Chemical Tags Mediate the Orthogonal Self-Assembly of DNA Duplexes into Supramolecular Structures**

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Self-assembly is an important functional property of biological polymers, as illustrated by naturally occurring higher-order structures of proteins or nucleic acids[1–7] and artificial DNA and peptide assemblies.[8–15] In light of potential applications in nano-biotechnology, materials science[16,17] and synthetic biology,[15,16] there is great interest in approaches that are able to rationally engineer self-assembly properties into otherwise monomeric biopolymers. For polypeptides, self-assembly has been imparted via the engineering of protein surfaces,[18] the fusion of protein recognition modules,[19,20] or the bottom-up design of linear peptides.[21] In the case of DNA, self-assembly is attained by selection of complementary sequences[22] or the use of ligand–protein receptors.[23] Here, we develop a new generic chemical approach which achieves self-assembly independent of the re-engineering of the structural framework of the subunits, and which does not require bulky protein-based recognition modules. The flexible approach uses small chemical tags that are covalently attached to a single point within the subunits. The tags form reversible yet tight metal chelate complexes and thereby confer the ability to form designed supramolecular structures. Our approach is exemplified by the creation of nanoarrays of DNA duplexes, which are examined via atomic force microscopy (AFM) at the single-molecule level. The chemical assembly strategy is orthogonal to the innate ability of DNA strands to hybridize and may also be extended to create functional nanoarrays of proteins, which would not otherwise assemble.

Our strategy to induce self-assembly in biomolecules is based on the two chemical tags bisNTA and His$_6$, which are able to form a metal chelate complex (Figure 1). In the bisNTA–Ni$^{2+}$–His$_6$ complex,[24] two nitrilotriacetic acid (NTA) groups are bonded via two Ni$^{2+}$ ions to the imidazole moieties of a hexahistidine peptide (Figure 1). Multivalent chelator heads such as bisNTA have been developed by Tinazli et al. and Lata et al. to bind with a dissociation constant, $K_{d,o}$ of $2.7 \times 10^{-7}$ M to His$_6$.[24,25] In addition to high affinity, a linkage via bisNTA–Ni$^{2+}$–His$_6$ features several other beneficial properties useful in the design of supramolecular structures. These include controlled reversibility of formation by adding or removing Ni$^{2+}$, and directional binding as a bisNTA tag can only interact with His$_6$. Reflecting these advantages, our aim was to attach the two tags to single base positions within a DNA duplexes (Figure 1). As the affinity tags are positioned at

![Chemical structure of the bisNTA-Ni$^{2+}$-His$_6$ complex](image-url)
opposite sites of the duplex, we anticipated the assembly into nanoarrays composed of parallel aligned duplexes (Figure 2). Furthermore, the formation of a nanoarray was expected to be triggered by addition of Ni\(^{2+}\), which holds together the metal-chelate bridge (Figure 2).

Prior to generating DNA nanoarrays, a proof-of-principle experiment was conducted to demonstrate that two chemically tagged duplexes interact tightly via metal-chelate linkages. Two duplexes, 42-H and 63B-N (Figure 3A-1), were used for this experiment. The first duplex is 42 bp long (bp = base pair) and carries two His\(_6\) tags, while the second, 63-bp-long duplex is equipped with three bisNTA groups (Figure 3A-1). Based on the rational design, His\(_6\) and bisNTA tags are positioned on one side of the duplexes in order to facilitate the creation of heterodimers in the presence of Ni\(^{2+}\) (Figure 3A-1). Two duplexes of different length were selected to facilitate their gel-based discrimination in a gel-assay (see further below). Duplex 42-H was generated by hybridizing a 42-nt-long (nt = nucleotide) single-stranded DNA (ssDNA) oligonucleotide to two 21-mer strands carrying each a His\(_6\) peptide (Figure 3A-1). The His\(_6\)-tagged DNA strand was obtained by coupling a cysteine-terminated peptide to a pyridyl disulfide group at an internal base position (Supporting Information). Similarly, duplex 63B-N was prepared by association of a 63-nt-long oligonucleotide and three 21-mers carrying a bisNTA tag (Figure 3A-1). The chemical attachment was achieved by coupling the synthesized maleimide-functionalized bisNTA to an internal thiol group of the ssDNA (Supporting Information).

The successful synthesis of the single-stranded bioconjugates was confirmed by ion-exchange chromatography and mass spectrometry. Further analysis by gel electrophoresis provided evidence that duplexes 42-H and 63B-N had been generated (Supporting Information).

