



Liquid-liquid phase separation in heteroproteinsystems: a mini review

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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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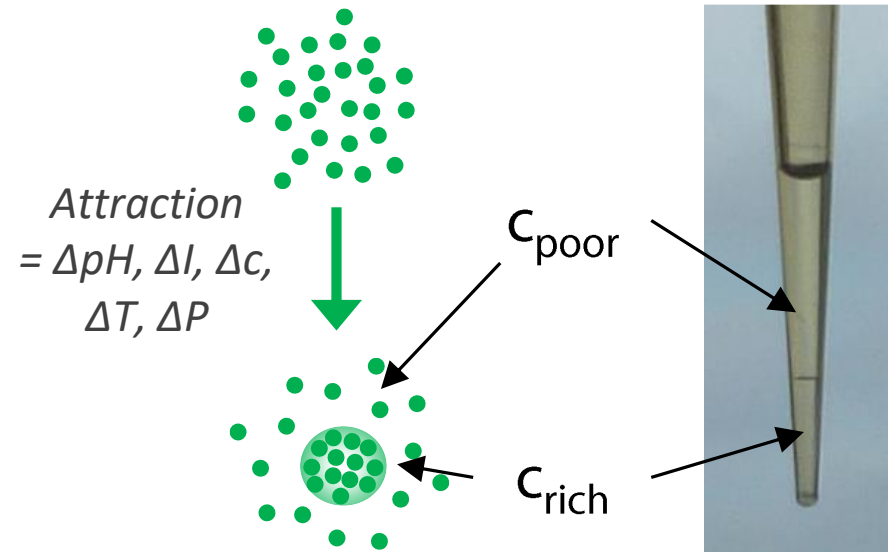
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➤ Liquid-liquid phase separation

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

2009

1729

Domaine très actif depuis une dizaine d'années!!

Exponential increase of number of publications

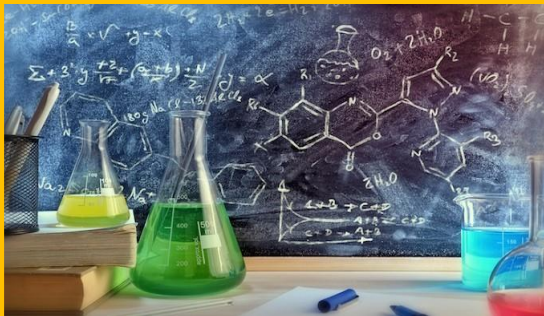
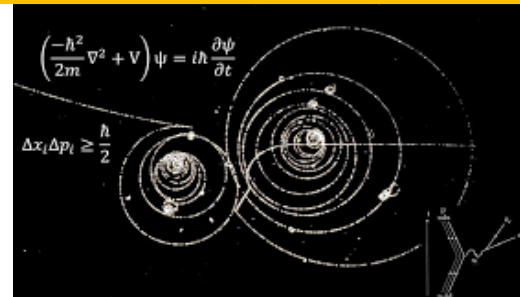
➤ Liquid-liquid phase separation

A universal phenomenon

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

Physics:

Soft matter/colloids/polymers
techniques & principles



Chemistry:

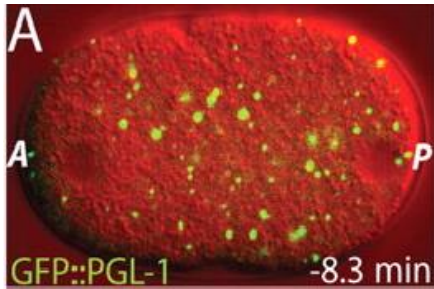
Synthesis, thermodynamic

Biology:

Molecular & cell biology /
genetic engineering /
labelling and microscopies

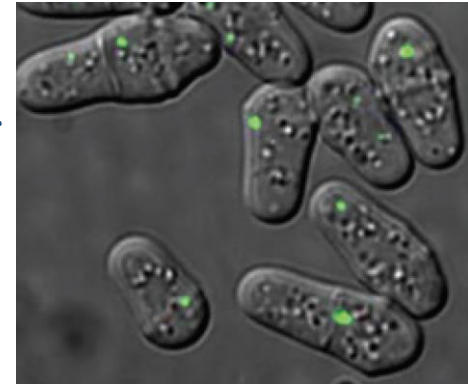


➤ Liquid-liquid phase separation *universal phenomenon*



Embryos germ cells P-granule
(RNA/proteins)

Dynamic reorganisation of
intracellular enzymes



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



« Sandcastle
worms »

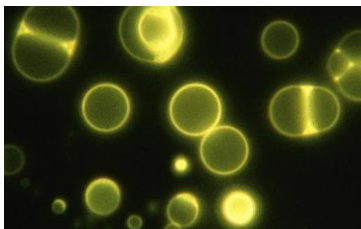
Mussels

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

**Adhesion; catalysis; regulation;
cell plasticity control**

Waite,, *The Journal of Adhesion*, **2005**

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

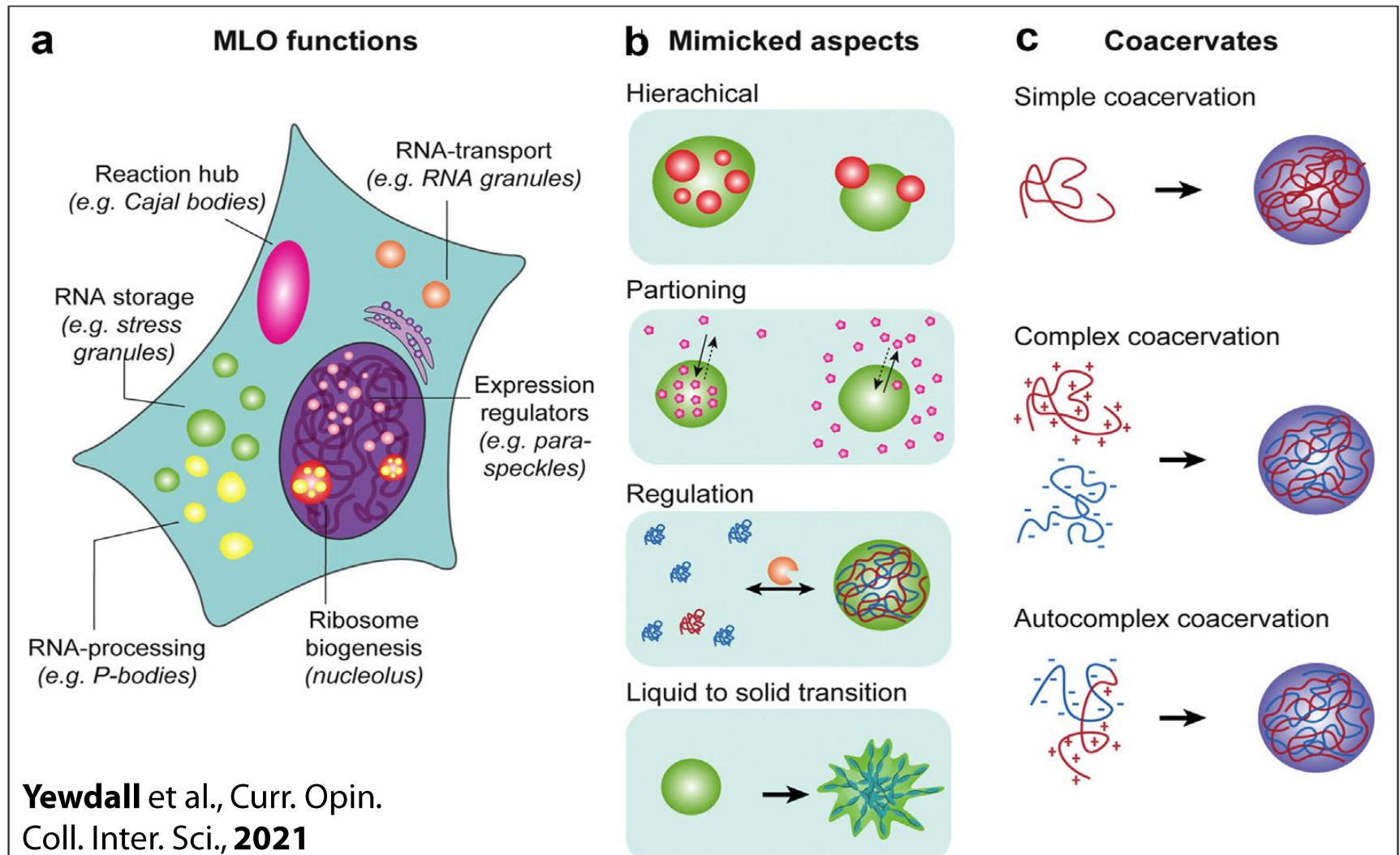


*Macromolecular phase separation is being recognized
for its potential importance and relevance as a driver of
spatial organization within cells*

