Supporting Information

Chaperone Assisted Host-Guest Interactions Revealed by Single-Molecule Force Spectroscopy

Shankar Pandey[1], Dilanka V.D. Walpita Kankanamalage[2], Xiao Zhou[2], Changpeng Hu[1], Lyle Isaacs[3], Janarthanan Jayawickramarajah[2,*], Hanbin Mao[1,4,*]

[1]Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio, 44242, USA.

[2]Department of Chemistry, Tulane University, 2015 Percival Stern Hall, New Orleans, Louisiana 70118, USA.

[3]Department of Chemistry and Biochemistry, University of Maryland, College Park,

Maryland 20742, USA.

[4]Advanced Materials and Liquid Crystal Institute, Kent State University, Kent, Ohio, 44242, USA.

*Corresponding authors,

Janarthanan Jayawickramarajah: jananj@tulane.edu,

Hanbin Mao: hmao@kent.edu.

Materials and Methods

General Experimental

DNA oligos were purchased from the IDT (Integrated DNA Technologies, IA). Enzymes were purchased from NEB (New England Biolab, England) for the preparation of DNA constructs. Polystyrene beads (coated with streptavidin or anti-digoxigenin) were purchased from Spherotech (Lake Forest, IL). Unless otherwise stated, all chemicals and solvents were purchased from Sigma Aldrich or Fisher Scientific and used without further purification. *N,N* dimethyladamantanamine was synthesized from adamantanamine hydrochloride using a previously reported literature protocol.[S1] *N,N*-dimethyl-*N*-(prop-2yn-1-yl)adamantan-1-aminium (positively charged adamantane complex) was synthesized using *N,N* dimethyladamantanamine according to a previously reported literature protocol.[S2] CB7-azide was prepared by Isaacs and co-workers.[S3] Adamantane-NHS ester was synthesized according to a previously reported literature protocol.[S4]

The core DNA sequences used to construct the host and guest functionalized single stranded DNA were prepared by the W. M. Keck Foundation Biotechnology Resource Laboratory (located at Yale University) using standard automated solid phase synthesis. All modifications used to prepare these ODNs, including 5′-Hexynyl Phosphoramidite, 3′-PT-Amino-Modifier C6 CPG, and 3' Amino Modifier Serinol CPG were purchased from Glen Research. The core DNA sequences were subsequently synthetically functionalized with host and guest molecules as described in the Synthesis of Host/Guest Functionalized DNA section (*infra*). All functionalized DNA sequences were purified via sephadex resin microspin G-25 columns (GE healthcare) followed by chromatographic separation using RP-HPLC. The RP-HPLC purification of the synthetic DNA samples were carried out using a Varian Prostar HPLC system, equipped with an Agilent PLRP-S 100 Å 5 μm 4.6 × 250 mm reverse phase column. The column was maintained at 65 °C for all runs. The flow rate was set at 1 mL/min. A gradient composed of two solvents (Solvent A is 0.1 M TEAA (aq) in 5% acetonitrile and solvent B is 100% acetonitrile) was used.

The concentrations of purified DNA samples were quantified based on their UV absorption at 260 nm and their molar extinction coefficients obtained by nearest neighbor calculations. All HPLC purified fractions were concentrated with a speedvac concentrator for 6-8 hrs and redissolved in ultrapure water prior to MALDI analysis. Mass spectral data were acquired using a Bruker Autoflex III matrix-assisted laser desorption time-of-flight mass spectrometer (MALDI-TOF MS) with positive ion and linear detection modes. For all synthetically functionalized DNA

samples, a solution of 3-hydroxypicolinic acid (50 mg/mL) and ammonium citrate (10 mg/mL) in 1:1 water/acetonitrile with 0.1% trifluoroacetic acid was used as the matrix. These MALDI samples were prepared by combining 1 μL of DNA solution (1 mM in H_2O) and 2 μL of matrix solution.

Synthesis of Host/Guest Functionalized DNA

Synthesis of CB7-DNA. A solution of 1 mg (0.8 μmol) CB7-N_3 in 100 μL H_2O was added to an Eppendorf tube containing 0.1 μmol hexynyl modified DNA in 100 μL H_2O. In a separate vial, 15 μL CuBr solution (100 mM in DMSO/tBuOH 3:1) and 30 μL Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) ligand solution (100 mM in DMSO/tBuOH 3:1) were vortexed and added to the DNA + CB7-N_3 solution. The solution was shaken at room temperature for 3 hours. A G25 micro spin column was used for desalting. The CB7-DNA conjugate was purified by RP-HPLC, and the structure was confirmed by MALDI-TOF (Figures S6, S7, and S18).

Synthesis of Ad-Hexyl-DNA. A solution of 1 mg (3.6 μmol) Adamantane-NHS-ester in 100 μL DMSO was added to an Eppendorf tube containing 0.1 μmol amino modified DNA in 100 μL H_2O and 10 μL DIEA. The solution was shaken at 50 ºC for 4 hours and then the solvents were removed using a speedvac concentrator. The resulting crude residue was dissolved in water, desalted, purified by RP-HPLC, and analyzed by MALDI-TOF (Figures S8, S9, and S18).

Synthesis of Ad^+-Hexyl-DNA. A solution of 2 mg (8.8 μmol) 4-azidobutyrate-NHS-ester in 20 μL DMSO was added to an Eppendorf tube containing 0.1 μmol amino modified DNA in 100 μL H_2O and 5 μL DIEA. The solution was shaken at 50 ºC for 3 hours and the resulting solution was desalted by using G-25 spin column, purified by RP-HPLC, and analyzed by MALDI-TOF to result in the azido-DNA conjugate (Figure S11).

A solution of 2 mg (9.1 μmol) *N,N*-dimethyl-*N*-(prop-2yn-1-yl)adamantan-1-aminium (Ad^+) in 50 μL H_2O was added to an Eppendorf tube containing the azido-DNA conjugate in 50 μL H_2O. In a separate vial, 15 μL CuBr solution (100 mM in DMSO/tBuOH 3:1) and 30 μL Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) ligand solution (100 mM in DMSO/tBuOH 3:1) were vortexed and added to the solution containing azido-DNA and Ad^+. The resulting solution was shaken at room temperature for 3 hours. A G25 micro spin column was used for desalting. The final Ad^+-Hexyl-DNA product was purified by RP-HPLC and the structure was confirmed by MALDI-TOF (Figures S10, S12, and S18).

Synthesis of Ad-Serinol-DNA. A solution of 1 mg (3.6 μmol) Adamantane-NHS-ester in 100 μL DMSO was added to an Eppendorf tube containing 0.1 μmol amino modified serinol DNA in 100 μL H_2O and 10 μL DIEA. The solution was shaken at 55 ºC for 5 hours and then the solvents were removed using a speedvac concentrator. The resulting adamantane-DNA conjugate was dissolved in water, desalted, purified by RP-HPLC, and analyzed by MALDI-TOF (Figures S13, S14, and S18).

Synthesis of Ad^+-Serinol-DNA. A solution of 2 mg (8.8 μmol) 4-azidobutyrate-NHS-ester in 20 μL DMSO was added to an Eppendorf tube containing 0.1 μmol amino modified serinol DNA in 100 μL H_2O and 5 μL DIEA. The solution was shaken at 50 ºC for 3 hours and the resulting solution was desalted by using G25 spin column, purified by RP-HPLC, and analyzed by MALDI-TOF to afford an azido-DNA conjugate (Figure S16).

A solution of 2 mg (9.1 μmol) *N,N*-dimethyl-*N*-(prop-2yn-1-yl)adamantan-1-aminium (Ad^+) in 50 μL H_2O was added to an Eppendorf tube containing azido-DNA conjugate in 50 μL H_2O. In a separate vial, 15 μL CuBr solution (100 mM in DMSO/tBuOH 3:1) and 30 μL Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) ligand solution (100 mM in DMSO/tBuOH 3:1) were vortexed and added to the solution containing azido-DNA and Ad^+. The solution was shaken at room temperature for 3 hours. A G25 micro spin column was used for desalting. The final product was purified by RP-HPLC and the structure was confirmed by MALDI-TOF (Figures S15, S17, and S18).

Synthesis of βCD-DNA. A solution of 2 μL (250 μM) hexynyl modified DNA was mixed with 18 μL (100 μM) azide-modified beta-cyclodextrin (β-CD). Then the 20 μl mixture was treated with the click solution containing 2 μL of CuBr solution (0.01 g of CuBr in 700 μL of DMSO/t-BuOH in 3:1) and 4 μL Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) ligand solution. The solution was purged with N_2 gas and shaken for 14 hrs in dark. Ethanol precipitation was performed to remove unreacted reactants while the precipitant was dissolved in 15 μl water. The product was analyzed with the 15% denature PAGE gel (Figure S20). The upper band was purified using 15% denaturing PAGE gel (see Figure S21 for purified product). The gel purified final product was confirmed by MALDI-TOF (Figures S19 and S22).

