Supplemental Information

Measurement of Single-Molecule Forces in Cholesterol and Cyclodextrin Host-guest complexes

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1. Nonequilibrium steered molecular dynamics (SMD) simulation

In nonequilibrium steered molecular dynamics (SMD) simulations, simulation runs are performed at 296 K and 1 atm pressure in an *NPT* ensemble. All SMD simulations start from the configurations around the free energy minima (i.e., i and ii for pulling from the alkyl tail; and iii for pulling from the hydroxyl head), as shown in Figure 3B and 4C. For each (meta)stable state, 25 independent SMD simulations were performed. Periodic boundary conditions are applied in three dimensions. The particle−particle particle−mesh solver is employed to calculate the long-range electrostatic interactions.[S1] The cutoff distance for the short-range Lennard-Jones interactions is set to 10 Å. The temperature is controlled at 296 K using the Nosé−Hoover thermostat.[S2] The equations of motion of the particles are propagated through the velocity Verlet algorithm with a time step of 1 fs in a constant-*NPT* ensemble. We use the consistent valence force field (CVFF)[S3] to describe the interatomic interactions between all atoms. Atomic partial charges in cyclodextrins and cholesterol are further recalibrated by quantum mechanical density functional theory calculations using B3LYP/6-31G** functional and basis set and by the CHarges from ELectrostatic Potentials using a Grid (CHELPG)-based method[S4]. We use a flexible simple-point charge (SPC) water model[S5-6], which is consistent with the CVFF force field parameters. For the purpose of verification, other popular force fields, such as general AMBER force field (GAFF)[S7] and q4md-CD,[S8] are also used to check against the CVFF. Negligible effects are found on the

dissociation force or free energy profiles from all three methods. Consequently, only the results from the CVFF are included in this paper.

2. Umbrella sampling

For umbrella sampling, the total sampling distance, d, is divided into N independent simulation segments. In each segment i, a constrained potential is applied to the system to obtain a biased probability distribution, $P(d_i)$. The unbiased distribution, $P(d)$, is then reconstructed for the free energy $G(d)$ calculations through the histogram reweighting method.[S9-10] This approach can obtain the realistic equilibrium free energy of the system. The free-energy value at global minimum is used as the reference point zero. A total of 6000 water molecules are included in the system. In each case, a total of N = 55 succeeding sampling intervals are arranged. A harmonic spring with a spring constant of 2 kcal/(mol Å^2) is used in the umbrella sampling. Molecular configurations are sampled by gradually changing the equilibrium distance of the spring within each sampling interval of 0.2 Å. The simulation time for each interval is 2 ns.

