



Soleil Synchrotron illuminates our knowledge on gastric digestion

Juliane Floury

► To cite this version:

Juliane Floury. Soleil Synchrotron illuminates our knowledge on gastric digestion. STLOpendays, Institut National de Recherche Agronomique (INRA). UMR UMR INRA / AgroCampus Rennes : Science et Technologie du Lait et de l'œuf (1253)., Mar 2019, Rennes, France. hal-02097226

HAL Id: hal-02097226

<https://hal.science/hal-02097226>

Submitted on 5 Jun 2020

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illuminates our knowledge on gastric digestion



STLOpen Days
19-21 March 2019



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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

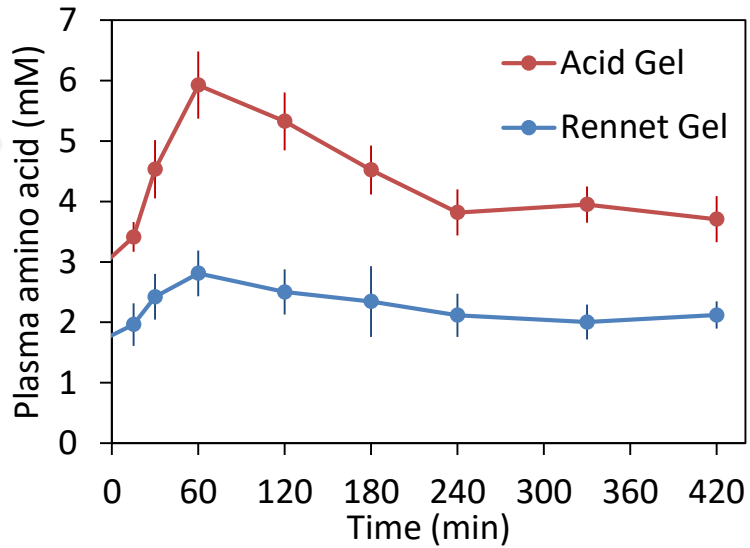


In vitro digestion



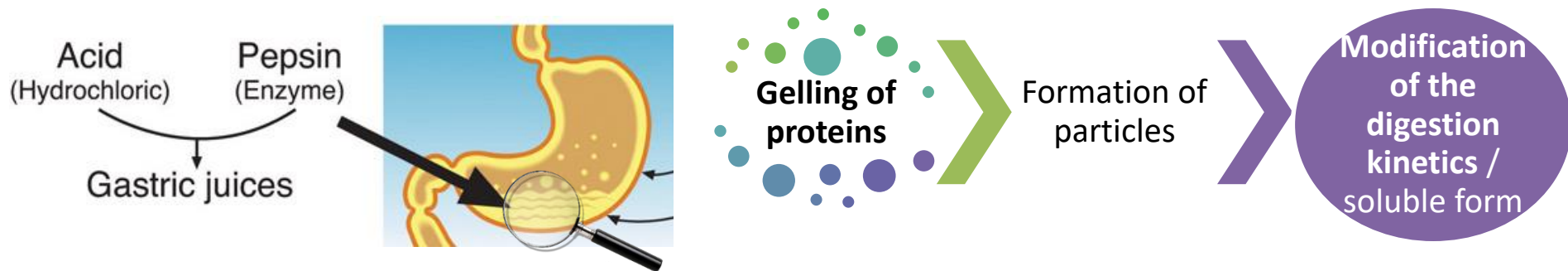
The rennet gel forms a compact coagulum in the stomach due to acid gastric fluids
→ the gastric emptying of caseins is slowed down

In vivo digestion

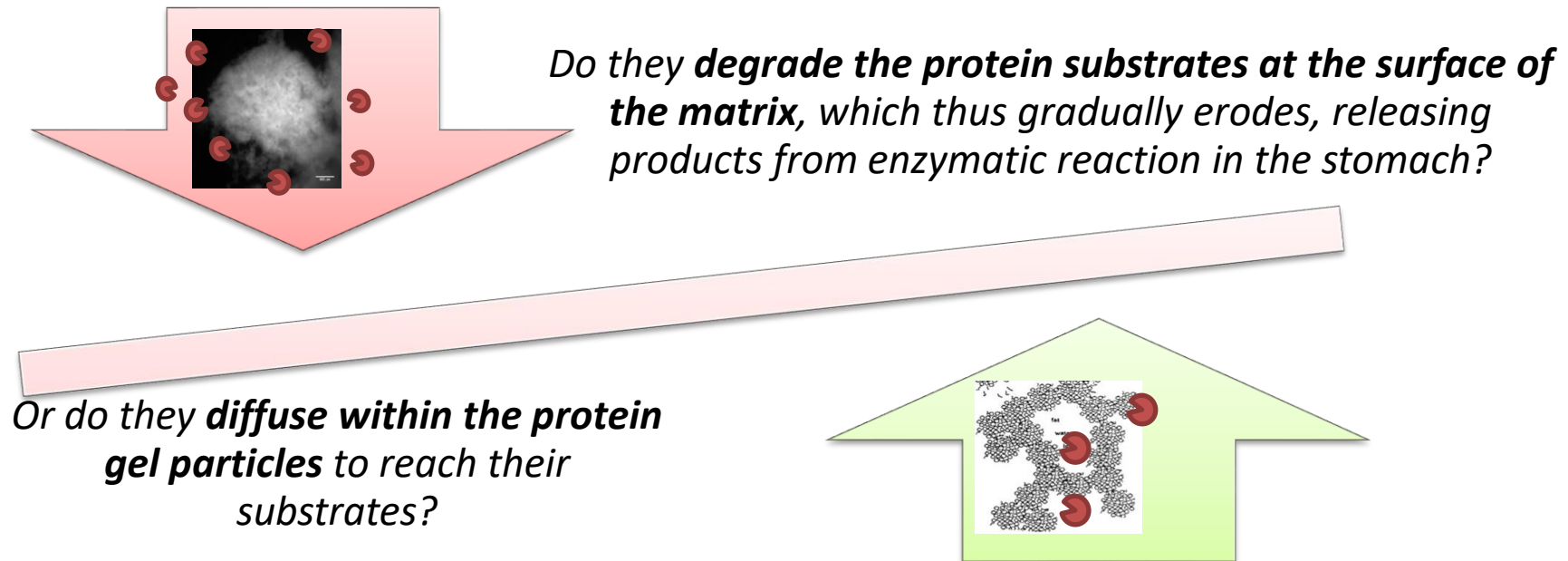


The structure of dairy products regulates the kinetics of protein digestion and the release of amino acids in the bloodstream.

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

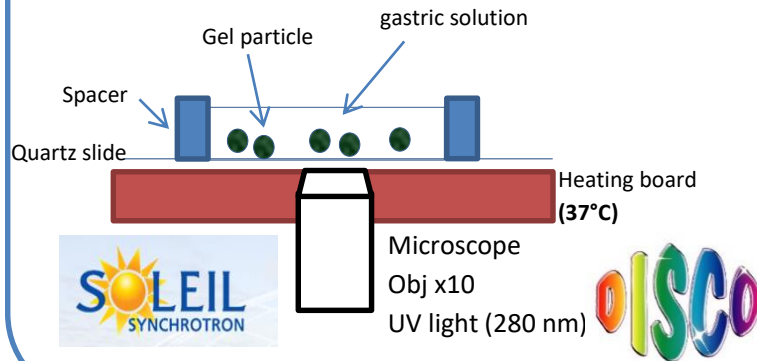


How do gastric acid secretion and pepsin reach their substrates in the stomach?



