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Prusty, Deepak K.; Kwak, Minseok; Wildeman, Jur; Herrmann, Andreas

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Modular Assembly of a Pd Catalyst within a DNA Scaffold for the Amplified Colorimetric and Fluorimetric Detection of Nucleic Acids

Deepak K. Prusty, Minseok Kwak, Jur Wildeman, and Andreas Herrmann*

Catalytic signal amplification is a powerful tool for the detection of chemical and biological analytes. In chemistry it has been employed in sensing various toxic metal ions (e.g. Pd^{2+}, Pb^{2+}, Cu^{2+}, and Hg^{2+}) as well as small molecules such as carbon monoxide and thiols. In a biological context, catalytic reactions have enabled the highly sensitive detection of scarce analytes. They have been widely utilized, for instance, in the detection and assay of proteins, antibodies, and nucleic acids.

In the case of nucleic acids, DNA-templated catalytic processes in particular have been successful. Fluorogenic transformations of this type have been exploited for the amplified detection of deoxyribonucleotides (ODNs) both in homogeneous solutions and in living cells. In this approach, DNA probes are labeled with poorly fluorescent precursors and assembled with target nucleic acids into catalytic hybrids. These then chemically convert the precursors into fluorescent reporters (e.g. through the Staudinger reaction) or aminolysis. The turnover rate and detection signal can be further improved by repeated thermal cycling. Besides variation of temperature, another external stimulus for signal amplification is light. Photochemical reactions have been employed to trigger the photocatalytic formation of singlet oxygen for the generation of fluorescent reporters. While such systems have resulted in the amplified detection of DNA, RNA, and peptide nucleic acids (PNAs), the approach remains limited by hurdles such as the covalent attachment of profluorescent molecules or photosensitizers to probe ODNs and the need for external stimuli to achieve multiple turnovers.

A potential solution to these drawbacks is grounded in the use of DNA as a structural component, rather than an analyte, in catalytic systems. ODNs on their own are versatile components for catalysis, able to adopt complex three-dimensional structures to catalyze DNA/RNA ligation.

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cence quantum yield (FQY), 2) high extinction coefficient, and 3) photostability.\[21\] We investigated mono- and diiodinated BODIPY precursor chromophores (1 and 2, respectively; see Figure 1a). To ensure water solubility, which is essential for applications in biological systems, a precursor was modified with four triethylene glycol chains. Both profluorescent compounds 1 and 2 proved highly soluble in aqueous media (> 10 mg mL\(^{-1}\)) and were synthesized in 10 and 30% overall yield, respectively (see pp. 2–3 in the Supporting Information for synthetic details and structural characterization). In both compounds, iodine atoms were incorporated at the C2 and/or C6 positions of the BODIPY core, to favor intersystem crossing to the triplet manifold.\[22\] The photophysical properties of the precursors and their deiodinated products (3 and 4) were initially investigated to confirm their suitability as substrates for fluorogenic reactions in water. Compounds 1 and 2 were individually subjected to palladium-catalyzed deiodination by dissolution in sodium acetate (NaOAc) buffer (0.5 m, pH 5.0) in the presence of a water-soluble Pd catalyst (Na-PdCl\(_2\)/TPPTS; see pp. 2–3 in the Supporting Information for details). The UV/Vis absorption and fluorescence spectra of the products (3 and 4) obtained after 4 h of shaking at room temperature differed markedly from those of the corresponding precursors. As anticipated, the absorption maxima of the dehalogenated products were strongly blue-shifted (Figure 1b; Figures S1 and S2). Indeed, the color change of the aqueous solution from red to yellow upon deiodination was evident to the naked eye, which implies the possibility for colorimetric detection (similar to Figure 2c). The fluorescence emission maxima of 3 and 4 also exhibited a blue shift, with fluorescence intensities increasing 35- and 80-fold, respectively, relative to the profluorescent substrates. The significant difference in the fluorescence intensity increase was anticipated in light of the higher background intensity of monoiodo precursor 1, which we attribute to its having fewer heavy atoms than the diiodo precursor 2. Moreover, the fluorescence quantum yields ($\Phi_\text{fl}$) of fluorophore 3 ($\Phi_\text{fl} = 0.68$) and 4 ($\Phi_\text{fl} = 0.81$) were 22 and 40 times greater than those of their precursors (Table S1). At this stage, it is important to note that the fluorogenic reaction does not proceed in the absence of any of three reaction components: phosphine ligands and Pd, which form the catalytic complex, and iodo-BODIPY.

