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"Cap-and-Catch" Purification for Enhancing the Quality of Libraries of DNA Conjugates

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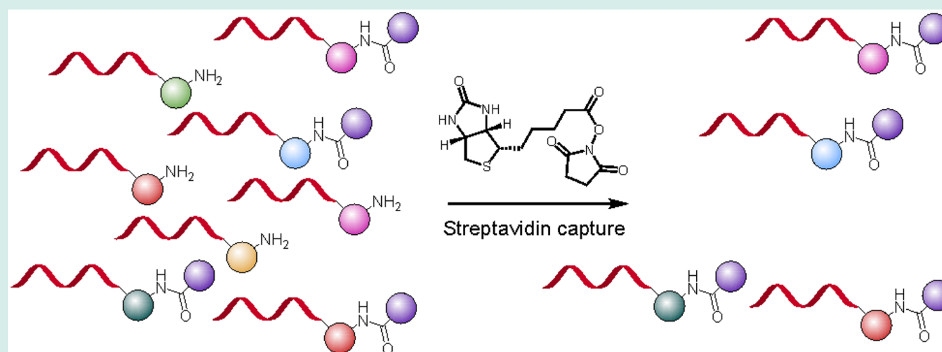
Supporting Information



ABSTRACT: The potential of DNA-encoded combinatorial libraries (DECLs) as tools for hit discovery crucially relies on the availability of methods for their synthesis at acceptable purity and quality. Incomplete reactions in the presence of DNA can noticeably affect the purity of DECLs and methods to selectively remove unreacted oligonucleotide-based starting products would likely enhance the quality of DECL screening results. We describe an approach to selectively remove unreacted oligonucleotide starting products from reaction mixtures and demonstrate its applicability in the context of acylation of amino-modified DNA. Following an amide bond forming reaction, we treat unreacted amino-modified DNAs with biotinylating reagents and isolate the corresponding biotinylated oligonucleotides from the reaction mixture by affinity capture on streptavidin-coated sepharose. This approach, which yields the desired DNA-conjugate at enhanced purity, can be applied both to reactions performed in solution and to procedures in which DNA is immobilized on an anion exchange solid support.

KEYWORDS: *DNA-encoded chemical libraries, purification, biotin, bioconjugation*

Encoding small-molecule compounds by the attachment of distinctive DNA recognition sequences allows for screening large combinatorial libraries in "one-pot" affinity selection protocols. Such DNA-encoded combinatorial libraries (DECLs) are increasingly being used for the discovery of pharmaceutical lead compounds.[1,2] In addition to enabling the discovery of hit compounds, DECL screening experiments may reveal structure−activity relationships important for ligand−protein interactions. For instance, conserved structural features were observed among series of hit compounds,[3−6] and simple structure−activity patterns could be identified from DECL screening experiments.[7] Indeed, a correlation between sequence enrichment and binding affinity may provide valuable guidance for subsequent lead optimization. However, the identification of semiquantitative structure−activity relationships using DECLs requires libraries of good quality. Heterogeneous distributions of the amounts of DNA-conjugates in a DECL, as well as variable synthetic yields for the different steps in library construction, are likely to represent confounding factors in DECL decoding procedures and to obscure structure−activity information.

Several strategies for the preparation of DECLs have been reported, including the synthesis of DECLs on beads,[8] DNA-directed synthesis,[9] hybridization-based self-assembly,[10,11] covalent self-assembly,[12,13] and DNA-guided sorting.[14] A widely used approach for library constructions consists in the preparation of DECLs by a split-and-pool strategy, in which compound synthesis and DNA-encoding steps are performed iteratively.[3,7,15−17] In this method, it is possible to purify DNA conjugates by HPLC after the incorporation of the first set of building blocks. However, at subsequent steps in library construction, the synthetic intermediates are present as mixtures of conjugates, which prevents their chromatographic purification. Consequently, chemical reactions used for DECL synthesis should ideally proceed with near-quantitative yields and with minimal formation of side-products. Nevertheless,

