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DNA Architectures

Bifunctional DNA Architectonics: Three-Way Junctions with Sticky Perylene Bisimide Caps and a Central Metal Lock

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Abstract: A new type of a bifunctional DNA architecture based on a three way junction is developed that combines the structural motif of sticky perylene bisimide caps with a tris-bipyridyl metal ion lock in the center part. A clear stabilizing effect was observed in the presence of Fe^{3+}, Ni^{2+} and Zn^{2+} by the formation of corresponding bipyridyl complexes in the branching part of the DNA three way junctions. The dimerization of the 5′-terminally attached perylene diimides (PDI) chromophores by hydrophobic interactions can be followed by significant changes in the UV/Vis absorption and steady-state fluorescence. The PDI-mediated DNA assembly occurs at temperatures below the melting temperature and is not influenced by the metal-ion bipyridyl locks in the central part. The corresponding AFM images revealed the formation of higher-ordered structures as the result of DNA assemblies mediated by the PDI interactions.

The double-helical DNA attracts an increasing interest as a unique structural scaffold for supramolecular architectures,[1] origami,[2] nanosized objects[3] and molecular devices.[4] DNA architectonics are consideed to be the next generation of bioinspired materials.[5] The predictable geometry, the self-assembly properties and the well-ordered structure provide the basis for such highly programmable structural architectures. The conjugation with artificial functionalities would make DNA an even more attractive structural tool.[6–9] In the bottom-up approach artificial functionalities can be introduced synthetically into DNA-based architectures by providing the corresponding artificial DNA building blocks and/or by postsynthetic modification strategies.[10] The combination of DNA architectures with synthetically introduced functionalities currently opens up a highly promising research field for the future.

Until now, mainly organic chromophores[6,8] and metal–ligand complexes[7,9] have been introduced in great variety as artificial functionalities into DNA architectonics.[6,10] This is not surprising since chromophore–chromophore interactions and metal ion–ligand interactions provide important and (in the chemical sense) orthogonal non-covalent binding motifs that can be added to the standard Watson–Crick-type hydrogen-bonding of natural DNA base pairs and hence to non-modified DNA architectures.

Among the different types of organic chromophores, perylene diimides (PDIs) have a significant potential for DNA architectures since it is known that PDIs undergo π-stacking interactions with themselves and DNA bases.[11] Moreover, their chemical resistance, unique fluorescence and self-assembling as well as electronic properties provide a good basis for the construction of supramolecular architectures with DNA.[12–16] We established a synthetic protocol to incorporate PDIs as artificial DNA base surrogates at internal positions or as 5′-terminal caps of oligonucleotides.[17] Based on this synthetic access to PDI–DNA conjugates we studied PDI–PDI aggregation inside and on top of DNA double helices.

DNA three way junctions represent a thoroughly studied type of branching structure for DNA origami[2] and DNA nano-objects.[3] In the sense of DNA architectonics we further developed these constructs by PDIs that are placed as caps on top of DNA three way junctions and thereby serve as "sticky" ends.[18] That means that the PDI caps assemble the DNA three way junctions to larger structures by stacking interactions which occurs spontaneously, reversibly, without any enzymatic ligation process and without the use of overhanging DNA as conventional sticky ends. It is important to mention here that PDI dimerization does not occur in the DNA single strands, hence the assemblies can be destroyed by thermal dehybridization and reassembled by reannealing of the three way junction as the underlying DNA scaffold. Herein, we present the development of new bifunctional DNA architectures based on three way junctions that combine the orthogonal structural motif of sticky perylene bisimide caps with a metal lock in the center part. Thereby, not only the thermal stability of the three-way junctions could be increased but also the PDI-mediated DNA aggregate gets equipped with metal ions in distinct positions as a structural prerequisite for future applications of DNA-based materials (e.g., electron transfer).

