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DNA micelles as nanoreactors: Efficient DNA functionalization with hydrophobic organic molecules

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We report a micelle-templated method to enhance the reactivity of DNA with highly hydrophobic molecules. Lipids, chromophores and polymers can be conjugated to DNA in high yield and under mild conditions. This method expands the range of DNA-templated reactions for DNA-encoded libraries, oligonucleotide and drug delivery, nanopore mimetics and DNA nanotechnology.

The conjugation of nucleic acids (DNA) to hydrophobic molecules, polymers or drugs can generate an important class of bio-hybrid materials that lend themselves to a broad range of biomedical applications such as gene therapy,[1-4] drug delivery[5-8] and biosensing[9-12] as well as material science.[13-18] To date, there are two main approaches to conjugate DNA to molecules, relying either on solid-phase or on solution-phase synthesis. While solid-phase approaches that incorporate non-natural moieties into DNA are versatile and powerful,[19-21] the molecules to be introduced need to be adequately modified for phosphoramidite synthesis, and importantly, they need to be stable to the relatively harsh deprotection conditions used. The yields for incorporation of long lipidic or polymeric chains using this method are also typically low.[7, 22, 23] Hydrophilic molecules and polymers can be efficiently attached to post-synthesized DNA strands in water.[24-27] However, attaching hydrophobic moieties to DNA in aqueous solution remains a challenge, in large part because of solvent incompatibility between DNA and hydrophobic molecules.[28-30]

DNA-templated reactions are a powerful method to enhance reaction rates, and have been used in biological sensing, controlled organic synthesis and DNA-encoded combinatorial library generation.[31-34] However, the reactions used typically need to be compatible with aqueous conditions, which can limit their range.[31] Micellar reactions constitute an important methodology for efficient and green synthetic organic transformations.[35] These rely on the use of surfactants to form micellar aggregates in aqueous solution, and take advantage of the hydrophobic effect to bring together reactants within the core of these micelles. The reactant molecules experience a significant increase in effective concentration,[36] and can undergo a variety of reactions with enhanced yields[37-41] and often altered regio- and stereo-selectivity.[42-44] Here, we report a simple DNA micelle-templated method to enhance the reactivity of a range of hydrophobic molecules and polymers with DNA, in aqueous buffer and under mild conditions, with excellent yields, short reaction times, and ready purification and separation of the final products. This method expands the range of DNA-templated reactions, because it allows them to occur in an organic micellar core with increased reactant effective concentration.

The method relies on a simple, commercially available DNA strand conjugated to 1,12-dodecanediol phosphoramidite units (hexaethylene, or HE) (see Supporting Information SI-III). This amphiphilic conjugate self-assembles in tris-acetate buffer containing magnesium ions (TAMg) to form highly monodisperse DNA micelles.[45] We show that these micelles can be used as reaction auxiliaries: a complementary, non-hydrophobically modified DNA strand can hybridize to them, thus orienting its reactive group towards the micelle core, and this significantly improves the conjugation yields **(Scheme 1)**. Reactions in these micelles are highly efficient: a range of molecules can be attached directly to the DNA strand as well as to the amphiphile components of the micelle, including activated N-hydroxysuccinimide (NHS) esters of palmitic acid (C_{16}), stearic acid (C_{18}), behenic acid (C_{22}), a branched (bis-C_{10}) N,N'-didecyl chain (NDS), the chromophore pyrene and pre-formed polystyrene (PS) via amide formation (for the functionalization of DNA amphiphiles, see Supporting Information SI-VI). Because of the fine polymer sequence control (e.g number of HE units and position of functional group) of the DNA-hydrophobic conjugates used for templation, we examined the position-dependent reactivity of the functional group inside the micellar HE core. This gave us

