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## Image analysis of cheese microstructure and diffusion properties of solutes: An important link to understand cheese ripening mechanisms

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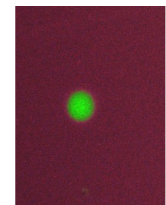
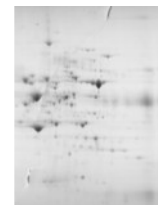
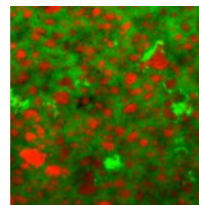
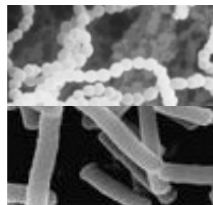
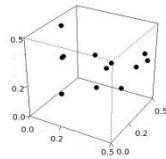
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“Image analysis of cheese microstructure and diffusion properties of solutes: An important link to understand cheese ripening mechanisms”



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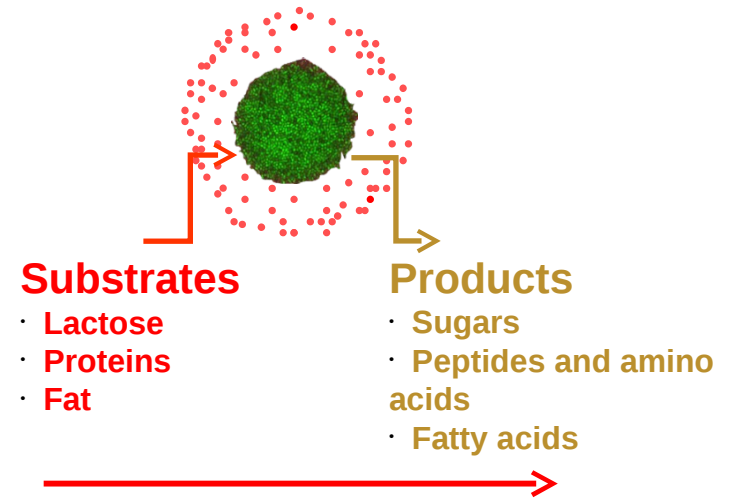
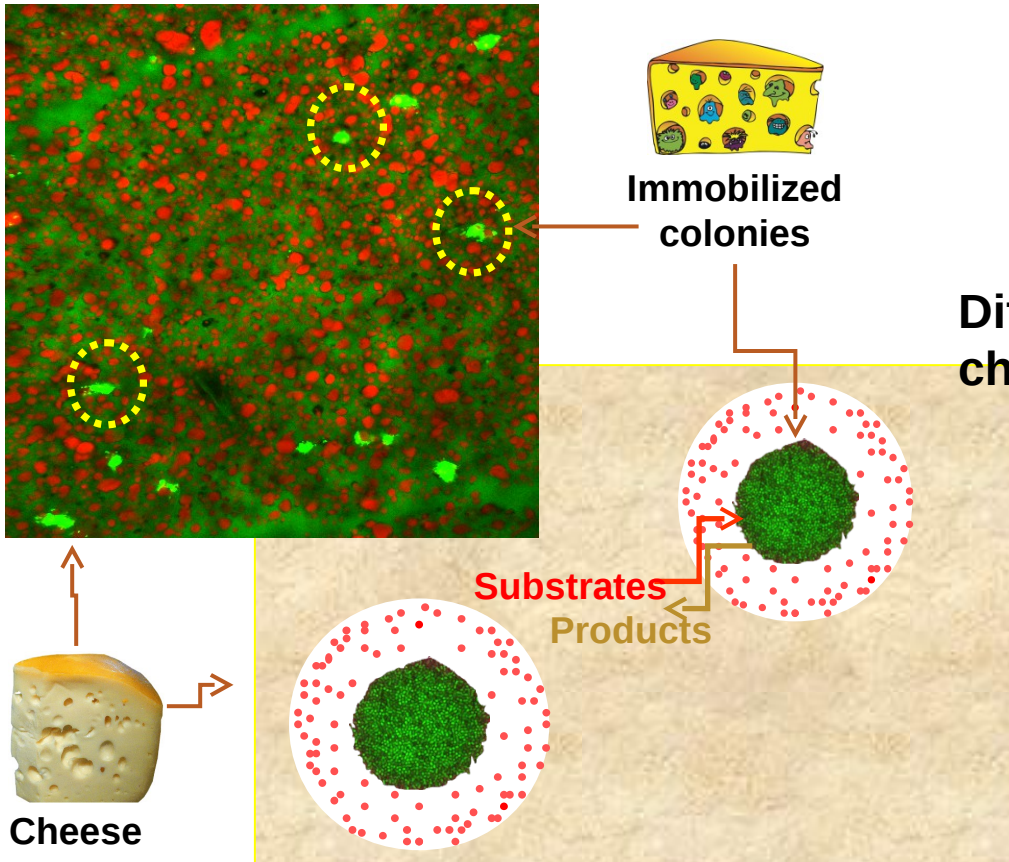
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<sup>3</sup>Agrocampus-Ouest, UMR1253, Science and Technology of Milk and Eggs, Rennes, France

