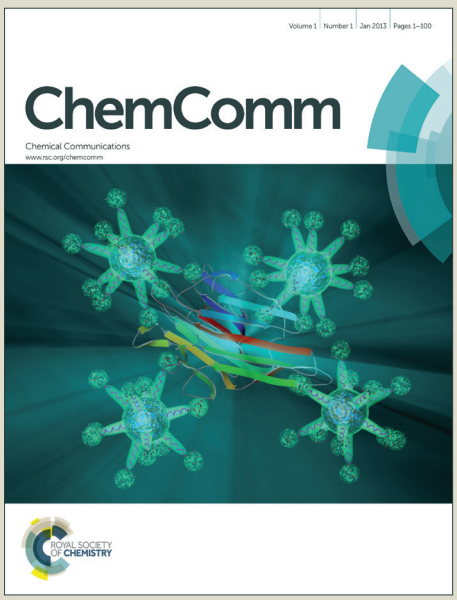
ChemComm

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Journal Name

RSCPublishing

COMMUNICATION

Aniline-Terminated DNA Catalyzes Rapid DNA-Hydrazone Formation at Physiological pH

Cite this: DOI: 10.1039/x0xx00000x

Dylan W. Domaille[a]* and Jennifer N. Cha[a]*

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

We report the effect of DNA-templation on aniline-catalyzed *N*-acylhydrazone formation. The reaction occurs in both two-component and three-component systems. Through systematic catalyst modifications, we are able to increase the efficiency of the DNA-templated variant to 85-fold above that of the uncatalyzed reaction at physiological pH.

The reaction of a hydrazine or an alkoxyamine with an aldehyde to form the corresponding hydrazone or oxime is a commonly used bioconjugation reaction, as it benefits from a high equilibrium constant and shows good bioorthogonality and compatibility with endogenous cellular functionality.[1–5] Recent studies have shown that aniline and related compounds can signifncantly increase the rate of product formation over that of the uncatalyzed variant.[6–8] Mechanistically, aniline acts as a nucleophilic catalyst and reacts with the aldehyde to form an aniline Schiff base intermediate in the rate limiting step;[6,9] however, high concentrations of aniline (10-100 mM) are required to realize a significant catalytic effect, and such a high concentration precludes the use of this in certain biological applications, though biocompatible aniline-derivatives have recently been reported.[10]

DNA-templated synthesis is a powerful method that has found applications in a diverse range of fields, from the construction of complex molecules[11–14] to the identification of novel chemical reactions[15] to the detection of biomolecules.[16–20,12,21–24]Typically, DNA-templation relies on DNA hybridization to bring two functionalized DNA strands together: the resulting increase in local concentration prompts the rapid and selective reaction between the two functional groups, effectively ligating the DNA strands together.[25,26] However, few reports have focused on DNA-templated organocatalysis with untethered reagents, the notable exception being a report from Tang *et al*., in which it was demonstrated that prolinamide-DNA could effect a proline-mediated aldol reaction between a hybridized aldehyde-DNA and an untethered aldehyde at a rate more than 170-fold above that of the uncatalyzed reaction.[27]

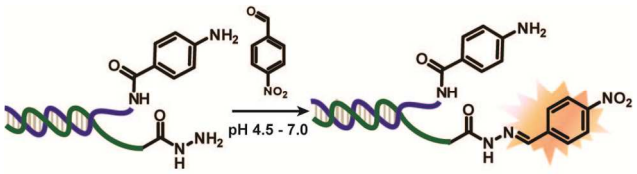


Figure 1. T15-acyl hydrazine DNA (green) hybridizes to A15-aniline DNA (blue), and the high local concentration of aniline enhances the rate of acyl hydrazone formation. If 4-nitrobenzaldehyde is the aldehyde substrate, the resulting hydrazone formation can be monitored by UV-visible spectroscopy in a region distinct from the large DNA absorption.

The successful integration of DNA-templation with sequence-specific DNA-hydrazone formation may allow us to realize a sensitive and catalytic system for the detection of bacterial ribosomal RNA or provide a system that increases the efficiency of DNA-hydrazone formation for bioconjugation. We reasoned that we could use a DNA templating strategy to organize an aniline nucleophilic catalyst in close proximity to an acyl hydrazine nucleophile. Because this approach would increase the effective molarity of the organocatalyst, we could avoid using high concentrations of an exogenous nucleophilic catalyst. We postulated that a direct consequence of the favorable placement of the organocatalyst would be an increase in the rate of hydrazone formation between the acyl hydrazine-derivatized DNA and an untethered aldehyde. Herein, we report our studies directed toward extending DNA-templated synthesis to the formation of DNA-*N*-acylhydrazones.

