A Two-Ring Interlocked DNA Catenane Rotor Undergoing Switchable Transitions Across Three States

Xiu-Juan Qi,[a,b‡] Chun-Hua Lu,[b‡] Alessandro Cecconello,[b] Huang-Hao Yang,[a] and Itamar Willner[b]*

[a] The Key Lab of Analysis and Detection Technology for Food Safety of the MOE, College of Chemistry and Chemical Engineering, Fuzhou University, Fuzhou 350002, China

[b] Institute of Chemistry and The Center for Nanoscience and Nanotechnology, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

‡ These authors contributed equally to this work.

*Address correspondence to: willnea@vms.huji.ac.il

Tel: +972-2-6585272

Fax: +972-2-6527715

Table S1. Sequences of the oligonucleotides

Name	Sequence* (5' to 3')
L_α	CACTGTGT GA/**Cy3**/T **CCCG*T*CCTG**T ATTAATTCAATTAGAAGT/**Cy5**/C **CCCG*T*CCTG**TC ACC TAC ACC AAT ACA CTTA **CCCGACCTG** ATGA GTTTCTAG
C_α	ACACAGTG CTAGAAAC
L_β	AGCAGACG TTTTTT TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT **CAGGTCGGG** A CAGAGCTT
C_β	CGTCTGCT AAGCTCTG
1	AAAAA ACG TCT GCT AAG CTC T - BHQ2
F_1	CAGGGTC AACTCATCAGGTCGGG AAGCTAT
aF_1	ATA GCT TCC CGA CCT GAT GAG TTG ACC CTG
F_2	ACTCTCC ATT GAA TTA ATA CAG G TGCATAT
aF_2	ATA TGC ACC TGT ATT AAT TCA ATG GAG AGT
F_3	ACAGAGG TGG TGT AGG TGA CAG G TTAGGAG
aF_3	CTC CTA ACC TGT CAC CTA CAC CAC CTC TGT
2	$(HS)_2$- AAAAAAAAAAAAACGTCTGCTAAGCTCT
2-comp	TTA ATA TAC CTG ATC GTC ATG ATT CGT TAC TAG TAG ACT TTA CACGGA TAG CAG AGC TTA GCA GAC GTT TTT TTT TTT TT
2-comp*	AAA AAA AAA AAA ACG TCT GCT AAG CTC TGC TAT CCG TGT AAA GTC TAC TAG TAA CGA ATC ATG ACG ATC AGG TAT ATT AA
STAB	$(HS)_2$-TTTTT

* The bases that interlocked the rings are in underlined and bold format. The base "***T***" of the L_α in italic, underlined and bold format is the mismatch base of the L_β.

Synthesis of the two-ring catenane rotor α/β

The DNA L_α and DNA L_β were first phosphorylated by using T4 polynucleotide kinase, respectively. The phosphorylated nucleic acids L_α and L_β were mixed in the quick ligation reaction buffer. For the hybridization of L_α and L_β, the mixture was incubated at 90 °C for 5 min, and instantly cooled down to 25 °C, and allowed to react for 40 min. Subsequently, caps C_α and C_β were added to the solution to yield the

hybridization of cap C_α with DNA L_α and cap C_β with DNA L_β (allowed to interact for 40 min at 25 °C). Finally, the quick T4 DNA ligase was added to the solution, resulting in the ligation of the 3' and 5' ends of DNA L_α and the 3' and 5' ends of DNA L_β to form the catenated rings α/β.

The volume of the reaction solution was 50 μL, and the concentrations of L_α, C_α, L_β and C_β used in the reaction were 2 μM, 10 μM, 2 μM and 10 μM, respectively. The DNA phosphorylation and ligation processes followed the protocols provided by NEB.

Purification of the two-ring catenane rotor α/β

The purification of the two-ring catenane α/β followed the QIAGEN protocol: extraction of DNA fragments from polyacrylamide Gels. The solution that contained the catenated rings α/β was loaded on the denatured polyacrylamide electrophoresis gel (10% polyacrilamide; acrylamide/bis-acrylamide 29:1, 8.0 M urea). After electrophoresis, the gel slice that included the catenated rings was excised. The excised gel slice was incubated in the diffusion buffer (0.5 M ammonium acetate; 10 mM magnesium acetate; 1 mM EDTA, pH 8.0; 0.1% SDS) at 50 °C for 30 min. The diffusion buffer was centrifuged, and the supernatant was collected. Buffer QX1 and QIAEX II were added to the supernatant and incubated for 10 min. After centrifugation, the resulting pellet was washed with a PE buffer (80 vol.% ethanol) twice. The pellet was air-dried to yield a white color. The catenated rings were extracted from the pellet with MES buffer (50 mM, pH 7.2), and their concentration was determined spectroscopically.

