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Guest-controlled aggregation of cavitand gold nanoparticles and N-methyl pyridinium-terminated PEG.

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**General Methods.**

All reagents and chemicals were obtained from commercial sources and used without further purification. Dry pyridine was distilled from KOH before use. Column chromatography was performed using silica gel 60 (MERCK 70-230 mesh). Electrospray ionization ESI-MS experiments were performed on a Waters ZMD spectrometer equipped with an electrospray interface. Exact masses were determined using a LTQ ORBITRAP XL Thermo spectrometer equipped with an electrospray interface. \(^1\)H NMR spectra were obtained using a Bruker AVANCE-300 (300 MHz) or a Bruker AVANCE 400 (400 MHz). All chemical shifts (δ) were reported in ppm relative to the proton resonances resulting from incomplete deuteration of the NMR solvents. \(^{31}\)P NMR spectra were obtained using a Bruker AVANCE-400 (162 MHz) spectrometer. All chemical shifts (δ) were recorded in ppm relative to external 85% H\(_3\)PO\(_4\) at 0.00 ppm.

Fluorescence spectra in solution were obtained with a modular UV/visible NIR spectrofluorimeter, Edinburgh, equipped with a 450 W Xe lamp. All solvents employed are CHROMASOLV, for HPLC grade.

**Gold Nanoparticles TEM analysis**

![HR TEM image of the dodecanthiol AuNPs.](image)

**Figure S1** HR TEM image of the dodecanthiol AuNPs.
Synthesis

Scheme S1  Synthesis of the molecular receptor Tiiii[disulfide, CH₃, Ph].

S3
Tetrahydroxy silylcavitand (II)

To a solution of I (2.5 g, 3.27 mmol) in pyridine (20 mL) dimethylchlorosilane was added (4.13 g, 3.85 mL, 32.7 mmol) at once. The resulting mixture was heated to 55°C for 12 hours. The solvent was removed under vacuum and the resulting residue was suspended in water. After filtration compound II was obtained in pure form as a white solid (2.46 g, 2.45 mmol, 84% yield). $^1$H NMR (CDCl$_3$, CD$_3$OD, 400 MHz): δ (ppm) 7.11 (s, 4H, ArH); 4.45 (t, 4H, ArCH$_3$, J = 7.8 Hz); 3.51 (t, 8H, CH$_2$CH$_2$O, J = 5.7 Hz); 3.21 (s, 4H, CH$_2$CH$_2$COH); 2.14 (bs, 8H, ArCHCH$_2$); 1.77 (s, 12H, ArCH$_3$); 1.36 (bs, 8H, CH$_2$CH$_2$CH$_2$OH); 0.37 (s, 12H, SiCH$_3$)$_2$; $^3$ESI-MS: calculated for C$_{52}$H$_{72}$O$_{12}$Si$_4$: 1177.67. Found: 1200.12 [M+Na]$^+$. 

MOM-protected silylcavitand (III)

To a solution of II (2.02 g, 2.02 mmol) in DMF (20 mL), diisopropylethylamine (5.30 mL, 30.3 mmol) and chloromethyl methyl ether (1.54 mL, 20.2 mmol) were added. The resulting mixture was heated at 50°C for 24 hours. The solvent was removed under vacuum and the resulting residue was suspended in water. After filtration compound III was obtained in pure form as white solid (2.01 g, 1.70 mmol, 85% yield). $^1$H NMR (CDCl$_3$, 300 MHz): δ (ppm) 7.17 (s, 4H, ArH); 4.59 (s, 8H, OCH$_2$O); 4.58 (t, 4H, ArCH$_3$, J = 7.8 Hz); 3.51 (t, 8H, CH$_2$CH$_2$COH, J = 5.7 Hz); 3.34 (s, 12H, OCH$_3$); 2.30 (q, 8H, CH$_2$CH$_2$CH$_2$O, J = 7.5 Hz); 1.87 (s, 12H, ArCH$_3$); 1.58 (q, 8H, CH$_2$CH$_2$CH$_2$O, J = 7.5 Hz); 0.47 (s, 12H, SiCH$_3$)$_2$; -0.72 (s, 12H, SiCH$_3$)$_3$; $^3$ESI-MS: calculated for C$_{60}$H$_{88}$O$_{16}$Si$_4$: 1177.67. Found: 1200.12 [M+Na]$^+$.

