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Protein recognition via oligonucleotide-linked small molecules: Utilisation of the hemin-binding aptamer

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The development of oligonucleotide-linked small molecules for protein recognition is a worthy undertaking with potential applications in drug discovery and diagnostics. Although the ground work for this novel approach to protein recognition is still being laid, a number of promising systems have recently been developed. Herein, we discuss a selection of these systems focusing on the distinct tactics that can be employed for protein binding. Also reported is a new example that underscores the unique versatility of linking aptamers to protein-binding small molecules. In particular, we discuss the development of a chimeric aptamer that in the 'on' state can bind and signal the presence of a target protein. Furthermore, we demonstrate that the activity of this sensing supramolecule can be switched 'off' on cue.

Keywords: aptamers; supramolecular chemistry; DNA self-assembly; protein recognition; DNAzyme

1. Introduction

Critical cellular processes such as chromosome packaging, gene expression and DNA replication exploit molecular recognition events that occur between oligonucleotides (ODNs) and proteins (*1*). While Nature has evolved specific proteins (including transcription factors, polymerases and nucleases) (*2*) to recognise and manipulate nucleic acid sequences, chemists have demonstrated that this formal receptor/ligand role can be readily reversed. Indeed, much effort has been devoted to developing single-stranded ODNs that can be selected against nearly any protein (or other target molecules) via an *in vitro* selection and amplification technique (*3*). These ODNs, termed aptamers, are short DNA or RNA sequences that bind to selected targets by accessing distinct 3D folds that position relevant nucleobase, sugar and phosphate units in an arrangement that complements the functionality and topology of the protein surface being recognised. An example of a DNA aptamer that binds to its target protein via a discrete, folded conformation is illustrated schematically in Figure 1(A). Here, the thrombin-binding aptamer (TBA) forms an intramolecular quadruplex upon binding to human α-thrombin, resulting in the inhibition of thrombin-catalysed fibrin-clot formation (*4*). Since the advent of aptamer technology, numerous functional aptamers have been prepared for biotechnology, diagnostics and therapeutic applications (*5*).

While aptamers selected against proteins harness the natural constituents of nucleic acids as the molecular recognition modality, an alternative approach to protein binding using ODNs linked to small molecules has been introduced. Here, the designed synthetic fragments act as the protein recognition functionality (Figure 1(B)), while the ODN moiety serves as a barcode (for selection and amplification purposes) that encodes the synthetic fragment. Furthermore, the ODN domain can also be utilised as a self-assembling motif to project multiple synthetic fragments. For instance, the smallest such self-assembled species can lead to the projection of two protein-binding molecules in a pseudo-parallel orientation (as a result of complementary single strands annealing to form a double helix; Figure 1(C)). This article will commence by highlighting the potential of ODN-linked small molecules in protein recognition, by discussing selected examples wherein the appended synthetic fragments play a predominant role in protein binding. The second portion of this paper will focus on a specific aspect of ODN-linked small molecules, where the ODN domain is not a traditional nucleic acid sequence, but, instead, is a judiciously chosen aptamer.

In particular, we detail the development of a chimeric aptamer that contains a core hemin-binding sequence tethered on the 5′-terminus to biotin. As a consequence of incorporating two separate moieties capable of non-genetic molecular recognition, this bifunctional agent can bind to streptavidin as well as hemin (Figure 1(D)), and thereby signal the protein recognition event via a colorimetric response. In addition, we illustrate that this streptavidin-sensing supramolecule can be turned 'off' by the addition of a specific external stimulus. It is expected that the generalised concept of using aptamer-linked small molecules for protein-binding can be extended to

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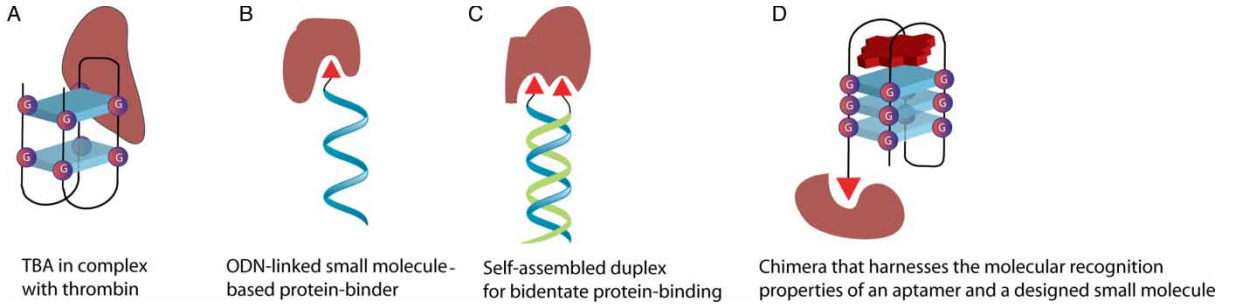

