

Liquid-liquid phase separation in heteroprotein systems: 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



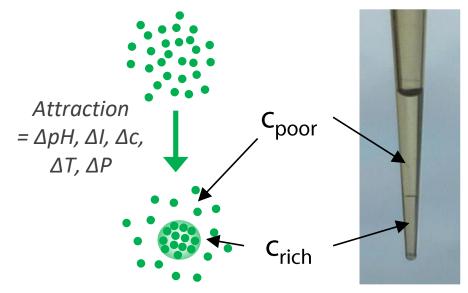






> Liquid-liquid phase separation

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

over close to the costs

there was a flux of P granules into the anterior that tude to the nosteriorly di-

natched the overall flow behavior of cytoplasmi

material such as yolk granules (6), quantified by

dividual P granules during localization. We found

(Fig. 2A). We determined the average rate of rel

ative & indicates P granule dissolution (i.e., shrink

posterior prowth rate in and 5(RNA) embryos was

etry breaking, & stayed negative in the

tive intensity change of a population of P granules. ints in space and time (9); may

ext examined inte

rior by convection in the surroundir un alone. Thus, flows have little or no role nule localization (9).

sity cha

caking, 5 was across the entire embryo, indicating over on (fig. S1). After the

n (Fig. 2,

ive in the nosterior of the

rate from the an-

was of similar maen

A universal phenomenon



2009

- Her, A. Rus, Pratein Sci. 15

A. W. Farthidge, R. A. Melopk, D. Yang, J. U. Bowie, C. M. Deber, J. Bial. Chem. 228, 22056 (2003). 26. J. P. Walds, L. D. ner, R. M. Bell, J. Biol. Chem. 242

L.K. Nary, F. W. Lau, L.U. Bowie, C. R. Sanders,

, many of the mutants used in this work, and much ion; M. Veehler, B. Gozelle, P. Power, C. Kang, Jacob for technical assistance; and K. Daenoid, urr, M.-D. Tusi, T. Ineruon, J. Medice inte, W. Charin, and o

to thank 1. Bowie for providing the DACK error

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

wynne,^{1,2,5} Christian R. Eckmann,¹ David S. Courson,⁸ Agata Rybarska,¹ Jöbin Gharakhani,^{2,3} Frank Jülicher,^{2,3} Anthony A. Hyman^{1,3}*

ryos specify germ cells, which tely gen habditis elements the first eerm cell is established when RNA and omtein-rich s localize to the posterior of the one-cell embryo. Localization of P granules and their sical nature remain poorly understood. Here we show that P granules exhibit liquid-like restored restored restored restored wetting, which we used to estimate their viscosity and tension. As with other liquids, P granules rapidly dissolved and condensed. Localization pured by a biased increase in P granule condensation at the posterior. This process reflects a on, in which polarity proteins vary the co ation point across the cell ons may represent a fur ental physicochemical mechanism for structuring

this por

or GLH-1 (9, 17), both o

ng from the fortilized egg, the cells of a op (3), the polarity proteins PAR-1 and PAR-2 ing embryo differentiate to give ucs, as well as maintain tal earn line that will concrate sports otein granules assembled om RNA and RNA binding proteins, although elegant, the party un (1.2) In Cosmorbal called P granules. P gr anales are in ly throughout the unp d one-cell embryo. Upon symmetry breaking

