Dynamic Combinatorial Chemistry

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Localized Template-Driven Functionalization of Nanoparticles by Dynamic Combinatorial Chemistry**

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Abstract: *We have developed a method for the localized functionalization of gold nanoparticles using imine-based dynamic combinatorial chemistry. By using DNA templates, amines were grafted on the aldehyde-functionalized nanoparticles only if and where the nanoparticles interacted with the template molecules. Functionalization of the nanoparticles was controlled solely by the DNA template; only amines capable of interacting with DNA were bound to the surface. Interestingly, even though our libraries contained only a handful of simple amines, the DNA sequence influenced their attachment to the surface. Our method opens up new opportunities for the synthesis of multivalent, nanoparticle-based receptors for biomacromolecules.*

Control over the relative arrangement of matter at the atomic level is one of the crucial requirements for the development of nanotechnology.[1] Organic chemists can now synthesize molecules of almost arbitrary complexity[2] and design them to perform useful work at the nanoscale, including directional movement,[3] programmed synthesis of other molecules,[4] regulation of catalytic activity,[5] self-assembly,[6] and even self-replication.[7] However, it is still very difficult to control the relative positioning of functional groups on the surface of nanoparticles, in stark contrast to the elaborate control possible for their organic counterparts. When metallic nanoparticles are functionalized with different ligands, the resulting mixed monolayers can typically be distributed statistically, form domains, or separate completely, thereby leading to Janus nanoparticles.[8]

Although randomly functionalized nanoparticles or nanoparticles functionalized with a single ligand have found applications in nanomedicine,[9] imaging,[10] sensing,[11] and as building blocks in controlled self-assembly,[12] the efficient and selective multivalent binding of biomacromolecules requires a complementary pattern of small recognition units on the surface of the nanoparticle.[11a,13] A promising approach to obtain a receptor complementary to its target is to synthesize it under thermodynamic control in the presence of the target that acts as a template. This approach has been successfully utilized in dynamic combinatorial chemistry (DCC)[14] to make receptors for small molecules,[15] ligands binding to pockets of biomacromolecules,[16] and multivalent polymers.[14n,17] We reasoned that the same approach may be utilized to achieve the selective functionalization of nanoparticle surfaces. DCC involving nanoparticles and small building blocks is so far unexplored, but should lead to multivalent receptors for biomacromolecules (Figure 1 A) if certain criteria are met. First of all, the nanoparticles should be stabilized by ligands that can form dynamic covalent bonds with other building blocks. Another necessary condition is the compatibility of the whole system with physiological conditions, which necessitates that the nanoparticles and building blocks are soluble in water. It is also desirable to use nanoparticles with a well-defined shape and suitable size that are able to interact with the target biomacromolecules across an extended surface area.

Not all dynamic covalent reactions are suitable for achieving the localized template-induced functionalization of surfaces. Reversible reactions for which the equilibrium lies on the side of the product always lead to functionalization of the nanoparticles, regardless of whether or not the template is present. For example, in the thiolate-ligand exchange reported by Rotello and co-workers,[13a,b] the gold nanoparticles always contain a mixture of attached ligands, also in the absence of the template. The addition of template only induces the rearrangement of the ligands on the surface. Nonspecific functionalization can be avoided if, in the absence of template effects, the covalent bond forming equilibrium lies far towards the starting materials, so that the building blocks are not attached to the surface of the nanoparticles in the absence of a template stabilizing them. Thus, the nanoparticles are only functionalized if the reversible covalent bonds are stabilized externally. Cooperative effects can stabilize weak covalent bonds[18] on a multivalent nanoparticle if multiple small building blocks are interfaced between the nanoparticle and the multivalent template. Imine chemistry[7c,19] meets the above requirements exceptionally well. The reaction between aldehydes and amines proceeds under thermodynamic control, thereby providing an error correction mechanism that proof-reads any wrongly attached building blocks. In an aqueous environment, the equilibrium for imine formation is strongly disfavored and imines are formed to a negligible extent if aldehydes and amines are present in (sub)millimolar concentrations.[20]

