Study of Interactions and Protein Structure Determination by NMR

For Application to Protein Characterization

by
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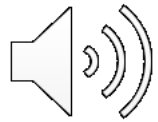
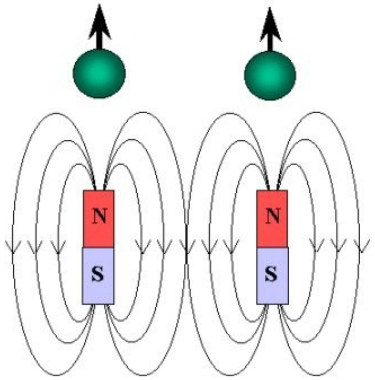
This project received funding by the European Commission under the GA n 2019-1-IT02-KA203-063391

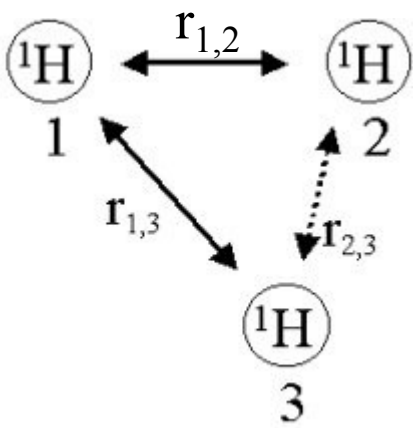


NMR as a tool for study **structure**, **dynamics** and **interactions** of biomolecules

0) AA/NA sequence, resonance assignment, standard chemical shifts
1) Structure determination of proteins/NAs
2) NMR can provide detailed information about the structure at the atomic level resolution relying on the spatial proximity of two interacting protons – nuclear Overhauser enhancement (NOE)
3) Additional structural information can be obtained (residual dipolar couplings – RDCs, *J*-couplings, backbone chemical shifts - CSI)

NOE:



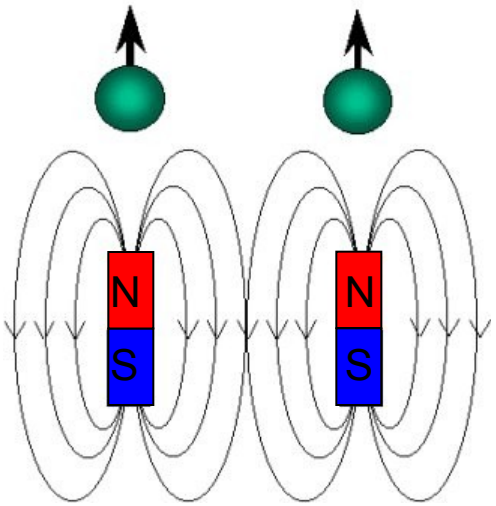


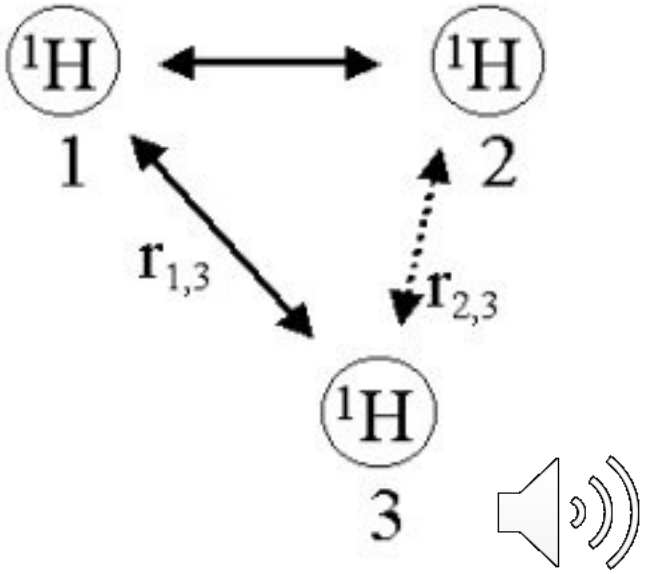
$r_{1,2}; r_{1,3}; r_{2,3} \leq 6$ Å

1Å=1.10^{-10}m

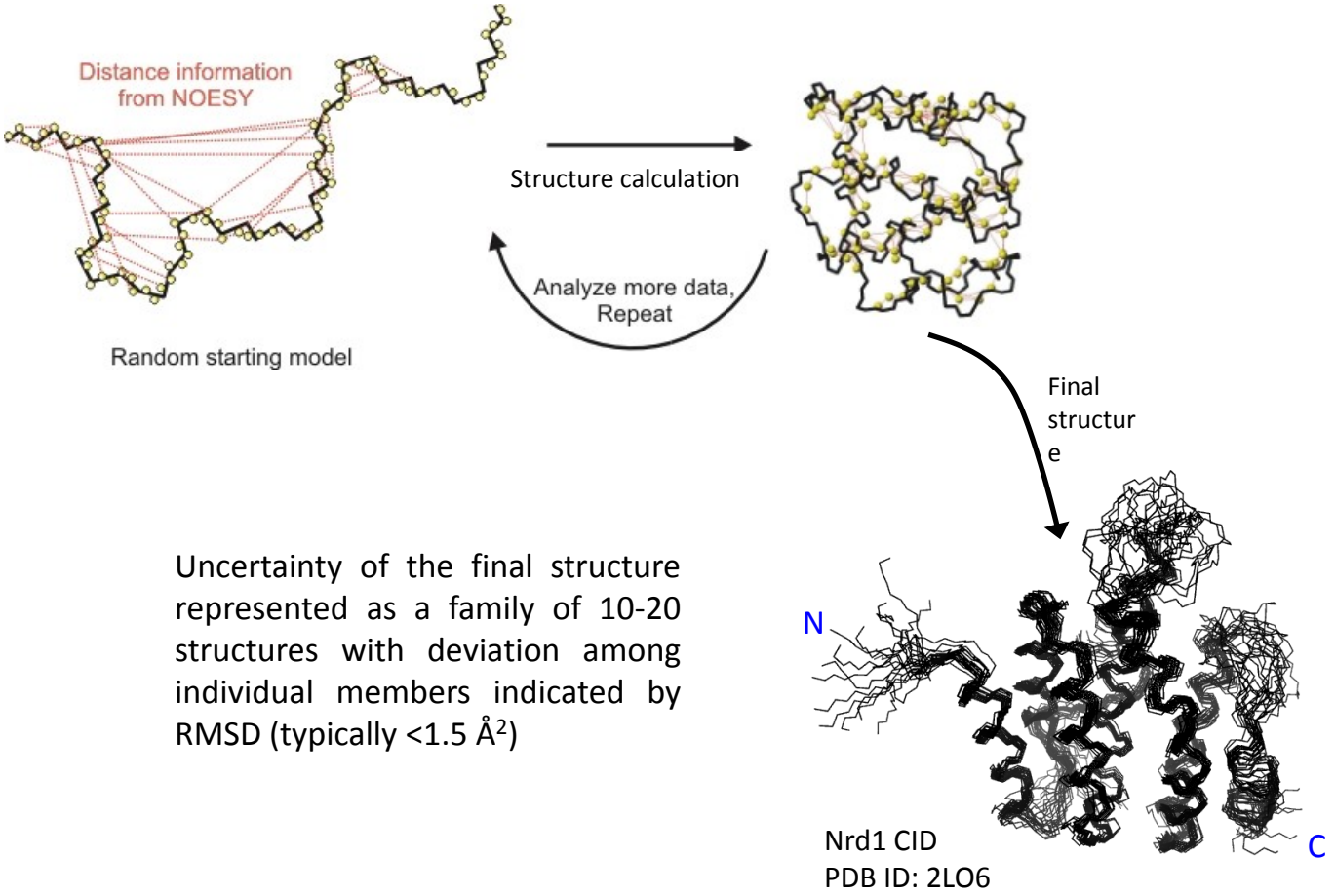
Nuclear **O**verhauser **E**ffect (**S**pectroscop**Y**) = **NOE**(**SY**)

i) caused by dipolar coupling between nuclei.
ii) the local field at one nucleus is affected by the presence of another nucleus.
iii) the result is a mutual modulation of resonance frequencies.
iv) the NOE operates through space.
v) the intensity of the interaction is a function of the distance between the nuclei according to the following equation: **$I = A(1/r^6)$**, **I** is the intensity, **A** is a scaling constant, and **r** is the distance between the nuclei
vi) the NOE provides a link between an experimentally measurable quantity, I, and internuclear distance
vii) NOE is only observed up to ~6Å





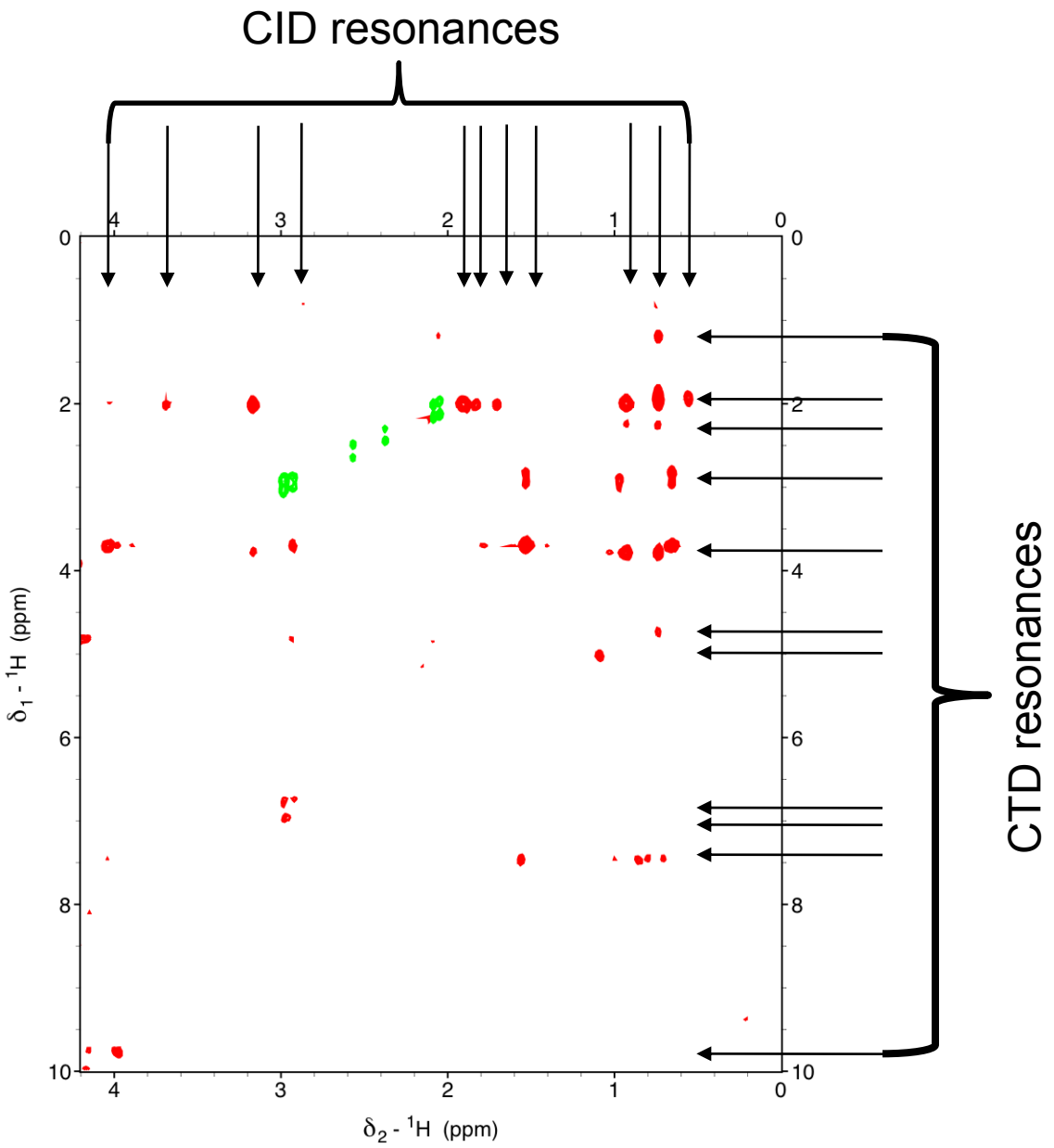
Iterative procedure of structure determination by NMR



