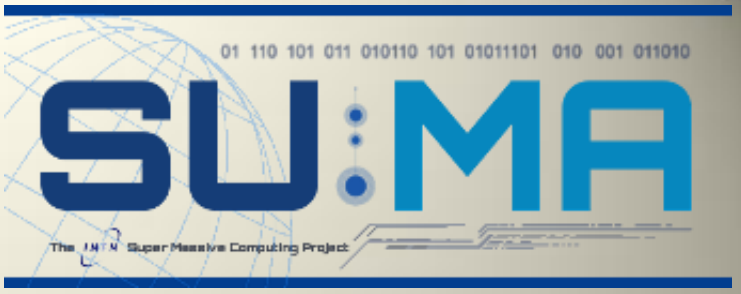
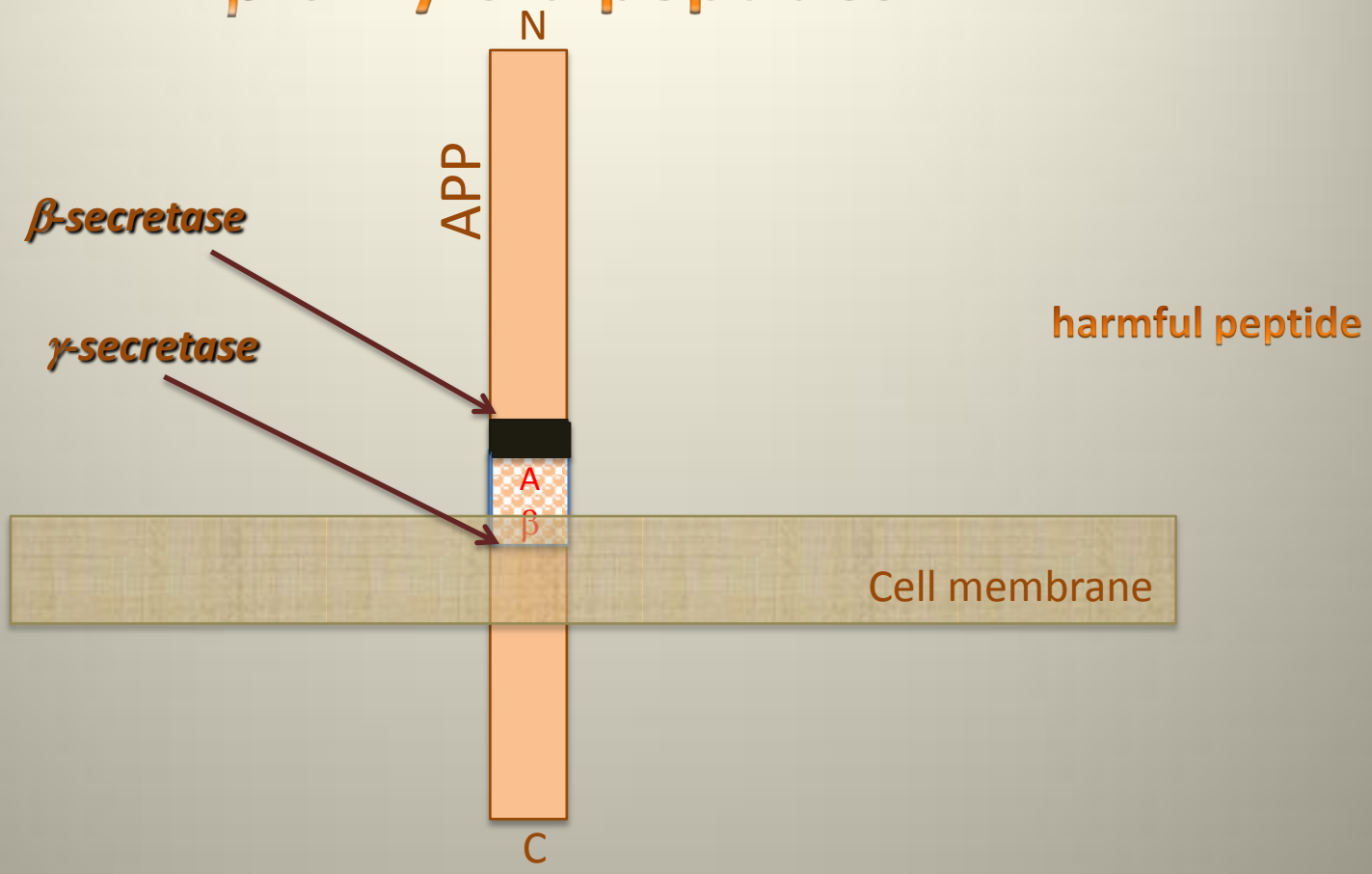


Zn induced structural aggregation patterns of β-amyloid peptides by first-principle simulations and XAS measurements