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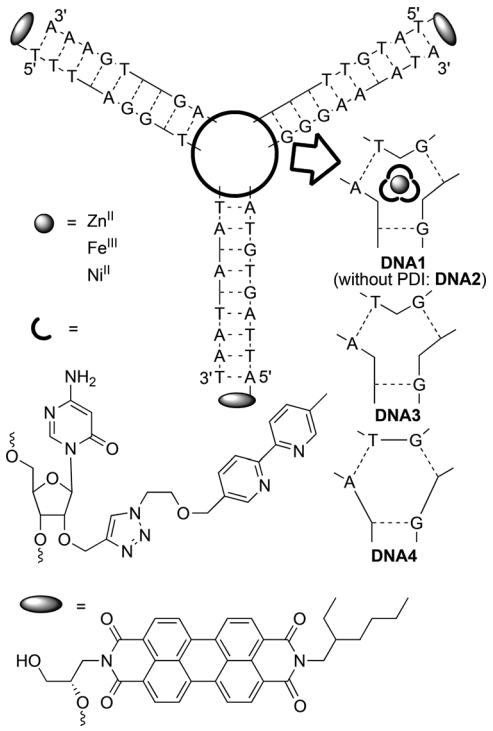
Accordingly to our published examples[18] we designed a bifunctional DNA three way junction (**DNA1**) containing three oligonucleotides; each bear a 5′-terminal PDI chromophore and a cytidine derivate in the middle of the sequence which is additionally modified at the 2′-position by a bipyridyl ligand (Scheme 1). The corresponding DNA building block of the 2′-propargylated cytidine is commercially available and the synthetic azide-modified bipyridyl precursor has been attached to the oligonucleotides through a postsynthetic click-type acetylene–azide cycloaddition (see Supporting Information). The PDI modification of the triangular DNA should induce the formation of larger DNA assemblies which can be followed by optical spectroscopy. The three bipyridyl ligands (bpy) in the center should allow placing an octahedral coordinated metal ion into the branching part of the DNA construct. The other three way junctions serve as control constructs to evaluate influences of the two different functional modifications on each other. **DNA2** lacks the 5′-PDI chromophore whereas **DNA3** contains unmodified cytidines in the middle. **DNA4** completely lacks the cytidines in the center part.

Based on published results[19] it was assumed that the coordination of metal ions that preferentially form tris-bipyridyl octahedral complexes yield an increase of thermal stability of the PDI-capped three way junction **DNA1**. Accordingly **DNA1** and, for comparison, **DNA2** were "locked" by Fe^{3+}, Zn^{2+} and Ni^{2+} ions ($M^{3+/2+}$). Measurements in the presence of excess EDTA serve as reference. The formation of the $[M(bpy)_3]^{3+/2+}$ complexes can primarily be followed by the increasing absorption band at approximately 308 nm. After addition of 1.1 equiv Zn^{2+} (relative to the DNA concentration in the sample) and of 1.1 equiv Ni^{2+}, respectively, the absorption band reached a saturation plateau. At this metal ion concentration, a significant positive effect on the thermal stability of **DNA1** was observed (Figure 1 left, and melting temperatures T_m in Table 1). The stabilization by Ni^{2+} is higher ($\Delta T_m = 10.0\,^{\circ}C$) than that of the weaker binding metal ion Zn^{2+} ($\Delta T_m = 7.2\,^{\circ}C$). Both hybridizations of the three single oligonucleotides to the metal ion-locked three way junctions are fully reversible. In case of Fe^{3+} the melting curve has shown a two-step transition in the presence of only 1.1 equiv of metal ion. This indicates the presence of two different species, probably the metal-free and the more stable metal-containing three way junction. Careful absorption band analysis during the titration of **DNA1** with Fe^{3+} revealed that saturation was observed only after addition of 6.0 equiv (Figure 1 right). And in fact, the T_m measurement of **DNA1** in the presence of 6.0 equiv Fe^{3+} shows a single transition and gave a stabilizing effect ΔT_m of 8.3 °C. In contrast to Zn^{2+} and Ni^{2+}, however, the annealing is not reversible after heating up the sample again to 90 °C.



Scheme 1. Bifunctional three way junction **DNA1** and the three control constructs **DNA2**-**DNA4**, structure of the bipyridyl 2′-modification and the 5′-PDI-modification cap.