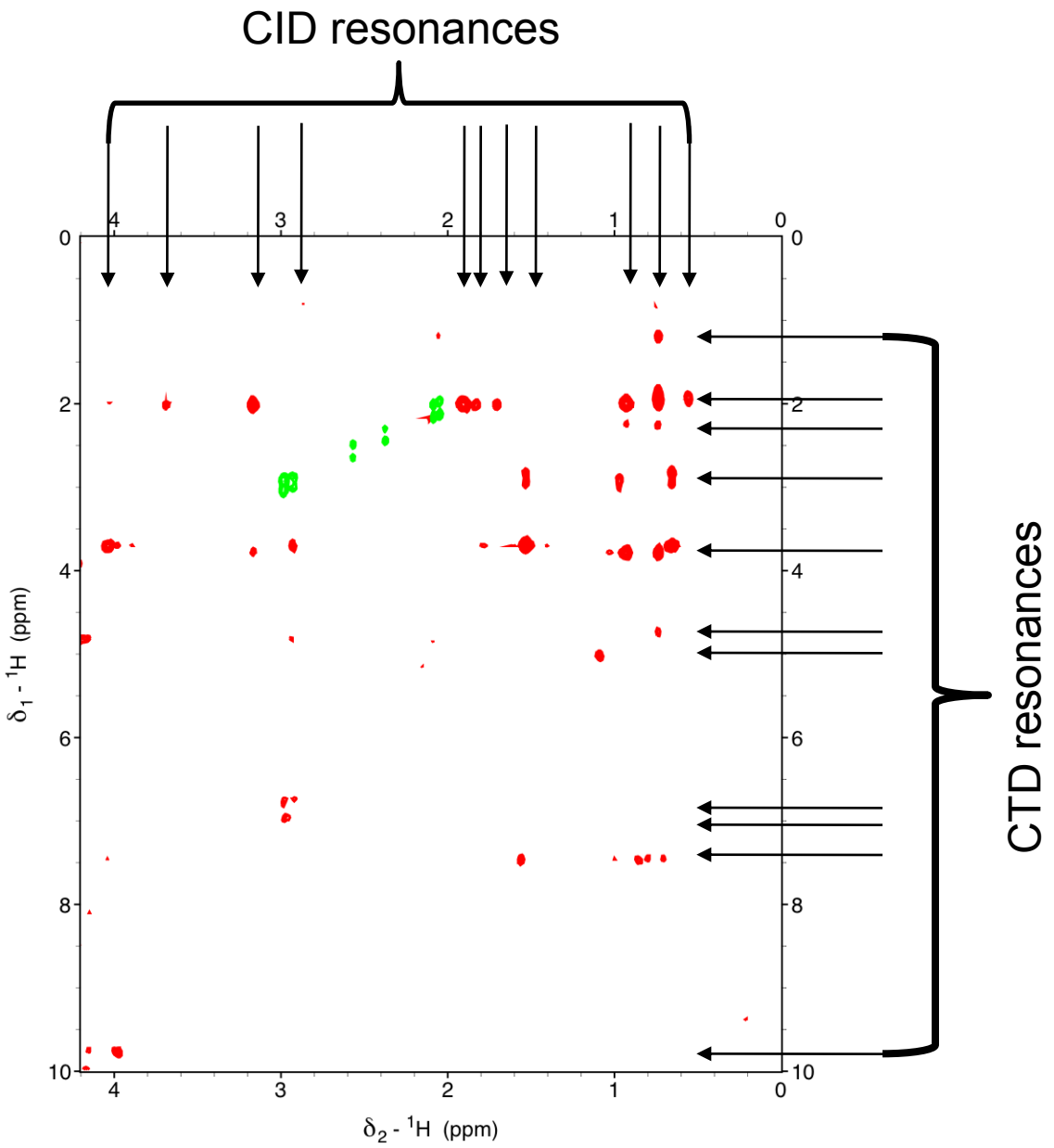
Uncertainty of the final structure represented as a family of 10-20 structures with deviation among individual members indicated by RMSD (typically <1.5 Å^2)

http://www.fbreagents.com/basics_nmr/9proteins.htm

Interligand NOEs between CID and CTD – 900MHz, 150ms, 293K



Interligand NOEs between CID and CTD – 900MHz, 150ms, 293K

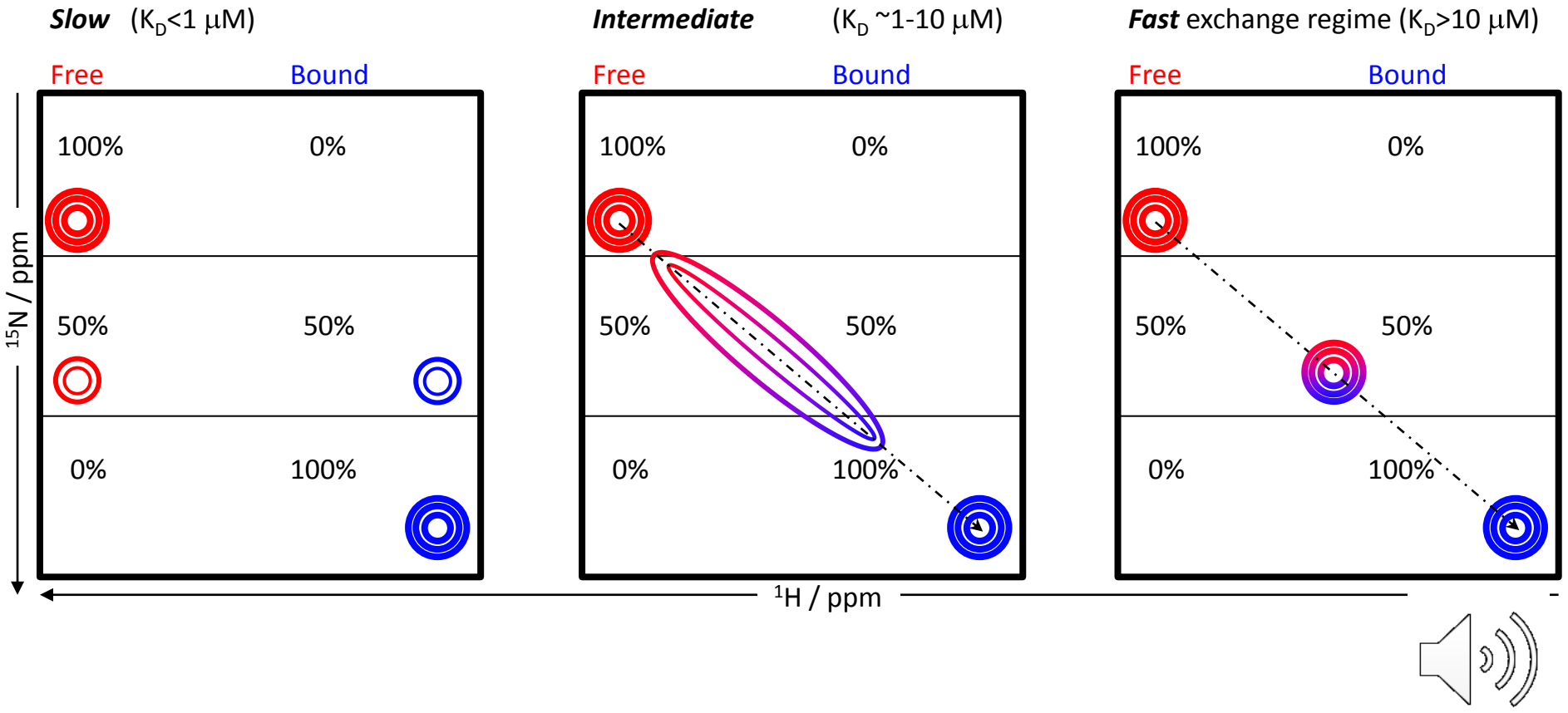


Studying interactions by NMR titration

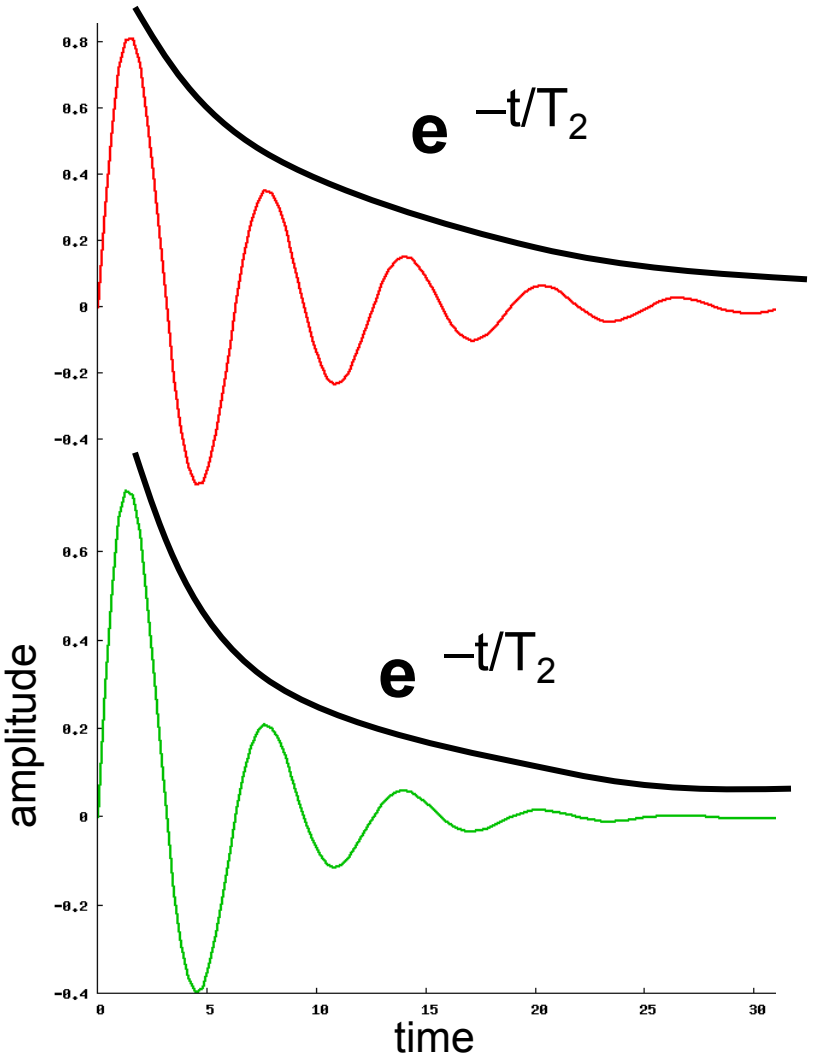
1) **Slow** exch. regime (on the NMR timescale) – individual peaks for each of the studied states (e.g. free / complexed forms of a protein), peak intensity representing population of a given state

