Supplemental data to the paper:

Structure of the Disulfide Bond Generating Membrane Protein DsbB in the Lipid Bilayer

Ming Tang, Anna E. Nesbitt, Lindsay J. Sperling, Deborah A. Berthold, Charles D. Schwieters, Robert B. Gennis & Chad M. Rienstra

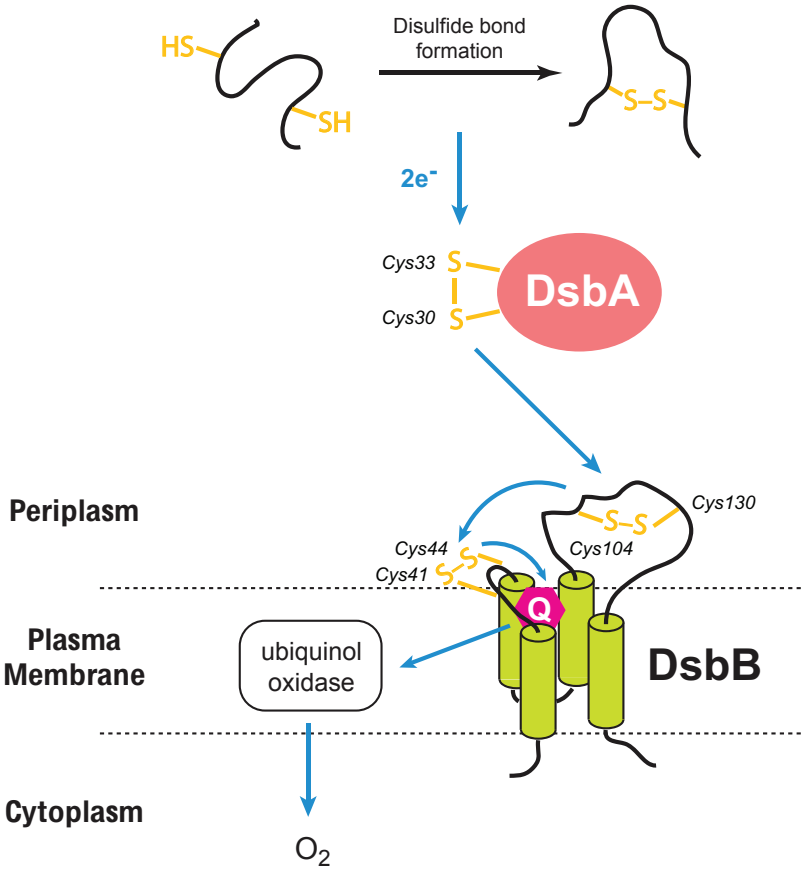

Figure S1. Schematic overview of disulfide bond formation system in *E. coli*. Essential cysteines of DsbA and DsbB are highlighted and the blue arrows indicate the directions of electron transfer.

