Electronic Supplementary Information

Fluorescence Turn-On Response of Conjugated Polyelectrolyte with Intramolecular Stack Structure to Biomacromolecules

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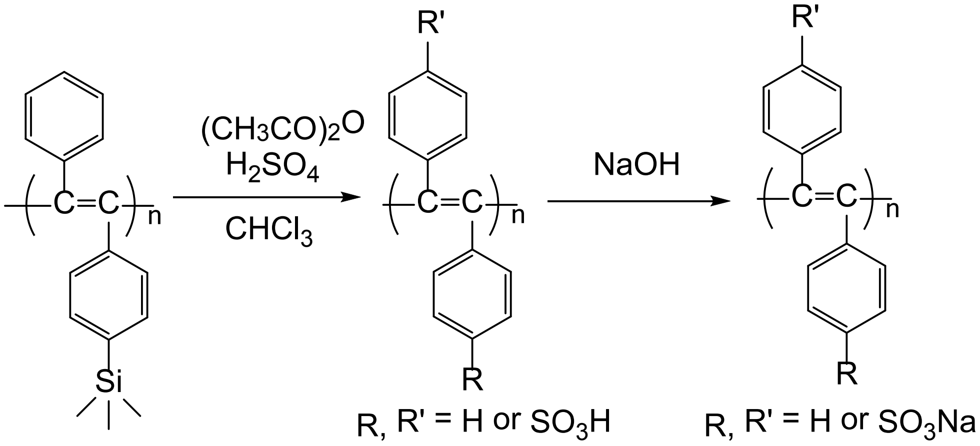
Experimental Methods

(1) Materials

The PTMSDPA was donated from NOF Co. Ltd., Japan and used as received. The synthetic method was described.[S1] The PTMSDPA used in this study has high weight-average molecular weights (M_w) of 1.23×10^6 g/mol and a polydispersity index ($PDI = M_w/M_n$) of 2.1. The chemicals and proteins used in this study were purchased from Sigma-Aldrich Co. Ltd.. The proteins were used as received. THF was purified by distillation over calcium hydride. The buffer solutions were purchased from Samchun Chemicals (Korea) and used without further purification.

(2) Sulfonation

Sulfonation of PTMSDPA was conducted according to the literature method.[S2] Acetyl sulfate was prepared by a reaction of acetic anhydride and sulfuric acid in $CHCl_3$. PTMSDPA was dissolved in $CHCl_3$. To obtain a water-soluble sulfonated PDPA (SPDPA), a 50 fold equimolar amount of acetyl sulfate was added slowly to the PTMSDPA solution with stirring. The reaction was terminated by precipitation in a large amount of THF after 1 h. The precipitate was washed several times with THF and dried in desiccator under vacuum at room temperature for 24 h. The product was characterized by Fourier transform infrared (FT-IR) spectroscopy (**Figure S9**). The degree of sulfonation was calculated by chemical titration : SPDPA (0.05 g) was dissolved in methanol (100 mL) and the solution was titrated with 0.05 mol/L-ethanolic potassium hydroxide or mol/L-aqueous potassium hydroxide using phenolphthalein and pH meter (YK-2001PH, LUTRON Electronics Inc.) as indicators. The sulfonic acid at the side chain of the product was converted to sodium sulfonate by a potentiometric titration with 0.1M NaOH. The excess NaOH was removed by dialysis. The final product, SPDPA, was obtained from lyophilization. **Scheme S1** shows the synthetic route and acid-base titration of SPDPA.



Scheme S1. Synthesis of SPDPA.

(3) Aggregation methods

The polymers were dissolved in toluene and water to prepare highly dilute solutions with a concentration of 1.0×10^{-5} M. To aggregate the polymers, water and methanol was added slowly to PTMSDPA in THF and SPDPA in water, respectively, with vigorously stirring.

