Imperial College London

CECAM Workshop
The self-organised cytoplasm
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Spatial organisation of the cell cytoplasm: P granule localisation by phase separation

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"Spatial organization of the cell cytoplasm by position dependent phase separation." *Physical Review Letters* **111**, 088101.

A cell

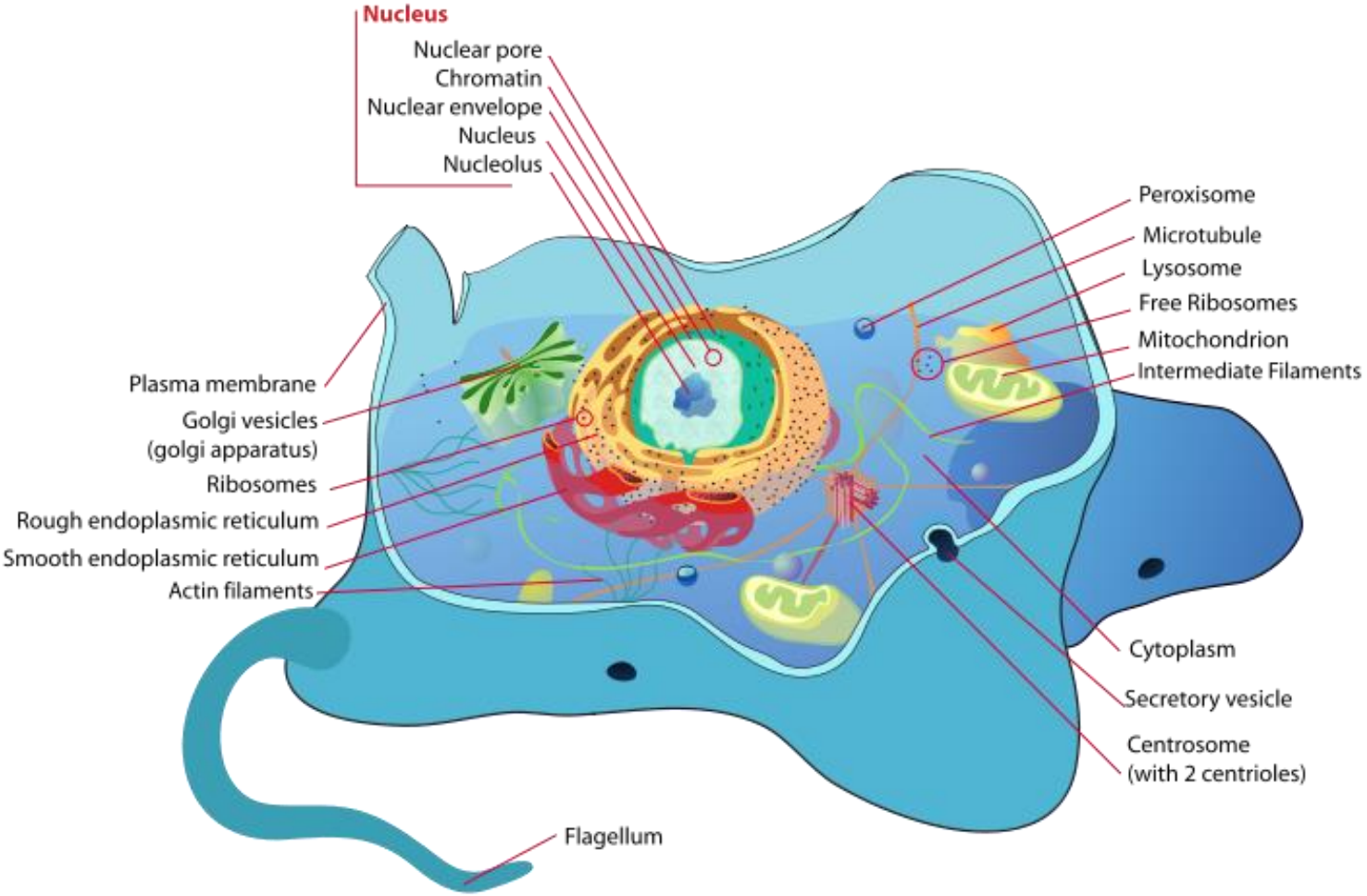
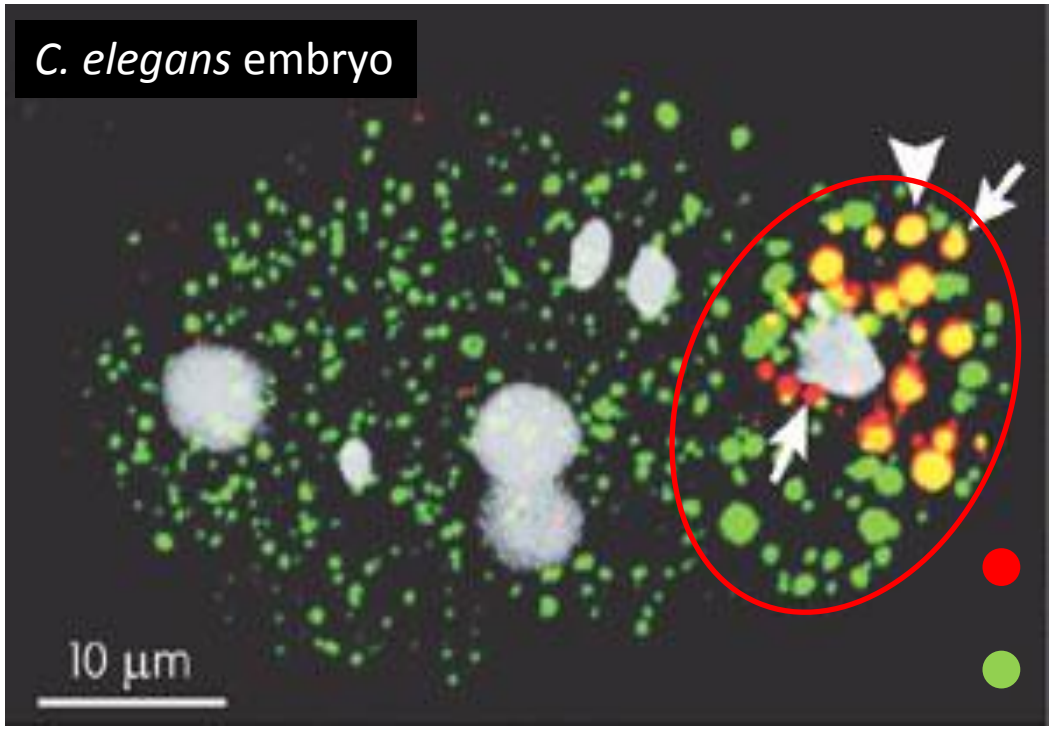


