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Pattern-Based Recognition of Thiols and Metals Using a Single Squaraine Indicator

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Abstract: The design of a sensor array that uses a single entity as both the host and the indicator (squaraine dye, **SQ**) to differentiate a series of metal ions and a series of thiols is reported. The metal ions and thiols act as both analytes and "modulators" of the squaraine response allowing pattern-based discrimination. Mercury(II), palladium(II), copper(II), iron(II), and nickel(II) can be discriminated when combining **SQ** with five thiols: propane thiol (**PT**), 3-mercaptopropionic acid (**MPA**), naphthalene-2-thiol (**NT**), 2,3-dimercaptopropanol (**DMP**), and 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**). Likewise, the five thiols can be discriminated using **SQ** and the five metals. For example, **SQ** in combination with 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**) afforded very good differentiation of all five metal ions. However, propanethiol, 3-mercaptopropionic acid, and naphthalene-2-thiol produced very similar differentiation of the considered metal ions. On the other hand, all metal ions considered in this study are able to discriminate 2,3-dimercaptopropanol (**DMP**) and 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**) clearly and completely, both from one another and from the other three thiols (**PT**, **NT**, **MPA**). Importantly, mercury(II) is the only metal ion able to effect the discrimination of naphthalenethiol (**NT**) from **PT** and **MPA**, thus giving the best discrimination overall. The study shows that complex discrimination of widely diverse classes, metal ions and thiols, can be achieved via a single receptor/indicator.

1. Introduction

The analysis of individual structurally similar analytes routinely requires the synthesis of a unique, highly selective sensor for each type of analyte to be detected, which is often impractical and difficult.[1] As an alternative to highly analyte-specific synthetic receptors, trends in chemical sensing have shifted to the design of new materials and devices that rely on a series of differential chemo- or biosensors that are lacking high specificity. The recognition is achieved by the distinct pattern of responses produced from the combined effect of all the sensors in the array. To analyze the cumulative array responses, chemometric pattern recognition tools are employed to decrease the dimensionality of the array response to simple patterned responses that are comprised of all the individual responses. For example, Principle Component Analysis (PCA) is one such chemometric tool.[2] These array sensors take their inspiration from nature, by mimicking receptors in the mammalian tongue and nose for the senses of taste and smell. To this end much work has been done in both vapor phase (noses) and solution phase (tongues).[3−6]

There are numerous examples in the literature, including those from our own group, on arrays and assays for differential sensing of various analytes using synthetic and commercially available components. For example, we have exploited differential array studies involving the recognition and discrimination of nucleotide phosphates,[7] proteins and glycoproteins,[8] and tripeptides and tripeptide mixtures.[9] Sensor arrays have been used by Hamilton for pattern recognition of proteins.[10] The utility of molecular imprinted polymers in sensor arrays was demonstrated by Shimizu.[11] In this later case an eight-member sensor array was used to discriminate six different aryl amines. Quite recently Rotello has targeted bacteria effectively using gold nanoparticles and conjugated polymers.[12] His work has demonstrated the ability to differentiate between species of bacteria as well as between strains of a single species, without the use of antibodies or radioactive markers. In another approach Anzenbacher et al. utilized conjugated chromophores attached to 8-hydroxyquinoline to yield a different response to various metal ions.[13] One aspect of each of these studies is the use of a series of independent separate receptors for the discrimination of analytes.

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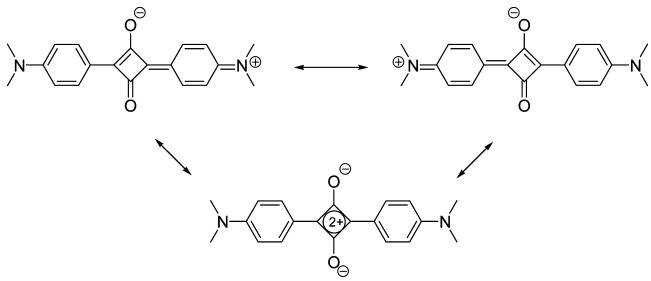
Scheme 1. Resonance Structures of Squaraine



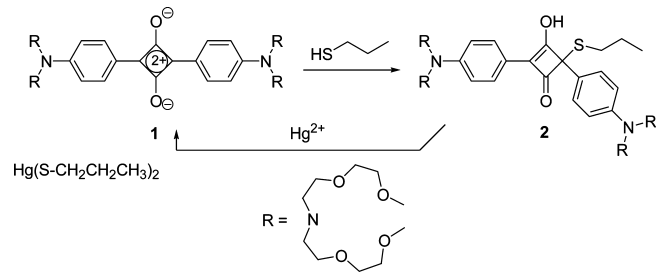
Lavigne has recently reported a method for stepwise detection and identification of metal ions and diamines using a carboxylate functionalized poly(thiophene) as a single receptor.[14−16] The use of a single receptor/indicator would simplify the array development. This could be accomplished with a binding/signaling unit which self-assembles with modulators, where the "modulator" imparts the diversity and differential recognition characteristics needed in array sensing. The advance embodied in this concept is the use of spontaneous assembly of the binding/signaling unit with the modulator, thereby lowering the amount of synthesis needed to create a suite of receptors. Herein we report that squaraine dyes can act as such a binding/signaling unit.

