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Nutrition of the neonate - How processing of milk powder affects protein digestion?

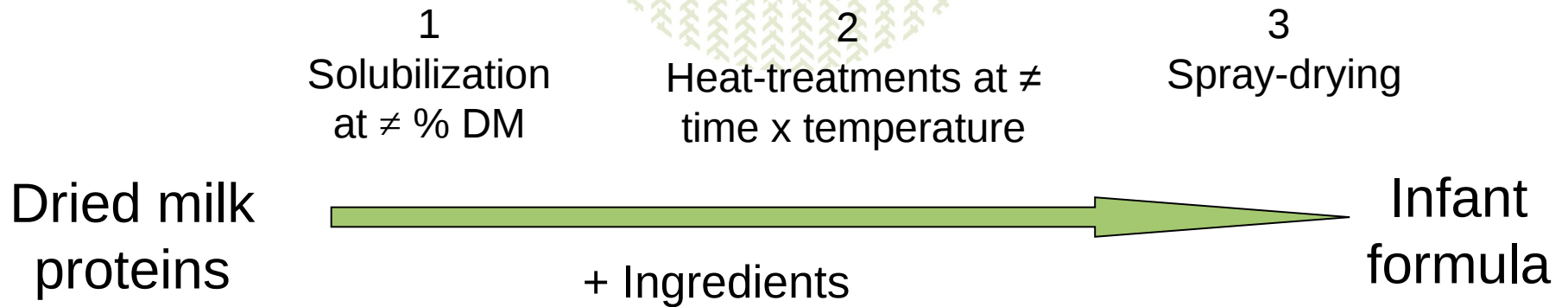
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Infant formula manufacture



↪ Interactions between proteins and other constituents

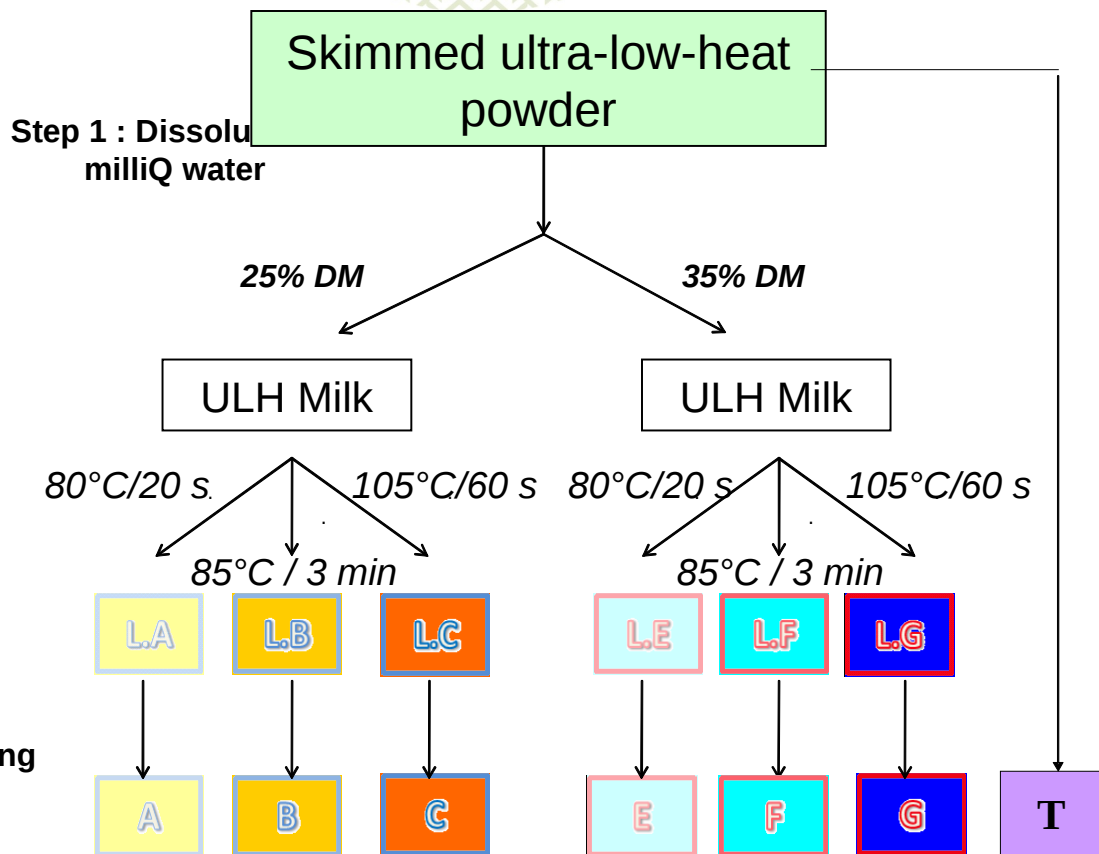
↪ Neo-formed components (protein aggregates, Maillard compounds...)

Does processing modify caseins digestibility in infant?