Operation of the two-ring catenane rotor α/β

The dynamic reconfiguration of the two-ring catenane rotor was examined in a MES buffer solution (50 mM, pH=7.2, 500 mM $NaNO_3$, 20 mM $Mg(NO_3)_2$ at 25 °C, that included the rotor α/β (0.1 μM) and the quencher nucleic acid (**1**) (0.5 μM). The two-ring catenane system was triggered by the addition of corresponding fuel(s) and

anti-fuel(s) for the cyclic reconfiguration of the system across the three states, S_1, S_2 and S_3. The concentrations of the respective fuel and anti-fuel were added at a 1:1 ratio, and were in excess compared to the concentration of the two-ring catenane rotor. The fluorophores Cy3 and Cy5 were excited at 540 nm and 640 nm, respectively. Fluorescence measurements were performed with a Cary Eclipse Fluorimeter (Varian Inc.), and fluorescence intensities of Cy3 and Cy5 were recorded at 565 nm and 665 nm, respectively.

Synthesis of the single nucleic acid (2)-modified 10 nm-sized Au NPs

1. *Pre-functionalization of the commercial Au NPs and preparation of the dithiol DNA stock solution.*

The 10 nm-sized Au NPs, as provided by the manufacturer, were mixed with an excess of Bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) and stirred for 8 hours. The resulting Au NPs were, then, concentrated using Amicon filtering tubes 100 kDa MWCO. The concentration of the Au NPs was determined spectroscopically and the Au NPs were stored at 4°C for the further use.

The dithiolated modified DNA (**2**) was diluted to a final concentrations of 15 μM with 15 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP), respectively. The DNA solutions were stored at -20°C for the further use.

2. *Conjugation of the appropriate nucleic acid to the Au NPs.*

10 nm-sized AuNPs (3 μM) were prepared in a solution that included 0.5 × TBE, 0.4 mg/ml BSPP, 100 mM NaCl. The dithiolated DNA (**2**), 2 μM, was mixed with the AuNPs. An auxiliary strand (**2-comp**), 10 μM, was added to improve the subsequent separation of the modified AuNPs by gel electrophoresis.

3. *Purification of the single strand modified AuNPs.*

The single strand-modified AuNPs functionalized with (**2**) was separated from the polyconjugated DNA-AuNPs existing in the mixture and from unreacted AuNPs on a 3% w/v agarose gel (MetaPhor). The samples were mixed with 12 % v/v glycerol

(final concentration), and loaded on the wells of the gel. The gels were run at a constant voltage of 80 V. When satisfactory visual separation was observed, the respective bands were cut, and subjected to a voltage of 100 V inside a closed dialysis membrane filled with 0.5 × TBE buffer (in order to release the desired modified NPs from the gel). Then, the solution trapped in the dialysis membrane was collected, filtered with a 0.22 μm syringe filter (Whatman), and concentrated with a 100 kDa MWCO Amicon filtering tube.

4. *Stabilization of the single strand modified AuNPs.*

The separated single strand modified AuNPs of DNA (**2**) revealed limited stability, and precipitated with time. To eliminate this difficulty, the different single strand-modified AuNPs were stabilized with an excess of a short thiolated DNA (**STAB**) in a solution that included 100 mM NaCl, 0.5 × TBE and 0.4 mg/ml BSPP. Concomitantly, to the stabilization of the AuNPs, a fully complementary strand **2-comp***, to the auxiliary strand **2-comp**, was added in excess to the solution in order to stimulate the stand displacement process of the auxiliary stand (**2-comp**) from the single strand modifying the AuNPs. The resulting mixtures were incubated at 25°C for 48 h and then washed four times using 100 kDa MWCO Amicon filtering tubes, in order to remove (**2-comp**), and (**2-comp***). At the end, the concentration of the stabilized-single strand modified-AuNPs of DNA (**2**) was determined spectroscopically by using the appropriate extinction coefficient of the AuNPs.

