Liquid-liquid phase separation in heteroproteinsystems: a mini review

Adeline Boire, Denis Renard, Antoine Bouchoux, Said Bouhallab

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

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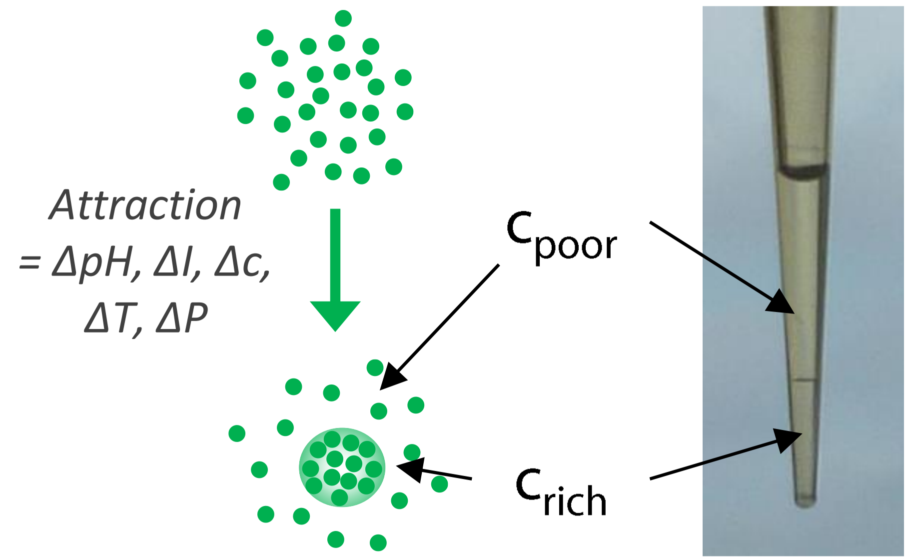
Liquid-liquid phase separation

A universal phenomenon



General features:

- Spontaneous phenomenon (ΔG < 0)
- Reversible
- Involves one or **mixture** of macromolecules

Protein / polysaccharides
Protein / polyelectrolytes
Protein / DNA - RNA
Strong or weak polycations, polyanions

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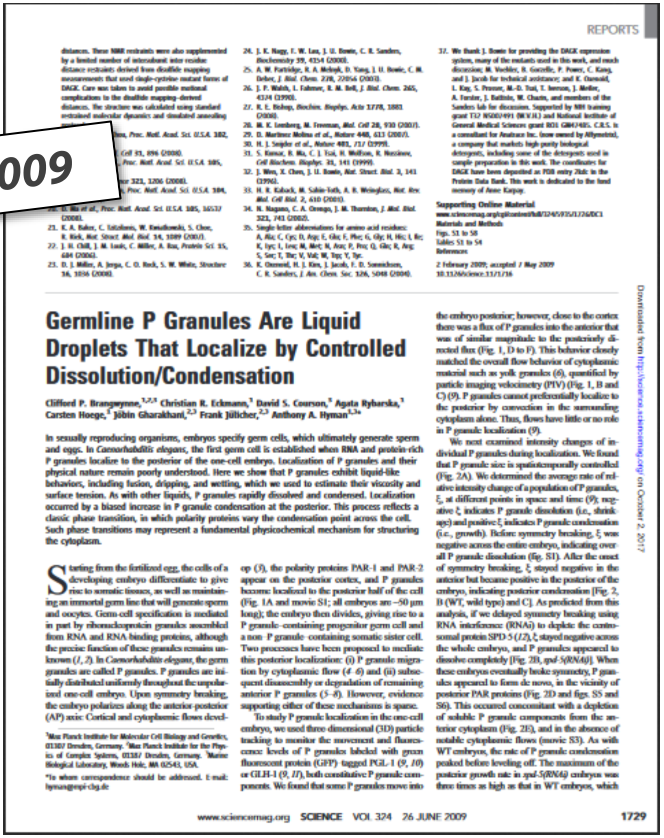
Liquid-liquid phase separation

A universal phenomenon

2009



REPORTS

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

Clifford P. Brangwynne,[1,2,3] Christian R. Eckmann,[1] David S. Courson,[1] Agata Rybarska,[1] Carsten Hoege,[1] Jöbin Gharakhani,[2,3] Frank Jülicher,[2,3] Anthony A. Hyman[1,3]*

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 remain poorly understood. Here we show that P granules exhibit liquid-like behaviors, 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.

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

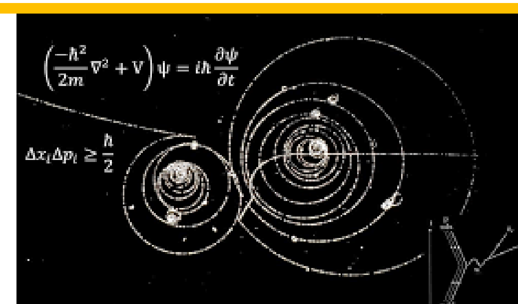
Exponential increase of number of publications

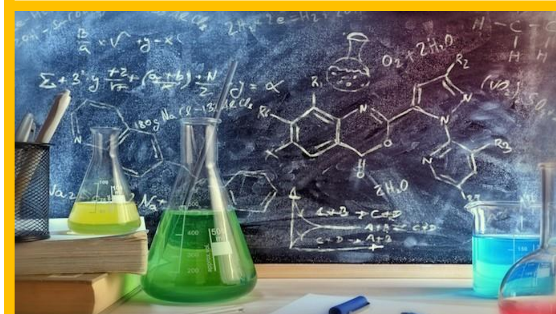
Liquid-liquid phase separation

A universal phenomenon

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

Physics:
Soft matter/colloids/polymers
techniques & principles

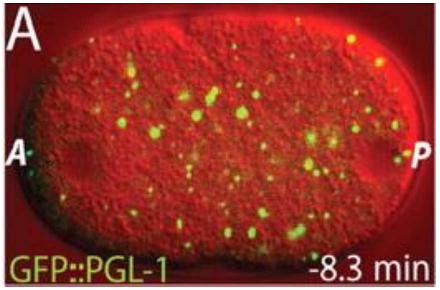




Chemistry:
Synthesis, thermodynamic

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

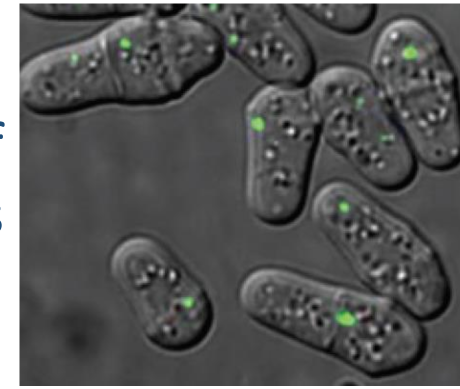
Liquid-liquid phase separation *universal phenomenon*



Embryos germ cells P-granule (RNA/proteins)

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

Dynamic reorganisation of intracellular enzymes



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

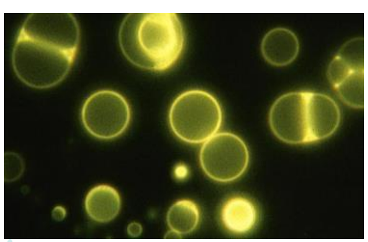
« Sandcastle worms »

