# Supplementary Material (ESI) for Chemical Communications


# Supplementary Information

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Experimental:

Gold films (100nm) were coated on freshly cleaved mica surfaces using (Balzer BAE 250) at rate of 0.2 nm/s and under a pressure of 5 x $10^{-6}$ mbar. Film thickness was monitored by a quartz crystal oscillator. After evaporation, the mica supported gold films were placed on a stainless steel plate which was kept inside a glass tube of a tube furnace. The gold films were annealed at 600°C under a constant flow of $N_2$ for 10 minutes. Finally the gold films were quenched and rinsed with methanol and dried with $N_2$. Gold (111) coated glass substrates were placed in an ethanolic solution of NTA thioalkane (3 mmol) for a period of 12 hours. After self assembly, the surfaces were washed with ethanol to remove unbound NTA. The quality of monolayer was ascertained by scanning force microscopy (SFM) and surface plasmon spectroscopy (SPS). To immobilize the protein, $Ni^{II}$ was bound to NTA group, the gold slides functionalized with NTA thioalkane were treated with 1 mmol NaOH for 10 minutes, followed by dipping of slides in a solution of $NiSO_4$ (40 mmol) for 1 hour. Then slides were taken out, washed with (150 mmol) solution of NaCl and deionized water and dried in a stream of $N_2$. Then silicatein solution (30 nmol) in 3-(N-morpholino)propane sulfonic acid (MOPS) buffer was introduced to the $Ni^{II}$ bound self assembled monolayers (SAMs) for 1 hour. Then slides were washed with MOPS buffer and deionized water to get rid of unbound protein and dried by $N_2$. The immobilization of silicatein was monitored by SFM and SPS. The silicification from monomeric tetraethoxysilicic acid (TEOS) was carried out at room temperature and neutral pH. The silicatein immobilized substrates were placed facing down into a reaction vessel containing 200 ml of TEOS (4.5 mmol, 135 ml) and MOPS buffer (75 ml). The reaction vessel was transferred into a desiccator. The polymerisation was stopped after 6 hrs. Then substrates were washed with MOPS and deionised water and dried by stream of $N_2$.

For SPS and (scanning electron microscopy (SEM) glass slides (3.5 x 2.5 cm) were used. A quartz crystal with an evaporated gold electrode was used as a substrate for the QCM measurements. A quartz crystal evaporated with gold and glass slides were cleaned with aq.$NH_3$ / $H_2O_2$ / $H_2O$ (1:1:5) for 10 minutes at 80 °C, washed with water and isopropanol and dried in stream of $N_2$. These glass slides were coated with gold using a Balzer BAE 250, vacuum coating unit under pressure of less than 5x$10^{-6}$ hPa, typically depositing 50 nm of gold after first depositing 2 nm of Cr. The slides were exposed to NTA thiol (1) solution (1 mmol) for 12 hours.

**Scanning Force Microscopy**

Samples were imaged at room temperature with a commercial SFM (Nanoscope IIIa, Digital Instruments, Santa Barbara, California) employing TappingModeTM using rectangular silicon cantilevers (Nanosensors, 125µm long, 30µm wide, 4µm thick) with an integrated tip, a nominal spring constant of 42 N m-1, and a resonance frequency of 330 kHz. To control and enhance the range

of the attractive interaction regime the instrument was equipped with a special active feedback circuit, called Q-control (Nanoanalytics, Germany) as described in *ref. 1*. The quality factor Q of this oscillating system is increased up to one order of magnitude. As a consequence, the sensitivity and lateral resolution are enhanced, allowing us to prevent the onset of intermittent repulsive contact and thereby to operate the SFM constantly in the attractive interaction regime.

## SPR Experimental

SPR measurements were performed in the Kretschmann configuration against ethanol. Optical coupling was achieved with a LASFN 9 prism, n= 1.85 at λ = 632.8 nm and index matching fluid n = 1.70 between prism and the BK270 glass sildes. The plasmon was excited with P-polarized radiation using a He/Ne laser (632.6 nm, 5 mW).

## Synthesis of chelating thioalkane NTA

### Synthesis of $N^{\alpha}$, $N^{\alpha}$-Bis(tert-butyloxycarbonyl)methyl]-$N^{\varepsilon}$-benzyloxy carbonyl-L-Lysine tert-butyl ester.

