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Screening and Purification of Metal Chelating Peptides in Hydrolysates for their Antioxidant Properties

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Societal context

Reactive Oxygen Species (ROS) induced oxidation

- ➔ Ageing and various pathologies in human related to oxidative stress, in particular inflammatory bowel diseases with iron anemia complication
- ➔ Degradation of consumption products (food, cosmetic matrix)

Living organisms developed their own **antioxidant defenses** against these ROS

Imbalance ↔ **Oxidative stress**

Importance to discover natural antioxidants and evaluation of their bioactivity

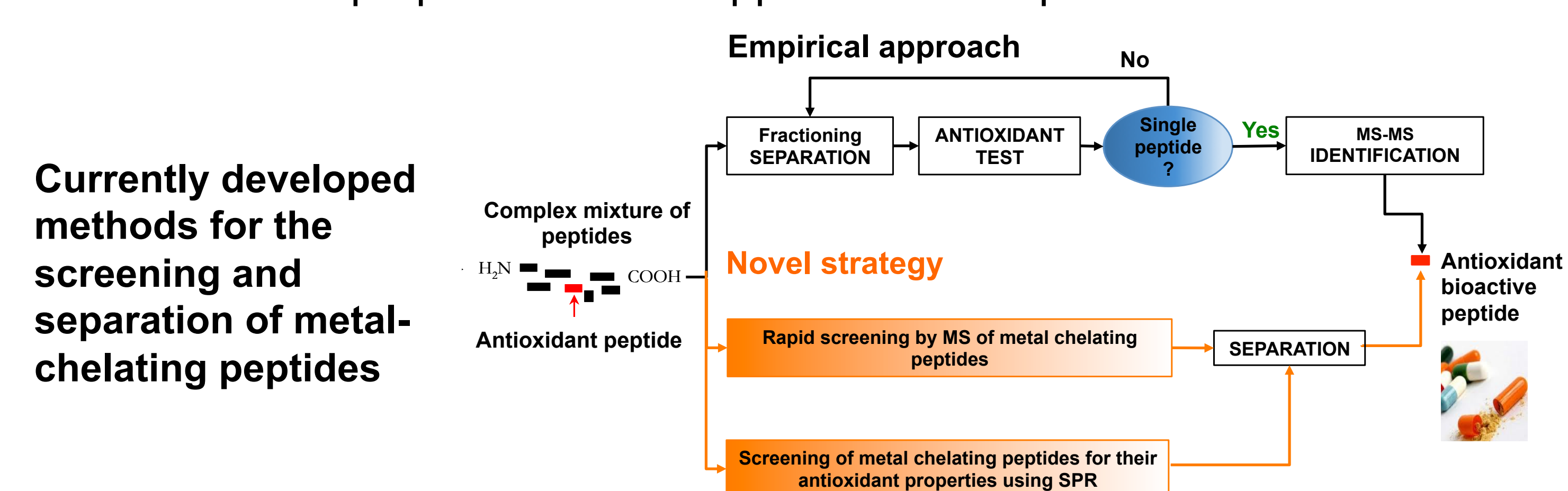


Introduction

Metal-chelating peptides obtained after protein hydrolysis

- Antioxidant properties related to the inhibition of Fenton reaction, responsible of ROS production
- Used in pharmaceuticals, foods and cosmetic

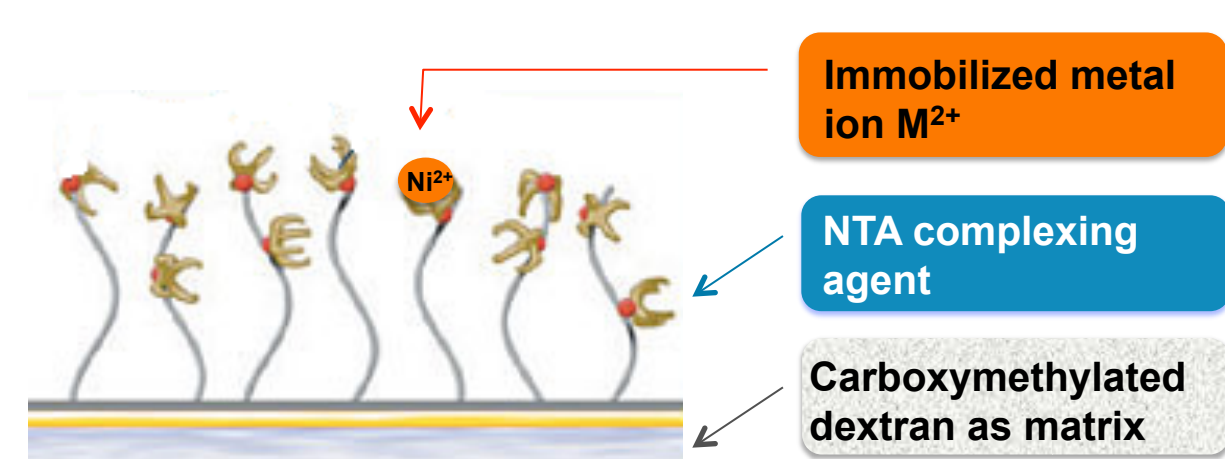
Novel strategy for the screening and purification of the peptides to target their antioxidant properties. Novel approach developed in order to save time.