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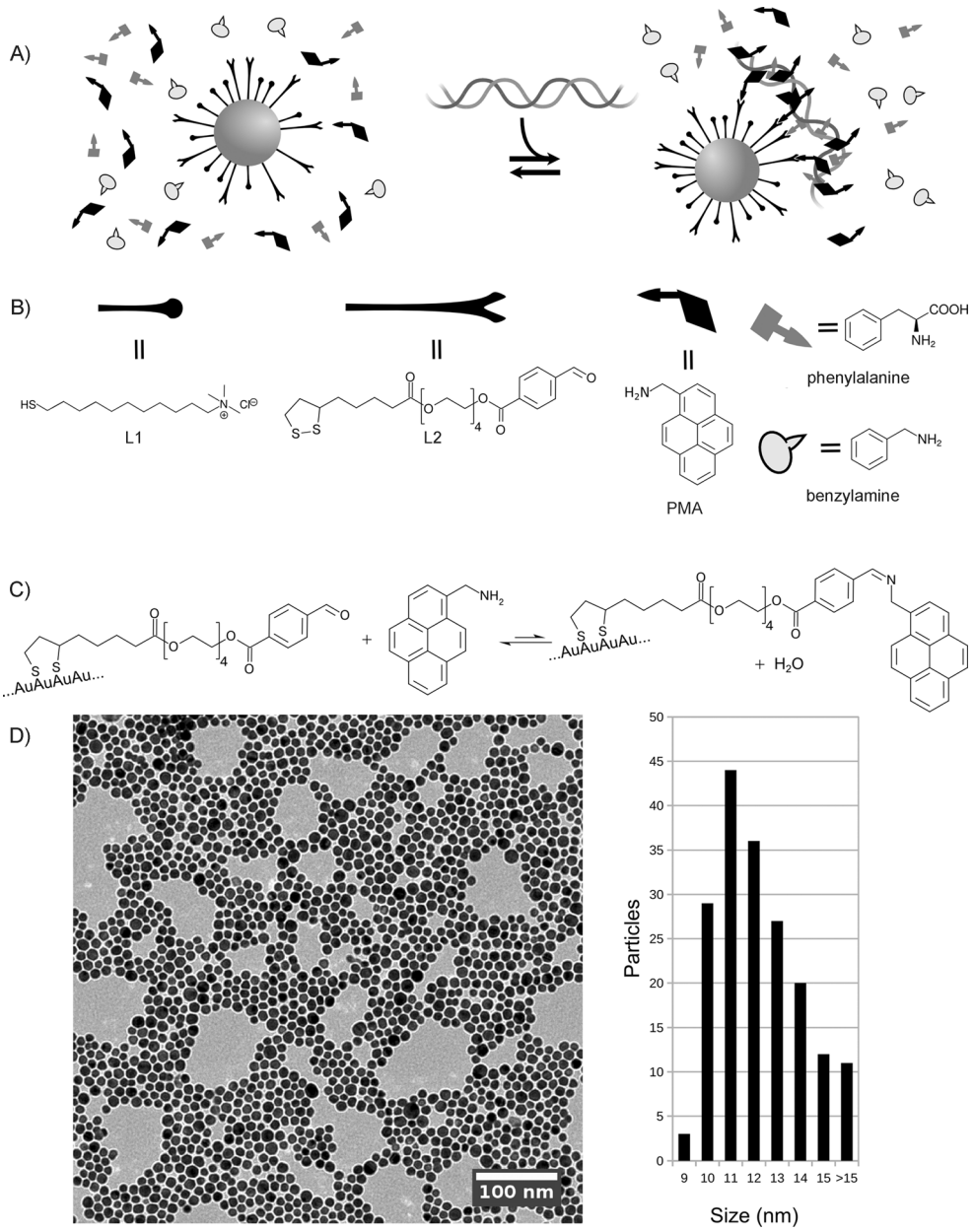

Figure 1. A) A DNA template directs the surface functionalization of the nanoparticles, which leads to the formation of a complementary receptor. B) Ligands used to stabilize the nanoparticles and recognition units. **L1** provides water solubility, while **L2** is the reactive ligand. C) Reaction between **L2** and PMA under thermodynamic control. D) TEM analysis of nanoparticles modified with **L1** and **L2** and the corresponding size histogram.

