Self-assembly and selective exchange of oligoanions on the surface of monolayer protected Au nanoparticles in water†

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Self-assembled monolayers on Au nanoparticles terminating with TACN-Zn\textsuperscript{II} head groups are attractive scaffolds for the formation of multivalent supramolecular structures at submicromolar concentrations in water.

Multivalency is a key concept in biorecognition and a strong interest exists in the development of synthetic multivalent systems able to interact with biological targets.\textsuperscript{1,2} Monolayer protected Au clusters (Au MPCs) have emerged as highly attractive multivalent systems because of their ease of preparation, stability and compatibility with physiological conditions.\textsuperscript{3} Although heterofunctionalized surfaces on Au MPCs can be simply prepared by using two or more different thiols for nanoparticle coverage, the obtained mixed self-assembled monolayers (SAMs) have generally only a limited complexity.\textsuperscript{4} Challenges are the purification of the resulting Au MPCs and the characterization of the mixed SAMs both in terms of composition and morphology. Here, we propose an alternative strategy towards heterofunctionalized multivalent surfaces relying on the self-assembly of small molecules on the surface of Au MPCs.\textsuperscript{5} We show that the cationic surface of Au MPCs can bind quantitatively up to around 18 oligoanions even at low \textmu M concentrations in water (Fig. 1a and b). The surface composition can be controlled simply by changing the ratio of added oligoanions. Seminal studies by Rotello \textit{et al.} have shown that Au MPCs containing terminal cationic ammonium groups have a very high affinity for polyanions, such as protein\textsuperscript{6} and polymers.\textsuperscript{7} This property has been used for the development of a series of highly innovative bioassays.\textsuperscript{8} Nonetheless, binding studies with small peptides revealed relatively low surface saturation concentrations with a maximum of around 5–6 peptides bound per Au MPC.\textsuperscript{9} Here, it will be shown that the presence of Zn(II)-metal ions on the surface of Au MPCs offers significant advantages for the interaction with small molecules: a higher binding affinity, a higher surface saturation concentration, and the possibility to discriminate between phosphate and carboxylate binding. It is shown that the latter property can be used to selectively displace one of the surface bound components, which is an attractive feature for the development of responsive surfaces.

Previously, we have shown that Au MPCs 3 highly efficiently catalyze the transphosphorylation of 2-hydroxypropyl-4-nitrophenyl phosphate (HPNPP).\textsuperscript{10,11} It was observed that Au MPCs 3 have a high affinity for oligoanions, such as ATP or Ac-DDD. This permitted a modulation of the catalytic activity of the Au MPCs through competition with the HPNPP-substrate, which led to the development of a new enzyme assay.\textsuperscript{12} In addition, the fluorescence quenching of \textit{ATP} \textit{F} \textit{ex} = 305 nm, \textit{em} = 370 nm) upon binding to the Au MPC surface permitted an assessment of the morphology of mixed monolayers.\textsuperscript{13} The fact that all these binding studies were performed under physiological conditions even at submicromolar concentrations of both nanoparticles and oligoanions made us consider these Au MPCs as potential scaffolds for the self-assembly of multivalent structures in water.

Au MPCs 2 were prepared according to literature procedures\textsuperscript{14,15} using a new thiol 5, which is accessible in only 4 steps from commercially available components. Au MPCs 1 were prepared \textit{in situ} by adding Zn(NO\textsubscript{3})\textsubscript{2} to a solution of Au MPCs 2 in a stoichiometric ratio compared to the number of triazacyclononane (TACN)-head groups. In addition, Au MPCs 4 containing an ammonium-head group were prepared as a reference cationic scaffold. Both Au MPCs 2 and 4 were characterized by NMR, TEM, TGA, UV-Vis and DLS (ESI†). All analyses were consistent with a diameter of the Au core equal to 1.8 ± 0.5 nm.
and 1.8 ± 0.4 nm, respectively, implying that around 70 thiols are present on the surface of each nanoparticle.16

