



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

Didier Dupont, Rachel Boutrou, Julien Jardin, Daniel Mollé, Gaëlle Tanguy-Sai, Frédéric Gaucheron, Pierre Schuck, Joëlle Léonil, S. Ahmad, B. Haab

► To cite this version:

Didier Dupont, Rachel Boutrou, Julien Jardin, Daniel Mollé, Gaëlle Tanguy-Sai, et al.. Nutrition of the neonate - How processing of milk powder affects protein digestion?. International Conference on Recent Advances in Human Nutrition with Special Reference to Vulnerable Groups, Feb 2010, Faisalabad, Pakistan. hal-01482815

HAL Id: hal-01482815

<https://hal.science/hal-01482815>

Submitted on 4 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - ShareAlike 4.0 International License

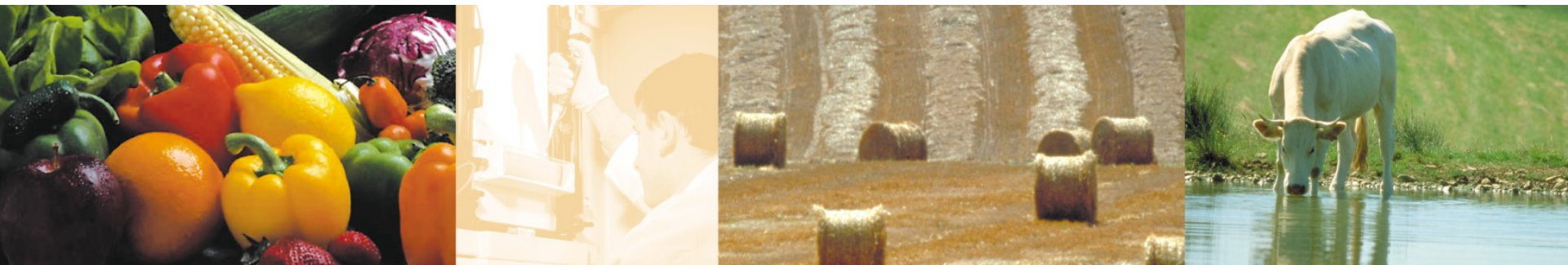
Nutrition of the neonate - How processing of milk powder affects protein digestion?

Dupont D, Boutrou R, Jardin J, Molle D, Tanguy G, Gaucheron F, Schuck P, Léonil J, Ahmad S & Haab B*

