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

Ghazi Ben Messaoud

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

**Ghazi Ben Messaoud**

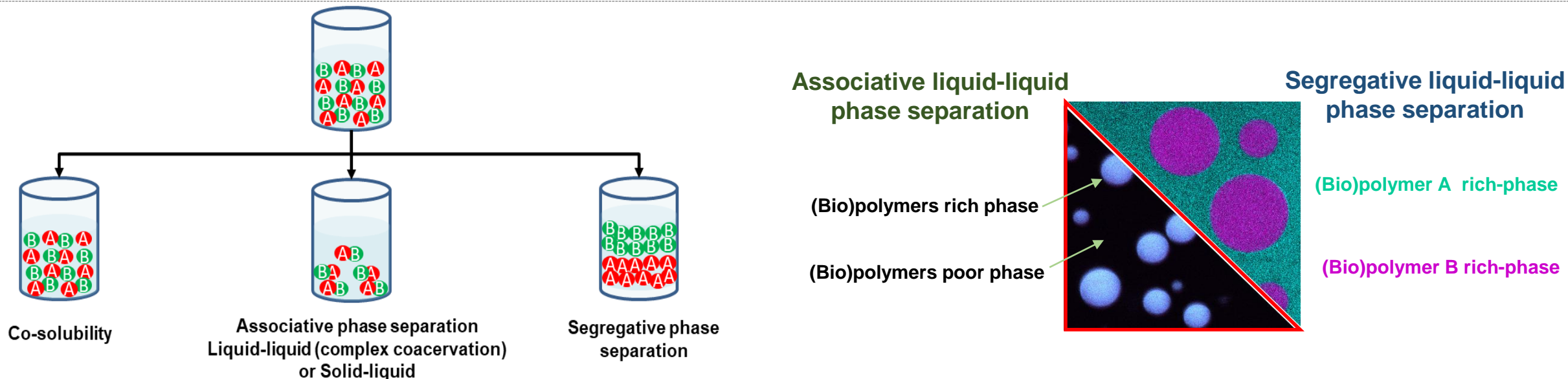
**RBPGO10**

Rennes 2024-06-25

# > Introduction

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

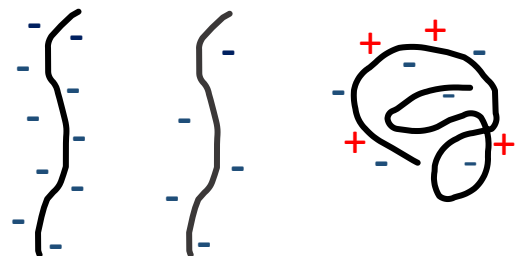
## PHASE BEHAVIOR OF TERNARY SYSTEM: (BIO)POLYMER A / (BIO)POLYMER B / SOLVANT



## COMPLEX COACERVATION

### Polyelectrolytes

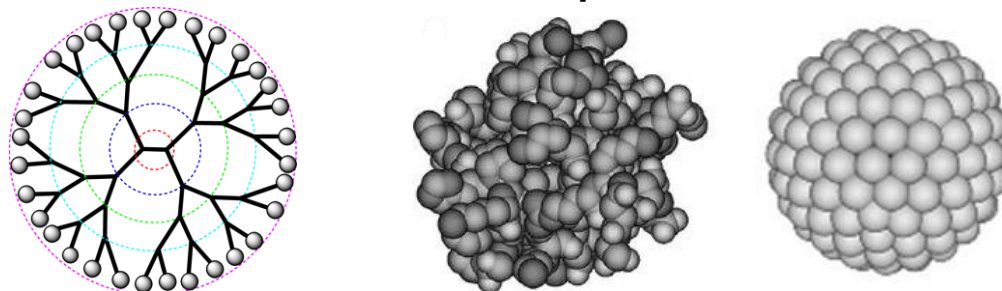
**Strong**   **Weak**   **Polyampholyte**



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

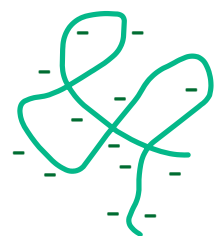


Polyoxometalate

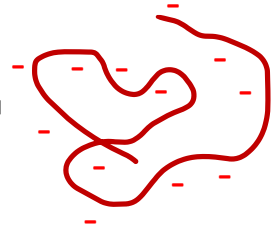
Jing et al., *Soft Matter*, 2017

# Complex coacervation...a generic process

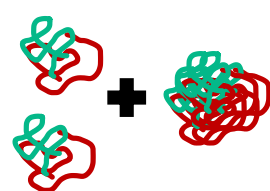
Anionic PEC



Cationic PEC



Primary units



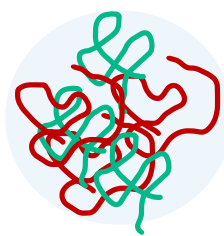
( $\Delta H$  driven)

Electrostatic interactions

Counterions release

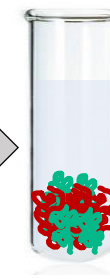
( $\Delta S$  driven)

Coacervates



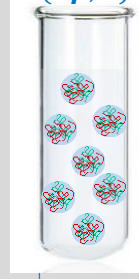
Main route

Coalescence

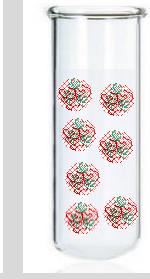


Stable

( $\Delta\rho, \sigma$ )



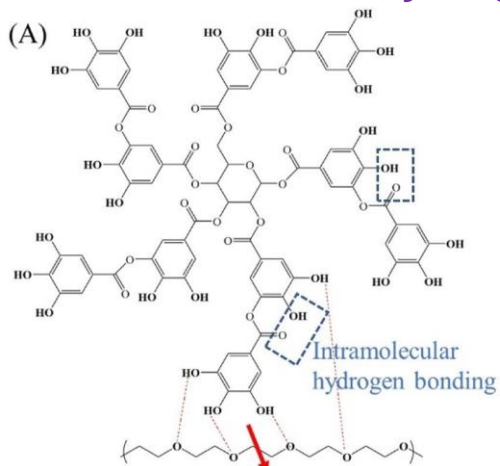
Gelation



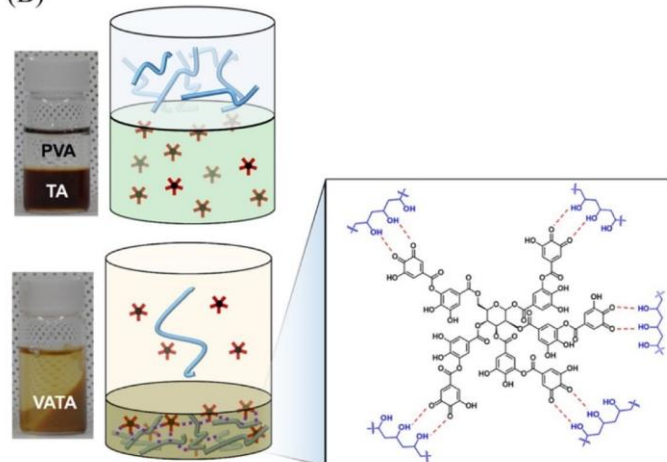
Alternative routes

Often...but not only!

## Hydrogen bonding



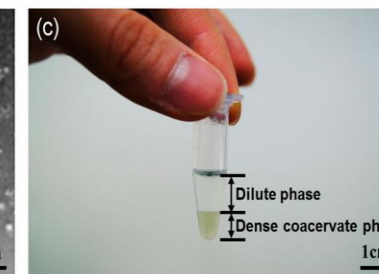
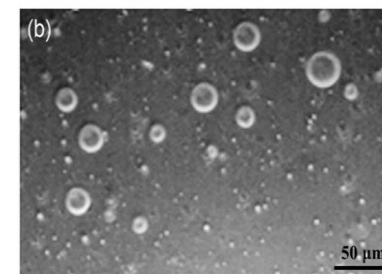
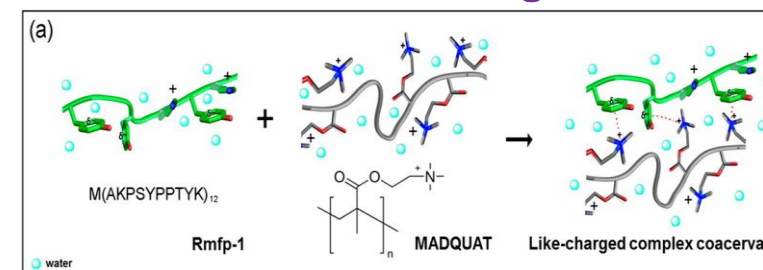
(B)



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

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

## $\pi - \pi$ Stacking

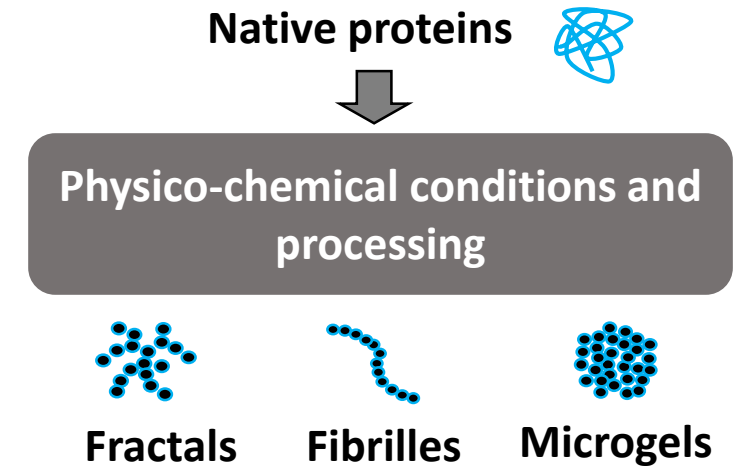


Kim, et al., *PNAS*, 113(7), 2016.

