

Supporting information for the paper:

**Metal Nanoparticle-Functionalized DNA Tweezers: From Mechanically
Programmed Nanostructures to Switchable Fluorescence Properties**

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Materials and Methods

Materials:

Sodium chloride, ammonium acetate, Bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt and Tris(2-carboxyethyl)phosphine hydrochloride were purchased from Sigma–Aldrich. Gold colloid 5 nm and 10 nm were purchased from British Biocell. Metaphore agarose was purchased from Lonza. Dialysis tubing snakeskin was purchased from Thermo Scientific. Amicon ultra 100 kDa was purchased from Millipore. Disposable filter units were purchased from Whateman GmbH. All DNA oligonucleotides were purchased from Integrated DNA Technologies. Ultrapure water from a NANOpure Diamond (Barnstead) source was used in all experiments. Tris Borate EDTA 5X was purchased from Biological Industries Beit Haemek.

Table S1: DNA Sequences used to construct and operate the DNA tweezers.

Name	Sequence
(1)	5'-S-CCAGCGCGTGGTCCAGTGCTGCAGTG-3'
(2)	5'-CATGTACCGATAAGCGTATGCCGACC-S-3'
(2a)	5'-CATGTACCGATAAGCGTATGCCGACC-Cy3-3'
(2b)	5'-CATGTACCGATAAGCCy3GTATGCCGACC-3'
(2c)	5'-CATGTACCGATAAGCGTATGCCGACC-3'
(3)	5'-S-ATACGCTTATCGGTACATGAGCACTGCAGCACTGGACCAC-3'
(3a)	5'-ATACGCTTATCGGTACATGAGCACTGCAGCACTGGACCAC-3'
(3b)	5'-ATACGCTTATCGGTACATGAGCACTGCAGCACTGGACCAC-Cy3-3'
(4)	5'-CTGGGTCGGCGCGCTGGGTC-3'
(5)	5'-GACCCAGCGCGCCGACCCAG-3'
(6)	5'-GTACCTTGTACCTTGTACCTTGTACCTTCACTGCAGCACTGGAC CACGCGCTGGA-3'
(7)	5'-TCCAGCGCGTGGTCCAGTGCTGCAGTGAAGGTACAAGGT ACAGGTACAAGGTAC-3'
(8)	5'-GGTCGGCATACGCTTATCGGTACATGGTACCTTGTACCTTG TACCTTGTACCTT-3'
(9)	5'-AAGGTACAAGGTACAAGGTACAAGGTACCATGTACCGATAA GCGTATGCCGACC-3'
(10)	5'-GTACCTTGTACCTTGTACCTTGTACCTTGTGGTCCAGTGCTGCA GTGCTC ATGTACCGATAAG-3'
(11)	5'-CTTATCGGTACATGAGCACTGCAGCACTGGACCACAAGGTACAA GGTACAAGGTACAAGGTAC-3'
(12)	5'-S- TTTTT-3'

* All thiol- functionalized DNA sequences contain a dithiol group.

Methods

Synthesis of nucleic acid functionalized Au NP

Au NPs 5nm and 10 nm were modified with one – or two nucleic acids, by implementing the reported method with some modifications⁶⁰. The synthesis and separation of the modified NPs are presented in Figure S1. The synthesis of the nucleic acid functionalized Au NPs involves:

Step 1 - Pre-functionalization of the commercial Au NPs and the deprotection of the thiolated nucleic acid for the conjugation reactions;

Step 2 - The conjugation of the appropriate nucleic acids to the Au NPs;

Step 3 - The purification of the single/double nucleic acid-functionalized Au NPs;

Step 4 - Stabilization of the single/double nucleic acids modified Au NPs;

Step 5 - Assembly of the final Au NP/Cy3 modified DNA tweezers.

Step 1- Pre-functionalization of the commercial Au NPs and the deprotection of the thiolated nucleic acid for the conjugation reactions.

5 and 10 nm Au NPs, as provided by the manufacturer, were mixed with an excess of Bis(*p*-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP) and stirred overnight. The resulting Au NPs were, then, concentrated using Centricon filtering tubes 100kDa, for 5 min under 3000g to final concentrations of 1-10 μ M (If necessary, a second concentration step was performed using Centricon filtering tubes). The concentration of the Au NPs was estimated by diluting the resulting solution (1:1000), recording the absorption spectrum and using the reported extinction coefficient of the respective Au NPs. The resulting Au NP solution was stored at +4°C and used for several weeks.

Dithiol (two –SH groups) protected DNA sequences functionalized at their 3' or 5' end were diluted to the final concentrations of 15 μ M with 15 mM Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) (added to preserve the thiol in the reduced form). The DNA solutions were stored at -20°C and used for several weeks.