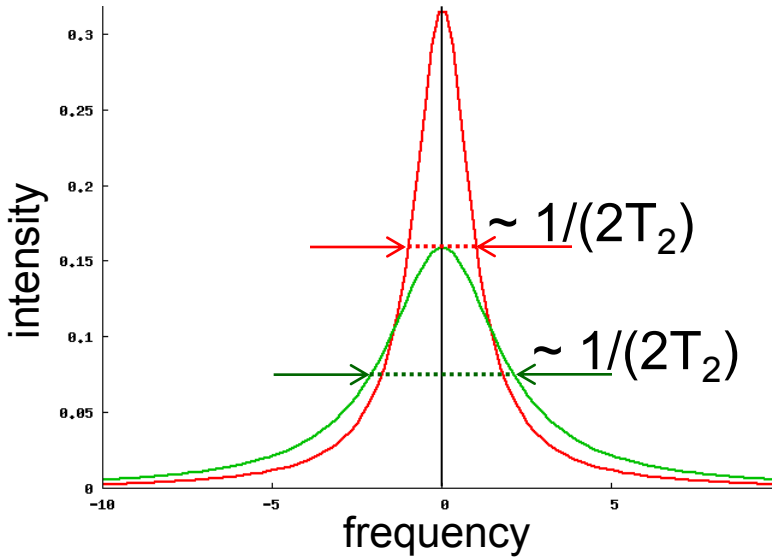
2) **Intermediate** exchange regime

3) **Fast** exchange regime – single peak whose chemical shift position is given by the molar ratio of the states present in solution



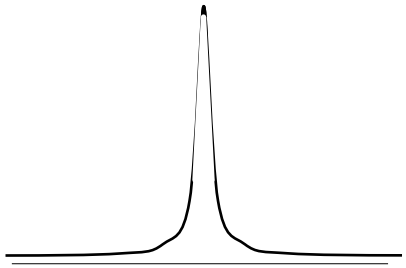
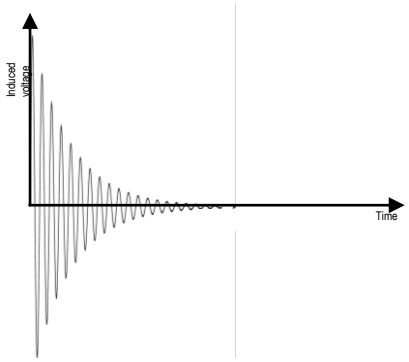
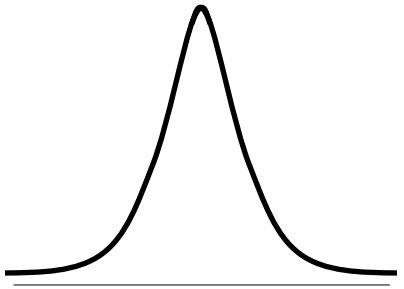
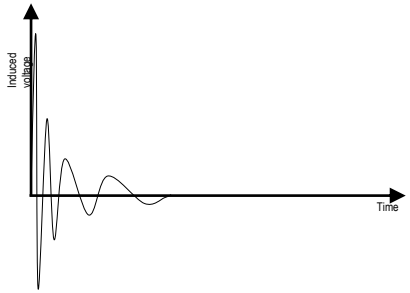
Not all molecules relax (decay) with the same rate

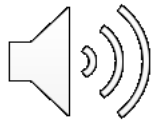


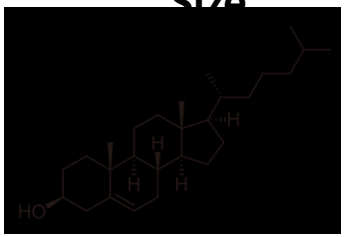
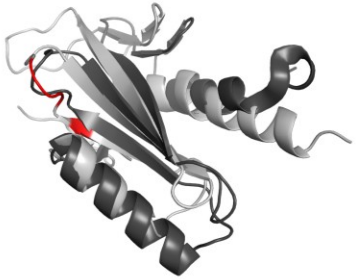
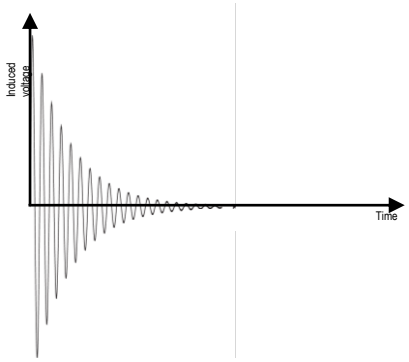
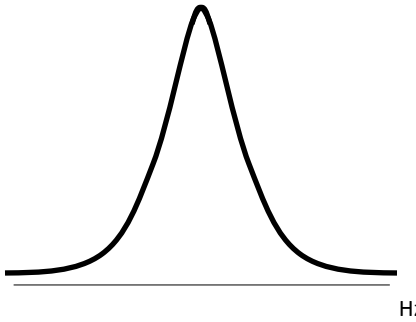
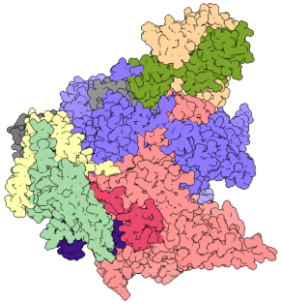
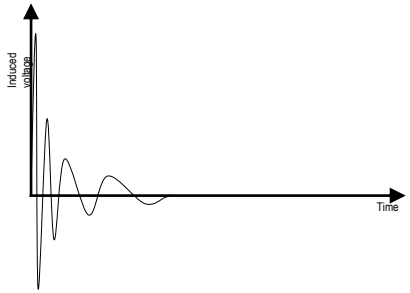


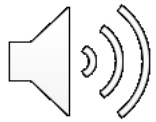
Bigger molecules (higher molecular weight) relax faster ⇒ **broad peaks**

Small molecules relax slower ⇒ **narow peaks**

Size	Relaxation	FID	NMR line(width) after FT
	slow (i.e. long t_2 time)		 Hz
	medium		 Hz
	fast		

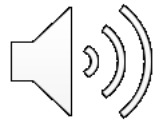


Size	Relaxation	FID	NMR line(width) after FT
 e.g. Cholesterol	slow (i.e. long t_2 time)		
 Biomolecules 5-30 kDa	medium		
 Large molecules 50+ kDa	fast		

