Rheological properties of the enzymatic casein gel: dependence with the ion distribution in casein micelles

Julien Bauland, Marie-Hélène Famelart, Fanny Guyomarc'H, Antoine Bouchoux, Thomas Croguennec

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PhD project

"Role of intra and inter casein micelle interactions for the structure and rheology of the enzymatic milk gel"

Introduction

Milk is a colloidal suspension

Caseins = phosphoproteins = main protein fraction of milk

κ

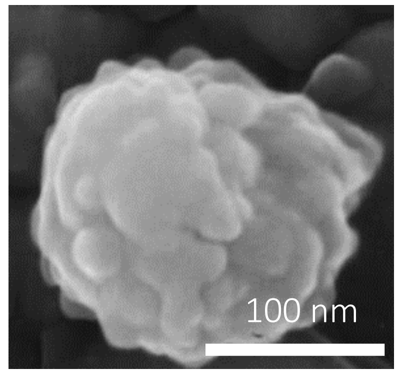
β

α_{s2}

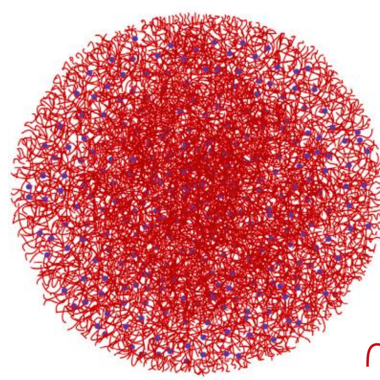
α_{s1}

19-23 KDa

Casein “micelles” = casein + $CaHPO_4$ + H_20



~200 000 KDa

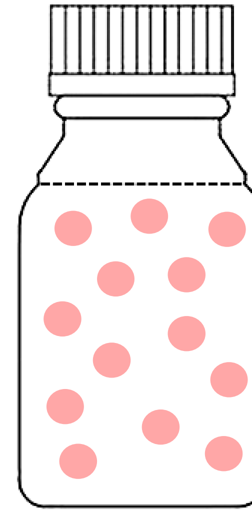


casein

$CaHPO_4$

schematic view

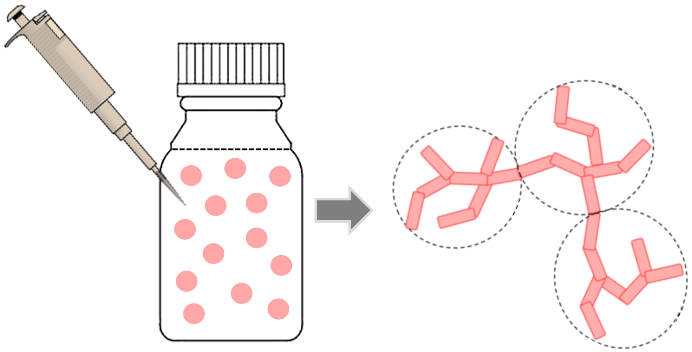
Milk = suspension



$\varphi = 0.1$

Introduction

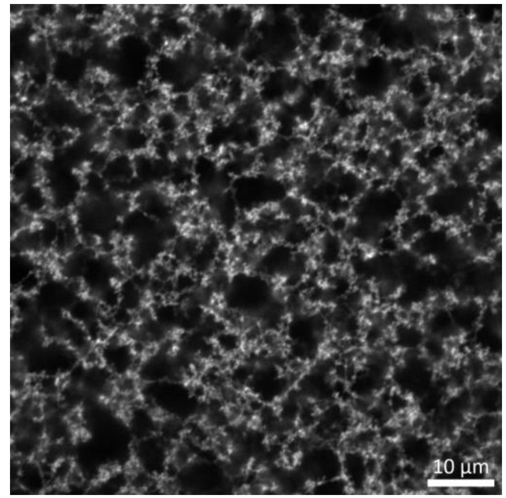
The enzymatic casein gel



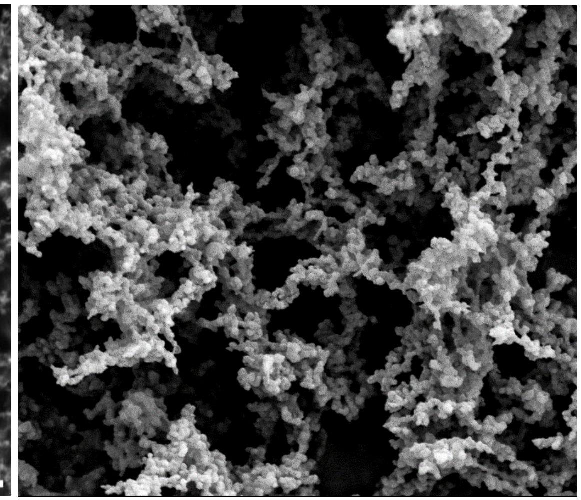
- cluster-cluster aggregation
- fractal organization (D_f = 2.2-2.3)
- physical (non-covalent interactions)
- transient / aging /

  microsyneresis (phase separation)

