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## Development of a workflow to apprehend dynamic digestion of a mix of proteins: application of meat product for elderly

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**Objective :** Designing food is a major issue for improving protein absorption in elderly as well as robust methods to evaluate the protein digestion efficiency. “Knacks”, which is restructured meat, was used to assess a streamlined workflow allowing to study how the digestive functions impact protein digestion efficiency. The main objective of this work is to create a workflow relevant for analyzing peptides from a complex meal.

## Global Peptidomic Workflow : from a complex food to a multi-protein digestive model

### Laboratory workflow – from food to peptides

**Aliments**  
Sausage “knacks”



**In vitro mastication with masticatory apparatus**

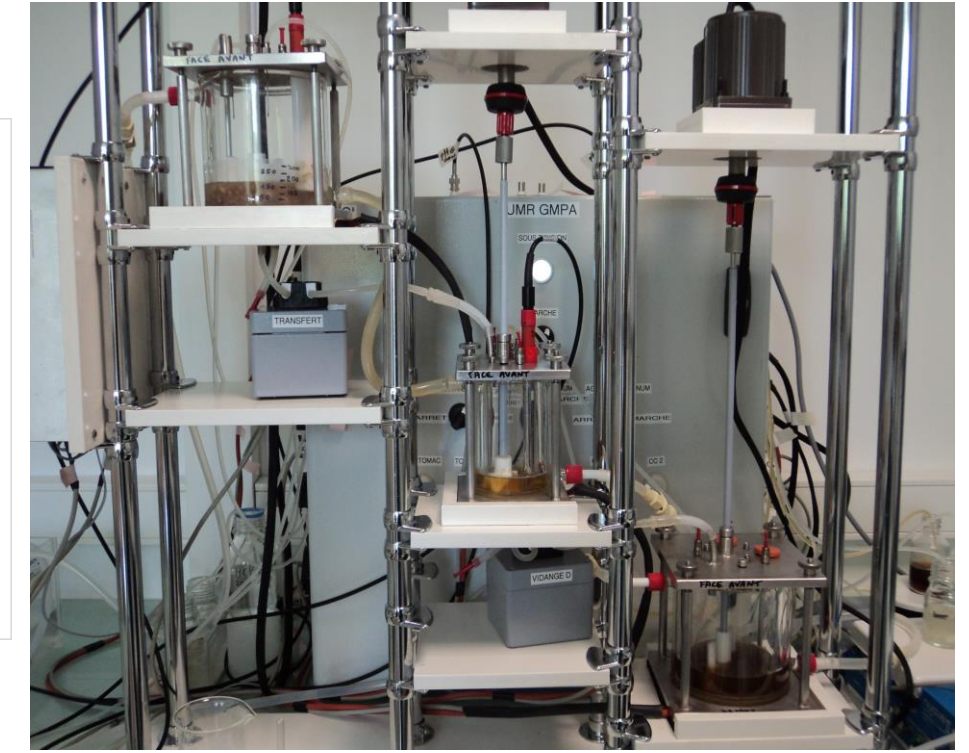
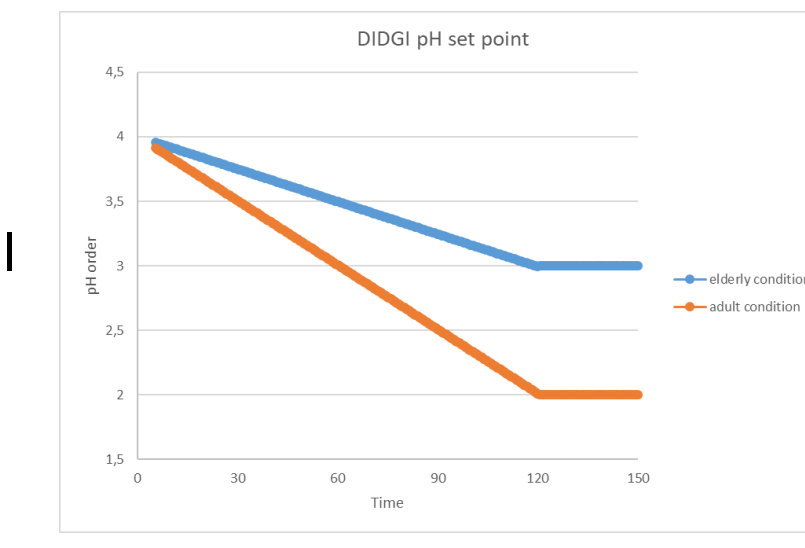