(4) Measurements

The weight-average molecular weight (M_w) and number-average molecular weight (M_n) of *para*-PTMSDPA were evaluated by gel permeation chromatography (GPC, Shimadzu A10 instruments, Polymer Laboratories, PLgel Mixed-B (300 mm in length) as a column, and HPLC-grade THF as eluent at 40 °C), based on a calibration with polystyrene standards. The fluorescence (FL) emission spectra and UV-vis absorption spectra of polymers in solutions were measured on *JASCO* ETC-273 spectrofluorometer equipped with a Xenon lamp as an excitation light and a *JASCO* V-650 spectrophotometer. The FL quantum efficiencies of the polymers in solution were obtained relative to quinine sulfate in 1N H_2SO_4 (FL quantum efficiency = 54.6%, excited at 365nm).[S3] Time-Correlated Single Photon Counting (TCSPC) was performed to measure the exciton lifetimes. The second harmonic (SHG = 420 nm) of a tunable Ti:sapphire laser (Mira900, Coherent) with an ~150 fs pulse width and 76MHz repetition rate was used as the excitation source. The FL emission was resolved spectrally using some collection optics and a monochromater (SP-2150i, Acton). The TCSPC module (PicoHarp, PicoQuant) with a MCP-PMT (R3809U-59, Hamamatsu) was used for ultrafast detection. The total instrumental response function (IRF) for FL decay was less than 140 ps and the temporal time resolution was less than 10 ps. Deconvolution of the actual FL decay and IRF was performed by using fitting software (FlouFit, PicoQuant) to deduce the time constant associated with each exponential decay. Dynamic light scattering (DLS) was performed by a Malvern Zetasized Nano Zs90 spectrometer equipped with a He-Ne laser operating at a wavelength of 633 nm and a collecting angle of 90°.

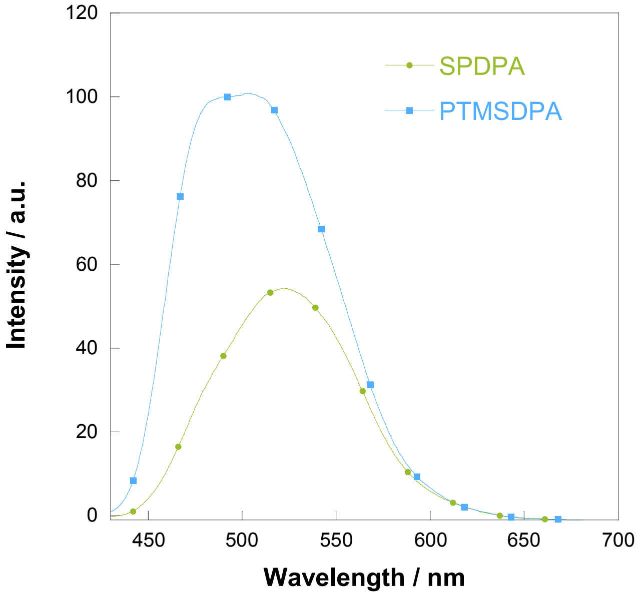


Figure S1. FL emission spectra of a) PTMSDPA in a toluene and b) SPDPA in water (1 × 10^{-5} M, excited at 420 nm).

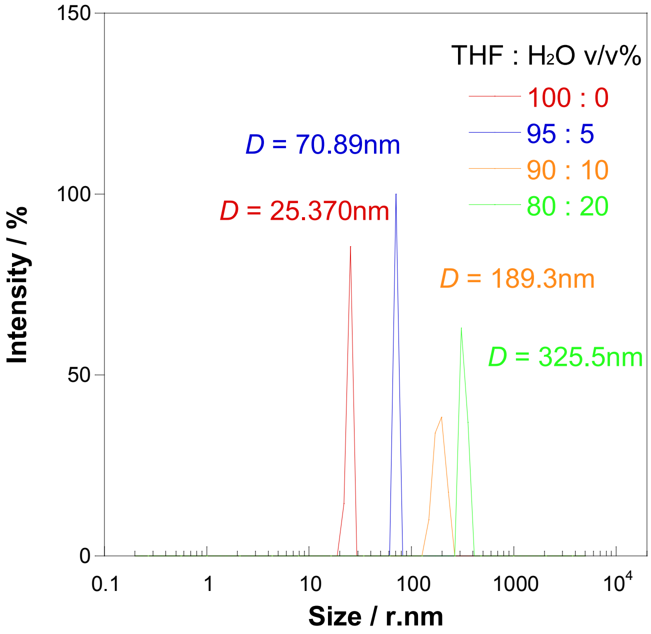


Figure S2. Intensity averaged size distribution and mean particle size (*D*) of PTMSDPA in THF/water mixtures.

