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Static in vitro digestion tests to assess allergenic risk of novel proteins - Use of a peptidomics workflow based on free softwares

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► To cite this version:

Julien Jardin, Valérie Briard-Bion, Amélia Torcello Gomez, Didier Dupont, Alan Mackie. Static in vitro digestion tests to assess allergenic risk of novel proteins - Use of a peptidomics workflow based on free softwares. SMAP 2019, Sep 2019, Strasbourg, France. , 2019. hal-02294941

HAL Id: hal-02294941

<https://hal.science/hal-02294941>

Submitted on 23 Sep 2019

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Introduction

- Allergenic risk assessment of proteins:
 - Safety to consume:
 - Evaluate **persistence of intact protein and large protein fragments** in gastrointestinal (GI) tract
 - Potential sensitisation via duodenum and eventual allergic reaction
- In vitro* digestion as a first screening step
 - Current pepsin resistance test (PRT) is not physiologically relevant
 - Naegeli, H., et al., Guidance on allergenicity assessment of genetically modified plants. EFSA Journal, 2017, 15(6), 4862-4911.

Objectives

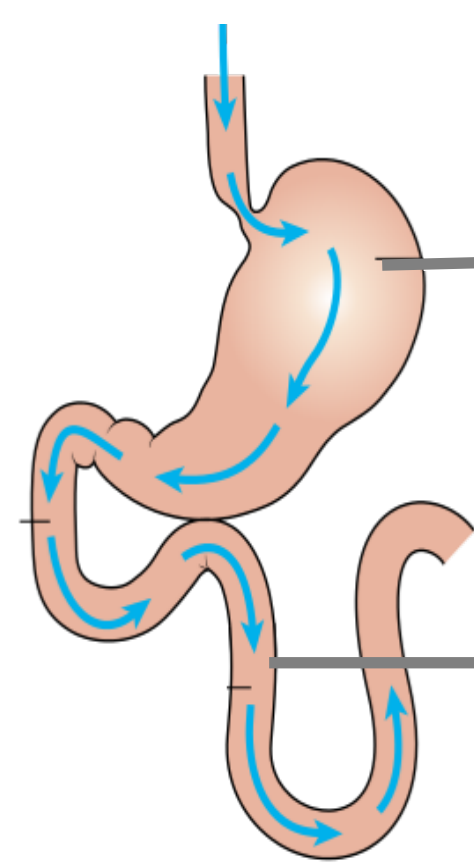
- Development and validation of a robust physiologically relevant, *in vitro* digestion protocol to assess stability of purified proteins
- Increase productivity of peptidomics data interpretation using only free softwares

Methodology

Static *in vitro* digestion models

- Shaking incubator:
 - T = 37 °C
 - 100 rpm
- Digestion run in triplicate

- Sampling :
 - 0.5, 2, 5, 10, 20, 30 and 60 min
 - Gastric and intestinal phase
 - Three digestion protocols
- Proteolysis stopped with protease inhibitors
 - Gastric: Pepstatin A
 - Intestinal: Pefabloc®



- Stomach:**
- Dilution ratio with simulated gastric fluid (SGF)
 - Acidic pH
 - Enzyme activity (pepsin)
- Small intestine:**
- Dilution ratio with simulated intestinal fluid (SIF)
 - Neutral pH
 - Enzyme activity (trypsin/chymotrypsin 4/1)
 - Biosurfactants: fixed total bile salt concentration

