

Supplementary Information

for

Selective Dehybridization of DNA-Au Nanoconjugates Using Laser Irradiation

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1. Characterization of Au nanostructures

The size distributions of these particles are shown in Fig. S1; the diameter is 24 ± 4 nm for AuNP and 253 ± 5 nm for AuNS, as measured by the TEM and processed by Image J software (as shown in Figure 1 of the main text).

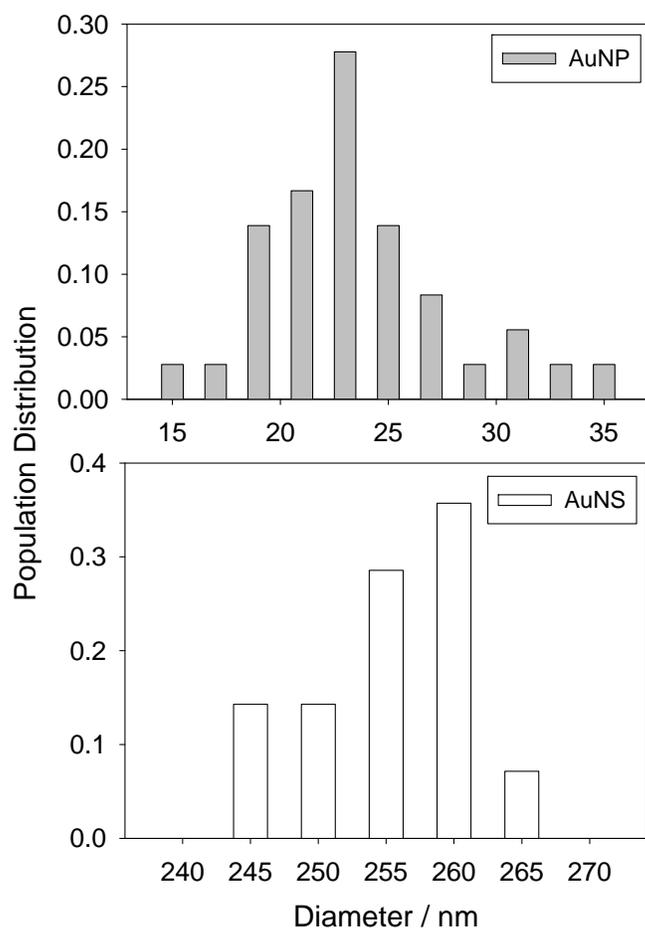


Figure S1. Size distribution of AuNP (a) and AuNS (b) as obtained by using Image J software to analyse the respective TEM images. AuNSs are very uniform (253 ± 5 nm) while AuNPs had a slightly broader size distribution (24 ± 4 nm).

2. Preparation and purification of dsDNA modified AuNP and AuNS

In order to reduce the disulfide bonds and generate thiol-terminated ssDNA, 20 μ L of 100 μ M DNA oligonucleotide was treated with 20 μ L of 10 mM tris(2-carboxyethyl) phosphine hydrochloride (TCEP, Aldrich-Sigma) in phosphate buffered saline (PBS: 100 mM NaCl, 10 mM phosphate buffer, pH=7.4) at room temperature for 4 hours. The function of TCEP is to cleave the disulfide bond. This solution was then desalted in an illustra MicroSpin G-25 Column (GE Healthcare, Buckinghamshire, UK). This G-25 column is used to separate DNA from the cleaved DNA labeling reagents;² it contains Sephadex™ G-25 DNA grade F resin, which allows DNA purification via gel filtration. Molecules larger than the largest pores in the Sephadex are

excluded from the gel and elute first (purified DNA oligonucleotide). Intermediate size molecules penetrate the matrix to varying extents, depending on their size and the resin used. Penetration of the matrix retards progress through the column; very small molecules elute last (excess TCEP and HS-(CH₂)₆-OH). To purify DNA, first the resin in the column was suspended by vortexing, followed by centrifuging the column for 1 minute. The DNA solution was carefully loaded to the top-center of the resin, and then centrifugation (3000 rpm, 735g) was carried again for 2 minutes to elute.