Synthesis of Host/Guest functionalized DNA

Synthesis of Me-β-CD-DNA or β-CD-DNA

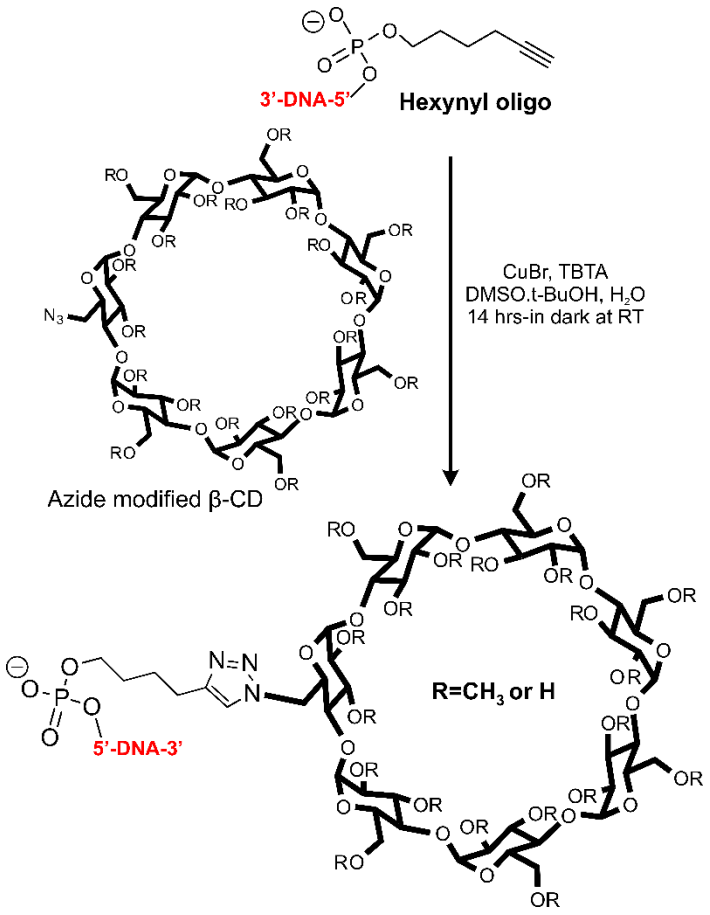

Figure S1. *Synthetic scheme for the Me-β-CD-DNA (R=CH₃) or β-CD-DNA (R=H).*

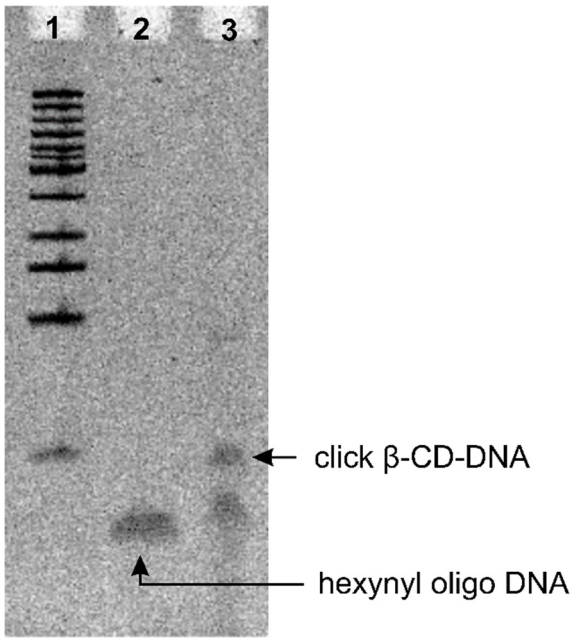

Figure S2. *The 15% denaturing PAGE gel showing the reaction mixture of the CD-DNA preparation by click reaction (see Materials and Methods). Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: the hexynyl oligo (Table S1); Lane 3: crude reaction mixture containing the β-CD-DNA. The reaction mixture was treated with ethanol precipitation.*

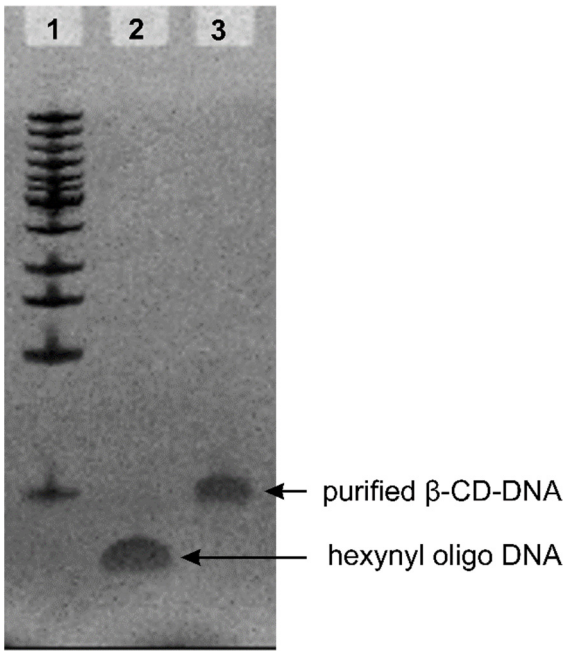

Figure S3. *15% denaturing PAGE gel showing purified β-CD-DNA. Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: the hexynyl oligo (Table S1); Lane 3: the gel-purified click product β-CD-DNA.*