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

> Liquid-liquid phase separation

A universal phenomenon

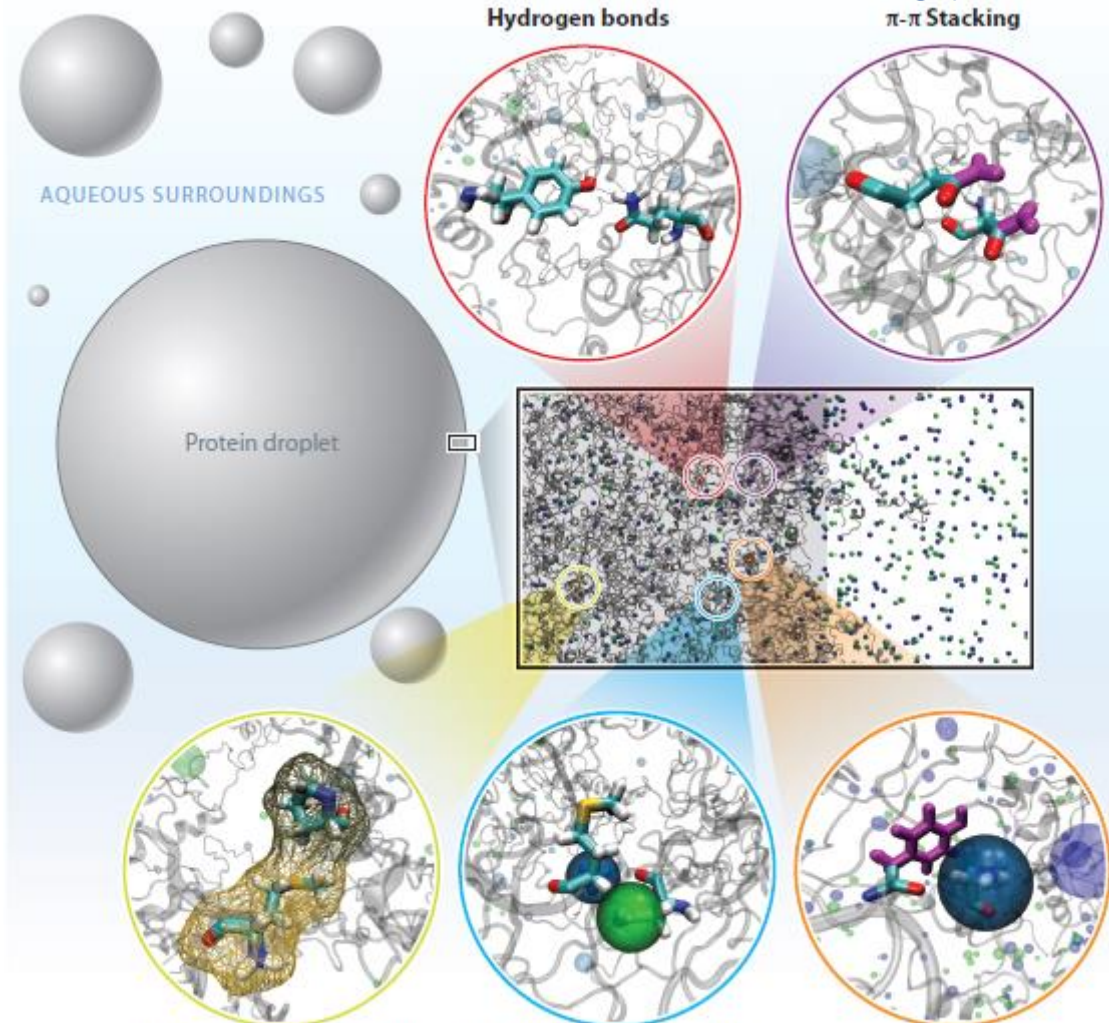


Understanding cell membraneless organelles
.... by mimickingusing coacervates.

