

Core Technologies for Education and Innovation in Life Sciences



Study of Interactions and Protein Structure Determination by NMR

For Application to Protein Characterization

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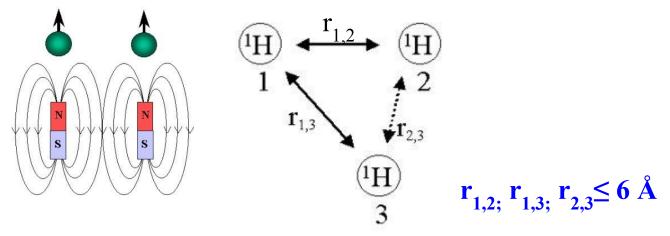
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NMR as a tool for study **structure**, **dynamics** and **interactions** of biomolecules

- 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:

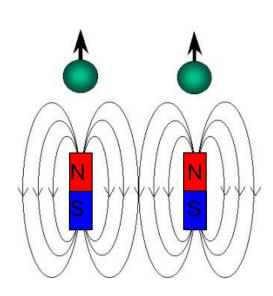


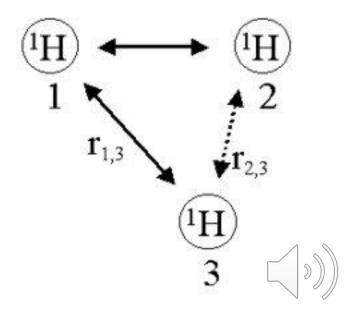
1Å=1.10⁻¹⁰m



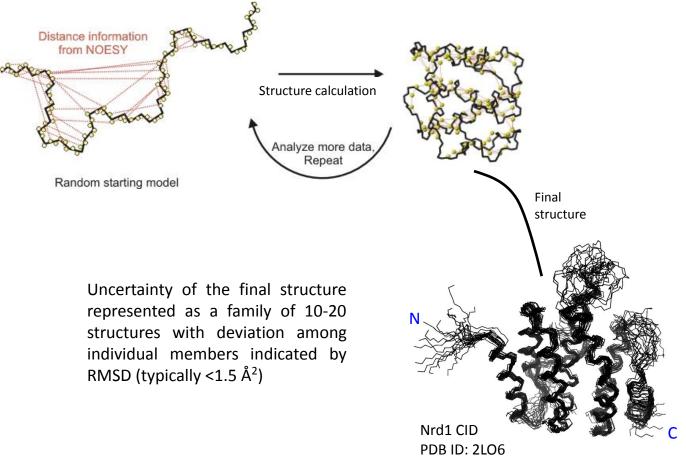
Nuclear Overhauser Effect (SpectroscopY) = NOE(SY)

- 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





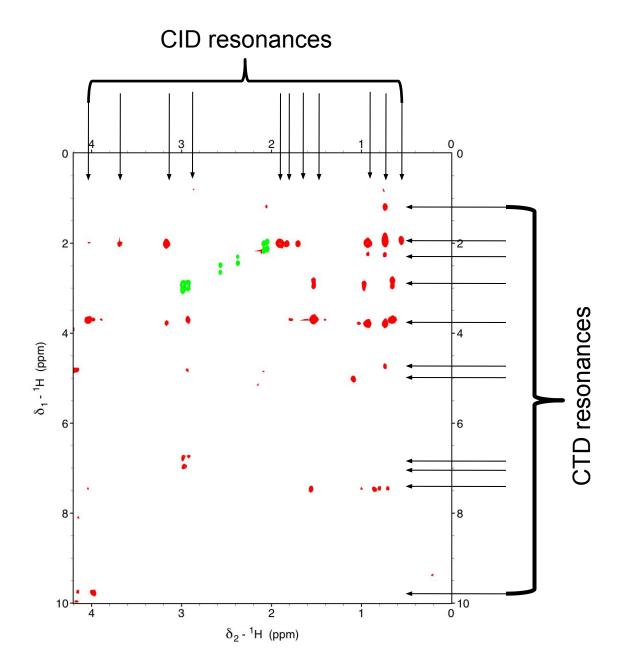
Iterative procedure of structure determination by NMR



