A Crystallographic Approach for Understanding the Recognition Mechanism of Thrombin and G-quadruplex Aptamers

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Human-thrombin, a serine protease that maintains blood hemostasis by balancing pro- and anti-coagulant actions is an example of protein with multiple binding sites[1]. In addition to the active site, the enzyme possesses two electropositive regions, in near-opposition on the protein surface, known as exosite I and exosite II, respectively. These two regions have a primary role in the regulation of enzymatic activity since they can bind molecules with diverse functions[2-4]. Given its central role in the clot formation, thrombin is an attractive target for the development of agents that effectively interfere with thrombogenesis. A special class of thrombin synthetic ligands is represented by nucleic acid aptamers adopting G-quadruplex structures. HD1, a 15-mer oligonucleotide recognizing exosite I[5], and HD22, a 29-mer binding exosite II[6], are the most studied thrombin binding aptamers and show high affinity toward their target (K_d (HD1)≈ 100 nM; K_d(HD22) ≈0.7 nM). The increased interest in the use of DNA aptamers as drugs has stimulated the search of HD1 and HD22 variants with improved properties. In particular, the bimodular oligonucleotides RE31[7] and NU172[8], which have been obtained by addition of a duplex motif to the HD1 quadruplex module, show higher affinity for thrombin and anticoagulant activity, and a slower disappearance rate in human plasma in comparison with HD1.
Here I will present the most relevant results regarding the elucidation of the interactions, which govern the recognition between thrombin and DNA G-quadruplex aptamers[9-14].

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