Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Directed arrangement of siRNA via polymerization-induced electrostatic self-assembly

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Abstract: Herein, polymerization-induced electrostatic self-assembly (PIESA) is conducted to mediate the self-assembly behavior of short interfering RNA (siRNA) for the first time. In PIESA, siRNA not only formed a simple electrostatic polyplex with positively charged polycations, but also facilitated directed self-assembly due to the molecular rigidity of siRNA, leading to appealing nanotubes.

Experimental Procedures

Materials

Poly (ethylene glycol) methyl ether ($mPEG_{113}$-OH, 5000 g/mol), 3-acrylamidopropyl trimethylammonium chloride (APTAC), calf thymus DNA (CT-DNA) and lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LPTP) were purchased from J&K Scientific. Diethyl pyrocarbonate (DEPC) water was purchased from Shanghai Aladdin Bio-Chem Technology. SYBR safe DNA gel stain was purchased from Thermo Fisher Scientific. siRNA-scramble: sense (5'-UUC UCC GAA CGU GUC ACG UTT-3'), antisense (5'-AGG UGA CAC GUU CGG AGA ATT-3') was purchased from Shanghai Genepharma Scientific. Macromolecular chain transfer agent (CTA) PEG_{113}-CTA was synthesized according to a previously published procedure.[1]

Characterization

^{1}H NMR analysis was performed to confirm the monomer conversions on a Bruker AV 600 MHz spectrometer. Size distributions and zeta potentials were carried out a Malvern ZS90 with a He Ne laser (633 nm, 4 mW) at a 90° angle. Gel permeation chromatography (GPC) measurement of PEG_{113}-CTA were performed on a Waters Alliance e2695 GPC system equipped with a Styragel guard column (WAT054415, 30 × 4.6 mm), two Org separation columns consisting of D2500 (300 × 8 mm) and D5000 (300 × 8 mm). Detection was made with a 2414 refractive index detector (Waters Alliance), a Viscotek 302/305 UV detector (Malvern Instruments), and a Viscotek TDA 305-020 LALS/RALS detector (Malvern Instruments). *N*, *N*-dimethylformamide (DMF, HPLC grade, containing 1.75 mg/mL LiBr) was used as the eluent at a flow rate of 0.7 mL/min. Molecular weight distributions (polydispersity index, PDI = M_w/M_n) of PEG_{113}-CTA and PEG_{113}-$PAPTAC_x$ block copolymers were determined by aqueous GPC measurements on a Waters 1515 HPLC and a 2410 refractive index detector, equipped with PL aquagel-OH MIXED-M columns. 0.1 M acetate buffer (pH 2.8) was used as eluent at a flow rate of 1.0 mL/min. The sample concentration was 2.0 mg/mL, and polyethylene glycol of a different molecular weight (2 mg/mL) was used as the standard for determination of the calibration curve. Morphology of nano-objects was characterized by transmission electron microscopy (TEM) on a Jeol 200CX microscope and SEM on a SU8010 microscope. Typically, a small drop of a sample solution (0.1% dispersed in DEPC water) was carefully dropped onto a carbon-coated copper grid (for TEM) or a silicon wafer (for SEM) and dried overnight at 25 °C under vacuum. The siRNA-containing nano-objects were analyzed by electrophoretic mobility-shift assays in a 2% agarose gel in 1 × TAE buffer and run for 20 min at a voltage of 120 V. The siRNA bands were stained by SYBR safe DNA gel stain and detected by a WD-9413B CCD camera.

Synthesis protocol of siRNA-based PIESA

Before polymerization, the glass vials were soaked by RNA enzyme scavenger and washed by DEPC water. siRNA-based PIESA was conducted as follow: a certain amount of PEG_{113}-CTA, APTAC, siRNA and LPTP were added into a glass vial and dissolved in DEPC water. Then the glass vial was sealed without being degassed. The illumination under purple light (5 mW/cm^2, maximum intensity at 406 nm) lasted for 16 h at 20 °C with a stirring speed of 300 rpm. The glass vial was fixed 0.4 cm above the lamp. After polymerization of 16 h, the glass vial was removed away from the lamp and the solution was exposed to air. DNA-based PIESA was also performed using the same protocol.

