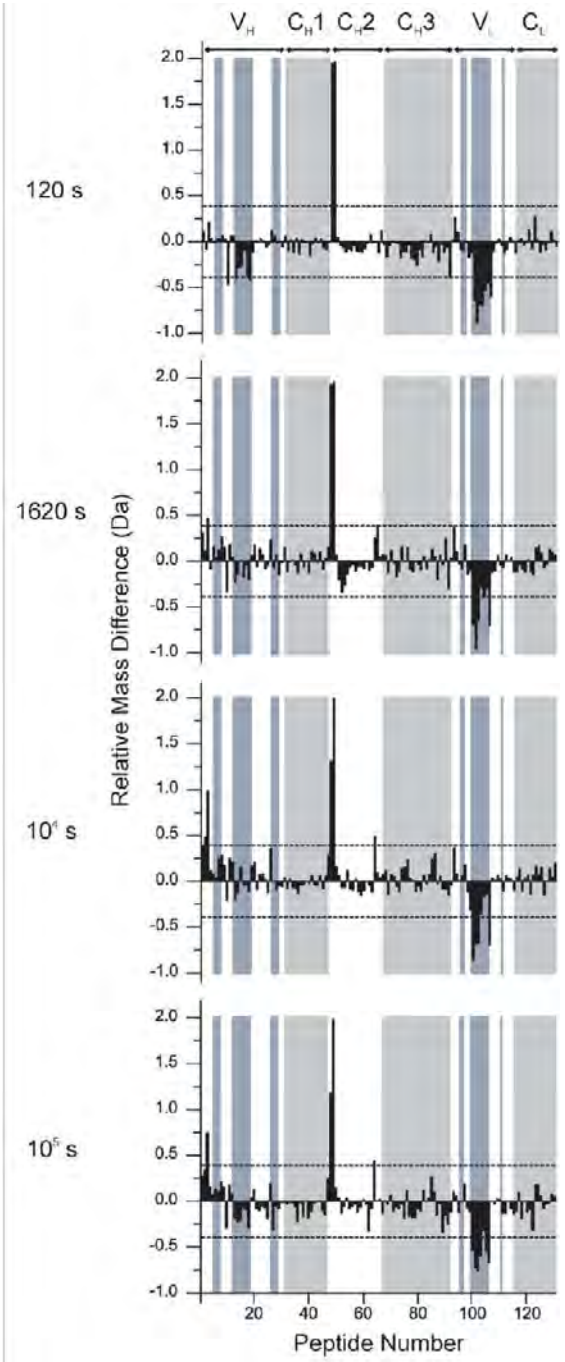
Promoted by high pH and sulfate

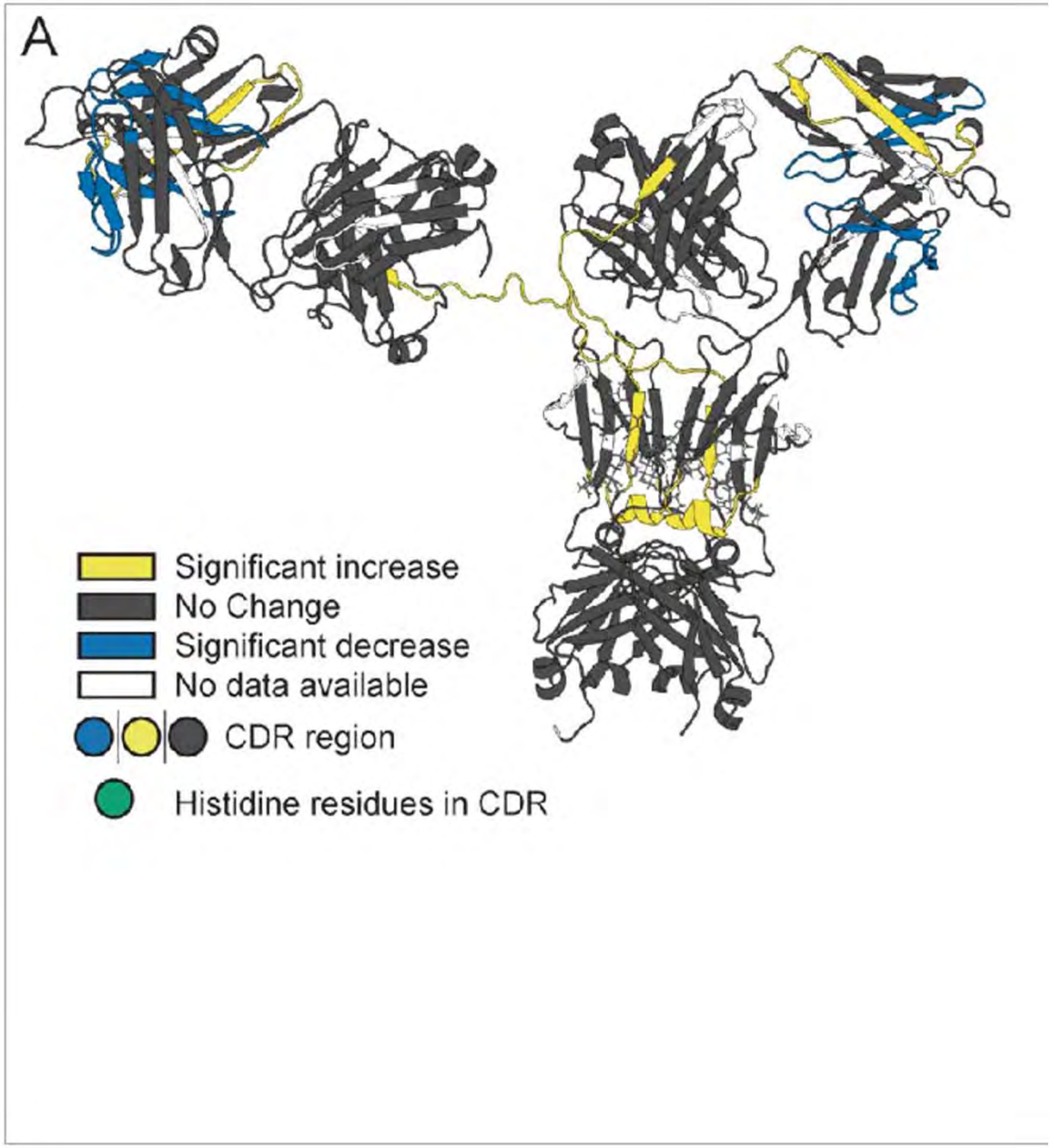
Sulfate promotes association



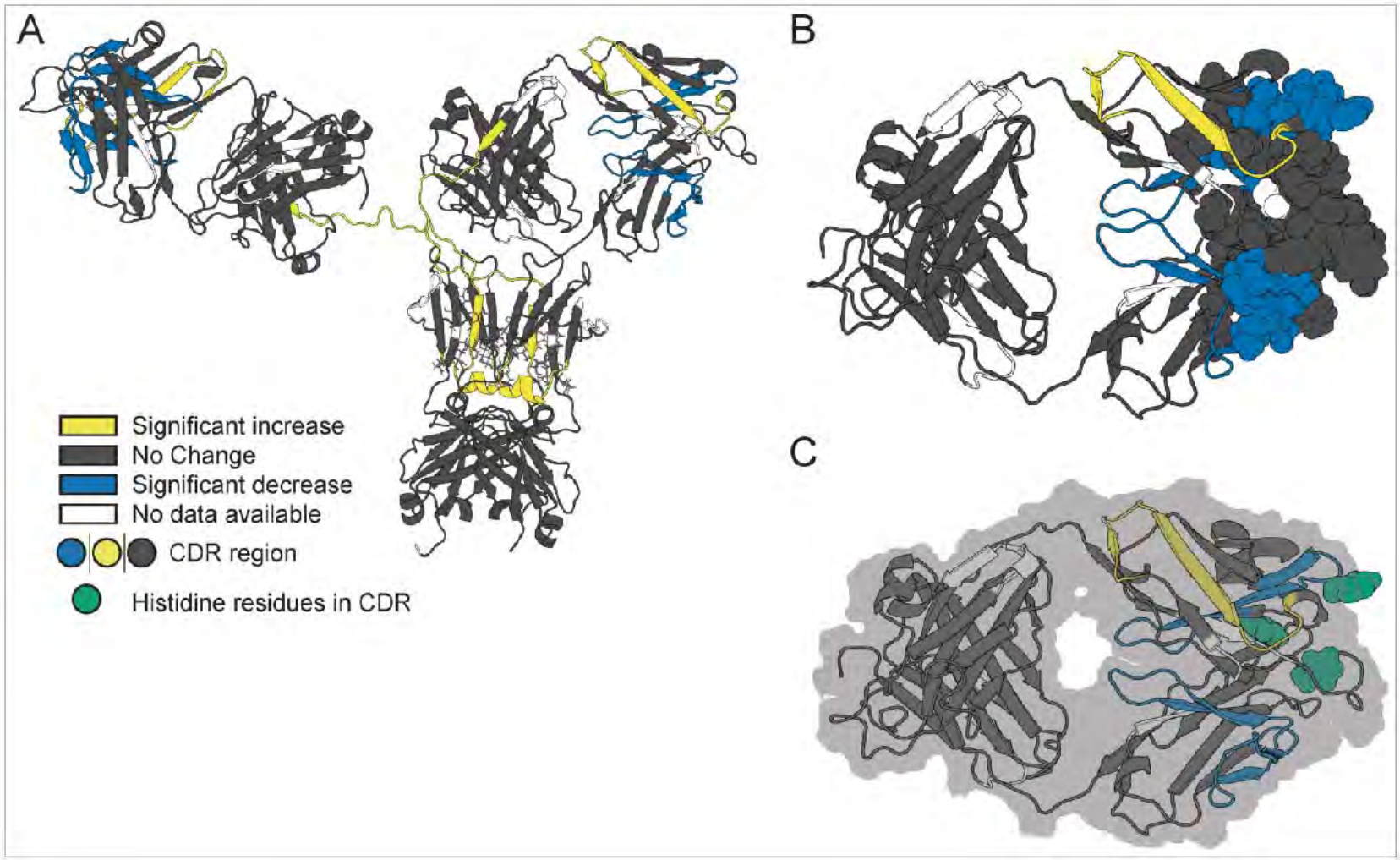
Mapping protected interface with HX-MS




V_H
C_H1
C_H2
C_H3
V_L
C_L
120 s
1620 s
10^4 s
10^5 s
Relative Mass Difference (Da)
Peptide Number


A
Significant increase
No Change
Significant decrease
No data available
CDR region
