Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Design of Polyhedral Oligomeric Silsesquioxane (POSS) Based Thermo-Responsive Amphiphilic Hybrid Copolymers for Thermally Denatured Protein Protection Applications

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S1.1. Molar Ratios Calculation of PEPS Copolymers

The chemical compositions of the copolymer in molar ratios, represented by x, y, and z values in the macromolecules structure (in Fig. 1), was calculated from the integral data Sa, Sb and Sc, respectively, according to equations (1), (2) and (3) as shown below. These equations were derived from the three equations established for the proton integral regions of Sa, Sb and Sc, respectively. The repeat unit numbers in PEGMA and PPGMA prepolymers were calculated from the molecular weight as stated in section 2.1.

$24.8\ x + 15\ y + 2z = Sa$ (1)

$2x + 2y + 3z = Sb$ (2)

$3x + 18y + 45z = Sc$ (3)

S1.2. Thermally Denatured Protein Protection Efficiency Assay

Green Fluorescent Protein (GFP): The thermal protection of GFP by PEPS hybrid copolymers were evaluated by the intrinsic fluorescence of GFP which can be correlated to folded and unfolded structure of GFP.[1] The fluorescence spectra were recorded by using the excitation and emission wavelength of 485 and 538 nm, respectively. During the protein protection test, GFP solutions (1 μg/mL) containing different concentrations of PEPS hybrid copolymers were first heated at 40 ºC for 1 h. The heating would induce the collapse of PPG segments in PEPS copolymers into hydrophobic domains on the shell of the micelles. Next, the solutions were further heated up to 70 ºC and kept at this temperature for another 30 min. The mixture was then cooled down to room temperature and the fluorescence was recorded. The GFP thermal protection efficiency was calculated from the fluorescence intensity ratios of the cooled GFP with that of native GFP.

Lipase: The relative protection efficiency of lipase was evaluated by comparing the enzyme activities of lipase using *p*-nitrophenyl acetate (*p*NPA) as the substrate at 30 ºC.[2] During the

measurements, 1.0 mL lipase in phosphate buffer solution (pH 7.2, 25 μg/mL), with or without PEPS hybrid copolymers, were quickly mixed with 0.01 mL MeCN solution containing 130 mM pNPA. The consumption of *p*NPA was determined by monitoring the production of *p*-nitrophenol as a function of time with a UV-Vis spectrophotometer at 400 nm. The lipase protection efficiencies were obtained by comparing the initial rates of *p*NPA hydrolysis reactions catalysed by different lipase solutions. The lipase-copolymer mixed solutions were incubated at 40 ºC for 1 h prior to the thermal denaturation of lipase carried out at 70 ºC for 30 min to trigger the collapse of PPG segments into the hydrophobic domains. Subsequent protein renaturation process was initiated by cooling the mixture to room temperature.

Lysozyme: Lysozyme activities were determined from the optical dispersion decrease of micrococcus lysodeikticus cell suspension.[3] Briefly, a 0.3 mg/mL cell suspension was prepared in phosphate buffer solution, pH 7.2. To 1.0 mL of this suspension, 0.1 mL of lysozyme solution (50 μg/mL), with or without PEPS copolymers, were added and the decrease in time of absorbance at 450 nm were monitored. The slope of the linear part of the curve was related to the slope of a standard sample, and the result was expressed as the lysozyme protection efficiencies. The lysozyme denaturation and possible renaturation process was carried out under the same protocol as the lipase activity assay.

