Biophysical Journal, Volume *123*

Supplemental information

Dark nanodiscs for evaluating membrane protein thermostability by differential scanning fluorimetry

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Supplemental Materials for Dark nanodiscs for evaluating membrane protein thermostability by differential scanning fluorimetry

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Table S1

Sample	[free thiol]	free thiol (%)
DsbB – DPC micelle	2.15 +/- 0.29 μM	2.25 %
DsbB – dark nanodisc	1.52 +/- 0.19 μM	1.59 %

Table S1. Quantification of free thiol content for DsbB in DPC micelles compared to dark nanodiscs. The Measure-IT™ Thiol Assay Kit was utilized to quantify the free thiol content of DsbB in micelles versus DsbB reconstituted in dark nanodiscs. Each sample was run in triplicate (n=3) at a DsbB protein concentration of 1 mg/mL. The dark membrane scaffold protein does not contribute to the thiol measurement because there is no cysteine content in the dark nanodisc construct.

Figure S1

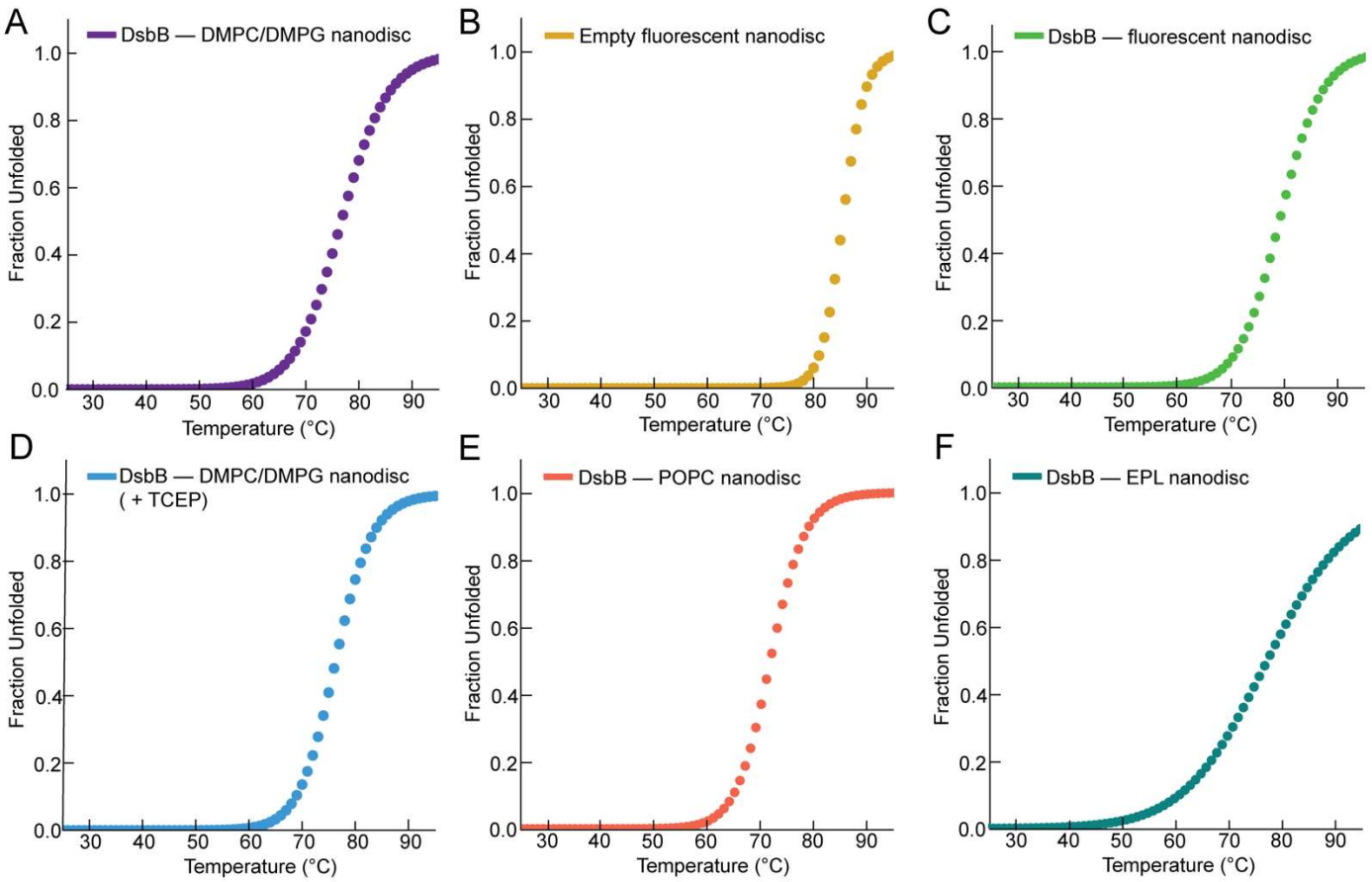

Figure S1. Fraction unfolded plotted as a function of temperature for the nanodisc samples evaluated in this study. For visualization, baseline-corrected experimental curves were calculated from the rate constant of the unfolding transition, the rate constant of the baseline transition, baseline noise, and baseline offset. Fraction unfolded plots are shown for (**A**) DsbB in a dark nanodisc (DMPC/DMPG), (**B**) empty fluorescent nanodisc (DMPC/DMPG), (**C**) DsbB in an MSP1D1 fluorescent nanodisc (DMPC/DMPG), (**D**), DsbB in a dark nanodisc (DMPC/DMPG) under reducing conditions. (**E**) DsbB in a dark nanodisc (POPC), (**F**) DsbB in a dark nanodisc (*E. coli* Polar Lipid Extract).