Figure 1. Different formats for ODN-derived protein binding. (A) TBA complexed with thrombin via a well-defined intramolecular quadruplex conformation. (B) A non-aptamer-based single-stranded ODN used for monodentate protein binding. (C) A self-assembled duplex used for bidentate protein binding. (D) A chimera that harnesses the protein-binding ability of a synthetic fragment in conjunction with the non-genetic molecular recognition property of an aptamer. Note: the red arrowheads represent protein-binding synthetic fragments. The circles containing a 'G' represent guanines and the squares between the guanines denote guanine quartets.

alternative aptamer systems as well as other protein-binding small molecules, resulting in well-controlled functional chimeras for a variety of healthcare-related applications.

2. Protein recognition via monodentate interactions

The utility of preparing synthetic molecules linked to DNA, wherein the nucleic acid sequence serves as an identification code, for selecting protein binders was proposed by Brenner and Lerner (*6*) in the early 1990s. These researchers realised that traditional genetic repertoires, although amenable to selection and amplification, do not possess the diversity of synthetic chemical libraries. Thus, they postulated that a large chemical library, each member of which is attached to a unique DNA coding sequence, could be built via a 'split-and-pool' approach. In particular, they proposed parallel and alternating syntheses of peptides and ODNs on a resin, such that one member of a library contains a single peptide sequence as well as its unique ODN identifier. These authors also recognised that such libraries could be exposed to a target protein of choice and the strong binders could then be amplified, enriched and, finally, decoded via cloning and sequencing. Subsequent to this hallmark theoretical paper, the viability of such an approach has been demonstrated experimentally by Gallop and co-workers (*7*), who prepared a library of $\sim 10^6$ unique heptapeptide sequences on beads that include distinct ODN barcodes. Exposure of this library to an anti-peptide antibody, followed by the isolation of strong binders (via fluorescence-activated bead sorting), amplification (by PCR) and sequencing, resulted in the identification of high-affinity peptide sequences.

The use of ODN-linked small molecules as modules for protein binding, *in lieu* of polypeptides, has been advanced by Liu and colleagues. These researchers attached small molecules onto single-stranded DNA barcodes and have clearly shown that the resultant conjugates can be preferentially enriched in the presence of their cognate protein targets (*8*). For instance, in one experiment, a mixture of DNA-linked phenylsulphonamide **1** (Figure 2(A)) and negative control **2** (using a 1000-fold excess of **2**) were selected for binding to carbonic anhydrase (CA). After only one round of selection (consisting of incubation in the presence of CA followed by washing of non-binders and PCR amplification of bound ODNs), an enrichment factor of 330-fold for ODN **1** was obtained. Analogous experiments testing DNA-linked biotin **3** (against streptavidin) and DNA-linked glutathione amide **4** (against glutathione s-transferase) resulted in dramatic enrichment factors of 4400 and 2500, respectively. These findings not only demonstrate the power of *in vitro* selections using DNA-linked small molecules, but also clearly indicate that the conjugation of synthetic fragments onto ODNs does not adversely impact the protein-binding ability of the synthetic head groups.

In addition to harnessing the ODN domain for encoding purposes, the reorganisation properties of ODNs upon binding to a complementary sequence can also be exploited. As illustrated in Figure 2(B), Ghadiri and co-workers (*9*) have prepared an allosteric enzyme (cereus neural protease, CNP) that is inactive as a result of being intrasterically regulated by a flexible single-stranded DNA attached to a small-molecule inhibitor of CNP (phosphoramidite-based dipeptide derivative **5**). Upon addition of a target strand that is complementary to the ODN tethered to the enzyme, an allosteric activation step takes place resulting in the formation of a rigid double helix. This conformational change, in turn, removes the small-molecule head group from the enzyme active site leading to an activated enzyme. The authors followed the protease activity of CNP by the introduction of a fluorescent-quenched enzyme substrate. The potential of this system is underscored by the fact that very low concentrations of DNA can be detected. For instance, the lower detection limit of complementary DNA was 10 pM.

