Supporting Information

Thermo- and oxidation-responsive homopolypeptide: Synthesis, stimuli-responsive property and antimicrobial activity

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Materials

Acryloyl chloride (98%), 4-(chloromethyl)benzoyl chloride (98%), 3-chloro-1-propanol (98%), N,N,N',N'-tetramethylguanidine (TMG, 98%), triphosgene (99.5%), NaI (98%), and $NaBF_4$ (98%), 1-butylimidazole (98%) were purchased from Energy Chemical. 2-Mercaptoethanol (98%) was purchased from Alfa Aesar. $_L$-Glutamic acid copper salt was synthesized by a reported procedure.[1] Anhydrous tetrahydrofuran (THF, 99%) and *N*,*N*-dimethylformamide (DMF, 99.9%) were dried over molecular sieves before use. Chloroform-*d* ($CDCl_3$, D.99.8%) was purchased from Cambridge Isotope Laboratories, Inc. Dimethyl sulfoxide-d_6 (DMSO-d_6, 99.9 atom % D, contains 0.03% v/v TMS), and $_L$-glutamic acid (99%) were purchased from Sigma-Aldrich. Deionized water (DI-H_2O) was obtained from Aquapro AR1-100L-P11 water-purification system (Ever Young Enterprises Development Co., Ltd., P. R. China). Luria−Bertani (LB) broth and gram-positive *Staphylococcus aureus* (*S. aureus*; ATCC 6538) were purchased from Dingguo Biotechnology Co., Ltd., R. R. China. Propargyl functionalized oligo(ethylene glycol) (Pr-OEG_7) was prepared according to a reported procedure.[2]

Instrumentation

^{1}H nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a Bruker ARX400 MHz spectrometer at room temperature. Chemical shifts (δ) were reported in the unit of ppm and referred to the protonic impurities. The polymer solution with concentrations of ~10 $mg \cdot mL^{-1}$ for ^{1}H NMR test was prepared by directly mixing and shaking at room temperature. Gel permeation chromatography (GPC) measurements were performed on a PL-GPC120 setup equipped with a column set consisting of two PL gel 5 μm MIXED-D columns (7.5 mm × 300 mm, effective molar mass range of 0.2-400.0 kg·mol^{-1}) and PL-RI differential refractive index (DRI) detector. DMF containing 0.01 M LiBr was used as the eluent at 80 °C at a flow rate of 1.0 mL·min^{-1}. Narrowly distributed polystyrene standards in the molar mass range of 2.95-871 kg·mol^{-1} (PSS, Mainz, Germany) were utilized for calibration. Polymer solutions for the GPC test with a concentration of 5 mg·mL^{-1} in 0.01 M LiBr/DMF were prepared by directly mixing and shaking at room temperature. FTIR spectra were recorded on a Thermo Scientific Nicolet 6700 FTIR spectrometer equipped with an attenuated total reflection (ATR) sample holder. Solid samples were placed on the diamond crystal window and pressed with a metal probe. Spectral measurements were carried out in the transmittance mode (scan range = 4000-600 cm^{-1}, resolution = 2 cm^{-1}, number of scans = 2, 25 °C). Circular dichroism (CD) measurements were carried out on a Jasco J820 CD spectrometer (Japan Spectroscopic Corp.). The polymer aqueous solutions were prepared at concentrations of 2 mg·mL^{-1} by directly ultrasonic dissolving at room temperature. Then, the above solutions (2 mg·mL^{-1}) were diluted to 0.2 mg·mL^{-1} for CD measurement. The solution was placed in a quartz cell with a path length of 1.0 cm. CD data were collected with the high tension voltage (i.e., the voltage applied to the photomultiplier) less than 600 V. Three scans were conducted and averaged between 190-250 nm with a resolution of 0.5 nm. The data were processed by subtracting the solvent (i.e., PBS) background and smoothing with FFT-Filter method with points of window of 8. The CD spectra were reported in mean residue ellipticity (MRE) (unit: deg·cm^2·dmol^{-1}) which was calculated by the equation $[\theta]_\lambda = \mathrm{MRW} \times \theta_\lambda/10 \times \mathrm{d} \times \mathrm{c}$, where MRW is the mean residue weight (MRW = the molecular weight of polypeptide repeating unit), θ_λ is the observed ellipticity (mdeg) at the wavelength λ (i.e., 222 nm), d is the path length (mm) and c is the concentration (mg·mL^{-1}).[3] The fractional helicity (f_H) of the polypeptides was calculated using the equation $f_H = (-[\theta]_{222} + 3{,}000)/39{,}000$ to allow for a quantitative comparison of the relative helical content, where $[\theta]_{222}$ is the mean residue ellipticity at 222 nm.[4] Ultraviolet–visible (UV–vis) spectra were measured using an Agilent Cary 100 spectrometer. The polymer solutions were prepared by stirring at temperatures above respective UCST-type cloud point temperature (T_{cp}) or below respective LCST-type T_{cp} and then placed in a quartz cell with a path length of 1.0 cm. The solutions were cooled from high temperatures to low temperatures for UCST-type transitions or from low temperatures to high temperatures for LCST-type transitions with initial stabilization of 20 min. The transmittance at 500 nm was recorded at every 2 °C decrement after a 5 min thermal equilibration at each measurement. T_{cp} was determined at 50% of transmittance in the cooling cycle for UCST-type transitions or in the heating cycle for LCST-type transitions.

