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Complex coacervation in heteroprotein systems: formation mechanism, rheology, and potential applications.

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Rousseau, Pascaline Hamon, Saïd Bouhallab

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➤ **Complex coacervation in heteroprotein systems: formation mechanism, rheology, and potential applications.**

Ghazi Ben Messaoud

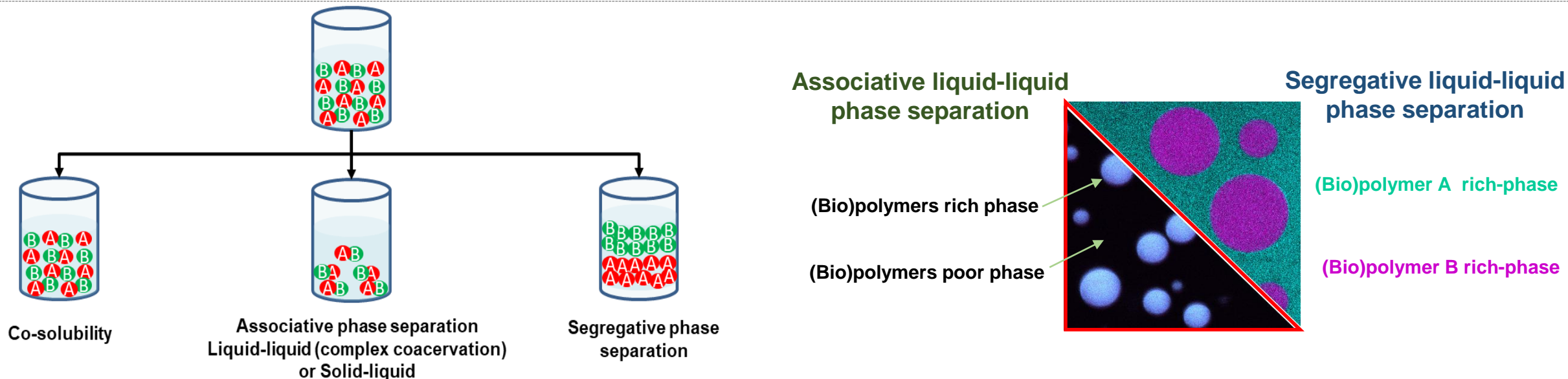
RBPGO10

Rennes 2024-06-25

> Introduction

- Food matrix: complex systems (Mixture of macro- and micro-nutriments)

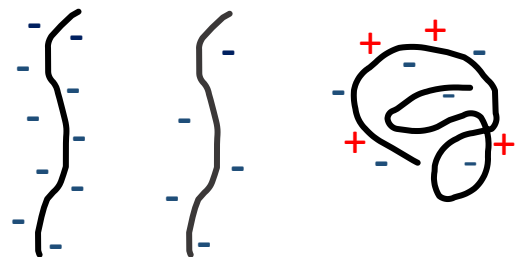
PHASE BEHAVIOR OF TERNARY SYSTEM: (BIO)POLYMER A / (BIO)POLYMER B / SOLVANT



COMPLEX COACERVATION

Polyelectrolytes

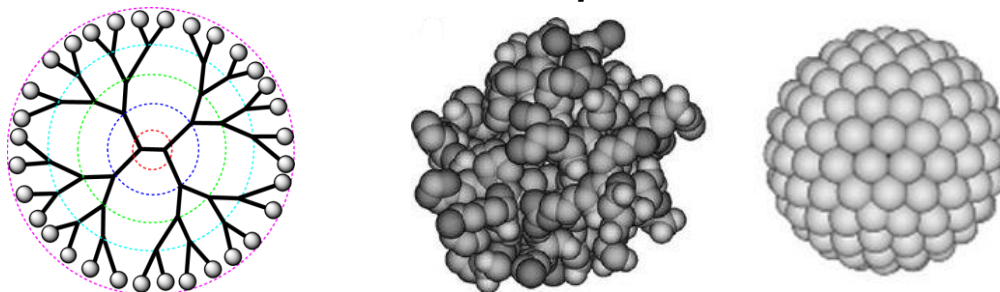
Strong **Weak** **Polyampholyte**



Schlenoff et al., *Macromolecules*, 2019

Colloids

Dendrimers **Globular proteins** **Micelles**



Kaup et al., *ACS Nano*, 2021

Croguennec et al., *Adv. Coll. Int. Sci.*, 2017

G. Ben Messaoud et al., *Green Chem*, 2018

Inorganic macroions

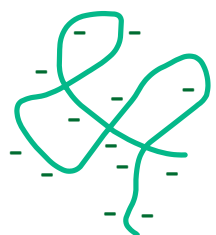


Polyoxometalate

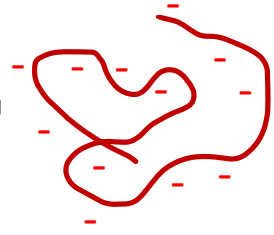
Jing et al., *Soft Matter*, 2017

Complex coacervation...a generic process

Anionic PEC



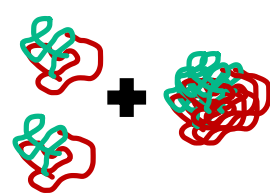
Cationic PEC



+



Primary units



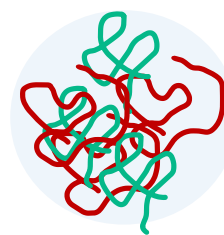
(ΔH driven)

Counterions release

(ΔS driven)



Coacervates



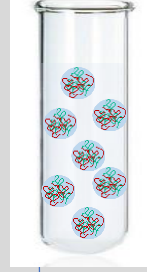
Main route

Coalescence



Stable

($\Delta\rho, \sigma$)



Gelation

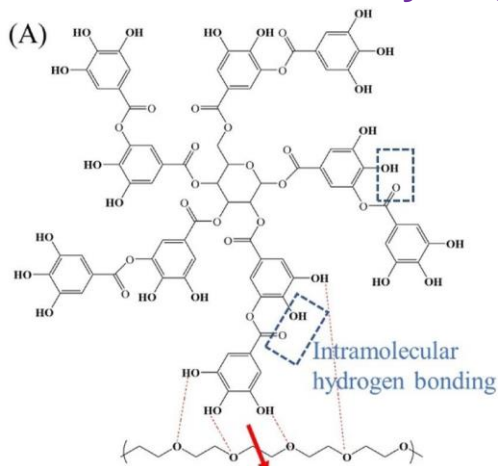


Alternative routes

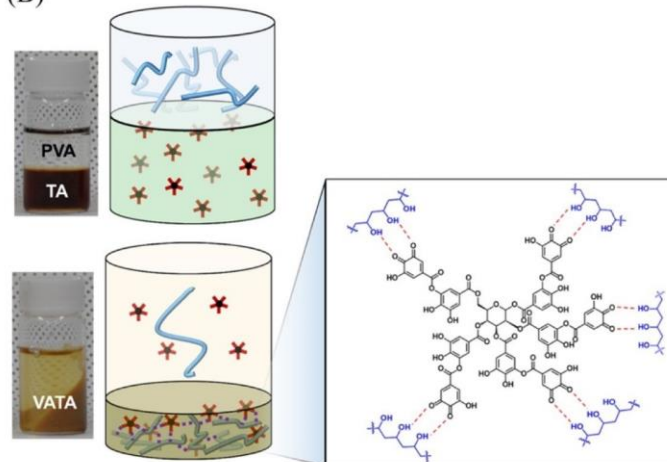
Electrostatic interactions

Often...but not only!

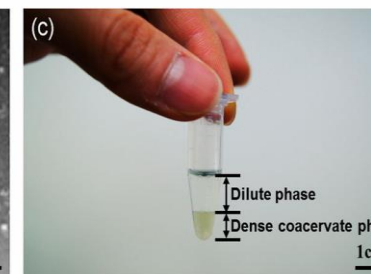
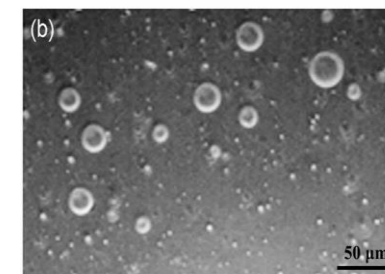
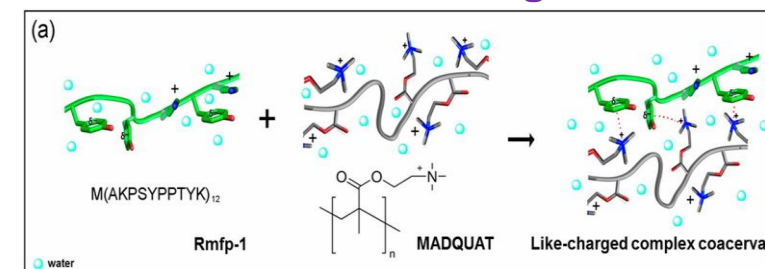
Hydrogen bonding



(B)



$\pi - \pi$ Stacking



Kim et al., *Adv Funct Mater*, 25 (2015)

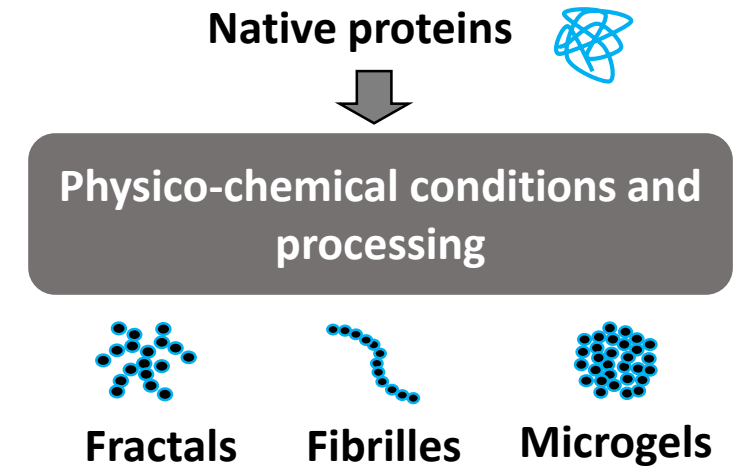
Lee et al., *ACS Appl Mater Interfaces*, 12 (2020)

Kim, et al., *PNAS*, 113(7), 2016.

