Supramolecular Assembly of Comb-like Macromolecules Induced by Chemical Reactions that Modulate the Macromolecular Interactions in-situ

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EXPERIMENTAL SECTION

General. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DMX 500 MHz spectrometer for polymer characterization. Tandem gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 18-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara, CA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100 Å, 500 Å, 10^3 Å and 10^4 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for external polymer standards and can be used for the determination of the absolute molecular weights of comb-like polymers. CD measurements were carried out on a JASCO J-700 spectrometer using a 0.2 mm path length cuvette. Morphologies of the supramolecular structure were characterized with JEOL 6335 field emission scanning electron microscopy (FESEM) with an accelerating voltage of 10 kV. Samples were deposited on pre-cleaned glass slides and coated with palladium before imaging. Laser confocal fluorescence microscopy (LCFM) experiments were performed on an Andor Confocal Microscope. The excitation wavelength was chosen at 488 nm and the detection wavelength at 509 nm. Before the experiments, the supramolecular polymers were stained with ThT. Asylum Research MFD-3D atomic force microscopy (AFM), was used to characterize the polymer membranes and filaments deposit on the glass or Si substrate. The scans were performed in non-contact mode using Si_3N_4 tips with a force constant of 45 N/m and a typical resonant frequency of 329 ± 20 kHz. Dynamic

light scattering (DLS) studies were carried out on an ALV compact goniometer system with multi-detectors (CGS-3MD) (Germany), which consists of a 22 mW He-Ne laser (emitting vertically polarized light with a wavelength of 632.8 nm). Light scattering data were obtained by in-situ monitoring the scattering intensity at 90° from different polymer concentrations and glutamic acids (Glu) / benzyl amine (BA) ratios at 25°C (Glu/DMTMM=1/1). For Fourier transform infrared spectroscopy (FTIR) study, supramolecular structures were collected by centrifugation, washed with D_2O for multiple times, and re-suspended in D_2O and transferred into a diamond attenuated total reflectance (ATR) accessory for data collection. Fluorescence spectroscopy data were obtained by monitoring the emission at 482 nm (excited at 440 nm) of the reaction medium at 25°C, with the presence of 20 μM of ThT in the solution. Control experiments were conducted by incubating aqueous solution of poly(acrylic acids) (Sigma-Aldrich) with BA/carboxylate ratio of 1/1, 2/1 and 5/1 at 25°C for 12 hours.

Synthesis of PN-*g*-PLG comb-like macromolecules and other chemical compounds. PN-*g*-PBLG comb-like macromolecules were prepared by following the procedure developed previously.[1,2] Poly(norbornene-endo-2,3-dicarboximide) (PN) bearing *N*-TMS groups was prepared through ROMP in THF or dichlorormethane. After the removal of the solvent under vacuum, the PN was used to initiate the Glu-NCA polymerization in DMF in situ without further purification. PN-*g*-PLG comb macromolecules were usually obtained in 80-95% yield. The molecular weight (MW) and molecular weight distribution (M_w/M_n) of the comb-like macromolecules PN-*g*-PBLG were determined by GPC and summarized in Table S1. To deprotect PBLG to obtain PN-*g*-PLG, PN-*g*-PBLG (500 mg) was dissolved in TFA (10 mL) in an ice bath and HBr (33 wt% in acetic acid, 2-3 mL) was added to the solution dropwise during a

time period of 5 min. The solution was stirred in the ice bath for 2 h. The reaction solution was poured into 40 mL ice-cold dry ether (4 °C). The precipitated polymer was collected by centrifugation, washed by dry ether twice (2 × 30 mL) and dried under vacuum. The crude product was dissolved in NaOH (2 M, 10 mL) and stirred at room temperature overnight. The resultant solution was cooled in an ice bath and its pH was adjusted to 1-2 by adding HCl (1 M). The suspension was dialyzed against DI water for two days and lyophilized to give the PN-*g*-PLG in almost quantitative yield.

Benzylamine (>99.5%) was purchased from Sigma-Aldrich. 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) was prepared according to a modified procedure based on the literature.[3] A solution of *N*-methylmorpholine (NMM) (212.4 mg, or 2.1 mmol) in 2 mL THF was added dropwise to a solution of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) (351.2 mg, or 2 mmol) in 10 mL THF at room temperature and stirred for 1 h. The white precipitate was collected by suction filtration and dried under vacuum overnight. The final product (491 mg, yield 88.7%) was stored in 4 °C before use. The NMR of the purified DMTMM is shown in Figure S1.

Solution small angle X-ray scattering analysis. PN_{10}-*g*-PLG_{102} samples with concentration of 4, 2, 1 and 0.5 mg/mL in 10 mM PBS buffer (pH = 7) were measured in the solution X9 beamline in National Synchrotron Light Source (Brookhaven National Laboratory, Upton, NY). Using the automated sample loader and capillary cleaner system, for each experiment using about 90 μL of sample and buffer, SAXS images were acquired for 30 s each while flowing the sample through a quartz flow cell. The scattered X-rays were recorded on charge-coupled device (CCD), corrected for flares close to beam-stopper, and circularly averaged about the coordinates

of the main beam to obtain SAXS intensity profiles as a function of q, using python script-based programs. Two sets of data from the same sample were averaged. The q range is covered from 0.008-0.4 Å⁻¹. Here, q is defined as the scattering vector that $q \equiv \frac{4\pi}{\lambda} sin\frac{\theta}{2}$ where θ is the scattering angle and λ is the X-ray wavelength.

SASView 4.0.1 (www.sasview.org) was used to analyze the 1-D scattering patterns. In the study, the scattering intensity, I(q), is expressed as $I_1(q)$ + $I_2(q)$ + BKG, where $I_1(q)$ and $I_2(q)$ represent the scattering contributions from the whole PN$_{10}$-g-PLG$_{102}$ macromolecule and the grafted PLGs, whose the form factors are $P_1(q)$ and $P_2(q)$, respectively, and are derived from the scattering functions of oblate spheroids and rectangular slabs, respectively. BKG represents for the incoherent scattering background. The form factor of the oblate spheroid is expressed as the following equation:

$$I_1(q) = \frac{\Phi}{V} \times \Delta\rho_1^2 \times P_1(q)$$

$$P_1(q) = \int_0^{\frac{\pi}{2}} F^2(q, \alpha) \sin \alpha d\alpha$$

$$F(q, \alpha) = \frac{3\{\sin[qr(R_p, R_e, \alpha)] - \cos[qr(R_p, R_e, \alpha)]\}}{[qr(R_p, R_e, \alpha)]}$$

$$r(R_p, R_e, \alpha) = (R_e^2 \sin^2 \alpha + R_p^2 \cos^2 \alpha)^{1/2},$$

where Φ is the volume fraction of the spheroids in the solution, α is the angel between the symmetric axis of the spheroid and q, $V = (4/3)\pi R_p R_e^2$ is the volume of the spheroid, R$_p$ is the polar radius along the rotational axis of the spheroid, R$_e$ is the equatorial radius perpendicular to the rotational axis of the spheroid and $\Delta\rho_1$, contrast, is the scattering length density difference between the oblate and the solvent.

