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Liquid-liquid phase separation of FG-nucleoporins

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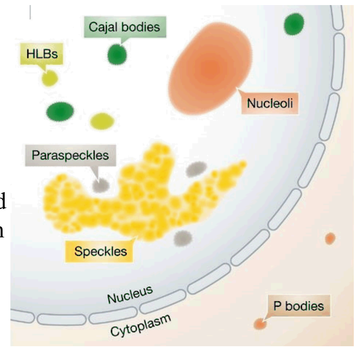
Liquid-liquid phase separation of proteins

Eukaryotic cells contain multiple membraneless organelles that originate through liquid-liquid phase separation of specific disordered proteins, in a manner similar to polymer condensation. The interior of these membraneless organelles is a liquid-protein phase that is maintained by weak, multivalent interactions between the disordered proteins. The liquid separated droplets provides spatiotemporal organization and creates a different physicochemical microenvironment from the surrounding fluid that is optimized to suit the organelle's task. The assembly of these organelles is often triggered in response to stress, with assembly controlled via disordered protein concentration, post-translational modifications, or generation of nucleating agents, such as mRNA seeds in the case of P bodies.



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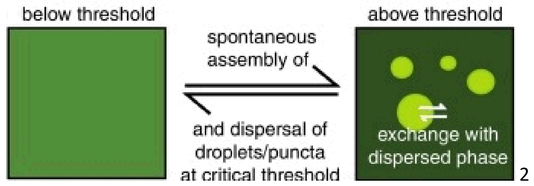

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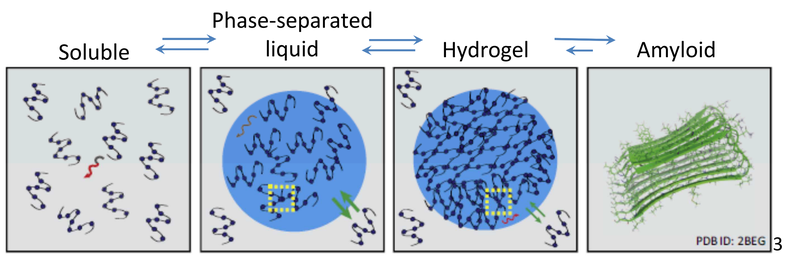
The phase separated protein organelles and droplets display liquid-like physical properties. Droplets can fuse together and deform under shear flow. The spherical shape of the droplet is driven by surface tension. Exchange between proteins inside the droplets and dispersed phase in solution is evidenced by fluorescence recovery after photobleaching.



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Proteins involved in forming liquid droplets contain intrinsically disordered protein regions (IDRs: characterized by a lack of stable secondary or tertiary structure) that have repeating motifs enriched in arginine, serine, glycine, glutamine, asparagine, and/or aromatic residues. Based on bioinformatic analysis of protein sequences, 33% of eukaryotic proteins are expected to contain IDRs. The plasticity of IDRs is often essential to their function. Many of these proteins can aggregate to form amyloids, which are implicated in various diseases, such as alzheimers and Parkinson's disease. In addition to forming amyloids, many IDRs have been shown to form hydrogels under certain conditions. Very recently, it has been discovered that the same IDRs that form hydrogels also form liquid-liquid phase separated droplets. Evidence is building that IDRs can convert between different positions on the soluble – phase separated liquid – hydrogel – amyloid spectrum.

Nuclear pore complex

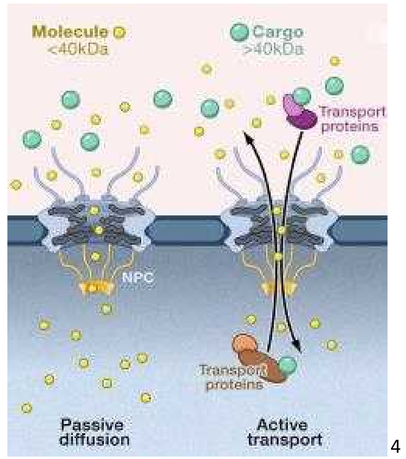
The nuclear pore complex (NPC) is a giant (>100 MDa, 120 nm wide) protein complex that controls transport of species across the nuclear envelope.