Mussels

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

Waite,, *The Journal of Adhesion*, **2005**
Kim *et al.*, *PNAS*, **2016**

Adhesion; catalysis; regulation; cell plasticity control

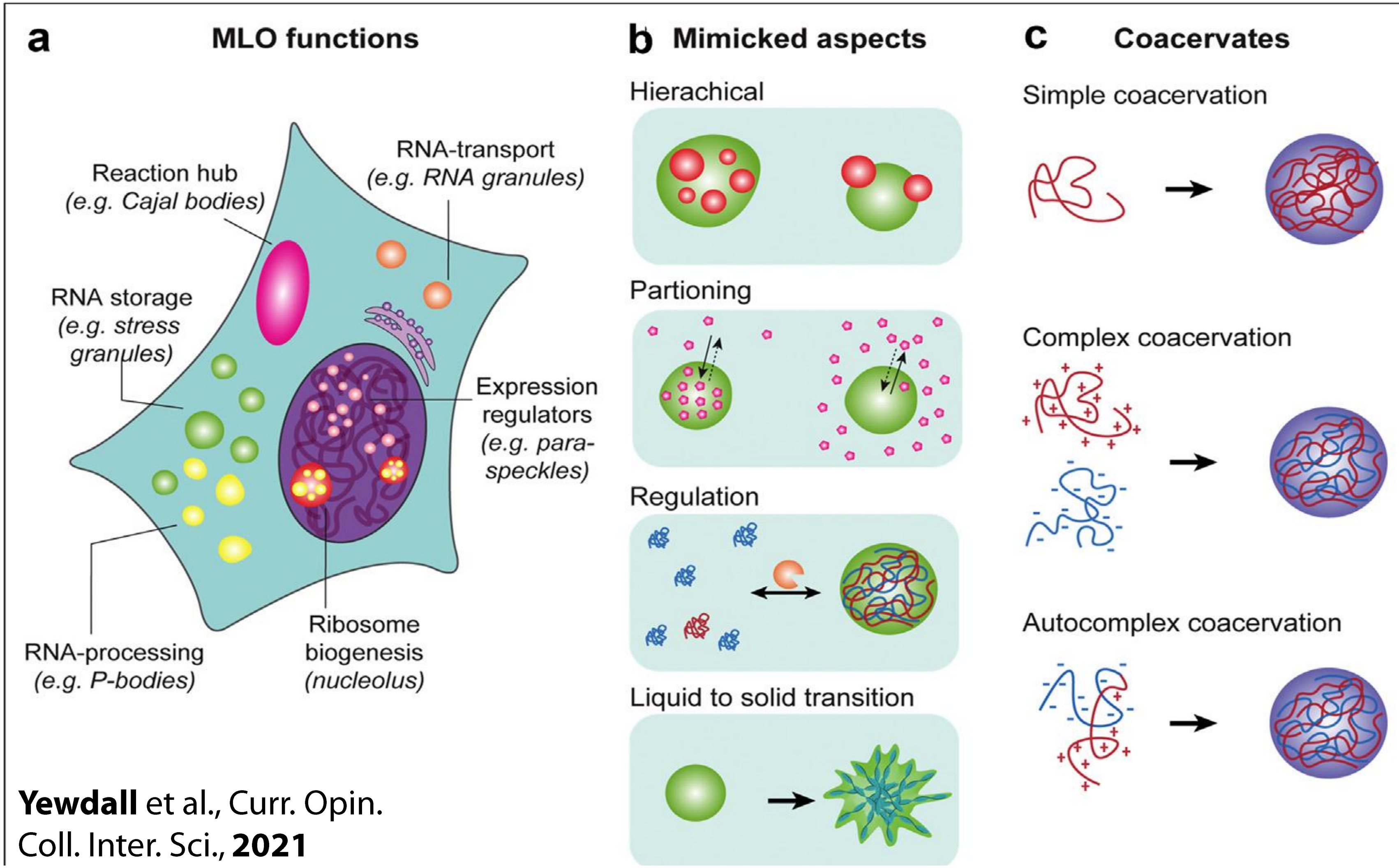


Macromolecular phase separation is being recognized for its potential importance and relevance as a driver of spatial organization within cells

Mittag & Pappu, Molecular Cell, **2022**

Liquid-liquid phase separation

A universal phenomenon



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

Understanding cell membraneless organelles
…. by mimicking …..using coacervates.

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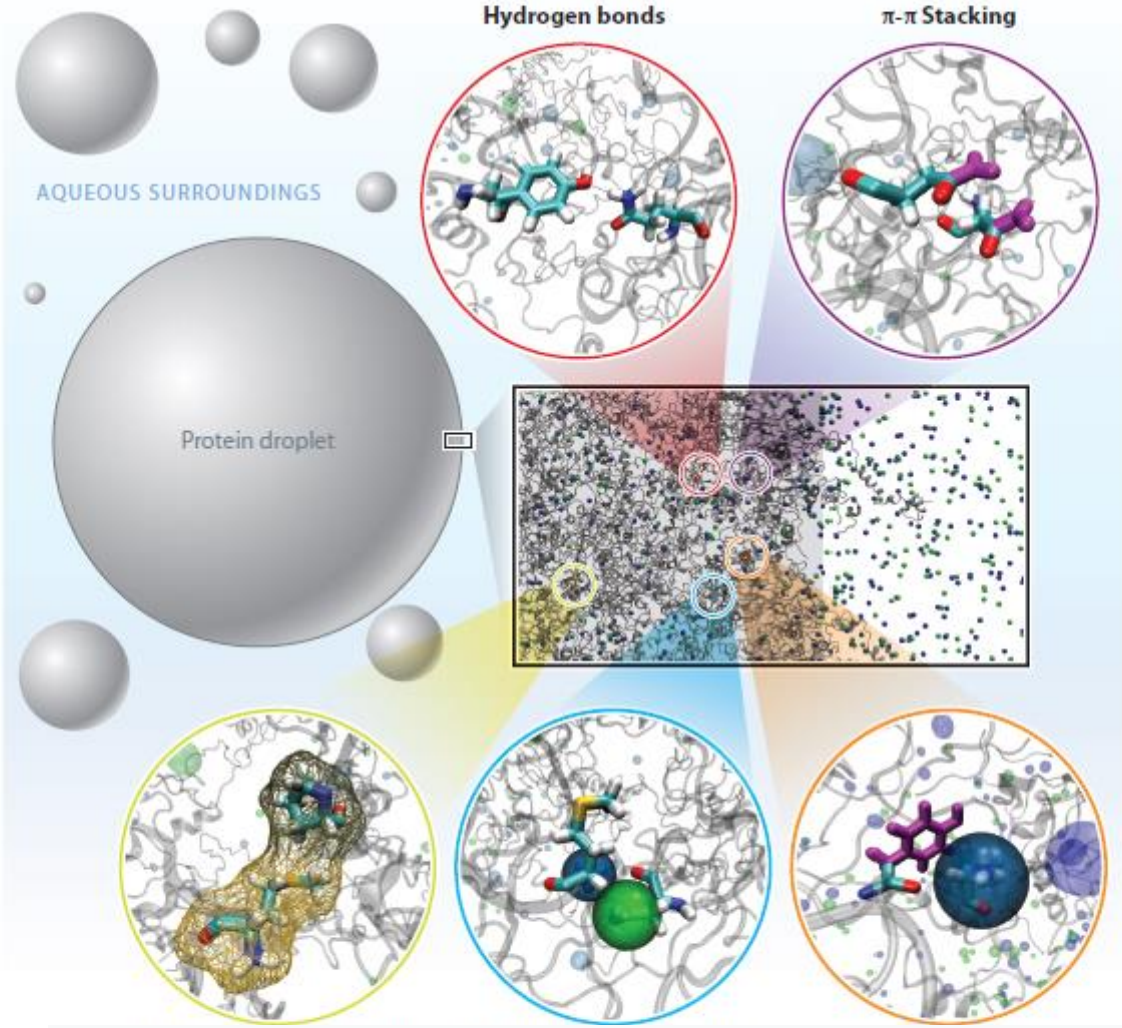
Liquid-liquid phase separation

