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Soleil Synchrotron illuminates our knowledge on gastric digestion

Juliane Floury

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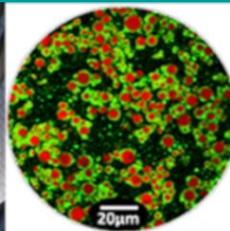
AGRO
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illuminates our knowledge on gastric digestion



STLOpen Days
19-21 March 2019



Juliane Flourey – Bioactivity & Nutrition team

J.Thévenot¹, D.Dupont¹, F.Jamme², E.Lutton³, M.Panouillé³, F.Boué³, S.Le Feunteun^{3→1}

(1) UMR INRA-AGROCAMPUS OUEST, Rennes, France

(2) SOLEIL synchrotron, Gif-sur-Yvette, France

(3) UMR INRA-AGROPARISTECH, Grignon, France.



Context Food structure affects nutritional properties and possible health effects of nutrients

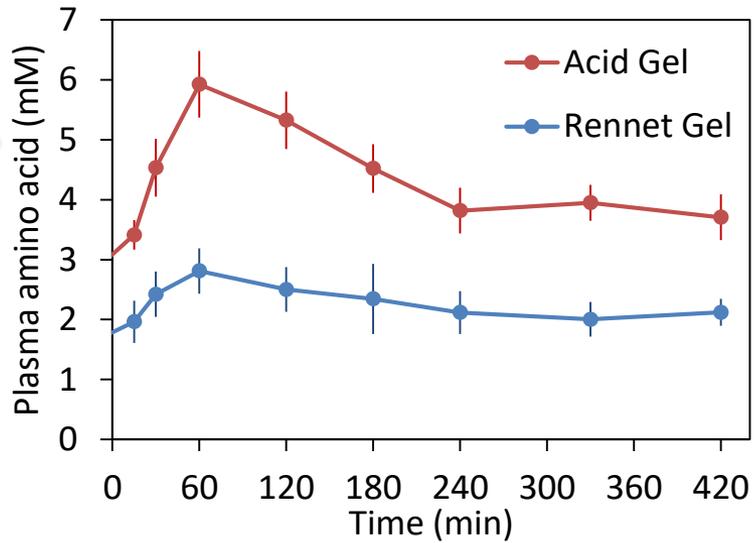
Curd-type milk gel using rennet
pH 6.6



Yogurt acid-type gel
pH 4



In vivo digestion



In vitro digestion



The rennet gel forms a compact coagulum in the stomach due to acid gastric fluids
→ the gastric emptying of caseins is slowed down

The structure of dairy products regulates the kinetics of protein digestion and the release of amino acids in the bloodstream.

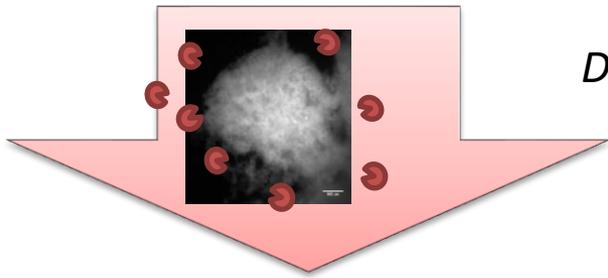
Understanding the mechanisms of food particle breakdown in the stomach is critical to control the structure that a food will adopt in gastric conditions.

Objective

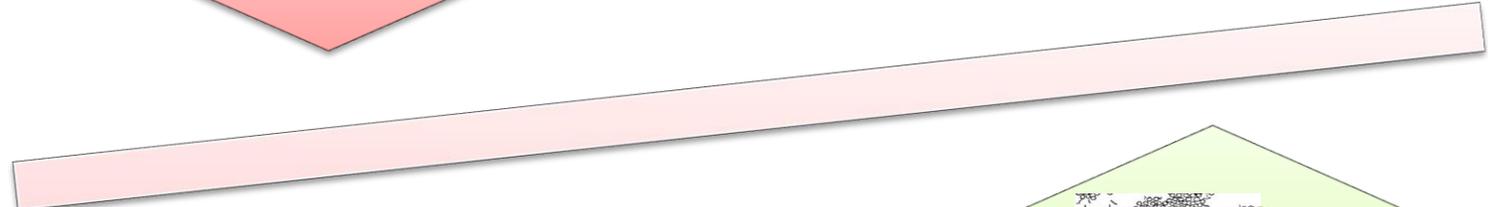
Protein is one of the most important macronutrients in food



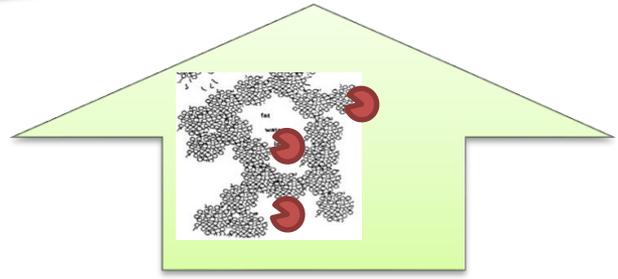
How do gastric acid secretion and pepsin reach their substrates in the stomach?



Do they **degrade the protein substrates at the surface of the matrix**, which thus gradually erodes, releasing products from enzymatic reaction in the stomach?

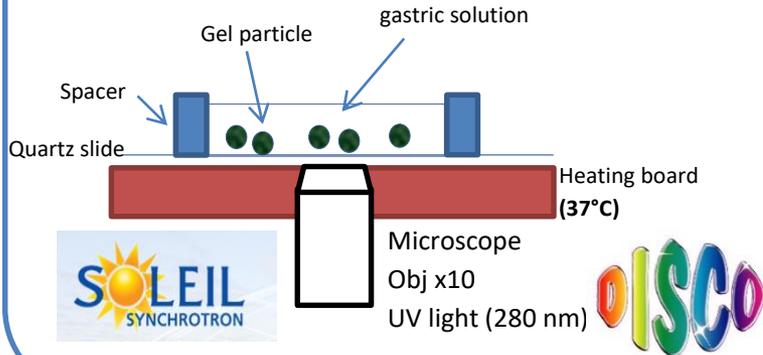


Or do they **diffuse within the protein gel particles** to reach their substrates?



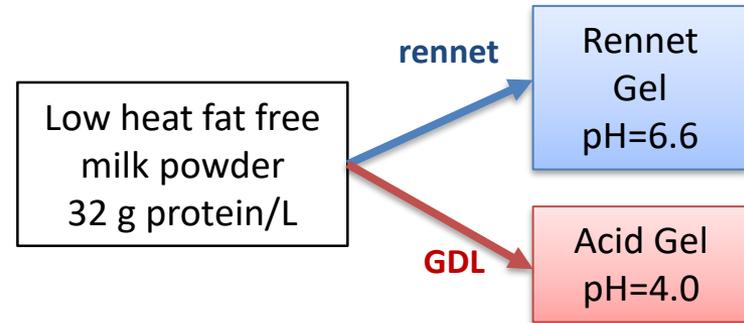
Monitoring the disintegration kinetics of dairy protein gel upon digestion by time-lapse synchrotron deep-UV microscopy