In comparison to **DNA1**, **DNA2** that lacks the PDI caps shows a similar stabilizing tendency (especially with Fe^{3+} and Zn^{2+}) which indicates that the PDI modifications as caps have

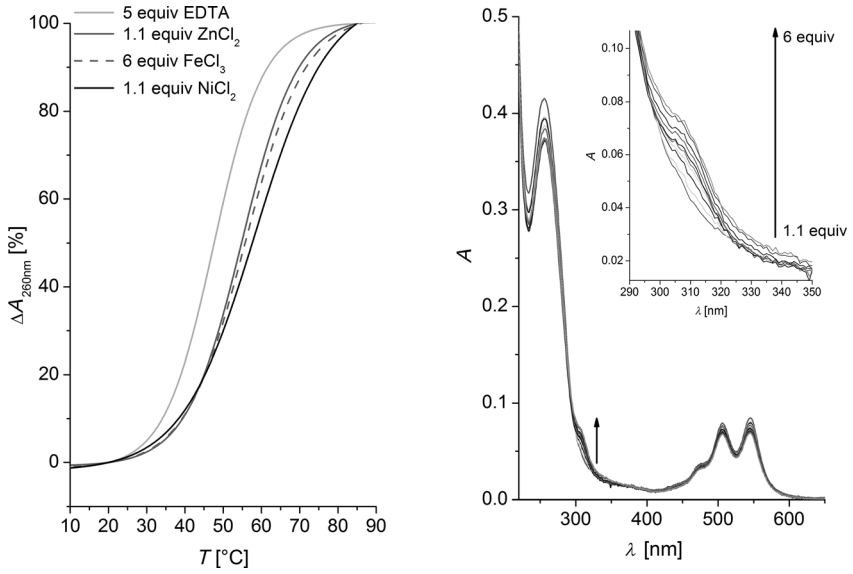


Figure 1. Optical measurements of **DNA1** (2.5 μM) in NaP_i buffer (10 mM, pH 7) with 250 mM NaCl. Left) Denaturation curves of **DNA1** in the presence of EDTA, Zn^{2+}, Fe^{3+} and Ni^{2+}. Right) Representative UV/Vis absorption spectra of **DNA1** demonstrating the increasing absorption during the titration with Fe^{3+}.

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Table 1. Melting temperatures (T_m) of **DNA1**-**DNA4**.

Metal ion	DNA1		DNA2		DNA3		DNA4
	T_m [°C]	ΔT_m [°C]	T_m [°C]	ΔT_m [°C]	T_m [°C]	ΔT_m [°C]	T_m [°C]
No[a]	47.6	–	45.5	–	46.3	–	49.3
Zn^{2+}[b]	54.8	+7.2	51.9	+6.4	46.3	±0.0	
Fe^{3+}[c]	55.9	+8.3	62.2	+16.7	47.0	+0.7	
Ni^{2+}[b]	57.6	+10.0	62.1	+16.6	45.9	+0.4	

[a] In the presence of EDTA. [b] 1.1 equiv. [c] 6.0 equiv.

only a minor influence on the metal ion lock in the center part. Interestingly, **DNA2** in the presence of Ni^{2+} yielded a three way junction with a $\Delta T_m = 16.6\,^{\circ}C$ that is significantly more stable than the metal free construct. This special effect cannot be explained at the moment.

According to our[17,18,20] and others' previous studies[14,21–24] the dimerization of the 5′-terminally attached PDI chromophores in DNA can be followed by significant changes in the UV/Vis absorption and fluorescence spectroscopy (Figure 2 top). The A_{506}/A_{544} as well as $F_{553}/_{680}$ ratios show the formation of excitonically interacting PDI dimers. It is established and does not need further comments that both ratios proof the assembly and finally aggregation of the constructs **DNA1**, **DNA3** and **DNA4** mediated by the PDI caps.[14,17,18,20–24] Accordingly, the PDI-mediated assembly of **DNA1** and **DNA3** in the presence of different metal ions (Fe^{3+}, Ni^{2+}, Zn^{2+}) and in the presence of EDTA (to exclude any metal ion complexation) has been investigated by both UV/Vis absorption and fluorescence spectroscopy at different temperatures (90→10 °C) (Figure 2 bottom and Supporting Information). In the presence of Fe^{3+} and Zn^{2+}, the absorption ratios A_{506}/A_{544} of **DNA1** at temperatures higher than T_m are around 0.7–0.8 (typical for PDI monomers) and increase to values of >1 for temperatures below T_m. The latter ratios can be assigned to PDI dimers and thereby to formation of assemblies of **DNA1**. Similar experiments with **DNA3** (see Supporting Information) showed that there is only a minor influence of the bipyridyl modification (in the center part of **DNA1**) on the assembly of the PDI dyes. The fluorescence measurements support these results. When excited at 505 nm the fluorescence of **DNA1** and **DNA3** at temperatures below T_m is dominated by a red-shifted, structureless and excimer-type band with a broad maximum at ~680 nm. The characteristic fluorescence at this wavelength confirms an aggregated PDI dimer that undergoes a structural relaxation process after photoexcitation.[25] Moreover, the spectra of **DNA1** in the presence of Fe^{3+} and Zn^{2+} at temperatures higher than T_m exhibit fluorescence at 553 nm that is typical for PDI monomers. Hence, the ratios F_{553}/F_{680} give information about the relative amount of monomeric and dimeric PDI ensembles in the sample.

