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DNA-Directed Control of Enzyme–Inhibitor Complex Formation: A Modular Approach to Reversibly Switch Enzyme Activity

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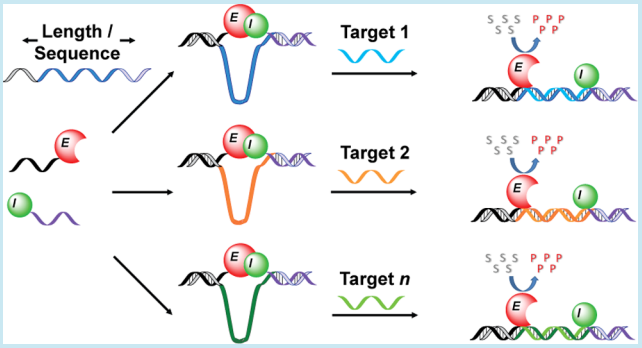
Supporting Information

ABSTRACT: DNA-templated reversible assembly of an enzyme–inhibitor complex is presented as a new and highly modular approach to control enzyme activity. TEM1-β-lactamase and its inhibitor protein BLIP were conjugated to different oligonucleotides, resulting in enzyme inhibition in the presence of template strand. Formation of a rigid dsDNA linker upon addition of a complementary target strand disrupts the enzyme–inhibitor complex and results in the restoration of enzyme activity, enabling detection of as little as 2 fmol DNA. The noncovalent assembly of the complex allows easy tuning of target and template strands without changing the oligonucleotide-functionalized enzyme and inhibitor domains. Using a panel of eight different template sequences, restoration of enzyme activity was only observed in the presence of the target viral DNA sequence. The use of stable, well-characterized protein domains and the intrinsic modularity of our system should allow easy integration with DNA/RNA-based logic circuits for applications in biomedicine and molecular diagnostics.



KEYWORDS: *bionanotechnology, reporter enzyme, molecular switch, β-lactamase, self-assembly*

The development of robust design strategies for the construction of biomolecular switches is one of the key challenges in bionanotechnology, providing essential tools for molecular imaging, synthetic biology, molecular diagnostics, and biomolecular computing.[1−3] Inspired by the modular architecture of many natural signaling networks, researchers have started to explore similar principles in semisynthetic systems.[1,4,5] Oligonucleotide-based circuits are attractive in this respect because their highly modular and predictable nature allows the construction of complex circuits using a limited set of principle logic gates and building blocks.[2,6,7] The application of these DNA/RNA-based logic circuits in biomedicine and molecular diagnostics is limited by a lack of generic approaches to interface them with protein activity, however.[8−13] Most previously reported approaches to control protein activity by DNA are based on the templated assembly of two protein (fragments), either via semisynthetic DNA-hybrids or zinc-finger fusions recognizing certain double stranded DNA-stretches. Several examples of DNA-directed complementation of split proteins have been reported, including the use of split fluorescent proteins and split luciferase,[12,14−17] split β-lactamase,[18] and split murine dihydrofolate reductase.[19] Although split reporter enzymes have the advantage of providing low background signal, split enzymes tend to be thermodynamically instable, and protein complementation is often not reversible.[20]

An alternative approach is the use of multiple protein domains that complement each other's function. Examples include the assembly of two enzyme domains that catalyze two consecutive reactions (cascade catalysis) such as glucose oxidase (GOx) and horseradish peroxidase (HRP). Although assembly of enzyme cascades on hierarchical self-assembled DNA-structures has been shown to enhance the efficiency of the overall catalytic process, these systems often show a substantial background activity in the absence of DNA-template due to diffusion processes.[10,21−25] A related strategy is to use split multidomain enzymatic systems. An example is the work of Niemeijer and co-workers who separated the reductase and hydroxylase domains of cytochrome P450 BM3 and conjugated each domain to different 20-mer single stranded oligodeoxynucleotides.[26] Hydroxylase activity was only observed upon hybridization with a complementary template strand and could be further modulated by tuning the length of the dsDNA linker in between the two domains. An advantage of this design is that it does not depend on diffusion of intermediate substrates, but the approach is not easily extended to more common reporter enzymes. A more generic approach is to control the interaction between an enzyme and an inhibitor, an approach that was used by Ghadiri and co-workers to modulate the activity of cereus neutral protease (CNP).[27,28] Either a phosporamidite inhibitor was tethered directly to this enzyme via an oligonucleotide linker or an inhibitor-oligonucleotide conjugate was connected to the oligonucleotide-function-

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alized CNP via hybridization. This system allowed the detection of pM concentrations of ssDNA and enabled the construction of logic-gate architectures. However, strategies such as these that rely on direct conjugation of a target binding oligonucleotide to a reporter enzyme require synthesis and purification of new protein−oligonucleotide conjugates for each new target sequence, hampering high throughput applications and making system optimization time and labor intensive.

