Physical Mechanisms of Cell Organization on Micron Length Scales

Michael Rosen
UT Southwestern Medical Center

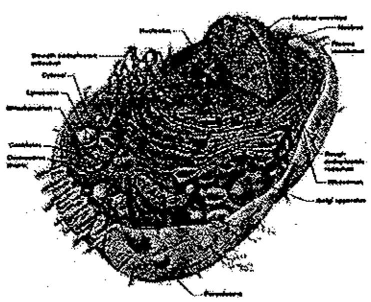
UTSW STARS Program
September 9, 2013

Eukaryotic Cells are Highly Compartmentalized

Best understood compartments are bounded by membranes: nucleus, ER, Golgi, mitochondria, lysosome, peroxisome

Most structures found in most eukaryotic cells

Structures persistent over time, but often show dynamic rearrangements through membrane remodeling machineries

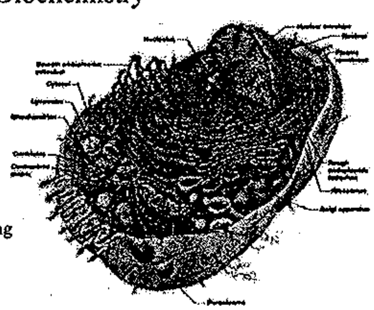


Compartmentalization Enables Segregation of Biochemistry

Concentration of enzymes and substrates:
throughput, selectivity

Maintenance of different environment (pH, redox potential)

Protection of cell from damaging activities (degradation, oxidation)

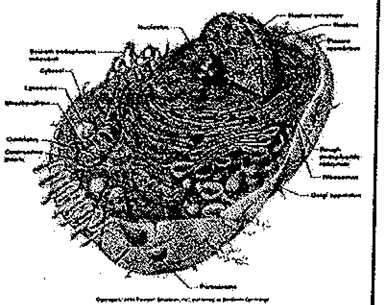


The Membrane Boundary Imparts Specific Requirements and Opportunities

Entry and exit require molecular transporters: channels, pumps

Fusion and fision of compartments requires complex machineries

Membrane itself is site of many reactions



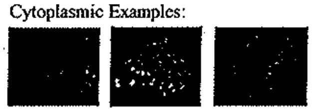
Some Compartments—Cellular Bodies—Are Not Bounded by Membranes

- Most found in most eukaryotic cells
- Persistent over time
- Not surrounded by lipid membrane, but appear as distinct, micron-sized compartments
- Each type concentrates 100s of unique species--protein and RNA
- Molecules enter and exit freely and rapidly, without need for transporters
- Some behave as liquid droplets--Fusion and fission do not require specialized machineries

Nuclear Examples:

Cajal Body: Spliceosome assembly | PML Body: Transcription, DNA Repair | Nuclear Speckle: Splicing

Cytoplasmic Examples:



P Bodies: RNA metabolism | Stress Granules: Translation suppression | Purinosome: Purine biosynth. (?)

Unlike Membrane-Bounded Compartments, Cellular Bodies Are Not Well Understood

- Physical nature—what allows them to be persistent, yet dynamic?
- Chemical nature—Molecular composition, and factors that control it
- Biochemical functions, and how these arise from physical properties and molecular composition
- Cellular functions, and how they arise from biochemistry and interactions with cellular structures

Nuclear Examples:

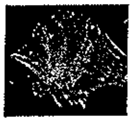
Cajal Body: Spliceosome assembly | PML Body: Transcription, DNA Repair | Nuclear Speckle: Splicing

Cytoplasmic Examples:

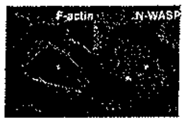
P Bodies: RNA metabolism | Stress Granules: Translation suppression | Purinosome: Purine biosynth. (?)

