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19.7. Nuclear magnetic resonance (NMR) spectroscopy

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19.7.1. Complementary roles of NMR in solution and X-ray crystallography in structural biology

X-ray diffraction in crystals and NMR in solution can both be used to determine the complete three-dimensional structure of biological macromolecules, and to date a significant number of globular protein structures have been independently determined in crystals and in solution (Billeter, 1992). Particularly detailed comparisons of the two states have been made for the α-amylase inhibitor Tendamistat, which also included solving the crystal structure by molecular replacement with the NMR structure (Braun *et al.*, 1989). The dominant impression is one of near-identity of the molecular architecture in solution and in single crystals, which holds for the polypeptide backbone as well as the core side chains.

Although the presently available results show that there is usually close coincidence between both the global molecular architecture and the detailed arrangement of the molecular core in corresponding X-ray and NMR structures of globular proteins, there is also extensive complementarity in the information that is accessible with the two methods: X-ray diffraction can provide the desired information for big molecules and multimolecular assemblies, whereas NMR structure determination is limited to smaller systems [recently introduced new experiments enable solution NMR measurements for molecular weights of 100000 and beyond (Pervushin *et al.*, 1997; Riek *et al.*, 1999)]. NMR measurements in turn provide quantitative information on both very rapid motions on the subnanosecond timescale (Otting *et al.*, 1991; Peng & Wagner, 1992) and slower dynamic processes (Wüthrich, 1986) which are not manifested in the X-ray data. Examples of low-frequency intramolecular mobility are the ring flips of phenylalanine and tyrosine (Wüthrich & Wagner, 1975), exchange of interior hydration water molecules with the bulk solvent (Otting *et al.*, 1991), and interconversion of disulfide bonds between the *R* and *S* chiral forms (Otting *et al.*, 1993). NMR studies of amide proton exchange and *cis–trans* isomerization of *Xxx*—Pro peptide bonds (Wüthrich, 1976, 1986) afford additional insight into conformational equilibria in the protein core. Finally, in all instances where a biological macromolecule cannot be crystallized, NMR is currently the only method capable of providing a three-dimensional structure.

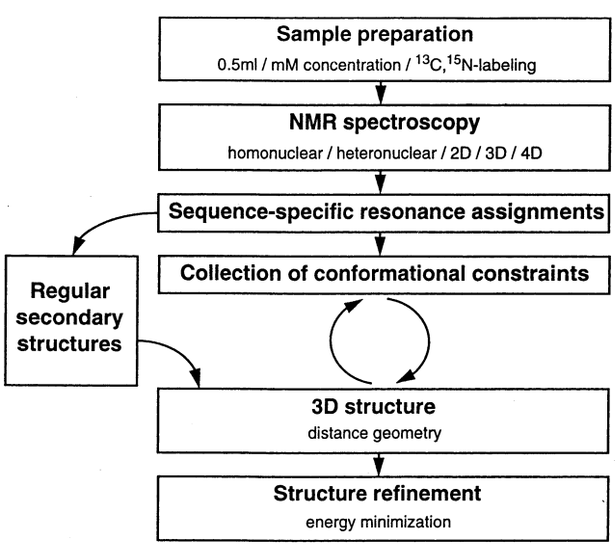

Fig. 19.7.2.1. Diagram outlining the course of a macromolecular structure determination by NMR in solution.

Overall, X-ray crystal structures and NMR solution structures provide qualitatively different information on the molecular surface. In the crystals, a sizeable proportion of surface amino-acid side chains are subject to similar packing constraints in protein–protein interfaces as the interior side chains in the protein core, and therefore they are rather precisely defined by the X-ray diffraction data. In NMR solution structures determined according to a standard protocol (Wüthrich, 1995), the surface is usually largely disordered. Surface disorder in NMR structures may in part arise from scarcity of nuclear Overhauser effect (NOE) distance constraints and packing constraints near the protein surface, but, in turn, scarcity of NOE constraints is often a direct consequence of dynamic disorder. Additional NMR experiments that are not part of a standard structure determination protocol can provide information needed for more detailed characterization of the molecular surface, but care must be exercised in the data analysis because of the presence of a multitude of equilibria between two or multiple transient local conformational states, of which the relative populations are usually not independently known.

19.7.2. A standard protocol for NMR structure determination of proteins and nucleic acids

An NMR structure determination involves sample preparation, NMR measurements, assignment of the NMR lines to individual atoms in the polymer chain, collection of conformational constraints, and structure calculation and refinement, where in present practice the sequence of steps usually corresponds to the flow diagram of Fig. 19.7.2.1. As is also indicated in Fig. 19.7.2.1, it is a special feature of protein structure determination by NMR that the secondary polypeptide structure, including the connections between individual segments of regular secondary structure, may be known early on from the data used for obtaining the resonance assignments, *i.e.* before the structure calculation is even started.

For the sample preparation, homogeneous macromolecular material is dissolved at about 1 m*M* concentration in 0.5 ml of water. The ionic strength, pH and temperature, and possibly the concentration of additives, may then be adjusted, for example, to ensure near-physiological conditions, or denaturing conditions *etc*. The NMR study will often include the preparation of compounds enriched with ^{15}N and/or ^{13}C, and possibly with ^{2}H (Kay & Gardner, 1997). Uniformly isotope-labelled recombinant proteins are routinely obtained by expression in *Escherichia coli* bacteria grown on minimal media. For RNA and DNA, isotope-labelling techniques are more involved, but labelled nucleic acids will also be commonly available in the future. Being able to work with solutions is generally considered to be a great asset of the NMR method, but there are also potential inherent difficulties. For example, in the course of an investigation it may be nontrivial to achieve identical solution conditions in different NMR samples of the same compound, the absence of which typically results in small chemical-shift differences that slow down the combined analysis of different NMR spectra.

The demands on NMR experiments for macromolecular structure determination are currently met by multidimensional NMR at high polarizing magnetic fields (Wüthrich, 1986; Ernst *et al.*, 1987; Cavanagh *et al.*, 1996). With increasing molecular size and concomitant increase of the number of NMR peaks, it becomes more and more difficult to resolve and assign the individual resonances. In heteronuclear three- or four-dimensional (3D or 4D) spectra recorded with compounds that are uniformly labelled with ^{15}N and/or ^{13}C, the peaks are spread out in a third and possibly

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