

Supporting Information for

Highly stable and biocompatible gold nanorod- DNA conjugates as NIR probes for ultrafast sequence-selective DNA melting

Verena Baumann,^{1,3} Peter Johan Friedrich Röttgermann,^{2,3} Frederik Haase,^{1,3,#} Katalin Szendrei,^{1,3,#} Priyanka Dey,^{1,3} Katja Lyons,^{1,3} Regina Wyrwich,⁵ Matthias Gräbel,^{1,3,4} Joachim Stehr,⁴ Lars Ullerich,⁴ Federico Bürsgens,⁴ Jessica Rodríguez-Fernández^{1,3}*

¹Photonics and Optoelectronics Group, Department of Physics and Center for NanoScience (CeNS), Ludwig-Maximilians-Universität München, Amalienstr. 54, 80799 Munich (Germany)

²Soft Condensed Matter Group, Department of Physics and Center for NanoScience (CeNS), Ludwig-Maximilians-Universität München, Geschwister-Scholl-Platz 1 80539 Munich (Germany)

³Nanosystems Initiative Munich (NIM), Schellingstr. 4, 80799 Munich (Germany)

⁴GNA Biosolutions GmbH, Am Klopferspitz 19, 82152 Martinsried (Germany)

⁵Department of Chemistry, Ludwig-Maximilians-Universität München, Butenandtstr. 5-13 (E), 81377 Munich (Germany)

[#]Current address: Max Planck Institute for Solid State Research, Heisenbergstr. 1, 70569 Stuttgart, Germany.

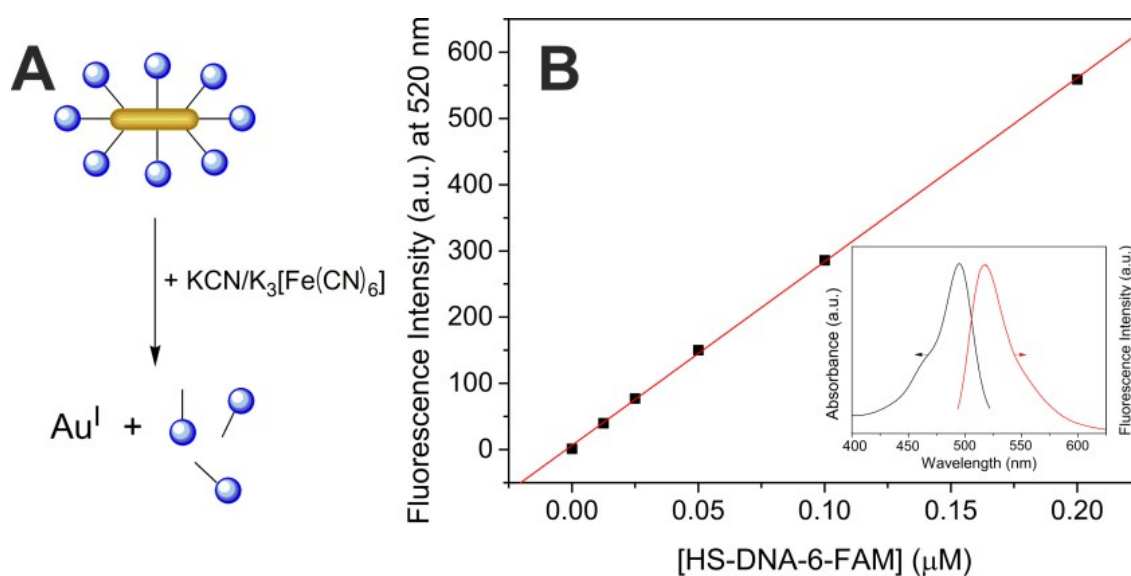


Figure S1. (A) Sketch showing the oxidation reaction of gold nanorods with cyanide in order to quantify the number of released HS-DNA-6-FAM strands (the blue spheres depict the fluorescent 6-FAM moiety). (B) Calibration curve for DNA quantification: linear fit of the fluorescence intensity of HS-DNA-6-FAM at 520 nm *vs.* the concentration of HS-DNA-6-FAM. Note that the experimental conditions (medium, concentration of oxidizing agents, etc.) used herein for the determination of the calibration curve were the same as the ones during the oxidation experiments. Inset: absorption (black) and emission (red) spectra of HS-DNA-6-FAM. The emission spectrum was measured upon excitation at $\lambda = 485$ nm. The emission maximum is centered at 520 nm.

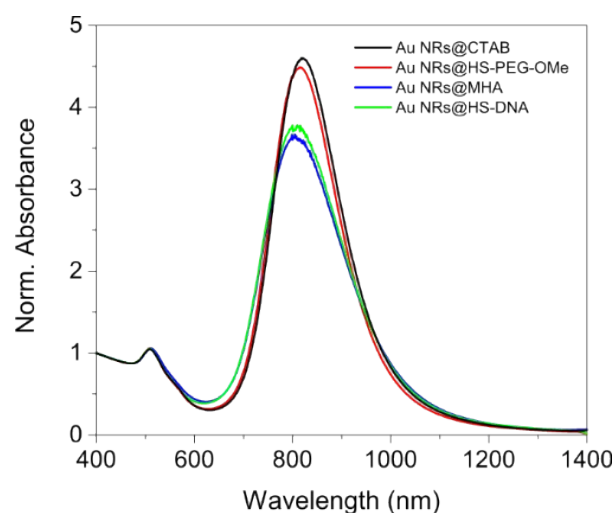


Figure S2. Vis-NIR spectra of Au NRs@CTAB in water (black), Au NRs@HS-PEG-OMe in water (red), Au NRs@MHA in TBE 1× containing 0.001 % tween 80 (blue), and Au NRs@HS-DNA-6-FAM in TAE 1× containing 0.001 % tween 80 (green). All spectra were normalized at 400 nm. The specific HS-DNA sequence used herein was 5'-thiol-C6-TTTTTTTTTTTTTTTTTTTT-3'. Colloidal stability is preserved during all functionalization steps.