MOM-protected resorcinare (IV)

An aqueous 36% HF solution (0.65 mL) was added to III (1.6 g, 1.36 mmol) dissolved in DMF (20 mL). The mixture was heated at 50°C overnight. The solvent was removed in vacuo and the product was washed with water. Vacuum filtration afforded pure IV (1.30 g, 1.36 mmol, quantitative yield). $^1$H NMR (DMSO-D$_6$, 300 MHz): δ (ppm) 8.68 (s, 8H, ArOH); 7.29 (s, 4H, ArH); 4.53 (t, 8H, OCH$_2$O); 4.22 (t, 4H, ArCH$_3$, J = 7.8 Hz); 3.48 (t, 8H, CH$_2$CH$_2$CH$_2$O, J = 6.0 Hz); 3.24 (s, 12H, OCH$_3$); 2.27 (m, 8H, CH$_2$CH$_2$CH$_2$O); 1.94 (s, 12H, ArCH$_3$); 1.58 (m, 8H, CH$_2$CH$_2$CH$_2$O); $^3$ESI-MS: calculated for C$_{52}$H$_{72}$O$_{16}$: 953.12. Found: 976.2 [M+Na]$^+$.

MOM-protected tetraphosphonate cavitand (V)

To a solution of IV (1.345 g, 1.41 mmol) in freshly distilled pyridine (15 mL), dichlorophenylphosphine (0.8 mL, 5.9 mmol) was added slowly, at room temperature. After 3 hours of stirring at 70 °C, the solution was allowed to cool at room temperature and 10 mL of a mixture of aqueous 35% H$_2$O$_2$ and CHCl$_3$ (1:1) was added. The resulting mixture was stirred for 3 hours of stirring at 70 °C, then the solvent was removed in vacuo. Addition of water resulted in the precipitation of a white powder, which is filtered to give pure V (1.77 g, 1.23 mmol, 87% yield). $^3$P NMR (CDCl$_3$, 300 MHz): δ (ppm) 8.06 (m, 8H, P(O)Ar$_{H_{ORTO}}$); 7.56 (m, 4H+8H,P(O)Ar$_{H_{PARA}}$ + P(O)Ar$_{H_{META}}$); 7.17(s, 2H, ArH$_{DOWN}$); 4.82 (bt, 4H, ArCH$_2$); 4.62 (s, 8H, OCH$_2$O); 3.62 (t, 8H, CH$_2$CH$_2$CH$_2$O, J = 6.0 Hz); 3.35 (s, 12H, OCH$_3$); 2.56 (m, 8H, CH$_2$CH$_2$CH$_2$O); 2.10 (s, 12H, ArCH$_3$); 1.60 (m, 8H, CH$_2$CH$_2$CH$_2$OH); $^3$P NMR (CDCl$_3$, 161 MHz): δ (ppm) 8.58 (s, P(O)); $^3$ESI-MS: calculated for C$_{70}$H$_{72}$O$_{14}$P$_4$: 1441.36. Found: 1464.36 {[M+Na]$^+$}. 

Tetrahydroxy-footed tetraphosphonate cavitand (Tiiii[OH, CH$_3$, Ph])

To a solution of V (1.77 g, 1.22 mmol) in CHCl$_3$(15 mL) and methanol (32mL) an aqueous 36% HCl solution was added. The resulting suspension was heated at 50°C overnight and the resulting residue was suspended in water. After filtration, pure compound VI was obtained as yellow light solid (1.26 g, 0.99 mmol, 81% yield). $^1$H NMR (acetone-D$_6$, 300 MHz): δ (ppm) 8.29 (s, 2H, ArH$_{DOWN}$); 8.20 (m, 8H, P(O)ArH$_{ORTO}$); 7.73 (m, 4H+8H,P(O)Ar$_{H_{PARA}}$ + P(O)Ar$_{H_{META}}$); 4.81 (t, 4H, ArCH$_3$, J = 7.6 Hz); 3.69 (t, 8H, CH$_2$CH$_2$CH$_2$OH, J = 5.3 Hz); 2.91 (m, 8H, CH$_2$CH$_2$CH$_2$O); 2.16 (s, 12H, ArCH$_3$); 1.60 (m, 8H, CH$_2$CH$_2$CH$_2$OH); $^3$P NMR (acetone-
**Tetralipoic ester-footed tetraphosphonate cavitand (Tiiii[disulfide, CH₃, Ph])**

To a solution of Tiiii[OH, CH₃, Ph] (395 mg., 0.312 mmol) in CH₂Cl₂ (2 mL), DCC (546 mg, 2.65 mmol), DMAP (95 mg, 0.78 mmol), and lipoic acid (514 mg, 2.49 mmol) were added. The resulting suspension was heated at 50°C overnight. The solvent was removed in vacuo and a 3 : 1 dichloromethane / ether solution was added. The white solid was filtrated and washed with 2 : 1 dichloromethane / ether solution. The filtrate was collected and the solvent was evaporated in vacuo. The resulting solid was washed with ether (2 x 20 mL). The crude was purified by column chromatography (0.1-10% MeOH / CH₂Cl₂) (127 mg, 0.062 mmol, 20% yield).