A universal phenomenon

A myriad of multiple interactions in a strongly aqueous medium…

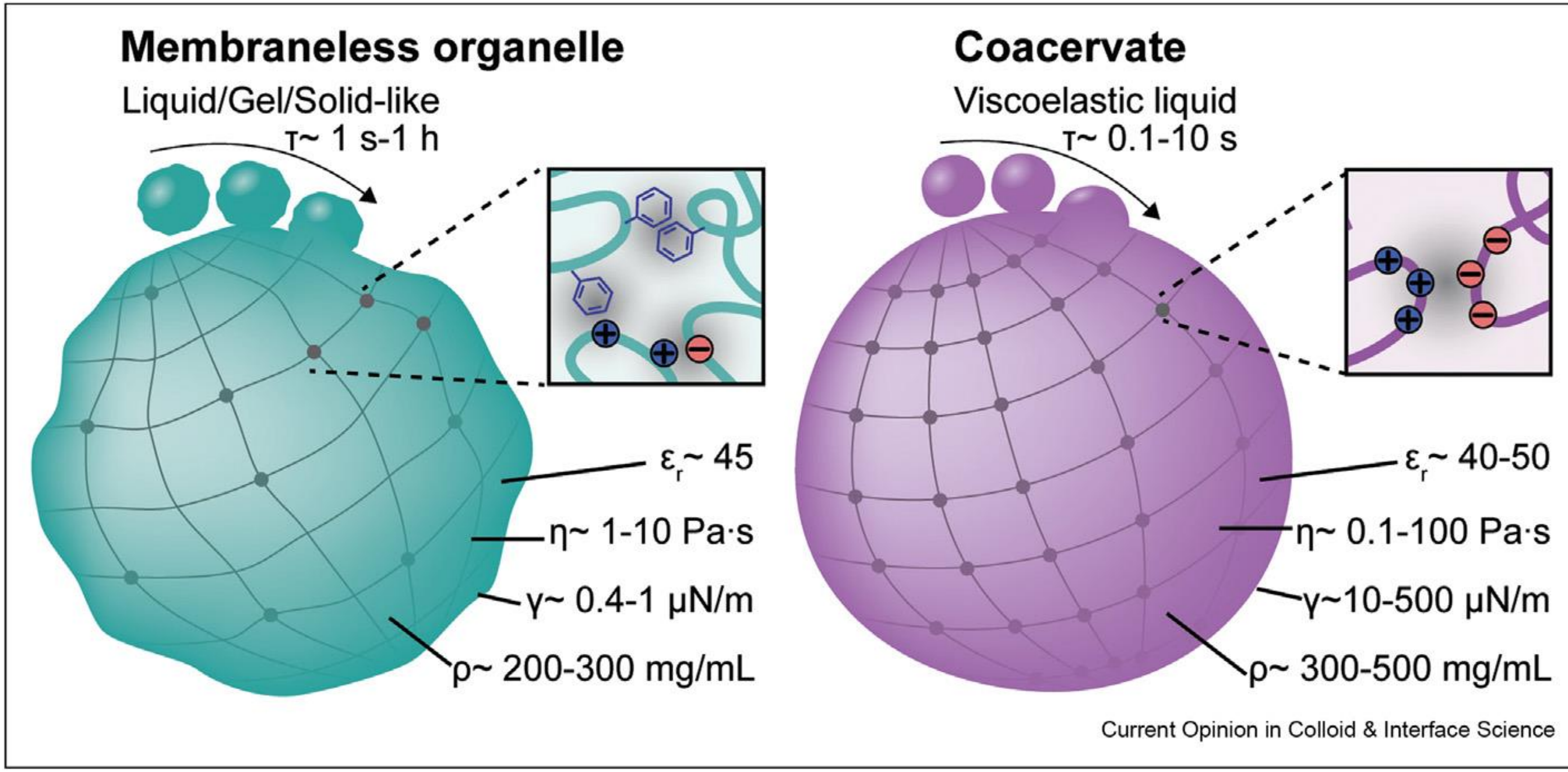


Dignon et al.,
Annu. Rev. Phys. Chem. **2020**

From simple ‘complex’ coacervates *in vitro* to complex MLOs *in vivo*

Liquid-liquid phase separation

A universal phenomenon



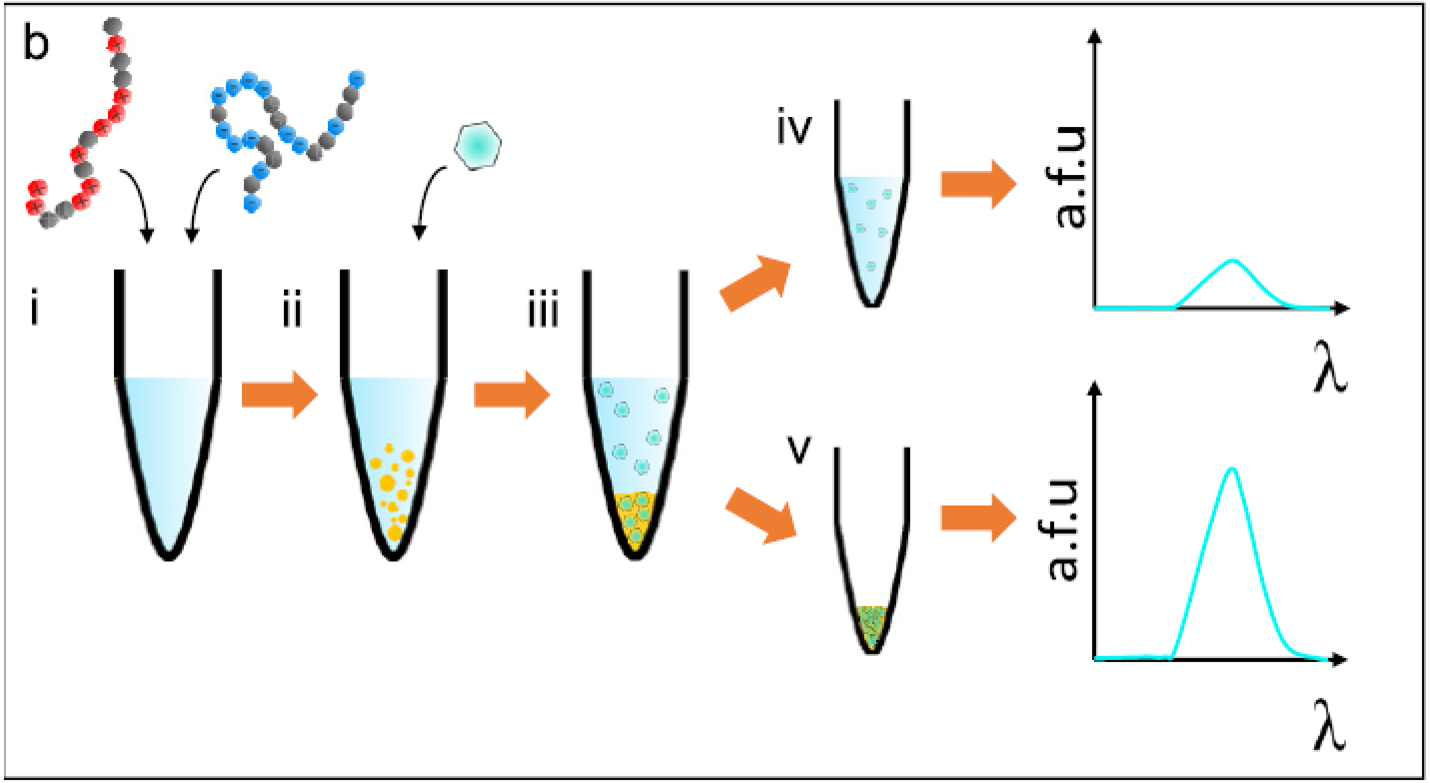
Relevant physicochemical properties of MLOs versus coacervates:

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

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

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

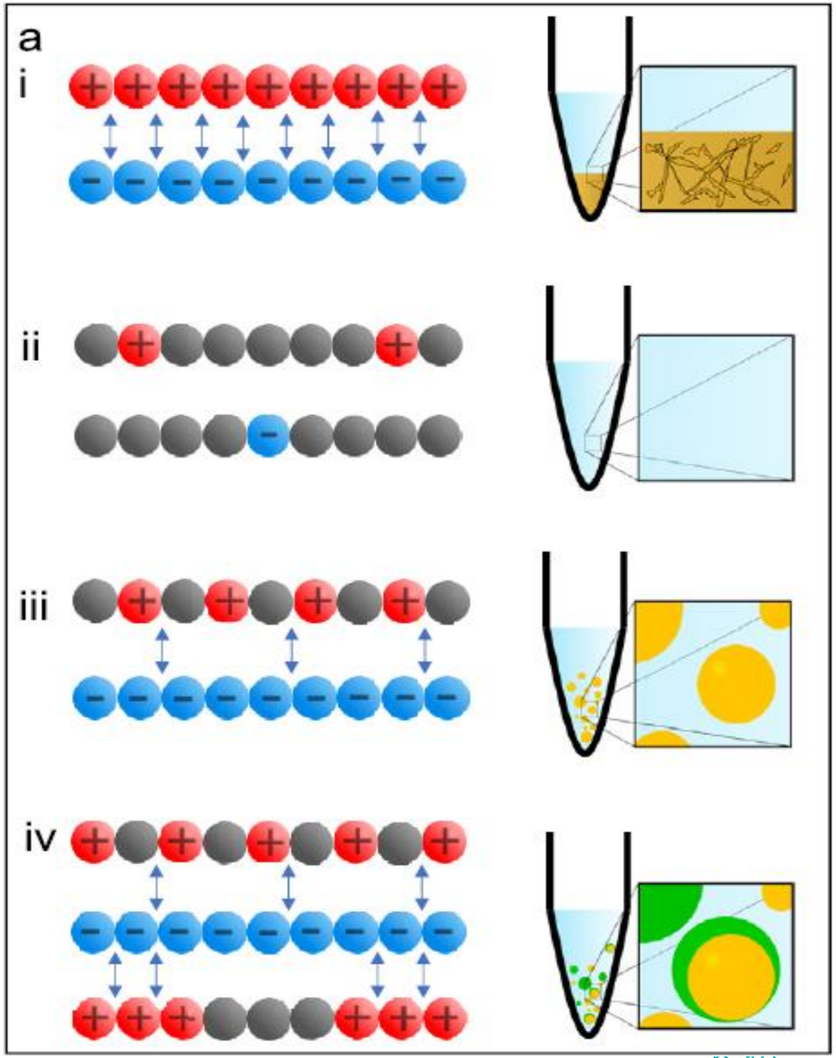
The strength of electrostatic interactions govern whether coacervates can be formed or not

