Intra-Droplet Patterning of RNA-protein Bodies (RNPs) in Phase Separated Systems

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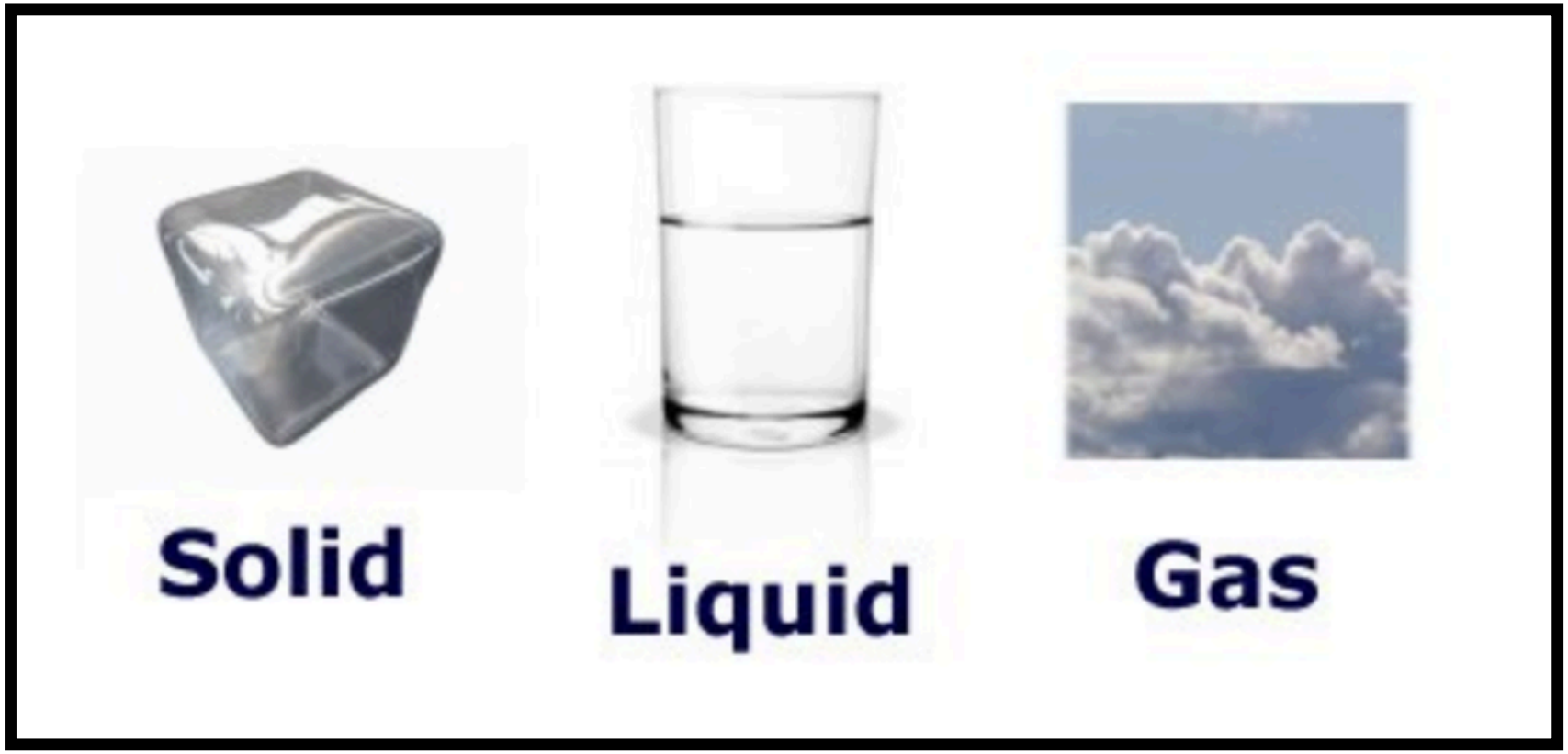
Phase Transitions Exist Throughout Nature

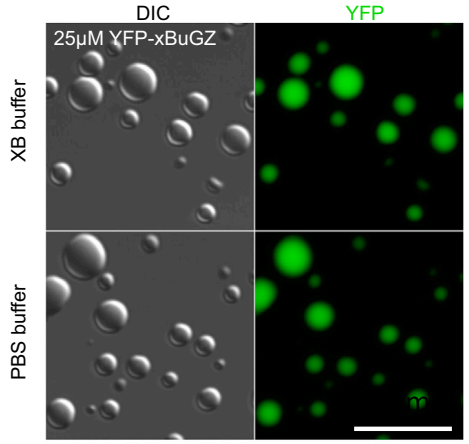


Liquid De-mixing

Oil + Vinegar

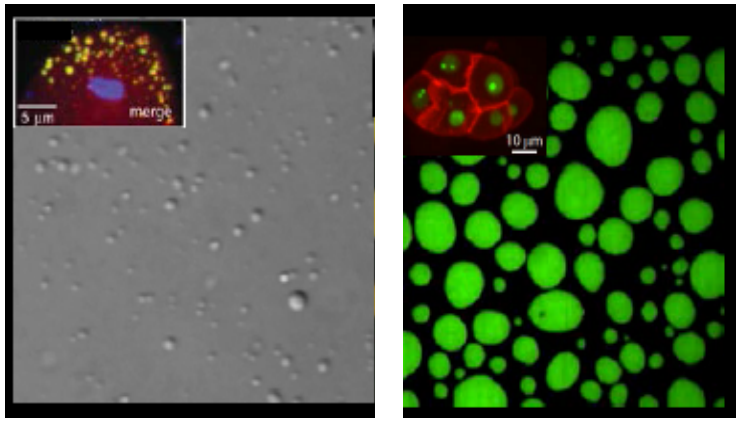
Phase Transitions are observed in a variety of cells

BuGZ: spindle regulatory protein conserved from *Drosophila* to humans



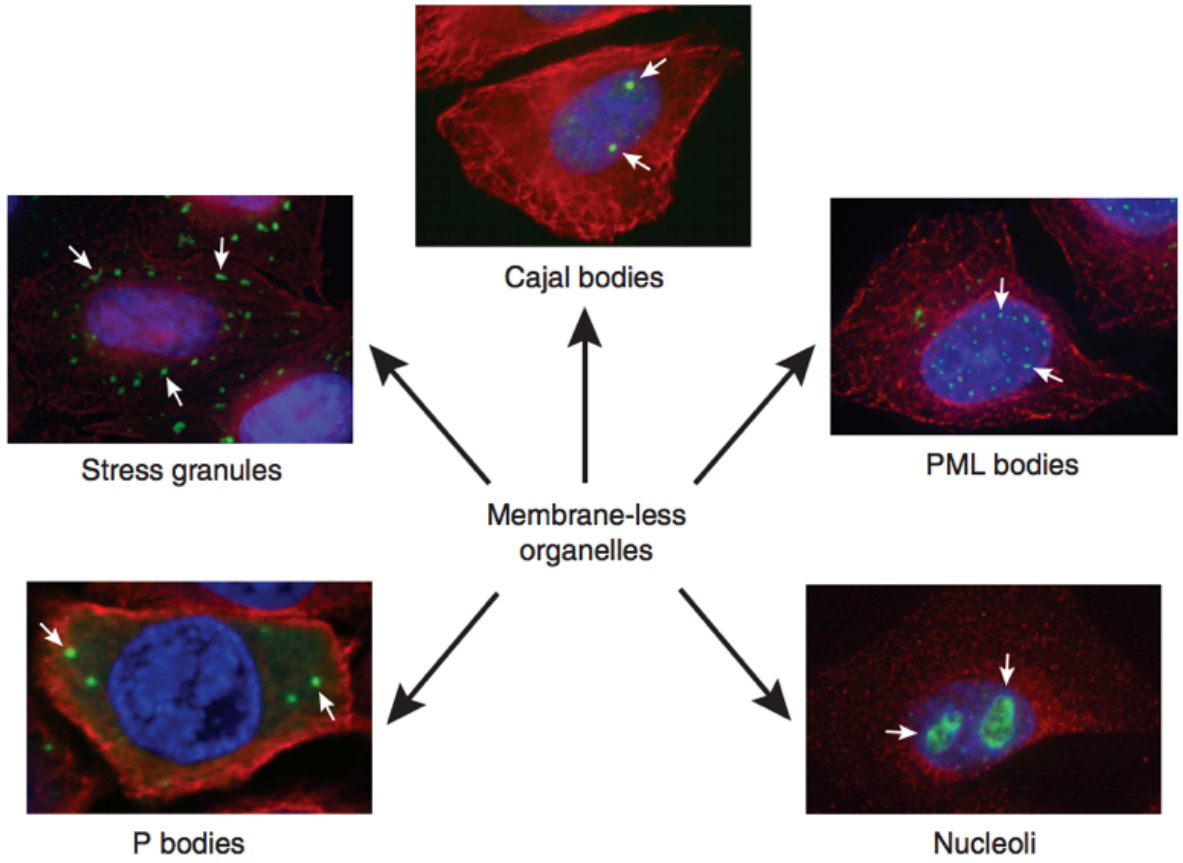
Jiang *et al.*, 2015

Disordered proteins (IDP/LCS)