Effect of the HS-PEG-OMe dose on the DNA loading capacity

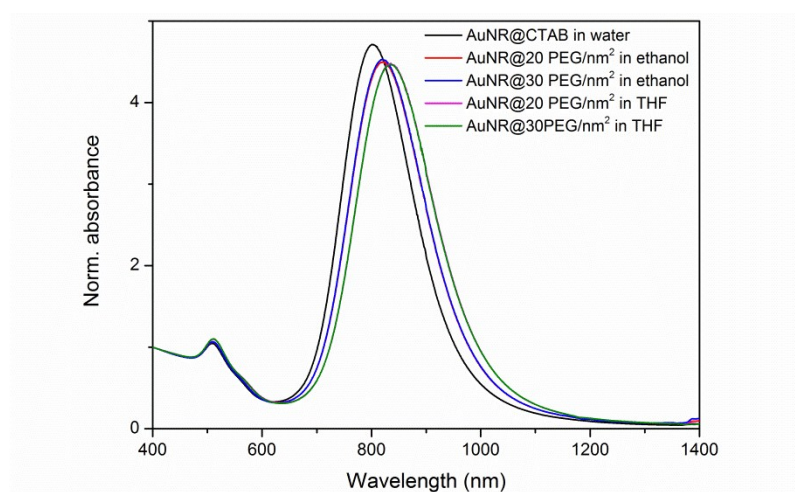


Figure S3. Vis-NIR spectra of Au NRs@HS-PEG-OMe after functionalization with a PEG dose of 20 and 30 PEG molecules / Au nm². The spectra are shown for both samples in ethanol

and in THF. The spectrum of the Au NRs@CTAB in water (black curve) is also provided for comparison. Note that the red spectrum is overlaid by the blue spectrum, indicating no significant differences between both samples at the ethanol redispersion step. The magenta spectrum is also overlaid by the green spectrum, which further confirms that there are no significant differences between both samples after redispersion in THF. All spectra were normalized at 400 nm for comparison.

Table S1. Effect of the HS-PEG-OMe dose used (20 *vs.* 30 molecules / Au nm²) on the DNA loading achieved for Au NRs@HS-DNA-6-FAM (samples shown in Figure S3). The MHA dose used was 300 molecules / Au nm², while the molar ratio HS-DNA-6-FAM/Au NRs was set to 10000 in both cases. The quantification results show the detrimental effect (lower DNA loading) of PEGylating at doses larger than 20 molecules / nm². Even though the difference is not dramatic for 20 *vs.* 30 HS-PEG-OMe molecules / Au nm², larger doses lead to very low DNA loadings (results not shown).

HS-PEG-OMe dose	DNA strands/particle	DNA strands / Au nm ²
20 PEG / Au nm ²	184	0.050
30 PEG / Au nm ²	158	0.043

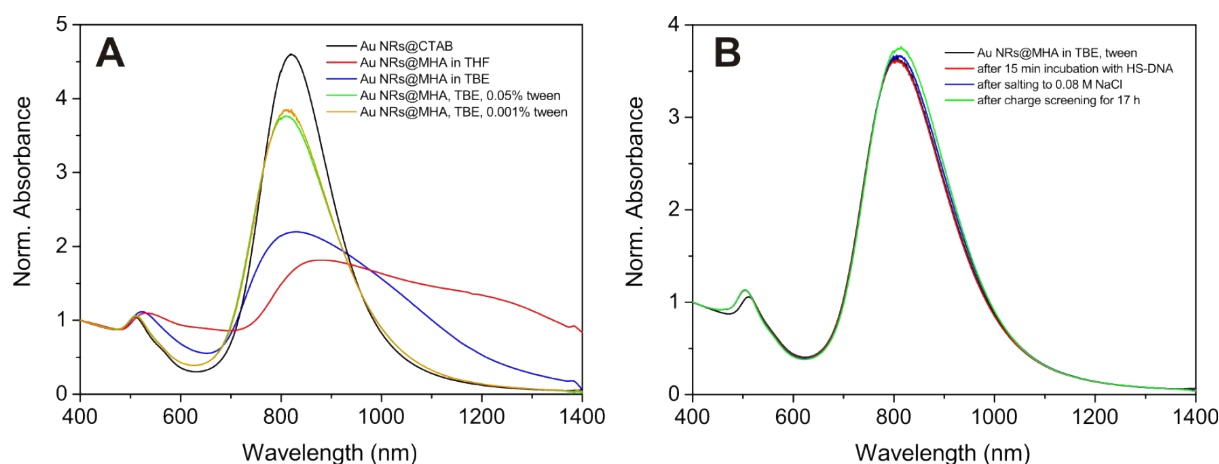


Figure S4. (A) Vis-NIR spectra showing the effect of the absence or presence of tween 80 in TBE 1× buffer upon redispersion of flocculated Au NRs@MHA. The graph includes the spectra of the Au NRs@CTAB in water plotted as reference (black), the flocculated Au NRs@MHA in THF (red), the Au NRs@MHA after redispersion in TBE 1× buffer (blue), in TBE 1× containing 0.05 % tween 80 (green) and in TBE 1× containing 0.001 % tween 80 (orange). All spectra were normalized at 400 nm. It can be seen that in the absence of tween 80 the Au NRs@MHA aggregate significantly. (B) Vis-NIR spectra of Au NRs@MHA in TBE 1× containing 0.001 % tween 80 (black) after incubation with the HS-DNA oligonucleotides for 15 min (red), after stepwise addition of NaCl to a final concentration of 0.08 M (blue), and after charge screening for 17 h (green). All spectra were normalized at 400 nm. The specific HS-DNA sequence used herein was 5'-thiol-C6-TTTTTTTTTTTTTTTTTTTT-3'. Colloidal stability was maintained throughout all steps.