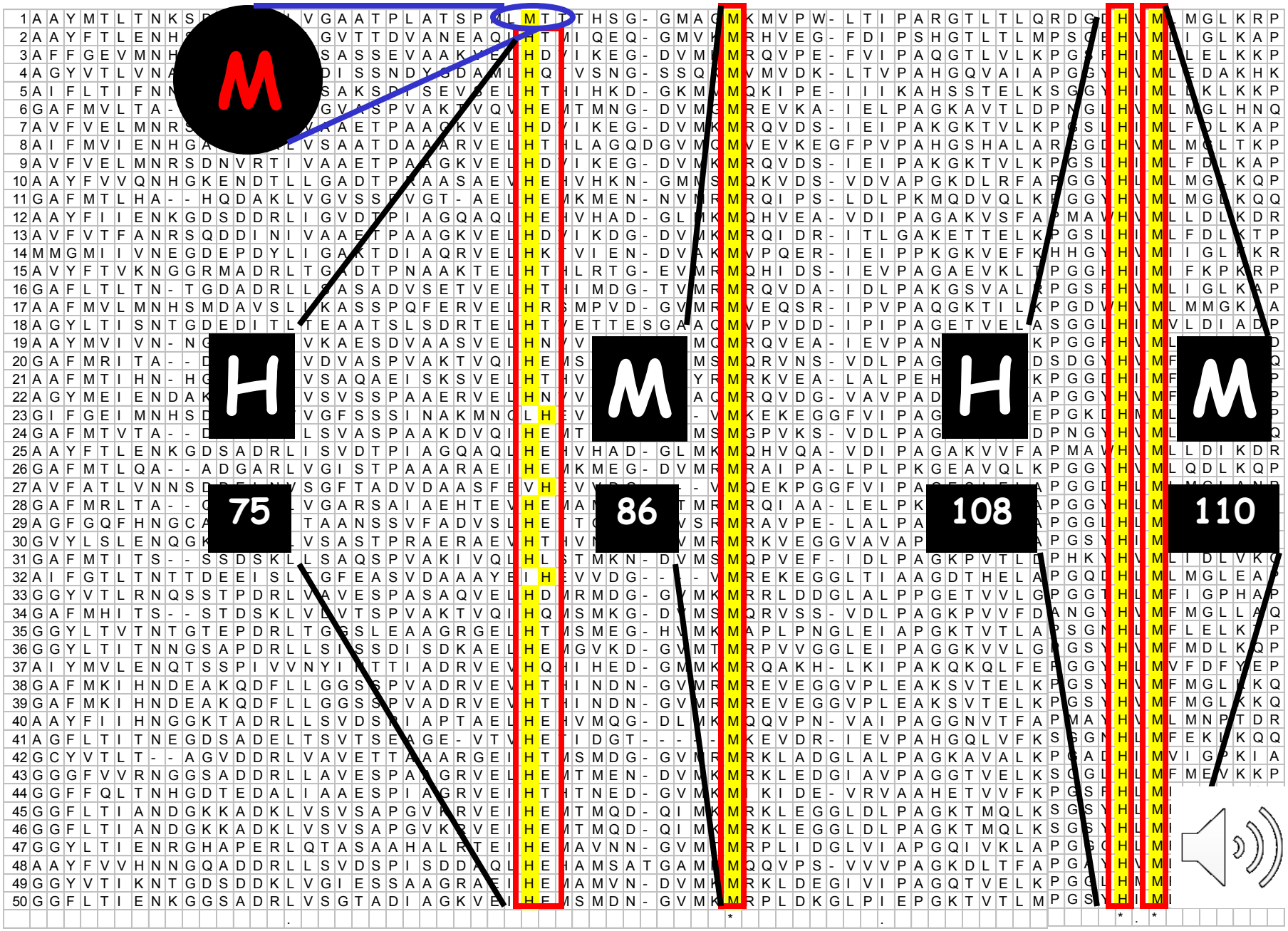


Protein – metal ion interaction

slow exchange case



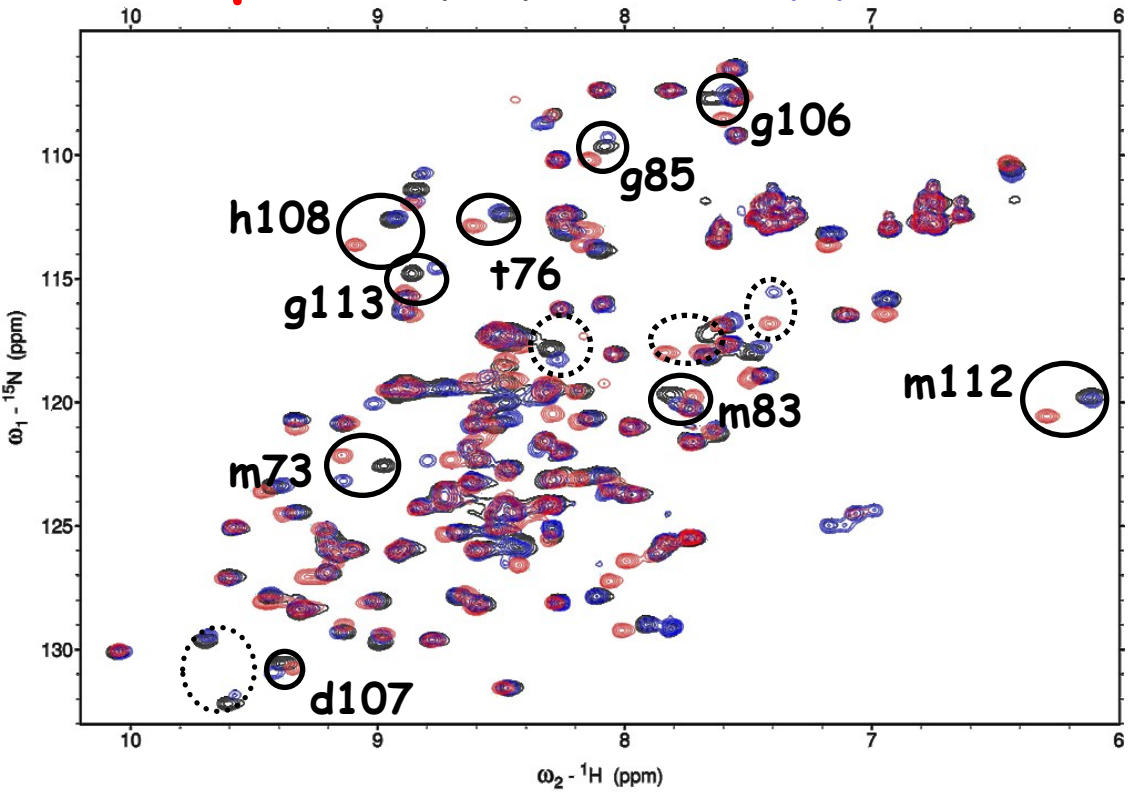
DR1885 from *deinococcus radiodurans*

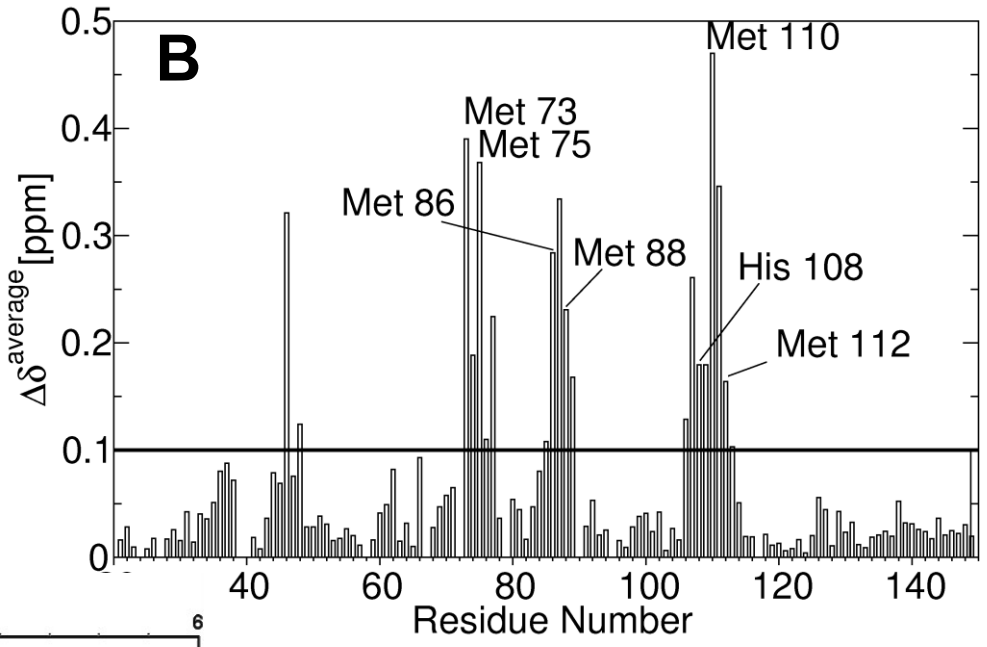


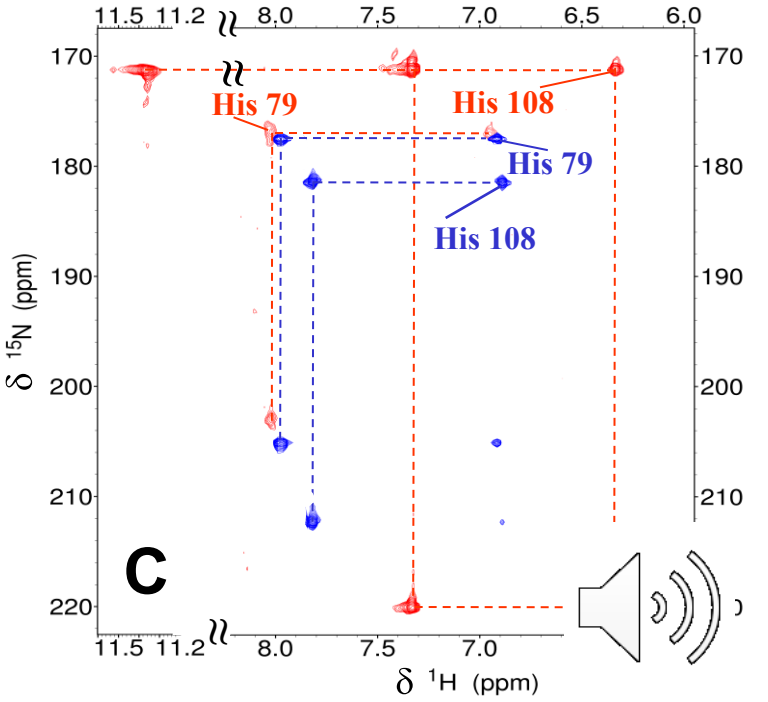
Interaction of DR1885 with copper

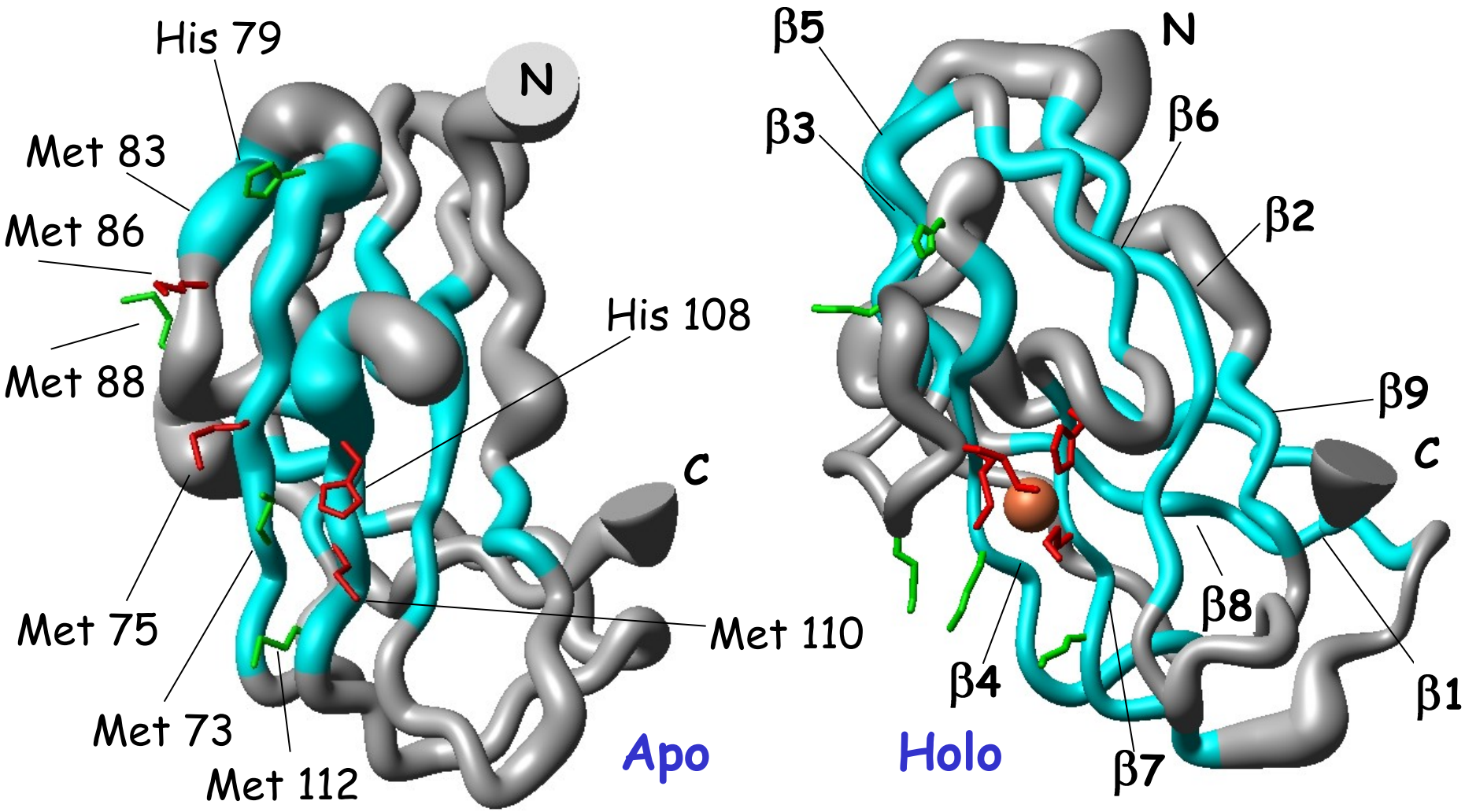
-titration (A,B)
-^{2}J HSQC (C)