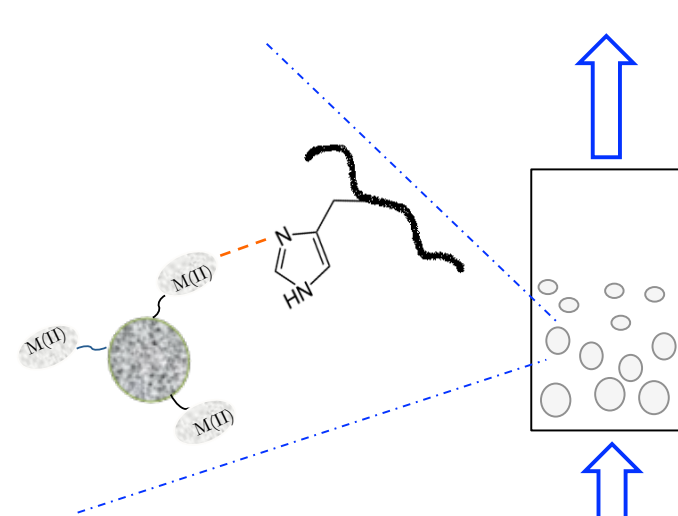
An interdisciplinary approach

Screening of metal-chelating peptides

by Surface Plasmon Resonance (SPR)