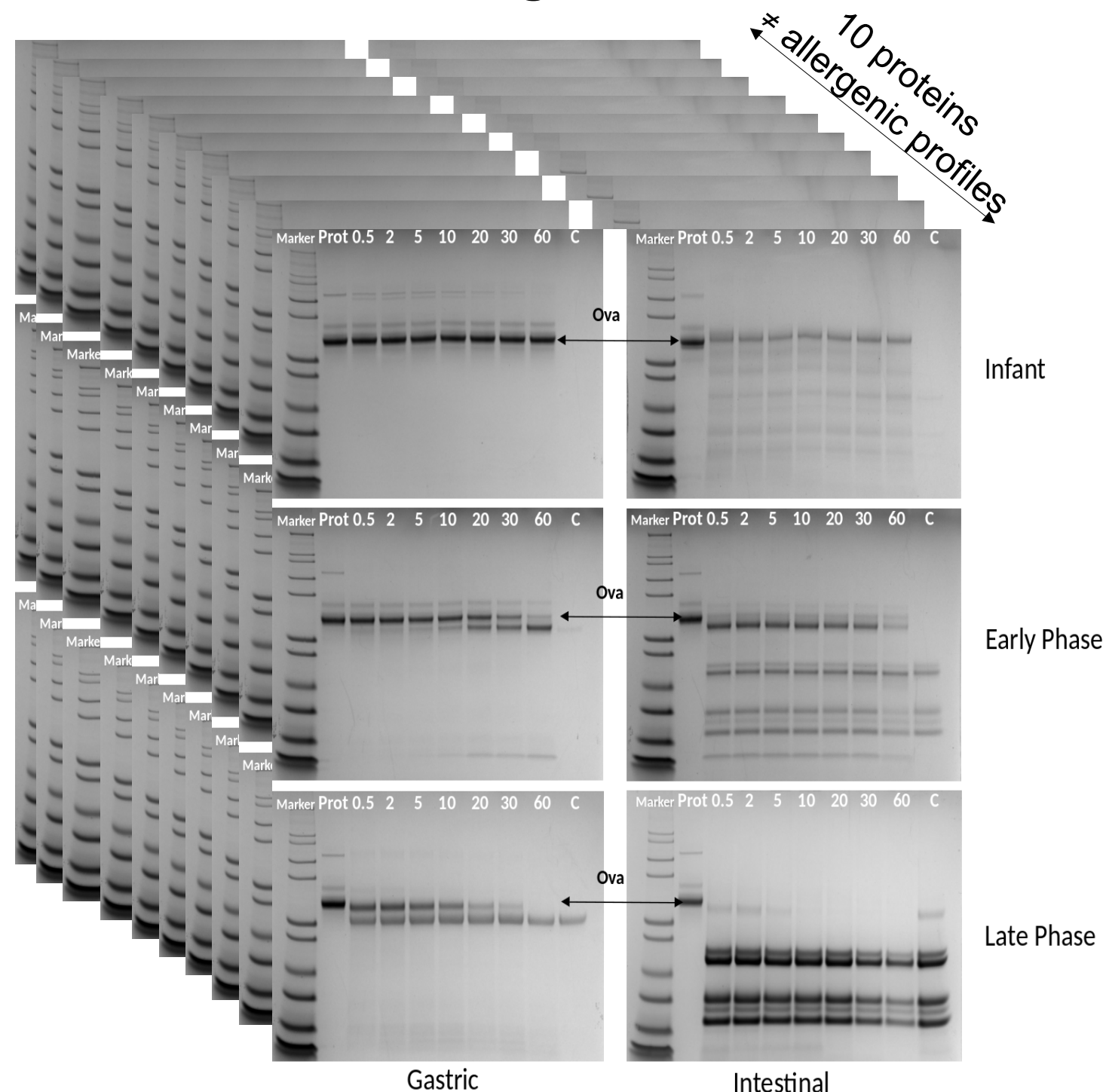
Mild conditions (Bile Salts, pH, Enzyme Ratios) → Harsh conditions

