Electronic Supplementary Information (ESI) for:

One-Step Fabrication of LSPR-Tuneable Reconfigurable Assemblies of Gold Nanoparticles Decorated with Biotin-Binding Proteins

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Contents:

Experimental Section

1. Assembly of gold nanoparticles. Citrate-capped gold nanoparticles with an average diameter of 20 nm and concentration of ca. 1 nM were obtained by adding 37.5 mg of sodium citrate dissolved in 1 mL of water to 250 mL of a boiling solution containing 23.5 mg of gold (III) chloride hydrate for 15 min under vigorous stirring. Proteins were prepared to a final concentration of 1 mg mL^{-1} in water and preserved as single use aliquots at -20°C. The day of the experiments the aliquots were thawed at room temperature and diluted 100 times in the required buffered solution with a concentration of 50 mM. Experiments performed at different pH values were prepared with 50 mM sodium citrate solutions buffered at pH 4, 5, 6, or 7, and with 50 mM sodium bicarbonate solutions buffered at pH 9 or 11. The protein solution was further diluted in the same buffer to obtain solutions with concentrations 20 times higher than the desired final concentration. Nanoparticle assemblies were obtained by adding 500 μL of nanoparticles to 500 μL of a solution containing 50 μL of protein and 450 μL of deionized water. Vis-NIR spectra and TEM images were obtained after 30 min (Fig. S2) with a Cary 300 Bio spectrophotometer and a Hitachi H-600 ABS TEM, respectively. TEM samples were prepared by depositing 5 μL of sample on a grid for 1 min and removing the excess solution with a piece of filter paper followed by washing with a drop of water and removing the excess liquid with a piece of filter paper.

2. Conjugation of biotin to glucose oxidase. The carboxylate group in biotin was transformed into a NHS ester that reacted with primary amines in GOx with the following procedure. 10 mg of GOx was diluted in 900 μL of PBS**.** 100 mM biotin was prepared in DMSO. 1.9 mg of N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC, Sigma) and 1.2 mg of N-hydroxysuccinimide (NHS, Sigma) was dissolved in 0.9 mL of 0.1 M MES buffer pH6. 100 μL of 100 mM biotin was added to the former solution for 5 min. 100 μL of the reaction mix was added to the GOx solution for 2-3 h. Subsequently 10 μL mL of 1 M glycine was added for 30 min in order to cap any unreacted NHS ester groups. Unreacted biotin, EDC and NHS were removed with a P-10 desalting column. GOx-rich fractions were identified by their characteristic yellow color. They were kept as single-use aliquots at -20°C until needed.

The following experiment was performed in order to demonstrate that GOx was successfully biotinylated and still active after bioconjugation:

ELISA plates were modified with streptavidin (5 μg/mL in PBS) or BSA overnight at 4°C. The next day the plates were emptied and blocked with PBS-BSA (1 mg/mL) for 1 h followed by washing 3 times with PBS-Tween (0.1%). Biotin-GOx was diluted 1:10 from 1:100 to 1:100000 in PBS-BSA. 100 μL of these solutions was added to each well coated with either streptavidin or BSA. After 1 h the plates were washed with PBS-Tween 5 times and 100 μL of a solution containing 0.1 mg/mL TMB, 0.1M citrate buffer pH 6, 0.1 M glucose and 1 μg/mL horseradish peroxidase (HRP) was added to the wells. HRP uses the hydrogen peroxide produced by GOx as a cofactor to oxidize TMB and produce a blue-colored molecule. After ca. 20 min the reaction was stopped with 100 μL of 2M sulfuric acid, which changed the color of the solution to yellow. The absorbance at 450 nm was measured with a Cary UV-vis spectrophotometer. In Figure S1 the colorimetric signal was only observed in wells modified with streptavidin, and the signal depended on the GOx dilution. These experiments prove that biotin was successfully attached to GOx.

