

RMN de proteínas de cobre de transferencia electrónica: procesos redox y reconocimiento molecular

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¿Como hacemos RMN de proteínas?

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Biomolecular NMR

FROM ASSIGNMENT TO STRUCTURE

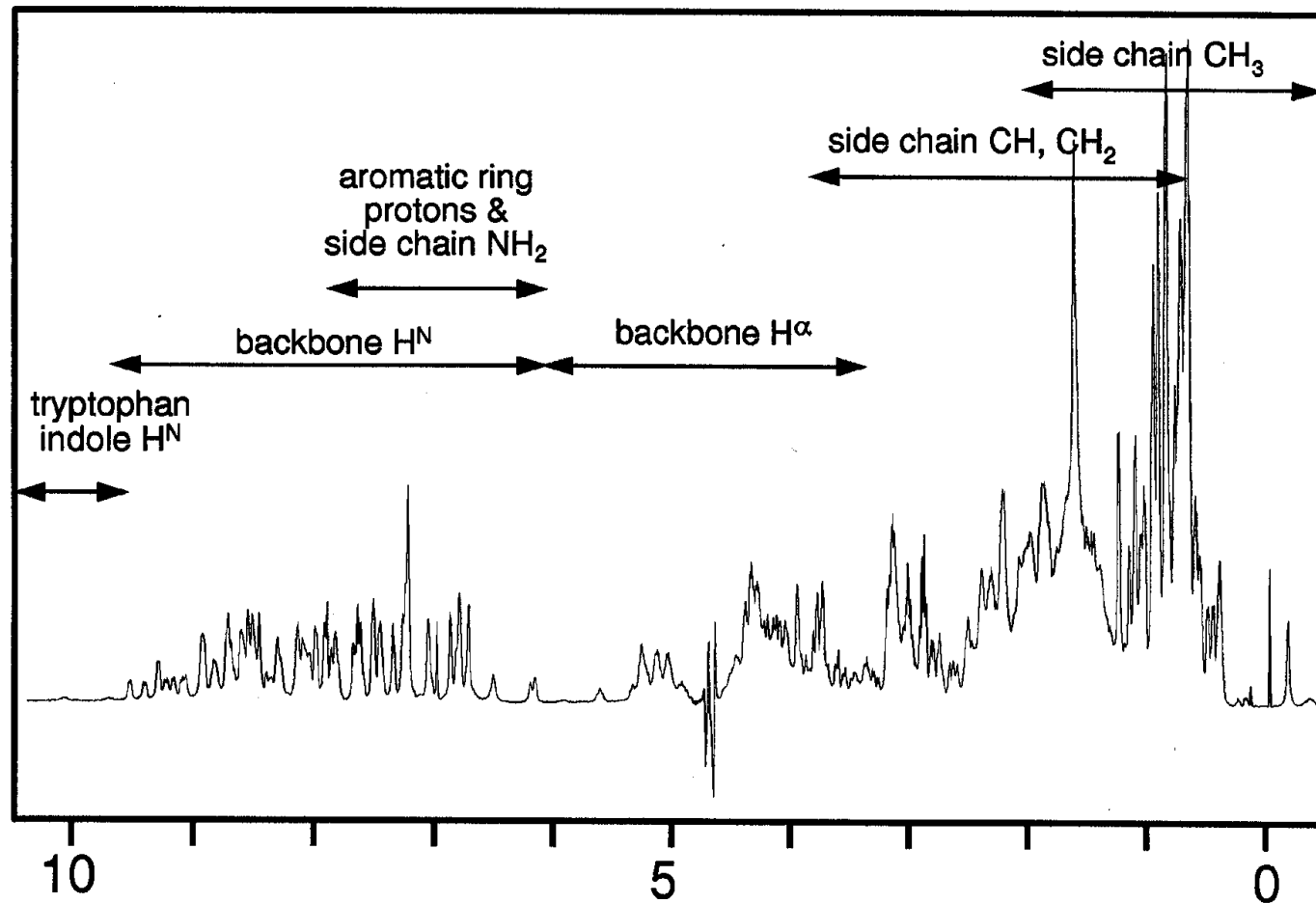
Sequential resonance assignment
strategies

NMR data for structure determination

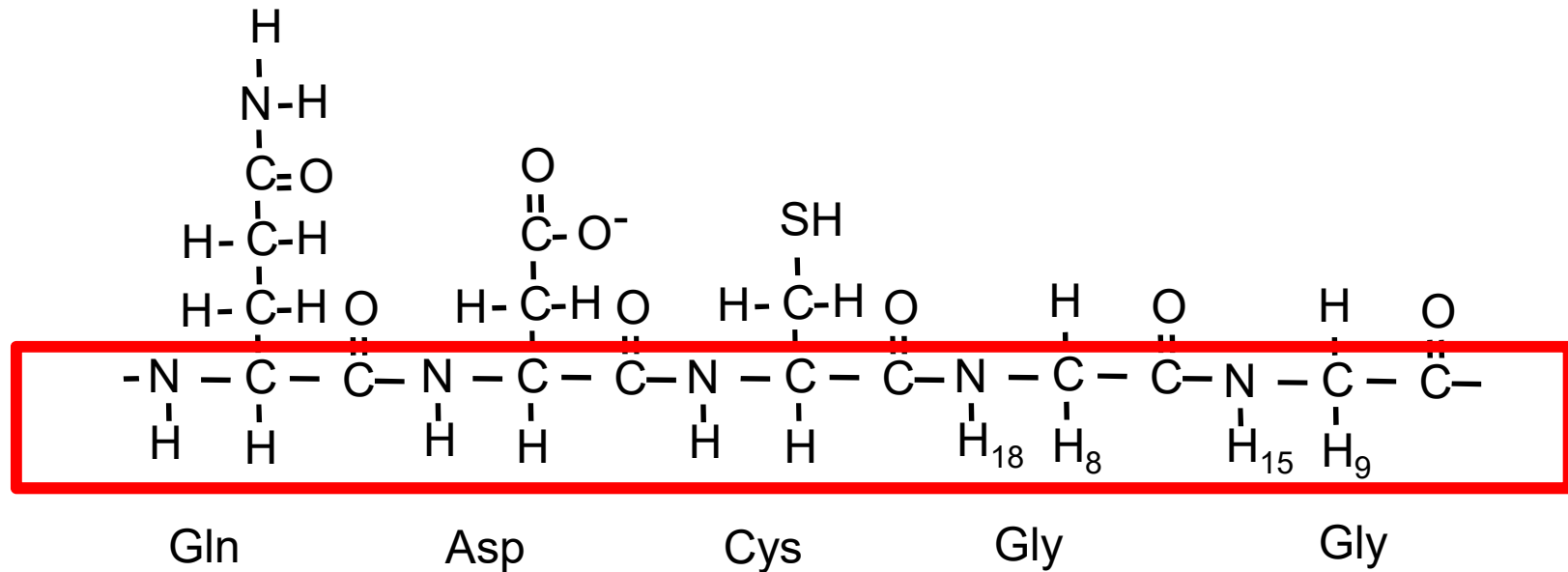
Structure calculations

Properties of NMR structures

^1H NMR Spectrum of a Small Protein

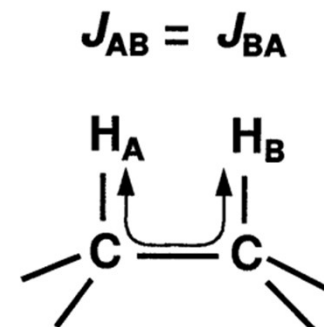
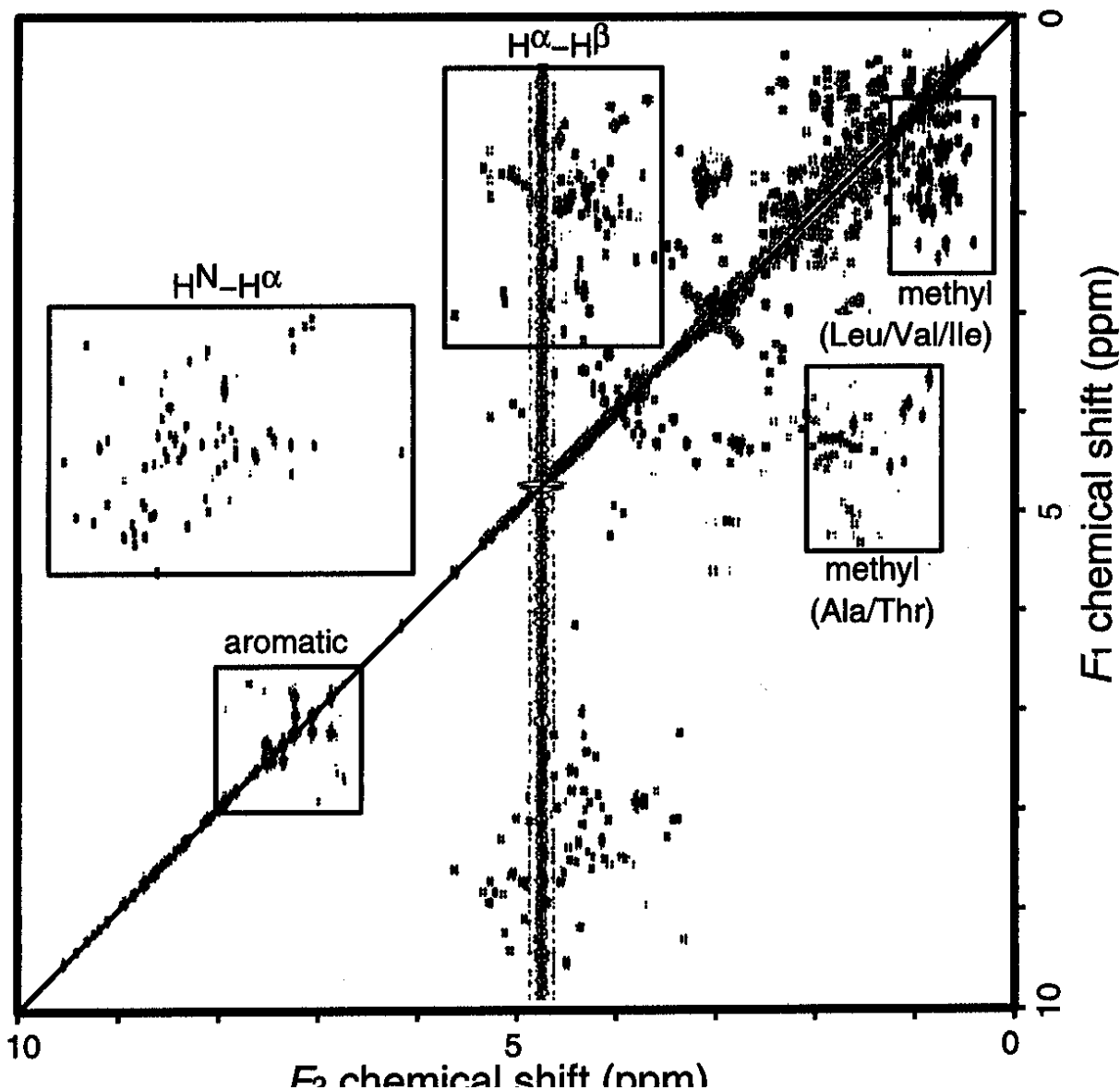


Proteins are polymers of known covalent structure

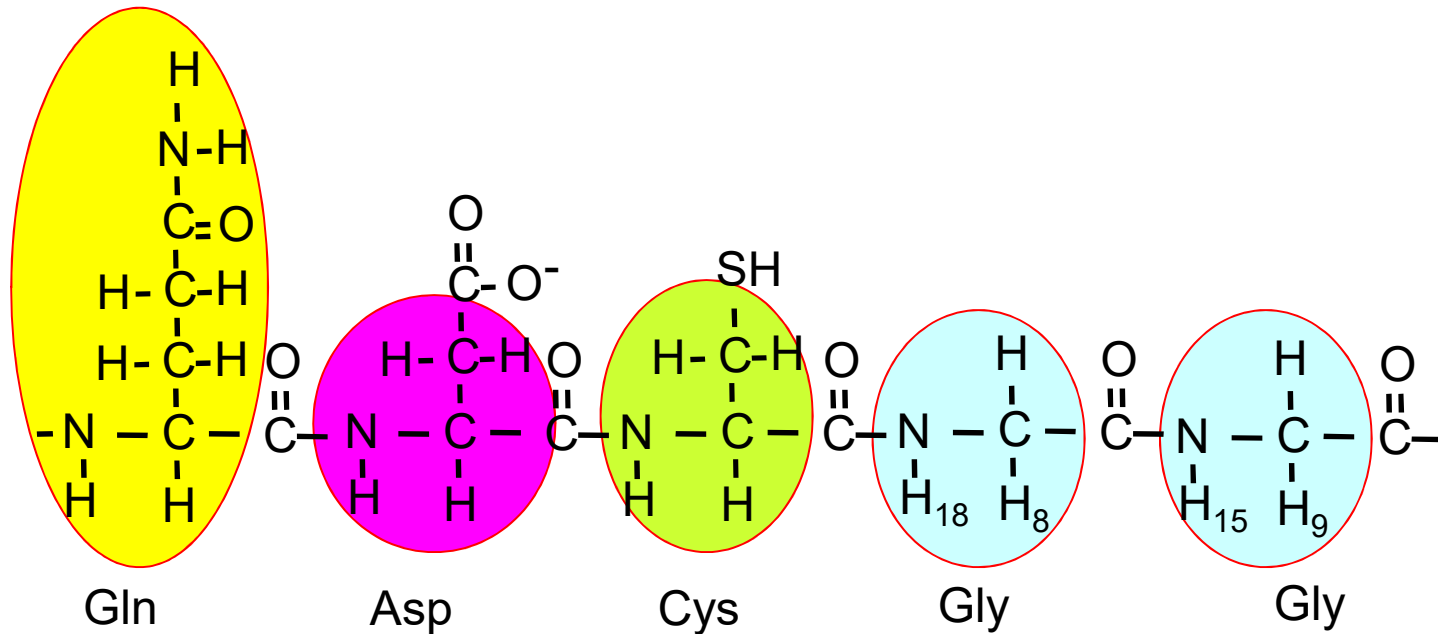


Each amino acid gives rise to an independent sub-spectrum (**SPIN SYSTEM**)

COSY: ^1H , ^1H Scalar Couplings (Governed by the chemical structure)

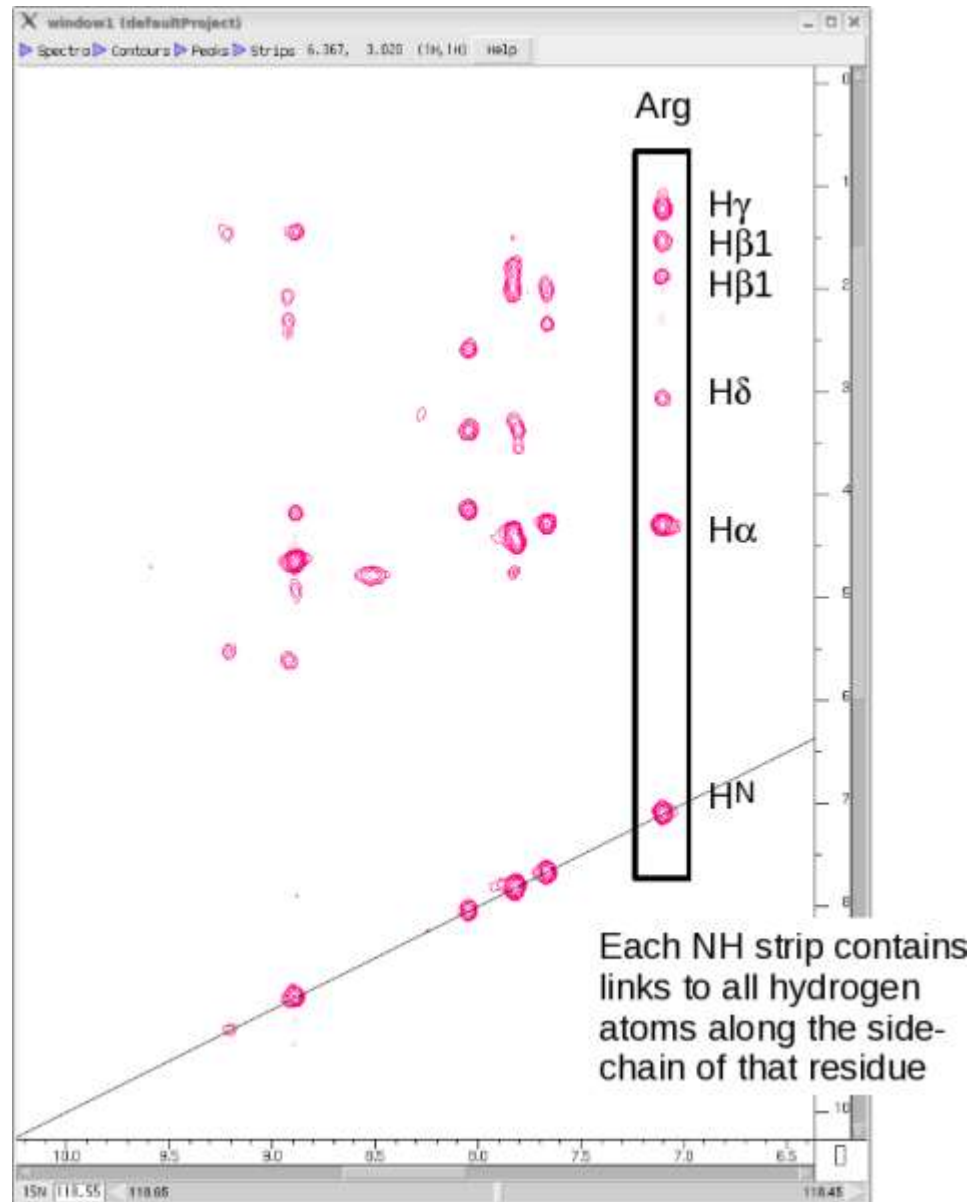


Proteins are polymers of known covalent structure



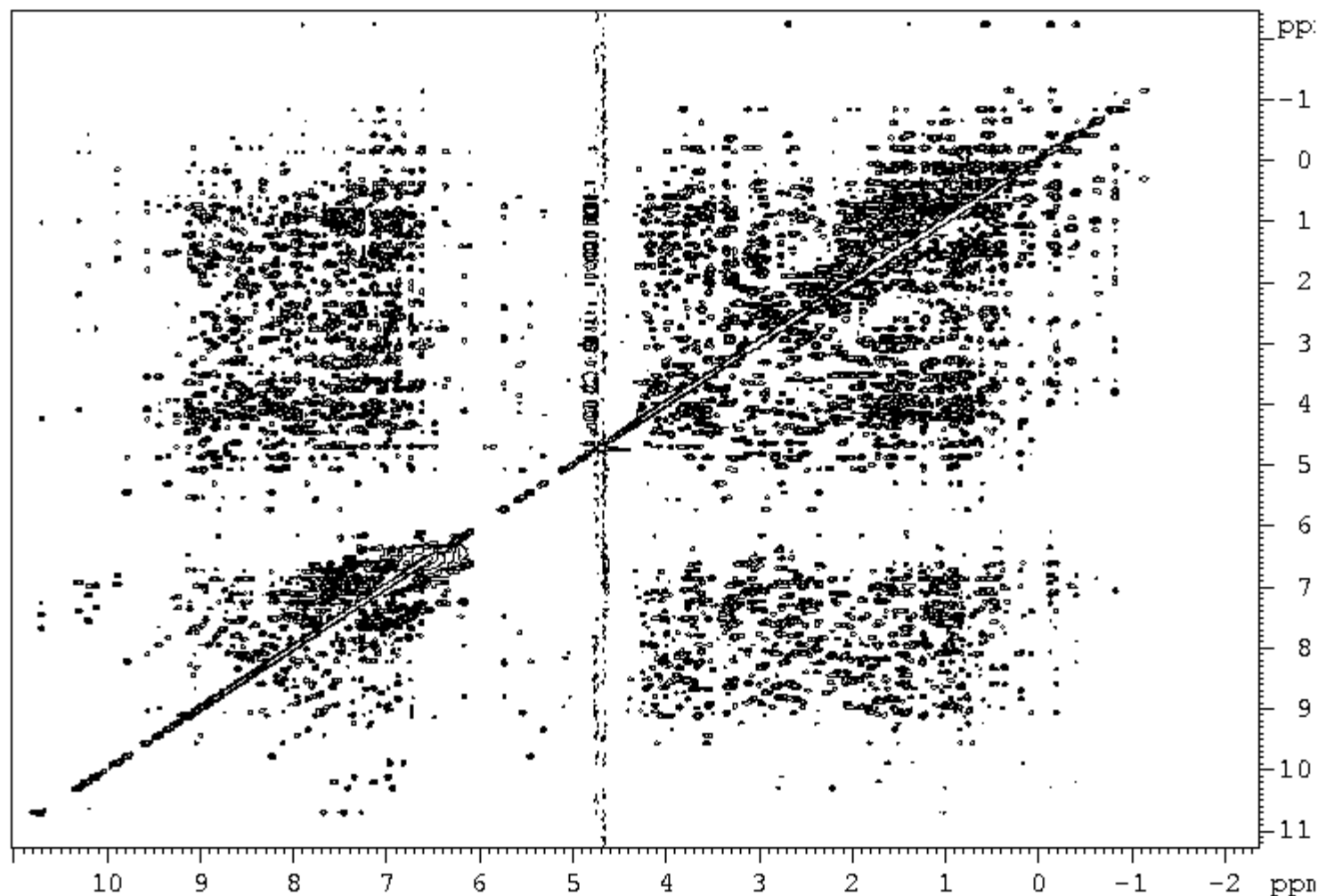
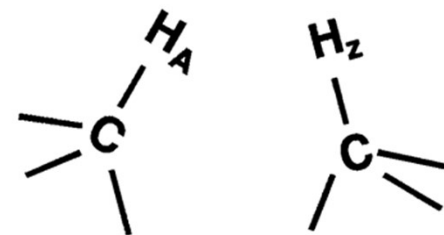
Each amino acid gives rise to an independent sub-spectrum (**SPIN SYSTEM**)

TOCSY: ^1H Spin Systems = Amino acid side chains



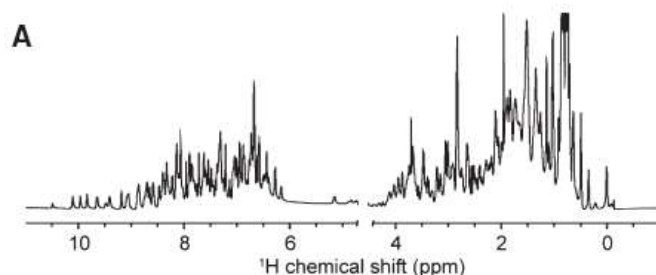
NOESY: ^1H , ^1H Dipolar couplings
(All types of signals may be coupled)

$$n.O.e \propto (1 / r_{az})^6$$

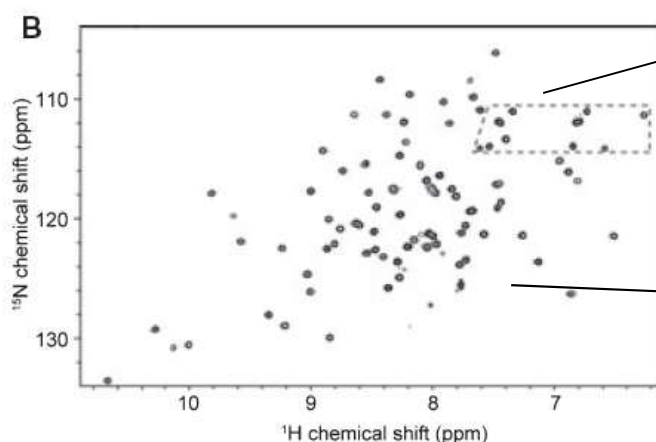


HETERONUCLEI ALLOW MORE RESOLUTION!!