Deep-UV microscopy using natural fluorescence of tryptophan

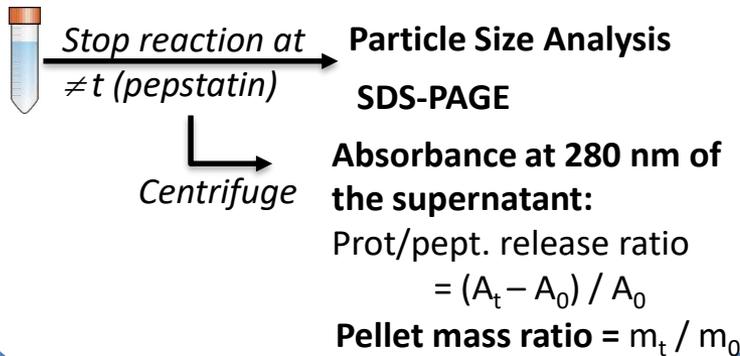


2 milk gels

➔ same composition but different microstructures

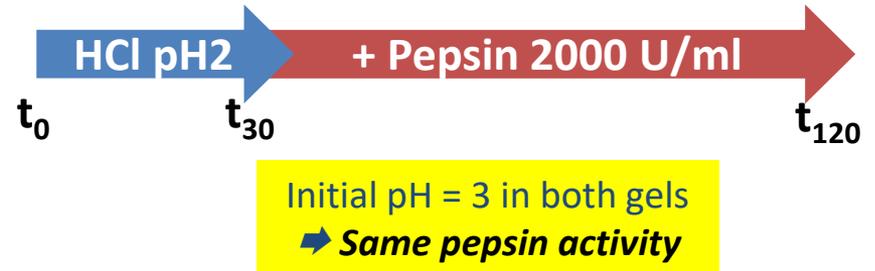


Static digestion in batch reactor → biochemical analysis

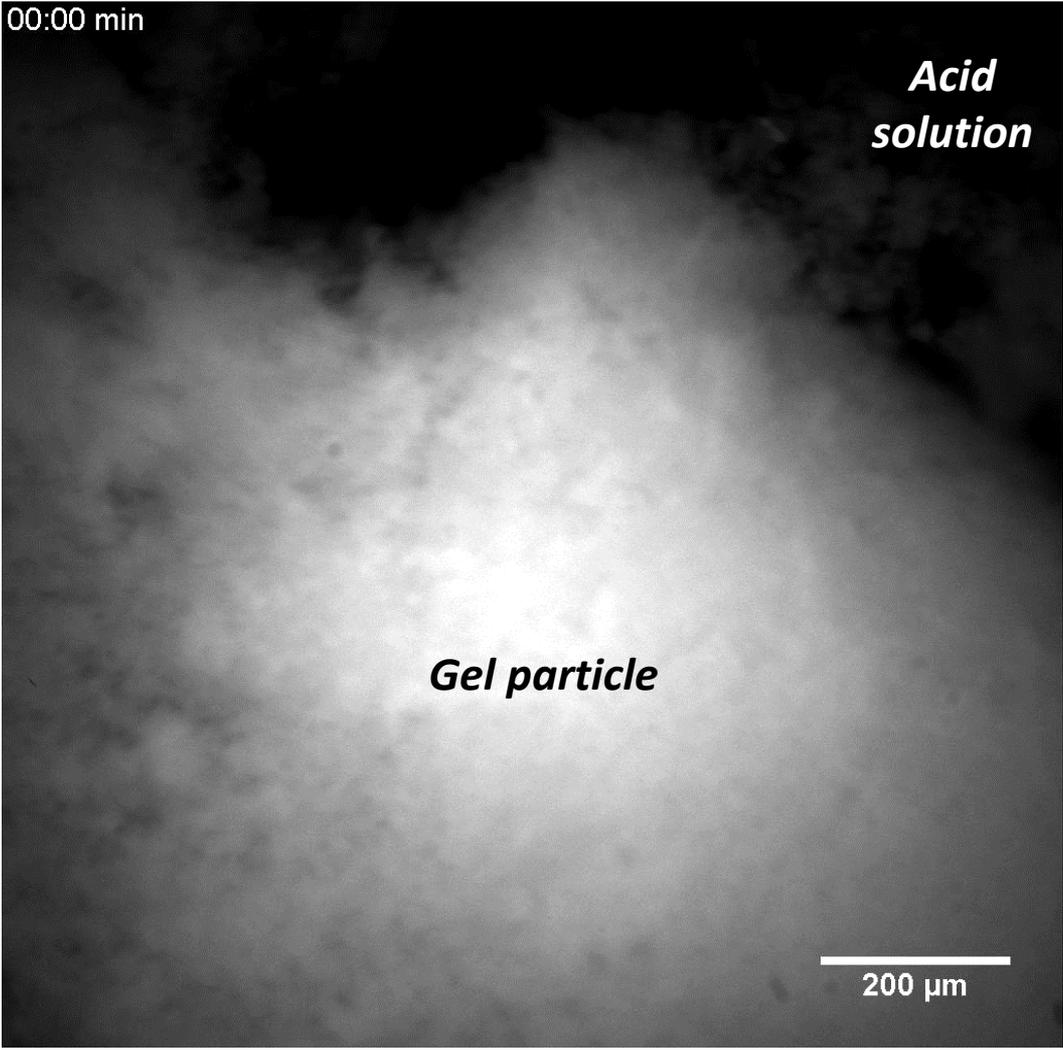


Static in vitro digestions

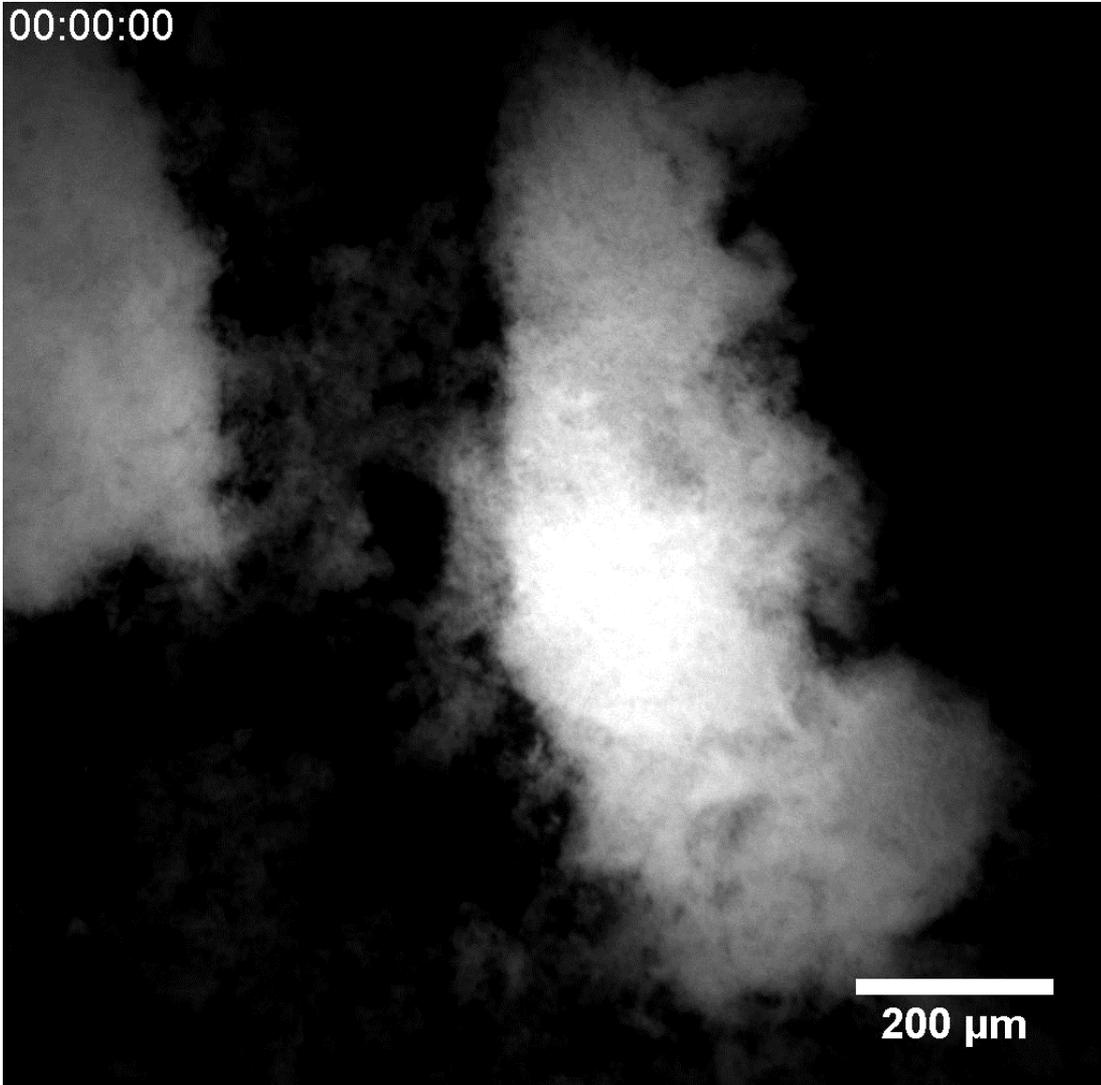
➔ Decoupling acidification from enzymatic reaction



