Supporting Information

Cell-Free Expressed Bacteriorhodopsin in Different Soluble Membrane Mimetics: Biophysical Properties and NMR Accessibility

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- Figure S3: Additional (NMR) data recorded on bR in an amphipol and nanodiscs environment. This figure refers to Figure 3.
- Figure S4: Residue-specific analysis of chemical shift perturbations of bR between the different environments. This figure refers to Figure 4.

Supplemental Figures

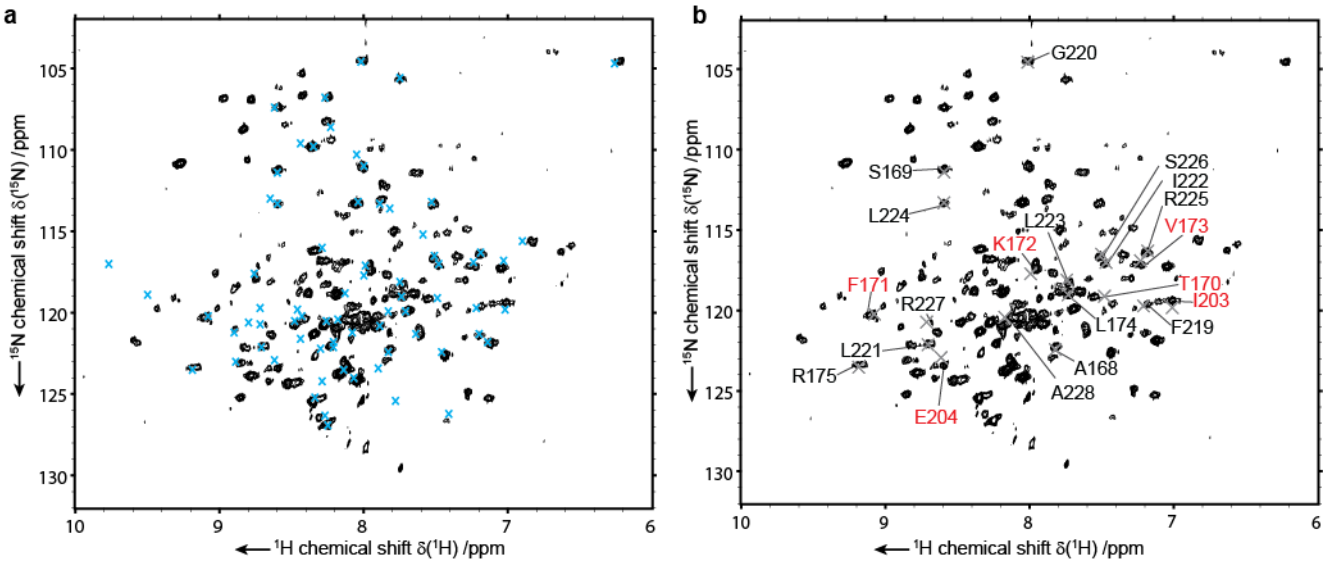

Figure S1: Comparison of cell-free expressed bR (cf-bR) and bR extracted from purple membranes (pm-bR). In a) all resonance assignments reported for pm-bR (Schubert et al., 2002) are overlaid on a TROSY-HSQC spectrum recorded on cf-bR. Note that the data needed to be slightly shifted to compensate for differences in chemical shift calibration and that peaks missing in cf-bR in a) can be explained by different isotope labeling patterns. In b) only assigned residues which are at least one helix turn within the TM helices are shown. Residues with the largest chemical shift perturbations ($|\Delta(\delta(^{1}H))|$ * 10 + $|\Delta(\delta(^{15}N)|$ > 0.4 ppm) are highlighted in red in b) and Figure 1. All other assigned residues labeled in b) are highlighted in blue in Figure 1.

