Poly(oligonucleotide)

Carrie R. James¹, Anthony M. Rush¹, Thomas Insley², Lela Vuković², Lisa Adamiak¹, Petr Král²,³, and Nathan C. Gianneschi*¹

¹Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0303, United States
²Department of Chemistry, University of Illinois at Chicago, Chicago, IL, 60607, USA.
³Department of Physics, University of Illinois at Chicago, Chicago, IL, 60607, USA.
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1. General Methods

All reagents were purchased from commercial sources and used without further purification unless otherwise indicated. N-phenyl-cis-5-norbomene-exo-dicarboximide\(^1\) \((1)\), 2-(2,5,8,11-tetraoxatridecan-13-yl)-3a,4,7,7a-tetrahydro-1H-4,7-methanoisoindole-1,3(2H)-dione\(^2\) \((2)\) N-benzyl-2-(1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisoindol-2-yl)-N,N-dimethylethan-1-aminium\(^3\) \((3)\), (peg) N-(glycine)-cis-5-norbomene-exo-dicarboximide\(^4\) \((4)\) were prepared as described. All PNA sequences were made using NovaPEG Rink Amide resin, purchased from EMD Millipore, with a loading of 0.49 mmol/g and a swelling volume of 8.8 mL/g in CH\(_2\)Cl\(_2\). The synthesis of all PNA oligomers used Fmoc/Bhoc protected monomers (Fmoc-A(Bhoc)-aeg-OH, Fmoc-C(Bhoc)-aeg-OH, Fmoc-G(Bhoc)-aeg-OH, and Fmoc-T-aeg-OH) purchased from Panagene. PNA sequences were manually synthesized in house using standard solid-phase peptide synthesis conditions. RP-HPLC analysis of PNAs was performed on a Hitachi-Elite LaChrom L-2130 pump with a step-wise gradient. Detection was at 260 nm using an in-line UV-Vis detector (Hitachi-Elite LaChrom L-2420). For analysis, an analytical scale Phenomenex Jupiter 4\(\mu\)m Proteo 90Å column \((150 \times 4.60 \text{ mm})\) was utilized. For purification, a semi-preparative Phenomenex Jupiter 4\(\mu\)m Proteo 90Å column \((250 \times 10.0 \text{ mm})\) was utilized. Mass spectra were obtained at the UCSD Chemistry and Biochemistry Molecular Mass Spectrometry Facility. Low-resolution mass spectra were obtained using a Thermo LCQdeca mass spectrometer. Concentrations of oligonucleotides and peptide nucleic acids were determined using a Thermo Scientific NanoDrop 2000c spectrophotometer. Modified 2nd Generation Grubbs’ Ruthenium initiator \((\text{IMesH}(\text{Cl})_2(\text{Cl})_2\text{Ru}=\text{CHPh})\) was prepared as described by Sanford et. al.\(^5\) Sealed ampules of \((\text{CD}_3)_2\text{NCOD (DMF-d})\) used in polymerization reactions was purchased from Cambridge Isotope Laboratories Inc. and was distilled and degassed with 3 freeze-pump-thaw cycles prior to use. \(^1\)H \((400 \text{ MHz})\) NMR spectra were recorded on a Varian Mercury Plus spectrometer. Chemical shifts \(\delta \) (ppm) relative to the DMF-d\(_6\) residual proton peaks \((8.03, 2.92, \text{ and } 2.75 \text{ ppm})\). Polymer molecular weight and dispersity were determined via size-exclusion chromatography \((\text{Phenomenex Phenogel 5}\mu\m 10, 1\text{K}-75\text{K}, 300 \times 7.80 \text{ mm in series with a Phenomenex Phenogel } 5\mu\m 10, 10\text{K}-1000\text{K}, 300 \times 7.80 \text{ mm (mobile phase: } 0.05 \text{ M LiBr in DMF)})\) using a Hitachi-Elite LaChrom L-2130 pump equipped with a DAWN HELEOS multi-angle light scattering (MALS) detector (Wyatt Technology) and a refractive index detector (Hitachi L-2490) normalized to a 30,000 g/mol polystyrene standard. The dn/dc values used were 0.179. Hydrodynamic diameter (D\(_h\)) of nanoparticles was measured via DLS using a DynaPro NanoStar (Wyatt Technology). TEM samples were deposited on carbon/formvar-coated copper grids (Ted Pella Inc.), stained with 1% w/w uranyl acetate, and imaged using a Tecnai G2 Sphera operating at an accelerating voltage of 200 kV. Complementary and non-complementary DNA sequences were purchased from Integrated DNA Technologies (purified by HPLC, confirmed by ESI-MS). DNA melting temperature analysis was conducted using a Cary Series 100 UV-Vis spectrophotometer equipped with a Cary temperature controller.
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2. PNA Monomer Synthesis

