Electronic Supplementary Material (ESI) for Nanoscale.


# Supporting Information:

## Effective PEGylation of Gold Nanorods

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## Content

**TEM analysis of extracted and etched AuNR@PEGMUA**

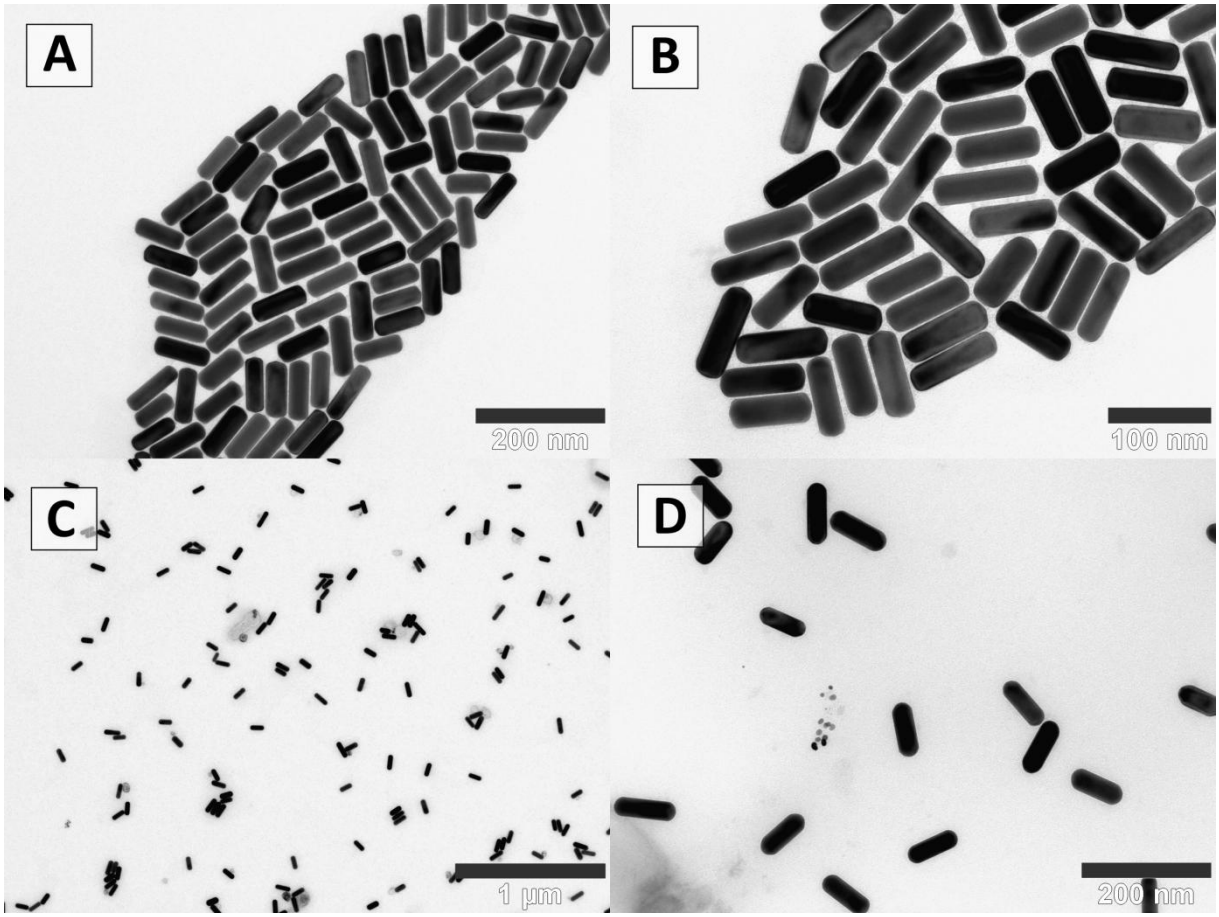


**Fig. S1** TEM-measurements of AuNR before and after etching. AuNR@PEGMUA after extraction with chloroform (A and B) show no signs of aggregation or morphological changes. They were reacted with 25 µM PEGMUA before extraction. A sample of the same batch was reacted with 25 µM PEGMUA before extraction and 50 mM PEGMUA after extraction and then etched with 25 mM KCN. Only very little etching was observed even after 2 weeks and the etching kinetics indicate that a steady state was reached (Fig. 4 in the main text, c(PEGMUA) = 75 µM as the sum of PEGMUA concentrations before and after extraction). TEM measurements of the sample after 2 weeks (C and D) reveal that most AuNR seem practically unchanged, whereas some residues of nearly completely etched AuNR were observed. These findings are in accordance with the interpretation of the UV-vis data discussed in the main text. Randomly analyzing 20-60 AuNR, no statistical significant differences in length and width of the AuNR@PEGMUA before and after etching with 25 mM was observed.

## Aggregation of AuNR after CTAB removal

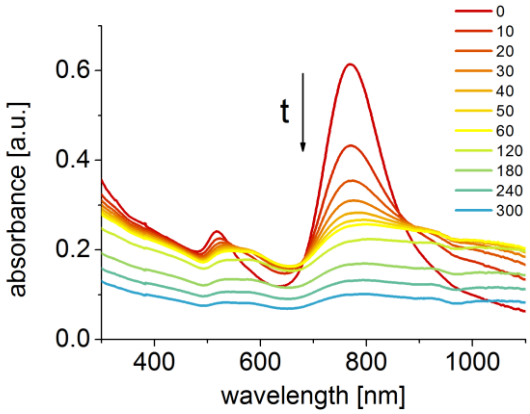


**Fig. S2** Monitoring of the aggregation of AuNR after removal of CTAB by chloroform extraction. The broadening of the transversal and longitudinal localized surface plasmon resonance band (LSPRB) indicate aggregation and the decrease of absorbance indicates concentration loss due to sedimentation of aggregates.[1,2] The time after extraction with chloroform is indicated in minutes by the color code.

