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Dipeptide recognition in water mediated by mixed monolayer protected gold nanoparticles

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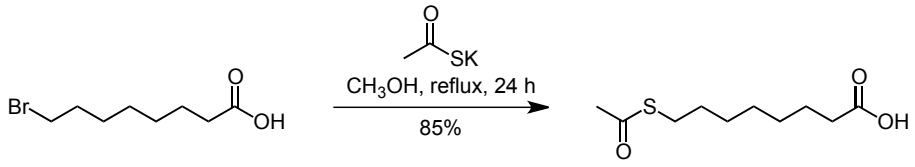
CONTENT

Ligand Syntheses

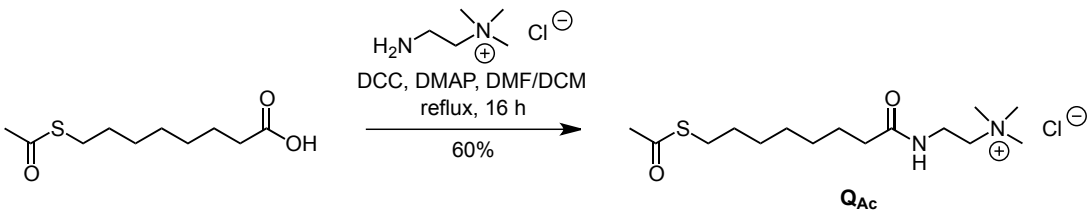
General details. Analyses were carried out as follows: melting points, Müller SPM-X 300; NMR, Bruker DPX 400; MALDI-TOF-MS, Bruker Ultraflex TOF/TOF; elemental analysis, Elementar vario Micro cube; IR, FT-IR System Spectrum BX, Perkin-Elmer; Silica gel 60 A (0.06-0.20 mm) Acros Organics, Silica gel 60 (flash) (0.04-0.06 mm) Macherey-Nagel. All starting materials are commercially available and were used without further purification.

Synthesis of 8-(Acetylthio)octanoic Acid.[1]



To a solution of 8-bromooctanoic acid (3 g, 13.4 mmol) in methanol (100 mL), potassium thioacetate (3 g, 26 mmol) was added and the mixture was refluxed for 24 h. After cooling, the solvent was evaporated and the crude product was dissolved in water (50 mL). The solution was extracted three times with dichloromethane, the organic layer was dried over Na_2SO_4, and the solvent was evaporated. The obtained material was purified by column chromatography (SiO_2, dichloromethane/hexane, 1:1) to afford the product as a beige solid. Yield: 2.5 g, 85%; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ = 2.86 (t, 2H, ^3J = 7.3 Hz, SCH_2), 2.35 (t, 2H, ^{3}J = 7.6 Hz, $COCH_2$), 2.33 (s, 3H, $SCOCH_3$); 1.65-1.55 (m, 4H, 2 × CH_2), 1.38-1.34 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (100.6 MHz, 25 °C, $CDCl_3$) δ = 196.9, 180.2, 34.6, 31.1, 30.1, 29.8, 29.6, 29.4, 29.3, 25.3 ppm; IR wavenumber/cm^{-1}: 3474 (m), 2931 (m), 2858 (w), 1723 (w), 1660 (s), 1487 (w), 1438 (m), 1408 (m), 1386 (s), 1255 (m), 1090 (s), 1063 (w), 658 (s).

Synthesis of Protected Ligand Q_{Ac}.[2]



To a solution of 8-(acetylthio)octanoic acid (600 mg, 2.7 mmol) in dichloromethane (50 mL) and dimethylformamide (5 mL), *N*,*N*'-dicyclohexylcarbodiimide (800 mg, 3.9 mmol) and 4-dimethylaminopyridine (50 mg, 0.4 mmol) were added, followed by 2-amino *N*,*N*,*N*-trimethyl ethanaminium chloride (500 mg, 3.6 mmol). The reaction mixture was refluxed for 16 hours under a nitrogen atmosphere. Afterwards, the solvent was evaporated, cold acetone (2 mL) was added to the residue, and the solution was filtered to remove residual DCU. The filtrate was evaporated and the addition of acetone, filtration, and evaporation was repeated twice more. The crude product was thus obtained as a light yellow sticky oil. Further purification was achieved by flash column chromatography (SiO_2, methanol). Fractions containing the product were collected and evaporated to dryness. The residue was dissolved by addition of dichloromethane (2 mL), and the resulting suspension was filtered to remove silica gel. The filtrate was evaporated to afford the product as a colourless sticky oil. Yield: 650 mg, 60%; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ = 8.69 (s, 1H, NH), 3.74 (m, 4H, 2 × NCH_2), 3.38 (s, 9H, $N(CH_3)_3$), 2.77 (t, 2H, ^3J = 7.5 Hz, SCH_2), 2.25 (s, 3H, $SCOCH_3$), 2.22 (t, 2H, ^3J = 7.7 Hz, $COCH_2$,), 1.60-1.42 (2 × m, 4H, 2 × CH_2), 1.18-1.29 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (100.6 MHz, 25 °C, $CDCl_3$) δ = 196.2, 174.8, 65.8, 54.2, 36.2, 34.4, 30.7, 29.4, 29.1, 29.1, 28.8, 28.6, 25.3 ppm; IR wavenumber/cm^{-1}: 3364 (m) 3252 (m), 3026 (w), 2928 (s), 2855 (m), 1687 (s), 1654 (s), 1541, 1480, 1353, 1133, 955; MALDI-TOF MS *m/z* (%): 303.1 (100) $[M\text{-}Cl]^+$; elemental analysis calcd (%) for $C_{15}H_{31}ClN_2O_2S \cdot 0.5H_2O$: C 51.78, H 9.27, N 8.05, S 9.22; found C 51.64, H 9.00, N 7.91, S 9.13.