The presence of Ni^{2+} plays a special role. **DNA1** in the presence of Ni^{2+} at temperatures above T_m exhibits ratios A_{506}/A_{544} of 1.05 and ratios F_{553}/F_{680} of 7 indicating a partial PDI dimerization of this three way junction. This DNA assembly must be driven by Ni^{2+} since it is known that the dimerization of the PDI caps needs the double helical DNA construct as a structural prerequisite.[17,18] We assume that PDI-modified single strands are folded in such a way that the PDI caps are shielded and PDI dimerization is inhibited. The changes of the ratios A_{506}/A_{544} from 90 °C down to 10 °C support this interpretation. The ratio 1.05 at 90 °C decreases to about 0.85 when the T_m value is reached and increases again up to 1.15 at temperatures lower than T_m. This indicates that the complexation of the bipyridyl ligands of **DNA1** with Ni^{2+} might be stable even when the DNA branches of the three way junction are not annealed (above T_m). Thereby, this Ni^{2+}-mediated preassembly leads an unfolding or different folding that unshields the PDI caps of the singles strands and thereby allows PDI dimerization. This kind of construct first breaks down when the samples reaches the T_m value and the DNA branches start to anneal. At temperatures lower than T_m the full hybridization of the oligonucleotide branches leads to PDI-mediated assembly of **DNA1** in the way how it was previously observed in the presence of Zn^{2+}/Fe^{3+} and in the absence of metal ions, as well as with **DNA3** (not metal ion-dependent).

In order to study the influence of the unbound cytidines in the middle of the construct (mainly **DNA3**) **DNA4** was synthesized lacking those cytidine spacers. **DNA4** revealed an increased T_m value ($\Delta T_m = 3\,^{\circ}C$) compared to **DNA3** but it is still lower than the corresponding T_m values of metal ion-complexated **DNA1** (and **DNA2**). This is an interesting result since it shows that the stability of PDI-capped three way junctions can be controlled to a certain extent by the sequential design of the branching part. Nevertheless, the incorporation of the bipyridyl modified and metal complexating cytidines in the middle lead to the most stable constructs (**DNA1**).

To obtain additional information on the PDI-mediated assembly of the DNA three way junctions we wanted to apply atomic force microscopy (AFM) on mica surfaces. The sequences of **DNA1**-**DNA4** contain arms with 10 base pairs that correspond to a length of 3.4 nm. These three way junctions clearly are below the resolution limit of AFM application in air. Hence, **DNA5** was synthesized as a bigger three way construct. Each branch of **DNA5** bears three times the sequence of the corresponding branches of **DNA1** (see Supporting Information) and is equipped with the PDI capped at the terminus. Hence **DNA5** contains 30 base pair per arm which corresponds to a length of 10.2 nm. **DNA6** serves as a non-aggregating reference since it bears the same sequence as **DNA5** but lacks the PDI modifications. The corresponding AFM images of samples with **DNA5** (Figure 3) immobilized by $NiCl_2$ on freshly cleaved mica[26] clearly show the formation of higher-ordered structures as the result of DNA assemblies using concentrations of 1 and 0.02 μM. AFM images of oligomeric (tetrameric) assemblies of **DNA5** (see Supporting Information) help to interpret and visualize the formation of the ordered polymeric aggregates (Figure 3, top). These structures must be PDI-mediated since they are not observed in similar experiments with the control construct **DNA6** that lacks the PDI caps. In contrast to our previous results, where we obtained primarily PDI-mediated dimers,[18] the increased size of **DNA5** obviously allows the formation and observation of higher ordered structure on the mica surface.