S1.3. Cellular Uptake and Cytotoxicity Assay

The cellular uptake of the prepared copolymer micelles was investigated by confocal laser scanning microscopy FV1000 (CLSM, Olympus Japan) using the green fluorescence in GFP as probe. PEPS hybrid copolymers were directly dissolved in the cell culture medium containing high concentration of GFP. The C6 Glioma cells (ATCC, USA) at densities of 4×10^4 cells/well were cultured in complete DMEM medium within confocal imaging

chamber (Lab-Tek) at 37 °C, 5% CO_2. The cell culture medium was supplemented with 10% fetal bovine serum (FBS), 100 units/mg penicillin and 100 μg/mL streptomycine. After 80% confluence, the medium was removed and the adherent cells were washed twice with 1 × PBS buffer. Fresh cell culture medium containing free GFP (10 μg/mL) or GFP mixed with PEPS hybrid copolymer at concentration of 1.0 mg/mL in the cell culture medium were then added to the chambers, respectively. After 24 h incubation, the cells were washed three times with 1 × PBS buffer followed by 4% paraformaldehyde for 15 min, which were further washed twice with 1 × PBS buffer. The cell monolayer was imaged by CLSM with imaging software under the same experimental conditions.[4]

The *in vitro* cytotoxicity test of PEPS hybrid copolymers were carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyl tetrazolium bromide (MTT) assay in metabolic activity of human dermal fibroblasts (HDFs) cell lines. HDFs cells were seeded in 96-well plates (Costar, IL, USA) at a density of 1×10^4 cells/well. After 24 h incubation, the culture media was replaced with serum-supplemented culture media containing known concentrations of the PEPS copolymers, and the cells were further incubated for 72 h. At pre-determined time intervals, 100 μL of sterile-filtered MTT stock solution in PBS (0.5 mg/mL) was added to each well, reaching a final MTT concentration of 0.5 mg/mL. After 3 h, the unreacted dye was removed by aspiration. The formazan crystals were dissolved in DMSO (100 μL/well), and the absorbance was measured using a microplate reader (Genios Tecan) at a wavelength of 570 nm. The wells containing the cells and culture medium served as controls. The relative cell viability (%) related to the control cells was calculated using the following equation; $[A]_{test}/[A]_{control} \times 100\%$, where $[A]_{test}$ is the absorbance of the wells with polymers and $[A]_{control}$ is the absorbance of the control wells.[5] All experiments were conducted with six repetitions and average values are reported.

