Supplementary information for

DNA hybridization as a general method to enhance the cellular uptake of nanostructures

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I. DNA sequences used

We benchmarked the DNA sequences used against following criteria in our selection process: 1. The DNA should ideally be comparatively short without too many guanosine units to warrant a high yield achievable using a solid phase DNA synthesizer; 2. A short linker motif was introduced to the DNA sequence to separate the NP surface from the binding sequence and to enable flexibility for facilitated strand hybridization; 3. Self-complementarity in each strand was avoided. 4. T_m is at least several degrees higher than 37 °C to allow strong binding. This was predicted by the software "OligoCalc". 5. The number of lipid tails was optimized by us in an earlier work and we noticed a higher cellular toxicity when more lipid tails were employed. Thus, we her used 4 lipid-modified nucleobases. Detailed information of all DNA used in this work is listed in **Table S1**.

Name	Sequences (5´ to 3´)	Modification
A	**GCGGATTCGTCTGC**TTT TTT T ACA TTC CTA AGT CTGAAA CAT TAC AGC TTG CTA CAC GAG AAG AGC CGC CAT AGT A	6-FAM at 5´
B	**GCGGATTCGTCTGC**TTT TTT T TAT CAC CAG GCA GTTGAC AGT GTA GCA AGC TGT AAT AGA TGC GAG GGT CCA ATA C	None
C	**GCGGATTCGTCTGC**TTT TTT T TCA ACT GCC TGG TGATAA AAC GAC ACT ACG TGG GAA TCT ACT ATG GCG GCT CTT C	None
D	**GCGGATTCGTCTGC**TTT TTT T TTC AGA CTT AGG AATGTG CTT CCC ACG TAG TGT CGT TTG TAT TGG ACC CTC GCA T	None
CrU4T	GCAGACGAATCCGC**UUUU**	None
U4T	**UUUU**GCGGATTCGTCTGC	None
CU4T	**UUUU**GCAGACGAATCCGC	None
Cr	GCAGACGAATCCGC	None
C-FAM	GCGGATTCGTCTGC	6-FAM at 5´
thiol-U4T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCGGATTCGTCTGC	thiol C6 at 5´
biotin-U4T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCGGATTCGTCTGC	biotin at 5´

Table S1. Sequences and modifications of DNA used in this work. **U** represents dodecyne modified deoxyuridine nucleotide. Sequences in red are free overhangs of DNA tetrahedron. Sequences highlighted are linkers of the sequences.

II. Lipid DNA synthesis and characterization

5-(dodec-1-ynyl) deoxyuracil and 5-(dodec-1-ynyl) deoxyuracil phosphoramidite were synthesized as follows. In short, the modified uracil phosphoramidite was dissolved in acetonitrile to reach a concentration of 0.15 M, in the presence of 3 Å molecular sieves. Then the prepared solution was directly connected to a DNA synthesizer (ÄKTA OligoPilot Plus, GE Healthcare (Uppsala, Sweden)). Oligonucleotides were synthesized on a 50 μmol scale using standard β-cyanoethylphosphoramidite coupling chemistry. Deprotection and cleavage from the PS support was carried out by incubation in concentrated aqueous ammonium hydroxide solution overnight at 65 °C. Following deprotection, the oligonucleotides were purified by using reverse-phase chromatography, using a C15 RESOURCE RPC 3 mL reverse phase column (GE Healthcare) through a custom gradient elution (A: triethylammonium acetate (TEAAc, 100 mM) and acetonitrile (5%), B: TEAAc (100 mM) and acetonitrile (65%)). Fractions were desalted using centrifugal dialysis membranes (MWCO 3000 $g{\cdot}mol^{-1}$). Oligonucleotide concentrations were determined by UV absorbance using extinction coefficients. Finally, the successful synthesis of the lipid DNA was confirmed by MALDI-TOF mass spectrometry.