In order to probe the effect of using DNA hybridization to direct an aniline organocatalyst in close proximity to an acyl hydrazine, we synthesized DNA (A15) with a 3'-aniline group (A15-aniline) and monitored its ability to increase the rate of hydrazone formation when hybridized to a complementary strand of DNA bearing a 5'-acyl hydrazine group (T15-acyl hydrazine). We used 4-nitrobenzaldehyde as the aldehyde substrate because the absorption and extinction coefficient of the resulting hydrazone are suitable for monitoring the rate of product formation by UV-visible spectroscopy (ε_{340} = 13,500 M^{-1} cm^{-1}).[28]

The kinetics of the reaction were measured at different pH values and in the presence and absence of complementary A15-aniline under pseudo-first order conditions (5.0 μM DNA, 500 μM aldehyde). We observed a pH dependence on the rate of hydrazone formation, in line with previous mechanistic investigations of hydrazone formation.[6,29] The rate was slower at pH 7.0 (k_{obs} = 3.91 ± 0.09 × 10^{-5} s^{-1}) than at pH 6.0 (k_{obs} = 1.04 ± 0.05 × 10^{-4} s^{-1}), and the reaction at pH 4.5 was the fastest (k_{obs} = 1.64 ± 0.02 × 10^{-2} s^{-1}) (Table 1; Fig. S1 A-C, blue traces). Upon the addition of 1.0 equivalent of A15-aniline, we saw a significant rate enhancement at each pH value (Table 1; Fig. S1 A-C, red traces). Whereas the reaction at pH 4.5 was ca. 80% complete in roughly 20 minutes in the absence of A15-aniline, in the presence of 1 equivalent of A15-aniline, the reaction was 100% complete within 5 minutes. Likewise, at pH 6.0, the reaction was ca. 30% complete after one hour, whereas the hydrazone conjugation was >90% complete in the presence of A15-aniline at the same timepoint. The corresponding rate enhancement factors range from a 3-fold rate enhancement at pH 7.0 to a 7.5-fold rate enhancement at pH 4.5 (Table 1; Fig. S1 D). Control experiments established that the Schiff base intermediate formed between aniline and 4-nitrobenzaldehyde contributes a negligible amount of signal to the measured absorbance (Fig. S2). Additional control experiments with complementary alkyl amine-terminated DNA and with non-complementary A15-aniline did not produce rate constants significantly different from the uncatalyzed variant (Fig. S3). Thus, both the aniline moiety and the correct basepairing are necessary for the rate enhancement of hydrazone formation.

Table 1. Rates for hydrazone formation between T15 acyl hydrazine DNA and 4-nitrobenzaldehyde in the absence and presence of DNA-aniline[a]

pH	k_{uncat} (s^{-1})	k_{cat} (s^{-1})	k_{cat}/k_{uncat}
4.5	1.64(2) × 10^{-3}	1.23(9) × 10^{-2}	7.5
6.0	1.04(5) × 10^{-4}	5.80(4) × 10^{-4}	5.6
7.0	3.91(9) × 10^{-5}	1.22(1) × 10^{-4}	3.1

[a] All spectroscopic measurements were carried out in phosphate buffer (50 mM) containing NaCl (150 mM) with T15 acyl hydrazine DNA (5.0 μM), 4-nitrobenzaldehyde (500 μM), and in the case of DNA-templated organocatalysis, A15-aniline DNA (5.0 μM).

Encouraged by these results, we reasoned that it may be possible to use an underivatized DNA tempate to organize the DNA-reagents in an arrangement shown in Figure 2A. In this case, an unmodified 30-mer DNA oligonucleotide that is complementary to both the 15-mer DNA-aniline and 12-mer DNA-acyl hydrazine effectively anchors the catalyst in close proximity to the nucleophile. This arrangement should allow the aniline to form a Schiff base intermediate with an exogenous aldehyde, and the high local concentration of DNA acyl hydrazine should facilitate rapid DNA-hydrazone formation. However, when we employed DNA-aniline in this system, we saw that the DNA-aniline only increased the rate of DNA-hydrazone formation by a little more than 2-fold compared to that of the uncatalyzed reaction (Fig. 2B, **1'**). Thus, we initiated a systematic search to find more efficient DNA-anchored organocatalysts.

