Devenir des protéines dans un système modèle de digestion simulée assisté par spectrométrie de masse

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4: Inserm U1011, « Récepteurs nucléaires, maladies cardiovasculaires et diabète », Lille

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- **Gastrointestinal digestion**

1883

"Shall I refuse my dinner because I do not fully understand the process of digestion?"

Oliver Heaviside (1850-1925)

[**Bunt**, B.J., *Phys. Today 2012, 65, 48*].

- Partial hydrolysis by pepsin (*Stomach*)
- proteases (trypsin, chymotrypsin, carboxypeptidases) and microorganisms peptidases (*small intestine lumen*)
- brush border membrane peptidase (*microvilli of epithelial cells*)

Free amino acids

+

Various molecular weight peptides with potential bioactivities

Peptidome

Sources and roles of bioactive peptides

Sources of bioactive peptides

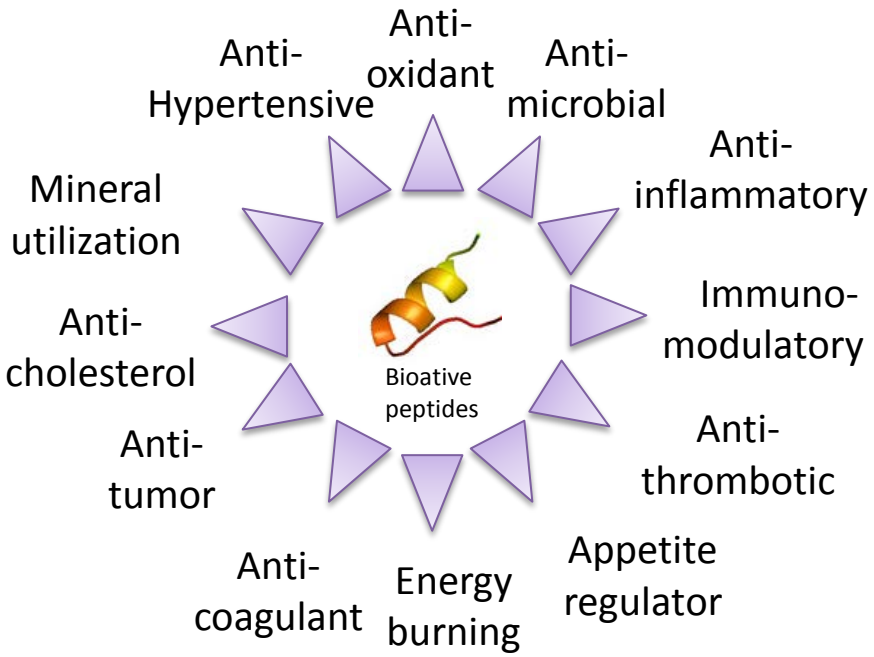
All proteins

- Animals
- Plants

→ Cow-milk proteins

benefit / harmful

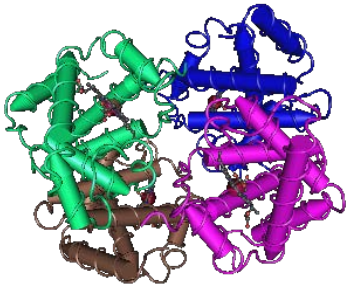
Health –promoting roles of bioactive peptides



Nagpal, R. *et al.* Food Funct. **2011**, 2, 18–27. ; **Sharma, S.** *et al. Int.J.Bioautomation* **2011**, *15*, 223–250.

- **Hemoglobin (Hb) as a potential source of bioactive peptides ?**

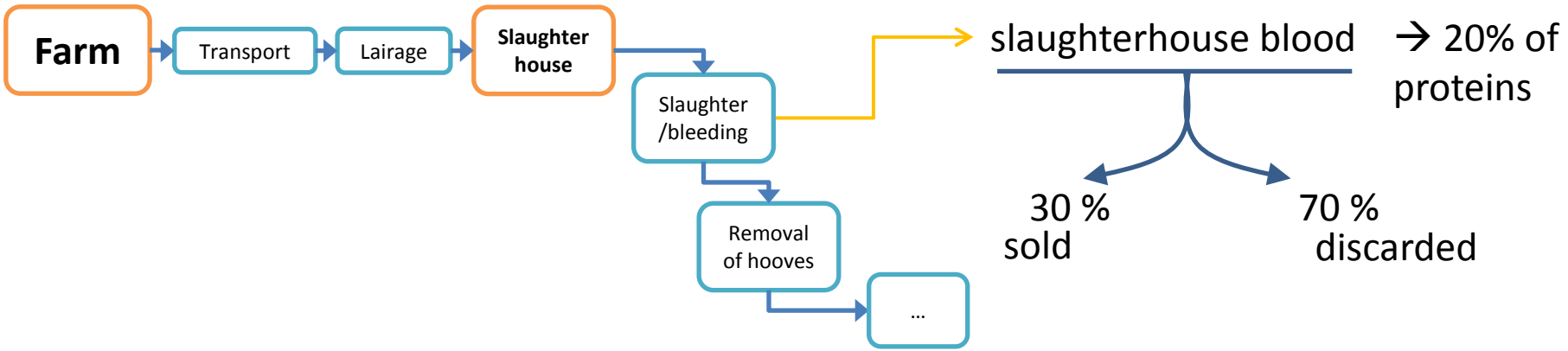
Hb ($\alpha 2/\beta 2$): a model protein



Bovine hemoglobin (Hb)

Source: NCBI

Meat production food chain



Hoorfar, J., et al., eds. *Food chain integrity: A holistic approach to food traceability, safety, quality and authenticity*. **2011,** Elsevier; **Bah, C. S. F.** *et al. Compr. Rev. Food Sci. Food Saf.* **2013**, *12*, 314–331.; **Ofori, J., Hsieh, Y.**, In *Food Additive*, In Tech, **2012**, pp. 230–256.

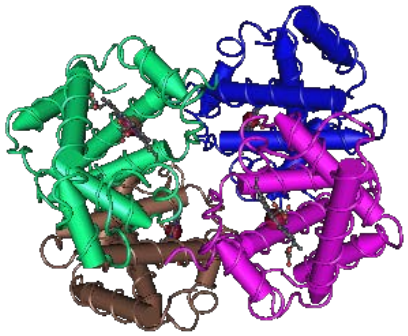
Objectives

1

- Development of an *in vitro* human digestion model to study protein digestion
- Characterization of peptidomes (GI digestion-derived peptides)

2

- Protein digestion and energy homeostasis: impact of generated peptides on intestinal hormones

