SAXS to study biomolecular phase separation

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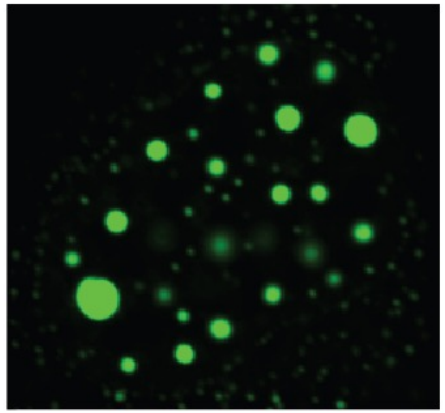
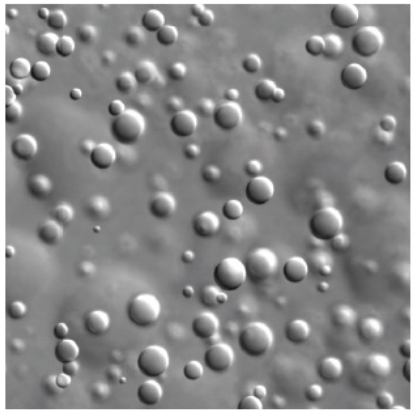
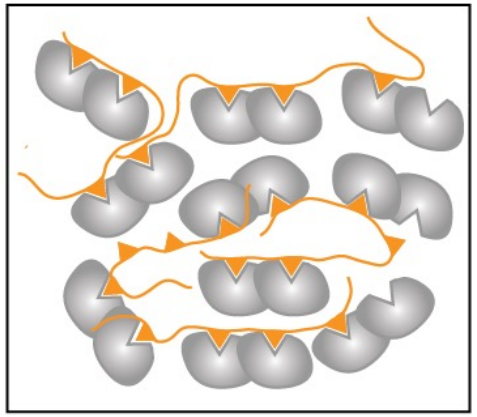
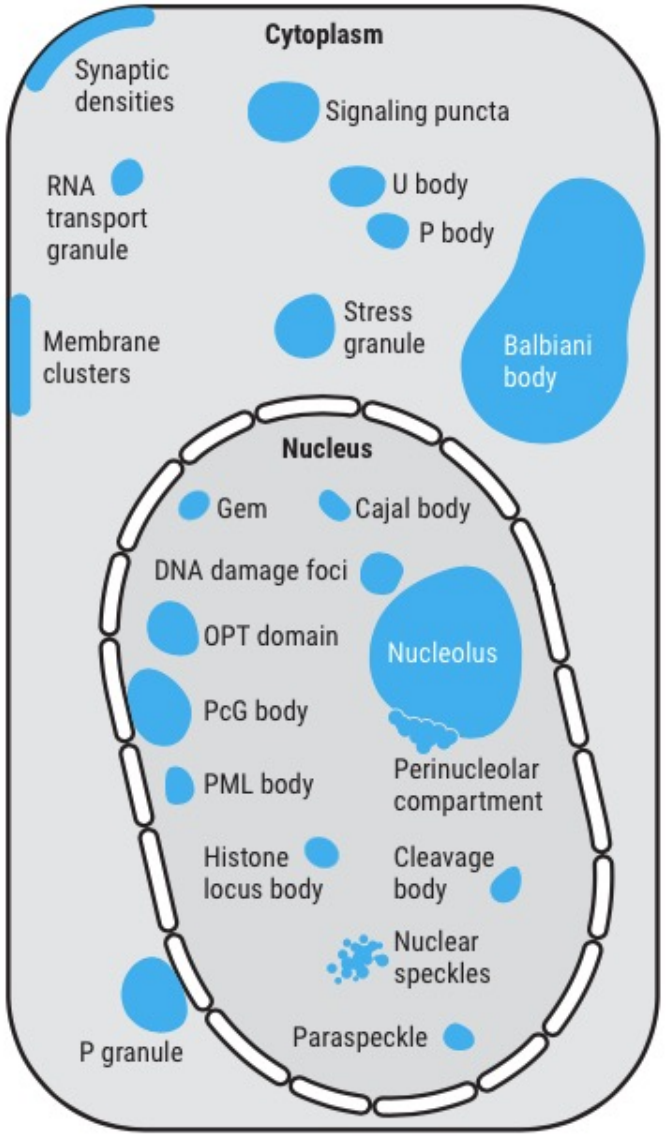


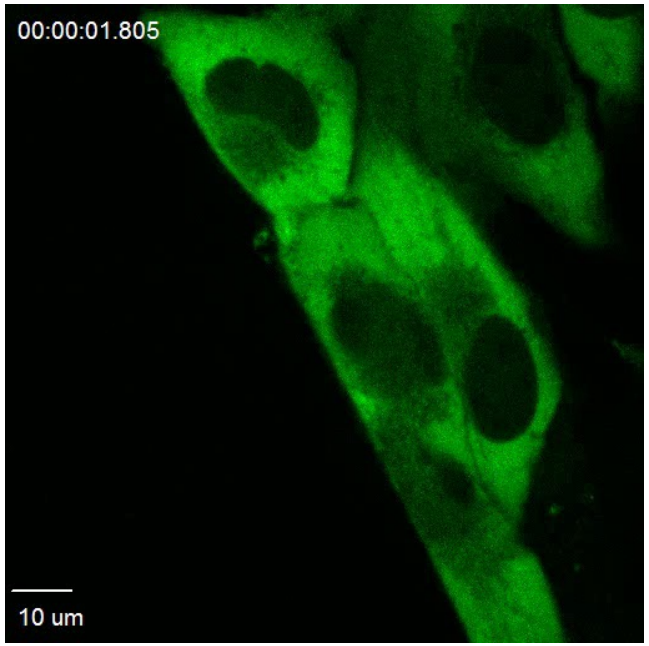


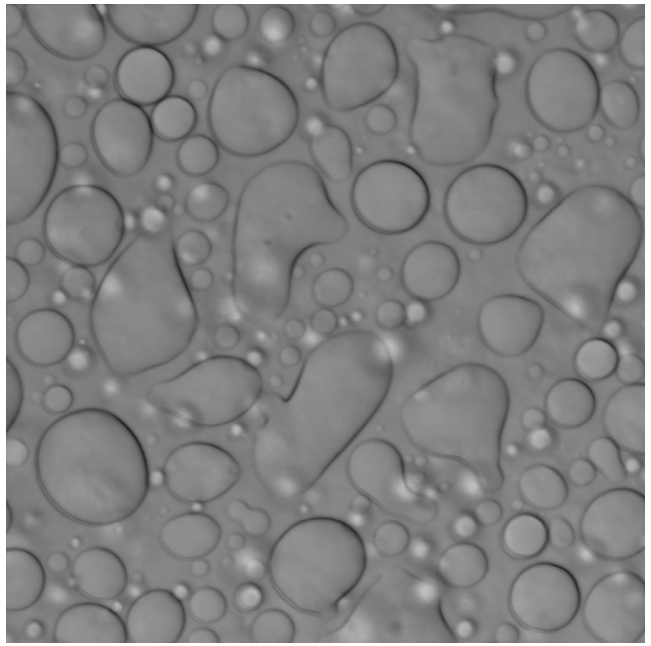


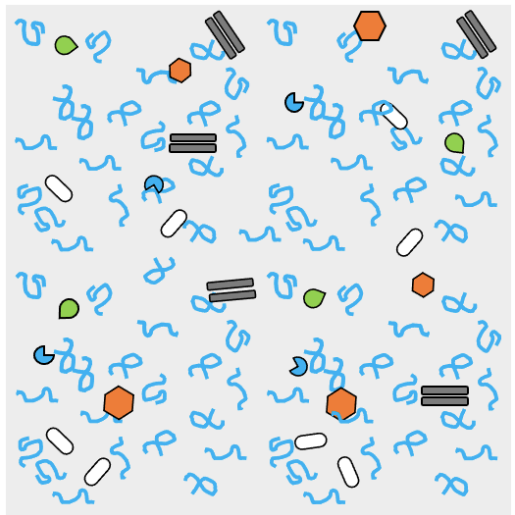
Phase separation mediates cellular compartmentalization



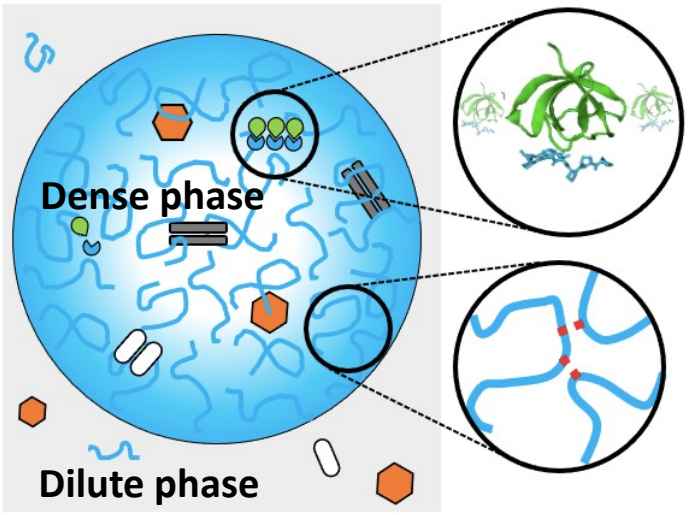
Dozens of biomolecular condensates







Phase separation



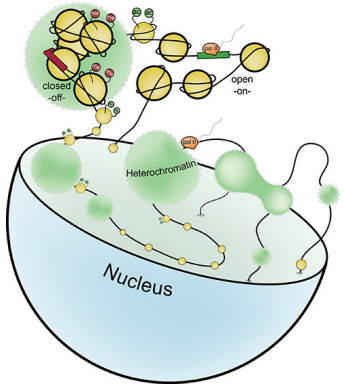
multivalent interactions

domain-motif

IDP-IDP

Phase separation governs critical processes throughout cells: Cell biology 2.0

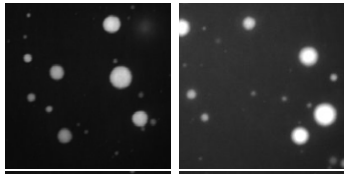
Heterochromatin

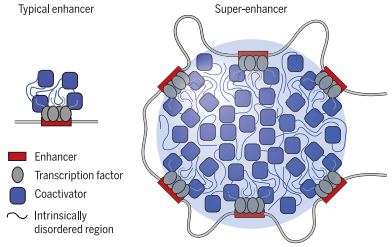


Liquid droplet formation by HP1α suggests a role for phase separation in heterochromatin.
Larson … Narlikar, *Nature* 2017
Phase separation drives heterochromatin domain formation.
Strom … Karpen. *Nature* 2017.

Super-enhancer function

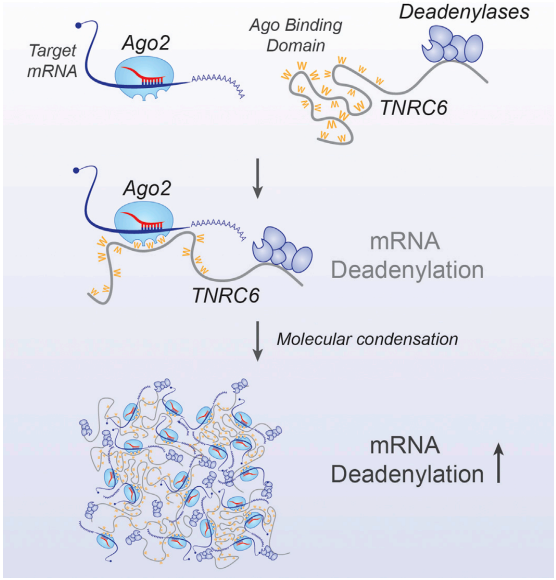
BRD4-IDR (10 μM) MED1-IDR (10 μM)





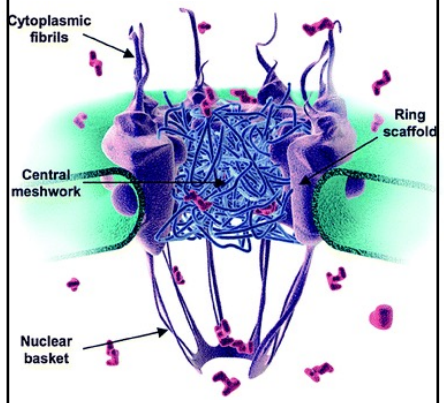
Coactivator condensation at super-enhancers links phase separation and gene control.
Sabari … Young. *Science* 2018

miRNA-mediated degradation



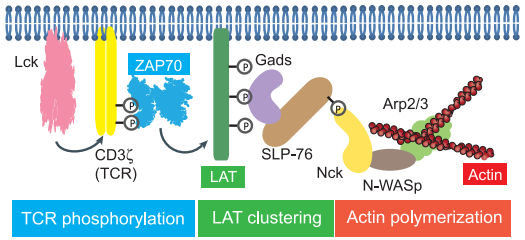
Phase Transitions in the Assembly and Function of Human miRISC
Sheu-Gruttadauria and MacRae. *Cell* 2018

Selectivity filter in NPC



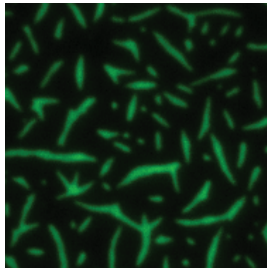
Nup98 FG domains from diverse species spontaneously phase-separate into particles with nuclear pore-like permselectivity.
Schmidt, Gorlich, *eLife* 2015

Membrane receptor clustering



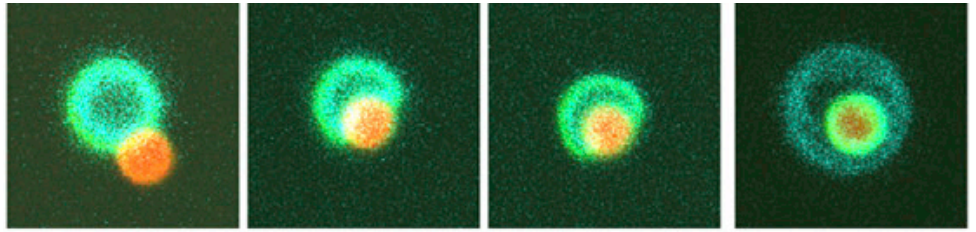
w/ Actin

LAT



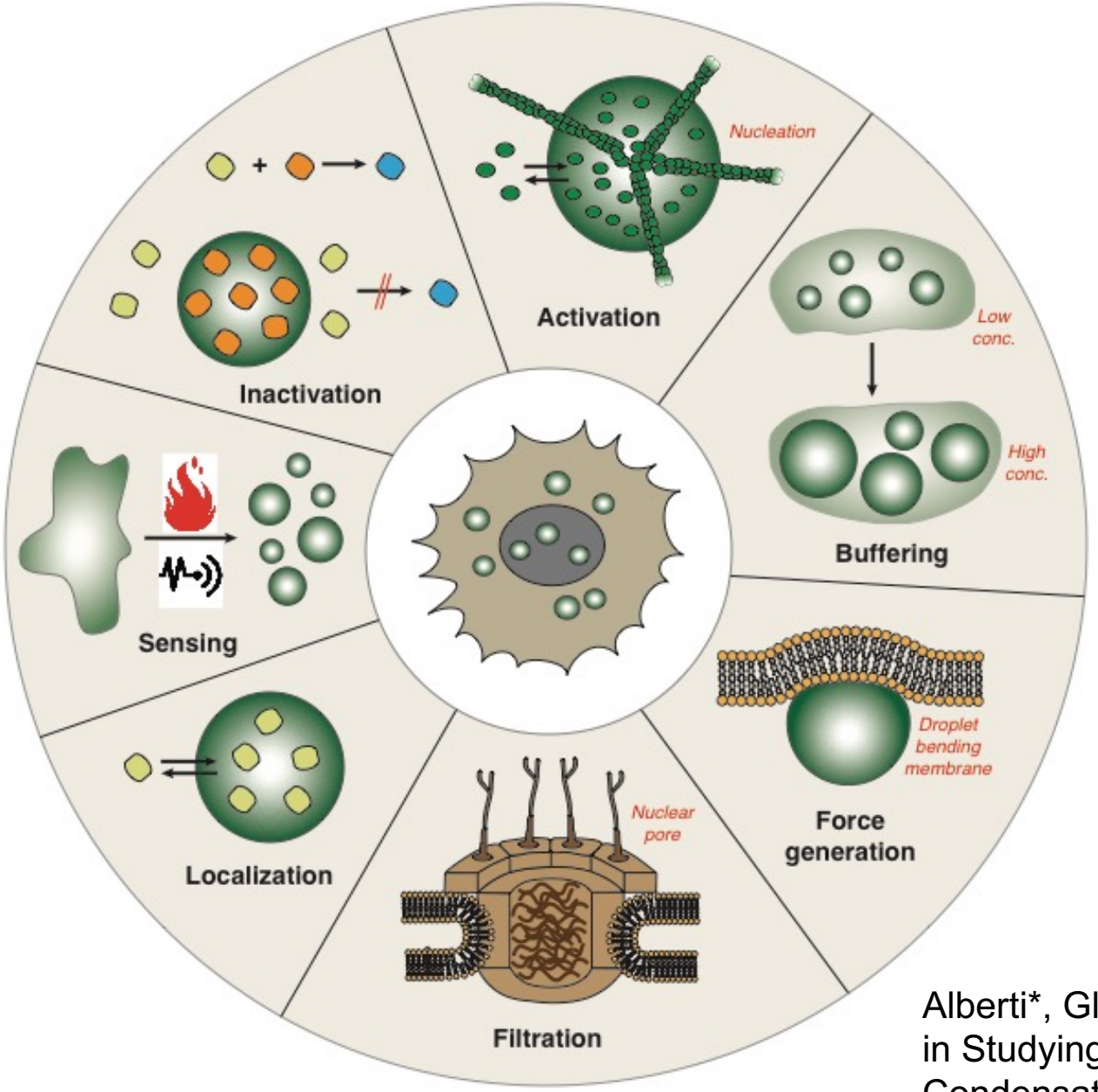
Phase separation of signaling molecules promotes T cell receptor signal transduction. Su et al. *Science* 2016

Autophagy



Liquidity Is a Critical Determinant for Selective Autophagy of Protein Condensates. Yamasaki et al. *Mol Cell* 2020

An initial repertoire of cellular functions mediated by phase separation

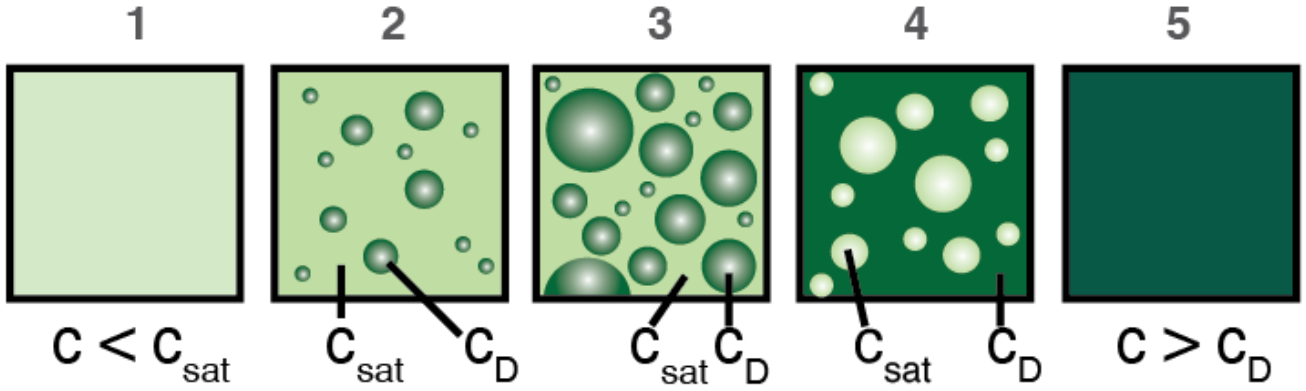


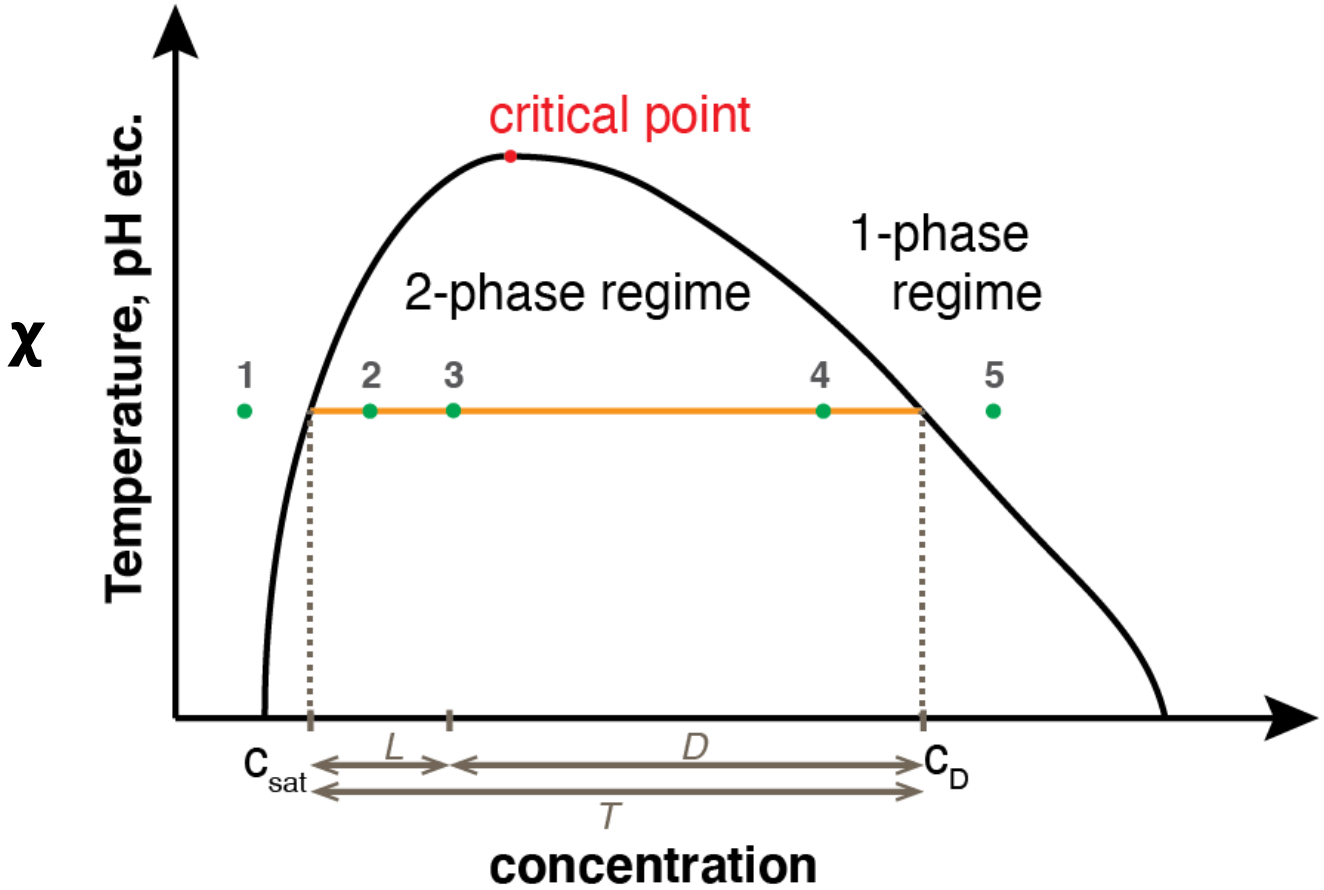
Alberti*, Gladfelter*, **Mittag***. Considerations and Challenges in Studying Liquid-Liquid Phase Separation and Biomolecular Condensates. *Cell* 2019

Phase separation promises to explain the biophysical basis of fundamental biological processes.