2140 mg (5.74 mmol) of $N^{\varepsilon}$-benzyloxycarbonyl-L-lysine tert-butyl ester hydrochloride, 5.32 ml of triethylamine and 12.8 g (8.25 mmol) of bromoacetic acid tert-butyl ester was stirred in 80 ml of DMF for four days at 50°C. The solvent and excess of bromoacetic acid tert-butyl ester was evaporated in vacuum and remaining very thick oil was extracted 4 times with hexane. The combined organic phases were combined and the solvent was removed in a rotary vacuum evaporator. The purification were carried out by column chromatography (pet.ether : MeOH 1000 : 1). Yield: 3237 mg (71%) $^1$H NMR (400 MHz, $CDCl_3$): δ=1.43 (s,18H), δ=1.45 (s,9H), δ=1.50 (m,4H), δ=1.65 (m, 2 H), δ=3.19 (m, 2 H), δ=3.33 (t,1 H), δ=3.48 (dd, 4H), δ= 5.08 (s,2 H), δ=7.35 (m,5 H),

### Synthesis of $N^{\alpha}$, $N^{\alpha}$-Bis(tert-butyloxycarbonyl)methyl]-L-Lysine tert-butyl ester

150 mg of Pd/C (10%) was suspended in 120 ml of dried ethanol. The $N^{\alpha}$, $N^{\alpha}$-Bis(tert-butyloxycarbonyl)methyl]-$N^{\varepsilon}$-benzyloxy carbonyl-L-lysine tert-butyl ester (1800 mg) was added and hydrogenated at 30 °C temperature and normal pressure for 4 hours. The catalyst was filtered off and solvent was removed in vacuum. Yield: 1220 mg (91%) $^1$H NMR (400 MHz, $CDCl_3$): δ=1.41 (s,18H), δ=1.43 (s,9H), δ=1.21 (q,2H), δ=1.65 (m, 2 H), δ=2.98 (t, 2 H), δ=3.39 (t,1 H), δ=3.67 (dd, 4H), δ=1.84 (bm, 2H),

### Synthesis of 11-(Acetyl Thio) undecanoic acid

The 11-bromoundecanoic acid (954 mg) was dissolved in 20 ml of dried DMF at 0 °C. After that 1172 mg of potassium thioacetate was added. The deep red mixture was stirred for 30 minutes at room temperature, diluted with $CH_2Cl_2$ (60 ml) and washed three times with water. The organic phase was dried over $MgSO_4$ and the solvent was coevaporated with toluene. The light yellow crude product was

purified by recrystallisation in pet. ether. Yield: 824 mg (88%) $^1$H NMR (400 MHz, $CDCl_3$): δ=2.33 (s,3H), δ=2.86 (t,2H), δ=1.53-1.67 (m,4H), δ=1.26-1.36 (m, 4 H), δ=2.37 (t, 2 H),

**Synthesis of 6-(11-acetyl sulfanyl-undecanoylamino)-2-(bis-tert-butyloxy carbonyl methyl-amino)-hexanoic acid tert-butyl ester**

The 11-(Acetyl thio) undecanoic acid (693 mg 2.66 mmol) were dissolved in 55ml of absolute $CH_2Cl_2$ and 550 mg of dicyclohexylcarbodiimide (DCC), 308 mg of N-hydroxy succinimide and 41 mg of 4-dimethylaminopyridine in 27 ml of acetone were added. The solution was stirred for 4 hours at room temperature, the precipitated urea was filtered off and (1150 mg 2.66 mmol) of $N^{\alpha}$, $N^{\alpha}$-Bis(tert-butyloxycarbonyl)methyl]-L-Lysine tert-butyl ester and 1065 µl of triethylamine in 50 ml of absolute $CH_2Cl_2$ were added. The reaction was stirred for 5 hours. Thereafter the solvent was removed in vacuum. The product was purified by re-crystallization in acetone. Yield: 1526 mg 85% $^1$H NMR (400 MHz, $CDCl_3$): δ=3.69 (dd, 4H), δ=3.43 (t, 1H), δ=3.11 (m,2H), δ=2.81 (m, 2 H), δ=2.63 (t, 2 H), δ=2.30 (s,3 H), δ=1.84 (m, 2H), δ=1.50-1.73 (m, 4H), δ= 1.42-1.44 (bs, 27H), δ=1.20-1.37 (m, 6H)

**Synthesis of 2-[$N^{\alpha}$-$N^{\alpha}$-bis(hydroxycarbonylmethyl)-amino-6-[11-Acetylthio-undecanoyl amino]-hexanoic acid.**