➤ Heteroprotein Complex coacervation (HPCC) in food science

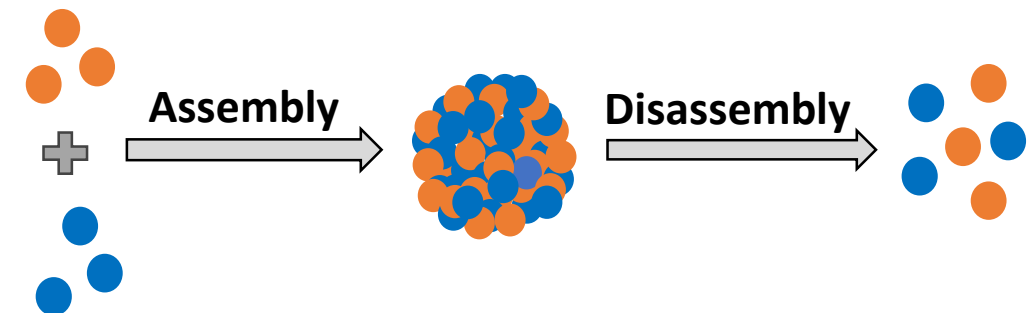
Food protein interactions & assemblies

- Formed after protein denaturation (heat, pressure, drying)
- Irreversible association reactions (non-covalent and covalent bounds)
- Inactive structures (biologically speaking) but relevant on a techno-functional point of view (Texturization, Stabilization of interfaces,..)

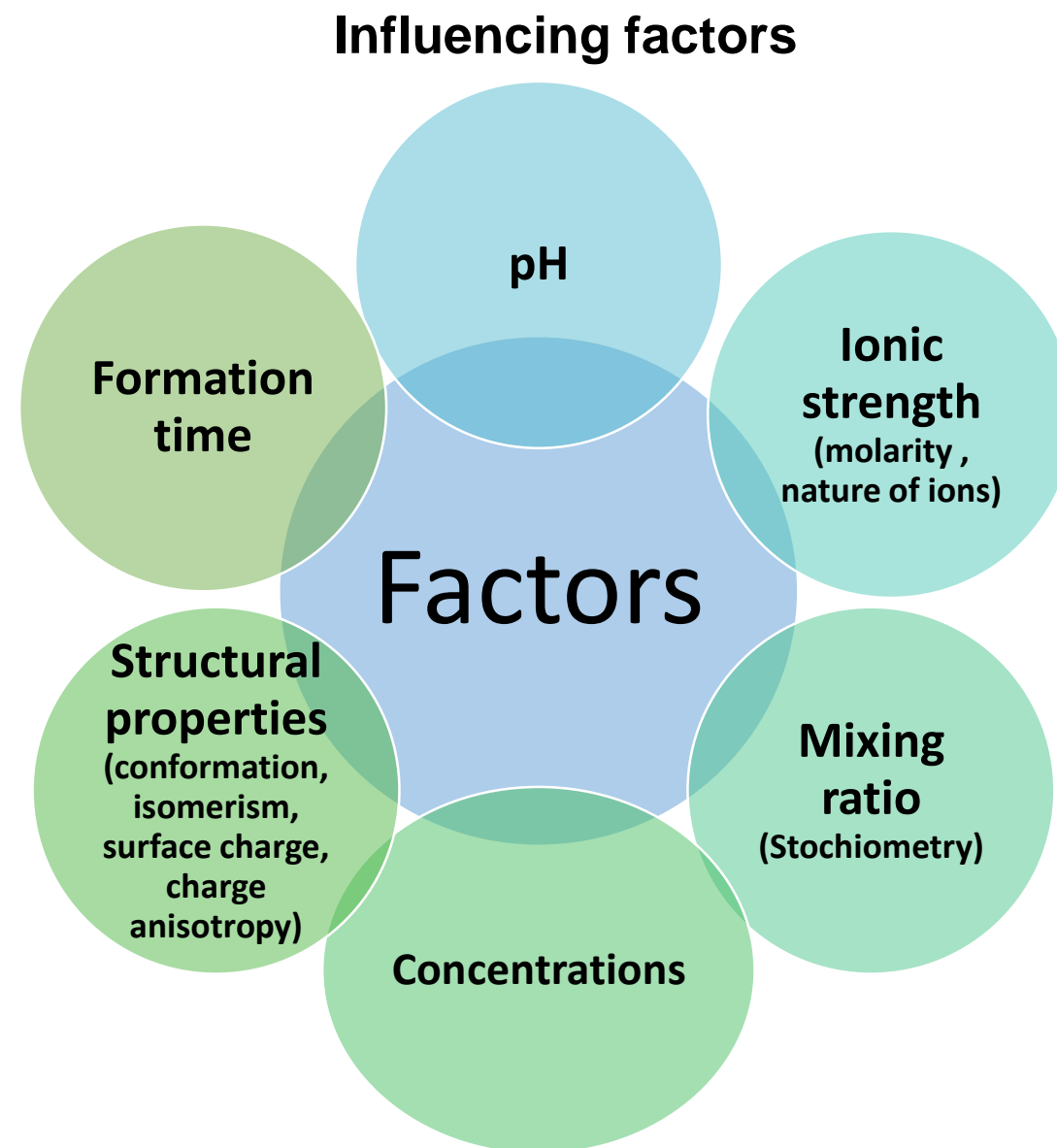
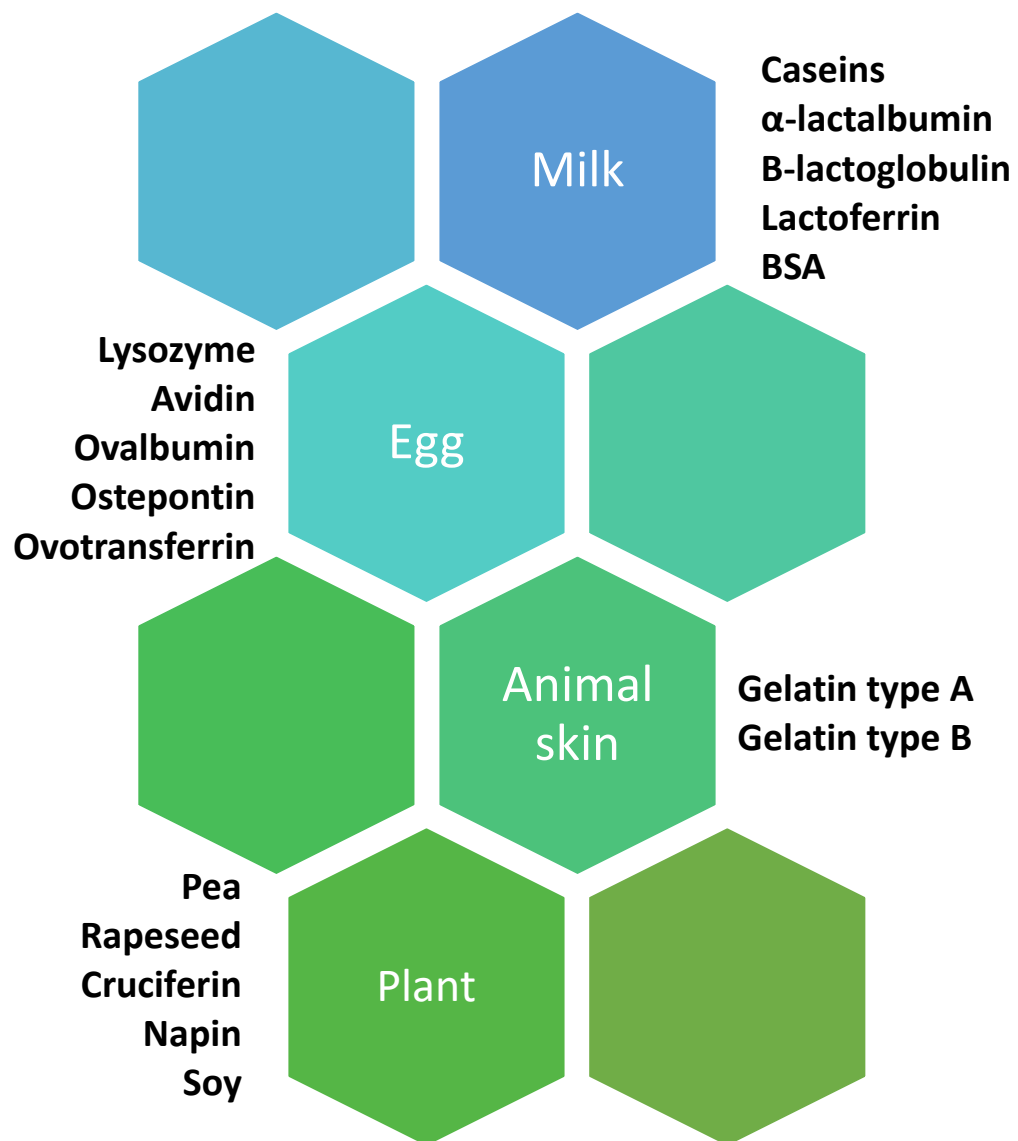