We need a quantitative framework for phase separation of biomolecules.

Phase separation is a density transition.

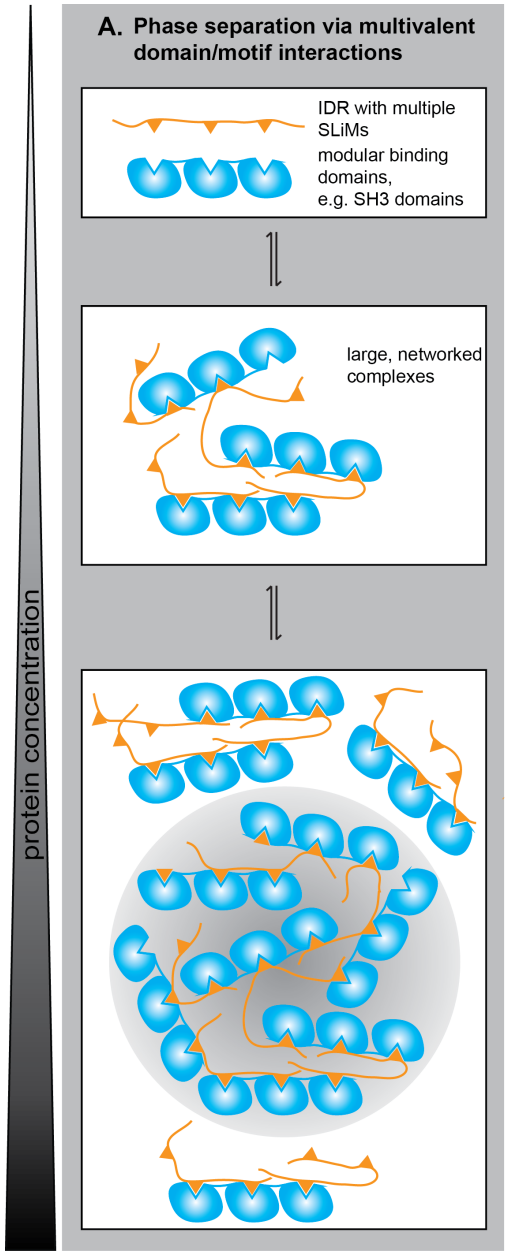




$$\chi \propto \frac{\left(2u_{PS} - u_{PP} - u_{SS}\right)}{kT}$$

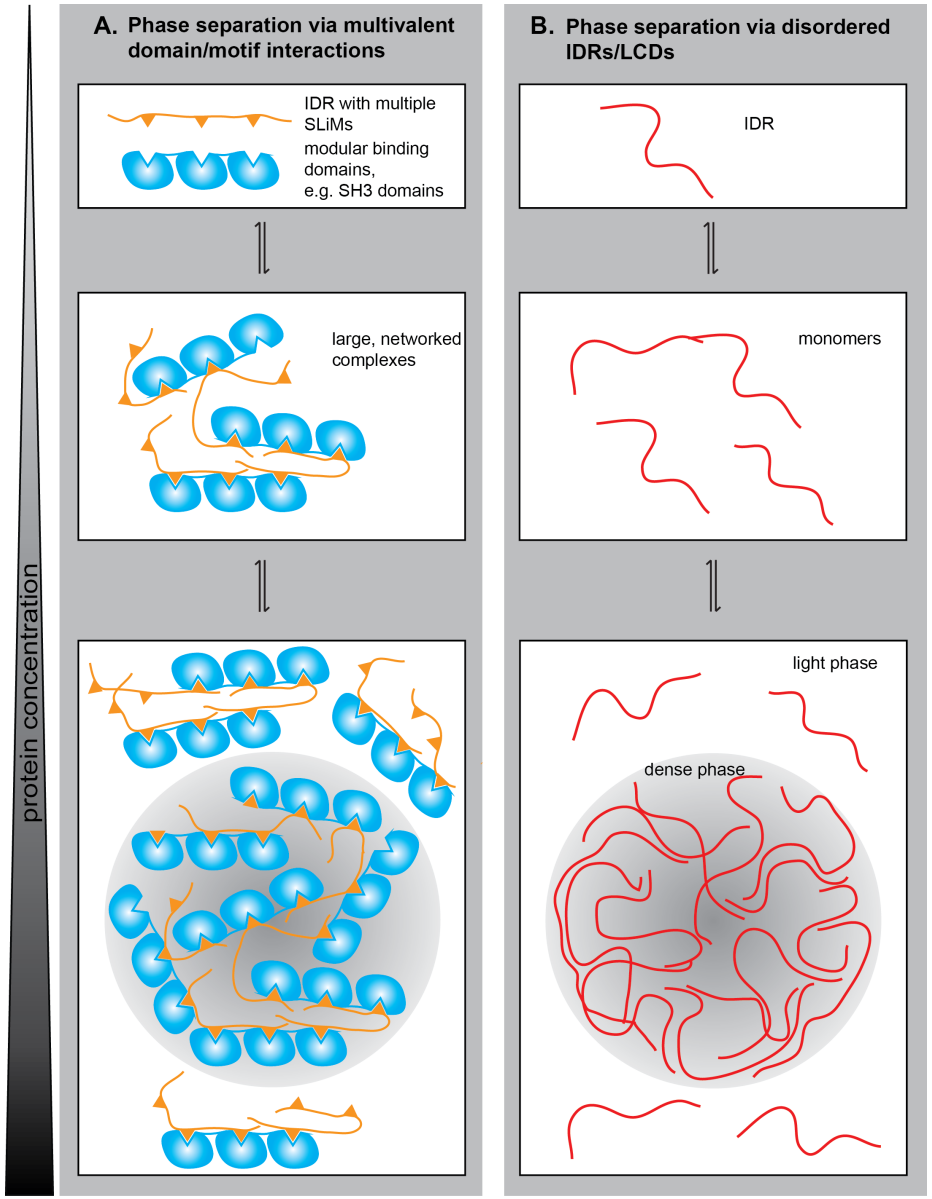
Alberti*, Gladfelter*, **Mittag***. *Cell* 2019

Which types of interactions mediate phase separation?



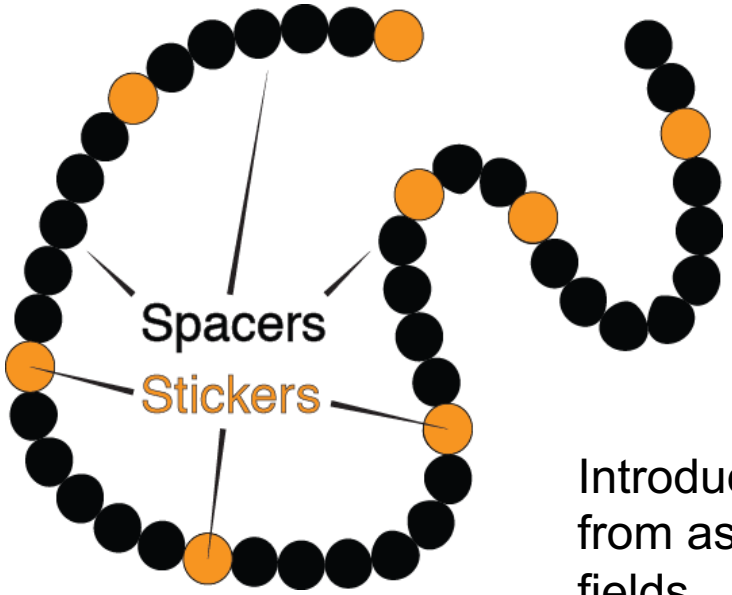
Mittag and Parker. *J Mol Biol* 2018

Which types of interactions mediate phase separation?



Mittag and Parker. *J Mol Biol* 2018

Stickers-and-spacers framework

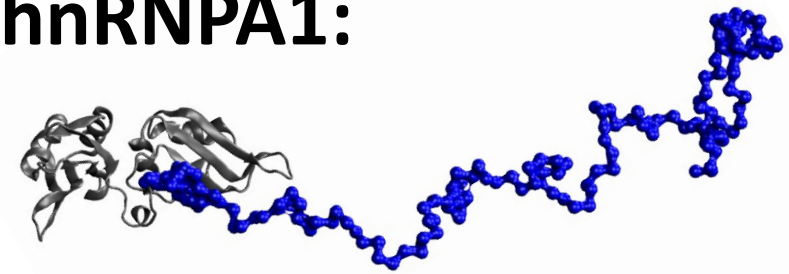


Introduced by Rohit Pappu from associative polymers fields

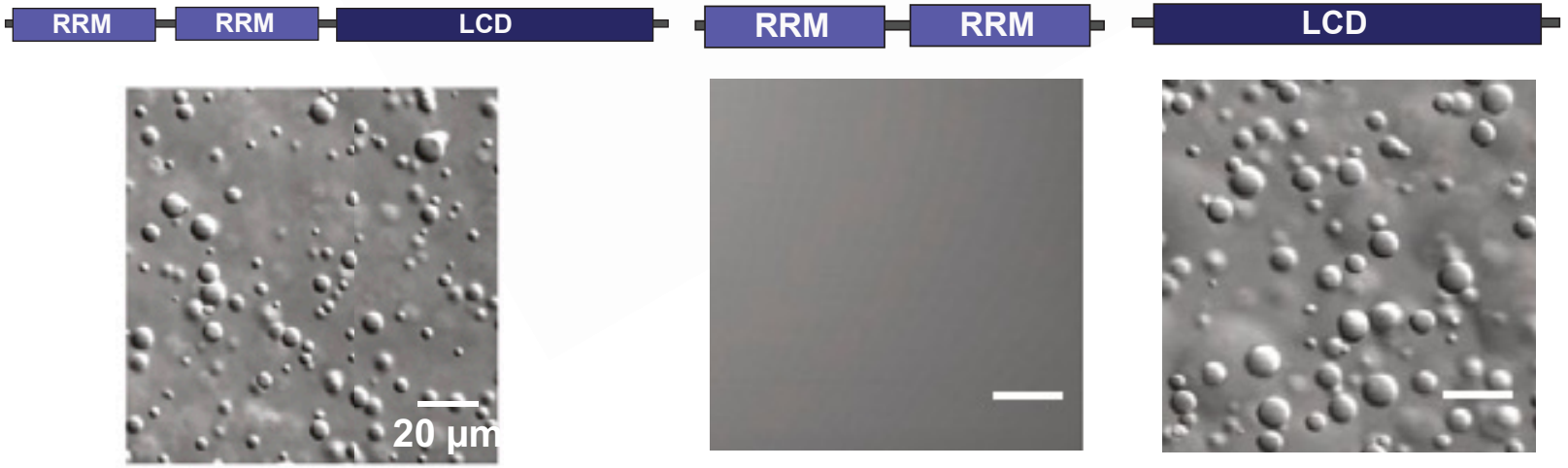
- **Stickers** are adhesive elements that form crosslinks.
- **Spacers** connect stickers and influence their ability to interact with each other.

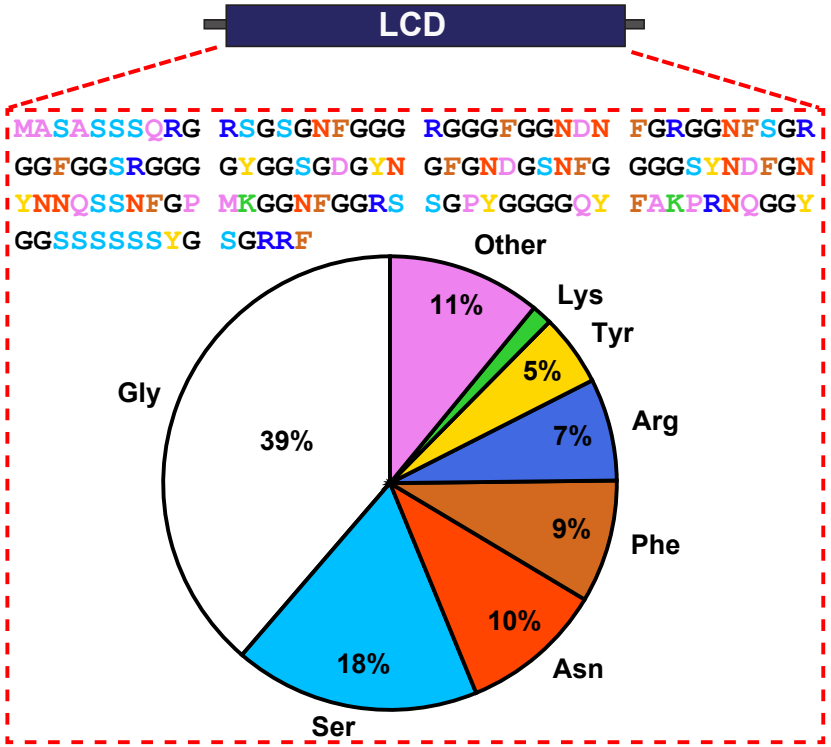
How is phase behavior encoded in prion-like low-complexity domains?

hnRNPA1:



Mutations in LCD can lead to ALS/FTD
Fusion of FET family LCDs can lead to cancer

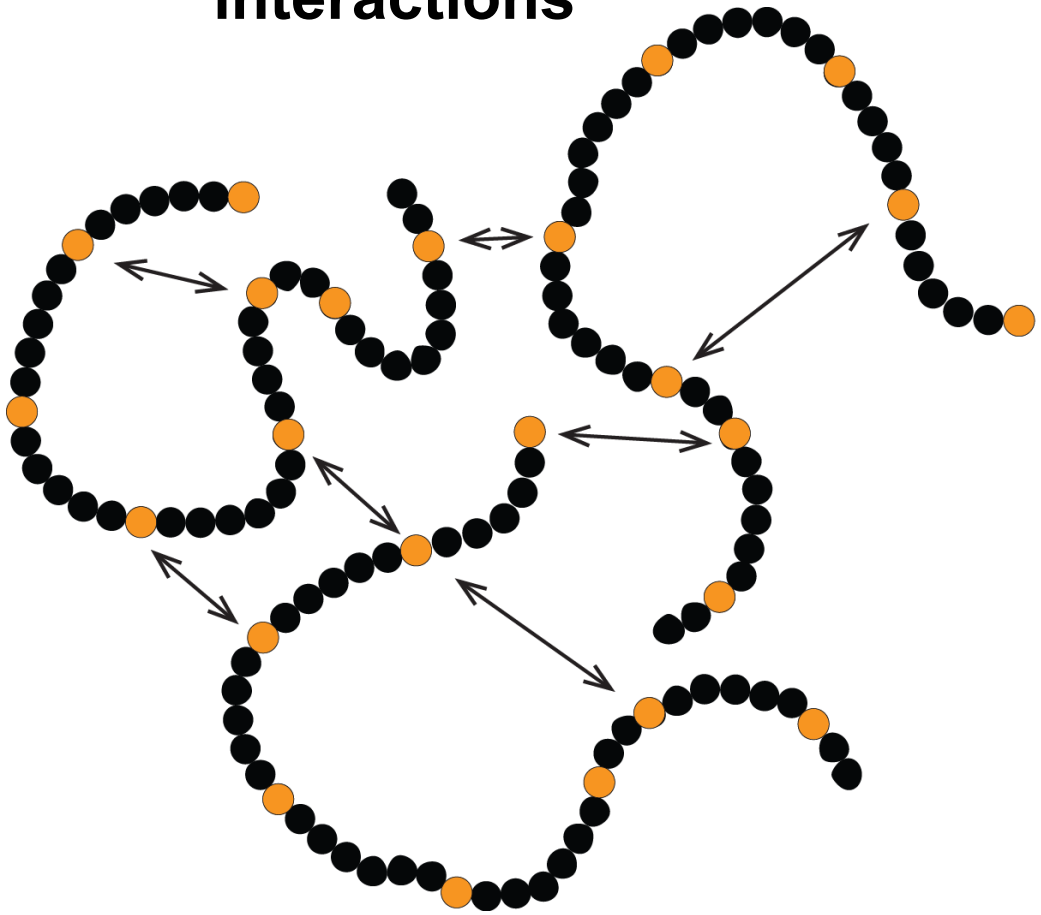




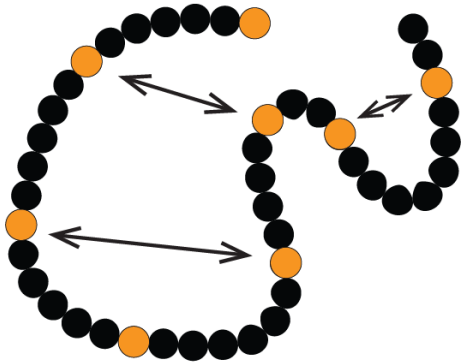
Molliex, Temirov, Lee, Coughlin, Kanagaraj, Kim, Mittag*, Taylor*. *Cell,* 2015

Can we identify stickers in dilute protein solutions?