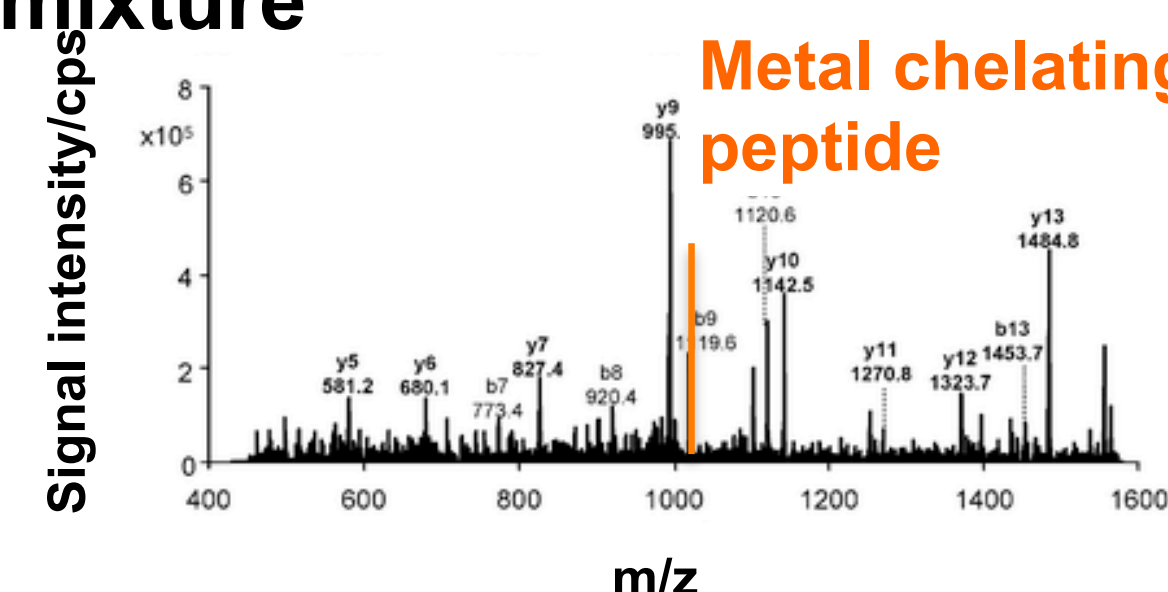


Processes and Modelling

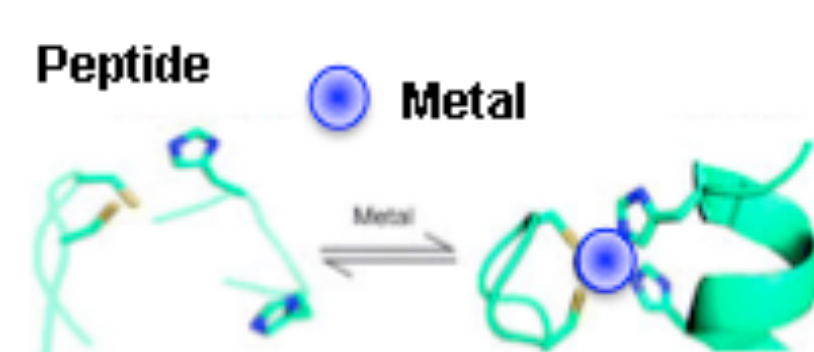


Integration of these SPR data for separation prediction in Immobilized Metal ion Affinity Chromatography (IMAC)

by mass spectrometry (MS) : identification of the peptide(s) of interest present in the complex mixture

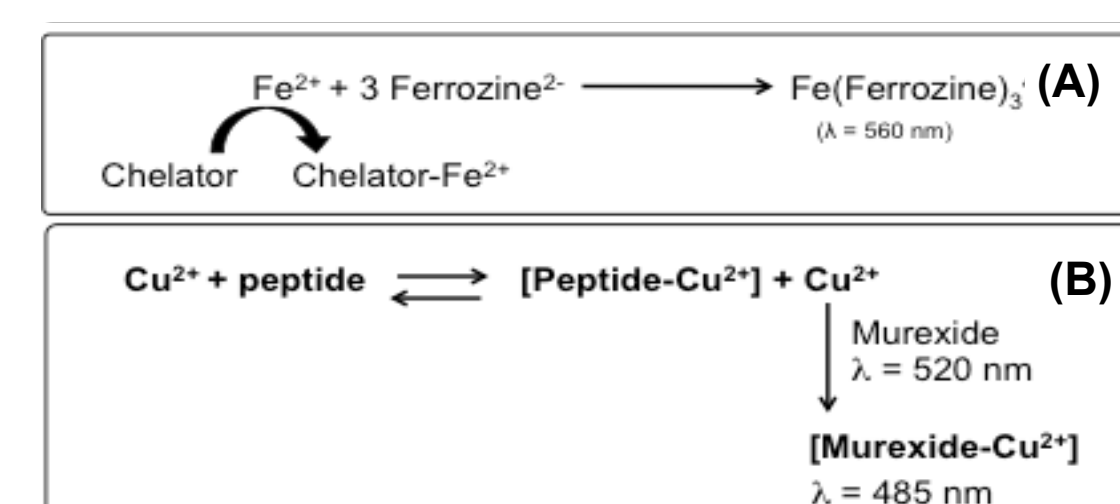


Coordination chemistry



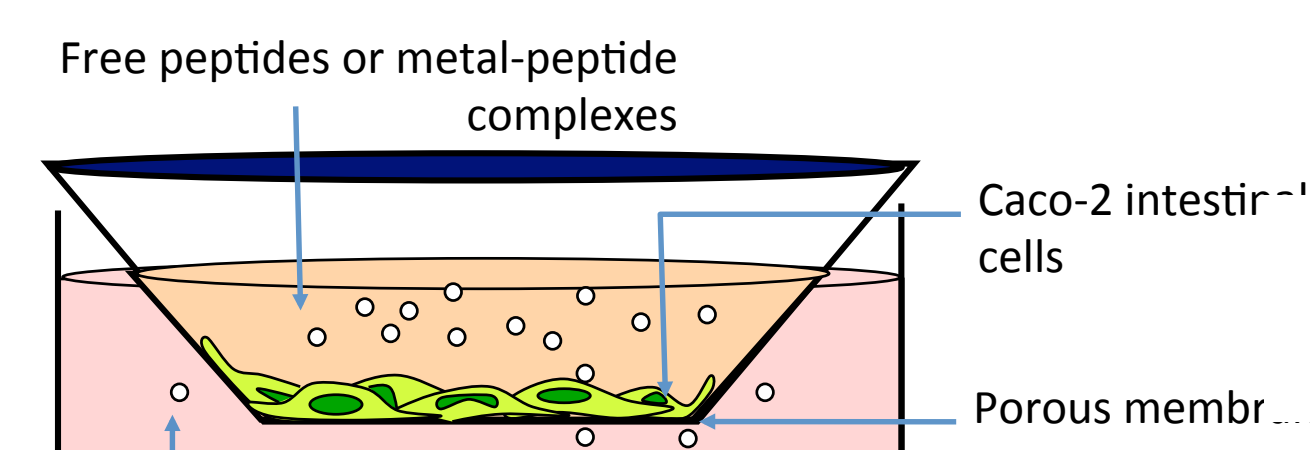
Understanding of peptide-metal complexation, SPR and MS results