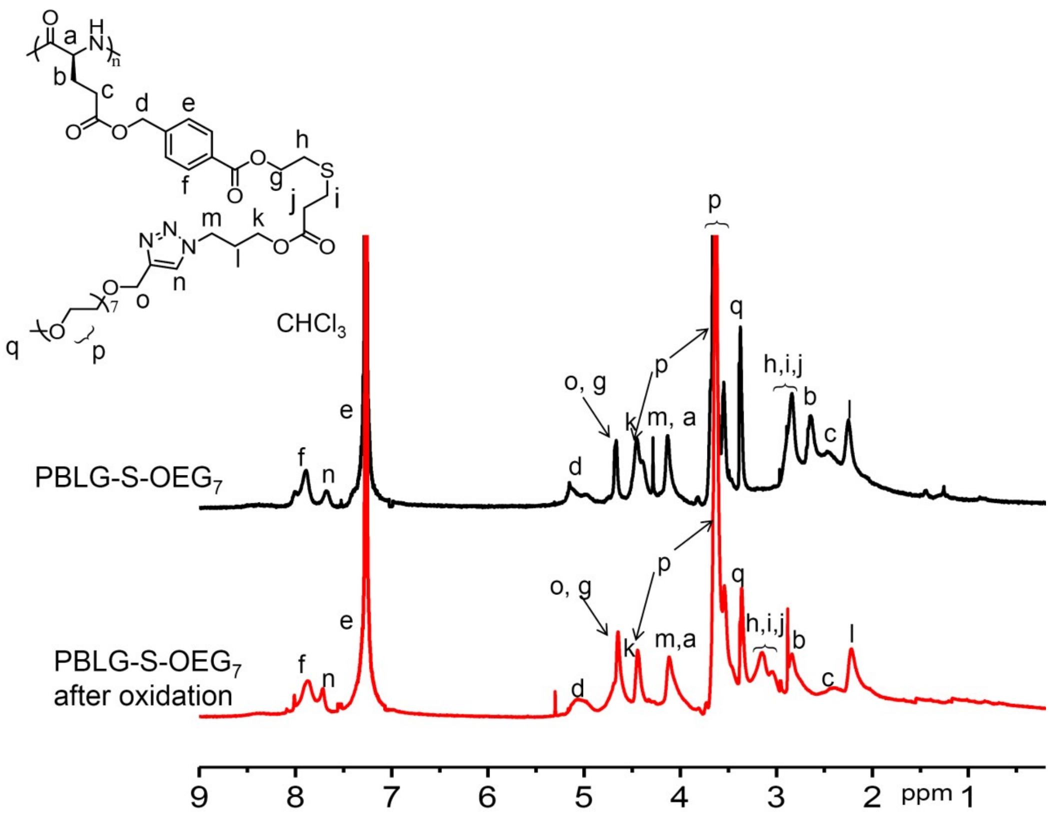

Figure S1. ^{1}H NMR spectra of PBLG-S-OEG$_7$ and H_2O_2 oxidized PBLG-S-OEG$_7$ in $CDCl_3$.