Figure S2

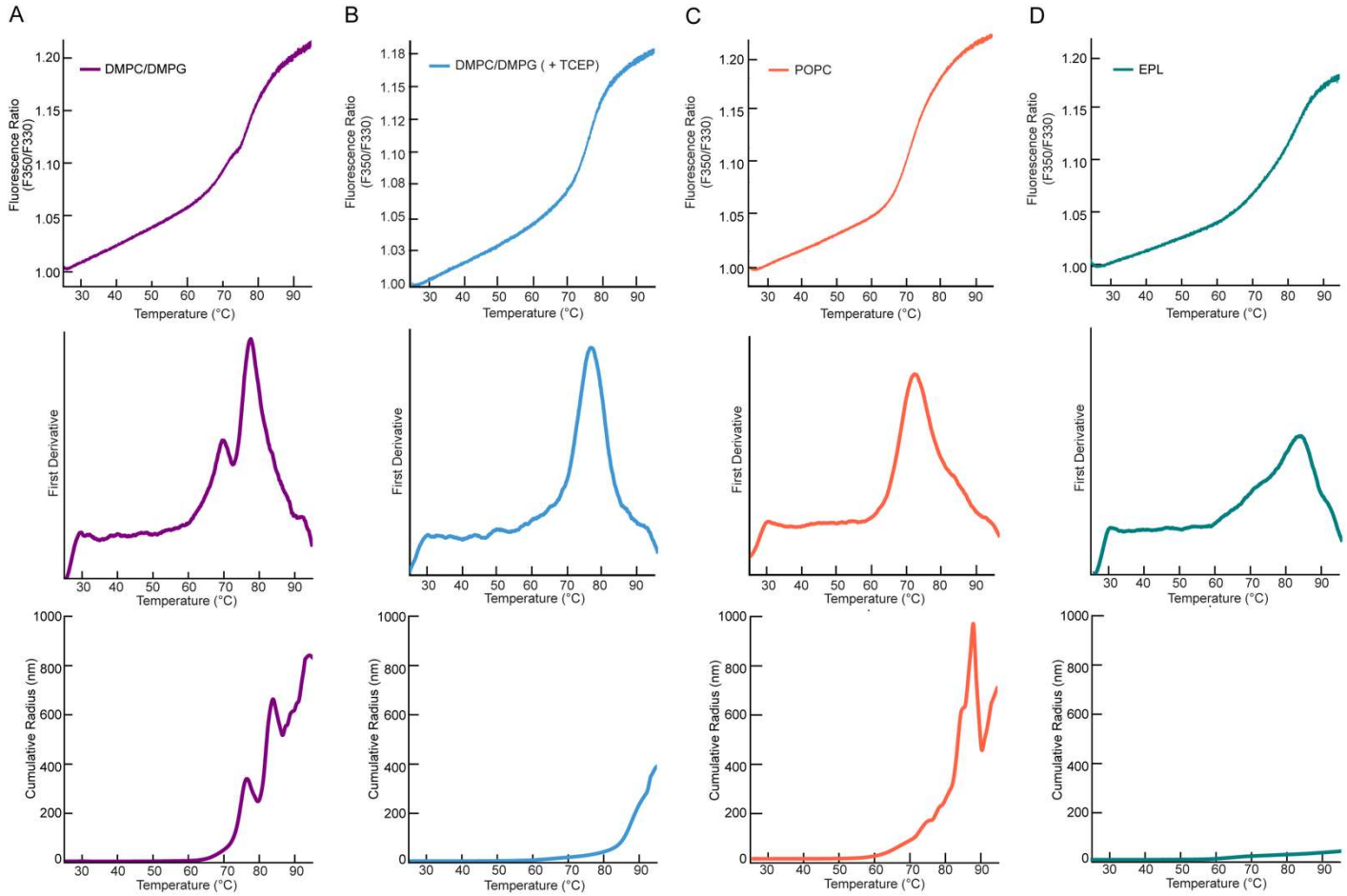

Figure S2. NanoDSF unfolding curves, first derivative, and cumulative radius plots for each dark nanodisc condition. (**A**) DsbB in a dark nanodisc (DMPC/DMPG), (**B**) DsbB in a dark nanodisc (DMPC/DMPG) under reducing conditions. (**C**) DsbB in a dark nanodisc (POPC), and (**D**) DsbB in a dark nanodisc (*E. coli* Polar Lipid Extract). **Top row:** The F350/F330 thermal unfolding curves for each of the nanodisc samples. **Second row:** The first derivative plots for each nanodisc sample. **Third row:** The cumulative radius plots from dynamic light scattering measurements collected in tandem with the fluorescence measurements. All samples were run in triplicate (n=3) and the average is plotted.