Effect of the molar ratio HS-DNA-6-FAM / Au NRs on the DNA loading capacity

Table S2. Effect of the molar ratio $n(\text{HS-DNA-6-FAM}) : n(\text{Au NRs})$ on the DNA loading. In the experiments shown below the PEG dose used was set to 30 PEG/nm² and DNA functionalization was carried out by performing the salting step up to a final concentration of $[\text{NaCl}] = 0.08 \text{ M}$.

n(HS-DNA) : n(Au NR)	DNA strands/particle	DNA strands/nm²
3700	158	0.043
8000	107	0.029
20000	105	0.028

Compared to our standard conditions, where the $n(\text{HS-DNA}) : n(\text{Au NR}) = 3700$, *i.e.*, the added dose is set to 1 HS-DNA strand / Au nm², a slightly lower DNA loading is obtained for $n(\text{HS-DNA}) : n(\text{Au NR}) = 8000$ (2.2 HS-DNA strands / Au nm²), and 20000 (5.4 HS-DNA strands / Au nm²). The most plausible explanation for this, *a priori*, counterintuitive effect relates to the high electrostatic repulsion of the negatively charged DNA backbones. The [NaCl] used (0.08 M) is enough to compensate for this repulsion at lower $n(\text{HS-DNA}) : n(\text{Au NR})$ ratios. However, it does not suffice when the relative concentration of DNA backbones is much larger ($n(\text{HS-DNA}) : n(\text{Au NR}) = 8000$ and 20000) and, hence, it cannot give rise to any increase in DNA loading. Therefore, unless the NaCl concentration is concomitantly increased along with the $n(\text{HS-DNA}) : n(\text{Au NR})$ ratio, increasing the latter ratio alone does not give rise to any DNA loading increase.

Effect of sonication during charge screening on the DNA loading capacity

Table S3. Effect of performing a 15 s sonication after each salting step on the final DNA loading. In the experiments below the final salting concentration was set to [NaCl] = 0.4 M, while the molar ratio $n(\text{HS-DNA}) : n(\text{Au NRs})$ was set to 10000. These results demonstrate the significant, positive, effect of sonication on the DNA loading capacity of the Au NRs. See the corresponding spectra in Figure S5.

15 s sonication after each salting step	DNA strands/particle	DNA strands/nm ²
no	352	0.095
yes	409	0.111

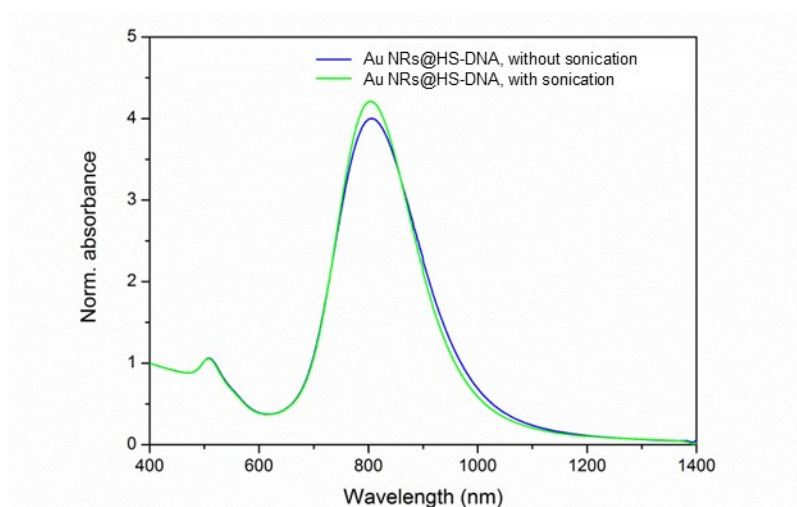


Figure S5. Vis-NIR spectra of Au NRs@HS-DNA in 1× TAE containing 0.001% tween 80 (washed samples). The Au NRs were functionalized with HS-DNA by performing no sonication during salting (blue curve), or by carrying out a 15 s sonication at the end of each salting step (green curve), see DNA loading data in Table S3 above. Both spectra are normalized at 400 nm.

Table S4. Influence of the number of centrifugation steps and the presence of tween 80 on the $[\text{HS-DNA-6-FAM}]_{\text{free}} / [\text{HS-DNA-6-FAM}]_{\text{total}}$ ratio of Au NRs@HS-DNA-6-FAM after washing. Note that here “centrifugation cycle” refers to a whole centrifugation and redispersion cycle.

Number of centrifugation cycles	$[\text{HS-DNA-6-FAM}]_{\text{free}} / [\text{HS-DNA-6-FAM}]_{\text{total}}$ (%)
2 (no tween 80)	34.4
2 (in the presence of 0.001 % tween 80)	8.75
3 (no tween 80)	0.352
3 (in the presence of 0.001 % tween 80)	0.006

We optimized the washing conditions of our DNA-functionalized Au NRs so that the amount of free (unbound) DNA strands present after washing is minimal ($[\text{HS-DNA-6-FAM}]_{\text{free}}/[\text{HS-DNA-6-FAM}]_{\text{total}} < 0.01$). The results in Table S4 clearly indicate that by increasing the number of centrifugation cycles from 2 to 3, the percentage ratio $[\text{HS-DNA-6-FAM}]_{\text{free}}/[\text{HS-DNA-6-FAM}]_{\text{total}}$ drops from 34.4% to 0.352 %. Furthermore, by adding 0.001 % tween 80, the ratio $[\text{HS-DNA-6-FAM}]_{\text{free}}/[\text{HS-DNA-6-FAM}]_{\text{total}}$ further decreases from 8.75 % (for 2 centrifugation cycles) down to 0.006 % (for 3 centrifugation cycles), as compared to the results in the absence of tween. In general, we observed that the surfactant decreases the affinity of the Au NRs@HS-DNA-6-FAM to the walls of the centrifugation tube. This enables the formation of a more compact pellet and thus, allows for a better removal of the supernatant, which contains unbound DNA strands. Based on these results the optimized washing conditions for DNA-functionalized Au NRs were set to 3×, in the presence of 0.001 % tween 80.

Table S5. Influence of the incubation temperature during the 17 h charge screening. Note that the temperature (23 °C vs. 50 °C) was strictly controlled for 17 hours once a final $[\text{NaCl}] = 0.08 \text{ M}$ was reached. In all cases, for quantification purposes, DNA functionalization was performed with HS-DNA-6-FAM oligonucleotides.