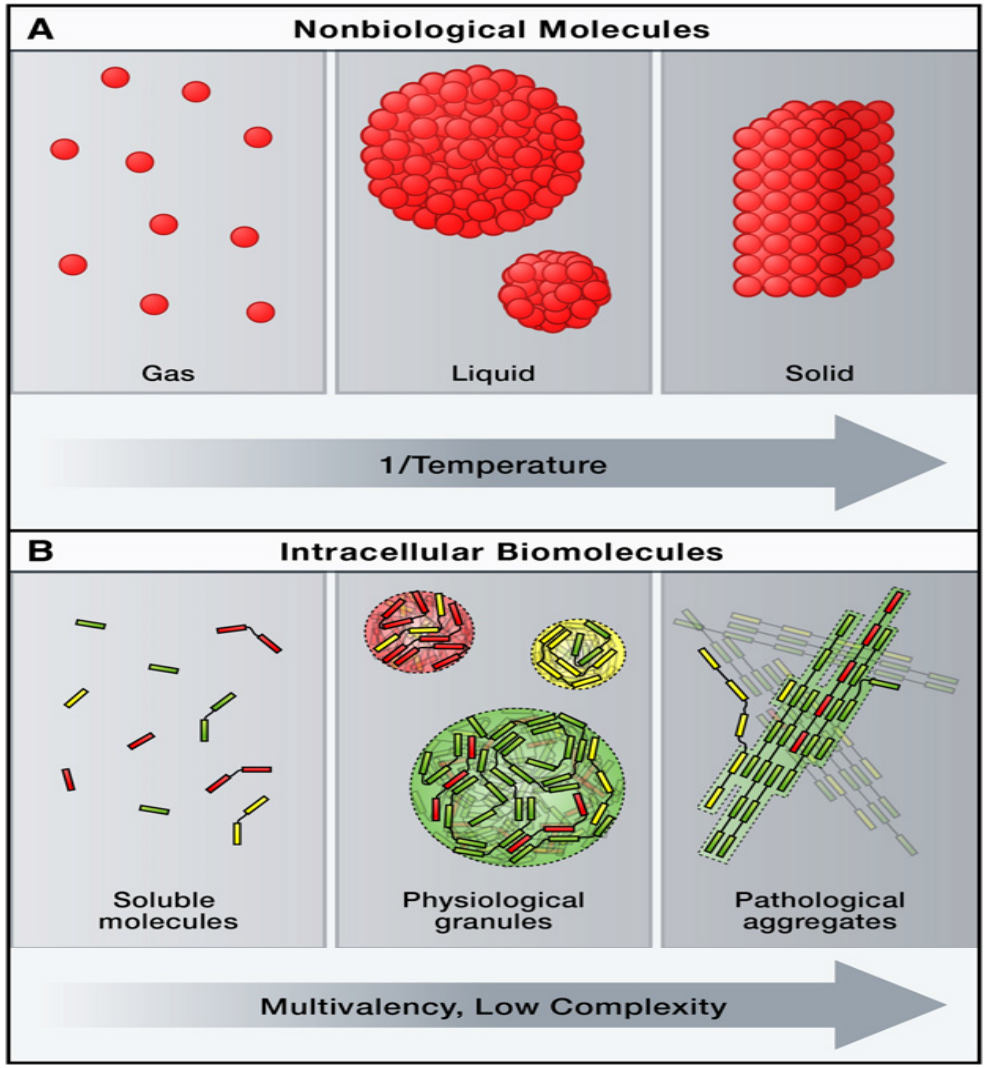
Elbaum et al. 2015; Berry et al. 2015

Membrane-less Organelles in HeLa Cells

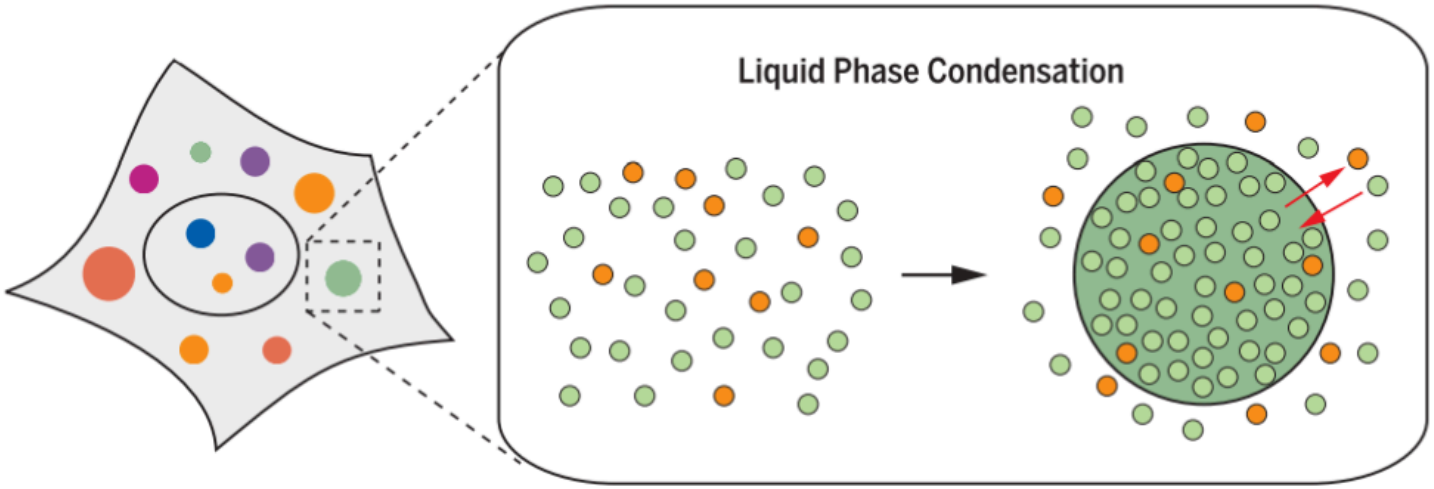


Alberti, 2017

Intracellular Organization can occur via Phase Separations



Weber & Brangwynne
Cell, 2012



Shin & Brangwynne 2017

Different protein characteristics can drive liquid-liquid phase separations

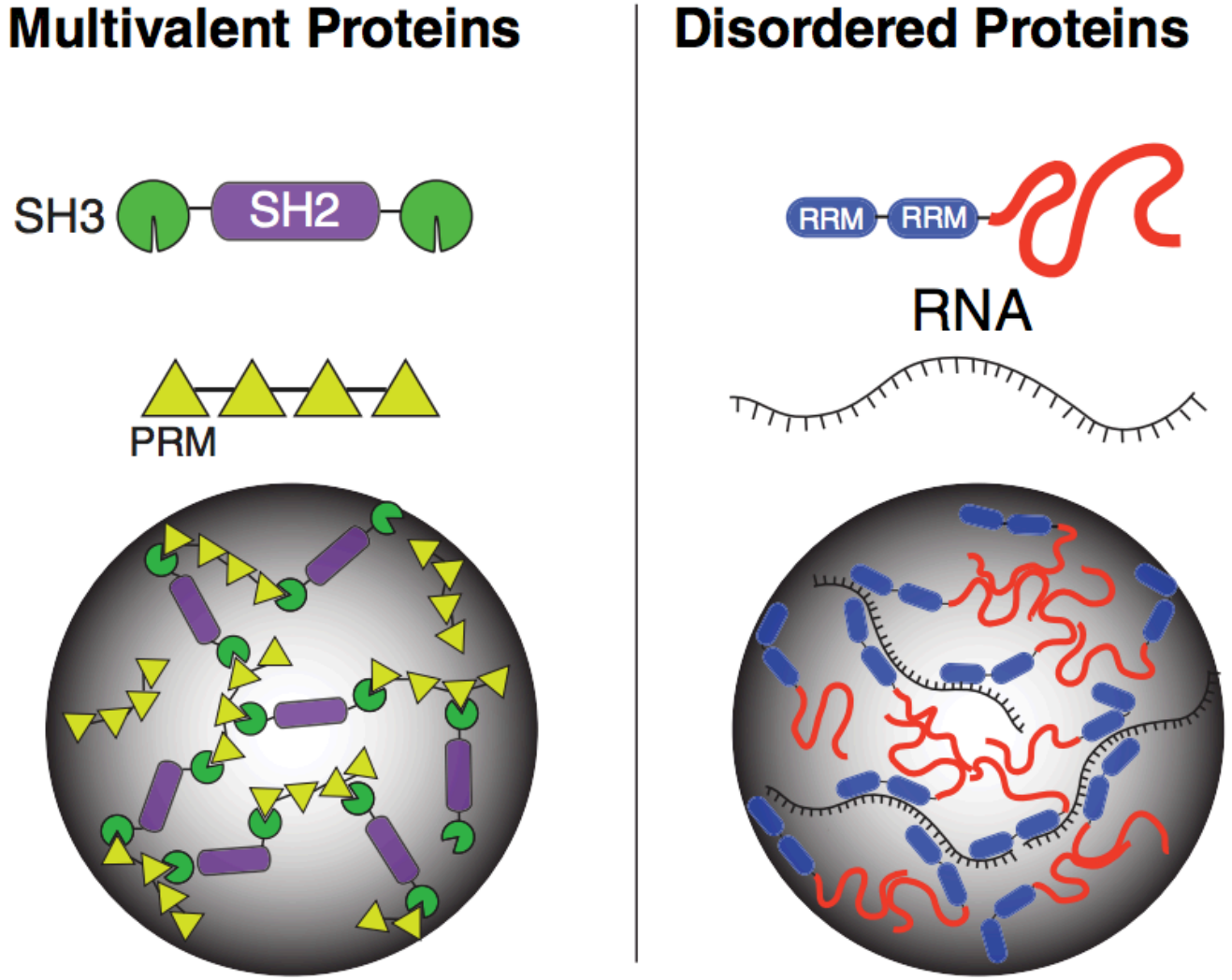
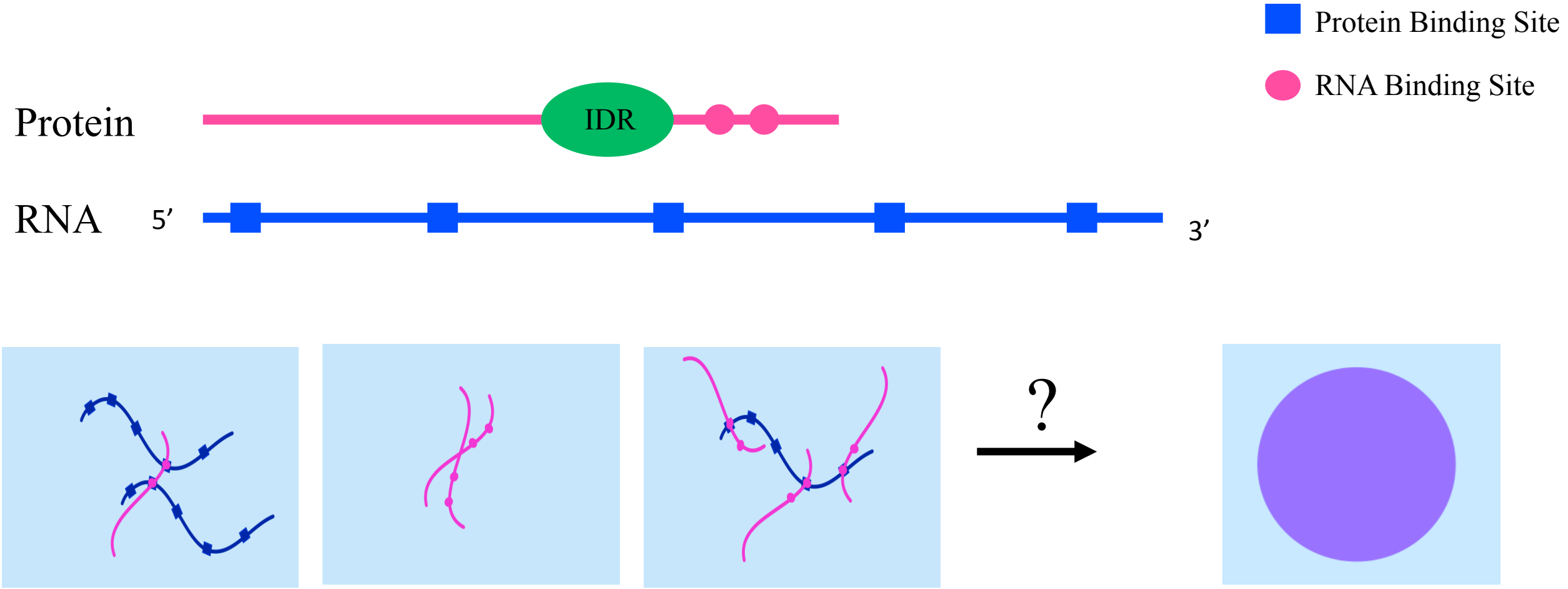