The form factor of a rectangular slab is expressed as the following equation:

$$P_2(q) = \int_0^1 \int_0^1 A_P^2 \, du \, d\sigma$$

$$A_p(q) = \frac{\sin(\frac{qC}{2})}{\frac{qC\sigma}{2}} \times \frac{\sin(\frac{\sqrt{1-\sigma^2}}{2}qA\sin\frac{\pi u}{2})}{\frac{\sqrt{1-\sigma^2}}{2}qA\sin\frac{\pi u}{2}} \times \frac{\sin(\frac{\sqrt{1-\sigma^2}}{2}qB\cos\frac{\pi u}{2})}{\frac{\sqrt{1-\sigma^2}}{2}qB\cos\frac{\pi u}{2}} \,,$$

where A, B and C are the lengths of three sides of the rectangular slab and must fulfill A ≤ B ≤ C. The parameters u and σ were applied to integrating all possible orientations of the slabs w.r.t. the scattering vector q.

Therefore, the scattering intensity contributed from the slab can be derived:

$$I_2(q) = \Phi_{slab} \times V_{slab} \times \Delta\rho_2^2 \times P_2(q)$$

where Φ_{slab}, V_{slab} are the volume fraction (w.r.t. total volume) and volume of the slab, respectively, $\Delta\rho_2$ is the difference in the scattering length densities between the slab and the oblate.

Reaction of PN-*g*-PLGs with benzyl amine in solution. In a typical experiment, we first introduced premeasured amount of BA into the aqueous solution of PN-g-PLGs, carefully adjusted the pH to the targeted value by using 1M NaOH and 1M HCl solution, then we filtered the solution before DMTMM (DMTMM/Glu=1/1 molar ratio) was introduced to trigger the reaction. Different spectroscopy and microscopy methods were then used to analyze the reaction and assembly process. If NMR is involved in the experiments, then D_2O and deuterated compounds (e.g., NaOD, DCl) were used.

Data reduction for the LS-based kinetics in the early stage of growth of supramolecular structures. For the individually dispersed macromolecules in the solution, the scattering intensity is:

$$I_0 = Im_{tot} \quad \text{(S1)}$$

where I is the unit scattering intensity for monomers, and m_{tot} is the total monomer concentration. For small oligomers in the very early stage of aggregation, $qR_g < 1$. Following the Guinier approximation, the scattering intensity of i-mers is proportional to i^2N_i, where N_i is the number concentration of i-mers. By summing the scattering intensity from the monomers and the oligomers (to be consistent with the classic models, we omit the oligomers that have aggregation size less than the primary nucleus size, n_c), the scattering intensity at time t (I_t) is:

$$I_t = \left(N_1 + n_c^2 N_{n_c} + (n_c + 1)^2 N_{n_c+1} + (n_c + 2)^2 N_{n_c+2} + \cdots\right)I \quad \text{(S2)}$$

Combining the two equations, we have:

$$\frac{(n_c - 1)N_1 - (n_c + 1)N_{n_c+1} - 2(n_c + 2)N_{n_c+2} - \cdots}{m_{tot}} = n_c - \frac{I_t}{I_0} \quad \text{(S3)}$$

Since the number concentration of monomers (N_1) is much greater than the number concentration of oligomers (N_{n_c+1}, N_{n_c+2}, etc) in the early stage of the aggregation, we can approximate the Eq. S3 with:

$$\frac{N_1(t)}{m_{tot}} = \frac{n_c - \dfrac{I_t}{I_0}}{n_c - 1} \quad \text{(S4)}$$

or

$$\frac{M(t)}{m_{tot}} = 1 - \frac{n_c - \dfrac{I_t}{I_0}}{n_c - 1} \quad \text{(S5)}$$

Eq. S5 was used to reduce the light scattering profiles in the early stage of the assembly and incorporate into the model analysis based on the MATLAB programs.

Table S1. GPC Characterizations of the comb-like macromolecules.

Entry	Comb-like macromolecules	x (x*)[a]	n (n*)[b]	Mn (M_n*)[c] ($\times 10^3$ g/mol)	M_w/M_n
1	PN$_{10}$-g-PBLG$_{51}$	10 (10)	51 (50)	113 (111)	1.06
2	PN$_{10}$-g-PBLG$_{102}$	10 (10)	102 (100)	225 (221)	1.08