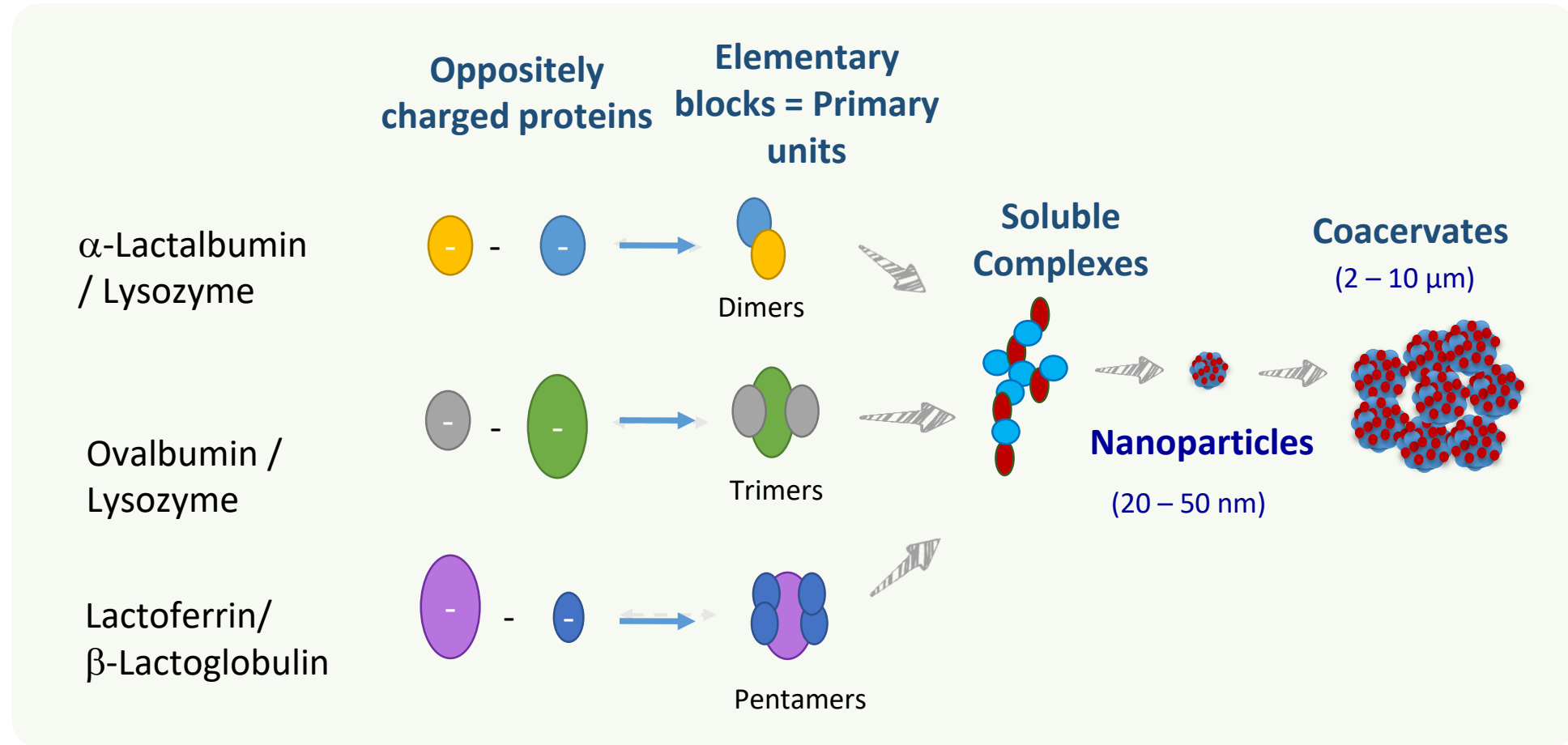
HPCC

- Self- or co-assembly under “mild” conditions:
- Reversible associations between proteins (non-covalent interactions)
- Flexible design (microscale, bulk, assembly-disassembly of food products, functional and nutritional properties)



➤ Studied food heteroprotein systems



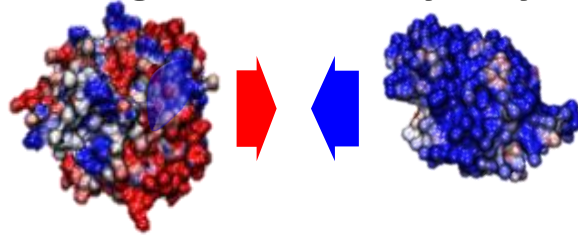


Croguennec et al., Adv. Coll. Int. Sci., 2017
Salvatore et al. Biomacromolecules, 2011
Dubin et al., Biomacromolecules, 2014

➤ Specificity over other macromolecular systems....Charge patchiness

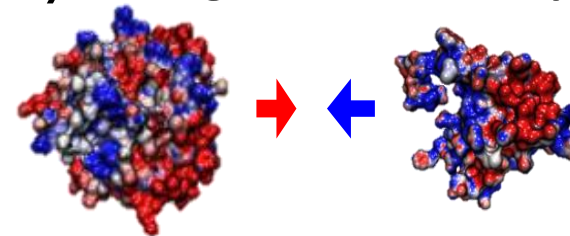
LYS and NAP with similar charge but:

β-lactoglobulin *Lysozyme*



Potentiel Zeta = -50 mV Potentiel Zeta = +20 mV
 Phase separation: > μm

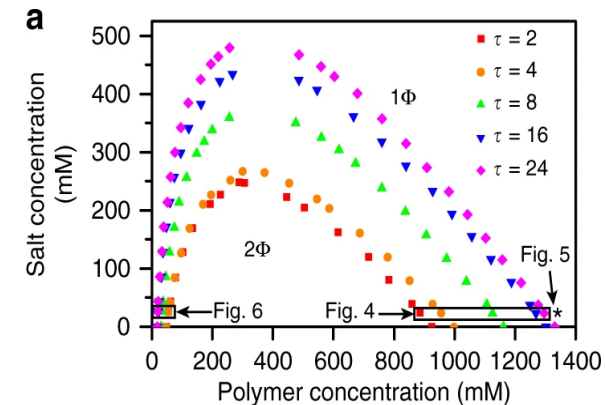
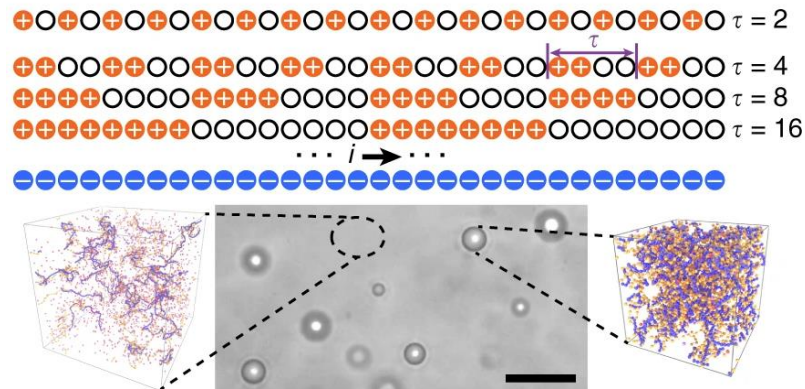
β-lactoglobulin *Napin*



Potentiel Zeta = -50 mV Potentiel Zeta = +20 mV
 Soluble complexes: < 20 nm

Ainis et al., Langmuir, 2019

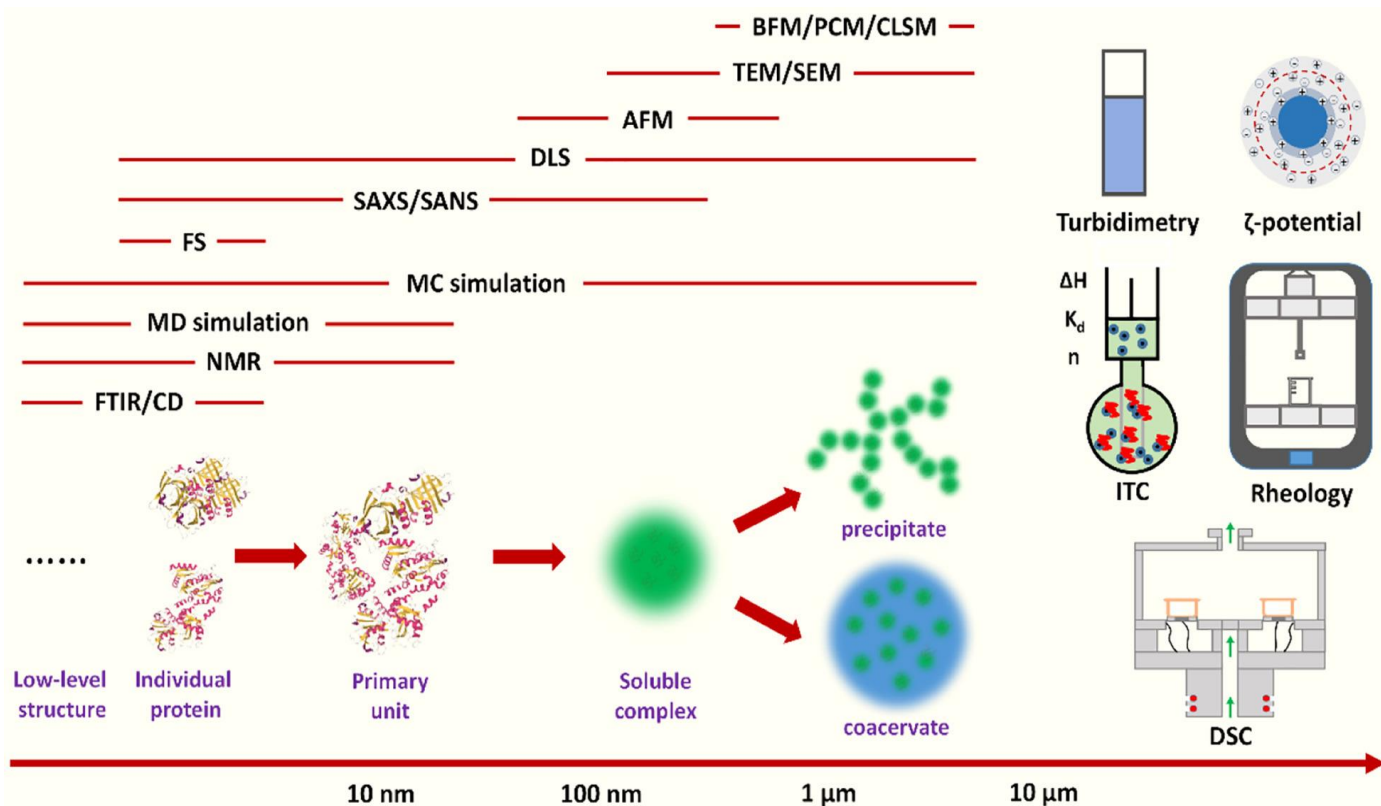
Specific to proteins... but in a good agreement with...