(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



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

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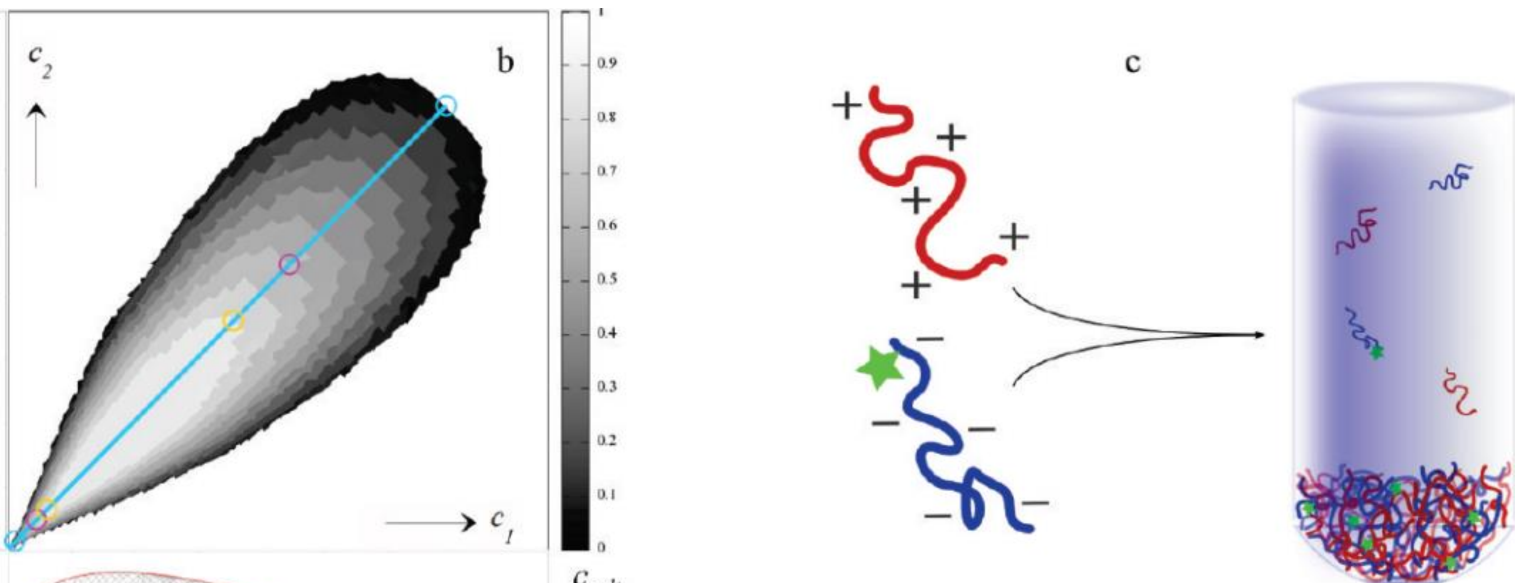
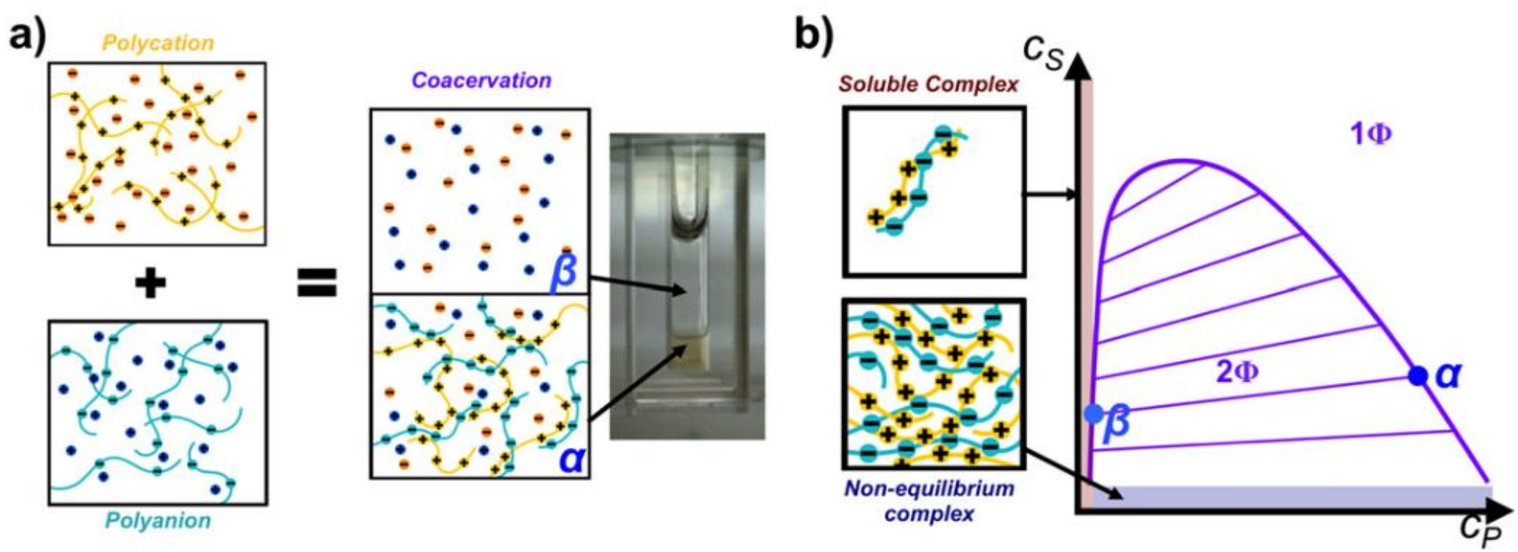
Chemical determinants of phase separation: phase diagram



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



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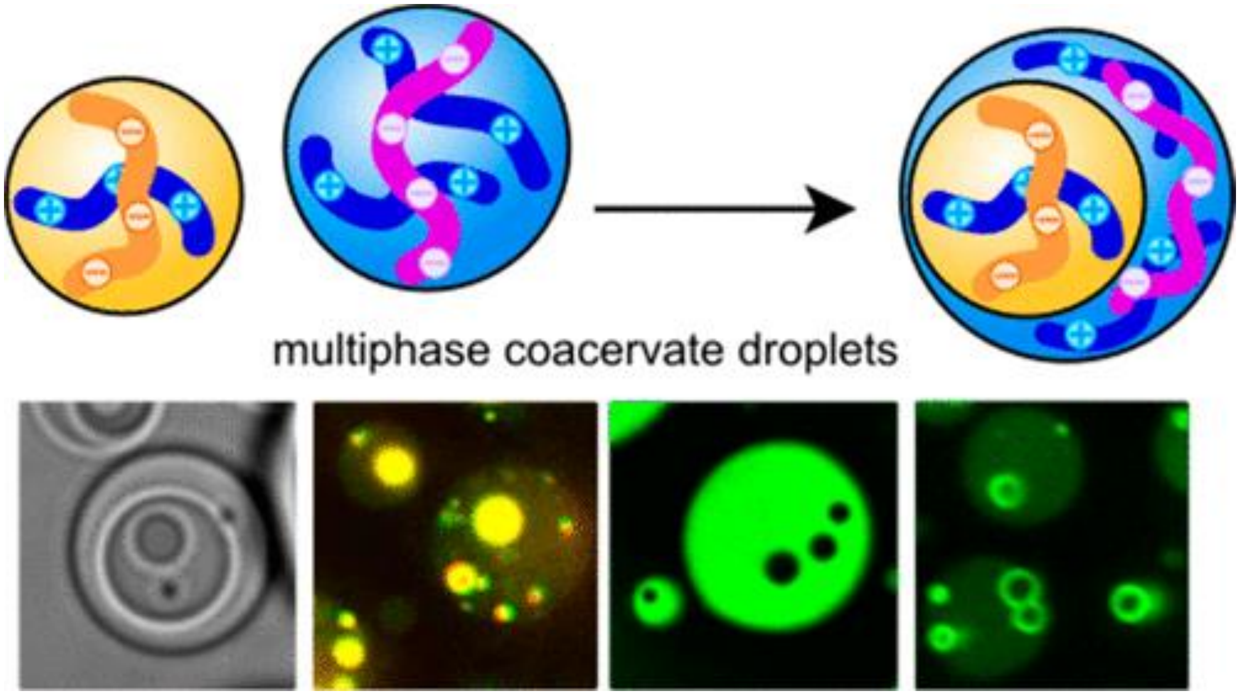
Spruijt et al. Macromolecules, **2010**

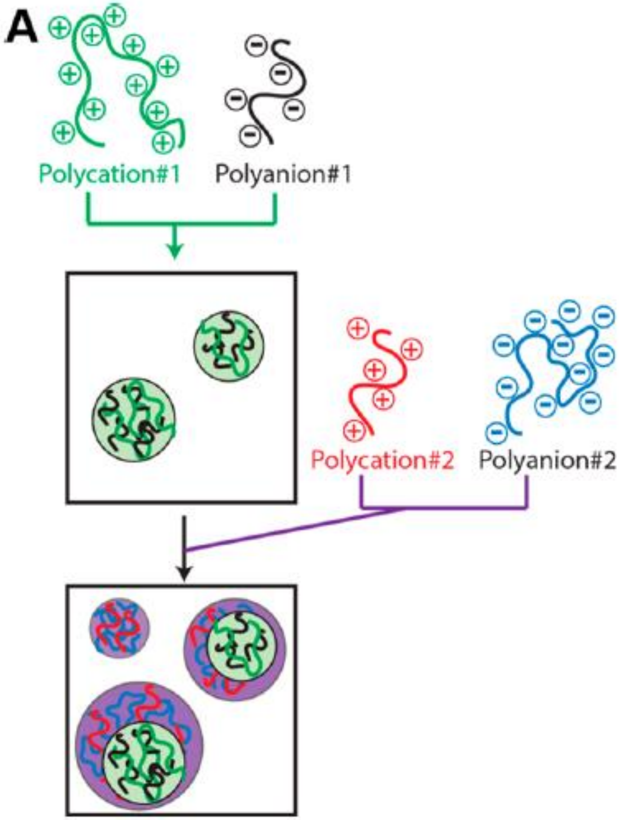
Control formation of multiphase liquid droplets .....
Partitioning in vivo

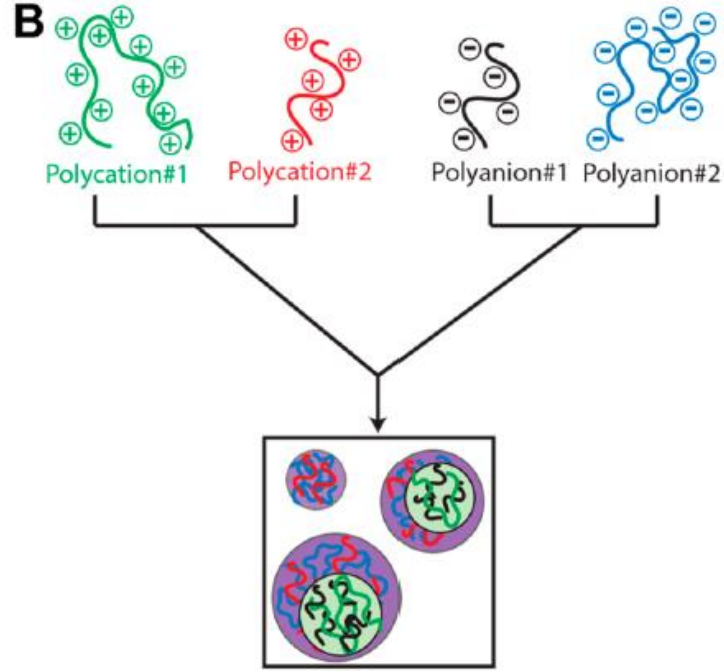


Multicomponent, multiphase droplets:
→ Surface tension
→ Macromolecular density between mixed polymers,
→ Sensitivity to salt ions

Lu & Spruijt, JACS, **2020**

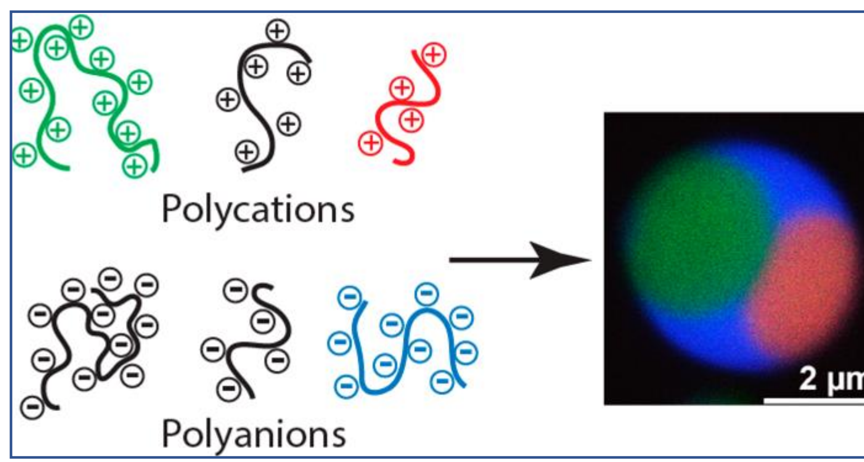
Control formation of multiphase complex coacervates .....





