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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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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

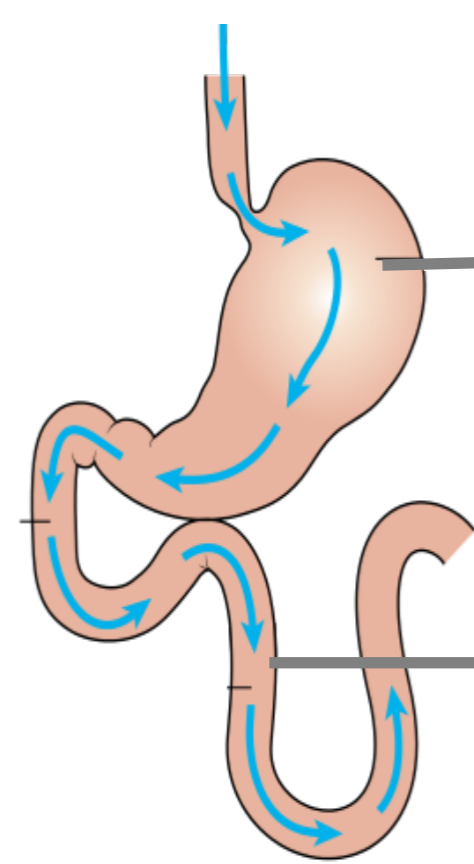
- Development and validation of a robust physiologically relevant, *in vitro* digestion protocol to assess stability of purified proteins
- Increase productivity of peptidomics data interpretation using only free softwares

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

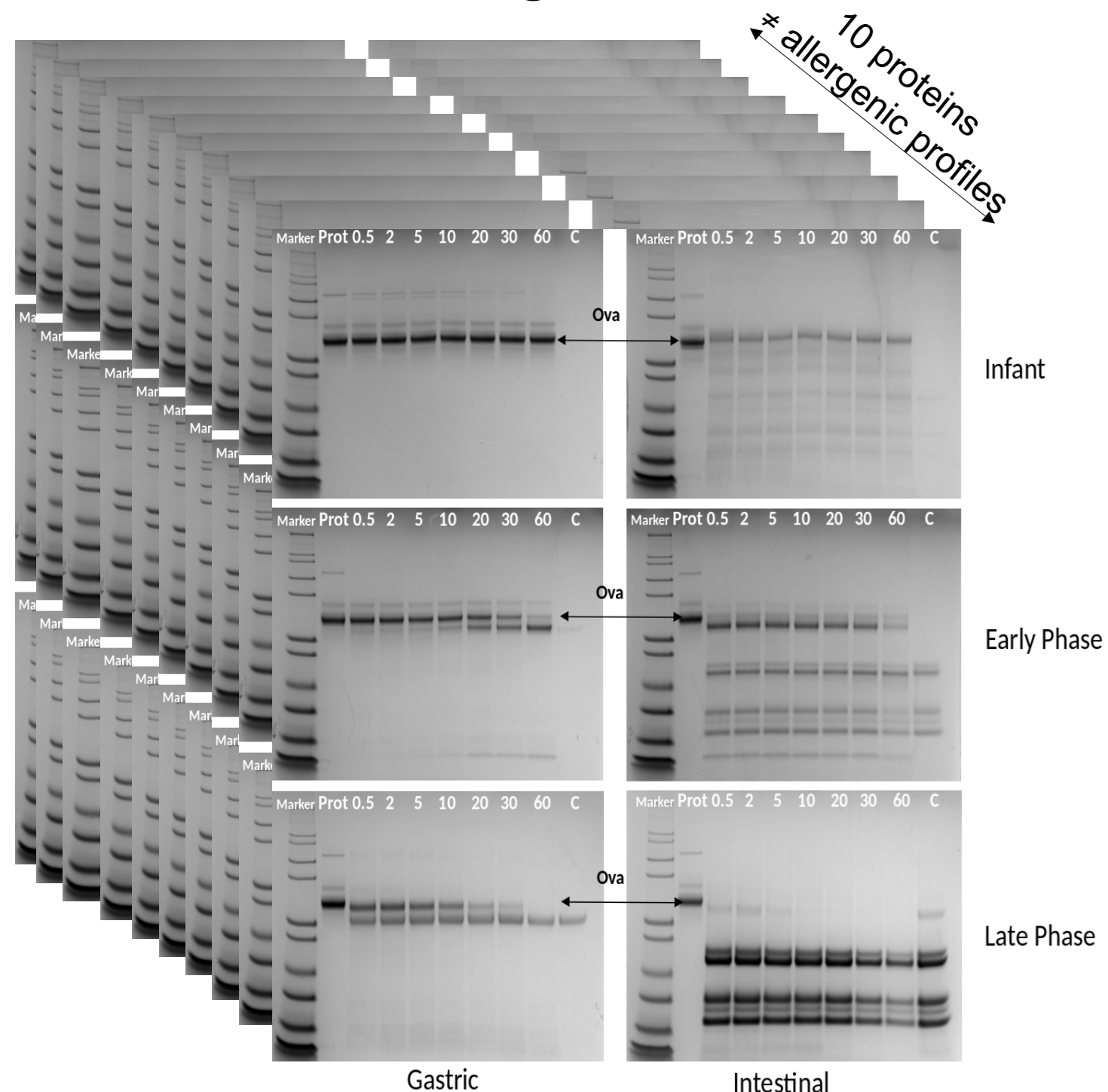
Mild conditions (Bile Salts, pH, Enzyme Ratios) → Harsh conditions

