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Liquid-liquid phase separation in heteroprotein systems: a mini review

A. Boire¹, D. Renard¹, A. Bouchoux², S. Bouhallab³

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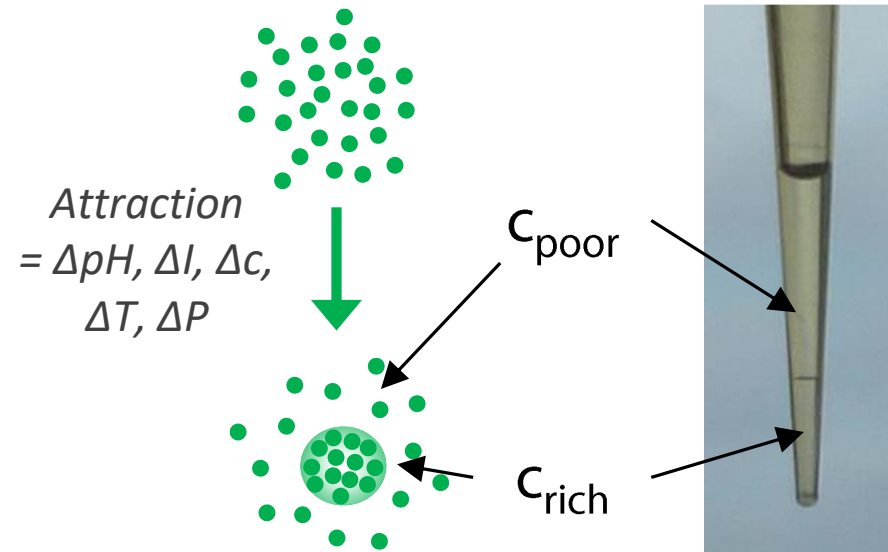
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Bio & Chemical Engineering

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STLO
Rennes

➤ Liquid-liquid phase separation

A universal phenomenon



General features:

- Spontaneous phenomenon ($\Delta G < 0$)
- Reversible
- Involves one or **mixture** of macromolecules

Protein / polysaccharides

Protein / polyelectrolytes

Protein / DNA - RNA

Strong or weak polycations, polyanions

Liquid-liquid phase separation

A universal phenomenon

2009

Germline P Granules Are Liquid Droplets That Localize by Controlled Dissolution/Condensation

Clifford P. Brangwynne,^{1,2,3} Christian R. Eckmann,⁴ David S. Courson,⁴ Agata Rybarska,¹ Carsten Hooge,¹ Jöhn Gharahkhani,^{2,3} Frank Jülicher,^{2,3} Anthony A. Hyman^{1,2,4}

In sexually reproducing organisms, embryos specify germ cells, which ultimately generate sperm and eggs. In *Caenorhabditis elegans*, the first germ cell is established when RNA and protein-rich P granules localize to the posterior of the one-cell embryo. Localization of P granules and their physical nature remains poorly understood. Here we show that P granules exhibit liquid-like behavior, including fusion, dripping, and wetting, which we used to estimate their viscosity and surface tension. As with other liquids, P granules rapidly dissolved and condensed. Localization occurred by a biased increase in P granule condensation at the posterior. This process reflects a classic phase transition, in which polarity proteins vary the condensation point across the cell. Such phase transitions may represent a fundamental physicochemical mechanism for structuring the cytoplasm.

Starting from the fertilized egg, the cells of a developing embryo differentiate to give rise to somatic tissues, as well as maintaining an immortal germ line that will generate sperm and oocytes. Germ cell specification is mediated in part by ribonucleoprotein granules assembled from RNA and RNA binding proteins, although the precise function of these granules remains unknown (1, 2). In *Caenorhabditis elegans*, the germ granules are called P granules. P granules are initially distributed uniformly throughout the unopulated one-cell embryo. Upon symmetry breaking, the embryo polarizes along the anterior-posterior (AP) axis: Cortical and cytoplasmic flows devel-

op (3), the polarity protein PAR-1 and PAR-2 appear on the posterior cortex, and P granules become localized to the posterior half of the cell (Fig. 1A and movie S1; all embryos are ~50 μm long); the embryo then divides, giving rise to a P granule-containing progenitor germ cell and a non-P granule-containing somatic sister cell. Two processes have been proposed to mediate this posterior localization: (i) P granule migration by cytoplasmic flow (4–6) and (ii) subsequent assembly or deceleration of remaining posterior P granules (5–7). However, evidence supporting either of these mechanisms is sparse.

To study P granule localization in the one-cell embryo, we used three-dimensional (3D) particle tracking to monitor the movement and fluorescence levels of P granules labeled with green fluorescent protein (GFP) tagged PGL-1 (5, 10) or GFP-1 (9, 10), both constitutive P granule components. We found that some P granules move into

the embryo posterior; however, close to the cortex there was a flux of P granules into the anterior that was of similar magnitude to the posteriorly directed flux (Fig. 1, D to F). This behavior closely matched the overall flow behavior of cytoplasmic material such as yolk granules (8), quantified by particle imaging velocimetry (PIV) (Fig. 1, B and C) (9). P granules cannot preferentially localize to the posterior by convection in the surrounding cytoplasm alone. Thus, flows have little or no role in P granule localization (9).

We next examined intensity changes of individual P granules during localization. We found that P granule size is spatiotemporally controlled (Fig. 2A). We determined the average rate of relative intensity change of a population of P granules, ξ , at different points in space and time (Fig. 2); negative ξ indicates P granule dissolution (i.e., shrinkage) and positive ξ indicates P granule condensation (i.e., growth). Radial symmetry breaking, ξ was negative across the entire embryo, indicating overall P granule dissolution (Fig. S1). After the onset of symmetry breaking, ξ turned negative in the anterior but became positive in the posterior of the embryo, indicating posterior condensation (Fig. 2, B (WT, wild type) and C). As predicted from this analysis, if we disrupted symmetry breaking using RNA interference (RNAi) to deplete the centrosomal protein SPD-5 (7, 8), ξ stayed negative across the whole embryo, and P granules appeared to dissolve completely (Fig. 2B, and S100A10). When these embryos eventually broke symmetry, P granules appeared to form *de novo*, in the vicinity of posterior PAR proteins (Fig. 2D and fig. S5 and S6). This occurred concomitant with a depletion of soluble P granule components from the anterior cytoplasm (Fig. 2E), and in the absence of visible cytoplasmic flows (movie S3). As with WT embryos, the rate of P granule condensation peaked before leveling off. The maximum of the posterior growth rate in *spd-5(RNAi)* embryos was three times as high as that in WT embryos, which

we used three-dimensional (3D) particle tracking to monitor the movement and fluorescence levels of P granules labeled with green fluorescent protein (GFP) tagged PGL-1 (5, 10) or GFP-1 (9, 10), both constitutive P granule components. We found that some P granules move into

REPORTS

distances. These NMR restraints were also supplemented by a limited number of interstrand nitro-oxide distance restraints derived from double-nitrogen measurements that used deuterio-cysteine instead of methionine. Cross-peaks in the 2D NOESY spectra were assigned to the double-nitrogen restraints. The structure was calculated using standard restrained molecular dynamics and simulated annealing. *Proc. Natl. Acad. Sci. U.S.A.* 106, 10372–10377 (2009).

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22. A. W. Partridge, R. A. Mink, D. Tang, H. Bock, C. M. DeRube, *J. Mol. Biol.* 388, 2054 (2005).

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27. H. I. Sigler, *Acta Cryst.* 34, 117 (2008).

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31. N. Nagata, C. A. Oomen, J. M. Thornton, *J. Mol. Biol.* 372, 741 (2008).

32. Single-letter abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

33. E. Drenth, R. L. Suck, J. Drenth, *Acta Cryst.* 35B, 348 (2008).

34. S. Suck, *J. Mol. Biol.* 376, 1068 (2008).

Downloaded from www.sciencemag.org on October 2, 2007



Domaine très actif depuis une dizaine d'années!!

Key words: LLPS, Condensates; Membraneless organelles; liquid droplets; Complex coacervates

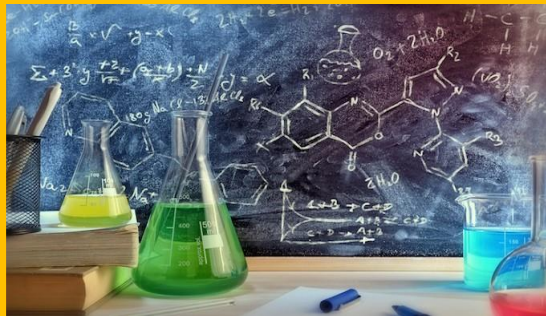
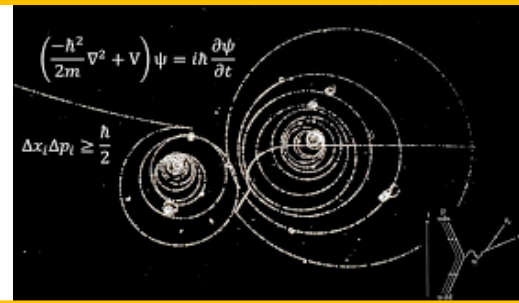
Exponential increase of number of publications

➤ Liquid-liquid phase separation

A universal phenomenon

Active field of research where the three communities meet ...
with equivalent / complementary approaches

Physics:
Soft matter/colloids/polymers
techniques & principles

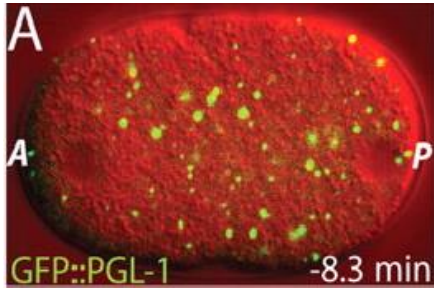


Chemistry:
Synthesis, thermodynamic

Biology:
Molecular & cell biology /
genetic engineering /
labelling and microscopies

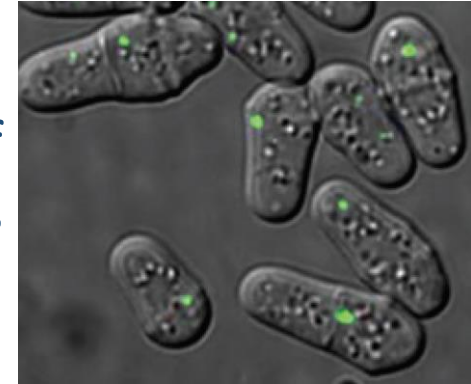


➤ Liquid-liquid phase separation *universal phenomenon*



Embryos germ cells P-granule (RNA/proteins)

Dynamic reorganisation of intracellular enzymes



O'Connell et al; Ann; Rev. Cell Dev. Biol., 2012

Brangwynne *et al.*, *Science*, 2009



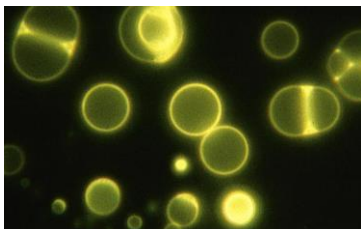
Marine organisms: A high adhesion (a glue!!)