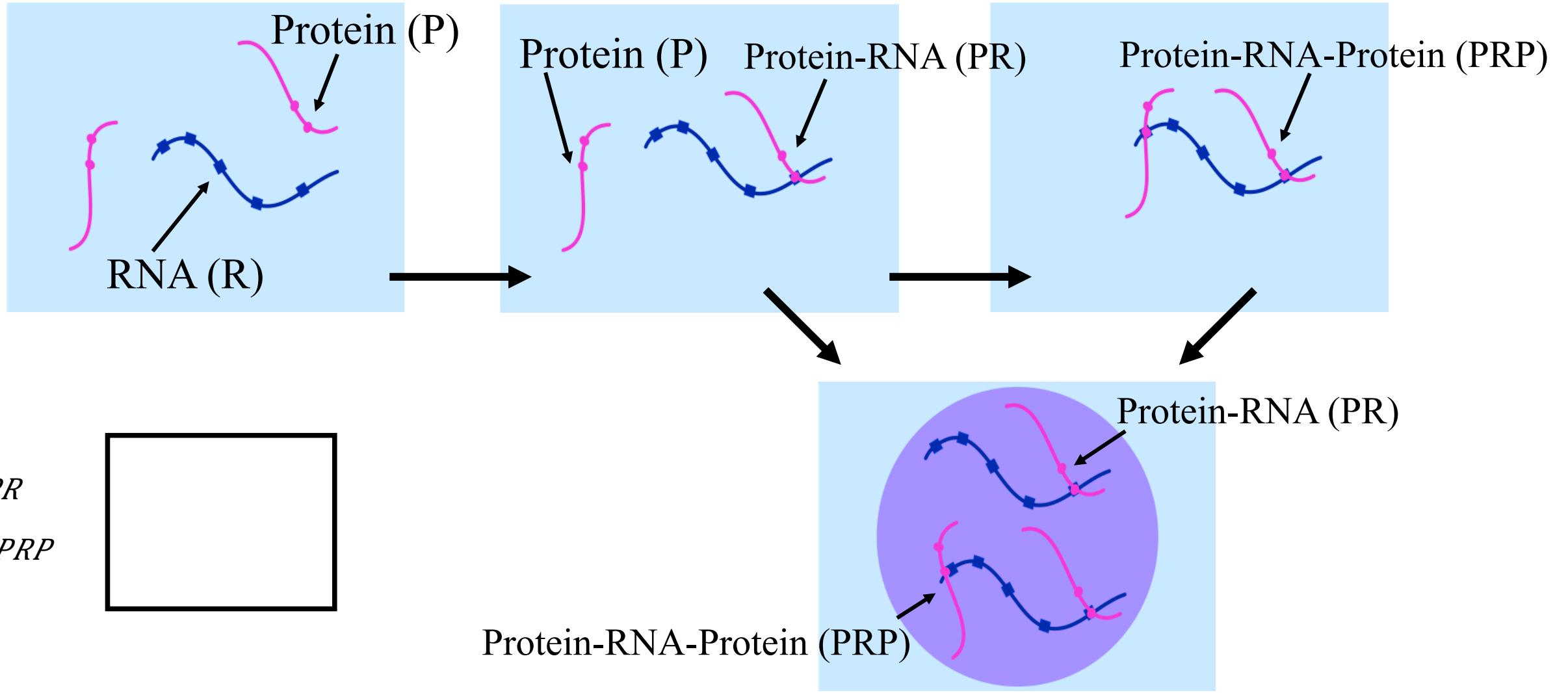
Image courtesy of *Shin & Brangwynne* 2017

Different Multivalent Interactions Can Exist within the Same Droplet



How do different protein-RNA complexes promote variable biophysical properties of droplet populations?

Modeling Protein-RNA Complexes Driving Droplet Formation



$+R \rightleftarrows PR$

$R+P \rightleftarrows PRP$

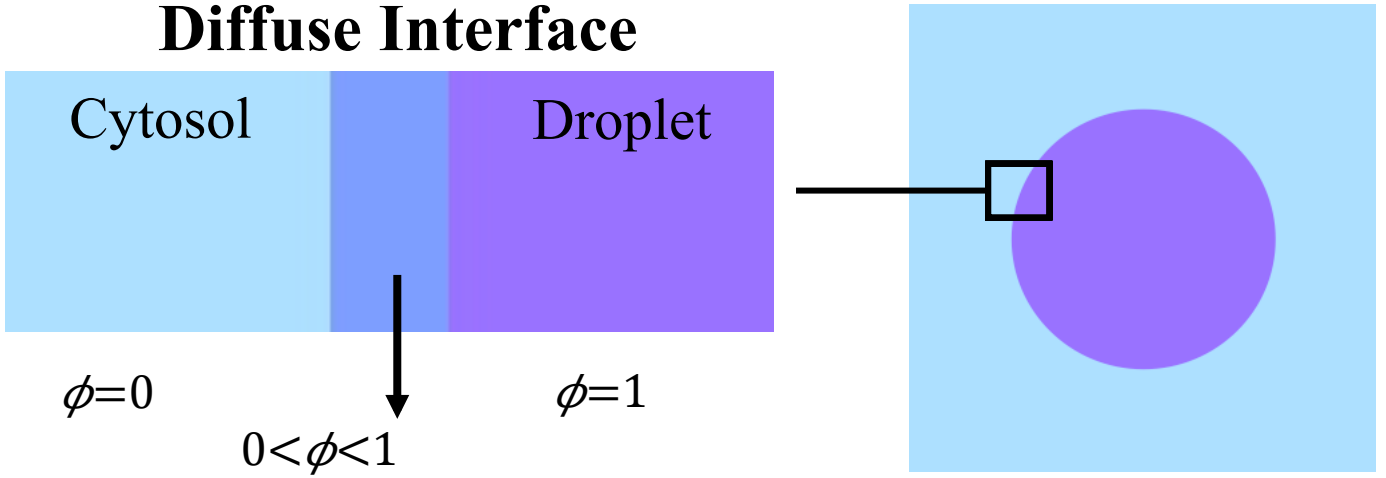
Phase Field Method and Cahn Hilliard Equation

$$\partial\phi/\partial t = \nabla\cdot(\lambda\nabla\delta F/\delta\phi)$$

Diffusion Parameter

Functional derivative of the free energy with respect to ϕ

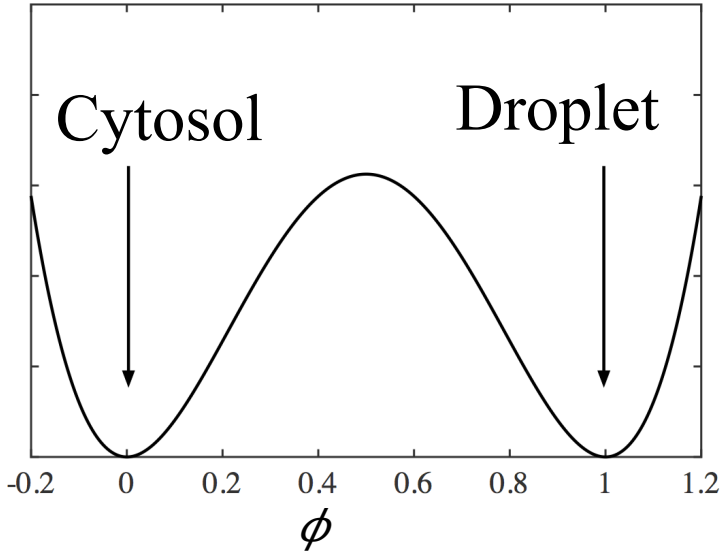
Diffuse Interface



Free Energy:

$$F = \int \gamma \left[\frac{\varepsilon}{2}|\nabla\phi|^2 + \frac{1}{\varepsilon}(\phi^2(1-\phi)^2)\right] dx$$

Double Well Potential



Current Model

$$\partial(PR)/\partial t = \nabla\cdot(\lambda_{PR}\,\nabla(\delta F/\delta(PR)\,)) + c_1\,P\cdot R - c_2\,(PR) - c_3\,(PR)P + c_4\,(PRP)$$

$$\partial(PRP)/\partial t = \nabla\cdot(\lambda_{PRP}\,\nabla(\delta F/\delta(PRP)\,)) + c_3\,(PR)P - c_4\,(PRP)$$

$$\partial P/\partial t = \nabla\cdot(\lambda_{P}\,\nabla P) - c_1\,P\cdot R + c_2\,(PR) - c_3\,(PR)P + c_4\,(PRP)$$

$$\partial R/\partial t = \nabla\cdot(\lambda_{R}\,\nabla R) - c_1\,P\cdot R + c_2\,(PR)$$

