

743 **Supplemental Materials**

744 **Table S1**

<b>Experimental Condition</b>	<b>T<sub>m1</sub> (°C)</b>	<b>T<sub>m2</sub> (°C)</b>
DsbB – dark nanodisc	70.5 ± 0.3	77.5 ± 0.1
	70.0 ± 0.1	79.1 ± 0.2
	70.3 ± 0.6	79.7 ± 0.5
empty fluorescent nanodisc	86.6 ± 0.6	
<i>dark loop</i> DsbB – dark nanodisc	85.3 ± 0.6	91.7 ± 0.3
DsbB – dark nanodisc + TCEP	76.6 ± 0.5	
DsbB – DDM	55.6 ± 0.2	
DsbB – LMPG	45.4 ± 0.1	
DsbB – LMNG	41.0 ± 1.2	
DsbB – DM	40.1 ± 0.9	
DsbB – LDAO	36.7 ± 0.2	

745 **Table S1. Table summarizing the measured inflection points (T<sub>m</sub>).**

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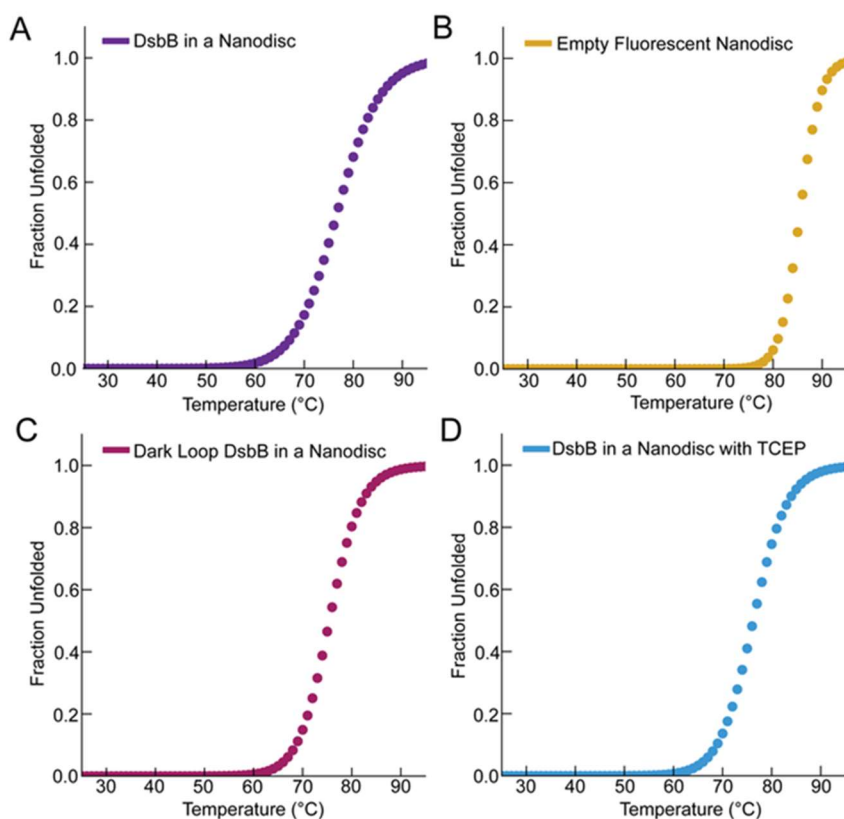
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## Figure S1



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**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, (B) empty fluorescent nanodisc, (C) *dark loop* DsbB in a dark nanodisc, and (D) DsbB in a dark nanodisc under reducing conditions.