# ➤ Heteroprotein Complex coacervation (HPCC) in food science

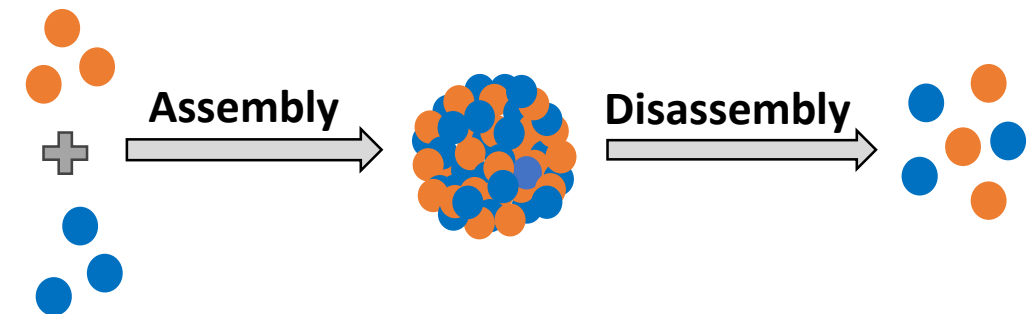
## Food protein interactions & assemblies

- 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,..)

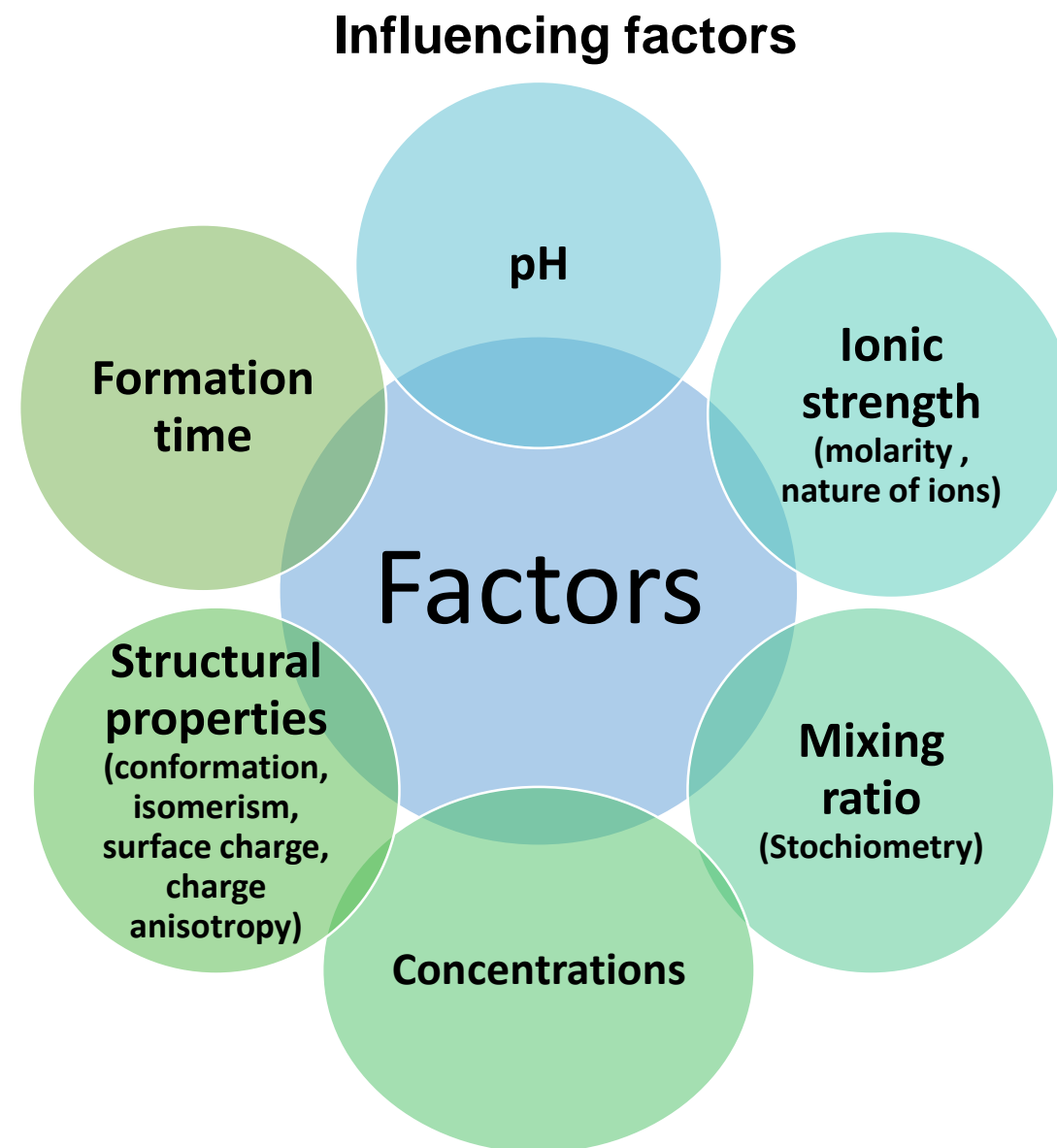
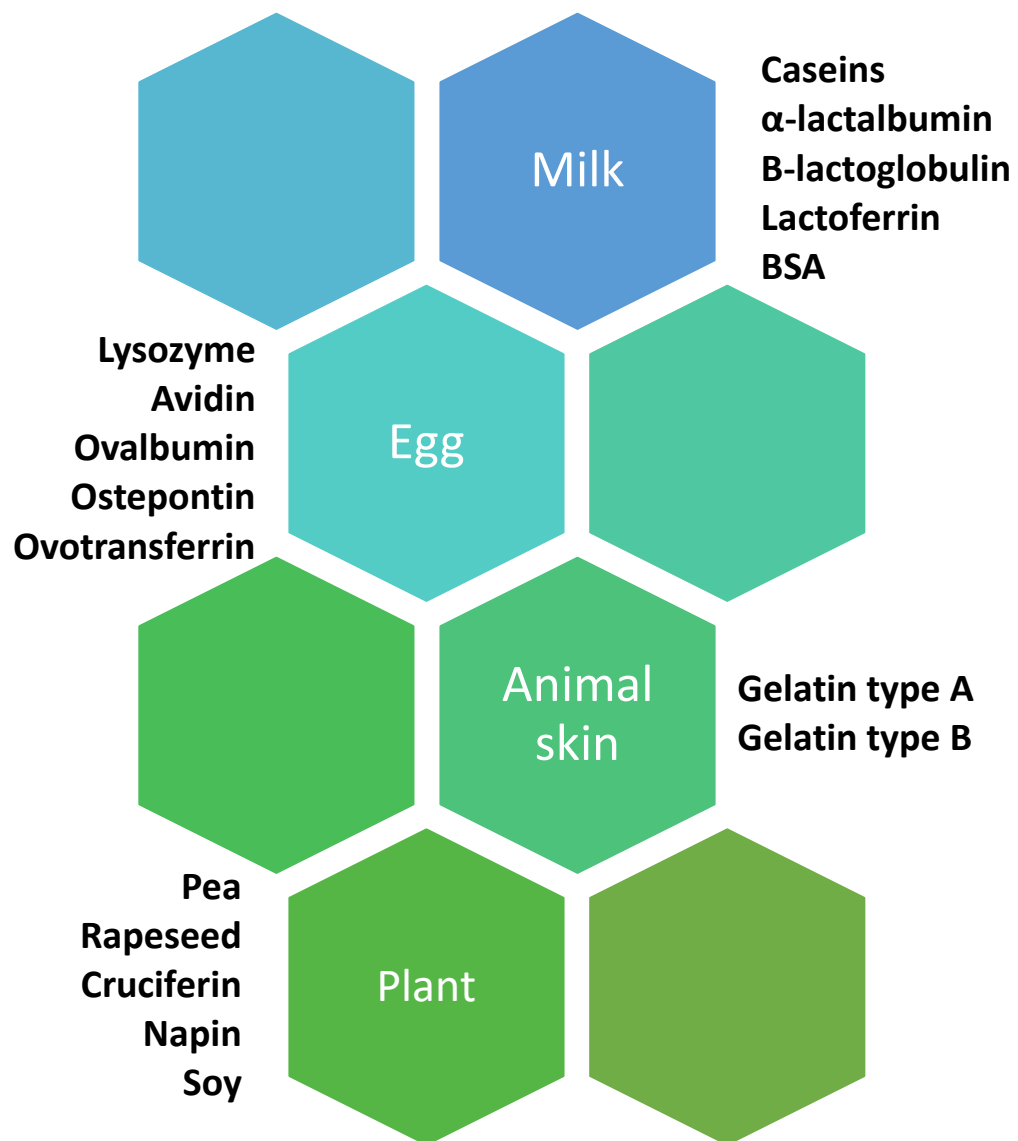