Here, we report a new approach to control the activity of a reporter enzyme that is based on the reversible assembly and disassembly of a complex between an enzyme and inhibiting protein domain. The well-studied reporter enzyme TEM1-β-lactamase and its inhibitor domain BLIP were each conjugated to an oligonucleotide strand to allow their interaction in the presence of a template strand, rendering the enzyme inactive (Figure 1). Subsequent formation of a rigid dsDNA complex

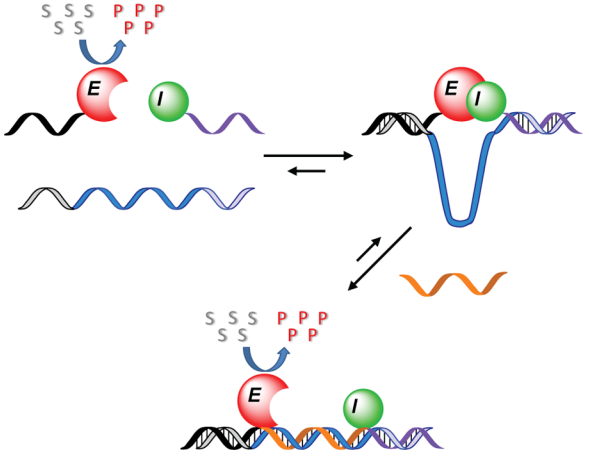

Figure 1. Concept of DNA-mediated assembly and disassembly of a complex between oligonucleotide-functionalized β-lactamase and its inhibitor protein BLIP. Formation of the enzyme−inhibitor domains requires the presence of a template strand consisting of complementary sequences and a flexible ssDNA linker. Subsequent hybridization of a complementary strand to the single stranded loop in between the proteins results in a rigid helix, which disrupts the enzyme−inhibitor complex and restores the enzymatic activity.

between the template and target strand disrupts this enzyme−inhibitor complex and results in enzyme activation. The noncovalent assembly of the inhibited complex allows easy tuning of the optimal linker/target recognition sequence in the template strand, using only a single pair of oligonucleotide-functionalized enzyme and inhibitor domains.

RESULTS AND DISCUSSION

TEM1-β-lactamase is a well-established reporter enzyme for which a broad range of colorimetric and fluorescent substrates is available. The interaction between TEM1-β-lactamase and its inhibitor protein BLIP has been structurally characterized using X-ray crystallography and has served as a model system for understanding and tuning protein−protein interactions.[29,30] Ideally, the interaction between TEM1-β-lactamase and BLIP should be strong enough to ensure complete intramolecular complex formation when both are linked by the template oligonucleotide strand, but weak enough such that the interaction is easily disrupted upon hybridization with the target DNA sequence.[31,32] Although the thermodynamic driving force provided by DNA hybridization is more than sufficient to disrupt the interaction between wild type TEM1-β-lactamase and BLIP (K_i = 0.5 nM), the use of such a strong enzyme−inhibitor interaction may also promote intermolecular enzyme−inhibitor interactions, which would suppress enzyme activation. We therefore used the E104D mutant of TEM1-β-lactamase, as this mutant shows the same enzymatic activity as wt TEM1-β-lactamase but binds BLIP with a 1000-fold weaker affinity (K_i = 1500 nM). A similar attenuation of affinity was found to be optimal in a related approach recently reported by our group in which antibody-induced disruption of an intramolecular interaction between TEM1-β-lactamase and its inhibitor protein BLIP was used to construct switchable antibody reporter enzymes.[31]

To prevent the use of bulky tags or fusion proteins between the oligonucleotides and the proteins, thiol-maleimide chemistry was chosen to allow site-specific conjugation of 21-mer oligonucleotides to each protein. A cysteine was introduced at the C-terminus of β-lactamase and near the N-terminus of BLIP, as shown in Figure 2a. These positions were chosen because conjugation at the flexible ends of the proteins is unlikely to perturb protein folding or interfere with complex formation. The distance between these positions in the complex is ∼42 Å. Both proteins were expressed in *E. coli* with a periplasmic leader sequence to allow proper formation of intramolecular disulfide bonds and an N-terminal His-tag for Ni-affinity purification (Supporting Information Figure S1 and S2). To allow conjugation of β-lactamase to the 3′-end of ODN1 and BLIP to the 5′-end of ODN2 (Supporting Information Table S2), these 5′- or 3′-end amine-functionalized 21-mer oligonucleotides were first reacted with a 20-fold excess of the bifunctional cross-linker Sulfo-SMCC. After removal of unreacted Sulfo-SMCC, the maleimide-functionalized ODNs were reacted in a 3-fold molar excess to the cysteine-functionalized proteins (Figure 2b). To prevent the formation of disulfide-bridged protein dimers, the proteins were stored in the presence of TCEP, which was removed by buffer exchange prior to the addition of the ODNs. Typical conversions of the reaction ranged between 50 and 95% and were monitored using seminative SDS-PAGE gels (Figure 2c and d). The ODN−protein conjugates were purified by Ni^{2+}-affinity chromatography to remove excess ODN. An additional purification step, anion-exchange chromatography, was necessary for BLIP-ODN2 in order to remove unreacted protein (Supporting Information Figure S3). Both conjugates were obtained with an overall yield of ∼35% and determined to be >90% pure based on seminative SDS-PAGE and analytical size exclusion chromatography (SEC) (Figure 2e and f).

