Enabling rapid liquid and freeze-dried formulation design for the manufacture and delivery of novel biopharmaceuticals

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Introduction

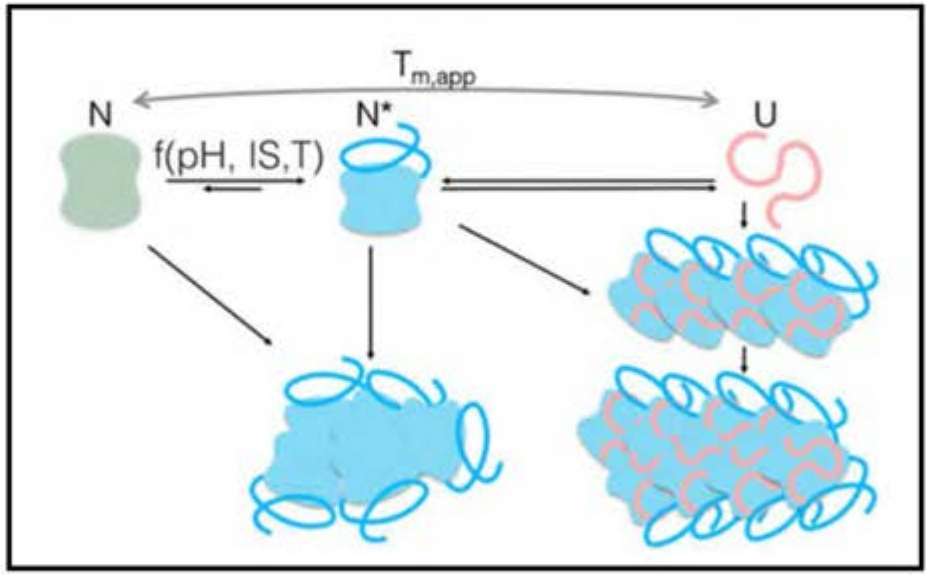
- Biological medicines account for >25% of all new drug approvals: 8 of the top 10 selling drugs
- Biopharmaceuticals market is rapidly growing with reported sales of £197 billion in 2016 (compared with total drug market of £816 billion)
- Next generation therapies are increasingly complex and engineered for biological activity at the expense of physical and chemical stability (eg protein fusions, fragments, conjugates with small drug molecules)
- Formulation development of biopharmaceuticals:

1. Dosage formulations fixed quickly - in time for clinical trials. Not much material is available. Shelf life over 2 years not known until mid-way through clinical trials.
2. Formulations require stability, potency, and ease of delivery to patient
3. Many therapeutics require high concentrations which leads to increased physical degradation, poor rheological properties, and phase separation

Protein aggregation

- Predicting and controlling aggregation is an outstanding challenge:

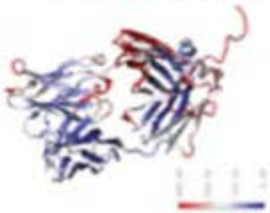
1. Key intermediates are transient, have low populations, and are difficult to isolate / study
2. Multiple mechanisms for aggregation, depend on protein and environment (solvent properties, temperature)



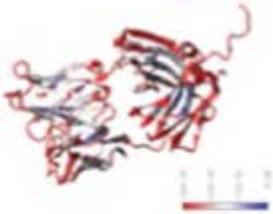
- Rapid experimental screens are too indirect:

1. Unfolding temperature or free energy, colloidal stability (eg aggregation temperatures and protein-protein interaction measurements)
2. Accelerated (eg. high T) aggregation assumes Arhenius-type extrapolations

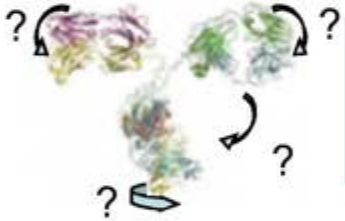
Aim1: Understand factors affecting aggregation in formulation



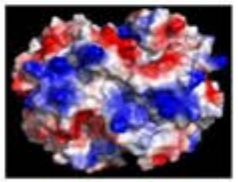
Local Dynamics / Partial unfolding



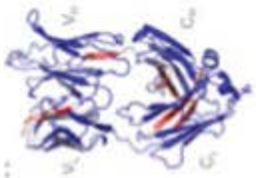
Global Conformational Stability



3° / 4° conformation



Surface properties: charge/ hydrophobicity



Sequence features eg. APRs

Aggregation

Fragmentation

Solubility

Excipient crowding

Excipient binding

Viscosity & diffusion

Aim2: How can we predict better formulations?

Do conformational and colloidal stabilities correlate to aggregation rates?

Does forced degradation at high temperature predict shelf-life?

Can alternative methods be developed for predicting aggregation rates?

Aim3: How can we engineer based on predictions?

Can we engineer lower aggregation rates?

Can we develop novel (GRAS-based) excipients?

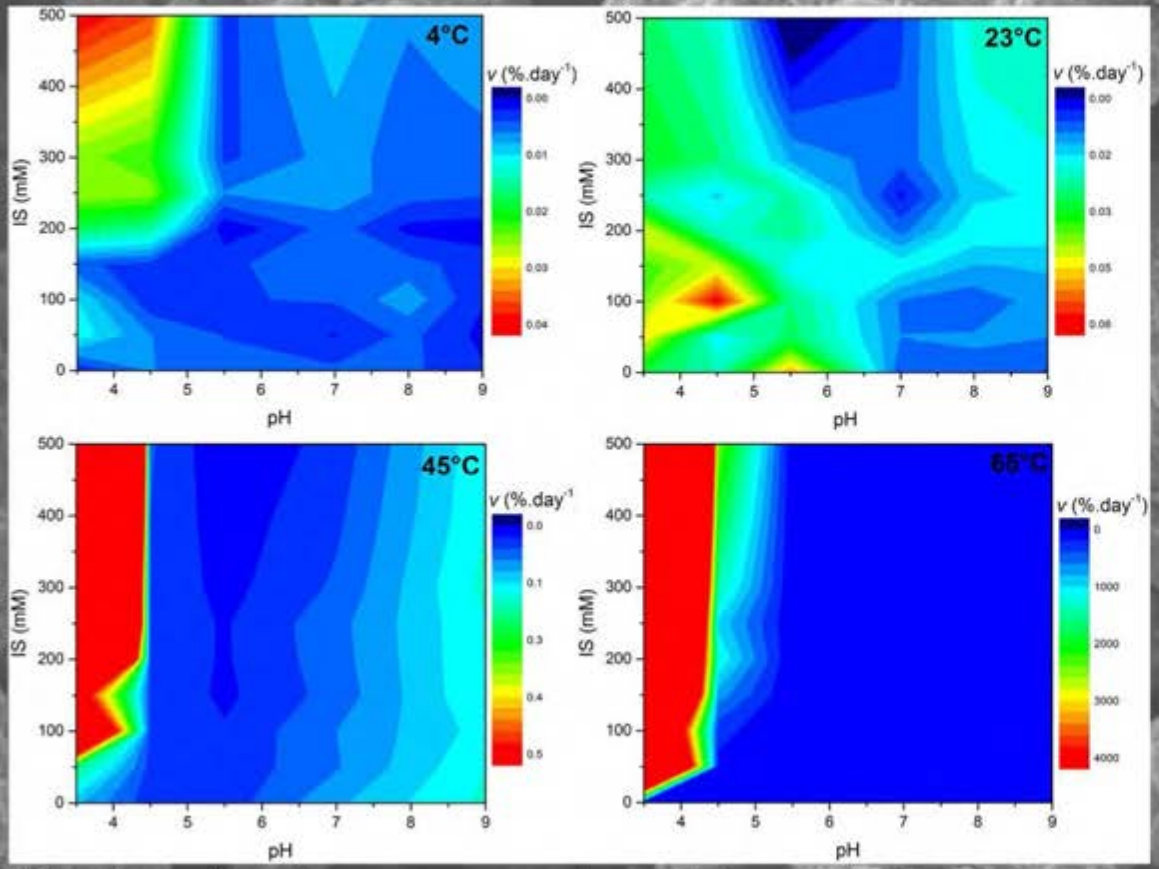
Objectives

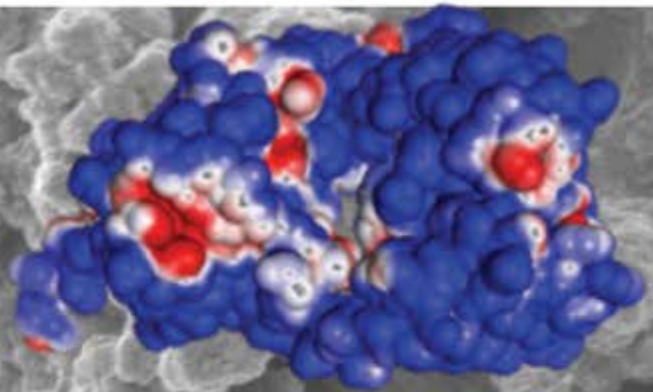
O1. Use high-throughput automation to generate a large experimental formulation dataset for protein:excipient combinations, that will include aggregation kinetics, conformational stability, colloidal stability, phase behaviour, and rheology measurements.

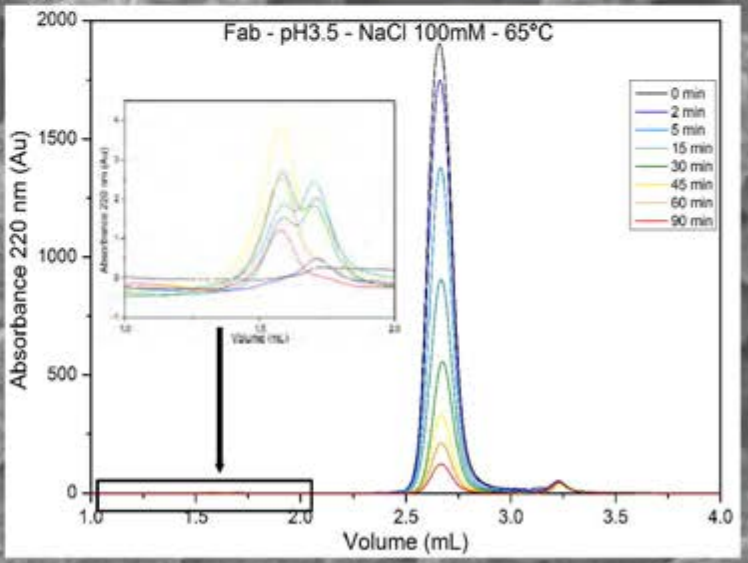
O2. Molecular informatics and modelling will improve predictability of formulation attributes and excipient effects

O3. Analytical advances will enable earlier, more sensitive, and lower-volume assessments of formulated protein degradation kinetics.