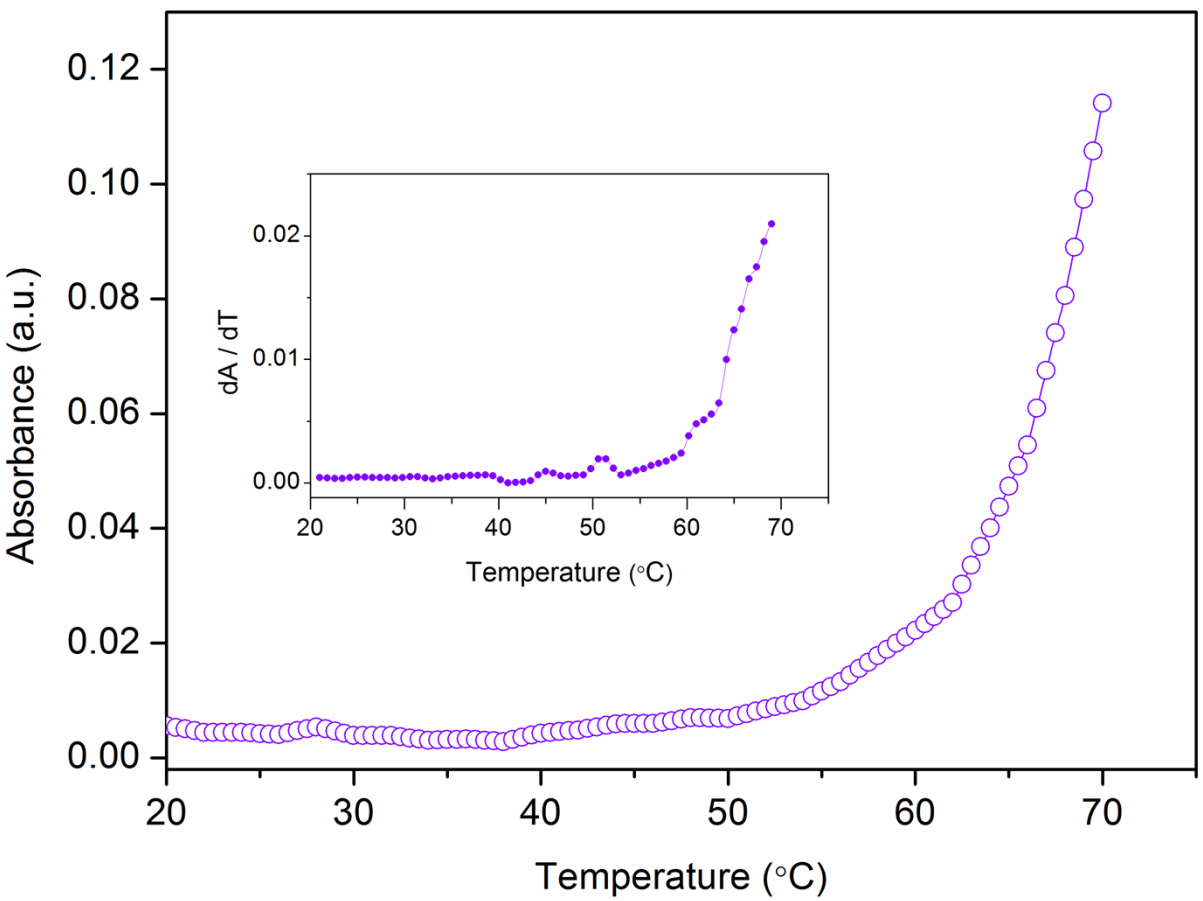

Fig. 1S. Thermo-responsive behaviour of PEPS-3 hybrid copolymer aqueous solution at 2.0 mg/mL, measured by UV-Vis spectroscopy at 530 nm. Inserted graph shows the derivative absorbance curve as a function of temperature.