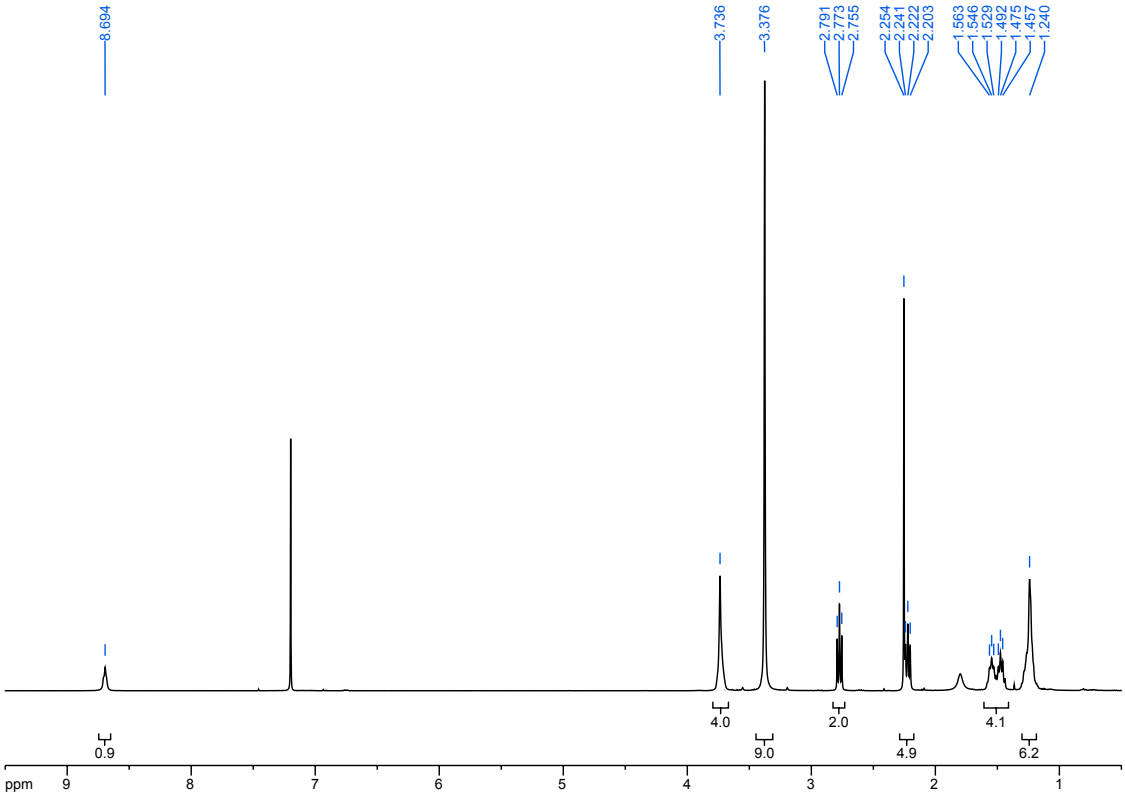


*Figure S1: ^{1}H NMR spectrum of ligand **Q_{Ac}** in $CDCl_3$.*

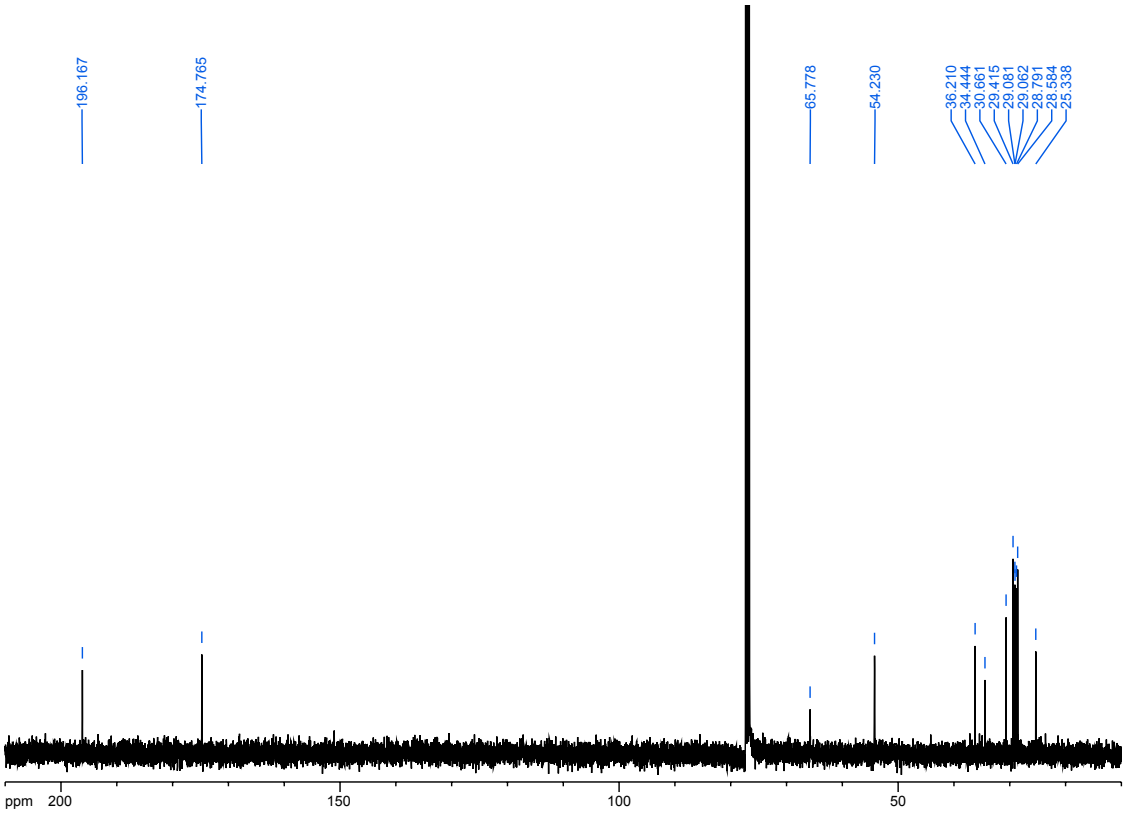
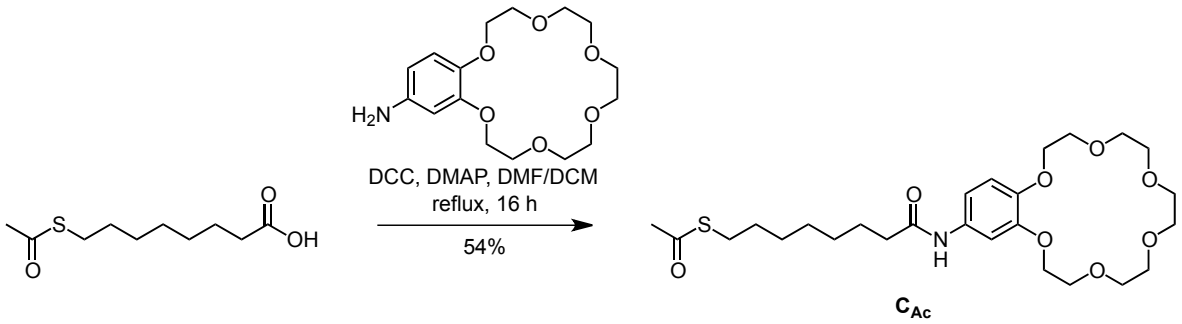


*Figure S2: ^{13}C NMR spectrum of ligand **Q_{Ac}** in $CDCl_3$.*

Synthesis of Protected Ligand C_{Ac}.



To a solution of 8-(acetylthio)octanoic acid (160 mg, 0.7 mmol) in dichloromethane (30 mL) and dimethylformamide (3 mL), *N*,*N*'-dicyclohexylcarbodiimide (185 mg, 0.9 mmol) and 4-dimethylaminopyridine (12 mg, 0.1 mmol) were added, followed by 4'-aminobenzo-18-crown-6 (200 mg, 0.6 mmol). The resulting reaction mixture was refluxed for 16 h under a nitrogen atmosphere. Afterwards, the solvent was evaporated and the product was purified chromatographically (SiO_2, ethyl acetate) to afford a white powder. Yield: 170 mg, 54 %; mp. 113-115 °C; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ = 7.29 (s, 1H, PhH), 6.97 (s, 1H, NH), 6.75 (s, 2H, PhH); 4.04-4.10 (2 × m, 4H, 2 × OCH_2), 3.81-3.85 (m, 4H, 2 × OCH_2), 3.60-3.70 (m, 12H, 6 × OCH_2), 2.78 (t, 2H, ^3J = 7.3 Hz, SCH_2), 2.21-2.26 (m, 5H, $COCH_2$, $SCOCH_3$), 1.70-1.61 (m, 2H, CH_2), 1.55-1.44 (m, 2H, CH_2), 1.25-1.35 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (100.6 MHz, 25 °C, $CDCl_3$) δ = 196.2, 171.1, 149.2, 145.4, 132.2, 114.8, 112.1, 106.9, 70.8, 70.8, 70.7, 70.6, 69.7, 69.6, 69.5, 68.9, 37.5, 30.7, 29.4, 29.0, 28.7, 28.5, 25.4 ppm; IR wavenumber/cm^{-1}: 3285 (m), 3075 (w), 2929 (m), 2854 (m), 1681 (s), 1650 (s), 1601 (w), 1544 (m), 1513 (m), 1410, 1247, 1126 (s), 1086, 1055, 947, 845, 721; MALDI-TOF MS *m/z* (%): 527.4 (63) $[M+H]^+$, 550.5 (100) $[M+Na]^+$, 566.5 (68) $[M+K]^+$; elemental analysis calcd (%) for $C_{26}H_{41}NO_8S$: C 59.18, H 7.83, N 2.66, S 6.08; found C 59.16, H 8.06, N 2.62, S 5.67.

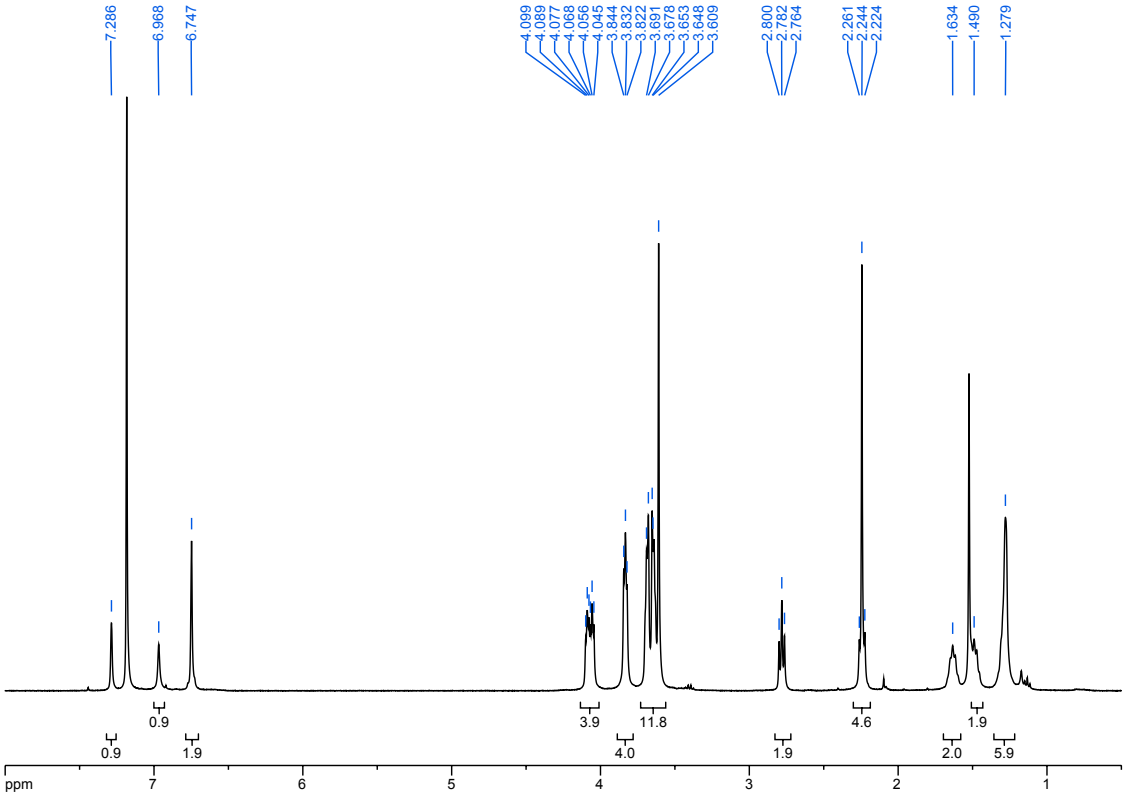


Figure S3: ^{1}H NMR spectrum of ligand $\mathbf{C}_{Ac}$ in $CDCl_3$.