Like Charge Polymers Are Premixed

Sequential Formation of
Multiphase Coacervates



Mountain and D. Keating,
Biomacromolecules , ***2020***

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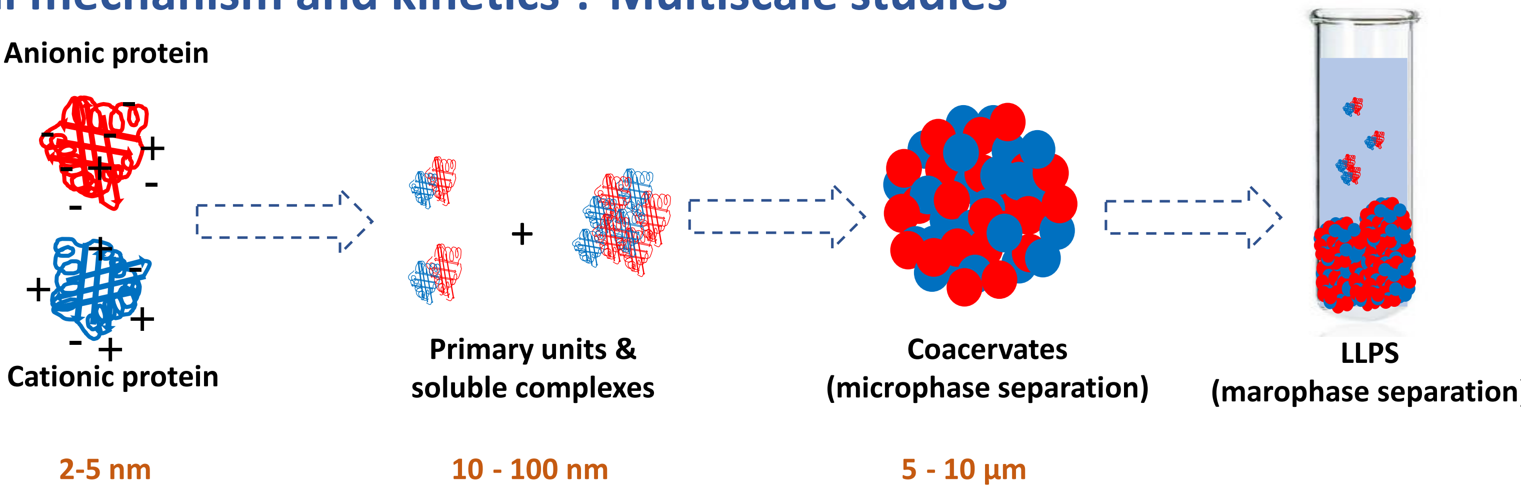
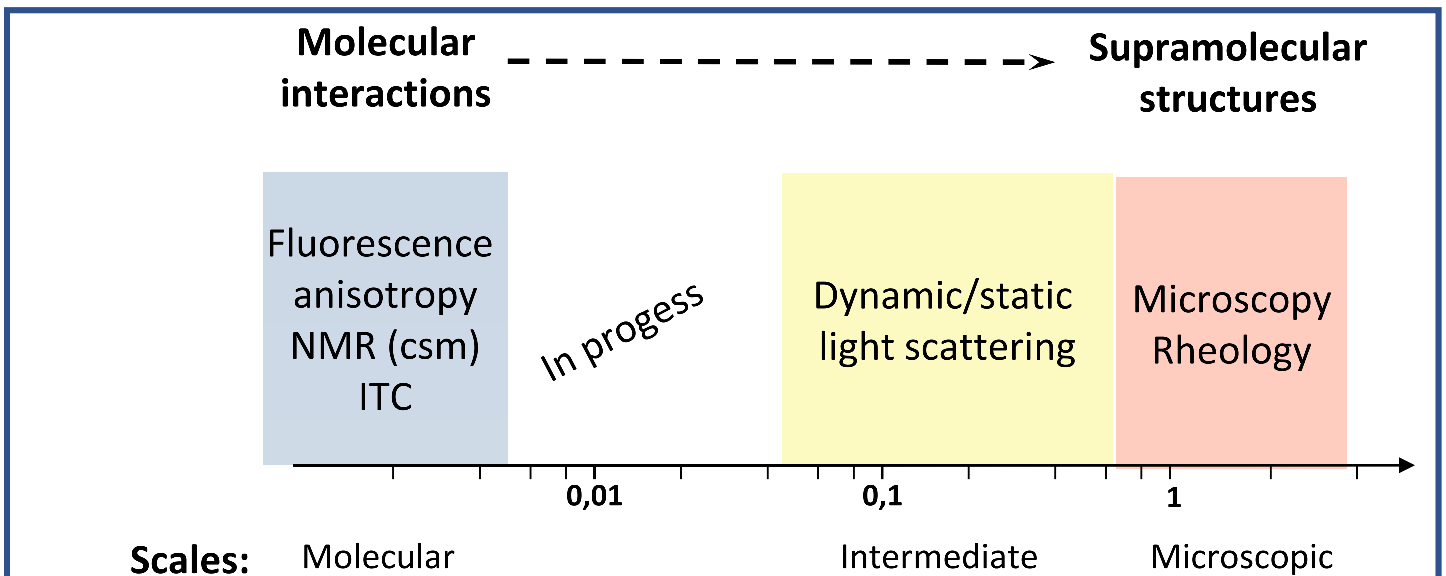
Liquid-liquid phase separation
Focus on heteroprotein complex coacervation

Overall mechanism and kinetics ? Multiscale studies





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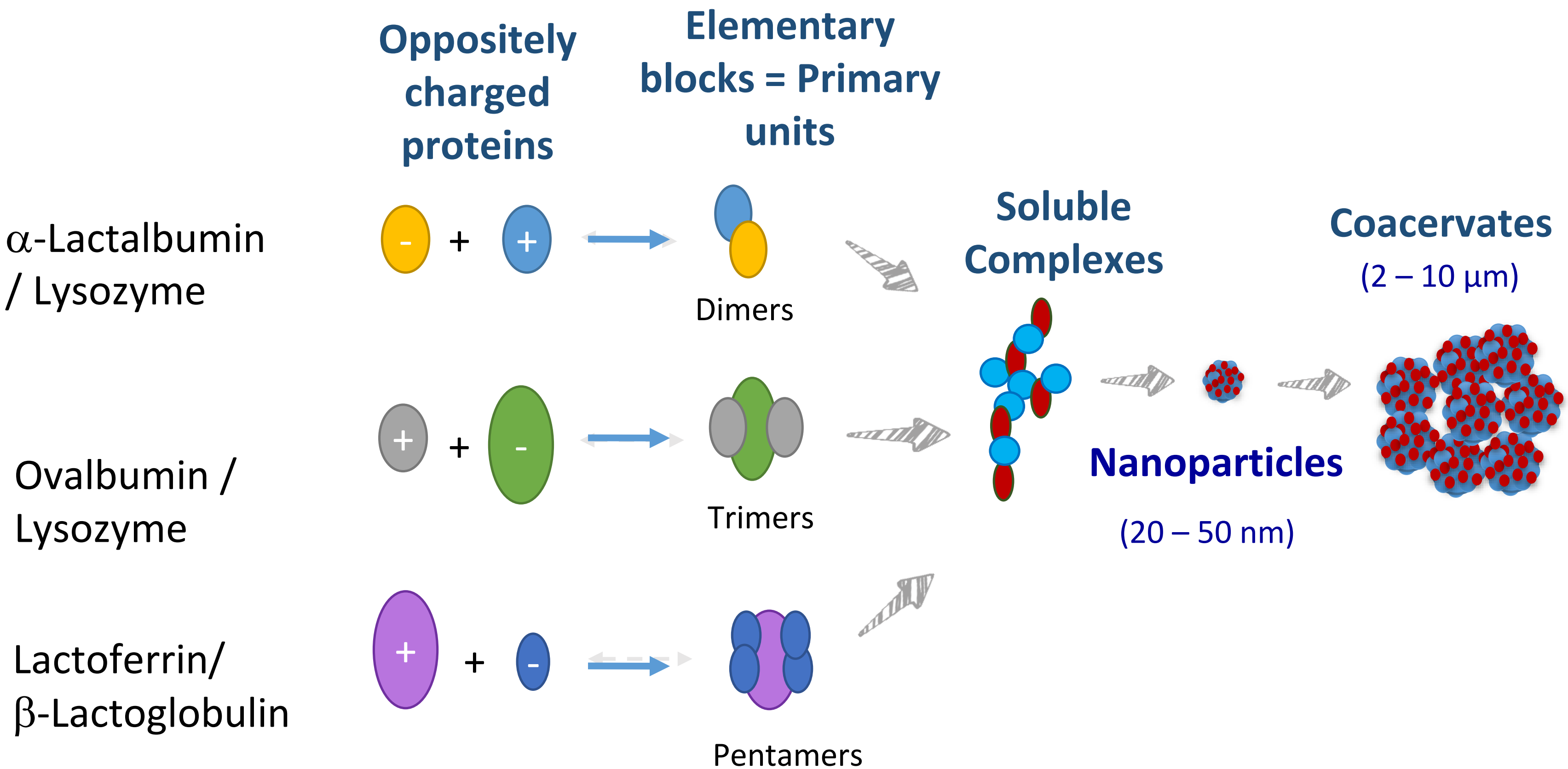
Heteroprotein complex coacervation : Molecular scale