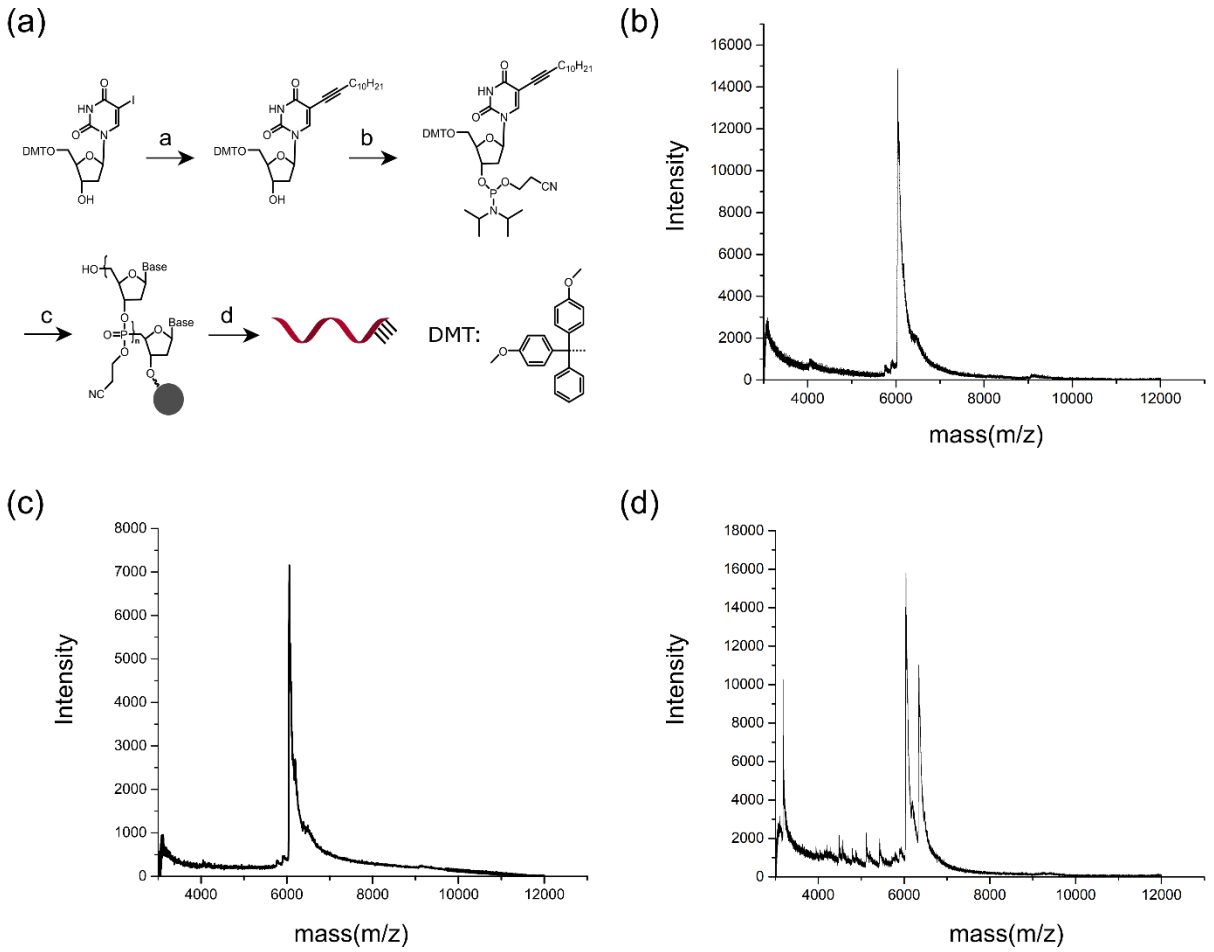

Fig. S1. (a) Synthetic steps towards lipid DNA. a: Dodec-1-yne, $Pd(PPh_3)_4$, CuI, iPr_2NH, DMF, RT; b: 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite, DIPEA, CH_2Cl_2, RT; c: DNA synthesizer; d: Purification. (b) MALDI-TOF mass spectra of U4T (calcd.: 6033 $g \cdot mol^{-1}$, found: 6050 $g \cdot mol^{-1}$), (c) MALDI-TOF mass spectra of CU4T (calcd.: 6020 $g \cdot mol^{-1}$, found: 6075 $g \cdot mol^{-1}$) and (d) MALDI-TOF mass spectra of CrU4T (calcd.: 6020 $g \cdot mol^{-1}$, found: 6038 $g \cdot mol^{-1}$).

III. Anchoring lipid DNA on the cellular membrane

To confirm anchoring and hybridization of CrU4T on the cellular membrane, HeLa cells were seeded in a µ-Slide 8 well at a density of 6×10^4 per well and grown for 24 h. Then, 300 µL of CrU4T (5 µM) was added to the cells and incubated for 20 min. After rinsing three times with PBS buffer, cells were further incubated with 300 µL C-FAM (5 µM) for 15 min. Images were acquired on a confocal laser scanning microscope (STP8, Leica) and analysed by ImageJ.