# Context

## Cheese

- Key step of cheese making technology
- Complex and specific of each variety of cheese



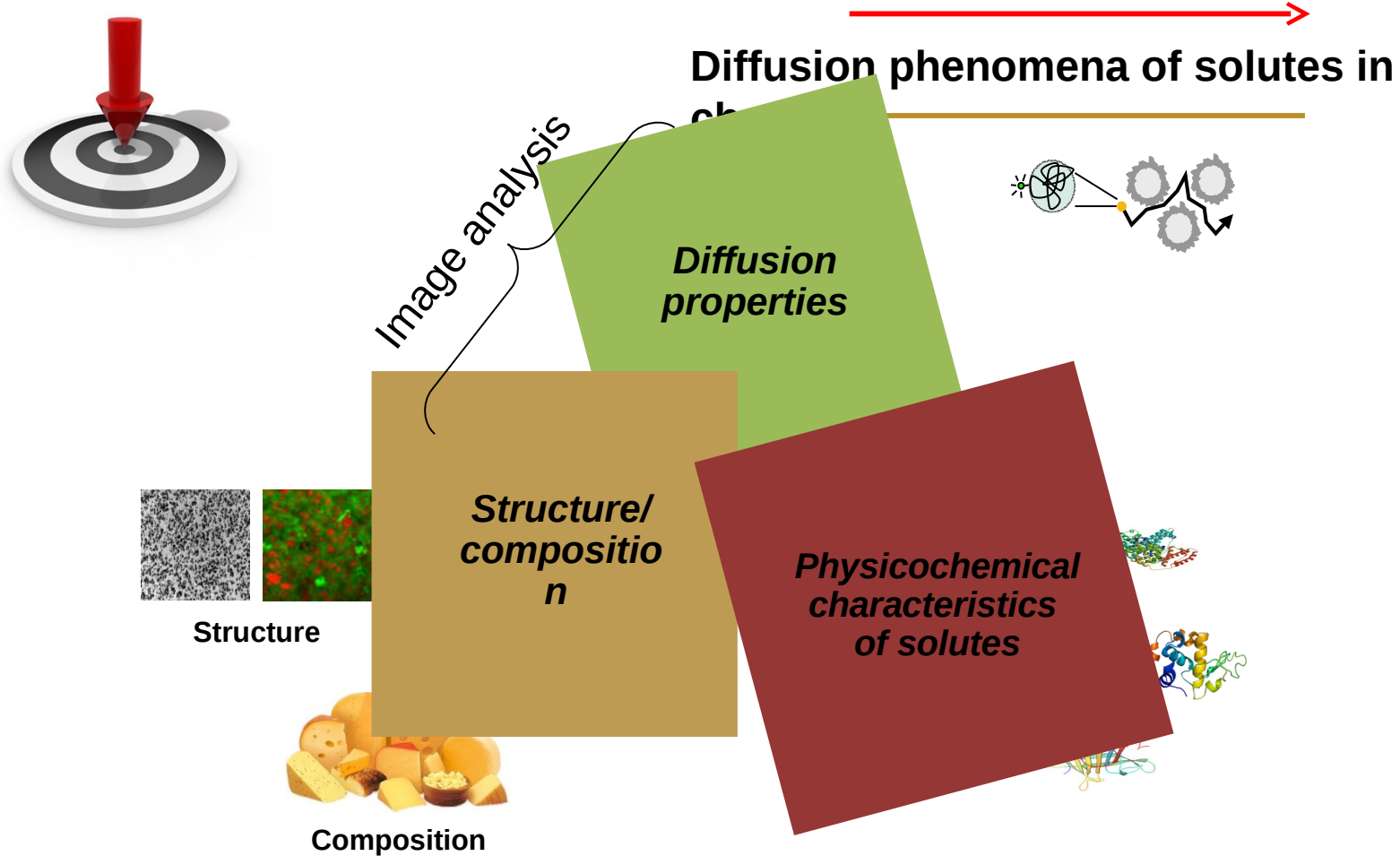
## Diffusion phenomena of solutes in cheese