## ATR-FTIR spectra of AuNR@PEGMUA

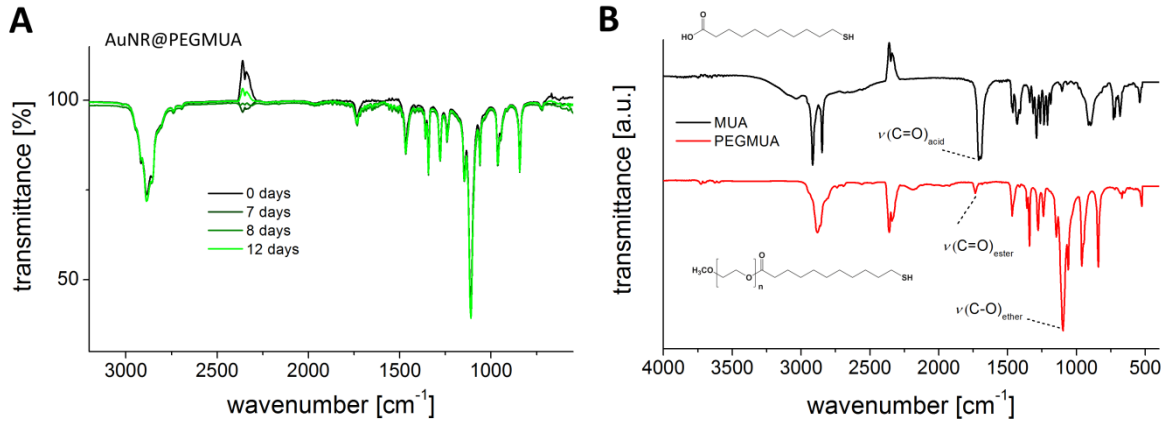


**Fig. S3** ATR-FTIR spectra of AuNR@PEGMUA after different storage times as indicated by the color code (A). The sample was stored at room temperature in a closed container, the pH was ~ 3. No signs of hydrolysis of PEGMUA were observed. ATR-FTIR spectra of the potential hydrolysis product 11-mercaptoundecanoic acid (MUA) and PEGMUA are shown for comparison with their structures and some vibrations indicated (B).

**Detailed study of the ligand exchange with assisting chloroform-extraction**

First, we compared the stabilizing effect of different PEGMUA concentrations adjusted before extraction, with no PEGMUA addition after extraction. The reaction time with the AuNR was $t_1$ = 22 hours. We found a higher stabilization for $c_1$(PEGMUA) = 25 µM and 50 µM compared to 10 µM (Fig. S4).

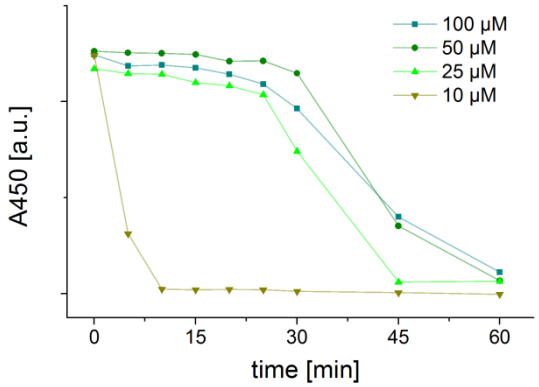


**Fig. S4** Etching kinetics of AuNR@PEGMUA reacted with different amounts of PEGMUA before extraction. The concentrations $c_1$(PEGMUA) are indicated by the color code. No PEGMUA was added after extraction. Lines are guide to the eye.

100 µM PEGMUA provided no significantly higher stability compared to 25 µM and 50 µM. Based on these observations we decided to compare AuNR@CTAB reacted with 25 µM or 50 µM PEGMUA before extraction. The reaction time before extraction was $t_1$ = 22 hours for all samples discussed in the following to allow for some ligand exchange. The samples were then extracted with chloroform. No migration of AuNR into the chloroform phase was observed, neither for AuNR@CTAB without nor with PEGMUA added before extraction. In extracts of different AuNR-batches incubated with 25 µM PEGMUA we identified 70-90 mol% CTAB, 8-26 mol% oleate and 0.5-3.5 mol% PEGMUA by NMR-analysis. Thus, CTAB is efficiently removed but also unbound PEGMUA. After extraction with chloroform the AuNR were washed by centrifugation (4 x 20 min, 9000 g) and incubated with different concentrations of PEGMUA (reaction 2 with reaction time $t_2$ and PEGMUA concentration $c_2$(PEGMUA)). We tested the effect of the second reaction time $t_2$ for both AuNR, reacted with 25 µM or 50 µM before extraction. The AuNR were reacted with $c_2$(PEGMUA) = 50 µM for different times $t_2$ = 30-300 minutes. Then KCN was added ($c$ = 25 mM) and the etching reaction monitored. Only very little differences in stabilization were observed (Fig. S5) and $t_2$ was set to 120 minutes for the following experiments.