w. Woods Hole: MA 02543, US

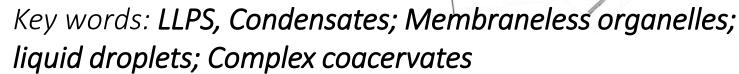
appear on the posterior cortex, and P granules become localized to the posterior half of the cell mbryo, indicating po (Fig. 1A and movie S1: all embryos are -50 µm. B (WT, wild type) and C1. As predicted from this ong); the embryo then divides, giving rise to a nalysis, if we delayed a RNA interference (RNAi) to deplete the centre P granule-containing progenitor germ cell and a non-Peranule-cor ine somatic sister cell. somal protein SPD 5 (12) 2 staved negative across en proposed to mediat terior localization: (i) P granule migra dissolve completely [Fig. 2B, and-5(RNA0]. When tion by cytoplasmic flow (4-6) and (ii) subse ably or deg ation of rema ules appe ning postorior PAR proteins (Fig. 2D and figs. S5 and anterior P granules (5-8). However, evidence ber of the S6). This To study P enable localization in the one-cell of soluble P granule of ree-dimensional (3D) particle terior cytopi n (Fig. 2E), and in the absence of r the m ic flows (movie S3). As with cence levels of P granules labeled with green WT embryos, the rate of P granule condensation peaked before leveling off. The maximum of the cin (GFP) tagged PGL-1 (9, 10)

e P granule com ments. We found that some P granul three times as high as that in WT embryos, which

of sy

ior but her

www.sciencemag.org SCIENCE VOI. 324 26 JUNE 20



Exponential increase of number of publications

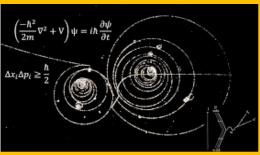
p. 3

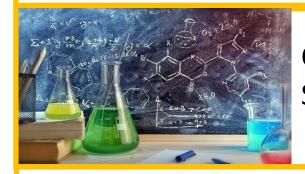


A universal phenomenon

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

Physics: Soft matter/colloids/polymers techniques & principles



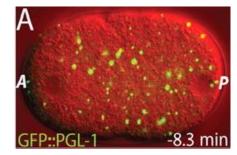


Chemistry: Synthesis, thermodynamic

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



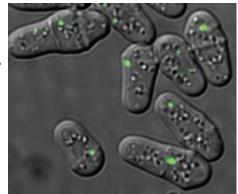
> Liquid-liquid phase separation *universal phenomenon*



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

Embryos germ cells P-granule (RNA/proteins)

Dynamic reorganisation of intracellular enzymes

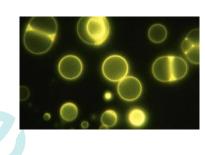




Marine organisms: A high adhesion (a glue!!)

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

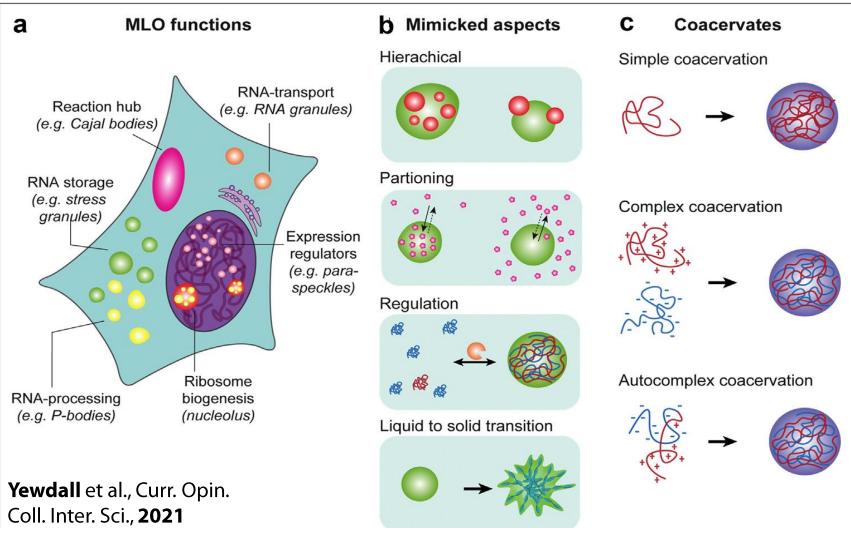
Waite,, *The Journal of Adhesion*, **2005** Kim *et al.*, *PNAS*, **2016** Adhesion; catalysis; regulation; cell plasticity control