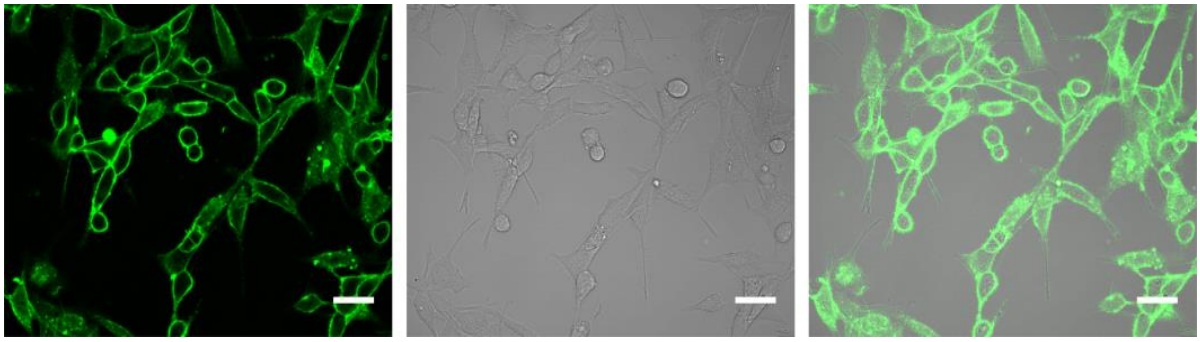

Fig. S2. CLSM micrograph of C-FAM distribution on CrU4T anchored HeLa membranes. C-FAM incubation time with CrU4T anchored HeLa was 15 min. Green: C-FAM. Scale bar: 40 µm.

IV. DNA tetrahedron nanostructure degradation in cell culture medium

10 µL fresh undiluted FBS was added to 80 µL DMEM. To this solution, 10 µL DNA tetrahedron solution (100 µM) was added. After incubation at 37 °C for 0, 1, 2, 5, 12, and 24 h, 6 µL aliquot were taken from each sample and run on 2% agarose gel at 100 V for 30 min.

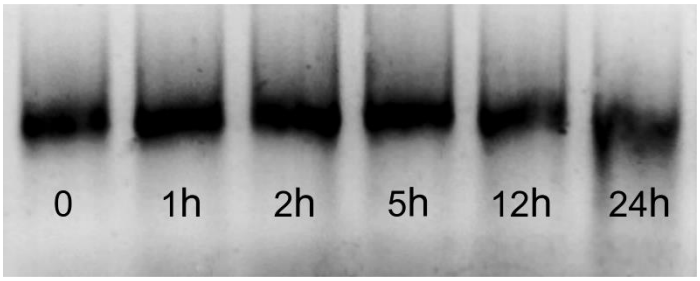

Fig. S3. Electrophoretic analysis of the DNA tetrahedral nanostructure stability in cell culture media for different periods of time.

V. AuNP synthesis, conjugation, and characterization

AuNPs were synthesized by reduction of tetrachloroauric acid ($HAuCl_4$) with trisodium citrate. Briefly, 225 mL of $HAuCl_4$ (1 mM, 88.61 mg) in Milli-Q water were placed into a round bottom two-neck flask. Then the solution was heated to reflux. Afterwards, 25 mL of 38.8 mM trisodium citrate (285 mg) was quickly added to the solution and allowed to reflux for 30 min with strong agitation. Subsequently, heating was stopped, and the system was cooled down to room temperature under stirring. Concentration of Au solution was determined by absorption at 520 nm with a corresponding molar absorptivity of 2.33×10^8 $M^{-1}cm^{-1}$.

To conjugate thiol-U4T to the AuNP surface, 300 µL AuNP solution (33.3 µM) were mixed with 12 µL thiol-U4T (200 µM in Milli-Q water) for 10 min at room temperature. Then 108.3 µL 100 mM Tris buffer (pH = 3) were quickly added and incubated at room temperature for 60 min. After that, the solution was subjected to 30 min centrifugation (15000 rpm, 4 °C). Then, the supernatant was removed, and AuNP pellets were rinsed three

times with PBS buffer to remove any unconjugated thiol-U4T. Finally, AuNPs pellets were redispersed in 1 mL PBS buffer.