Infant¹

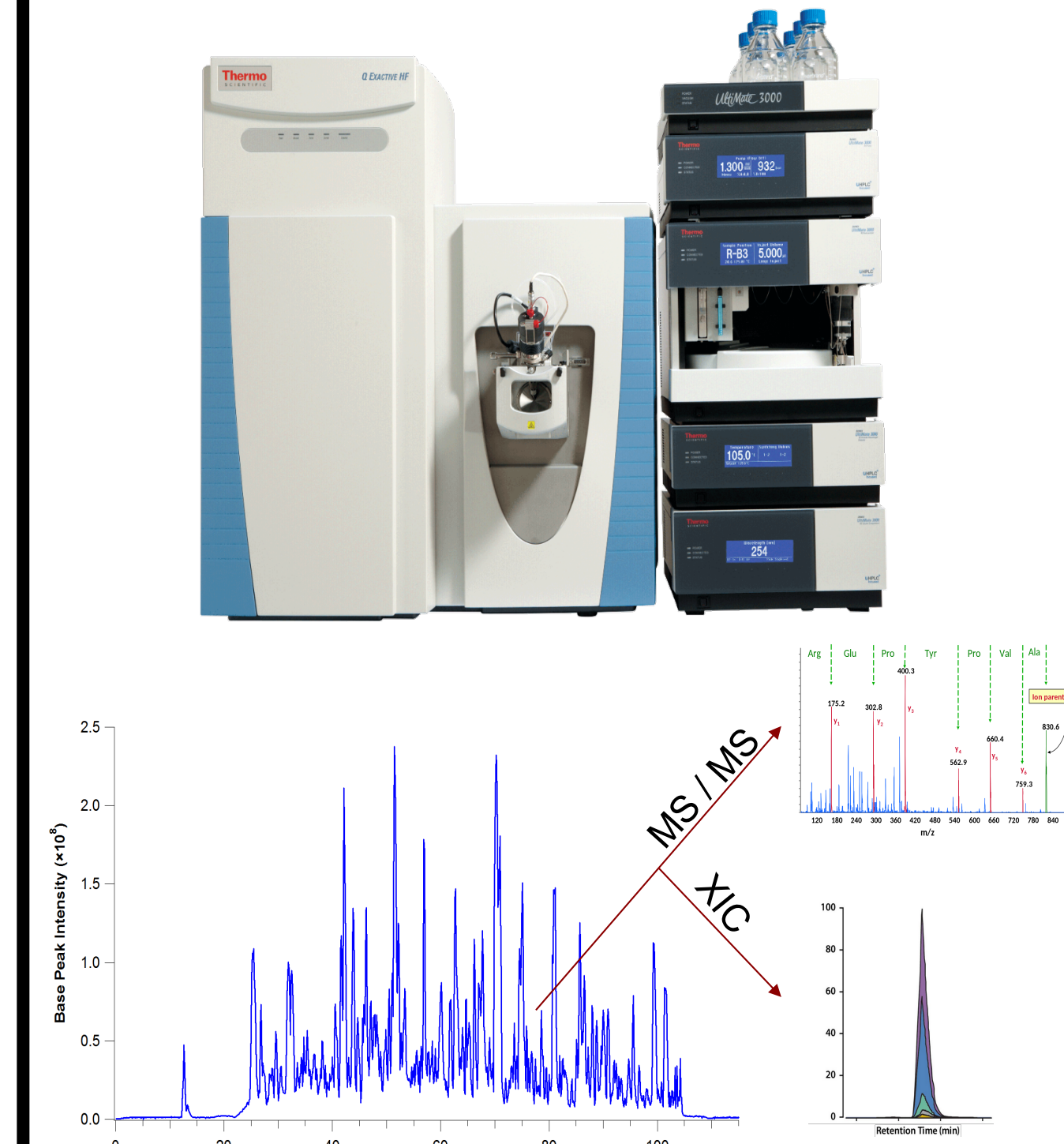
Early Phase Adult (INFOGEST)²

Late Phase Adult (PRT)³

SDS-PAGE: Evaluation of protein degradation

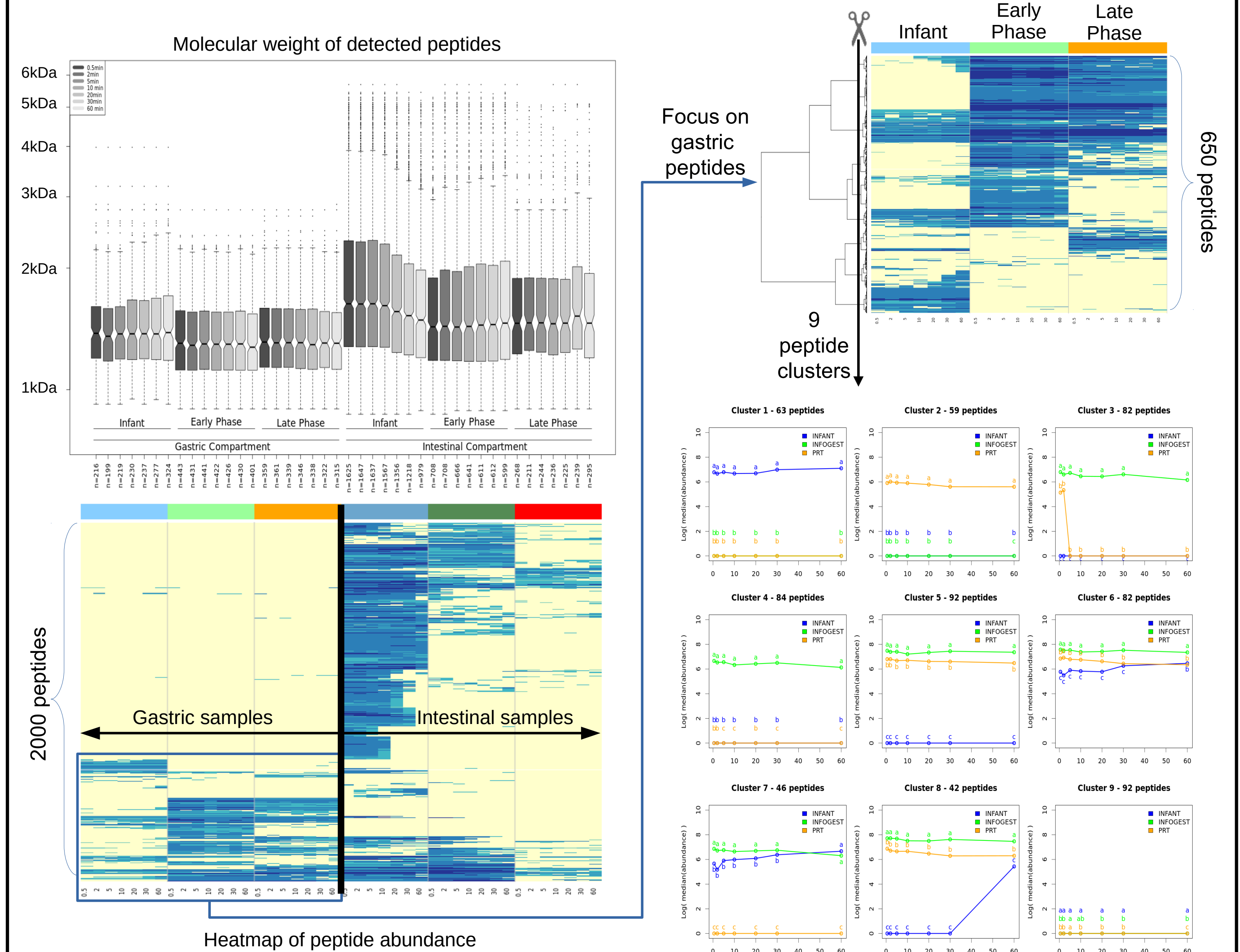


NanoLC-MS/MS: Peptide identification and quantification



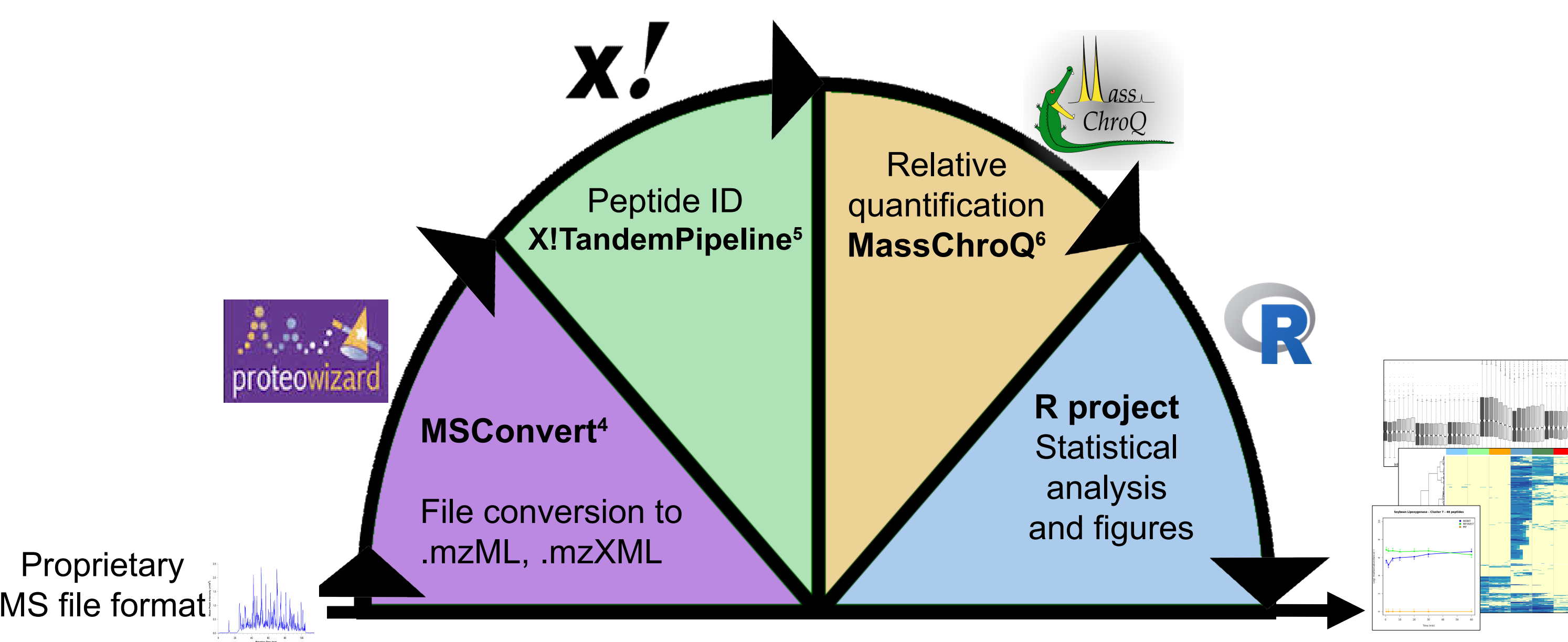
Preliminary results

- Proteins that are not pepsin resistant (Beta-Casein, Soybean Lipoxygenase, Acid Phosphatase) show slower kinetics of proteolysis for the infant model of digestion
- Intact protein and protein fragments >> 9 amino acids remain throughout the intestinal phase for Kunitz Trypsin Inhibitor and Concanavalin A regardless of the digestion model
- Peptidomics allow for a precise description of peptides with specific behaviours according to the digestion model



Conclusions