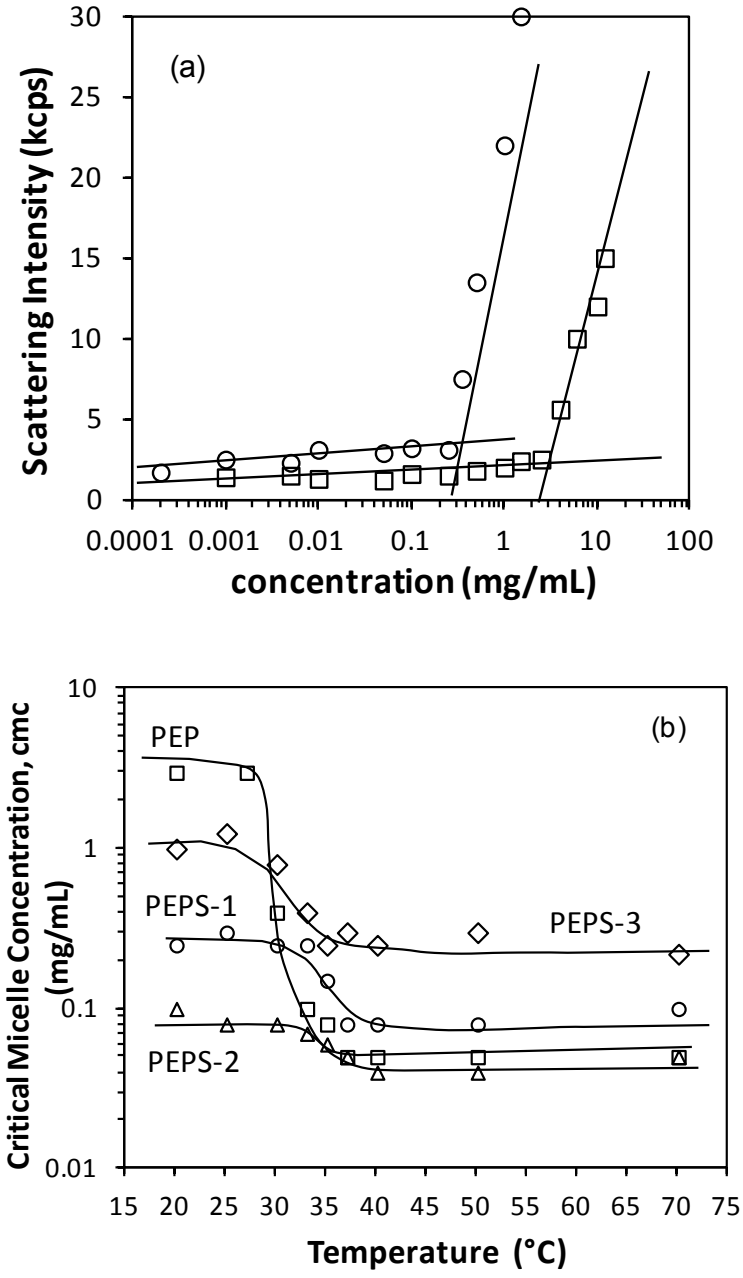

Fig. 2S: (a) Scattering intensities from DLS measurements as a function of copolymer concentration (mg/mL) in aqueous solution of PEP (square symbols) and PEPS-1 (circle symbols) respectively at 25°C. The solid lines are added to obtain the critical micelle concentration (CMC); (b) the critical micelle concentration (CMC) in aqueous solution as a function of temperature for PEP (square symbols), PEPS-1 (circle symbols), PEPS-2 (triangle symbols) and PEPS-3 (diamond symbols) respectively. The solid lines are included to guide the eye.

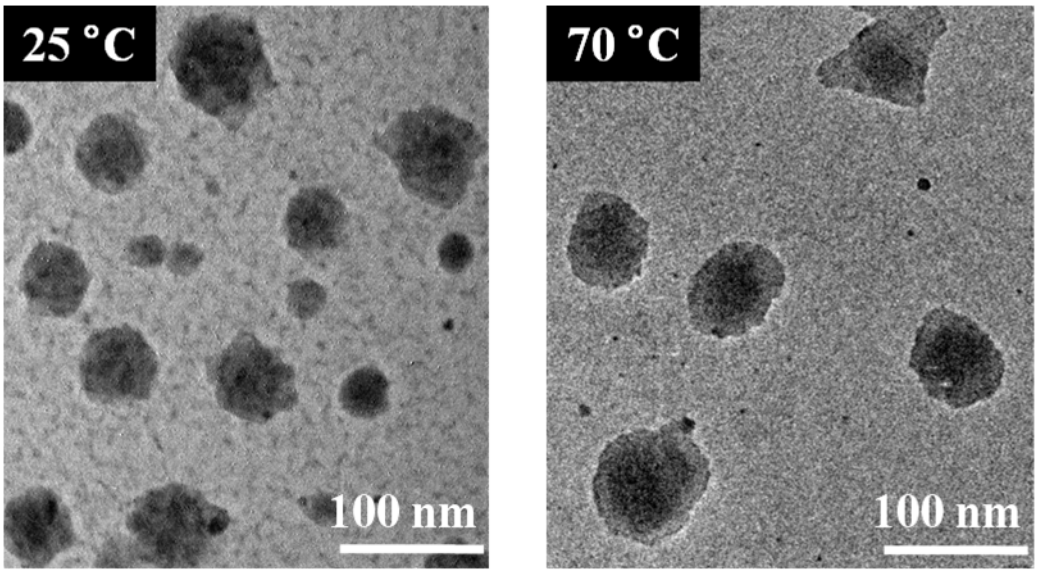

Fig. 3S TEM micrographs of particles formed in PEPS-2 copolymer aqueous solution at concentration of 0.5 mg/mL. Sizes recorded on the TEM micrographs are in good agreement with the sizes obtained using DLS.

