Supplementary Information

Layer-by-layer assembly of charged poly(phenylacetylene)s with induced macromolecular helicity

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Experimental Section

Materials. Deionized, distilled water was degassed with nitrogen before use for all experiments. 3,3’-Dithiodipropionic acid (DTPA), (S)-phenylalaninol ((S)-3), and (S)-phenyllactic acid ((S)-4) were purchased from Tokyo Kasei (TCI, Tokyo, Japan). The sodium salt of (S)-4 was prepared by neutralization of (S)-4 with aqueous 1 N NaOH, followed by precipitation into a large amount of acetone. Cystamine dihydrochloride (CA) was obtained from Aldrich (Milwaukee, WI). Polyacrylic acid (PAA, M_n = 2.5 \times 10^4) and poly(allylamine hydrochloride) (PAH, M_n = 1.5 \times 10^5) were purchased from Wako (Osaka, Japan) and Aldrich, respectively. (3-Aminopropyl)triethoxysilane (APS) was obtained from Nacalai Tesque (Kyoto, Japan).

Cis-transoidal poly-1^1 and poly-2-HCl^2 were prepared according to the previously reported methods. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) of poly-1 were estimated to be 1.9 \times 10^4 and 3.5, respectively, as its methyl ester by size exclusion chromatography (SEC) using poly(ethylene oxide) (PEO) and poly(ethylene glycol) (PEG) standards in DMF containing 10 mM lithium chloride as the eluent. The M_n and M_w/M_n of poly-2-HCl were 3.4 \times 10^5 and 2.2, respectively, as determined by SEC using PEO and PEG standards in water containing 9
mM tartaric acid and the 0.1 mM tartaric acid disodium salt as the eluent. The stereoregularities of poly-1 and poly-2-HCl were confirmed to be *cis-transoidal* based on their $^1H$ NMR and Raman spectra.

**Instruments.** The solution pH was measured with a B-211 pH meter (Horiba, Japan). NMR spectra were taken on a Varian INOVA-700 operating at 700 MHz for $^1H$ in D$_2$O using acetonitrile as the internal standard. SEC measurements were performed with a Jasco PU-980 liquid chromatograph (Jasco, Hachioji, Japan) equipped with a RI (Jasco RI-930) detector. Tosoh (Tokyo, Japan) TSKgel α-3000 (30 cm) and α-5000 (30 cm) SEC columns were connected in series and DMF containing 10 mM lithium chloride was used as the eluent at a flow rate of 1.0 mL min$^{-1}$ for the methyl ester of poly-1. A Tosoh TSKgel SuperAWM-H SEC column (15 cm) was used for the SEC measurement of poly-2-HCl, and 9 mM tartaric acid and the 0.1 mM tartaric acid disodium salt was used as the eluent at a flow rate of 0.6 mL min$^{-1}$. The molecular weight calibration curves were obtained with PEO and PEG standards (Tosoh). IR spectra were recorded with a Jasco Fourier Transform IR-620 spectrophotometer. Laser Raman spectra were taken on a Jasco RMP-200 spectrophotometer. CD and absorption spectra were measured on a Jasco J-725 or J-820 spectropolarimeter and a Jasco V-570 spectrophotometer, respectively. Linear dichroism (LD) spectra were measured using a Jasco J-725 spectropolarimeter with an LD attachment. AFM measurements were performed on a Nanoscope IIIa microscope (Digital Instruments, Santa Barbara, CA) in air at ambient temperature using standard silicon tips (NCH-10V) in the tapping mode.

**Preparation of Modified Quartz Plates.** Quartz plates (2 cm diameter) were cleaned with piranha solution (H$_2$SO$_4$/H$_2$O$_2$ = 3/1, v/v) at 60 °C for 1 h and rinsed with deionized water. CAUTION: Piranha solution is highly corrosive and reacts violently with most organics. Extreme care should be taken when handling piranha solution, and only small quantities should be prepared. The quartz plates were further cleaned and hydrophilized with RCA solution (H$_2$O/H$_2$O$_2$/NH$_3$ = 5/1/1, v/v/v) at 70 °C for 0.5 h, followed by
rinsing with deionized water and drying with a stream of nitrogen. The quartz surface was then coated with APS according to the literature procedure. The above precleaned quartz plates were initially conditioned by successive rinses in methanol, methanol/toluene (1/1, v/v), and toluene. They were immersed in a 0.42 M solution of APS in toluene at room temperature for 15 h and then ultrasonically agitated for 5 min each in toluene, methanol/toluene (1/1, v/v), methanol, and deionized water, in this turn. Finally, the modified quartz plates were treated with 0.1 N HCl to convert the amino groups to the positively charged ammonium groups, washed with distilled water, and dried in a stream of nitrogen.