Heat maps of Fab aggregation kinetics at 4-65 °C







- Kinetics of native monomer loss determined for >1 year
- Range of pH, incubation T, and ionic strength

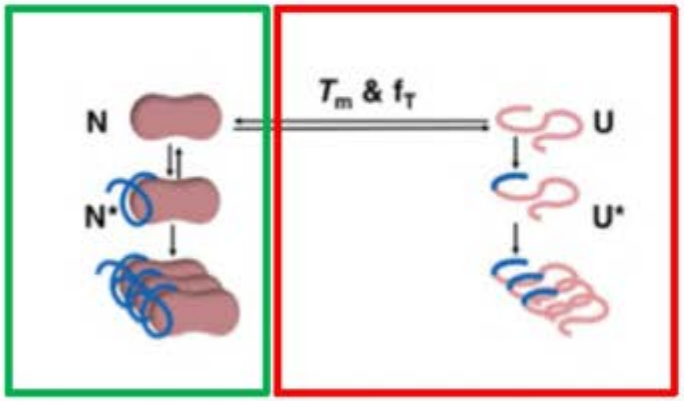
Nesrine Chakroun, David Hilton, Shahina S. Ahmad, Geoffrey W. Platt and Paul A. Dalby (2016) Molecular Pharmaceutics

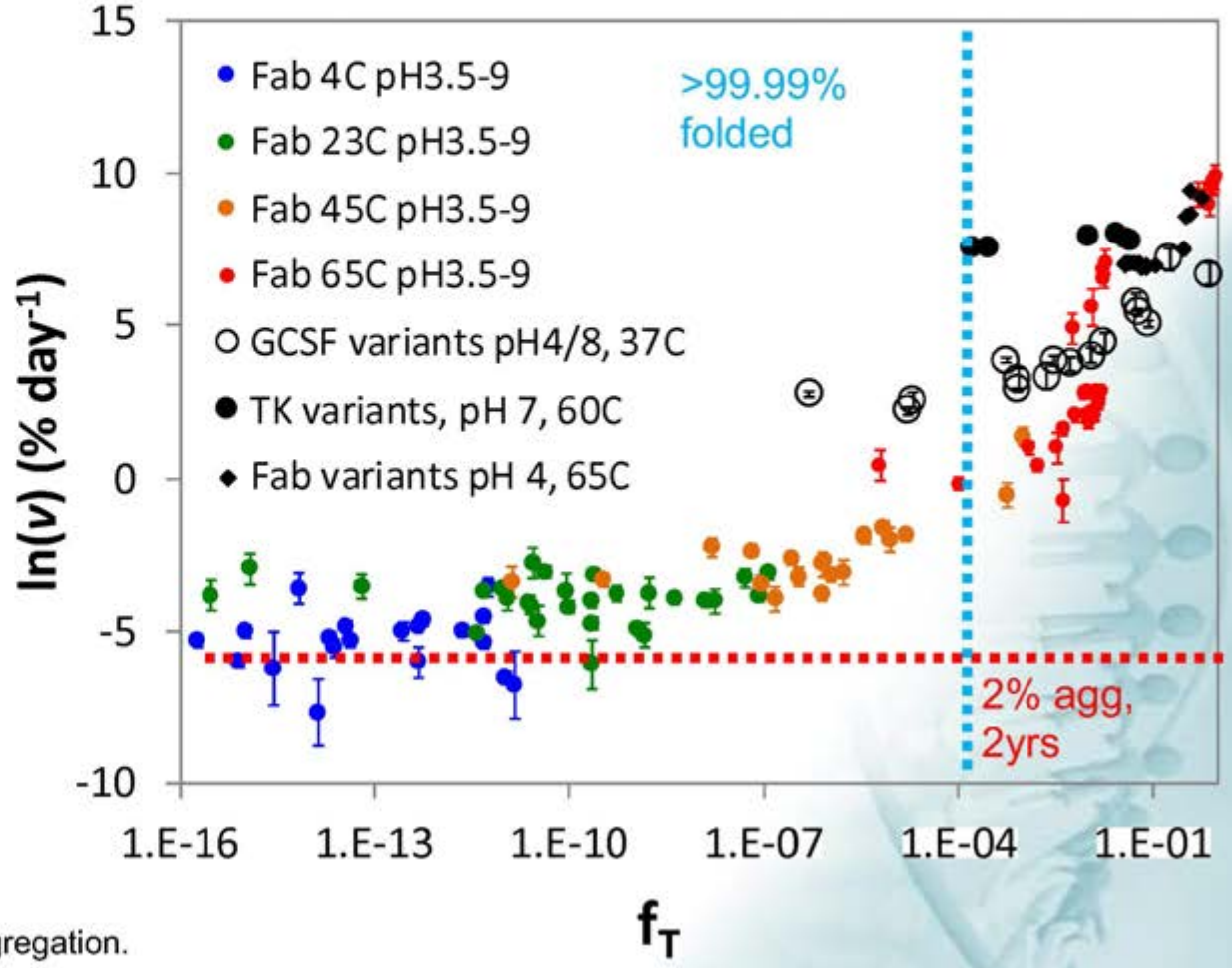
Kinetics at low T_{inc} often dont correlate with melting temperature

Where T_{inc} << T_m, fraction unfolded is <<0.0001:

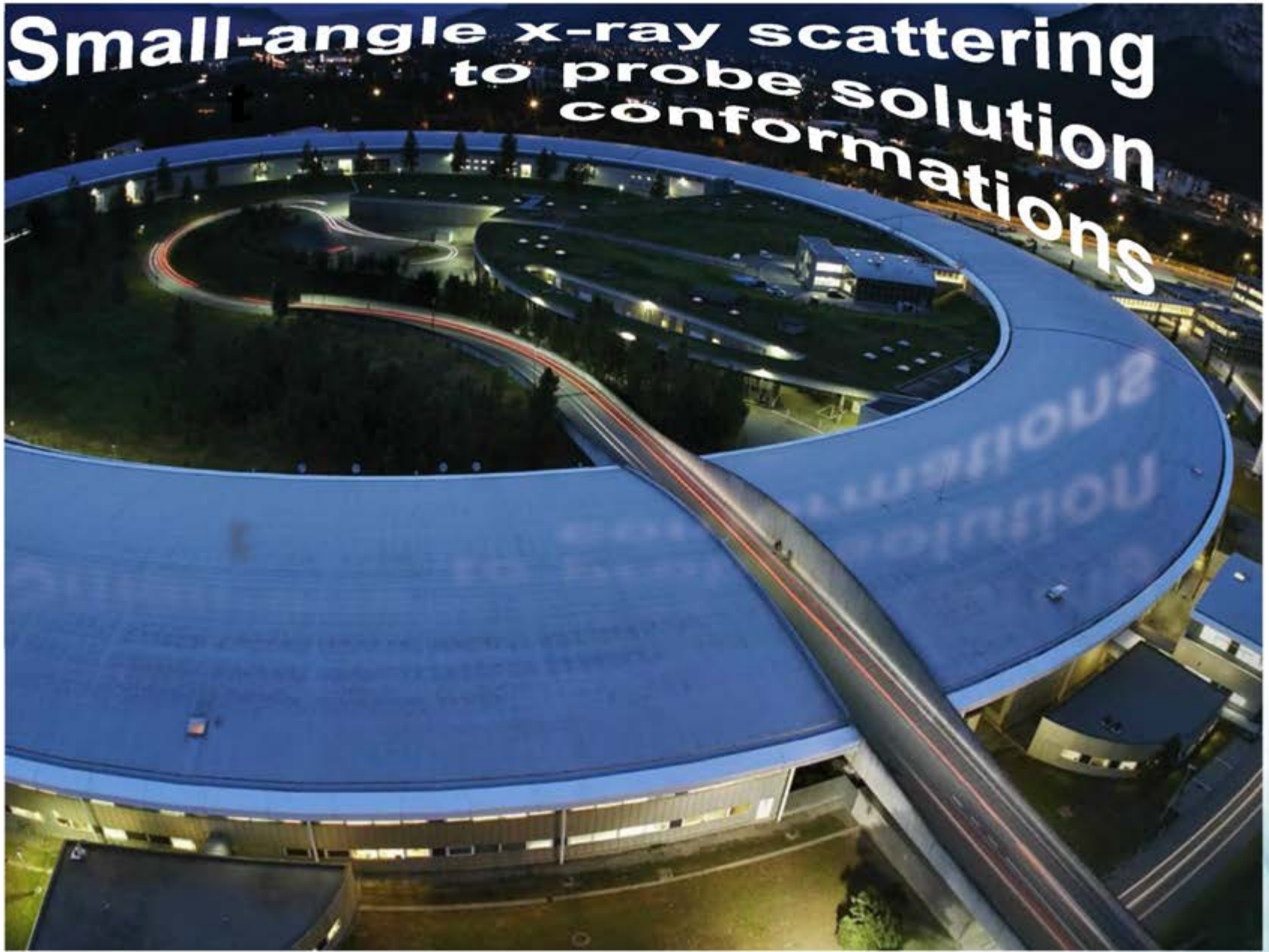
Global unfolding (and hence T_m) is not relevant

Native ensemble dynamics & colloidal stability control aggregation kinetics.

