

## Supplementary Information

### Protein Storage with Perfluorinated PEG Compartments in a Hydrofluorocarbon Solution

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#### Contents

<b>Experimental Section</b>	S2
<b>Supporting Data</b>	
<b>Figure S1.</b> <sup>1</sup> H and <sup>19</sup> F NMR spectra of <b>P1</b>	S6
<b>Figure S2.</b> DLS intensity distribution of <b>P1</b>	S7
<b>Figure S3.</b> Circular dichroism spectra of lysozyme	S7
<b>Figure S4.</b> Enzymatic activity of lysozyme	S8
<b>Scheme S1.</b> Enzymatic Hydrolysis of NBLTN by $\alpha$ -chymotrypsin	S8
<b>Figure S5.</b> Enzymatic activity of chymotrypsin	S9

## Experimental Section

### Materials

Ethyl-2-chloro-2-phenylacetate (ECPA: Aldrich, purity > 98%) was purified by distillation under reduced pressure before use. Poly(ethylene glycol) methyl ether methacrylate [PEGMA:  $\text{CH}_2=\text{C}(\text{CH}_3)\text{CO}_2(\text{CH}_2\text{CH}_2\text{O})_9\text{CH}_3$ , Aldrich,  $M_n = 500$ ] and 1*H*,1*H*,2*H*,2*H*-perfluorooctyl methacrylate (13FOMA: Wako, purity > 95%) were purified by column chromatography charged with inhibitor remover (Aldrich) and purged by argon before use.  $[\text{RuCp}^*(\mu_3\text{-Cl})_4$  (Cp\*: pentamethylcyclopentadienyl), prepared according to the literature,<sup>1</sup> and 1,2-bis(diphenylphosphino)-ethane monooxide (PO-2, Aldrich, purity > 97%) were handled in a glove box under a moisture- and oxygen-free argon atmosphere ( $\text{H}_2\text{O} < 1$  ppm,  $\text{O}_2 < 1$  ppm). *n*-Bu<sub>2</sub>NH (TCI, purity > 98%) was purged by argon before use. Tetralin (1,2,3,4-tetrahydronaphthalene, TCI, purity > 98%) as an internal standard to determine monomer conversion in <sup>1</sup>H NMR was dried over calcium chloride overnight and distilled from calcium hydride. Toluene (Kishida Chemical, purity > 99%) was purified by passing it through a purification column (Glass Contour Solvent Systems by SG Water, USA). 2*H*,3*H*-perfluoropentane [ $\text{CF}_3(\text{CHF})_2\text{CF}_2\text{CF}_3$ : 2HPFP, TCI, purity > 98% including isomers] was used as received. Lysozyme from chicken egg white (Aldrich, lyophilized powder, protein > 90%, > 40,000 units/mg protein) and  $\alpha$ -chymotrypsin from bovine pancreas (Aldrich, Type II, lyophilized powder, protein  $\geq 85\%$ ,  $\geq 40$  units/mg protein) were used as received. *Micrococcus lysodeikticus* (Aldrich) and *N*-benzoyl-*L*-tyrosine-*p*-nitroanilide (NBLTN, Aldrich) were used as received. For a phosphate buffer solution (PBS), disodium hydrogenphosphate (Wako, purity > 99%), sodium dihydrogen phosphate dehydrate (Wako, purity > 98%), and ultrapure water (Wako, for LC/MS) were used as received.

### Characterization

Number-average molecular weight ( $M_n$ ) and molecular weight distribution ( $M_w/M_n$ ) of polymers were measured by size exclusion chromatography (SEC) in DMF containing 10 mM LiBr at 40 °C (flow rate: 1 mL/min) on three linear-type polystyrene gel columns (Shodex KF-805L: exclusion limit =  $4 \times 10^6$  g/mol; particle size = 10  $\mu\text{m}$ ; pore size = 5000 Å; 0.8 cm i.d.  $\times$  30 cm) that were connected to a Jasco PU-2080 precision pump, a Jasco RI-2031 refractive index detector, and a Jasco UV-2075 UV/vis detector set at 270 nm. The columns were calibrated against 10 standard poly(methyl methacrylate) samples (Polymer Laboratories:  $M_n = 1000$ –1200000;  $M_w/M_n = 1.06$ –1.22). <sup>1</sup>H and <sup>19</sup>F nuclear magnetic resonance (NMR) spectra were recorded in D<sub>2</sub>O, CD<sub>2</sub>Cl<sub>2</sub> and

