Table S1. **(A)** Bacterial strains, plasmids and **(B)** primers utilized in this study.

A

Strains	Relevant Genotype	Source
SF100	F- Δ*lacX74 galE galK thi rpsL* (*strA*) Δ*phoA* (*PvuII*) Δ(*ompT- entF*)	Ref (1)
MC1000	*araD139* Δ(*araA-leu*)*7679* Δ(*codB-lac*)*X74 galE15 galK16 rpsL150 relA1 thi*	Lab Collection
BL21(DE3)pLysS	F- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cam^R)	Novagen
PB351	SF100 Δ*degP* Δ*dsbC*	Ref (2)
PB403	SF100 Δ*degP dsbA::kan*	Lab Collection
LM106	MC1000 *dsbA::kan5*	Lab Collection
LM102	MC1000 *dsbB::kan5*	Ref (3)

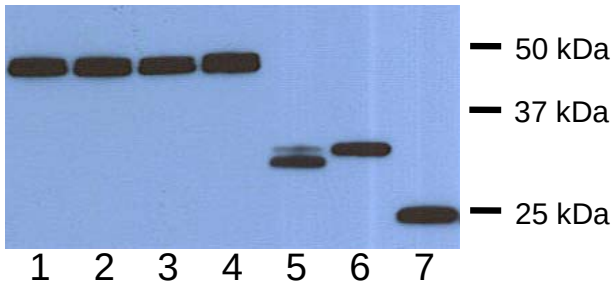
Plasmids	Relevant Genotype	Source
pET-28(a)	T7 expression vector, C-terminal 6x histidine tag	Novagen
pTrcStIIvtPA	*tPA* (Δ6-175) with StII leader in pTrc99A	Ref (4)
pBADdsbC	*dsbC* from *Escherichia coli* in pBAD33	Lab Collection
pBAD-mDsbC-mDsbC	two *dsbC* genes fused via $(GGGS)_3GSA$ linker in pBAD33	This work
pBAD-DsbC[C98A]	*dsbC* C98A in pBAD33	This work
pBAD-mDsbC-mDsbC[C98A]	*dsbC* fused to *dsbC* C98A via $(GGGS)_3GSA$ linker in pBAD33	This work
pBAD-DsbC[C98A/C101A]	*dsbC* C98A C101A in pBAD33	This work
pBAD-mDsbC-mDsbC[C98A/C101A]	*dsbC* fused to *dsbC* C98A C101A via $(GGGS)_3GSA$ linker in pBAD33	This work
pBAD-DsbC[C101A]	*dsbC* C101A in pBAD33	This work
pBAD-mDsbC-mDsbC[C101A]	*dsbC* fused to *dsbC* C101A via $(GGGS)_3GSA$ linker in pBAD33	This work
pBAD-mDsbC-dim	*dsbC* fused to *dsbC*(Δ65-216) via $(GGGS)_3GSA$ linker in pBAD33	This work
pBAD-mDsbC[H45D]-dim[D53H]	*dsbC* H45D fused to *dsbC* D53H (Δ65-216) via $(GGGS)_7SA$ linker in pBAD33	This work
pET28-DsbC	*dsbC* from *Escherichia coli* in pET-28(a)	Lab Collection
pET28-mDsbC-mDsbC	two *dsbC* genes fused via $(GGGS)_3GSA$ linker in pET-28(a)	This work
pET28-DsbC[C98A]	*dsbC* C98A in pET-28(a)	This work
pET28-mDsbC-mDsbC[C98A]	*dsbC* fused to *dsbC* C98A via $(GGGS)_3GSA$ linker in pET-28(a)	This work
pET28-DsbC[C98A/C101A]	*dsbC* C98A C101A in pET-28(a)	This work
pET28-mDsbC-mDsbC[C98A/C101A]	*dsbC* fused to *dsbC* C98A C101A via $(GGGS)_3GSA$ linker in pET-28(a)	This work
pET28-DsbC[C101A]	*dsbC* C101A in pET-28(a)	This work
pET28-mDsbC-mDsbC[C101A]	*dsbC* fused to *dsbC* C101A via $(GGGS)_3GSA$ linker in pET-28(a)	This work
pET28-mDsbC-dim	*dsbC* fused to *dsbC*(Δ65-216) via $(GGGS)_3GSA$ linker in pET-28(a)	This work
pET28-mDsbC[H45D]-dim[D53H]	*dsbC* H45D fused to *dsbC* D53H (Δ65-216) via $(GGGS)_7SA$ linker in pET-28(a)	This work
pET28-mDsbC[H45D]-dim[H45D]	*dsbC* H45D fused to *dsbC* H45D (Δ65-216) via $(GGGS)_7SA$ linker in pET-28(a)	This work

1. Baneyx, F., and G. Georgiou 1990. *J Bacteriol* **172,** 491-494 3.
2. Bessette, P. H., Qiu, J., Bardwell, J. C., Swartz, J. R. & Georgiou, G. (2001) *J Bacteriol* **183,** 980-8.
3. Masip, L., Pan, J. L., Haldar, S., Penner-Hahn, J. E., DeLisa, M. P., Georgiou, G., Bardwell, J. C. & Collet, J. F. (2004) *Science* **303,** 1185-9.
4. Bessette, P. H., Aslund. Beckwith & Georgiou, G. (1999) *Proc. Natl. Acad. Sci. U.S.A.* **96**, 13703-13708