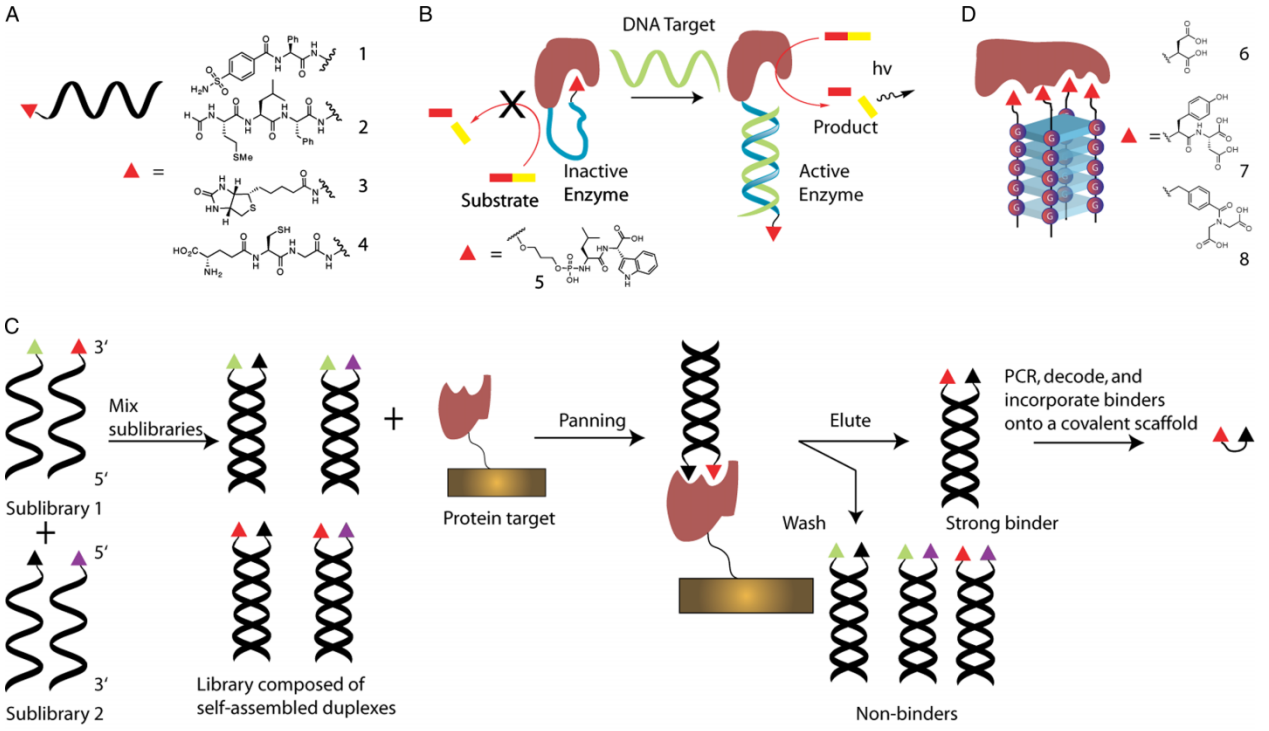

Figure 2. Examples of ODN-linked small molecules for protein recognition. (A) Known ODN-linked protein-binding synthetic fragments (phenylsulphonamide (**1**), biotin (**3**), glutathione amide (**4**) and negative control peptide fMet-Leu-Phe (**2**)) developed by Liu and co-workers to test for *in vitro* selection of protein binders. (B) An engineered allosteric enzyme capable of binding to a specific DNA sequence and converting the binding event to a catalytic signal. (C) DNA-ESAC libraries based on duplex formation. Mixing of two sublibraries results in a self-assembled library wherein each member has the potential to interact in a bidentate fashion with the target protein. Subsequent to biopanning, the enriched binders are PCR-amplified and decoded, leading to the identification of the individual protein recognition elements. These elements are imbued onto a covalent scaffold as potential pharmacophores. (D) Self-assembling intermolecular quadruplexes formed by a guanine-rich sequence (5′-TGGGGGGATCTTA-3′) can be used to project four synthetic fragments onto the surface of cytochrome *c*.

Furthermore, these researchers have recently shown that modulating enzyme activity by the addition of various external DNA inputs can lead to complex molecular devices capable of performing AND, OR and NOR logic operations (*10*).