« Sandcastle worms » Mussels

Adhesion; catalysis; regulation; cell plasticity control

Waite,, *The Journal of Adhesion*, 2005

Kim *et al.*, *PNAS*, 2016

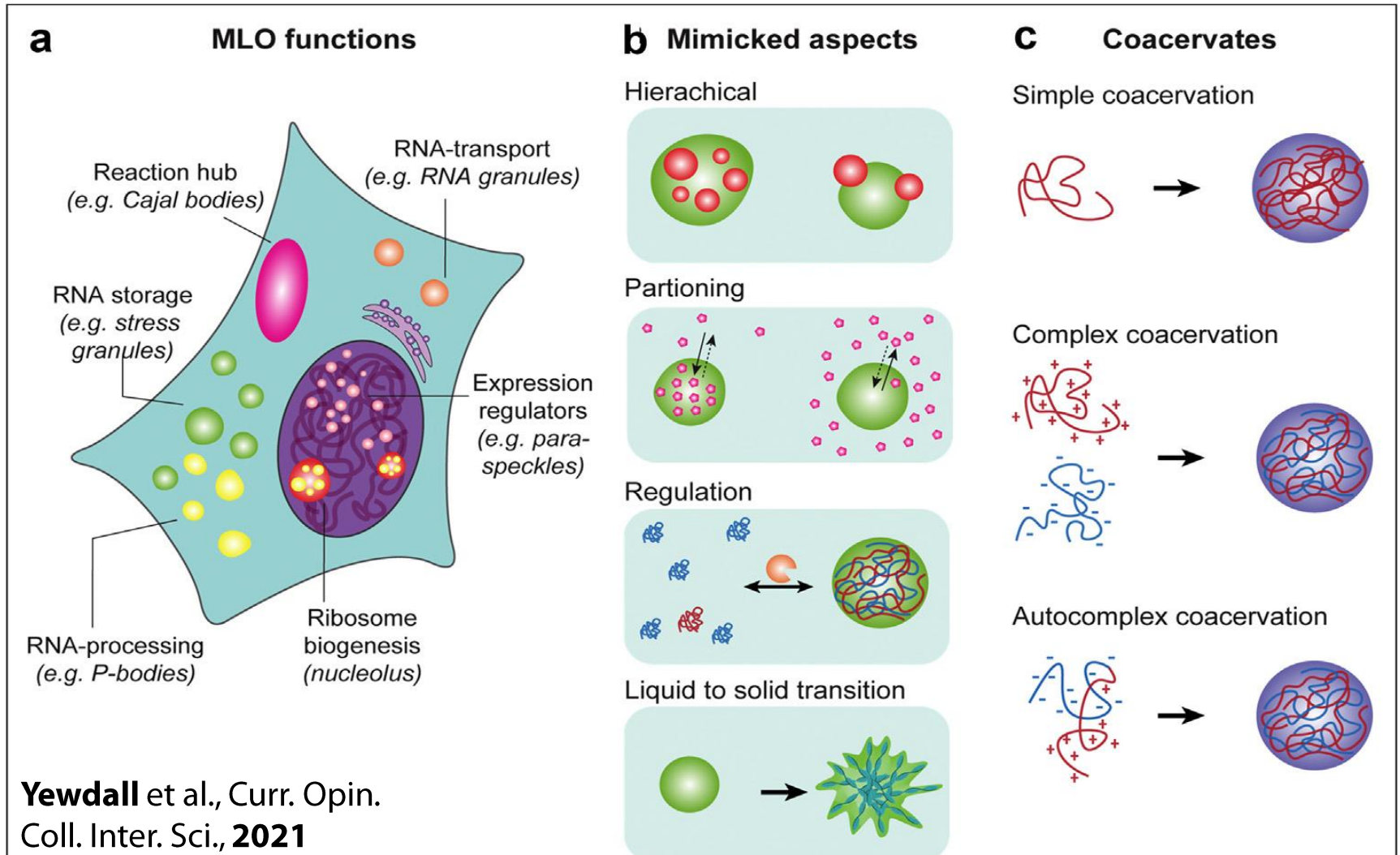


Macromolecular phase separation is being recognized for its potential importance and relevance as a driver of spatial organization within cells

Mittag & Pappu, *Molecular Cell*, 2022 p. 5

Liquid-liquid phase separation

A universal phenomenon



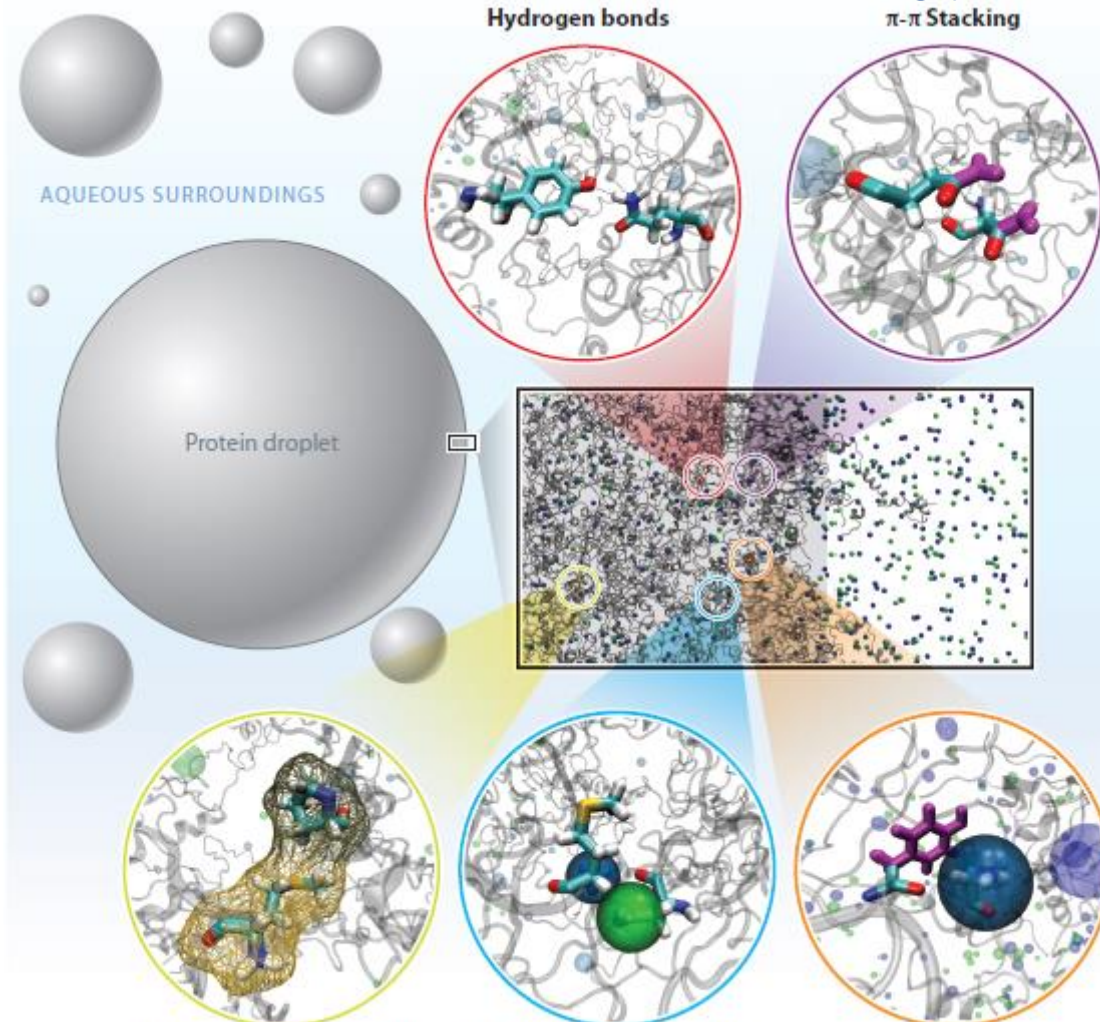
Yewdall et al., Curr. Opin. Coll. Inter. Sci., **2021**

Understanding cell membraneless organelles
.... by mimickingusing coacervates.

➤ Liquid-liquid phase separation

A universal phenomenon

A myriad of multiple interactions in a strongly aqueous medium...

