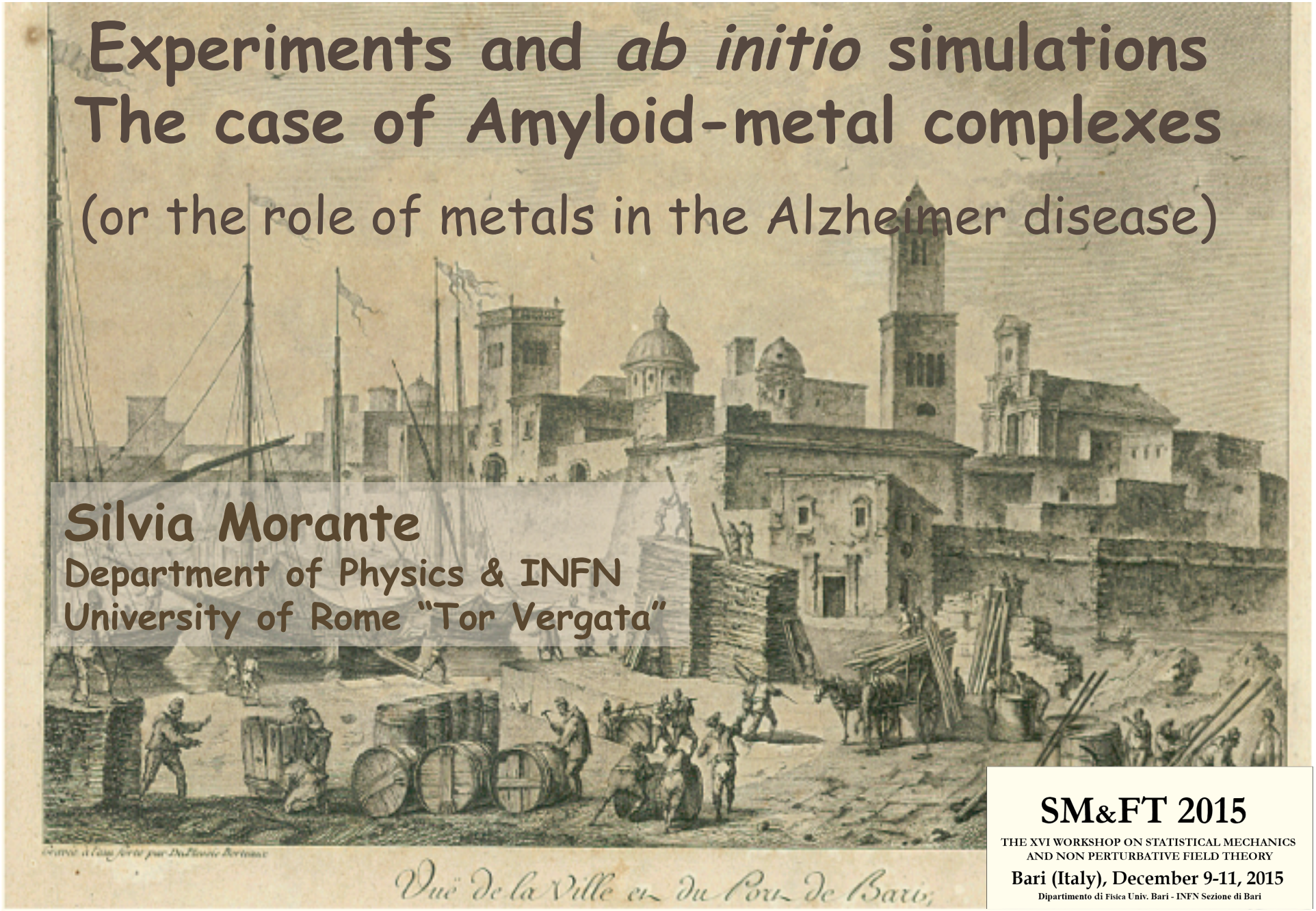
Experiments and *ab initio* simulations
The case of Amyloid-metal complexes

(or the role of metals in the Alzheimer disease)



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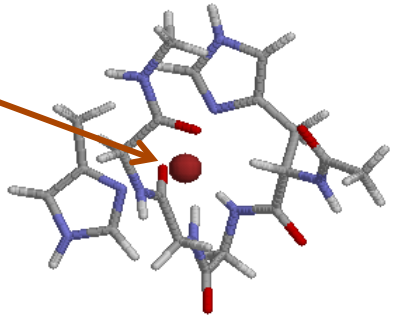
SM&FT 2015
THE XVI WORKSHOP ON STATISTICAL MECHANICS AND NON PERTURBATIVE FIELD THEORY
Bari (Italy), December 9-11, 2015
Dipartimento di Fisica Univ. Bari - INFN Sezione di Bari

XAS - EXAFS

Data analysis

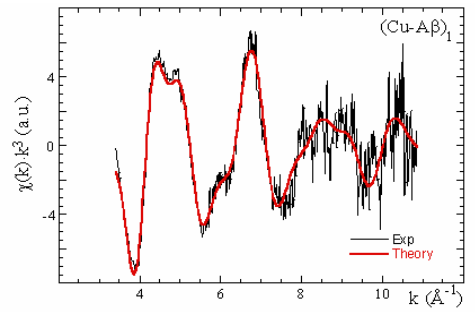
metal



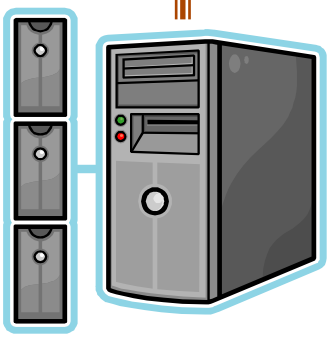
Data refinement

Structural information

Structural model



Ab initio calculations



Outline

Biological challenge

understanding the molecular basis of
Protein Conformational Diseases (PCD's)
among which the Alzheimer disease

- misfolding and aggregation
- the Aβ-peptide
- metal ions and brain

Scientific instruments

focused experiments + numerical approaches

- XAS spectroscopy
- classical and *ab initio* molecular dynamics

Results

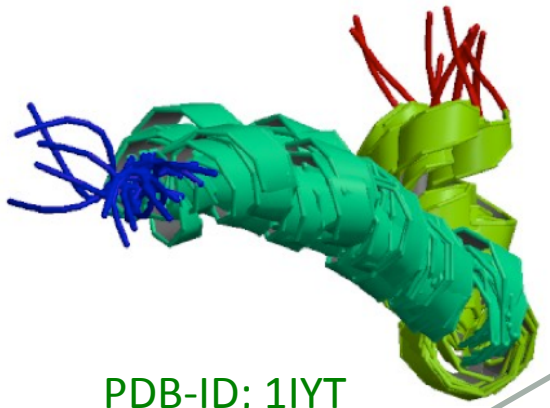
unveiling the (relevant) role of metal ions

Protein Conformational Diseases (PCD's)

Disease	Misfolded Protein
Alzheimer's Disease	Aβ-peptide
Transmissible Spongiform Encephalopathies (TSE)	Prion protein
Parkinson's disease	α-synuclein
Type II diabetes	Amylin
Thyroid carcinoma	Procalcitonin
Atrial amyloidosis	Atrial natriuretic factor
Amyotrophic lateral sclerosis	Superoxide dismutase
Huntington disease	Glutamine
Primary systemic amyloidosis	Ig light chains
Secondary systemic amyloidosis	Serum amyloid A
Senile systemic amyloidosis	Transthyretin (wild tipe)
Familial amyloidotic polyneuropathy I	Transthyretin (mutant)
Familial amyloidotic polyneuropathy II	Apolipoprotein A1
Familial Mediterranean fever	Serum amyloid A
Hemodialysis-related amyloidosis	b2-microglobulin
Finnish hereditary systemic amyloidosis	Gelsolin (mutant)
Lysozyme systemic amyloidosis	Lisozime
Insulin-related amyloidosis	Insulin
Fibrinogen systemic amyloidosis	Fibrinogen α chain

