

Nucleation-Controlled Polymerization of Nanoparticles into Supramolecular Structures

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Equations

By approximating the shape of particle aggregate by an ellipsoid with an axial ratio p , the change of standard chemical potential of the charged particles in an aggregate of N in relative to isolated particles in solution can be expressed as:

$$\Delta\mu_N^0(N, p) = k_B T \left[-n\varepsilon + \frac{(n-n')\varepsilon}{N^{1/3}} g(p) + \frac{N^{2/3} l_B z^2 f(p)}{d} \right] \quad (\text{S1})$$

where:

$$g(p) = \left(\frac{9\pi}{2}\right)^{1/3} p^{2/3} \left(1 + \frac{\text{ArcSin}\sqrt{1-p^2}}{p\sqrt{1-p^2}}\right) \quad (\text{S2})$$

$$f(p) = \frac{(36\pi)^{1/3}}{5} p^{2/3} \frac{\text{ArcTanh}\sqrt{1-p^2}}{\sqrt{1-p^2}} \quad (\text{S3})$$

The approach is similar to the theoretical method first proposed by Oosawa¹. However, the chemical potential of the particles in the aggregates are compared here rather than the free energy of the whole aggregates in Oosawa's paper¹.

Experimental Section

General. All chemicals were purchased from Sigma-Aldrich (St. Louis, Mo) and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF) was dried by columns packed with 4Å molecular sieves and stored in glove-box. Tetrahydrofuran (THF) and hexane were dried by columns packed with alumina and stored in a glove-box. γ -benzyl-L-glutamate *N*-carboxylanhydride (Glu-NCA) were prepared by following the previously reported procedures.²

Characterization. NMR spectra were recorded on a Varian UINB 500 MHz or a Bruker DRX 500 MHz spectrometer for polymer characterization. The NP-g-PLG₅₅ samples with different polymer contents were collected by centrifugation, dried in vacuum oven overnight, and dispersed in D₂O with 0.1 mg/ml DSS for NMR experiments. The absorbance spectra of NP-g-PLG in solution were detected from Nanodrop 1000. The solutions were kept at 4°C for the polymerization and the changes of Au particles' concentration in solutions during polymerization were tracked by the absorbance. The morphologies of Au nanoparticles and the supramolecular structures were characterized with a Tecnai T12 transmission electron microscopy (TEM) operating at an accelerating voltage of 120 kV. Samples were deposited on carbon-coated copper grids, blotted by filter paper and subsequently vacuum-dried. The supramolecular polymers poly(NP-g-PLG) were unstained. Field Emission Scanning Electron Microscopy (FESEM) was performed at various magnifications using a JEOL 6335 field-emission scanning electron microscope with an accelerating voltage of 10 kV. The samples were deposited on pre-cleaned glass slides and coated with palladium before imaging. Reflective Fourier-

transform infrared spectroscopy (FTIR) was performed on a Nicolet Magna 560 FTIR system equipped with 2x Spectra-Tech IR-Plan microscopes. The samples were deposited on gold-coated glass slides for the FTIR experiments in reflective mode. Laser confocal fluorescence microscopy (LCFM) experiments were performed on an Andor Confocal & TIRF Microscope. The excitation wavelength was chosen at 488 nm and the detection wavelength was at 509 nm. Before the experiments, the supramolecular polymers were stained with thioflavin T (ThT) for 15-min at 4 °C.

Synthesis of PLG_n-S-S-PLG_n polymers. PBLG_n-S-S-PBLG_n (PBLG represents poly(γ -benzyl-*L*-glutamate)) was synthesized by NCA polymerization using *N, N'*-bis(trimethylsilyl)cystamine as initiator using previously reported methods,^{2a} and the subsequent deprotection of PBLG using HBr gave PLG_n-S-S-PLG_n. The DP of PLG chains (*n*) were controlled by the monomer/initiator (M/I) ratios and determined by a combination of GPC and NMR.

