# Supplementary Information

# Protein stabilization by an amphiphilic short monodisperse oligo(ethylene glycol)

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**Contents**

## 1. General

Variable temperature circular dichroism (VT CD) spectra were recorded using JASCO J-820 spectropolarimeter. The optical path length of the cuvette was 1.0 mm. The temperature of the cuvette-holder was controlled with a Peltier-type temperature regulator JASCO PTC-4232, where the temperature was increased by 10 °C with an equilibrium time of 5 min before every measurement. UV-vis absorption spectra were recorded on JASCO V-530 UV-vis spectrophotometer. Differential scanning calorimetry (DSC) was performed using GE MicroCal VP-DSC ultrasensitive scanning calorimeter with the scan rate of 1.0 K $min^{-1}$. DSC data were analyzed with the ORIGIN software package of MicroCal. To obtain the denaturation temperatures ($T_m$), the contribution of the buffer was subtracted, the protein concentration was normalized and the resulting profile was fitted according to a two-state thermal transition model.

## 2. Reagents

Disodium hydrogen phosphate, DTT, EDTA, glutathione, glutathione disulphide, guanidinium chloride, NaOH, *M. Lysodeikticus* bacteria, sodium dihydrogen phosphate, and tris(hydroxymethyl)aminomethane were purchased from Nacalai Tesque (Kyoto, Japan). Chicken egg white lysozyme was purchased from Calbiochem (USA). Triethylamine ($Et_3N$) was purchased from Sigma-Aldrich (St. Louis, USA). These commercial reagents were used without purification. Deionized water (filtered through a 0.22 μm membrane filter, >18.2 MΩ cm) was purified in a Milli-Q system of Millipore. OEG, PhOEG and PhTEG were synthesized by the procedure reported in our previous paper.[1]

## 3. Quantitative lysozyme activity assay[2]

A bacteria suspension of *M. Lysodeikticus* (75 mg $mL^{-1}$) was freshly prepared in 66 mM sodium phosphate buffer (pH 6.2). A PBS solution of chicken egg white lysozyme (0.10 mL, pH 7.4) was rapidly mixed with 2.9 mL of the bacteria suspension in a 1.0-cm thick quartz cuvette at 25 °C. The rate of the turbidity change was monitored at 450 nm for 5 min using JASCO V-530 UV/Vis spectrophotometer. The enzymatic activity of lysozyme per minute was calculated from the slope between the absorbance of the initial 60 s and the time. The observed enzymatic activity was

expressed as the percentage of the activity of the native lysozyme evaluated under the same condition. The average of three independent measurements under the same condition was used as the activity of lysozyme. The concentration of native lysozyme in the sample was determined by GE NanoVue Plus spectrophotometer.

## 4. Thermal denaturation of lysozyme and aggregation suppression study[3]

Chicken egg white lysozyme (0.037 mM) and various concentrations of OEG, PhOEG or PhTEG were dissolved in PBS (pH 7.4). After being heated at 90 °C for 30 min, the samples were incubated at 25 °C for 30 min followed by centrifugation at 15000 rpm at 4 °C for 10 min. The enzymatic activity of the recovered lysozyme in the supernatant was evaluated by the turbidimetric bacteriolytic assay method as described in Section 3.

## 5. Oxidative refolding of lysozyme[4]

Chicken egg white lysozyme (1.4 mM) was denatured by incubating in a denaturation buffer (6.0 M GuHCl, 100 mM DTT, 1.0 mM EDTA and 100 mM Tris•HCl, pH 8.5) for overnight at 37 °C. The pH of the solution was adjusted to 4.0 with 1.0 M HCl. The resulting lysozyme solution was dialyzed twice for 8 h each at 4 °C, using a gently stirred solution of 4.0 M GuHCl, 1.0 mM EDTA and 50 mM acetic acid/NaOH (pH 4.5), to yield a stock solution of denatured and reduced lysozyme.

Oxidative refolding was initiated by rapid mixing of the denatured lysozyme with 60-fold volumes of a degassed renaturation buffer (3.0 mM glutathione, 0.30 mM glutathione disulfide, 1.0 mM EDTA, 0.10 M Tris•HCl; pH 8.2) containing 40 mM of OEG, PhOEG or PhTEG, under $N_2$ at 25 °C. The final concentration of lysozyme was 0.020 mM. The aggregation of denatured lysozyme in the absence and presence of additives (40 mM) are measured by optical density at 600 nm measured at every 2 min using a JASCO spectrophotometer V 650. The sample taken at 300 min after the initiation of refolding was subjected to the quantitative assay of the lysozyme enzymatic activity described in Section 3. The final refolding yields were expressed as percentage of the specific activity of native lysozyme.