Histidine residues in CDR

Mechanism of RSA



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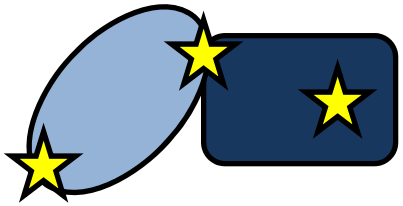
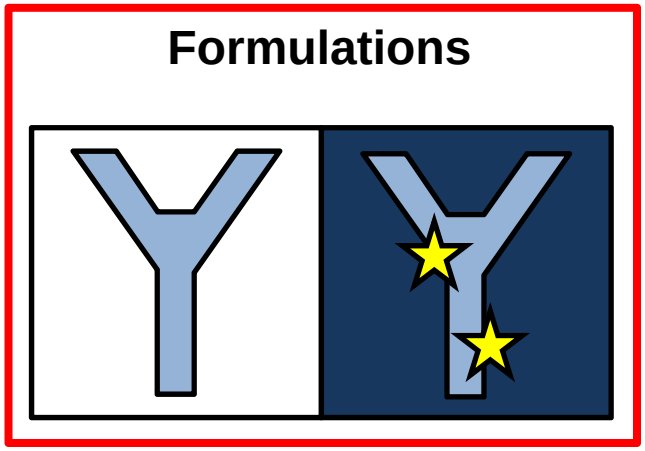
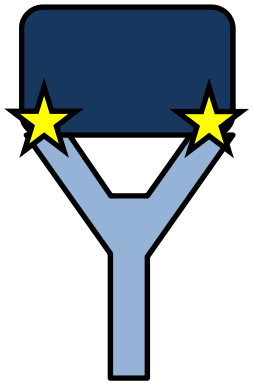


Formulations

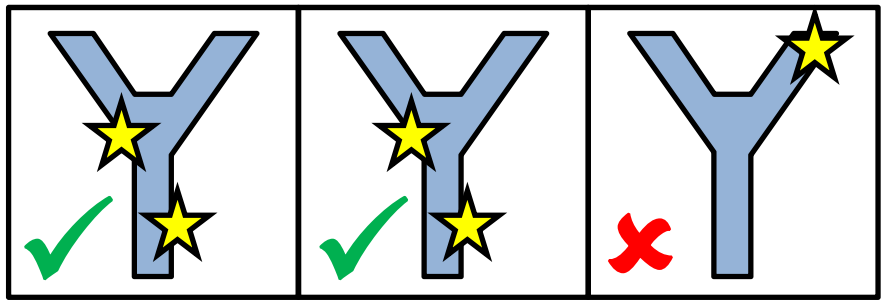


Disordered proteins

Epitope mapping



Comparability



Maintaining the physical stability of protein therapeutics is a critical problem.

