Kurt Wüthrich, the ETH Zürich, and the Development of NMR Spectroscopy for the Investigation of Structure, Dynamics, and Folding of Proteins

Harald Schwalbe*[a]

KEYWORDS:

history of science · NMR spectroscopy · Nobel prize · protein folding · protein structures

General George Patton was the military leader of the American Third Army in World War II. Starting in 1944, the Third Army advanced through Brittany, round Paris, up the Marne and the Moselle, across the Rhine, and eventually into Czechoslovakia in April 1945. Patton was known for his short and sharp addresses to his troops. In one of those speeches, he welcomed the 761st Black Panther Tank battalion, the first battalion to admit black soldiers to the army. He ended his speech for the soldiers with the following sentences:

> *"Everyone has their eyes on you and [is] expecting great things from you. Most of all, your race is looking forward to your success. Don't let them down, and damn you, don't let me down! If you want me you can always find me in the lead tank."*[1]

Nuclear magnetic resonance (NMR) spectroscopy is one of the most powerful techniques to study the structure, dynamics, and folding of proteins. This view has been recognized by the Nobel prize committee by awarding one half of the 2002 Nobel prize in Chemistry to Prof. Kurt Wüthrich, of the Eidgenössische Technische Hochschule (ETH) Zürich, "for his development of nuclear magnetic resonance spectroscopy for determining the three-dimensional structure of biological macromolecules in solution."

And indeed, when summarizing Wüthrich's scientific contributions to this field, one has to tell the story of the development of NMR spectroscopy for the study of biological macromolecules in general and of proteins in particular (Table 1). This development has been driven by a battalion of outstanding researchers, amongst which, like General Patton during the Allied invasion in 1944, Wüthrich could always be found in the lead tank. And similarly, Wüthrich's scientific orientation has been and continues to be focused, ambitious, and straight.

Wüthrich's first encounter with magnetic resonance was during his PhD research with Silvio Fallab in Basel, where he studied the mechanism of Cu^{2+}-catalyzed reactions[2] and VO^{2+} complexes[27] by using electron spin resonance spectroscopy. He joined Robert E. Connick as a postdoctoral fellow at Berkeley, California, where he published his first NMR spectroscopy paper entitled "Nuclear magnetic resonance relaxation of oxygen-17 in aqueous solutions of vanadyl perchlorate and the rate of elimination of water molecules from the first coordination sphere". The paper deals with ^{17}O NMR spectroscopy of paramagnetic vanadylate perchlorate.[28] Interestingly, Wüthrich later studied the dynamics of hydration in proteins—old questions investigated under a new angle.

From paramagnetic inorganic salts to paramagnetic proteins

The first NMR spectrum of the protein ribonuclease was studied by Saunders et al.[29] in 1957. Wüthrich's first protein NMR studies, together with Bob Shulman, dealt with the paramagnetic protein cyanometmyoglobin and were published in 1968.[3] In 1970, the young Privatdozent Wüthrich published an article in *Chimia* on the "study of the spatial structure of protein molecules by NMR spectroscopy".[4] This was seven years after Kendrew and Perutz had solved the first three-dimensional structures of the proteins myoglobin[30] and haemoglobin[31] by X-ray crystallography. By showing whether NMR investigations of proteins in solution and X-ray studies of proteins in their crystalline form would yield similar spatial structures of proteins under the widely different experimental conditions, Wüthrich intended to provide an important cross-validation for both techniques. In addition, he continued, NMR spectroscopy could be applied to investigate the structural properties of proteins under conditions that do not yield crystals.

What in hindsight turned out to be *the* topic of his life must have evoked some scepticism at the time of this initial publication. It took Wüthrich's courage and determination for over thirty years to prove that his claim to be able "to solve the structure of a protein", put forward in his unique and charming

[a] *H. Schwalbe*
Institute for Organic Chemistry and Chemical Biology
Center for Biomolecular Magnetic Resonance
Johann Wolfgang Goethe-University Frankfurt
Marie-Curie-Strasse 11, 60439 Frankfurt/M (Germany)
Fax: (+49) 69-798-29515
E-mail: schwalbe@nmr.uni-frankfurt.de

 © 2003 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 1439-4227/03/04/2-3 $ 20.00+.50/0

Table 1. Chronological overview of contributions in the field of protein NMR spectroscopy from the Wüthrich group.

Year	Contribution
	The heroic times of NMR: 1946 – 1975
1967	^{17}O relaxation in vanadyl perchlorate,[2] determination of hydration sphere
1968	NMR spectra of cyanometmyoglobin,[3] investigation of paramagnetic effects on NMR spectra
1970	Review: The study of the spatial structure of protein molecules with nuclear magnetic resonance spectroscopy[4]
1972	NMR spectroscopy of cyclic peptides, determination of conformational equilibria in cyclic peptides[5]
1975	Determination of activation free energies of aromatic ring flips in BPTI[6]
1975	NMR spectroscopy in living cells[7]
	2D NMR spectroscopy and sequential assignments: 1977 – 1985
1977	2D *J*-resolved spectroscopy[8]
1978 – 79	NOE difference spectroscopy, transient proton – proton Overhauser effects, truncated driven nuclear Overhauser effect[9]
1979	Investigation of random-coil chemical shifts[10]
1979	Hydrogen exchange studies[11]
1979	2D SECSY spectroscopy[12]
1980	2D NOESY spectroscopy[13]
1982	Sequential assignment procedure in proteins based on homonuclear spectroscopy[14]
1983	Phase-sensitive 2D COSY spectroscopy[15]
1984	Distance geometry for the calculation of protein structure from NMR data[16]
1984	Karplus parameterization for $^3J(N^H,H_\alpha)$ coupling constants in proteins to define the backbone angle ϕ[17]
	NMR protein structures: 1985 – present
1985	Protein structure determination by NMR spectroscopy, proteinase inhibitor IIA and tendamistat[18]
1985	Hydrogen exchange studies under various exchange regimes and in unfolded proteins[19]
1986	Time-resolved protein folding by combination of quench-flow hydrogen exchange experiments and NMR detection[20]
1988	Protein structure determination: comparison of NMR spectroscopy and X-ray crystallography for tendamistat[21]
1989	Protein hydration studied by NMR spectroscopy[22]
1990	Filtering technology to investigate symmetric protein structures (X-filter technology) and macromolecular complexes such as protein – DNA, –RNA, and –protein complexes[23]
1992	Resonance assignment of unfolded protein[24]
1997	TROSY spectroscopy[25]
2001	NMR of membrane proteins: Resonance assignment of OmpX[26]

English with a Swiss accent on behalf of the whole NMR community, could indeed be fulfilled: NMR spectroscopy can yield structures of proteins.[32] In fact, NMR spectroscopy goes far beyond a purely static description of proteins; it is able to describe complete studies of the dynamics of a polypeptide chain from its random coil state to intermediates populated during folding, to the different manifestations of proteins from soluble monomeric forms, to insoluble fibrillic aggregates. And ultimately, the size barriers that restricted NMR spectroscopy to comparatively small systems in the past may finally be pushed back with the TROSY technology that was introduced by the Wüthrich group in 1997.[25]

"If you want to solve the structure of a protein…"[78]

Protein structure determination by NMR spectroscopy involves two steps. Each atom in the molecule generates a signal in the NMR spectrum. Even in a small protein such as ubiquitin whose one-dimensional spectrum is shown in Figure 1 A, there are more than a thousand hydrogen atoms, all of which need to be assigned. The resonance assignment then forms the basis for the extraction of conformational dependent parameters such as through-space-mediated NOEs and through-bond-mediated scalar *J* couplings that can be used to obtain distance and torsion angle restraints.