The affinity of the cationic surface of Au MPCs 1 for ATPF was studied by measuring the fluorescence intensity as a function of the amount of ATPF added to a solution of Au MPCs 1 (Fig. 2a). This titration relies on the ability of Au nanoparticles to highly efficiently quench the fluorescence of bound fluorophores.17 The resulting graph is characteristic of complex formation under saturation conditions: ATPF is quantitatively bound to the surface up to the saturation concentration and remains free in solution afterwards. A surface saturation concentration of 2.5 ± 0.1 μM was calculated by fitting of the data to a simplified model assuming the formation of a 1 : 1 complex between ATPF and a fictitious binding site (ESI†). The saturation concentration of 2.5 ± 0.1 μM corresponds to a total of around 18 molecules of ATPF bound to the surface at saturation. Strikingly, the surface saturation concentration dropped to just 0.5 ± 0.1 μM when the fluorescence titration was performed on Au MPCs 2, i.e. the identical system but in the absence of Zn(ii) (Fig. 2a). Likewise, a surface saturation concentration of 1.7 ± 0.1 μM was determined for Au MPCs 4 equipped with cationic ammonium groups (Fig. 2a). In addition, the shallow saturation curve for the latter nanoparticles is indicative of a lower binding affinity of ATPF for the surface. These results clearly demonstrate that the presence of the Zn(ii)-metal ion on the surface of Au MPCs 1 drives the formation of a multicomponent complex of significantly higher valency compared to the positively charged surfaces of either Au MPCs 2 or 4. The impressive ability of Au MPCs 1 to bind ATPF is further evidenced by the fact that binding could be detected at a concentration of TACN-ZnII down to 0.5 μM, which corresponds to a nanoparticle concentration of around 10 nM. From these measurements an apparent binding constant, $K_{\text{app}}$ of $2.4 \times 10^9$ M$^{-1}$, was calculated (Fig. 2b).

Working under saturation conditions implies that all added oligoanions are localized on the surface of Au MPC 1. We investigated the possibility of creating a heterofunctionalized multivalent surface through the self-assembly of different oligoanions (Fig. 3a). For that purpose, a second probe NBD-GDDD was prepared, in which the NBD (7-nitro-2,1,3-benzoxadiazole) fluorophore ($\lambda_{\text{ex}} = 484$ nm, $\lambda_{\text{em}} = 545$ nm) was attached to the N-terminus of the tetrapeptide GDDD. The advantage of this probe is, first, that its fluorescence can be measured independently from the ATPF probe and, second, that the negative charges originate from carboxylate rather than phosphate groups. A titration of the NBD-GDDD probe to Au MPCs 1 reveals a saturation curve which is practically superimposable on the curve obtained for ATPF (Fig. 3b, ■). Curve fitting yielded a surface saturation concentration of 2.7 ± 0.1 μM indicating that both ATPF and NBD-GDDD saturate the surface to the same extent. Next, the NBD-GDDD probe was titrated to Au MPCs 1 ‘preloaded’ with increasing amounts of ATPF (ranging from 0 to 2.5 μM). Interestingly, the observed surface saturation concentration of NBD-GDDD decreases regularly as the amount of ATPF on the surface increases (Fig. 3a). No fluorescence emission originating from a displacement of ATPF (see below) from the surface was observed. Importantly, summing the concentrations of bound NBD-GDDD and ATPF probes gave a constant concentration of 2.6 ± 0.2 μM over the complete range of probe ratios studied (Fig. 3c). This shows that the surface composition can be varied simply by changing the ratio of added oligoanions without affecting the valency of the complex. The noncovalent nature of the multivalent complexes provides the additional possibility of exchanging the surface bound components. This is highly attractive for the development of responsive multivalent surfaces.18 The dynamic nature of the system is illustrated by the displacement of the ATPF probe (2.0 μM) from the surface of Au MPCs 1 upon the addition of increasing amounts of competing ATP. The displacement can be simply monitored by measuring the fluorescence intensity originating from free ATPF (Fig. 4a). A displacement of 50% of ATPF requires the addition of 3.3 μM ATP, corresponding to a relative binding affinity of 3.3 ($K_{\text{app,ATP}}$ : $K_{\text{app,ATPF}}$).