Temperature during DNA functionalization	DNA strands / Au NR	DNA strands / Au nm ²
23	151	0.04
50	322	0.09

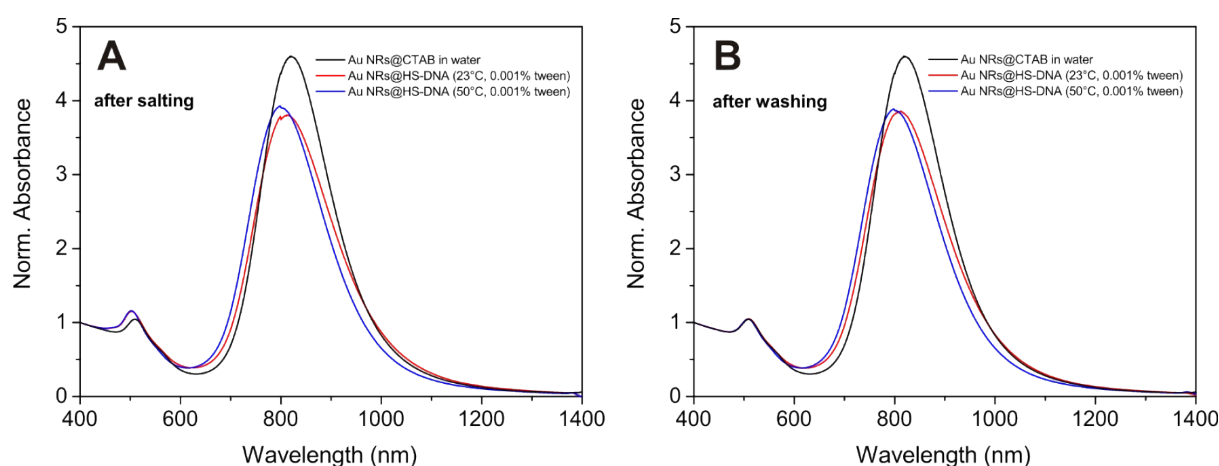


Figure S6. Effect of the incubation temperature on the vis-NIR spectra of Au NRs upon DNA functionalization. (A) Extinction spectra of the Au NRs@HS-DNA after salting overnight for 17 h at 23°C and 50°C in the presence of 0.001% tween 80. (B) Extinction spectra of the same batches after 3× washing with TAE 1× containing 0.001% tween 80. The spectrum of Au NRs@CTAB in water (black) is included as reference. All spectra were normalized at 400 nm. In all cases the stability of the Au NRs is preserved. Nevertheless, for the samples incubated at 50 °C a small blue-shift of the longitudinal plasmon band of the Au NRs can be observed. This is due to a slight decrease in the aspect ratio of the Au NRs resulting from partial reshaping at higher temperatures, as previously reported.^{1,2}

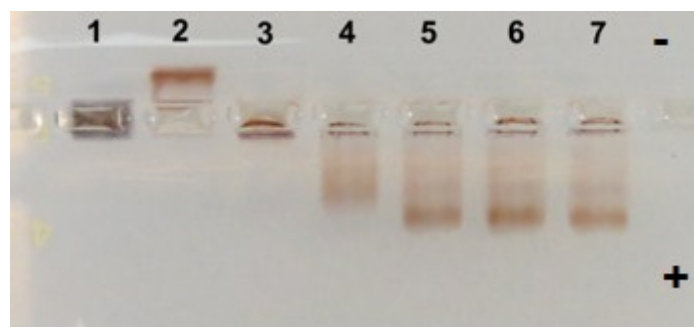


Figure S7. Photograph illustrating the gel electrophoresis results for a batch of Au NRs (length $\times$ width = $(64 \pm 12) \times (16 \pm 3)$ nm) bearing different functionalities on their surface. The samples correspond to: Au NRs@CTAB (1), Au NRs@HS-PEG-OMe (2), Au NRs@MHA (3), Au NRs@HS-DNA loaded with 0.050 strands / Au nm² (4), Au NRs@HS-DNA loaded with 0.115 strands / Au nm² (5), Au NRs@HS-DNA loaded with 0.123 strands / Au nm² (6), and Au NRs@HS-DNA loaded with 0.116 strands / Au nm² (7). The number of DNA strands loaded was controlled by adjusting the [NaCl] from 0.08 M to 0.7 M, from (4) to (7), respectively. In general, the larger the DNA loading, the more homogeneous the bands. Indeed, the sample with the lowest DNA loading (4) shows the strongest band smearing. These gel electrophoresis results indicate that the loading on the Au NRs' surface gets more homogeneous as larger NaCl concentrations are used during the salting step.

Effect of Au NRs@MHA aging on the DNA loading capacity

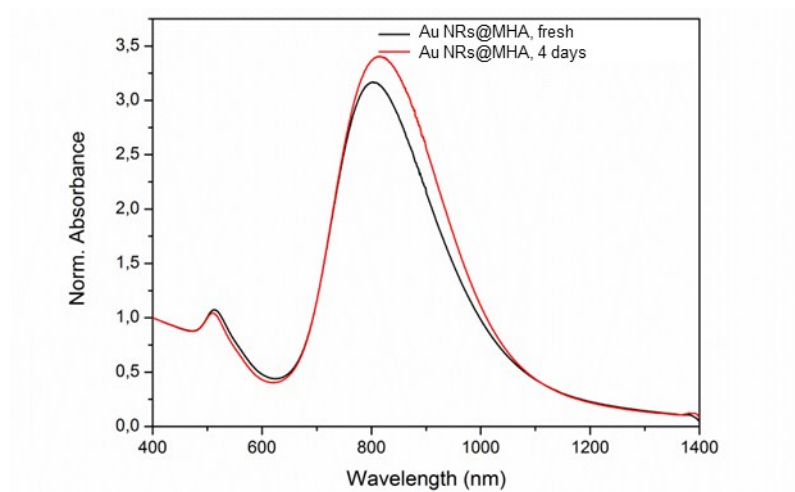


Figure S8. Vis-NIR spectra of MHA-functionalized Au NRs in 1× TBE containing 0.001% tween 80. Spectra measured immediately after MHA functionalization (black curve) and after 4 days storage at room temperature (red curve). The spectra are normalized at 400 nm. A slight increase of the relative longitudinal plasmon band intensity points to a slight improvement in colloidal stability upon aging for 4 days. This could be due to two reasons: (i) upon desorption of some MHA molecules from the Au NRs' surface, tween 80 may physisorb and lead to a slightly better sample stability; (ii) it can also be that a small portion of the Au NRs@MHA remain flocculated after redispersion in buffer and that these redisperse (thus, increasing the overall stability slightly) upon longer incubation in the tween-containing buffer.

