In Situ Structural Studies of *Anabaena Sensory Rhodopsin* in the *E.coli* Inner Membrane

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Introduction

Membrane proteins comprise approximately a third of cellular proteins and perform many vital functions. Interaction with the membrane environment, e.g., lipids, other proteins, etc., is one of the important factors which often influences both the conformation and function of membrane proteins [1,2]. While the lipid bilayer represents a good experimentally tractable model for structural and functional studies of membrane proteins, the natural cellular membrane includes a diverse array of lipids and other transmembrane (TM) proteins, and is invariably more complex. Here, we applied 2D and 3D solid-state NMR to characterize the conformation of a 7TM microbial photosensor *Anabaena* Sensory Rhodopsin (ASR) in the inner membrane of *E. coli*.

Experimental

The 900 MHz solid state NMR spectrometer equipped with a low-E $^{1}H/^{13}C/^{15}N$ MAS probe at the NHMFL was used to perform 3D NCACB and CANCO experiments on a uniformly $^{15}N,^{13}C$-labeled samples of ASR reconstituted in *E.coli* membrane (IM-ASR). Spectra have been processed with NMRpipe and analyzed using CARA software.

Results and Discussion

We have found that ASR is organized into trimers in the *E.coli* membrane, similar to those found in the proteoliposome environment (PL-ASR) [3,4]. Site-specific analysis of chemical shifts for approximately 40% of residues reveals that the overall structure of ASR is largely conserved in the *E. coli* membrane, with many of the important structural features of rhodopsins remaining intact. Specifically, the retinal binding pocket and the trimeric interface look very similar in both environments (Fig. 1). Subtle changes corresponding to the chemical shift changes of 0.5 ppm and 1 ppm in ^{13}C and ^{15}N dimensions, respectively, are mostly detected on the faces of helices facing the interior of a monomer or the bilayer (Fig 1).

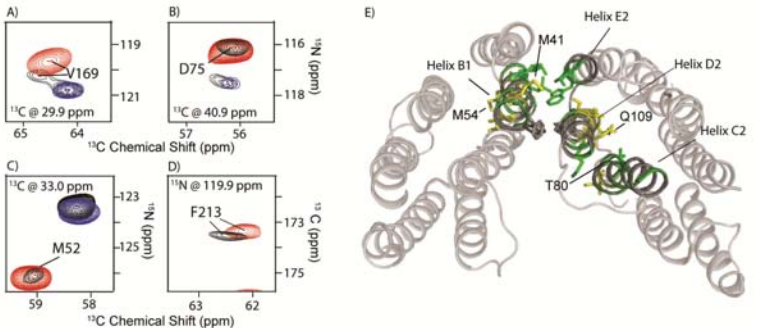

Fig 1. 2D planes of **A-C)** NCACB and **D)** CANCO experiments performed on PL-ASR (red), IM-ASR (black), and on a control *E.coli* membrane sample without ASR (IM-NIC, blue). PL-ASR and IM-NIC spectra were recorded on a 800 MHz spectrometer at Guelph, while IM-ASR spectra were recorded on the 900 MHz spectrometer at NHMFL. **E)** Extracellular view of the intermonomer interface. Green and yellow are unperturbed and perturbed residues, respectively.

Conclusions

SSNMR can be used to detect small, site-specific perturbations in large proteins which occur as a result of the membrane environment changes. Our results indicate that ASR subtly adapts to its environment without large structural rearrangement.

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References

[1] Andersen, O.S., *et al.*, Annu. Rev. Biophys. Biomol. Struct., **36**, 107–30 (2007).
[2] Lee, A.G., Biochim. Biophys. Acta - Biomembr., **1666**, 62–87 (2004).
[3] Wang, S., *et al.*, J. Am. Chem. Soc., **17**, 16995-16998 (2012).
[4] Wang, S., *et al.*, Nature Methods, **10**, 1007-1012 (2013).