The acid-type gel remains stable during the acidification phase



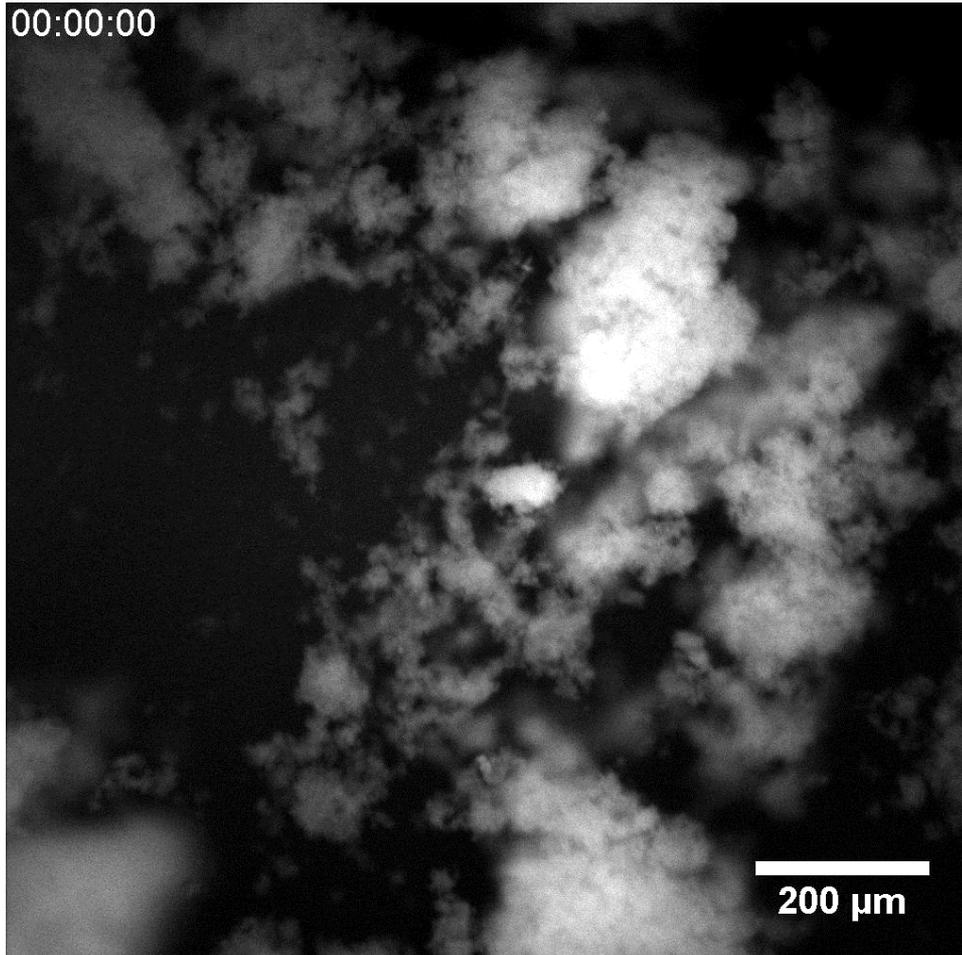
The rennet gel undergoes major structural modifications during acid incubation



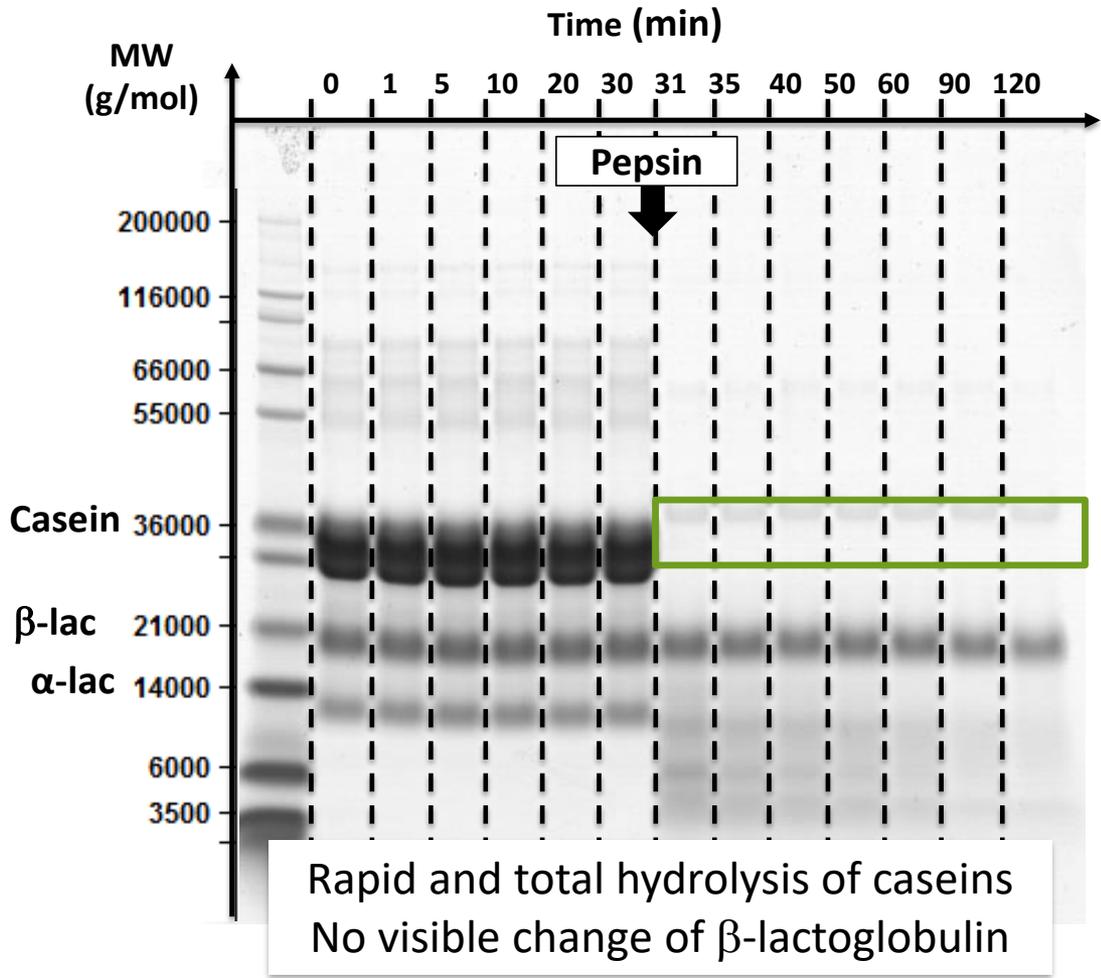
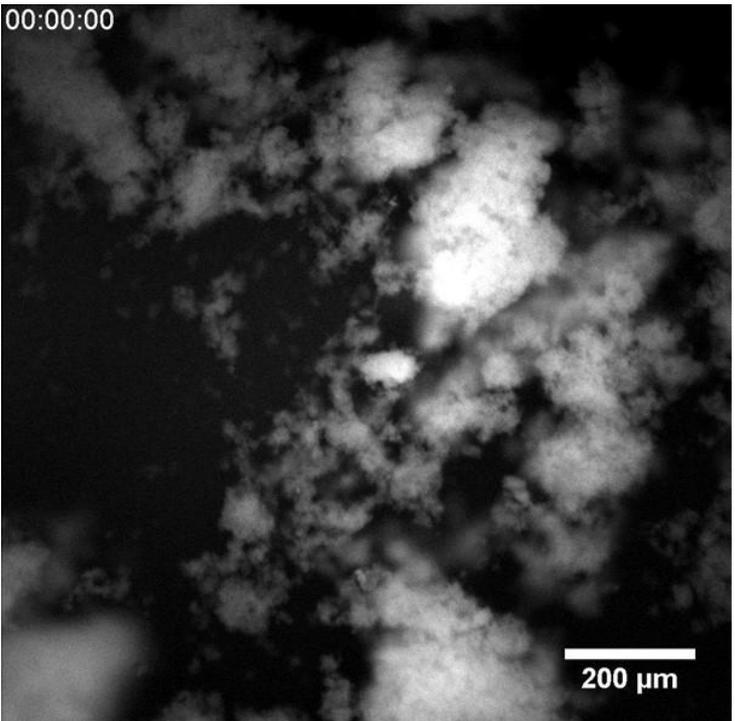
- initial swelling of the particle periphery
- contraction of the inner part