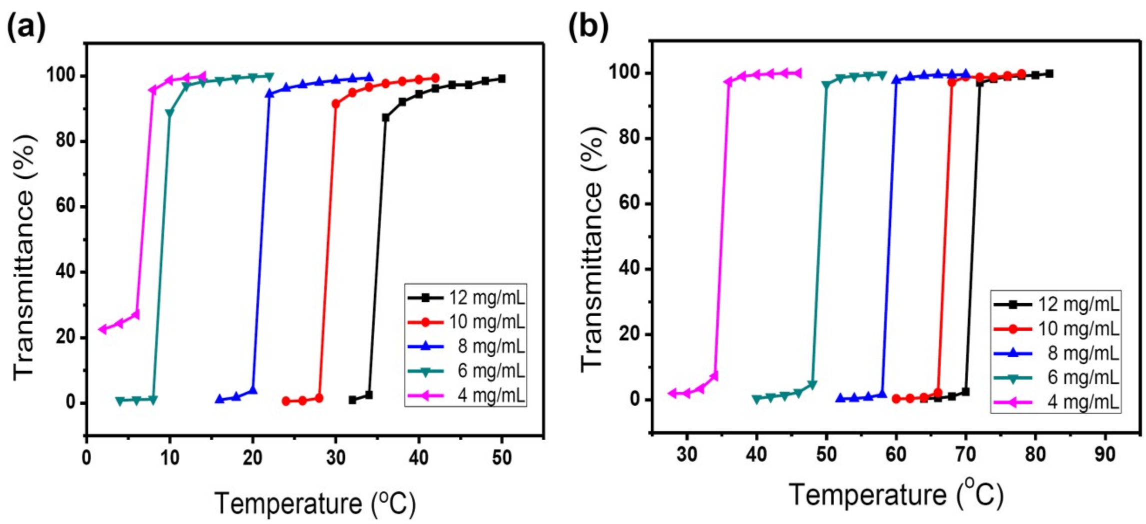

Figure S2. Plots of transmittance at λ = 500 nm versus temperature for PBS solutions of (a) PBLG-S-ImI and (b) PBLG-S-LmBF$_4$ at different polymer concentrations (DP = 40).

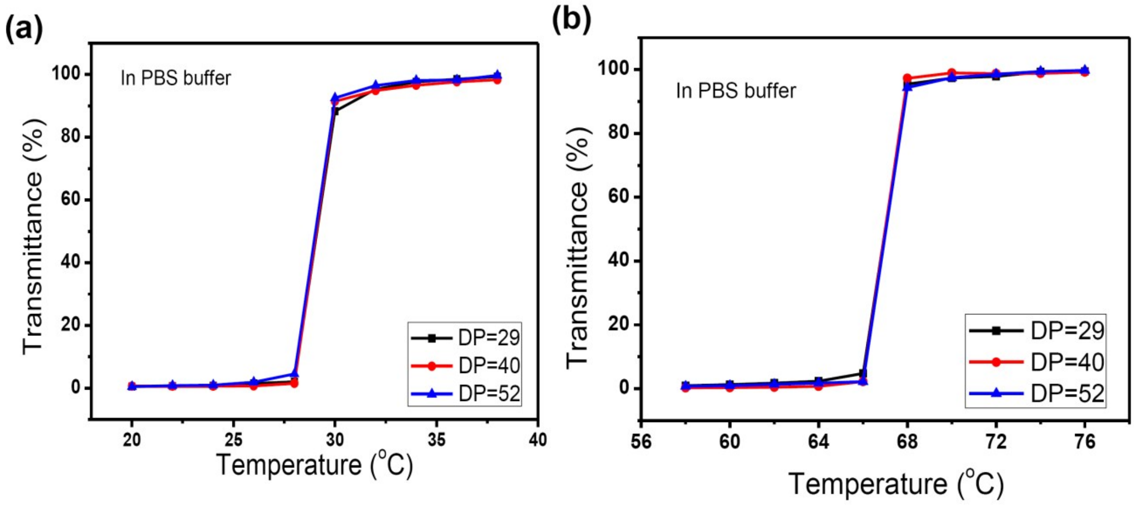

Figure S3. Plots of transmittance at λ = 500 nm versus temperature for (a) PBLG-ImI and (b) PBLG-S-ImBF$_4$ with different DPs at 10 mg·mL^{-1}.

