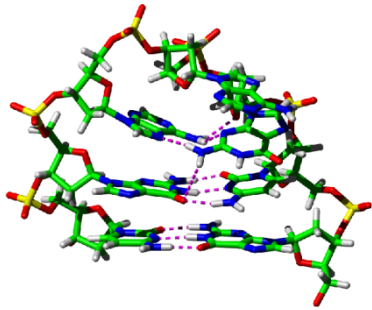
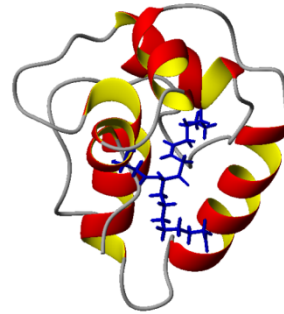


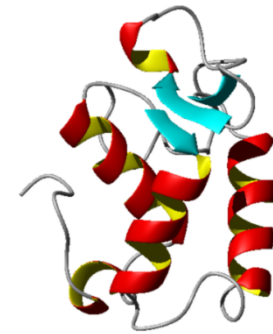
Quo Vadis NMR?



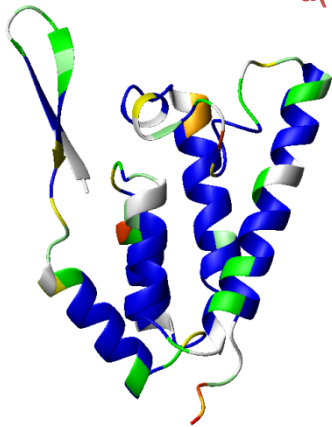
d(GCGAAGC)



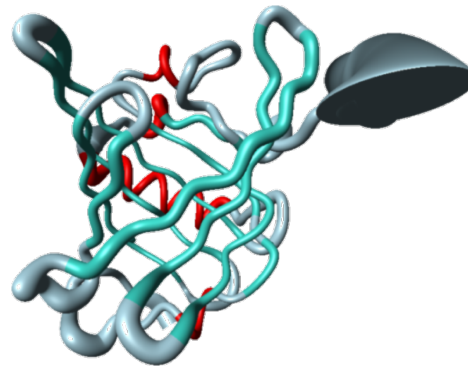
LTP-1



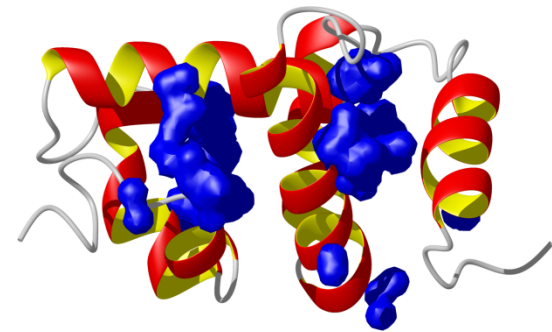
δ subunit of RNA polymerase



CANT



MUP-I



CSP-1

NMR

$$E = h \cdot \nu$$

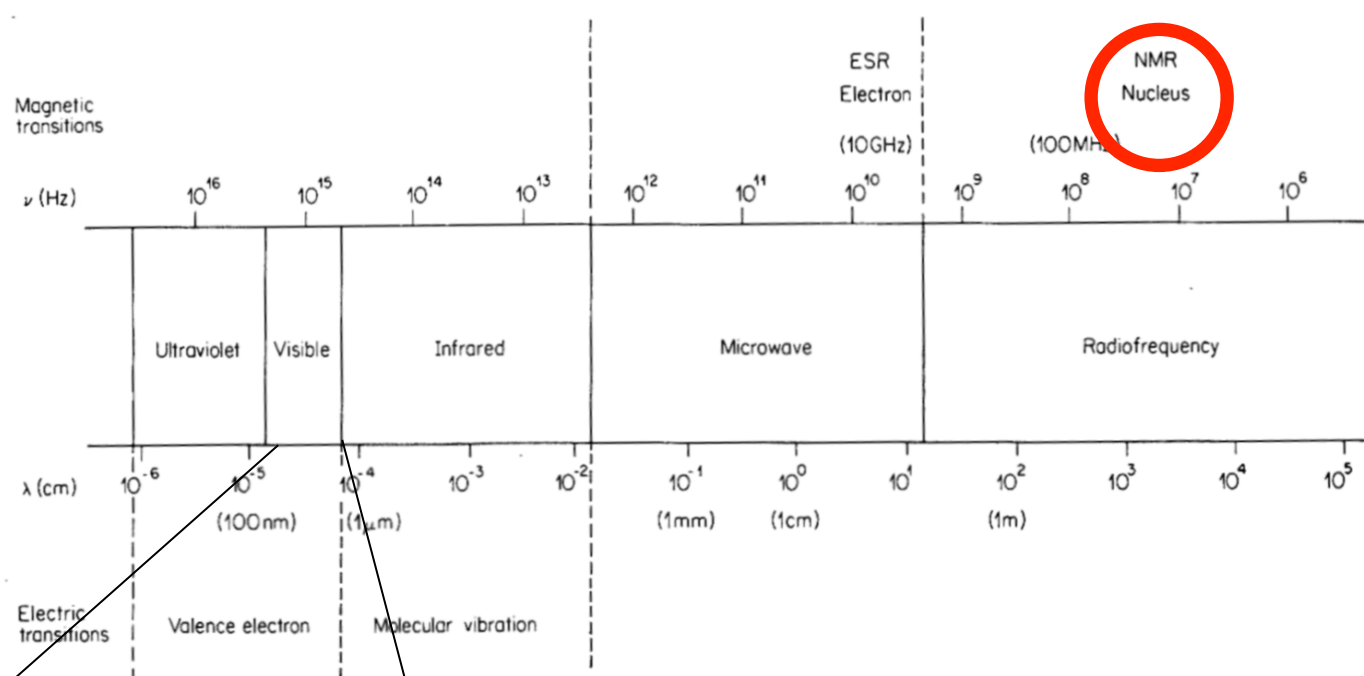
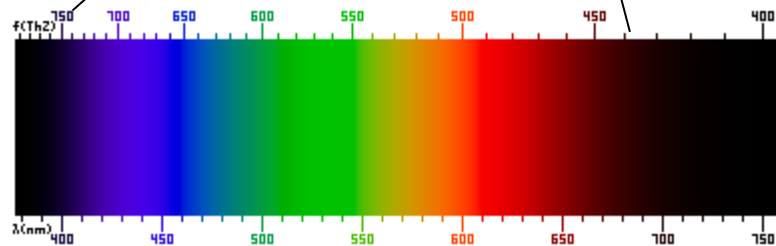
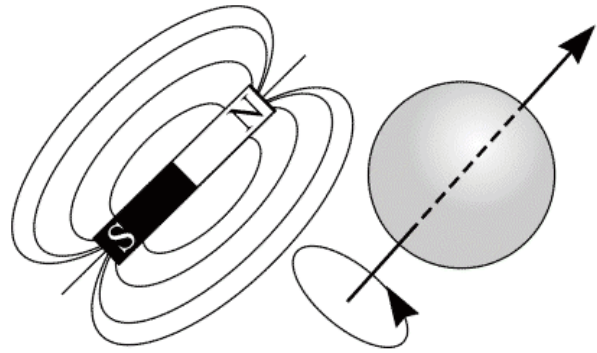


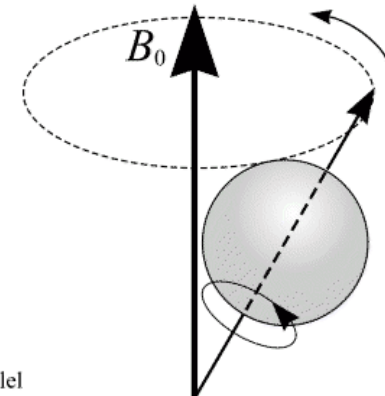
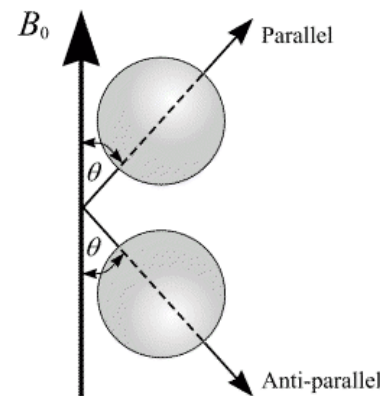
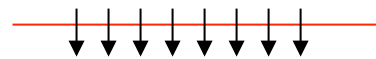
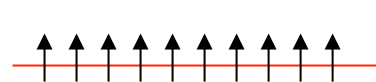
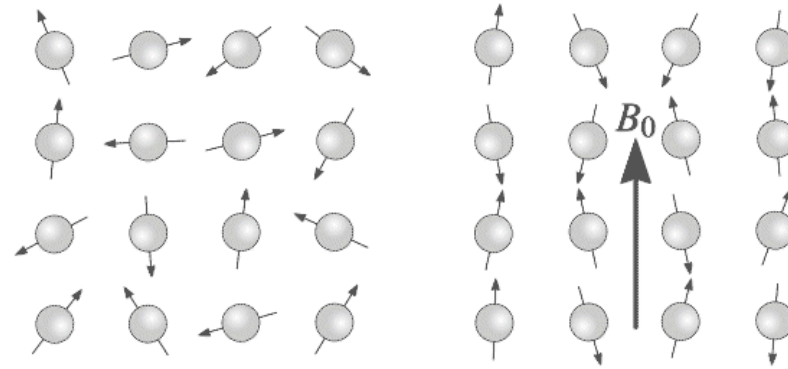
Figure 1.2 Spectral regions of the electromagnetic spectrum of interest in biological investigations



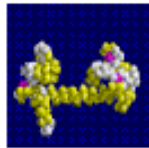


1945 · · · · 1952

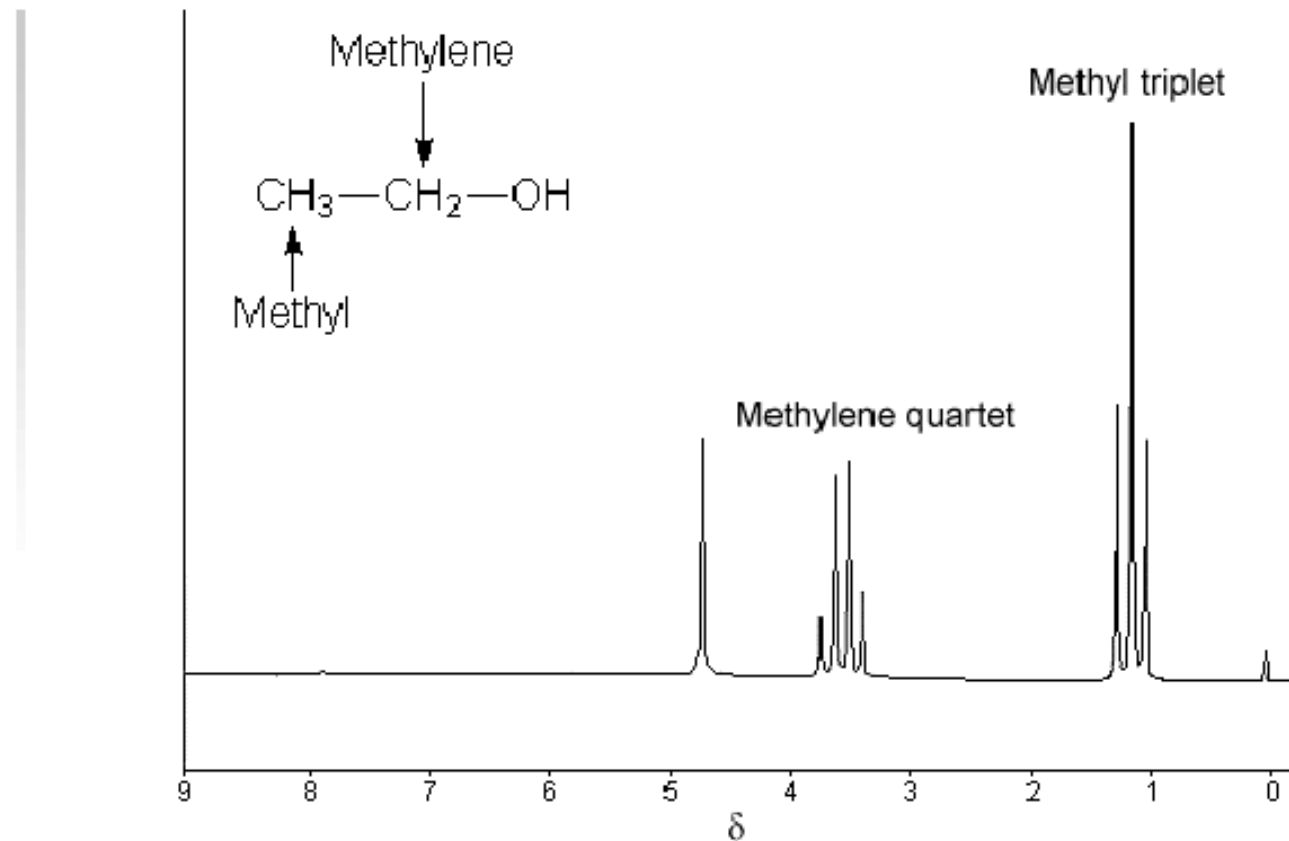
$$\omega = \gamma \cdot B_0$$



$$\nu = \gamma \cdot B_0 / 2\pi \cdot (1 - \sigma) \sim 10 - 1000 \text{ MHz}$$

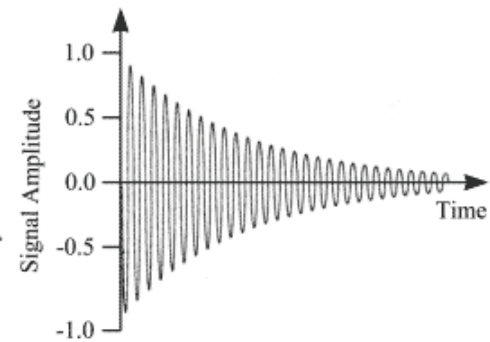
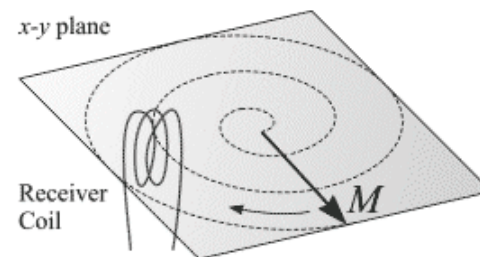
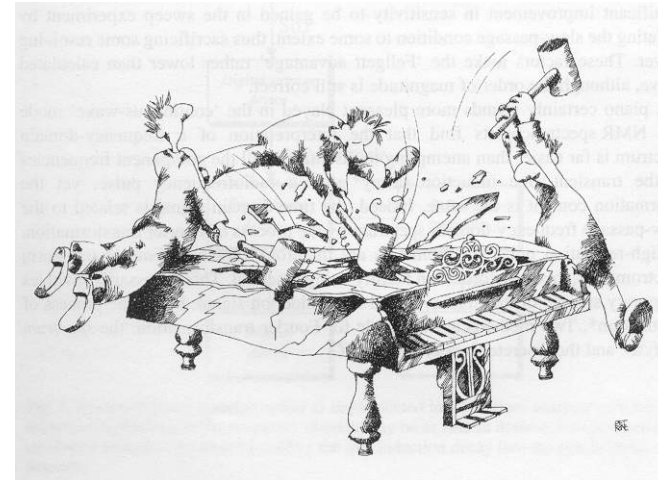


The NMR spectrum of ethanol



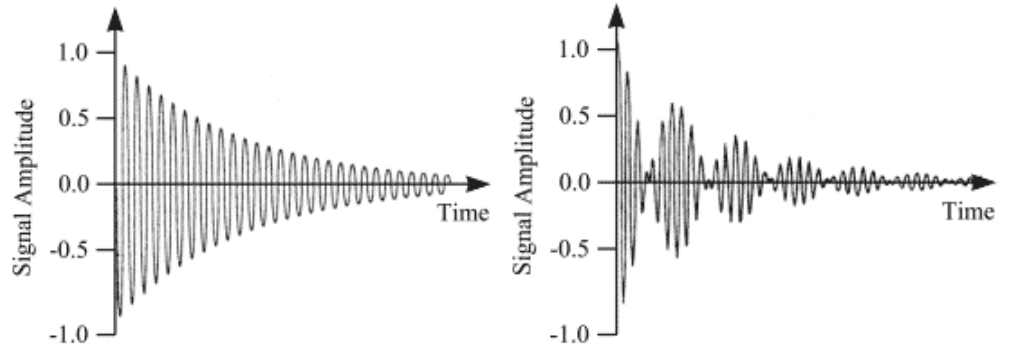
Classical Spectroscopy: dv/dt or dB/dt

$$\nu = \gamma \cdot B_0 / 2\pi \cdot (1 - \sigma)$$

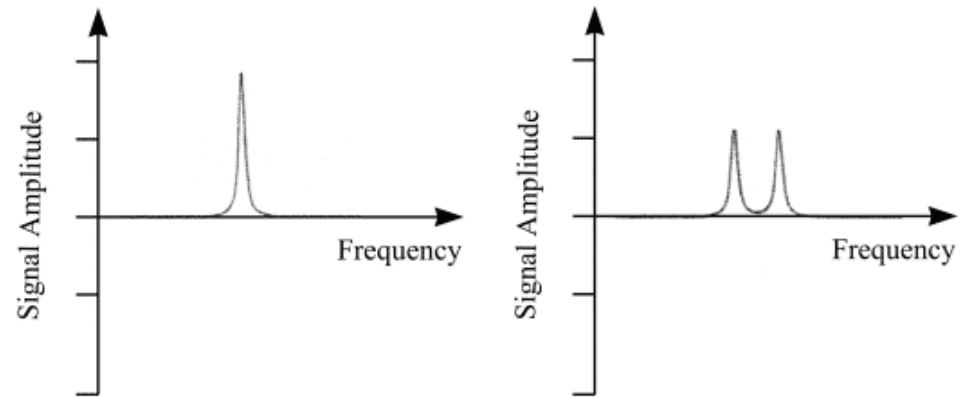




Joseph Fourier
(1768 – 1830)



Fourier Transform



Jean Baptiste Joseph Fourier
born Auxerre, March 21, 1768
died, Paris, May 16, 1830

He took a prominent part in his own district in promoting the revolution, and was rewarded by an appointment in 1795 in the Normal school, and subsequently by a chair in the Polytechnic school.

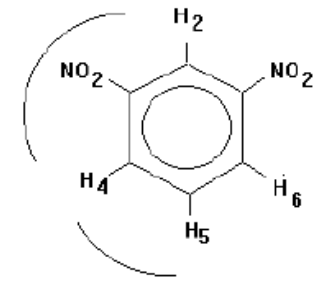
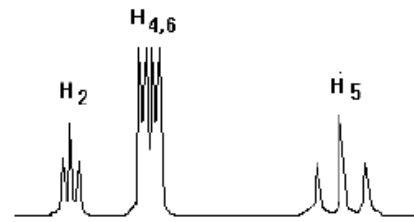
Fourier went with Napoleon on his Eastern expedition in 1798, and was made governor of Lower Egypt.

After the British victories and the capitulation of the French under General Menou in 1801, Fourier returned to France, and was made prefect of Grenoble, and it was while there that he made his experiments on the propagation of heat. He moved to Paris in 1816. In 1822 he published his *Théorie analytique de la chaleur*, in which he shows that **any functions of a variable, whether continuous or discontinuous, can be expanded in a series of sines of multiples of the variable** - a result which is constantly used in modern analysis.