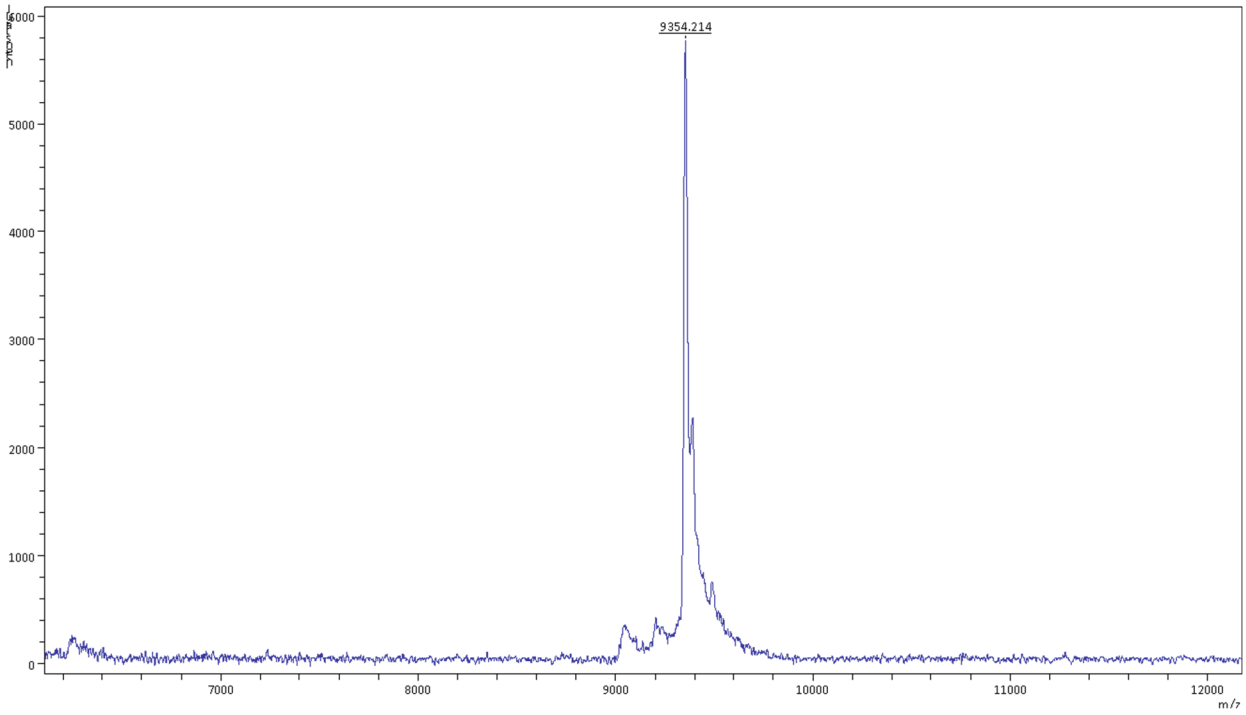

Figure S4. *MALDI-TOF spectrum for Me-β-CD-DNA, found MW = 9354.21 Da, calculated MW = 9353.73 for* $[M+Na]^+$.

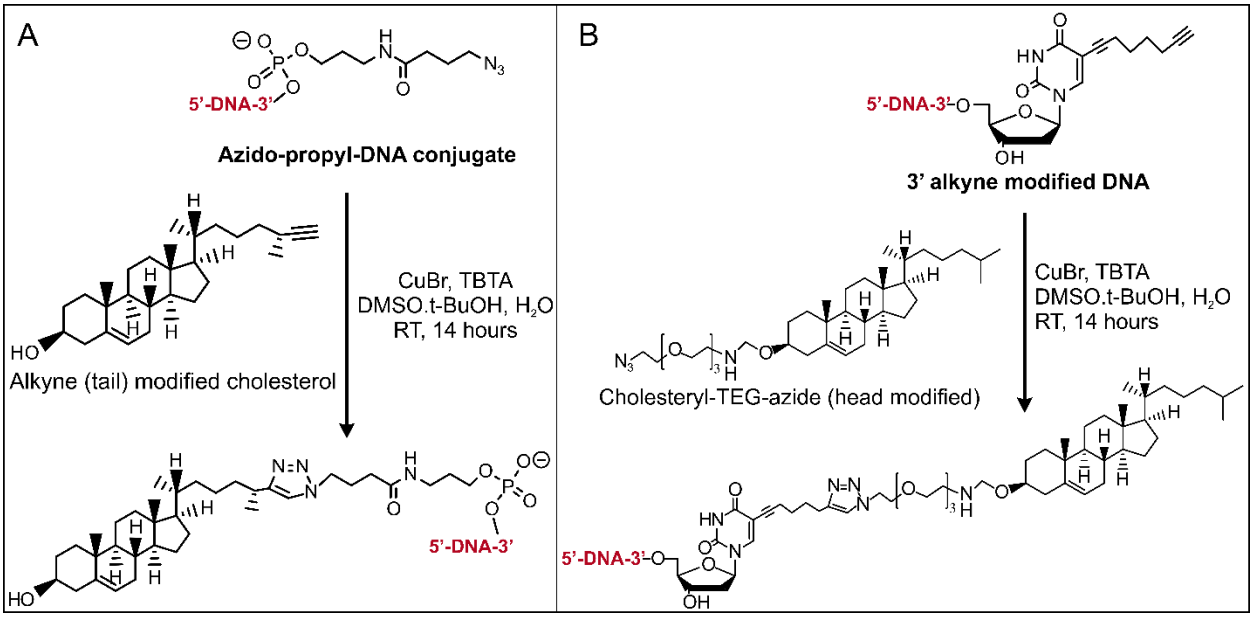

Figure S5. *Synthetic scheme for the 3' cholesterol-propyl-DNA* ***(A)*** *and 3' cholesterol-TEG-DNA* ***(B)***.

