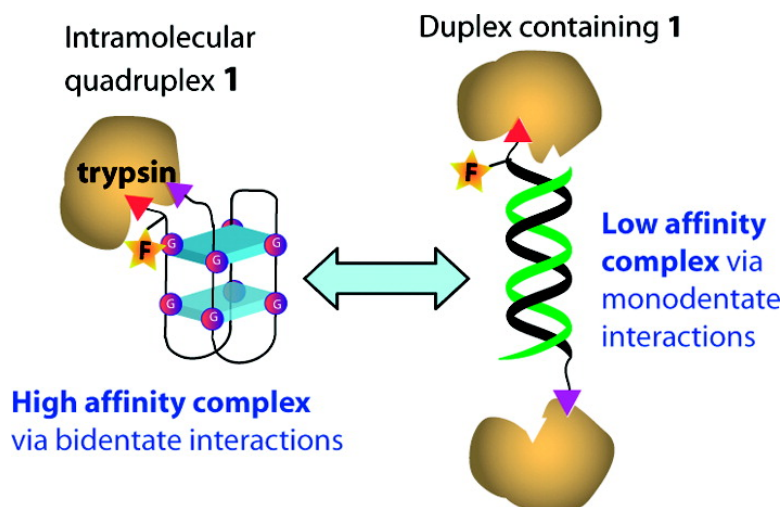# JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

DNA-Small Molecule Chimera with Responsive Protein-Binding Ability

D. Calvin Harris, Xiaozhu Chu, and Janarthanan Jayawickramarajah

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

ACS Publications
High quality. High impact.

DNA-Small Molecule Chimera with Responsive Protein-Binding Ability

D. Calvin Harris, Xiaozhu Chu, and Janarthanan Jayawickramarajah*

Department of Chemistry, Tulane University, 2015 Percival Stern Hall, New Orleans, Louisiana 70118

Received August 21, 2008; E-mail: jananj@tulane.edu

Oligonucleotides (ODNs) tethered to protein-binding small molecules have received much interest,[1] *inter alia*, as a result of the unique self-assembly capabilities of the ODN domains. For instance, the research groups of Neri and Hamilton have independently shown that ODN-linked small molecules can form duplexes that project synthetic protein-binding fragments in a bidentate manner, leading to selective sequestration of protein targets (including serum albumin,[1c] carbonic anhydrase,[1c] trypsin,[1e] and streptavidin[1d]).

Since chelate interactions play a central role in enhancing the affinity of the abovementioned bidentate binders,[1g] we reasoned that a single molecule capable of switching between bidentate and monodentate protein-binding modes (*via* addition of external stimuli) could result in a generalizable strategy for developing agents with regulable protein-binding ability. Herein we demonstrate this proof of concept using a DNA-small molecule chimera (DC) **1** that targets the serine protease trypsin. DC **1** (Scheme 1) reversibly projects bidentate or monodentate synthetic trypsin-binding fragments (*via* an intramolecular quadruplex-to-duplex transition and vice versa)[2] in response to specific ODN stimuli. Moreover, the bidentate conformation exploits chelate interactions, leading to a 20-fold enhancement in trypsin-binding potency (with a 0.8 μM dissociation constant, K_d) versus the monodentate form. Such DCs may have application as responsive pharmacophores with potency that can be modulated after administration,[3] potentially leading to safer therapeutic agents.

The design of DC **1** includes an 18-mer core ODN domain, derived from a sequence (underlined in Scheme 1A),[4] capable of folding into a chair-type quadruplex conformation in the presence of potassium ions. The core domain also incorporates a spectroscopic handle (fluorescein-linked deoxythymidine) on the 5′-terminus for fluorescence polarization (FP) based binding studies. The 5′- and 3′-termini flanking the core domain are functionalized with spacers-linked to the trypsin-binding groups benzamidine (**ba**) and iodophenylthiourea (**ipt**), respectively.

These synthetic fragments were chosen because **ba** is a known active site (S1 pocket) inhibitor of trypsin,[5] and the potency of small molecules containing a **ba** moiety can be dramatically increased by covalent attachment, *via* appropriate linkers, to aromatic hydrophobic elements such as **ipt**.[1e] While not conclusive, literature precedence[5b] and preliminary modeling studies (SI-8) indicate that **ipt** potentially binds to the S4 pocket in a similar manner to other aromatic hydrophobic modules. Based on these putative trypsin-binding pockets, C10 and C6 spacers were chosen as appropriate linkers for DC **1** (Figure 2A). In addition to preparing **1**, control ODNs **2** and **3** lacking the 3′-**ipt** or 5′-**ba** arm, respectively, were synthesized. The double mutant **4** containing neither binding arm was also prepared.