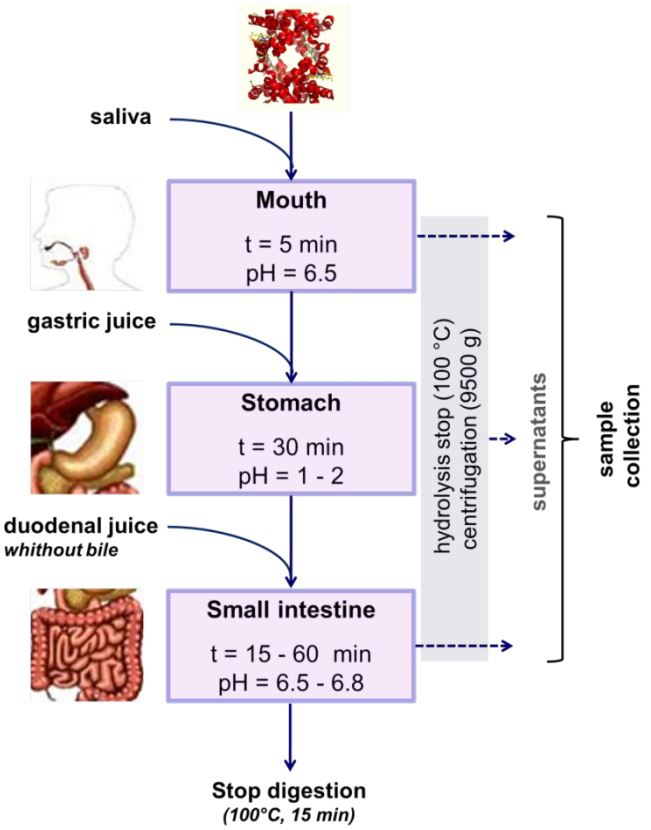


Bovine hemoglobin

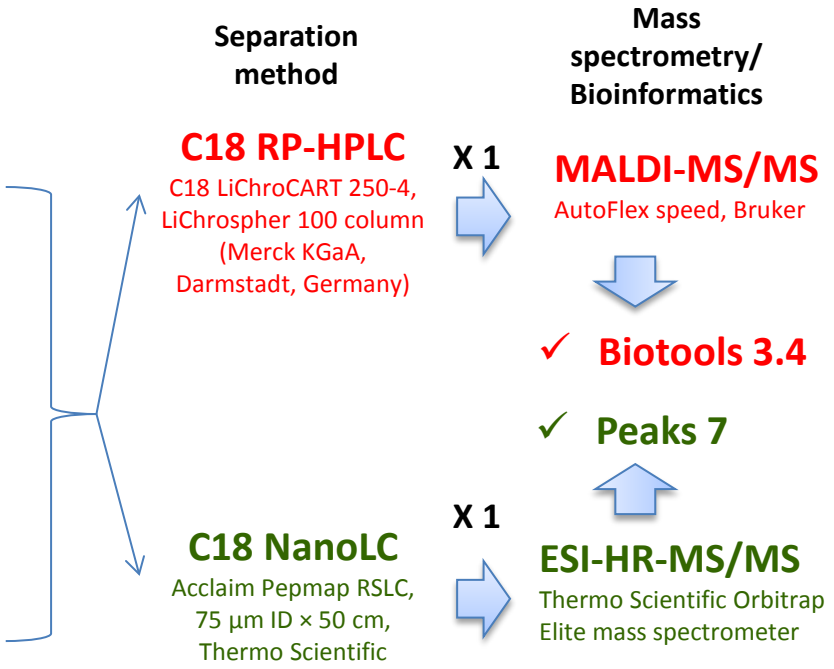
Source: NCBI

In vitro GI digestion X 3

No heating over 37°C

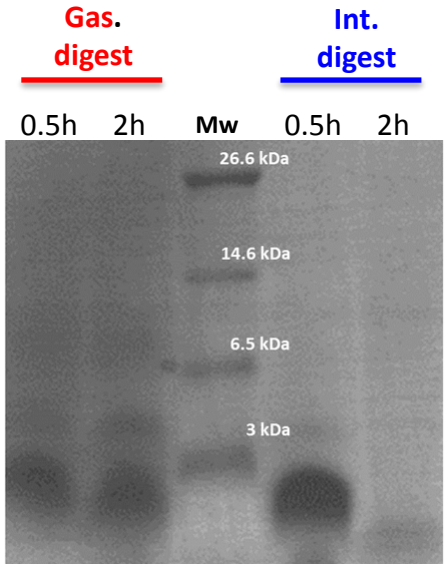


Analytical strategy

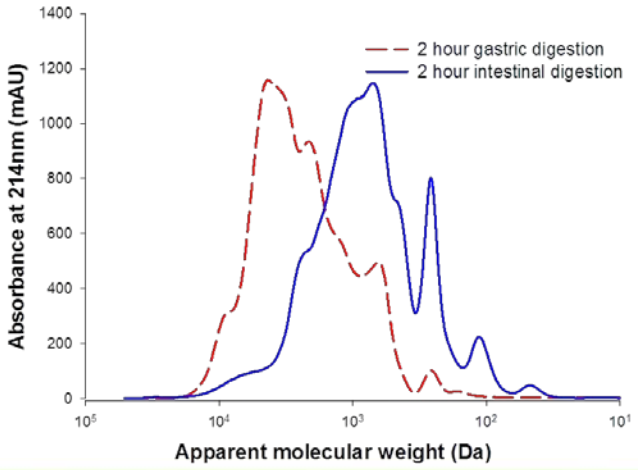


- **Hemoglobin (Hb): a highly digestible protein**

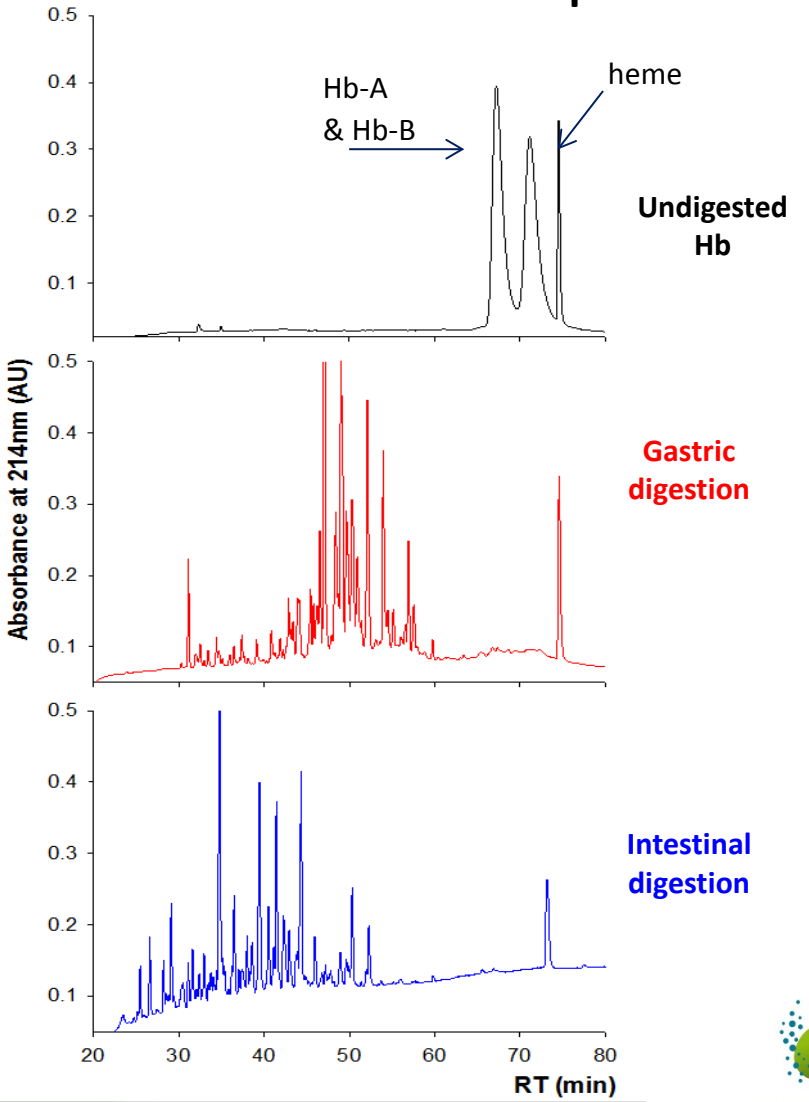
SDS-PAGE: 16.5%



SEC-chromatography



Normal bore C18-HPLC profiles



Results - Low resolution approach

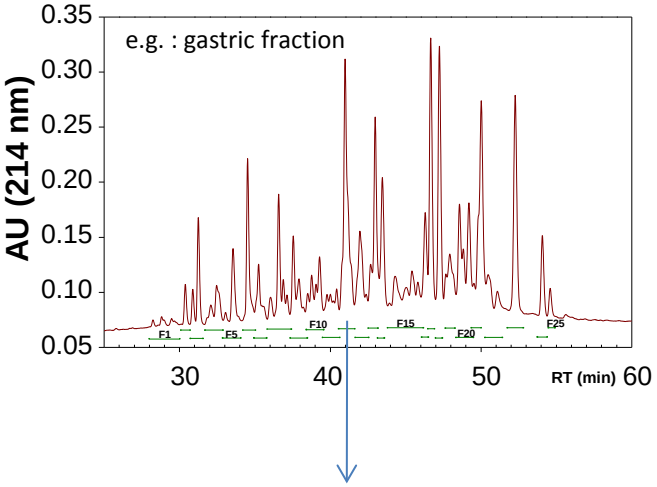
- **Normal bore C18 HPLC-MALDI-MS (off-line)**