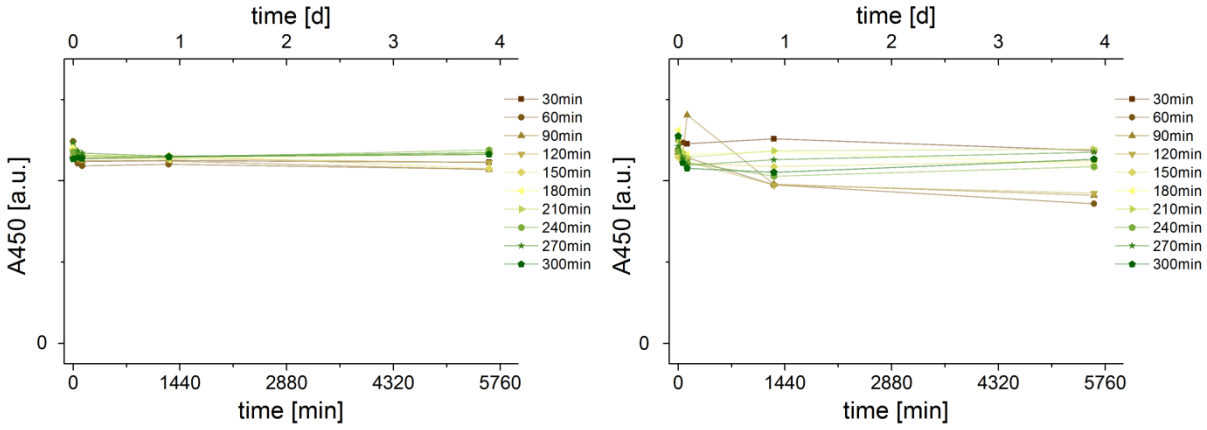


**Fig. S5** A450 as a function of etching time ($c$(KCN) = 25 mM) for different AuNR@PEGMUA samples. AuNR@PEGMUA were reacted with 25 µM (left) or 50 µM (right) PEGMUA before extraction ($t_1$ = 22 h) and 50 µM PEGMUA after extraction for different times $t_2$ as indicated by the color codes. No significant influence of the reaction times $t_2$ within the tested range was observed. The samples reacted for $t_2$ > 120 min seem slightly more stable in the case of AuNR reacted with 50 µM before extraction (right). Lines are guide to the eye.

Fig. 4 in the main text shows the results obtained for AuNR reacted with $c_1$(PEGMUA) = 25 µM before and different concentrations $c_2$(PEGMUA) after extraction ($t_1$ = 22 hours, $t_2$ = 2 hours). The plots A450 vs. etching time are shown in Fig. S6.

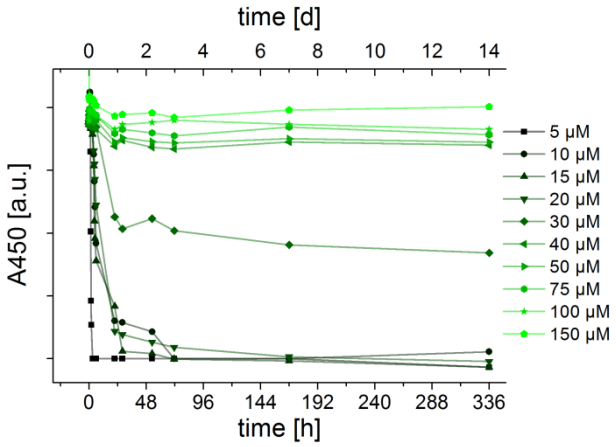


**Fig. S6** A450 as a function of etching time ($c$(KCN) = 25 mM) for different AuNR@PEGMUA samples. AuNR@PEGMUA were reacted with 25 µM PEGMUA before extraction ($t_1$ = 22 hours) and different amounts of PEGMUA, $c_2$(PEGMUA), after extraction ($t_2$ = 2 hours) as indicated by the color code. Lines are guide to the eye.

For maximum stabilization, the minimal PEGMUA concentration added in total ($c_1$(PEGMUA)+ $c_2$(PEGMUA)) was 65 µM in this experiment. Similar results were obtained in a reproduction (Fig. S7) and for AuNR reacted with 50 µM before extraction (Fig. S8).

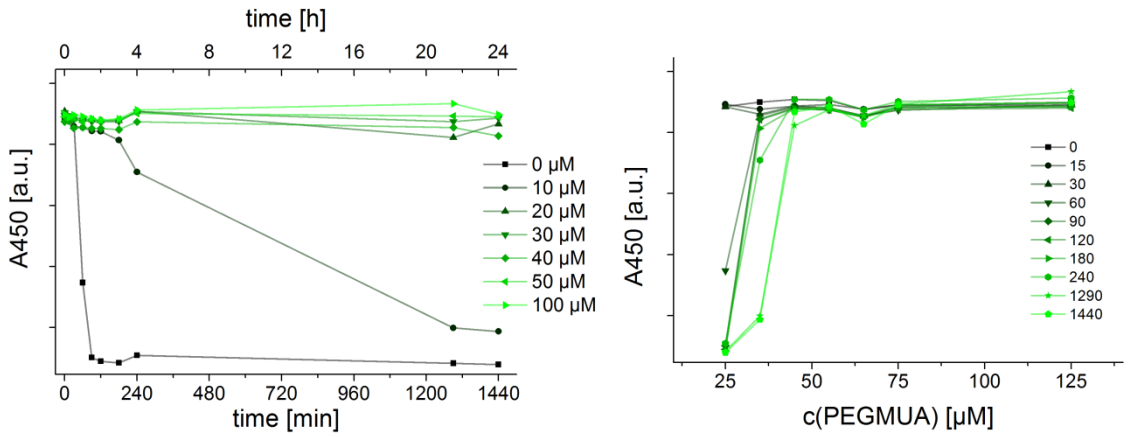


**Fig. S7** Reproduction of the experiments represented in Fig. 4 in the main text and in Fig. S6. A450 is plotted as a function of etching time (left) or of *c*(PEGMUA) (right) for different AuNR@PEGMUA samples reacted with *c*(KCN) = 25 mM. AuNR@PEGMUA were reacted for $t_1$ = 22 hours with 25 µM PEGMUA before extraction and with different amounts of PEGMUA, $c_2$(PEGMUA), as indicated by the color codes (left) for $t_2$ = 2 hours after extraction. In the plot at the right the etching time is indicated by the color codes in minutes and c(PEGMUA) is the sum of PEGMUA concentrations added before and after extraction ($c_1$(PEGMUA)+ $c_2$(PEGMUA)). In this reproduction, slightly less PEGMUA was necessary for maximum stabilization. Lines are guide to the eye.