RNase A assay

siRNA-containing nano-objects were incubated in the presence of 0.25 μg/mL RNase A at 37 °C for 0, 5.10, 30, 60, 120 and 240 min. Then 2.0% SDS solution was added and incubated at 4.0 °C to release siRNA. Samples were analyzed by electrophoretic mobility-shift assays in a 2% agarose gel in 1 × TAE buffer and run for 20 min.

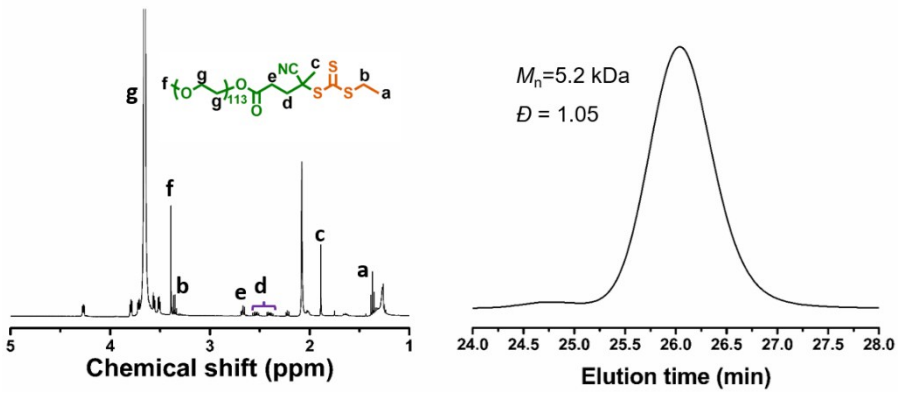


Figure S1. A) ^{1}H NMR spectrum of PEG_{113}-CTA in $CDCl_3$; B) GPC trace of PEG_{113}-CTA using DMF (HPLC grade, containing 1.75 mg/mL LiBr) as the eluent.