Micron-Sized Two-Dimensional Puncta Are Also Present on Membranes

Immunological synapse (T cell)



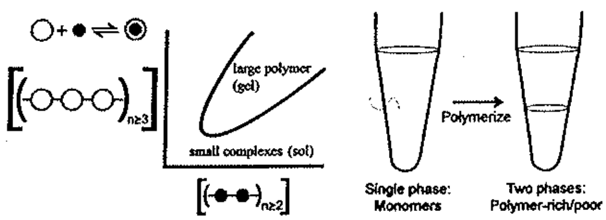
Focal adhesions (migrating cells)



Invadopodia (cancer cells)

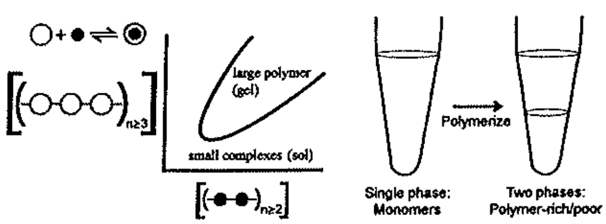
- Similar to cellular bodies (3D puncta), physical and chemical nature not well understood, nor how these relate to biochemical and cellular functions
- Unclear whether related or distinct mechanisms govern 2D vs 3D puncta
- Both 2D and 3D puncta provide cellular organization on micron scales

A Hypothesis Born From Polymer Chemistry



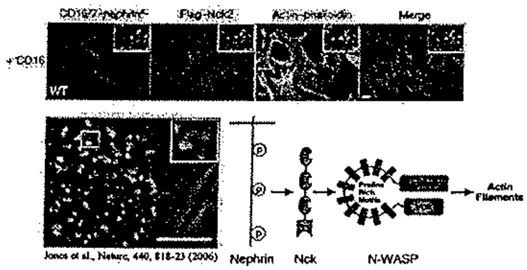
Interactions between multivalent proteins and multivalent ligands can drive polymerization and phase separation

A Hypothesis Born From Polymer Chemistry



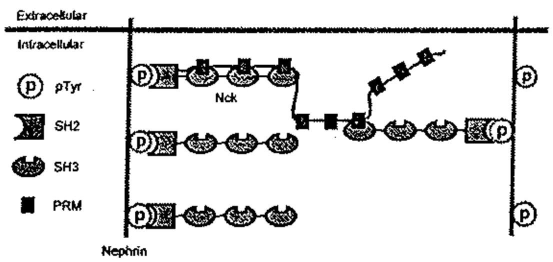
Provides a mechanism to translate from molecular (nm) to cellular (μm) scales of organization

The Nephrin Pathway Provides a Potential Example in Actin Regulatory Signaling



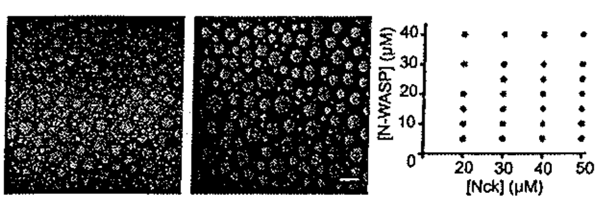
Nephrin and Nck form micron-sized puncta at the plasma membrane
These puncta are sites where actin filaments are assembled

The Nephrin Pathway Provides a Potential Example in Actin Regulatory Signaling



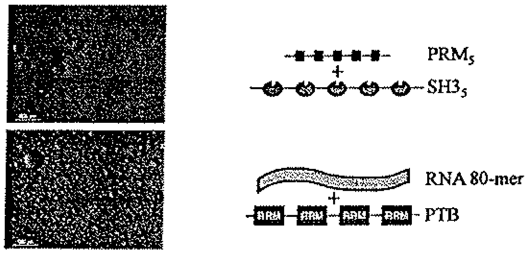
Very hard to understand from classical biophysical standpoint, but was greatly simplified by viewing through lens of polymer science

Multivalent Interactions Between Nck and N-WASP Drive a Phase Transition



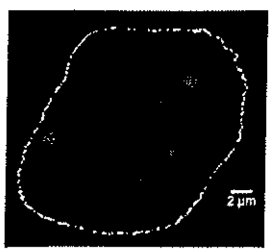
Droplets are 1~50 μm in diameter, coalesce over time
Proteins are concentrated in the droplets ~100-fold
Fluorescence recovers quickly after photobleach ($t_{1/2}$ ~ 30s)

