ORGANIC LETTERS

2007
Vol. 9, No. 17
3217−3220

A Food Freshness Sensor Using the Multistate Response from Analyte-Induced Aggregation of a Cross-Reactive Poly(thiophene)

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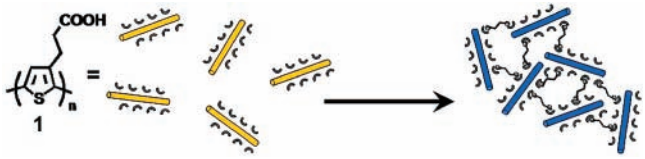
Received May 16, 2007

ABSTRACT



A single cross-reactive conjugated polymer generates a multidimensional response capable of identifying and differentiating between 22 structurally similar and biologically relevant amines with 97% accuracy in a highly competitive aqueous environment. Statistical analysis on an array of wavelengths was used to assess the viability of this approach. In a separate investigation, the multidimensional response from a single cross-responsive poly(thiophene) has been analyzed using a different ratiometric method to quantify the amount of biogenic amine present in a fish matrix, thereby evaluating the quality of the food.

There is an increasing need for fast, accurate, reproducible, and economical sensors for the detection of biologically relevant analytes. Electronic nose[1] and tongue[2] technologies have found great utility in this pursuit. While most of these systems allow for identification of different classes of compounds, little information is available about discrimination between compounds within a given class. We have previously used the multidimensional response from a functional conjugated polymer to differentiate between structurally similar α,ω-diamines in organic solvent.[3] Herein we *extend* this paradigm to a highly competitive aqueous media and *expand* the scope to detect and differentiate between 22 structurally similar and biologically relevant amines with 97% accuracy. Furthermore, this approach has been *applied* to detect biogenic amines in fish. The innovation of these analyses lies within the inherent supramolecular response of polymer **1** producing a multidimensional spectral response. Statistical analysis on an array of wavelengths from this single polymer provided excellent classification accuracies in *highly competitive aqueous media*. Additionally, use of a ratiometric analysis on the multidimensional response from the polymer allowed determination of its *utility toward assessing food freshness*.

Biogenic amines have been associated with rapid cell proliferation, and consequently, they can serve as indicators of various health risks, including cancer,[4] bacterial infection,[5] and food poisoning.[6] However, the detection and classification of amines in water is difficult, and existing methods

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10.1021/ol071065a CCC: $37.00
Published on Web 07/18/2007

have only been shown to work for a small number of analytes. Previous approaches have used MIPs,[7] enzymes,[8] antibodies,[9] single molecule[10] and array sensors,[11] and most commonly complicated chromatographic methods.[12] While there is considerable precedence for using conjugated polymers as optical sensors in solution,[13] most of these analyses focus on a single transduction event, most commonly, planarization/deplanarization of the polymer backbone upon interaction of analyte with side-chain functionality.[13c,e] As a result, this single-dimensional response is often expressed as a function of the change over one or two wavelengths in the relevant polymer spectra.

An alternate and underutilized approach relies on analyte-induced aggregation of the polymer[3,14] taking advantage of not only intrachain conformational changes but also interchain interactions producing analyte specific optical fingerprints that are dependent on the size, shape, valency, and rigidity of the analyte. Figure 1 schematically depicts the different color response with differing analyte (A−I) structures.

This analysis does not rely on a single perturbation to define the sensor response. Rather this multistate, multidimensional response derives from numerous dynamic polymer−analyte interactions causing main-chain conformational changes, $\pi-\pi$ stacking between polymer chains and scattering of visible light caused by the solution stable polymer−analyte aggregates. Given the many dynamic equilibria established as part of the analyte induced aggregation, multiple assemblies are formed in solution, each having their own unique spectral properties. Thus, it is the collective response from all of these interactions that characterize the overall shape of the absorbance curve across the entire spectrum and is, therefore, responsible for discrimination between analytes. The variations in the optical response of the polymer may be dramatic or quite subtle. The changes in the absorption spectra upon addition of amine are shown in Figure 2.[15]