Microscopic structure

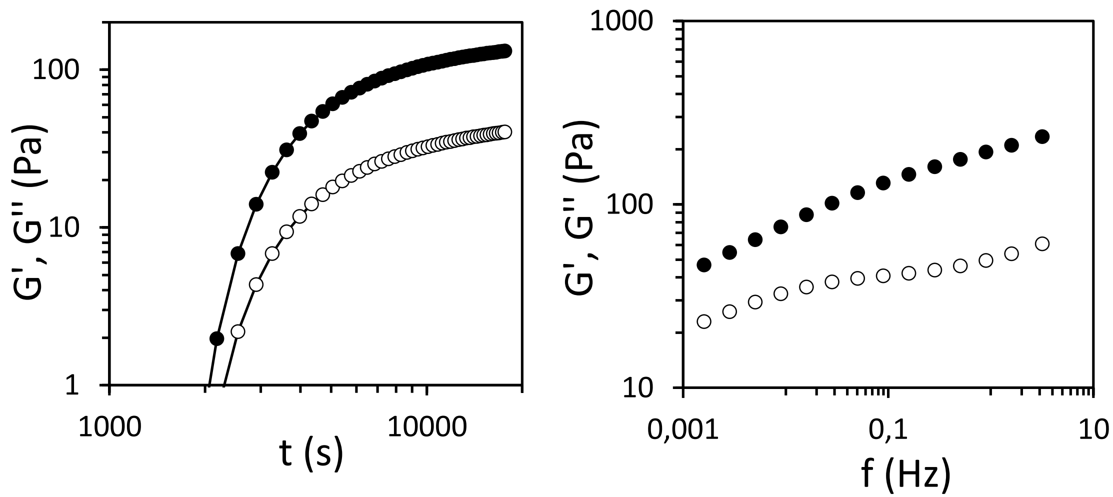


LS confocal



SEM

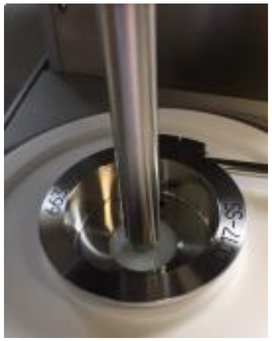
Linear rheology



f=0.1 Hz ; γ=0.01 ; T=30°C

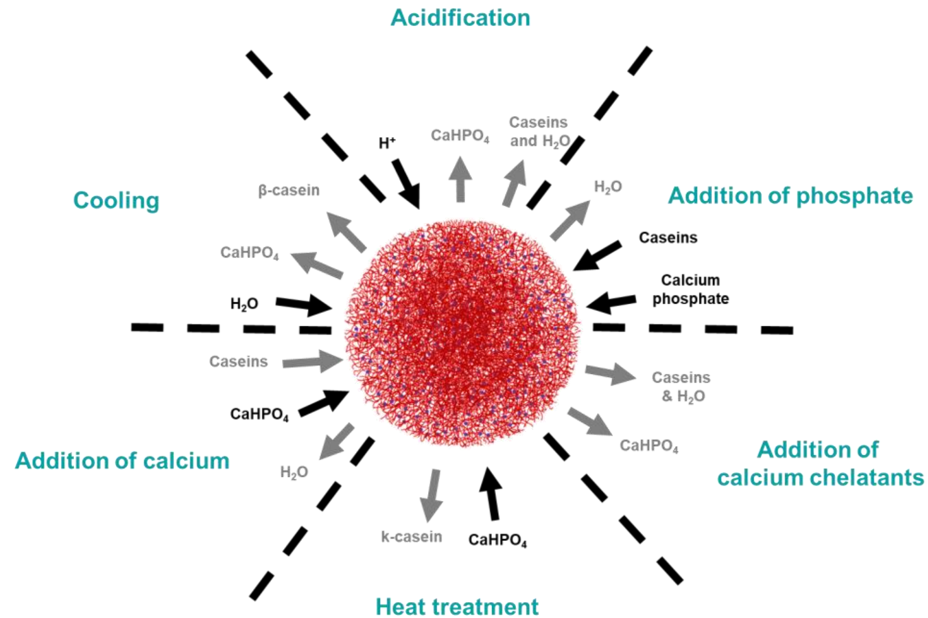
Introduction

Casein micelle is a dynamic object



Gel rheology

Mineral environment of casein micelle



Introduction

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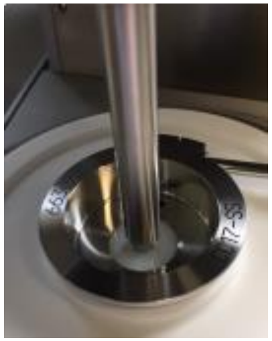
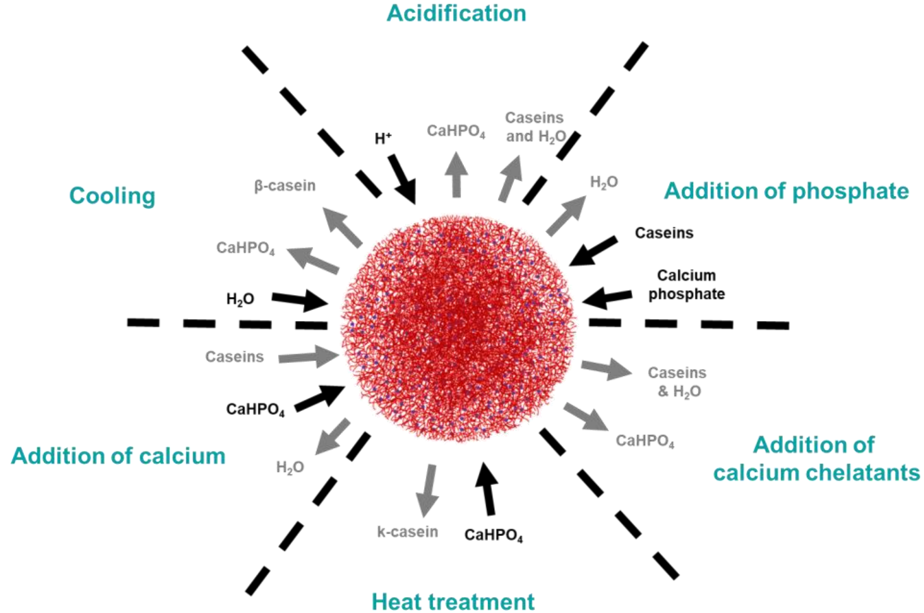