To quantify the amount of thiol-U4T on the AuNPs surface, 5 µL AuNP solution was diluted with 90 µL Milli-Q water and then mixed with 5 µL DTT solution (1 M in Milli-Q water). After incubation at 60 °C for 1 h, the solution was centrifuged for 30 min (15000 rpm, 4 °C) and UV-Vis absorption of the supernatant at 260 nm was measured to calculate the amount of thiol-U4T conjugated on the AuNP surface.

For TEM measurements, 5 µL AuNP solution was deposited on a glow-discharged holey carbon coated grid and dried overnight. The morphology was recorded by Libra 120 Transmission Electron Microscope (Carl Zeiss, Germany) with 120 kV accelerating voltage.

For DLS measurements, AuNPs solutions (with or without conjugated thiol-U4T) were filtered with a 0.45 µm syringe filter and measured with a Zetasizer Ultra (Malvern Instruments) at T = 25 °C and the diameters were averaged from number distributions of three measurements.

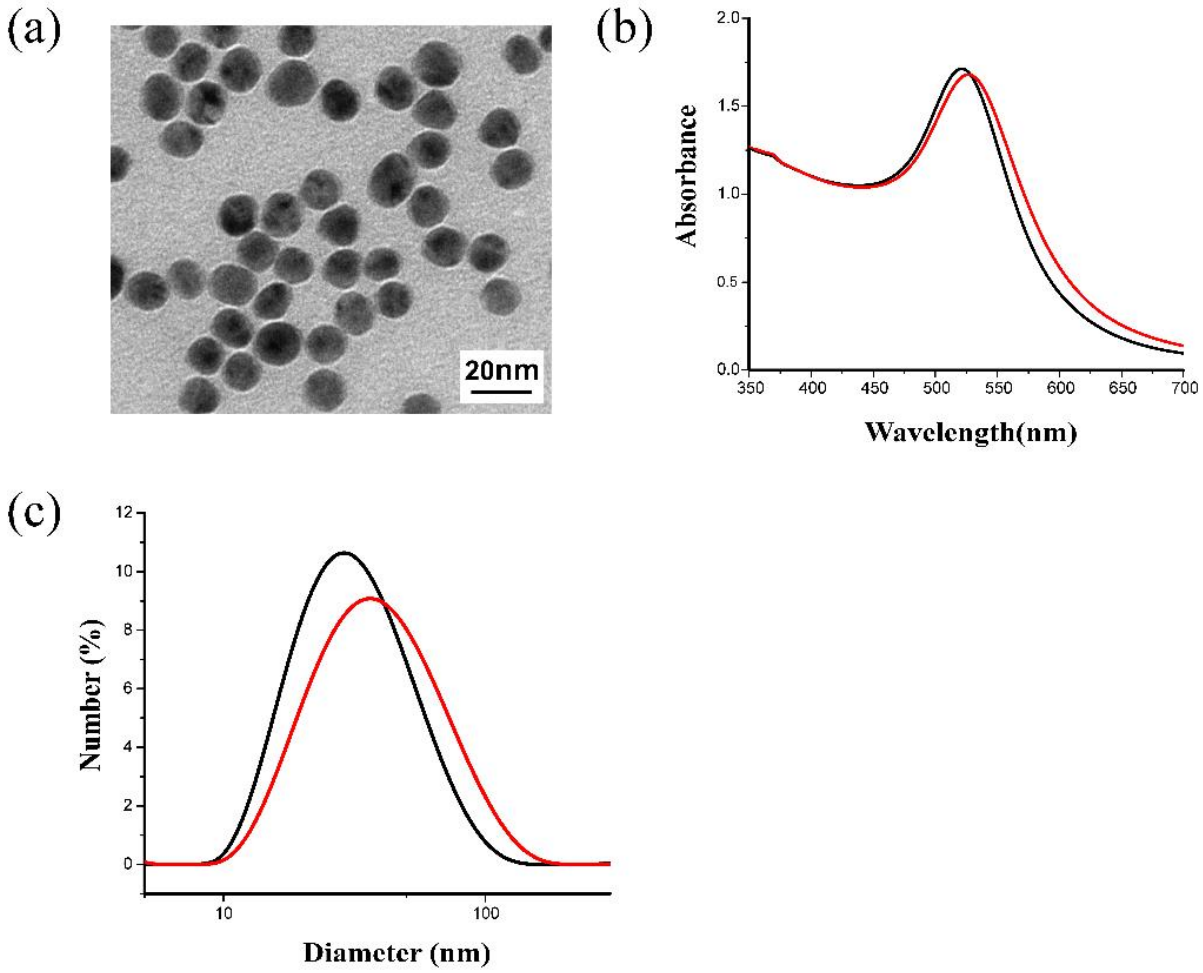

Fig. S4. Characterization of AuNPs. (a) Representative TEM image showing diameter and spherical morphology of AuNPs. (b) UV-Vis absorption spectra of AuNPs before and after DNA conjugation; Black: before; red: after. (c) DLS histogram comparison between bare AuNPs and DNA-conjugated AuNPs. Black: bare AuNPs; red: DNA-conjugated AuNPs.