$$P + R \rightleftarrows PR$$

$$PR + P \rightleftarrows PRP$$

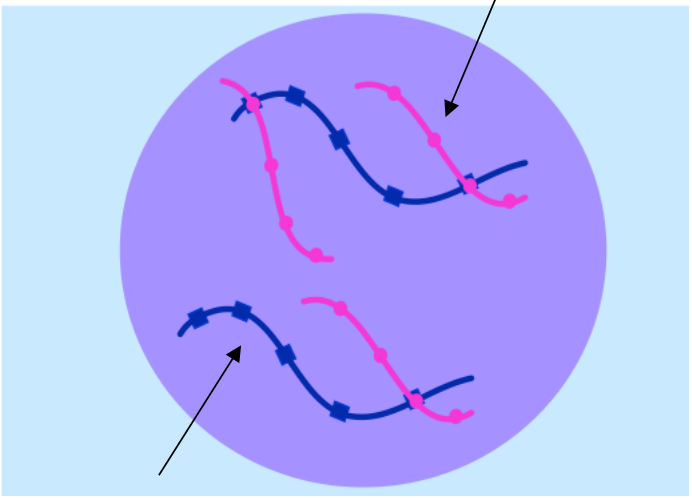
$$\phi = PR + PRP$$

P, R, PR, PRP= Unit-less Volume Fractions

Parameters

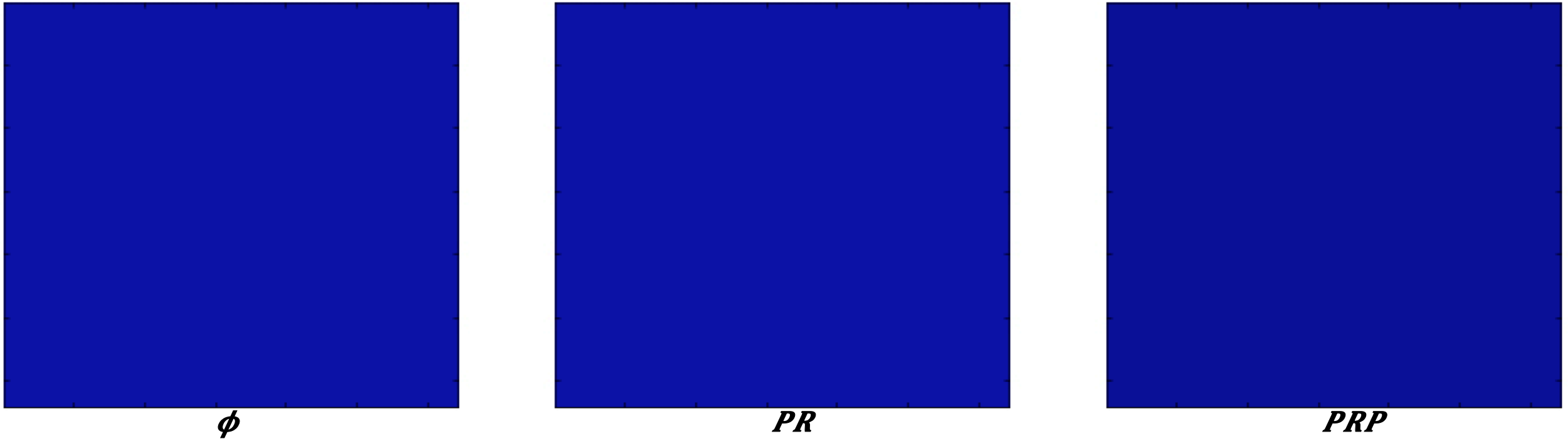
- λ = Diffusion Parameters
- c_1 = Rate of Protein and RNA association to form PR
- c_2 = Rate at which PR disassociates
- c_3 = Rate of Protein and PR association to form PRP
- c_4 = Rate at which PRP disassociates, giving PR and free Protein

Protein-RNA-Protein (PRP)



Protein-RNA (PR)

Quickly forming protein-RNA (PR) complexes and slowly forming protein-RNA-protein (PRP) complexes result in droplets dominated by the protein-RNA (PR) complex at the onset of phase separation



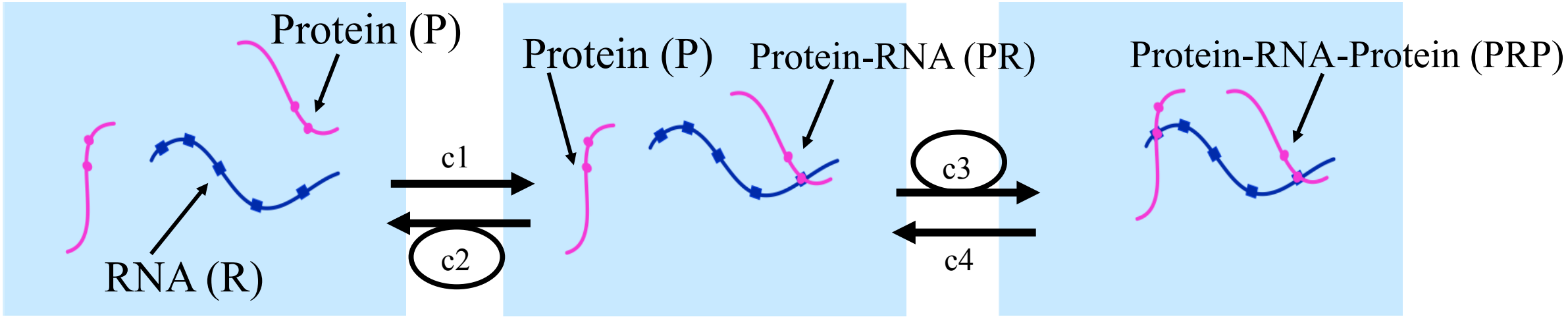
Reversible Molecular Interactions

c1 = Rate of association of protein and RNA to form PR complex

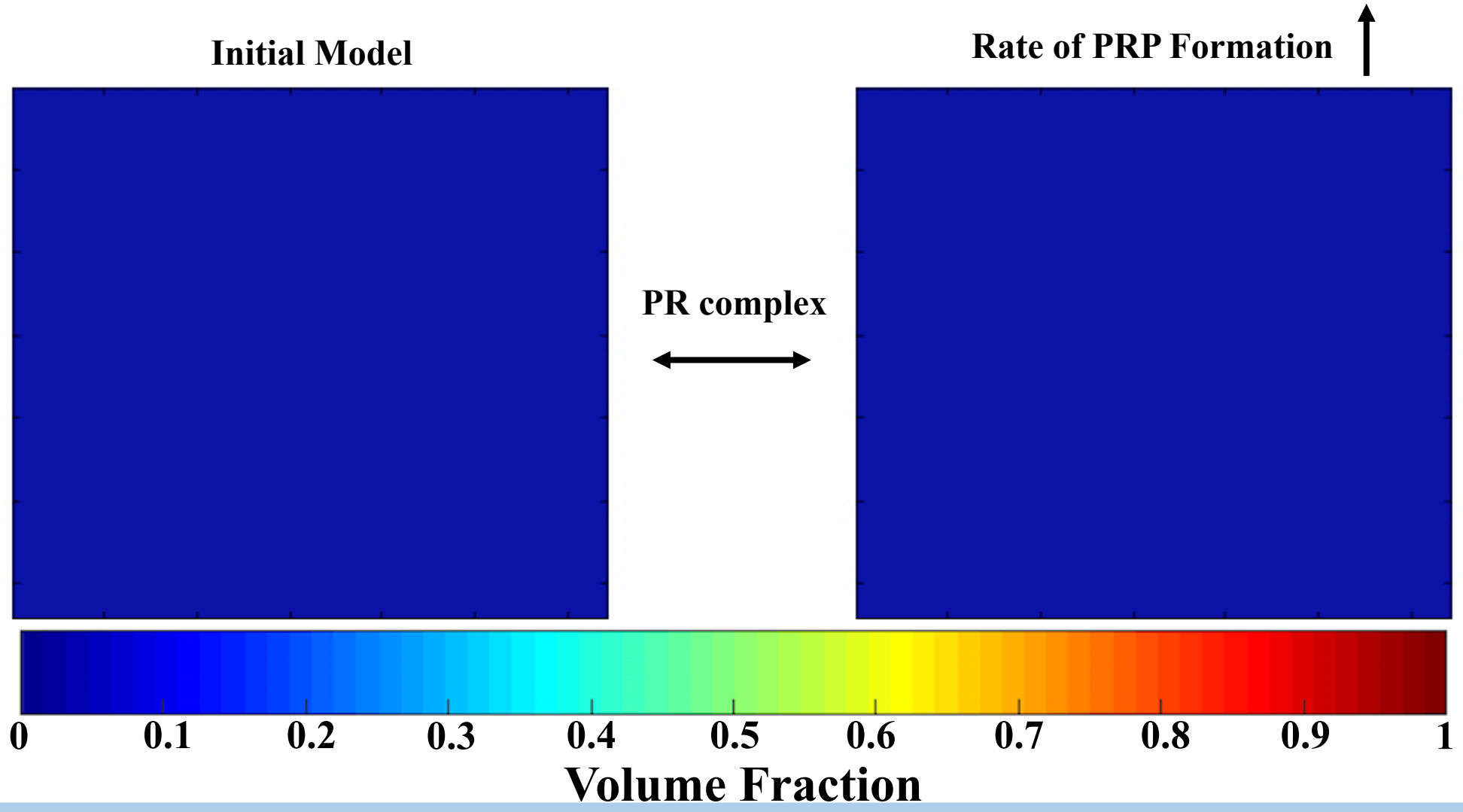
c2 = Rate of disassociation of PR complex resulting in free protein and RNA