*inter*molecular sticker-sticker interactions



*intra*molecular sticker-sticker interactions



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CSELS Center for Science & Engineering of Living Systems

Pappu Lab

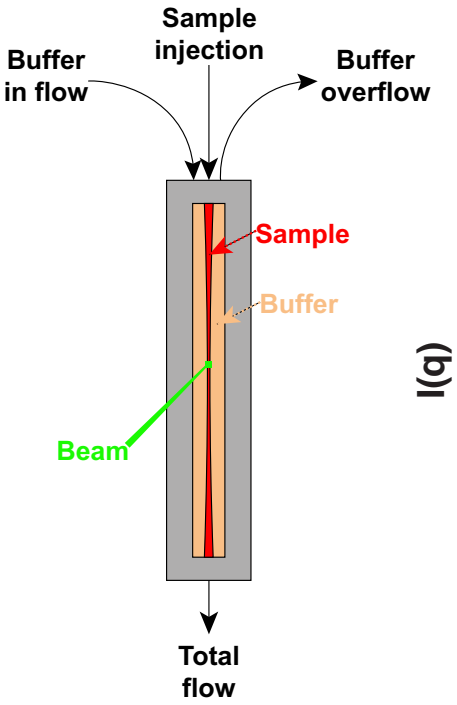

Mina Farag

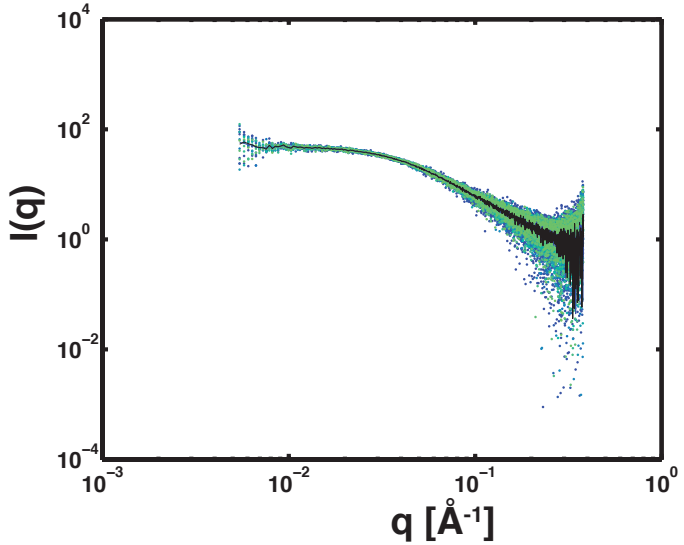

Alex Holehouse


Rohit Pappu

Combination of biophysical techniques

Co-flow SEC-SAXS



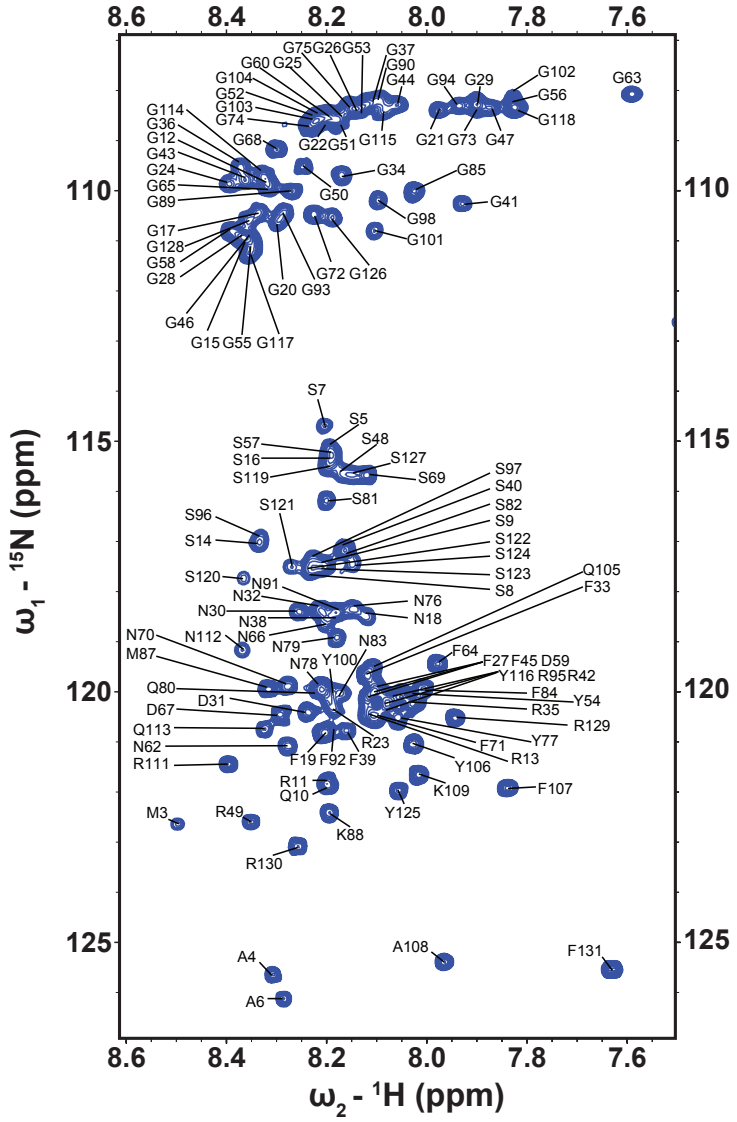


Martin, Hopkins and **Mittag**.
Methods in Enzymology 2020

Erik Martin

Ivan Peran

NMR spectroscopy

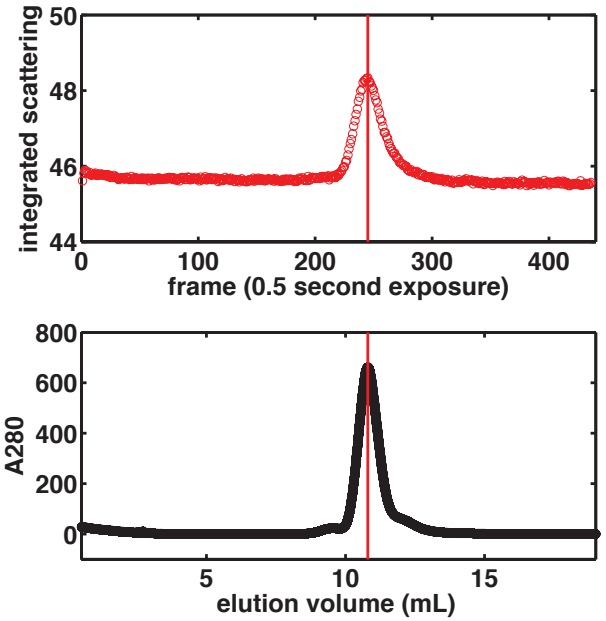


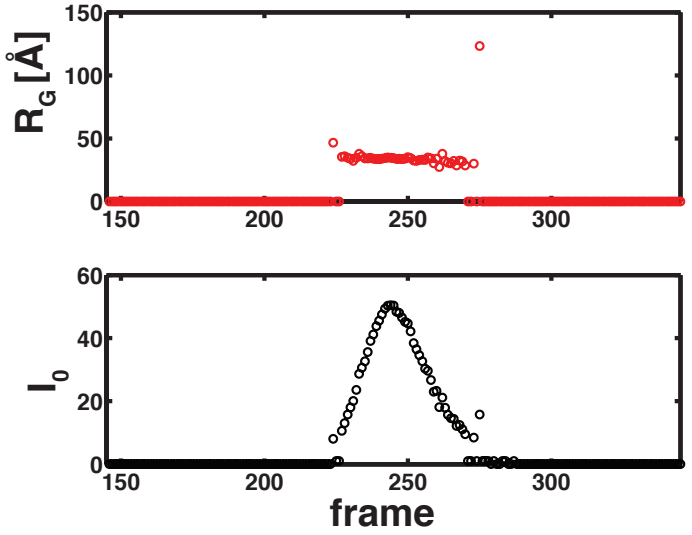
At BioCAT at the Advanced Photon Source

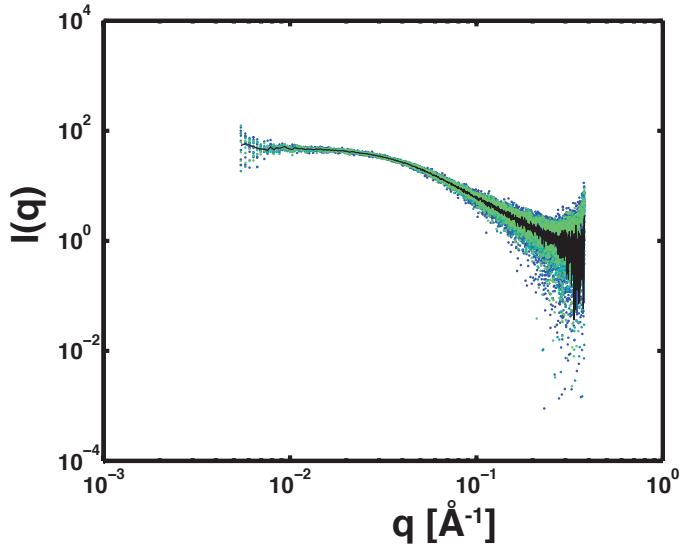
Reports on size and shape:
LCD is unusually compact

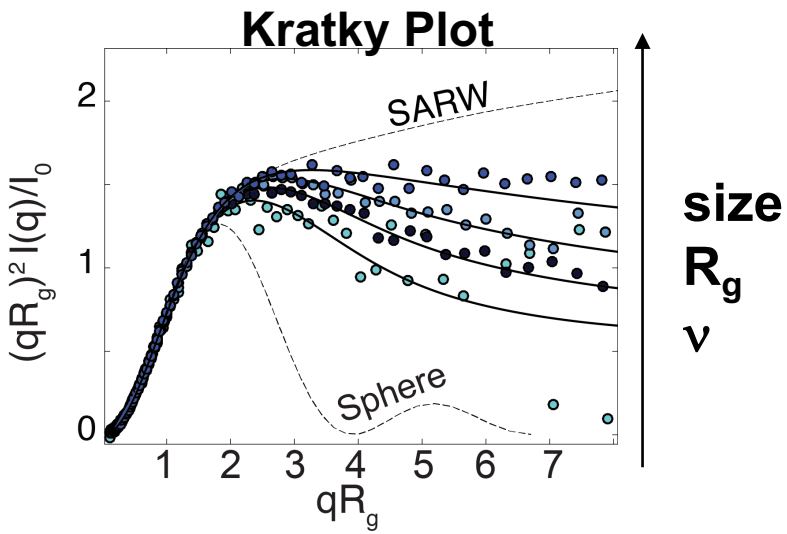
MASASSSQRG RSGSGNFGGG RGGGFGGNDN FGRGGNFSGR GGFGGSRGGG
GYGGSGDGYN GFGNDGSNFG GGGSYNDFGN YNNQSSNFGP MKGGNFGGRS
SGPYGGGGQY FAKPRNQGGY GGSSSSSSYG SGRRF

The LCD adopts compact intrinsically disordered conformations

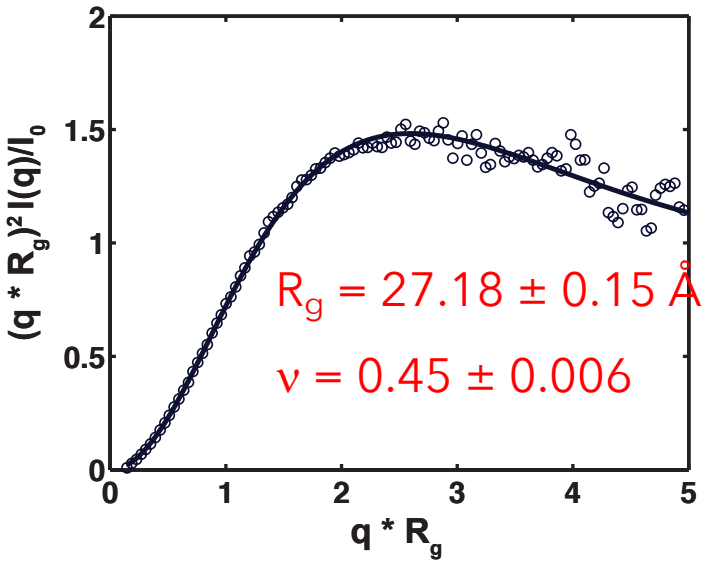








size
R_g
ν

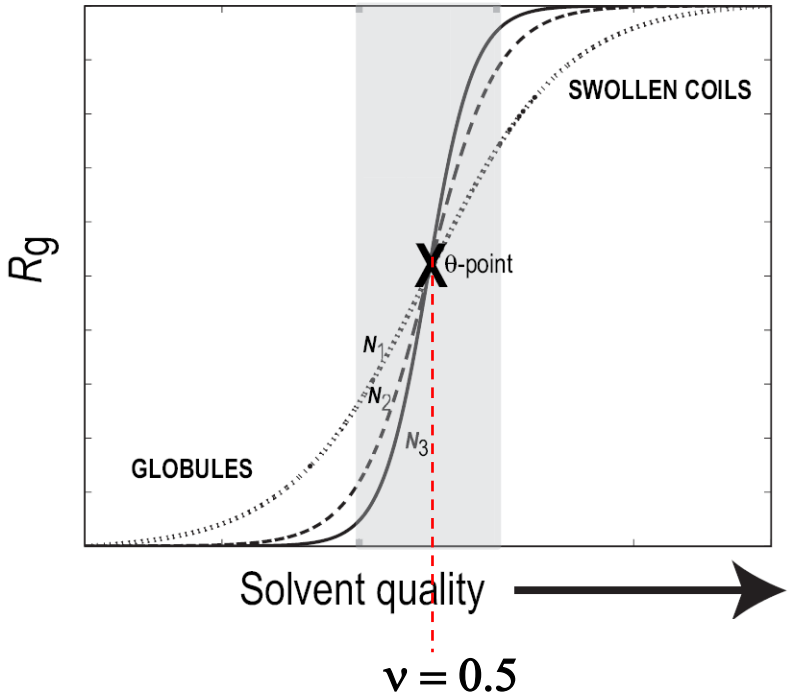


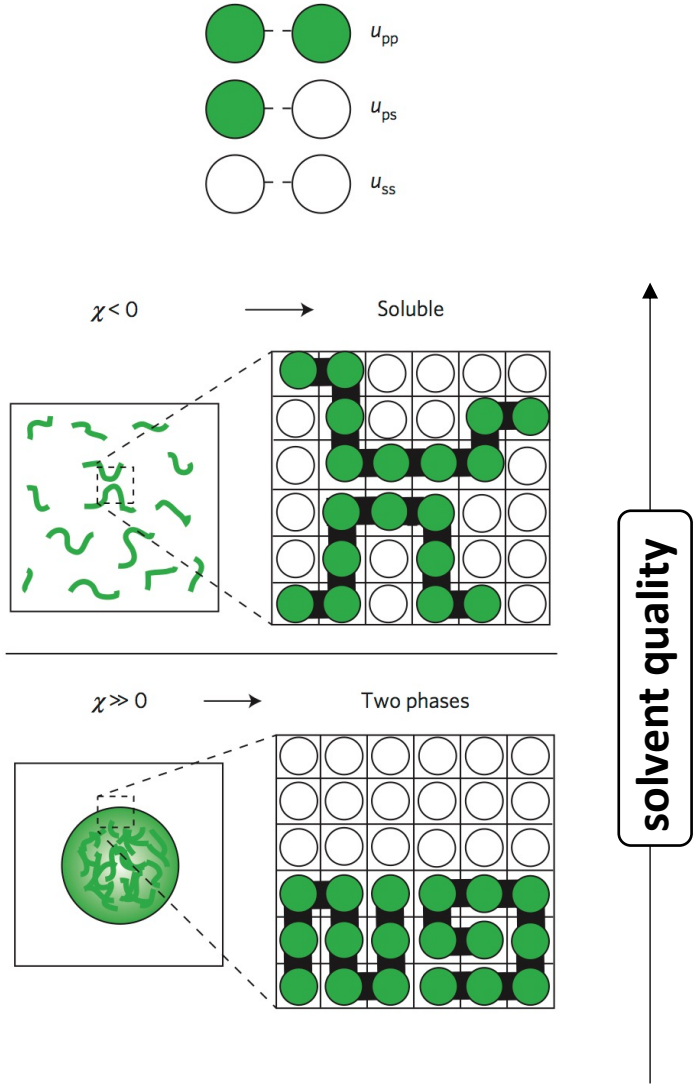
Erik Martin

Global dimensions of disordered chains report on solvation

$$R_g \sim N^{\nu}$$

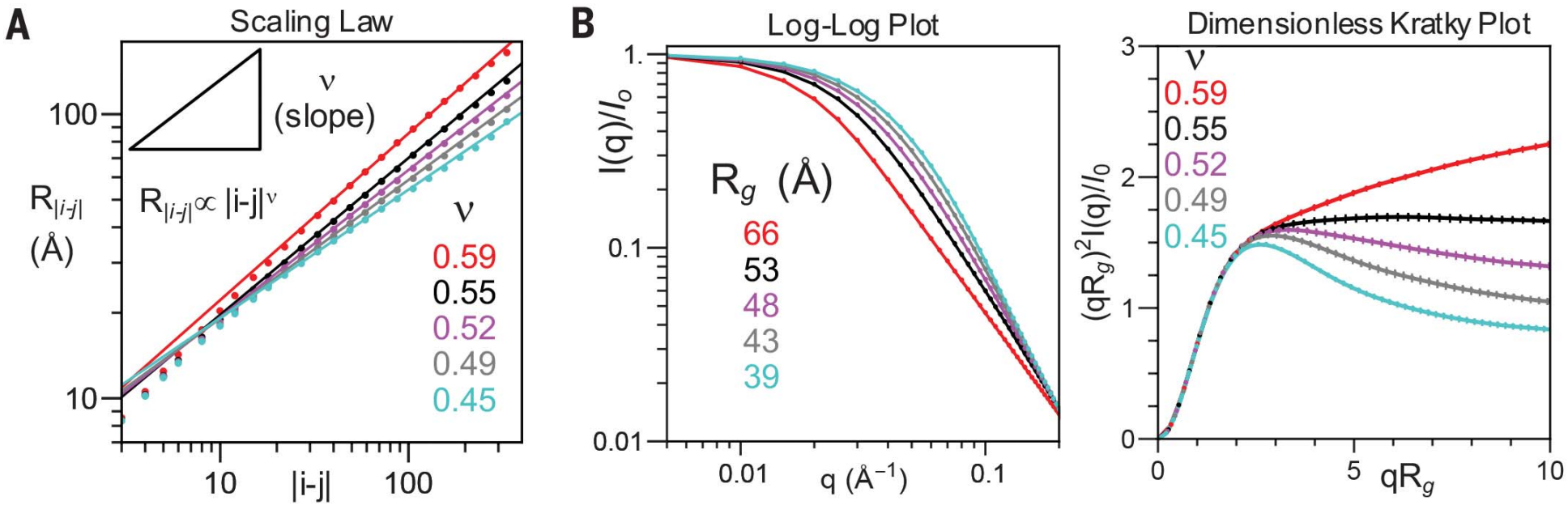
The radius of gyration of polymers scales with the number of residues and a scaling exponent ν, which reflects solvent quality.





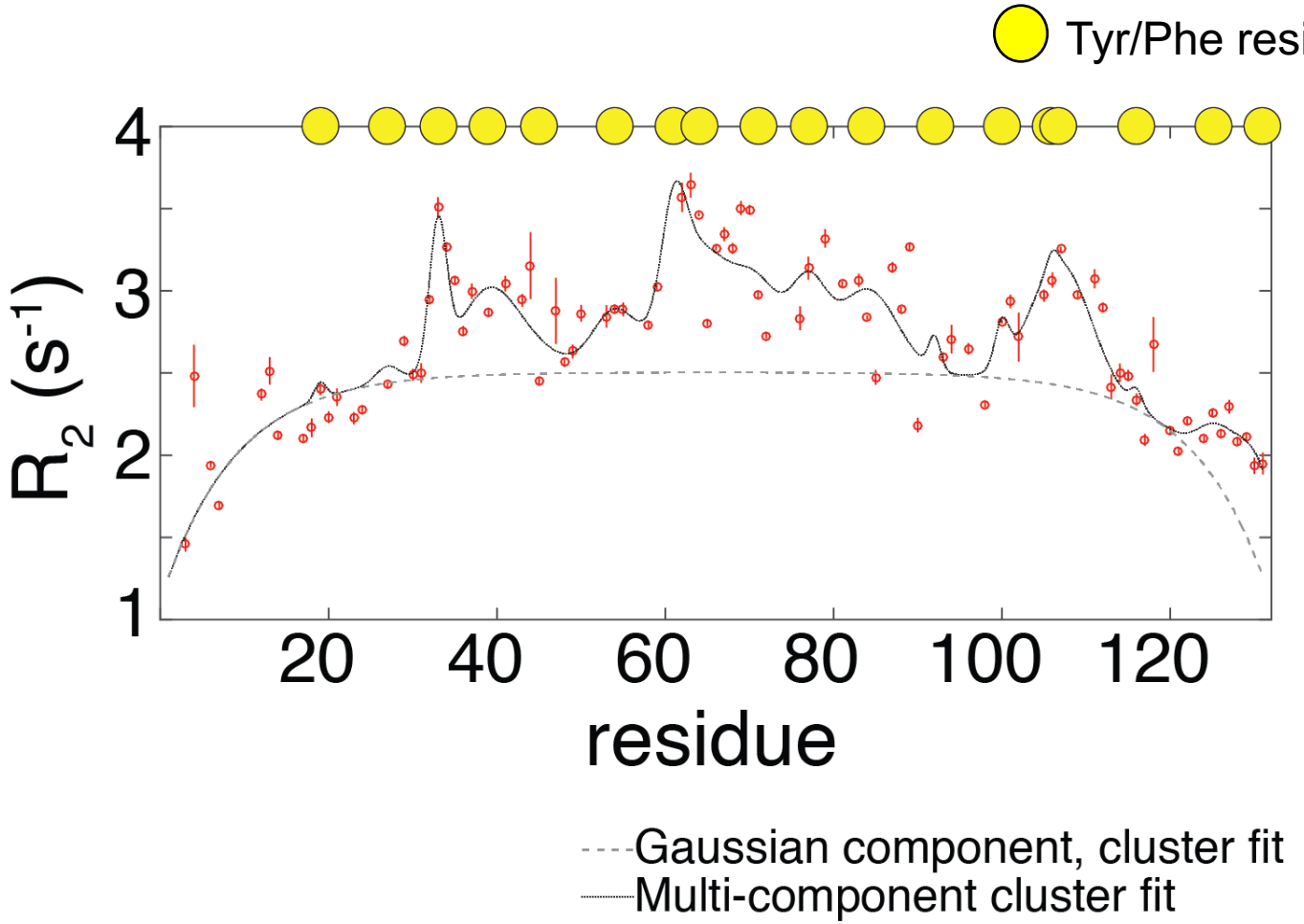
Pappu et al. Arch Biochem Biophys 2008
Ruff et al. JMB 2018