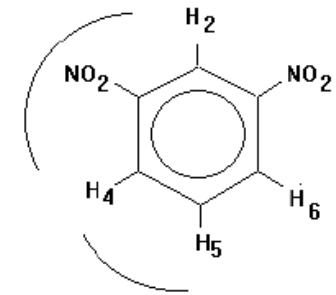
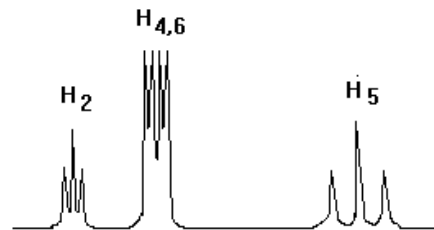


**J.W.Cooley and J.W.Tukey, *Math. Comp.* 1965, 19, 297
Fast Fourier Transform**

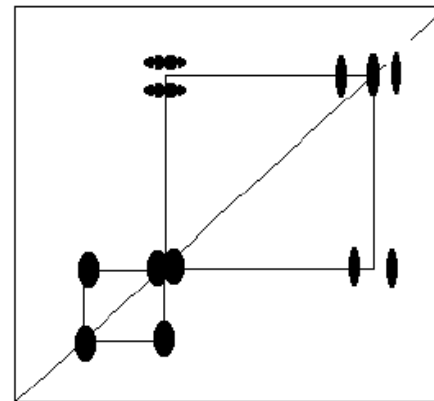
1D NMR



2D NMR



3D NMR



nD NMR



66

NMR



Felix Bloch
(1905-1983)



Edward M. Purcell
(1912-1997)

Nuclear Induction
F. BLOCH, W. W. HANSEN, AND MARTIN PACKARD
Stanford University, Stanford University, California
January 29, 1946

THE nuclear magnetic moments of a substance in a constant magnetic field would be expected to give rise to a small paramagnetic polarization, provided thermal equilibrium be established, or at least approached. By superposing on the constant field (z direction) an oscillating magnetic field in the x direction, the polarization, originally parallel to the constant field, will be forced to precess about that field with a latitude which decreases as the frequency of the oscillating field approaches the Larmor frequency. For frequencies near this magnetic resonance frequency one can, therefore, expect an oscillating induced voltage in a pick-up coil with axis parallel to the y direction. Simple calculation shows that with reasonable apparatus dimensions the signal power from the pick-up coil will be substantially larger than the thermal noise power in a practicable frequency band.

We have established this new effect using water at room temperature and observing the signal induced in a coil by the rotation of the proton moments. In some of the experiments paramagnetic catalysts were used to accelerate the establishment of thermal equilibrium.

By use of conventional radio techniques the induced voltage was observed to produce the expected pattern on an oscillograph screen. Measurements at two frequencies showed the effect to occur at values H of the order of 100 gauss that the ratio H/ν had the same value. Within the experimental error this ratio agreed with the value for protons, as determined by Kellogg, Rabi, and Zacharias.¹

We have thought of various investigations in which this effect can be used fruitfully. A detailed account will be published in the near future.

¹J. M. B. Kellogg, I. I. Rabi, N. F. Ramsey, and J. R. Zacharias, *Phys. Rev.* **56**, 738 (1939).

Figure 9 The first report of nuclear induction.²² (Reproduced by permission of the American Physical Society)

Physical Review 69, 37 (1946)

THE DEVELOPMENT OF NMR 9

Reprinted from THE PHYSICAL REVIEW, Vol. 69, Nos. 1 and 2, 37-38, January 1 and 15, 1946
Printed in U. S. A.

Resonance Absorption by Nuclear Magnetic Moments in a Solid
E. M. PURCELL, H. C. TOUSSAINT, AND R. V. POUPP^{*}
Radiation Laboratory, Massachusetts Institute of Technology,
Cambridge, Massachusetts
December 24, 1945

IN the well-known magnetic resonance method for the determination of nuclear magnetic moments by molecular beams,¹ transitions are induced between energy levels which correspond to different orientations of the nuclear spin in a strong, constant, applied magnetic field. We have observed the absorption of radiofrequency energy, due to such transitions, in a solid material (paraffin) containing protons. In this case there are two levels, the separation of which corresponds to a frequency, ν , near 30 megacycles/sec., at the magnetic field strength, H , used in our experiment, according to the relation $h\nu = 2\mu_N H$. Although the difference in population of the two levels is very slight at room temperature ($h\nu/kT \sim 10^{-6}$), the amount of nuclear taking part is so large that 7×10^{23} spins are to be expected providing thermal equilibrium can be established. If one assumes that the fields of importance are caused by the moments of neighboring nuclei, one can show that the inhomogeneous part of the magnetic permeability, at resonance, would be of the order $h\nu/kT$. The absorption of energy is explained by the fact that the sum of these factors upon absorption cross section per nucleus and density of nuclei is just cancelled by their influence on the width of the observed resonance.

A crucial question concerns the time required for the establishment of thermal equilibrium between spins and lattice. A difference in the populations of the two levels is a prerequisite for the observed absorption, because of the relation between absorption and stimulated emission. Moreover, unless the relaxation time is very short the absorption of energy from the radiofrequency field will equalize the population of the levels, more or less rapidly, depending on the strength of this r -f field. In the expectation of a long relaxation time (several hours), we chose to use so weak an oscillating field that the absorption would persist for hours regardless of the relaxation time, once thermal equilibrium had been established.

A resonant cavity was made in the form of a short section of coaxial line loaded heavily by the capacity of an end plate. It was adjusted to resonate at about 30 mc/sec. Input and output coupling loops were provided. The inductive part of the cavity was filled with 850 cm³ of paraffin, which remained at room temperature throughout the experiment. The resonator was placed in the gap of the large cosmic-ray magnet in the Research Laboratory of Physics, at Harvard. Radiofrequency power was introduced into the cavity at a level of about 10^{-10} watts. The radiofrequency magnetic field in the cavity was everywhere perpendicular to the steady field. The cavity output was balanced in phase and amplitude against another portion of the signal generator output. Any residual signal, after amplification and detection, was indicated by a microammeter.

With the r -f circuit balanced the strong magnetic field

was slowly varied. An extremely sharp resonance absorption was observed. At the peak of the absorption the deflection of the output meter was roughly 20 times the magnitude of fluctuations due to noise, frequency, instability, etc. The absorption reduced the cavity output by 0.4 percent, and as the loaded Q of the cavity was 670, the imaginary part of the permeability of paraffin, at resonance, was about $3 \cdot 10^{-4}$, as predicted.

Resonance occurred at a field of 7100 oersted, and a frequency of 29.8 mc/sec., according to our rather rough calibration. We did not attempt a precise calibration of the field and frequency, and the value of the proton magnetic moment inferred from the above numbers, 2.75 nuclear magnetons, agrees satisfactorily with the accepted value, 2.7896, established by the molecular beam method.

The full width of the resonance, at half value, is about 10 oersteds, which may be caused in part by inhomogeneities in the magnetic field which were known to be of this order. The width due to local fields from neighboring nuclei had been estimated at about 4 oersteds.

The relaxation time was apparently shorter than the time (\sim one minute) required to bring the field up to the resonance value. The types of spin-lattice coupling suggested by I. Waller² fail by a factor of several hundred to account for a time so short.

The method can be refined in both sensitivity and precision. In particular, it appears feasible to increase the sensitivity by a factor of several hundred through a change in detection technique. The method seems applicable to the precise measurement of magnetic moments (strictly, gyromagnetic ratios) of most moderately abundant nuclei. It provides a way to investigate the interesting question of spin-lattice coupling. Incidentally, as the apparatus required is rather simple, the method should be useful for standardization of magnetic fields. An extension of the method in which the r -f field has a rotating component should make possible the determination of the sign of the moment.

The effect here described was sought previously by Gorter and Broer, whose experiments are described in a paper³ which came to our attention during the course of this work. Actually, they looked for dispersion, rather than absorption, in LiCl and KCl. Their negative result is perhaps to be attributed to one of the following circumstances: (a) the applied oscillating field may have been so strong, and the relaxation time so long, that thermal equilibrium was destroyed before the effect could be observed—(b) at the low temperatures required to make the change in permeability easily detectable by their procedure, the relaxation time may have been so long that thermal equilibrium was never established.

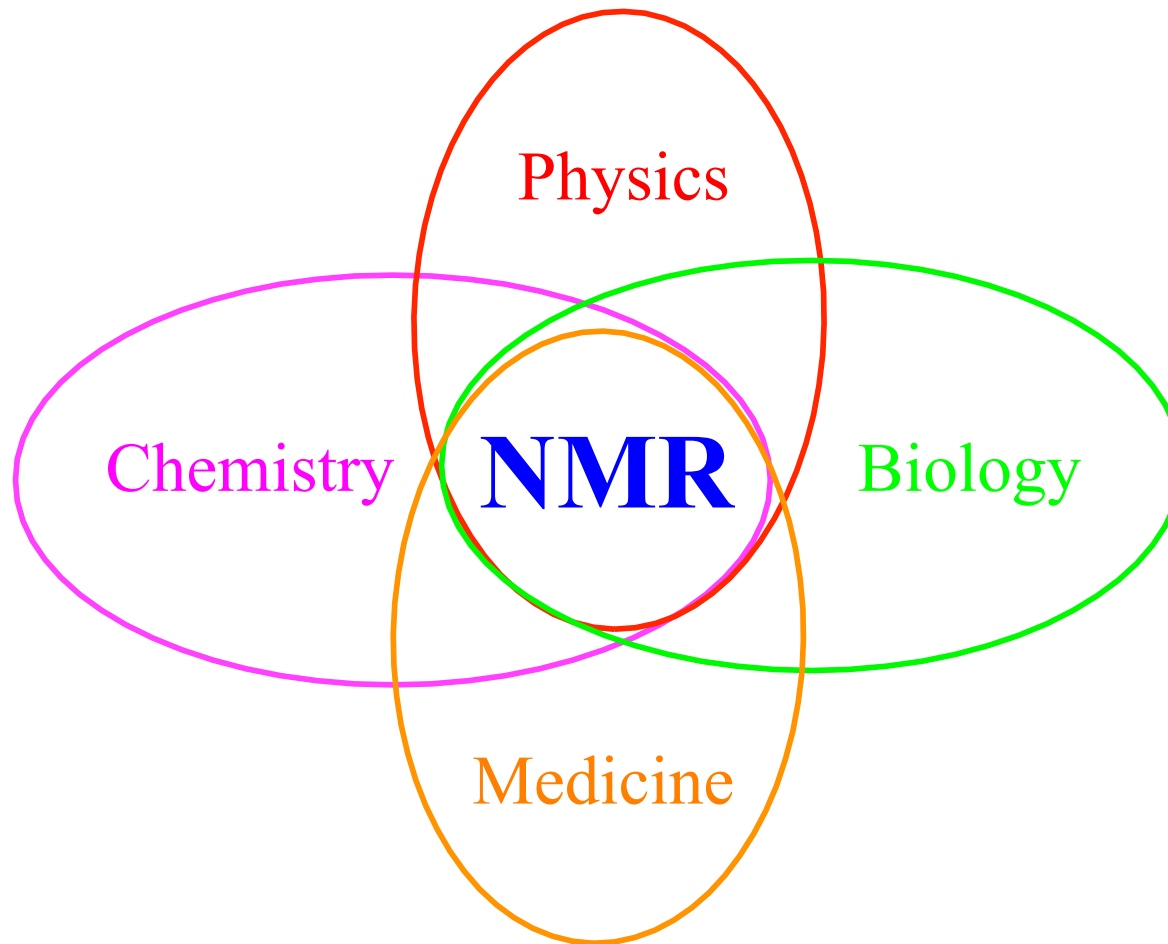
^{*} Harvard University, Society of Fellows (on leave).
¹ I. Rabi, Z. Zacharias, M. Millman, and K. Koch, *Phys. Rev.* **53**, 318 (1938).
² I. Waller, *Zeits. f. Physik* **79**, 370 (1932).
³ Gorter and Broer, *Physica* **9**, 591 (1942).

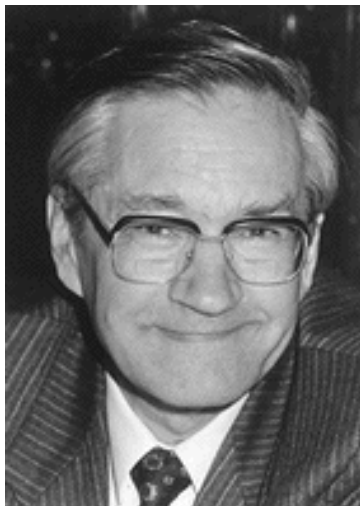
Figure 8 The first report of a nuclear magnetic resonance in a bulk material.²¹ (Reproduced by permission of the American Physical Society)

Physical Review 69, 127 (1946)

For References see p. 143

Nuclear Magnetic Resonance
64 years
1945 – 2009





Nobel Prize in Chemistry 1991

Richard R. Ernst (1933 - *)

Swiss Federal Institute of Technology (ETH), Zürich, Switzerland

“for his contributions to the development of the methodology of high resolution nuclear magnetic resonance (NMR) spectroscopy”



Nobel Prize in Chemistry 2002

Kurt Wüthrich (1938 - *)

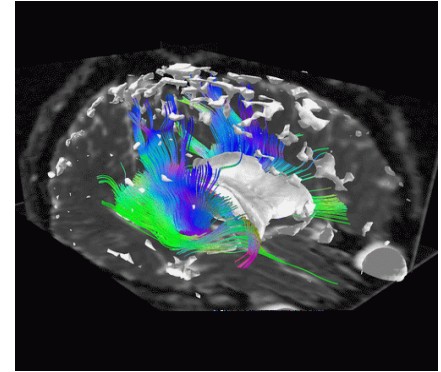
Swiss Federal Institute of Technology (ETH), Zürich, Switzerland
and The Scripps Research Institute, La Jolla, USA

“for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution”.

Nobel Prize in Physiology or Medicine 2003

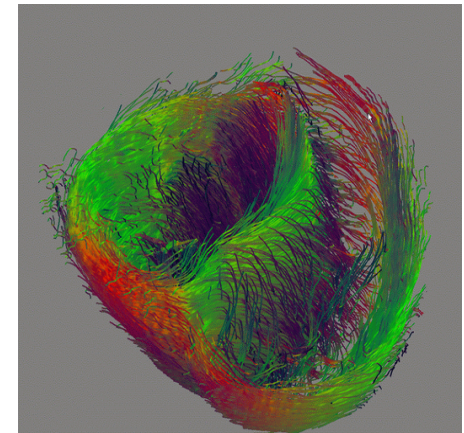


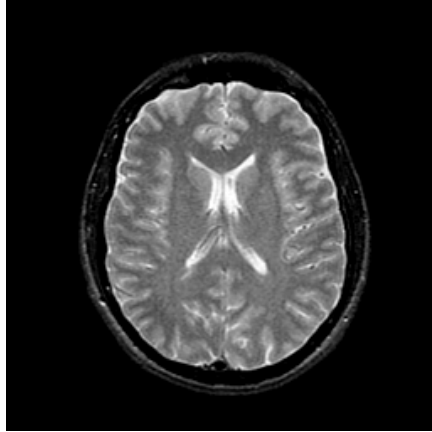
Paul C. Lauterbur (1929 - 2007)
Biomedical Magnetic Resonance Laboratory, University of Illinois, Urbana, USA



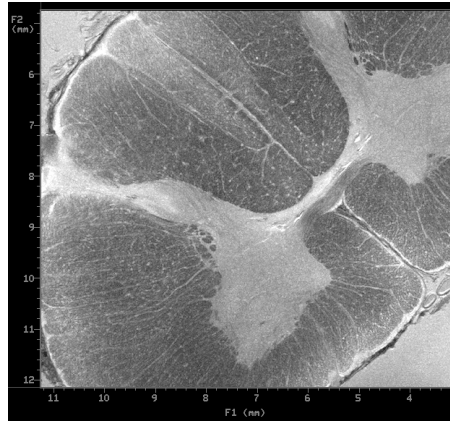
Sir Peter Mansfield (1933 - *)
Department of Physics, University of Nottingham, UK

for their discoveries concerning "magnetic resonance imaging"

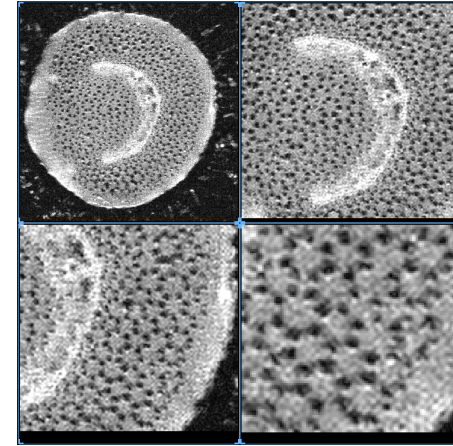




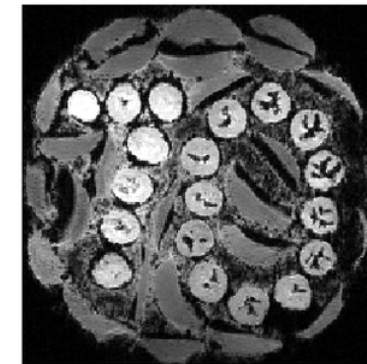
**Human Brain
AIDS dementia**



**Spinal cord
 μm resolution**



Plants



Birthday cake

Milestones of NMR History

- 1945 Detection of NMR signals in bulk materials
- 1949 Discovery of chemical shift
- 1950 Discovery of spin-spin coupling
- 1952 Bloch and Purcell receive Nobel Prize
- 1953 First commercial NMR spectrometer (Varian 30MHz)
- 1966 Fourier transform (FT) techniques introduced – R.R. Ernst
- 1971 Two-dimensional (2D) NMR concept suggested – J. Jeener
- 1973 Zeugmatography: first two-dimensional NMR image - P. Lauterbur
- 1974 2D-NMR techniques developed – R.R. Ernst
- 1985 First 3D structures of proteins from NMR data – K. Wüthrich
- 1991 Ernst receives Nobel Prize
- 2002 Wüthrich receives Noble Prize
- 2003 Lauterbur and Mansfield receive Nobel Prize
- 2009 1 GHz spectrometer (Bruker)

Magnet history



1967
90 MHz



1970
270 MHz



1979
400 MHz



1983
500 MHz



1995
800 MHz



1998
700 MHz



2009
1000 MHz



2005
950 MHz



2001
900 MHz

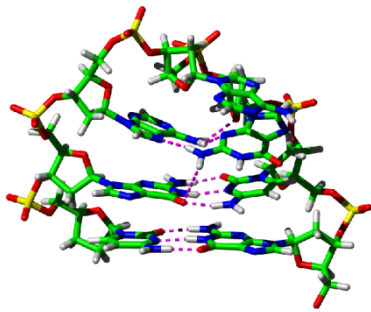


2000
800 US²

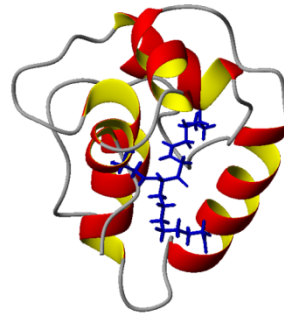


1999
750 WB

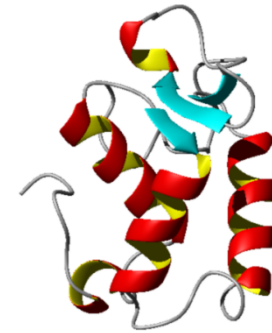
NMR as an eminent tool for structural and system biology



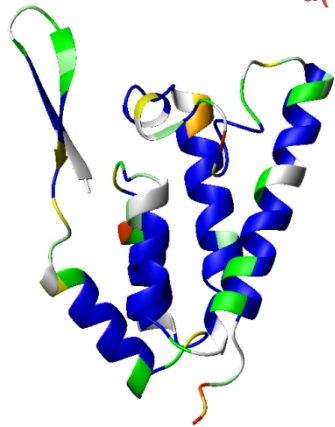
d(GCGAAGC)