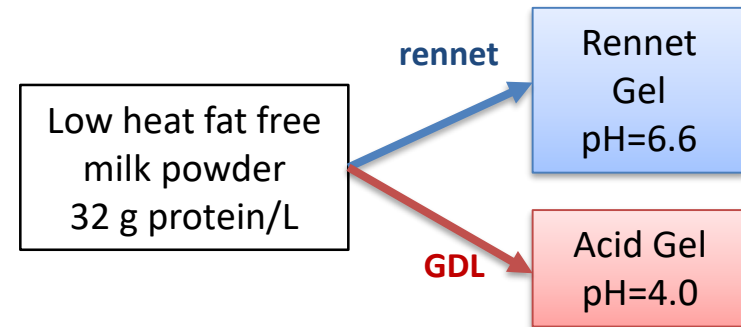
Monitoring the disintegration kinetics of dairy protein gel upon digestion by time-lapse synchrotron deep-UV microscopy

Deep-UV microscopy using natural fluorescence of tryptophan

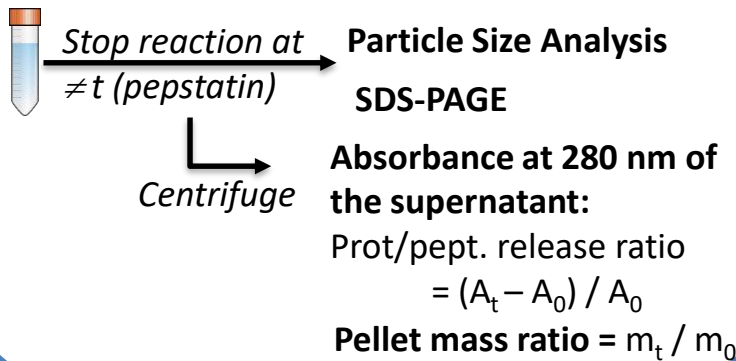


2 milk gels

➔ same composition but different microstructures



Static digestion in batch reactor → biochemical analysis



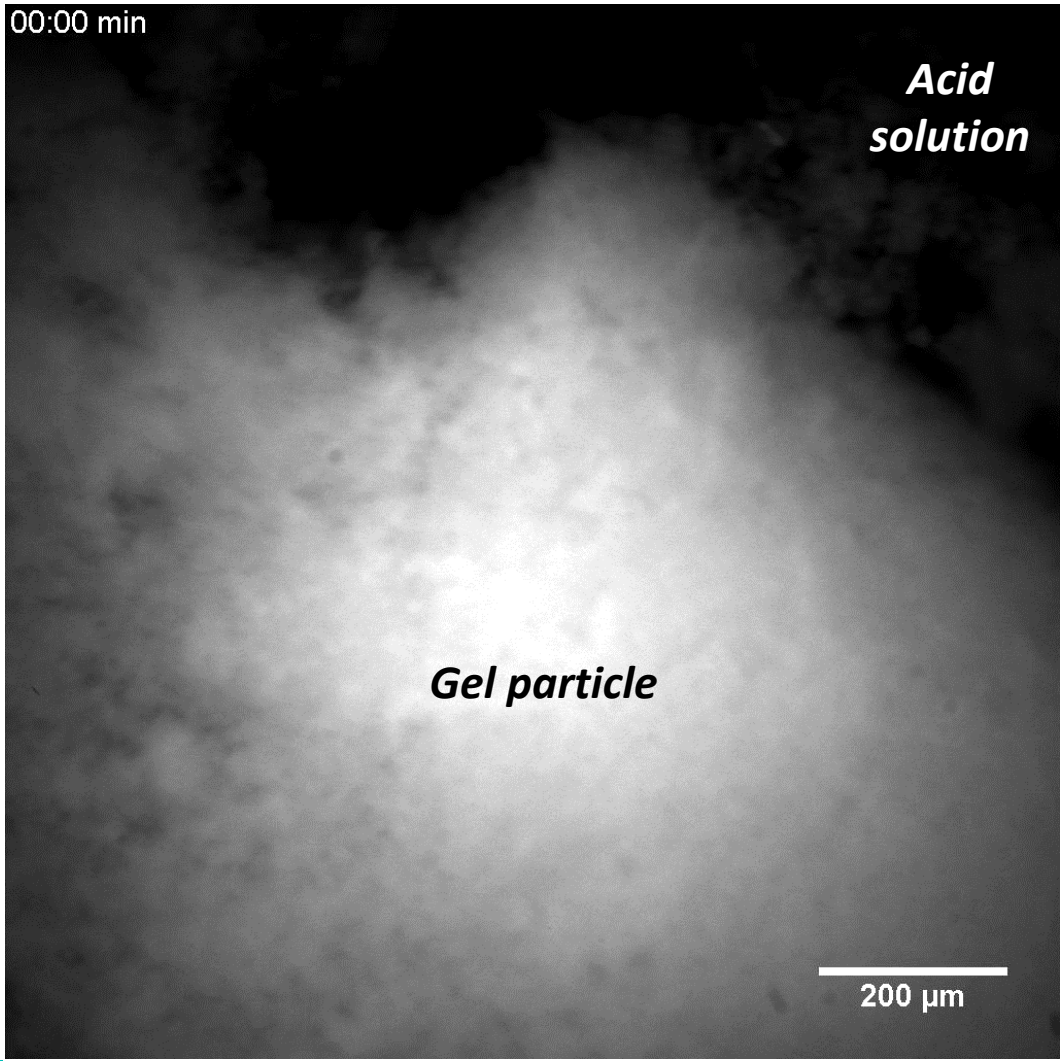
Static in vitro digestions

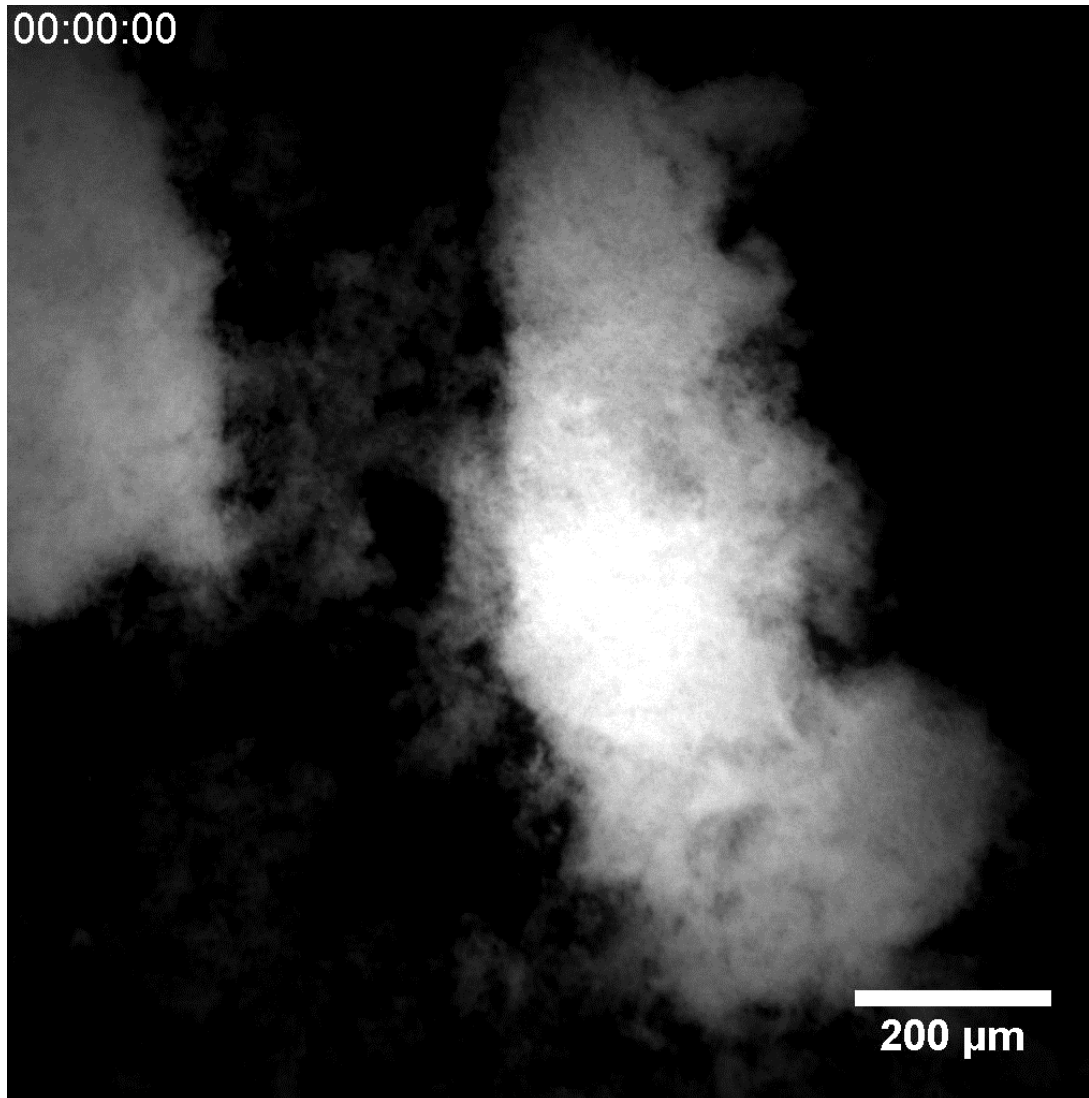
➔ Decoupling acidification from enzymatic reaction