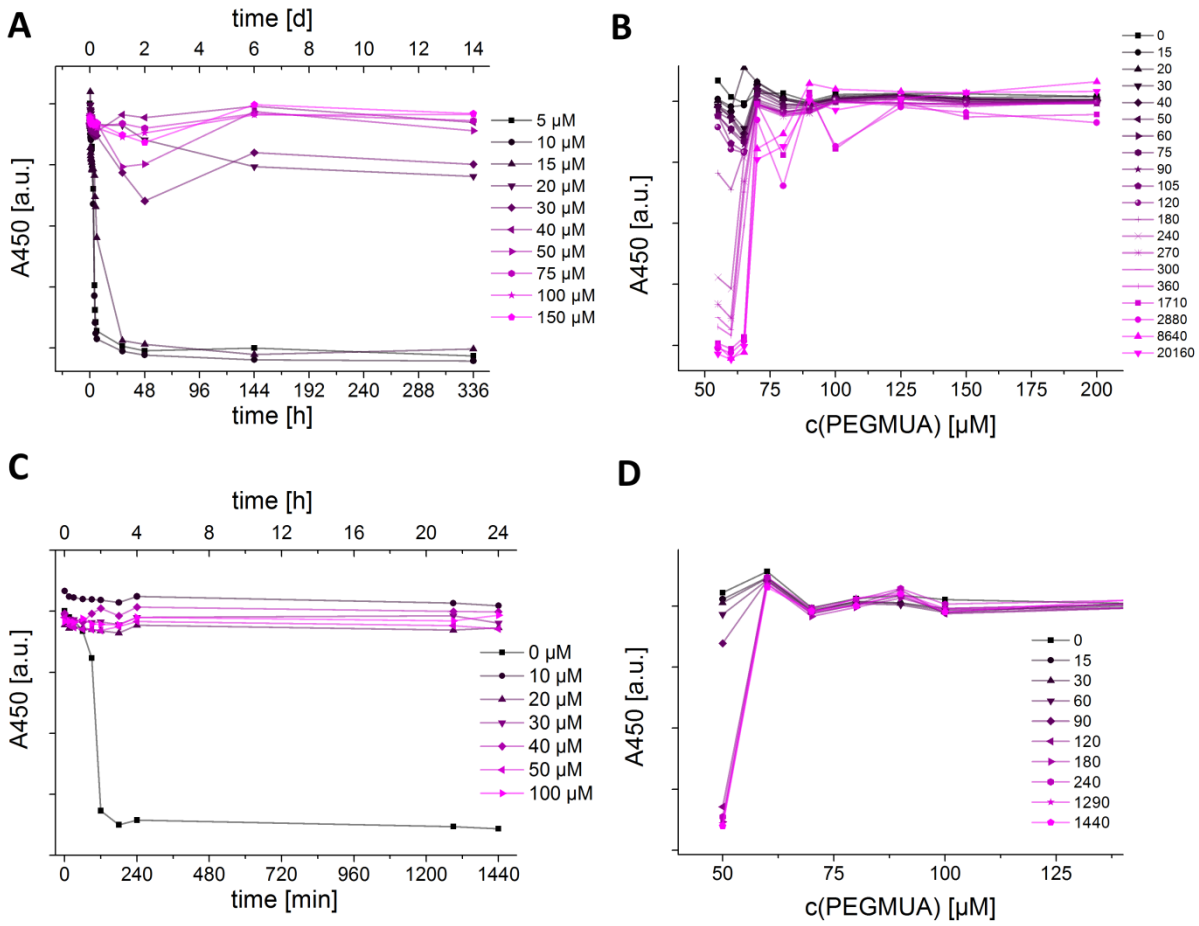


**Fig. S8** A450 as a function of etching time (A and C) or of *c*(PEGMUA) (B and D) for different AuNR@PEGMUA samples reacted with *c*(KCN) = 25 mM. AuNR@PEGMUA were reacted for $t_1$ = 22 hours with 50 µM PEGMUA before extraction and different amounts of PEGMUA, $c_2$(PEGMUA), as indicated by the color codes (A and C) for $t_2$ = 2 hours after extraction. In B and D the etching time is indicated by the color codes in minutes and c(PEGMUA) is the sum of PEGMUA concentrations added before and after extraction ($c_1$(PEGMUA)+ $c_2$(PEGMUA)). The set of experiments represented in C and D is a reproduction of the set represented in A and B. The reproducibility is good but not excellent; in the reproduction slightly less PEGMUA was necessary for maximum stabilization. Lines are guide to the eye.

The minimal amount of PEGMUA for maximum stabilization was 45-70 µM in all cases. We concluded that an addition of 25 µM PEGMUA before extraction and a total addition of 75 µM was sufficient to provide maximum stabilization for these AuNR. Adding 50 µM PEGMUA before extraction brought no benefit in terms of stabilization or minimum PEGMUA amount. To substantiate our results, we compared the reproducibility of the experiments for the same batch and for different batches of AuNR. The AuNR were reacted with a total of *c*(PEGMUA) = 75 µM with or without extraction. For the extracted samples $c_1$(PEGMUA) = 25 µM were added before and $c_2$(PEGMUA) = 50 µM after the extraction, the reaction time 1 was $t_1$ = 22 hours and $t_2$ = 2 hours for all samples. As expected, the stability of the extracted samples was much higher than that of the non-extracted ones (Fig. S9). Moreover, the reproducibility was much better for extracted samples of the same batch and of different AuNR-batches.