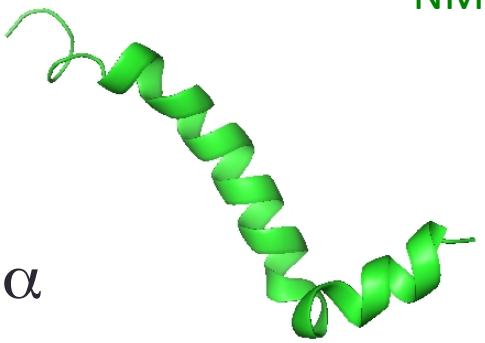
The Aβ-peptide aggregation

Crescenzi et al. (2002) Eur. J. Biochem. 269, 5642–5648



PDB-ID; 1IYT

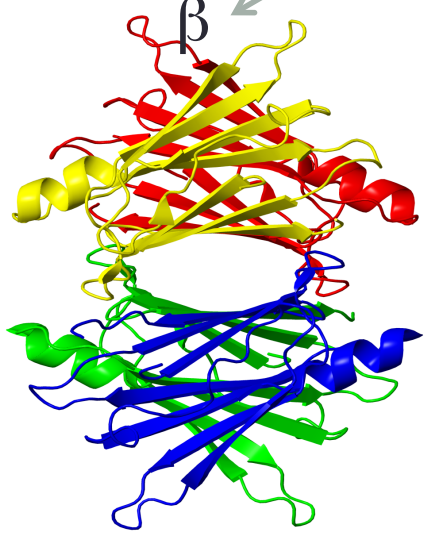
NMR



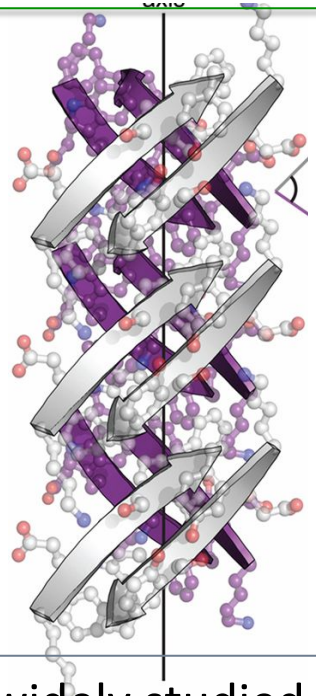
α

β

Oligomer

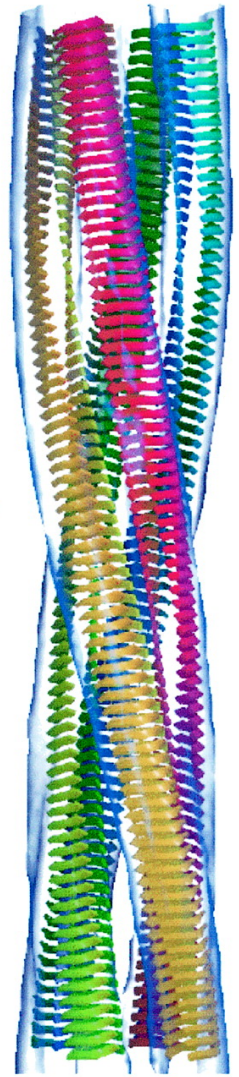


Proto-filament



Cross-beta fibril

⦸70-150 Å



Amyloid-like aggregates are now widely studied also in **non-medical** contexts
food science, pharmacology, nanomaterials …

Metal ions are essential cell components

They can easily and quickly form complexes with enzymes and other biological molecules

They are involved in a wide range of biological processes

≈ ¼ ÷ ⅓ of all proteins are estimated to require metals to function

Metalloproteins have many different functions in cells

- storage and transport
- enzymatic activity
- signalling and transduction
- …
- …

BUT METALS HIGH REACTIVITY CAN EASILY BECOME HIGHLY TOXIC FOR THE CELL

Metals should cross the blood-brain barrier

Central Nervous System

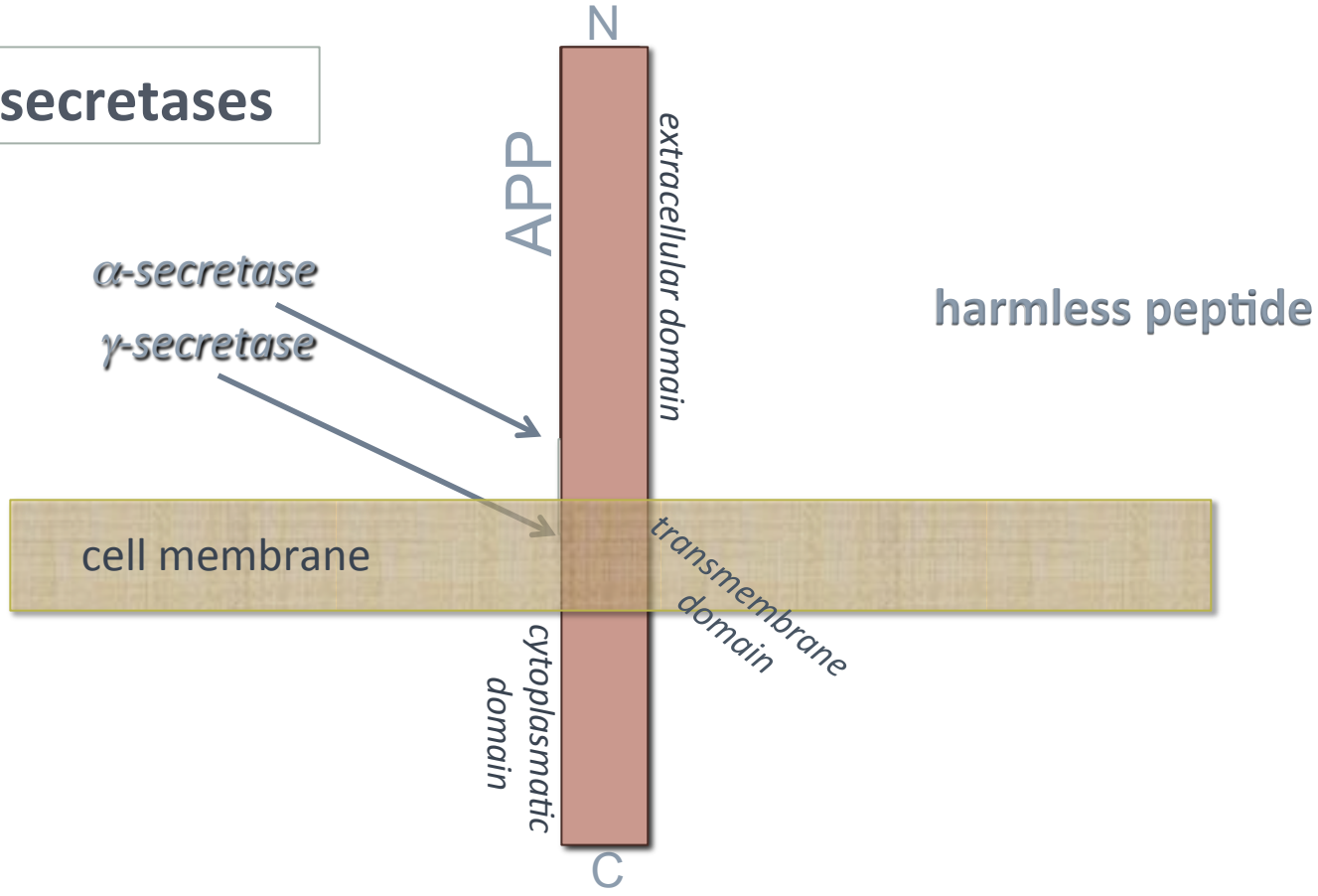
- Metals are **essential** for neurochemical activity
- **Fe**, **Cu** and **Zn** are fairly abundant in **grey matter**
- during neuro-transmission processes, high concentrations of Zn (~300 μM) and Cu (~30 μM) are normally released
- **Fe**, **Cu** and **Zn** are continuously **trafficked** whithin the cell and also into and out of it
- Metals **deficiency** may cause **dysfunctionality and** metal **excess may** lead to **toxicity**
- **Failure of metal trafficking (dyshomeostasis)** is known to occur both in AD and in Parkinson diseases

In presence of dyshomeostasis, metal ions may

- interfere with normal nervous cells activities
- disrupt brain electrochemical balance
- •••
- **promote protein misfolding and aggregation?**