Objective

☞ Determine if heat treatment affects casein digestion

☞ Milk powders

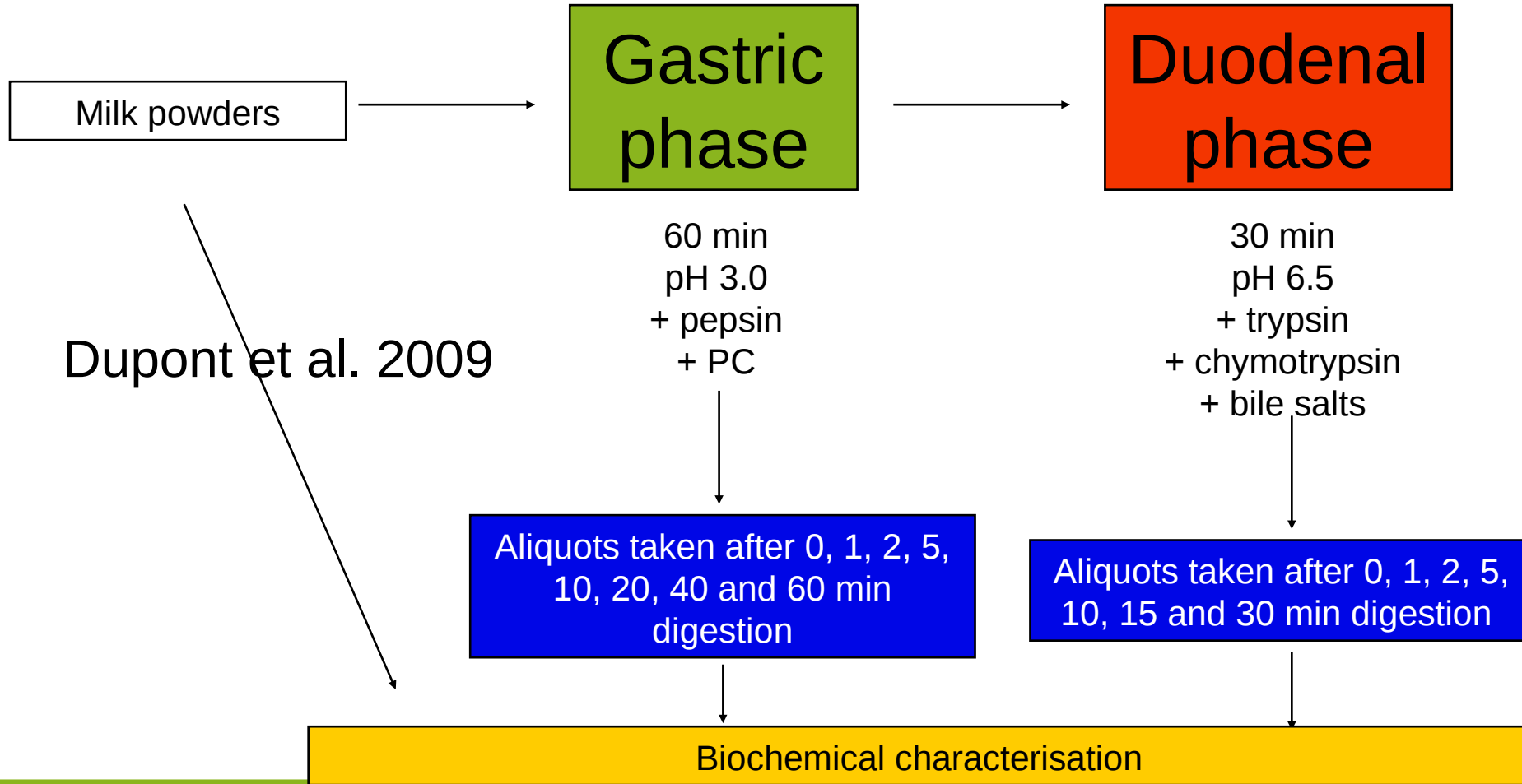


In vitro digestion



Infant gut Model

Modification of the adult gut model (Eiwegger *et al.* 2006)

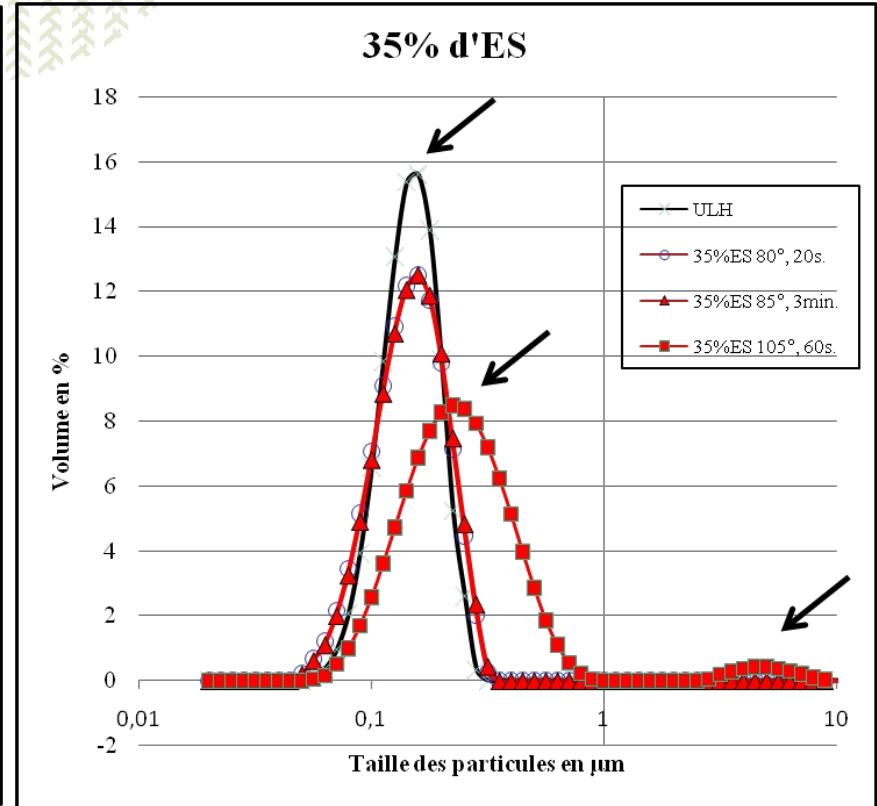
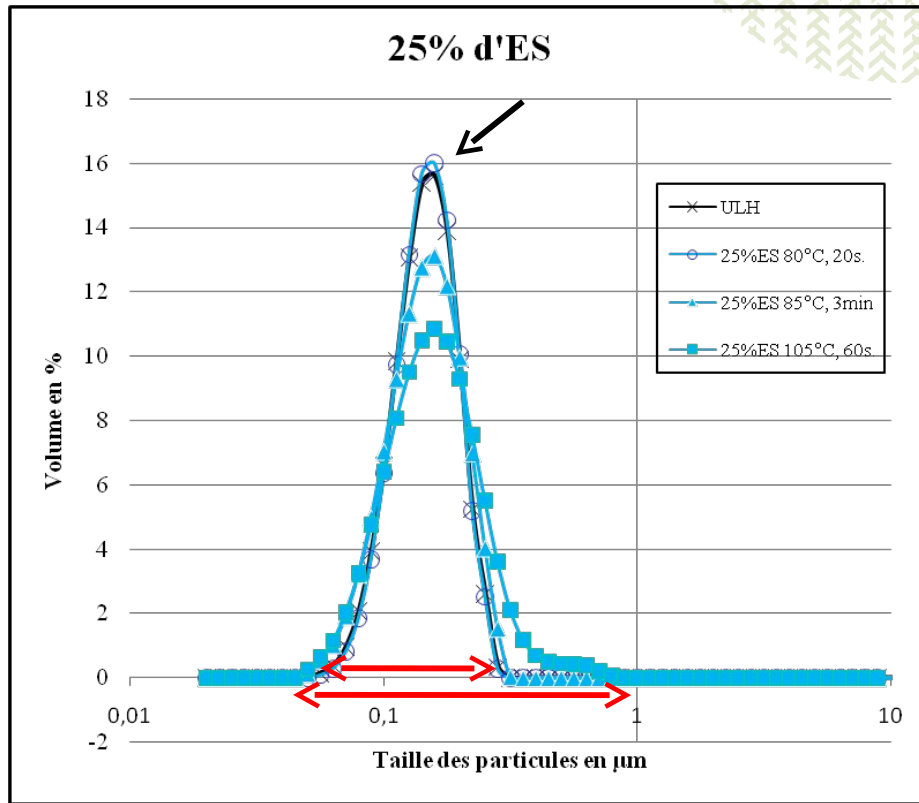