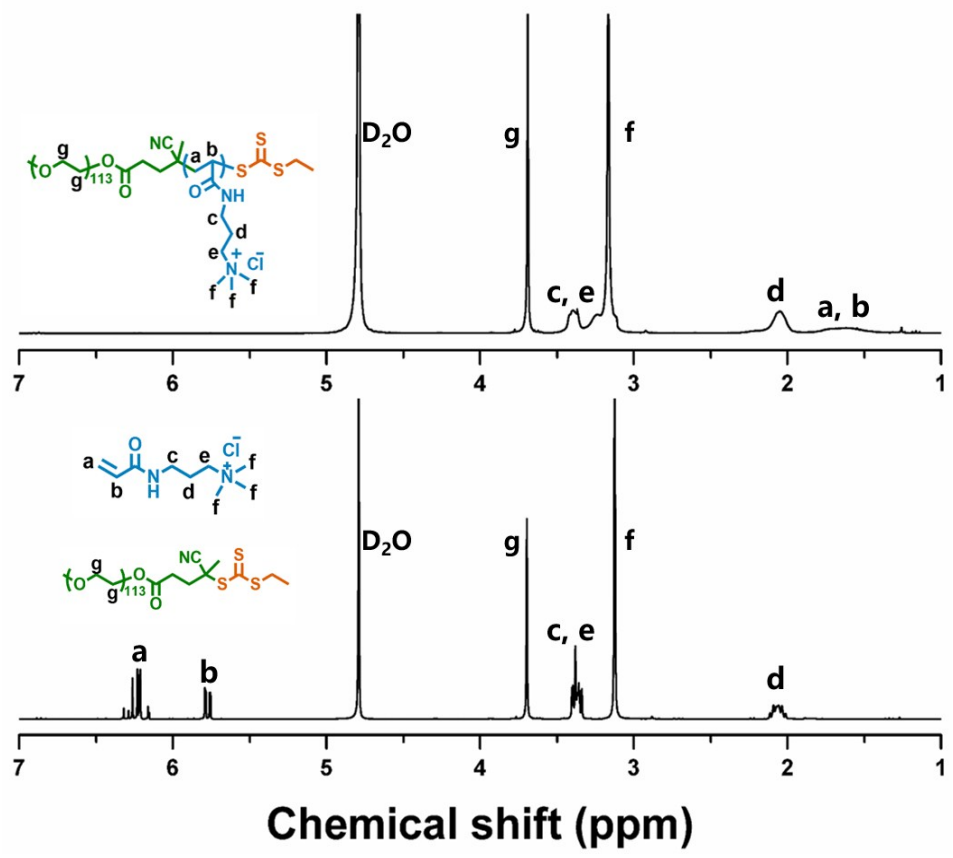


Figure S2. ^{1}H NMR spectra of polymerization system: PEG_{113}-CTA@siRNA@APTAC in D_2O before (bottom) and after (top) polymerization. [PEG_{113}-CTA]/[siRNA]/[APTAC]=1:0.73:50.

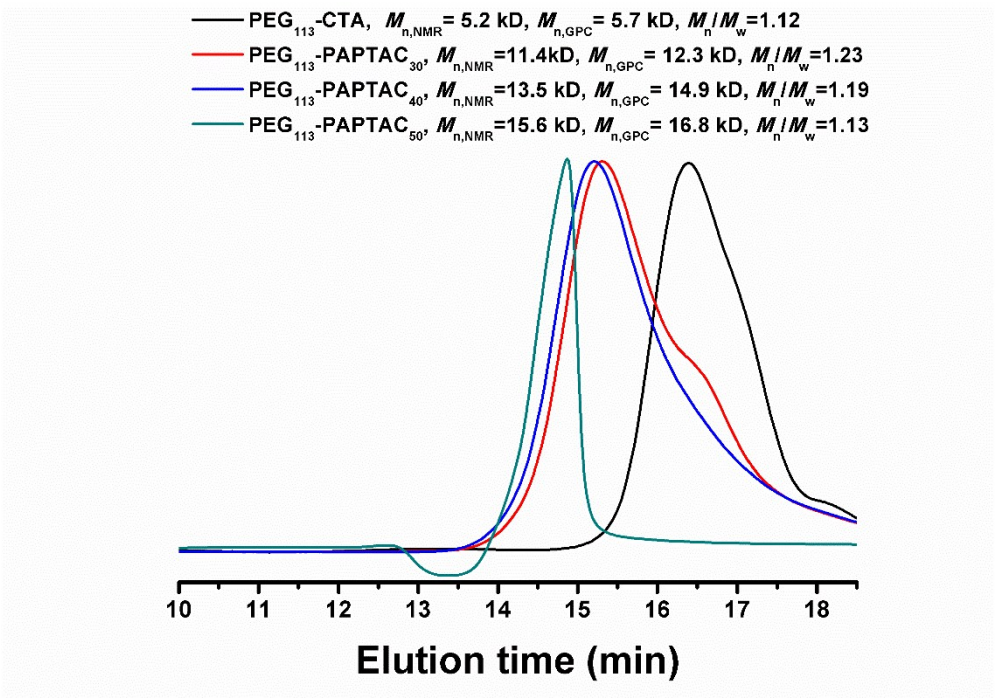


Figure S3.GPC traces of PEG_{113}-$PAPTAC_x$ block copolymers synthesized in the siRNA-based PIESA.

