ORGANIC LETTERS

2013
Vol. 15, No. 13
3456–3459

# Pattern-Based Sensing of Peptides and Aminoglycosides with a Single Molecular Probe

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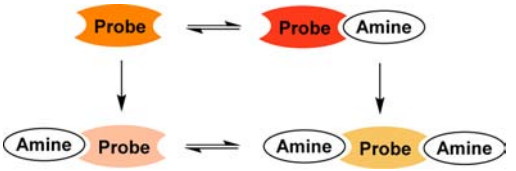
**Received May 31, 2013**

## ABSTRACT




**A coumarin-based molecular probe can be used for the sensing of peptides and aminoglycoside antibiotics. The probe reacts with the primary amine group(s) of the analytes to give a mixture of covalent adducts with distinct colors. Each analyte gives rise to a characteristic UV−vis spectrum. A pattern-based analysis of the spectra allows identifying structurally related analytes. Furthermore, it is possible to obtain information about the quantity and the purity of the analytes.**

Cross-reactive sensor arrays can be used for the pattern-based sensing of analytes in solution.[1] These arrays are composed of spatially separated and chemically distinct sensors. The individual sensors respond differently to the analytes, thereby creating a characteristic signal pattern for each compound (Scheme 1a). Signal readout is typically achieved by fluorescence or UV−vis spectroscopy. The identity, quantity, or purity of the analyte can then be evaluated with the help of pattern-recognition tools such as principal component analysis (PCA). Sensor arrays have been used successfully for the analysis of sugars,[2] amino acids,[3] peptides,[4] proteins,[5] cell surfaces,[5] odorants,[6] small ions,[7] and explosives,[8] among others.[1]

Instead of using multiple sensors in an array format, it is also possible to use a pattern-based sensing approach for the analysis of just one solution containing an analyte and a chemosensor. A prerequisite is the formation of a characteristic spectral pattern upon addition of the analyte (Scheme 1b). Different strategies have been described in this context. The groups of Li, Shi, and Lavigne have used the analyte-induced aggregation of polythiophene polymers for the detection of nucleotides[9] and amines.[10]

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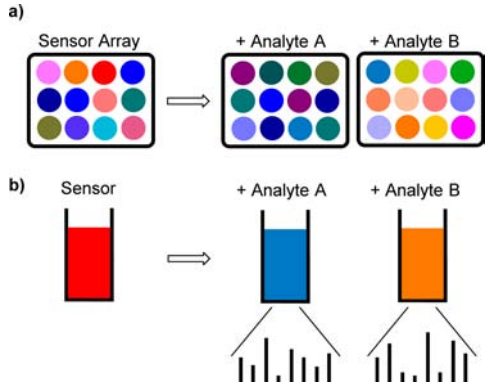
10.1021/ol401495c 
**Published on Web 06/24/2013**

Margulies and Hamilton have used a dynamic mixture of fluorescent DNA G-quadruplexes for the sensing of proteins,[5c,11] whereas Anslyn et al. have explored dynamic mixtures of synthetic receptors and dyes for the colorimetric detection of polycarboxylic acids.[12] A sensing system based on NMR spectroscopy was introduced by the Bode group.[13] They have used a $^{13}$C-labeled bullvalene with porphyrin side chains. The bullvalene core is able to undergo sequential Cope rearrangements, resulting in a rapidly interconverting population of structural isomers. Fullerenes were shown to alter the isomer distribution, and a characteristic $^{13}$C NMR signal pattern was observed for different fullerenes. A synthetic receptor containing three boronic acid recognition units and four different fluorescent groups was synthesized by Margulies et al.[14] A pattern-based analysis of the fluorescence emission spectrum allowed the identification of common drugs. A multicomponent sensing system based on self-assembled monolayers on gold nanoparticles terminating with $Zn^{2+}$ complexes and polyanionic fluorescence dyes was described by Prins et al.[15] Dye displacement reactions were used to detect nucleotides in a quantitative manner at micromolar concentrations. Our group has used dynamic mixtures of transition metal complexes for the colorimetric detection of peptides,[16] nucleotides,[17] and glycosaminoglycans.[18,19] Below, we describe a novel approach for the pattern-based detection of amine-containing bioanalytes using a single molecular probe.