[a] x = the obtained degree of polymerization (DP) of ROMP, x* = the expected DP of ROMP; [b] n= the obtained DP of polypeptides, n* = the expected DP of polypeptides; [c] Mn = the obtained Mn; Mn* = the expected Mn.
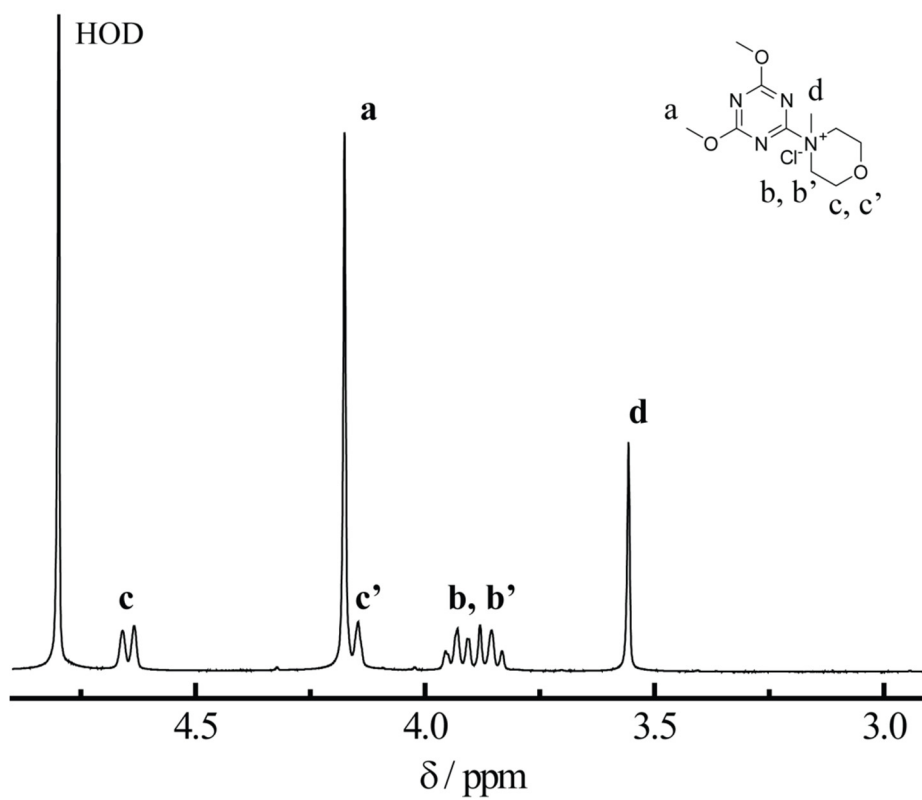
Figure S1. ^{1}H-NMR spectra of DMTMM in D2O.
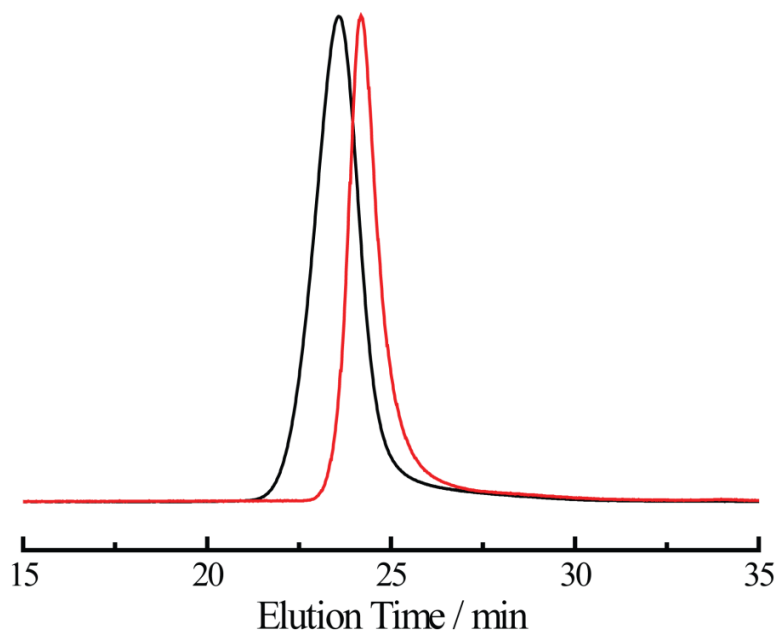
Figure S2. Overlay of the GPC curves (reflective index signals) of PN$_{10}$-g-PBLG$_{51}$ (in red) and PN$_{10}$-g-PBLG$_{102}$ (in black).
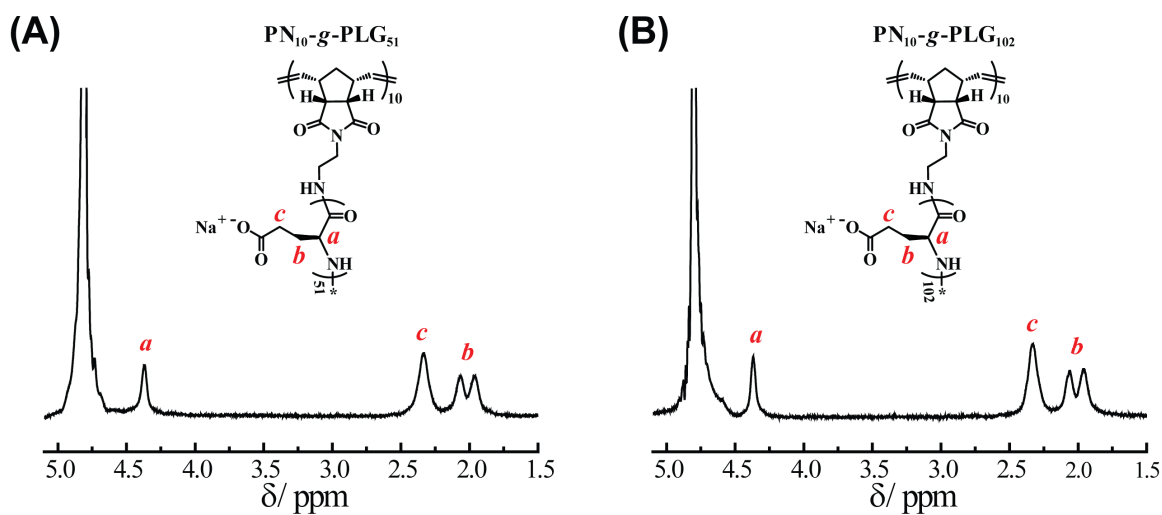
Figure S3. ^{1}H-NMR spectra of PN$_{10}$-g-PLG$_{51}$ (A) and PN$_{10}$-g-PLG$_{102}$ (B) in D$_2$O, pD adjusted to 7 by using NaOD.
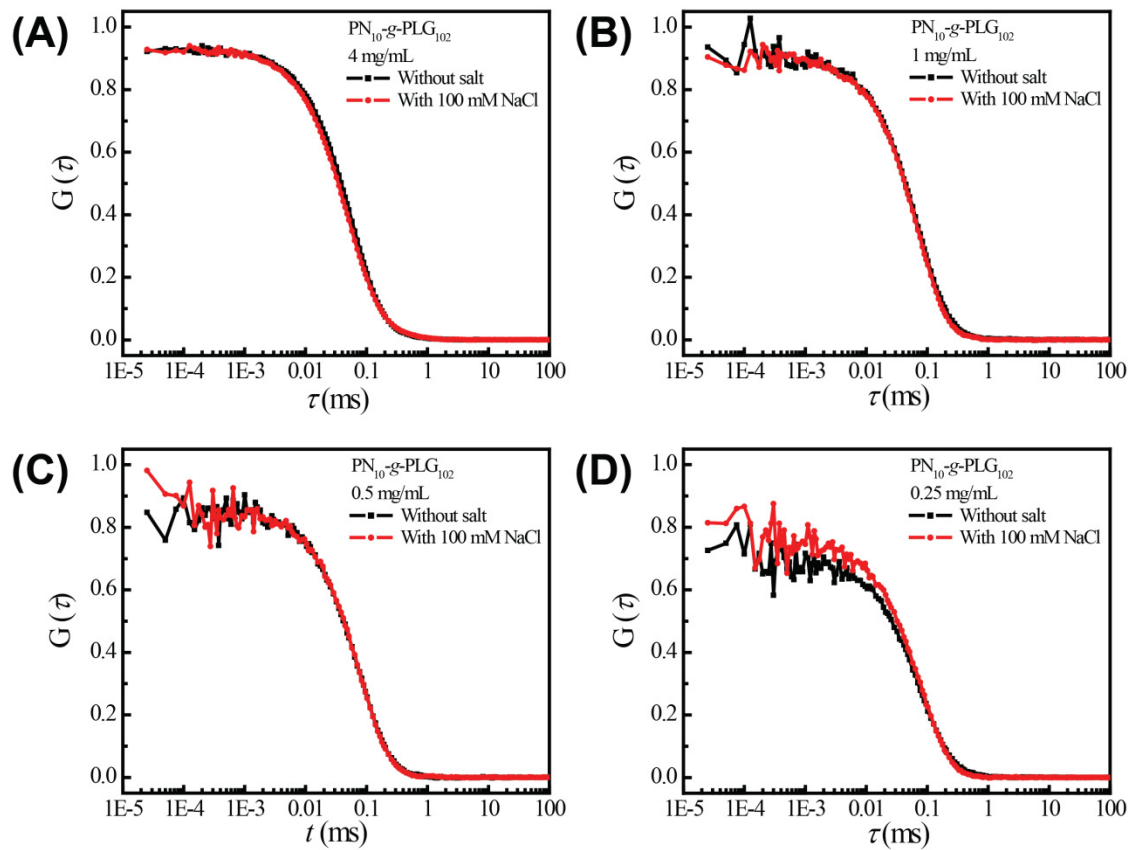