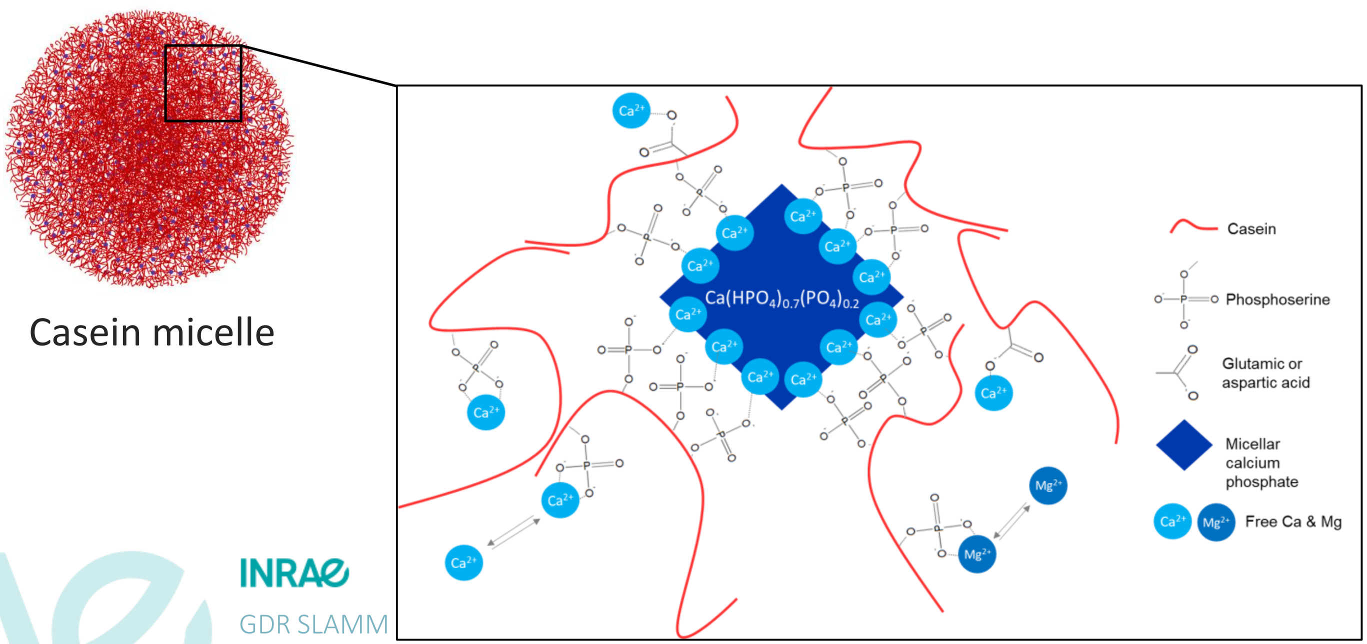


Gel rheology

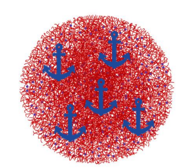
Mineral environment of casein micelle





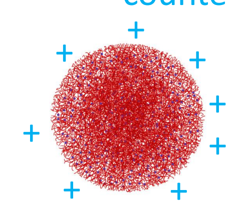
Calcium distribution in casein micelle

Micellar calcium phosphate (MCP) ~crosslink intra



Bound cations (C-bound)

~counter ions



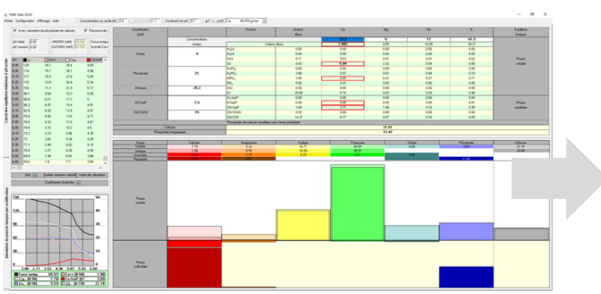
Results

Direct link between cations distribution and rheology

CaCl$_2$
MgCl$_2$
Na$_2$HPO$_4$
CitNa$_3$
HCl

Salt addition
(19 conditions)