Compaction and densification of the protein network = *syneresis*



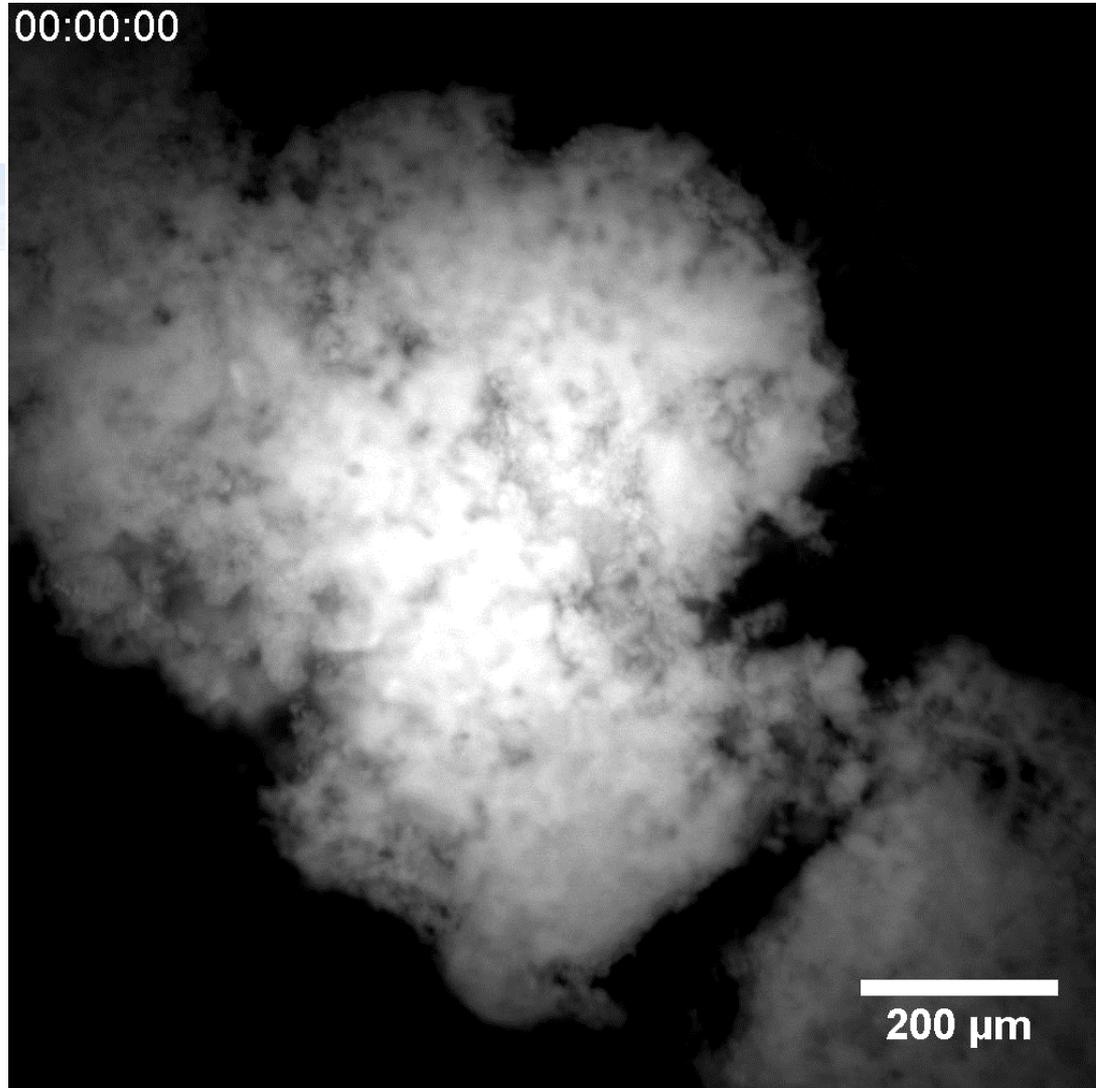
The particles are disappearing very quickly of the focal plane from the microscope, because of their **extremely rapid digestion by pepsin**.