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

Liquid-liquid phase separation

A universal phenomenon

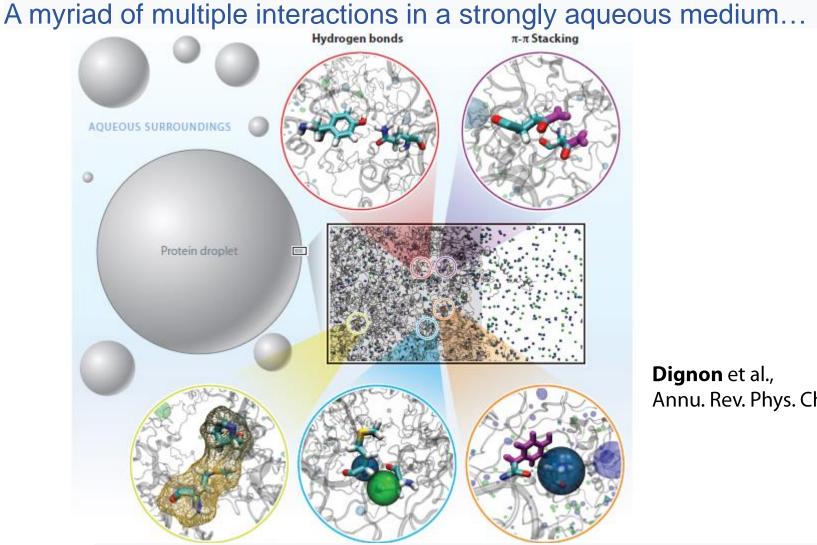


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Understanding cell membraneless organelles by mimickingusing coacervates.

> Liquid-liquid phase separation

A universal phenomenon

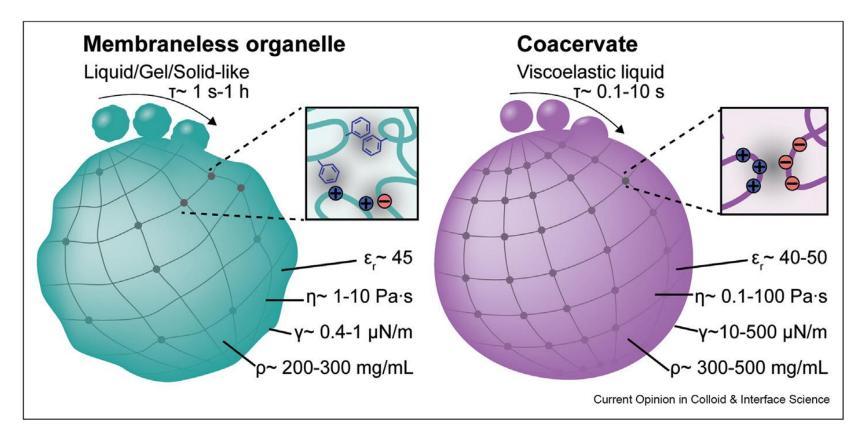


Dignon et al., Annu. Rev. Phys. Chem. 2020

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

Liquid-liquid phase separation

A universal phenomenon



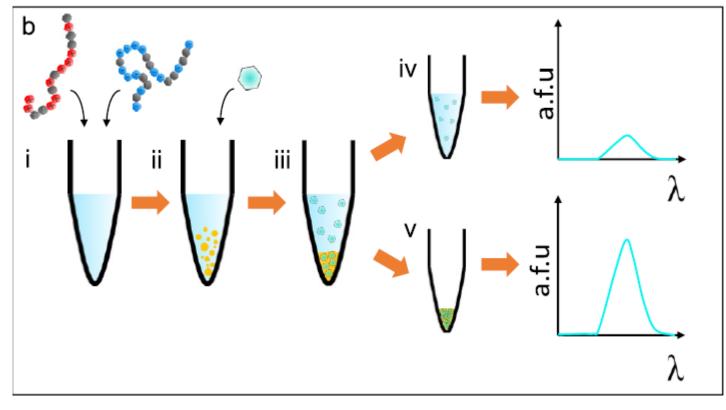
Relevant physicochemical properties of MLOs versus coacervates:

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)

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> Chemical determinants of phase separation

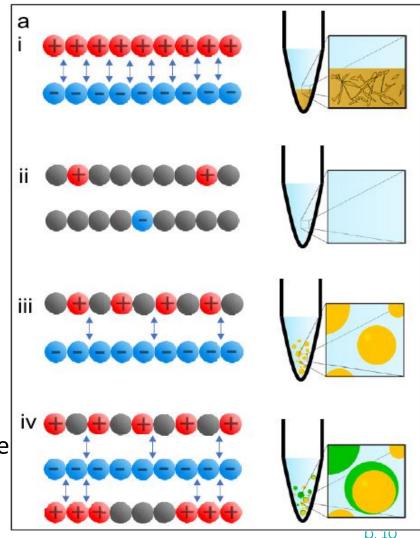
The strength of electrostatic interactions govern whether coacervates can be formed or not

(i) If a very strong attraction \rightarrow precipitates

(ii) If too weak interactions \rightarrow no phase-separation

(iii) Optimal strength of the interactions \rightarrow Coacervates

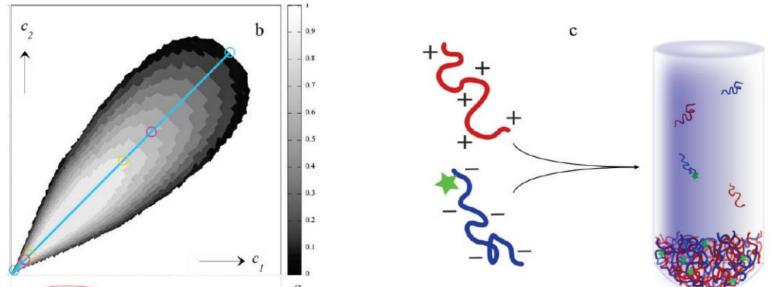
(iv) If multiple polyelectrolyte species, possible to form multiphasic coacervates



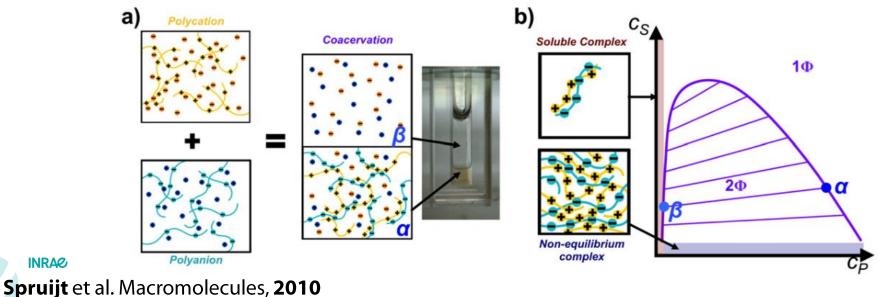
Ghosh et al., Curr. Opin. Coll. Inter. Sci. 2021



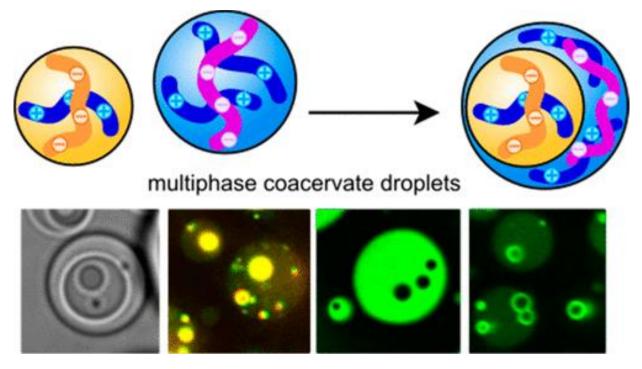
Chemical determinants of phase separation: phase diagram