Step 2- Conjugation of the appropriate nucleic acids with the Au NPs

- 5 nm NPs (ca. 3 μ M) were prepared in a solution that included 0.5 X TBE, 0.4 mg/ml BSPP, 100 mM NaCl. The thiolated DNA sequence (**3**) was mixed with the Au NPs (molar ratio 1:1 for the single modified Au NPs and 2:1, respectively, for the double modified Au NPs). An appropriate, longer, partly complementary strand (**10**) was added in excess to provide higher resolution in the separation step. The mixture was, then, incubated for 12 hours at room temperature.
- 10 nm nanoparticles (ca. 0.5 μ M) were prepared in a solution that included 0.5 X TBE, 0.4 mg/ml BSPP, 100 mM NaCl. The thiolated DNA {(1) or (2)} was mixed with the Au NPs (molar ratio 1:1 for the single modified Au NPs and 2:1, respectively, for the double modified Au NPs). An excess of a partly complementary strand {(6) or (8), respectively,} was added to provide higher resolution in the separation step. The mixture was, then, incubated for 12 hours at room temperature.

Step 3- Purification of the single/double nucleic acid-functionalized Au NPs

The mono-or bis-conjugated Au NPs were separated from the polyconjugated DNA-Au NP mixture on a 3% w/v agarose gel (MetaPhor). The different samples were mixed with 12 % v/v glycerol (final concentration), and loaded in the wells of the gels. The gels were run at 80 V, constant voltage. When satisfactory separation was observed, the bands of interest were cut and subjected to a voltage of 100 V inside a closed dialysis membrane filled with 0.5 X 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 μ m syringe filter, and concentrated with a 100 kDa Centricon filtering tube.

Step 4-Stabilization of the single/double nucleic acid modified NPs

The mono- or bis-conjugated Au NPs were stabilized with an excess of a short thiolated DNA strand (**12**) in a solution that included 100 mM NaCl, 0.5X TBE and 0.4 mg/ml BSPP. A DNA sequence {(7), (9) or (11)} was added in excess to the respective solution in order to allow the strand-displacement process of the auxiliary DNA strand, respectively from the conjugated Au NPs. The resulting mixtures were incubated at 25°C for 48h and then washed four times using 100 kDa Centricon filtering tubes 5' x 3000g, diluting the samples to 15 ml in water each time. At the end, the stabilized mono- or bis-conjugated nanoparticles were

diluted (1:400) and their concentrations were determined by recording their absorption spectra and using the appropriate extinction coefficients.

Step 5- Assembly of the final Au NP/Cy3 modified DNA tweezers.

The resulting stabilized Au NPs modified with the appropriate nucleic acids were added to a TBE buffer solution (0.1 X TBE, 0.4 mg/ml BSPP, 500 mM NaCl) in order to assemble the desired tweezers nanostructure for a time interval of 12 hours. The nanostructure was separated from the excess DNA-Au NP mixture on a 3% w/v agarose gel (MetaPhor). The sample was mixed with 12 % v/v glycerol (final concentration), and loaded in the wells of the gel. The gel was run at 50 V, constant voltage, at 10 °C. When satisfactory separation was observed, the bands of interest were cut and added to a TBE buffer solution (0.1 X TBE, 0.4 mg/ml BSPP, 500 mM NaCl) in order to let the nanostructure diffuse out from the gel. Then, the solution was concentrated with a 100 kDa Centricon filtering tube.

Figure S1: Synthesis and purification of the DNA-Au NP hybrids and assembly of the final tweezers

