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

Ghazi Ben Messaoud

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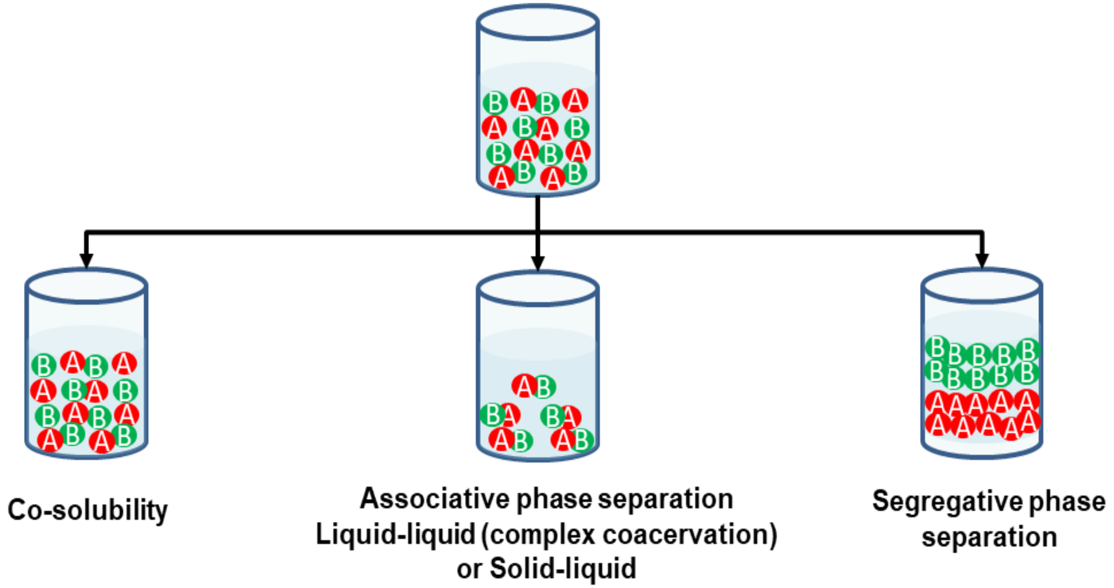
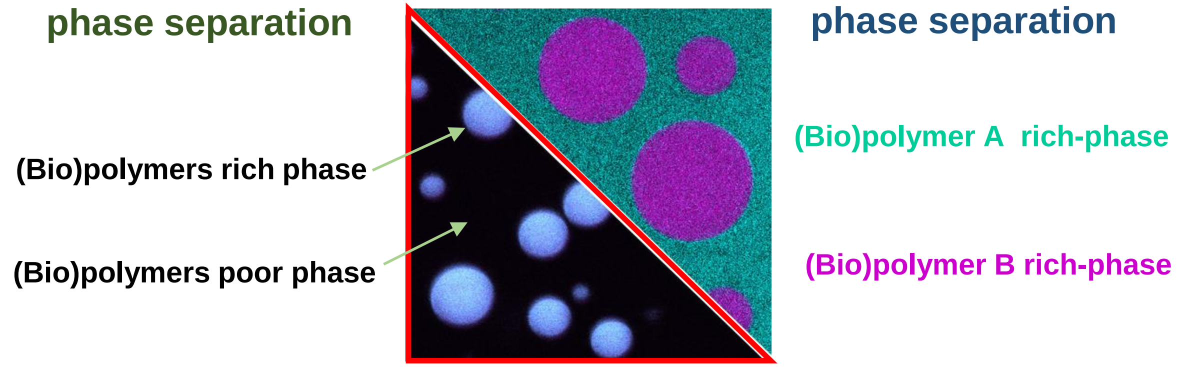
Introduction

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

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

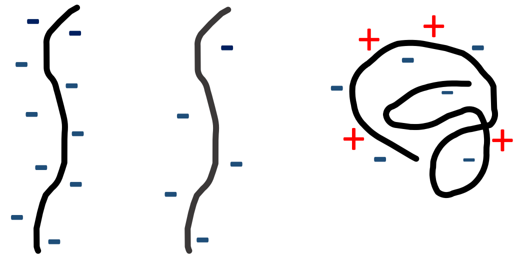




COMPLEX COACERVATION

Polyelectrolytes

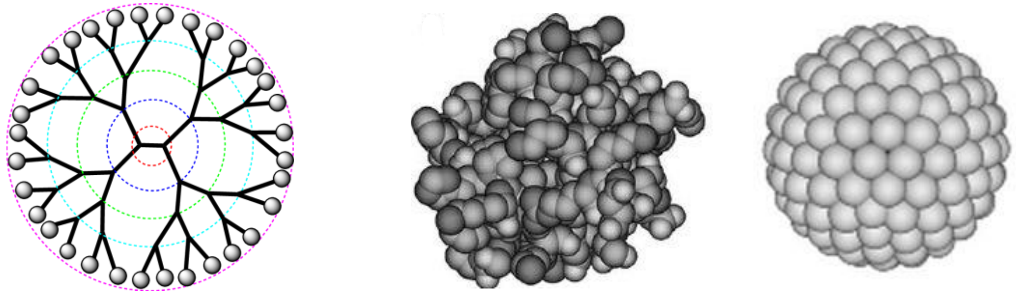
Strong *Weak* *Polyampholyte*



Schlenoff et al., *Macromolecules.*, 2019

Colloids

Dendrimers *Globular proteins* *Micelles*



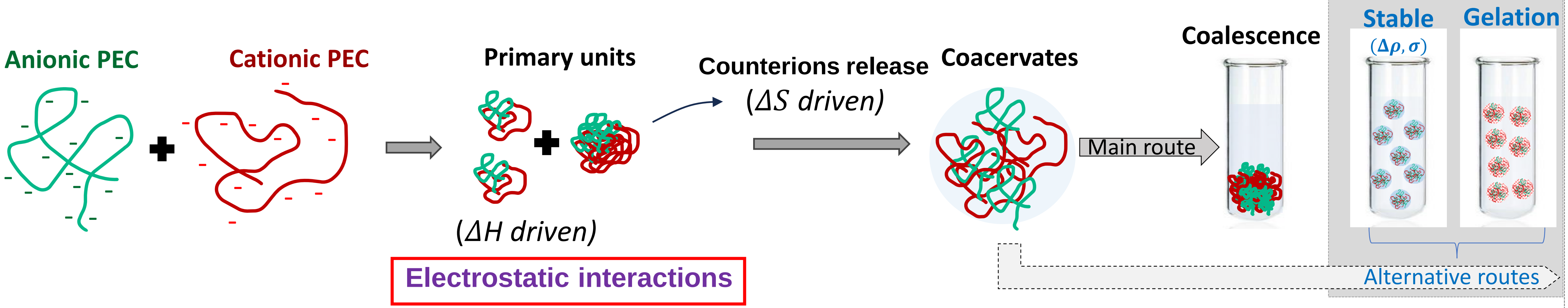
Kaup et al., *ACS Nano*, 2021 Croguennec et al., *Adv. Coll. Int. Sci.*, 2017 G. Ben Messaoud et al., *Green Chem*, 2018

Inorganic macroions

Polyoxometalate

Jing et al., *Soft Matter*, 2017

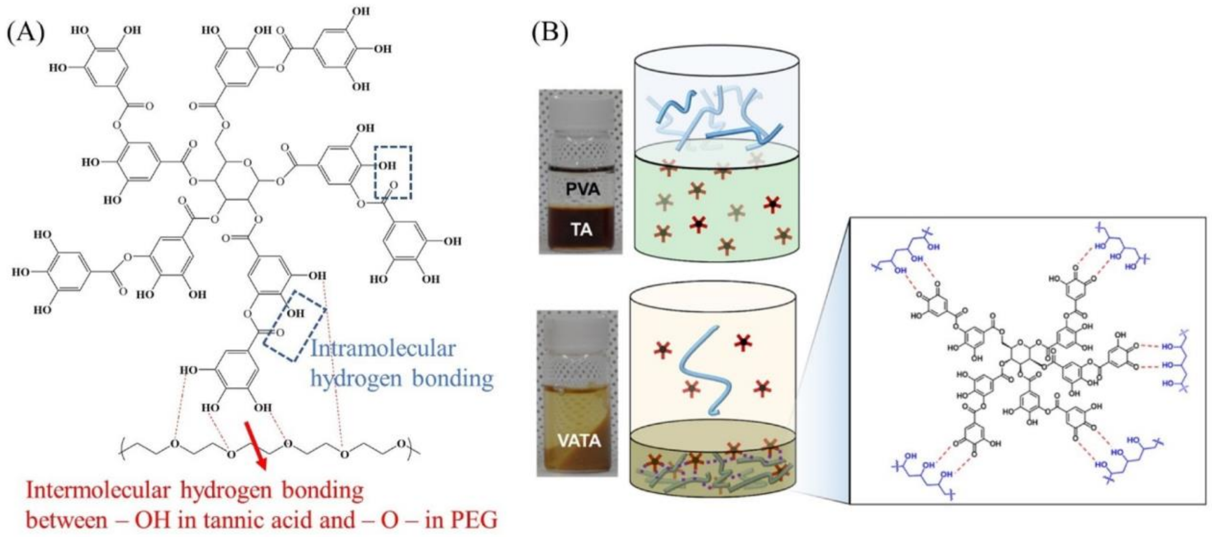
Complex coacervation…a generic process



Electrostatic interactions

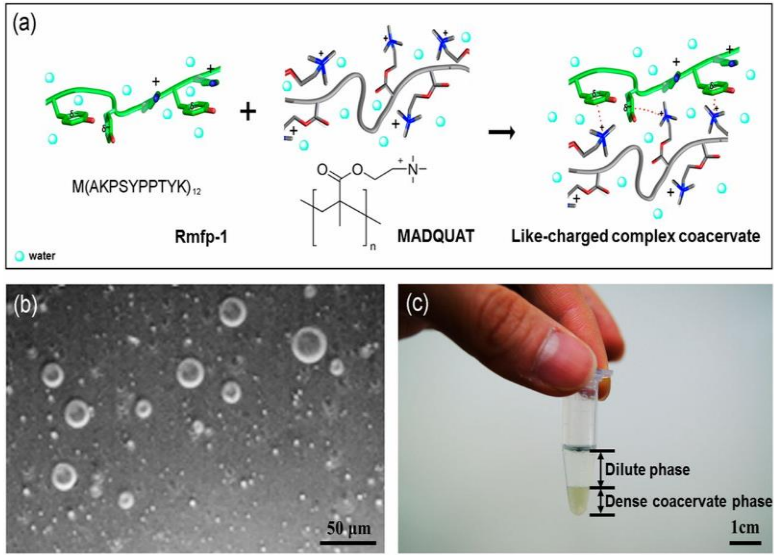
Often…but not only!

