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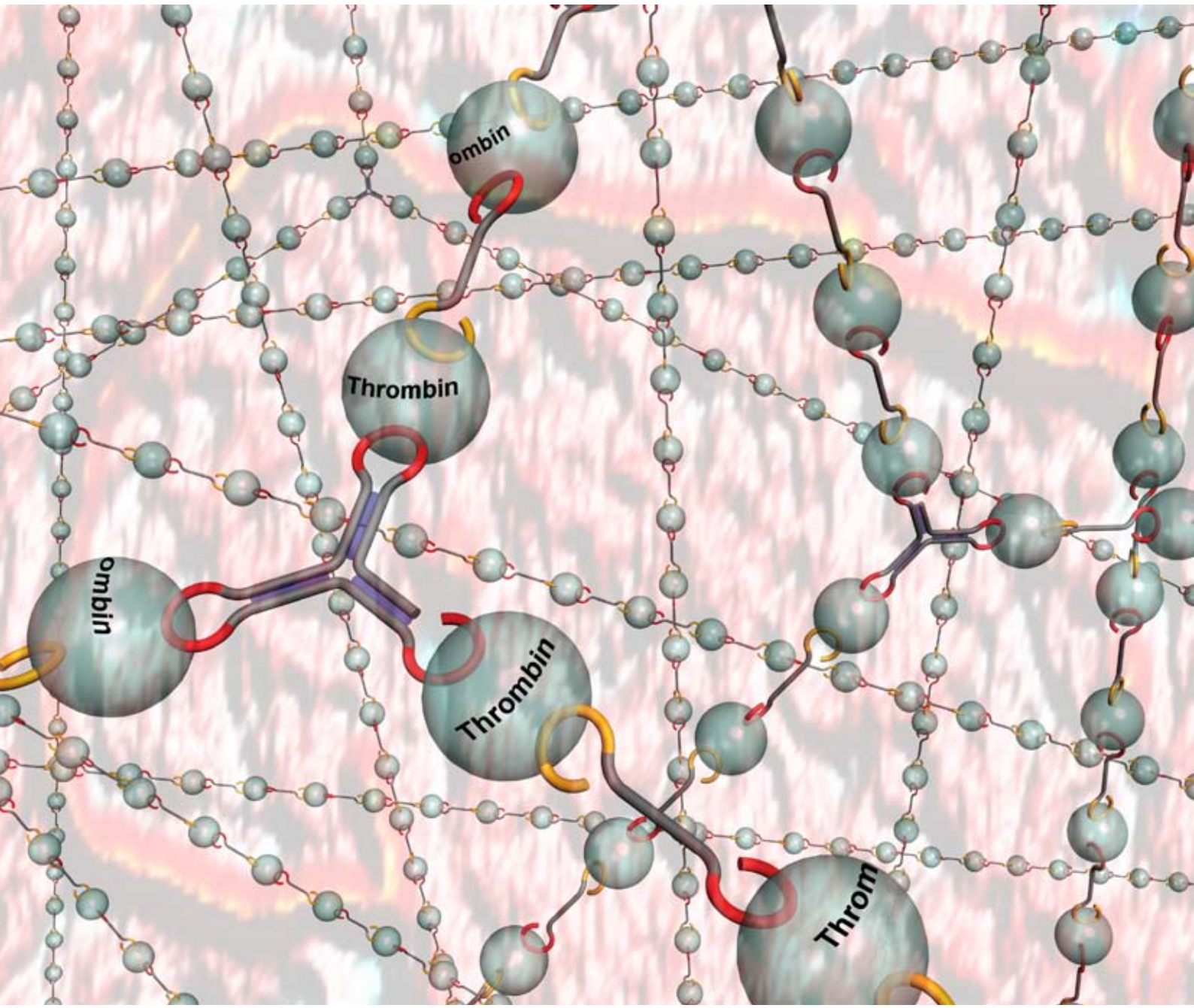

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Supramolecular aptamer–thrombin linear and branched nanostructures