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



Control formation of multiphase liquid droplets Partitioning in vivo



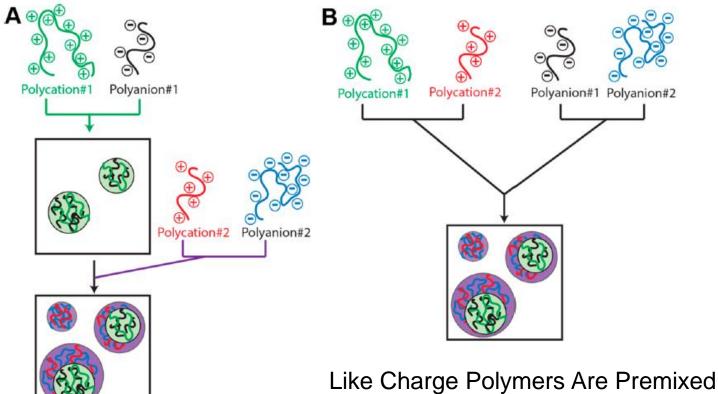
Multicomponent, multiphase droplets:

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

Lu & Spruijt, JACS, 2020



Control formation of multiphase complex coacervates

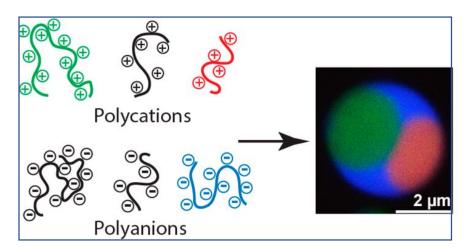


Sequential Formation of Multiphase Coacervates

Mountain and D. Keating,

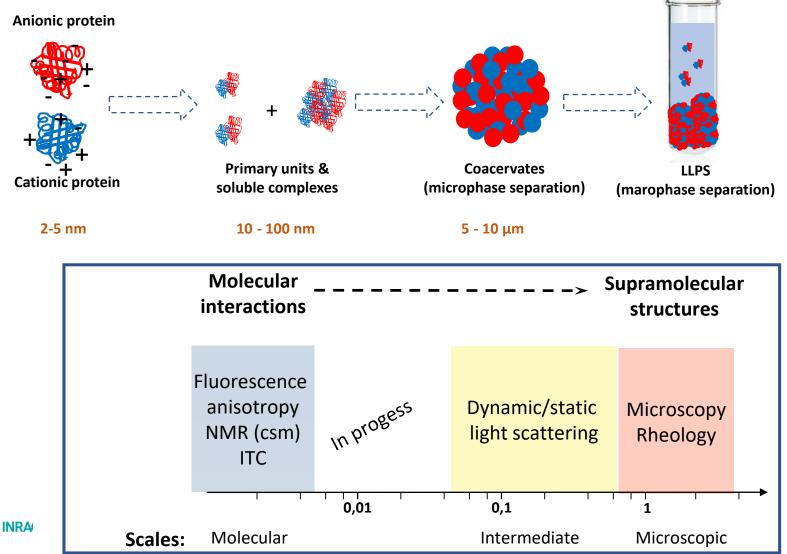
Biomacromolecules, 2020

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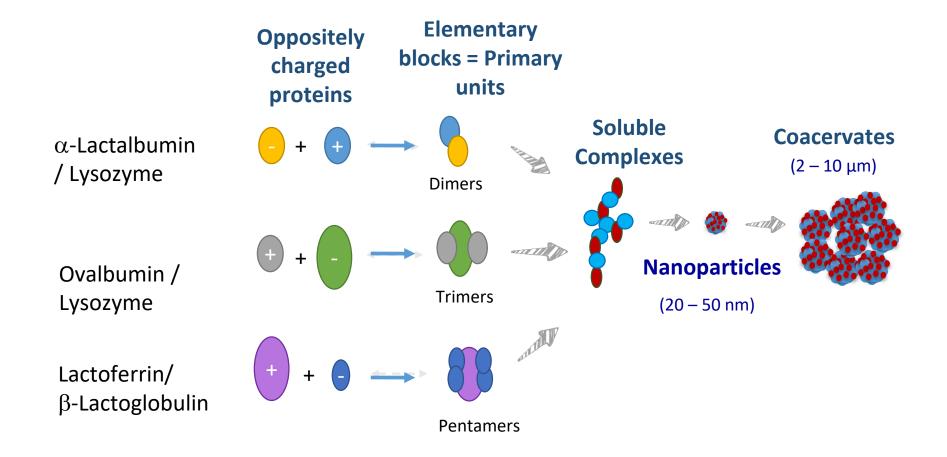


