Supplementary Material
Unique Photobleaching Phenomena of the Twin-Arginine Translocase Respiratory Enzyme Chaperone DmsD

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Table S1. Peptides observed in MALDI-TOF spectrum obtained from chymotrypsin digested DmsD.

Observed Mass (m/z)	Error (Da)	Peptide Sequencea	Fragment Position	Trp[b]	UV Irradiated	R_t Range	s/n	Modificationc	Signal Peptide Binding[d]
620.6	-0.34	(m)/tggqqm/(g)	-21 to -16e	- f	+	19-21	6.05	-	-
2270.3	-0.38	(m)/tggqqmgrdlyddddkdrw/(g)	-21 to -3e	-3e	-	17-19	4.06	-	-
1886.3	0.31	(l)/yddddkdrwgsMTHF/(S)	-11 to 4e	-3e	+	21-23	8.03	-	-
1338.9	-0.39	(y)/ddddkdrwgsM/(T)	-10 to 1e	-3e	-	17-19	5.06	-	-
580.2	0.01	(L)/TSDGW/(E)	38-42	42	+	23-25	6.02	W42: +16	-
1001.8	-0.23	(W)/QRLFVGPW/(A)	73-80	(72), 80	-	21-23	44.7	-	(72), 80
620.6	-0.30	(L)/FVGPW/(A)	76-80	80	+	19-21	6.05	W80: +16	80
1195.8	-0.20	(W)/ALPSPPWGSVW/(L)	81-91	(80), 87, 91	-	23-25	4.04	-	(80), 87, 91
2008.4	-0.40	(W)/ALPSPPWGSVWLDRESVL/(F)	81-98	(80), 87, 91	-	23-25	5.03	-	(80), 87, 91
2155.5	-0.41	(W)/ALPSPPWGSVWLDRESVLF/(G)	81-99	(80), 87, 91	-	23-25	17.02	-	(80), 87, 91
2628.8	-0.50	(W)/ALPSPPWGSVWLDRESVLFGDSTL/(A)	81-104	(80), 87, 91	-	23-25	51.4	-	(80), 87, 91
560.5	-0.16	(W)/GSVWL/(D)	88-92	(87), 91	-	23-25	9.04	-	(87), 91
1451.1	-0.36	(W)/LDRESVLFGDSTL/(A)	92-104	*(91)*	-	19-21	6.00	-	(91)
2105.5	-0.40	(W)/LDRESVLFGDSTLALRQW/(M)	92-109	(91), 109	-	21-23	4.01	-	(91)
704.6	-0.23	(L)/ALRQW/(M)	105-109	109	+	17-19	8.01	W109: +32	-
1886.3	0.41	(F)/EMKQNEPEDHFGSLLL/(M)	118-133	- f	+	21-23	8.03	-	-
3032.9	-0.43	(W)/LAENGRQTECEELLAWHLFPWSTRF/(L)	138-162	(137), 153, 158	+	17-19	3.4	-	-
3032.9	-0.43	(L)/AENGRQTECEELLAWHLFPWSTRFL/(D)	139-163	153, 158	-	17-19	3.4	-	-
2528.3	-0.04	(L)/FPWSTRFLDVFIEKAEHPFY/(R)	156-175	158	-	17-19	12.03	-	-

Table S1. Contd....

Observed Mass (m/z)	Error (Da)	Peptide Sequencea	Fragment Position	*Trpb*	UV Irradiated	Rt Range	s/n	Modificationc	Signal Peptide Bindingd
1935.4	-0.37	(W)/STRFLDVFIEKAEHPF/(Y)	159-174	*(158)*	-	21-23	4.05	-	-
2098.4	-0.38	(W)/STRFLDVFIEKAEHPFY/(R)	159-175	*(158)*	-	21-23	5.09	-	-
2151.5	-0.25	(L)/TLAQWQSQLLIPVAVKPLF/(R)	185-203	189	+	23-25	7.04	-	-
1708.4	-0.34	(W)/QSQLLIPVAVKPLFR	190-204	*(189)*	-	21-23	4.00	-	-

[a]Residues with modifications are underlined, preceding or following the cleaved fragments are in brackets and the border of the cleavage fragment indicated by /, part of the N-terminal His_6-T_7 tag are in lowercase.
[b]Numbers in brackets indicate the position of a Trp cleaved before the identified fragment.
[c]Mass addition in Da.
[d]Trp residues from the identified fragment were observed to be involved in DmsA signal peptide binding as defined by Chan *et al*. [20] and Stevens *et al.* [18].
[e]Negative values indicate amino acid positions preceding DmsD and are part of the N-terminal His_6-T_7 tag.
[f]Fragments without Trp, but showing the same mass of fragments containing Trp reported in this table.