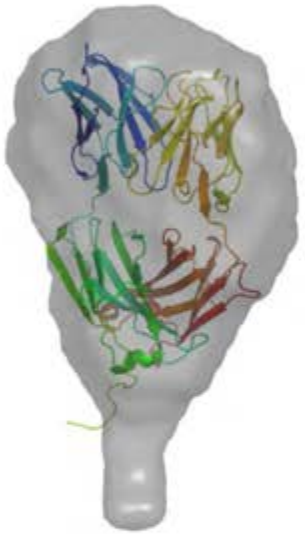




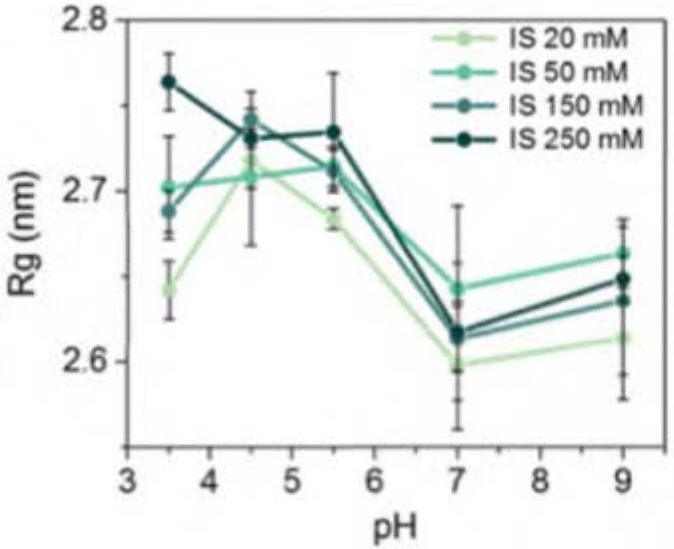
Zhang et al. (2018) Molecular Pharmaceutics. 15, 3079-3092
Robinson et al. (2018) Molecular Pharmaceutics. 15, 256-267.
Chakroun et al. (2016) Molecular Pharmaceutics. 13, 307-319.
See also Roberts (2013) – review on non-Arrhenius protein aggregation.


Small-angle x-ray scattering
to probe solution
conformations

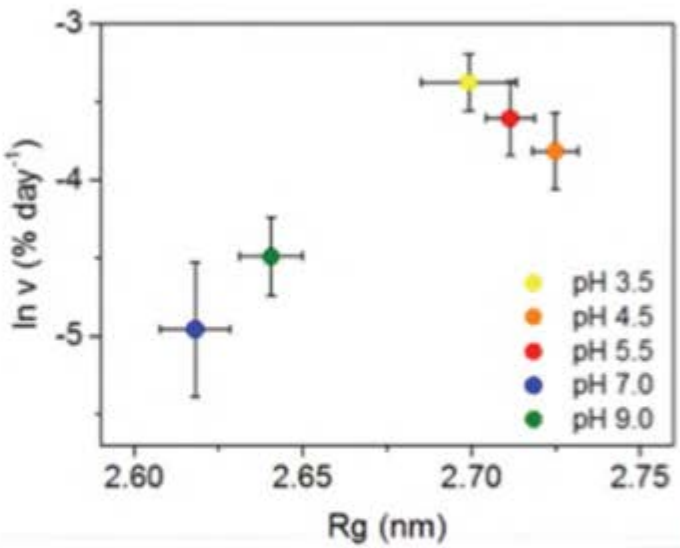
Small-angle x-ray scattering of Fab under native conditions



pH-dependent conformation



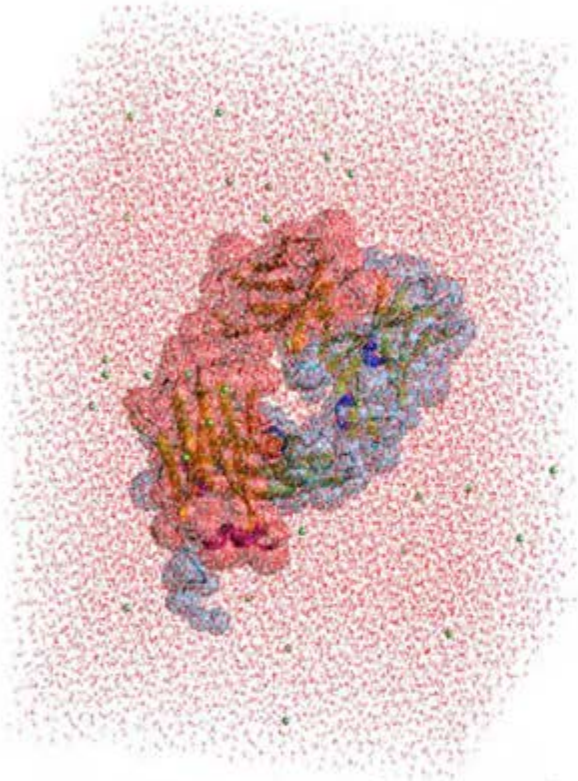
Aggregation kinetics correlate

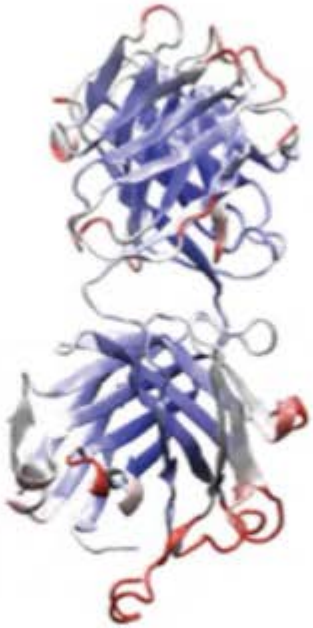


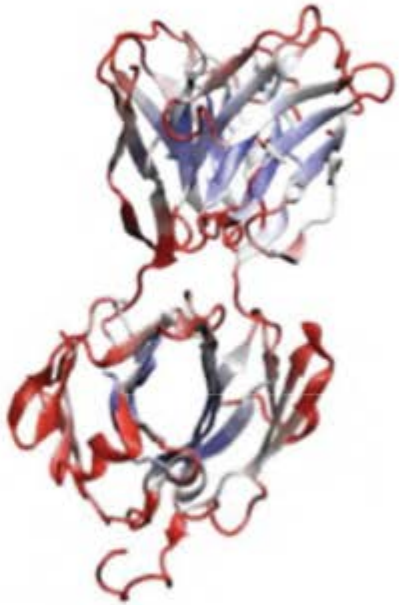
- Conformational change with pH correlates with aggregation kinetics, at 23 °C

Codina at al. 2019 *JMB*

Molecular dynamics simulation for Fab







Equilibrium RMSF (300K)
- pH7, 25°C, 50ns, 50mM IS
- pH3.5, 25°C, 50ns, 50 mM IS
- OPLS-AA/L force field & SPC/E water
- Triplicated

