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

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UMR STLO, Rennes, France

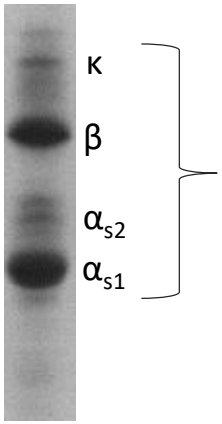
PhD project

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

Introduction

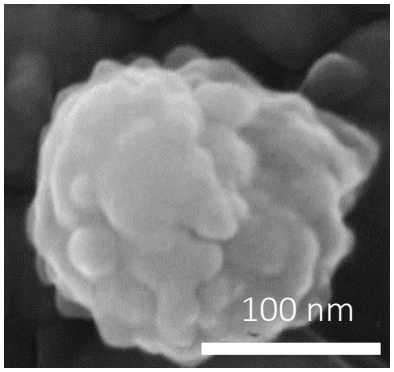
Milk is a colloidal suspension

Caseins = phosphoproteins = main protein fraction of milk

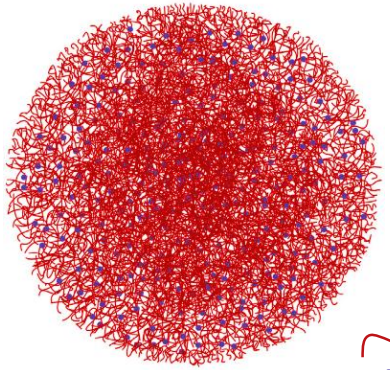


19-23 KDa

Casein "micelles" = casein + $CaHPO_4$ + H_20

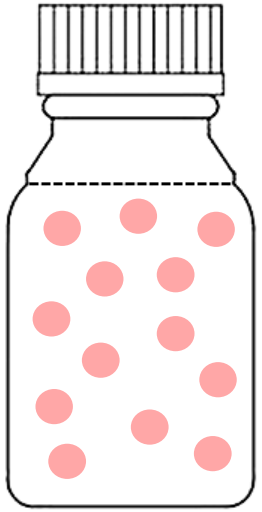


~200 000 KDa



schematic view

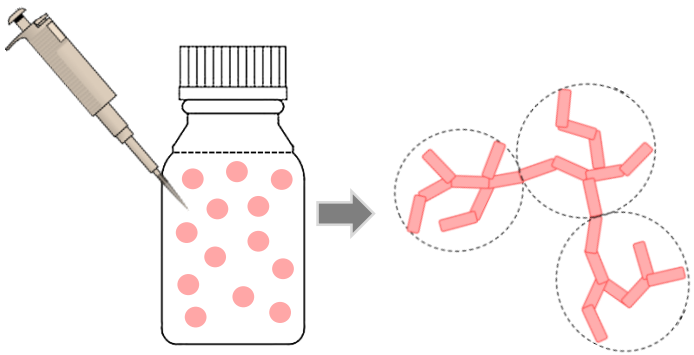