P Giannozzi, K Jansen, G La Penna, V Minicozzi, S Morante, GC Rossi, F Stellato

β-amyloid peptides



APP: transmembrane 770 a.a.'s protein

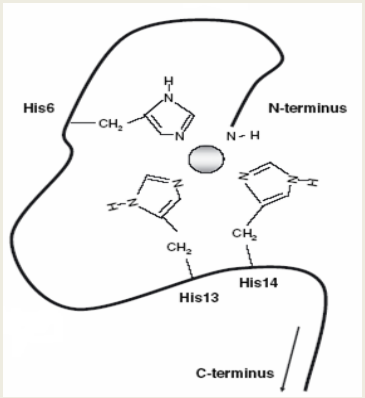
672 699 710 712 723

…KMDAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIATVIVITLVMLKKKQY…

β cut

γ cuts

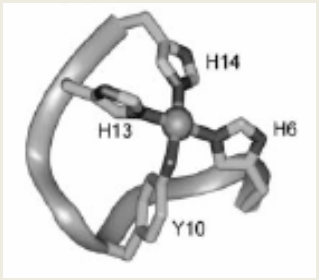
Cu^{2+}

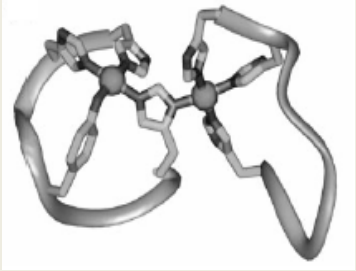


NMR

J. Danielsson et al. (2007) *FEBS* 27[illegible]6

Cu^{2+} *dimer*



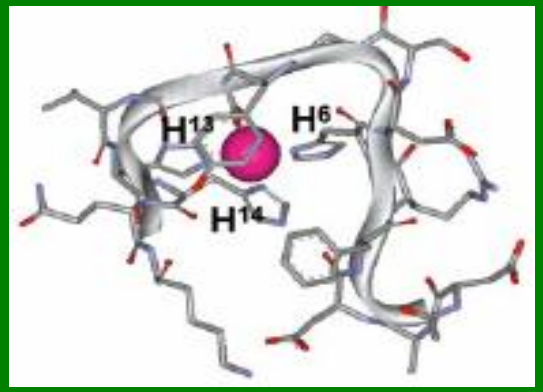


EPR

A.K. Tickler et al. (2005) *JBC* 280:13355

XAS

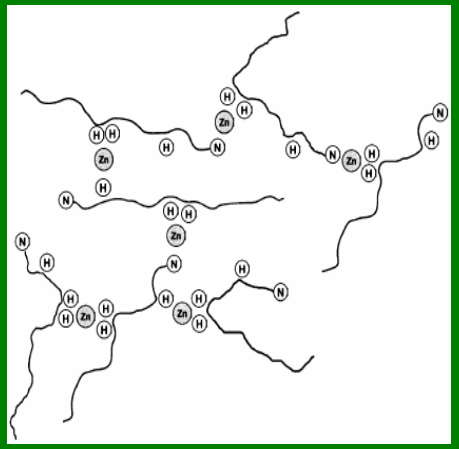
Zn^{2+}



NMR

S. Zirah et al. (2006) *JBC* 281:2151

Zn^{2+}



NMR

Syme & Viles (2006) *BBA* 1764:246

Amino acidic sequence of the five measured Aβ fragments

Oligopeptide	a.a. Sequence
$A\beta_{1\text{-}16}$	H_3N^+-DAEFRHDSGYEVHHQK-COO^-
$A\beta_{1\text{-}28}$	H_3N^+-DAEFRHDSGYEVHHQKLVFFAEDVGSNK-COO^-
$A\beta_{5\text{-}23}$	H_3N^+-RHDSGYEVHHQKLVFFAED-$COOH_3N^+$
$A\beta_{17\text{-}40}$	H_3N^+-LVFFAEDVGSNKGAIIGLMVGGVV-COO^-
$A\beta_{1\text{-}40}$	H_3N^+-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVV-COO^-

1 DAEFRHDSGYEVHHQK 16 — containing all three His's

17 LVFFAEDVGSNKGAIIGLMVGGVVIA 40 — containing no His

1 DAEFRHDSGYEVHHQKLVFFAEDVGSNK 28 — hydrophobic region

5 RHDSGYEVHHQKLVFFAED 23 — N-terminal region

XAS results

Cu^{2+}/Zn^{2+}-$A\beta_{17-40}$ = Cu^{2+}/Zn^{2+}- buffer

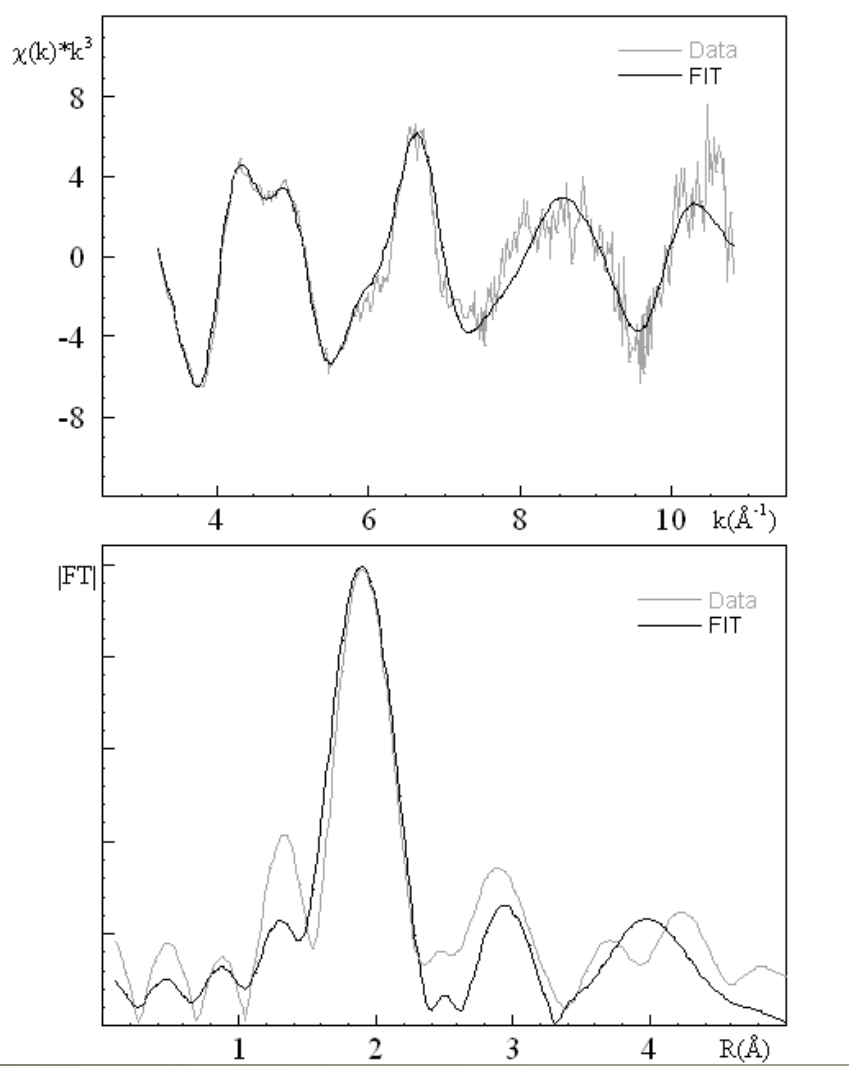
Cu^{2+}-$A\beta_{1-16}$ = Cu^{2+}- $A\beta_{1-28}$ = Cu^{2+}-$A\beta_{1-40}$

Zn^{2+}-$A\beta_{1-16}$ = Zn^{2+}-$A\beta_{1-28}$ = Zn^{2+}-$A\beta_{5-23}$ = Zn^{2+}-$A\beta_{1-40}$

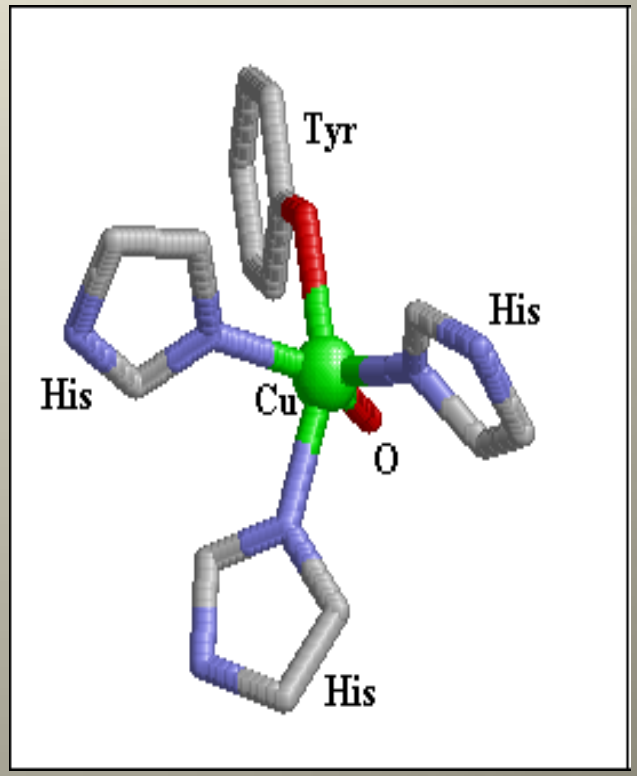
Cu^{2+}-$A\beta_{5-23}$ ≠ Cu^{2+}-$A\beta_{1-40}$