All PNA sequences were manually synthesized. All reactions and washes of the resin were performed in a fritted glass peptide synthesis vessel, with the exception of the cleavage and deprotection of the oligomer from the resin, which was done in a polypropylene Poly-Prep Chromatography Column, purchased from Bio-Rad Laboratories. Unless otherwise stated the following standard protocol was used:

1) Swelling of the NovaPEG Rink Amide resin in CH₂Cl₂ for 2 hours. Deprotection of the resin is not required as it is sold without protecting groups.
2) Resin is washed with a steady flow of DMF (30 seconds) followed by a steady flow of with CH₂Cl₂ (30 seconds).
3) Activation of PNA monomer (5 equivalents with respect to total active sites on the resin) occurred by addition of 4.5 equivalents N,N,N,N-Tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate (HATU) (slightly less equivalents were used to ensure total activation of monomer with HATU and to prevent occurrence of unreacted HATU from reacting with resin amine or unprotected amines in the growing PNA sequence. Unprotected amines can form a guanidine moiety with HATU that blocks further elongation) and 10 equiv. of diisopropylethylamine (DIPEA) in DMF. The final concentration of the monomer was 0.2M in DMF. Monomer activation was allowed to proceed for 10 minutes before being added to the resin.
4) A steady stream of N₂(g) was bubbled through the peptide synthesis vessel while coupling occurred. Coupling time was 60 minutes.
5) Upon completion of coupling, the activating solution was vacuumed off the resin, and the resin was washed with a steady stream of DMF for 30 seconds (3 times), followed by CH₂Cl₂ for 30 seconds (3 times). No capping steps were necessary for the PNA sequences chosen.
6) Deprotection of the Fmoc was done using a solution of 20% piperidine in DMF for 5 minutes.
7) Steps 2-6 were repeated until chain length was complete.
8) Following the removal of the final Fmoc group, and subsequent washings of the resin with DMF and CH₂Cl₂, the HATU-activated carboxylic acid-substituted norbornene (4) was added and a steady stream of N₂(g) bubbled through the vessel for 60 minutes (the carboxylic acid-substituted norbornene was activated using the same protocol used for the PNA monomers in step 3).
9) Upon completion of coupling, the activating solution was vacuumed off the resin, and the resin was washed with a steady stream of DMF for 30 seconds (3 times), followed by CH₂Cl₂ for 30 seconds (3 times).
10) Step 8 was repeated (carboxylic acid-substituted norbornene (1) coupling to the resin).
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11) Upon completion of coupling, the activating solution was vacuumed off the resin, and the resin was washed with a steady stream of DMF for 30 seconds (3 times), followed by CH₂Cl₂ for 30 seconds (3 times).

12) The resin was dried under vacuum for several hours.

13) The removal the Boc protecting groups and cleavage from the resin was accomplished by treatment with a solution of TFA:m-cresol (80:20) for 90 minutes. The cleavage was performed in a polypropylene Poly-Prep Chromatography Column. After cleavage, the TFA:cresol solution was separated from the resin by centrifugation. The TFA:cresol solution was then evaporated until near dryness by applying a stream of air to the solution for several hours.

14) The crude PNA-norbornene oligomer crashed out of the TFA:cresol solution upon addition of 5 equivalents of diethyl ether with respect to the TFA:m-cresol solution, yielding an off-white powdery solid.

15) Reverse-phase preparatory HPLC was used to purify all sequences, and masses were confirmed by Matrix Assisted Laser Desorption-Time of Flight (MALDI-TOF)

3. HPLC Purification of PNA Sequences

RP-HPLC analysis of PNA was performed using 0.1% TFA/H₂O as solvent A, and 0.1% TFA/CH₃CN as solvent B. Gradient: 0% solvent B in 2 min, 0% to 5% solvent B in 3 min, 5% to 20% solvent B in 10 min, and 20% to 100% solvent B in 20 min.
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4. Homopolymer Synthesis via Ring-Opening Metathesis Polymerization (ROMP)