Table S6: Results summarizing the effect of Au NR@MHA aging on the final DNA loading.

The samples used herein are those whose spectra are shown in Figure S8.

Au NRs@MHA storage	DNA strands/particle	DNA strands/nm ²
0 days	627	0.170
4 days	852	0.231

When DNA functionalization was performed straight after the preparation of the Au NRs@MHA, a loading of 627 DNA strands/particle (0.170 DNA strands/nm²) was obtained. In contrast, when DNA functionalization was carried out with the aged sample (4 days in this case), a notable larger loading was achieved (852 DNA strands/particle, *i.e.*, 0.231 DNA strands/nm²). Thus, it can be concluded that the aging time of the Au NRs@MHA sample has a strong (positive) effect on the DNA loading capacity. As indicated above, some MHA molecules may desorb from the nanorods' surface, allowing the tween 80 molecules to physisorb. The physisorbed tween 80 molecules are weakly grafted on the NRs' surface. Therefore, their replacement by HS-DNA is more favorable than the replacement of MHA molecules, whose Au-S binding is much stronger. This can explain the higher DNA loadings obtained for the aged Au NRs@MHA sample.

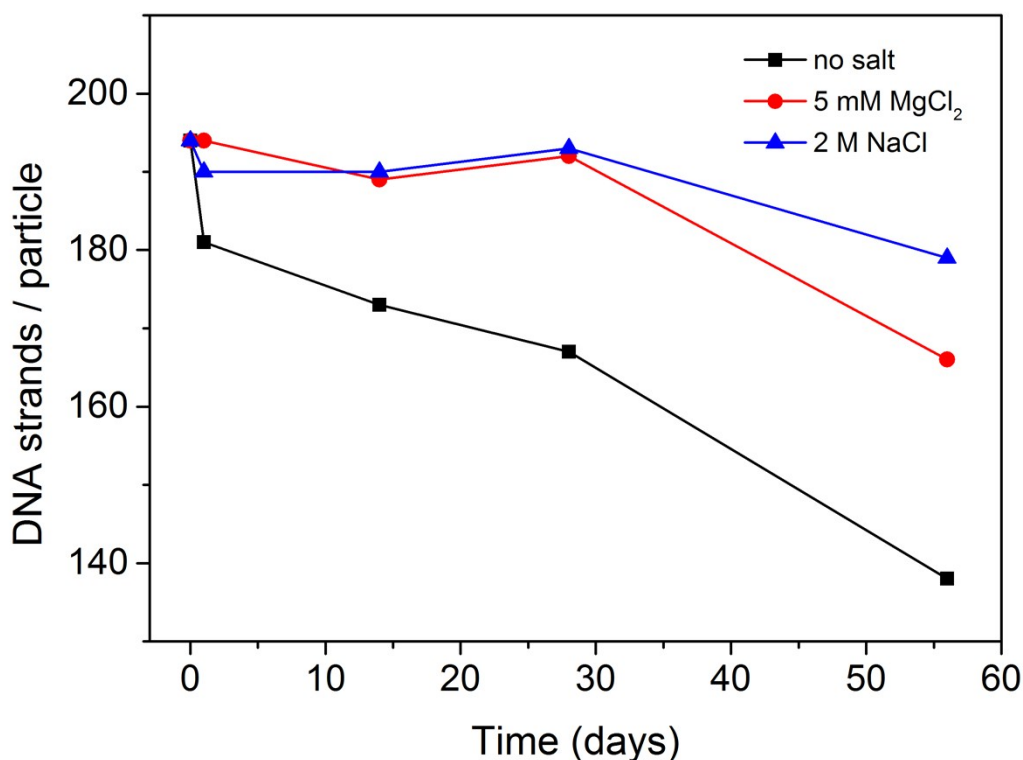


Figure S9. Assessment of DNA detachment from the DNA-functionalized Au NRs shown in Figure 2A, 2B, and 2C, upon incubation in salt-containing media. Evolution of the number of DNA strands *per* Au NR as a function of time for an Au NR@HS-DNA-6-FAM sample in TAE 1× (containing 0.001 % tween 80) upon storage without addition of any extra salt (black curve), and upon addition of 5 mM MgCl₂ (red curve), and 2 M NaCl (blue curve). The sample containing no extra salt is the one presenting the most significant and gradual DNA detachment over time (29 % after 56 days). However, when the ionic strength of the medium is increased by addition of mono- (NaCl) or divalent (MgCl₂) salts, *i.e.*, when the electrostatic repulsion between the negatively charged DNA backbones is conveniently screened, DNA detachment from the Au NRs' surface is significantly reduced. For the sample in 2 M NaCl, DNA detachment is reduced to ~ 8 % after 56 days. For the sample in 5 mM MgCl₂, the result is slightly worse (~ 14 %), which may be related to the slightly lower stability of the Au NRs@HS-DNA-6-FAM in 5 mM MgCl₂ *vs.* in 2 M NaCl after 56 days (see spectra in Figure S10). Nevertheless, over a time period of 56 days the salt containing samples show a

relatively stable amount of loaded DNA and, in either case (NaCl and MgCl₂), the stability of the DNA-modified Au NRs is maintained (see Figure 2 and Figure S10).

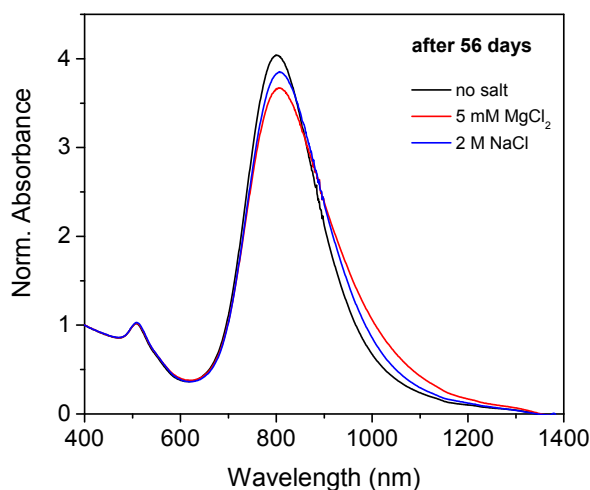


Figure S10. Vis-NIR spectra of the Au NRs@HS-DNA-6-FAM shown in Figure 2A, 2B, and 2C (grafting density: 194 DNA strands / Au NR, *i.e.*, 0.05 DNA strands / Au nm²) in 1× TAE containing 0.001 % tween 80. The spectra were taken after 56 days storage in the above medium (no extra salt added), in 5 mM MgCl₂, and in 2 M NaCl. All spectra were normalized at 400 nm for comparison. The overall concentration of HS-DNA-6-FAM in those samples (after having taken into account the actual concentration of Au NRs@HS-DNA-6-FAM and their corresponding grafting density) is $\sim 4.9 \times 10^{-8}$ M. The absorption of HS-DNA-6-FAM is low at this concentration and gets overlapped by the stronger absorption of Au. This explains why the characteristic FAM absorption is not detected in our extinction spectra.