Figure from Wikipedia

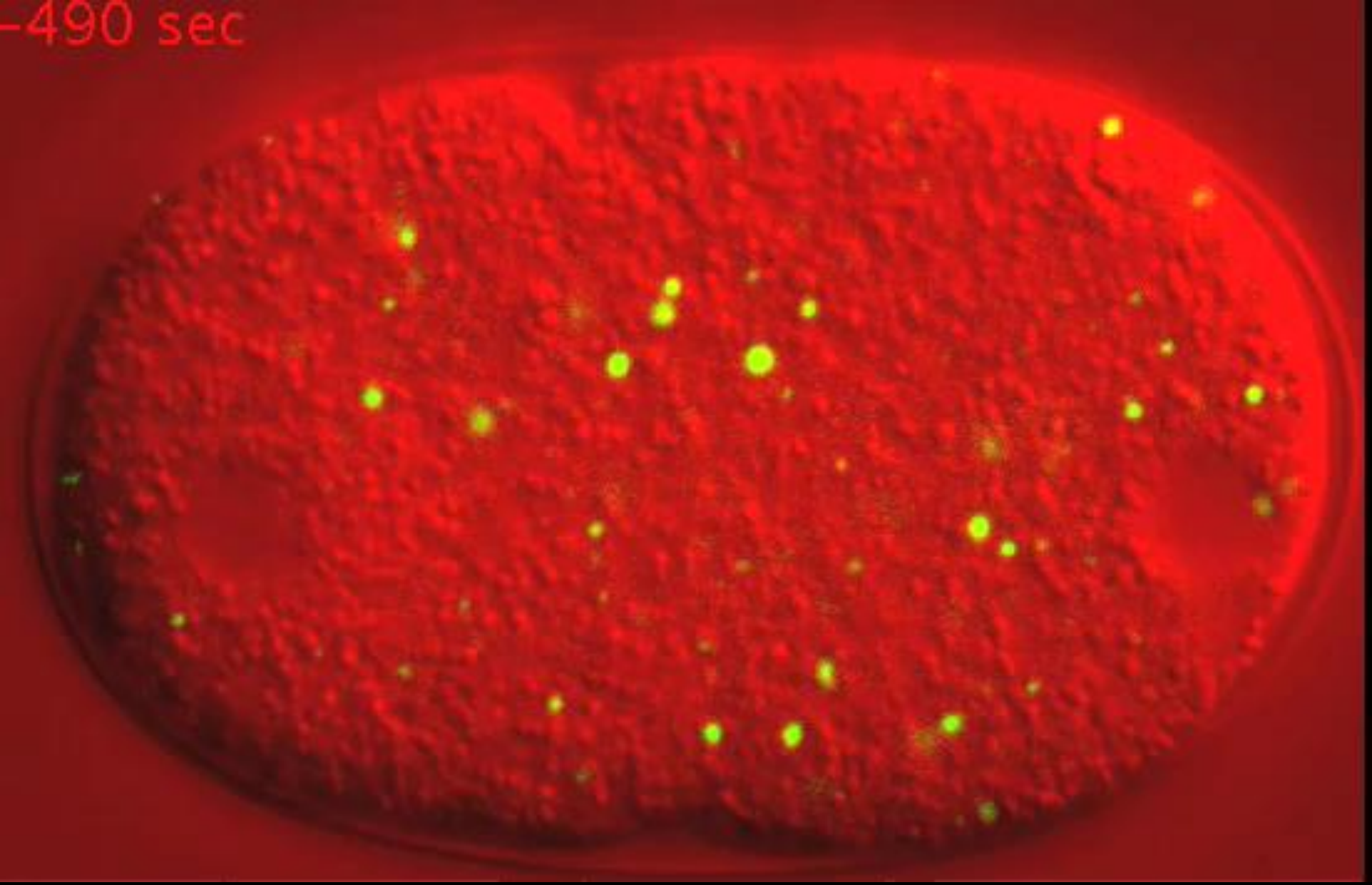
Nonmembrane bound organelles



Germ granules (coined "cloud" by the discoverers)

Processing bodies (P bodies)

Fig. adapted from Anderson & Kedersha (2009) *Nature Rev. Mol. Cell Biol.*



Brangwynne (Hyman Lab, MPI-CBG)

Plan

Three questions:

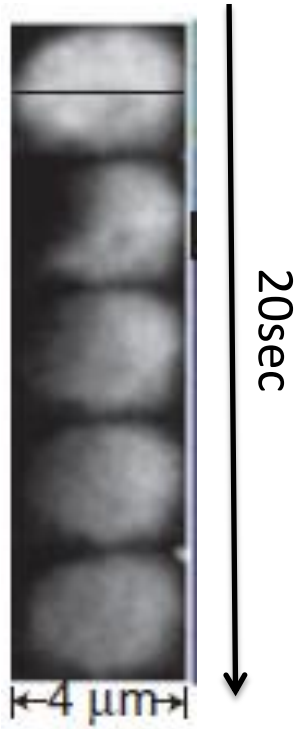
1. How do P granules form?
2. How do they regulate their interior contents?
3. How do they get localised?