Hydrogen bonding



Kim et al., *Adv Funct Mater*, 25 (2015)
Lee et al., *ACS Appl Mater Interfaces*, 12 (2020)

$\pi - \pi$ Stacking

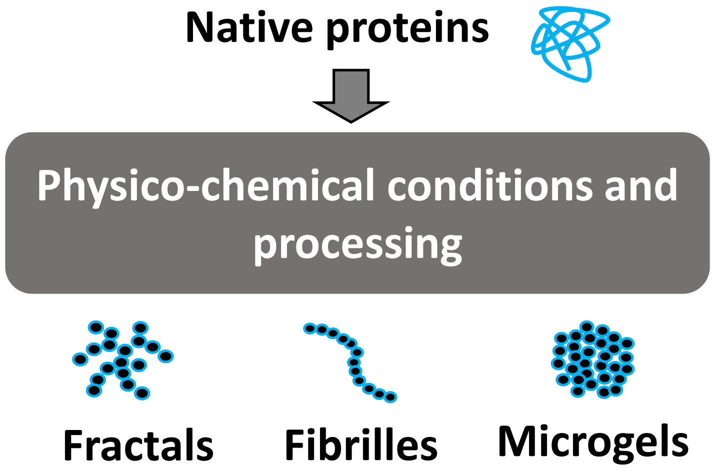


Kim, et al., *PNAS*, 113(7), 2016.

Heteroprotein Complex coacervation (HPCC) in food science

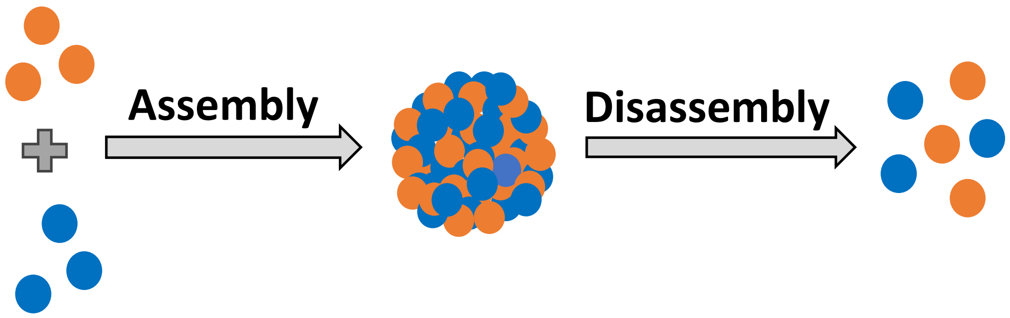
Food protein interactions & assemblies

- 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,..)

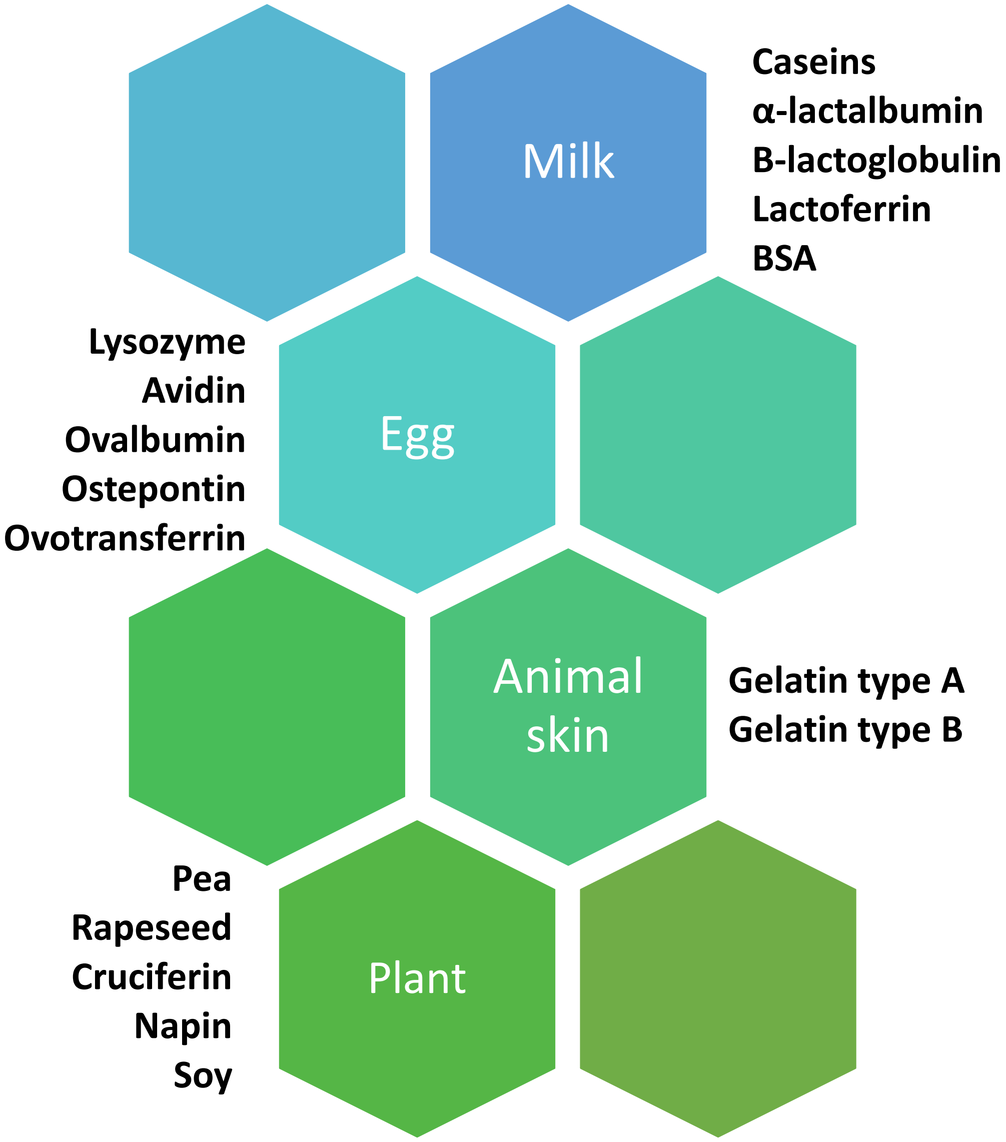


HPCC

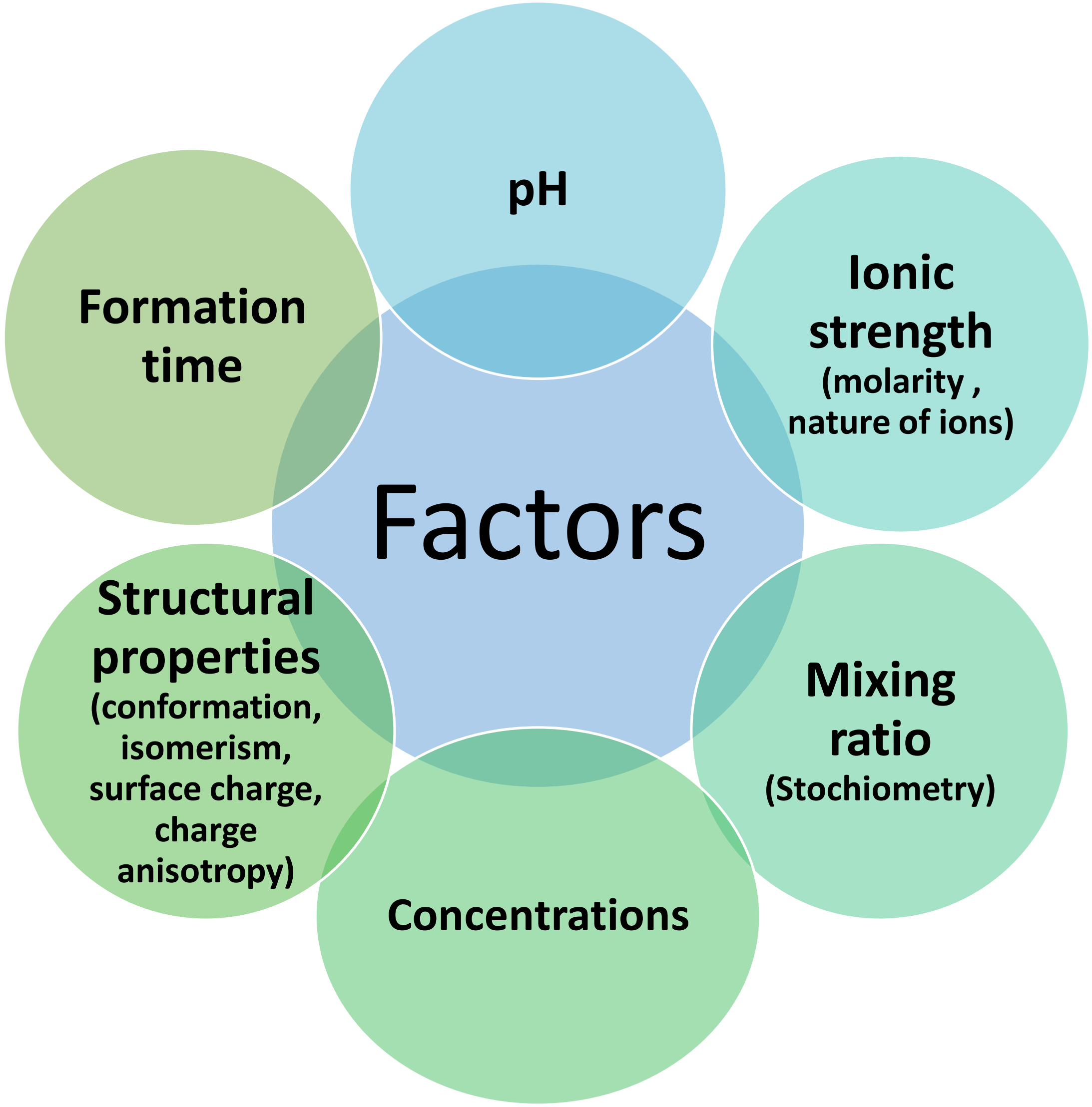
- Self- or co-assembly under "mild" conditions:
- Reversible associations between proteins (non-covalent interactions)
- Flexible design (microscale, bulk, assembly-disassembly of food products, functional and nutritional properties)