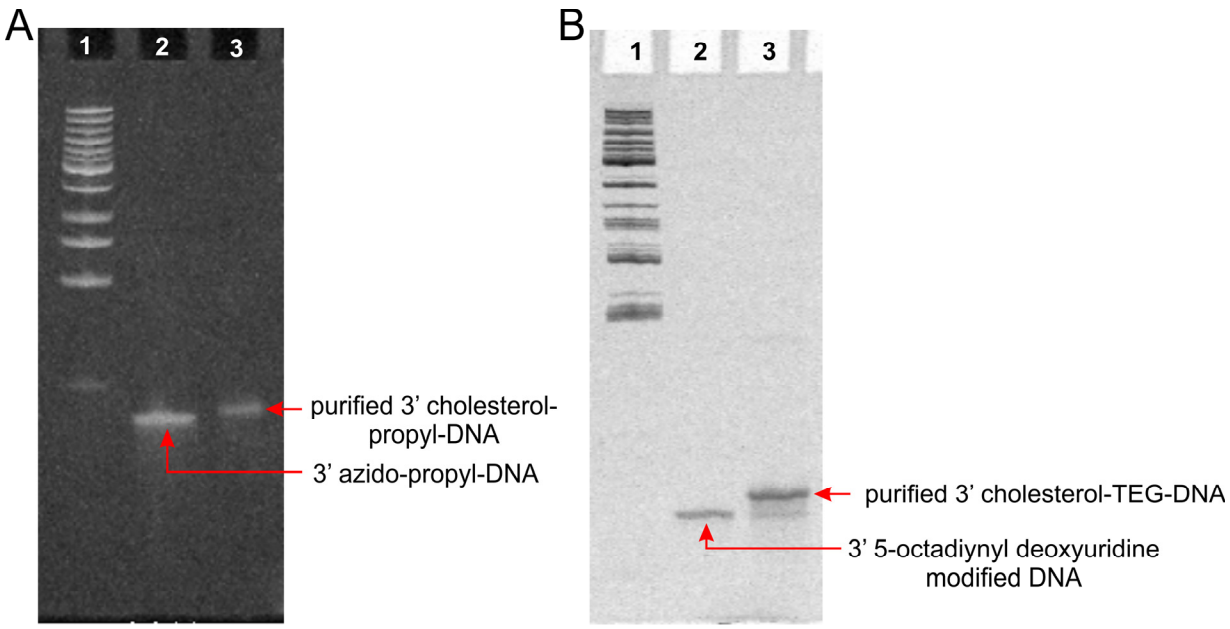

Figure S6. *Denaturing PAGE gels showing purified 3' modified cholesterol-DNA compounds.* ***(A)*** *12% denaturing PAGE gel showing purified 3' cholesterol-propyl-DNA. Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: the azido-propyl-DNA (Table S1); Lane 3: gel-purified click product 3' cholesterol-propyl-DNA.* ***(B)*** *15% denaturing PAGE gel showing purified 3' cholesterol-TEG-DNA. Lane 1: Low Molecular Weight DNA Ladder (NEB); Lane 2: 3' 5-octadinyl deoxyuridine modified DNA (Table S1); Lane 3: gel-purified click product 3' cholesterol-TEG-DNA.*