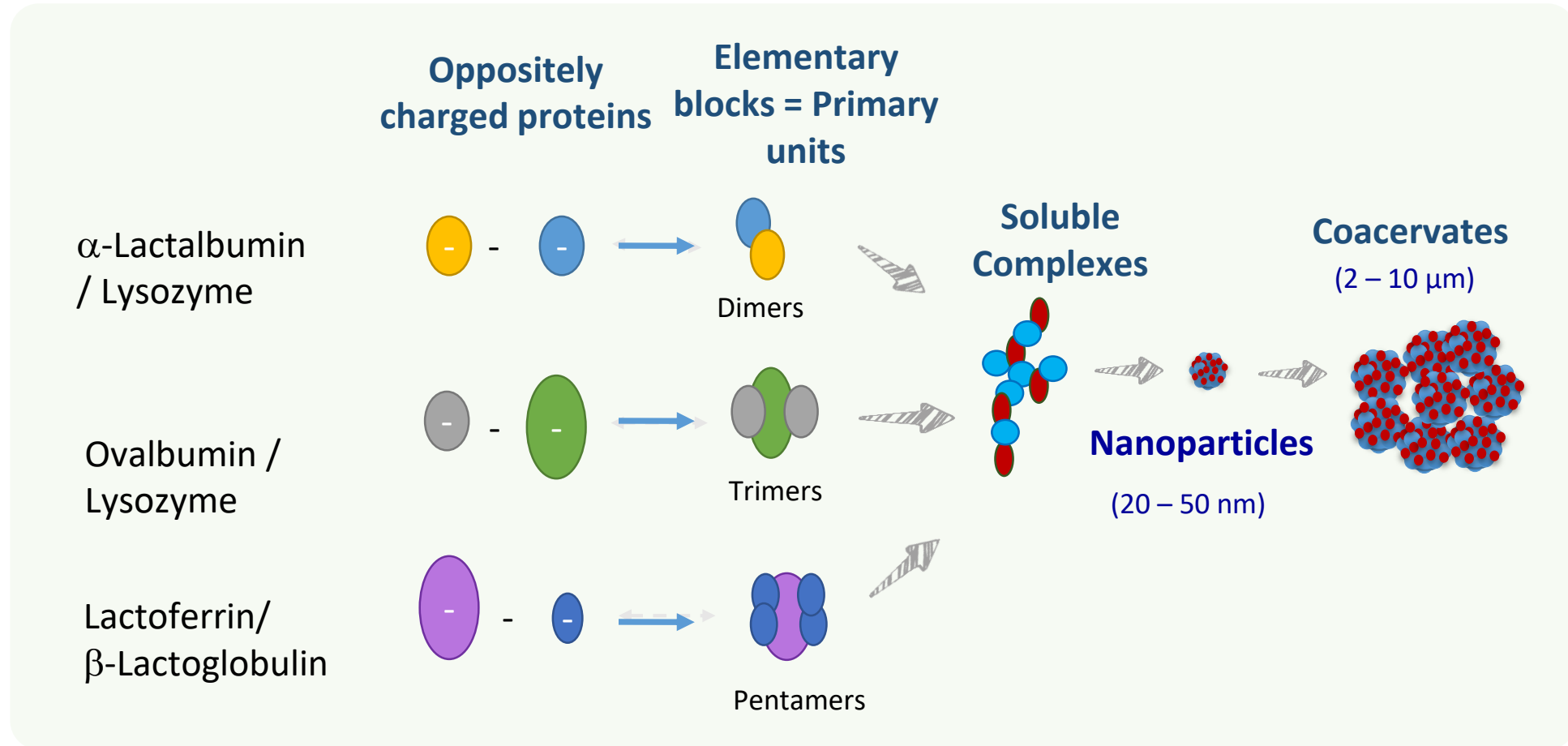
## HPCC

- 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



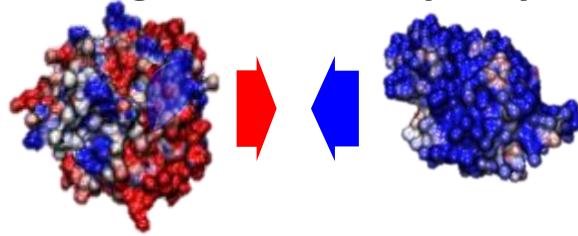


*Croguennec et al., Adv. Coll. Int. Sci., 2017*  
*Salvatore et al. Biomacromolecules, 2011*  
*Dubin et al., Biomacromolecules, 2014*

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

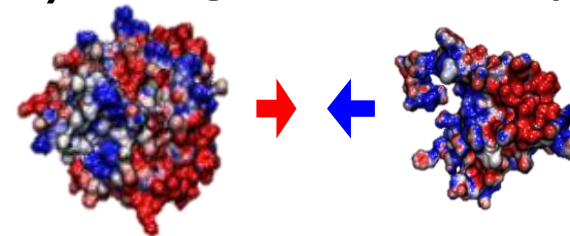
LYS and NAP with similar charge but:

***β-lactoglobulin***      ***Lysozyme***



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

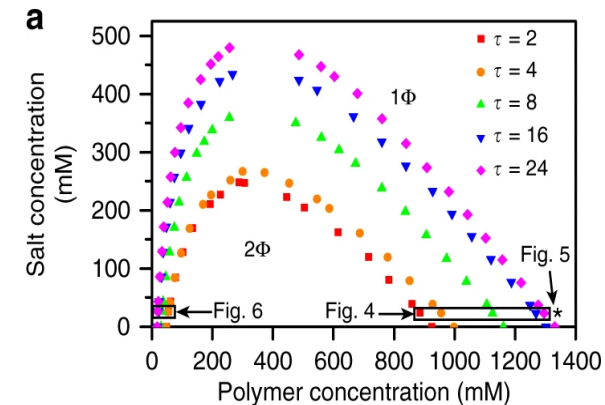
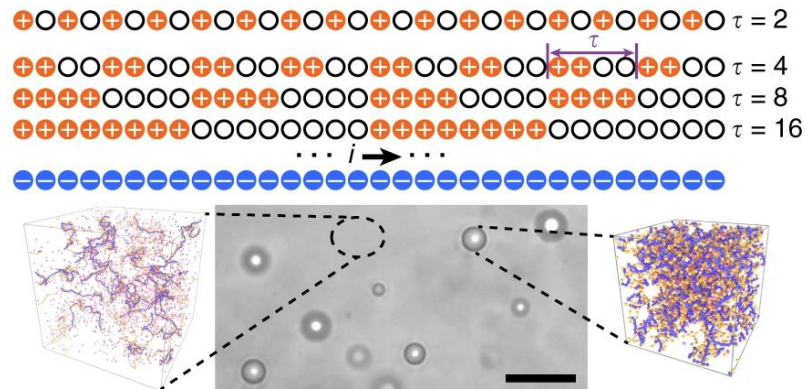
***β-lactoglobulin***      ***Napin***



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...