Infant¹

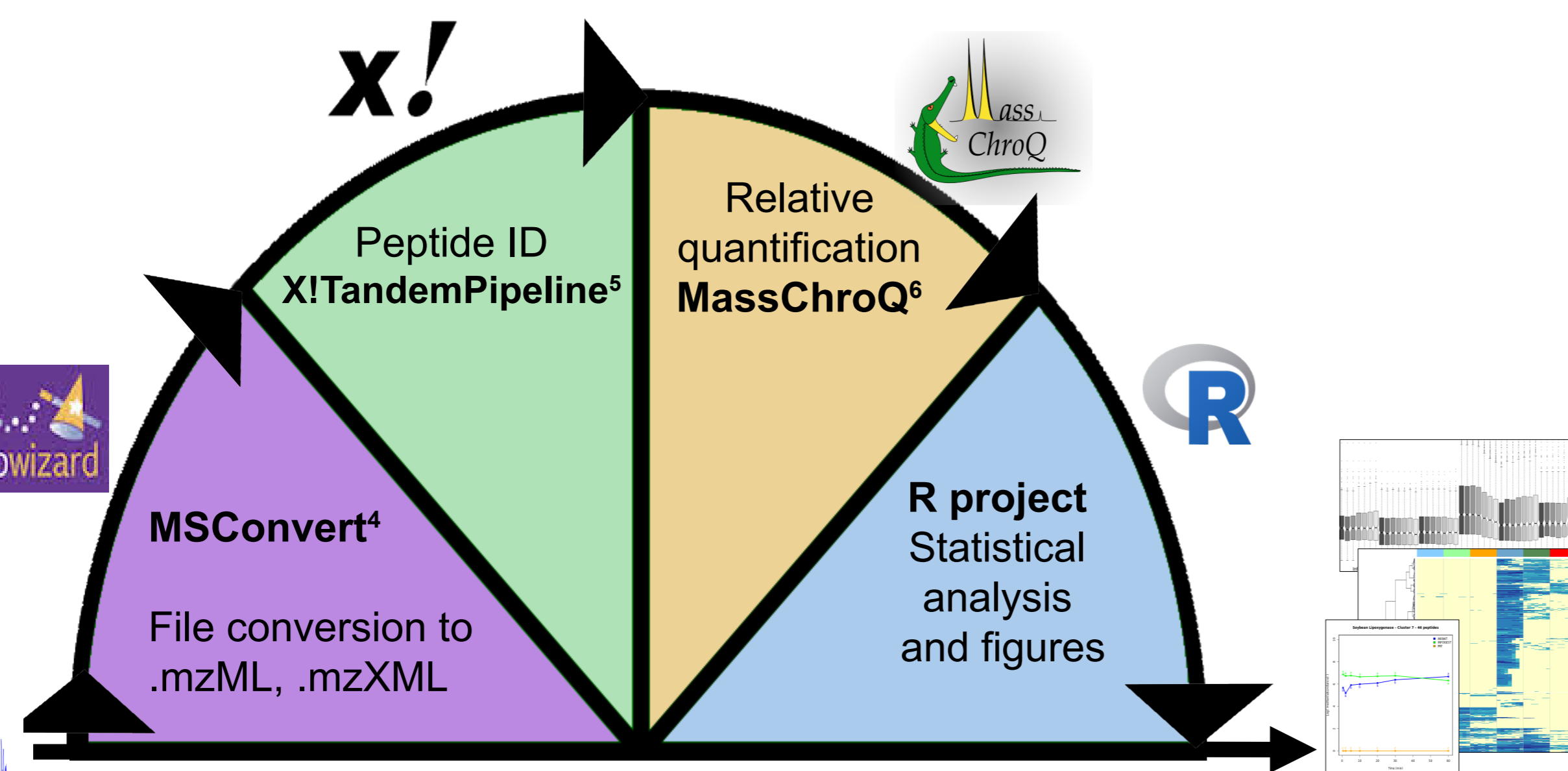