1. NMR Resonance Assignment Procedure: 2D FT-NMR Spectroscopy and the COSY – NOESY Sequential Assignment Approach

With a thousand hydrogen atoms, it is impossible to resolve all resonance lines in a one-dimensional spectrum. It was clear to Wüthrich early on that the two-dimensional[33] methodologies, such as the COSY[34] and NOESY[35, 36] experiments developed by his colleague Richard R. Ernst[37] in the Department of Physical Chemistry at the ETH Zürich, would have to be transferred from small molecules and their applicability proven for large proteins. At the time, the research situation at the ETH of two groups with complimentary focus, each pushing the limits of the novel NMR technology, was enormously successful. In 1980, the groups of Wüthrich and Ernst published the first application of the novel two-dimensional experiments for the study of proteins.[12, 13] From an NMR point of view, the hydrogen atoms of amino acids in polypeptides form spin systems of coupled protons that resonate at a unique and characteristic chemical shift. Spins two or three bonds apart can be correlated through scalar ^2J and ^3J coupling constants in COSY experiments (Figure 1 B). Such correlation leads to the assignment of resonances within a given amino acid. Connection of individual residues can be obtained from sequential, through-space-mediated transfers in NOESY experiments that reveal cross peaks between protons that are closer than 5 Å together (Figure 1 C).

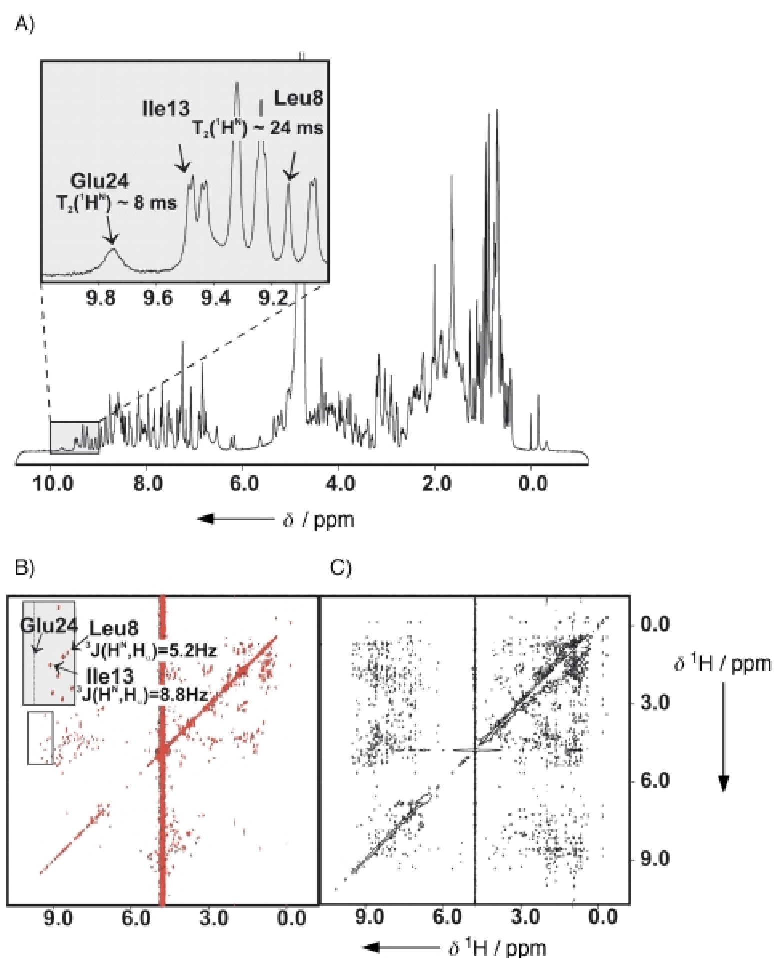

Figure 1. A) One-dimensional NMR spectrum, B) two-dimensional COSY spectrum, C) two-dimensional NOESY spectrum of the protein ubiquitin in H_2O at 600 MHz.

2. Protein Structure Calculation

On the basis of resonance assignment and the analysis of NOE patterns, Wüthrich first turned to the question of defining the conformational dependence of NMR parameters such as chemical shifts,[10] short and medium-range NOEs,[14b] and scalar *J* coupling constants[17] by comparing the two extremes of states that a protein can adopt (the native folded state and the random coil state of a protein), using model peptides GGXGG with assumed random-coil conformational averaging.

In 1979, it could be shown that distance geometry algorithms can yield structures of proteins,[38] and in 1985, the laboratories of Wüthrich[18a] and of Kaptein[39] solved the first experimental NMR spectroscopy protein structures, which were soon followed by the first NMR spectroscopy structure of a DNA by Hare and Reid.[40] The distance geometry method may be compared with a neatly woven three-dimensional spider's web. In the interior of a protein, each proton is surrounded by a large number of protons less than 5 Å apart, just as a knot in a spider's web is connected through short strings to the next layer of the web with corresponding knots. Each knot in the second sphere is the center of a new network of connectivity, and the uniform distribution of distances in globular proteins allows for the calculation of protein structure based on a large number of short and locally defined NOEs. This procedure is the essence of the distance geometry algorithm. In this algorithm, the connections between the knots in the web are the experimental restraints (such as NOE-derived distances and torsion-angle restraints from scalar coupling constants) which are taken together with the covalent structure of the protein to create a matrix of interatomic distances between all atoms. Starting from random coordinates that only fulfill the covalent constraints, the experimental restraints are embedded.