**In vitro gastric digestion using a dynamic artificial digestive system: DIDGI® (INRA, GPMA, France)**

Two digestive conditions studied

**Adult (blue curve)**  
pH decrease 4 – 2 [Pepsin] 2080 U/ml

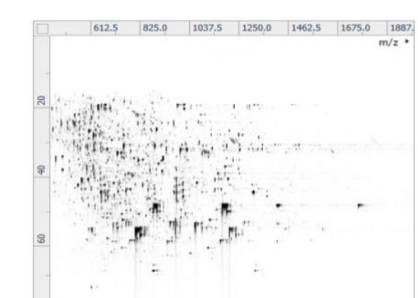
**Elderly (red curve)**  
pH decrease 4 – 3 [Pepsin] 1248 U/ml



### Reprocessing mass data workflow: from the peptides to protein hydrolysis intensity

**Progenesis-QI (waters).**

Identification results were import in progenesis and peptide were assigned to their respective intensity



**Progenesis-QI (waters).**  
Quantification detection of features

**Progenesis-QI (waters).**  
the sum of peptide intensity from a single protein accession number, permit to obtain a global protein intensity

**Selected features were export on MASCOT** used for database search using the Uniprot Taxonomy sus scrofa protein database with searching parameter “no enzyme

A total of 1419 peptides were identified, among them 610 were unique and used for quantification. These unique peptides were assigned to 68 proteins with at least 2 peptides.

**High protein intensity means = High protein digestion extent**

Sampling time at 30, 60, 90, 120 and 150 min.

**Mass spectra acquisition :**



Acquisition of mass spectra using a LC/MS/MS (capLC-QexactivePlus(thermofisher scientific)).

**Extraction of peptides :**

Peptides extraction using ACN, after acidic precipitation, and captured using silica.

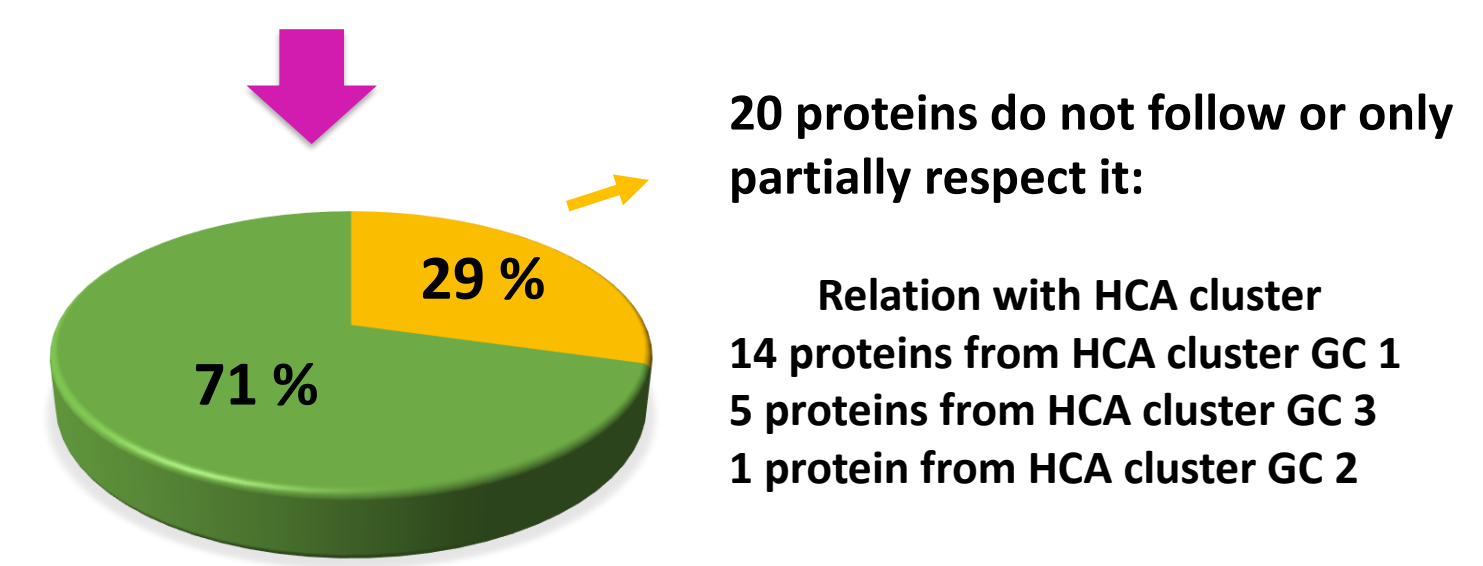
### Modeling kinetic workflow: from protein intensity to a model kinetic law