Synthesis of DNA constructs for single-molecule investigations. The poly T (T_{50}) containing oligo was annealed with an oligo that was linked with a host molecule at the 5′ end and another

oligo that was linked with a guest molecule at the 3′ end (Figure 1B). The annealed construct was phosphorylated and then ligated between two double-stranded DNA handles with 2028 bp and 2391 bp in length. The 2028 bp DNA handle was synthesized from a pBR322 plasmid template by PCR using two primers, 5′-biotin- GCA TTA GGA AGC CCA GTA GG and 5′ AAA CCA TAG AGG CTA CAC TAG AAG GAC AGT ATT TG 3′. The purified PCR product was digested by XbaI enzyme to generate a cohesive end compatible with the polyT containing fragments prepared above. The other dsDNA handle (2391 bp) was prepared by PCR using the λ-DNA template with two primers, 5′ AAA AAA AAG AGC TCC TGA CGC TGG CAT TCG CAT CAA AG and 5′ AAA AAA AAG GTC TCG CCT GGT TGC GAG GCT TTG TGC TTC TC. The purified PCR product was digested by using SacI, which was followed by terminal transferase labeling of the 3′ end SacI overhang with digoxigenin-dUTP for 8 hrs at 37 °C. The digoxigenin labelled product was digested by BsaI enzyme, which generated an overhang that is compatible with the polyT containing fragment. Finally, the two dsDNA handles and the polyT containing fragment were ligated by T4 DNA ligase and stored in the -20 ºC in 1 mM Tris-HCl buffer (pH 7.4) after the agarose gel purification and ethanol precipitation (see Figure S4 for gel images).

Single molecule force ramping experiments.

Single-molecule experiments were performed by a dual-beam laser-tweezers instrument.[S5] All the experiments were performed in 10 mM Tris buffer containing 100 mM KCl (pH 7.4) at 23 ºC. Polystyrene beads coated with digoxigenin antibody (diameter 2.1 μm) were incubated with so prepared DNA construct. Two laser traps were used to separately capture the bead immobilized with the DNA construct and a streptavidin coated bead (diameter 1.87 μm). After bringing two beads in contact with each other, the DNA construct was tethered between the two beads via affinity linkages of biotin/streptavidin and digoxigenin-antibody/digoxigenin. By controlling the direction of laser beam, one of the laser foci was moveable while the other was fixed. Upon moving the two beads apart, the tethered DNA was stretched, and the tension produced on the host-guest complex was calculated based on the spring constant of each trap (calibrated by the thermal motion of the bead in the trap) and the displacement of the bead from the center of the trap (measured by position sensitive photodetectors). The resulting force-extension (F-X) curves were recorded through LabView program (National Instruments, Austin, TX) at 1 KHz with loading rate of 5.5 pN/s (in the 10-30 pN range). The presence of single molecule was confirmed by the ~65 pN

plateau at F-X curve due to the phase transition of single duplex DNA molecules.[S6] Alternatively, it is confirmed by the single breakage event of the DNA tether during the mechanical unfolding experiments.

The collected data were filtered by using Savitzky-Golay function at a time constant 10 ms in the Matlab program (the MathWorks, Nattick, MA). The mechanical stability of the host-guest interaction was determined by the unfolding forces revealed in the F-X curves.

Supplementary figures and captions

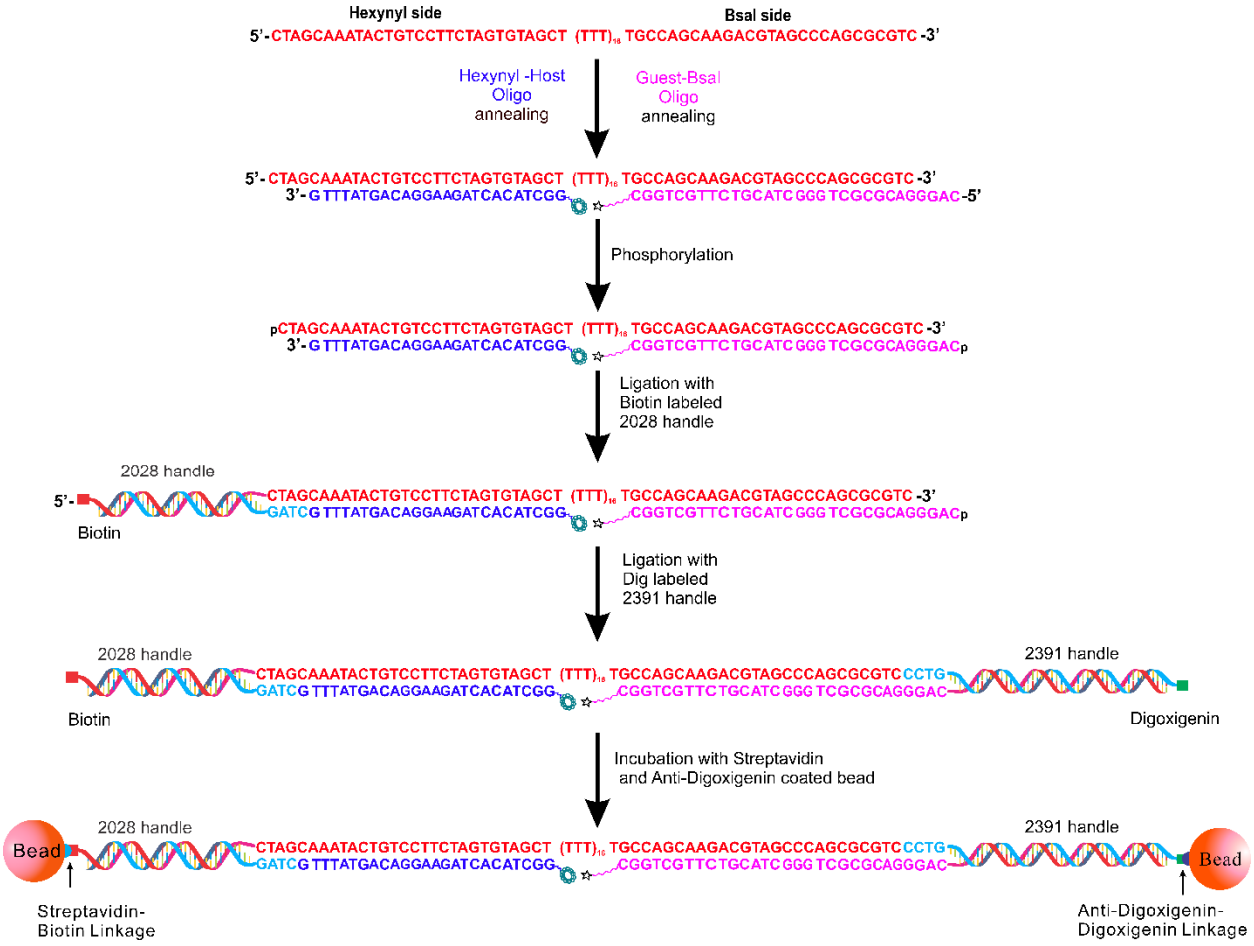

Figure S1. *Flow chart for the synthesis of the DNA constructs to investigate host-guest interactions.*