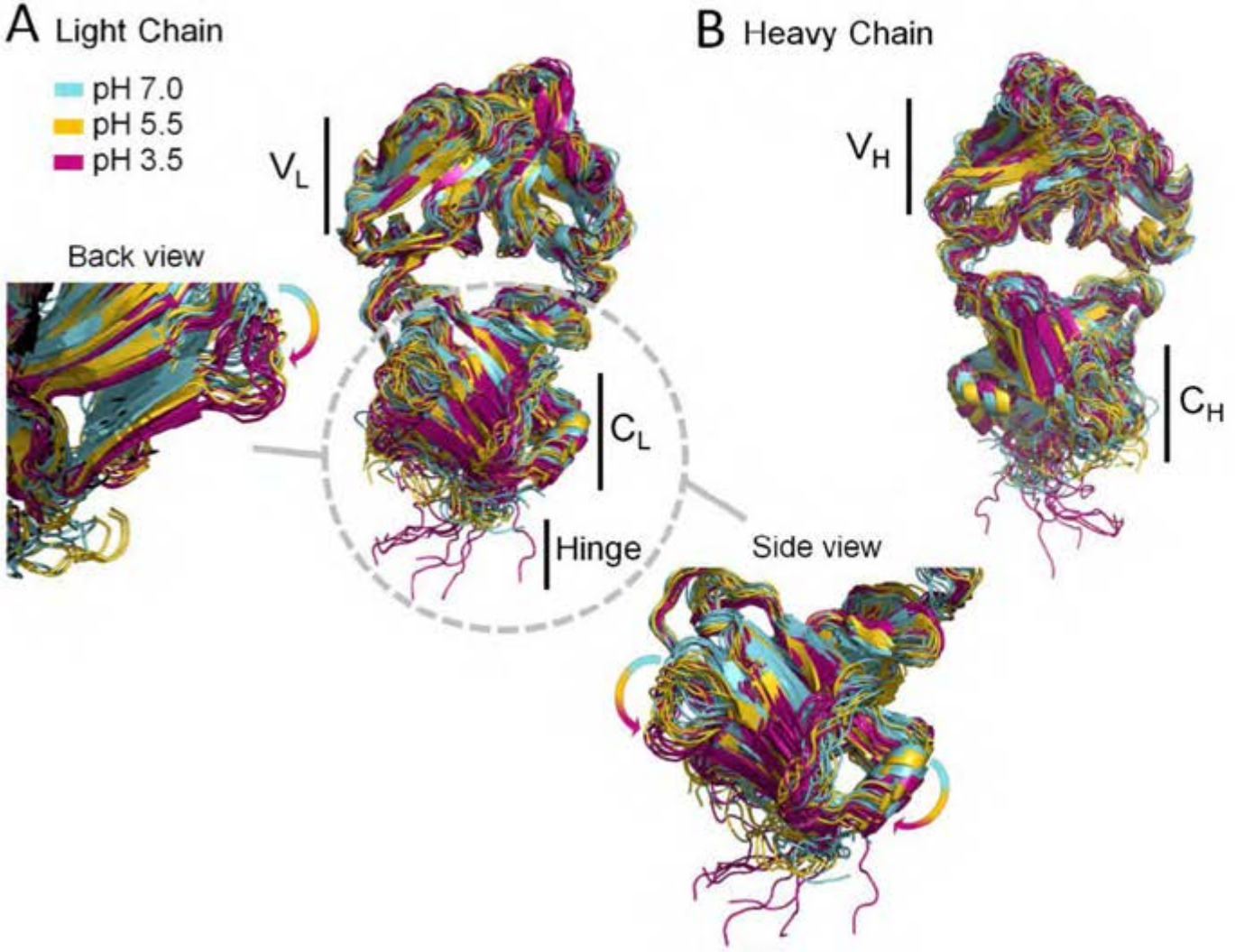
pH 7

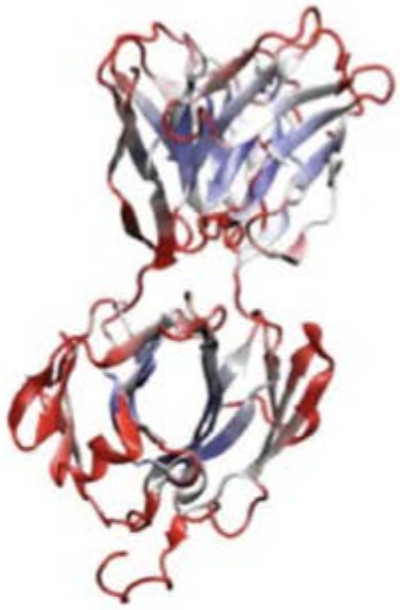
pH 3.5

CL domain displacement

Codina at al. 2019 *JMB*

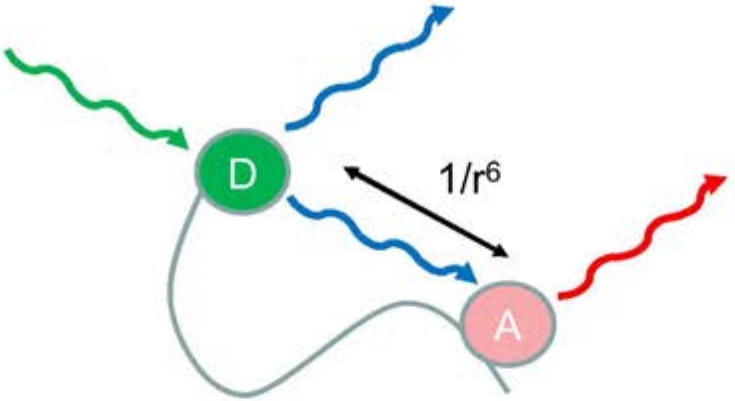
Which molecular dynamics simulation structures explain SAXS?





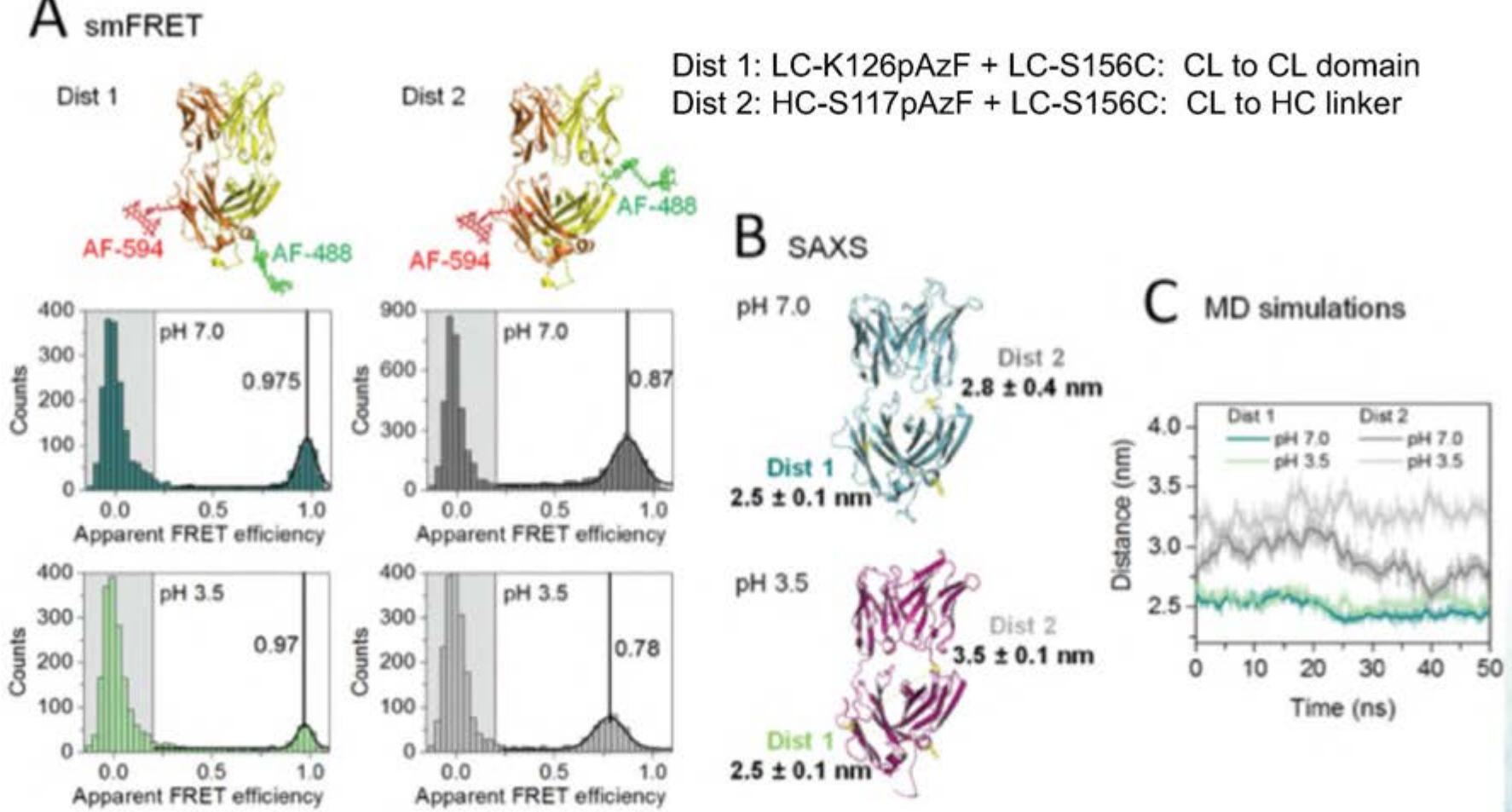
CL domain displacement

Codina at al. 2019 *JMB*



Single-molecule fluorescence

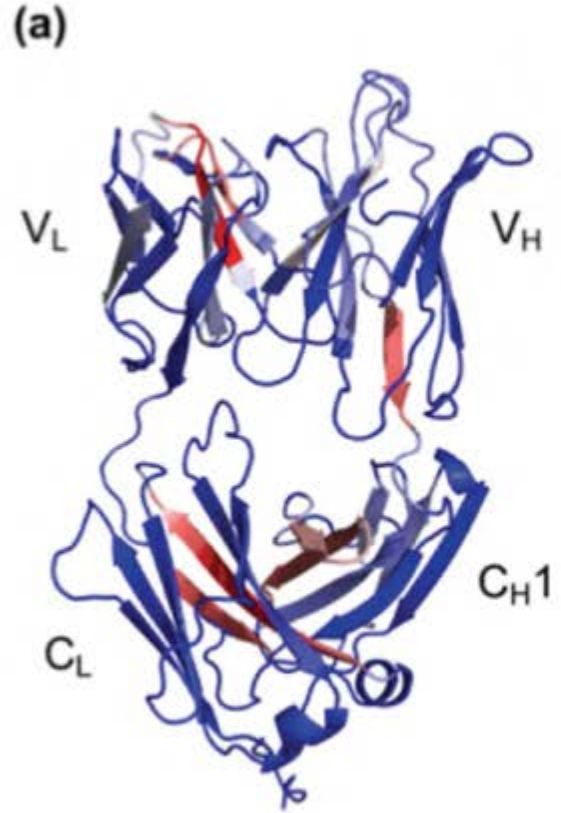
smFRET analysis of pH-dependent Fab conformations

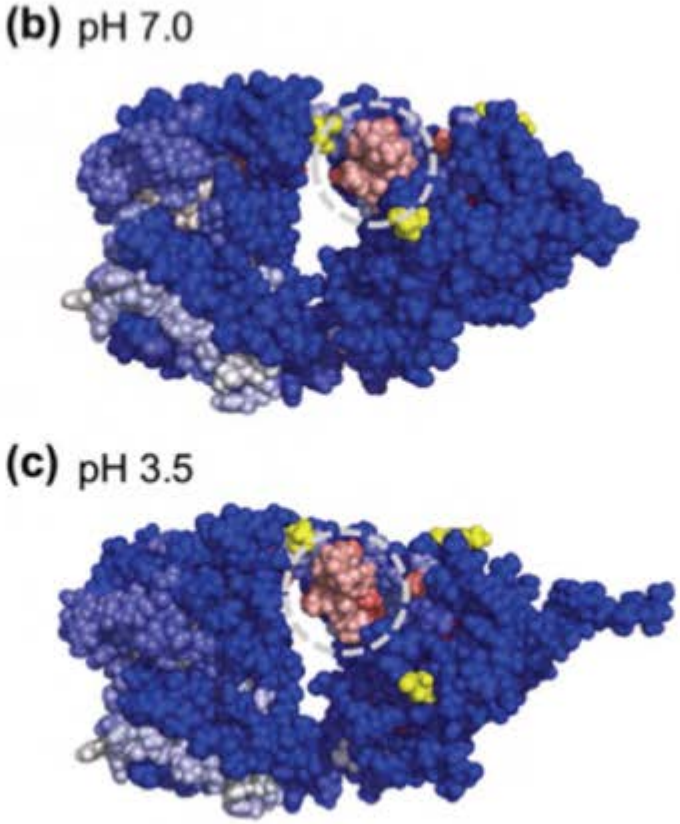


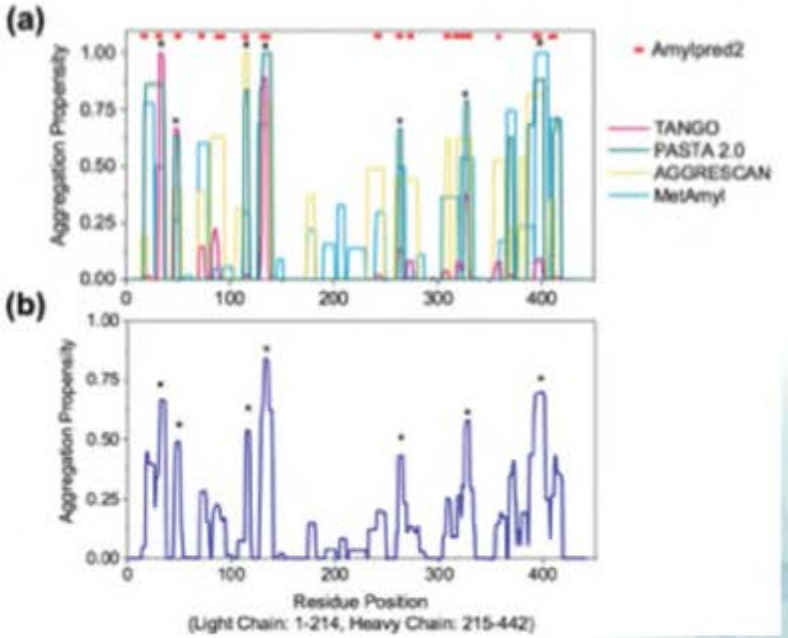
smFRET, SAXS and MD reveal the same dynamics and conformational shift with pH

