Drug Release

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Triggered Release of an Active Peptide Conjugate from a DNA Device by an Orally Administrable Small Molecule**

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In recent years there has been a dramatic increase in the number of therapeutic peptides and proteins that have been approved for use or are in advanced clinical trails.[1–3] Methods for their delivery are still primarily based on injectable formulations, often with inconvenient dosing regimes.[1] For example, an important barrier to the ideal management of diabetes is the complex regimen of injections for insulin delivery, which combines a constant background (basal) release of insulin and increased dosages (bolus) after meals. We demonstrate herein multiple triggered releases of an

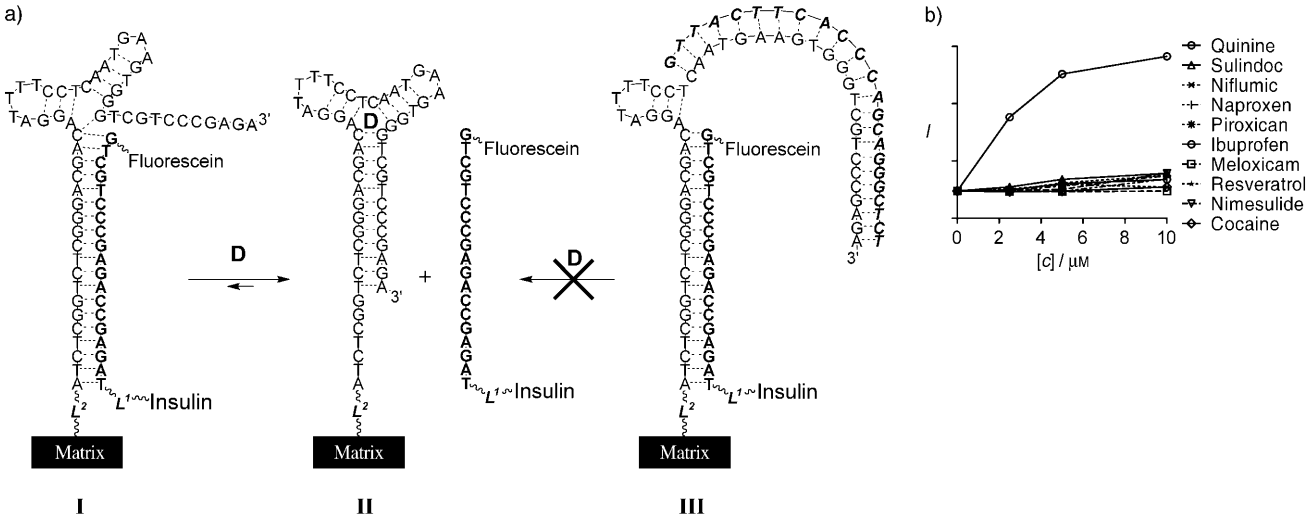

Figure 1. a) A DNA device for small-molecule-triggered release of an insulin conjugate, in which **D** is a small-molecule drug (L^1, L^2 = polyethylene glycol linker): the equilibrium shifts from **I** to **II** in the presence of **D**; triggering can be blocked with an inhibitor oligonucleotide (curved strand of complex **III**). b) Screening results for binding of FDA-approved drugs to the aptamer MNS-1.

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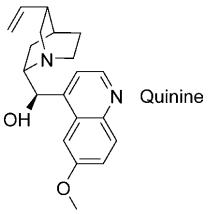
insulin analogue from a DNA device[4] that is sensitive to a small molecule, quinine. Our method could substitute frequent injections with oral administrations of therapeutic tonic water at meal times to trigger releases of the peptide from an implanted depot.

Our approach hinged on, among other things, our ability to identify a small-molecule-trigger–device pair (**D** and **I**, respectively, in Figure 1a), such that 1) the small molecule is not commonly present in food, to minimize chances for accidental hypoglycemic events; 2) the small molecule must have negligible physiological activity on its own at the escalating concentrations used to trigger multiple releases (the increases in concentrations are required in order to allow for identical pulses, if required, over several administrations of the small molecule; it is for this reason that a glucose aptamer would be useless); 3) the small molecule should trigger release at serum concentrations readily achievable through its oral administration; 4) interactions between the device and the small molecule have to be sufficiently selective to avoid accidental release, which would result in hypoglyce-

mia; 5) interactions between the device and the small molecule have to be adjustable to fit the actual therapeutic need; and 6) patients should be able to inhibit the release if they change their mind about a meal or in the case of accidental overdose of the small molecule.