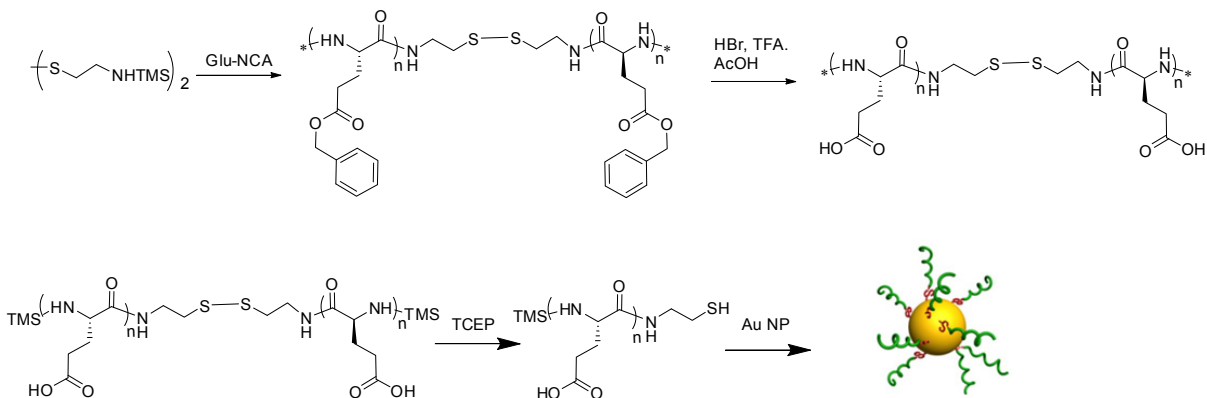
Synthesis of gold nanoparticles (Au NPs). The Au NPs (~40 nm and ~20 nm) were prepared by the Frens' method.³ Sodium citrate solution (1% (w/v)) was added to a boiling solution of HAuCl₄ (290 mL, 0.015% (w/v)) with vigorous stirring. The color of the solution turned wine-red after 3 min, indicating the formation of Au NPs. The solution was boiled for another 10 minutes, cooled to room temperature and the Au NPs were used for the preparation of PLG-grafted gold nanoparticles (NP-g-PLG). The size of Au NPs is controlled by the adding amount of sodium citrate.

Synthesis of PLG-grafted gold nanoparticles (NP-g-PLG) and quantify the average numbers of PLGs bounded on each particle. NP-g-PLG was synthesized by grafting PLG-SH on gold nanoparticles. The NP-g-PLG used in this study is denoted as NP_x-g-PLG_n, where *x* is the core of nanoparticles (in nanometers), and *n* is the degree of

polymerization (DPs) of PLGs. The PLG-SH ligands were obtained by cleaving the disulfide (S-S) bond in $\text{PLG}_n\text{-S-S-PLG}_n$ with the addition of tris(2-carboxyethyl)phosphine hydrochloride (TCEP). 2 mL of $\text{PLG}_{55}\text{-S-S-PLG}_{55}$ (4 mg/mL) was incubated with 24 μL of TCEP (10 mM) for 1 hr with stirring. 1 mL, 0.2 mL and 0.05 mL of the freshly made PLG-SH solutions were added to 30 mL Au NPs solutions (0.22 nM, NP core size 40 nm) to make three $\text{NP}_{40}\text{-g-PLG}_{55}$ samples ($\text{NP}_{40}\text{-g-PLG}_{55}\text{-I}$, II and III, respectively). In the preparation $\text{NP}_{20}\text{-g-PLG}_{55}$ samples, 0.3 and 0.03 mL of freshly made PLG-SH solutions were added to 3 mL of Au NPs solutions (5 nM, NP core size 20 nm) to make $\text{NP}_{20}\text{-g-PLG}_{55}\text{-I}$ and II, respectively. The solutions were incubated from 3 hours to overnight. Careful steps were taken to remove unbound PLGs from the solution after the synthesis of NP-g-PLG and quantify the PLG grafting densities on NPs. Multiple centrifugation-washing steps were carried out at pH 9, a condition selected to avoid PLG aggregation. The concentration of dispersed PLGs in the supernatant was monitored at each centrifugation-washing step (Figure S1), in order to determine the effectiveness of washing. The unbounded PLGs became out of detection limits after three centrifugation-washing steps (Figure S1). After removal of excess ligands, the amount of PLGs bounded on NPs was then determined from their ^1H NMR spectroscopy by the addition of 0.1 mg/ml of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) into the D_2O solution as the internal calibration standard. The ligand coverage was found to be tunable by controlling the initial amount of PLG-SH added into the synthesis of PN-g-PLG and the incubation conditions.

Supplementary Schemes, Tables and Figures

Scheme S1. Synthesis of PLG_n-S-S-PLG_n by ROP-NCA and the cleavage of disulfide bonds in PLG_n-S-S-PLG_n to obtain PLG_n-SH for the synthesis of PLG grafted Au-NP nanoparticles.