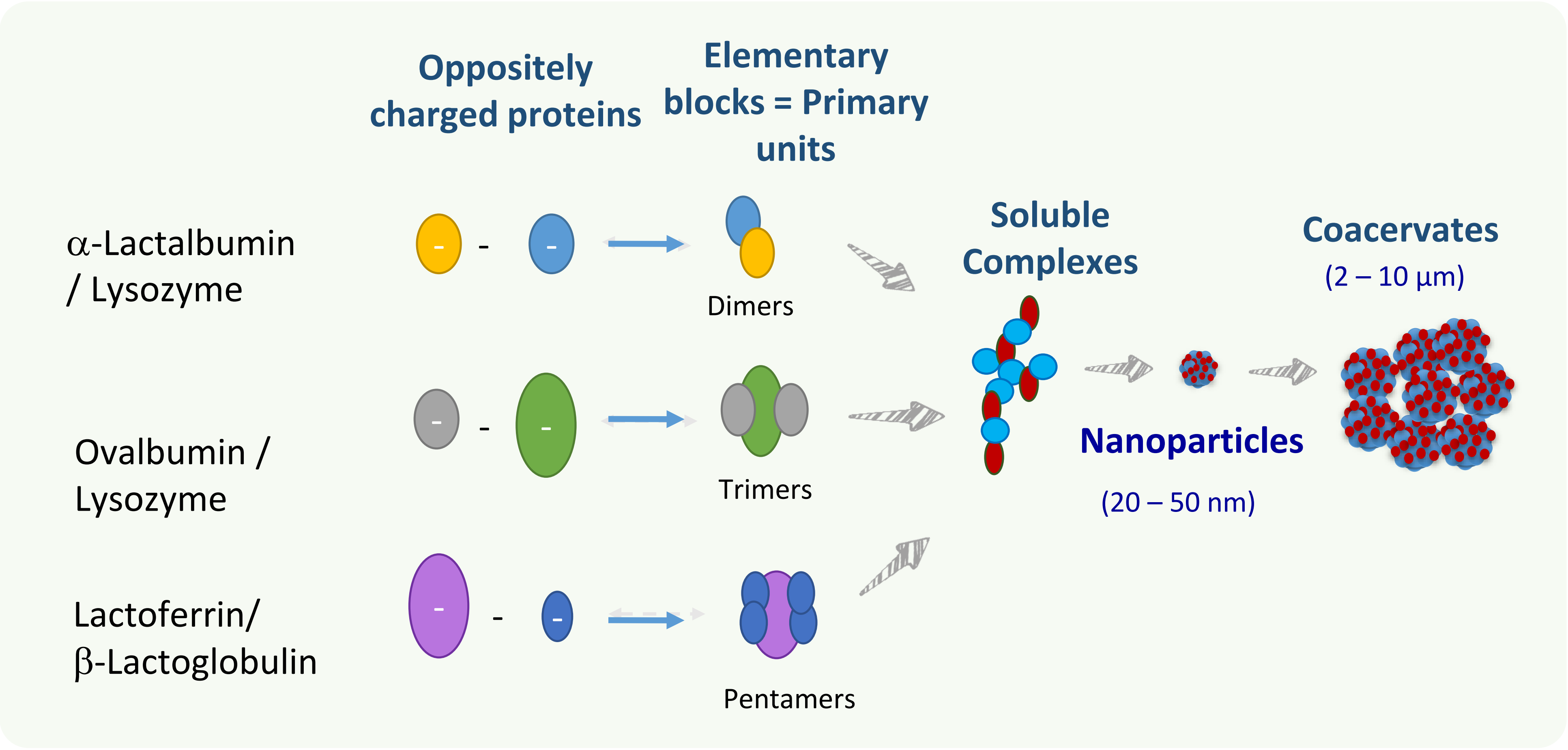
Studied food heteroprotein systems



Influencing factors



Formation dynamic

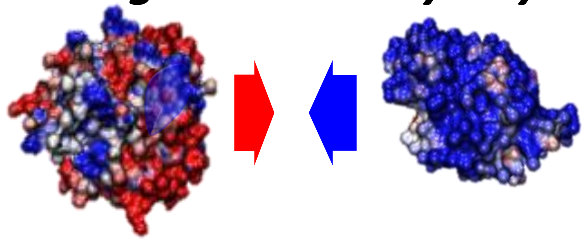


Croguennec et al., Adv. Coll. Int. Sci., 2017
Salvatore et al. Biomacromolecules, 2011
Dubin et al., Biomacromolecules, 2014

Specificity over other macromolecular systems….Charge patchines

LYS and NAP with similar charge but:

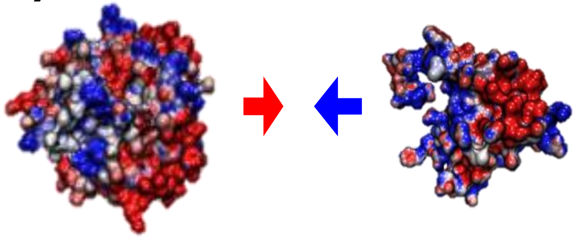
β-lactoglobulin ***Lysozyme***



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

Phase separation: > µm

β-lactoglobulin ***Napin***

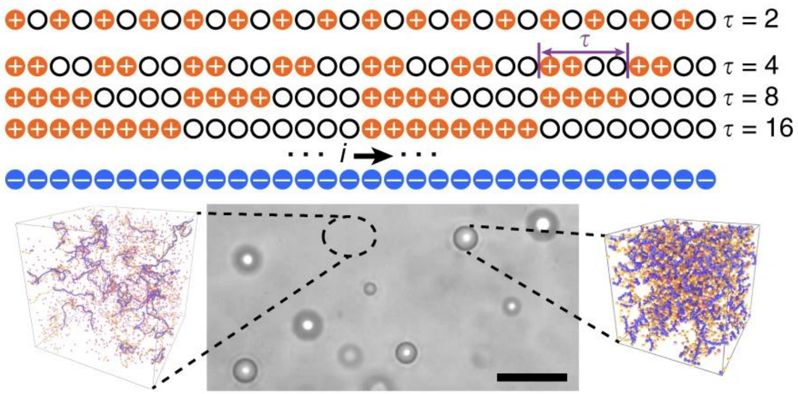


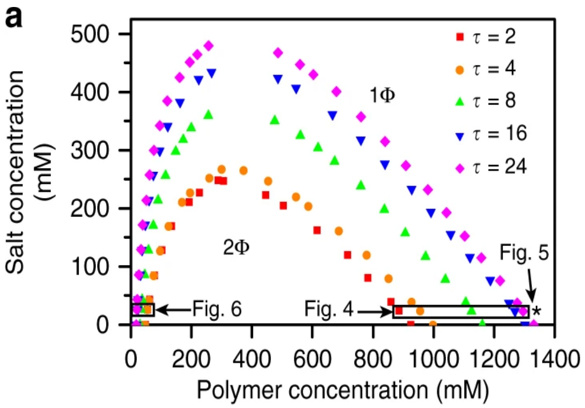
Potentiel Zeta = - 50 mV *Potentiel Zeta = + 20 mV*