Much of what I say should be relevant to other RNA granules

1. How do P granules form?

Characteristics of P granules

- Granules diffuse and are moved under flow
- Two granules fuse when touched
- Contents are in dynamical equilibrium with the cytoplasm – quick FRAP recovery (in seconds)



Brangwynne et al. Science (2009)

Suggest that P granules are droplets of condensed P granule material resulted from phase separation, just like oil droplets in water

Self-assembly of RNA granules *via* phase separation

[Sear (2008) Faradays Discussions; Brangwynne *et al.* (2009) Science]

What is phase separation? A two-slide review

A physical system tends to lower its free energy:

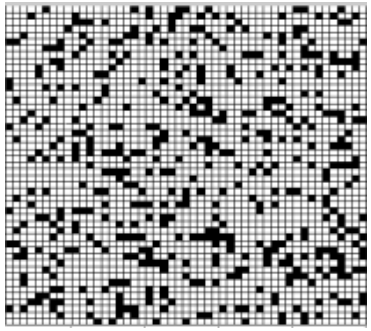
Free energy = Energy – Temperature × Entropy

Water and oil repels

High energy

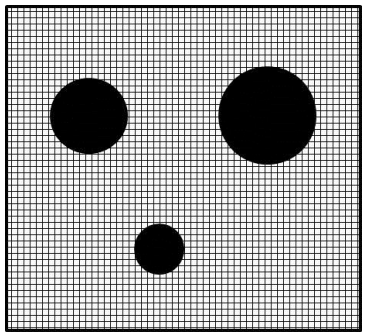
Low energy

High energy
High entropy



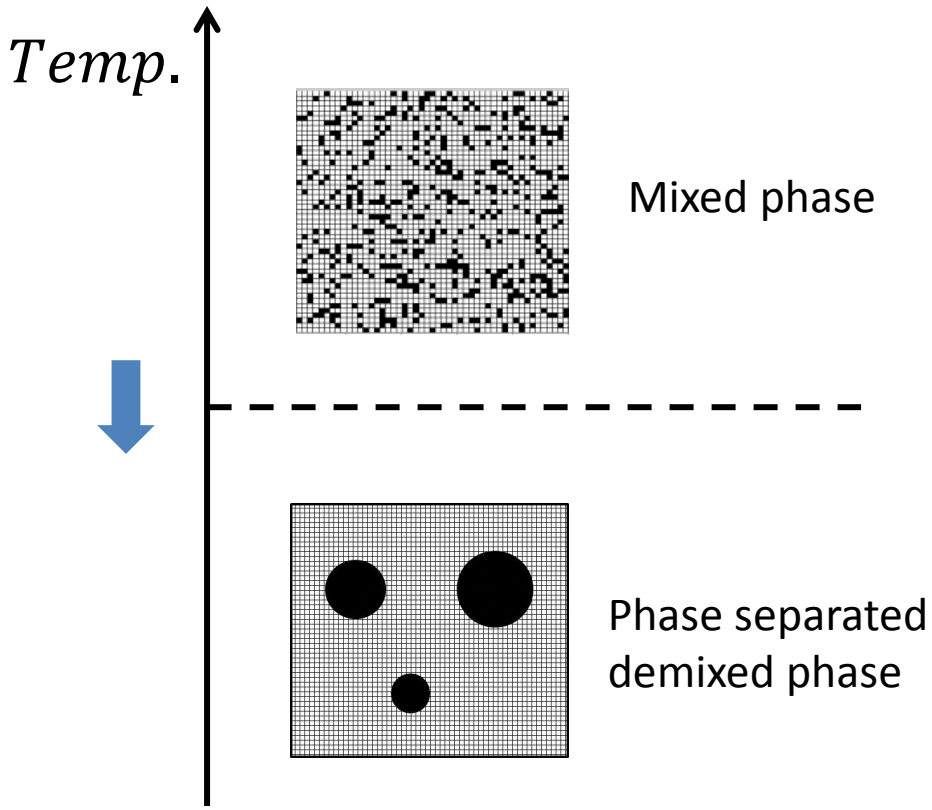
High temperature configuration

Low energy
Low entropy



Low temperature configuration

Phase diagram



Phase transition exhibits a switch-like behaviour

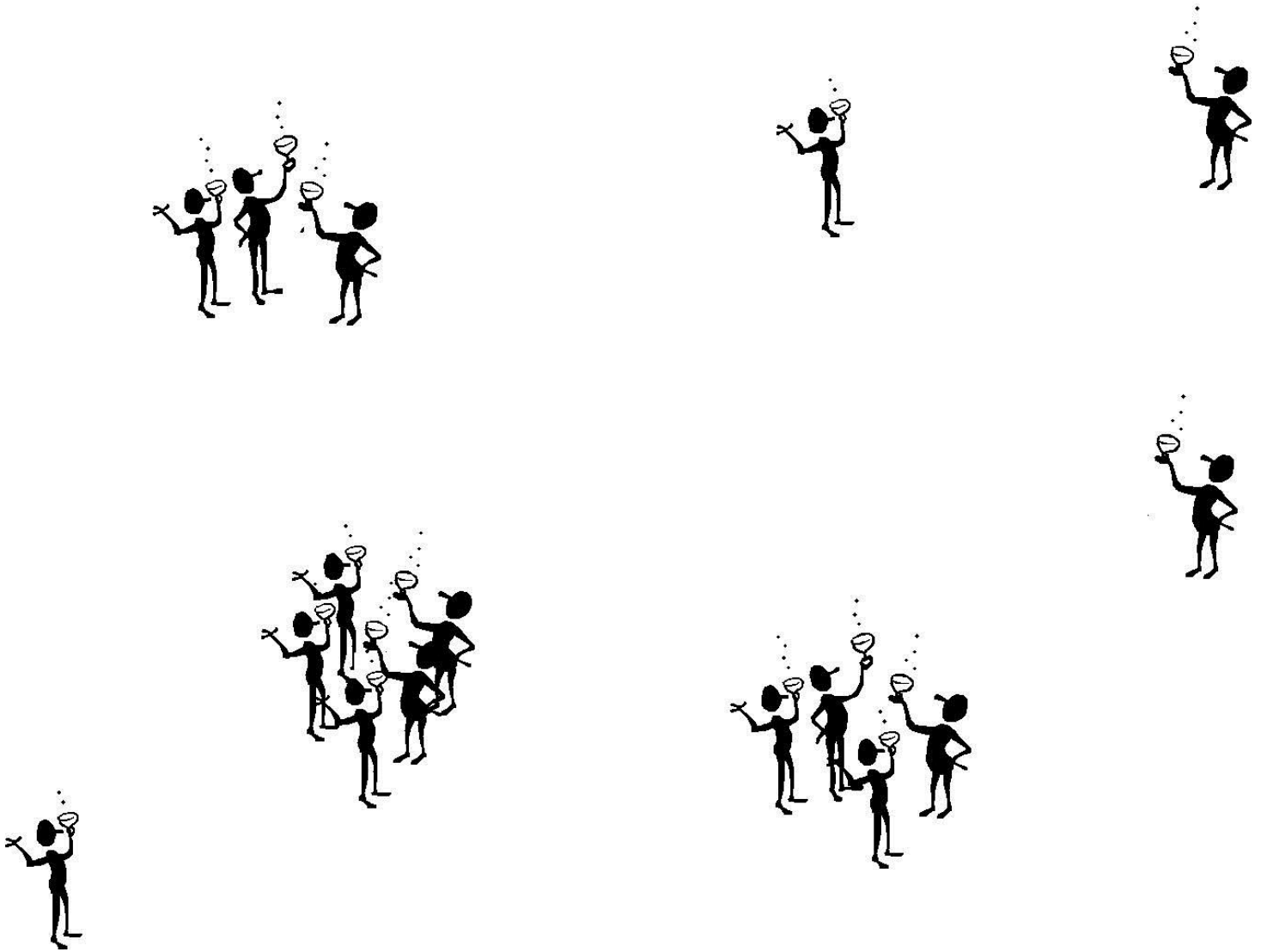
Phase separation is the partitioning of the system into Subsystems with distinct macroscopic properties

2. How do P granules regulate their contents?

Content control

- High turnover of material -> passive control via weak and potentially unspecific interactions and rely on diffusion to move things around

Analogy – a drink reception



Many components

- There are multiple components in a granule, not all components are phase separating!