Initial pH = 3 in both gels
 ➔ Same pepsin activity

The acid-type gel remains stable during the acidification phase

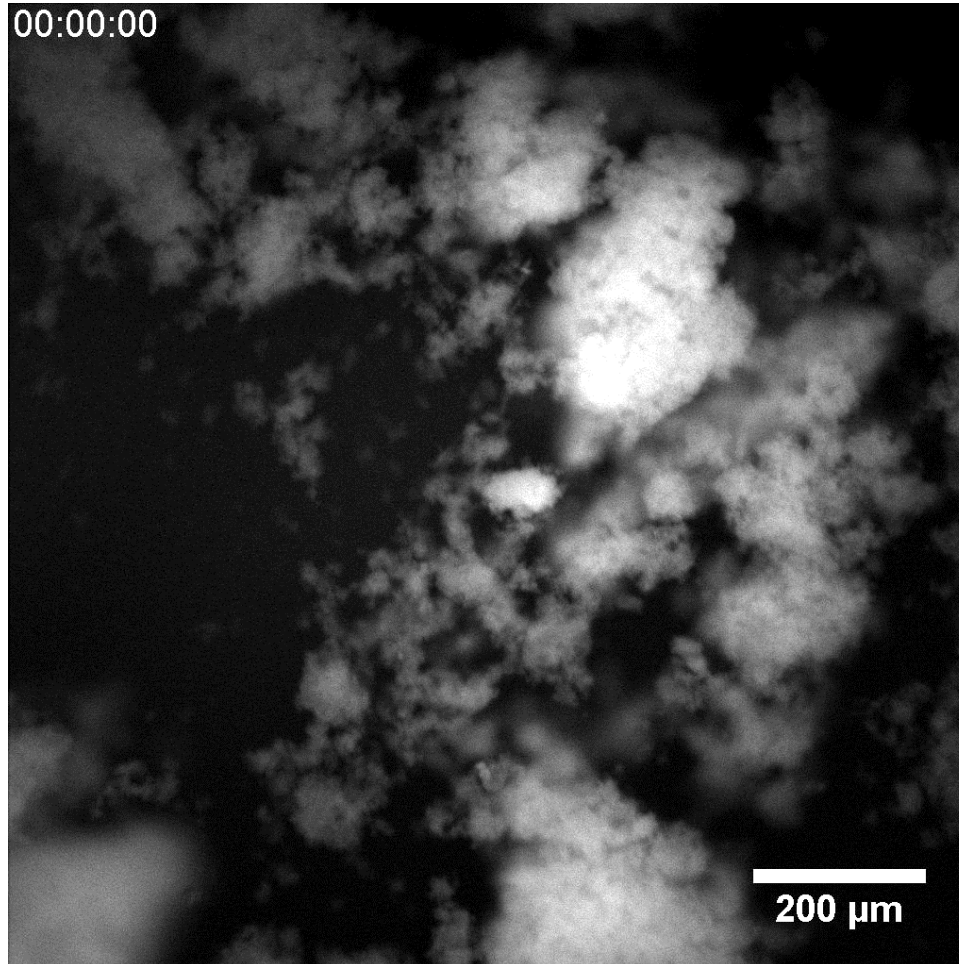




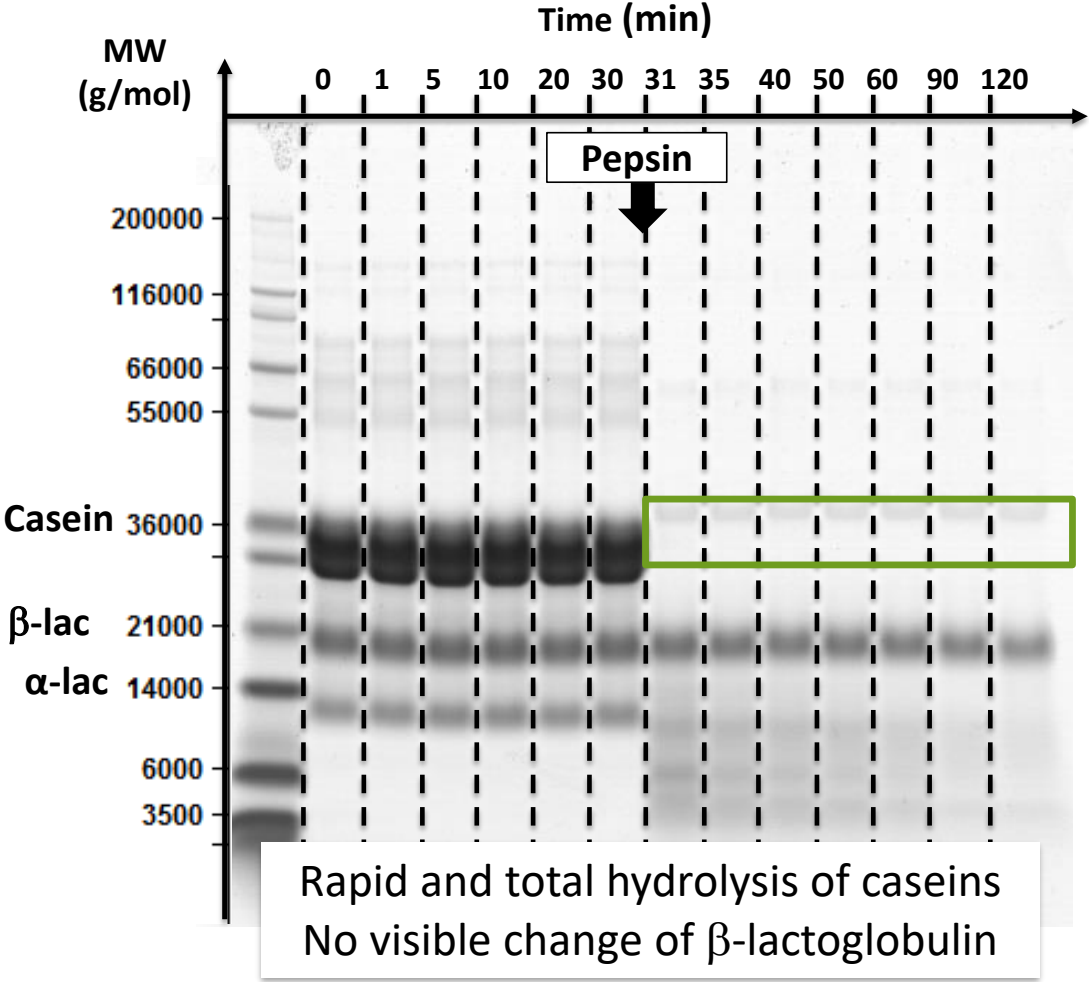
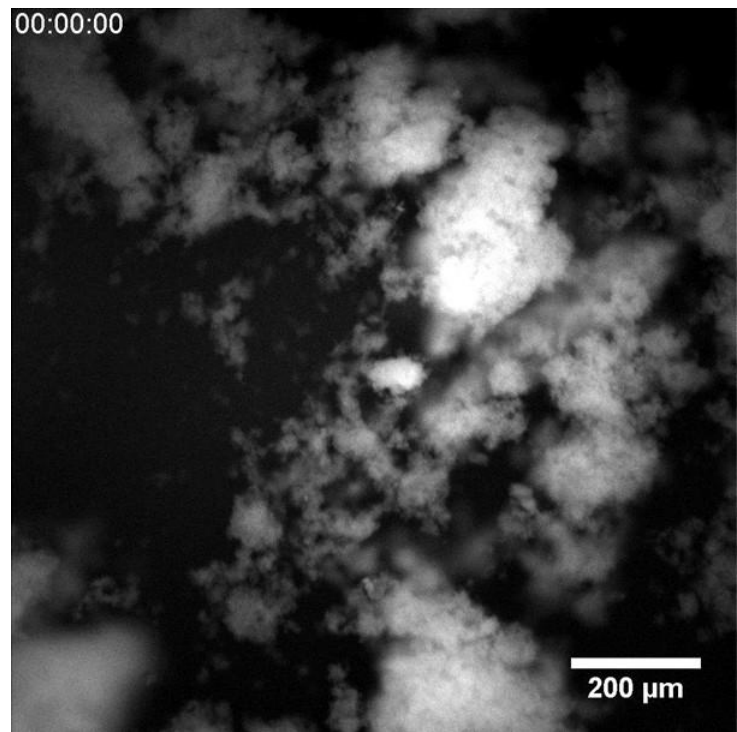
- initial swelling of the particle periphery
- contraction of the inner part