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

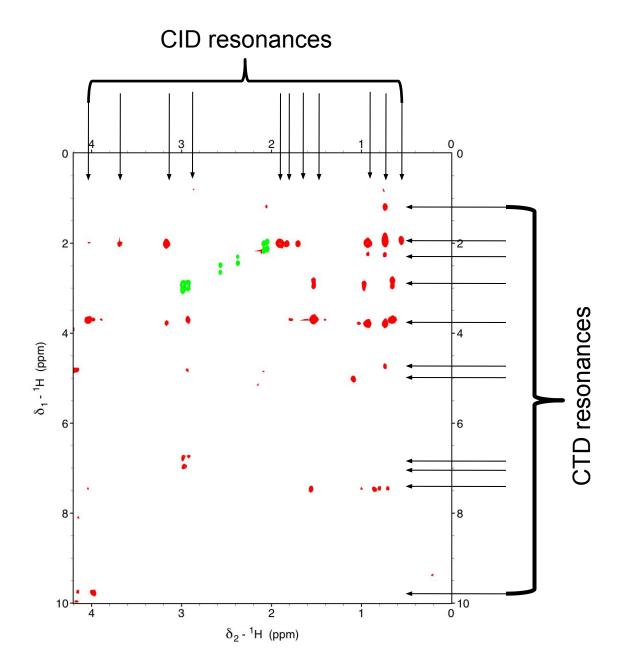


Interligand NOEs between CID and CTD – 900MHz, 150ms, 293K





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



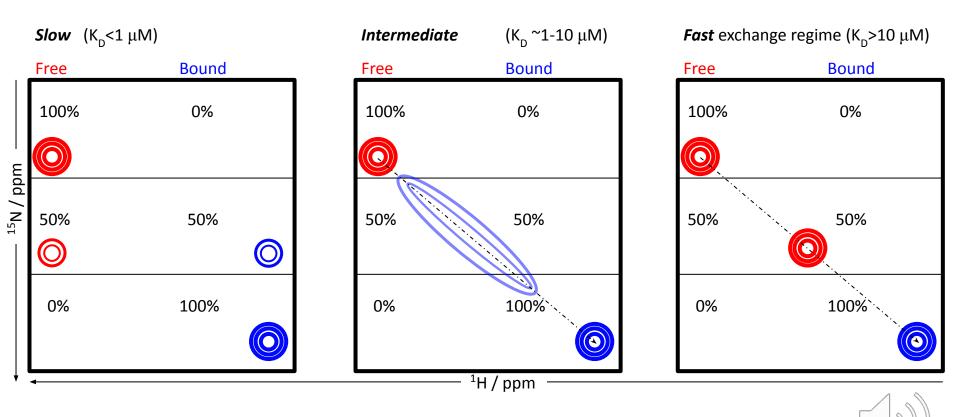


Studying interactions by NMR titration

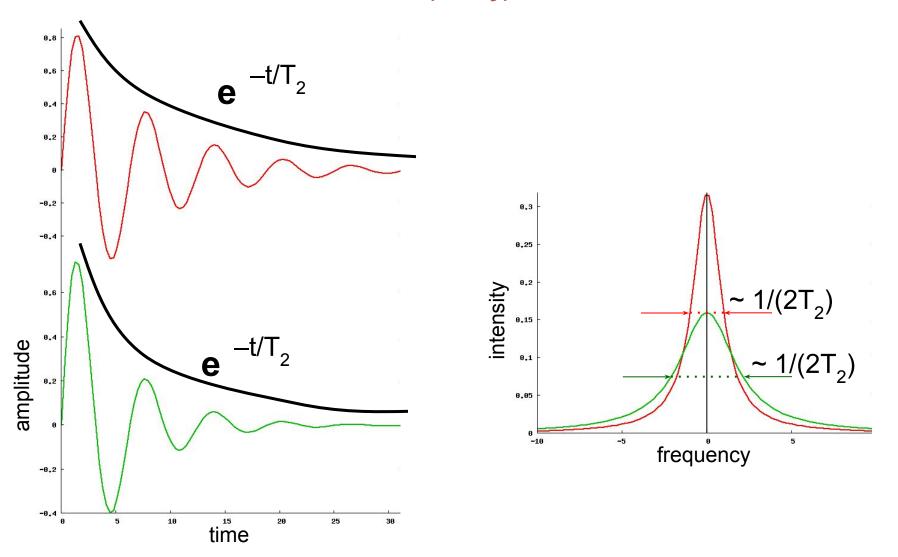
- 1) Slow exch. regime (on the NMR timescale)
- 2) Intermediate exchange regime
- 3) Fast exchange regime

 – individual peaks for each of the studied states (e.g. free / complexed forms of a protein), peak intensity representing population of a given state

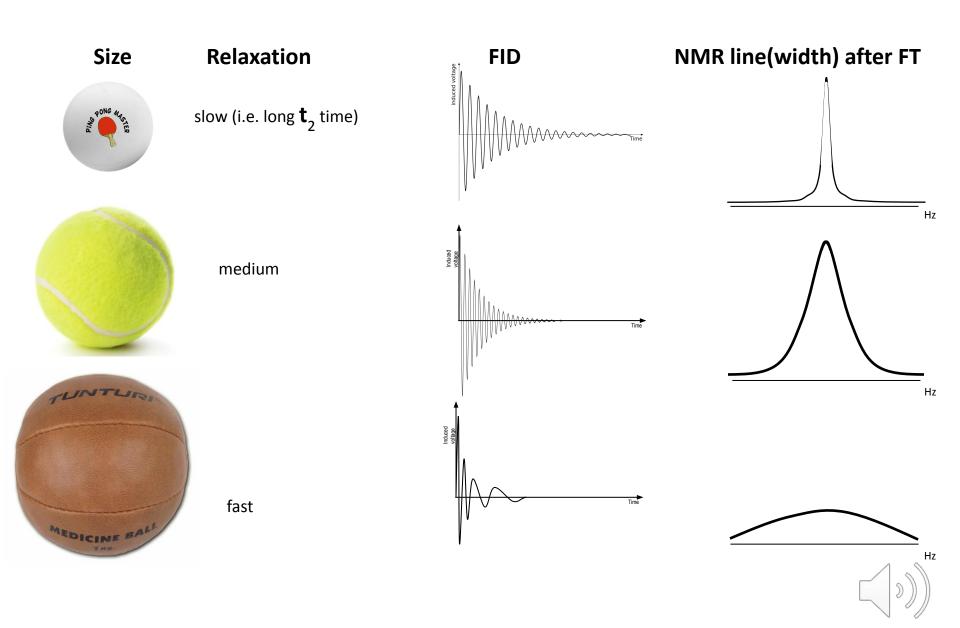
 – single peak whose chemical shift position is given by the molar ratio of the states present in solution