- Microbial and enzyme activities
- Development of flavor and texture

## Final quality of cheese



# Objective

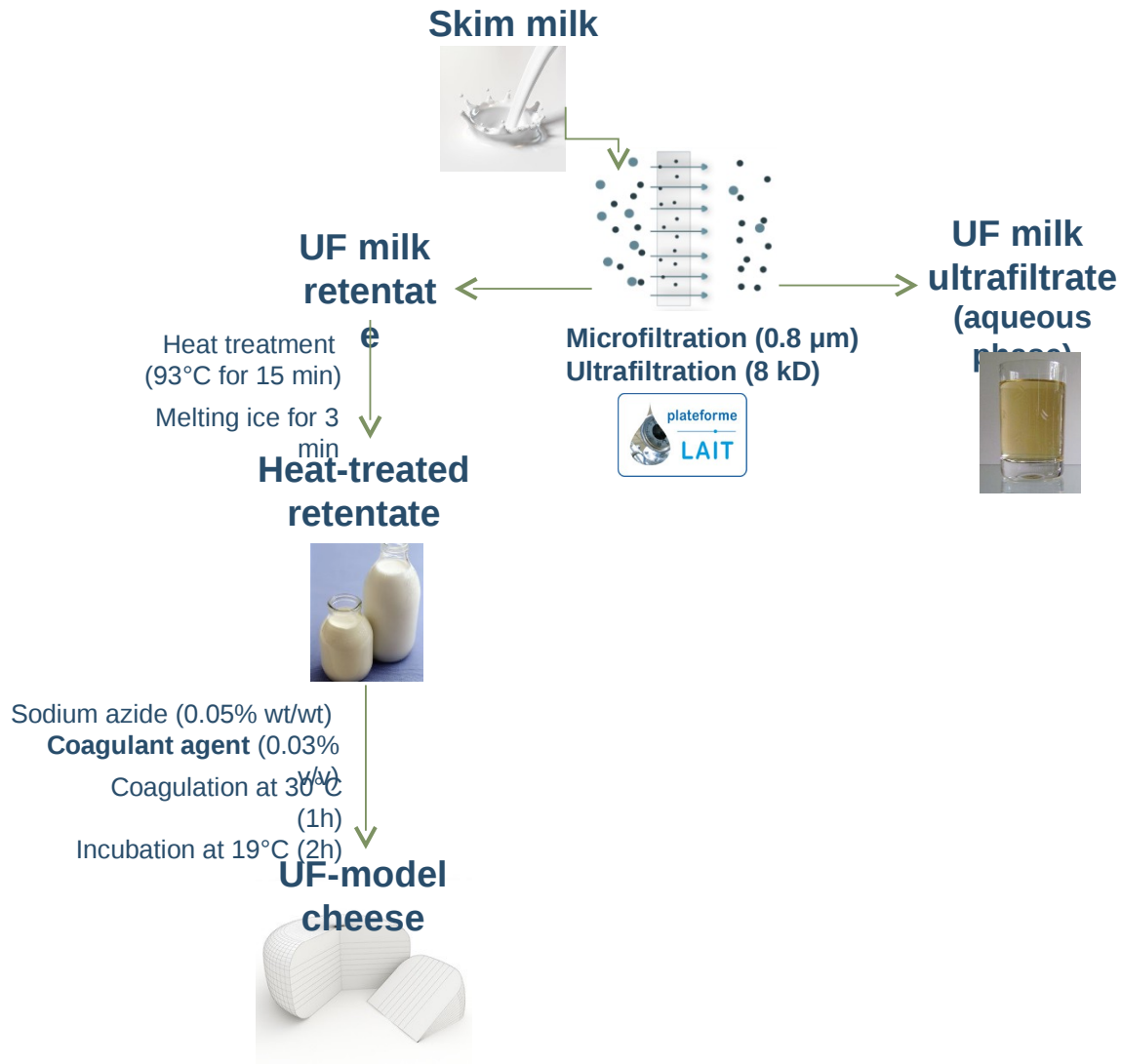