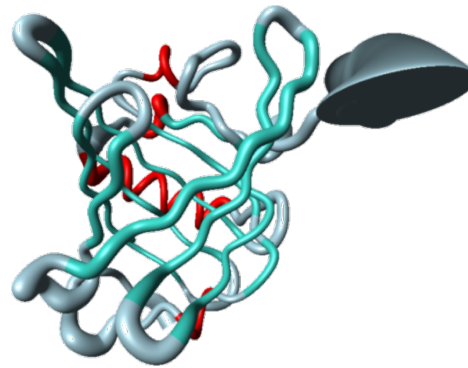
LTP-1



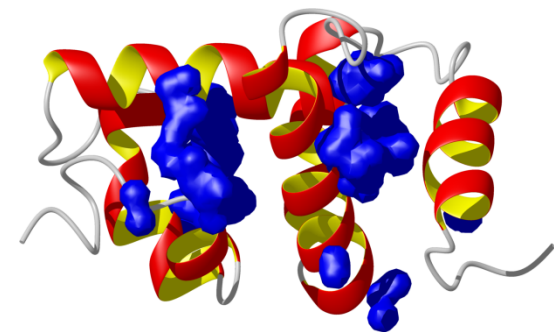
δ subunit of RNA polymerase



CANT

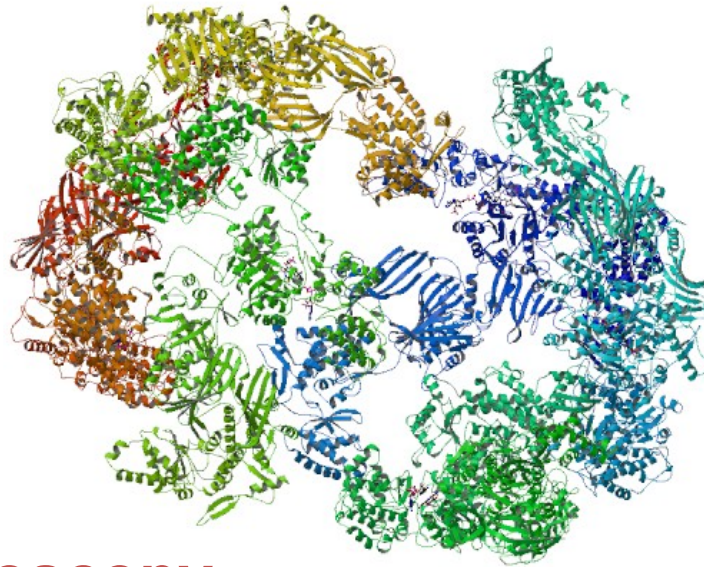


MUP-I



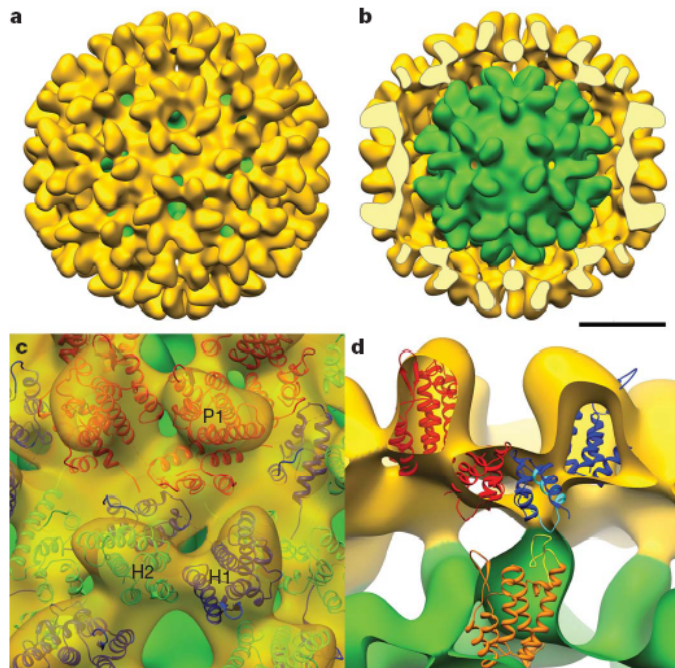
CSP-1

X-ray



2.6-MD $\alpha_6\beta_6$ heterododecameric fatty acid synthase from *Thermomyces lanuginosus* at 3.1 Å resolution

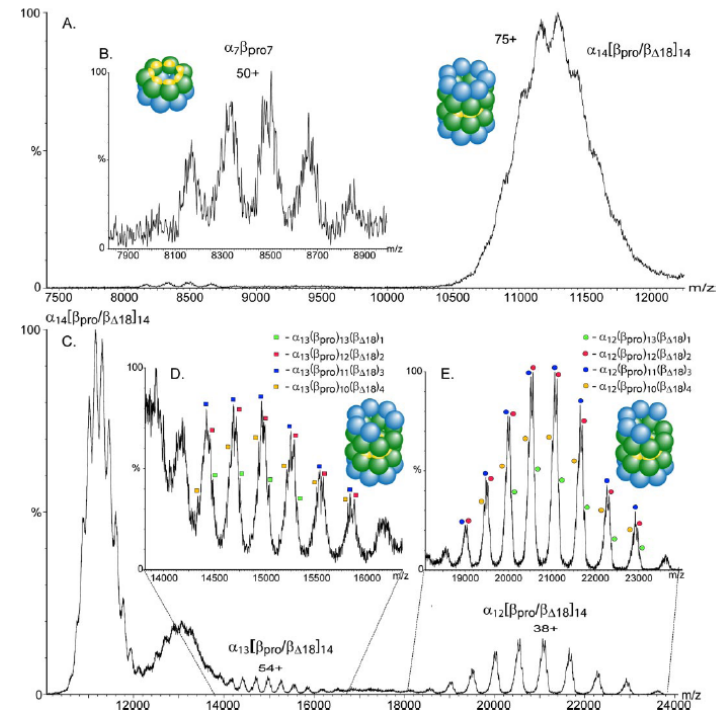
EM microscopy



Features of Rous Sarcoma Virus capsid revealed by cryo electron microscopy and image reconstruction of the virion.

MS spectroscopy

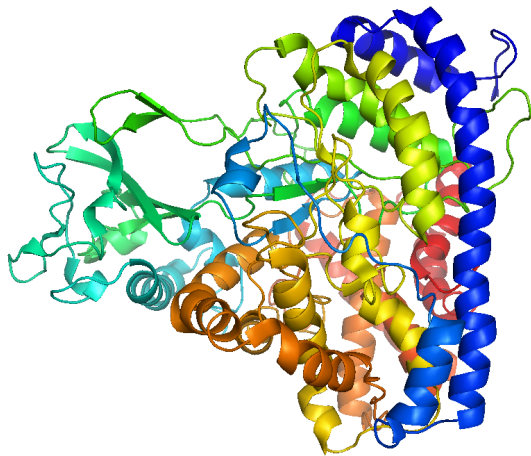
Monitoring the Assembly of the 20 S Proteasome



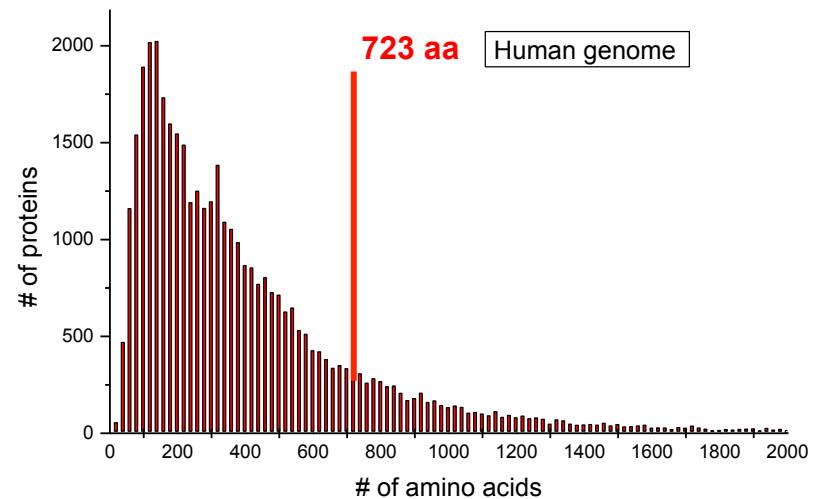
A real-time mass spectrometry approach to capture transient species along the assembly pathway of the 20 S proteasome

PDB Current Holdings Breakdown

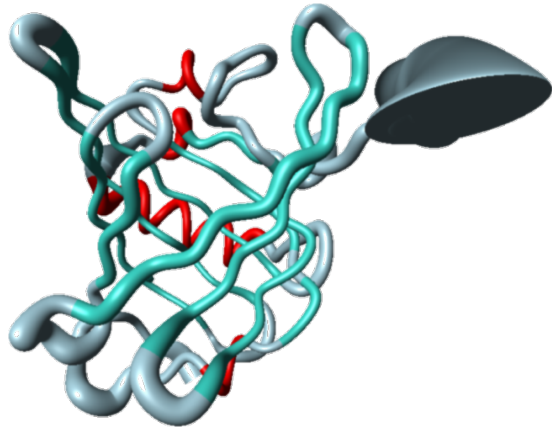
X-RAY	<u>61868</u>	<u>1313</u>	<u>2987</u>	<u>3</u>	<u>66171</u>
NMR	<u>7909</u>	<u>958</u>	<u>179</u>	<u>7</u>	<u>9053</u>
ELECTRON MICROSCOPY	<u>258</u>	<u>22</u>	<u>96</u>	<u>0</u>	<u>376</u>
HYBRID	<u>41</u>	<u>3</u>	<u>1</u>	<u>1</u>	<u>46</u>
Other	<u>133</u>	<u>4</u>	<u>5</u>	<u>13</u>	<u>155</u>
Total	<u>70209</u>	<u>2300</u>	<u>3268</u>	<u>24</u>	<u>75801</u>



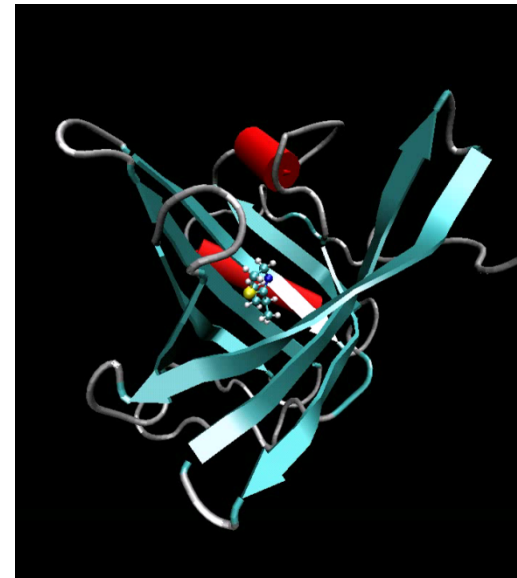
Solution global fold of the monomeric 723-residue (82-kDa) enzyme malate synthase G from *Escherichia coli* (Tugarinov V, Choy WY, Orekhov VY, Kay LE., *Proc Natl. Acad. Sci U.S.A.*, 18, 102:622-7, 2005).



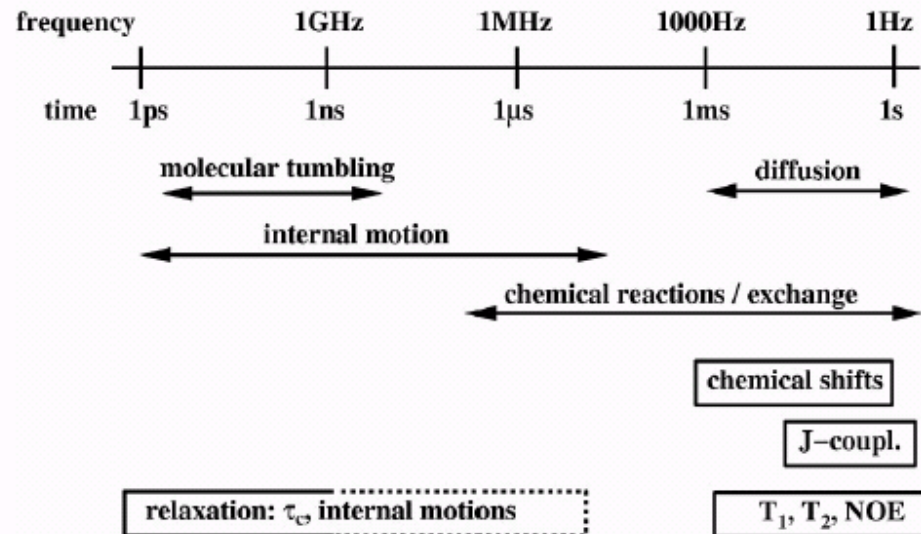
NMR



MD simulation

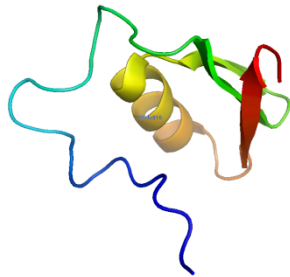


Time scales of high-resolution NMR



NMR of Biomacromolecules

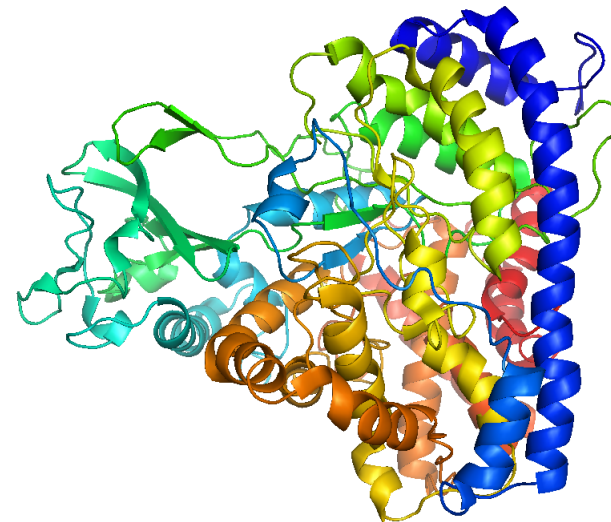
1985 – 6.2 kDa



Williamson, M.P. Havel, T.F. Wuthrich, K.

Solution conformation of proteinase inhibitor IIA from bull seminal plasma by ^1H nuclear magnetic resonance and distance geometry. *J.Mol.Biol.* 182. 295-315, 1985

2005 – 82 kDa



Vitali Tugarinov, Wing-Yiu Choy, Vladislav Yu. Orekhov, and Lewis E. Kay
Solution NMR-derived global fold of a monomeric 82-kDa enzyme, PNAS
2005 102: 622-627; published online before print January 6 2005,
10.1073/pnas.0407792102

Natural Abundance of NMR isotopes

^1H	99.98 %	active in NMR
--------------	---------	---------------

^{12}C	98.9 %	non-active in NMR
-----------------	--------	-------------------

^{13}C	1.1 %	active in NMR
-----------------	-------	---------------

^{15}N	0.3 %	active in NMR
-----------------	-------	---------------

^{31}P	100.0 %	active in NMR
-----------------	---------	---------------

Isotope labeling by molecular biology methods

Isotope labeling of RNA

In vitro transcription with T7 RNA polymerase using isotopically labeled 5'-triphosphates (NTPs) as substrates

Production of ^{13}C , ^{15}N -labeled RNA

E. coli fermentation with N-15 ammonium sulfate and/or C-13 glucose



Isolation of rRNA



Nuclease P-1 degradation of rRNA to rNMPs



Enzymatic or chemical phosphorylation to rNTPs



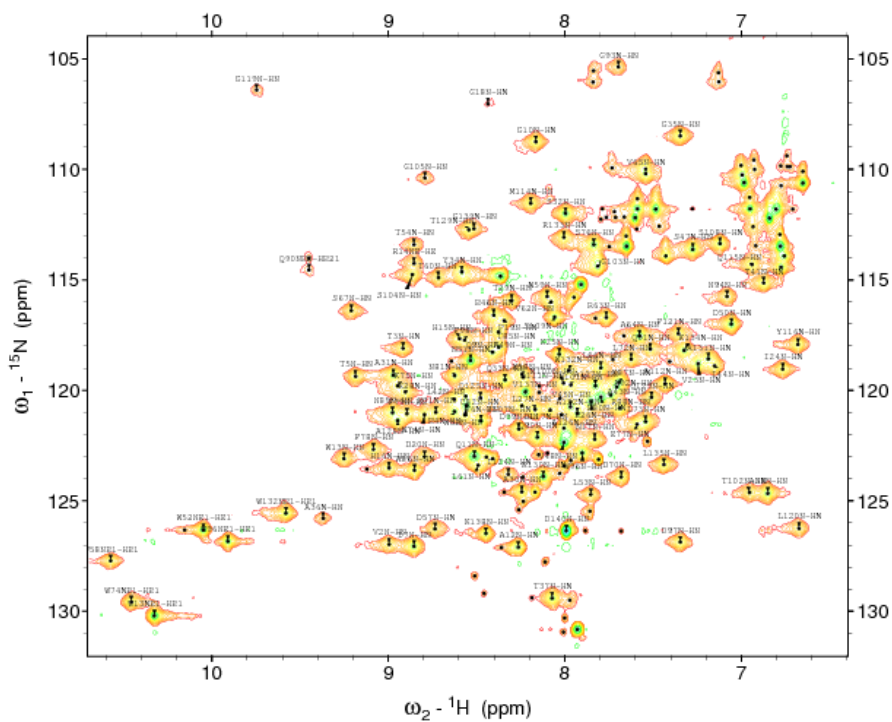
ATP GTP UTP CTP



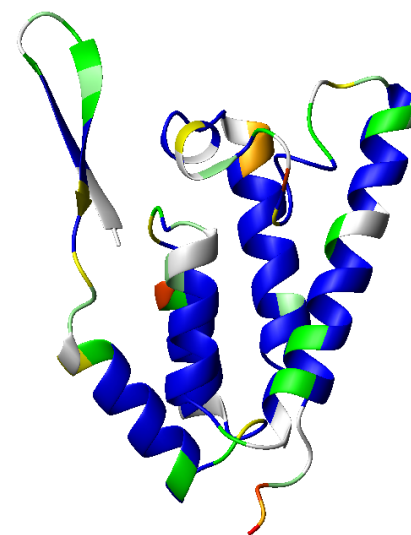
RNA oligomer synthesis on a DNA template using T7 RNA polymerase

Basic principles of 3D structure determination by NMR

How Do We Go From Spectra to A Structure?