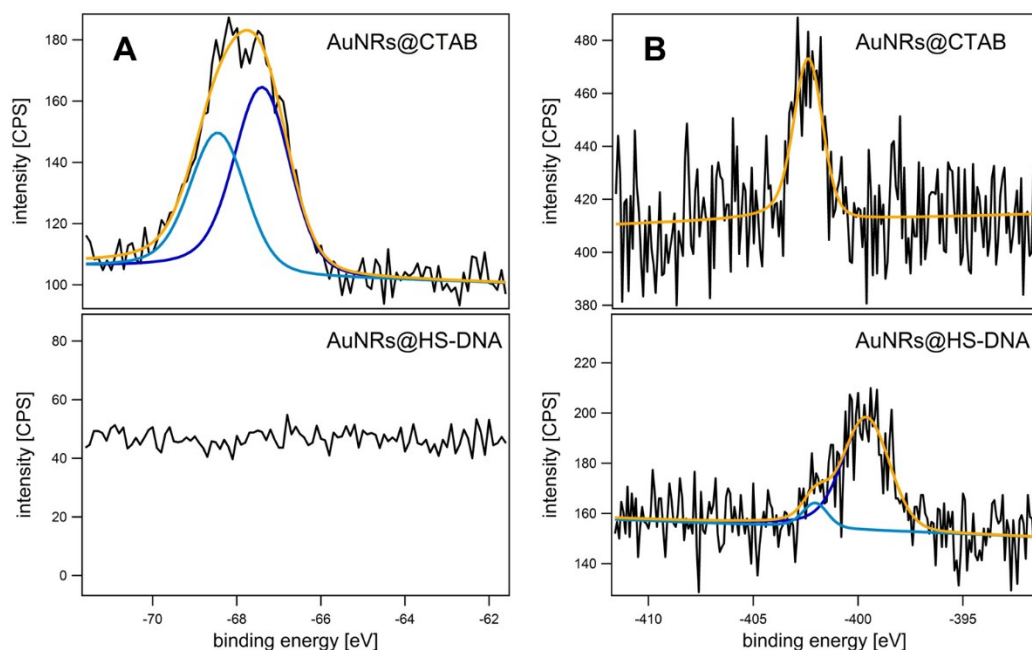


Figure S11. XP spectra of (A) Br3d and (B) N1s for the AuNRs@CTAB and for the Au NRs@HS-DNA. In (A) the peaks correspond to the $3d_{5/2}$ and $3d_{3/2}$ photoelectron lines of anionic Br. These peaks are present in the Au NRs@CTAB, but not in the Au NRs@HS-DNA, which indicates that the DNA functionalization worked well regarding CTAB removal. In (B), the N 1s spectrum for the Au NRs@CTAB consists of a peak at ~ 402 eV, which indicates the presence of N in an ammonium salt (CTAB). For the Au NRs@HS-DNA, the N 1s spectrum can be mainly fitted by a peak at ~ 400 eV, ascribed to the presence of N in an organic matrix. The spectrum also contains a weak shoulder at ~ 402 eV, ascribed to the presence of N in an ammonium salt. The almost complete vanishing of the peak at ~ 402 eV in the Au NRs@HS-DNA as compared to the Au NRs@CTAB is due to the CTAB removal; while the appearance of the new strong peak at ~ 400 eV stems from the presence of N in the organic DNA matrix. The dimensions of the analyzed Au NRs were: length $\times$ width = 72×19 nm. For the Au NRs@HS-DNA sample, the DNA grafting density was 221 DNA strands / Au NR, *i.e.*, 0.046 DNA strands / Au nm².

Table S7. Relative N/Au/Br/S/C/O and N/Au/Br atomic ratios determined from the XP spectra of the Au NRs@CTAB and of the sputter-cleaned Au NRs@HS-DNA sample shown in Figure S11. The atomic ratios are normalized to 1 with respect to Au.

	N/Au/Br/S/C/O (normalized to Au=1)	N/Au/Br
Au NRs@CTAB	1.6/1/2/40.5/4	1.6/1/2
Au NRs@HS-DNA	62.5/1/~0/71.6/424.8/27.3	62.5/1/~0

The peaks were fitted using a Doniach-Sunjic function³ convoluted with a Gaussian after a linear background subtraction. Intensities of surface species were corrected by the specific cross sections.⁴ For the analyzer, the apparatus specific transmission function was determined and peak areas were corrected accordingly.

For the sample Au NRs@CTAB, a clear peak is visible in the Br 3*d* spectrum, indicating the presence of Br⁻. Also in the N 1*s* spectrum a clear peak can be seen. Based on its binding energy of ~402 eV, one would assign it to the presence of ammonium salt, or in this case, CTAB.⁵ When we measured the Au NRs@HS-DNA sample as prepared, there was no gold detectable in the Au 4*f* spectrum. Probably the DNA strands around the Au NRs were too large and XPS was challenged with its limited penetration depth. Because of this, the Au NRs@HS-DNA sample was then sputter-cleaned twice (1 kV, 4 μ A, 15 min) to investigate deeper layers. After the second sputter-cleaning process we could identify a clear peak in the Au 4*f* spectrum. In the Br 3*d* spectrum of the cleaned sample there is no peak detectable, which demonstrates the absence of Br⁻ (also in the spectra of the sample as prepared and after the first cleaning step there was no Br visible). The detection limit of the instrument is in this case ~ 1 %, so this result proves that the Br content of the sample Au NRs@HS-DNA is below the detection limit. The N 1*s* peak of ammonium salt decreased compared to the Au NRs@CTAB sample, and

there is a second peak at ~ 400 eV that demonstrates the presence of N in an organic matrix environment (DNA).⁵