Chang et al., Nat Commun 8, 1273, 2017.

➤ Experimental techniques

Length Scale



Aspects

Formation

- Turbidimetry (Qualitative formation analysis)
- Dynamic light scattering (Particle size and polydispersity)
- Laser Doppler electrophoresis (Surface charge)
- Isothermal titration calorimetry (Thermodynamic driving force)
- Molecular dynamics simulation (Formation process and assembly structure)
- Molecular docking (Binding sites and assembly structure)

Structure

- Fourier transform infrared spectroscopy (Secondary structure, hydrogen bond, and electrostatic interaction)
- Far-UV circular dichroism (Secondary structure)
- Fluorescence spectrum (Molecular conformation)
- Differential scanning calorimetry (Molecular conformation and bonding water)
- Small angle x-ray scattering (Multiscale assembly structure)
- Small angle neutron scattering (Multiscale assembly structure and single component structure)
- Nuclear magnetic resonance (Interacting amino acid residues, hydrodynamic radius, and relative abundance)
- Scanning electron microscopy (Network structure)
- Transmission electron microscopy (Morphologic structure)
- Atomic force microscopy (Morphologic structure)
- Laser confocal scanning microscopy (Structural co-localization)

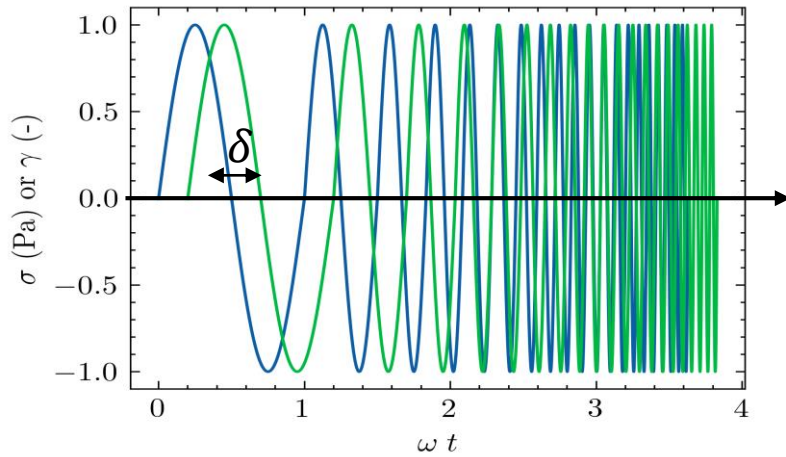
Properties

- Bright-field microscopy (Phase behavior)
- Phase-contrast microscopy (Phase behavior)
- Monte Carlo simulation (Assembly and phase boundary)
- Rheology (Viscoelasticity and macroscopic properties of interactions)

> Rheology



➤ Linear viscoelasticity (SAOS, SR...)



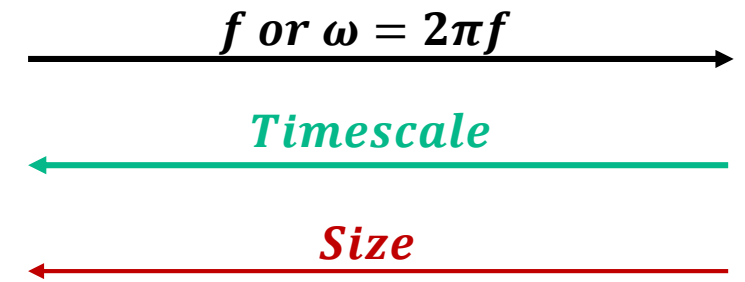
$$\gamma(t) = \gamma_0 \sin(\omega t)$$

$$\sigma(t) = \sigma_0 \sin(\omega t + \delta)$$

$$0^\circ < \delta < 90^\circ$$

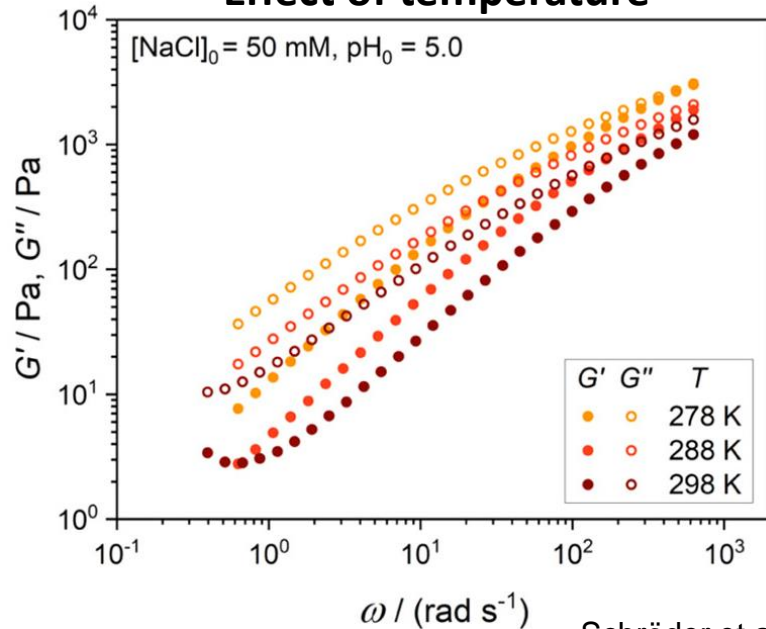
$$G' = \sigma_0 / \gamma_0 \cos(\delta)$$

