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High-field CW electron paramagnetic resonance spectroscopy with Gd(III) tags for structure-dynamics studies of proteorhodopsin[1]
JESSICA A. CLAYTON, CHUNG-TA HAN, C. BLAKE WILSON, University of California, Santa Barbara, MIAN QI, ADELHEID GODT, Bielefeld University, DANIELLA GOLDFARB, Weizmann Institute of Science, MARK S. SHERWIN, SONGI HAN, University of California, Santa Barbara — Proteorhodopsin (PR) is a seven-helical transmembrane protein that functions as a light-activated proton pump. Much of the structure of PR has been mapped by solution-state NMR and X-ray crystallography, however it remains difficult to study protein associations and conformational changes. Here we report development of 240 GHz CW electron paramagnetic resonance (EPR) as a tool to determine inter- and intra-protein distances in the range of 1 4 nm under biologically relevant conditions, using S = 7/2 Gd(III)-based complexes as an EPR-active paramagnetic tag. The dipolar coupling between Gd(III) pairs is determined via the width of the central transition in the CW EPR spectrum, allowing for the inference of an interspin distance. Proof-of-principle experiments are demonstrated on Gd-ruler molecules, from cryogenic temperatures up to room temperature. First results applying this method to inter-protein measurement of Gd(III) tagged PR oligomers reveals distances consistent with the penta- or hexameric organization determined by crystal structure. Finally, we present progress towards development of measurement methods that will enable observation of light-induced conformational changes in the EF-loop region of PR at temperatures above the protein dynamical transition.

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