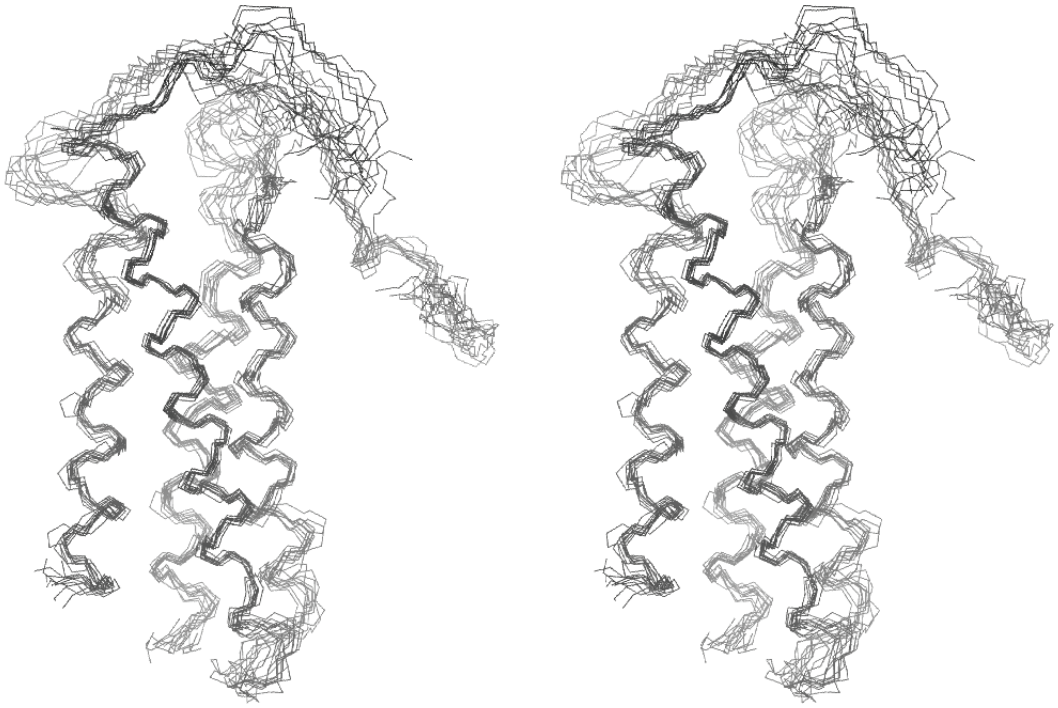

Figure S2. Stereo view of a superposition of ten lowest energy structures of DsbB (Cys41Ser) calculated against X-ray reflections and SSNMR restraints.

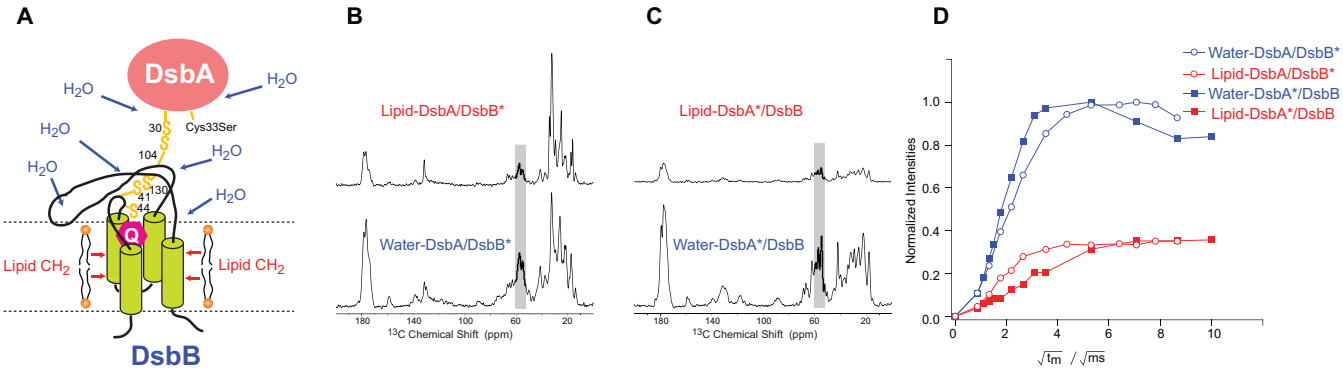

Figure S3. Membrane topology of DsbB-DsbA complex. (A) Schematic view of proton spin diffusion from lipid or water to DsbB-DsbA in the lipid bilayers. (B) ^{13}C 1D spectra of selective lipid or water proton spin diffusion to DsbA/DsbB*. (C) ^{13}C 1D spectra of selective lipid and water proton spin diffusion to DsbA*/DsbB. Gray rectangles highlight the peaks with integration

for plotting the buildup curves. (D) Buildup curves of proton spin diffusion from lipid (red) or water (blue) to DsbA/DsbB* (open circles) and DsbA*/DsbB (filled rectangles).

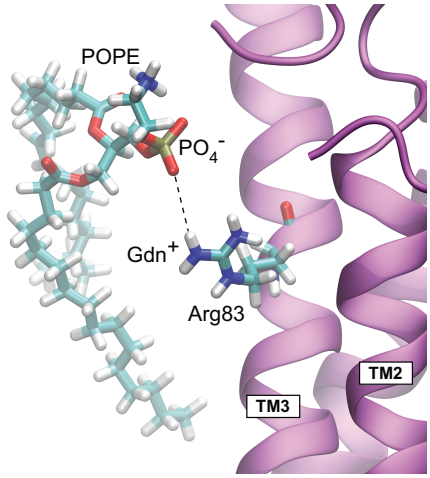

Figure S4. Stabilization of DsbB Arg83 in the middle of TM3 by a POPE phosphate headgroup. Possible hydrogen bond between Arg83 guanidinium (Gdn^+) and POPE phosphate (PO_4^-) is highlighted by the dashed line.