**Compaction and densification of the protein network
= *syneresis***



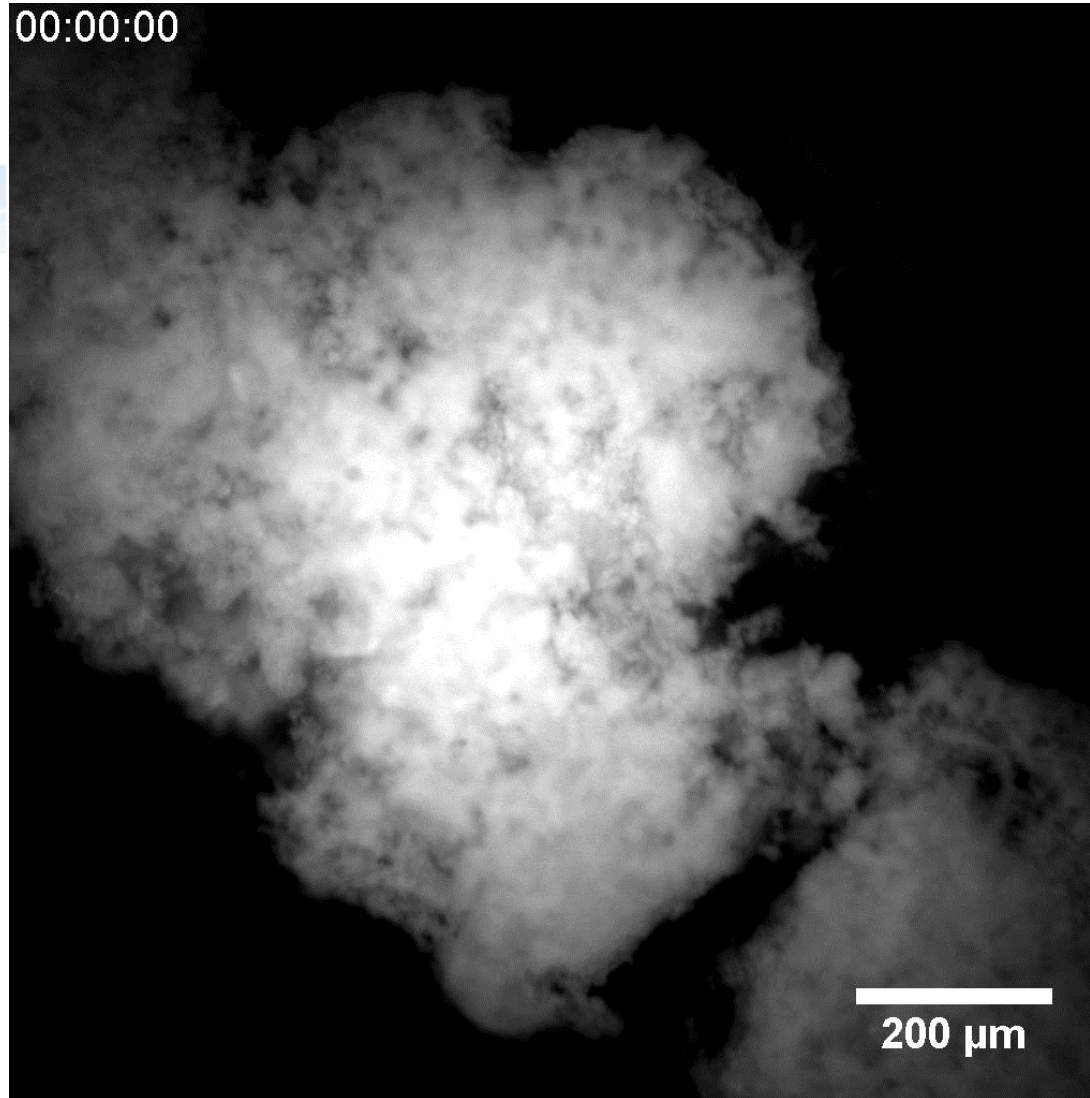
The particles are disappearing very quickly of the focal plane from the microscope, because of their **extremely rapid digestion by pepsin**.