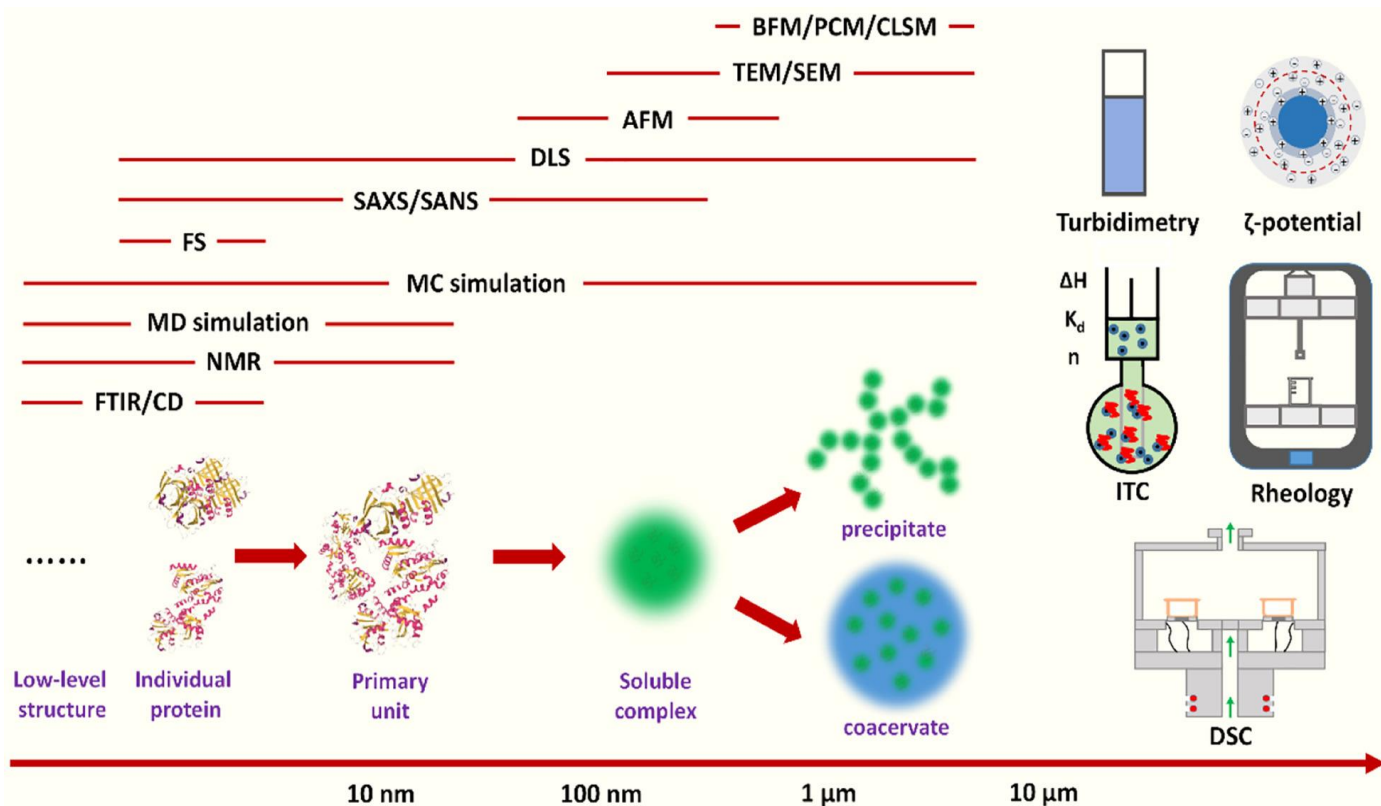


*Chang et al., Nat Commun 8, 1273, 2017.*



# ➤ 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)

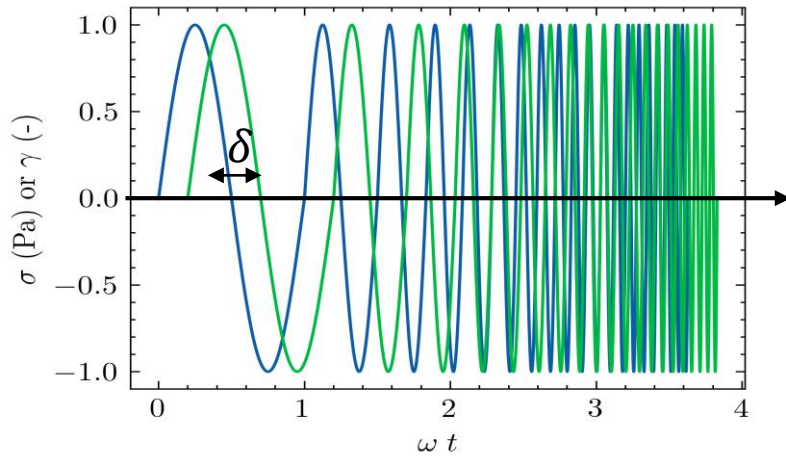
**Properties**

- 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...)



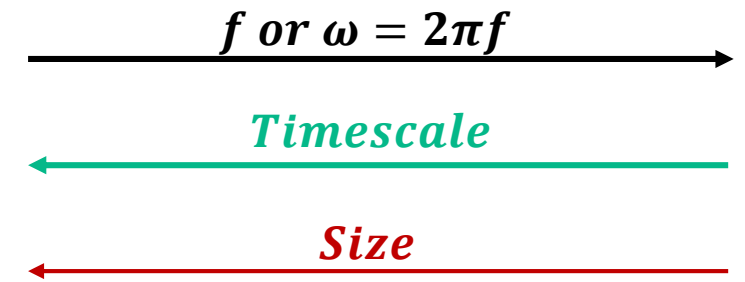
$$\gamma(t) = \gamma_0 \sin(\omega t)$$

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

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

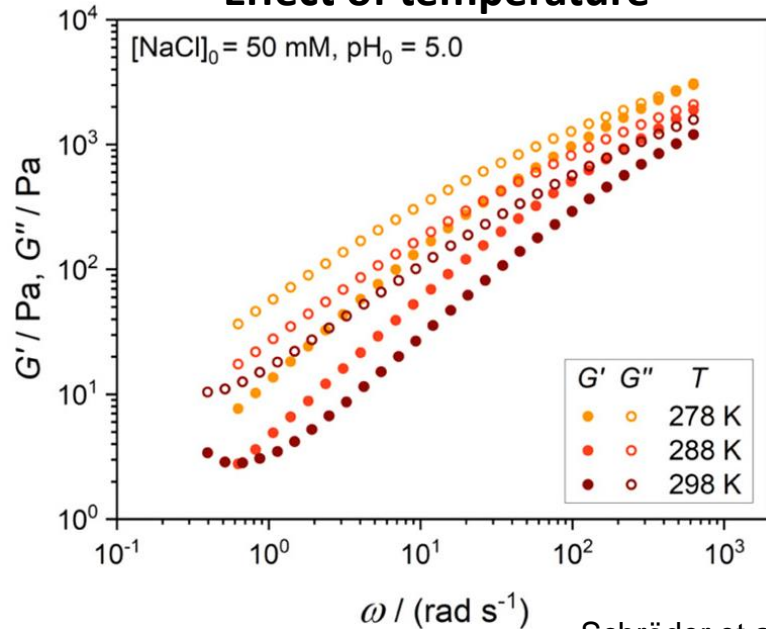
$$G' = \sigma_0 / \gamma_0 \cos(\delta)$$