Figure S4. DLS correlation functions collected from a series of different concentrations of PN_{10}-g-PLG_{102} in solution (pH=7, adjusted by NaOH), with and without the addition of 100 mM NaCl.
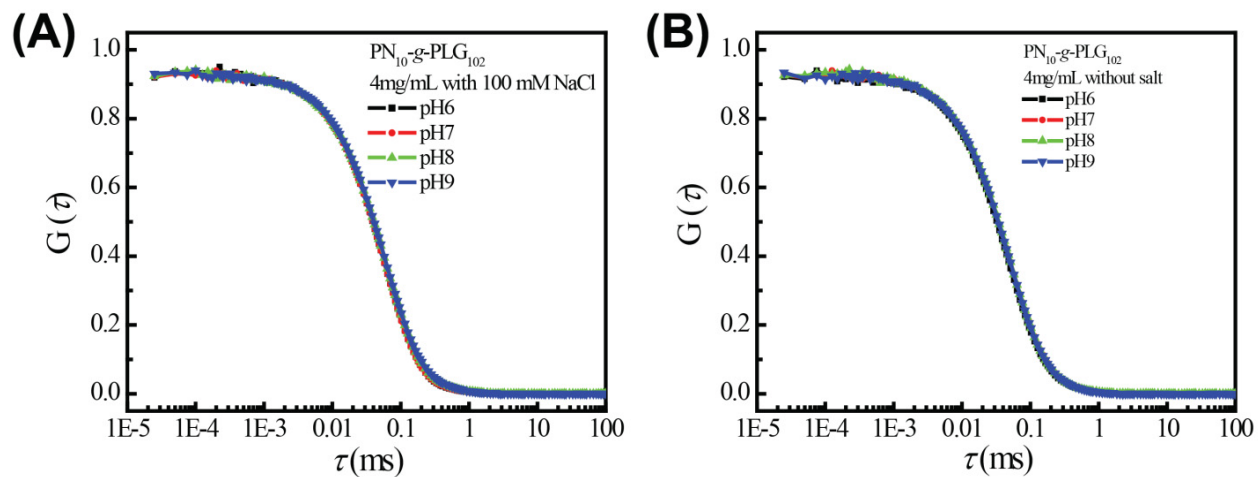
Figure S5. DLS correction functions collected from a series of PN_{10}-g-PLG_{102} (4 mg/mL) solutions with different pHs. pH of the solutions was adjusted by titration with 1M NaOH and 1M HCl.
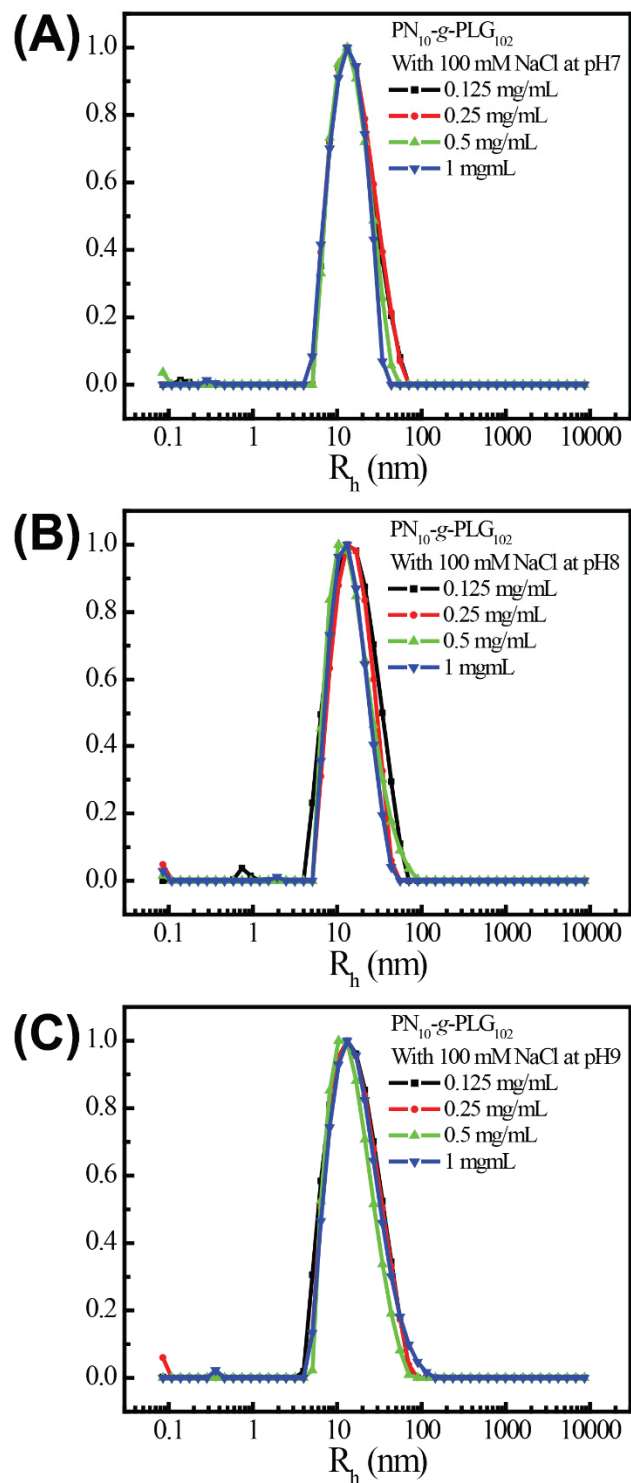
Figure S6. DLS analysis of PN$_{10}$-*g*-PLG$_{102}$ solutions (with 100 mM NaCl) at different polymer concentrations, and at (A) pH 7, (B) pH 8, and (C) pH 9.
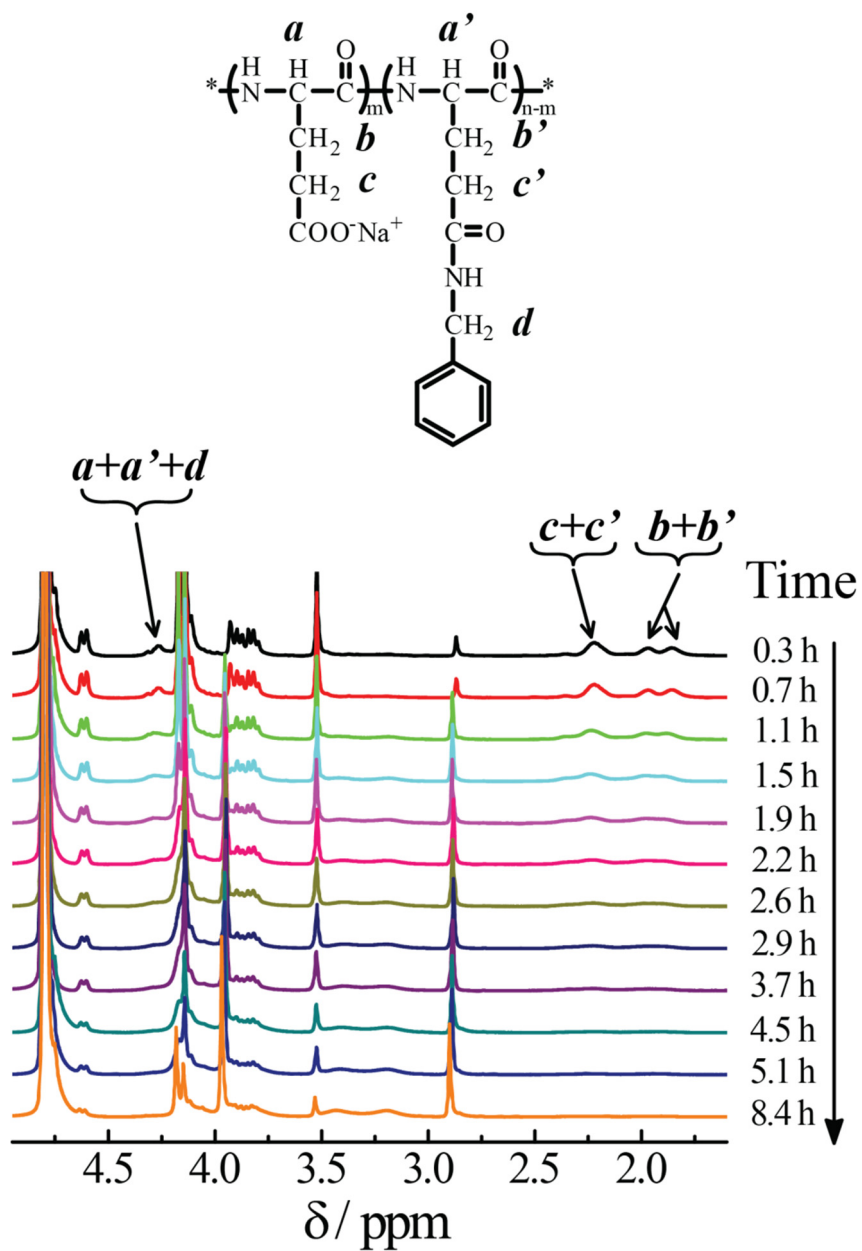
