Pattern-based sensing of short oligodeoxynucleotides with palladium–dye complexes†

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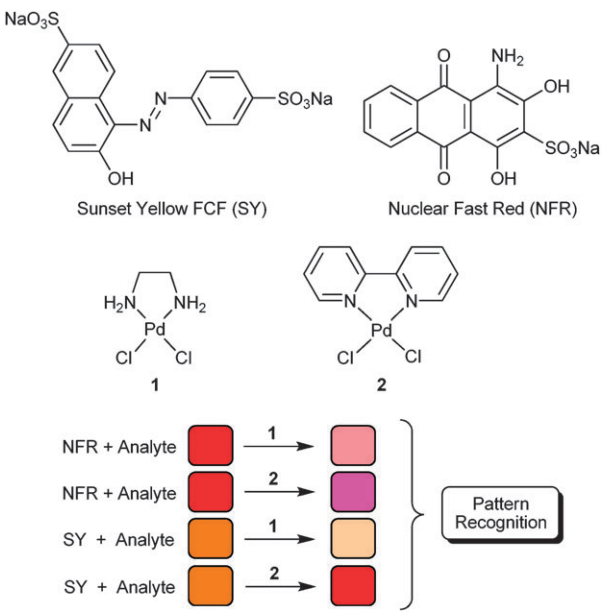
The colorimetric response of a sensor array composed of palladium–dye complexes can be used to identify different hexadeoxynucleotides and to distinguish mixtures of sequence-isomeric hexadeoxynucleotides.

Small-molecule optical probes for the detection of oligonucleotides in solution have found numerous applications in chemistry and biology.[1] In most cases, the optical response is due to a change of absorption or fluorescence properties of the probe upon its intercalation into the stack of nucleobases or binding in the grooves of DNA.[2] This approach has proven to be quite powerful, and sub-nanogram detection limits of double-stranded DNA in solution or in electrophoresis gels have been achieved with proprietary dyes such as PicoGreen or SYBR Green, respectively.[3] However, most probes of this kind are less sensitive towards single-stranded DNA.[4] Probes optimized for detection of single-stranded DNA, such as the proprietary OliGreen stain, are rare[5] and incapable of detecting short oligonucleotides of six bases or less.[5b] At the same time, it was shown that even short oligonucleotides (less than 10 bases long) may find practical application, *e.g.* as PCR primers or as therapeutically important antisense oligonucleotides.[6] Below we describe a conceptually new type of colorimetric assay for sensing of short oligonucleotides, which is based on the analyte-induced displacement of dyes from Pd(II) complexes. As representative applications, we demonstrate that it is possible to identify different hexadeoxynucleotides and to distinguish mixtures of sequence-isomeric hexadeoxynucleotides.

Over the last years, sensor arrays based on metal–dye complexes have been developed for many types of analytes.[7,8] Each sensor of such an array is composed of a transition metal complex and a dye. The complexation of the dye to the metal leads to a change of its color and/or fluorescence. Upon addition of the analyte, a displacement of the dye occurs. The observed optical response of the sensor will depend on the relative affinities of the dye and the analyte for the metal complex, as well as on their concentrations. A pattern-based analysis of the response of the entire array can be used to obtain information about the identity, the quantity, or the purity of the analyte.

To create a sensing array for oligonucleotides, metal complexes with the following characteristics were needed: (1) they should be soluble in water at neutral pH, (2) they should display high affinity for nucleotides, and (3) they should exhibit reasonably fast ligand exchange reactions. Square planar Pt(II) complexes such as *cis*-$[PtCl_2(NH_3)_2]$ are of course well known for their ability to bind to nucleic acids, but ligand exchange is typically slow.[9] We thus decided to focus on analogous Pd(II) complexes, which tend to be more labile.[10] Mass-spectrometric studies had shown that $[PdCl_2(en)]$ (**1**, en = ethylenediamine) can bind to short oligonucleotides.[11] This finding prompted us to investigate whether complex **1** and the related $[PdCl_2(bipy)]$ (**2**, bipy = 2,2′-bipyridine) could be used for colorimetric displacement assays.[12]

Looking for suitable dyes, we turned our attention to anionic colorants, which were expected not to interact with the negatively charged oligonucleotides *per se*. We found that Sunset Yellow FCF (SY, Scheme 1), a commonly used food additive, is able to bind to the complexes **1** and **2**. Most likely, the dye forms an N,O-chelate by coordination to the binding sites opposite to the N-donor ligands en and bipy. In both cases, the complexation is accompanied by a strong change in color (see ESI†). However, the spectral changes are different



Scheme 1 Molecular structures of the sensor components and basic principle of the colorimetric assay.