Liquid-liquid phase separation Focus on heteroprotein complex coacervation

Overall mechanism and kinetics ? Multiscale studies



> Heteroprotein complex coacervation : Molecular scale



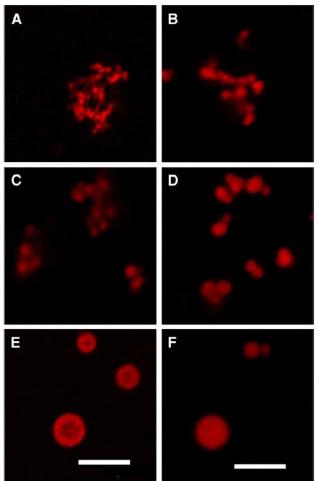
Croguennec et al., Adv. Coll. Int. Sci., **2017 Salvatore et al.** Biomacromolecules, **2011**



> 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 ($B \rightarrow E$).
- 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

 r
 Δ
 A
 B

 1μm
 2μm
 D

 0.5 μm
 2μm

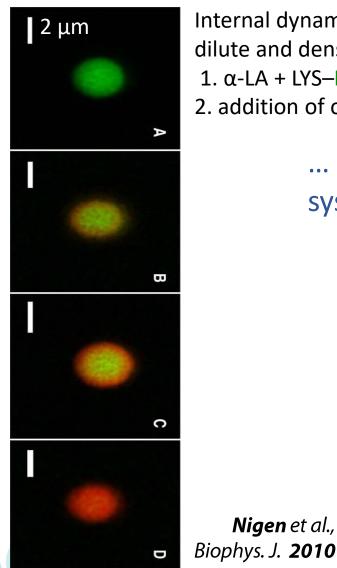
Temperature > 30°C

- **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



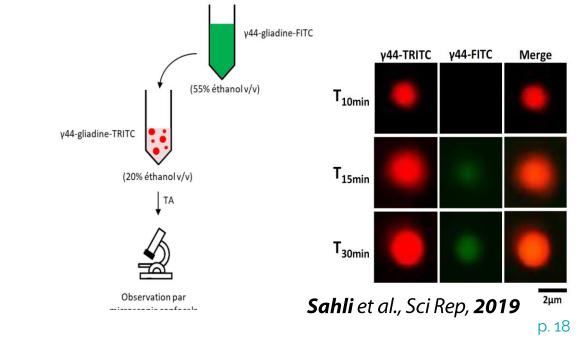
Internal dynamic of heteroprotein coacervation: exchange between dilute and dense phase.

1. α -LA + LYS-**FITC**;

Nigen et al.,

2. addition of of LYS-RBITC. Evolution from 0 to 75 min

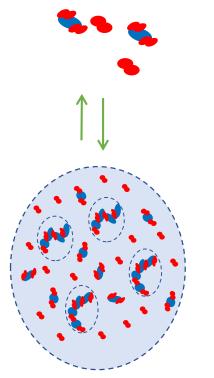
... Similar of what happens in mono-protein LLPS system: the case of γ - gliadin



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
- \rightarrow Evidences from FRAP, ¹H NMR and simulation