In 1988, Wüthrich and his colleague Robert Huber, an X-ray crystallographer from the Max Planck Institute, Martinsried, used tendamistat, an α-amylase inhibitor whose structure was first solved independently,[41, 18c] as a model to compare the results of the two independent structure determinations by NMR spectroscopy and X-ray crystallography (Figure 2).[21b] The result was overwhelmingly positive for both techniques: In the interior of the protein the two structures were nearly identical, while only on the protein surface could a small number of local differences be identified. For most residues on the surface of the protein, the solution structure "appeared more disordered than the crystal structure, with the exception of tyrosine-14, which was not observed in the X-ray diffraction." In a subsequent publication, Huber and Wüthrich also showed that the NMR spectroscopy solution structure could be used as model to solve the X-ray crystallographic phases with similar quality to isomorphous replacement techniques.[21c]

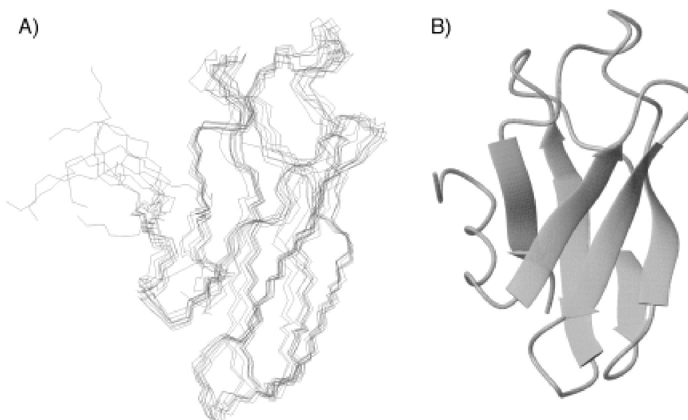

Figure 2. A) Family of structures of tendamistat, an α-amylase inhibitor determined by NMR spectroscopy, B) ribbon diagram of the structure with lowest energy.

3. Protein Dynamics and Beyond

It is a particular strength of NMR spectroscopy to investigate more than the static three-dimensional structure of proteins. Dynamic applications range from the internal dynamics of

individual atomic groups, to interactions of the protein with solvent molecules such as water or chemical denaturants, to the process of protein folding. One early example from the Wüthrich group is the determination of the free activation energies of aromatic ring flips in bovine pancreatic trypsin inhibitor (BPTI).[42, 6] The systematic investigation of hydrogen-exchange dynamics as a function of pH value, temperature, and concentration of denaturants[11, 19] laid the ground work for the investigation of the kinetics of protein folding itself with atomic resolution by Roder and Wüthrich,[20] a highly influential experiment that at the time may not have received its due recognition. Lastly, NMR spectroscopy can be used to map out the hydration sphere of proteins[22] and oligonucleotides[43] and to provide bounds for residence life times of individual water molecules.

4. The Protein Structure Gallery

NMR spectroscopy has made a major contribution to the determination of structures of proteins and oligonucleotides: 20% of the approximately 14000 structures deposited in the protein data bank (PDB) to date have been solved by NMR spectroscopy. Over the years, Wüthrich's group has contributed with more than 50 structures (summarized in Table 2 and Figure 3). The three-dimensional structures of biological macromolecules are fundamental for our understanding of cell biology. Again, NMR spectroscopy provides important insight beyond the description of only the conformationally rigid part of proteins: the structure of the benign cellular form of the prion protein[62, 70–72] solved by Wüthrich's group shows that half of the prion protein backbone is well-ordered whereas the other half is a highly mobile, extended coil.

5. NMR Spectroscopy for Large Proteins

For a long time, NMR spectroscopy could only solve structures of relatively small proteins. In 1997, Pervushin, Wüthrich, and coworkers[25] published a communication in which they showed that for amide sites in a very large protein, in which all hydrogen atoms but the exchangeable ones such as the amide hydrogen have been replaced with deuterium, the effect of cross-correlated relaxation of different relaxation mechanisms such as NH dipole–dipole relaxation and N chemical shift anisotropy[76] leads to enhanced relaxation for the downfield component of the H^N doublet, while for the upfield component, the two relaxation processes can mutually cancel. Since the chemical shift anisotropy is field dependent and the dipole–dipole relaxation is field independent for large molecules, the two

Table 2. *Chronological overview of protein and DNA structures solved in the Wüthrich group and deposited in the PDB.*

PDB accession Code	Protein
1BUS[18a]	Proteinase inhibitor IIA from bull seminal plasma
1MRB[44]	Metallothionein-2a
1NTX[45]	α-Neurotoxin from *Dendroaspis polylepis*
AIT[18b]	α-Amylase inhibitor tendamistat
1ATX[46]	Neurotoxin ATX Ia from *Anemonia sulcata*
1HOM, 2HOA, 1AHD[47]	Antennapedia homeodomain and antennapedia homeodomain–DNA complex
1EGR, 1EGO[48]	Reduced *Escherichia coli* glutaredoxin, oxidized *E. coli* glutaredoxin
1PBA[49]	From porcine procarboxypeptidase B
1EGF[50]	Murine epidermal growth factor
1HIC[51]	Hirudin(1–51)
1PIT[52]	Bovine pancreatic trypsin inhibitor
1PRA[53]	DNA-binding domain (residues 1 to 69) of the 434 repressor
1DTK[54]	Dendrotoxin K from the venom of *Dendroaspis polylepis polylepis*
1ERP, 1ERC, 1ERD, 1ERY, 1HA8, 1HD6[55]	pheromones Er-1, Er-2, Er-10, Er-11, Er-23, and Er-22 from the ciliated protozoan *Euplotes raikovi*
1SHP[56]	Kunitz-type proteinase inhibitor from the sea anemone *Stichodactyla helianthus*
1ADR[57]	DNA-binding domain of the P22 c2 repressor (residues 1 to 76)
1FTZ[58]	Fushi tarazu homeodomain from *Drosophila*
1SPF[59]	Pulmonary surfactant-associated polypeptide SP-C
1TAP[60]	Recombinant tick anticoagulant protein (rTAP)
3CYS, 1OCA[61]	Cyclophilin A–cyclosporin A complex, unligated human cyclophilin A
1AG2[62]	Mouse prion protein domain PrP(121–231)
1WKT[63]	Ancestral $\beta\gamma$-crystallin precursor structure
1XBL[64]	J-domain and the Gly/Phe-rich region of the *Escherichia coli* DnaJ chaperone
1CFE[65]	Protein P14a
2LFB[66]	Homeodomain from the rat liver LFB1/HNF1 transcription factor
1BF8[67]	Periplasmic chaperone FimC
1QJK, 1QJL[68]	Sea urchin (*Strongylocentrotus Purpuratus*) metallothionein Mta
2FNB[69]	Human oncofoetal fibronectin ED-B domain
1DWY, 1DWZ, 1DX0, 1DX1[70]	Bovine prion protein
1E1G, 1E1J, 1E1P, 1E1S, 1E1U, 1E1W[71]	Three single-residue variants of the human prion protein
1QLX, 1QLZ, 1QM0, 1QM1, 1QM2, 1QM3[72]	Human prion protein
1QND[73]	Sterol carrier protein-2
1HHN[74]	Calreticulin P-domain
1LS8[75]	Unliganded *Bombyx mori* pheromone-binding protein