➤ Liquid-liquid phase separation

A universal phenomenon

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

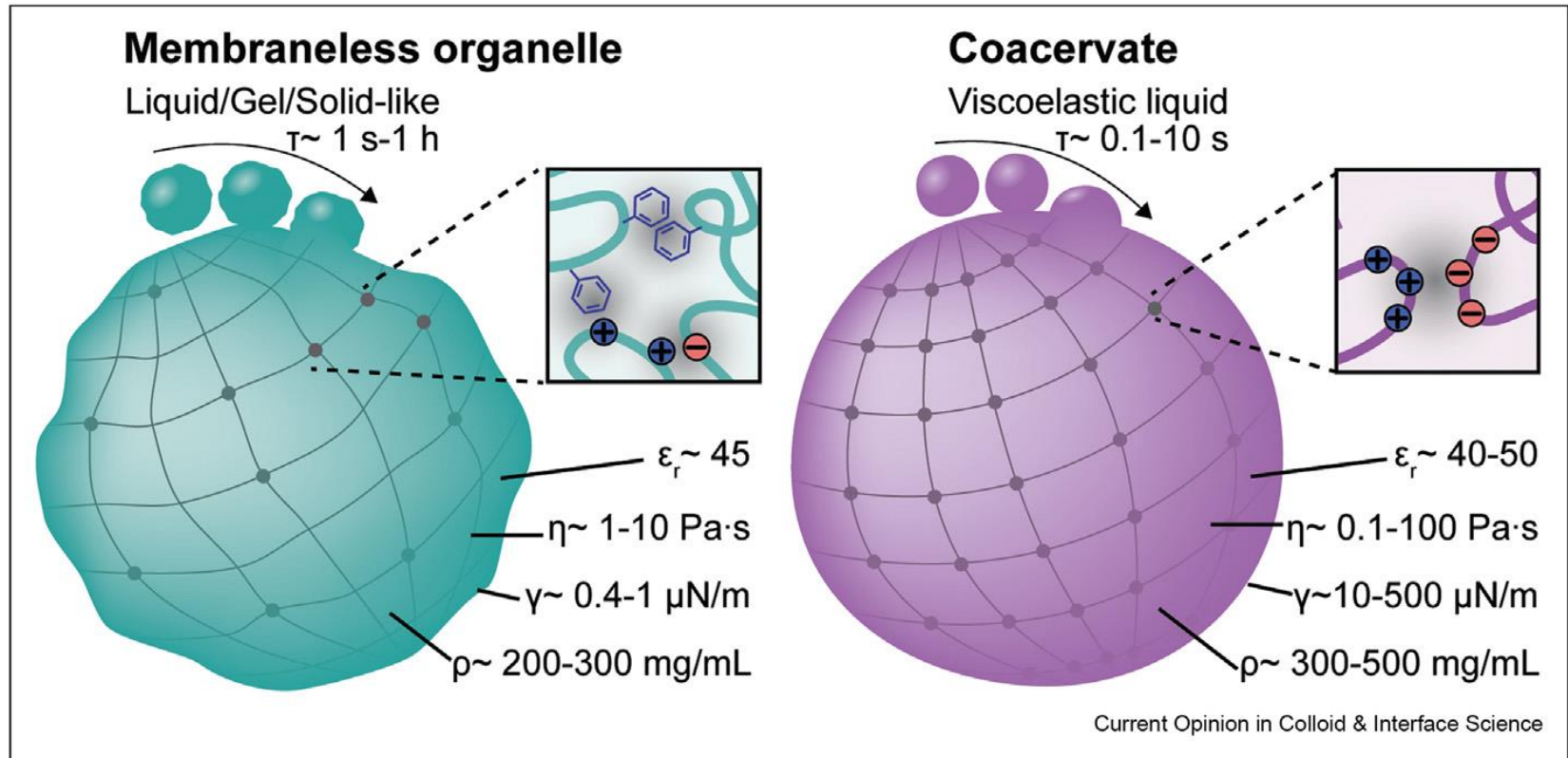


Dignon et al.,
Annu. Rev. Phys. Chem. **2020**

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

➤ Liquid-liquid phase separation

A universal phenomenon

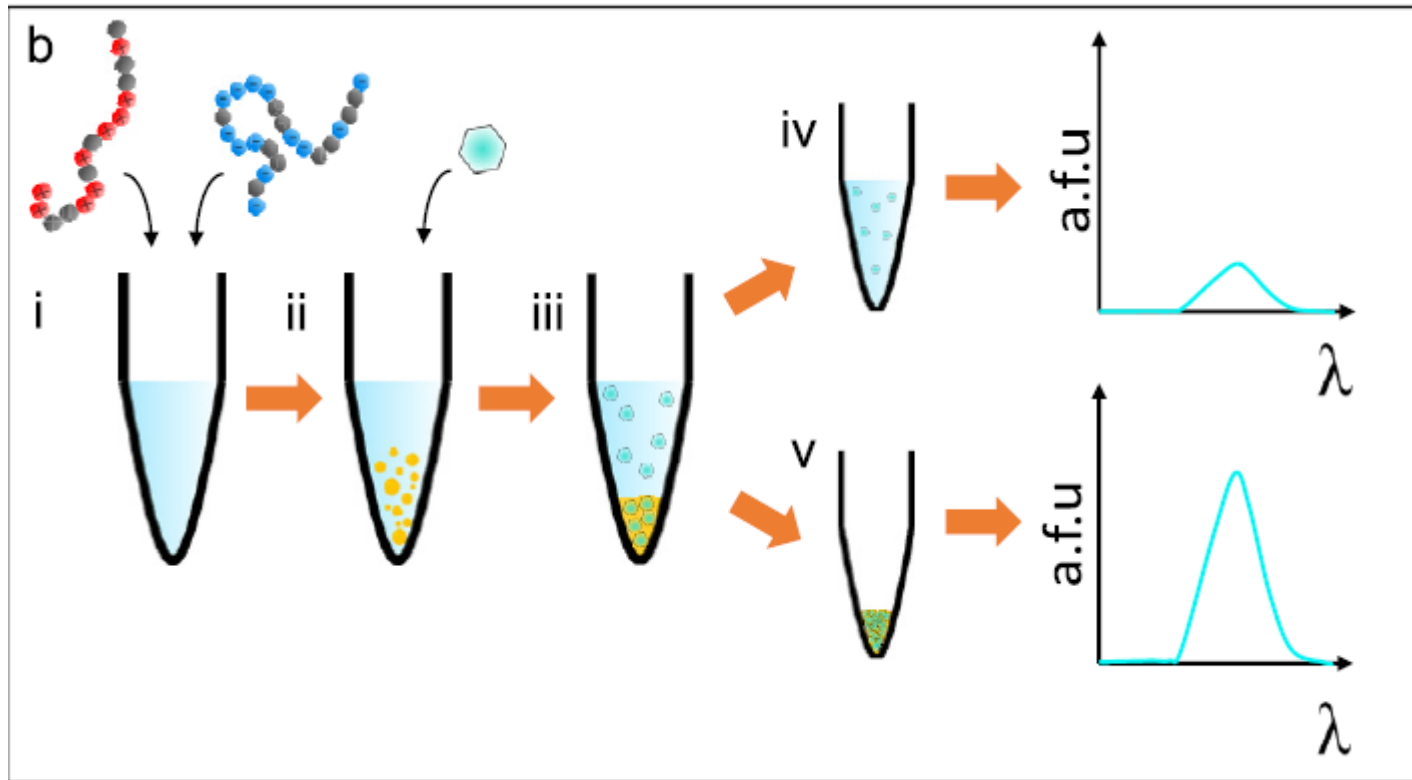


Relevant physicochemical properties of MLOs versus coacervates:

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

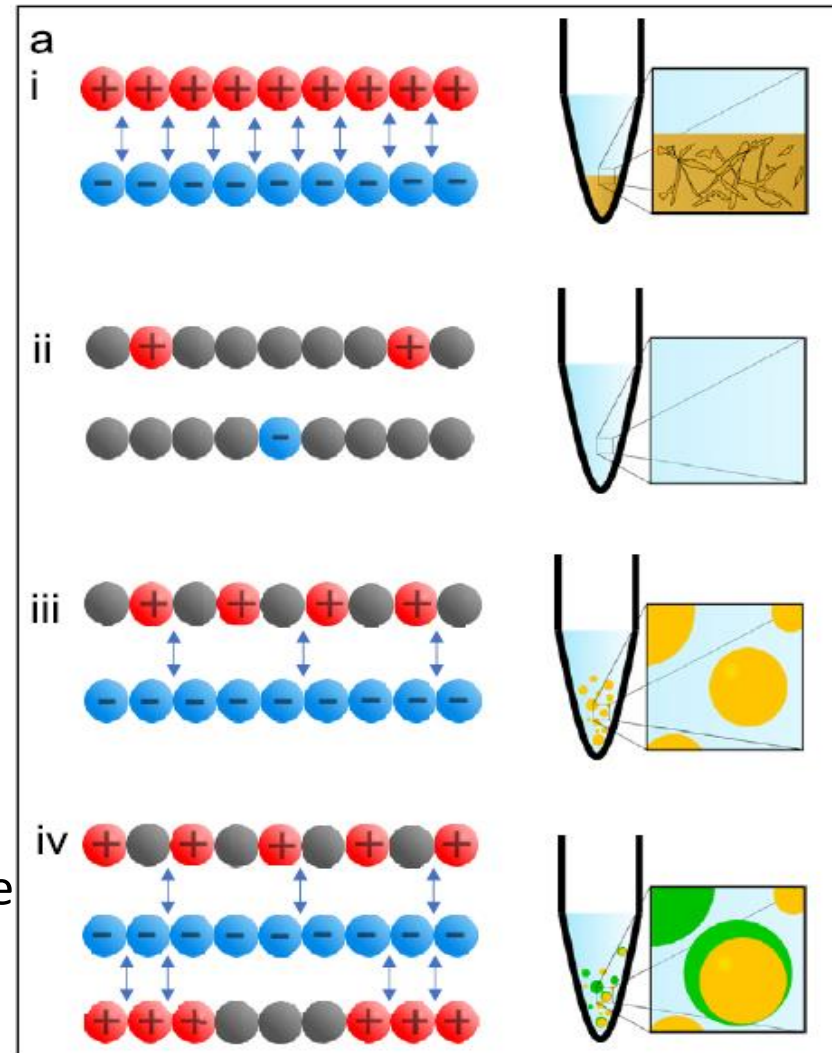
The strength of electrostatic interactions govern whether coacervates can be formed or not

(i) If a very strong attraction → precipitates

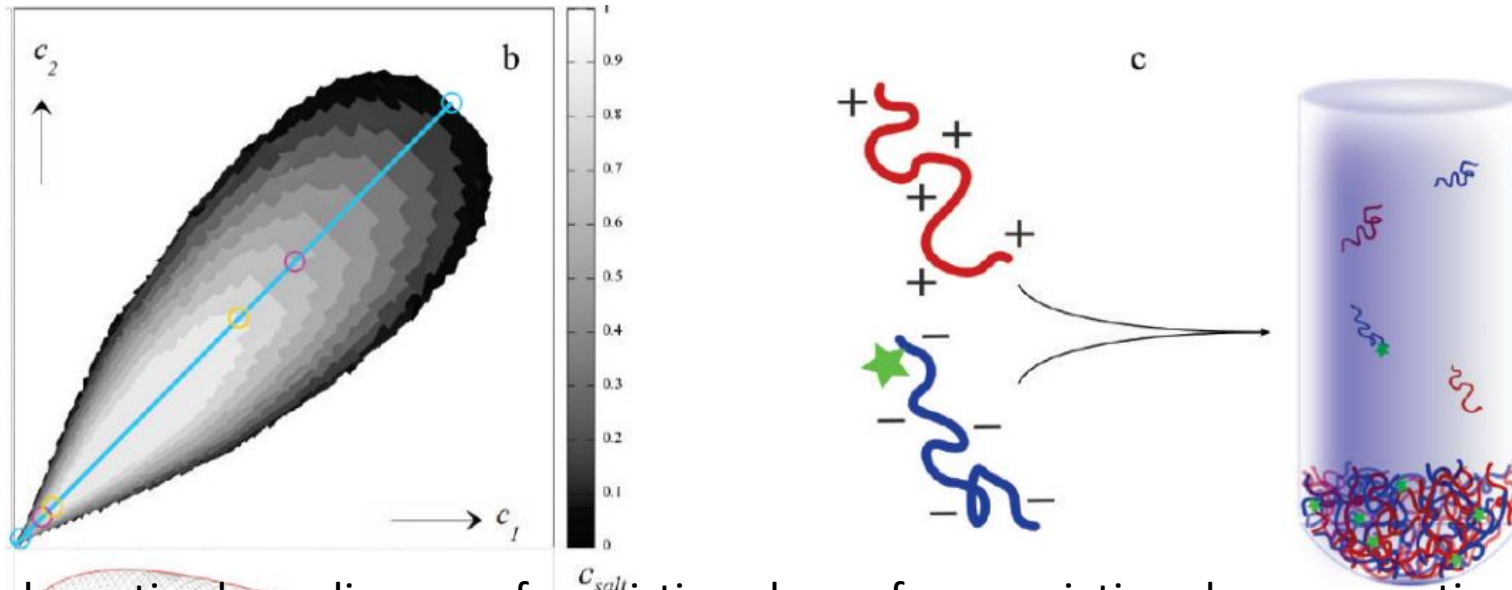
(ii) If too weak interactions → no phase-separation