Soluble complexes: < 20 nm

Ainis et al., Langmuir, 2019

Specific to proteins… but in a good agreement with…

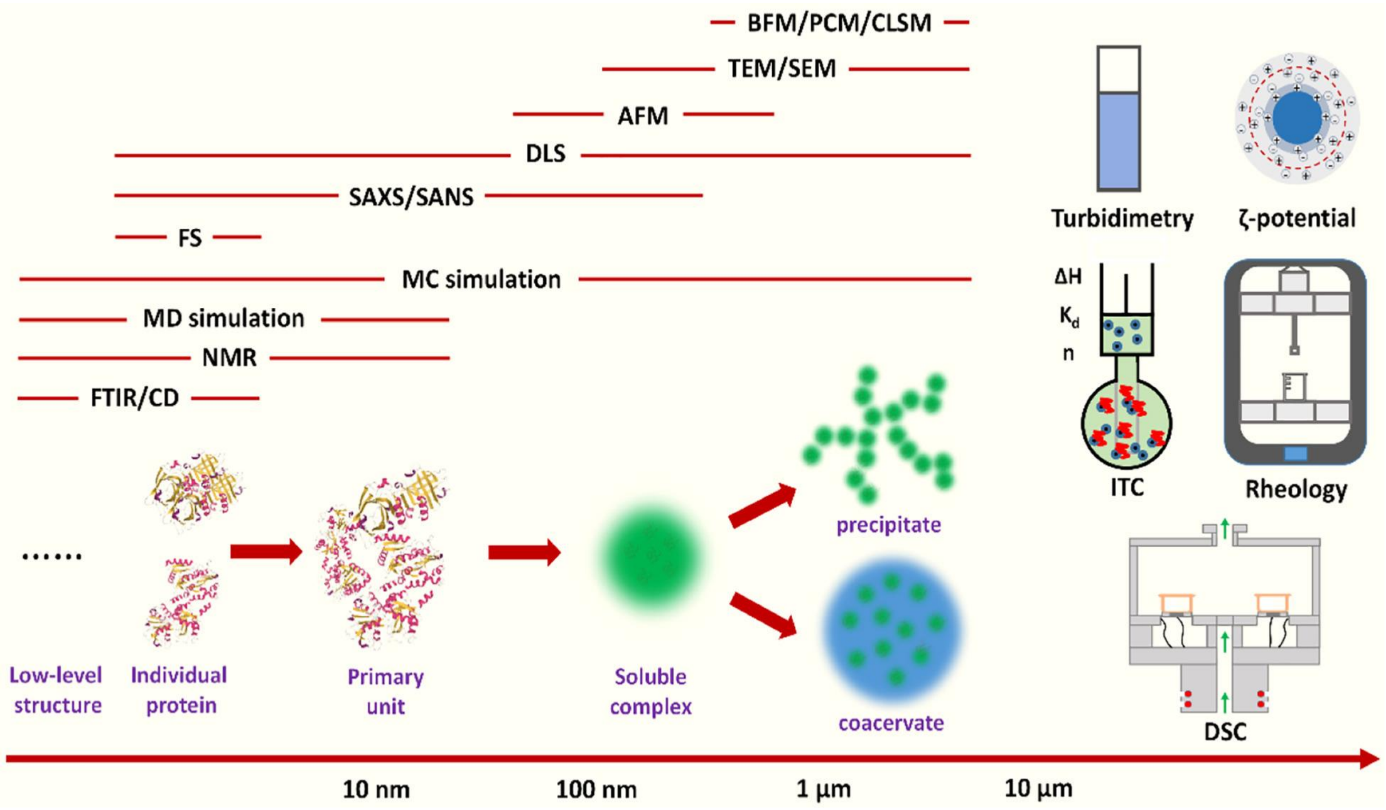




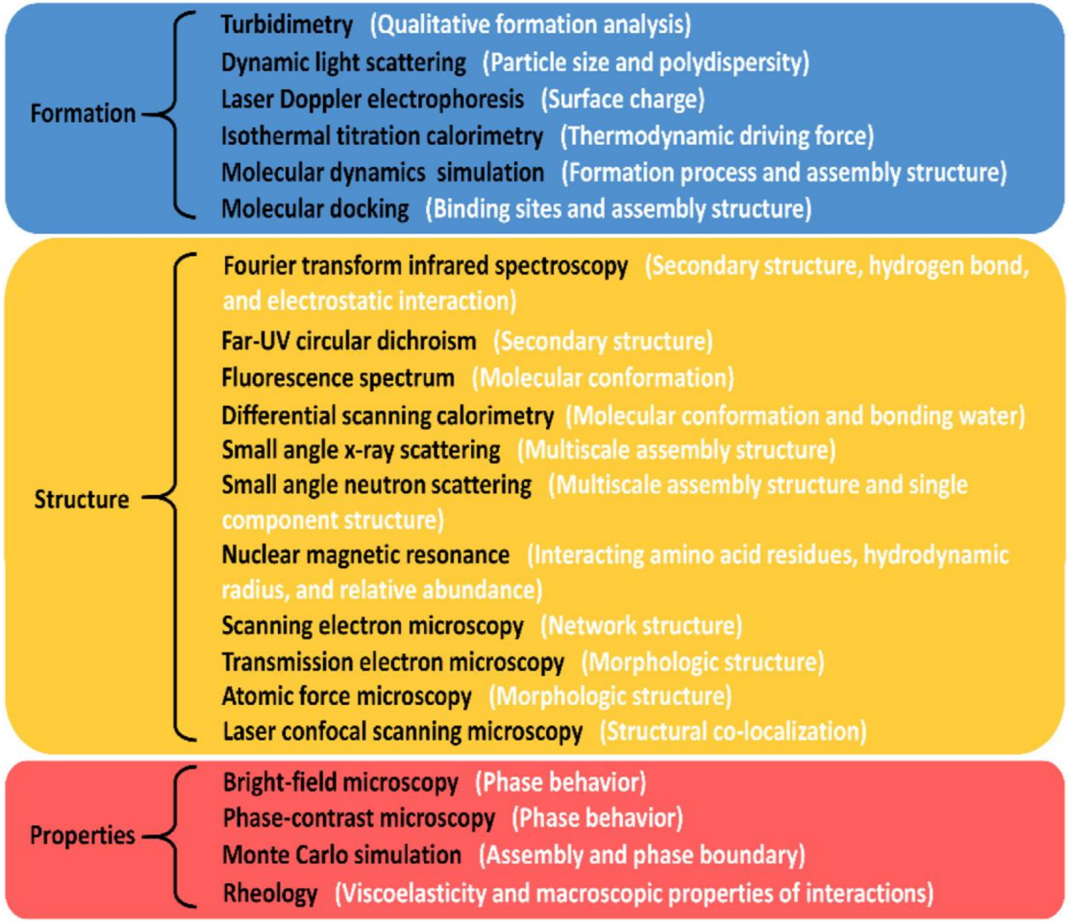
Chang et al., *Nat Commun* 8, 1273, 2017.

Experimental techniques

Length Scale



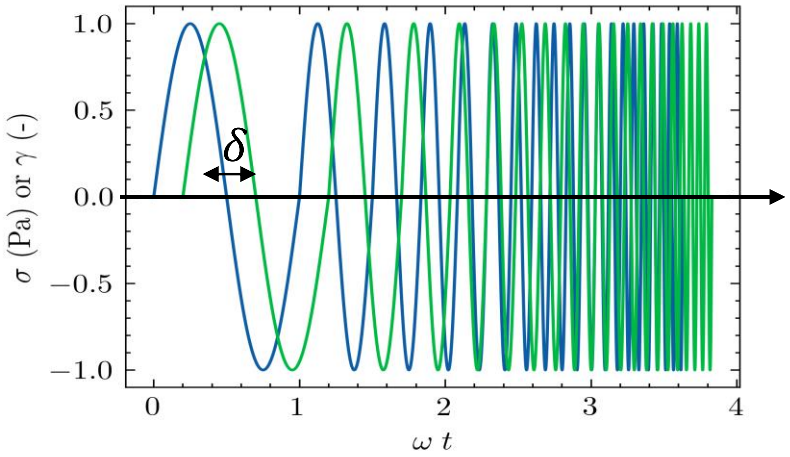
Aspects



Zheng et al., *Adv in Coll & Inter Sci*, 2020

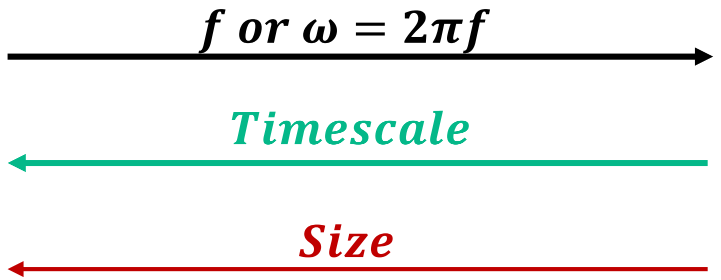
Rheology

Linear viscoelasticity (SAOS, SR...)