PNA-Nb monomer (CGAGTCATT-Tb) was polymerized via ROMP using Grubbs’ modified 2nd generation catalyst (IMesH)(C5H5N)(Cl)2Ru=CHPh in a glove box. The PNA-Nb monomer (3mg, 1 µmol) in a J-Young NMR tube was dissolved in 250 µL of anhydrous and degassed DMF-d7. The tube was removed from the glove box and a 1H NMR spectrum (t = 0) was taken. The tube was returned to the glove box and the catalyst (0.2 µmol or 0.1 µmol) was added to the reaction solution. 1H NMR spectra were recorded at the indicated time points until consumption of the olefin. It was observed that as the PNA monomer olefin disappeared, the corresponding DMF-d7 solutions became cloudy, and that the polyolefin peaks typically seen at ~5.5 ppm (see Figure S4) were absent, indicating that the resultant polymer had limited to no solubility in DMF. In addition, the homopolymers were insoluble in H2O, MeOH, and DCM solutions and had limited solubility in a solution of 0.05 M LiBr in DMF. Upon consumption of the olefin, the tube was returned to the glove box and termination agent ethyl vinyl ether (100 µL, excess) was added to the reaction mixture, and the mixture was allowed to sit at room temperature for 20 minutes. The crude polymer was precipitated from cold methanol and analyzed by SEC-MALS.

5. ROMP timescale 1H NMR for homopolyPNAs:

Figure S1. Integrations based on 10 eq of PNA olefin at t=0. DMF residual proton is then integrated accordingly for the ensuing time points.
**Figure S2.** Integrations based on 5 eq of PNA olefin at t=0. DMF residual proton is then integrated accordingly for the ensuing time points.

**Figure S3.** SEC-MALS for I. The $M_r$ was determined to be 13,790 with a PDI of 1.388, giving a degree of polymerization of 5 by LS, as opposed to 10 by $^1$H NMR. This discrepancy can be attributed to error in the assignment of the dn/dc. The dn/dc used to calculate the $M_r$ was 0.179, the known dn/dc for polystyrene in DMF. In addition, a large LS peak can be seen at 16 minutes. This peak corresponded to a $M_r$ of $7.3 \times 10^6$ and indicated polymer aggregation in DMF. SEC-MALS for III could not be obtained due to polymer insolubility in DMF.
6. Block Copolymer Synthesis via Ring-Opening Metathesis Polymerization (ROMP)

Figure S4. General scheme of ROMP synthesis of block copolyPNA using Grubb’s 2nd generation modified catalyst. A small aliquot of block 1 was terminated using III (ethyl vinyl ether) for SEC-MALS analysis before adding PNA. This provided an accurate M_n and degree of polymerization for the first block. After polymerization of the PNA block, the complete block copolymer was again analyzed using SEC-MALS.

7. Timescale 1H NMR of ROMP of PNA block copolymers

Figure S5. 1H NMR timescale for II. To a live catalyst on the end of a polyphenyl was added PNA (5 eq w.r.t. catalyst). The timescale shown is after 17 hours of reaction, at which point the polymer was terminated. The integrals shown
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are based on the amount of added phenyl-functionalized norbornene (35 eq. w.r.t catalyst, 5 protons/phenyl plus 5 protons of phenyl alkylidene for a total of 180 protons) and the amount of added PNA-Nb (5 eq w.r.t. catalyst).

Synthetic procedure details for ROMP of \( \text{II} \)

N-phenyl-cis-5-norbornene-exo-dicarboximide\(^1\) (1) in a J-Young NMR tube (5 mg, 0.02 mmol) was dissolved in 250 µL anhydrous and degassed DMF-\(d_7\) in a glove box. Catalyst \((\text{IMesH}_2)(\text{C}_5\text{H}_5\text{N})_2(\text{Cl})_2\text{Ru=CHPh} \) (0.411 mg, 0.56 µmol) was added and the tube was removed from the glove box and \(^1\text{H} \) NMR spectra were recorded until complete consumption of olefin. After olefin consumption, the tube was returned to the glove box and PNA-Nb \((8.2 \text{ mg, 2.8} \text{ µmol, 5 equivalents w.r.t. catalyst}) \) was added in 100 µL anhydrous and degassed DMF-\(d_7\). The tube was removed from the glove box and \(^1\text{H} \) NMR spectra were recorded at the indicated time points. Upon consumption of the olefin, the tube was returned to the glove box and termination agent ethyl vinyl ether \((100 \text{ µL, excess}) \) was added to the reaction mixture, and the mixture was allowed to sit at room temperature for 20 minutes. The crude polymer was precipitated from cold methanol and analyzed by SEC-MALS.