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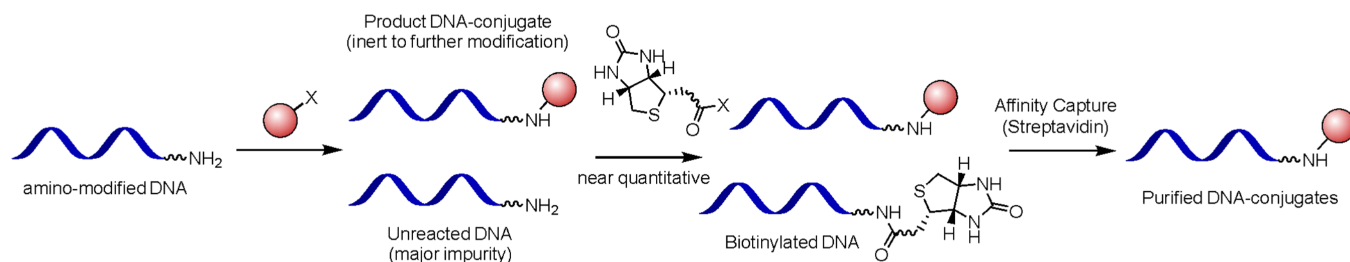


Figure 1. General scheme of "cap-and-catch" method for the removal or unreacted DNAs, following DNA-conjugation reactions, based on a biotinylation procedure and a subsequent streptavidin-assisted purification step.

even for well-established reactions used for the preparation of DNA conjugates,[18−20] it can be challenging to achieve high conversion rates for large sets of structurally diverse building blocks.[19]

Unreacted DNAs generally represent the major side products in DECL construction and methods for their separation could significantly enhance the purity and homogeneity of the library. Here, we present an approach to remove unreacted DNA conjugates during DECL synthesis, to enhance overall library purity. The method, termed "cap-and-catch" purification, includes the modification of unreacted functional groups on the starting DNA with a capping agent that contains a tag amenable to affinity capture. Related strategies have been reported for the solid-phase synthesis of peptides,[21−23] oligonucleotides,[24−26] and oligosaccharides.[27] We demonstrate the concept of "cap-and-catch" purification for the attachment of carboxylic acids to amino-modified DNAs, a frequently used reaction for the preparation of DECLs.[19] In this protocol, following the synthesis step, electrophilic biotin reagents cap unmodified amino-DNAs and the resulting biotinylated DNAs can be removed by affinity capture on streptavidin-coated sepharose (Figure 1). Several tested reaction protocols provided excellent biotinylation yields and enabled the straightforward purification of DNA-conjugates from unreacted amino-DNA.

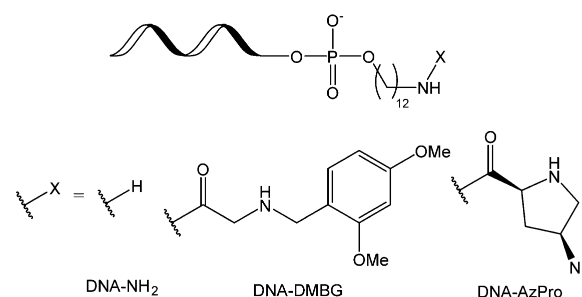
Initially, we tested a series of biotinylation conditions (Table 1) using a pseudosolid phase reaction protocol, a convenient method for DECL preparation. In pseudosolid phase reactions, the DNA is immobilized on DEAE anion exchange resin, which allows for repeated reaction and washing cycles with simple elution of reagents similar to solid-phase synthesis.[19,28] All tested coupling conditions led to biotinylation of 5′-amino-modified DNA (DNA-NH_2, Scheme 1) in good yields, although prolonged incubation times were necessary for some coupling reagents (e.g., EEDQ, IIDQ) to reach >90% conversion (Table 1). LC-MS analysis of the reaction product of biotin activated by the coupling reagent DMT-MM was indicative of multibiotinylation of DNA-NH_2 possible by side reactions with the nucleobases. We selected the *N*-hydroxysuccinimide ester of biotin (B-NHS) for further studies because apart from providing good conversion rates this reagent is commercially available, bench-stable, and generally provides clean reaction products.

