



Biophysics Interdepartmental Group

PhD. Thesis Defence

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“SOLID STATE NMR STUDY OF PROTEORHODOPSIN IN THE LIPID ENVIRONMENT: SPECTRAL ASSIGNMENTS AND STRUCTURAL INSIGHTS”

ABSTRACT

Proteorhodopsin (PR) is a recently discovered ubiquitous, eubacterial retinal-binding light-driven proton pump. Almost a thousand PR variants are widely distributed in species of marine and freshwater bacteria, suggesting its important photobiological role. PR is a typical seven-transmembrane (7TM) α -helical membrane protein and as such, poses a significant challenge to structural studies. Attempts to crystallize PR have not been successful, and its three-dimensional structure remains unknown. We show that PR reconstituted in lipids gives well-resolved magic-angle spinning (MAS) NMR spectra of high signal-to-noise ratio. We have achieved sequential assignment of ^{13}C and ^{15}N backbone and side chain chemical shifts by three-dimensional chemical shift correlation experiments. The first set of experiments performed on FLY (Phe, Leu and Tyr reversely labelled) and WHYIF (Trp, His, Tyr, Ile and Phe reversely labeled) samples has lead to assignments of 103 out of the 238 PR residues, the second set of experiments performed on the UCN (Uniformly- ^{13}C , ^{15}N labeled) sample has not only confirmed and corrected the assignment results obtained on the FLY and WHYIF samples, but also gave additional assignments for 50 more residues. Last, experiments on PR grown on 1,3- ^{13}C glycerol and 2- ^{13}C glycerol yielded assignments for another 8 residues. At the completion of this thesis, the assignments for PR total 161 residues or 795 atoms, with particularly high density in the transmembrane regions (~80% of residues).

The extent of assignments permitted a detailed examination of the secondary structure and dynamics in PR. The chemical shift analysis gives a number of important structural insights not available from other studies: we have established protonation states of several carboxylic acids, identified the boundaries and distortions of transmembrane α -helices, and detected secondary structure elements in the loops. Finally, we present experimental evidence of mobility of the protein's termini and of the A-B, C-D, and F-G loops, the latter being possibly coupled to the PR ion-transporting function.

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