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insights into the nature of this hydrophobic environment. The DNA amphiphiles can be re-used for a variety of chemical reactions. Considering the wealth of micelle-promoted reactions and micellar catalysis in organic chemistry, we predict that this will be a general method to attach molecules to DNA, as well as increase their complexity through subsequent reactions within the micellar core, thereby increasing the range of applications of DNA conjugates in biomedicine and nanotechnology.
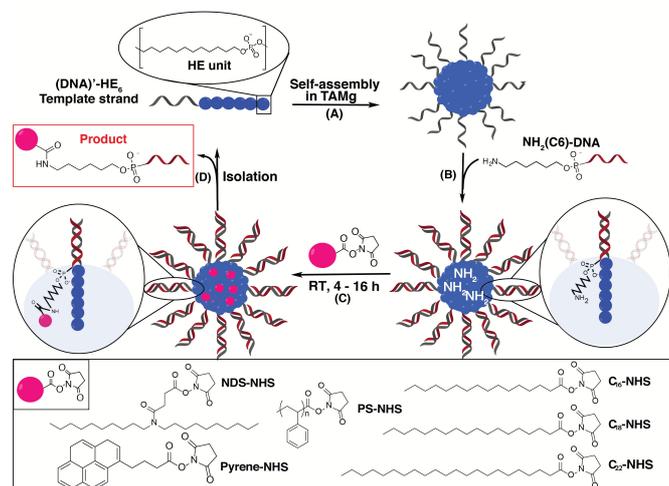
Scheme 1. Synthetic methodology for DNA micelle templated conjugation. **(A)** Self-assembly of DNA amphiphiles ((DNA)'-HE$_6$) into micelles in aqueous buffer **(B)** Hybridizing amino-modified DNA (complementary to (DNA)') **(C)** Adding activated hydrophobic NHS esters separately and incubating at room temperature. **(D)** Using RP-HPLC to separate starting material, template strand and conjugated product.

We functionalized a 19-mer DNA strand (we name the DNA sequence "**DNA**") with three different commercially available amino modifiers, namely **NH$_2$**, **NH$_2$(C3)** and **NH$_2$(C6)** (see Supporting Information SI-XII for the chemical structures). A DNA amphiphile strand (DNA)'-HE$_6$ was designed as template, containing 6 HE units and a DNA sequence complementary to the amino-DNA. The amino modified DNA strands and (DNA)'-HE$_6$ template strands were mixed and assembled into double-stranded micelles in TAMg buffer by thermally annealing from 95°C to 4°C for 1 hour. This arrangement will direct the amino group towards the hydrophobic core of the micelle and thus bring this group into closer proximity to the NHS guest molecules. Separately, hydrophobic molecules functionalized with a NHS ester group, were dissolved in a small amount of organic solvent (THF or DMSO), then added to the DNA micelles solution. The reaction mixture was shaken at room temperature for 4 to 16 hours. After the reaction, the products conjugated with hydrophobic molecules were isolated by reverse-phase HPLC (RP-HPLC) and the template strand can be recovered and subsequently recycled for another conjugation process **(Scheme 1** and see Supporting Information SI-XII for more details on recycling process).

With the micelle-templated approach, we observed significantly improved conjugation yields. The amino DNA **NH$_2$(C6)**-DNA gave 60% conjugation yield with C$_{16}$-NHS, up from 36% without templation. We expanded the library of hydrophobic molecules to further validate this approach. The strategy was extremely effective for conjugating DNA to C$_{18}$-NHS (64% yield, 16% without template), a branched molecule containing two C$_{10}$ chains NDS-NHS (83% yield), the chromophore pyrene-NHS (95% yield), and it was moderately effective for the very long C$_{22}$-NHS (30%, up from 0% without template). (**Scheme 1**, **Figure 1** and see Supporting information SI-XII). All these molecules give poor to no yields without the template micelle. If the amino DNA is made using other shorter amino modifiers, the yields are lower (18% of C$_{22}$-NHS for **NH$_2$**-DNA and 22% for **NH$_2$(C3)**-DNA, see Supporting Figure SF27 and SF36). This suggests that with a smaller spacer, the terminal amino group on this DNA strand may not be able to reach into the micellar core to react with the hydrophobic molecules. Conjugation yields are summarized in **Figure 1**. The template amphiphile can be recovered in 85% isolated yield by HPLC, and we estimate that it can be re-used ~15 times under our conditions (Supporting Information SI-XII). Thus, simple hybridization of amino-DNA to the micelle auxiliary can significantly increase the coupling yields to a variety of hydrophobic units.
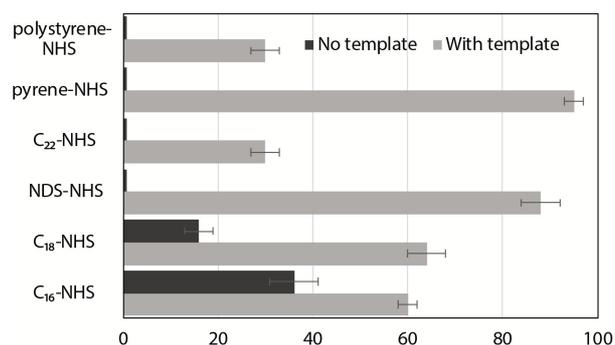
Figure 1. Conjugation yields of **NH$_2$(C6)**-DNA with hydrophobic molecules with and without **(DNA)'-HE$_6$** template. All conjugation reactions were carried out at least in triplicate.