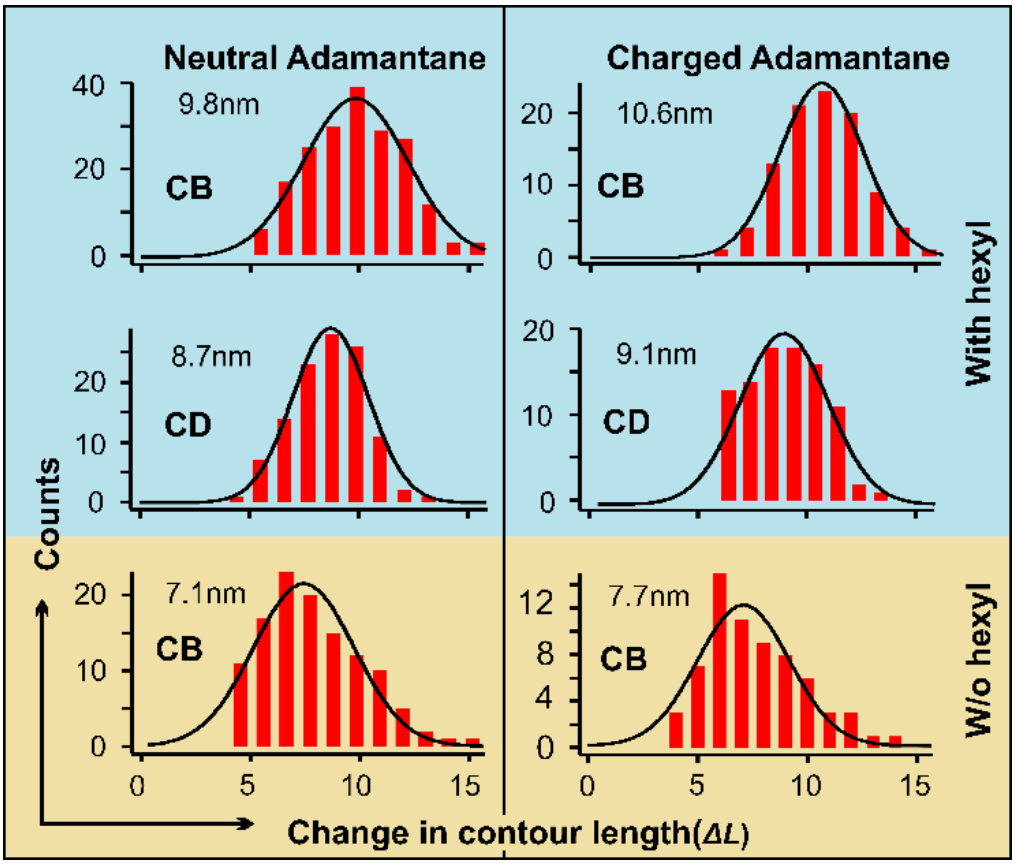

Figure S2. *Histograms of the change-in-contour length (ΔL) during the rupture events of host-guest interactions with and without hexyl chain in the guest molecules (upper cyan panels for the guest that contains hexyl chain and the bottom panel for the guest molecule without hexyl chain). Solid curves in the ΔL histograms represent the Gaussian fittings. The ΔL of each rupture event was determined by the equation* $\Delta x/\Delta L = 1 - 1/2(k_BT/FP)^{1/2} + (F/S)$, [S7] *where Δx is the change in the end-to-end distance,* k_B *is the Boltzmann constant, T is absolute temperature, P is the persistent length of dsDNA (50.8 nm* [S8]*), and S is the stretching modulus of dsDNa (1243 pN* [S8]*). Of note, without the hexyl group, the ΔL values are smaller compared to those with hexyl group when interacting with CB. This supports the interaction of hexyl group with the CB, which causes smaller end-to-end distance x (see Figure S3), resulting in bigger ΔL values (ΔL=L-x, here L is the total contour length of 50T, which is a constant value). The contour length L can be estimated as* $L=L_{nt} \times 50$ *nt = ~20 nm, where* L_{nt} *is the contour length per nucleotide, 0.42-0.45 nm/nt* [S9-11]*. The end-to-end distance x can be estimated from the combination of the length of the 5' end linker (~4 nm), the length of the 3' end linker (~4 nm), and the outer diameter of the cucurbit[7]uril barrel (~2 nm) or the β-cyclodextrin (~2 nm), which gives a total ~10 nm. Therefore,* $\Delta L = L - x \cong$ *10 nm, which falls into the observations (see histograms above).*

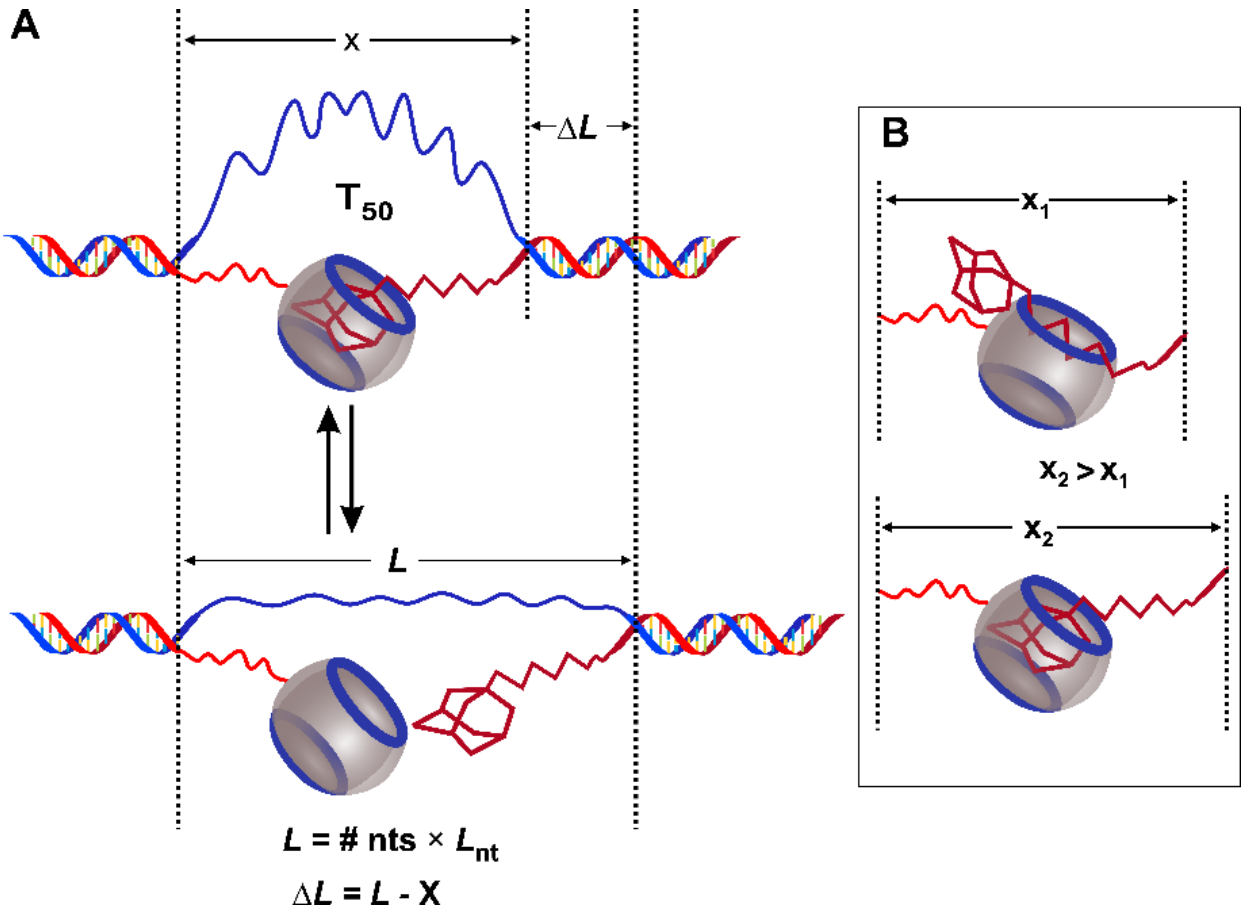

Figure S3. *(A) The relationship between the total contour length (L), change in contour length (ΔL) during the unfolding of host-guest interaction, and the end-to-end distance (x). (B) Difference in the end-to-end distance for the binding between adamantane host and guest molecules with (x_1) and without (x_2) hexyl group. The drawing in (B) represents the compound pair* ***iii*** *(with hexyl linker, see Figure 1) and* ***v*** *(without hexyl linker), or the compound pair* ***iv*** *(with hexyl linker) and* ***vi*** *(without hexyl linker). Since $x_2 > x_1$, the $\Delta L_2 < \Delta L_1$ (the ΔL comparison is based on the equation, $\Delta L = L - x$, where L is determined by the T50 linker). The observation in Figure S2 (compare the first and the third row) is in full agreement with current model.*

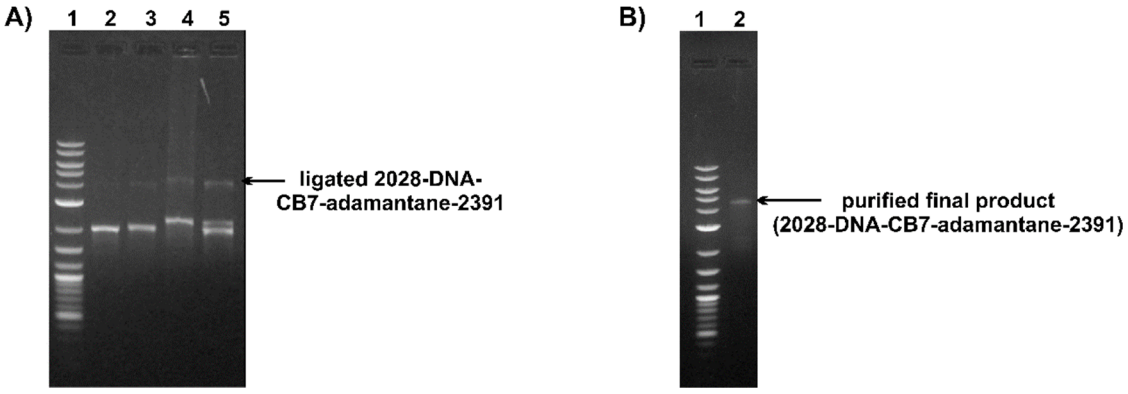

Figure S4: *Native agarose gel images of the final DNA construct preparation. (A) DNA final construct that contains the host-guest pair (compound* ***iii*** *and the CB7; see Figure 1) before purification. Lanes 1–5 represent the 2-log DNA ladder, the 2028 bp handle, the 2028bp-DNA-CB7-adamantane, the 2391 bp handle, and the final ligated product before purification, respectively. The faint bands in the lanes 3 & 4 are the dimers for the 2028 bp handle and 2391 bp handle, respectively. (B) Purified final product (lane 2) in 0.8% native agarose gel. Lane 1 contains the 2-log DNA ladder.*

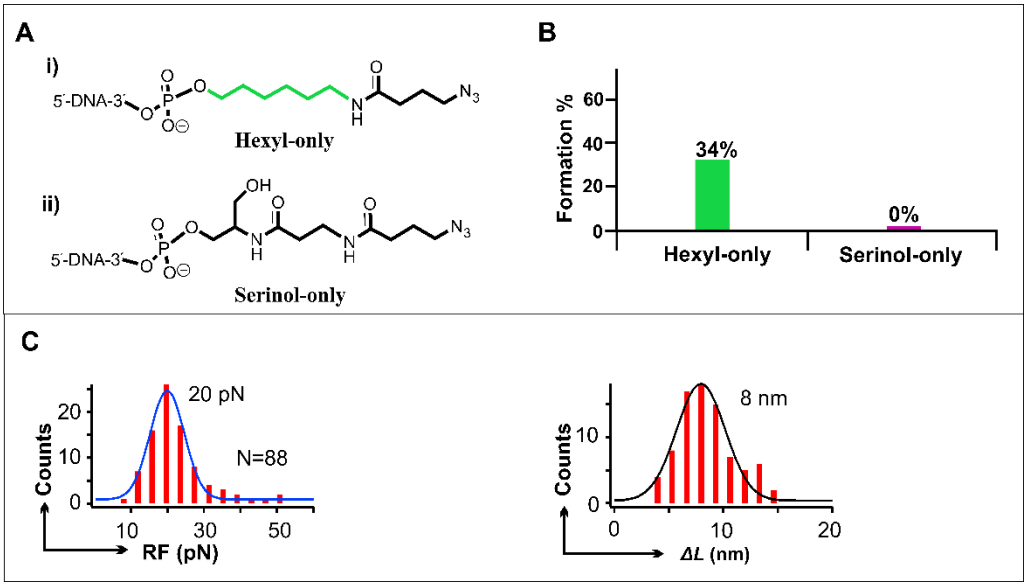

Figure S5. *(A) Structure of the guest molecules used in control experiments. (B) Comparison of the formation % between host (CB7)-guest pairs. (C) Histograms of the rupture force (RF) and the change-in-contour-length (ΔL) during the unfolding of the complexes between the hexyl-only guest and the CB7 host.*