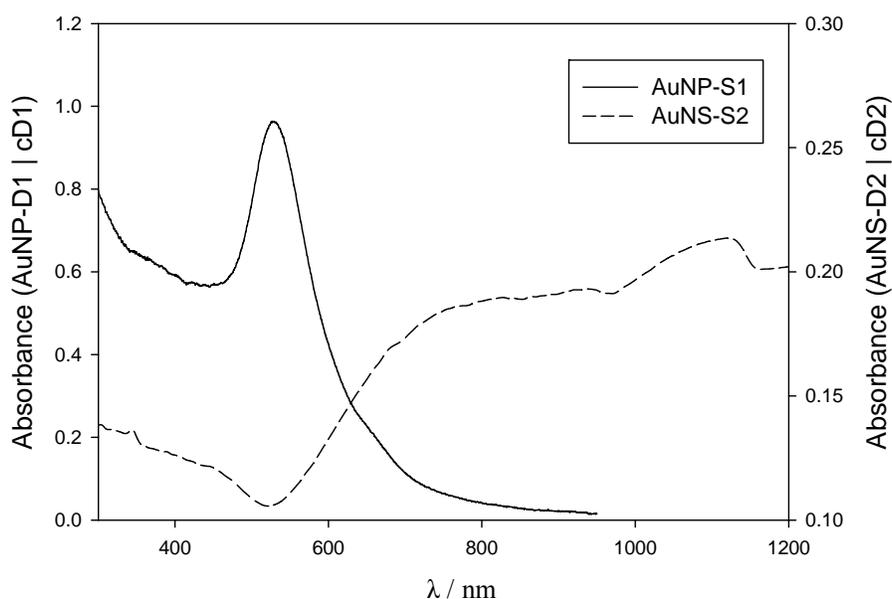


Figure S2. Optical absorbances of typical samples of AuNP-D1|cD1 (solid line) and AuNS-D2|cD2 (dotted line).

The purified thiol-terminated ssDNA strands were then hybridized with 2 nmol of their complementary strands by heating at 85 °C for 5 minutes and allowed to slowly cool to room temperature in 1 hour. Coupling of DNA oligonucleotides and Au nanostructures was performed following the procedure reported by Rosi et al.³ Hybridized dsDNAs were incubated with 0.8 mL of argon-purged AuNP or AuNS solution. After 20 minutes, 10 μL of sodium dodecyl sulphate (SDS, 10% solution in water), 100 μL of phosphate buffer (0.1 M; pH = 7.4) and 50 μL of 2.0 M NaCl were added. This solution was sonicated for 10 seconds and then mounted on a vortexer and shaken gently for 30 minutes. Afterwards addition of 50 μL of 2.0 M sodium chloride and 10 seconds of sonication were repeated twice in a 30 minutes interval. This final solution was gently

shaken overnight to complete the oligonucleotide functionalization. The product was then centrifuged at 13,000 rpm for 20 minutes, so that gold nanostructures precipitated from solution. After centrifugation, the supernatant was discarded and fresh PBS solvent was added to the precipitate. The precipitate was then re-suspended by vortexing and 10 seconds of sonication.⁴ This centrifugation was repeated twice to separate DNA-Au nanoconjugates from remaining free DNAs in the immobilization solution. After centrifugation, only immobilized DNAs (FL was quenched) were expected to present, while free DNAs (high FL) were discarded.

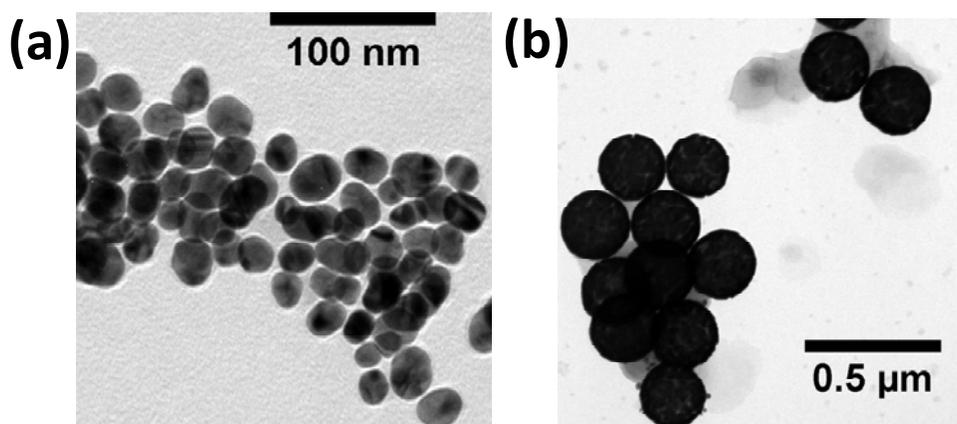


Figure S3. TEM images of (a) AuNP-D1|cD1 and (b) AuNS-D2|cD2.