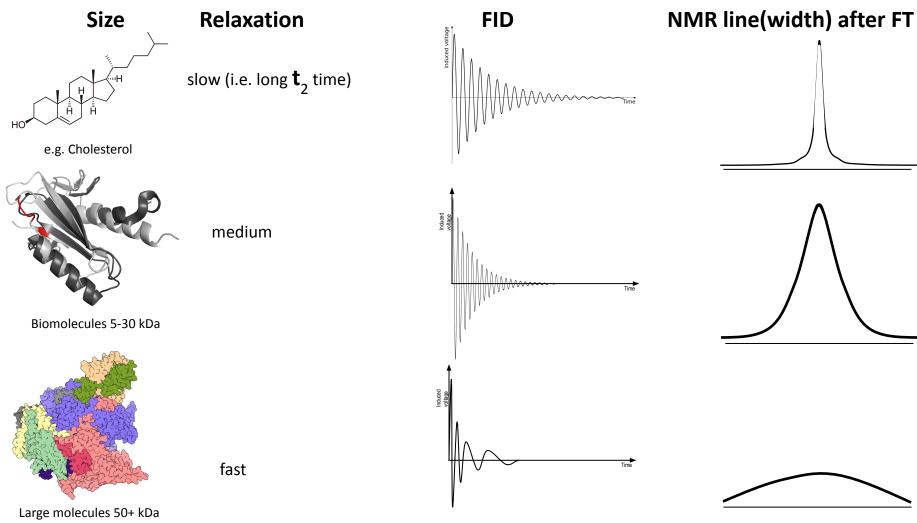


Not all molecules relax (decay) with the same rate



Bigger molecules (higher molecular weight) relax faster ⇒ broad peaks Small molecules relax slower ⇒ narow peaks