fitted spectra

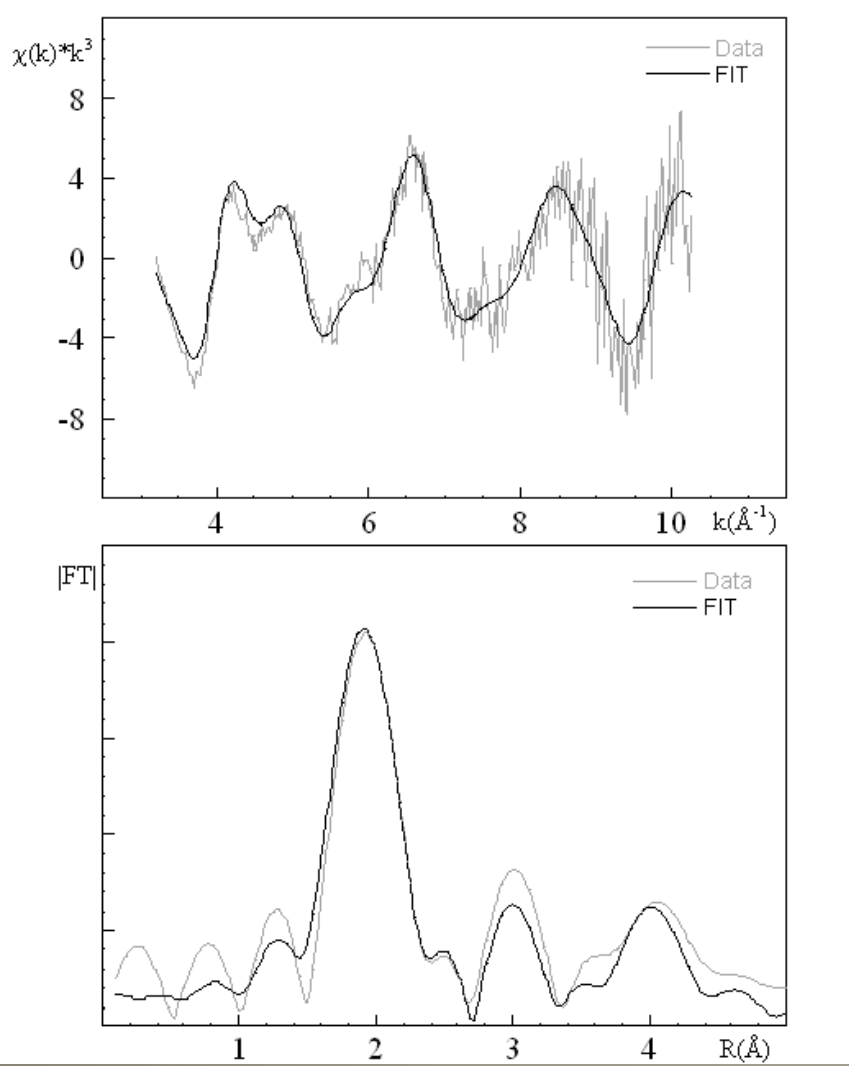
Cu-$A\beta_{1-16}$



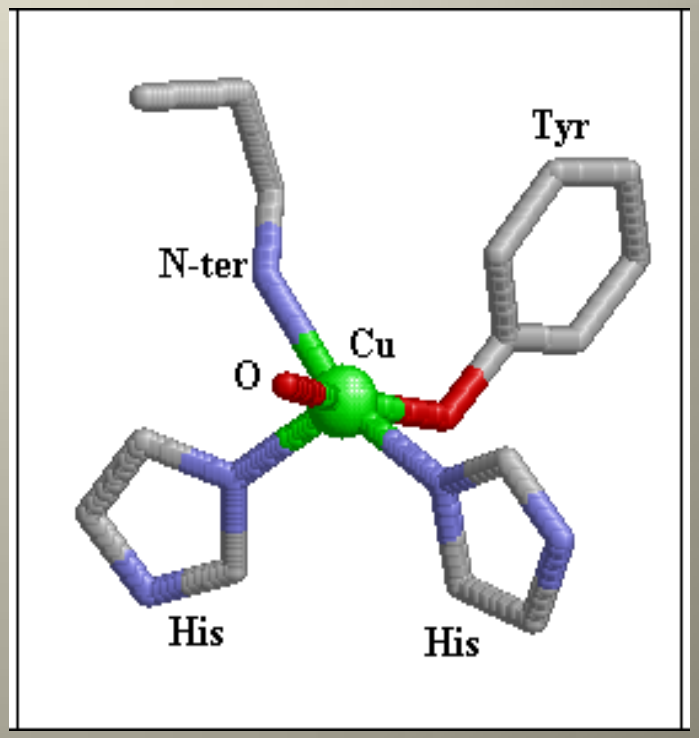
- 3 Histidines
- 1 Tyrosine
- 1 OH^-



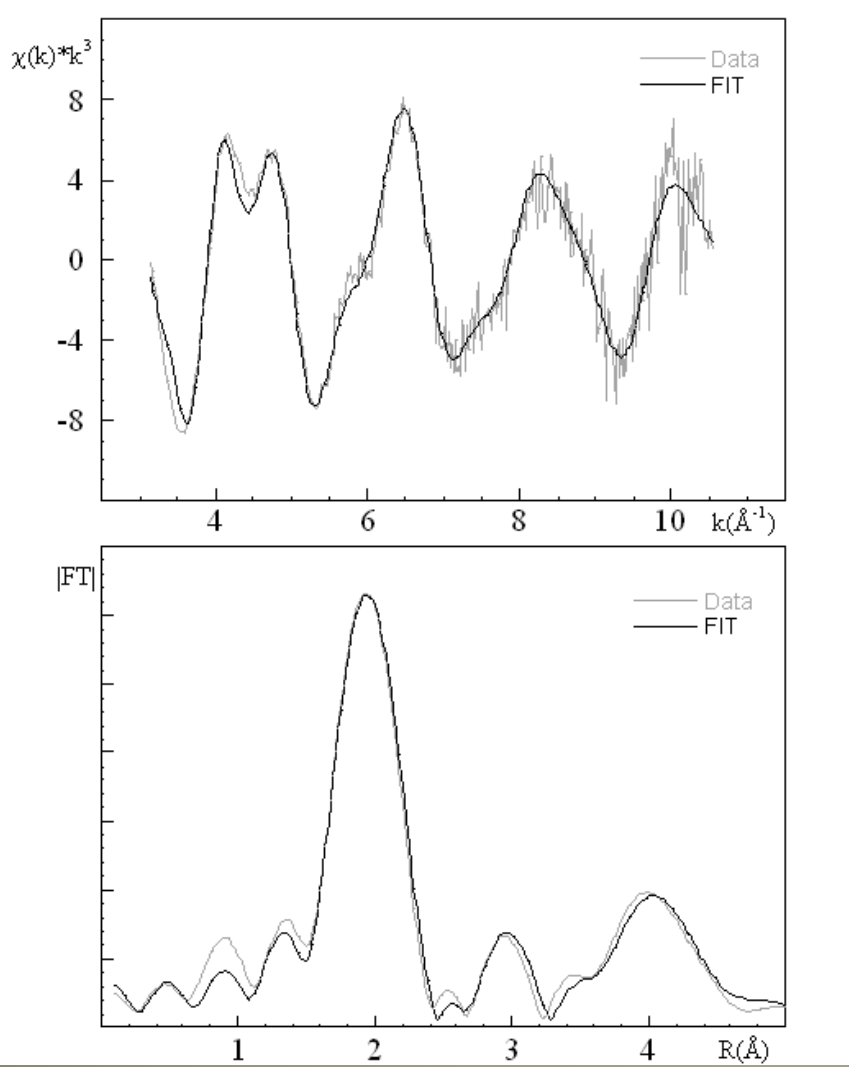
Cu-$A\beta_{5-23}$



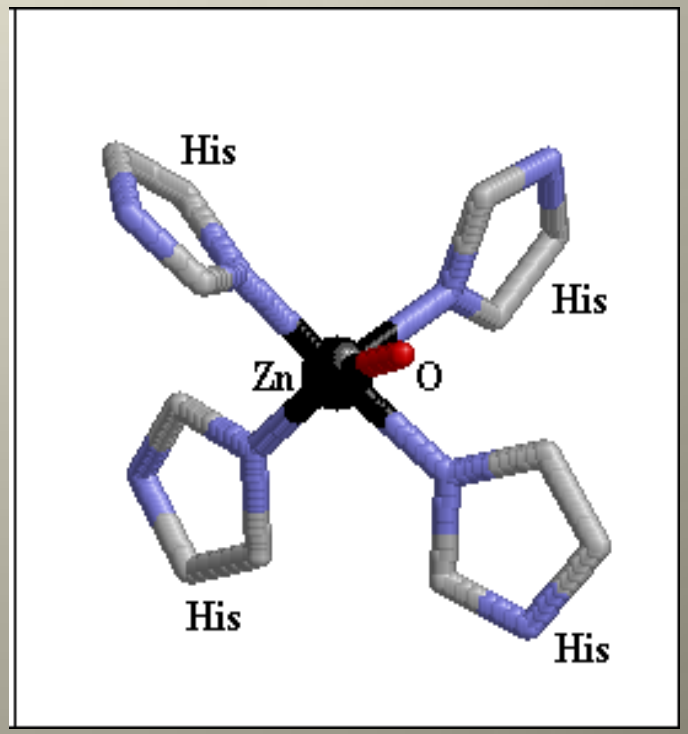
- 2 Histidines
- 1 N-term
- 1 Tyrosine
- 1 OH^-



Zn-$A\beta_{1-16}$



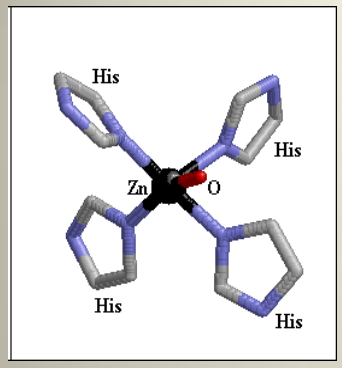
- 4 Histidines
- 1 OH^-



Conclusions from XAS data analysis

- **Metal binding site lies within the first 16 aminoacids**
- **Cu^{2+} and Zn^{2+} have different binding geometry**
- **Cu^{2+}-Aβ intra-molecular binding**
- **Zn^{2+}-Aβ inter-molecular binding, suggesting aggregation**
- **Very crowded Zn coordination**

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



DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Which structure stabilizes this (very crowded) 4 His's coordination?

Out of 5854 PDB entries containing at least one Zn
only in

- 1PB0 (monomer)
- 1HWT and 1QP9 (dimer)

Zn is coordinated to 4 His's

In our case two pairs of consecutive His's residues