Supramolecular aptamer–thrombin linear and branched nanostructures†

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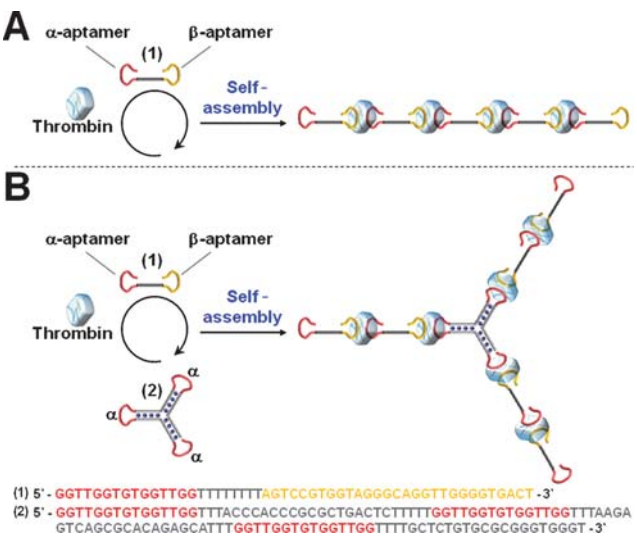
α and β conjugated bis-aptamers against thrombin act as bidentate "glue" for the self-assembly of thrombin nanowires; mixing the bidentate aptamer with a tripodal tridentate α aptamer construct yields branched thrombin nanowire structures.

The interplay between covalent and non-covalent bonding in supramolecular polymers is the source of the architectural, structural and functional diversity in these macromolecules.[1] One important paradigm for the synthesis of supramolecular polymer nanostructures involves the use of polydentate macromonomers that include complementary binding interactions. The reversible, supramolecular binding of complementary macro-monomer units that include oppositely positioned association sites yields, then, linear polymers.[2] Similarly, the use of macro-monomers with three or more supramolecular binding sites may lead to branched two- or three-dimensional nanostructures.[3] Hydrogen bonding,[4] π–π interactions,[5] host–guest interactions,[6] hydrophilic–hydrophobic interactions,[7] nucleobase pairing,[8] and metal–ligand coordination[9] have been employed as driving forces for the self-assembly of polymer nanostructures. Among these binding motifs, the metal–ligand coordination is unique, as the metal complexes act as "glues" for identical ligand-functionalized macromonomers, rather than complementary interactions persisting in the other organized polymers that are brought together by complementary macromonomers. Among the supramolecular polymer nanostructures, DNA-based assemblies organized by dictated, complementary oligonucleotide sequences,[10] protein–protein nanostructures,[11] and self-organized nucleic acid–protein architectures[12] attract substantial recent research efforts. Such self-assembled biomolecule-based supramolecular polymers hold great promise in the field of nanotechnology or nanomedicine, and the use of the polymers as templates for the fabrication of nanodevices, drug delivery or therapeutic systems was suggested.

Aptamers are sequence-specific nucleic acids, selected from a pool of 10^{15}–10^{16} oligonucleotides, and they reveal selective binding to low-molecular-weight substrates or macromolecules such as proteins.[13] For example, two aptamers that bind to two different domains on thrombin, were discovered through library screening.[14] In the present study we demonstrate that predesigned aptamer–oligonucleotide macro-monomers act as "glues" for the synthesis of linear or branched protein (thrombin) nanostructures.