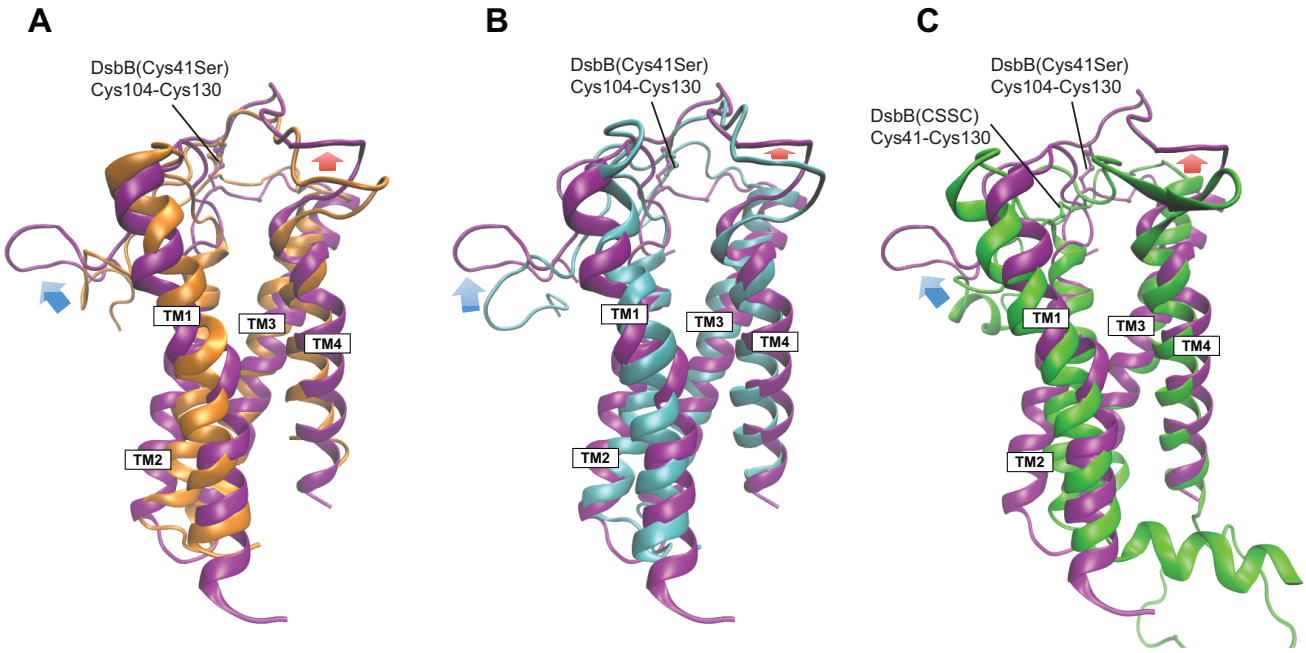

Figure S5. Comparison of DsbB model from molecular dynamics simulation with DsbB structures from X-ray (PDB ID: 2ZUQ), joint X-ray/SSNMR (PDB ID: 2LTQ) and solution

NMR (PDB ID: 2K74). (A) Overlay of DsbB crystal structure (orange) and molecular dynamics model with transmembrane helices aligned. (B) Overlay of DsbB structure (cyan) from joint X-ray/SSNMR calculation and molecular dynamics model with transmembrane helices aligned. (C) Overlay of DsbB solution NMR structure (green) and molecular dynamics model with transmembrane helices aligned. Blue and red arrows indicate the conformational changes of DsbB loops due to the presence of the lipid bilayers.