The proof-of-principle experiment on the formation of a metal-chelate-bridged dimer of duplexes is schematically summarized in Figure 3A-1. In the assay, 42-H and 63B-N form a heterodimer that can bind via a biotin moiety at the terminus of 63B-N to streptavidin-coated beads. Given that capturing by the beads depletes 42-H from the solution, the absence of this duplex was used as a read-out for the successful formation of the dimer. The pull-down assay with beads was performed by first confirming the ability of streptavidin to capture a biotinylated DNA duplex. As shown by gel electrophoresis analysis of the cleared supernatant, biotinylated duplex 63B was depleted due to capture by the beads (Figure 3B, lane 2; corresponding to Figure 3A-1), while non-biotinylated duplex 63 migrated as a defined band (Figure 3B, lane 3; corresponding to Figure 3A-1). Gel electrophoretic analysis also demonstrated that the 63-mer and 42-mer duplex do not interact in the absence of bisNTA and His\(_6\) tags because the nontagged 42 duplex migrated in the gel (Figure 3B, lane 2). By contrast, when the fully tagged duplexes 63B-N and 42-H were incubated with beads in the presence of Ni\(^{2+}\), the intensity of the 42-H band was reduced by over 95% (Figure 3B, lane 1) suggesting successful formation of a heterodimer via two bisNTA-Ni\(^{2+}\)-His\(_6\) complexes (Figure 3A-1). The weakened band of remaining 42-H (Figure 3B, lane 1) was slightly upshifted compared to the other bands of duplex 42 in lanes 2 and 3 because the former is modified and of higher mass. Additional gel analysis in the absence of Ni\(^{2+}\) confirmed that the dimerization of duplexes 42-H and 63B-N was specific for the formation of a metal-chelate complex (Supporting Information).

The convincing results of the pull-down assay prompted the construction of larger supramolecular structures as illustrated in Figure 2. For the design of the nanoarray, two different DNA duplexes 50-N and 50-H were generated. The two 50-mers contain either four bisNTA or four His\(_6\) tags, respectively (Figure 2). The duplexes were obtained from smaller units in a multistep process. The procedure involved coupling the
chemical tags to 25-nt-long ssDNA oligonucleotides, followed by the ligation of each two 25-mers to 50-mer strands carrying two chemical tags, and the subsequent hybridization of two complementary strands to yield duplexes with four tags each (Supporting Information). Chemical analysis by MS and chromatography confirmed the successful coupling of the tags to the single-stranded DNA, as well as their ligation into 50-mer strands. The hybridization to form the corresponding duplexes was checked by gel electrophoresis (Supporting Information) and thermal melting-point analysis. The melting temperature ($T_m$) of the modified duplexes was slightly lower (75 °C) than the $T_m$ of unmodified duplex (79 °C) suggesting that the tags only weakly affected the stability of the duplex. An important feature in the rational design of the modified duplexes is that all tags within a duplex are aligned within a plane to facilitate assembly into a planar nanoarrays (Figure 2).

The formation of the ordered DNA structure was investigated by dynamic AFM. DNA was assembled by incubating a 1:1 mixture of 50-H and 50-N in Ni$^{2+}$ containing buffer on mica surfaces, followed by removal of unbound DNA by rinsing with buffer. The incubated mica surface (Figure 4) showed $\approx 100$-nm-long features, which were parallel aligned into regular 2D sheet. The height of the elongated lines (Figure 4) was 1.35 ± 0.15 nm similar to the diameter of DNA duplexes, as found in other AFM studies.[8–12] Furthermore, the center-to-center distance between the parallel DNA strands was $3.0 \pm 0.2$ nm (Figure 4). After accounting for the diameter of the DNA duplex, this distance is consistent with the expected length of the linkers between DNA and the bisNTA and His$_6$ tags (1.3 nm when fully extended, time-average end-to-end distance of 0.8 nm deduced from a model linker molecule).[26]

In line with assembly supported by metal-chelate bridges, no nanostructures were observed in the absence of Ni$^{2+}$ or with DNA duplexes lacking the chemical tags (Supporting Information). Taken together, these data strongly support the interpretation of the parallel structures as a nanoarray composed of interlinked DNA duplexes as schematically illustrated in the inset of Figure 4. Due to the multiple metal-chelate bridges between the DNA strands, it is very likely that the combined affinity of the interaction is tighter than the individual bisNTA-Ni$^{2+}$-His$_6$ bridge. Based on the latter’s $K_d$ of $2.7 \times 10^{-7}$ M$^{24,25}$ and the known binding enhancement of other multivalent interactions[27] the $K_d$ for the combined interaction should be at least two orders of magnitude lower than the monomeric value.

In conclusion, we have employed covalently attached His$_6$ and bisNTA tags to endow DNA duplexes with the ability to self-assemble via reversible chelate bonds. The work extends our research interest to expand nanobiotechnology with chemical tags.[28,29] The chemical assembly strategy is orthogonal to the innate ability of DNA strands to hybridize and thereby provides a route to achieve tunable self-assembly caused by chemical triggers. In addition, the novel approach towards nanoscale biomolecular entities is of relevance for peptide and protein arrays as it does not require redesigning the structural framework of the constituent polypeptides. Based on its modularity, the chemical strategy may be applied to create functional protein or peptide nanoarrays form otherwise non-assembling subunits.

**Experimental Section**

Experimental procedures are described in the Supporting Information with details on the synthesis of bisNTA and of the His$_6$-peptide, the coupling of the tags to the DNA, the formation of the DNA 42- and 63-bp duplexes, the use of the duplexes for the metal-chelate pull-down assay, the design and preparation of His$_6$- and bisNTA-tagged DNA duplexes 50-N and 50-H for the formation of a self-assembled nanoarrays, melting point analysis of 50-N and 50-H duplexes, the self-assembly of 50-N and 50-H into nanoarrays, and their analysis via AFM.

**Keywords:** nanobiotechnology · DNA · peptides · chemical tagging · atomic force microscopy

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