CtBP-THAP (10.6 kDa)



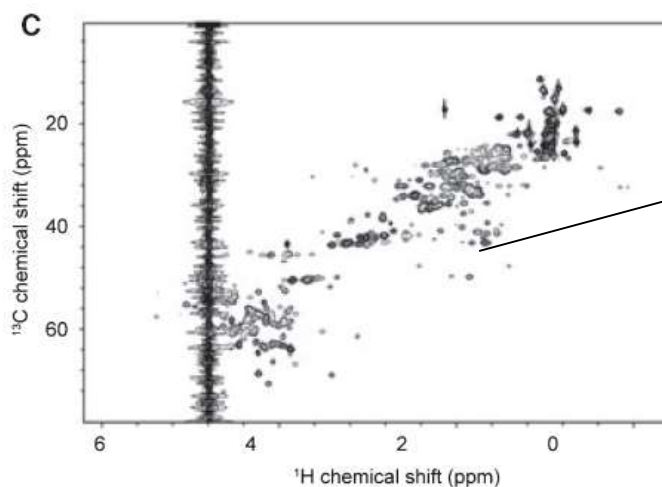
^1H , ^{15}N
HSQC



Asn, Gln side chains

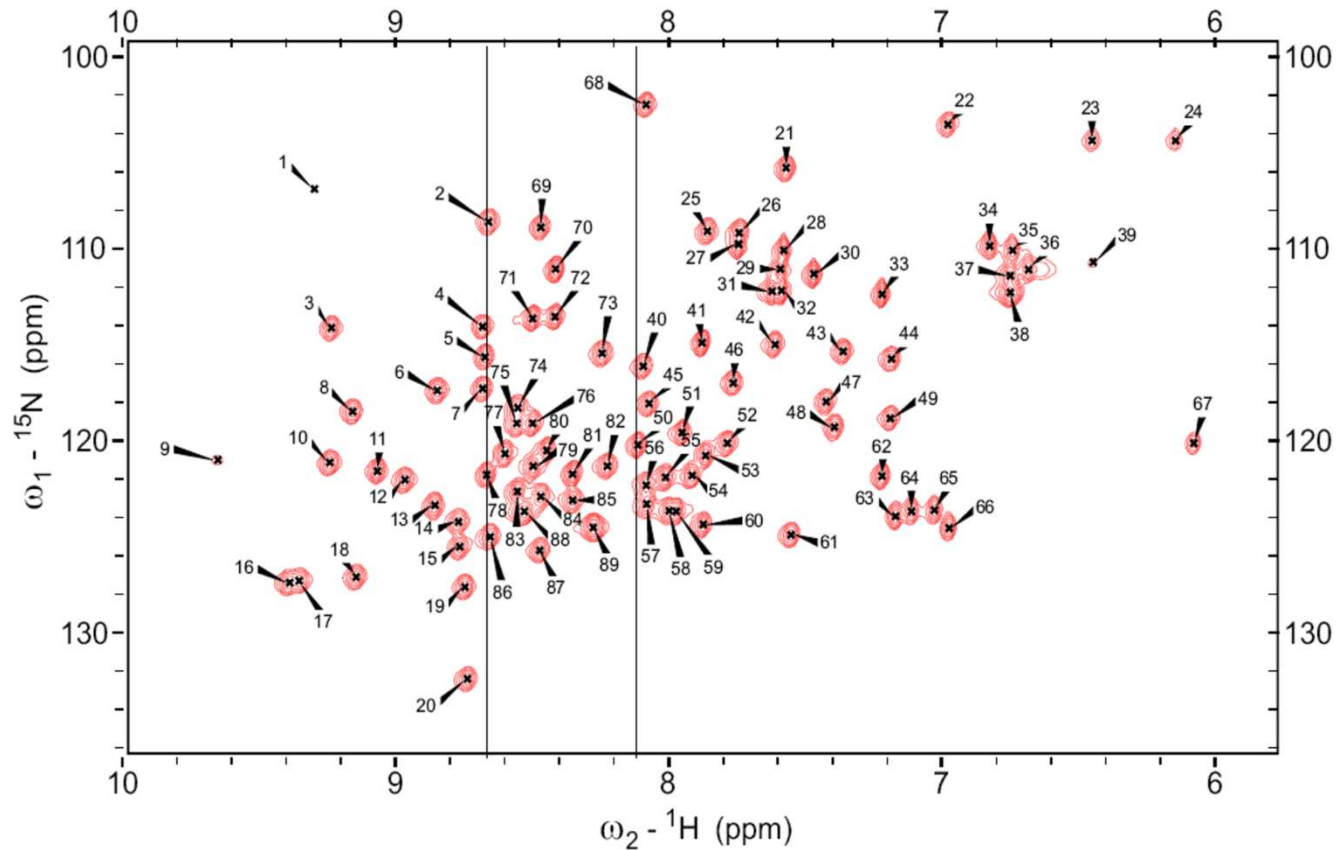
Peptide NH:
Fingerprint

^1H , ^{13}C
HSQC

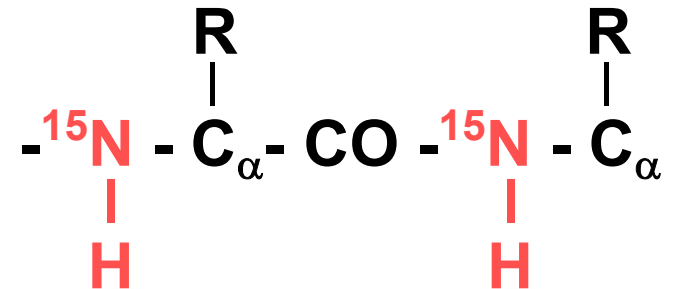


Better than 1D,
worse than ^1H , ^{15}N .
Shifts sensitive to
secondary structure

^1H - ^{15}N HSQC: 1J couplings

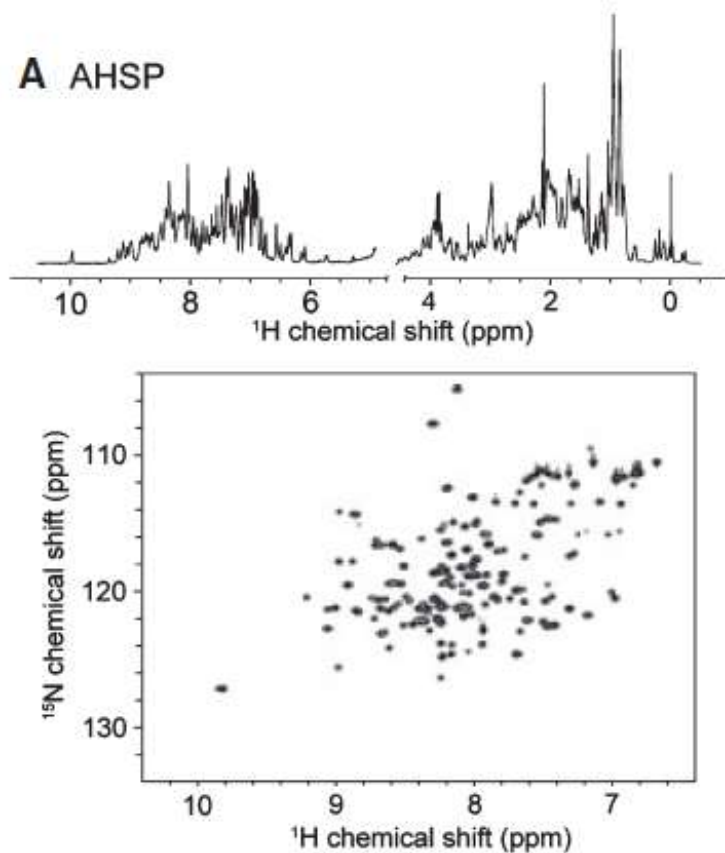


2D ^{15}N / ^1H correlation ($J = 90$ Hz)
 ^{15}N - ^1H , one per residue



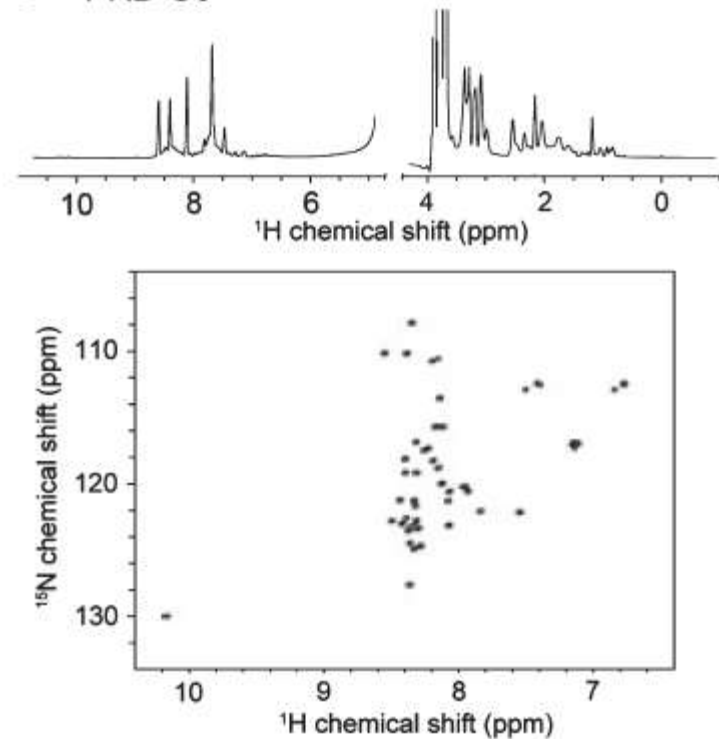
Is my protein folded?

A AHSP



**A 10 kDa
all α -helical protein**

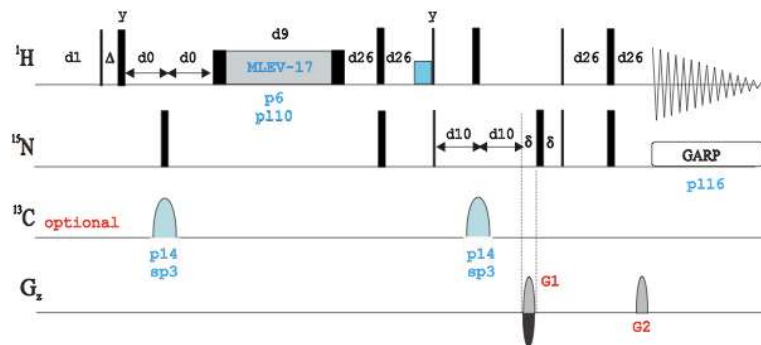
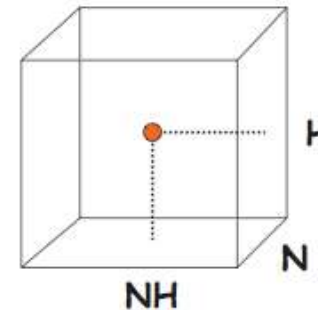
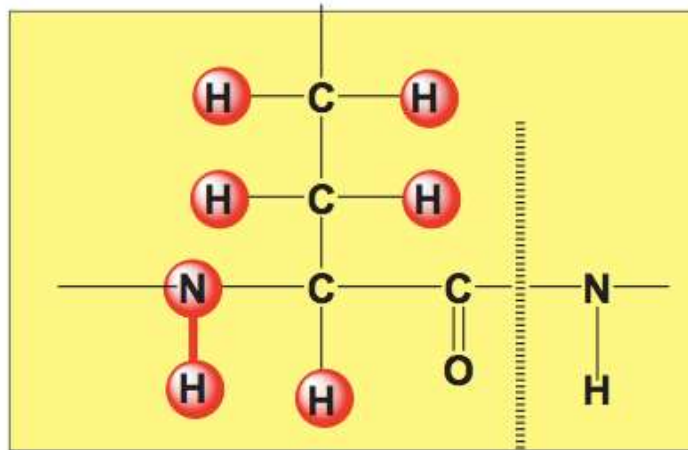
C PRD-C6



**A disordered
6 kDa polypeptide**

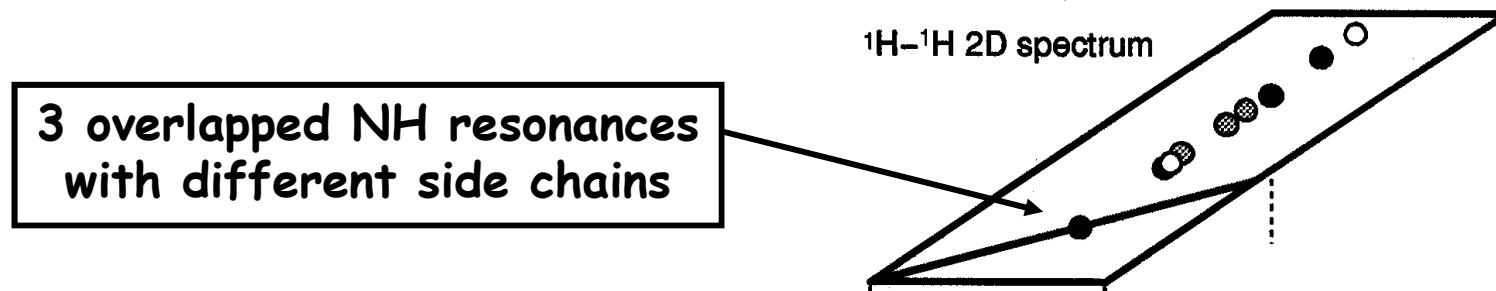
Experimentos 3D heteronuclear

- ▶ Extensión del HSQC a otra dimensión
- ▶ Cada señal HN-N “ve” las resonancias de ^1H acoplados



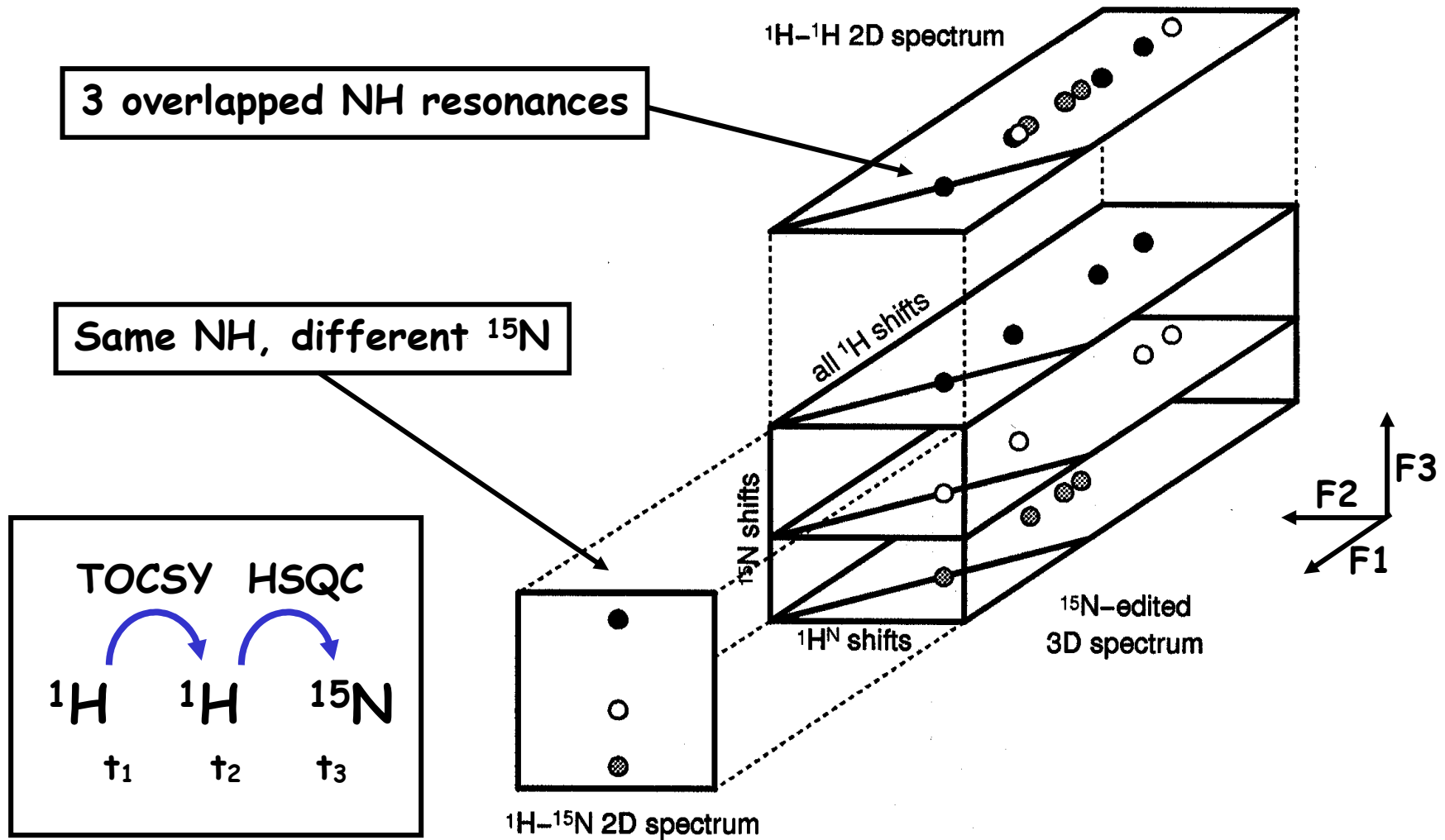
TOCSY-HSQC

^{15}N Dispersed ^1H - ^1H TOCSY

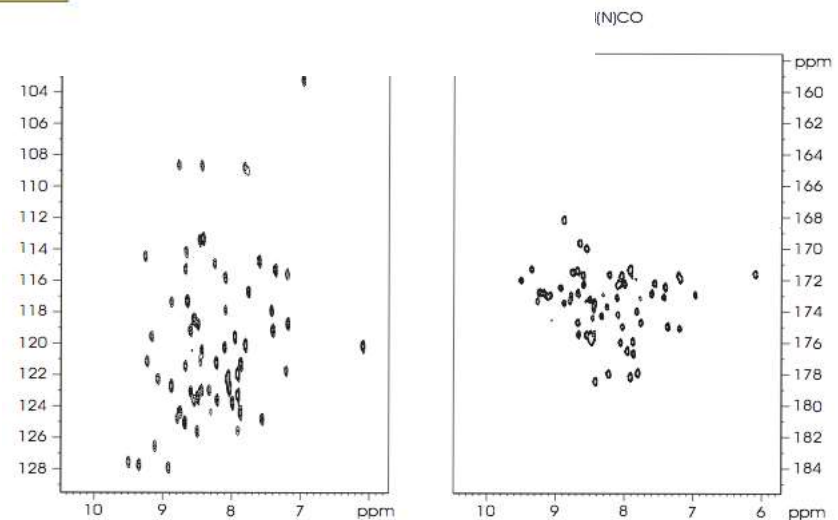
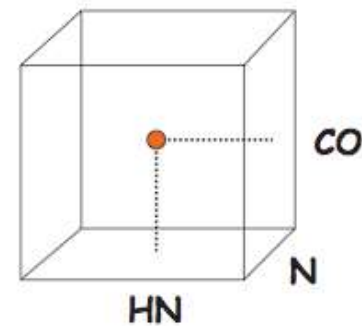


Add a 3rd dimension separating
out H^{N} overlaps by their ^{15}N
frequency

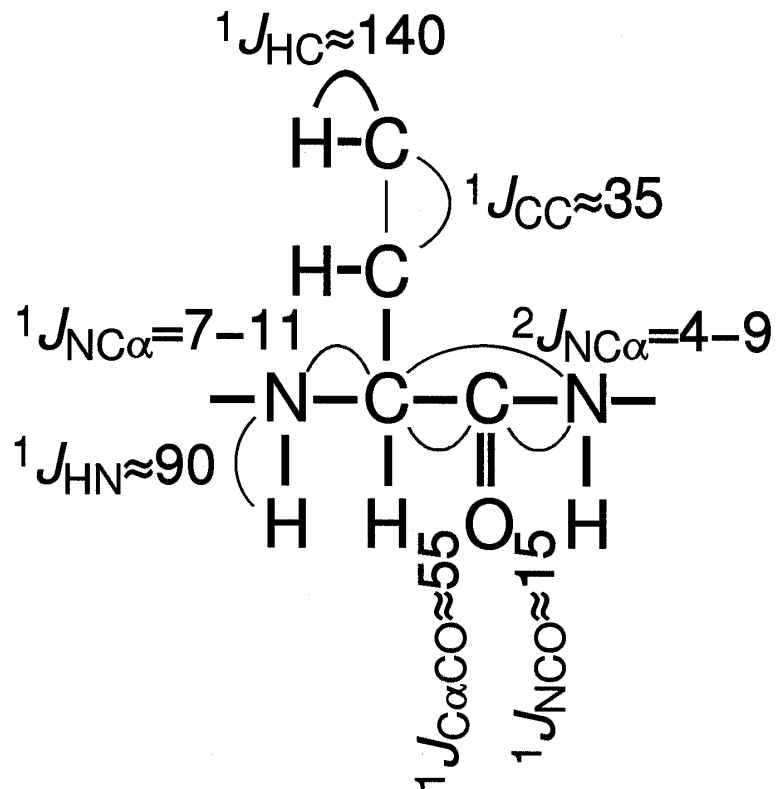
^{15}N Dispersed ^1H - ^1H TOCSY



► Se transfiere magnetización entre ^1H , ^{15}N y ^{13}C

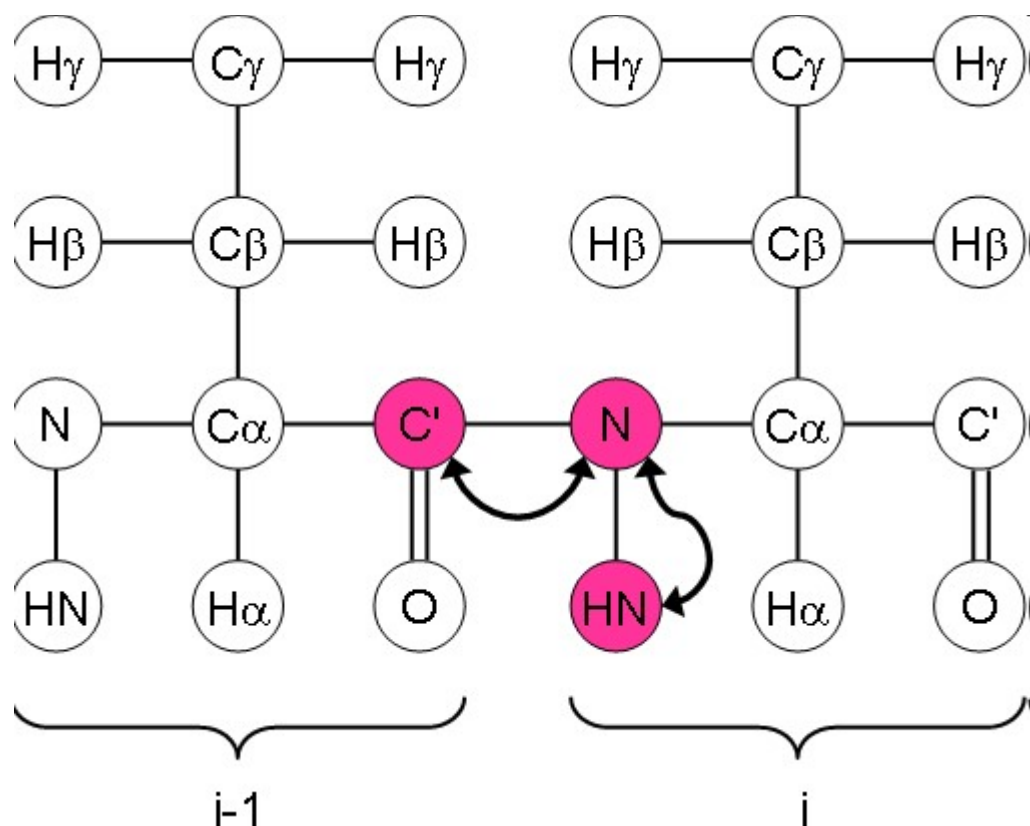


Large Scalar Couplings → Less Sensitive to the Protein size



- Superior to ^1H homonuclear NMR: $\text{H}-\text{H}$ couplings < 20 Hz
- Mixing is faster so less time for signal to relax
- These couplings are *uniform* throughout peptides/proteins
- These couplings are virtually *conformation independent*