The suitability of this fluorogenic deiodination reaction for DNA detection was tested with a DNA-templated catalyst (Figure 2a). Triphenylphosphine ligands were individually coupled through amide bonds to the 5'-end of probe L and 3'-end of probe R (see Scheme S4 and p. 7 in the Supporting Information for details on the purification and characterization). After HPLC purification, the phosphine-labeled probes were annealed with the target strand (or template, T) in a hybridization buffer (see p. 8 in the Supporting Information). Subsequent addition of Na\(_2\)PdCl\(_2\), with the reducing agent NaBH\(_4\), resulted in an active catalytic complex owing to the close proximity of the ligands attached to L and R. Low probe concentrations (≤ 1 μM) were chosen so that Pd complex formation would only occur through hybridization to the template. It should be noted that in initial experiments we investigated different templates T with various nucleotide gaps (0–4 nt) between the two annealing sites of L and R. When the two probes were separated by 0, 1, and 2 nt an active catalyst was formed; at greater distances (3 and 4 nt) dehalogenation did not occur (data not shown). Since the highest activity was achieved with a single nucleotide gap (see Figure 2a), this hybrid catalyst in combination with profluorescent BODIPYs was used to detect the presence of a target DNA sequence, which we investigated in terms of reaction kinetics, scope, and detection limit.

The DNA-directed catalyst for the fluorogenic reaction was tested at a range of concentrations of the target strand T (from 1 nM to 1 μM) and iodo-BODIPY 1 or 2, with fixed concentrations of the probes R and L, and catalyst Na\(_2\)PdCl\(_2\) and NaBH\(_4\) (see p. 8 in the Supporting Information). The reactions were performed in NaOAc buffer at pH 5 under inert conditions. It is noteworthy that the reaction required an acidic pH probably to prevent complexation of Pd with the nucleobases. After gentle mixing for 4 h, the reaction mixtures exhibited the expected intense fluorescence resulting from multiple turnovers of the deiodination reaction (Figure 2b). As negative controls, all reactions were also performed without the template or catalyst, or with a template containing a single-base mismatch (T-sbm). When the fully complementary template was used, 90% of the fluorescence maximum was reached after 4 min for a template concentration of 1 nM, and saturation was achieved within 10 min (Figure 3a, curve 1). This rate of reaction is twice that of a DNA-templated Heck reaction used to deiodinate an analogous BODIPY-DNA conjugate.\[23\] A possible explanation for this improvement is that the dehalogenation reaction entails fewer intermediates than the Heck cross-coupling.\[23\] It should be also noted that at lower template concentrations (100 pM and 10 pM) the reaction is so rapid (Figure 3a, curves 2 and 3) that detection assays could be performed within only several minutes. In contrast, the use of T-sbm in identical conditions slowed the reaction dramati-
Figure 3. Fluorimetric determination of the reaction kinetics and the limit of detection of the catalytic conversion. a) Evolution of fluorescence intensity over time at target concentrations \( C_T \) of 1 nM to 10 pM (curves 1–3), in the presence of T-sbm with catalyst (curve 4), in the presence of T without catalyst (curve 5), and without T (curve 6) for the conversion of 1 into 3. b) Comparison of the reaction kinetics for the conversion of 1 into 3 under an argon atmosphere (curve 1) and under ambient air (curve 2) in the presence of \( C_T = 1 \) nM and 10 nM monoiodo substrate. c) Fluorescence intensities corresponding to the conversion of 1 into 3 for \( C_T = 1, 10, 100, 500, \) and 1000 fm (squares) and without template (circles) at a fixed concentration of the monoiodo substrate of 500 fm. Magnified sub-picomolar range of the graph (inset). All fluorogenic dehalogenations were monitored at 510 nm \( (\lambda_{\text{ex}} = 500 \text{ nm}) \).

However, the amplified conversion to reporter dyes in our system and the high extinction coefficient of BODIPY analogues made such simple and immediate visual detection possible without any additional instruments.

In summary, we have developed a novel and modular DNA–transition-metal hybrid catalyst based on the powerful DNA hybrid catalyst.

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class of Pd-catalyzed reactions. At the same time a concept for amplified DNA detection combining iodo-BODIPYs with the DNA–Pd complex was successfully realized, allowing the generation of multiple signals from a single hybridization event. This simple assay is suitable for rapid colorimetric or fluorometric detection of nucleic acid targets. Phosphine ligands for the catalytic complex, rather than dye precursors, are conjugated directly to the DNA probes, yielding an active catalytic complex upon hybridization to the target sequence. As such, each hybridization event can catalyze the fluorogenic conversion of many precursor dye molecules, producing hundreds of fluorophores even in sub-picomolar target concentration. This is an obvious improvement over the standard method of conjugating reporter dye precursors to the probes, which is limited to one fluorophore per target molecule. Each threshold was determined by the complete conversion of the dye added in various amounts (30–100 equiv) at fixed C_r = 10, 100, 500, and 1000 fm. At C_r = 10 fm, complete conversion of diiodo-BODIPY 2 could not be detected at any dye concentration (N/D). All fluorogenic dehalogenations were monitored at 510 nm (λ_ex = 500 nm).

Figure 4. Equivalents of di-iodoiodo (black/gray) dye molecules converted per target molecule. Each threshold was determined by the complete conversion of the dye added in various amounts (30–100 equiv) at fixed C_r = 10, 100, 500, and 1000 fm. At C_r = 10 fm, complete conversion of diiodo-BODIPY 2 could not be detected at any dye concentration (N/D). All fluorogenic dehalogenations were monitored at 510 nm (λ_ex = 500 nm).

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