2HPFP with  $\text{CDCl}_3$  at 30 °C on a JEOL JNM-ECA500 spectrometer, operating at 500.16 ( $^1\text{H}$ ), 470.62 ( $^{19}\text{F}$ ) MHz. When  $^1\text{H}$  and  $^{19}\text{F}$  NMR measurements of polymers were conducted in 2HPFP, the capillary containing the 2HPFP solution of polymers was placed inside a NMR tube including  $\text{CDCl}_3$  (0.5 mL). The absolute weight-average molecular weight ( $M_w$ ) of polymers was determined by multi-angle laser light scattering (MALLS) equipped with SEC (three linear-type polystyrene gel columns: Shodex KF-805L) in DMF containing 10 mM LiBr at 40 °C on a Dawn HELEOS II instrument (Wyatt Technology, semiconductor laser,  $\lambda = 663$  nm). The refractive index increment ( $dn/dc$ ) was measured in DMF at 40 °C on an Optilab DSP refractometer (Wyatt Technology,  $\lambda = 690$  nm,  $c < 4.4$  mg/mL). Dynamic light scattering (DLS) measurements were conducted on Otsuka Photal ELSZ-0 equipped with a semiconductor laser ( $\lambda = 658$  nm) at 25 °C ( $[\text{polymer}] = 50$  mg/mL in  $\text{H}_2\text{O}$  or 2HPFP). The measuring angle was 165°, and the data were analyzed by CONTIN method. Circular dichroism (CD) measurements were performed on Jasco J-1500 CD spectrometer in  $\text{H}_2\text{O}$ , PBS, and 2HPFP at 25 °C (optical path length = 0.1 cm, sensitivity: standard, response: 1.0 sec, band width: 1.0 nm, data pitch: 0.2 nm, scanning speed: 20 nm/min). Transmission electron microscopy measurements of polymers were performed on HT7700 (Hitachi) at an accelerating voltage of 100 kV. The samples were prepared by drop cast from the 2HPFP solutions of polymers (10 mg/mL) and lysozyme (0.05 mg/mL) on carbon coat grids (OKENSHOJI; C20-C10 STEM Cu100P); the cast samples were then stained with vapor of the 1% aqueous solution of  $\text{OsO}_4$ . Ultraviolet-visible (UV/Vis) spectra were recorded PBS at 25 °C UV-1800 (Shimadzu, optical path length = 0.1 or 1.0 cm) for the activity study of lysozyme.

## Polymer synthesis

The synthesis of polymers was carried out by syringe technique under argon in baked glass tube equipped with a three-way stopcock.

**P1** (PEGMA/13FOMA random copolymer):<sup>S1</sup> A ruthenium complex stock solution was first prepared by mixing  $[\text{RuCp}^*(\mu_3\text{-Cl})_4]$  (0.025 mmol, 27 mg) and PO-2 (0.20 mmol, 82 mg) in toluene (3.3 mL) in a baked glass tube at 60 °C for 12 h. Toluene (7.0 mL), tetralin (0.4 mL), a 400 mM toluene solution of  $\text{Bu}_2\text{NH}$  (0.18 mmol, 0.45 mL), PEGMA (5.4 mmol, 2.5 mL), 13FOMA (3.6 mmol, 1.04 mL), and a 670 mM toluene solution of ECPA (0.036 mmol, 0.054 mL) were sequentially added into a glass tube in this order at 25 °C under argon. Into the mixture, the Ru stock solution (0.6 mL) was added into the solution at 25 °C (total volume: 12 mL). The flask with the mixture was placed in an oil bath kept at 80 °C. After 33 h, the reaction was terminated by cooling the mixture to -78 °C. The conversion of PEGMA and 13FOMA was determined as 72% and 81%, respectively, by  $^1\text{H}$  NMR with tetralin as an internal standard. The crude product was purified by silica gel column chromatography with toluene as an eluent and was dialyzed