To enable a better understanding of the **ripening mechanisms...**



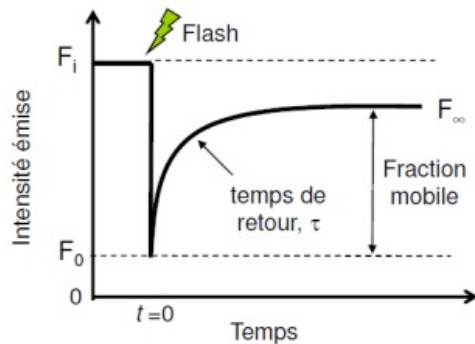
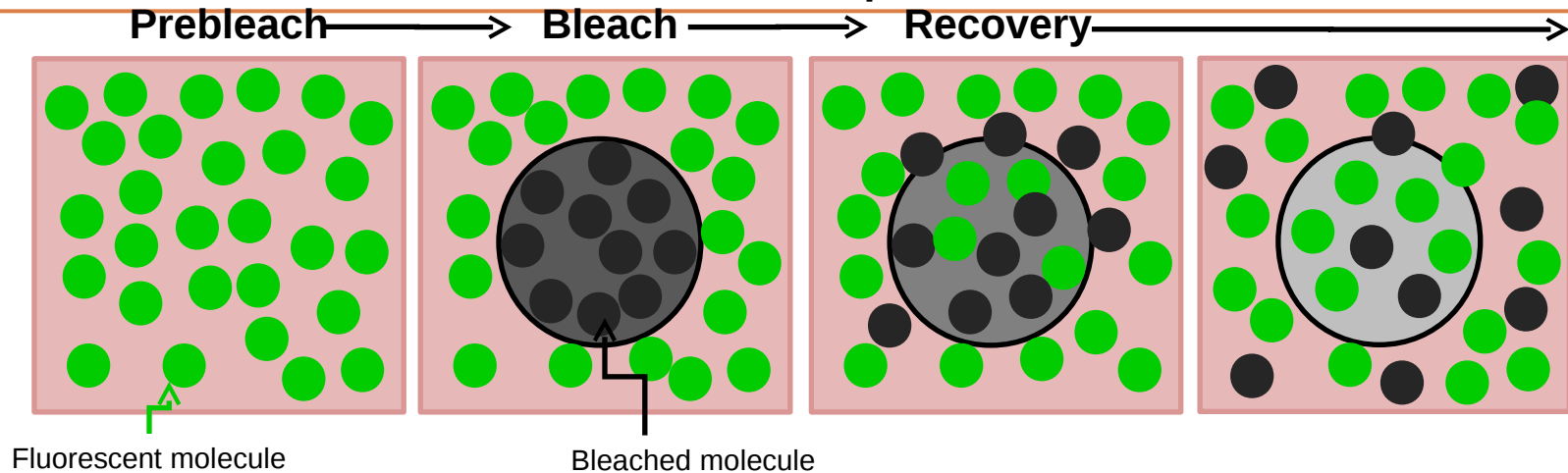
# Materials and Methods