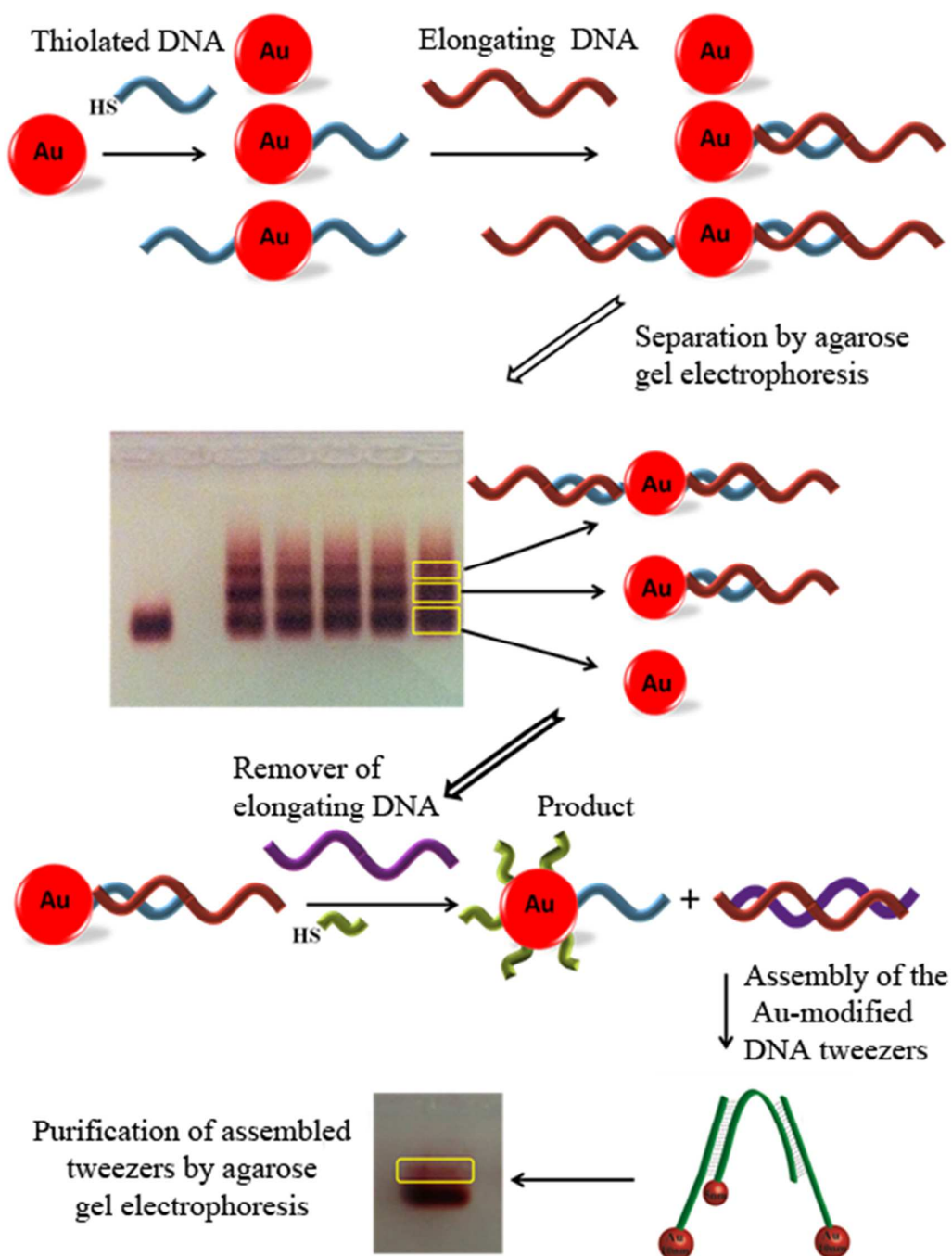


Figure S1: Preparation of the single nucleic acid-modified Au NPs, double nucleic acid-modified Au NPs and their electrophoretic separation and purification.

Fluorescence experiments where fluorophore/Au NP-functionalized tweezers systems were used.

Fluorescence measurements were performed in a 100 μ l quartz cuvette (Hellma Analytics) using a Cary Eclipse Varian. The systems included a solution composed of 20 nM of the DNA tweezers machine containing: 10 nm sized monoconjugated Au NPs and Cy3 modified nucleic acids (**2a**, **2b** or **3b**) in a TBE buffer solution (0.1 X TBE, 0.4 mg/ml BSPP, 500 mM NaCl). For the operation of the DNA tweezers machine the appropriate fuel/anti-fuel strands were added in excess (20-fold excess compared to the tweezers concentration). All fluorescence experiments were performed at 25°C using a temperature control unit. The fluorophore (Cy3) was excited at 540 nm, and the emitted fluorescence was recorded at 564 nm.

STEM experiments

Assemblies of the Au NPs/DNA tweezers machine for STEM analyses were prepared in a solution containing 0.4 mg/ml BSPP, 0.1X TBE, 500 mM NaCl. The resulting nanostructures were diluted to a final concentration of 1nM in a 200 mM NaCl solution, and deposited on the copper grids. The drop was dried at air, at room temperature for two hours. Pictures were taken with an Extra High Resolution Scanning Electron Microscope *Magellan(TM) 400L* with scanning-transmission electron microscopy detector. Settings: 20-27 kV, 0.1-0.2 nA.

Figure S2. Representative large area domains of STEM images consisting of the three Au NP-tweezers structure (bar= 100 nm). Red triangles indicate the closed tweezers structure, the blue triangle points to a “wrong” open configuration.