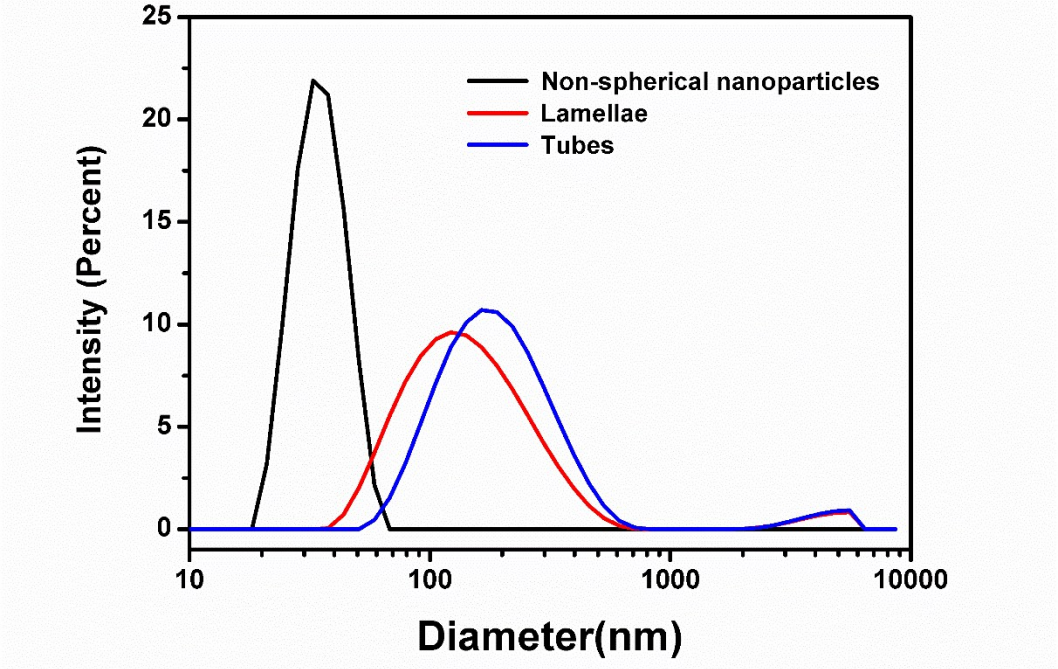


Figure S4. DLS analysis of siRNA-containing nanostructures through PIESA in DEPC water at 20 °C under purple light illumination: non-spherical nanoparticles for PEG_{113}-$PAPTAC_{30}$@siRNA; lamellar structures for PEG_{113}-$PAPTAC_{40}$@siRNA; nanotubes for PEG_{113}-$PAPTAC_{50}$@siRNA.

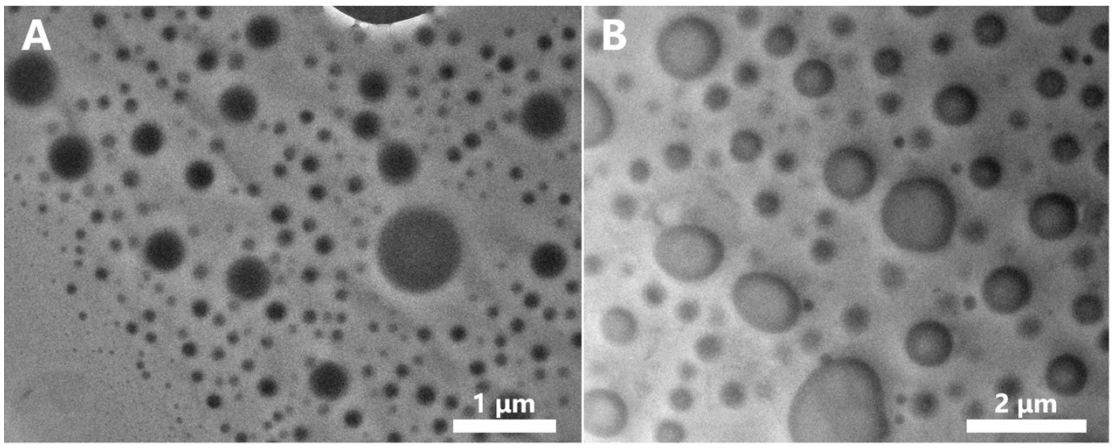
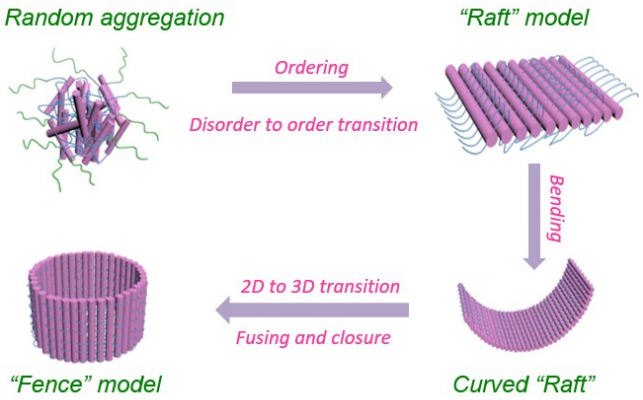


Figure S5. TEM images of the siRNA-containing nanostructures through PIESA in DEPC water at 20 °C under purple light illumination: A) image for PEG_{113}-$PAPTAC_{60}$@siRNA self-assemblies; B) image for PEG_{113}-$PAPTAC_{70}$@siRNA self-assemblies.