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for the two complexes. For complex **1**, the complexation results in a gradual weakening of the main SY absorption band at λ = 482 nm. For the bipy complex **2**, on the other hand, one can observe the formation of a new band at λ = 520 nm along with the decreased absorption at λ = 482 nm. Spectrophotometric titrations were performed by adding increasing amounts of **1** or **2** to a buffered aqueous solution of SY ([SY] = 10 μM; 20 mM phosphate buffer, pH 7.0). The resulting UV-Vis absorption data could be fitted to a 1 : 1 binding model using the following binding constants: K(**1**–SY) = 2.9(±0.3) × 10^6 M^{-1} and K(**2**–SY) = 1.4(±0.1) × 10^7 M^{-1} (see ESI†). As a second dye, we decided to employ Nuclear Fast Red (NFR, Scheme 1). We had previously observed that complex **1** has a very high affinity for NFR.[7] As in the case of SY, the complexation of NFR to [$PdCl_2$(en)] or [$PdCl_2$(bipy)] results in a weakening of the two main absorption bands at λ = 504 and 535 nm. For [$PdCl_2$(bipy)], the complexation is accompanied by the appearance of new bands at λ = 573 and 626 nm. Fitting of the UV-Vis absorption data to a 1 : 1 binding model gave the following binding constants: K(**1**–NFR) = 4.8(±0.5) × 10^7 M^{-1} and K(**2**–NFR) > 1 × 10^8 M^{-1} (see ESI†). In all cases, the exclusive formation of complexes with a 1 : 1 stoichiometry was demonstrated by a Job plot analysis (Fig. S9, ESI†).

With the two dyes and the two Pd complexes we created a mini array comprised of four individual sensors. As representative examples of short oligonucleotide analytes, we decided to employ single-stranded hexadeoxynucleotides of identical composition but with different non-self-complementary sequences, namely 5′-d(ACCGTA)-3′, 5′-d(GACATC)-3′, 5′-d(CTCAGA)-3′, and 5′-d(CAAGTC)-3′. The experimental procedure of the assay is outlined in Scheme 1. First, solutions of the respective hexanucleotide and dye were mixed; this resulted in no change of color, which shows that the dyes do not interact with the oligonucleotides. The competition reactions were then started by adding aqueous solutions of the respective Pd complex (final concentrations: [oligonucleotide] = 17.5 μM, for sensors with **1**: [dye] = [Pd] = 40 μM; for sensors with **2**: [dye] = [Pd] = 25 μM, 20 mM phosphate buffer, pH 7.0).‡ After equilibration for 15 h, the four sensors were analyzed by measuring the absorption at λ = 503 (**1**–SY), 478 (**2**–SY), 535 (**1**–NFR), and 626 nm (**2**–NFR). Six independent measurements were performed for each sensor–analyte combination. All hexanucleotides that we have tested gave optical changes, indicating that the analytes can compete with the dyes for the complexation to the Pd complexes. Isosbestic points were found for the superposition of spectra of dyes, Pd–dye complexes, and Pd–dye–oligonucleotide mixtures (ESI†, Fig. S10–S13). This observation provides evidence that the spectral changes in the presence of oligonucleotides are caused by a displacement of the dyes from the Pd complexes and not due to formation of ternary complexes. A graphic representation of the sensor response for four sequence-isomeric hexanucleotides is shown in Fig. 1. It is apparent that the individual sensors respond differently to the four analytes. Furthermore, one can observe a characteristic signal pattern for each nucleotide.

A pattern-based analysis of sensor array data can be achieved with statistical tools such as linear discriminant

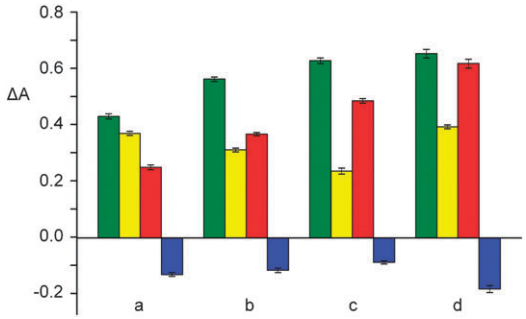

Fig. 1 Changes in absorption of the four sensors for the hexanucleotide analytes 5′-d(ACCGTA)-3′ (a), 5′-d(GACATC)-3′ (b), 5′-d(CTCAGA)-3′ (c), and 5′-d(CAAGTC)-3′ (d). The sensors are color coded as follows: **1**–SY: green, **2**–SY: yellow, **1**–NFR: red, **2**–NFR: blue. The assay was performed as described in the main text.