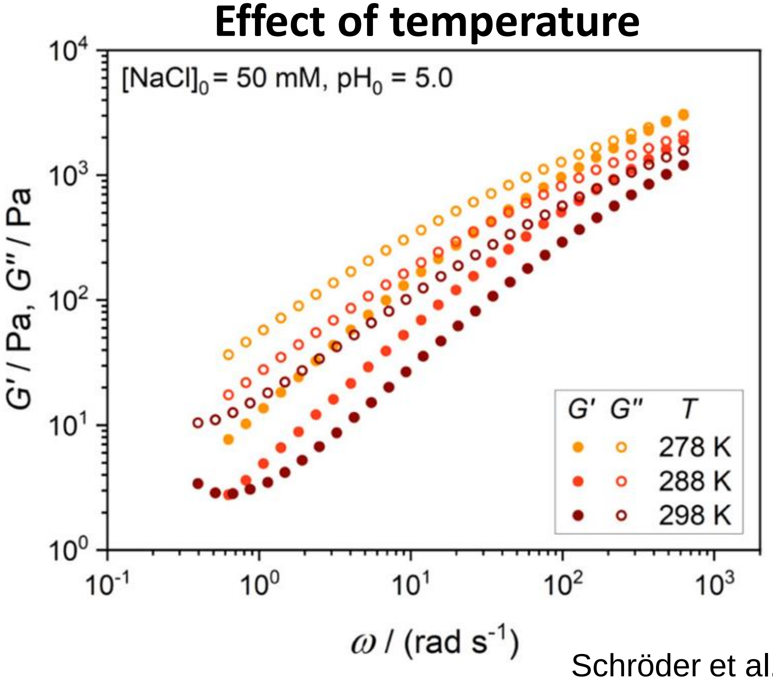


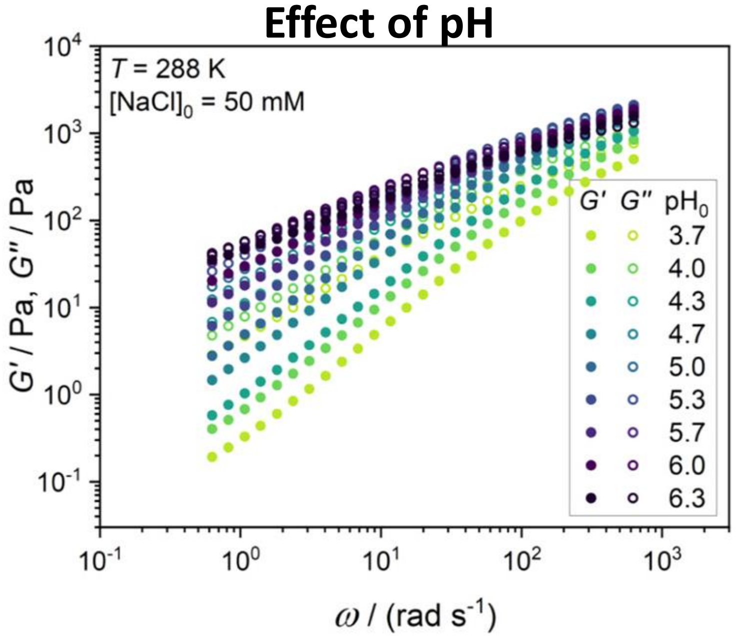
$$\gamma(t) = \gamma_0 \sin(\omega t)$$
$$\sigma(t) = \sigma_0 \sin(\omega t + \delta)$$
$$0° < \delta < 90°$$

$$G' = \sigma_{0/\gamma_0} \cos(\delta)$$
$$G'' = \sigma_{0/\gamma_0} \sin(\delta)$$