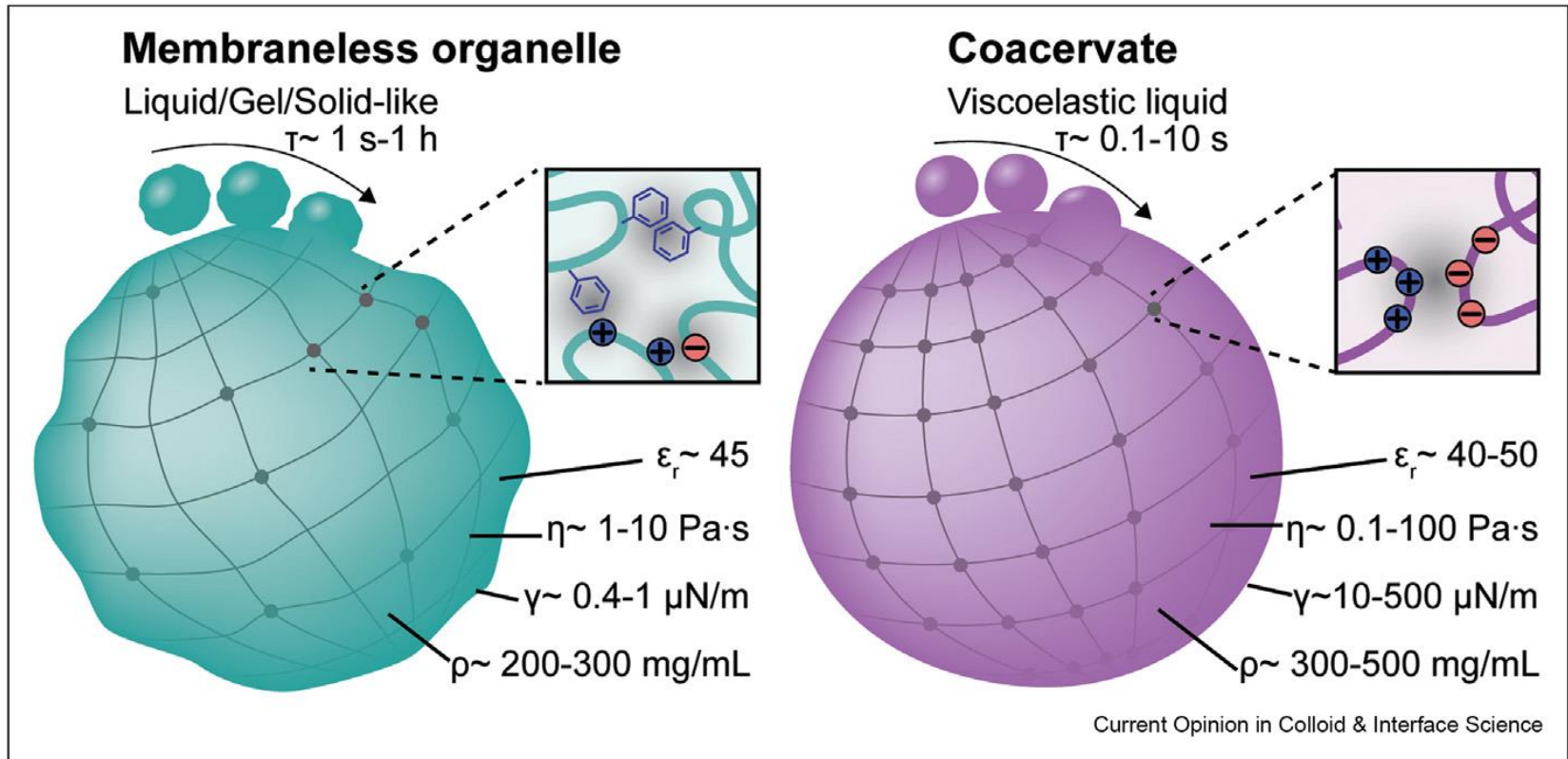


Dignon et al.,
Annu. Rev. Phys. Chem. **2020**

From simple 'complex' coacervates *in vitro* to complex MLOs *in vivo*

➤ Liquid-liquid phase separation

A universal phenomenon

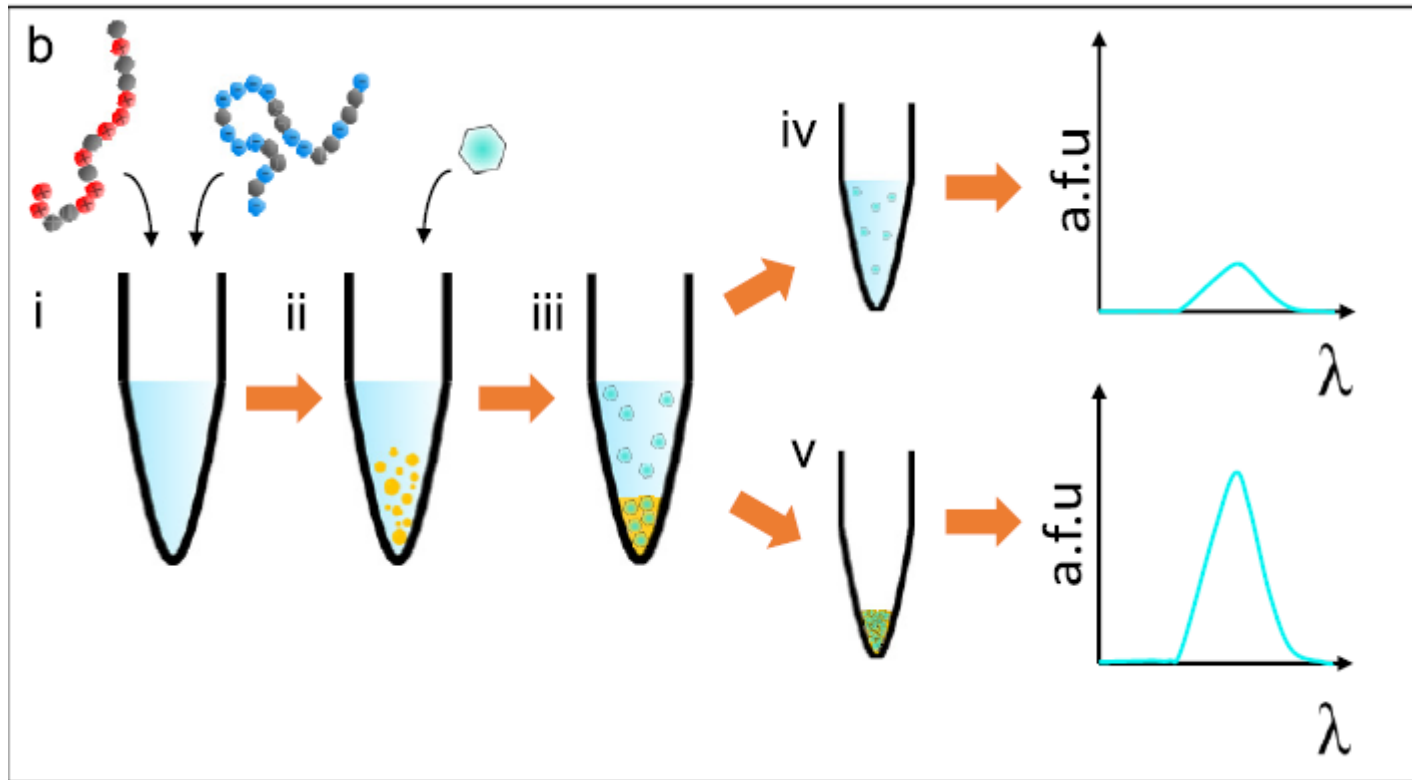


Relevant physicochemical properties of MLOs versus coacervates:

High charge screening for coacervates compared to more variable interaction for MLOs

➤ Liquid-liquid phase separation

A universal phenomenon



Schematic representation of a bulk experimental methodology

(i) Coacervates are formed by mixing oppositely charged polyelectrolytes.

(ii-iii) A molecule of interest is added, and the mixture is centrifuged. Quantification in the dilute aqueous phase (iv) and in the dense coacervate phase (v)

➤ Chemical determinants of phase separation

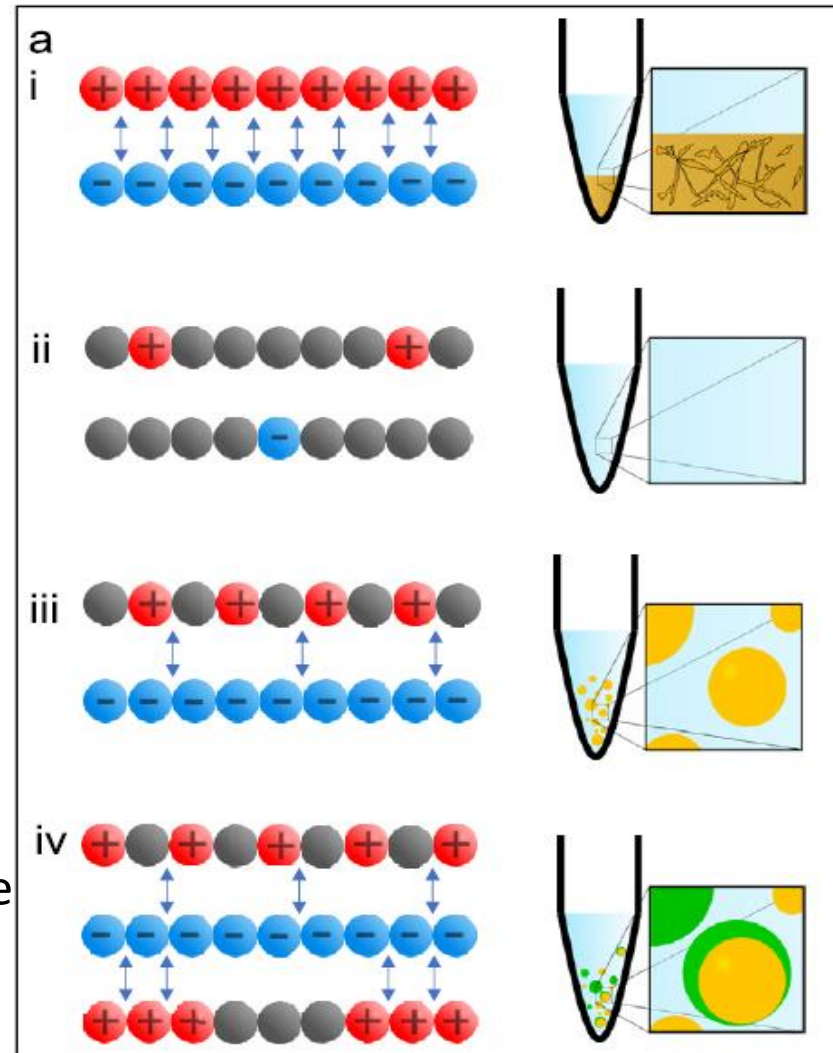
The strength of electrostatic interactions govern whether coacervates can be formed or not