$$G'' = \sigma_0 / \gamma_0 \sin(\delta)$$



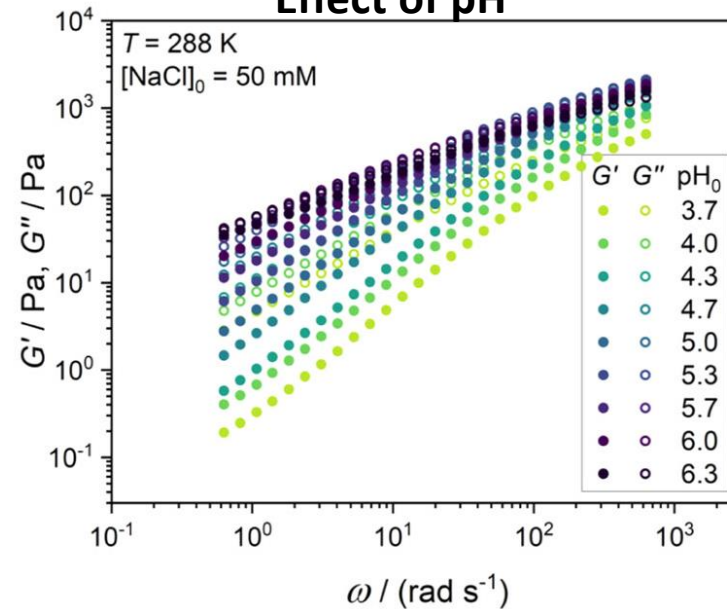
Chitosan-Gum Arabic

Effect of temperature



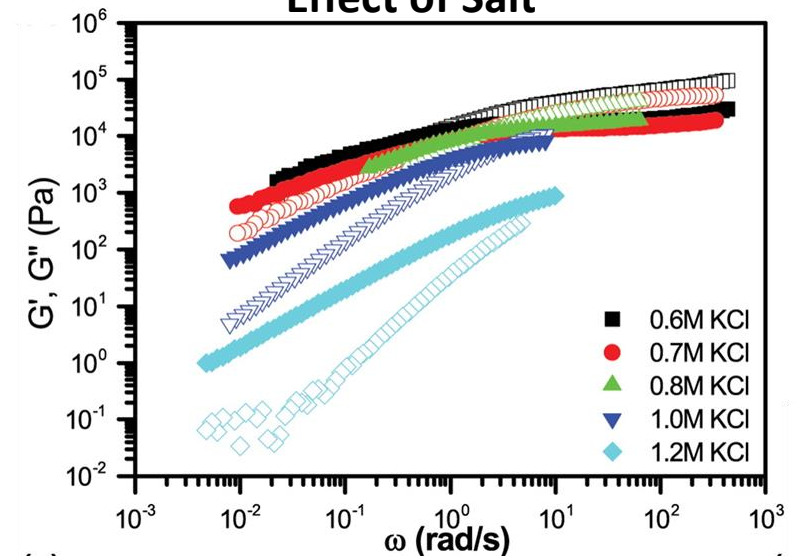
Schröder et al., *Macromolecules* 2023

Effect of pH

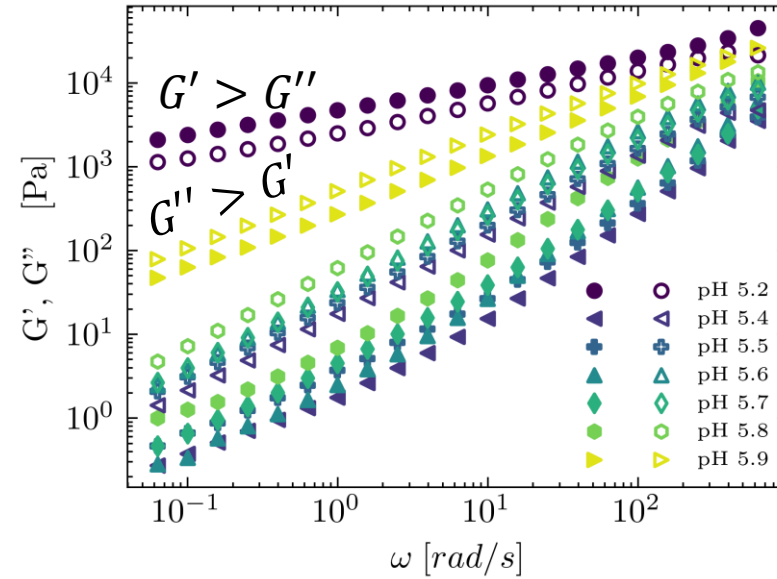
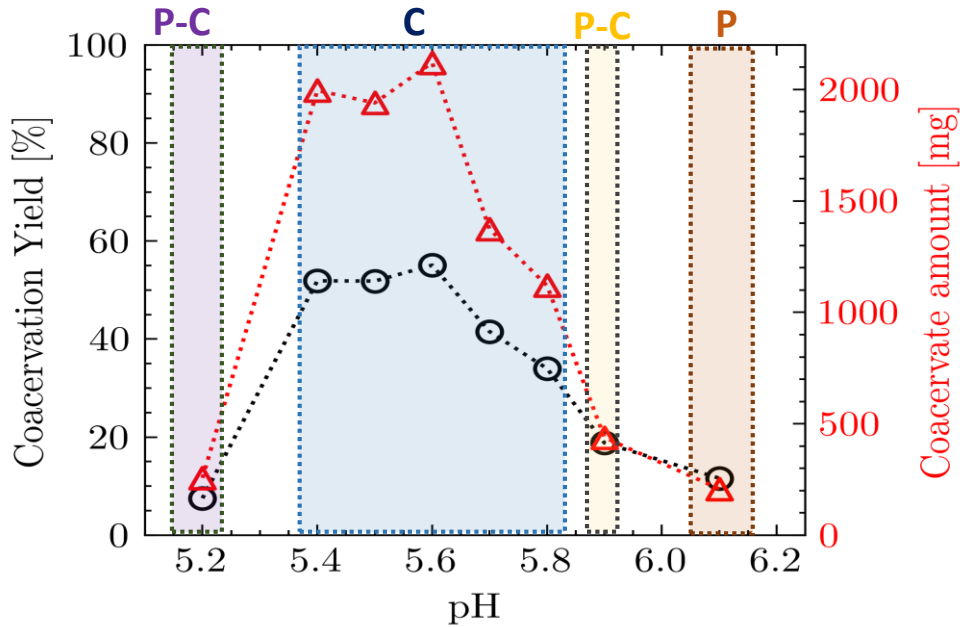
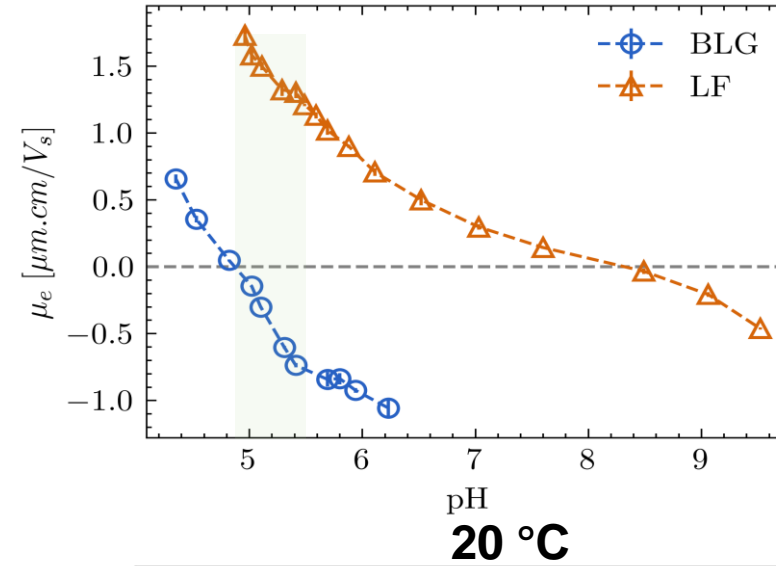
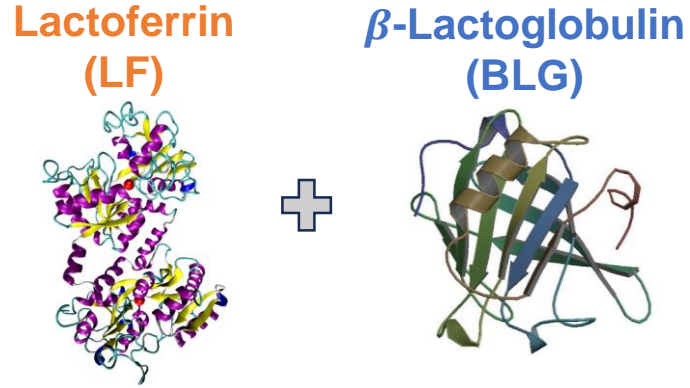


PDMAEMA - PAA

Effect of Salt

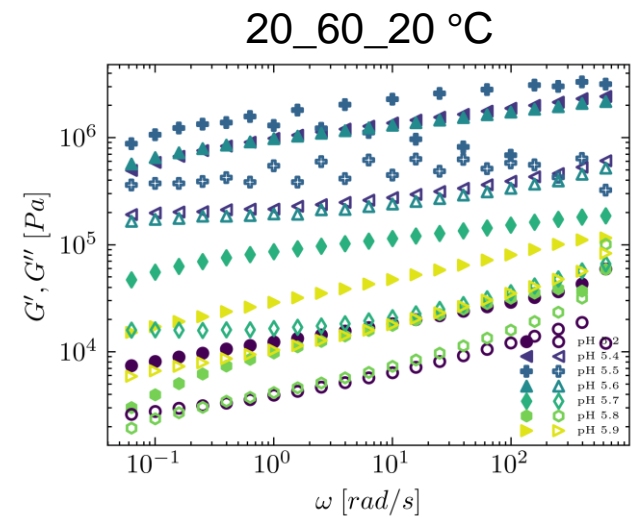
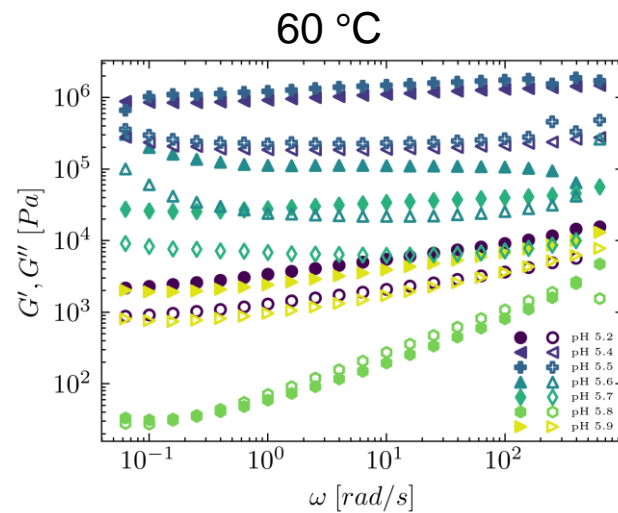
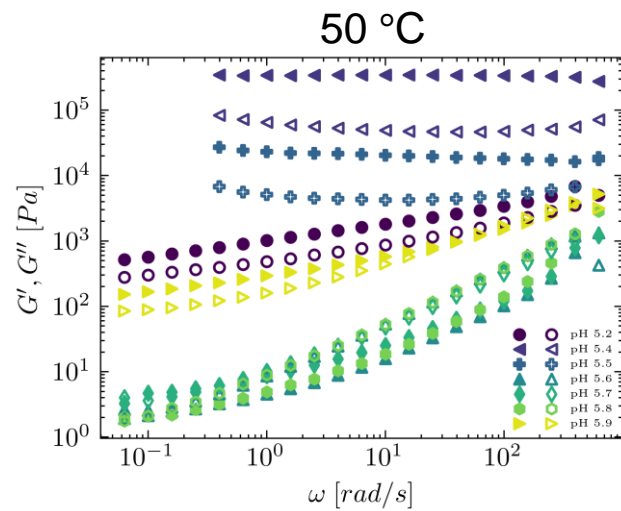
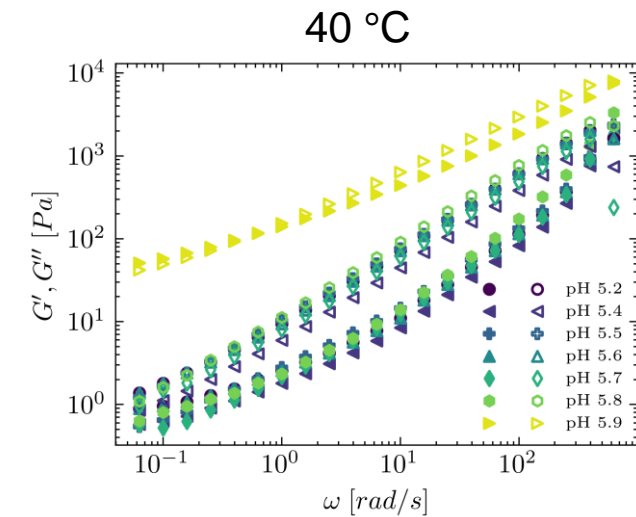
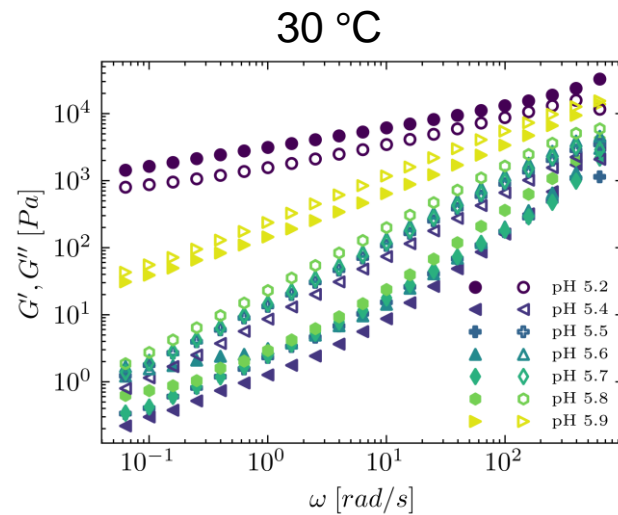
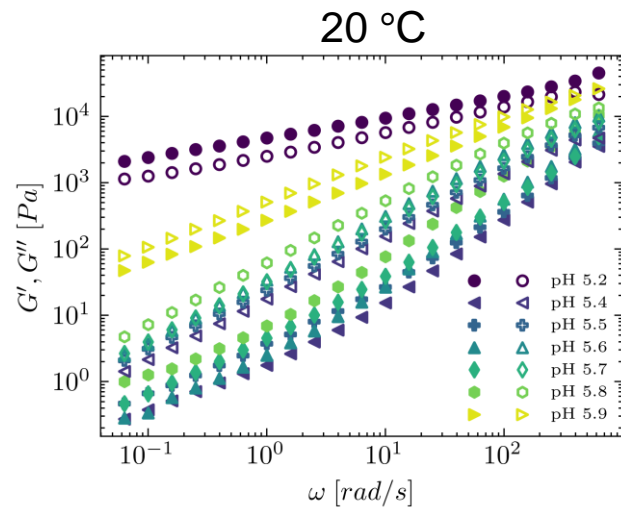