To ascertain that both proteins remained functional after ODN-conjugation, their activities were first compared to their nonconjugated counterparts. Michaelis−Menten plots showed similar K_M and k_{cat} values for β-lactamaseE104D (K_M = 75 ± 9 μM, k_{cat} = 504 ± 18 s^{-1}) and ODN1-β-lactmaseE104D conjugate (K_M = 88 ± 13 μM, k_{cat} = 602 ± 24 s^{-1}) using nitrocefin as the colorimetric substrate (Supporting Information Figure S4). These values are also similar to those reported in literature[33] and show that neither substrate binding nor substrate turnover are affected by conjugation. To assess whether ODN-functionalization of BLIP affected its interaction with β-lactamase, the activity of wt β-lactamase was measured at a fixed nitrocefin concentration and increasing concentrations of BLIP and BLIP-ODN2. Similar K_i-values were obtained for BLIP and BLIP-ODN2 (K_i of 1.41 ± 0.14 nM and a K_i of 1.44 ± 0.06 nM, respectively), which are also consistent with previously reported values.[34]

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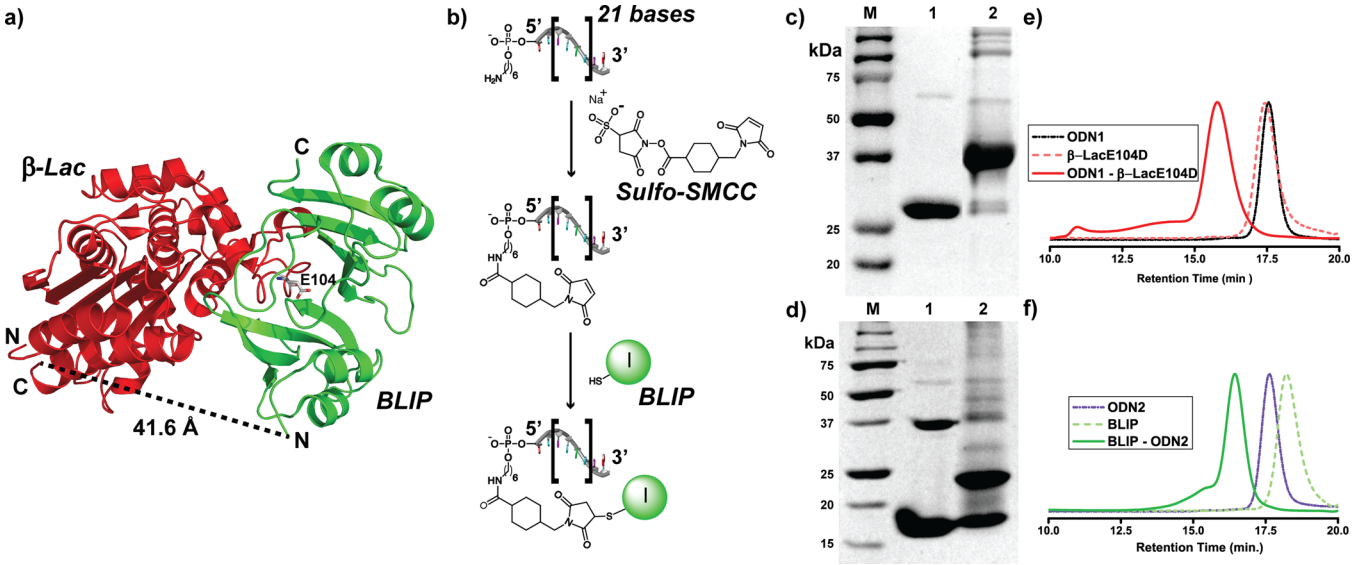

Figure 2. (a) Crystal structure of wild type β-lactamase (red) in complex with BLIP (green). The distance between the N-terminus of BLIP and the C-terminus of β-lactamase was determined using PyMol (PDB: 3C7V). (b) Synthetic strategy for site-specific oligonucleotide (ODN) conjugation. The heterobifunctional linker Sulfo-SMCC was reacted with a 5′- or 3′-end amine-functionalized ODN. The maleimide moiety reacts with a cysteine introduced at the termini of β-lactamase and BLIP. (c, d) Seminative 12% SDS-PAGE analysis of the conjugation of the maleimide-activated ODN and β-lactamaseE104D containing a C-terminal cysteine (c) and BLIP containing an N-terminal cysteine (d). Lane 1 shows the protein before the reaction, and lane 2 shows the protein after conjugation to the maleimide-activated ODN. (e, f) SEC-traces (monitored at $A_{280\ nm}$) of ODN, protein, and purified protein−ODN conjugates for ODN1-β-lactamaseE104D (e) and BLIP-ODN2 (f).