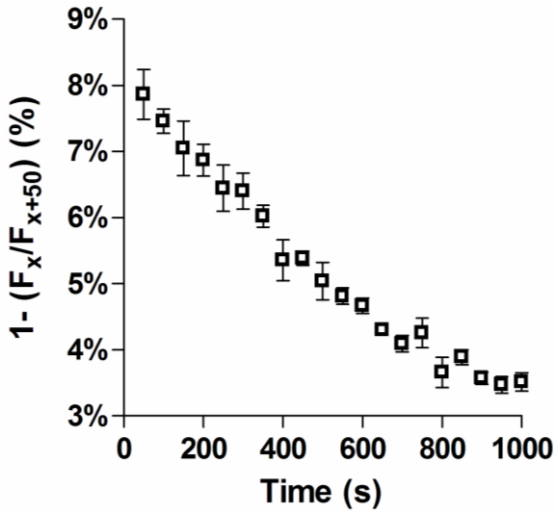

Fig. (S1). Percentage fluorescence decrease of DmsD upon steady state irradiation. The DmsD initial intensity (F) was measured, and then irradiated with steady-state UV light. The intensity was measured at a rate of 1 point/second. The percentage fluorescence decrease was calculated as a ratio between the fluorescence at time x (Fx) and the fluorescence measured 50 seconds later (Fx+50).

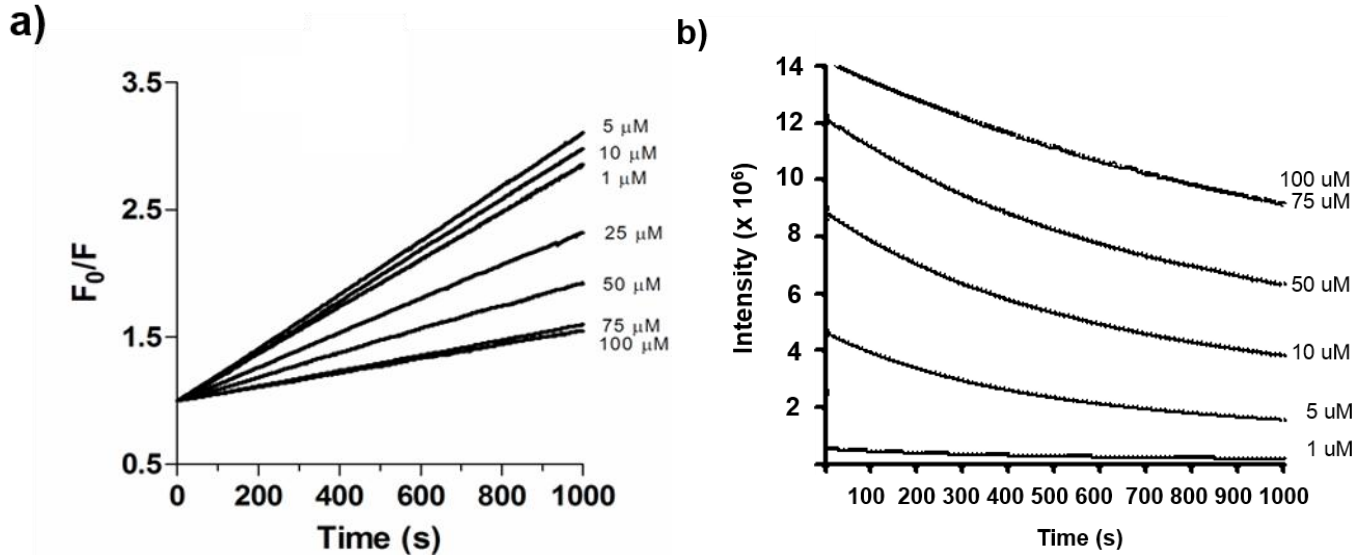

Fig. (S2). Contd....

