

Supporting Information

Misfolding of a single disulfide bonded globular protein into a low-solubility species conformationally and biophysically distinct from the native one

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Name	Est. Mw (Da)	Cal. Mw (Da)	Error (%)
DEN4 ED3-1st sup	11376.01	11383.06	6.19×10^{-2}
DEN4 ED3-3rd ppt	11376.01	11386.62	9.32×10^{-2}

Table S1. The estimated molecular weight (Est. Mw) by MALDI-MS and calculated molecular weight (Cal. Mw) by ProtParam in DEN4 ED3-1st sup and 3rd ppt. The error was calculated by using the following formula ($|Est. Mw - Cal. Mw| / Est. Mw \times 100$).

Name	Concentration (mg/ml)	Volume (ml)	Estimated yield (mg)	A_{260}/A_{280}
1st sup	1.08	15	16.2	0.65
1st ppt	2.16	4	8.64	0.73
2nd sup	0.29	6	1.74	0.70
2nd ppt	1.94	3	5.82	0.73
3rd sup	0.17	6	1.02	0.86
3rd ppt	2.52	2	5.04	0.74

Table S2. The yield of DEN4 ED3 in the supernatant (sup) and precipitation (ppt) after dialysis, three times. The protein concentrations were measured by NanoDrop 2000 using an extinction coefficient of 280 nm. The supernatant fractions were measured as they were, and the precipitated fractions were measured by dissolving in 6 M GuHCl with 10% acetic acid (pH 2.7).

	DEN4 ED3-1st sup	DEN4 ED3-3rd ppt
pH 5.0	1.97	7.91
pH 8.0	1.61	747.2

Table S3. Hydrodynamic radius (nm, R_h) of DEN4 ED3-1st sup and 3rd ppt at pH 5.0 and 8.0 after the cleavage of a 6×histidine-tag by TEV protease-C9R. The R_h values were calculated from DLS size-volume graphs.

Peak 1	Peak 2
3.78	5.04

Table S4. Hydrodynamic radius (nm, R_h) of Peak 1 and Peak 2 obtained at RP-HPLC of DEN4 ED3-3rd ppt at pH 5.0 and 20°C with a 6×histidine-tag. The R_h values were calculated from DLS size-volume graphs.

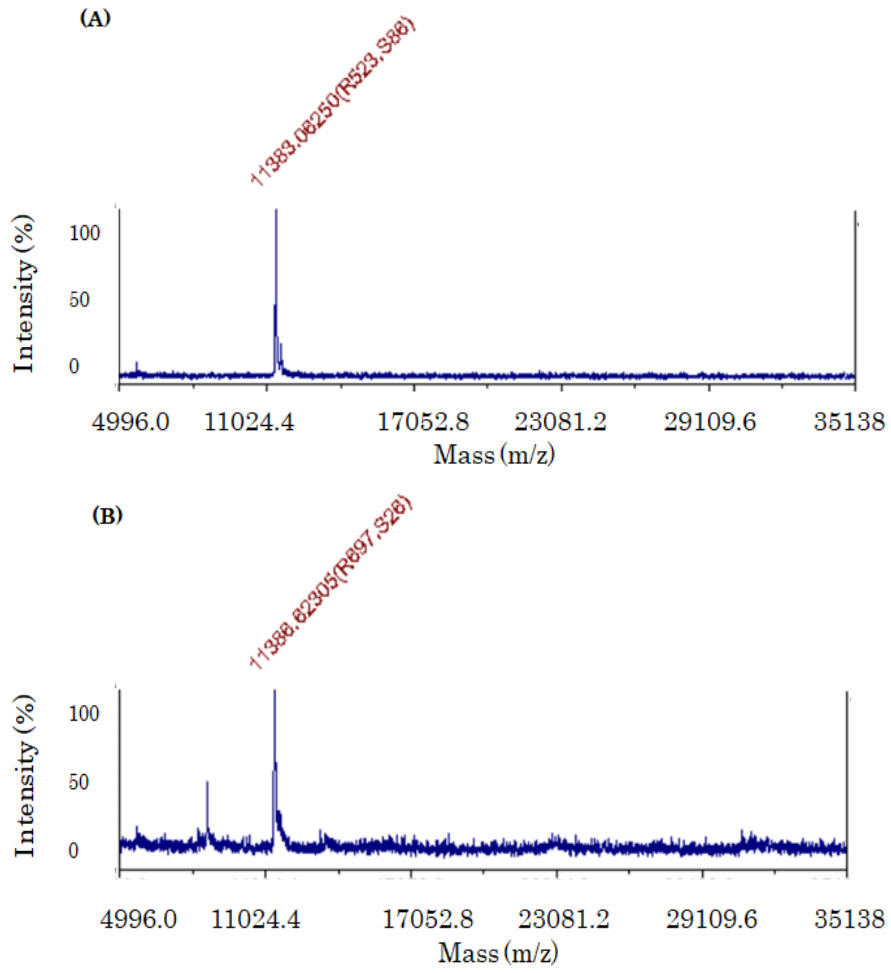


Figure S2. The MALDI-MS spectra of (A) DEN4 ED3-1st sup and (B) DEN4 ED3-3rd ppt at the protein concentration of 1 μ M.