Ηz

Hz

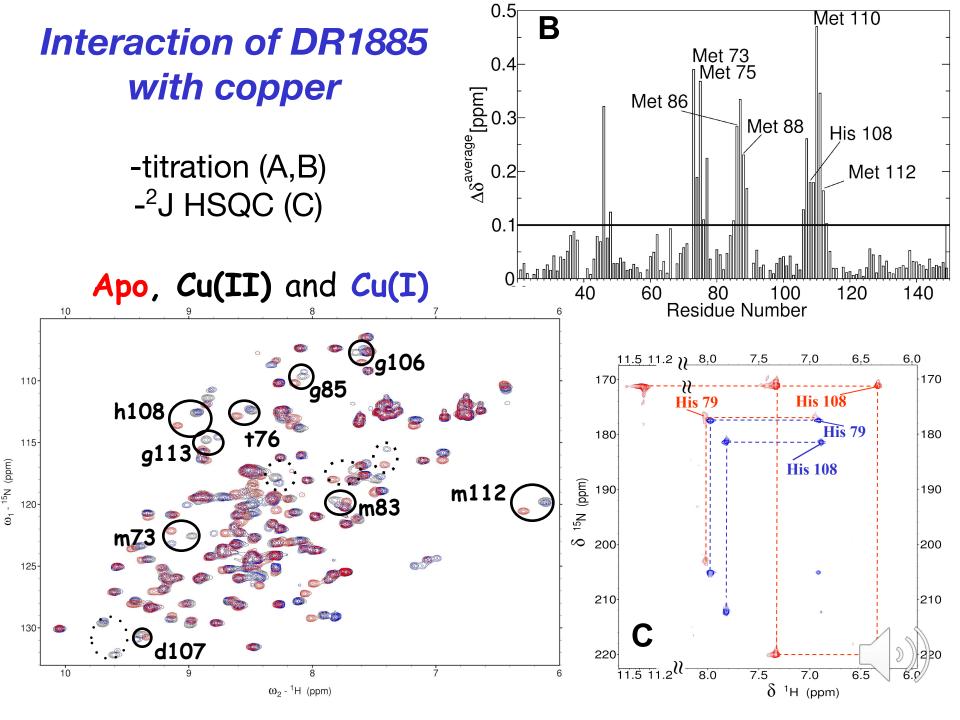
Protein – metal ion interaction

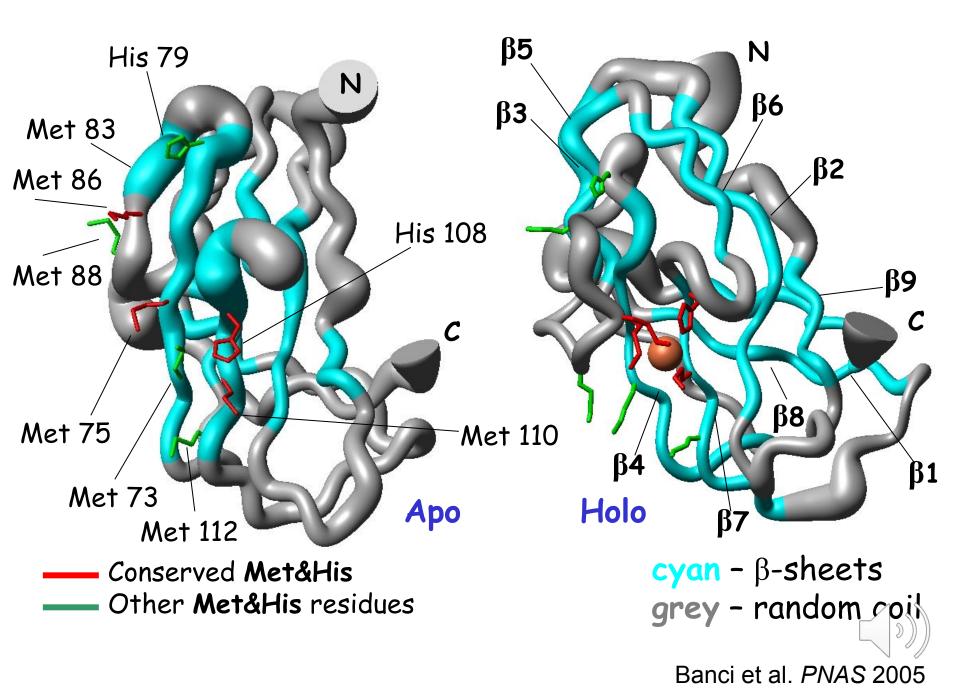
slow exchange case



DR1885 from deinococcus radiodurans

1 A A Y M T L T N K S P 4 V G A A T P L A T S P 4	_ <mark>M</mark> T 🗋	🖸 THSG-GMA (MKMVPW-LTIPARGTLTLQRDGIHVMLMGLKRP
	T T	II QEQ-GMVI	MRHVEG-FDIPSHGTLTLMPSCEHVMLIGLKAP
3 A F F G E V M N H	H D	I KEG-DVM	MRQVPE-FVI PAQGTLVLKPG FHIMLLELKKP
	HQ	VSNG-SSQ	MVMVDK-LTVPAHGQVALAPGGYHVMLEDAKHK
5AIFLTIFNN SAKSDISEV JEL	НТ	II HKD- GKM	MQKIPE-IIIKAHSSTELKSGGYHIML KLKKP
	HE	1 T M N G - D V M	MREVKA-IELPAGKAVTLDPNGLHVMLMGLHNQ
	HD		MRQVDS-IEIPAKGKTVLKPGSLHIMLFDLKAP
8AIFMVIENHGA			MVEVKEGFPVPAHGSHALARGGLHVMLMGLTKP
	HD	ILAGQDGVWC	
	HE		MQKVDS-VDVAPGKDLRFA PGG`HUMLMGLKQP
	HE	1 K M E N - N V N F	MRQIPS-LDLPKMQDVQLK FGG`H\M L MG KQQ
	HE		MQHVEA-VDI PAGAKVSFA PMAVHVMLLDLKDR
13 A V F V T F A N R S Q D D I N I V A A F T P A A G K V E L	HD	I K D G - D V A P	MRQIDR-ITLGAKETTELKPGSLHIMLFDLKTP
14 M M G M I I V N E G D E P D Y L I G A K T D I A Q R V E L	HK	VIEN-DVAK	MVPQER-IEIPPKGKVEFKHHG <mark>\H\M</mark> IIGLKKR
15 A V Y F T V K N G G R M A D R L T G A D T P N A A K T E L	H T	ILRTG-EVMF	MQHIDS-IEVPAGAEVKL TPGGHHIMIFKPKRP
16 G A F L T L T N - T G D A D R L L T A S A D V S E T V E L	ΗT	IIMDG-TVMF	MRQVDA-IDLPAKGSVAL RPGSFHVM LI GLKAP
17 A A F M V L M N H S M D A V S L L K A S S P Q F E R V E L	HR	MPVD-GVMP	MVEQSR-IPVPAQGKTILKPGDVHVMLMMGKAA
18 A G Y L T I S N T G D E D I T L T E A A T S L S D R T E L	HT	/ E T T E S G A A O	MVPVDD-IPIPAGETVELASGGLHIMVLDIADP
19 A A Y M V I V N - N C	H N	/ V 🗖 🚽 M (
20 G A F M R I T A E O V D V A S P V A K T V Q I	HE		MQRVNS-VDLPAG O DSDGNHNMF
	HT	IV YF	
	HN		
	HE	M S	M G P V K S - V D L P A G D P N G Y H Y M L Q
25 A A Y F T L E N K G D S A D R L I S V D T P I A G Q A Q L	비브	IVHAD-GLMM	MQHVQA-VDIPAGAKVVFAPMAVHYMLLDIKDR
26 G A F M T L Q A A D G A R L V G I S T P A A A R A E I	HE	1 K M E G - D V M F	M R A I P A - L P L P K G E A V Q L K P G G Y H Y M L Q D L K Q P
27 A V F A T L V N N S D S S V Y S G F T A D V D A A S F E	∨н	: V Y 2 2	MQEKPGGFVIPACECLELAPGGLHLMLMCAP