Figure S7. Time-dependent ^{1}H-NMR spectra of the reaction of benzylamine (BA) (2.88 mg/ml) with PN$_{10}$-g-PLG$_{102}$ (2 mg/ml) (molar ratio of BA:Glu:DMTMM = 2:1:1) in D$_2$O at pH 7 and RT.
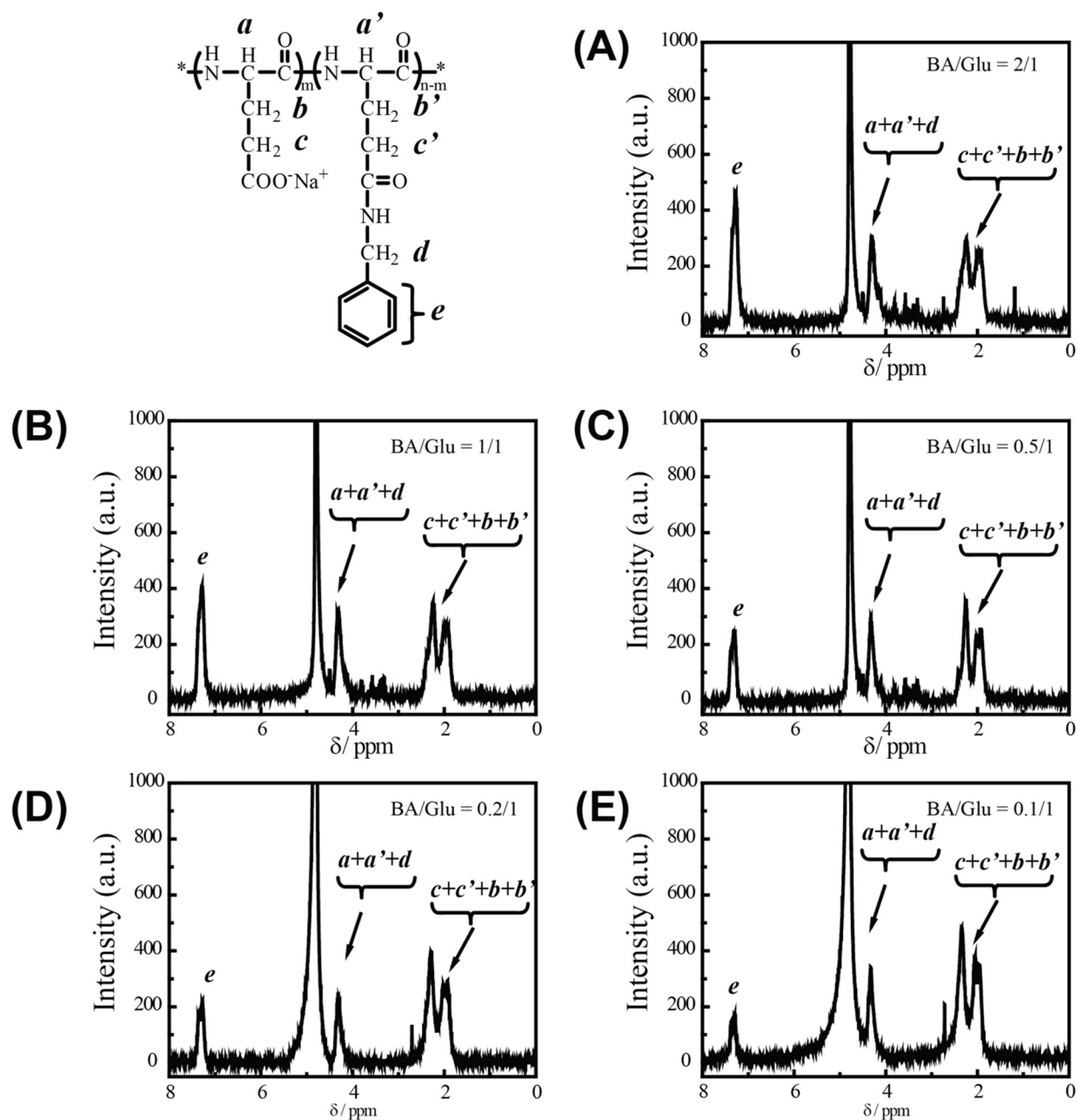
Figure S8. ^{1}H-NMR spectra from the BA-substituted PN_{10}-g-PLG_{102} samples in D_2O. The reaction products were obtained from overnight reactions of 0.5 mg/mL PN_{10}-g-PLG_{102}, with BA/Glu ratio = 2/1 (**A**), 1/1 (**B**), 0.5/1 (**C**), 0.2/1 (**D**) and 0.1/1 (**E**).
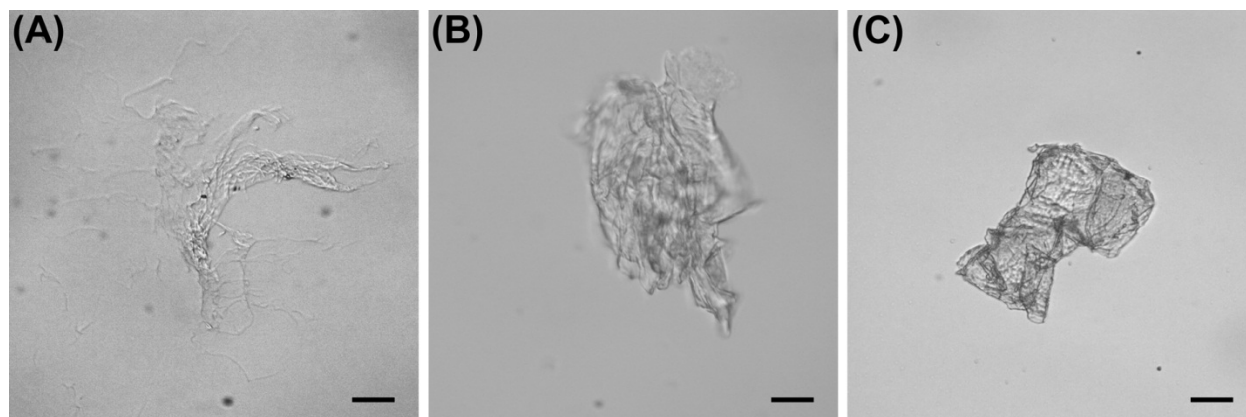
Figure S9. Optical microscope images of thin membrane supramolecular structures assembled from BA substituted PN_{10}-g-PLG_{102} samples with BA/Glu ratio of (A) 0.5/1, (B) 1/1, and (C) 2/1 after overnight incubation in pH 7 solution at RT (scale bar 20 μm).