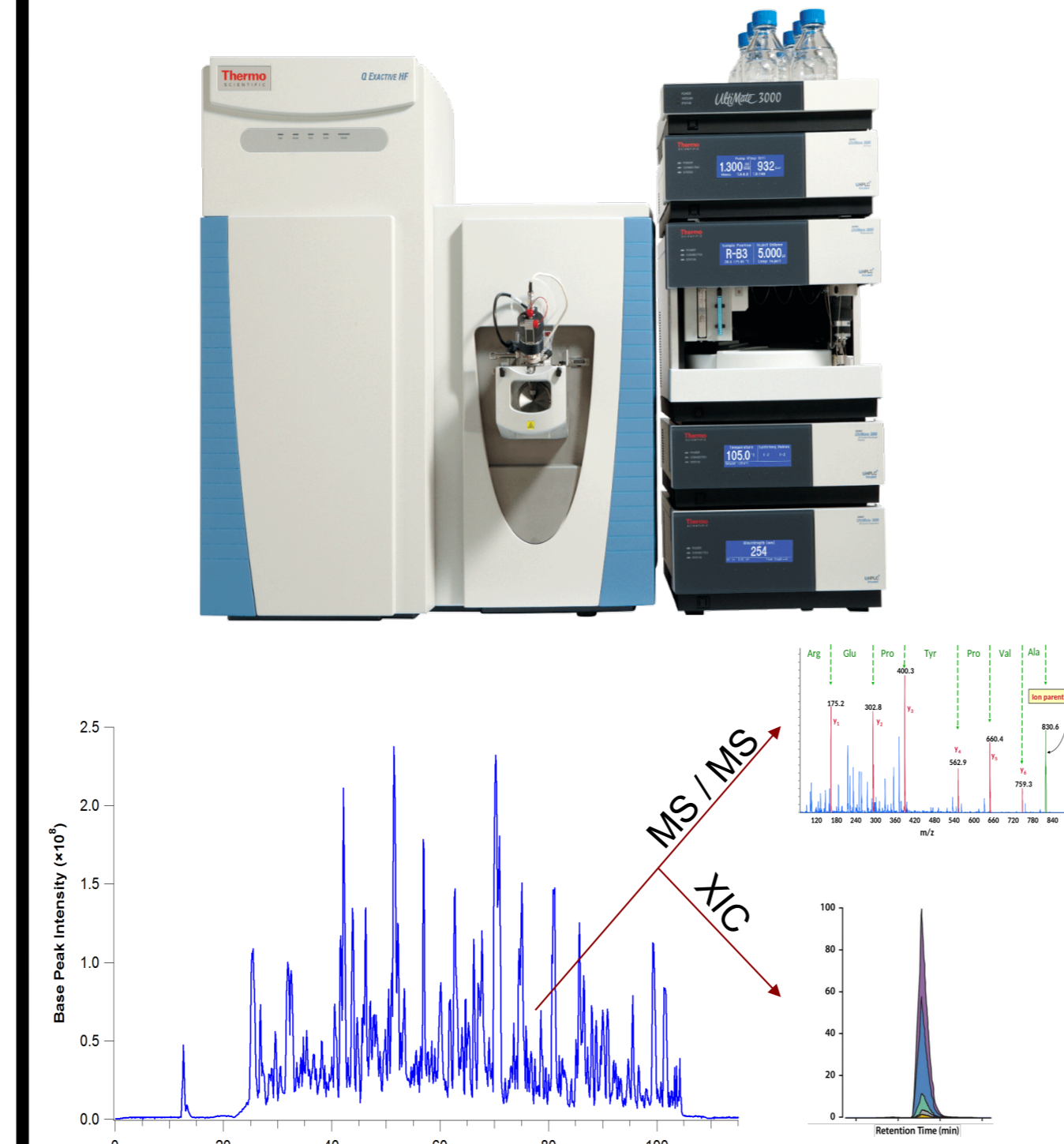
Early Phase Adult (INFOGEST)²