Fitting of protein intensities of adult or senior (divided by emptying ratio).

$$\text{Kinetic law : } f(t) = a \cdot t^b$$

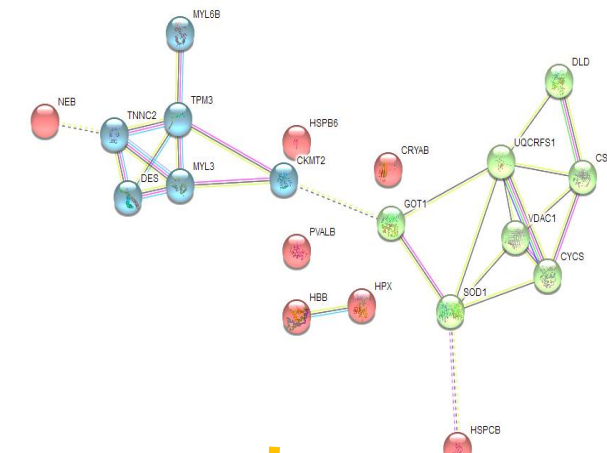
For each protein, determination of a and b for adults and seniors.

**Are the 68 proteins intensities following the kinetic law ?**



20 proteins do not follow or only partially respect it:

Relation with HCA cluster  
14 proteins from HCA cluster GC 1  
5 proteins from HCA cluster GC 3  
1 protein from HCA cluster GC 2



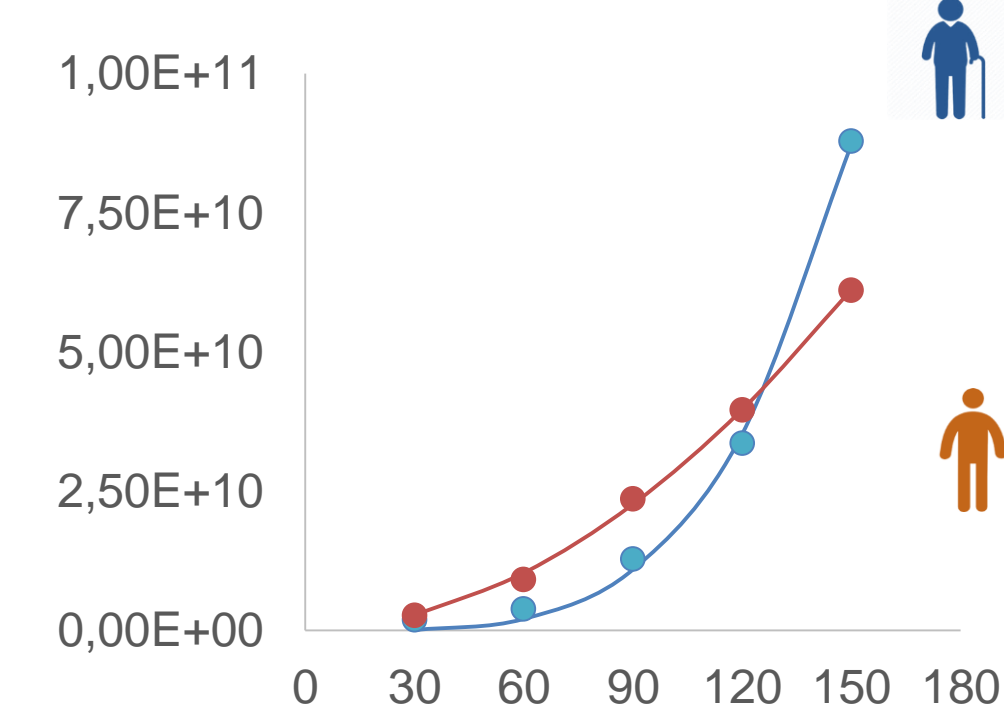
They are located in blood, mitochondria or myofibrils.

These 20 proteins follow other kinetic law(s).

**48 protein hydrolysis correctly simulated** for the two digestive conditions (adult & elderly).

For each protein kinetic: can we correctly assign whether it is from an adult or an elderly ?