(iii) Optimal strength of the interactions → Coacervates

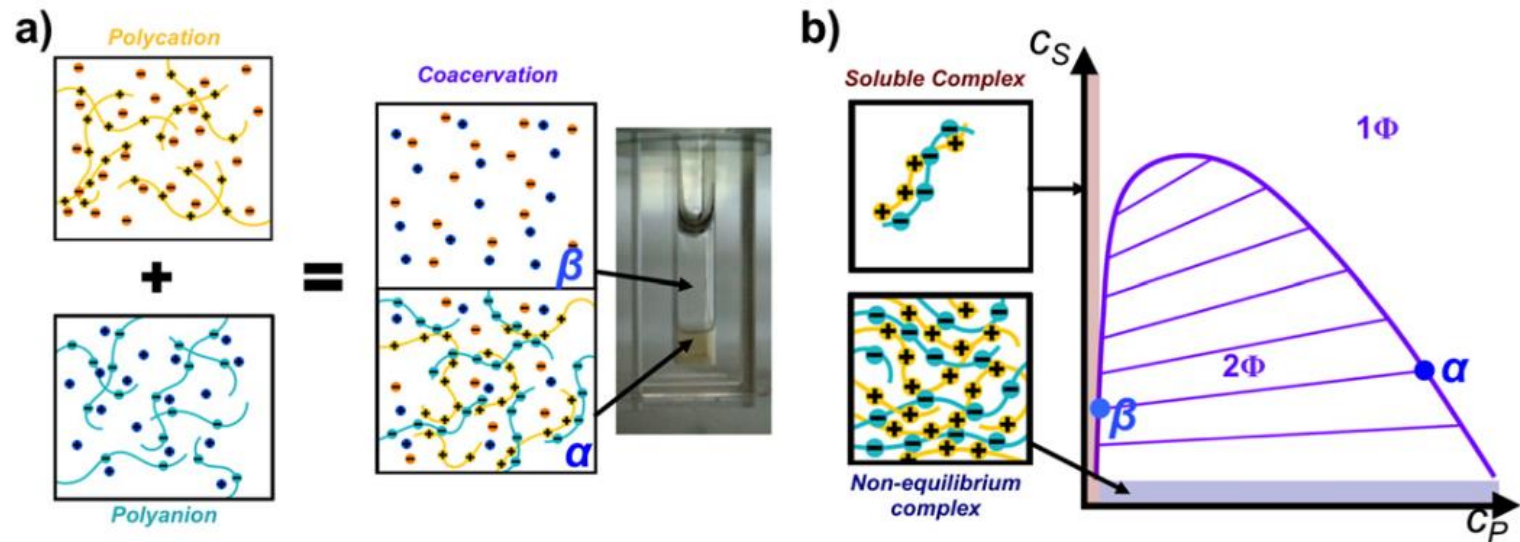
(iv) If multiple polyelectrolyte species, possible to form multiphasic coacervates



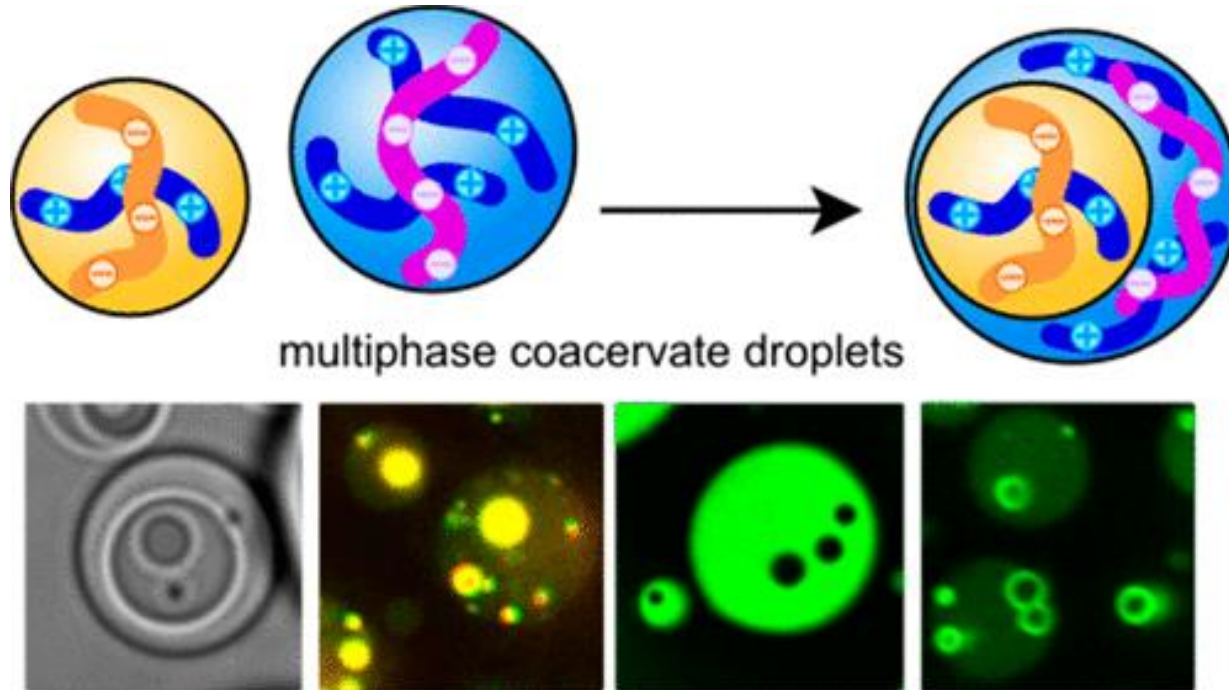
➤ Chemical determinants of phase separation: phase diagram



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



➤ Control formation of multiphase liquid droplets Partitioning in vivo

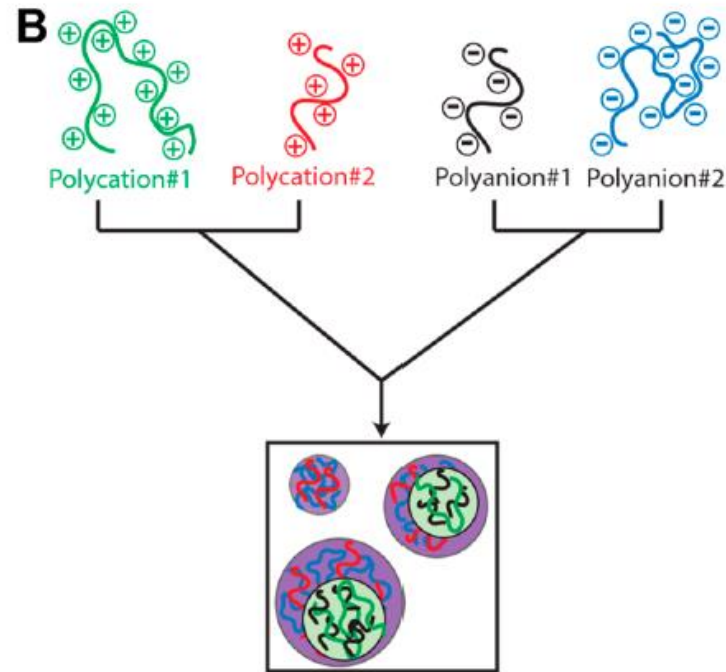
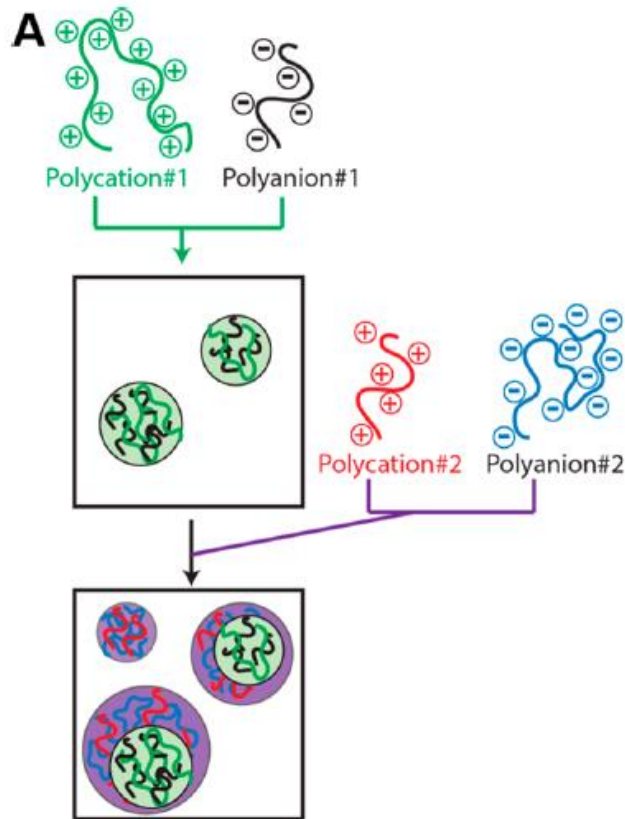


Multicomponent, multiphase droplets:

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

Lu & Spruijt, JACS, 2020

➤ Control formation of multiphase complex coacervates

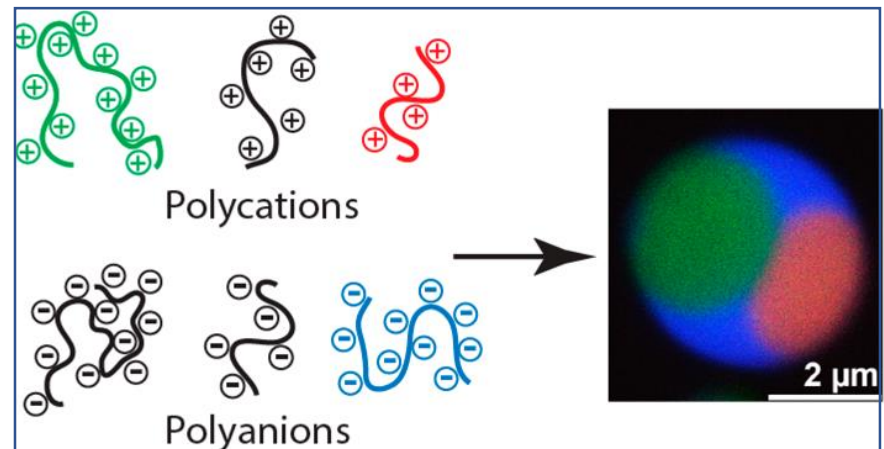


Like Charge Polymers Are Premixed

Sequential Formation of
Multiphase Coacervates

Mountain and D. Keating,
Biomacromolecules, 2020

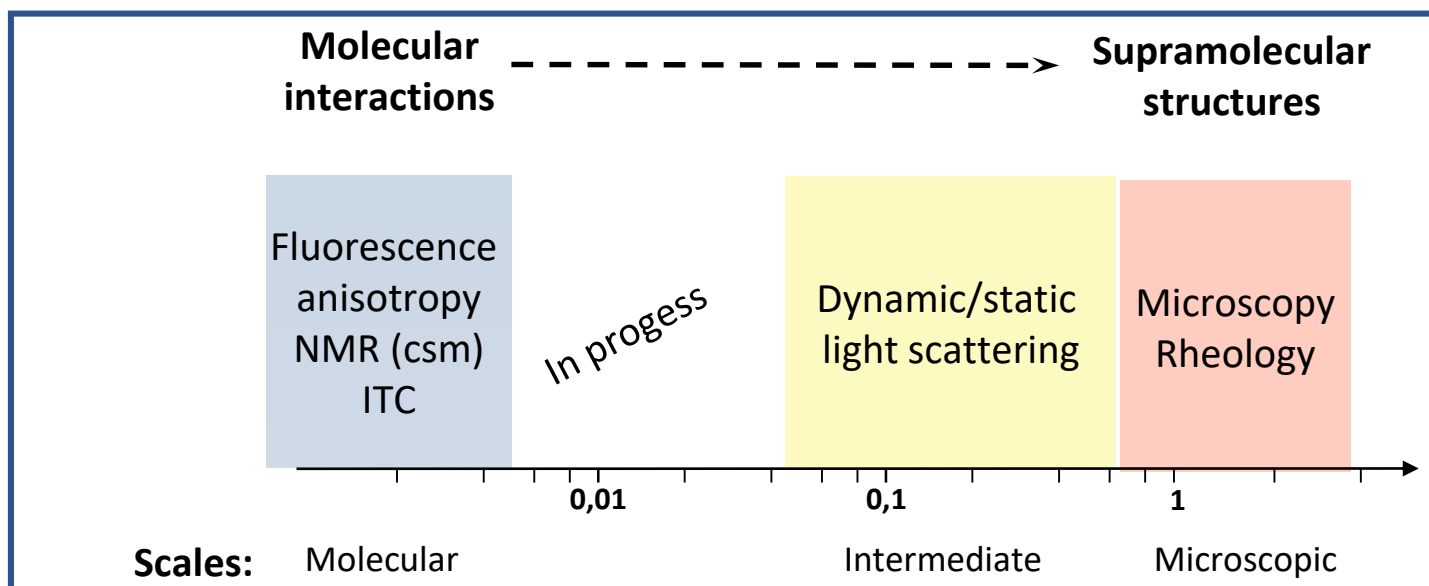
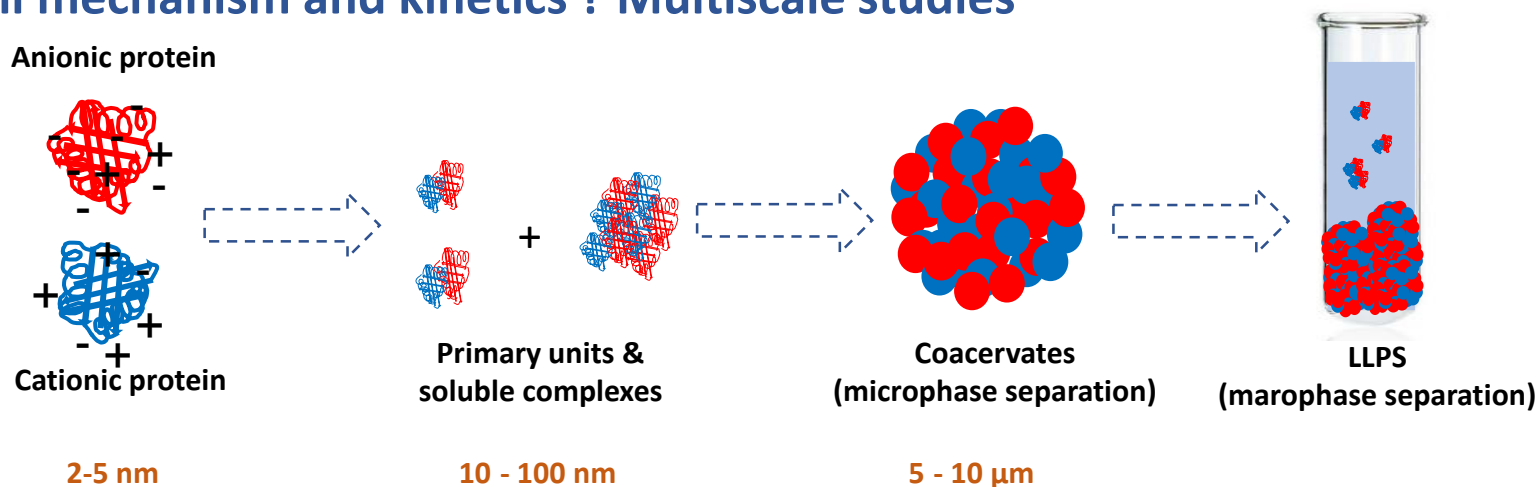
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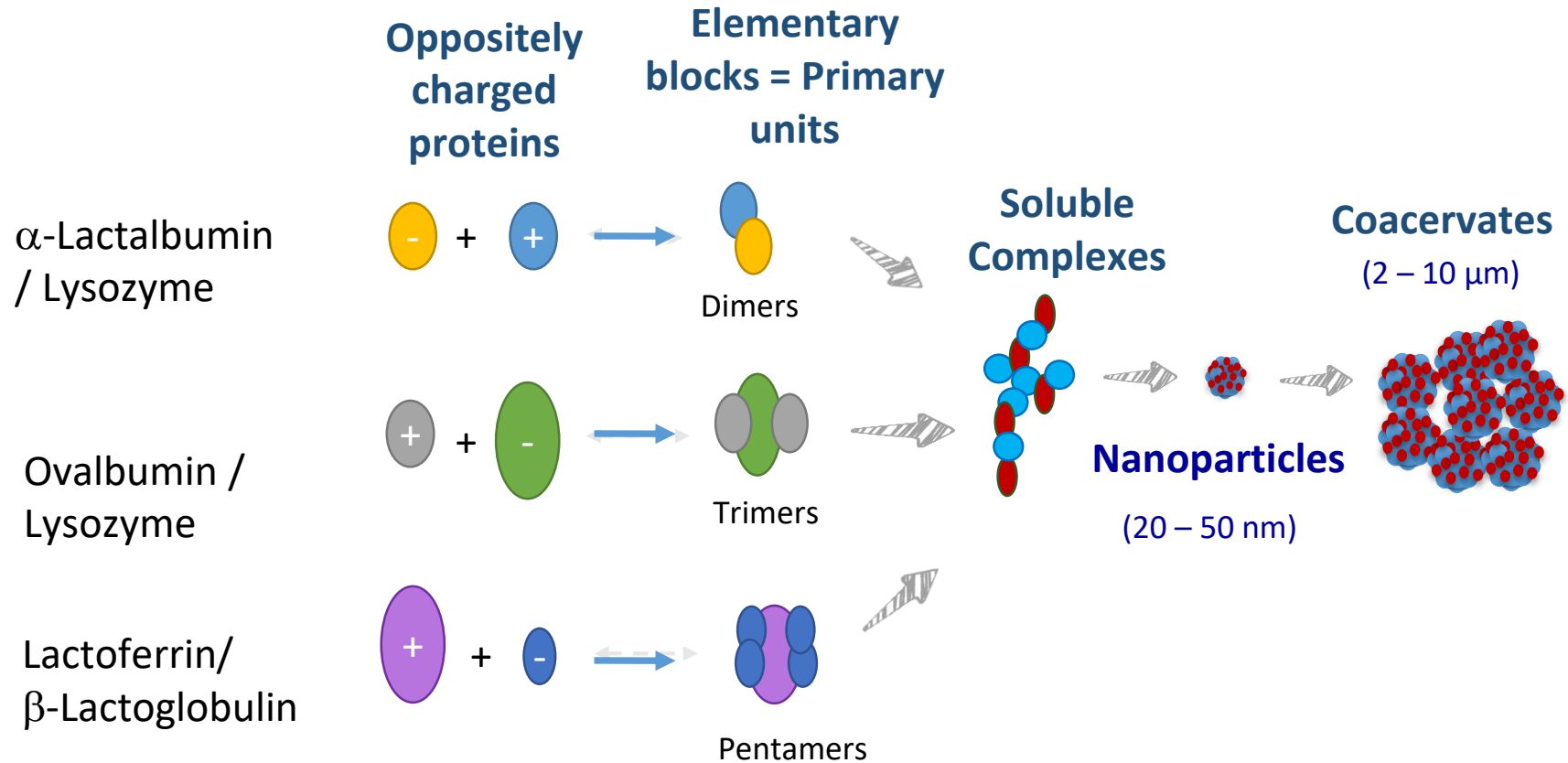
Liquid-liquid phase separation