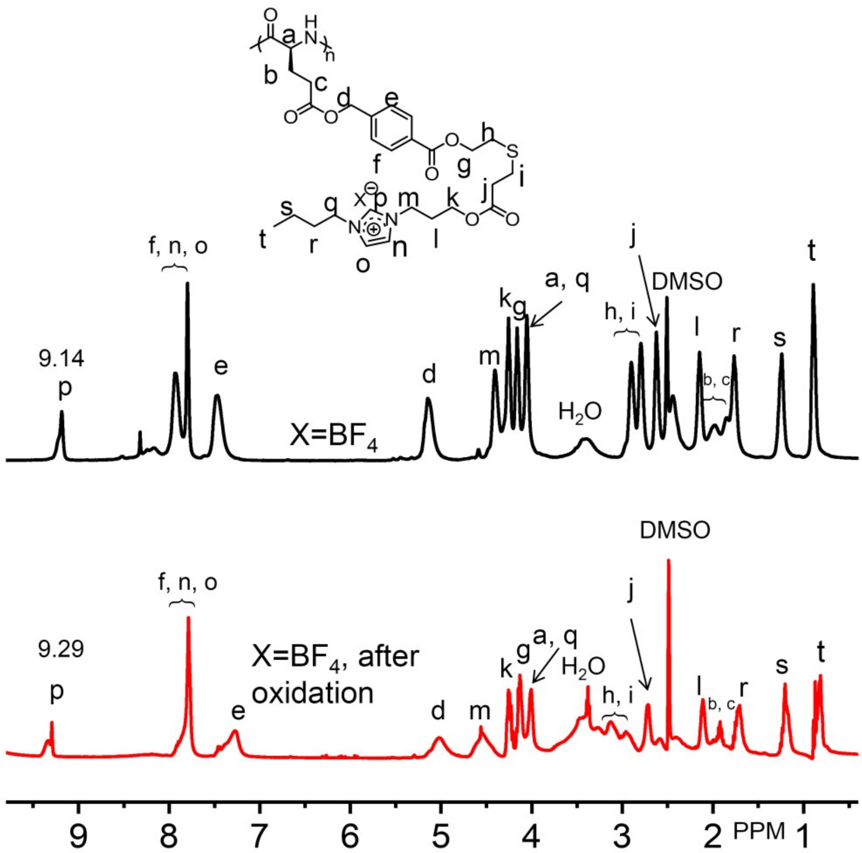

Figure S4. ^{1}H NMR spectra of PBLG-S-ImBF$_4$ and H_2O_2 oxidized PBLG-S-ImBF$_4$ in DMSO-d$_6$.