Prior to performing any trypsin-binding studies, circular dichroism (CD) experiments were undertaken to confirm the ability of **1** (and controls **2**−**4**) to fold into an intramolecular quadruplex.[6] Indeed, a characteristic CD profile for an antiparallel quadruplex

Scheme 1[a]
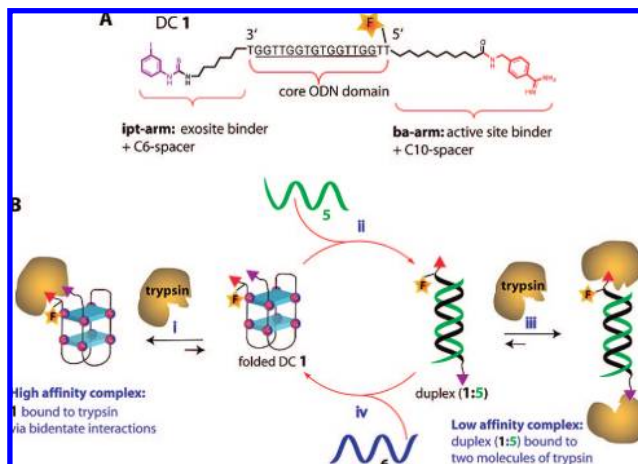
[a] (A) Design of DC **1**. (B) Controlled trypsin-binding through addition of external ODN-derived stimuli.

with a positive ellipticity at 292 nm and a negative ellipticity at 266 nm was observed (SI-4).[7] ODN controls **2**−**4** gave similar profiles.

FP titrations were undertaken to probe whether the folded structure of **1** leads to enhanced trypsin binding as a result of exploiting bidentate interactions (Scheme 1Bi). Hence folded ODNs **1**−**4** were incubated with increasing concentration of trypsin (20 mM KCl, 200 mM NaCl, 25 mM Hepes, pH 7.4) at 25 °C for 30 min. The anisotropy associated with the fluorescein emission was followed at 525 nm (excitation at 495 nm). The resultant binding isotherms (Figure 1) were fitted using nonlinear regression analysis to a 1:1 binding mode. As the inset in Figure 1 indicates, the K_d for bidentate DC **1** ((8.1 ± 1.2) × 10^{-7} M) is substantially tighter (a binding enhancement of 100- and 30-fold over ODNs **3** and **2**, respectively) than that for the monodentate controls. Importantly, the negative control, ODN **4**, which lacks both binding arms displayed only weak binding to trypsin ($K_d = (2.9 \pm 1.8) \times 10^{-3}$ M). Taken together, these results clearly indicate that a bidentate interaction facilitated by the quadruplex fold dramatically enhances the affinity of **1** to trypsin.

In addition to forming a quadruplex structure in the presence of potassium, we hypothesized that incubation of folded **1** with an appropriate complementary strand should result in the formation of a duplex that necessarily projects the **ba** and **ipt** arms on opposite sides (Scheme 1Bii). This conformational switch should significantly decrease the trypsin-binding ability of DC **1**. Thus DC **1** was annealed with 1 equiv of ODN **5** (5′-ACCAACCACAC-CAACCA-3′), capable of forming Watson−Crick base pairs with all except the terminal 5′ (fluorescein containing dT) residue on **1**, followed by incubation with trypsin (Scheme 1Biii). The titration of duplex containing **1** (i.e., **1:5**)[8] resulted in a binding profile that was fitted to the Hill equation.[9] From this fit, a Hill coefficient (h)
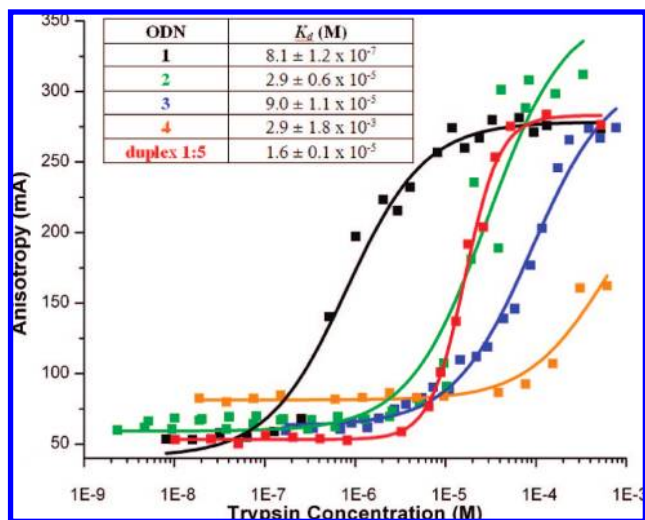
COMMUNICATIONS