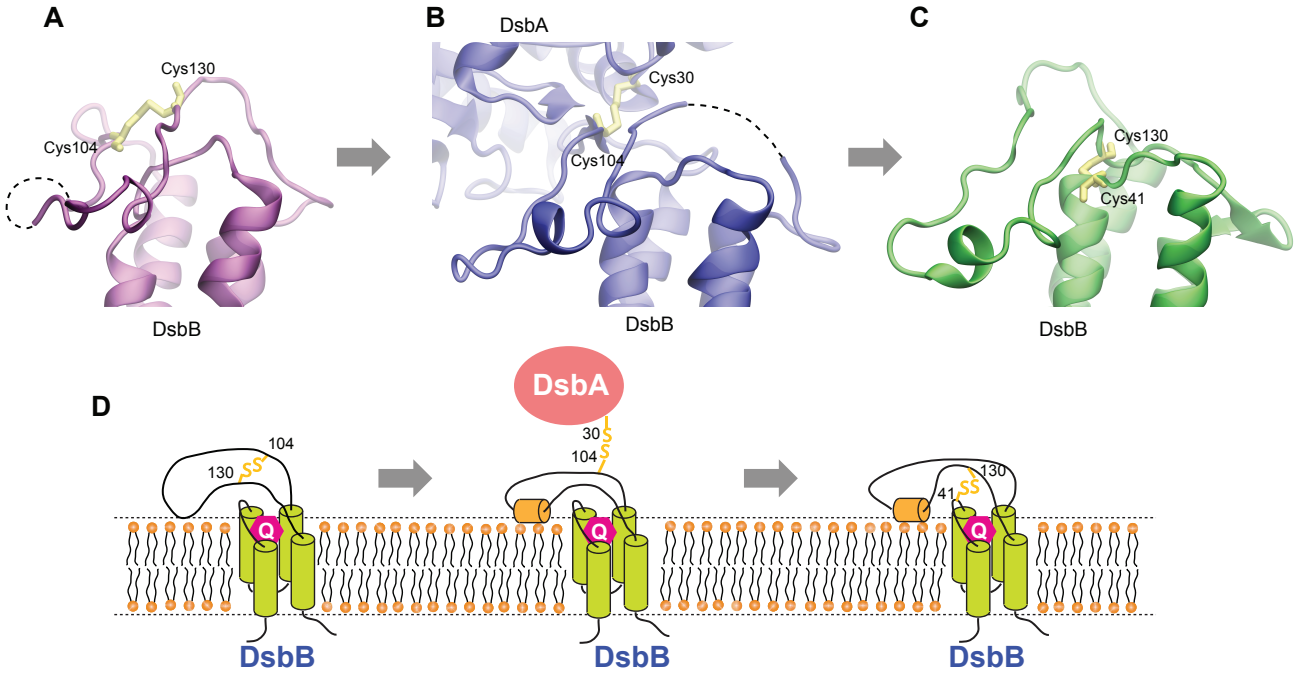

Figure S6. Conformational changes of DsbB periplasmic loop upon disulfide-bond rearrangements. (A) Loop region of DsbB molecular dynamics model. The disulfide bond between Cys104 and Cys130 is highlighted. (B) Loop region of DsbB-DsbA crystal structure (PDB ID: 2ZUP). The disulfide bond between DsbB Cys104 and DsbA Cys30 is highlighted. (C) Loop region of DsbB solution structure. The disulfide bond between DsbB Cys41 and Cys130 is highlighted. (D) Schematic diagram representing that DsbB loop changes from flexible loop to horizontal helix upon the breakage of DsbB Cys104–Cys130 and the disulfide-bond formation of either DsbB Cys104 – DsbA Cys30 or DsbB Cys41–Cys130.

Table S1. Crosspeak assignments of DsbB residues correlated with water or lipids in proton spin diffusion experiments.

Water-DsbB	^{13}C F1 (ppm)	^{13}C F2 (ppm)	Lipid-DsbB	^{13}C F1 (ppm)	^{13}C F2 (ppm)
S9CA-CB	59.6	63.2	L17CB-CA	41.7	57.6
G11CA-C	43.9	172.6	M18CG-CA	33.6	60.4
A14CB-CA	18.0	55.3	A19CB-CA	18.0	55.4
L16CB-CA	41.3	58.3	T21CG2-CA	22.8	66.0
L17CB-CA	41.2	57.8	T21CG2-CB	22.7	69.3
V35CG2-CA	21.1	64.3	A22CB-CA	17.7	54.8
L37CG-CA	27.1	56.6	L23CB-CA	41.4	57.7
L38CG-CA	27.3	54.9	L23CG-CA	26.9	57.8
V42CG2-CA	21.9	67.3	A24CB-CA	17.5	55.4
I45CD1-CD1	8.3	8.3	L27CB-CA	41.9	57.8
Y46CB-CA	35.3	59.4	L27CB-CA	41.6	58.0
R48CB-CA	30.3	60.4	L27CG-CA	27.2	57.9
R48CG-CD	27.7	44.3	A29CB-CA	16.8	56.1
G61CA-C	45.2	174.0	L30CB-CA	41.1	57.6
A64CB-CA	19.7	52.9	L30CG-CA	26.4	57.7
P65CG-CA	27.2	65.5	L43CB-CA	41.2	57.1
K66CB-CA	30.5	57.2	L43CG-CA	26.6	57.3
T67CG2-CA	22.3	60.1	Y46CB-CA	35.3	59.2
T67CG2-CB	22.0	70.0	V49CB-CA	31.4	66.6
L69CB-CA	42.2	57.4	A50CB-CA	16.8	55.9
L69CG-CA	26.3	57.3	L51CG-CA	26.3	57.3
L94CB-CA	41.4	57.8	G53CA-C	47.8	174.9
L94CD2-CA	23.3	57.5	V54CB-CA	31.2	67.4
L94CG-CA	27.1	57.7	L55CB-CA	42.1	58.4
D117CB-CA	41.3	54.3	L55CG-CA	27.0	58.4
V120CB-CA	31.9	59.0	G56CA-C	47.5	174.6
P121CB-CD	31.9	50.7	A57CB-CA	18.0	55.6
P121CG-CD	28.2	50.5	A58CB-CA	18.1	54.8
V123CB-CA	35.2	62.0	L59CB-CA	41.2	57.6
V123CG2-CA	21.5	61.6	L59CG-CA	26.1	57.7
V125CB-CA	32.7	61.9	I60CG2-CA	17.1	64.4
V125CG2-CA	21.5	61.6	G61CA-C	45.8	174.0
A126CB-CA	17.3	56.0	A62CB-CA	19.4	53.4
V160CG2-CA	22.1	67.7	A73CB-CA	19.5	55.8
V161CB-CA	30.9	67.4	M74CG-CA	35.7	61.4
V161CG2-CA	21.8	67.6	V75CB-CA	31.6	67.5
A168CB-CA	19.1	51.3	I76CD1-CA	15.0	65.5
			I76CG2-CA	17.2	65.7