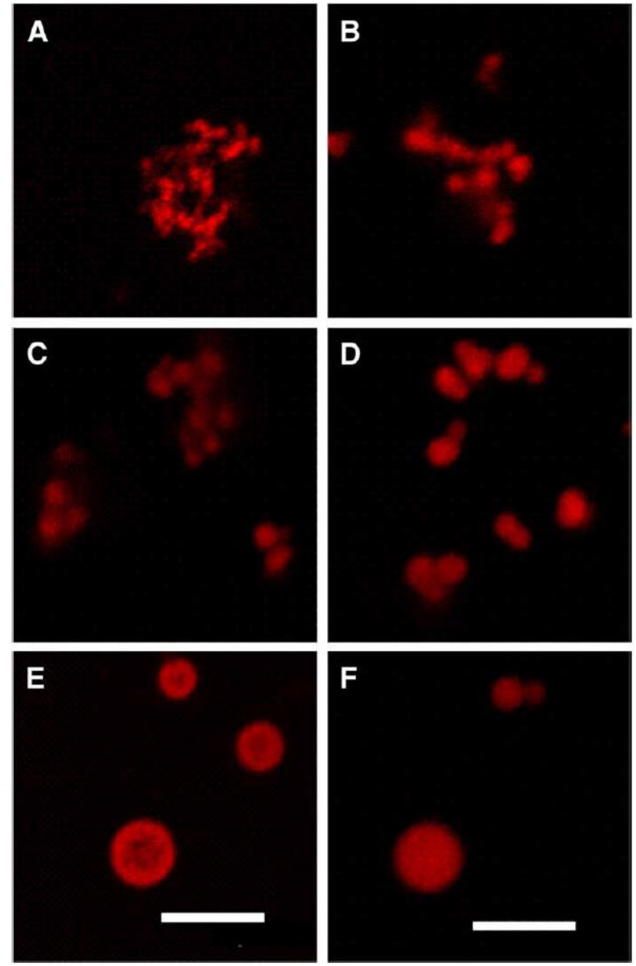


Croguennec *et al., Adv. Coll. Int. Sci.,* ***2017***
Salvatore et al. *Biomacromolecules,* ***2011***

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).

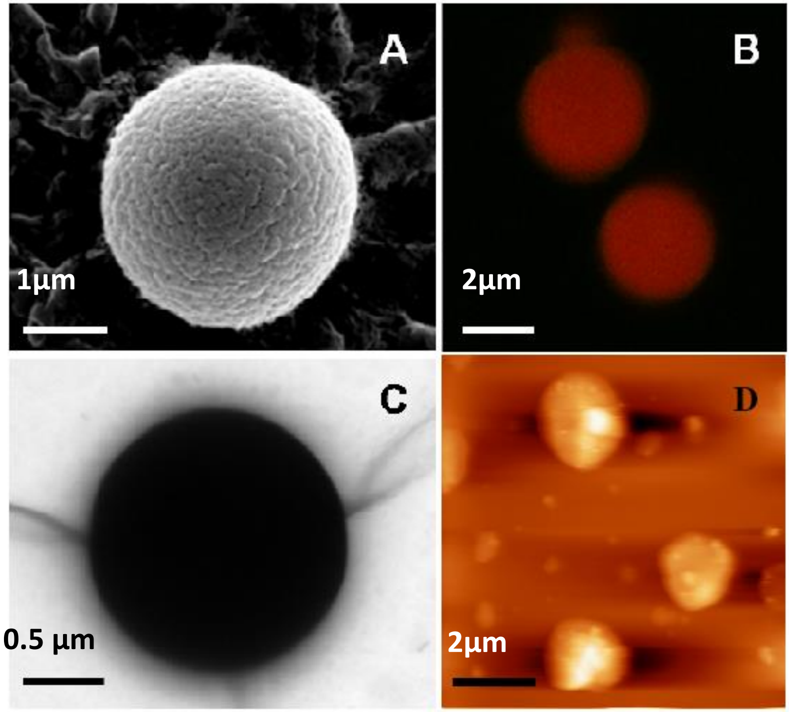


Nigen *et al., FEBS Journal,* ***2010***

Heteroprotéin complex coacervation : macroscopic scale

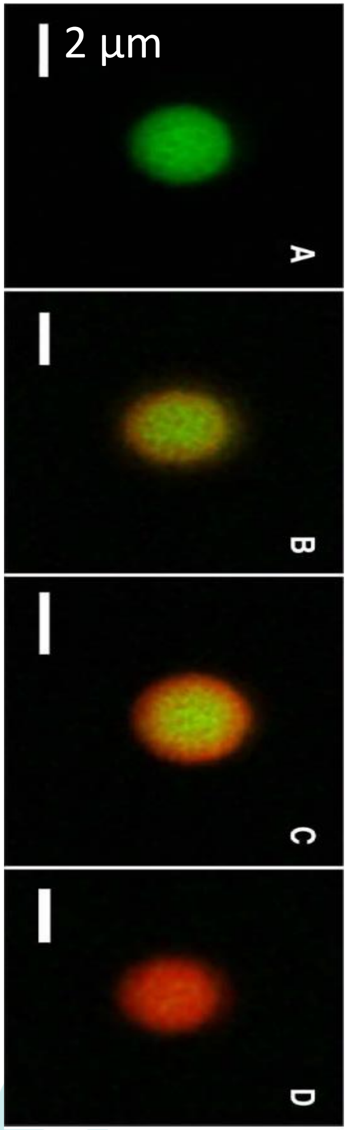
Temperature > 30°C

- ❑ **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***

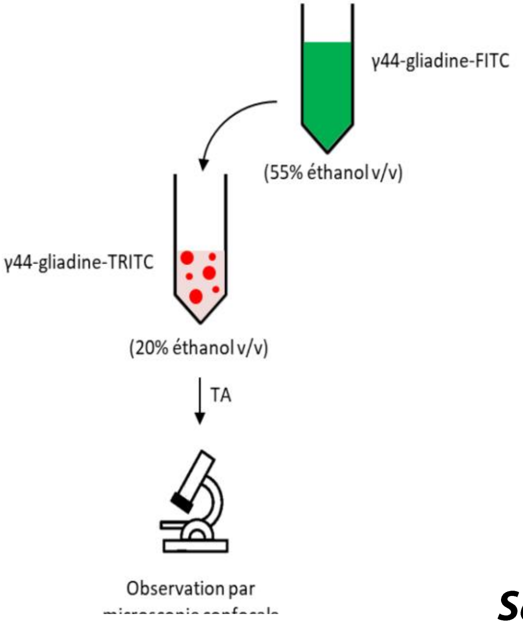
Heteroprotein complex coacervation : Dynamic process between dilute and dense phase

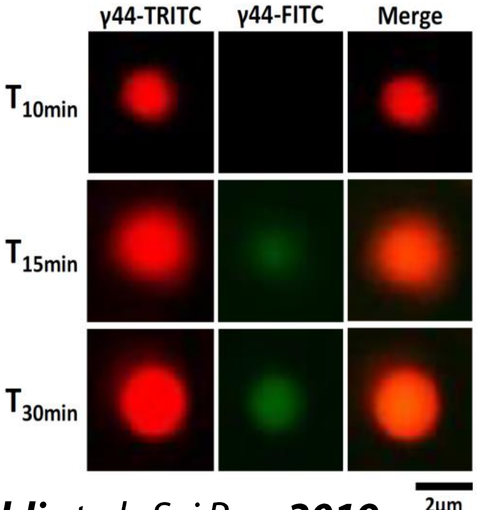


Internal dynamic of heteroprotein coacervation: exchange between dilute and dense phase .