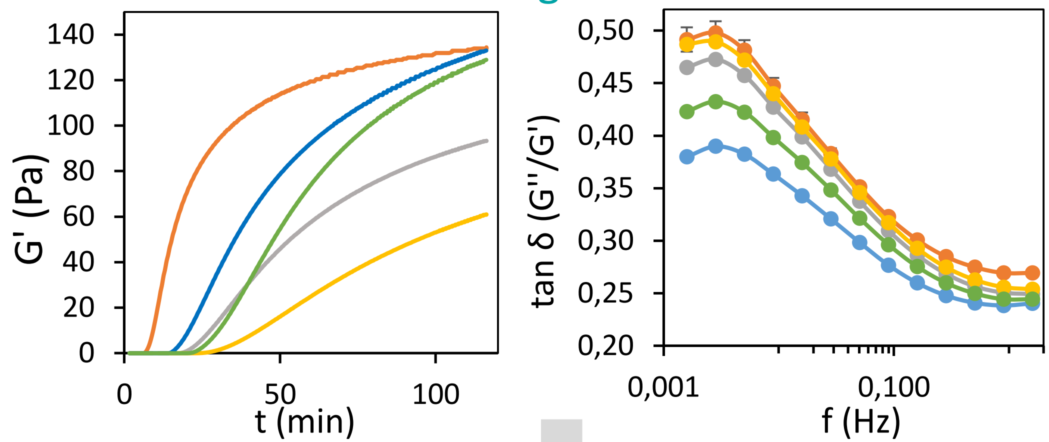
A_i , C_i

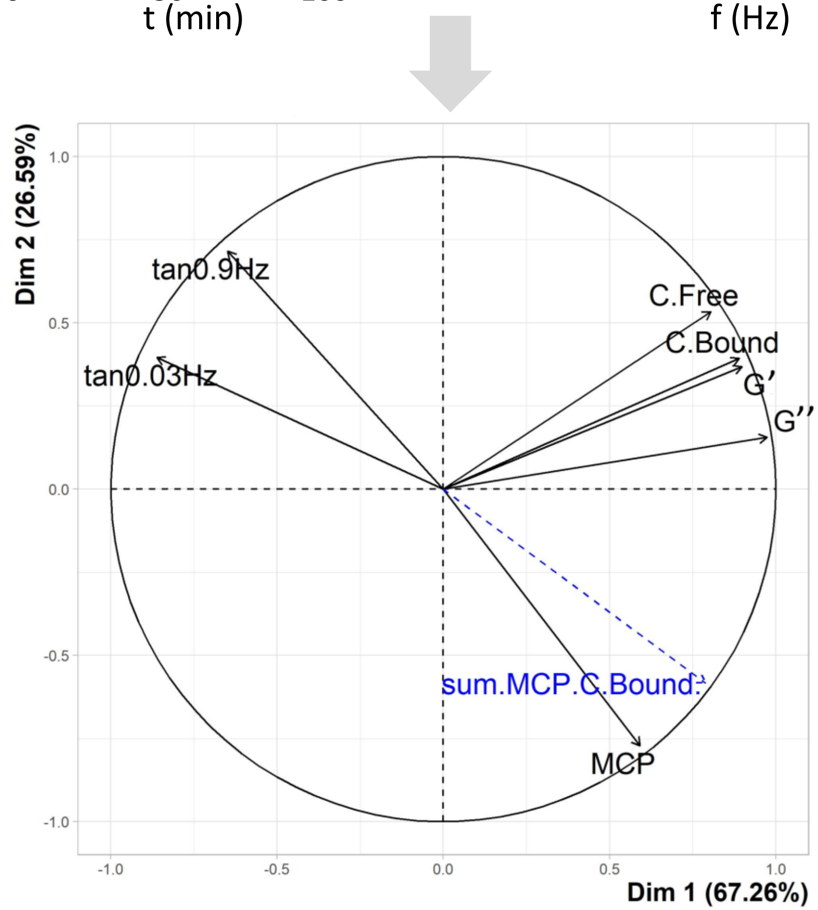


Calculation of mineral distribution

Holt (2004), Mekmene et al. (2009)

Rheological data



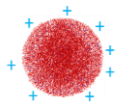


Principal component analysis

Results

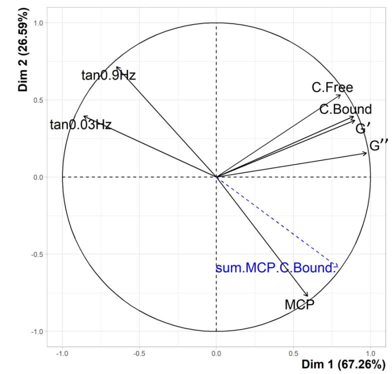
Direct link between cations distribution and rheology

- G'_{max} , G''_{max} = positively correlated to the bound-C

$N \propto \zeta$ 

- $\tan\delta$ = negatively correlated to the MCP content

? 

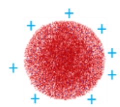


→ Can we explain the link between the gel viscoelasticity and intra-colloid interactions?