Thus, we designed nanoparticles that are water soluble, complementary in size to their DNA targets, and contain aldehyde groups for the reversible formation of imines.[7c,19] The nanoparticles were stabilized by two ligands: **L1** for water solubility and **L2** for imine formation (Figure 1B). The nanoparticles were prepared in two steps. First, the inorganic cores were prepared according to a literature procedure[21] and these were subsequently functionalized with ligands **L1** and **L2**. The resulting AuNPs were spherical and relatively monodisperse with a mean diameter of 11.7 nm (Figure 1D). We studied the surface functionalization using thermogravimetric analysis (TGA) and quantified the concentration of surface aldehyde on the basis of hydrazone formation (see the Supporting Information for details). The nanoparticles contained mostly ligand **L1**; on average there were more than 100 aldehyde ligands per nanoparticle, which gives a distance of less than 2 nm between two aldehydes (assuming a statistical distribution of the ligands on the surface; for details on AuNP synthesis and characterization see the Supporting Information, sections 3 and 4).

To test if the nanoparticles can be functionalized with amines, we prepared simple libraries composed of pyrenemethylamine, AuNPs, and 16-meric double-stranded DNA $(CG)_8$ as a template. Pyrenemethylamine (PMA) is a good DNA intercalator and a minor-groove binder[22] and has an amine group for imine formation. To analyze the libraries we devised a procedure based on separation of the nanoparticles together with species attached to them from the free amines in solution by using centrifugal filtration (Figure 2A). We then quantified the amines in the filtrate using HPLC (to yield the concentration of amines not attached to the AuNPs). The imine bonds at the nanoparticle surface were subsequently cleaved using hydroxylamine, which displaces the amines with formation of more-stable oximes.[23] Afterwards, the nanoparticle solution was filtered once more to quantify the amines which were attached to the nanoparticles.

By using this approach we investigated the degree of surface functionalization for a series of libraries with different PMA or DNA concentrations. In the presence of the DNA template and low amine concentrations, essentially all the amines are attached to the nanoparticles and only a trace amount remains free in the solution. As the binding sites in the DNA become saturated, the excess amine cannot attach to the nanoparticles and the concentration of PMA bound to the AuNPs reaches a plateau (Figure 2B). When varying the

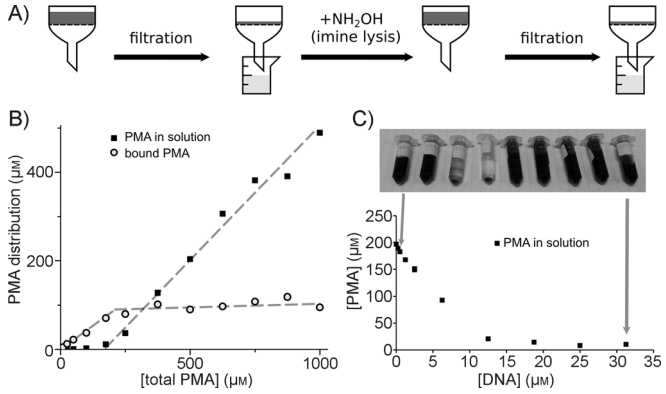

Figure 2. A) Analysis of the dynamic combinatorial libraries (DCLs). HPLC analysis of the first filtrate gives the concentration of the free amines in a DCL solution. Subsequent cleavage of the imines with hydroxylamine liberates the amines that had formed imines on the NP surface, which were quantified after a second filtration. B) Analysis of DCLs with different total PMA concentrations. [AuNP] = 80 nM, [DNA] = 12.5 μM (CG-repeat hexadecamer). C) Analysis of DCLs with different DNA concentrations. [AuNP] = 80 nM, total [PMA] = 200 μM. The inset shows the appearance of the AuNP solutions with increasing (from left to right) DNA concentrations.

DNA concentration, the amount of the amine bound to the nanoparticles changes linearly with the template concentration, starting with no binding for a template-free library to almost complete binding when the PMA/template ratio reaches approximately 1:15 (Figure 2C). The amount of PMA attached to the nanoparticles (either directly or indirectly, through DNA)[24] depends solely on the template concentration with no binding without the DNA present.