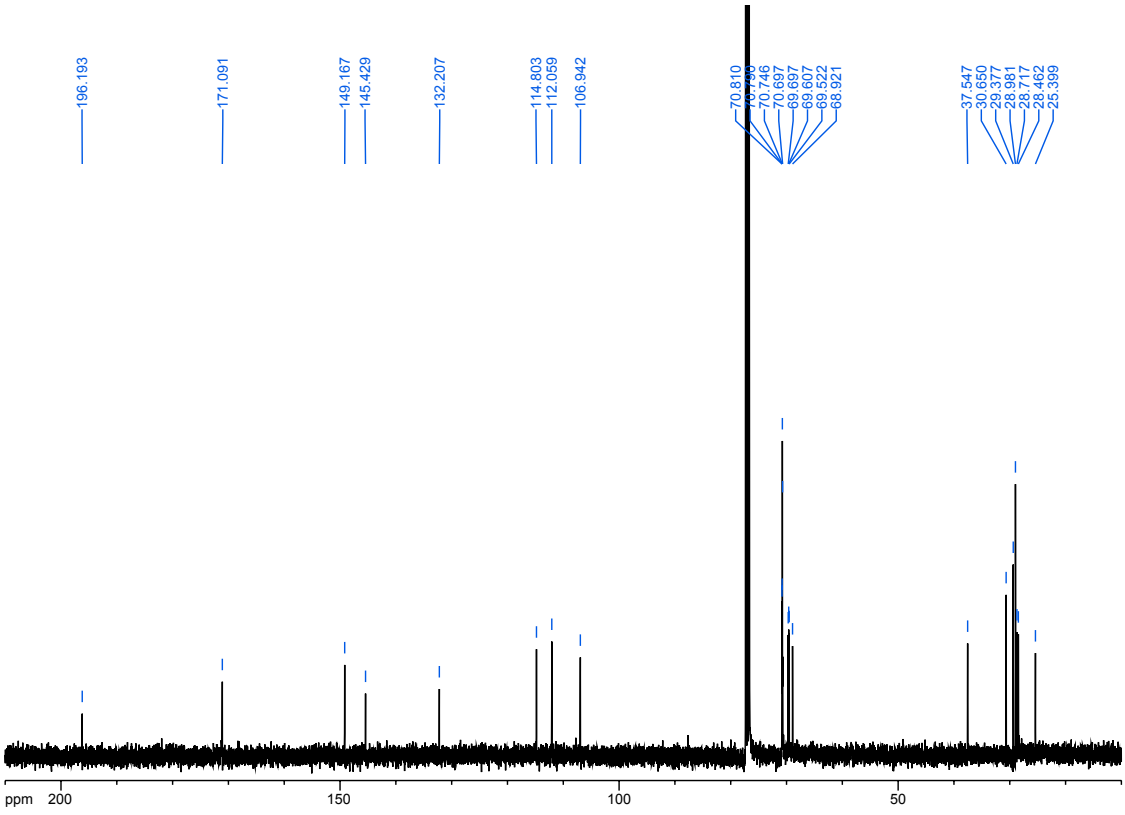
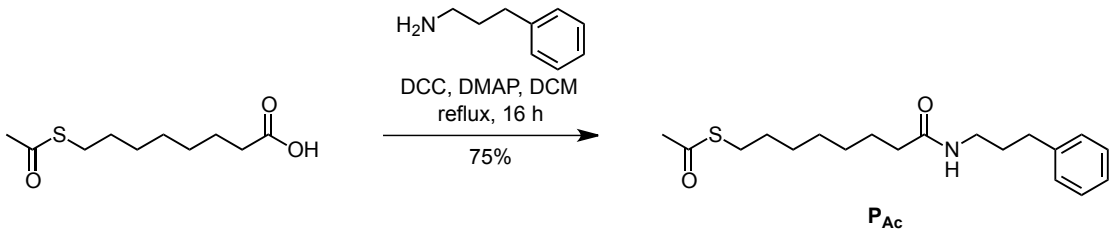


Figure S4: ^{13}C NMR spectrum of ligand $\mathbf{C}_{Ac}$ in $CDCl_3$.

Synthesis of Protected Ligand P_{Ac}.



P_{Ac}

To a solution of 8-(acetylthio)octanoic acid (500 mg, 2.3 mmol) in dichloromethane (30 mL), *N*,*N*'-dicyclohexylcarbodiimide (700 mg, 3.4 mmol) and 4-dimethylaminopyridine (70 mg, 0.6 mmol) were added, followed by 3-phenylpropan-1-amine (0.27 mL, 1.9 mmol). The resulting reaction mixture was refluxed for 16 h under a nitrogen atmosphere. Afterwards, the solvent was evaporated and product was purified chromatographically (SiO_2, acetone/hexane, 1:1). The yellow solid collected after evaporation of the product fractions was dissolved in dichloromethane and precipitated in cold ether. The beige product was collected by filtration, washed with ether, and dried. Yield: 480 mg, 75 %; mp. 205-207 °C; ^{1}H NMR (400 MHz, 25 °C, $CDCl_3$) δ = 7.33-7.04 (m, 5H, PhH), 5.32 (s, 1H, NH), 3.22 (q^{app}, 2H, ^3J = 6.6 Hz, NCH_2), 2.78 (t, 2H, ^3J = 7.2 Hz, SCH_2), 2.59 (t, 2H, ^3J = 7.6 Hz, $PhCH_2$), 2.25 (s, 3H, $SCOCH_3$), 2.04 (t, 2H, ^3J = 7.5 Hz, $COCH_2$), 1.77 (q, 2H, ^3J = 7.3 Hz, CH_2), 1.43-1.54 (m, 4H, 2 × CH_2), 1.19-1.24 (m, 6H, 3 × CH_2) ppm; ^{13}C NMR (100.6 MHz, 25 °C, $CDCl_3$) δ = 196.1, 173.1, 141.4, 128.4, 128.3, 126.0, 39.2, 36.6, 33.3, 31.2, 30.6, 29.4, 29.0, 28.7, 28.5, 25.6 ppm; IR wavenumber/cm^{-1}: 3325 (m), 3063 (w), 3029 (w), 2926 (m), 2851 (m), 1682 (m), 1640 (s), 1544 (s), 1452 (w), 1244 (m), 1115 (m), 1088 (m), 744, 698, 634; MALDI-TOF MS *m/z* (%): 336.3 (100) $[M+H]^+$, 358.3 (56) $[M+Na]^+$, 374.3 (10) $[M+K]^+$; elemental analysis calcd (%) for $C_{19}H_{29}NO_2S$: C 68.02, H 8.71, N 4.18, S 9.56; found C 68.10, H 8.94, N 4.39, S 9.86.

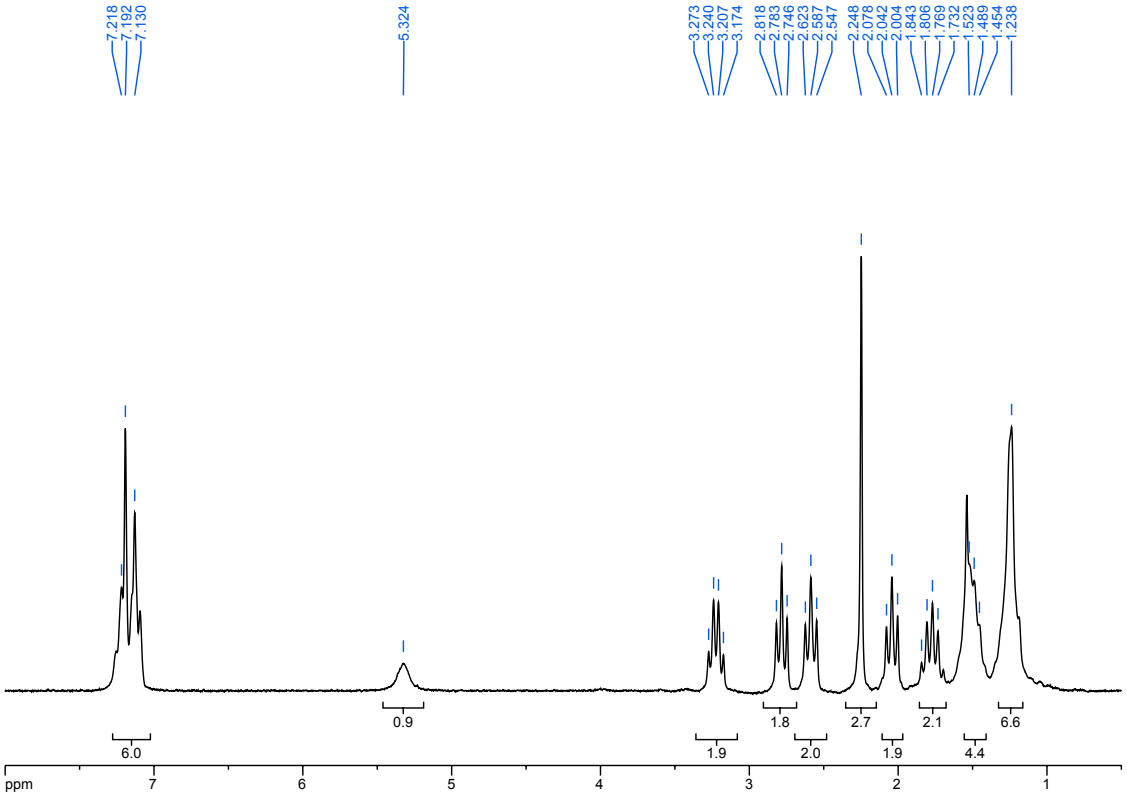


*Figure S5: ^{1}H NMR spectrum of ligand **P_{Ac}** in $CDCl_3$ (the multiplet at ca. 7.2 ppm contains the signal of residual $CHCl_3$).*