1. Characterisation of the rehydrated powders



Particle size



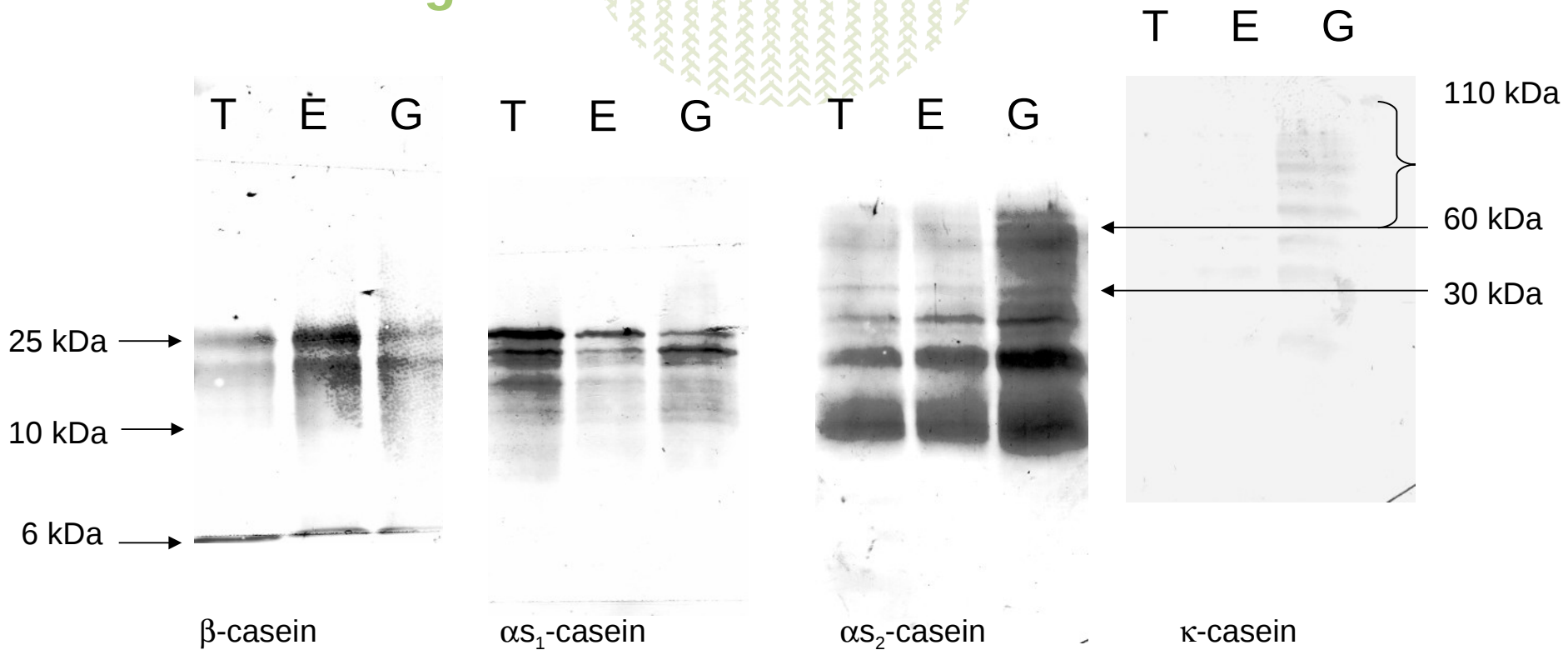
➡ heat-treatment at 105°C during 60s on 35% DM increases particle size



2. Characterisation of the digested samples by Western-blotting using αs_1 -, αs_2 -, β -, κ -casein specific antibodies



Western-blotting



High Mw compounds detected by α_{S_2} - and κ -casein specific Pabs

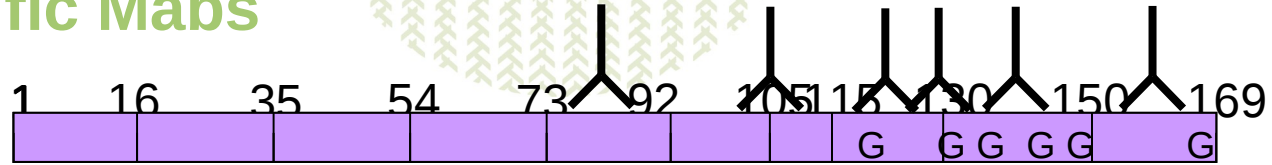
Thermal-induced aggregates with β -lactoglobulin resistant to digestion ?
(high Mw compounds observed with β -lg specific antibodies)

3. Characterisation of the digested samples by ELISA using αS_1 -, αS_2 - β -, and κ -casein specific monoclonal antibodies