Figure 1. Fluorescence anisotropy titrations of ODNs **1** (black), **2** (green), **3** (blue), **4** (orange), and duplex **1:5** (red) in the presence of increasing trypsin concentration in 20 mM KCl, 200 mM NaCl, 25 mM Hepes, pH 7.4. The concentration of all ODNs was 2 nM.
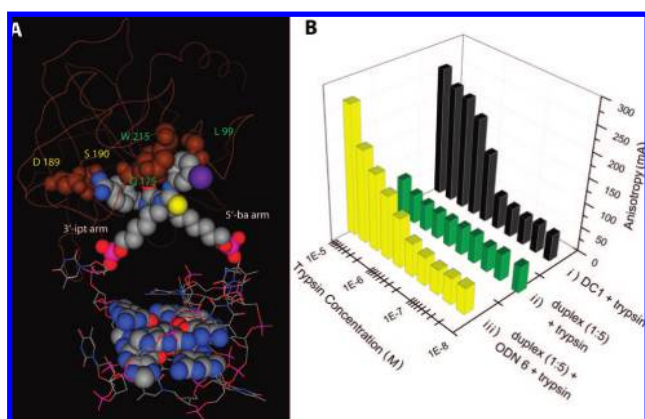

Figure 2. (A) Schematic illustrating possible bidentate interactions between the quadruplex **1** and trypsin (shown in brown). The 5′-**ba** arm and 3′-**ipt** arm (space-filling models) are bound to residues in the trypsin S1 and S4 pockets, respectively (key residues labeled in yellow and green). The two stacked guanine quartets formed by **1** are depicted as space-filling models. The model of **1** bound to trypsin was derived from the X-ray structures of the ODN sequence underlined in Scheme 1A and trypsin (PDB codes: 1HUT and 1f0u). For clarity the fluorescein moiety has been omitted. (B) Bar graphs illustrating the increase in fluorescence anisotropy as trypsin binds to (i) DC **1**, (ii) duplex (**1:5**), and (iii) duplex (**1:5**) incubated with ODN **6**. All binding studies were performed in 20 mM KCl, 200 mM NaCl, 25 mM Hepes, pH 7.4. The concentration of ODNs **1**, **5**, and **6** was 2 nM.

of 2.2 was obtained, a finding that supports the notion that duplex **1:5** binds to two molecules of trypsin (i.e., one binding site on each end of the duplex) with positive cooperativity. Furthermore, the concentration of trypsin that yields half-maximal binding ($[\text{trypsin}]_{1/2}$) was determined to be $(1.6 \pm 0.1) \times 10^{-5}$ M. This value indicates that the quadruplex form of **1** is ca. 20 times more potent in binding to trypsin than the double helical structure.

A further versatility of DC **1** is that the structure-switching mechanism can be reversed (Scheme 1Biv). For instance, incubation of duplex **1:5** with 1 equiv of ODN **6** (5′-TGGTTGGTGTGGT-TGGT-3′), capable of forming a perfect duplex with **5**, followed by incubation in the presence of trypsin, leads to an anisotropy profile (Figure 2B, Bar graph iii) that closely matches the one

obtained for preformed quadruplex **1**. These results clearly suggest that incubation of duplex **1:5** with ODN **6** reverts a significant amount of DC **1** back to the quadruplex form, which in turn, can sequester trypsin in a bidentate fashion.

In conclusion, we have developed a DC that can switch between a folded quadruplex conformation and a duplex form. The former structure exploits bidentate interactions, mediated *via* two designed synthetic molecules, to dramatically enhance trypsin binding. On the other hand, the duplex of **1** presents its binding arms on opposite sides and cannot bind as tightly to trypsin. Since a diversity of bivalent synthetic fragments can be prepared against salient protein targets,[10] and because alternative structure-switching ODNs[11] and stimuli molecules[12] can be readily developed, it is envisioned that such DCs may find application as safe and regulable therapeutic agents.[13]

Acknowledgment. This work was supported by the Tulane Research Enhancement Fund (Grant 546738G1). D.C.H. acknowledges the Louisiana BOR for a graduate fellowship.