Scheme 1(A) depicts the method used to synthesize linear DNA–thrombin nanostructures. The nucleic acid, **1**, includes at its two ends the two aptamers that bind to thrombin, α and β. This bifunctional oligonucleotide yields, in the presence of thrombin, a supramolecular nanowire formed by the polymeric association of the aptamer units to thrombin units with a 1 : 2 stoichiometry. Fig. 1(A) shows the AFM image of the resulting aptamer-bridged protein wires. Linear nanowires with lengths in the range of 80 to 450 nm were observed. The cross section of wire #1, Fig. 1(B), indicates that the height of the nanowire is *ca.* 2.2 nm, in agreement with the height of other thrombin–DNA nanostructures.[15,16] The method to design branched aptamer-bridged thrombin nanowires is shown in Scheme 1(B). A tridentate aptamer linking nucleic acid was designed, such that a component of the tripodal nucleic acid "glue" consisted of three aptamers of composition α, **2**. The tridentate (**2**) was mixed with **1**, and thrombin at a ratio of 1 : 5 : 5 that corresponded to 5, 50 and 50 nM, respectively (see details in ESI†). A branched supramolecular thrombin nanostructure is anticipated to be formed, where **2** acts as a branching site. Fig. 2(A) shows the AFM image of the resulting branched nanowires. In addition to the linear nanowires that are formed (for example, nanowire #1 that is *ca.* 350 nm long), branched thrombin nanostructures



Scheme 1 Self-assembly of the supramolecular aptamer–thrombin linear or branched nanostructures.

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† Electronic supplementary information (ESI) available: Experimental protocols for the supramolecular nanostructures, and confocal image of the nanowires bundles. See DOI: 10.1039/b812486h

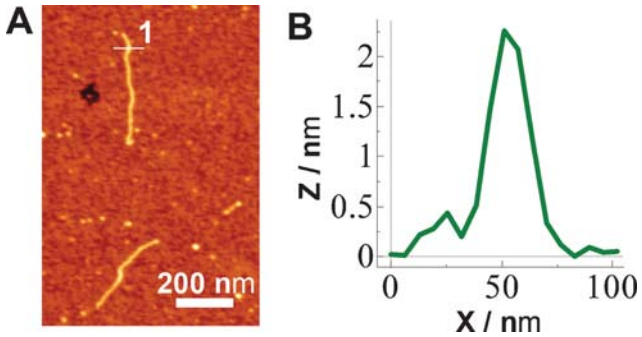


Fig. 1 (A) AFM image of the linear supramolecular thrombin nanostructures. (B) The height profile analysis of wire #1.

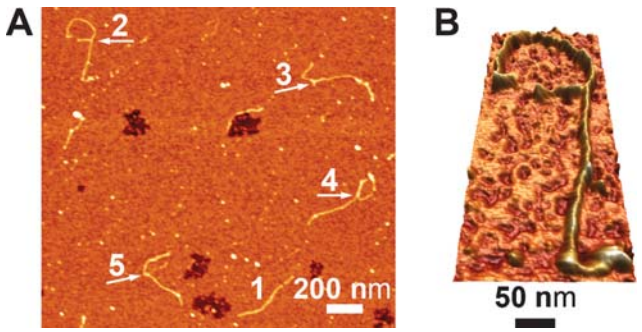
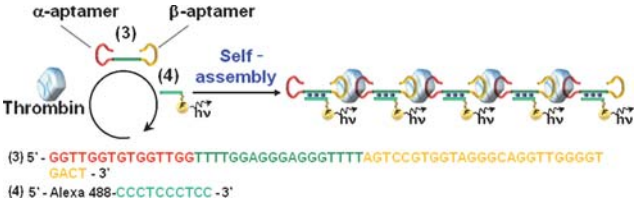


Fig. 2 (A) AFM image of a branched supramolecular thrombin nanostructures. (B) Zoom view of structure #2 from A.

are predominantly observed. The branching points that result from the inclusion of **2** are marked with arrows for different wires. Fig. 2(B) shows the enlarged image of the branched thrombin nanowire #2. Three nanowires with lengths that correspond to *ca.* 375, 250 and 80 nm originated from the branching site generated by **2**. The height of each of these branched thrombin nanowires is 2.0–2.2 nm, consistent with the formation of the supramolecular thrombin–aptamer nanostructure.