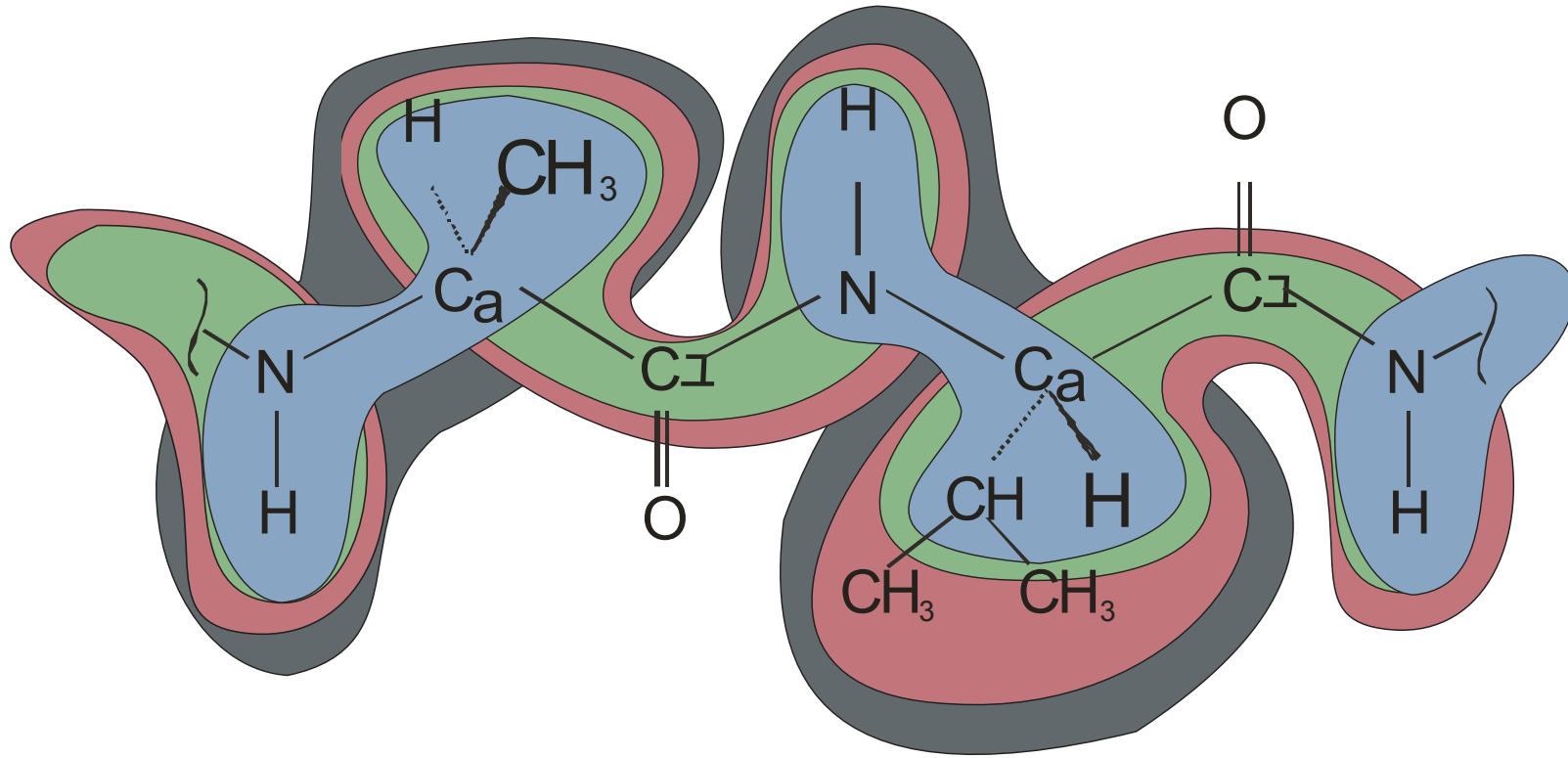


2D HSQC spectra



N-terminal domain of M-PMV capsid protein

Resonance Assignments



2D HSQC

3D CBCA(CO)NH

3D CBCANH

3D HCC(CO)NH-TOCSY

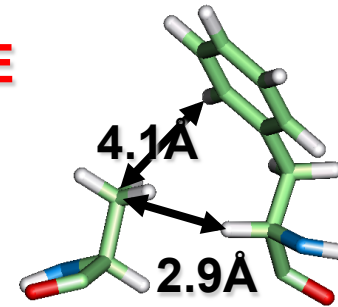
3D TOCSY-HSQC

Basic principles of 3D structure determination by NMR

NOE

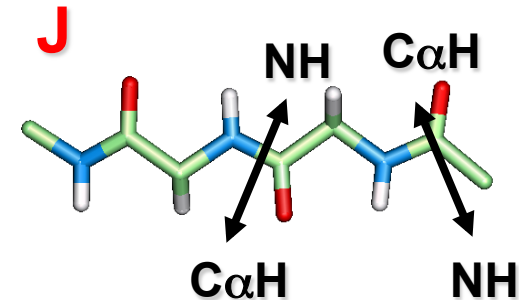
- a through space correlation ($<5\text{\AA}$)
- distance constraint

NOE



Coupling Constant (J)

- through bond correlation
- dihedral angle constraint



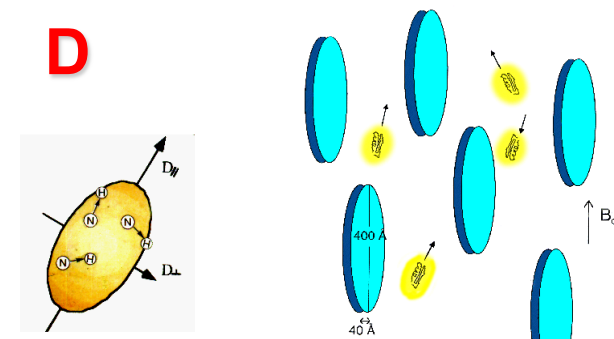
Chemical Shift

- very sensitive to local changes in environment
- dihedral angle constraint

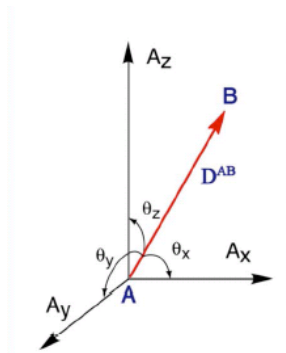
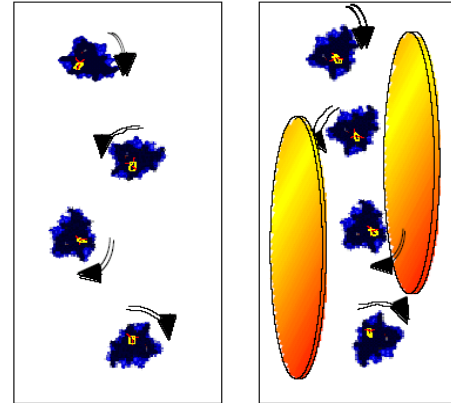
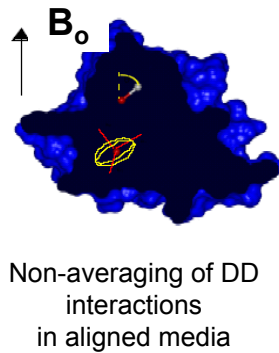
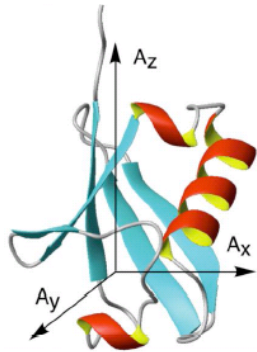
Dipolar coupling constants (D)

- bond vector orientation relative to magnetic field
- alignment with bicelles or viruses

D



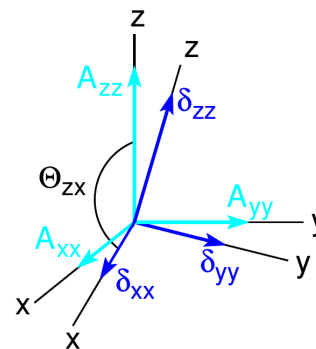
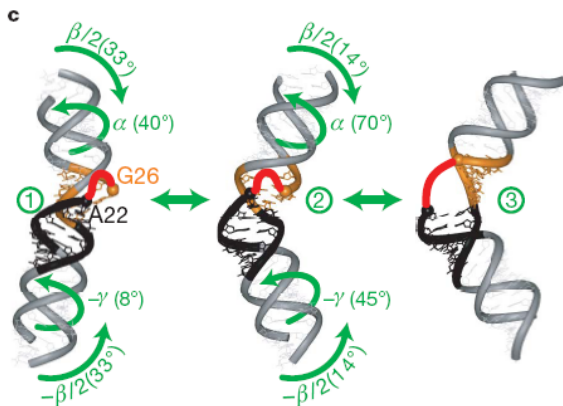
➤ Dipolar couplings and induced changes of chemical shifts
(relative orientation of atom-atom vectors, dynamics)



RDC - residual dipolar couplings

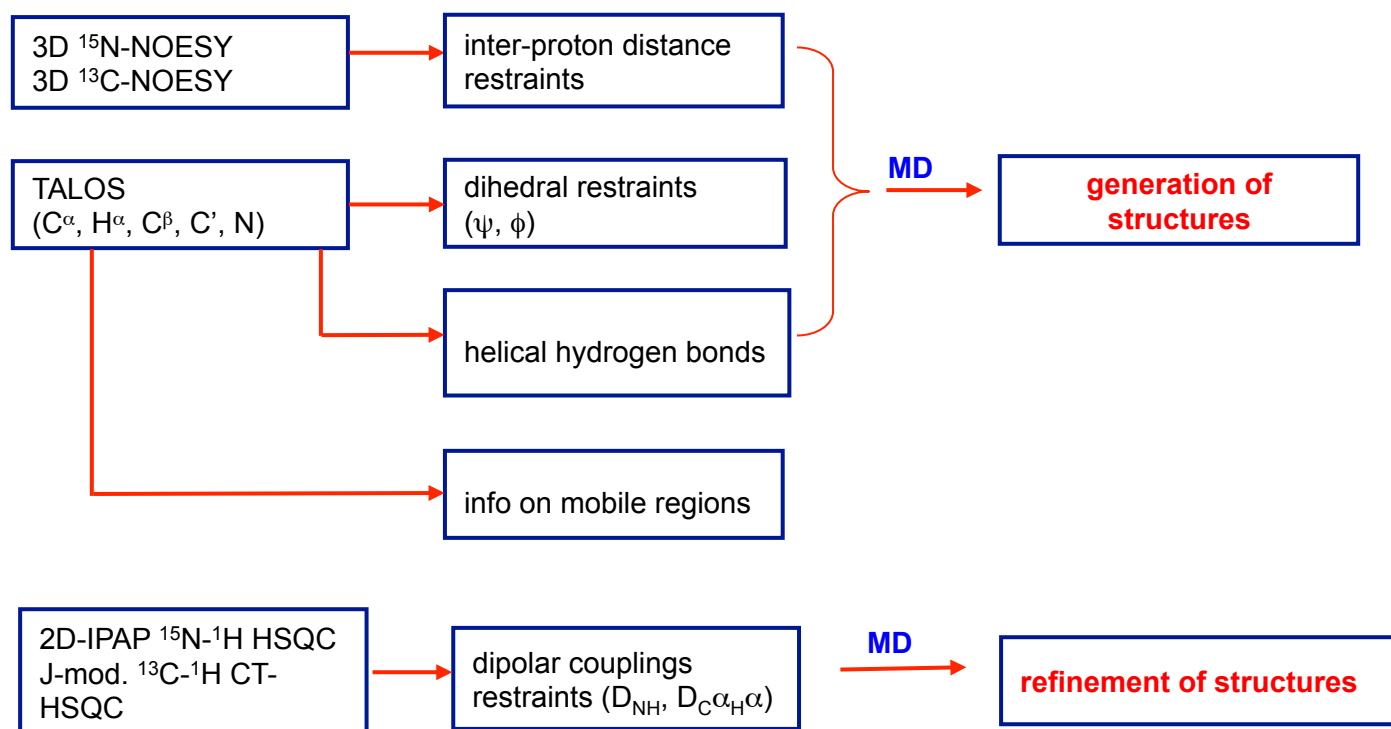
$$D^{AB}(\theta, \phi) = D_a(3\cos^2\theta - 1) + 3/2 D_r(\sin^2\theta \cos^2\phi)$$

CSI - induced changes of isotropic chemical shifts



$$\Delta\delta = \frac{1}{3} \sum_{i=x,y,z} \sum_{j=x,y,z} A_{ij} \cos^2\theta_{ij} \delta_{jj}$$

Example of NMR structure determination

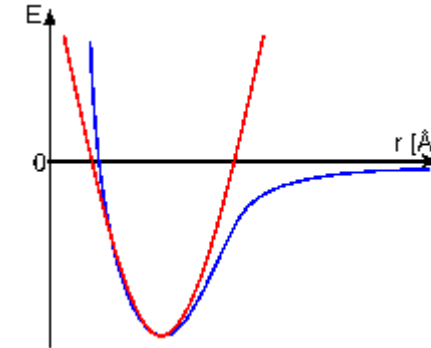


Potential Energy Terms

$$E_{\text{TOTAL}} = E_{\text{chem}} + W_{\text{exp}} E_{\text{exp}}$$

$$E_{\text{exp}} = E_{\text{NOE}} + E_{\text{torsion}} + E_{\text{H-bond}} + E_{\text{gyr}} + E_{\text{rama}} + E_{\text{RDC}} + E_{\text{CSA}} + E_{\text{para}}$$

$$E_{\text{chem}} = E_{\text{bond}} + E_{\text{angle}} + E_{\text{dihedral}} + E_{\text{vdw}} + E_{\text{electr}}$$



- E_{chem} : (*a priori* knowledge) primary structure, topology, covalent bonds, dihedral angles (harmonic), etc.
 - non-covalent van-der-Waals forces: Lennard-Jones potential
 - electrostatic interactions - Coulomb potential etc.
- E_{exp} : experimental constraint terms

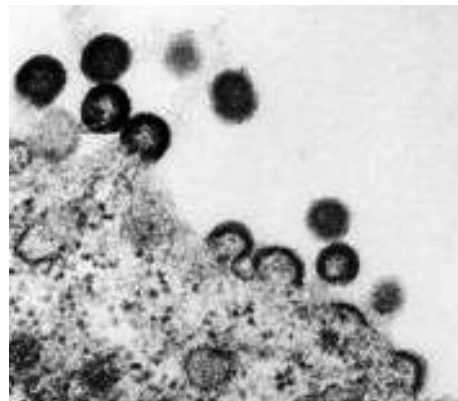
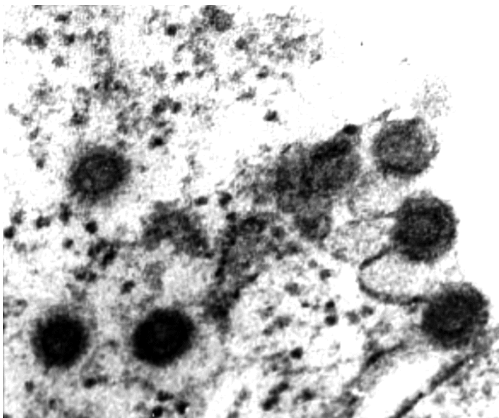
Recent potential energy terms used in MD:

- dipolar couplings (E_{rdc})
- radius of gyration (E_{gyr})
- CSA (E_{CSA})
- side chain conformational database torsion angle potentials (E_{rama})
- paramagnetic relaxation enhancement module (E_{para})

Mason-Pfizer Monkey Virus

M-PMV

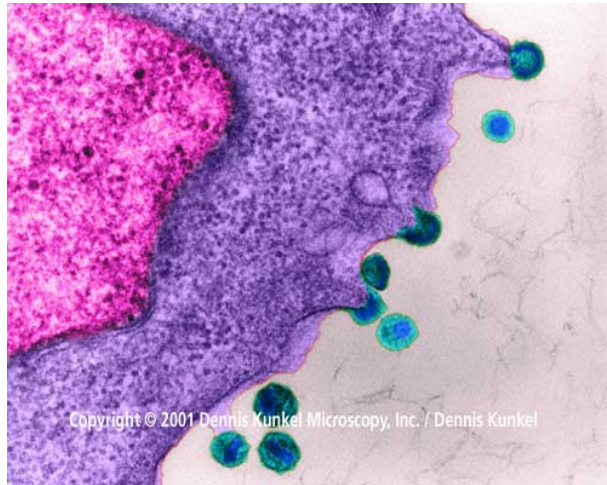
- ❑ 1970: Chopra, H.C., Mason, M.M. (mammary carcinoma of a female rhesus monkey - *Macaca mulatta*)
- ❑ Retroviridae, Oncovirinae, Betaretrovirus
- ❑ D type / C type (HIV)



Assembly of immature viral particles

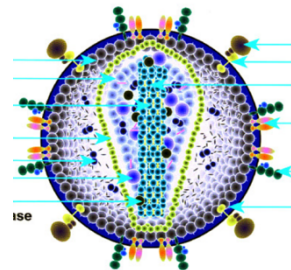
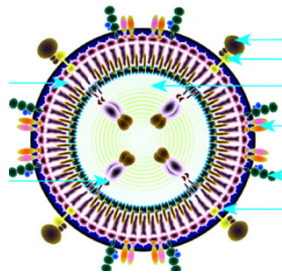
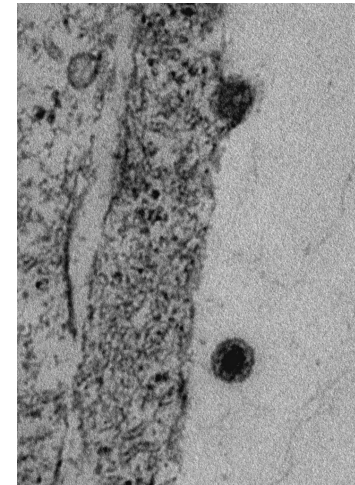
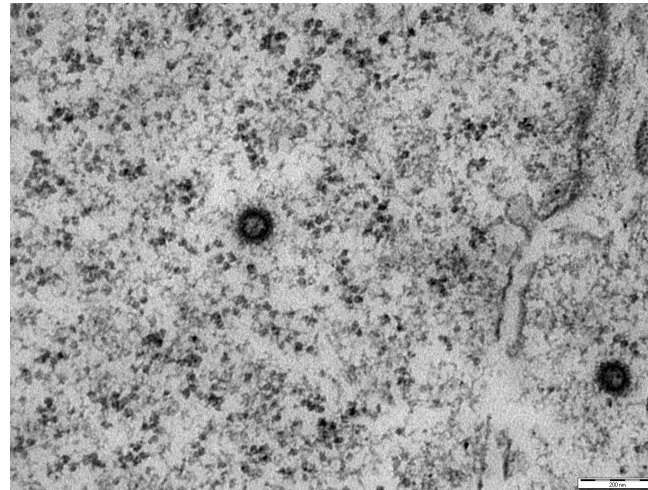
C-type

HIV-1

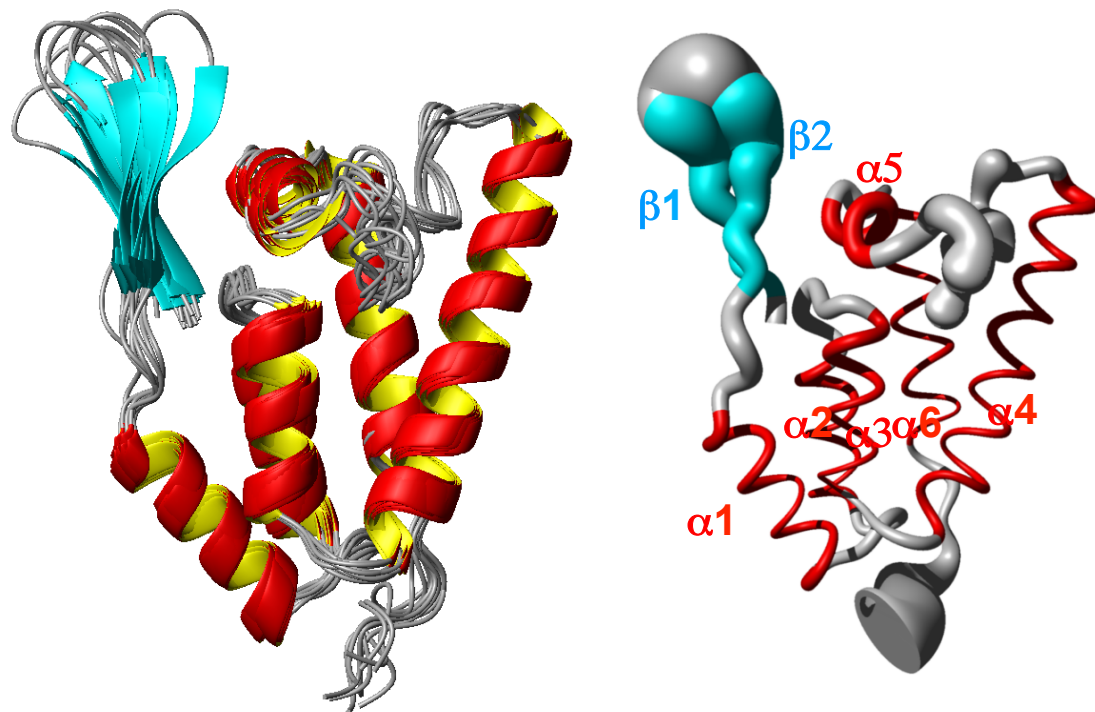


D-type

M-PMV



N-terminal domain of M-PMV capsid protein



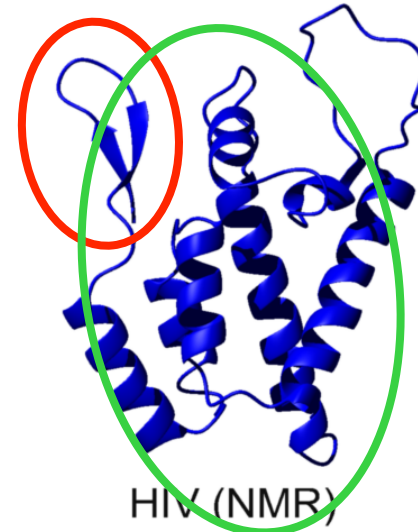
Restraint Information		
Distance restraints	2246	
Hydrogen bonding restraints	124	
Torsion Angle restraints (ϕ/ψ)	107/107	
Residual Dipolar Couplings	130	
Average rms deviation from experimental restraints		
Distance restraints (Å)	0.023 ± 0.001	
dihedral angle restraints ($^\circ$)	0.552 ± 0.067	
residual dipolar couplings (Hz)	1.811 ± 0.061	
RDCs Q factor	0.225 ± 0.008	
Violations		
distance violations > 0.5Å	0	
dihedral angle violations > 5°	0	
Pairwise Cartesian RMS deviation (Å)		
ordered all heavy atoms	1.60 ± 0.23	
ordered backbone heavy atoms	1.11 ± 0.23	
ordered all heavy atoms	1.24 ± 0.10	helices only
ordered backbone heavy atoms	0.74 ± 0.11	helices only



