Role of DNA in Condensation and Combinative Self-Assembly

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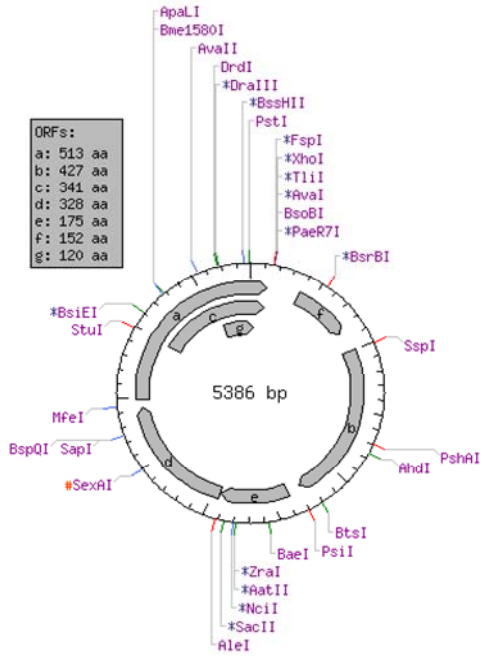
Supporting Information

Materials

The double-stranded negative-supercoiled, relaxed-circular and single-stranded circular ΦX174DNA, supplied in 1XTE buffer pH 8.0, were purchased from New England Biolabs. The 1XTE buffer was exchanged for 18.2MΩ water using Qiagen QIAquick gel extraction kit. The quantity of the DNA was determined by spectrophotometric analysis at 260nm. DNA was diluted to a final concentration of 20ug/mL in 18.2uΩ water for further studies.

Obtaining the linear double-stranded DNA by linearization of the negative-supercoiled ΦX174 plasmid DNA



ΦX174 plasmid DNA

Double-stranded supercoiled ΦX174 plasmid DNA was linearized with the restriction enzyme *SspI* and purified using Qiagen QIAquick gel extraction kit according to supplier's protocol. Briefly, 5 units of *SspI*/ug DNA in 1X NEBuffer were incubated at 37°C for 3 hours, followed by inactivation at 65°C for 30 min. Three volumes of Buffer QC and one volume of isopropanol was added to the reaction mixture, placed in the spin column, centrifuged and washed with Buffer PE. Linearized DNA was eluted from the column with 18.2uΩ water. Full linearization of the DNA was determined by agarose gel electrophoresis (1.2% agarose, 100V, 60 minutes).

SspI Recognition Site

5'…AAT ATT…3'
3'…TTA TAA…5'

Obtaining the linear single-stranded DNA by denaturation of the linear double-stranded DNA

Denaturation of the linear double-stranded DNA was performed by heating the DNA solution to 95^{o}C for 4 minutes, followed by immediate incubation on ice. The degree of denaturation of the single-stranded linear ΦX174 RFI was assed by agarose gel electrophoresis (1.2% agarose, 100V, 60 minutes). The quantity of double-stranded linear ΦX174 RFI and single-stranded linear ΦX174 RFI was determined by spectrophotometric analysis at 260nm. The DNA was diluted to a final concentration of 20ug/mL in 18.2MΩ water for further studies.

Grafting of gene-binding oligopeptide onto PBD-b-PEO diblock copolymer

The amphiphilic diblock copolymer scaffold (PBD_{14}-*b*-PEO_{93}, subscripts denote the average number of repeating units) with a narrow molecular-weight distribution (M_n ~ 4900 g/mol) polydispersity index PDI = 1.05) was prepared by the well-established sequential anionic polymerization of buta-1,3-diene and ethylene oxide.[S1] Thiol-containing Cysteine (Cys) was attached to the gene-binding oligopeptide sequence KWK_4 as the linker terminus for grafting purpose. Peptide $CKWK_4$ was synthesized using the standard Fmoc Solid Phase Peptide Synthesis[S2] procedure with HOBT, HBTU, and DIPEA couplings, followed by N-capping with acetylation and C-capping with amidation. The synthesized peptides were analyzed by ESI-MS and ^{1}H NMR before grafting. $CKWK_4$ was then grafted to PBD_{14}-*b*-PEO_{93} to produce **PP40** according to the procedure published elsewhere, utilizing the free radical addition of the thiol group of the cytseine to the double bonds of PBD.[S3, S4] Characterization of **PP40** by NMR and SEC has been published elsewhere.[S4] Briefly, NMR analysis confirms that eight butadiene units of PBD_{14}-*b*-PEO_{93} have been grafted with the $CKWK_4$ peptide in **PP40** (M_n ~ 12,800 g/ml) , and SEC shows that **PP40** has similar narrow PDI as the PBD_{14}-*b*-PEO_{93} scaffold.