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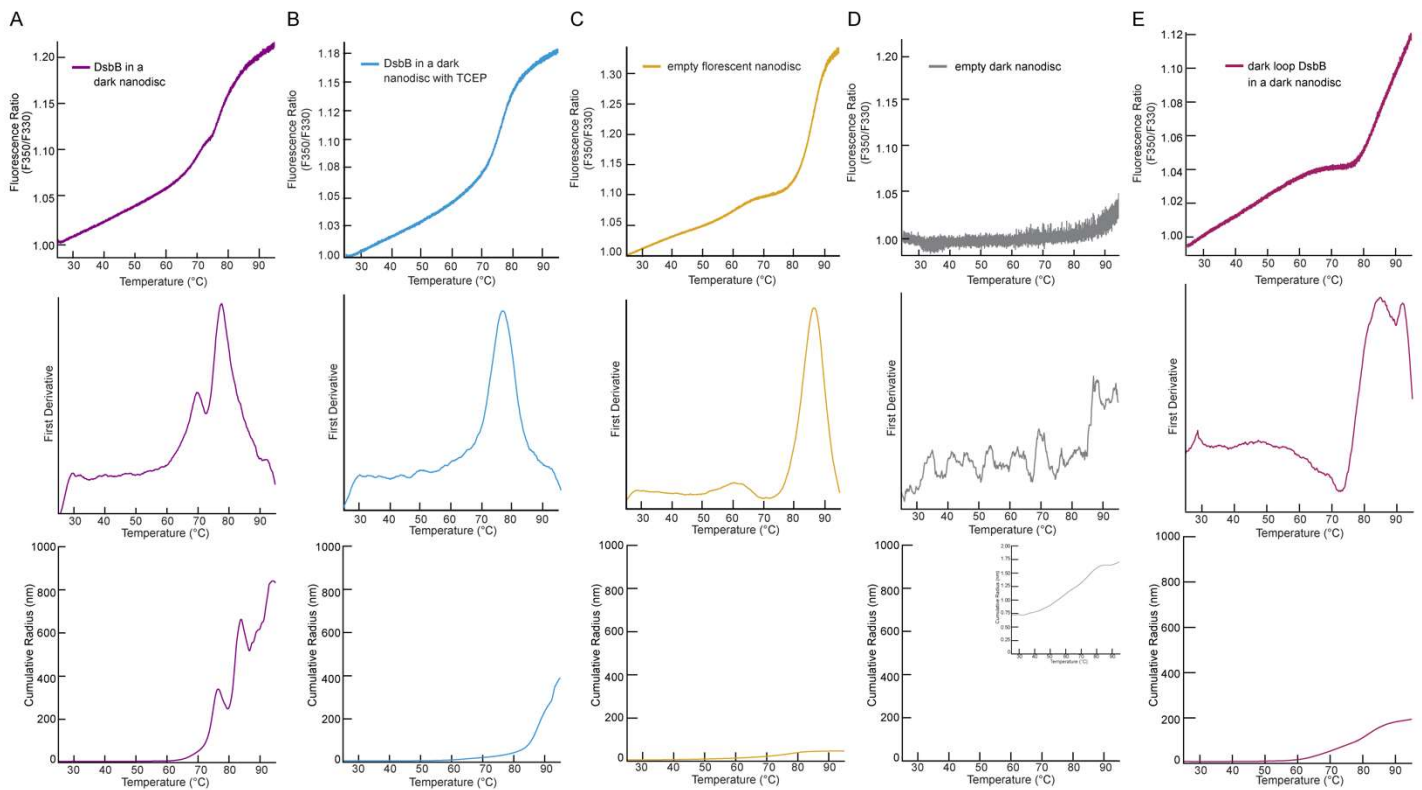
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## Figure S2



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**Figure S2. NanoDSF unfolding curves, first derivative, and cumulative radius plots for all nanodisc samples. (A)** DsbB in a dark nanodisc, **(B)** DsbB in a dark nanodisc under reducing conditions, **(C)** empty fluorescent nanodisc, **(D)** empty dark nanodisc, and **(E)** *dark loop* DsbB in a dark nanodisc. **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) with the exception that DsbB in a dark nanodisc was run for three independent sample preparations in triplicate (n=9), the average was plotted.