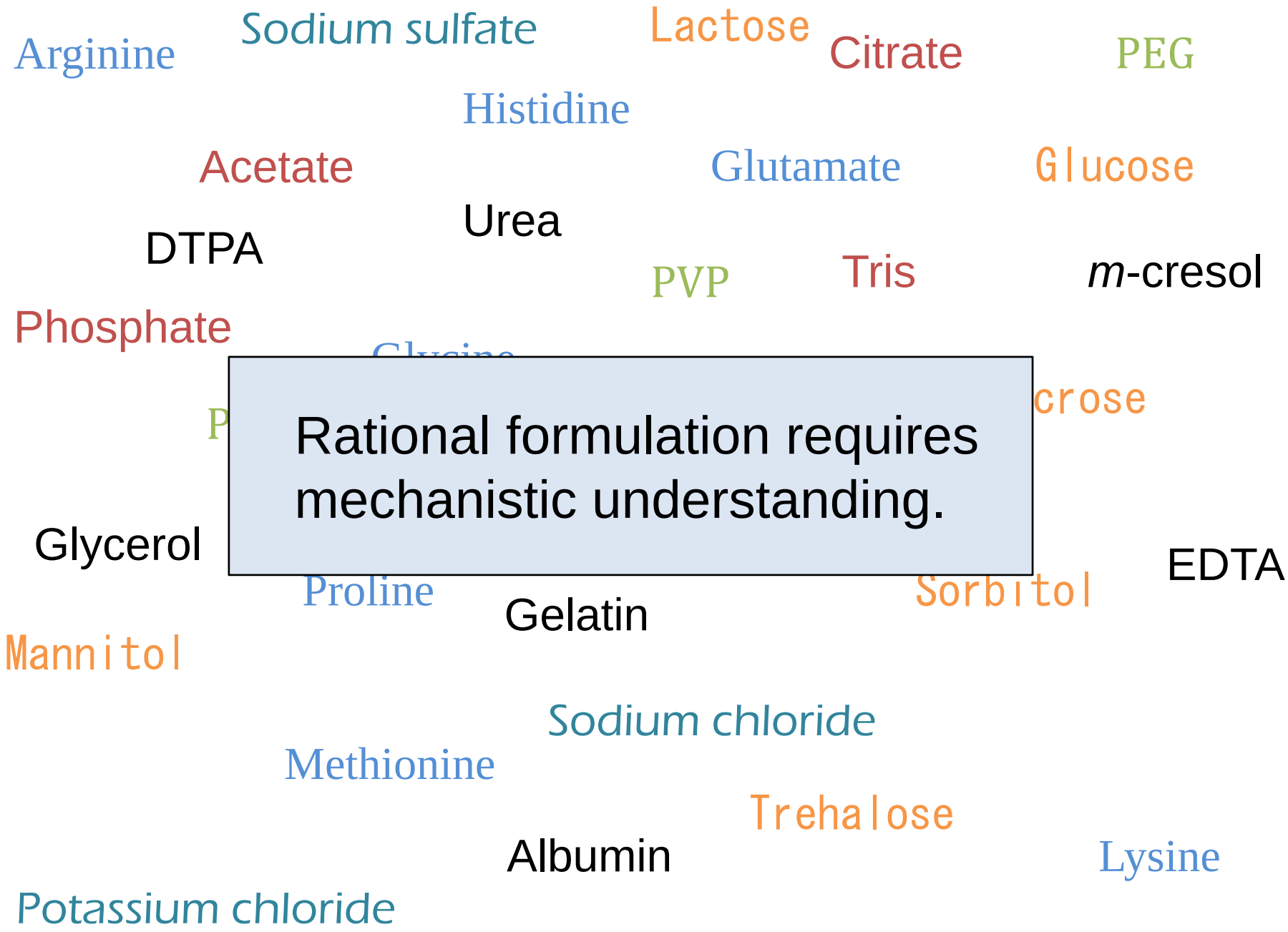
Conformational instability

Aggregation

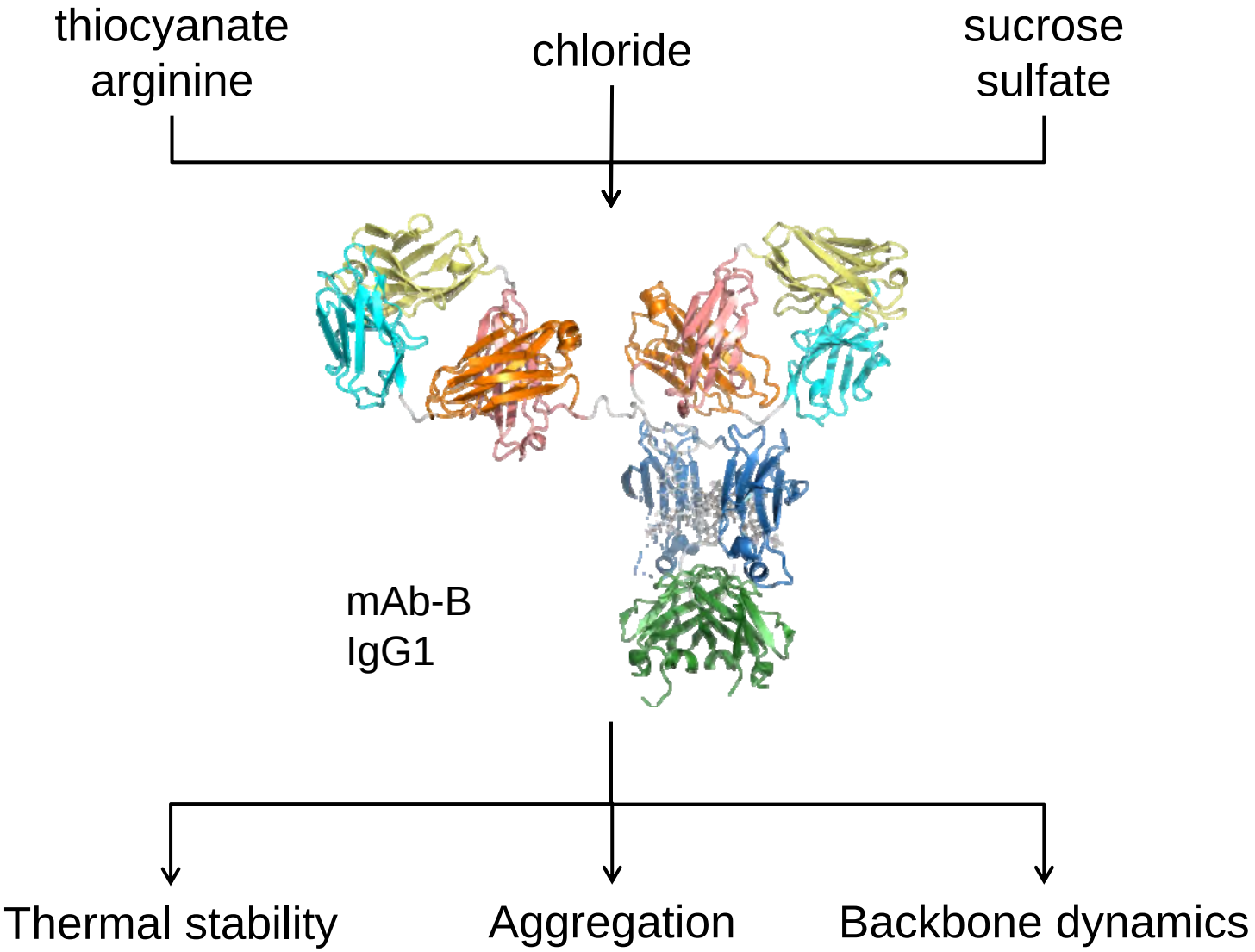
Loss of efficacy

Immunogenicity

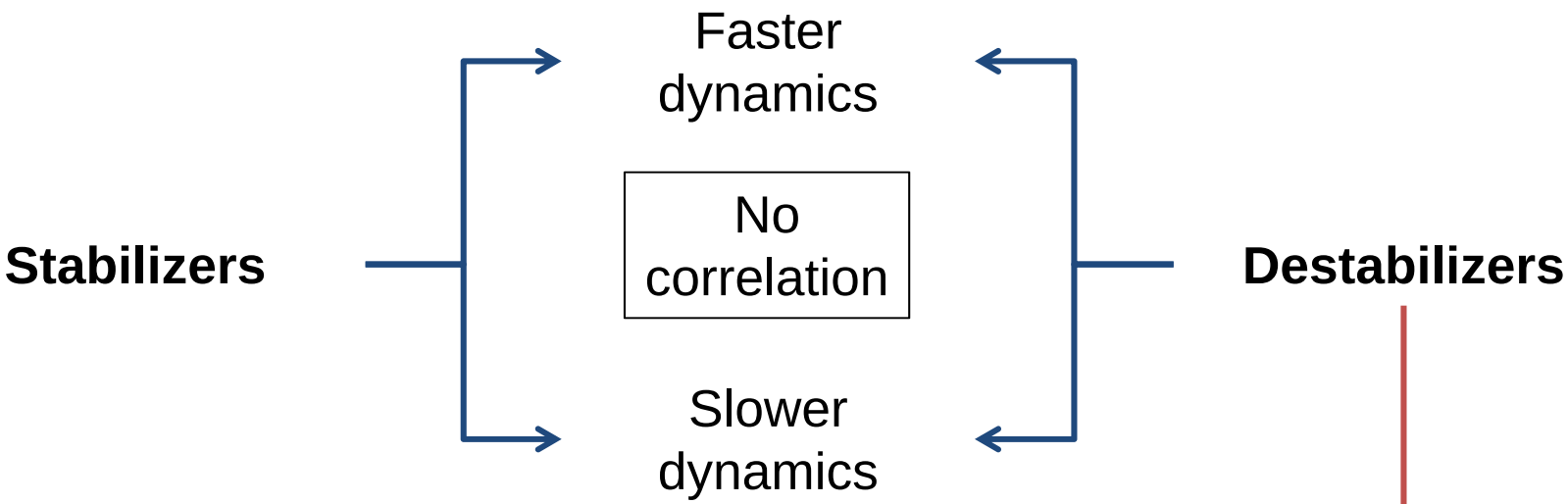
Solution:
Develop a stabilizing formulation.


Arginine
Sodium sulfate
Lactose
Citrate
PEG
Histidine
Acetate
Glutamate
Glucose
Urea
DTPA
PVP
Tris
m-cresol
Phosphate
crose
Rational formulation requires mechanistic understanding.