Synthesis of Host/Guest Functionalized DNA

Synthesis of CB7-DNA

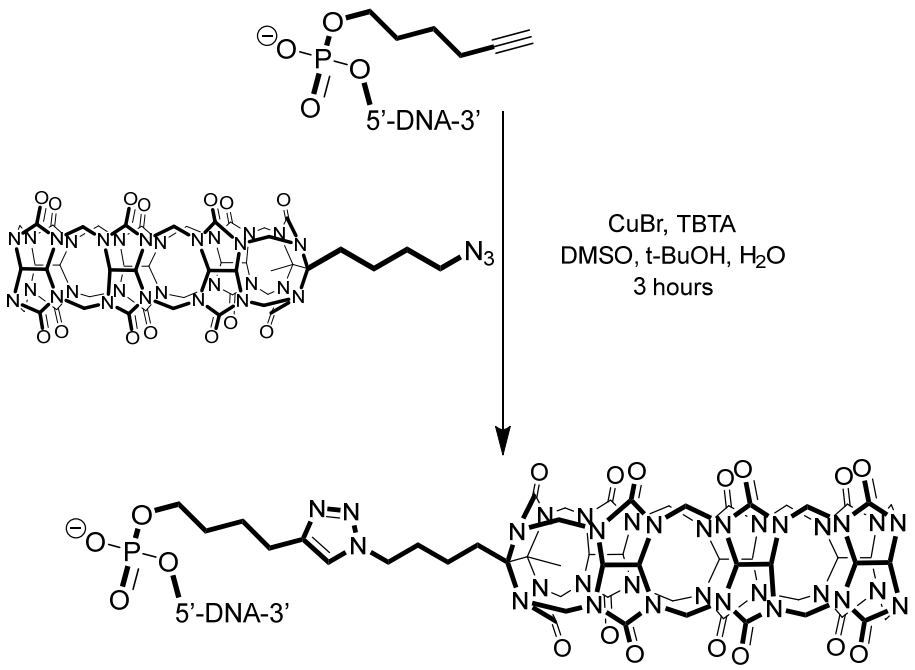

Figure S6*. Synthetic scheme for CB7-DNA.*

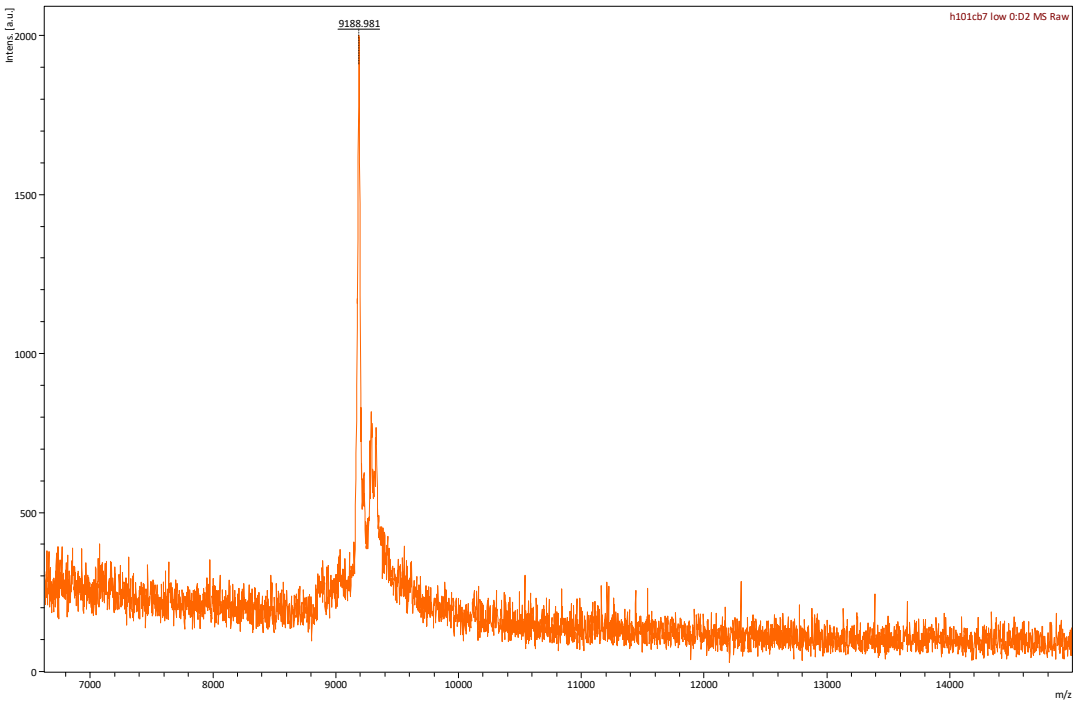

Figure S7*. MALDI-TOF spectrum for CB7-DNA, Found MW = 9188.98 Da, calculated MW = 9187.31 Da for* $[M+ Na]^+$.

Synthesis of Ad-Hexyl-DNA

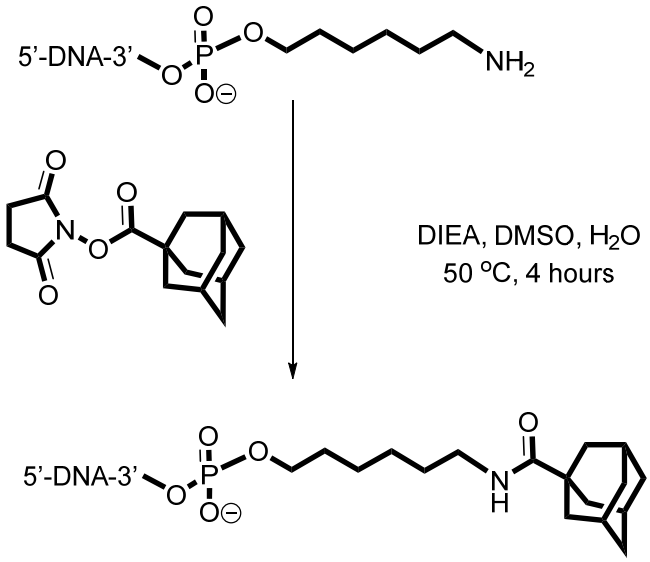

Figure S8*. Synthetic scheme for Ad-Hexyl-DNA.*

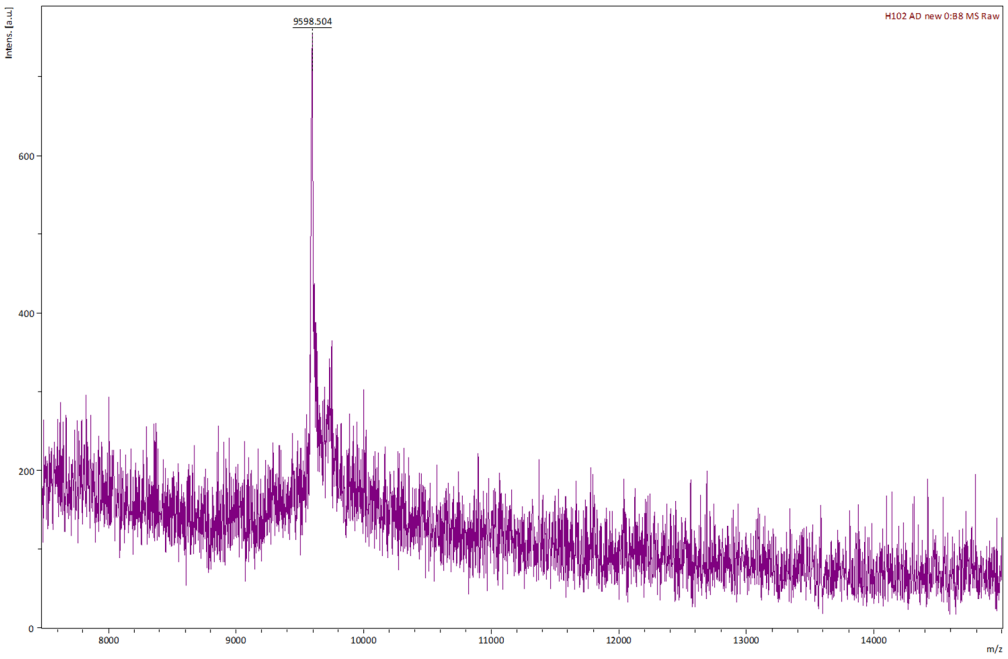

Figure S9. *MALDI-TOF spectrum for Ad-Hexyl-DNA, found MW = 9598.50 Da, calculated MW = 9596.35 Da, for* $[M+Na]^+$.