Lack of functionalization in the absence of the template is consistent with imine bonds being too labile to be formed without external stabilization provided by the multivalent template. Addition of the template promotes functionalization of the nanoparticles thanks to the cooperative effect[18] induced by interfacing two multivalent surfaces that are bridged by the amine building blocks. Thus, we conclude that functionalization of the nanoparticles is localized: it only occurs in the vicinity of the DNA template. The degree of functionalization depends on the AuNP/DNA ratio, which determines how much of the nanoparticle surface is covered by the DNA template and thus how much of the surface is functionalized with PMA.

We verified by two different experiments that PMA uptake by the nanoparticles is caused by imine formation and not merely by electrostatic interactions between the positively charged AuNPs and negatively charged DNA. Firstly, imine formation is evident from the observed release of amines from the DNA-templated nanoparticles upon treatment with hydroxylamine (Figure 2A), which is known to react with imines to form a more stable oxime,[23] thereby displacing the amine. Secondly, we performed a control experiment with nanoparticles that did not contain the aldehyde ligand. **L1**-only nanoparticles, while otherwise identical to the **L1**/**L2** nanoparticles, cannot form imine bonds with PMA and are capable of interacting with DNA only electrostatically. This system resulted in very limited PMA uptake (13% for the control nanoparticles and 85% for the aldehyde nanoparticles, for details see the Supporting Information, section 6).

We noticed that at a certain range of DNA concentrations, the nanoparticles aggregated and precipitated, as shown in Figure 2C. While it is well-known that mixtures of cationic and anionic nanoparticles show similar behavior,[25] in this case it is also possible that the aggregation occurs because of cross-linking of the nanoparticles by DNA and PMA. At low DNA/AuNP ratios, only a limited number of nanoparticles can be cross-linked by DNA and PMA. At high DNA/AuNP ratios, the entire nanoparticle surface is likely to be covered with DNA and PMA, effectively preventing any aggregation. Only at intermediate DNA/AuNP ratios can extensive cross-linking occur. Note that when nanoparticles are aggregated, treatment with hydroxylamine does not lead to efficient imine cleavage and release of the amines from the aggregates; therefore, in such cases only analysis of the first filtrate provides reliable results.

Finally, we investigated whether DNA binders can be selectively incorporated on the nanoparticle surface from a pool of different amines. We first prepared libraries containing pyrenemethylamine, L-phenylalanine, or benzylamine (Figure 1B). Table 1 shows that no amines were incorporated into the AuNPs without the DNA template, but in its presence the systems showed almost complete uptake of PMA and L-phenylalanine, which is also a known minor-groove binder.[26] Unsurprisingly, benzylamine did not show significant binding. Performing a similar experiment in the presence of all three amines led to the selective uptake of PMA, while also some L-phenylalanine was bound but no benzylamine. These results show that it is possible to use DNA to selectively functionalize the nanoparticles only with amines which are capable of attractive interactions with the templates. In the case of salmon testes DNA (on average 2000 base pairs), we observed nonspecific binding of benzylamine, presumably because of the observed significant aggregation of the nanoparticles with the DNA. Therefore, care must be taken to use relatively short DNA sequences which do not lead to significant cross-linking and aggregation of the nanoparticles.

Table 1: Selective incorporation of amine ligands induced by DNA templation.[a]

DNA sequence	Amines used	BA[b]	Phe[c]	PMA[d]
none	BA, Phe, PMA	0	0	0
$(CG)_8$	PMA	–	–	186
$(CG)_8$	Phe	–	200	–
$(CG)_8$	BA	2	–	–
$(CG)_8$	BA, Phe, PMA	0	86	181
stDNA[e]	BA, Phe, PMA	52	44	199

[a] The total concentration of each amine (if present) was 200 μM. The concentration of AuNPs was 80 nM, while the total DNA concentration was 25 μM with respect to single-stranded $(CG)_8$. [b] NP-bound benzylamine (μM). [c] NP-bound L-phenylalanine (μM). [d] NP-bound pyrenemethylamine (μM). [e] Salmon testes DNA, concentration equivalent to 25 μM 16-mer concentration.