Molecular form factor for the interpretation of SAXS data of IDPs



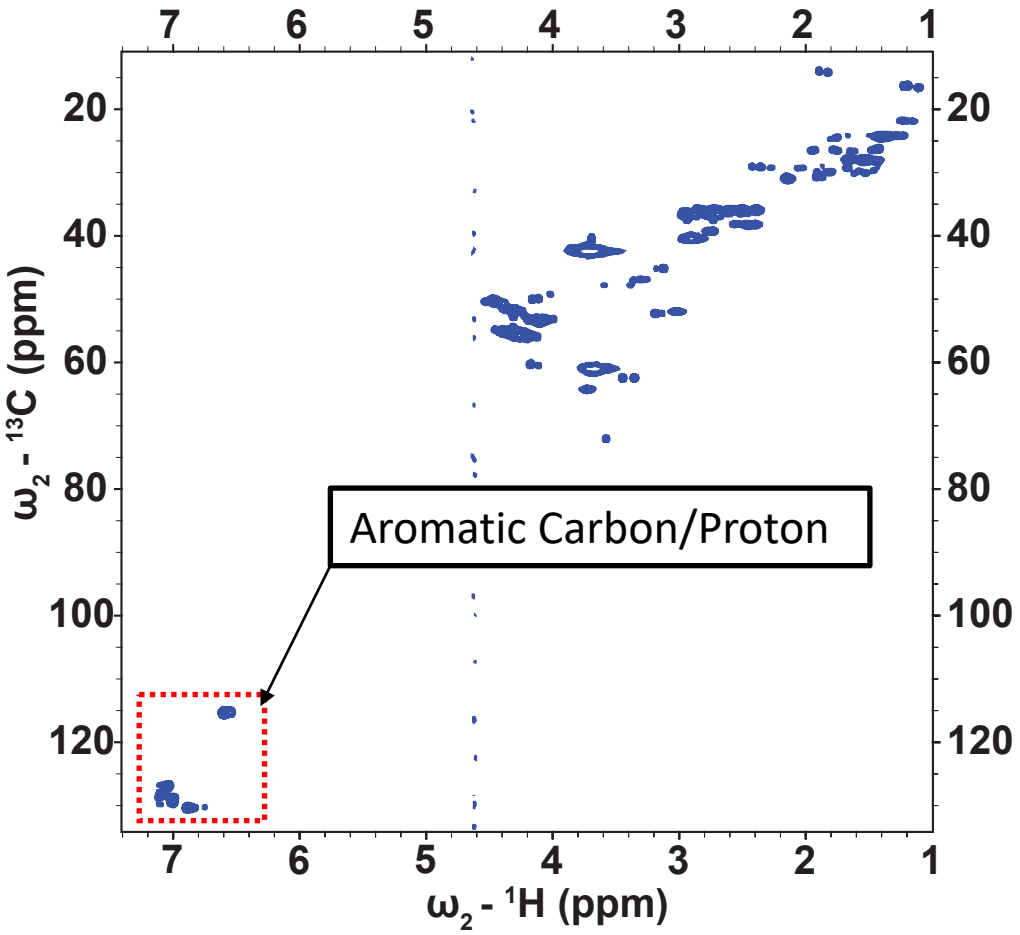
Riback et al. Sosnick. Science 2017

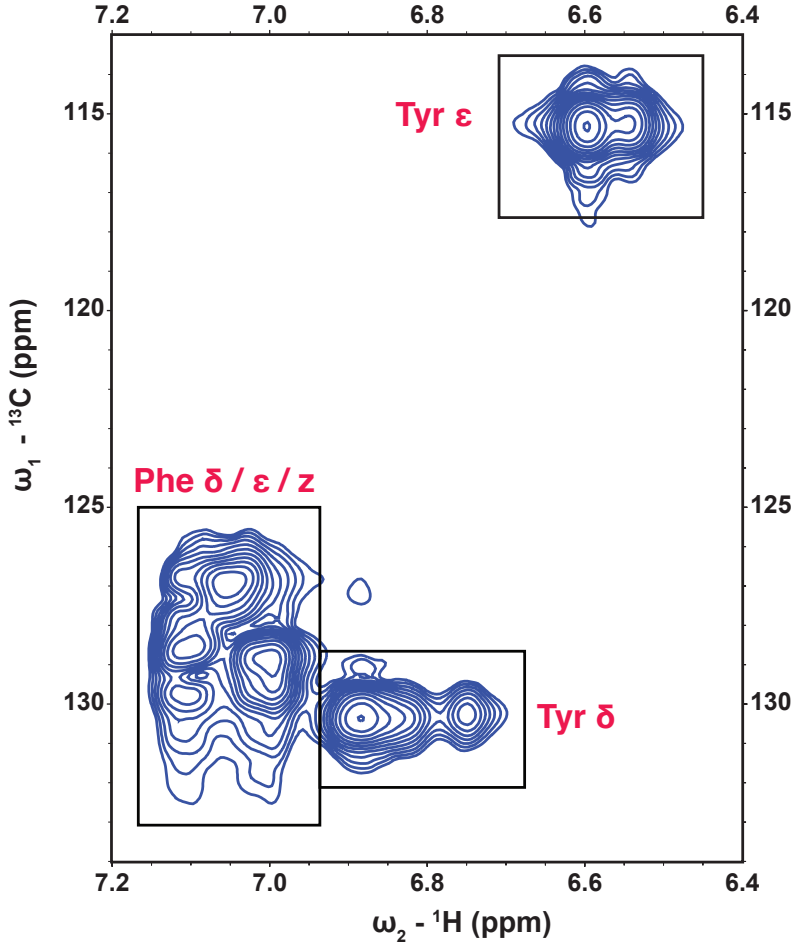
R_2 relaxation rates report on slowed dynamics around aromatics residues



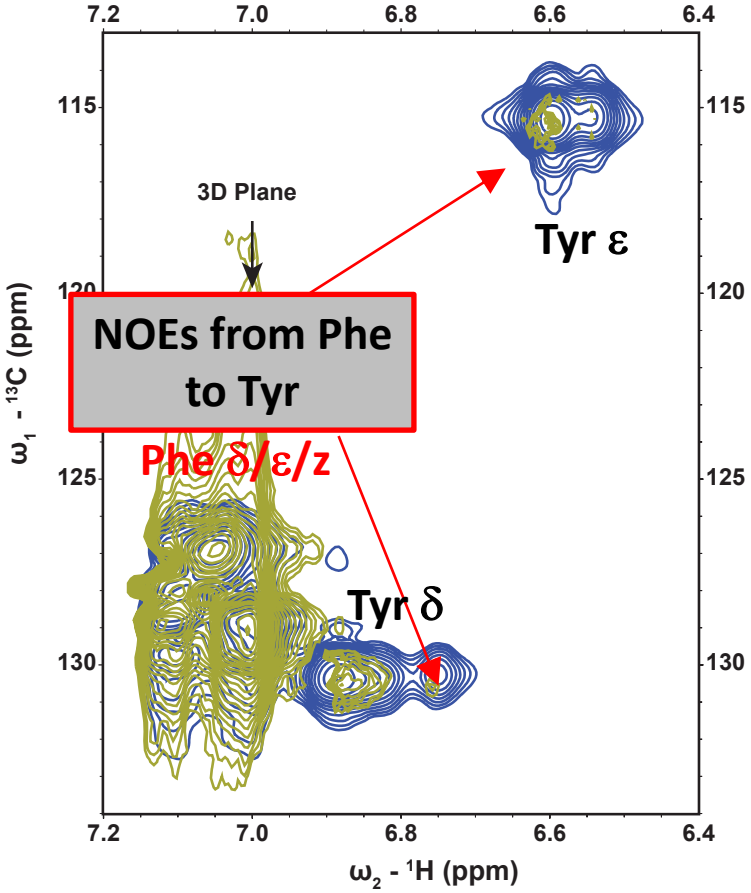
These R_2 rates are consistent with clustering of aromatics side chains.

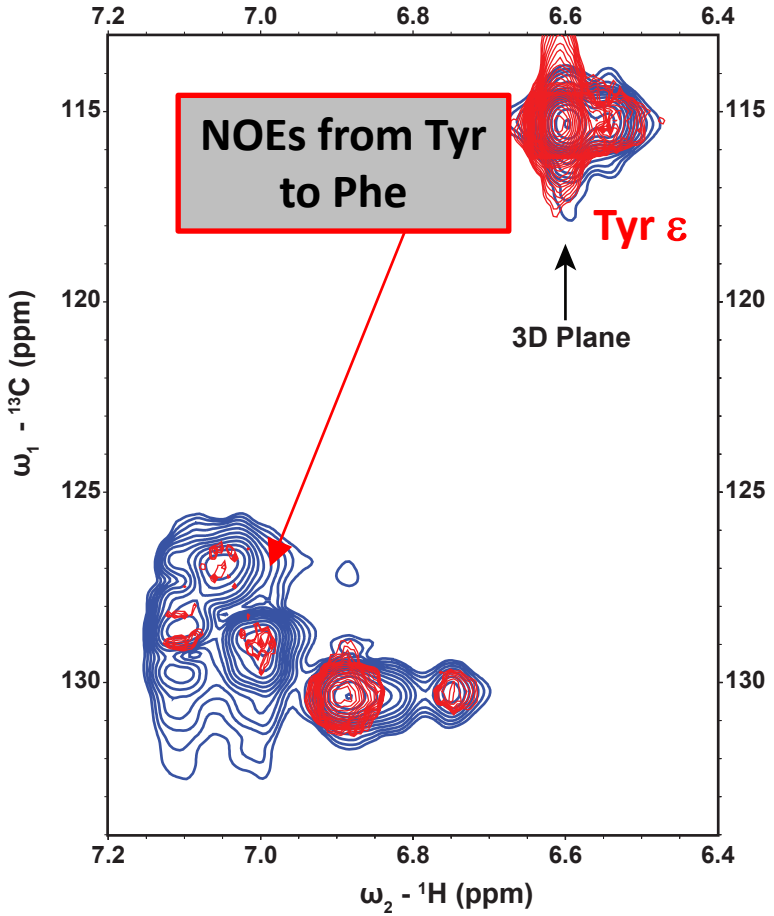
Can we see direct contacts between aromatic sidechains by NMR? (i.e. are there NOEs?)





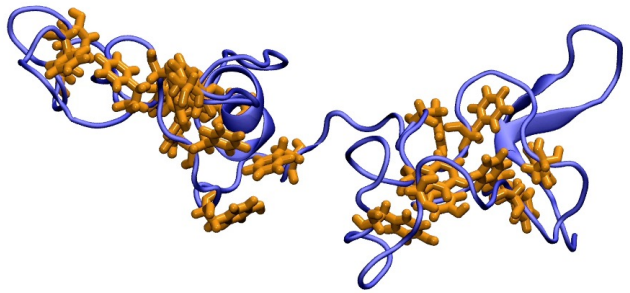
NOEs between Phe and Tyr in disordered LCD demonstrate direct contacts

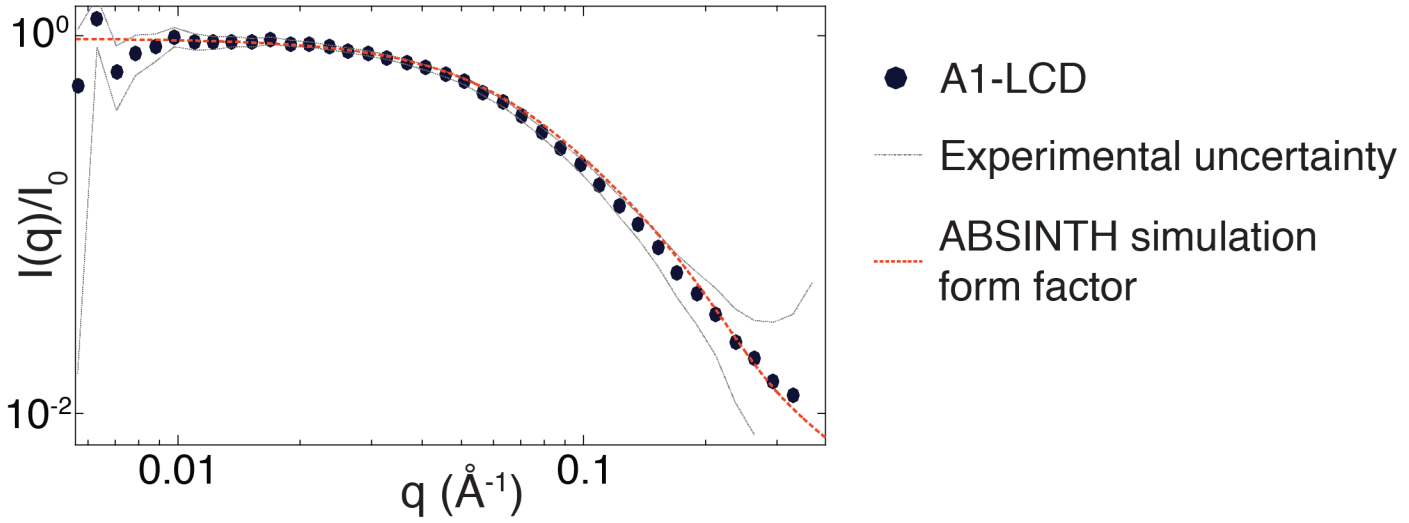




All-atom simulations recapitulate the compact dimensions of the LCD

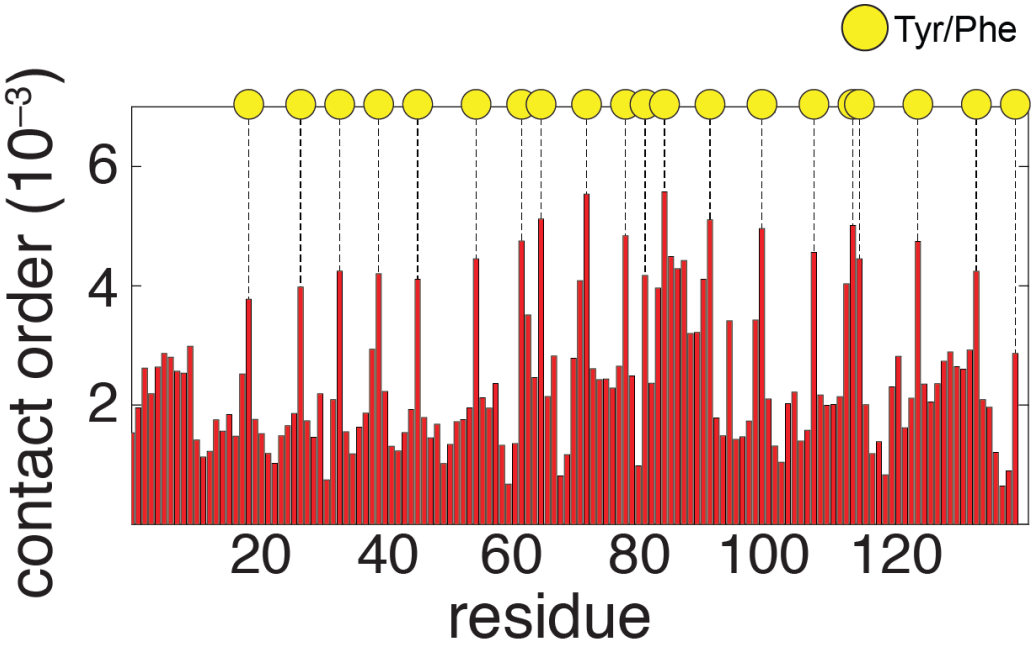
Alex Holehouse

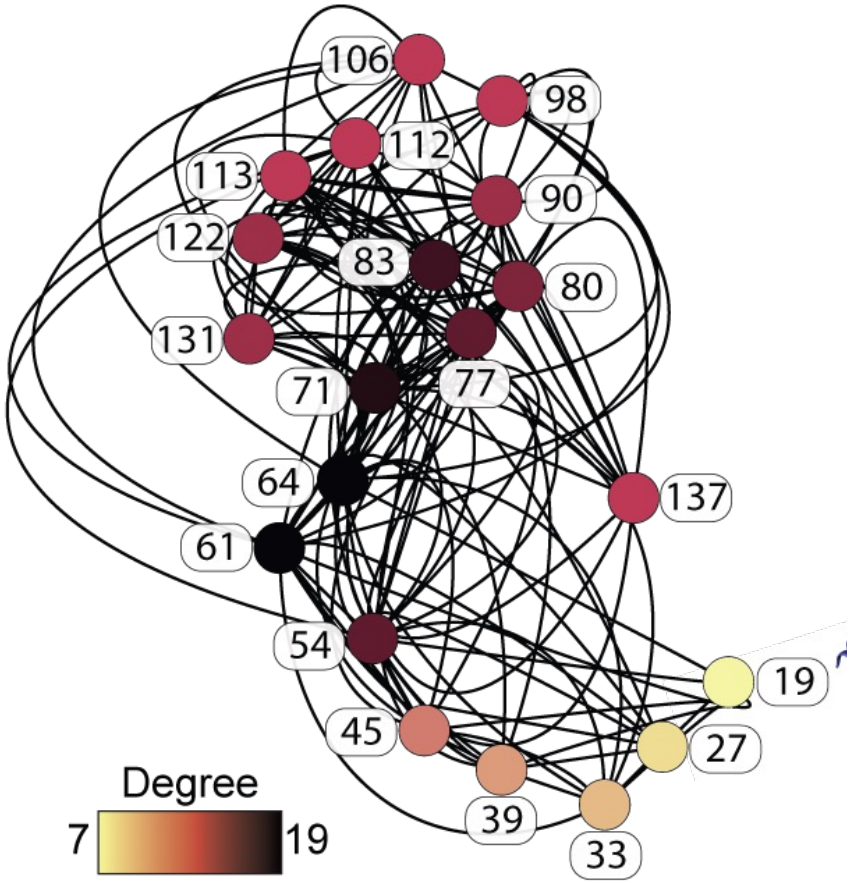




ABSINTH implicit solvent model
Vitalis & Pappu, *J. Comp. Chem*. (2009)

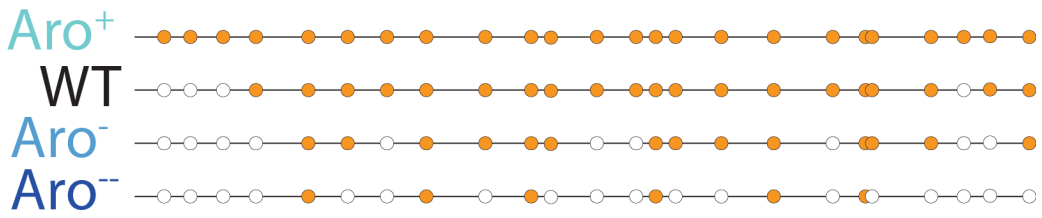
Aromatic residues engage in distributive, transient interactions