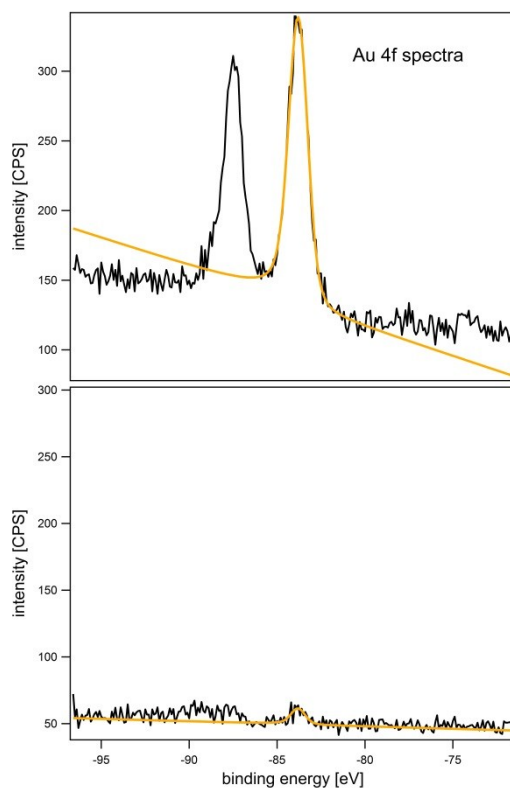


Figure S12. XP spectrum of Au 4f for the Au NRs@CTAB (top) and for the AuNRs@HS-DNA (bottom). The peak position in both cases indicates that gold is present as Au(0). The peak in the spectrum of the Au NRs@HS-DNA is significantly smaller because it gets attenuated by the large DNA strands surrounding the gold.

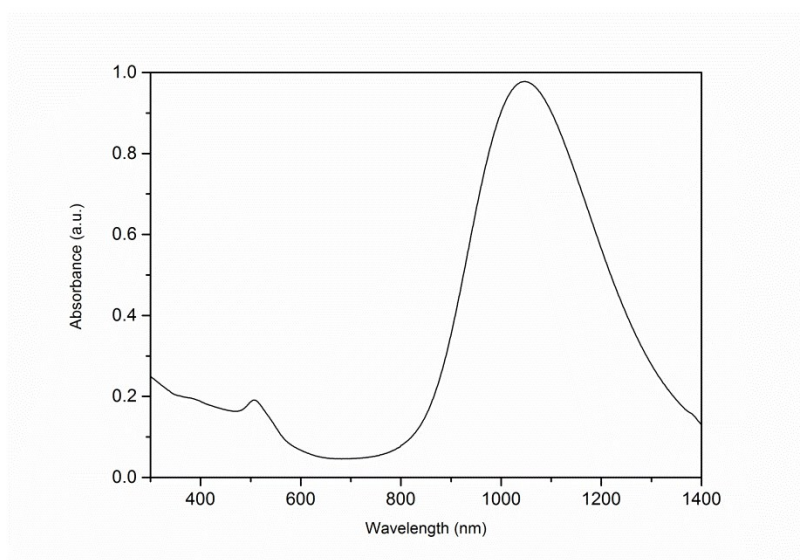


Figure S13. UV-vis-NIR spectra of high aspect ratio Au NRs (length $\times$ width = 93×14 nm, AR = 6.65) functionalized with a 5'-thiol- C_6 - A_{20} GGTTCAGGCACAGCACATCAA-3'-6-FAM DNA sequence. The grafting density on the Au NRs' surface is 0.016 strands/Au nm².

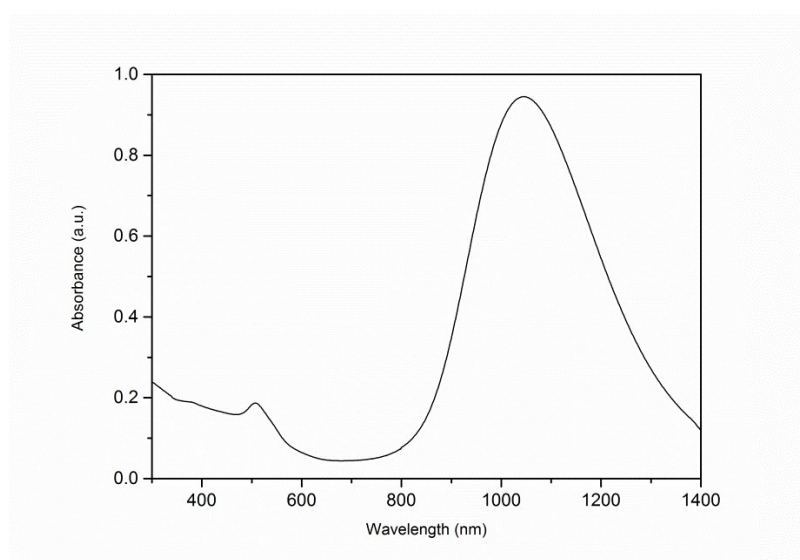


Figure S14. UV-vis-NIR spectra of high aspect ratio Au NRs (length $\times$ width = 93×14 nm, AR = 6.65) functionalized with a 5'-thiol- C_6 - A_{10} CTGTGCC-3'-6-FAM sequence. The grafting density on the Au NRs' surface is 0.025 strands/Au nm².