**Multilayer Film Formation.** A typical experimental procedure for the layer-by-layer (LbL) assembly between poly-1 and PAH is described below. Stock solutions of (S)-3 (10.8 mg mL⁻¹, 71.4 mM) and NaCl (0.70 M) in water were prepared. Poly-1 (3.0 mg, 0.014 mmol monomer units) was suspended in 27 mL of water and to this were added 1 mL aliquots of the stock solutions of (S)-3 and NaCl. The solution pH was then adjusted at pH 4.0 by adding 1.0 and 0.1 N HCl aqueous solutions and diluted with water to keep the poly-1 and NaCl concentration at 0.1 mg mL⁻¹ (0.48 mM) and 0.024 M, respectively, and the molar ratio of (S)-3 to monomer units of poly-1 was 5 (poly-1—(S)-3 complex solution). A stock solution of PAH (1.0 mg mL⁻¹, 10.7 mM) in water was also prepared and the pH was adjusted at pH 8.0 by adding 1.0 and 0.1 N NaOH aqueous solutions. The quartz plate modified with APS was immersed in the poly-1—(S)-3 complex solution for 20 min, rinsed with water (10 mL x 3), and dried in a stream of nitrogen. The quartz plate was then immersed in the PAH aqueous solution for 10 min, and the same procedure was repeated. This alternating dipping cycle was performed in a refrigerator at 4 °C and repeated until the desired layer number was reached. In each step, each polymer was deposited on both sides of the quartz plate. The absorption and CD spectra of multilayer films assembled on the quartz plate were recorded at room temperature after each assembly cycle. LD spectra of the films were also measured under the same measurement conditions, because CD spectra of films are sometimes
affected by macroscopic anisotropy and they often include components derived from LD.\textsuperscript{4} The optical densities of the LbL assembled films were much smaller than those arising from CD, indicating that the LD contributions caused by macroscopic anisotropy could be negligible. In the similar way, the LbL assemblies between poly-2-HCl and PAA or poly-1 and poly-2-HCl were performed using poly-2-HCl—(S)-4 ([poly-2-HCl] = 0.5 mg mL\textsuperscript{-1} (2.0 mM), [(S)-4]/[poly-2-HCl] = 0.5) and PAA (1.0 mg mL\textsuperscript{-1}, 10.6 mM) aqueous solutions adjusted at pH 3.8 and 5.0, respectively.

**QCM Measurements.** The quartz crystal microbalance (QCM) measurements were performed using an *AffinixQ* QCM2000 (Initium Co. Ltd., Tokyo, Japan) equipped with a magnetic stirrer bar with the temperature controlling system.\textsuperscript{5} AT-cut QCM plates with a parent frequency of 27 MHz (8 mm diameter of a quartz plate and an area of 4.9 mm\textsuperscript{2} of gold electrode) are available from Initium. The amount of the adsorbed polymers (\(\Delta m\)) was measured by following the frequency shift of the QCM (\(\Delta F\)) with time, using the following Sauerbrey's equation for the AT-cut shear mode QCM,\textsuperscript{6}

\[
-\Delta F = \frac{2F_0^2}{A\sqrt{\rho_q\mu_q}} \Delta m
\]

where \(F_0\) is the fundamental frequency of the QCM (27 \(\times\) \(10^6\) Hz), \(A\) is the electrode area (4.9 mm\textsuperscript{2}), \(\rho_q\) is the density of quartz (2.65 g cm\textsuperscript{-3}), and \(\mu_q\) is the shear modulus of quartz (2.95 \(\times\) \(10^{11}\) dyn cm\textsuperscript{-2}).