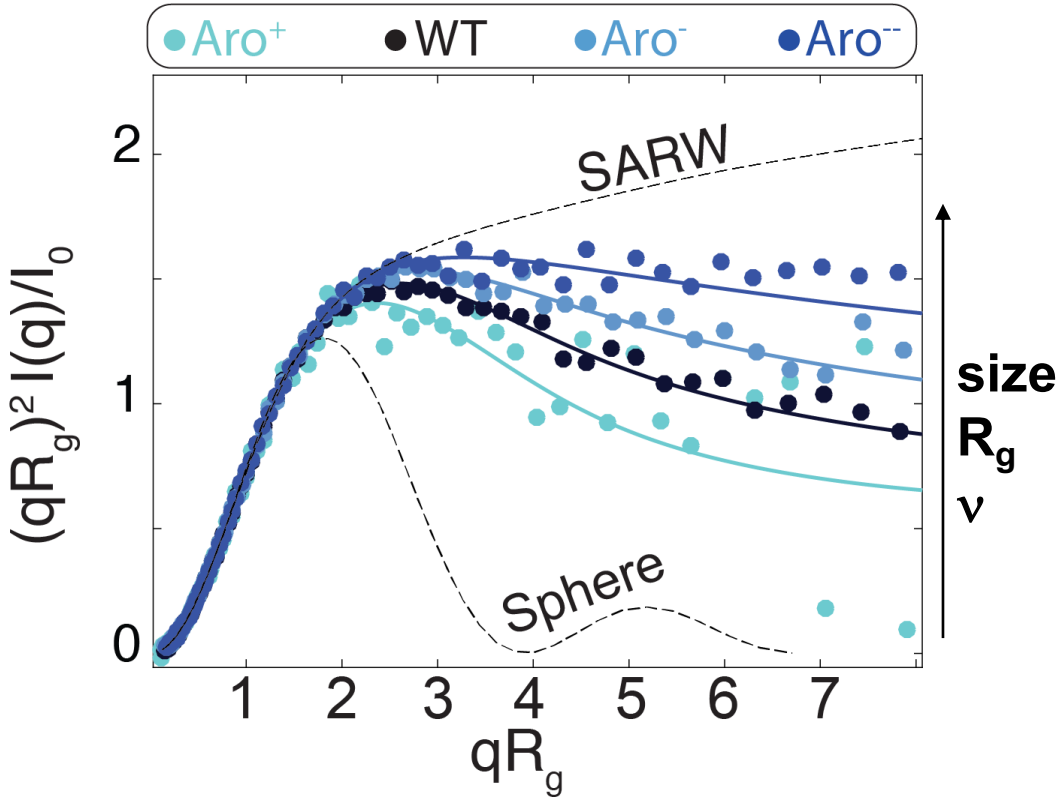


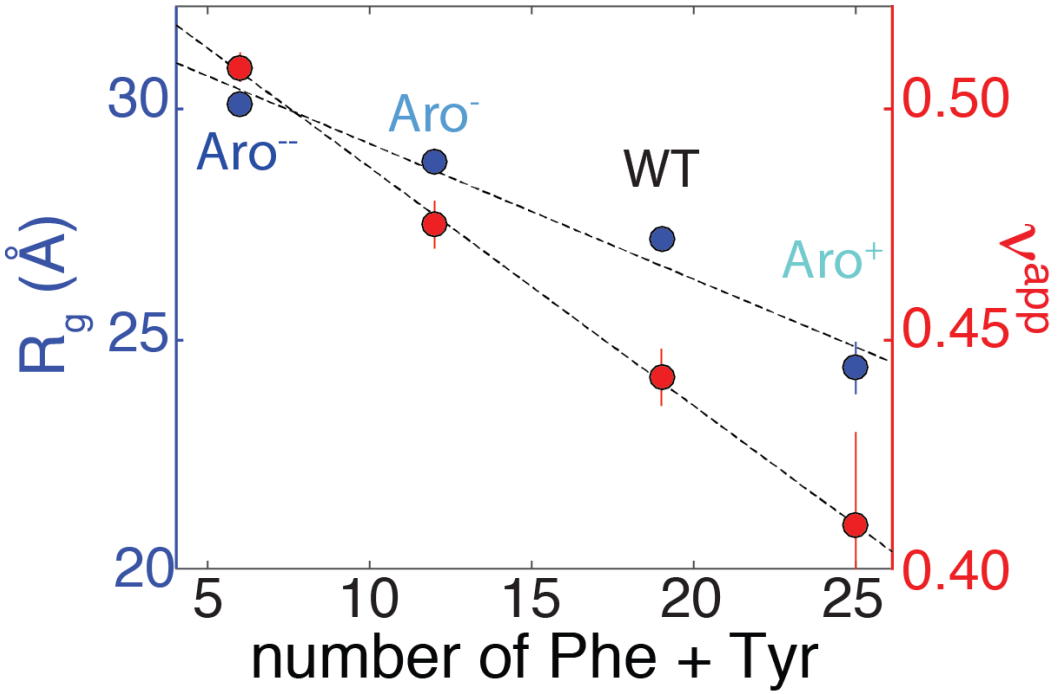


Are these the *cause* or a *consequence* of the compact dimensions of this IDP?

Test whether removal of aromatics expands the LCD



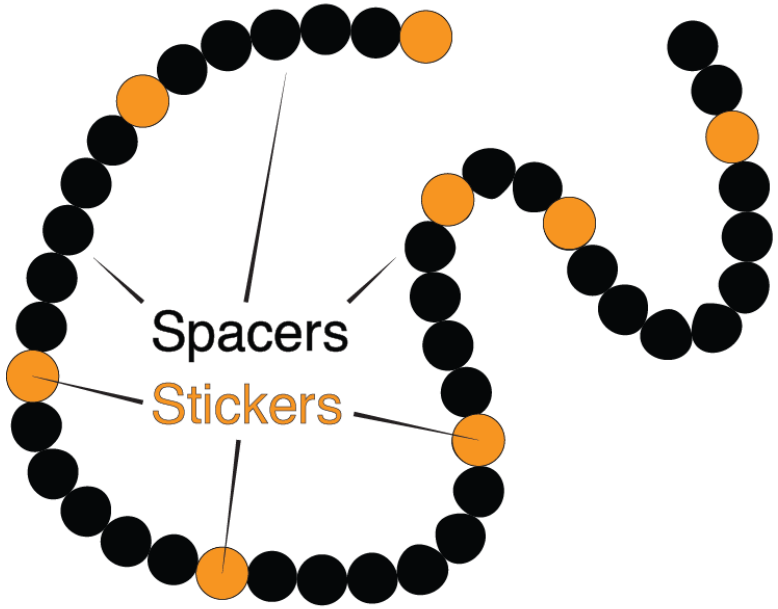


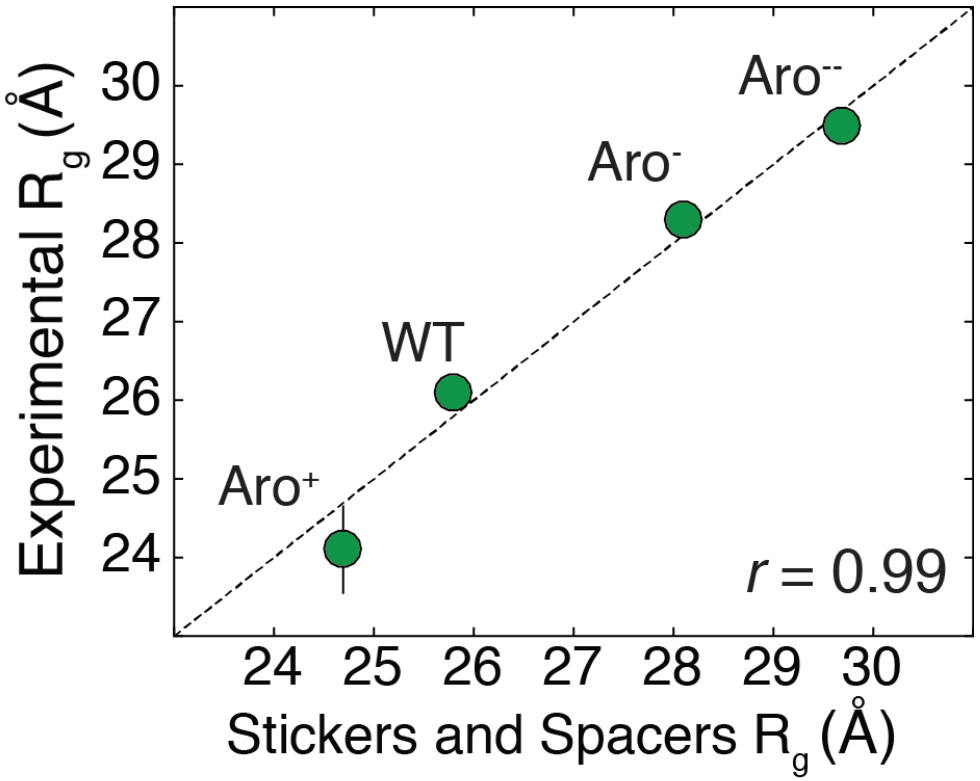


Aromatic stickers give rise to cohesive interactions that determine chain dimensions.

Martin, Holehouse, Peran, Farag, Incicco, Bremer, Grace, Soranno, Pappu, Mittag; *Science* 2020

Parameterization of the *stickers*-and-*spacers* model



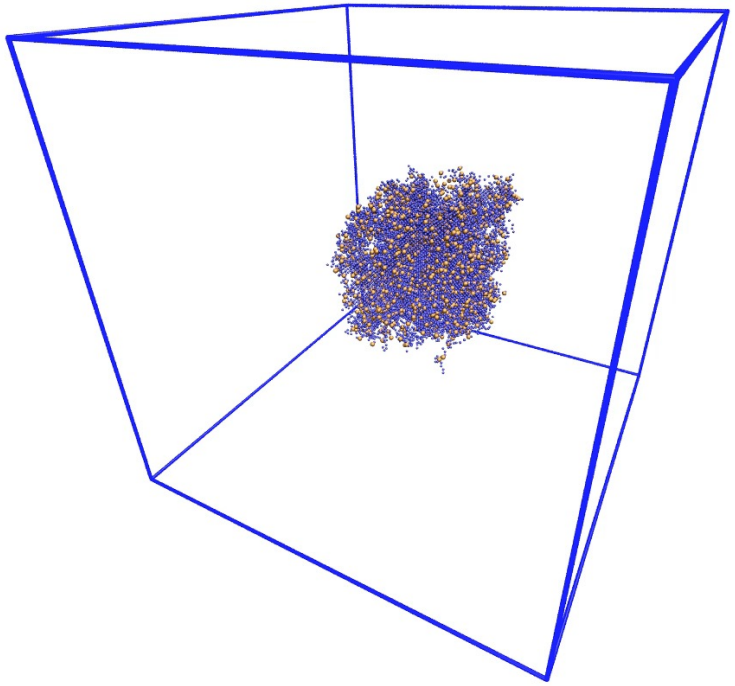


lattice-based coarse-grained model that uses a single bead per residue

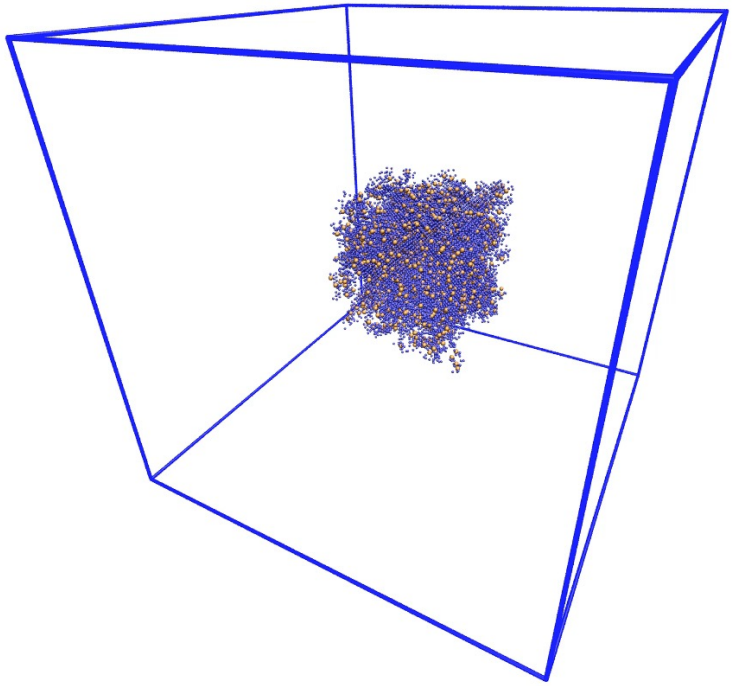
parameterizing the strengths of the sticker-sticker, sticker-spacer, and spacer-spacer interactions to reproduce the experimental R_g values and R_g distributions from all-atom simulations

Martin, Holehouse, Peran, Farag, Incicco, Bremer, Grace, Soranno, Pappu, Mittag; *Science* 2020

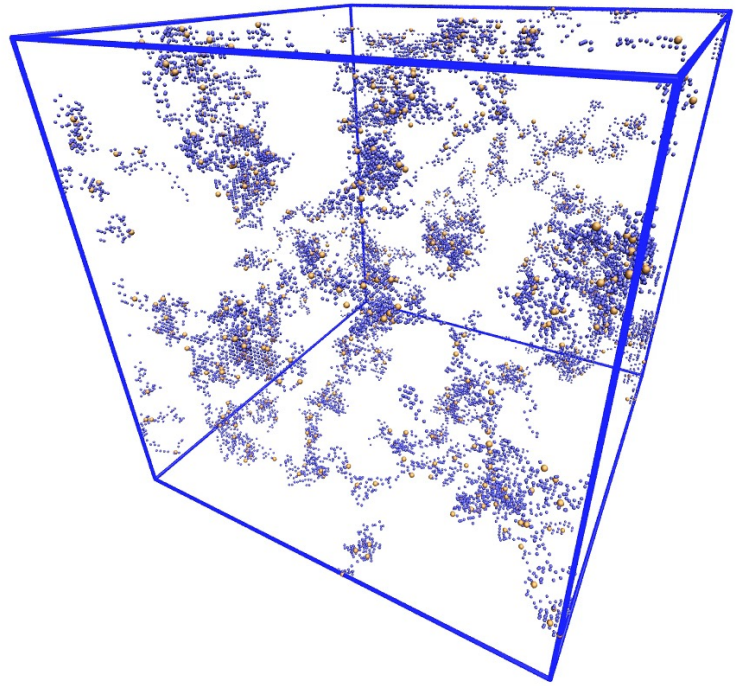
Sticker-and-spacer lattice simulations allow determination of full phase diagrams



