Electric Supplementary Information for

Doubly spin-labeled nanodiscs to improve structural determination of membrane proteins by ESR

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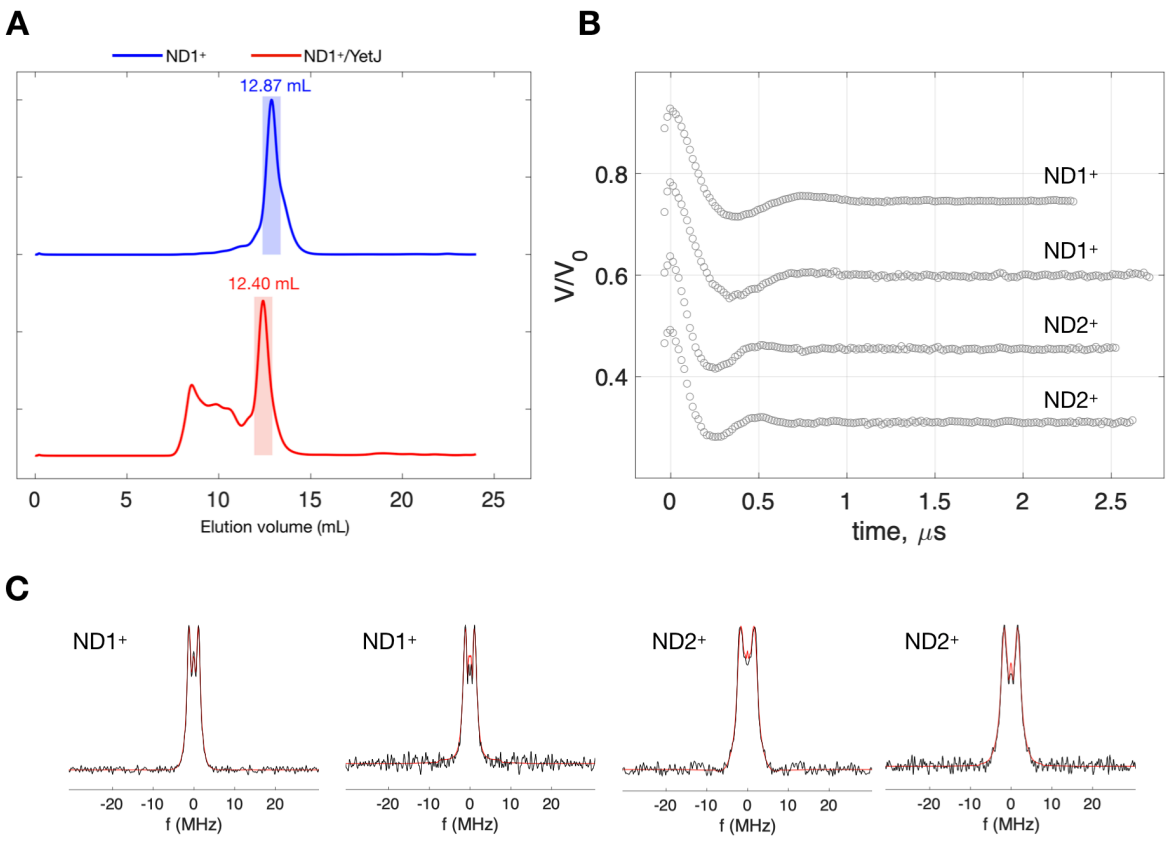

Figure S1. Sample preparation and reproducibility. (**A**) Representative purification results for empty ND1$^+$ and ND$^+$/YetJ samples by size exclusion chromatography (SEC-FPLC). The homogeneity, as highlighted by shaded regions, is better for ND$^+$/YetJ than ND1$^+$ owing to the passage through nickel column after nanodisc formation. The shaded areas also indicate the fractions collected for DEER measurements. (**B**) Representative background-corrected DEER traces of the samples ND1$^+$ and ND2$^+$ at pH 7. It shows that these ND$^+$ samples can be reliably reproduced with the protocol reported in this study such that the experimental DEER time-domain traces are reproducible in replicate experiments. Same average distances are obtained by TIKR for the replicate measurements of ND1$^+$ or ND2$^+$. Pake doublet plots of these replicate measurements are shown in (**C**), confirming that the same dipolar frequency in the Pake doublets for ND1$^+$ (or ND2$^+$) is obtained in the replicate measurements. The Pake doublets were generated using DeerAnalysis.