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**Figure S6.** \(^1\text{H} \) NMR timescale for ROMP of 3 identical block copolymers \( \text{IV-VI} \). One lot of polyphenyl was distributed evenly for the synthesis of the 3 block copolymers. The amount of PNA-Nb added was identical \((7.5 \text{ eq w.r.t. catalyst}) \). The timepoint shown is after 12 hours of reaction, at which point all three polymers were terminated. The integrals shown are based on the SLS value determined for the polyphenyl block \((M_n \text{ was 7,546 g/mol, giving a degree of polymerization of 30}) \) and the amount of added PNA-Nb. Based on this value, the total degree of polymerization should be 37.5.
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Synthetic procedure details for ROMP of IV-VI

N-phenyl-cis-5-norbornene-exo-dicarboximide\(^1\) (1) was polymerized by dissolving 13.57 mg (0.05 mmol) in 200 µL DMF-\(d_7\) and mixing with 1.3 mg (.0017 mmol) of catalyst dissolved in 50 µL DMF. After complete polymerization (10 min), 6.4 µl of this reaction (0.044 µmol w.r.t. catalyst) was taken on and added to 1 mg (0.34 µmol) of PNA nor-bornyl monomer (this reaction described below). The remaining phenyl homopolymerization reaction mixture was quenched with excess ethyl vinyl ether, precipitated and analyzed by SEC-MALS (DP = 30).

For incorporation of PNA nor-bornyl monomer as the second block in a phenyl-PNA block copolymer, 3.5 mg (1.2 µmol) PNA monomer was dissolved with heating in 15 µL DMSO-\(d_6\) and diluted to 65 µL with 50 µL of DMF-\(d_7\) to yield a stock solution of PNA monomer at a concentration of 18.5 mM. 18.6 µL of this PNA stock solution (1mg, 0.34 µmol) was added to 6.4 µl of phenyl homopolymer solution with live ruthenium catalyst (as described above) for a total reaction volume of 25 µL. This exact protocol was followed for 3 identical 25 µL reactions. Each of the three reactions was heated in a glass HPLC insert at 40°C on a heat block with sand used to facilitate efficient heat transfer between the heating block and the glass HPLC vial insert. After 1 hour of heating, all three reactions were removed from heat and subsequently diluted to 80 µL total volume with DMF-\(d_7\) and added to a 3mm O.D. NMR tube via a heat-pulled glass pipette in order to provide enough volume for NMR analysis while keeping the concentration at a maximum. After 12 hours, an NMR spectrum was acquired for each of the three samples. Following this, the tube was returned to the glove box and termination agent ethyl vinyl ether (100 µL, excess) was added to the reaction mixture, and the mixture was allowed to sit at room temperature for 20 minutes. The crude polymer was precipitated from cold methanol and analyzed by SEC-MALS.
Figure S7. $^1$H NMR timescale for VII-X

The timescale shown is after 12 hours of reaction, at which point all polymers were terminated. The integrals shown are based on the SLS value determined for the first block ($M_n$ was 11,900 g/mol, giving a degree of polymerization of 33 for peg, $M_n$ was 13,430 g/mol, giving a degree of polymerization of 41 for NR4, and the $M_n$ was 8,827 g/mol, giving a degree of polymerization of 35 for ph) and the amount of added PNA-Nb. Based on this value, the total degree of polymerization should be 45 for each block copolymer.

ROMP conditions for VII-X

N-phenyl-cis-5-norbornene-exo-dicarboximide (1) (3.47 mg, 13.7 μmole), 2-(2,5,8,11-tetraoxatridecan-13-yl)-3a,4,7a-tetrahydro-1H-4,7-methanoisindole-1,3(2H)-dione (2) (4.8 mg, 13.7 μmol), and N-benzyl-2-(1,3-dioxo-1,3,3a,4,7,7a-hexahydro-2H-4,7-epoxyisindol-2-yl)-N,N-dimethylethan-1-aminium (3) (4.47 mg, 13.7 μmol) in 85.5 μL DMF-d$_7$ were polymerized by mixing with 0.274 mg (0.38 μmol) of catalyst (14.5 μL of a 0.026 M soln. in DMF-d$_7$) for a total volume of 100 μL DMF-d$_7$. After complete polymerization, 10 μL of this reaction (0.038 μmol w.r.t. catalyst) was taken out and added to 1 mg (0.34 μmol) of PNA norbornyl monomer (this reaction described below). The remaining polymerized first block reaction mixture was quenched with excess ethyl vinyl ether, precipitated and analyzed by SEC-MALS.

For incorporation of PNA norbornyl monomer as the second block in a phenyl-PNA block copolymer, 4.2 mg (1.4 μmol) PNA monomer was dissolved with heating in 25 μL DMSO-d$_6$ to yield a stock solution of PNA monomer at a concentration of 57.5 mM. 5.95 μL of this PNA stock solution (1mg, 0.34 μmol) was added to 10 μL of phenyl homopolymer solution with live ruthenium catalyst (as described above) for a total reaction volume of 15.95 μL. After 12 hours at r.t., all four reactions were diluted to 90 μL total
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volume with DMF-d$_7$ and added to a 3mm O.D. NMR tube via a heat-pulled glass pipette in order to provide enough volume for NMR analysis while keeping the concentration at a maximum.