			L78CB-CA	44.2	58.0
			A81CB-CA	17.8	55.2
			V85CB-CA	31.0	67.0
			L87CB-CA	42.1	57.8
			L87CG-CA	27.0	57.8
			L146CB-CA	41.6	57.7
			L146CG-CA	26.1	58.0
			L147CB-CA	41.1	58.0
			L147CG-CA	27.5	57.8
			G148CA-C	48.2	174.7
			I149CD1-CA	14.6	66.1
			I149CG2-CA	18.0	66.2
			A152CB-CA	18.0	56.2
			L154CB-CA	42.0	57.9
			L154CG-CA	27.2	58.0
			I155CB-CA	38.4	66.0
			I155CG2-CA	18.0	65.8
			V156CB-CA	31.7	67.7
			A157CB-CA	16.2	55.0
			V158CB-CA	31.3	67.1
			L159CB-CA	41.4	57.4
			L159CG-CA	26.6	57.6

Table S2. Summary of solid-state NMR experiments.

Sample	Amount	Experiment	Mixing time	Time	Spectrometer	Purpose
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	2D ^{13}C-^{13}C DARR	25 ms	1 d	Agilent 750 MHz	Assignment
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	3D NCACX	90 ms	11.6 d	Agilent 500 MHz	Assignment
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	3D NCOCX	90 ms	9.5 d	Agilent 500 MHz	Assignment
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	2D selective ^{1}H spin diffusion	^{1}H-^{1}H 7 ms ^{13}C-^{13}C 50 ms	4.8 d	Agilent 500 MHz	Water-protein correlations
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	2D selective ^{1}H spin diffusion	^{1}H-^{1}H 7 ms ^{13}C-^{13}C 50 ms	4.8 d	Agilent 500 MHz	Lipid-protein correlations
[2-^{13}C-glycerol,^{15}N] DsbB(Cys41Ser)	4 mg	2D ^{13}C-^{13}C DARR	500 ms	2.6 d	Agilent 750 MHz	Distance restraints
[1,3-^{13}C-glycerol,^{15}N] DsbB(Cys41Ser)	3 mg	2D ^{13}C-^{13}C DARR	300 ms	2.9 d	Agilent 750 MHz	Distance restraints
DsbA(Cys33Ser)/ [U-^{13}C, ^{15}N]DsbB	4 mg	1D selective ^{1}H spin diffusion	^{1}H-^{1}H 0.75 ~ 75 ms	1.2 d	Agilent 500 MHz	Water/lipid-protein correlations
[U-^{13}C, ^{15}N] DsbA(Cys33Ser)/ DsbB	4 mg	1D selective ^{1}H spin diffusion	^{1}H-^{1}H 0.75 ~ 100 ms	2.8 d	Agilent 500 MHz	Water/lipid-protein correlations
[U-^{13}C, ^{15}N] DsbB(Cys41Ser)	7 mg	2D ^{13}C-^{13}C DARR with ^{15}N REDOR dephasing	500 ms	7 d	Agilent 600 MHz	Confirmation of Glu26–Tyr153 hydrogen-bond