Table 1. Evaluated Biotinylation Conditions

activating or biotinylating agent[a]	conversion [%][b] 2 h	18 h
EDC, HOAt	93	97
DMT-MM, NMM	88	>99[c]
EEDQ	62	95
IIDQ	51	96
IIDQ, DMAP	52	95
isobutylchloroformate, NMM	84	82
isobutylchloroformate, NMM, DMAP	93	97
biotin-NHS	52	68
biotin-NHS, DMAP	91	>99

[a]Biotin concentration was 100 mM except for EDC sample (50 mM). Detailed coupling conditions are summarized in the Supporting Information; HPLC traces are shown in Supporting Information Figure S1. [b]Degree of conversion was estimated by analytical HPLC; values are averages of triplicate measurements. [c]LC-ESI-MS analysis was indicative of biotinylation of nucleobases.

Scheme 1. Amino-Modified DNA Oligonucleotides Used for Biotinylation and "Cap-and-Catch" Purification Experiments[a]



[a]The sequences are provided in the Supporting Information.

Incubation of resin-immobilized DNA-NH_2 and B-NHS afforded the biotinylation product in moderate yields (Table 2). Addition of substoichiometric quantities of 4-dimethylaminopyridine (DMAP) substantially enhanced biotinylation (Tables 1 and 2). The fraction of biotinylated conjugates reached 78%, 84%, and 90% in the presence of 5, 10, and 20 mM DMAP, respectively (50 mM B-NHS, 2 h, room temperature in DMSO). For comparison, the conversion reached only 44%, when B-NHS alone was used. Increasing the B-NHS concentration and the reaction time also had a positive effect on biotinylation. Moderate heating (37 °C) additionally increased the reactivity of B-NHS to DNA-NH_2. LC-ESI-MS analysis of certain samples with long incubation times (18 h) or elevated B-NHS concentrations (500 mM) revealed the formation of overbiotinylated DNA-NH_2 although the fraction of such side products was generally low. Other protocols, providing the product in near-quantitative conversion, showed no presence of such side products and, several reaction conditions provided near-quantitative biotinylation of DNA-NH_2 suitable for "cap-and-catch" purification. We used 4 h incubation at 37 °C with 200 mM B-NHS and 20 mM DMAP

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Table 2. Evaluation and Optimization of Biotinylation Conditions Using the *N*-Hydroxysuccinimide Ester of Biotin (B-NHS)

c(B-NHS) [mM]	*c*(DMAP) [mM]	time [h]	temp [°C]	conversion[a] [%]
50		1	RT	28 ± 4
50		2	RT	44 ± 5
50		4	RT	53 ± 5
50		18	RT	69 ± 1
50	5	1	RT	66 ± 5
50	5	2	RT	78 ± 5
50	5	2	37	89 ± 4
50	5	4	RT	88 ± 2
50	**5**	**4**	**37**	**>99**
50	5	18	RT	96 ± 1
50	10	2	RT	84 ± 4
50	10	18	RT	98 ± 1
50	20	2	RT	90 ± 1
100		2	RT	52 ± 12
100	10	2	RT	91 ± 4
100	**10**	**18**	**RT**	**>99**
200	20	2	RT	94 ± 2
200	**20**	**18**	**RT**	**>99**
500	**50**	**2**	**RT**	**>99**

[a]Estimated by HPLC; average of triplicate experiments. HPLC traces are shown in Supporting Information Figure S2.

as standard conditions for further experiments unless stated differently.