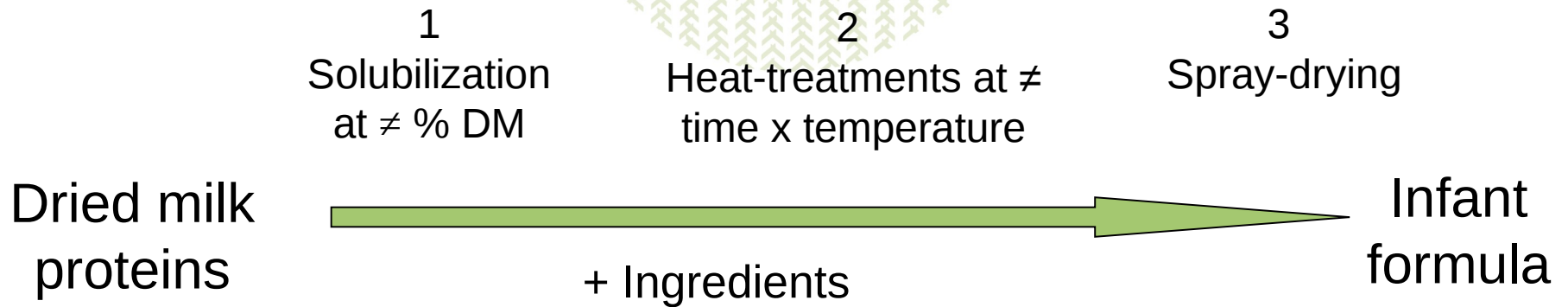
didier.dupont@rennes.inra.fr

INRA – Agrocampus Ouest, UMR STLO, 65 rue de St Brieuc 35042 Rennes France

* VAN ANDEL Research Institute, 333 Bostwick NE, Grand Rapids, MI 49503, USA



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



Step 1 : Dissolve in
milliQ water

Skimmed ultra-low-heat
powder

25% DM

35% DM

ULH Milk

ULH Milk

80°C/20 s

105°C/60 s

80°C/20 s

105°C/60 s

85°C / 3 min

85°C / 3 min

Step 2 :
Heat
treatment

L.A

L.B

L.C

L.E

L.F

L.G

Step 3 :
Spray drying

A

B

C

E

F

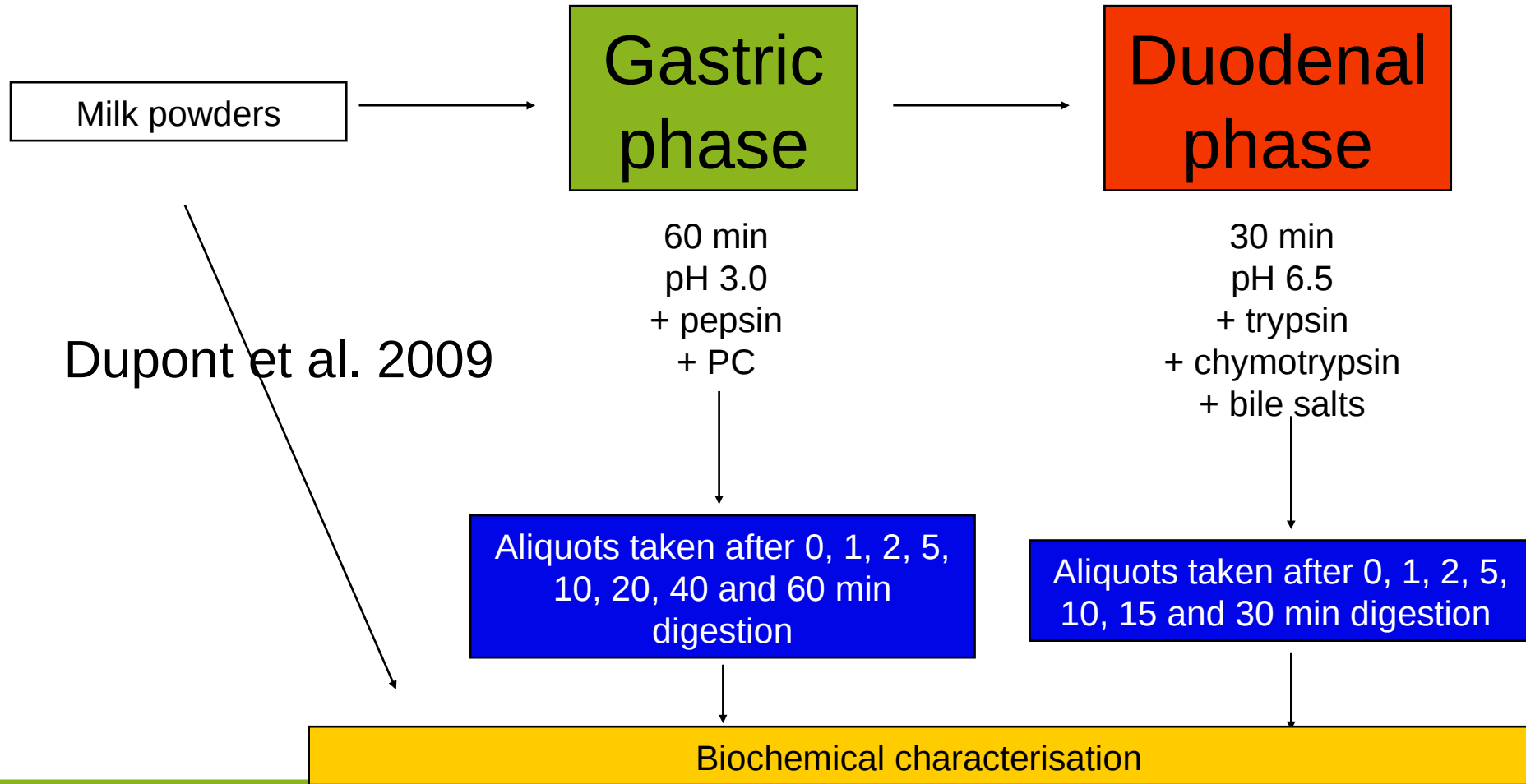
G

T

In vitro digestion

Infant gut Model

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



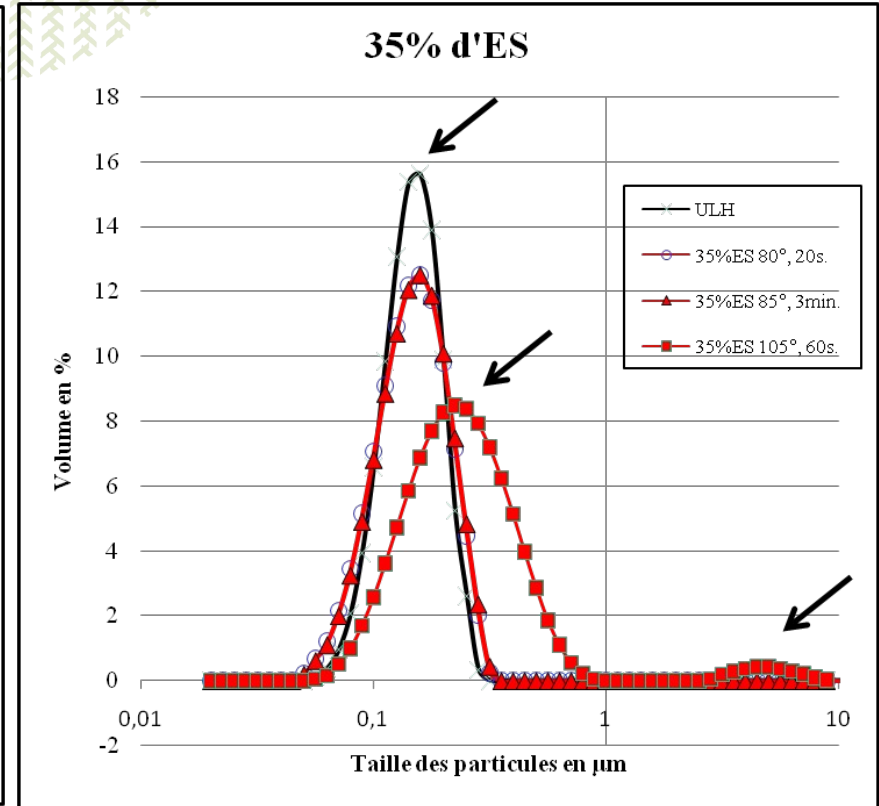
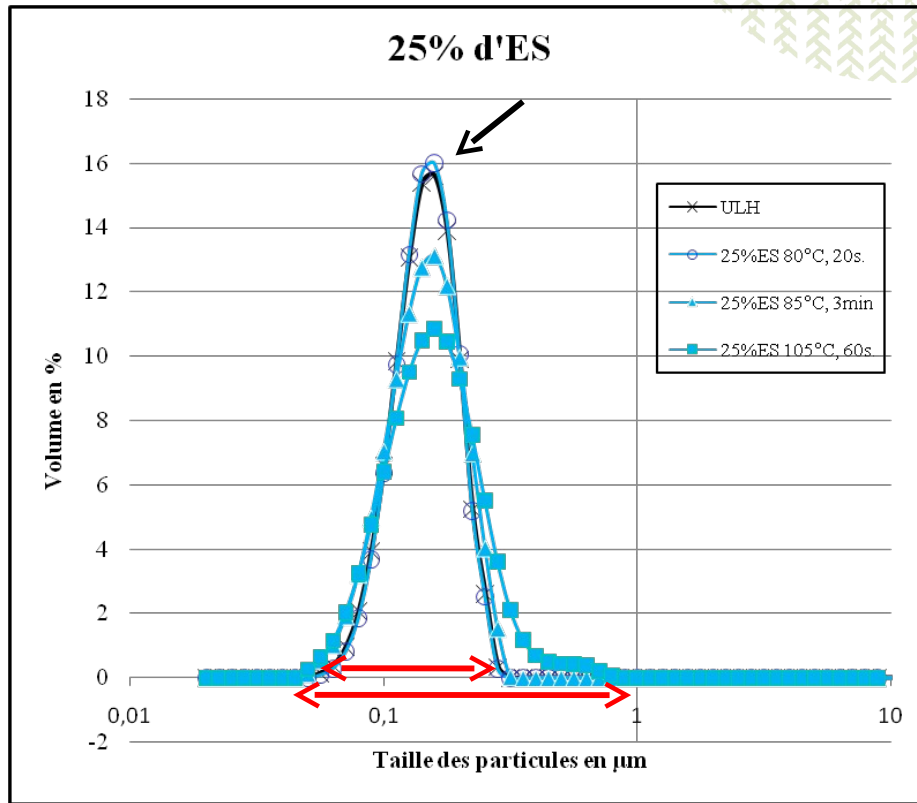
DIET
AGRICULTURE
ENVIRONMENT

