- 4篇文献
- 每篇3-4张幻灯片
- 尽量简化了，只抓重点
- 哪个地方讲得不够细了请老师同学指出后面再补充

LLPS and Simulations

Li Jiaqi

Presented on Sept 5th, 2020

Literatures

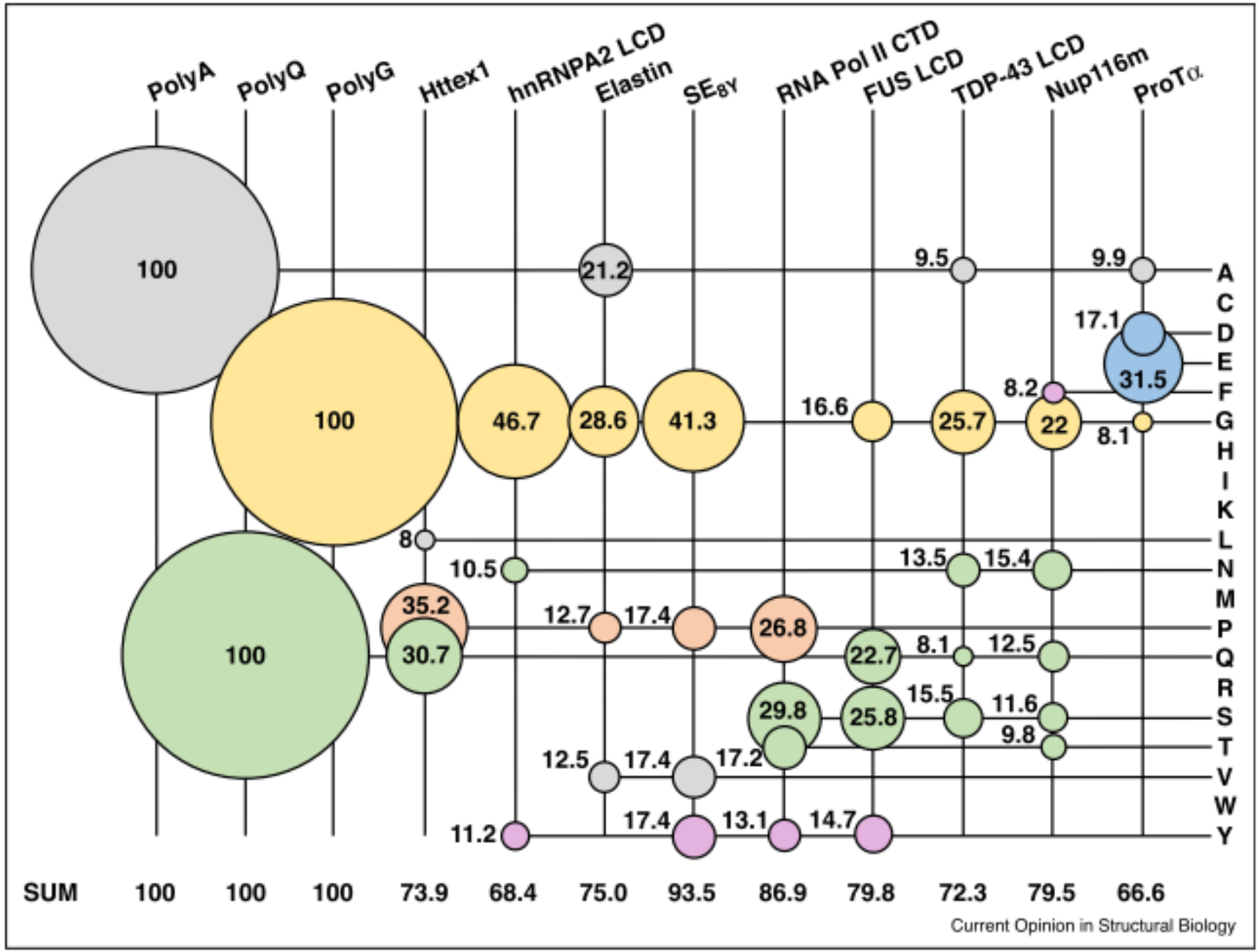
1. Conformational preferences and phase behavior of intrinsically disordered low complexity sequences: insights from multiscale simulations
2. Why Do Disordered and Structured Proteins Behave Differently in Phase Separation?
3. Three archetypical classes of macromolecular regulators of protein liquid-liquid phase separation
4. Valence and patterning of aromatic residues determine the phase behavior of prion-like domains

1. Conformational preferences and phase behavior of intrinsically disordered low complexity sequences: insights from multiscale simulations