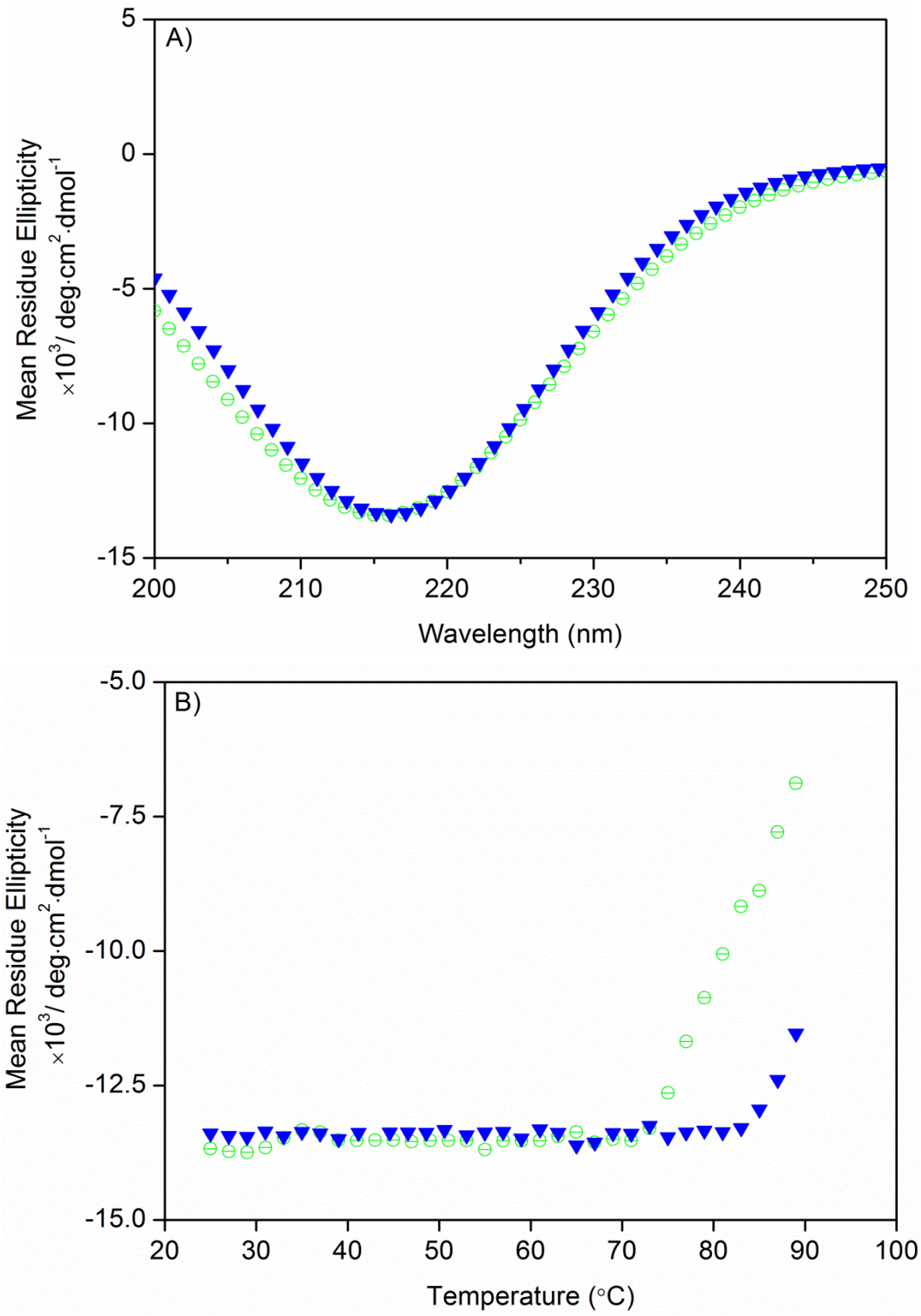

Fig. 4S. (A) Far UV-CD spectra of free lipase (open circle) and lipase mixed with PEPS-1 hybrid copolymer (close triangle) in phosphate buffer at 25 ºC. (B) Mean residue ellipticity (θ) for free lipase (open circle) and lipase mixed with PEPS-1 hybrid copolymer (close triangle) as a function of temperature in phosphate buffer.