The amyloid β-peptide

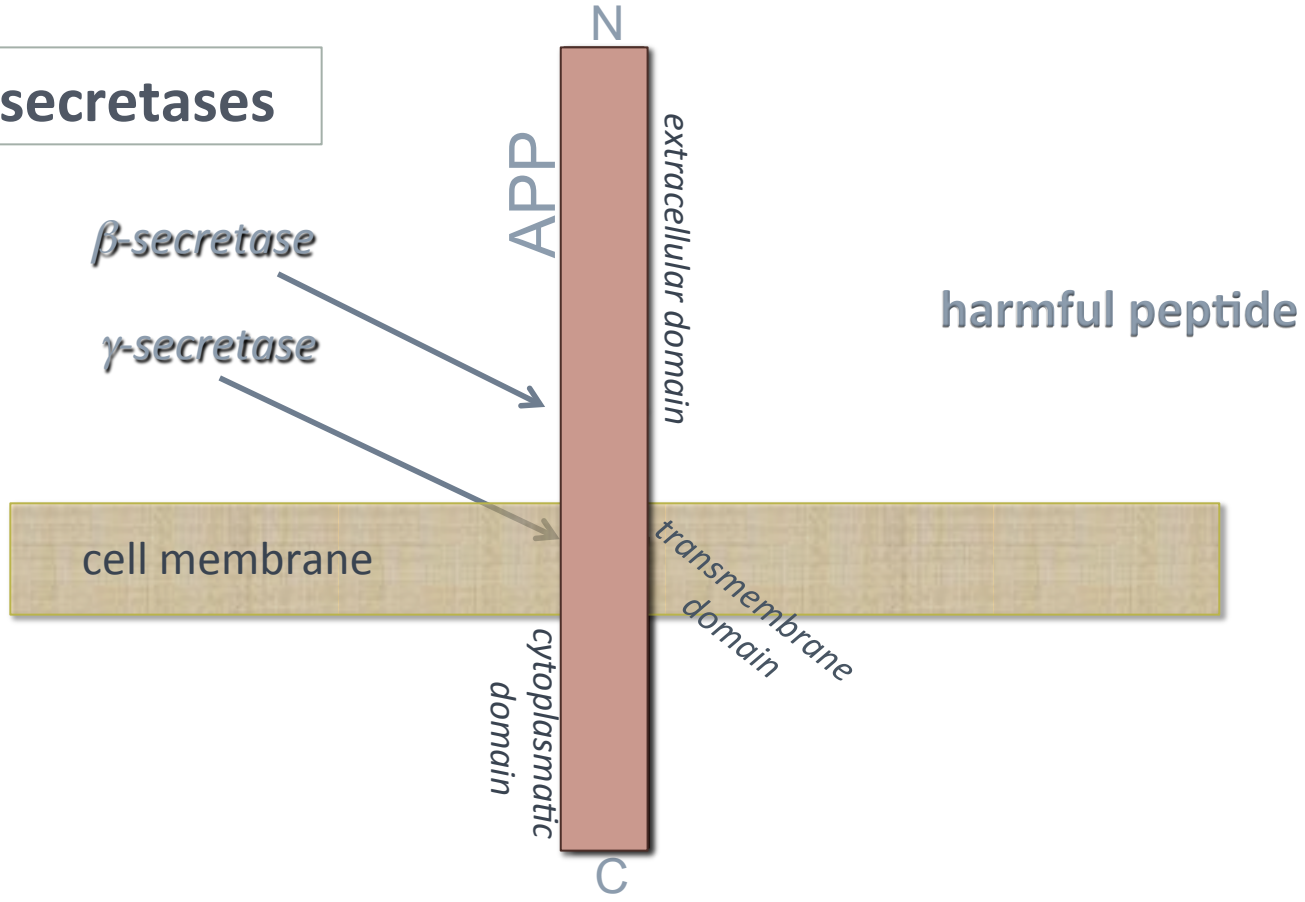
α-, β- and γ-secretases



APP: transmembrane 770 a.a.'s protein

The amyloid β-peptide

α-, β- and γ-secretases



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The amyloid β-peptide

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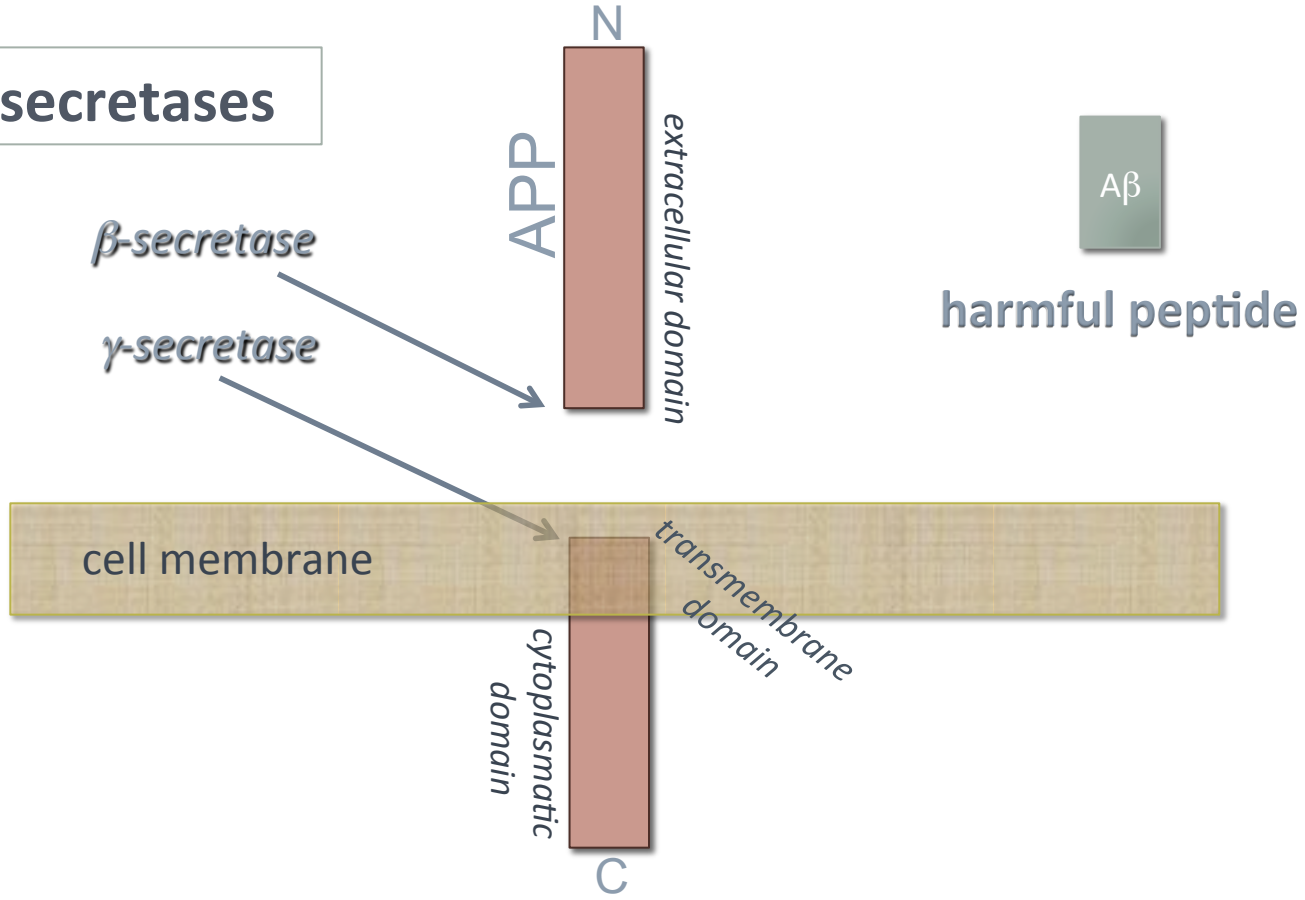
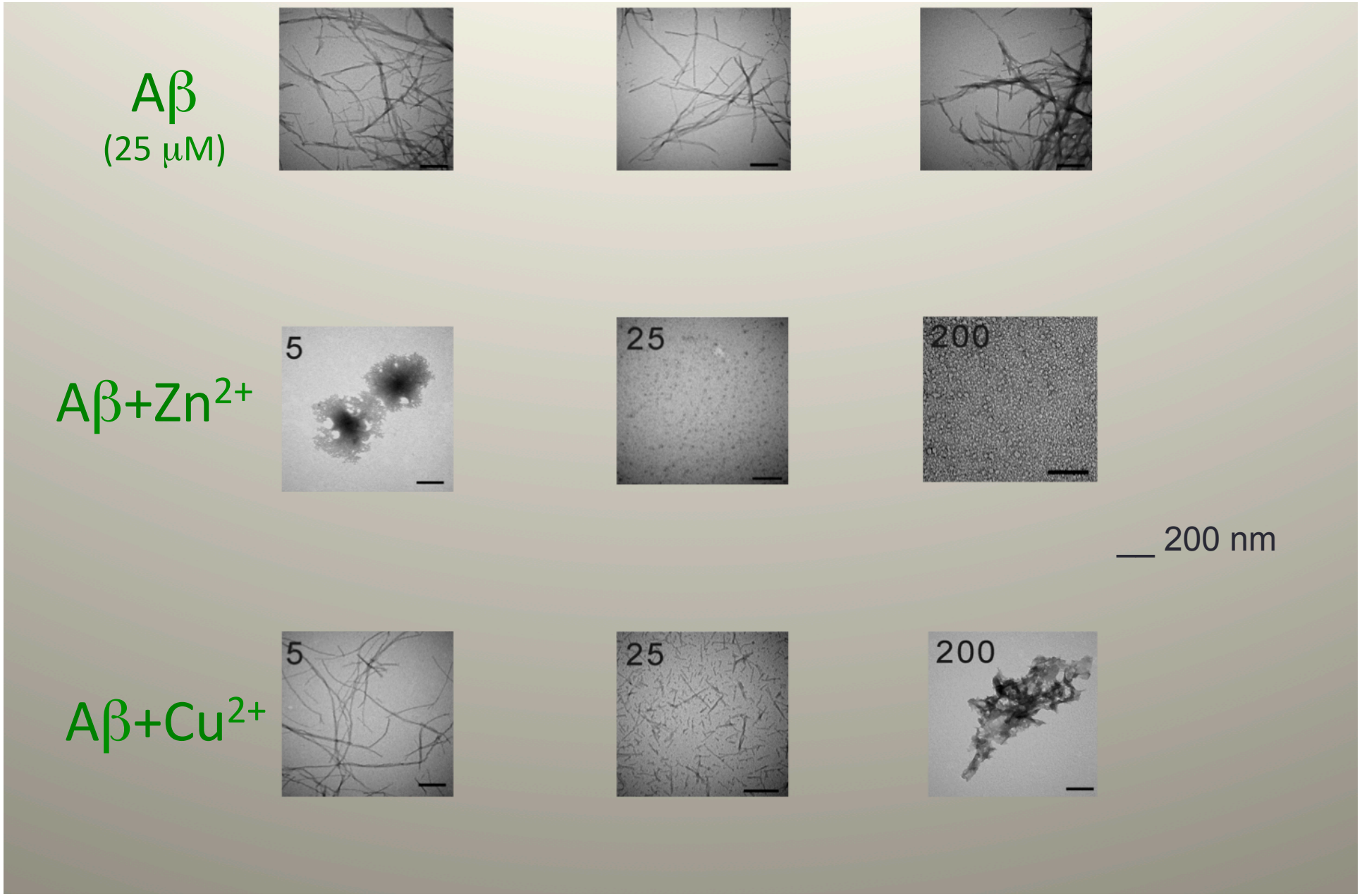