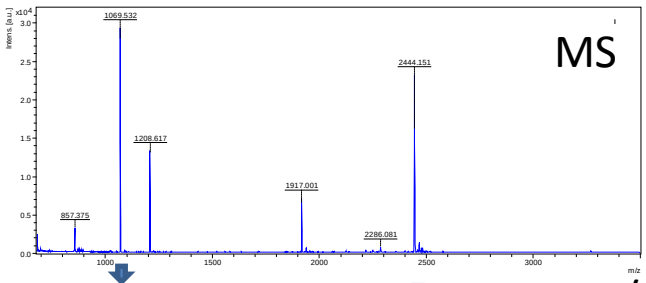
Digestome samples X3

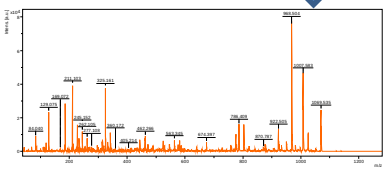
C18-HPLC separation X3

Manual collect (26 fractions) X3

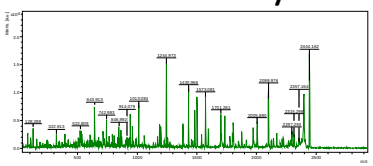
Automatic MALDI-MS and MS/MS X3







MS/MS



Gathering of all XML files

Combined XML file
+ renumbering of detected peaks

Enzyme : none
Missed cleavage : 36

Hb-A and Hb-B sequences

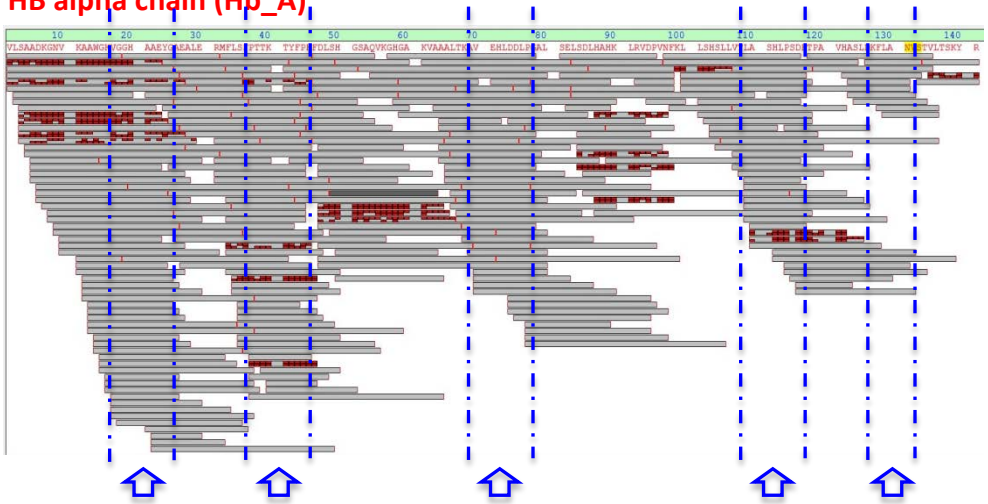
Peptides Matching

Results - Low resolution approach

e.g. : gastric fraction

- **Peptide heterogeneity (HPLC-MALDI-MS)**

HB alpha chain (Hb_A)



Digestion resistant sequences ?

HB beta chain (Hb_B)

*Ion parent error tolerance **30 ppm** and fragment mass error tolerance **0.5 Da**.*

317 matching peptides (based on MS-data)

→ sequence coverage (MS) = 100 %

19 peptides unambiguously identified by MS/MS

→ sequence coverage (MS/MS) = 77 %

339 matching peptides (based on MS-data)

→ sequence coverage (MS) = 100 %

26 peptides unambigously identified by MS/MS

→ sequence coverage (MS/MS) = 66 %

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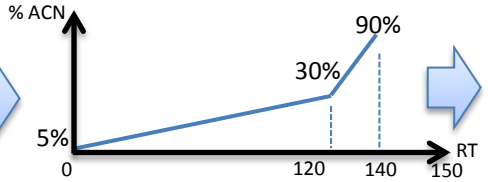
Results – High resolution approach

- **nanoLC-ESI-HR-MS/MS**

e.g. : intestinal fraction

X3
Digestome samples

zip-tip



Orbitrap Elite mass spectrometer

MS param.

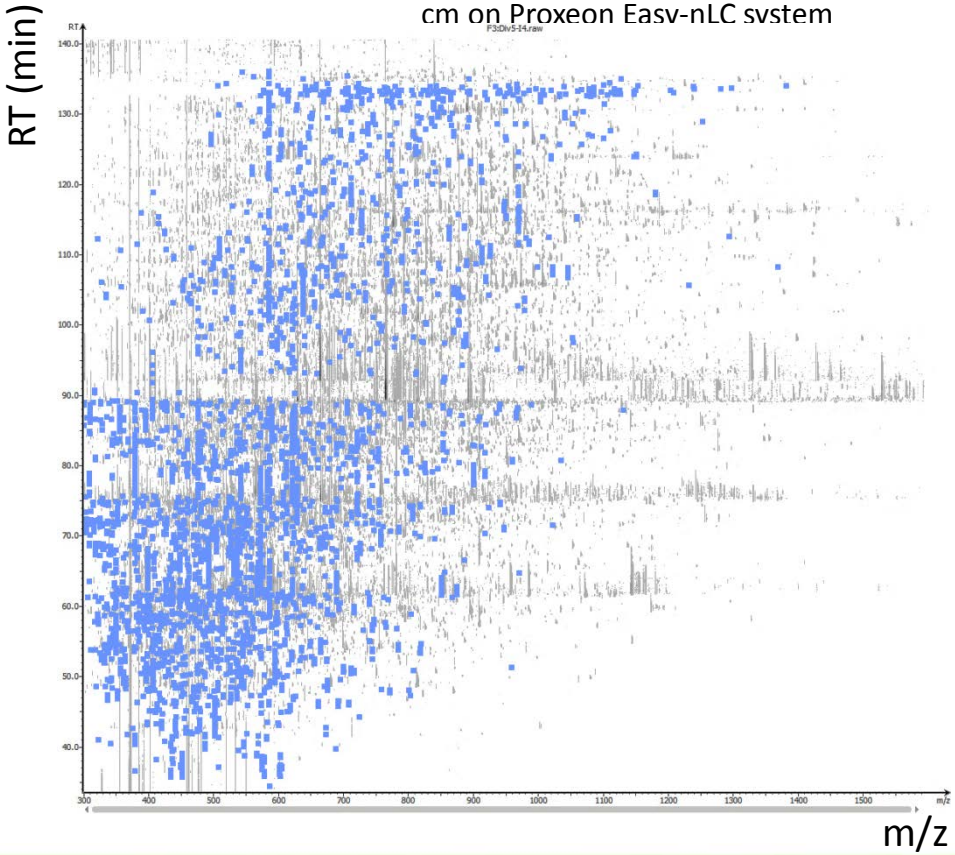
- data-dependent mode
- RP = 70,000 (FWHM)
- 300–1600 m/z → 3^E6 ions

MS/MS param.

- 500 counts
- isolation window of 4 amu
- normalized collision energy of 35%.
- 5000 ions and max. inj. time = 60 ms
- 300–2000 m/z.

X3

Acclaim Pepmap RSLC, 75 µm ID × 50 cm on Proxeon Easy-nLC system



Peptide identifications

Database : bovine proteins

Hb_A

306 peptides unambiguously identified by MS/MS

→ sequence coverage (MS/MS) = 100 %

False discovery rate (FDR) = 0%

Hb_B

420 peptides unambiguously identified by MS/MS

→ sequence coverage (MS/MS) = 100 %

726 peptides

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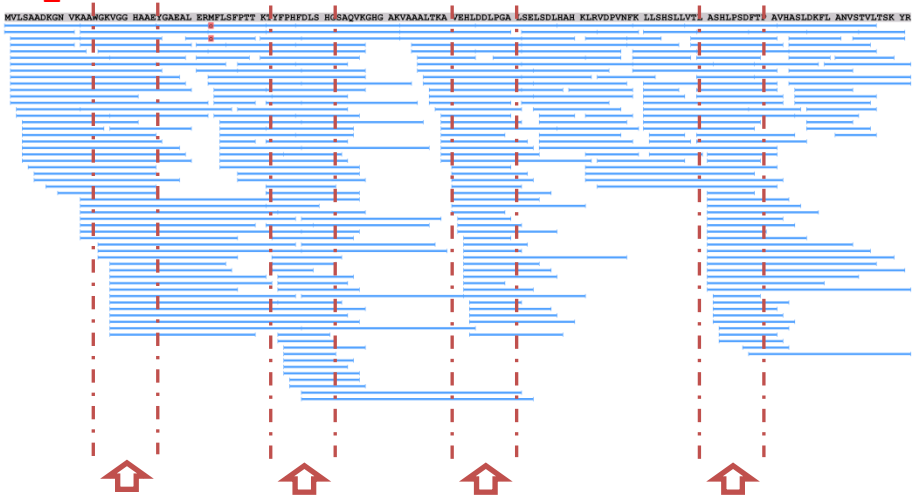
Results – High resolution approach