Glycerol
EDTA
Proline
Gelatin
Sorbitol
Mannitol
Sodium chloride
Methionine
Trehalose
Albumin
Lysine
Potassium chloride

Does backbone flexibility correlate with stability?

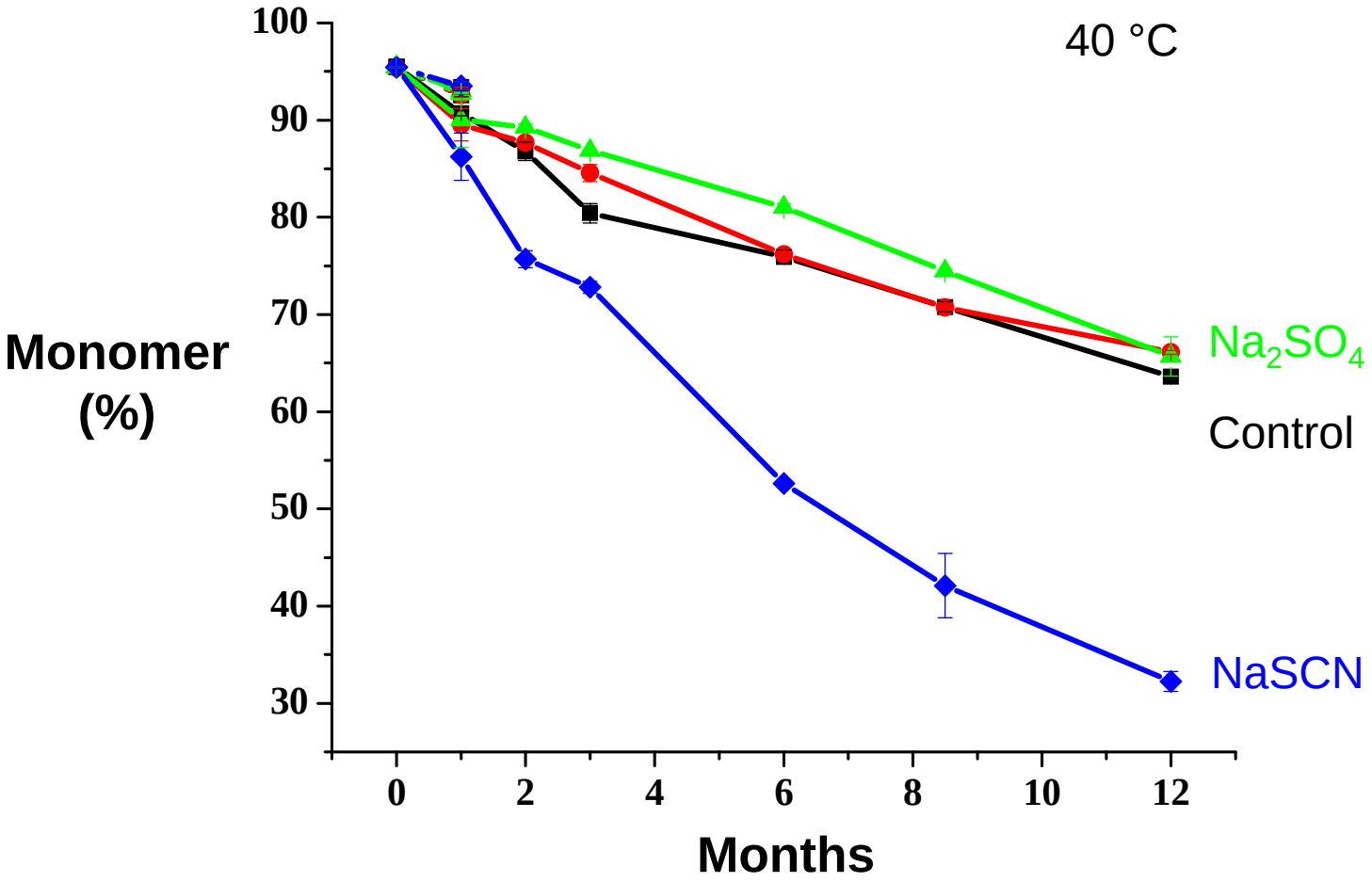


The connection between flexibility and stability was not obvious.



Loss of stability correlated with increased flexibility in C_H2 domain.

Sulfate slowed aggregation.
Thiocyanate accelerated aggregation.