**1H NMR (CDCl₃, 300 MHz):** δ (ppm) 8.08 (m, 8H, P(O)ArHORTO); 7.73 (m, 4H+8H+4H, P(O)ArHPARA + P(O)ArHMETA + ArHDOWN); 4.81 (t, 4H, ArCH, 3J = 7.6 Hz); 4.25 (t, 8H, CH₂CH₂CH₂O, 3J = 6.2 Hz); 3.52 (m, 4H, CH₂(S)CH₂C); 3.13 (m, 8H, CH₂(S)CH₂CH₂); 2.42 (m, 8H, CH₂(S)CH₂O); 2.11 (s, 12H, ArCH₃); 1.94-1.57 (m, 32H, CH₂CCH₂O + O(CO)CH₂CH₂CH₂ + O(CO)CH₂CH₂CH₂CH₂); 31P NMR (CDCl₃, 161 MHz): δ (ppm) 7.31 (s, P(O)).

**HR ESI-MS:** calculated for the complex Tiiii[disulfide, CH₃, Ph]•N-methylbutyl ammonium iodide C₁₀₅H₁₃₀N₁O₂₀P₄S₈ [M+C₅H₁₄N]+: 2104.58968. Found: 2104.60183.

**Preparation of AuNPs**

AuNPs were synthesised by a two-phase method: nanoparticles were prepared by dissolving 0.400 g (1.02 mmol) of hydrogen tetrachloro-aurate(III) trihydrate in 30 mL of deionized water. The solution was then shaken in a separatory funnel with 80 mL of toluene solution containing 2.00 g (3.56 mmol) of tetra-n-octylammonium bromide (TOAB). The toluene phase was then recovered and combined with 0.018 mL (0.015 g, 0.07 mmol) of dodecyl mercaptane. A freshly prepared aqueous solution of sodium borohydride (25 mL, 0.386 g) was slowly added under vigorous stirring. After further stirring for 3 h, the organic phase was separated, concentrated to 10 mL and mixed with 70 mL of ethanol. The mixture was cooled overnight at -20°C and the dark precipitate was then recovered by filtration. The crude product was then purified by soxhlet extraction with acetone as cleansing solvent to remove all the unbound free thiol and residual TOAB impurities.

Elemental analysis: found (%) C = 24.7; H = 4.2; N = 0.0.

**Preparation and characterization of Tiiii Au NPs**

Dodecanthiol stabilized gold nanoparticles (20 mg) were mixed in dry chloroform with tetraphosphonate cavitand (13.52 mg, 0.0067 mmol) at room temperature, for 5 days. The solvent was evaporated under N₂ flux and the resulting solid was suspended in a toluene / ethanol mixture (2:1). The suspension was then ultracentrifugated at 15000 rpm (2 runs of 15 minutes each). The supernatant was decanted off, the solid was collected and dried under vacuum. The washing procedure was repeated twice.

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Figure S2  A) $^1$H NMR of dodecanthiol-stabilized gold nanoparticles in CDCl$_3$; B) $^1$H NMR of TiIII-AuNPs in CDCl$_3$ (diagnostic cavitand signals)

Figure S3  $^{31}$P NMR of TiIII Au NPs in CDCl$_3$
Fluorimetric Titration

3 mL of a 1x10^{-6} M solution of fluorescent pyrene guest derivative 2 in dichloromethane were prepared by repeated dilution of a concentrated mother solution (1x10^{-3} M) and a starting point spectra (blue line of Figure 1 in the text) was recorded. This solution was then titrated adding 10 µl aliquots of a dichloromethane solution of Tiili-AuNPs prepared dissolving 1.5 mg of Tiili-AuNPs in 1 mL of dichloromethane. The solution was left without any agitation for 5 minutes after each addition in order to achieve the equilibrium, then an emission spectra was recorded (λ exc = 345 nm). The aliquots were added until no more changes in the spectrum were observed, i.e. until the achievement of the equivalent point. This required the addition of 130 µl of Tiili-AuNPs solution corresponding to the addition of 0.2 mg of Tiili-AuNPs. The experiment was repeated three times. The Tiili-AuNPs aliquots added ranged between 130 and 140 µl.

To a saturated solution of Tiili-AuNPs • 2 complex increasing amount of competitive guest 3 were added, as 10 µl aliquots (0 -1.3 eq. of a solution 1x10^{-5} M). In this case the solution was left with any further agitation for 30 minutes after each addition before acquiring the spectra. The competitor guest 3 was added until no further change was visible, i.e., until all the Tiili present on the nanoparticles was complexed.

Figure S4 Fluorescence spectra (λ exc =345 nm) of a CH₂Cl₂ solution of Tiili-AuNPs•2 complex upon addition of an increasing (0–1.3 eq.) amount of competitive guest 3.
UV/Vis spectra

![UV/Vis spectra graph]

**Figure S5:** Plasmon Band Resonance shift of Dodecanthiol AuNPs (blue line); Tiiii Au NPs (red line); Tiiii-AuNPs•1 network (green line).

