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Cross-Reactive Conjugated Polymers: Analyte-Specific Aggregative Response for Structurally Similar Diamines

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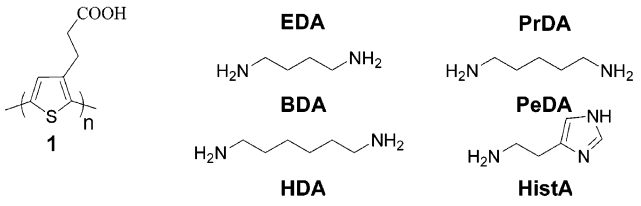
Conjugated polymers have been evaluated for their utility as chemical and biological sensors because of their unique optical and electronic properties,[1] and they have been used for the detection of simple inorganic ions,[2] small organic targets,[3] DNA,[4] and proteins,[5] and even for the determination of enantioselectivity.[6] Traditionally, the principal signal transduction mechanism for these materials has focused on the planarization/deplanarization of the polymer backbone upon interaction of the side-chain functionality with analyte. An additional transduction mechanism, driven by analyte-induced aggregation, results from the interactions between polymer main chains.[7] This approach takes advantage of the multivalent nature of the polymer to assemble aggregate structures not available to small-molecule sensors. This concept has been demonstrated for single-analyte detection[7c] and is applicable for any analyte capable of making multiple contacts with the polymer (i.e., multi-topic analytes). Given the versatility of this latter approach, it is surprising that virtually no other sensory systems are designed on this premise. The approach described herein takes advantage of this analyte-directed polymer aggregation, using the cross-reactive carboxylic acid functionalized poly(thiophene) **1**,[8] to identify and classify structurally similar diamines. Pattern recognition protocols were used to discriminate between similar α,ω-diamines on the basis of the unique optical response from the polymer-diamine assembly, schematically depicted in Figure 1.

Cross-reactive receptors are designed to interact with a certain class of compounds but lack specificity for any one member of the group. Traditionally, cross-reactive sensors are incorporated into sensor arrays for convenient analyses.[9] Polymer **1** is cross-reactive and binds to all basic amines; however, the optical response of the polymer to each analyte is different. Given this analyte-selective response from polymer **1**, a sensor array is not required to differentiate diamine analytes. Instead, the entire spectral response serves as an array of wavelengths that can be used to identify the target analyte.

To illustrate the applicability of this approach, five α,ω-diamines were chosen: 1,2-ethylenediamine (EDA), 1,3-propylenediamine (PrDA), 1,4-butylenediamine (BDA), 1,5-pentylenediamine (PeDA), and 1,6-hexylenediamine (HDA). This series of α,ω-diamines vary successively by only one methylene unit separating the amines. Additionally, histamine (HistA) was used which has the same number of carbons separating the amines as PrDA but is more rigid.



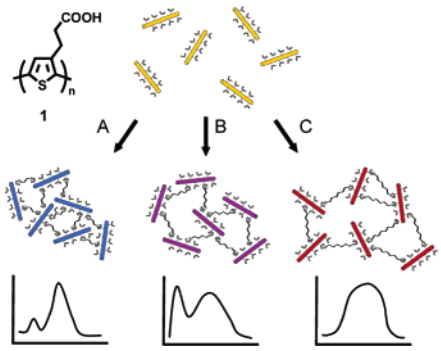


Figure 1. Schematic representation of the aggregative interactions between polymer **1** (colored rods) and different diamine analytes (A−C). Different colored aggregates are formed depending on the added diamine, depicted by the different colored rods and simulated absorbance traces.

The unique chromic response from **1** toward structurally similar diamines results from multiple inter- and intramolecular contacts between the many carboxylic acid side chains on the polymer and the divalent amines via electrostatic and/or hydrogen-bonding interactions. This pairing causes main-chain twisting as one source of signal transduction. Moreover, the polymers may be cross-linked through the bifunctional diamines, causing $\pi-\pi$ interactions between polymer main chains.[10] The length and degree of flexibility of the tether between the two amines will determine the extent of communication between polymer chains.[7a] Both of these interactions influence the absorbance maximum for the polymer. This assembly process also leads to the formation of aggregates capable of scattering light and thereby providing an alternate transduction mechanism.[11] Dynamic light scattering measurements have confirmed the formation of nanometer-size aggregates. The solvent system selected solubilizes the diamine−polymer aggregates, maintaining a homogeneous assay solution while preserving the aggregate response. The spectral variations for the different diamines are subtle, yet there are obvious differences in the polymer response for each analyte (Figure 2a), particularly near the absorbance maximum (due to main-chain twisting and π-stacking) and at long wavelengths (due to aggregative scattering). Consequently, the subtle variations in the overall shape of the absorbance curve, resulting from the collective response of these interactions, are responsible for discrimination between analytes.