Results

Direct link between cations distribution and rheology

- G'_{max} , G''_{max} = positively correlated to the bound-C

$N \propto \zeta$ 

- tanδ = negatively correlated to the MCP content

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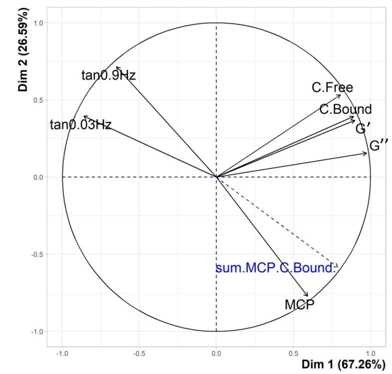
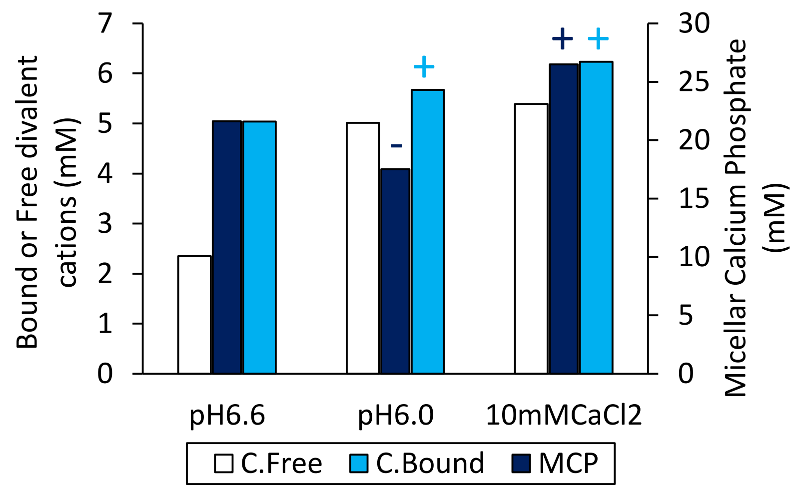
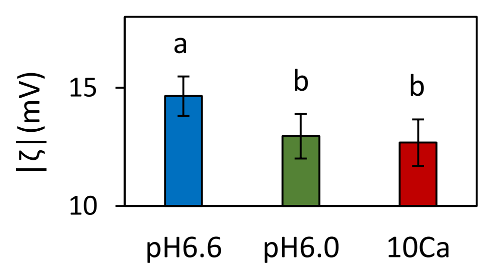


→ Can we explain the link between the gel viscoelasticity and intra-colloid interactions?

- 3 samples with different MCP content
  - pH6.6 *control*
  - pH6.0 *MCP-depleted*
  - 10 mM $CaCl_2$ (pH6.3) *MCP-enriched*



Cations distribution in casein micelle

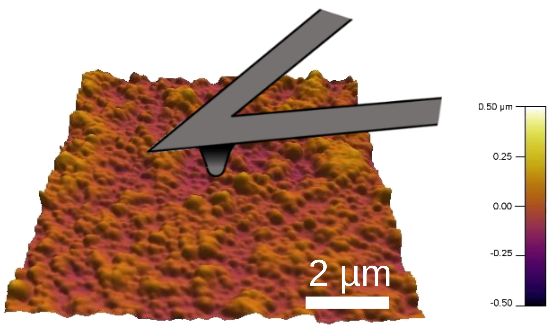


Zeta potential

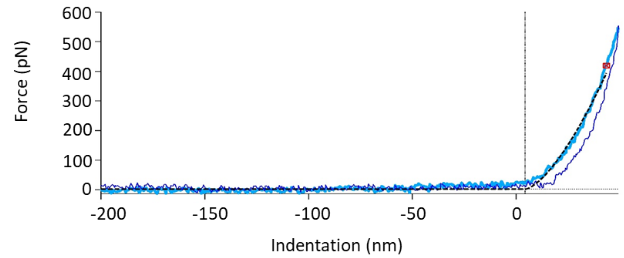
INRAE

Results

Mechanical properties of individual colloids studied by AFM



Single layer of casein micelles



Force curve

N=10,000 force curves over 4-7 chips	MCP-depleted	Control	MCP-enriched	p-value
Young modulus E* (MPa)	0.36 ± 0.24[a]	0.44 ± 0.35[b]	0.56 ± 0.40[c]	2.2×10^{-16}

Average value of Young moduli

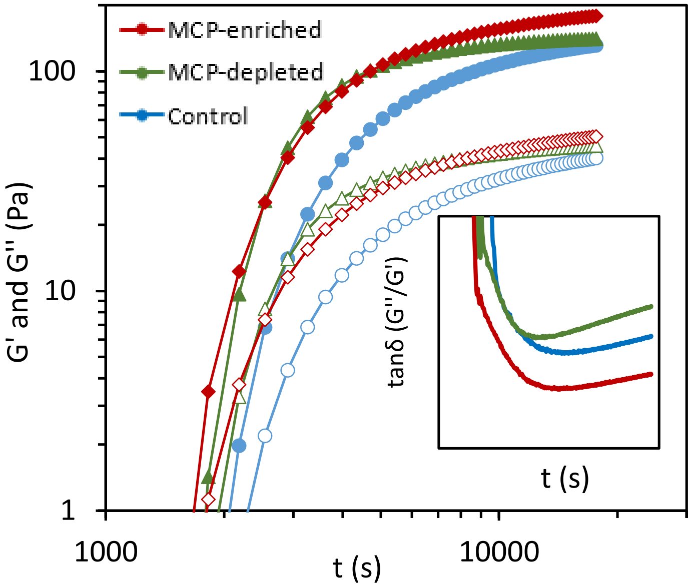
- E* is correlated with the MCP content

E*= 0.27 MPa   pH6.6   *Bahri et al. 2017*
E* ϵ [0.05-0.5] MPa   pH5.2   *Uricanu et al. 2004*