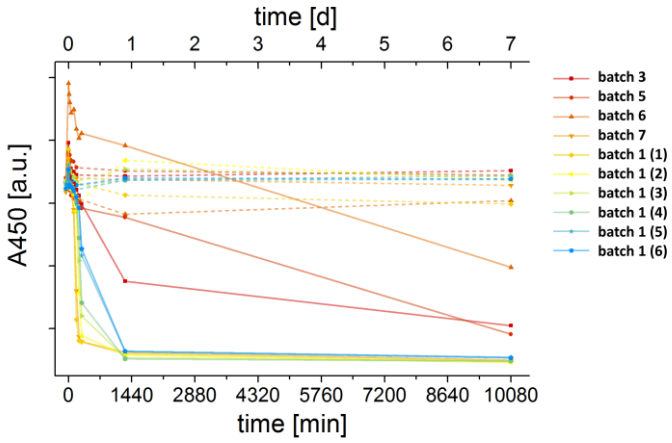


**Fig. S9** Etching kinetics (A450 vs. etching time) for extracted (dashed lines) and non-extracted (solid lines) samples prepared with the same total amount of PEGMUA as described in the text. Five different batches of AuNR were used and five reproductions with one and the same batch were tested as indicated by the color code. The stability of the extracted samples is always higher and the reproducibility of the experiments is better. The increases of A450 for some non-extracted samples in the initial phase of the etching reaction are caused by aggregation and possibly adhesion. Lines are guide to the eye.

## CTAB removal by chloroform extraction and by centrifugation

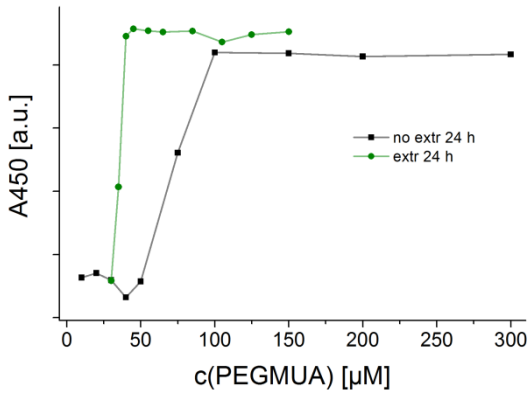


**Fig. S10** Comparison of the etching of AuNR@PEGMUA functionalized with assisting extraction (extr; green circles) and without assisting extraction (no extr; dark grey squares) but removal of CTAB by two centrifugation steps before reaction with different amounts of PEGMUA. The reaction time was $t_1$ = 16 hours. For the extracted samples, the reaction time after extraction was $t_2$ = 1 hours. The A450 values after 24 hours etching reaction are plotted versus the total PEGMUA concentration. For the extracted samples 40 µM PEGMUA (25 µM before and 15 µM after extraction) was sufficient for maximum stabilization, for the non-extracted samples 100 µM PEGMUA was necessary for maximum stabilization.

**CTAB removal with ethanol**

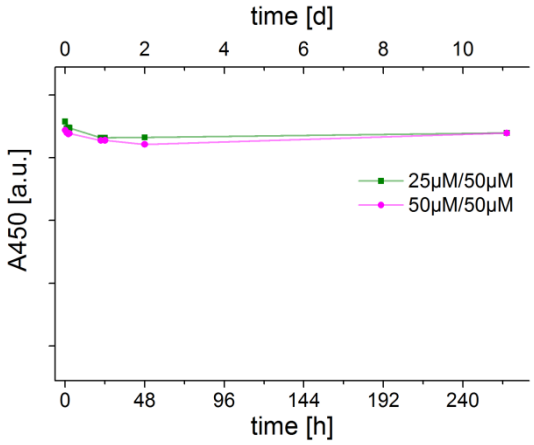


**Fig. S11** A450 as a function of etching time (*c*(KCN) = 25 mM) for different AuNR@PEGMUA samples. AuNR@PEGMUA were reacted with 25 µM or 50 µM PEGMUA for $t_1$ = 22 hours. Then CTAB was removed by repeated centrifugation, replacing the supernatants with ethanol. After the CTAB removal the samples were reacted with 50 µM PEGMUA for $t_2$ = 2 hours. No significant etching was observed, indicating efficient removal of CTAB leading to efficient ligand exchange and a high stability of the final samples. Lines are guide to the eye.

**Effect of reaction time for AuNR@PEGSH**

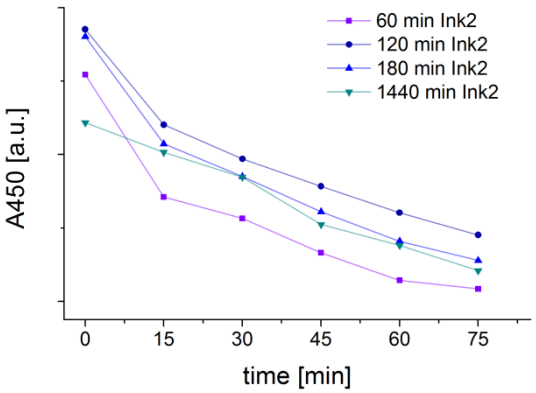


**Fig. S12** A450 as a function of etching time (*c*(KCN) = 5 mM) for different AuNR@PEGSH samples. AuNR@CTAB were reacted with 25 µM PEGSH for $t_1$ = 22 hours before extraction with chloroform and for different reaction times $t_2$, as indicated by the color code, with 50 µM PEGSH. No stabilizing effect of longer reaction times with PEGSH was observed.