LC的模拟按尺度分有几种?
每种有什么优势和缺点?
有什么要注意的问题?
以及一些应用的例子

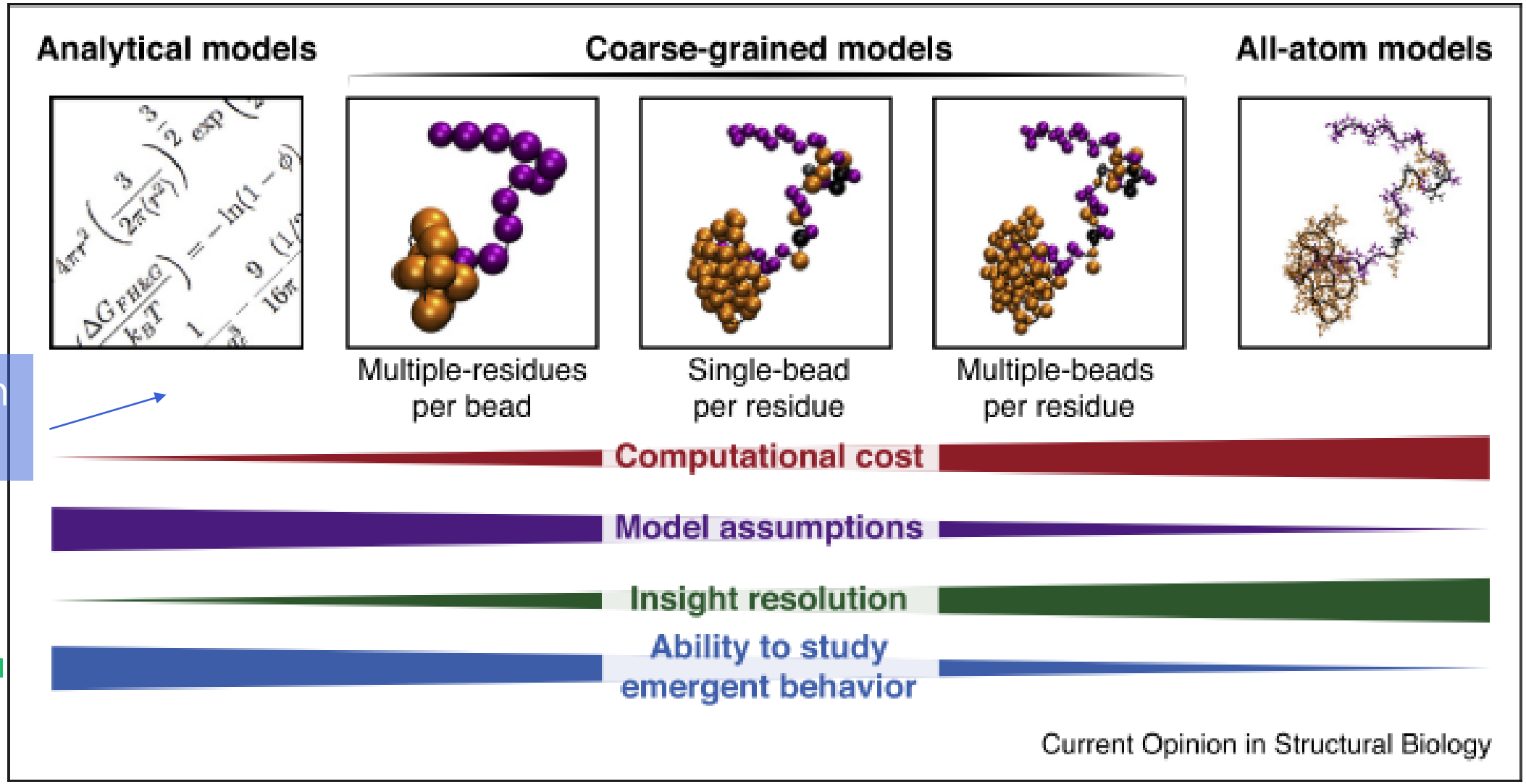
Research Objects --- Low Complexity (LC) Sequences

- IDRs are not necessarily low complexity and LC sequences are not necessarily intrinsically disordered.



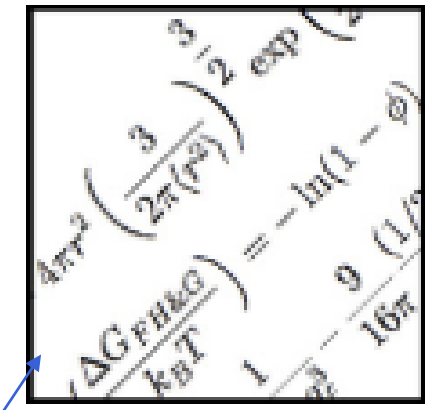
Comparison of Different Methods

huntingtin exon 1



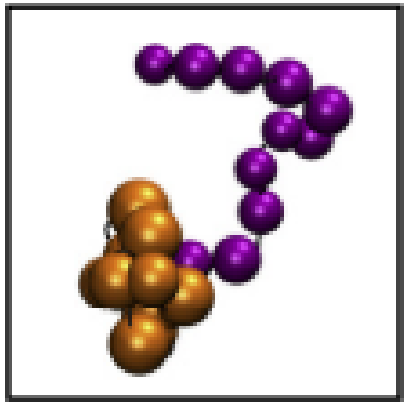
Some Details about the Methods

Analytical models



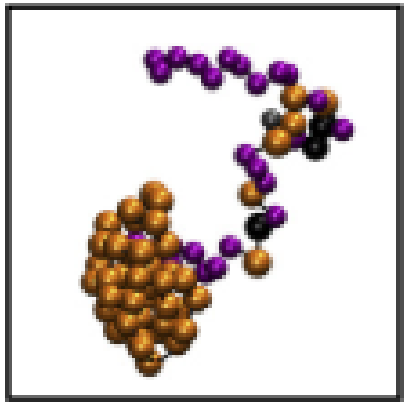
Homopolymer higher-complexity sequence

Coarse-grained models



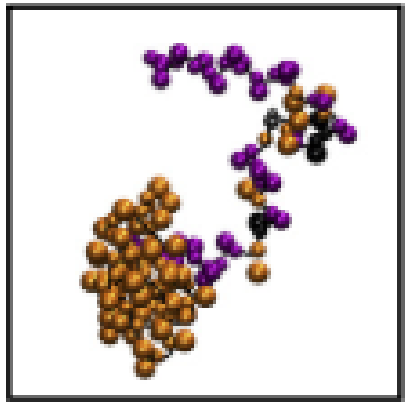
Multiple-residues per bead

1. homoproteins or perfect repeats of a short peptide
2. mapping and parameterization usually rely on results from atomistic simulations



Single-bead per residue

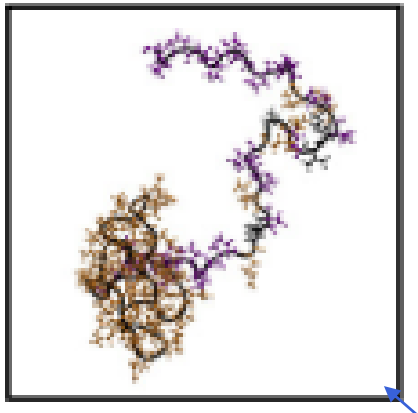
1. hydrophobicity scales
2. highly charged



Multiple-beads per residue

1. transferable models and system-specific models
2. two approaches for systematically determining parameters: structure-based and force-matching methods

All-atom models



1. 'infinite resolution' at infinite dilution
2. extracting
3. Limitations: computational cost and underlying forcefield

Why Do Disordered and Structured Proteins Behave Differently in Phase Separation?

为什么无序蛋白比结构蛋白更容易相分离?