Milk = suspension



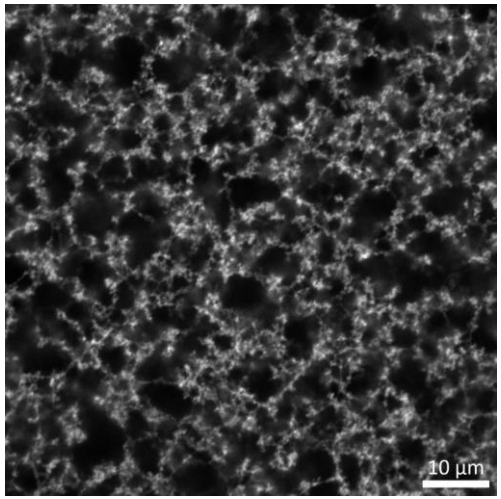
φ = 0.1

Introduction

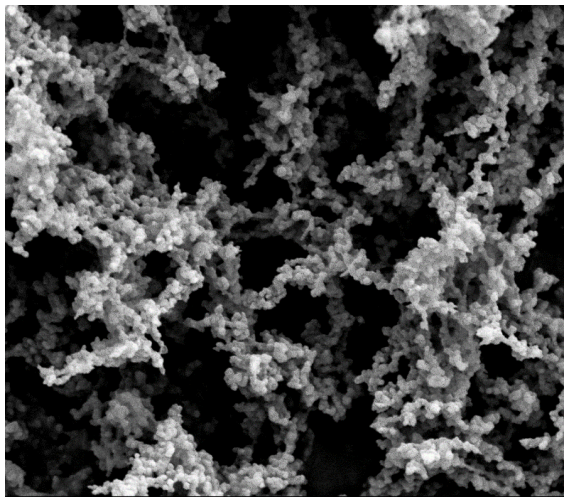
The enzymatic casein gel



Microscopic structure



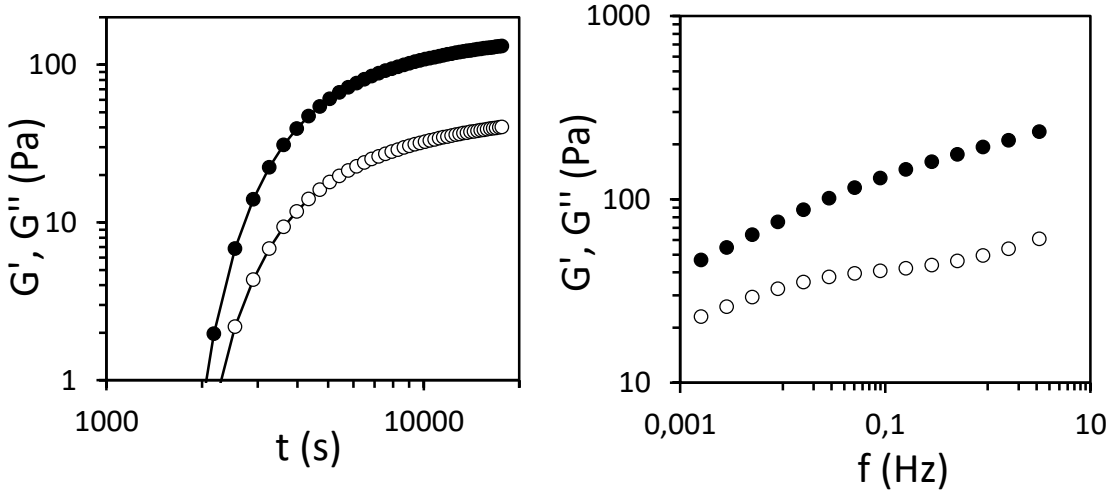
LS confocal



SEM

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

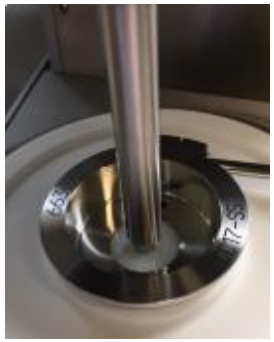
Linear rheology



f=0.1 Hz ; γ=0.01 ; T=30°C

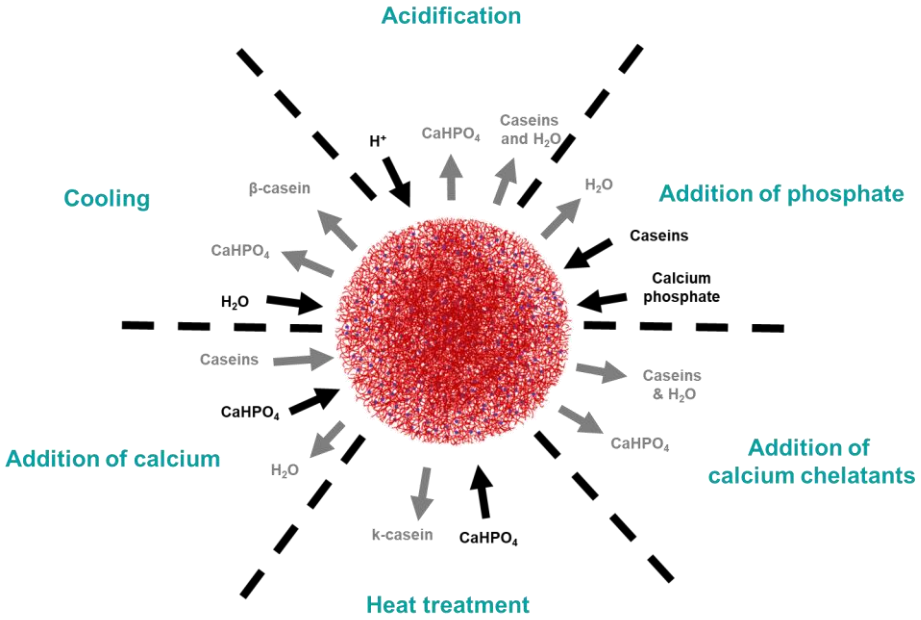
Introduction

Casein micelle is a dynamic object



Gel rheology

Mineral environment of casein micelle



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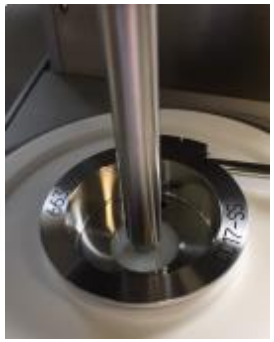