Apo, **Cu(II)** and **Cu(I)**









Conserved **Met&His**

Other **Met&His** residues

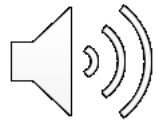
cyan – β-sheets

grey – random

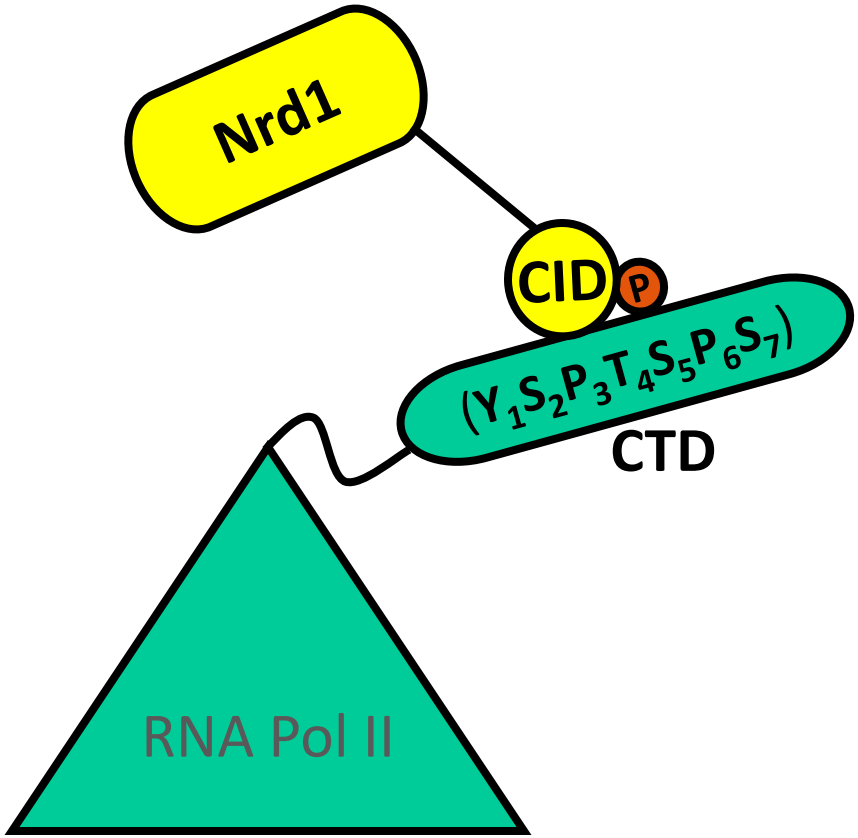
Banci et al. *PNAS* 2005

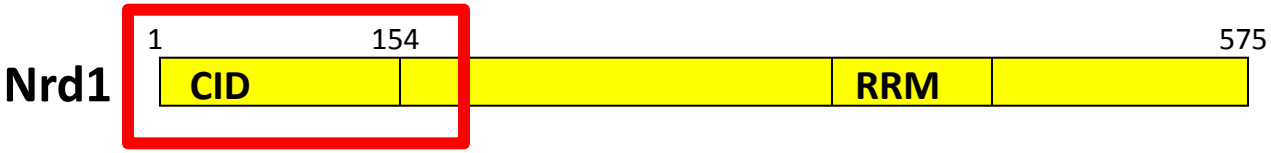
Protein – peptide interaction

fast exchange case



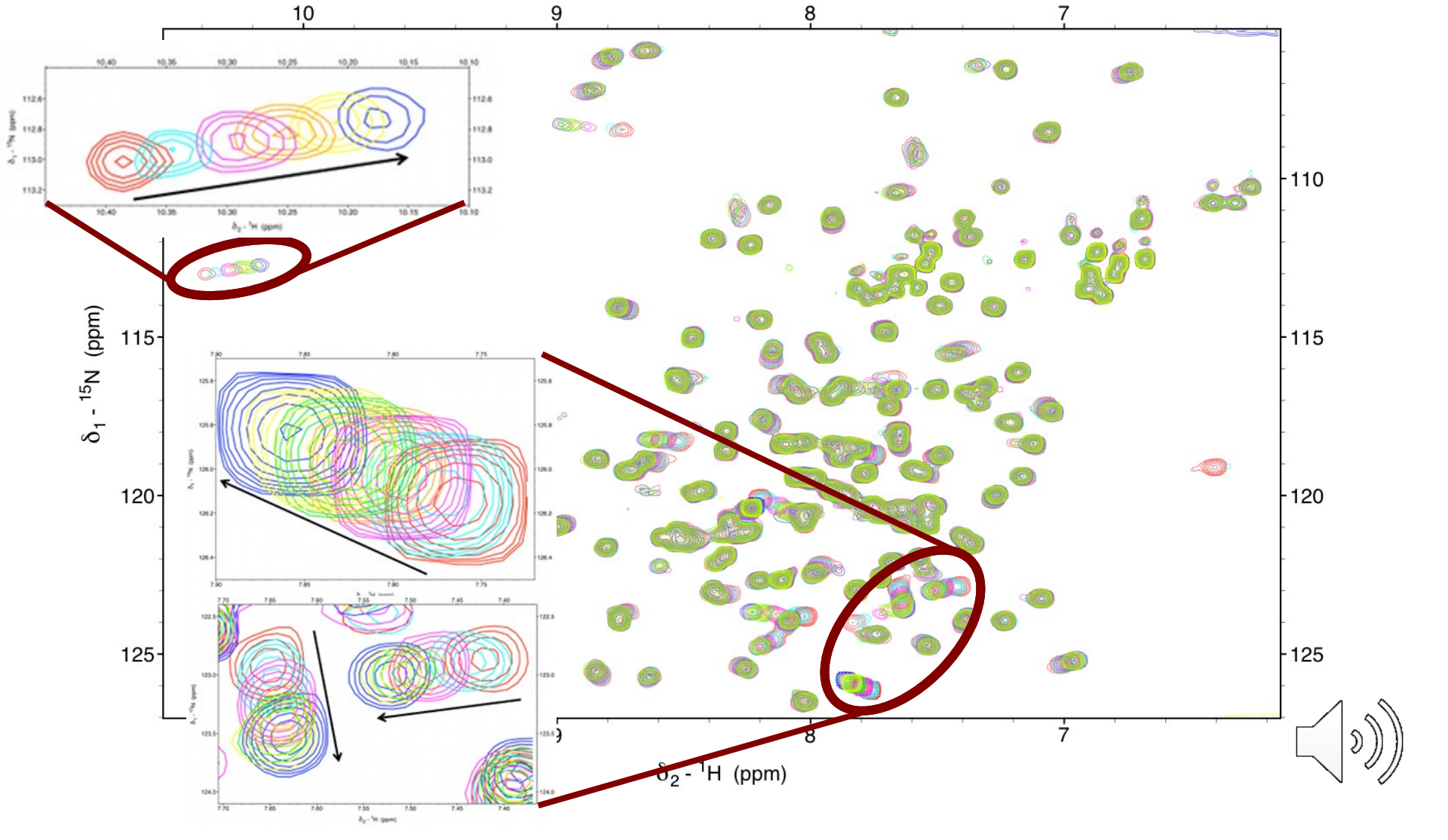
Interaction of Nrd1-CID with CTD





RRM: RNA recognition motif; **CID**: CTD interaction domain; **CTD**: C-terminal domain

Interaction of **^{15}N enriched CID** with **unlabeled CTD-Ser5P** in *n*-steps, *n*=6 in our case - peaks corresponding to the interacting residues of CID change their chemical shift (position in the spectrum) =>interaction surface, binding constant, stoichiometry



Interaction of Nrd1-CID with CTD

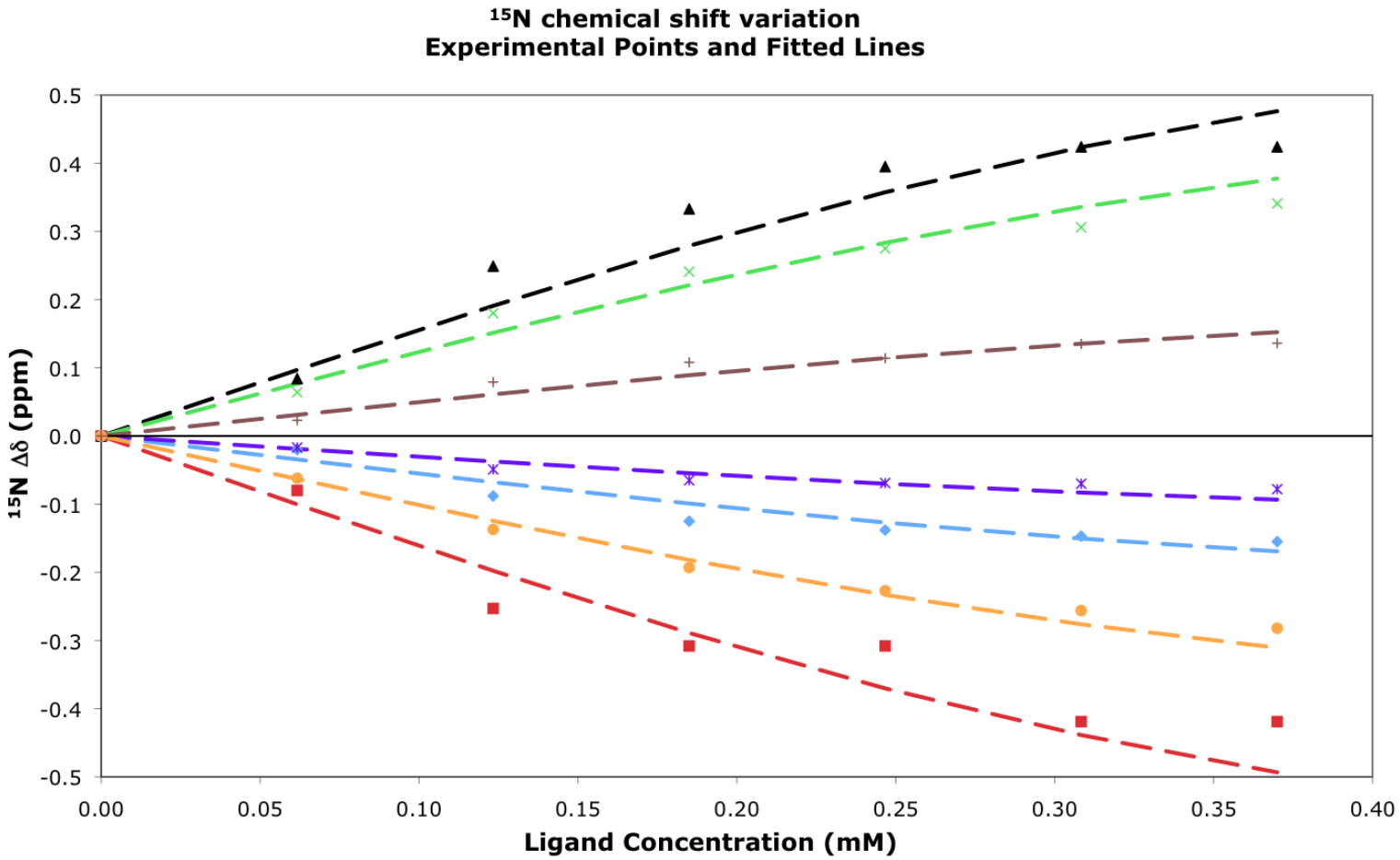
NMR Titration: ~ 0.6 mM ^{15}N enriched CID + ~ 0.8mM (YSPTpSPS)$_2$