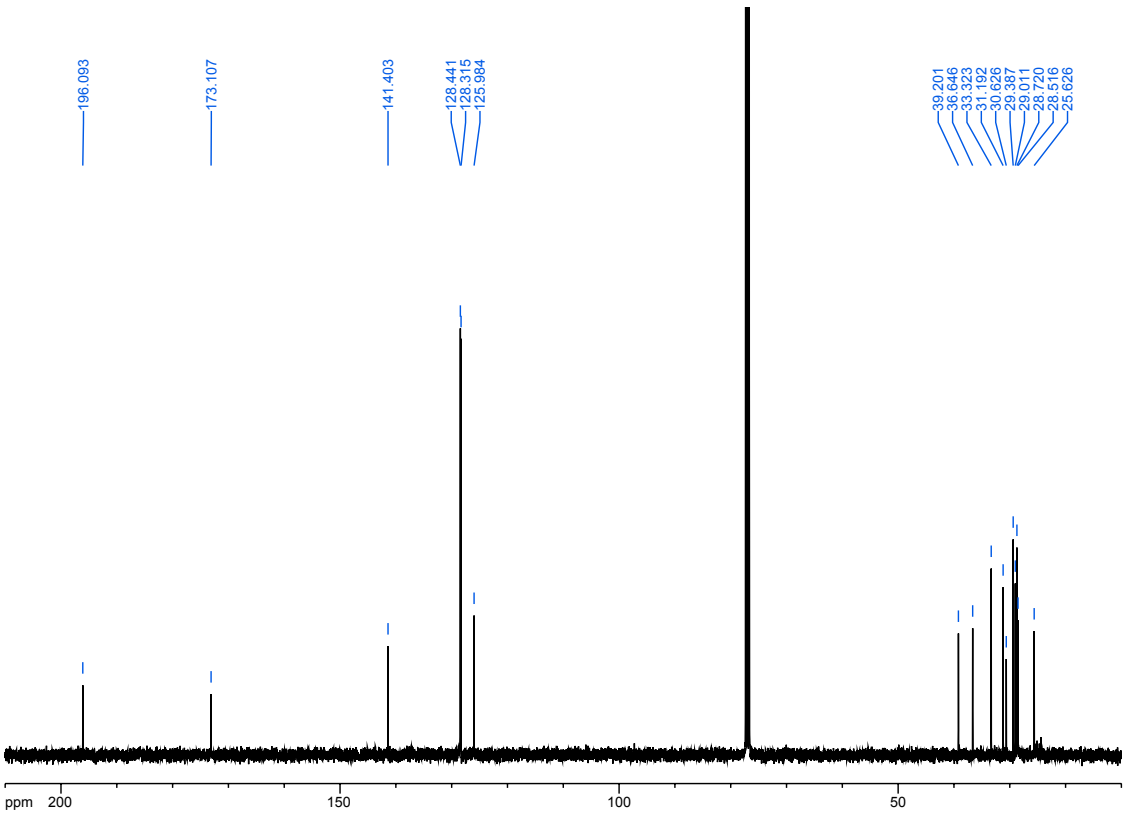


*Figure S6: ^{13}C NMR spectrum of ligand **P_{Ac}** in $CDCl_3$.*

Nanoparticle Synthesis and Characterization

General strategy. Nanoparticle syntheses involved preparation of a solution of dioctylamine-protected gold nanoparticles $\mathbf{NP_A}$ in toluene. The individual ligands required for the respective nanoparticles were independently deprotected by treatment with HCl in methanol.

The reaction conditions of this deprotection step were optimized in a separate study during which the course of reference reactions were followed by ^{1}H NMR spectroscopy. Monitoring the progressive disappearance of the methyl signals of the thioacetate groups indicated that deprotection is complete after stirring for 4 h at room temperature. This method was then used throughout the following syntheses.

The acidic ligand solutions obtained after completion of the reactions were evaporated and the remaining free thiols immediately dissolved in degassed methanol under argon to avoid disulfide formation. Aliquots of these solutions were mixed and then added to an aliquot of the nanoparticle solution. This procedure should ensure that the extent of disulfide formation of the thiols was kept at a minimum. The general strategy is schematically shown in Figure S7 by using the example of the synthesis of nanoparticle $\mathbf{NP_{QPC}}$.

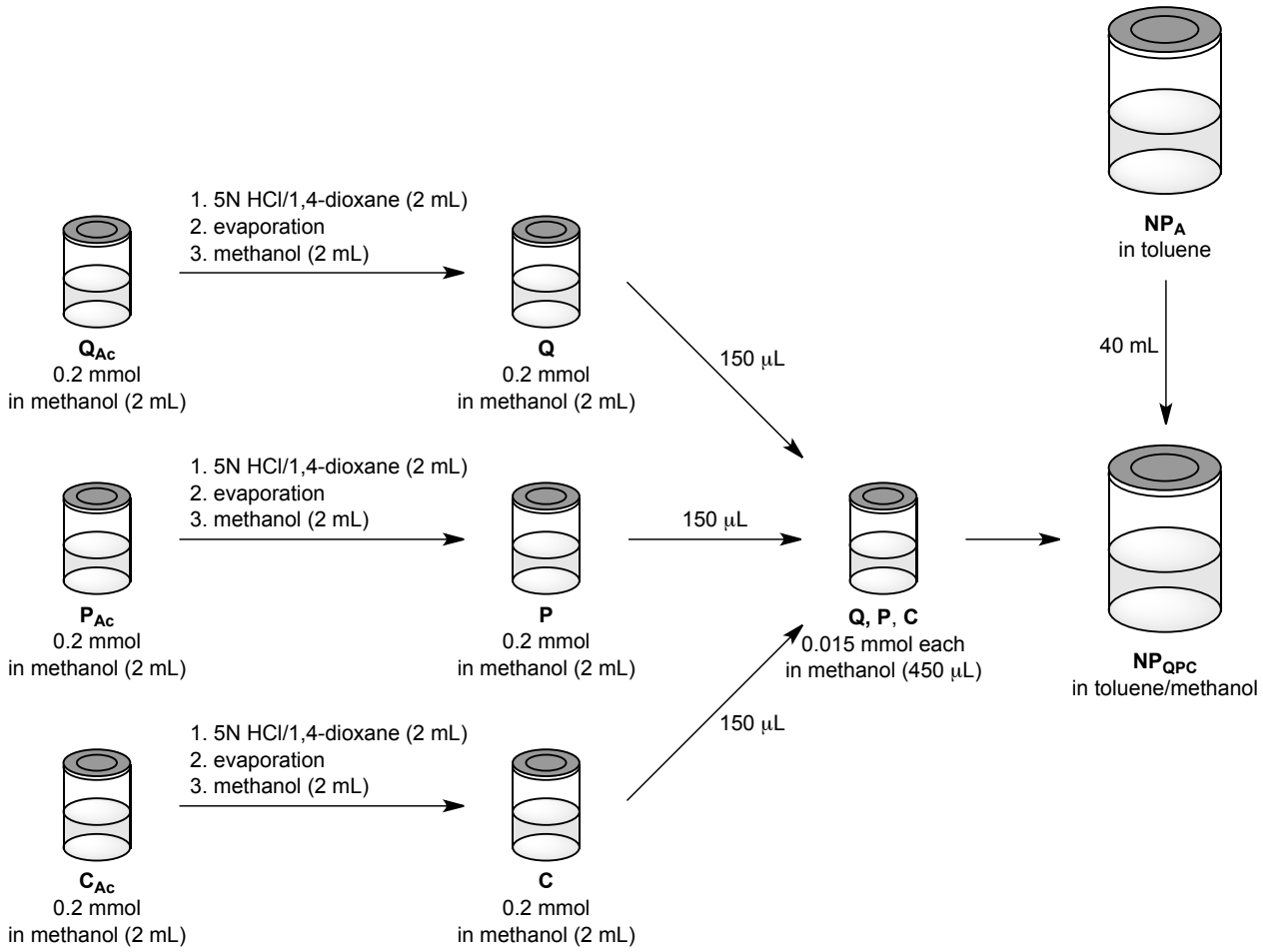


Figure S7: Graphical representation of nanoparticle synthesis by using the example of ***NP_{QPC}***.

Synthesis of Dioctylamine-protected Gold Nanoparticles (NP_A).[2] A solution of $HAuCl_4 \cdot 3\ H_2O$ (40 mg, 0.12 mmol) in water (15 mL) was mixed with a solution of tetraoctylammonium bromide (2.18 g, 4 mmol) in degassed toluene (100 mL). The yellow aqueous solution turned colourless and the organic layer became reddish-orange. Di-*n*-octylamine (2.78 mL, 9.2 mmol) was added and the mixture was vigorously stirred for 40 min while the color disappeared. Afterwards, a solution of $NaBH_4$ (37 mg, 0.97 mmol) in H_2O (10 mL) was added within 20 s under vigorous stirring. The solution was stirred for another 3 h. Then, the aqueous layer was separated and the remaining toluene solution of the nanoparticles was kept under a nitrogen atmosphere for 24 h at 10 °C.

Ligand Deprotection. For the syntheses of the functionalized nanoparticles, the required ligands were deprotected independently. To this end, the individual ligands (0.2 mmol) were dissolved in degassed methanol (2 mL) in separate vials and the solutions were purged thoroughly with argon. Afterward, HCl in dry 1,4-dioxan (5 N, 2 mL) was added to each solution and the resulting

mixtures were stirred at 25 °C under argon. After 4 h, each sample was evaporated under vacuum and the residue dissolved in degassed methanol (2 mL) under argon.

Synthesis of Functionalized Nanoparticles. Subsequently, aliquots of these solutions containing the molar amounts of ligands specified in Table S1 were transferred with a syringe under argon into a vial closed with a septum and mixed. The resulting solution was transferred to the toluene solution of $\mathbf{NP_A}$ (40 mL) under an argon atmosphere in another vial. This reaction mixture was stirred for 2 h at 25 °C, water (2 mL) was added, and the nanoparticles were transferred to the aqueous layer by stirring for 30 min. The aqueous layer was separated, the solvent was removed, and the nanoparticles were purified by dissolving them in methanol and removing the solvent by centrifugation through a molecular weight cutoff membrane (Hydrosart membrane, 5K). This procedure was repeated four times. Finally, the nanoparticle layer was collected and dried. Purity was checked by ^{1}H NMR spectroscopy using D_2O as solvent. The amounts of the obtained nanoparticles are specified in Table S1.