Figure S3

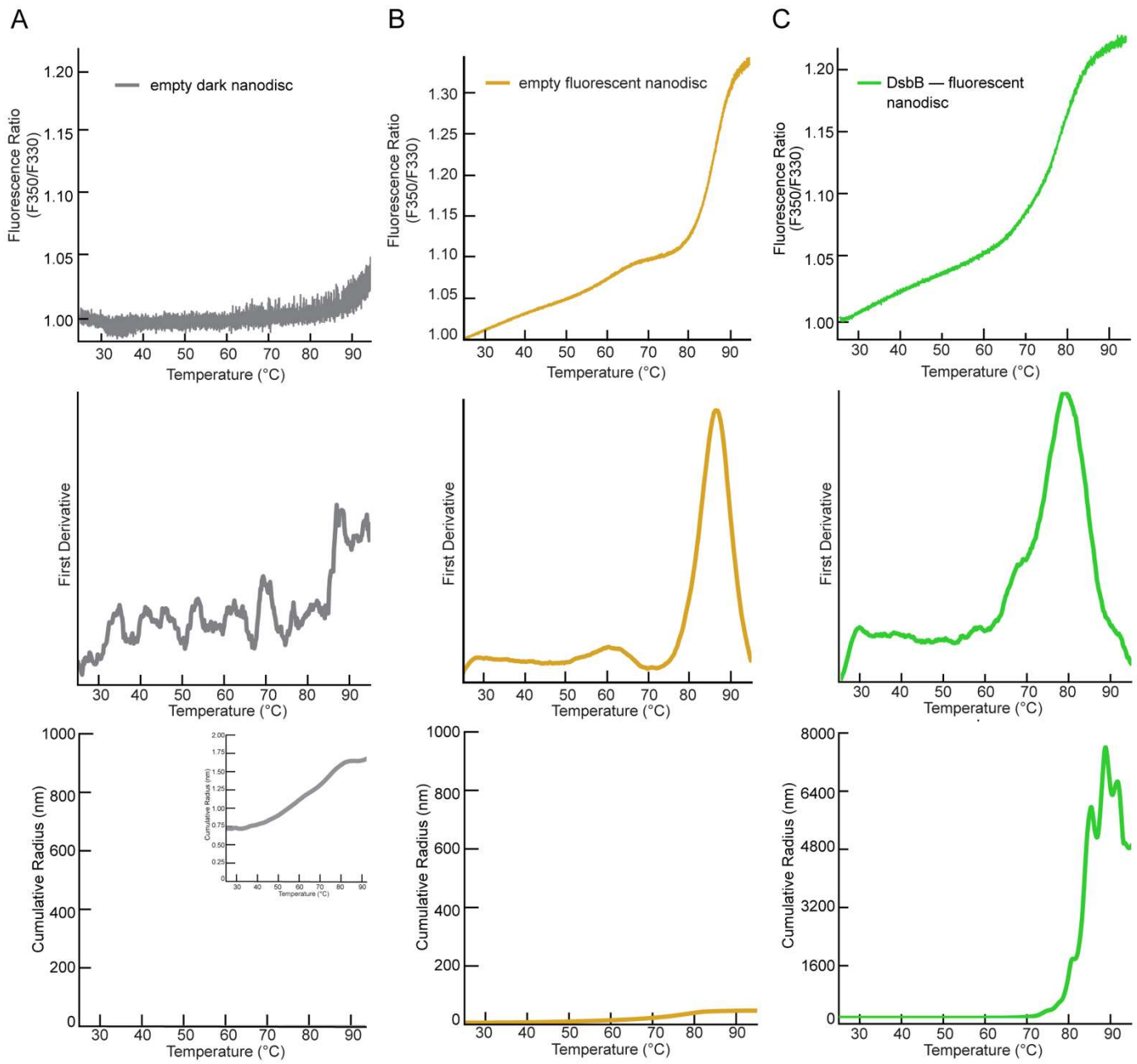

Figure S3. NanoDSF unfolding curves, first derivative, and cumulative radius plots for all nanodisc control samples. (**A**) empty dark nanodisc, (**B**) empty fluorescent MSP1D1 nanodisc, and (**C**) DsbB in a fluorescent MSP1D1 nanodisc. All control nanodisc samples were loaded with DMPC/DMPG lipids. **Top row:** The F350/F330 thermal unfolding curves for each of the nanodisc samples. **Second row:** The first derivative plots for each nanodisc sample. **Third row:** The cumulative radius plots from dynamic light scattering measurements collected in tandem with the fluorescence measurements. All samples were run in triplicate (n=3) and the average is plotted.