We then tried to conjugate DNA directly to hydrophobic, fully formed polymers in aqueous buffer. As a proof of concept, we chose polystyrene (PS with M$_n$ = 900, PDI = 1.5) terminated with an NHS moiety (PS-NHS, **Scheme 1**). Using the previously described method, **NH$_2$(C6)**-DNA can be conjugated with polystyrene with a 30% yield using template, up from 0% without templation (see Supporting Figure SF34). Several peaks in RP-HPLC between retention time 18 min and 31 min were separated and identified by LC-MS, as the conjugate products with polystyrene-NHS and increasing styrene units. Interestingly, although the starting polymer has a relatively high polydispersity index (PDI=1.5), the monodispersity of our DNA template allowed the identification and separation of DNA conjugates with different lengths of polystyrene (see Supporting Figure SF35).

One of the important design elements of this approach is to aim the reactive amino group towards the hydrophobic micellar core in order to improve the conjugation efficiency. Therefore, we were interested to examine the dependence of conjugation efficiency on two parameters: *1. the position of the amino group inside the micellar core* and *2. the size of the*

Figure 2. Varying the DNA amphiphile length and position of amino monomer **(NH₂)** on DNA amphiphiles and yield of each strand with C_{22}-NHS (N.R: No reaction).

micellar core, to gain better understanding on how DNA micelles enhance the conjugation efficiency. We synthesized DNA amphiphiles that were themselves substituted with an amino group (**Figure 2,** see Supporting Information SI-III). Our synthesis allows the placement of the amino group in precise positions along the hydrophobic HE backbone, and a detailed assessment of its reactivity. Four classes of DNA amphiphiles were prepared: DNA amphiphiles with **12 HE**, **6 HE**, **1** and **0 HE** repeats (**Figure 2**). The first two classes of molecules form stable micelles, while the third and fourth do not assemble in aqueous solution and were used as a control. Within these, the position of the amino group was varied by placing it at the end of the hydrophobic chain, in the middle, or at the interface between the DNA strand and the hydrophobic block **(Figure 2)**. This position was chosen such that there are at least 6 HE contiguous repeats, in order not to disrupt the micelle formation. The DNA amphiphiles were assembled into micelles in TAMg buffer and the assembly was verified by dynamic light scattering and atomic force microscopy (see Supporting Information SI-VII and SI-VIII for synthesis and characterization). Our investigations were carried out using the hydrophobic NHS molecules described earlier. They allowed us to extract reactivity trends, summarized here:

1. *The yields are significantly higher with micelle formation*. For example, for C_{16}-NHS and C_{22}-NHS, DNA amphiphile **(4)** achieved 87% and 74% yield respectively, while non-micelle-forming **(7)** resulted in 0% in both cases (see Supporting Figure SF9 and **Figure 2** for C_{22}-NHS).

2. *The yields are higher for the HE_6 than for the HE_{12} amphiphiles*. For example, for C_{16}-NHS and C_{22}-NHS, amphiphile **(4)** achieved 87% and 74% yields, while **(1)** gave 40% and 18% yields, respectively (see Supporting Figure SF9 and **Figure 2** for C_{22}-NHS). This is possibly due to increased rigidity of the micellar core with a higher number of HE repeats, slowing down the diffusion of the small molecules to the reactive units.