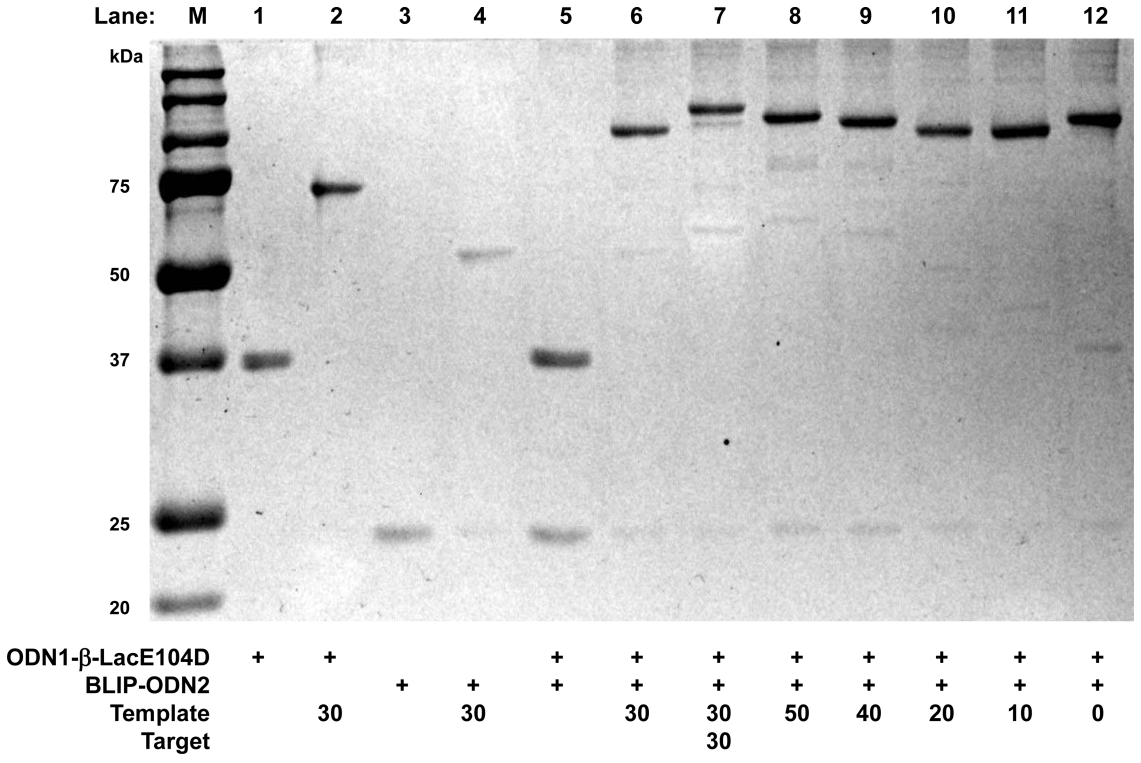

Figure 3. Monitoring the assembly of protein−DNA complexes using seminative 12% SDS-PAGE stained with Coomassie Blue. Components were sequentially mixed at low micromolar concentrations and at a ratio of 1:1.2:2 of ODN1-β-lactamaseE104D/Template/BLIP-ODN2. The complex formed in lane 7 was also incubated with 10 equiv (with respect to Template) of 30-base Target strand. Lanes 6, 8, 9, 10, 11, and 12 show the hybridization with both conjugates and different lengths of template strand (30, 50, 40, 20, 10, and 0 bases, respectively). Sequential hybridization steps were performed for 30 min at room temperature.

Having established that ODN conjugation did not affect the enzymatic activity of the reporter enzyme or interfere with the interaction between β-lactamase and BLIP, we next tested the DNA-mediated assembly of the system by monitoring the interactions of various components using seminative PAGE (Figure 3). Addition of a template strand with a 30-mer linker sequence between the two complementary sequences (Templ-30), resulted in a clear shift to higher molecular weight complexes