$E^*_{colloid} >> G_{gel}$

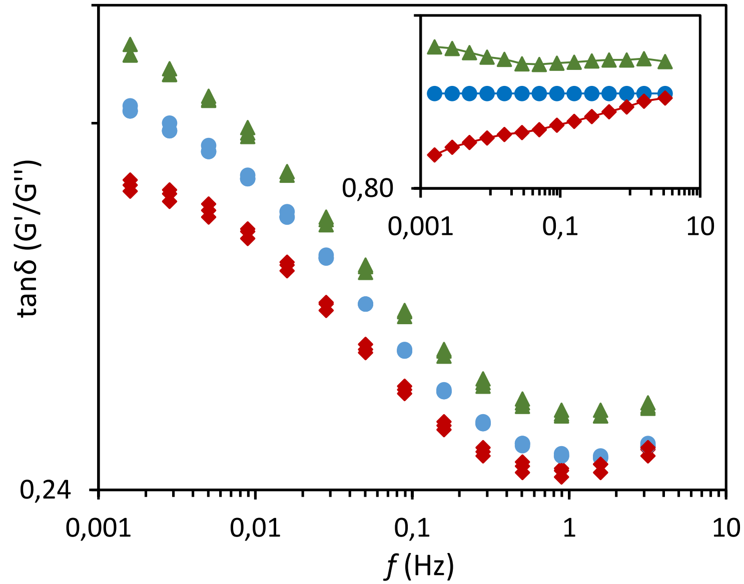
Results

Rheological properties



Kinetics of gelation

f=0.1 Hz ; ɣ=0.01 ; T=30°C

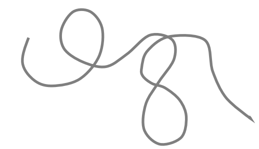


Frequency sweep

ɣ=0.01 ; T=30°C

Ferry (1980)

$H(\tau).d\ln(\tau)$

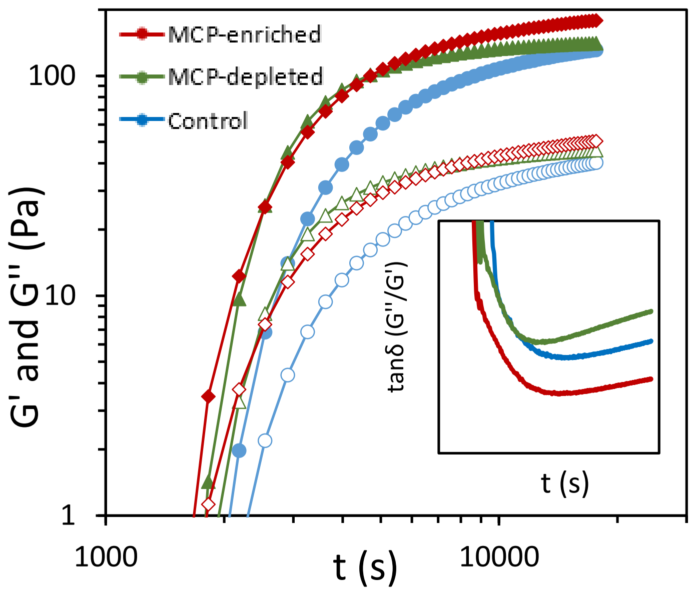


van Vliet et al. (1991)

$\tan\delta \sim H(\tau)_{\tau=1/\omega}$

Results

Rheological properties



Kinetics of gelation

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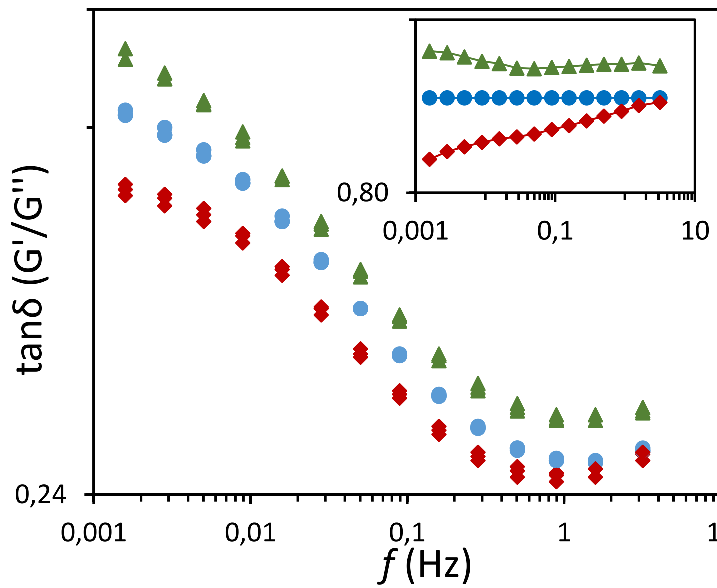

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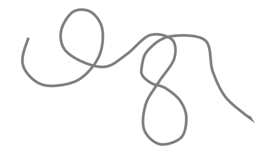