Codina at al. 2019 *JMB*

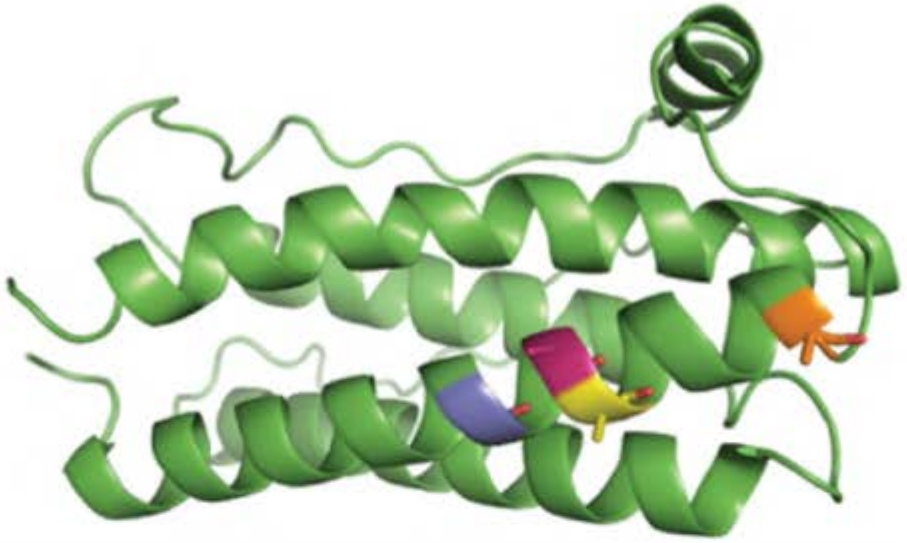
Best-fit SAXS structures reveal APR exposure at low pH



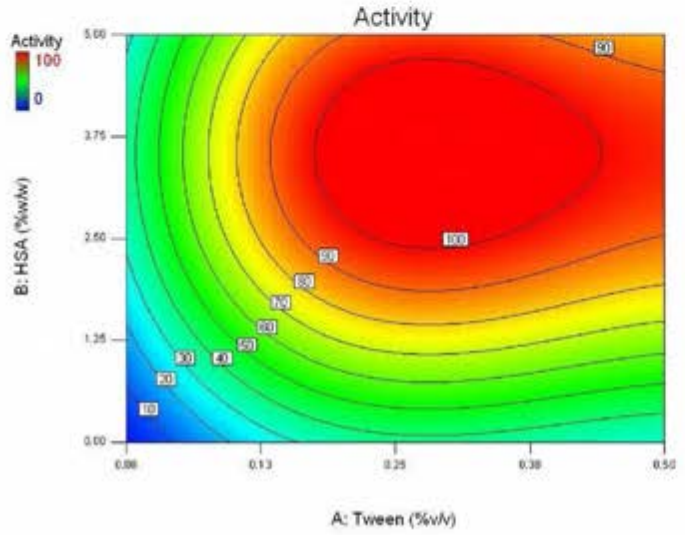


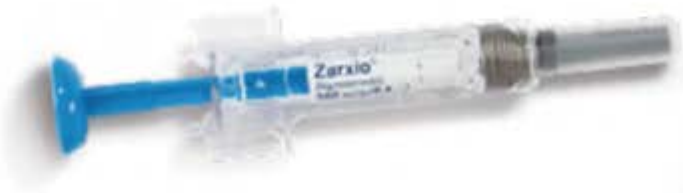


Codina at al. 2019 *JMB*



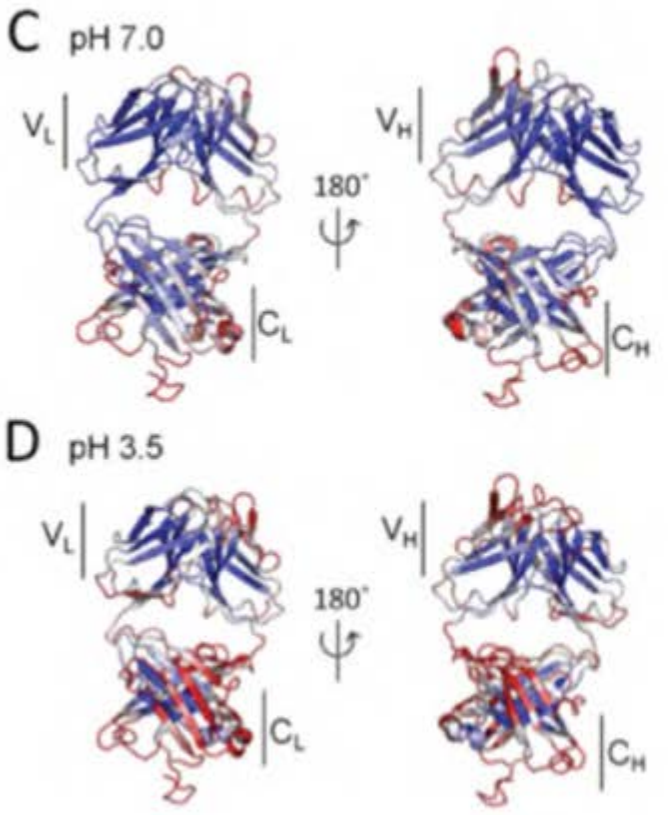
Protein engineering and formulation

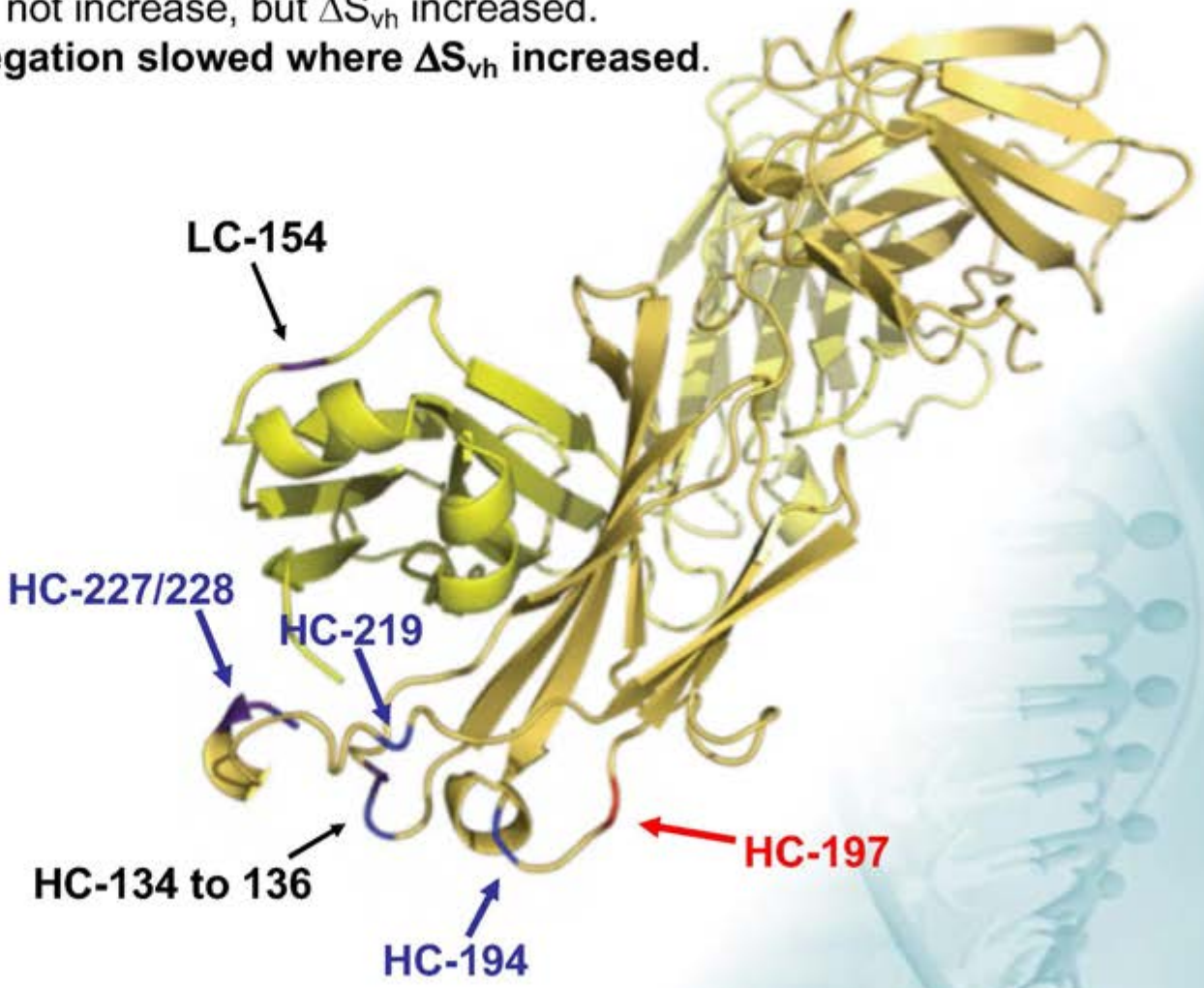




MD-guided protein engineering to slow aggregation

T_m did not increase, but ΔS_{vh} increased.
Aggregation slowed where ΔS_{vh} increased.

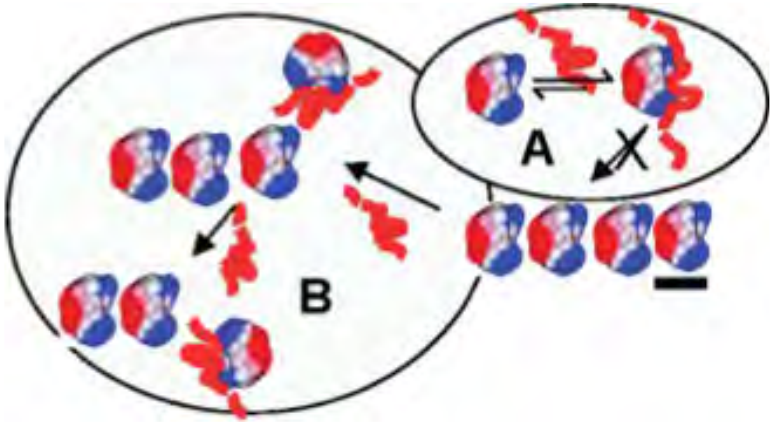




Designed 12 stabilising and 5 destabilising mutations using ROSETTA at flexible sites.