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for both ODN1-β-lactamaseE104D (lanes 1 and 2) and BLIP-ODN2 (lanes 3 and 4). Complete complex formation was observed upon addition of a slight excess (1.2 equiv) of the template strand. As expected, no complex formation was observed between ODN1-β-lactamaseE104D and BLIP-ODN2 in the absence of a complementary template strand (lane 5). Subsequent addition of Templ-30 to ODN1-β-lactamaseE104D and BLIP-ODN2 resulted in the clean formation of the ternary ODN1-β-lactamaseE104D/Templ-30/BLIP-ODN2 complex (lane 6). To ensure complete inhibition of the enzyme activity in the presence of template and reduce background enzymatic activity a 1:1.2:2 ratio of ODN1-β-lactamaseE104D/Templ-30/BLIP-ODN2 was chosen for all activity assays. Using a slights excess of BLIP-ODN2 is not a problem, since the concentration of noncomplexed BLIP-ODN2 is too low to inhibit ODN1-β-lactamaseE104D. Importantly, under these conditions, no bands corresponding to ODN1-β-lactamaseE104D or ODN1-β-lactamaseE104D/Templ-30 were present, showing that all reporter enzyme was bound to its inhibitor protein. A further gel-shift was observed upon addition of the 30-mer complementary target oligonucleotide, consistent with the formation of a 4-component complex in which β-lactamase and BLIP are separated by a rigid 30 bp dsDNA linker. The length and composition of the flexible oligonucleotide linker can be easily tuned in our approach by using a different template strand (Supporting Information Table S2), without having to synthesize new protein-ODN conjugates. Lanes 7−12 show that the length of the flexible linker can be varied between 0 and 50 bases without compromising complex formation. A consistent trend of smaller complexes is observed with decreasing linker length, except for Templ-0. The latter shows a slight increase in apparent hydrodynamic radius compared to Templ-10, suggesting the formation of a different, less compact complex.

Next the effect of template strand addition and subsequent addition of target ODN on the enzymatic activity was monitored by varying both the length of the linker in the template strand and the length of the complementary target strand between 0, 10, 20, 30, 40, and 50 bases. ODN1-β-lactamaseE104D and BLIP-ODN2 were first incubated with the template strand at low micromolar concentration for 30 min to ensure complete formation of the inhibited complex. Subsequently, the complexes were diluted to a final concentration of 1.2 nM, followed by the addition of a 10-fold excess (12 nM) of target strand. After 1 h incubation at room temperature, enzymatic activity was measured by monitoring the hydrolysis of the colorimetric substrate nitrocefin (Figure 4 and Supporting Information Figure S5). As expected, the enzymatic activity of ODN1-β-lactamaseE104D was not affected by the addition of BLIP-ODN2 in the absence of template strand, confirming the absence of complex formation. Enzymatic activity was strongly inhibited upon the addition of any of the template strands, with the strongest apparent inhibition observed for the longer linker lengths. At first, it may seem surprising that inhibition is also observed for the template with no oligonucleotide linker separating the enzyme and inhibitor domains (Templ-0), since the distance between the two attachment points in the X-ray structure of the β-lactamase-BLIP complex is ~42 Å. The observed inhibition suggests that this distance can be bridged by the combination of the hexyl spacer on the DNA, the SMCC-linker and the flexible residues at the termini of both proteins and/or that complex formation between the template strand and the ODN1 and ODN2 is somewhat strained. Such strain would be released when increasing the length of the oligonucleotide linker, which

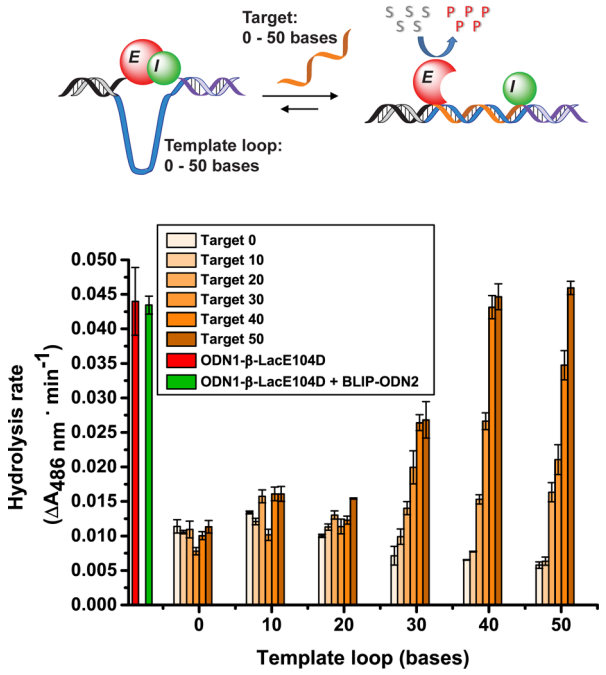

Figure 4. Influence of template-linker length and target length on sensor performance. Template strands containing a flexible loop of 0, 10, 20, 30, 40, or 50 bases were each combined with complementary target strands varying in length between 0, 10, 20, 30, 40, or 50 bases. ODN1-β-LacE104D, BLIP-ODN2, and different templates were preincubated (at low micromolar-concentrations, similar to Figure 3) and diluted to final concentrations of 1 nM ODN1-β-LacE104D, 2 nM BLIP-ODN2, and 1.2 nM Template (1:2:1.2). Ten equivalents of Target (12 nM) were added and incubated for 1 h at room temperature prior to the addition of substrate. Assays were performed using 50 μM Nitrocefin in 50 mM phosphate, 100 mM NaCl, and 1 mg/mL BSA, pH 7.0. ODN-sequences used in this assay for Template and Target strands are displayed in Supporting Information Table S2.

explains the more complete inhibition for the longer template sequences.

