Solution NMR Spectroscopy

DOI: 10.1002/anie.201107639

First Solution Structures of Seven-Transmembrane Helical Proteins**

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Keywords:
cell-free expression · membrane proteins · NMR spectroscopy · structural biology

Seven-transmembrane (7-TM) helical proteins constitute a biologically important class of membrane proteins. The most prominent members of this class are the G-protein coupled receptors (GPCRs). These proteins are involved in transmitting the signal from the extracellular space into cells, and therefore play a pivotal role in biology. Not astonishingly, about 30% of all drugs target GPCRs. Despite their prime pharmacological importance still little high-resolution structural knowledge is available, although we have witnessed significant progress in this area over the last decade. Following the structure of bovine rhodopsin in 2000[1] a number of other GPCR structures were determined by crystallography, including the β1 and β2 adrenergic receptors, the dopamine D3, the adenosine A_{2A}, the histamine H1 and the chemokine CXCR4 receptors.[2]

NMR spectroscopy does not require single crystals and therefore in principle can deal with proteins that contain flexible moieties. Therefore many expect that this technique significantly contributes to our understanding of these precious receptors. Moreover, NMR spectroscopy in addition allows to study protein dynamics, an aspect that is intimately connected to biological function. However, despite the potential of NMR spectroscopy, only few membrane protein structures have been solved so far. The problems are routed in many technical issues that confound the experiments, deteriorate the quality of spectra, and complicate their analysis.

Technically speaking membrane proteins must be imbedded in a suitable membrane mimetic to enable their study by NMR spectroscopy. Detergent micelles, bicelles, or the so-called minidisks can be used for that purpose.[3] The lipids substantially increase the molecular weight and hence complicate and deteriorate NMR spectra. Previous work on GPCRs using solution NMR techniques indicated that signals from the TM helix segments often remained invisible whereas signals from the loops or termini could often be assigned.[4,5]

Last year the group of Nietlispach reported on the solution structure of sensory rhodopsin.[6,7] Sensory rhodopsin is not coupled to a G-protein but possesses a 7-TM helix bundle architecture reminiscent of GPCRs. This work indicated that structure determination of 7-TM helix membrane proteins by NMR spectroscopy is possible, and in quality similar to X-ray crystallography.

Recently, Dötsch et al. reported the solution structure of proteorhodopsin.[8] Proteorhodopsin, only discovered in 2000, represents the photoreceptor in marine bacterioplanktons and eukaryotes, and is homologous to bacteriorhodopsin. Both proteins act as light-driven proton pumps. Surprisingly, no X-ray coordinates are available for proteorhodopsin despite considerable efforts. The new structure represents a very significant achievement in the field of structural membrane protein biology. Almost complete assignments for backbone and for a large fraction of side-chain resonances were obtained. Additional photolysis experiments were performed to show that the protein is in a biologically relevant state. The overall fold is in agreement with expectations from homologous proteins. Interestingly, the first extracellular loop connecting the second and third TM helix is devoid of secondary structure whereas a short antiparallel beta strand is observed in bacteriorhodopsin, sensory rhodopsin, and xanthorhodopsin underlining the importance of structural information even in the presence of such information from homologues.

This work likely presents the effort of many researchers over a substantial amount of time. So, why are solution structures of these molecules so difficult to obtain? First of all, expression and purification of helical membrane proteins is cumbersome and yields are usually very low.[3,9] In addition, solution NMR studies require a number of different samples using different flavors of isotope labeling. The necessity for ^{2}H, ^{13}C and ^{15}N labeling essentially precludes the use of insect cells that have successfully been exploited as expression hosts for GPCRs by the crystallography community. High levels of deuteration in vivo are only possible in *E. coli*[10] and *Pichia Pastoris*[11] hosts. In this work, however, cell-free expression (CFE) methods were utilized that allow the rapid in vitro expression of constructs bypassing issues of, for example, cell toxicity and overloading of the translocon system commonly encountered in vivo.[12] In addition CFE methods permit incorporation of specially labeled amino acids without isotope

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[**] Dr. Reto Walser is acknowledged for valuable comments on this manuscript.