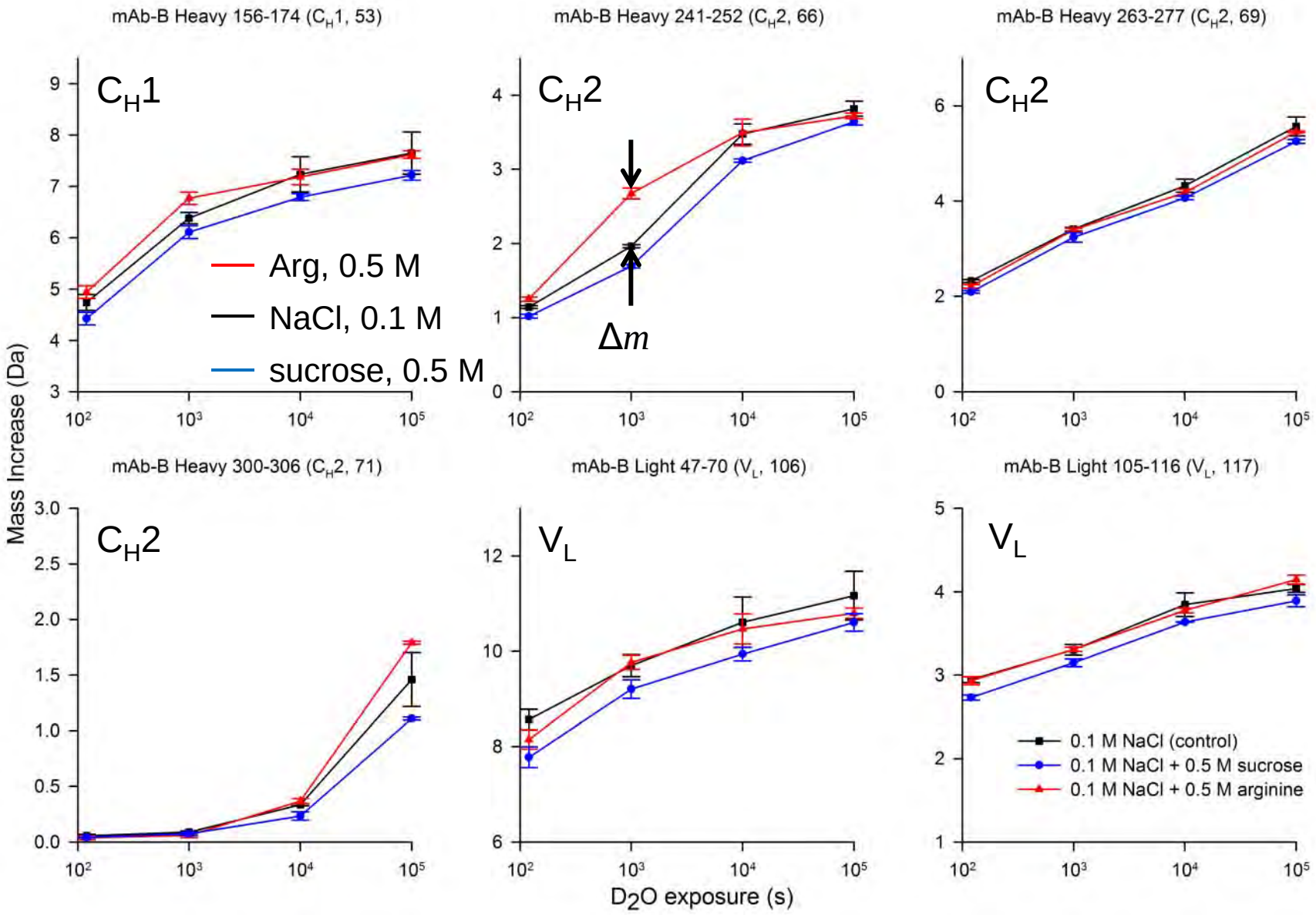


Stabilizers and destabilizers acted as expected.

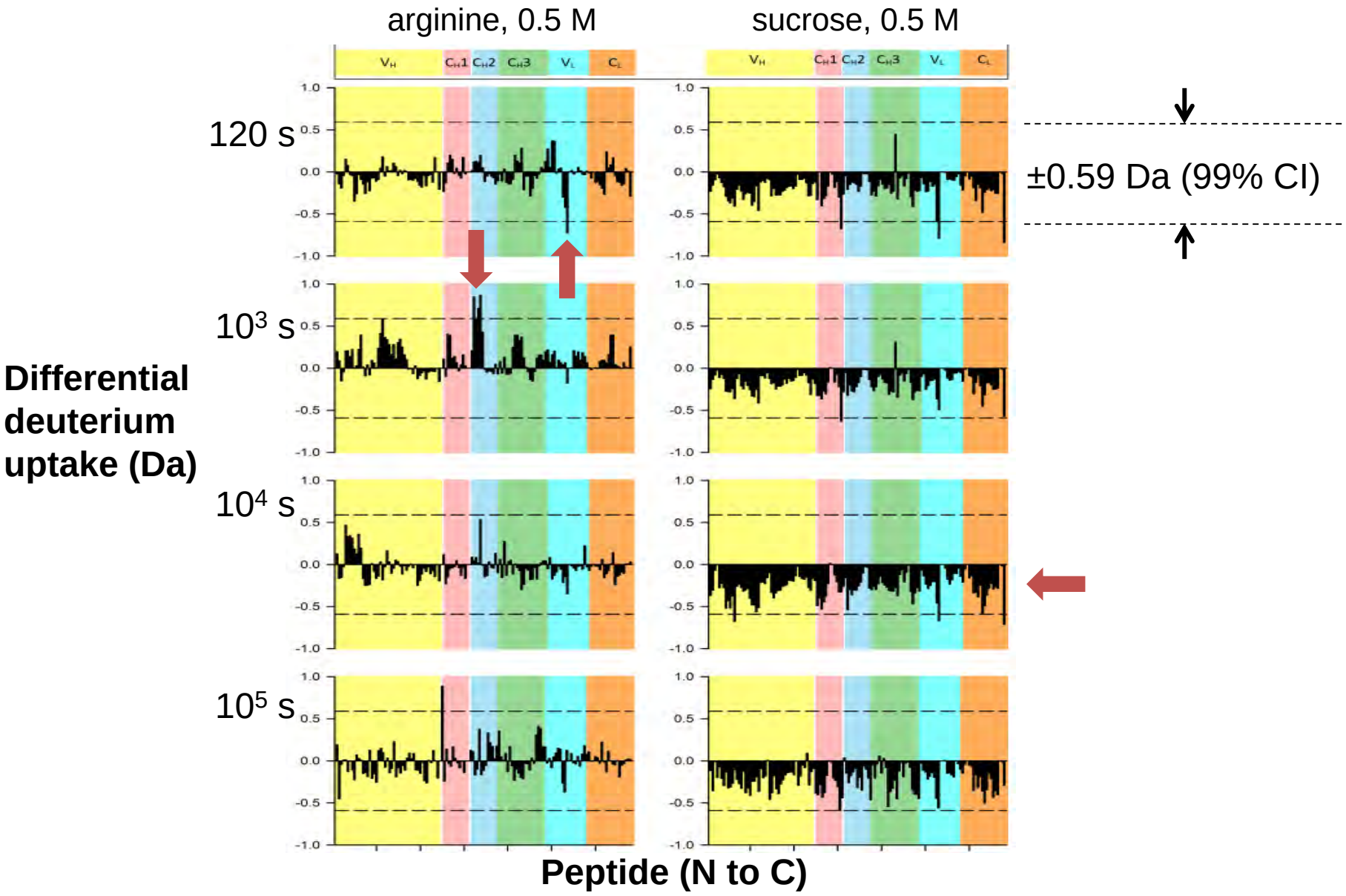
	$\Delta T_m 1$	**Aggregation**
thiocyanate	−9.0 °C	Faster (++)
arginine	−1.9 °C	Faster (+)
chloride	+0.3 °C	Negligible
sucrose	+1.5 °C	Slower (−)
sulfate	+1.8 °C	Slower (−)

How do these excipients work at the molecular level?