(i) If a very strong attraction → precipitates

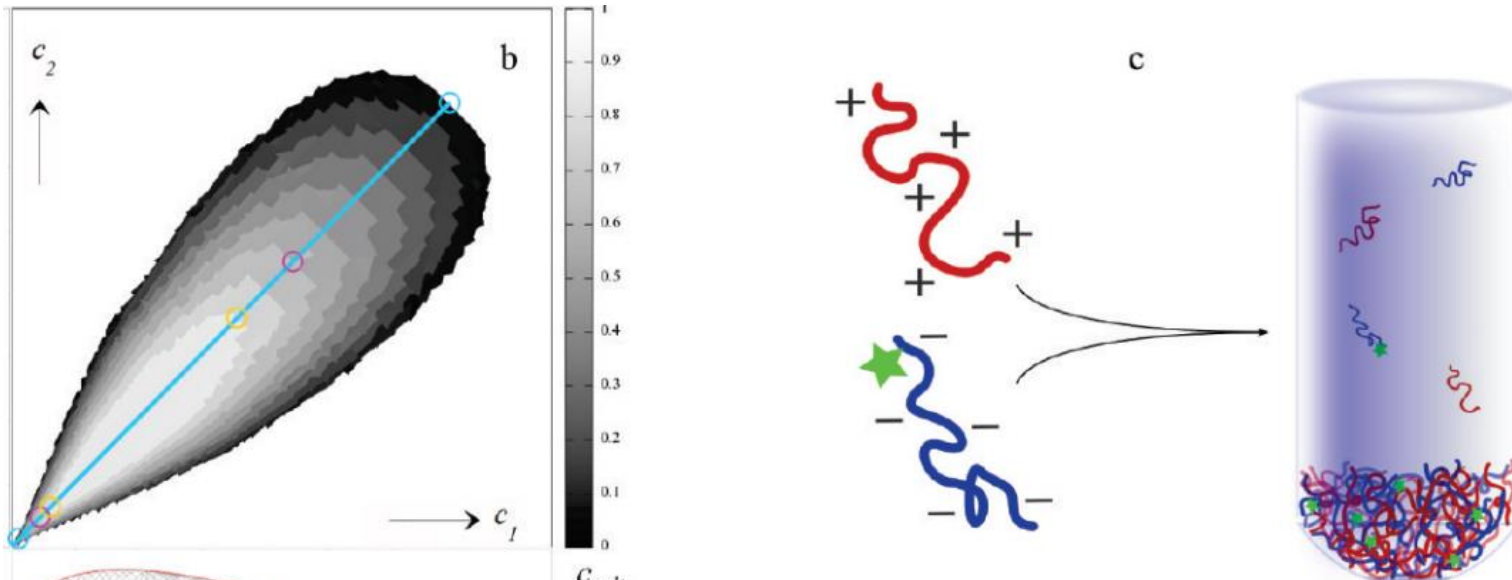
(ii) If too weak interactions → no phase-separation

(iii) Optimal strength of the interactions → Coacervates

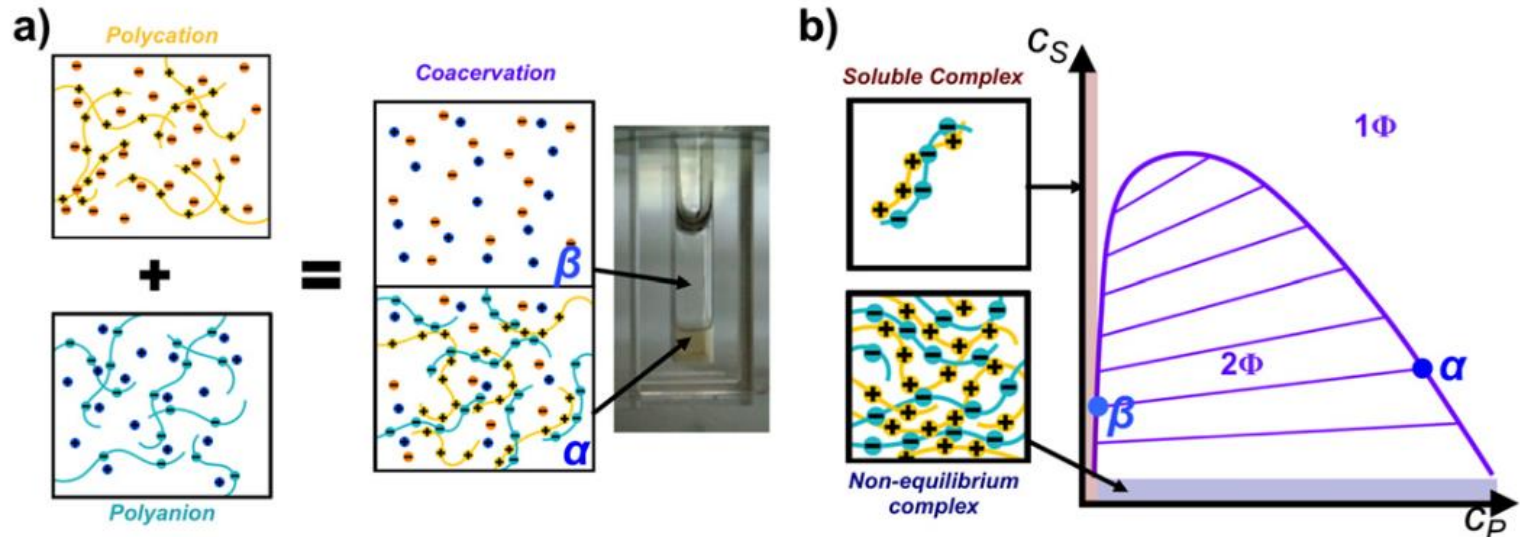
(iv) If multiple polyelectrolyte species, possible to form multiphasic coacervates



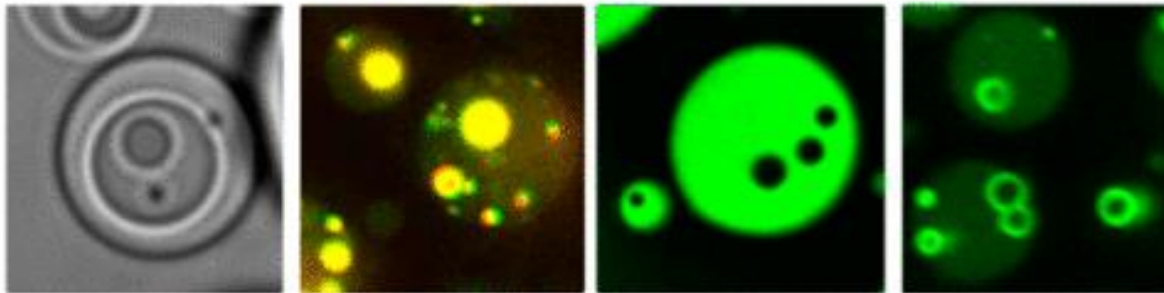
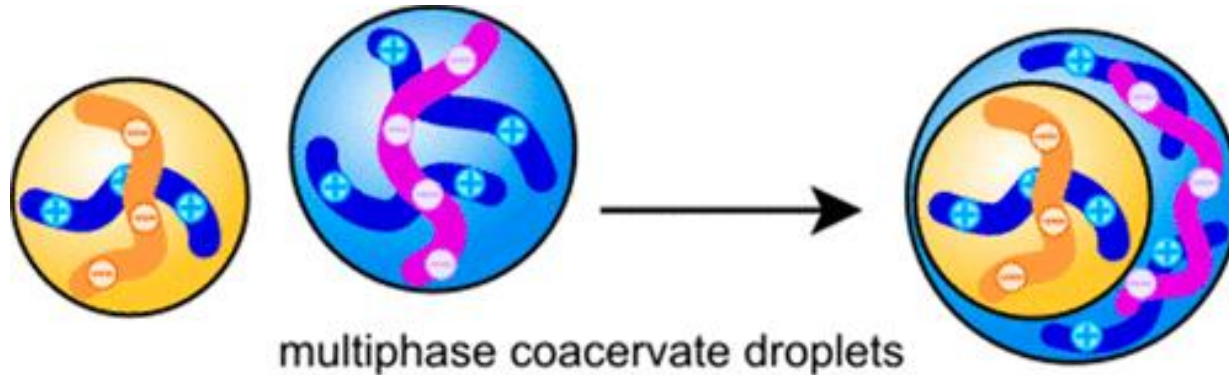
➤ Chemical determinants of phase separation: phase diagram



Schematic phase diagram of coexisting phases for associative phase separation and schematic associative phase separation