Bare gold QCM electrodes were cleaned with a freshly prepared piranha solution for 5 min, followed by rinsing with distilled water and drying in a stream of nitrogen. After this cleaning procedure was repeated three times, the gold surface was modified with DTPA or CA according to the previously reported method.\textsuperscript{7} The surface of the gold electrode was covered with a 150 \(\mu\)L of DTPA solution (5 mM) in water-ethanol (19/1, v/v) for 2 h, followed by rinsing with distilled water and drying in a stream of nitrogen. Then the electrode was covered with a 150 \(\mu\)L of NaOH aqueous solution (0.01 M) for 10 min to convert the carboxy groups to the carboxylate groups, and washed with distilled water and dried in a stream of nitrogen to give a DTPA-modified QCM plate.
The CA-modified QCM plate was prepared by covering the surface of the gold electrode with a 150 μL of CA solution (5 mM) in water-ethanol (19/1, v/v) for 2 h, followed by rinsing with distilled water and drying in a stream of nitrogen.

The LbL assembly of polyelectrolytes on the modified gold electrode was carried out in the same way as that on quartz plates. The CA-modified QCM plate was used for the LbL assembly between a helical poly-1 induced by (S)-3 ([poly-1] = 0.1 mg mL⁻¹, [(S)-3]/[poly-1] = 5) and PAH (1 mg mL⁻¹). The QCM plate was initially immersed in the poly-1—(S)-3 solution at 25 °C under a stirring and the frequency changes after immersion were followed with time. The plate was taken out and washed with water, and dried in a stream of nitrogen. The plate was then immersed in the PAH aqueous solution at 25 °C, and the same procedure was repeated. This alternating cycle was repeated for the LbL assembly until the desired layer number was reached (Fig. S1a). The same procedure was done for the LbL assembly between poly-2-HCl and PAA, and poly-1 and poly-2-HCl by using the DTPA- and CA-modified QCM plates, respectively (Fig. S1b and c); (S)-3 ([(S)-3]/[poly-1] = 5) and (S)-4 ([(S)-4]/[poly-2-HCl] = 0.5) were used for helicity induction on poly-1 and poly-2-HCl, respectively.

**SEC Measurements of the LbL Films.** To check if a certain amount of (S)-3 is still present in the LbL assembled poly-1–PAH film, SEC analysis of the LbL film was performed using a Jasco PU-980 liquid chromatograph equipped with a UV (220 and 300 nm; Jasco UV-970) detector. Tosoh TSKgel SuperAWM-H SEC column (15 cm) was connected, and a 9 mM NaOH / 0.1 mM potassium chloride buffer solution (pH 12.0) was used as the mobile phase at a flow rate of 0.6 mL min⁻¹. Standard solutions of poly-1 and PAH ([poly-1] = 1.0 mg mL⁻¹, [poly-1]/[PAH] = 1) containing various amounts of (S)-3 ([(S)-3]/[poly-1] = 0.1, 0.05, and 0.01) were prepared. The solutions were injected into the SEC system. The polymers were eluted first, followed by the (S)-3 (Fig. S2b-d). As shown in Fig. S2b, the peak due to the 0.01 equiv. of (S)-3 was clearly detected. The LbL assembled poly-1—PAH film (40 layers) was dissolved in a 200 μL of alkaline water (pH 12) and the solution was injected into the SEC system.
using the same mobile phase. In the chromatogram of the LbL film, the peak due to (S)-3 was barely detected (Fig. S2a). These results indicate that the LbL film exhibiting the intense ICD contains very little (S)-3 (less than 0.001 equiv. based on the detection limit) and (S)-3 must be almost completely removed during the LbL assembly process. The LbL film consisting of poly-2—(S)-4 and PAA was soluble neither in acidic nor basic water. Therefore, it was difficult to perform the same experiment.

**1H NMR Measurement of the LbL Assembled Poly-1–PAH Film.** The poly-1—(S)-3 complex ([poly-1] = 10 mg mL⁻¹, [(S)-3]/[poly-1] = 5) and PAH (10 mg mL⁻¹) solutions were prepared in the same way described above. The poly-1—(S)-3 solution was then added to the PAH solution under vigorous stirring. Yellow polyion complexes immediately precipitated and the precipitate was collected by centrifugation, washed with water, and dried in vacuo over night. The obtained precipitate, which can be regarded as a model polyion complex of the LbL assembled poly-1—(S)-3–PAH film, was analyzed by ¹H NMR spectroscopy to see if a detectable amount of (S)-3 is present in the obtained LbL film. The precipitate was dissolved in 0.01 M NaOD in D₂O and its ¹H NMR spectrum was measured at 60 °C (Fig. S3a). Standard samples consisting of poly-1 and PAH ([poly-1]/[PAH] = 1/1) in the absence and presence of (S)-3 ([poly-1]/[PAH]/[(S)-3] = 1/1/0.01) in 0.01 M NaOD in D₂O were separately prepared and their ¹H NMR measurements were also conducted (Fig. S3b and c). The poly-1–PAH solution containing 0.01 equiv. (S)-3 showed clear resonances due to the (S)-3, whereas no detectable peak was observed at all in the ¹H NMR spectrum of the precipitate. These results also support the absence of (S)-3 in the LbL film.