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



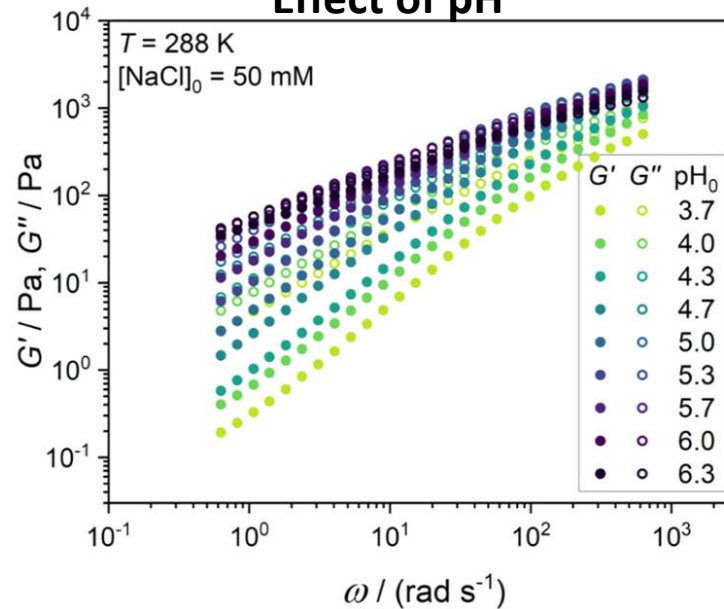
## Chitosan-Gum Arabic

### Effect of temperature



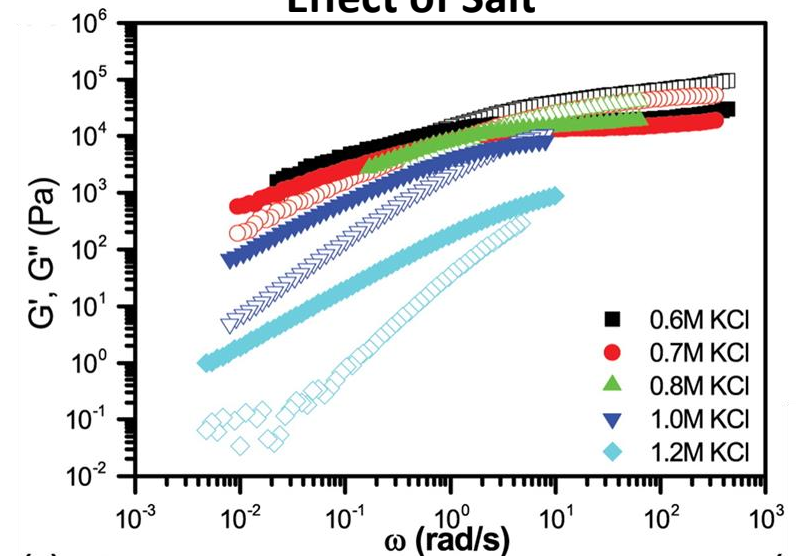
Schröder et al., *Macromolecules* 2023

### Effect of pH

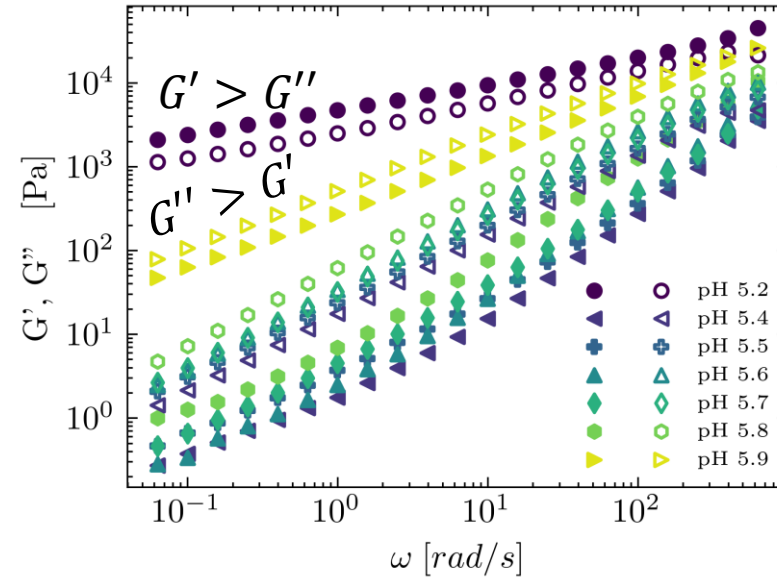
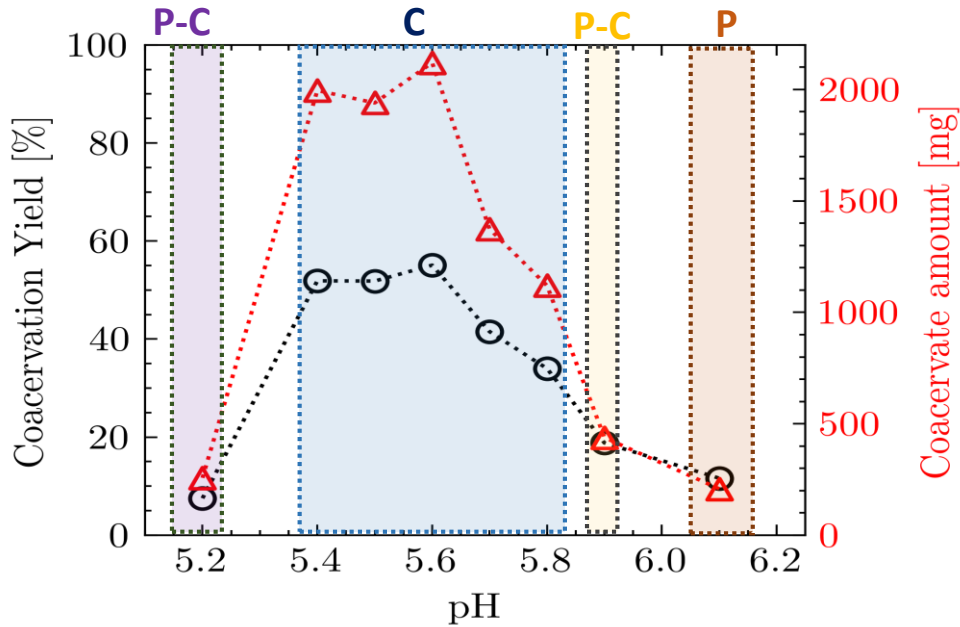
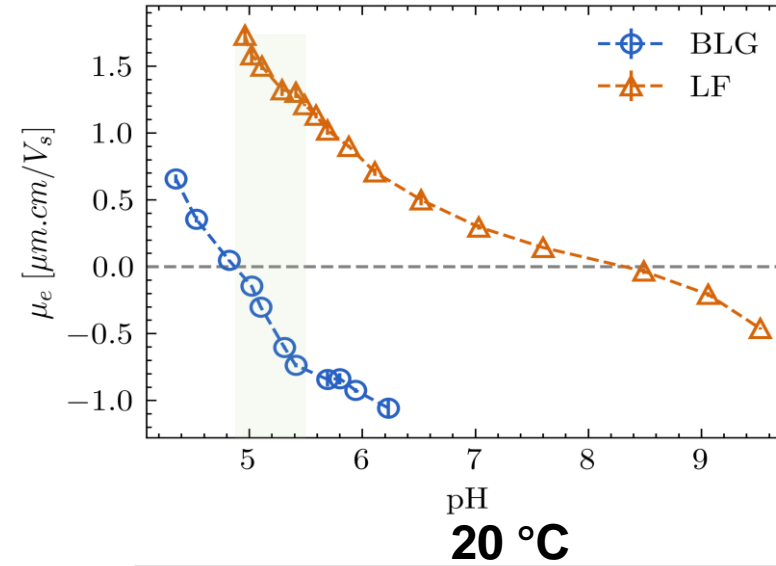
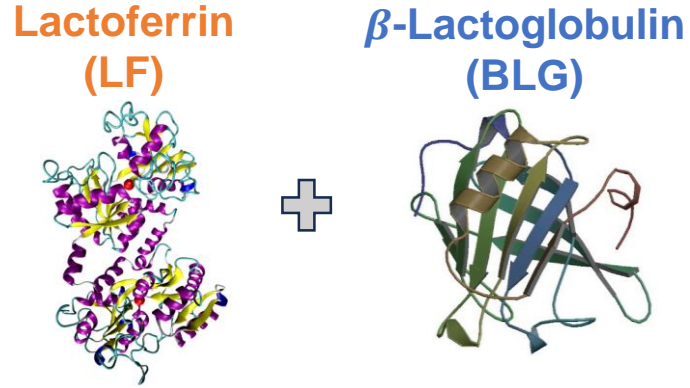


## PDMAEMA - PAA

### Effect of Salt

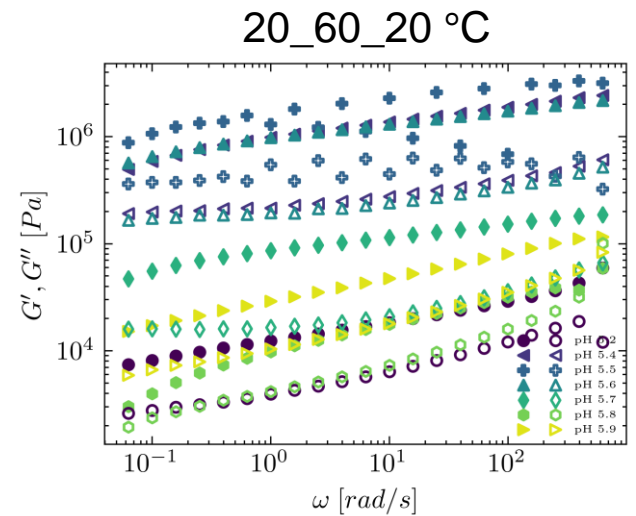
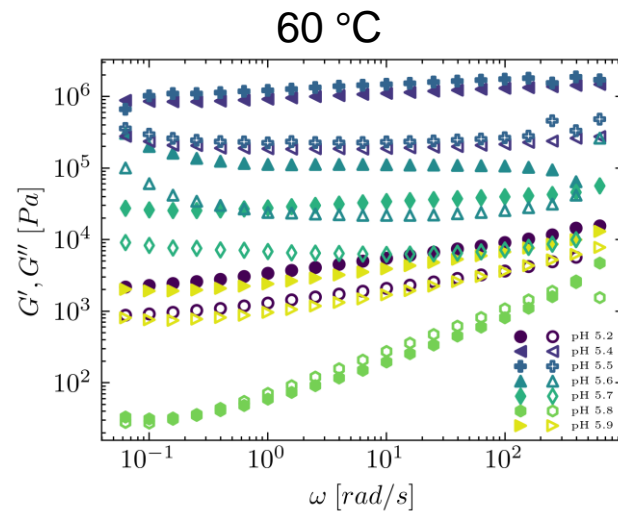
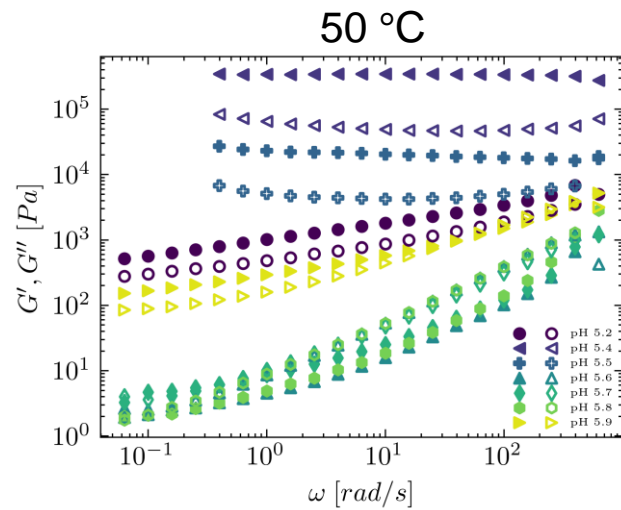
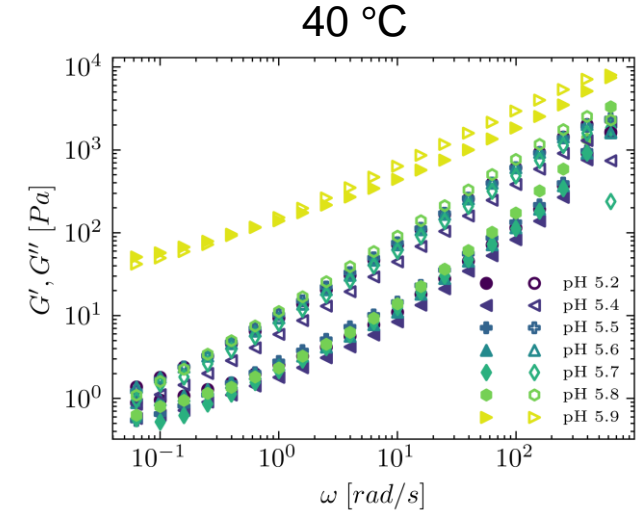
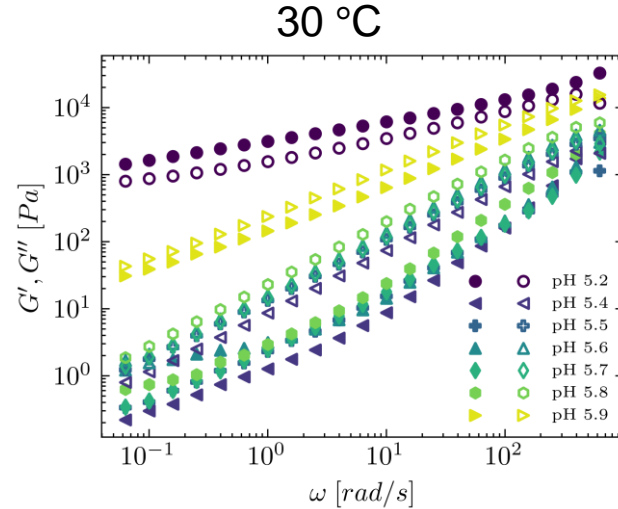
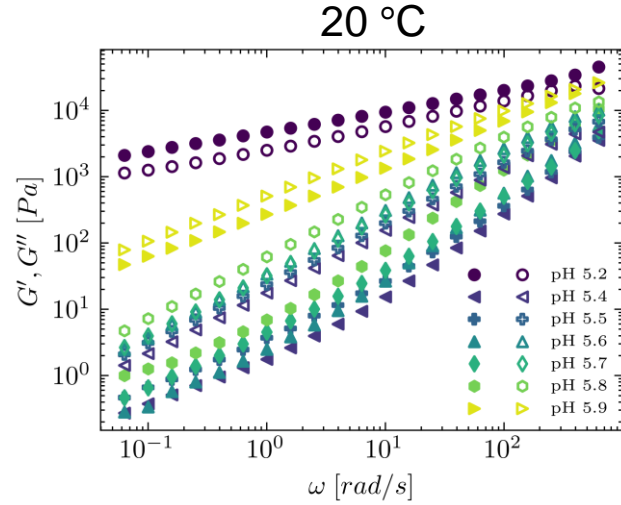