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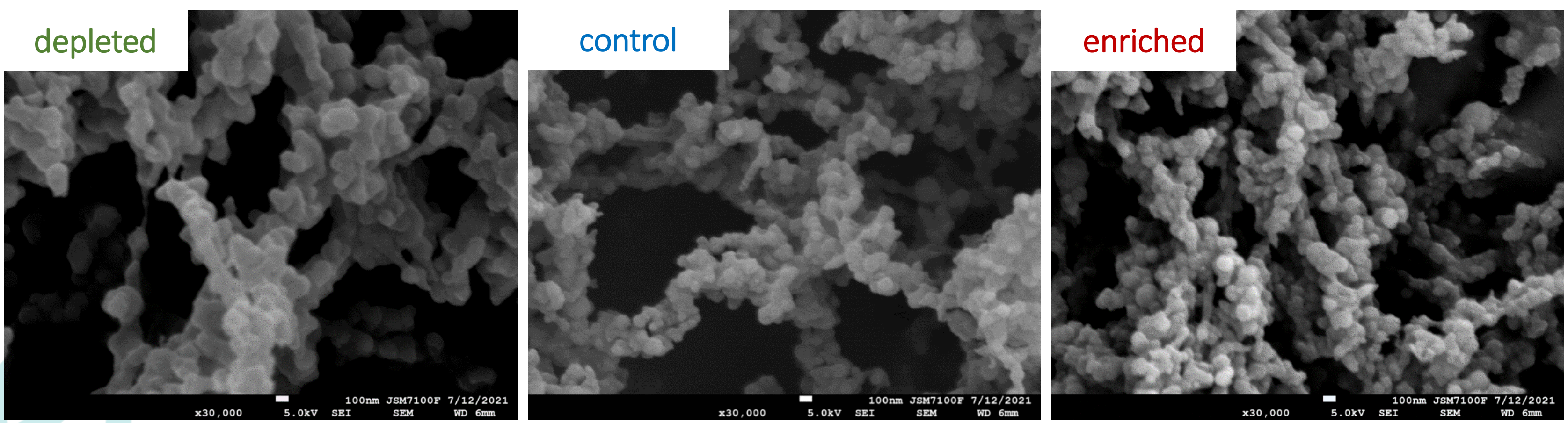


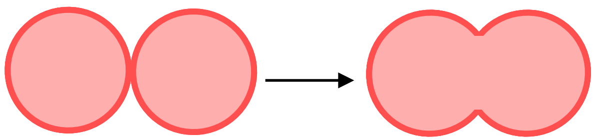
SEM images (t = 9,000 s)

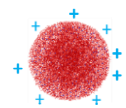
Results

Rheological properties

- Fusion = motor of rearrangement → stress in network

van Vliet et al. (1991)
Mellema et al. (2002)



+ fusion (i) Strength attraction ⟷ C-bound 

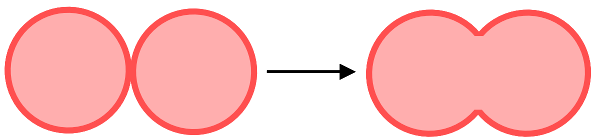
- fusion (ii) Rigidity (E*) ⟷ MCP 

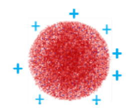
Results

Rheological properties

- Fusion = motor of rearrangement → stress in network

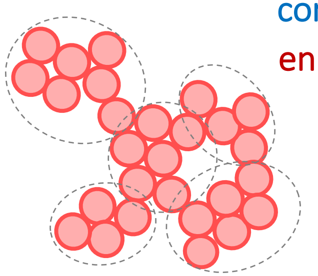
van Vliet et al. (1991)
Mellema et al. (2002)

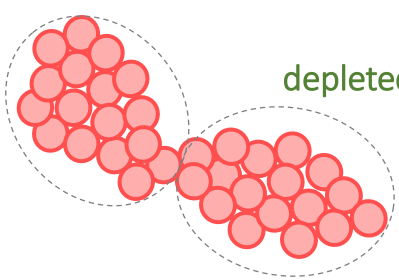


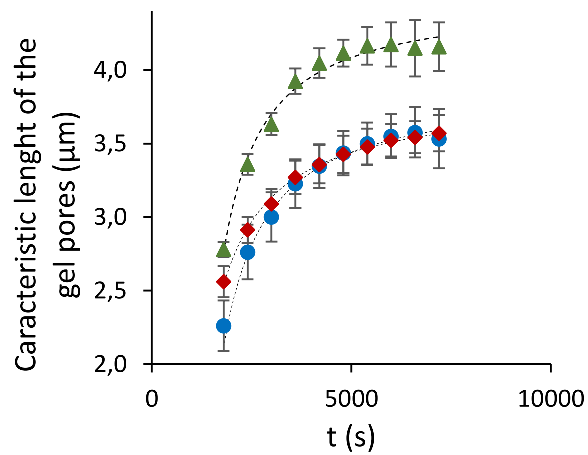
+ fusion (i) Strength attraction ↔ C-bound 