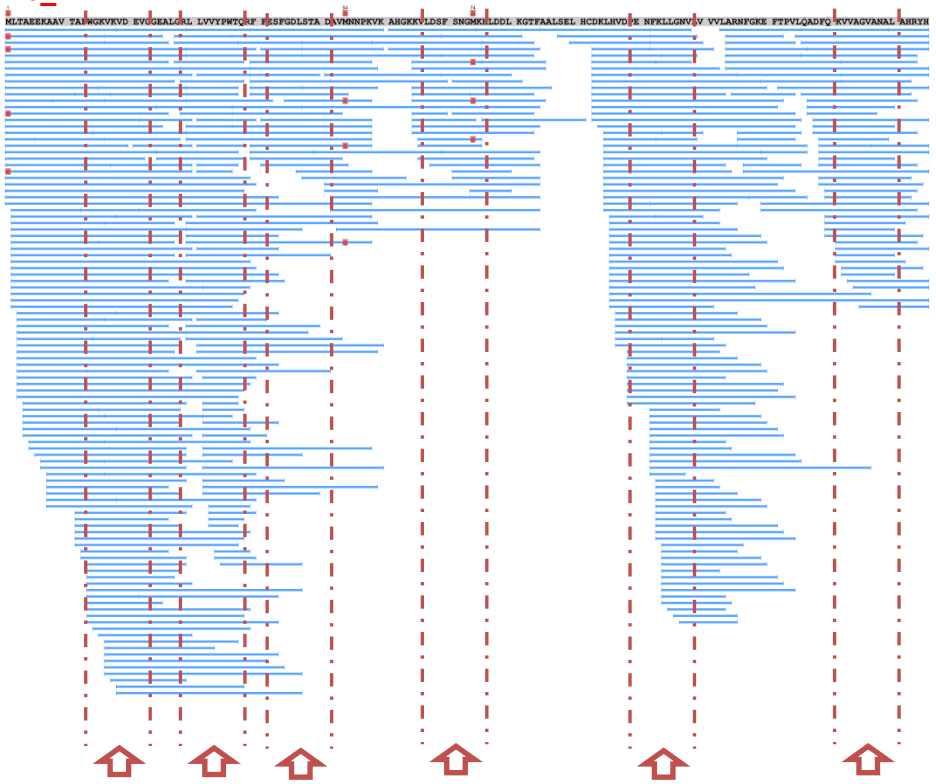
- **Peptide heterogeneity(nanoLC-ESI-HR-MS/MS)**

e.g. : intestinal fraction

Hb_A



Hb_B



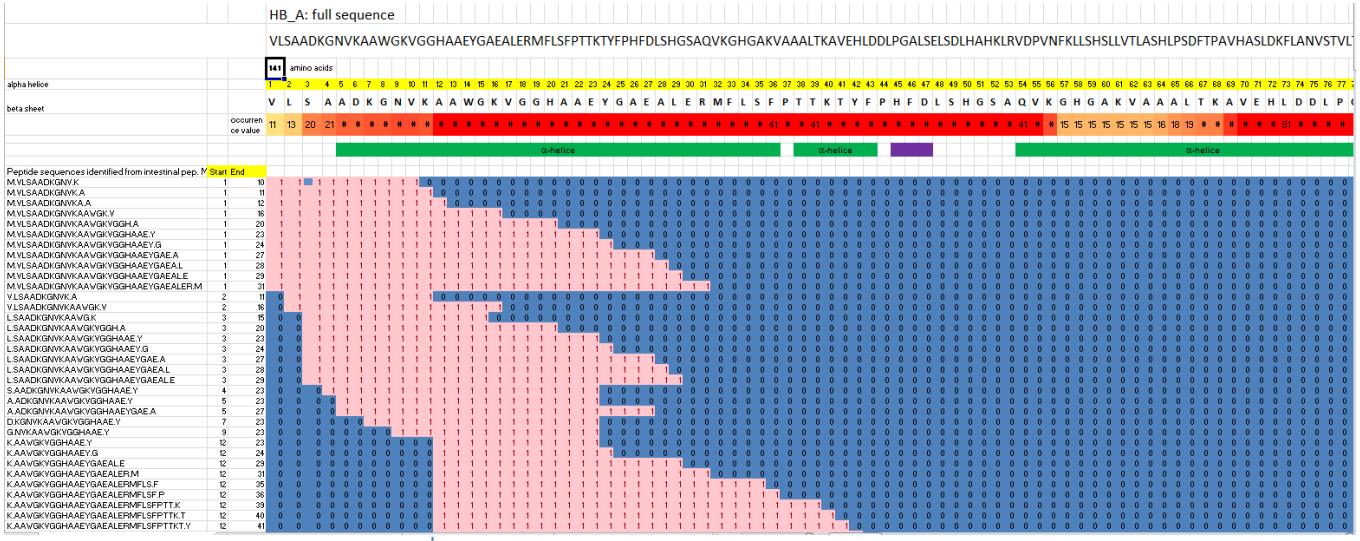
GI digestion-resistant sequences

*Ion parent error tolerance **10 ppm** and fragment mass error tolerance **0.2 Da**.*

Results – The GI digestion-resistant sequences

Hb_A – intestinal maps

Hb_B – intestinal maps

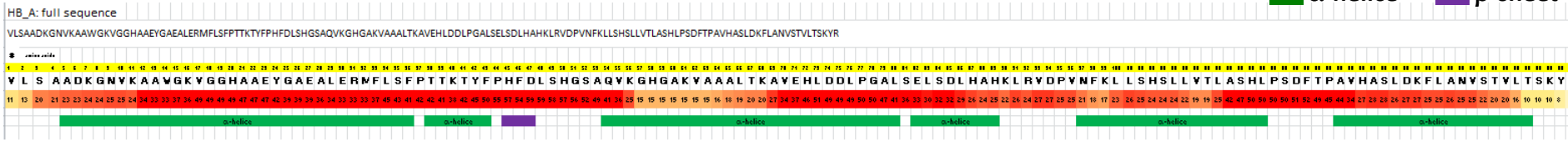


K | A

Preferential cleavage site for trypsin

HB-A: heat map of AA occurrence frequency

α-helice β-sheet



HB-B: heat map of AA occurrence frequency

Objectives

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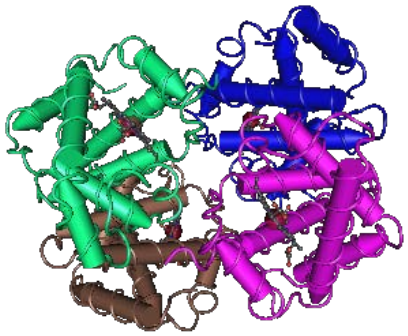
- Protein digestion and energy homeostasis: impact of generated peptides on intestinal hormones