## UF-model cheese manufacture



# Materials and Methods

## Diffusion of solutes through the UF-model cheese and in the ultrafiltrate using the FRAP technique



Typical recovery curve of fluorescence in function of time

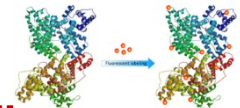
Def: effectif diffusion coefficient in the UF-model cheese ( $\mu\text{m}^2/\text{s}$ )

Daq: diffusion coefficient in the aqueous phase (ultrafiltrate)

wM: profile width in the radial direction (m)

$\tau$ : characteristic time of diffusion

### Fluorescent solutes:

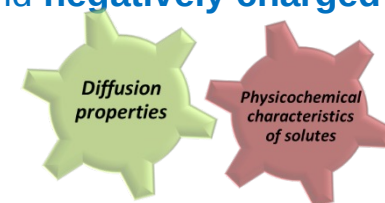


#### FITC-dextrans (4 kDa to 2 MDa)

- Flexible and neutral solutes

#### FITC-labeled dairy proteins ( $\alpha$ -LA, $\beta$ -LG and BSA)

- Rigid and negatively charged solutes





# Materials and Methods

## Study of the **microstructure** of the **UF-model cheese** using **TEM**

### Freeze-substitution and low-temperature embedding protocol:

- Protects the structure of dairy constituents



Leica EM PACT

### Cryo-fixation



#### High-pressure freezing:

- Samples are frozen in liquid nitrogen (-196°C) at high-pressure (2100 bars)
- Advantage: at 2 100 bars the melting point of water decreases because the water becomes 1 500 times more viscous. The increase in viscosity reduces the ice crystal growth rate.



Leica EM AFS2

### Freeze-substitution



- The sample is immersed in a **solvent**
- Substitution of **ice** by the **solvent**

### Embedding



The **solvent** is replaced with a low-temperature **resin**:

- Ethanol 3:1 resin
- Ethanol 1:1 resin
- Ethanol 1:3 resin
- 100% resin

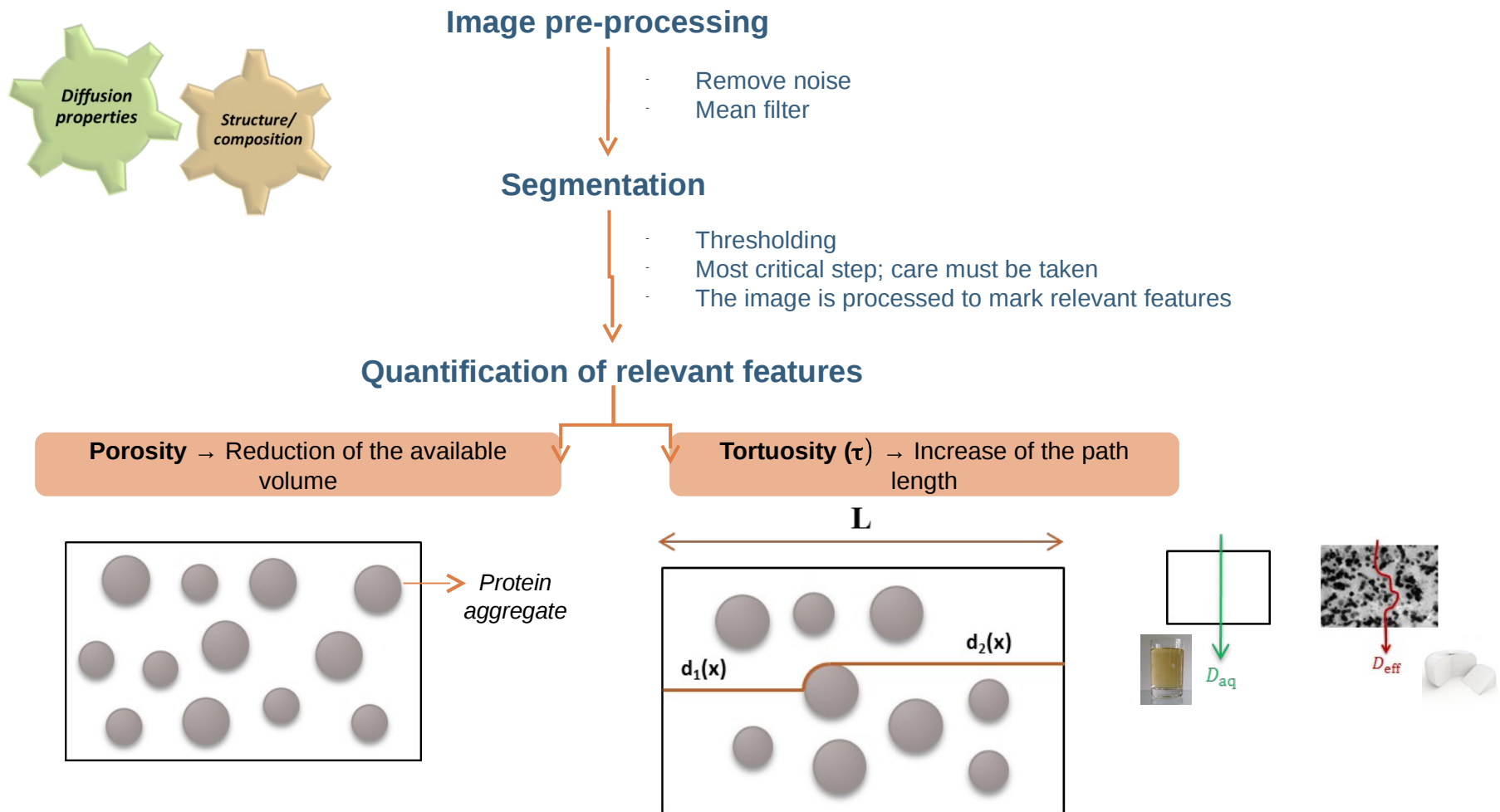
### Sectioning

Thin sections (**90nm thick**) of embedded material are cut with a diamond knife using a Reichert ultramicrotome



# Materials and Methods

## Quantitative analysis of the microstructure of the UF-Model Cheese



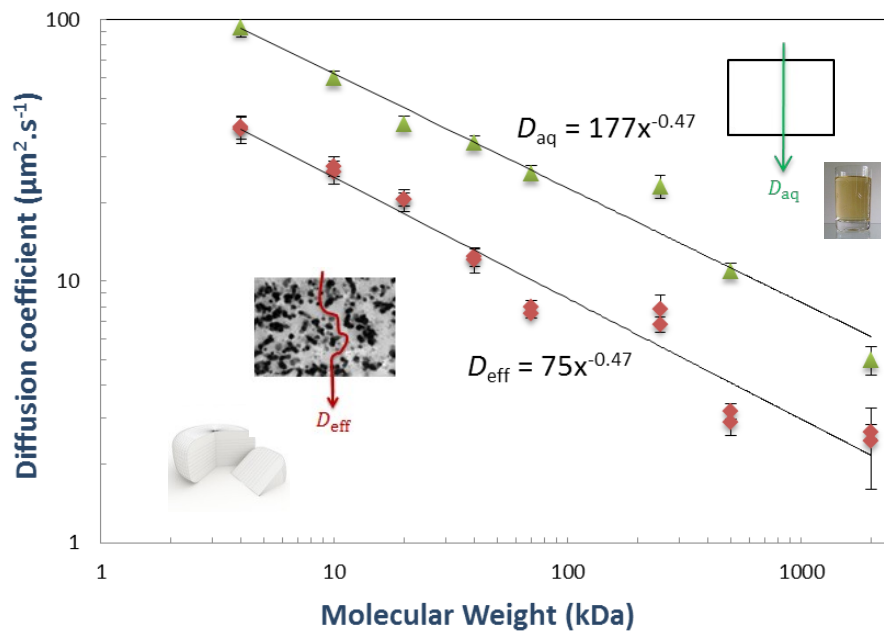
Shortest path containing a point  $x$  in the matrix, connecting two opposite edges of the image (Delarue et al. , 2003).