In our first-round screen for more efficient catalysts, we examined the effect of regioisomeric positioning of the amino group on the rate of DNA-hydrazone formation. To this end, DNA was elaborated with the amino group in either a *meta* or *ortho* position with respect to the amide linkage to the DNA; this provided **2'** and **3'**, respectively (Fig. 2A). As expected, the 2-amino derivative showed poor catalytic efficacy, most likely owing to steric congestion around

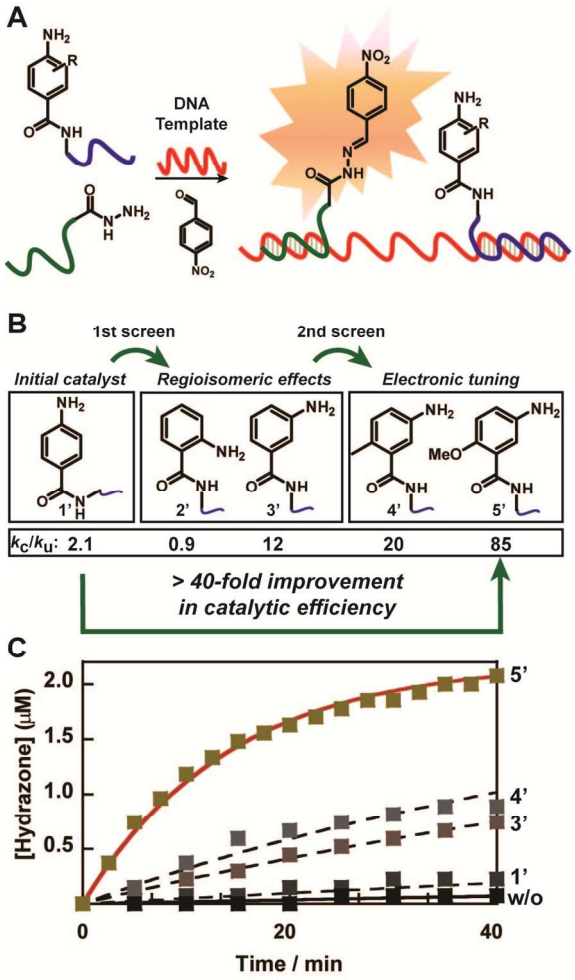


Figure 2. (A) DNA-anchored aniline organocatalyst derivatives and their associated catalytic rate enhancements compared to that of the uncatalyzed variant. k_c/k_u refers to the ratio of the catalyzed reaction (k_c) to that of the uncatalyzed reaction (k_u). (B) Time-course UV-visible spectroscopy study that shows the DNA-templated formation of the DNA-hydrazone between a DNA-5'-acyl hydrazine (2.2 μM) and 4-nitrobenzaldehyde (500 μM) with a complementary DNA template (2.5 μM) in the absence ("w/o") or the presence of the indicated DNA-anchored organocatalyst (2.2 μM, 1.0 equiv.). Spectra were collected in PBS, pH 7.4 by monitoring the signal at 340 nm. Data were fitted to a first order reaction to obtain the apparent rate constants.

the organocatalytic amino group. However, we were pleased to see that the **3'** derivative was capable of enhancing the rate of hydrazone formation to 13-fold above that of the uncatalyzed reaction (k_{obs} = 1.7 ± 0.2 × 10^{-4} s^{-1}), an improvement of more than 5-fold above that of the initial catalyst, **1'** (Figure 2B, **3'**).

We hypothesized that **3'** had a higher catalytic activity than the initial catalyst **1'** because the moderately withdrawing amide group was no longer electronically coupled to the amino; thus the amino group in **3'** was more nucleophilic than the other derivatives, and this contributed to its enhanced catalysis. Several recent comprehensive publications by Crisalli *et al* support this line of reasoning.[7,8] To investigate this hypothesis, we installed several electron-donating groups *para* to the amino group and measured their corresponding catalytic efficiencies.