28 GAFMRLTAC75-VGARSALAEHTEN	HE		
29 A G F G Q F H N G C A T A A N S S V F A D V S L	HE		W RAVEL-LALEA
	Н Т	IVI VMF	MRKVEGGVAVAP APGS\HIM
31 G A F M T I T S S S D S K L L S A Q S P V A K I V Q L	HL	TMKN-DVMS	M Q P V E F - I D L P A G K P V T L D P H K Y H V M L I D L V K Ø
32 A I F G T L T N T T D E E I S L T G F E A S V D A A A Y E	I H	. V V D G	MREKEGGLTIAAGDTHELAPGQ IHLMLMGLEA P
33 G G Y V T L R N Q S S T P D R L VA V E S P A S A Q V E L	HD	IRMDG-GYMP	MRRLDDGLALPPGETVVLG PGG⁻HLM FI GPHA P
34 G A F M H I T S S T D S K L V N V T S P V A K T V Q I	ΗQ	1 S M K G - D Y M S	MQRVSS-VDLPAGKPVVFDANGYHVMFMGLL/Q
35 G G Y L T V T N T G T E P D R L T G G S L E A A G R G E L	HT	ISMEG-HNMP	
36 G G Y L T I T N N G S A P D R L L S I S S D I S D K A E L	ΗĒ	IGVKD-GVM1	MRPVVGGLEIPAGGKVVLGIGS HVMFMDLKQP
37 A I Y M V L E N Q T S S P I V V N Y I N T T I A D R V E N	НQ	IIHED-GMM	
	НТ		MREVEGGVPLEAKSVTELKPGSVHVMFMGLKKQ
	HT		MREVEGGVPLEAKSVTELKPGSTHTMFMGLKKQ
40 A A Y F I I H N G G K T A D R L L S V D S N I A P T A E L	HE	IVMQG-DLVI	MQQVPN-VALPAGGNVTFAPMANHNMLMNFTDR
41 A G F L T I T N E G D S A D E L T S V T S E A G E - V T N	HE	IDGT	M K E V D R - I E V P A H G Q L V F K S B G I H L M F E K K Q Q
	ΗT		M R K L A D G L A L P A G K A V A L K P G A L H I M V I G P K I A
43 G G G F V V R N G G S A D D R L L A V E S P A G R V E L	HE	1 T M E N - D V N P	M R K L E D G I A V P A G G T V E L K S C G L H L M F M E V K K P
44 G G F F Q L T N H G D T E D A L I A A E S P I A G R V E I	ΗT	ITNED-GVM	
45 G G F L T I A N D G K K A D K L V S V S A P G V A R V E I	ΗE	ITMQD-QIM	MRKLEGGLDLPAGKTMQLKSGSYHLMFIE/PEKP
46 G G F L T I A N D G K K A D K L V S V S A P G V K V E I	ΗE	1 T M Q D - Q I M	MRKLEGGLDLPAGKTMQLKSG6 HLMFL PEH?
47 G G Y L T I E N R G H A P E R L Q T A S A A H A L R T E I	ΗE	1 A V N N - G V M	MRPLIDGLVIAPGQIVKLAPGGCHUMF
48 A A Y F V V H N N G Q A D D R L L S V D S P I S D D Q L		IAMSATGAM	MQQVPS-VVVPAGKDLTFAPGANHVMLM PKOK
49 G G Y V T I K N T G D S D D K L V G I E S S A A G R A E I	and the second se	1 A M V N - D V M I	
50 G G F L T I E N K G G S A D R L V S G T A D I A G K V E I		1 SMDN-GVM	