Bovine hemoglobin

Source: NCBI

Background - Food intake regulation: the brain/gut axis

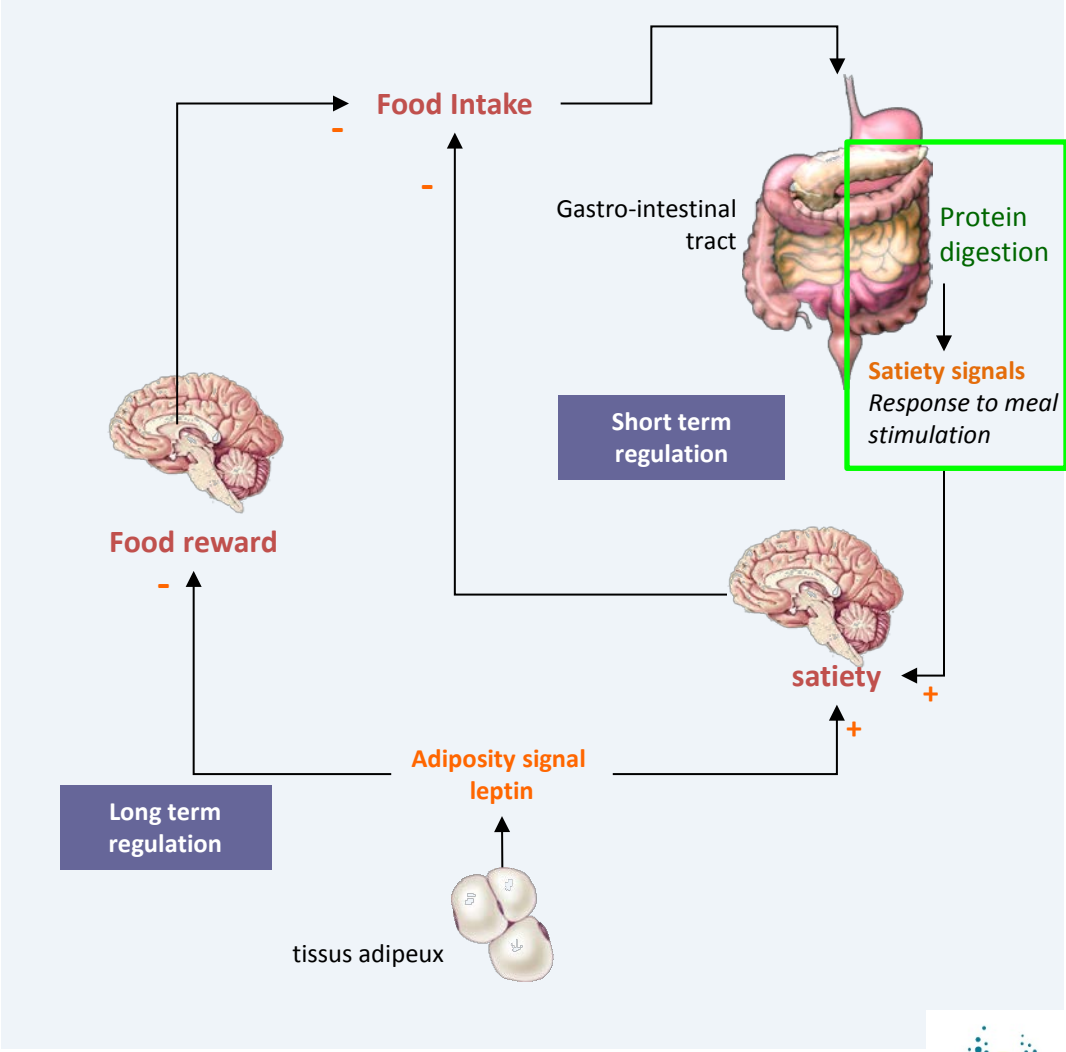
- **Energy Homeostasis:**

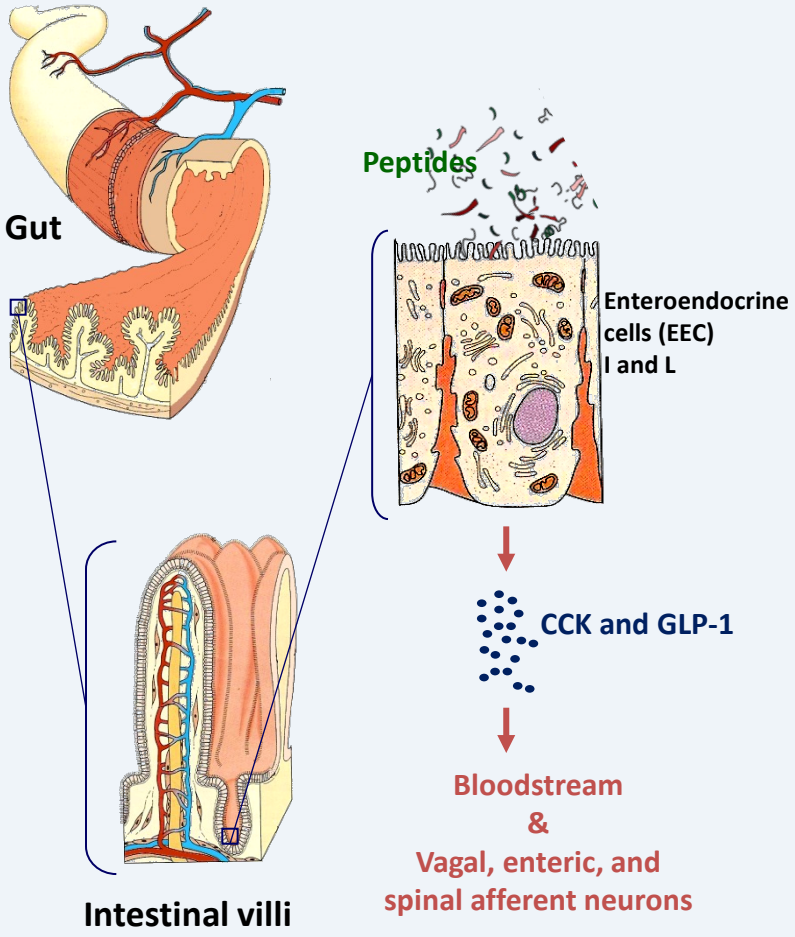
Energy expenditure *vs* caloric intake:
need to ensure a balance

- **Regulation mechanisms**

Long term - adiposity signal:
To maintain body weight
« adiposity negative feedback »
(*Leptin*)

Short term - satiation signal:
(*gut hormones, gastric distension*)





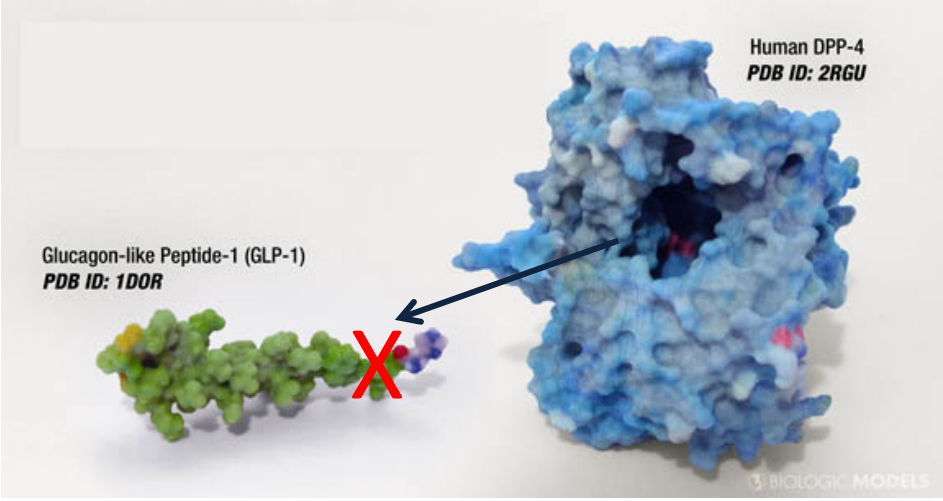
- Cholecystokinin (**CCK**)

Produced by **I cells** (duodenum) in response to lipids and **proteins**.
Promotes **satiation**: increase gastric secretion, decrease gastric emptying, induces satiety feeling by vagal afferents

- Glucagon-like Peptide 1 (**GLP-1**)

Produced by **L cells** (ileum and colon)
One of the proglucagon products
Promotes satiation by various pathways
Incretin: stimulates glucose-dependant insulin secretion

GLP-1 inactivation by dipeptidyl peptidase IV (**DPP-IV**).
Only 10-20% plasmatic GLP-1 remains



- Dipeptidyl peptidase 4 **(DPP-IV)**

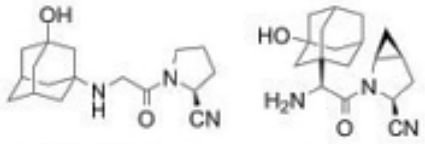
DPP-IV rapidly degrades GLP-1 → decrease in plasma

DPP-IV inhibition → indirect increase of GLP-1 activity
→ indirect impact on food intake

DDP-IV – GLP-1:

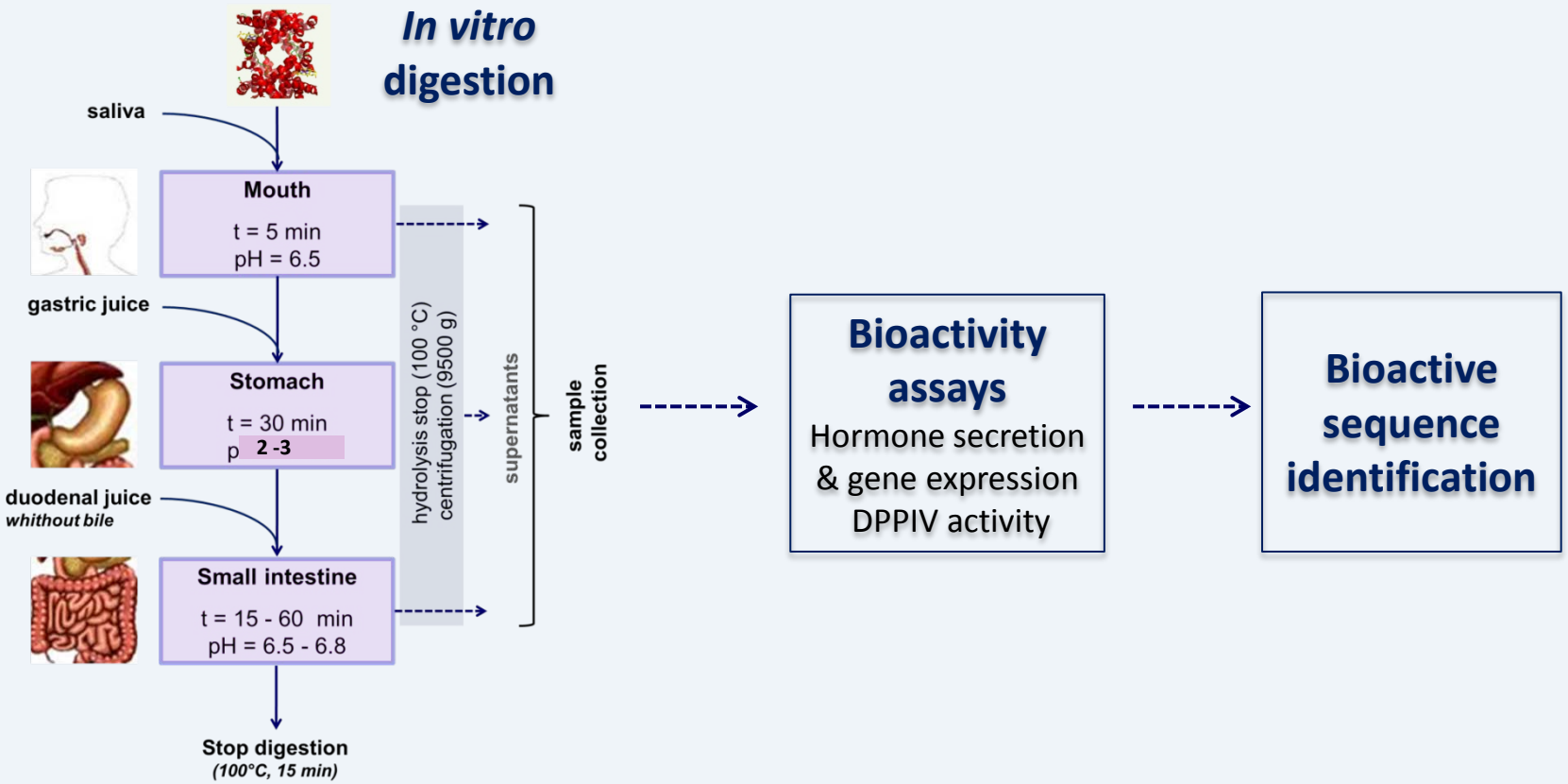
Inhibiting DPP-IV extends GLP-1 incretin activity
New target for type-2 diabetes therapy

Ex: Gliptins (e.g. Vidagliptin and saxagliptin)



→ Dietary proteins: promising sources as "natural" DPP-IV inhibitors

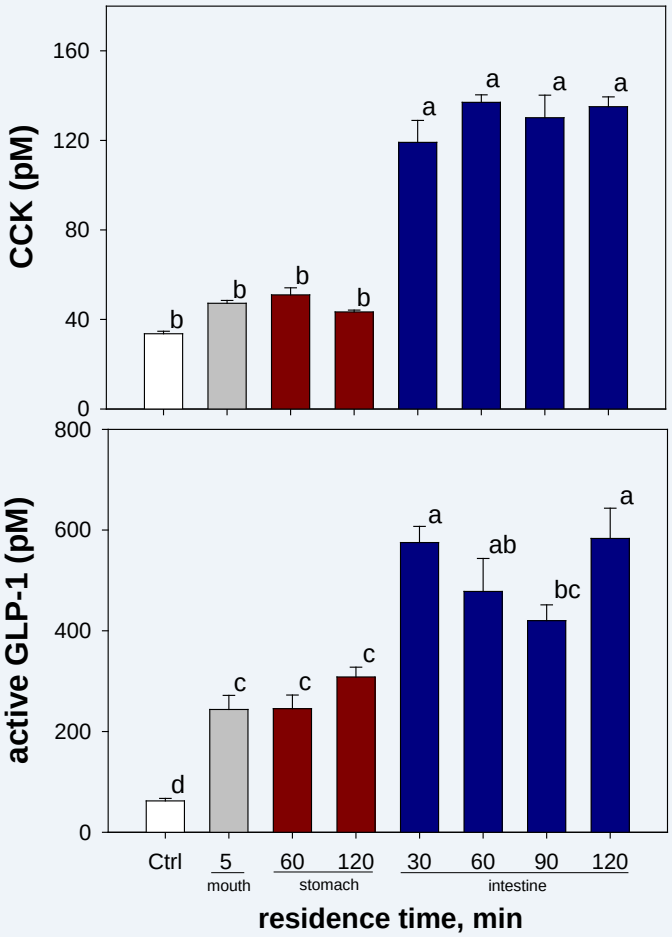
Experimental design



Hormones secretion

STC-1
Enteroendocrine cells

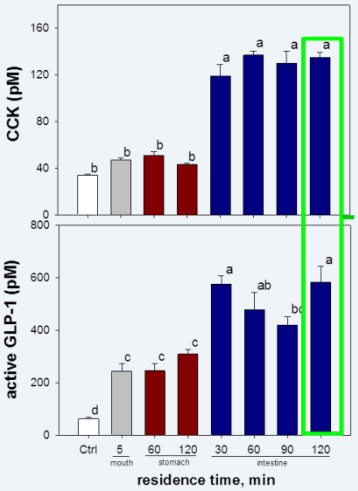
2h → CCK & GLP-1 secretion
RIA detection



Significant increase of both CCK and GLP-1 secretion in presence of intestinal samples

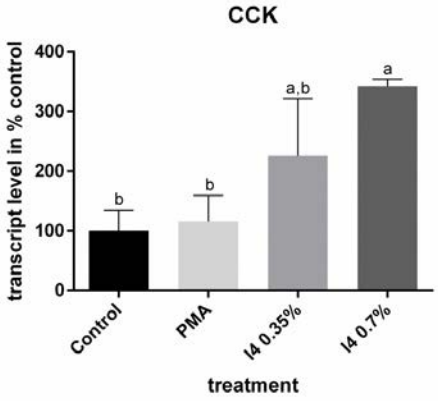
→ Beneficial effect of **intestinal enzymes** on peptide potential bioactivity

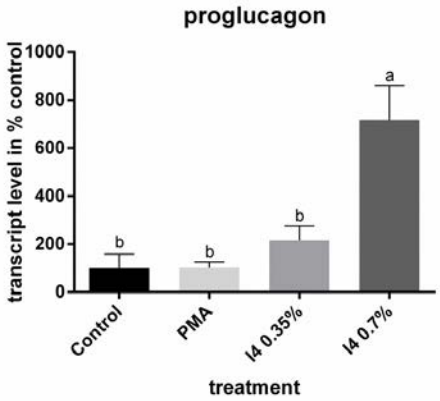
• Regulation of hormone gene expression



24h → CCK & Proglucagon genes expression *qPCR*

Intestinal hydrolysate (I4) significantly induces both CCK and proglucagon gene expression





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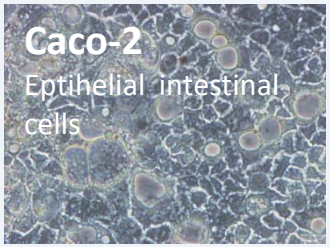
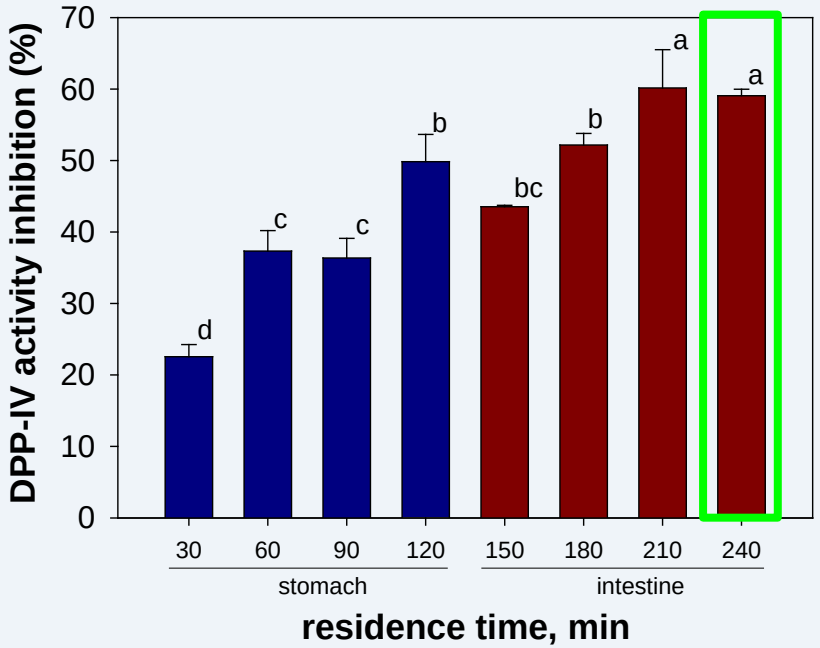
Results – DPP-IV activity inhibition