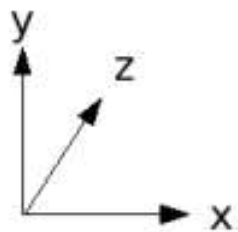
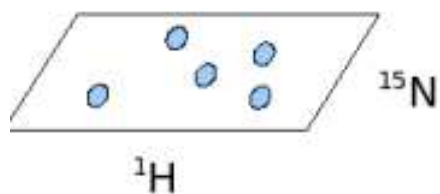
Heteronuclear Assignments: HNCO Experiment



Visualising 3D Spectra in Planes

HSQC

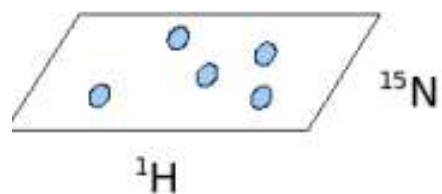
(a)



Visualising 3D Spectra in Planes

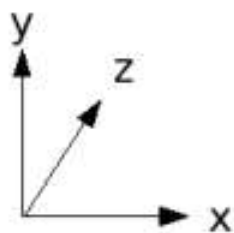
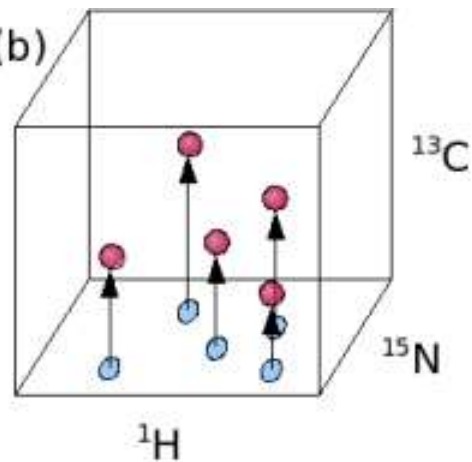
HSQC

(a)



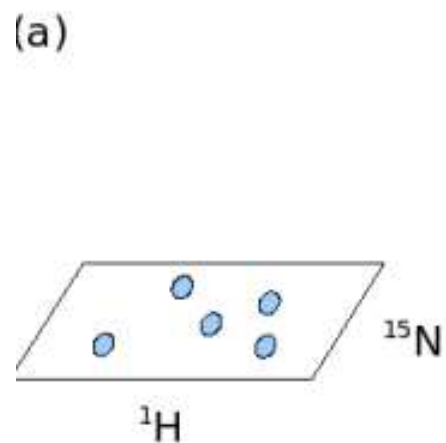
HNCO

(b)

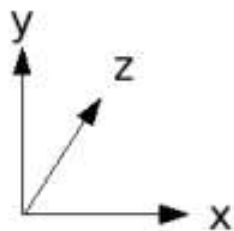
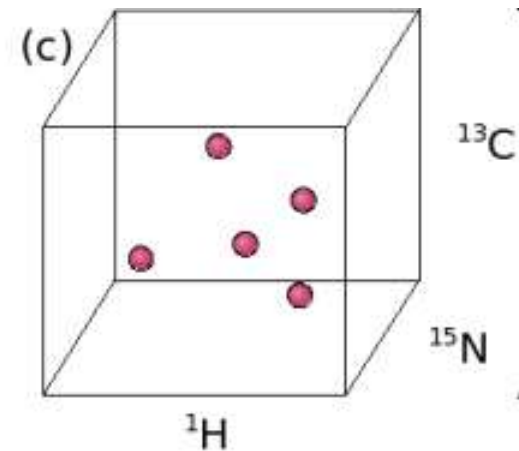
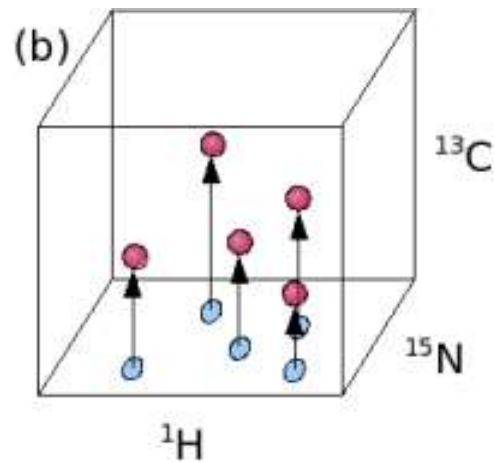


Visualising 3D Spectra in Planes

HSQC

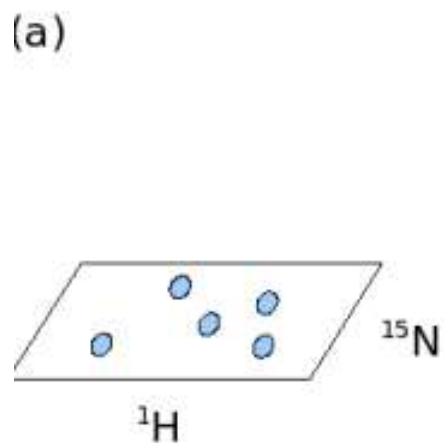


HNCO

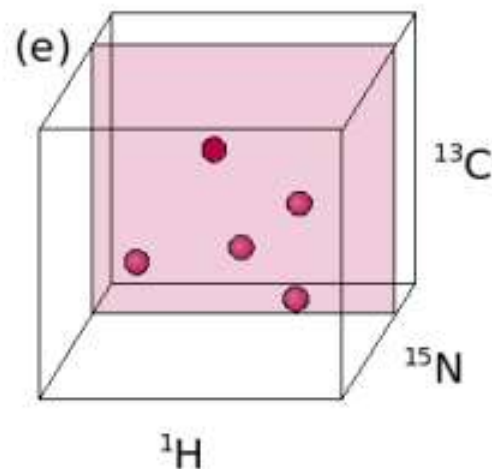
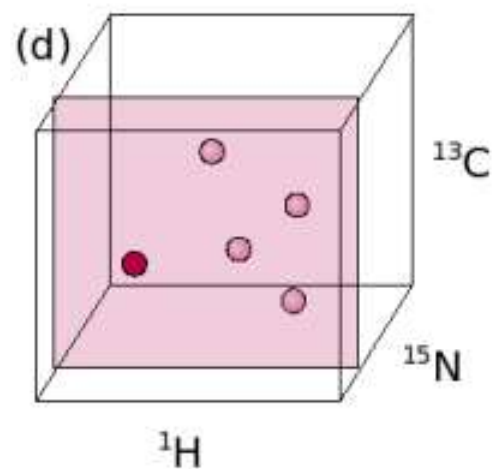
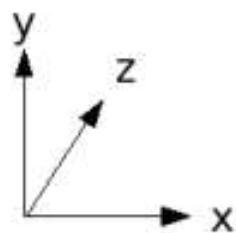
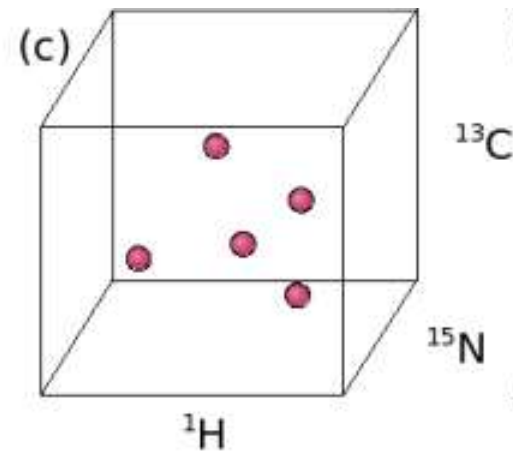
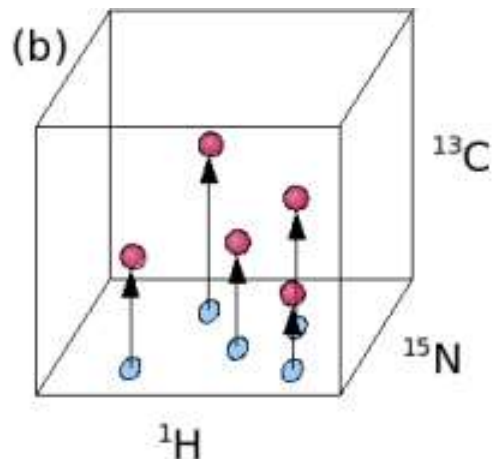


Visualising 3D Spectra in Planes

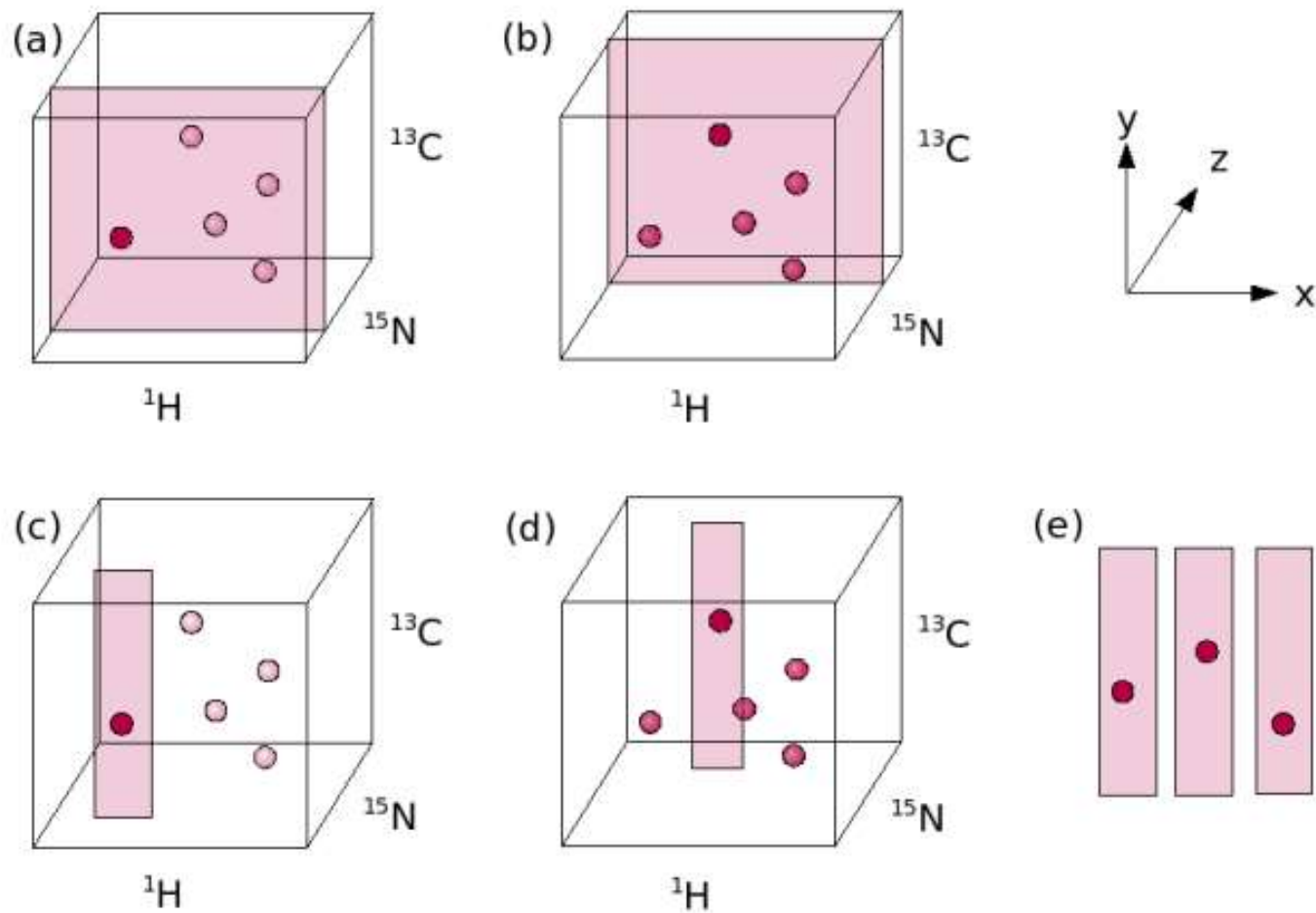
HSQC



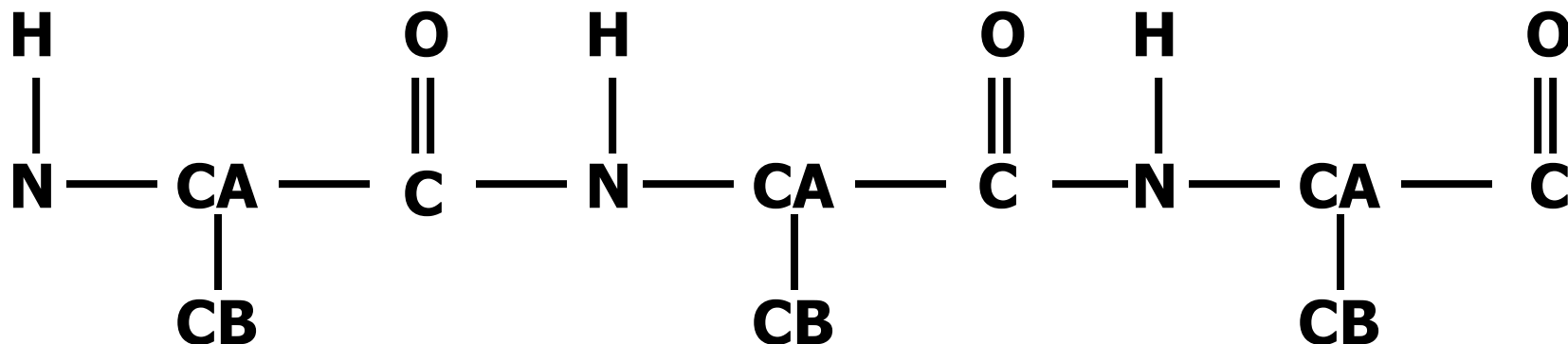
HNCO



Visualising 3D Spectra in Strips



Backbone and $C\beta$ assignment

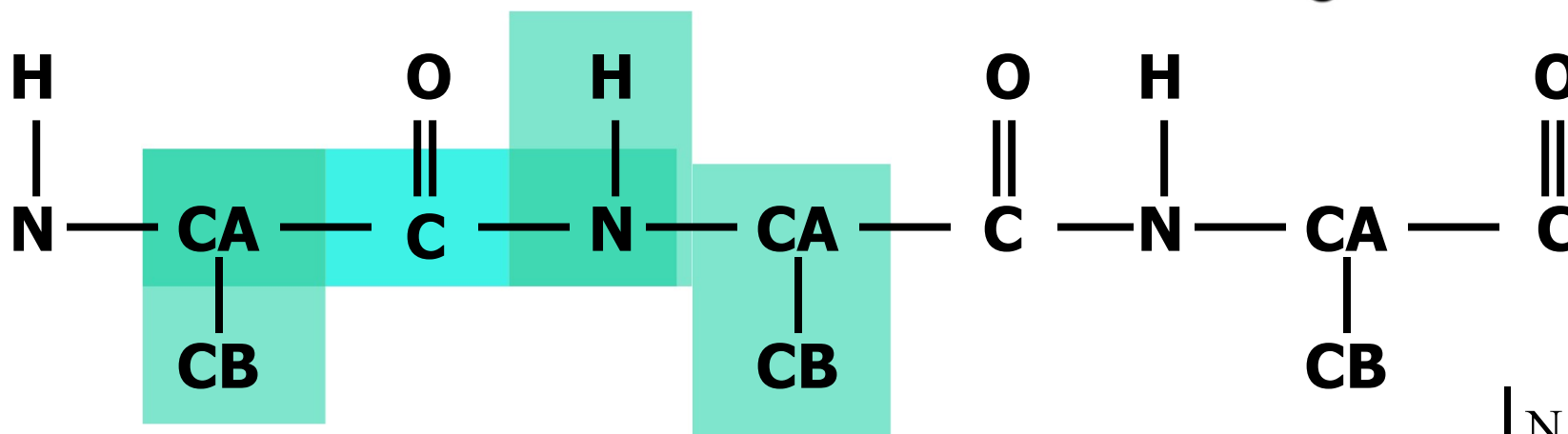
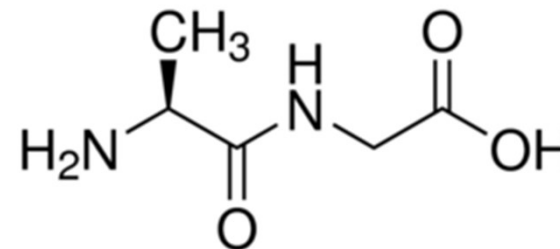


In principle, it can be done ONLY with two experiments:

CBCA(CO)NH

CBCANH

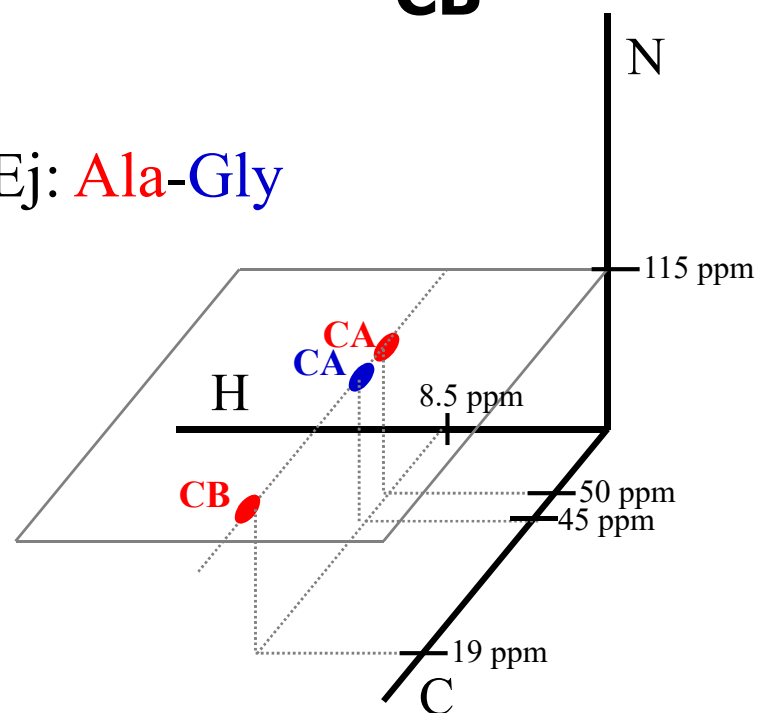
CBCANH



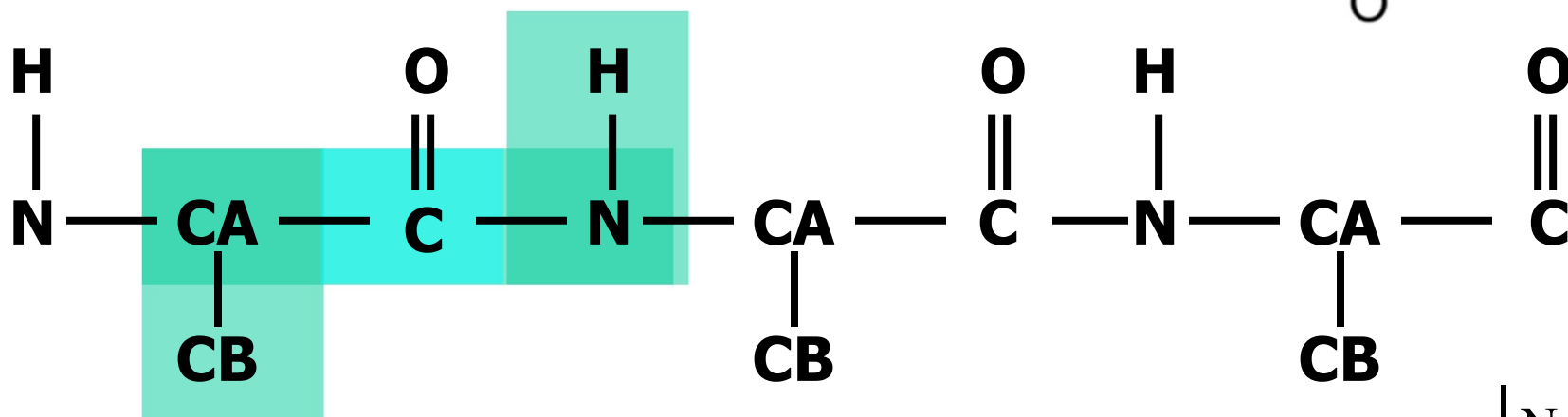
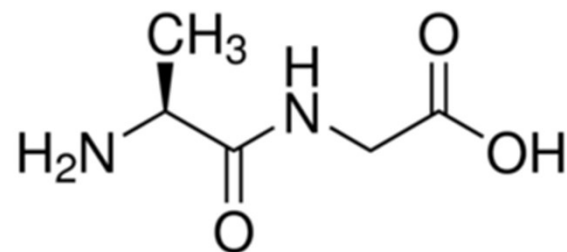
Correlates:

- H_i in one dimension
- N_i in other dimension
- CB_{i-1} , CB_i , CA_{i-1} and CA_i in the third dimension

Ej: **Ala**-**Gly**



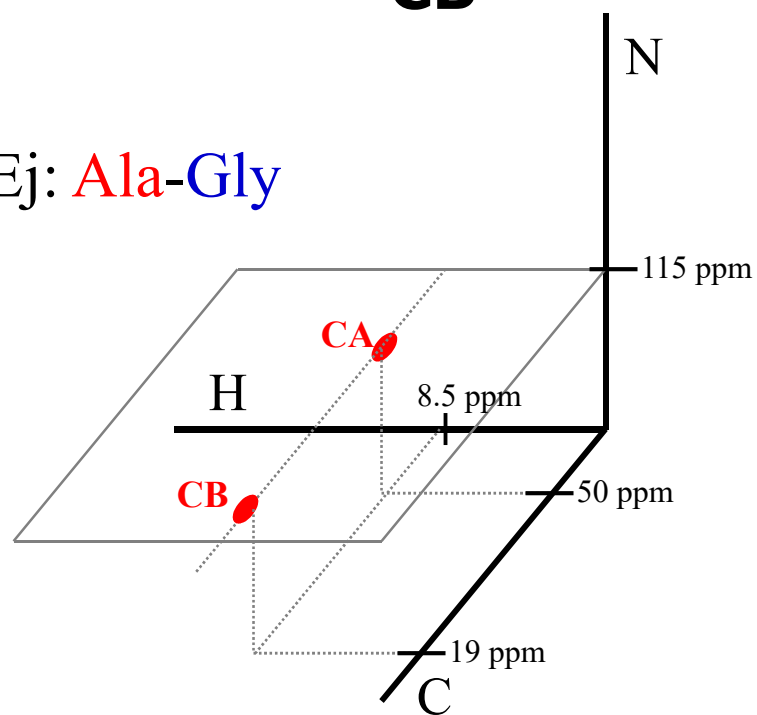
CBCA(CO)NH



Correlates:

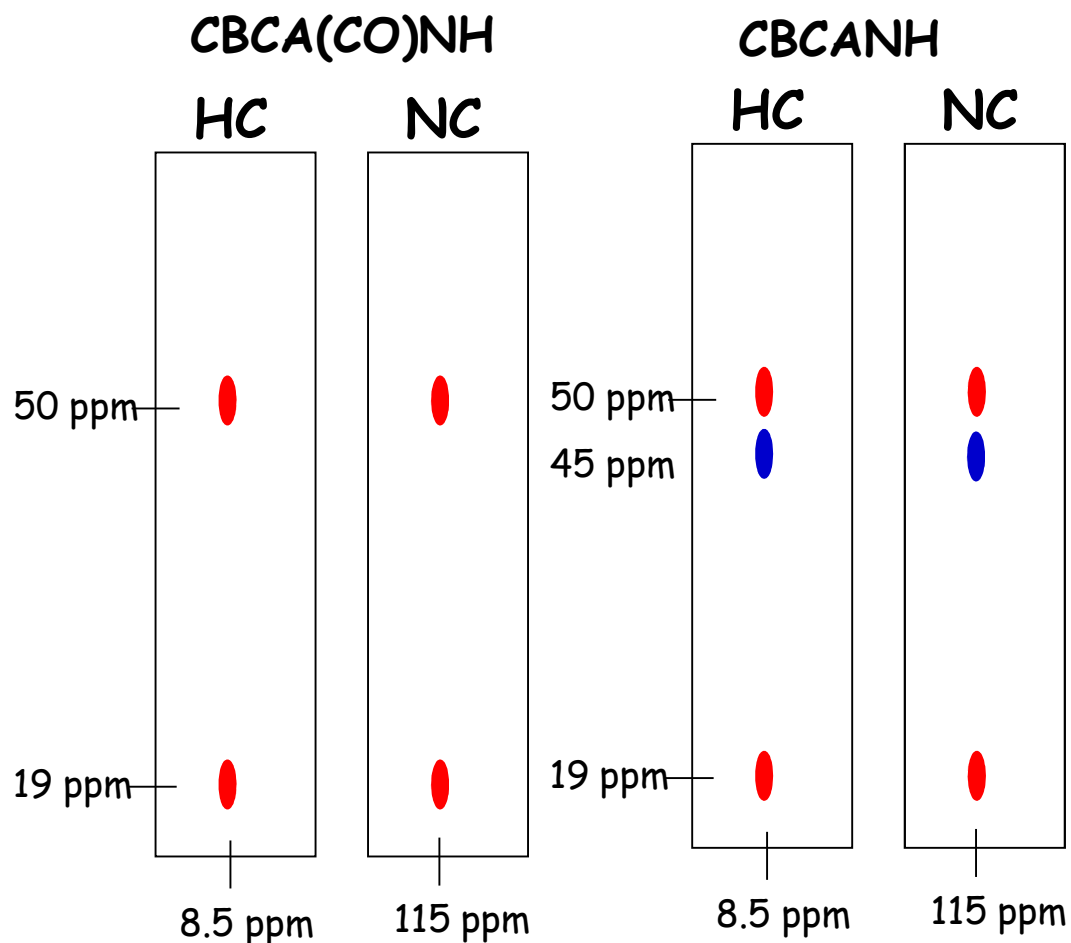
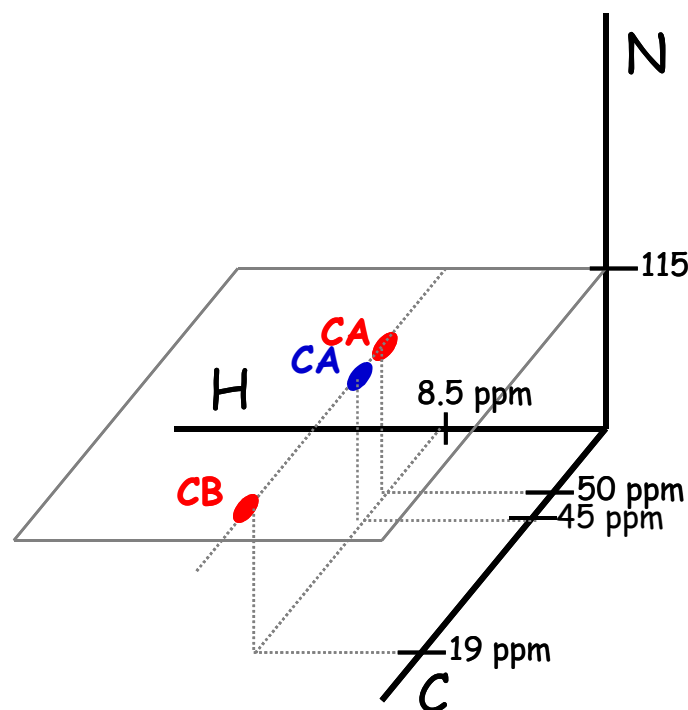
- H_i in one dimension
- N_i in other dimension
- CB_{i-1} , and CA_{i-1} in the third dimension

Ej: **Ala**-**Gly**

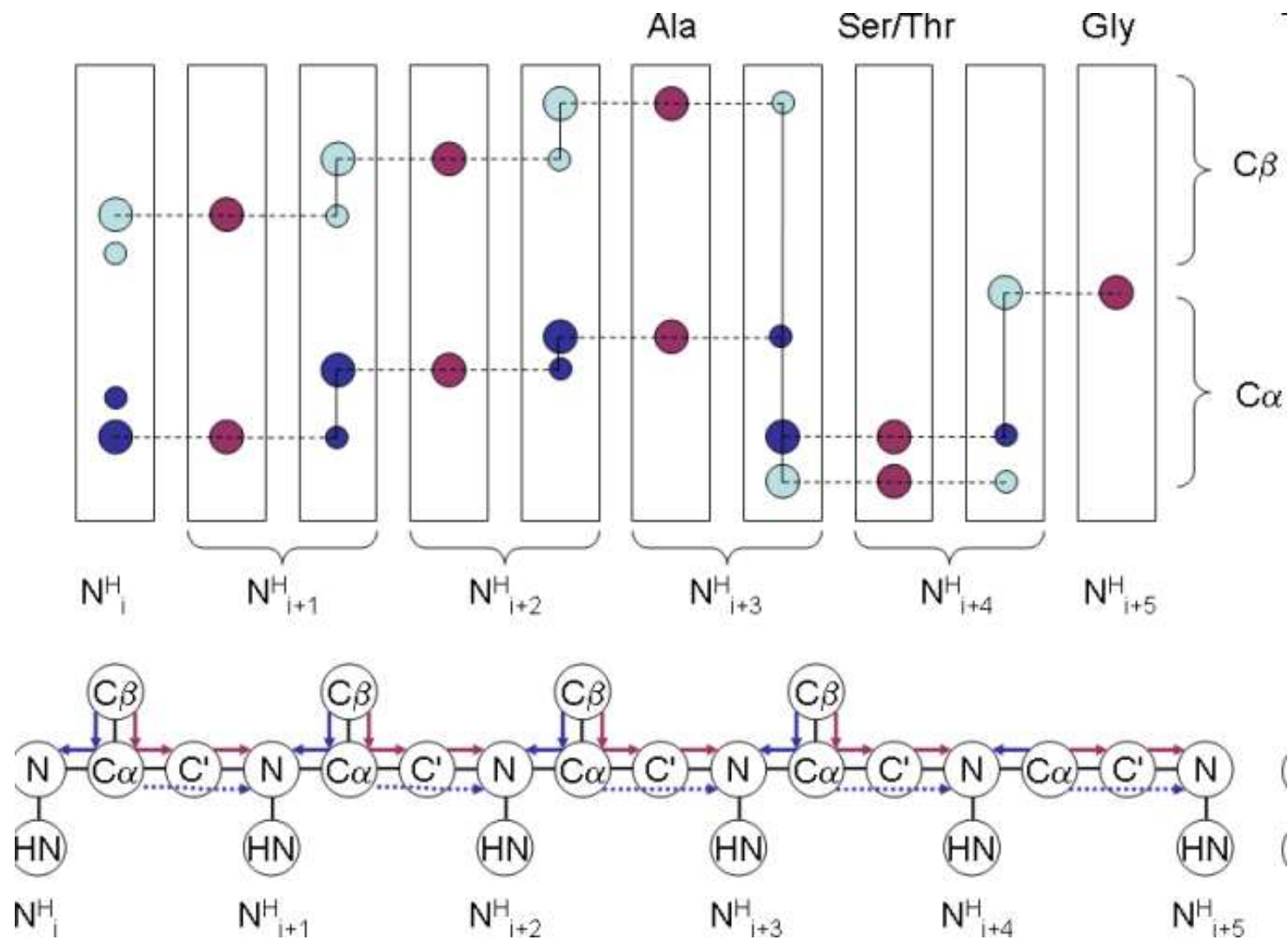


Looking at the HC or NC planes

Ej: **Ala**-**Gly**



Alternation between CBCANH and CBCA(CO)NH spectra ($C\alpha$ s in dark blue, $C\beta$ s in light blue)



HNCO

HN(CA)CO

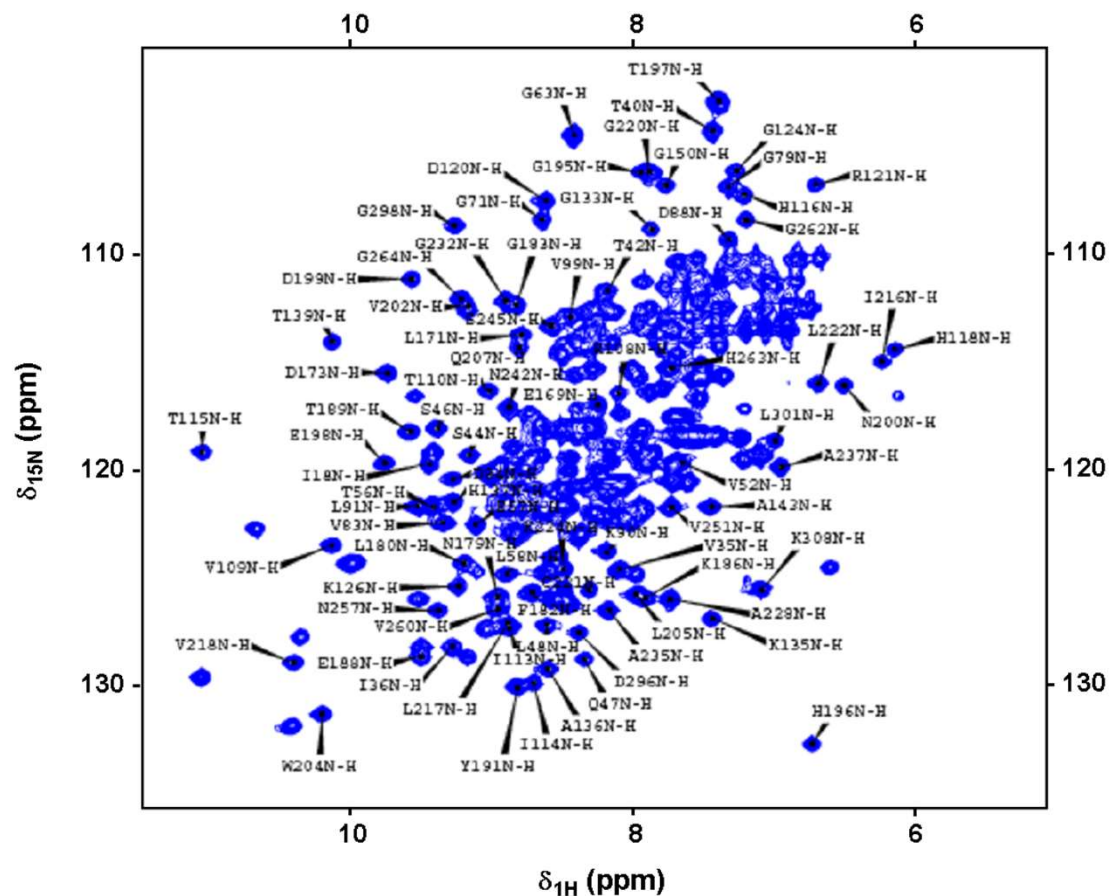
HN(CO)CA

HNCA

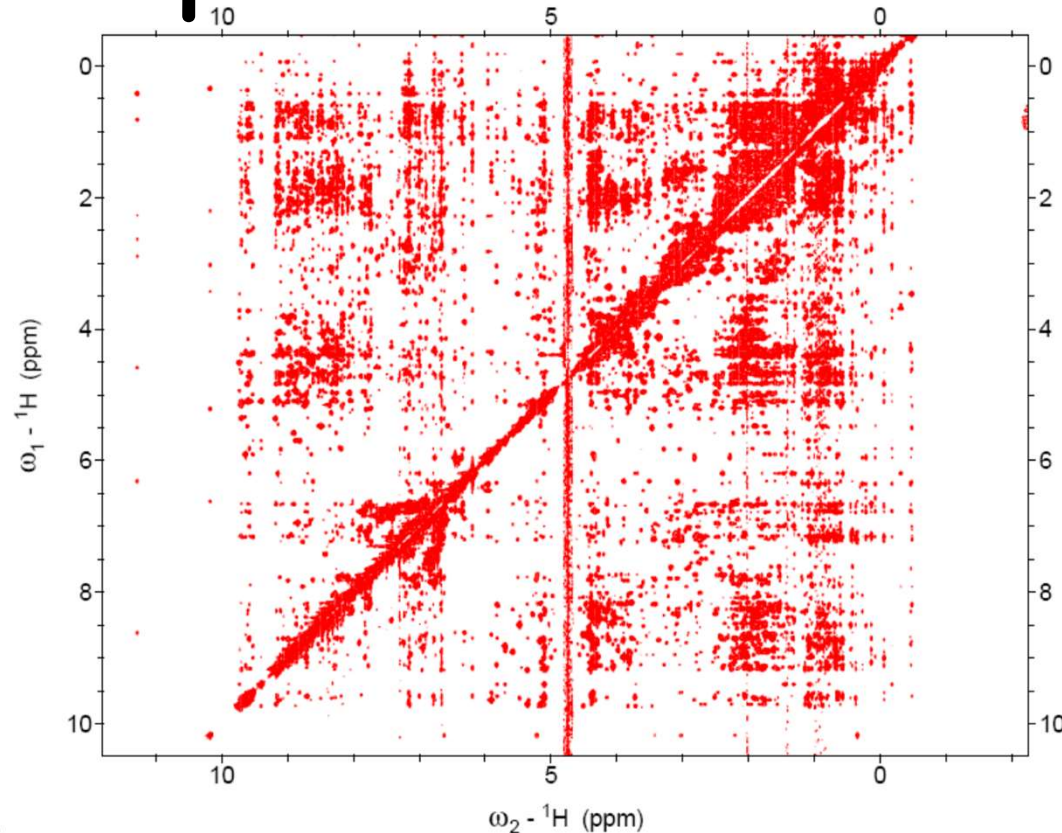
HN(CO)CACB

HNCACB

HNHA

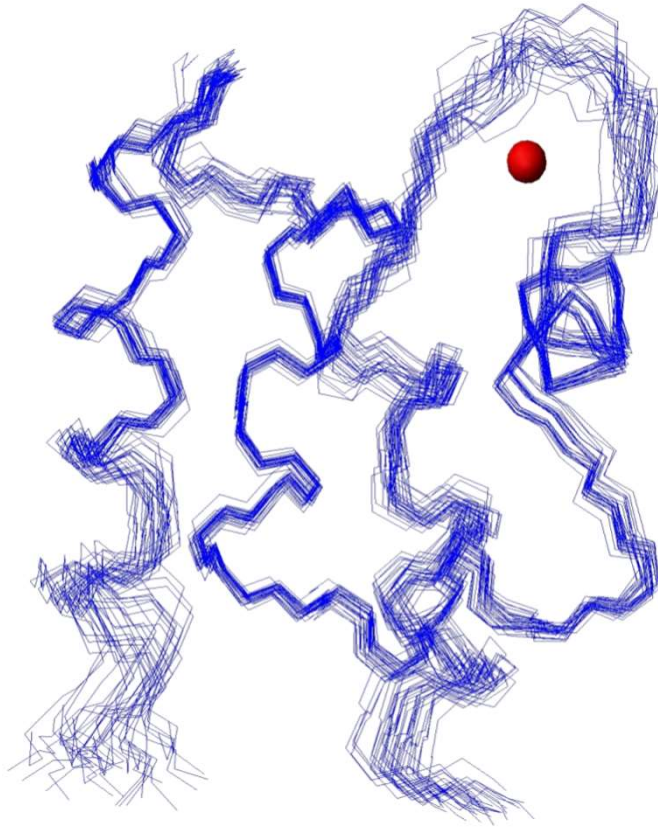


NOEs are needed to solve a protein structure

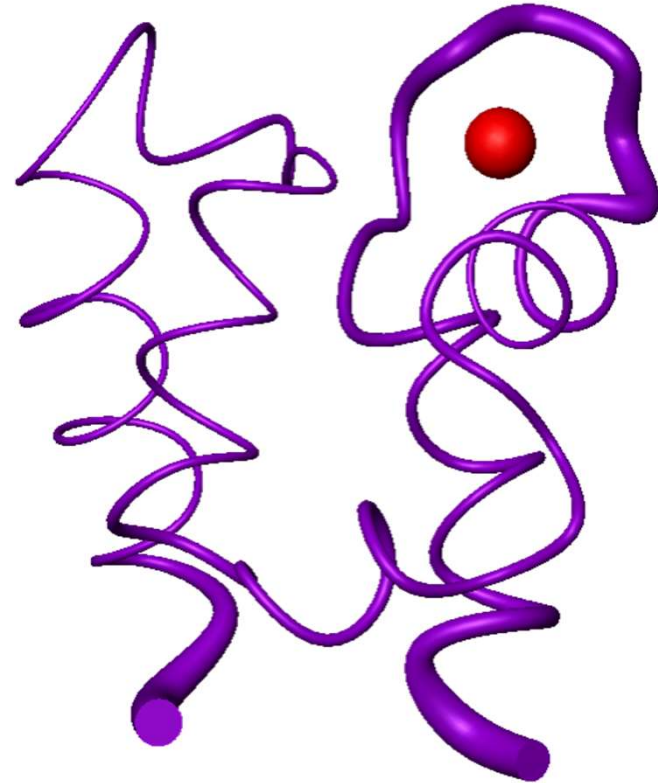


ATNOS: automated NOESY peak picking

Family of NMR-based Structures

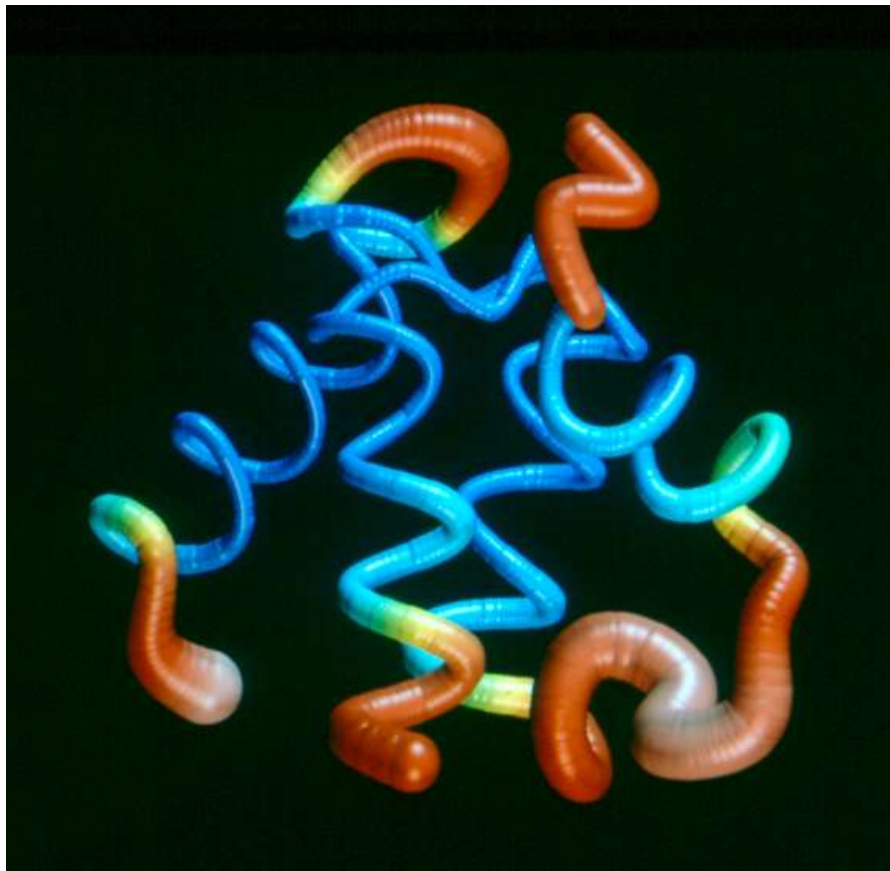


Family of structures



Sausage representation

**Is NMR useful only for determining
Protein 3D Structures?**



The structure is important, but...

*"The picture of a horse does not
tell you how the horse runs"*

(Jeremy Knowles)

Relaxation occurs mostly by dipolar coupling

¹⁵N as the dynamic probe:

$$\begin{aligned}\frac{1}{T_1} &= d^2 \frac{1}{10} [J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H + \omega_N)] + \frac{2}{15} \omega_N^2 \Delta\sigma^2 J(\omega_N) \\ \frac{1}{T_2} &= \frac{1}{20} d^2 [4J(0) + J(\omega_H - \omega_N) + 3J(\omega_N) + 6J(\omega_H) + 6J(\omega_H + \omega_N)] + \frac{1}{45} \omega_N^2 \Delta\sigma^2 [3J(\omega_N) + 4J(0)] + R_{ex} \\ NOE &= 1 + T_1(\gamma_H/\gamma_N) d^2 \frac{1}{10} [6J(\omega_H + \omega_N) + 4J(\omega_H - \omega_N)]\end{aligned}$$

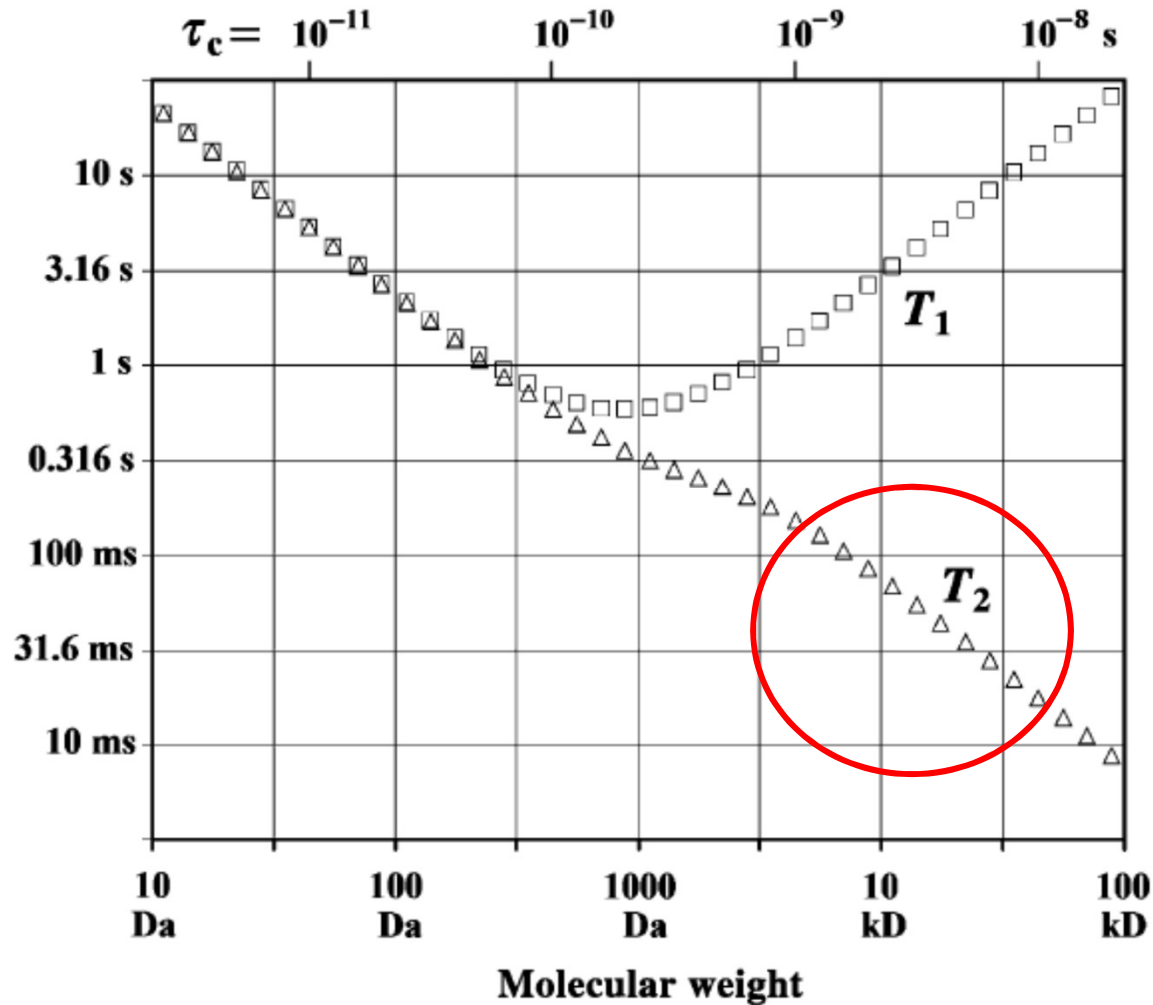
Proteins

$$J(\omega) = \frac{2\tau_c}{1 + \omega^2 \tau_c^2} \quad d^2 = (\hbar^2 \gamma_I^2 \gamma_S^2 / r^6)$$