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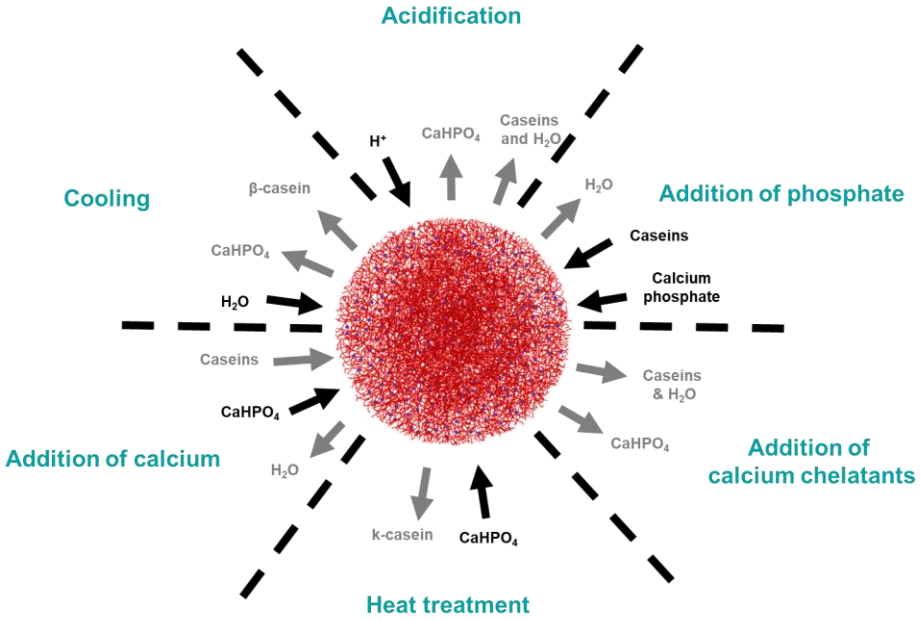
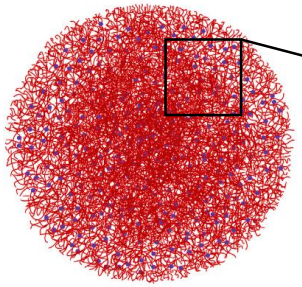
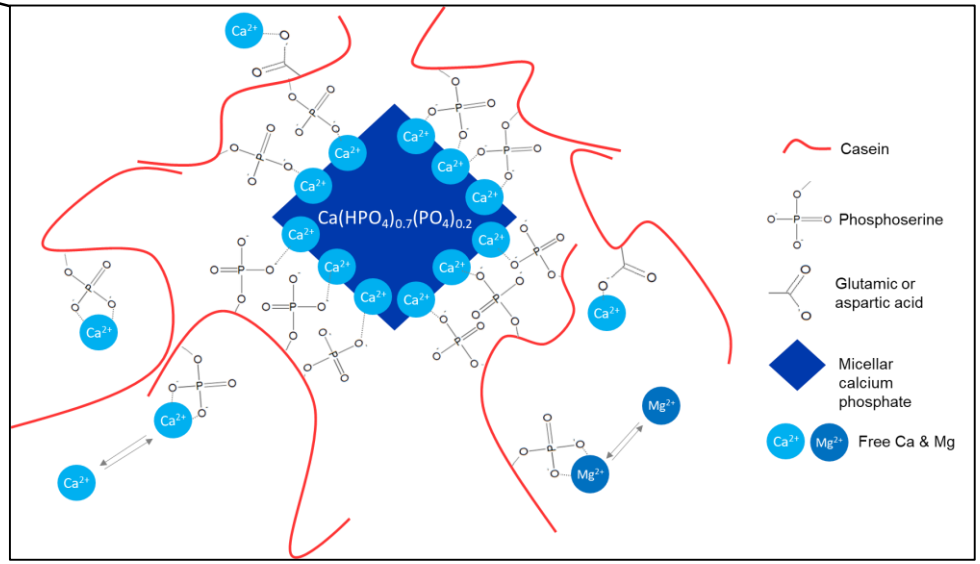


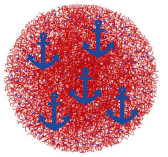


Casein micelle



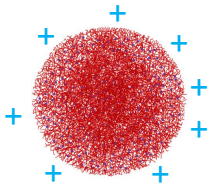
Calcium distribution in casein micelle

Micellar calcium phosphate (MCP) ~crosslink intra



Bound cations (C-bound)

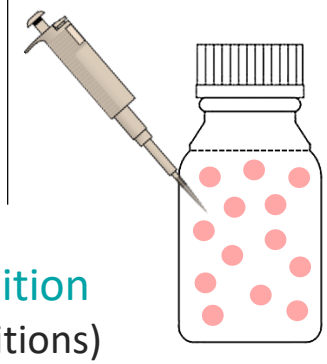
~counter ions



Results

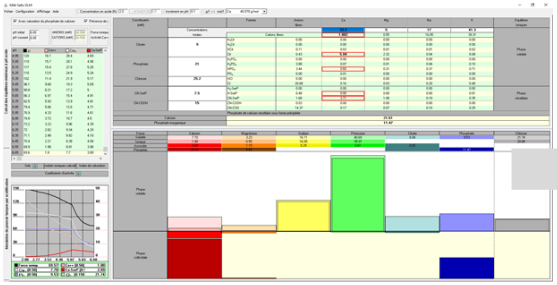
Direct link between cations distribution and rheology

$CaCl_2$
$MgCl_2$
Na_2HPO_4
$CitNa_3$
HCl



Salt addition
(19 conditions)