Devices based on oligonucleotide-based receptors or aptamers that bind small molecules may fulfil all six requirements listed above. Both others and ourselves have demonstrated several "proof-of-principle" DNA devices for triggered release of active forms of proteins (for example, thrombin or Taq polymerase)[5,6] from their complexes with inhibitors, but these devices had dissociation rates (k_{off}) that were too high for practical applications. Instead, in order to achieve precise control of the release rate, we decided to focus on the release of oligonucleotide–insulin conjugates from their "structure-switching" complexes[7] with DNA receptors. Importantly, such release can be stopped through administration of a complementary oligonucleotide to the sequence of the aptamer that is responsible for displacing the oligonucleotide–insulin conjugate (**III** in Figure 1a).[8] Cross-linking of polymers with antibody–antigen pairs, with circulating antigens disrupting the cross-links, has been previously proposed for triggered release,[9] and a similar principle has recently been applied with a structure-switching aptamer.[10]

We screened drugs approved by the American Food and Drug Administration (FDA) for their ability to interact with a mismatched DNA-based three-way junction that was previously reported to bind cocaine and steroids (at concentrations above those typically achievable in serum).[11,12] The screening was performed at the low concentrations required for negligible physiological activity, with the goal of identifying a molecule with high selectivity (Figure 1b). We implemented a screening assay based on the fluorescein-labeled cocaine aptamer and a competitor oligonucleotide with a 4-(4′-(dimethylamino)phenylazo)benzoic acid (Dabcyl) quencher (see the Supporting Information for details). We identified quinine as a suitable compound (dissociation constant (K_d) ≈ 0.6 μM). Quinine is used to provide a characteristic bitter taste in tonic water and is also used as an oral therapeutic agent for malaria, with rapid absorption and a recommended serum therapeutic concentration range of 14–46 μM.[13]



We synthesized an insulin–oligonucleotide conjugate suitable for release from the aptamer. We selected the B1(Phe) ε-amino group position for attachment of the oligonucleotide and a spacer, based on previous activity studies on poly(ethylene glycol) conjugates.[14,15] Our synthesis was performed in three steps by starting from Boc-protected A1(Gly) ε-amine and B29(Lys) γ-amine human insulin,[16] as depicted in Figure 2a. Finally, the full activity of the insulin conjugate was confirmed in rat hepatoma cells (Figure 2b; see also the Supporting Information).

Release of the insulin conjugate (**I**→**II** in Figure 1a) was studied, under continuous flow, from a dextran matrix with surface plasmon resonance (SPR) monitoring (for release of the insulin conjugate from micro-beads with fluorescence