SEC-MALS of copolyPNAs:

**Figure S8 A** SEC-MALS of II. SEC-MALS of the polyphenyl block was not observed due to an insufficient aliquot removal of the polyphenyl block. However, complete consumption of the phenyl-norbornene backbone was observed by $^1$H NMR. After complete polymerization of the block copolymer, the $M_n$ of the total polymer (28,270) and the PDI (1.035) were determined by SEC-MALS. $M_n$ gives a degree of polymerization of 6 for PNA (mass fraction of this RI peak is 97.9%). The phenyl-norbornene monomer (1) was added in 35 equivalents w.r.t. to PNA. A large LS peak, but small RI (corresponding to a mass fraction of 2.1%) peak can be seen at 20 minutes. This peak corresponded to a $M_n$ of $3.3 \times 10^5$ and indicated block copolymer aggregation in DMF. **B** $^1$H NMR for the polymerization of the phenyl-norbornene block of p$_{35}$PNA$_5$. The red star indicates the olefin peak at 6.32 ppm that is observed to disappear upon polymerization. The peak corresponds to the 2 protons also indicated by a star in the chemical structure.
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Figure S9. SEC-MALS of polyphenyl block of as well as SEC-MALS of 3 identical block copolymers of PNA (IV-VI). After complete polymerization of phenyl-norbornene monomer (1), a predetermined volume was removed from the solution and terminated with ethyl vinyl ether to obtain the Mₙ of the polyphenyl block, which was determined to be 7,546, giving a degree of polymerization of 30 for the phenyl block, with a PDI of 1.03. After termination of the complete block copolymer, the Mₙ was determined for all 3 identical polymers and for all peaks that could be analyzed. A) Mₙ was 7.7×10⁶, PDI 2.06, and mass fraction was 26% for peak between 15.4-20.5 min by RI. Mₙ was 14,810, PDI 1.54, and mass fraction was 48% for peak between 20.6-24.8 min by RI. This peak was used to determine degree of polymerization for the PNA, as it most likely corresponds to the disaggregated block copolymer. Mₙ was 1,860, PDI 1.41, and mass fraction was 26% for peak between 25-26.5 min by RI, corresponding to unpolymerized PNA-Nb (2922 g/mol). B) Mₙ was 1.5×10⁶, PDI 1.45, and mass fraction was 10% for peak between 16-20.6 min by RI. Mₙ was 18,700, PDI 1.34, and mass fraction was 54% for peak between 20.9-24.9 min by RI. This peak was used to determine degree of polymerization for the PNA, as it most likely corresponds to the disaggregated block copolymer. Mₙ was 3,850, PDI 1.11, and mass fraction was 36% for peak between 25.1-27.3 min by RI, corresponding to unpolymerized PNA-Nb (2922 g/mol). C) Mₙ was 1.6×10⁵, PDI 1.23, and mass fraction was 32% for peak between 25-26.5 min by RI, corresponding to unpolymerized PNA-Nb (2922 g/mol). D) Expanded view of LS peaks for all 3 block copolymers.
**Figure S10** SEC-MALS of polyphenyl block as well as SEC-MALS of VII.  
A) After complete polymerization of phenyl norbornene monomer (1), a predetermined volume was removed from the solution and terminated with ethyl vinyl ether to obtain the $M_n$, which was determined to be 8,827, giving a degree of polymerization of 35, with a PDI of 1.02. After termination of the complete block copolymer, the $M_n$ was determined for all peaks that could be analyzed. $M_n$ was $6.2 \times 10^5$, PDI 2.66, and mass fraction was 25% for peak between 14.6-20.2 by RI. $M_n$ was 16,840, PDI 1.33, and mass fraction was 51% for peak between 20.7-25.1 min by RI. This peak was used to determine degree of polymerization for the PNA, as it most likely corresponds to the disaggregated block copolymer. The $M_n$ for peak between 25.6-27.3 min by RI could not be determined due to inadequate LS. The mass fraction for this peak was 24%.  
B) Expanded view of RI peak showing 3 populations.