Figure S4

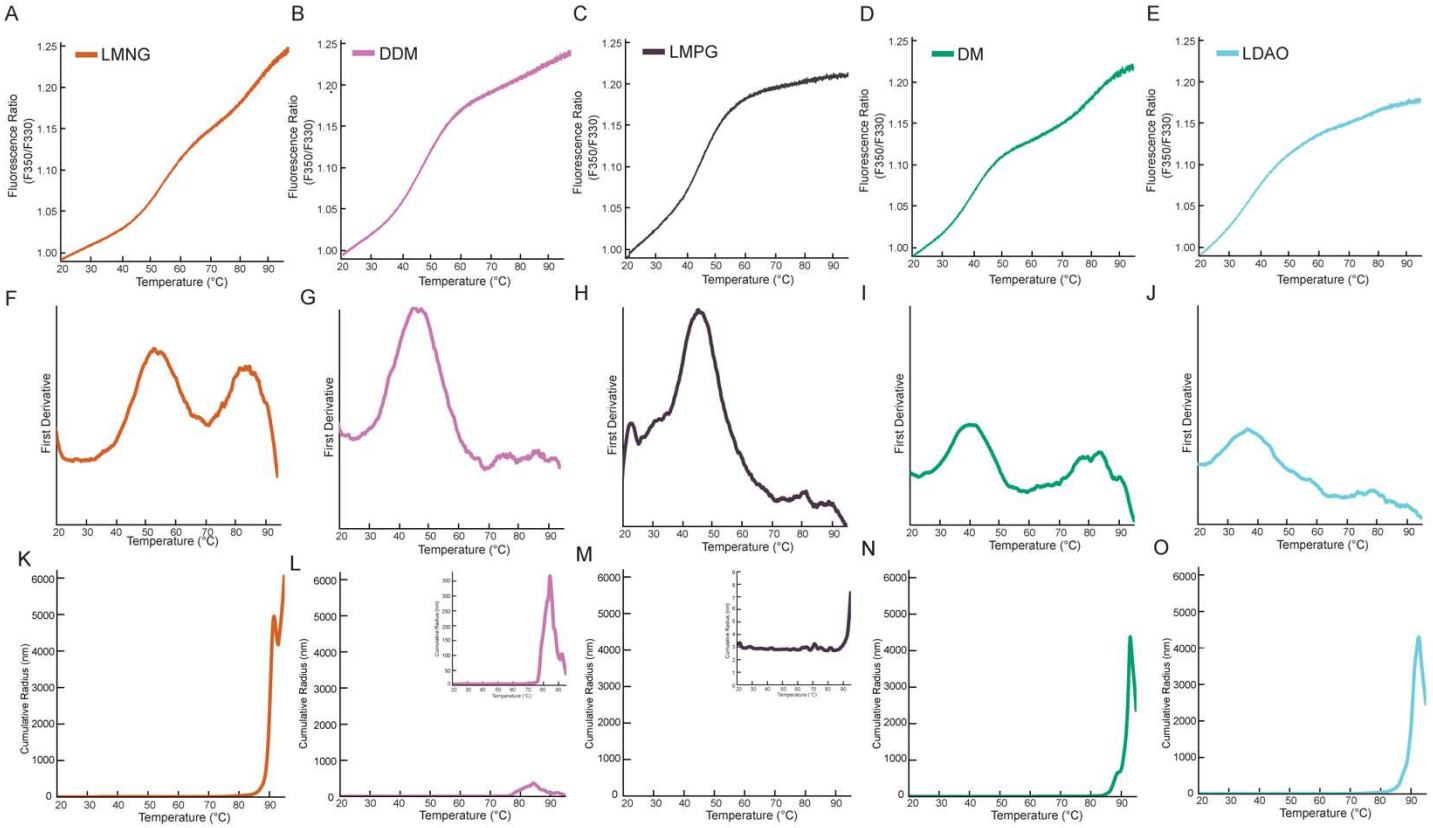

Figure S4. NanoDSF unfolding curves, first derivative, and cumulative radius plots for detergent-solubilized DsbB under a panel of micelle conditions. (**A**) LMNG, (**B**) DDM, (**C**) LMPG, (**D**) DM, and (**E**) LDAO. **A-E:** The F350/F330 thermal unfolding curves for detergent-solubilized DsbB under a panel of different micelle conditions. **F-J:** The first derivative plots for detergent-solubilized DsbB. The inflection points correspond to T_m values of 56.4 ± 0.9 °C (LMNG, orange), 49.5 ± 1.0 °C (DDM, pink), 45.4 ± 0.1 °C (LMPG, dark purple), 40.1 ± 0.9 °C (DM, green), and 36.7 ± 0.2 °C (LDAO, cyan). **K-O:** The cumulative radius