➤ Control formation of multiphase liquid droplets Partitioning in vivo

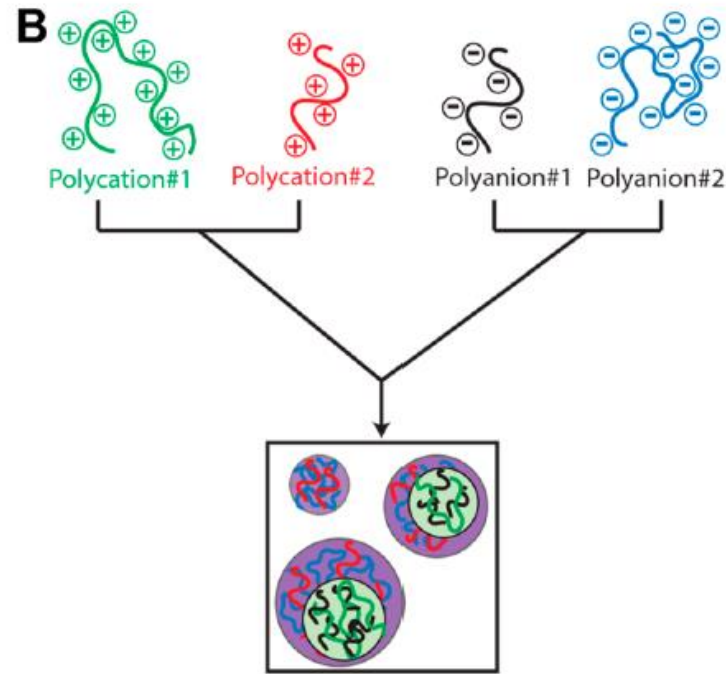
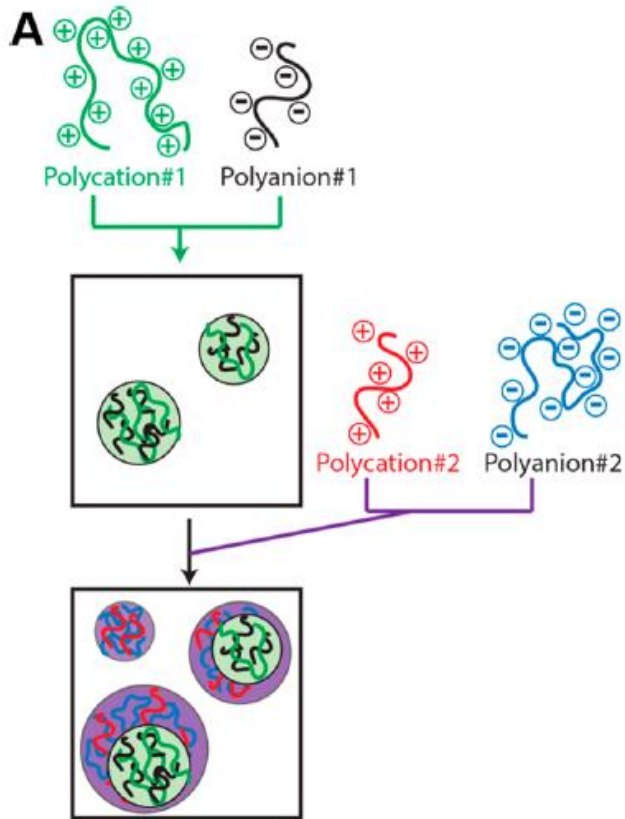


Multicomponent, multiphase droplets:

- Surface tension
- Macromolecular density between mixed polymers,
- Sensitivity to salt ions

Lu & Spruijt, JACS, 2020

Control formation of multiphase complex coacervates

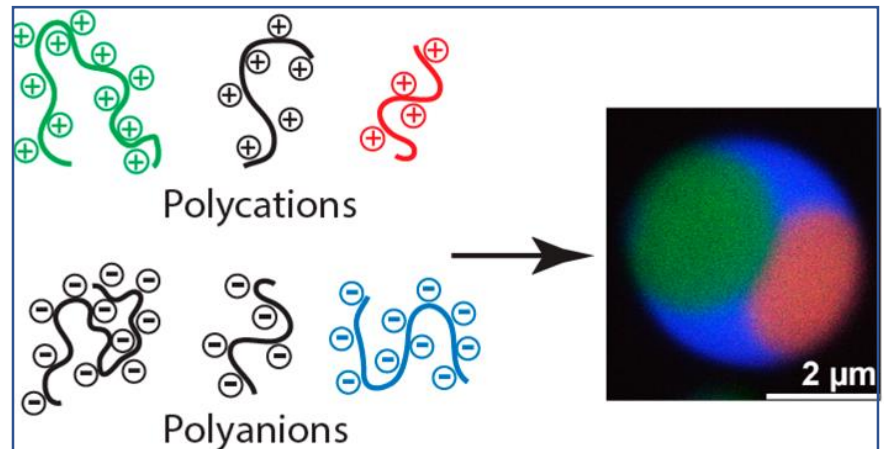


Like Charge Polymers Are Premixed

Sequential Formation of
Multiphase Coacervates

Mountain and D. Keating,
Biomacromolecules, 2020

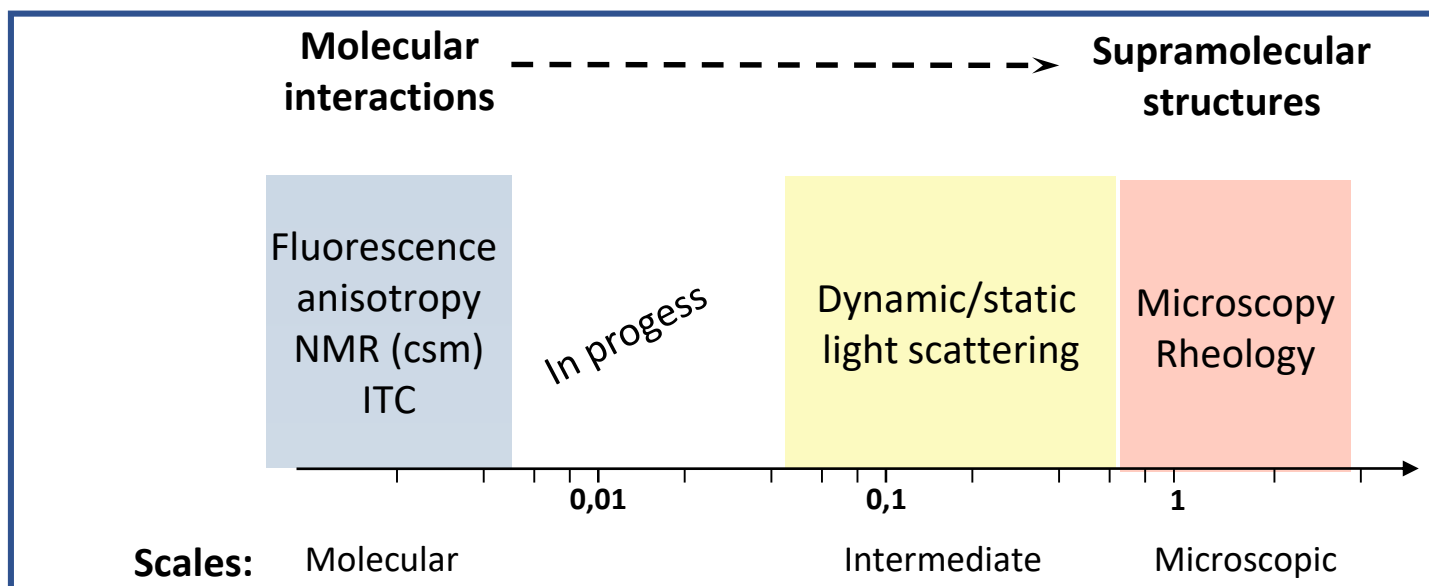
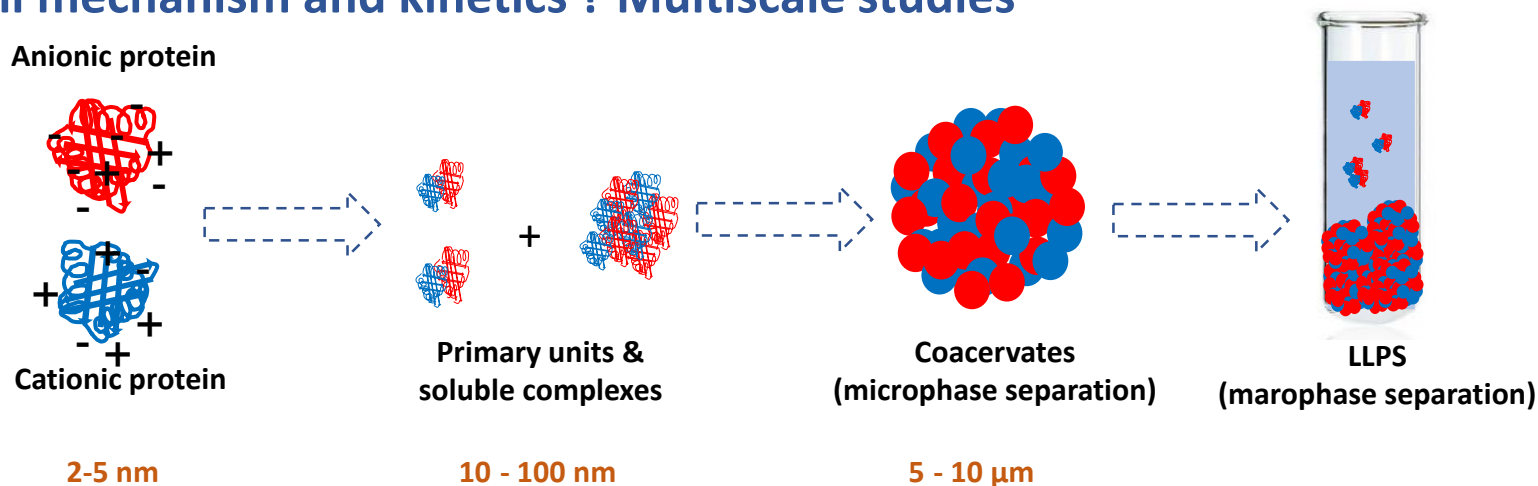
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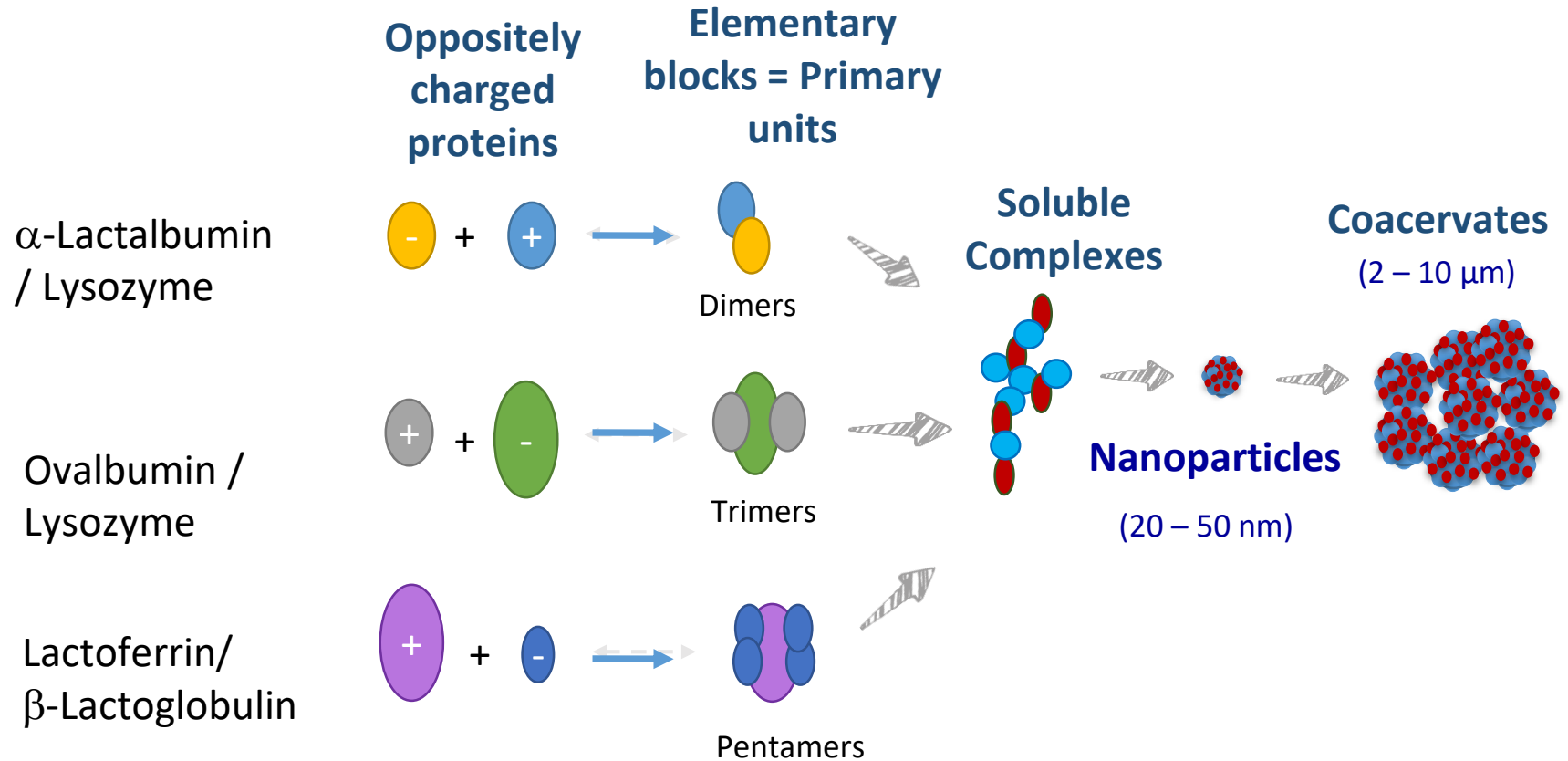
Liquid-liquid phase separation