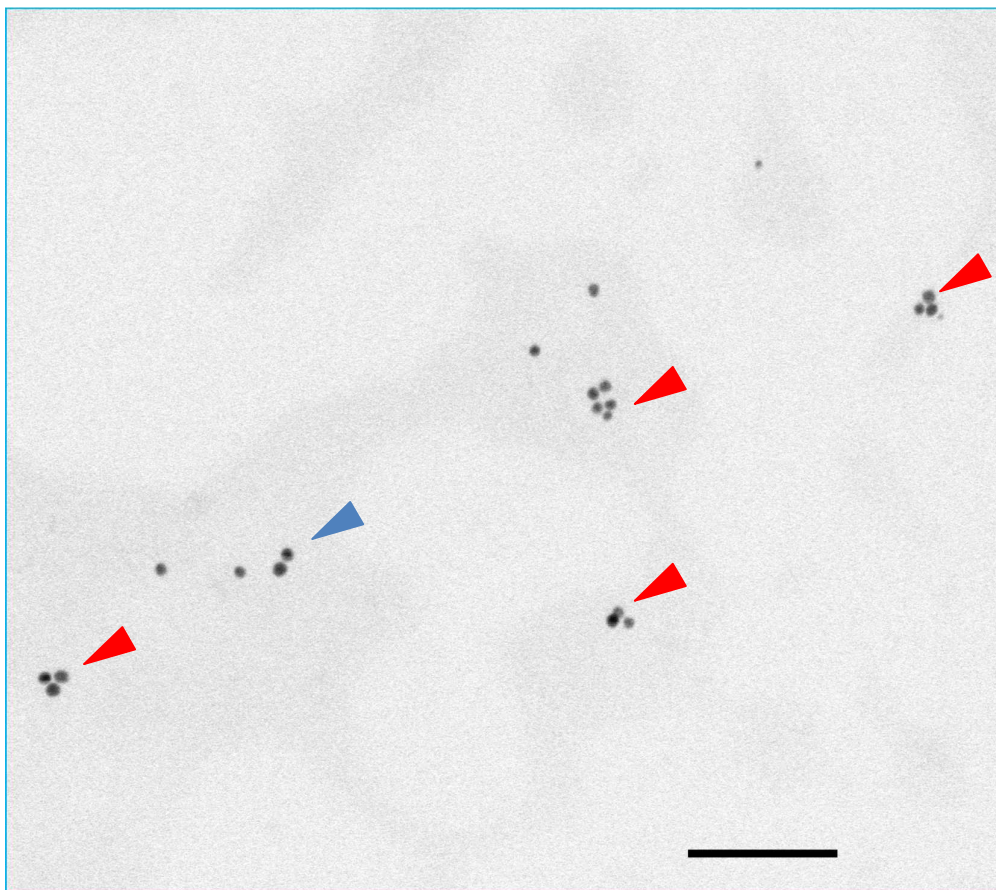


Figure S3. Representative large area domains of STEM images consisting of the three Au NP-tweezers structure (bar = 100 nm). Red triangles indicate the closed tweezers structure, the blue triangle points to a “wrong” open configuration.

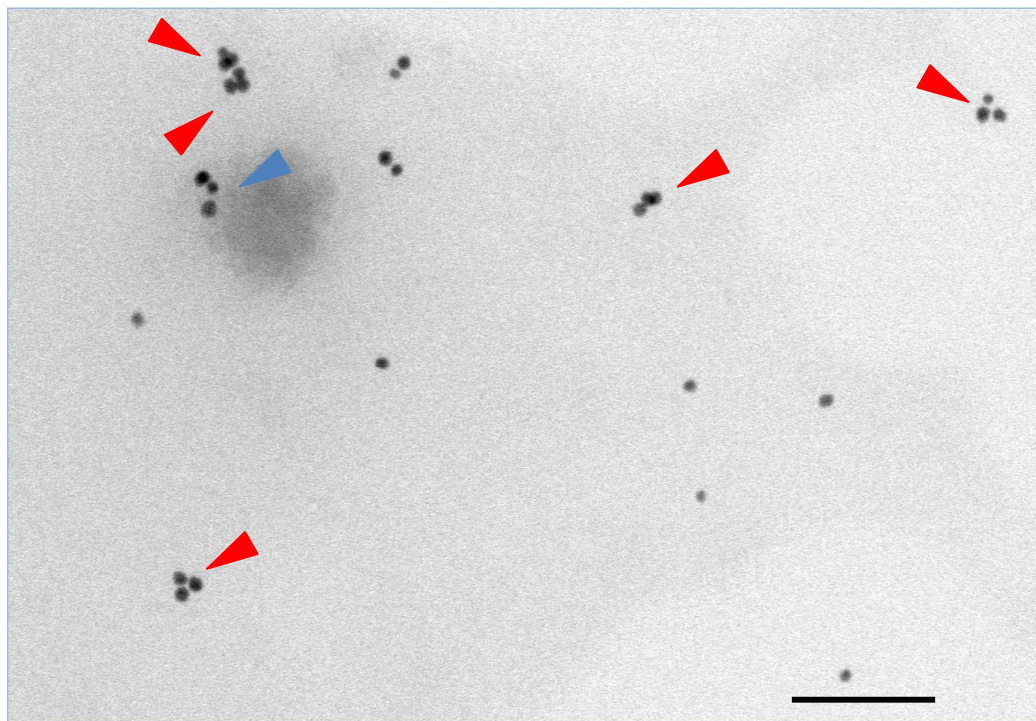


Figure S4. Representative large area domains of STEM images consisting of the three Au NP-tweezers structure (bar = 100 nm). Red triangles indicate the closed tweezers structure.

