In vitro digestibility: application of a sequential protocol (gastric/duodenal) to pairs of proteins from the same protein family but with different allergenicity.

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Background of the project:

- **Pepsin digestion** assays have been part of the **weight-of-evidence approach** for evaluating the allergenic potential of proteins expressed in GM crops since **protein stability** has been suggested to correlate with **allergenicity** of proteins.

- EFSA has provided guidance that **more physiologically relevant digestion conditions** should be evaluated for their potential to support the allergenicity risk assessment, i.e. **different pH and pepsin concentrations** during gastric digestion and **addition of duodenal digestion**.

Major research questions:

1) **Do more/different physiological conditions change the outcome of digestibility assays?**

2) **Do they help in better discrimination of allergens and non-allergens?**

Some considerations with respect to “more physiological conditions”:

pH

- Normal gastric pH lies between 1.5 and 3.5 (circadian rhythm)
- Food intake influences gastric pH
- The use of PPIs increases pH to 4.0 - 5.0

Pepsin

- pepsin concentration in healthy volunteers is probably around a few hundred units/ml
- PPR10 (at 50 µg/ml protein) would be 500 U/ml, i.e. the highest pepsin conc used in this study is still within the range of normal pepsin concentrations.

PPR= pepsin (U/ml) to protein (µg/ml) ratio

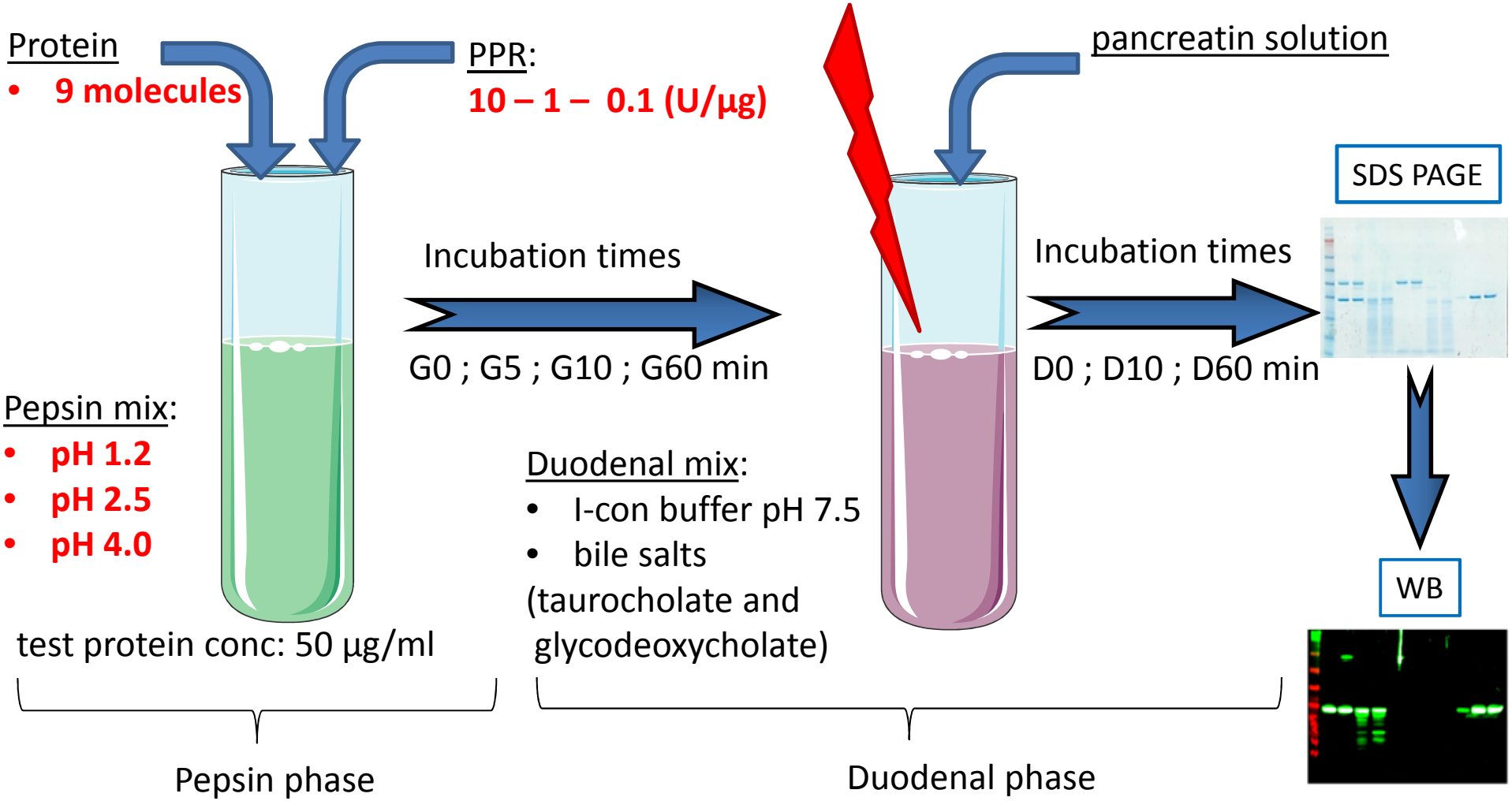
Pairs of proteins to compare

Protein family	Allergenic proteins	Non-/Weakly Allergenic proteins	% identity
Seed albumins	Peanut Ara h 2	Pea albumin	5,2
Tropomyosins	Shrimp Pen a 1	Porcine tropomyosin	55,0
Parvalbumins	Carp Cyp c 1	~~Swordfish Xyp g 1~~	77,8
Collagens	Fish collagen	Bovine collagen	55-75
lipid transfer proteins	Peach Pru p 3	Strawberry Fra a 3	66,6