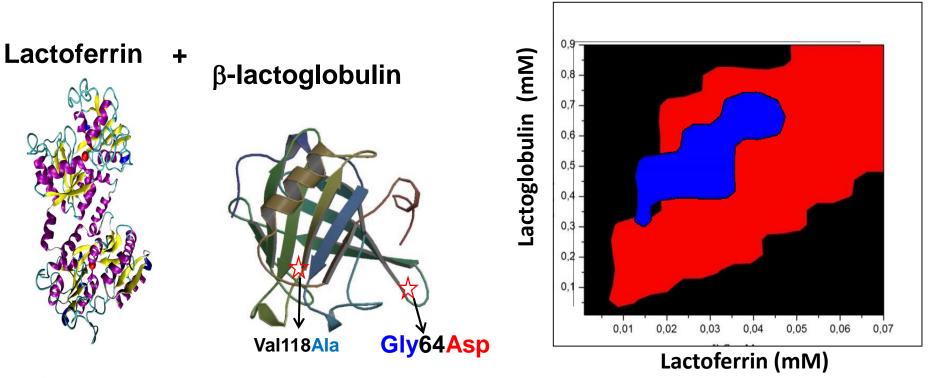




- Heterogeneity: coexistence of dynamic different species :
 - <mark>β-LG</mark>2 (5 nm),
 - LF(βLG₂)₂ (10-12 nm),
 - LF (β-LG₂)_n (30-40 nm)
- \bullet Change in the proportion of these structures could explain the variation of the $\beta\text{-LG/LF}$ molar ratio in the coacervate phase

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

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



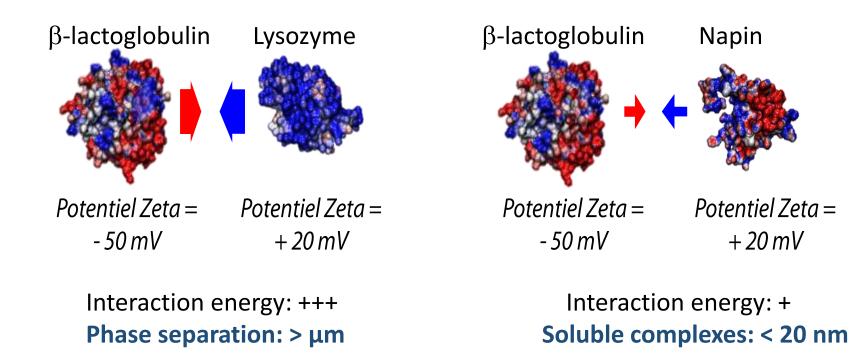
Coacervation domains of Lf and βLG isoforms

INRAe

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.