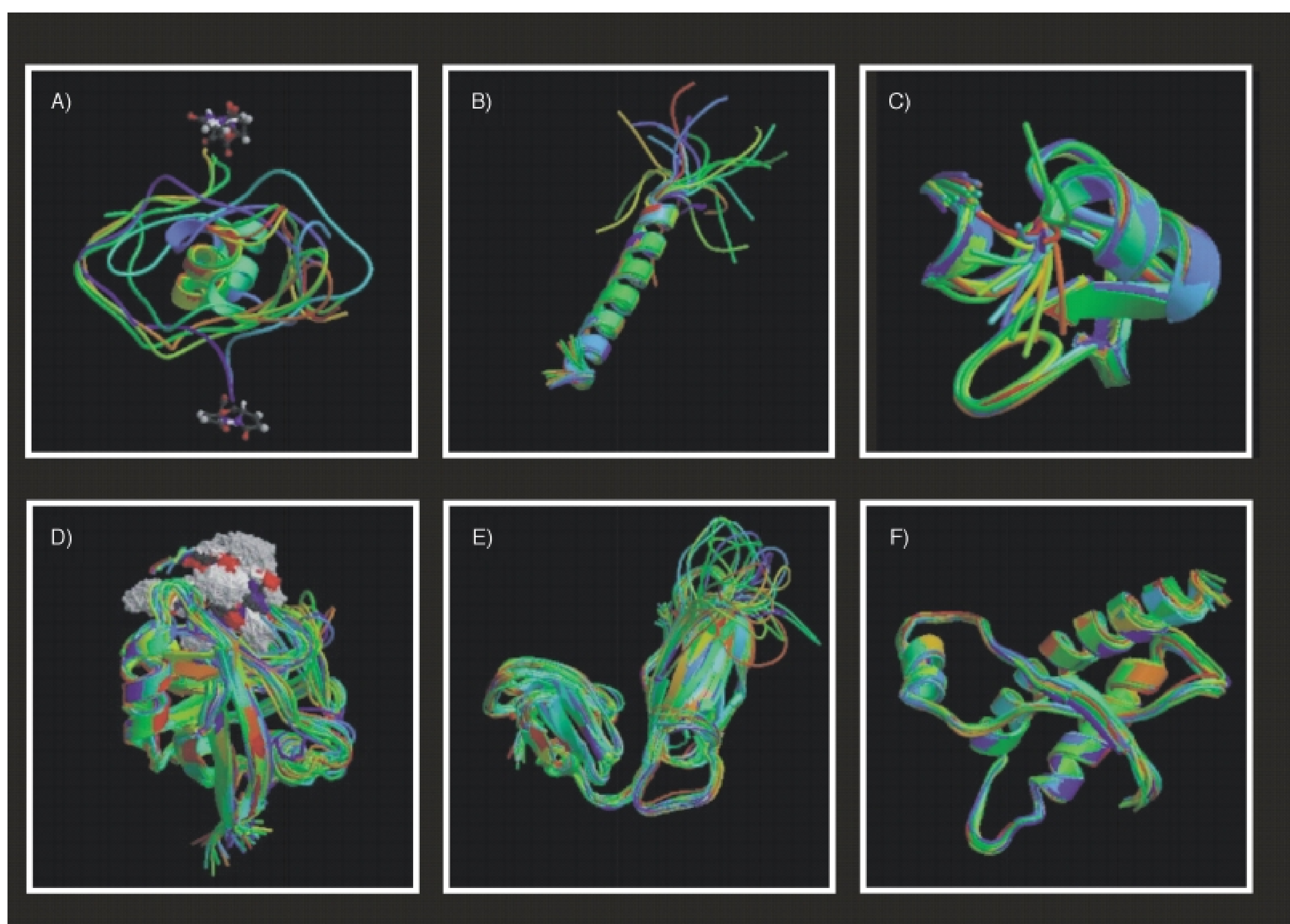

Figure 3. Gallery of selected protein structures solved by the Wüthrich group: A) Proteinase inhibitor IIA from bull seminal plasma (PDB accession code: 1BUS),[18a] B) pulmonary surfactant-associated polypeptide SP-C (1SPF),[59] C) bovine pancreatic trypsin inhibitor (1PIT),[52] D) the complex of cyclosporin A bound to cyclophilin A (3CYS),[61] E) the periplasmic chaperone FimC (1BF8),[67] G) the structured part of the human prion protein (1QLX).[72]

effects will mutually cancel at a magnetic field of approximately 950 MHz (Figure 4). It was again the Wüthrich group who clearly recognized the importance of this observation and its tremendous impact for the NMR spectroscopy of larger protein complexes—with the latest record documented in a study of the molecular chaperone complex GroEL–GroES with a molecular weight of 900 K.[77]

Final Remarks

The development of NMR spectroscopy as one of the two major tools for the study of structure, dynamics, and folding of proteins and their interaction with small and large molecules from drugs to molecular chaperones has been and continues to be intimately linked with the research of Kurt Wüthrich. His school is now spread throughout the world and is continuing to advance this exciting field, unique and interdisciplinary with applications and influences to and from physics, chemistry, biology, and medicine.

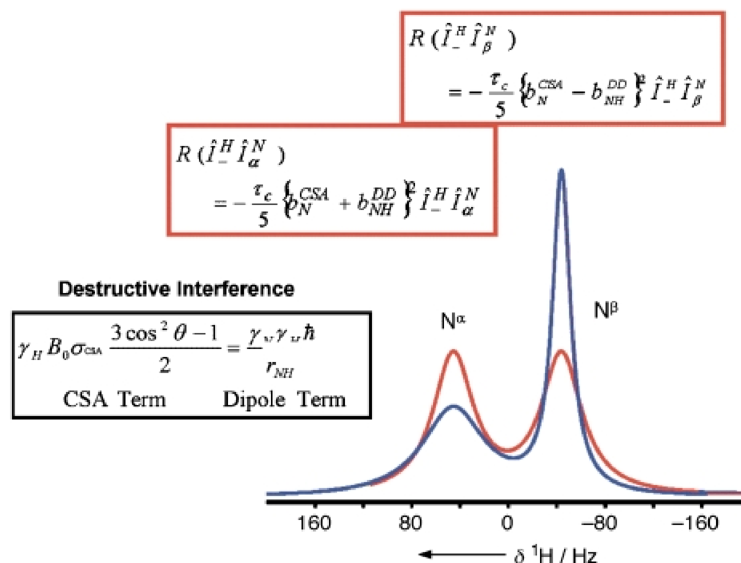

Figure 4. The TROSY effect.[25] In a ^{15}N-labeled protein, the amide resonance line is split into a doublet due to the ^1J(N,H) coupling constant of approximately 90 Hz. Due to cross-correlated relaxation of the dipole–dipole relaxation and the chemical shift anisotropy (CSA) relaxation mechanism, the two components of the doublet have different line width (blue line). The CSA contribution is field dependent and the condition at which destructive interference of the two relaxation mechanisms leads to mutual cancellation is given.