672 DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV 711

$A\beta_{1\text{-}40}$

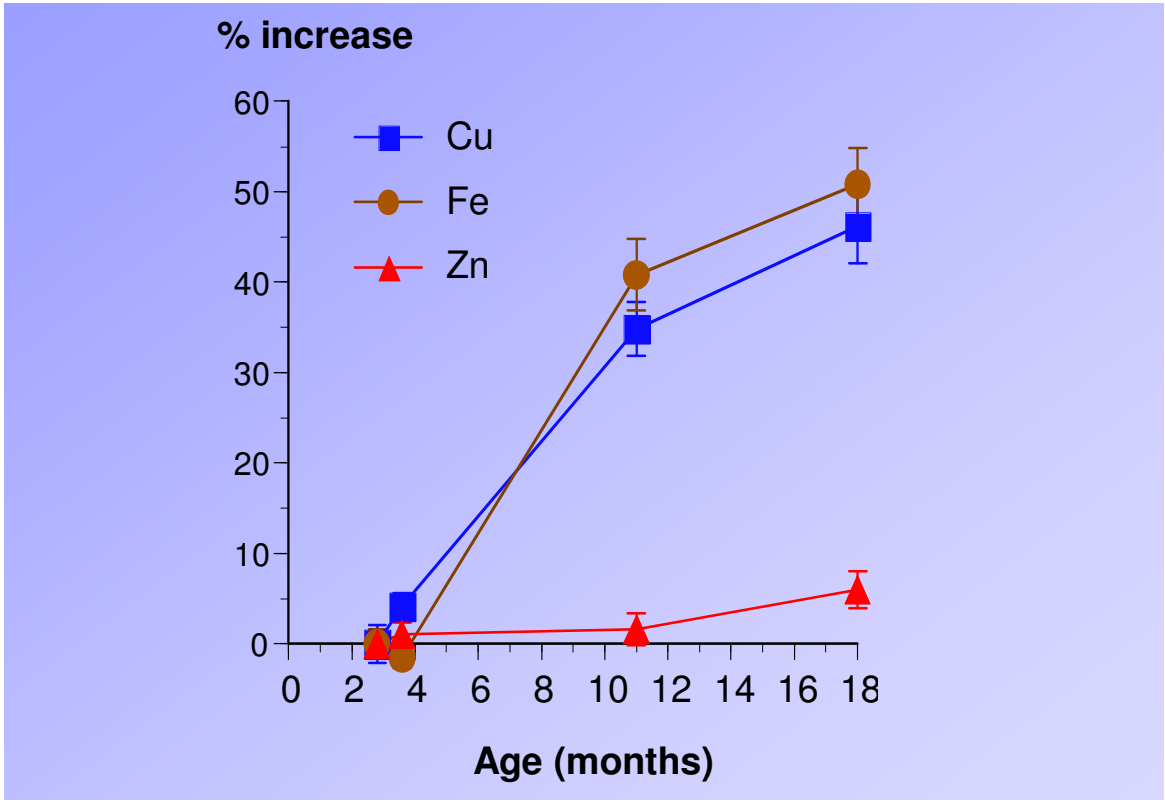
Metals affect Aβ-peptide aggregation



Chen et al. *J. Biol. Chem. 2011, 286:9646-9656.*

Normal aging and increased brain metal level

Maynard et al. (2002) *JBC*, 277: 44670



The triggering event is possibly metal dyshomeostasis

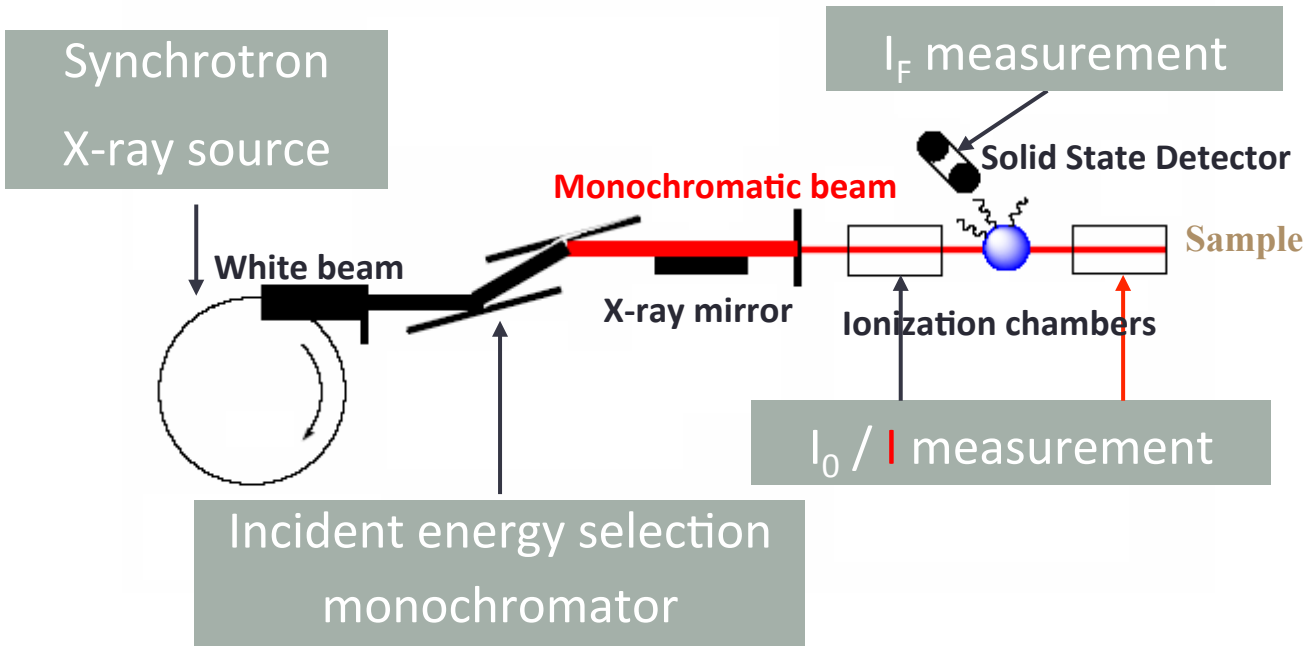
- The number of people aged 60 and over has doubled since 1980
- The number of people aged 80 years or older will be almost quadrupled to 395 millions between 2000 and 2050
- The risk of dementia (like Alzheimer disease) rises sharply with age with an estimated 25-30% of people aged 85 or older having some degree of cognitive decline.

Horizon 2020: improve healthy ageing

The challenge is:
understand, control and (possibly) stop PCD's evolution

X-ray Absorption Spectroscopy - XAS

Photons are produced by synchrotrons



X-ray Absorption Spectroscopy - XAS

- It may be used to study samples in any aggregation state (not necessarily crystallized, frozen or transparent)

  proteins can be studied in physiological or controlled solution condition

- There are no selection rules for absorption

  a XAS spectrum can always be measured

- Spectra registered in a relatively short time (few min's to few msec's)

  limited radiation damage

- High chemical selectivity -

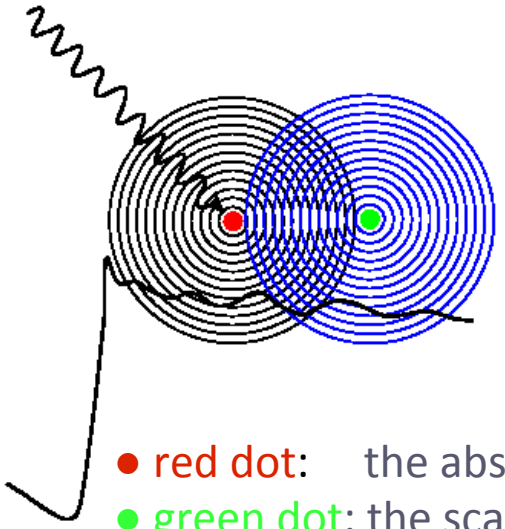
  local atomic environment around a selected absorber

  1s ionization energy (K-edge)