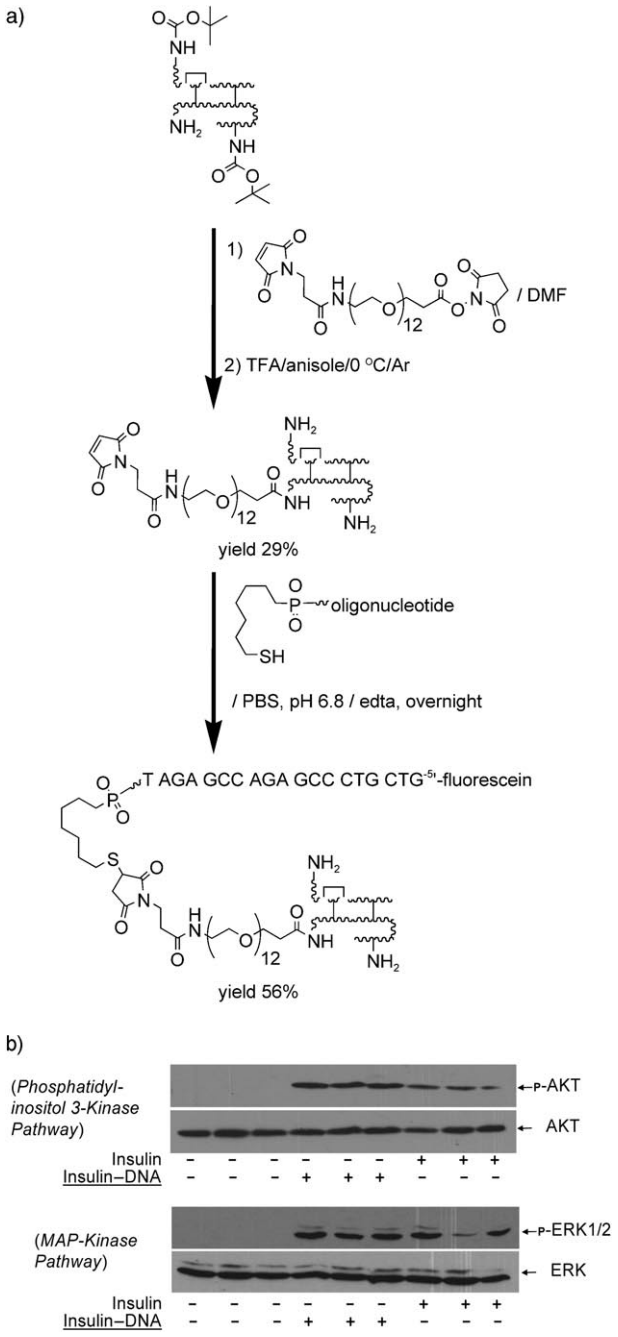


Figure 2. a) Synthesis of the insulin–oligonucleotide conjugate, with insulin represented as two chains displaying three amino groups (two of which are protected). Yields determined by HPLC are provided. DMF = dimethylformamide; TFA = trifluoroacetic acid; PBS = phosphate buffered saline, edta = ethylenediaminetetraacetate. b) Effects of the insulin–oligonucleotide conjugate on rat hepatoma cell insulin signaling pathways (in triplicate).

monitoring see the Supporting Information). The biotinylated quinine-sensor oligonucleotide was deposited ,at saturating concentrations, on a Biacore SA chip, which is a surface consisting of a carboxymethylated dextran matrix preimmobilized with streptavidin for binding of biotinylated molecules. We then adjusted, through several iterations, the number of bases in the sensor oligonucleotide and insulin

conjugate in order to be able to mimic a typical daily pattern of insulin secretion for three meals; that is, a basal rate of release (that is, without quinine) over 24 hours, combined with three pulses of quinine-induced release at 5 hour intervals. The system was nonresponsive to other hydrophobic molecules (including cocaine and steroids) at concentrations far exceeding those realistically found in serum (see the Supporting Information).

We focused on actually mimicking the normal insulin-release profile (Figure 3d)[17] while using amounts of quinine that are readily achievable with oral ingestion. For example, we accomplished the desired profile with three postprandial boluses (that is, after breakfast, lunch, and dinner) by using quinine at escalating concentrations of 0, 2, and 8 μM (Figure 3a, black trace) or, after prewashing the chip, by using 1.5, 6, and 18 μM concentrations (Figure 3c). The chip could be reloaded with insulin conjugate, with reproducible results (Figure 3a, gray trace). Importantly, without insulin, the same oligonucleotide was released much more rapidly, which resulted in the third bolus being unachievable. This observation is consistent with the additional stabilization through insulin in the matrix. One possibility is that the oligomerization capabilities of insulin lead to hindered release due to multivalent interactions;[18] however, further experiments are needed to shed some light on this process.

Figure 3 also illustrates that we could control the amount of insulin conjugate released for "lunch" by adjusting the quinine concentration: 0 μM quinine was used for a missed-lunch scenario (Figure 3b, gray trace) or 1 μM quinine used for a small-lunch scenario (Figure 3a, dashed-line trace), with 2 μM quinine used for a standard-lunch scenario (Figure 3a, solid black and gray traces). We also demonstrated that if a hypoglycemic event is threatening, an oligonucleotide inhibitor[9] strand could block the release of the conjugate in the presence of quinine (Figure 3b, solid black trace with inhibitor, compare with gray trace without inhibitor).

In conclusion, we used harmless subtherapeutic concentrations of an orally administrable small molecule to release an active form of a peptide drug from a supporting matrix. Most importantly, we readily achieved a complex release profile that matched a challenging therapeutic need. Our results are significant for several reasons: 1) We demonstrated in vitro the feasibility of multiple injections being substituted by orally triggered release from a depot, with release profiles that mimicked those required to achieve good glycemic control and that could be otherwise obtained only