Table S1: Conditions used for the preparation of nanoparticles $\boldsymbol{NP_Q}$, $\boldsymbol{NP_{QC}}$, $\boldsymbol{NP_{QP}}$, $\boldsymbol{NP_{QPC}}$.

nanoparticles	**Q** / mmol	**C** / mmol	**P** / mmol	yields / mg
$\mathbf{NP_Q}$	0.04	-	-	17
$\mathbf{NP_{QC}}$	0.02	0.02	-	20
$\mathbf{NP_{QP}}$	0.02	-	0.02	15
$\mathbf{NP_{QPC}}$	0.015	0.015	0.015	19

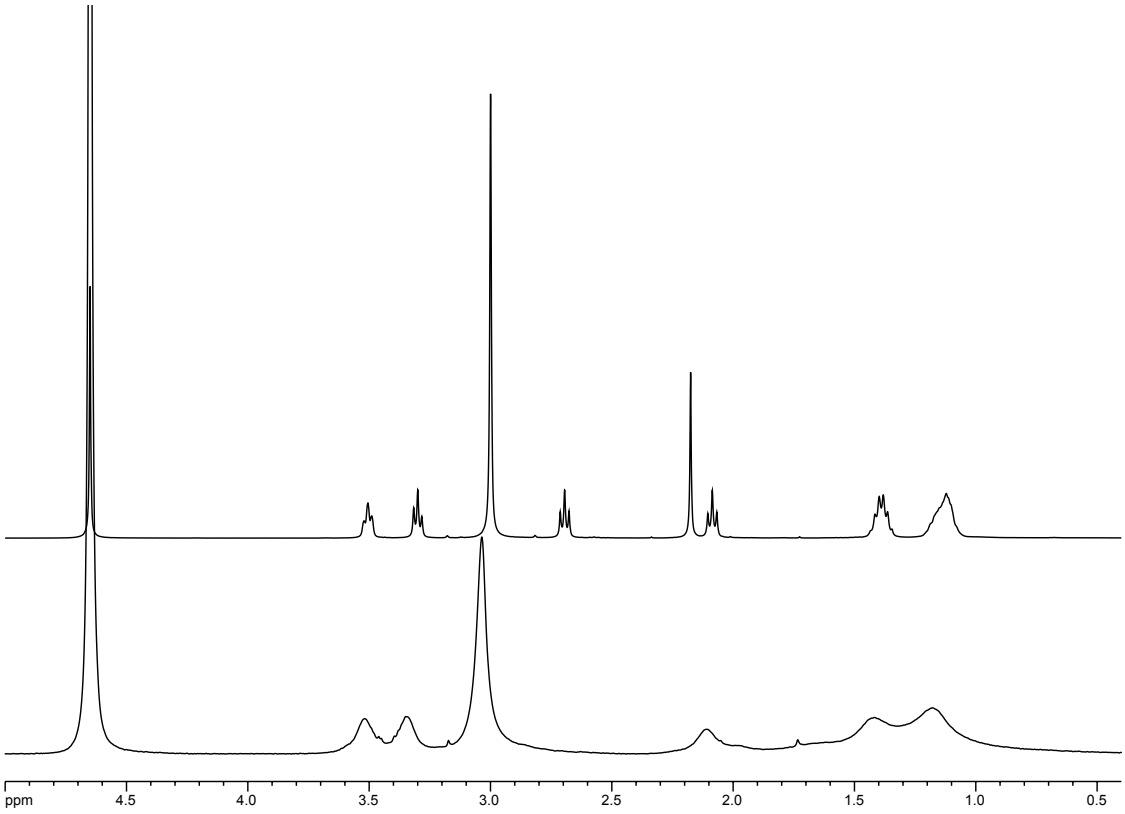


Figure S8: ^1H NMR spectrum of $\boldsymbol{NP_Q}$ *in* D_2O *(bottom) in comparison with the spectrum of the protected ligand* $\boldsymbol{Q_{Ac}}$ *(top) in the same solvent.*

UV/vis Spectroscopy. $\mathbf{NP_Q}$ was characterized by UV/vis spectroscopy (Varian Cary 100 Conc UV/vis Spectrophotometer) to estimate the average size of the nanoparticles. To this end, a solution of $\mathbf{NP_Q}$ (2 μM) in water was prepared and the UV/vis spectrum recorded in the range 200-800 nm. The spectrum exhibits a very weak absorption band at ca. 515 nm indicating that the average diameter of gold core of $\mathbf{NP_Q}$ is around 2 nm.[3]

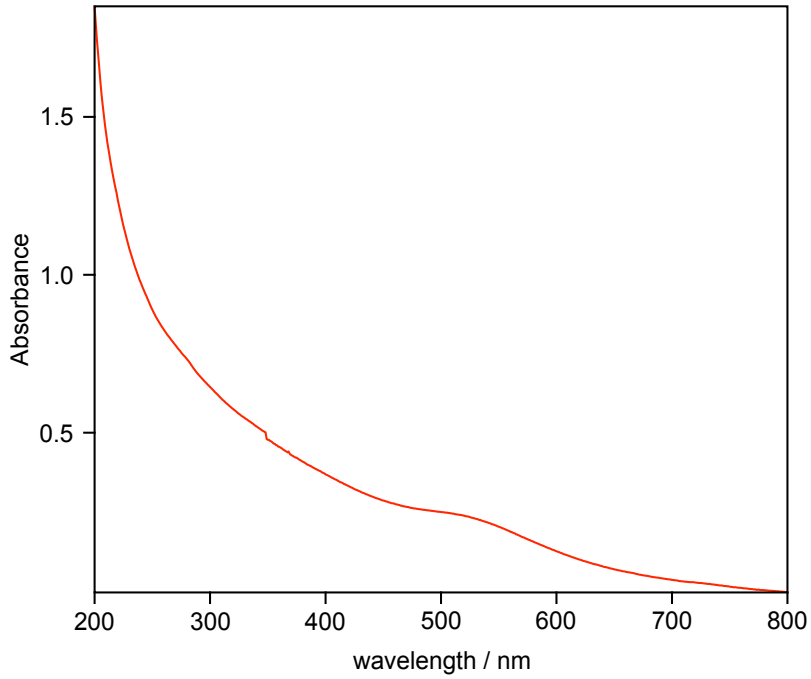


Figure S9: UV/vis spectrum of ***NP$_Q$*** *(0.2 µM) in water.*

Transmission Electron Microscopy. TEM images of the nanoparticles were recorded by using a JEOL JEM1011 microscope with an acceleration voltage of 80 kV. The images were processed with the program *ImageJ* to determine the average diameters of the nanoparticles.

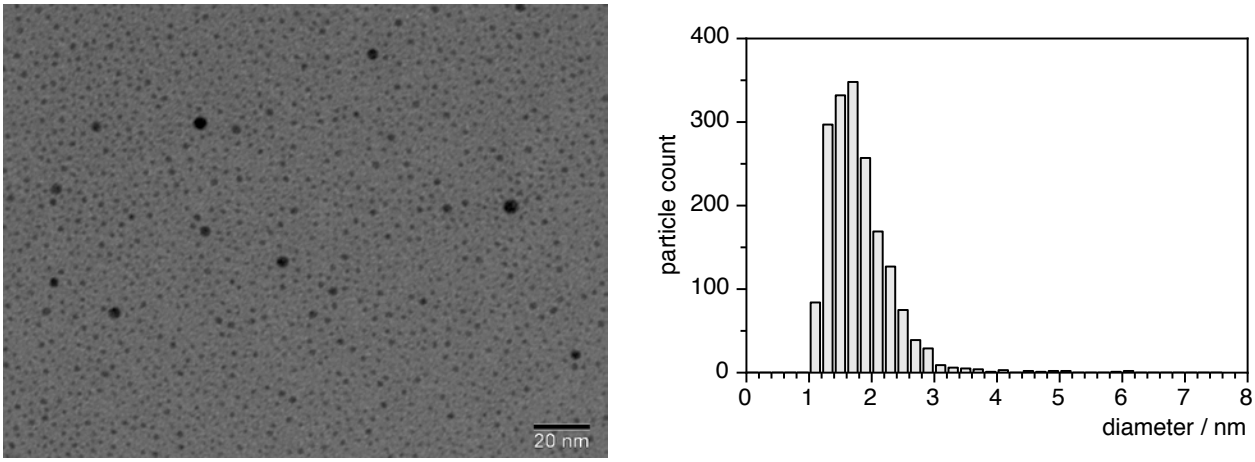


Figure S10: TEM image of ***NP$_Q$****.*

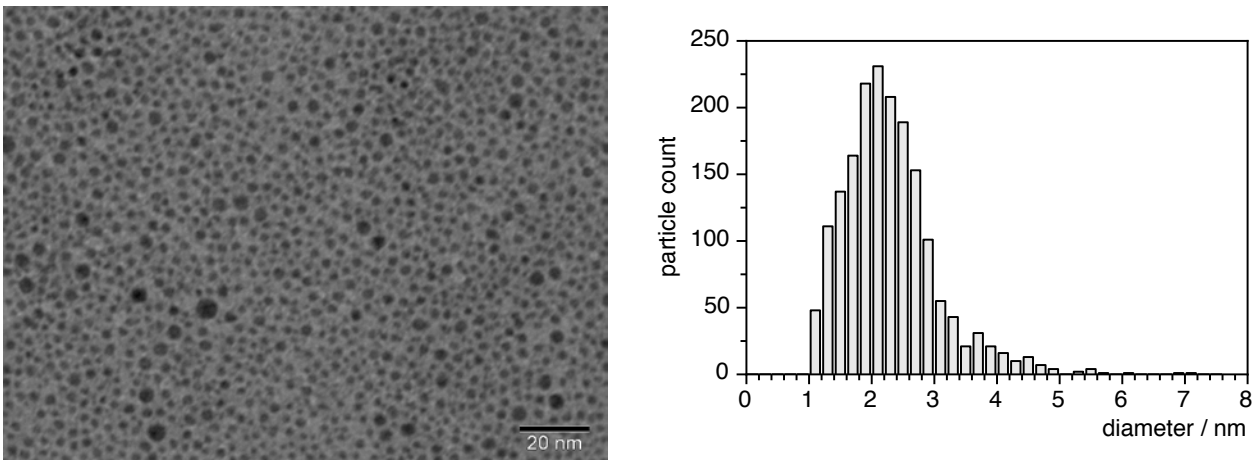


Figure S11: TEM image of $\boldsymbol{NP_{QC}}$.