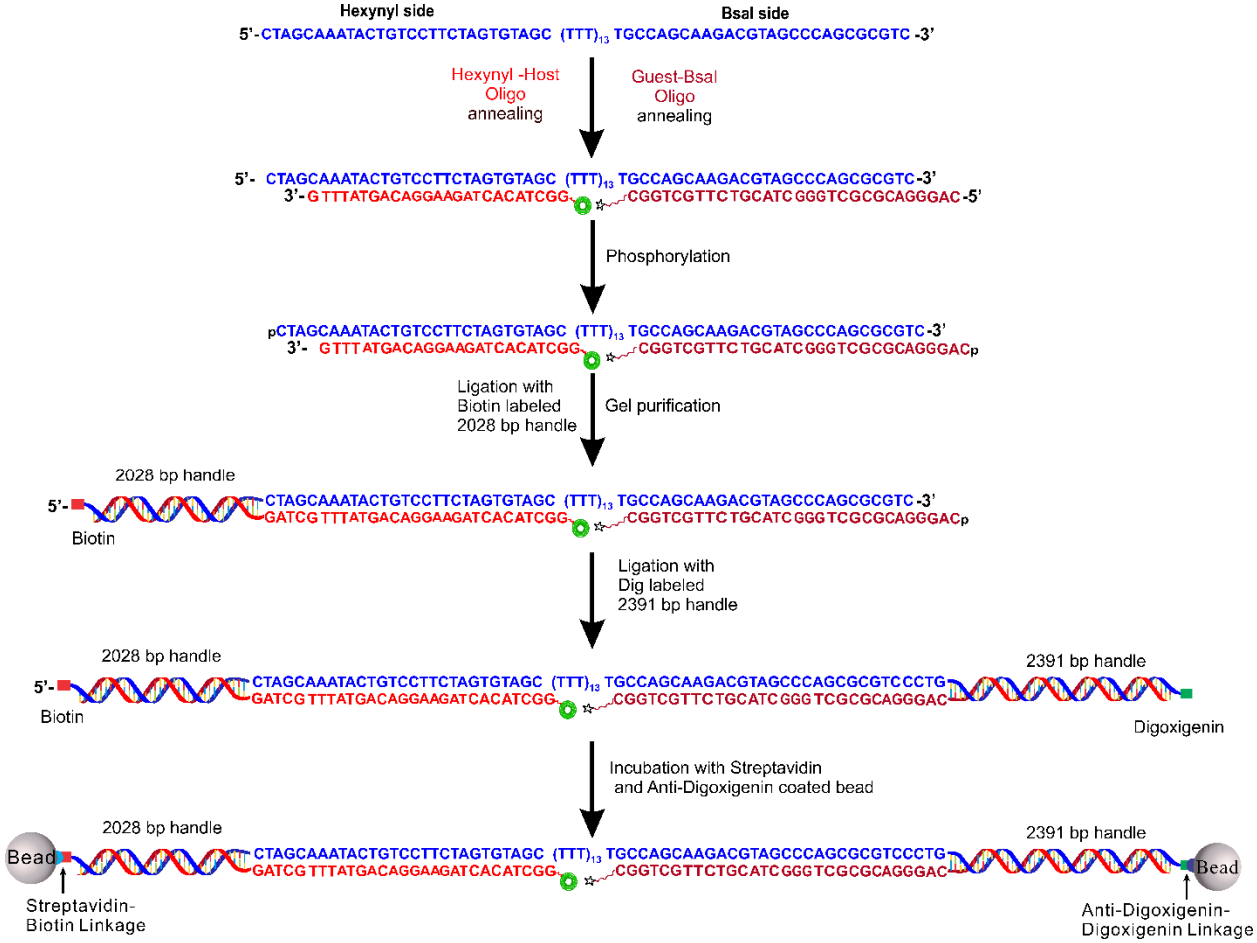

Figure S7. *Flow chart for the preparation of the construct for single molecular mechanical unfolding experiments.*

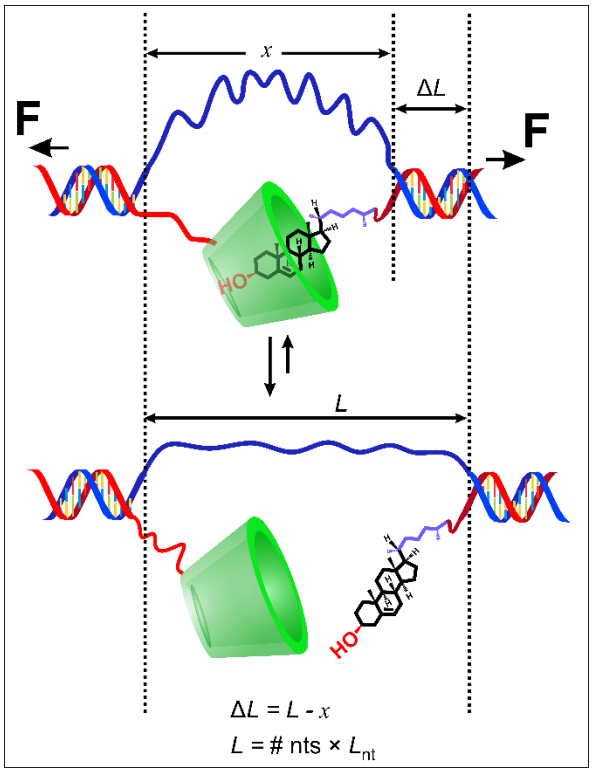

Figure S8. *The relationship between the total contour length (L), change in contour length (ΔL) during the unfolding of host-guest interaction, and the end-to-end distance (x).*

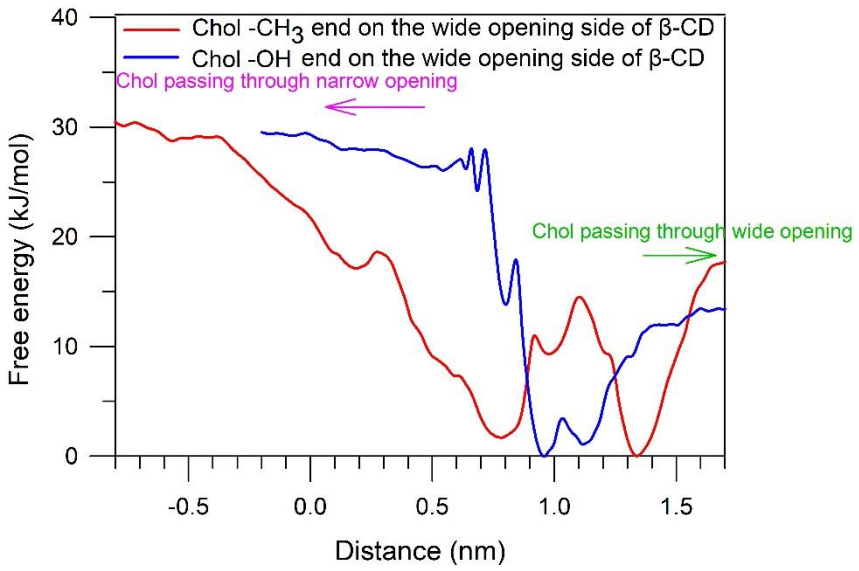

Figure S9. *Variations of the free energy versus the distance, d, between β-CD and cholesterol (Chol) molecules along different pulling directions. The distance, d, is defined as the length between the center of mass of the β-CD and the 3rd (blue line, hydroxyl end) or 25th carbon atom (red line, alkyl end) of the cholesterol molecule as shown in Figure 3A. The free energy curves are extensions of Figure 3B, focusing on the cholesterol molecule passing through both wide and narrow openings of the β-CD molecule.*