from two adjacent Aβ-peptides

Cu,Zn-superoxide dismutase (Cu,Zn-SOD)

- XAS data provide structural information only within about 5-6 Å from the absorber
- *numerical* simulations can give information about the whole system

Building a model system

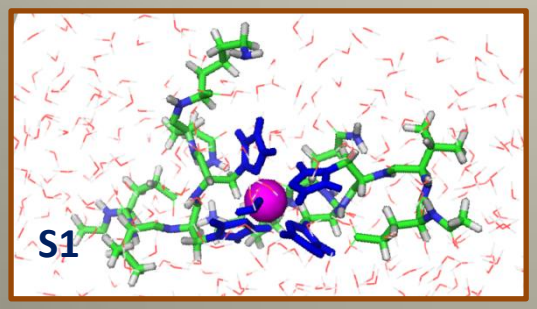
- 4 initial configurational models, formed by
- 2 identical $A\beta_{1\text{-}16}$ chains, A&B, with Zn bound to Nδ of $His14^{(A)}$&$His14^{(B)}$
- all others dihedral angles are randomly tried (MCRW)
- many configurations produced, few selected ← structural constraints
- configurations with ‘‘bad’’ contacts automatically discarded

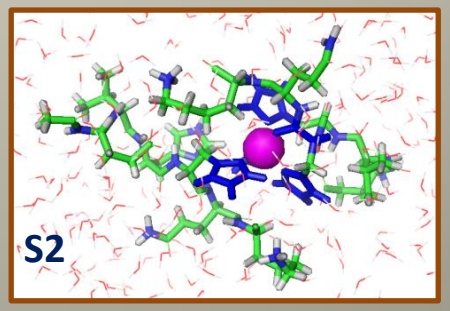
P. Giannozzi, et al. (2012) Metallomics, 4, 156-165

Name	Starting configuration

S1 and S2: 11-16 truncated peptide chains
+ 376 TIP3P water molecules

S2: (3+½) His



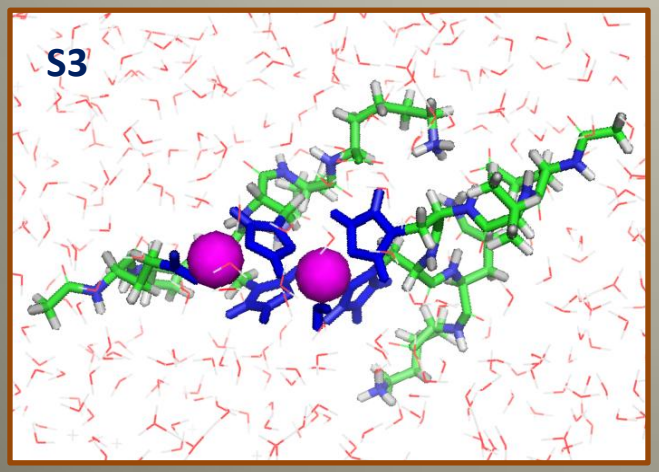


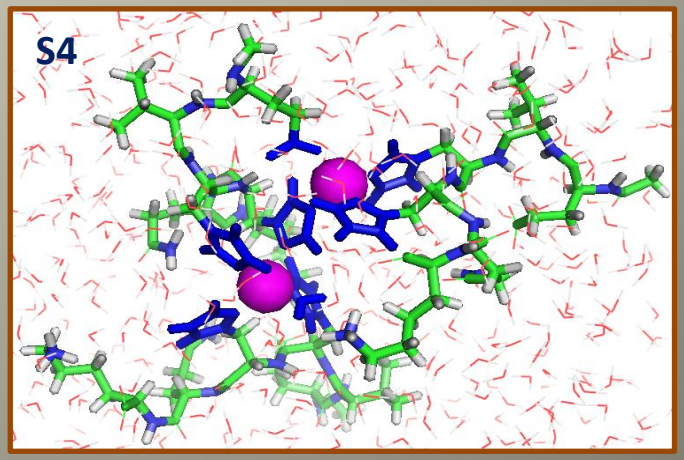
S. Furlan & G. La Penna Phys Chem Chem Phys 11: 6468 (2009)
S. Zirah et al. J Biol Chem 281: 2151-2161 (2006)