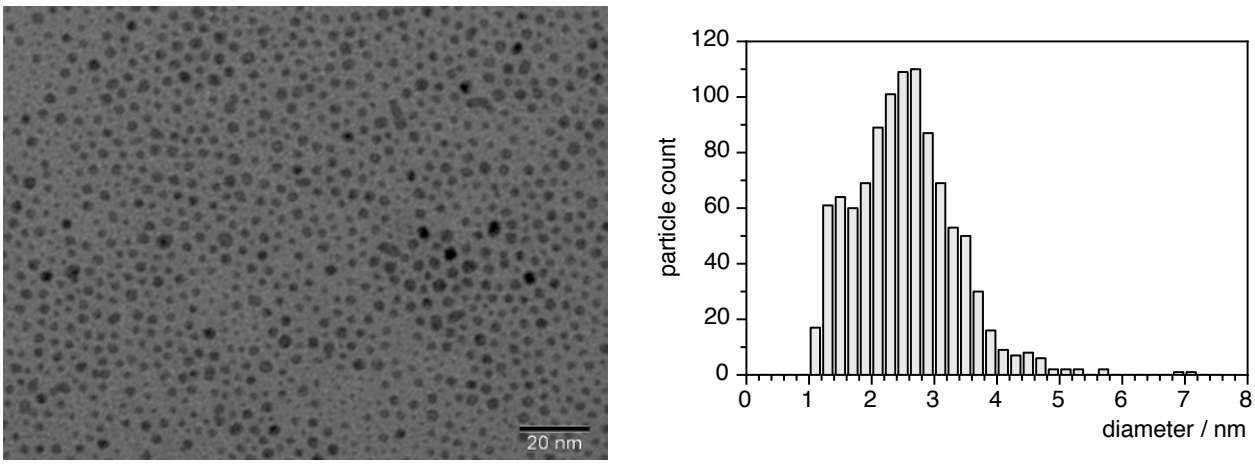


Figure S12: TEM image of $\boldsymbol{NP_{QP}}$.

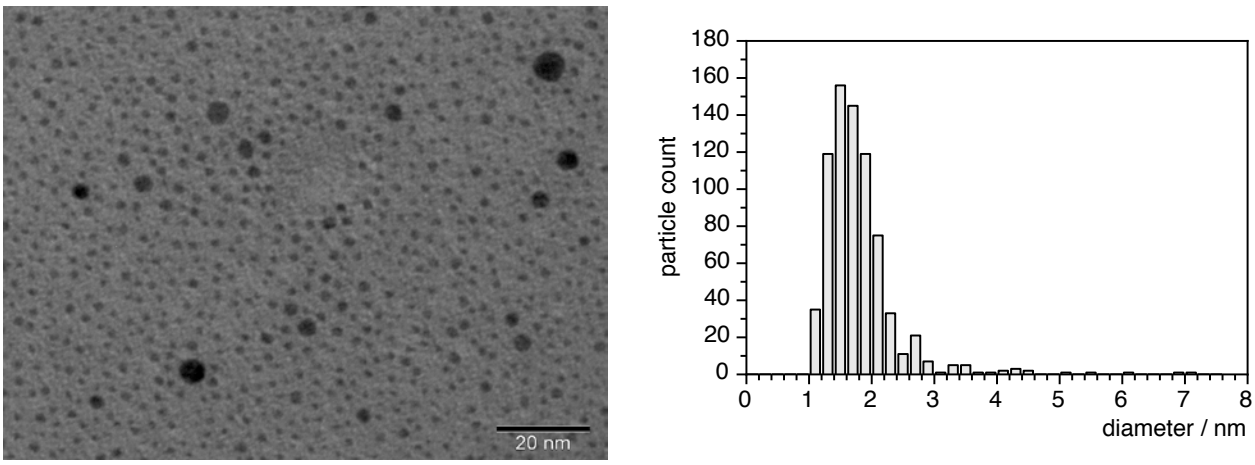


Figure S13: TEM image of $\boldsymbol{NP_{QPC}}$.

Table S2: Average diameters of nanoparticles ***NP$_Q$, NP$_{QC}$, NP$_{QP}$,*** *and* ***NP$_{QPC}$****.*

nanoparticles	d / nm	# gold atoms[a]
NP$_Q$	1.9	211
NP$_{QC}$	2.5	481
NP$_{QP}$	2.6	541
NP$_{QPC}$	1.9	211

[a] The number of gold atoms was estimated by using the theoretical model described by Leff *et al*.[4]

DOSY NMR Spectroscopy. All DOSY NMR measurements were performed on a 500 MHz Bruker Avance III NMR spectrometer, proton frequency 500.137 MHz, equipped with a 5 mm TXI probehead at 300 K. The pulse sequence was a stimulated echo bipolar gradient pulse (stebpgp1s) with the DOSY spectra acquired for each sample having 32 increments (exponential array), 32 scans, gradient pulse length (δ) 6.0 ms and big delta (Δ) 150.0 ms. The diffusion coefficients of the individual species in solution were corrected by considering the diffusion coefficient of water (2.18 $\times$ 10^{-9} m^2 s^{-1}). In the case of overlapping signals of nanoparticle and peptide peaks (for **NP$_{QP}$** and **NP$_{QPC}$**) D_{obs} values were obtained from resolving the decay of the overlapping peaks by biexponential fitting and using the independently determined nanoparticle diffusion coefficient as a known value.[5] Diffusion of HOD exhibited excellent reproducibility throughout all measurements. Diffusion coefficients of AuNPs were used to estimate the hydrodynamic radii of the nanoparticles by using the Stokes-Einstein equation (1) where k is the Boltzmann's constant, T is the temperature (300 K), η is the viscosity of the solvent (D_2O), D is the diffusion coefficient of the AuNPs, and R_h is the hydrodynamic radius.

$$D = \mathrm{k}\, T / 6\, \pi\, \eta\, R_h \qquad (1)$$

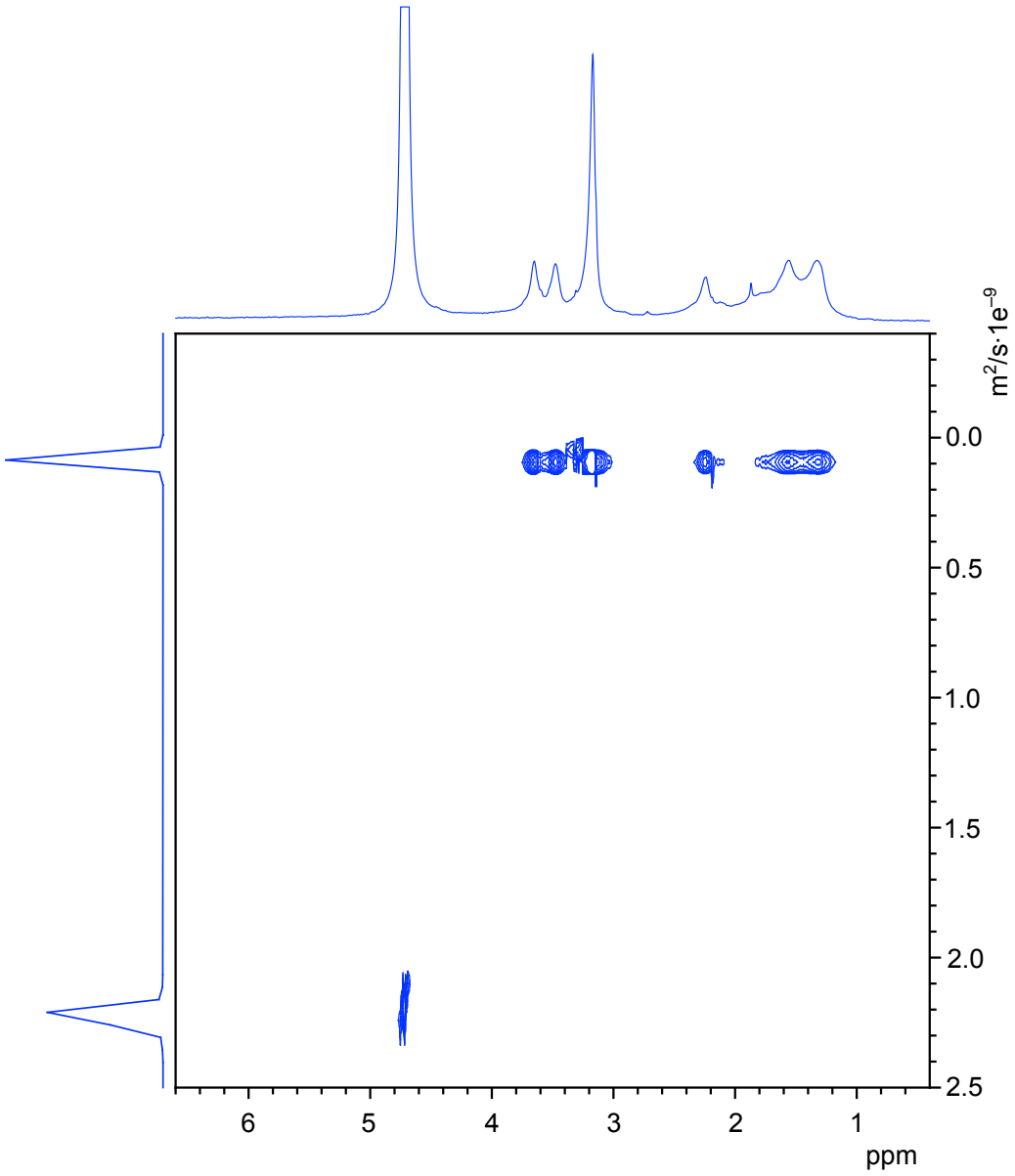


Figure S14: DOSY NMR spectrum of ***NP*** $_{\boldsymbol{Q}}$ *in* D_2O.

Iodine Decomposition.[6] Iodine decomposition of the nanoparticles in the presence of an internal standard (2,4,6-trimethoxy-1,3,5-triazine) followed by ^{1}H NMR spectroscopic investigation of the resulting solution was used to determine the ratio of ligands bound to nanoparticle core and the absolute amount of ligands on each nanoparticle.