E. Spruijt, *Macromolecules*, 2013



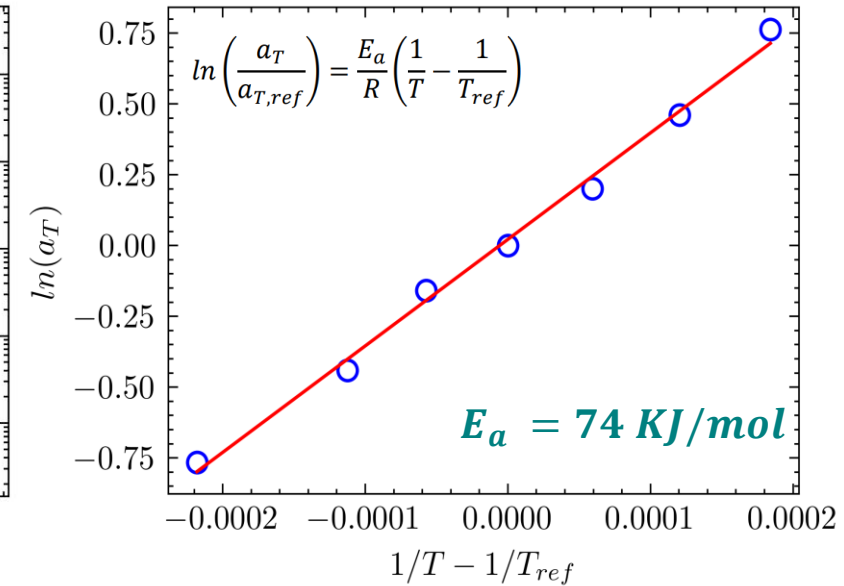
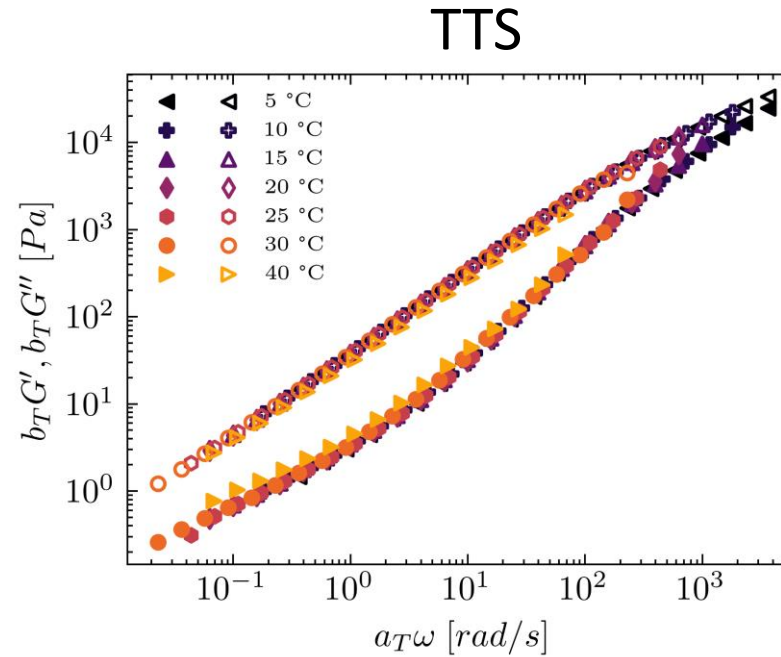
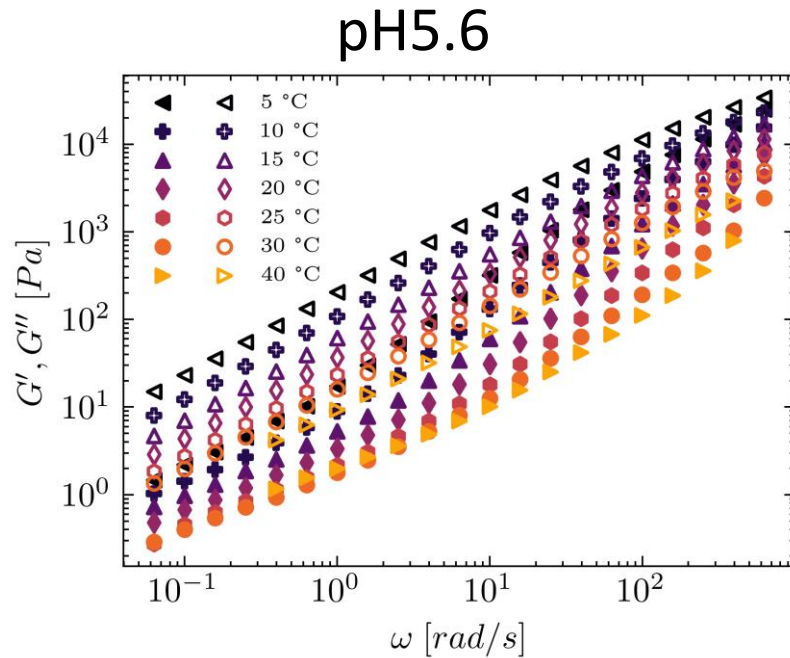
- High sensitivity of the system towards slight change of pH (0.1 unit).
- pH 5.2: Precipitate-coacervates: BLG precipitation close to the isoelectric point ( $pH \sim 5$ )
- pH 5.9 and pH 6.1: precipitation due to strong electrostatic interaction between BLG and LF

# ➤ Influence of temperature for different pH-values



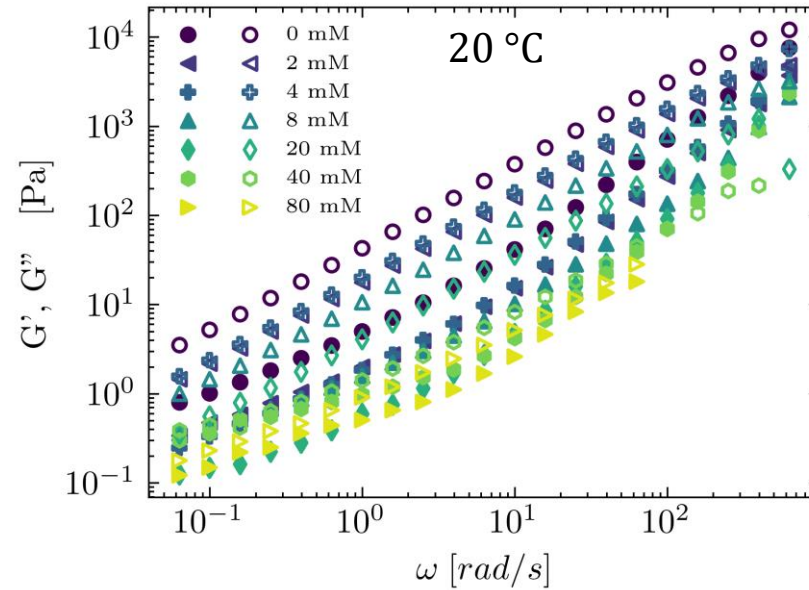
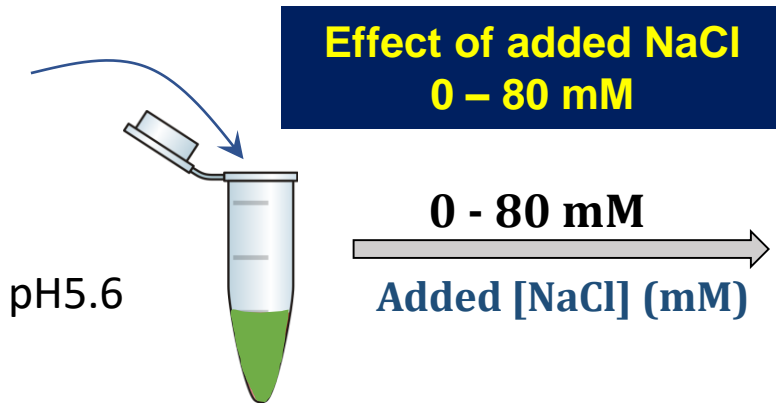
- Absence of thermal gelation at pH5.2
- Thermal behavior: Softening from 20 to 40 (or 50 °C as a function of pH) followed by irreversible gelation at 60 °C (or 50°C).
- Gelation of the coacervate phase lead to stiff materials ( 1 KPa <  $G'$  < 1 MPa )