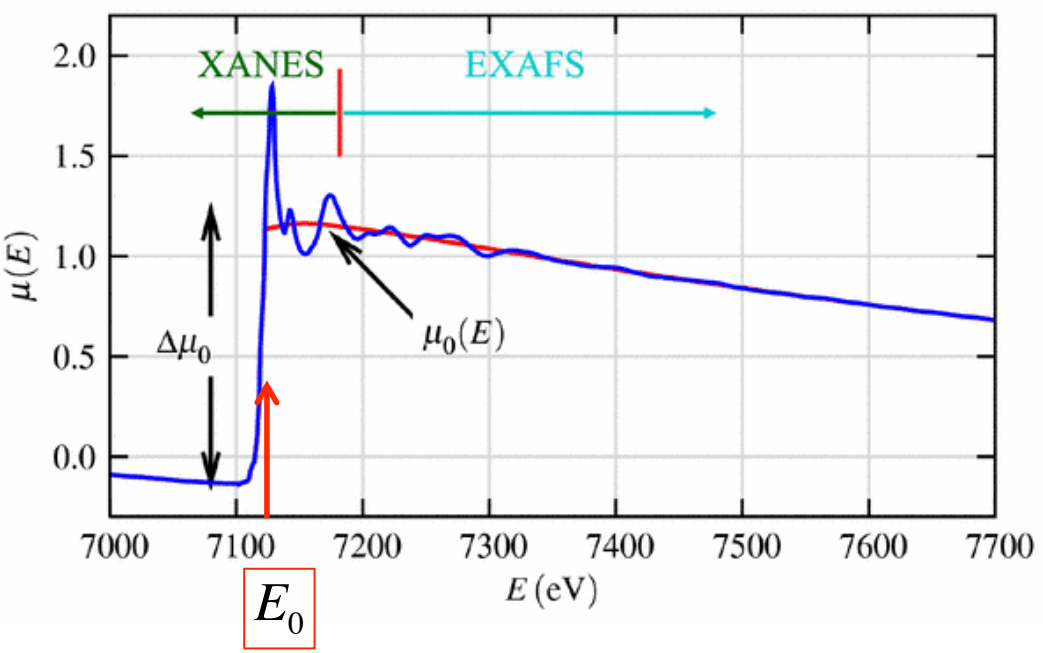
  $_{26}$Fe ≈ 7112 eV ; $_{29}$Cu ≈ 8993 eV; $_{30}$Zn ≈ 9673 eV

XAS of a multi atomic system

Absorption coefficient, μ, behaviour as a function of emitted photon energy



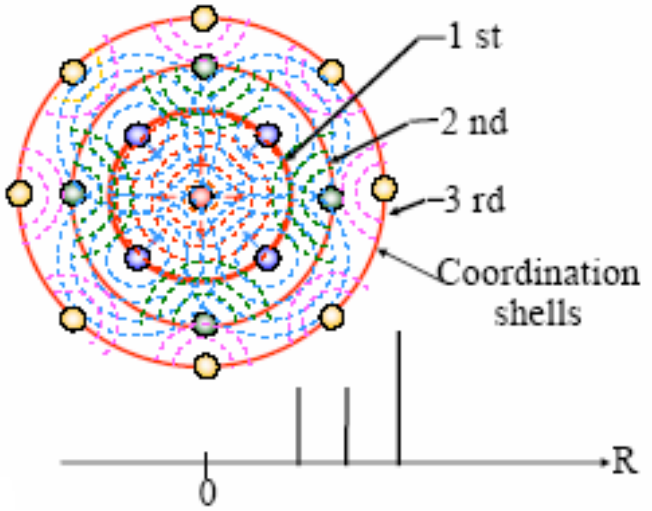
- red dot: the absorber
- green dot: the scatterer



$\hbar\omega >> E_0$ Single Scattering approximation

$$\chi(k) = \frac{\sigma_a - \sigma_0}{\sigma_0}$$

$$\chi(k) = S_0^2 \sum_i \frac{N_i}{kR_i^2} |f_i(k,\pi)| e^{-2k^2\sigma_i^2} e^{-2R_i/\lambda(k)} \sin\left[2kR_i + \Phi_i(k)\right]$$



Car Parrinello Molecular Dynamics based on ***Density Functional Theory*** and ***Classical MD***

CPMD DFT

Density Functional Theory

The electronic energy is a functional of the electronic density, $n(\vec{r})$

$$E_{DFT} = \Gamma_{HK}\left[n(\vec{r})\right] + \int n(\vec{r}) V(\vec{r}) d\vec{r}$$

that is at its minimum when *n(r)* is the ground-state electronic density.

The latter is determined by solving the Kohn-Sham equations

$$\left[-\frac{\hbar^2}{2m}\nabla^2 + V(\vec{r}) + V_H(\vec{r}) + V_{xc}(\vec{r})\right]\psi_i(\vec{r}) = \varepsilon_i \psi_i(\vec{r})$$

$$V_H(\vec{r}) = e^2 \int \frac{n(\vec{r}')}{|\vec{r} - \vec{r}'|} d\vec{r}' \quad \text{Hartree potential}$$

$V_{xc}(\vec{r})$ Exchange-correlation potential

$$n(\vec{r}) = (2)\sum_i |\psi_i(\vec{r})|^2 \qquad \langle \psi_i | \psi_j \rangle = \delta_{ij}$$

The simulation strategy

Quantum-ESPRESSO package
www.quantum-espresso.org

1 "prepare" the initial state of the system (classical MD)
   type of atoms: number of electrons and bands → pseudopotentials
   atomic coordinates
   dimension of system cell
   cut-offs → plane wave expansion
   FFT grid spacing
2 electronic minimization (Steepest Descent, SD) with fixed ions → ground state
3 ionic positions minimization (SD or damped dynamics) → to relax the system
4 start the Car-Parrinello dynamics

NOTE: in order to reach the desired temperature
ions are coupled to Nosè-Hoover thermostat

QM key feature: bonds not imposed (like in MD)
but derived from solving the Schroedinger equation

1. Zn-ion-induced aggregation pattern

XAS allows to identify the Zn^{2+}-binding site sequence in $A\beta_{1-40}$

1 6 13 14 40

DAEFR**H**DSGYEV**HH**QKLVFFAEDVGSNKGAIIGLMVGGVV

Besides the whole peptide, we measured the XAS spectra of the following samples

Zn^{2+}- $A\beta_{1-16}$	minimal fragment containing His_6, His_{13}, and His_{14}, suggested to be involved in metal binding
Zn^{2+}- $A\beta_{17-40}$	complementary to the previous sequence: no His is present
Zn^{2+}- $A\beta_{1-28}$	besides the three His's, it contains a long hydrophobic region believed to be relevant in the aggregation process

V. Minicozzi … S.M. et al., *J Biol Chem* (2008) **283**: 10784
F. Stellato … S.M. et al., *Eur Biophys J* (2006) **35**: 340

1° Zn-ion-induced aggregation pattern

Synopsis of XAS results: comparing spectra

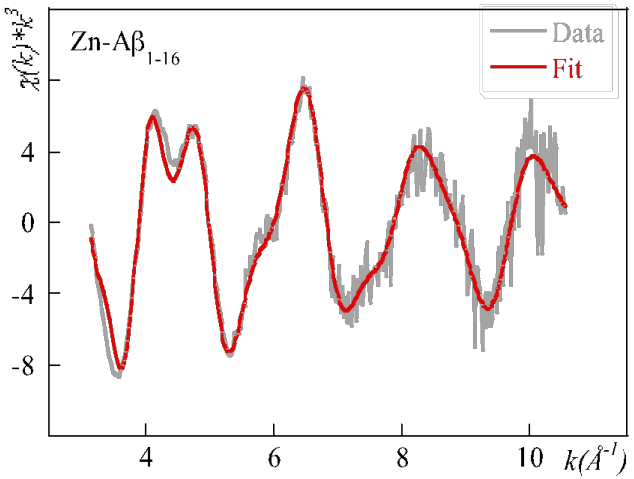
Zn^{2+}- $A\beta_{17-40}$ = Zn^{2+}- buffer → Zn^{2+} is unable to bind $A\beta_{17-40}$

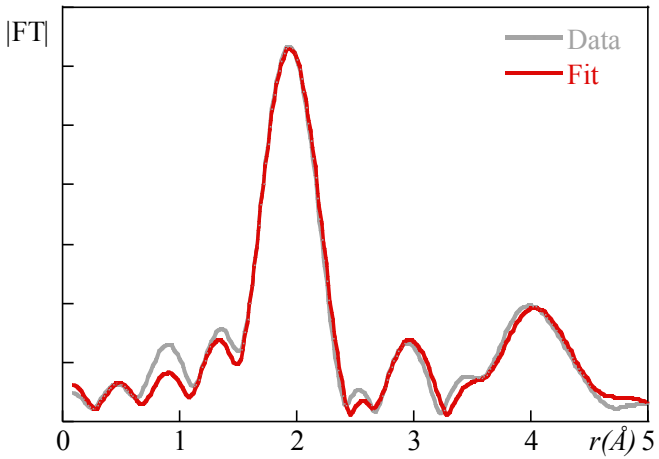
Zn^{2+}- $A\beta_{1-16}$ = Zn^{2+}- $A\beta_{1-28}$ = Zn^{2+}- $A\beta_{1-40}$ → Zn^{2+} always bind to the first 16 a.a. fragment

1 DAEFR**H**DSGYEV**HH**QKLVFFAEDVGSNKGAIIGLMVGGVV 40
6 1314

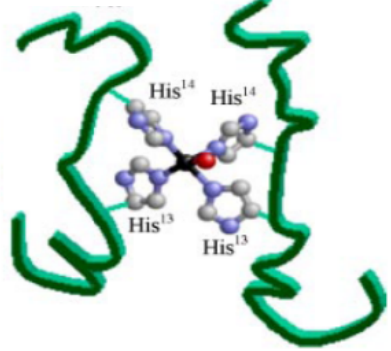
Zn^{2+} always binds with the same (*very crowded*) binding structure to the first 16 a.a.'s

Zn^{2+}-$A\beta_{1-16}$





Zn^{2+}-$A\beta_{1-16}$



- 4 histidines
- 1 water

1° Zn-ions-induced aggregation pattern

Models and *ab-initio* calculations

Question:

which structure stabilizes this (very crowded), 4 His's - Zn^{2+} coordination?