To a sample of a functionalized nanoparticle (3 mg) in an NMR tube, a solution of iodine (25 mg) in methanol-d_4 (0.5 mL) followed by a stock solution of 2,4,6-trimethoxy-1,3,5-triazine (73 mM, 0.1 mL) in methanol-d_4 were added. The resulting mixture was sonicated for 30 min at 40 °C to decompose the nanoparticles. Afterwards, a ^{1}H NMR spectrum was recorded and the ratio of the

integrals of characteristic signals from the individual ligands were estimated by considering the respective number of absorbing protons. In general, at least two signals of each ligand were used to increase reliability of the results. Specifically, the following signals were considered:

Ligand **Q**: 3.09 ppm (9H), 3.34 ppm (2H)

Ligand **C**: 7.20 ppm (1H), 6.90-6.77 ppm (2H), 4.03 ppm (4H), 3.75 ppm (4H)

Ligand **P**: 7.16-7.02 ppm (5H), 1.7 ppm (2H)

By relating the integrals of the ligand signals to the one of the internal standard at 3.95 ppm and considering the concentration of the internal standard, the total amount of ligands per mg of nanoparticle could be calculated. This information together with the calculated number of gold atoms per nanoparticle (Table S2) allowed estimation of the ligand/gold ratio and the composition of the nanoparticles. The obtained results are in good agreement with the expected composition on the basis of the Leff model.[4] Surface coverage of the prepared AuNPs is therefore typically lower than the one reported by Murray.[7]

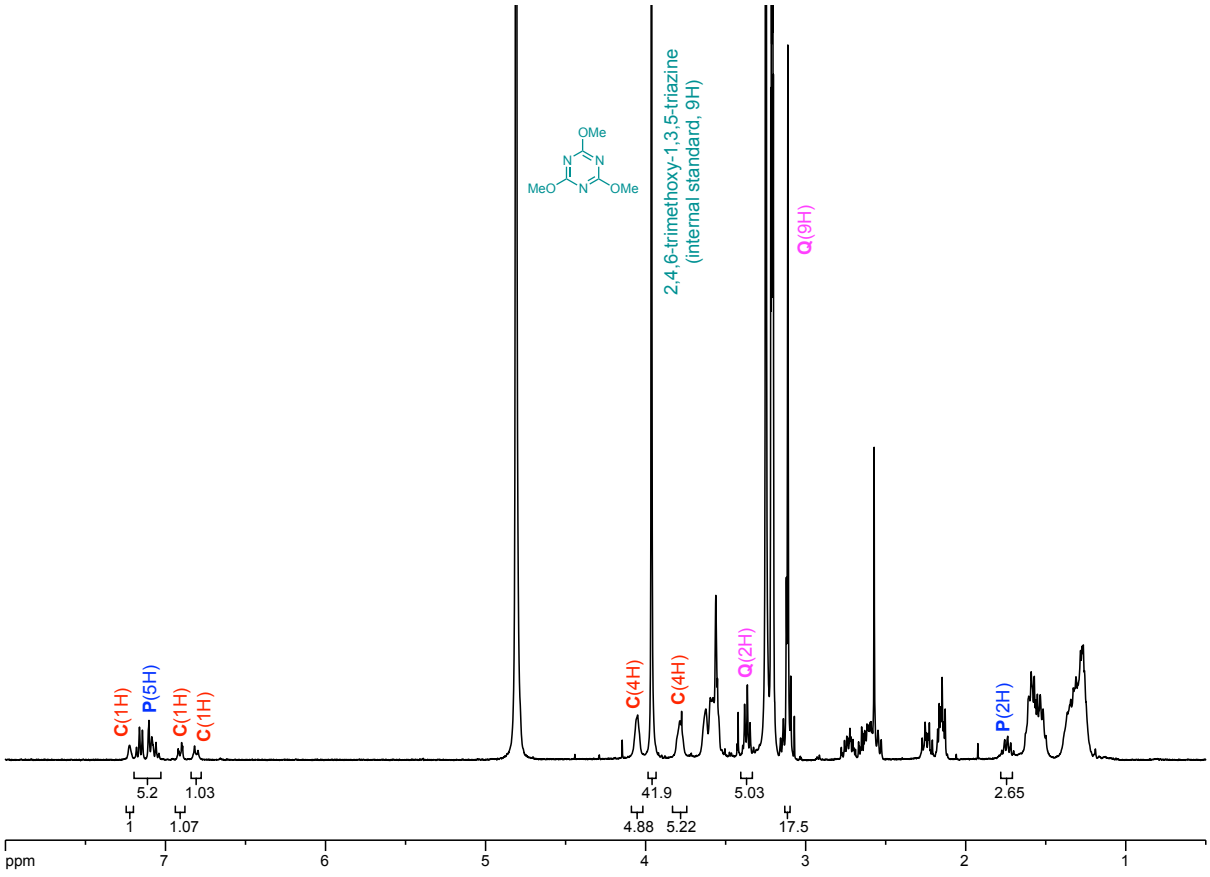


Figure S15: ^{1}H NMR spectrum of ***NP$_{QPC}$*** *in methanol-*d_4 *after* I_2 *decomposition.*

*Table S3: Relative amounts of ligands **Q**, **C,** and **P** on the surfaces of **NP_Q**, **NP_{QC}**, **NP_{QP}**, and **NP_{QPC}**. The error of the results was estimated from repeated measurements to ca. ± 5 %.*

nano-particles	**Q** / %	**C** / %	**P** / %	total amount of ligands per nanoparticle / mol mg^{-1}	# of ligand molecules / # Au atoms	# of ligands on surface	# of ligands calcd.[a]	average composition
NP_Q	100			8.76×10^{-7}	4.29	49	53	$Au_{211}Q_{49}$
NP_{QC}	53	47		7.47×10^{-7}	4.84	99	92	$Au_{481}Q_{52.5}C_{46.5}$
NP_{QP}	47		53	8.21×10^{-7}	4.69	115	99	$Au_{541}Q_{54.0}P_{61.0}$
NP_{QPC}	48	25	27	8.57×10^{-7}	4.17	51	53	$Au_{211}Q_{24.5}C_{12.8}P_{13.7}$

[a] The number of ligands was estimated by using the theoretical model described by Leff *et al*.[4]

Binding Studies

NMR titration.

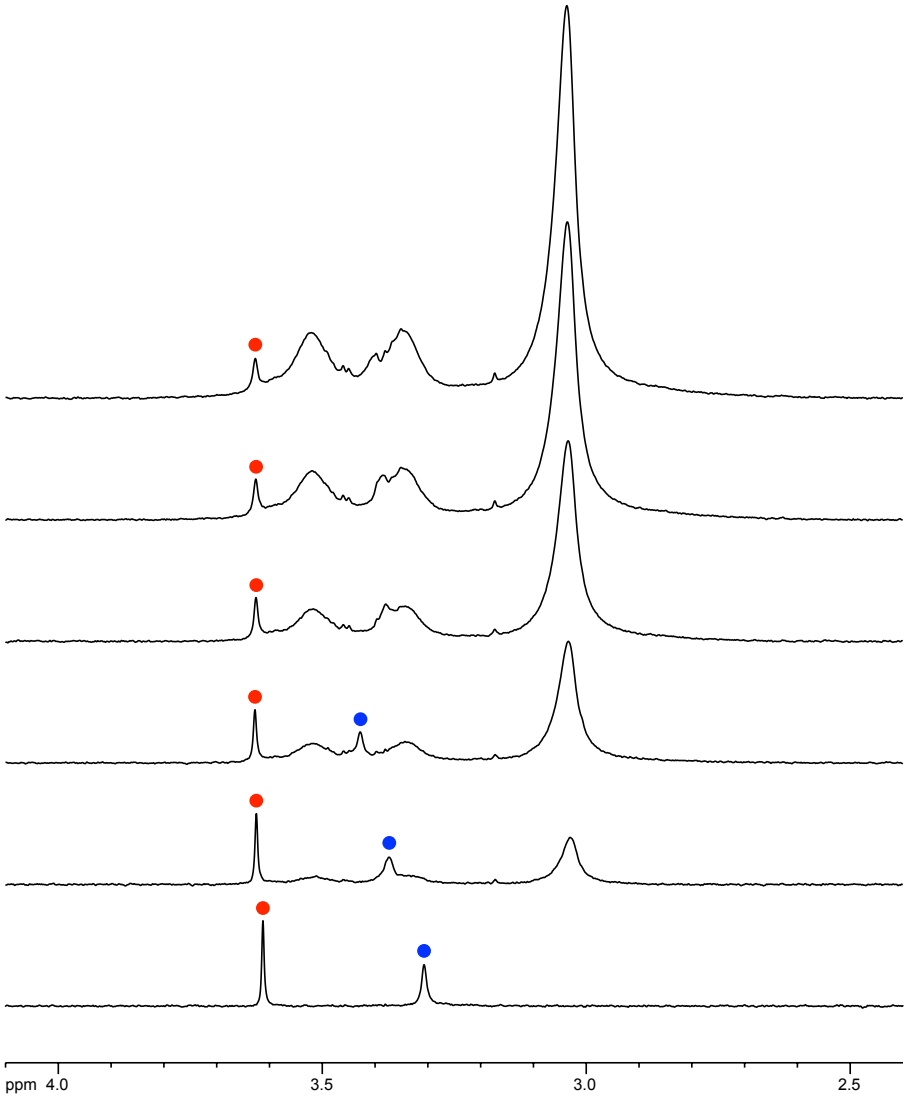


Figure S16: Influence of the addition of an increasing amount of ***NP$_Q$*** *on the ^{1}H NMR spectrum of Gly-Gly (0.05 mM) in D_2O, from bottom to top: 0 mg, 0.12 mg, 0.36 mg, 0.60 mg, 0.90 mg, 1.20 mg. The Gly-Gly signals are denoted with the red and blue circles.*

Estimation of the fraction χ of bound peptides by DOSY NMR spectroscopy. As peptide binding can be expected to take place on the surface of the nanoparticles and controlled by the type and spatial arrangement of the different surface-bound ligands, these measurements were performed at a constant ratio of peptide over total ligand concentration in solution. Thus, it was ensured that each peptide molecules can interact with the same number of ligands independent of the size of the nanoparticles.