Scheme S2. The preparation of PLG grafted Au-NP nanoparticles, and the schematic illustration of the repeated precipitation-washing steps used in sample preparation.

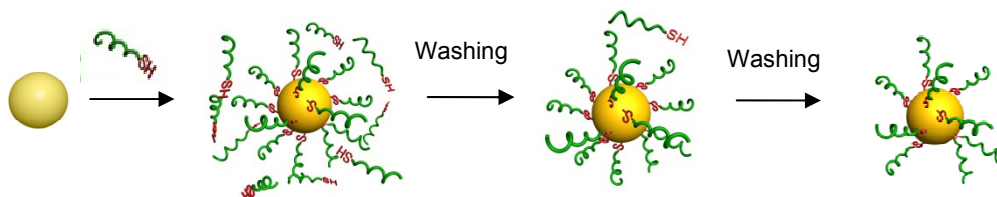


Table S1. Characterizations of the PBLG_n-S-S-PBLG_n polymers.

entry	polymer	n(n*) ^a	$M_n(M_n^*)^b$ ($\times 10^3$ g/mol)	MWD (M_w/M_n)
1	PBLG ₉₇ -S-S-PBLG ₉₇	97(93)	45.9(43.8)	1.02
2	PBLG ₅₅ -S-S-PBLG ₅₅	55(46)	25.9(21.9)	1.06

^a n = the obtained DP of polypeptides, n* = the expected DP of polypeptides;

^b M_n = the obtained M_n ; M_n^* = the expected M_n .

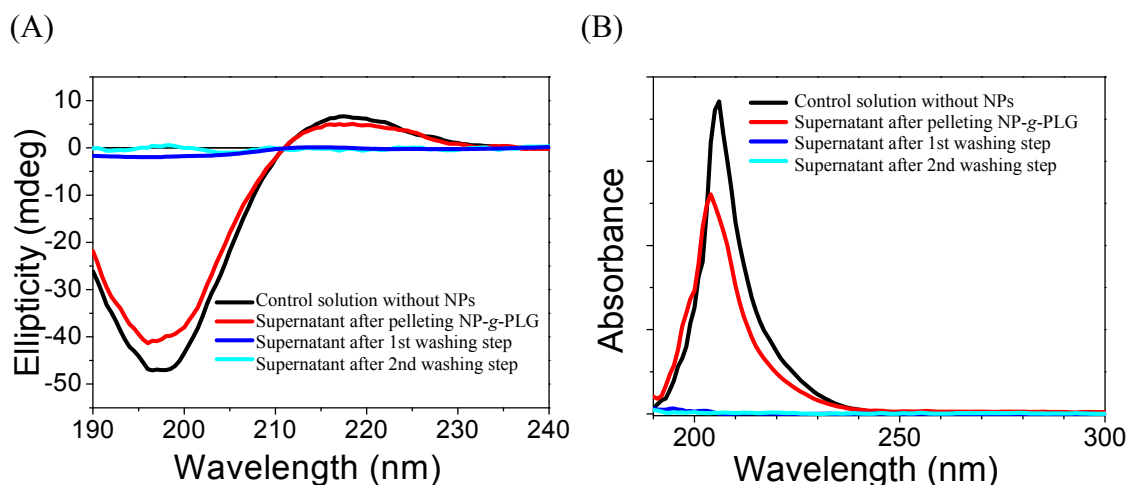


Figure S1. Removal of unbound PLG₅₅-SH in the NP₄₀-g-PLG₅₅ solution with repeated precipitation-washing steps, as monitored by (A) ultraviolet-visible (UV-Vis) spectroscopy, and (B) circular dichroism (CD) spectroscopy. The PLG₅₅-SH ligands were obtained by cleaving the disulfide (S-S) bond in PLG₅₅-S-S-PLG₅₅ with the addition of tris(2-carboxyethyl)phosphine hydrochloride (TCEP). PLG₅₅-S-S-PLG₅₅ was incubated with TCEP for 1 hr with stirring before the addition of Au NPs solution. The mixture was then incubated overnight. NP₄₀-g-PLG₅₅ was collected by centrifugation, washed by ultrapure water three times to remove the excess PLG-SH, and re-dispersed in water. At each step, the supernatants (red, blue and magenta lines) were collected for the analysis. For the control solution (black line), PLG₅₅-SH was mixed with the same amount of water instead of Au NPs solution. pH was adjusted to 7 just before the characterizations. Thiol groups have strong affinity to Au surface, and the majority of unbound PLG-SH in solution can be removed by precipitation and washing with water repeatedly (blue and magenta lines).