Penetration of acid into gel particles

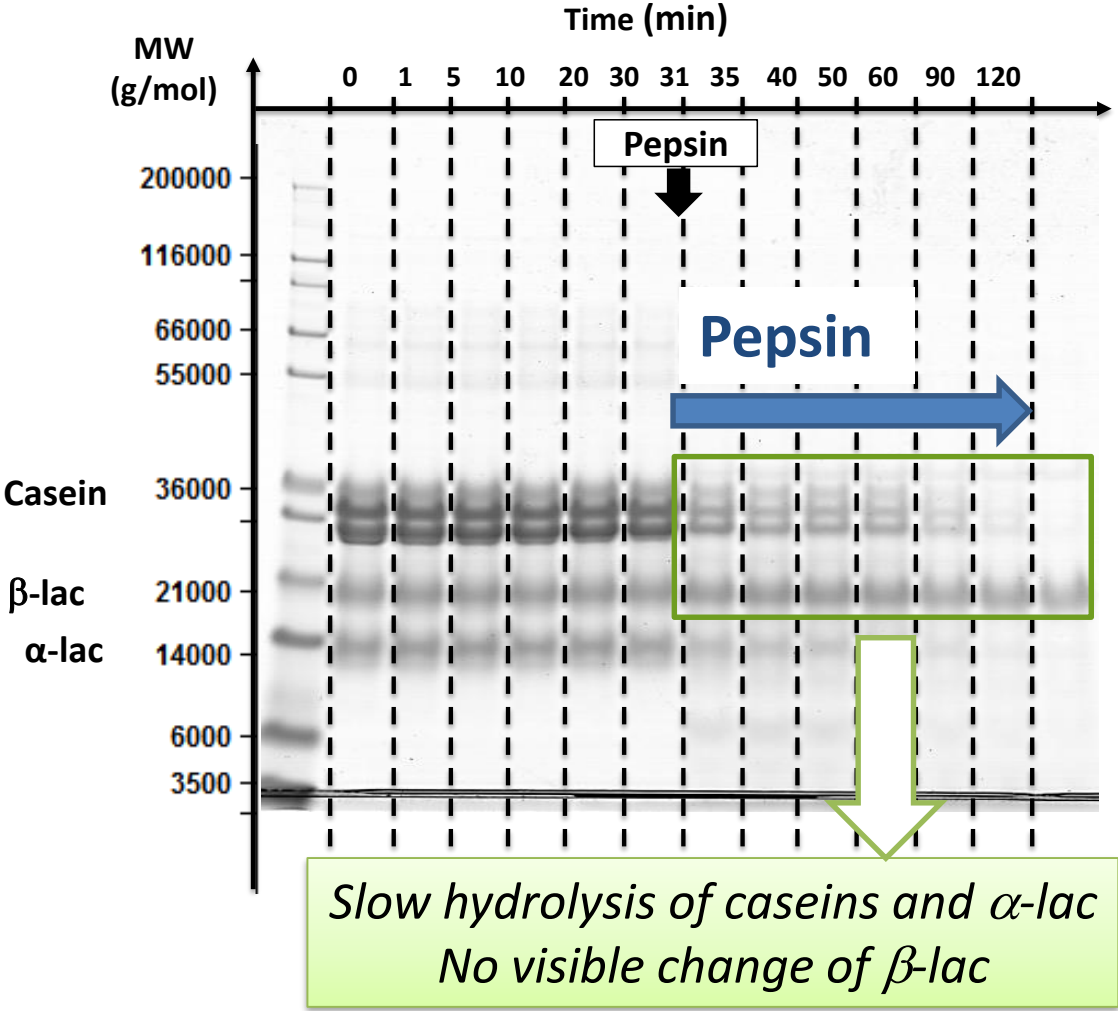
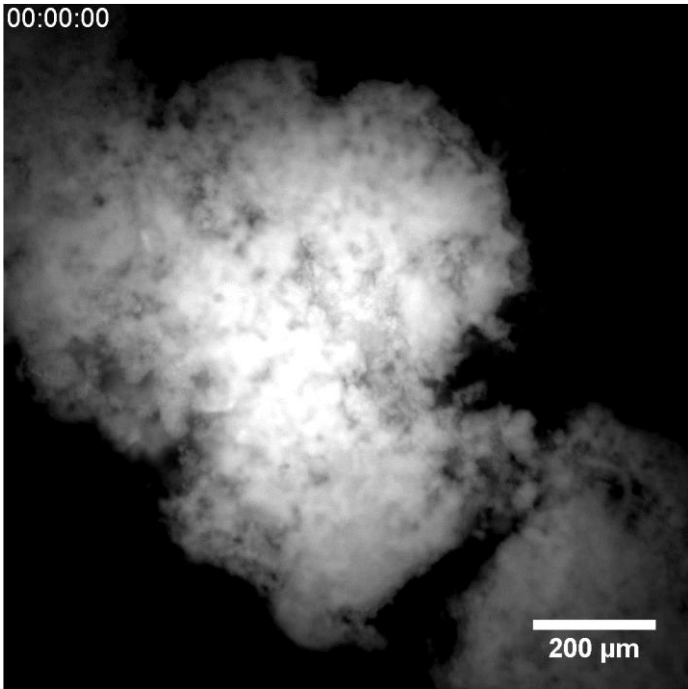
Internal bonds between casein micelles are weakened

Facilitated and fast proteolysis by pepsin

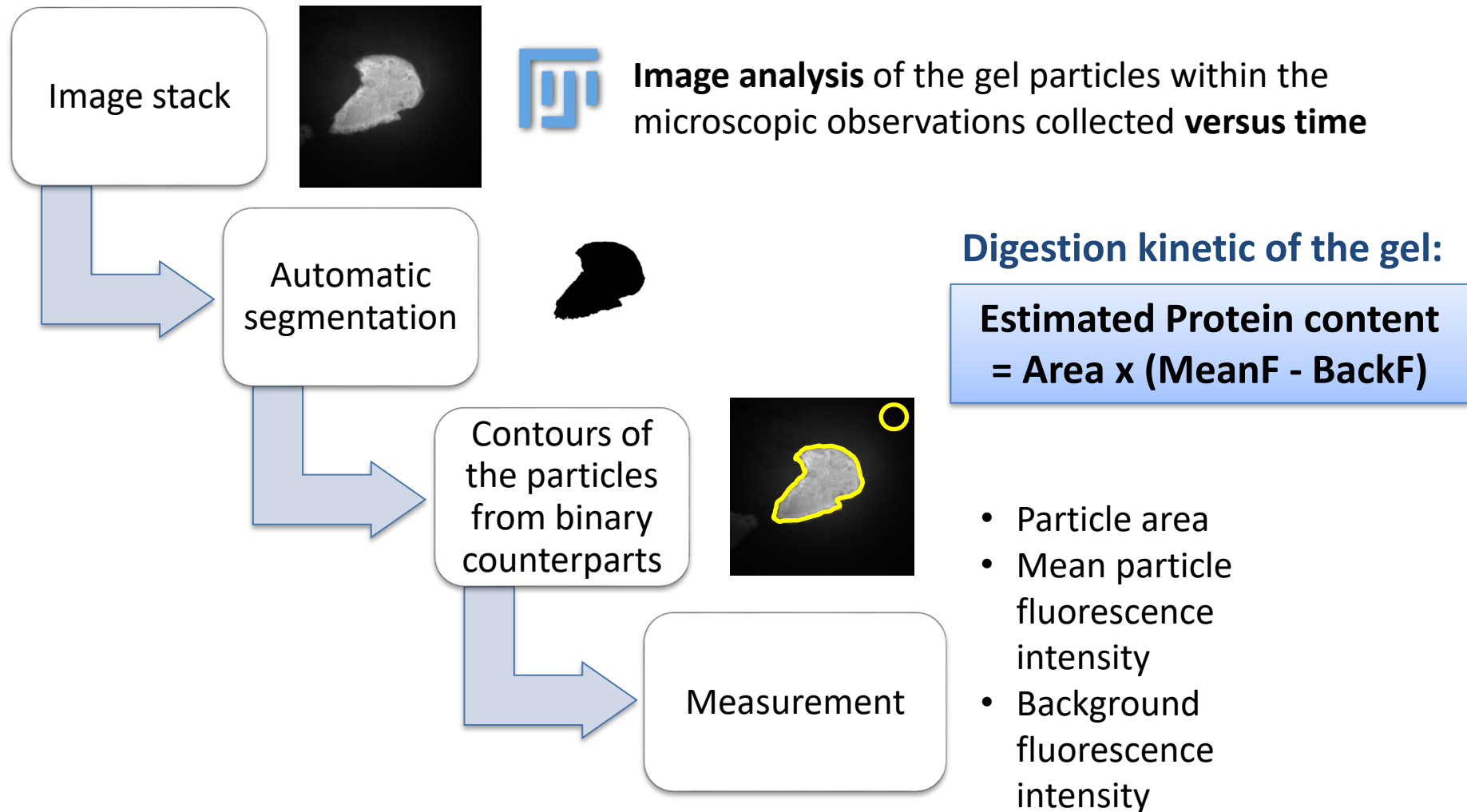


The size of the particle is decreasing over time, but the particle is still present after 2 hours of enzymatic digestion

Rennet gel is digested much slower than acid gel

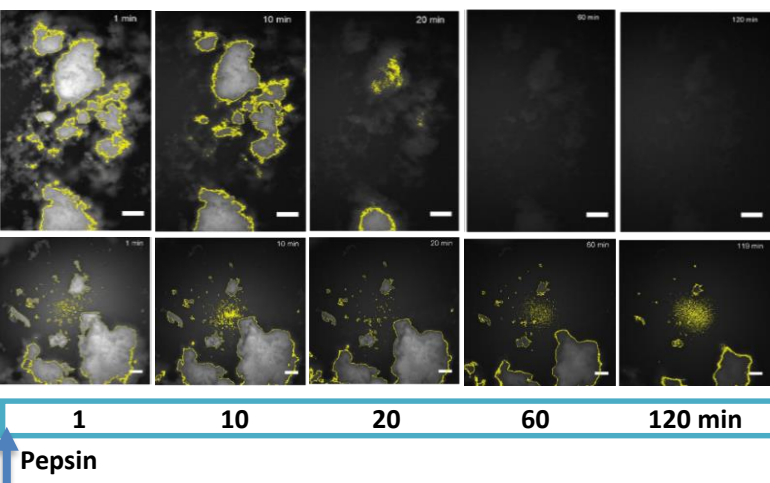


How to go further in the exploitation of the videos obtained in DUV microscopy ?