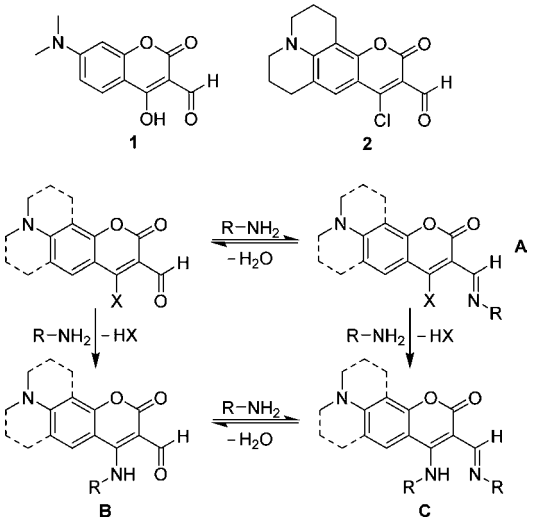
**Scheme 1.** Different Strategies for the Pattern-Based Sensing of Analytes in Solution[a]




[a](a) A sensor array composed of spatially separated sensors gives characteristic patterns for different analytes; (b) distinct spectral patterns are observed upon addition of different analytes to a single sensing system.

In a recent communication, we reported that the coumarin derivative **1** can be used for the detection of biogenic amines in micellar aqueous solutions by UV−vis or fluorescence spectroscopy.[20] Analyses of reaction mixtures had shown that the amines undergo condensation reactions with the aldehyde function and/or the enol function of the dye to give the condensation products **A**−**C** and/or their corresponding tautomic forms (Scheme 2).

**Scheme 2.** Molecular Probes **1** and **2** Form Different Covalent Adducts with Amines




In our previous study, the analyses were performed at a fixed wavelength using simple primary amines.[20] We realized, however, that the system is potentially suited for the pattern-based analysis of more complex amine-containing bioanalytes. The basic requirements for such an analysis would be as follows: (a) the various analytes result in a different product distribution after a given time, and (b) the probe and the products **A**−**C** show distinct absorption spectra.

To evaluate the feasibility of such an approach, we have examined reactions of probe **1** with four different

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aminoglycoside antibiotics: Paromomycin, Kanamycin A, Gentamicin, and Neomycin. In addition to dye **1**, we have used the structurally related probe **2**.[21,22] Test reactions with the simple monoamines tryptamine and tyramine had shown that dye **2** undergoes analogous condensation reactions to give adducts of type **A**–**C** (analyses by HPLC-MS and X-ray crystallography; see Supporting Information (SI)). Importantly, we observed that the product distribution was indeed different for tryptamine and tyramine.

Probes **1** and **2** were then allowed to react with the four different antibiotics. The reactions were performed in buffered aqueous solutions (HEPES, 50 mM, pH 7.4) at room temperature with probe concentrations of 20 $\mu$M and analyte concentrations of 50 $\mu$M. The surfactant sodium dodecyl sulfate (SDS) was added to solubilize the dyes and to facilitate the condensation reactions.

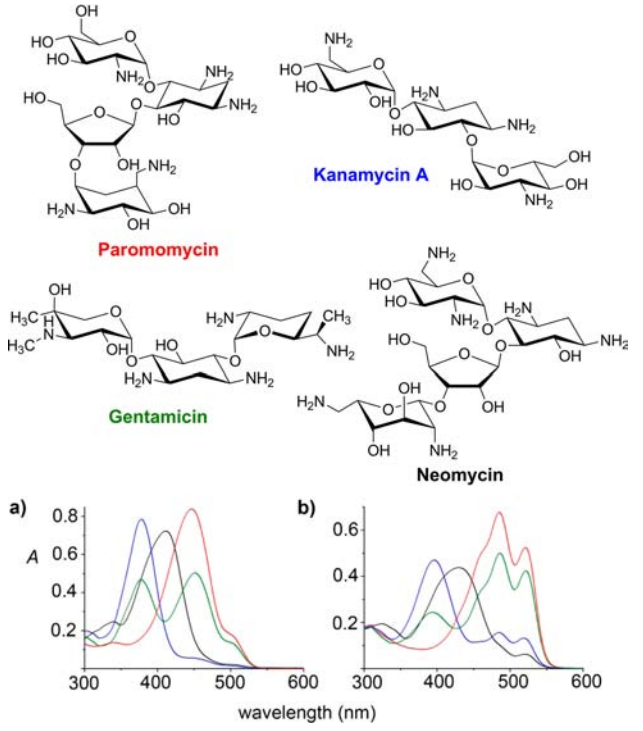


**Figure 1.** Absorption spectra of buffered aqueous solutions (50 mM, HEPES, pH 7.4) containing SDS (6.0 mM), different aminoglycosides (50 $\mu$M) and (a) probe **1** (20 $\mu$M) or (b) probe **2** (20 $\mu$M). The measurements were performed 2 h after addition of the respective amine.