**Stability of AuNR during multiple centrifugation steps**

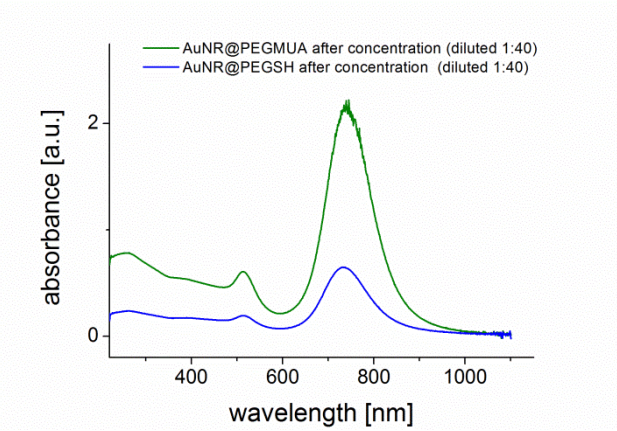


**Fig. S13** Absorbance spectra of AuNR@PEGMUA and AuNR@PEGSH after concentration by repeated centrifugation. The theoretical concentration factor was 160 for both samples. In the case of AuNR@PEGSH, ~80 % of the sample were lost because of irreversible adhesion to vessel walls and possibly aggregation. In the case of AuNR@PEGMUA < 20 % were lost and no adhesion was observed.

**STEM-EDX-mapping analysis of gold and silver in AuNR**

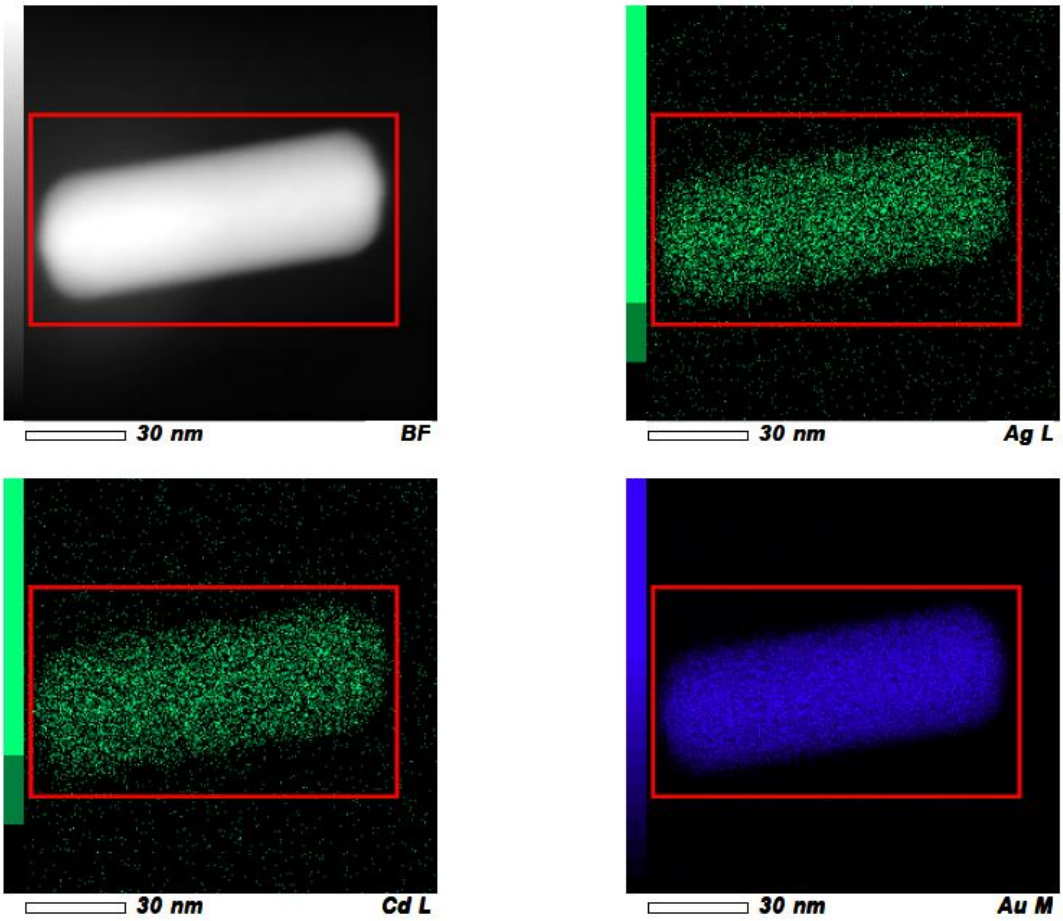


**Fig. S14** STEM-EDX-mapping of a single AuNR. The contributions of Ag-L, Cd-L and Au-M were mapped. The signal intensities were integrated over the areas indicated by the red lines. Note the different color scales for the intensity. The Cd-L contribution was tested as an indicator of the noise, that is higher in the area of the AuNR because more Bremsstrahlung is produced. The sample contained no Cd. The integrated Ag-L signal is just slightly above the noise level, indicating low amounts (< 3 atom%) of silver. The results are summarized in Table S1.