Penetration of acid into gel particles

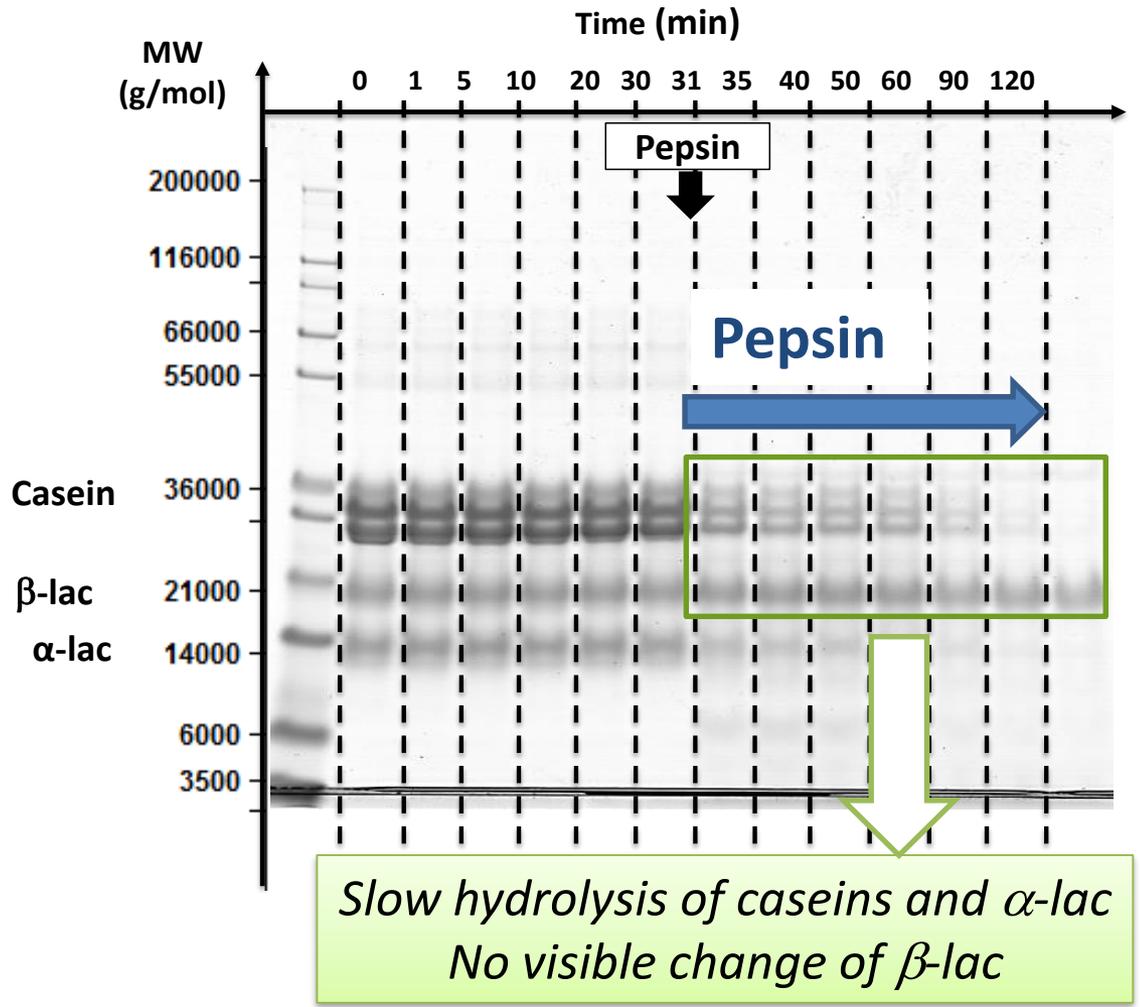
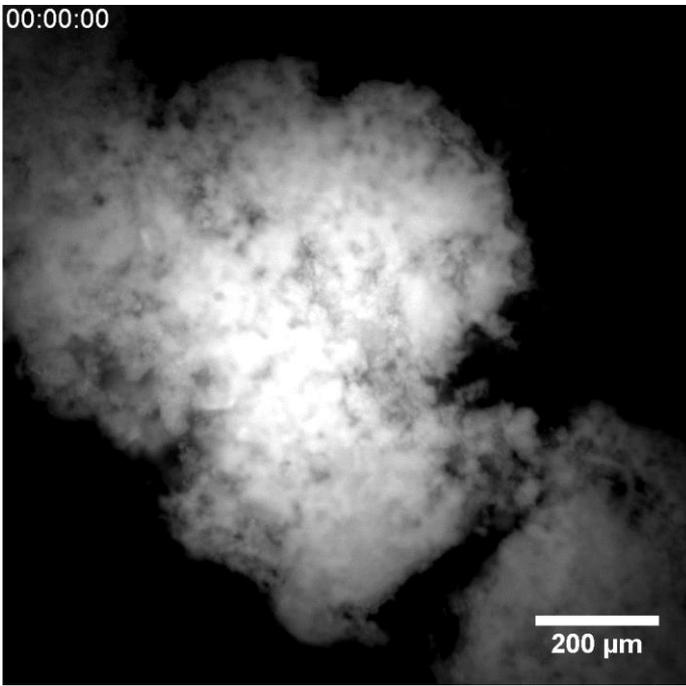
Internal bonds between casein micelles are weakened

Facilitated and fast proteolysis by pepsin



The size of the particle is decreasing over time, but the particle is still present after 2 hours of enzymatic digestion

Rennet gel is digested much slower than acid gel



How to go further in the exploitation of the videos obtained in DUV microscopy ?

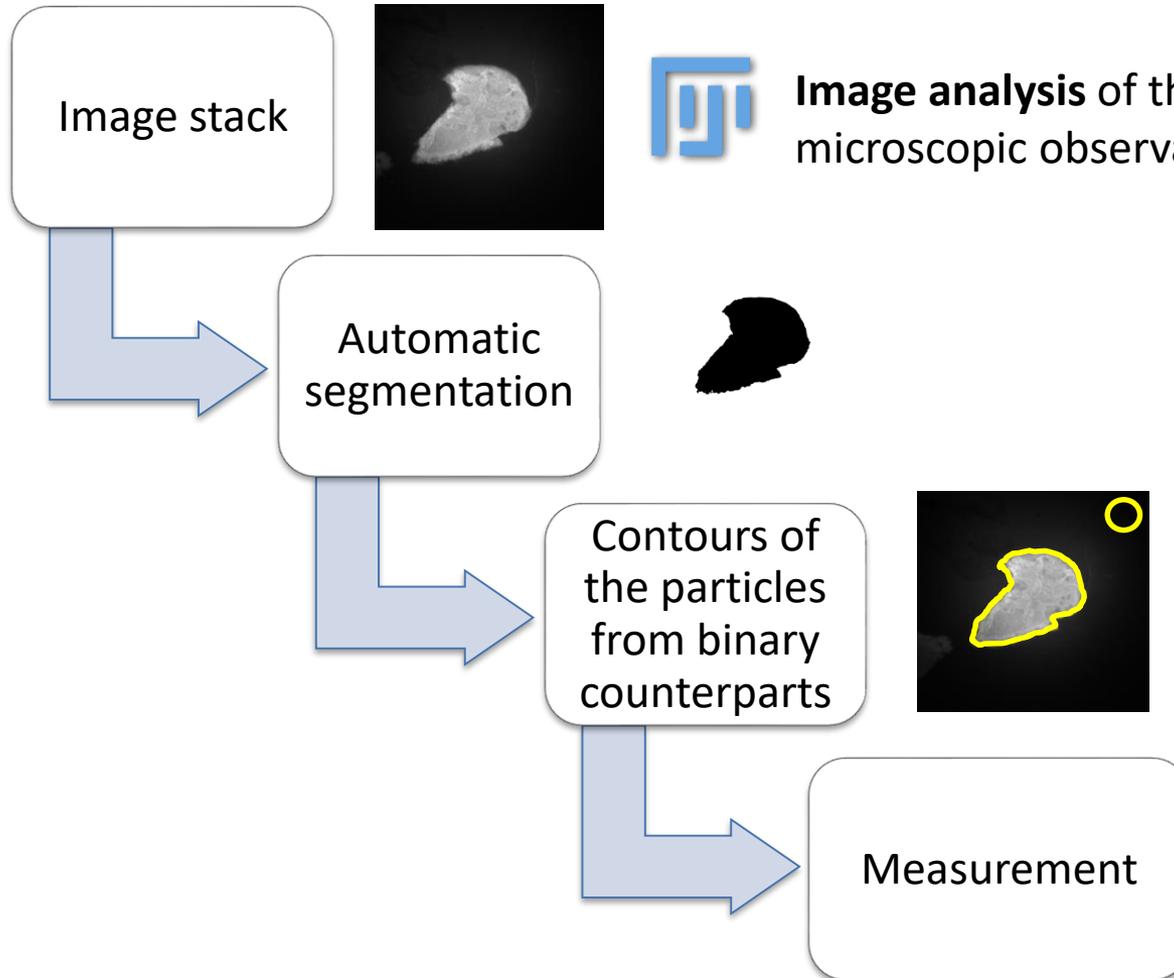


Image analysis of the gel particles within the microscopic observations collected **versus time**