INRA

1. Characterisation of the rehydrated powders



Particle size

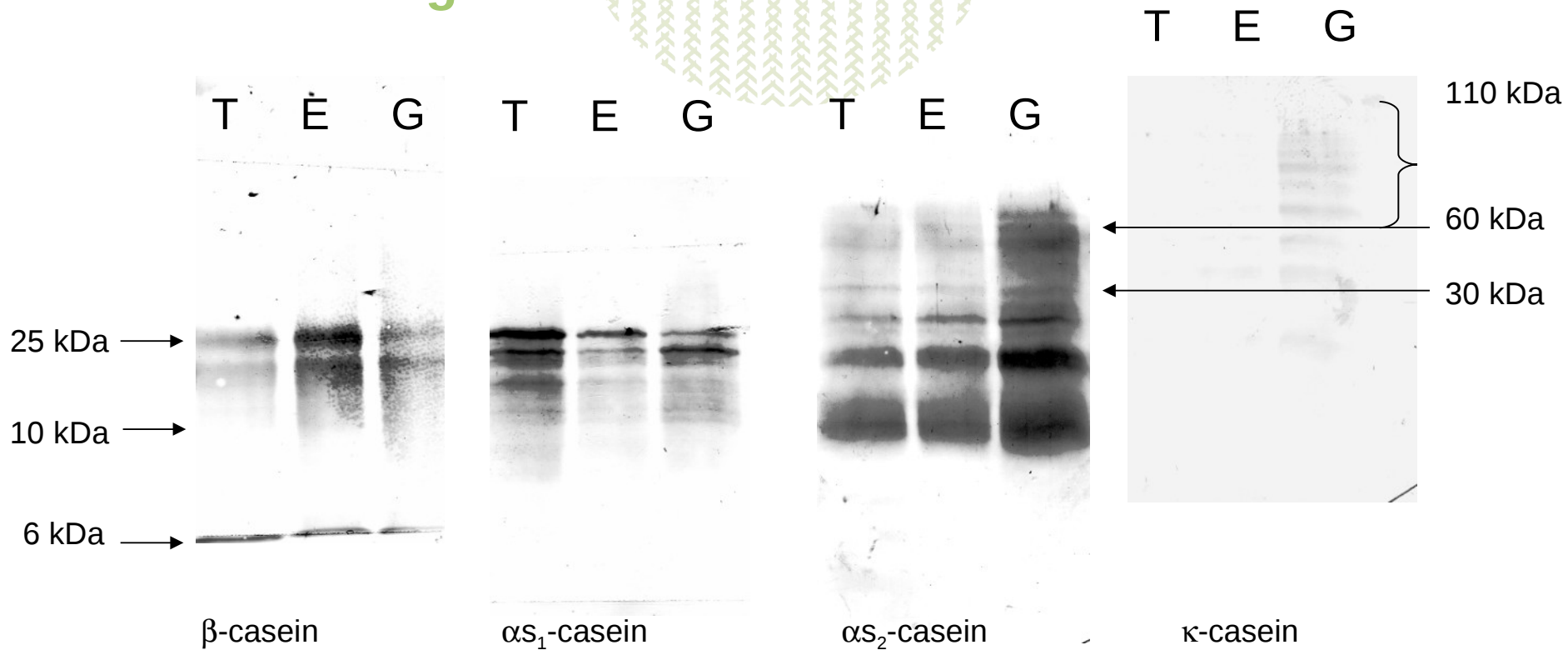


➡ heat-treatment at 105°C during 60s on 35% DM increases particle size

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_{S2} - and κ -casein specific Pabs

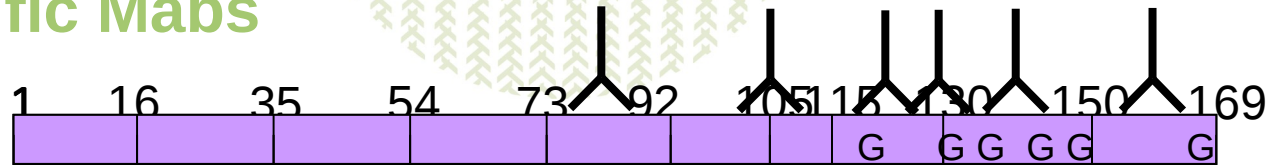
Thermal-induced aggregates with β -lactoglobulin resistant to digestion ?
(high Mw compounds observed with β -lg specific antibodies)