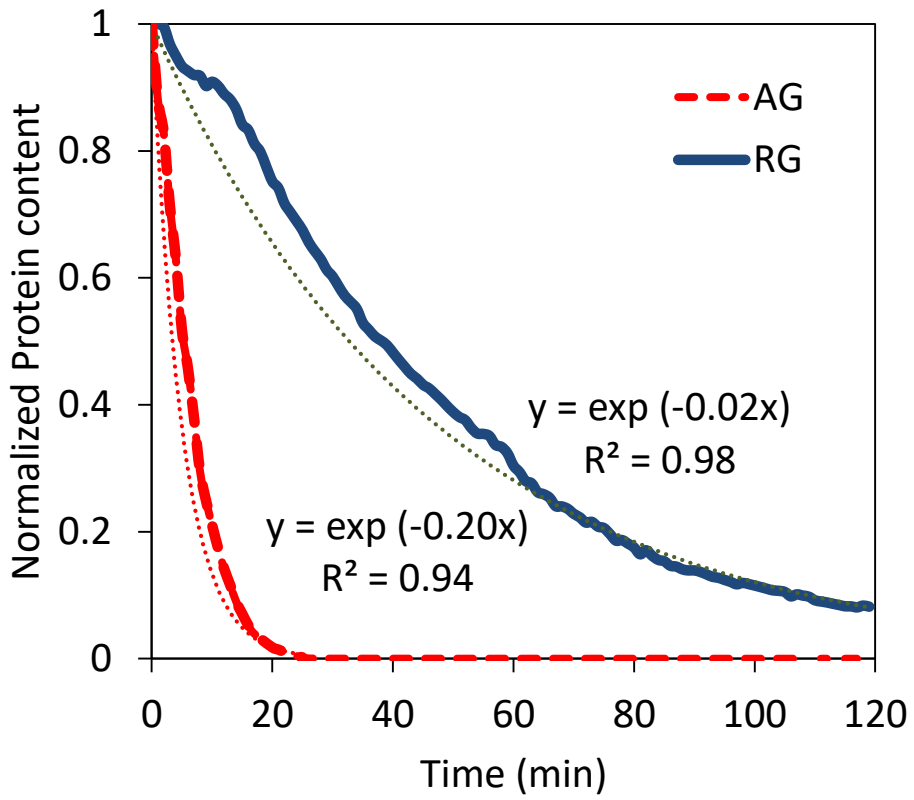
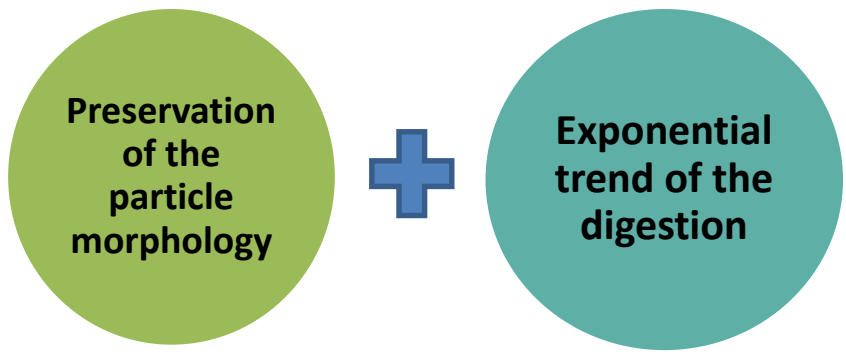
Results

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

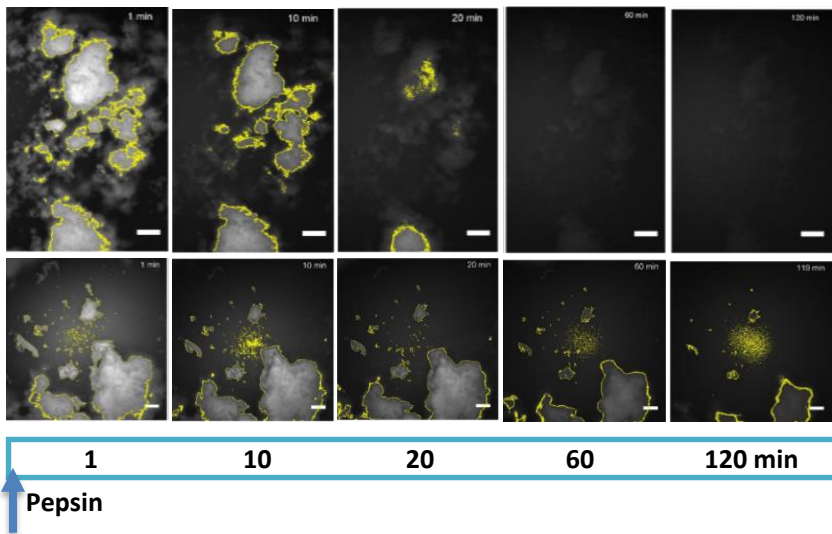


Acid Gel
⇒ Fast proteolysis

Rennet Gel
⇒ Slow incomplete proteolysis



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

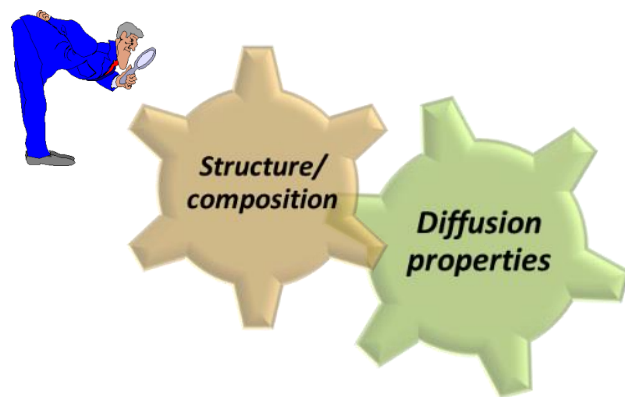


Acid Gel
➡ Fast proteolysis

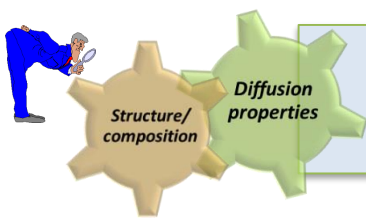
Rennet Gel
➡ Slow & incomplete proteolysis



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



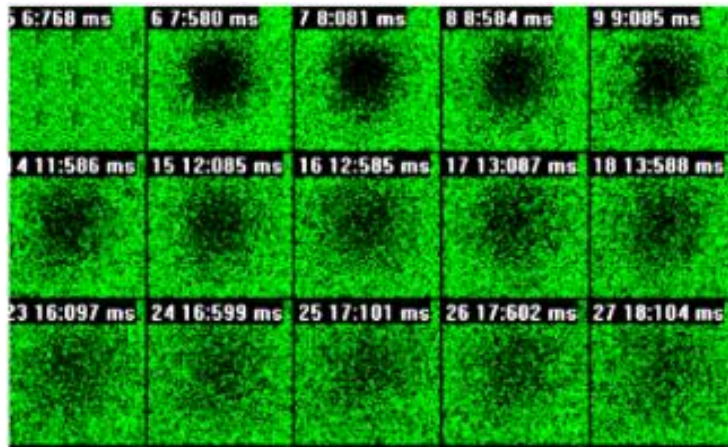
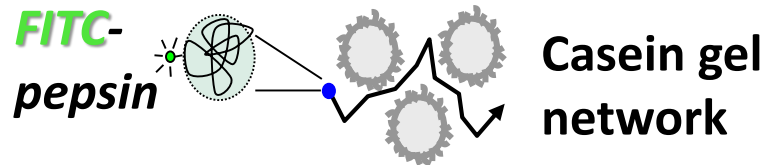
How does the microstructure of the protein gel affect the diffusion behavior of pepsin ?