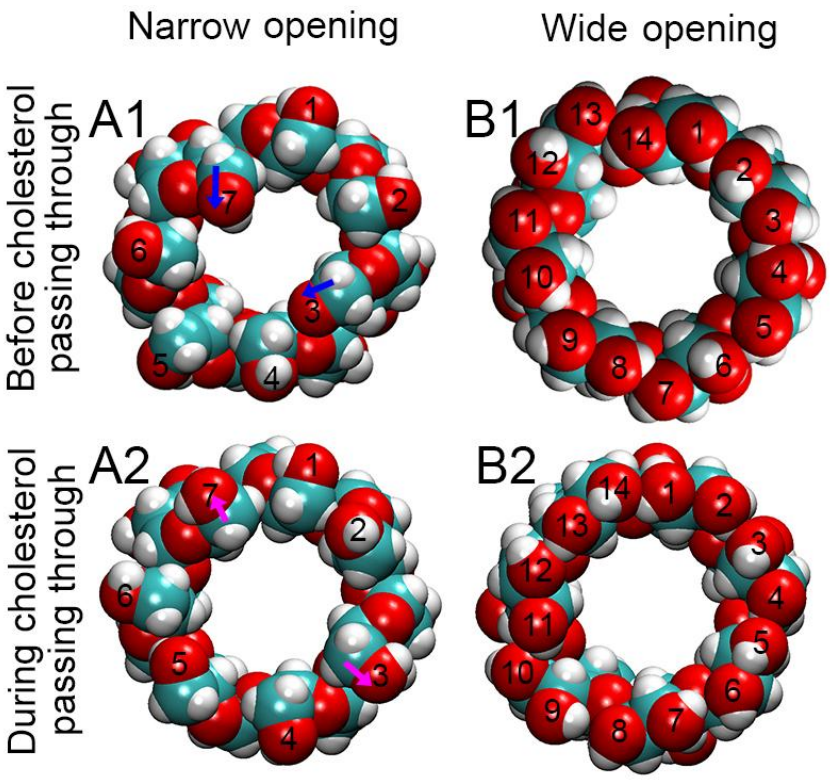

Figure S10. *Conformation changes of the β-CD molecule before (A1) and during (A2) the cholesterol molecule (pulled from the hydroxyl end) passing through the narrow opening (viewed from the narrow (primary) hydroxyl rim), and before (B1) and during (B2) the cholesterol molecule (pulled from the hydroxyl end) passing through the wide opening (viewed from the wide (secondary) hydroxyl rim). Colors: red, O; white, H; light blue, C. Water molecules are not shown for clarity.*

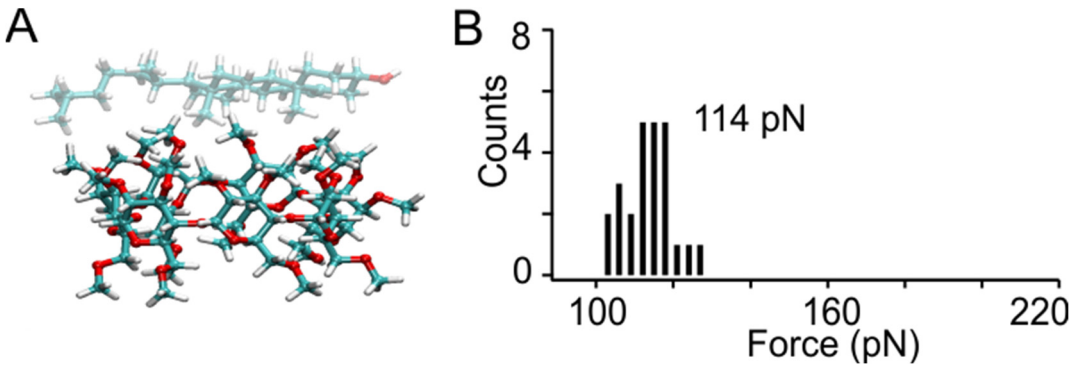

Figure S11. A) *The third stable conformation between the Me-β-CD and the cholesterol molecule, in which the latter adopts a direct parallel contact on the rim of the Me-β-CD.* ***B)*** *The dissociation force histogram corresponding to conformation A.*