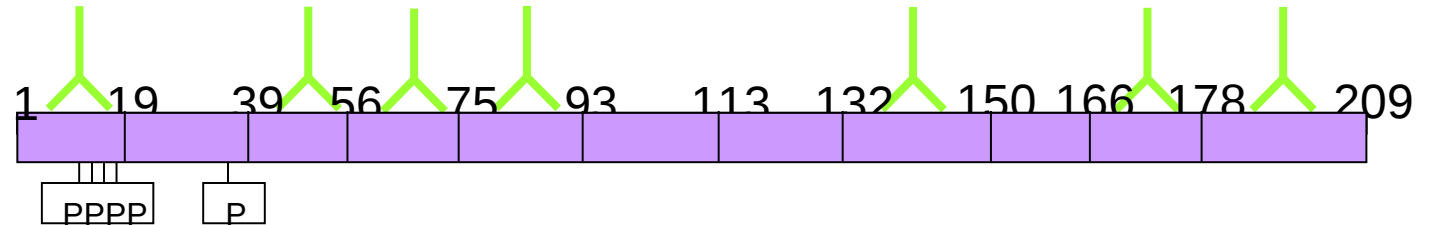


Identification of epitopes resistant to digestion by ELISA using specific Mabs

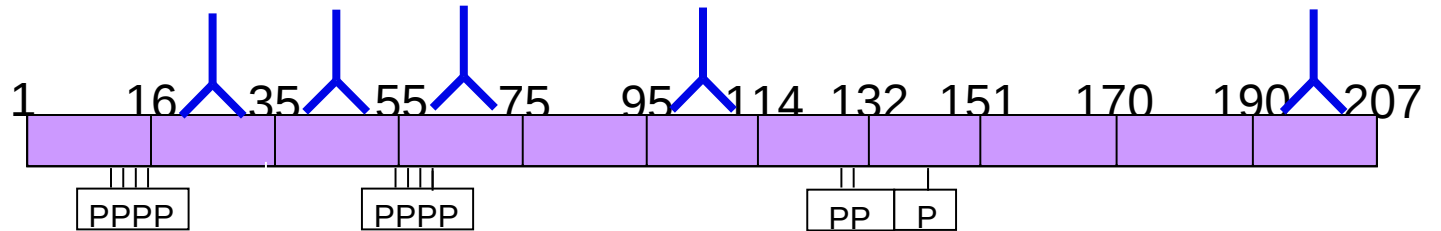
**κ -
casein**



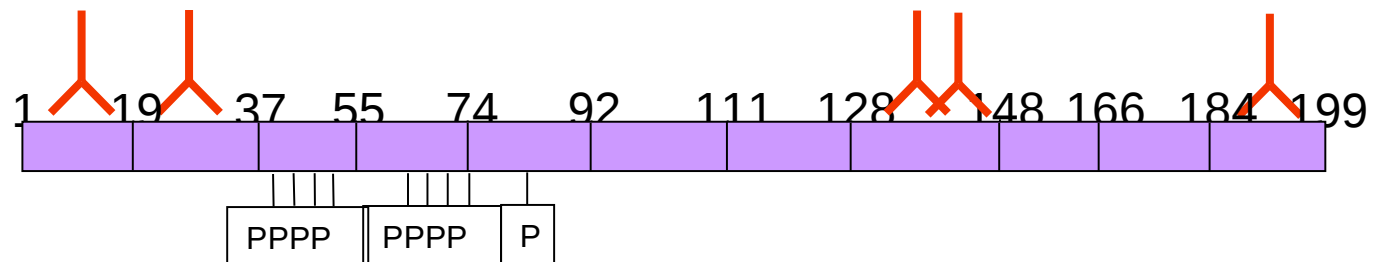
**β -
casein**



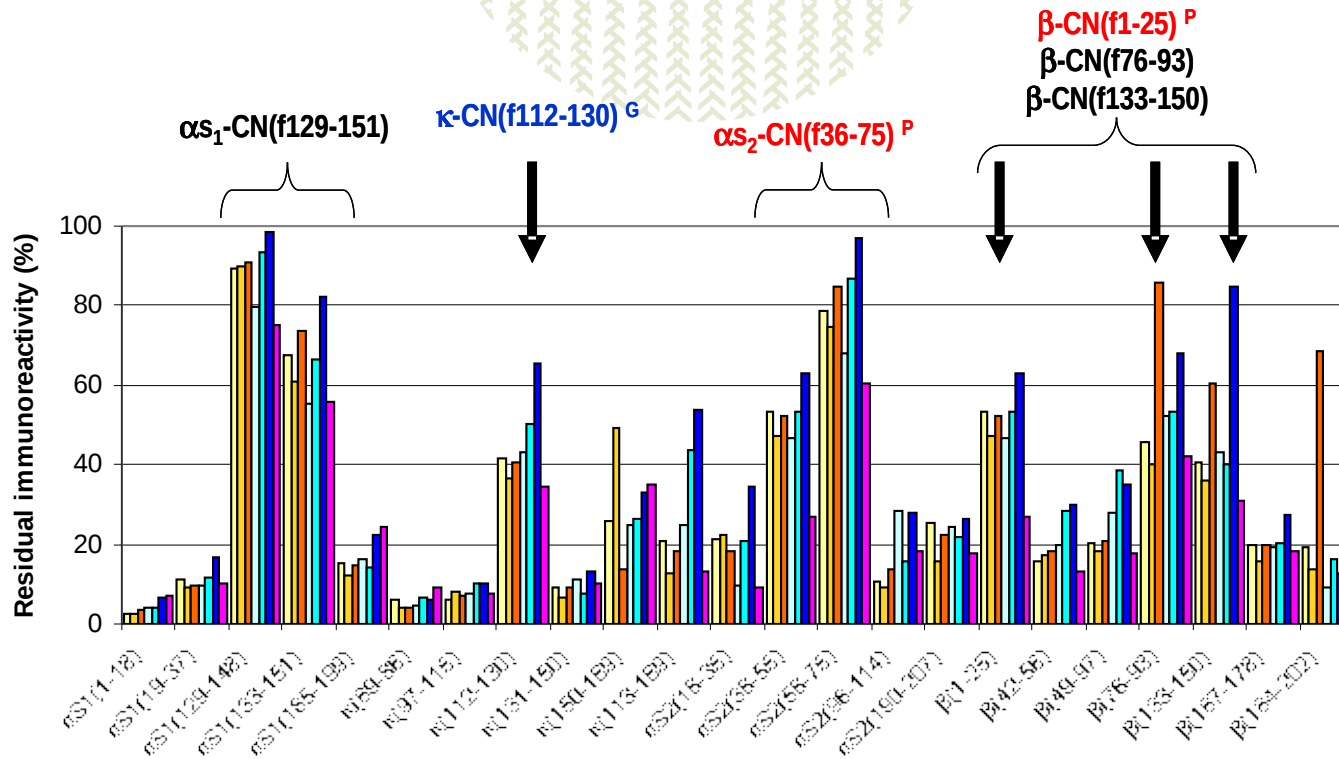
**α_{S_2} -
casein**



**α_{S_1} -
casein**



Residual immunoreactivity of casein fragments



Factor	<i>p</i> value	Significance
T	0.00001	***
% DM	0.00555	**
T:%DM	0.03262	*

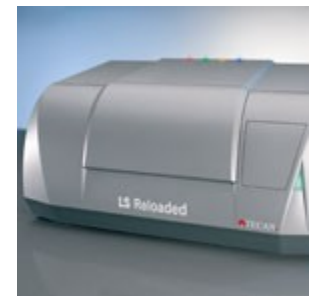
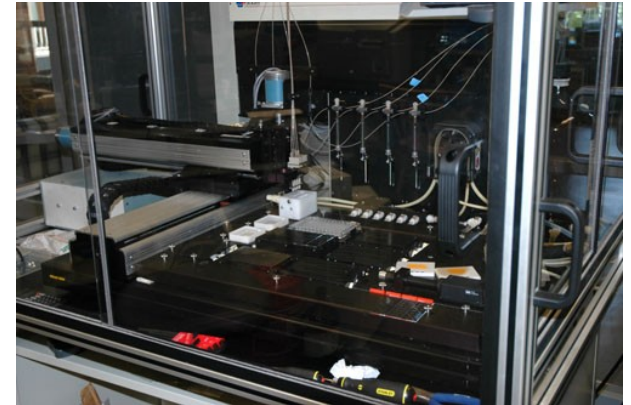
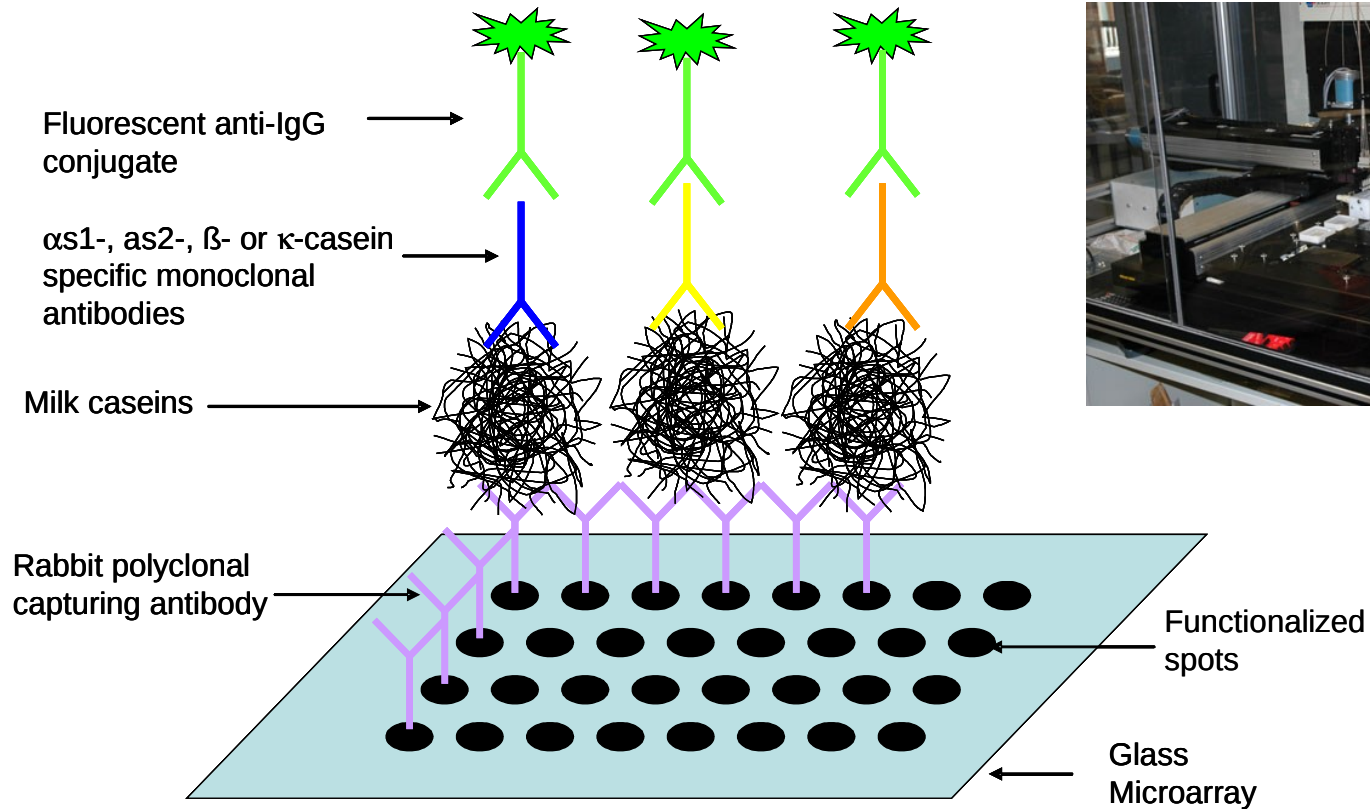
A
 B
 C
 E
 F
 G
 T