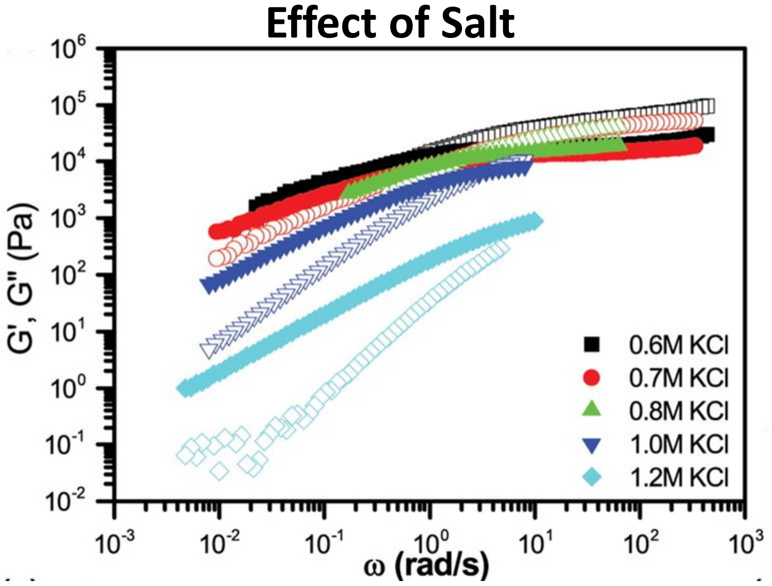
Chitosan–Gum Arabic





Schröder et al., *Macromolecules* 2023

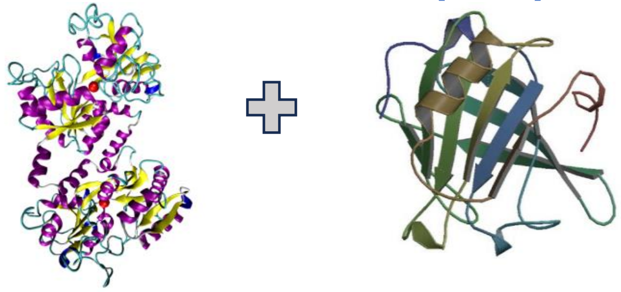
PDMAEMA - PAA

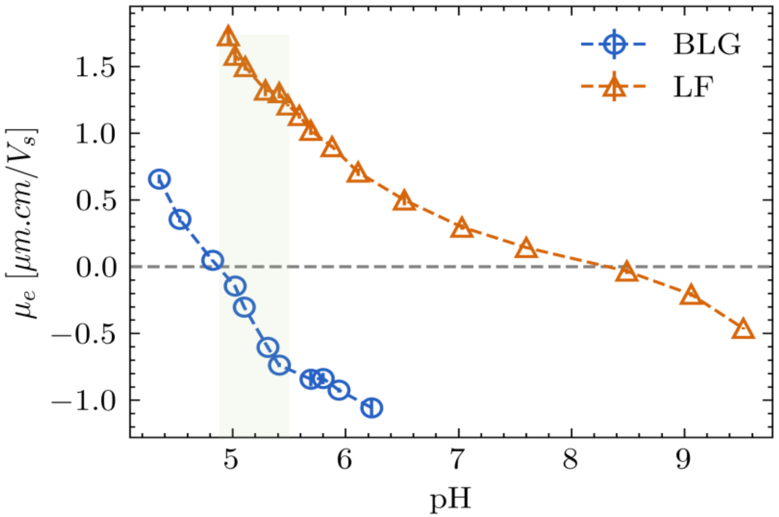


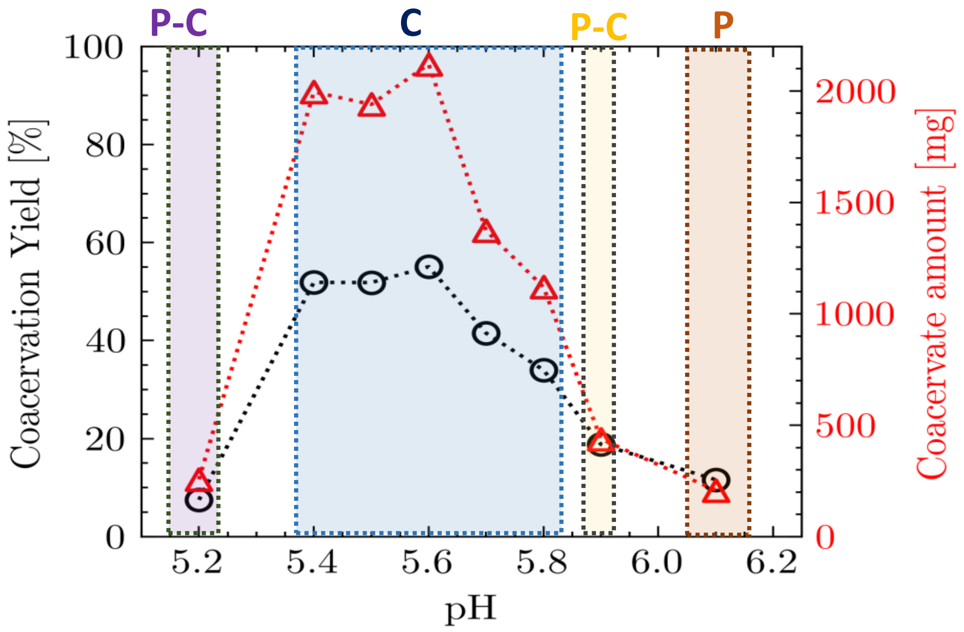
E. Spruijt, *Macromolecules*, 2013

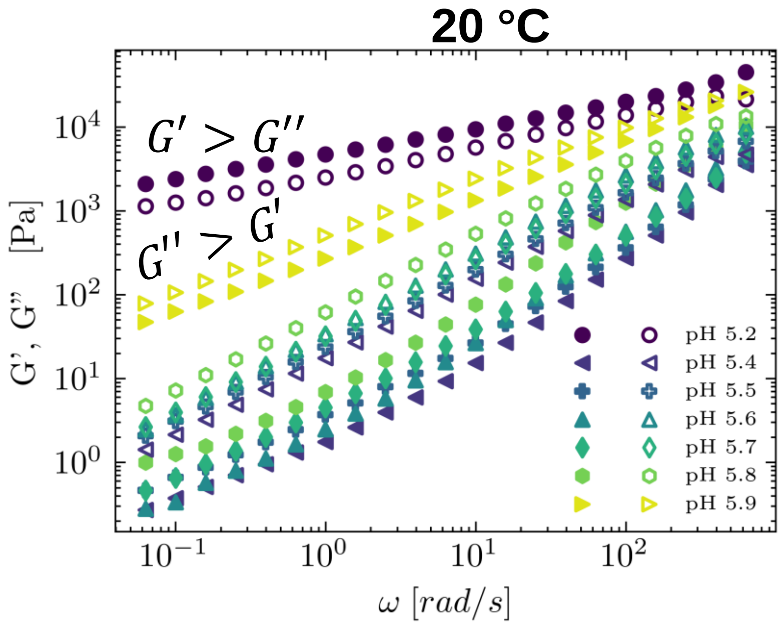
HPCC

Lactoferrin (LF) **β-Lactoglobulin (BLG)**



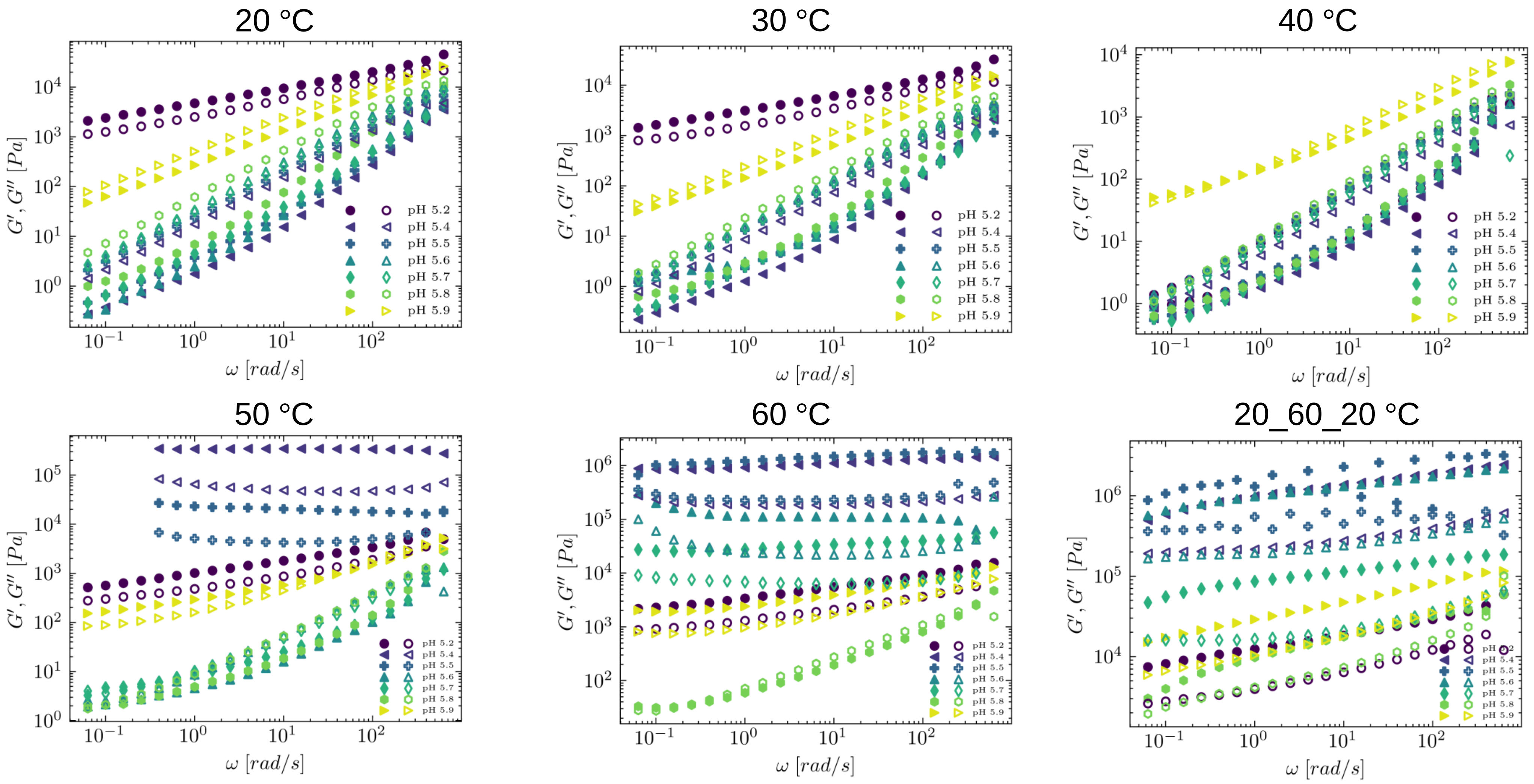






- High sensitivity of the system towards slight change of pH (0.1 unit).
- pH 5.2: Precipitate-coacervates: BLG precipitation close to the isoelectric point ($pH \sim 5$)
- pH5.9 and pH 6.1: precipitation due to strong electrostatic interaction between BLG and LF

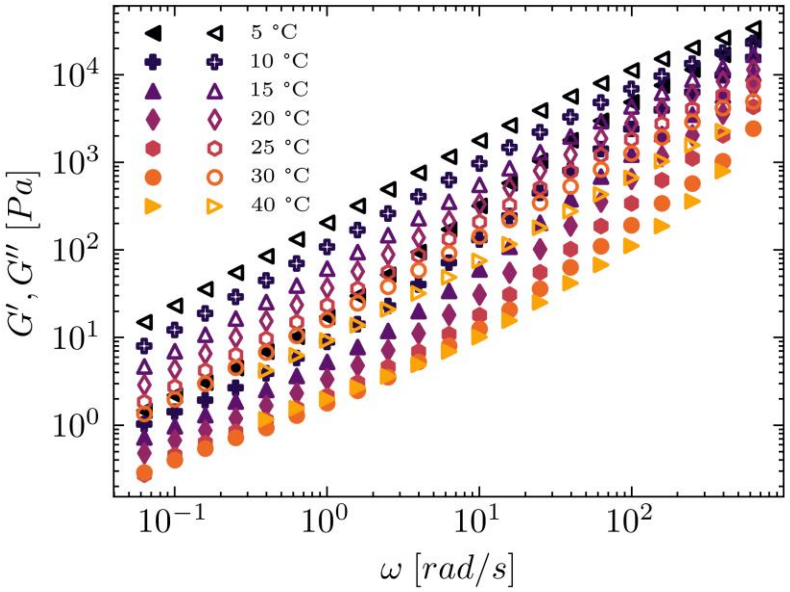
Influence of temperature for different pH-values



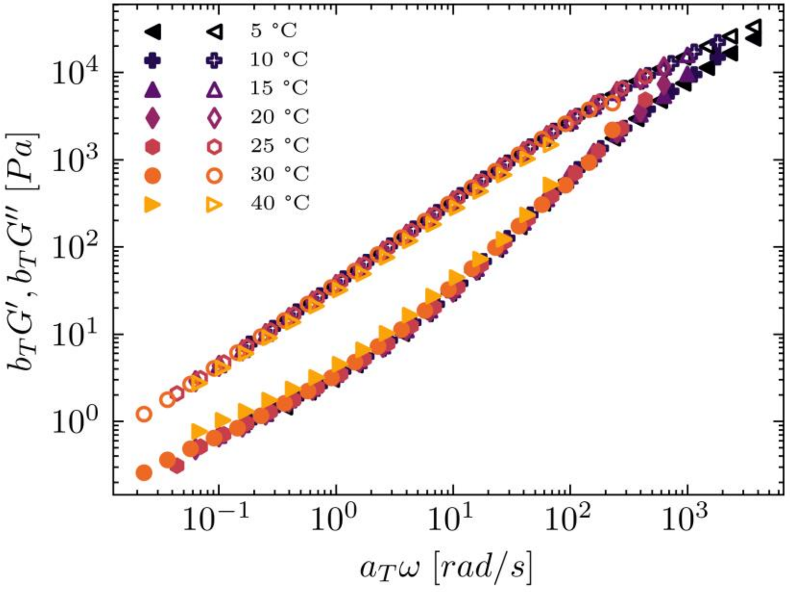
- Absence of thermal gelation at pH5.2
- Thermal behavior: Softening from 20 to 40 (or 50 °C as a function of pH) followed by irreversible gelation at 60 °C (or 50°C).
- Gelation of the coacervate phase lead to stiff materials (1 KPa < G' < 1 MPa)

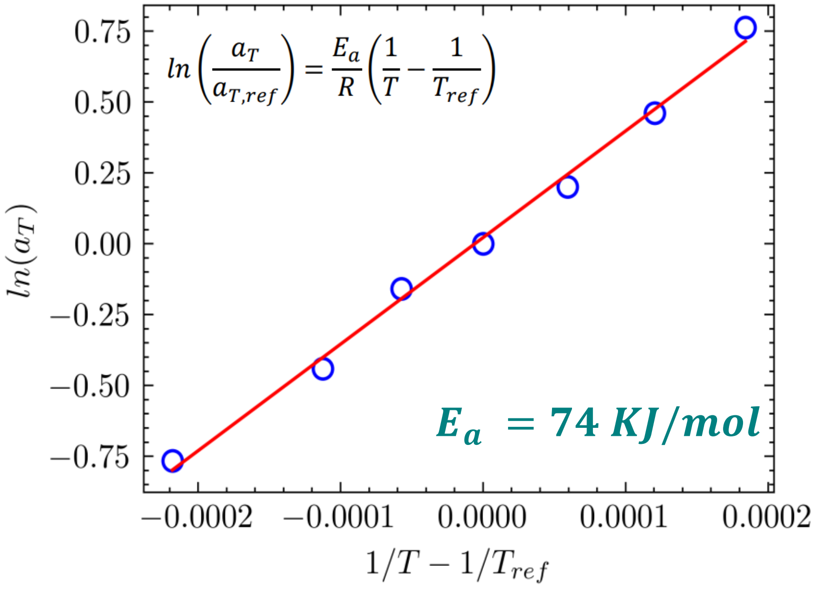
Time temperature superposition

pH5.6



TTS

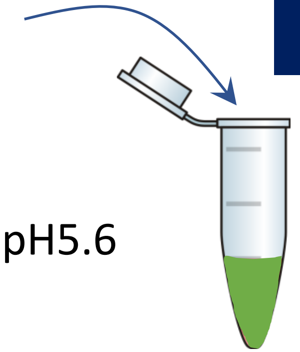




- Heating: accelerate the coacervates dynamics
- TTS principle applies in the limited range of T°C ($5 \leq T < 50$ °C):
  - Both proteins show the same *T*-dependent monomer friction
  - The dynamics of the coacervate is dominated by BLG/LF interactions
  - The dynamics of the two proteins are strongly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