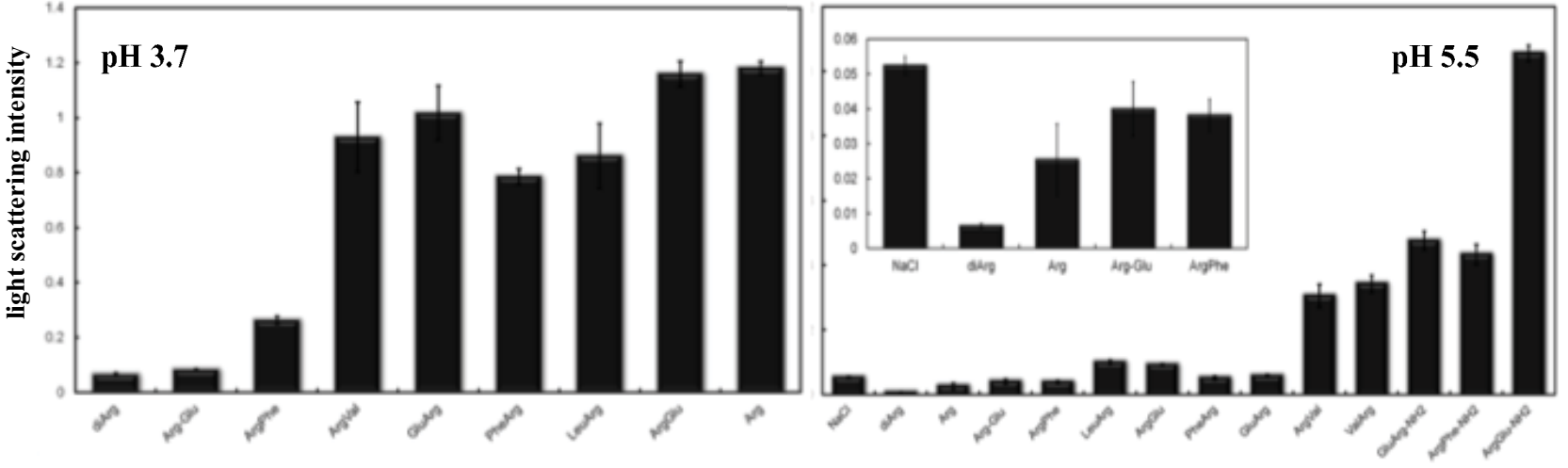
Zhang et al. (2018) Computational-design to reduce conformational flexibility and aggregation rates of an antibody Fab fragment. *Molecular Pharmaceutics*. 15, 3079-3092

Dipeptides as novel excipients



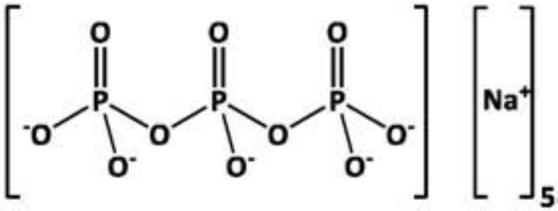
- *At isoelectric pH, diArg is most effective at reducing insulin self association versus all other additives reflecting ability to neutralize electrostatic attraction.*

- *At pH 3.7, diArg, ArgPhe and mixtures of Arg and Glu equally effective at neutralizing hydrophobic interactions between insulin molecules*

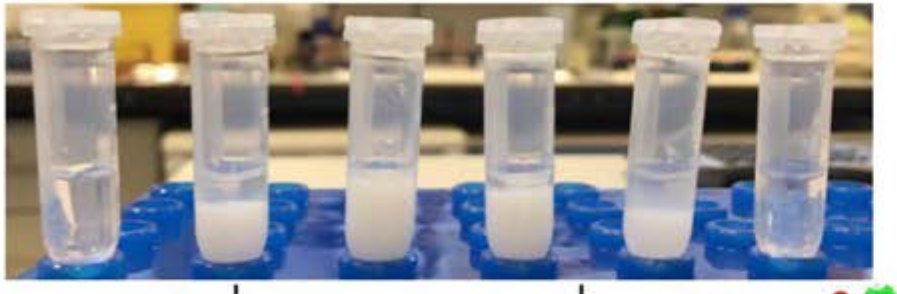


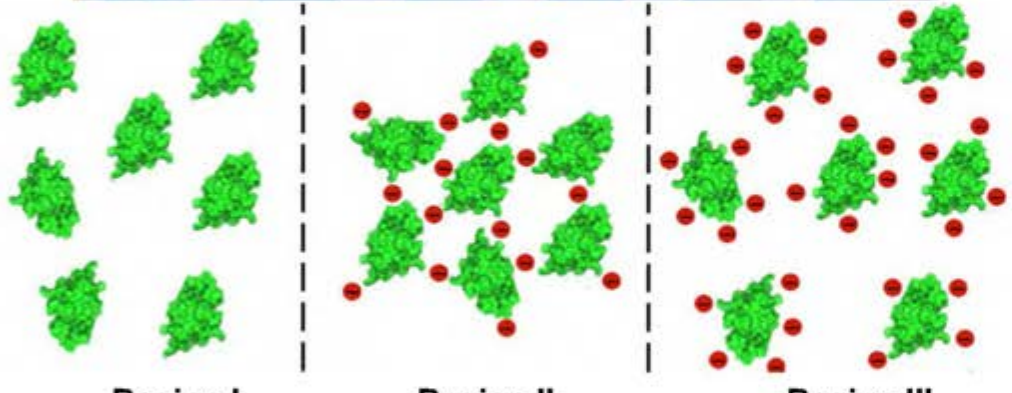
STPP for tuning protein phase behaviour

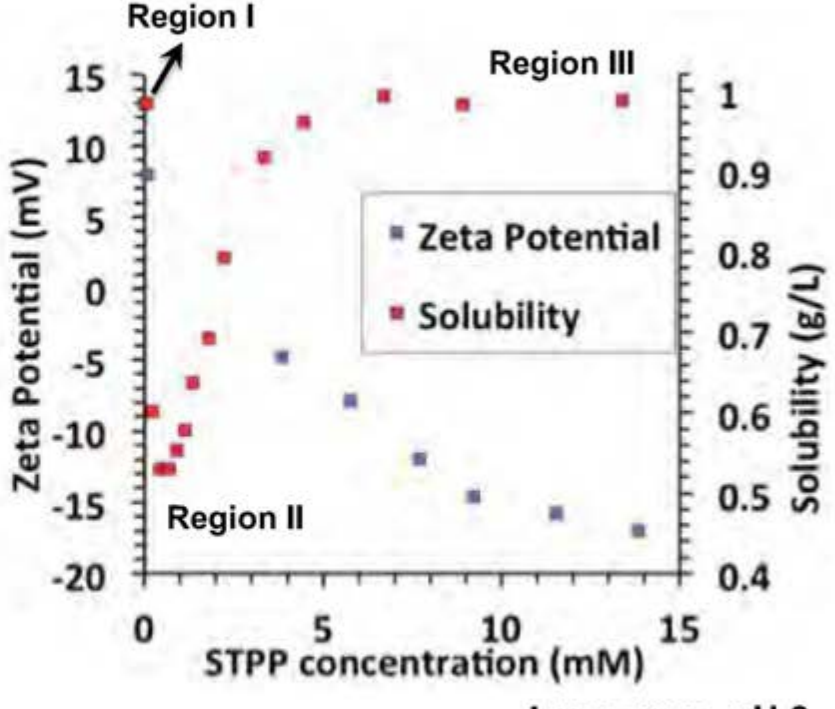
sodium tripolyphosphate (STPP)



