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Multivalent Protein Binding and Precipitation by Self-Assembling Molecules on a DNA Pentaplex Scaffold

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Cooperative binding of multimeric synthetic agents to multivalent protein targets remains challenging.[1] An elegant solution in nature is exemplified by the IgM family of antibodies, which function as homopentameric assemblies that bind to selected targets with high avidity.[2] Use of variable regions in antibodies allows for rapid selection of protein binding epitopes, a feature utilized in the development of antibody-based diagnostic, biochemical, and even therapeutic applications. Organic synthesis allows for a greater scope of functional group diversity in multimeric binding agents and for rationally designed targeting of substrate binding pockets. Examples include the multimeric STARFISH[3] and polymeric mannose-derivatized protein-binding molecules.[4] Surprisingly few self-assembling examples of such molecules exist. Recent examples include efforts to target proteins using DNA duplex[5] and quadruplex[6] scaffolds of two and four molecular fragments. A strategy that combines the facile, multimeric self-assembly observed in the IgM family of antibodies, and the active or binding site specificity of synthetic organic molecules would represent an intriguing, hybrid approach to targeting multivalent proteins. Here we present a self-assembling, pentameric structure that, when functionalized with protein substrates, binds cooperatively to a pentavalent protein target. This structure utilizes a spontaneously assembling DNA scaffold with phosphocholine (PC) head groups (Figure 1A) to target human C-reactive protein (CRP). We observe cooperative binding in the functionalized pentameric assembly, which binds CRP with 350-fold higher affinity than its monomeric counterpart. The functionalized DNA pentaplex also mimics the biological function of these higher order biological structures by inducing coprecipitation with CRP. This study suggests a general structure of these pentaplexes with a "reporter" face and a "target binding" face for potential use in diagnostic applications involving CRP.

CRP is a homopentameric protein produced in the liver in response to infection and inflammation.[7] It is one of the most rapidly produced infection biomarkers and has the potential to serve as a diagnostic in therapeutic settings.[8] It functions as a PC-binding protein and is thought to target the lipid membranes of foreign matter in serum. Recently, CRP has been implicated in heart disease.[9] Both bidentate and polydentate[10] PC, on polymethylene and BSA protein, respectively, have been shown to target CRP with increased binding affinity compared to monovalent substrate. Pentadentate substrates have targeted pentavalent proteins, such as the five-armed STARFISH inhibitor of Shiga-like toxin.[3] At a supramolecular level, even templation of multidentate arrays by a complementary protein has shown an increase in binding efficacy to multivalent protein targets, such as the pentavalent Shiga and cholera toxins.[11] To our knowledge no discretely pentameric binding structure for CRP has been described.

Higher-order, hydrogen-bonded assemblies of DNA have been extensively documented.[12] The DNA pentaplex structure has been described by Switzer and Seela to form in the presence of Cs^+.[13]

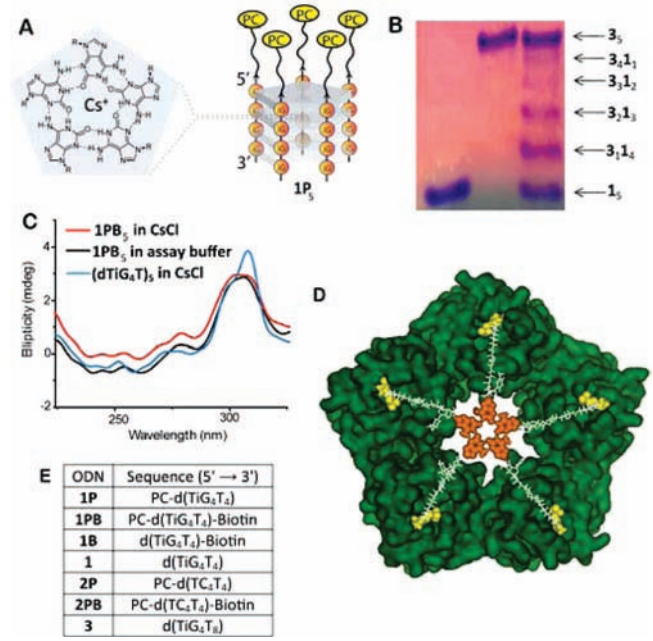

Figure 1. (A) Pentaplex-assembled PC,$\mathbf{1P}_5$ with illustration of iG pentad (left). (B) PAGE and (C) CD of assembled pentaplexes. (D) Docking of $\mathbf{1P}_5$ to CRP (CRP = green; PDB = 1B09). (E) ODN sequences prepared.

The structure is an assembly of five oligodeoxynucleotide (ODN) strands containing a sequence of at least four tandem isoguanine bases. The strands assemble in a parallel orientation, in stacked pentads arranged by hydrogen-bonds (Figure 1A). When termini of these individual strands are functionalized, either one or both "faces" of the resulting pentaplex will present five small molecules in proximal positions, making these architectures an appealing choice to target complementary protein surfaces such as the pentameric CRP. As illustrated in the docking model (Figure 1D) the PC-funtionalized pentaplex, with an appropriate tethering arm, can simultaneously reach the binding pocket of each CRP subunit.