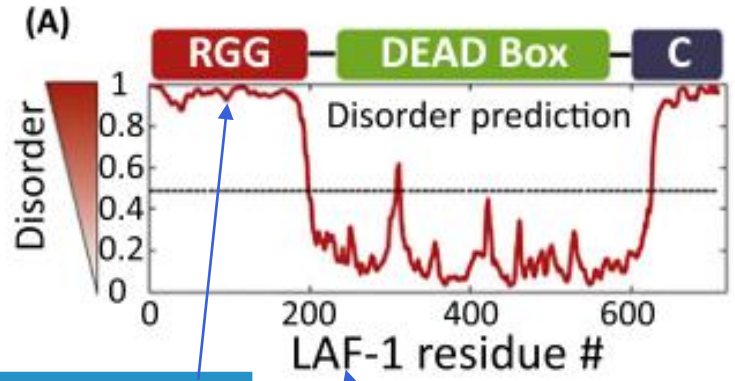
- 结构蛋白容易直接结晶
- 无序蛋白不能结晶，但是可以形成胶状或者纤维状沉聚物
- 都可以发生相分离的情况下，通常无序蛋白的临界温度更高（UCST）、饱和浓度更低

Basic Knowledge

- Phases and Determination of Coexistence Curves
- Free Energy Calculation: Lattice Models and Mean-Field Treatment (Flory-Huggins)
- Free Energy Calculations: Perturbation Theory for Colloids（胶质物）——有吸引力、有刚性体积的小球
- Free Energy Calculations: Perturbation Theory for Polymers —— 考虑单体之间成键

Disordered Regions and RNA

intrinsically disordered regions (IDRs) sometimes have RNA-binding ability



The RNA Binding Region

LAF-1: a disordered driver protein for P granules

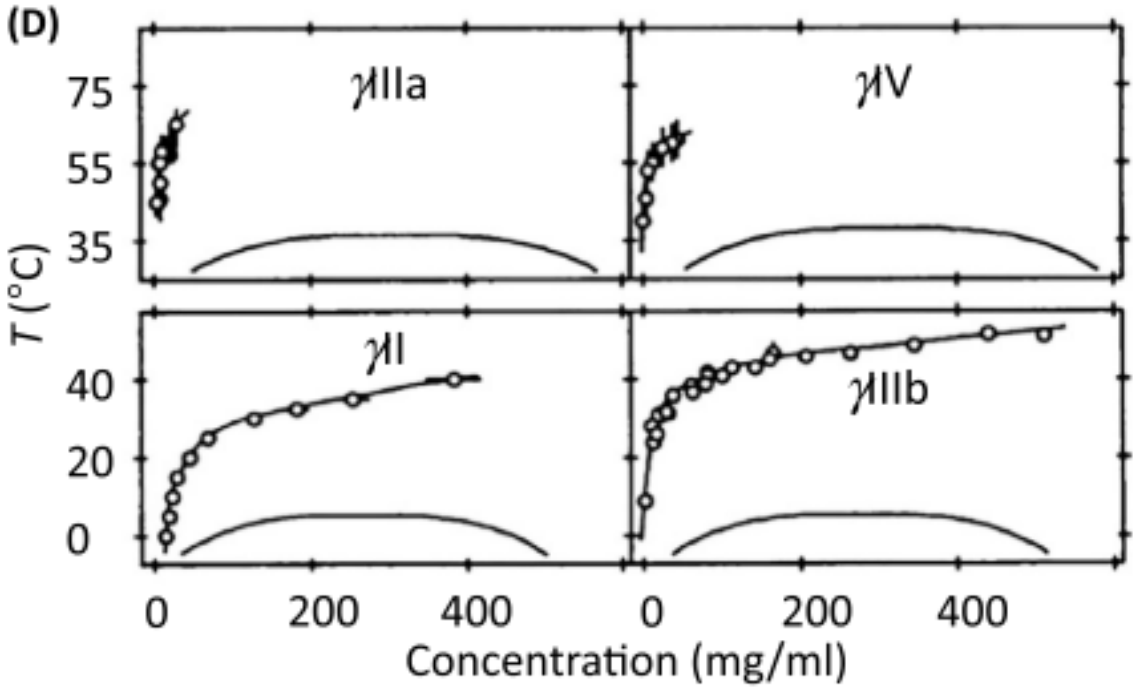
Table 1. Disordered Regions and RNA-Binding Domains of Proteins That Drive the Formation of Membraneless Organelles

Driver protein[a]	IDRs[b]	Enriched amino acids	RNA binding	MLOs	Refs
DDX3_CAEEL (708) D0PV95 LAF-1/DDX3	1–202 620–708	9–168: R/G 624–691: G	N-term IDR (RGG repeats)	P granule	[13,29]
DDX4_HUMAN (724) Q9NQI0 DDX4	1–257	58–234: G	N-term IDR?	Nuage	[17]
FBRL_CAEEL (352) Q22053 FIB1/fibrillarin	1–122	8–114: DMA/G	N-term IDR? Fibrillarin domain (119–346)?	Nucleolus	[10]
FUS_HUMAN (526) P35637 FUS	1–286 356–526	1–165: Q/G/S/Y 166–267: G 371–526: R/G	RRM (287–365)	Stress granule Paraspeckle DNA damage site	[12,14,16,47]
IF4F2_YEAST (914) P39936 eIF4G2	1–104 111–389 454–581 812–914	32–97: N 459–510: R/S 840–863: R/S	MIF4G (567–810)	Stress granule	[14]
LSM4_YEAST (187) P40070 LSm4	88–187		LSM (5–81)	P body	[14]

RNA had little effects on the threshold protein concentration and critical salt concentration, but significantly reduced the protein concentration in the droplet phase.

Structured Regions

1. A metastable step on the way to crystallization.
2. The metastability (Figure 1D) can be attributed to the small ratio between the ranges of attractive interactions and the diameters of the proteins, as demonstrated on spherical models of colloidal particles.
3. Kinetically, the metastable droplets rich in proteins can facilitate nucleation and thereby accelerate crystallization