An alternative approach for DECL synthesis performs the acylation reactions of amino-modified DNAs in liquid phase. We therefore tested the reaction of DNA-NH_2 and B-NHS in a buffered solution (4 h, 37 °C, 0.5 M TEA-HCl, pH 10, 84% DMSO). Biotinylation of DNA-NH_2 proceeded efficiently under the tested conditions. DNA-biotin was formed near-quantitatively at B-NHS concentrations that provided only partial conversion on pseudosolid phase. For instance, incubation of DNA-NH_2 with 50 mM B-NHS without DMAP for 4 h at 37 °C provided the biotinylated DNA without detectable traces of residual DNA-NH_2 (Figure 2a). LC-ESI-MS analysis results for samples prepared using these conditions showed the presence of a minor DNA-product with two biotin molecules attached (approximately 10%), possibly by the modification of exocyclic amines of the nucleobases. Such a side product would be eliminated during streptavidin capture and lower the recovery yield. Overbiotinylation became more pronounced with increasing B-NHS concentrations (Supporting Information Figure S4) and careful control of the biotinylation conditions may be advised when implementing "cap-and-catch" procedures for library synthesis. Thus, when suitable conditions (50 mM B-NHS for 4 h at 37 °C) are used, also biotinylation procedures in liquid phase represent effective methods for "cap-and-catch" cleanup of DNA-acylation reactions.

We further aimed at evaluating the biotinylation of DNAs with terminal secondary amines. The generation of secondary amines by either reductive amination or nucleophilic substitution followed by acylation represents an attractive avenue to combinatorial libraries of structurally compact and diverse compounds[29] compatible with DECL synthesis.[19] However, yields for acylation reactions of secondary amines tend to be lower than for primary amine oligonucleotide conjugates.[19] Test biotinylations with two DNA conjugates bearing secondary amines (DNA-DMBG and DNA-AzPro, Scheme 1) provided lower modification yields than for DNA-NH_2. In a test reaction on pseudosolid phase (200 mM B-NHS, 20 mM DMAP, 2 h, room temperature) biotinylation yields were 33% and 73% for DNA-DMBG and DNA-AzPro, respectively (Supporting Information Figure S3). Harsher conditions increased product formation (76% for DNA-DMBG and 95% for DNA-AzPro; 500 mM B-NHS, 50 mM DMAP, 4 h, 37 °C; Supporting Information Figure S3) without reaching complete conversion. Formation of overbiotinylated DNA products was further a problem under these conditions. In light of the higher biotinylation efficiency observed for reactions in solution, we also tested biotinylation of DNA-DMBG and DNA-AzPro using the liquid phase protocol. The reactions yields were 84% for DNA-DMBG and 85% for DNA-AzPro at 50 mM B-NHS (Figures 2b and 2c) and reached 95% and >95% at 100 mM B-NHS (data not shown). Conclusively, biotinylation and "cap-and-catch" purification may in principle be applied to DNA-conjugates with secondary amines. However, the incomplete degree of conversions and the formation of biotin adducts at harsher reaction conditions may limit the practical use of this approach for secondary amines and further optimization of reaction conditions may be required.

As the next step, we tested the "cap-and-catch" method for the cleanup of bioconjugation reactions of DNA-NH_2 with a representative set of carboxylic acids (Figures 3 and Supporting Information S5). Two peaks were visible in the HPLC traces of