NMR seems to be an expensive technique for determining molecular tumbling rates

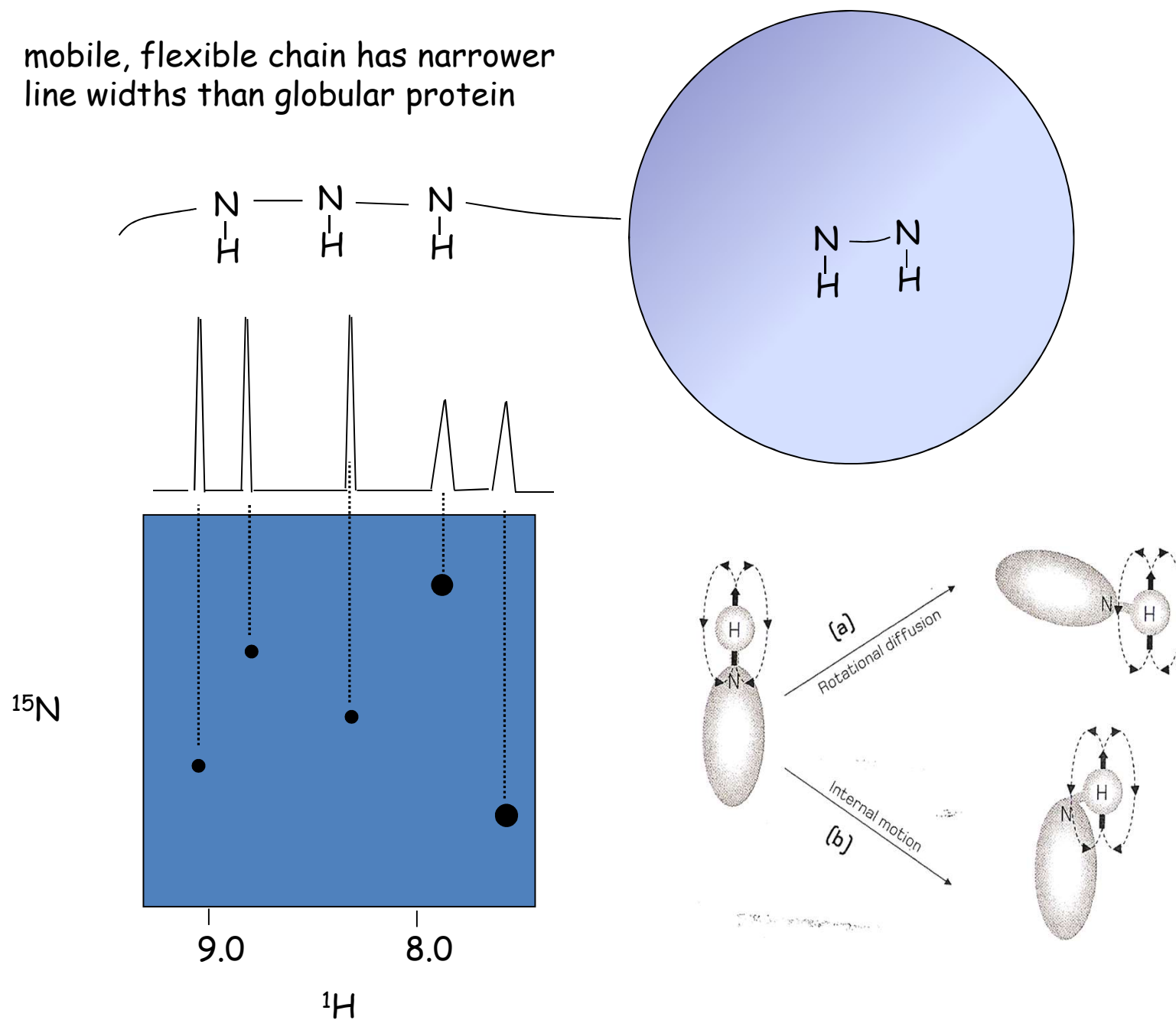
But...if the protein presents internal motions,
 τ_c will not be uniform

Relaxation occurs mostly by dipolar coupling

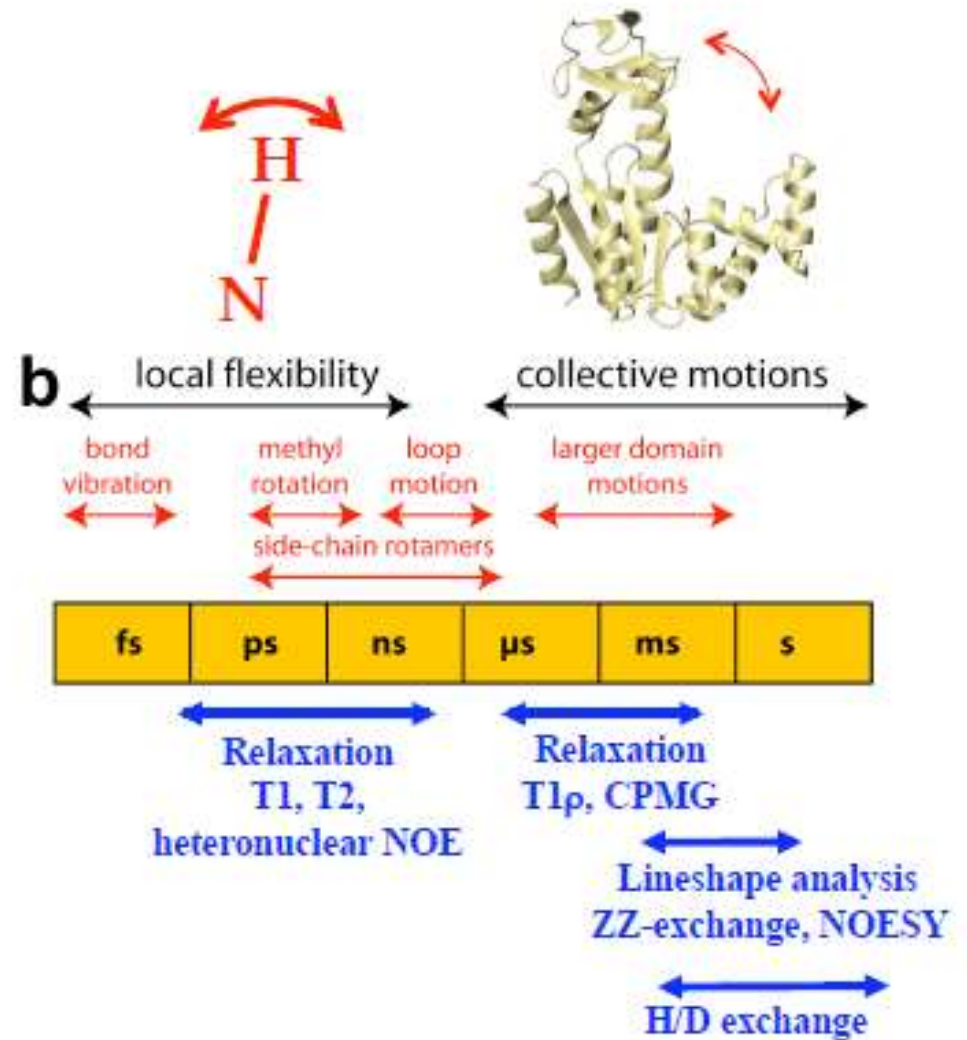
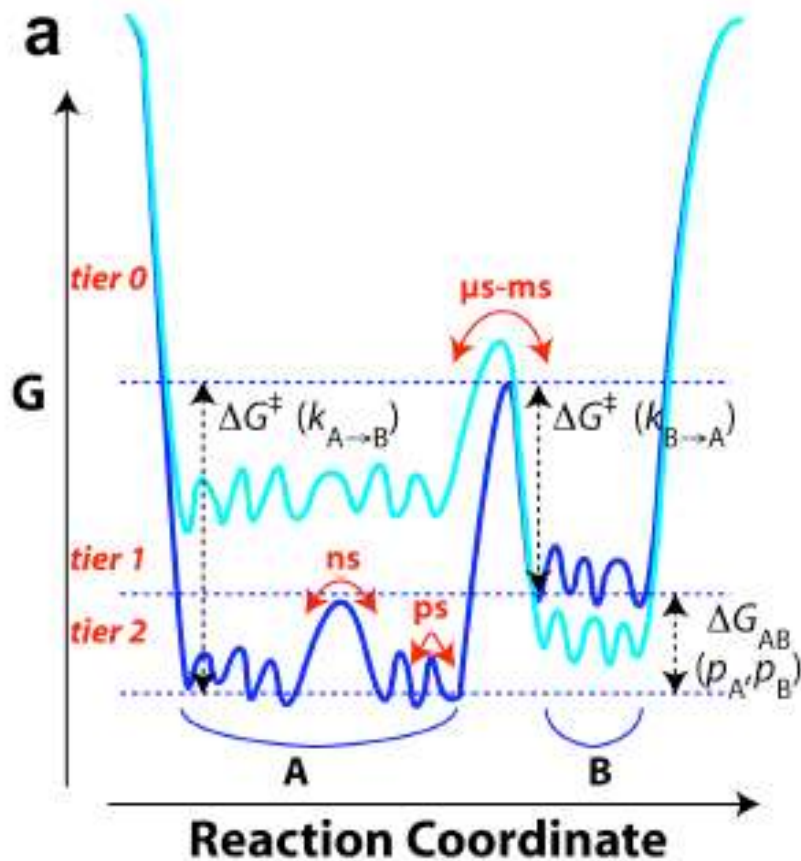


Oscillating magnetic fields arise from molecular tumbling
Relaxation efficiency depends on molecular mass

mobile, flexible chain has narrower line widths than globular protein



Protein Dynamics by NMR



NMR can sample dynamics from 2 regimes: fast and slow

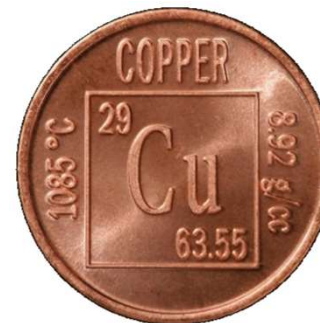
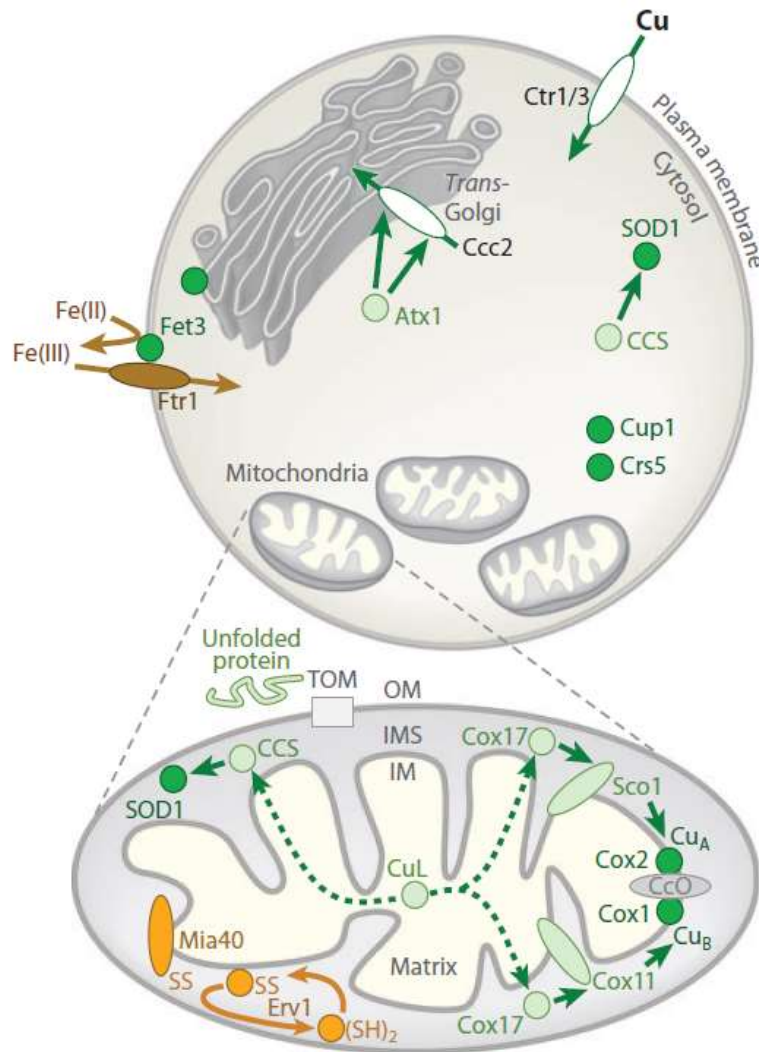
How protein motions affect NMR parameters depend on whether they are faster or slower than the rotational correlation time

- **Fast Timescale dynamics (ps-ns)**
 - limited by rotational correlation time of protein
 - parameters describe distribution of states
- **Slower Timescale dynamics (μ s-ms)**
 - require chemical shift difference
 - measured more directly

Copper in the cell

Copper is essential but toxic at the same time

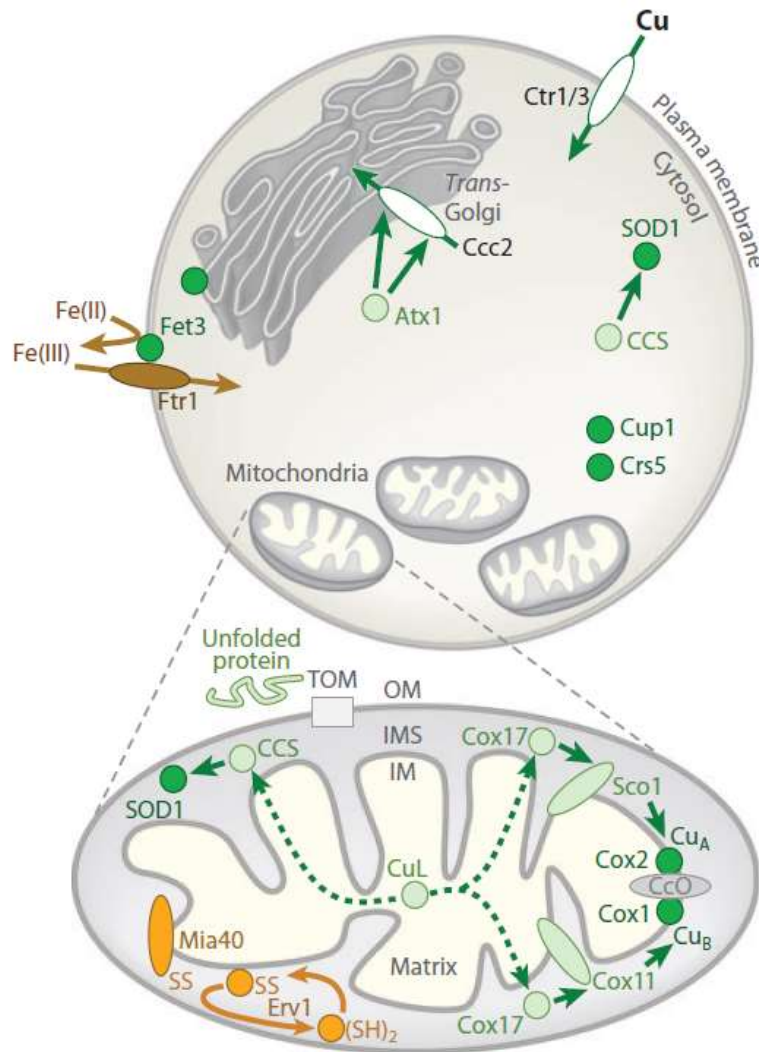
Copper levels should be regulated within the cell



Robinson N.J., Winge D.R. *Annu. Rev. Biochem.* (2010)

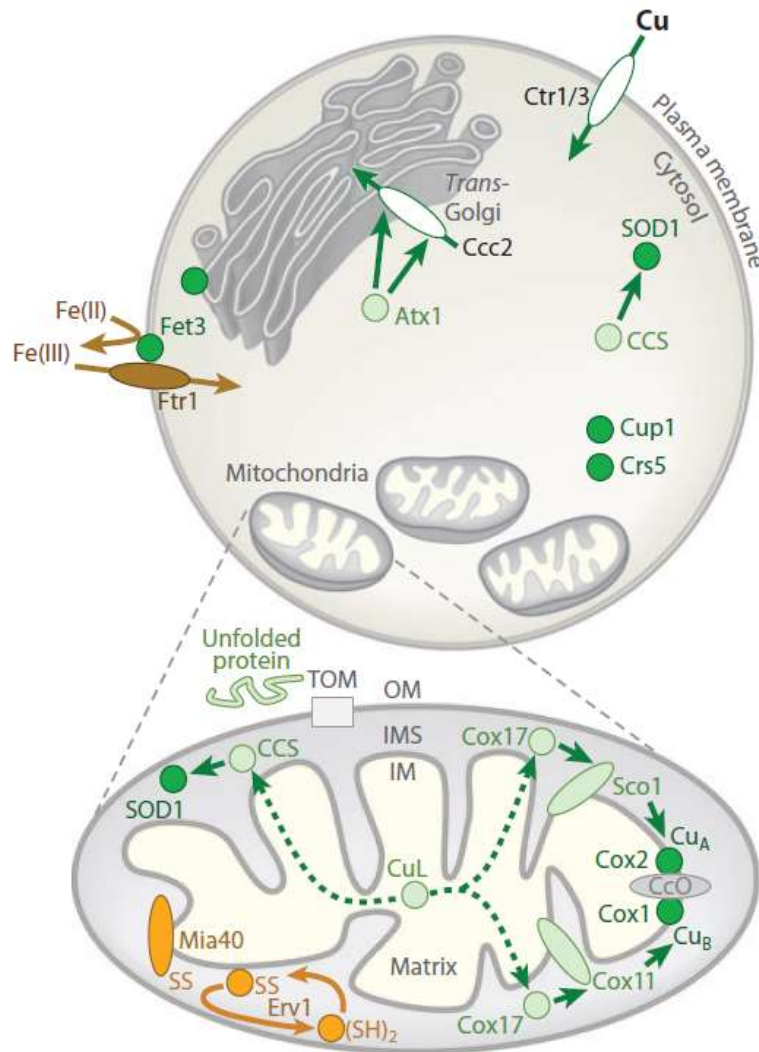
Copper in the cell

There's no free copper in the cell!
(O'Halloran)



Robinson N.J., Winge D.R. *Annu. Rev. Biochem.* (2010)

Copper in the cell

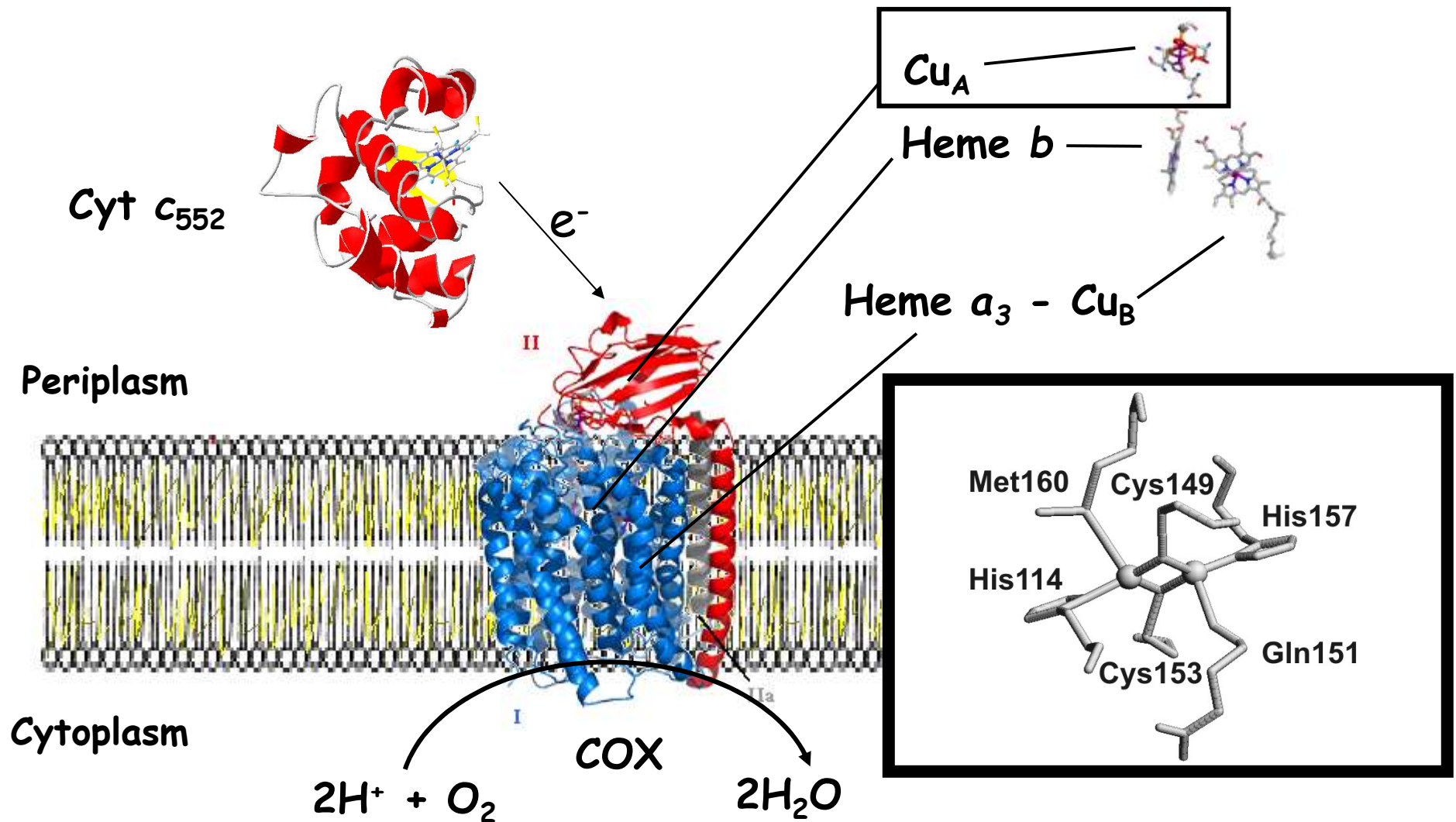


Metallochaperones:
Involved in the transport
and delivery of copper ions
to their target proteins

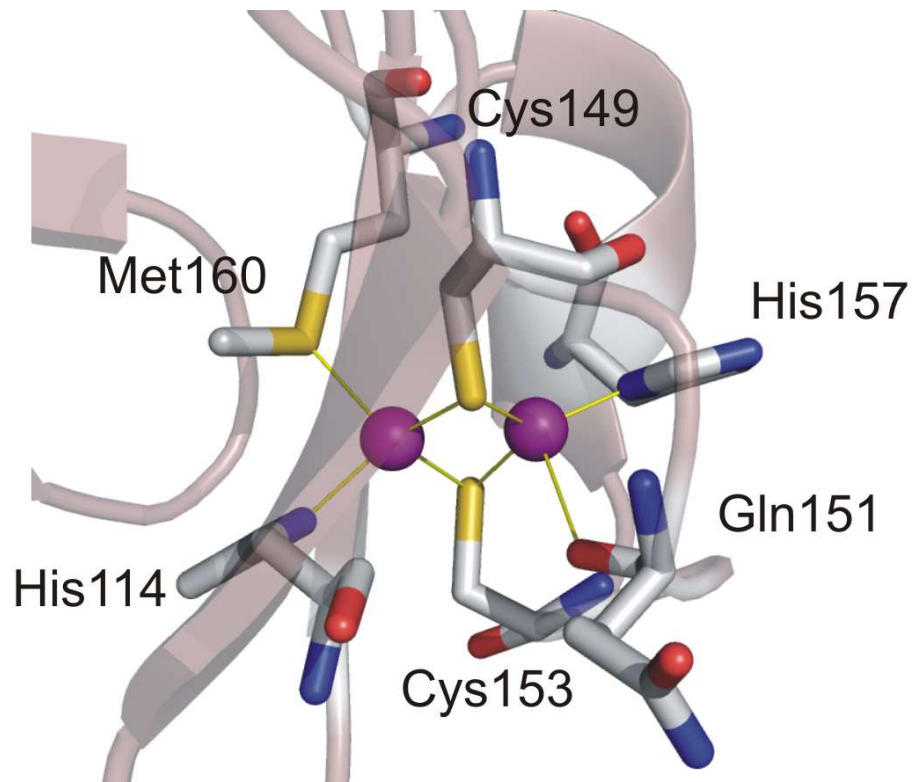


Robinson N.J., Winge D.R. *Annu. Rev. Biochem.* (2010)