c3 = Rate of association of protein and PR complex to form PRP complex

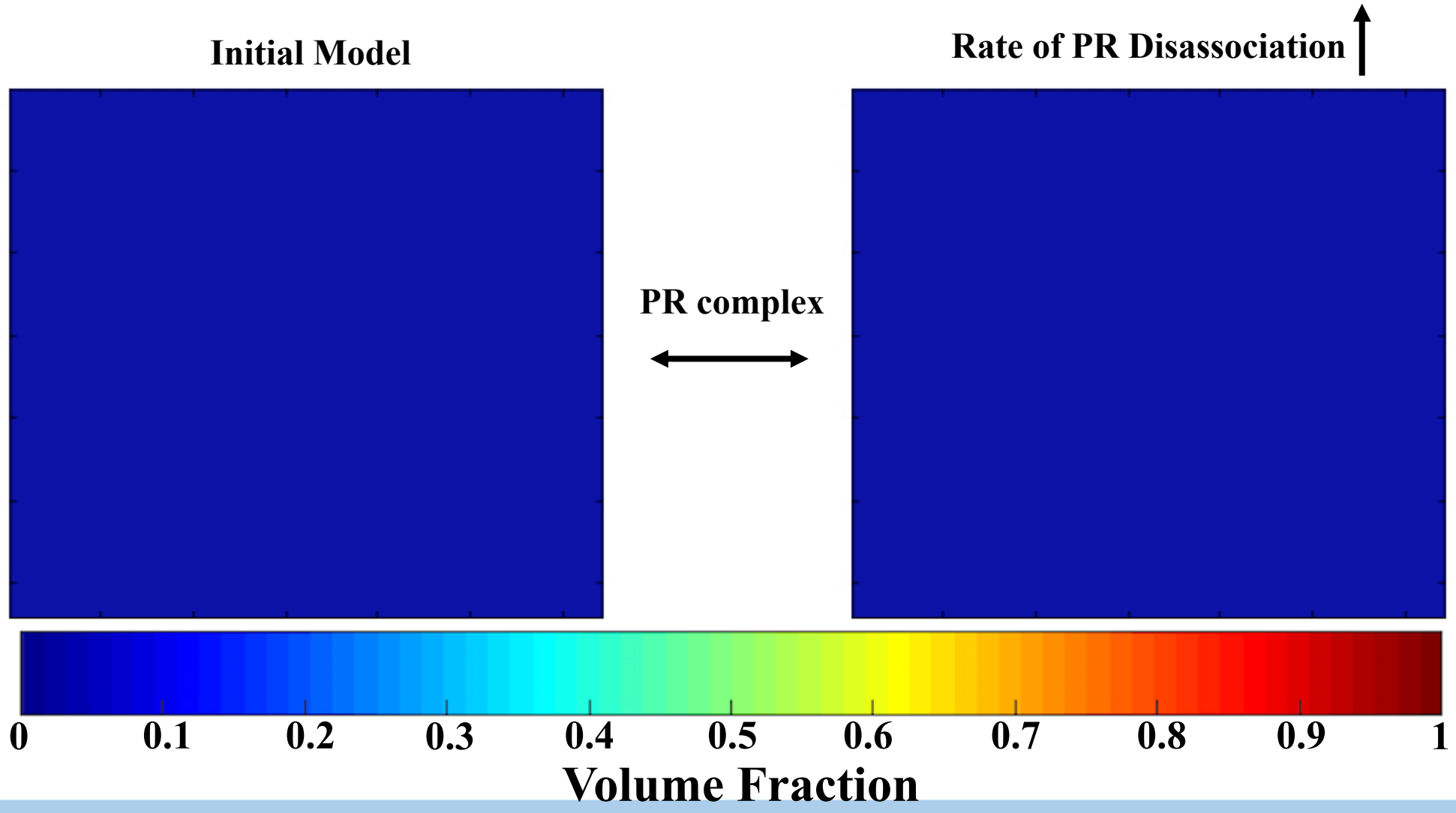
c4 = Rate of disassociation of PRP complex resulting in free protein and PR complex



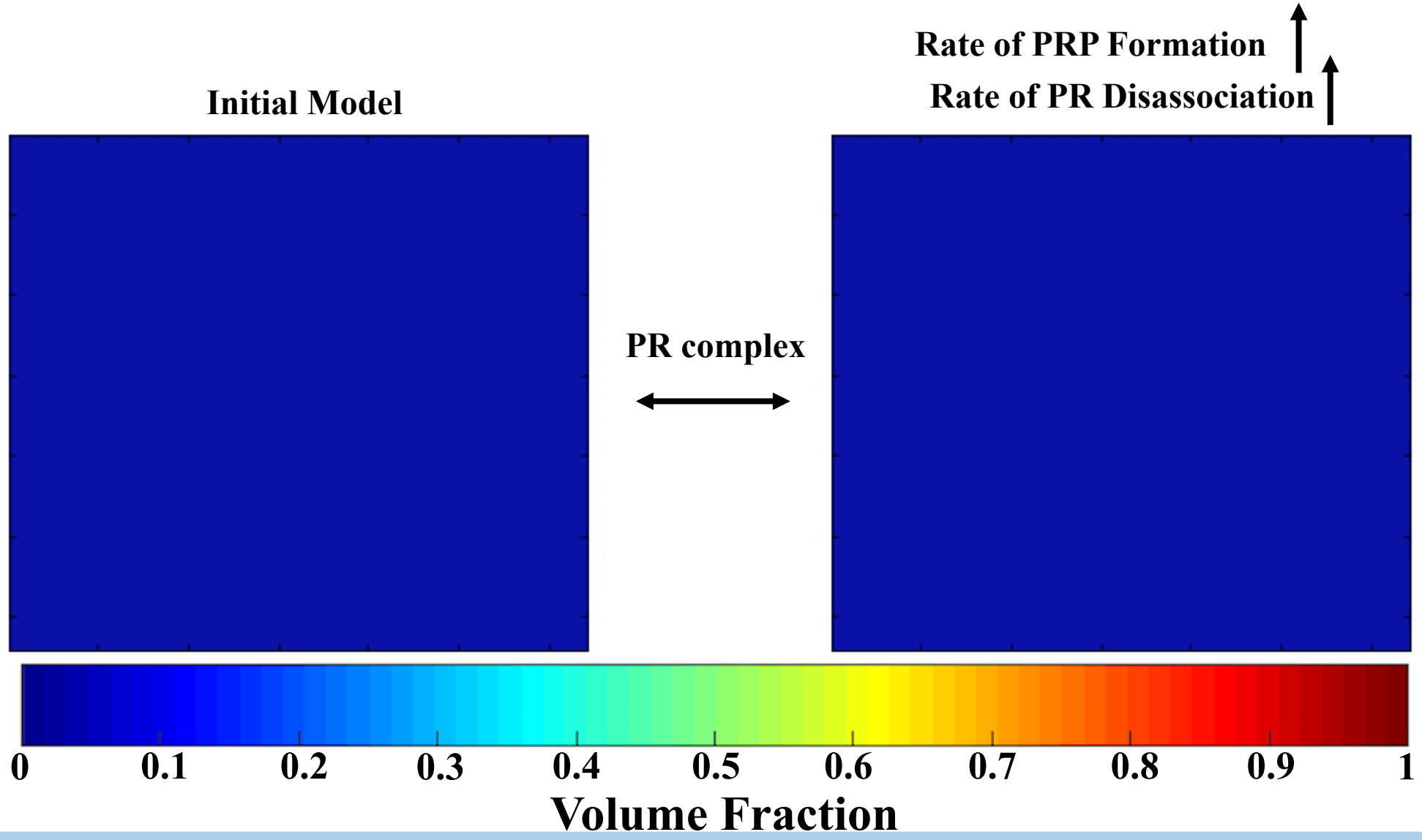
Increasing the rate of the protein-RNA-protein (PRP) complex formation (c3) results in droplets dominated by the protein-RNA (PR) complex initially, but with an increase in PRP concentration as the system evolves



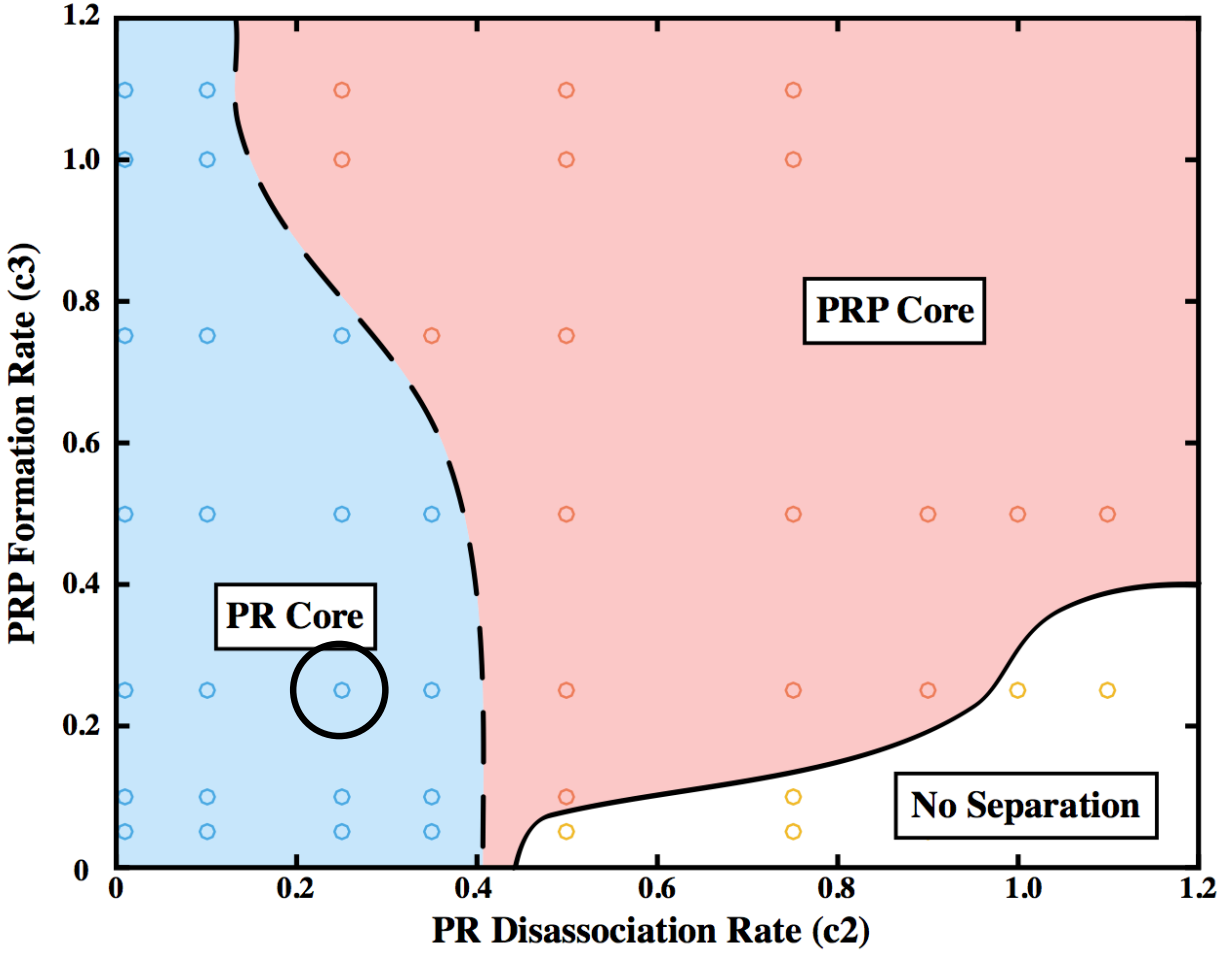
Increasing the rate of protein-RNA (PR) complex disassociation (c2) results in a system that is slow to phase separate and is slightly dominated by the protein-RNA-protein (PRP) at the onset of phase separation