Focus on heteroprotein complex coacervation

Overall mechanism and kinetics ? Multiscale studies



➤ Heteroprotein complex coacervation : Molecular scale

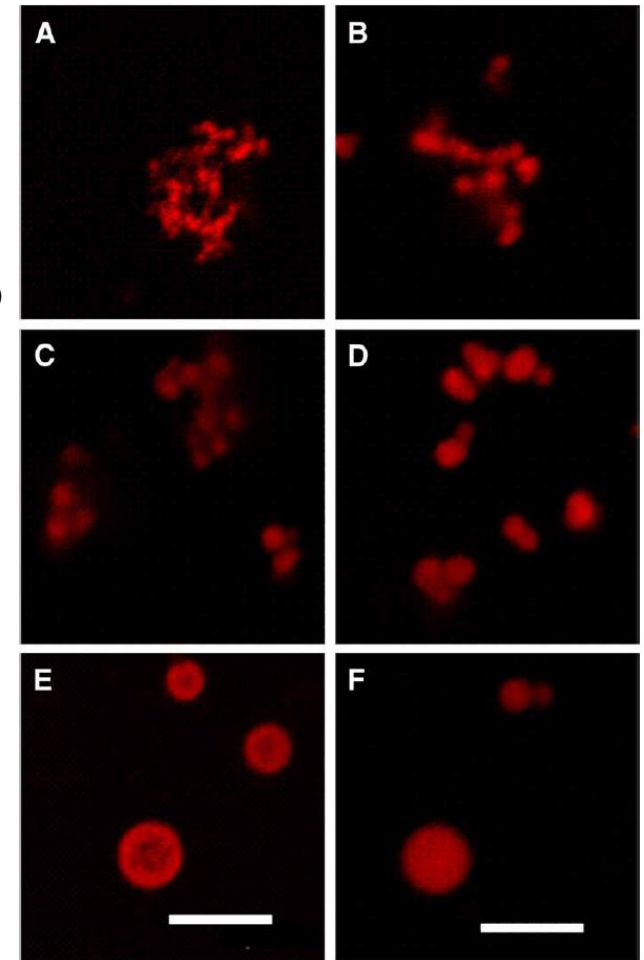


Croguennec et al., Adv. Coll. Int. Sci., 2017
Salvatore et al. Biomacromolecules, 2011

➤ 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).

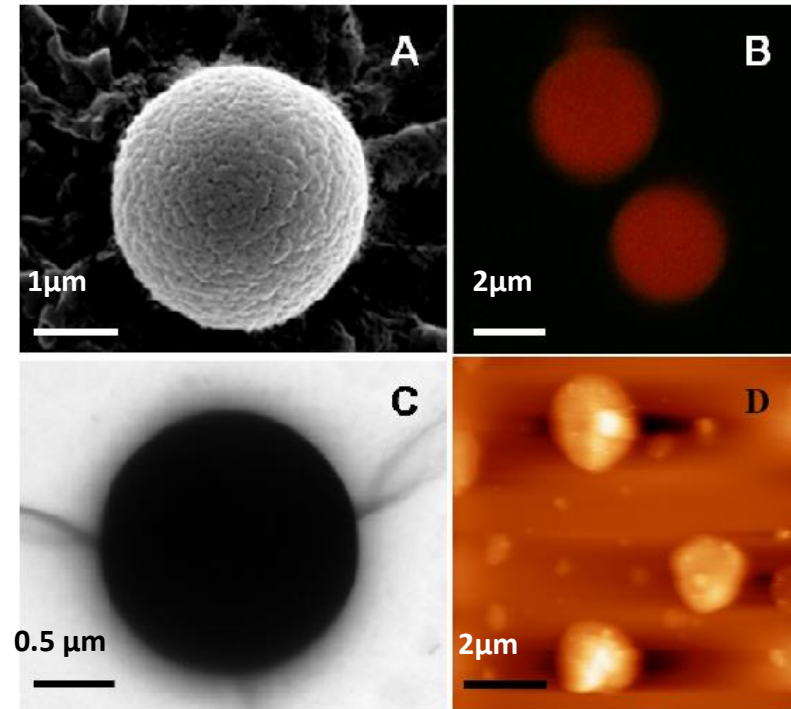


Nigen et al., FEBS Journal, 2010

➤ Heteroprotéin complex coacervation : macroscopic scale

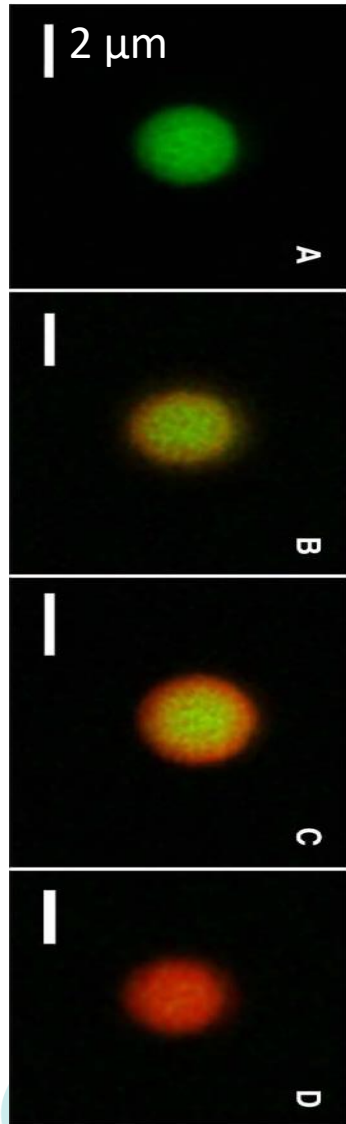
Temperature > 30°C

- ❑ 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**

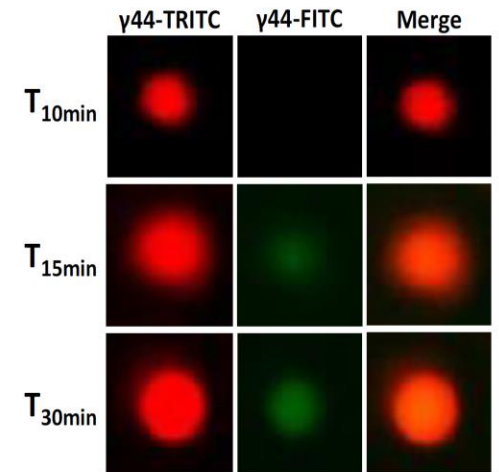
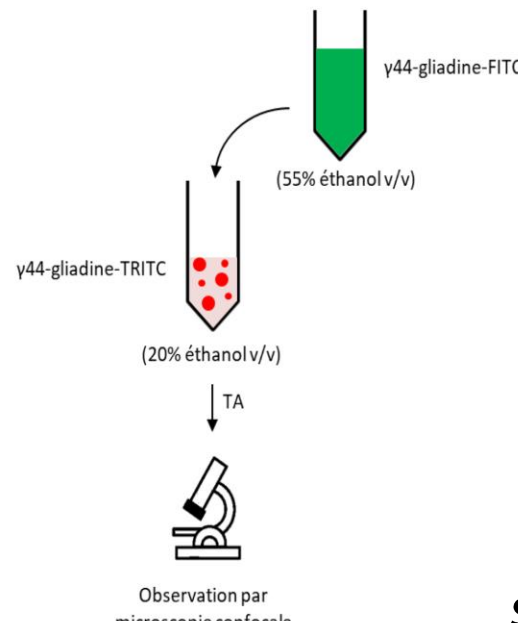
➤ Heteroprotein complex coacervation : Dynamic process between dilute and dense phase



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

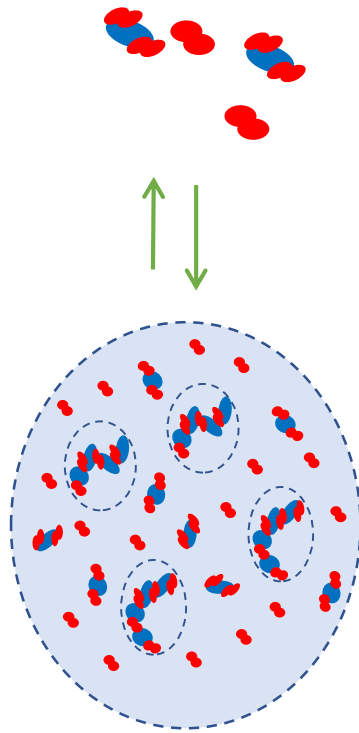


**Nigen et al.,
Biophys. J. 2010**

Sahli et al., Sci Rep, 2019

➤ 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 $\beta\text{-LG/LF}$ molar ratio in the coacervate phase