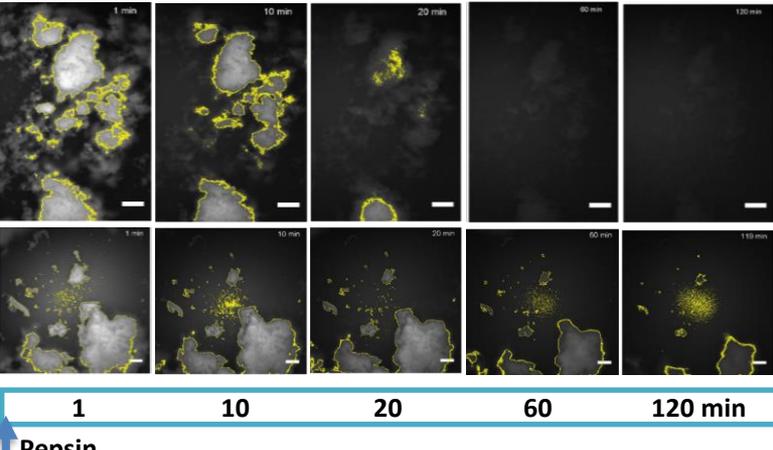
Digestion kinetic of the gel:

$$\text{Estimated Protein content} = \text{Area} \times (\text{MeanF} - \text{BackF})$$

- Particle area
- Mean particle fluorescence intensity
- Background fluorescence intensity

Results

Hydrolysis kinetics of the rennet gel by pepsin is much slower than that of the acid gel



Acid Gel
 ➔ Fast proteolysis

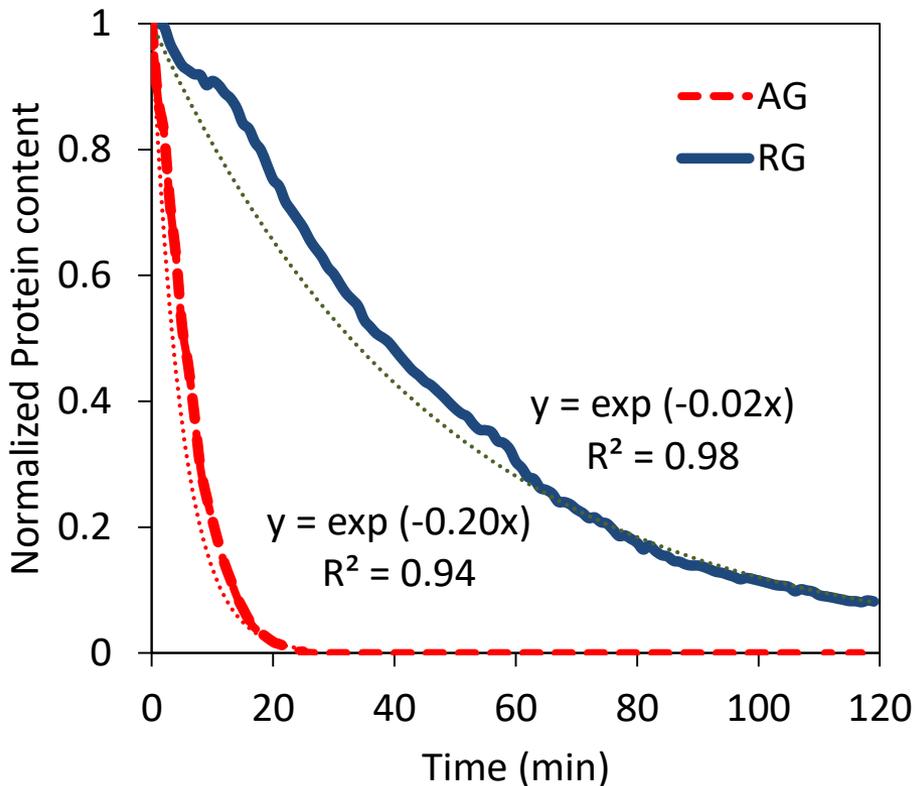
Rennet Gel
 ➔ Slow incomplete proteolysis



Preservation of the particle morphology

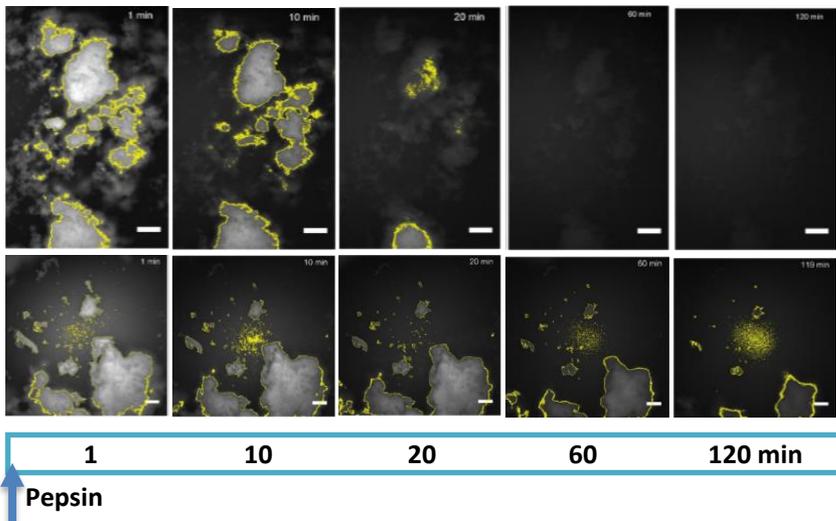


Exponential trend of the digestion



Results

Hydrolysis kinetics of the rennet gel by pepsin is much slower than that of the acid gel

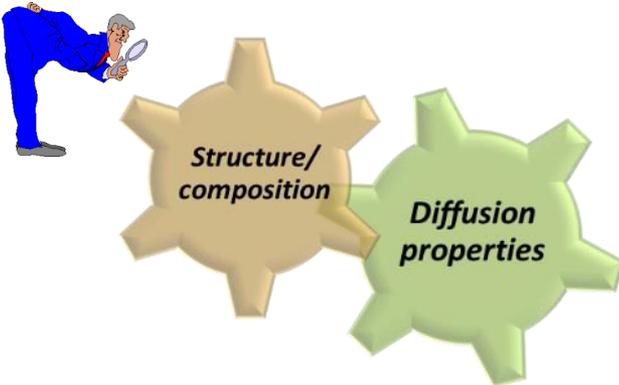


Acid Gel
➔ *Fast proteolysis*

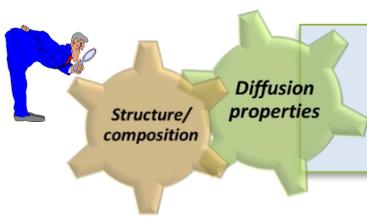
Rennet Gel
➔ *Slow & incomplete proteolysis*



Syneresis of RG during acidification ↗ the resistance of RG protein network to pepsin action ?