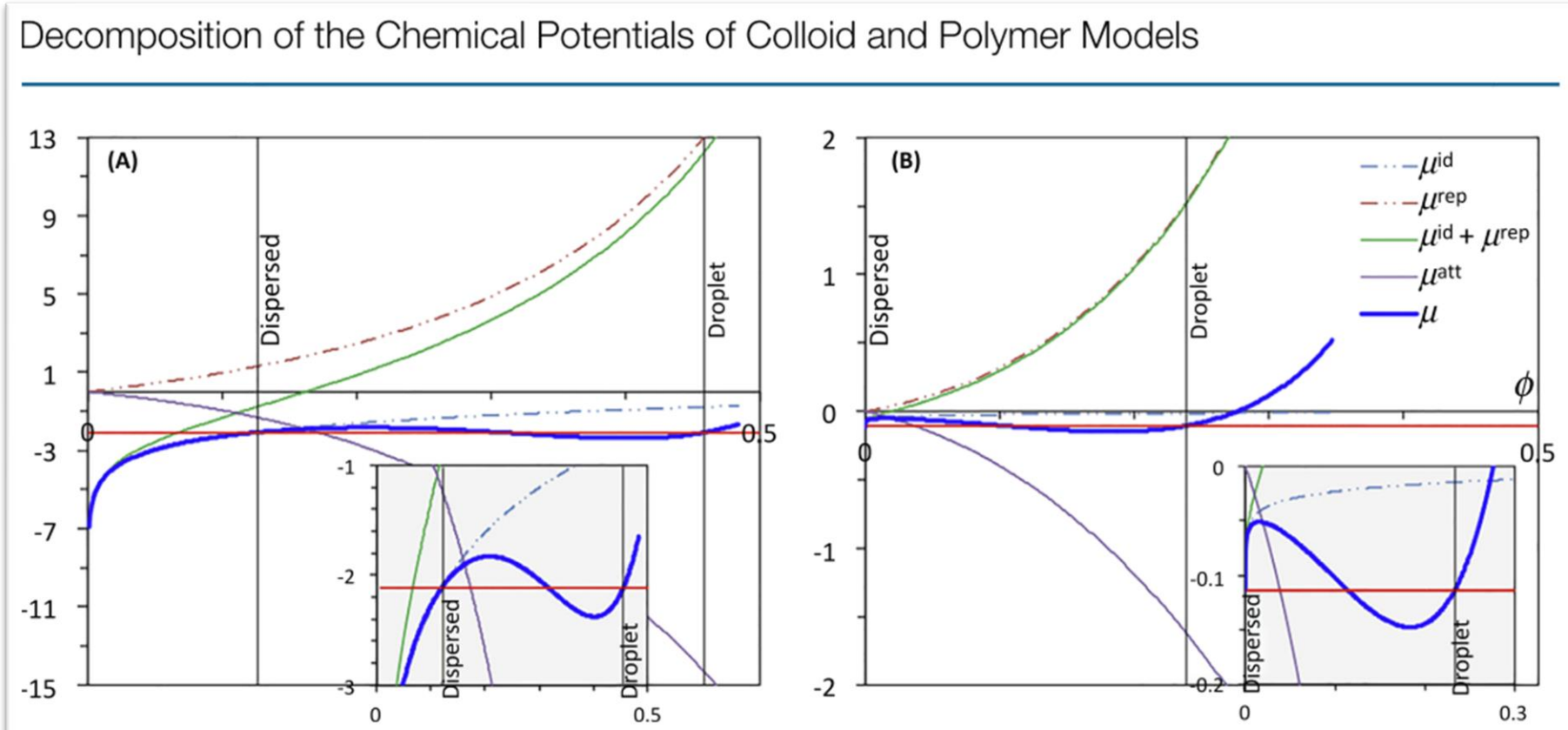


(D) Phase diagrams of four types of g-crystallins. Upper curves represent the fluid branch of the fluid–solid phase diagram, and lower curves are fits to liquid–liquid coexistence data.

Explanation for Contrasting Phase Behaviors of Disordered and Structured Proteins

- 横轴是ϕ，纵轴是μ。左边是Colloid，右边是Polymer。$\mu = \mu^{id} + \mu^{rep} + \mu^{att}$（理想、排斥、吸引）。Id和rep都是递增的，favor低浓度，而att favor高浓度。
- 结构蛋白类比Colloid，无序蛋白类比Polymer。
- Polymer的化学势低，因为一般成键是放能的。注意两边纵轴有数量级上的差别。

- Colloid的id在一开始升的很快，然后rep也跟着升，att一直到比较高浓度才能赶上，所以如果相分离的话，droplet相浓度就很高，而且dispersed相浓度不能太低，不然太稳定，没有与之对应的droplet相。Polymer的id就很小，rep也升得慢，att很快就赶上了。
- 总的来说，还是因为Polymer里每个单体之间都可以相互作用。链的柔性(flexibility)越好（像IDPs），越易形成高价相互作用。



3. Three archetypical classes of macromolecular regulators of protein liquid-liquid phase separation

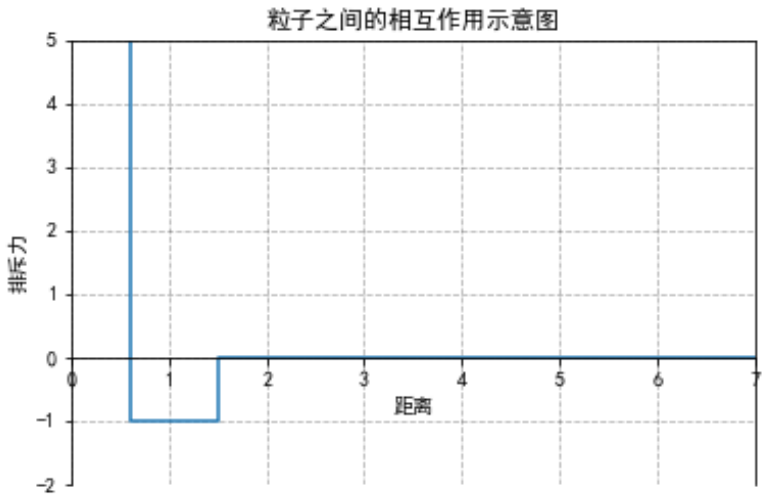
实验+模拟(Coarse-Grained)