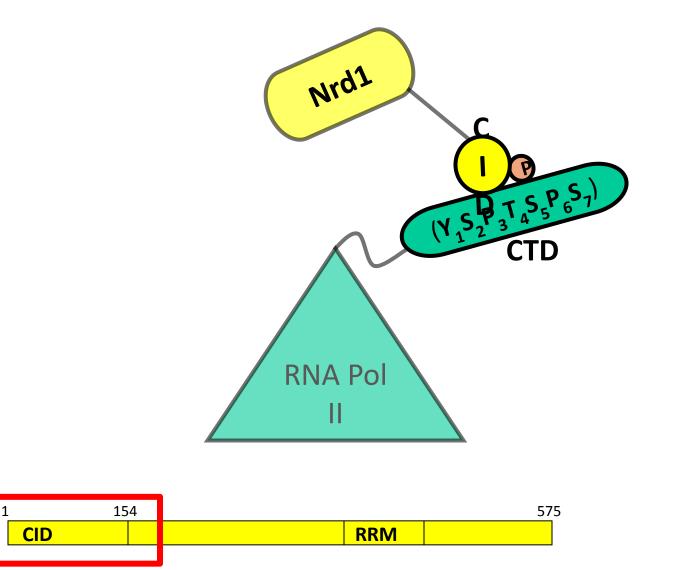


Protein – peptide interaction

fast exchange case



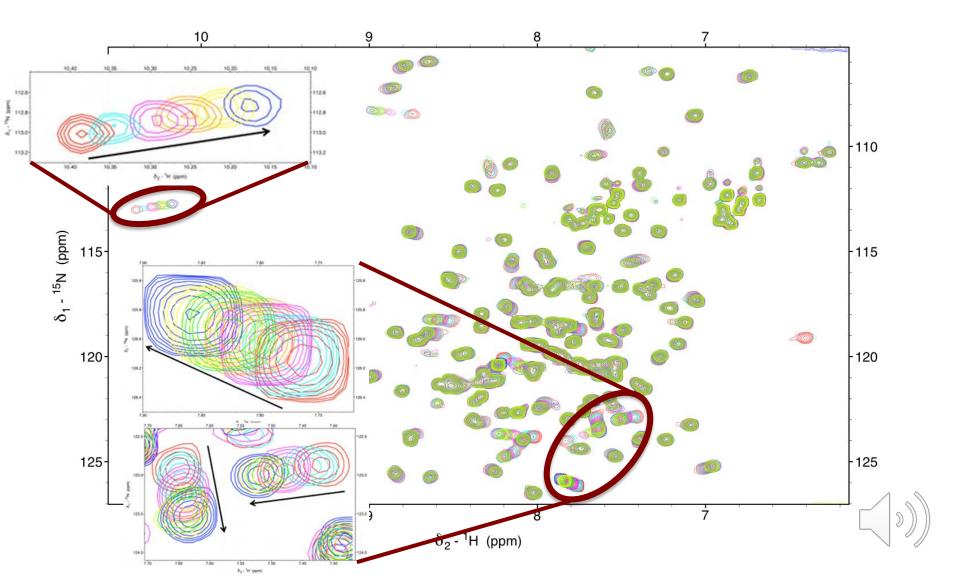
Interaction of Nrd1-CID with CTD



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

Nrd1

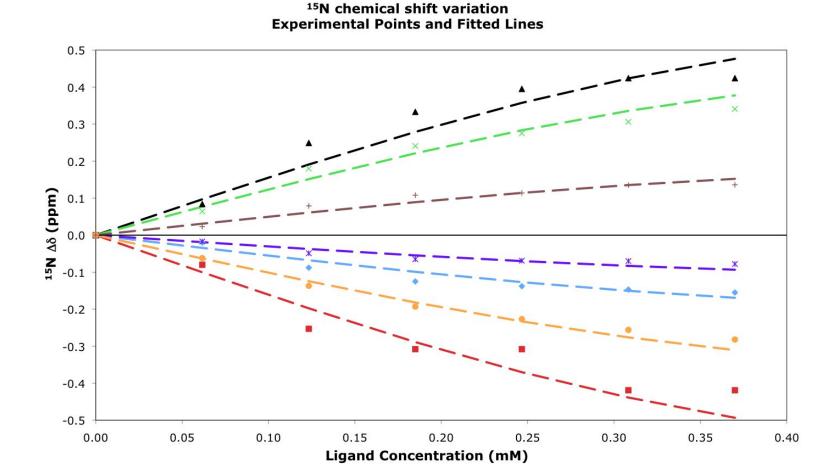
Interaction of ¹⁵N enriched CID with unlabeled CTD-Ser5P in *n*-steps, *n*=6 in our case - peaks corresponding to the interacting residues of CID change their chemical shift (position in the spectrum) =>interaction surface, binding constant, stoichiometry



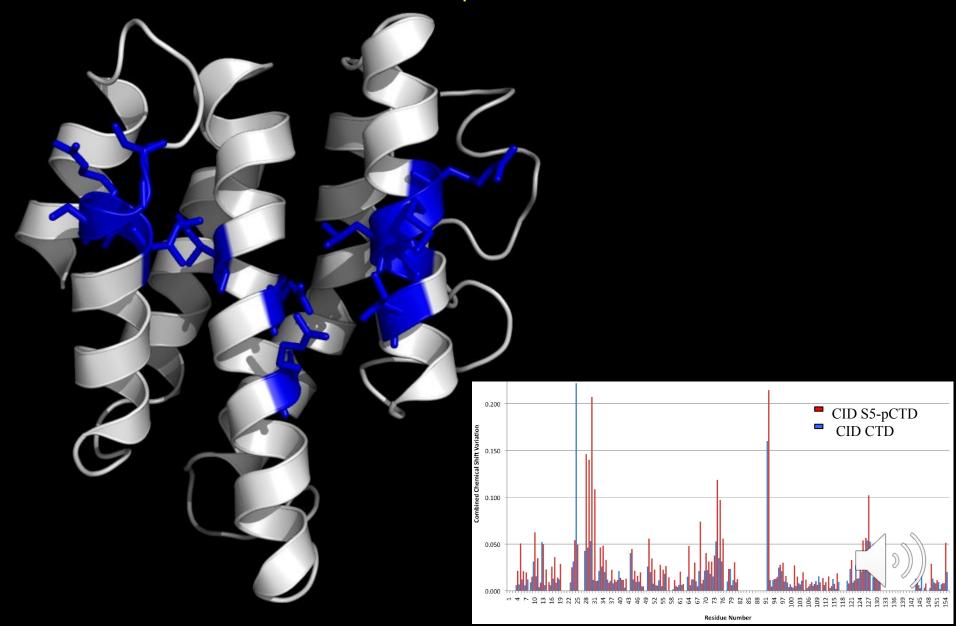
Interaction of Nrd1-CID with CTD

NMR Titration: ~ 0.6 mM ¹⁵N enriched CID + ~ 0.8 mM (YSPT<u>pS</u>PS)₂

- ~ 0.6 mM ¹⁵N enriched CID + ~ 0.8mM (YSPT<u>S</u>PS)₂
- µM-mM range of interaction ->
 - -> fast exchange regime on NMR time-scale
- NMR-derived $K_d = 0.080 \text{ mM}$ and 35 mM