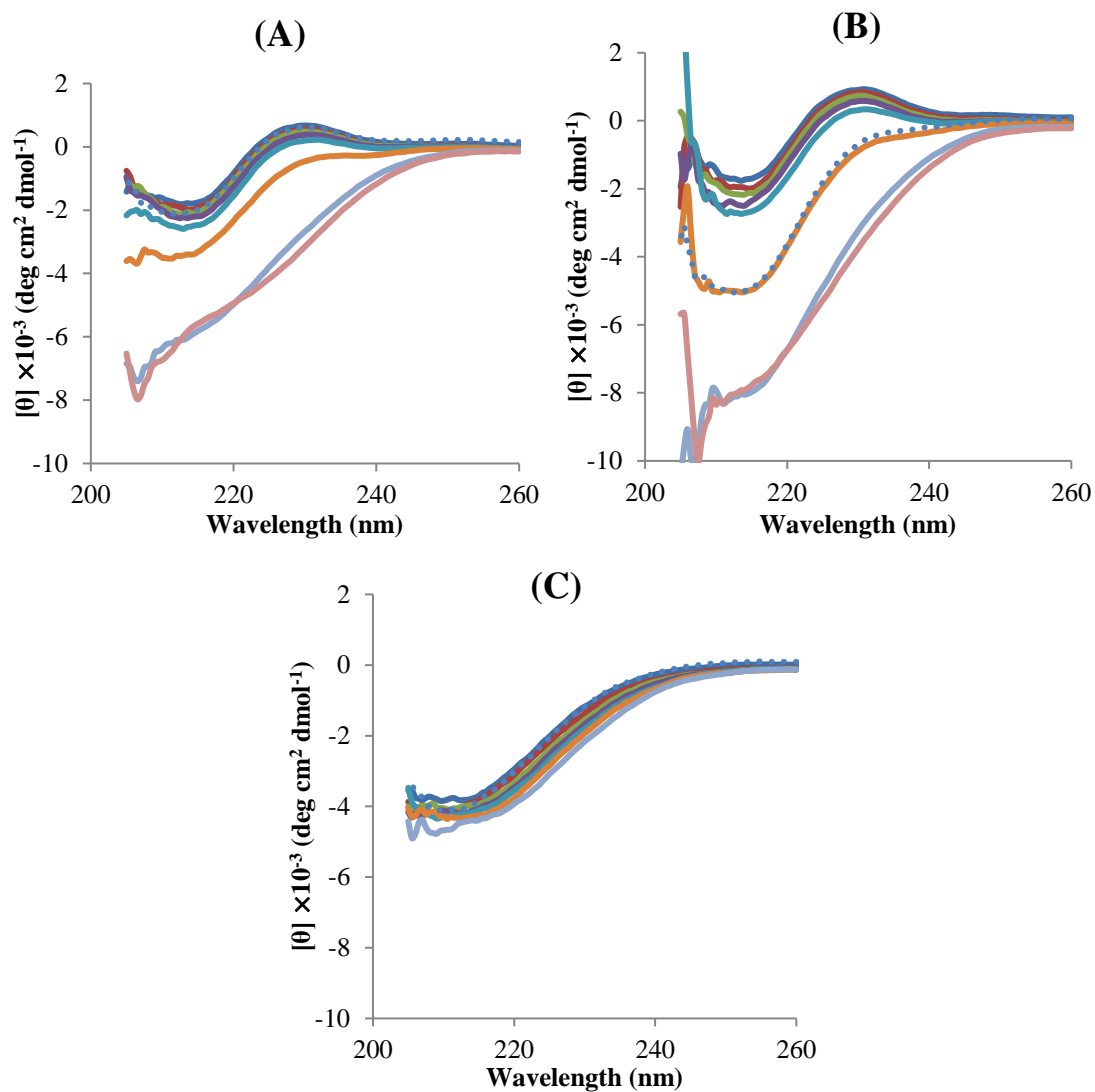


Figure S3. The temperature effect on CD spectra of (A) DEN4 ED3-1st sup (pH 5.0) (B) DEN4 ED3-1st sup (pH 8.0) (C) DEN4 ED3-3rd ppt (pH 5.0) at 0.2 mg/ml, between 20°C and 90°C. 20°C (Blue); 30°C (Red); 40°C (Green); 50°C (Purple); 60°C (Dark blue); 70°C (Orange); 80°C (Sky blue); 90°C (Pink); 20°C after heating (Blue dotted line).

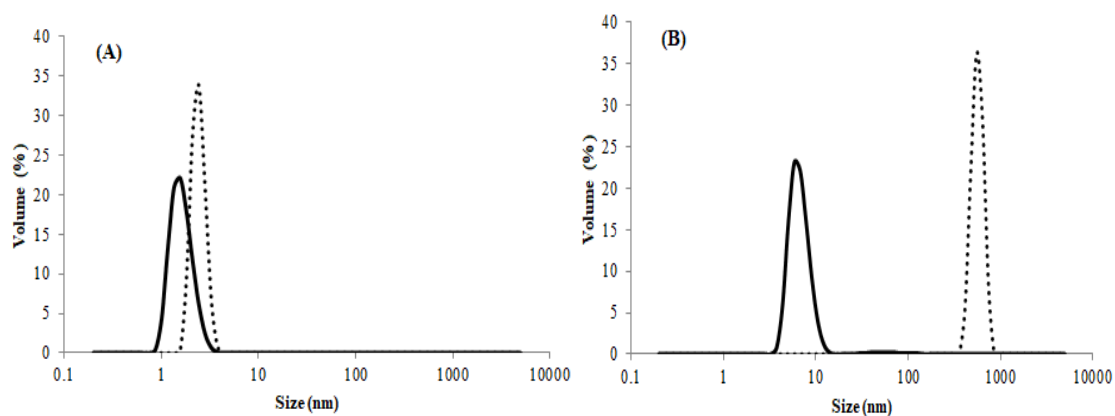


Figure S4. DLS size-volume graphs of (A) DEN4 ED3-1st sup; (B) DEN4 ED3-3rd ppt at pH 5.0 (solid line) and pH 8.0 (dotted line). The 6×histidine-tags attached to N-terminus of DEN4 ED3 were cleaved by TEV protease-C9R just before DLS measurements.