Cytochrome c Oxidase: paradigm for LRET

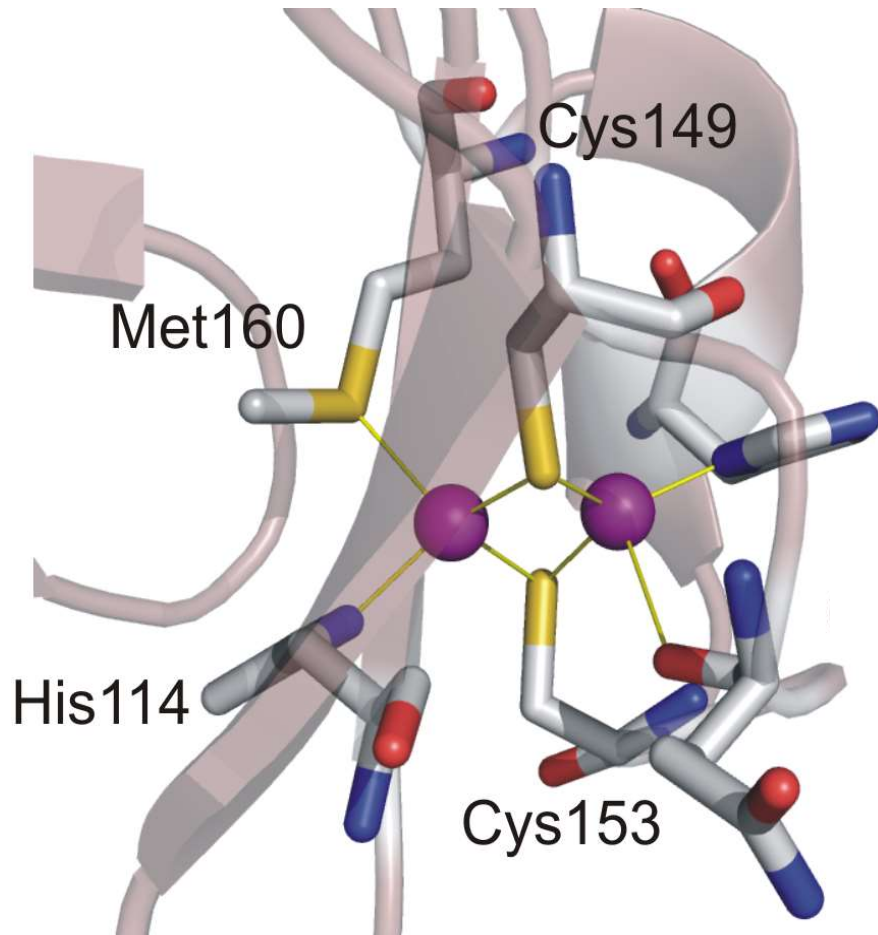


The Cu_A Site



- *How are the metal ions inserted in vivo in the protein?*

Cu_A and Sco proteins



Yeast

Cox17 and Sco are involved in
Cu_A assembly
Glerum et al. (1996) JBC

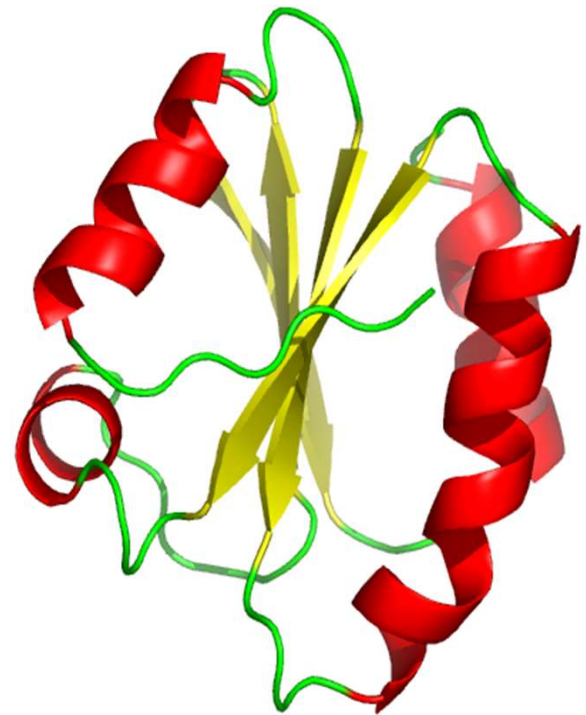
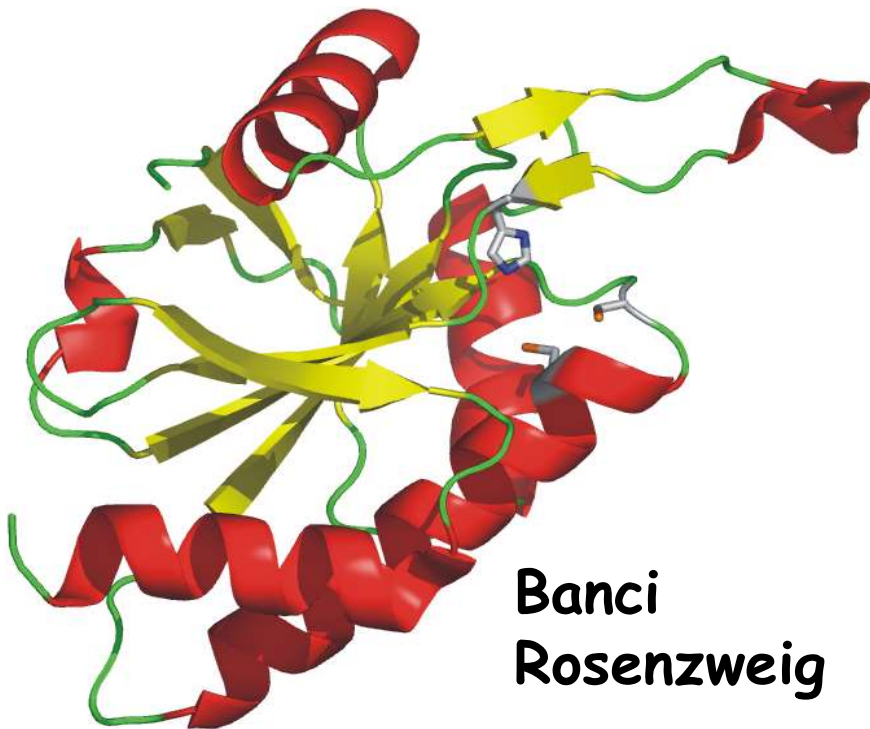
B. subtilis

Sco is essential for
Cu_A uptake in COX
Mattatall et al. (2000) JBC

Humans

Mutations in Sco1 and Sco2 result
in severe COX deficiencies
related to copper availability
Winge, Leary

Which is the role of Sco?



Thioredoxin fold - **CXXXCP** motif

Additional loop: **His** residue

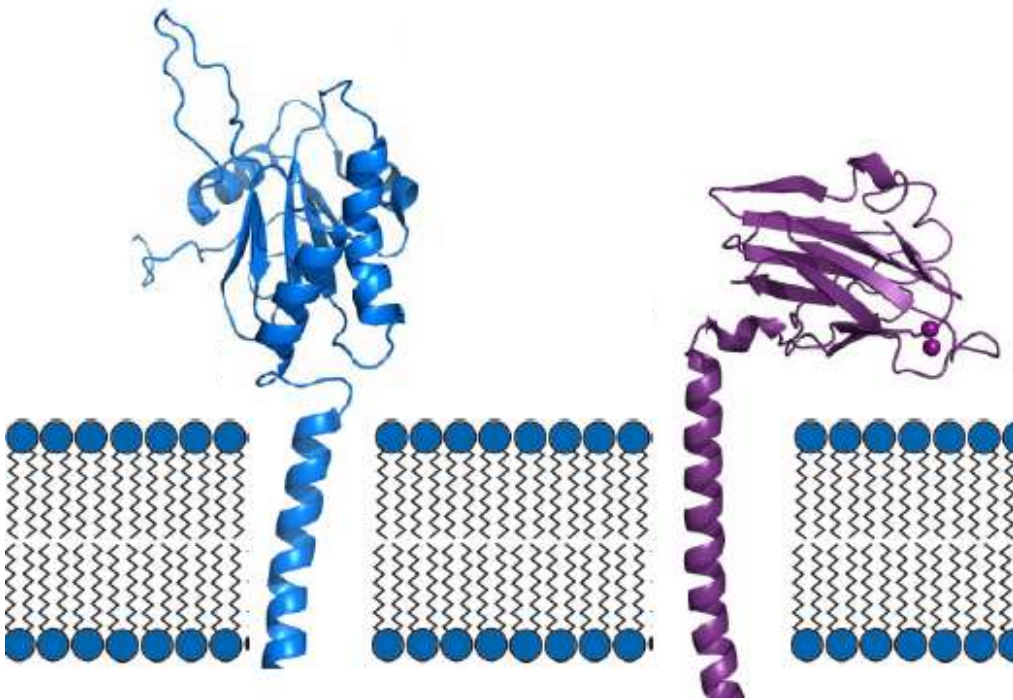
Sco proteins bind Cu(I)

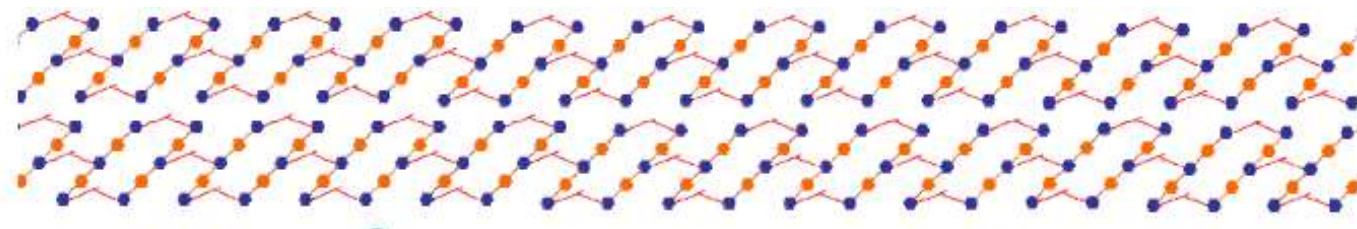
Which is the role of Sco?

A simple system to
test copper transfer
in vitro

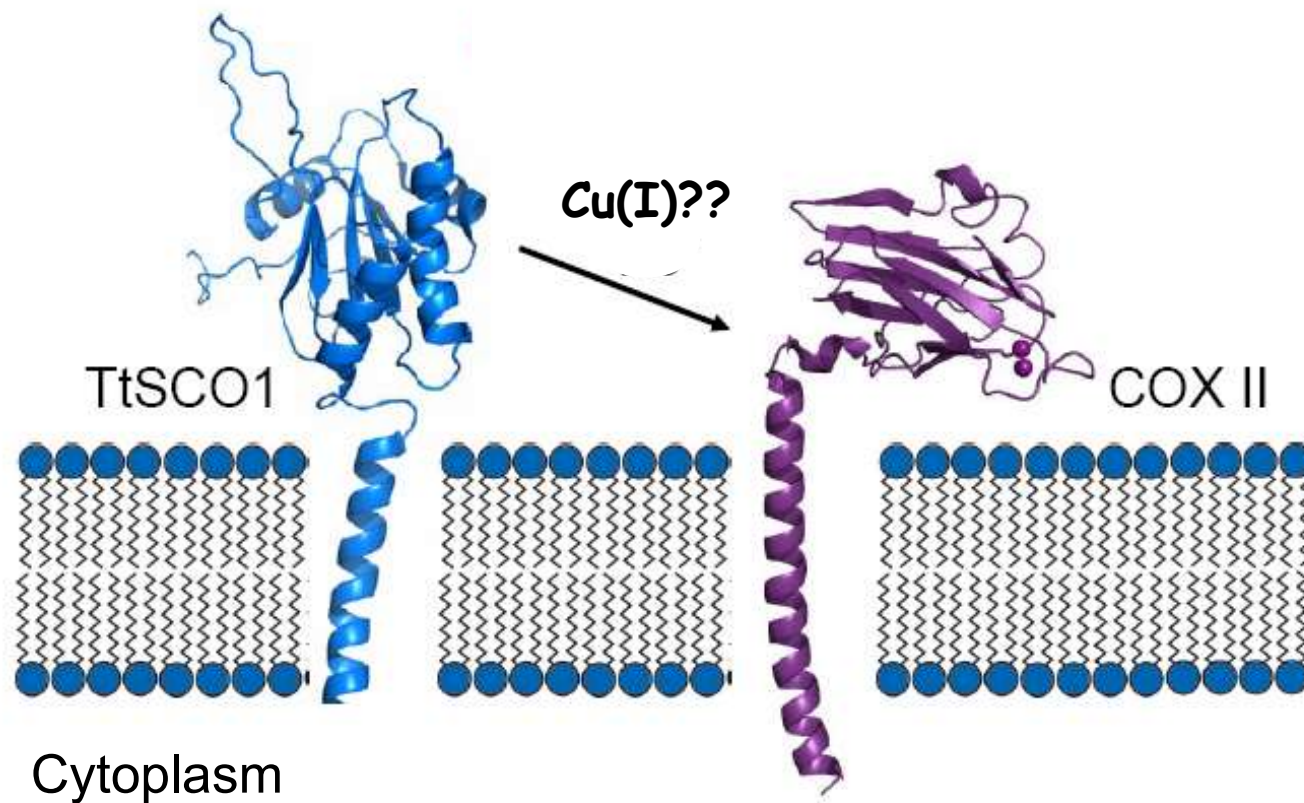
Soluble Sco and
COX II domains

NMR





Periplasm



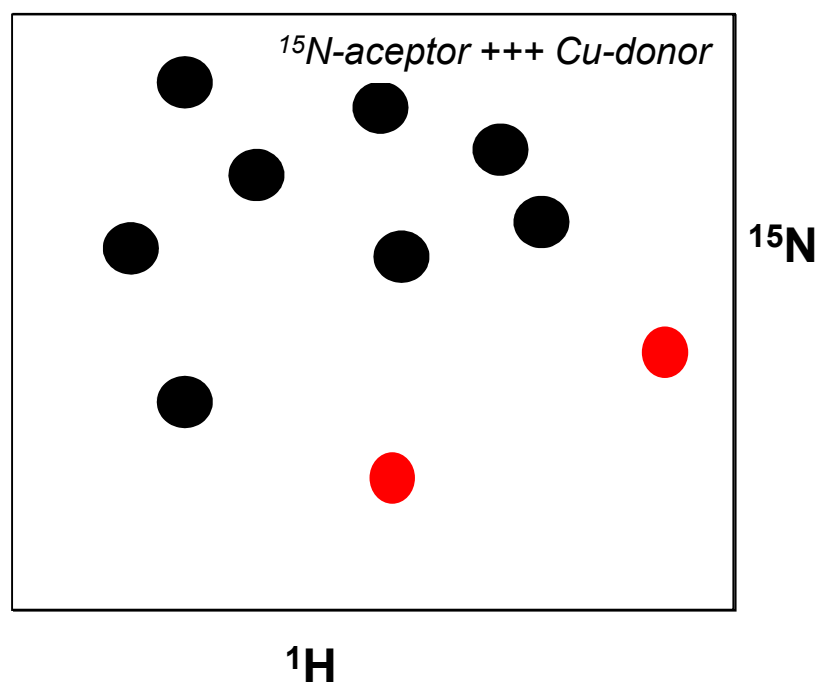
Why NMR?

- Identify the copper transfer process with atomic resolution
- Assess copper binding to the native binding sites
- Monitor protein folding-unfolding and order-disorder events
- Selective labeling: Look one protein at the time in complex mixtures
- We do NMR!

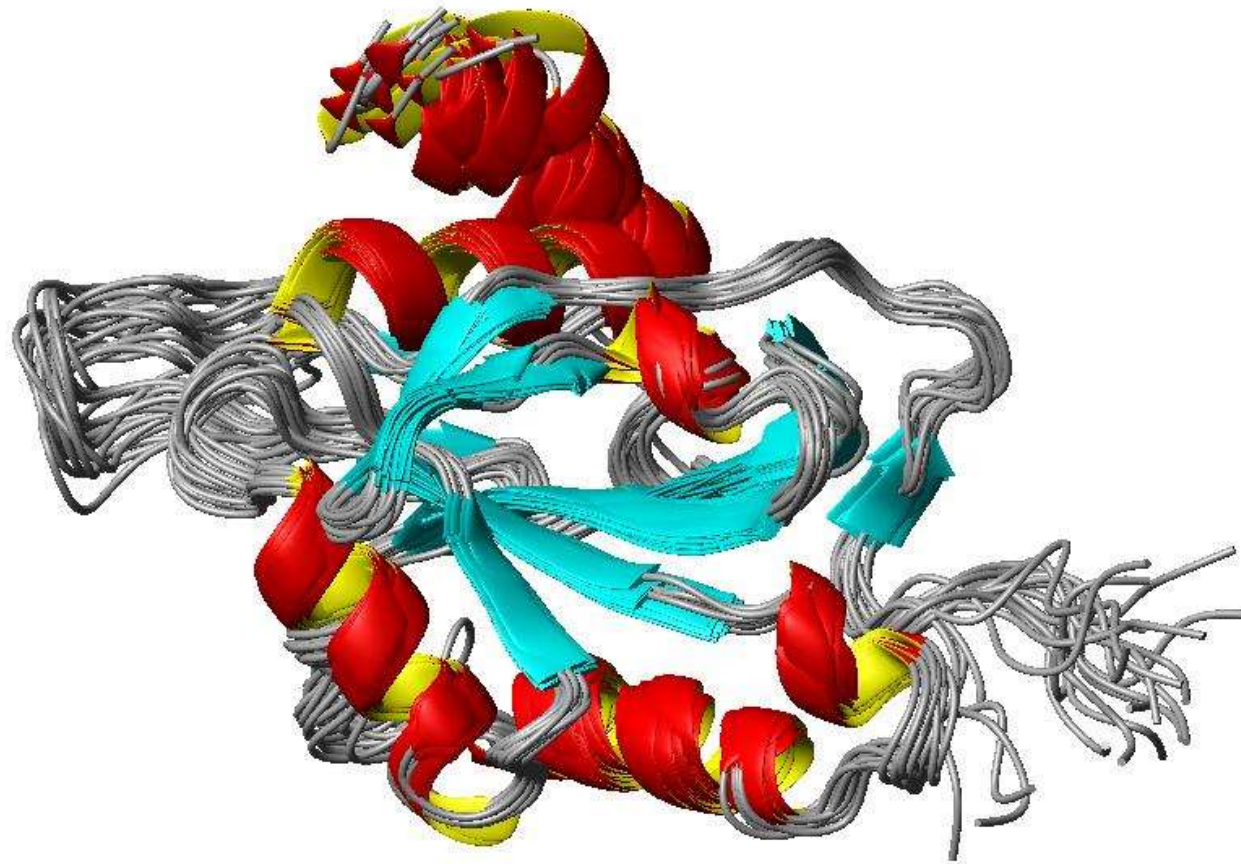
Copper Transfer followed by NMR

We can exploit NMR to follow the copper uptake or release for a specific protein at a residue level