Highest resistance at 35% DM heated at 105°C /60s

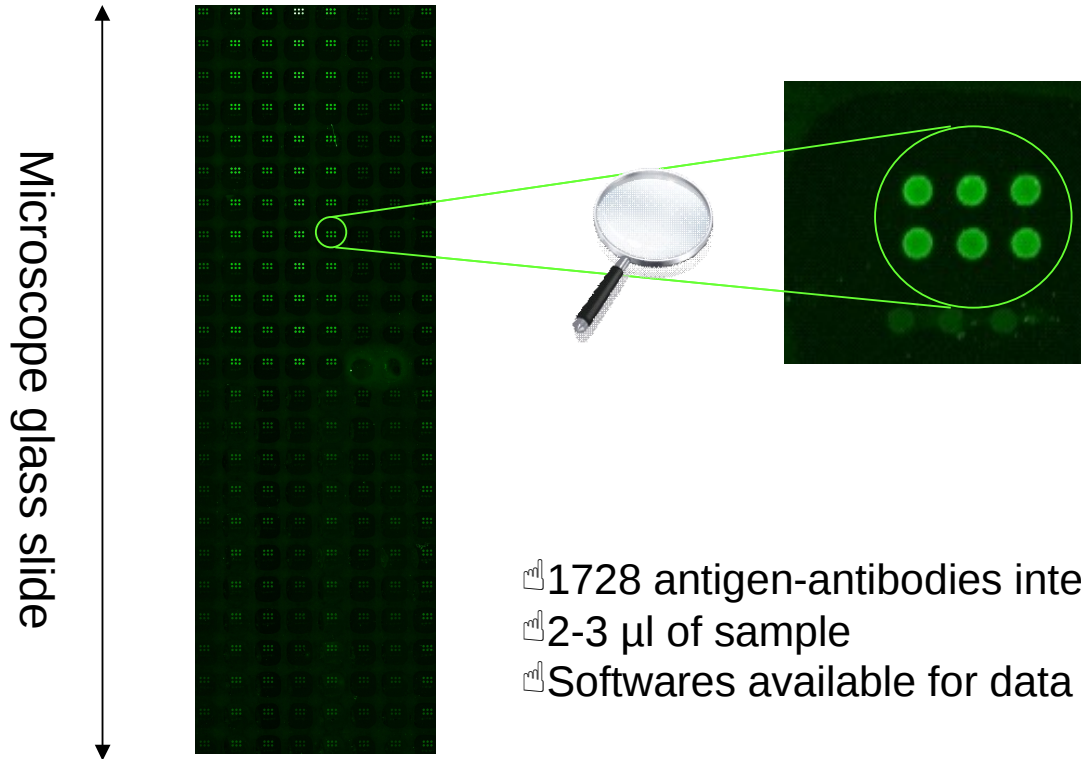
4. Kinetic of β -casein digestion using antibody microarrays



Principle of the technique

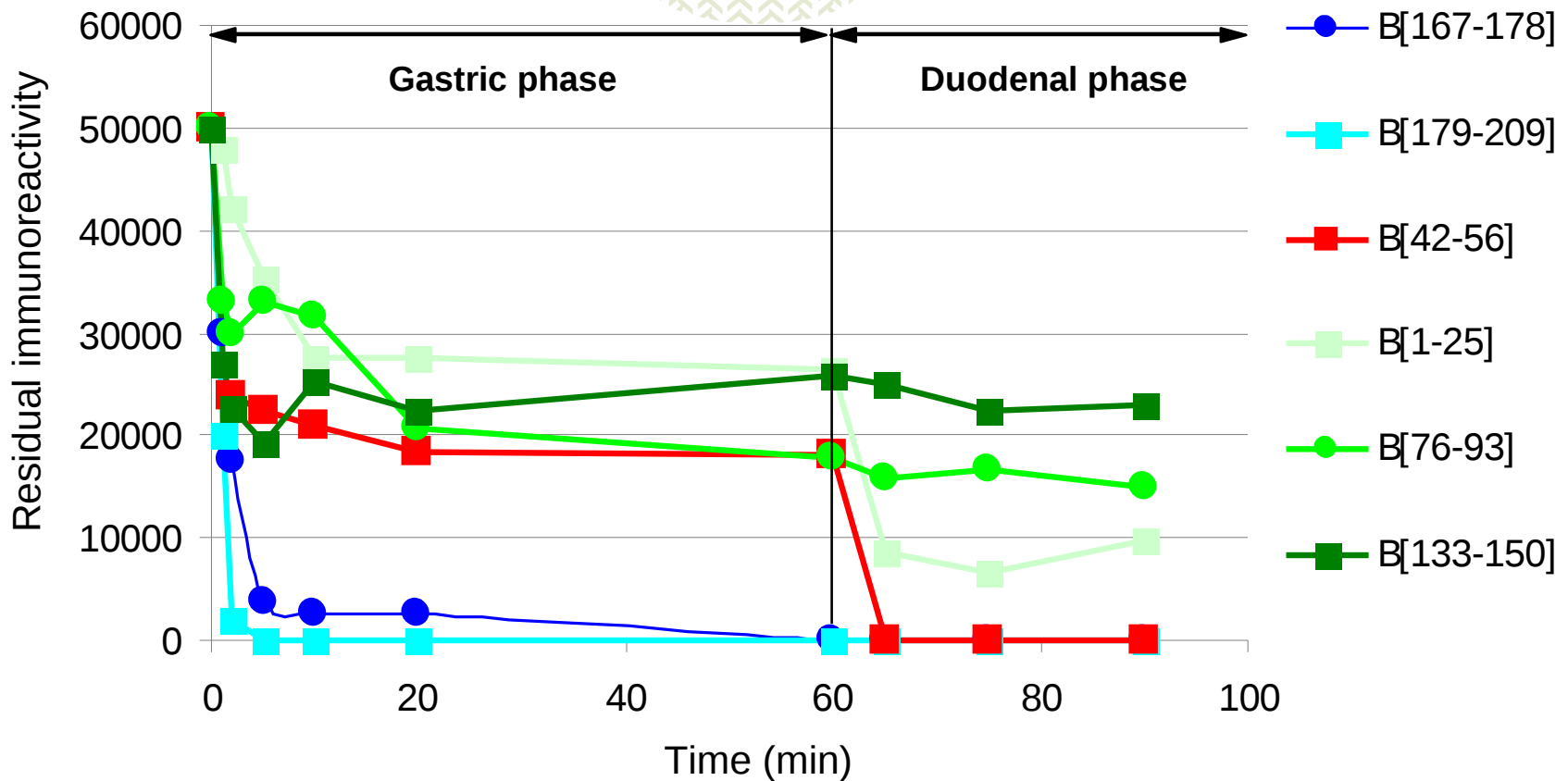


Antibody arrays – an example of results



- 👉 1728 antigen-antibodies interactions studied simultaneously
- 👉 2-3 μ l of sample
- 👉 Softwares available for data integration