3. *The yields increase in the following order: NH_2 in the middle of the hydrophobic chain < NH_2 at the interface between the polymer and the DNA < NH_2 at the end of the hydrophobic chain.* For instance, for conjugation with C_{22}-NHS, **(1)** gave 18% yield, while **(2)** and **(3)** gave 13% and 9% respectively; **(4)** (74%) is more reactive than **(5)** (33%) **(Figure 2)**. This is likely because of the decreased accessibility of the NH_2 moiety to the reactive hydrophobic molecules when it is in the middle of the chain as compared to the chain end.

We noted above that the NH_2 group at the chain end is more reactive than the NH_2 at the micelle interface (e.g., **(4)** and **(5)** in **Figure 2**, see Supporting Information SI-X for more details). This implies that the two groups reside in different locations, and that there is a degree of chain folding of the long alkyls in the core. (If the chains were completely unfolded then the NH_2 end-group would be oriented near the interface, and the two groups would have similar reactivity). We are currently examining the internal structure of the micelle core in greater detail.

Another important insight obtained from the site-specific labeling with NH_2 was to ascertain that micelle formation is essential to the rate acceleration. We have previously shown that micelle formation in these structures is dependent on the presence of Mg^{2+}, most likely needed to overcome the repulsion in bringing the phosphate units within the core, and that it does not occur in pure water without these ions. We thus compared the reaction of **(4)** with C_{22}-NHS in Mg-containing buffer and in pure H_2O. Indeed, the reaction in water showed significantly lower yield (20%) compared to the Mg^{2+}-buffer (74%) (see Supporting Information SI-XI). We also observed that the conjugation yields are very poor (~0%) in pure organic solvents, such as DMSO or DMF. (Supporting Figure SF29) Thus, DNA micelle formation is a key factor in the increased conjugation yields observed here. Overall, these studies reveal the polymer sequence-controlled reactivity characteristics of these DNA micelles. By changing the number of HE repeats and the position of the reactive group, the reactivity of molecules with DNA can be finely controlled.

With this information, we turned our attention back to the templated micelle approach described earlier **(Scheme 1, Figure 2, in red)**. For the **NH₂-**DNA **(7)** with a short serinol amino modification, micelle-templated coupling to C_{22}-NHS gives only 18% yield (**Figure 2**, see Supporting Figure SF36). Recall that the longer C_6-amino modification gives a higher yield (30%, **Scheme 1 and Figure 1**). Interestingly, introducing a single HE spacer between the DNA strand and the amino group in **(7)** significantly increases the yield of this coupling reaction to 62% (**Figure 2**, see Supporting Figure SF38). Thus, directing the amino group deeper into the micellar core by

using an amino modification with a longer spacer, or introducing an alkyl spacer allows coupling DNA to extremely hydrophobic units in good yields. For C_{20} or less, the coupling of regular amino-modified DNA occurs with high efficiency.

In conclusion, we have demonstrated a facile methodology to conjugate hydrophobic molecules to DNA strands in aqueous solution with high yield and under mild conditions. This method combines a DNA-templated reaction with the ability of a micellar core to increase the effective concentration of reactants, and thus enhance the reaction rates. The DNA micelle is assembled from a commercially available strand, which can be re-used for multiple conjugations. This will potentially open the door to the introduction of many new reaction classes into DNA structures. A number of hydrophobic units were attached to DNA, ranging from long alkyl chains, branched long alkyls, chromophores like pyrene to polymers like polystyrene. In mechanistic work, we positioned the reactive groups in different locations within the micelle. This allowed us to optimize the structure of the micelle auxiliary and the location of this reactive group. With a wide range of hydrophobic carboxylic acids from commercially available as well as natural sources, this method has the potential to be applied to numerous research problems, from DNA and small molecule therapeutic delivery to diagnostics, nanopore formation, DNA nanotechnology and material science.

Notes and references

Acknowledgements

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