3. Protein recognition via multidentate interactions

The above-mentioned examples highlight the potential of using DNA-linked synthetic protein binders in a monodentate fashion, where only one small molecule is used to bind to a distinct site on a target protein. However, the ability of DNA to self-assemble into double helices or other higher order structures is an attractive means of projecting multiple synthetic fragments for more effective protein sequestration. Indeed, the groups of Hamilton and co-workers (*11*) and Neri and co-workers (*12*) have separately developed such a strategy termed encoded self-assembling chemical (ESAC) libraries.

In one embodiment of this approach (outlined in Figure 2(C)), two sublibraries are prepared wherein each sublibrary member consists of a specific identifying DNA sequence flanked by two conserved regions. The 3′ termini of members of one sublibrary (and the 5′ termini of the second sublibrary) are functionalised with unique small molecules. The Hamilton procedure utilises the formation of bulged duplexes when the two sublibraries are mixed. Here, both the conserved regions (surrounding the central identifying sequence) on one sublibrary are complementary to the conserved regions of the second sublibrary (*13*). On the other hand, the Neri protocol involves the self-assembly of only one of the conserved regions (the constant region that is directly attached to the synthetic fragment), resulting in a duplex with frayed tails (*14*). In both cases, however, mixing of the two sublibraries leads to a double helical structure at the terminus projecting the small molecules.

It is important to note that this bioinspired assembly process results in the generation of a large library in a rapid manner. For instance, from a mixture of two sublibraries, each containing x members, a dynamic combinatorial library of x^2 DNA duplexes can be assembled.

Furthermore, the resultant library members (each now containing two protein-binding moieties) are expected to possess enhanced affinity and selectivity for a target protein as a result of chelating interactions (*15*). Exposure of this latter library against an immobilised protein target, followed by the separation of the binding members from the non-binding library members (via a washing step) and the subsequent enrichment of the binders using PCR, should enable the identification of *de novo* high-affinity agents. Subsequently, the two small-molecule moieties can be unified onto an appropriate covalent framework as a potential antagonist against the targeted protein. An important version of this generalised strategy is to exploit a fragment-based discovery approach, where one of the library members is a previously established protein-binder. Hence, ESAC libraries can be used as an affinity maturation procedure to generate bidentate binders with exceptionally high affinity to their target proteins. Indeed, Neri and colleagues have successfully converted hits from such a fragment-based approach to develop potent inhibitors of matrix metalloproteinase-3 (IC_{50} improved from >300 to 9.9 μM) (*16*), trypsin (IC_{50} improved from 90 μM to 98 nM) (*17*) and CA (IC_{50} improved from 1 μM to 25 nM) (*12*).

Hamilton and co-workers (*18*) have further advanced the concept of projecting multiple small molecules in a directed manner by exploiting tetramolecular DNA self-assembly. In particular, intermolecular quadruplex formation, driven by the cyclic self-assembly of guanine quartets, can lead to the presentation of four protein-binding elements (Figure 2(D)). These researchers have demonstrated that parallel quadruplexes functionalised with specifically designed anionic (e.g. aspartate **6**) and hydrophobic modules (e.g. tyrosine–aspartate **7** and iminodiacetate **8**) can bind to a central hydrophobic patch on cytochrome *c* (cyt *c*) flanked by basic lysine residues. In addition to binding to cyt *c*, these supramolecular assemblies possess the remarkable ability of inducing protein denaturation, possibly as a result of binding to a non-native fold of the protein. For instance, 1 equiv of the quadruplex presenting four units of **8** decreases the melting temperature (T_m) of cyt *c* by 45 K (a decrease in T_m from 82 to 37°C) (*18*). Importantly, equimolar single-stranded DNA congeners not capable of forming such a defined self-assembly (or unfunctionalised quadruplexes) are ineffective in inducing such a pronounced reduction in the T_m of cyt *c*. From these results, it is clear that the higher order self-assembly of DNA-linked small molecules can produce novel and emergent protein recognition properties.