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

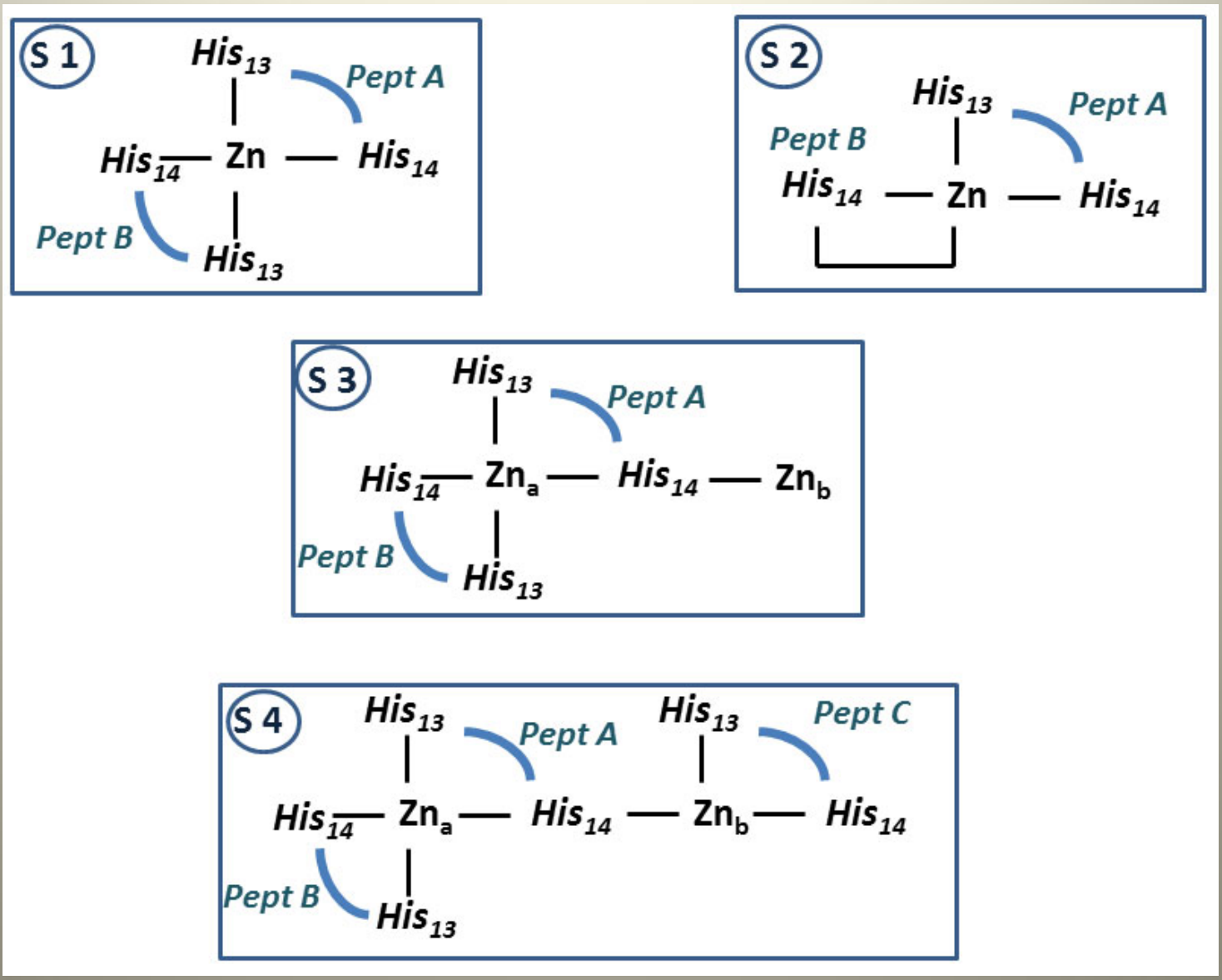
S3: from S1→replace Hε of His $14^{(A)}$ → Zn_b

S4: from S3 add an extra chain C (11-16 fragment)

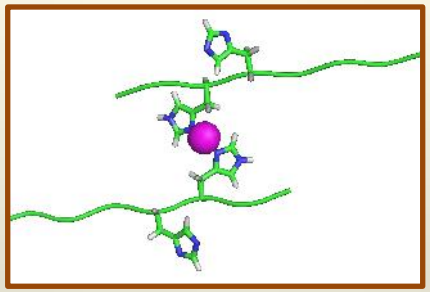




In summary

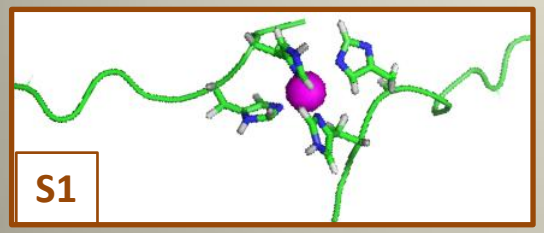

S 1
His13
Pept A
His14
Zn
His14
Pept B
His13
S 2
His13
Pept A
Pept B
His14
Zn
His14
S 3
His13
Pept A
His14
Zn a
His14
Zn b
Pept B
His13
S 4
His13
Pept A
His13
Pept C
His14
Zn a
His14
Zn b
His14
Pept B
His13