Kinetics of β -casein digestion



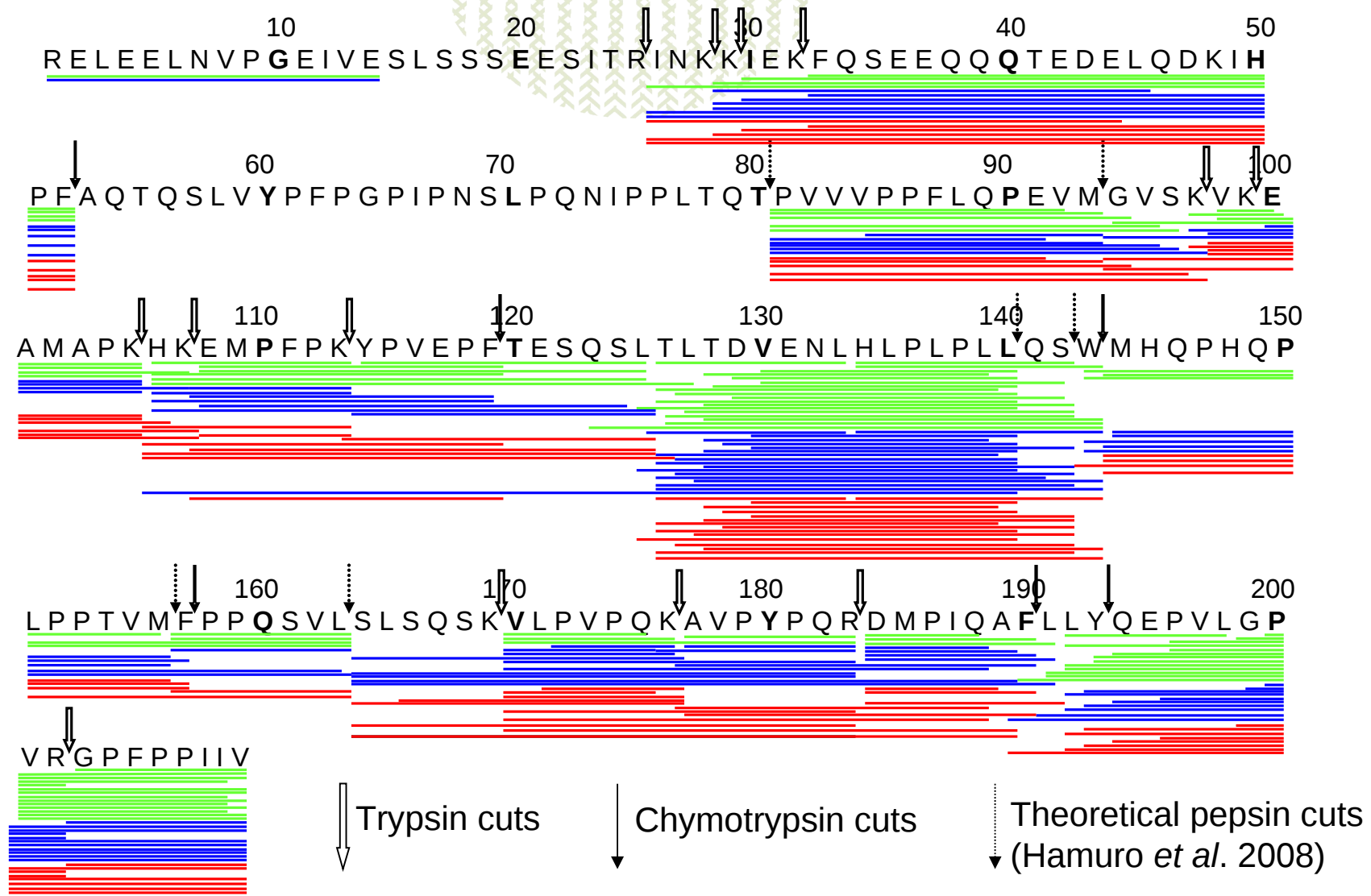
📄 Kinetics of β -casein digestion differ according to the area studied



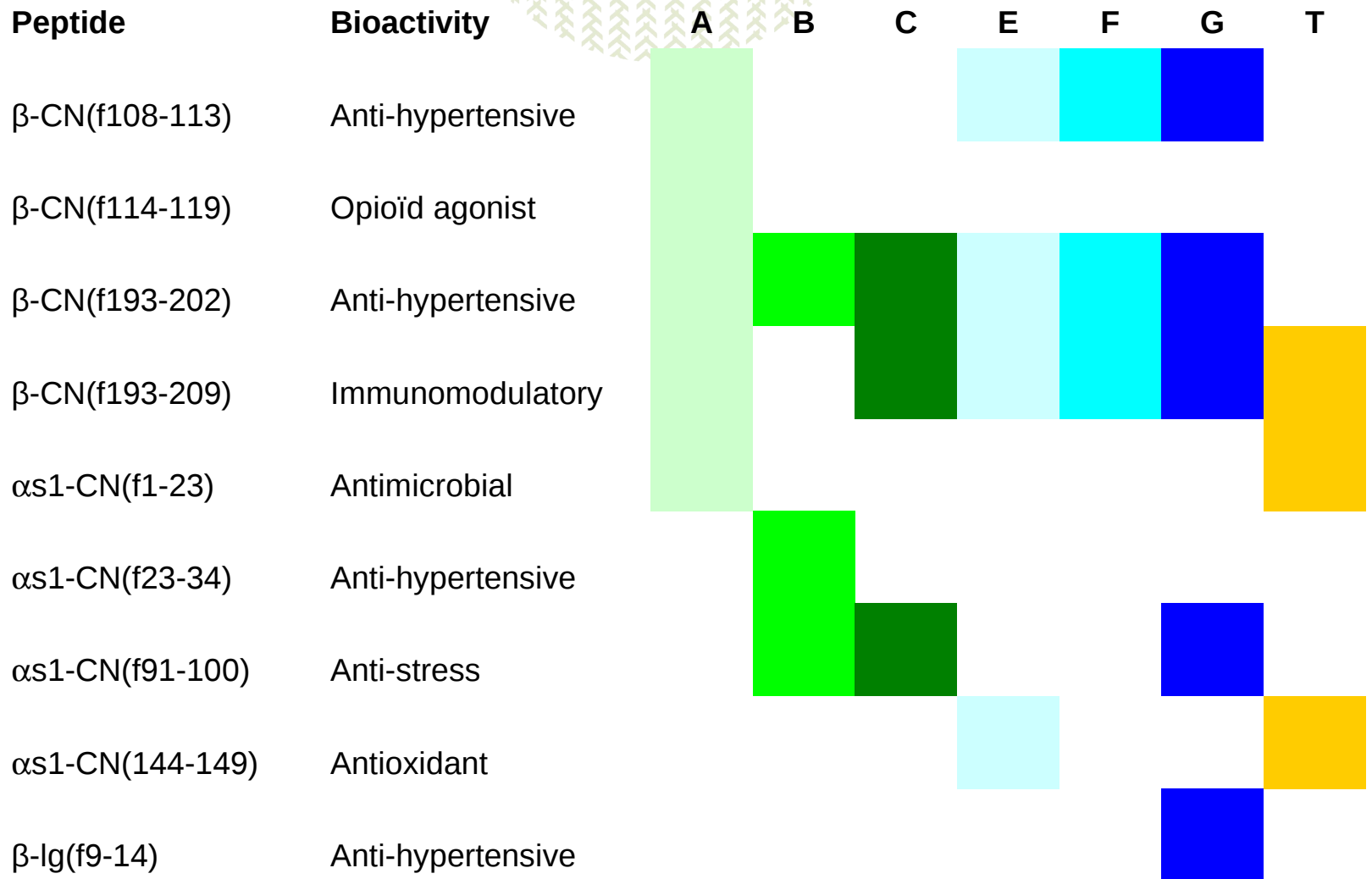
5. Characterisation of the digested samples by LC-MS-MS