- fusion (ii) Rigidity (E*) ↔ MCP 

- Differences of fusion rate / particle distribution

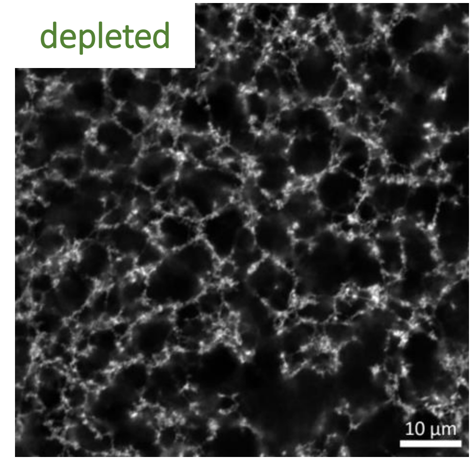


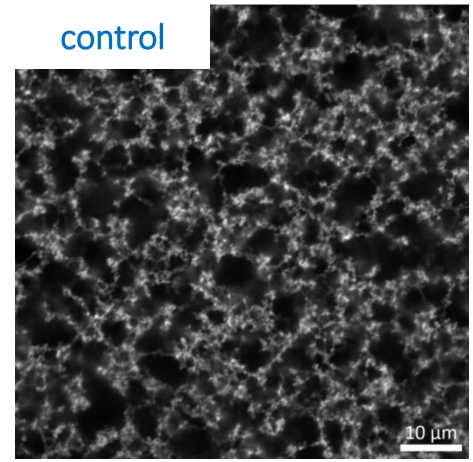


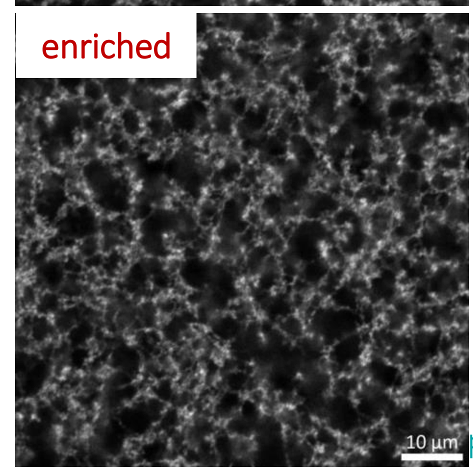


Size of the gel pores

- Heterogeneous = Stress concentration = further reorganization



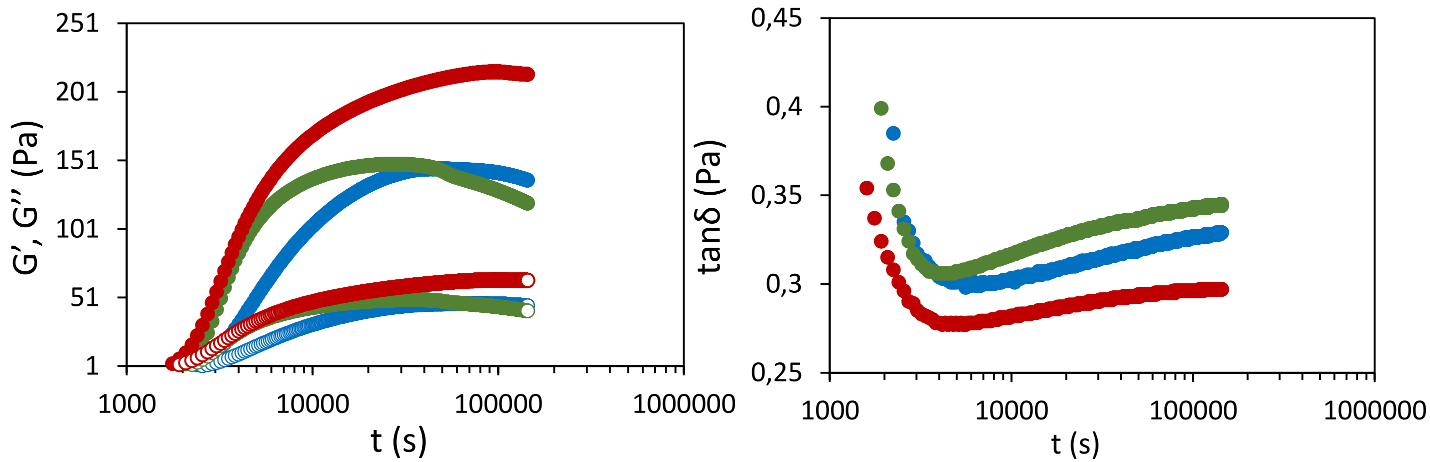




Results

Discussion

- Is this picture consistent? / Lack of theoretical framework



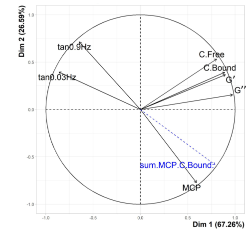
- Different kinetics of phase separation
    - Creation and relaxation of internal stress?

- What can be learned from the aging dynamic? ⇄ Glassy soft matter systems

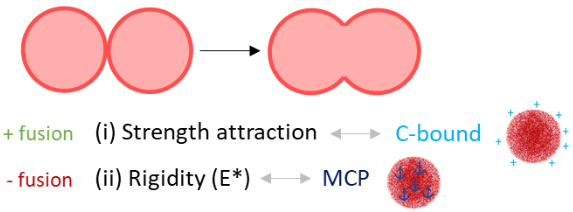
Cipelletti and Ramos (2005)

Conclusion

- Industrial window for enzymatic casein gel processing
  → Gel rheology correlated to 2 cation forms in casein micelle



- 2 cations form → different effect on particle fusion → structure heterogeneity



- Need of theoretical work to study aging dynamics / internal stress relaxation

Thanks for your attention!

Thanks to Lazhar Benyahia and Mathieu Leocmach!

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