I thank Dr. Jens Wöhnert, Sergey Ilin, Dr. Aphrodite Anastasias-Pool, Dr. Emily Collins, Dr. Uwe Reinscheid, Prof. Horst Kessler (TU München), and Prof. Christian Griesinger (MPI Göttingen) for valuable comments about the manuscript and help with preparation of the figures.

[1] General George Patton, quoted from *Speeches*, 2nd ed. (Ed.: B. MacArthur), Penguin Books, London, **1999**.
[2] K. Wüthrich, H. Loeliger, S. Fallab, "Elektronenspinresonanzmessungen zur Untersuchung von Kinetik und Mechanismus von Cu^{2+}-katalysierten Reaktionen", *Experientia* **1964**, *20*, 599 – 601.
[3] K. Wüthrich, R. G. Shulman, J. Peisach, "High resolution proton magnetic resonance spectra of sperm whale cyanometmyoglobin", *Proc. Natl. Acad. Sci. USA* **1968**, *60*, 373 – 380.
[4] K. Wüthrich, "Studien der räumlichen Struktur von Proteinmolekülen mit magnetischer Kernresonanzspektroskopie", *Chimia* **1970**, *24*, 409 – 418; the title of this German publication is translated as follows by the Chemical Abstracts Service online: "Three-dimensional structure of protein molecules studied by nuclear magnetic resonance". The article is the written form of a lecture given at the Swiss chemists' association symposium about biopolymers in Bern, 26 – 28 August, 1970.
[5] K. Wüthrich, A. Tun-Kyi, R. Schwyzer, "Manifestation in the ^{13}C NMR spectra of two different molecular conformations of a cyclic pentapeptide", *FEBS Lett.* **1972**, *25*, 104 – 108.
[6] K. Wüthrich, G. Wagner, "NMR investigations of the dynamics of the aromatic amino acid residues in the basic pancreatic trypsin inhibitor", *FEBS Lett.* **1975**, *50*, 265 – 268.
[7] M. Llinás, K. Wüthrich, W. Schwotzer, W. von Philipsborn, "^{15}N nuclear magnetic resonance of living cells", *Nature* **1975**, *257*, 817 – 818.
[8] a) K. Nagayama, K. Wüthrich, P. Bachmann, R. R. Ernst, "Two-dimensional J-resolved ^{1}H NMR spectroscopy of biological macromolecules", *Biochem. Biophys. Res. Commun.* **1977**, *78*, 99 – 105; b) K. Nagayama, K. Wüthrich, P. Bachmann, R. R. Ernst, "Two-dimensional NMR spectroscopy: a powerful tool for the investigation of biopolymers in solution", *Naturwissenschaften* **1977**, *64*, 581 – 582.
[9] a) R. Richarz, K. Wüthrich, "NOE difference spectroscopy: a novel method for observing individual multiplets in proton nmr spectra of biological macromolecules", *J. Magn. Reson.* **1978**, *30*, 147 – 150; b) S. L. Gordon, K. Wüthrich, "Transient proton – proton Overhauser effects in horse ferrocytochrome c", *J. Am. Chem. Soc.* **1978**, *100*, 7094 – 7096; c) G. Wagner, K. Wüthrich, "Truncated driven nuclear Overhauser effect (TOE): a new technique for studies of selective ^{1}H – ^{1}H Overhauser effects in the presence of spin diffusion", *J. Magn. Reson.* **1979**, *33*, 675 – 680.
[10] A. Bundi, K. Wüthrich, "^{1}H NMR parameters of the common amino acid residues measured in aqueous solutions of the linear tetrapeptides H-Gly-Gly-X-L-Ala-OH", *Biopolymers* **1979**, *18*, 285 – 297.
[11] G. Wagner, K. Wüthrich, "Correlation between the amide proton exchange rates and the denaturation temperatures in globular proteins related to the basic pancreatic trypsin inhibitor", *J. Mol. Biol.* **1979**, *130*, 31 – 37.
[12] K. Nagayama, K. Wüthrich, R. R. Ernst, "Two-dimensional spin echo correlated spectroscopy (SECSY) for ^{1}H NMR studies of biological macromolecules", *Biochem. Biophys. Res. Commun.* **1979**, *90*, 305 – 311.
[13] Anil-Kumar, R. R. Ernst, K. Wüthrich, "A two-dimensional nuclear Overhauser enhancement (2D NOE) experiment for the elucidation of complete proton – proton cross-relaxation networks in biological macromolecules", *Biochem. Biophys. Res. Commun.* **1980**, *95*, 1 – 6.
[14] a) K. Wüthrich, G. Wider, G. Wagner, W. Braun, "Sequential resonance assignments as a basis for determination of spatial protein structures by high resolution proton nuclear magnetic resonance", *J. Mol. Biol.* **1982**, *155*, 311 – 319; b) M. Billeter, W. Braun, K. Wüthrich, "Sequential resonance assignments in protein ^{1}H nuclear magnetic resonance spectra: computation of sterically allowed proton – proton distances and statistical analysis of proton – proton distances in single crystal protein conformations", *J. Mol. Biol.* **1982**, *155*, 321 – 346; c) G. Wagner, K. Wüthrich, "Sequential resonance assignments in protein ^{1}H nuclear magnetic resonance spectra: basic pancreatic trypsin inhibitor", *J. Mol. Biol.* **1982**, *155*, 347 – 366.
[15] D. Marion, K. Wüthrich, "Application of phase sensitive two-dimensional correlated spectroscopy (COSY) for measurements of ^{1}H – ^{1}H spin – spin coupling constants in proteins", *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967 – 974.
[16] T. F. Havel, K. Wüthrich, "A distance geometry program for determining the structures of small proteins and other macromolecules from nuclear magnetic resonance measurements of intramolecular ^{1}H – ^{1}H proximities in solution", *Bull. Math. Biol.* **1984**, *46*, 673 – 698.
[17] A. Pardi, M. Billeter, K. Wüthrich, "Calibration of the angular dependence of the amide proton – Cα proton coupling constants, $^{3}JHN\alpha$, in a globular protein: use of $^{3}JHN\alpha$ for identification of helical secondary structure", *J. Mol. Biol.* **1984**, *180*, 741 – 751.
[18] a) M. P Williamson, T. F. Havel, K. Wüthrich, "Solution conformation of proteinase inhibitor IIA from bull seminal plasma by 1H nuclear magnetic resonance and distance geometry", *J. Mol. Biol.* **1985**, *182*, 295 – 315; b) A. D. Kline, K. Wüthrich, "Secondary structure of the α-amylase polypeptide inhibitor Tendamistat from S*treptomyces tendae* determined in solution by ^{1}H nuclear magnetic resonance", *J. Mol. Biol.* **1985**, *183*, 503 – 507; c) A. D. Kline, W. Braun, K. Wüthrich, "Studies by ^{1}H nuclear magnetic resonance and distance geometry of the solution conformation of the α-amylase inhibitor Tendamistat", *J. Mol. Biol.* **1986**, *189*, 377 – 382.
[19] a) H. Roder, G. Wagner, K. Wüthrich, "Amide proton exchange in proteins by EX_1 kinetics: studies of the basic pancreatic trypsin inhibitor at variable $p^{2}H$ and temperature", *Biochemistry* **1985**, *24*, 7396 – 7407; b) H. Roder, G. Wagner, K. Wüthrich, "Individual amide proton exchange rates in thermally unfolded basic pancreatic trypsin inhibitor", *Biochemistry* **1985**, *24*, 7407 – 7411.
[20] H. Roder, K. Wüthrich, "Protein folding kinetics by combined use of rapid mixing techniques and NMR observation of individual amide protons", *Proteins* **1986**, *1*, 34 – 42.
[21] a) A. D. Kline, W. Braun, K. Wüthrich, "Determination of the complete three-dimensional structure of the α-amylase inhibitor Tendamistat in aqueous solution by nuclear magnetic resonance and distance geometry", *J. Mol. Biol.* **1988**, *204*, 675 – 724; b) M. Billeter, A. D. Kline, W. Braun, R. Huber, K. Wüthrich, "Comparison of the high-resolution structures of the α-amylase inhibitor Tendamistat determined by nuclear magnetic resonance in solution and by X-ray diffraction in single crystals", *J. Mol. Biol.* **1989**, *206*, 677 – 687; c) W. Braun, O. Epp, K. Wüthrich, R. Huber, "Solution of the phase problem in the X-ray diffraction method for proteins with the nuclear magnetic resonance solution structure as initial model", *J. Mol. Biol.* **1989**, *206*, 669 – 676.
[22] G. Otting, K. Wüthrich, "Studies of protein hydration in aqueous solution by direct NMR observation of individual protein-bound water molecules", *J. Am. Chem. Soc.* **1989**, *111*, 1871 – 1875.
[23] G. Otting, K. Wüthrich, "Extended heteronuclear editing of 2D ^{1}H NMR spectra of isotope-labeled proteins, using the $X(\omega_1,\omega_2)$-double-half-filter", *J. Magn. Reson.* **1989**, *85*, 586 – 594.
[24] a) D. Neri, G. Wider, K. Wüthrich, "Complete 15N and 1H NMR assignments for the amino-terminal domain of the phage 434 repressor in the urea-unfolded form", *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4397 – 4401; b) D. Neri, G. Wider, K. Wüthrich, "1 H, 15N and 13C NMR assignments of the 434 repressor fragments 1 – 63 and 44 – 64 unfolded in 7 M urea", *FEBS Lett.* **1992**, *303*, 129 – 135.
[25] K. Pervushin, R. Riek, G. Wider, K. Wüthrich, "Attenuated T_2 relaxation by mutual cancellation of dipole-dipole coupling and chemical shift anisotropy indicates an avenue to NMR structures of very large biological macromolecules in solution", *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 12 366 – 12 371.
[26] a) C. Fernandéz, K. Wüthrich, "Transverse relaxation-optimized NMR spectroscopy with the outer membrane protein OmpX in dihexanoyl phosphatidylcholine micelles", *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 2358 – 2363; b) C. Fernández, C. Hilty, S. Bonjour, K. Pervushin, K. Wüthrich, "Solution NMR studies of the integral membrane proteins OmpX and OmpA from *Escherichia coli*", *FEBS Lett.* **2001**, *504*, 173 – 178.
[27] K. Wüthrich, "Elektronenspinresonanz-Untersuchungen von VO^{2+} Komplexverbindungen in wässeriger Lösung", *Helv. Chim. Acta* **1965**, *48*, 779 – 790.
[28] K. Wüthrich, R. E. Connick, "Nuclear magnetic resonance relaxation of oxygen-17 in aqueous solutions of vanadyl perchlorate and the rate of elimination of water molecules from the first coordination sphere", *Inorg. Chem.* **1967**, *6*, 583 – 590.

