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history

The way to NMR structures of proteins

Kurt Wüthrich

In 1998 Kurt Wüthrich was awarded the Kyoto Prize in Advanced Technology for having "developed a method of determining the conformations of proteins, nucleic acids and other biomacromolecules in solutions or biomembranes, where they exhibit their function"[1].

Wüthrich has used nuclear magnetic resonance (NMR) techniques to study proteins and nucleic acids since 1967. In a series of four papers his group outlined a framework for NMR structure determination of proteins in 1982, and in 1984 the first de novo structure of a globular protein in solution was determined. The Wüthrich group went on to solve more than 60 protein structures in solution, including the Antennapedia homeodomain, the cyclophillin A–cyclosporin A complex, and the human and bovine prion proteins.

What follows is a personal recollection by Kurt Wüthrich of how he and his associates arrived at the first view of a protein structure through the NMR eye.

In the 1950s, magnetic resonance spectroscopy evolved into a useful tool in chemistry. During the period of 1962–1967, my graduate and postdoctoral research, with Professor Silvio Fallab at the University of Basel and Professor Robert E. Connick at the University of California, Berkeley, focused on the use of electron paramagnetic resonance (EPR) and nuclear magnetic resonance (NMR) spin relaxation measurements to study metal complexes in solution. With this background, I joined the Biophysics Department of Dr. Robert G. Shulman at Bell Telephone Laboratories in Murray Hill, New Jersey, where a superconducting high resolution ^{1}H NMR spectrometer operating at 220 MHz was available for 'research on protein structure and function'. At that time I was aware of exactly 10 papers on NMR observations of proteins and nucleic acids, which had all been published during the period of 1957–1965[2]. Prominent figures in the small community of spectroscopists that ventured into direct NMR observation of biological macromolecules were William D. Phillips[3], Oleg Jardetzky[4] and Robert G. Shulman[5]. Based on the observation of empirical correlations between protein unfolding and NMR spectra[2–4], there was much enthusiasm about the future of NMR for *de novo* protein structure determination. Nonetheless, true to my background, I initially focused on the metal ion coordination in the active sites of hemoproteins and on the electronic structure of the heme groups[6].

At the time, Swiss scientists who landed a job at the famous Bell Telephone Laboratories were automatically considered prime candidates for academic positions back home. In 1969, I moved to the Eidgenössiche Technische Hochschule (ETH) in Zürich, where my startup package included an EPR and three NMR spectrometers — all the instrumentation that had been available to me at Bell Telephone Laboratories. I assembled a small research group, and, with time, I was promoted to Professor of Biophysics, which is also my present position at ETH. During the first years at Zürich, my research continued to focus primarily on the metal ions in the active centers of hemoproteins[2–6], and I developed a mild infatuation with polypeptide chains only in connection with the discovery of aromatic ring flipping[2]. My primary research interest changed in 1975, when I took some time to write a monograph on the early years of biomacromolecular NMR[2]. These reflections on the state of the field turned out to have been the starting point for our subsequent work on *de novo* protein structure determination by NMR[7].

Four principal elements are combined in the NMR method for protein structure determination[8,9]: (i) the nuclear Overhauser effect (NOE) as an experimentally accessible NMR parameter in proteins that can yield the information needed for *de novo* global fold determination of a polymer chain; (ii) sequence-specific assignment of the many hundred to several thousand NMR peaks from a protein; (iii) computational tools for the structural interpretation of the NMR data and the evaluation of the resulting molecular structures; and (iv) multidimensional NMR techniques for efficient data collection. During the period 1976–1980, my research group at the ETH Zurich had grown to more than 20 scientists, all of whom made great contributions toward the structure determination method. In particular, I worked with Regula M. Keller, Sidney L. Gordon and Gerhard Wagner on developing techniques to measure NOEs for the collection of conformational constraints in proteins, and with Martin Billeter, Werner Braun and Gerhard Wagner on the sequential resonance assignment strategy and

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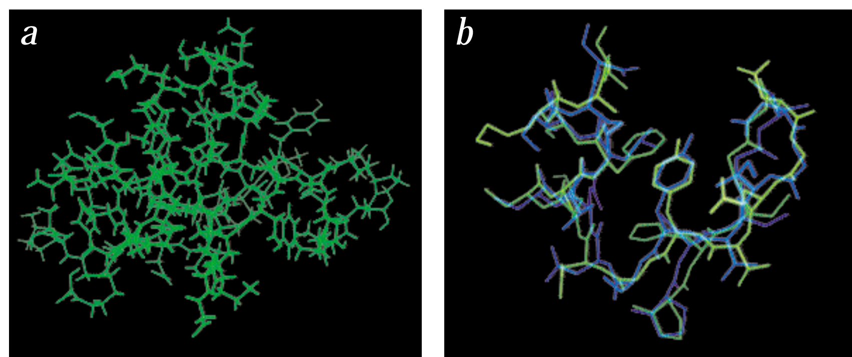