用分子模拟遍历LLPS的三种调控类型

The Model

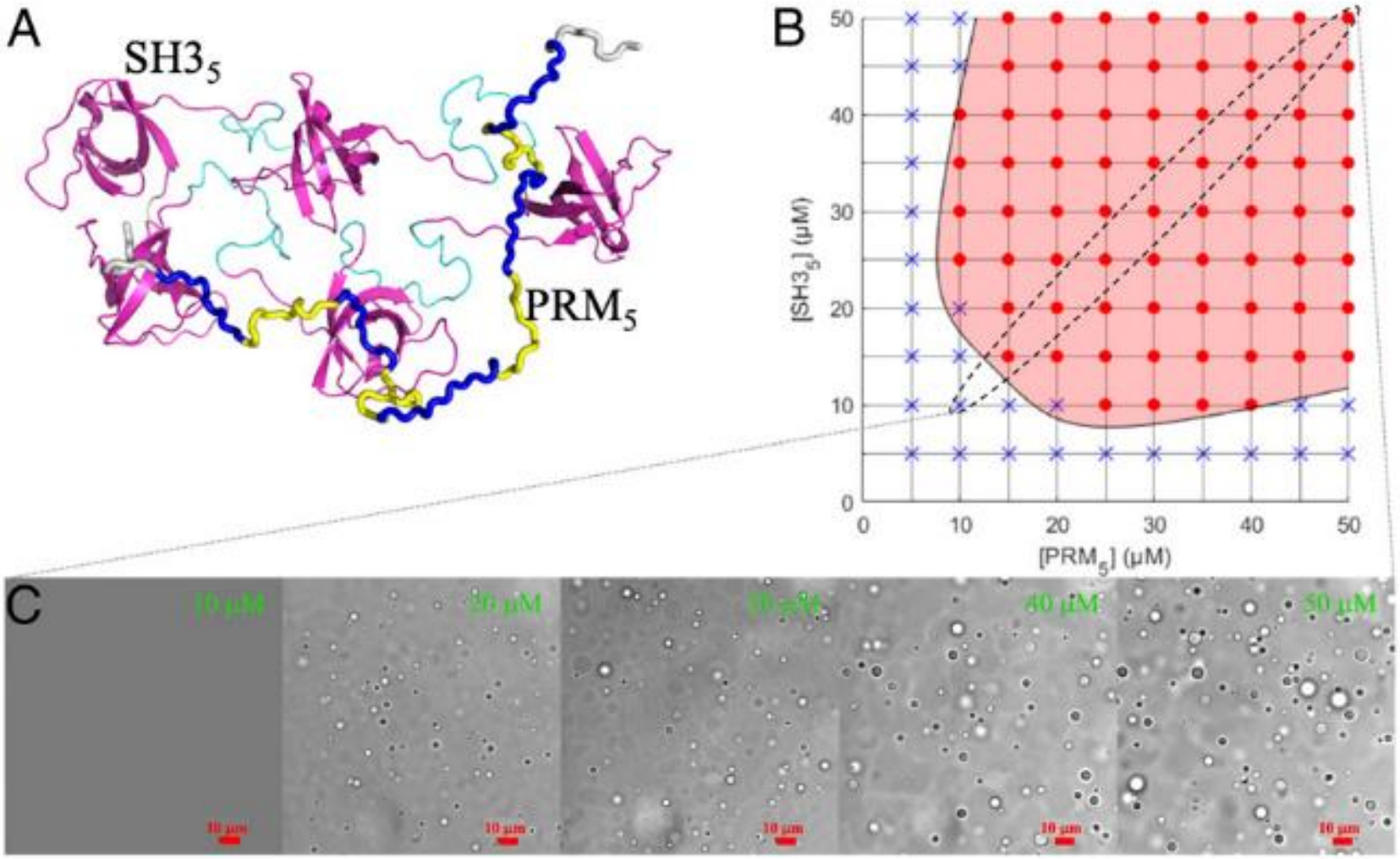
- The effects of regulators:
  1. critical temperature (Tc) or threshold concentration (Cth)
  2. partition coefficient (PC), i.e., the ratio of the regulator's concentration in the droplet phase to that in the bulk phase
- Drivers and regulators were represented as patchy particles:
  1. Steric repulsion: too close
  2. Attraction: patches are in contact.

- Strength of driver–regulator attraction: ε_{PR}(sweeping)
- Strength of driver-driver attraction: ε_{PP}(fixed)



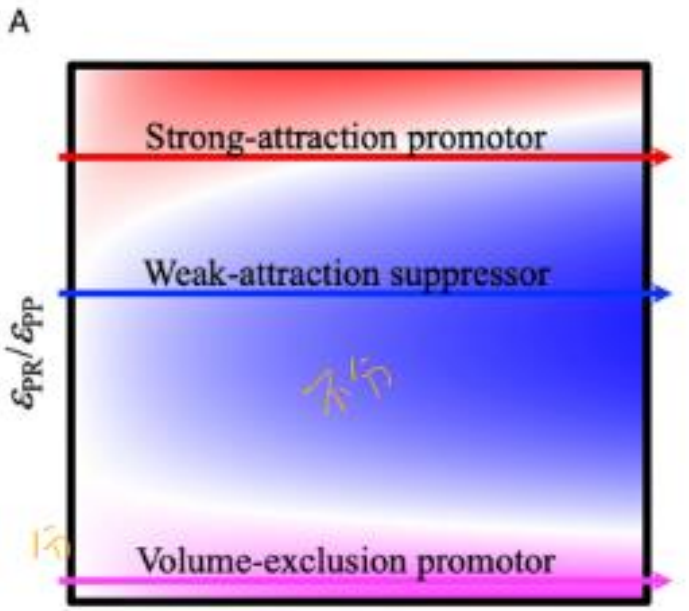
Simulated System

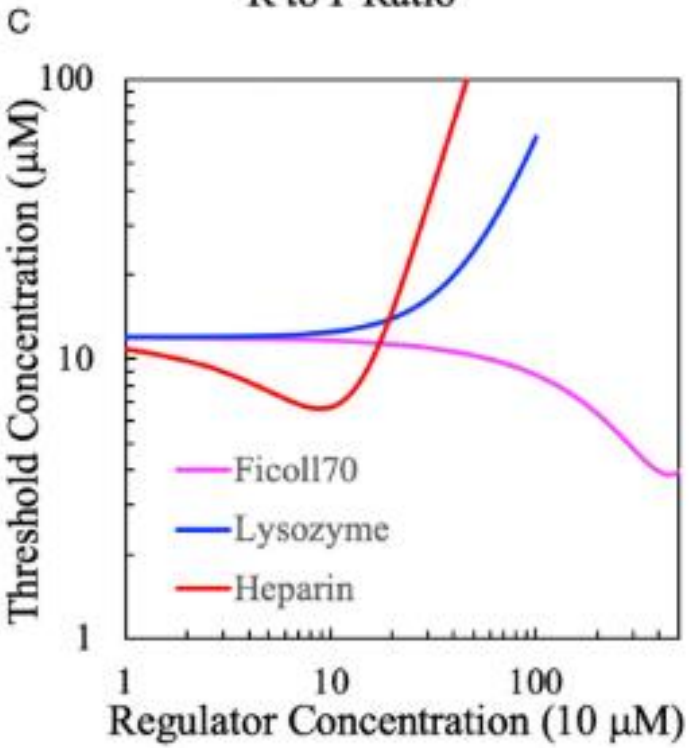
- pentamers of SH3 domains and proline-rich motifs (SH35 and PRM5)