B

Primers	Sequence
XbaIDsbCss.f	GAGCTCGAATTCTCTAGATTAAAGAGGAGAAAGGTACCCATGATGAAGAAAGGTTTTAT
DsbCHisHindIII.r	TTTTTAAGCTTTTAGTGGTGGTGGTGGTGGTGTTTACCGCTGGTCATTTTTTG
dimstopHindIII.r	TTTTTAAGCTTTTAGTGGTGGTGGTGGTGGTGATTGGTGACATTGACCGGAGCCG
DsbC-AGYC.f	GATATTACCGCGGGTTACTGCCACAAACTG
DsbC-AGYC.r	CAGTTTGTGGCAGTAACCCGCGGTAATATC
DsbC-AGYA.f	CCGTGTTTACTGATATTACCGCGGGTTACGCGCACAAACTGCATGAGCAAATGGC
DsbC-AGYA.r	GCCATTTGCTCATGCAGTTTGTGCGCGTAACCCGCGGTAATATCAGTAAACACGG
DsbC-CGYA.f	CCGTGTTTACTGATATTACCTGTGGTTACGCGCACAAACTGCATGAGCAAATGGC
DsbC-CGYA.r	GCCATTTGCTCATGCAGTTTGTGCGCGTAACCACAGGTAATATCAGTAAACACGG
DsbC-H45D.r	GGCCCCTGAATGATATCTTTACCATCATC
DsbC-D53H.r	GTGCCACTAACATGATACATTGGCCC
LinkDsbCa.f	GGCGGTGGCAGCGGTGGAGGCTCCGGCGGAGGTAGCGGTTCAGCTGATGACGCGGCA
LinkDsbCb.f	GGTAGCGGTTCAGCTGATGACGCGGCAATTCAACAAAC
DsbCLink.r	GCCGGAGCCTCCACCGCTGCCACCGCCTTTACCGCTGGTCATTTTTTGGTGTTCGTC
Linker30a.r	CCGCTCCCGCCACCGGATCCGCCTCCAGAACCTCCGCCGGAGCCTCCACCGCTGCCACC
Linker30b.r	CGCGTCATCAGCTGAACCGCTACCGCCAGAGCCACCTCCGCTCCCGCCACCGGATCCGC

Figure S1. Expression level of the covalently linked DsbC variants as shown by western blot analysis. Overnight cultures of PB351 (SF100 *ΔdegP ΔdsbC*) cells grown in LB medium supplemented with 25 μg/ml of chloramphenicol at 37 ºC were sub-cultured, grown to OD_{600} ~ 0.8 and induced with arabinose (0.2% final concentration). After 2 hours of induction, samples were collected, mixed with SDS-PAGE loading buffer and boiled, an equal number of A_{600} units were then loaded on each lane. Western blot analysis was carried out using a monoclonal antipolyhistidine HRP-conjugated antibody.



1. mDsbC-mDsbC
2. mDsbC-mDsbC[C98A]
3. mDsbC-mDsbC[C98A/C101A]
4. mDsbC-mDsbC[C101A]
5. mDsbC-dim
6. mDsbC[H45D]-dim[D53H]
7. DsbC

Figure S2. Gel filtration FPLC of mDsbC-dim. Purified proteins were run on a Superdex^{TM} 200 column in PBS-10% glycerol buffer. Fractions corresponding to peaks A and B were collected and further analyzed by re-running them independently under identical conditions.