- This project highlights the importance of a multi-test protocol to assess protein digestibility. Future studies should include the effect of the food processing and/or food matrix on protein digestion.
- A workflow based on free softwares can successfully be used to quickly :
 - convert proprietary MS file formats to open .mzML or .mzXML
 - perform database search → **peptide identification**
 - align chromatograms and automatically extract peak areas on large datasets → **relative quantification**
 - perform **statistical treatment** and automate **figure production**



¹Menard, O., et al., A first step towards a consensus static *in vitro* model for simulating full-term infant digestion. Food Chemistry, 2018, 240, 338-345.
²Minekus, M., et al., A standardised static *in vitro* digestion method suitable for food-an international consensus. Food & Function, 2014, 5, 1113-1124
³Thomas, K., et al., A multi-laboratory evaluation of a common *in vitro* pepsin digestion assay protocol used in assessing the safety of novel proteins. Regulatory Toxicology and Pharmacology, 2004, 39, 87-98.
⁴Chambers, M.C., et al., A cross-platform toolkit for mass spectrometry and proteomics. Nature Biotechnology 30, 918-920 (2012)
⁵Langella O, et al., X!TandemPipeline: A Tool to Manage Sequence Redundancy for Protein Inference and Phosphosite Identification. J. Proteome Res., 2 (16) 494-503 (2017)
⁶Valot B, et al., MassChroQ: a versatile tool for mass spectrometry quantification. Proteomics, 17 (11) 3572-3577 (2011)

Acknowledgements :

- Olivier Langella and the PAPPISO platform
- Antonio Fernández Dumont
- Javier Moreno