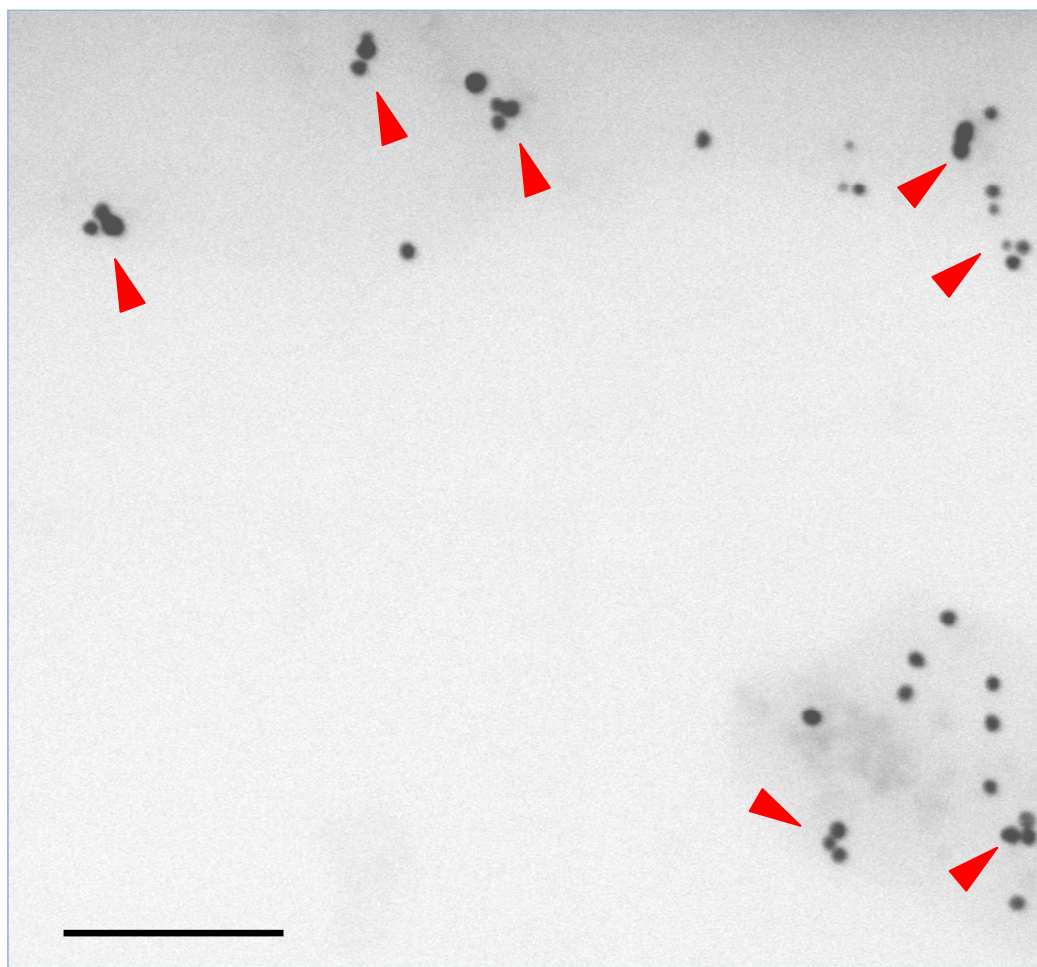


Figure S5. Representative large area domains of STEM images consisting of the open three Au NP-tweezers structure (bar = 100 nm). Blue triangles indicate the open tweezers structure.