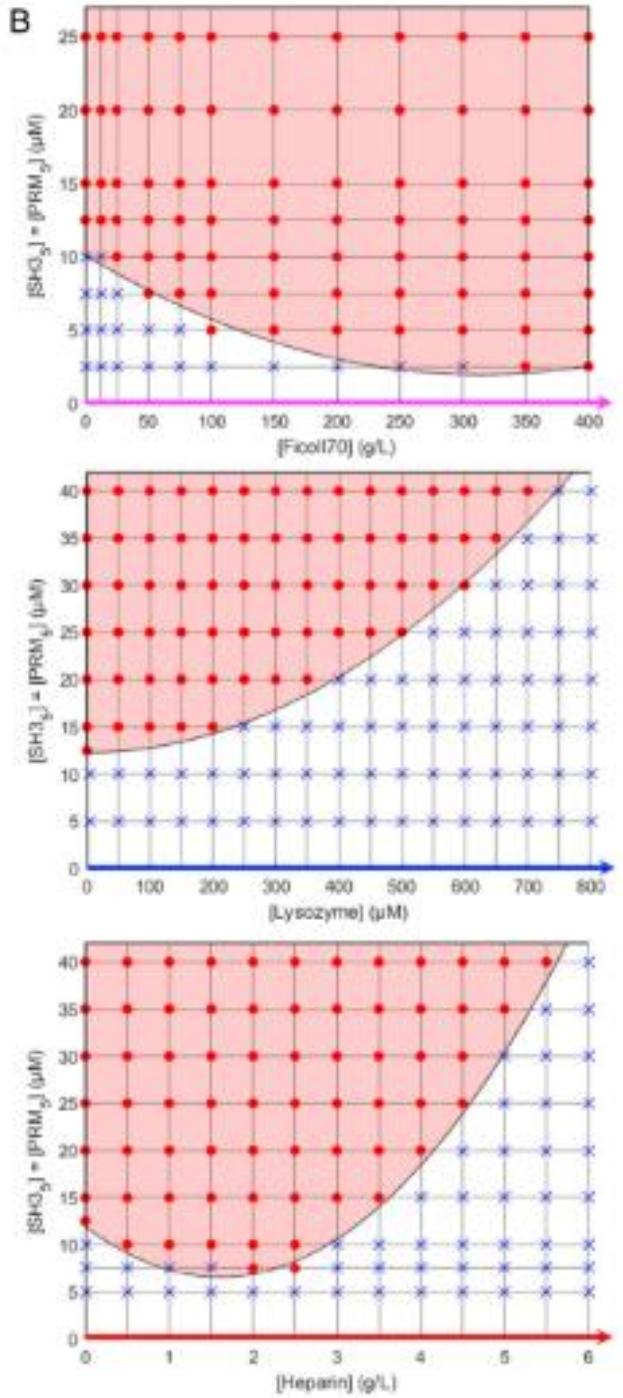


Results (Threshold Concentration)

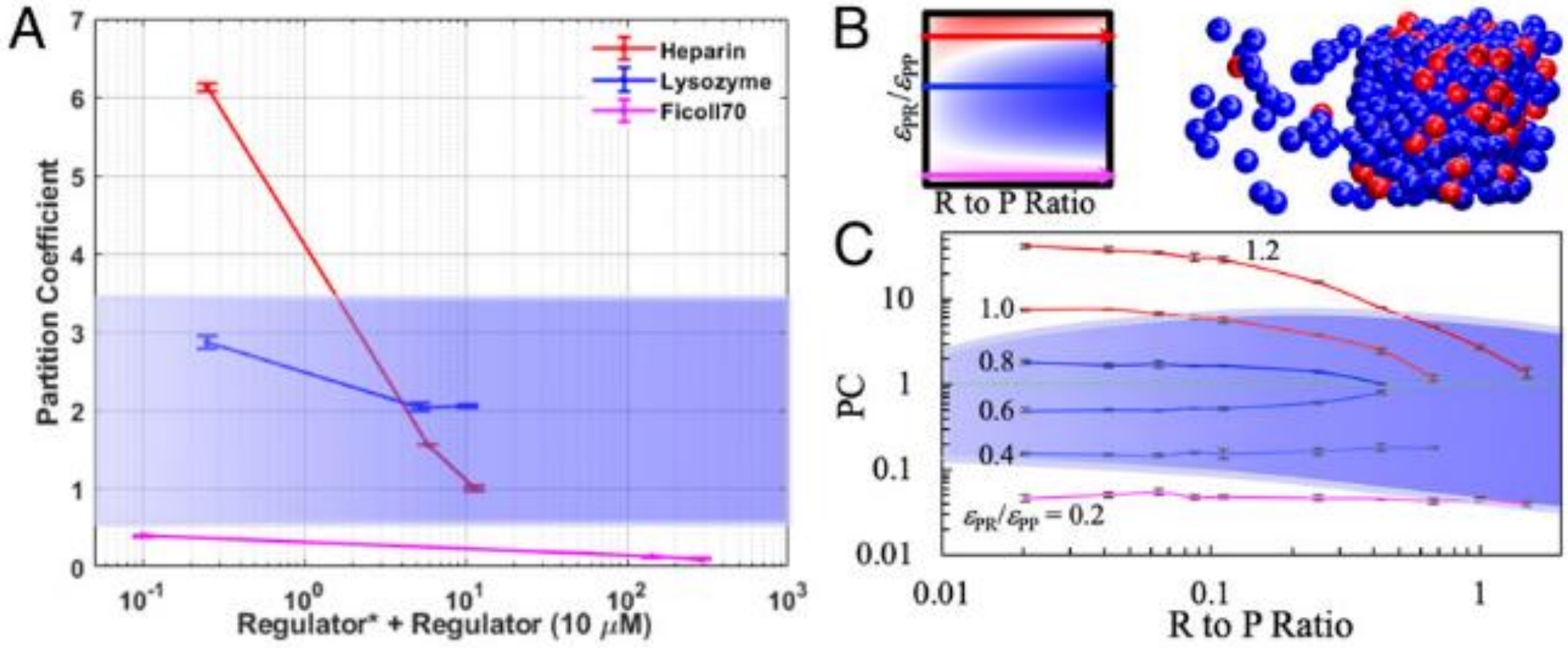
- Ficoll 70： 与Driver之间相互作用弱，促进相分离；因为可以占据聚集相以外的体积，促使Driver进入液滴
- lysozyme: 阳离子；与Driver之间有弱的吸引力，抑制相分离；因为吸引力会使得Regulator进入液滴，但是吸引的程度又没有本身Driver之间强，所以Driver更难凝聚了
- Heparin: 阴离子；与Driver之间有强的吸引力，低浓度促进，高浓度抑制；低浓度增强吸引，高浓度体积排阻







Results (Partition Concentration)



蓝色：抑制
蓝色上面：strong-attraction promotion
蓝色下面: volume-exclusion promotion

本文：
模拟的好处是可以确定只有这三种调控类型
还有一点关于改变两种Driver比例的结果

Valence and patterning of aromatic residues determine the phase behavior of prion-like domains