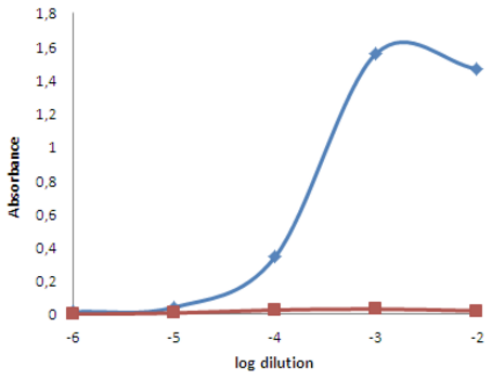

Figure S1. *Absorbance at 450 nm obtained after adding the biotinylated GOx to a substrate modified with streptavidin (blue) or BSA (red).*

3. Reconfiguration of nanoparticle assemblies. Nanoparticle assemblies were prepared as described in section 1. Reconfiguration experiments in the presence of thiolated PEG (MW 2 kDa, Sigma) proceeded as follows. 100 μL of thiolated PEG was added to a final

concentration of 5, 1.5, 0.5, 0.15, 0.05 or 0 μM to 1 mL of the nanoparticle assemblies. Vis-NIR spectra were taken at intervals of 15 min for 180 min. TEM samples were prepared after 180 min.

For pH-triggered reconfiguration studies, 100 μL of 20 mg mL^{-1} bovine serum albumin (Sigma) in water was first added to the 1 mL suspension containing nanoparticle assemblies for 10 min followed by the addition of 50 μL of NaOH to a final concentration of 12.5, 6.2, 3.1, 1.6, 0.8 or 0 mM. The resulting solutions had a pH of 13, 12.8, 12.6. 12.3, 12.1 and 6, respectively. Vis-NIR spectra were taken at intervals of 15 min for 180 min.

The process of reconfiguration in Figures 4c and 4d was followed by calculating the parameter $\Delta E_{NIR}/\Delta E_{vis}$ from extinction spectra in Figures S5 and S6. In this calculation ΔE_{NIR} is the difference in extinction at the initial NIR LSPR with respect to the extinction at 800 nm, whereas ΔE_{vis} is the difference in extinction at 525 nm with respect to the extinction at 800 nm. This ratio was chosen rather than the extinction of the NIR LSPR alone because the latter also changes when the large nanoparticle assemblies sediment. Calculating the ratio enables differentiating changes in extinction originating from sedimentation or from reconfiguration. Calculating differences with respect to the extinction value at 800 nm allows to compensate for small differences in the baseline.

Figures S2 to S8:

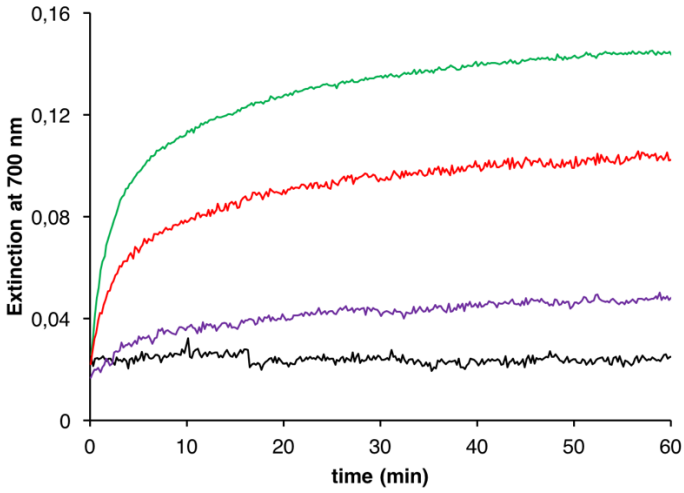

Figure S2. *Time-dependent evolution of the extinction at 700 nm of a suspension of citrate-capped nanoparticles after adding avidin at different final concentrations; black: 0 nM, purple: 0.08 nM, red: 0.15 nM; green: 0.22 nM. In these experiments, the NIR LSPR increases with time during the first 10 min and then plateaus. Consequently, extinction spectra shown in this manuscript were taken 30 min after the addition of proteins, when changes in the NIR LSPR are minimal.*