~ 0.6 mM ^{15}N enriched CID + ~ 0.8mM (YSPTSPS)$_2$

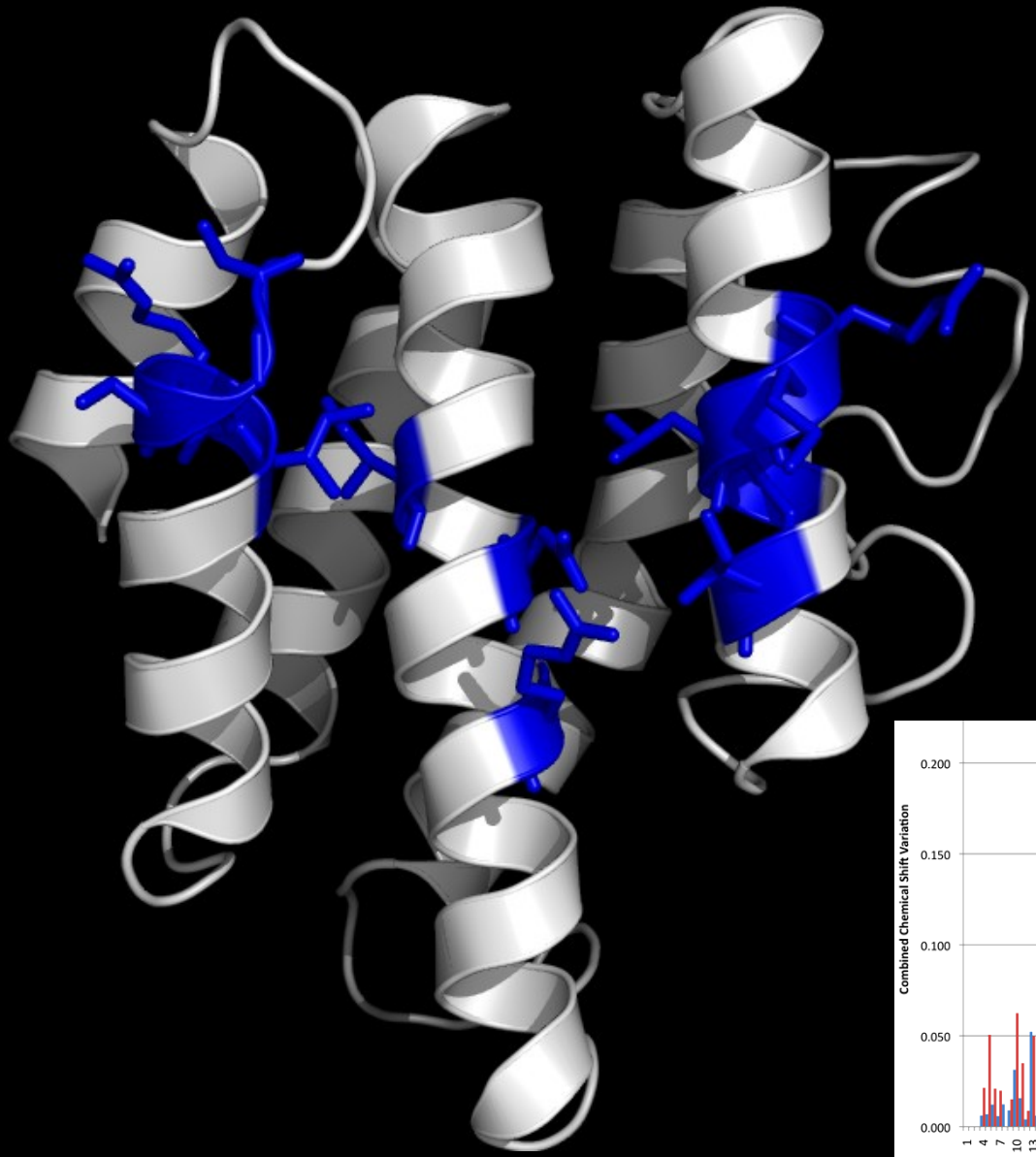
- μM-mM range of interaction ->

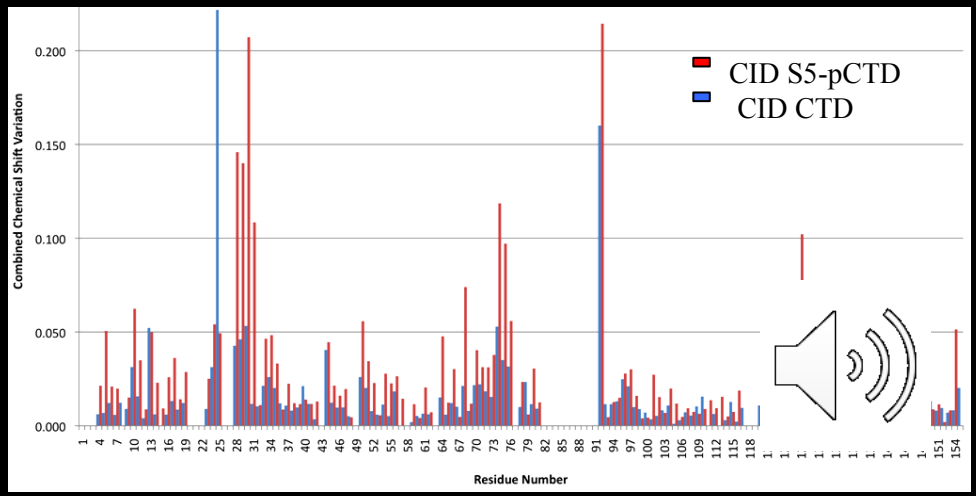
-> fast exchange regime on NMR time-scale

- NMR-derived K_d=0.080mM and 35mM



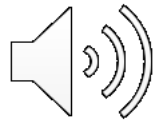
Nrd1 CID interaction surface – CID residues experiencing the largest chemical shift variations upon the interaction with 5-phospho-Ser CTD shown in blue with side-chains in stick representation