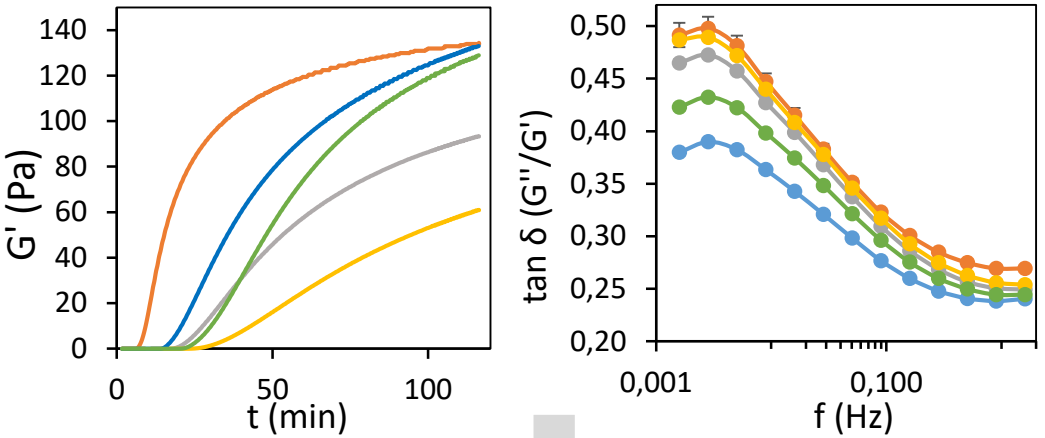
A_i , C_i

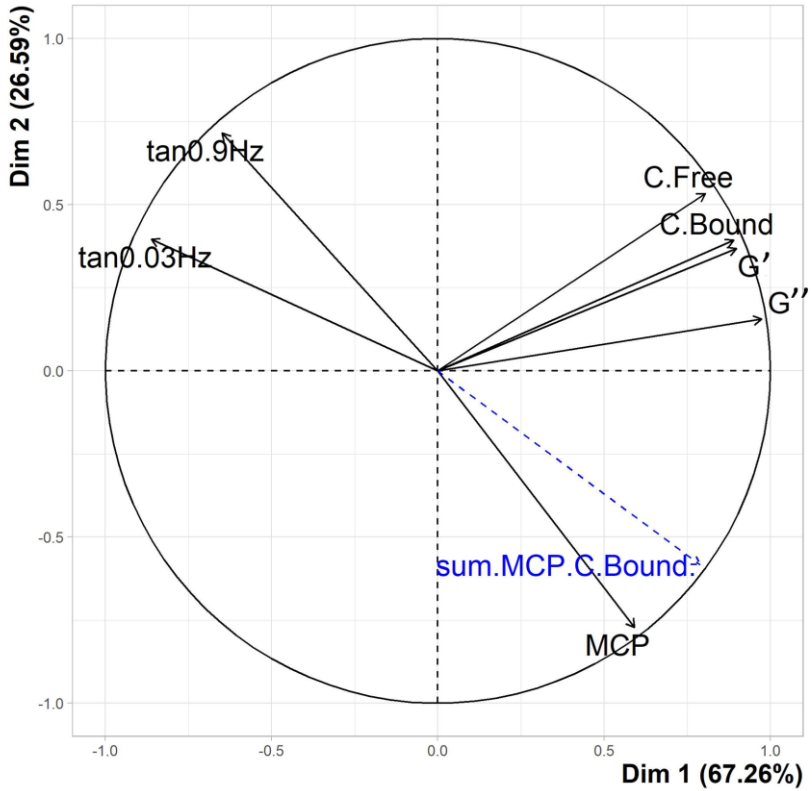


Calculation of mineral distribution

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

Rheological data



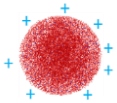


Principal component analysis

Results

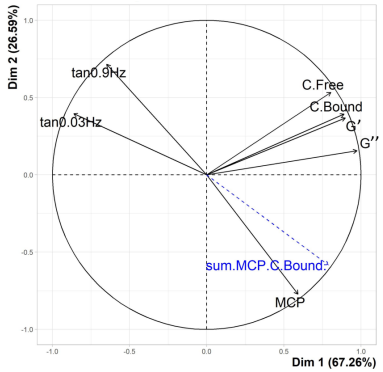
Direct link between cations distribution and rheology

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

$N \propto \zeta$ 

- tanδ = negatively correlated to the MCP content

? 



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

Results

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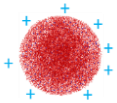
$N \propto \zeta$ 