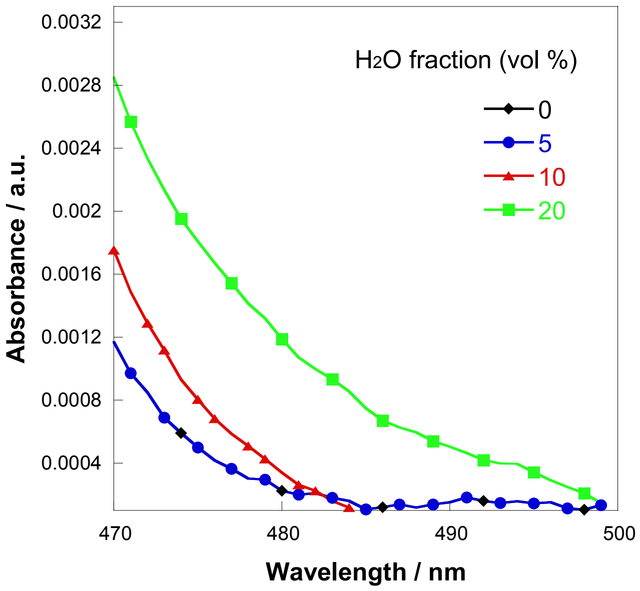


Figure S3. UV-vis absorption spectra of PTMSDPA in THF/water mixtures.

The effects of polymer concentration to the FL properties of aqueous solution were investigated. If the SPDPA self-assembled nano-structures are affected by polymer concentration in aqueous solution, the FL properties will significantly change according to the polymer concentration.[S4] In SPDPA, no significant change in FL spectra was observed in aqueous solutions with different concentration. It means that the SPDPA nanostructure is independent to polymer concentration. The concentration-invariable FL emission feature is a common characteristic of conjugated polymer with intramolecular excimer emission.[S5] Therefore, Fig. S4 suggests that the FL emission of SPDPA originated from an intramolecular excimer. This emission feature is similar to polystyrene[S6] and polyvinylcarbazole[S7]. These polymers are well known to have intramolecular excimer emission due to the stacking structures between the side aromatic rings.

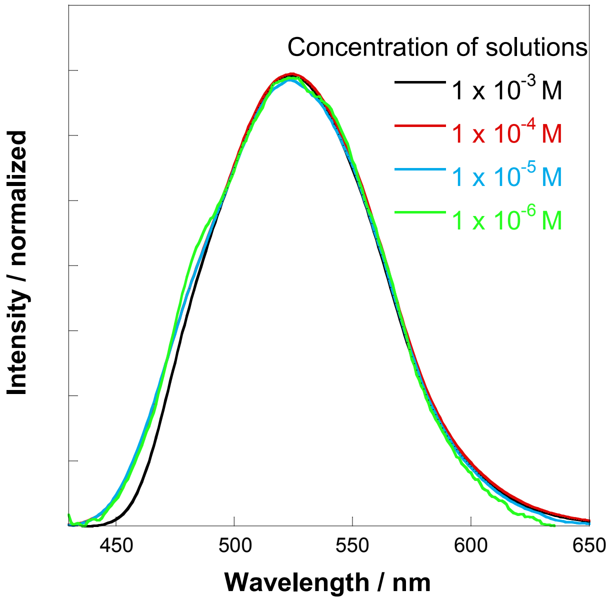


Figure S4. FL emission spectra of SPDPA in aqueous solutions with different concentration.

The mean particle size of SPDPA in methanol/water mixtures was measured by using DLS. As expected, the SPDPA formed nano-sized aggregates in water due to strong hydrophobic backbone. The particle size gradually decrease from 108.6 nm to 34.63 with increasing methanol content over a wide range of 0 ~ 40 vol%. Aggregates of SPDPA in methanol/water mixtures with high methanol content over 60% could not be measured because the size of aggregates is too small to be measured by DLS. It shows that methanol act as surfactant and the polymer chain is completely solubilized.