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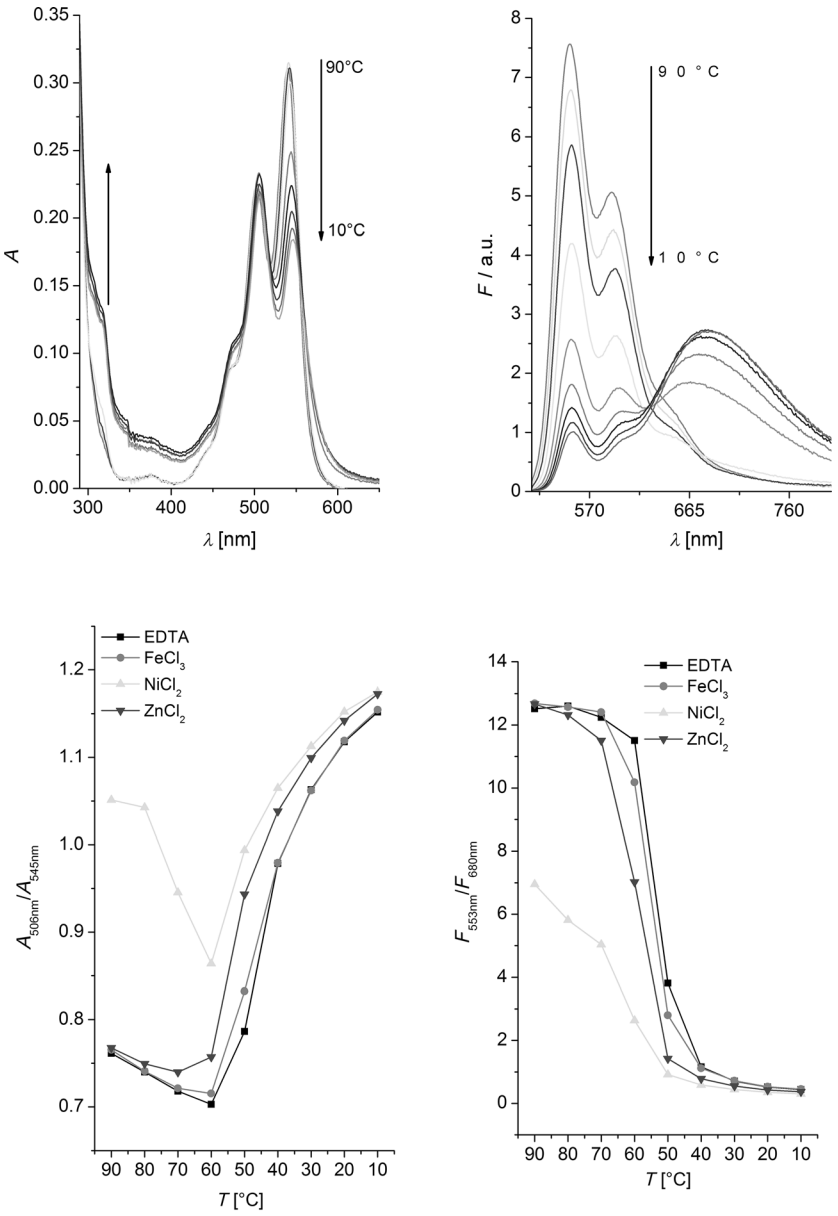

Figure 2. Top: UV/Vis absorption spectra (left) and fluorescence spectra (right) of **DNA1**, 2.5 μM in 10 mM NaP_i buffer, pH 7, 250 mM NaCl, representatively shown in the presence of Zn^{2+}. Bottom: Temperature dependence of the absorption ratios A_{506}/A_{544} and fluorescence ratios F_{553}/F_{680} of **DNA1** in the presence of Fe^{3+}, Ni^{2+}, Zn^{2+} and EDTA (to exclude any metal complexation).