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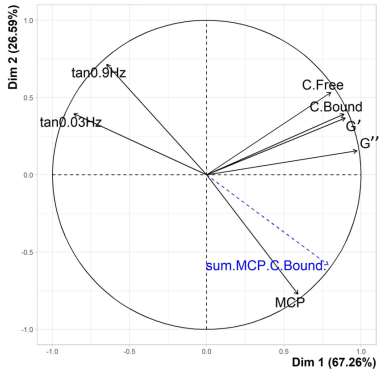
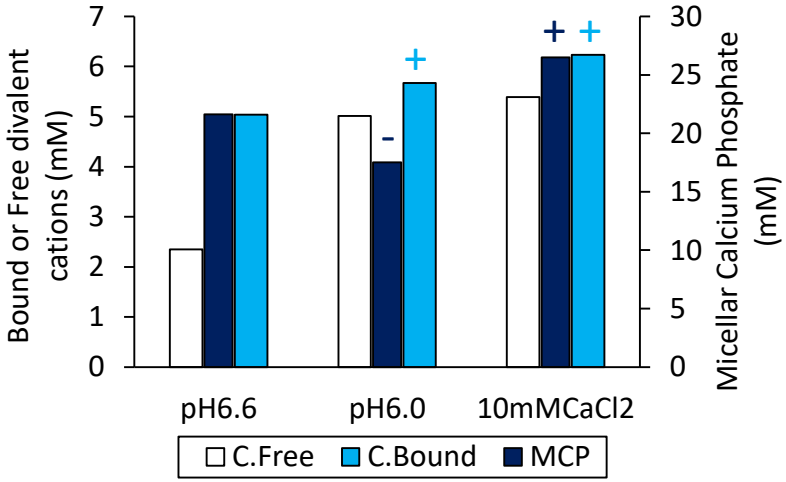
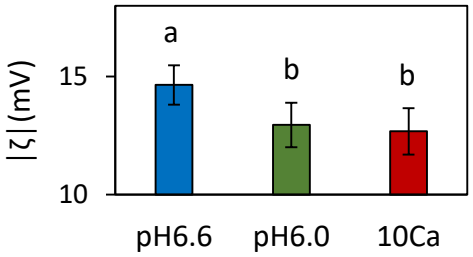


→ Can we explain the link between the gel viscoelasticity and intra-colloid interactions?

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



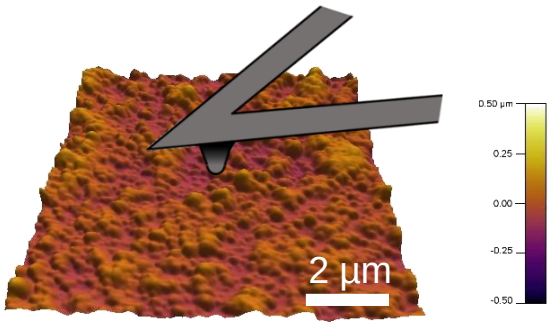
Cations distribution in casein micelle



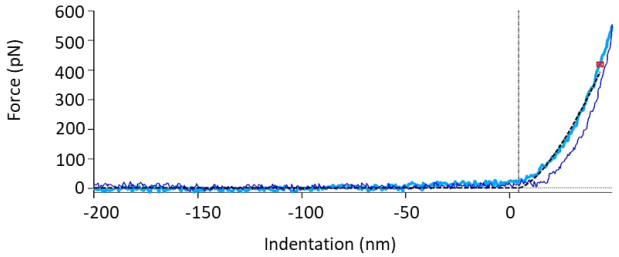
Zeta potential

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

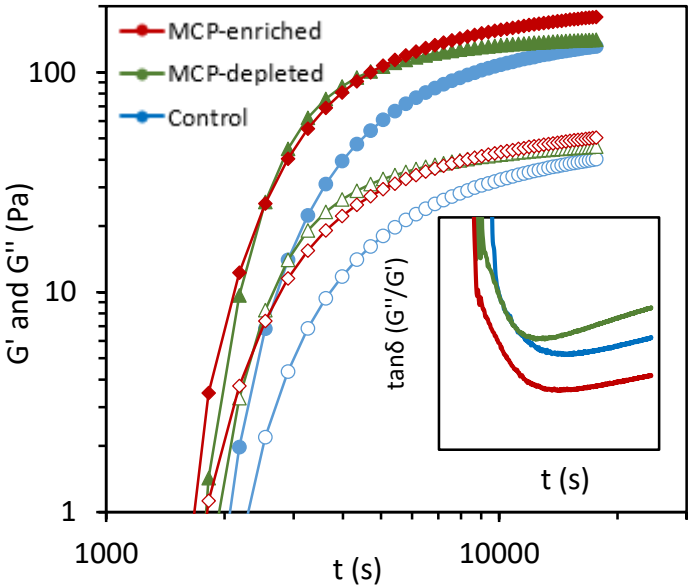
- E* is correlated with the MCP content

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

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

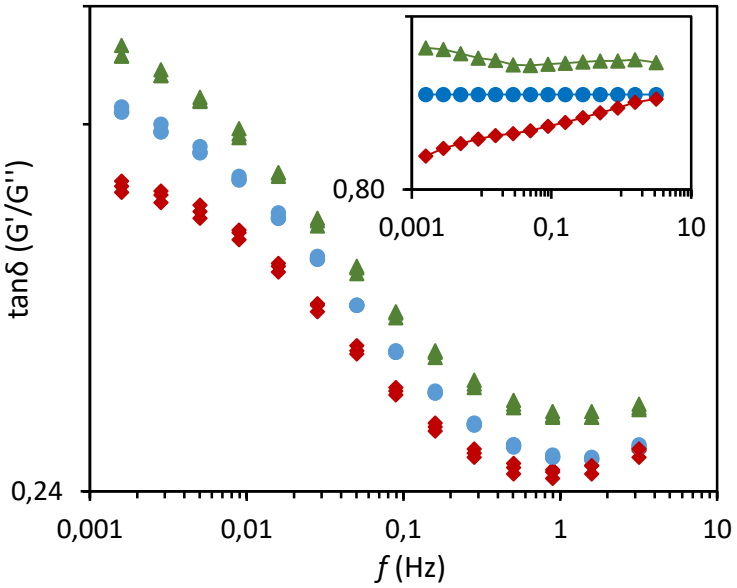
Results

Rheological properties



Kinetics of gelation

f=0.1 Hz ; γ=0.01 ; T=30°C

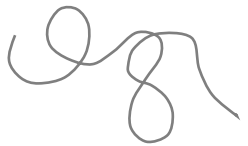


Frequency sweep

γ=0.01 ; T=30°C

Ferry (1980)