Fig. 1 The first protein structure determined by NMR. ***a***, All heavy-atom presentation of the NMR structure of the proteinase inhibitor IIA from bull seminal plasma (BUSI IIA)[12]. ***b***, Superposition of the core region of residues 23–42 in the NMR structure of BUSI IIA (green) with the corresponding polypeptide segment in the X-ray crystal structure of the homologous porcine pancreatic secretory trypsin inhibitor (PSTI) (blue)[13]. The drawings were prepared from the atomic coordinates obtained in refs 12,13.

algorithms for structure calculation from NMR data. This technology passed its initial tests when we obtained partial structure determinations of the bovine pancreatic trypsin inhibitor (BPTI), cytochrome b_5 and the polypeptide hormone glucagon based on data collection with one-dimensional (1D) NMR experiments.

In a parallel project from 1976 to 1980, Richard R. Ernst (Nobel Prize in Chemistry, 1991), who also worked at the ETH Zürich, and I joined forces to develop two-dimensional (2D) NMR techniques for applications with biological macromolecules. Kuniaki Nagayama used 2D correlation spectroscopy for amino acid spin system identification in a protein, and Anil Kumar recorded the first 2D NOE spectra during the Christmas break 1979, when he was allotted two weeks of the precious measuring time on our highest-field spectrometer operating at a proton NMR frequency of 360 MHz[10]. By 1981 we routinely applied a group of four homonuclear 2D ^{1}H NMR experiments, known under the acronyms COSY, SECSY, FOCSY and NOESY[9], in the protein structure determination project. This resulted in complete resonance assignments of several small proteins in 1982 and 1983[11], and in the first *de novo* atomic resolution NMR structure determination of a globular protein, the bull seminal protease inhibitor (BUSI)[12], by Timothy F. Havel and Michael P. Williamson in 1984.

The completion of the first protein NMR structure brought new, unexpected challenges. When I presented the structure of BUSI (Fig. 1*a*)[12] in some lectures in the spring of 1984, the reaction was one of disbelief, and because of the close coincidence (Fig. 1b) with results from an independent crystallographic study of the homologous protein PSTI (porcine pancreatic secretory trypsin inhibitor)[13] it was suggested that our structure must have been modeled after this crystal structure. In a discussion following a seminar in Munich on May 14, 1984, Robert Huber (Nobel Prize in Chemistry, 1988) proposed that we settle the matter by independently solving a new protein structure by X-ray crystallography and by NMR. For this purpose, each one of us received an ample supply of the α-amylase inhibitor tendamistat from scientists at the Hoechst company. Virtually identical three-dimensional structures of tendamistat were obtained in our laboratory by NMR in solution and in Robert Huber's laboratory by X-ray diffraction in single crystals.

The refined tendamistat structure was published in *Journal of Molecular Biology* as a 50-page report[14], and the addendum to that paper clearly illustrated the impact of structure determination by NMR. I quote: "Editor's Note: We have taken the step of publishing this paper with full supporting data since it is the first high resolution structure worked out in detail by 2D NMR. We therefore think that in this one instance everything should be published in full, but it does not set a precedent, since it is hoped that in the future, such supporting data can be deposited in a data bank, as is the practice in X-ray protein crystallography". Considering that over 2,000 NMR structures have since been deposited in the Protein Data Bank, the Editor should be commended for his vision.

At that time his kind comments were comforting in the context of our structure determinations of mammalian metallothioneins, which are a class of small, metal-rich proteins that we studied in collaboration with Jeremias H.R. Kägi at the University of Zürich. In June 1985 I presented the structure of rabbit metallothionein at Yale University, where I learned about a manuscript accepted for publication in *Proc. Nat. Acad. Sci. USA*, which described a completely different metallothionein 'NMR structure', and at the University of Pittsburgh, where I was confronted with a rat metallothionein crystal structure that was again very different from our NMR structure. In both instances the structural differences were very clearcut, since they involved different polypeptide folds as well as different coordinating ligands to the metals. Metallothionein had been a tough challenge for all of us involved[15], and my initial reaction was to spend two nights on the phone in my US motel room rechecking step by step the sequential resonance assignments with Gerhard Wagner in Zürich. All the assignments were, of course, correct, and I am afraid that Gerhard still bears a grudge against me for ever having doubted his spectral analysis. The crystal structure, which included erroneous chain tracing and identification of 11 out of a total of 20 metal-coordinating amino acid residues, eventually appeared as a feature article in *Science*, whereas *Nature* rejected our NMR structure paper. In 1992, the crystal structure of rat metallothionein was redetermined, a correction of the first structure was published, and the correct crystal structure was found to be identical with the NMR structures of the rabbit, rat and human metallothioneins that we had solved from 1985 to 1990[16].