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

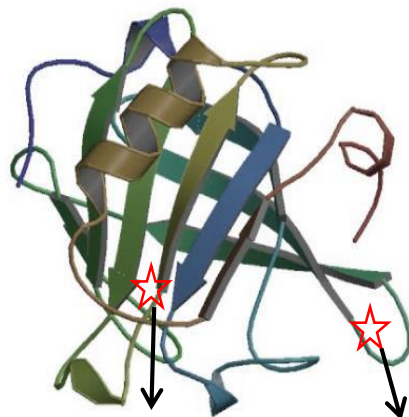
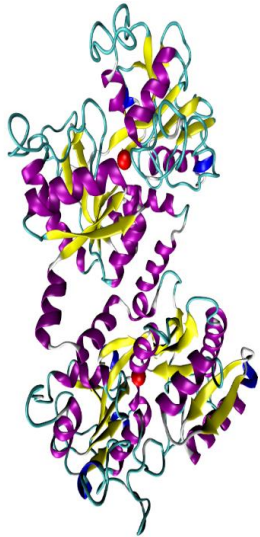
Behind classical parameters, critical role of surface charge and charge 'patchiness'

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 +

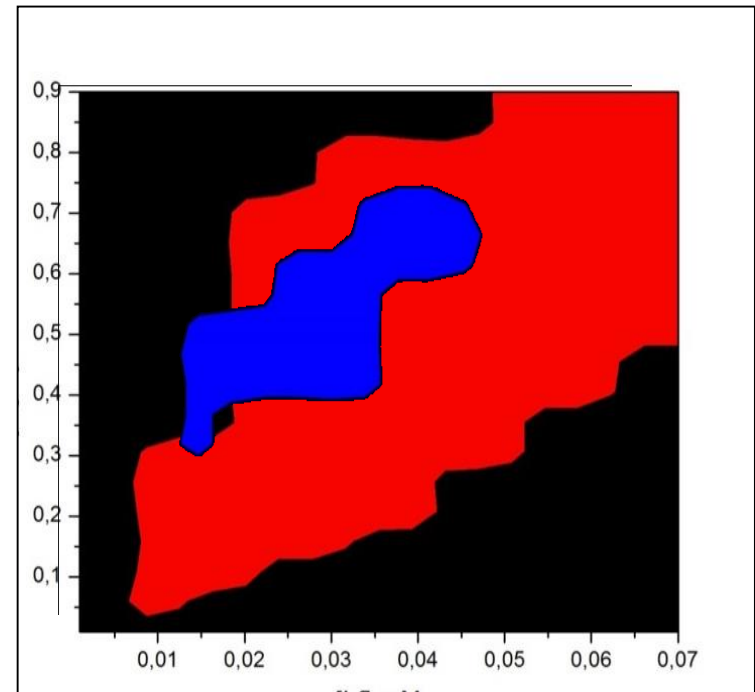
β -lactoglobulin



Val118Ala

Gly64Asp

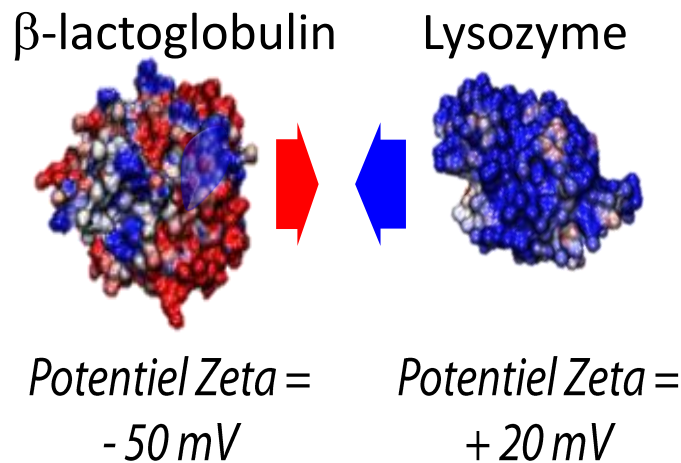
Lactoglobulin (mM)



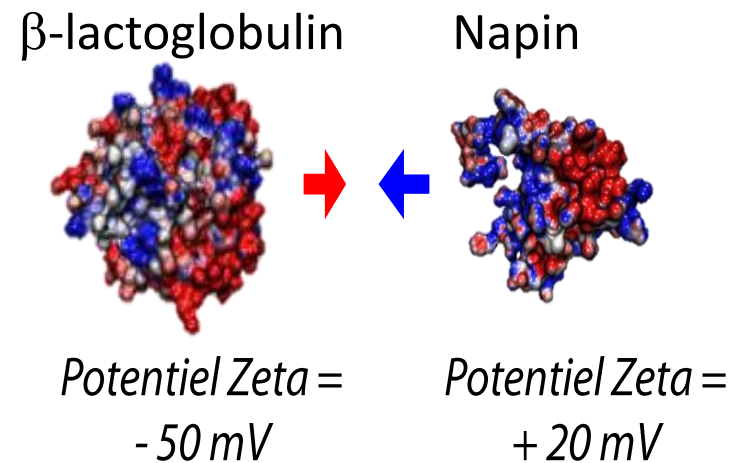
Lactoferrin (mM)