Using a rule on the calculated values of {a, b} to attribute the kinetics to adults or seniors:



**SENIOR : a < 1131 AND b > 3,441**

42 senior protein kinetics correctly classified  
6 proteins misclassified

**ADULT : a > 1308 AND b < 3,138**

44 adult protein kinetics correctly classified  
4 proteins misclassified

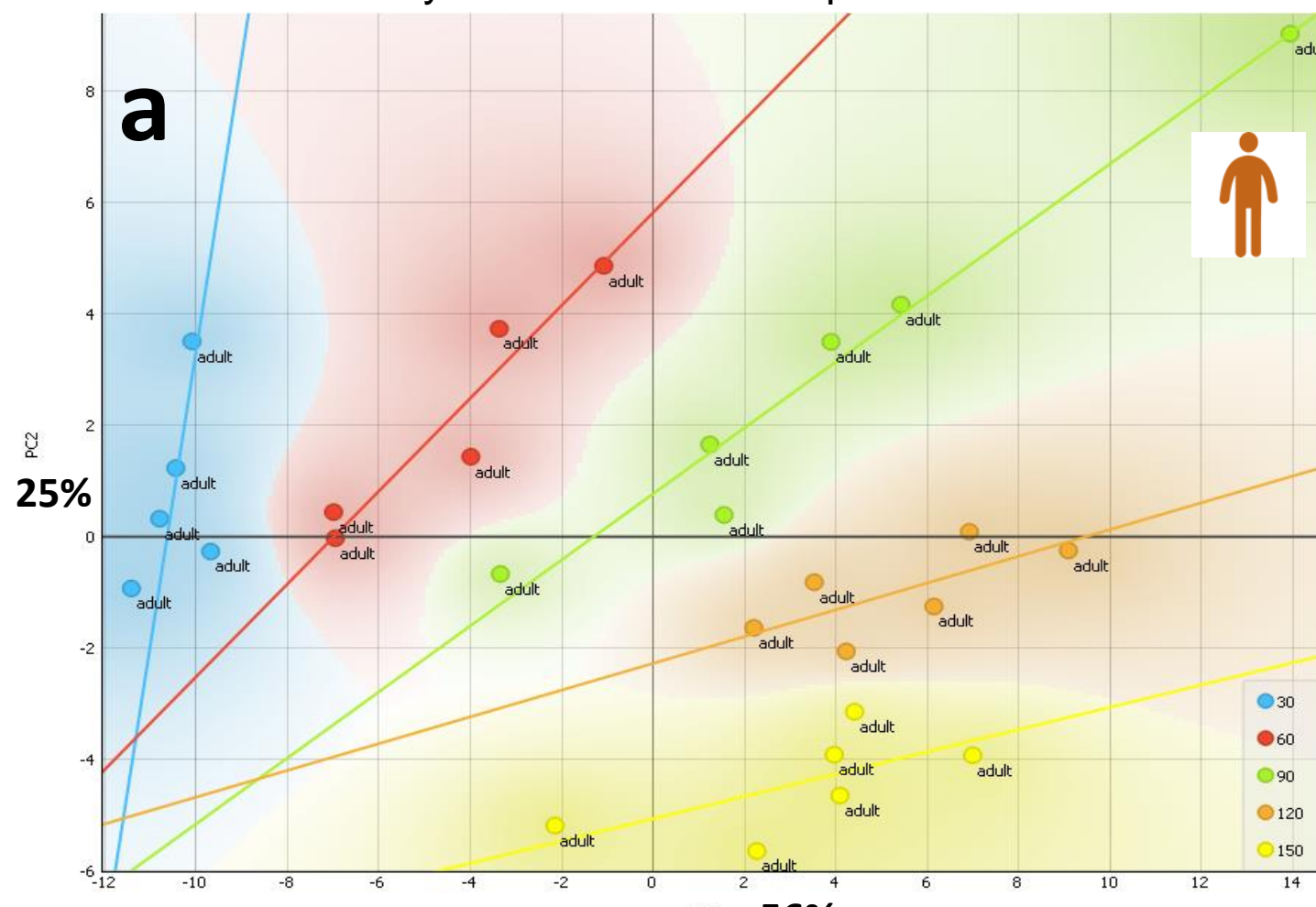
**56 % of the 68 quantified and identified digested proteins follow a power-type law with specific parameters for adult and elderly conditions.**

**44 % of the 68 quantified and identified digested proteins may follow another law or have no specific model kinetic parameters.**