Fig. 2 (a) Fluorescence intensity at 370 nm (a.u.) as a function of the amount of ATPF added to a solution of Au MPCs 1 (■), 2 (□), and 4 (○) with a headgroup concentration of 10 μM. (b) Fluorescence intensity (370 nm) as a function of the amount of ATPF added to Au MPCs 1 at different concentrations of TACN-ZnII (■: 1 μM; ○: 0.5 μM; □: 0.1 μM). Experimental conditions: [HEPES] = 10 mM, pH 7.0, $T = 25$ °C. Solid lines are obtained by fitting the data to a model (ESI†).

Fig. 3 (a) Schematic representation of the sequential addition of two probes for the controlled formation of a heterofunctionalized surface on Au MPCs 1. (b) Fluorescence intensity at 545 nm (a.u.) as a function of the amount of NBD-GDDD added to Au MPCs 1 in the presence of different amounts of ATPF: 0% (■), 13% (□), 26% (●), 32% (○), 53% (△), 66% (△), 80% (*) (c) Surface composition as a function of the amount of ATPF present. Experimental conditions: [HEPES] = 10 mM, pH 7.0, $T = 25$ °C.
the concentration of ATP and ADP in a 1:1 ratio (1.0 mM NBD-GDDD*: Ac-DDD) added. Experimental conditions: (a) [ATP] = 2.0 μM, (b) [ATP] = 1.4 μM, [HEPES] = 10 mM, pH 7.0, T = 25 °C.

In the same manner, relative binding affinities of 120 and 580 were determined for ADP and Ac-DDD, respectively. Remarkably, the same displacement studies performed on ammonium-terminatated Au MPCs 4 gave nearly identical relative binding affinities of 10 and 14 for ATP and Ac-DDD, respectively (Fig. 4b).† This strong difference between the two systems illustrates the importance of the ZnII-metal ion in differentiating between phosphate and carboxylate binding. For the Au MPCs 1 presented here, this creates an attractive opportunity to selectively exchange a surface bound carboxylate in the presence of a phosphate (Fig. 5a). In order to verify that hypothesis, the displacement of the phosphate probe ATP and carboxylate probe NBD-GDDD by the intermediate competitor ADP was compared. The observed relative binding affinities of 120 for ATP and 15 for NBD-GDDD indeed show that ADP displaces NBD-GDDD more effectively than ATP by a factor of 8 (Fig. 5b). Next, the ability to selectively exchange one of the surface bound components was demonstrated by monitoring the release of both probes upon the addition of increasing amounts of ADP to a solution of Au MPCs 1 covered with ATP and NBD-GDDD in a 1:1 ratio (1.0 μM each). The obtained displacement curves confirm that the NBD-GDDD probe is released at lower concentrations compared to ATP and the 6.7 ratio between the relative binding affinities (120 and 18, respectively) indicates that the two probes exchange independently (Fig. 5c).

In conclusion, our results show that Au MPCs 1 are highly attractive supramolecular building blocks for the self-assembly of multivalent nanostructures. The high affinity of both oligo-phosphates and oligocarboxylates for the multivalent TACN·ZnII surface allows the complexation of up to 18 probe molecules at low micromolar concentrations in water. The surface composition can be modulated in a straightforward manner simply by changing the ratio of the anionic probes. The surface of Au MPCs 1 has a higher affinity for phosphates compared to carboxylates and this can be used for the selective exchange of surface bound components. Financial support from the ERC (SIR-239898) and COST (CM0703) is acknowledged.

Notes and references
† All displacement studies were performed at 80% of the surface saturation concentrations in order to avoid the presence of even a minimal amount of fluorescent probe in solution. The weaker binding of the ATP probe to Au MPCs renders determination of the relative binding affinities less precise.