E. Spruijt, *Macromolecules*, 2013



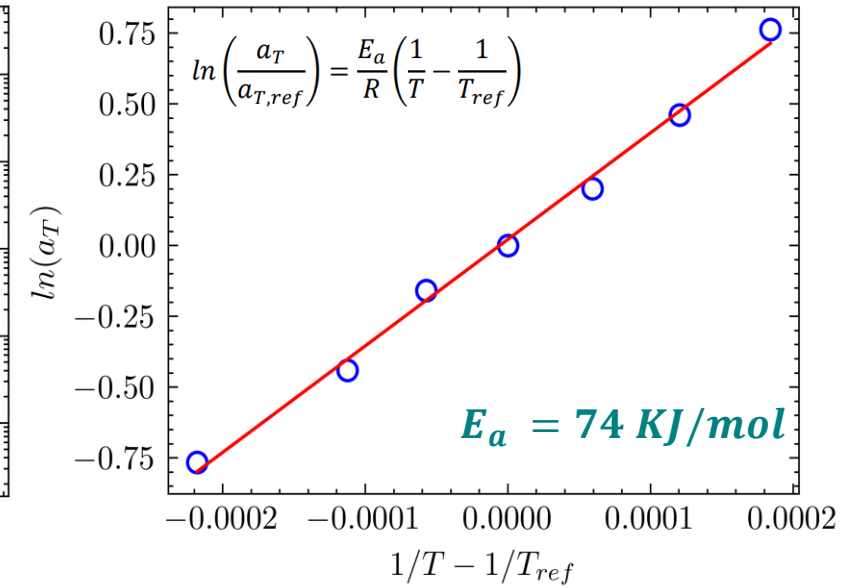
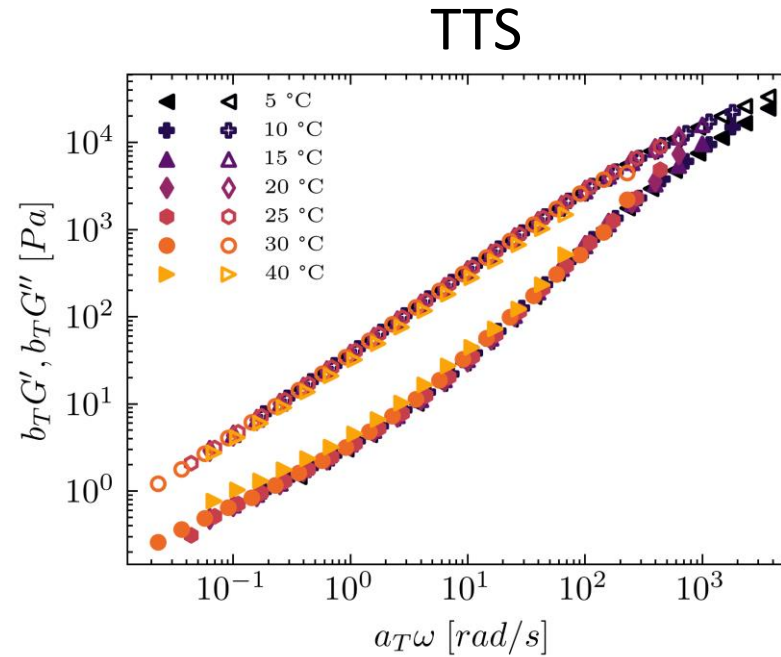
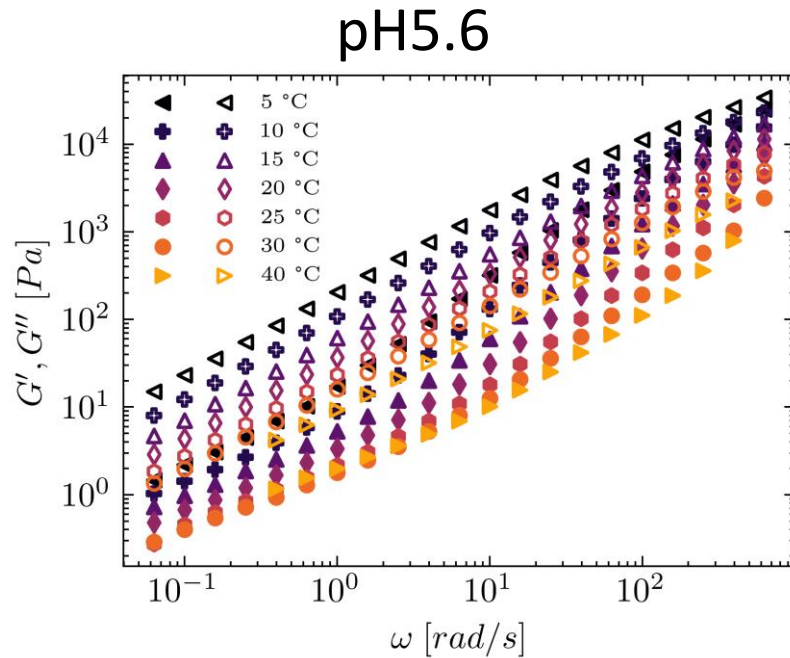
- High sensitivity of the system towards slight change of pH (0.1 unit).
- pH 5.2: Precipitate-coacervates: BLG precipitation close to the isoelectric point ($pH \sim 5$)
- pH 5.9 and pH 6.1: precipitation due to strong electrostatic interaction between BLG and LF

➤ Influence of temperature for different pH-values



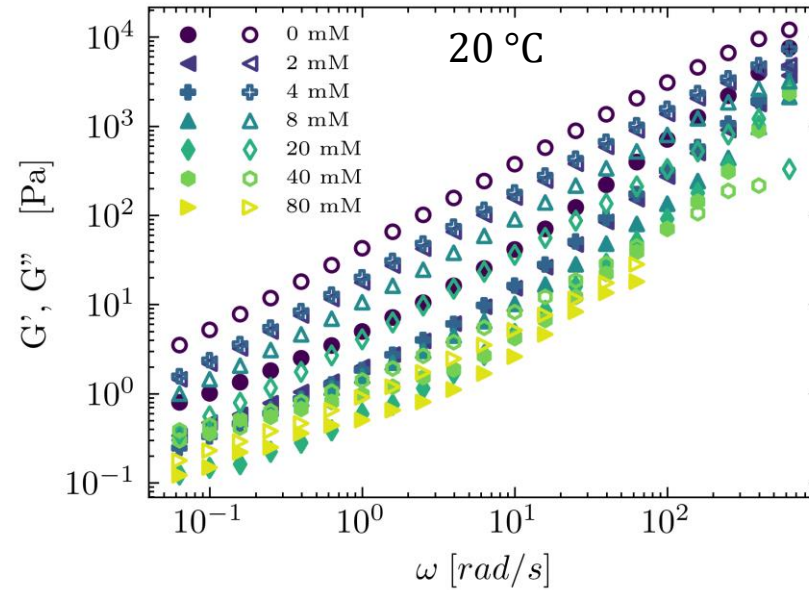
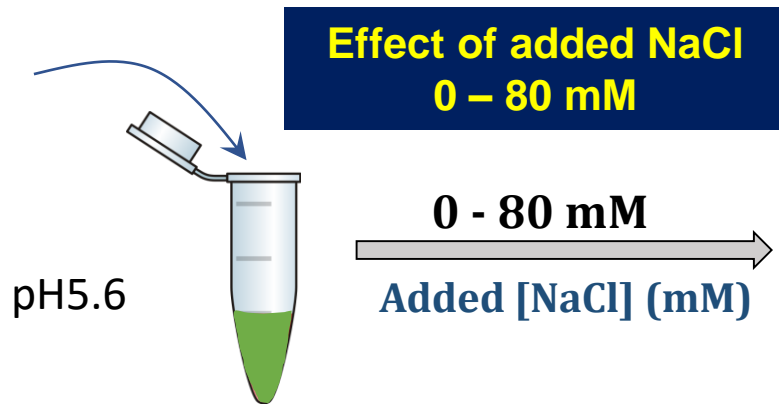
- Absence of thermal gelation at pH 5.2
- Thermal behavior: Softening from 20 to 40 (or 50 °C as a function of pH) followed by irreversible gelation at 60 °C (or 50 °C).
- Gelation of the coacervate phase lead to stiff materials (1 KPa < G' < 1 MPa)