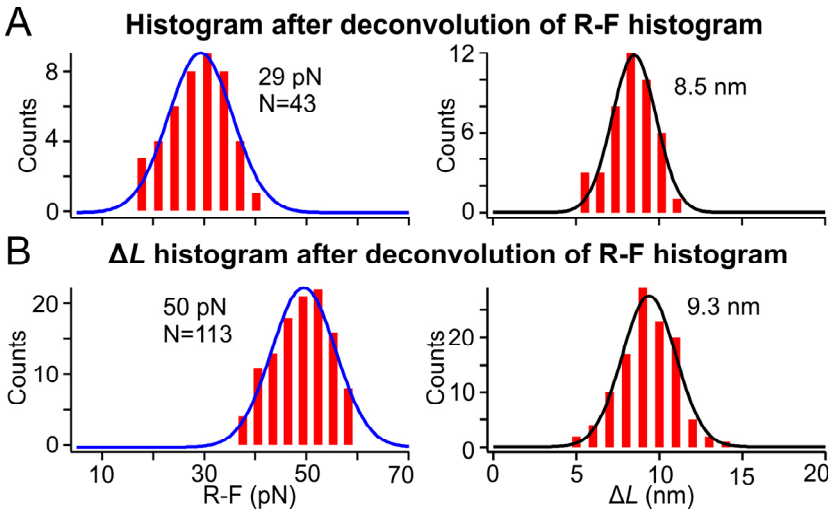

Figure S12. *Deconvolution based on Figure 4A results.* ***A)*** *The deconvoluted rupture force (R-F) histograms (left, smaller force population) and corresponding change-in-contour-length (ΔL) histogram (right) for the Me-β-CD and cholesterol complex pulled from the alkyl end of the cholesterol.* ***B)*** *The deconvoluted rupture force (R-F) histograms (left, higher force population) and corresponding change-in-contour-length (ΔL) histogram (right) for the Me-β-CD and cholesterol complex pulled from the alkyl end of the cholesterol.*