Squaraines are a class of dyes with a resonance stabilized zwitterionic structure.[17] These dyes are symmetric conjugated polymethine dyes formed by a central four-member squaric acid ring and two substituted aniline moieties at the 1 and 3 positions of the cyclobutadienyl ring. A representative example is shown in Scheme 1. Squaraines typically contain an electron-deficient central four-membered ring and two electron-donating groups arranged in a donor−acceptor−donor (D−A−D) pattern.

Squaraines exhibit strong absorption ($\epsilon > 10^5$ L mol^{-1} cm^{-1}) in the visible region and are also fluorescent.[17] The squaraine dye color results from a charge transfer band between the donor anilinium moieties and the central acceptor four-membered ring. Most of these dyes emit in the visible to near-IR region.[18,19] NIR absorbing organic dyes are important in the imaging of biological specimens.[20] Therefore, squaraine dyes are good candidates for biological applications.[21] These organic dyes have been studied and used intensely for a wide variety of applications. Some of the applications are imaging,[22] nonlinear optics,[23] and ion sensing.[24]

The electron-deficient central cyclobutene ring of squaraine dyes is susceptible to nucleophilic attack which breaks the conjugation of the dye, resulting in bleaching. Martinez-Manez and Rurack have extensively investigated the use of squaraine derivatives for the development of chromogenic sensors for cations and anions by exploiting the electron-deficient central ring that undergoes nucleophilic attack. Based on this principle, chemodosimeters for mercury, cyanide, and thiols have been developed.[25] In the approach to develop a chemodosimeter assay for Hg^{2+} ions, Martinez-Manez designed a method where squaraine **1** was allowed to participate in a reaction with a thiol. The thiol acts as a "spectroscopic inhibitor" that switches off the absorption and fluorescence of the squaraine (Scheme 2).[21] Herein we define each thiol as a "modulator". This addition product is the chemodosimeter. The target ion Hg^{2+} then reacts with the inhibitor, liberating the dye. The signaling was accomplished by a metal-induced dye release method. The method is based on the thiophilic nature of the Hg^{2+}, and as a result, it regenerates the original dye which allows recycling of the squaraine. Like mercury, palladium is a sufficiently thiophilic metal that it can also easily scavenge the thiol. We have previously shown that palladium salts, $Pd(OAc)_2$ and $PdCl_2(PPh_3)_2$ which are extensively used in cross-coupling reactions, will likewise switch "on" the color of the squaraine.[26]

Scheme 2. Chemodosimeter for the Detection of Hg^{2+}



2. Results and Discussion

2.1. Design Criteria. Our goal in this research project was to develop a sensor array using a single squaraine dye, which could differentiate both metal ions and a series of thiols in an array format. A chemodosimeter is based on the use of a selective, irreversible reaction, which is induced by the target species and gives rise to an observable signal in fluorescence or in color. The general strategy we use in the individual elements of our assay is shown in Figure 1. In essence, there are two analytes that modulate the signal of the squaraine. One is via a reversible covalent interaction with a thiol (shown as a pink box), which turns off the signal. The metal analyte (shown as a green triangle) undergoes reversible binding with the thiol and, hence, also modulates the squaraine signal by turning it on. Scheme 3 shows the specific chemical identity of the modulators and receptor (**SQ**). Unlike the previous studies introduced above, we choose to study the fluorescence response because of the potential increase in sensitivity. Palladium, mercury, nickel, copper, and iron were used in the sensor array as proof of principle metals, because each has different

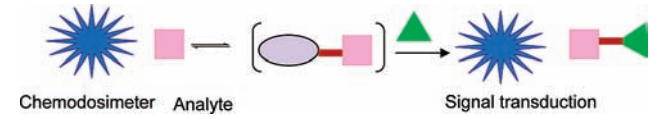


Figure 1. Schematic representation of how the chemodosimeter functions. Blue star = receptor with blue color, pink square = thiol, green triangle = metal.