**Table S1** Results of the STEM-EDX mapping shown in Fig. S14.

| element | (keV) | counts | mass% | error% | atom% |
|---|---|---|---|---|---|
| Ag-L | 2.984 | 1027.75 | 1.24 | 5.16 | 2.24 |
| Cd-L | 3.133 | 206.29 | 0.25 | 25.48 | 0.43 |
| Au-M | 2.12 | 78242.63 | 98.51 | 0.08 | 97.32 |

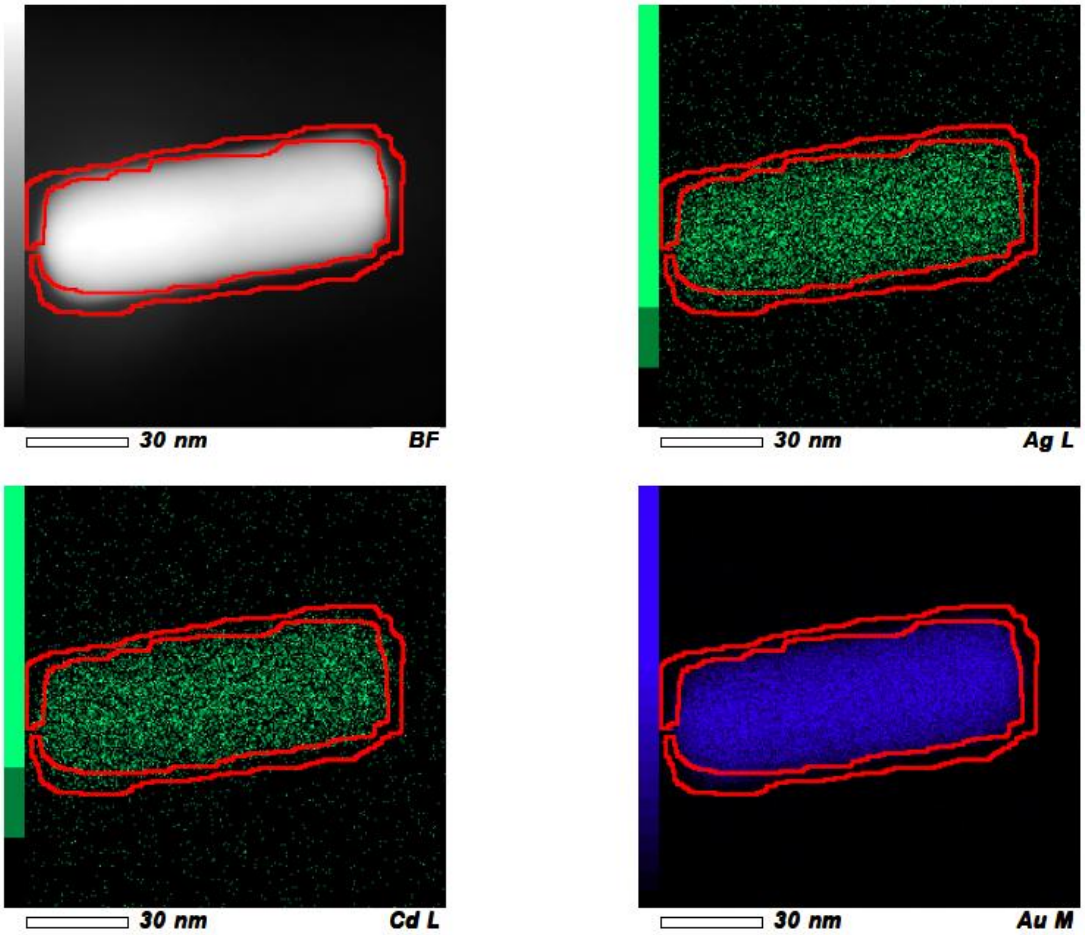


**Fig. S15** STEM-EDX mapping as in Fig. S14. Here, the signal was integrated only near the AuNR-surface as indicated by the red lines. A significantly higher contribution of Ag-L is obtained, suggesting that Ag is located near or at the AuNR-surface. The Cd-L contribution was not above the noise level. The results are summarized in Table S2.

**Table S2** Results of the STEM-EDX-mapping shown in Fig. S15.

| element | (keV) | counts | mass% | error% | atom% |
|---|---|---|---|---|---|
| Ag-L | 2.984 | 394.89 | 7.44 | 2.91 | 12.8 |
| Cd-L | - | - | - | - | - |
| Au-M | 2.12 | 4720.35 | 92.56 | 0.3 | 87.2 |

**Residual CTAB**

Comparing the IR-spectra in Fig. 6C and Fig. 6B in the main text it appears that the amounts of CTAB differ between the AuNR@PEGMUA samples. This is possible because of different experimental conditions which are described in detail in the methods section of the main text. However, a quantitative analysis of the IR-spectra is not possible. Firstly, the AuNR cause strong backgrounds (which have been corrected in the spectra shown) and the relative amounts of ligands are very low (even at very high coverage). Secondly, as can be clearly seen in Fig. 6A, the relative signal intensities of CTAB vary significantly when it is bound to AuNR, possibly because of surface enhancement effects in the dried AuNR films.[3] The intensities of the methylene asymmetric and symmetric stretching vibrations (at $\nu$ = 2918 $cm^{-1}$ and 2848 $cm^{-1}$) are enhanced relatively to all other vibrations in the presence of AuNR (Fig. 6A). These vibrations, however, are not unique for CTAB, but also occur in oleate and in the $C_{10}$-alkylene spacer of PEGMUA. Probably the most sensitive and straightforward technique for CTAB detection is MALDI-TOF,[4] sometimes also termed NALDI-TOF (nanostructure assisted laser desorption ionization-time of flight mass spectrometry),[5] depending on whether a standard matrix is used or the nanoparticles themselves to assist ionization. CTAB is ionized very well, even without any matrix, and thus can be detected in very low concentrations.[6] We detected CTAB in all samples prepared for IR and NMR-analyses, AuNR@PEGMUA and AuNR@PEGSH. It has to be noted that oleate has a mass similar to the $CTA^+$-ion that is detected in MALDI-TOF, but in contrast to CTAB it is not ionized easily under MALDI-conditions. MALDI-TOF is not suitable for quantitative analysis though. In the NMR analysis, CTAB could not be identified unambiguously (Fig. S16). Signals at $\delta$ = 0.88 ppm and $\delta$ = 1.25 ppm indicated the presence of methyl- and methylene groups, but again, these occur not only in CTAB but also in oleate (and oxidation products of oleate), PEGMUA and possibly in degradation products of the harsh etching procedure. Thus, a confident estimation of the relative amount of residual CTAB was not possible, but especially MALDI-TOF experiments suggested that residual CTAB was present in all purified samples studied. Kinnear et al. suggested for PEGylated AuNR that some CTAB remained interchelated into the PEG-brush.[7] In the case of PEGMUA, the attractive interactions of CTAB and especially oleate with the ligand layer might be even stronger due to attractive hydrophobic interactions (compare Scheme 1 in the main text). In future studies it should therefore be tested if quantitative CTAB and oleate removal is possible with advanced purification strategies.