实验+模拟

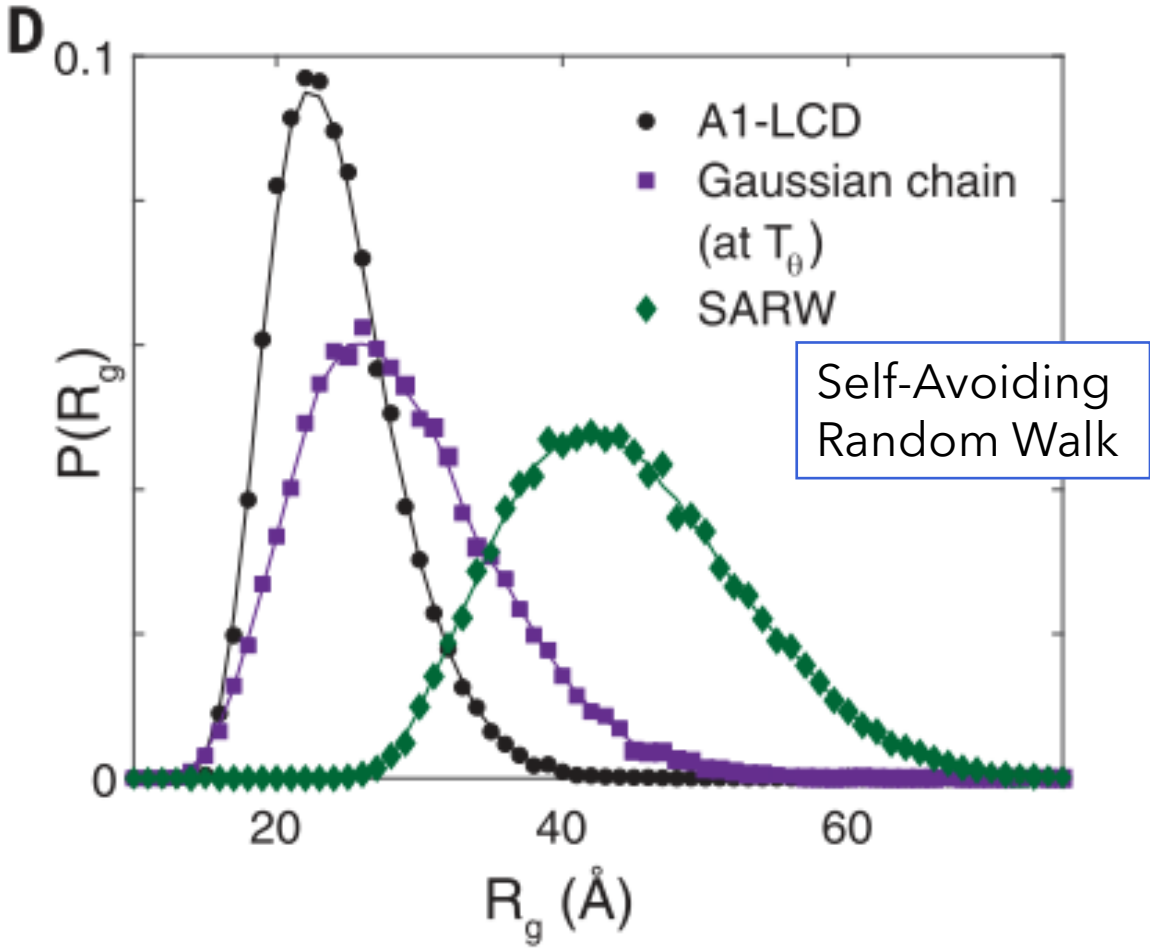
芳香族氨基酸的影响：价态（个数）和排列方式

Prion-Like Domains

- PLDs: prion-like domains; intrinsically disordered; exist in many phase-separation-driving proteins
- PLDs have distinctive amino acid compositions: They are enriched in polar amino acids and are often punctuated by aromatic residues.
- hnRNPA1 (A1-LCD): PLD or LCD

Sticker-Spacer Model

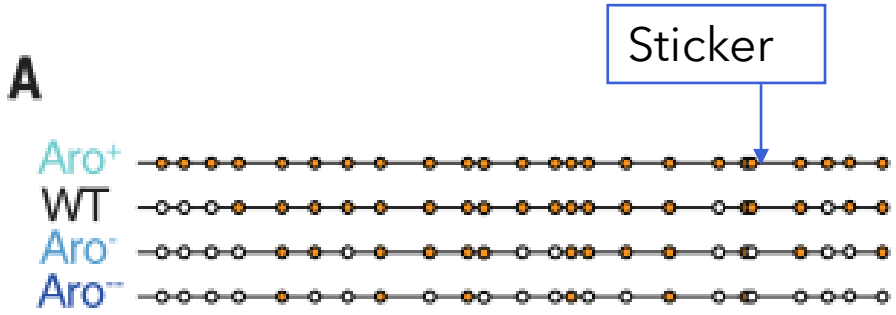
- All-atom simulations based on the ABSINTH
- Stickers can be patches on folded domains or sequence motifs within disordered regions that can be as small as individual residues.
- Spacers are residues that are interspersed between stickers.
- Aromatic residues are stickers in this study.