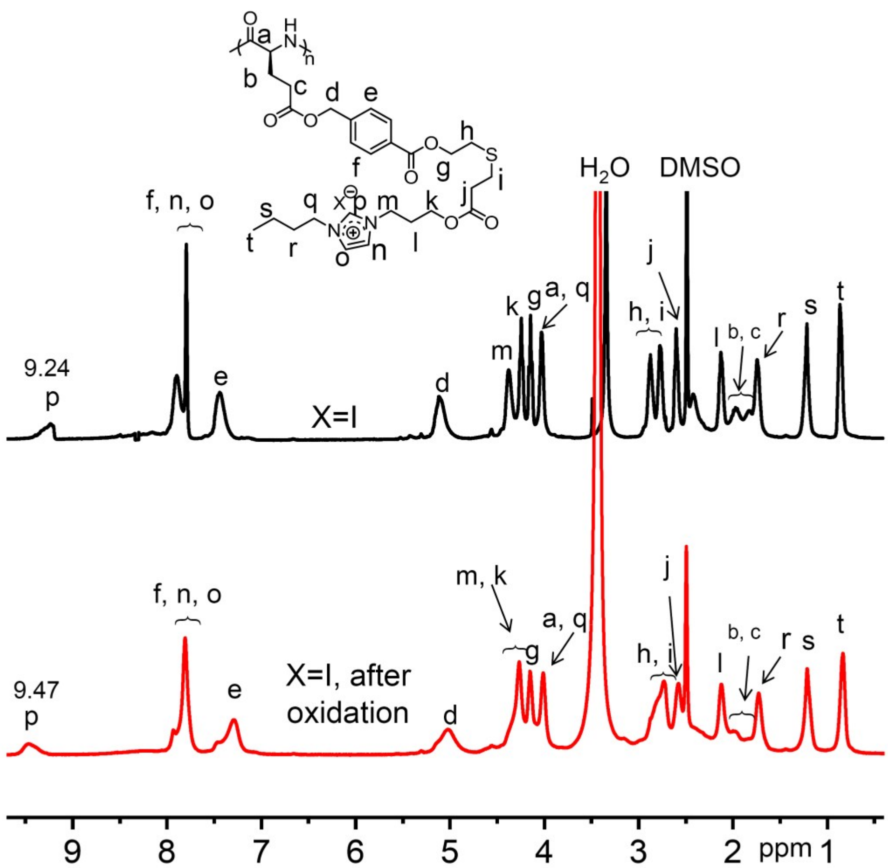

Figure S5. ^{1}H NMR spectra of PBLG-S-ImI and H_2O_2 oxidized PBLG-S-ImI in DMSO-d_6.

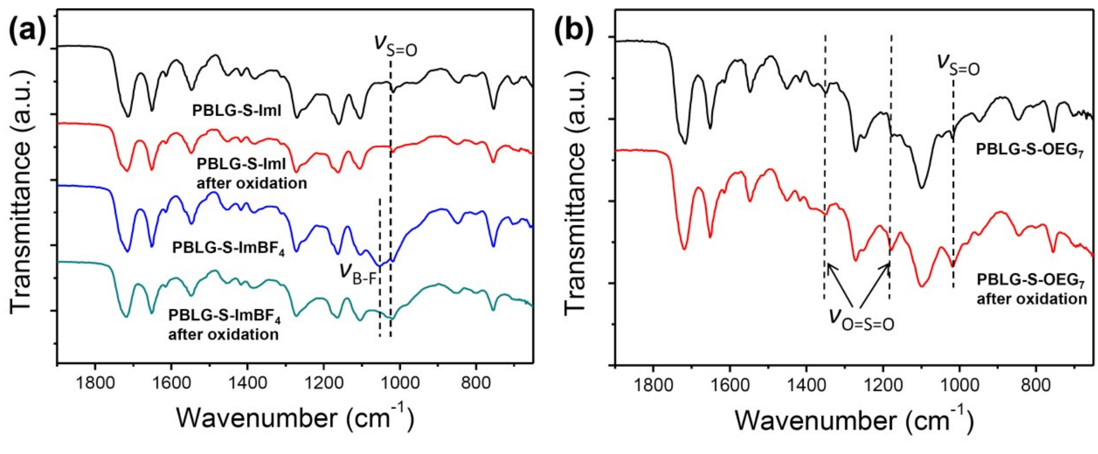

Figure S6. FTIR spectra of PBLG-S-ImX (X = I, and BF_4), PBLG-S-OEG_7, and the H_2O_2 oxidized polymers in the solid-state.

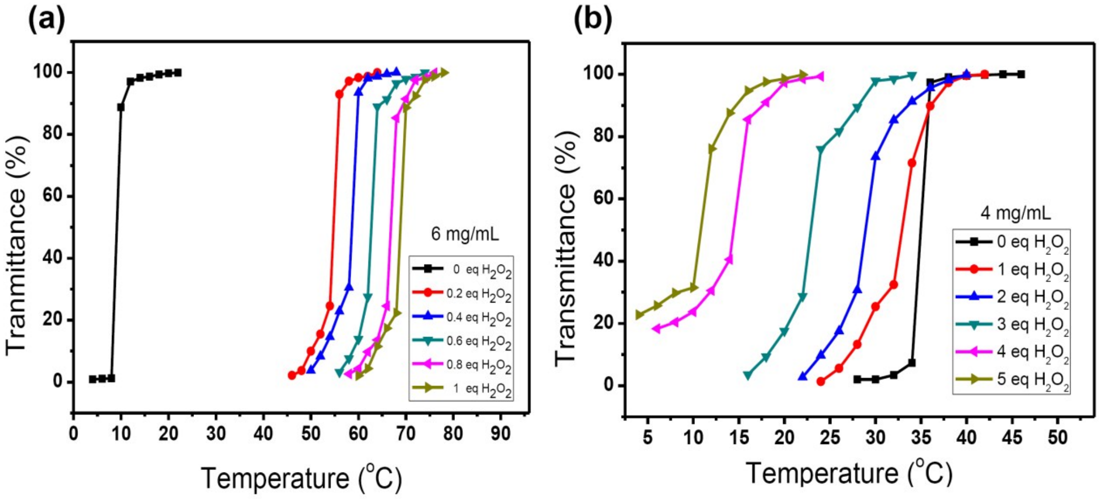

Figure S7. Plots of transmittance at λ = 500 nm versus temperature for PBS solutions of (a) PBLG-S-ImI and (b) PBLG-S-ImBF$_4$ different amounts of H_2O_2 (DP = 40).