PC-conjugated ODNs were prepared on solid phase by labeling NHS-ester activated ODN with an amine-modified PC derivative (Figure S1). The resulting conjugate tethered the PC to the DNA via a ~30 Å linker. Self-assembly was achieved by heating ODNs in sterile, deionized water containing 80 mM CsCl at pH 6.5 for 10 min at 95 °C, followed by slow cooling to room temperature and incubation at 4 °C for 48 h. Assembly and stability of the pentaplexes were monitored by both polyacrylamide gel electrophoresis (PAGE) and circular dichroism (CD). To monitor formation of pentaplex by PAGE, ODNs **1** and **3** (Figure 1E) were mixed.[13] The resulting solution was predicted to contain six possible pentaplexes of distinct sizes: $\mathbf{3}_5$, $\mathbf{3}_4\mathbf{1}_1$, $\mathbf{3}_3\mathbf{1}_2$, $\mathbf{3}_2\mathbf{1}_3$, $\mathbf{3}_1\mathbf{1}_4$, and $\mathbf{1}_5$. As

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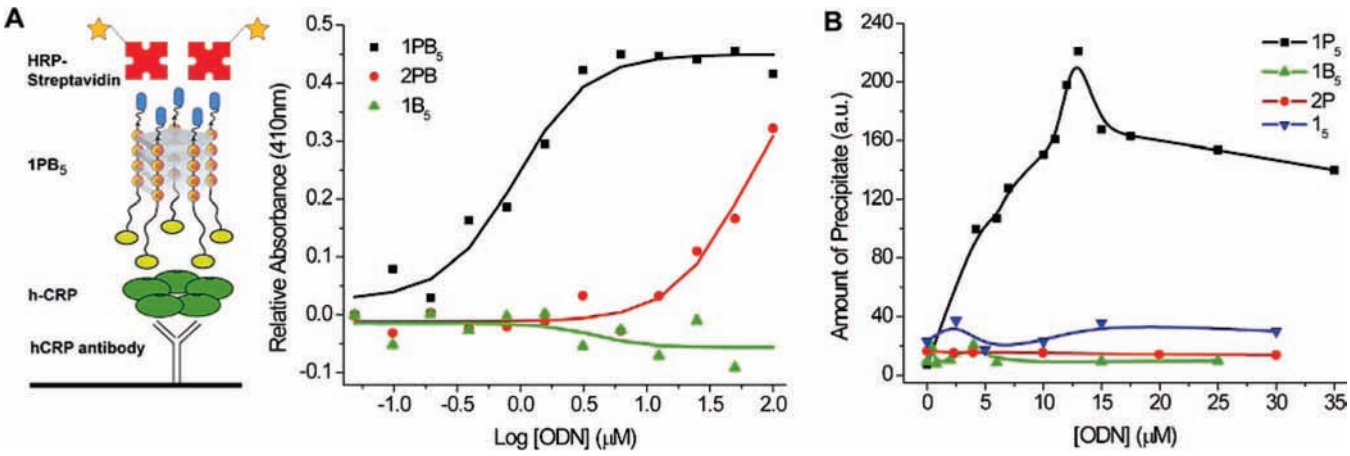

Figure 2. (A) ELISA experimental multiplex (left) with binding curves for each construct (right). (B) Light scattering experiments demonstrating that only functionalized, assembled pentaplex (**1P**$_5$) is capable of coprecipitating with CRP (2.0 μM). Data shown is representative of experiments conducted in triplicate.

shown in Figure 1B, there are six distinct bands discernible by PAGE. Furthermore, the CD spectrum of PC-conjugated pentaplex **1PB**$_5$ formed in 80 mM CsCl exhibits a signal characteristic of isoguanine aggregation, with a maximum at 309 nm (Figure 1C, red).[14] This signature peak is present in the CD of known pentaplex $(dTiG_4T)_5$ (blue), and the signal is not affected by change of buffer conditions necessitated (see SI section 3) by binding assays with CRP (black).

To examine the effectiveness of the self-assembled iG-pentaplexes as multivalent binders of pentameric CRP, an enzyme-linked immunosorbent assay (ELISA) was employed. Unless otherwise noted, well plates were coated with polyclonal rabbit IgG anti-CRP, blocked with BSA, and loaded with CRP. ODNs were then added in 2-fold dilution, and binding was monitored using HRP-conjugated streptavidin. The resulting dose−response curves are shown in Figure 2A. The binding affinity of pentaplex-assembled PC **1PB**$_5$ to CRP is 3 orders of magnitude greater than that of single-stranded PC ODN **2PB** (K_d = 141 nM vs 50 μM). To demonstrate that the observed binding of **1PB**$_5$ is dependent on the presence of PC, non-PC conjugated pentaplex **1B**$_5$ was assayed and it exhibited no significant affinity for CRP by ELISA. To preclude false signal caused by nonspecific binding of functionalized DNA to ELISA well plates lacking CRP, pentaplex **1PB**$_5$ was screened in ELISA wells that were coated solely with rabbit IgG anti-CRP and blocked with BSA (Figure S2). In this case as well no signal was observed.

Light scattering experiments were conducted to quantify the extent of precipitation caused by solution-phase binding of PC-functionalized pentaplex to CRP.[15] If **1P**$_5$ binding to CRP mimics a physiological function, we would expect precipitation in a solution based assay. Maximum precipitation was observed at a 1.3:1 molar ratio of **1P**$_5$:CRP, giving a characteristic peak followed by a slow decrease in scattering (Figure 2B). Precipitation is only induced by fully assembled substrate. PC in solution, nonfunctionalized pentaplex **1**$_5$, and nonassembling conjugate **2P** do not induce any observable precipitation.

Functional self-assembled oligomers appended with both a binding and a reporting face hold great promise in the area of multivalent protein targeting. Here we have established a functionalized pentameric DNA that exhibits a multivalent biological interaction and has 3 orders of magnitude greater affinity than in a monomeric form. Furthermore, we have validated this interaction using both surface-immobilized and solution-based techniques to quantify affinity and biological mimicry. Further studies include use of noncovalent self-assembled DNA probes for detection of CRP in complex solutions, as well as extending this scaffold to target other multivalent proteins and protein surfaces.

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Supporting Information Available: Complete ref 9; experimental and synthetic details. This material is available free of charge via the Internet at http://pubs.acs.org.

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