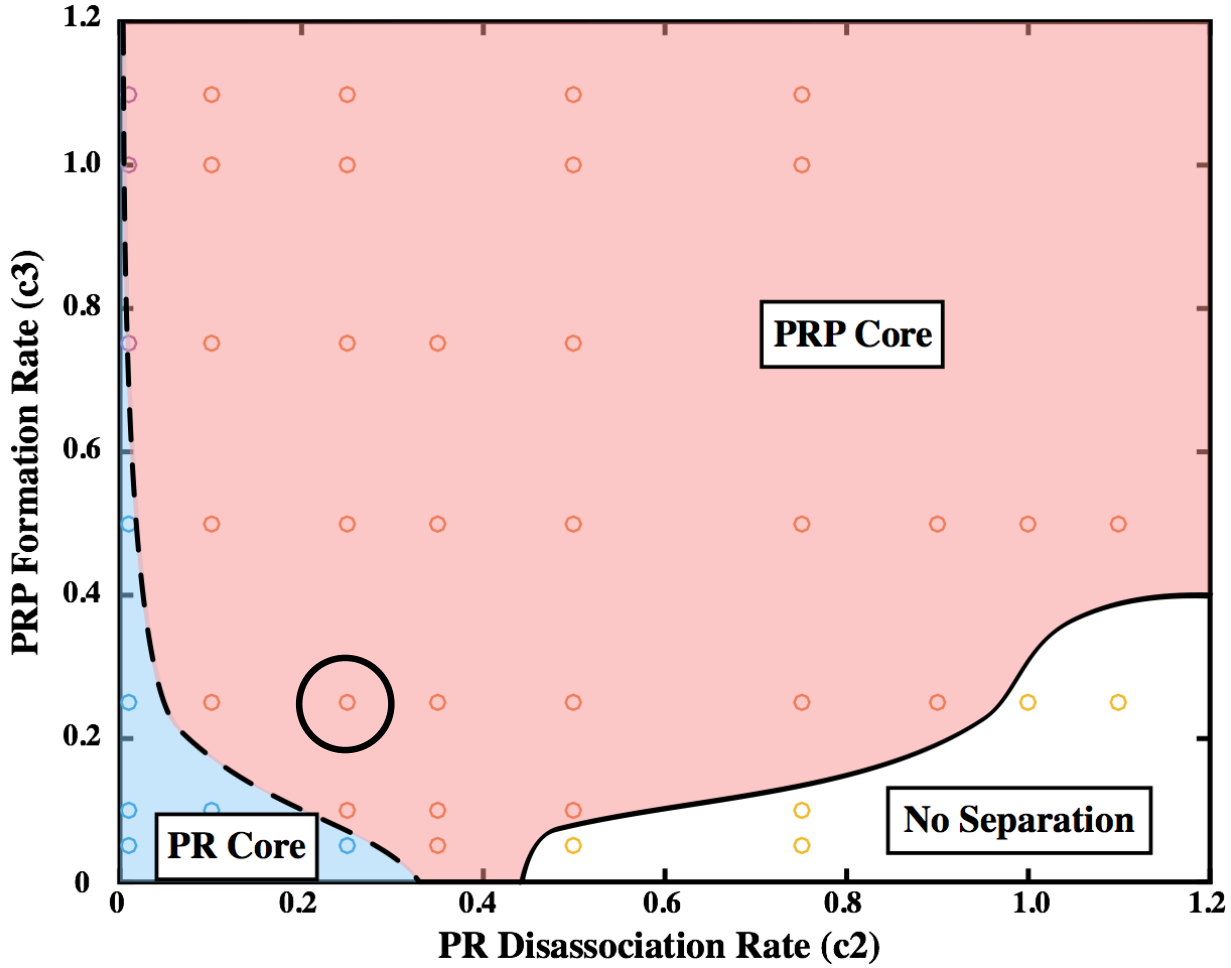
Increasing the rate of protein-RNA (PR) complex disassociation (c2) AND the rate of protein-RNA-protein (PRP) formation (c3) results in a system where phase separation is driven by the protein-RNA-protein (PRP) complex



Changes in Droplet Composition Occur due to Changes in Protein-RNA (PR) Disassociation and Protein-RNA-Protein (PRP) Formation

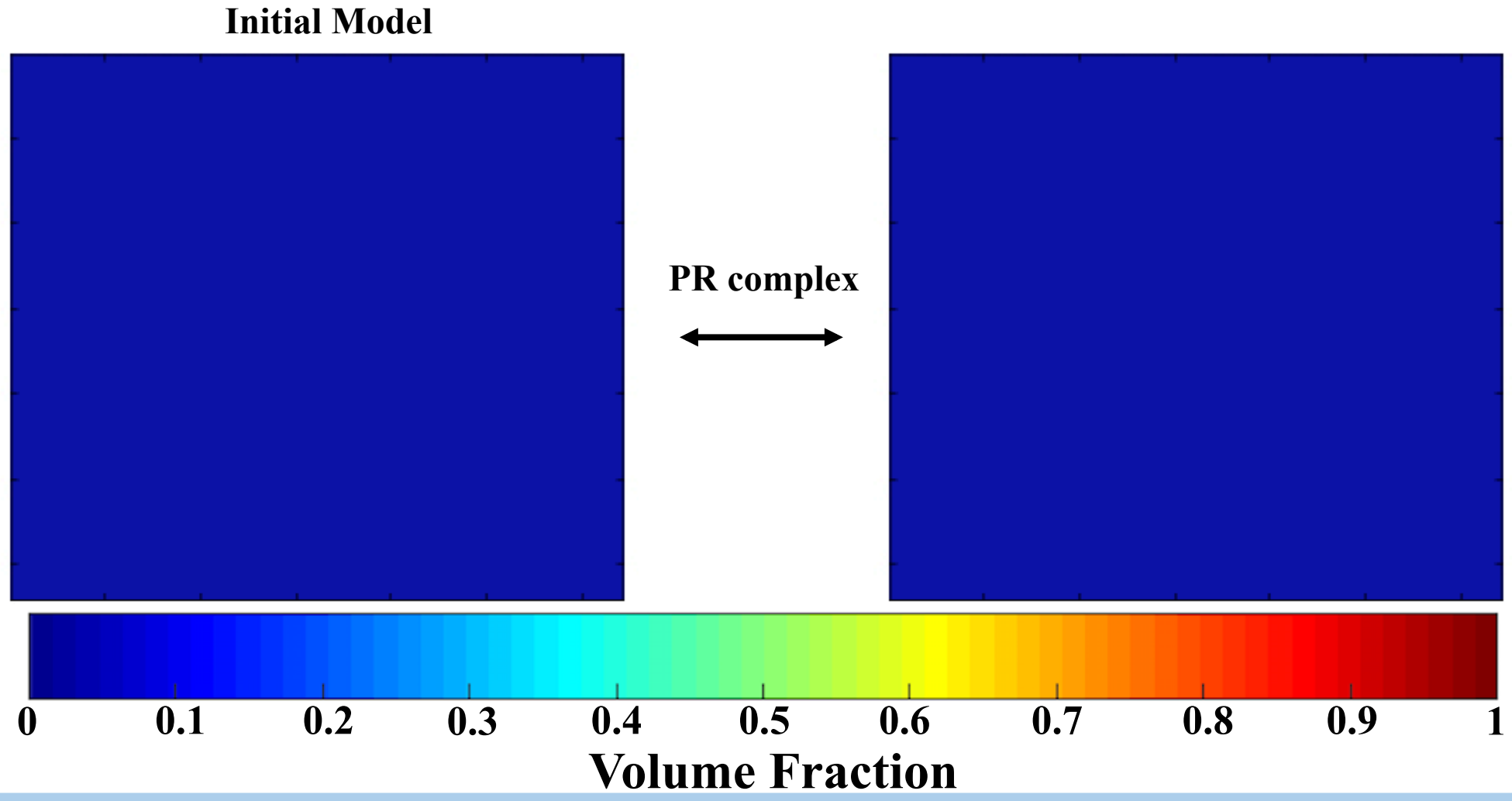


At Time of Phase Separation



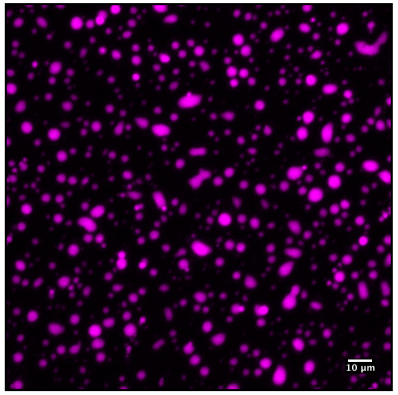
At t = 1000

If the stability of the protein-RNA (PR) complex is reduced slightly and the rate at which the protein-RNA-protein (PRP) complex is increased, the separation will be driven by the PR complex but droplets will be dominated by the PRP complex as the system evolves

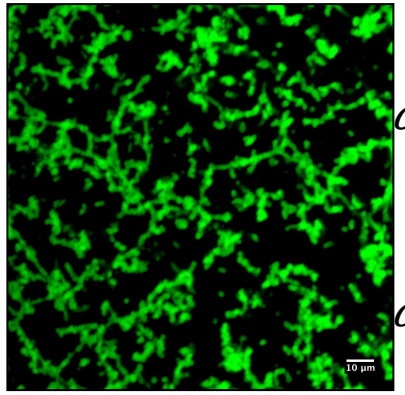


Phase Dependent Diffusion

Phase Dependent Diffusion



Whi3 and CLN3



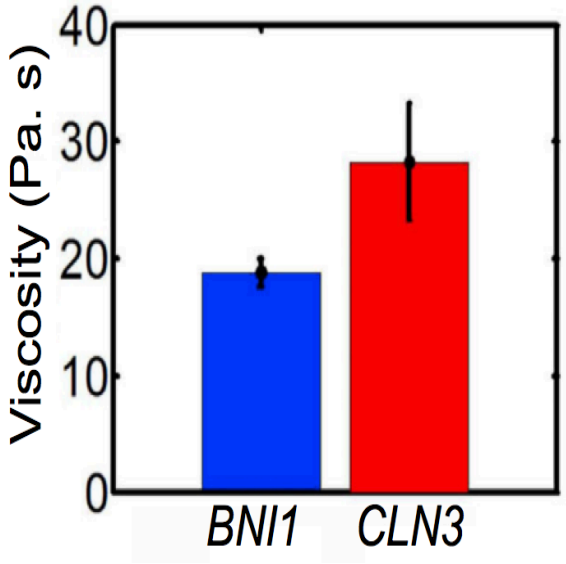
Whi3 and BNI1

$$\partial(PR)/\partial t = \nabla\cdot(\lambda_{PR} D_\phi \nabla(\delta F/\delta(PR))) + c_1 P\cdot R - c_2 (PR) - c_3 (PR) P + c_4 (PR$$

$$\partial(PRP)/\partial t = \nabla\cdot(\lambda_{PRP} D_\phi \nabla(\delta F/\delta(PRP))) + c_3 (PR) P - c_4 (PRP)$$