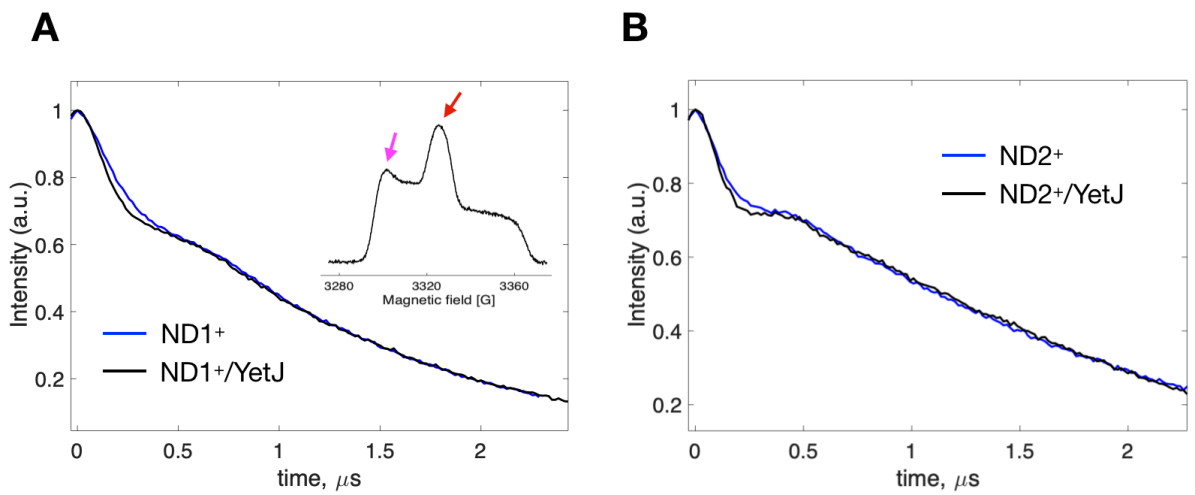

Figure S2. Sensitivity of spin-labeled ND to the change in ND geometry. (**A**) A comparison of the DEER experimental data of ND1$^+$ with/without the incorporated YetJ. For the sake of a clear comparison, the raw data are plotted with the modulation-depth scaling using the DeerAnalysis program. The subtle differences in the DEER traces between the ND1$^+$ and the ND1$^+$/YetJ are clearly revealed, demonstrating the high sensitivity of ND1$^+$ to a change in the ND geometry. Specifically, the time-domain signal for ND1$^+$/YetJ decays distinctly faster than that for ND1$^+$, supporting a shorter interspin distance in the former. Inset: Hahn echo-detected field swept spectrum of ND1$^+$ and the positions of the pump and observe pulses (red and magenta arrows, respectively). The comparison of the DEER experimental data of ND2$^+$ with/without the incorporated YetJ is provided in (**B**), which leads to the same conclusion about the high sensitivity of doubly spin-labeled ND.

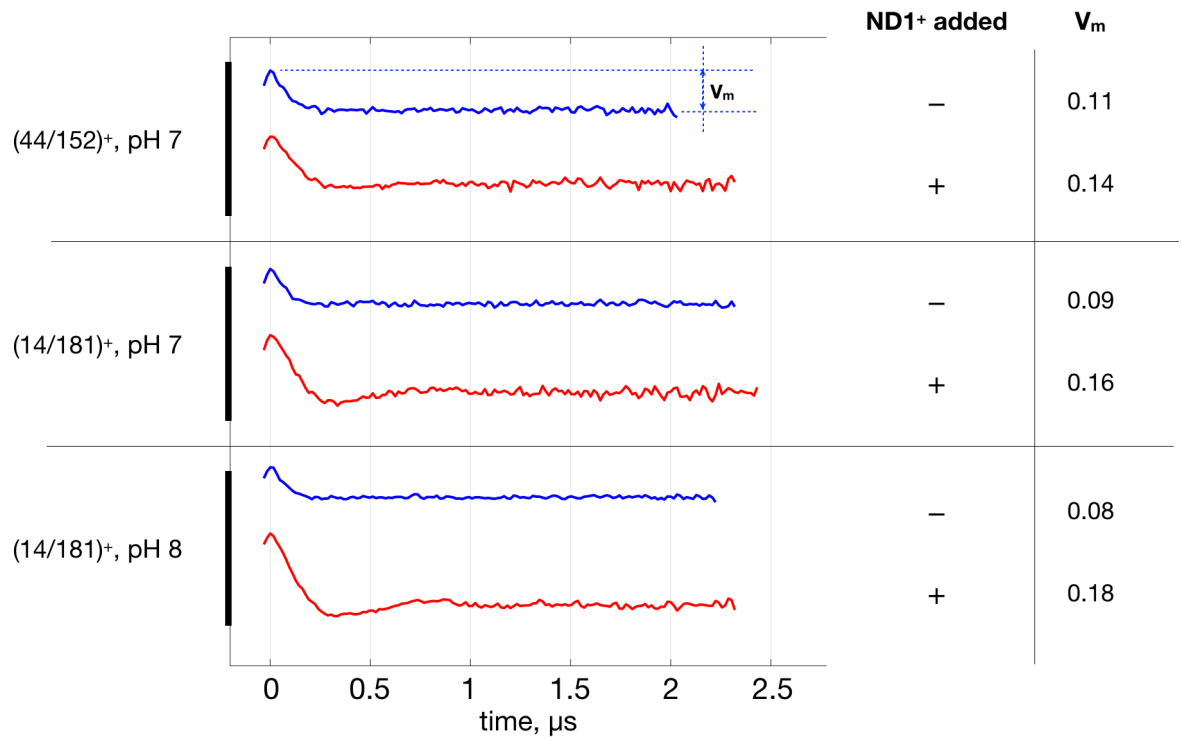

Figure S3. DEER time-domain traces after the removal of background signals. Data are displayed without normalization to the same magnitude so as to emphasize that when ND1$^+$ are present in the YetJ$^+$ (e.g., (44/152)$^+$ or (14/181)$^+$) solutions, the overall dipolar modulation depth (V_m, as illustrated) is more clearly revealed in the background-corrected DEER traces. All samples shown have the same spin concentration, as described in Experimental. The presence of ND$^+$ samples, whose spin-labeling locations are more solvent-exposed, is useful to enhance the overall modulation depth of DEER signals and thus effectively improve the SNR of DEER data. Note that as the SNR of DEER data is defined by a ratio of the peak intensity to the root-mean-square of noise, it is directly proportional to the peak intensity (i.e., V_m) of the background-corrected data when all of the experimental data have a similar noise level. As shown, we observed an improvement of SNR in the range of 27% and 125%. With the SNR improvement, it directly leads to an enhancement in the distance resolution obtainable by TIKR, hence a distinct reduction in the distance distribution width as compared to the pure YetJ$^+$ results (Figs. 3 and 4).