The effect of target sequence length on the restoration of enzymatic activity depends on the length of the template strand. No restoration of enzymatic activity is observed for complexes containing 0, 10, or 20 base template strands. For 0 and 10 base template strands, this can be simply explained by the absence of stable duplex formation between template and target under these conditions. The 20-base template and its complementary 20-base target sequence would be expected to result in formation of a stable double helix. Apparently, the length of this rigid linker alone (20 bp ≈ 68 Å) is not sufficient to disrupt the complex between β-lactamaseE104D and BLIP, which we again attribute to the inherent flexibility contributed by the linkers of the protein domains and the conjugation-site of the DNA. Consistent with this hypothesis, restoration is observed upon further increasing the lengths of the template strand and target strands. Partial restoration of enzymatic activity was observed for the 30-mer template strand in combination with 30-, 40-, or 50-mer target strands. The observation that activity is only partially restored, suggests that the 10 nm 30-mer linker can still be partially accommodated by fully stretching the linkers between the protein cores and the dsDNA, and possibly by partial destabilization of the DNA duplex at its ends. Complete release of enzyme inhibition and full restoration of enzyme activity was only observed for template strands with 40- or 50-mer template

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strands with their full complementary target sequences. While in these experiments enzymatic activity was monitored after 1 h incubation with target strand, the response time of the system is actually much faster, as the same enzymatic activity was observed when the enzymatic assay was started 1 min after target addition (Supporting Information Figure S6). An alternative explanation for the increase in enzyme activation with increasing length of template strand is an enhanced thermodynamic driving force. However, the theoretical Gibbs free energies for hybridization of the 30-mer sequence is −39 kcal/mol, which should already be more than enough to completely disrupt the interaction between β-lactamaseE104D and BLIP (see thermodynamic model in ESI, Supporting Information Figure S10).

Figure 4 shows that the optimal system, consisting of ODN1-β-lactamaseE104D, BLIP-ODN2, and 40-mer template and targets strands, displays an 8-fold increase in enzymatic activity in the presence of the target strand. To challenge the analytical performance of the system further, we decreased the concentration of the reporter enzyme to 100 pM and used the more sensitive fluorescent substrate CCF2-FA. CCF2-FA is a FRET probe in which the coumarin donor is released upon hydrolysis by β-lactamase resulting in an increase in fluorescence at 447 nm. The increased sensitivity of CCF2-FA compared to nitrocefin allows it to be used at a concentration of only 2 μM, which is far below its K_M. At this concentration, the substrate will compete less with BLIP for binding to the β-lactamase active site, which should further suppress enzymatic activity in the absence of template. In addition, unlike nitrocefin, CCF2-FA also shows no background hydrolysis in the absence of the enzyme.

Figure 5 shows a near linear increase in enzymatic turnover up to the addition of 1 equiv of template strand, when the enzyme is

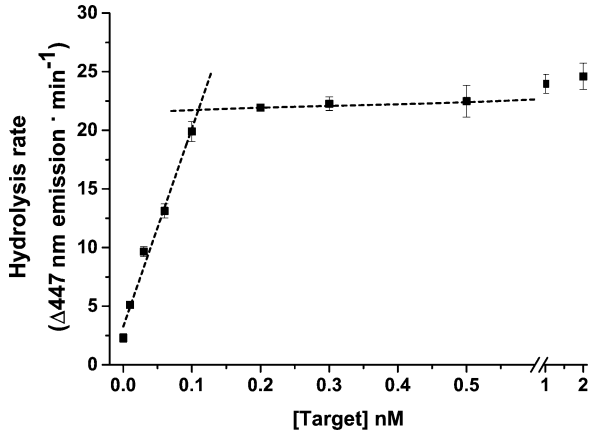

Figure 5. Enzymatic activity as a function of target concentration. The assay was performed in duplo using 100 pM ODN1-β-LacE104D, 200 pM BLIP-ODN2, 120 pM Templ-40, 2 μM CCF2-FA, 50 mM phosphate, 100 mM NaCl, and 1 mg/mL BSA, pH 7.0. All components including the substrate were preincubated for 45 min, after which the hydrolytic activity was assessed by monitoring the increase in fluorescence at 447 nm.

fully activated. Even 10 pM concentrations of template, corresponding to 2 fmol of target DNA, can be distinguished from background activity under these conditions. Please note that reliably measuring these very low hydrolysis rates required pre-equilibration of all components including substrate for 30–45 min (Supporting Information Figure S7).