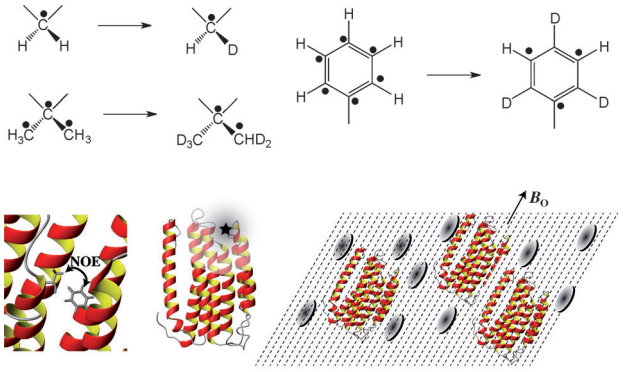

Figure 1. Top: Modifications of labeling according to the SAIL method. Black dots indicate locations of ^{13}C labels. Bottom: Type of restraints used: NOEs (left), PREs (middle), and RDCs (right)

scrambling or metabolic changes. The latter is compatible with SAIL labeling,[13] a recently introduced method that utilizes stereoselectively chemically synthesized amino acids, in which protons of one of the two prochiral methyl groups in Val or Leu residues, one of the two methylene protons, or most of the ring protons from aromatic moieties are replaced by deuterium (Figure 1). Thereby, the content of protons is substantially lowered, resulting in reduced transverse relaxation rates and hence narrower lines. Moreover, stereospecific assignments become possible improving precision of the computed structures. In the case of proteorhodopsin restraints derived from classical nuclear Overhauser effects (NOEs) were augmented by distance restraints derived from paramagnetic relaxation enhancements (PREs)[14] using site-specifically introduced paramagnetic tags, and from residual dipolar couplings (RDCs).[15]

The combined usage of these methods enabled solving this challenging structure. Nevertheless, these methods have been known for some time now, and therefore it is fair to ask why this is only the second structure of a 7-TM protein that is known presently? The reason is certainly not a lack of efforts in this field. Rather, the specific nature of these proteins complicates their spectroscopic study. First the particular mode by which these proteins are activated requires substantial flexibility of the polypeptide chain. This feature may broaden signals beyond detection through conformational exchange, a feature that was also observed for resonances from loop residues in this case. Many NMR groups working in that field have noticed this frustrating behavior. Second, our knowledge on membrane protein–lipid (or detergent) interactions is limited, and third the substantial difficulties for obtaining sufficient amounts of the protein for screening various detergents certainly does not make this task easier. The fact that solution structures were determined for sensory rhodopsin previously and now for proteorhodopsin is related to some extent to the fact that these two proteins are better behaved than true GPCRs. In crystallographic studies GPCRs required substantial modifications to enable crystallization, for example replacing the rather long and flexible third cytosolic loop by T4 lysozyme, stabilization by antibodies, or thermostabilization through extensive mutagenesis.[16] It is often claimed that NMR spectroscopy can handle proteins that contain flexible parts and therefore are not directly amenable to crystallization. Unfortunately, this is not always the case, and often those proteins that readily crystallize result in good-quality NMR spectra and those that don't frequently also display low-quality spectra. The structures of sensory rhodopsin and proteorhodopsin convincingly show that NMR spectroscopy is capable of delivering structures, though significant effort and expertise are required in NMR data acquisition and interpretation, once spectra of sufficient quality have been obtained. Using mutants, in which the ground or activated state have been particularly stabilized either through mutations or through binding of a ligand may therefore present an avenue to circumvent the issues associated with conformational exchange, and allow to also obtain solution data for true GPCRs.

The work of Reckel et al. nicely shows what can be achieved by solution NMR methods in the field of helical membrane proteins. If we could improve our understanding of how to modify the systems so that spectra of comparable quality can be recorded we are potentially looking into a bright future for NMR spectroscopy in this area of structural biology.

Received: October 30, 2011
Published online: December 16, 2011

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