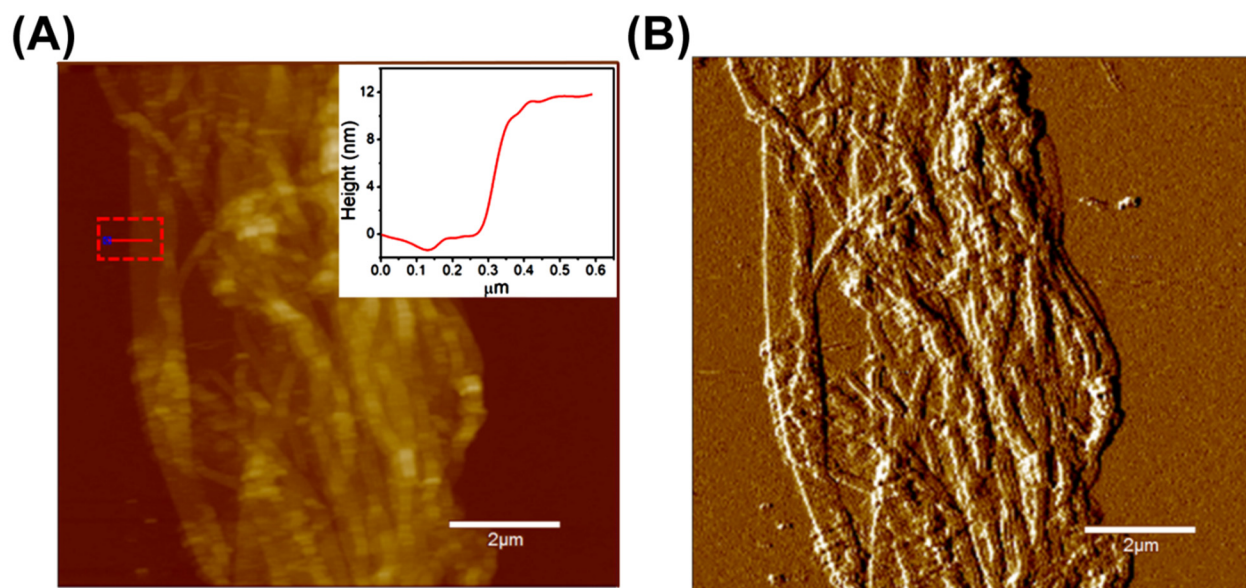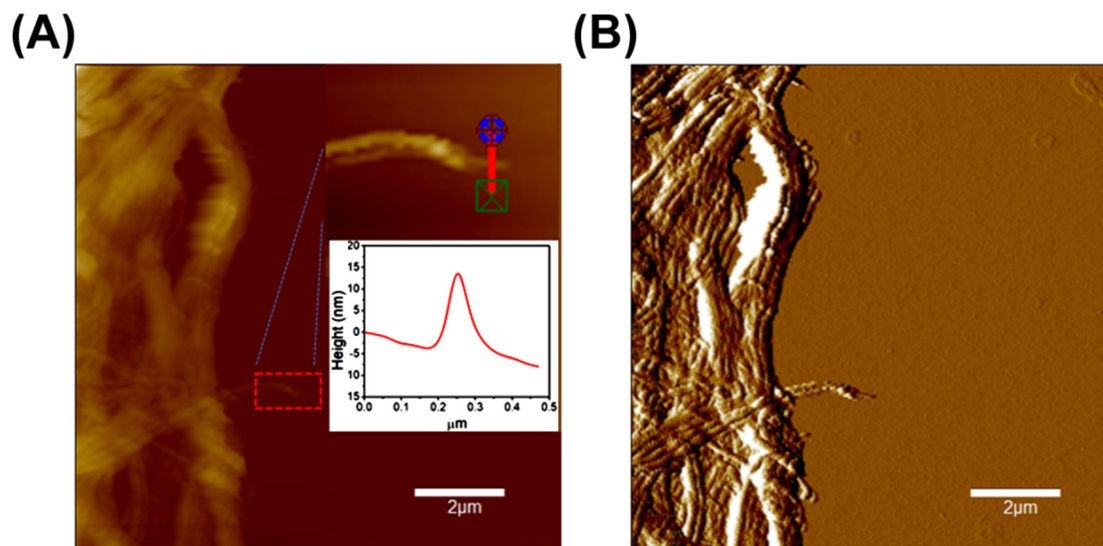
Figure S10. AFM images of thin membrane supramolecular structures based on the **(A)** height, and **(B)** amplitude profile, respectively. The membrane was assembled from BA substituted PN$_{10}$-*g*-PLG$_{102}$ samples at pH 7 and RT (BA/Glu=0.5/1 in the reaction).
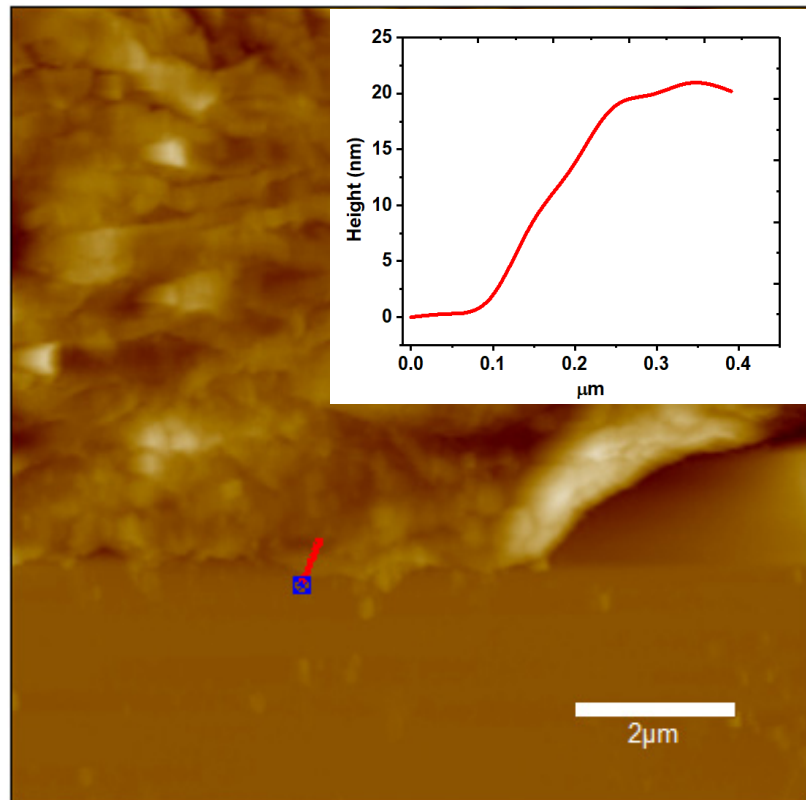
Figure S11. AFM images of thin membrane supramolecular structures based on the **(A)** height, and **(B)** amplitude profile, respectively. The membrane was assembled from BA substituted PN_{10}-*g*-PLG_{102} samples at pH 7 and RT (BA/Glu=0.5/1 in the reaction).