against methanol and water with a regenerated cellulose membrane (Spectra/Por<sup>®</sup> 7; MWCO 15000). The inner aqueous solution was lyophilized to give a colorless solid product (**P1**). SEC (DMF, PMMA std.):  $M_n = 68200$  g/mol;  $M_w/M_n = 1.30$ .  $dn/dc$  (DMF) = 0.012. SEC-MALLS (DMF, 0.01 M LiBr):  $M_w = 177000$  g/mol. <sup>1</sup>H NMR [500 MHz, acetone-*d*<sub>6</sub>, 30 °C,  $\delta = 2.05$  (acetone)]:  $\delta$  7.3–7.1 (aromatic), 6.2, 5.7 (olefin), 4.5–4.2 (-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CF<sub>2</sub>-), 4.2–4.0 (-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO-), 3.8–3.7 (-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>O-), 3.7–3.6 (-OCH<sub>2</sub>CH<sub>2</sub>O-), 3.6–3.4 (-CH<sub>2</sub>OCH<sub>3</sub>), 3.4–3.3 (-OCH<sub>3</sub>), 2.8–2.6 (-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CF<sub>2</sub>-), 2.2–1.4 (-CH<sub>2</sub>-), 1.4–0.7 (-CCH<sub>3</sub>). <sup>19</sup>F NMR [470 MHz, acetone-*d*<sub>6</sub>, 30 °C,  $\delta = -76.5$  ppm (CF<sub>3</sub>COOH in CDCl<sub>3</sub>)]:  $\delta$  -81.4 – -82.0 (-CF<sub>3</sub>), -113.3 – -114.4 (-CH<sub>2</sub>CF<sub>2</sub>-), -122.0 – -122.7 (-CH<sub>2</sub>CF<sub>2</sub>CF<sub>2</sub>CF<sub>2</sub>-), -123.1 – -123.7 (-CF<sub>2</sub>CF<sub>2</sub>CF<sub>3</sub>), -123.7 – -124.4 (-CH<sub>2</sub>CF<sub>2</sub>CF<sub>2</sub>-), -126.4 – -127.1 (-CF<sub>2</sub>CF<sub>3</sub>). A PEGMA homopolymer was synthesized with the same initiating/catalytic system as **P1** in the absence of 13FOMA.

**References:** (S1) Koda, Y.; Terashima, T.; Sawamoto, M. *Macromolecules* **2016**, *49*, 4534-4543.

### Enzymatic Activity Study of Lysozyme

**Original Lysozyme:** A PBS (pH = ~ 7.0) solution of *Micrococcus lysodeikticus* (0.17 mg/mL, pH = ~ 7.0) substrate was prepared, where the absorbance at 450 nm was about 0.6. The substrate solution (2.9 mL) was mixed with the PBS (pH = ~ 7.0) solution of lysozyme (25 µg/mL, 0.1 mL). Without any exposure, the mixture ([lysozyme] = 0.83 µg/mL) was immediately analyzed by UV/vis spectroscopy where the decrease in absorbance at 450 nm was monitored at 25 °C for two minutes ( $Abs_{2min} - Abs_{0min}$ ). One activity unit (AU) was defined as a change in absorbance of 0.001 per minute:  $1AU = (Abs_{2min} - Abs_{0min}) / (0.001 \times 2)$ . The activity study was repeated 5 times and the average ( $\langle AU \rangle_{original}$ ) was determined.

**Lysozyme Extracted from 2HPFP:** Lysozyme was dissolved in ultrapure water ([lysozyme] = 25 mg/mL). The aqueous solution (0.01 mL) was mixed with the 2HPFP solution of **P1** in (5.0 mg/mL, 9.99 mL) in a 50 mL centrifuge tube ([lysozyme]/[**P1**] = 0.025/5.0 mg/mL, 10 mL) and the mixture was kept at room temperature for 24 or 48 h. Into the solution, PBS (pH = ~ 7.0, 10 mL) was then added to extract lysozyme. The bilayer solution was centrifuged to give transparent PBS upper layer containing lysozyme and 2HPFP lower layer (6000 rpm, 30 min, r.t.). The upper lysozyme solution (25 µg/mL, 0.1 mL) was then added into the PBS solution of *Micrococcus lysodeikticus* (0.17 mg/mL, 2.9 mL, absorbance at 450 nm = ~ 0.6). The mixture ([lysozyme] = 0.83 µg/mL) was immediately analyzed by UV/Vis spectroscopy where the decrease in absorbance at 450 nm was monitored at 25 °C for two minutes ( $Abs_{2min} - Abs_{0min}$ ). One activity unit (AU) was defined as a change in absorbance of 0.001 per minute:  $1AU = (Abs_{2min} - Abs_{0min}) / (0.001 \times 2)$ . The activity study was repeated 5 times and the average ( $\langle AU \rangle$ ) was determined. Then, the enzymatic activity ( $\langle AU \rangle$ ) was normalized by  $\langle AU \rangle_{original}$ . Similarly, lysozyme extracted from

CH<sub>2</sub>Cl<sub>2</sub> was also applied to activity study. All the *p*-values were calculated using the independent Student's t-test. Significance was accepted at *p* < 0.001.