5. *Electrophoretic characterization of the nucleic acid modified Au NPs and the purification and stabilization of single-nucleic acid-functionalized Au NPs.*

(a) In the first step the optimized conditions for the synthesis of the single nucleic acid (**2**)-functionalized Au NPs were identified. In the second step the single nucleic acid-modified Au NPs were separated and purified.

In the first step, the BSPP-stabilized 10 nm-sized Au NPs were functionalized with different concentrations of (**2**), according to section 2. Note that single-, double-, and triple-(**2**)-modified Au NPs can be formed. Figure S1 shows the electrophoretic

images of the resulting (**2**)-functionalized Au NPs generated in the presence of different concentrations of (**2**), lanes 2-4. Evidently as the concentration of (**2**) increases, the bands are smeared to larger molecular weight products of the modified particles. In fact, in lane 2 the nucleic acid unmodified BSPP-functionalized Au NPs are almost depleted. In lane 4, in addition to the nucleic acid unmodified BSPP-functionalized Au NP a single intense product is observed, attributed to the single (**2**)-functionalized Au NPs. This section of the gel that includes the (**2**)-modified Au NPs was cut-out, and the Au NPs were extracted, as described in section 3.

(b) The long strand (**2**)-comp assisting the separation of the Au NPs was removed, and the resulting (**2**)-functionalized Au NPs were stabilized with the short strand, **STAB** as described in section 4. For the different sequences comprising the different strands see Table S1. The gel electrophoresis of the resulting single (**2**)-functionalized Au NPs, stabilized with the short strand STAB is presented in lane 5 of Figure S1.

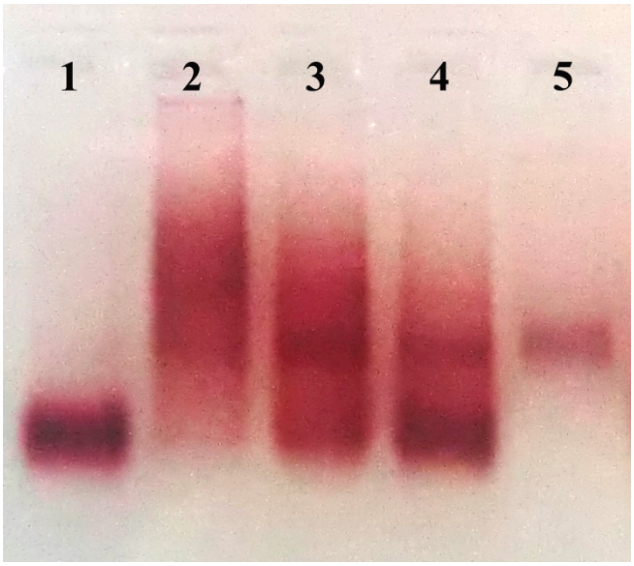

Figure S1. Lane 1 corresponds to BSPP-stabilized 10 nm-sized Au NPs. Lanes 2, 3 and 4 correspond to the BSPP-functionalized Au NPs treated with different concentrations of (**2**) (in lane 2, 3 μM; in lane 3, 2 μM; in lane 4, 1 μM). Lane 5 corresponds to the pure single nucleic acid (**2**)-functionalized 10 nm-size Au NPs.

Operation of the 10 nm-sized Au NPs-functionalized two-ring catenane rotor

The dynamic reconfiguration of the 10 nm-sized Au NPs-functionalized two-ring catenane system was examined in a MES buffer solution (50 mM, pH=7.2, 500 mM $NaNO_3$, 20 mM $Mg(NO_3)_2$ at 25 °C, that included the rotor α/β (20 nM) and (**2**)-functionalized Au NPs (20 nM). The 10 nm-sized Au NPs-functionalized two-ring catenane system was triggered by the addition of corresponding fuel(s) and anti-fuel(s) for the cyclic reconfiguration of the system across the four states, S_1, S_2 and S_3. The concentrations of the respective fuel and anti-fuel were added at a 1:1 ratio, and were in excess compared to the concentration of the two catenaned rings rotor. The fluorophores Cy3 and Cy5 were excited at 540 nm and 640 nm, respectively, using a Cary Eclipse Fluorimeter (Varian Inc.), and their fluorescence were recorded at 565 nm and 665 nm, respectively.