**DLS and UV-Vis measurements**

Nanoparticles hydrodynamic diameter distributions were obtained through Dynamic Light Scattering measurements employing a Malvern Nano ZS instrument with a 633 nm laser diode. Samples were housed in semi-micro (1400 µL volume) quartz cells of 1 cm optical path length, using chloroform as solvent (CHROMASOLV® Plus, for HPLC, ≥99.9%, amylene stabilized). All DLS measurements were carried on 400 µL solutions. NPs suspensions were prepared through sonication in chloroform at room temperature (10 min). Solvent and suspensions used were thoroughly filtered (PTFE membrane syringe filter, 0.20 µm) before any DLS measurement.

The width of DLS hydrodynamic diameter distribution is indicated by PdI (Polydispersion Index). In case of a mono-modal distribution (gaussian) calculated by means of cumulant analysis, \( \text{PdI} = \left( \frac{\sigma}{Z_{\text{avg}}} \right)^2 \), where \( \sigma \) is the width of the distribution and \( Z_{\text{avg}} \) is average diameter of the particles population respectively.

The hydrodynamic diameter values determined during the titrations with ditopic guest 1 (concentration of nanoparticle bound \( T\text{iii} \approx 5 \times 10^{-5} \text{ M} \)) are mediated upon three different measurements, while the hydrodynamic diameter of the assembly-disassembly experiments with guest 1 and 3 (d_{III} Volume Mean, Table 1) are the mean values (± standard deviation) of ten measurements of the same suspension.

The concentration of nanoparticle bound \( T\text{iii} \approx 5 \times 10^{-5} \text{ M} \) was selected to make the system suitable for DLS measurements, to be fairly close to the one used in the NMR experiments and to promote an almost quantitative Tiiii-AuNPs•1 network formation during the titration.
Figure S6: Size volume distribution of dodecanethiol coated Au nanoparticles, $d_H = (8 \pm 1)$ nm, (chloroform, 20°C).

Figure S7: Size volume distribution of Tiiliii Au NPs, $d_H = (13 \pm 3)$ nm, (chloroform, 20°C).

Table S1 Volume mean hydrodynamic diameter during the assembly-disassembly experiments of the system Tiiliii-AuNPs with guest 1 and 3. (concentration of nanoparticle bound Tiiliii $\approx 5x10^{-5}$M, chloroform 20°C); (a) [1] = 150 µM, (b) [1] = 150 µM, [3] = 280 µM.

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<th>$d_H$ Volume Mean (nm)</th>
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<td>Pristine</td>
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<tr>
<td>(a)</td>
<td>75±15</td>
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<td>(b)</td>
<td>29±5</td>
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The measured size volume distribution for Tiili-AuNPs (chloroform, 20°C) in diluted conditions (concentration of nanoparticle bound Tiili ≈ 2x10^{-6} M) was d_l = (13 ± 3) nm, a value that is consistent with the presence of Tiili[disulfide,CH₃,Ph] on the AuNPs surface (see Fig. S7†). The slight increase of d_l measured for Tiili-AuNPs at higher concentration (concentration of nanoparticle bound Tiili ≈ 5x10^{-5} M) during the pristine and final parts of the aggregation experiments carried out with guests 1 and 3, are attributed to non-specific reversible interactions between nanoparticles.

Figure S8: Sample size volume distributions during the assembly-disassembly experiments of Tiili-AuNPs with guests 1 and 3. (concentration of nanoparticle bound Tiili ≈ 5x10^{-5} M, chloroform, 20°C).
Absorption spectra of Tiili-AuNps, during the aggregation experiments with ditopic guest 4 were measured using a 400 µL Tiili-AuNps suspension housed in a micro quartz cuvette of 2 mm optical length. This experimental set up allowed to carry out DLS and UV-VIS experiments on identical solution at the same time.

**Figure S9** Absorption spectra of Tiili-AuNps (concentration of nanoparticle bound Tiili ≈ 1x10⁻⁵ M in CHCl₃, black line, λ_max=527nm), and of Tiili-AuNps with ditopic guest 4 ([4]=0.34x10⁻⁵ M, methanol, green line, λ_max=569nm, 1300 min.) showing a 42 nm plasmon band resonance red shift caused by the formation of NPs network.

**Figure S10:** Sample size volume distribution during the assembly experiments of Tiili-AuNPs in CHCl₃ with guest 4 (concentration of nanoparticle bound Tiili) ≈ 1x10⁻⁵ M, [4]=0.34x10⁻⁵ M, 1300 min., methanol, 20°C). Hydrodynamic diameters: d_H (average)=170 nm (PDI=0.29), single distributions d_H small=(64±10) nm, d_H large=(270±80) nm.