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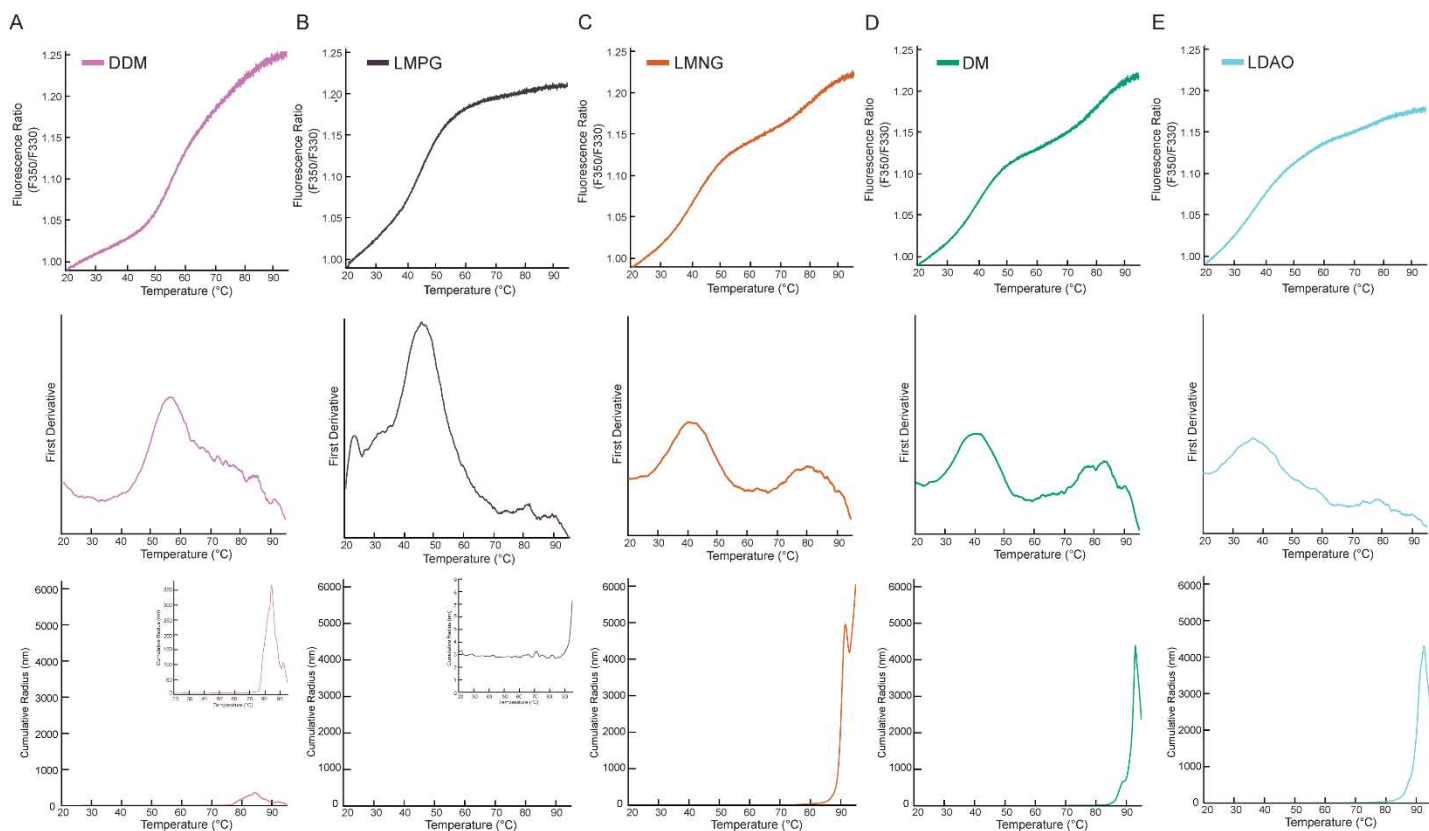
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803 **Figure S3**



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805 **Figure S3. NanoDSF unfolding curves, first derivative, and cumulative radius plots for detergent-**  
 806 **solubilized DsbB under a panel of micelle conditions. (A) DDM, (B) LMPG, (C) LMNG, (D) DM, and (E)**  
 807 **LDAO. Top row:** The F350/F330 thermal unfolding curves for detergent-solubilized DsbB under a panel of  
 808 different micelle conditions. **Second row:** The first derivative plots for detergent-solubilized DsbB. The inflection  
 809 points correspond to  $T_m$  values of  $36.7 \pm 0.2$  °C (LDAO, cyan),  $40.1 \pm 0.9$  °C (DM, green),  $41.0 \pm 1.2$  °C (LMNG,  
 810 orange),  $45.4 \pm 0.1$  °C (LMPG, purple), and  $55.6 \pm 0.2$  °C (DDM, pink). **Third row:** The cumulative radius plots  
 811 from dynamic light scattering measurements collected in tandem with fluorescence measurements. All samples  
 812 were run in triplicate ( $n=3$ ) and the average was plotted.