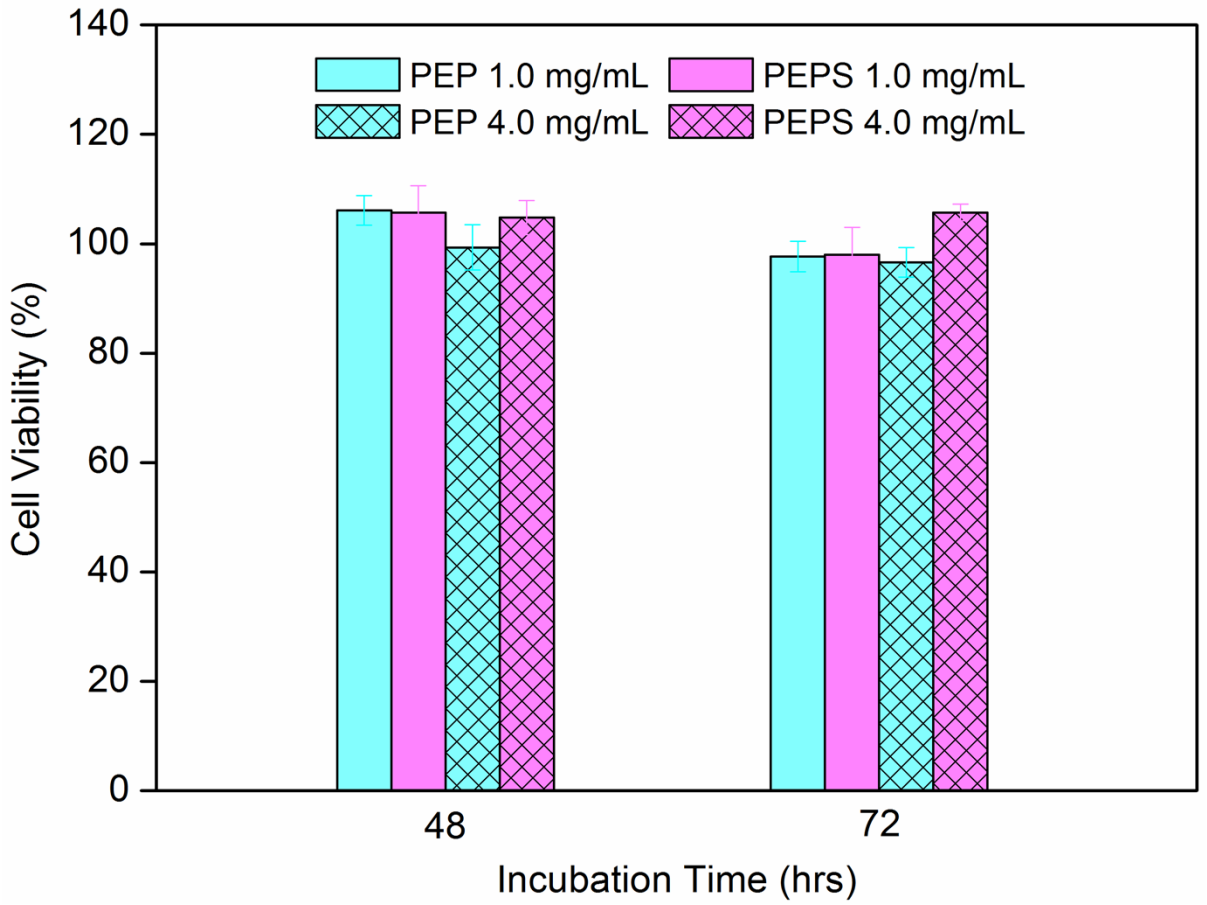

Fig. 5S. Cell viability of human dermal fibroblast cells incubated at known concentrations of PEP copolymer and PEPS-1 hybrid copolymer. Cells in blank samples are considered as 100% viable. Each column presents the average value of three independent measurements. The error bar presents the standard deviation.

Reference

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