Figure S12. AFM image of the thin membrane supramolecular structure assembled from BA substituted PN_{10}-*g*-PLG_{102} samples at pH 7 and RT (BA/Glu=1/1 in the reaction).
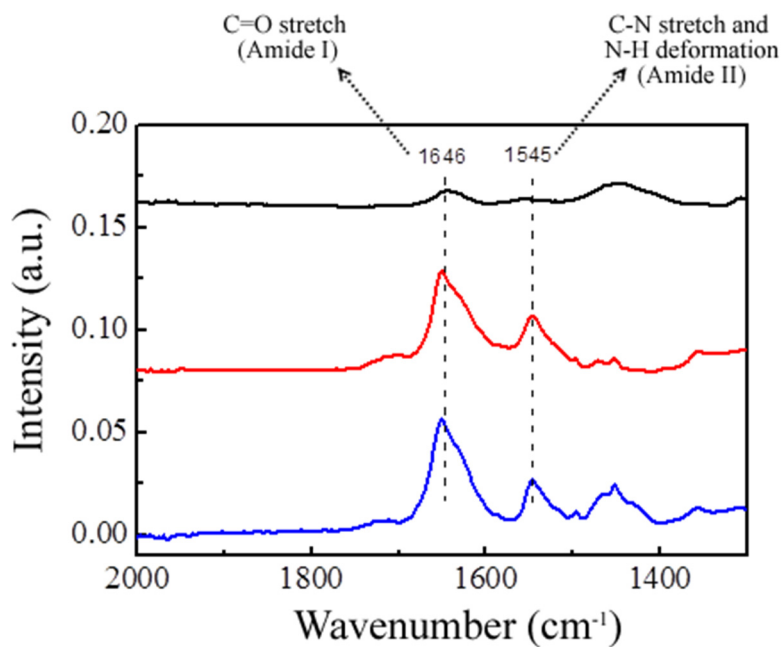
Figure S13. ATR-FTIR of reaction products from the BA-substitution of PN_{10}-*g*-PLG_{102} with BA/Glu ratio of 0.5 /1 (in black), 1/1 (in red), and 2/1 (in blue), respective. The reactions were carried out in the presence of DMTMM (DMTMM/Glu=1/1) at pH 7 and RT.
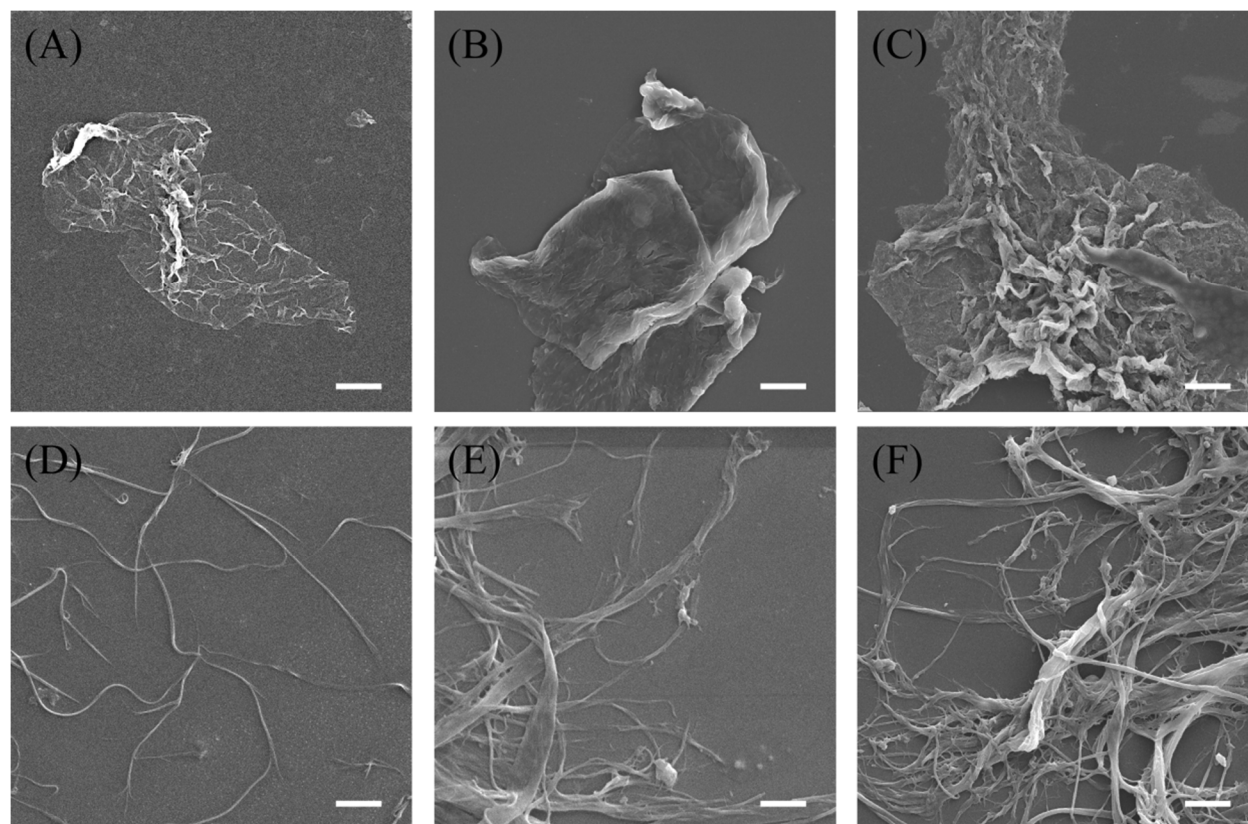
Figure S14. (A)-(C) FESEM images of thin membrane supramolecular structures assembled from BA substituted PN_{10}-g-PLG_{51} samples with BA/Glu ratio of 0.5/1 (A), 1/1 (B) and 2/1 (C), respectively. The samples were prepared after overnight reactions at pH 7 at RT (scale bar 2 μm). **(D)-(F)** FESEM images of fibrous supramolecular structures formed by incubating the membrane structures in (A)-(C) in a solution of pH 10 for about 6 h (scale bar 5 μm).