Bioactivity tests



Determination of metal chelation capacity using UV-visible spectrophotometry

Health applications



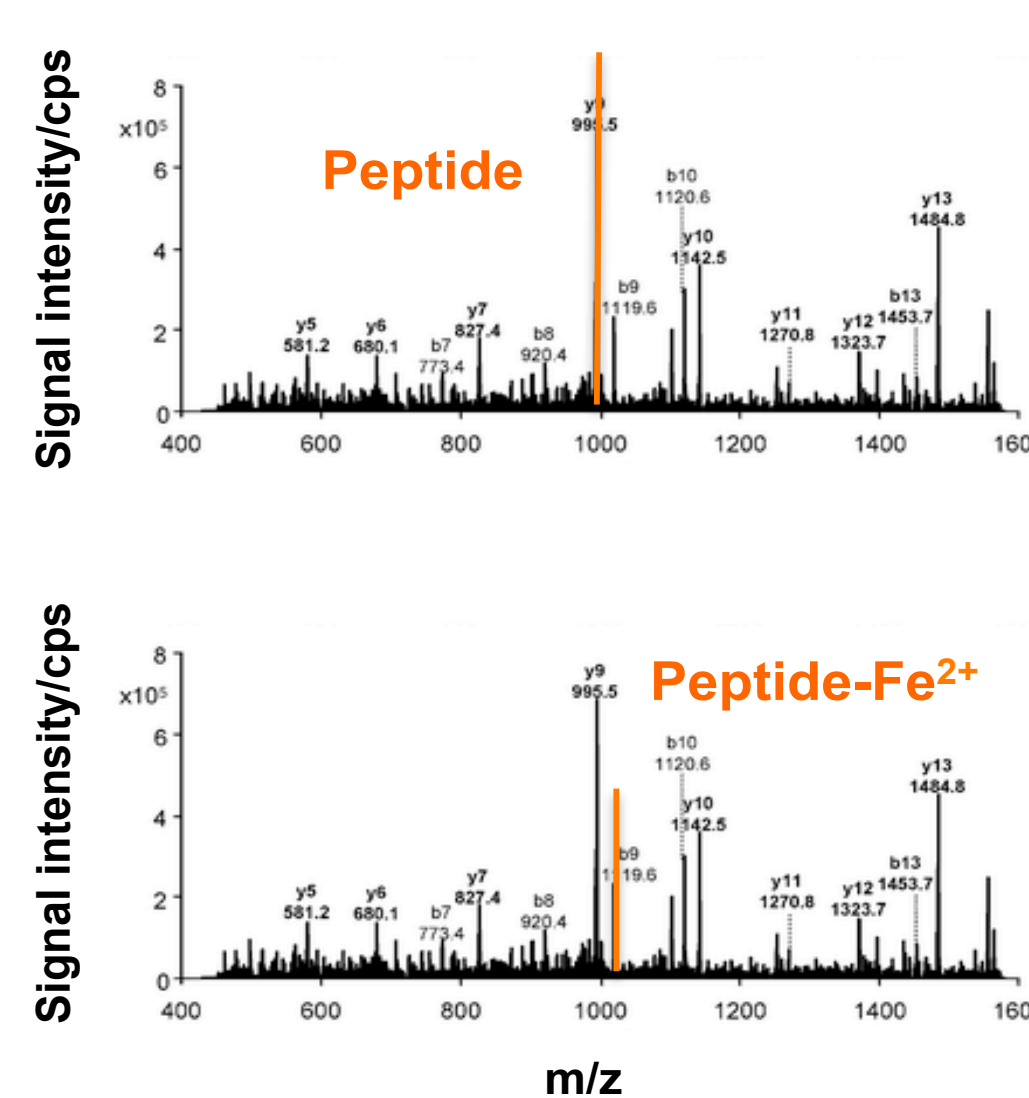
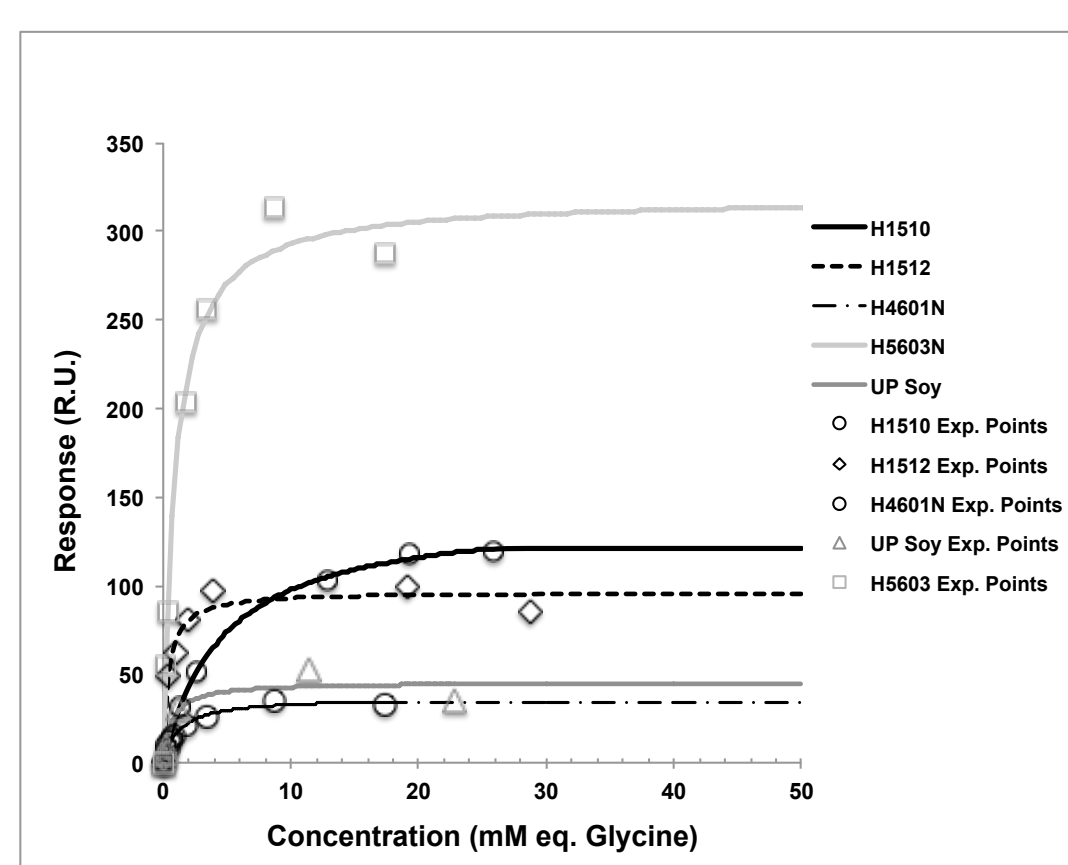
Analysis of absorbed peptides
Quantification of free amine using OPA test
Capillary electrophoresis → peptides separation and identification