The number of protein-binding fragments can be significantly enhanced not only by the self-assembly of higher order ODN strands but also through the incorporation of multiple copies on an appropriate surface. For instance, Mirkin and co-workers (*19*) have decorated 13 nm gold nanoparticles with single-stranded ODNs (~110 ODNs per nanoparticle). These ODN-modified nanoparticles were hybridised with complementary ODNs linked to haptens (e.g. dinitrophenyl). The resultant complex projects the haptens outwards from the nanoparticle surface. Upon addition of anti-dinitrophenyl immunoglobulin E, multivalent protein–small molecule interactions are formed resulting in the aggregation of the nanoparticles and a corresponding red-to-purple colour change (which can be used to signal the presence of the target protein of interest) and precipitation. Furthermore, the ODN sequence linked to the hapten is itself a biobarcode that encodes for the hapten. Thus, isolation of the nanoparticle–protein aggregate, followed by de-hybridisation of the duplex, and decoding (via an ODN microarray) leads to enhanced sensitivity in terms of protein detection. In addition to sensing applications, ODN-linked small molecules can also be used to build nanostructures with unique architectures. For example, Niemeyer et al. (*20*) have prepared supramolecular nanocircles by using the self-assembly properties of bisbiotinylated duplex DNA in the presence of streptavidin.

4. Aptamer-linked small molecules for protein recognition

The hitherto mentioned examples have shown that the self-assembly and coding properties (i.e. genetic molecular recognition) of ODNs tethered at their extremity with the synthetic fragments can lead to versatile protein recognition agents. These results beg the question of whether aptamer-based molecular recognition properties of ODNs can also be utilised in a similar manner to prepare novel bifunctional agents. Although aptamers have been conjugated to a variety of molecules (including quantum dots (*21*), single-walled carbon nanotubes (*22*), fluorophores (*23*) and proteins (*24*)), there has been little systematic development of aptamers as scaffolds for projecting protein-binding small molecules (*25*). However, aptamers linked to biotin (exploiting the high-affinity biotin–streptavidin interaction, $K_d = 10^{-15}$ M) (*26*) have been utilised in biotechnology protocols including for surface immobilisation (*27*). Given this high-affinity constant and the ease of synthesis of biotinylated aptamers, we reasoned that the biotin–streptavidin interaction can be used, not for its robust biotechnology applications, but rather as a model that illustrates a simple proof of concept; that is, tethering of a protein-binding small molecule onto the terminus of an aptamer can lead to a bifunctional supramolecule with controllable protein detection capability.

In an effort to illustrate this concept, we have developed a straightforward aptamer chimera (ODN **9**)