Selective uptake of amines capable of binding with the DNA templates encouraged us to investigate to what extent the DNA sequence affects surface functionalization. We prepared AuNP libraries with several non-intercalating amines and templated them with double- and single-stranded hexadecameric DNAs with different sequences. The results are summarized in Figure 3. L-Tryptophan (Trp), L-phenylalanine (Phe), 2-(2-pyridyl)ethylamine (PEA), and tryptamine (TA) all contain aromatic rings for hydrophobic contacts with DNA grooves. Trp and Phe have additional carboxylate groups, thus allowing them to accept up to two hydrogen bonds with DNA bases.[27] Similar to the previous experiments, no functionalization occurred in the absence of the templates. When DNA templates were present, we observed significant differences between amine incorporation induced by different DNA sequences. The single-stranded C/G-rich templates promoted amine incorporation most efficiently, presumably because of a larger template surface available for interaction with the amines. Most DNA sequences showed a pronounced preference for Trp, followed by Phe, while PEA and TA were in most cases incorporated less efficiently. This observation is surprising, as one might expect the carboxylate groups of Phe and Trp to repel the negatively charged DNA. It is noteworthy that double-stranded $(AAAT)_4/(ATTT)_4$ and single-stranded $(AAAT)_4$ induced markedly different template effects compared to the other sequences we tested. Remarkably, the former template has the same overall nucleobase composition as the double-stranded $(AT)_8$ but the amine uptake is significantly different. Apparently, the system shows selective uptake of amines, not induced by the overall nucleobase content, but by the sequence of the bases in the DNA strand.

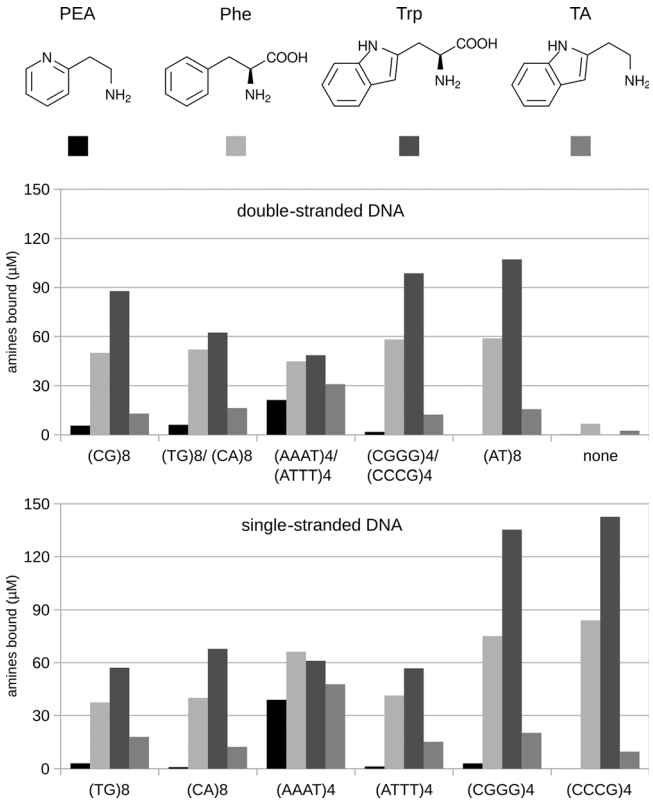

Figure 3. Influence of the DNA sequence on the functionalization of nanoparticles with different amines. The concentration of each amine was 200 μM. The concentration of AuNPs was 80 nM, while the total DNA concentration was 12.5 μM for single-stranded DNA and 6.25 μM for double-stranded DNA. Relative standard deviations did not exceed 10% of the measured free amine value, as determined for representative libraries.

In conclusion, we developed a method to locally functionalize gold nanoparticles using dynamic combinatorial chemistry and DNA templates. Imine formation under thermodynamic control allows for unprecedented control of the placement of the recognition groups thanks to the combination of cooperative template effects and the inherent instability of imines in aqueous solutions. Thereby, the nanoparticles are functionalized only in the direct proximity of the template, forming patches of recognition units complementary to the template molecules. Remarkably, in libraries containing even very simple amines, ligand incorporation is significantly influenced by the DNA sequence. Current investigations are focused on fixation of the labile imine bonds by reduction,[19c,l] expanding the set of DNA binding ligands with the aim of achieving sequence-selective recognition, and extending the method to other nanoparticle platforms.

Keywords: dynamic combinatorial chemistry · gold nanoparticles · multivalent interactions · surface functionalization · systems chemistry

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