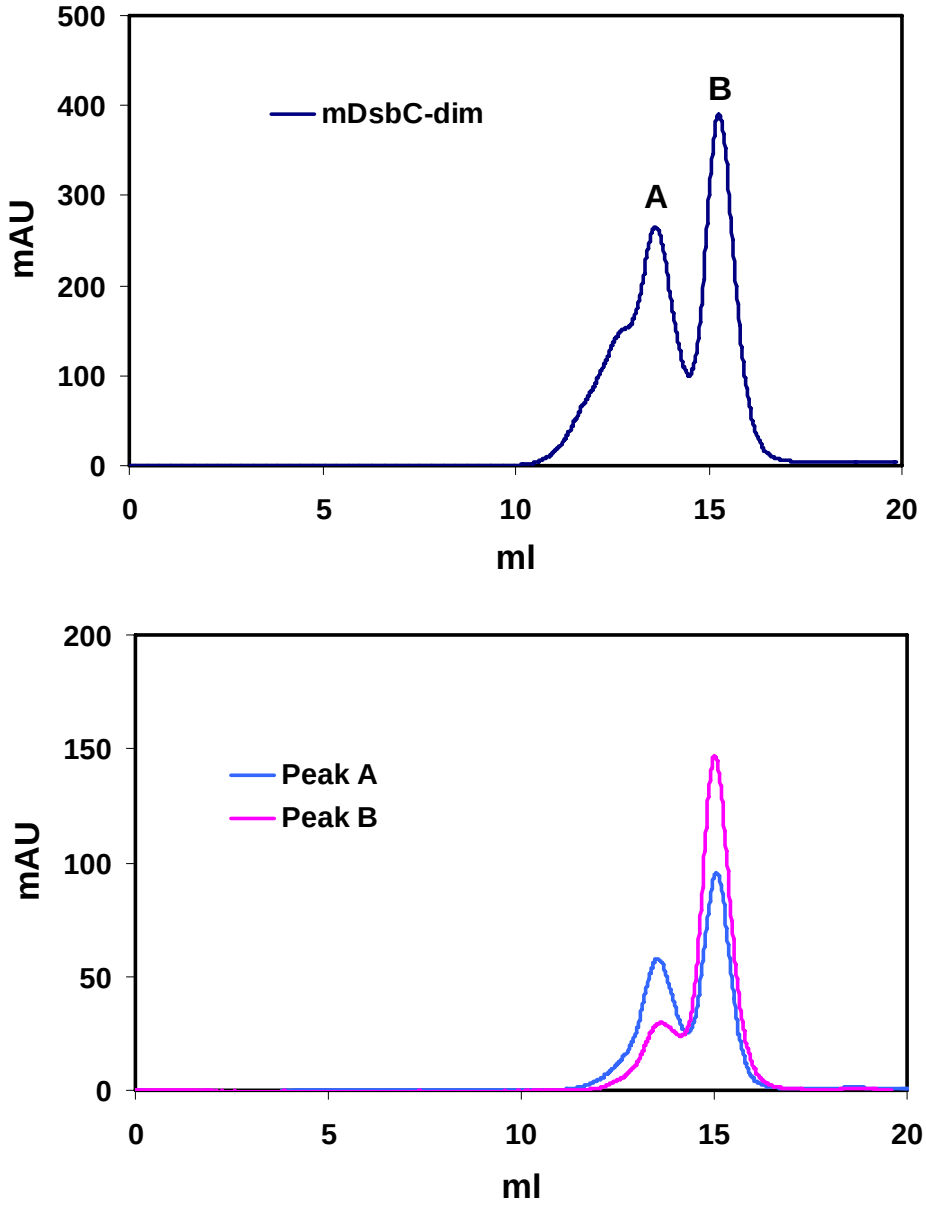


Fig S3. Gel filtration FPLC of mDsbC-dim derivative proteins containing 17, 19, and 30 amino-acid glycine-serine linkers.

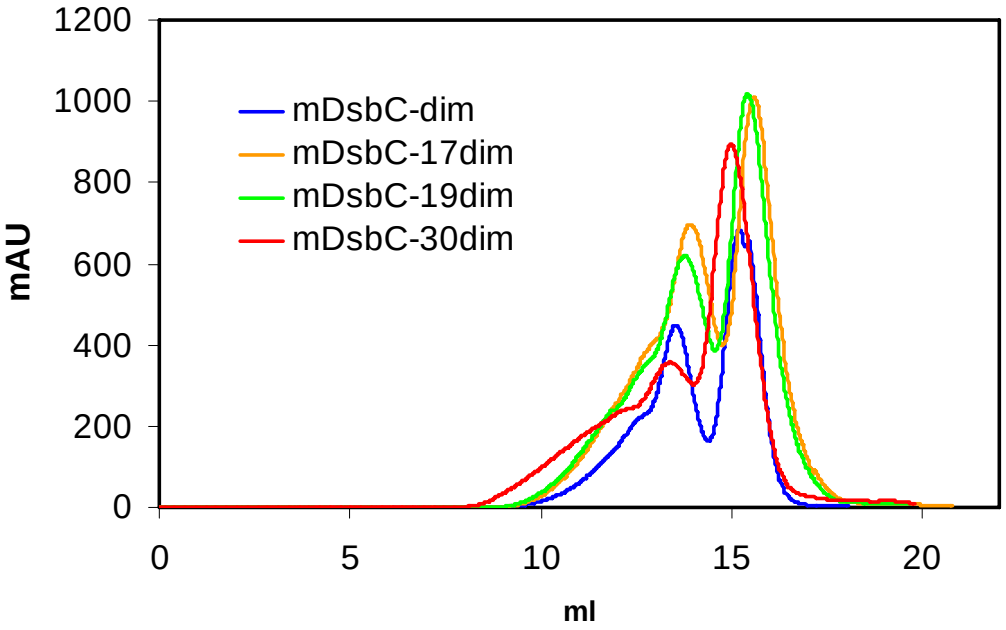


Fig S4. *In vivo* redox state of DsbC and covalently linked proteins. PB351 (SF100 *ΔdegP ΔdsbC*) cell samples were precipitated with trichloroacetic acid (TCA). Derivatization of free thiols was performed using 15mM AMS. The reduced and oxidized standards were generated by the same procedure using 0.02 mg of purified protein. For the reduced standard, the protein was previously incubated with 100mM DTT. For the oxidized standard, addition of AMS was omitted. Following SDS-PAGE in a 12% gel, the proteins were detected by western blot using antihistidine HRP-conjugated antibody.