Over the years a variety of applications of the NMR structure determination method have been pursued in my laboratory. The following three examples may convey some of the excitement that was thus generated in our professional life and further indicates the wide range of NMR applications in structural biology. Studies on the structural foundations of transcriptional regulation in higher organisms pursued in collaboration with Walter J. Gehring at the Biocenter of the University of Basel, Switzerland, yielded the NMR structure of the *Antennapedia* homeodomain[17], and provided entirely novel insights into the role of hydration water in protein–DNA recognition[18]. An NMR structure determination of the human cyclophilin A–cyclosporin A complex was obtained in collaboration with two of my former graduate students, Hans Senn and Hans Widmer, who had subsequently joined the Sandoz company in Basel, Switzerland. This structure determination not only introduced me to the field of

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immune suppression but also had an immediate practical impact on cyclosporin research, since the structure of the bound drug molecule was found to be turned inside-out when compared with the structure of free cyclosporin A[19]. Barely 10 days after the bovine spongiform encephalopathy (BSE) crisis in Great Britain had broken into the open in March 1996, we completed the NMR structure determination of the murine prion protein[20] in a collaboration with Rudi Glockshuber, who had joined our institute at the ETH Zürich as an Assistant Professor in 1994. The observation of a long flexible tail in prion proteins[21] presents on the one hand a striking illustration of the unique power of NMR to characterize partially structured polypeptide chains in physiological milieus, and on the other hand indicates novel possible avenues for the transition of the benign cellular form of prion proteins to the disease-related scrapie form. With the introduction of TROSY (transverse relaxation-optimized spectroscopy)[22], the molecular weight limit for solution NMR spectroscopy has extended to ~500 kDa, and we may soon be able to obtain information on the structure of the disease-related, aggregated form of the prion protein.

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picture story

A force to be reckoned with

Bacteriophage DNA is packaged into protein capsids to near crystalline density. It was originally thought that the DNA was condensed first and the protein shell was built around it, until about 30 years ago when empty phage capsids, or proheads, were found to form first. This discovery presented the difficult question: how does a virus force its DNA into the tiny capsid? For the well-studied *Bacillus subtilis* bacteriophage ϕ29, ~19 kilobases of double stranded DNA (6.6 µm long) must fit into a prohead of 42 x 54 nm. The portal complex, the ATP-dependent protein and RNA motor responsible for this feat, must overcome substantial energetic barriers to package the DNA so tightly, but exactly how this is accomplished is not clear.

As reported in a recent issue of *Nature* (**413**, 748–752; 2001), Bustamante and colleagues use optical tweezers to measure the rates and forces involved in packaging ϕ29 DNA into individual phage heads. The unpackaged end of the DNA is attached to a polystyrene bead, which is held in an optical trap (left). At the other end of the DNA, the partly packaged phage head is attached to another bead and held in place with a pipette. Packaging resumes upon the addition of ATP, and the beads move closer together. The experiment can be done in a 'constant force feedback' mode, keeping a predetermined tension in the DNA by moving the bead position, or the 'no feedback' mode, where the force is allowed to change but the beads are held in place.

The authors show that packaging is highly processive and efficient, with few pauses and slips. Despite this efficiency, the rate of packaging decreases as more DNA is packed into the head (middle), suggesting that pressure builds up inside the capsid. Using the 'no feedback' mode, the authors measured the decreasing rate of packaging as the tension between the tethered ends built up, allowing them to quantitatively estimate the internal force produced by the DNA as it is condensed and packaged (right).

Interestingly, the internal force is quite small until ~50% of the genome is packaged, indicating that the DNA is initially packed fairly loosely, not in its condensed final state. The force then increases, reaching ~50 pN as the entire genome is packaged and making the packaging machinery one of the strongest molecular motors reported. As the authors point out, building up so much internal force may be useful for the phage during infection; the pressure may be used to partially inject the DNA into the host cell.

Julie Hollien