Consistent with our hypothesis, we found that an electron-donating methyl group para to the amino group (**4'**) led to a further rate enhancement to 20-fold above that of the uncatalyzed reaction

(k_{obs} = 2.6 ± 0.1 × 10^{-4} s^{-1}). Finally, the inclusion of a strongly electron-donating methoxy group in the organocatalyst yielded a large rate increase to more than 85-fold above that of the uncatalyzed reaction (k_{obs} = 1.2 ± 0.2 × 10^{-3} s^{-1}). This represents >90% conversion to the hydrazone in 40 minutes at pH 7.4 with 1.0 equivalent of DNA-aniline, which is especially notable because hydrazone conjugation is typically performed at acidic pH (e.g., pH 4.5) or at neutral pH in the presence of many equivalents of aniline (e.g., >50-fold excess). These results track well with studies of exogenous small-molecule catalysts for organocatalytic formation of hydrazones, suggesting that trends observed in the non-templated variant persist in the DNA-templated version of hydrazone formation.[7,8]

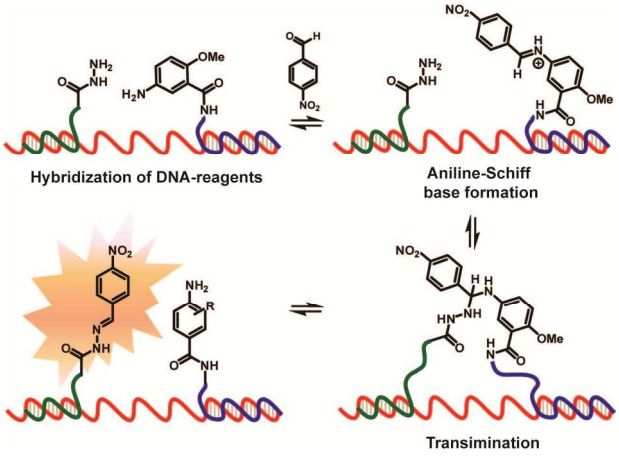


Figure 3. Proposed mechanism of DNA-templated hydrazone formation. Hybridization of the DNA-reagents to the DNA template organizes the organocatalyst in close proximity to the nucleophilic acyl hydrazine. Aniline-Schiff base formation activates the aldehyde for subsequent nucleophilic attack by the DNA-acyl hydrazine. Collapse of the tetrahedral intermediate produces the DNA-*N*-acyl hydrazone and results in an absorbance increase.

A proposed mechanism is shown in Figure 3. Hybridization of the DNA-reagents anchors the aniline derivative in close proximity to the acyl hydrazine. The organocatalyst activates the aldehyde to form the anilinium Schiff base intermediate, which is rapidly attacked by the DNA-acyl hydrazine. Completion of the transimination process yields the DNA-*N*-acyl hydrazone, and consequently, an increase in absorbance signal.

To close, we have presented the effects of DNA-templation on the rate of DNA-hydrazone formation. We show that DNA with a 3'-aniline derivative is capable of accelerating the rate of DNA-hydrazone formation when directly hybridized to a complementary DNA bearing a 5'-acyl hydrazine. Further, our data indicate that this rate enhancement persists when the reagents are organized by a third oligonucleotide. A systematic search of more efficient DNA-anchored aniline derivatives revealed an exceptionally efficient catalyst that is capable of increasing the rate of DNA-hydrazone formation to more than 85-fold above that of the uncatalyzed reaction. Our approach obviates the need for acidic pH conditions, eliminates high concentrations of added organocatalysts, and synthesizes the product in a highly DNA-sequence dependent manner. Taken together, these characteristics suggest that this DNA-templated variant of organocatalytic hydrazone formation should find extensive application in the fields of bioassay development and stimuli-responsive materials.

This work was supported by an NSF Career Award (DMR-1056808) and the Office of Naval Research (Award Number: N00014-09-01-0258). We are greatly indebted to Dr. Subhadeep Roy (Caruthers lab) for help with analytical and preparative HPLC. We also thank Dr. Jeremy Balsbaugh for help with ESI-MS analysis and Kirsten Fitch (Goodwin lab) for assisting with IR data collection.