Simulation strategy
S1 and S2 models

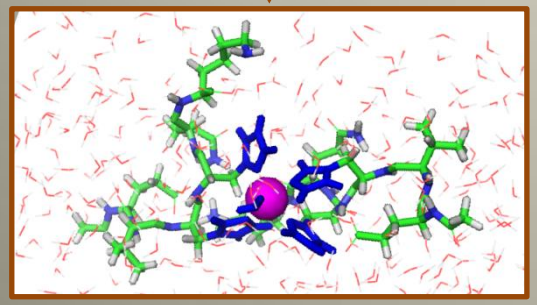


MD

MC



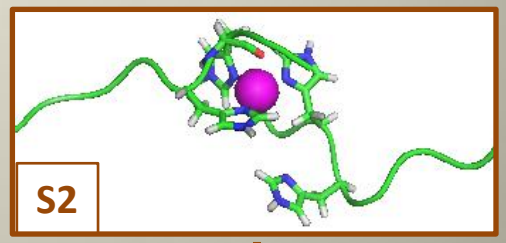
MD



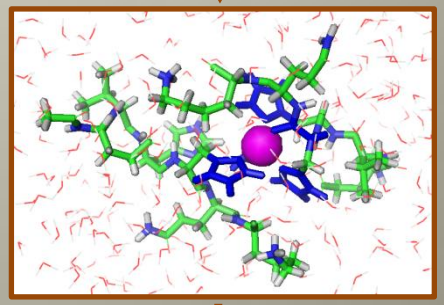
MD to relax water

TB & BOMD

CPMD



MD



MD to relax water

TB & BOMD

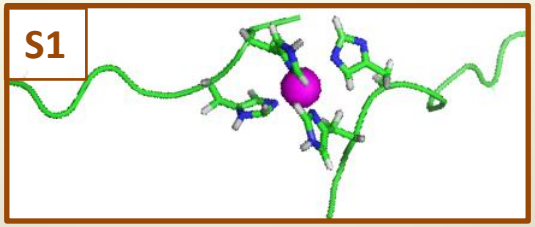
CPMD

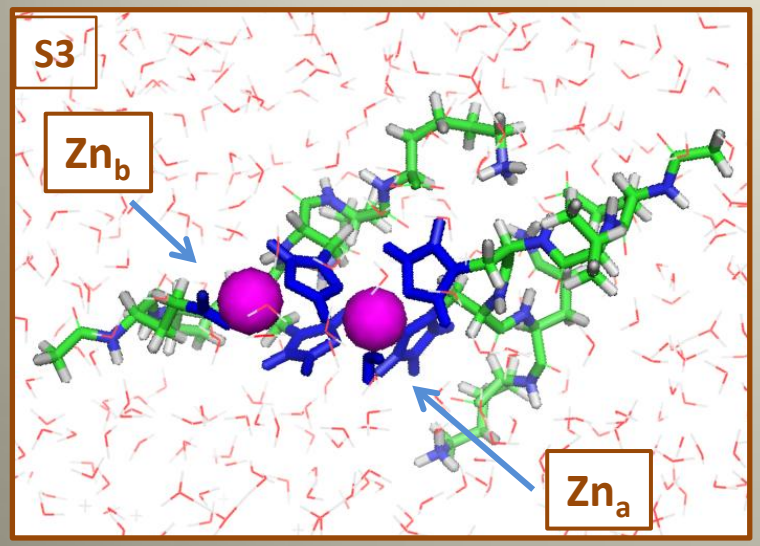
Step 1

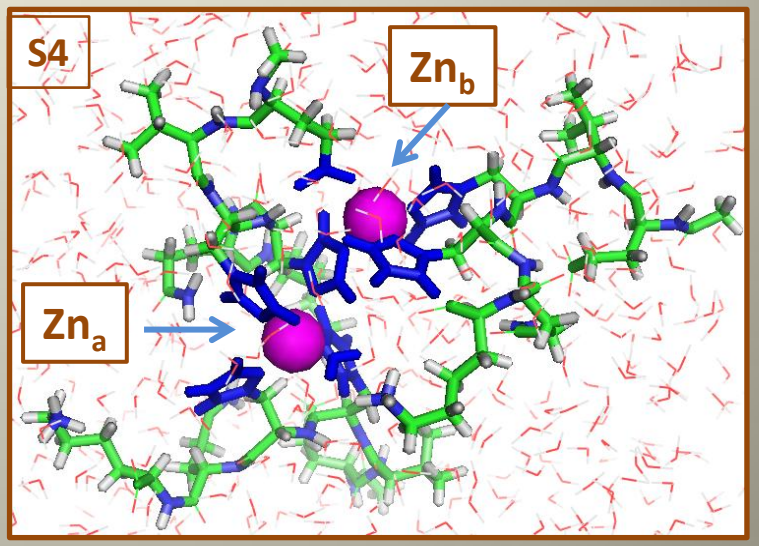
Step 2

Step 3

Simulation strategy
S3 and S4 models







MD to relax water

TB & BOMD

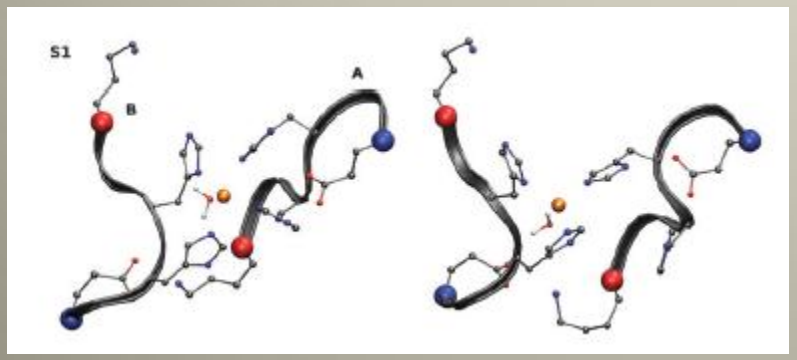
CPMD

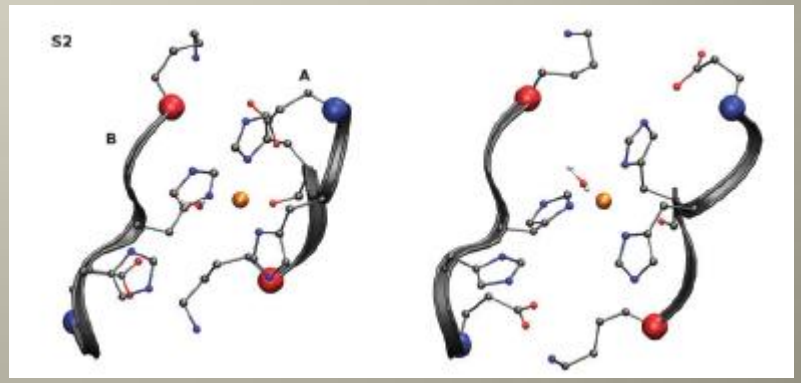
MD to relax water

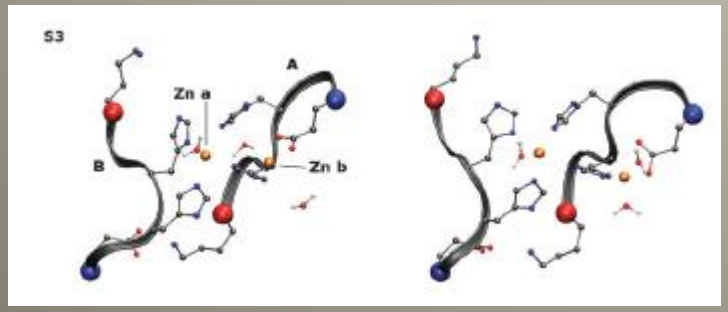
TB & BOMD

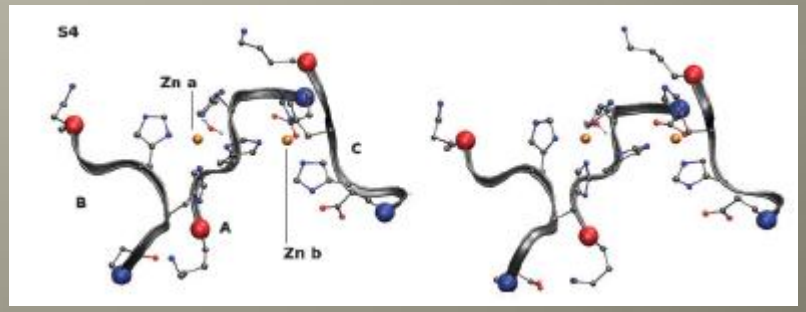
CPMD IF NEW MACHINE!