- XAS: structure information only within 5-6 Å from the absorber
- *ab-initio* simulations: structural information up to a longer distance

Model building

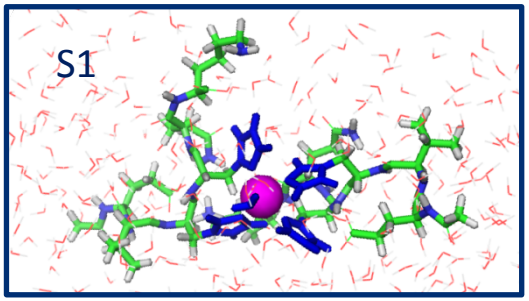
1. XAS → 4 His's coordination → two $A\beta_{1\text{-}16}$ –peptides (denoted A and B) with a single Zn atom bridging $N_\delta(His_{14}(A))$ and $N_\delta(His_{14}(B))$

2. minim + Monte Carlo Random Walk (MCRW) ("bad" contact discarded) → only configurations that fulfill structural constraints of point 1. are retained

3. $A\beta_{1\text{-}16}$ truncated to $A\beta_{11\text{-}16}$ and put in a cell with periodic conditions filled with TIP3P water molecules

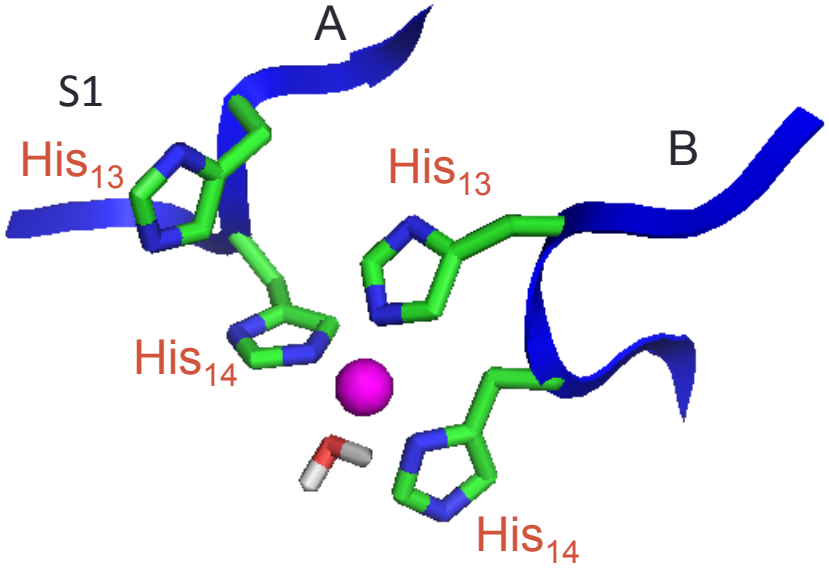
4. Four trial binding geometries are chosen for *ab initio* computations

1° Zn-ion-induced aggregation pattern



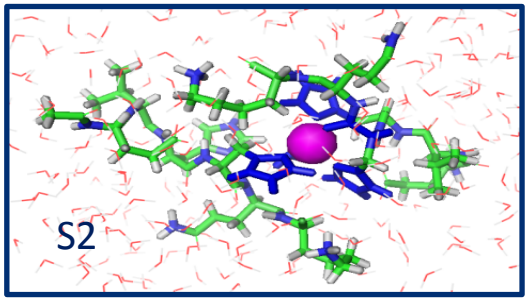
Besides the two His_{14}, also the two His_{13} happen to be within 3 Å from Zn

Name	Starting configuration
S1	Zn: $4N_{His} + 1O_W$



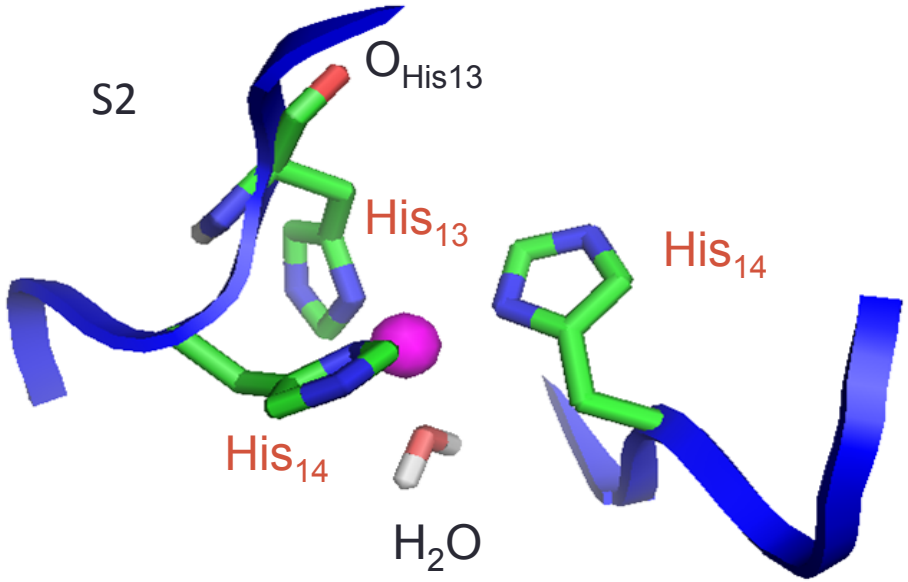
Name	Final configuration
S1	Zn: $3N_{His} + 1O_W$

1° Zn-ion-induced aggregation pattern



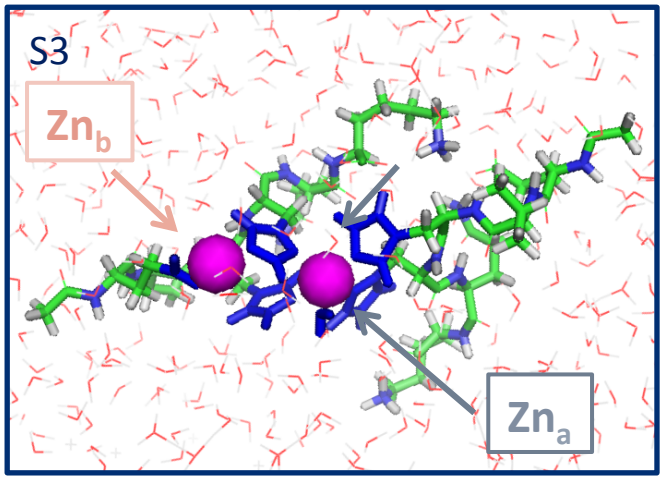
Only three His's. One of the His's is also through a main chain oxygen

Name	Starting configuration
S2	Zn: $3N_{His}$ +$1O_{His}$ + $1O_W$



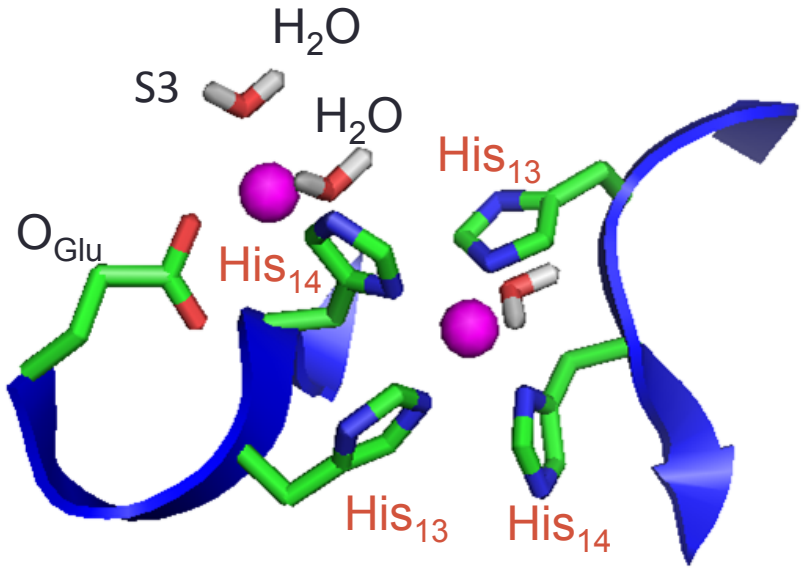
Name	Final configuration
S2	Zn: $3N_{His}$ + $1O_W$