When the reaction mixtures were analyzed after 2 h by UV–vis spectroscopy, we observed pronounced spectral differences for the four aminoglycosides (Figure 1). The complexity of the spectra obtained with dye **1** was rather low, with a single absorption band dominating for three of the four analytes (Figure 1a). For dye **2**, however, the spectra were all rather complex with two or more bands for each analyte (Figure 1b). Furthermore, the spectral range was larger with strong absorption bands in the region 500–550 nm. Since an increased complexity is advantageous for a pattern-based analysis, we have performed all further analyses with the new probe **2**.

First, we have examined whether probe **2** can be used to distinguish a larger number of aminoglycosides.[23] In addition to the four analytes shown in Figure 1, we have used Apramycin and Kanamycin B. It should be noted that Kanamycin B is structurally very similar to Kanamycin A (exchange of one OH for a $NH_2$ group). The experiments were performed as described above: a buffered aqueous solution of probe **2** (20 $\mu$M) and SDS (6.0 mM) was mixed with the respective aminoglycoside (50 $\mu$M). After incubation for 2 h, a UV–vis spectrum was recorded. Eight independent measurements were performed for each analyte. The spectra were then analyzed with the help of the statistical software SYSTAT 11. First, a small subset of relevant wavelengths was identified with the help of a stepwise variable selection algorithm (see SI). The absorption at the four selected wavelengths (530, 515, 465, and 455 nm) was then used as input for a PCA. The corresponding score plot shows that the data for the different aminoglycosides appear in well separated clusters (Figure 2). The UV–vis spectra can thus be used for a pattern-based recognition of these antibiotics.

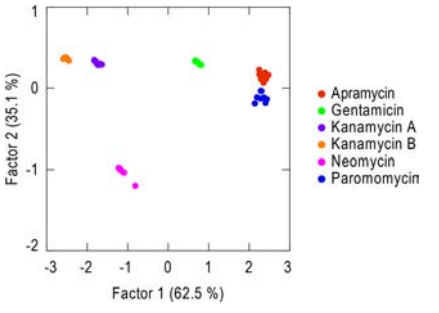


**Figure 2.** Two-dimensional PCA score plot for the analysis of six different aminoglycosides. UV–vis spectra (eight independent measurements) of buffered aqueous solutions (HEPES, 50 mM, pH 7.4) containing probe **2** (20 $\mu$M), SDS (6.0 mM), and the respective aminoglycosides (50 $\mu$M) were used as input for the statistical analysis. The spectra were recorded after incubation for 2 h.

A similar approach can be used to distinguish pure aminoglycoside samples from mixtures. This fact was demonstrated by an analysis of the spectra obtained for Apramycin (A), Kanamycin B (KB), Neomycin (N), and equimolar mixtures of A+N, KB+N, KB+A, and

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KB+A+N. The resulting PCA score plot reveals again a very good separation of the data clusters (SI, Figure S6). The possibility of distinguishing pure samples from mixtures could be interesting for quality control measurements.

Next, we have investigated whether the probe can be used for the analysis of peptides. The following 11 peptides containing between five and nine amino acid residues were chosen for this study: Delta Sleep Inducing Peptide (**3**), Bradykinin (**4**), 5-Met-Enkephalin (**5**), 5-Leu-Enkephalin (**6**), Dynorphin A (13−17), porcine (**7**), Adipokinetic Hormone G (**8**), Mega-3-antigen (**9**), Experimental Allergic Encephalitogenic Peptides **A**−**C** (**10**−**12**), and HCV-core protein (107−114) (**13**) (Figure 3). To simplify the syntheses, all peptides contain amide groups at the C-terminus, except the purchased peptides **4**, **5**, and **6**. It should be noted that the nonapeptides **10**−**12** are sequence isomers, which differ in the position of only one tryptophan residue. Furthermore, the content of the reactive primary amine groups is in most cases low. Therefore, the peptide analysis was expected to be more challenging than the analysis of the aminoglycosides.