Increasing STPP concentration



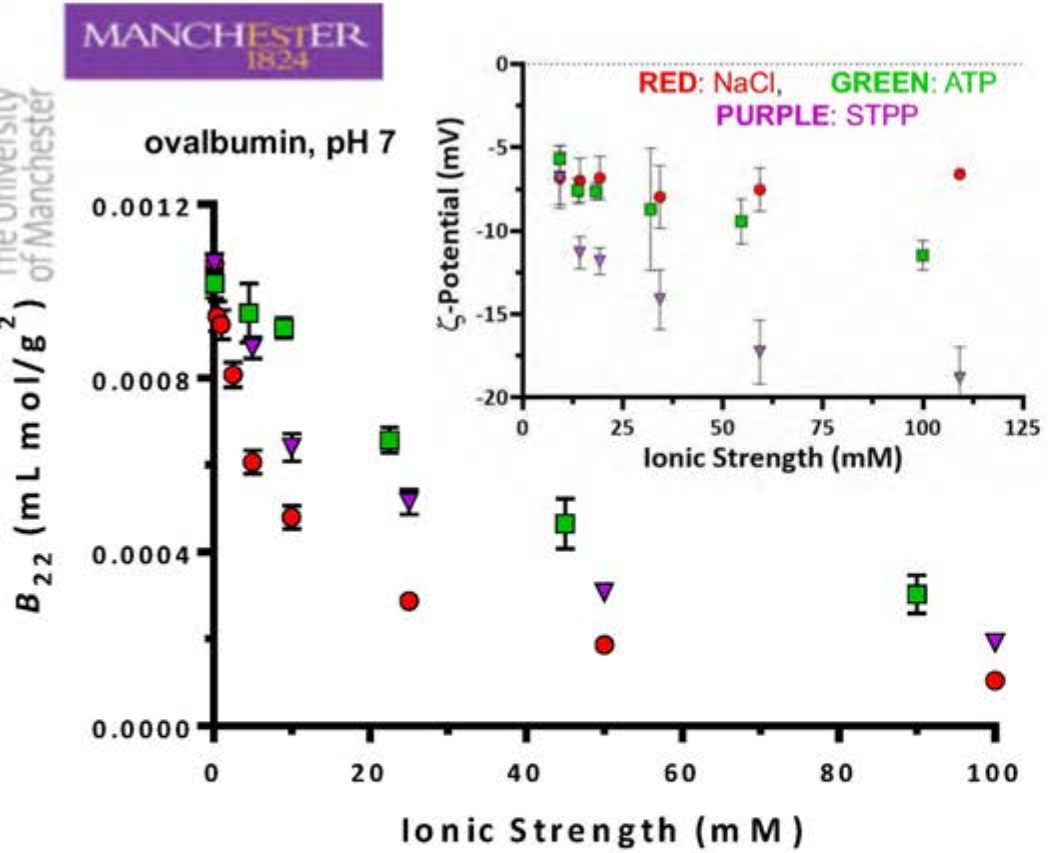




Lysozyme, pH 8

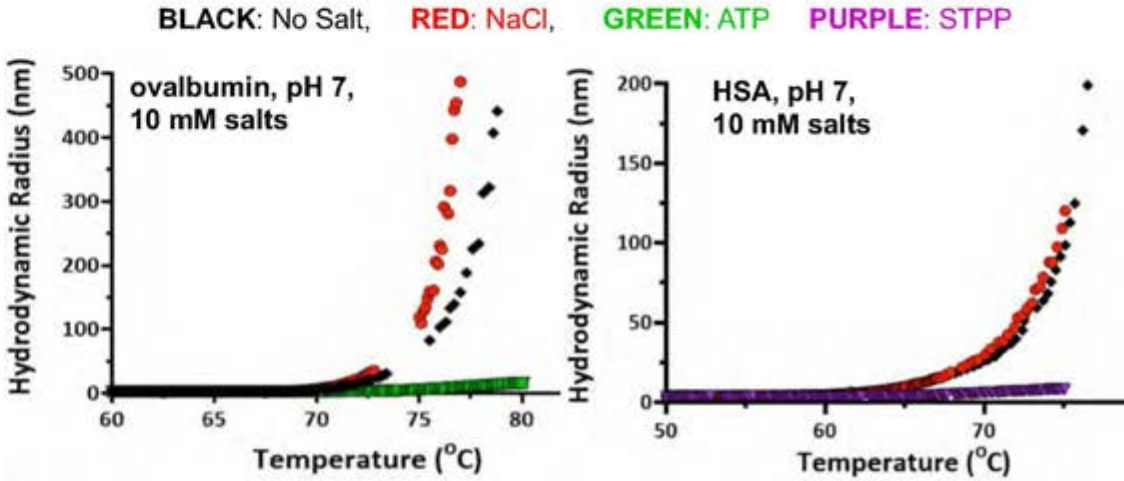
- Reentrant condensation phenomena observed with positively charged proteins and STPP (or ATP)
- Initial [STPP] causes protein precipitation through forming ion bridges across proteins
- Resolubilization at higher [STPP] due to overcharging protein
- STPP can direct formation of reversible colloidal gels with glassy dynamics for formulating proteins

ATP and STPP prevent protein aggregate growth



- Multivalent anions ATP and TPP supercharge negatively charged proteins through ion binding
- Supercharging protein with ATP or TPP increases protein-protein repulsion and colloidal stability as reflected by increase in B_{22} values at fixed IS

- ATP and TPP prevent thermal-induced aggregation of negatively charged proteins.
- Stabilization is due to reduction in aggregate growth rates through electrostatic stabilization

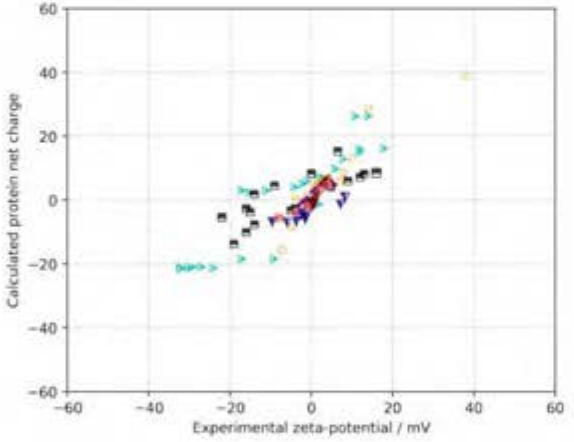


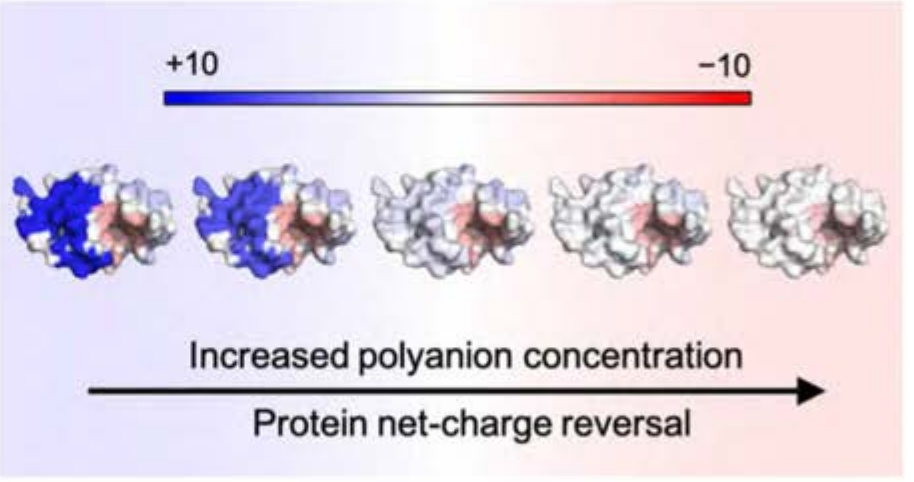
Polyvalent anion binding and solubility

A model (not a web tool), following on from Bye & Curtis:

Controlling Phase Separation of Lysozyme with Polyvalent Anions

Jordan W. Bye and Robin A. Curtis*





model for anion binding fits measured zeta potl

model applied to human proteins suggests that over-charging and potential solubilisation is general

Bye and Curtis (2019) J Phys Chem B 123:593; Kalayan et al (2020) Mol Pharm 17:595.

Solubility prediction server

Sequence Prediction

The protein-sol software will take a single amino acid sequence and return the result of a set of solubility prediction calculations, compared to a solubility database.

Please enter a single sequence of single letter amino acid codes in the FASTA format.

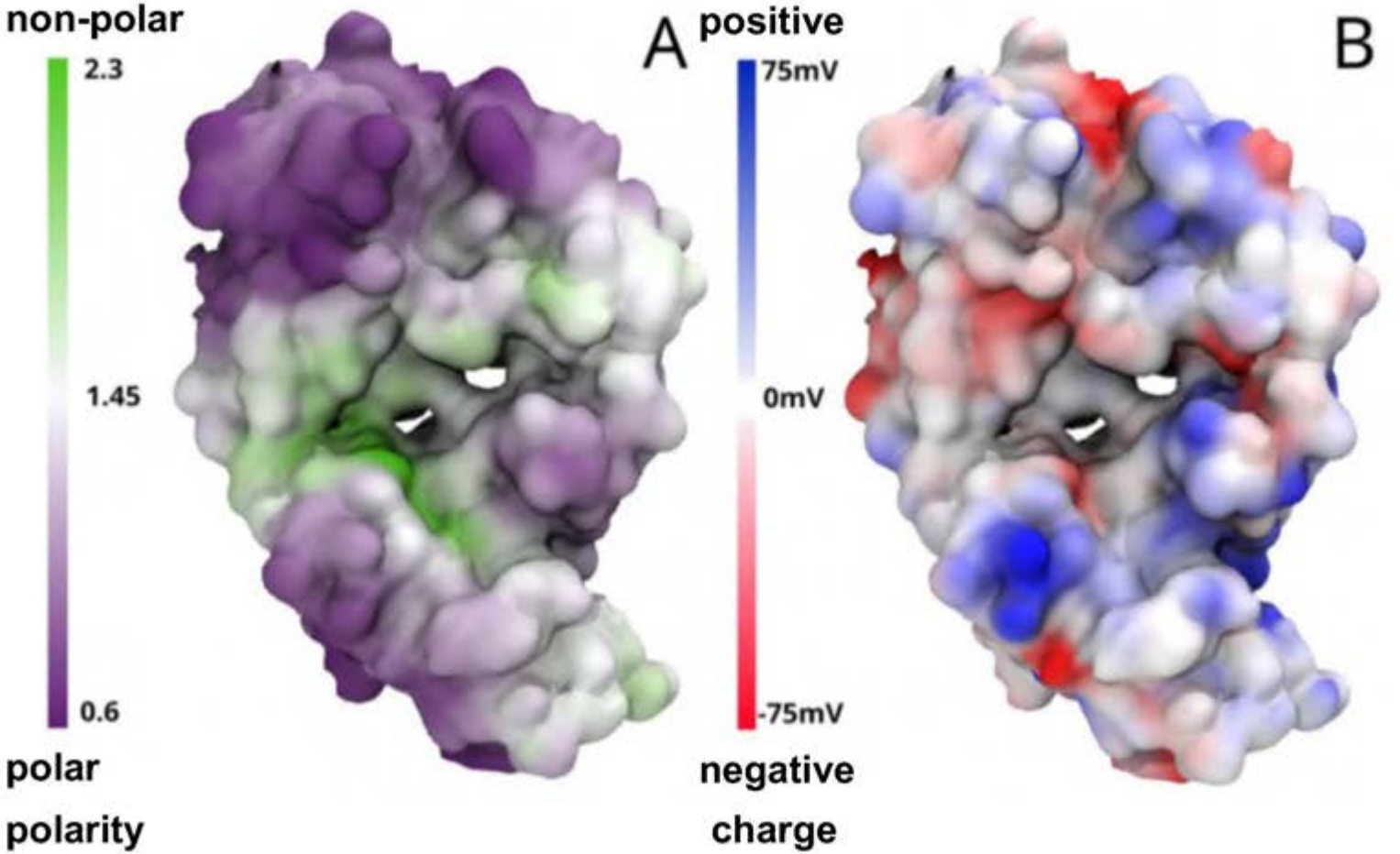
For example

```
> P00547
MVKVYAPASSANMSVGFDVLGAAVTPVDGALLGDVVTVEAAETFSLNNLGRFADKLPSEPRENIVYQCWERFCQELGKQI
PVAMTLEKNMPIGSGLGSSACSVVAALMAMNEHCGKPLNDTRLLALMGELEGRISGSIHYDNVAPCFLGGMQLMIEENDI
```