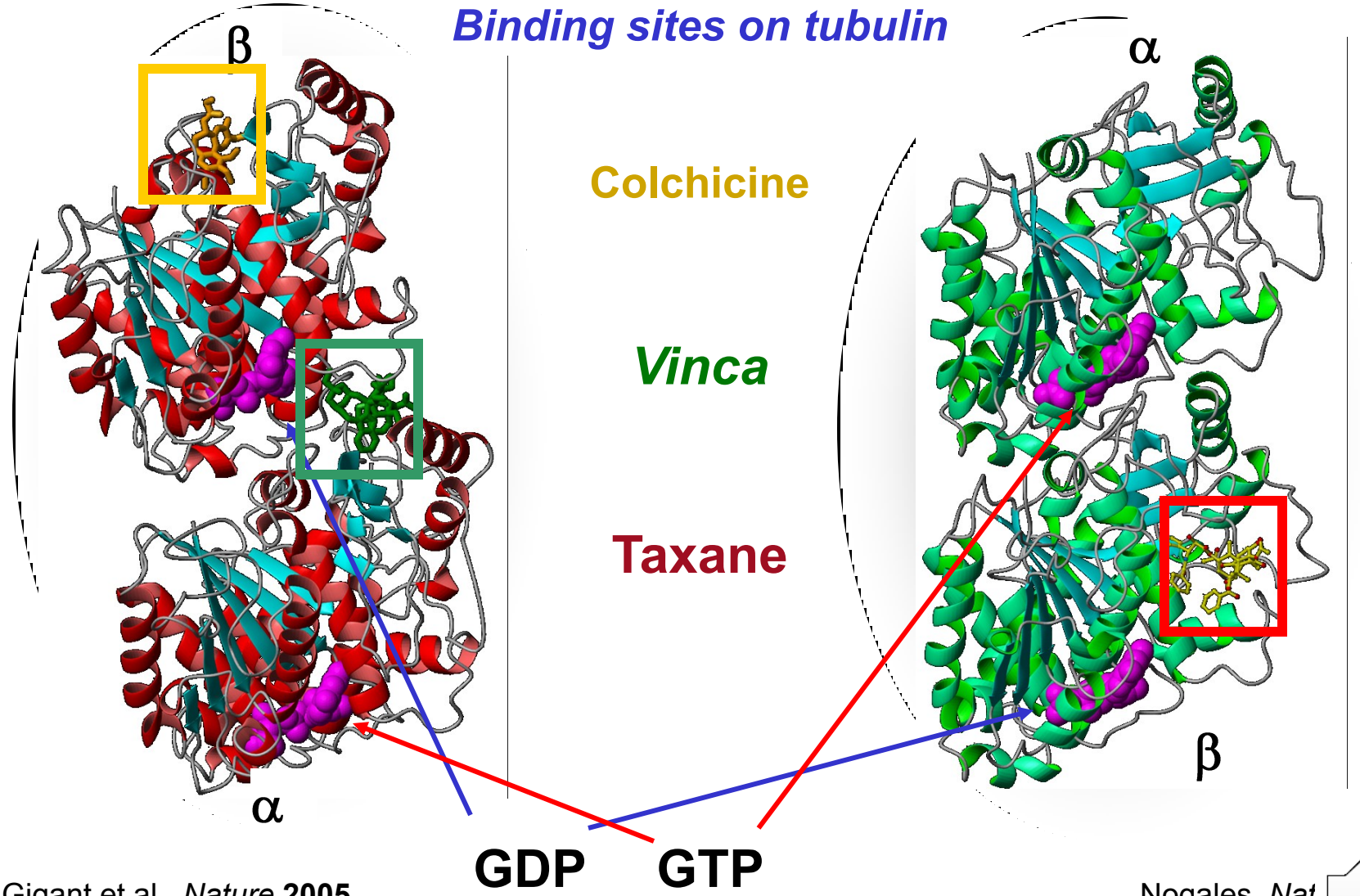


Protein – peptide interaction

drug-receptor case



Tubulin - successful target for anticancer therapy



Gigant et al., *Nature* **2005**

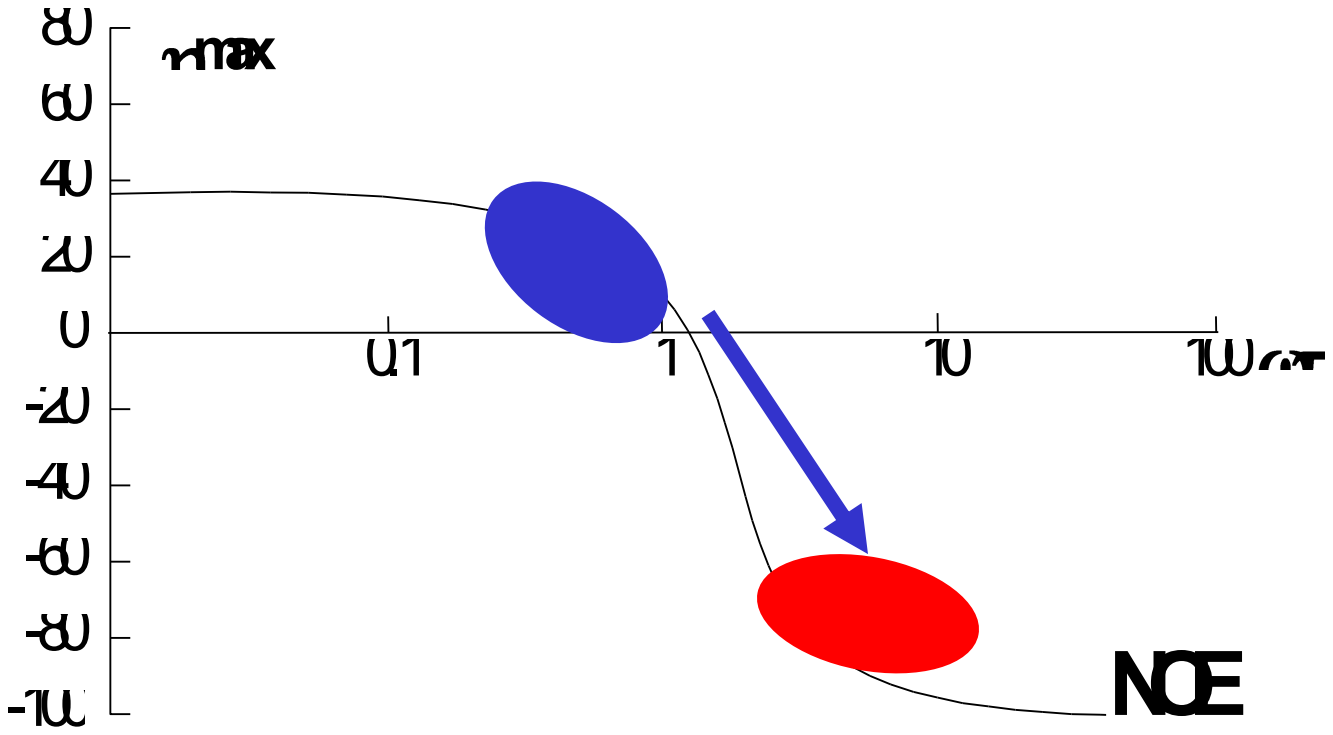
Nogales, *Nat*

Transferred-NOE

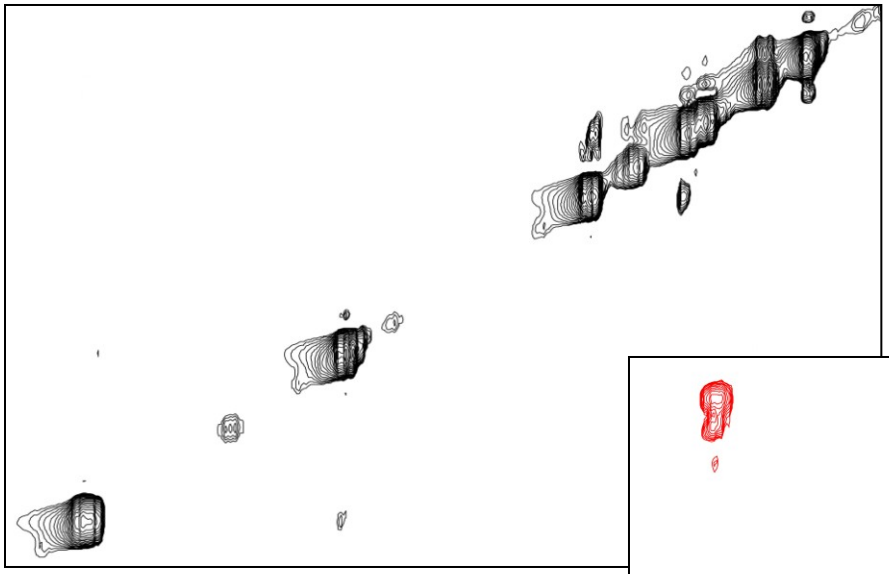
$$NOE = p_{bound} \cdot NOE_{bound} + p_{free} \cdot NOE_{free}$$

$$\tau_{c,bound} >> \tau_{c,free} \quad (\text{and} \quad p_{L,free} >> p_{L,bound})$$

$$NOE_{bound} > NOE_{free}$$

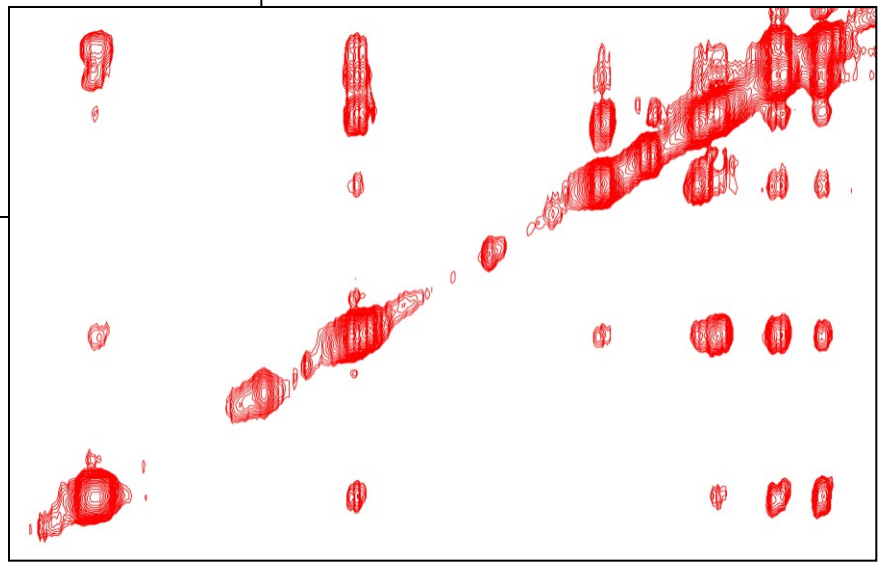


tr-NOESY~500μM tubulysin (TBS) **without** and **with ~10μM tubulin**



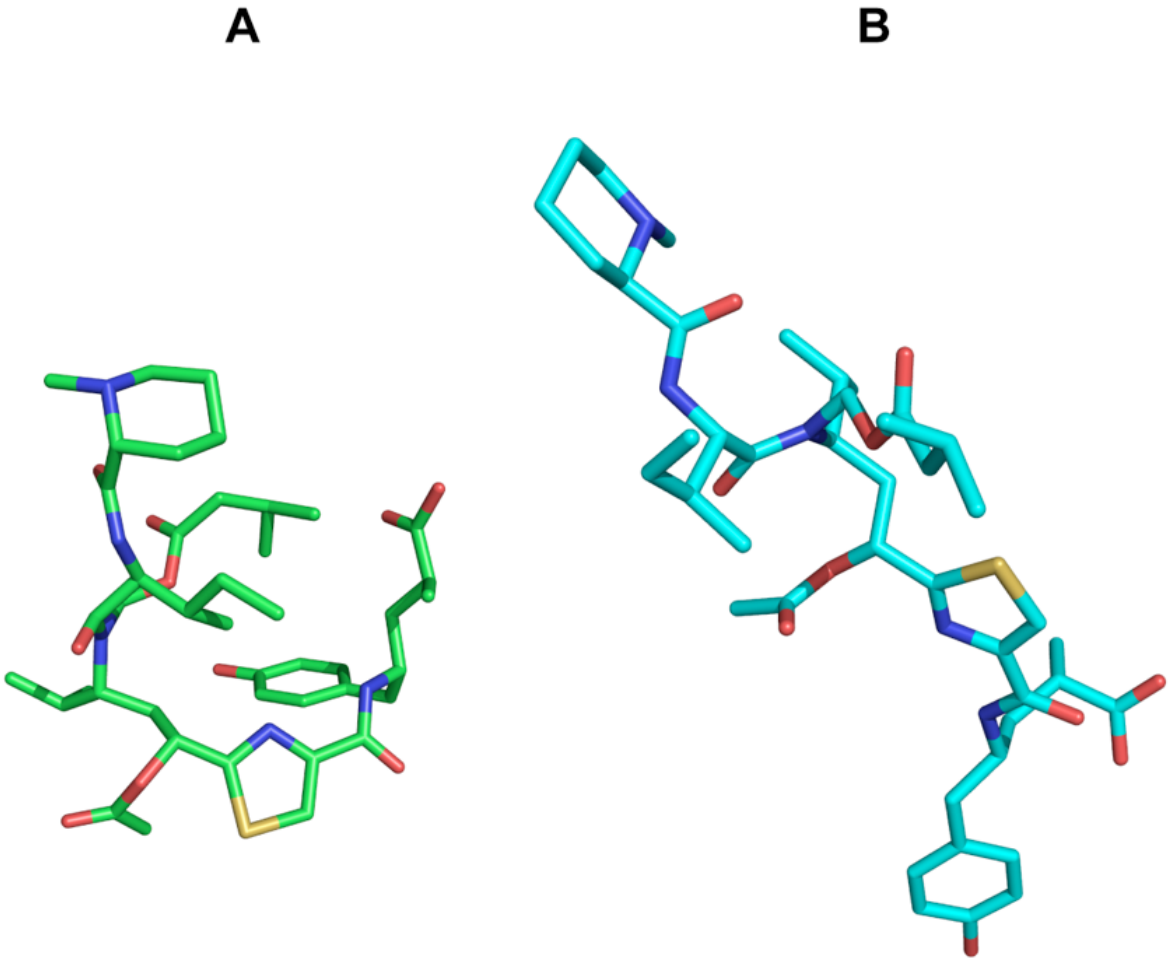
900MHz,

mixing time=100ms



tbs w/o tub

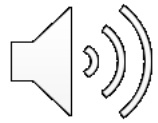
tbs:tub 50:1



Conformation of the tubulin-bound - NMR (**A**) and free – X-Ray TB (**B**)