VI. Quantification of cellular AuNPs by ICP-MS

HeLa cells were seeded in the 12 well plate and grown for overnight. 500 µL U4T (10 µM), LR (10 µM) or cell culture media were incubated with cells for 20 mins. Afterwards, 400 µL AuNPs in cell culture media (37.5 nM) were added and incubated with cells for 18 h. Then, the solution was removed, and the remaining cells were carefully washed 3 times with PBS to remove AuNPs that were not internalized. 400 µL of cell lysis buffer (1x) was then added to each well for 20 mins incubation and the plate was maintained at -20 °C for further 20 mins. then, the cells in one well were totally transferred to metal ion-free glass tube by scratch, and gold was then dissolved by 600 µL freshly prepared Aqua Regia solution (HCl: HNO_3= 1:3, V/V) for 15 min. Then, 10 mL MQ water was added and the content of gold in the samples was analyzed by ICP-MS.

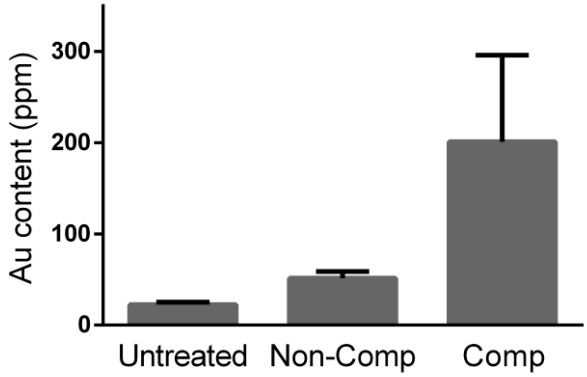

Fig. S5. Determination of internalized AuNPs by ICP-MS. Error bars indicate SD from the mean (*N* = 3). Untreated: cells were not treated with lipid DNA; Non-Comp: cells were anchored with LR; Comp: cells were anchored with U4T.

VII. PSNP characterization

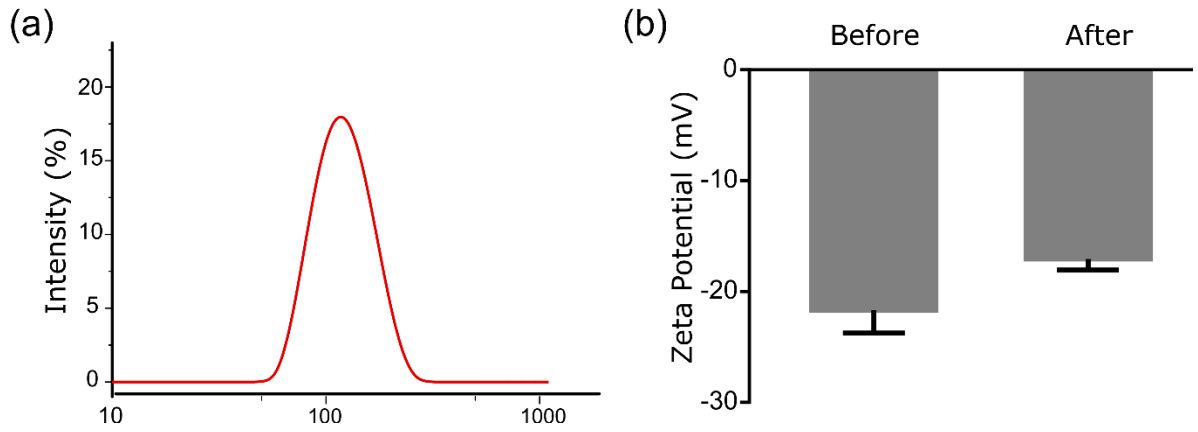

Fig. S6. Characterization of PSNPs. (a) DLS distribution of PSNPs. (b) Zeta potential of PSNPs before and after DNA conjugation.