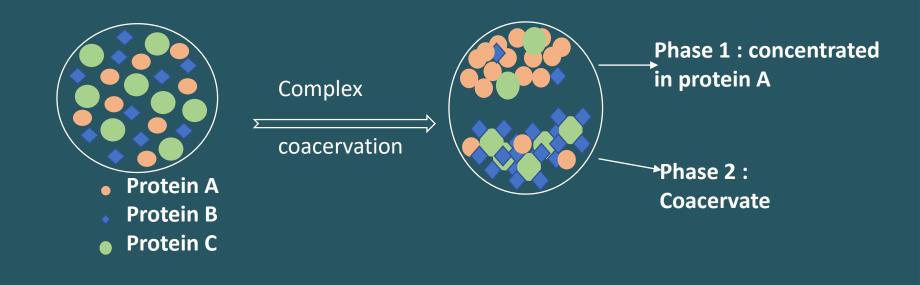


Ainis et al., Langmuir, 2019



> Heteroprotein complex coacervation : Applications

Protein purification

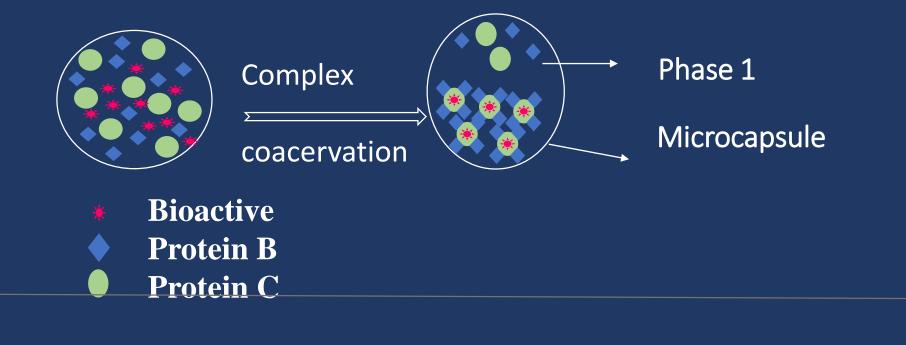


Shunuan et al., J. Agric. Food Chem., 2020



> Heteroprotein complex coacervation : Applications

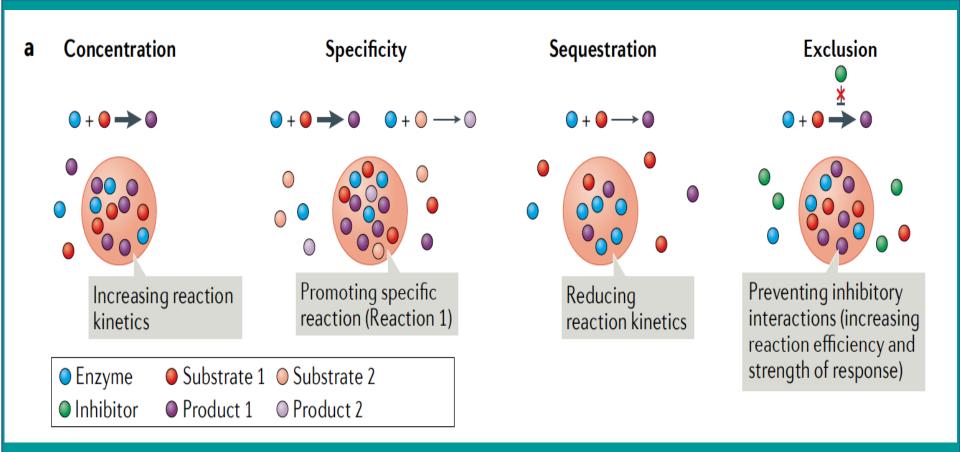
Protein purification Encapsulation : Vitamins, oils, drogues ...



Chapeau et al., Food Hydrocolloids, 2016



> Heteroprotein complex coacervation : Applications

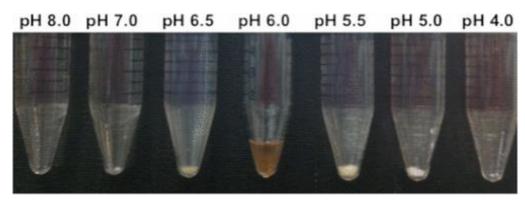


Lyon et al., Nature Reviews, 2020

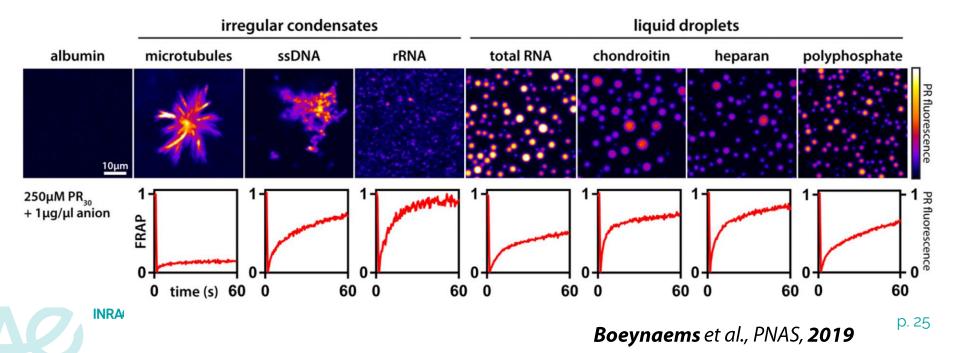


Enjeu majeur : Competition agregation & LLPS

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