# Results and Discussion

## Influence of different physicochemical characteristics of solutes on diffusion in the UF-model cheese

### > Influence of the solute size...

**FITC-dextrans (4 kDa to 2 MDa)**  
- Flexible and neutral solutes




**Figure 1.** Logarithmic plots of experimental diffusion coefficients of FITC-dextrans in the UF-model cheese (□) and in the ultrafiltrate (□) as a function of their molecular weight.

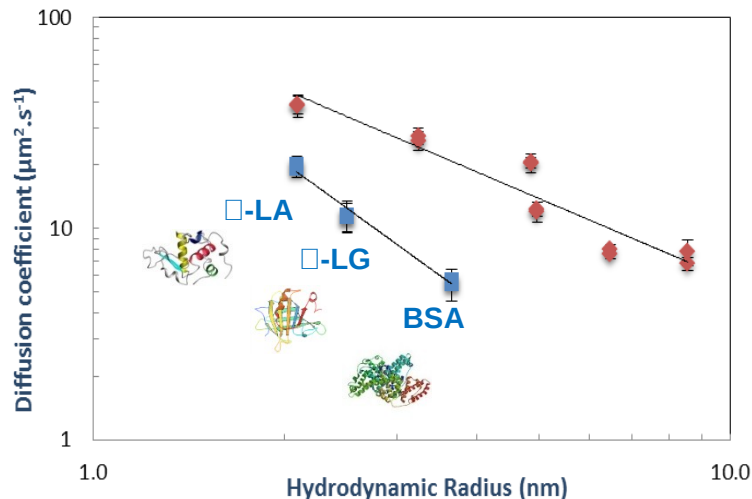
- The **larger FITC-dextrans** were **no more hindered** by the structure of the UF-model cheese when compared to the smaller ones.
- The mobility of FITC-dextrans in the UF-model cheese was reduced by a **constant obstruction factor** ( $k = 0.42$ ).
- The **pore sizes** of this model cheese were believed to be sufficiently large when compared to the **size of the solute molecules**.

# Results and Discussion

## Influence of different physicochemical characteristics of solutes on diffusion in the UF-model cheese

- > Influence of the **shape** and **charge** of solutes...

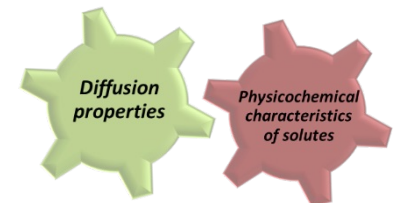
FITC-labeled dairy proteins ( $\alpha$ -LA,  $\beta$ -LG and BSA)   
 - Rigid and negatively charged solutes



**Figure 2.** Logarithmic plots of experimental diffusion coefficients of FITC-dextrans ( $\square$ ) and FITC-dairy proteins ( $\square$ ) in the UF-model cheese as a function of their hydrodynamic radius.

**Rigid and negatively charged solutes** were **hindered more** than the **flexible and neutral solutes** in the UF-model cheese

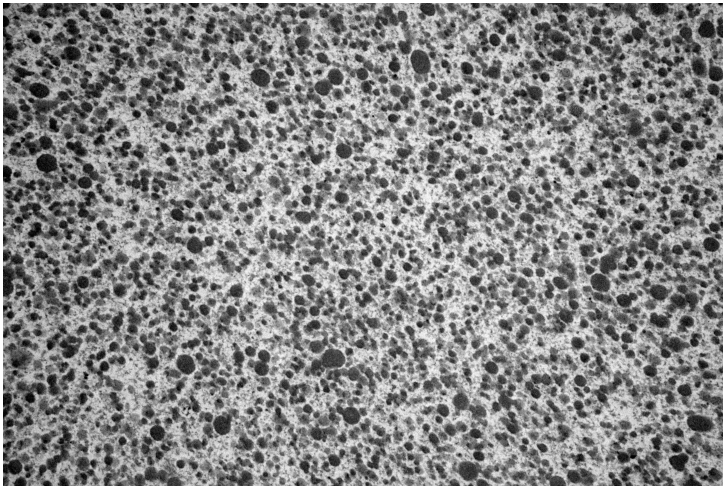
- The existence of steric and electrostatic interactions between the **protein matrix of the UF-model cheese** and the **FITC-dairy proteins** is suggested.
- The **diffusion through the protein aggregates** could play an important role in the overall diffusion of the **FITC-dairy proteins**, retarding their movement when they diffuse in the UF-model cheese.



