Supporting Information

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69451 Weinheim, Germany
Design of Molecular Logic Devices Based on a Programmable DNA-Regulated Semi-Synthetic Enzyme

Nathan C. Gianneschi and M. Reza Ghadiri*

Departments of Chemistry and Molecular Biology and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA, 92037

General methods
All reactions were carried out at room temperature. The anhydrous solvents used in oligonucleotide synthesis were bought from Aldrich Chemical Company and used as received. Unmodified DNA oligonucleotides \( D^1, D^2, D^3, D^4, D^5, D^6, D^7 \) were purchased from Sigma-Genosys and purified by polyacrylamide gel electrophoresis followed by extraction of the crushed gel with a tris/EDTA (1 mM/1mM) solution and desalting with a reverse phase C18 Sep-Pak cartridge. Modified cereus neutral protease (CNP\(_{E151C}\)), fluorogenic peptide substrate and phosphoramidate inhibitor were prepared as previously reported [Saghatelian, A.; et al. J. Am. Chem. Soc. 125, 344, (2003)]. Fluorescence measurements were performed with a microplate reader (Genios, Tecan Instruments, \( \lambda_{ex} = 365 \text{ nm}, \lambda_{em} = 460 \text{ nm} \)) using black 96 well plates with measurements taken approximately every 28 seconds (for graphical clarity, only data points at 2.5 min intervals are shown). Total reaction volumes of 100 \( \mu \text{L} \) were used in all enzyme reactions. HPLC purifications of DNA-Inhibitor strands (DI) and DNA strands for enzyme modifications were performed on a Zorbax extend C18 column using an binary gradient (Sovent A: 0.1 M triethylammonium acetate, pH 7.5; Solvent B: A + 80% acetonitrile; gradient: 0-25% B over 30 minutes, Flow rate: 1 mL/min). Anion exchange chromatography was used to purify the DNA-Enzyme (DE) conjugates using Mono Q HR 5/5, ÄKTA purifier, Pharmacia, (Binary gradient – Solvent A: 100 mM Tris, 50 mM CaCl\(_2\), 2.5 \( \mu \text{M Zn(CIO}_{4}\)\(_2\); Solvent B: A + 1 M NaCl; 0% B 30 minutes, 0-100% B 30 minutes, flow rate 1 mL/min). MALDI-TOF mass spectrometry was performed using THAP matrix (2,4,6-trihydroxyacetophenone monohydrate) (97 \( \mu \text{mol} \)), ammonium citrate (29 \( \mu \text{mol} \)), acetonitrile/water (1 mL, 1:1).

Preparation of disulfide activated DNA (1) for reaction with CNP\(_{E151C}\) to form DE (see main text for DNA sequence)
The 3'-sulphydryl DNA strand (Ultramild DNA synthesis, Glen Research) was prepared using a 3'-disulphide modified column (C3-SS-CPG, Glen Research) on an automated synthesizer. The oligonucleotide was deprotected and cleaved from the solid support using K\(_2\)CO\(_3\)/methanol (0.5 M, 0.5 mL methanol) for 4 hr. Following deprotection and cleavage the solvent was removed and the dried material was redissolved in H\(_2\)O/methanol (60% methanol, 0.3 mL) and treated with an aqueous solution of tricarboxyethylphosphine (50 \( \mu \text{L} \), 10 \( \mu \text{mol} \), pH 8.0) for 30 min. The resulting solution was concentrated (3000 MW, Micron YM-3 spin column, 45 min, 14,000 g) and treated with an acetonitrile/H\(_2\)O (1:1) solution of 2,2'-dithiopyridine (300 \( \mu \text{L} \), 15 \( \mu \text{mol} \)) and concentrated again (3000 MW, Micron YM-3 spin column, 60 minutes). The modified oligonucleotide was purified by polyacrylamide gel electrophoresis followed by extraction of the crushed gel with a tris/EDTA (1 mM/1mM) solution, for 18 hr, and then desalted with a reverse phase C18 Sep-Pak cartridge. The oligonucleotide was further purified by RP-HPLC (retention time = 20 min), desalted (reverse phase C18 Sep-Pak cartridge) and characterized by MALDI-MS. Mass calcd: 13698.7, Mass obs: 13711.9.