In wet acetonitrile solution, the addition of each diamine to a solution of polymer **1** resulted in an immediate change in the solution color from purple to different shades of red. Similar spectral patterns were differentiated and random noise was filtered out using multivariate statistics. Analysis was performed on the response of the polymer across the entire spectrum using nine different wavelengths (i.e., inputs or dimensions) between 420 and 740 nm every 40 nm.[12] This approach reduces the nine-dimensional data set to three dimensions while still containing as much of the

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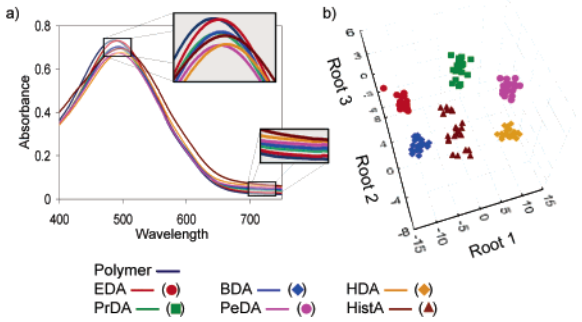

Figure 2. (a) Absorption spectra of polymer **1** (0.4 mM) responding to six diamines (0.5 mM each). Insets: expansion of absorbance plots near the λ_{max} and in the long-wavelength region, highlighting the variation in polymer response to different diamines. (b) Three-dimensional LDA plot of the response of polymer **1** for discriminating these six diamines.

distinguishing features of the original data as possible. Specifically, linear discriminant analysis (LDA), through commercially available software,[13] was used. LDA fundamentally minimizes variation within each diamine group while maximizing differences between each different diamine.[14]

The analysis was performed on spectral data obtained from a constant concentration of polymer (0.4 mM) responding to the six different diamines at five different concentrations (0.5, 1.5, 2.5, 3.5, and 5.0 mM[15]) at a constant temperature for a total of 24 measurements for each amine and 144 measurements overall. All spectral data were normalized such that the area under each absorption spectrum was equal to one. This preprocessing minimizes variation between absolute absorbance values resulting from different analyte concentrations, while at the same time preserving the general shape of the absorption spectrum. Figure 2b shows the projection of the LDA results in three dimensions. Each axis of the LDA plot represents weighted combinations of the nine-dimensional data. Therefore, each point in the plot contains information from the nine wavelengths taken from the spectrum for the specific diamine.

To evaluate the accuracy of this approach, the existing data set was treated as if one measurement was an unknown and a new training set was created. The excluded data was then reintroduced to the data set and classified.[13,16] Each analyte response can then be used as an unknown and the classification accuracy determined for the entire data set. Using this "leave-one-out cross-validation" method, conjugated polymer **1** correctly classified 143 out of 144 measured samples (>99% accuracy). This result is consistent with our hypothesis that identification is not influenced by concentration. It is the shape of the curve (the relative absorbance at multiple wavelengths), not the absolute intensity at any one wavelength, that is responsible for differentiation.

There is also a concentration dependence to the polymer response. At the absorbance maximum for the polymer−analyte complex, the response does not follow a linear trend due to the combined inter- and intramolecular interactions. However, by performing multiple linear regression on the unnormalized absorbance data, the concentration of an unknown sample could be predicted within 15% of the expected value. Diamines have been detected and identified in the nanomolar range (~100 nM).

In summary, we report a simple cross-reactive carboxylic acid-substituted poly(thiophene) capable of producing different and unique chromic responses for a series of structurally similar diamines. Using multivariate statistics to deconvolute subtle variations in the resulting spectra, the analyte can be accurately identified >99% of the time. The spectral response is determined by planarization/deplanarization of the polymer backbone, $\pi-\pi$ interactions between polymer chains, and scattering of visible light. All of these phenomena are caused by multiple polymer−diamine interactions and dictated by the length and rigidity of the tether between the amine moieties. Regardless of the amount of diamine added, identification can be achieved with high accuracy because the response relies on the composite response from the assembly across the entire spectrum and not on variation in a single wavelength. Current efforts are focused on expanding this general approach for the discrimination of biogenic amines associated with numerous disease states including cancer, bacterial infections, and food-borne illnesses.

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Supporting Information Available: Synthesis, assay conditions, and analysis protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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