# Time temperature superposition

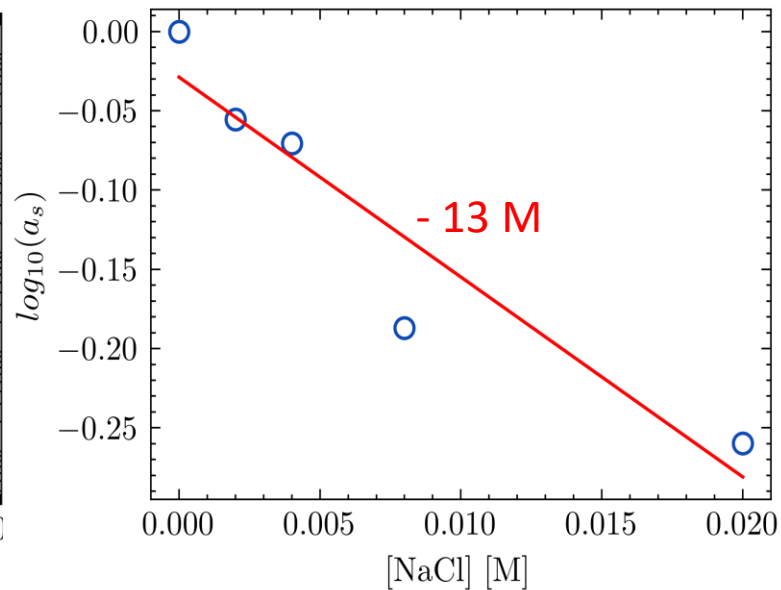
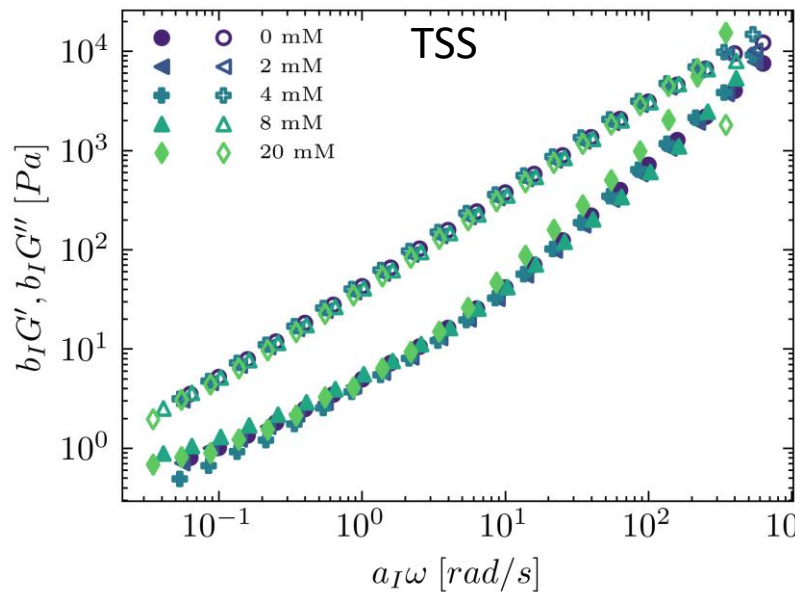


- Heating: accelerate the coacervates dynamics
- TTS principle applies in the limited range of  $T$ °C ( $5 \leq T < 50$  °C):
  - Both proteins show the same  $T$ -dependent monomer friction
  - The dynamics of the coacervate is dominated by BLG/LF interactions
  - The dynamics of the two proteins are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