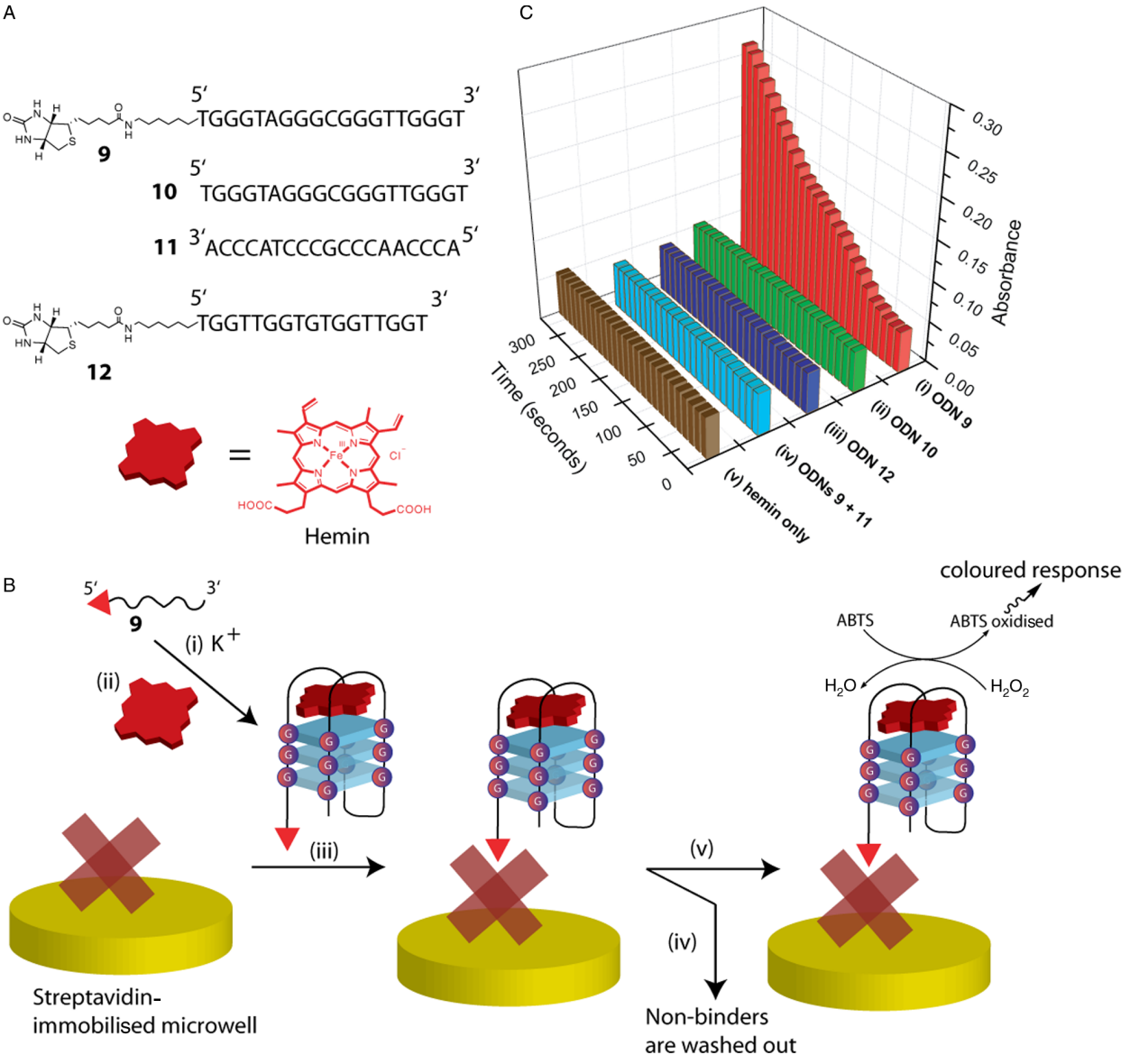

Figure 3. Hemin-binding aptamer linked to biotin for the detection of streptavidin. (A) Illustration of ODN sequences used in the current study as well as the structure of hemin. (B) General schematic for streptavidin detection: (i)–(ii) ODN **9** is pre-annealed and complexed with hemin. (iii) The resultant supramolecule is introduced into streptavidin containing microwell plates. (iv) Subsequent to an incubation step, non-binders are eluted out by washing with the PBS buffer. (v) The streptavidin-bound aptamer chimera **9** is visualised by the addition of ABTS and peroxide. (C) Bar graphs depicting the time-dependent absorbance changes of streptavidin-immobilised microwells (measured at 414 nm) upon incubation with various pre-annealed ODNs in the presence of hemin (5 μM), ABTS (2 mM) and hydrogen peroxide (2 mM) in the HEPES buffer (50 mM HEPES, 20 mM KCl, 200 mM NaCl, 1% DMSO, 0.05% Triton X-100, pH 8.0). The ODNs used in the current study are: (i) ODN **9** (red), (ii) ODN **10** (green), (iii) ODN **12** (dark blue), (iv) ODNs **9** + **11** (light blue) and (v) the hemin-only control (brown). All ODN concentrations were 5 μM.

designed to bind and signal the presence of streptavidin (Figure 3(A)). Here, it is important to note that the biotin head group is used purely as a model protein-binding fragment. It is expected that biotin can be replaced with other small molecules for the detection of their cognate protein targets. We chose the hemin-binding aptamer because this DNA sequence is capable of binding to hemin via guanine quadruplex formation (facilitated by templating potassium cations), resulting in horseradish peroxidase-like catalysis (*28*). Indeed, the DNAzyme activity of this particular aptamer can be used to oxidise a non-coloured 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; ABTS) to a coloured radical ion product ($ABTS_{oxidised}$) (*29*). As a negative control, DNA strand **10**, which contains the hemin-binding aptamer sequence but lacks the biotin head group, was prepared. A second

control DNA strand (ODN **12**) that incorporates a biotin head group as well as an alternative aptamer domain (based on the TBA, Figure 1(A)) was also chosen. While this latter chimera is capable of forming an intramolecular quadruplex structure in the presence of potassium cations, it does not, however, possess hydrogen peroxidase activity.