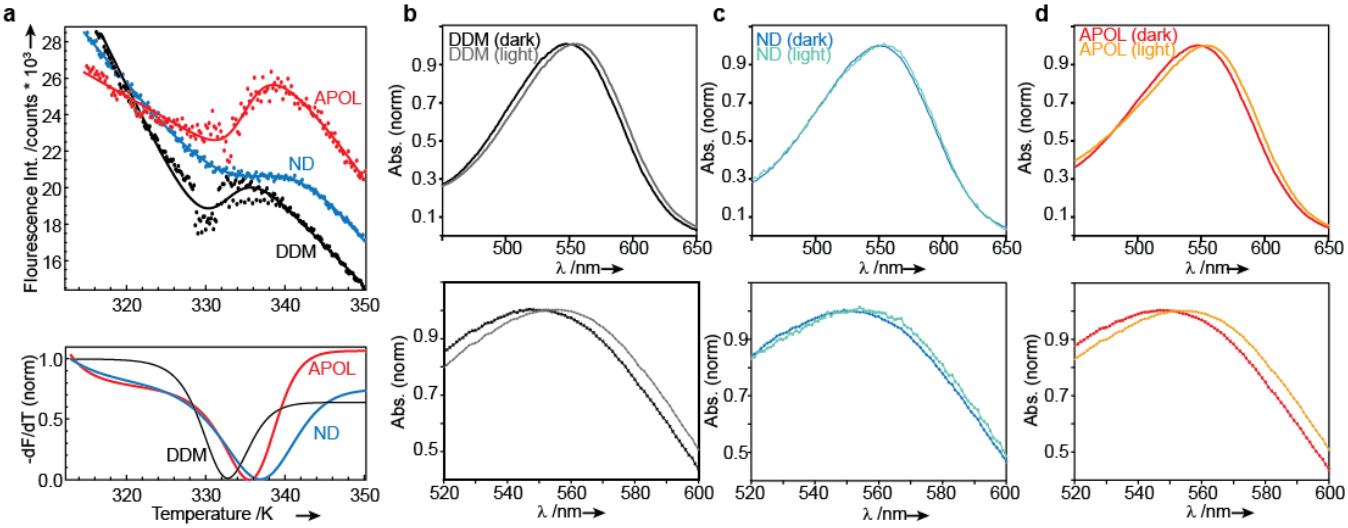

Figure S2: Thermal denaturation and absorption profiles of dark- and light-adapted bR in the different environments. (a) Experimental data of thermal denaturation and respective data fits are shown. Data fitting and first derivatives (lower part) were calculated using Mathematica (Wolfram Scientific). (b-d) Absorption profiles of dark- and light-adapted bR in the indicated

environments. A shift of the absorption maximum can be identified in all environments. However, the shift difference is significantly smaller for bR-ND than for bR-DDM and bR-APOL. Lower panels show magnification of peak maxima. The measurements were recorded at pH 7.3 after 48 h in the dark or 20 min illumination, respectively. Illumination was carried out using an incandescent light source and continued during data acquisition. This figure provides additional data for Figure 2.