All but the LTPs are from source tissues / LTPs are E.coli recombinants

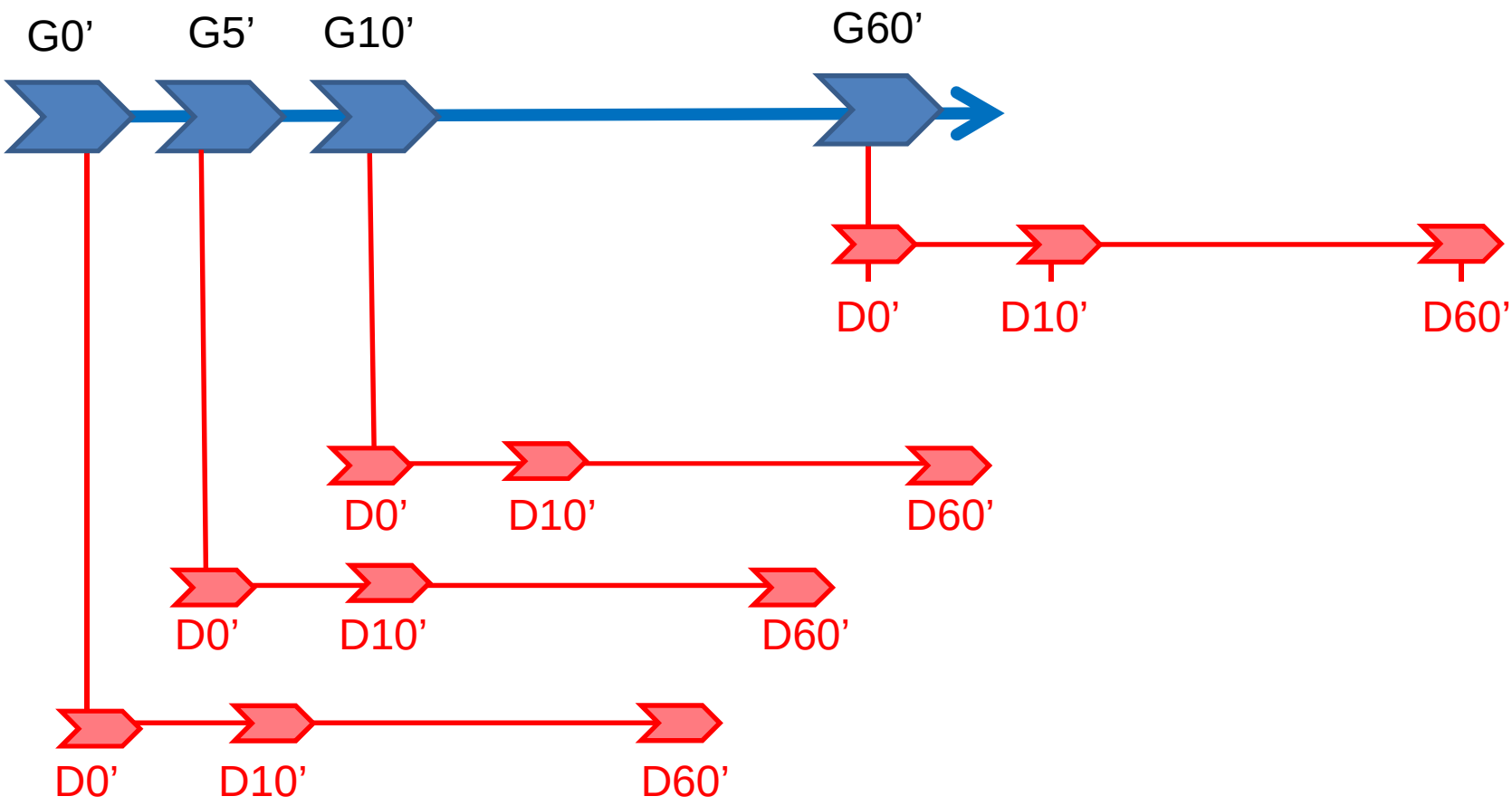
Combined Pepsin + Duodenal phases

Method development based on the paper from Mandalari et al., 2009



Combined Pepsin + Duodenal phases: sampling

Digestion & sampling in pepsin phase:
3 pHs (pH 1.2, 2.5 and 4.0) & 3 PPR(10, 1 and 0.1)



Digestion & sampling in duodenal phase (pH 7.5)

- At optimal pH for pepsin good distinction between allergen and non/weak allergen
- “More physiological” conditions increase resistance protein / decrease potency pepsin
- “More physiological” conditions: no discrimination between allergen and weak/non-allergen

As purified proteins in solution, even established strong allergens do not survive duodenal digestion!

Explanation?
- we do not eat purified proteins; matrix is decisive?
- the concept of allergens being resistant is invalid?

By immunoblot, gastric digestion seems to discriminate allergen and weak allergen, however on SDS-PAGE both LTPs both seem very resistant to gastric digestion

- “more physiological” conditions (higher pH/lower PPR plus duodenal) do not help in discrimination: weak allergen more resistant
- strange (but robust) observation: Ara h 2 recognition re-appears??

Conclusions from observations with tested proteins:

- Pepsin digestion at low pH performs best in discriminating allergens and non-allergens
- At pH4 this discriminatory power disappears
- Inclusion of duodenal digestion does not help: strong allergens are readily digested

Discussion:

- Intuitively, resistance to digestion increases risk for sensitization and symptoms
  It therefore seems most appropriate to continue its inclusion in weight-of-evidence approach as it has been done so far: optimal pepsin conditions
- In particular the observation that strong allergens are readily digested during duodenal phase warrants investigations into the role of food matrices
- It is important to realize that for sensitization, the route may not always be the gastro-intestinal route (skin or nose/lungs)

Future plans: study the role of food matrices in digestion assays

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