Time temperature superposition

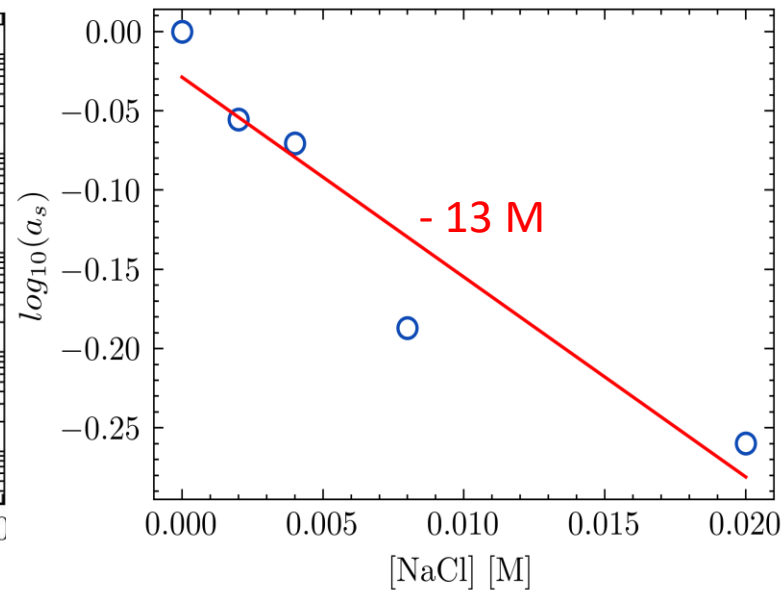
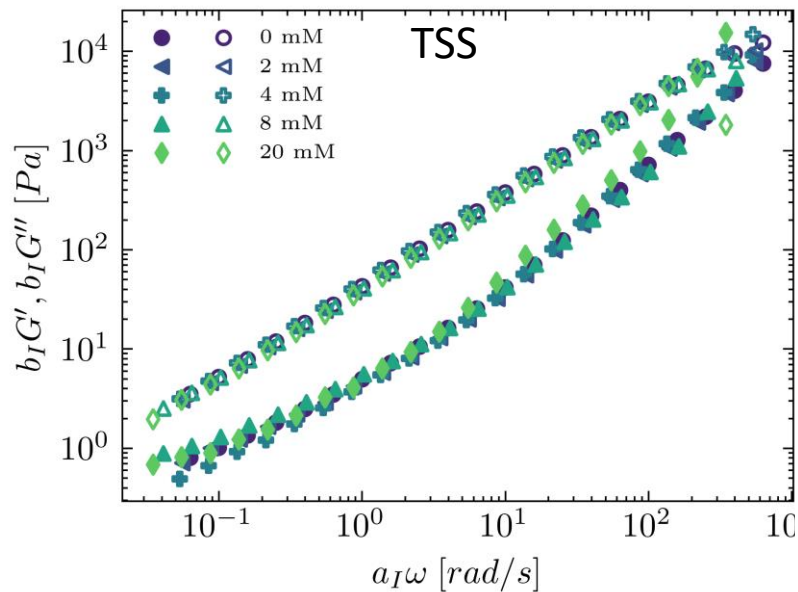


- Heating: accelerate the coacervates dynamics
- TTS principle applies in the limited range of T °C ($5 \leq T < 50$ °C):
 - Both proteins show the same T -dependent monomer friction
 - The dynamics of the coacervate is dominated by BLG/LF interactions
 - The dynamics of the two proteins are strongly coupled.

➤ Time salt superposition



➤ **Accelerates the coacervates dynamics of the system.**



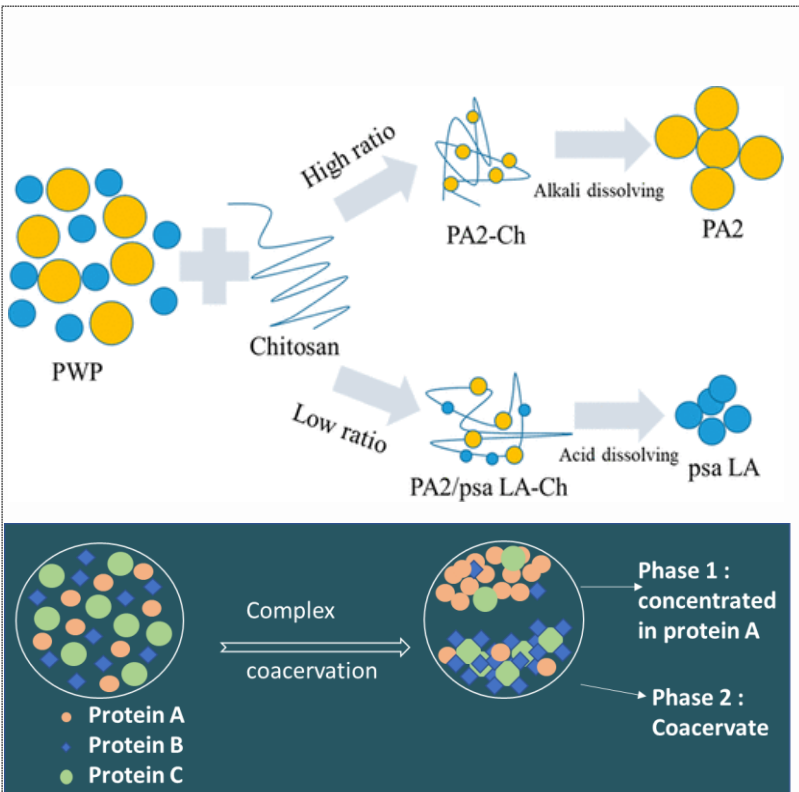
- Decrease of the number of intrinsic ion pairs
- Decrease of the energy of their dissociation (E_a)
- Reduced local friction

➤ Potential applications



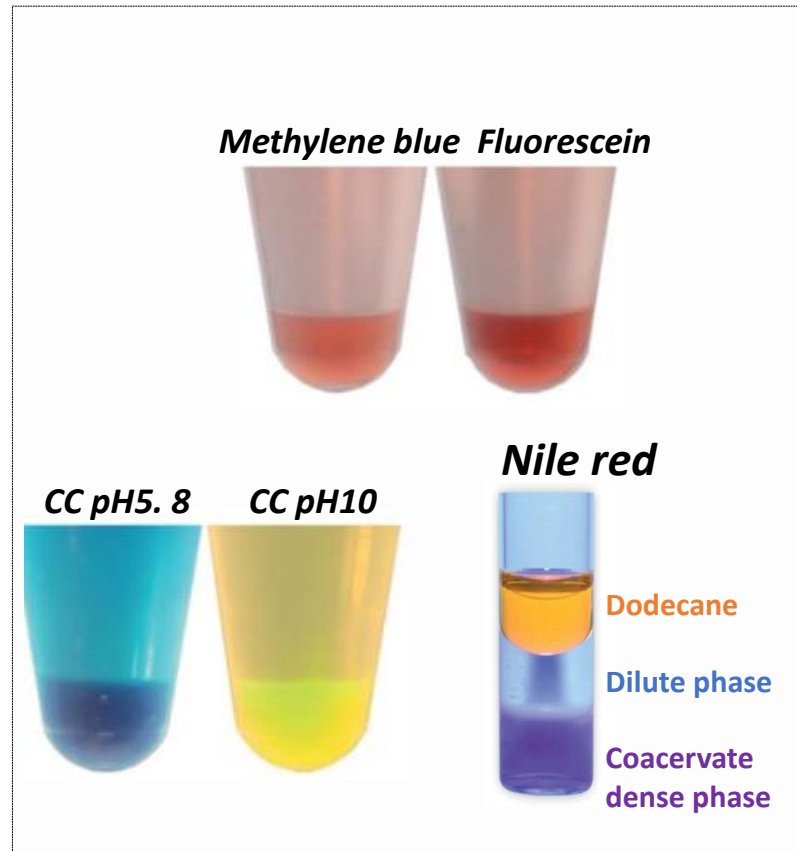
Purification & Encapsulation

Selective Complex Coacervation



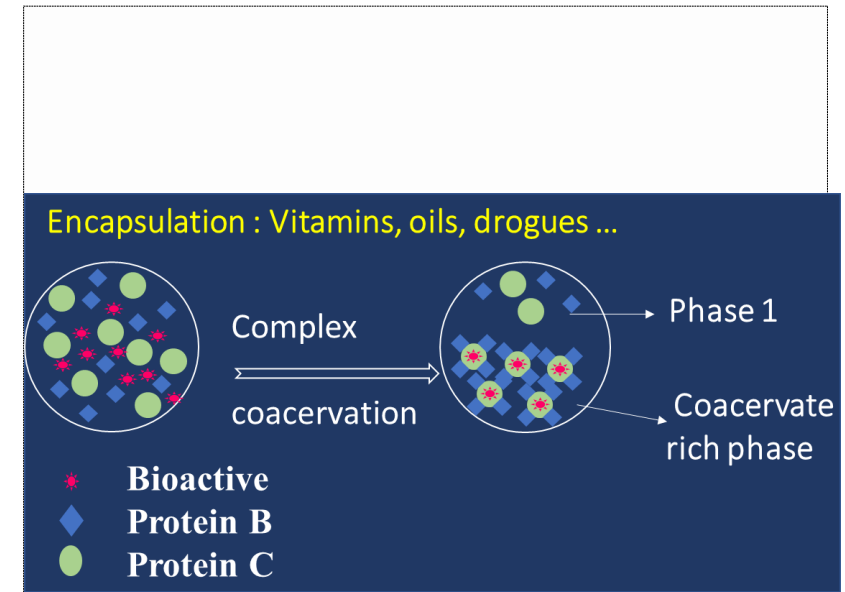
Yang et al., J. Agric. Food Chem. 2020

Sequestration properties (preferential partitioning)