[29] M. Saunders, A. Wishnia, K. G. Kirkwood, "The nuclear magnetic resonance spectrum of ribonuclease", *J. Am. Chem. Soc.* **1957**, *79*, 3289 – 3290.
[30] J. C. Kendrew, "Myoglobin and the structure of proteins", *Science* **1963**, *139*, 1259 – 1266.
[31] M. F. Perutz, "X-ray analysis of hemoglobin", *Science* **1963**, *140*, 863 – 869.
[32] About 20% of the atomic coordinates deposited in the protein data bank have been determined by NMR spectroscopy.
[33] J. Jeener, *Ampère International Summer School* (Basko Polje, Yugoslavia), **1971**, unpublished results.
[34] W. P. Aue, E. Bartholdi, R. R. Ernst, "Two-dimensional spectroscopy. Application to nuclear magnetic resonance", *J. Chem. Phys.* **1976**, *64*, 2229 – 2246.
[35] S. Macura, R. R. Ernst, "Elucidation of cross relaxation in liquids by two-dimensional NMR spectroscopy", *Mol. Phys.* **1980**, *41*, 95 – 117.
[36] a) J. Jeener, B. H. Meier, P. Bachmann, R. R. Ernst, "Investigation of exchange processes by two-dimensional NMR spectroscopy", *J. Chem. Phys.* **1979**, *71*, 4546 – 4553; b) B. H. Meier, R. R. Ernst, "Elucidation of chemical exchange networks by two-dimensional NMR spectroscopy: The heptamethylbenzenonium Ion", *J. Am. Chem. Soc.* **1979**, *101*, 6441 – 6442.
[37] R. R. Ernst, "Kernresonanz-Fourier-Transformations-Spektroskopie (Nobel-Vortrag)", *Angew. Chem.* **1992**, *104*, 817 – 836; "Nuclear Magnetic Resonance Fourier Transform Spectroscopy (Nobel Lecture)", *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 805 – 823.
[38] T. F. Havel, I. D. Kuntz, G. M. Crippen, "Effects of distance constraints on macromolecular conformation. II. Simulation of experimental results and theoretical predictions", *Biopolymers* **1979**, *18*, 73 – 82.
[39] R. Kaptein, E. R. P. Zuiderweg, R. M. Scheek, R. Boelens, W. F. A. van Gunsteren, "A protein structure from nuclear magnetic resonance data. Lac repressor headpiece", *J. Mol. Biol.* **1985**, *182*, 179 – 182.
[40] D. R. Hare, B. R. Reid, "Three-dimensional structure of a DNA hairpin in solution. Two-dimensional NMR studies and distance geometry calculations on $d(CGCGTTTTGCGC)_2$", *Biochemistry* **1986**, *25*, 5341 – 5350.
[41] J. Pflugrath, E. Wiegand, R. Huber, L. Vertesy, "Crystal structure determination, refinement and molecular model of the α-amylase inhibitor Hoe-467A", *J. Mol. Biol.* **1986**, *189*, 377 – 382.
[42] G. Wagner, A. DeMarco, K. Wüthrich, "Dynamics of the aromatic amino acid residues in the globular conformation of the basic pancreatic trypsin inhibitor (BPTI). I. Proton NMR studies", *Biophys. Struct. Mech.* **1976**, *2*, 139 – 158.
[43] E. Liepinsh, G. Otting, K. Wüthrich, "NMR observation of individual molecules of hydration water bound to DNA duplexes: direct evidence for a spine of hydration water present in aqueous solution", *Nucleic Acids Res.* **1992**, *20*, 6549 – 6553.
[44] a) A. Arseniev, P. Schultze, E. Wörgötter, W. Braun, G. Wagner, M. Vasak, J. H. R. Kägi, K. Wüthrich, "Three-dimensional structure of rabbit liver [Cd7] metallothionein-2a in aqueous solution determined by nuclear magnetic resonance", *J. Mol. Biol.* **1988**, *201*, 637 – 657; b) P. Schultze, E. Wörgötter, W. Braun, G. Wagner, M. Vasak, J. H. R. Kägi, K. Wüthrich, "Conformation of [Cd7]-metallothionein-2 from rat liver in aqueous solution determined by nuclear magnetic resonance spectroscopy", *J. Mol. Biol.* **1988**, *203*, 251 – 268.
[45] A. M. Labhardt, E. H. Hunziker-Kwik, K. Wüthrich, "Secondary structure determination for a neurotoxin from *Dendroaspis polylepis* based on sequence-specific ^{1}H-nuclear-magnetic-resonance assignments", *Eur. J. Biochem*. **1988**, *177*, 295 – 305.
[46] H. Widmer, M. Billeter, K. Wüthrich, "Three-dimensional structure of the neurotoxin ATX Ia from *Anemonia sulcata* in aqueous solution determined by nuclear magnetic resonance spectroscopy", *Proteins* **1989**, *6*, 357 – 371.
[47] a) M. Billeter, Y. Q. Qian, G. Otting, M. Müller, W. J. Gehring, K. Wüthrich, "Determination of the three-dimensional structure of the *Antennapedia* homeodomain from *Drosophila* in solution by 1H nuclear magnetic resonance spectroscopy", *J. Mol. Biol.* **1990**, *214*, 183 – 197; b) M. Billeter, Y. Q. Qian, G. Otting, M. Müller, W. J. Gehring, K. Wüthrich, "Determination of the nuclear magnetic resonance solution structure of an *Antennapedia* homeodomain – DNA complex", *J. Mol. Biol.* **1993**, *234*, 1084 – 1093.
[48] a) P. Sodano, K. V. R. Chary, O. Björnberg, A. Holmgren, B. Kren, J. A. Fuchs, K. Wüthrich, "Nuclear magnetic resonance studies of recombinant *Escherichia coli* glutaredoxin: sequence-specific assignments and secondary structure determination of the oxidized form", *Eur. J. Biochem.* **1991**, *200*, 369 – 377; b) T. Xia, J. H. Bushweller, P. Sodano, M. Billeter, O. Björnberg, A. Holmgren, K. Wüthrich, "NMR structure of oxidized *Escherichia coli* glutaredoxin: comparison with reduced *E. coli* glutaredoxin and functionally related proteins", *Protein Sci.* **1992**, *1*, 310 – 321.