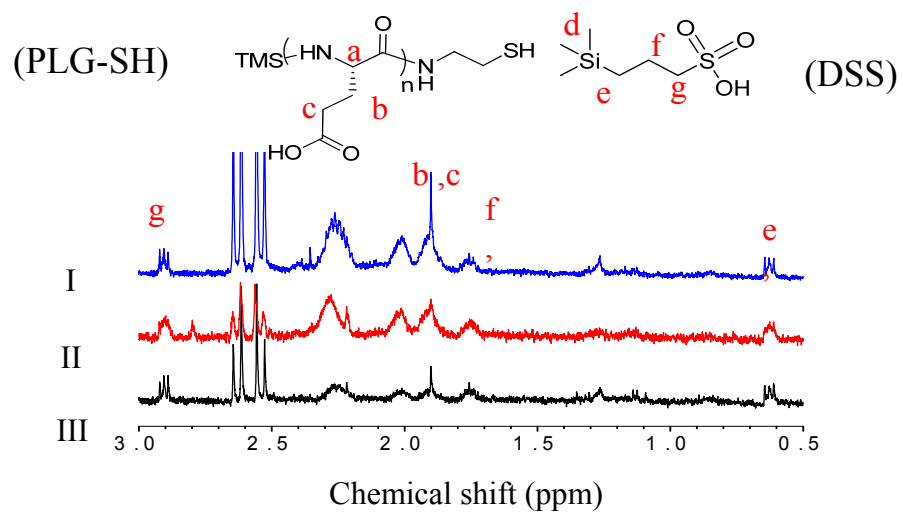


Figure S2. ^1H NMR spectra of three $\text{NP}_{40}\text{-g-PLG}_{55}$ samples in D_2O ($\text{NP}_{40}\text{-g-PLG}_{55}\text{-I}$, II and III with different numbers of bounded PLG ligands per particle). 0.1mg/ml of DSS is added as internal calibration standard for the calculation of PLG contents.

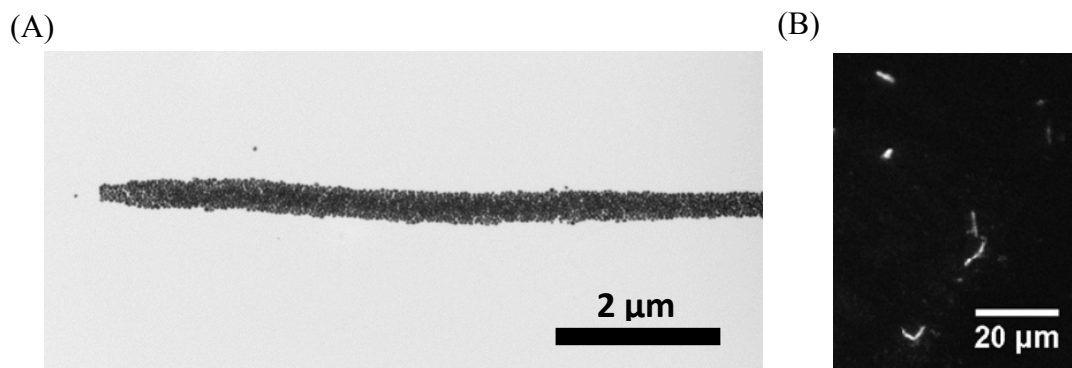


Figure S3 (A) TEM image of a fibrous supramolecular structure assembled from NP₄₀-g-PLG₅₅-III in solution after incubation for 14 days at pH 6.5 and 4 °C. (B) Fluorescence microscopy image of a number of poly(NP₄₀-g-PLG₅₅-III) in solution after stained with thiofavin T dye (ThT).