M-PMV
PDB 2KGF



MLV (X-ray)
PDB 3BP9



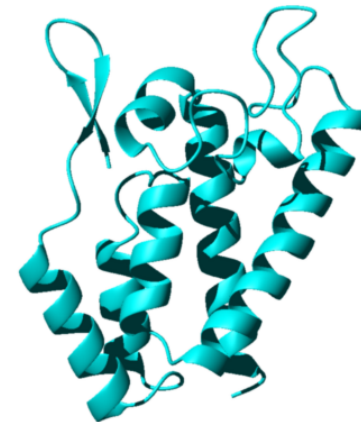
HIV (NMR)
PDB 1GWP



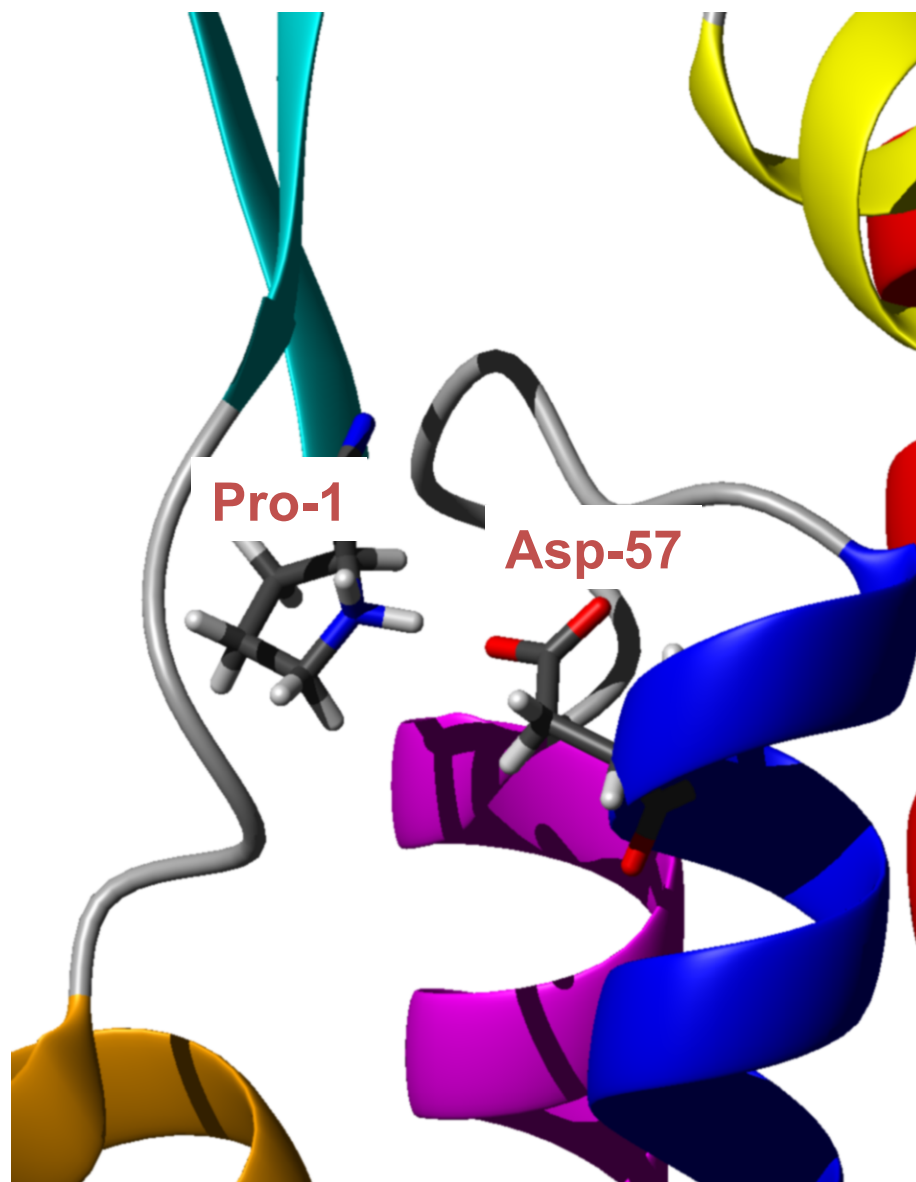
HIV (X-ray)
PDB 1AK4

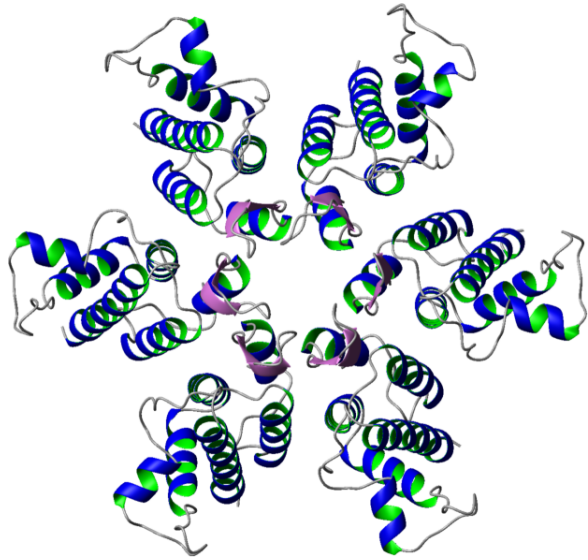


HTLV (NMR)
PDB 1G03

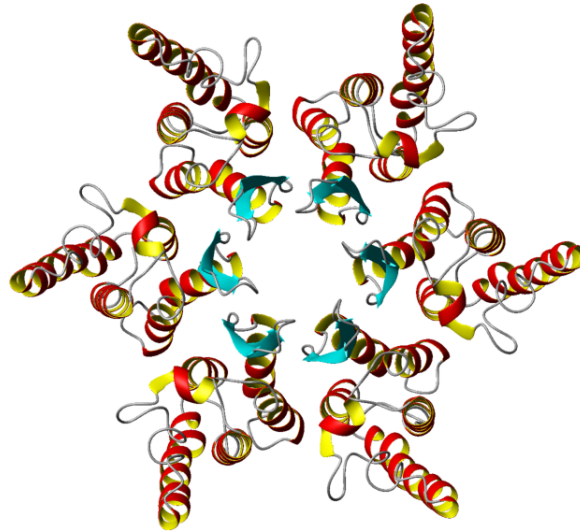


RSV (X-ray)
PDB 1EM9

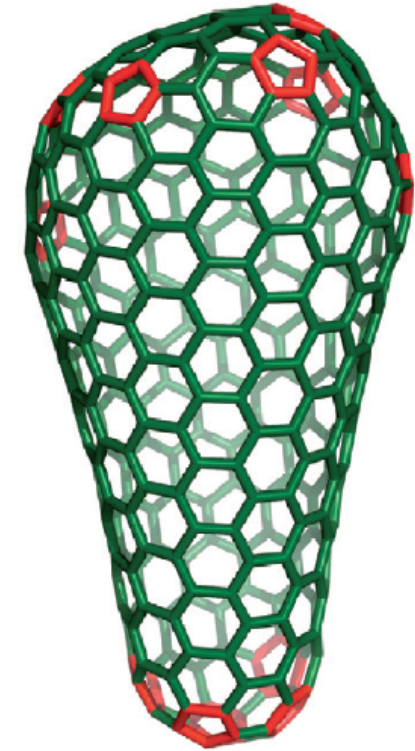
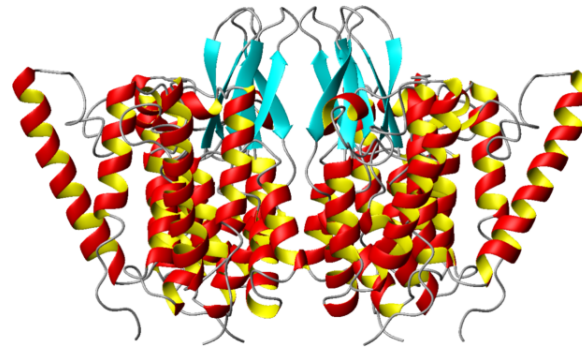
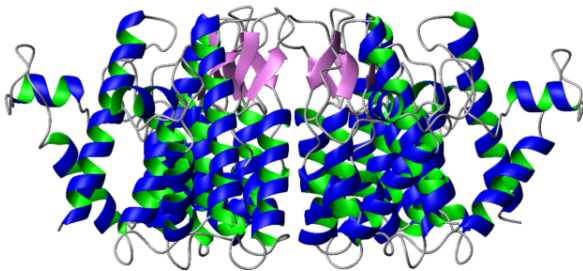




MLV CA-NT hexamer by x-ray
(Mortuza, G.B. et al. JMB 376, 1493-1508, 2008)

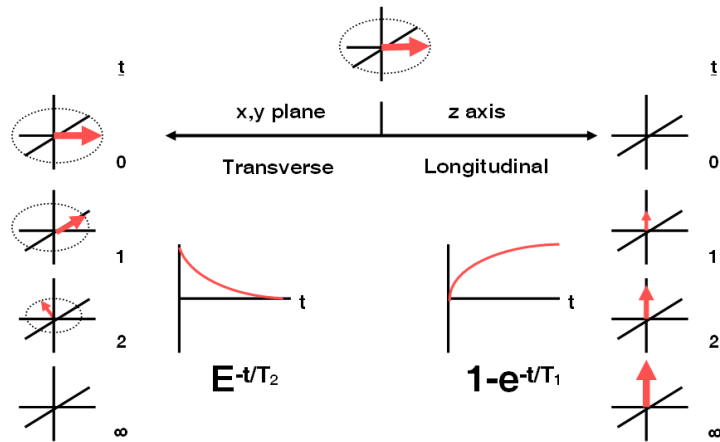


M-PMV CA-NT hexamer
model from nmr data



Fullerene model for the conical
capsid, with CA hexamers
(Ganser-Pornillos, B.K. et al. COSB, 18,
203-217, 2008)

Dynamics and molecular motions



Heteronuclear relaxation ^{15}N , ^{13}C

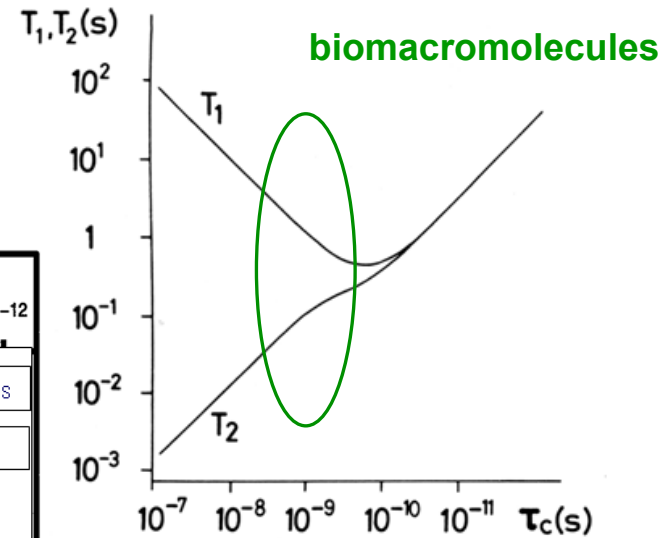
T_1 – spin-lattice relaxation time – fast motions

T_2 – spin-spin relaxation time – fast & slow motions

^1H - ^{15}N NOE – fast and some slow motions

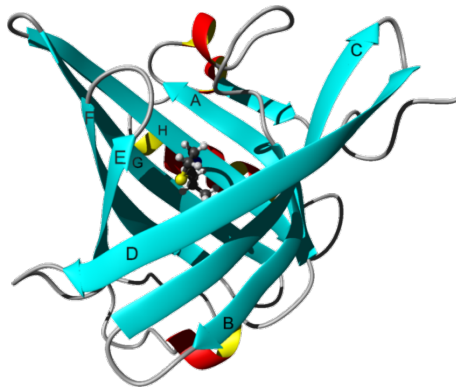
	Timescale (s)					
	10^3	10^0	10^{-3}	10^{-6}	10^{-9}	10^{-12}
Type of motion	Global unfolding		Local unfolding		overall tumbling	bond librations
	Chemical kinetics		Slow loop reorientation		Fast loop reorientation	
	S-S flipping			Side-chain rotation/re-orientation		
	Aromatic ring flips					
NMR parameter	HN exchange		$T_2, T_{1\rho}$			T_1, T_2, NOE
	δ		J			

relaxation

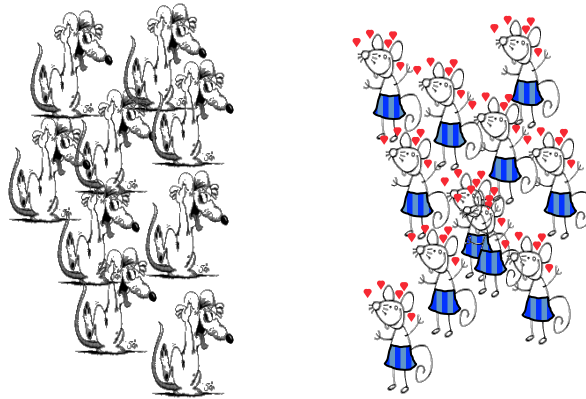


large ---- small molecules

Quo vadis? Molecular motions on ps -ns time scale

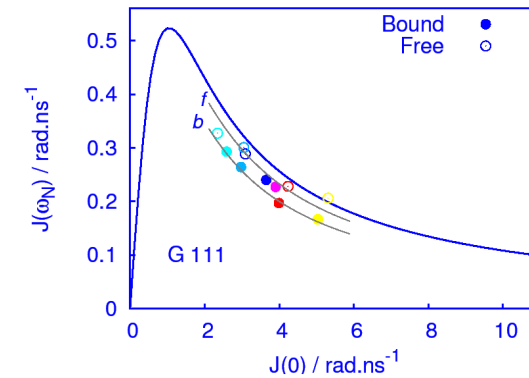
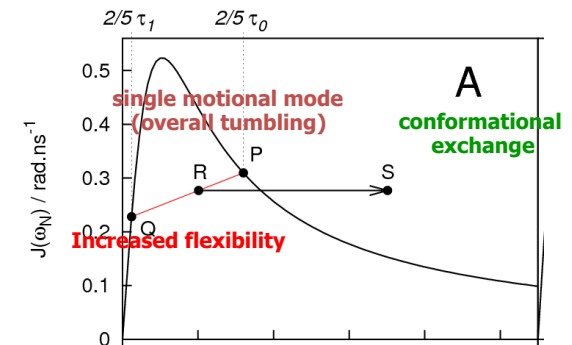
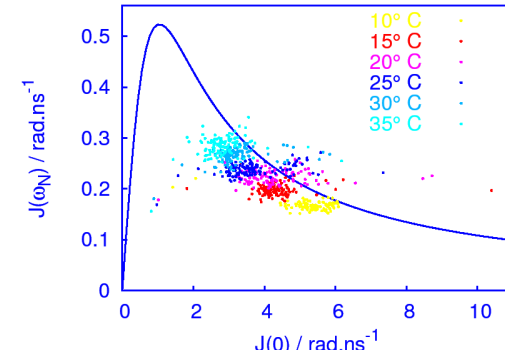
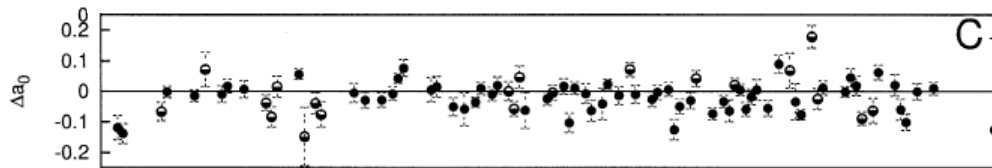


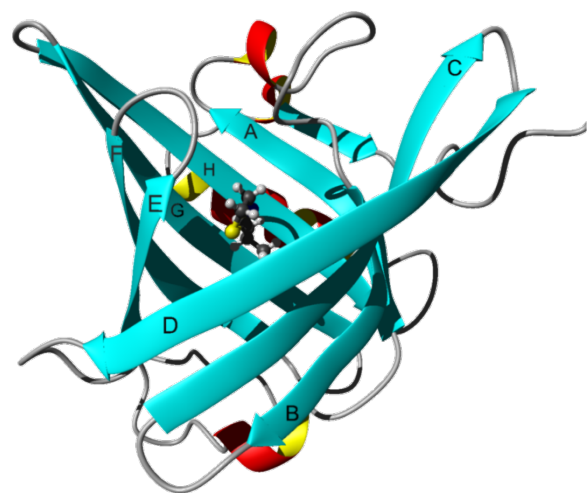
MUP – mouse urinary protein



Pheromones

$$\begin{pmatrix} J(0) \\ J(\omega_N) \\ J(0.87\omega_H) \end{pmatrix} = \begin{pmatrix} \frac{-3}{4(3d^2+c^2)} & \frac{3}{2(3d^2+c^2)} & \frac{-9}{10(3d^2+c^2)} \\ \frac{1}{(3d^2+c^2)} & 0 & \frac{-7}{5(3d^2+c^2)} \\ 0 & 0 & \frac{1}{5d^2} \end{pmatrix} \begin{pmatrix} \frac{1}{T_1} \\ \frac{1}{T_2} \\ \frac{\gamma_N}{\gamma_H} \frac{NOE-1}{T_1} \end{pmatrix}$$