Focus on heteroprotein complex coacervation

Overall mechanism and kinetics ? Multiscale studies



➤ Heteroprotein complex coacervation : Molecular scale

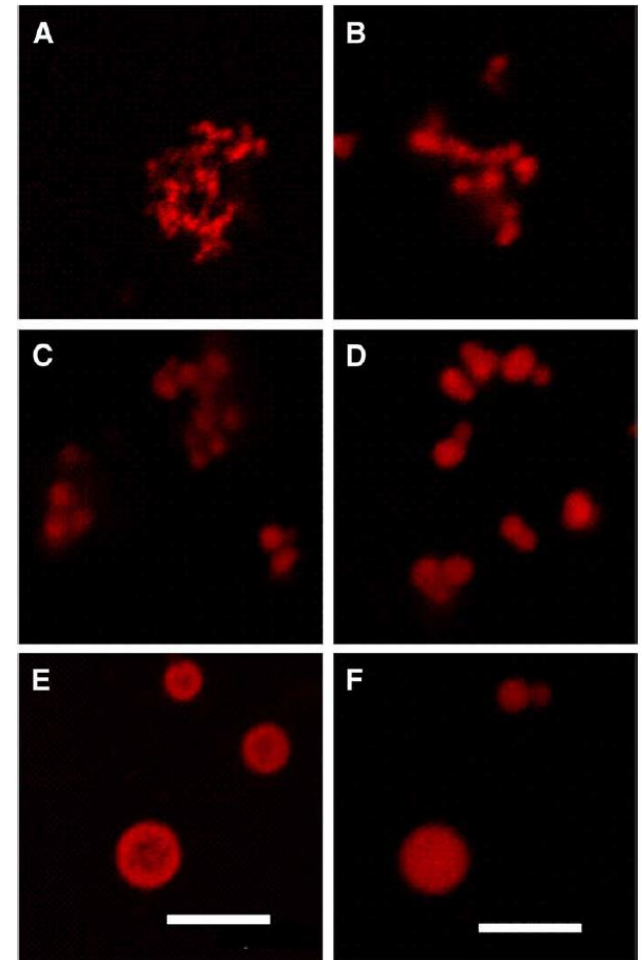


Croguennec et al., Adv. Coll. Int. Sci., 2017
Salvatore et al. Biomacromolecules, 2011

➤ Heteroprotein complex coacervation : In between

Early observations under confocal microscopy

- Immediately after mixing the proteins self-assemble into a large number of small entities.
- Some of which form clusters of irregular shape (aggregation of small entities is faster than self organisation of the cluster) (A)
- The clusters self-organize into larger entities with more regular shape (B→E).
- As the number of entities is reduced, the collision and fusion steps are casual.
- It seems that the coacervates densify with time (F).

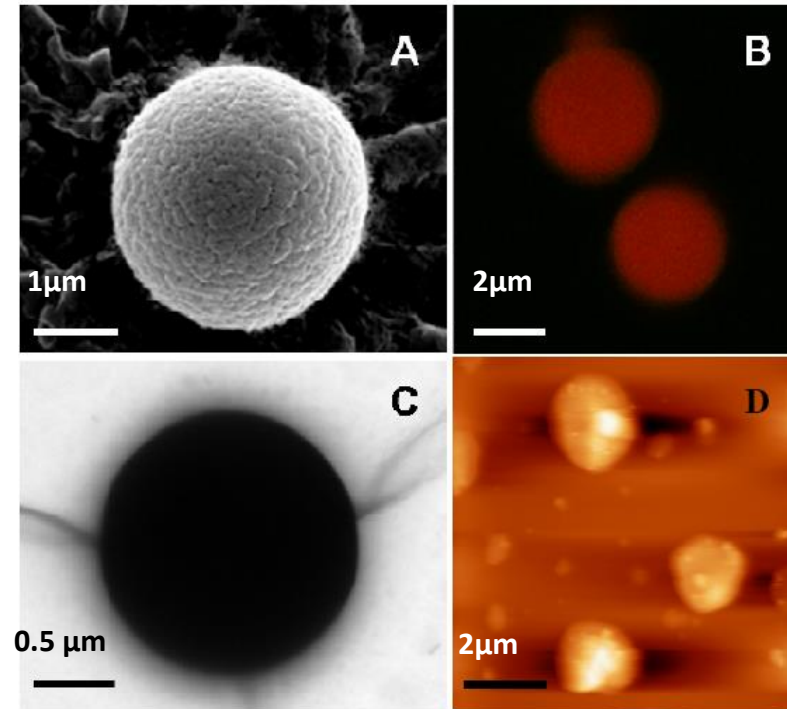


Nigen et al., FEBS Journal, 2010

➤ Heteroprotéin complex coacervation : macroscopic scale

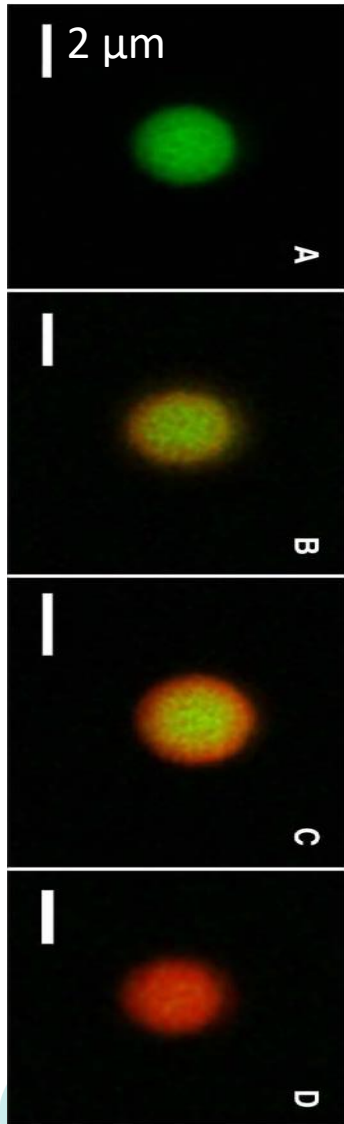
Temperature > 30°C

- ❑ Heteroprotein coacervates (1 - 5 μm)
- ❑ Rapid formation (spontaneous process under optimal conditions)
- ❑ **Specific stoichiometry** of proteins in the coacervates
- ❑ **Co-localization** of the proteins in the coacervates (FRET experiments)



Salvatore et al., Biomacromolecules, 2011
Nigen et al., FEBS Journal, 2007

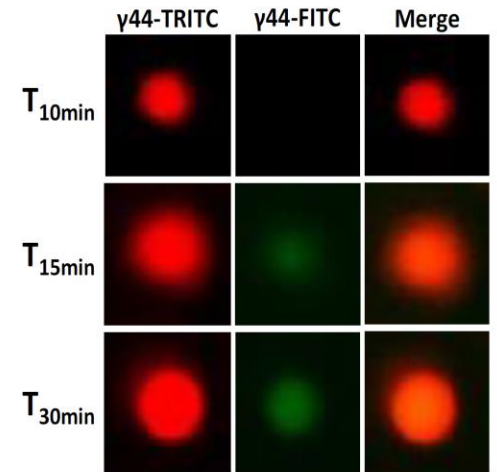
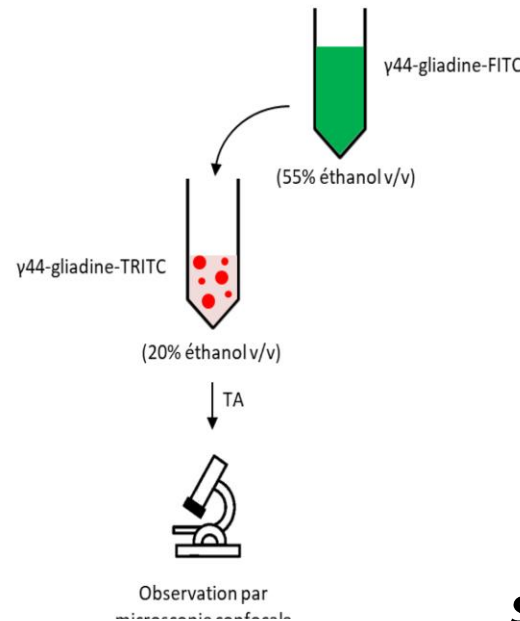
➤ Heteroprotein complex coacervation : Dynamic process between dilute and dense phase



Internal dynamic of heteroprotein coacervation: exchange between dilute and dense phase .