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

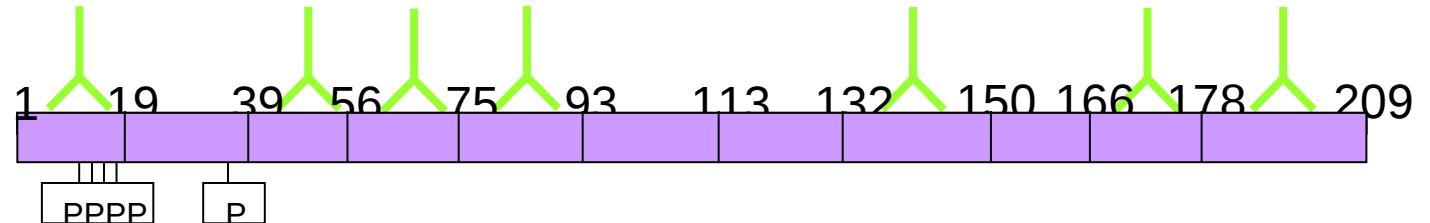


Identification of epitopes resistant to digestion by ELISA using specific Mabs

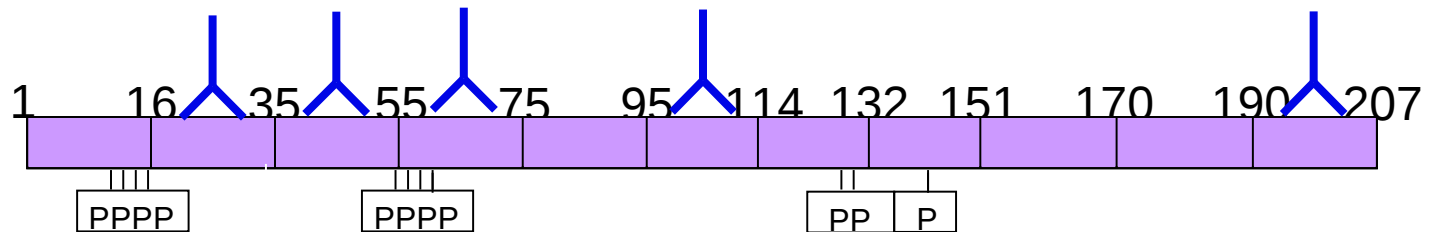
κ -
casein



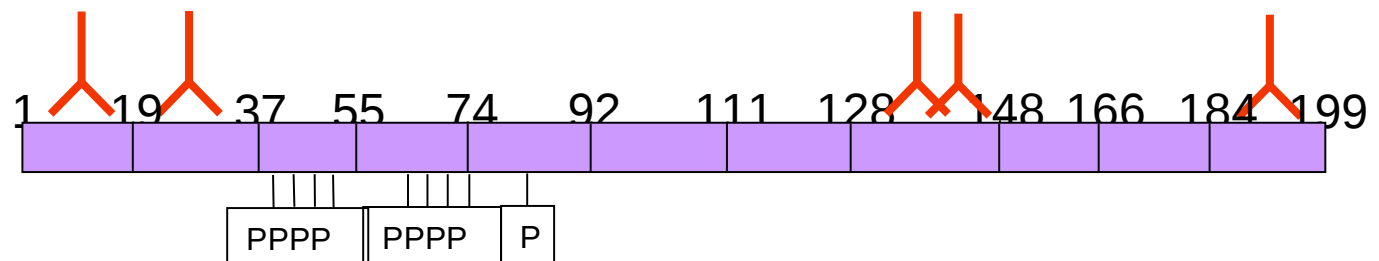
β -
casein



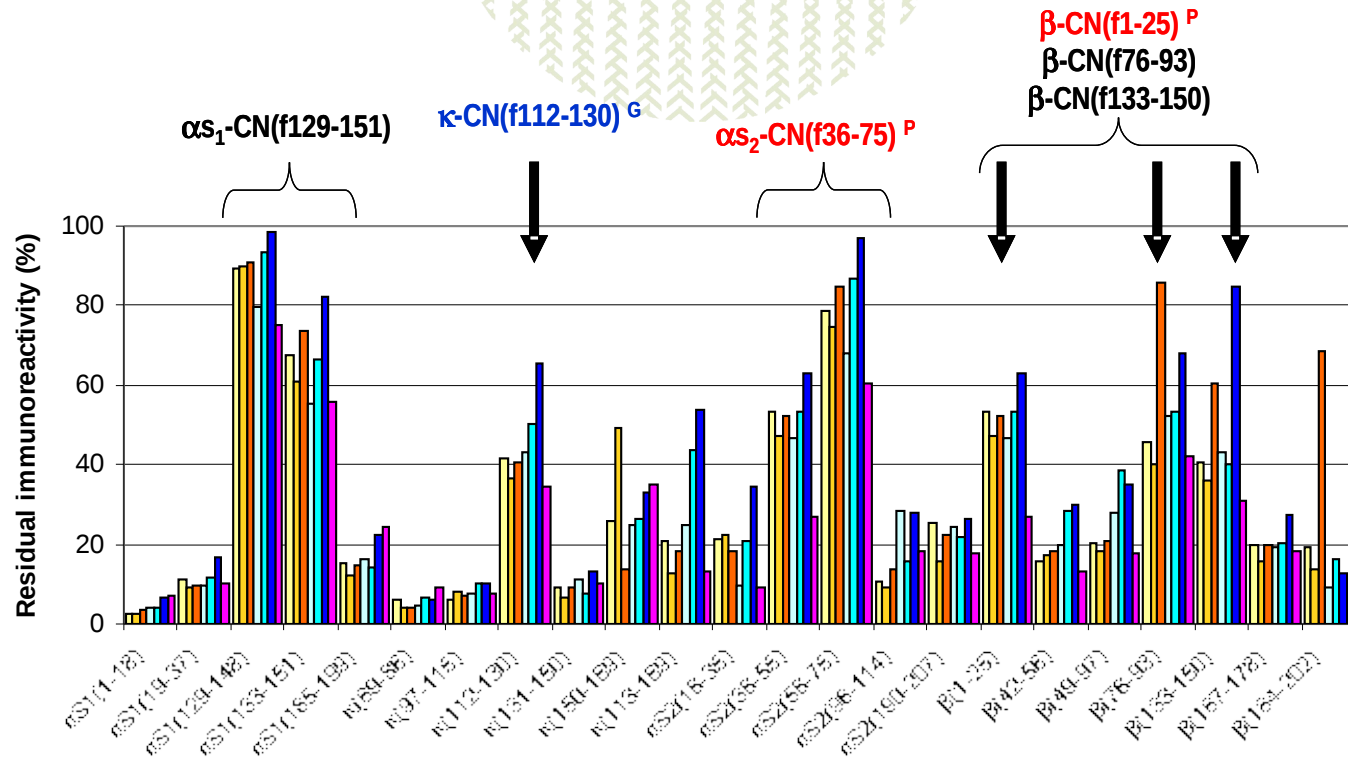
α_{S_2} -
casein



α_{S_1} -
casein