X-ray

Side chains
N35 and R60

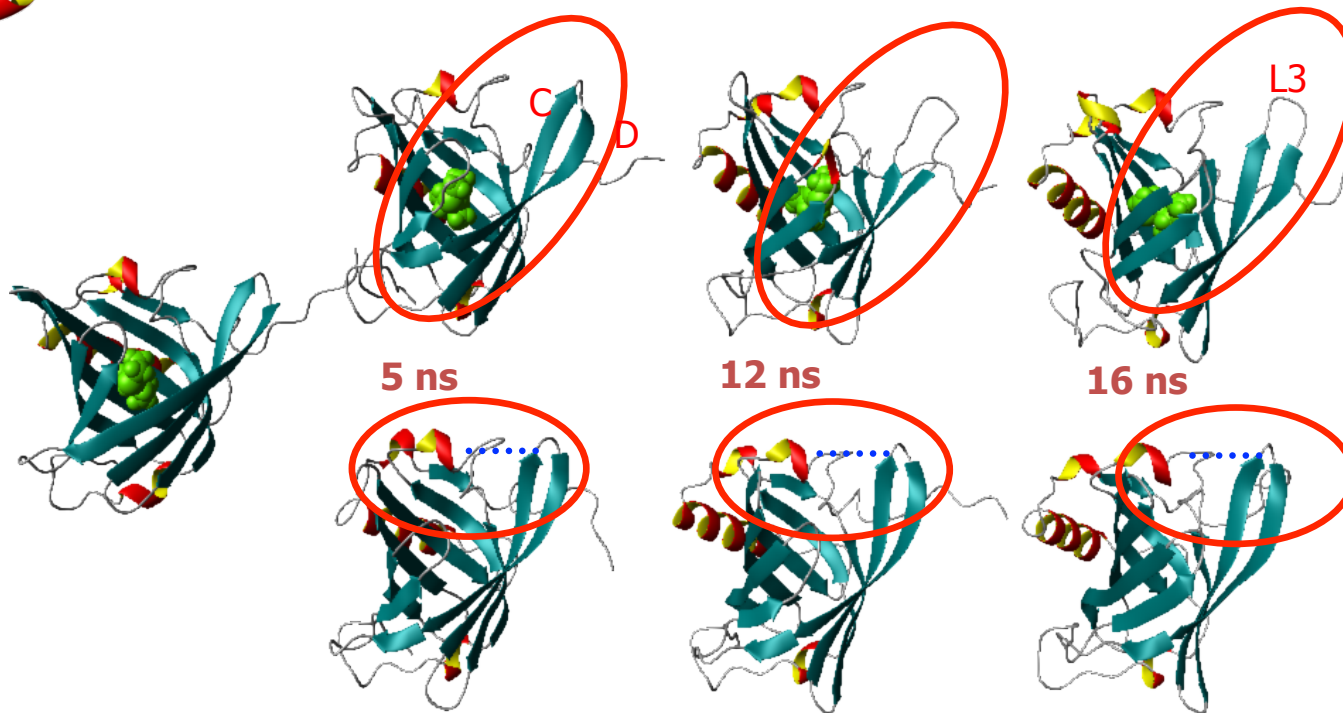
MUP (120F) – PDB: 1YP7



10 MUP complexes



PDBs: 1QY0, 1QY1, 1QY2, 1YP6, 1ZND, 1ZNE, 1ZNG, 1ZNH, 1ZNK, 1ZNL)



Start X-ray

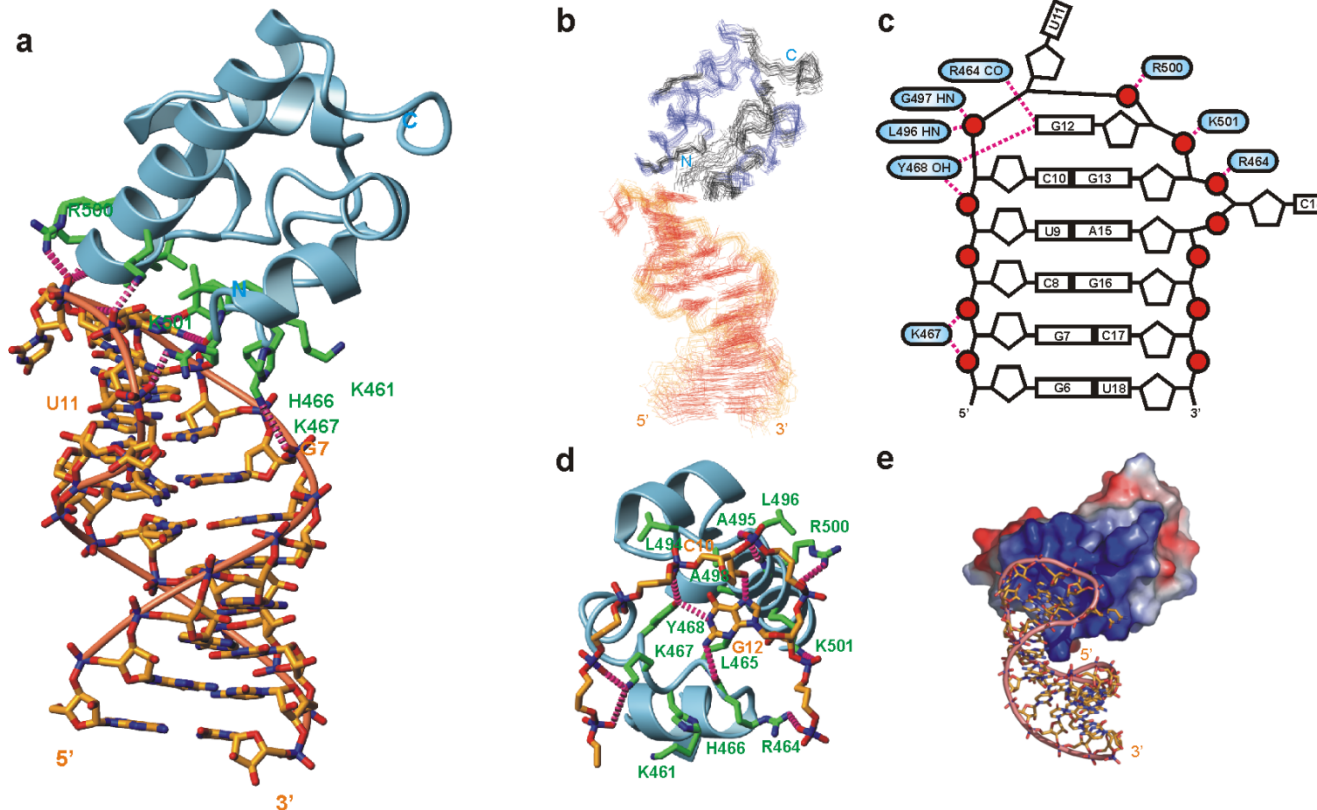
Macek, P., Novák, P., Křížová, H., Židek, L., and Sklenář, V.: *FEBS Letters*, **2006**, 580, 682-684: Molecular Dynamics Study of Major Urinary Protein – Pheromone Interactions: A Structural Model for Ligand-Induced Flexibility Increase.

Macek, P., Novák, P., Židek, L., and Sklenář, V.: *J. Phys. Chem. B* **2007**, 111, 5731-5739: Backbone Motions of Free and Pheromone-Bound Major Urinary Protein Studied by Molecular Dynamics Simulation.

Quo vadis?

Molecular complexes of increasing size and complexity

structure of SAM^{Vts1p} with RNA: a shape specific recognition



Quo vadis?

3D protein structure generation from NMR chemical shift data

CHESHIRE (CHEmical SHift REstraints)

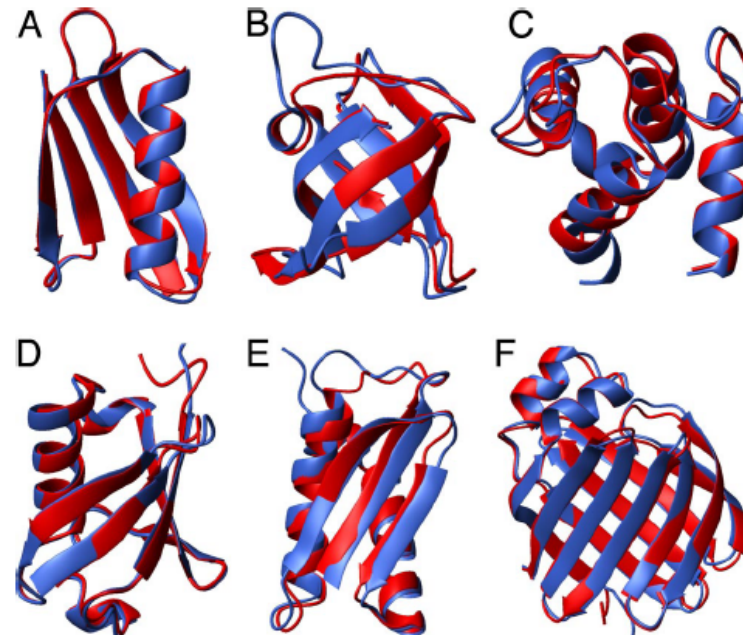
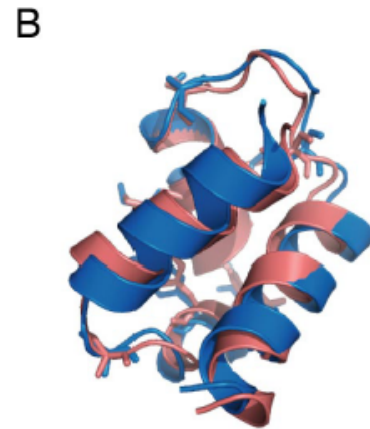
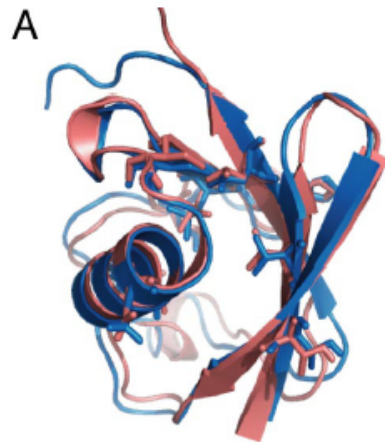
Michele Vendruscolo, Oxford

11 proteins in the size range of 46–123 residues, yielded results remarkably close (**1.3–1.8 Å backbone atom rmsd**; 2.1–2.6 Å rmsd for all atoms) to structures previously determined using conventional x-ray crystallography or NMR methods.

CS-ROSETTA

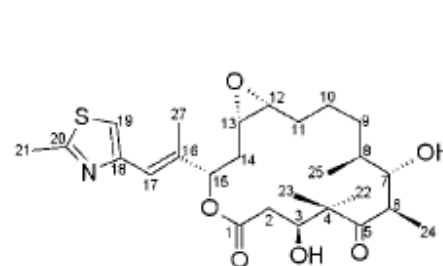
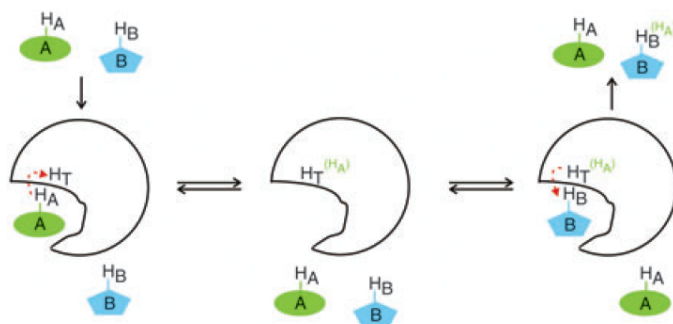
Ad Bax, Bethesda

16 proteins with known structures;
9 proteins with unknown structures for which only chemical shift assignments but no structural coordinates were available. **56 to 129 residues** full-atom models that have **0.7–1.8 Å backbone atom rmsd** to the experimentally determined x-ray or NMR structures.

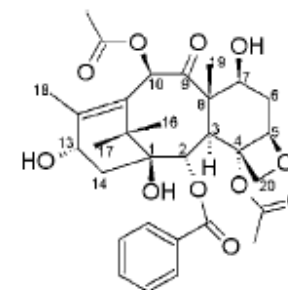


Quo vadis? Characterization of weakly interacting molecular networks

INPHARMA method

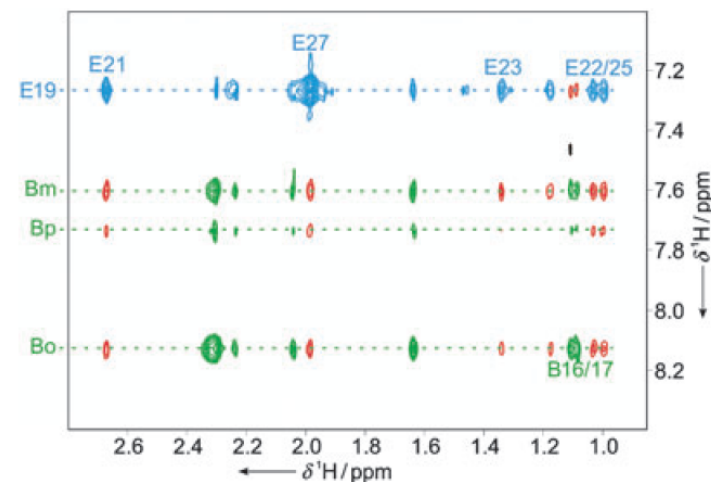


epothilone A (E)

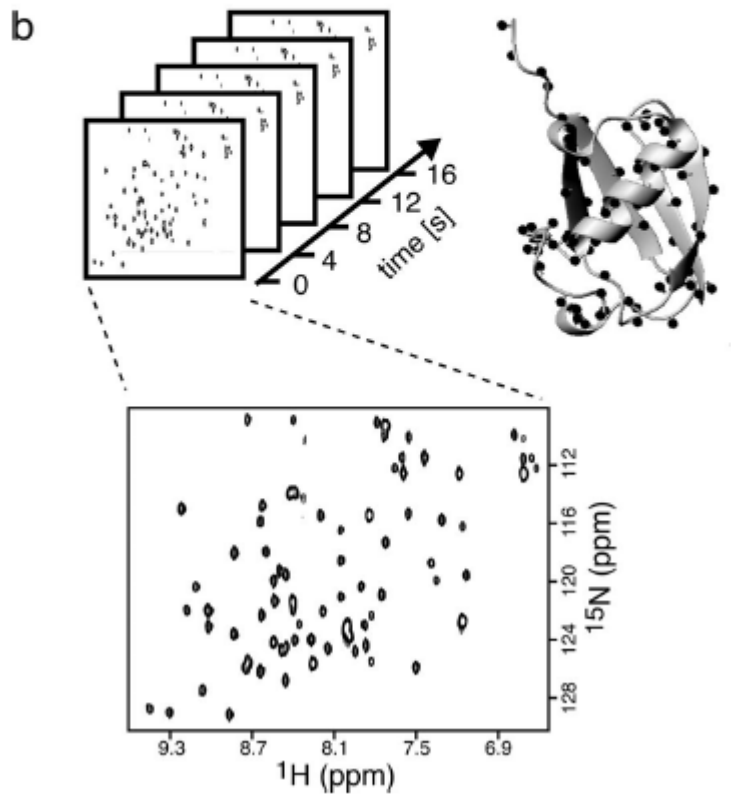
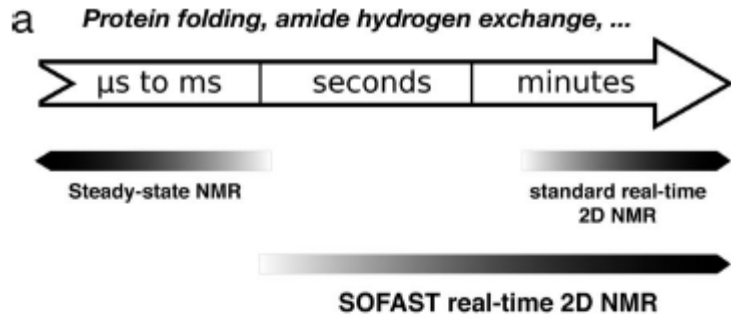


baccatin III (B)

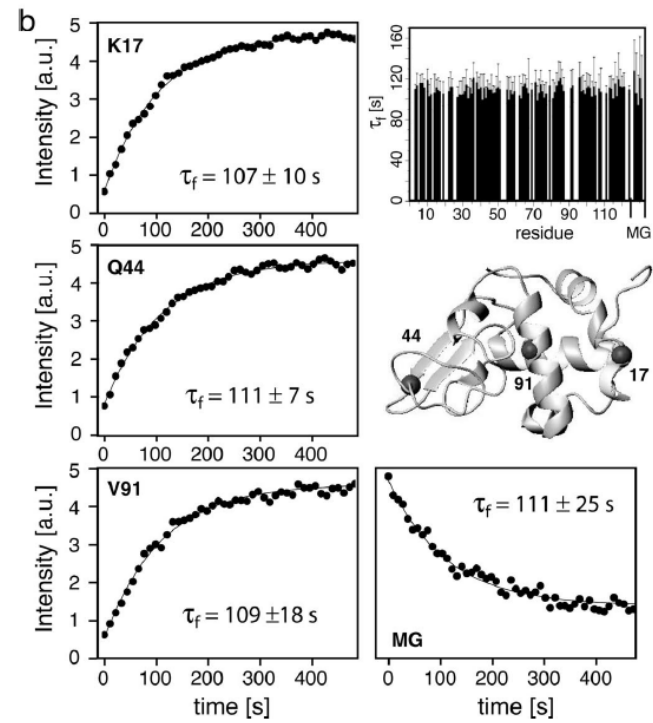
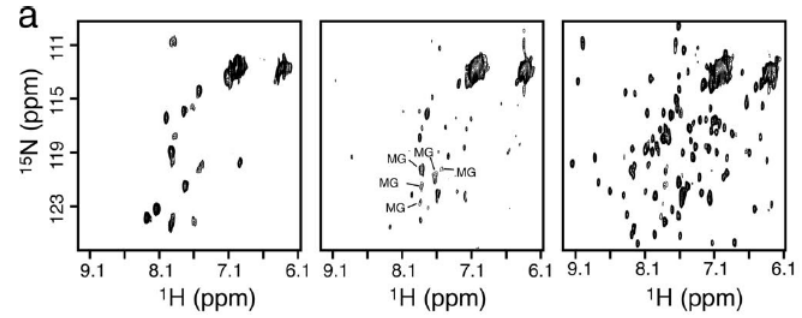
Tubulins are targets for anticancer [drugs](#) like [Taxol](#) and the "Vinca alkaloid" drugs such as [vinblastine](#) and [vincristine](#). The anti-gout agent [colchicine](#) binds to tubulin and inhibits microtubule formation, arresting [neutrophil](#) motility and decreasing [inflammation](#). The anti-fungal drug [Griseofulvin](#) targets microtubule formation and has applications in cancer treatment.