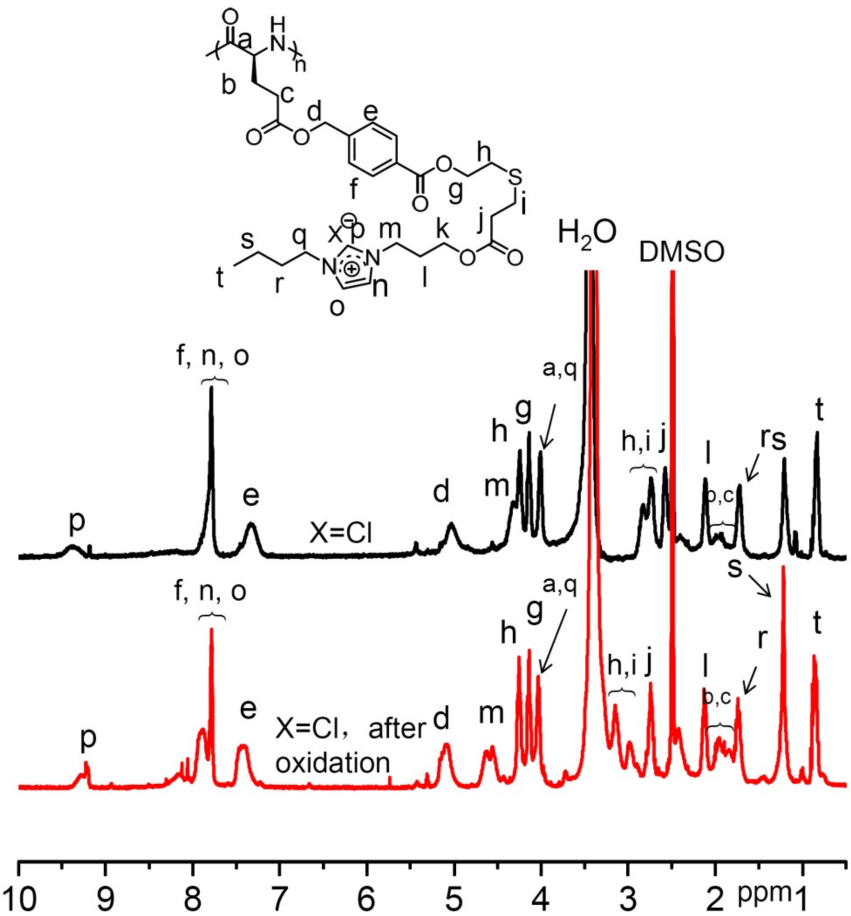

Figure S8. ^{1}H NMR spectra of PBLG-S-ImCl and the H_2O_2 oxidized PBLG-S-ImCl in DMSO-d$_6$.

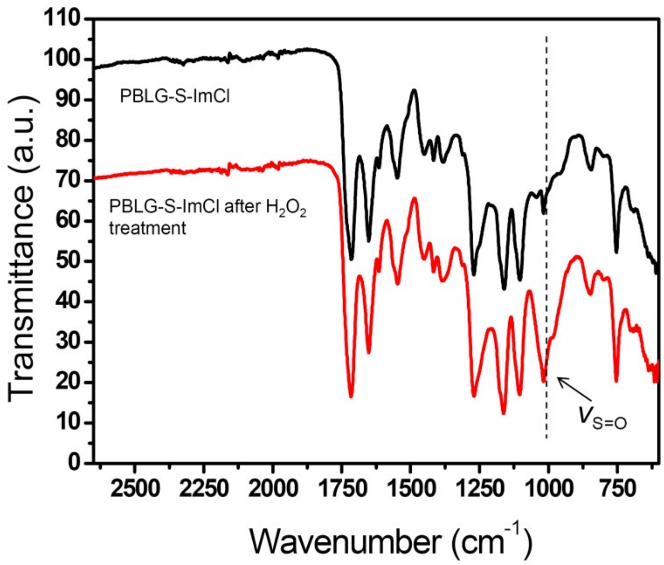

Figure S9. FTIR spectra of PBLG-S-ImCl and the oxidized PBLG-S-ImCl treated with H_2O_2.