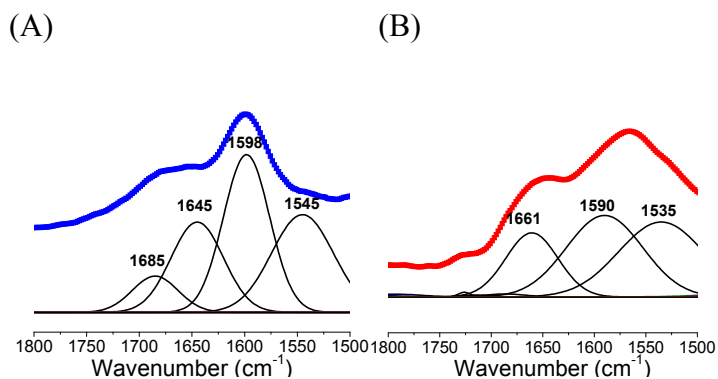


Figure S4. (A) FTIR spectrum of isolated poly(NP₄₀-g-PLG₅₅-III) and assignment of the absorption peaks. The amide absorption peaks indicate the formation of some β -sheet structures in the assemblies on the basis of the assignment of the absorption peaks at 1685 cm⁻¹ (amide I, antiparallel), 1645 cm⁻¹ (amide I, parallel) and 1545 cm⁻¹ (amide II, parallel). The peak at 1598 cm⁻¹ was due to the ionized carboxylate groups. In comparison, (B) a film of NP-g-PLG₅₅-III casted from freshly made solution exhibited the absorption peaks at 1661 cm⁻¹ (amide I, coil), 1590 cm⁻¹ (ionized carboxylate) and 1535 cm⁻¹ (amide II, coil). The structural change of the grafted PLGs on the NPs during the assembly process allow for thermodynamically favored growth of supramolecular structure.

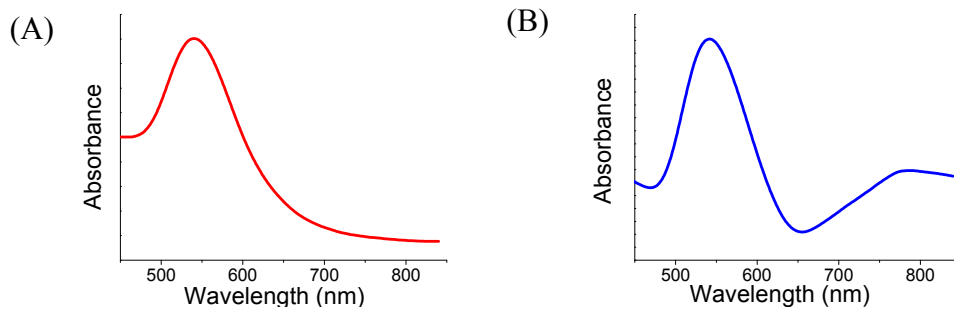


Figure S5. Comparison of optical spectra of the NP₄₀-g-PLG₅₅-III solution (A) before and (B) after the formation of fibrous poly(NP₄₀-g-PLG₅₅-III) supramolecular structures in solution.

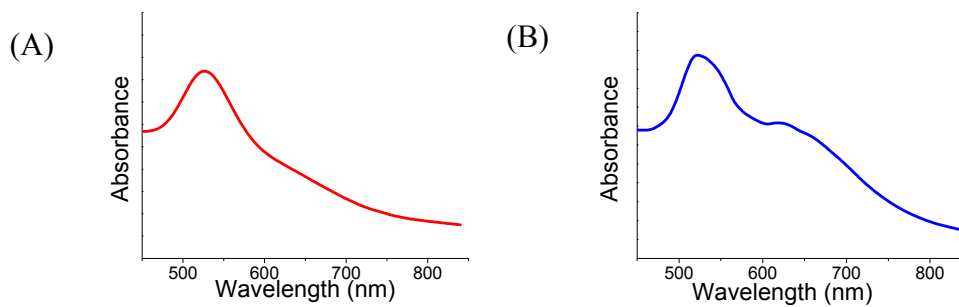


Figure S6. Comparison of optical spectra of the NP₂₀-g-PLG₅₅ (~100 ligands per particle) solution (A) before and (B) after the formation of tubular poly(NP₂₀-g-PLG₅₅) supramolecular structures in solution.

References:

- (1) Oosawa, F. *Journal of Polymer Science* **1957**, *26*, 29-45.
- (2) (a) Lu, H.; Cheng, J. J. *J. Am. Chem. Soc.* **2008**, *130*, 12562-12563; (b) Lu, H.; Wang, J.; Lin, Y.; Cheng, J. J. *J. Am. Chem. Soc.* **2009**, *131*, 13582-13583; (c) Wang, J.; Lu, H.; Kamat, R.; Pingali, S. V.; Urban, V. S.; Cheng, J. J.; Lin, Y. *J. Am. Chem. Soc.* **2011**, *133*, 12906-12909.
- (3) Frens, G. *Nature-Phys. Sci.* **1973**, *241*, 20.