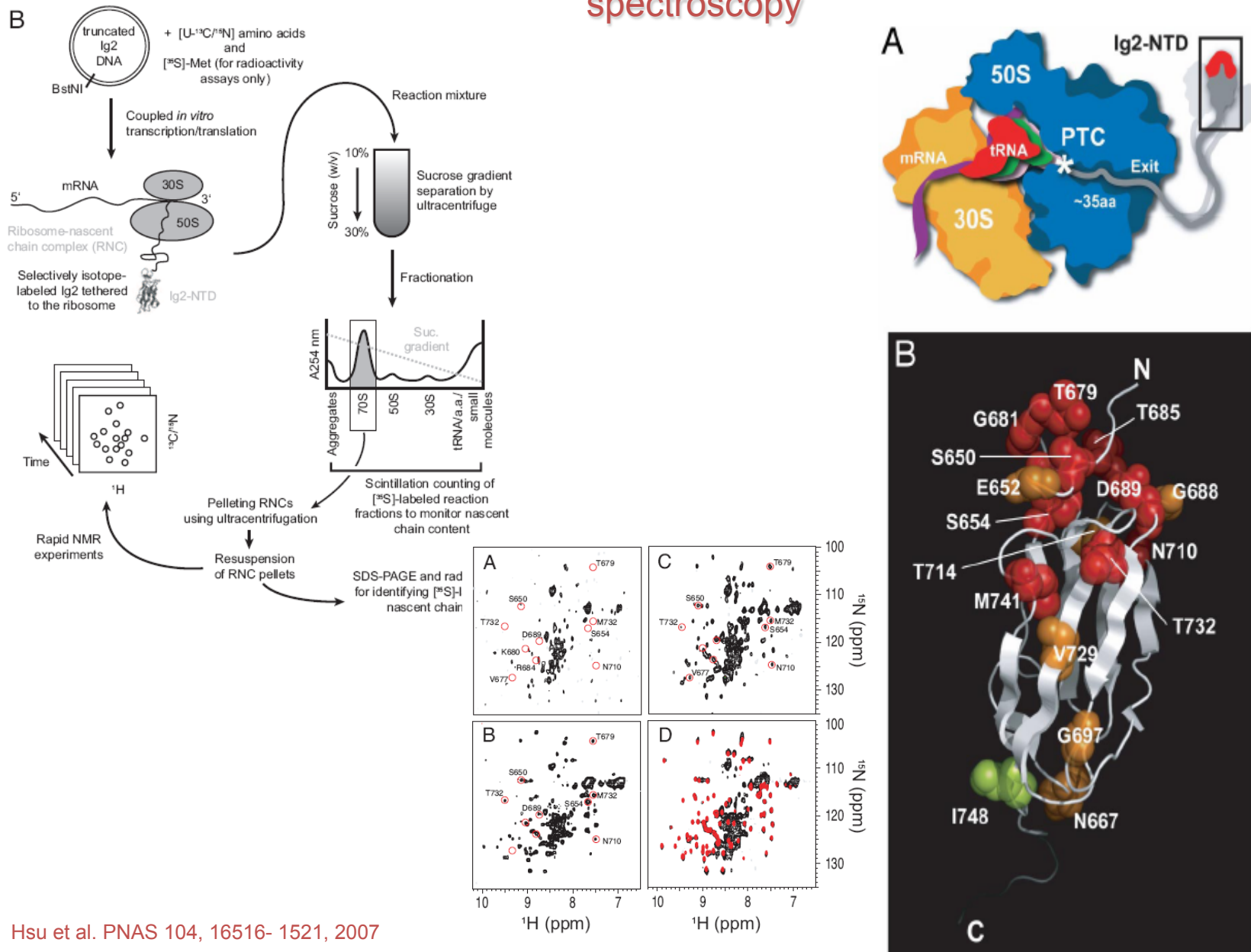
Quo vadis? Kinetic processes - Folding of α -lactalbumin



Schanda et al. PNAS 104, 11257- 11262, 2007

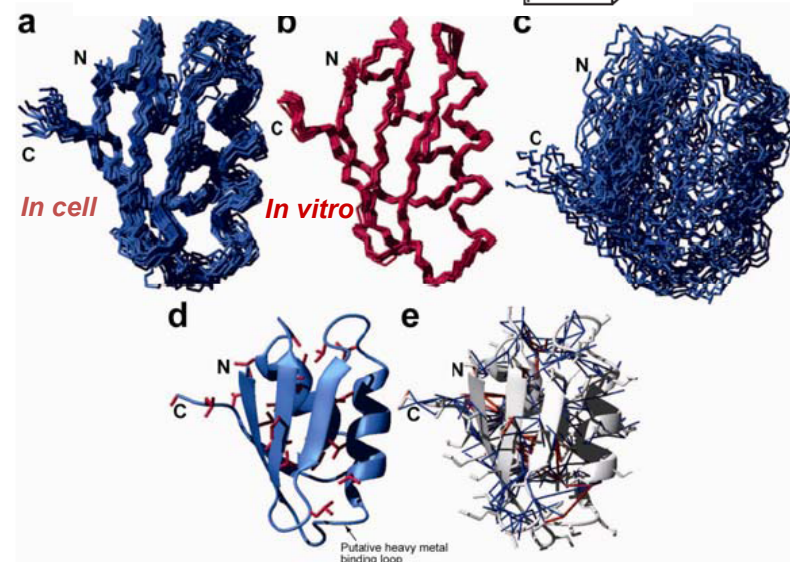
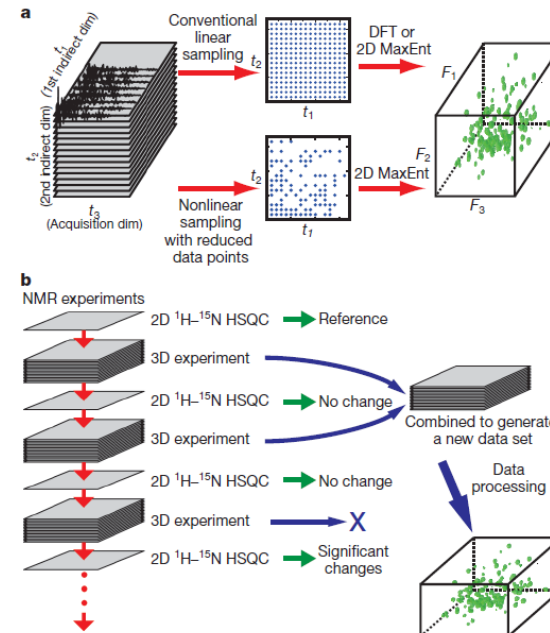
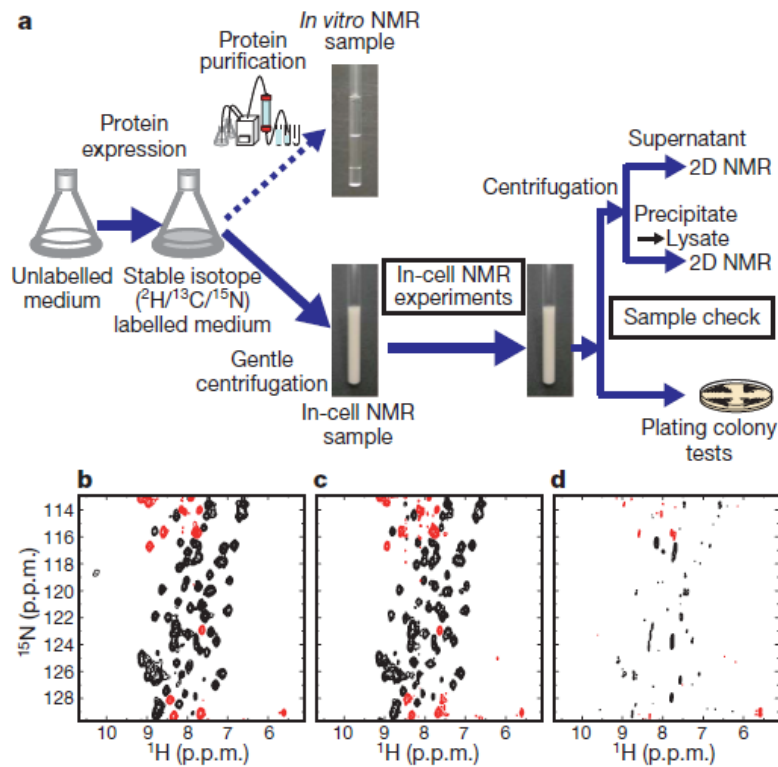


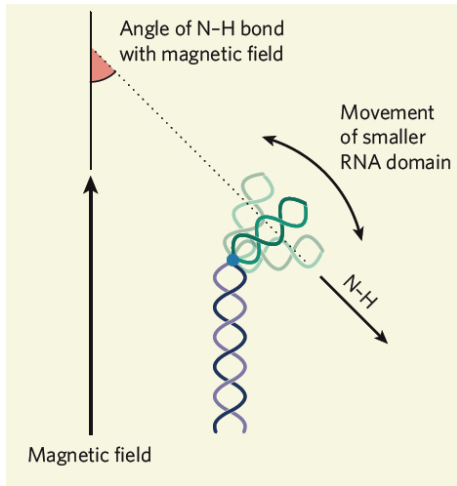
Quo vadis? Structure and dynamics of a ribosome-bound nascent chain by NMR spectroscopy



Quo vadis?

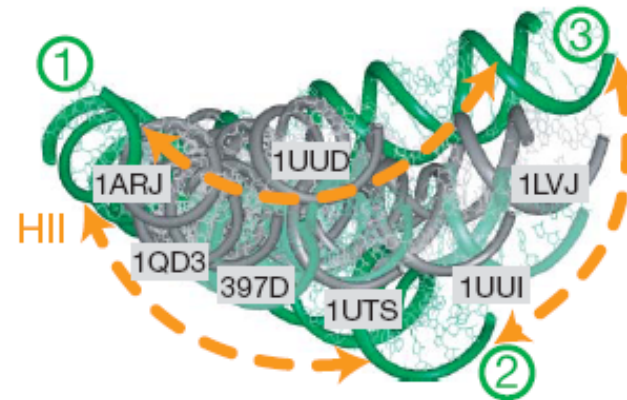
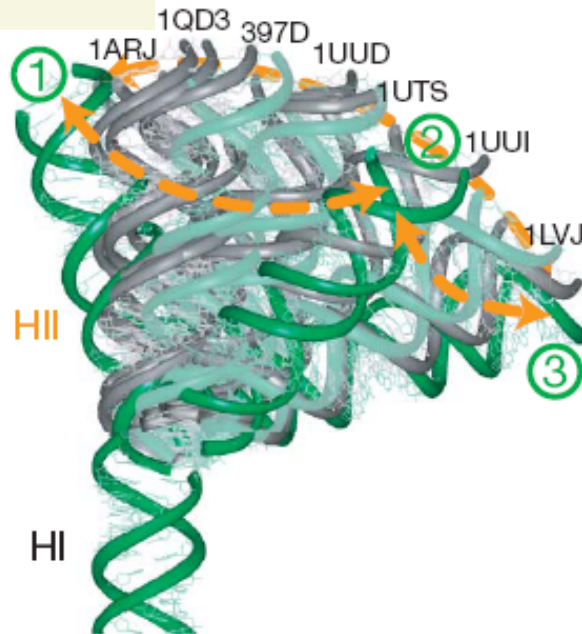
Structure and function of biomacromolecules in living cells or cell extracts





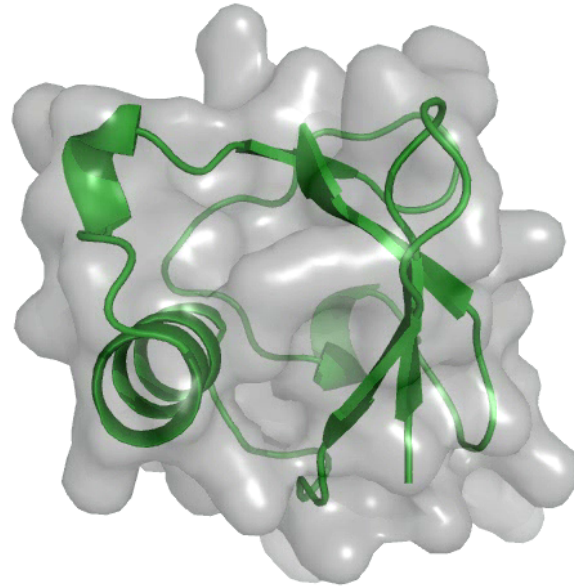
Quo Vadis Dynamics from Residual Dipolar Couplings Molecular motions on μs time scale

HIV TAR



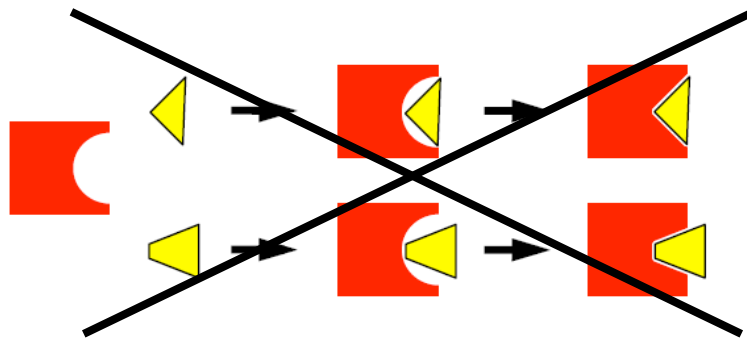
Comparison of the three TAR dynamical conformers (green) and ligand-bound TAR conformations (grey). Sub-conformers along the linear pathway linking conformers 1R2, 2R3 and 3R1 are shown in light green, and the direction of the trajectory is shown with arrows.

Quo Vadis
Dynamics from Residual Dipolar Couplings
Molecular motions on μs time scale

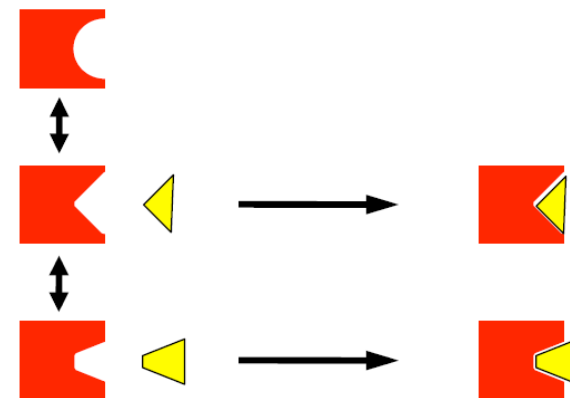


46 ubiquitin structures

Induced Fit

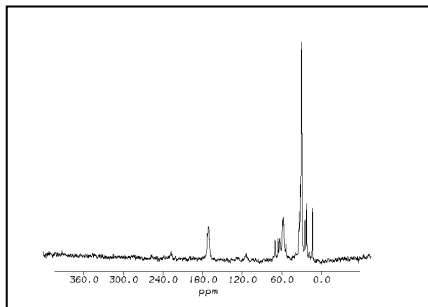
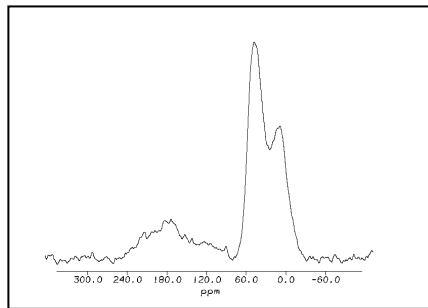
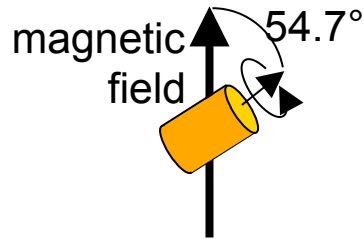


Conformational Selection

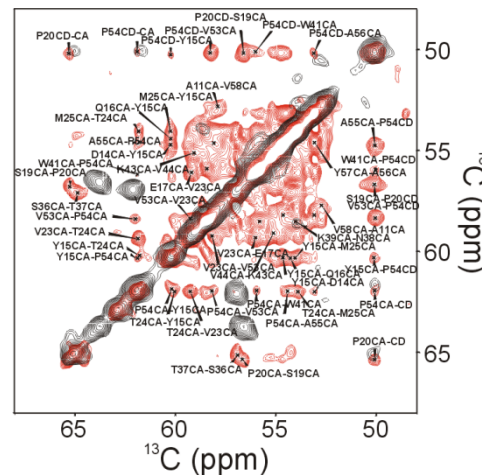
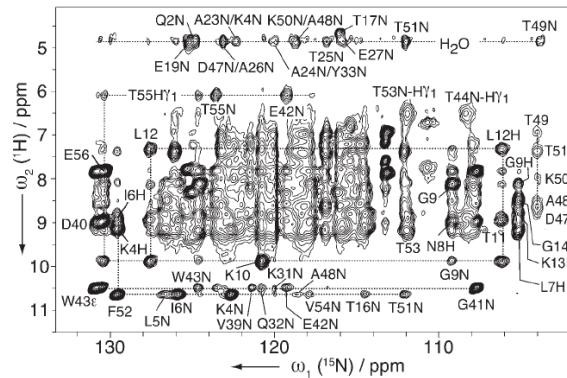




Recognition mechanisms

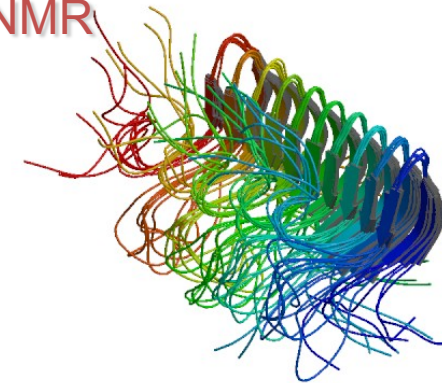
Quo vadis? 3D protein structures by solid-state NMR



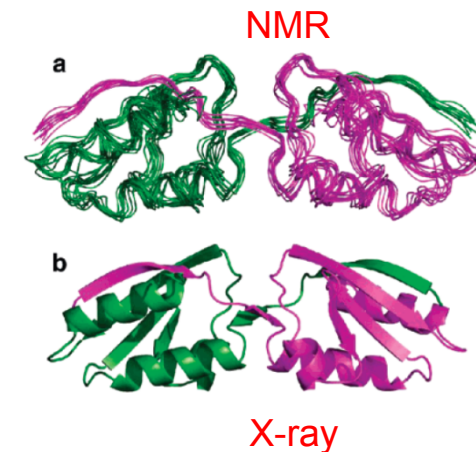
MAS: ^{13}C –NMR of ^{13}C , ^{15}N labelled peptide in hydrated DMPC bilayer with and without sample spinning



-  [U- ^{13}C]glycerol SH3
-  [2- ^{13}C]glycerol SH3

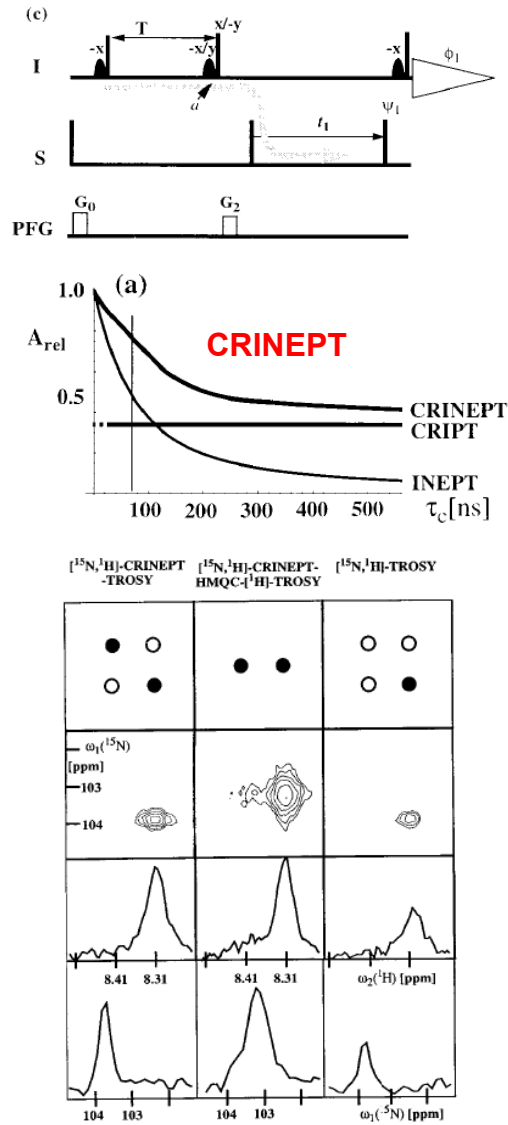
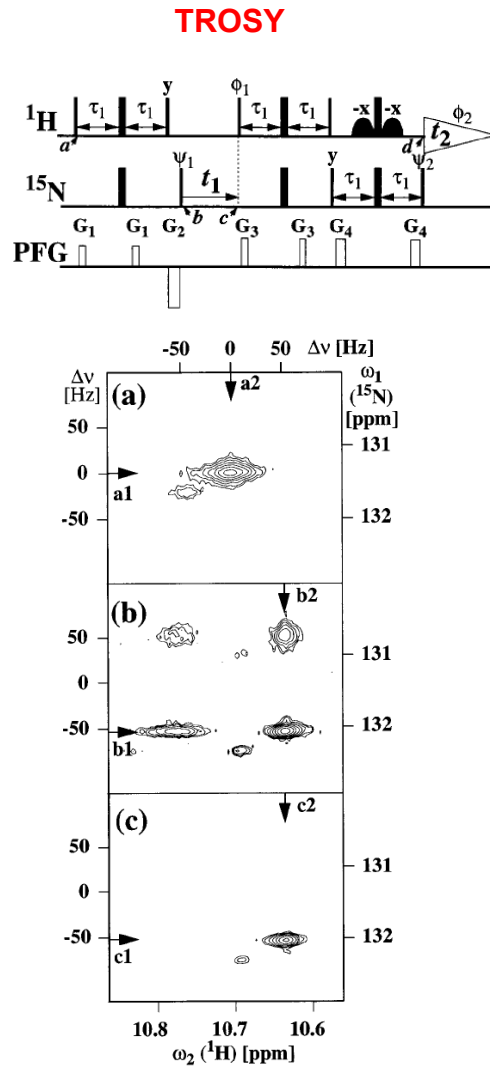


[Wasmer, C., Lange, A., Van Melckebeke, H., Siemer, A.B., Riek, R., Meier, B.H.](#) Amyloid fibrils of the HET-s(218-289) prion form a beta solenoid with a triangular hydrophobic core
Science **319**, 1523-1526, 2008



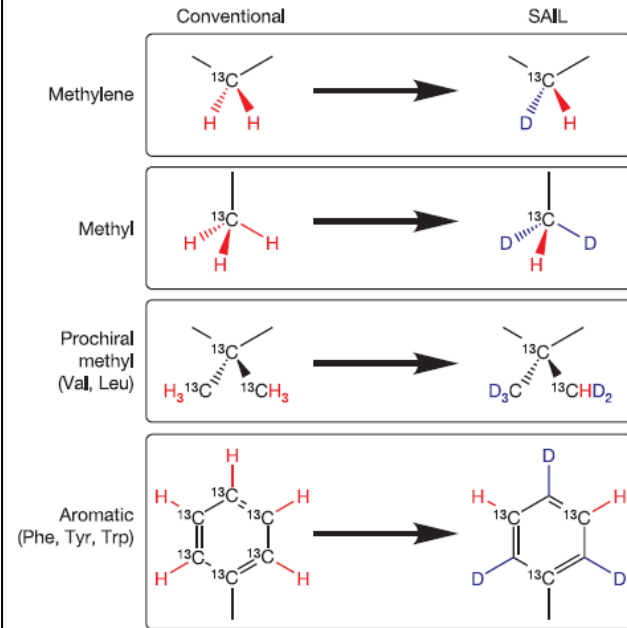
[Loquet, A., Bardiaux, B., Gardienet, C., Blanchet, C., Baldus, M., Nilges, M., Malliavin, T., Bockmann, A.](#)
3D structure determination of the Crh protein from highly ambiguous solid-state NMR restraints. *J. Am. Chem. Soc.* **130**, 3579-3589, 2008.