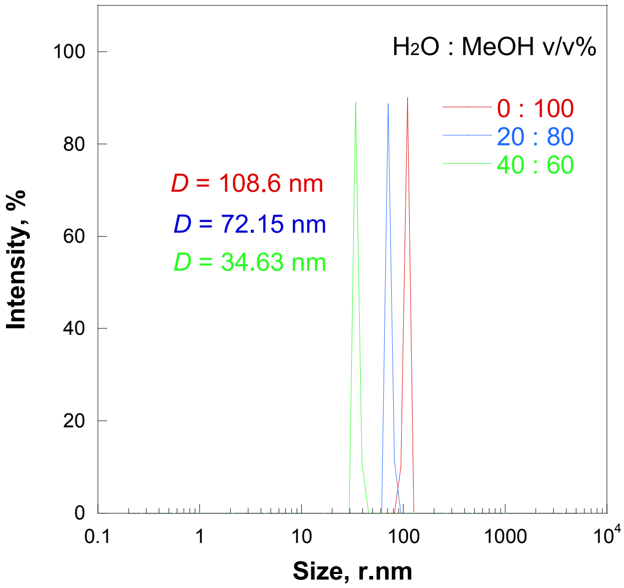


Figure S5. Intensity averaged size distribution and mean particle size (*D*) of SPDPA in methanol/water mixtures.

The FL intensities of SPDPA in buffer solutions dramatically increased according to positively charge proteins content. Interestingly, the FL emission of SPDPA combined with a positively charged BSA was more intense than that of the polymer combined with positively charged lysozyme. This show that surface activity of BSA is stronger than that of lysozyme.

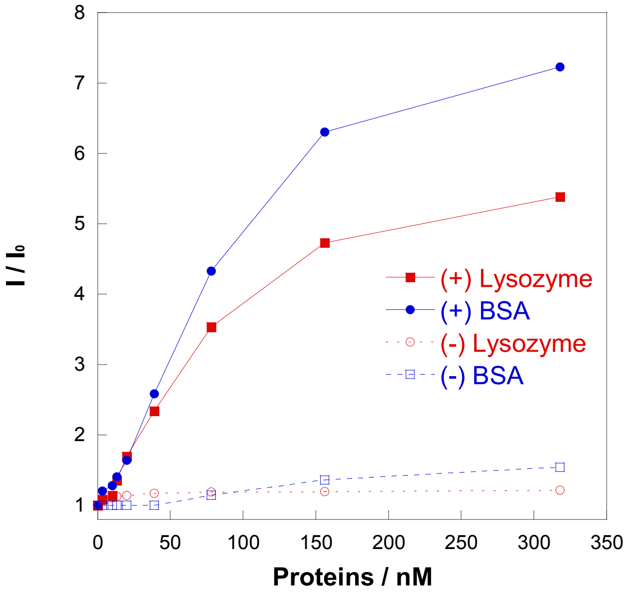


Figure S6. Relationship between FL intensity of SPDPA and proteins concentration in buffer solutions.

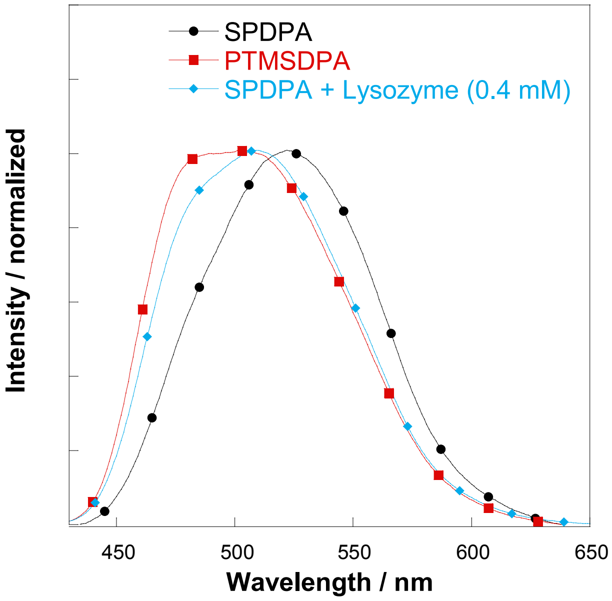


Figure S7. Normalized FL emission spectra of PTMSDPA in a toluene, SPDPA in water, and SPDPA in pH 7 buffer solution with excess lysozyme (0.4 mM), respectively. (1×10^{-5} M, excited at 420 nm).