Residual immunoreactivity of casein fragments



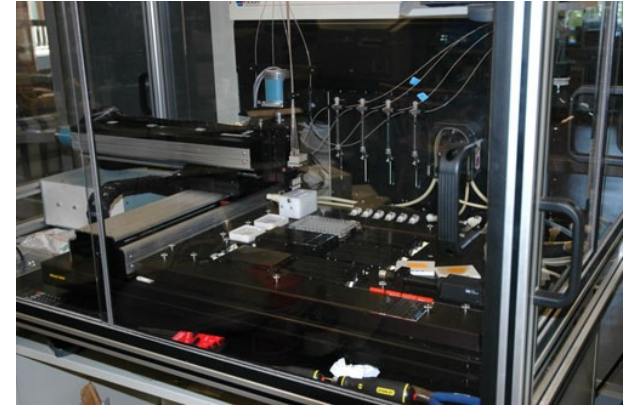
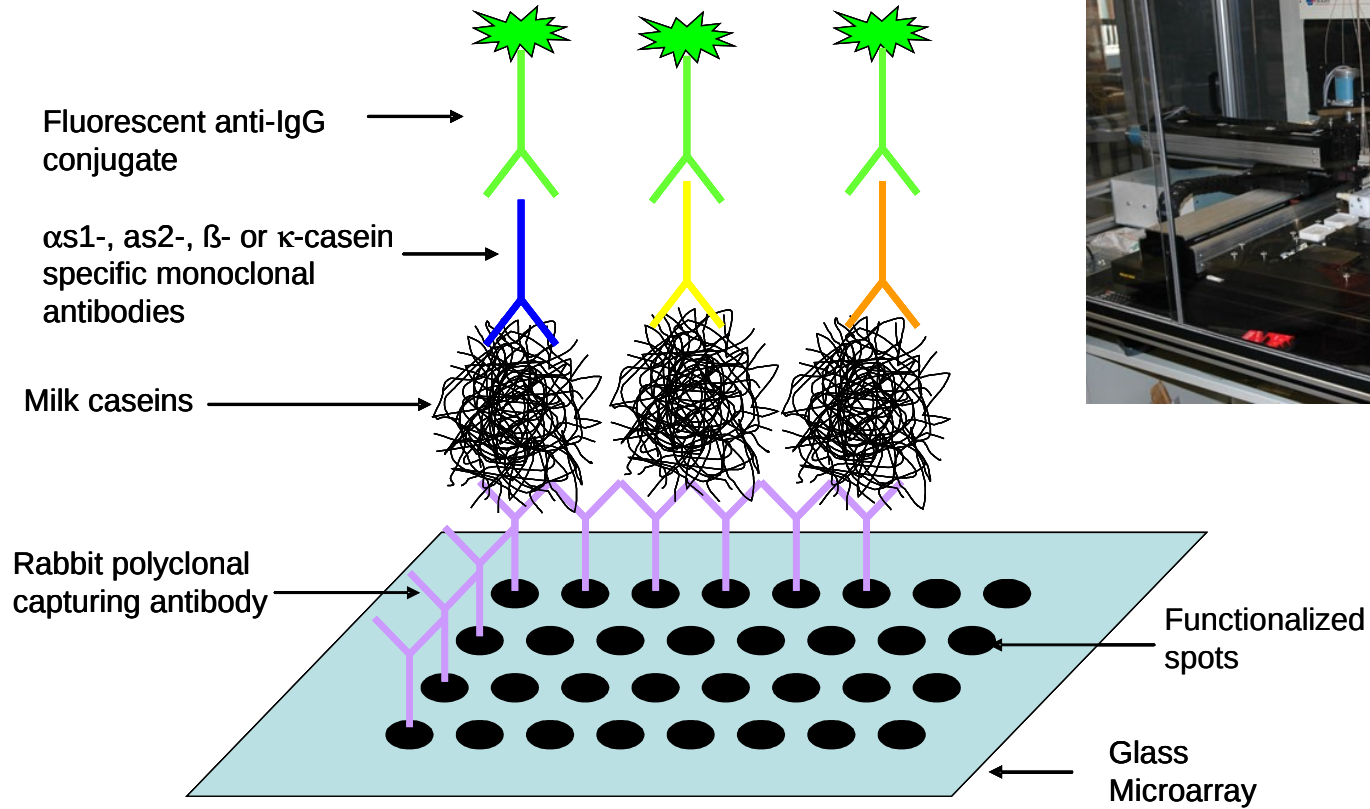
Factor	<i>p</i> value	Significance
T	0.00001	***
% DM	0.00555	**
T:%DM	0.03262	*

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

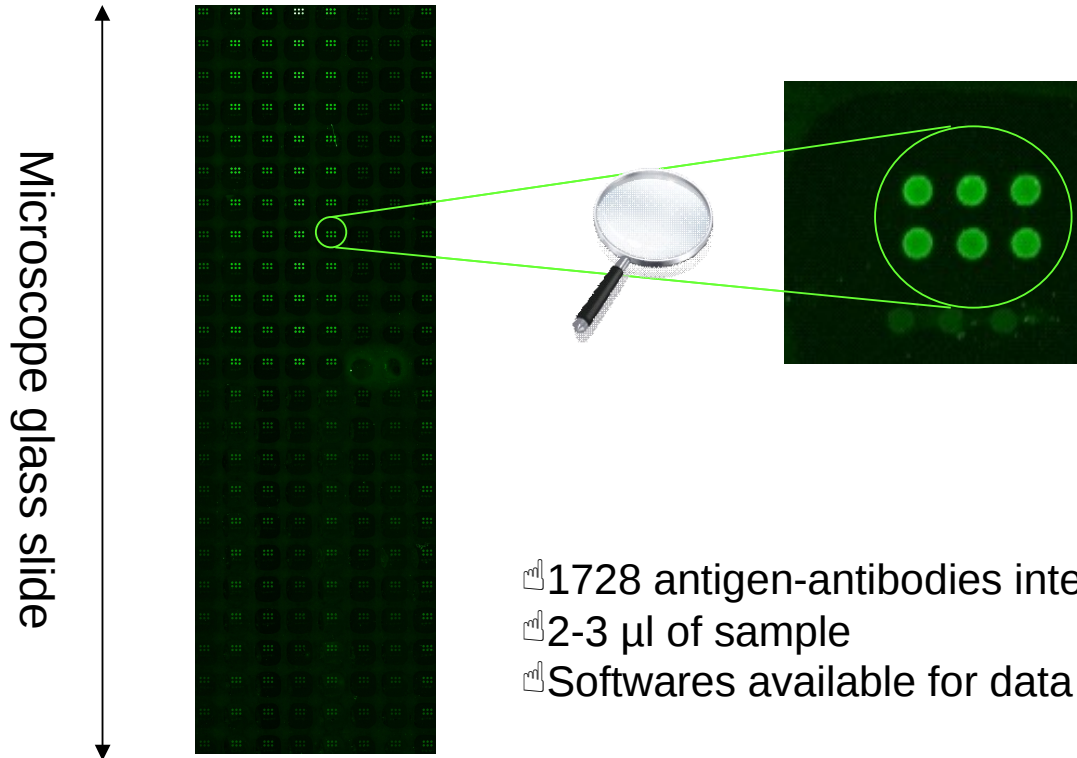
4. Kinetic of β -casein digestion using antibody microarrays