Further evidence for the formation of the polymer nanostructures was obtained by optical imaging. Towards this end, we modified thrombin with 5-carboxyfluorescein *N*-succinimidyl ester (FAM) and attempted to induce the formation of the nanowires by bridging the labeled thrombin with (**1**). This experiment failed, however, and no wire formation could be detected either by AFM or confocal microscopy. Presumably, the modification of thrombin with the dye perturbs the protein structure and prohibits the supramolecular aptamer-induced polymerization of thrombin. In further experiments, we attempted to hybridize a FAM-labeled nucleic acid to the single-stranded nucleic acid region linking the α/β aptamer units of (**1**) that bridges the thrombin units. Similarly, this experiment was unsuccessful, and no dye-labeled supramolecular thrombin nanostructures could be imaged. We assumed that the short nucleic acid chain linking the α and β aptamer units and the resultant steric crowding generated by the protein units prevents the hybridization of the labeled nucleic acid. Accordingly, we examined the possible generation of the linear thrombin nanowires by the "glue", **3**, that includes a chain consisting of 18 bases that links



Scheme 2 Self-assembly of the supramolecular aptamer–thrombin linear fluorescent nanostructures.

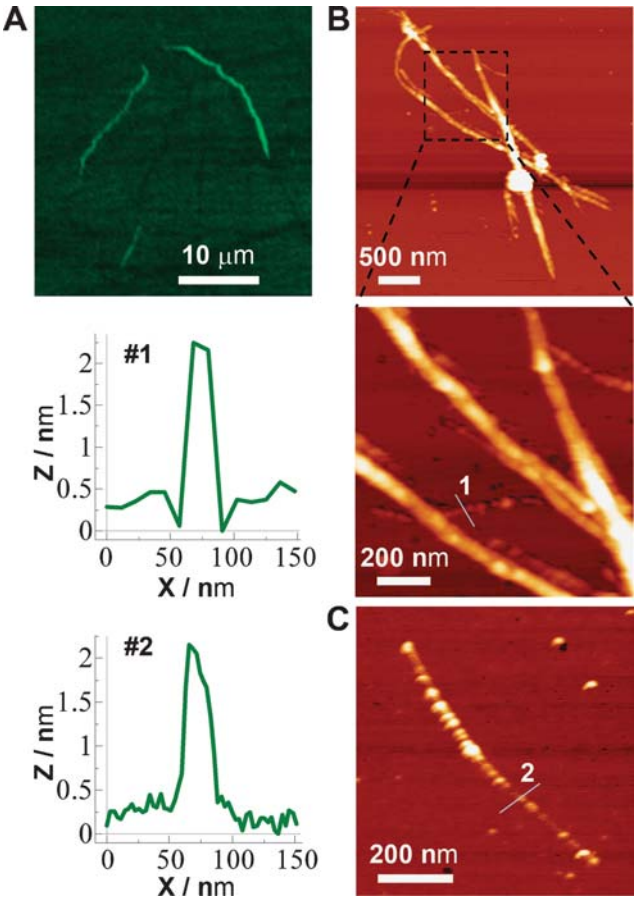


Fig. 3 (A) Confocal image of linear supramolecular thrombin nanostructures. (B, C) AFM images of linear thrombin nanostructures in the form of bundles or single wires. Profile height of wires #1 and #2 shown at the left.

the α and β aptamer sites, Scheme 2. We tried to image optically the formation of the resulting structures by the hybridization of the Alexa 488-labeled nucleic acid, **4**, that is complementary to part of the chain that links the α and β aptamer units, Scheme 2. Fig. 3(A) shows the confocal microscopy image of the resulting nanostructures. Wires as long as 20 μm were observed. Complementary AFM images confirmed the formation of the bis-aptamer-bridged nanostructures. Although many of the nanowires appear as bundles, Fig. 3(B), individual nanowires are also detected (for example, the nanowires #1 and #2 exhibit heights that correspond to *ca.* 2 nm). The AFM image of a *ca.* 1.2 μm long thrombin–aptamer nanowire is shown in Fig. 3(C).

In conclusion, the present study demonstrates a new recognition motif to assemble supramolecular bionanostructures by the

bridging of protein units (thrombin) with aptameric oligonucleotides. Additionally, the strategy employed is modular and will lead to diverse architectures and properties by modifying the length and composition of the oligonucleotide segments.

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