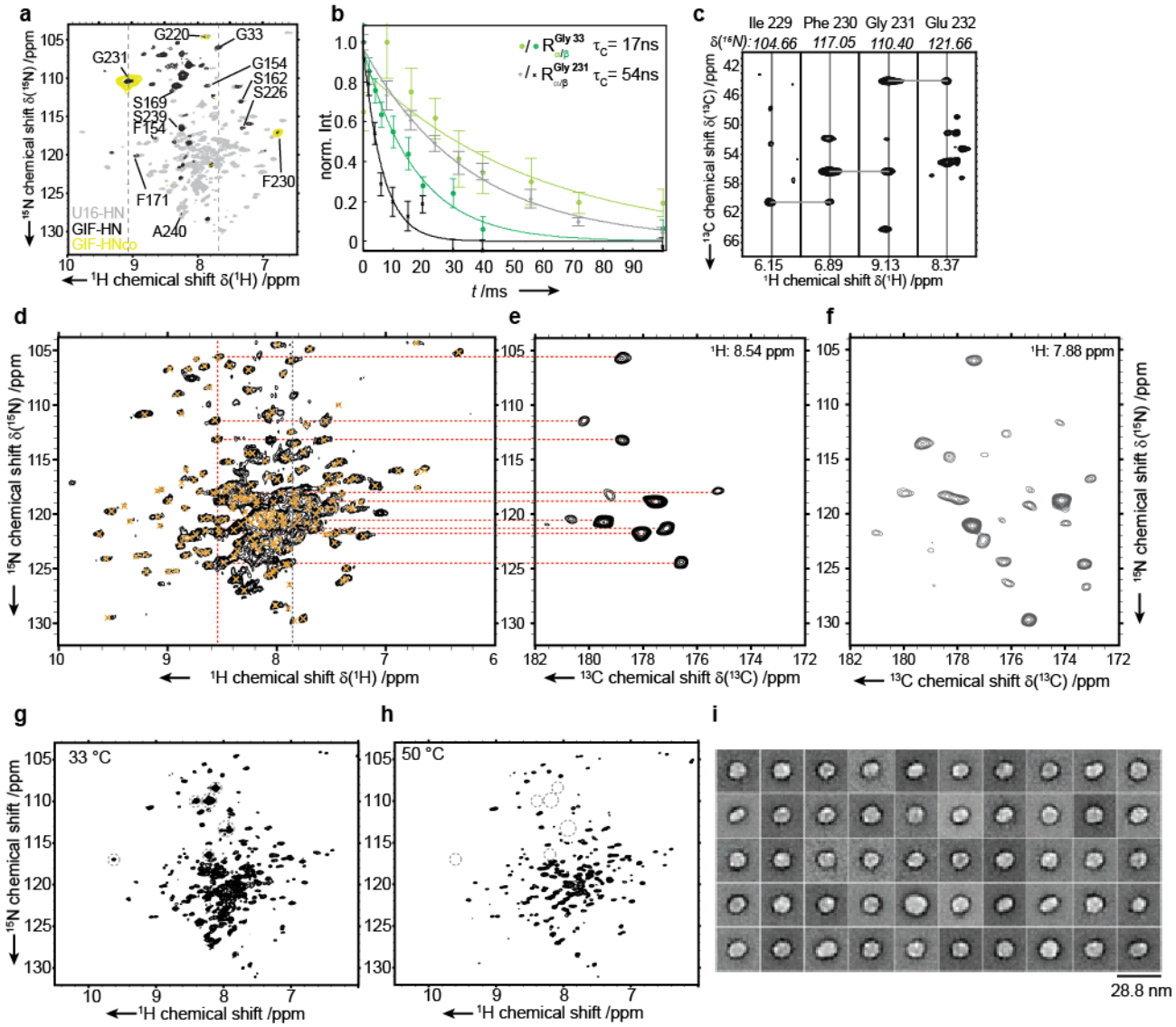

Figure S3: Additional (NMR) data recorded on bR in an amphipol (a-c) and nanodiscs (d-i) environment. a) TROSY-HSQC spectra of GIF-bR-APOL (black) (see Experimental Procedures for details on labeling) on top of ALGAL-bR-APOL (grey). Additionally a 2D TROSY-HNco spectrum of GIF-bR-APOL is shown (yellow). Dotted vertical lines indicate regions used to analyse residue-specific rotational correlation times in GIF-bR-APOL using a conventional TRACT analysis (b). c) Strip plot of 3D TROSY-HNCA spectrum recorded on ALGAL-bR-APOL. The same region as shown in Figure 3g-i) for ALGAL-bR-ND is shown. Note that the spectra in a) confirm the assignments shown in c).

d) 2D TROSY-HSQC spectrum of bR-ND. Orange crosses indicate resolved peaks identified in a 3D TROSY-HNCO spectrum. e) Example of a NCO plane (both indirect dimensions) in the 3D HNCO data set at a proton frequency of 8.54 ppm (red dotted line in d). Mostly residues with helical secondary chemical shifts are found. f) Same as e) but at a proton frequency of

7.88 ppm (black dotted line in a). The data were recorded at 48°C and show on the one hand that the transmembrane region of bR-ND is accessible by solution-state NMR and on the other that even in crowded regions of the 2D HSQC data most peaks are well resolved in the 3D HNCO. Taking into account the occurrence of proline residues and the applied isotope labeling 211 cross peaks would be expected in the 3D HNCO. In total 195 (i.e. 92%) resolved peaks could be observed in the spectrum. However, factors such as peak splitting (which is expected for some residues) as well as reduced signal intensity due to proton exchange between water and residues in unstructured parts (which is observed for several residues in the protein termini (g) and h)), may decrease or increase the ratio of expected to observed peaks, respectively. g) and h) show 2D TROSY-HSQC spectra of bR-ND at 33°C (g) and 50°C (h). While the spectral resolution significantly increases with temperature, several peaks (highlighted with dotted circles) disappear at higher temperature, most likely due to chemical exchange with water (pH was set to 7.3). Overall we hence estimate that about 90% +/- 10% of all expected peaks are observed and resolved in the 3D HNCO spectrum. i) EM class average of negative stained bR-ND (fraction B in Figure 3a). The full set of 50 classes is shown. The figure provides additional experimental data supplementing Figure 3.