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

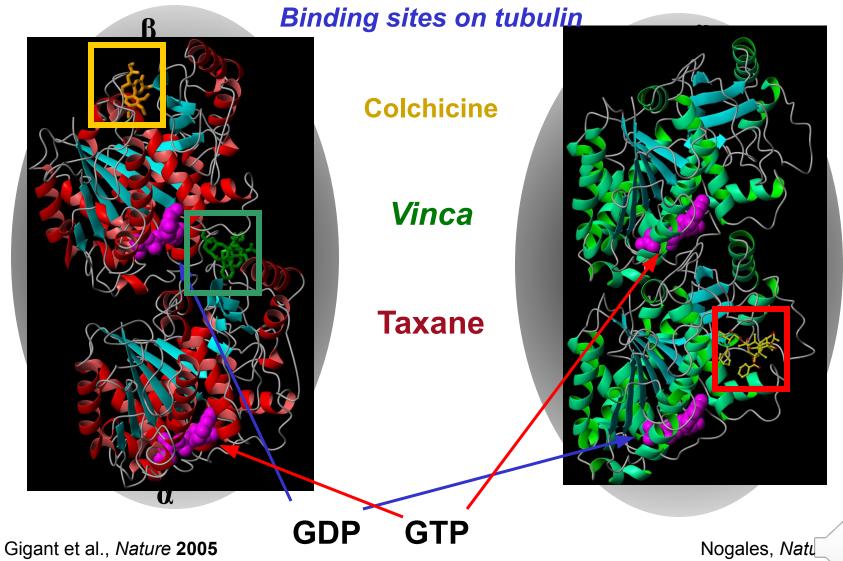


Protein – peptide interaction

drug-receptor case

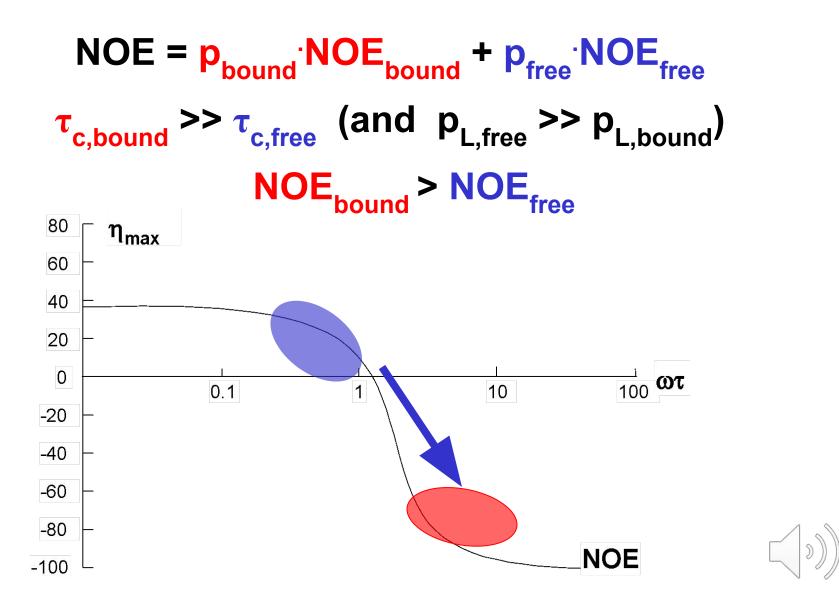


Tubulin - successful target for anticancer therapy

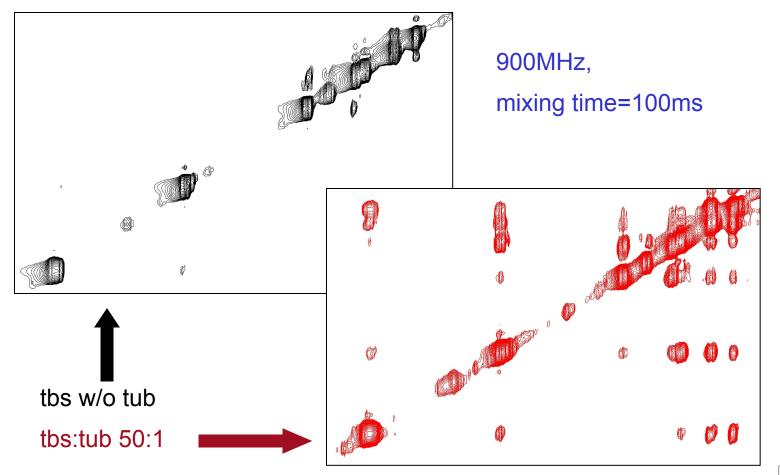


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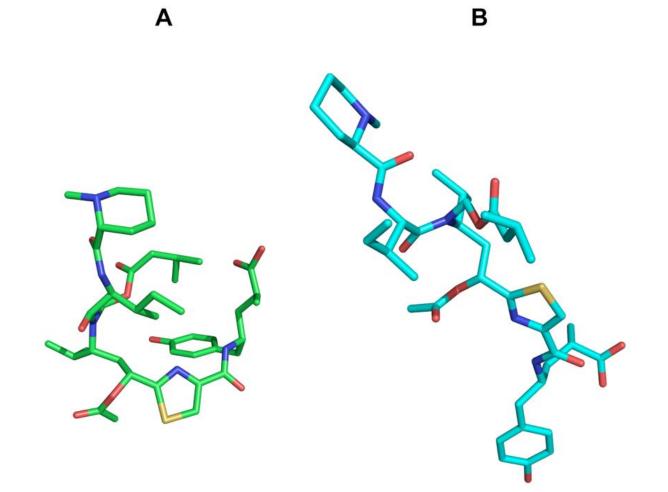
Transferred-NOE