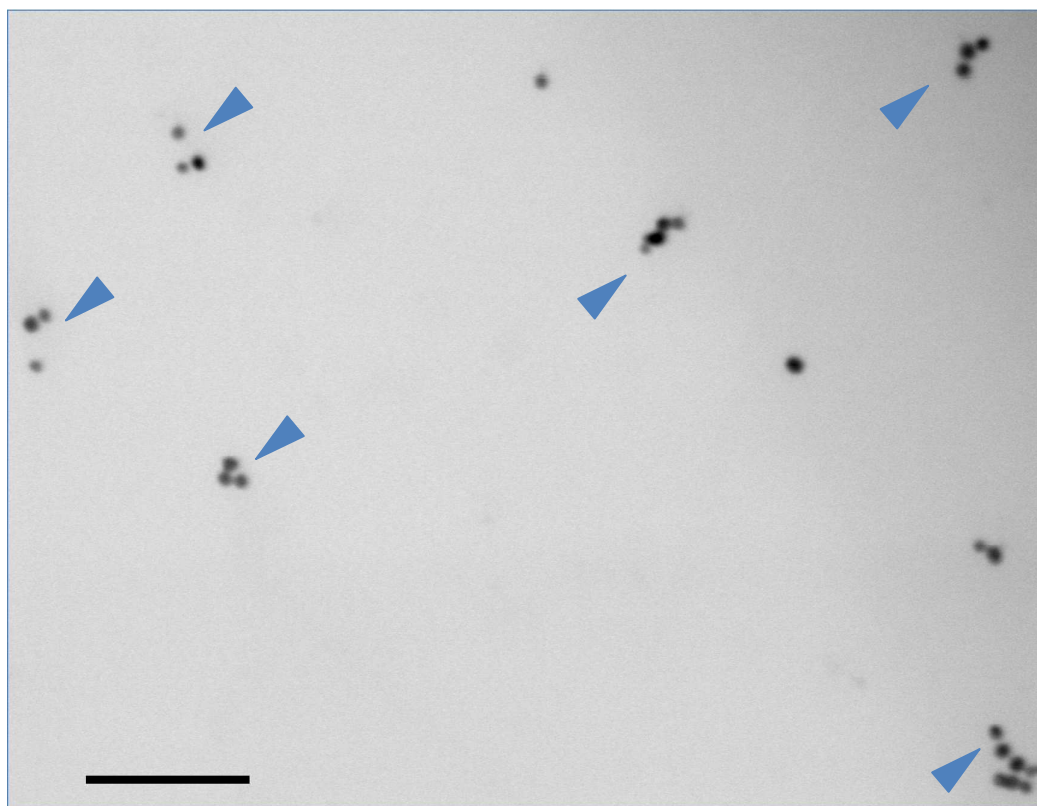


Figure S6. Representative large area domains of STEM images consisting of the open three Au NP-tweezers structure (bar = 100 nm). Blue triangles indicate the closed tweezers structure.

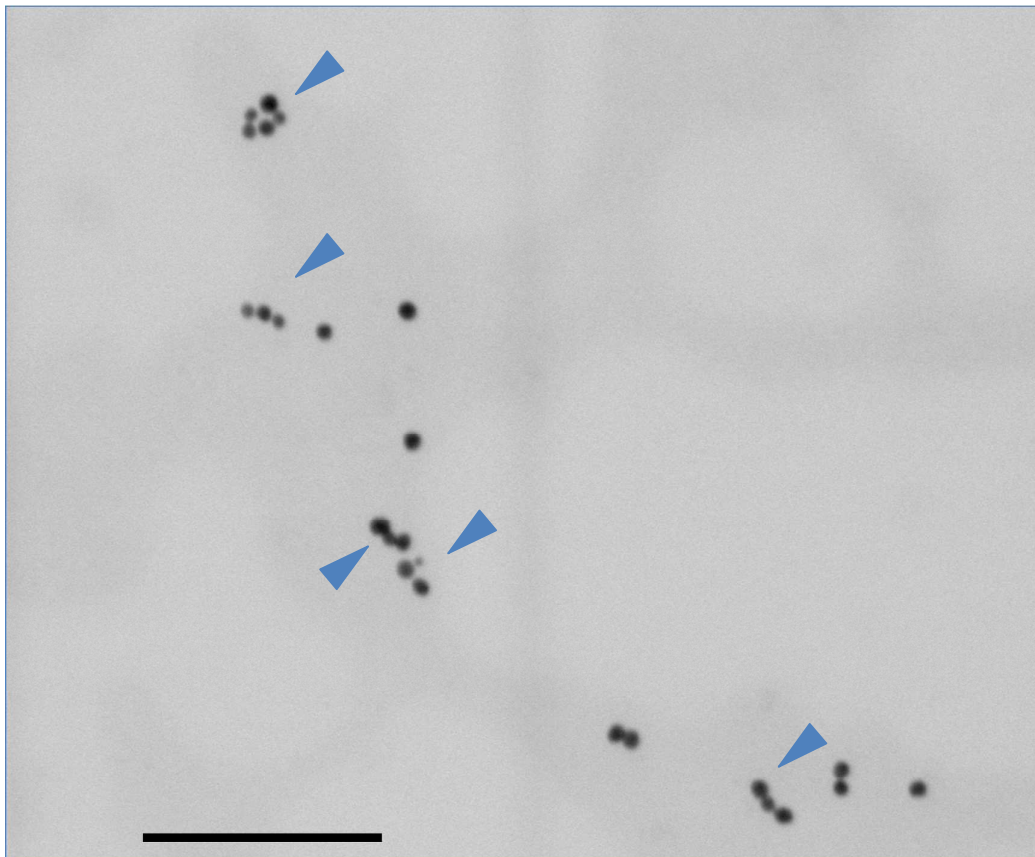


Figure S7: (A) Schematic opening and closure of a double tweezers containing a Au NP and two fluorophore units using fuel/antifuel DNA strands. (B) Time dependent fluorescence changes corresponding to the cyclic opening and closure of the tweezers structure: (a) high fluorescence, triggered closed configuration; (b) low fluorescence, triggered open configuration.