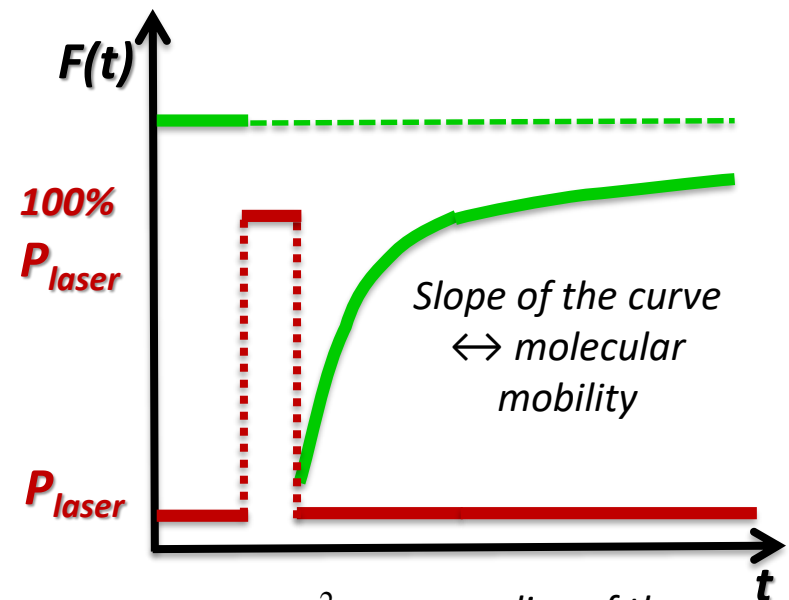
How does the microstructure of the protein gel affect the diffusion behavior of pepsin ?

FRAP technique (*Fluorescence Recovery After Photobleaching*)

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

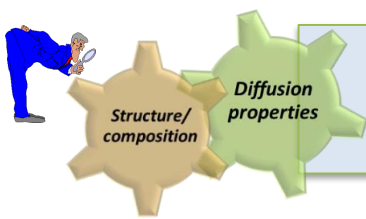


➡ In rennet gels of ↗ casein concentration (30 to 130 g/kg)

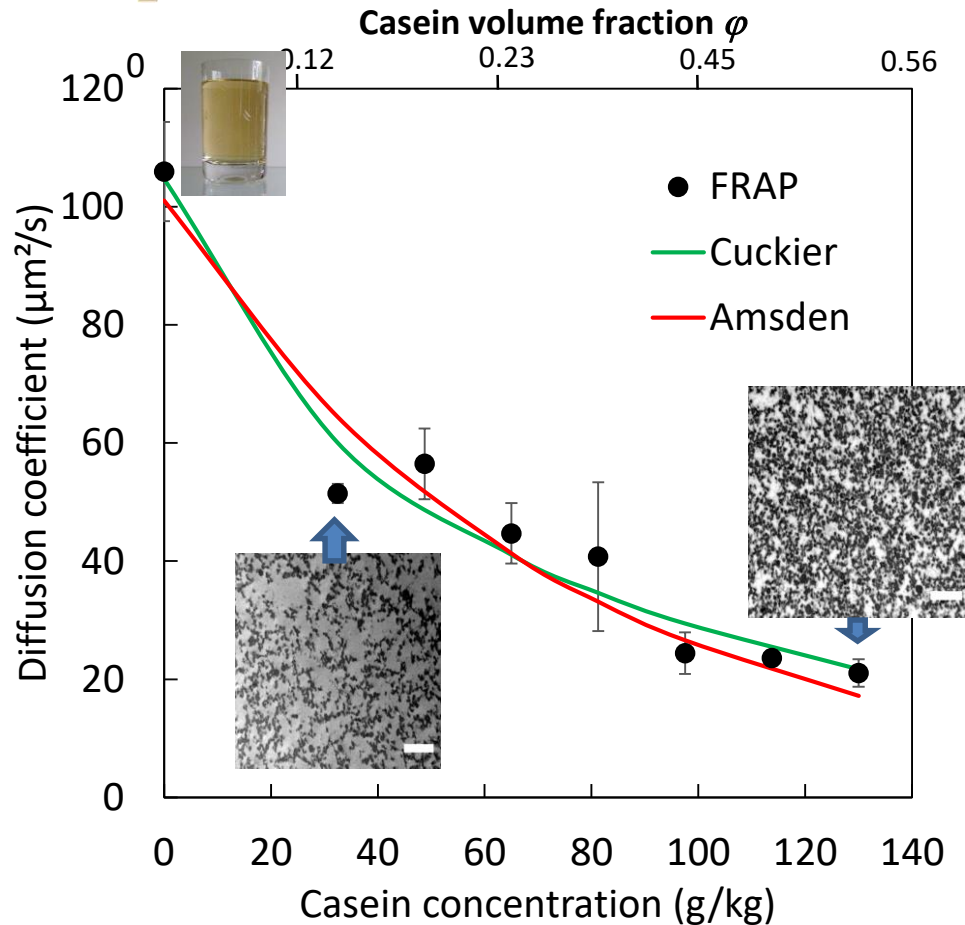


$$\tau_D = \frac{w_B^2}{4D}$$

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



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

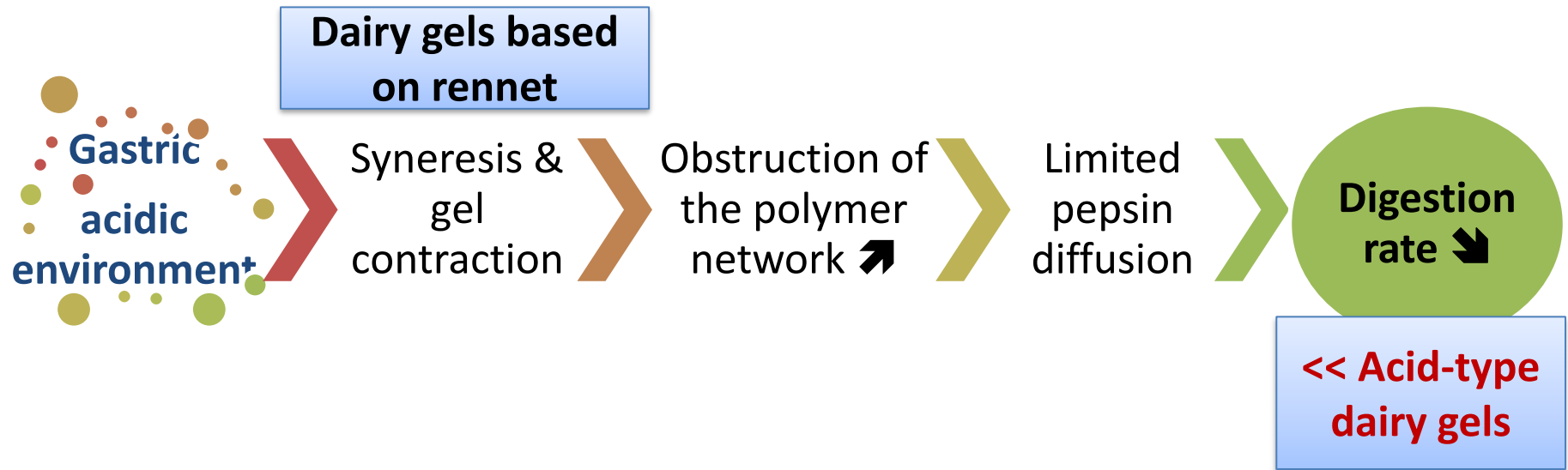
TEM Scale bars = 1 μm

Hydrodynamic model (Cuckier, 1984)