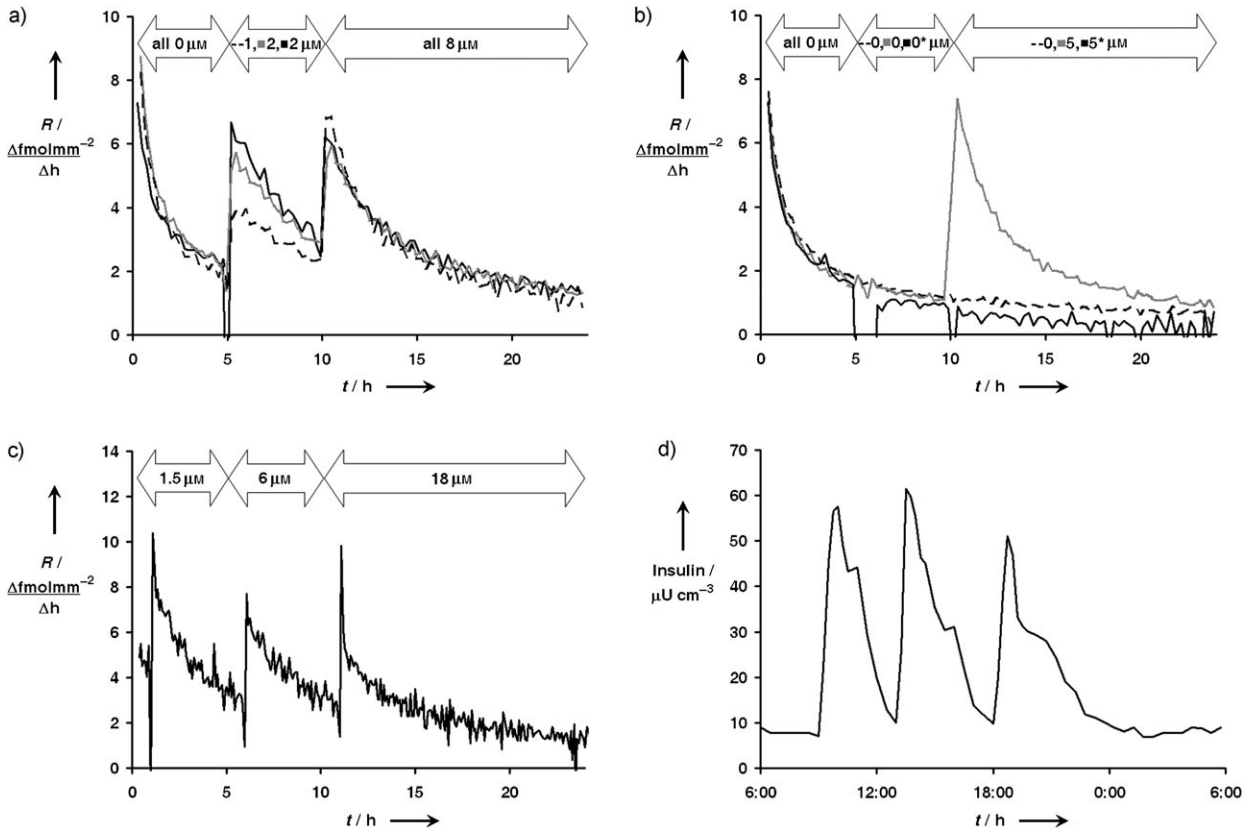


Figure 3. Surface plasmon resonance results as the amount of insulin conjugate released (*R*) from a dextran matrix per time period. All concentrations refer to quinine. a) Insulin-conjugate boluses produced by escalating concentrations of quinine: solid black and gray-line traces show reproducible release of conjugate with 0, 2, and 8 μM quinine after reloading of the matrix; the dashed line represents release with 0, 1, and 8 μM quinine and demonstrates the use of the quinine concentration to control the amount of the second bolus. b) The solid-line trace shows the profile of release with 1 μM of inhibitor added (indicated by asterisks) to prevent release of insulin conjugate in the presence of quinine (compared with the gray-line trace, which represents the same experiment but without inhibitor added); the dashed line represents the profile of release of the insulin conjugate when quinine is not added. c) Insulin-conjugate boluses produced by 1.5, 6, and 18 μM quinine. d) Typical human serum levels of insulin over a 24 h period in response to meals at 9:00 AM, 1:00 PM, and 6:00 PM (adapted from Ref. [18] with permission).

through a complex regimen; 2) the described principle seems general, and it could be used with other small molecules for which aptamers can be isolated and for other peptides that retain their activity as conjugates;[19] 3) the described DNA device is the first of its kind that transfers proof of concept to a relevant peptide and a relevant small molecule, with realistic potential for practical applications; 4) we demonstrated that the insulin–oligonucleotide conjugate retained its activity, which may eventually allow control of insulin retention and activity through DNA nanotechnology approaches.[20] In vivo success would depend on integration of the DNA device with suitable biologically compatible functionalized polymers.

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