Solutions were prepared by mixing stock solutions of the nanoparticles (total ligand concentration 3.2 mM, 300 µL) and of the peptides (12 mM, 3 µL) in D_2O in NMR tubes. The total volume of the NMR tubes was adjusted to 500 µL by adding 197 µL of D_2O. After recording the DOSY NMR spectrum of each sample, the diffusion coefficients of the peptides and of the nanoparticles were determined separately. The fraction χ of bound peptides was calculated by using equation (2).

$$\chi = (D_{\text{free}} - D_{\text{obs}})/(D_{\text{free}} - D_{\text{bound}}) \quad (2)$$

Table S4: Diffusion coefficients D_{free} *of peptides Gly-Phe and Gly-Gly and of the prepared mixed monolayer protected nanoparticles* D_{bound} *in water and diffusion coefficients* D*obs of the peptides in the presence of nanoparticles.*

	total ligand concentration / mM	D_{free} / × 10^{10} m^2 s^{-1}	D_{bound} / × 10^{10} m^2 s^{-1}	D_{obs} / × 10^{10} m^2 s^{-1}	χ / %
NP_Q + Gly-Phe	1.91	5.82	0.81	3.93	38
NP_{QC} + Gly-Phe	1.84	5.82	0.72	4.24	31
NP_{QP} + Gly-Phe	1.93	5.82	0.74	4.75	21
NP_{QPC} + Gly-Phe	2.02	5.82	0.80	1.91	78
NP_Q + Gly-Gly	1.91	7.49	0.81	5.36	32
NP_{QC} + Gly-Gly	1.83	7.49	0.72	5.94	23
NP_{QP} + Gly-Gly	1.81	7.49	0.74	6.22	19
NP_{QPC} + Gly-Gly	2.02	7.49	0.80	4.25	48

Quantitative evaluation of peptide affinity. For the quantitative estimation of the affinity of Gly-Phe to nanoparticles **NP_Q** and **NP_{QPC}** a series of solutions were prepared containing the same amount of nanoparticles and increasing concentrations of the peptide. Eight samples were prepared by mixing stock solutions of the nanoparticles (total ligand concentration 3.2 mM, 300 µL) and

peptide (12 mM, 3 to 120 µL) in D_2O in individual NMR tubes and adjusting the overall volume of each sample to 500 µL. The diffusion coefficients of the peptides D_{obs} were determined from the DOSY NMR spectra of these solutions and the corresponding fractions of bound peptides χ were calculated from equation (2) by considering the diffusion coefficient of free peptide D_{free} (5.82×10^{-10} m^2 s^{-1}) and of the respective nanoparticle D_{bound} (8.05×10^{-11} m^2 s^{-1} for **NP$_Q$** and 8.00×10^{-11} m^2 s^{-1} for **NP$_{QPC}$**). D_{free} and D_{bound} were determined from the DOSY NMR spectra of two additional samples obtained by mixing the nanoparticle stock solution (total ligand concentration 3.2 mM, 300 µL) with D_2O (200 µL) and the peptide stock solution (12 mM, 200 µL) with D_2O (300 µL). Plotting χ against the peptide concentration afforded curves that were fitted to Langmuir isotherms according to equation (3). The corresponding non-linear regression yielded the equilibrium constants K and the maximum fraction of bound peptide c_{max}. Relating this concentration to the one of the nanoparticle provided information about the average number of peptides bound to the AuNPs.

$$\chi = c_{max} \cdot K / (1 + K \cdot c_{Pep}) \quad (3)$$

*Table S5: Results of the titration of **NP$_Q$** with Gly-Phe.*

c_{Pep} / mM	D_{obs} / $\times 10^{10}$ m^2 s^{-1}	χ
0.072	3.93	0.377
0.144	4.58	0.248
0.288	4.88	0.188
0.567	5.17	0.131
0.960	5.41	0.083
1.200	5.39	0.086
1.440	5.46	0.073
2.160	5.44	0.076

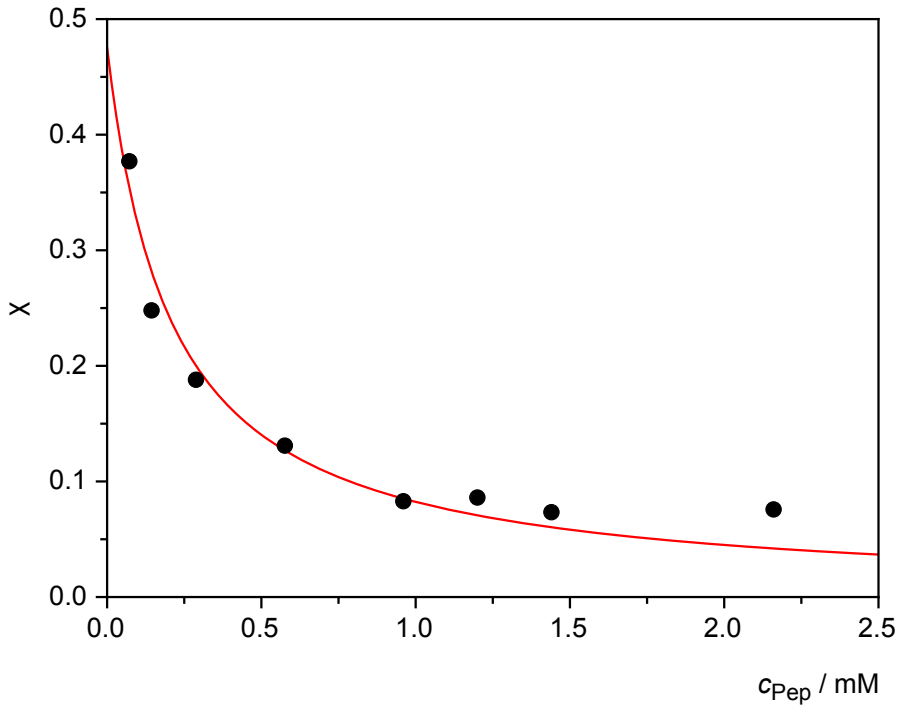


Figure S17: Dependence of the DOSY NMR spectroscopically determined fraction of bound Gly-Phe to ***NP$_Q$*** *on peptide concentration.*

Table S6: Results of the titration of ***NP$_{QPC}$*** *with Gly-Phe.*

c_{Pep} / mM	D_{obs} / × 10^{10} m^2 s^{-1}	χ
0.036	1.45	0.871
0.072	1.91	0.779
0.144	3.02	0.558
0.288	3.84	0.395
0.567	4.98	0.168
0.960	5.43	0.079
1.200	5.50	0.065
1.440	5.55	0.055
2.160	5.60	0.045

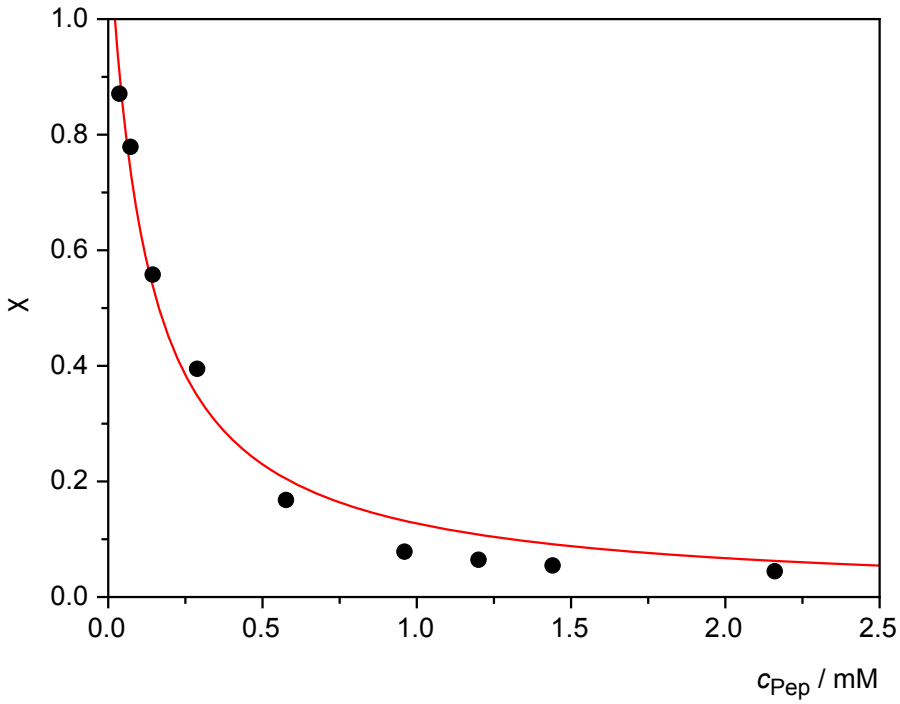


Figure S18: Dependence of the DOSY NMR spectroscopically determined fraction of bound Gly-Phe to ***NP$_{QPC}$*** *on peptide concentration.*

Table S7: Summary of the results of the titrations.

AuNP	K / M^{-1}	c_{max} / mM	$c_{lig\ tot}$ / c_{max}	# lig / NP	# pep / NP
NP$_Q$	4770 ± 1180[a]	0.100	19.2	49	2.6
NP$_{QPC}$	8260 ± 1480[a]	0.143	14.2	51	3.6

[a] The calculated errors describe the goodness of the fit of the regression curve and the experimental results.

Titrations using other batches of independently prepared nanoparticles were performed analogously. The equilibrium constant obtained for binding of Gly-Phe to **NP$_Q$** is the same as the one in Table S7 within the error limits (3880 ± 860 M^{-1}). The one for **NP$_{QPC}$** is slightly different (6090 ± 1380 M^{-1}), but confirms the higher affinity of this AuNP to the peptide.

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