In conclusion we presented the development of a new type of bifunctional DNA architectonics that is based on three way junctions that combine the structural motif of sticky PDI caps with a metal ion lock in the center part. The terminal PDI modifications were linked as DNA building blocks according to our published procedure. The bipyridyl ligands were attached by postsynthetic click-type acetylene-azide cycloaddition to central cytidines in the oligonucleotide sequences which were propargylated at the 2′-position. Recent studies by energy transfer probing showed that Y-shaped three way junctions adopt a pyramidal structure, in which the bases next to the branch point remain unpaired, even if full Watson–Crick is given by the sequence.[27] Based on this result it is clear that the incorporation of a metal ion complex into the branchpoint is key to stabilize the three way junction and thus to reduce its conformational flexibility. This was similarly observed by Shionoya et al.[19] A clear stabilizing effect was observed in the presence of Fe^{3+}, Ni^{2+} and Zn^{2+} by the formation of corresponding bipyridyl complexes in the branching part of the **DNA1** and **DNA2**. Our construct **DNA1** allows additional dimerization of the 5′-terminally attached PDI chromophores by hydrophobic interactions of the three way junctions which can be followed by significant changes in the UV/Vis absorption and fluorescence. The PDI-mediated DNA assembly occurs at temperatures below T_m and is not influenced by the metal-ion bipyridyl locks in the central part. Except Fe^{3+}, the hybridizations of the three single oligonucleotides to the metal ion-locked three way junctions are fully reversible if the sample was denatured at higher temperature and annealed again. The corresponding AFM images revealed the formation of higher-ordered structures as the result of DNA assemblies mediated by the PDI interactions. It is important to point out that the two different structural motifs (PDI and bipyridine) behave nearly independent from each other and hence be considered orthogonal (in the chemical sense). This is an important prerequisite for the development of DNA-inspired biomaterials for future applications, for example, molecular electronics or light harvesting antenna.

Experimental Section

For the synthesis of the azide modified bipyridyl ligand, the bifunctional DNA strands as well as additional optical spectra please see the Supporting Information.

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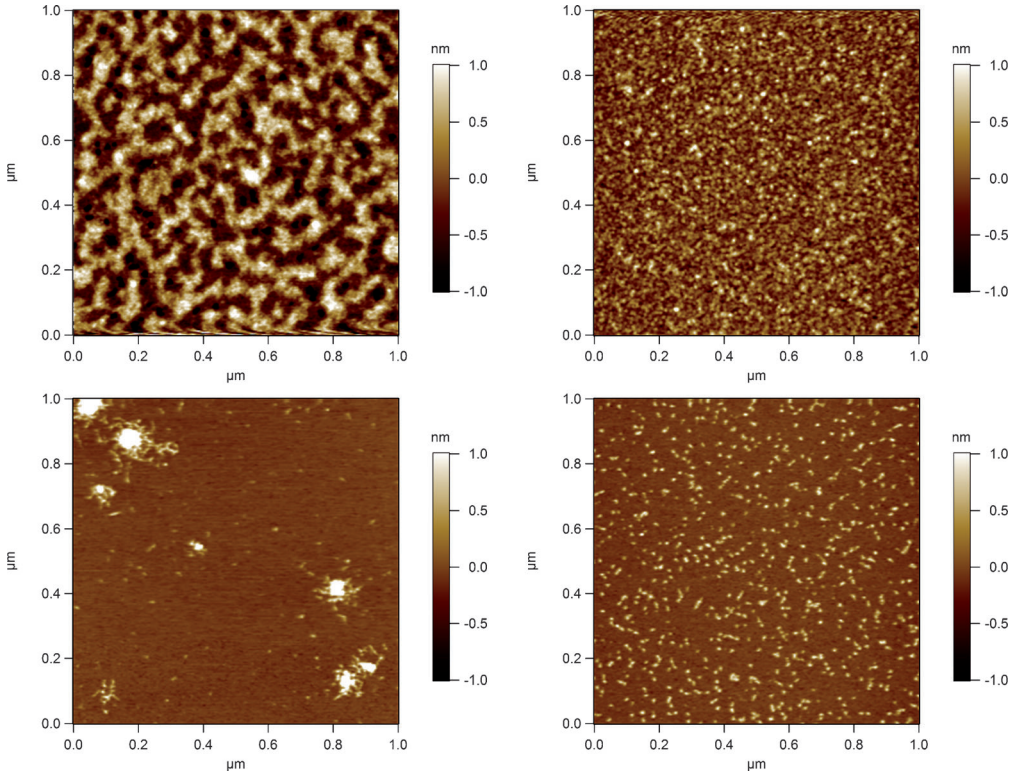

Figure 3. AFM hight images in air using diluted DNA in deionized water and upon addition of a 3 mM $NiCl_2$ solution on freshly cleaved mica. Top: **DNA5** 1 µM (left) and 0.02 µM (right). Bottom: **DNA6** 1 µM (left) and 0.02 µM (right).

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Keywords: bipyridines · DNA architectonics · exciton · fluorescence · oligonucleotides

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