Methods

Agarose Gel Electrophoresis

Fifteen microliters of each DNA sample was loaded onto a 1.2% agarose gel, and the electrophoresis was ran at 100V for 60 minutes in 40mM Tris-acetate, 1mM EDTA, pH 8 running buffer. Gels were stained with ethidium bromide (0.5ug/ml, 60 minutes) and visualized with a UV transilluminator.

AFM Analysis

10ug/mL of each DNA aqueous solution was mixed 1:1 with 20mM Tris, 2mM EDTA, 12.5mM $MgCl_2$. Two microliters of the mixed DNA solution was then deposited on to freshly cleaved mica and dried under a gentle flow of nitrogen gas. AFM imaging was then performed using tapping mode at ambient temperature on a Dimension 3100 AFM (Veeco), equipped with Nanoscope III software (Digital Instruments, Santa Barbara, CA). Silicon probes (Veeco RTESP, spring constant 40N/m, resonant frequency 300Hz) were used to obtain all images.

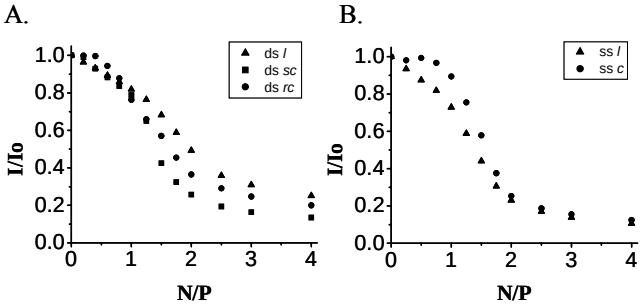
TEM Analysis

Equal volume of measured **PP40** (78 μM was added to each DNA aqueous solution (10ug/ml) to obtain final N/P ratio of 3 (N: nitrogen from positively charged lysine; P: phosphorous from the negatively charged DNA backbone). The DNA-PP40 complexes were allowed to equilibrate at room temperature for 1 hour and then deposited onto glowdischarged formvar coated copper grids. The complexes were then stained with 2% uranyl acetate for 1 minute, and the grids were blotted and air-dried. EM imaging was performed on a 200kV Tecnai 20 transmission electron microscope at a magnification of 10,000X.

Ethidium bromide (EB) displacement assay

The degree of DNA condensation was determined as a function of the N/P ratio by a ethidium bromide displacement assay. In the EB displacement assay, binding of an agent to DNA would displace the intercalated EB and subsequently quench the fluorescence caused by the EB-DNA complex. Before measurement, DNA (20ug/mL) was incubated with EtBr (0.8ug/mL) for 1 hour. Concentrated PP40 (78uM) was then titrated into the DNA-EtBr solution. The fluorescence intensity of samples at different N/P ratios were excited at 520nm, and the fluorecence was measured at 590nm at temperature of 25^{o}C, using a Jobin Yvon FluoroMax-3. Sample fluorescence was determined after subtracting the baseline fluorescence of EtBr in the absence of the DNA.

Figure S1. Binding of **PP40** with ΦX174 DNA's via EB displacement Assay. (A) ds ΦX174 DNA. (B) ss ΦX174. I_0 = fluorescence intensity of DNA-EB complex without addition of **PP40**.



For each form of DNA, **PP40** continuously binds to DNA and quenches the fluorescence as N/P increases. Upon N/P = 3, **PP40** is able to achieve nearly complete quenching ($I/I_0 \leq 0.3$), indicating complete binding between **PP40** and DNA.

Figure S2. More representative AFM images of the five different forms of ΦX174 DNA.