WT



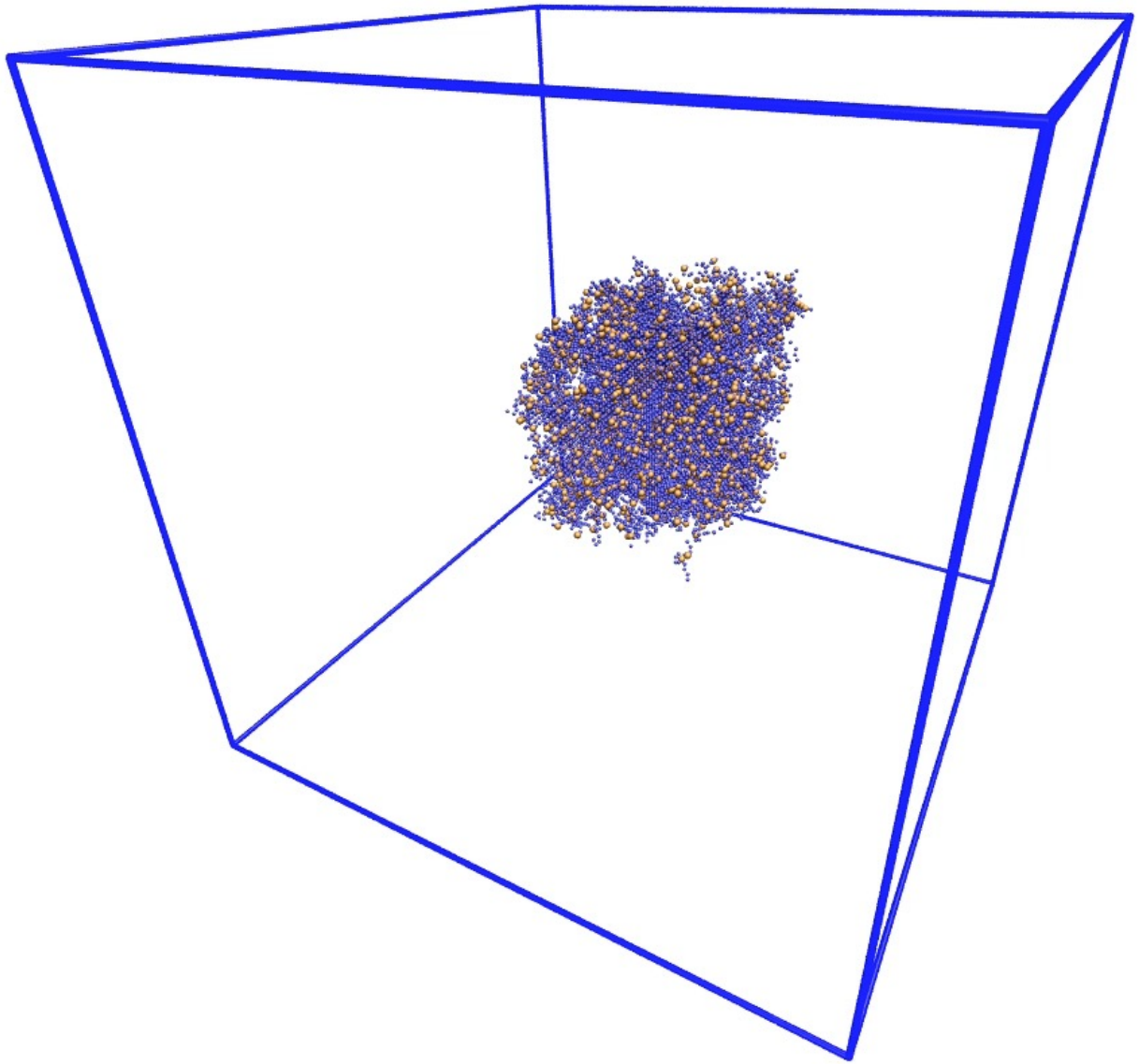
Aro^{-}

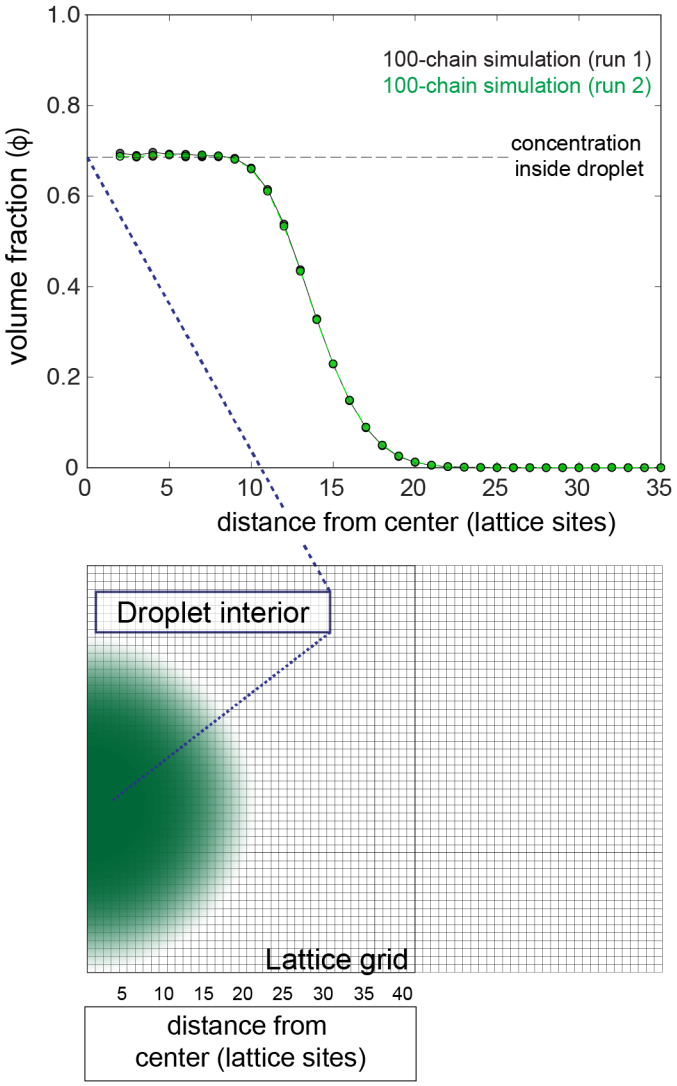


Aro^{--}

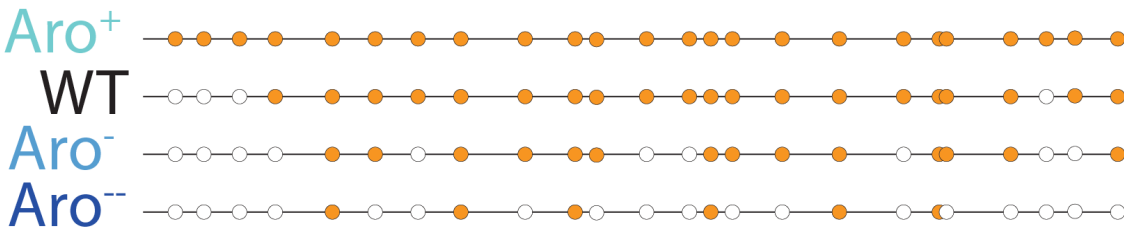
Alex Holehouse, Rohit Pappu

Stickers-and-spacers simulations to construct phase diagrams

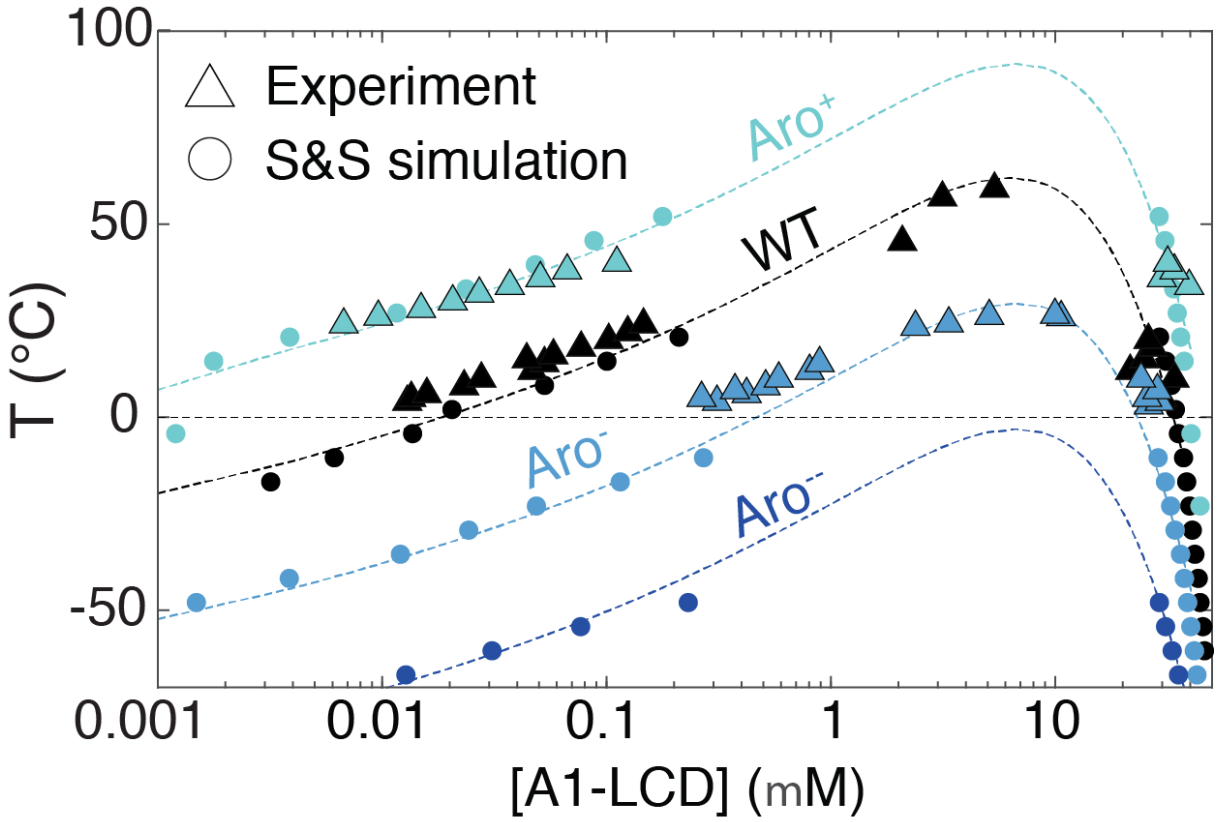




The stickers-and-spacers model is predictive of experimental phase behavior



Ivan Peran



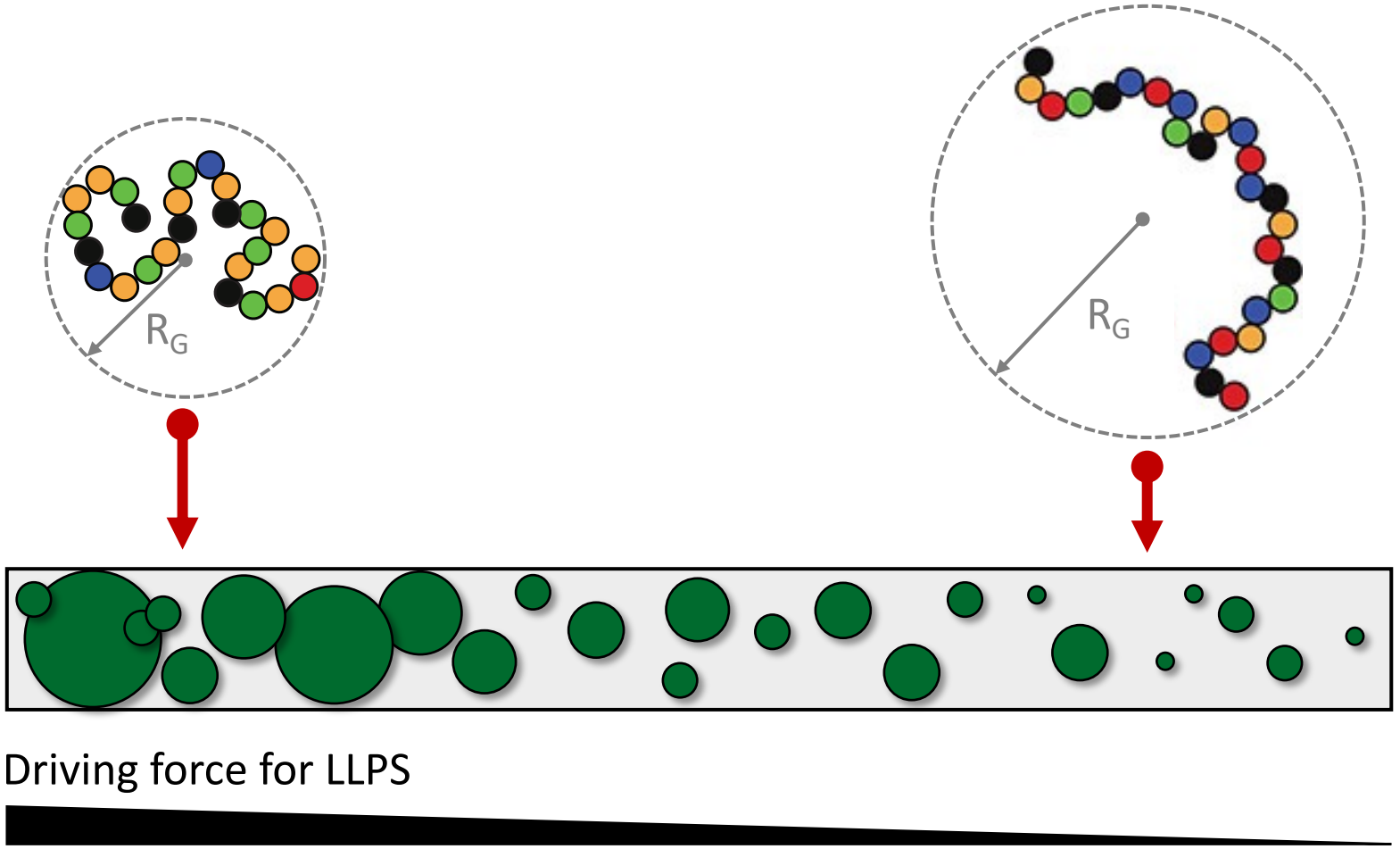
The higher the sticker valence, the larger is the driving force for phase separation.

Martin#, Holehouse, Peran#, Farag, Incicco, Bremer, Grace, Soranno, Pappu*, Mittag*; *Science* 2020

Conclusions

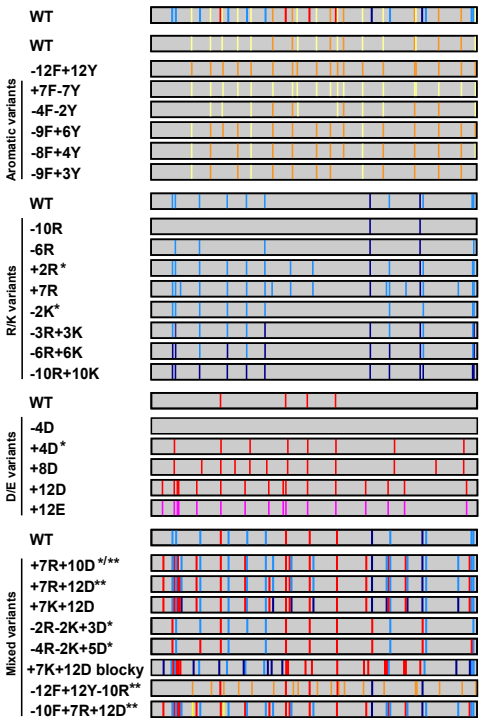
- Stickers in LCDs can be identified in an unbiased manner from experiments on dilute samples.
- Aromatic residues act as “stickers” that mediate distributive, cohesive interactions, both intra- and intermolecularly.
- Linearly distributed aromatic residues promote phase separation and avoid aggregation.

Do global dimensions report on the driving force for phase separation in LCDs?

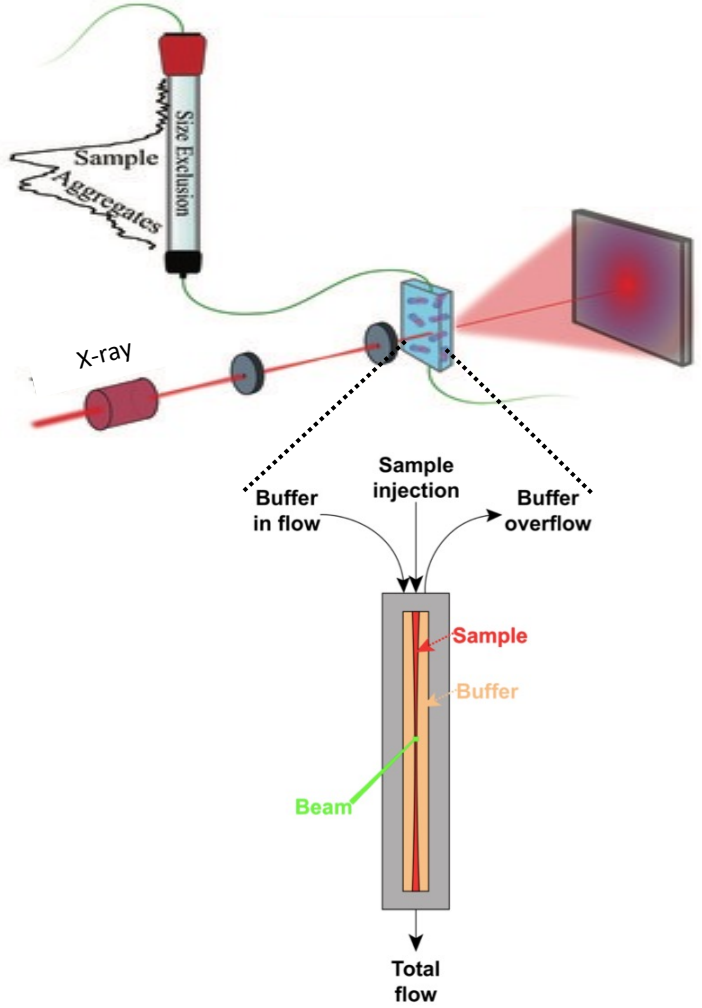


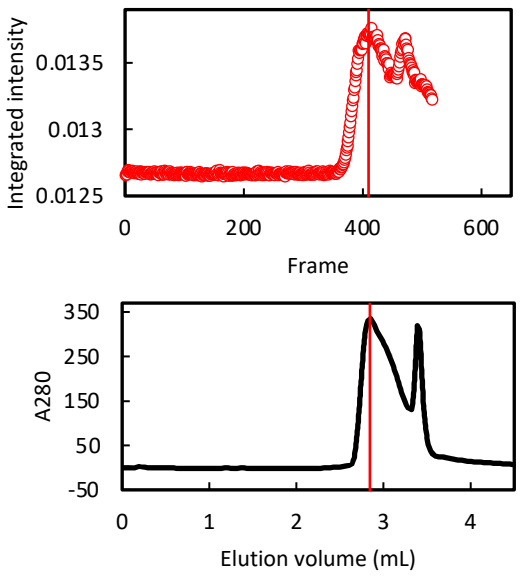
SEC-SAXS to measure single-chain dimensions

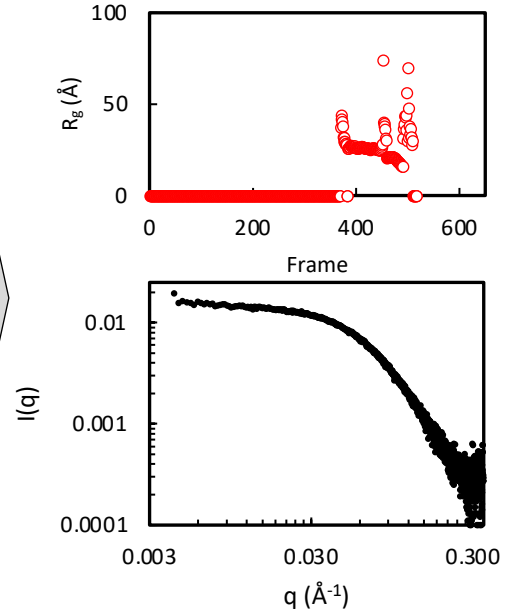
A1-LCD sequences (~30)



co-flow SEC-SAXS



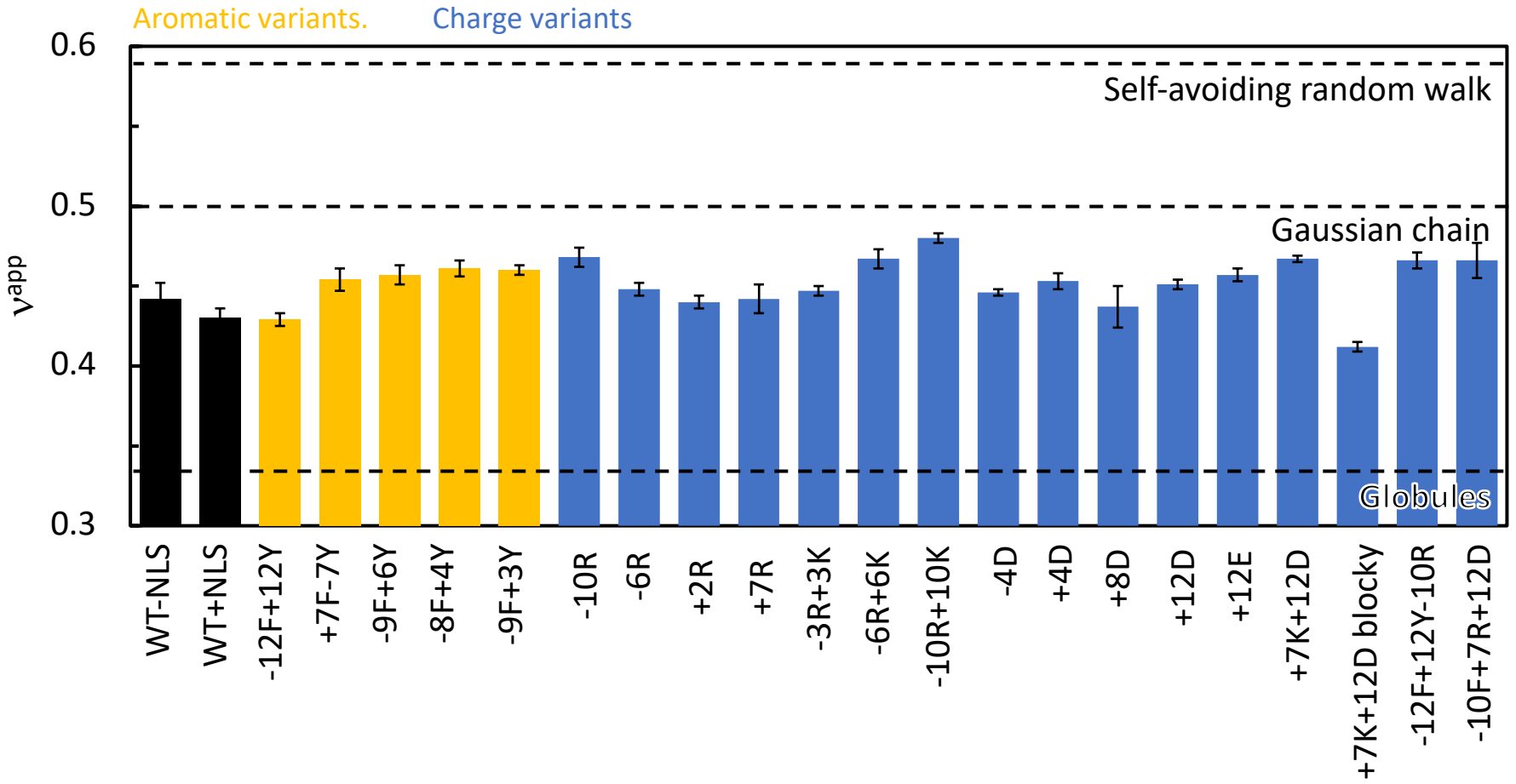




$R_g \sim N^{\nu}$

The radius of gyration of disordered proteins scales with the number of residues and a scaling exponent ν, which reflects solvent quality.

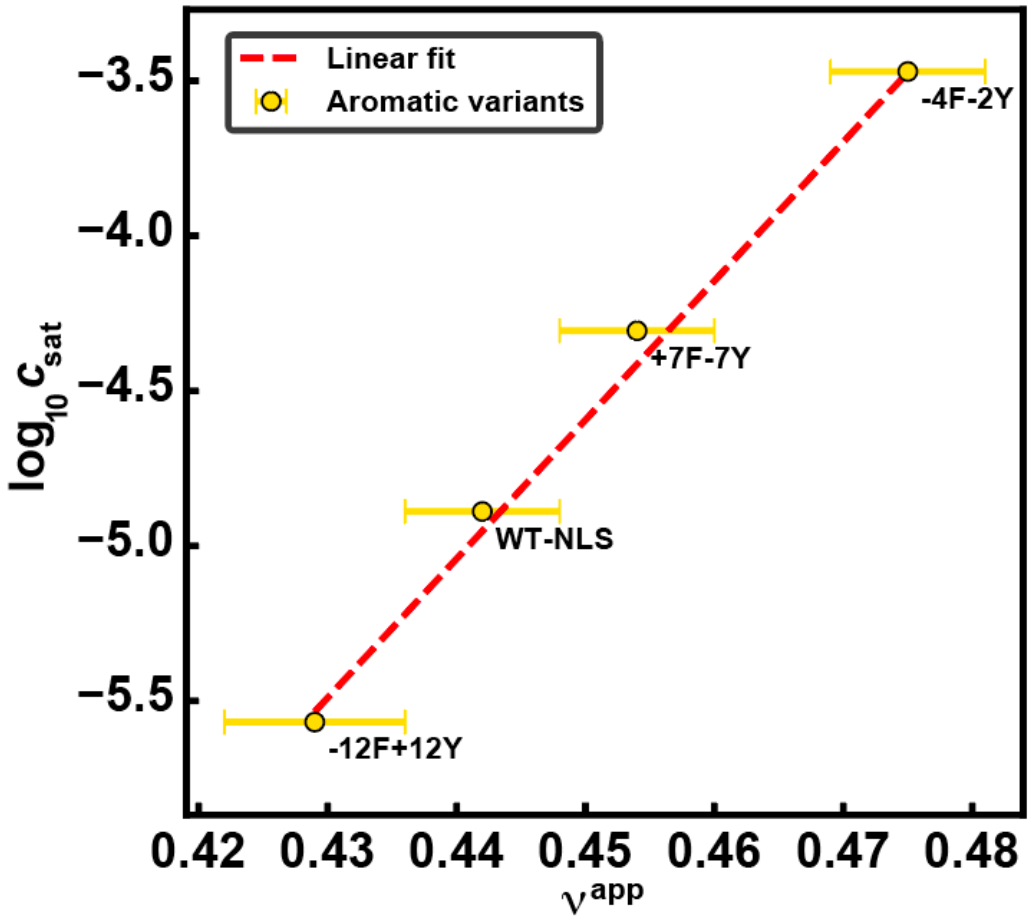
All hnRNPA1 LCD variants are compact



Buffer: 20mM HEPES pH 7.0, 150mM NaCl

Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

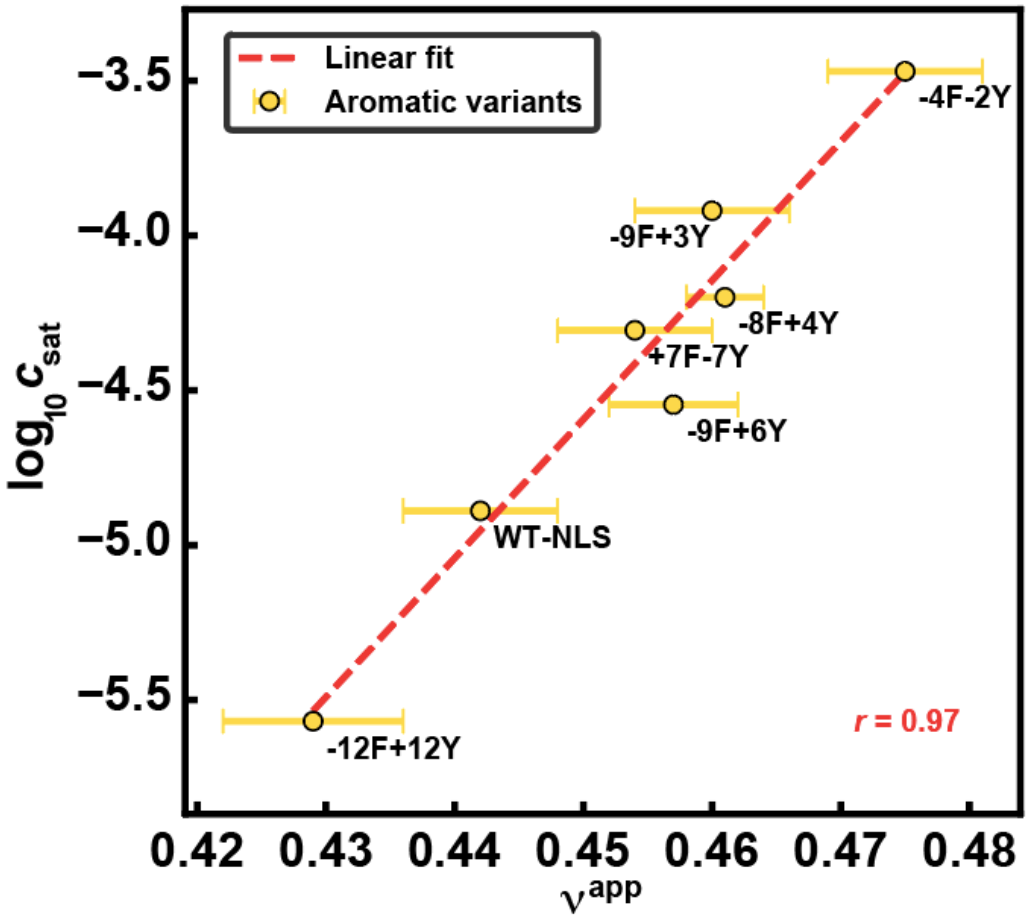
Global dimensions report on the driving force for phase separation in aromatic variants



The saturation concentration (c_{sat}) is the protein concentration above which droplets first appear at a certain condition.

Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

Global dimensions report on the driving force for phase separation in aromatic variants

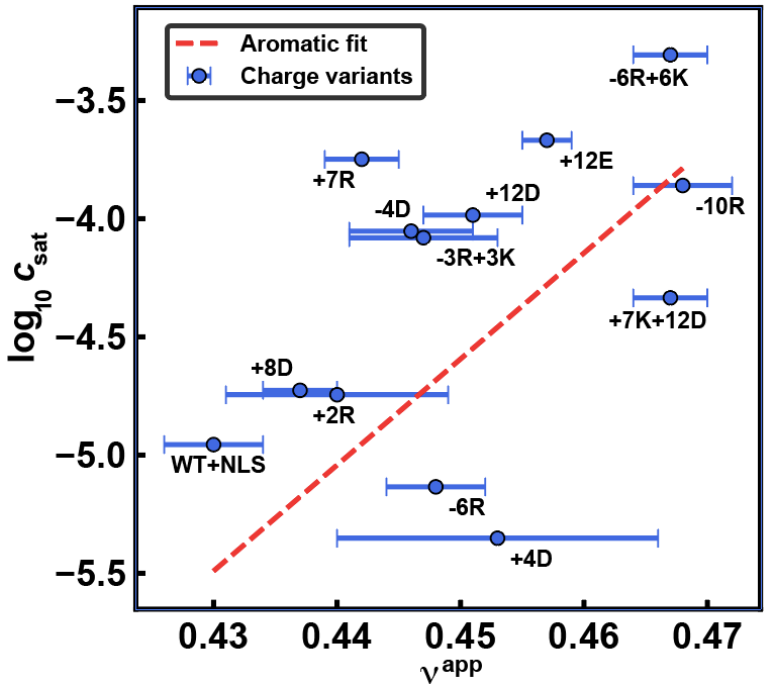


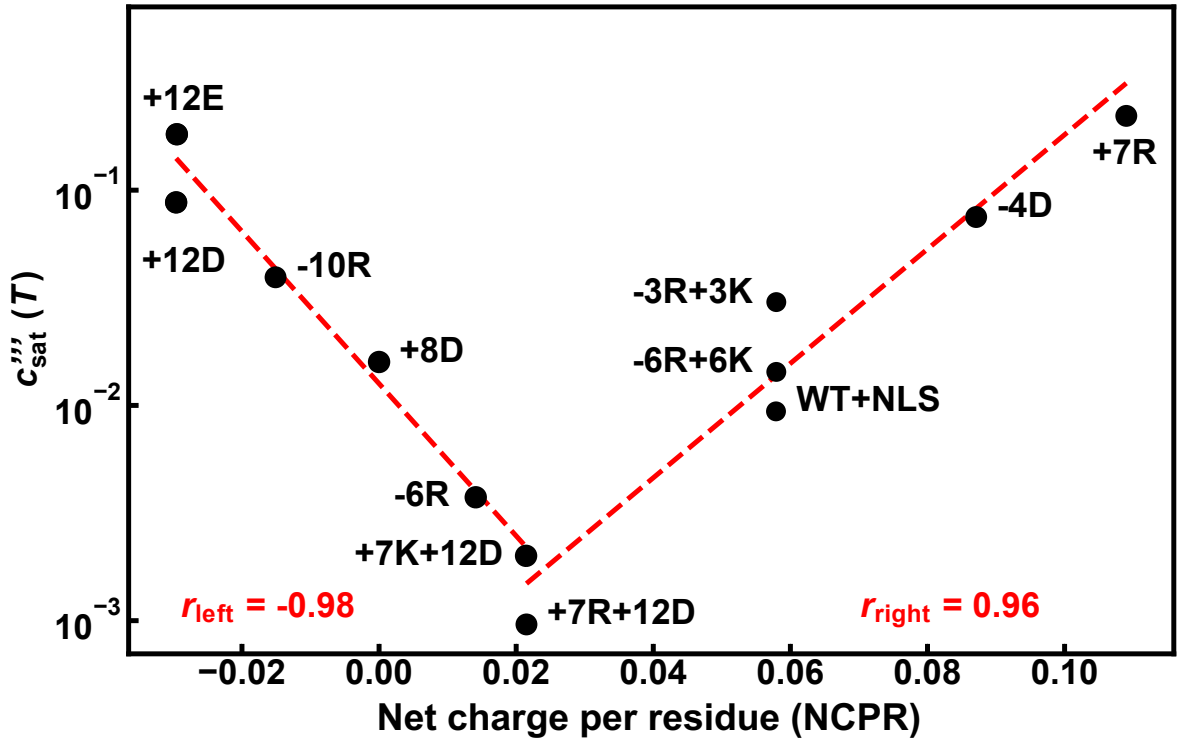
The saturation concentration (c_{sat}) is the protein concentration above which droplets first appear at a certain condition.

For aromatic variants, the global dimensions report on the driving force for phase separation.

Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

Decoupling of global dimensions and driving force for LLPS

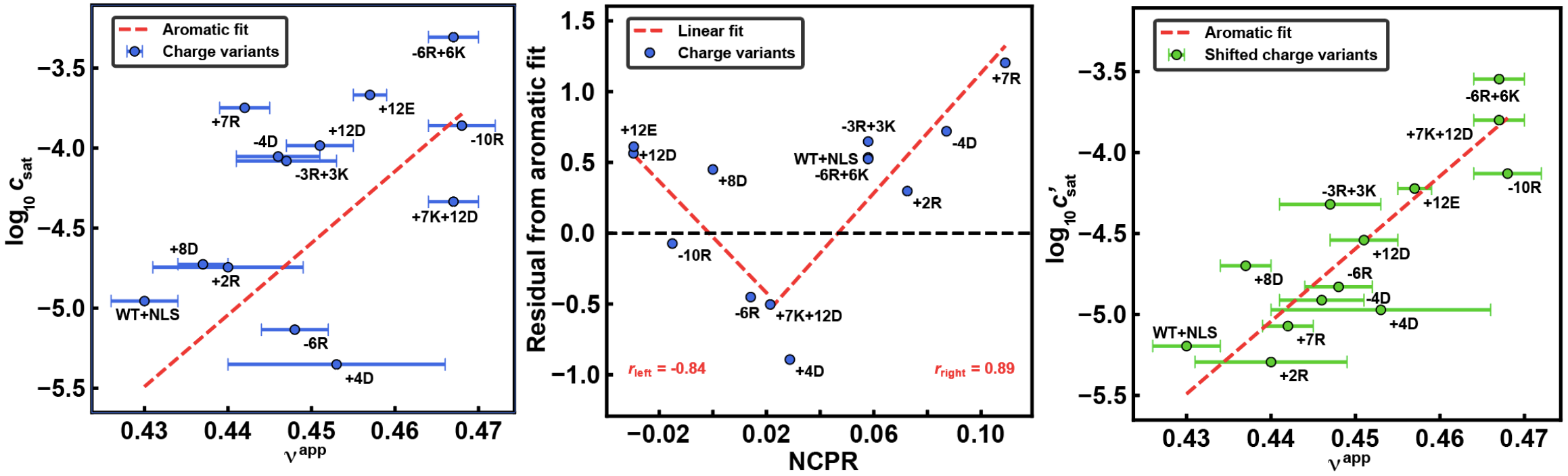




NCPR strongly modulates the driving force for phase separation.

Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

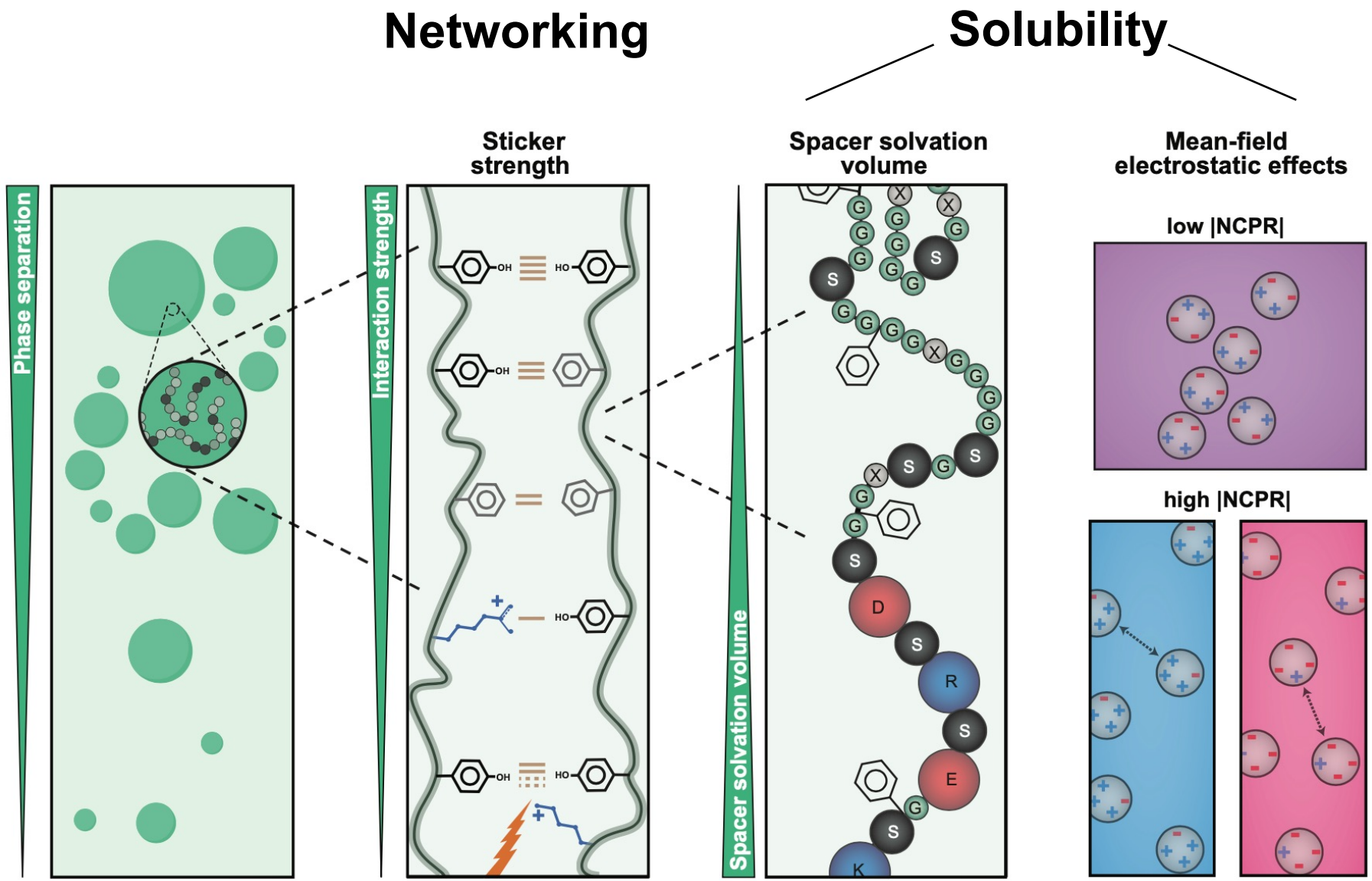
Decoupling of global dimensions and driving force for LLPS



Mean-field electrostatic interactions can decouple single-chain dimensions in IDRs and the driving force for phase separation.

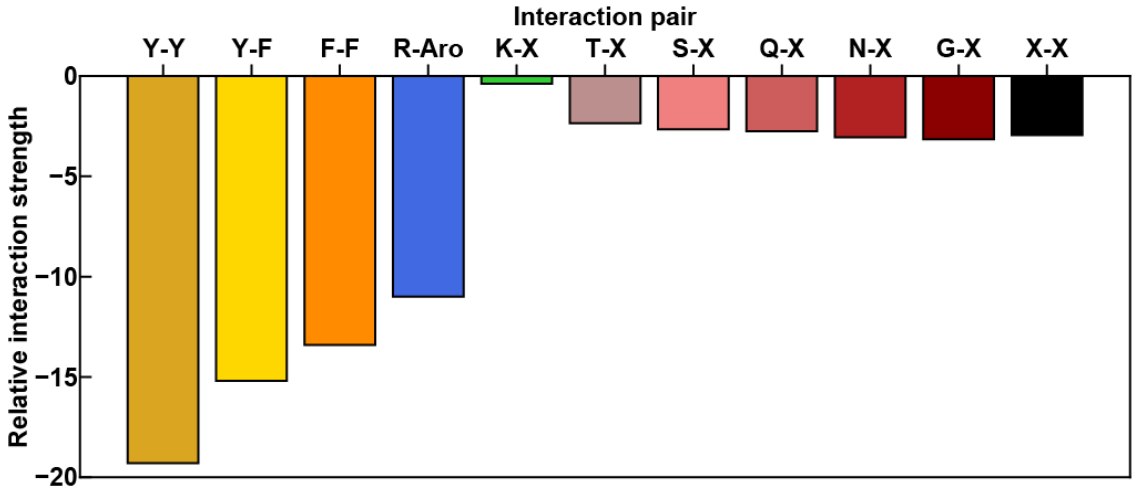
Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

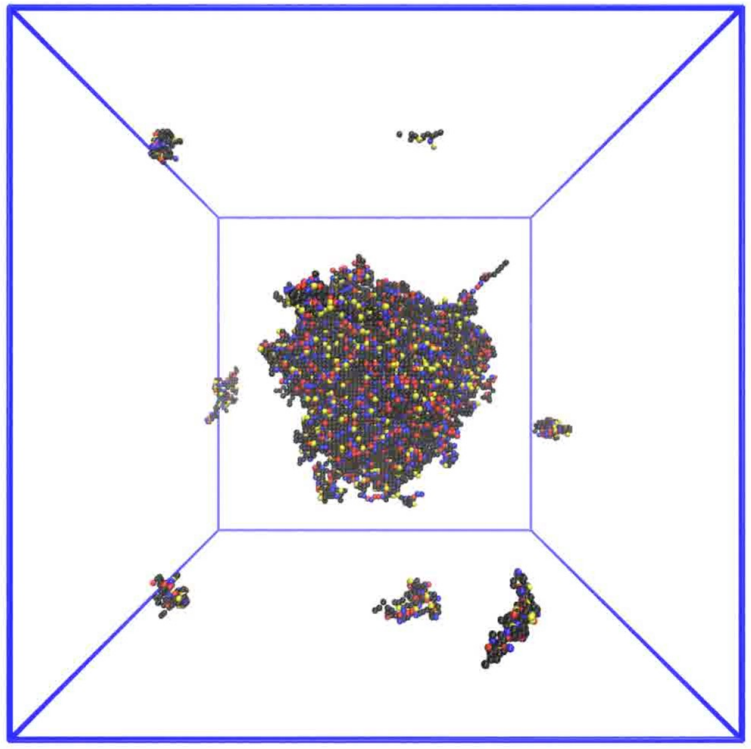
The composition of PLDs is very well conserved, not only the sticker valence.

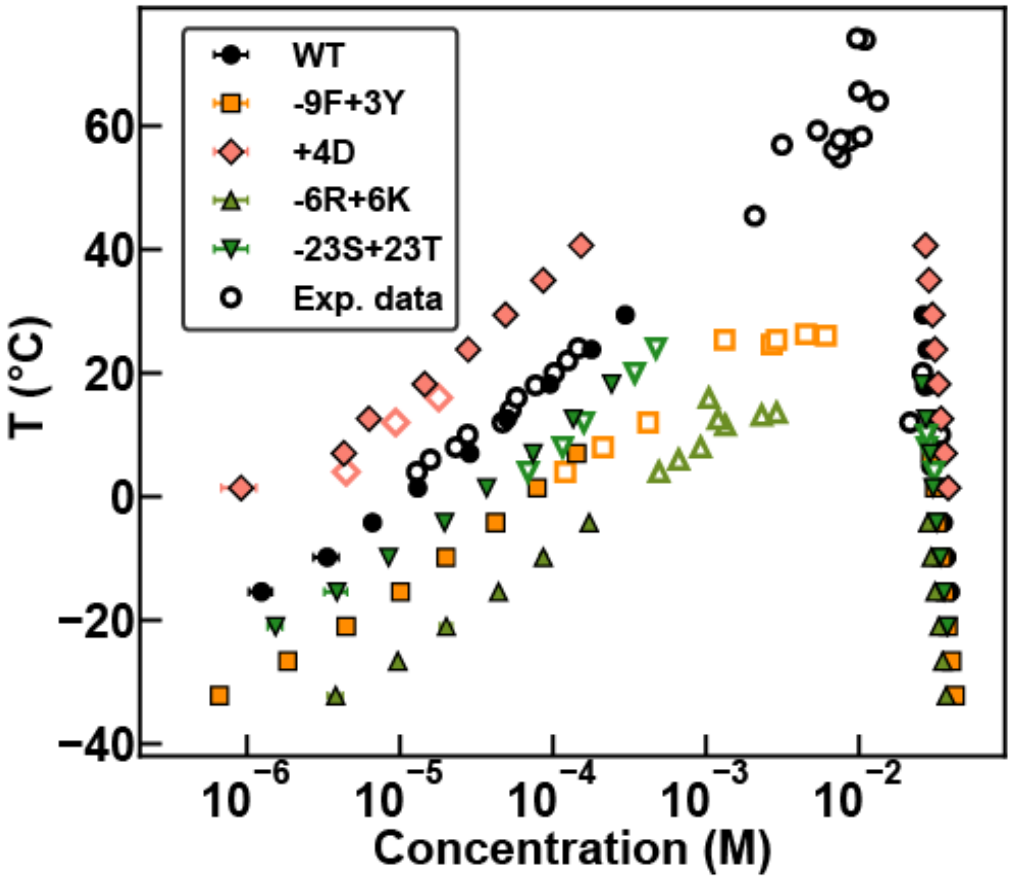


Bremer#, Farag#, **Borcherds**# , **Peran**, **Martin**, Pappu*, **Mittag***. *Nature Chemistry* 2022

Coarse-grained stickers-and-spacers model describes phase behavior of PLDs







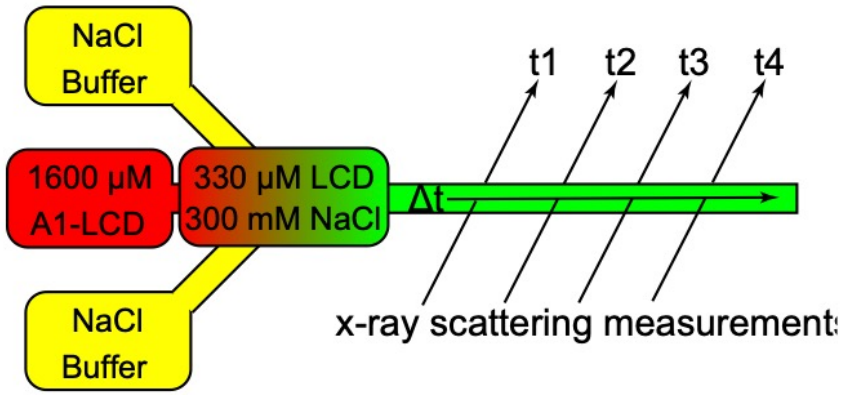
Bremer[#], Farag[#], Borcherds[#] , Peran, Martin, Pappu*, Mittag*. *Nature Chemistry* 2022