Originally (2017): Sequence-based solublity prediction, based *E. coli* data
Currently (2020), also: Patches, Heatmap, Abpred, pKa
In development (2020): Excipient predictions (for next meeting)

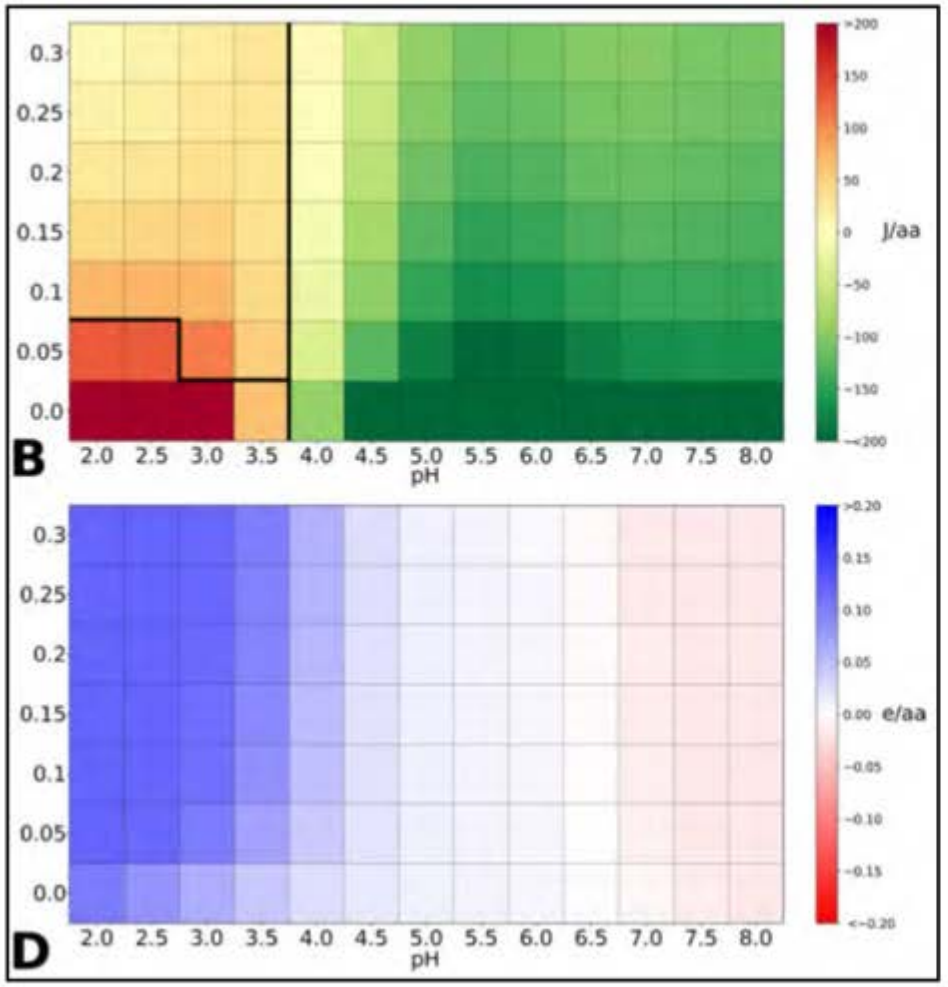
(2017) Bioinformatics (2017) 33:3098; protein-sol.manchester.ac.uk; PNAS (2009) 106:4201

Patches: with Fab fragment



Hebditch and Warwicker (2019) Sci Rep 9:1969

Heatmap: pH and ionic strength



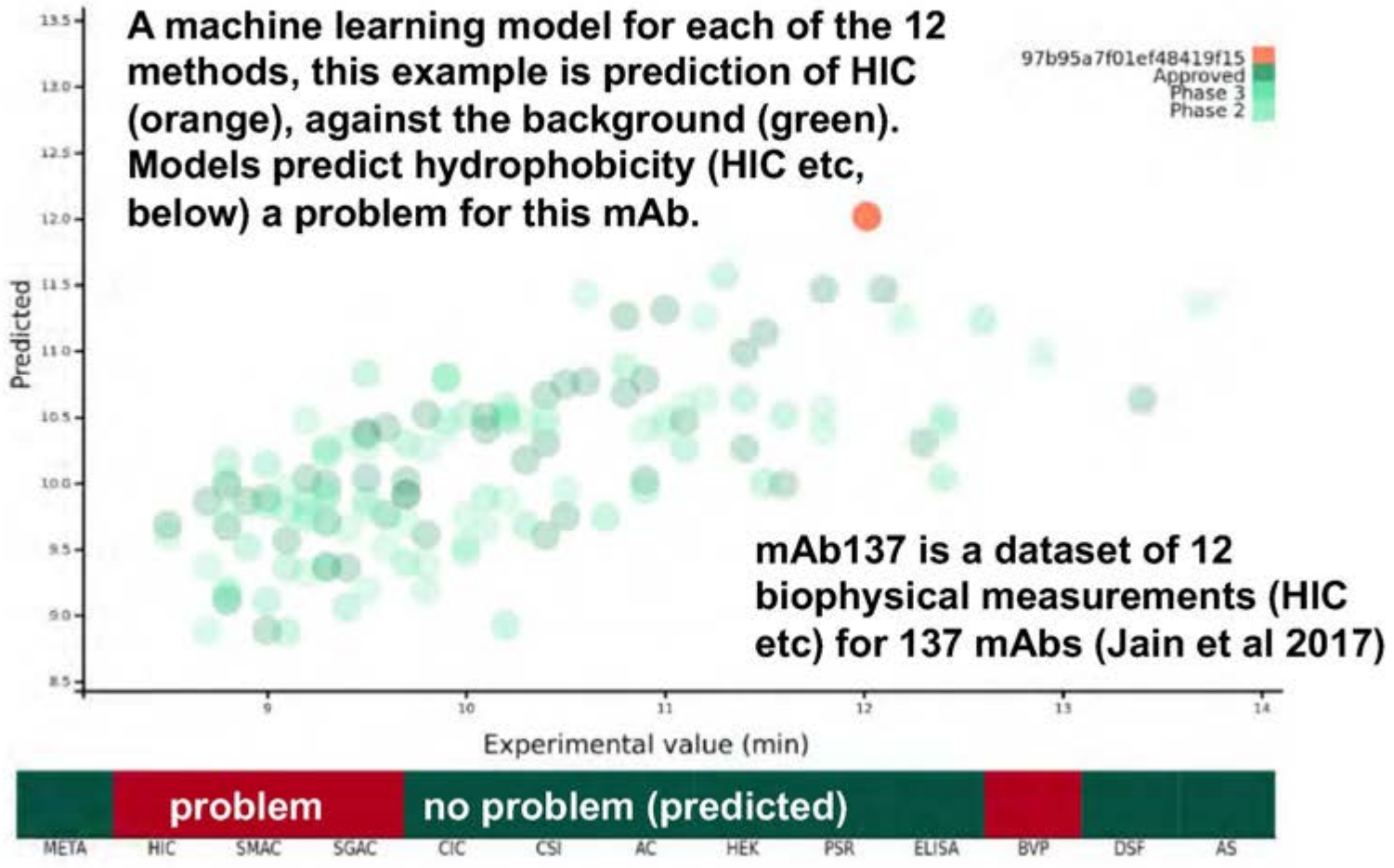
Predicted pH and ionic strength dependence of folded state stability (upper panel), and net charge (lower panel).

Upper panel shows 'phase diagram' fit to experimental data.

Method can be used to identify regions that lower stability in conditions such as a low pH of bioprocessing.
These regions can then be engineered out, e.g. switching Asp/ Glu for Asn/Gln.

Hebditch and Warwicker (2019) Sci Rep 9:1969; Hebditch et al (2020) Comp Struct Biol J 18:897

AbPred: ML models from mAb137



Hebditch and Warwicker (2019) PeerJ 7:e8199 ; Jain et al (2017) PNAS: 114:944.

Take-home messages

Aim1: Understand factors affecting aggregation in formulation

- local dynamics/unfolding
- exposure of aggregation hotspots (APRs)
- colloidal stability (net charge)
- Optimize formulations to increase T_m, decrease dynamics and increase net charge

Aim2: How can we predict better formulations?

- Local dynamics and aggregation hotspots can be predicted computationally
- Excipient interactions can be predicted by molecular docking or LCMS
- Net charge and effect of charged excipient binding can be predicted computationally
- see webserver at **protein-sol.manchester.ac.uk**

Aim3: How can we engineer based on predictions?

- Mutations that suppress dynamics sometimes decrease aggregation kinetics
- Novel (GRAS-based) excipients based on dipeptides, and supercharging with STPP

Acknowledgements

UCL

MANCHESTER 1824
The University of Manchester

UG/PhD/PDRA:	where are they now?
Yuki Abe	- Biopharm Services
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Nesrine Chakroun	- Merus (NL)
Teresa Barata	- IPSEN
Cheng Zhang	**- PDRA UCL**
David Hilton	- GSK
Mathew Robinson	- Lonza
Victoria Wood	- GSK
Akash Pandya	**- PhD UCL**
Nuria Codina	**- NeoLeukin (Seattle, USA)**
Maariyah Samad	- O4 Research

Collaborators:	
SAXS & AUC	Steve Perkins (UCL)
GCSF	Paul Matejtschuk – NIBSC
Mass Spec	Milena Quaglia - LGC
Fabs	UCB Pharma & Darren Nesbeth (UCL)
IgGs	Lonza, UCB, MedImmune, FujiDSB
Molecular docking	Mire Zloh (Univ Herts / UCL)

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PDRAs on EPSRC Formulation project

- John Hales – UCL
- Cheng Zhang – UCL
- Samir Aoudjane - UCL
- Jordan Bye – University of Manchester
- Max Hebditch – University of Manchester

EPSRC CDT PhD students aligned to Formulation project

- Nuria Codina – UCL
- Akash Pandya - UCL
- Nikita Vekaria – University of Manchester
- Jas Kalayan – University of Manchester

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UKRI Biotechnology and Biological Sciences Research Council

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