The transport channel is full of IDRs that are rich in phenylalanine-glycine repeat motifs (FG-Nups), which functions as a permeability barrier. The amino acid composition of FG-Nups indicates that they could be good hydrogel and droplet formers.

Small cargos (<40kDa) can pass through by passive diffusion. Large cargos can only go through when bound to nuclear transport receptor proteins – then they go through faster than predicted by passive diffusion.

Energy consumed in binding/unbinding cargo from transporters, but not in actually translocating through the pore.

There are multiple competing theories for how the FG-Nups create the permeability barrier.



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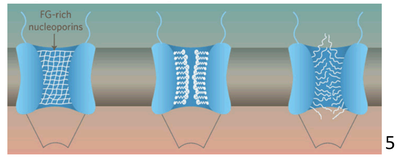
Competing theories of how the permeability barrier works:



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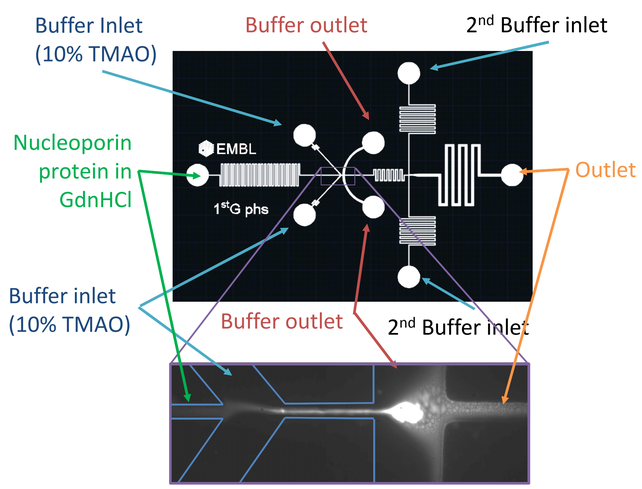
Selective phase model (hydrogel) | Forest model | Polymer brush model

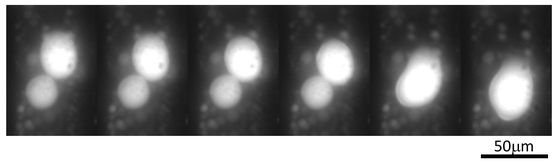
Microfluidic device

A microfluidic device was designed to form liquid droplets of FG-Nups:

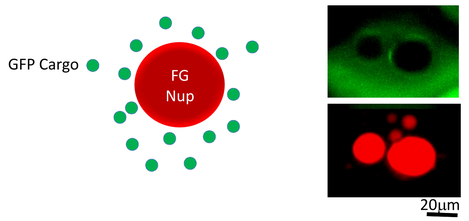


Results

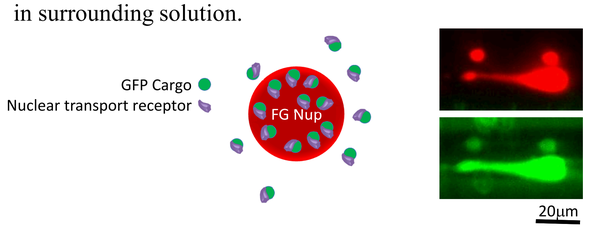
FG-Nucleoporins (Nup49) can form phase separated droplets that exhibit liquid characteristics, including deforming and merging.



Large cargos (GFP) by themselves cannot enter the liquid droplets:



Large cargos with nuclear transporters can enter the droplets. The concentration of the cargo in droplets is greatly enriched over concentration in surrounding solution.



The droplets are initially liquid, although over time they convert to gels, as observed with fluorescence recovery after photobleaching experiments. The microfluidic device makes it possible to examine the droplets immediately after formation before gelation occurs and to introduce reagents or cargo to the droplets.

The phase-separated liquid FG-Nup droplets reconstitute the transport properties of the nuclear pore complex, introducing a new theory for how the permeability barrier works.

Understanding phase-separated proteins could lead to future novel materials for selective barriers or chemical reaction centers.

References

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