Scheme S1. Schematic demonstration of possible mechanism for morphological evolution of siRNA-containing nanostructures through PIESA.

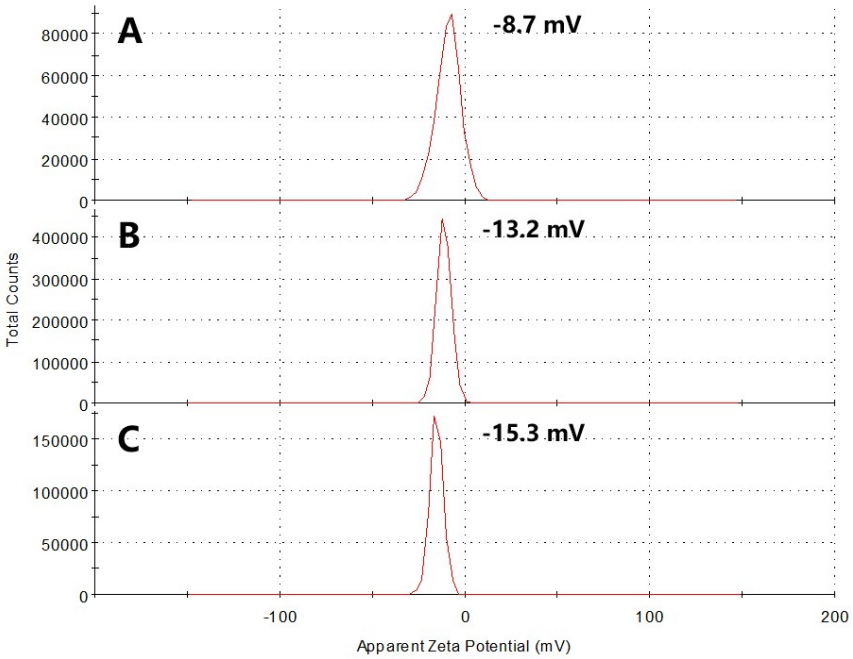


Figure S6. The zeta potential of siRNA-containing nano-objects: A) non-spherical nanoparticles; B) lamellas and C) nanotubes.

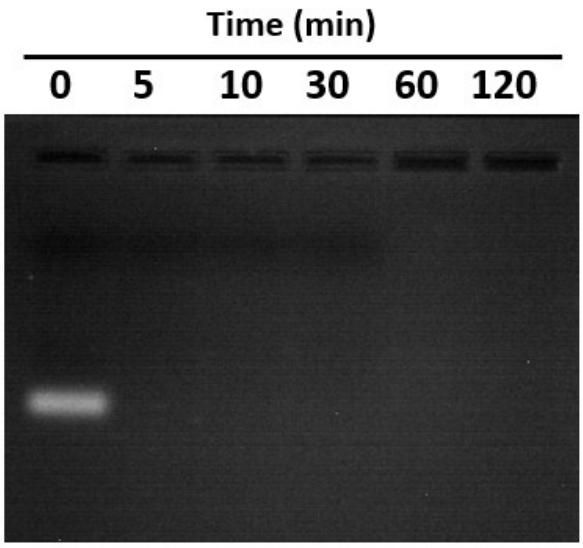


Figure S7. Naked siRNA was degraded rapidly by RNase within 5.0 min.

References

[1] W. Shen, Q. Qiu, Y. Wang, M. Miao, B. Li, T. Zhang, A. Cao, Z. An, *Macromol. Rapid Commun.*, 2010, **31**, 1444.