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

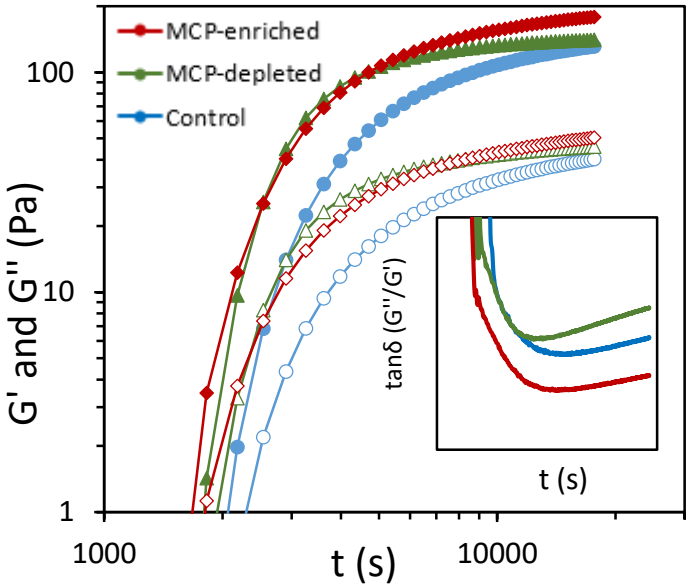


van Vliet et al. (1991)

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

Results

Rheological properties



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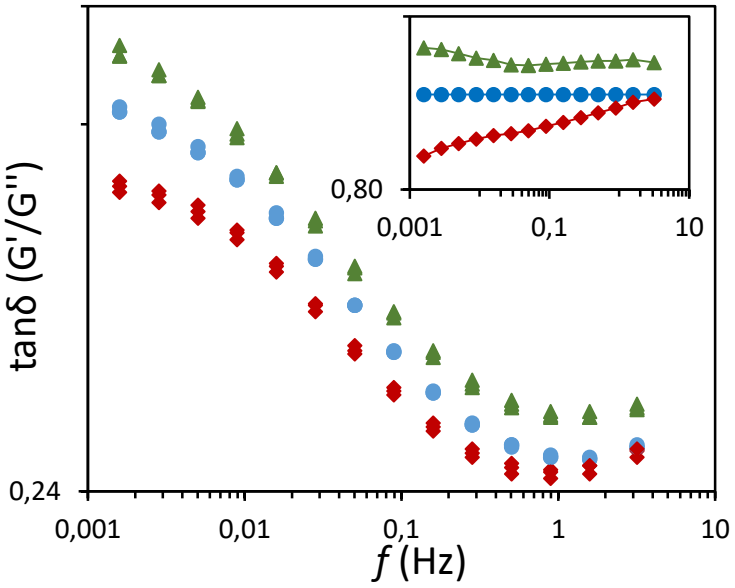

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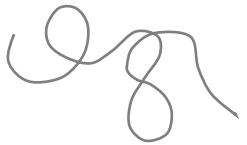

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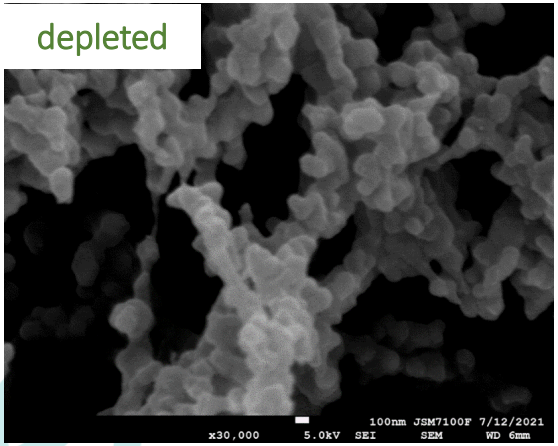
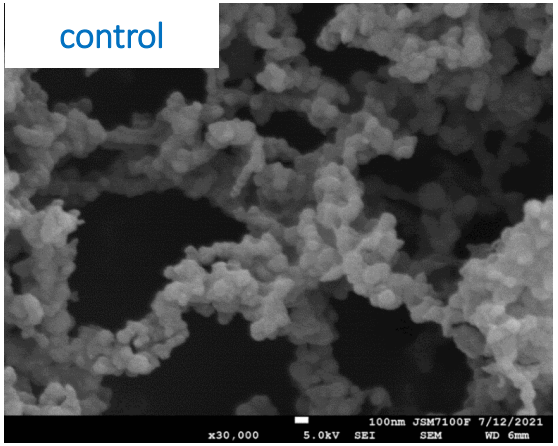
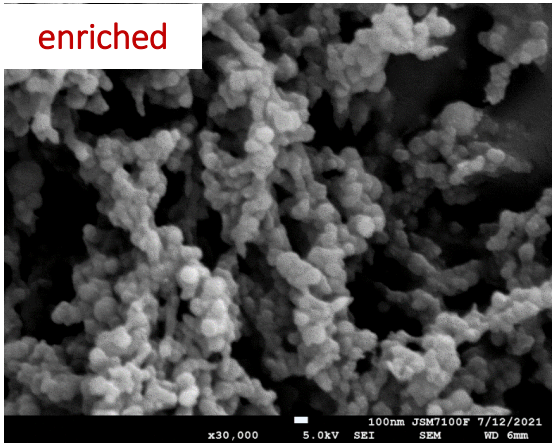