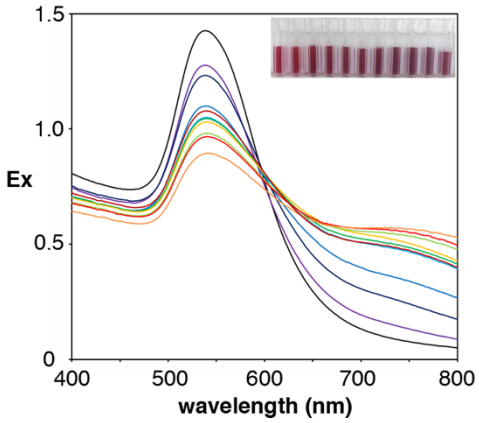

Figure S3. *Extinction spectra of suspensions of 40 nm gold nanoparticles upon addition of avidin with a final concentration of 0 (black), 0.04 (gray), 0.08 (purple), 0.15 (navy), 0.22 (dark blue), 0.30 (light blue), 0.38 (dark green), 0.45 (light green), 0.53 (yellow), 0.61 (orange), 0.68 (red) and 0.75 (maroon) nM; in 2.5 mM citrate buffer pH 6.*

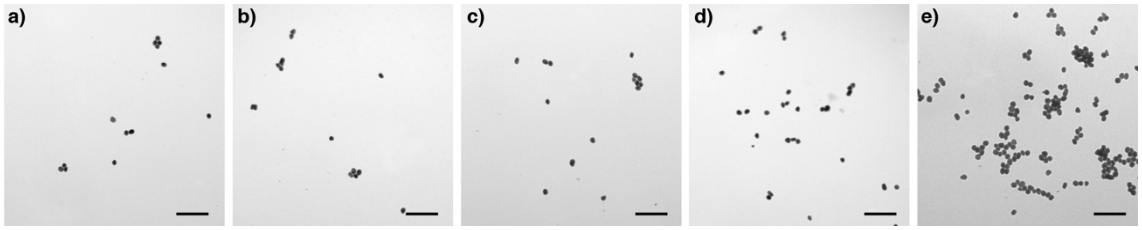

Figure S4*. TEM images of nanoparticle assemblies after adding avidin at different final concentrations to a solution containing 40 nm gold nanoparticles and 2.5 mM citrate buffer pH 6; a) 0.08 nM; b) 0.22 nM; c) 0.38 nM; d) 0.53 nM; e) 0.68 nM. Scale bar: 500 nm.*

Analysis of Figures S3 and S4. In these experiments nanoparticles were added to a final concentration of 0.25 nM rather than the 0.5 nM concentration used for 20 nm nanoparticles in order to avoid exceedingly large extinction values, since 40 nm nanoparticles have higher molar extinction coefficients compared to 20 nm colloids. In Figure S2, addition of avidin at protein/ nanoparticle ratios similar or higher than the ones shown in Figure 1a resulted in the formation of broader, less defined NIR LSPR. The resulting assemblies contained less nanoparticle building blocks (Figure S4). This outcome fits well with our hypothesis that the key factor behind the assembly of gold nanoparticles is to destabilize them via protein physisorption, since larger 40 nm nanoparticles had a weaker tendency to assemble when proteins covered a smaller fraction of their surface.