➤ 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

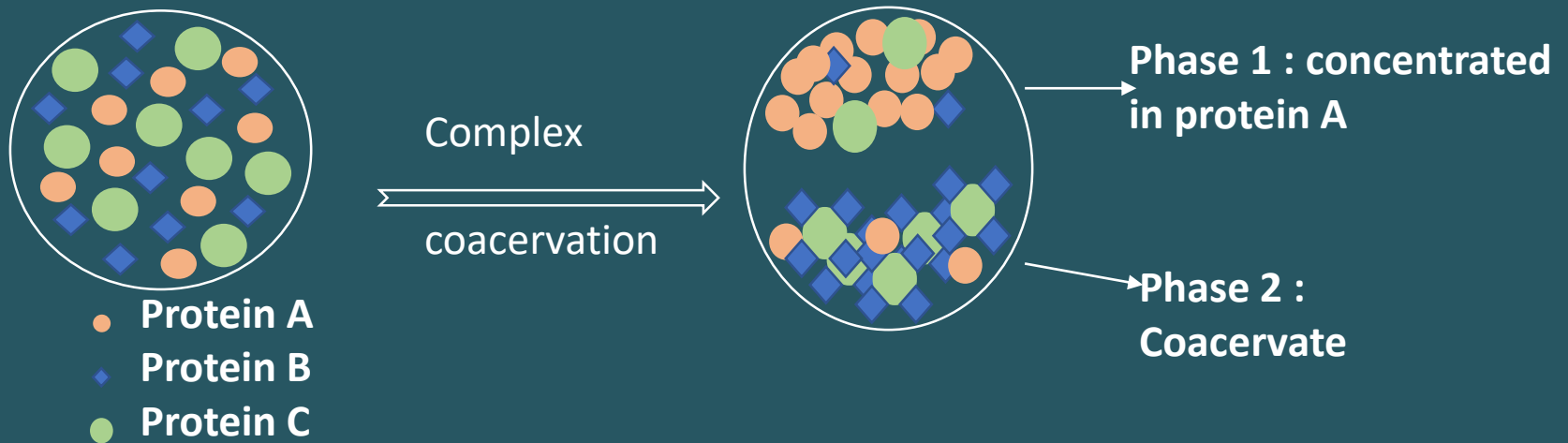


Interaction energy: +
Soluble complexes: < 20 nm

Ainis et al., Langmuir, 2019

➤ Heteroprotein complex coacervation : Applications

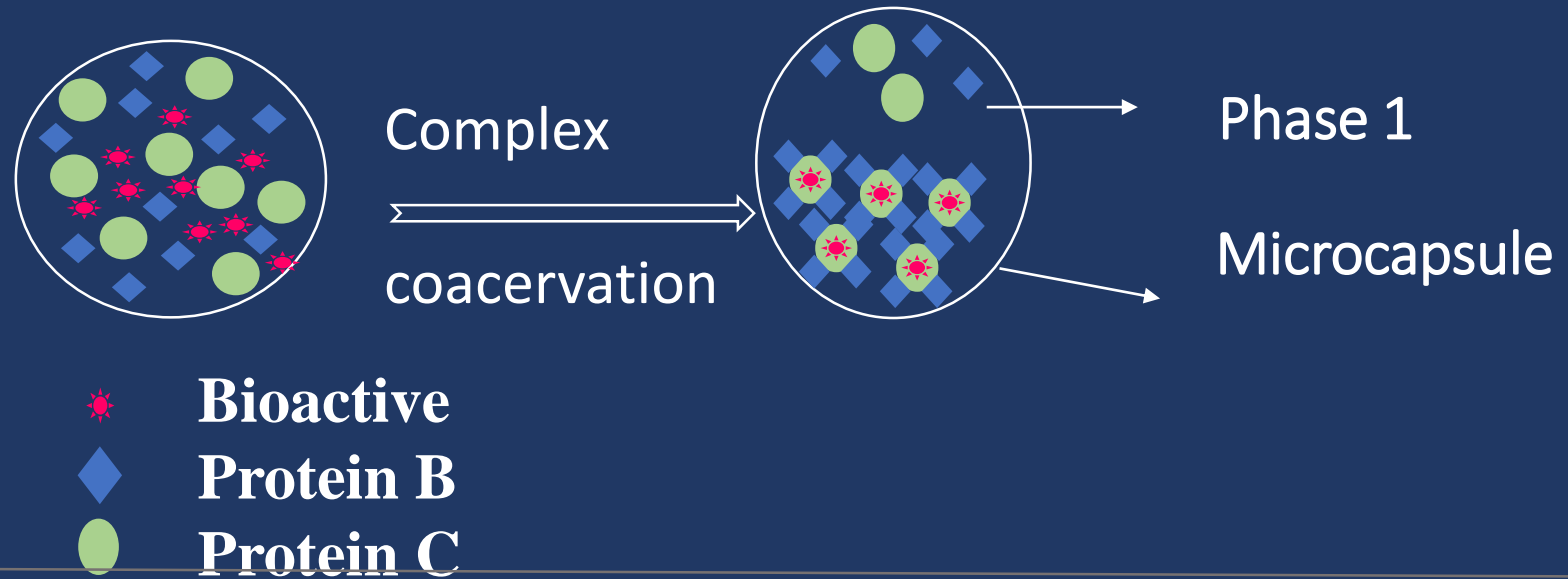
Protein purification



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

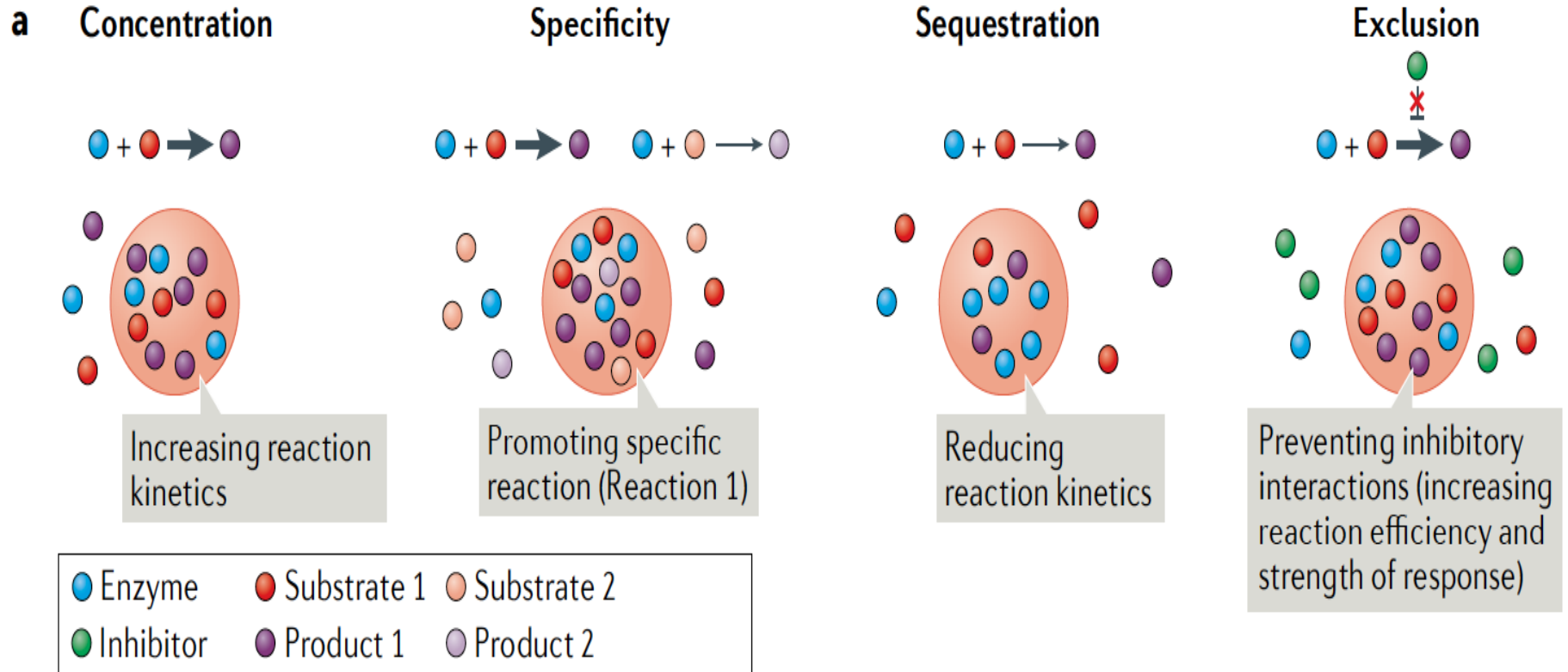
➤ Heteroprotein complex coacervation : Applications

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



Chapeau et al., Food Hydrocolloids, 2016

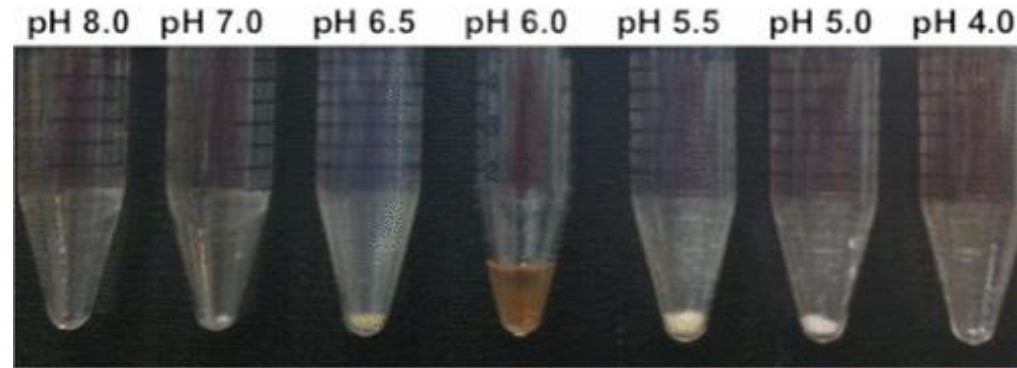
> Heteroprotein complex coacervation : Applications



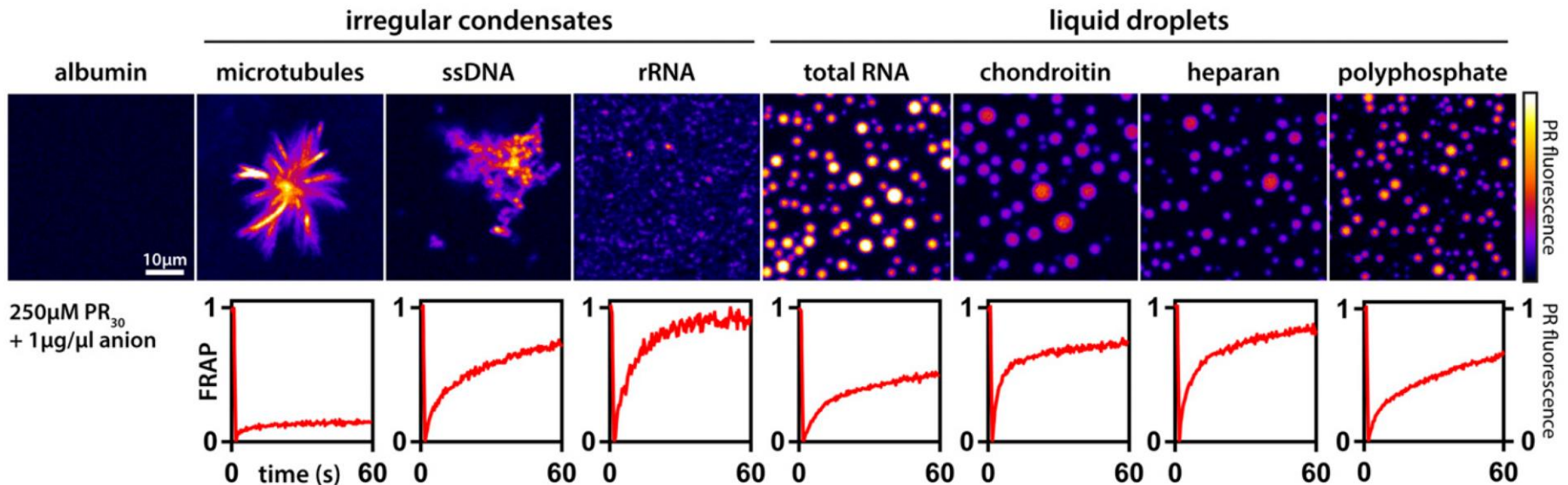
Lyon et al., Nature Reviews, 2020

➤ 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



For more information:

Polyelectrolytes - Coacervates and Membraneless Organelles

Edited by Christine Keating, Nicolas Martin, Maria Santore

Last update 12 March 2021



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