1° Zn-ion-induced aggregation pattern



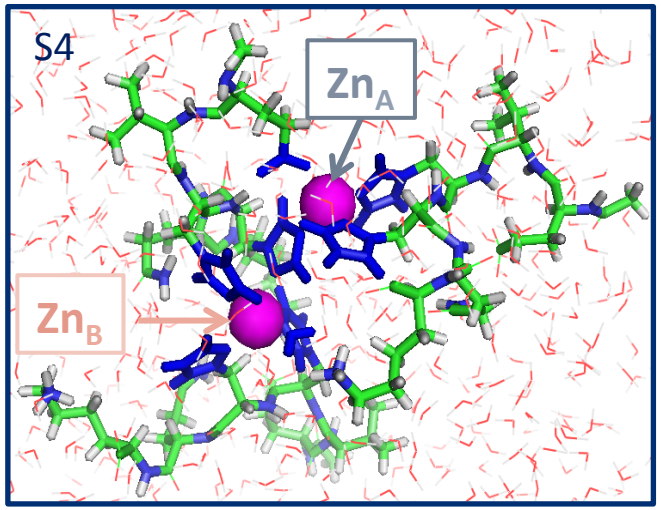
modified S1: the Hε of a His_{14} is substituted by a second Zn

Name	Starting configuration
S3	Zn_a: $4N_{His} + 1O_W$ Zn_b: $1N_{His} + 2O_{Glu}$



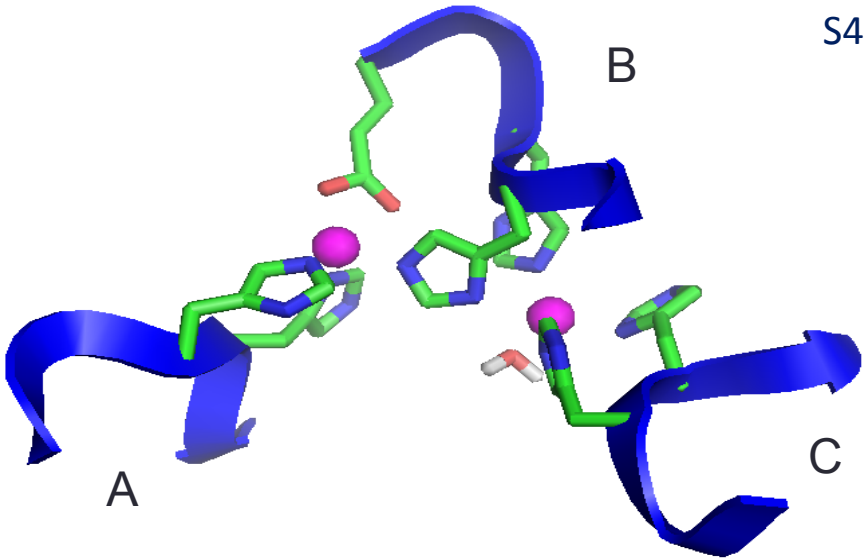
Name	Final configuration
S3	Zn_a: $4N_{His} + 1O_W$ Zn_b: $1N_{His} + 1O_{Glu} + 2O_W$

1° Zn-ion-induced aggregation pattern



modified S3: a third peptide is bound to the second Zn

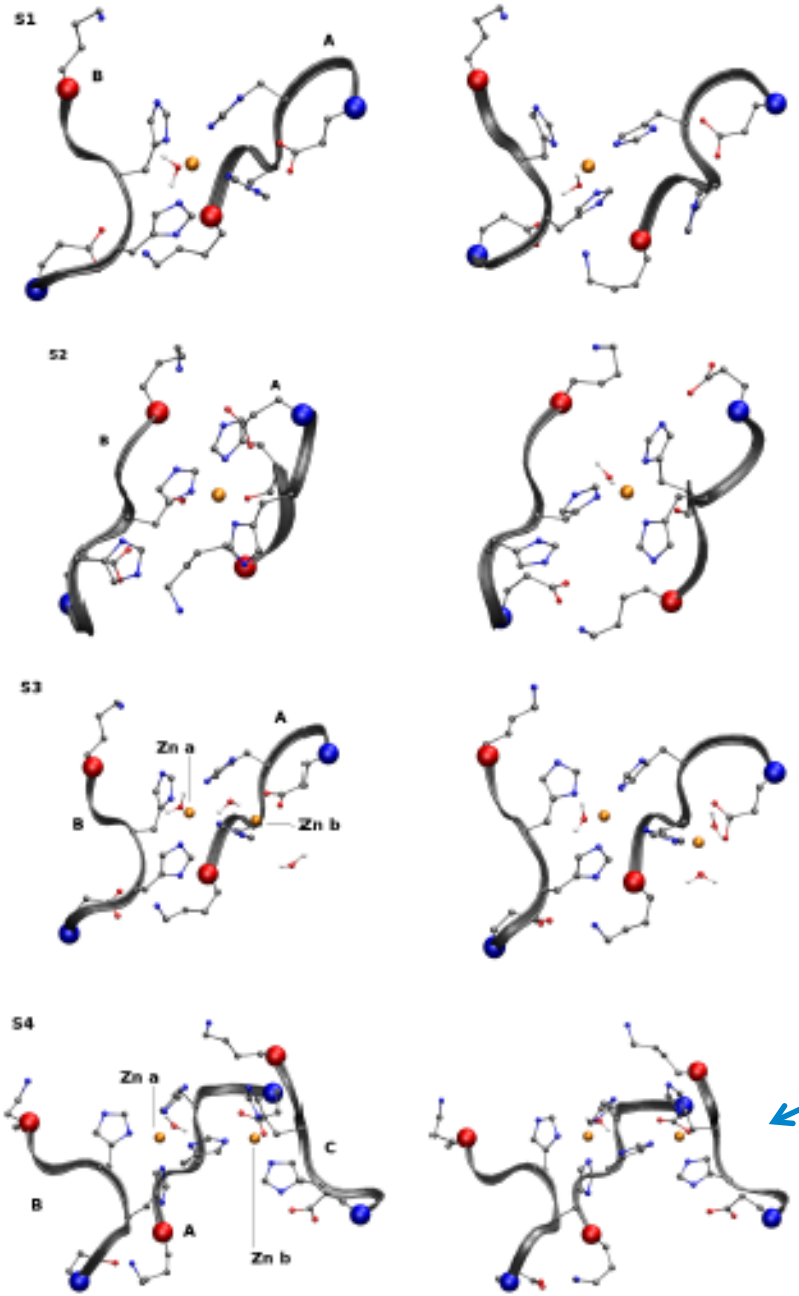
Name	Starting configuration
S4	Zn_a: $4N_{His} + 1O_W$ Zn_b: $3N_{His} + 1O_{Glu}$



Name	Final configuration
S4	Zn_a: $4N_{His} + 1O_W$ Zn_b: $3N_{His} + 1O_{Glu}$

1° Zn-ion-induced aggregation pattern

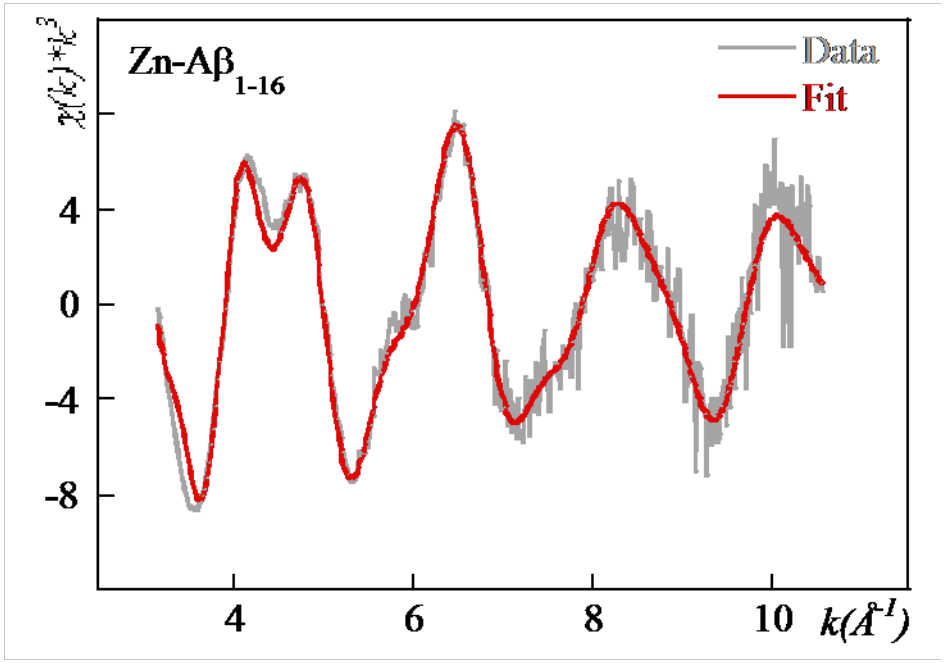
P. Giannozzi, K. Jansen, G. La Penna, V.Minicazzi, S. M., G.C. Rossi, F. Stellato. *Metallomics* (2012)