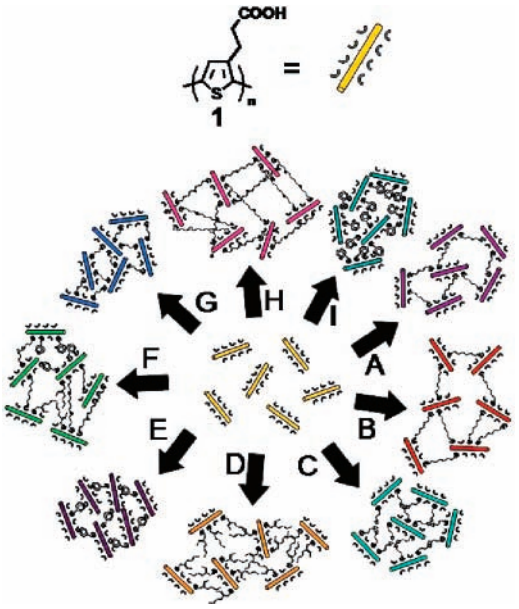

Figure 1. Schematic representation of the multidimensional response from polymer **1** (yellow rods) upon analyte-induced aggregation with different amines (A−I). Unique optical signatures are depicted by the different colored rods.

Pattern recognition protocols, specifically linear discriminant analysis (LDA),[16] were employed to reduce noise and simplify the spectral fingerprint from carboxy-functionalized poly(thiophene) **1**,[17] responding to the amine-containing compounds. This approach is analogous to many sensor array platforms,[1,2,11] where our array derives from the wavelengths used as inputs spanning the entire polymer spectrum.

To demonstrate the utility of this approach in highly competitive, buffered aqueous media, the multidimensional response from polymer **1** was used to discriminate between six structurally similar diamines (encircled in Figure 4). The analysis relied on the optical signature of a 1 mM aqueous solution of polymer **1** (based on monomer) responding to 1

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(15) Changes are shown by subtracting the polymer spectrum from each polymer-amine response; see: Buryak, A.; Severin, K. *Angew. Chem., Int. Ed.* **2005**, *44*, 7935−7938.

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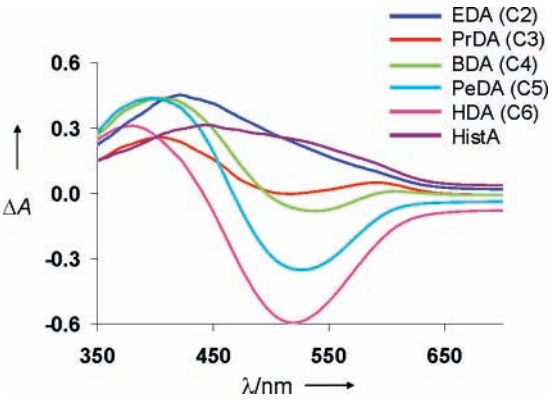

Figure 2. Changes in the absorption spectra of polymer **1** (1 mM) upon addition of different diamines (1 mM each) in aqueous HEPES buffer (40 mM, pH 7.4).

equivalent of amine in 40 mM HEPES buffer (pH 7.4) at a constant temperature (25 °C). The absorption spectra were recorded using a microtiter plate reader with the samples randomized on the plates to avoid systematic errors.[18] To further minimize systematic error, spectra for 47 replicates of each amine (282 total samples) were collected on different days, using different stock solutions.[19] The entire spectrum between 350 and 700 nm every 10 nm (36 wavelengths) was used for the analysis.[3] Absorbance values below 350 nm were excluded so that the assay would not be influenced by absorption from aromatic analytes. The pH was controlled to ensure that discrimination between amines was based upon polymer response to the amine rather than pH changes.