[49] J. Vendrell, M. Billeter, G. Wider, F. X. Avilés, K. Wüthrich, "The NMR structure of the activation domain isolated from porcine procarboxypeptidase B", *EMBO J.* **1991**, *10*, 11 – 15.
[50] G. T. Montelione, K. Wüthrich, A. W. Burgess, E. C. Nice, G. Wagner, D. Gibson, H. A. Scheraga, "Solution structure of murine epidermal growth factor determined by NMR spectroscopy and refined by energy minimization with restraints", *Biochemistry* **1992**, *31*, 236 – 249.
[51] T. Szyperski, P. Güntert, S. R. Stone, K. Wüthrich, "Nuclear magnetic resonance solution structure of hirudin(1 – 51) and comparison with corresponding three-dimensional structures determined using the complete 65-residue hirudin polypeptide chain", *J. Mol. Biol.* **1992**, *228*, 1193 – 1205.
[52] K. D. Berndt, P. Güntert, L. P. M. Orbons, K. Wüthrich, "Determination of a high-quality nuclear magnetic resonance solution structure of the bovine pancreatic trypsin inhibitor and comparison with three crystal structures", *J. Mol. Biol*. **1992**, *227*, 757 – 775.
[53] D. Neri, M. Billeter, K. Wüthrich, "Determination of the NMR solution structure of the DNA-binding domain 1 – 69 of the 434 repressor and comparison with the X-ray crystal structure", *J. Mol. Biol*. **1992**, *223*, 743 – 767.
[54] K. D. Berndt, P. Güntert, K. Wüthrich, "Nuclear magnetic resonance solution structure of dendrotoxin K from the venom of *Dendroaspis polylepis polylepis*", *J. Mol. Biol.* **1993**, *234*, 735 – 750.
[55] a) L. R. Brown, S. Mronga, R. A. Bradshaw, C. Ortenzi, P. Luporini, K. Wüthrich, "Nuclear magnetic resonance solution structure of the pheromone E*r*-10 from the ciliated protozoan *Euplotes raikovi*", *J. Mol. Biol.* **1993**, *231*, 800 – 816; b) S. Mronga, P. Luginbühl, L. R. Brown, C. Ortenzi, P. Luporini, R. A. Bradshaw, K. Wüthrich, "The NMR solution structure of the pheromone E*r*-1 from the ciliated protozoan *Euplotes raikovi*", *Protein Sci.* **1994**, *3*, 1527 – 1536; c) M. Ottiger, T. Szyperski, L. Luginbühl, C. Ortenzi, P. Luporini, R. A. Bradshaw, K. Wüthrich, "The NMR solution structure of the pheromone E*r*-2 from the ciliated protozoan *Euplotes raikovi*", *Protein Sci.* **1994**, *3*, 1515 – 1526; d) P. Luginbühl, J. Wu, O. Zerbe, C. Ortenzi, P. Luporini, K. Wüthrich, "The NMR solution structure of the pheromone Er-11 from the cilitated protozoan *Euplotes raikovi*" *Protein Sci.* **1996**, *5*, 1512 – 1522; e) R. Zahn, F. Damberger, C. Ortenzi, P. Luporini, K. Wüthrich, "NMR structure of the *Euplotes raikovi* pheromone E*r*-23 and identification of its five disulfide bonds", *J. Mol. Biol.* **2001**, *313*, 923 – 931; f) A. Liu, P. Luginbühl, O. Zerbe, C. Ortenzi, P. Luporini, K. Wüthrich, "NMR structure of the pheromone Er-22 from *Euplotes raikovi*", *J. Biomol. NMR* **2001**, *19*, 75 – 78.
[56] W. Antuch, K. D. Berndt, M. A. Chavez, J. Delfin, K. Wüthrich, "The NMR solution structure of a Kunitz-type proteinase inhibitor from the sea anemone *Stichodactyla helianthus*", *Eur. J. Biochem.* **1993**, *212*, 675 – 684.
[57] P. Sevilla-Sierra, G. Otting, K. Wüthrich, "Determination of the nuclear magnetic resonance structure of the DNA-binding domain of the *P22 c*2 repressor (1 to 76) in solution and comparison with the DNA-binding domain of the *434* repressor", *J. Mol. Biol.* **1994**, *235*, 1003 – 1020.
[58] Y. Q. Qian, K. Furukubo-Tokunaga, D. Resendez-Perez, M. Müller, W. J. Gehring, K. Wüthrich, "Nuclear magnetic resonance solution structure of the *fushi tarazu* homeodomain from *Drosophila* and comparison with the *Antennapedia* homoedomain", *J. Mol. Biol.* **1994**, *238*, 333 – 345.
[59] J. Johansson, T. Szyperski, T. Curstedt, K. Wüthrich, "The NMR structure of the pulmonary surfactant-associated polypeptide SP-C in an apolar solvent contains a valyl-rich α-helix", *Biochemistry* **1994**, *33*, 6015 – 6023.
[60] W. Antuch, P. Güntert, M. Billeter, T. Hawthorne, H. Grossenbacher, K. Wüthrich, "NMR solution structure of the recombinant tick anticoagulant protein (rTAP), a factor XA inhibitor from the tick *Ornithodoros moubata*", *FEBS Lett.* **1994**, *352*, 251 – 257.
[61] a) C. Spitzfaden, W. Braun, G. Wider, H. Widmer, K. Wüthrich, "Determination of the NMR solution structure of the cyclophilin A – cyclosporin A complex", *J. Biomol. NMR* **1994**, *4*, 463 – 482; b) M. Ottiger, O. Zerbe, P. Güntert, K. Wüthrich, "The NMR solution conformation of unligated human cyclophilin A", *J. Mol. Biol.* **1997**, *272*, 64 – 81.
[62] R. Riek, S. Hornemann, G. Wider, M. Billeter, R. Glockshuber, K. Wüthrich, "NMR structure of the mouse prion protein domain PrP(121 – 231)", *Nature* **1996**, *382*, 180 – 182.