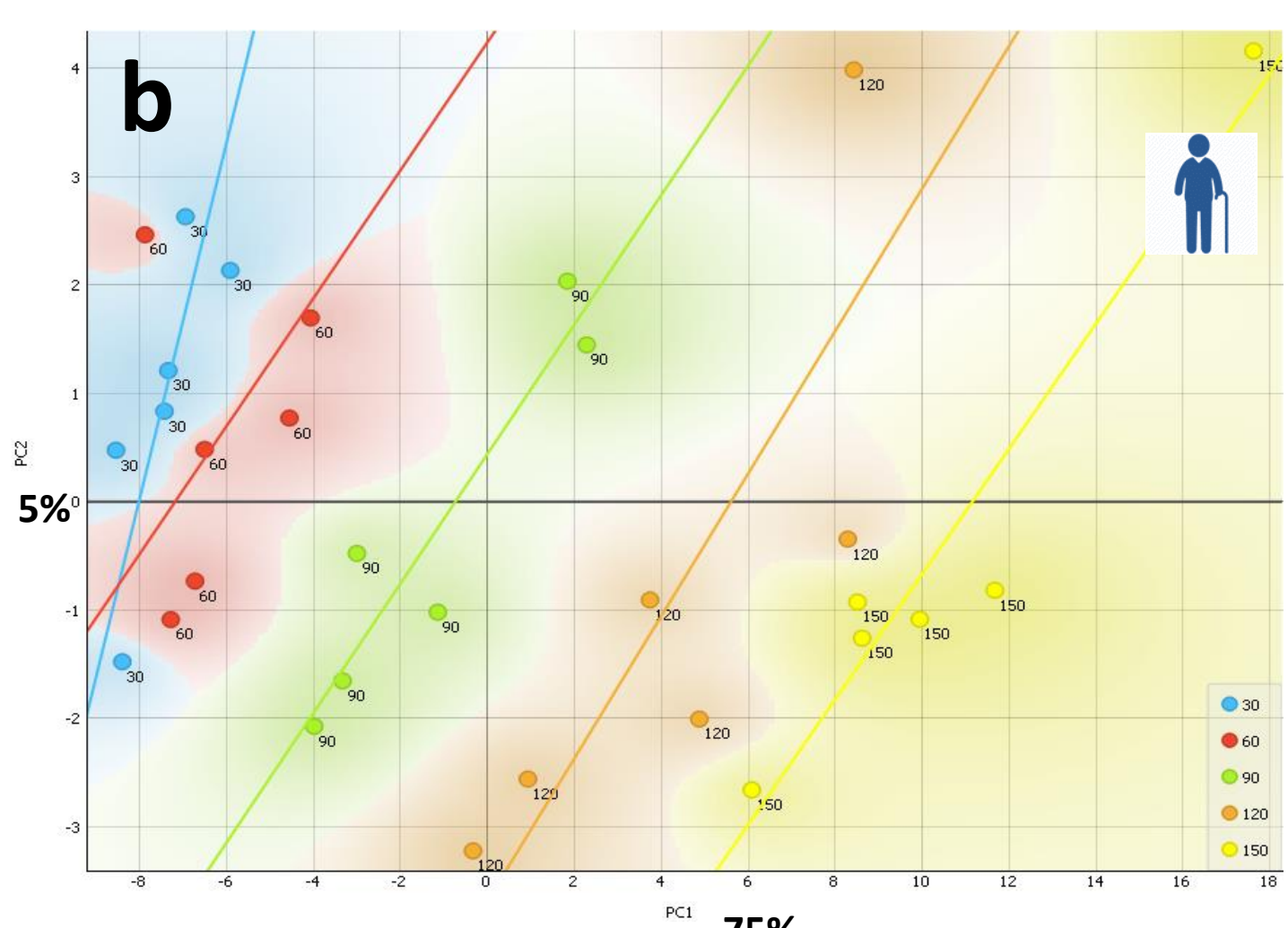
### Statistical workflow: from protein intensity to multivariate analysis

Multivariate analysis using PCA method to compare kinetic digestion (sampling time: 30, 60, 90, 120, 150 min) of adult or elderly:

The intensity of the 68 identified proteins were variables.