### Enzymatic Activity Study of $\alpha$ -Chymotrypsin

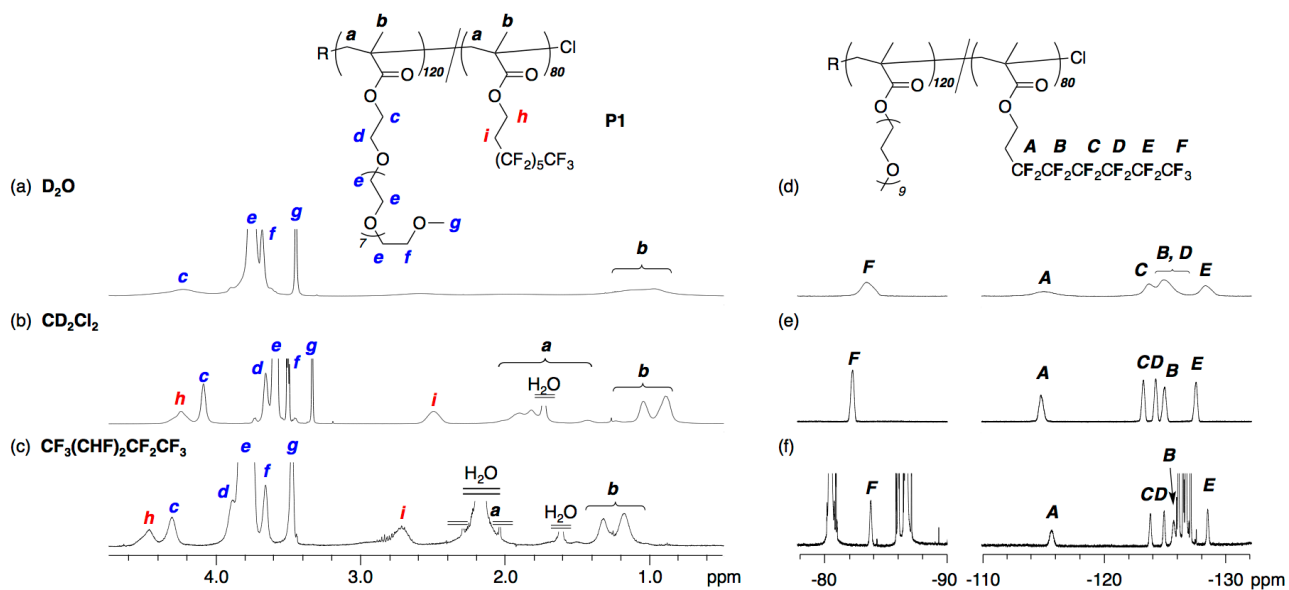
All tubes, solvents and a 2HPFP solution of **P1** (**[P1]** = 50 mg/mL) were cooled in a refrigerator (~ 4 °C) overnight before use.

**Original  $\alpha$ -Chymotrypsin:**  $\alpha$ -Chymotrypsin was solubilized in cold pure water (original aqueous solution; [ $\alpha$ -chymotrypsin] = 18 mg/mL). The solution was diluted 1000 times before the activity assay. A DMF solution of *N*-benzoyl-*L*-tyrosine-*p*-nitroanilide (NBLTN, 400 mg/mL, substrate solution) was prepared. The substrate solution (0.5  $\mu$ L) was mixed with the aqueous solution of  $\alpha$ -chymotrypsin (18  $\mu$ g/mL, 500  $\mu$ L); the mixture was then immediately analyzed by UV/vis spectroscopy where the absorbance at 410 nm was monitored at 37 °C for 300 seconds. The increase of absorbance per second was calculated as enzymatic activity between 50 sec and 300 sec [slope = (Abs<sub>300sec</sub> - Abs<sub>50sec</sub>)/250]. The activity tests were repeated 5 times and the average was determined. After kept at 4 °C (in a refrigerator) for 3 or 5 hr, the activity was evaluated.