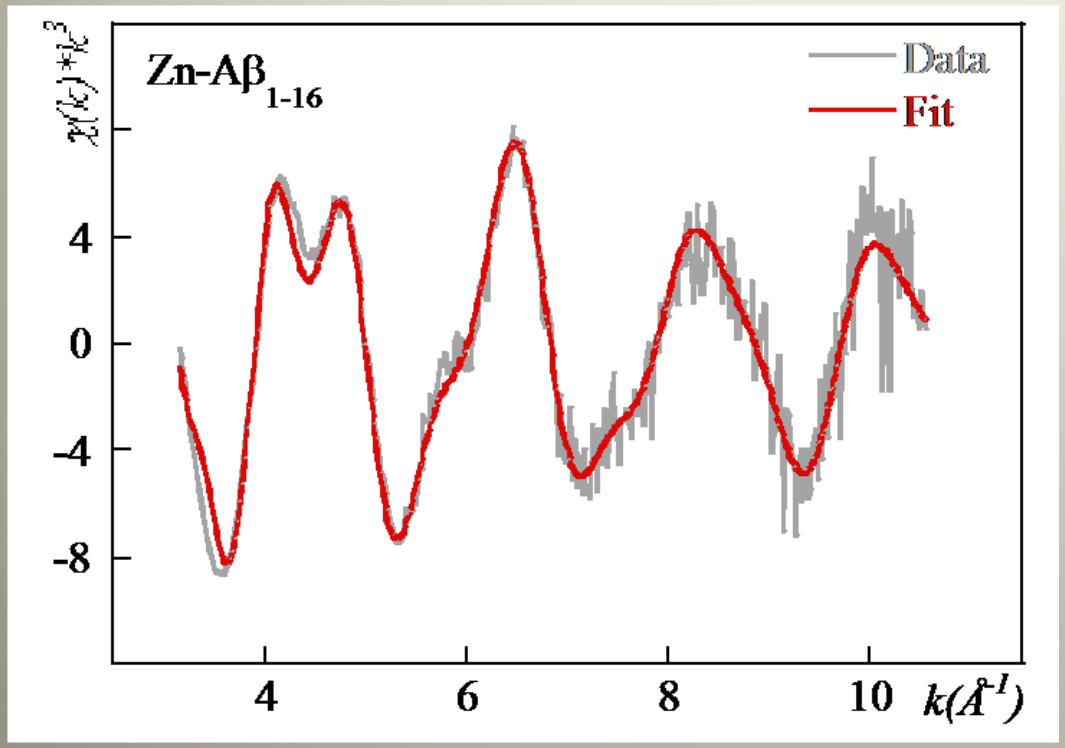
Name	Starting configuration	Zn-binding modification 0 - 250 K	300 K	Final Zn coordination
S1	Zn: $4N_{His} + 1O_W$	$-1N_{His}$	none	3N1O
S2	Zn: $3N_{His} + 1O_{His} + 1O_W$	$-1O_{His}$	none	3N1O
S3	Zn_a: $4N_{His} + 1O_W$ Zn_b: $1N_{His} + 2O_{Glu}$	Zn_b: $+1O_W$	Zn_b: $-1O_{Glu} + 1O_W$	Zn_a: 4N1O Zn_b: 1N3O
S4	Zn_a: $4N_{His} + 1O_W$ Zn_b: $3N_{His} + 1O_{Glu}$	none	none	Zn_a: 4N1O Zn_b: 3N1O

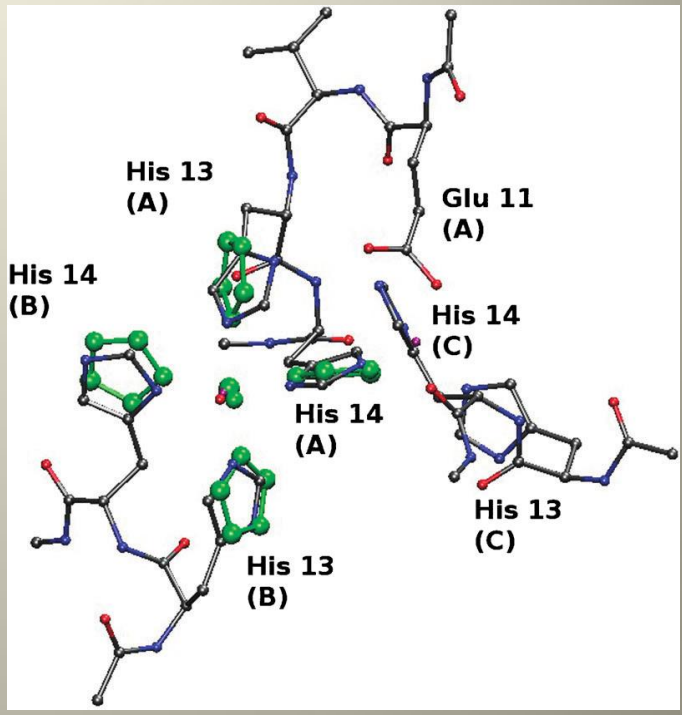









Zn-Aβ1-16
Data
Fit
χ(k)*k3
4
0
-4
-8
4
6
8
10
k(Å-1)


His 13
(A)
Glu 11
(A)
His 14
(B)
His 14
(C)
His 14
(A)
His 13
(C)
His 13
(B)

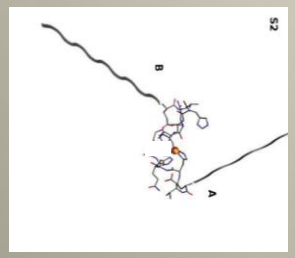
Effects of the long-range coordination topology

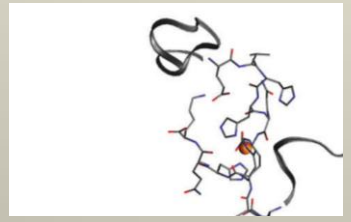
1–10 regions in the *all-trans* configuration is added

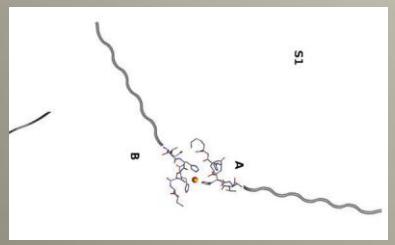
in vacuum

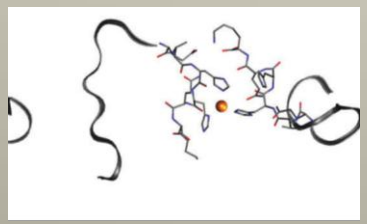
11–16 peptides and Zn atoms → were kept fixed