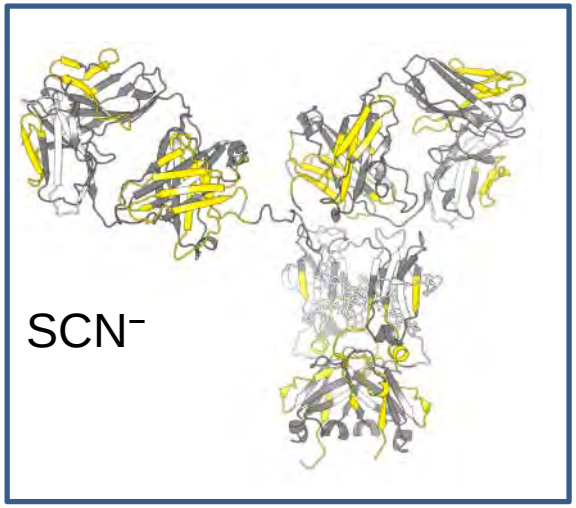
The effects of excipients are not uniform.

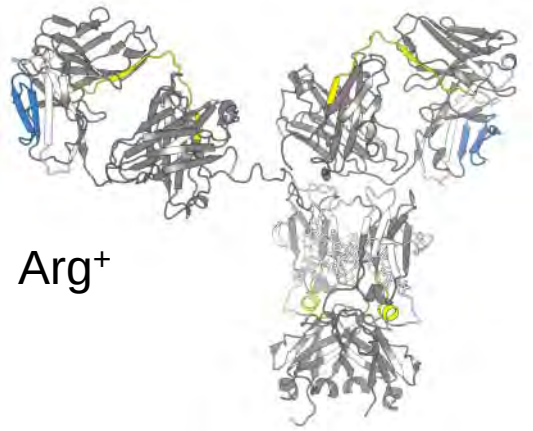


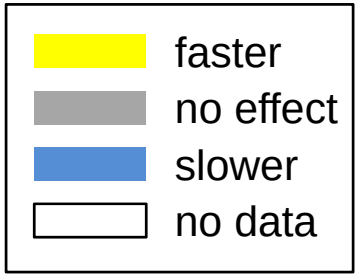
The effects are excipient-dependent.

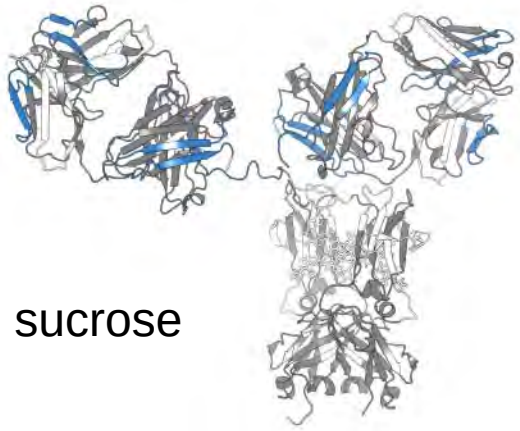


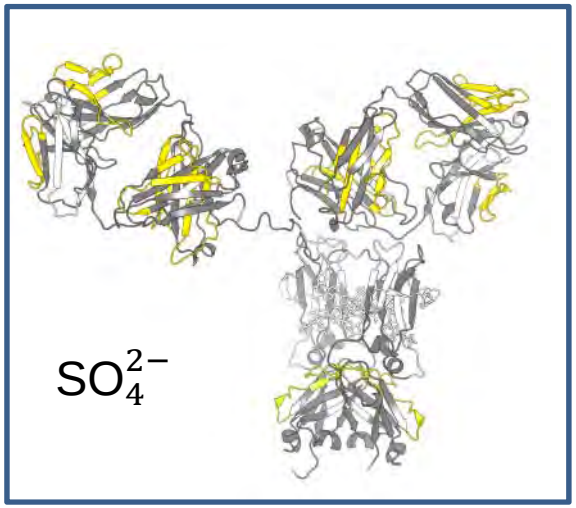
The correlation between stability and altered H/D exchange is not obvious.











Homology model based on [Saphire, 2001] 1HZH

Extremes have nearly-identical effects.