## 6. Visual and VT-CD spectroscopic studies of thermal response of lysozyme in the presence of 1.0 mM PhOEG

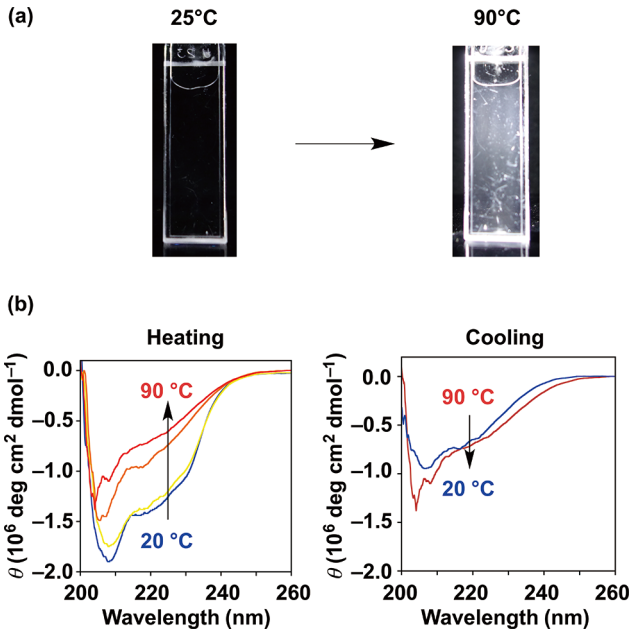


**Fig. S1** (a) Photographs and (b) VT-CD spectra of chicken egg white lysozyme (0.025 mM) in PBS (pH 7.4) containing 1.0 mM of PhOEG in a 1.0-mm thick quartz cell. Photographs were taken at 25 °C (left) and 90 °C (right). Arrows in (b) indicate the direction of spectral change upon heating (left; 20, 60, 80 and 90 °C (blue, yellow, orange, and red, respectively) and cooling (right; 90 and 20 °C (red and blue, respectively)).

## 7. DLS and TEM analyses of PhOEG

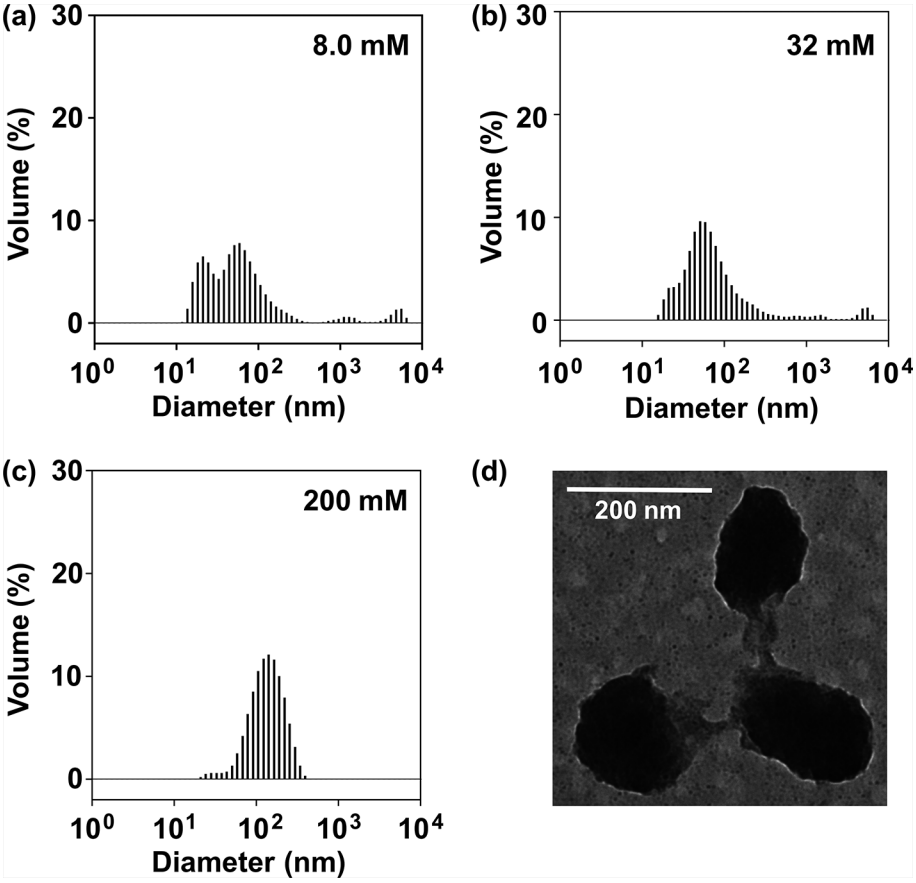


**Fig. S2** DLS size distribution profiles of PhOEG at (a) 8.0 mM, (b) 32 mM and (c) 200 mM in water at 25 °C. (d) TEM micrograph of PhOEG dried from 200 mM aqueous dispersion at 25 °C. Stain: $RuO_4$.

## 8. References


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