- E.g. soluble RNA can be concentrated in P granules through weak binding

  e.g., [Protein] + [RNA] <-> [Protein*RNA]

A reception with servers

RNA

Phase separating proteins

Many components

- There are multiple components in a granule, not all components are phase separating!

- E.g. soluble RNA can be concentrated in RNA granules through weak binding

  e.g., [Protein] + [RNA] <-> [Protein*RNA]

Phase separating components ↔ scaffold

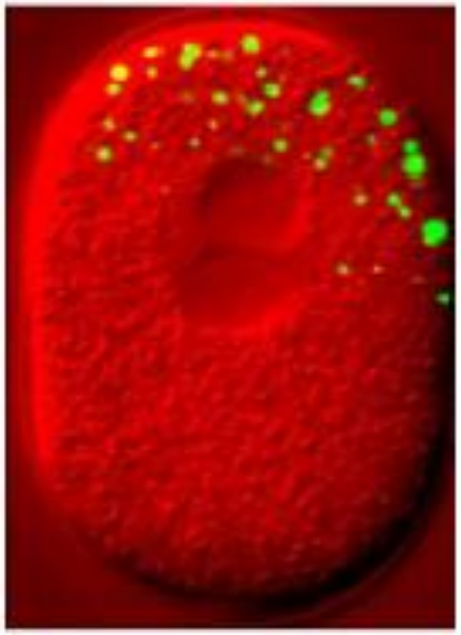
Other granule components ↔ cargoes

3. How do P granules get localised to the posterior?

P granules localise to the posterior

- Localisation?

Picture from
National Weather Service, FL

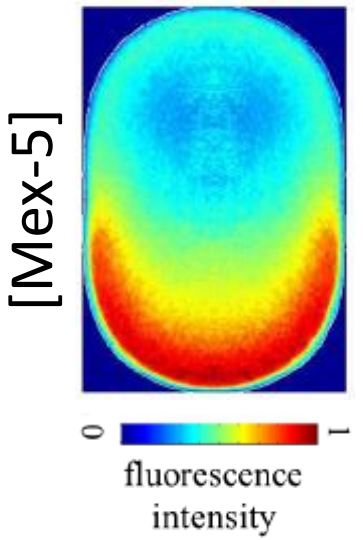


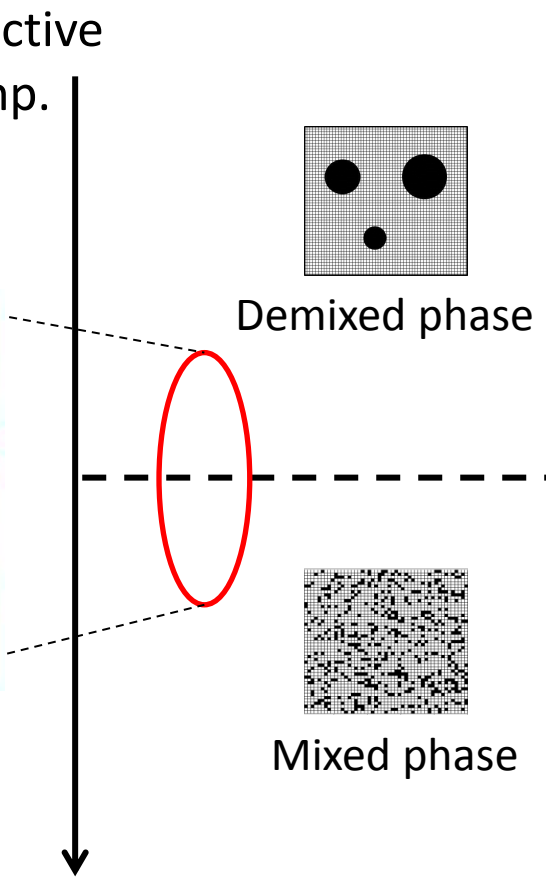
Effective temp.