Principle of the technique

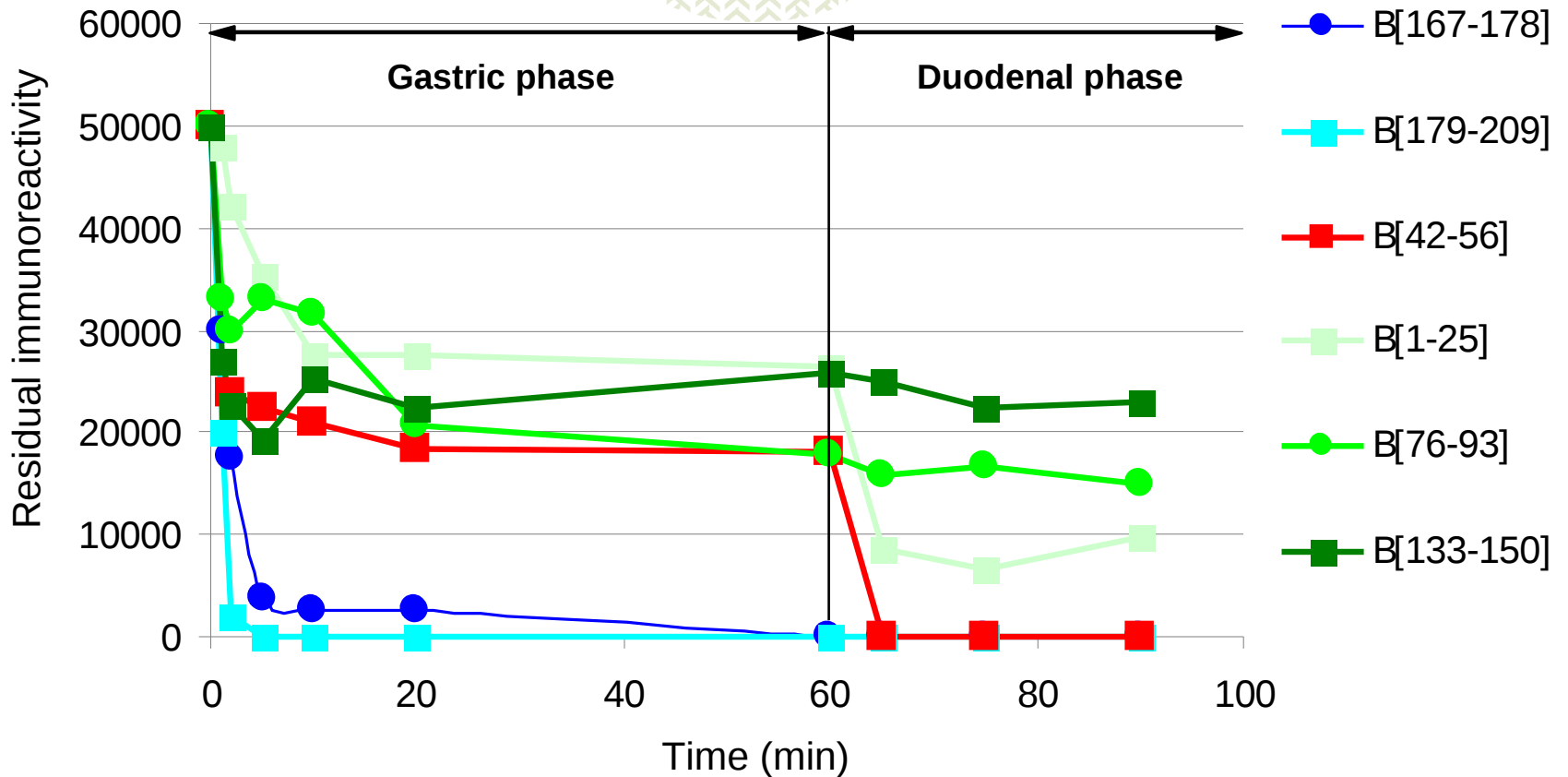


Antibody arrays – an example of results



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

Kinetics of β -casein digestion

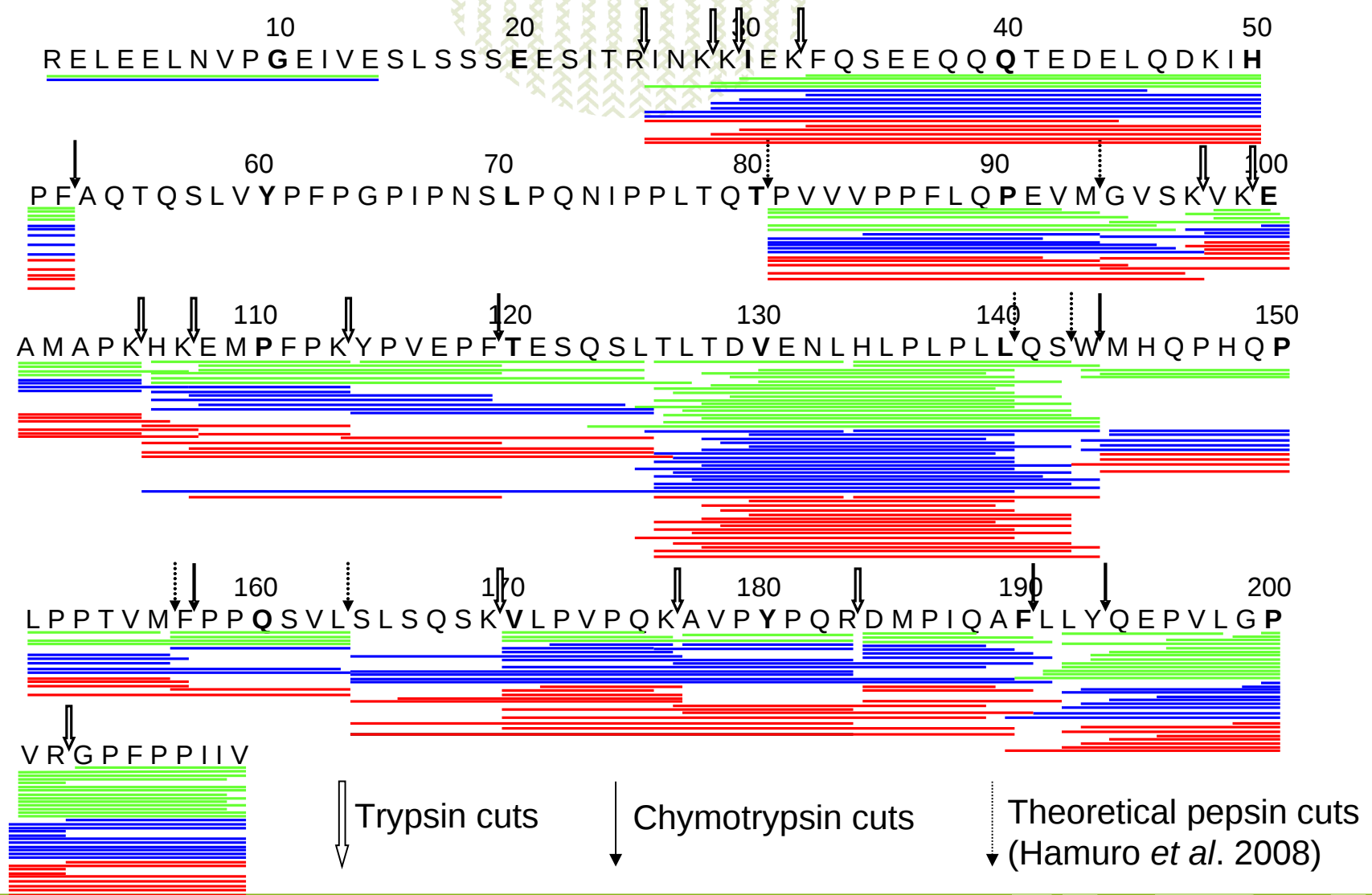


📊 Kinetics of β -casein digestion differ according to the area studied

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



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



Bioactive peptides detected

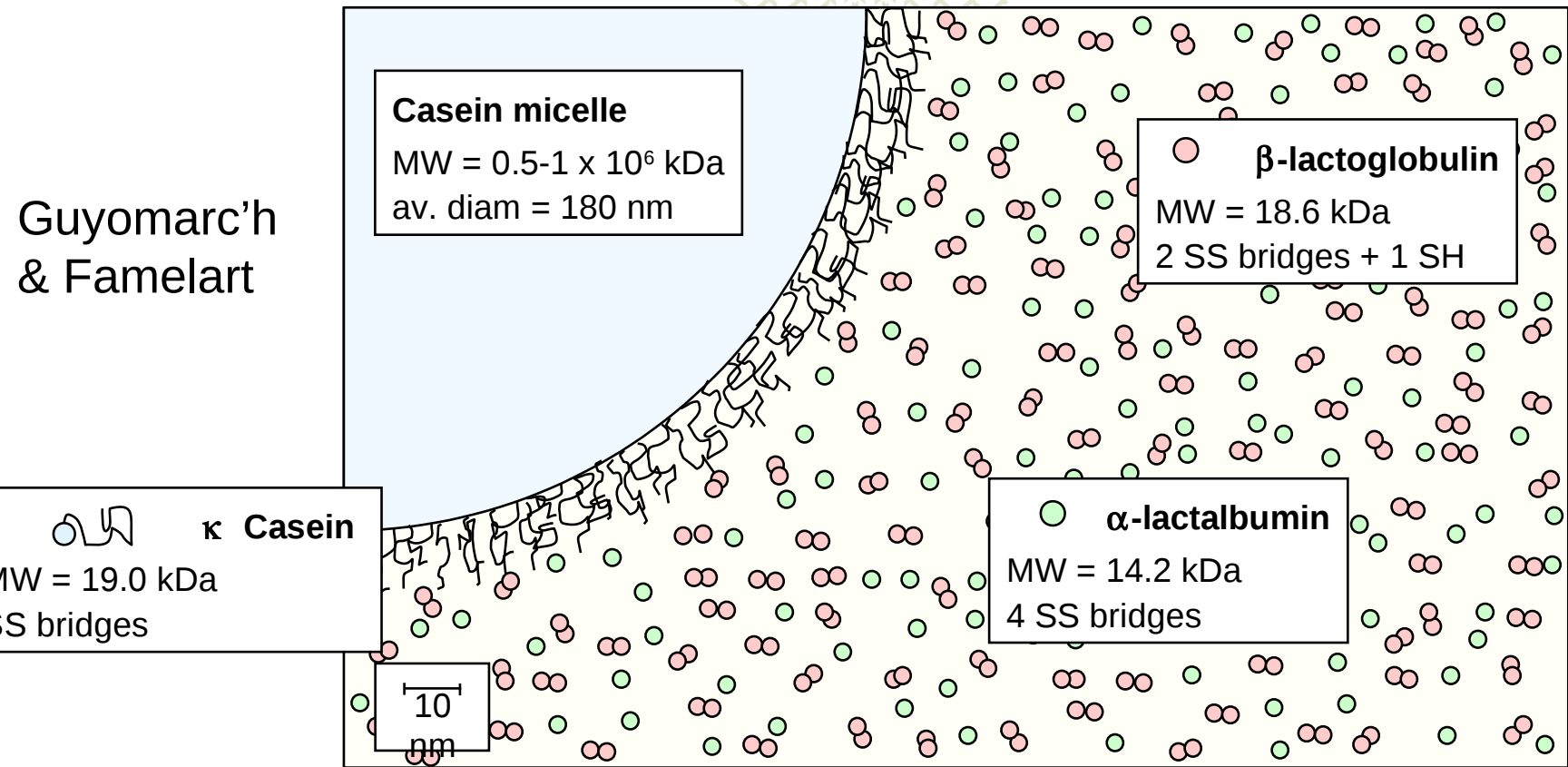
Peptide	Bioactivity	A	B	C	E	F	G	T
β -CN(f108-113)	Anti-hypertensive							
β -CN(f114-119)	Opioid agonist							
β -CN(f193-202)	Anti-hypertensive							
β -CN(f193-209)	Immunomodulatory							
α s1-CN(f1-23)	Antimicrobial							
α s1-CN(f23-34)	Anti-hypertensive							