Time salt superposition

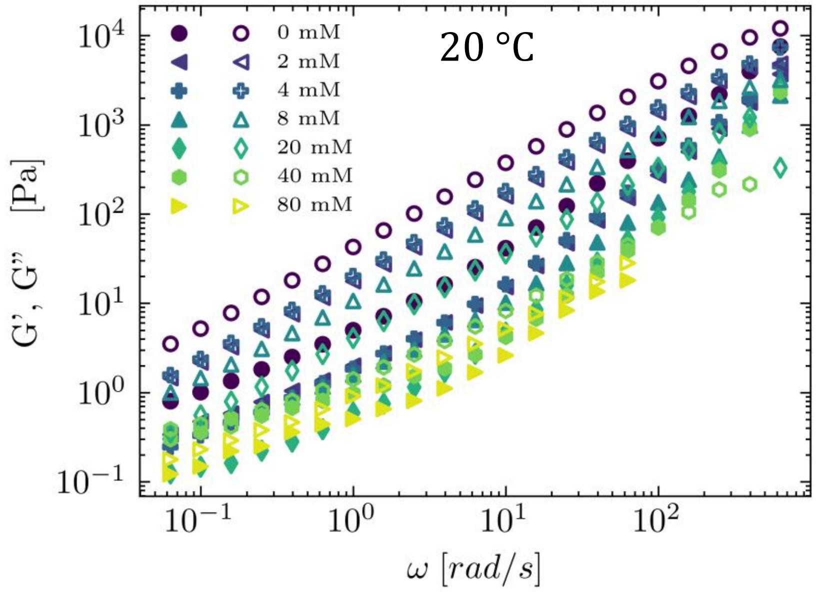
Effect of added NaCl
0 – 80 mM

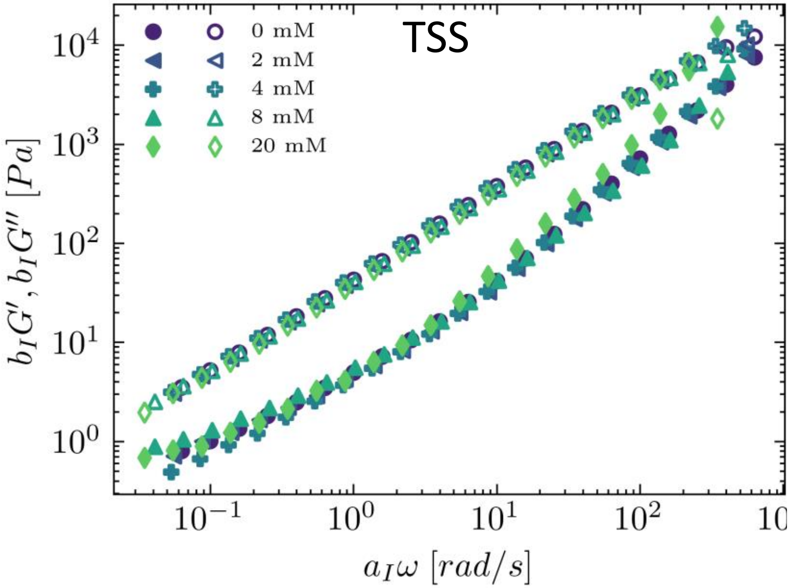


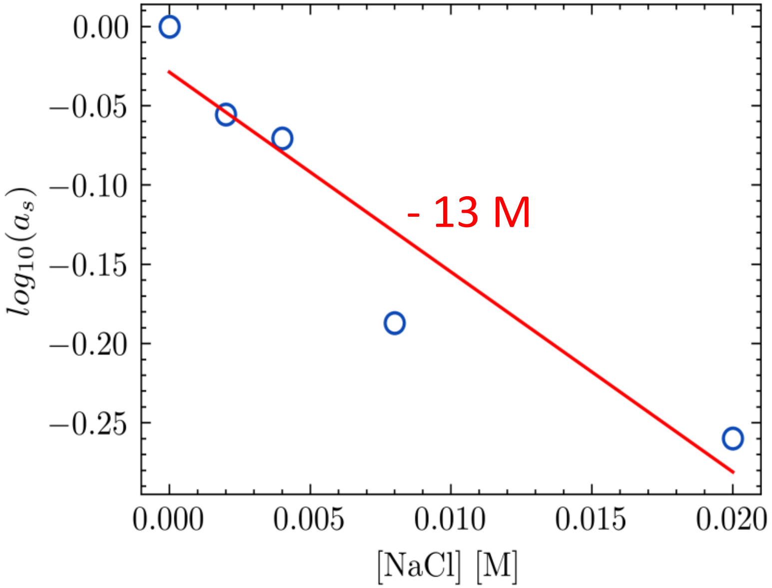
pH5.6

0 - 80 mM

Added [NaCl] (mM)





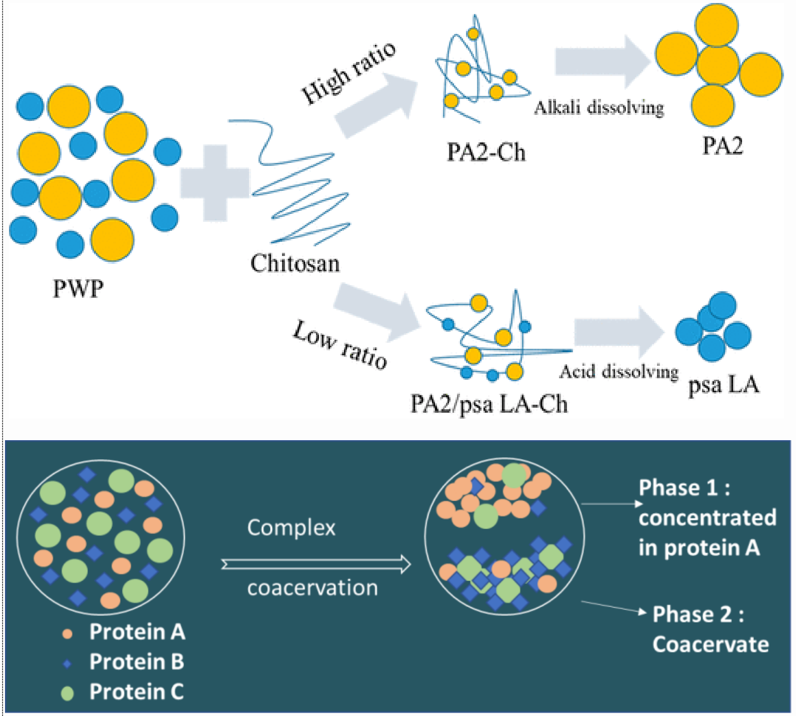


- **Accelerates the coacervates dynamics of the system.**
  - Decrease of the number of intrinsic ion pairs
  - Decrease of the energy of their dissociation (E_a)
  - Reduced local friction

Potential applications

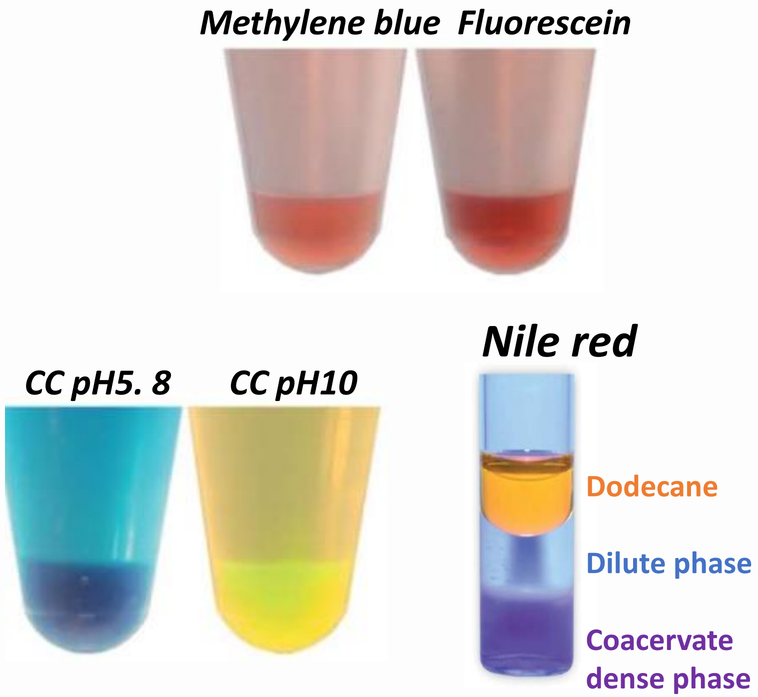
Purification & Encapsulation

Selective Complex Coacervation



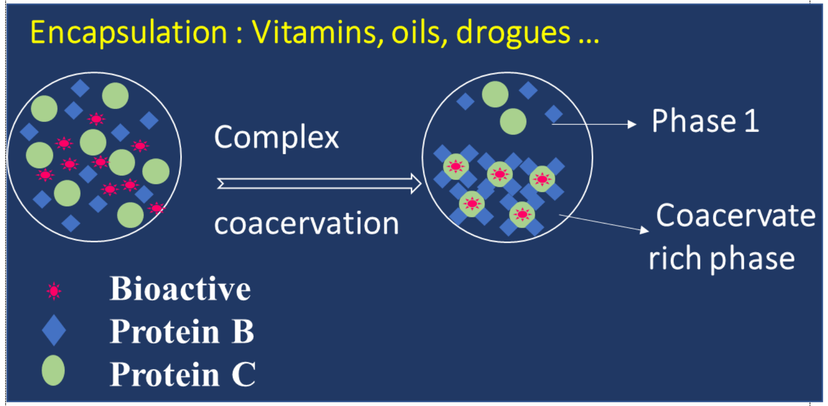
Yang et al., J. Agric. Food Chem. 2020

Sequestration properties (preferntial partionning)