β-casein peptides identified in T, E, G digested samples



Bioactive peptides detected

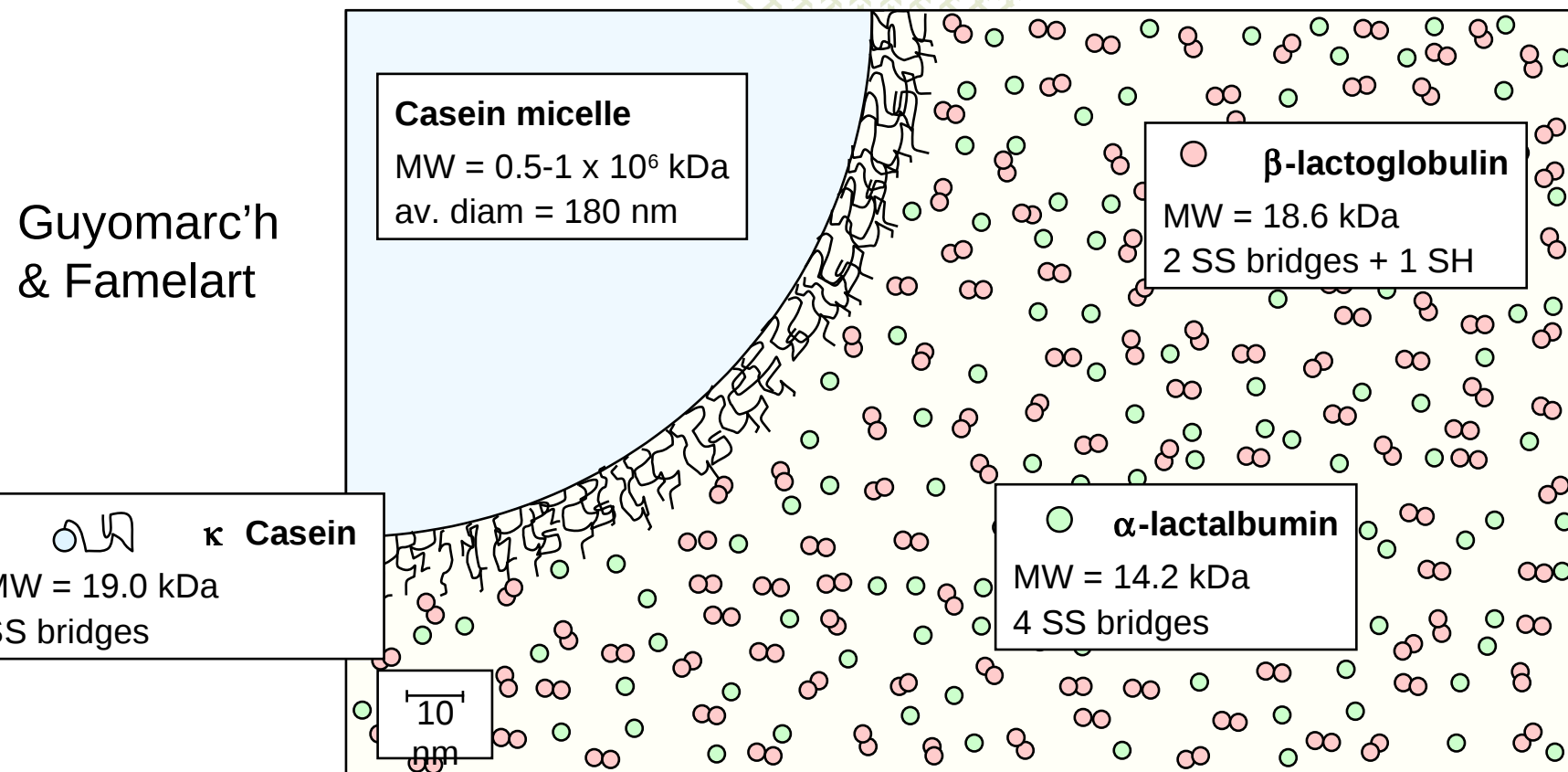


Discussion / Conclusion

- Caseins partly resist to *in vitro* infant digestion model
- Resistant area have been partly identified
- Post-translational modifications and hydrophobicity could explain the resistance of these fragments to digestion
- Bioactive peptides profile can be modified by processing
- Heat treatment has a significant influence on casein immunoreactivity in digested samples (increase)
Why?

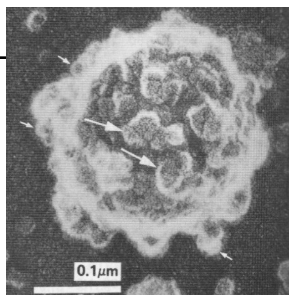
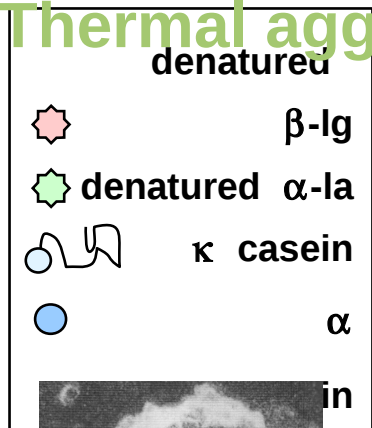
Thermal aggregation of whey proteins on the casein micelle

Guyomarc'h
& Famelart

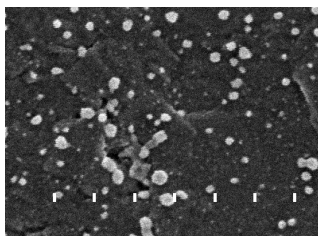


To scale schematic representation of the major whey proteins and of the casein micelle in unheated milk