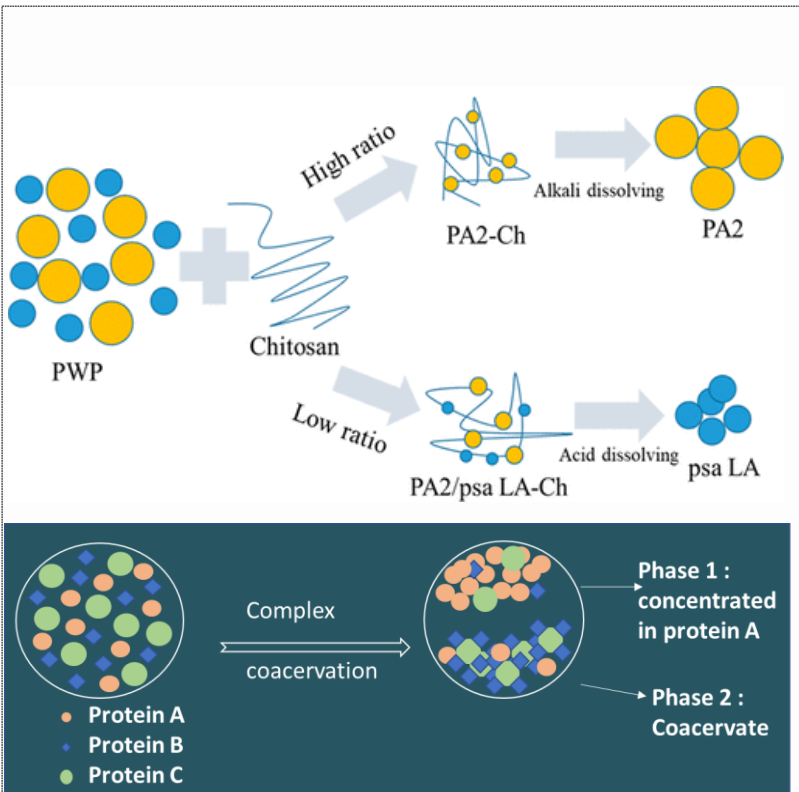
## ➤ Potential applications





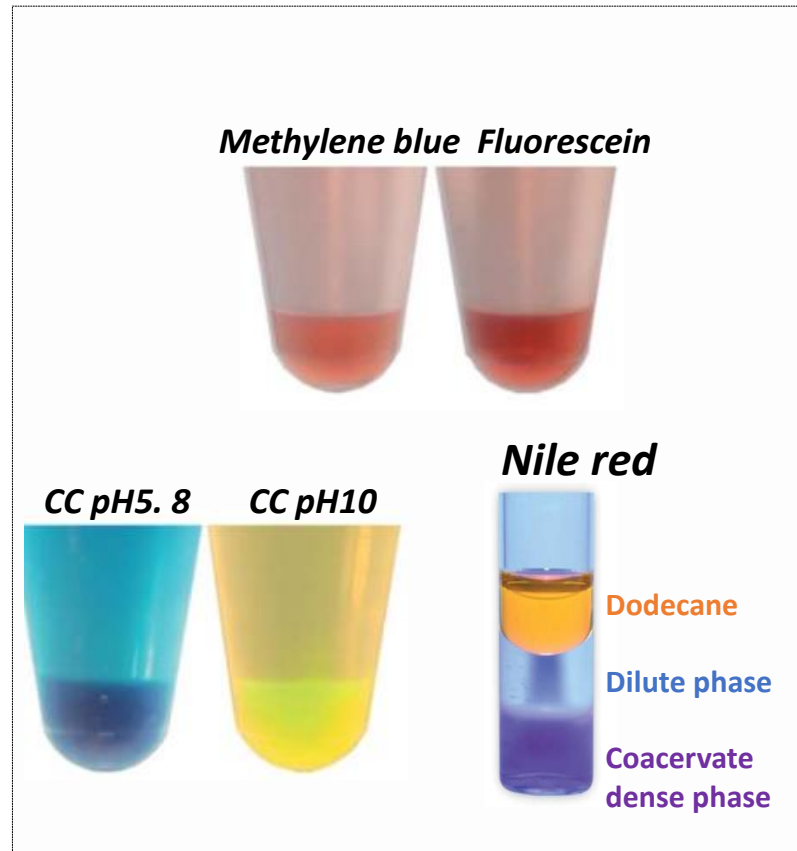
# Purification & Encapsulation

## Selective Complex Coacervation



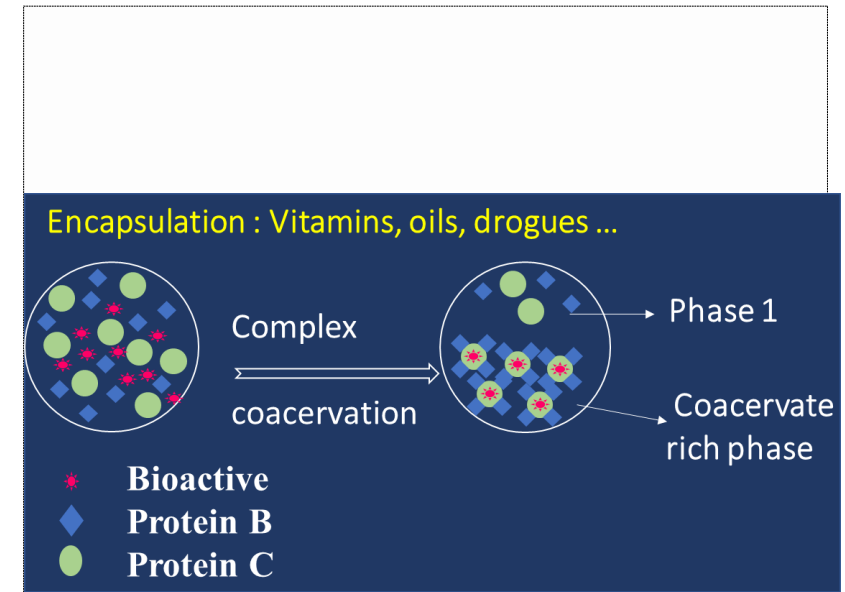
Yang et al., J. Agric. Food Chem. 2020

## Sequestration properties (preferential partitioning)



Williams et al., Soft Matter, 2012

## Weak attractive interactions

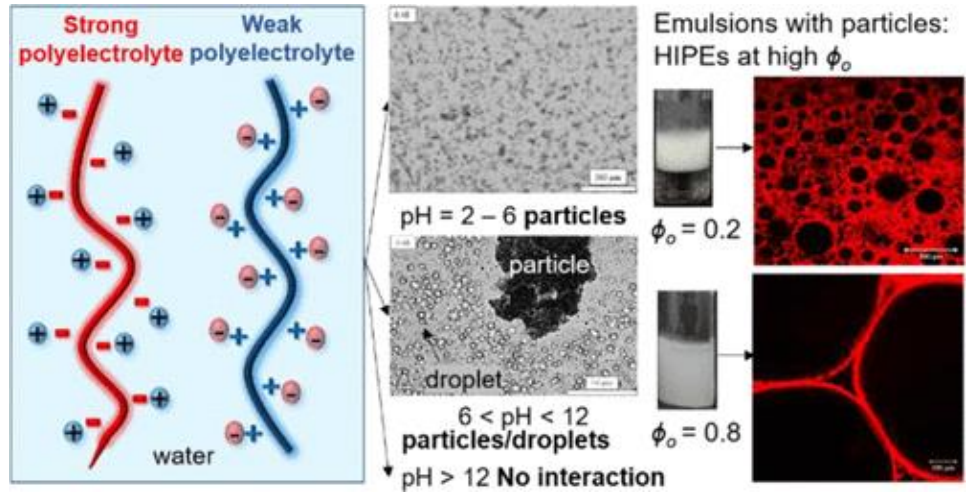


Chapeau et al., Food Hydrocolloids, 2016  
 McTigue et al., Soft Matter, 2019

### III. Emulsion stabilization

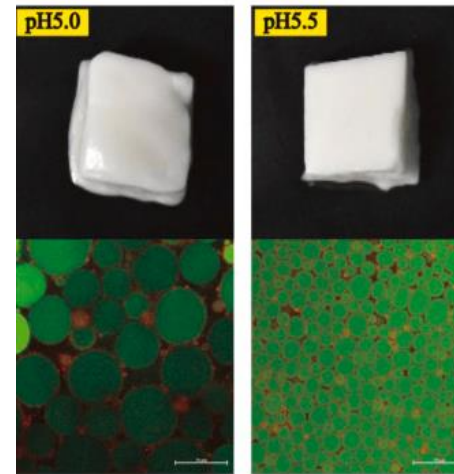
Up to date synthetic PEC/PEC or polysaccharide/proteins coacervates...

#### PSS / PAH



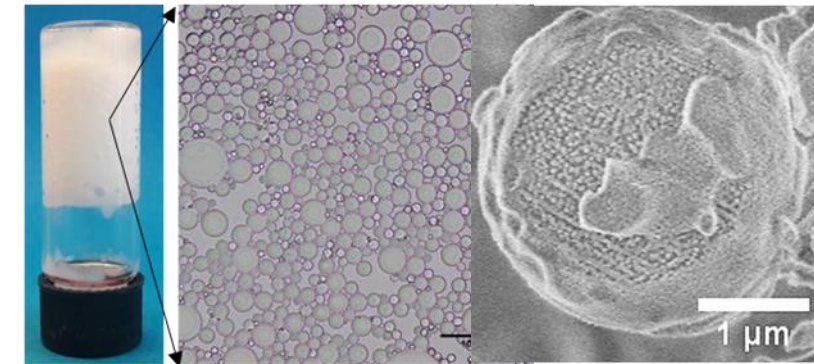
Bago et al., *Langmuir*, 2019

#### Soy protein isolate / $\kappa$ -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



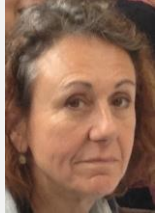
P. Hamon



M. Ganne



M.F. Famelart



R. Soussi

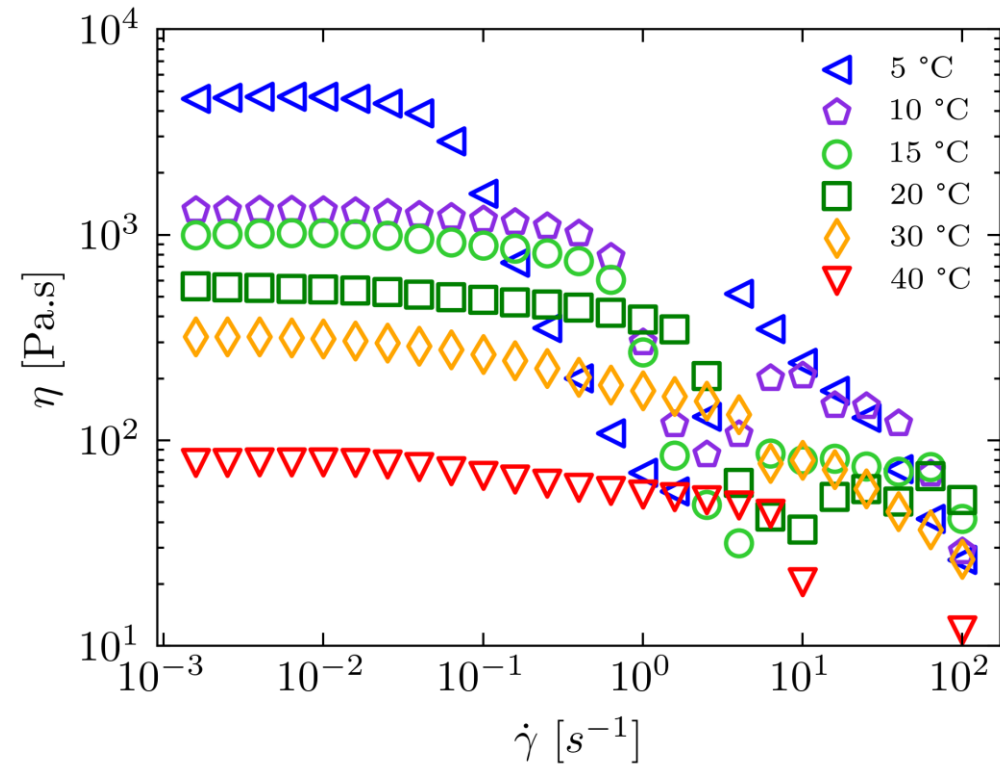


# Thank you for your attention

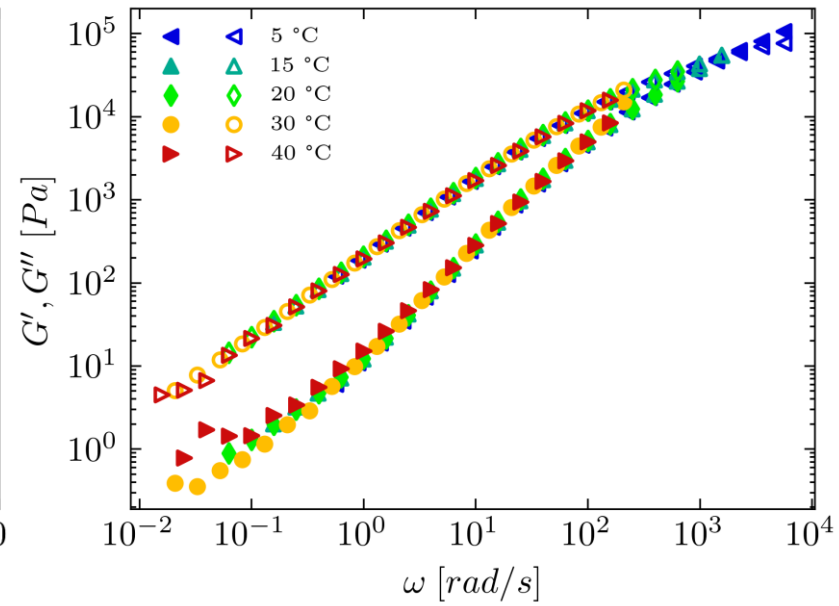
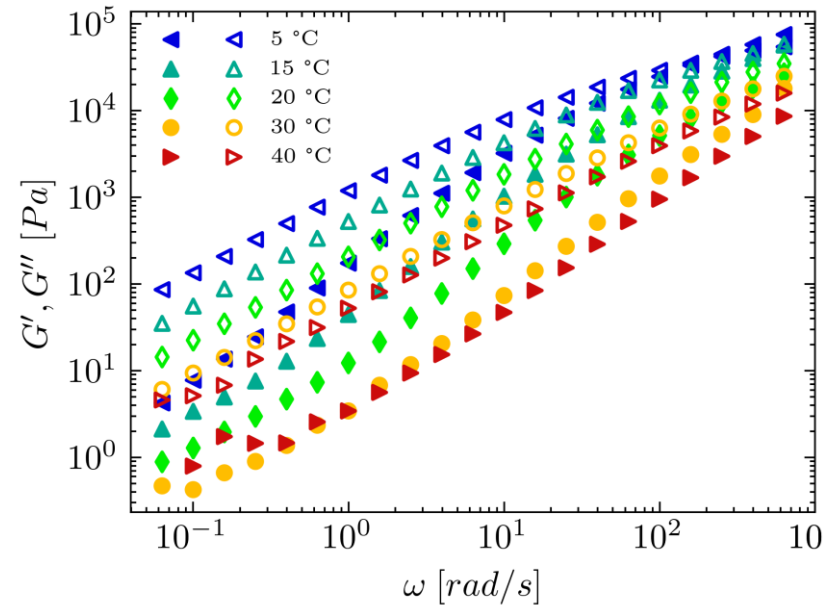


➤ Additional Slides

## ➤ New Sample Ghazi



## ➤ New sample Ghazi



## ➤ New sample Ghazi