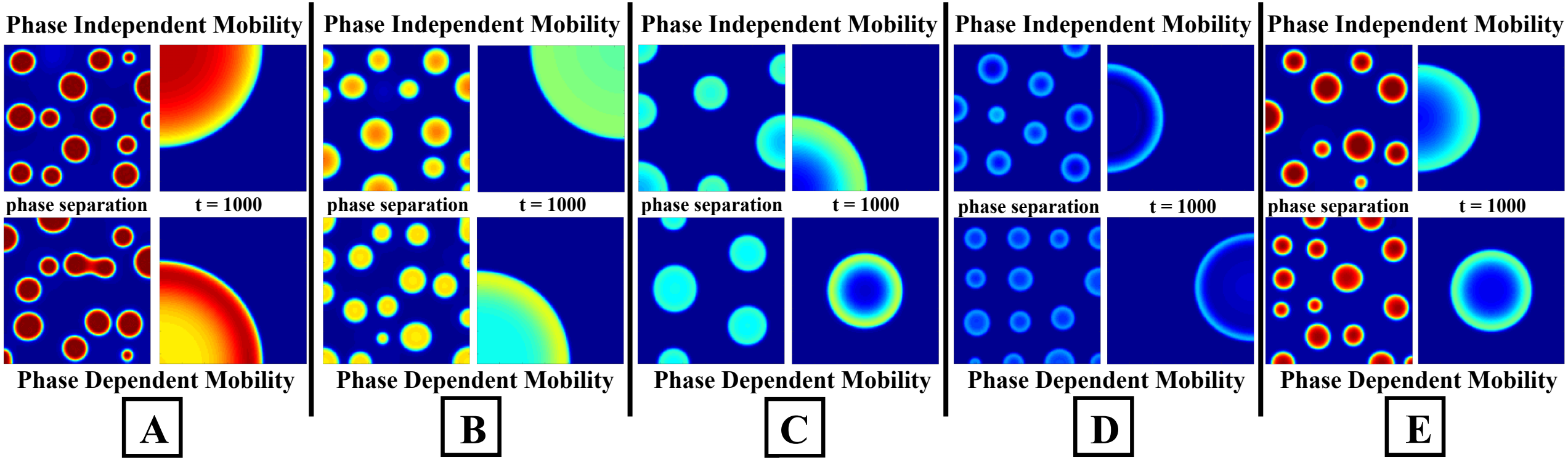
$$\partial P/\partial t = \nabla\cdot(\lambda_P D_\phi \nabla P) - c_1 P\cdot R + c_2 (PR) - c_3 (PR) P + c_4 (PRP)$$

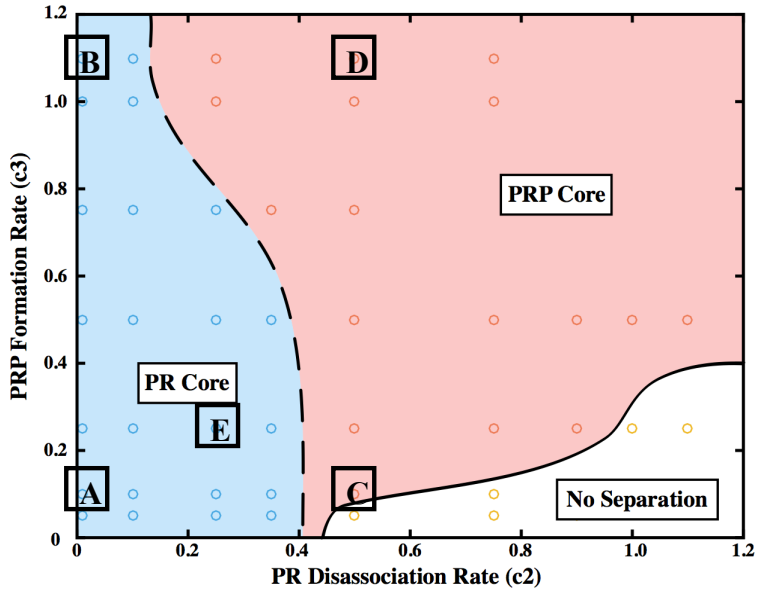
$$\partial R/\partial t = \nabla\cdot(\lambda_R D_\phi \nabla R) - c_1 P\cdot R + c_2 (PR)$$



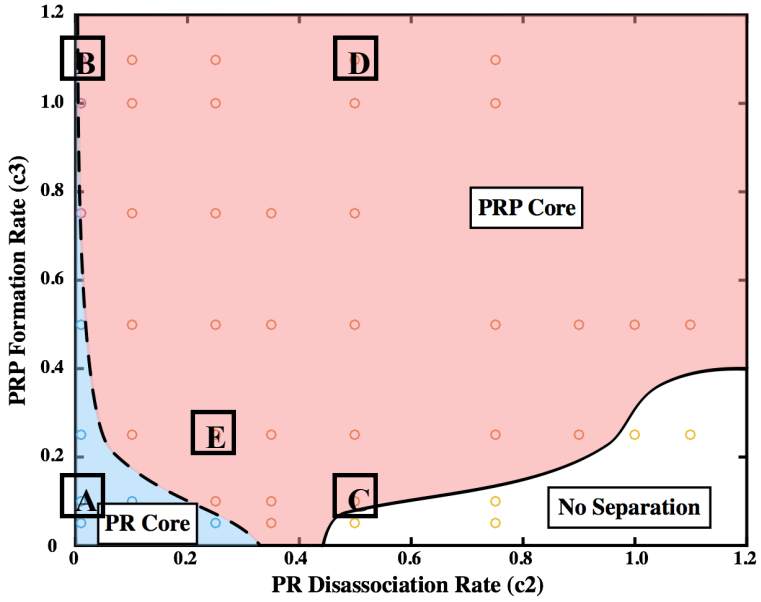
Images courtesy of Erin Langdon

Figure courtesy of Zhang et al.