1. α -LA + LYS-FITC;
2. addition of of LYS-RBITC. Evolution from 0 to 75 min

... Similar of what happens in mono-protein LLPS system: the case of γ - gliadin



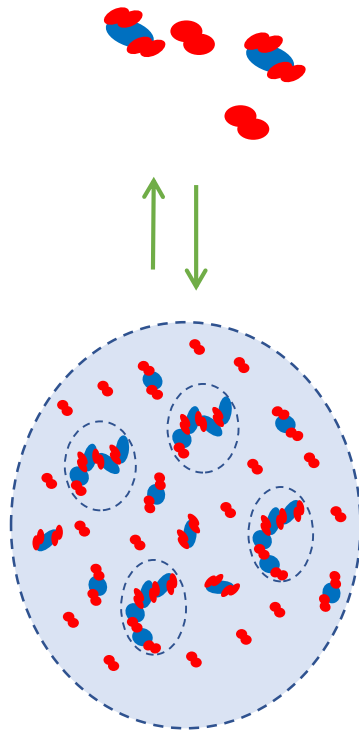
**Nigen et al.,
Biophys. J. 2010**

Sahli et al., Sci Rep, 2019

2 μ m

➤ Heteroprotein complex coacervation : properties of dense phase

- Highly hydrated, viscoelastic network (see R. Hachfi Soussi talk)
- Bicontinuous phase with some water rich phase in co-existence with material rich phase
- Evidences from FRAP, ^1H NMR and simulation



- **Heterogeneity:** coexistence of **dynamic** different species :
 - $\beta\text{-LG}_2$ (5 nm),
 - $\text{LF}(\beta\text{LG}_2)_2$ (10-12 nm),
 - $\text{LF}(\beta\text{-LG}_2)_n$ (30-40 nm)
- Change in the proportion of these structures could explain the variation of the $\beta\text{-LG}/\text{LF}$ molar ratio in the coacervate phase

➤ Heteroprotein complex coacervation : Specificity over other macromolecular systems (1. surface charge)

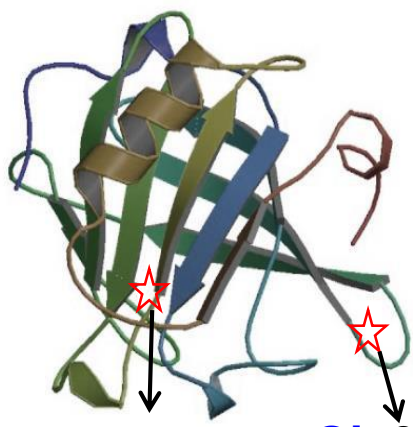
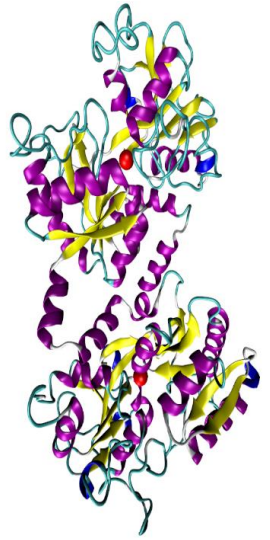
Behind classical parameters, critical role of surface charge and charge 'patchiness'

Phase diagram of HPCC between Lactoferrin (+) and β -Lactoglobulin isoforms **B** and **A** (with one more negative charge)

Coacervation domains of Lf and β LG isoforms

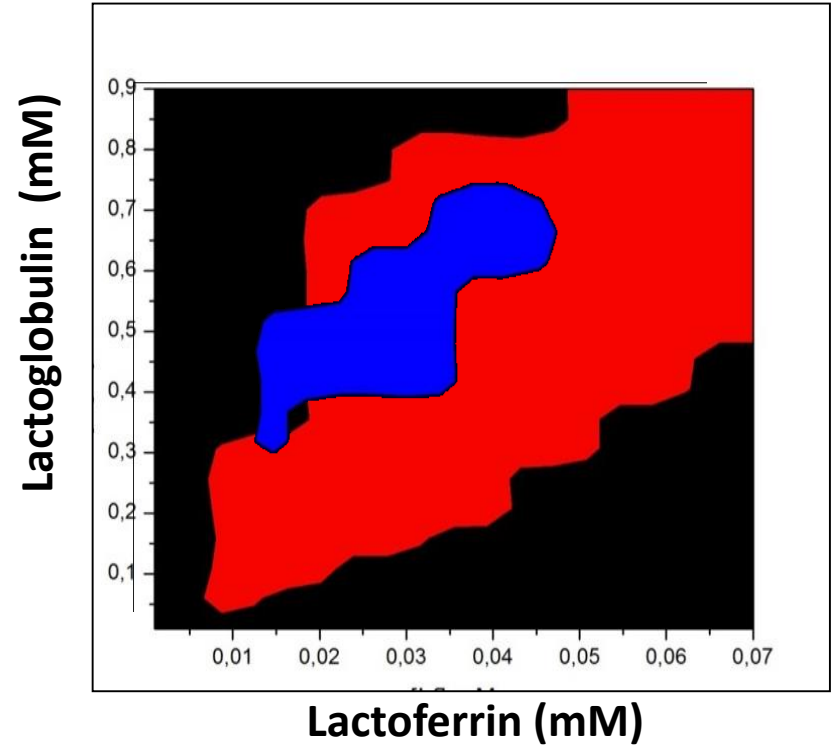
Lactoferrin +

β -lactoglobulin



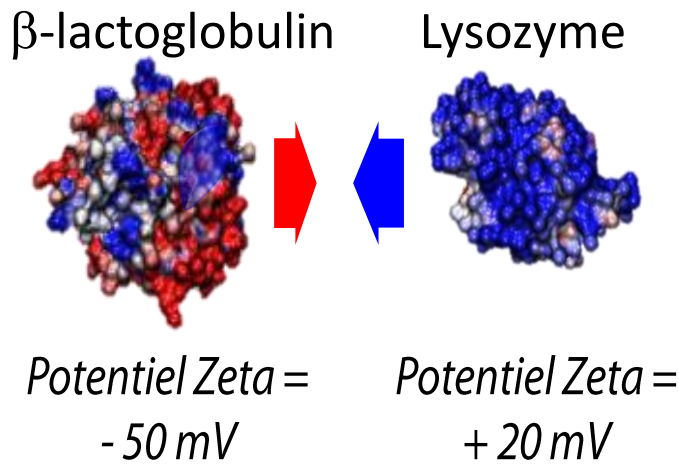
Val118Ala

Gly64Asp

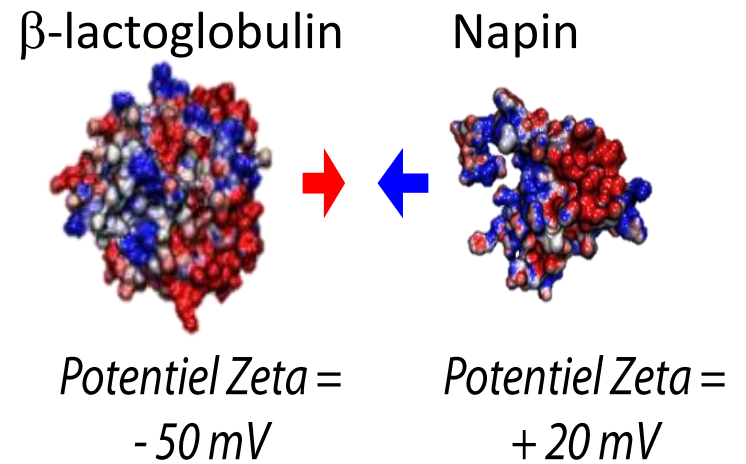


➤ Heteroprotein complex coacervation : Specificity over other macromolecular systems (2. Charge anisotropy)

LYS and NAP with similar charge but: Homogeneous charge distribution for LYS, relatively patchy charge distribution for NAP.