3-component mean-field free energy model:

$$f = k_B T\left[p \ln p + m \ln m + s \ln s\right] + \chi_{pm} pm + \chi_{ps} ps + \chi_{ms} ms$$

<0 >0 <0

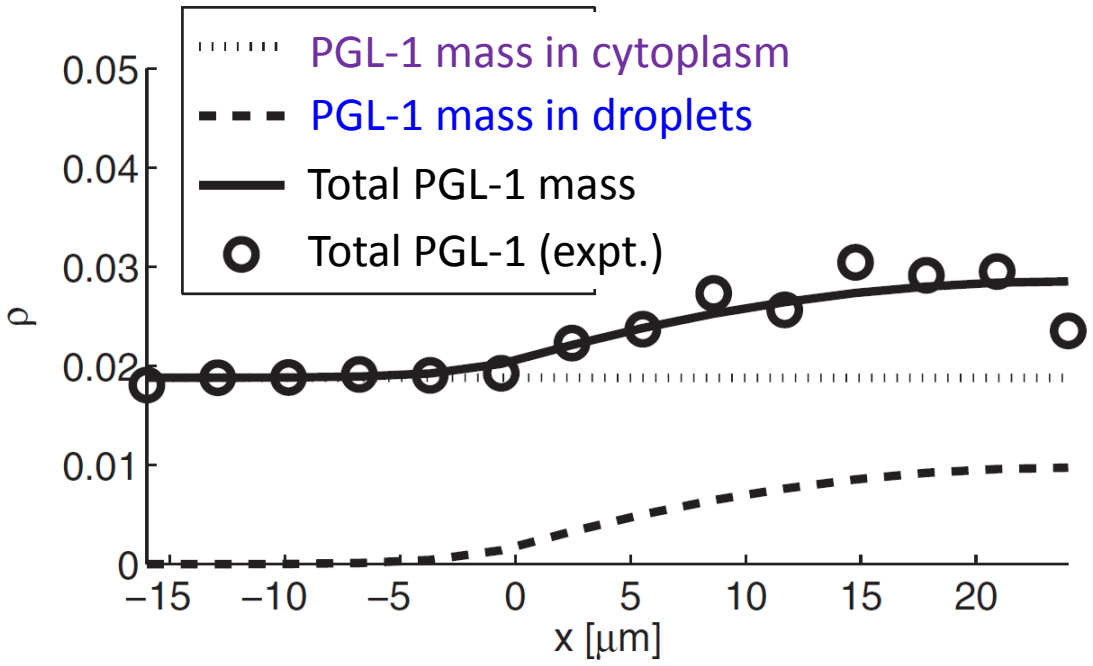


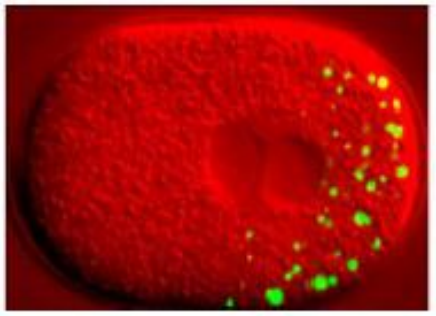


Mex-5 gradient serves as an effective temperature gradient

Lee, Brangwynne, Gharakhani, Hyman, Jülicher (2013) Physical Review Letters 111, 088101

Quantitative comparison





PGL-1 in the cytoplasm

PGL-1 in droplet form

Overall, there is only ~50% more P granule material (PGL-1) in the posterior side

But 50% more scaffold could lead to much more cargoes

e.g., [S] + [S] + [C] <-> [S*S*C]

Potentially, there can be much more cargoes in the posterior!

Conclusion

Three questions addressed

1. How do P granules form?
   - Phase separation
2. How does they regulate their interior contents?
   - Diffusion with weak and unspecific binding
3. How do they get localised?
   - Mex-5 gradient serving as an effective temperature gradient

Why does biology choose to assemble RNA granules via phase separation?

- Pros: Cheap, easy and fast to assemble
- Cons: lack of precise temporal and spatial control

Open question, can we do a proper cost-benefit analysis?

Acknowledgement

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Thank you!