analysis (LDA).[13] LDA is a supervised method that maximizes the ratio of between-class variance to within-class variance. To evaluate the analytical power of the senor array in combination with LDA, we have carried out two experiments. First, we measured the array response for four additional hexanucleotides, the homooligomers 5′-d(AAAAAA)-3′, 5′-d(GGGGGG)-3′, 5′-d(CCCCCC)-3′, and 5′-d(TTTTTT)-3′. An LDA was then performed with the data of all eight hexanucleotides and that of a blank sample without analyte. We found that we can identify the respective oligonucleotide without any misclassifications (see ESI†). A graphic representation

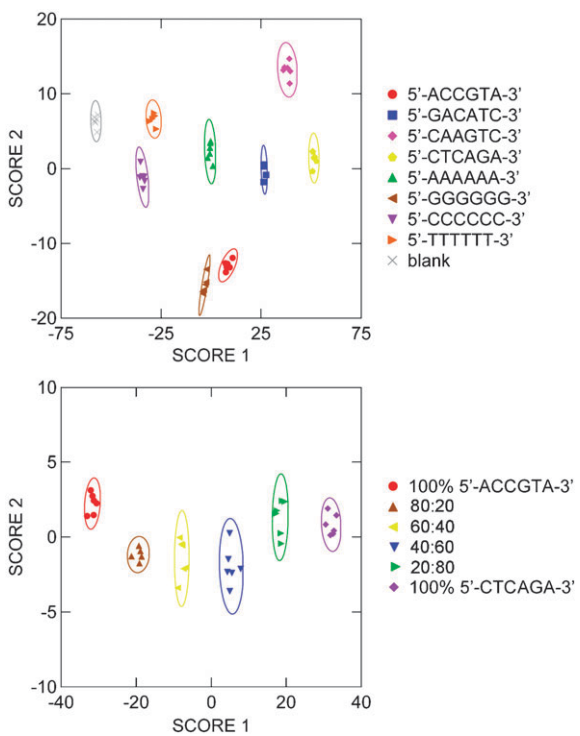

Fig. 2 Top: two-dimensional LDA score plot for the discrimination of eight different hexanucleotides (17.5 μM each) and a blank sample without analyte. Bottom: two-dimensional LDA score plot for the discrimination of mixtures of 5′-d(ACCGTA)-3′ and 5′-d(CTCAGA)-3′ ([hexanucleotide]$_{total}$ = 17.5 μM). The input data for the analyses were obtained from the sensor array described in the main text.

of the LDA in the form of a 2-dimensional score plot is shown in Fig. 2. Next, we examined the possibility of sensing mixtures of hexanucleotides. For that purpose we prepared samples containing a variable ratio of the sequence isomers 5′-d(ACCGTA)-3′ and 5′-d(CTCAGA)-3′ with a fixed total strand concentration of 17.5 μM. Remarkably, an LDA of the sensor array data gave again a correct classification in all cases (Fig. 2 and ESI†). It should be pointed out that this type of discrimination is not possible with a single Pd–dye sensor: examination of the response of the four sensors shows that there is signal overlap for at least two out of the six samples (ESI†, Fig. S14). A multidimensional, pattern-based analysis is thus crucial for the success of the analysis.

In conclusion, we have demonstrated that a small array made from two Pd complexes and two dyes can be used as a powerful colorimetric sensor for hexanucleotides at low micromolar concentrations. The sensor is neither based on hybridization events nor on direct interaction with probe molecules. Importantly, it is responsive to short, unfolded oligonucleotides. Our assay thus complements existing strategies for the optical detection of oligonucleotides. From a practical point of view it is important to note that all components of the sensor are commercially available. A disadvantage for potential applications is the fact that several hours are required to equilibrate the sensor. Furthermore, it is unlikely that such a sensor could be used to detect oligonucleotides in a complex biological matrix because the Pd complexes are expected to bind to proteins.[14] However, a sensor of this kind could be a valuable tool for sensing of oligonucleotides in a more controlled, abiotic environment (*e.g.* analysis of synthetic samples).

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Notes and references

‡ In a classical displacement assay, the analyte is added to a solution of the metal–dye complex. We found that our procedure (adding the metal last) resulted in faster equilibration. The metal–dye concentrations of sensors with complex **2** (25 μM) were lower than those with complex **1** (40 μM) because **2** displayed higher binding constants.

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