[63] W. Antuch, P. Güntert, K. Wüthrich, "Ancestral βγ-crystallin percursor structure in a yeast killer toxin", *Nat. Struct. Biol.* **1996**, *3*, 662 – 665.
[64] T. Szyperski, M. Pellecchia, D. Wall, C. Georgopoulos, K. Wüthrich, "NMR structure determination of the *Escherichia coli* DnaJ molecular chaperone: secondary structure and backbone fold of the N-terminal region 2 – 108 comprising the highly conserved J-domain", *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11 343 – 11 347.
[65] C. Fernández , T. Szyperski, T. Bruyère, P. Ramage, E. Mösinger, K. Wüthrich, "NMR solution structure of the pathogenesis-related protein P14a", *J. Mol. Biol.* **1997**, *266*, 576 – 593.
[66] O. Schott, M. Billeter, B. Leiting, G. Wider, K. Wüthrich, "The NMR solution structure of the non-classical homeodomain from the rat liver LFB1/HNF1 transcription factor", *J. Mol. Biol.* **1997**, *267*, 673 – 683.
[67] M. Pellecchia, P. Güntert, R. Glockshuber, K. Wüthrich, "NMR solution structure of the periplasmic chaperone FimC", *Nat. Struct. Biol.* **1998**, *5*, 885 – 890.
[68] R. Riek, B. Prêcheur, Y. Wang, E. A. Mackay, G. Wider, P. Güntert, A. Liu, J. H. R. Kägi, K. Wüthrich, "NMR structure of the sea urchin (*Strongylocentrotus purpuratus*) metallothionein MTA", *J. Mol. Biol.* **1999**, *291*, 417 – 428.
[69] R. Fattorusso, M. Pellecchia, F. Viti, P. Neri, D. Neri, K. Wüthrich, "NMR structure of the human oncofoetal fibronectin ED-B domain, a specific marker for angiogenesis", *Folding Des.* **1999**, *7*, 381 – 390.
[70] F. López Garcia, R. Zahn, R. Riek, K. Wüthrich, "NMR structure of the bovine prion protein", *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8334 – 8399.
[71] L. Calzolai, D. Lysek, P. Güntert, C. von Schroetter, R. Riek, R. Zahn, K. Wüthrich, "NMR structures of three single-residue variants of the human prion protein", *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 8340 – 8345.
[72] R. Zahn, A. Liu, T. Lührs, R. Riek, C. von Schroetter, F. López Garcia, M. Billeter, L. Calzolai, G. Wider, K. Wüthrich, "NMR solution structure of the human prion protein", *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 145 – 150.
[73] F. Lòpez Garcia, T. Szyperski, J. H. Dyer, T. Choinowski, U. Seedorf, H. Hauser, K. Wüthrich, "NMR Structure of the sterol carrier protein-2: implications for the biological role", *J. Mol. Biol.* **2000**, *295*, 595 – 603.
[74] L. Ellgaard, R. Riek, T. Herrmann, P. Güntert, D. Braun, A. Helenius, K. Wüthrich, "NMR structure of the calreticulin P-domain", *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 3133 – 3138.
[75] D. Lee, F. F. Damberger, R. Horst, P. Güntert, L.Nikonova, W. S. Leal, K. Wüthrich, "NMR Structure of the Unliganded Bombyx Mori Pheromone-Binding Protein at Physiological pH", *FEBS Lett.* **2002**, *531*, 314 – 318.
[76] R. H. Griffey, A. G. Redfield, "Proton-detected heteronuclear edited and correlated nuclear magnetic resonance and nuclear Overhauser effect in solution", *Q. Rev. Biophys.* **1987**, *19*, 51 – 82.
[77] J. Fiaux, E. B. Bertelsen, A. L. Horwich, K. Wüthrich, "NMR analysis of a 900 K GroEL-GroES complex", *Nature* **2002**, *418*, 207 – 211.
[78] Note added in proof: Kurt Wüthrich uses his belt to explain the process of NMR structure determination, as can be seen in the video recording of his lecture given on December 8, 2002, at the Magna Aula, Stockholm University, Sweden; www.nobel.se.

Received: November 27, 2002 [M 533]