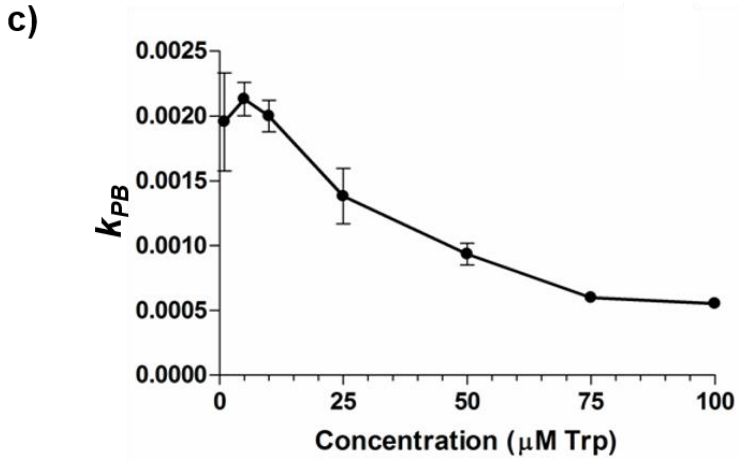

Fig. (S2). Photobleaching of DmsD is concentration dependent. **a**) Modified Stern-Volmer plot of DmsD fluorescence quenching based on different Trp concentrations over time. Scans were performed in ammonium bicarbonate buffer, pH 8.0 at 20 ^{O}C using 280 and 350 nm excitation and emission wavelengths, respectively. **b**) Fluorescence spectra of varying concentrations of DmsD-Trp. **c**) Photobleaching constant (*kPB*) at each concentration was also determined using the data in a).