Synthesis of Ad^+-Hexyl-DNA

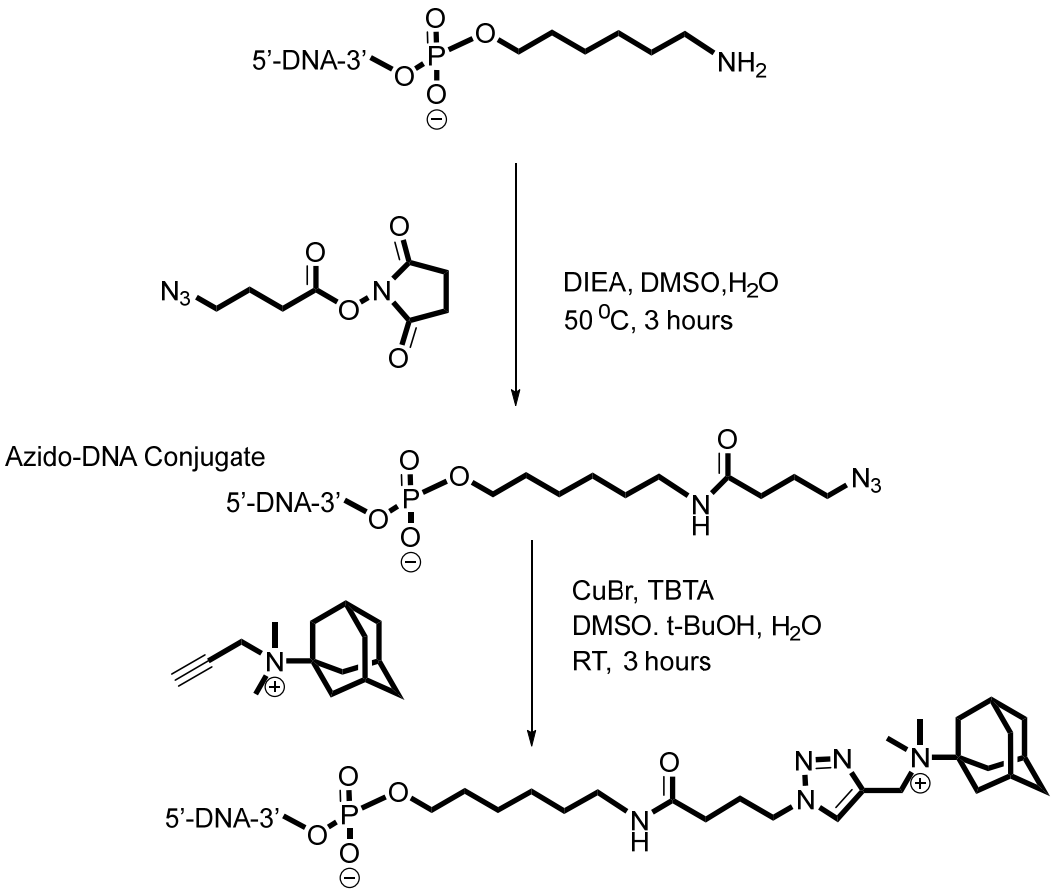

Figure S10. *Synthetic scheme for Ad^+-Hexyl-DNA.*

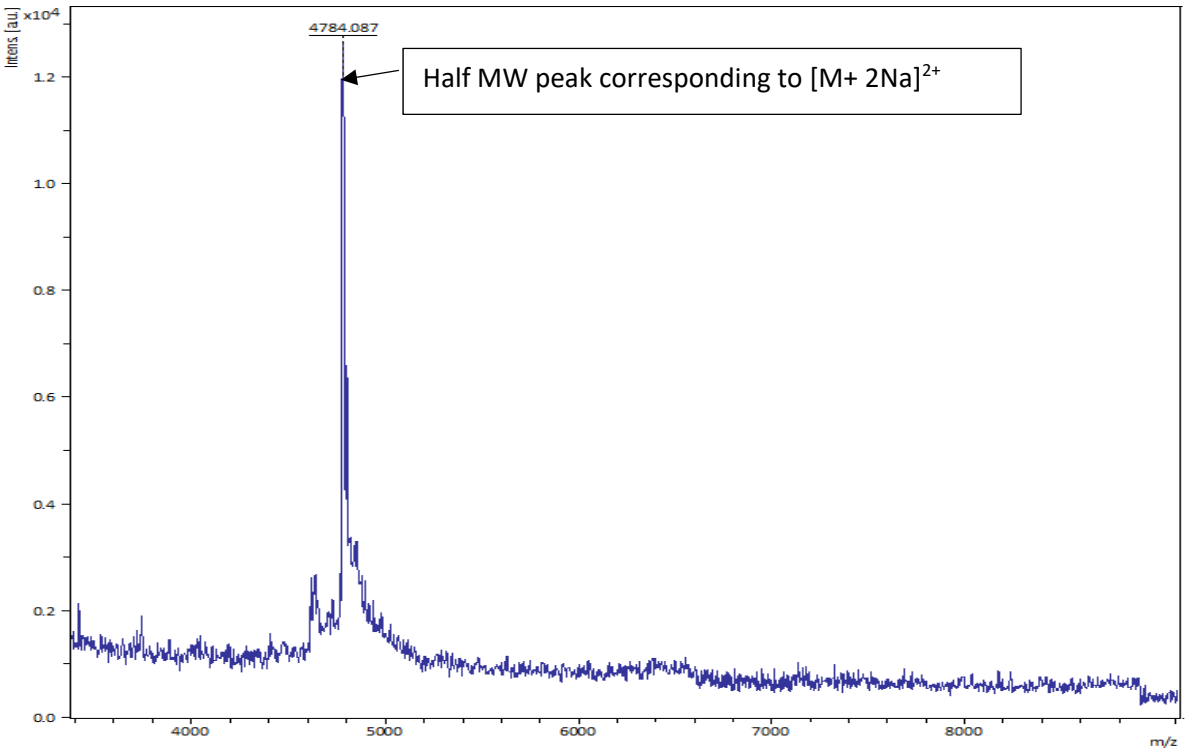

Figure S11. *MALDI-TOF spectrum for Azido-Hexyl-DNA conjugate, found MW = 4784.09 Da, calculated MW= 4784.50 for* $[M+ 2Na]^{2+}$.

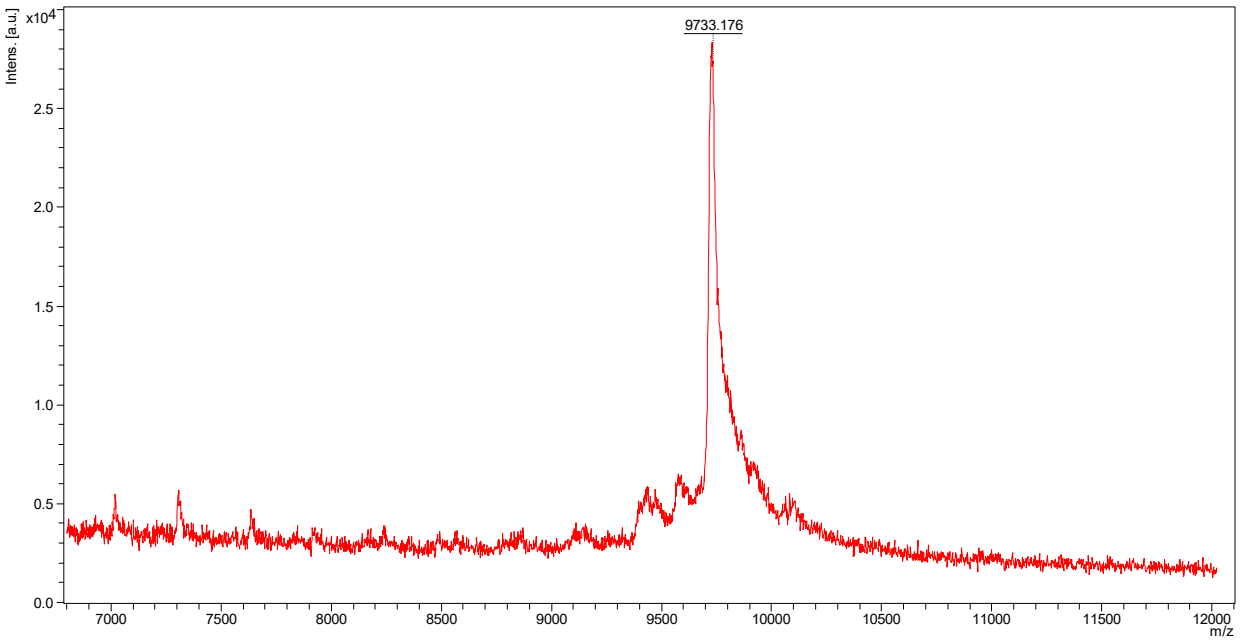

Figure S12. *MALDI-TOF spectrum for Ad^{+}-Hexyl-DNA, found MW = 9733.18 Da, calculated MW=9735.83 for $[M]^{+}$.*