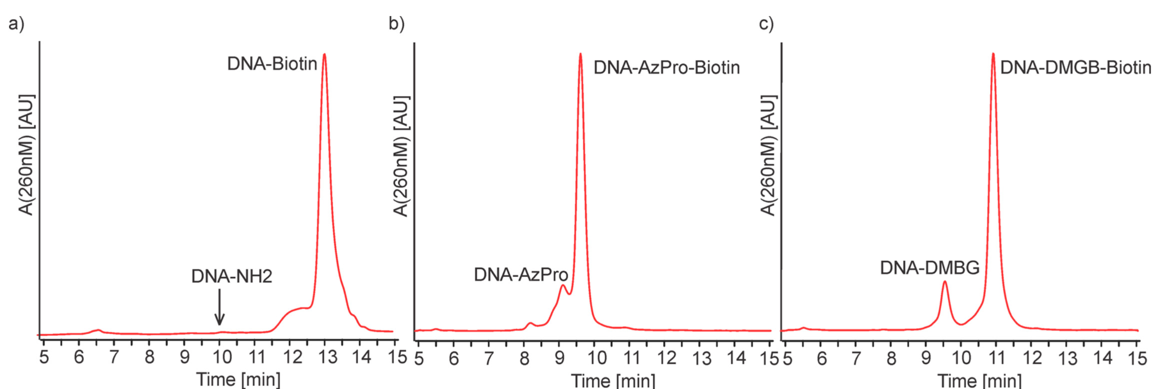

Figure 2. Biotinylation of amino-modified DNA in solution. HPLC traces for biotinylation reaction mixtures (50 mM B-NHS, 37 °C, 4 h) for (a) DNA-NH_2, (b) DNA-AzPro, and (c) DNA-DMBG. Arrow indicates anticipated elution time of DNA-NH_2.

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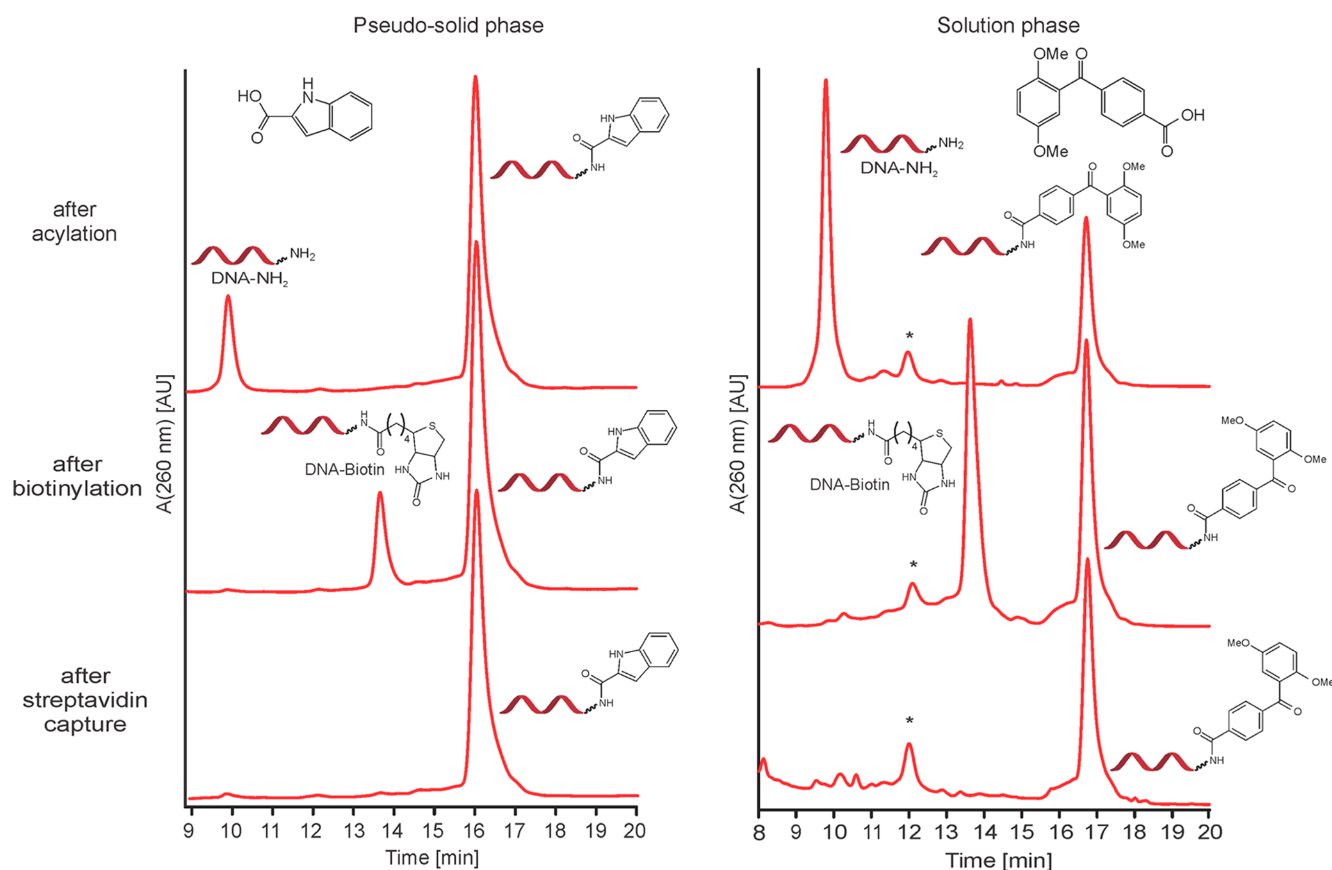