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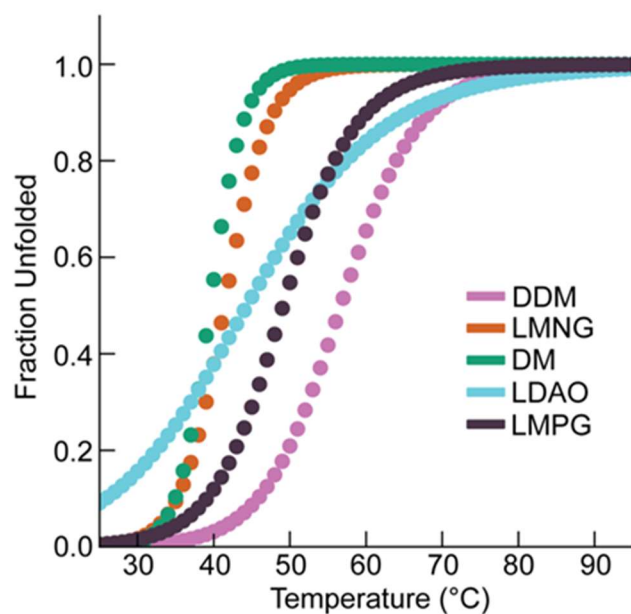
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823 **Figure S4**



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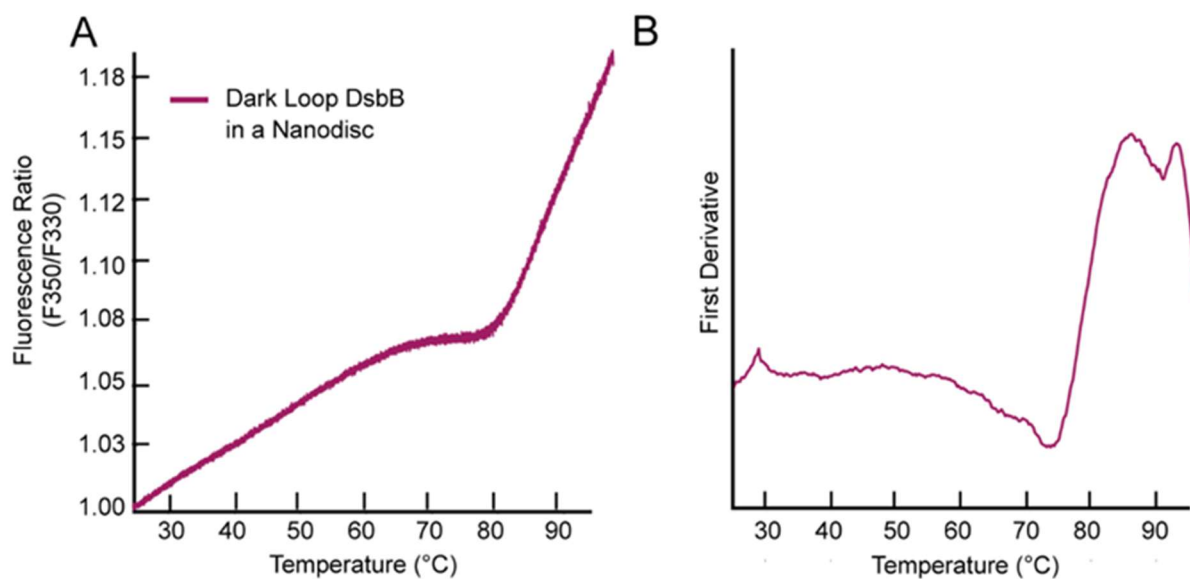
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**Figure S4. Fraction unfolded plotted as a function of temperature for detergent-solubilized DsbB under a panel of different detergent micelle conditions.** The fraction unfolded is plotted for DsbB under a panel of different detergent micelle conditions including DDM, LMNG, DM, LDAO, and LMPG. For visualization, baseline-corrected experimental curves were calculated from parameters derived after fitting the data to an equilibrium two-state unfolding model in MoltenProt<sup>42</sup>.