Synthesis of Ad-Serinol-DNA

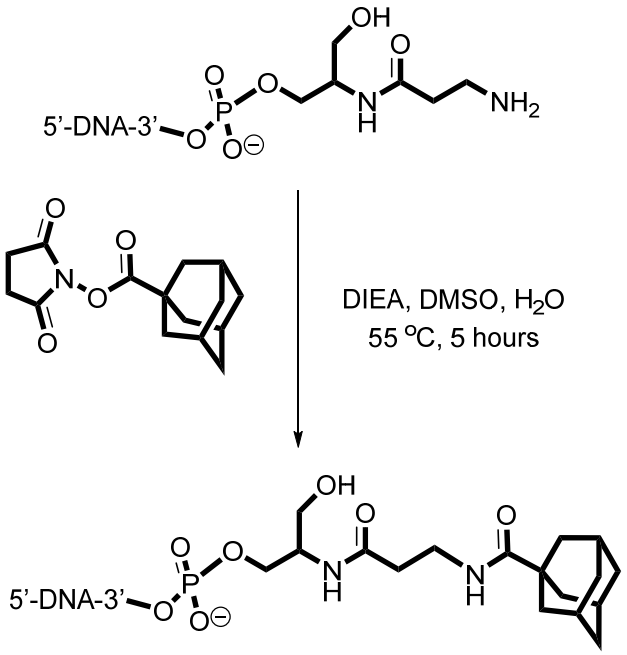

Figure S13*. Synthetic scheme for Ad-Serinol-DNA.*

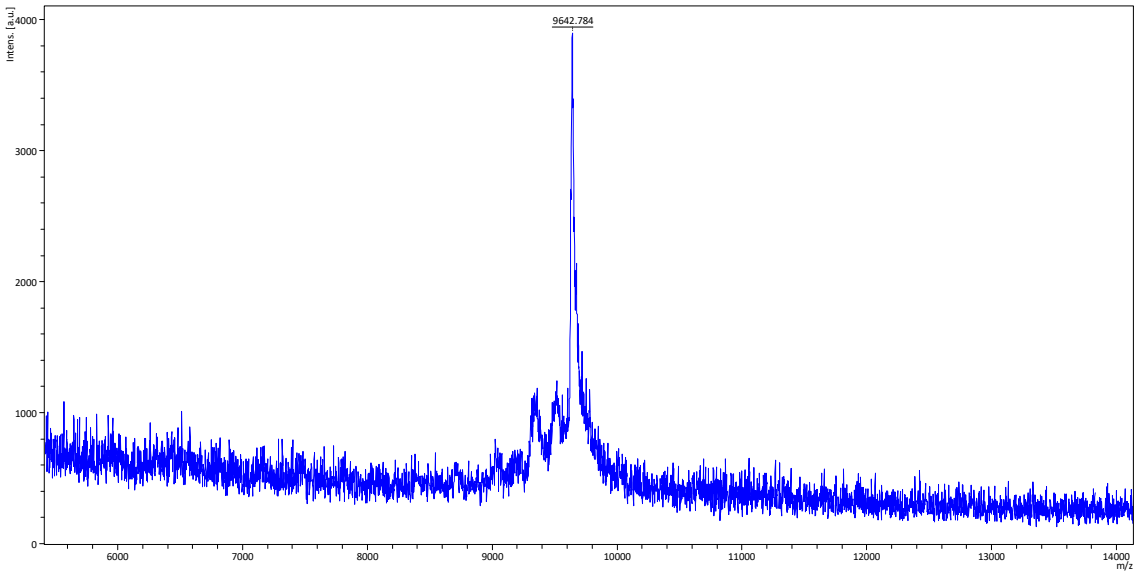

Figure S14. *MALDI-TOF spectrum for Ad-Serinol-DNA, found MW = 9642.78 Da, calculated MW = 9642.38 for* $[M+H]^+$.

Synthesis of Ad^+-Serinol-DNA

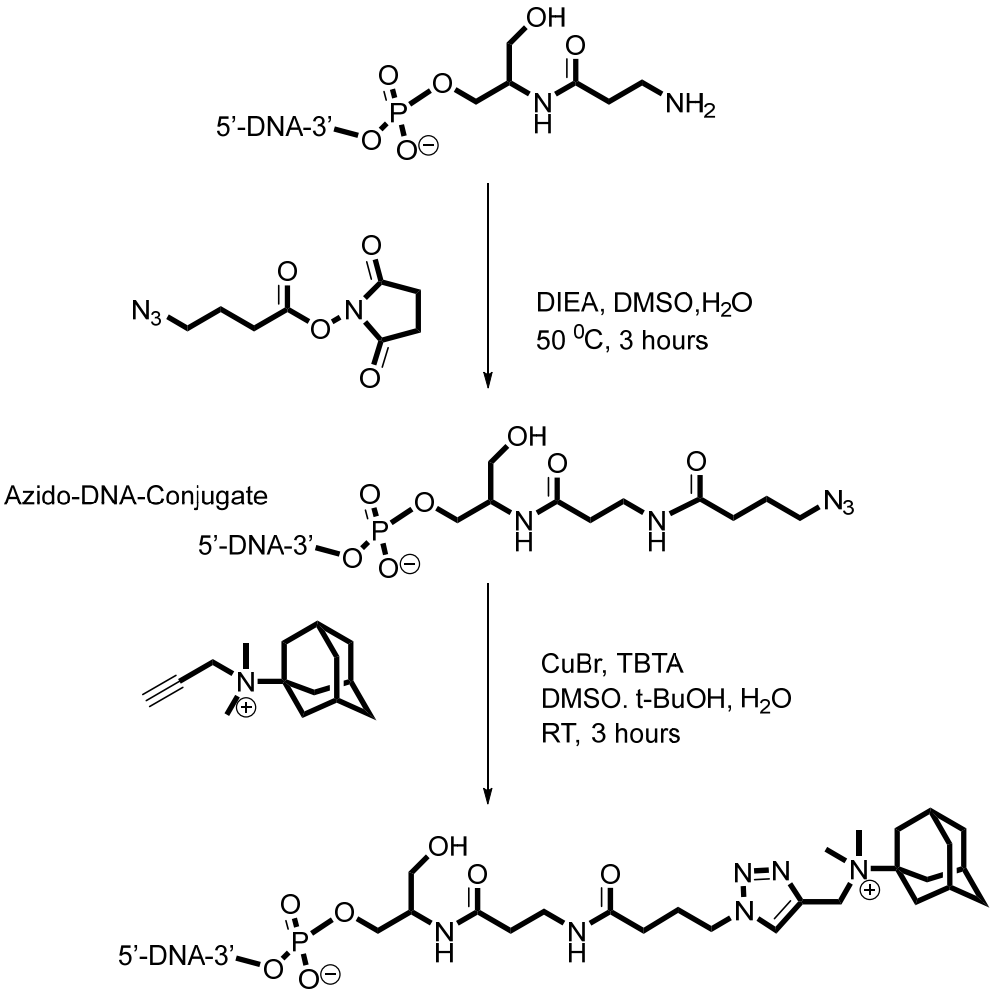

Figure S15. *Synthetic scheme for Ad^+-Serinol-DNA.*

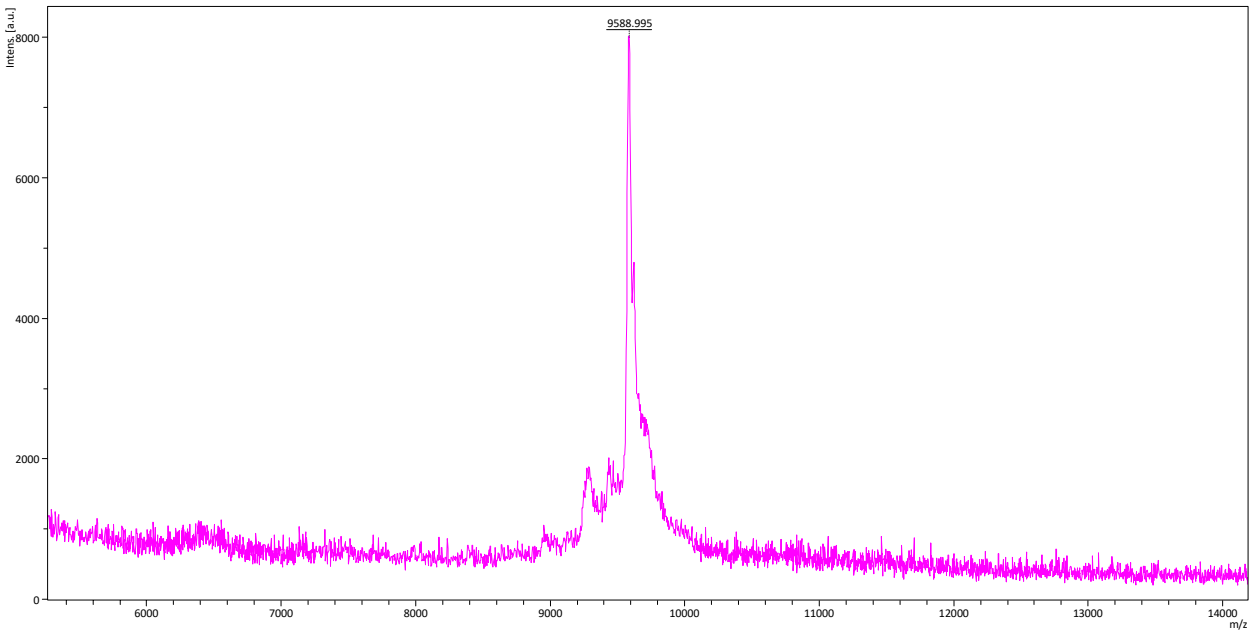

Figure S16. *MALDI-TOF spectrum for Azido-Serinol-DNA conjugate, found MW = 9588.99 Da, calculated MW = 9591.25 for* $[M+H]^+$.