Figure 3. Examples of "cap-and-catch" cleanup of DNA-acylation reactions. Purification performed for amide-bond forming reaction with DNA immobilized on a DEAE sepharose resin (pseudosolid phase; left figure) and in solution (right figure). Structures of carboxylic acid substrates are shown in the figure. Asterisks indicate an impurity in the starting DNA.

the reaction products prior to "cap-and-catch" cleanup. A first peak eluting at ∼10 min was identified as unmodified DNA-NH_2 and a second eluting several minutes later corresponded to the coupling product. Upon biotinylation (200 mM B-NHS, 20 mM DMAP, 4 h, 37 °C), the unmodified DNA completely disappeared and a new peak at 13.5 min emerged corresponding to the biotinylated DNA. Affinity capture with streptavidin-coated sepharose removed all biotinylated DNA and afforded the conjugation product in visibly enhanced purity. "Cap-and-catch" cleanup was successful for all tested carboxylic acids despite the variable coupling yields (Supporting Information Figure S5).

We further tested the "cap-and-catch" method for incomplete DNA-conjugation reactions in solution (Figure 3b and Supporting Information S6). DNA-NH_2 was incubated with activated carboxylic acids providing a partial conversion as indicated by two peaks in the HPLC trace. B-NHS (50 mM, 37 °C) was added directly to the reaction and the mixture was incubated for additional 4 h, which entirely converted the remaining unreacted DNA-NH_2 to biotinylated DNA. The biotinylated DNA could be quantitatively eliminated by affinity capture on streptavidin-coated sepharose. Conclusively, "cap-and-catch" purification enables to remove unreacted amino-modified DNAs from incomplete DNA-acylation reactions both on anion exchange resin and in solution affording the desired products with distinctly improved purities.

Finally, we assessed the recovery of DNA during "cap-and-catch" cleanup. Incubation of DEAE-immobilized DNA under biotinylation conditions (200 mM B-NHS, 20 mM DMAP, 4 h) provided the same quantities of DNA as samples incubated with DMSO alone (data not shown). Furthermore, untreated DNA-NH_2 eluted quantitatively from the streptavidin-coated sepharose although two elution steps were necessary for quantitative recovery (Supporting Information Figure S7). Moreover, the quantity of the product in the "cap-and-catch" samples (Figure 3 and Supporting Information Figures S5 and S6) was similar for untreated and biotinylated samples and those that have been purified by streptavidin capture. Conclusively, no loss of DNA was observed during "cap-and-catch" cleanup.

In summary, this Letter describes a method for the removal of unreacted amino-modified DNAs from DNA-acylation reaction mixtures by a sequence of biotinylation and streptavidin-affinity capture steps. This "cap-and-catch" method may find application for the preparation of DECLs with enhanced purities. DECL library purity likely is an important parameter to ensure the reliability of screening results.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and HPLC analysis of conjugation reactions. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscombsci.5b00072.

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Notes

The authors declare no competing financial interest.

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