Analogous Phase Separations are Observed in Several Model Systems

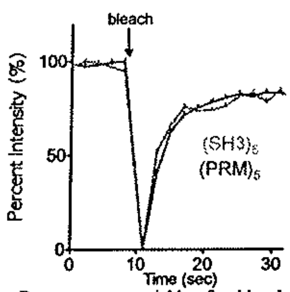


Model systems allow systematic analysis of physical parameters
Suggests this behavior may be general for intracellular systems

Co-Expression of $(SH3)_5$ + $(PRM)_5$ Produces Dynamic Puncta in HeLa Cells

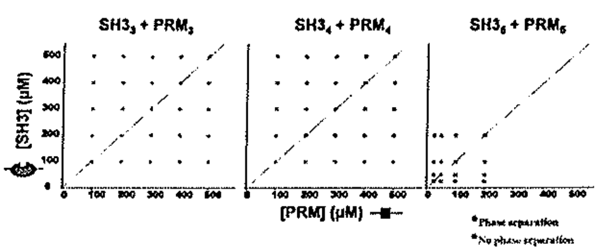


Require both $(SH3)_5$ and $(PRM)_5$
Not observed with $(SH3)_5$ + $(PRM)_3$
Puncta do not appear to be vesicular



Puncta recover quickly after bleach
Unlikely to be solid aggregates

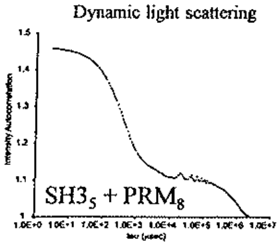
Higher Valency Favors the Phase Transition



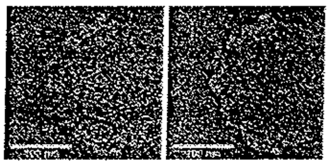
Suggests that oligomerization drives phase separation

The Droplet Phase is a Polymer

Dynamic light scattering

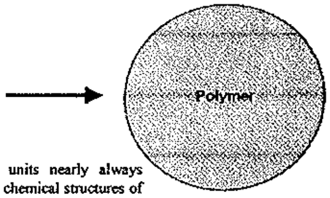


Cryo-EM



Macroscopic phase transition corresponds to molecular sol-gel transition to a polymer

Phase Transition Represents a Switch From nm-Scale Dimers to μm-Scale Polymer



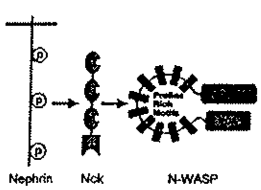
The presence of polyfunctional units nearly always presents the possibility of forming chemical structures of macroscopic dimensions...It is at this point that the concept of molecules as the primary chemical entities must be abandoned....

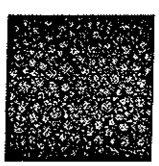
Paul Flory, 1953

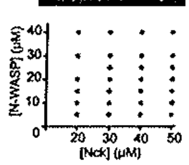
Droplet phase (Not to scale)

Provides a potential mechanism to achieve micron-scale organization of cellular processes

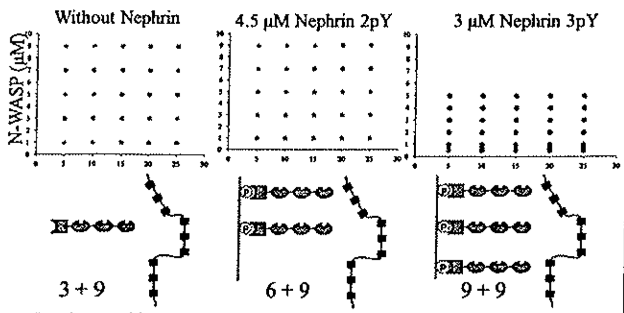
How Could Multivalent Phase Transitions be Regulated in Cells?