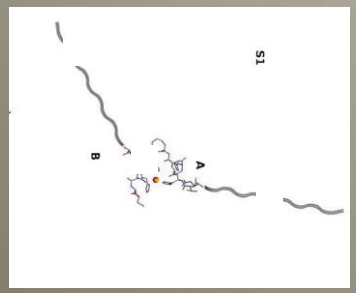
A few ps of classical simulations

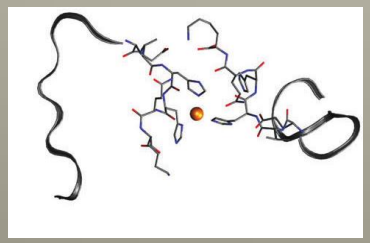










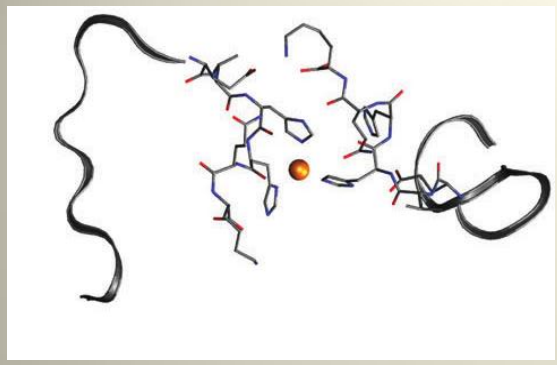


S1 Low mutual interaction

Intra-peptide collapse

S2

S4 enhanced chance of interaction

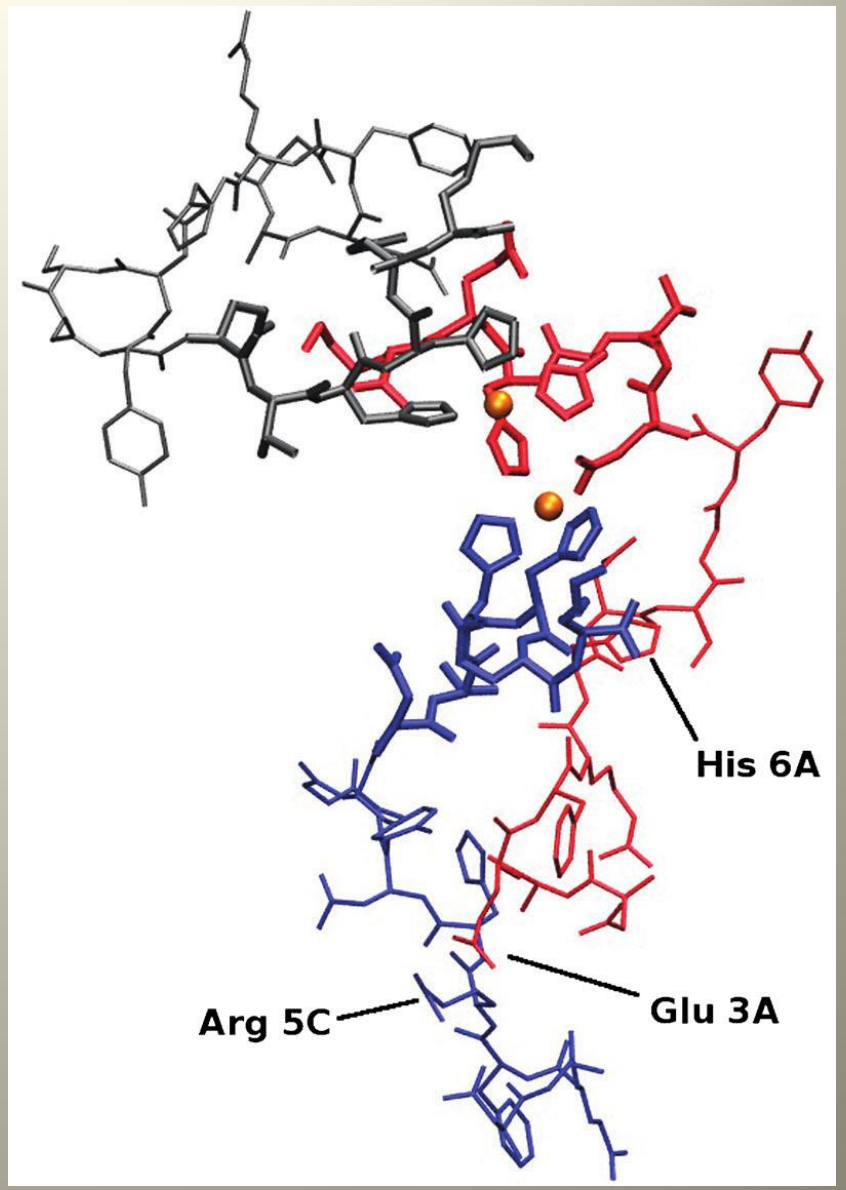


1739 empirical water molecules

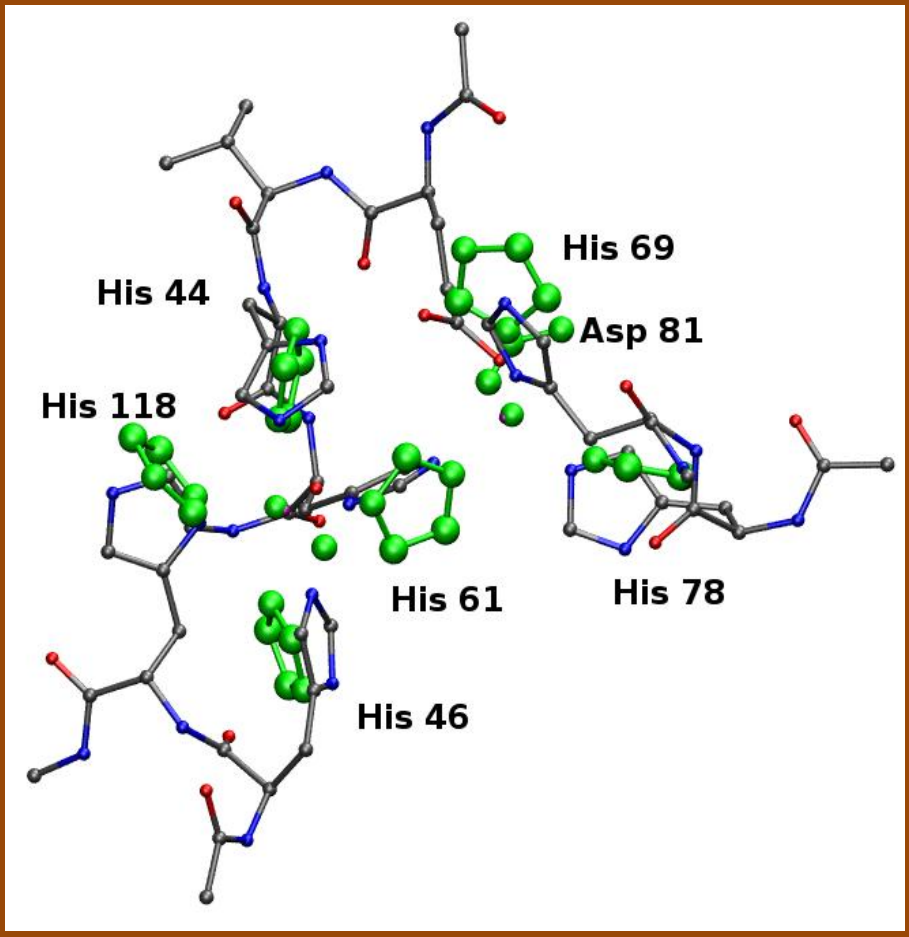
500 ps at T = 300 K

simulations confirm
what was found in vacuum

quite strong interactions stabilize
the mutual wrapping configuration
of two of the three chains



Stable structure is similar to that of Cu(I),Zn-SOD



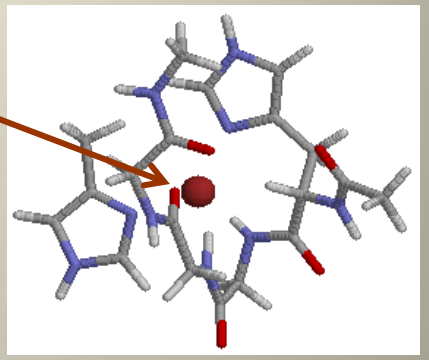
Comparison between the atomic structure of **reduced bovine SOD enzyme** (PDB 1SXA), in green, and the last S4 configuration

Conclusions

XAS - EXAFS

Data analysis

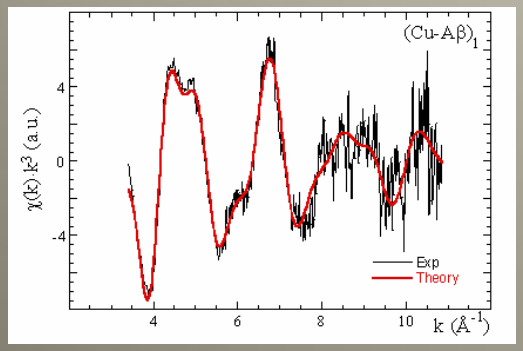
metal



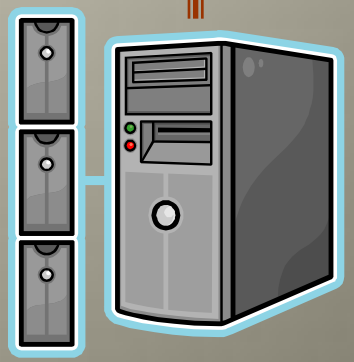
Data refinement

Structural information

Structural model



Ab initio calculations



Thank you