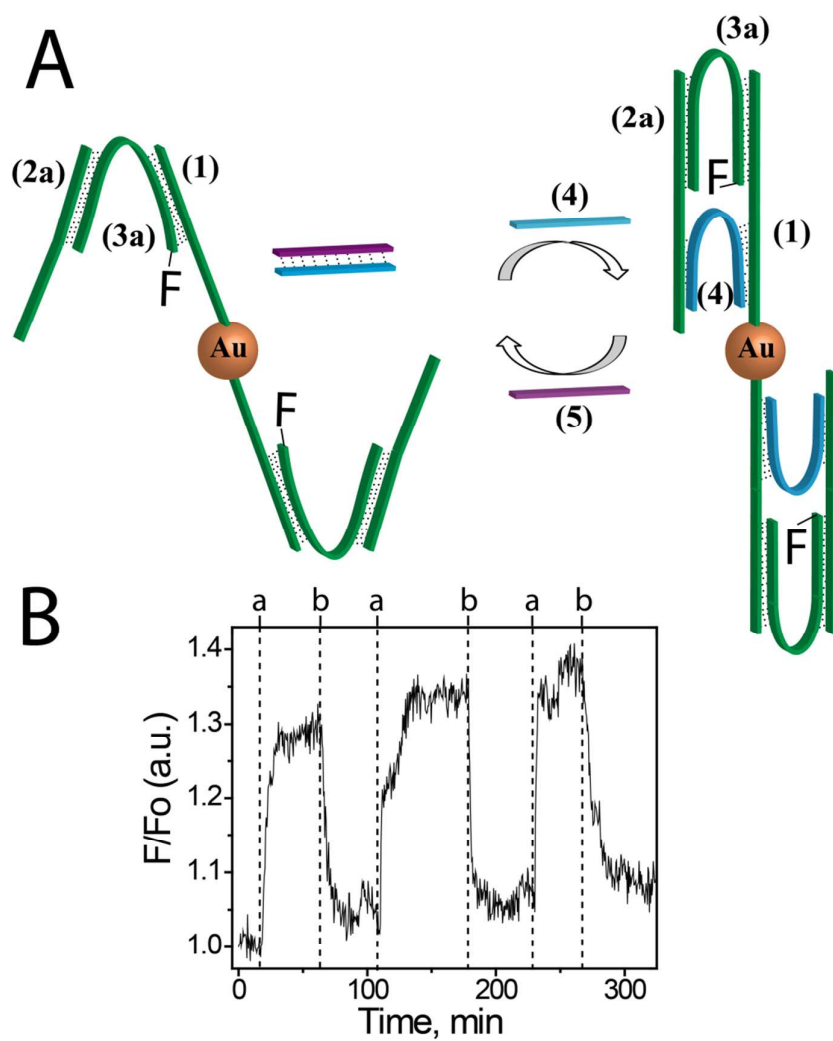
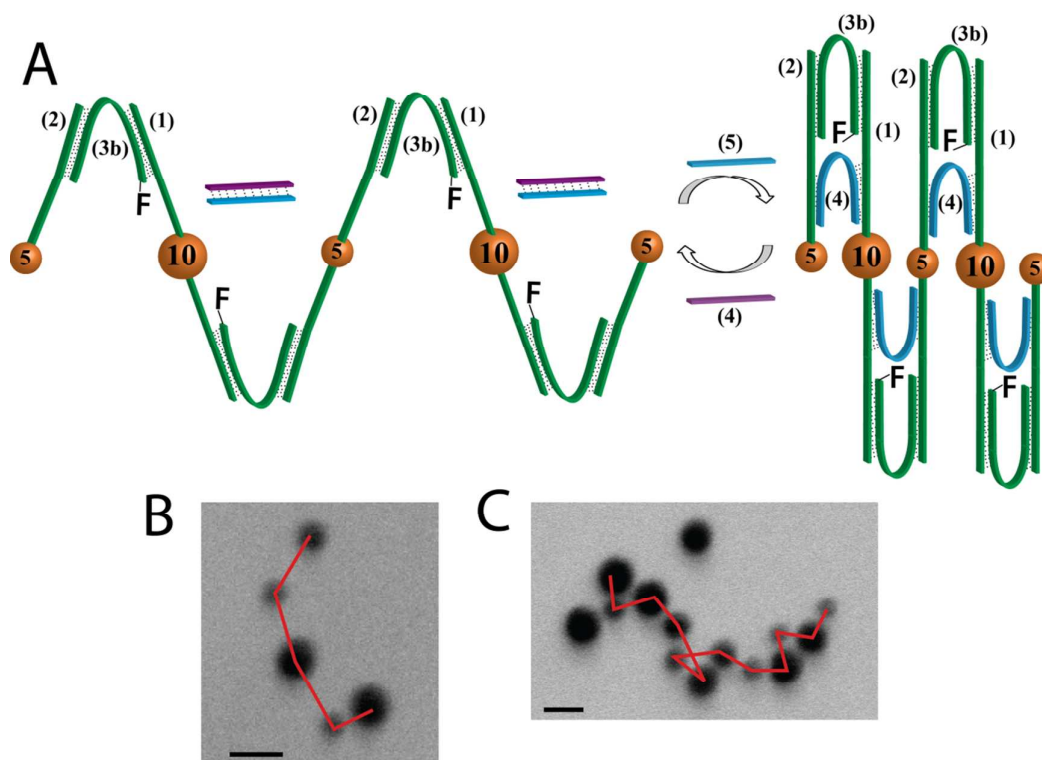


Figure S8 (A) Oligomeric nanostructures of Au NP-crosslinked multi tweezers; (B) A representative STEM image of the open tweezers oligomer; (C) a representative STEM image of the closed tweezers oligomer (insets B, C, bar = 10 nm).