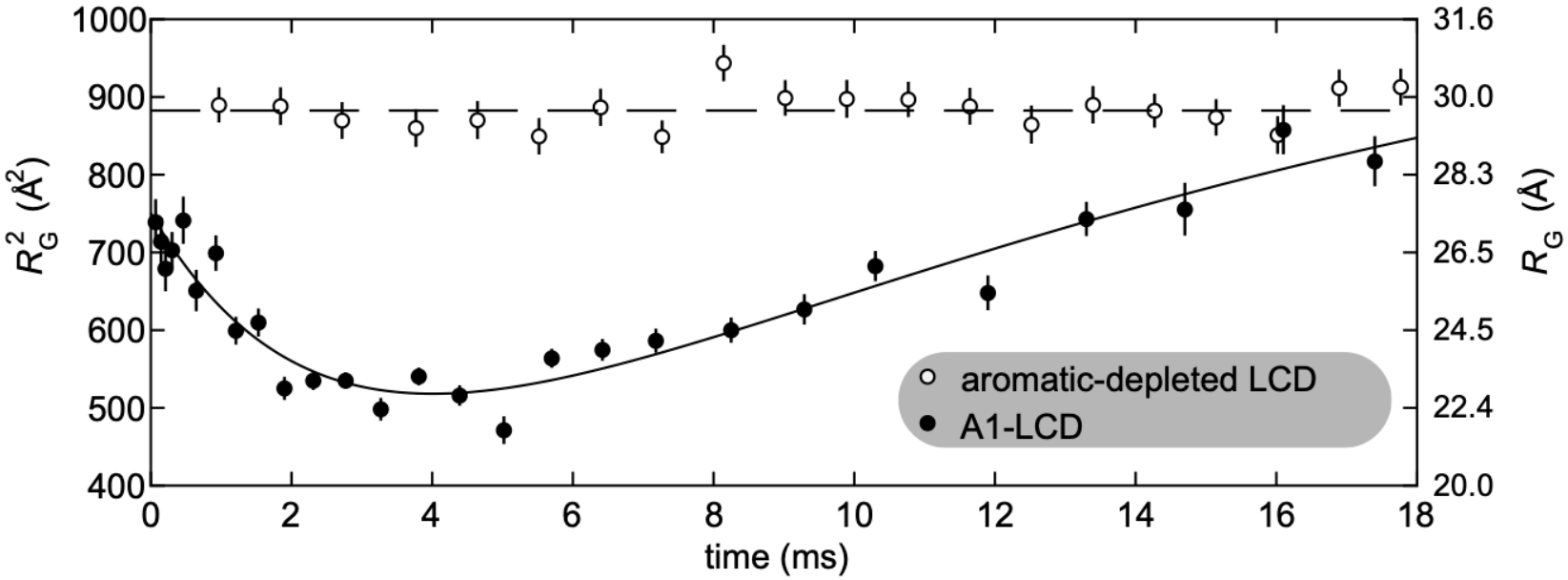
Farag, Cohen, Borcherds, Bremer, Mittag, Pappu. *Nat Commun* 2022

Kinetics of phase separation

The hnRNPA1 LCD collapses on the sub-millisecond timescale

Rapid-mixing, time-resolved SAXS experiments



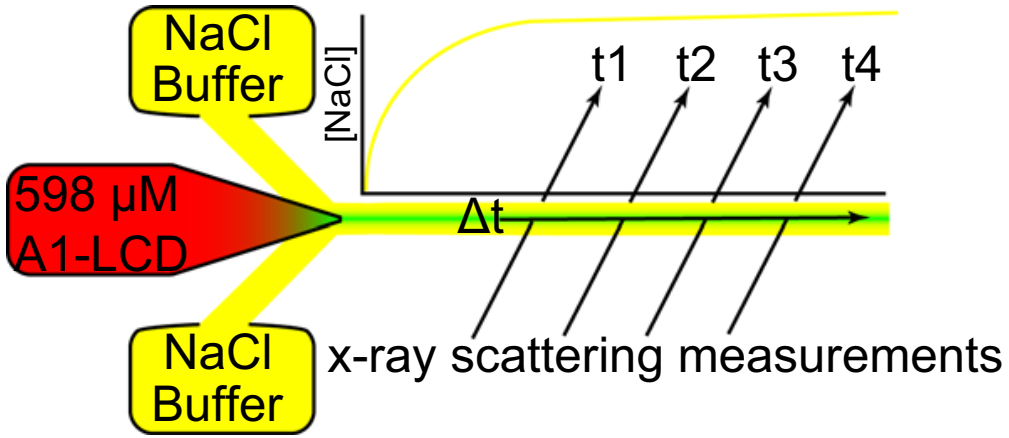


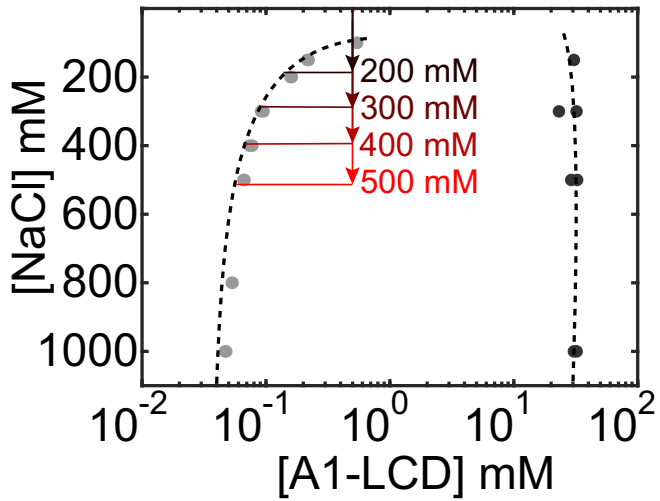
Difference in behavior between the phase separating and non-phase separating protein already after the fraction of a millisecond.

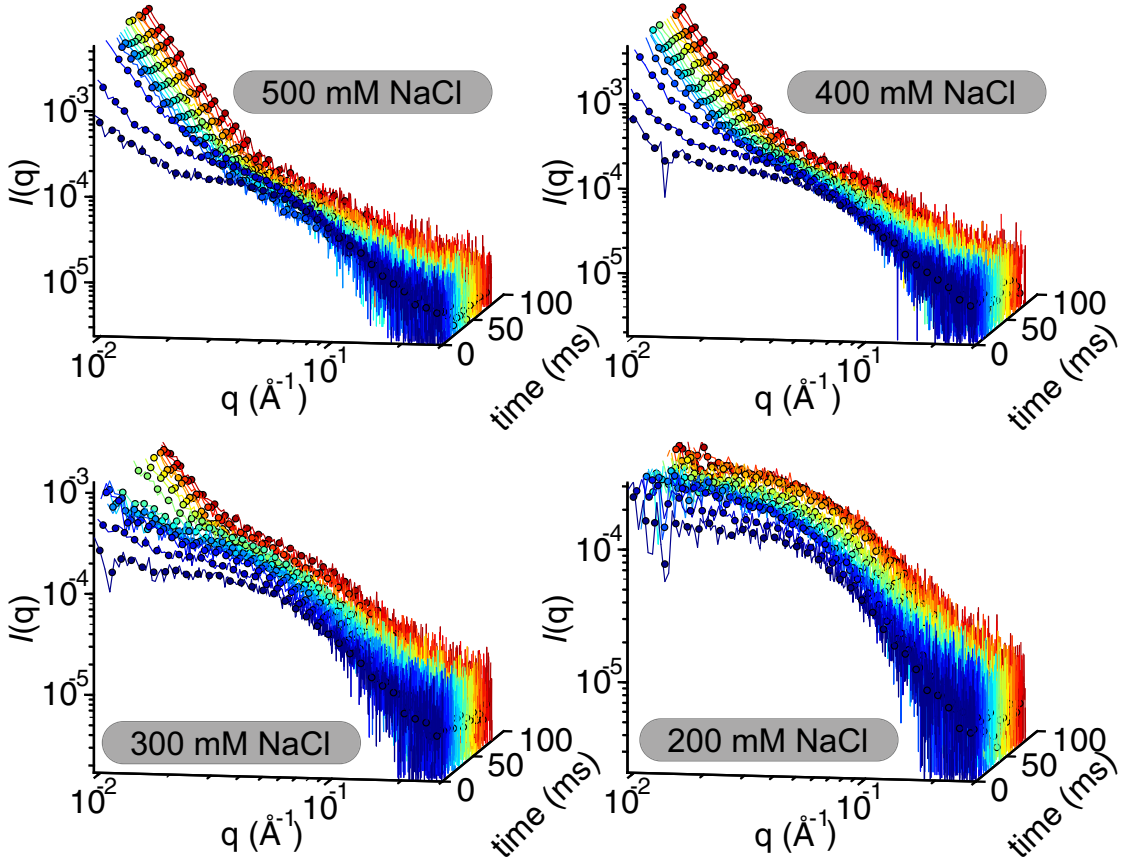
Martin, Harmon, Hopkins, Chakravarthy, Incicco, Schuck, Soranno, Mittag. *Nat Commun* 2021

Kinetics of phase separation depends on the quench depth

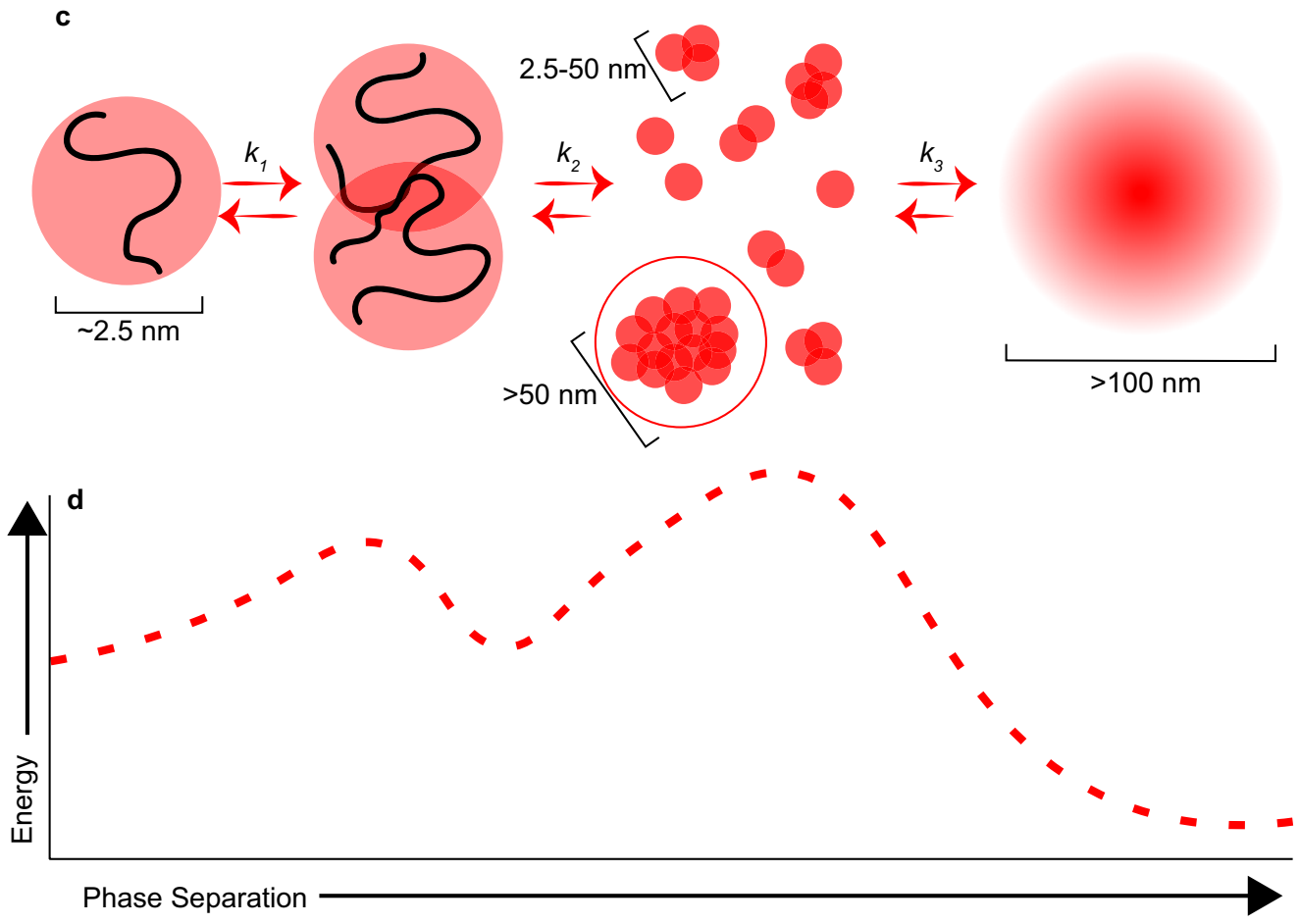
Rapid-mixing, time-resolved SAXS experiments







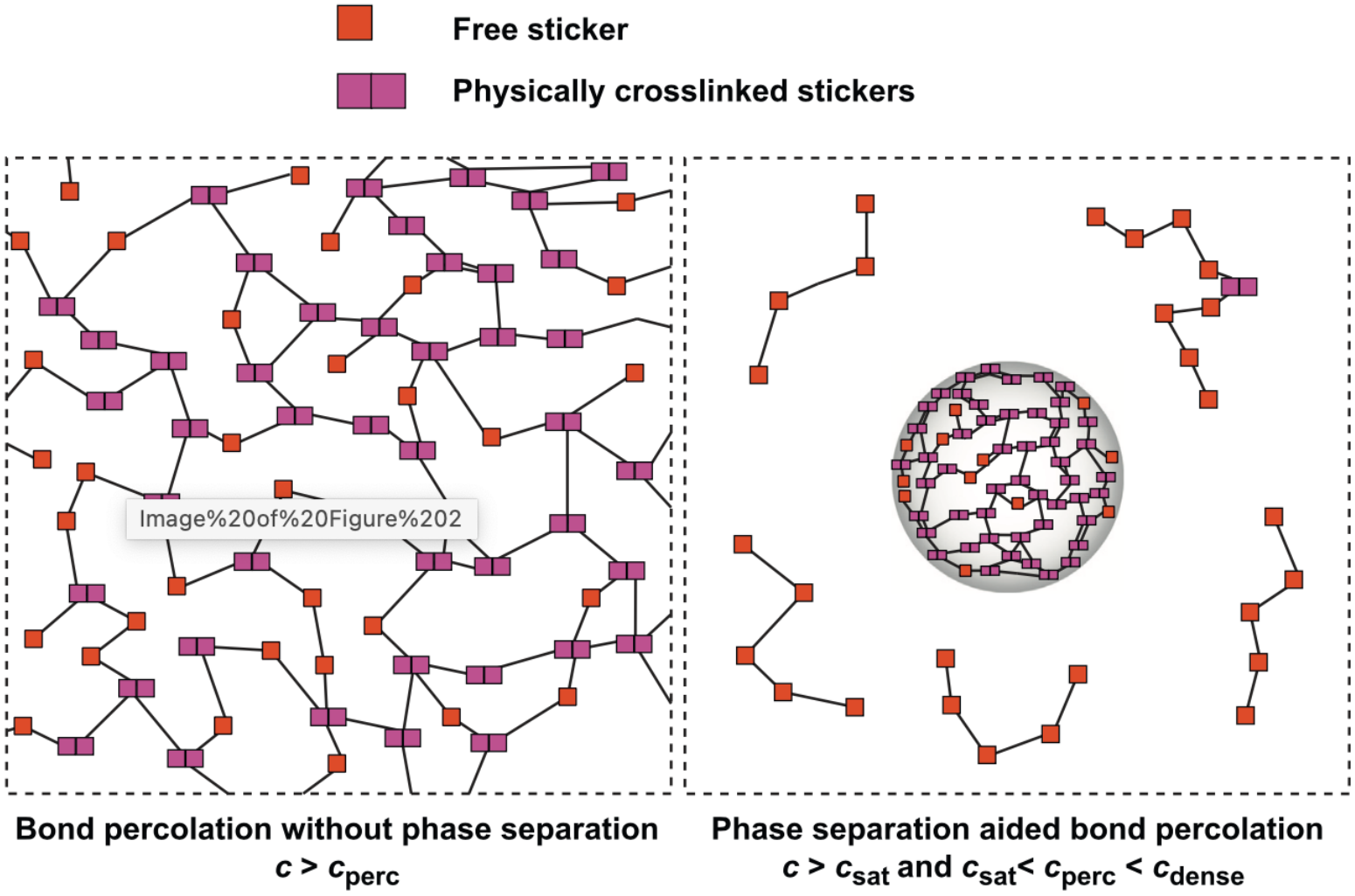
Martin, Harmon, Hopkins, Chakravarthy, Incicco, Schuck, Soranno, Mittag. *Nat Commun* 2021



Martin, Harmon, Hopkins, Chakravarthy, Incicco, Schuck, Soranno, Mittag. *Nat Commun* 2021

SAXS is useful to characterize phase separation!

Specific sticker interactions drive networking
Non-specific interactions drive the density transition



Phase separation coupled to percolation (PSCP)!

Condensate-spanning network enabled by physical crosslinking of stickers

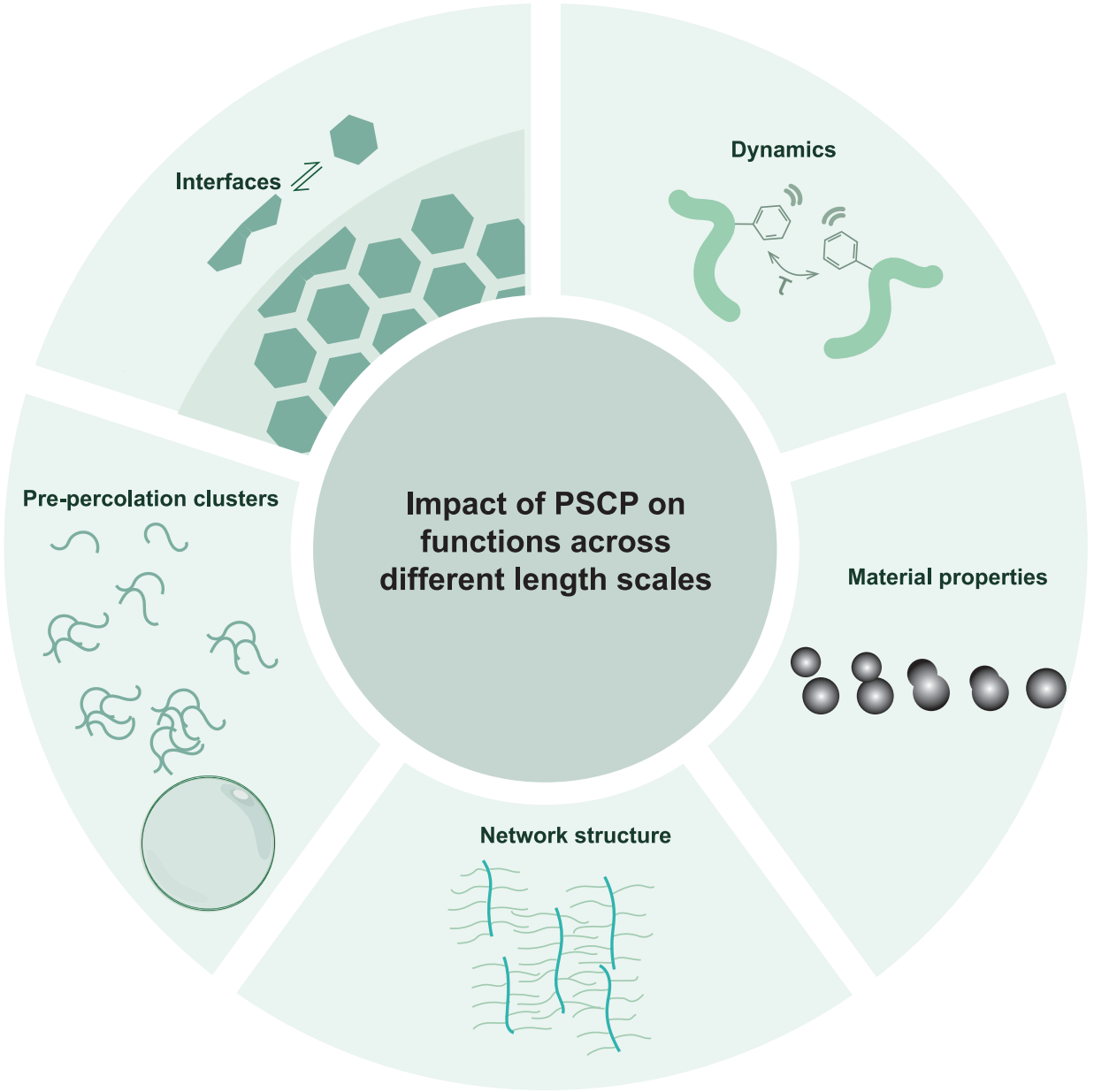
Network fluid!

Viscoelasticity


Silly putty!

Cohan & Pappu. *Trends Biochem Sci* 2020
Harmon et al. Elife 2017

Condensates as network fluids – what are properties that determine function?



PSCP: Phase separation coupled to percolation

Mittag & Pappu, Mol Cell 2022

Acknowledgements

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Rohit Pappu

Mina Farag

Andrea Soranno

Peter Schuck

Paul Taylor

Kresten Lindorff-Larsen

Former lab members

Melissa Marzahn

Wendy Pierce

Jihun Lee

Joel Otero

Nicole Milkovic

Jill Bouchard

Erik Martin

Ivan Peran

Postdoc positions available! Email me.

APS at Argonne National Lab

Jesse Hopkins

Srinivas Chakravarthy

Microscopy Facility

NMR Center

Molecular Interaction Analysis Facility

Cryo-EM Center

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder

National Institute of General Medical Sciences

Collaborative on Biology and Biophysics of RNP granules

THE V FOUNDATION for Cancer Research

National Institute of Neurological Disorders and Stroke