The modular, self-assembling properties of the system not only allow straightforward optimization of its architecture but enable the system to be easily adapted to different target sequences. To illustrate this property, we designed template sequences complementary to 8 different 40-mer viral DNA sequences, flanked by the constant 21-base regions required to hybridize to ODN1-β-lactamaseE104D and BLIP-ODN2. The DNA target sequences were selected in the protein region of interest of Hepatitis B (HBV), Hepatitis C (HCV), Avian influenza (H5N1), Smallpox, Human Immunodeficiency Virus (HIV), Measles, Ebola, and Rotavirus C (Supporting Information Table S3 and S4) without consideration of possible secondary structure formation in template and target strand. Complexes assembled using these 8 different template strands were incubated with all 8 target sequences or no target. The heatmap shown in Figure 6 shows that enzyme activation was only

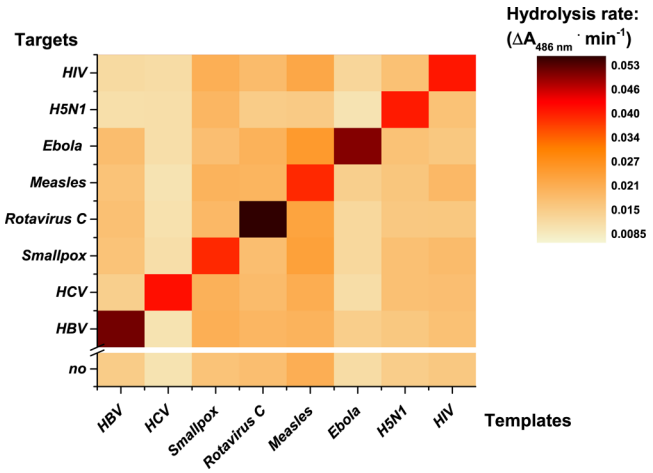

Figure 6. Modular sensor architecture allows straightforward tuning of target specificity. Components were preincubated at low μM-concentrations and diluted to final concentrations of 1 nM ODN1-β-LacE104D, 2 nM BLIP-ODN2, and 1.2 nM Template. Ten equivalents of Target (12 nM) were added and incubated for 1 h at room temperature prior to the addition of substrate. The respective assays were performed in duplo, 50 μM nitrocefin in 50 mM phosphate, 100 mM NaCl, and 1 mg/mL BSA, pH 7.0. ODN-sequences used in this assay for Template and Target strands are displayed in Supporting Information Table S3 and S4.

observed in the presence of the complementary target sequence. Although the dynamic range was somewhat smaller in this screening type of assay, these results show the excellent specificity of the system and illustrate the ease by which the system can be adjusted to any target sequence of interest (Supporting Information Figure S8).

Finally, we chose one of these target sequences (HCV) to show that enzyme activity can be reversible switched on and off by repeated addition of a toehold-containing target strand (TT) and a full complementary displacer strand. Nearly complete switching was observed between enzymatically active and inactive states for 3 cycles of target and displacer strand addition, respectively (Supporting Information Figure S9), demonstrating that DNA-directed control of enzyme activity is dynamic and fully reversible.

Conclusions. In this study, we introduced a new concept to control the activity of a reporter enzyme by DNA. Our system does not rely on DNA-templated assembly of a split enzyme system but is based on the reversible complex formation between a reporter enzyme and an inhibiting protein domain. By using an enzyme–inhibitor pair with an interaction strength in the low

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micromolar range, effective formation of an enzyme−inhibitor complex is only observed in the presence of a template strand. The noncovalent assembly of the inhibited complex allowed easy tuning of the optimal linker/target recognition sequence in the template strand, using a single pair of oligonucleotide-functionalized enzyme and inhibitor domains. Efficient disruption of the enzyme−inhibitor interaction and full restoration of enzyme activity was observed upon formation of a rigid double strand DNA helix of 40 bp or more. The present system consisting of TEM1-β-lactamaseE104D and BLIP allowed detection of as little as 2 fmol of ssDNA using a simple fluorescence assay and was shown to be easily adapted to any target sequence of interest. The use of stable, well-characterized protein components and the intrinsic modularity of the system makes it an attractive system to use in conjunction with DNA-based computing and extend the range of input targets beyond ssDNA/RNA, for example, using aptamers as ligand binding intermediates. Finally, the concept of reversibly controlling the interaction between two proteins could be easily extended to other output functions, such as self-assembling fluorescent protein domains or other enzyme−inhibitor pairs.

METHODS

Mutagenesis. DNA encoding for β-lactamase and BLIP were available in pET29a expression vectors (Genscript).[31] Single point mutations in β-lactamase and BLIP were introduced via QuickChange site-directed mutagenesis (Stratagene) according to the manufacturer's protocol using the primers listed in Supporting Information Table S1. All mutagenesis results were confirmed by DNA sequencing (StarSEQ).