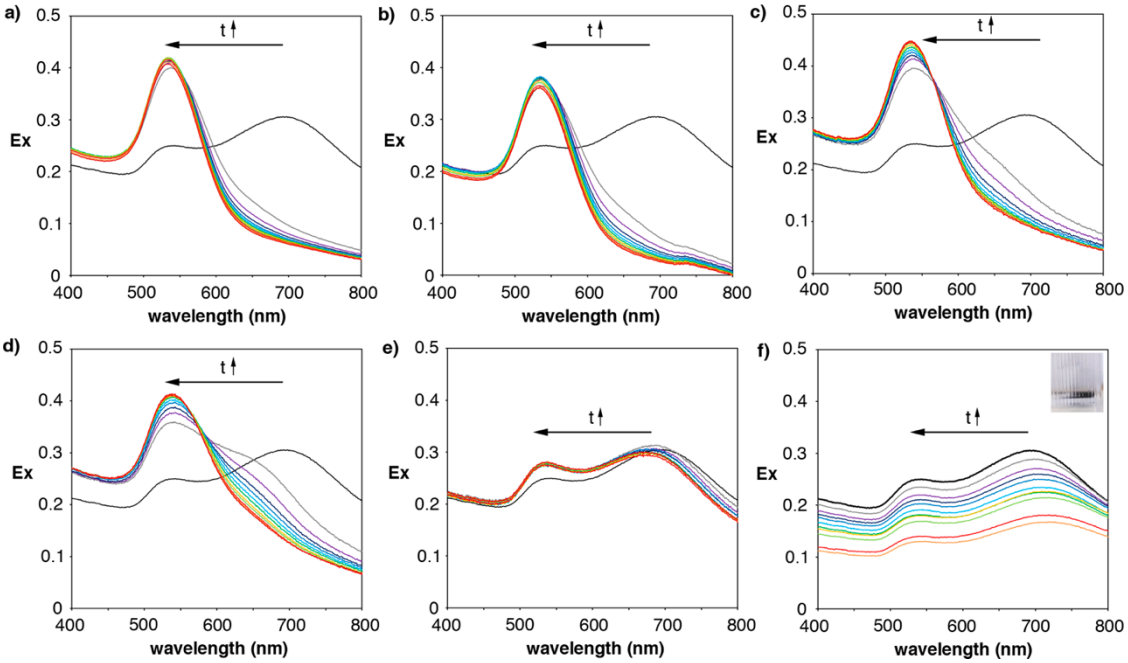

Figure S5*. Reconfiguration of nanoparticles assembled with 0.75 nM avidin after adding thiolated PEG to a final concentration of a) 5; b) 1.5, c) 0.5, d) 0.15; e) 0.05 and f) 0 (black) μM. Figures S5a-5e show that the NIR LSPR shifts blue and progressively disappears as a function of the concentration of thiolated molecules. Some spectra such as those in Figure S5d show a shoulder at 600 nm that indicates that the reconfiguration is not complete in the time scale under study. In Figure S5f the extinction at all wavelengths decreases with time in the control experiment without thiolated PEG because the large assemblies of nanoparticles sediment (a photograph of an overnight sediment is shown in the inset of Figure S5f). No blue-shift or disappearance of the NIR LSPR is observed in these spectra.*