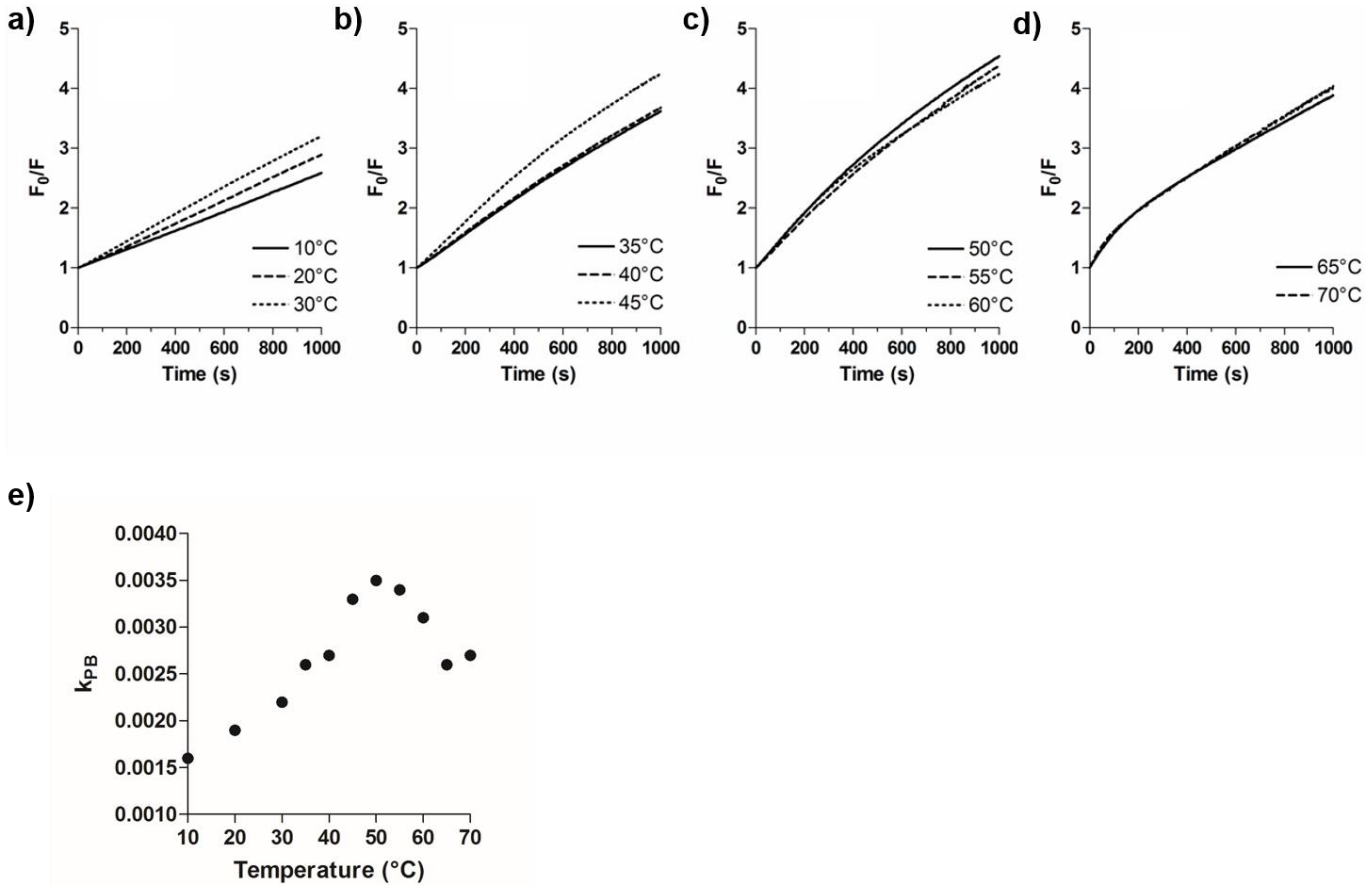

Fig. (S3). Effect of temperature on DmsD fluorescence. a-d) Modified Stern-Volmer plots of DmsD fluorescence at varying temperatures. Scans were performed with 10 μM DmsD-Trp in ammonium bicarbonate buffer, pH 8.0 at 20 ^{O}C using 280 and 350 nm excitation and emission wavelengths, respectively. e) Photobleaching constant (*kPB*) of DmsD at varying temperatures.

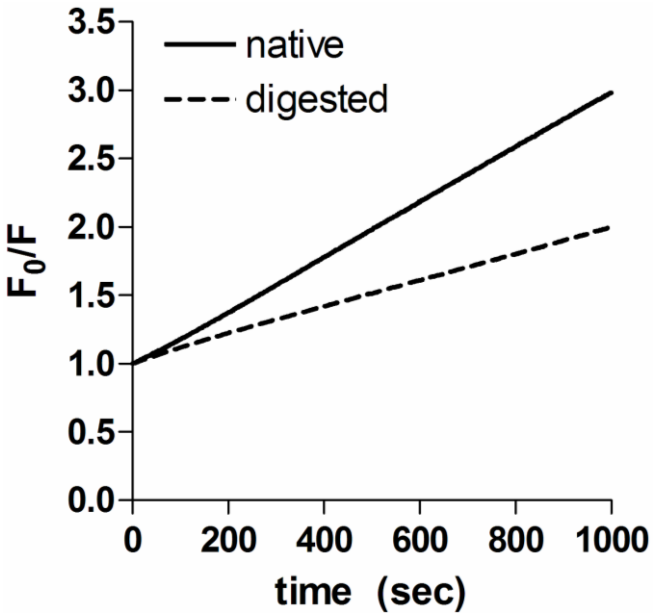

Fig. (S4). Modified Stern-Volmer plot of native versus proteinase K digested DmsD. Fluorescence quenching upon UV irradiation at 280 nm excitation and 350 nm emission wavelengths of 10 μM DmsD Trp at 20°C was measured over 1000 s. *kPB* of native DmsD was calculated as 1.98 × 10-3 s-1 and 0.999 × 10-3 s-1 for digested DmsD.