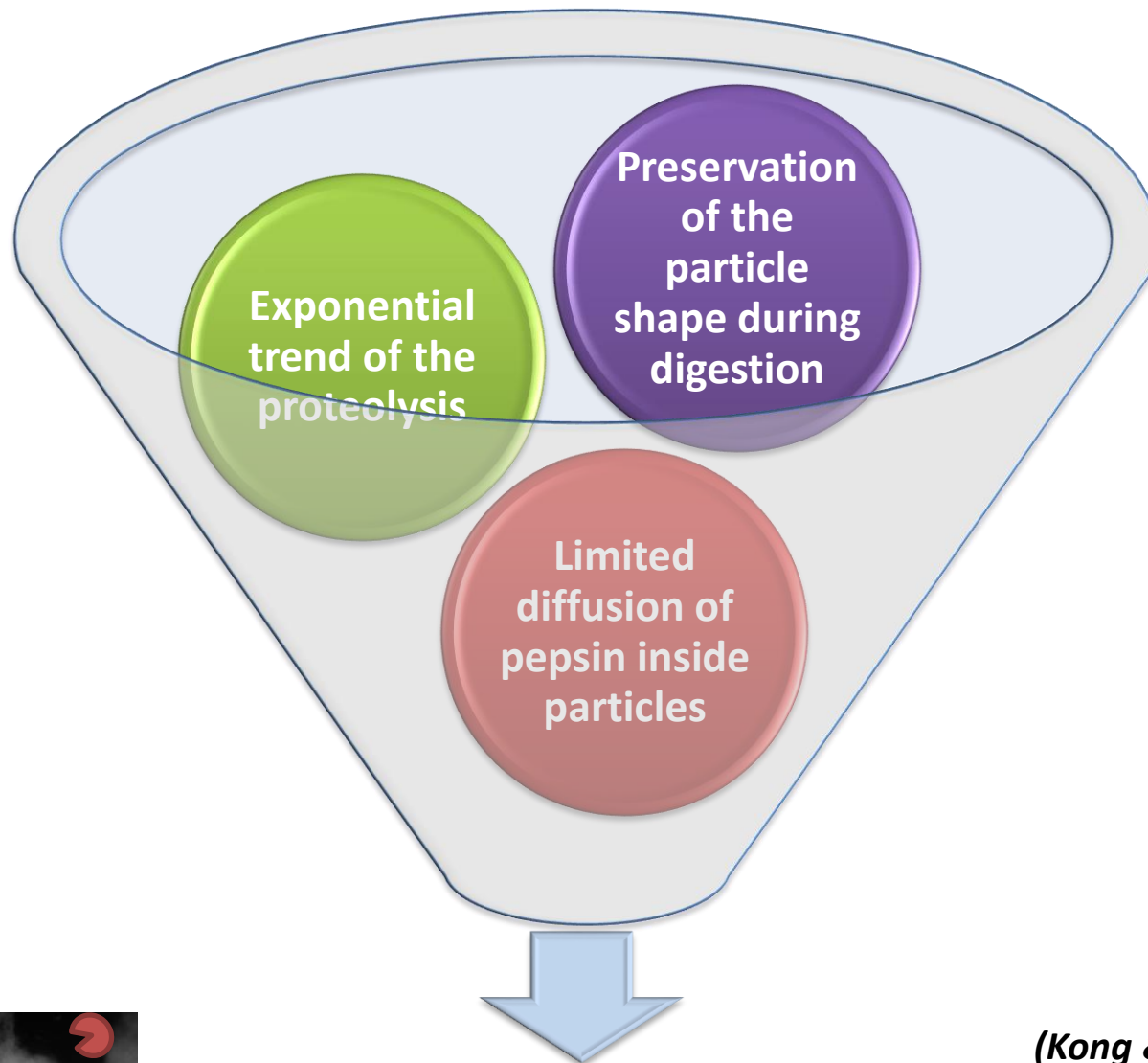
➡ frictions caused by the polymer chains slow down the solute

Obstruction-scaling model (Amsden, 1998)

➡ combines obstruction effects & hydrodynamic interactions

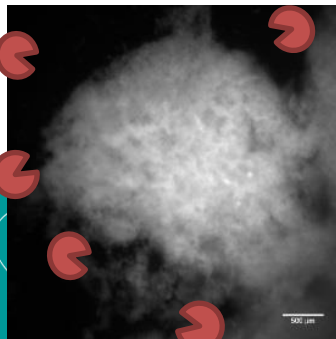


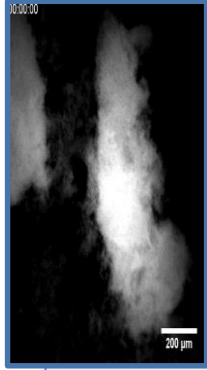
Controlling the gelling process of food proteins can lead to the formation of particles with specific structural properties that change the kinetic of digestion



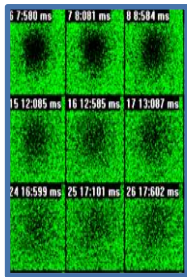
(Kong & Singh, 2009a, 2009b, 2011)

Surface erosion
= predominant mechanism of enzymatic breakdown of dairy gels

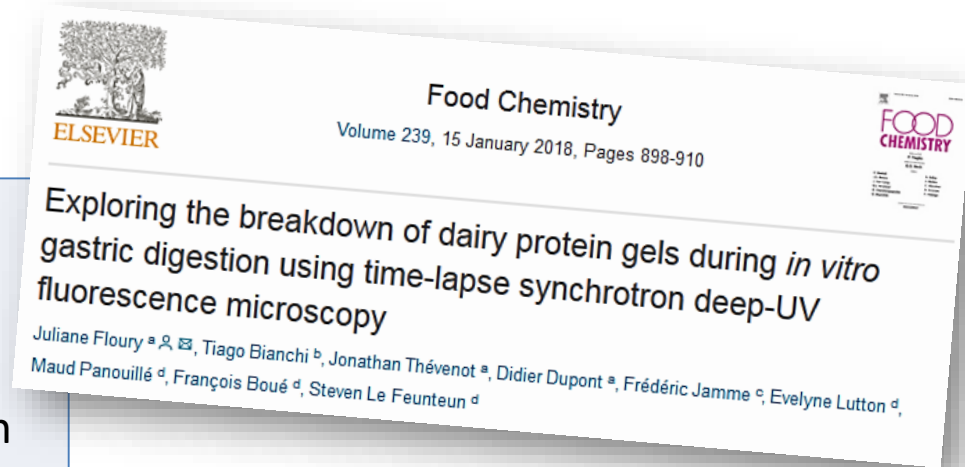




Time-lapse synchrotron deep-UV fluorescence microscopy enables the study in real time and with a high resolution of the changes in food particles during the digestion process



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

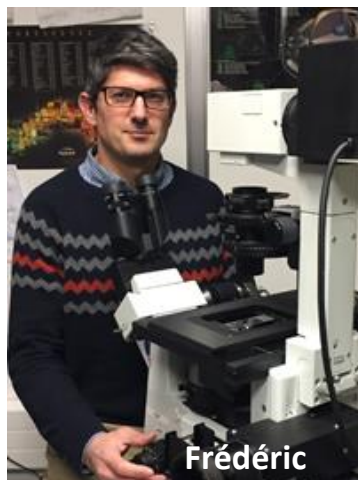




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Maude



Jonathan



Evelyne