• DPP-IV activity assay



Cell extract DPP-IV → DPP-IV activity inhibition of **Hb digests** *In vitro* colorimetric assays



Intestinal DPP-IV inhibition activity is enhanced during GI digestion

Final intestinal hydrolysate: best bioactivity like for intestinal hormone

------→ Physiological relevance, same peptides involved?

------→ Need to identify resistant active sequences

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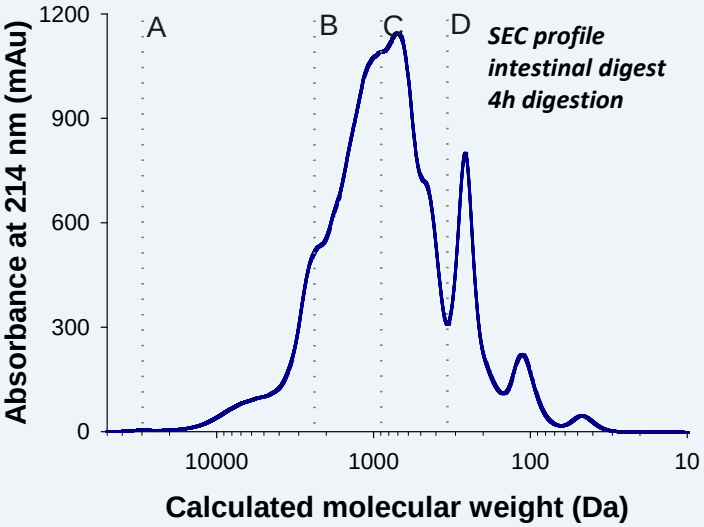
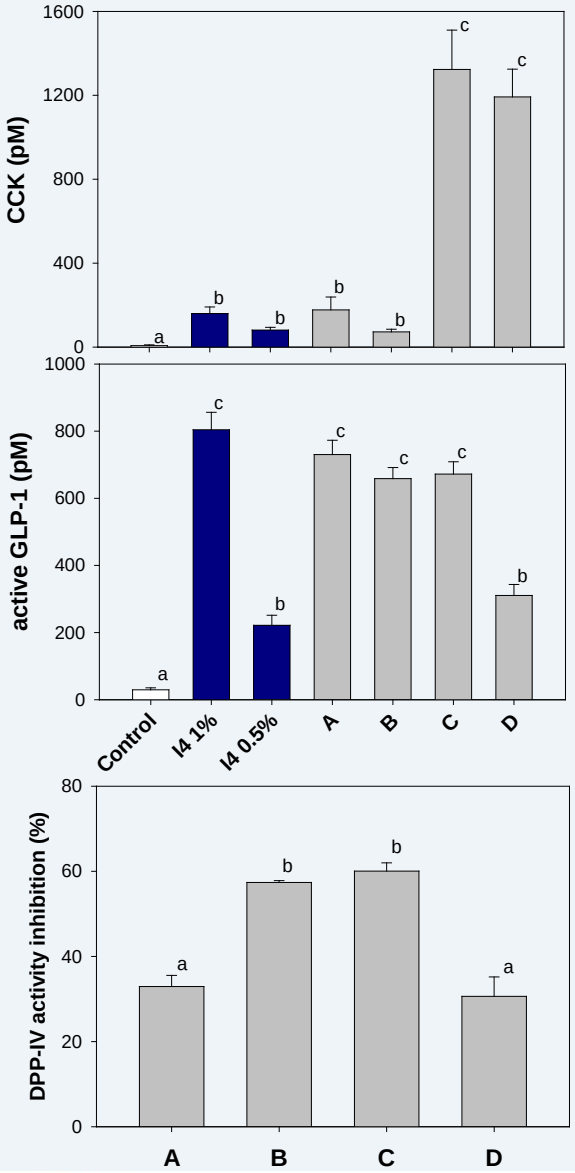
Results - peptide purification

- **Size exclusion chromatography**



bioactivities



CCK and GLP-1 different peptide groups involved

------→ DPP-IV and GLP-1 Same peptides involved?

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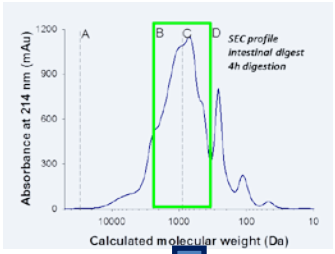
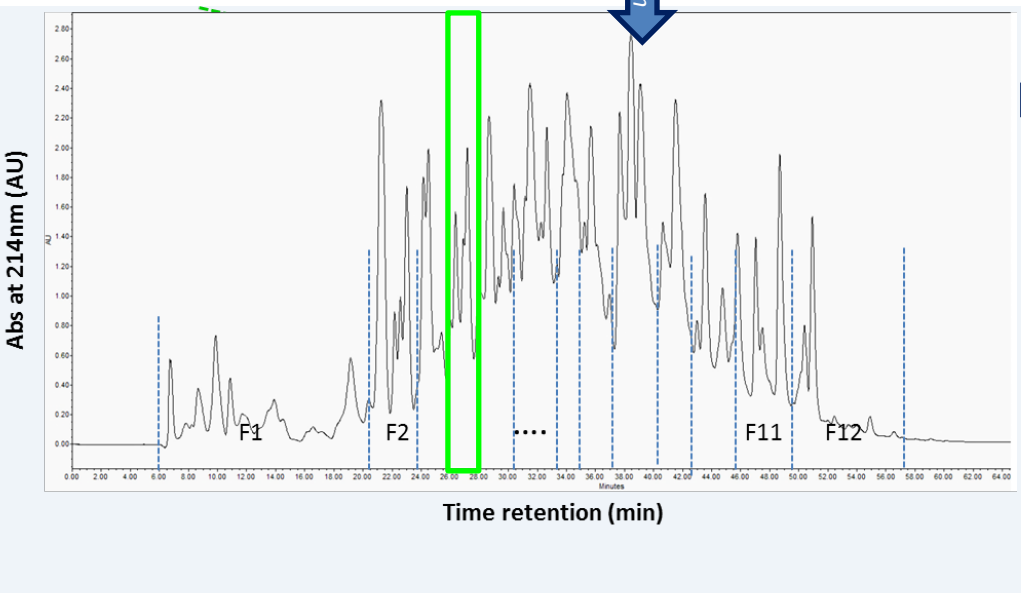
Results - peptide purification

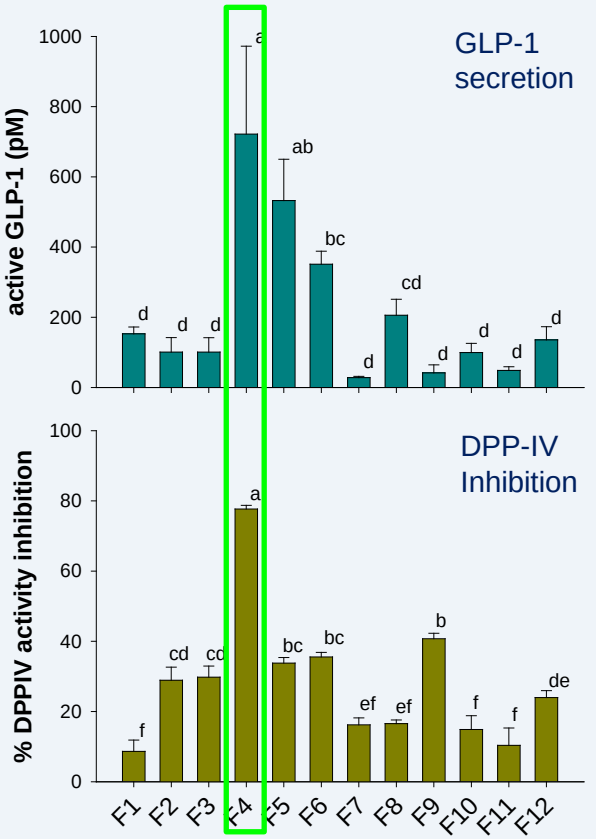
- **C18 RP-HPLC**



Separation



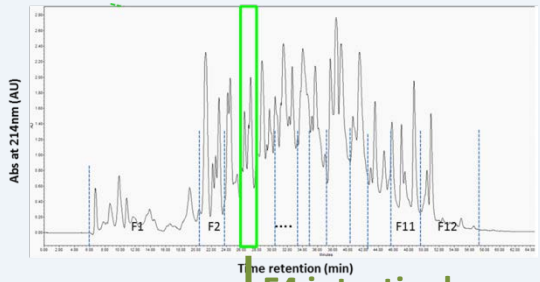
bioactivities



Peptides contained in F4 both stimulate GLP-1 secretion and inhibit DPP-IV activity