However, one may think that the observed intense CD of the LbL film could be originated not from macromolecular helicity memory effect, but from a helical poly-1 induced by a tiny amount of (S)-3 (less than 0.01 equiv.) that may be still present in the LbL film. Therefore, we checked if a small amount of (S)-3 (0.01 equiv. of (S)-3) could induce CD on poly-1 in the film state. However, no apparent CD was observed for the cast film prepared from the poly-1—(S)-3 complex solution (((S)-3)/[poly-1] = 0.01).
These results clearly showed that even if 0.01 equiv. of (S)-3 would be still present in the LbL film, such a small amount of (S)-3 could not induce any CD in poly-1 at all.

**CD Measurements of Cast Films.** In order to estimate the memory efficiency of the macromolecular helicity in the LbL assembled films, the poly-1—(S)-3 ([((S)-3)/poly-1] = 5) and poly-2-HCl—(S)-4 ([((S)-4)/poly-2-HCl] = 0.5) films were prepared on a quartz plate (2 cm diameter) by casting the dipping solutions used for the LbL assembly process, followed by evaporation of the solvent under reduced pressure. The absorption and CD spectra of the films cast on a quartz plate were then measured. We also measured the LD spectra of these cast films under the same measurement conditions and found that the LD contributions caused by the macroscopic anisotropy are negligible. The memory efficiencies of the LbL thin films were estimated based on the ICD intensities normalized with their absorption relative to those of the cast films, and were 78, 38, and 6% for the poly-1—PAH, poly-2—PAA, and poly-1—poly-2 systems, respectively.

**AFM Measurements.** The AFM measurements of LbL films deposited on quartz plates were performed at ambient conditions (in air at ca. 25 °C) and height and phase images were simultaneously measured at the resonance frequency of the tips with 125 μm long cantilevers (220—280 kHz). All the images were collected with the maximum available number of pixels (512) in each direction (1 μm). Scanning speed was at a line frequency of 1.0 Hz. The Nanoscope image processing software was used for image analysis. The surface roughness was evaluated by using the mean roughness \( (Ra)^8 \) values in given observed areas, which was obtained from the Nanoscope software (Fig. S4).

**References**


Fig. S1 Frequency shift of QCM by the stepwise assembly from polyanion and polycation aqueous solutions of (a) poly-1-(S)-3 complex and PAH, (b) PAA and poly-2-(S)-4 complex, and (c) poly-1-(S)-3 complex and poly-2-(S)-4 complex, respectively.

Fig. S2 (A) SEC chromatograms of the LbL thin film deposited from poly-1—(S)-4 complex and PAH solutions (a, —) and the standard solutions of poly-1 and PAH ([poly-1]/[PAH] = 1) containing (S)-4 ([(S)-4]/[poly-1] = 0.01 (b, —), 0.05 (c, —), and 0.1 (d, —)). (B) Expanded detail of the SEC chromatograms around the elution time of (S)-4.
Fig. S3 700 MHz $^1$H NMR spectra of a precipitate obtained from mixing the poly-$1$—(S)-3 complex ([S-3]/[poly-$1$] = 5, [poly-$1$] = 10 mg mL$^{-1}$) and PAH ([poly-$1$]/[PAH] = 1/1, 10 mg mL$^{-1}$) solutions (a) and mixtures of poly-$1$ and PAH ([poly-$1$]/[PAH] = 1/1) (b), poly-$1$, PAH, and (S)-3 ([poly-$1$]/[PAH]/[(S)-3] = 1/1/0.01) (c) in 0.01 M NaOD in D$_2$O.
Fig. S4 Tapping mode AFM images of the surfaces of the multilayer thin films deposited from polyanion and polycation aqueous solutions of poly-1-(S)-3 complex and PAH (a, b), PAA and poly-2-(S)-4 complex (c, d), and poly-1-(S)-3 complex and poly-2-(S)-4 complex (e, f), respectively. The observed surface is poly-1 (a and e) or PAA (d) of 21th layer, poly-2 (c and f) or PAH (b) of 20th layer.