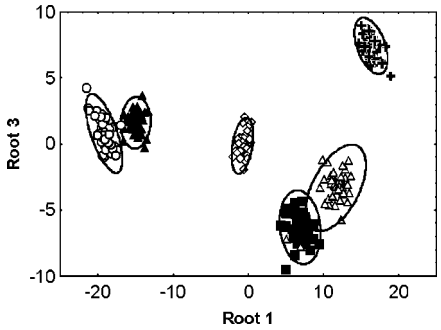

Figure 3. Two-dimensional LDA plot of polymer **1** response to EDA (■), PrDA (△), BDA (▲), PeDA (◇), HDA (○) and HistA (+).

Figure 3 shows the projection of the LDA results in two dimensions. Each axis of the LDA plot represents weighted combinations of the 36 dimensional data, where each point in the plot is an individual replicate that contains information from the 36 wavelengths from the relevant spectrum.[3,20] The circles around each cluster represent 95% confidence limits. Leave-one-out cross-validation[21] was used to estimate the predictive ability of the LDA model and showed excellent discrimination between amines accurately identifying the analyte 99% of the time (278/282 samples).

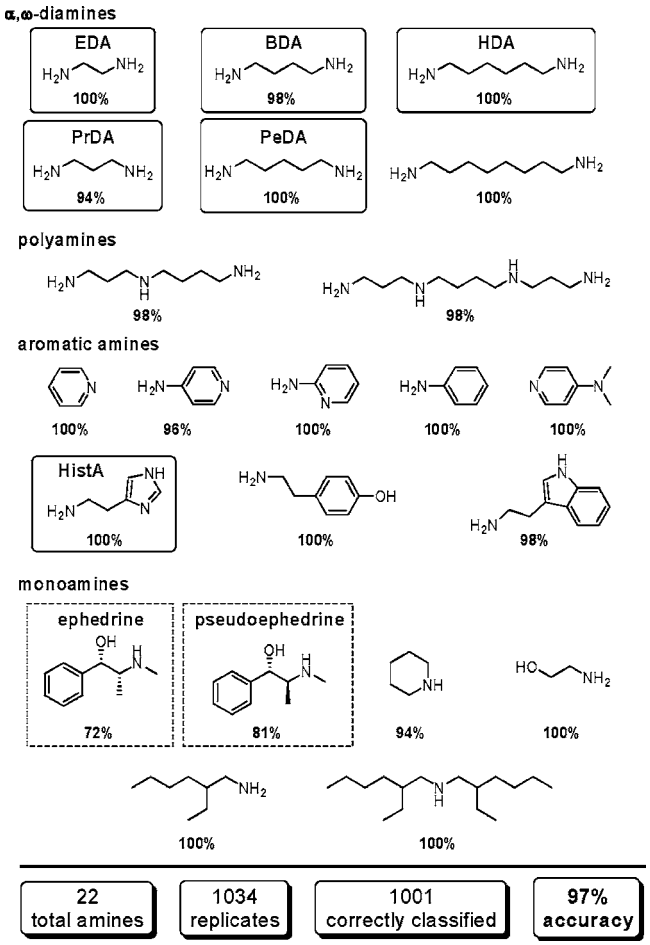

Figure 4. Classes and structures of the amine-containing analytes used in this study. Leave-one-out cross-validation accuracies for each analyte is given below the structure. The six structurally similar diamines used in the initial analysis are encircled, and the two diastereomers are in dashed squares.

To demonstrate the breadth of this system, the number of different amines studied was increased from 6 to 22, based on a range of commercially available aromatic and aliphatic mono-, di-, and polyamines (Figure 4). Forty-seven replicates of each of the 22 amines were examined as described above,

(18) Cuvet data could be combined with the microtiter plate analysis without compromising the quality of the analysis when all data was normalized.

(19) Polymers synthesized at different times, i.e. different batches, could be used without compromising the quality of the analysis when all data was normalized.