**Fig a:** Projection of adult samples with regression line calculate for each sampling time.

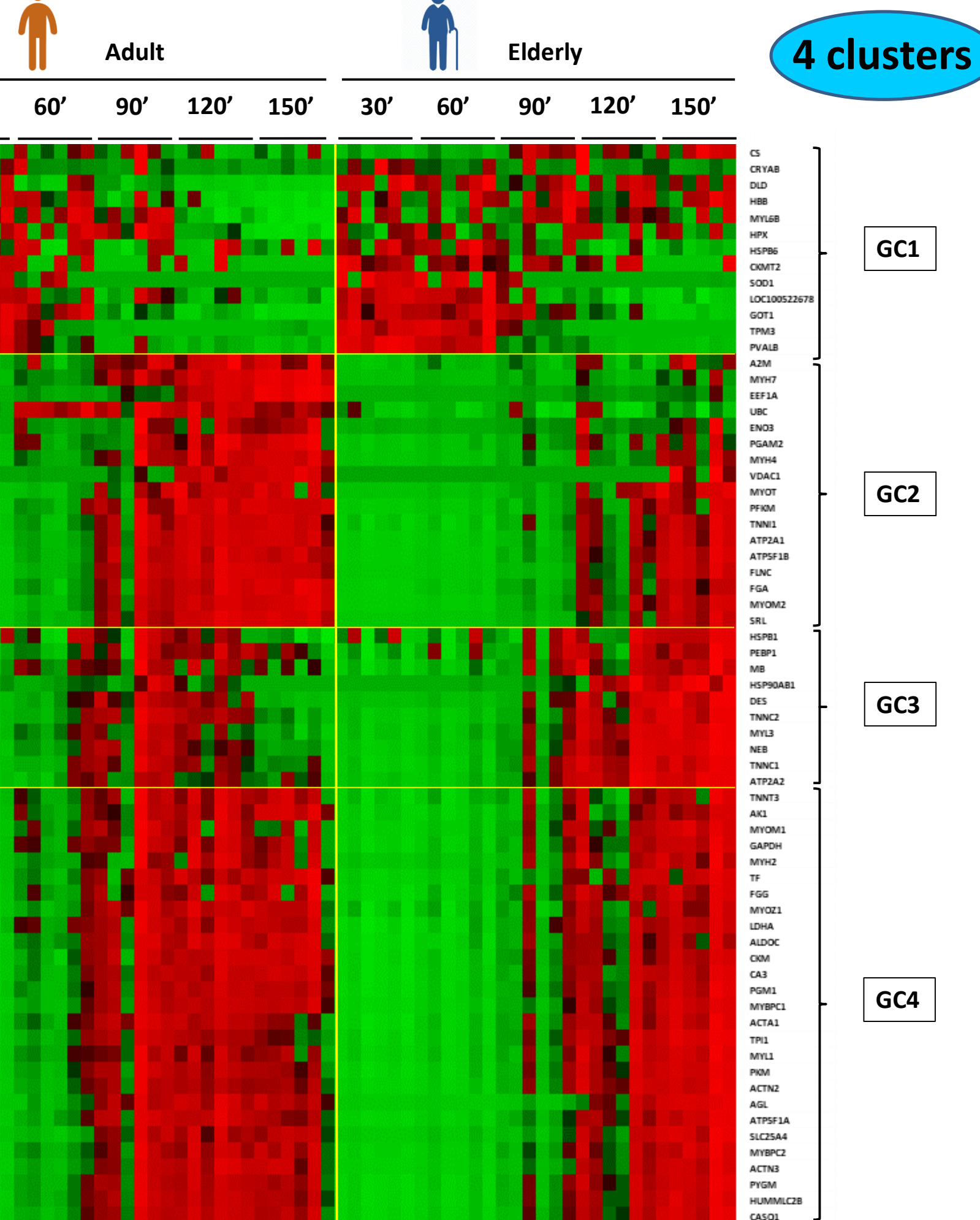


**Fig b:** Projection of senior samples with regression line calculate for each sampling time.

The protein digestions kinetics differ at the point 90 min between adult and senior.

Multivariate analysis using hierarchical clustering method (HCA) to create protein groups:

Hierarchical clustering analysis was performed to group proteins according to a correlation matrix.



Each line represents one protein identified  
Each column represents one sample of gastric digestion:  
- Digestive status: adult – elderly;  
- Sampling time: 30 – 60 – 90 – 120 – 150 min

High protein digestion (red)  
Low protein digestion (green)

The four clusters permitted to group proteins according the sampling kinetics and the age condition

### Conclusion :

1. We evidenced that the meat proteins are differently affected by enzymatic digestion in adult or elderly physiological digestive conditions
2. The majority of protein kinetics follow a power-type law specific for adult or elderly
3. Development of a streamlined workflow , a new tool for designing food for specific population