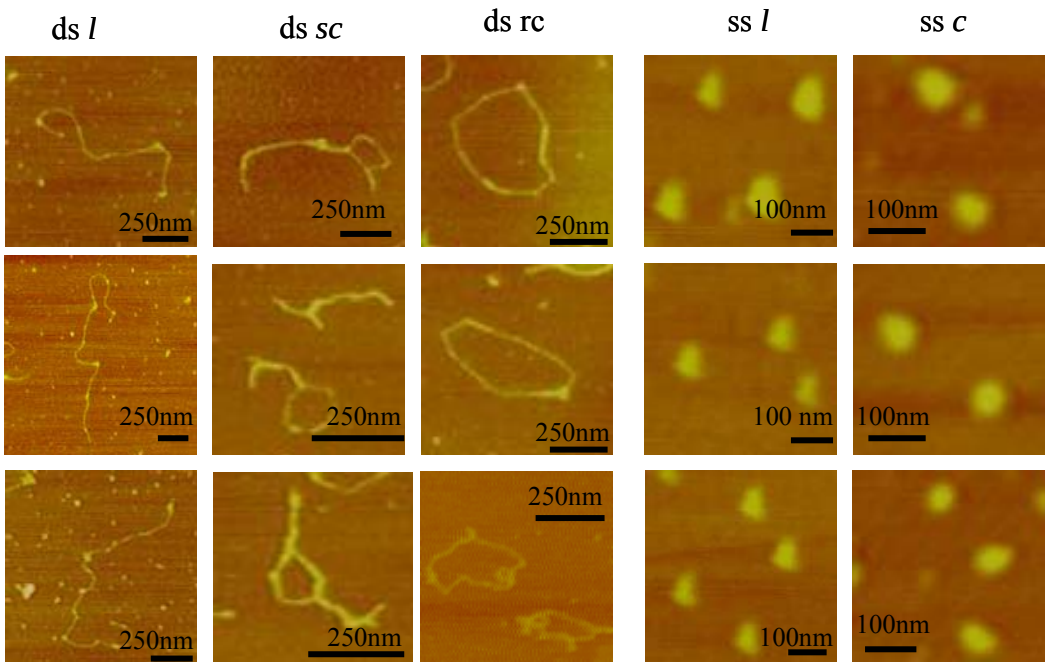
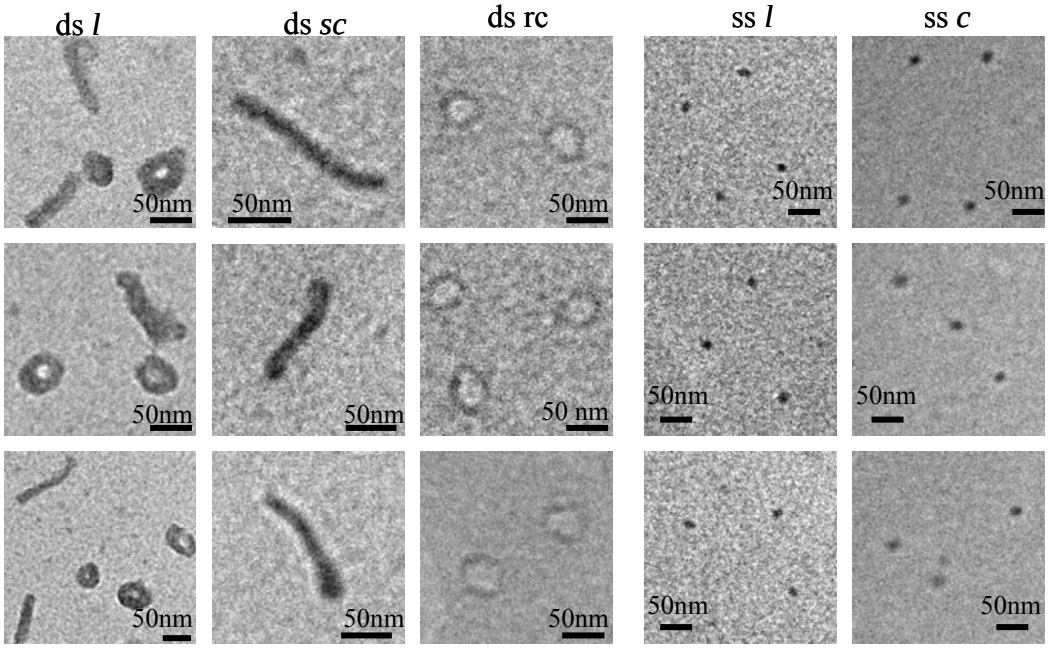


Figure S2. More representative TEM images of the five different forms of ΦX174 DNA condensates via the combinative self-assembly.



References:

(S1) Forster, S.; Kramer, E. *Macromolecules* **1999**, *32*, 2783-2785.

(S2) Merrifield, B. *The concept and development of solid-phase peptide synthesis*; American Chemical Society: Washington DC, 1993.

(S3) Geng, Y.; Discher, D.; Justynska, J.; Schlaad, H. *Angew. Chem.* **2006**, *45*, 7478-7581.

(S4) Haley, J.; Geng, Y. "Effect of clustered peptide binding on DNA condensation", *Molecular BioSystems*, 2009, DOI:10.1039/B908873C