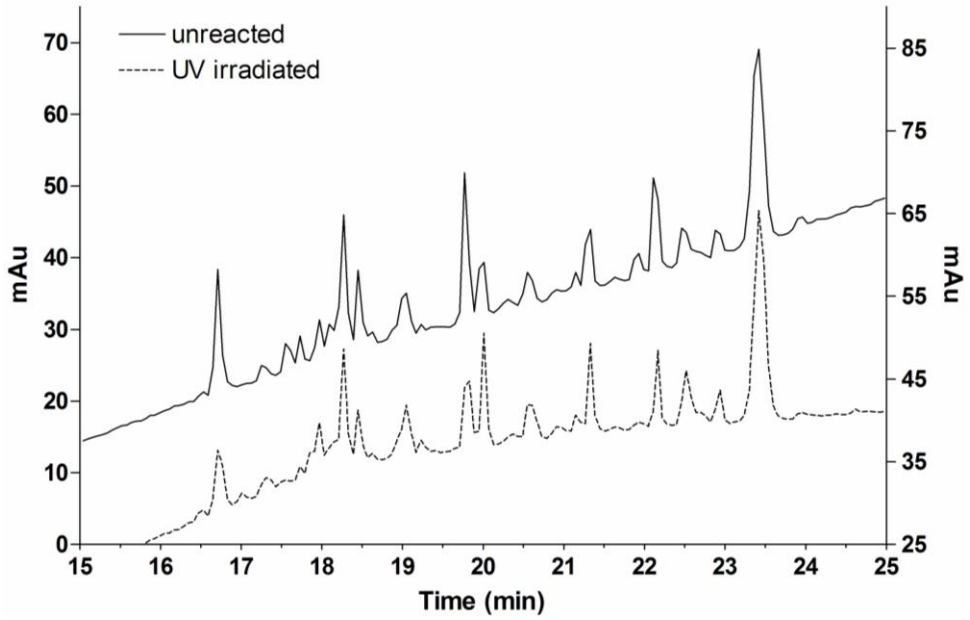

Fig. (S5). HPLC chromatogram of chymotrypsin digested DmsD. Non-irradiated samples were compared to those irradiated at 280 nm for 1000 s at 20 ^{O}C. For simplicity, only peaks eluting between 15 and 25 min are shown but peptides eluted up to 33 min.

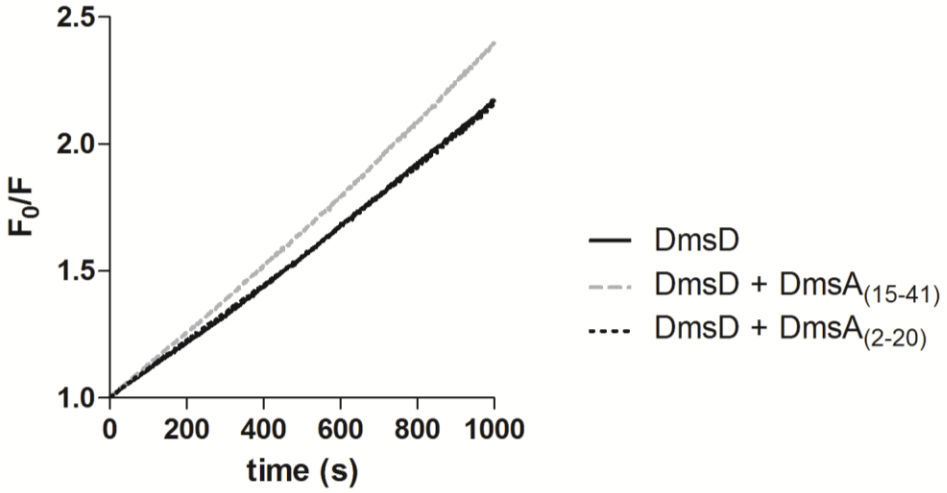

Fig. (S6). Modified Stern-Volmer plot of DmsD in the absence or presence of two-fold molar excess of DmsA signal peptides consisting of residues 2-20 and 15-41 of the *E. coli* DmsA sequence. Fluorescence quenching upon UV irradiation at 280 nm excitation and 350 nm emission wavelengths of 10 μM DmsD-Trp at 20°C was measured over 1000 s. F0/F ratios after 1000 s of irradiation was 2.1702 ± 0.0199, without peptide; 2.3966 ± 0.0111; with DmsA15-41, and 2.1559 ± 0.0311 with DmsA2-20.