Notes and references

[a]Department of Chemical and Biological Engineering, University of Colorado Boulder, CO 80303

*To whom correspondence should be addressed. Dylan.Domaille@colorado.edu; Jennifer.Cha@colorado.edu

Electronic Supplementary Information (ESI) available: Synthetic procedures and molecular characterization. See DOI: 10.1039/c000000x/

1. F. M. Brunel, J. D. Lewis, G. Destito, N. F. Steinmetz, M. Manchester, H. Stuhlmann, and P. E. Dawson, *Nano Lett.*, 2010, **10**, 1093–1097.
2. Z. M. Carrico, M. E. Farkas, Y. Zhou, S. C. Hsiao, J. D. Marks, H. Chokhawala, D. S. Clark, and M. B. Francis, *ACS Nano*, 2012, **6**, 6675–6680.
3. D. W. Domaille, J. H. Lee, and J. N. Cha, *Chem. Commun.*, 2013, **49**, 1759–1761.
4. Y. Zeng, T. N. C. Ramya, A. Dirksen, P. E. Dawson, and J. C. Paulson, *Nat Meth*, 2009, **6**, 207–209.
5. D. D. McKinnon, D. W. Domaille, J. N. Cha, and K. S. Anseth, *Adv. Mater.*, 2013, in press.
6. A. Dirksen, S. Dirksen, T. M. Hackeng, and P. E. Dawson, *J. Am. Chem. Soc.*, 2006, **128**, 15602–15603.
7. P. Crisalli and E. T. Kool, *J. Org. Chem.*, 2013, **78**, 1184–1189.
8. P. Crisalli and E. T. Kool, *Org. Lett.*, 2013, **15**, 1646–1649.
9. E. H. Cordes and W. P. Jencks, *J. Am. Chem. Soc.*, 1962, **84**, 826–831.
10. A. R. Blanden, K. Mukherjee, O. Dilek, M. Loew, and S. L. Bane, *Bioconjug. Chem.*, 2011, **22**, 1954–1961.
11. X. Li and D. R. Liu, *Angew. Chem. Int. Ed.*, 2004, **43**, 4848–4870.
12. P. J. Milnes, M. L. McKee, J. Bath, L. Song, E. Stulz, A. J. Turberfield, and R. K. O'Reilly, *Chem. Commun.*, 2012, **48**, 5614–5616.
13. Y. Li, M. Zhang, C. Zhang, and X. Li, *Chem. Commun.*, 2012, **48**, 9513–9515.
14. Y. Li, P. Zhao, M. Zhang, X. Zhao, and X. Li, *J. Am. Chem. Soc.*, 2013, **135**, 17727–17730.
15. R. E. Kleiner, C. E. Dumelin, and D. R. Liu, *Chem. Soc. Rev.*, 2011, **40**, 5707–5717.
16. S. Sando and E. T. Kool, *J. Am. Chem. Soc.*, 2002, **124**, 9686–9687.
17. S. Sando, H. Abe, and E. T. Kool, *J. Am. Chem. Soc.*, 2004, **126**, 1081–1087.
18. T. N. Grossmann and O. Seitz, *Chem. – Eur. J.*, 2009, **15**, 6723–6730.
19. S. Kummer, A. Knoll, E. Socher, L. Bethge, A. Herrmann, and O. Seitz, *Angew. Chem. Int. Ed.*, 2011, **50**, 1931–1934.
20. S. Kummer, A. Knoll, E. Socher, L. Bethge, A. Herrmann, and O. Seitz, *Bioconjug. Chem.*, 2012, **23**, 2051–2060.
21. K. Gorska and N. Winssinger, *Angew. Chem. Int. Ed.*, 2013, **52**, 6820–6843.
22. H. Saneyoshi, Y. Ito, and H. Abe, *J. Am. Chem. Soc.*, 2013, **135**, 13632–13635.
23. A. Shibata, T. Uzawa, Y. Nakashima, M. Ito, Y. Nakano, S. Shuto, Y. Ito, and H. Abe, *J. Am. Chem. Soc.*, 2013, **135**, 14172–14178.
24. A. Shibata, Y. Ito, and H. Abe, *Chem. Commun.*, 2013, **49**, 270–272.
25. I. Boll, E. Jentzsch, R. Kramer, and A. Mokhir, *Chem. Commun.*, 2006, 3447–3449.
26. C. Percivalle, J.-F. Bartolo, and S. Ladame, *Org. Biomol. Chem.*, 2013, **11**, 16–26.
27. Z. Tang and A. Marx, *Angew. Chem. Int. Ed.*, 2007, **46**, 7297–7300.
28. T. P. King, S. W. Zhao, and T. Lam, *Biochemistry*, 1986, **25**, 5774–5779.
29. W. P. Jencks, *J. Am. Chem. Soc.*, 1959, **81**, 475–481.