α s1-CN(f91-100)	Anti-stress							
α s1-CN(144-149)	Antioxidant							
β -Ig(f9-14)	Anti-hypertensive							



Discussion / Conclusion

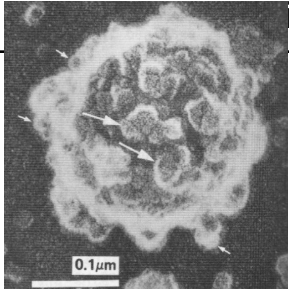
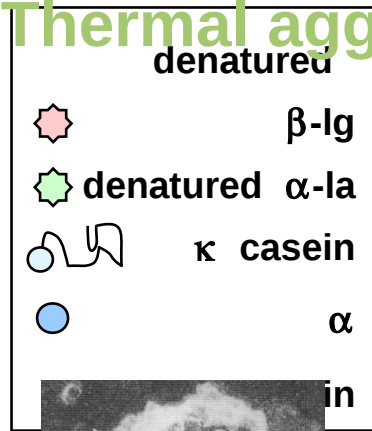
- Caseins partly resist to *in vitro* infant digestion model
- Resistant area have been partly identified
- Post-translational modifications and hydrophobicity could explain the resistance of these fragments to digestion
- Bioactive peptides profile can be modified by processing
- Heat treatment has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

Thermal aggregation of whey proteins on the casein micelle

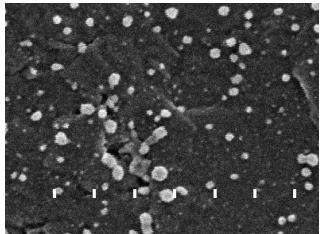


To scale schematic representation of the major whey proteins and of the casein micelle in unheated milk