847 **Figure S5**



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849 **Figure S5. NanoDSF thermal unfolding curve for dark loop DsbB in a dark MSP-based nanodisc. (A)** The  
850 F350/F330 thermal unfolding curve for dark loop DsbB reconstituted in a dark nanodisc model membrane system.  
851 **(B)** The first derivative plot of the F350/F330 ratio with respect to temperature for dark loop DsbB in a nanodisc.  
852 The inflection points correspond to  $T_m$  values of 85.3 °C and 91.7 °C, respectively. All samples were run in  
853 triplicate.

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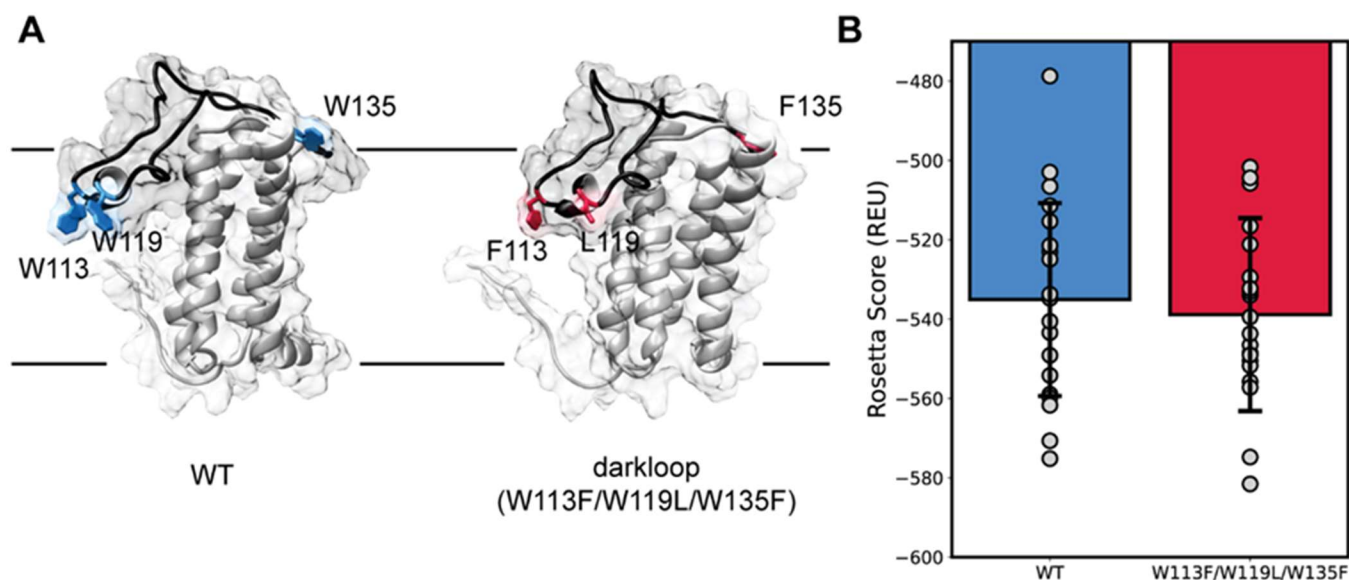
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872 **Figure S6**



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**Figure S6. Rosetta calculations of wt and dark loop DsbB based on the ensemble NMR model PDB ID: 2K73.** (A) Illustration of the residues that were modified to generate the dark loop DsbB construct, which was then relaxed and scored with a Rosetta membrane protein score function. Wt tryptophan residues are depicted in blue and corresponding dark loop residues are depicted in red. The periplasmic loop is shown in black. (B) Bar and whisker plot of the membrane score functions for both wt (blue) and dark loop (red) DsbB with a 4 Rosetta energy unit (REU) reduction from -535 REU in wt to -539 REU for dark loop DsbB. Grey dots represent an individual model from the 20-model ensemble and error bars indicate the standard deviations of the scores.