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



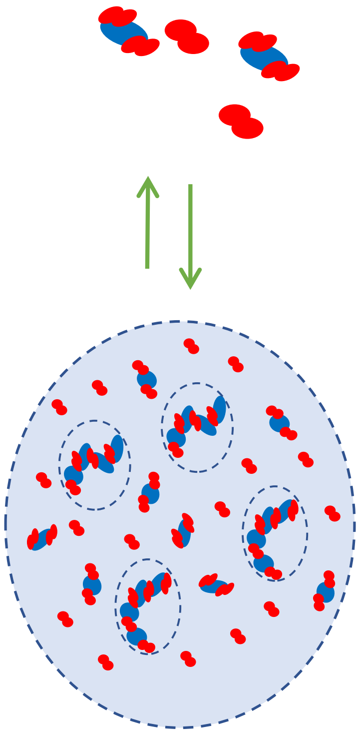


Nigen *et al., Biophys. J.* ***2010***

Sahli *et al., Sci Rep,* ***2019***

Heteroprotein complex coacervation : properties of dense phase

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



- **Heterogeneity**: coexistence of **dynamic** different species :
  - **β-LG$_2$ (5 nm),**
  - **LF(βLG$_2$)$_2$ (10-12 nm),**
  - **LF (β-LG$_2$)$_n$** (30-40 nm)

- Change in the proportion of these structures could explain the variation of the β-LG/LF molar ratio in the coacervate phase

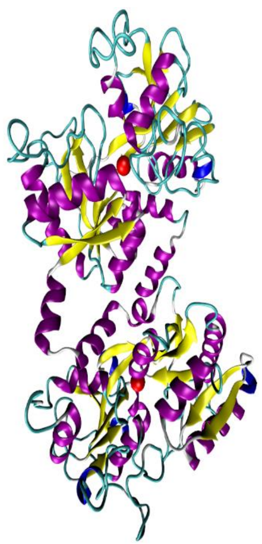
Peixoto *et al., Langmuir,**2016***

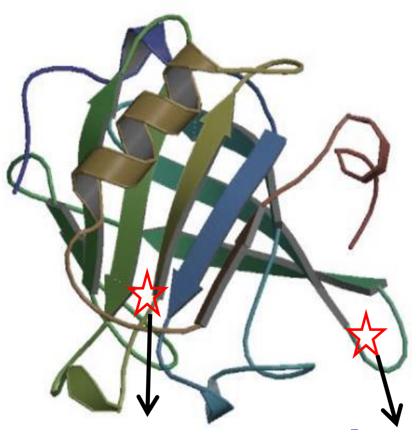
Heteroprotein complex coacervation : Specificity over other macromolecular systems (1. surface charge)

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

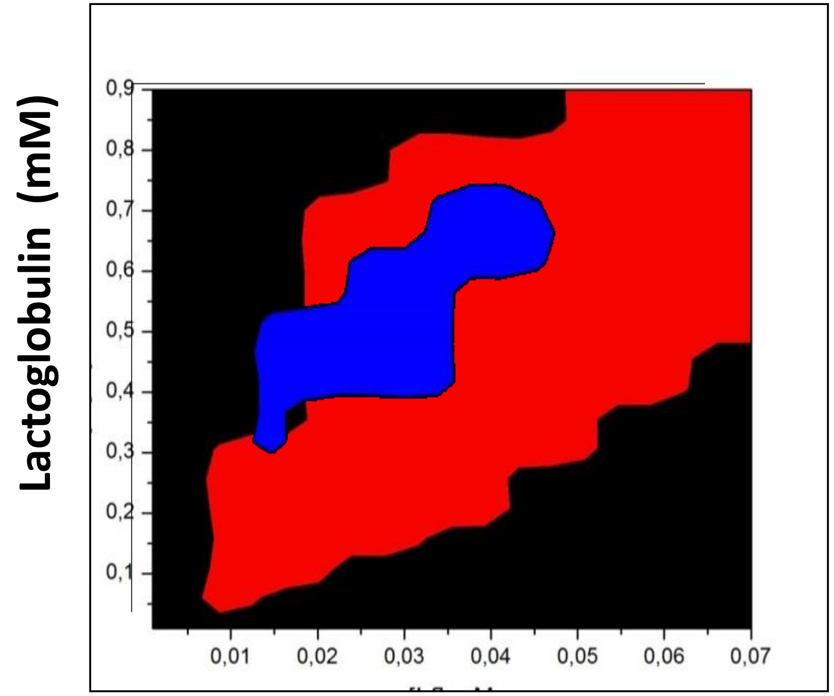
Phase diagram of HPCC between Lactoferrin (+) and β-Lactoglobulin isoforms **B** and **A** (with one more negative charge)

Lactoferrin + **β-lactoglobulin**





Coacervation domains of Lf and βLG isoforms



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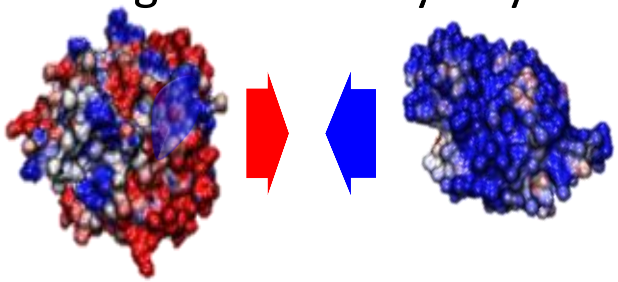
Tavares** et al. . Food Hydrocoll,* ***2015

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.

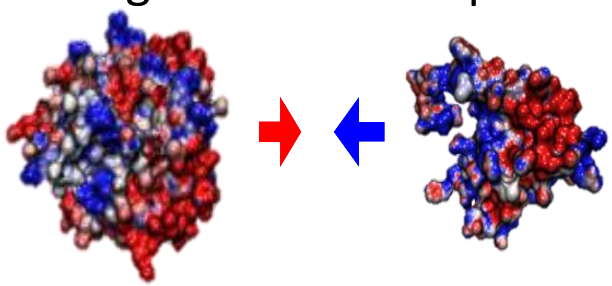
β-lactoglobulin Lysozyme



Potentiel Zeta = - 50 mV *Potentiel Zeta = + 20 mV*

Interaction energy: +++
Phase separation: > µm

β-lactoglobulin Napin



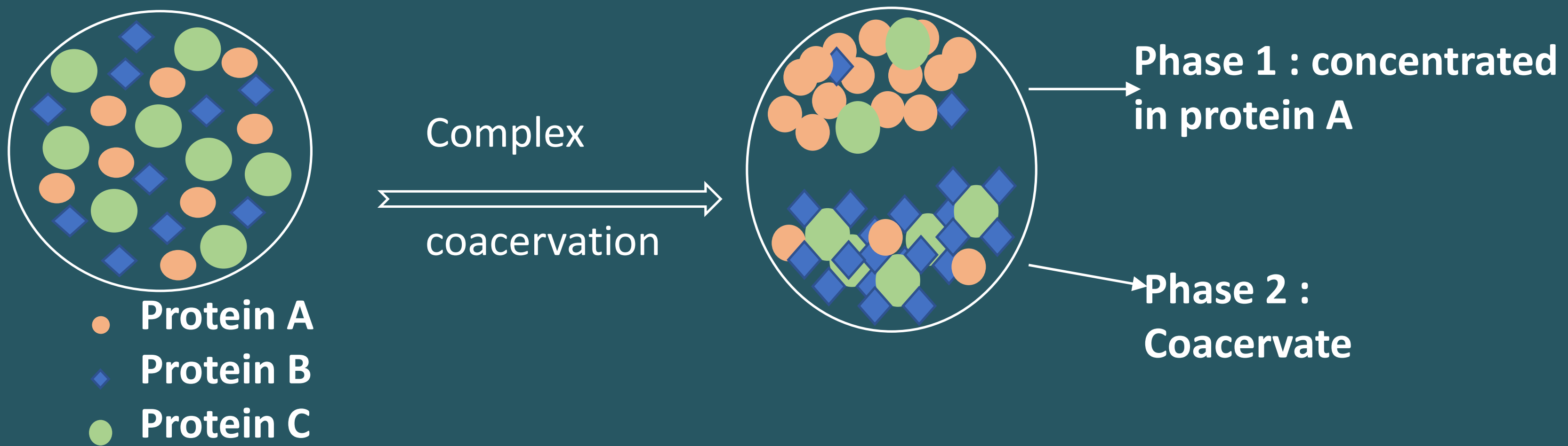
Potentiel Zeta = - 50 mV *Potentiel Zeta = + 20 mV*