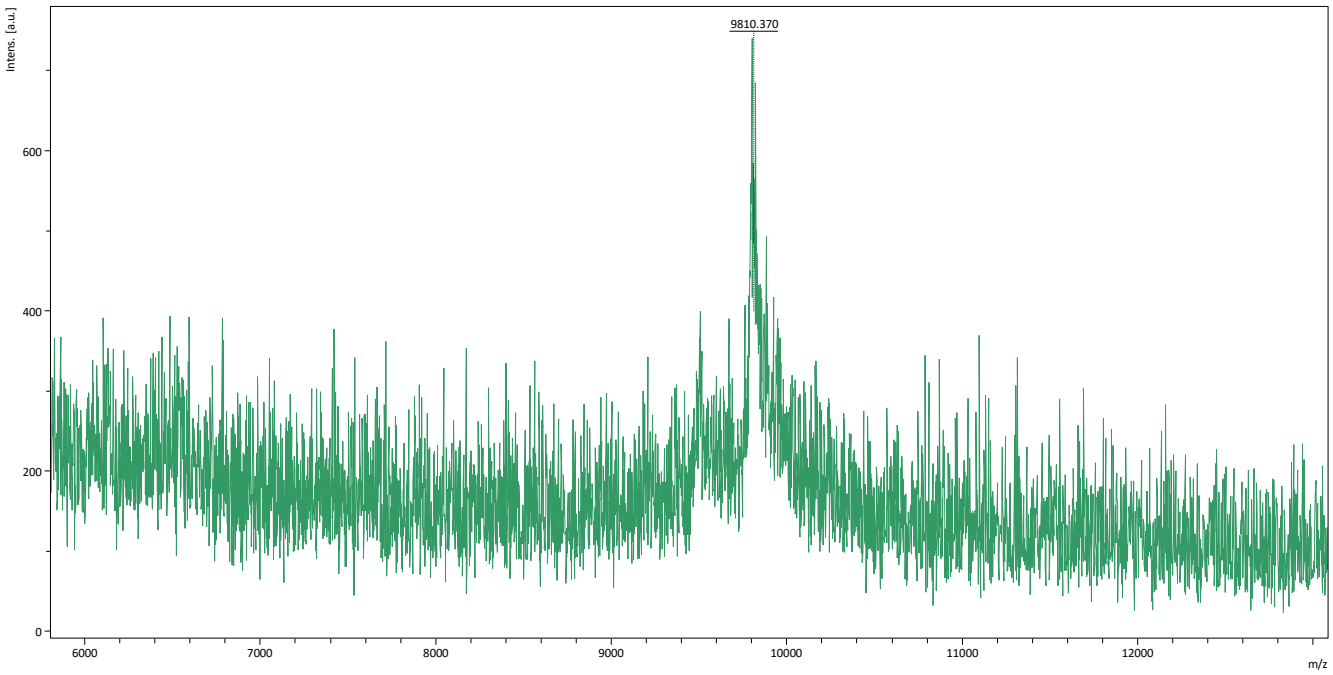

Figure S17. *MALDI-TOF spectrum for Ad^+-Serinol-DNA, found MW = 9810.37 Da, calculated MW=9809.61 for $[M]^+$.*

The purity of each final product was determined by further analysis of the RP-HPLC purified fractions using a Varian Prostar HPLC system and a PLRPS reverse phase column. The column was maintained at room temperature for all runs. The flow rate was set at 1 mL/min. A gradient composed of two solvents (Solvent A is 0.1 M TEAA (aq) in 5% acetonitrile and solvent B is 100% acetonitrile) was used. UV absorption was monitored at 260 nm.

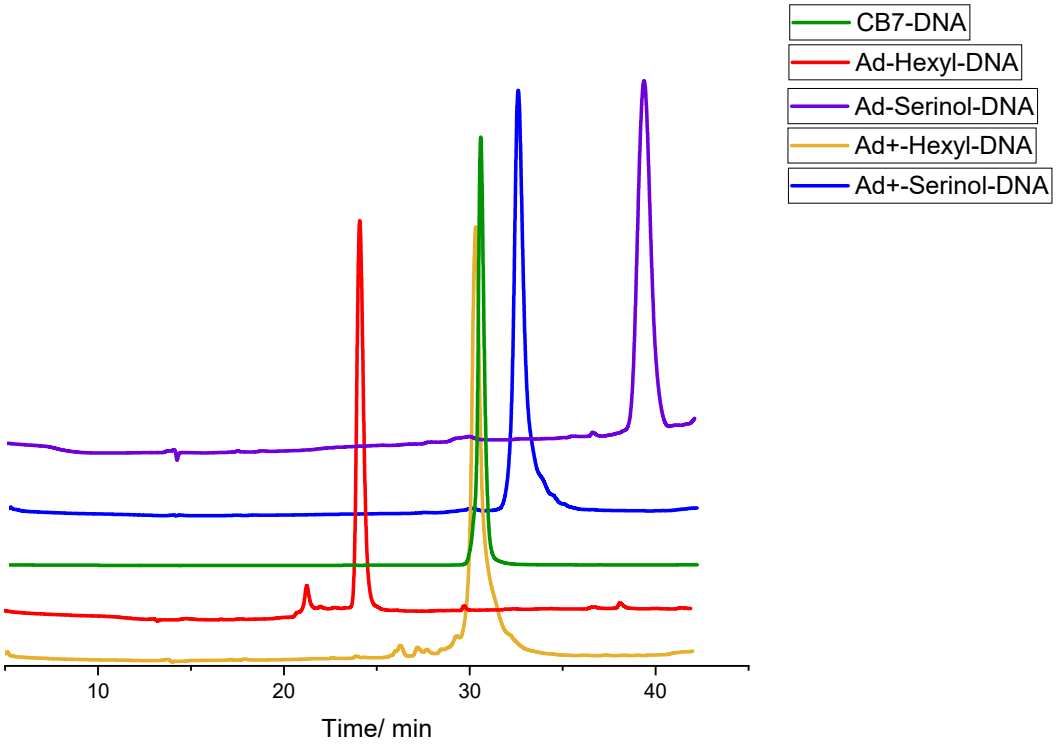

Figure S18. *HPLC purity data for CB7-DNA, Ad-Hexyl-DNA, Ad-Serinol-DNA, Ad^{+}-Hexyl-DNA, and Ad^{+}-Serinol-DNA were found to be 99%, 90%, 91%, 92% and 99% respectively.*