Results - peptide purification

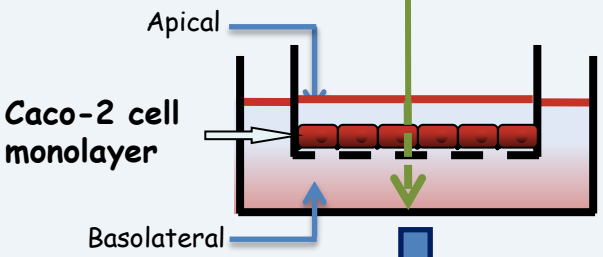
- **Peptide identification and passage across intestinal wall**



F4 intestinal sub-fraction

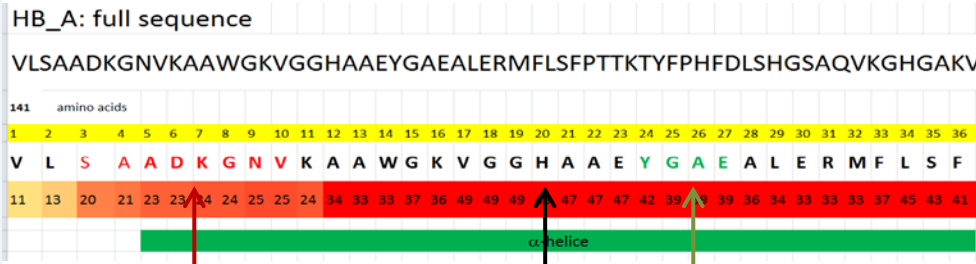
60 peptides

LC-MSMS



LC-MSMS

18 peptides



Protein	Sequence	Mass (Da)
HBA_BOVIN	ADKGNV	602.3024
HBA_BOVIN	ADKGNVK	730.3973
HBA_BOVIN	SAADKGNV	760.3715
HBA_BOVIN	SAADKGNVKA	959.5035
HBA_BOVIN	DLHAH	591.28
HBA_BOVIN	SDLHAHK	806.4035
HBB_BOVIN	SDLHAH	678.3085
HBA_BOVIN	DLSHGSAQ	813.3617
HBA_BOVIN	KAAVT	488.2958
HBA_BOVIN	MNNPK	602.2846
HBA_BOVIN	SLDK	461.24
HBA_BOVIN	VAAA	330.19
HBA_BOVIN	VDPVN	542.27
HBA_BOVIN	VGGHAAE	639.2976
HBA_BOVIN	YGAE	438.175
HBA_BOVIN	YGAEA	509.2122
HBB_BOVIN	ANVST	490.2387
HBB_BOVIN	LTAEEK	689.3596

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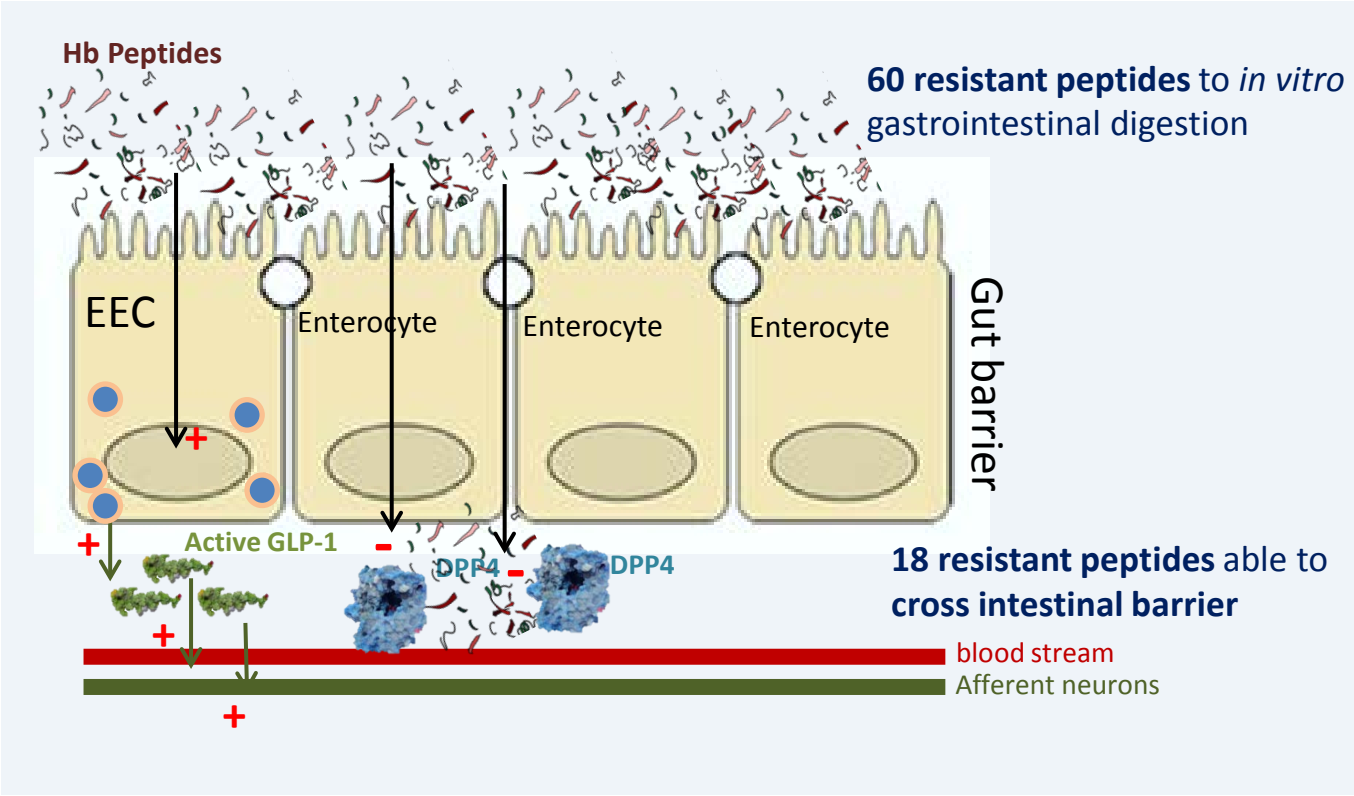
- **GLP-1 / DPP4**



→ Significant increase of both GLP-1 secretion and proglucagon gene expression

→ Inhibition of DPP-IV activity

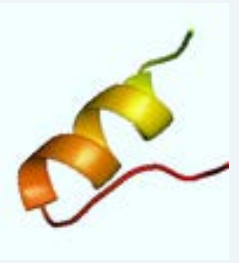
→ **Extending GLP-1 actions (food intake regulation and incretin effect)**

- **Hemoglobin peptidomes**

- More than **700 sequences unambiguously identified** in gastric and intestinal peptidomes
- **Specific cleaveages sites** identified
- Resistant sequences identified – **recurent patterns**

- These recurring patterns were made of amino acids that **could be potential preferential cleavage sites** with regard to enzyme specificity.
- **No particular link** between enzyme resistivity and isoelectric point or hydrophobicity index has been found out so far.
- **Peptide conformations** could prevent or slower enzyme activity. **Secondary structure implicated.**

Proteases and peptidases (GI environnement) → Emergence of **resistant bioactive sequences**



Peptidofoodomics → New tool for the study of this complexe phenomenom

→ **New tool for screening dietary protein bioactivities**

Thank you for your attention !

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