Supplementary Information

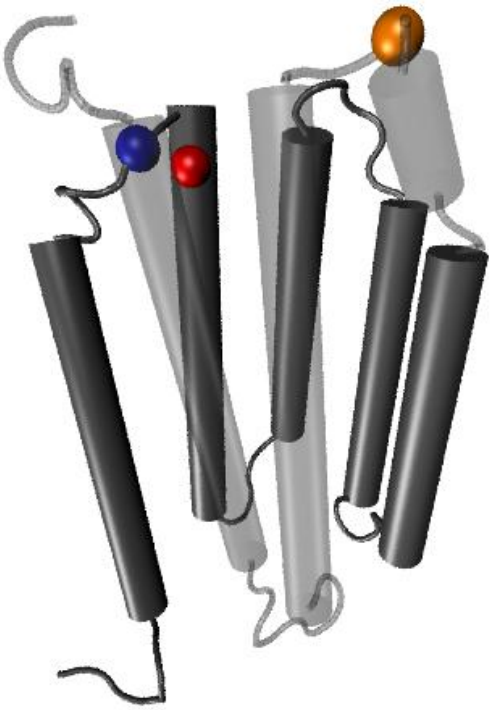

Figure S1. Althernate view of PR. The F and G helices are made transparent in order to more clearly show the positions of the residues 55 (blue), 58 (red) and 177 (orange). The NMR structure from reference 47 was used to make this representation.

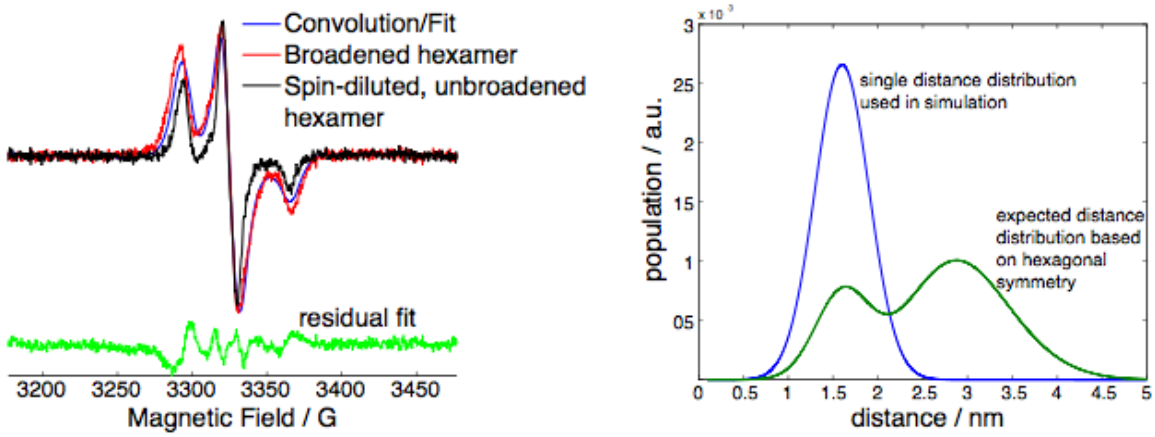

Figure S2. (A) The fully labeled hexameric complex (red),the spin-diluted hexamer with on average only one spin labeled PR per complex (black), and the resulting convolution of the spin-diluted hexamer with a distance centered at 16 Å and a Gaussian distance width, σ, of 3 Å. The difference between the convolution and the broadened hexamer is shown in the residual fit (green). (B) Single distance distribution used to calculate the convoluted spectrum (blue) and the distance distribution expected from the symmetry of

the hexagonal complex with all possible distances of 16, 27.2 and 32 Å and σ of 3 Å. The spin-spin interactions at distances 27.2 and 32 Å do not (or minimally) influence the dipolar broadened hexameric spectrum as they are outside of the measurable range for CW ESR.