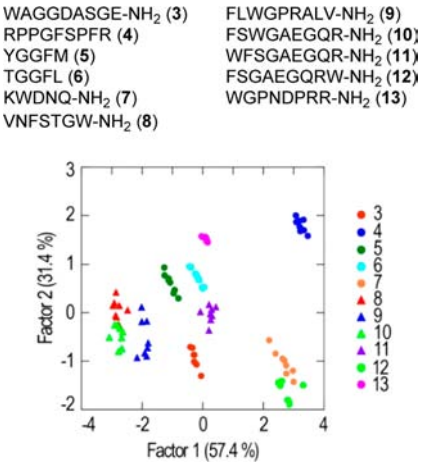


**Figure 3.** Two-dimensional PCA score plot for the analysis of 11 different peptides. UV−vis spectra (eight independent measurements) of buffered aqueous solutions (HEPES, 50 mM, pH 7.4) containing probe **2** (20 $\mu$M), SDS (6.0 mM), and the respective peptide (500 $\mu$M) were used as input for the statistical analysis. The spectra were recorded after incubation for 4 h.

A preliminary test reaction revealed that higher analyte concentrations (500 $\mu$M) were needed to induce a significant optical response. Furthermore, we decided to extend the incubation time from 2 to 4 h. Apart from these changes, the sensing experiments and the statistical analyses were performed as described above. Using the absorption at six different wavelengths (530, 445, 425, 410, 400, and 385 nm) as data input, we were able to achieve very good discrimination (Figure 3). Additionally, the same data set was used to perform a linear discriminant analysis (SI, Figure S8) and a jack-knife validation procedure. 50% of the data was randomly omitted, and the remaining data were used as a training set for the LDA. The omitted observations were then classified resulting in a correct classification in all cases (100%).

The sequence-isomeric peptides **10**−**12** are remarkably well separated in the PCA score plot (Figure 3). Encouraged by these results, we investigated whether it is possible to differentiate the isomeric peptides at four different concentrations (100, 300, 500, and 700 $\mu$M). A PCA of the spectral data revealed that the sensing system is indeed able to identify the peptide sequence *and* the concentration range of the sample (SI, Figures S9 and S10).[24]

The experiments with peptide analytes show that the analytical power of probe **2** is remarkably high. It should be noted that the presence of the surfactant SDS likely contributes to the good selectivity. Dye **2** partitions into the micellar subphase. Reactions with amines could occur inside the micelles or at the micelle−water interphase. Therefore, the kinetics of the reactions depend not only on the intrinsic reactivity of the substrates but also on their partitioning coefficients for the micellar subphase. Support for this assumption comes from control experiments with tryptamine and tryptophan. It was found that the reaction of dye **2** with the more lipophilic tryptamine gave rise to a very different UV−vis spectrum compared to that of reactions with the more polar amino acid tryptophan (SI, Figure S3).

In conclusion, we have shown that coumarin **2** can be used as a molecular probe for the sensing of aminoglycosides and peptides in the micromolar concentration range. Molecular diversity is generated by the formation of different covalent adducts in an analyte-specific fashion. The distinct absorption spectra of the resulting mixtures are the basis for the successful pattern-based analysis. So far, there are very few reports about the utilization of a single cross-reactive molecular probe for pattern-based sensing.[9,10,13,14] The basic concept can likely be extended to other types of probes, detection methods, and analytes. Work in this direction is currently pursued in our laboratory.

**Acknowledgment.** The work was supported by the Swiss National Science Foundation and by the Ecole Polytechnique Fédérale de Lausanne (EPFL). We thank Dr. E. Solari (EPFL) for help with the elemental analysis and crystallographic measurements and Dr. L. Menin (EPFL) for supporting the MS analyses.

**Supporting Information Available.** Experimental procedures and crystallographic data in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

(24) Despite this success, the analysis of complex mixtures with unknown concentrations of analytes remains a challenging task for pattern-based sensors.

The authors declare no competing financial interest.