Late Phase Adult (PRT)³

SDS-PAGE: Evaluation of protein degradation

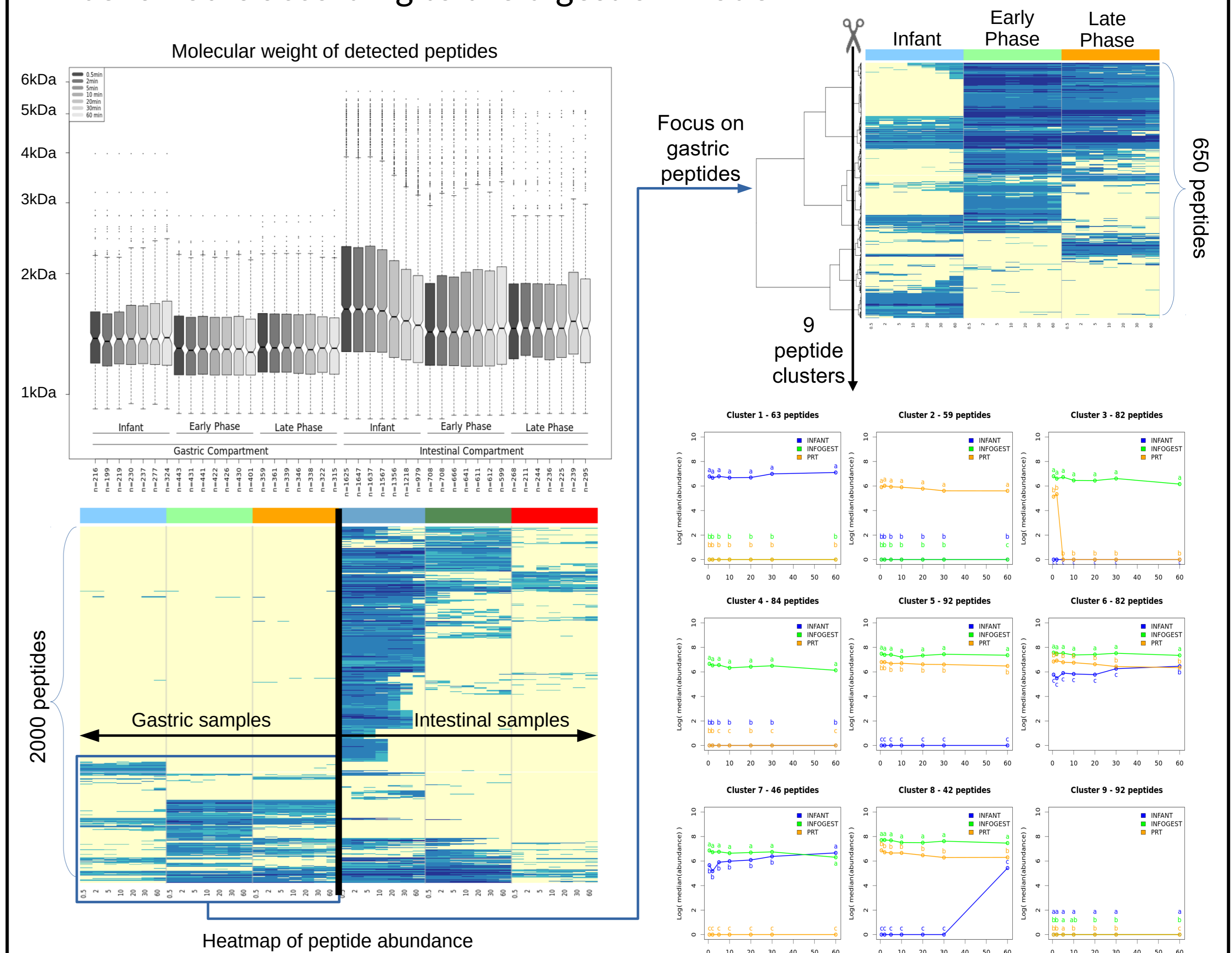


NanoLC-MS/MS: Peptide identification and quantification



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**

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