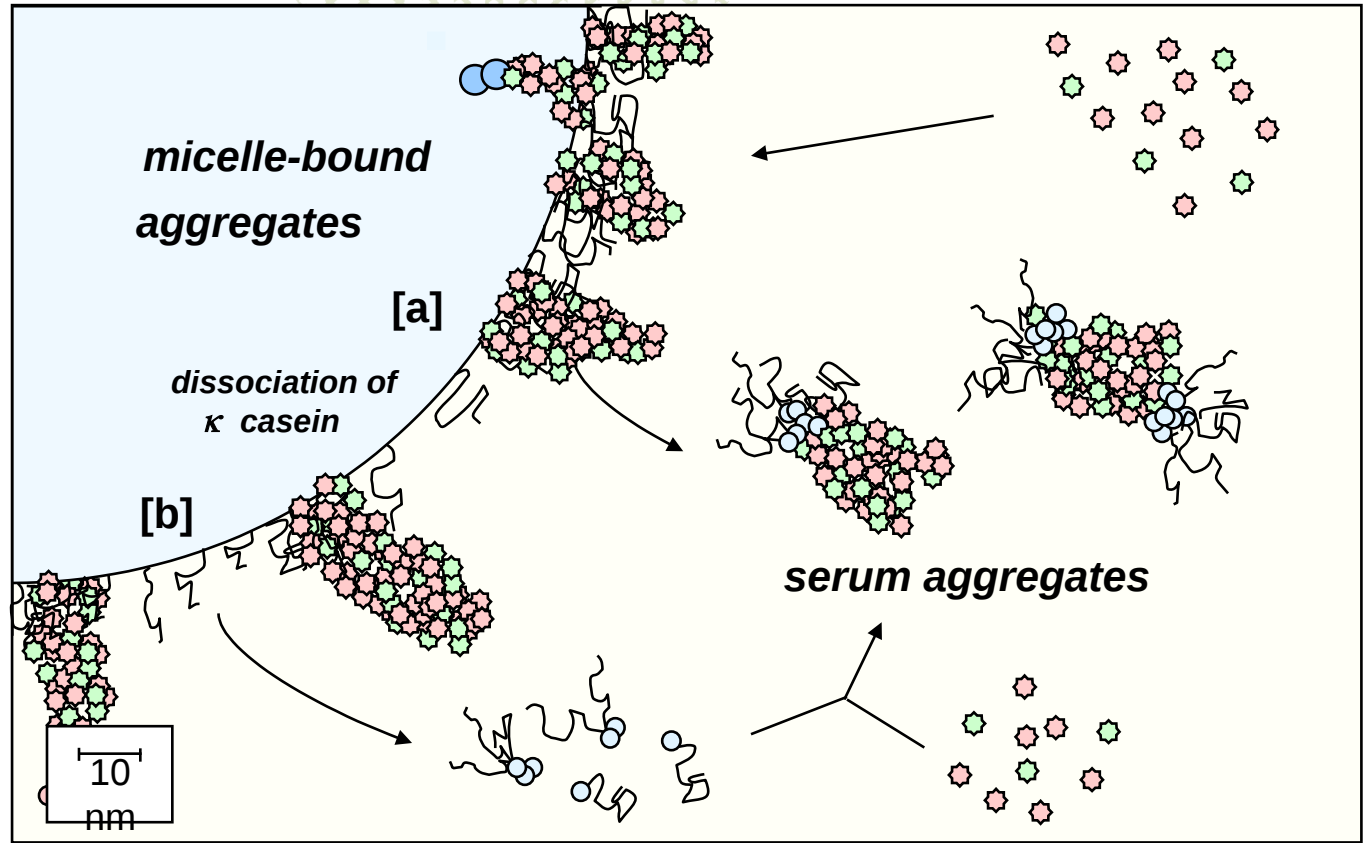
Thermal aggregation of whey proteins on the casein micelle



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

- 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

What's next?

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

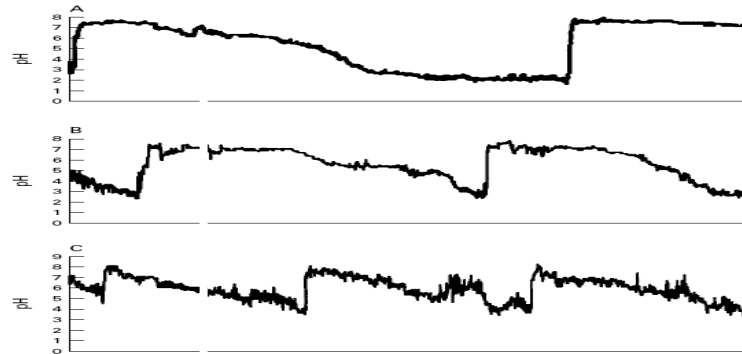
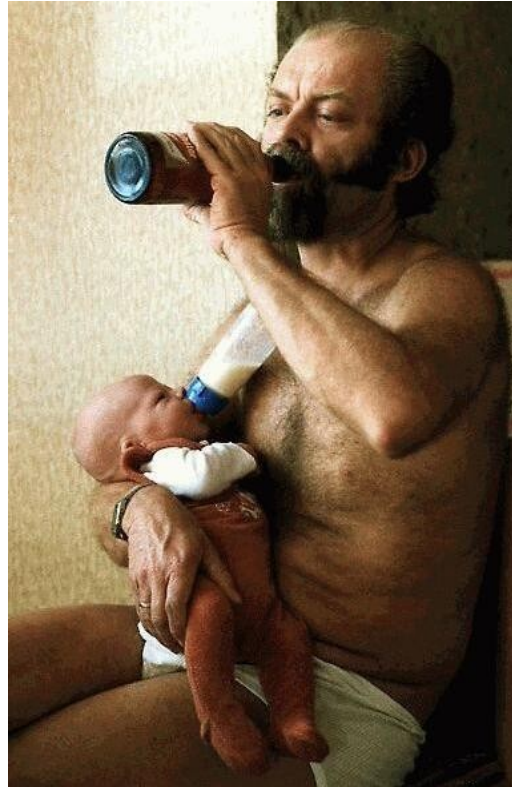


Figure 2 Examples of gastric pH records, six hour intervals: (A) four hourly feeds; (B) three hourly feeds; (C) 1/2 hourly feeds.

- 👉 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 !!!

DIET
AGRICULTURE
ENVIRONMENT

INRA