tr-NOESY~500 μ M tubulysin (TBS) without and with ~10 μ M tubulin





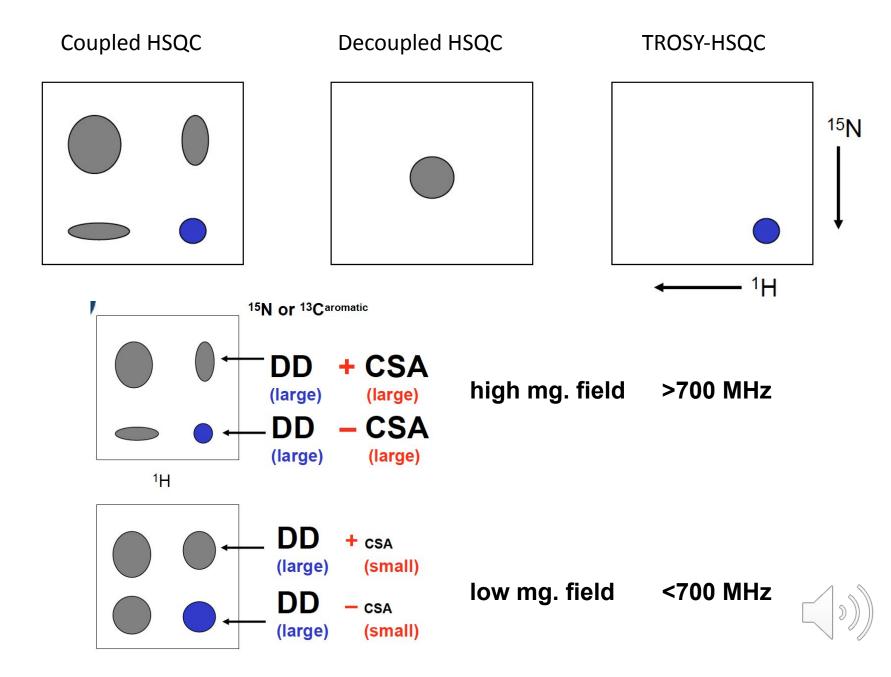


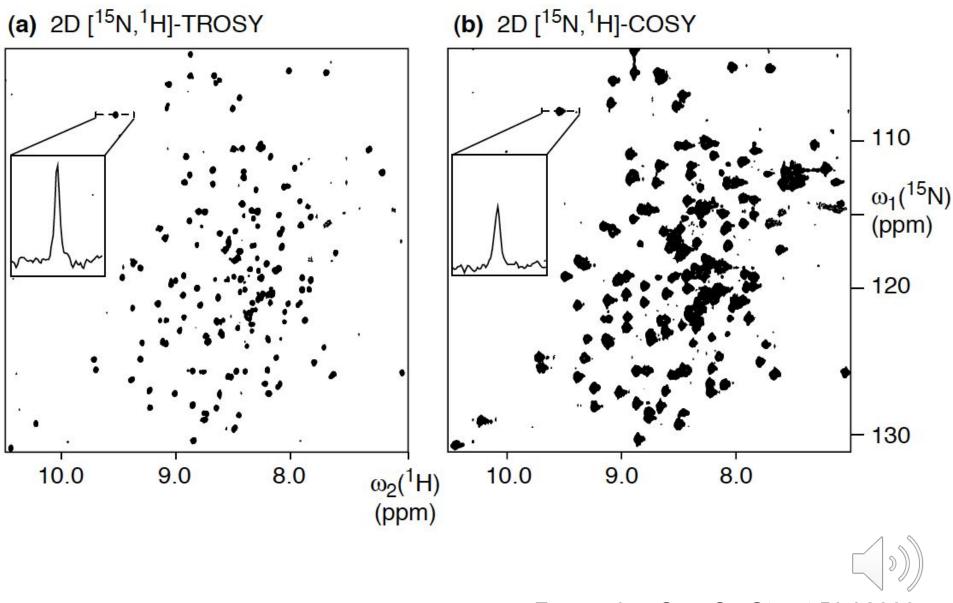
Conformation of the tubulin-bound - NMR (**A**) and free – X-Ray TBS (**B**)

Large biomolecules and their interactions

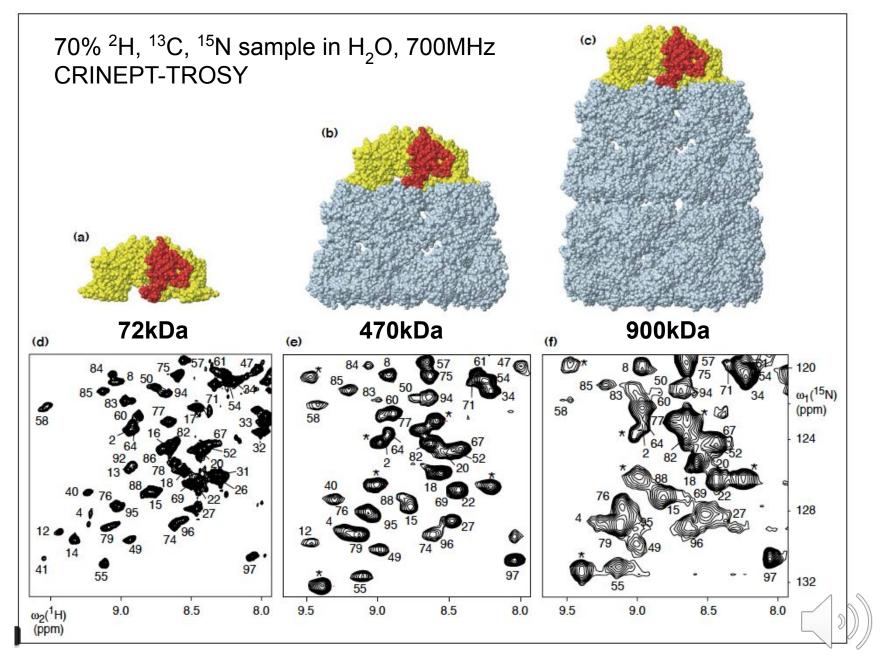


TROSY-based NMR experiments to study complexes up to 900kDa





Fernandez Curr Op Struct Biol 2003



Fernandez Curr Op Struct Biol 2003



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



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Thank you for your attention

For Application to Protein Characterization

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