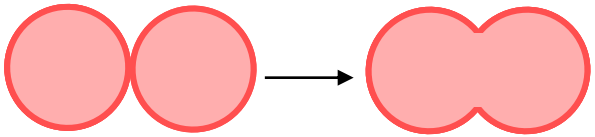
SEM images (t = 9,000 s)

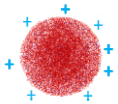
Results

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 

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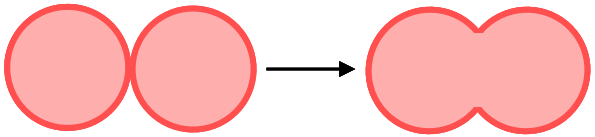

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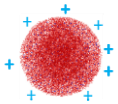
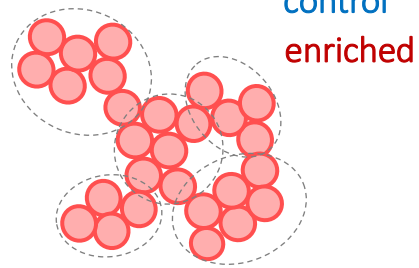
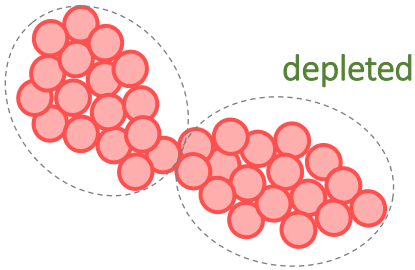
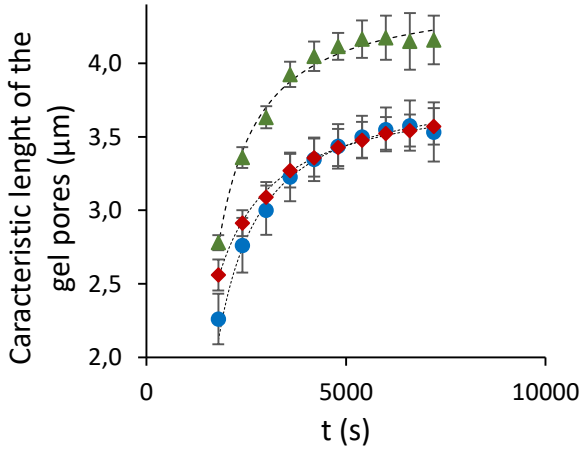