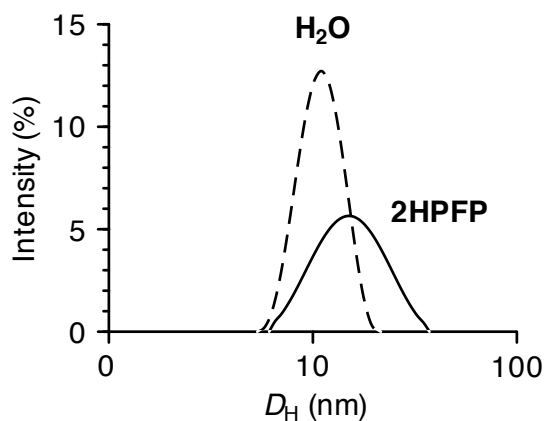
**Storage of  $\alpha$ -Chymotrypsin in H<sub>2</sub>O (Diluted Condition):** The original aqueous solution of  $\alpha$ -chymotrypsin (1.0  $\mu$ L, prepared above) was diluted with cold water (999  $\mu$ L), and the mixture was kept at ~ 4 °C ([ $\alpha$ -chymotrypsin] = 18  $\mu$ g/mL) for 3 or 24 hr. Into the aqueous solutions of  $\alpha$ -chymotrypsin (500  $\mu$ L), a DMF solution of NBLTN (400 mg/mL, 0.5  $\mu$ L) was added; the mixtures were immediately analyzed by UV/vis spectroscopy where the absorbance at 410 nm was monitored at 37 °C for 300 seconds. The increase of absorbance per second was calculated between 50 sec and 300 sec [slope = (Abs<sub>300sec</sub> - Abs<sub>50sec</sub>)/250]. The activity tests were repeated 5 times and the average was determined.

**$\alpha$ -Chymotrypsin Extracted from 2HPFP:** The original aqueous solution of  $\alpha$ -chymotrypsin (18  $\mu$ g/mL, 5.0  $\mu$ L) was mixed with the cold 2HPFP solution of **P1** (50 mg/mL, 4995  $\mu$ L) in a 15 mL centrifuge tube ([ $\alpha$ -chymotrypsin]/[**P1**] = 0.018/50 mg/mL, 5 mL), and the mixture was kept at ~ 4 °C (in a refrigerator) for 0, 3, or 24 hours. Into the mixtures, cold pure water (5 mL) was added to extract chymotrypsin. The bilayer solutions were centrifuged to give transparent H<sub>2</sub>O upper layer containing  $\alpha$ -chymotrypsin (6000 rpm, 30 min, 20 °C). Into the aqueous solutions of  $\alpha$ -chymotrypsin (18  $\mu$ g/mL, 500  $\mu$ L), a DMF solution of NBLTN (400 mg/mL, 0.5  $\mu$ L) was added; the mixtures were immediately analyzed by UV/vis spectroscopy where the increase of absorbance at 410 nm was monitored at 37 °C for 300 seconds. The increase of absorbance per a second was calculated between 50 sec and 300 sec [slope = (Abs<sub>300sec</sub> - Abs<sub>50sec</sub>)/250]. The activity tests were repeated 5 times and the average was determined. All the *p*-values were calculated using the independent Student's t-test. Significance was accepted at *p* < 0.05.