Williams et al., Soft Matter, 2012

Weak attractive interactions

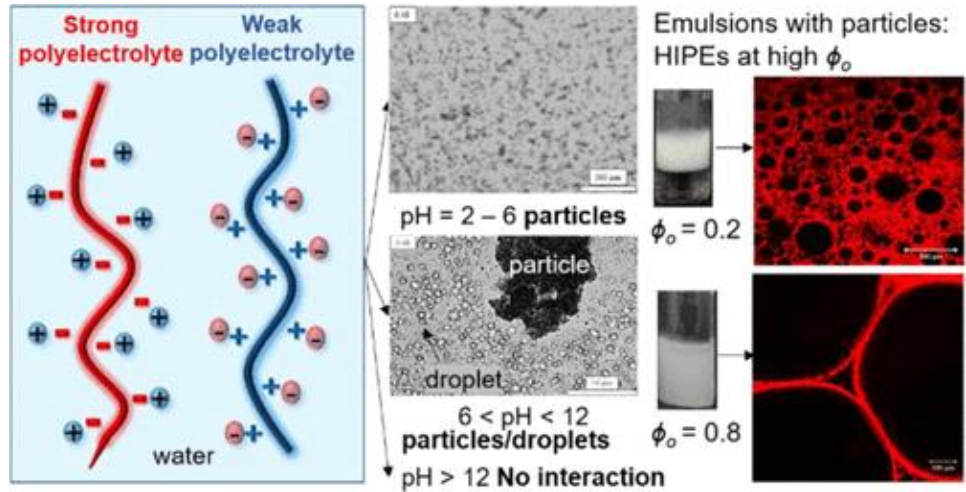


Chapeau et al., Food Hydrocolloids, 2016
McTigue et al., Soft Matter, 2019

III. Emulsion stabilization

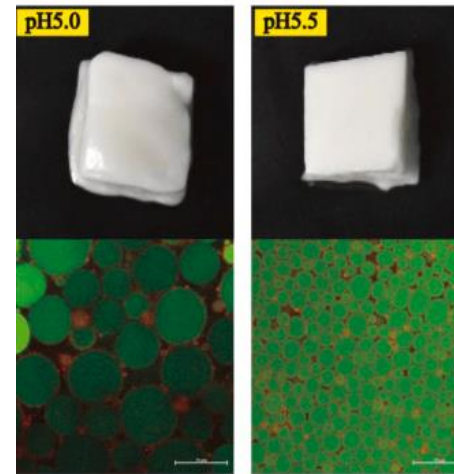
Up to date synthetic PEC/PEC or polysaccharide/proteins coacervates...

PSS / PAH



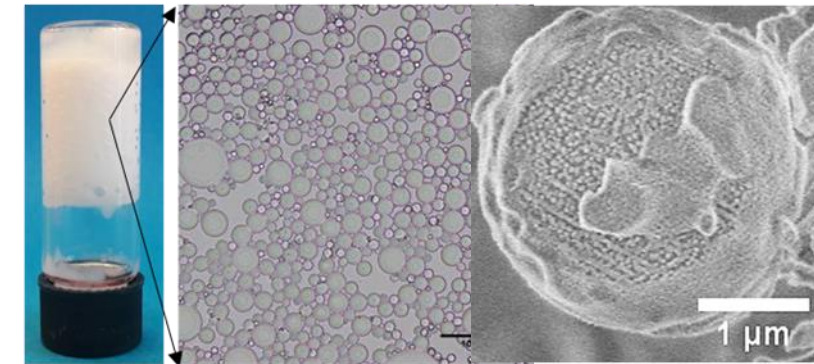
Bago et al., *Langmuir*, 2019

Soy protein isolate / κ -Carrageenan



Meng et al., *Food Hydrocolloids*, 2024

Biosurfactant / PEC



Laquerbe et al., *JCIS*, 2021

✓ Successful stabilization of oil/water emulsion and High internal phase emulsion (HIPE)

However...

Stabilization mechanism is still poorly understood: A pickering effect or a continuous layer around the droplets?

Evolution of coacervate structures from bulk solution to the interfaces: crowding at the interface, nature of oil/water interface?

➤ Conclusion

- Complex coacervation: Generic process
- Optimal conditions: specific for each mixture (Patchiness, Charge anisotropy...)
- “High” Sensitivity to: pH, Ionic strength, concentrations, stoichiometry
- Possible variability in the same system (isomerism, oligomerization)
- Rheology – structure relationship: still to be elucidated
- Promising applications in food industry (thickening agent, stabilizer...): Fundamental aspects still to be understood (stabilization mechanism of biphasic systems, preferential partitioning of active molecules,...)

Acknowledgements

S. Bouhallab



F. Rousseau



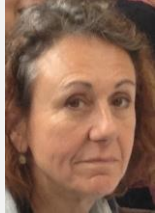
P. Hamon



M. Ganne



M.F. Famelart



R. Soussi

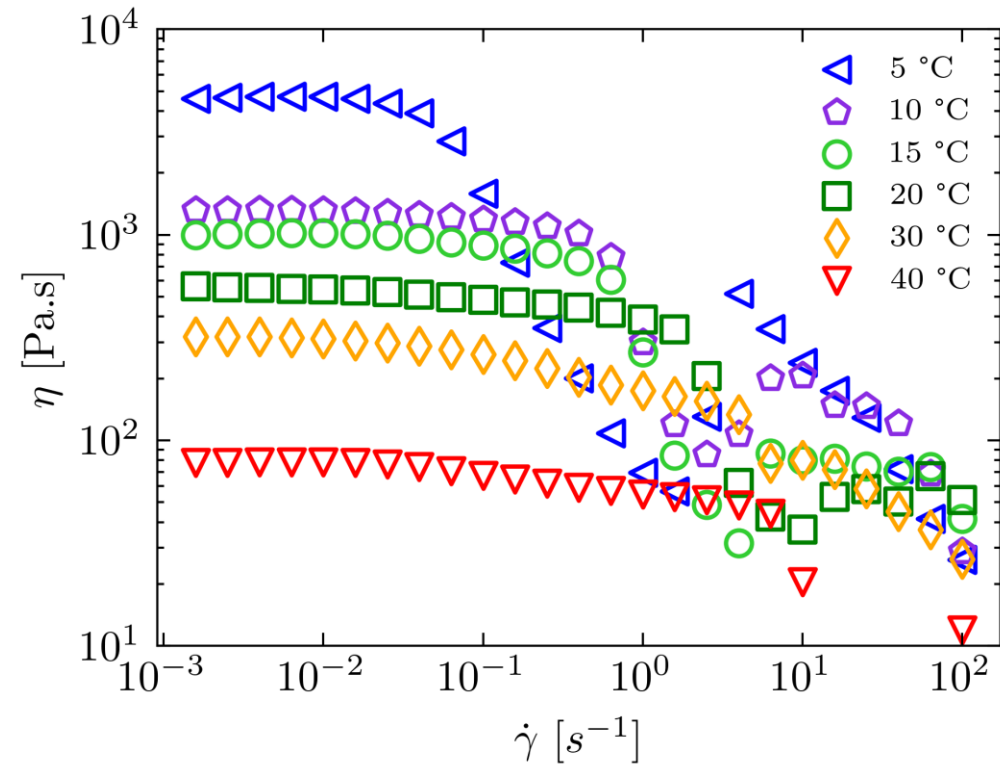


Thank you for your attention

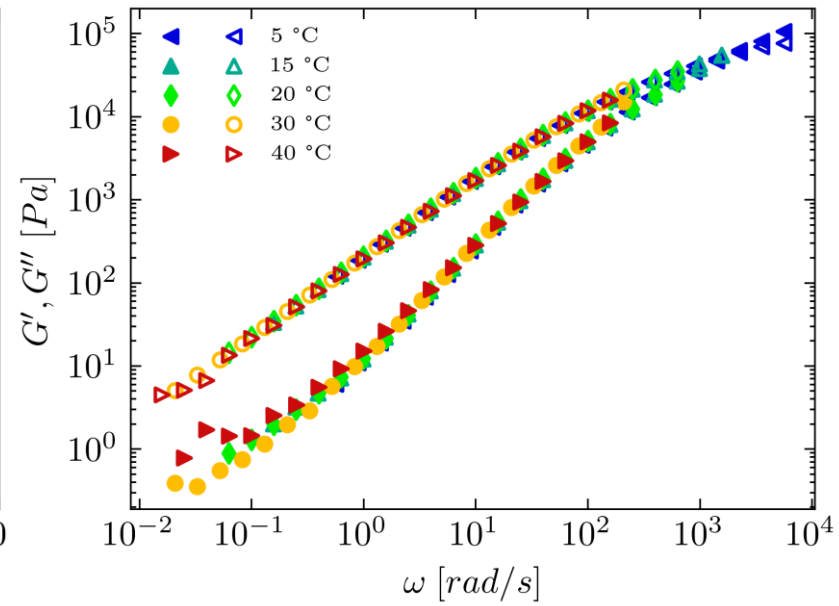
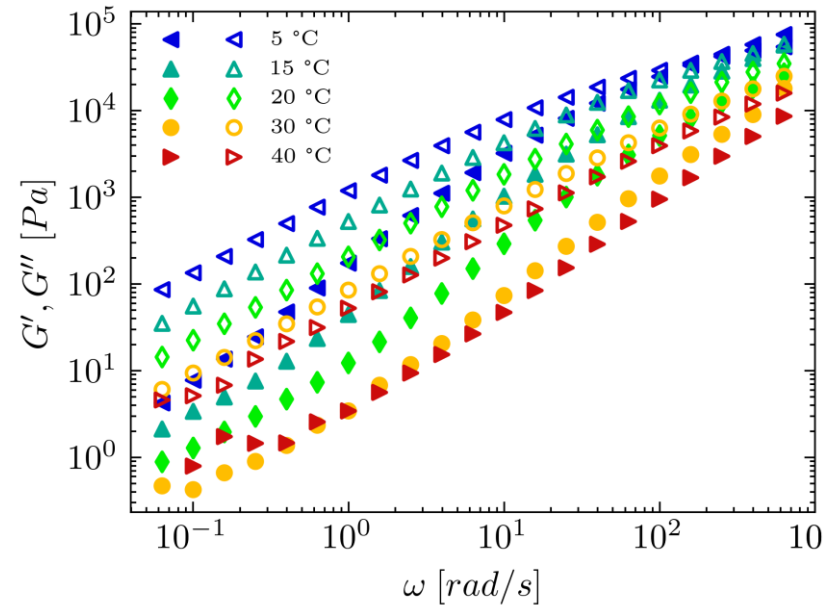


➤ Additional Slides

➤ New Sample Ghazi



➤ New sample Ghazi



➤ New sample Ghazi