Due to partial loss of gold nanoparticles in the immobilization procedure, the concentrations of both nanostructures decreased. The AuNS-D2|cD2 sample was concentrated four times by centrifugation and redispersed to maintain the concentration. Absorbance spectra of modified AuNP and AuNS are shown in Fig. S2. Compared with the unmodified nanostructures, AuNP peak shifted from 520 nm to 527 nm, AuNS from 1095 nm to 1120 nm. TEM images of these DNA-Au nanoconjugates (Fig. S3) show no discernible change in the monodispersity or the particle size. As shown in the TEM images, the gold nanostructures tend to stack on each other. The stacking is possibly caused by the increase in concentration during the process of gradual drying after being dropped onto the TEM grid.

3. Preparation of the mixed solution containing both AuNP-D1|cD1 and AuNS-D2|cD2

200 μL of AuNP-D1|cD1 solution and 200 μL of AuNS-D2|cD2 solution were mixed and centrifuged. The precipitate was redispersed in 200 μL of PBS. 5 μL of 10% SDS was added to 50 μL of this mixed solution in a fluorescence cuvette with interior footprint of $3 \times 3 \text{ mm}^2$. To minimize inconsistency, the cuvette was put in a holder in a fixed position in front of the laser system. Separate samples (containing only AuNP-D1|cD1 or AuNS-D2|cD2 conjugates) were also prepared by adding 5 μL of 10% SDS to 50 μL of respective solutions.

4. Time-dependence of the laser-induced dehybridization of AuNP-D1|cD1

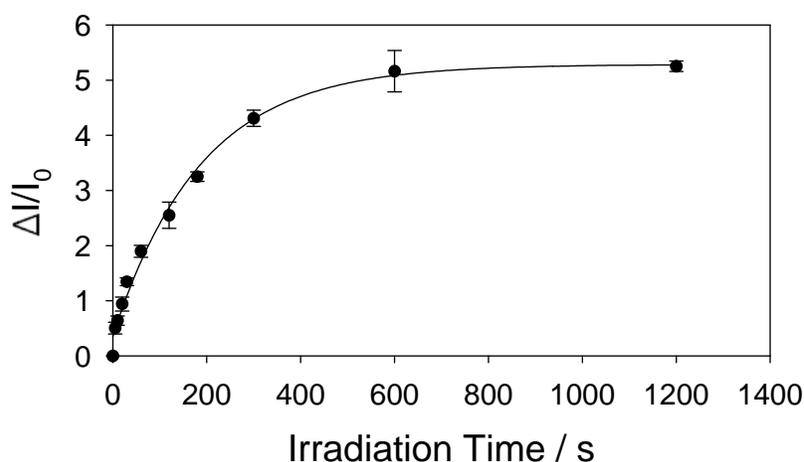


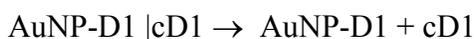
Figure S4. Irradiation time-dependence of AuNP-D1|cD1 dehybridization by 532-nm laser. The laser spot was 8 mm in diameter, and the laser power was measured at the sample position.

For a better control of dehybridization conditions, the effect of irradiation time was also studied. It has been proposed that gold nanostructures can be heated up within picosecond timeframe. However, in order to build up the temperature of the local environment to above T_m (60.1 $^{\circ}\text{C}$ in this case), multiple pulses might be needed, hence longer irradiation time. Furthermore, DNA dehybridization is a reversible process, which means that the released cD1 ssDNA may rehybridize with the surface-attached D1 strands. In this regard, the laser irradiation time should be long enough so that the released cD1 strands would be able to diffuse away from the AuNP surface (into the bulk solution). As shown in Fig. S4, initially the dehybridization percentage increases with increasing irradiation time, and remains constant after 300 seconds. In

this case, the FL intensity no longer increases when irradiated for a longer period of time. The time dependence indicates that the dehybridization might be dictated by the DNA diffusion into the bulk solution, instead of the heat transfer from AuNP to DNA. The relative FL intensity increase $\Delta I/I_0$ (defined as I') as function of irradiation time (t) fits in the equation:

$$I' = I'_0 + a(1 - e^{-kt}) \quad (3)$$

The fitting parameters are $I'_0 = 0.36$, $a = 4.9$, $k = 0.0053 \text{ s}^{-1}$ ($R^2 = 0.99$). This shows that the reaction:



can be treated as a first-order reaction on the AuNP surface. This is in accordance with the research of Wetmur et al.,⁵ where the kinetics of DNA dehybridization was discussed in detail. Temperature of the solution was measured before and after laser irradiation and found no change, which proves that only the local environment, not the entire solution, is heated by laser irradiation.

References:

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