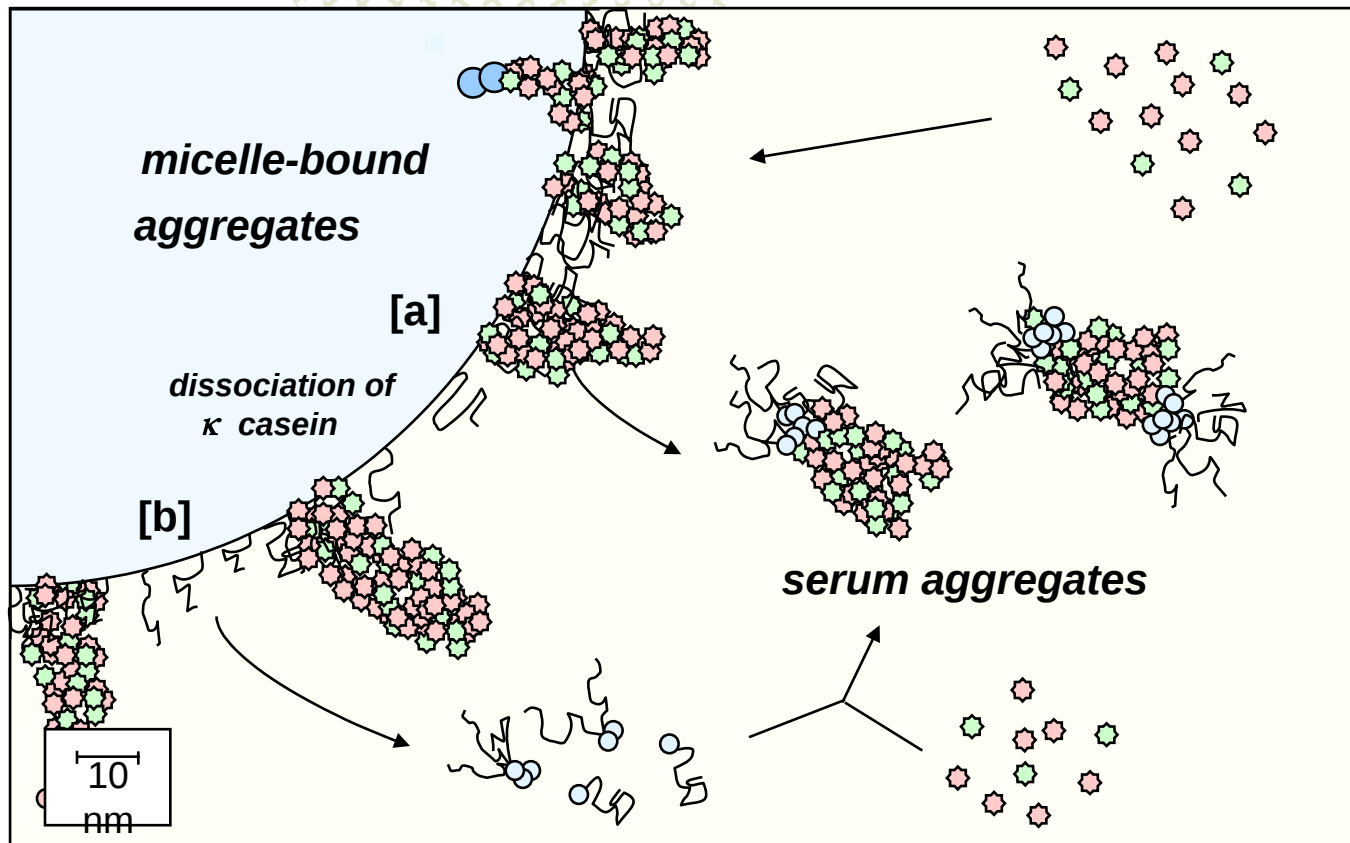
Thermal aggregation of whey proteins on the casein micelle



a (Harwalkar et al., 1989)



b (Rodriguez del Angel & Dalgleish, 2006)



Micelle-bound and serum aggregates are formed, containing denatured whey proteins, κ casein and possible traces of α_{s2} casein. [a] and [b] indicate the 2 possible ways for the formation of the serum aggregates.

Guyomarc'h & Famelart

Discussion / Conclusion

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Why?

? Thermal aggregation of whey proteins on the casein micelle

What's next?

- 👉 Development of a more sophisticated *in vitro* digestion model including the flows and the pH variation in the stomach

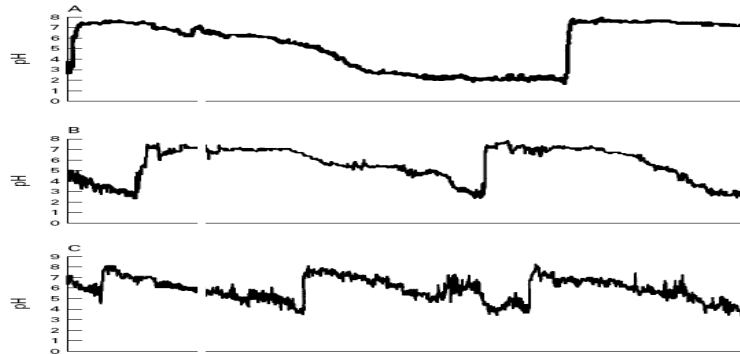
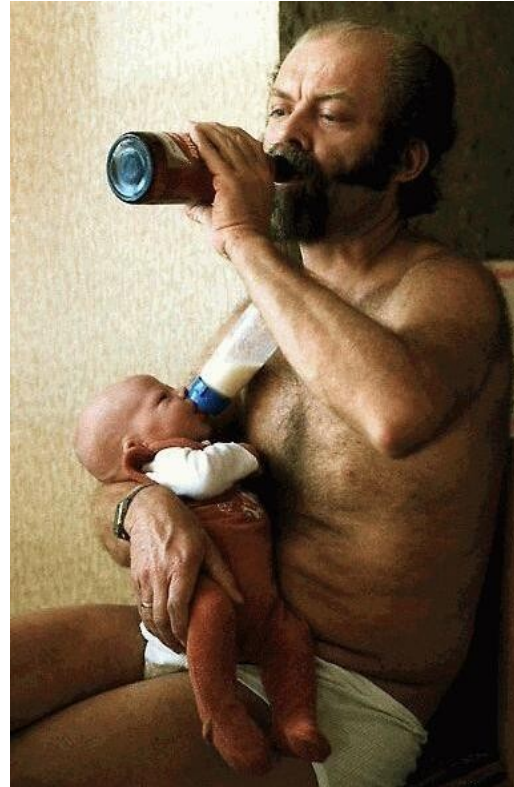


Figure 2 Examples of gastric pH records, six hour intervals: (A) four hourly feeds; (B) three hourly feeds; (C) 1 so hourly feeds.

- 👉 Comparison of *in vitro* data with those obtained on animal models (piglets)
- 👉 Validation of the gastric phase of the *in vitro* model towards human data (collection of gastric contents in pre-term neonates)
- 👉 Study of the influence of lipid-protein interactions on protein digestion

Improving infant formula for improving human life



Thanks for your kind attention !!!

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