Interaction energy: +++
Phase separation: > μm

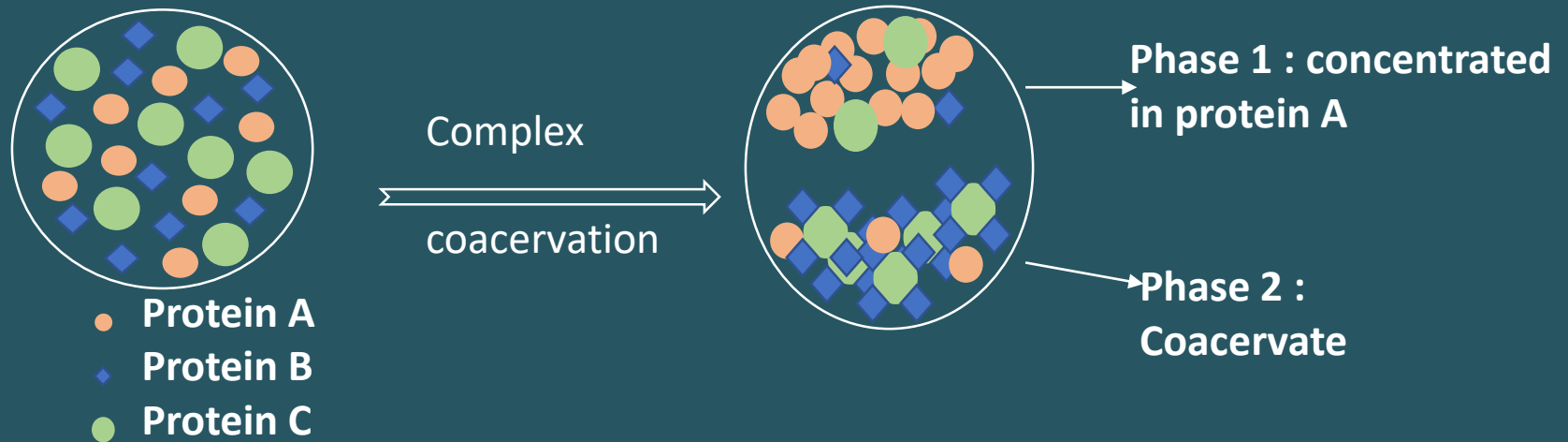


Interaction energy: +
Soluble complexes: < 20 nm

Ainis et al., Langmuir, 2019

➤ Heteroprotein complex coacervation : Applications

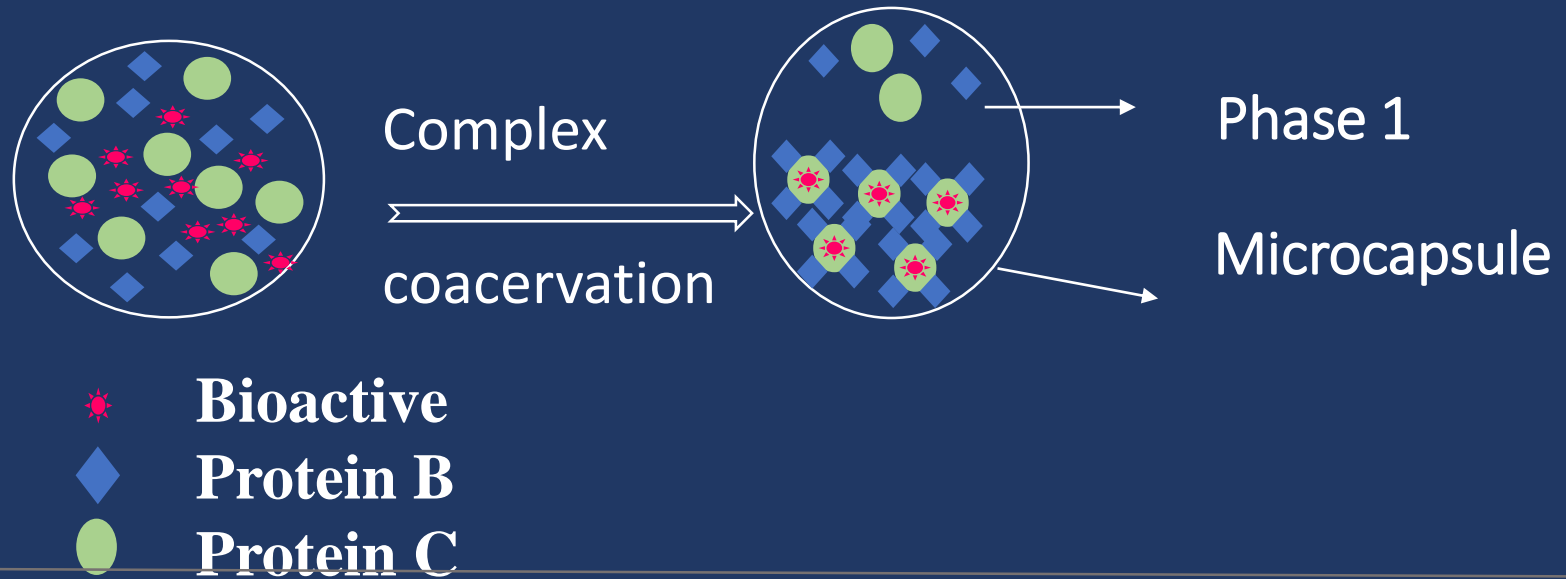
Protein purification



Shunuan et al., J. Agric. Food Chem., 2020

➤ Heteroprotein complex coacervation : Applications

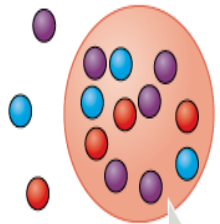
Protein purification **Encapsulation : Vitamins, oils, drogues ...**



Chapeau et al., Food Hydrocolloids, 2016

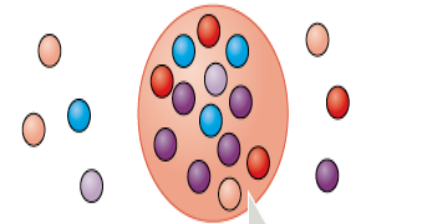
➤ Heteroprotein complex coacervation : Applications

a Concentration



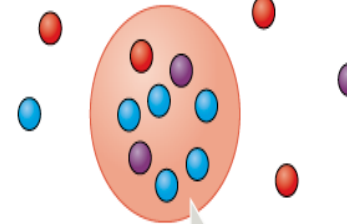
Increasing reaction kinetics

Specificity



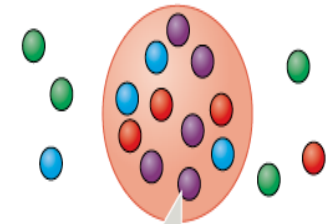
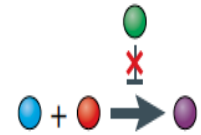
Promoting specific reaction (Reaction 1)

Sequestration



Reducing reaction kinetics

Exclusion



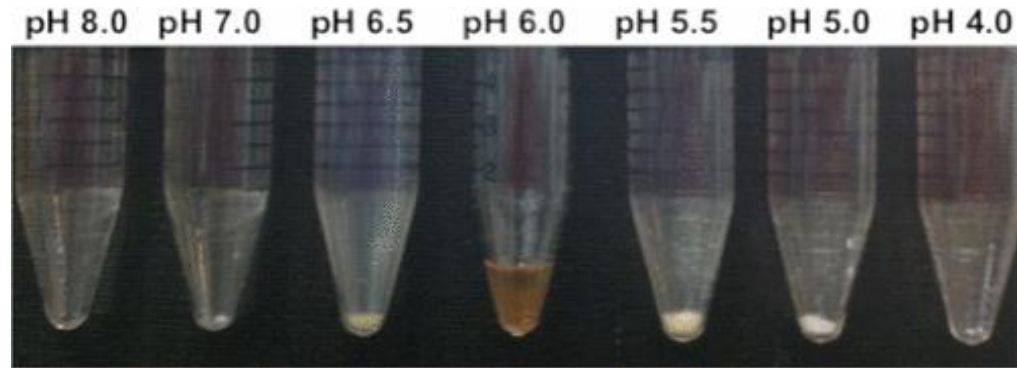
Preventing inhibitory interactions (increasing reaction efficiency and strength of response)

- | | | |
|-------------|---------------|---------------|
| ● Enzyme | ● Substrate 1 | ● Substrate 2 |
| ● Inhibitor | ● Product 1 | ● Product 2 |

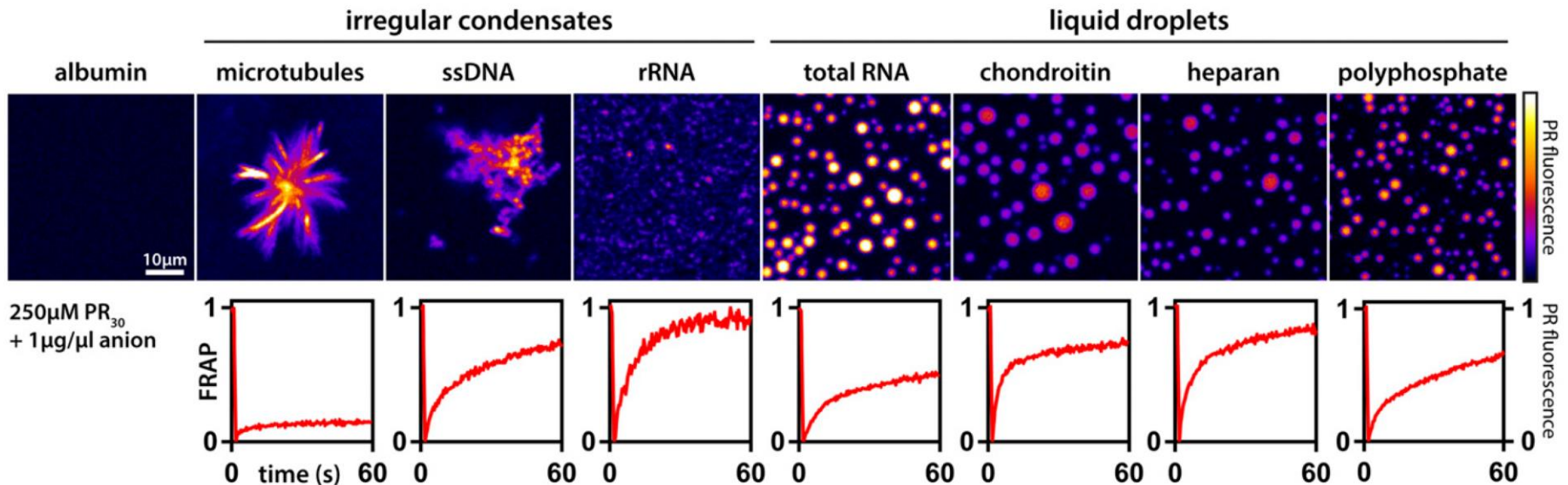
Lyon et al., Nature Reviews, 2020

➤ Enjeu majeur : Competition aggregation & LLPS

Comprendre le chemin thermodynamique des LLPS versus LSPS versus arrested SC.



Phase separation between basic protein and various anionic molecules : from Arrested soluble complexes to aggregates or coacervates



For more information:

Polyelectrolytes - Coacervates and Membraneless Organelles

Edited by Christine Keating, Nicolas Martin, Maria Santore

Last update 12 March 2021



Current Opinion in Colloid & Interface Science

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