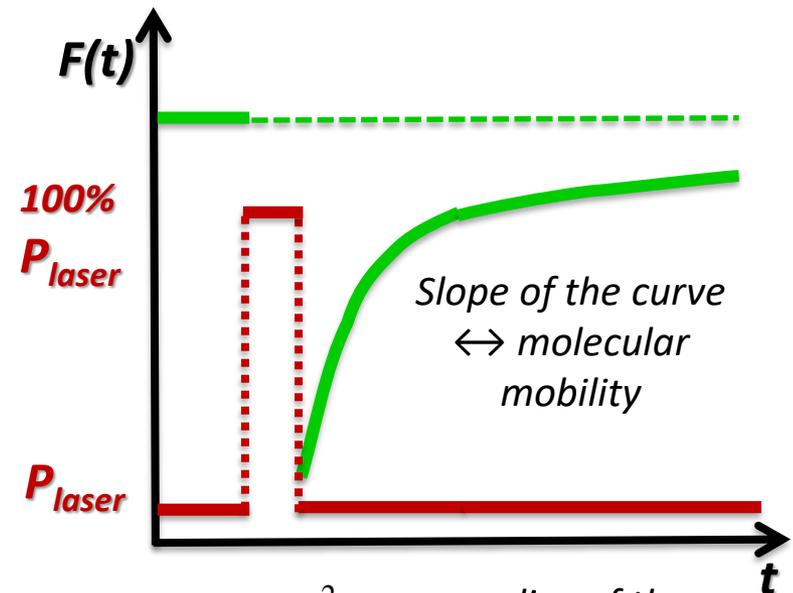
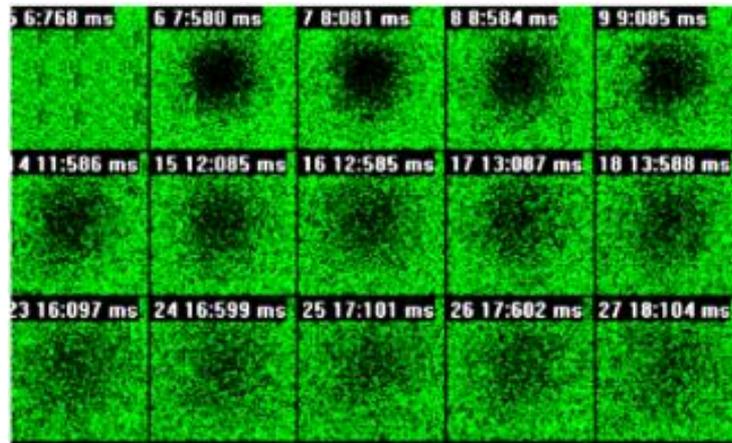
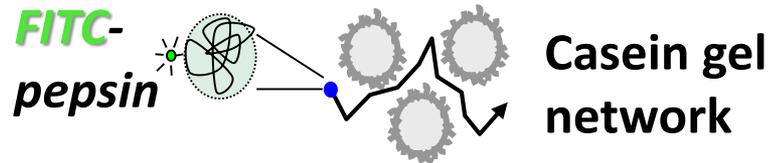
How does the microstructure of the protein gel affect the diffusion behavior of pepsin ?



How does the microstructure of the protein gel affect the diffusion behavior of pepsin ?

FRAP technique (*Fluorescence Recovery After Photobleaching*)

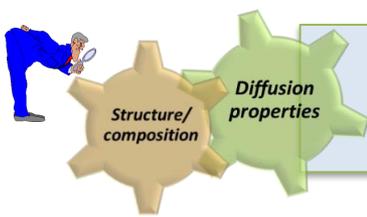
➔ based on dynamic measurements of fluorescent molecules by **confocal microscopy**



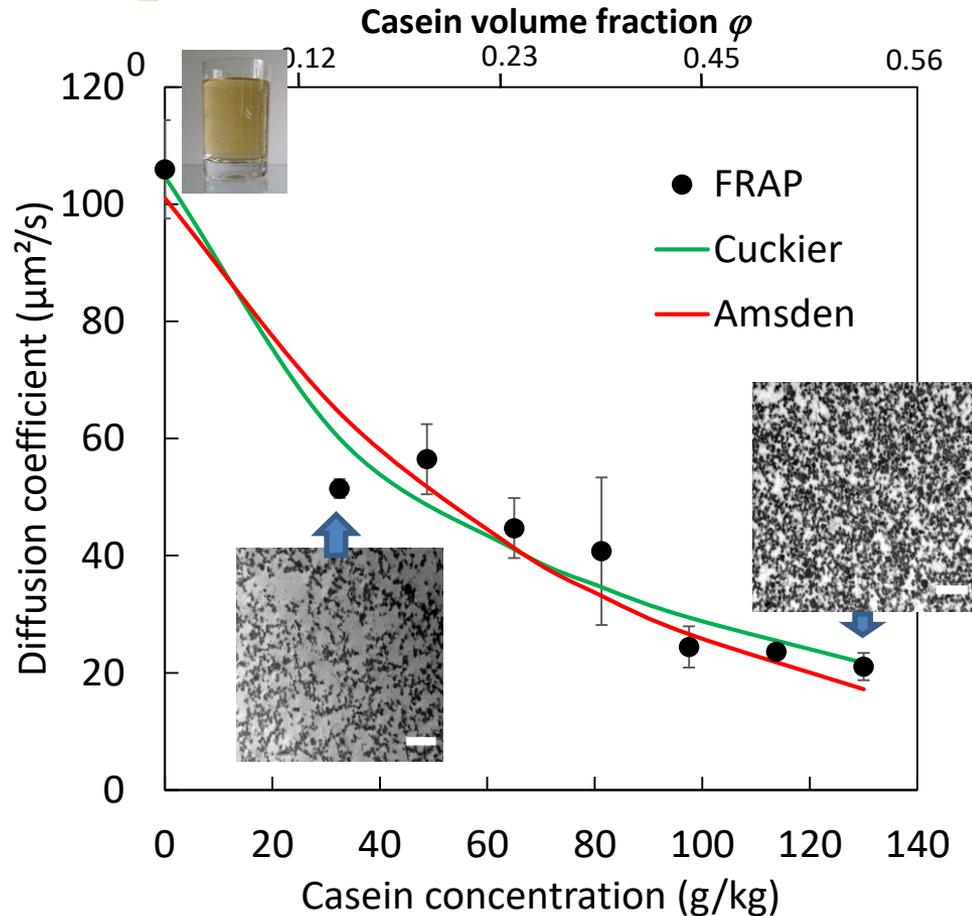
$$\tau_D = \frac{w_B^2}{4D}$$

w_B : radius of the Region Of Interest
 D : diffusion coefficient

➔ In rennet gels of ↗ casein concentration (30 to 130 g/kg)



How does the microstructure of the protein gel affect the diffusion behavior of pepsin ?



As protein concentration \nearrow ,
the free volume of solvent \searrow
& the steric hindrance of the
protein network \nearrow



**Diffusion
coefficient of
pepsin \searrow in the gel**

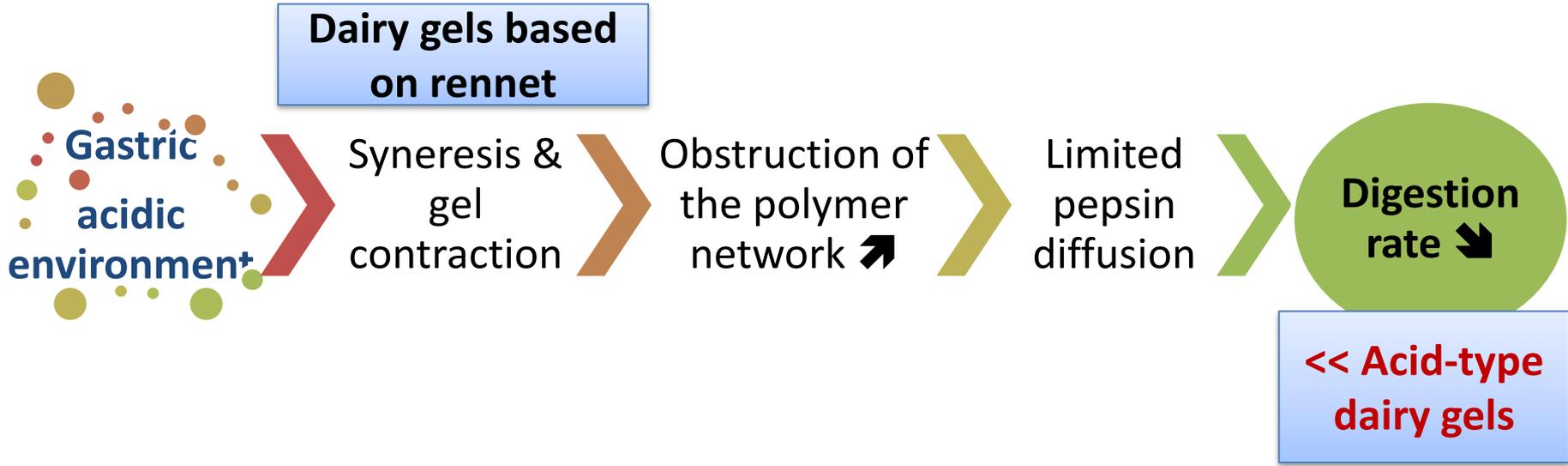
TEM Scale bars = 1 μm

Hydrodynamic model (Cuckier, 1984)