In order to demonstrate the effectiveness of ODN **9** as a detection molecule for streptavidin, we developed a direct ELISA-type microwell assay wherein streptavidin is immobilised on a plate and the detection agent is aptamer-linked biotin **9** (instead of a traditional antibody linked to a signal molecule). Specifically, a solution of ODN **9** in potassium containing HEPES buffer (50 mM HEPES, 20 mM KCl, 200 mM NaCl, 1% DMSO, 0.05% Triton X-100, pH 8.0) was annealed under quadruplex-forming conditions (i.e. incubating at 95°C followed by cooling to room temperature), followed by the addition of hemin (also in the HEPES buffer), such that the final concentrations of **9** and hemin were both 5 μM (Figure 3(B(i),(ii))). This solution was introduced into streptavidin-immobilised microwells (Figure 3(B(iii))) and the resulting mixture was incubated for 1 h. After washing with phosphate buffered saline (PBS) buffer to remove any non-binders (Figure 3(B(iv))), the presence of streptavidin was detected by the addition of ABTS and hydrogen peroxide in order to initiate the colorimetric response (Figure 3(B(v))).

As shown in Figure 3(C), a microwell containing ODN **9** exhibits an increase in absorbance at 414 nm over time corresponding to the formation of oxidised ABTS in a catalytic process. Furthermore, this colour evolution clearly signals the presence of streptavidin in the microwell. In marked contrast, an analogous experiment with control ODN **10** does not show an appreciable response since this ODN has no affinity for streptavidin (and thus is washed away in step (iv)). Similarly, control **12** also displayed no significant change in absorbance. Although **12** is capable of binding to streptavidin, it does not possess horseradish peroxidase-like catalytic activity in the presence of hemin. Taken together, these experiments not only indicate that judicious choice of small-molecule and aptamer moieties can lead to novel protein-sensing systems, but also that such aptamer chimeras can be readily incorporated into microwell-based assays necessary for rapid screening of samples.

A further advantage of using a single-stranded aptamer domain as the detection module is that upon annealing in the presence of a complementary sequence a duplex should form. Since the hemin-binding aptamer requires a quadruplex conformation to bind to hemin (*29*), this change in conformation should switch the supramolecular sensor to an 'off' state. Indeed, incubation of ODN **9** with complementary sequence **11**, followed by the addition of hemin and introduction into a streptavidin-containing microwell, resulted in dramatically attenuated absorbance values. An important rationale for developing detecting agents that can be selectively turned 'off' on cue is that such controlled sensors may have application in multi-plexed devices that contain multiple distinct sensors, each capable of detecting a different analyte. Here, the ability to turn 'off' certain sensors while keeping a particular sensor (for the analyte that is to be detected) in the 'on' position is attractive for minimising background noise. In addition, the supramolecular sensor illustrated in this work contains no proteinaceous components and as such is expected to circumvent a number of negative aspects associated with enzyme-based biosensors. These include the rapid denaturation and short shelf life of proteins (while DNAzyme-based systems can be easily renatured and typically possess longer shelf life). In addition, nucleic acid-based sensors are expected to be particularly useful for detecting a variety of proteases wherein protein-based sensors may undergo degradation.

5. Conclusion

It is clear that coupling of small molecules onto ODN scaffolds has led to a number of highly versatile protein recognition agents with promising therapeutic potential. For instance, ODN-linked synthetic fragments can be used to develop potent small-molecule inhibitors against a variety of salient target proteins. This same technology can also be utilised to prepare large self-assembled structures capable of selective protein denaturation. ODN-linked synthetic units are also important from a diagnostic standpoint since the unique encoding and amplification capability of ODNs can be exploited in a stand-alone manner or in conjunction with complementary techniques (such as nanoparticle-based detection). Furthermore, as the latter portion of this paper has outlined, non-traditional nucleic acid sequences such as aptamers can be tethered to small molecules resulting in bifunctional agents capable of non-genetic molecular recognition and protein detection. In addition, genetic molecular recognition (i.e. duplex formation) can be used as a control switch to modulate the activity of such sensing molecules. We are currently exploring the potential of alternative small molecule–aptamer conjugates as responsive agents for both therapeutic and diagnostic applications.