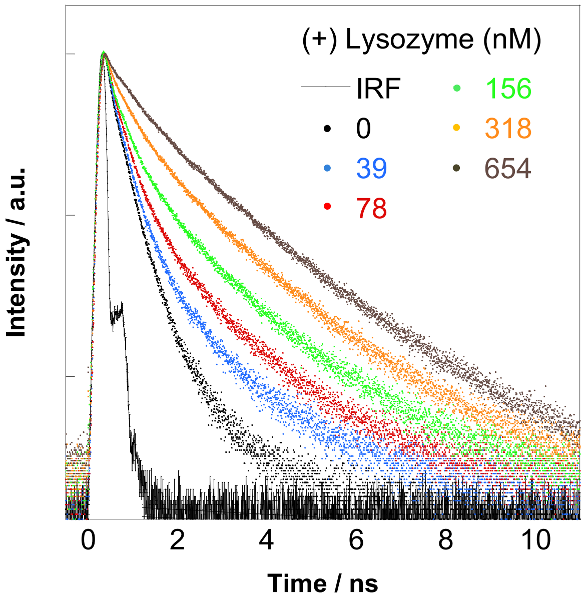


Figure S8. FL decay profiles of SPDPA (1 × 10^{-5} M) in pH 7 buffer solution according to the concentration of positively-charged lysozyme (excited at 420 nm, monitoring wavelength is 520 nm).

After sulfonation, broad absorption at approximately 1200 cm^{-1} and the sharp absorption at 1050 cm^{-1} was observed. The absorption at 1200 cm^{-1} was assigned to sulfonic acid groups,[S2] because absorptions of sulfonic acid is generally observed at 1350 and 1150 cm^{-1}, and sulfonic acid monohydrate was observed at 1200 cm^{-1}. The absorptions at 1250, 855, and 810 cm^{-1}, which were assigned to silyl groups, were not observed after sulfonation. This suggests that the silyl groups were converted to sulfonic acid groups or protons by sulfonation. The broad peak observed in the range from 3700 to 3200 cm^{-1} indicates the existence of OH groups derived from SO_3H, $[H_3O]^+[SO_3]^-$ and adsorbed H_2O. The absorption at 700 cm^{-1}, which is assigned to mono-substituted benzene, was reduced after sulfonation. This suggests that sulfonic acid groups were substituted at the *para*-position of side phenyl rings.

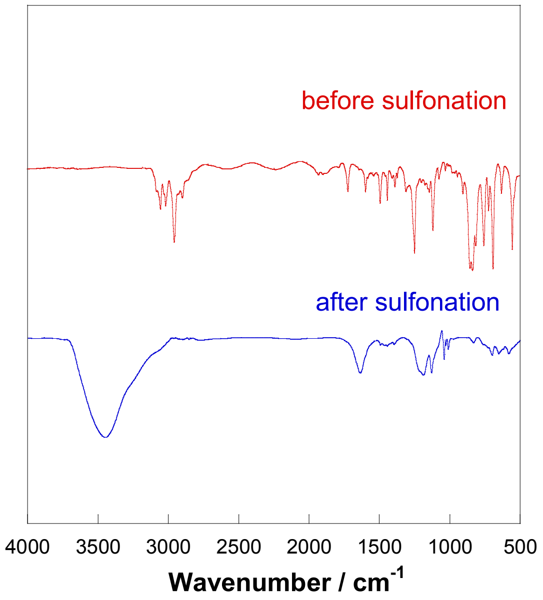


Figure S9. FT-IR spectra of PTMSDPA before and after sulfonation.

References

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