Protein Expression and Purification. β-Lactamase and BLIP were expressed and purified as previously described.[31] Pure protein fractions were pooled, and the buffer was exchanged to a storage buffer (20 mM Tris-HCl, 150 mM NaCl, 2 mM TCEP pH 7.9) using a PD-10 desalting column (GE-Healthcare). Protein aliquots were stored at −80 °C. SDS-PAGE analysis and mass spectrometry (Xevo G2 QToF mass spectrometer, Waters) was performed to confirm the purity and correct molecular weight of the proteins (Supporting Information Figure S1 and S2). Protein concentrations were determined by measuring the absorbance at 280 nm using extinction coefficients calculated with Vector NTI (TEM1-β-lactamase: $\varepsilon_{280\ nm}$ = 33,585 $M^{-1}\cdot cm^{-1}$ and BLIP: $\varepsilon_{280\ nm}$ = 28,670 $M^{-1}\cdot cm^{-1}$).

ODN−Protein Conjugation and Purification. Oligonucleotides functionalized with a primary amine via a C6 linker at their 5′-end (ODN1) or 3′-end (ODN2) were purchased from Bioneer (Supporting Information Table S2). The oligonucleotides were dissolved in PBS (100 mM NaPi, 150 mM NaCl, pH 7.2) to a final concentration of 1 mM and 20 equiv of Sulfo-SMCC (Thermo Scientific) were added. Following incubation for 2 h at room temperature with continuous shaking (850 rpm), excess sulfo-SMCC was removed by (repetitive) ethanol precipitation of the conjugate. The precipitated SMCC-oligonucleotides were pelleted by centrifugation (14 000 rpm for 15 min) and dried to air overnight at room temperature.

Prior to ODN ligation to the proteins, the storage buffer was exchanged to ligation buffer (100 mM NaPi at pH 7.0) using a PD-10 desalting column to remove TCEP. Subsequently, a 3-fold molar excess of dried ODN-SMCC was added to 20−60 μM of protein and incubated for 2 h at room temperature under continuous shaking (850 rpm). The reaction mixture was loaded on a prepacked His-bind resin column, and the column was thoroughly washed to remove unreacted oligonucleotide. Subsequently, the protein and protein−ODN conjugates were eluted using several column volumes of elution buffer (20 mM Tris-HCl, 500 mM NaCl, 400 mM imidazole, pH 7.9). An additional purification step was necessary for BLIP-ODN2 in order to remove nonfunctionalized protein (Supporting Information Figure S3), the (ODN-)protein mixture was loaded on a Strong Anion-exchange Spin Column (Thermo Scientific) and washed with 15 column volumes of a low ionic strength buffer (20 mM Tris-HCl, 50 mM NaCl, pH 8.0) by centrifugation. The ODN−protein conjugate was eluted in 200 μL fractions of high ionic strength buffer (20 mM Tris-HCl, 1 M NaCl, pH 8.0). The reaction yield was determined using extinction coefficients of $\varepsilon_{260\ nm}$ = 203 900 $M^{-1}\cdot cm^{-1}$ for ODN1 and $\varepsilon_{260\ nm}$ = 225 400 $M^{-1}\cdot cm^{-1}$ for ODN2 (UV spectrum calculator of Integrated DNA Technologies, IDT). ODN−protein conjugation and purification were preferably done on the same day to avoid repeated freeze and thaw cycles in order to maintain the enzymatic activity of β-lactamase. The purity of the ODN−protein conjugates was assessed by SDS-PAGE and by analytical size exclusion chromatography on a Superdex 200 column (GE), using a flow of 0.1 $mL\cdot min^{-1}$ (20 mM NaPi, 150 mM NaCl, 1 mM EDTA at pH 7.0).

Activity Assays. Activity assays were performed in a phosphate buffer at pH 7.0 (50 mM NaPi, 100 mM NaCl and 1 mg/mL BSA) in 96-well plates at 28 °C. All target and template strands were HPLC-purified and purchased from Eurofins MWG Operon and used as received (Supporting Information Tables S2−S4). Hydrolysis of nitrocefin (VWR) was monitored by measuring the increase in absorbance at 486 nm, whereas the hydrolysis of CCF2-FA (Invitrogen) was studied by measuring the increase in coumarine fluorescence (excitation = 409 nm; emission = 447 nm). Both absorbance and fluorescence were recorded on a Safire2 spectrofluorimeter (Tecan). Hydrolysis rates were obtained by calculating the slope of absorbance or fluorescence intensities in time (linear regime) and corrected for background hydrolysis of substrate in the absence of enzyme.

ASSOCIATED CONTENT

Supporting Information

Primer and oligonucleotide sequences, protein expression and purification, protein−ODN conjugation, enzyme kinetics assay of oligonucleotide-functionalized β-lactamaseE104D and BLIP, additional activity assay data, experiment showing reversible switching between on- and off state using toehold-mediated strand displacement, thermodynamic model, and protein sequence information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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