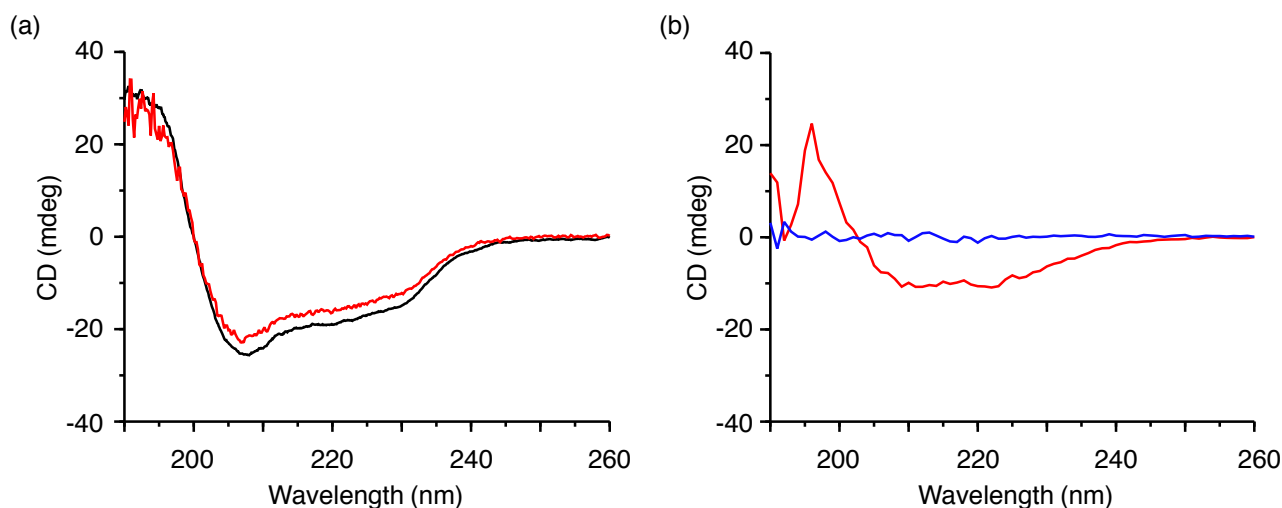
## Supplementary Data



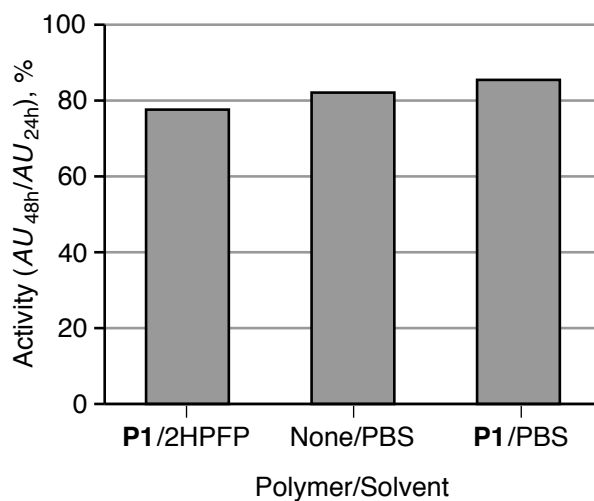
**Figure S1.** (a-c)  $^1H$  NMR (500 MHz) and (d-f)  $^{19}F$  NMR (470 MHz) spectra of **P1** in (a,d)  $D_2O$ , (b,e)  $CD_2Cl_2$ , and (c,f) 2HPFP at 30 °C.



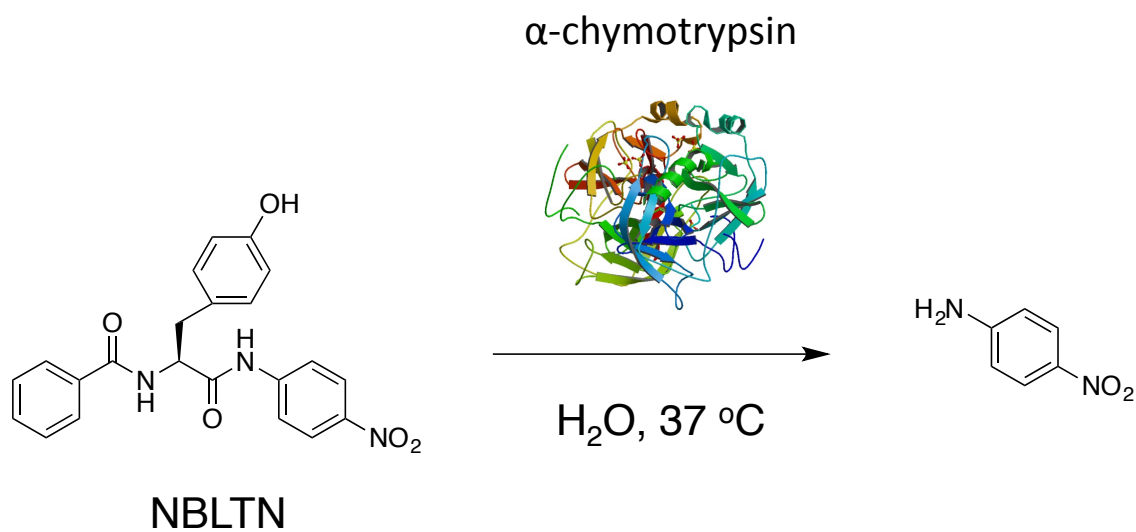
**Figure S2.** DLS intensity distribution of **P1** in H<sub>2</sub>O (dash) and 2HPFP (solid) at 25 °C: [Polymer] = 10 mg/mL in H<sub>2</sub>O or 2HPFP.