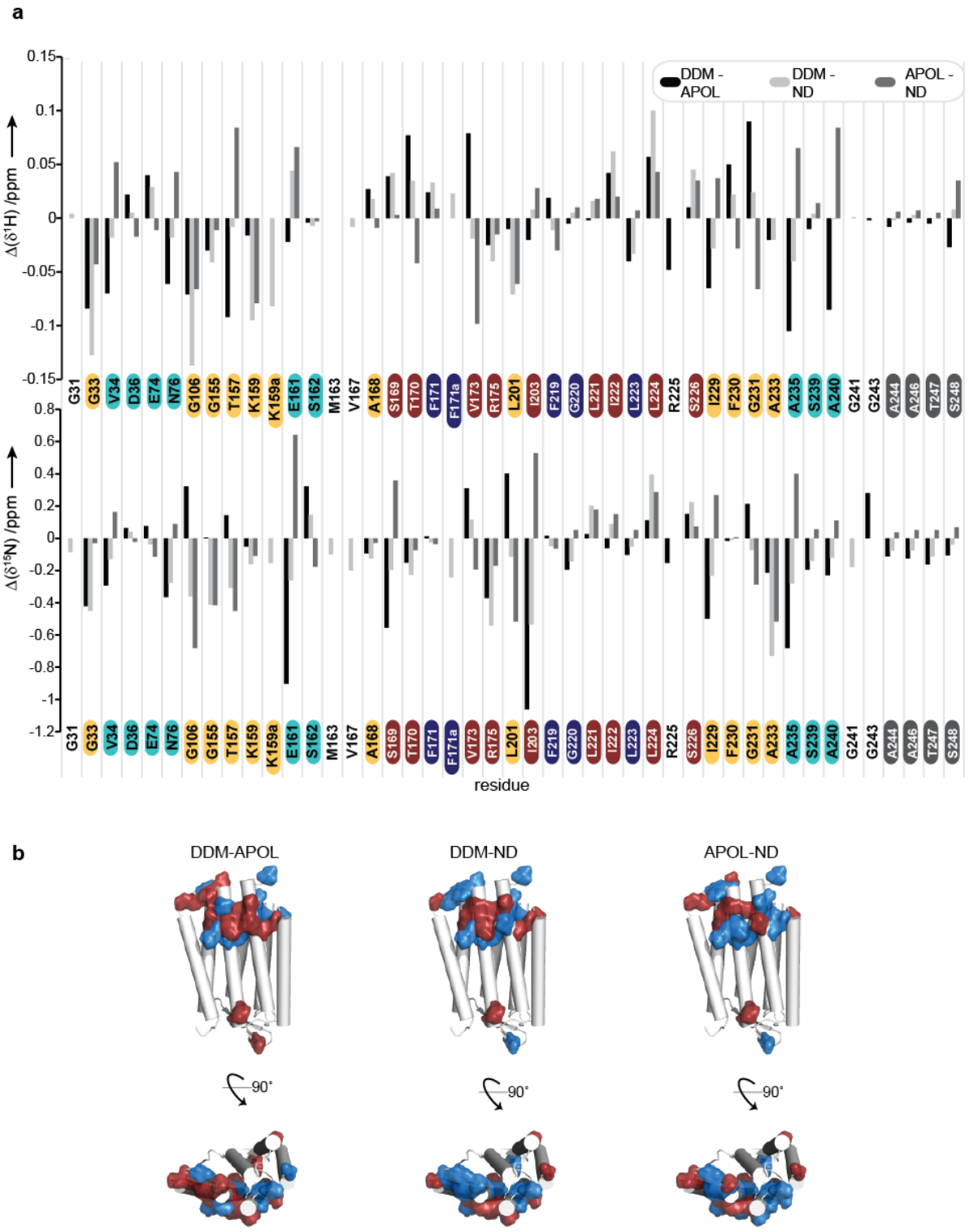

Figure S4: Residue-specific analysis of chemical shift perturbations of bR between the different environments. (a) Plot of pairwise (environment 1 - environment 2) chemical shift perturbations for the indicated 3 possibilities (upper part ^{1}H chemical shift differences, lower part ^{15}N chemical shift difference). Residues coloring indicate classification as given in Figure 5e. Residues were classified using the following conditions: Residues within the transmembrane core (i.e. at least 3 residues

from the end of the helices) with side chains facing the protein core or the surfactant were assigned to the TM – in or TM – out class, respectively. TM – loop residues are found within +/- 3 residues from the end of the helices, loop residues are > 4 residues apart from the end of a helix. The last five residues of bR belong to the C-terminal class. Only residues for which chemical shift assignments in all three environments could be obtained were considered. Thus residues without classification in a) could only be assigned in 2 environments. (b) Pairwise shift perturbations are mapped on the bR x-ray structure (Luecke et al., 1999) (blue = no/small differences, red = larger differences ($|\Delta(\delta(^{1}H))| * 10 + |\Delta(\delta(^{15}N)| > 0.4$ ppm).

Supplemental References

Luecke, H., Schobert, B., Richter, H.T., Cartailler, J.P., and Lanyi, J.K. (1999). Structure of bacteriorhodopsin at 1.55 A resolution. J Mol Biol *291*, 899-911.

Schubert, M., Kolbe, M., Kessler, B., Oesterhelt, D., and Schmieder, P. (2002). Heteronuclear multidimensional NMR spectroscopy of solubilized membrane proteins: resonance assignment of native bacteriorhodopsin. ChemBioChem *3*, 1019-1023.