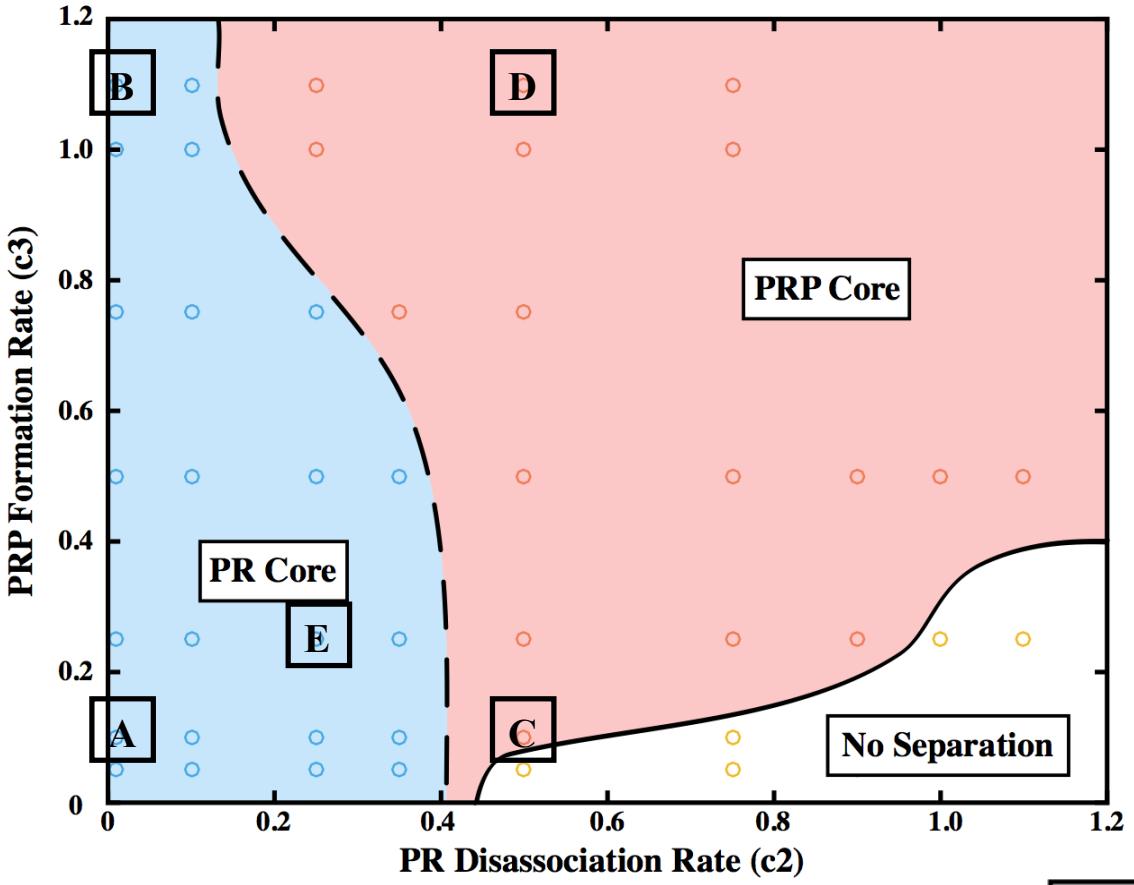


At Time of Phase Separation

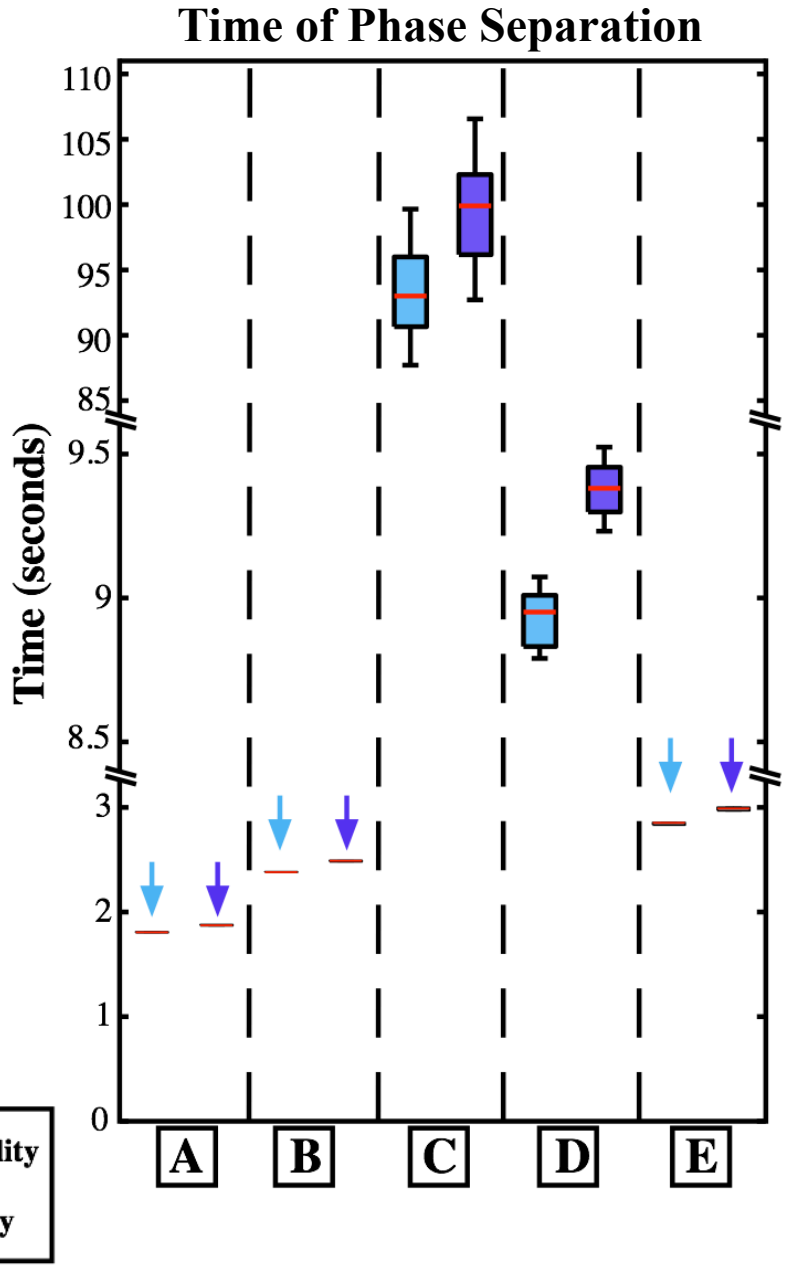


At t = 1000

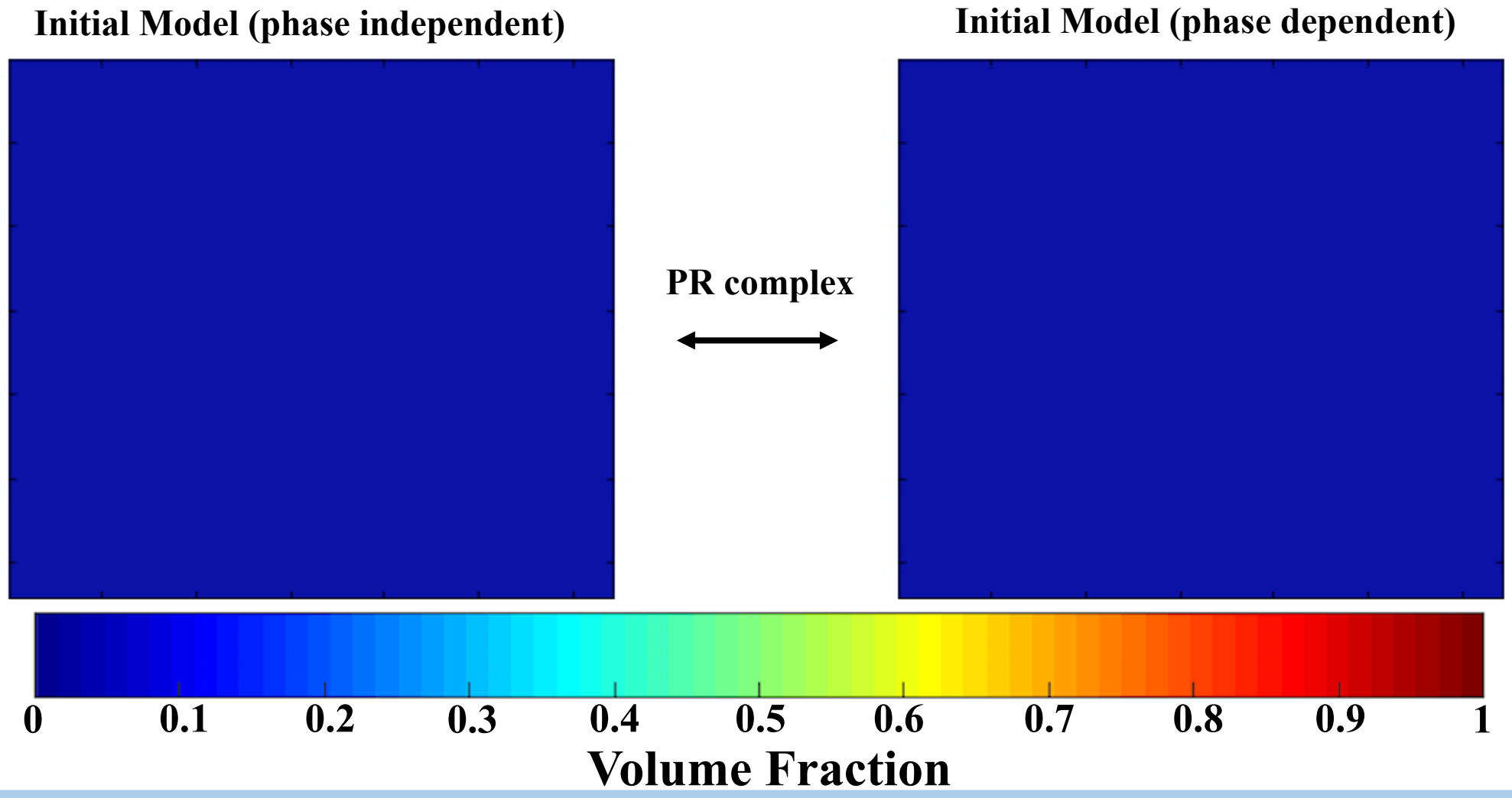
Phase-dependent mobility does influence the time of phase separation



At Time of Phase Separation



Phase-dependent mobility creates an environment that continues to promote the reversible molecular interactions that form the protein-RNA complexes

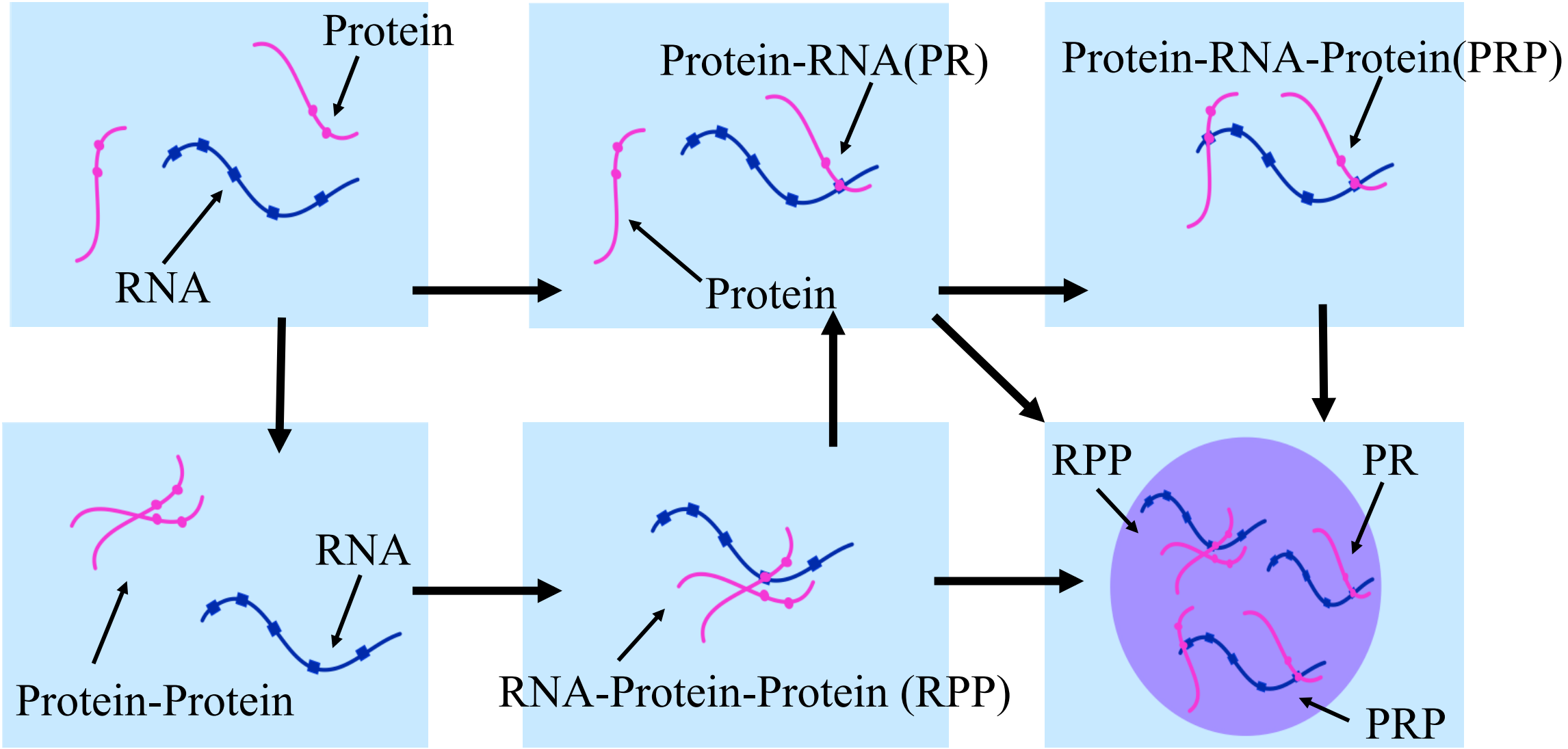


Final Thoughts

- Phase transitions are observed in lots of different cells.
- Proteins harboring intrinsically disordered regions are key for driving liquid-liquid phase separations. RNA can promote this phase separation by interacting with protein through RNA-binding domains.
- Reversible molecular interactions drive the phase separation.
- Competition for a shared resource can both alter the composition of the droplets at the time of phase separation and composition as the droplet system evolves.
- Phase-dependent mobility does not influence the initial properties of the droplets but instead creates smaller microenvironments that promote the existing molecular interactions.

Future Work

Future Work: Protein-Protein Interactions



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