**Figure S3.** (a) Circular dichroism (CD) spectra of (a) lysozyme in water (black) and lysozyme with **P1** after exposure to 2HPFP for 24 h (red) in PBS (pH = ~ 7.0). (b) CD spectra of the 2HPFP mixture (filtrate) of lysozyme and a PEGMA homopolymer (blue) or that of lysozyme and **P1** (red): [lysozyme] = 0.25 mg/mL.

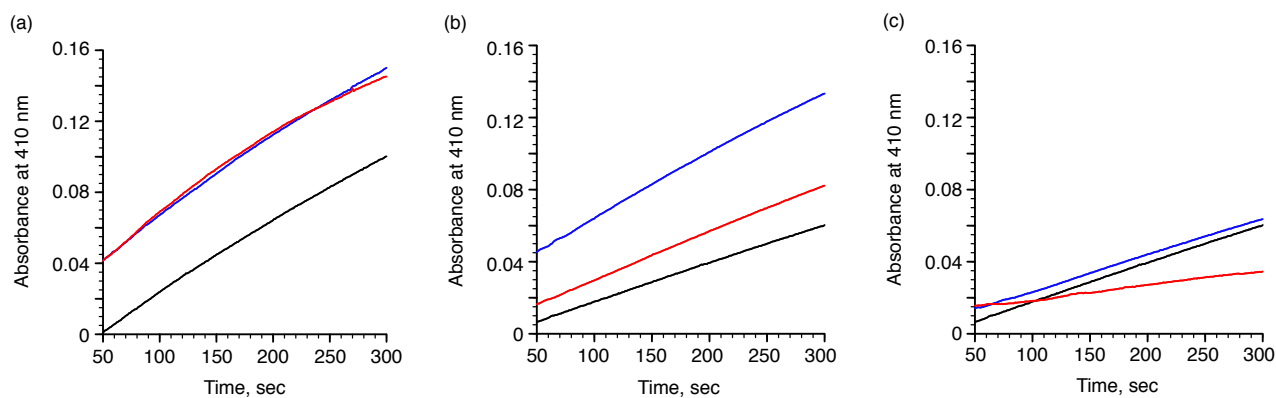


**Figure S4.** Enzymatic activity ( $\langle AU \rangle_{2\text{day}}/\langle AU \rangle_{1\text{day}}$ ) of lysozyme exposed to PBS (with or without **P1**) or 2HPFP for 48 h against that for 24 h.



**Scheme S1.** Enzymatic hydrolysis of *N*-benzoyl-*L*-tyrosine-*p*-nitroanilide (NBLTN) to *p*-nitroaniline with  $\alpha$ -Chymotrypsin in  $H_2O$  at  $37^\circ C$ .





**Figure S5.** Enzymatic activity of  $\alpha$ -chymotrypsin for the hydrolysis of *N*-benzoyl-*L*-tyrosine-*p*-nitroanilide (NBLTN) in H<sub>2</sub>O at 37 °C ( $\alpha$ -chymotrypsin = 18  $\mu$ g/mL, NBLTN = 986  $\mu$ M), monitored at 410 nm by UV/Vis spectroscopy. The  $\alpha$ -chymotrypsin was employed after storage (a) in 2HPFP with **P1** or (b, c) in H<sub>2</sub>O:  $\alpha$ -chymotrypsin/**P1** = (a) 0.018/50 mg/mL in 2HPFP for 0 (black), 3 (blue) or 24 (red) hr, (b) 0.018/0 mg/mL in water for 0 (black), 3 (blue) or 24 (red) hr, and (c) 18/0 mg/mL in water for 0 (black), 3 (blue) or 5 (red) hr.