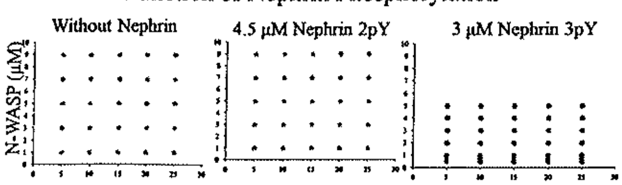


With N-WASP and Nck, Phase Transition Varies as a Function of Nephrin Phosphorylation



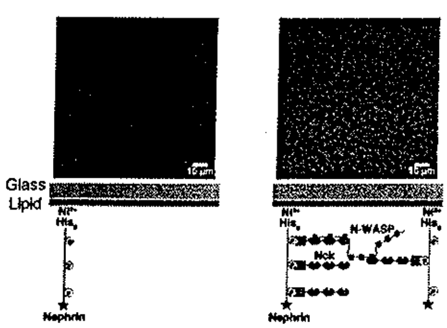
Consistent with polymer theory and our experiments with model systems

With N-WASP and Nck, Phase Transition Varies as a Function of Nephrin Phosphorylation



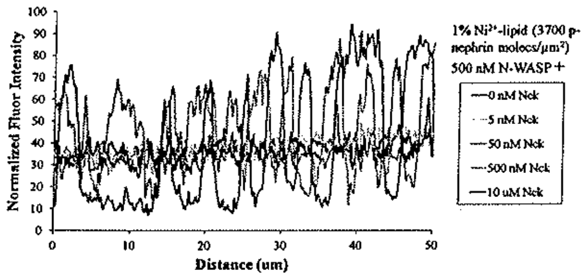
Kinase activity could control phase diagram in cells
Enables rapid control of N-WASP assembly by signaling
Also enables control of actin assembly activity (not shown)

Similar Behavior can be Observed in Two-Dimensions

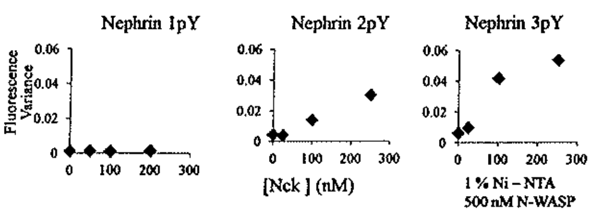


Membrane puncta show similar characteristics as 3D droplets
Multivalent phase transitions could drive clustering at membranes

Clustering Occurs at Nanomolar Nck Concentrations



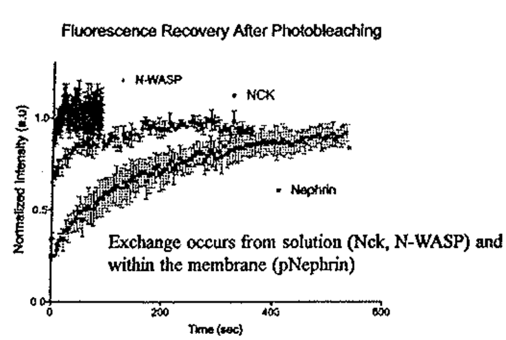
Critical Concentration Depends on Nephrin Phospho-Tyrosine Valency



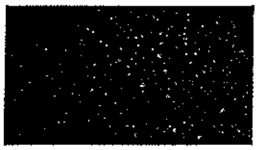
Critical concentration also decreases with increased SH2-pTyr affinity

Suggests that clusters are p-nephrin/Nck/N-WASP polymers

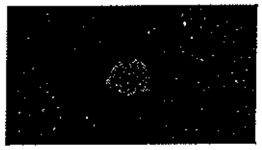
Components of the Membrane Clusters Exchange Rapidly



Clusters Coalesce over Time

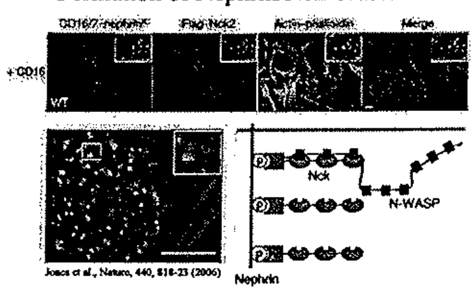


0.33 hours



24 hours

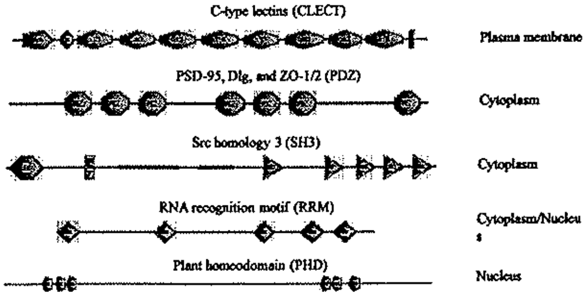
Intracellular Polymerization Could Contribute to Formation of Nephrin/Nck Clusters in Cells