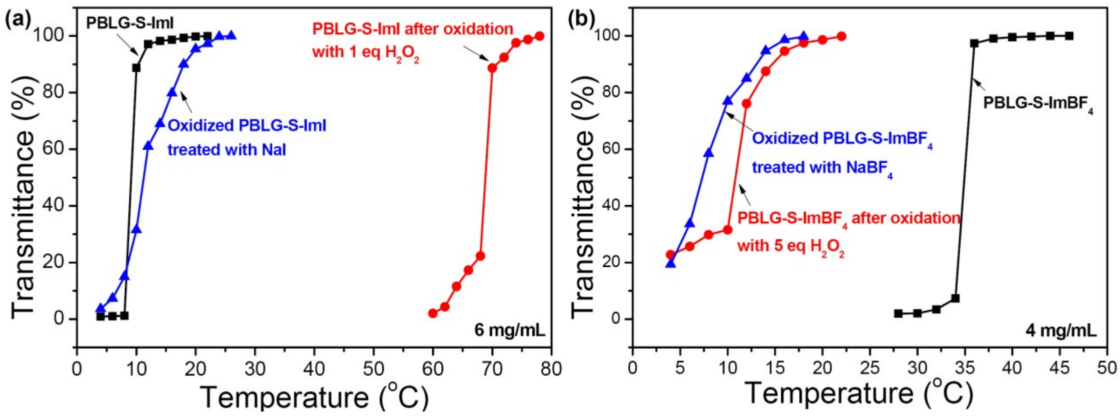

Figure S10. Plots of transmittance at λ = 500 nm versus temperature for PBS solutions of (a) PBLG-S-ImI and (b) PBLG-S-$ImBF_4$ treated at various conditions including oxidation with H_2O_2 or ion-exchange reaction with NaX (X = I, BF_4).

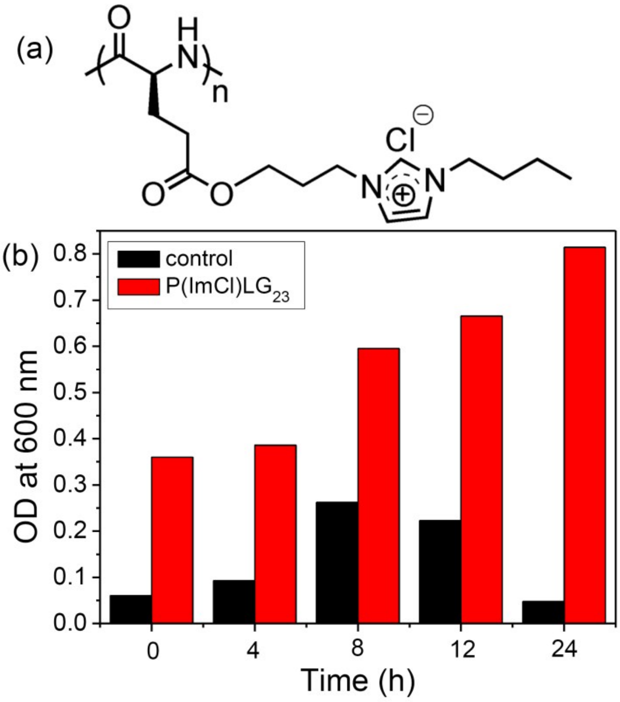

Figure S11. (a) Molecular structure of P(ImCl)LG$_{23}$. (b) Growth inhibition of *S. aureus* cells in the presence of P(ImCl)LG$_{23}$ at 37 °C. OD: optical density.

Reference

1. H. Yamamoto and T. Hayakawa, *Int. J. Biol. Macromol.*, 1982, **4**, 116-120.
2. Y. Deng, Q. Hu, Q. Yuan, Y. Wu, Y. Ling and H. Tang, *Macromol. Rapid Commun.*, 2014, **35**, 97-102.
3. S. M. Kelly, T. J. Jess and N. C. Price, *Biochim. Biophys. Acta*, 2005, **1751**, 119-139.
4. J. A. Morrow, M. L. Segall, S. Lund-Katz, M. C. Phillips, M. Knapp, B. Rupp and K. H. Weisgraber, *Biochemistry*, 2000, **39**, 11657-11666.