6. Experimental

6.1 General methods

ODNs **9**–**12** were synthesised by the Keck Foundation Biotechnology Resource Laboratory at Yale University using standard automated solid-phase synthesis. These ODNs were purified in our laboratory with Sephadex resin Microspin G-25 columns (GE Healthcare, Waukesha, WI, USA) and chromatographed with a Varian Prostar reverse-phase HPLC equipped with a Polymer Laboratories 100 Å

5 μm polystyrene/divinylbenzene reverse-phase column (complete with MetaTherm column heater). The column was maintained at 65°C for all runs, with an elution gradient of 5% (0.1 M triethylammonium acetate (TEAA)) to 100% acetonitrile over a period of 70 min. Concentrations of stock solutions of ODNs were quantified based on their respective electronic absorption at 260 nm and their molar extinction coefficients obtained by nearest-neighbour calculations. Streptavidin-immobilised, Nunc 96-well microplates were obtained from Thermo Fisher Scientific (Rockford, IL, USA). These plates feature anthraquinone-functionalised streptavidin coupled to the microplate well through an ethylene glycol spacer, yielding a biotin-binding capacity of 13 pmol/well. Hemin was purchased from Frontier Scientific (Logan, UT, USA). ABTS was obtained from AppliChem (Darmstadt, Germany). All other chemicals including salts, buffers and peroxide were purchased from Sigma Aldrich (St Louis, MO, USA).

6.2 ODN characterisation via matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF)

Purified ODNs were characterised by MALDI-TOF mass spectrometry using a Bruker Daltonics Autoflex III in the linear negative mode. ODN **9**: calculated MW = 6417.3 g/mol, observed 6415.9 g/mol; ODN **10**: calculated MW = 6011.9 g/mol, observed 6012.5 g/mol; ODN **11** calculated MW = 5607.7 g/mol, observed 5609.0 g/mol and ODN **12**: calculated MW = 5739.9 g/mol, observed 5743.9 g/mol.

6.3 Streptavidin detection assay

Each well of the streptavidin-immobilised plate was pre-washed three times with 100 μL of the PBS buffer (20 mM NaH_2PO_4, 30 mM Na_2HPO_4, 100 mM NaCl, pH 7.4). Next, each ODN in 98 μL of the HEPES buffer (50 mM HEPES, 20 mM KCl, 200 mM NaCl, 1% DMSO, 0.05% Triton X-100, pH 8.0) was incubated at 95°C for 5 min, and then allowed to cool slowly to room temperature. After the cooling to room temperature, 2 μL of hemin in the HEPES buffer (50 mM HEPES, 20 mM KCl, 200 mM NaCl, 1% DMSO, 0.05% Triton X-100, pH 8.0) was added, bringing the total concentration of hemin to 5 μM and the total concentration of ODN to 5 μM in 100 μL of the HEPES buffer. The ODN/hemin solutions were then allowed to incubate for 30 min at 25°C to ensure hemin complexation with ODNs. The resultant ODN/hemin solutions were then added to microwells containing immobilised streptavidin, and incubated with gentle agitation for 1 h. Next, non-binding ODN/hemin solutions were removed by inversion, and each well was washed three times with the PBS buffer. Plate wells were then refilled with 97.5 μL of the HEPES buffer containing ABTS. Hydrogen peroxide (2.5 μL of 80 mM solution) was then introduced to each well, bringing the total assay volume to 100 μL and the total ABTS and hydrogen peroxide concentrations to 2 mM. The visible light absorbance generated by oxidised ABTS (at 414 nm) was then immediately monitored on a Molecular Devices SpectraMax 190 microplate reader.

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(29) It should be noted that Willner and co-workers have elegantly demonstrated that the allosteric nature of the hemin-binding aptamer (when tethered to other nucleic acid sequences or proteins) can lead to bifunctional molecules capable of detecting various agents of import (including DNA, AMP, lysozyme and glucose). See: (a) Pavlov, V.; Xiao, Y.; Gill, R.; Dishon, A.; Kotler, M.; Willner, I. *Anal. Chem.* **2004**, *76*, 2152–2156. (b) Li, D.; Shlyahovsky, B.; Elbaz, J.; Willner, I. *J. Am. Chem. Soc.* **2007**, *129*, 5804–5805 (also see Ref. (*24*)). (c) In addition, Kolpashchikov has recently shown that an ODN containing the hemin-binding aptamer can be split into binary components that self-assemble in the presence of a specific DNA sequence and hence can be used as a probe for detecting single nucleotide polymorphism (see: Kolpashchikov, D.M. *J. Am. Chem. Soc.* **2008**, *130*, 2934–2935). However, to the best of our knowledge, there has been no exploration of using the hemin-binding aptamer linked to a synthetic fragment for targeted protein-binding and detection.