Quo vadis? Biomolecular NMR methodology



[u- ^{15}N , 75% ^2H]-labeled *S. aureus* aldolase
a 110 kDa protein, $\tau_c > 70$ ns

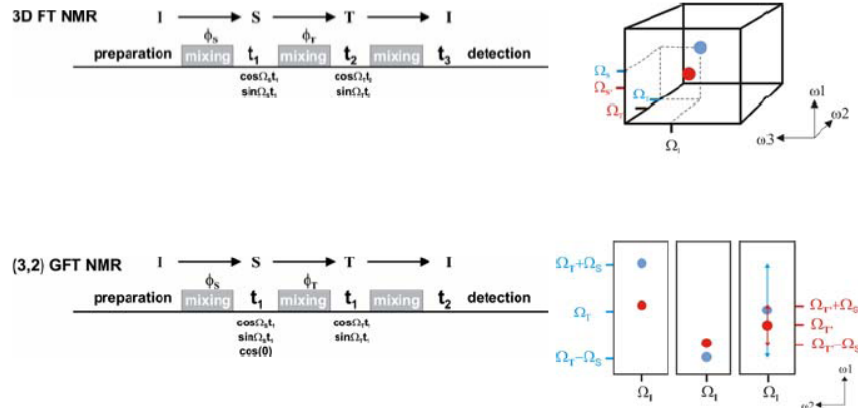
SAIL isotope labeling stereo-array isotope labeling cell-free expression



Quo vadis? Biomolecular NMR methodology

Reduced dimensionality, GFT NMR

- GFT NMR = reduced dimensionality with quadrature detection

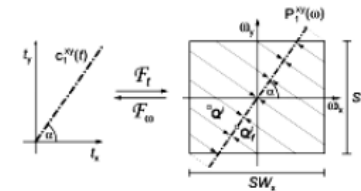


Projection reconstruction

- Record lower dimensionality projections of ND spectra → time saving
 - 1) Reconstruction of ND object (spectrum) from projections (as in tomography) i.e. using lower value algorithm (Kupce & Freeman)
 - 2) ND peak list from projections without reconstruction (APSY, Wider et al.)

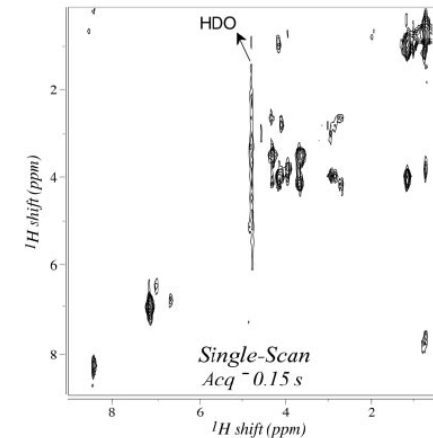
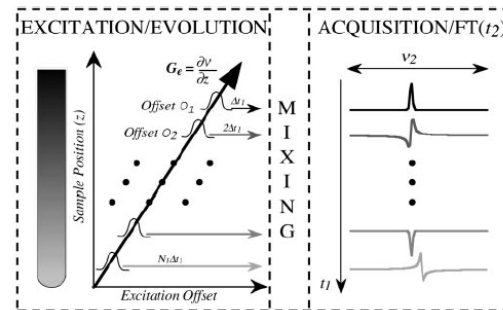
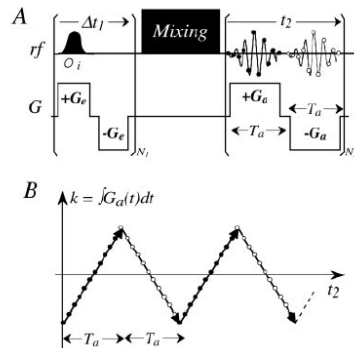
Projection reconstruction theorem

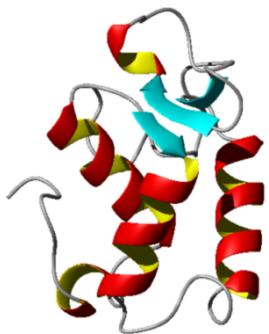
A skewed projection with angle α of a 2D frequency domain corresponds to the FT of a skew cross-section through the origin of the 2D time domain with angle α .



2D projection of the two indirect dimensions of a 3D experiment with angle α involves simultaneous evolution of the indirect time dimensions t_1, t_2 with increments: $\Delta t_2 \cos(\alpha)$ and $\Delta t_1 \sin(\alpha)$

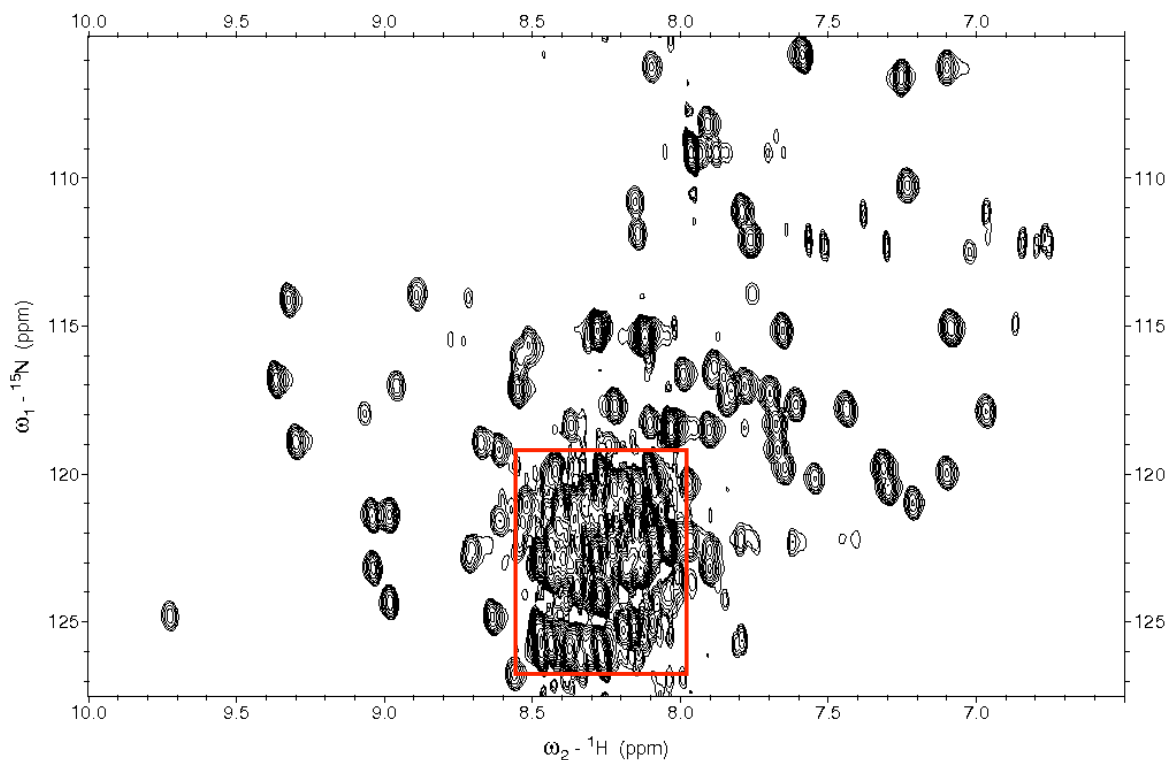
Single-scan nD acquisition



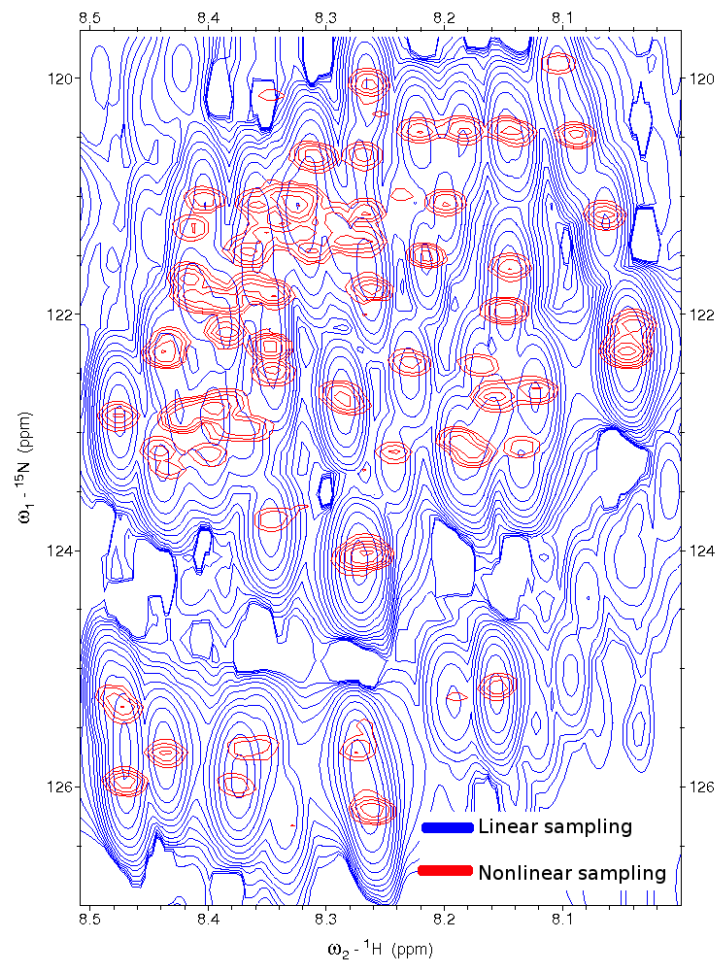


Quo vadis? Non-linear sampling

δ subunit of RNA polymerase
with an unstructured C-tail (57 aa)



2D projection of 3D HNCO
Linear sampling 20 hours



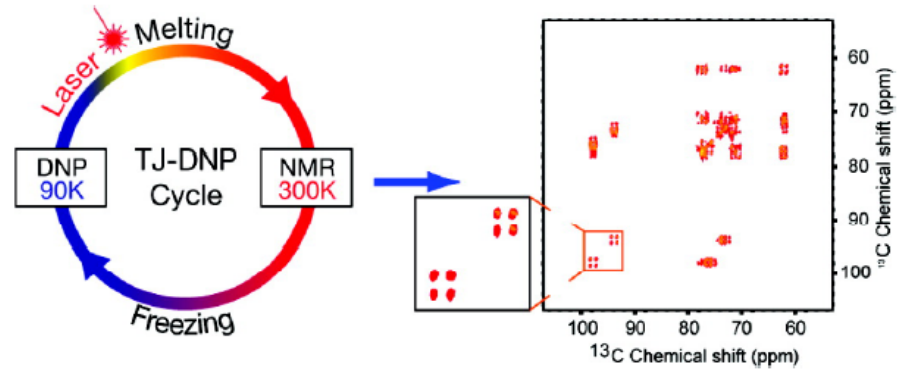
2D projection of 3D HNCO
Non-linear sampling 3 hours

Quo vadis? Biomolecular NMR methodology

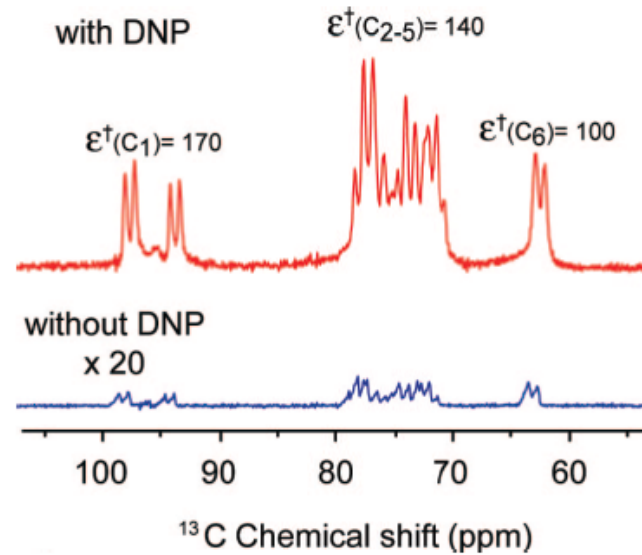
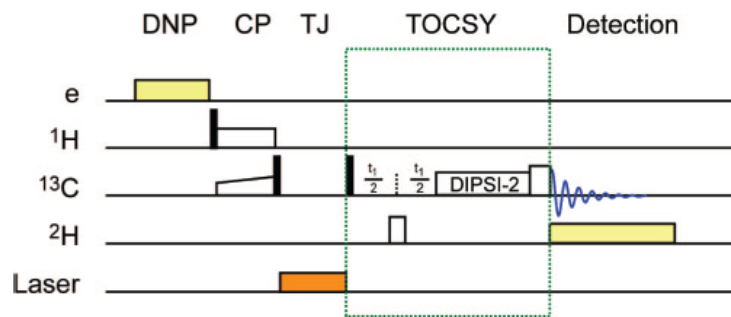
DNP – Dynamic Nuclear Polarization

800 ms laser pulse

In Situ Temperature-Jump DNP



40 s freezing by N₂ gas



Quo vadis NMR?

Current challenges

- study of molecular complexes of increasing size and complexity
- characterization of weakly interacting molecular networks
- investigation of structural preferences in natively unstructured proteins
- observation of kinetic processes and excited state conformations involved in protein folding, binding, and allosteric signal transduction
- studies of structure and function in living cells or cell extracts
- studies of molecular motions on μs -ms time scale in complex assemblies
- structure determination in solid state polycrystalline samples
- structure determination of membrane proteins using solid state technology

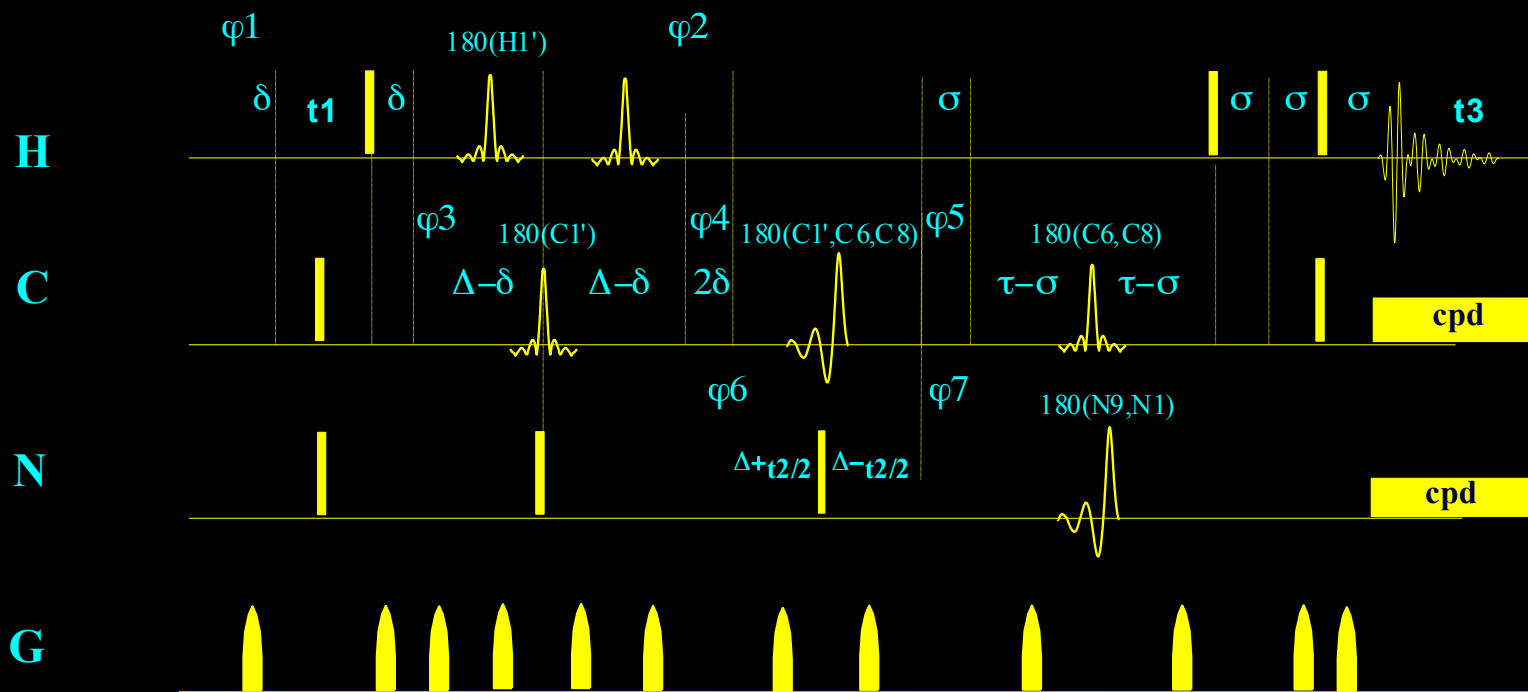
Quo vadis NMR?

Forward, future is bright



Magic Spin Gymnastics

3D HsCNbCHb experiment MQ-TROSY



time

~ 10 – 100 ms

NMR Laboratory

Bruker Avance

1995



500 MHz

1999



300 MHz

2001



600 MHz

2012

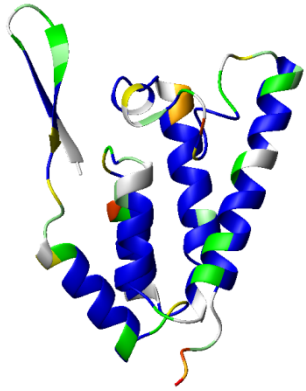


CEITEC NMR Core Facility

- 500 MHz
- 600 MHz
- 700 MHz
- 700 MHz
- 850 MHz
- 950 MHz



Acknowledgement



(also to those who are not here)

Pavel Macek
Pavel Kadeřávek

