Measurement of Aptamer-Protein Interactions with Back-scattering Interferometry

Ian R. Olmsted,[a] Yi Xiao,[b] Minseon Cho,[b] Andrew Csordas,[b] Jonathan H. Sheehan,[c] Jens Meiler,[a] H. Tom Soh,[b] Darryl J Bornhop[a]

[a]Department of Chemistry and the Vanderbilt Institute for Chemical Biology, Vanderbilt University, 4226 Stevenson Center, Nashville, TN 37235. [b]Materials Department and Department of Mechanical Engineering, University of California, Santa Barbara, CA 93106. [c]Vanderbilt Center for Structural Biology 5137 MRB III 465 21st Avenue South Nashville, TN 37232-8725

Supporting Information

A. PROTEIN-APTAMER STRUCTURAL COMPUTATION

We created a model to understand the allosteric effect of binding by the two aptamers to thrombin. Starting from the 2.9-Å resolution X-ray crystal structure of the Bock-thrombin complex[S1] (1HUT.pdb), we added a model of the Tasset aptamer, following the procedure of Tasset *et al*. 1997.[S2] We built the aptamer structure by modeling the G-quadruplex of Tasset (sequence: AGTCCGTGGTAGGGCAGGTTGGGGTGACT) on the corresponding coordinates from the Bock structure (sequence: GGTTGGTGTGGTTGG). The 11 additional nucleotides of Tasset at the 5' and 3' termini, which do not have corresponding structure in the Bock aptamer, were modeled as a duplex (B-form DNA) and relaxed using 1 nanosecond of annealing in AMBER 10[S3] with the ff99bsc0 force field and the generalized Born continuum solvent model. This structure was then docked to the complex using the cross-linking data, which imply a contact between residue Phe245 and nucleotide T12. The phosphate backbone was positioned to contact the positively-charged surface patch formed by Arg126, Lys236, Lys240 and Arg93. We refined the docking pose using RosettaDock[S4] in all-atom dock_pert mode to determine the lowest-energy configuration.

The model suggests that the binding sites of the two aptamers, which reside at opposite ends of the substrate-binding cleft, are connected by a framework of rigid secondary-structural elements. Previous studies have found evidence for allosteric linkage between exosites I and II using fluorescently-labeled hirudin and a prothrombin fragment[S5]. It is possible that the allostery observed here between the Bock and Tasset aptamers is mediated by the same mechanism, which we postulate to be transmission of motional signals through the rigid structure inside the protein from one exosite to the other [S6].

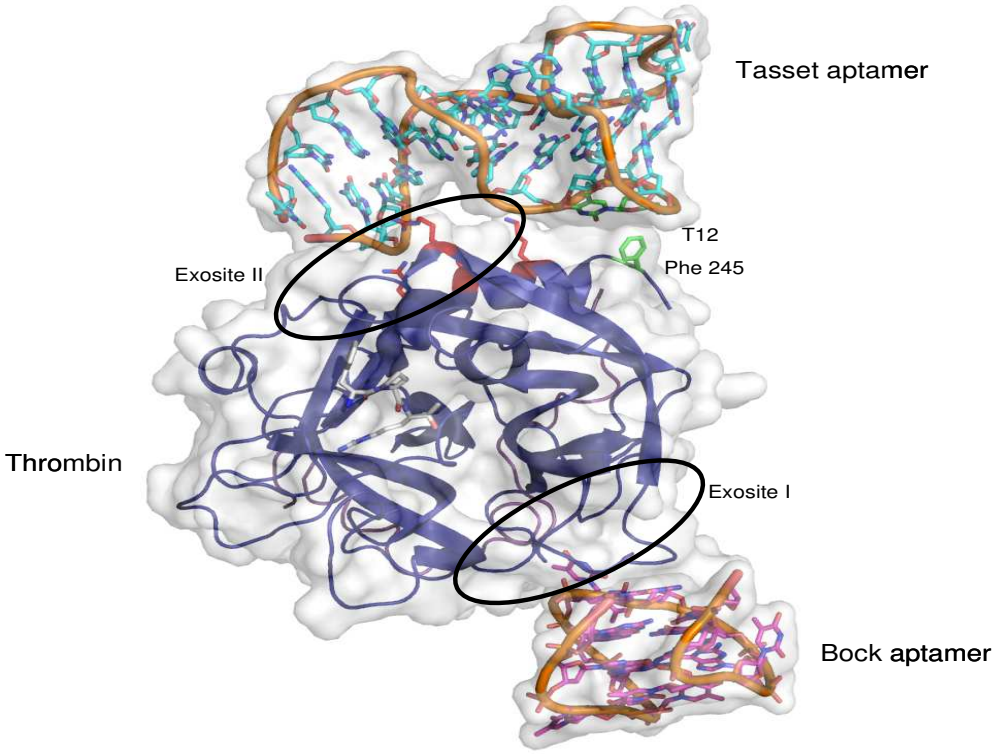

Figure S-1: An illustration for inter-site communication in thrombin. Human α-thrombin is shown with blue ribbons in standard orientation (per Bode 2006)[S6]. The inhibitor PPACK, displayed in gray sticks, occupies the central active-site cleft. Exosites I (fibrinogen recognition exosite) and II (heparin-binding site) are indicated with ovals. The Bock DNA aptamer is shown in magenta at exosite I, using the coordinates of the 2.9-Å resolution Bock-thrombin complex from Padmanabhan et al. 1993 (1HUT.pdb)[S1]. A model of the Tasset DNA aptamer is shown in cyan, positioned according to Tasset et al. 1997 to maintain a contact between the cross-linked residue Phe245 with nucleotide T12 (highlighted in green) [S2], and to appose the phosphate backbone with four positively-charged surface residues (in red) near exosite II.

(S1) Padmanabhan K. et al. *J Biol Chem*. **1993**. 268 (24) 17651-17654.
(S2) Tasset, D.M. et al. *J Mol Biol*. **1997.** 272 (5) 688-698.
(S3) Case, D.A. et al. **2008**. AMBER 10, University of California, San Francisco.
(S4) Gray, J.J. et al. *Proteins*. **2003.** 52 (1) 118-122.
(S5) Fredenburgh, J.C. et al. *J Biol Chem*. **1997.** 272 (41) 25493-25499.
(S6) Bode, W. *Blood Cells Mol. Dis*. **2006.** 36 (2) 122-130.