Williams et al., Soft Matter, 2012

Weak attractive interactions

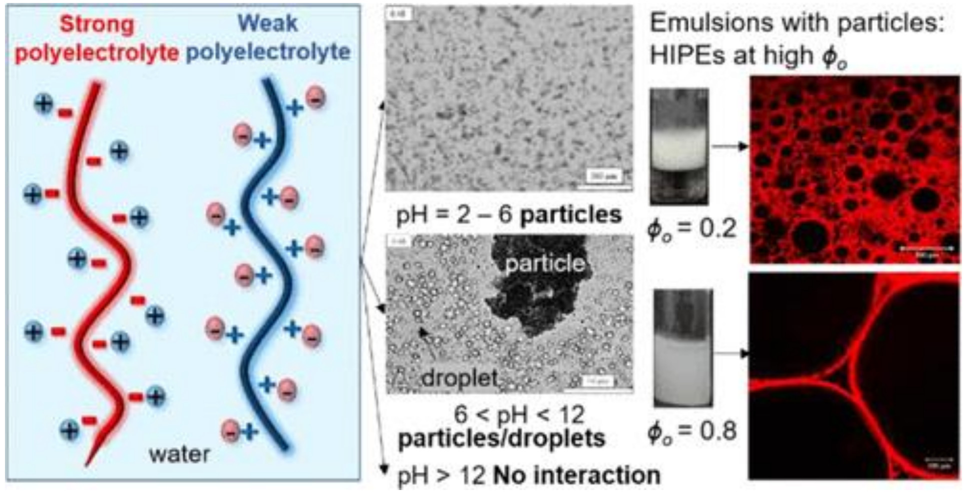


Chapeau et al., Food Hydrocolloids, 2016
McTigue et al., Soft Matter, 2019

III. Emulsion stabilization

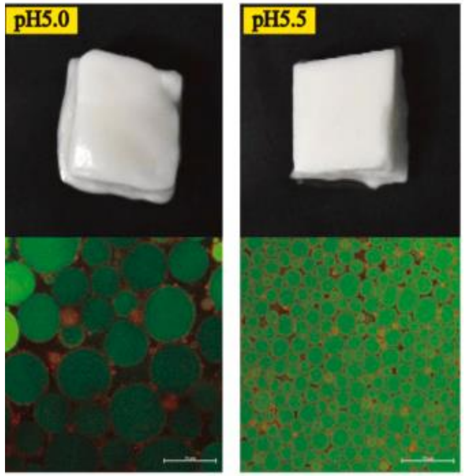
Up to date synthetic PEC/PEC or polysaccharide/proteins coacervates…

PSS / PAH



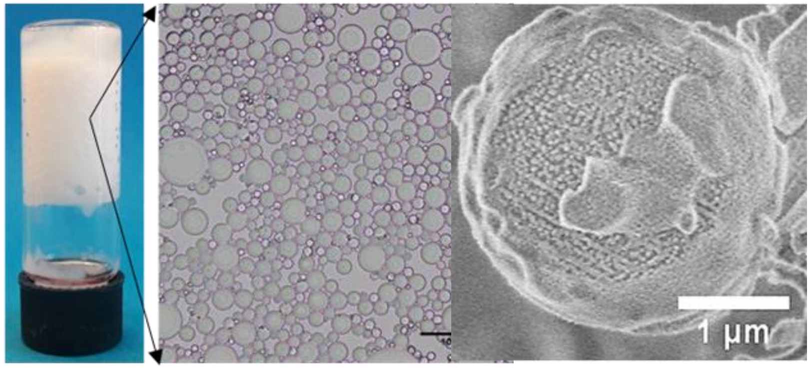
Bago et al., *Langmuir*, 2019

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 dorplets?

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…): Fondamental aspects still to be understood (stabilization mechanism of biphasic systems, preferential partitioning of active molecules,…)

Acknowledgements

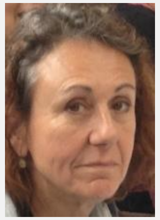
S. Bouhallab

F. Rousseau

P. Hamon

M. Ganne

M.F. Famelart

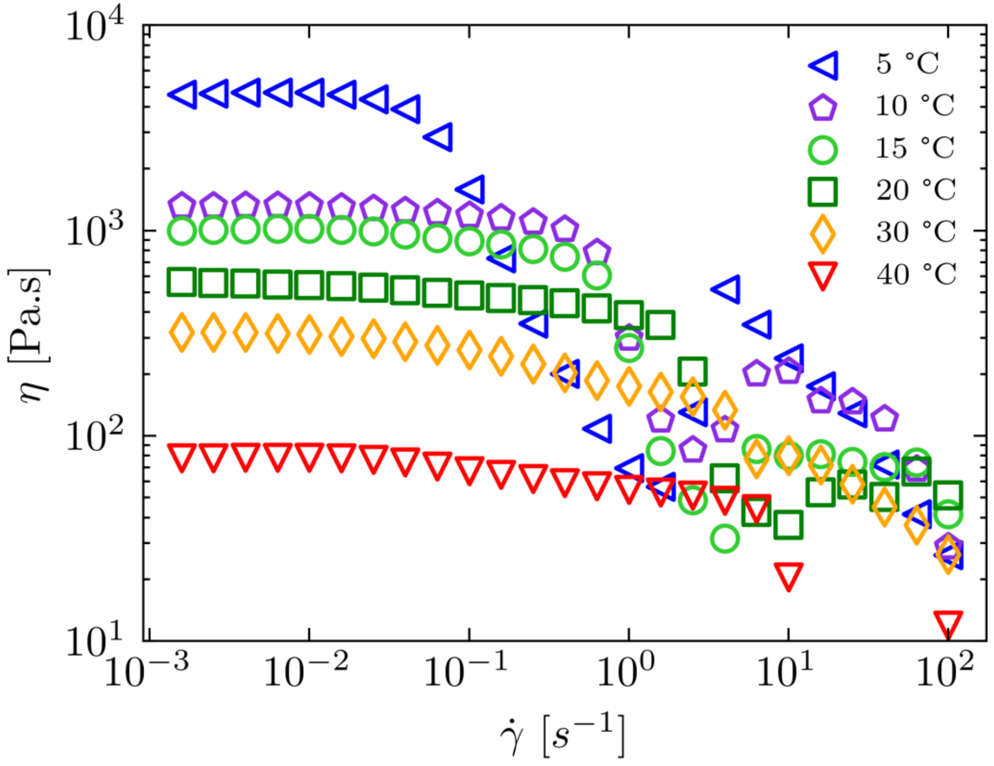


R. Soussi

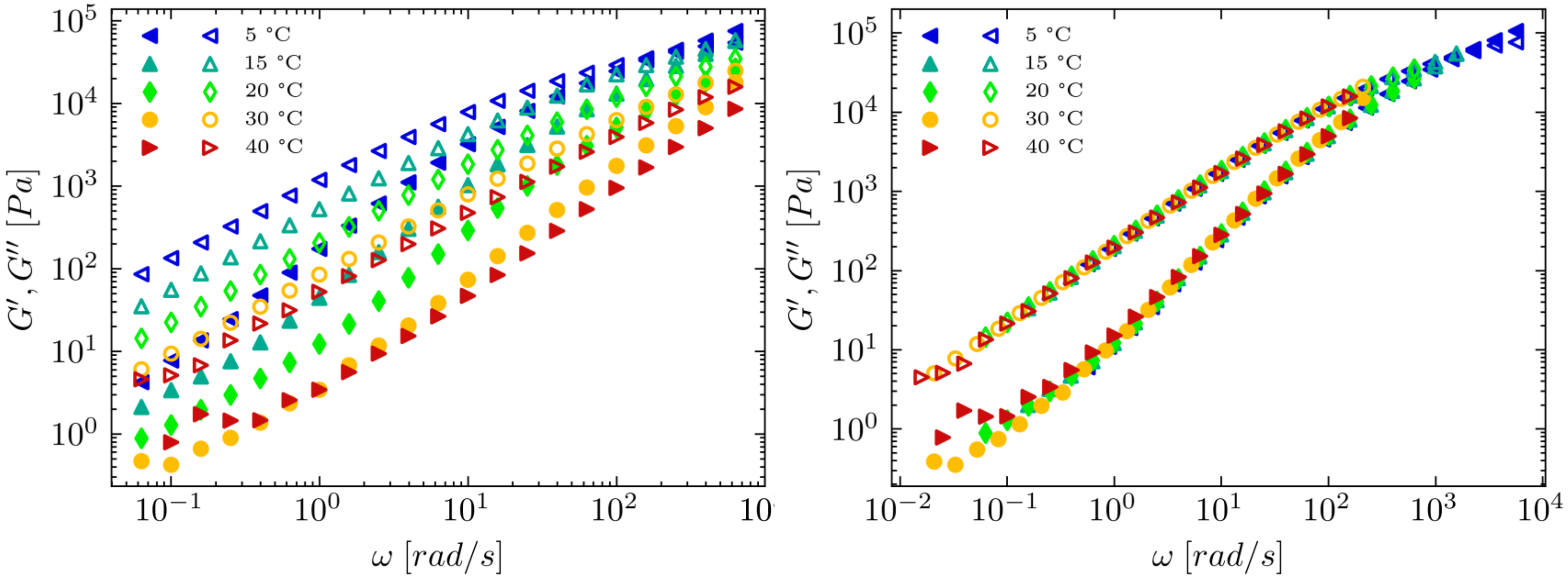
Thank you for your attention

Additional Slides

New Sample Ghazi



New sample Ghazi



New sample Ghazi