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**Figure S11** SEC-MALS of polyphenyl block as well as SEC-MALS of VIII.  
A) After complete polymerization of phenyl norbornene monomer (1), a predetermined volume was removed from the solution and terminated with ethyl vinyl ether to obtain the $M_n$, which was determined to be 8,827, giving a degree of polymerization of 35, with a PDI of 1.02. After termination of the complete block copolymer, the $M_n$ was determined for all peaks that could be analyzed. $M_n$ could not be determined for the LS peak between 13.9-19.3 min due to a lack of RI. $M_n$ for the peak between 22-25 min by RI could not be determined due to inadequate LS. This peak had a mass fraction of 45%. The $M_n$ for peak between 25.2-27.3 min by RI could not be determined due to inadequate LS. The mass fraction for this peak was 55%.  
B) Expanded view of RI peak showing 2 populations.
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Figure S12 SEC-MALS of first block as well as SEC-MALS of IX. A) After complete polymerization of peg-norbornene monomer (2), a predetermined volume was removed from the solution and terminated with ethyl vinyl ether to obtain the $M_n$ of the polypeg block, which was determined to be 11,900, giving a degree of polymerization of 33 for the peg block, with a PDI of 1.03. After termination of the complete block copolymer, the $M_n$ was determined for all peaks that could be analyzed. $M_n$ was $2.5 \times 10^6$, PDI 1.29, and mass fraction was 12% for peak between 15-20 by RI. Despite showing multiple peaks by RI, the $M_n$ could not be determined for any other peaks due to lack of an LS peak. The mass fraction for RI peak between 20.5-25 min was 61%, and 27% for the peak between 25.15-27.7 min B) Expanded view of RI peaks showing 3 populations.

Figure S13 SEC-MALS of quaternary amine block as well as SEC-MALS of X. A) After complete polymerization of quaternary amine-norbornene monomer (3), a predetermined volume was removed from the solution and terminated with ethyl vinyl ether to obtain the $M_n$, which was determined to be 13,430, giving a degree of polymerization of 41, with a PDI of 1.1. After termination of the complete block copolymer, the $M_n$ was determined for all peaks that could be analyzed. $M_n$ was $4.6 \times 10^5$, PDI 1.05, and mass fraction was 18% for peak between 13.9-19.8 by RI. Despite showing multiple peaks by RI, the $M_n$ could not be determined for any other peaks due to lack of an LS peak. The mass fraction for RI peak between 20.6-24.8 min was 43%, and 39% for the peak between 25.7-27.1 min B) Expanded view of RI peaks showing 3 populations.
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8. PNA-Polymer Micelle Formation

Synthesis

6 mg of II was dissolved into DMSO at a concentration of 2 mg/ml. This solution was transferred to 3,500 MWCO snakeskin dialysis tubing (Thermo Scientific) and 3 ml H2O was added. The resulting solution was dialyzed against 1.0 L of Nanopure H2O for 3 days, with the H2O being changed daily. UV confirmed the concentration of the final solution and a speedvac was used to concentrate solutions to ~0.1 mg/ml. Nucleic acid concentrations were determined by UV absorbance at 260 nm using a Thermo Scientific NanoDrop 2000c spectrophotometer. An extinction coefficient of 99,200 L/mol·cm⁻¹ was used. This coefficient was calculated as the extinction coefficient of the entire sequence.

9. TEM

Copper grids (formvar/carbon-coated, 400 mesh copper, Ted Pella # 01754) were prepared by glow discharging the surface at 20 mA for 1.5 minutes followed by treatment with 3.5 µL 250 mM MgCl₂ in order to prepare the surface for PNA nanoparticle adhesion. The MgCl₂ solution was wicked away with filter paper and 3.5 µL of PNA nanoparticle (ca 100 µM PNA) solution was deposited on the grid surface. This solution was allowed to sit for 5 minutes before being washed away with 4 drops of glass distilled H2O and subsequent staining with 3 drops of 1% w/w uranyl acetate. The stain was allowed to sit for 30 seconds before wicking away with filter paper. All grid treatments and sample depositions were on the dark/shiny/glossy formvar-coated face of the grid (this side face up during glow discharge). Samples were then imaged via TEM.
Figure S14. A) After dialysis into H₂O from DMSO, II forms nanoparticles that by DLS are aggregates on the size order of 50 nm in diameter. B) Autocorrelation function for nanoparticles formed from copolyPNA-3. C) Negative-stain TEM showing nanoparticles on the order of 10-30 nm. The majority of the material, once dried, showed no particle aggregation. D) Negative-stain TEM showing a zoomed-out section of the grid.
10. DNA Melting Temperature Analysis

Melting temperature analysis were performed by heating each sample from 20 °C (20 minute equilibration time) to 90 °C using a temperature gradient of 1 °C/minute. Melting temperatures were calculated as first derivatives of the curve. Nanoparticles formed from II, renamed as PNA-NP, were at a concentration of 1 µM in water. The mixture was made by adding Dulbecco’s 1X PBS to the nanoparticles followed by 100 nM-1 µM of the complementary DNA in H₂O. Final concentration of NaH₂PO₄ is 6.7 mM, NaCl is 113 mM, KCl is 22.2 mM, and KH₂PO₄ is 1.46 mM, all in a total volume of 50 µL. Annealing was done at room temperature for 2 hours. The sample was refrigerated at 8 °C for 15 minutes, after annealing, and subsequently analyzed.