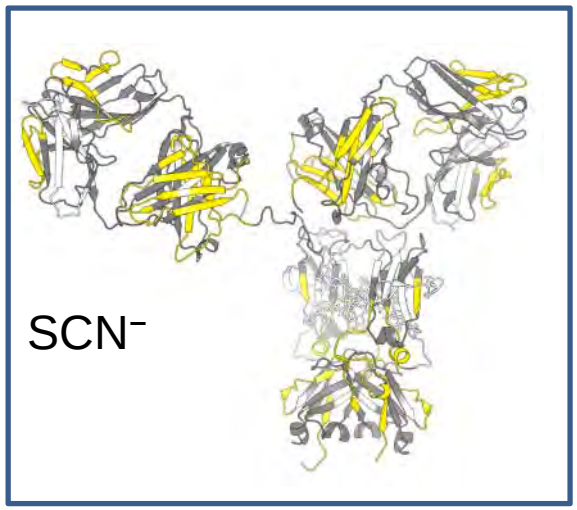
SCN^-

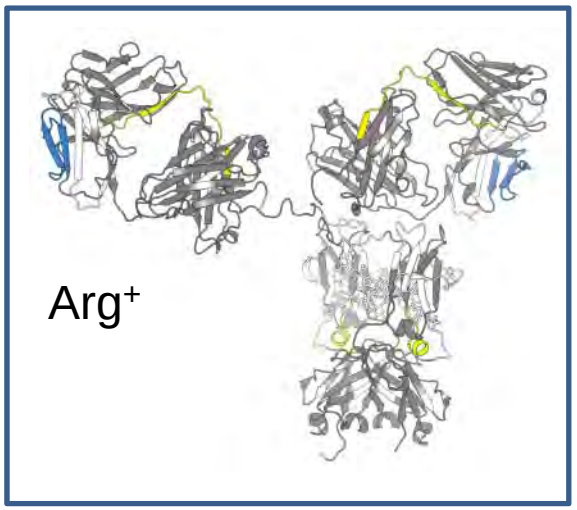
SO_4^{2-}

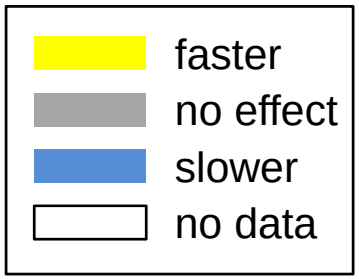
Destabilizer

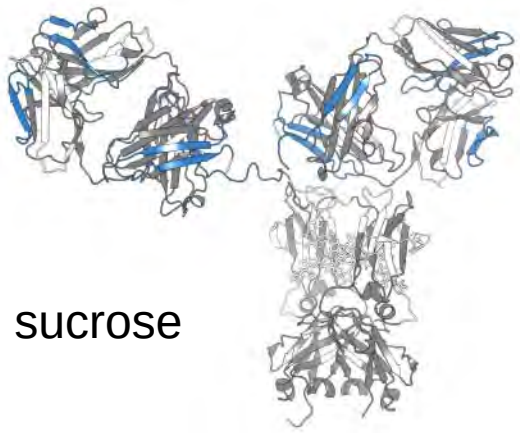
Stabilizer

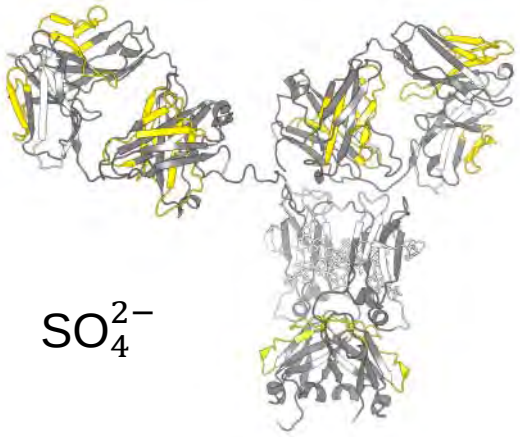
The correlation with altered hydrogen exchange is not obvious.



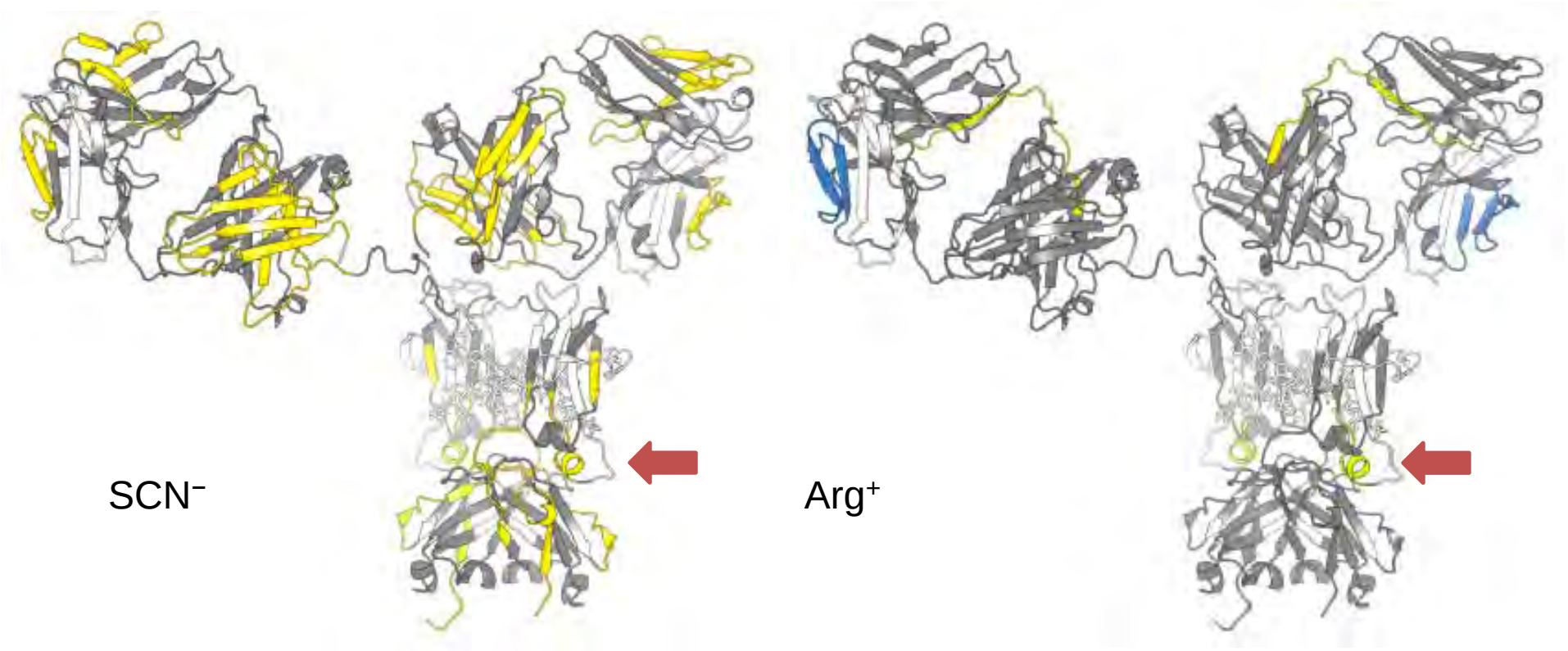




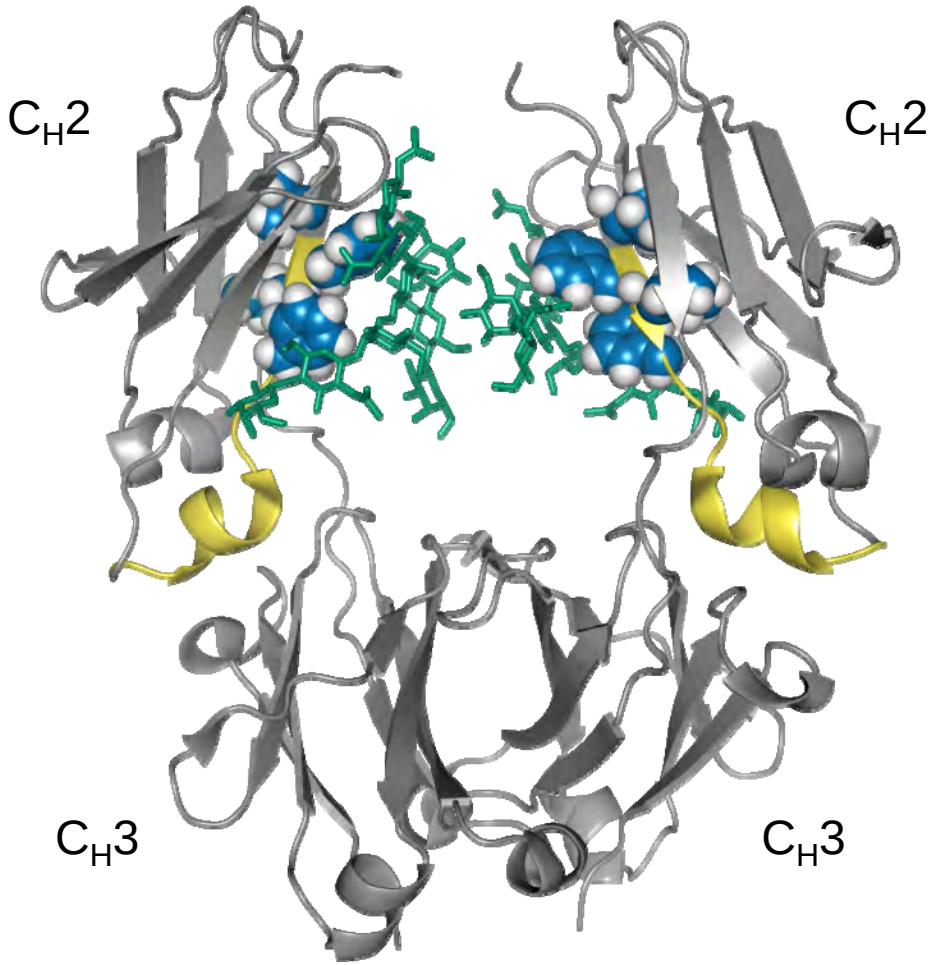




Destabilizers have very different effects.