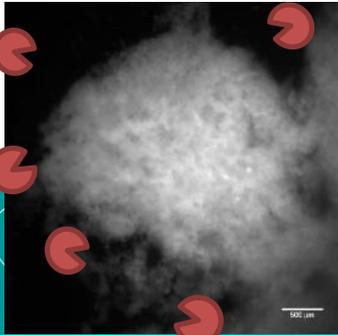
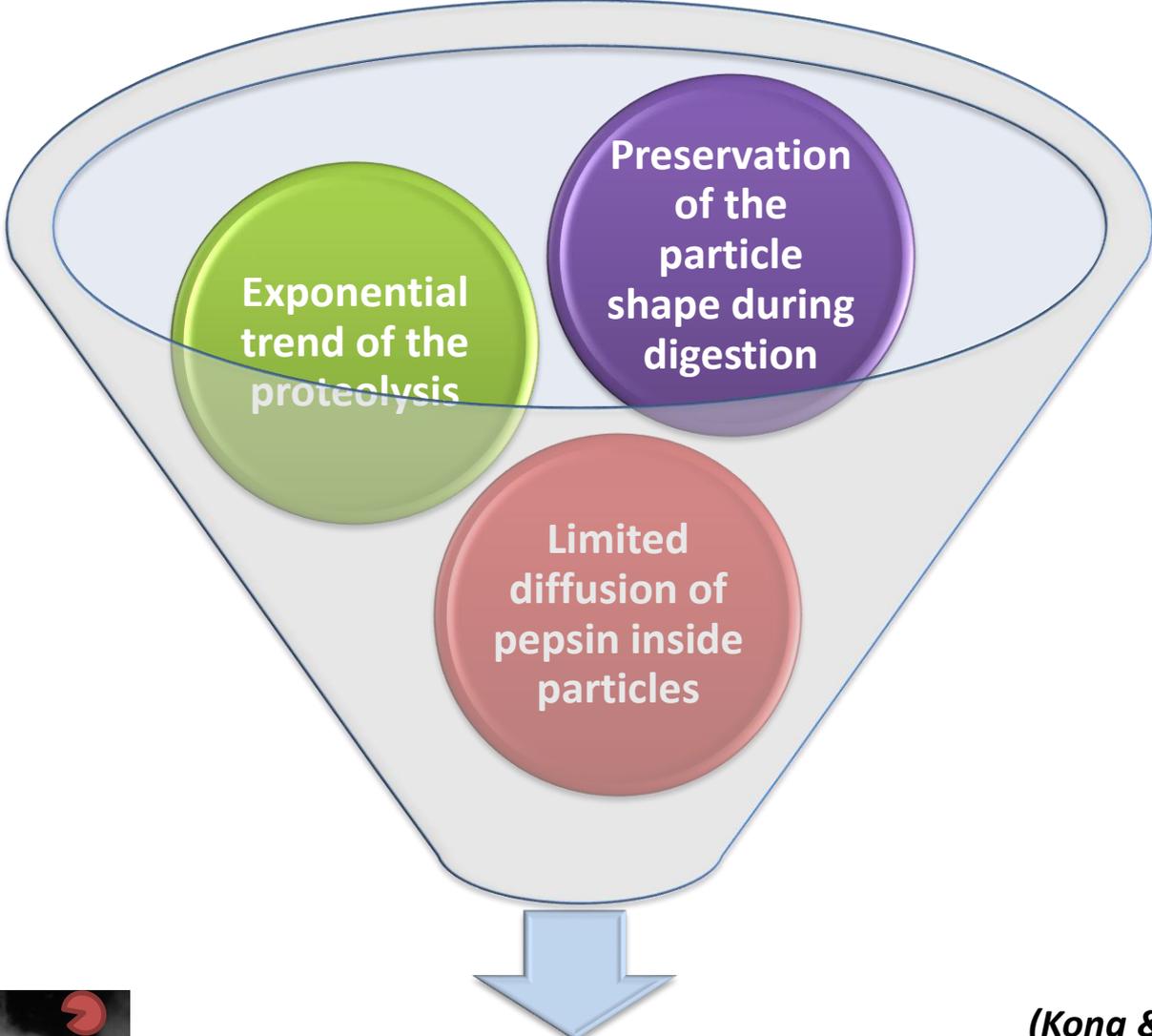
\blacktriangleright frictions caused by the polymer chains slow down the solute

Obstruction-scaling model (Amsden, 1998)

\blacktriangleright combines obstruction effects & hydrodynamic interactions



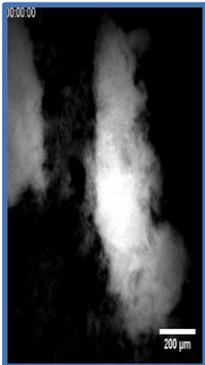
Controlling the gelling process of food proteins can lead to the formation of particles with specific structural properties that change the kinetic of digestion



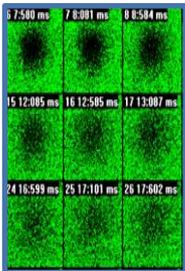
Surface erosion
= predominant mechanism of enzymatic breakdown of dairy gels

(Kong & Singh, 2009a, 2009b, 2011)

Summary



Time-lapse synchrotron deep-UV fluorescence microscopy enables the study in real time and with a high resolution of the changes in food particles during the digestion process



The **FRAP technique** is a powerful tool to quantify the diffusion properties of enzymes in food matrices of different microstructure



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Exploring the breakdown of dairy protein gels during *in vitro* gastric digestion using time-lapse synchrotron deep-UV fluorescence microscopy

Juliane Floury ^a, [✉], Tiago Bianchi ^b, Jonathan Thévenot ^a, Didier Dupont ^a, Frédéric Jamme ^c, Evelyne Lutton ^d, Maud Panouillé ^d, François Boué ^d, Steven Le Feunteun ^d



Food Chemistry
Volume 223, 15 May 2017, Pages 54-61

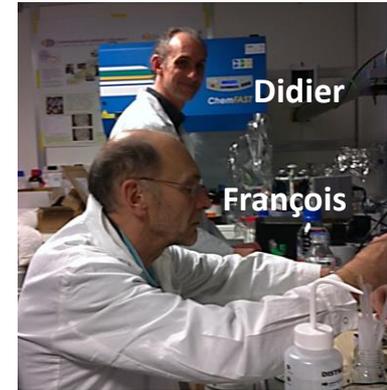


Pepsin diffusion in dairy gels depends on casein concentration and microstructure

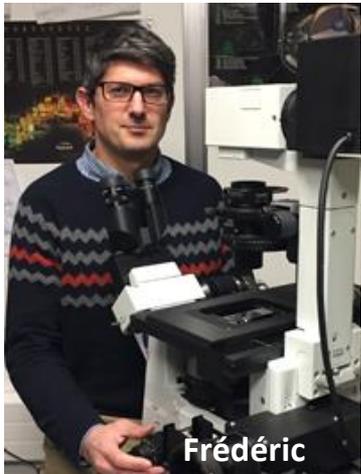
J. Thévenot ^a, [✉], C. Cauty ^a, D. Legland ^b, D. Dupont ^a, J. Floury ^a, [✉]



MERCI



**THANK YOU FOR
YOUR ATTENTION**



Maude



Jonathan



Evelyne