Figure S15. A) Raw Tₘ data for PNA-NP and complementary DNA sequence. Several buffers were tested to determine ideal conditions for hybridization between PNA-NP and its DNA complement at room temperature. 1 µM PNA-NP with 100 nM complementary DNA in PBS was subsequently chosen. B) A 10-pt FFT filter was applied to the raw data. C) Derivative plots for each of the Tₘ curves showing a 57.8°C average for PNA-NP and complementary DNA.
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Figure S16. A) Raw $T_m$ data for PNA-NP and complementary DNA, as well as non-complementary DNA sequence. The $T_m$ for the corresponding PNA sequence (identical to PNA that was polymerized) and complementary DNA was also determined. B) A 10-pt FFT filter was applied to the raw data. C) Derivative plots for each of the $T_m$ curves showing a 7.3$^\circ$C increase for PNA-NP over its identical PNA sequence. While a derivative can be taken of the $T_m$ curve corresponding to PNA-NP and its non-complementary sequence, the curve itself is more indicative of non-specific binding, as is implied by the broad derivative, and the almost linear $T_m$.

Figure S17. A) Raw $T_m$ data for PNA-NP without complementary DNA and complementary DNA without PNA in PBS showing no melting. B) A 10-pt FFT filter was applied to the raw data.
A single PNA-polyborbornyl unimer was initially built in fully extended conformation with GaussView software (6). 60 identical unimers, first relaxed in vacuum for 0.3 ns at the temperature of 300 K, were spherically distributed in space, with PNA ends oriented towards the outside. The resulting PNA-NP of 60 unimers was then immersed in a cubic (TIP3P) water box with solvate plugin in VMD (7); water molecules present within a 65 Å radius of PNA-NP center were deleted. The resulting unit cell with solvated 60-monomer PNA-NP contained 2,122,554 atoms.

MD simulations of solvated PNA-NP were performed with NAMD2 software (8), where the molecules were described using the CHARMM force field. The parameters for the unimer units (PNA, norbornyl) were obtained by analogy to molecules already parametrized in the CHARMM forcefield, using the ParamChem Server (9-11). All simulations were performed in NpT ensemble using periodic boundary conditions, at a constant temperature T= 300 K, a Langevin constant $\gamma_{\text{Lang}} = 0.001 \text{ ps}^{-1}$ (to ensure fast dynamics), and at a constant pressure p= 1.01325 bar. The particle-mesh Ewald (PME) method (12) was used for evaluation of long-range Coulombic interactions. The timestep was set to 1.0 fs, and long range interactions were evaluated every 1 (van der Waals) and 2 timesteps (Coulombic).

In the prepared system, water was minimized for 10 ps around the fixed PNA-NP, then for additional 8 ps around the constrained PNA-NP. The whole system was then heated to the temperature of 300 K and equilibrated at this temperature for 16 ns without constraints.
CHARMM parameters for all atoms were prepared by analogy to known molecules, using the ParamChem Server [4-6]. The obtained partial charges were slightly modified to ensure that the unimer had no net charge. Below are the partial atomic charges for each PNA base, a unit of the PNA peptide chain, and a unit of the hydrophobic chain.

![Figure 1: Partial Charges on Adenine Base in PNA](image)

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | CR64      | 0.499754              |
| C2         | CRC0      | 0.432754              |
| C3         | CRC0      | 0.280754              |
| C4         | CR64      | 0.456754              |
| C5         | CR53      | 0.345754              |
| N1         | NR62      | -0.741245             |
| N2         | NR62      | -0.748245             |
| N3         | NR50      | -0.709245             |
| N4         | NR51      | -0.072245             |
| N5         | N2S3      | -0.769245             |
| H1         | HR62      | 0.126754              |
| H2         | HGP4      | 0.378754              |
| H3         | HGP4      | 0.378754              |
| H4         | HR52      | 0.140193              |
Supporting Information

Guanine:

![Guanine Structure](image)

**Figure 2: Partial Charges of Guanine Base in PNA**

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | CR64      | 0.750754              |
| C2         | CRC0      | 0.269754              |
| C3         | CRC0      | 0.004754              |
| C4         | CR63      | 0.538754              |
| C5         | CR53      | 0.258754              |
| N1         | NR61      | -0.341245             |
| N2         | NR62      | -0.735245             |
| N3         | NR50      | -0.599245             |
| N4         | NR51      | -0.038245             |
| N5         | N2S3      | -0.677245             |
| O1         | O2D4      | -0.508245             |
| H1         | HGP1      | 0.262754              |
| H2         | HGP4      | 0.336754              |
| H3         | HGP4      | 0.336754              |
| H4         | HR52      | 0.140438              |
Supporting Information