# Results and Discussion

## Influence of the microstructure of the UF-model cheese on diffusion of solutes

### Image Analysis of the UF-model cheese

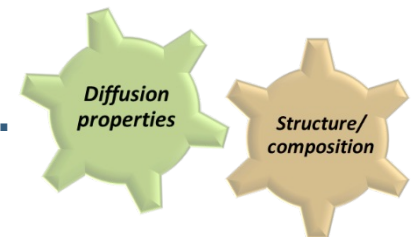


*TEM micrograph of the UF-model cheese*



*Segmented (binary) image of the UF-model cheese*

Quantification of relevant features...

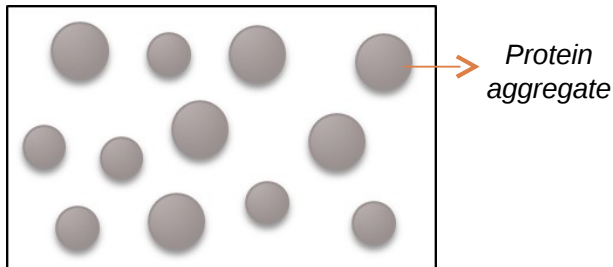


# Results and Discussion

## Influence of the microstructure of the UF-model cheese on diffusion of solutes

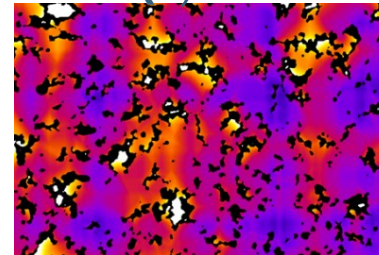
- Image Analysis of the UF-model cheese

### Porosity ( $\epsilon$ )



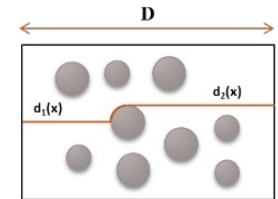
$$\epsilon = 0.638 \pm 0.007$$

### Tortuosity ( $\tau$ )



Tortuosity distribution in the UF-model cheese.  
Blue zones: low tortuosity. Yellow/orange zones: high tortuosity

$$\tau = 1.085 \pm 0.008$$

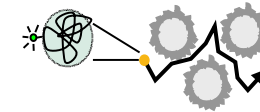


### Under way...

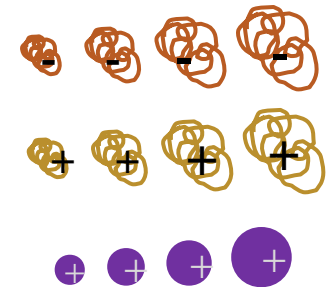
To establish the relationship between these **microstructural parameters** and **diffusion properties** by comparing results obtained in **model matrices** with **different microstructures**.

# Conclusions and Perspectives

- ❖ The **physicochemical characteristics** of solutes influence their diffusion in the UF-model cheese



- ❖ The **diffusion of other solutes** (flexible and negatively charged, rigid or flexible positively charged) may be studied to elucidate the **solute-matrix interactions**



- ❖ Studies of the **diffusion** and **structure** in **other model cheese matrices** are required to the development of **predictive models** of diffusion of solutes



- ❖ A better understanding of the influence of the **matrix microstructure** on the **diffusion of solutes** should allow a more generic view of **cheese ripening kinetics** for future innovations





# Acknowledgements

## My research team

### (MICRO):

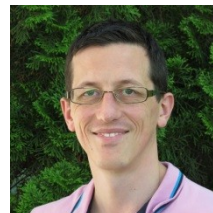
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- Anne-Thierry
- Sophie Jeanson
- Valérie Gagnaire
- Chantal Cauty



- Platform MRic



- David Legland







**Thank you for your attention!**

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