Jones et al., Nature, 440, 818-23 (2006)

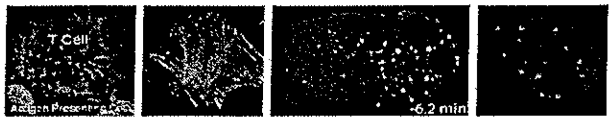
Multivalent phase transitions could provide a general mechanism of clustering at membranes

Multivalent Proteins are Found Throughout Biology



Suggests that phase transitions may be widespread

Multi-Valent Phase Transitions May Contribute to Various Cellular Puncta/Bodies



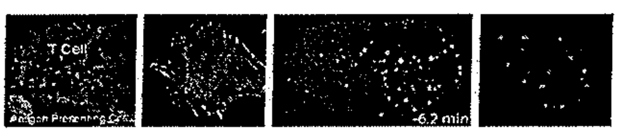
Immunological synapse (cell surface)	Focal adhesions (cell surface)	P granules (cytoplasm)	PML Bodies (nucleus)

Phase transitions of multi-valent proteins may provide a general mechanism to control macromolecular activity and cell organization on µm scales

Acknowledgements

Past:	Present:	Collaborators:
Behzad Aghazadeh	**Salman Banani**	Diana Tomchick (UTSW)
Zehan Abdul-Manan	**Sudeep Banjade**	Chad Brautigham (UTSW)
Zhucheng Chen	Stone Chen	John Leong (U Mass)
Hui-Chun Cheng	Lynda Doolittle	Brian Skehan (U Mass)
Ayman Ismail	Lisa Henry	Zbyszek Otwinowski (UTSW)
Annette Kim	Da Jia	Dominika Borek (UTSW)
Pilong Li	**Soyeon Kim**	**Tony Pawson (SLRI)**
Sanjay Panchal	**Yuan Lin**	**Tom Wandless (Stanford)**
	Shae Padrick	**Liang Guo (ANL)**
	Ben Peeples	**Tracy Nixon (Penn State)**
	Chi Pak	**Qiu-Xing Jiang (UTSW)**
	Ali Yunus	**Paul Russo (LSU)**
	Jacob Zahm	

Multi-Valent Phase Transitions May Contribute to Various Cellular Puncta/Bodies

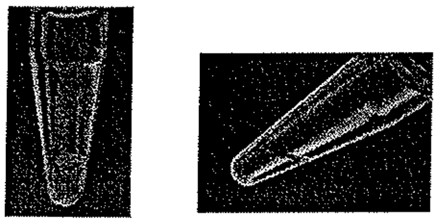


Immunological synapse (cell surface)	Focal adhesions (cell surface)	P granules (cytoplasm)	PML Bodies (nucleus)

2-D and 3-D puncta observed—membrane clustering & cellular bodies enriched in multi-valent interactions

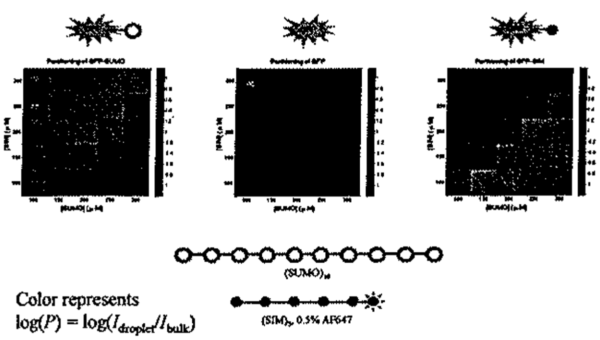
P Granules and nucleolus have been described as liquid droplets

The Droplet Phase Behaves as a Liquid



Collection of droplets enables their physical characterization

SUMO/SIM Ratio Affects Recruitment



Color represents
$\log(P) = \log(I_{droplet}/I_{bulk})$