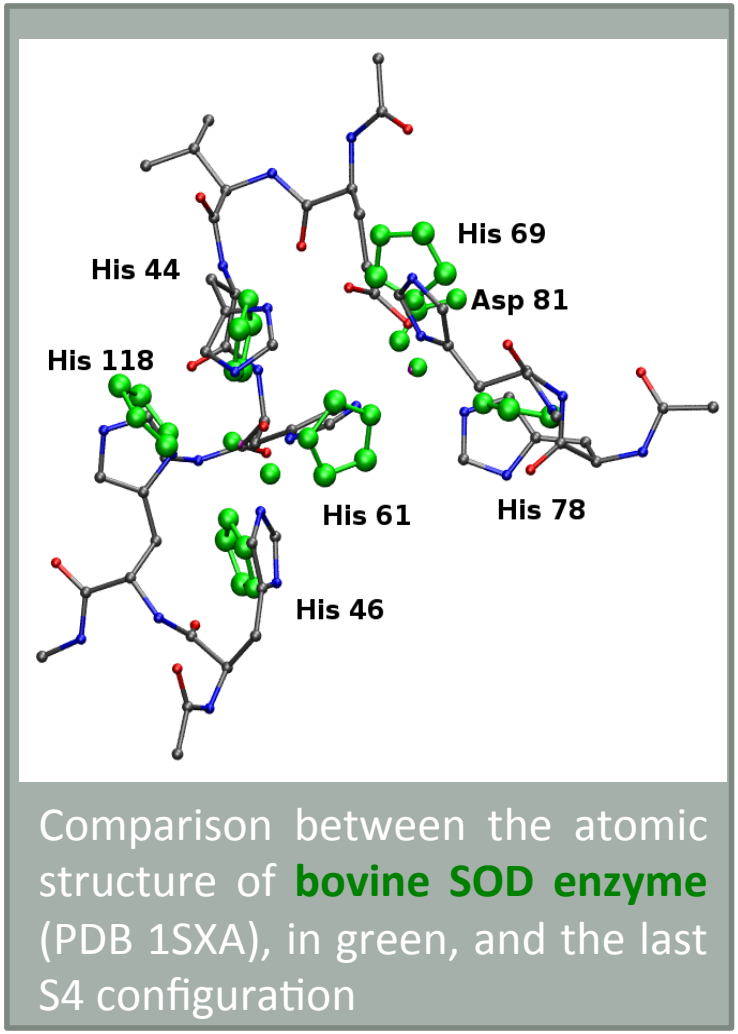
Zn site structures in the four models

Our favorite model

1° Zn-ion-induced aggregation pattern



Comparing the experimental XAS spectrum with the theoretical one of the S4 model



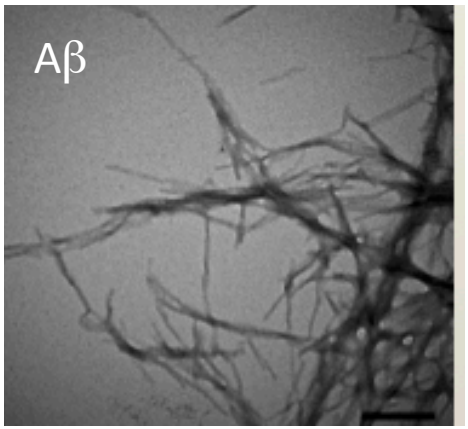
Comparison between the atomic structure of **bovine SOD enzyme** (PDB 1SXA), in green, and the last S4 configuration

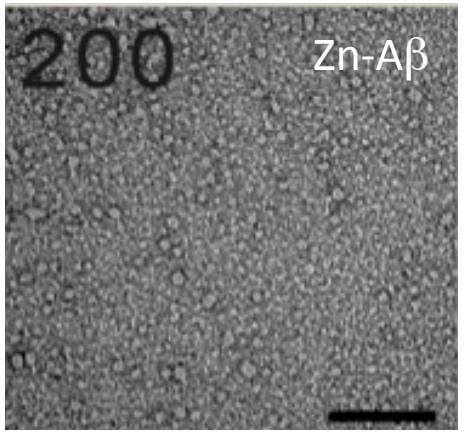
His's "crowding" in SOD enzymes is helped by the presence of two metal ions bridged by the His imidazole (in the unusual form of an imidazolate anion)

1° Zn-ion-induced aggregation pattern

Conclusions

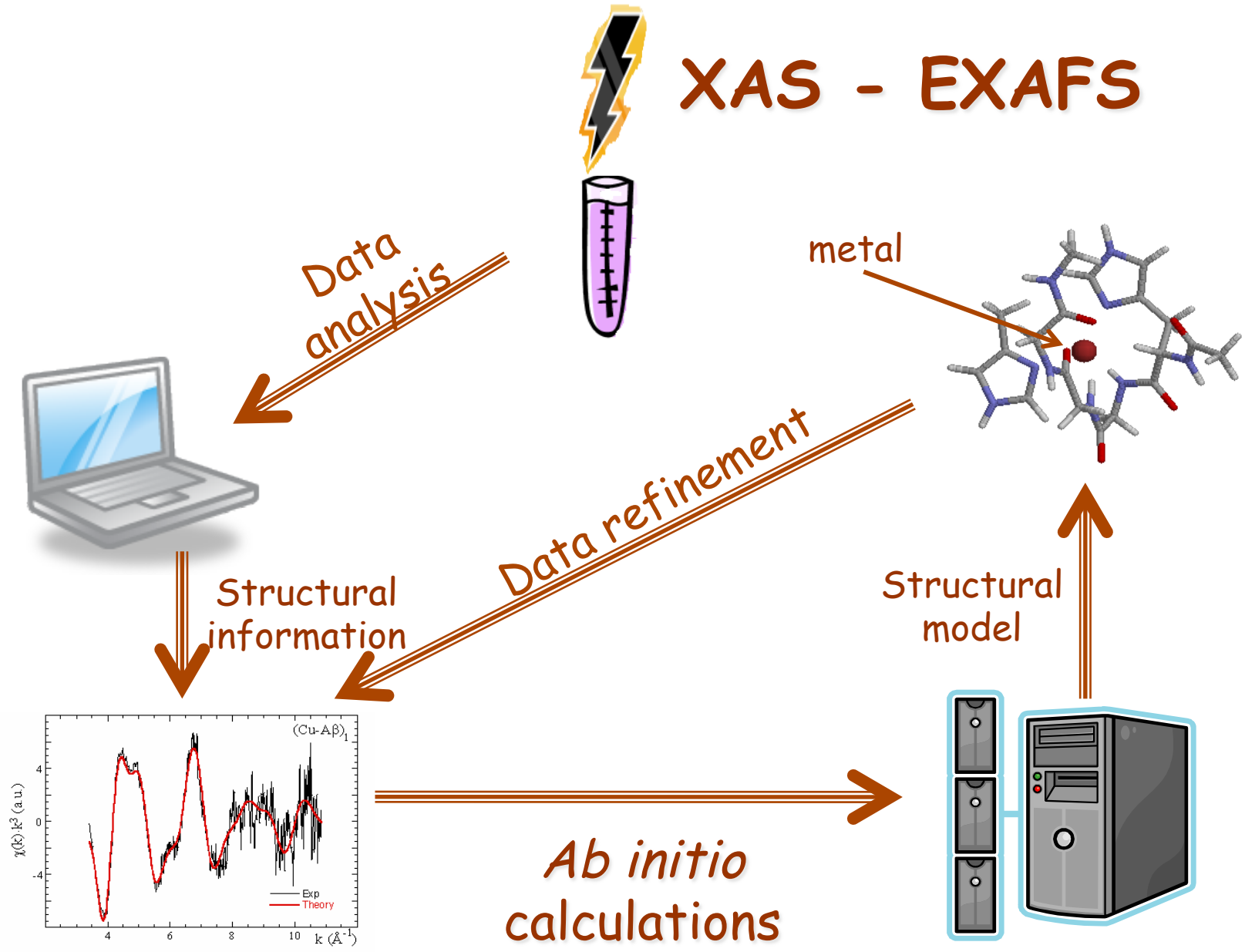
- Zn promotes oligopeptides formation





- XAS cannot discriminate among different structural models, but
- empirical and first-principle simulations → stable Zn-His-Zn structure (≈ SOD)

General conclusions



Further examples

2° - Cu^{2+}-Zn^{2+} cross-modulation in Aβ binding

XAS measurements: Aβ + Zn and/or Cu → they compete for the binding

De Santis, … S.M. *J. Phys. Chem. B* (2015) accepted

It would be of the utmost importance to know what happens to the whole peptide structure as a result of metal binding competition …

… we are planning to investigate this problem via MD and CPMD simulations

3° - Beta-sheet-breakers BSB's: targeting Aβ fibrils

MD of Aβ + BSB's that inhibit or delay Aβ conformational transition →
we devised a new, more efficient, BSB

It would be of great interest to study how BSB's affect metal-Aβ interaction …

… we are planning XAS of Aβ + BSB's in the presence of Cu and/or Zn

V. Minicozzi, …S. M. *J. Biol. Chem.* (2014) 2**89**, 11242-11252

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Thank you