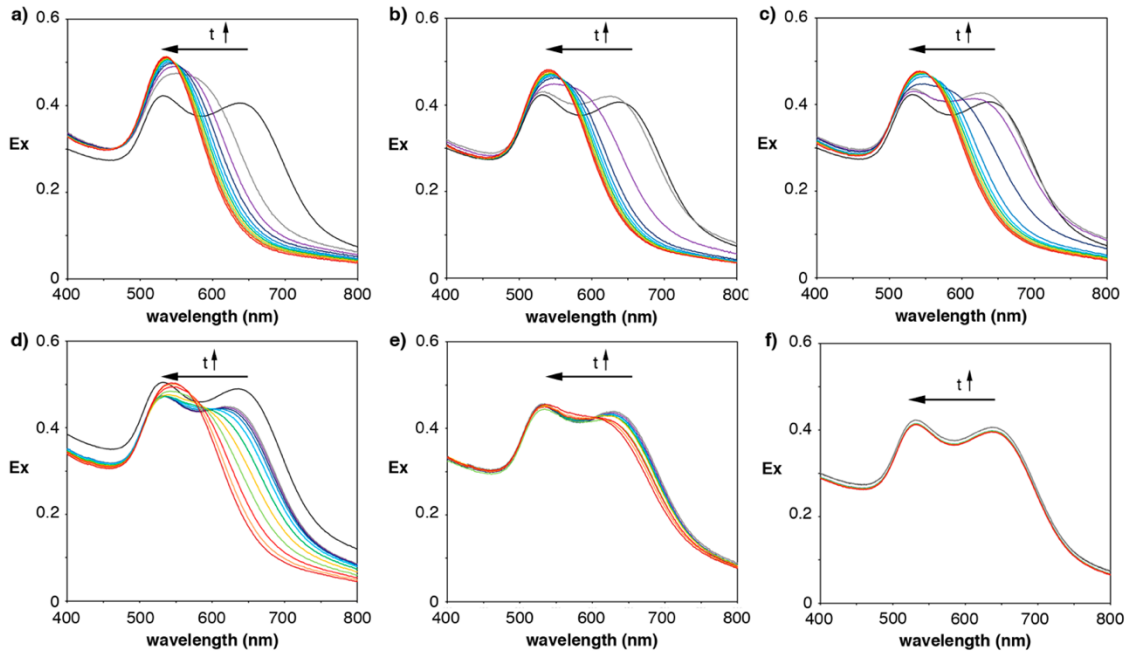

Figure S6*. Reconfiguration of gold nanoparticles assembled with 0.53 nM avidin after adding adding NaOH with the final concentration of a) 12.5; b) 6.2; c) 3.1; d) 1.6; e) 0.8; and f) 0 mM.*

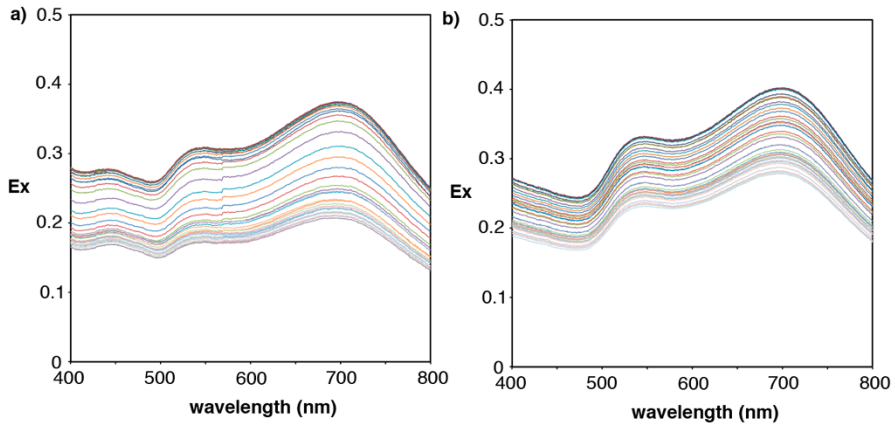

Figure S7. *Extinction spectra at 15 min intervals for 10 h in nanoparticle suspensions assembled with 0.75 nM avidin and after adding biotinylated GOx to a final concentration of a) 0.3 or b) 0 μM. The peak around 450 nm in a) is originated by the yellow-colored GOx. The spectra show no LSPR blue-shift, and the progressive decrease in extinction at all wavelengths with time is attributed to the sedimentation of the nanoparticle assemblies.*

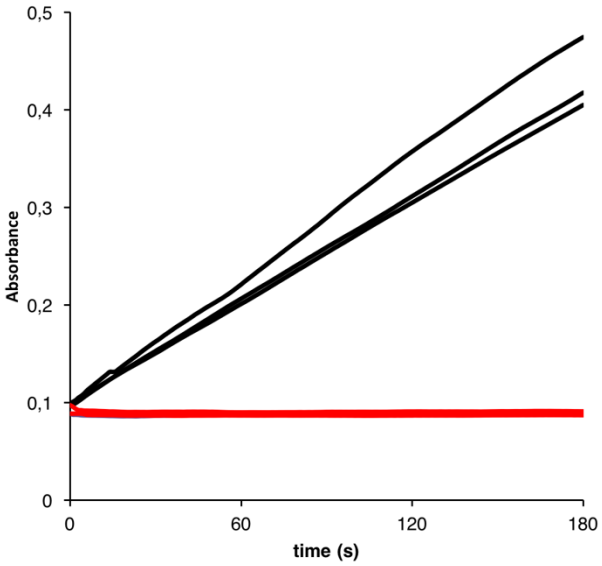

Figure S8. *HRP activity in avidin-covered nanoparticle assemblies modified with biotinylated HRP (black) or non-biotinylated HRP (red) with a concentration of 1 μg/mL. Samples were incubated with enzymes overnight followed by washing 3 times by centrifugation with PBS-BSA (1 mg/mL). HRP activity was measured by adding a solution containing 0.1 mg/mL TMB, 0.1 M acetate buffer pH 5 and 0.01% hydrogen peroxide and measuring the absorbance at 650 nm. HRP was biotinylated with the same procedure detailed for GOx above. Each experiment was performed as a triplicate.*