Thymine:

![Thymine Diagram]

*Figure 3: Partial Charges for Thymine Base in PNA*

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | CR63      | 0.515142              |
| C2         | CR63      | 0.516142              |
| C3         | CR62      | -0.127856             |
| C4         | CR62      | 0.181142              |
| C5         | C331      | -0.153856             |
| N1         | NR61      | -0.350856             |
| N2         | NR61      | -0.443856             |
| O1         | O2D4      | -0.392856             |
| O2         | O2D4      | -0.432856             |
| H1         | HR62      | 0.181142              |
| H2         | HR62      | 0.187142              |
| H3         | HGA3      | 0.107142              |
| H4         | HGA3      | 0.107142              |
| H5         | HGA3      | 0.107142              |
Supporting Information

Cytosine:

![Cytosine diagram]

Figure 4: Partial Charges of Cytosine Base in PNA

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | CR63      | 0.499754              |
| C2         | CR64      | 0.647754              |
| C3         | CR62      | -0.084298             |
| C4         | CR62      | 0.049754              |
| N1         | NR61      | -0.163245             |
| N2         | NR62      | -0.660245             |
| N3         | N2S3      | -0.748245             |
| O1         | O2D4      | -0.480245             |
| H1         | HR62      | 0.169754              |
| H2         | HR62      | 0.069754              |
| H3         | HGP4      | 0.349754              |
| H4         | HGP4      | 0.349754              |
Supporting Information

Peptide Chain Unit:

**Figure 5: Partial Charges for a Unit of the Peptide Chain in PNA**

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | C321      | -0.052245             |
| C2         | C321      | 0.040754              |
| C3         | C321      | 0.019754              |
| C4         | C201      | 0.530754              |
| C5         | C201      | 0.415754              |
| C6         | C321      | -0.037245             |
| N1         | N2S1      | -0.442245             |
| N2         | N2S0      | -0.415245             |
| O1         | O2D1      | -0.510245             |
| O2         | O2D1      | -0.533245             |
| H1         | HGP1      | 0.265422              |
| H2         | HGA2      | 0.089754              |
| H3         | HGA2      | 0.089754              |
| H4         | HGA2      | 0.089754              |
Hydrophobic Unit:

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| H5         | HGA2      | 0.089754              |
| H6         | HGA2      | 0.089754              |
| H7         | HGA2      | 0.089754              |
| H8         | HGA2      | 0.089754              |
| H9         | HGA2      | 0.089754              |

**Figure 6: Partial Charges for a Unit of the Hydrophobic Chain**

| Atom Label | Atom Name | Partial Atomic Charge |
|------------|-----------|-----------------------|
| C1         | CG2D      | -0.124833             |
| Supporting Information          |     |     |
|--------------------------------|-----|-----|
| C2                            | CG3C| -0.112833 |
| C3                            | CG3C| -0.174833 |
| C4                            | CG3C| -0.193833 |
| C5                            | CG2D| -0.124833 |
| C6                            | CG3R| 0.068166  |
| C7                            | CG3R| 0.085166  |
| C8                            | CG2R| 0.327666  |
| C9                            | CG2R| 0.327666  |
| C10                           | CG32| -0.014833 |
| C11                           | CG2R| -0.015833 |
| C12                           | CG2R| -0.113833 |
| C13                           | CG2R| -0.105833 |
| C14                           | CG2R| -0.110833 |
| C15                           | CG2R| -0.105833 |
| C16                           | CG2R| -0.113833 |
| N1                            | NG2R| -0.126833 |
| O1                            | OG2D| -0.508333 |
| O2                            | OG2D| -0.508333 |
| H1                            | HGA4| 0.154166  |
| H2                            | HGA1| 0.094166  |
| H3                            | HGA2| 0.094166  |
| H4                            | HGA2| 0.094166  |
| H5                            | HGA2| 0.094166  |
| H6                            | HGA5| 0.169507  |
| H7                            | HGA1| 0.094166  |
| H8                            | HGA1| 0.094166  |
| H9                            | HGA2| 0.094166  |
| H10                           | HGA2| 0.094166  |
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| H11 | HGR6 | 0.113966 |
|-----|------|----------|
| H12 | HGR6 | 0.113966 |
| H13 | HGR6 | 0.113966 |
| H14 | HGR6 | 0.113966 |
| H15 | HGR6 | 0.113966 |

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