Interaction energy: +
Soluble complexes: < 20 nm

Ainis *et al., Langmuir,* ***2019***

Heteroprotein complex coacervation : Applications

Protein purification

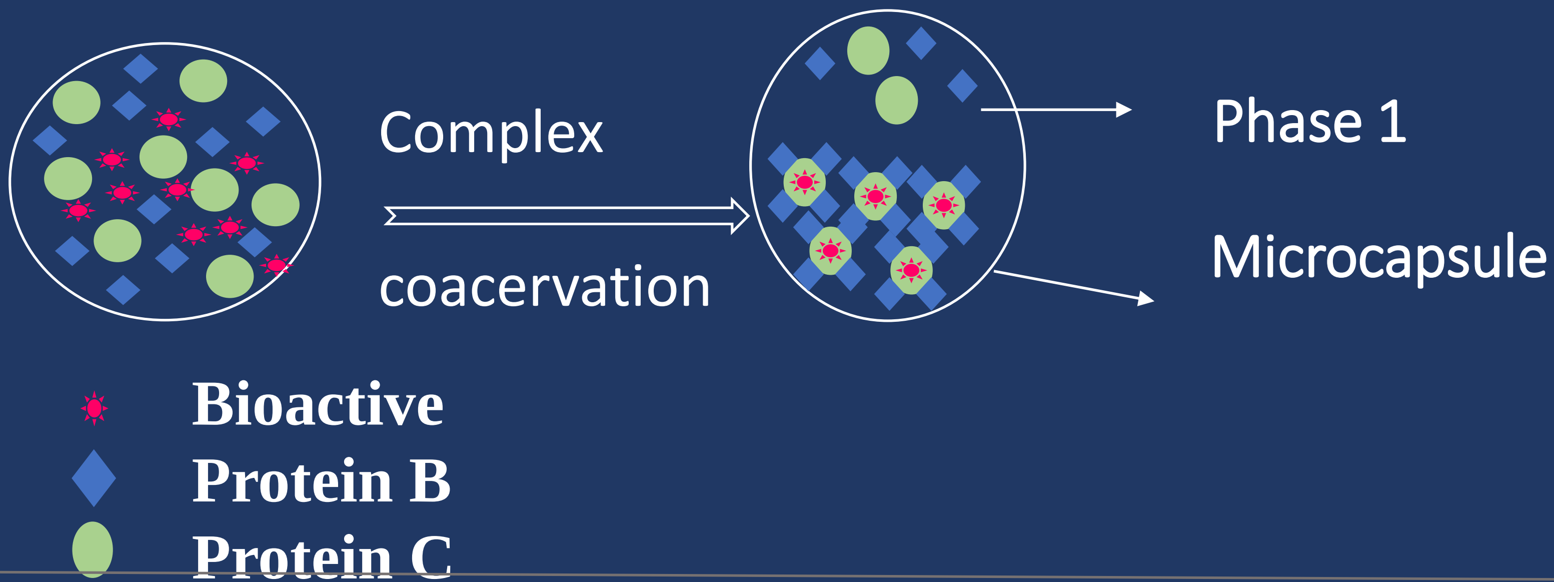


Shunuan *et al., J. Agric. Food Chem.,* ***2020***

Heteroprotein complex coacervation : Applications

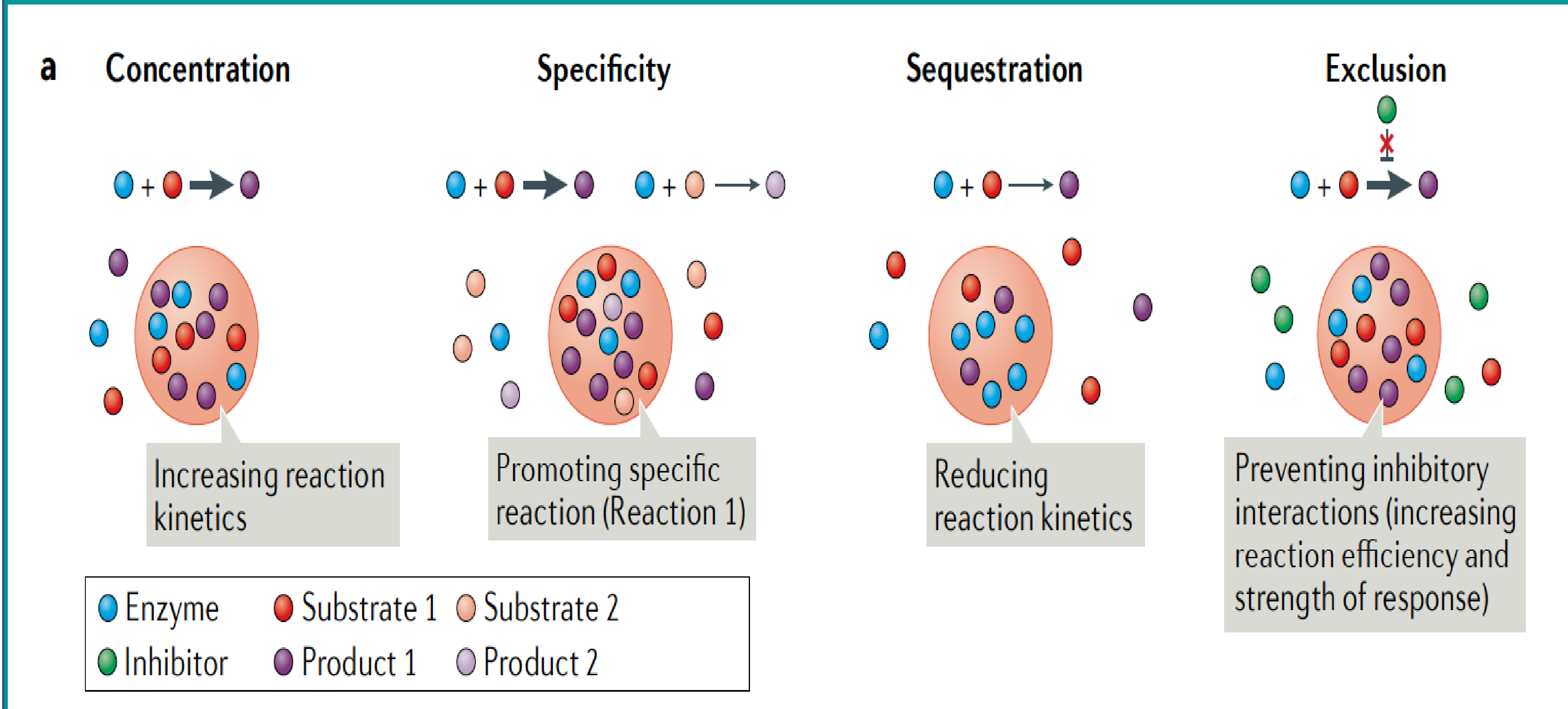
Protein purification

Encapsulation : Vitamins, oils, drogues …



Chapeau** et al., Food Hydrocolloids, **2016

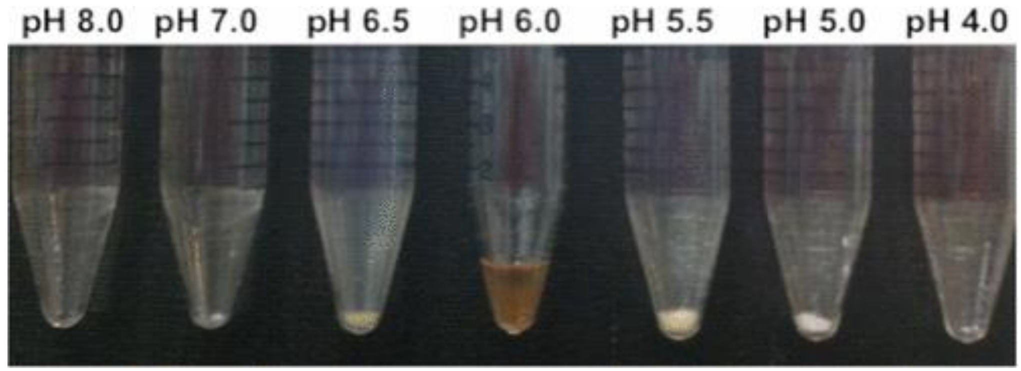
Heteroprotein complex coacervation : Applications



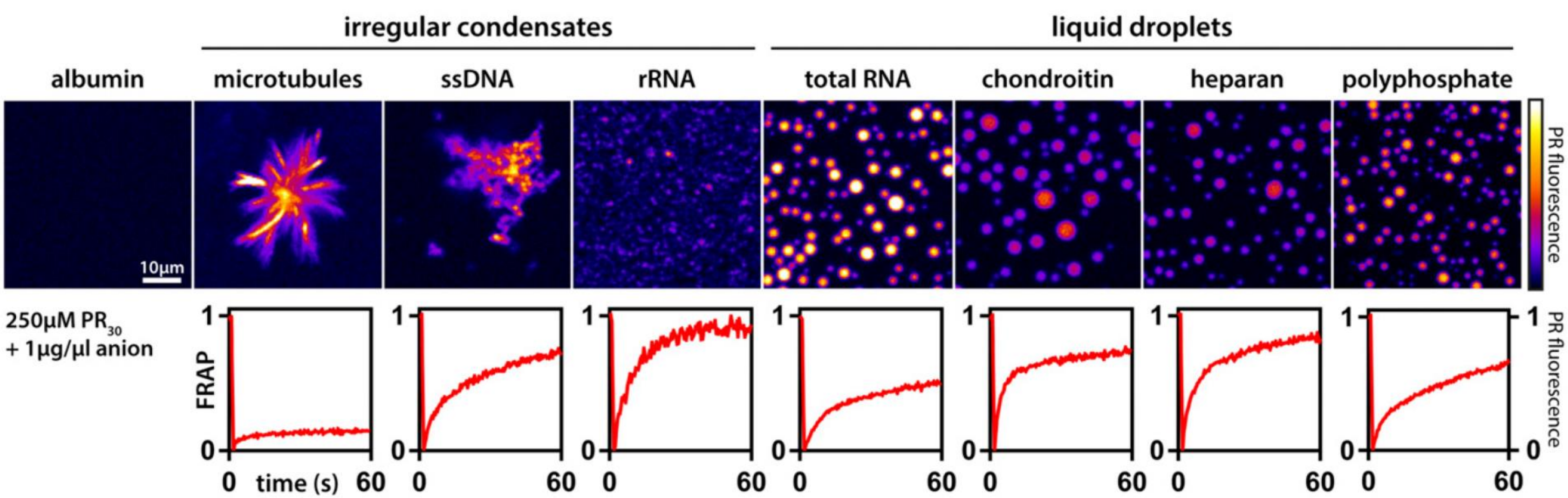
Lyon** et al., Nature Reviews, **2020

Enjeu majeur : Competition agregation & 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



Boeynaems *et al., PNAS,* ***2019***

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For more information:

Polyelectrolytes - Coacervates and Membraneless Organelles

Edited by Christine Keating, Nicolas Martin, Maria Santore

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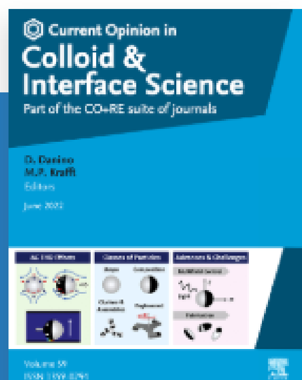

Current Opinion in Colloid & Interface Science

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