Kubíček et al. *Angew Chem Int* Ed 2010

Large biomolecules and their interactions

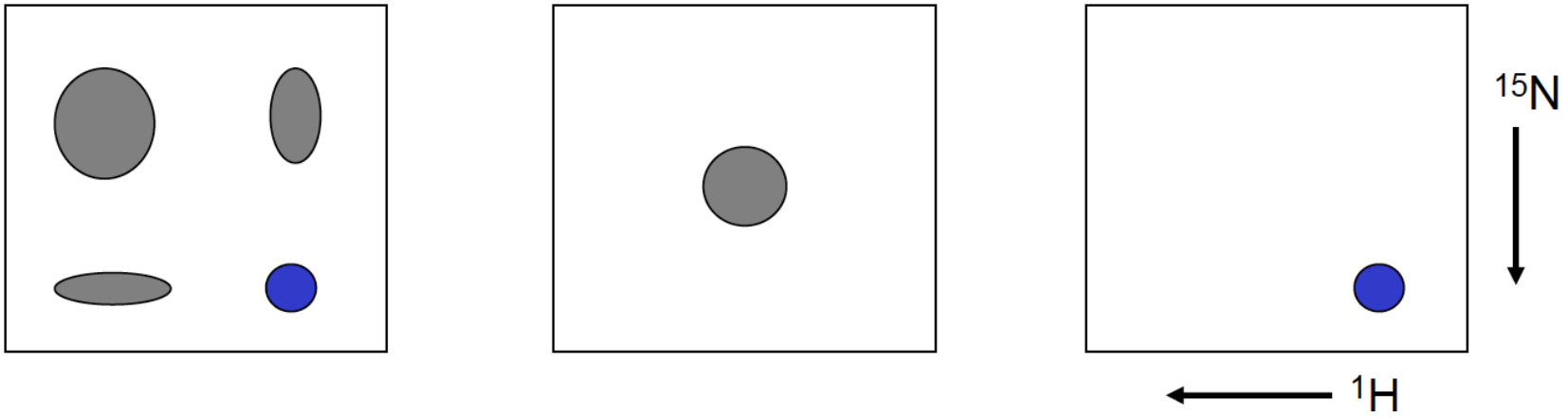


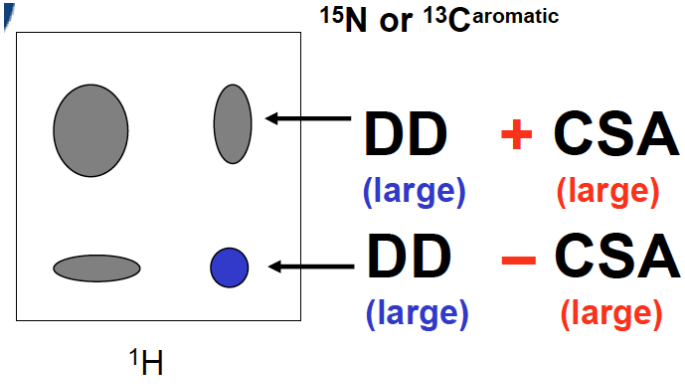
TROSY-based NMR experiments to study complexes up to 900kDa

Coupled HSQC

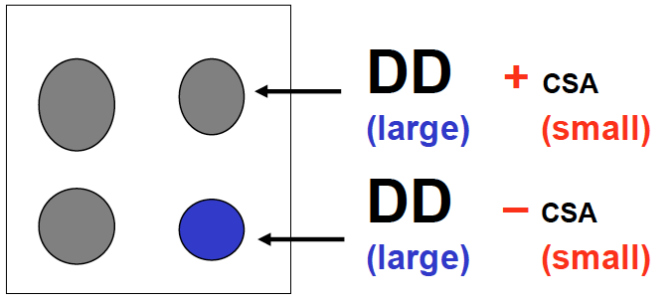
Decoupled HSQC

TROSY-HSQC

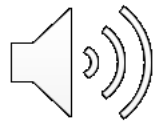


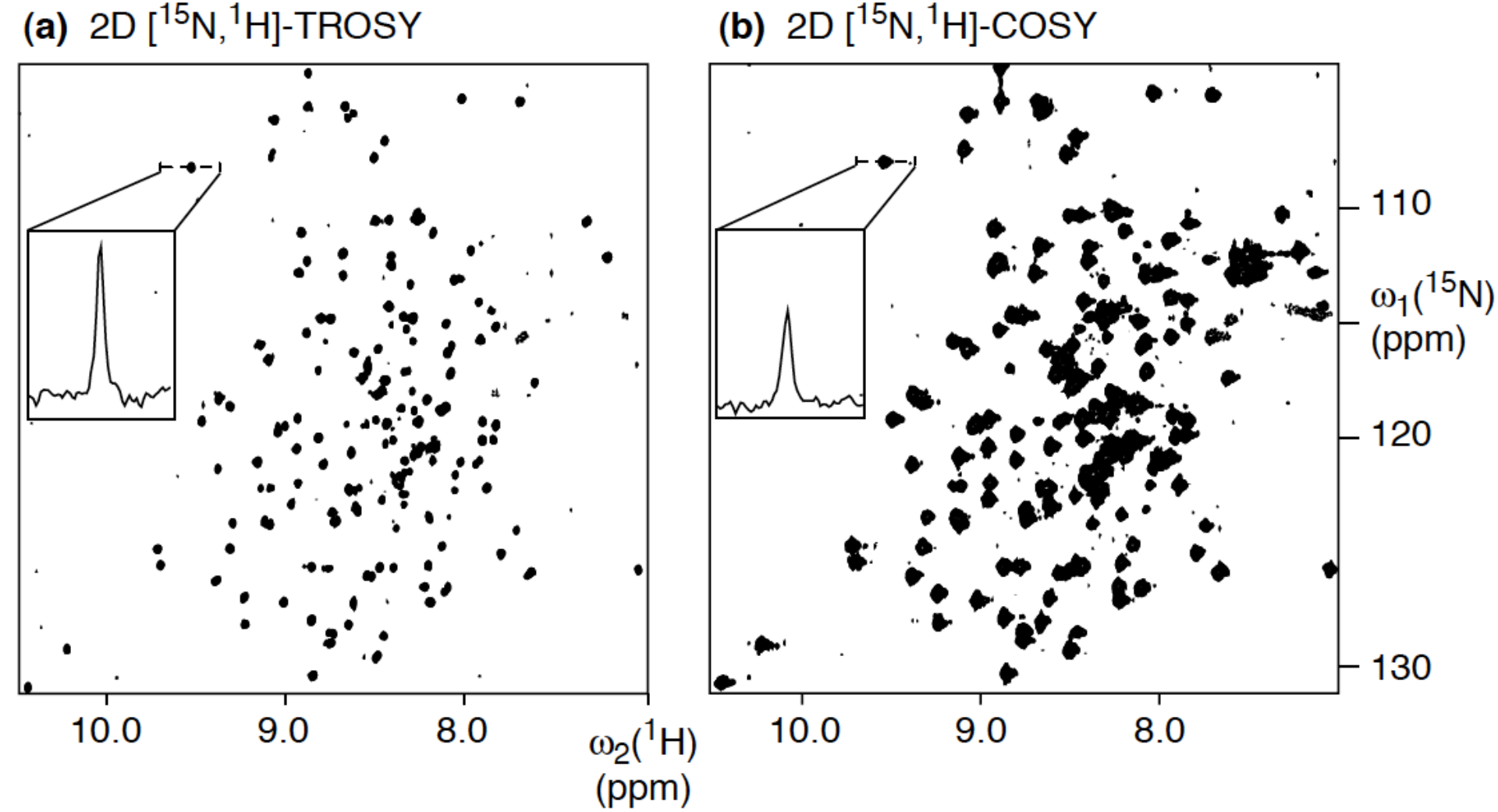


high mg. field >700 MHz



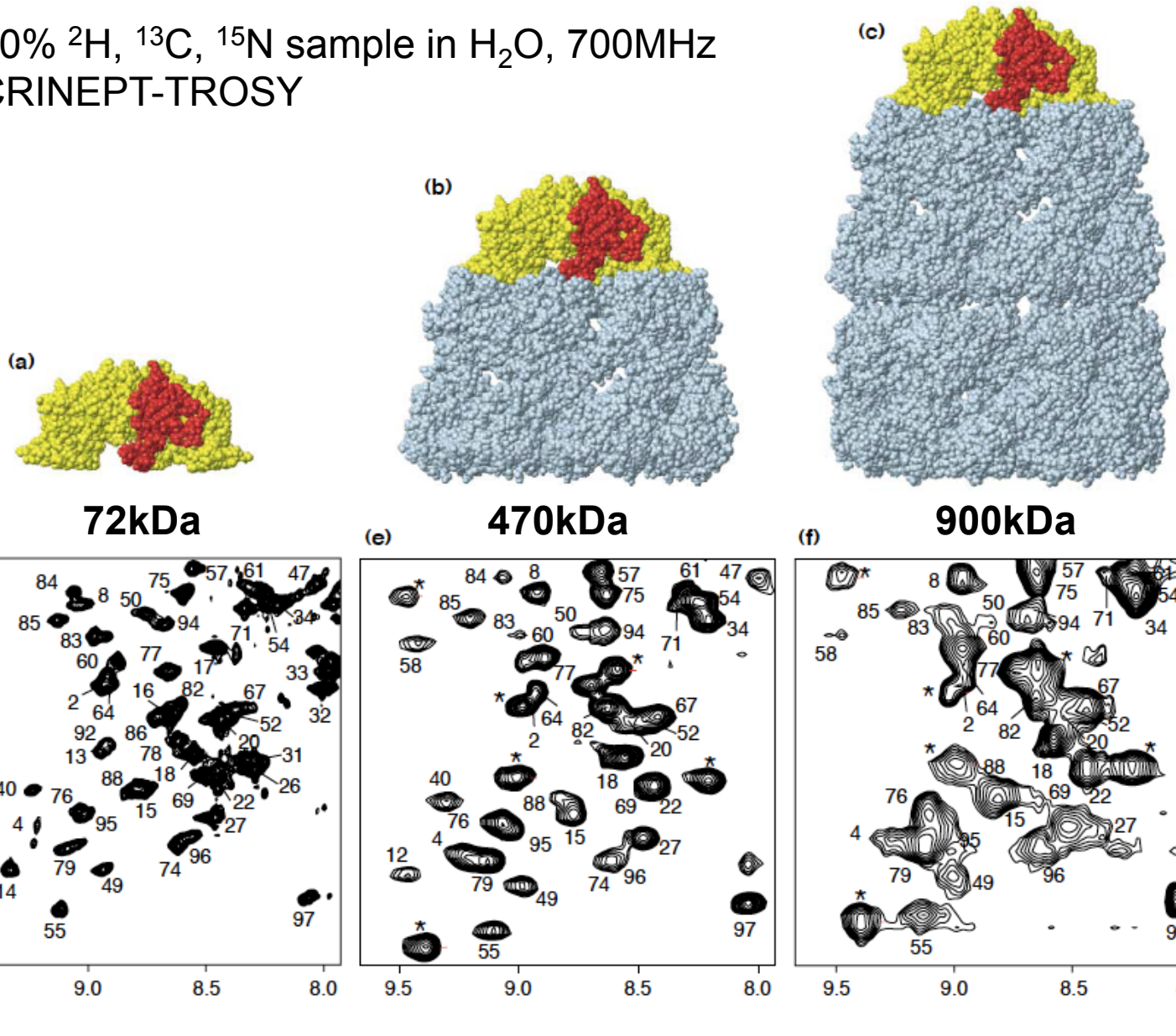
low mg. field <700 MHz





Fernandez *Curr Op Struct Biol* 2003

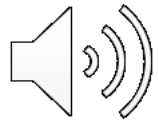
70% ^{2}H, ^{13}C, ^{15}N sample in H_2O, 700MHz
CRINEPT-TROSY



Fernandez *Curr Op Struct Biol* 2003

Summary

NMR is a robust tool for studying structural properties and interaction properties of biomolecules of variable molecular size at various levels of resolution.



Thank you for your attention

For Application to Protein Characterization

by
Radovan Fiala, Karel Kubicek, and Pavel Kaderavek
CEITEC, Masaryk University

This project received funding by the European Commission under the GA n 2019-1-IT02-KA203-063391