We need to assign the resonances of the apo and metallated forms

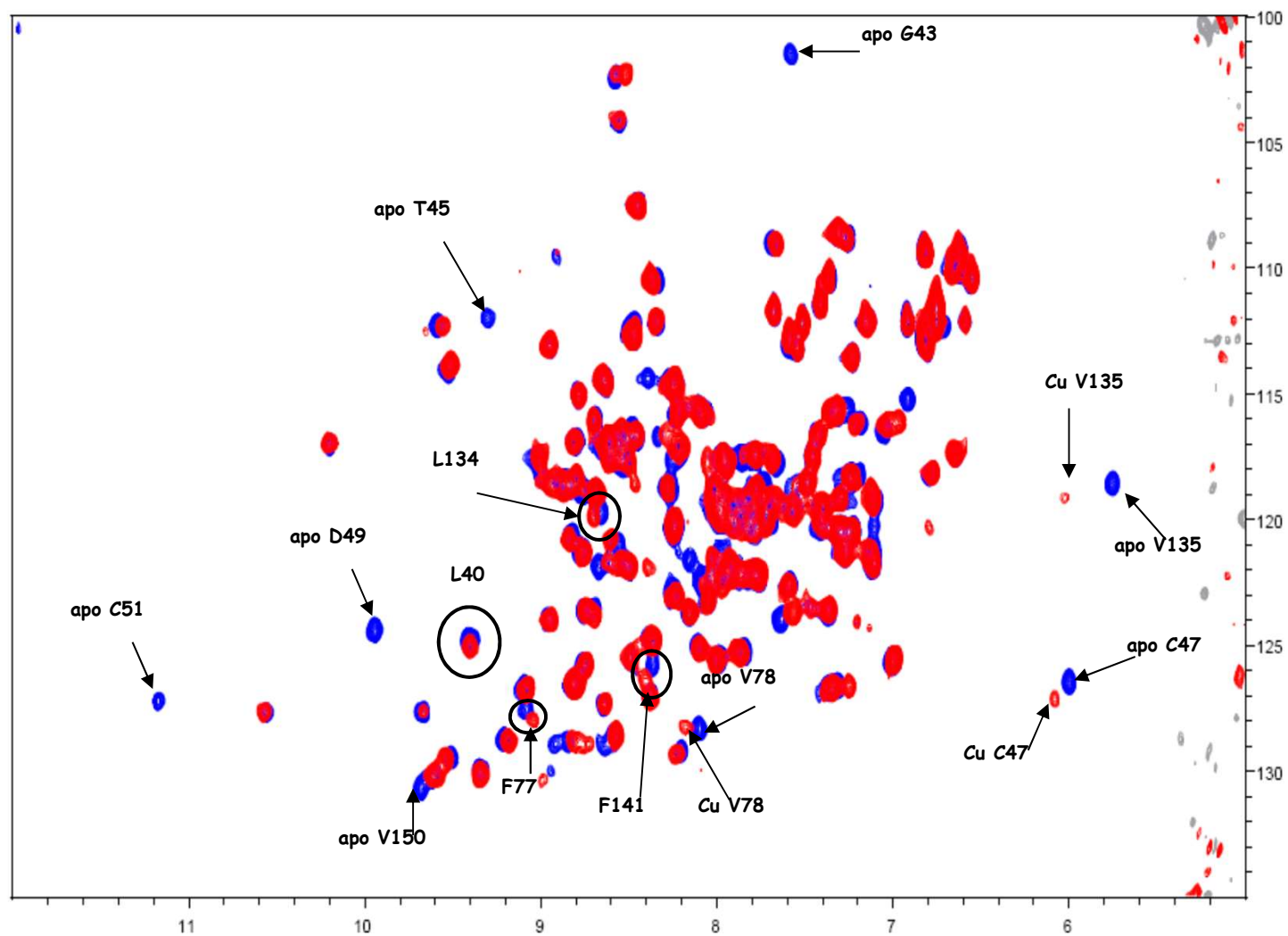


Sco1 from *T. thermophilus*

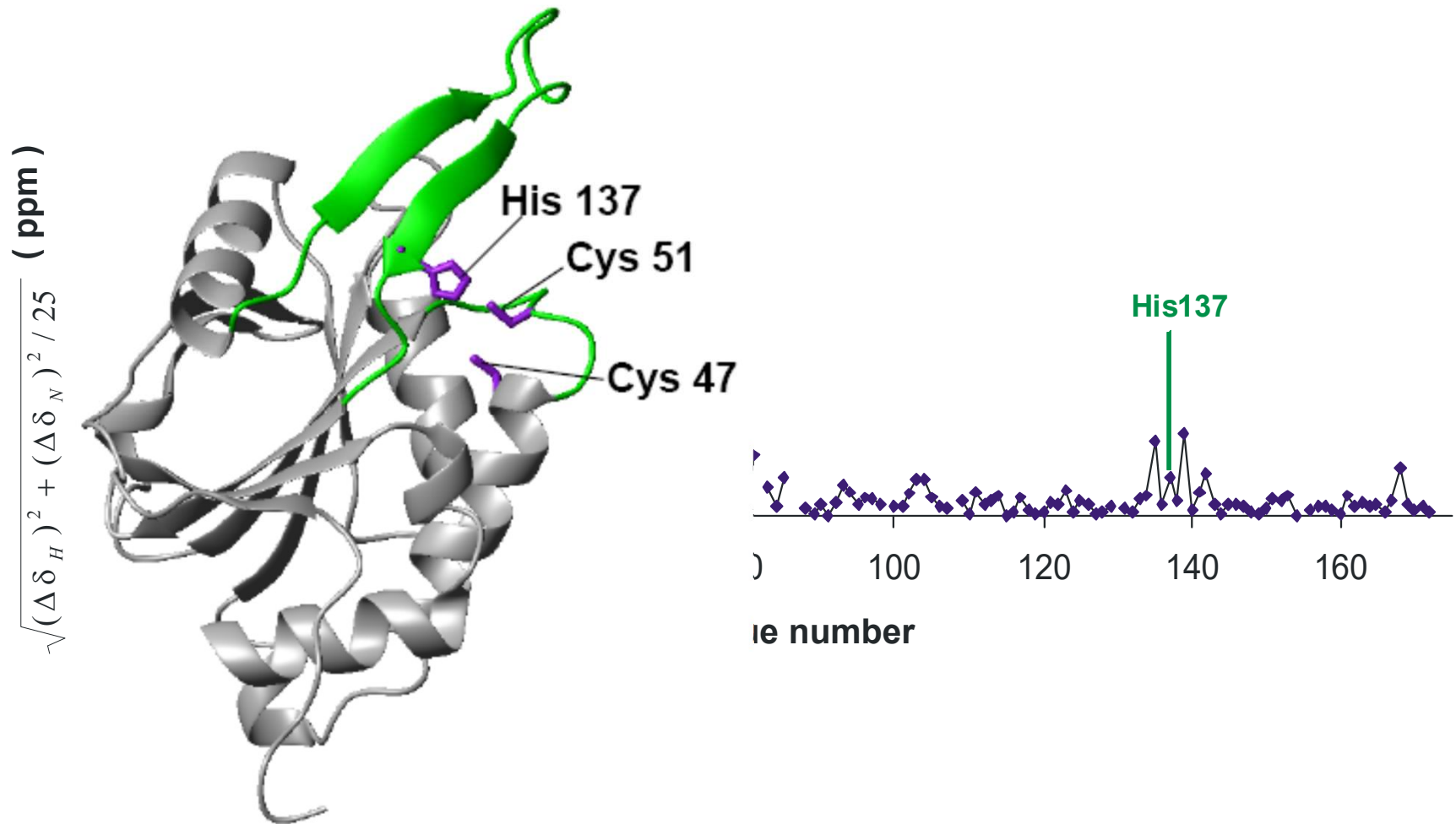


Abriata et al., *Nature Chem.Biol.*, **4**, 599-601 (2008).

ApoSco1 + Cu(I)



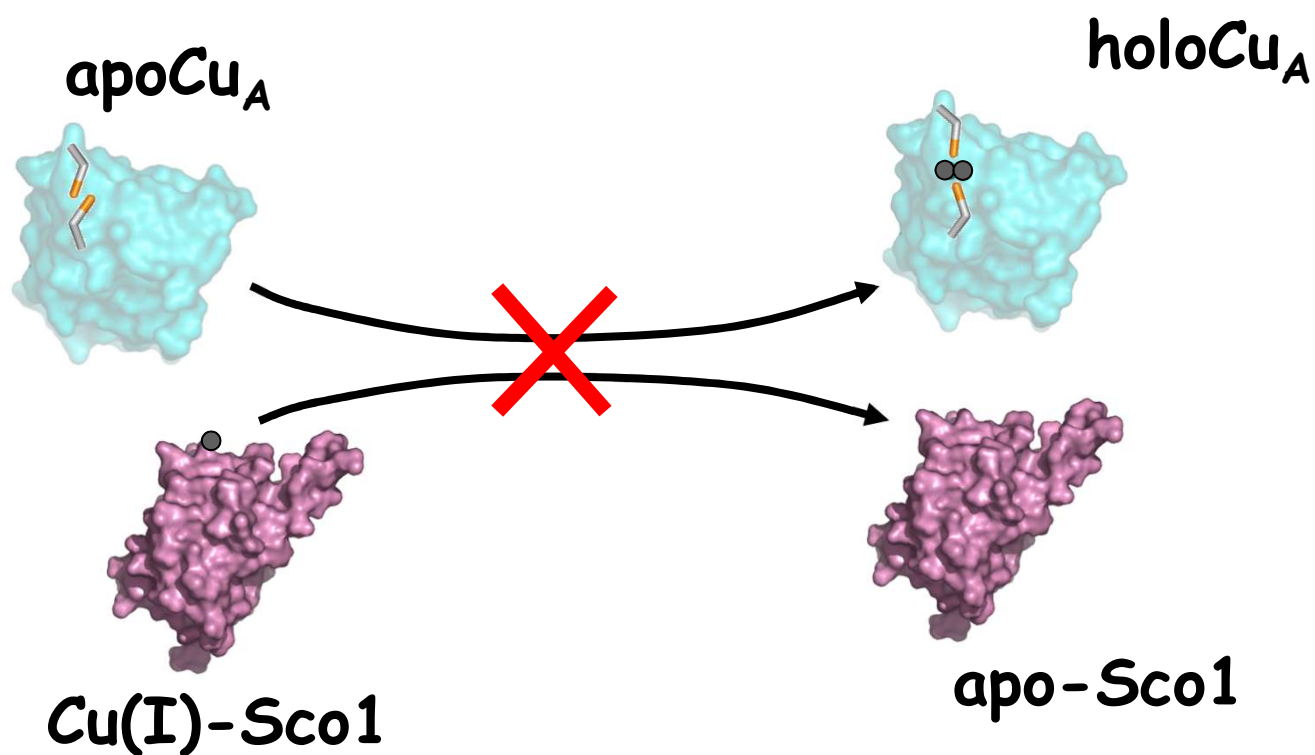
ApoSco1 + Cu(I)

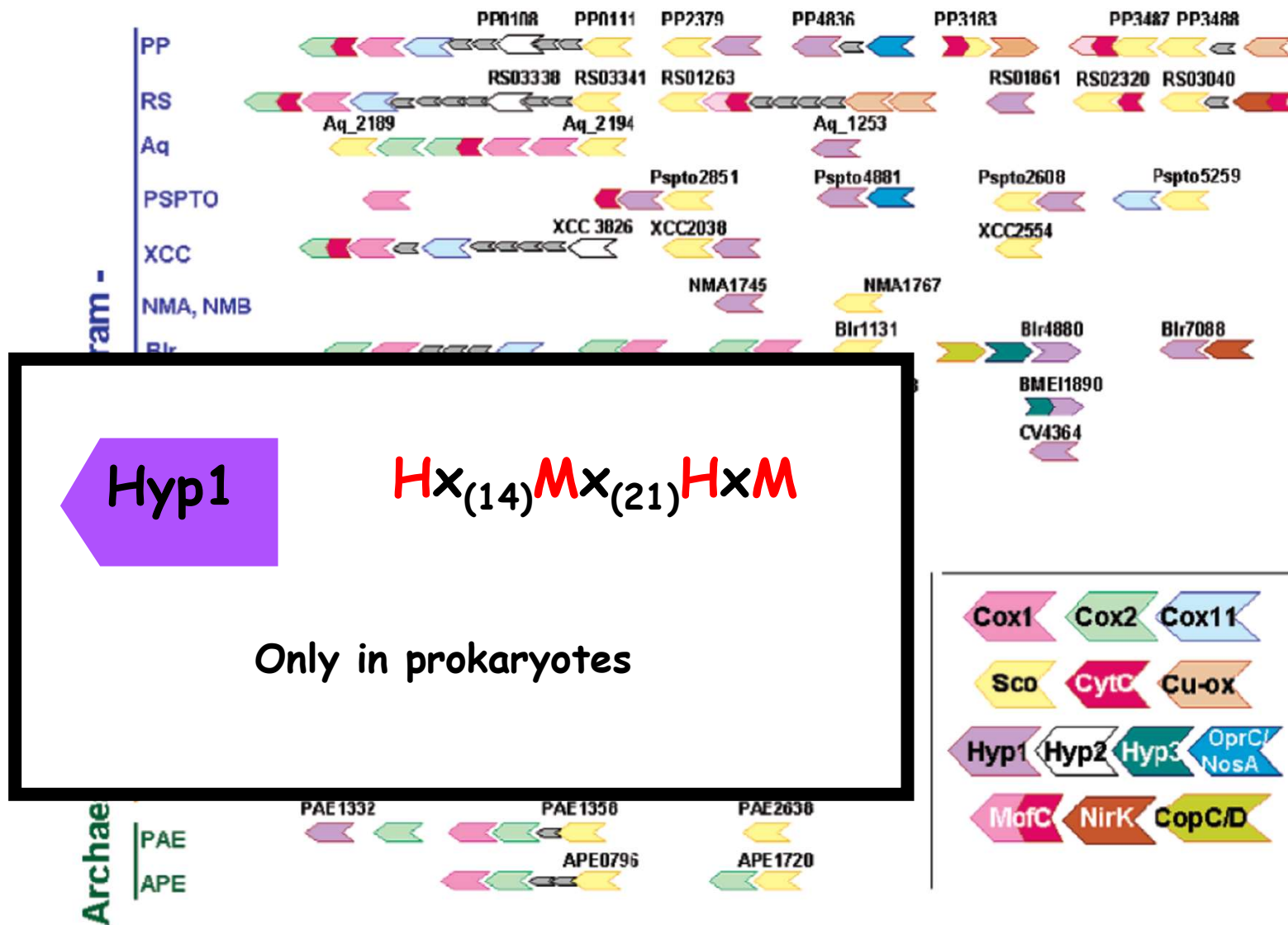


Titration with Cu(I) monitored by HSQC

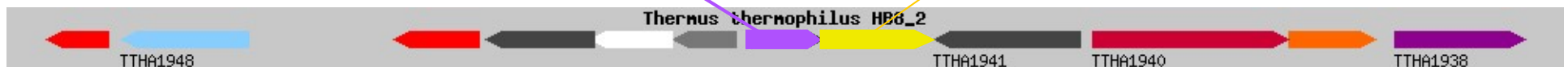
Tt-Sco1

Copper transfer followed by NMR

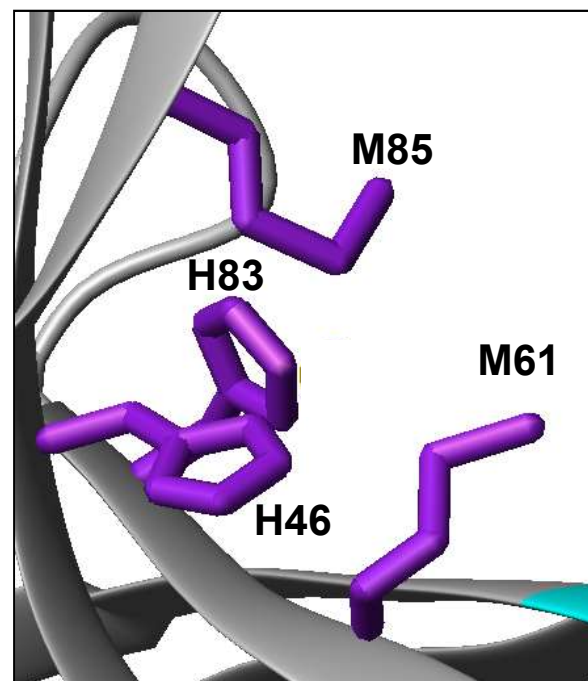
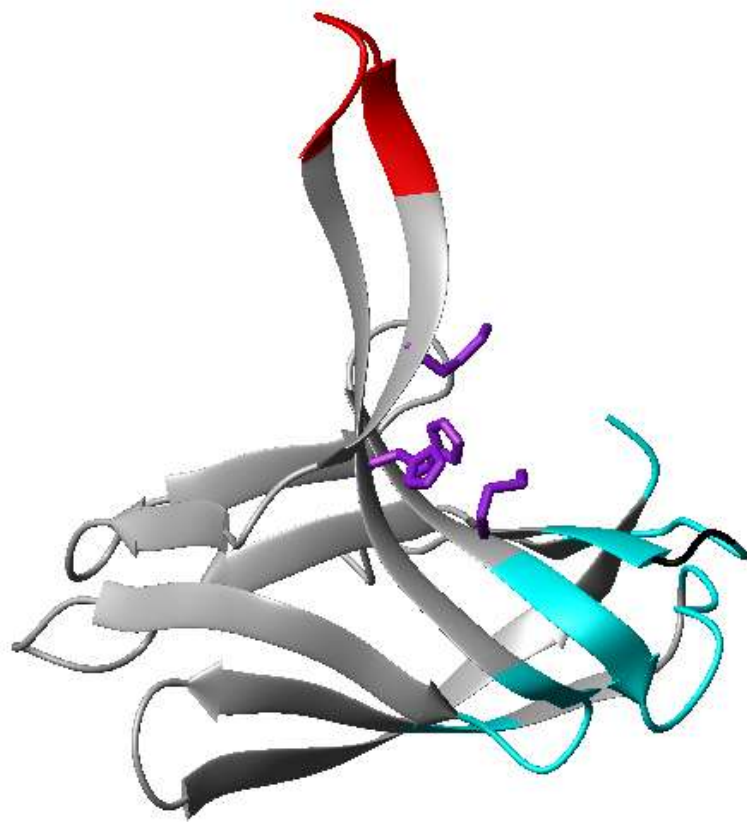




Arnesano F, Banci L, Bertini I, Martinelli M., *J Proteome Res.*;4, 63 (2005)
 (TTHA1943) Sco1 (TTHA1942)

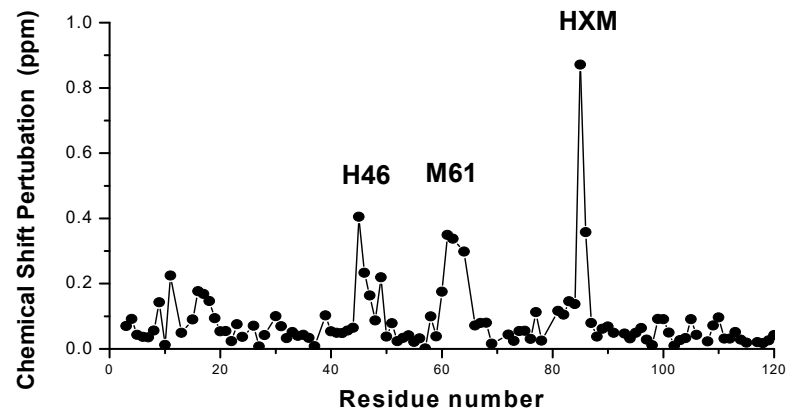
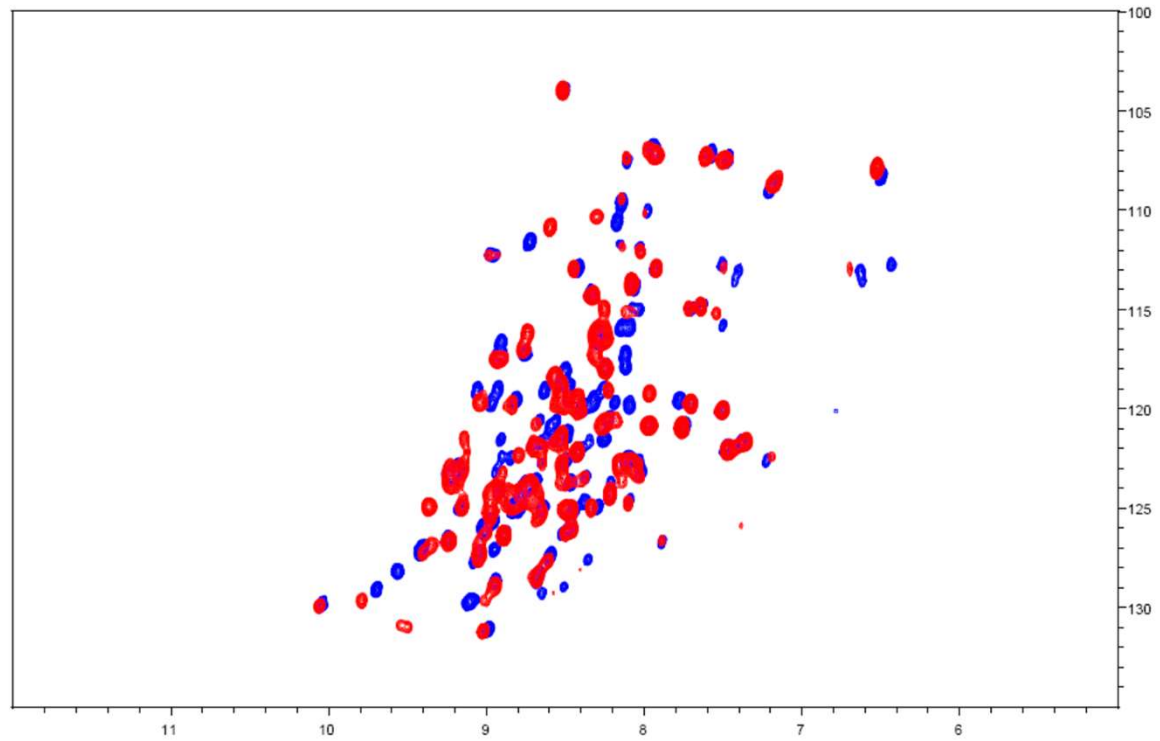
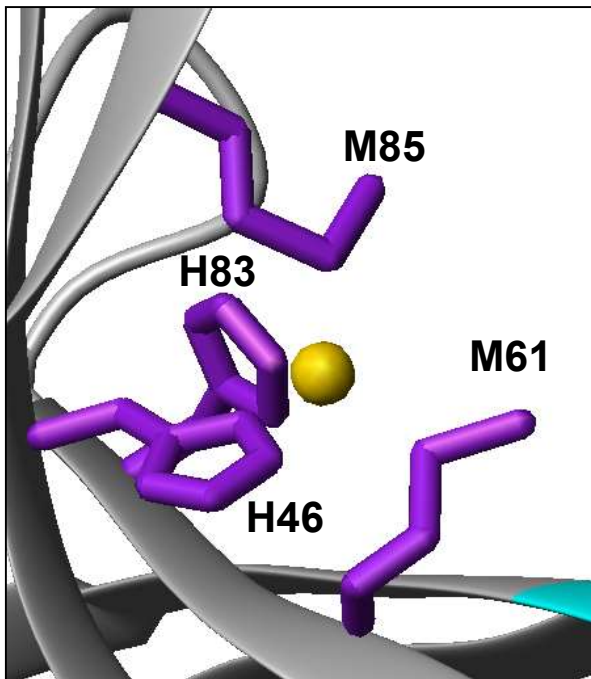


Does *Tt*-Hyp1 bind Cu(I)?



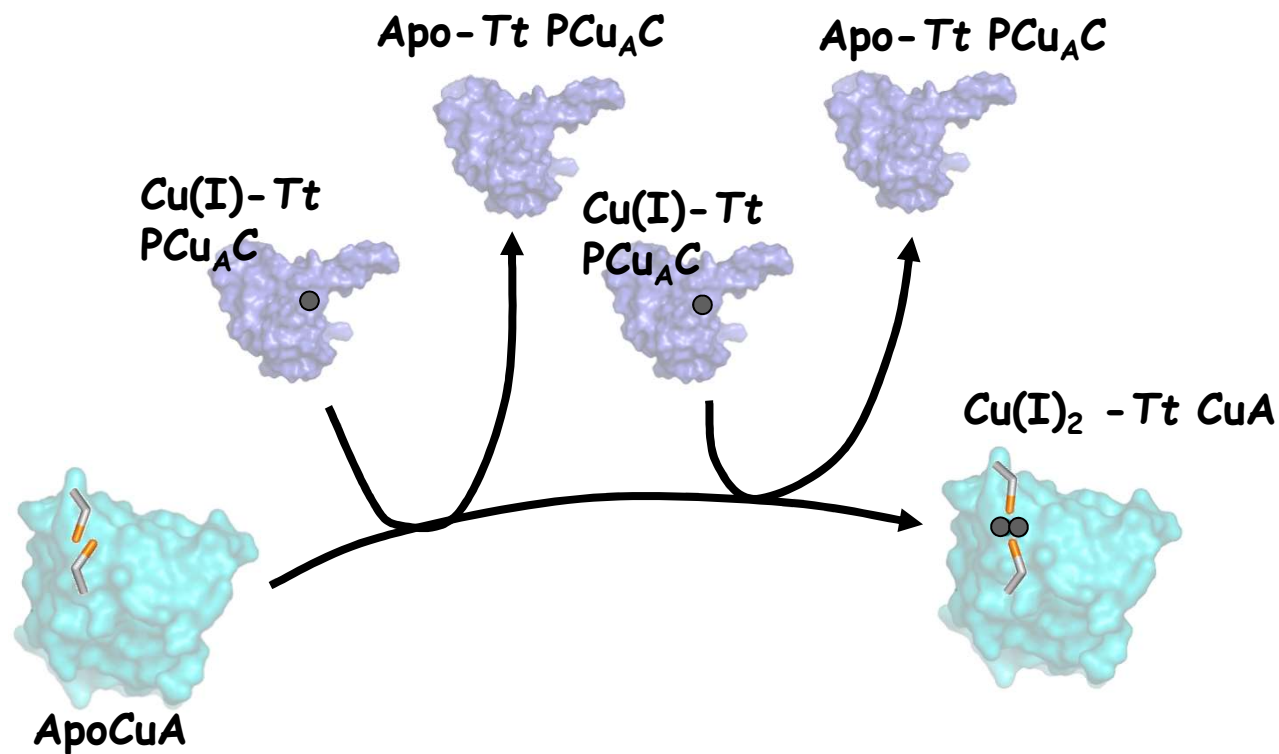
Cu(I) binding to *Tt* Hyp1

Apo-Hyp1
Hyp1 + Cu(I)