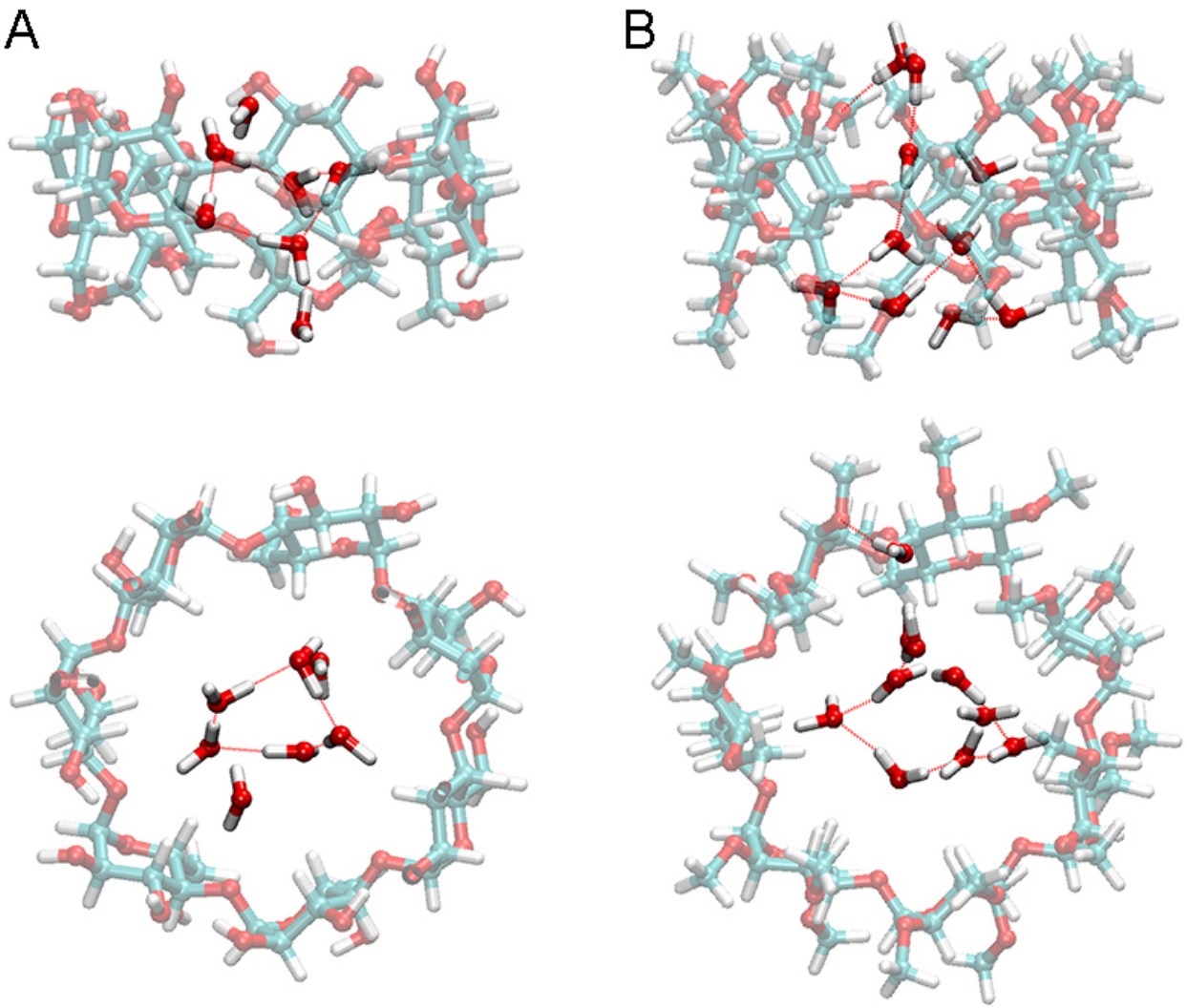

Figure S13. *Snapshots showing the water structures within the cavities of* ***A)*** *β-CD (side view, upper left; top view, lower left) and* ***B)*** *Me-β-CD (side view, upper right; top view, lower right). Red lines indicate the hydrogen bonds.*

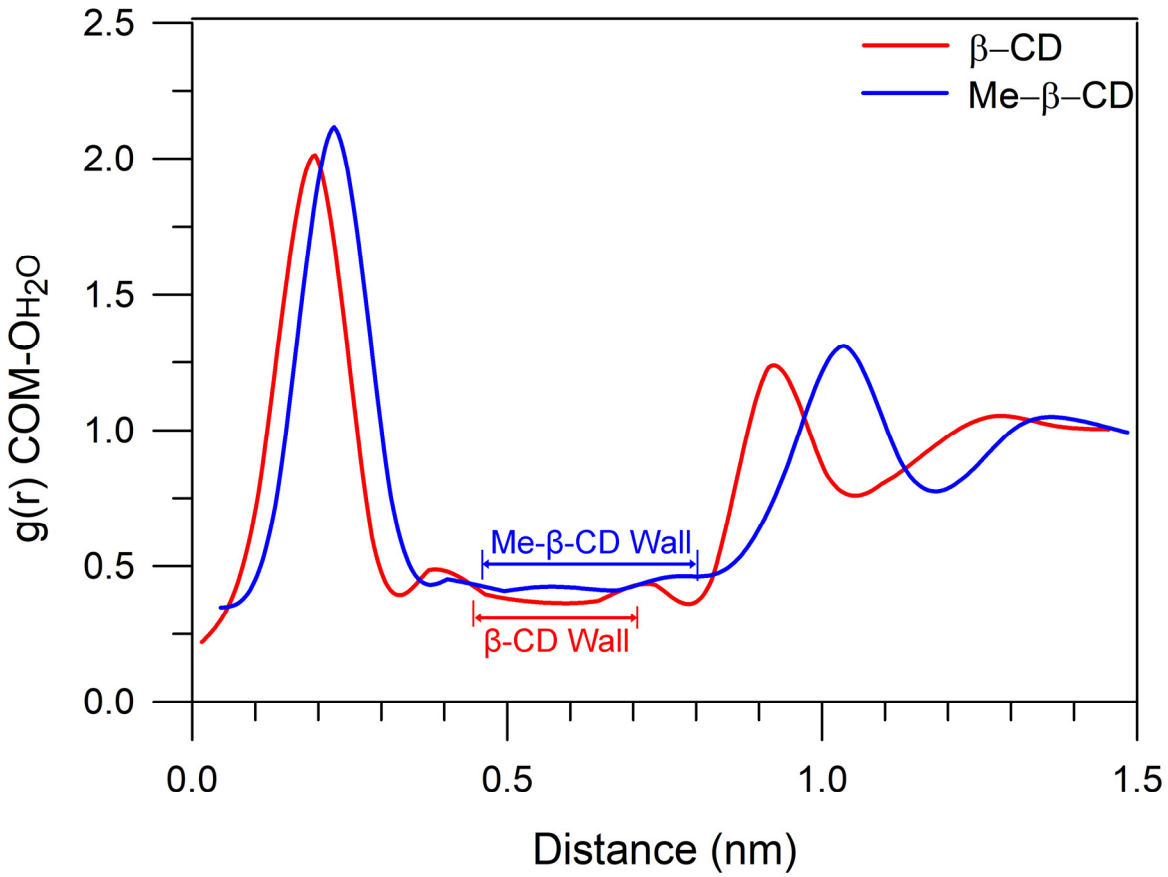

Figure S14. *Radial distribution functions of water oxygen around the center of mass of β-CD (red line) and Me-β-CD (blue line). The first high peak of each curve corresponds to the water molecules inside the cavity, other peaks outside (Me-)β-CD walls show the first and second hydration shells around the cavity.*

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

DNA Conjugate	DNA Sequence Used (5'-3')
β-CD-DNA	GGC TAC ACT AGA AGG ACA GTA TTT G
Me-β-CD-DNA	GGC TAC ACT AGA AGG ACA GTA TTT G
3'cholesterol-propyl DNA	CAG GGA CGC GCT GGG CTA CGT CTT GCT GGC
3'cholesterol-TEG-DNA	CAG GGA CGC GCT GGG CTA CGT CTT GCT GGC

SI References

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