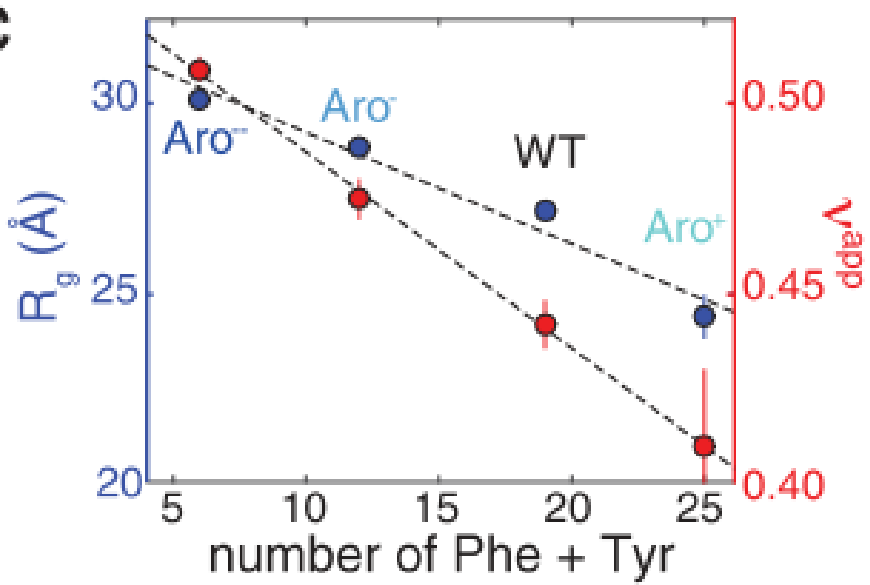


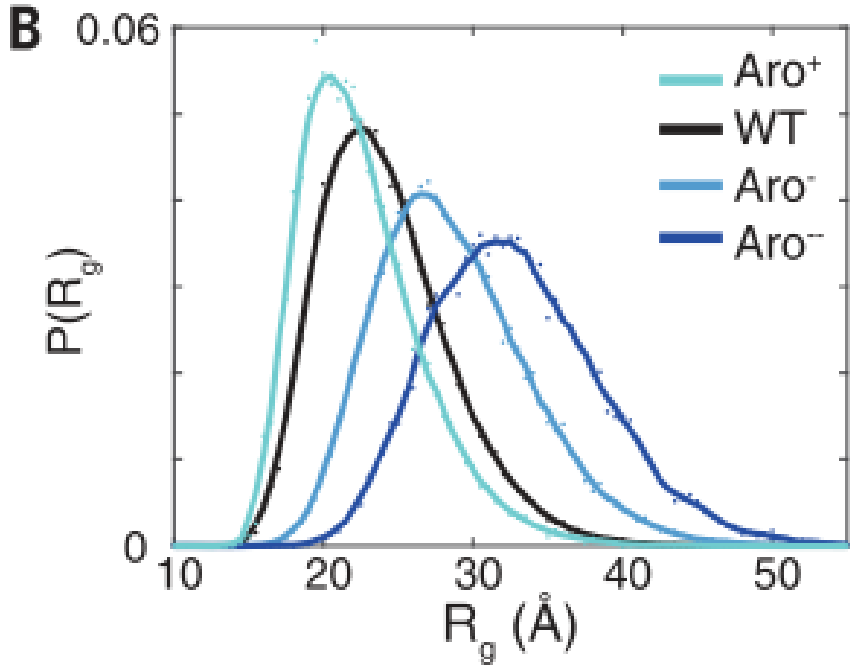
Number (valence) of aromatic residues

- All-atom simulations indicate a systematic chain expansion that accompanies a decrease in the valence of aromatic residues.
- SEC-SAXS measurements of the three variants confirm the simulation results.

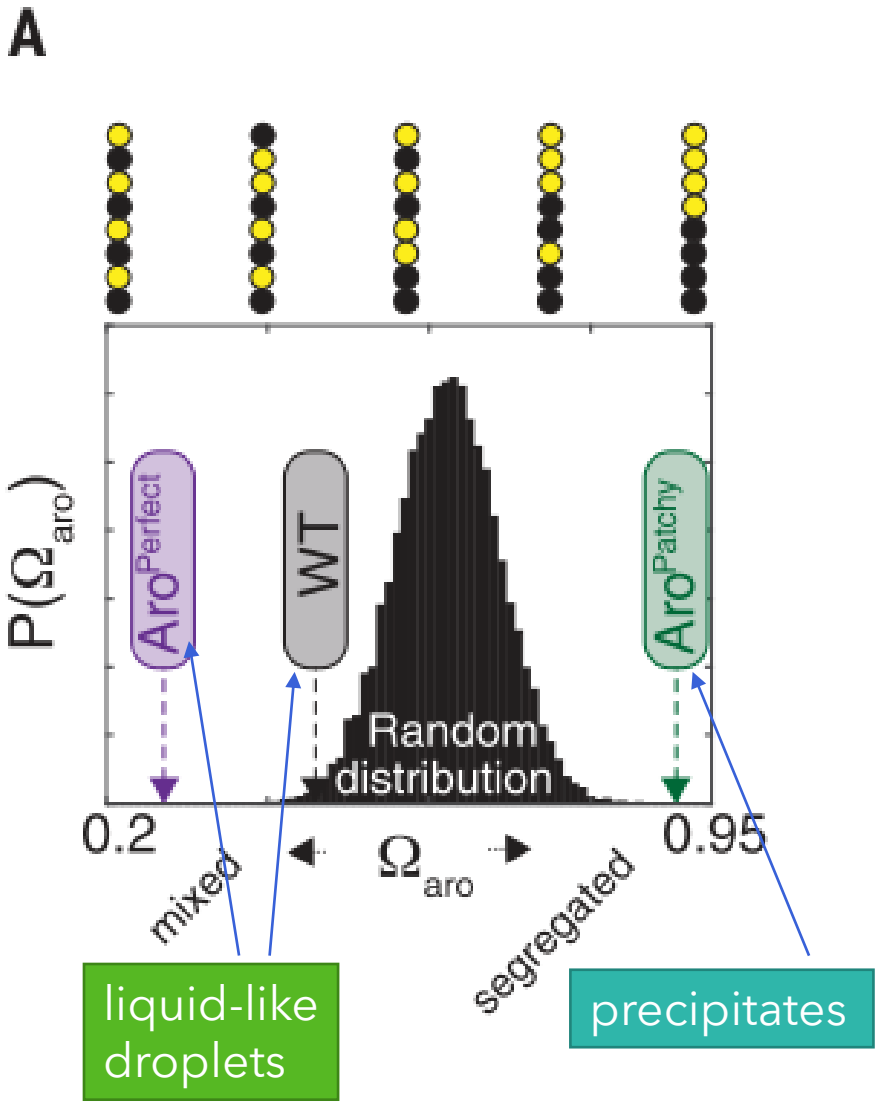
之后就根据这些结果建了一个one bead per residue的model，用来预测cloud temperature 和binodal diagram，该模型甚至可以推广至其他种类的蛋白







Preference for Uniform distribution of stickers



- The simulations show: increased linear clustering --- micellar substructures

$$\sigma_{Y/F/W}^{total} = (f_{Y/F/W} - f_{other})^2$$

$$\delta^{Y/F/W} = \frac{\sum_{i=1}^{N_{blobs}} (\sigma_{Y/F/W}^{local} - \sigma_{Y/F/W}^{total})^2}{N_{blobs}}$$

$$\Omega_{aro} = \frac{\delta^{Y/F/W}}{\delta_{MAX}^{Y/F/w}}$$

和κ差不多，
$0 \leq \Omega_{aro} \leq 1$

- Theories predict that these micelles can aggregate to form amorphous precipitates as opposed to liquid-like droplets because the increased linear clustering of stickers increases the apparent inter-sticker interaction strengths.

liquid-like droplets V.S. micellar substructures