- fusion (ii) Rigidity (E*) ⟷ MCP

- Differences of fusion rate / particle distribution

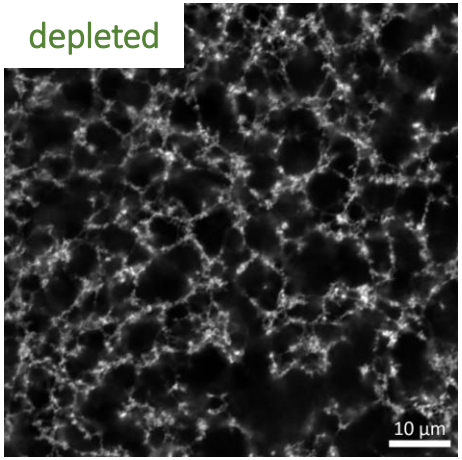


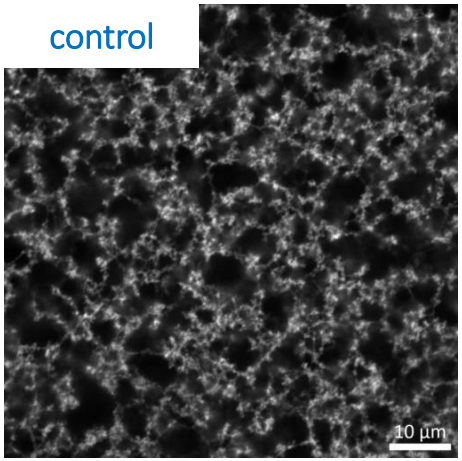


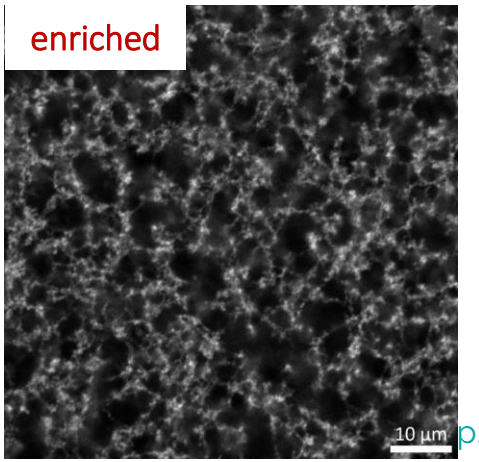


Size of the gel pores

- Heterogeneous = Stress concentration = further reorganization



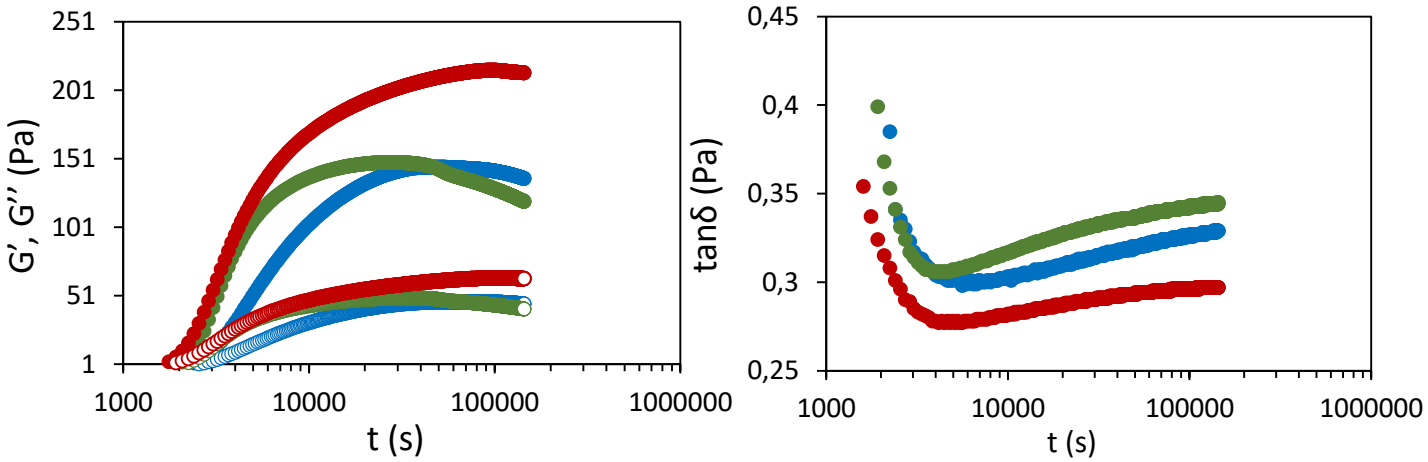




Results

Discussion

- Is this picture consistent? / Lack of theoretical framework



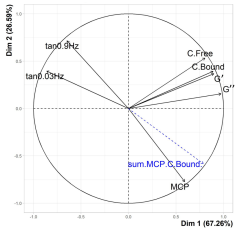
- Different kinetics of phase separation
  - Creation and relaxation of internal stress?

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

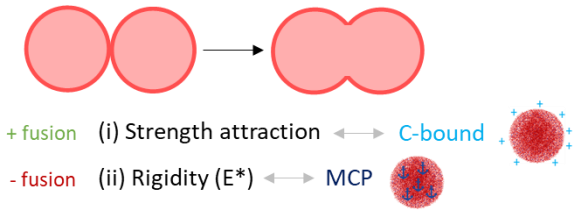
Cipelletti and Ramos (2005)

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!

References

Bahri, A., Martin, M., Gergely, C., Pugnière, M., Chevalier-Lucia, D., & Marchesseau, S. (2017). Atomic Force Microscopy Study of the Topography and Nanomechanics of Casein Micelles Captured by an Antibody. *Langmuir*, *33*(19), 4720–4728.

Cipelletti, L., Ramos, L. (2005). Slow dynamics in glassy soft matter. *J. Phys. Cond. Mat*. 17:R253-R85

Ferry, J. D. (1980). Viscoelastic properties of polymers, 3rd edition. *Wiley, New York*, 672.

Holt, C. (2004). An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. *European Biophysics Journal*, *33*(5), 421–434.

Mekmene, O., Le Graët, Y., & Gaucheron, F. (2009). A model for predicting salt equilibria in milk and mineral-enriched milks. *Food Chemistry*, *116*(1), 233–239.

Mellema, M., Walstra, P., van Opheusden, J. H. ., & van Vliet, T. (2002). Effects of structural rearrangements on the rheology of rennet-induced casein particle gels. *Advances in Colloid and Interface Science*, *98*(1), 25–50.

Uricanu, V. I., Duits, M. H. G., & Mellema, J. (2004). Hierarchical networks of casein proteins: An elasticity study based on atomic force microscopy. *Langmuir*, *20*(12), 5079–5090.

van Vliet, T., van Dijk, H. J. M. M., Zoon, P., & Walstra, P. (1991). Relation between syneresis and rheological properties of particle gels. *Colloid & Polymer Science*, *269*(6), 620–627.