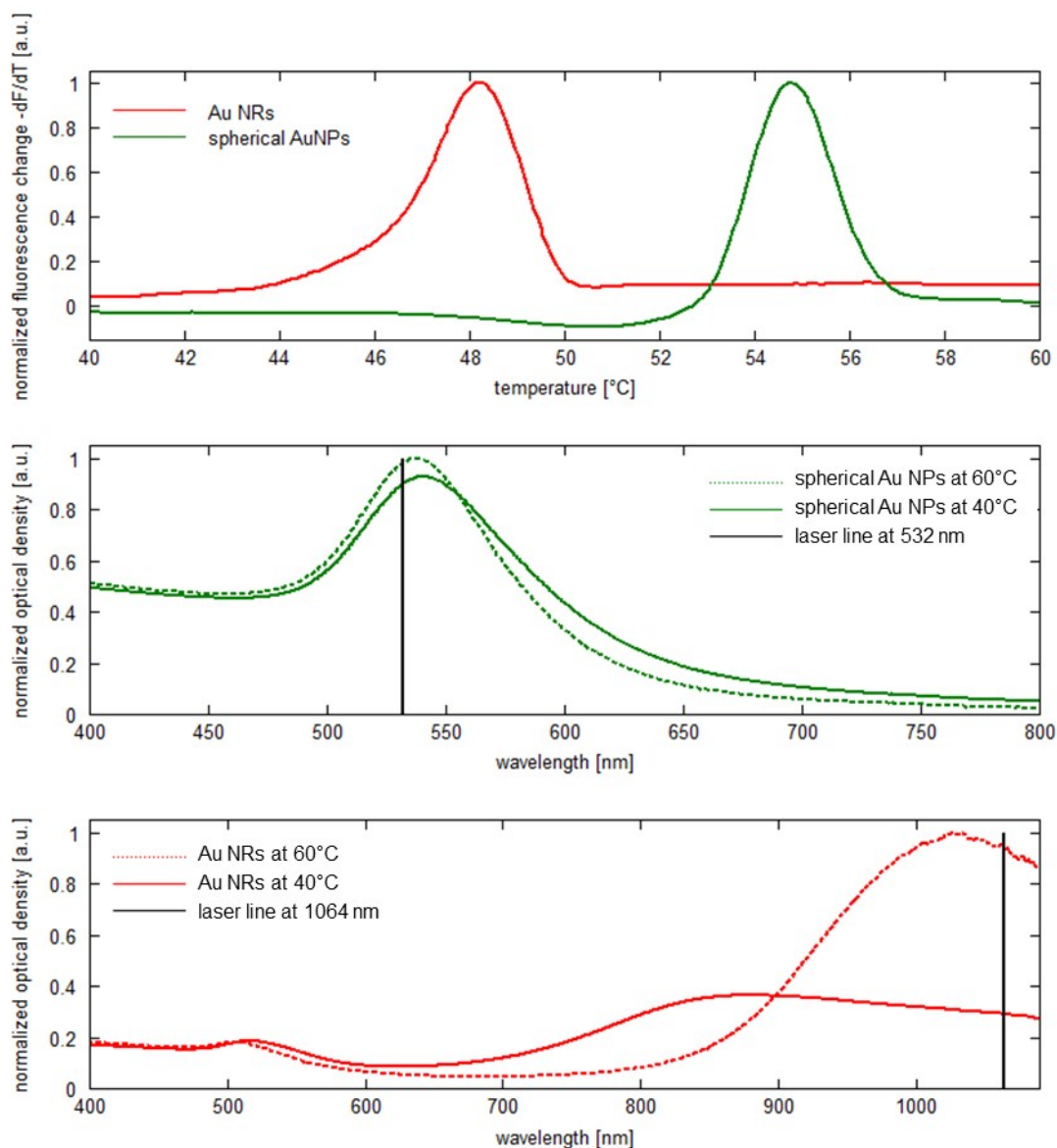


Figure S15. Top: melting curves of clusters of ssDNA-functionalized spherical Au NPs (green curve) and Au NRs (red curve) as prepared for the transmission experiments presented in Figure 6. The melting curves were obtained with a Roche Diagnostics LightCycler96 Real-Time PCR system. The graph shows the negative normalized change in fluorescence at 514 nm over temperature ($-dF/dT$) for the reporter fluorescent dye FAM (100 nM were added to the sample solution). The excitation wavelength was set to 470 nm. As the temperature rises, the

DNA strands connecting the NP clusters melt. This leads to an increased absorption of the NPs at the excitation and emission wavelength (see center and bottom panels) and, in consequence leads to a decreased fluorescence signal of the reporter molecule. The measurement shows a melting temperature of 54.7 °C for the sample containing clusters of spherical Au NPs and 48.1 °C, for the sample containing clusters of Au NRs. Center: extinction spectrum of clusters of ssDNA-functionalized spherical Au NPs at 40 °C (solid green curve) and at 60 °C (dashed green curve), as prepared for the transmission experiments shown in Figure 6. Both curves were normalized to the optical density of the plasmon resonance peak of the spherical Au NPs at a wavelength of 538 nm at 60°C. The normalized optical density shows an increase at 532 nm when the sample is heated to 60 °C (see vertical black line for reference). This can be attributed to the melting of DNA connecting the clusters at 54.7 °C, as shown in the top panel (green curve). Bottom: extinction spectrum of clusters of ssDNA-functionalized Au NRs at 40 °C (solid red curve) and at 60 °C (dashed red curve), as prepared for the transmission experiments presented in Figure 6. Both curves were normalized to the optical density of the longitudinal plasmon resonance peak of the Au NRs at a wavelength of 1027 nm at 60°C. The normalized optical density shows an increase at 1064 nm when the sample is heated to 60 °C (see vertical black line for reference). This can be attributed to the melting of DNA connecting the clusters at 48.1 °C, as shown in the top panel (red curve). The extinction spectra for the center and bottom panels were obtained with an Agilent Technologies Varian Cary 50 UV-Vis Spectrophotometer.

REFERENCES

1. R. Kofman, P. Cheyssac, A. Aouaj, Y. Lereah, G. Deutsch, T. Bendavid, J. M. Penisson and A. Bourret, *Surface Science*, 1994, **303**, 231-246.

2. H. Petrova, J. Pérez-Juste, I. Pastoriza-Santos, G. V. Hartland, L. M. Liz-Marzán and P. Mulvaney, *Phys. Chem. Chem. Phys.*, 2006, **8**, 814-821.
3. S. Doniach and M. Sunjic, *Journal of Phys. C*, 1970, **3**, 285.
4. J. J. Yeh and I. Lindau, *Atomic Data and Nuclear Data Tables*, 1985, **32**, 1.
5. J. F. Moulder, J. Chastain and R. C. King, *Handbook of X-Ray Photoelectron Spectroscopy: A Reference Book of Standard Spectra for Identification and Interpretation of XPS Data*, Physical Electronics Eden Prairie, MN, 1995.