Determining MW of a protein/detergent complex by SEC-LS/UV/RI

The mathematical basis for determining the subunit stoichiometries of trans-membrane proteins in a detergent solution is described in detail by Slotboom et al (14). Briefly, the molecular weight of the protein can be measured using the following relationship:

$$MW,_{protein} = \frac{LS}{K\left(\frac{dn}{dc}\right)^2_{apparent} c_{protein}} \quad (1)$$

where LS is the signal from the light scattering detector, K is a constant, $c_{protein}$ is the concentration of the protein in mg/mL, and $(dn/dc)_{apparent}$ is the apparent refractive index (RI) increment which describes the change in the refractive index of the solution as the concentration of the protein changes. $(dn/dc)_{apparent}$ includes contributions to the RI by both the protein and the detergent, and can be written as:

$$\left(\frac{dn}{dc}\right)_{apparent} = \left(\frac{dn}{dc}\right)_{protein} + \delta\left(\frac{dn}{dc}\right)_{detergent}$$

(2)

where δ is the amount of detergent in g/g bound to the protein. As is the case for proteorhodopsin, typically the value for δ is unknown. However, since the DDM detergent does not absorb light at 280 nm, $(dn/dc)_{apparent}$ can be directly determined by the relationship:

$$\left(\frac{dn}{dc}\right)_{apparent} = \frac{RI}{c_{protein}} = \frac{\varepsilon_{280,protein} * RI}{UV_{280}} \quad (3)$$

where $\varepsilon_{280,\ protein}$ is the molar extinction coefficient of the protein and UV_{280} is the light absorbed by the protein at 280 nm. Now eq. 3 can be inserted into eq. 1, and the molecular weight of the protein is expressed in terms of measureable signals from the three detectors, LS, RI and UV:

$$MW,_{protein} = \frac{LS * UV_{280}}{K * \varepsilon_{280,protein} * RI^2}$$

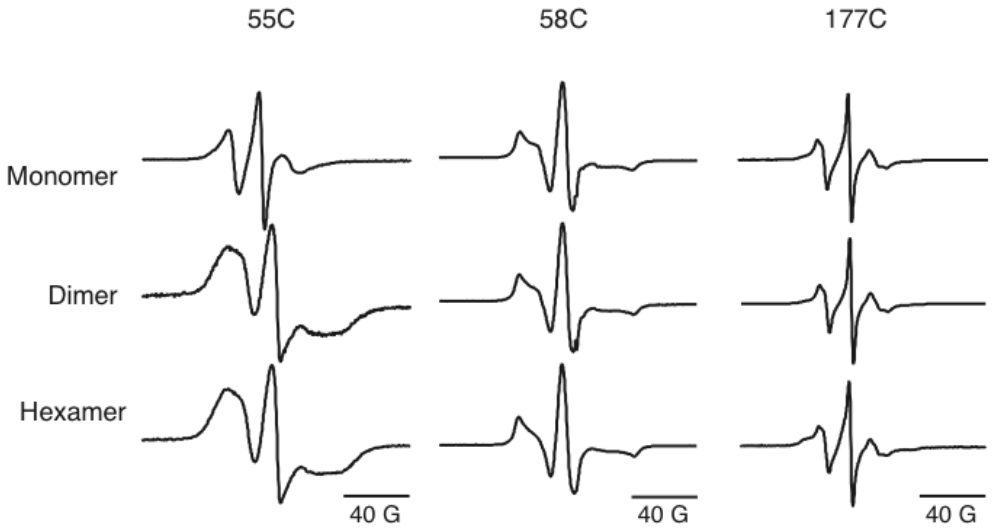

Figure S3. Room temperature CW spectra of hexameric, dimeric and monomeric fractions of 55R1, 58R1, and 177R1 in 1.0% DDM. Raising the detergent concentration did not affect the dipolar broadening of 55R1. The 58R1 spectra of the monomer, dimer and hexamer all have similar lineshapes in 1.0% DDM.