Preparation of DNA-enzyme conjugate (DE)
To a solution of CNP\(_{E151C}\) (20 \( \mu \text{L}, 3.2 \text{ nmol} \)) in buffer (20 \( \mu \text{L} \), 50 mM Tris, 300 mM NaCl, pH 8) was added I (16 \( \mu \text{L}, 3.8 \text{ nmol} \)). The mixture was placed at 4 °C for 18 hr and then purified by anion exchange chromatography. The resulting DE was identified by apparent molecular weight using SDS polyacrylamide gel electrophoresis (Fig. 1).
ON-OFF switch cycle (Fig. 3 – main text)

The five panels shown in Fig. 2S are plots of the fluorescence vs time data collected from five wells that were set up in the following fashion. Solution conditions: Tris/HCl (20 mM, pH 7.4), MgCl₂ (50 mM), DE (2 nM), input DNA (50 nM), fluorogenic substrate (80 µM), 100 µL total volume. Initially, DI² was added to all wells to establish the starting architecture DE–DI². These wells were allowed to incubate for 60 minutes. At t = 0 min (Fig. 2S: Panel 1) substrate was added to well 1. After 10 min of data acquisition, D⁶ was added to wells 2-5, which were allowed to incubate for 10 min. At t = 20 min (Fig. 2S: Panel 2), substrate was added to well 2. After 10 min of acquisition time, DI² was added to wells 3-5, which were allowed to incubate 10 min. At t = 40 min (Fig. 2S: Panel 3), substrate was added to well 3. After 10 min of acquisition time, D⁶ was added to wells 4 and 5, which were allowed to incubate for 10 min. At t = 60 min (Fig. 2S: Panel 4), substrate was added to well 4. After 40 min of acquisition time, DI² was added to well 5, which was allowed to incubate for 10 min. At t = 110 min (Fig. 2S: Panel 5), substrate was added to well 5. Therefore, the state of the enzyme at each cycle was read by rates of substrate turnover in individual wells to obtain the five separate states shown in Fig. 3 of the main text, and as panels 1 to 5 in Fig. 2S. Alternatively, experiments were performed in which the switching was performed in the presence of substrate (Fig. 3S).
Logic gates (OR, AND, NOR) – full time course plots and control studies

By definition the AND gate has a true (1) output only when both inputs are true (present in the reaction mixture). The following control reactions were performed to establish that only the correct combinations of DNA inputs would turn on the enzyme (Fig. 6S). For example, according to the DNA sequence programming, while D5 is required to turn the enzyme on from the DE–DI1 starting architecture, D6 should not. Control studies show that D6 could not turn on the DE–DI1 enzyme complex (formed in situ by incubation of DI1 with DE for 30 min). The opposite is observed for the DE–DI2 architecture, which was programmed for activation by D6 but not with D5.

An alternative AND gate architecture

To illustrate the potential generality of logic gate programming, we show here the results for an AND gate constructed using a different approach (Fig. 7S). For enzyme activation this method utilizes cooperative binding of two input strands to the allosteric $\alpha$-loop of DE2–DI1 enzyme complex. The DE2 used here in combination with DI1 is described by the following architectural parameters ($\alpha = 20, \beta = 2, \gamma = 18, \delta = 0, \varepsilon = 0$). The DE2 strand is 6 bases shorter than the one discussed in the main text, forming an $\alpha$-loop of 20 bases that interacts with the two incoming 10-mers, D7 and D8. The 10-mer input strands are designed to bind to the $\alpha$-loop at different non-overlapping positions. D7 binds the loop directly adjacent to the enzyme, and D8 binds next to the inhibitor.
**DNA-encoded intrasterically regulated enzymes as diagnostic tools for sensitive detection of label-free nucleic acids**

Because of the built-in signal amplification capacity (enzyme turnover), the DNA-encoded intrasterically regulated enzymes can be used for highly sensitive, rapid, and PCR-independent detection of label-free nucleic acid sequences. Figure 8S shows an example in which 50 pM (5 fmol) or 1 pM (100 amol) of an HIV target DNA were distinguished from background at 20 or 100 minutes of amplification/reaction time, respectively.

*Figure 8S.* Sensitivity of DE–DI\(^1\) in detecting targeted DNA sequences. Conditions: Tris/HCl (20 mM, pH 7.4), MgCl\(_2\) (250 mM), total assay volume is 100 µL, DE (2 nM), DI (50 nM), D\(^1\) various concentrations as indicated. The α-loop is programmed to respond to D\(^1\), a 26 base long ssDNA oligonucleotide derived from a sequence unique to the HIV viral genome. (A) Full time course data. (B) Subset of graph A, showing low concentrations from 100 min. With the detection limit set at 3σ; 50 pM (5 fmol) or 1 pM (100 amol) of target DNA can be distinguished from background at 20 or 100 minutes of amplification/reaction time, respectively.
Supporting Information