**NMR analysis of AuNR@PEGMUA**

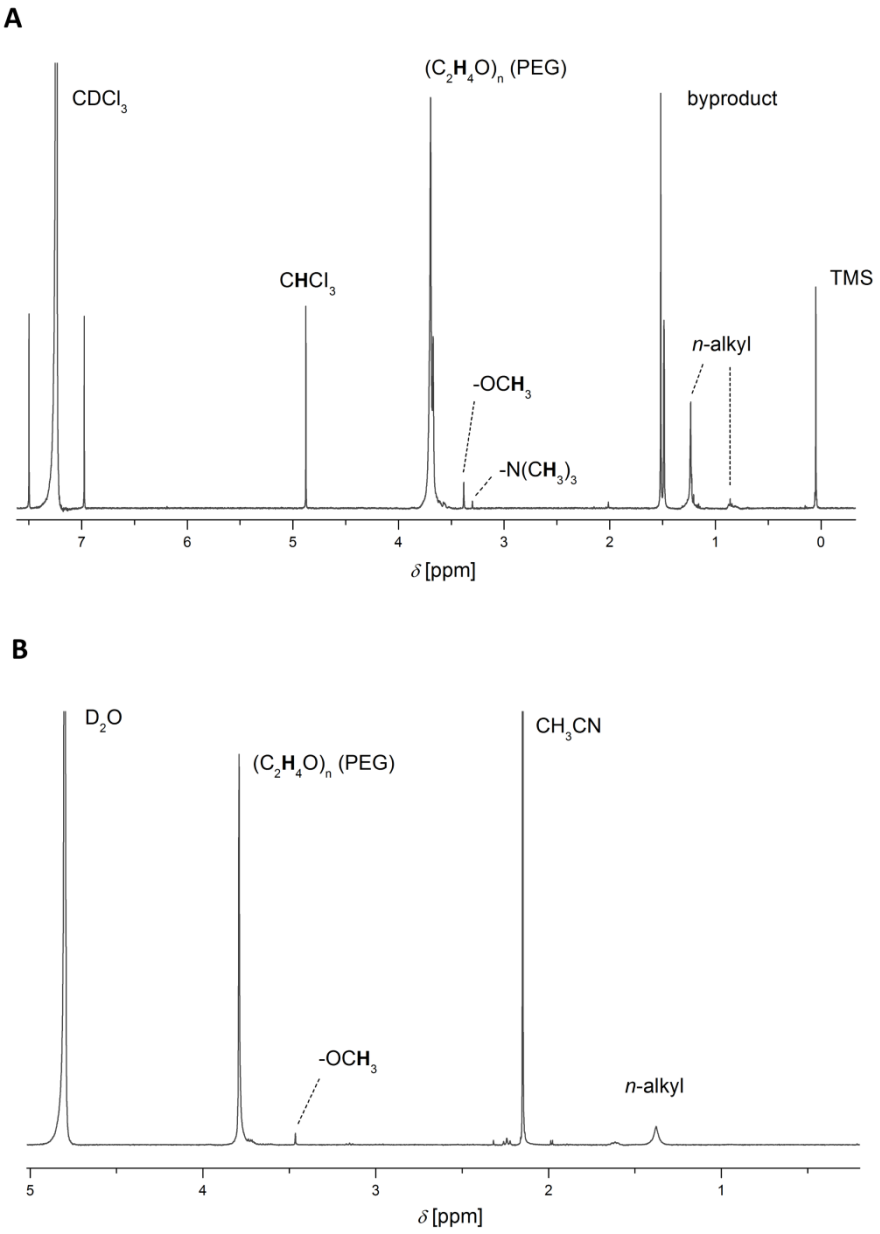


**Fig. S16** NMR-spectra of a highly concentrated and purified sample of AuNR@PEGMUA that was etched with 250 mM KCN. The residue after drying was solved in $CDCl_3$ and this sample was spiked with iodoform ($CHI_3$, s, $\delta$ = 4.88 ppm) for calibration (A). PEG could be identified, confirmed by MALDI-TOF analysis (data not shown). CTAB could not be identified but might be decomposed due to the harsh etching conditions. The signal at $\delta$ = 3.30 ppm might correspond to trimethylammonium-groups. The signals at $\delta$ = 1.50 ppm could not be identified but probably stem from byproducts of the etching. Signals of aliphatic methylene and methyl groups were also observed (*n*-alkyl at $\delta$ = 1.25 and 0.88 ppm). These could stem from MUA as a hydrolysis product of the etching reaction, from CTAB or oleate or oxidation products thereof. The residue that was not soluble in $CDCl_3$, mainly cyano-complexes of gold as confirmed by UV/Vis spectroscopy, was completely dissolved in $D_2O$ and also analyzed by NMR with acetonitrile spiking for calibration (B). PEG and methylene groups ($\delta$ = 1.25 ppm) could be identified but no methyl groups.

**References**


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