A hydrophobic segment of the C_H2 domain may mediate aggregation.



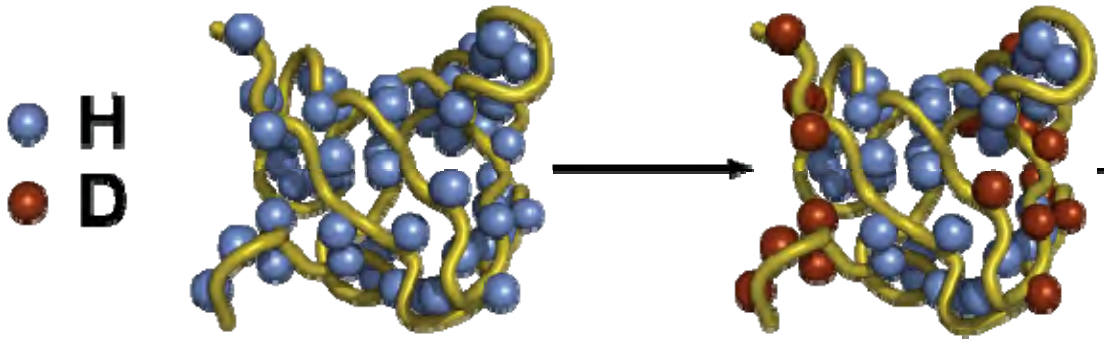
VFLFPPKPDTLMI

Destabilizers and oxidation increased backbone flexibility. [Houde, 2010]

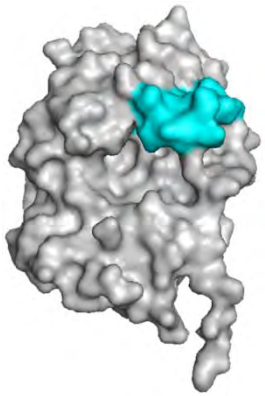
Protein A binding inhibits aggregation. [Zhang, 2012]

Disulfide bond increased thermal stability. [Gong, 2009]

Summary

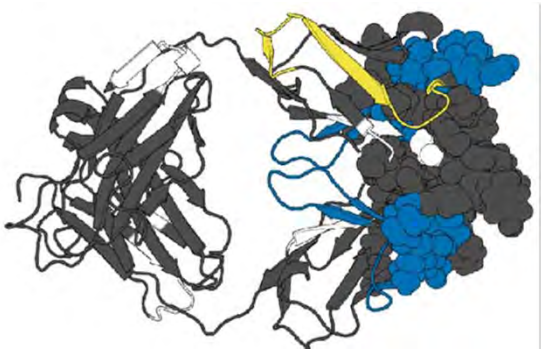


Epitope mapping



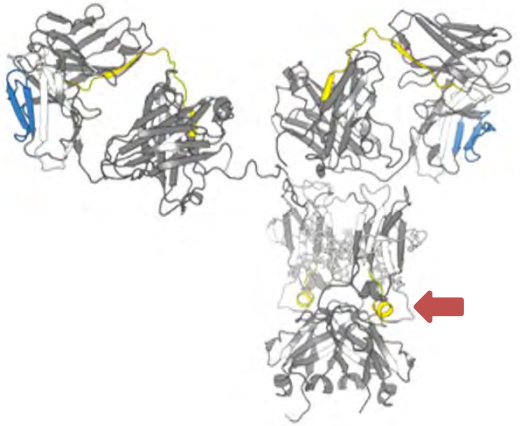
ricin antibody

Protein interactions

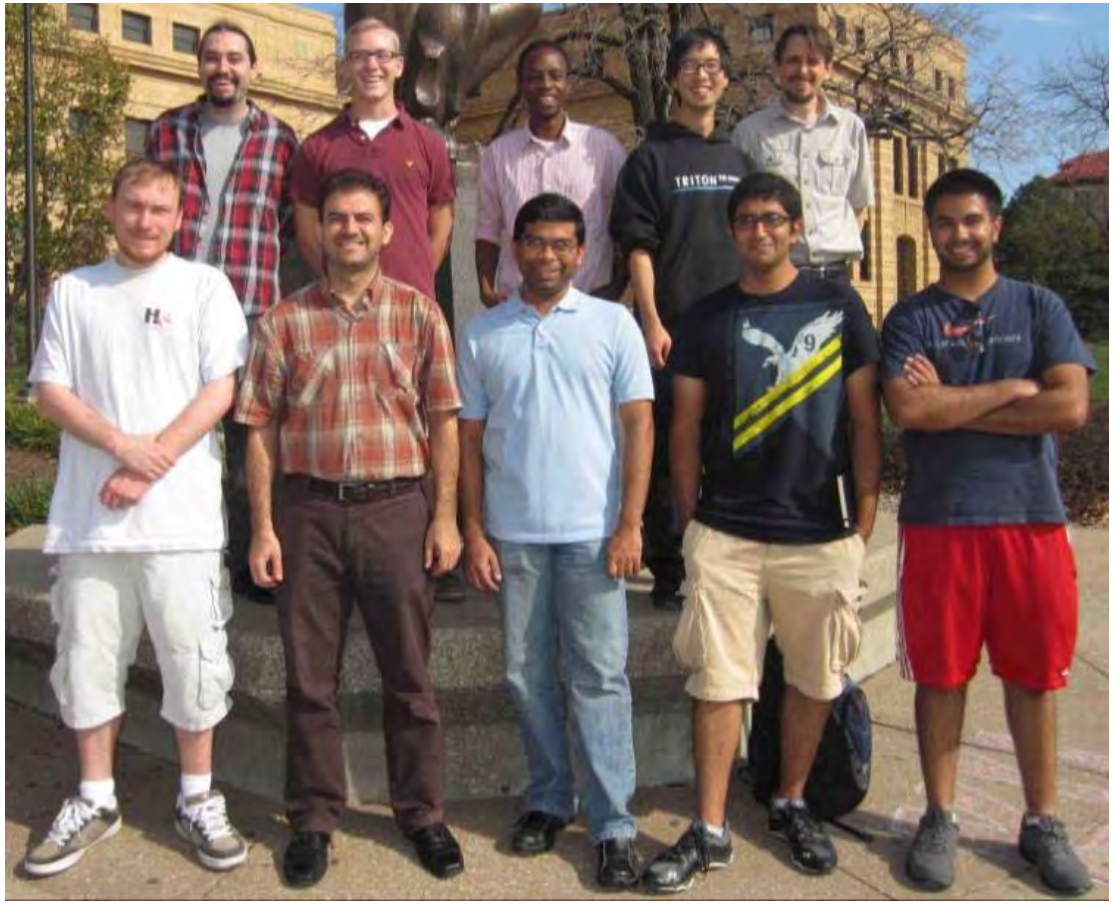


mAb self-association

Formulations



aggregation hotspots



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