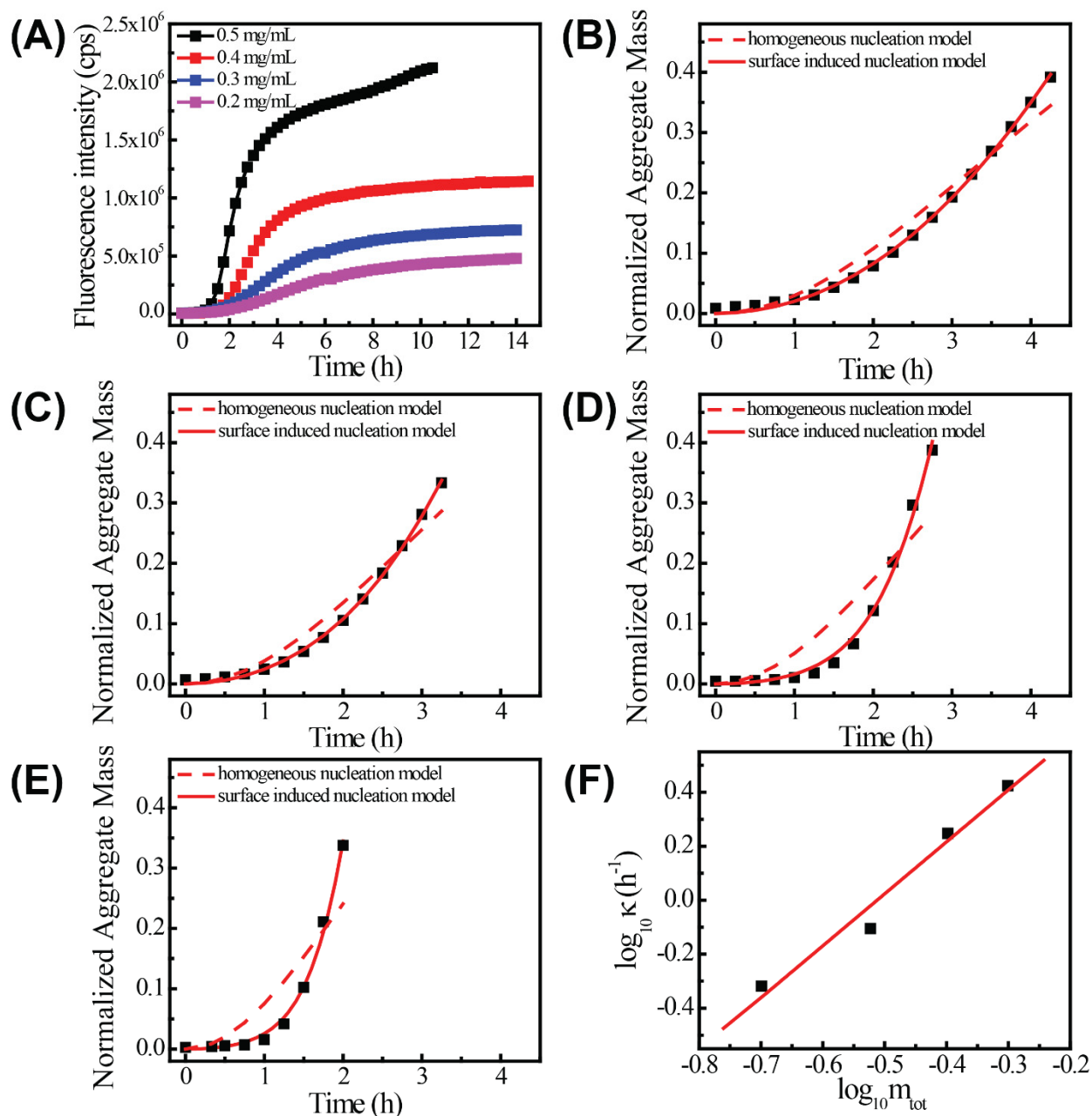
Figure S15. Model-based analysis of the assembly kinetics of PN_{10}-*g*-PLG_{51} with different polymer concentrations. (A) Time progress of ThT fluorescence of PN_{10}-*g*-PLG_{51} with different polymer concentrations from 0.1 mg/ml to 0.5 mg/ml. BA/Glu/DMTMM=5/1/1 in molar ratio. (B)-(E) Fitting of the early stage of kinetic process by the homogeneous nucleation model (in dash line) and the surface-induced nucleation model (in solid line). The concentration of PN_{10}-*g*-PLG_{51} is (B) 0.2 mg/mL, (C) 0.3 mg/mL, (D) 0.4 mg/mL, (E) 0.5 mg/mL, respectively. (F) Analysis of the dependence of fitted parameter κ on the initial polymer concentrations.
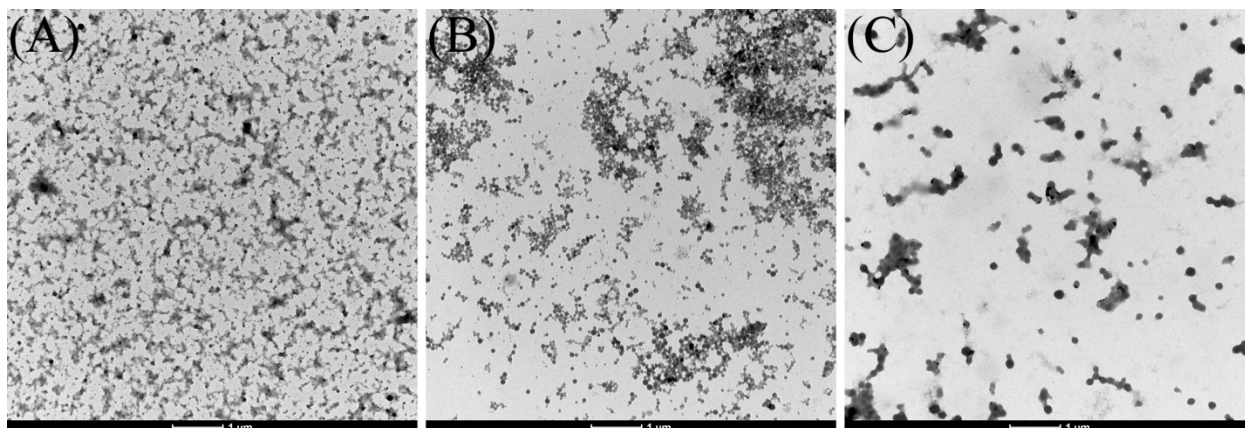
Figure S16. TEM images of spherical aggregates from BA substituted poly(acrylic acids) samples, with BA/ carboxylate ratio of 1/1 (A), 2/1 (B) and 5/1 (C) in the reaction.

Reference

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