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(21) Leave-one-out cross-validation successively removes each sample from the data set while recomputing discriminant functions based on the remaining samples. Classification errors for each sample are then obtained without using that sample to calculate the discriminant model and assessing the model's ability to correctly identify the removed sample.

producing 97% classification accuracy (1001/1034) based on leave-one-out cross-validation analysis. The classification accuracies for each amine are shown below the structure in Figure 4. The greatest error, 20 of the 33 misclassifications, was found in differentiating between the diastereomers ephedrine and pseudoephedrine (structures shown in dashed boxes in Figure 4). If these analytes were combined, and classified as the same target, the classification accuracy rose to 99% (1020/1034).

For final proof of principle, polymer **1** was used to assess the amount of biogenic amine present in a fish sample. The most prevalent biogenic amine found in tuna is histamine; we therefore spiked a tuna sample with histamine to assess whether our system could detect varying amounts of this biogenic amine in a consistent "fish matrix." Standard methods were used to process the fish sample, extracting biogenic amines present with trichloroacetic acid while simultaneously precipitating out undesired proteins.[22]

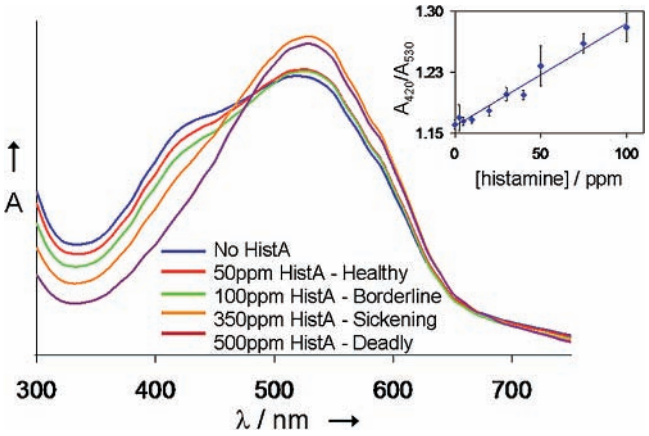

Figure 5. Polymer **1** response to histamine in a tuna fish matrix. Inset: Response factor (A_{420}/A_{530}) showing linearity in the polymer response in the relevant range to detect spoilage.

Figure 5 shows the absorption spectra of polymer **1** responding to increasing amounts of histamine in a "tuna fish matrix." The analysis here uses the same multidimensional response from the polymer, but the data manipulation is different than that described above because this determination does not require identification of the analyte but rather quantification. Recall that the normalization used above removed any concentration information. Therefore, this second analysis was carried out using the raw, unprocessed data. In addition, this analysis could default to more traditional means of ratiometric evaluation rather than relying on statistical models. The inset in Figure 5 shows a linear ratiometric response (A_{420}/A_{530}) with increasing histamine concentrations over the range most useful for detecting food spoilage associated with food poisoning. The sensitivity of the described assay is better than the typical mammalian sense of smell and is able to detect this nonvolatile amine at hazardous levels before the fish would begin to smell rancid.

In summary, the colorimetric, multidimensional response from a single functional conjugated polymer has been used to identify and discriminate between a set of 22 closely related and biologically relevant amines as well as in detecting these compounds in real life assays, such as in fish samples. These parallel studies require two different approaches. First, an array of wavelengths was statistically analyzed using pattern recognition protocols, to identify a large range of compounds, including: aromatic and aliphatic mono-, di-, and polyamines. Second, a ratiometric approach was used to determine the amount of amine present in a spiked tuna sample. We are currently investigating these approaches for the detection of biogenic amines in fish and other foods as a means to determine freshness and quality.

Acknowledgment. This work was supported by Research Corporation (RI1191) and the USC NanoCenter Seed Award. M.S.M. and T.L.N. thank SREB for a Dissertation Year Fellowship. We thank Dr. K. D. Shimizu, Dr. N. T. Greene, and Mr. C. J. Stephenson, all from USC, for helpful discussions.

Supporting Information Available: Procedures for carrying out the assays and data analysis protocols as well as detailed statistical outputs and spectral characterization of the polymers used in the assay. This material is available free of charge via the Internet at http://pubs.acs.org.

OL071065A

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