plots from dynamic light scattering measurements collected in tandem with fluorescence measurements. All samples were run in triplicate (n=3) and the average is plotted.

Figure S5

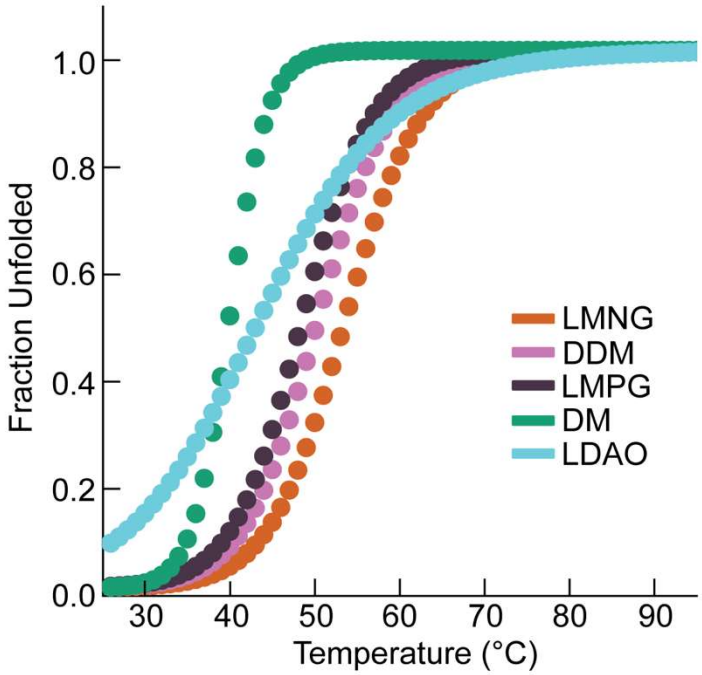

Figure S5. Fraction unfolded plotted as a function of temperature for detergent-solubilized DsbB under a panel of different detergent micelle conditions. The fraction unfolded is plotted for DsbB under a panel of different detergent micelle conditions including LMNG, DDM, LMPG, DM, and LDAO. For visualization, baseline-corrected experimental curves were calculated from parameters derived after fitting the data to an equilibrium two-state unfolding model in MoltenProt.