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Unusual binding ability of anticoagulant aptamers revealed by the crystallographic analysis of an engineered pseudo-cyclic TBA in complex with α-thrombin

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Since the development of the SELEX procedure [1,2], thrombin binding aptamers are among the most studied oligonucleotides with either therapeutics or diagnostics properties [3]. In particular, aptamers (as TBA [4] or NU172 [5]) able to compete with the protein substrate for the binding with exosite I have been or are currently being evaluated in clinical trials for their extraordinary anticoagulant activity. Other aptamers (as HD22 [6] or Toggle-25t [7]) that recognize with high affinity the heparin-binding site (exosite II) of thrombin have been extensively examined to develop effective diagnostic tools.
Despite the unquestionable properties of these aptamers, some drawbacks continue to hinder their applications encouraging various functionalization strategies to overcome them [8]. In this context, a *pseudo*-cyclic TBA analogue (named TBA-NNp/DDp), containing two naphthalene diimides and two dialkoxynaphthalene groups respectively at 5'- and 3'-ends of the 5'-GGTTGGTGTGGTTGG-3' sequence, was recently selected by some of us [9]. TBA-NNp/DDp exhibited a considerable improvement of the thermal stability and nuclease resistance, coupled with a moderate increase in anticoagulant activity with respect to the unmodified TBA [9].
To obtain a molecular view of the effects of these modifications on aptamers, we solved the crystal structure of this new engineered aptamer in complex with thrombin. Surprisingly, three of the four examined crystallographic structures are ternary complexes in which thrombin binds a TBA-NNp/DDp molecule at exosite II as well as at exosite I, highlighting the ability of this aptamer, differently from unmodified TBA, to also recognize a localized region of exosite II. Studies were also performed in solution to examine the properties of TBA-NNp/DDp in a crystal-free environment. Details will be discussed at the Meeting.

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