Supporting Information Available: Synthesis of ODNs **1**−**4**, MALDI-TOF, CD, FP, FRET, and modeling studies. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Doyon, J. B.; Snyder, T. M.; Liu, D. R. *J. Am. Chem. Soc.* **2003**, *125*, 12372–12373. (b) Saghatelian, A.; Guckian, K. M.; Thayer, D. A.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2003**, *125*, 344–345. For duplex DNA derived bidentate binders see: (c) Melkko, S.; Scheuermann, J.; Dumelin, C. E.; Neri, D. *Nat. Biotechnol.* **2004**, *22*, 568–574. (d) Sprinz, K. I.; Tagore, D. M.; Hamilton, A. D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3908–3911. (e) Melkko, S.; Zhang, Y.; Dumelin, C. E.; Scheuermann, J.; Neri, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 4671–4674. For higher-order tetradentate protein binders, see: (f) Tagore, D. M.; Sprinz, K. I.; Fletcher, S.; Jayawickramarajah, J.; Hamilton, A. D. *Angew. Chem., Int. Ed.* **2007**, *46*, 223–225. (g) Melkko, S.; Dumelin, C. E.; Scheuermann, J.; Neri, D. *Chem. Biol.* **2006**, *13*, 225–231.
(2) For reviews on quadruplexes, see: (a) Davis, J. T. *Angew. Chem., Int. Ed.* **2004**, *43*, 668–698. (b) *Quadruplex Nucleic Acids*; Neidle, S., Balasubramanian, S., Eds.; RSC Publishing: Cambridge, 2006; pp 1−301. For work on quadruplex-based nanomachines, see: (c) Alberti, P.; Bourdoncle, A.; Sacca, B.; Lacroix, L.; Mergny, J.-L. *Org. Biomol. Chem.* **2006**, *4*, 3383–3391. (d) Beissenhirtz, M. K.; Willner, I. *Org. Biomol. Chem.* **2006**, *4*, 3392–3401. (e) Alberti, P.; Mergny, J.-L *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 1569–1573. (f) Li, J. J.; Tan, W. *Nano Lett.* **2002**, *2*, 315–318.
(3) Aptamers with controllable protein-binding activity have been investigated. However, unlike the DNA-synthetic molecule chimeras detailed in this work, these earlier systems are based only on the protein recognition properties of ODNs. See: (a) Dittmer, W. U.; Reuter, A.; Simmel, F. C. *Angew. Chem., Int. Ed.* **2004**, *43*, 3550–3553. (b) Dyke, C. K.; Steinhubl, S. R.; Kleiman, N. S.; Cannon, R. O.; Aberle, L. G.; Lin, M.; Myles, S. K.; Melloni, C.; Harrington, R. A.; Alexander, J. H.; Becker, R. C.; Rusconi, C. P. *Circulation* **2006**, *23*, 2490–2497.
(4) Nagatoishi, S.; Tanaka, Y.; Tsumoto, K. *Biochem. Biophys. Res. Commun.* **2007**, *352*, 812–817.
(5) (a) Talhout, R. T.; Engberts, J. B. F. N. *Eur. J. Biochem.* **2001**, *268*, 1554–1560. (b) Maignan, S.; Guilloteau, J.-P.; Pouzieux, S.; Choi-Sledeski, Y. M.; Becker, M. R.; Klein, S. I.; Ewing, W. R.; Pauls, H. W.; Spada, A. P; Mikol, V. *J. Med. Chem.* **2000**, *43*, 3226–3232.
(6) Formation of an intramolecular quadruplex was achieved by heating in the presence of templating potassium ions (20 mM KCl, 200 mM NaCl, 25 mM Hepes, pH 7.4) at 95 °C for 5 min, followed by cooling to RT.
(7) Rachwal, P. A.; Findlow, I. S.; Werner, J. M.; Brown, T.; Fox, K. R. *Nucleic Acids Res.* **2007**, *35*, 4214–4222.
(8) Evidence for duplex formation came from FRET studies using a fluorescent congener of ODN **5** (SI-7).
(9) Fialcowitz-White, E. J.; Brewer, B. Y.; Ballin, J. D.; Willis, C. D.; Toth, E. A. *J. Biol. Chem.* **2007**, *282*, 20948–20959.
(10) M.; Chessari, G.; Tisi, D.; Woodhead, A. J. *J. Med. Chem.* **2008**, *51*, 3661–3680.
(11) For instance, hairpin-to-duplex transitions could also be used to switch between bidentate and monodentate protein binders.
(12) In theory, alternative DC-stimuli pairs can be developed by using an aptamer as the core domain. For e.g., ODN **1** should also be responsive to thrombin (see: Padmanabhan, K.; Padmanabhan, K. P.; Ferrara, J. D.; Sadler, J. E.; Tulinsky, A. *J. Biol. Chem.* **1993**, *268*, 17651–17654.). Thus, investigations are underway in fine-tuning the activity of DC **1** with thrombin.
(13) The requisite of two binding sites on a protein is a limitation of our approach; development of responsive DCs that target one site is ongoing.

JA806552C

J. AM. CHEM. SOC. ■ VOL. 130, NO. 45, 2008 **14951**