Fluorescence studies on the Tweezers system consisting of a Au NP (10nm) and the fluorophore Cy3. Switching fluorescence properties by the dynamically controlled opening and closure of nanostructures T1, T2 and T3

a) In order to simulate theoretically the fluorescence features of Cy3 in different configurations of the Au NP(10nm)/Cy3 tweezers systems, we had to estimate the fluorescence quantum yield of Cy3 in the Au NP-free Cy3-modified tweezers systems. We find that while Cy3 exhibits in its free state in water a quantum efficiency of 15%, the introduction of salts, buffer composition, binding to DNA, assembling of the Cy3-functionalized DNA tweezers and the specific modification site in respect to the DNA structure (internal or 3' terminal) have all effects on the resulting quantum yield of Cy3. Table S2 summarizes the fluorescence efficiencies of Cy3 subjected to the different environments. One might realize that Cy3 bound to the 3' end of a nucleic acid component of the DNA tweezers system and in the presence of the environmental conditions (buffer, salt, etc.) exhibits a quantum yield efficiency of ca. 1%, which is further decreased when using an internal Cy3 modification⁵⁷.

b) We then attempted to theoretically calculate the fluorescence quantum yield of Cy3 as a function of the distance separating the fluorophore from the NP surface. This calculation followed the procedure provided in reference⁵⁶.

Any effort to correlate the experimental results and the theoretical calculation should be considered with cautions:

- We use 10 nm sized NPs. Nonetheless, STEM analysis of the NPs indicates the existence of 8, 9 and 10 nm NPs.
- The calculation requires the assumption of a preferred orientation of the dipole of the fluorophore in respect to the NP surface, or the use of an averaged orientation. Using an averaged orientation and a quantum yield of 1%, we do not find any distance domain (for 10 nm NPs), where enhanced fluorescence is observed.

On the other hand, if we assume a fixed perpendicular orientation of the fluorophore in respect to the NPs' surface, we find that the couple of Au NP/Cy3 shows, at distinct distances, enhanced fluorescence and quenching. Figure 2 (CI-II-III) shows the calculated distance dependent quantum efficiencies for the different tweezers structures (T1, T2 and T3). In these calculations a reference quantum

efficiency of 1% for Cy3 was used in tweezers T1 and T3 (Cy3 at the 3' end of the respective nucleic acid **2a** and **3b**), while a 0.5% reference quantum efficiency was used for the theoretical model of T2 (Cy3 as internal modification of strand **2b**). One may realize that for all three sized NPs, spatially-separated regions for quenching or enhanced fluorescence are observed.

The boundary conditions for the orientation of the fluorophore should be, however, addressed and possibly supported. A literature search reveals that Cy3 undergoes specific stacking interaction with duplex DNA structures.^{61,62} Such stacking phenomenon might be the origin for a fixed preferred orientation of the fluorophore in respect to the NP's surface.

Several conclusions may be extracted from the calculated curves of Figure 2(CI-II-II):

Figure 2(CI) and 2(CIII):

- From a 10 nm Au NP and Cy3 as fluorophore, under the specific environmental conditions, assuming a 1% reference quantum efficiency, the maximum enhancement in the fluorescence quantum yield can be ca. 20% at a separation distance of 8 nm.
- At separation distances >8 nm or <8 nm the fluorescence quantum efficiency declines and while a constant fluorescence quantum efficiency (plateau) is observed at longer distances, the fluorescence quantum efficiency is quenched, versus the reference value, at distances <5.4nm.

Figure 2(CII):

- Assuming a reference quantum efficiency of 0.5%, due to the presence of the fluorophore (Cy3) as an internal modification of nucleic acid **2b**, the calculated curve shows quenching and enhancing regions shifted to shorter distances as opposed to the calculated model with 1% reference quantum efficiency.

Table S2. Estimated relative fluorescence efficiencies of Cy3 in the different environments.

Medium composition	Relative fluorescence efficiency of Cy3
H ₂ O	0.1500
H ₂ O; NaCl	0.0941
Tris/Borate/EDTA; bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt	0.1041
Tris/Borate/EDTA; bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt; NaCl	0.0738
Phosphate buffer	0.0396
Phosphate buffer; NaCl	0.0209
Tris/Borate/EDTA; bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt; NaCl; DNA tweezers (T1 & T3)	0.0102
Tris/Borate/EDTA; bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt; NaCl; DNA tweezers (T2)	0.0054

Supplementary References

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61. Spiriti, J.; Binder, J. K.; Levitus, M.; van der Vaart, A. *Biophys. J.* **2011**, *100*, 1049.
62. Norman, D. G.; Grainger, R. J.; Uhrin, D.; Lilley, D. M. J. *Biochemistry.* **2000**, *39*, 6317.