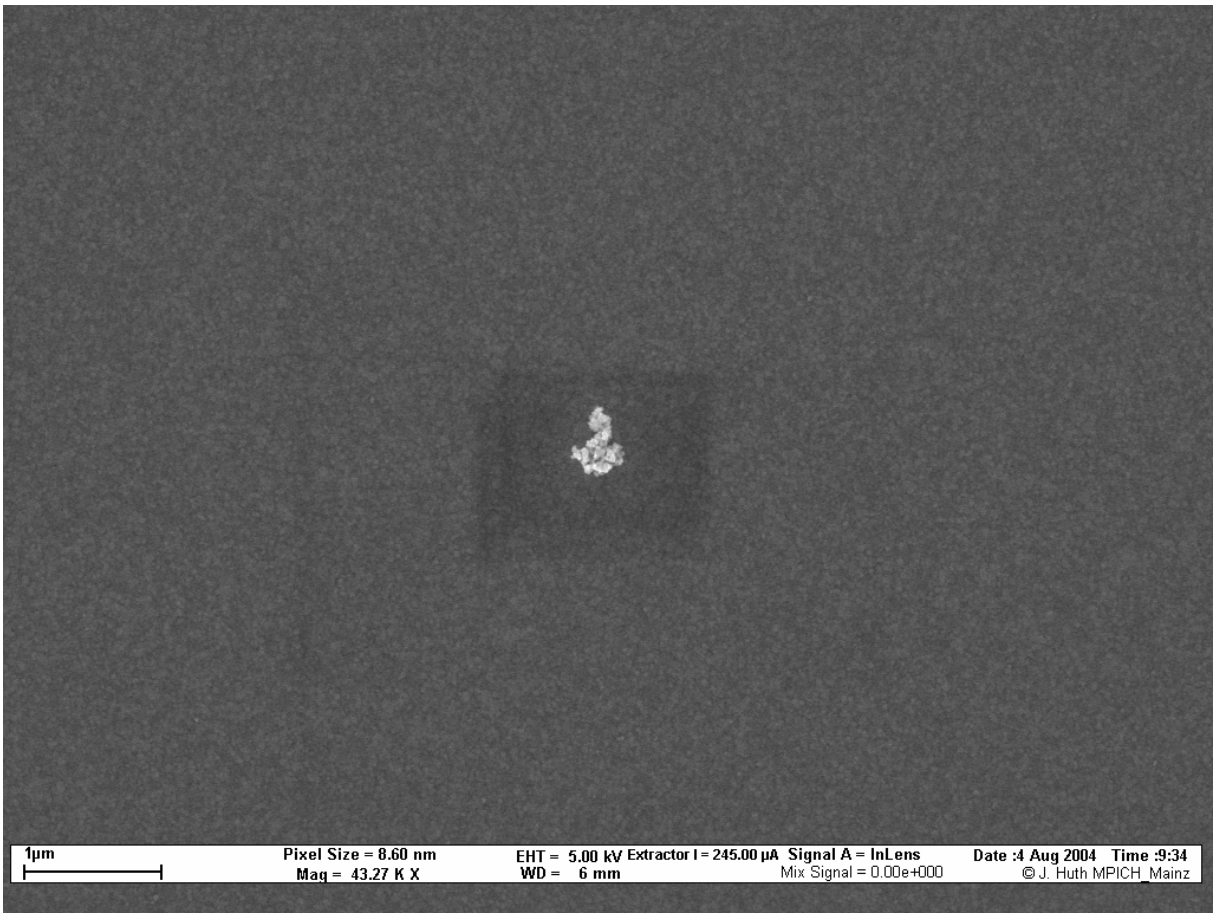
1200mg (1.78 mmol) of 6-(11-acetyl sulfanyl-undecanoylamino)-2-(bis-tert-butyloxy carbonyl methyl-amino)-hexanoic acid tert-butyl ester was dissolved in 180ml of chloroform/trifluoroacetic acid (5:1). The reaction was stirred for 20 hours at room temperature, extracted twice with 1N NaOH. The solvent was evaporated and the product was crystallised in acetone. Yield: 721 mg 80.76% $^1$H NMR (400 MHz, $CDCl_3$): δ=3.67 (dd, 4 H), δ=3.26-3.46 (bm, 6 H), δ=2.28 (t, 2 H), δ=2.12 (s, 3 H), δ=1.84 (m, 2 H), δ=1.57-1.70 (bm, 6 H), δ=1.04-1.34 (m, 6H)

**Synthesis of 2-[$N^{\alpha}$-$N^{\alpha}$-bis(hydroxycarbonylmethyl)-amino-6-[11-mercapto-undecanoyl amino]-hexanoic acid.**

The compound 2-[$N^{\alpha}$-$N^{\alpha}$-bis(hydroxycarbonylmethyl)-amino-6-[11-Acetylthio-undecanoyl amino]-hexanoic acid (690 mg 1.36 mmol) was dissolved in 50ml of $CH_2Cl_2$ and the solution was purged with $N_2$. then 355 µl of HCl was added and the mixture was refluxed under a $N_2$ atmosphere for 5 hours. The product was concentrated and washed with 18.2 MΩ water until neutral. The organic phase was dried over $MgSO_4$ and evaporated. The product was purified by recrystallisation from acetone. Yield: 600mg 95%.$^1$H NMR (400 MHz, $CDCl_3$): δ=3.74 (dd, 4 H), δ=3.22 (t, 2 H), δ=2.28 (t, 2 H), δ=2.55 (t, 2 H), δ=1.84 (m, 2 H), δ=1.20-1.48 (m, 4 H), δ=1.55-1.70 (m, 4H)

## References

[1] Wilhelm Tischer, Frank Wedekind, in Topics in Current Chemistry, Volume 200, 1999, pp. 95-126.




**Supplementary figure 1:**
SEM picture of $SiO_2$ polymerized on NTA functionalized surfaces with silicatein only present in solution. Hardly any $SiO_2$ was found and the picture shows an exceptional place where some $SiO_2$ was deposited in order to focus the picture.