Synthesis of βCD-DNA

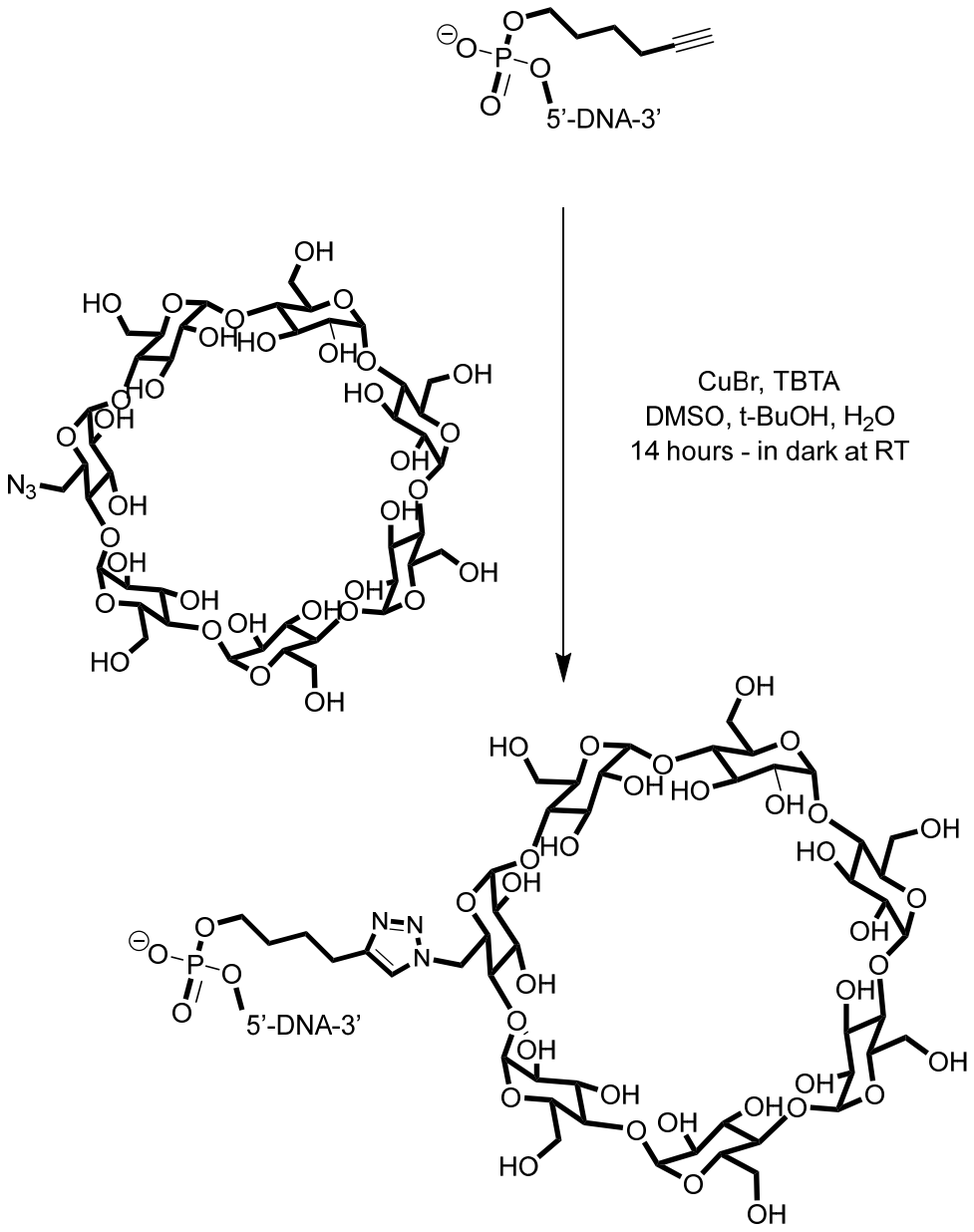

Figure S19. *Synthetic scheme for βCD-DNA.*

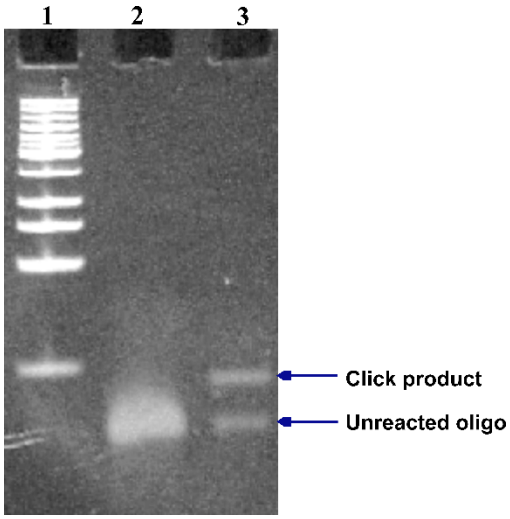

Figure S20. *The 15% denaturing PAGE gel showing the reaction mixture of the CD-DNA preparation by click reaction (see Materials and Methods). Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: the hexynyl oligo (Table S1); Lane 3: crude reaction mixture containing the βCD-DNA preparation. The reaction mixture was treated with ethanol precipitation before loading to the gel.*

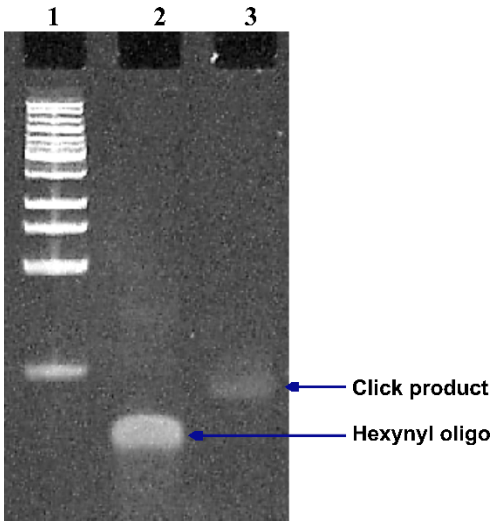

Figure S21. *Purified CD-DNA revealed by 15% denaturing PAGE gel. Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: the hexynyl oligo (Table S1); Lane 3: the gel-purified click product CD-DNA.*

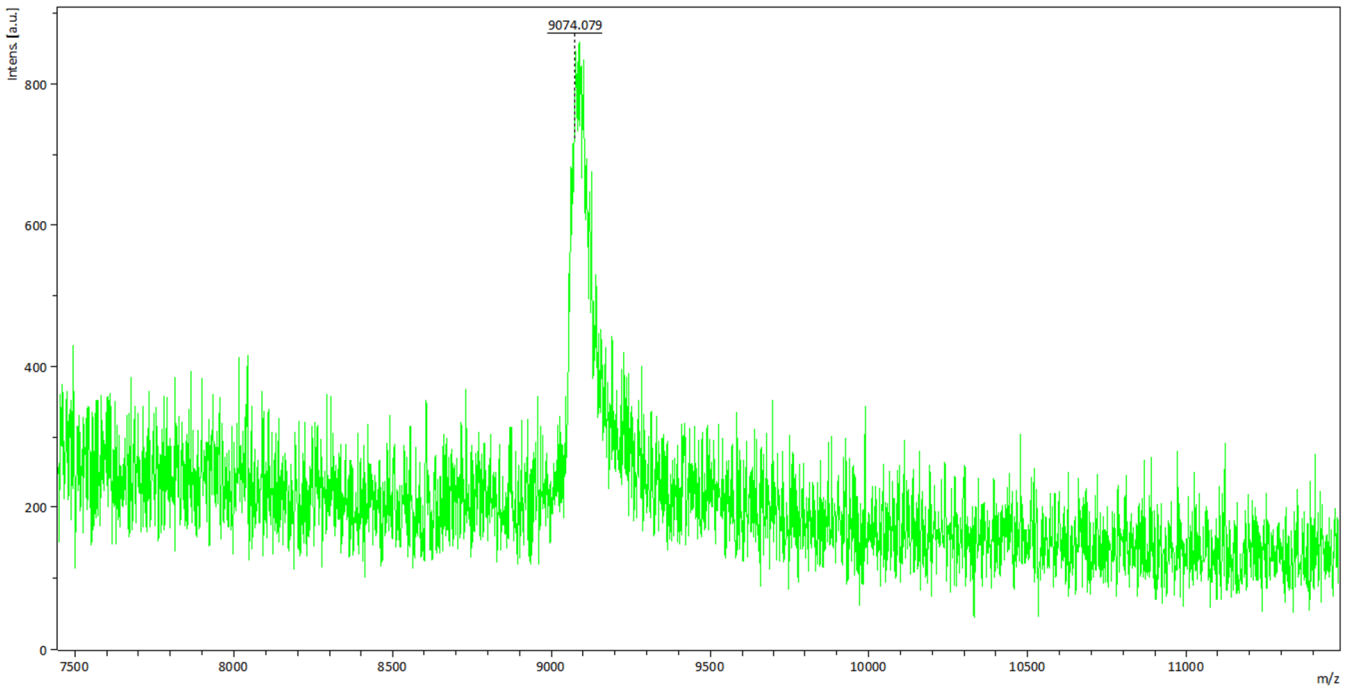

Figure S22. *MALDI-TOF spectrum for βCD-DNA, found MW = 9074.08 Da, calculated MW = 9073.19 for* $[M+Na]^+$.

Table S1. Sequences of DNA oligos used in this work.

DNA Conjugate	DNA Sequence Used (5'-3')
CB7-DNA	GGC TAC ACT AGA AGG ACA GTA TTT G
Ad-Hexyl-DNA	GGC CGA CGC GCT GGG CTA CGT CTT GCT GGC
Ad^{+}-Hexyl-DNA	GGC CGA CGC GCT GGG CTA CGT CTT GCT GGC
Ad-Serinol-DNA	CAG GGA CGC GCT GGG CTA CGT CTT GCT GGC
Ad^{+}-Serinol-DNA	CAG GGA CGC GCT GGG CTA CGT CTT GCT GGC
βCD-DNA	GGC TAC ACT AGA AGG ACA GTA TTT G

Supporting Reference

(S1) Chen, H.; Ma, H.; Chieng, Y. Y.; Hou, S.; Li, X.; Tan, Y. Aggregation and thermal gelation of N-isopropylacrylamide based cucurbit[7]uril side-chain polypseudorotaxanes with low pseudorotaxane content. *RSC Advances* **2015**, *5*, 20684.

(S2) Chen, H.; Huang, Z.; Wu, H.; Xu, J.-F.; Zhang, X. Supramolecular Polymerization Controlled through Kinetic Trapping. *Angew. Chem. Int. Ed. EngI.* **2017**, *56*, 16575.

(S3) Vinciguerra, B.; Cao, L.; Cannon, J. R.; Zavalij, P. Y.; Fenselau, C.; Isaacs, L. Synthesis and Self-Assembly Processes of Monofunctionalized Cucurbit[7]uril. *J. Am. Chem. Soc.* **2012**, *134*, 13133.

(S4) Zhou, M.; Haldar, S.; Franses, J.; Kim, J.-M.; Thompson, D. H. Synthesis and Self-assembly Properties of Acylated Cyclodextrins and Nitrilotriacetic Acid (NTA)-modified Inclusion Ligands for Interfacial Protein Crystallization. *Supramolecular Chemistry* **2005**, *17*, 101.

(S5) Mao, H.; Luchette, P. An integrated laser-tweezers instrument for microanalysis of individual protein aggregates. *Sens. Actuators, B* **2008**, *129*, 764.

(S6) Smith, S. B.; Cui, Y. J.; Bustamante, C. Overstretching B-DNA: The elastic response of individual double-stranded and single-stranded DNA molecules. *Science* **1996**, *271*, 795.

(S7) Yu, Z.; Mao, H. Non-B DNA structures show diverse conformations and complex transition kinetics comparable to RNA or proteins — a perspective from mechanical unfolding and refolding experiments. *Chem. Rec.* **2013**, *13*, 102.

(S8) Dhakal, S.; Cui, Y.; Koirala, D.; Ghimire, C.; Kushwaha, S.; Yu, Z.; Yangyuoru, P. M.; Mao, H. Structural and mechanical properties of individual human telomeric G-quadruplexes in molecularly crowded solutions. *Nucleic Acids Res.* **2013**, *41*, 3915.

(S9) Yu, Z.; Schonhoft, J. D.; Dhakal, S.; Bajracharya, R.; Hegde, R.; Basu, S.; Mao, H. ILPR G-Quadruplexes Formed in Seconds Demonstrate High Mechanical Stabilities. *J. Am. Chem. Soc.* **2009**, *131*, 1876.

(S10) Record, M. T. J.; Anderson, C. F.; Lohman, T. M. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Quart. Rev. Biophys.* **1978**, *11*, 103.

(S11) Mills, J. B.; Vacano, E.; Hagerman, P. J. Flexibility of single-stranded DNA: use of gapped duplex helices to determine the persistence lengths of poly(dT) and poly(dA). *J. Mol. Biol.* **1999**, *285*, 245.