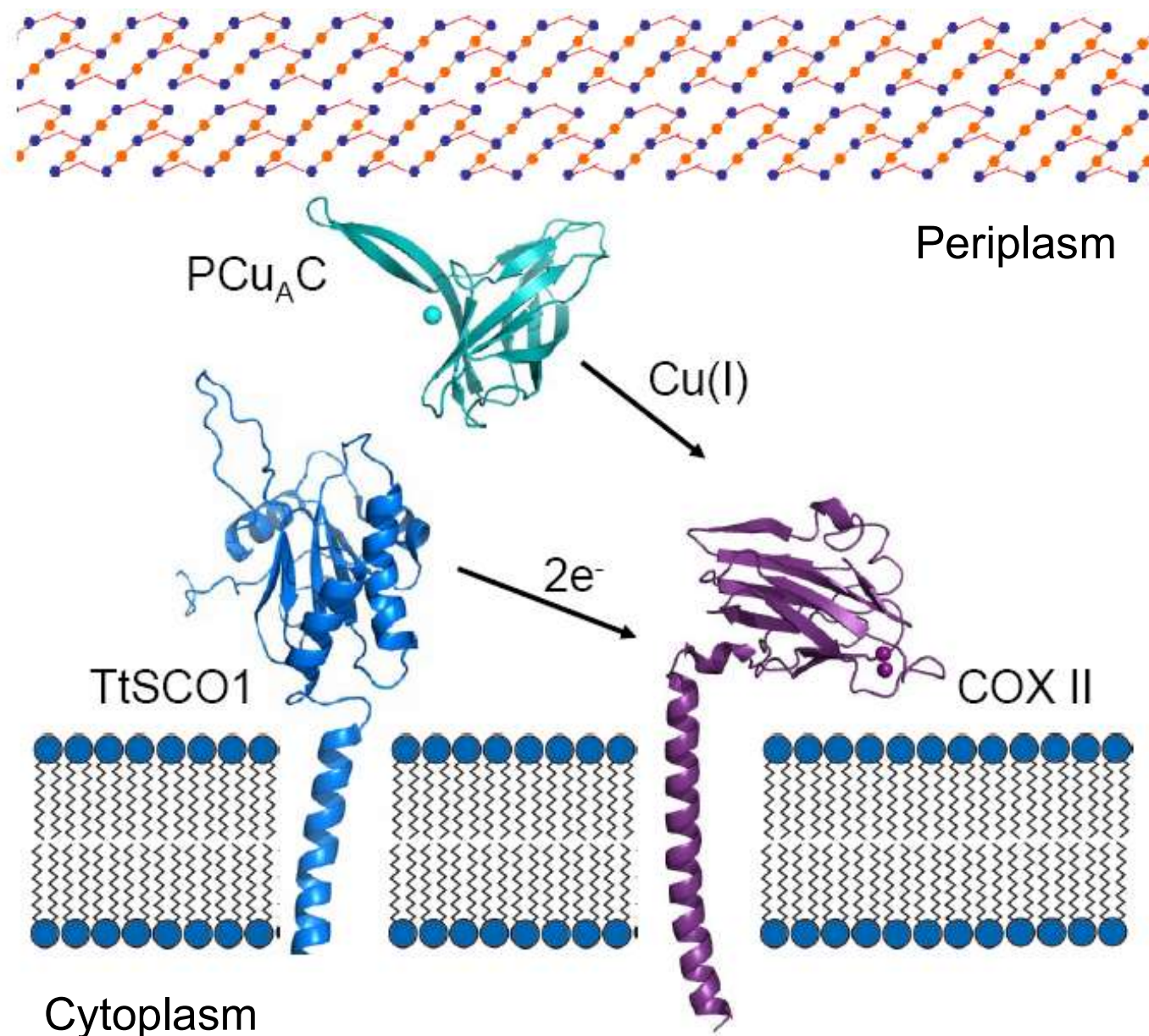


apoCuA + Cu(I)-Hyp1

- Hyp1 is able to transfer two Cu(I) ions to apo-CuA
- Cu(I) transfer is sequential

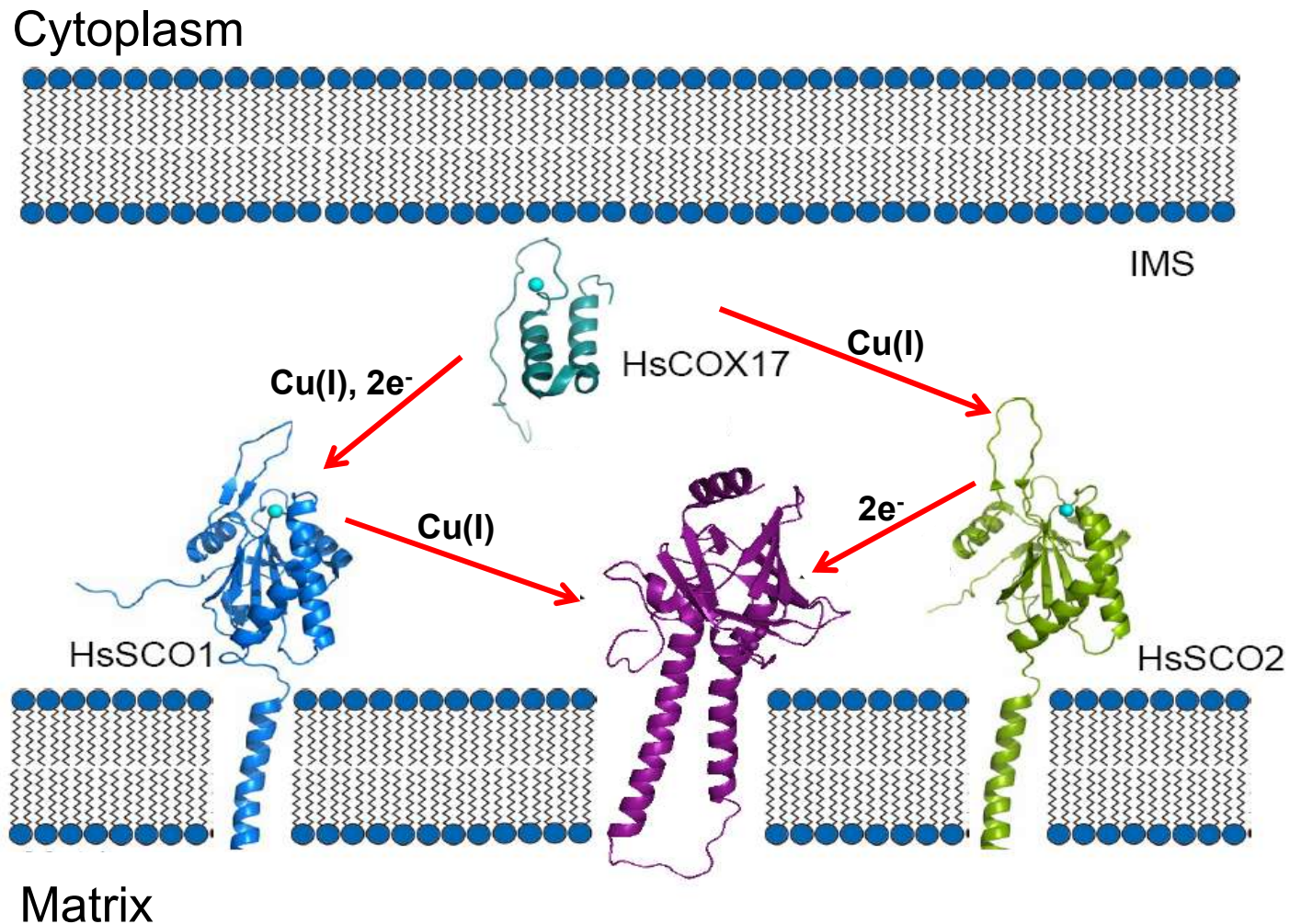


Hyp1: Periplasmic CuA Chaperone (PCuAC)



Abriata et al. *Nature Chem.Biol.*, 4, 599-601 (2008).

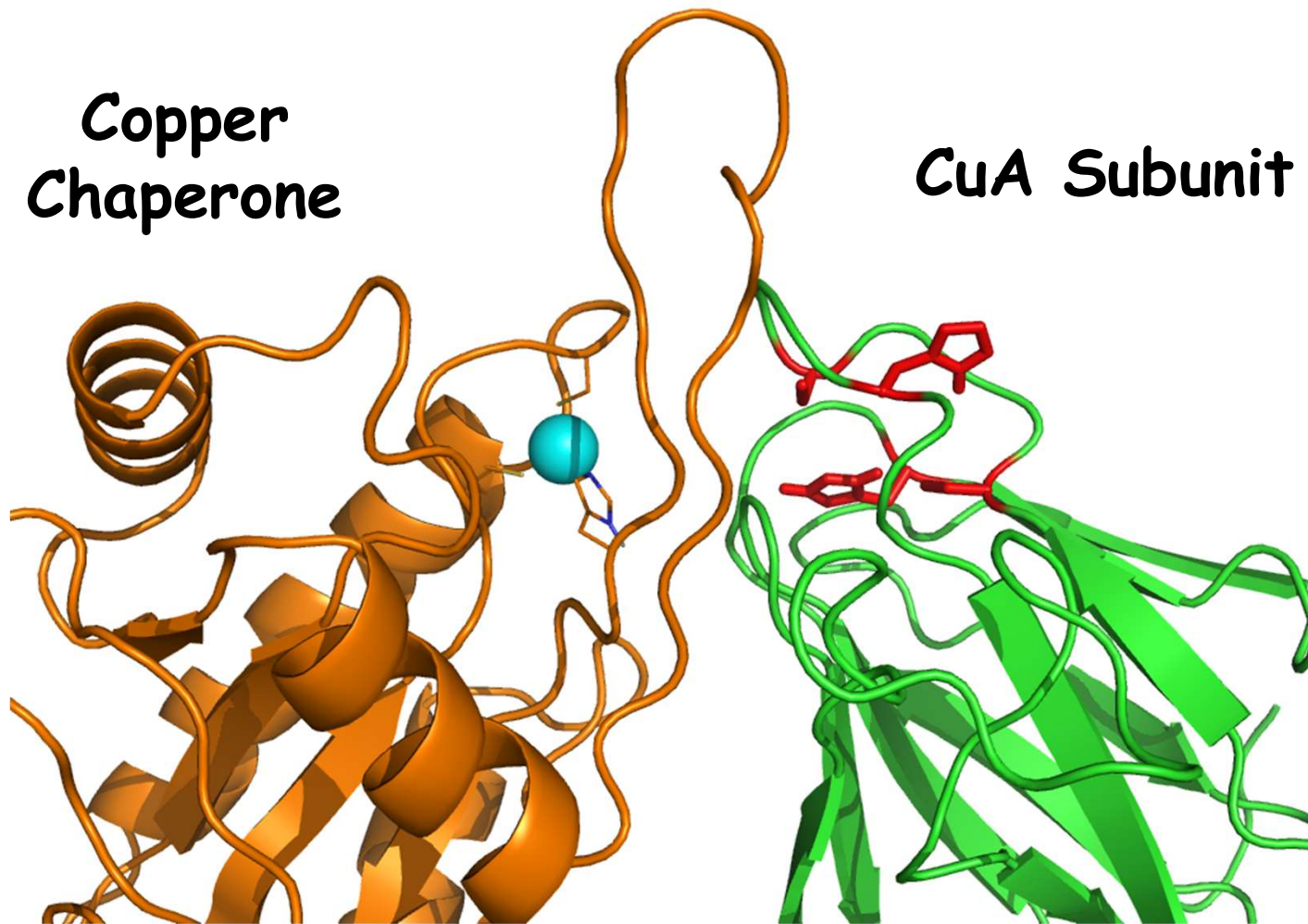
CuA assembly of human COX II



Morgada et al. (2014) *Angew. Chemie*

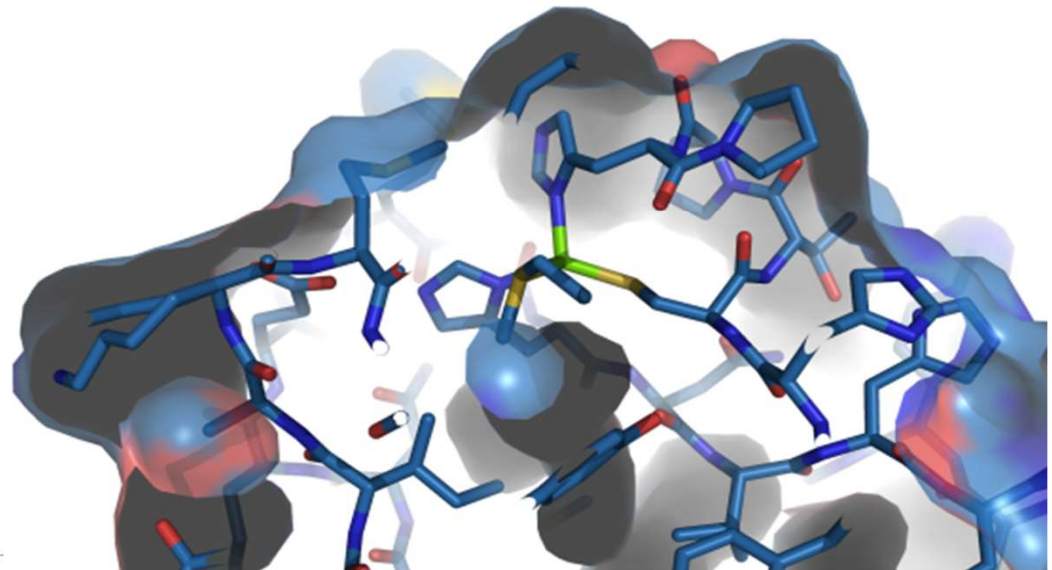
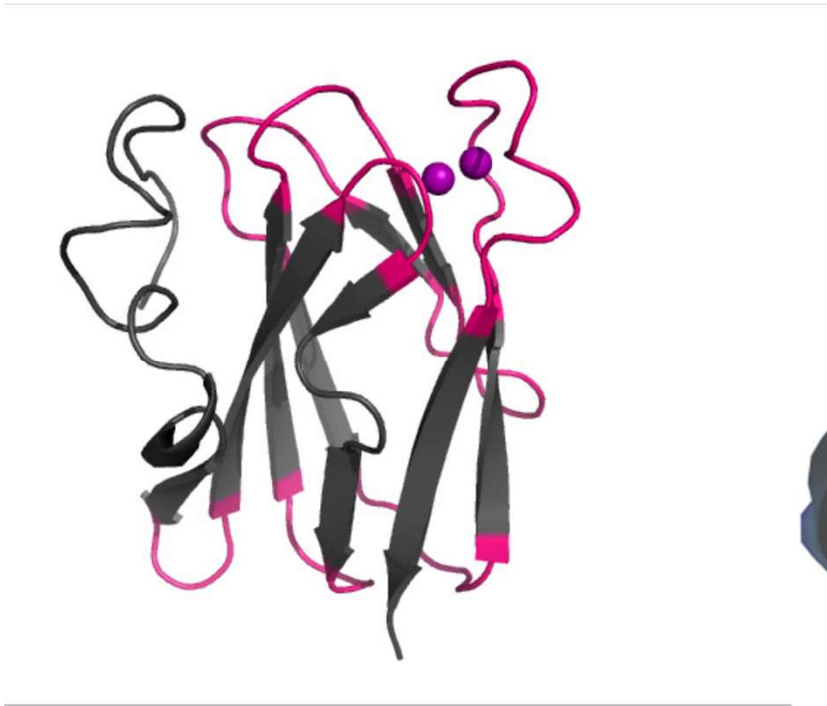
Morgada et al (2016) PNAS USA

How does copper binding occur?



Cupredoxin Fold (rigid)

Copper site occluded from
solvent

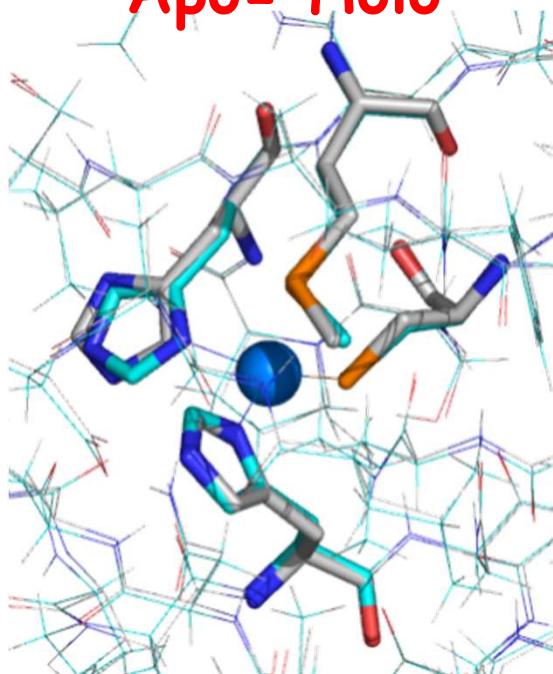


Malmstrom, RJP Williams, HB Gray

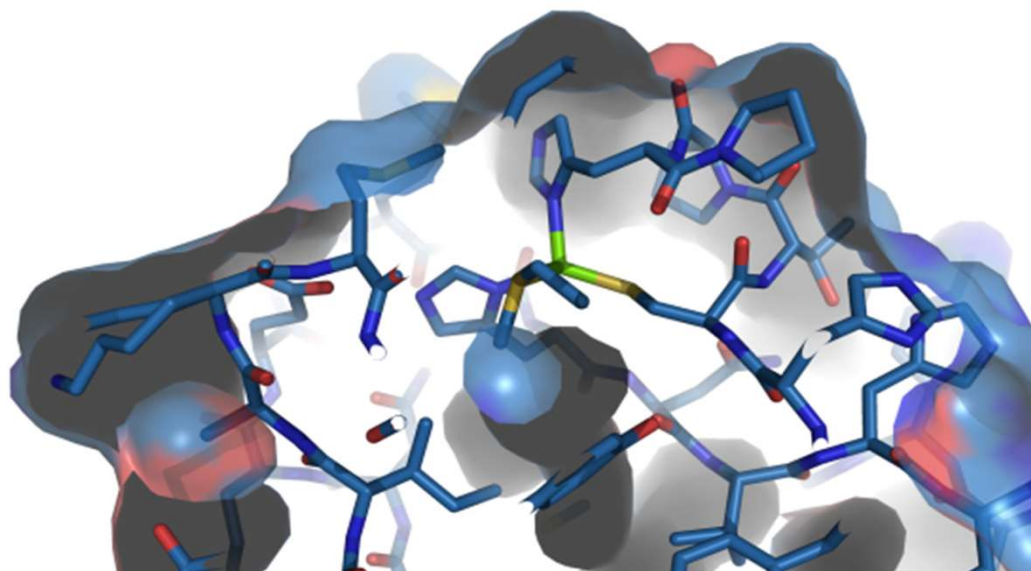
Cupredoxin Fold (rigid)

Copper site occluded from
solvent

Apo= Holo

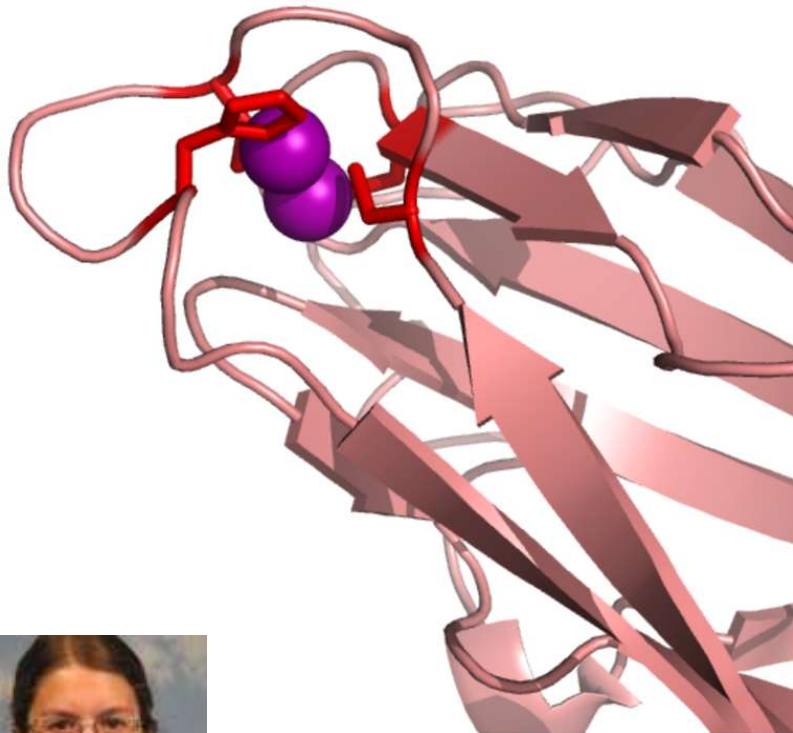


Preorganized
chelating site
(X-ray)



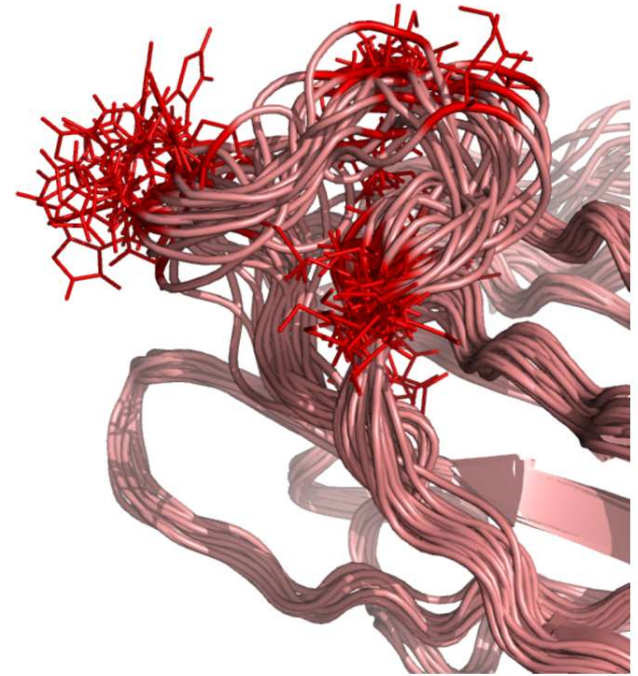
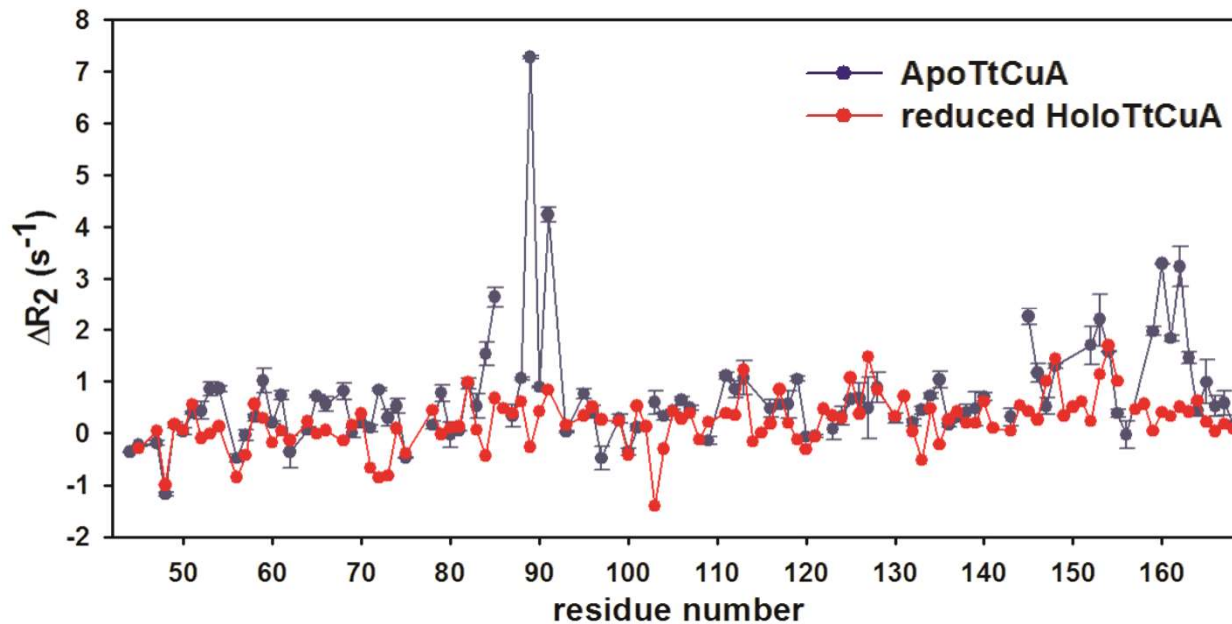
Malmstrom, RJP Williams, HB Gray

holoCu_A



Zaballa et al. *Proc.Natl.Acad.Sci USA*, **109**, 9254-9 (2012).

Protein dynamics by NMR



μ s to ms dynamics in the metal binding site

These loops may provide the chaperone recognition site

Zaballa et al. *Proc.Natl.Acad.Sci USA*, **109**, 9254-9 (2012).

Conclusions

- *NMR is not only a technique useful to solve protein structure*
- *NMR can be used to assess protein dynamics and interaction even when crystal structures are available*
- *NMR allows to design experiment to annotate protein function*