Pharmacology study of metal chelating peptides *in cellulo*: determination of their intestinal permeability

Nanoparticles for bioimaging

Some results

Screening of complex mixture of peptides by SPR



Identification by MS of the peptide(s) of interest present in the complex mixture. MS (Orbitrap, ESI, Direct infusion) solution. (A) without Fe²⁺. (B) with Fe²⁺.

Mass shift of +56 u and isotopic pattern = iron-containing ion.

Conclusion and Perspectives

SPR using a M²⁺-chip:

- a miniaturized approach of IMAC-type affinity chromatography
- screening of the most interesting sources of metal-chelating peptides.

Positive correlations evidenced between SPR screening and metal chelation tests using UV-Vis spectrometry on five sources of peptides.

The affinity parameters are determined by SPR on some synthetic model peptides and then used to predict the separation of peptides on IMAC chromatographic column.

Novel chromatography-MS method currently developed:

- Screening of metal-chelating peptides sequences in a given complex mixture
- Methodology successfully set-up on a mixture of synthetic peptides
- Further tests on real hydrolysates

Identified peptides currently assessed for their antioxidant activity by chemical metal chelation and by *in cellulo* tests.

Potential health application

- Treatment of ferriprive anemia related to MICI, or inversely to hematochromatosis
- Anchoring of these chelating peptides on nanoparticles for bioimaging or therapeutic applications.