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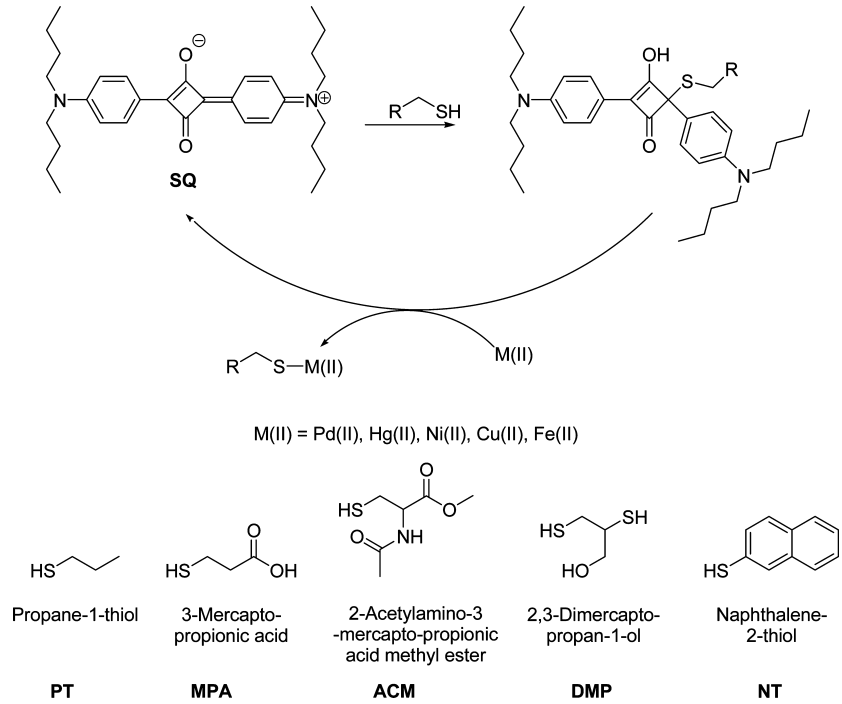
Scheme 3. Squaraine/Thiol/Metal Interaction and Metals and Thiols Used in the Array Design



thiophilicities. As the functional group of the amino acid cysteine, the thiol group plays an important role in biological systems. Recently, the ability of cysteine and cysteine derivatives to act as biomarkers has begun to be explored, and their determination in biological fluids has gained importance in clinical chemistry.[6] In this respect, the development of straightforward, low-cost, and undemanding probes for the determination of biomarkers such as total aminothiols in blood is attracting interest.[27]

The five different thiols used for this array are shown in Scheme 3: propanethiol, 3-mercaptopropionic acid, 2-acetylamino-3-mercaptopropionic acid methyl ester, 2,3-dimercaptopropanol and naphthalene-2-thiol. These thiols were picked among those commercially available, with the intention of increasing the chemical diversity. They were chosen such that each thiol is sufficiently different from one another in their structure and functionality to ensure variation among analytes: propanethiol having one thiol group; mercaptopropionic acid, a thiol with a acid functionality; 2-acetylamino-3-mercaptopropionic acid methyl ester, a thiol with a ester group; 2,3-dimercaptopropanol, having two thiol groups; and naphthalene-2-thiol, a thiol with an aromatic group.

2.2. Fluorescence Titrations with Thiols. The first goal was to demonstrate fluorescence turn off of the **SQ** response with added thiol. Two thiols, propanethiol and mercaptopropionic acid, were used for preliminary fluorescence titrations and construction of calibration curves to reveal potential differences among only the thiols. To maximize the nucleophilicity of the thiols, 1 equiv of Hunig's base was added to each thiol in DMSO to assist the nucleophilic attack, by scavenging the proton created. Each thiol solution was added in aliquots separately to a solution of **SQ** in DMSO. Fluorescence titrations and calibration curves for both propanethiol and mercaptopropionic acid are shown in Figures 2 and 3, respectively. They are almost identical, indicating that the affinities of thiols to **SQ** are essentially the same.

2.3. Fluorescence Titrations of Squaraine/Thiol Complexes with Metals. The second goal was to discover if different metals responded differently to a single **SQ**/thiol complex. However, as a third goal we sought to discover if different metals would respond differently to different **SQ**/thiol complexes. This third goal would lend credence to the postulate that **SQ**/thiol complexes could pattern metals and vice versa. Palladium and mercury metals were used as the test metals for the construction of calibration curves with **SQ**/thiol complexes. Propane thiol (**PT**) and mercaptopropionic acid (**MPA**) were used as the test thiols. First, a 1:1 complex of squaraine and propanethiol was prepared by adding 1 equiv of propanethiol and Hunig's base in DMSO to a solution of **SQ** (2×10^{-6} M) in DMSO. This complex will be referred to as **SQ/PT**. Complex formation was monitored by disappearance of the fluorescence signal of **SQ** after a 10 min waiting period. The **SQ/PT** complex was then titrated separately with two metal salts, $Pd(NO_3)_2$ and $Hg(OAc)_2$ (Figures 4 and 5 respectively). In a similar fashion the **SQ/MPA** (**MPA**, mercaptopropionic acid) complex was prepared and titrated with $Pd(NO_3)_2$ and $Hg(OAc)_2$ (Figures 6, 7), respectively.

Upon addition of the palladium(II) salt or mercury(II) salt to **SQ/PT**, the band at 680 nm increased, indicating the switching on of the analytical signal. The isotherms obtained for the titration experiments are sigmoidal in shape for both Pd(II) and Hg(II). In the equilibrium conditions prevalent at the start of the titration, thiol complexation to the squaraine is not complete; a small fraction of the thiol is free in solution. The initial aliquots of metal ion added thus are first bound by the free thiol in solution. As the metal ion scavenges the free thiol in solution,

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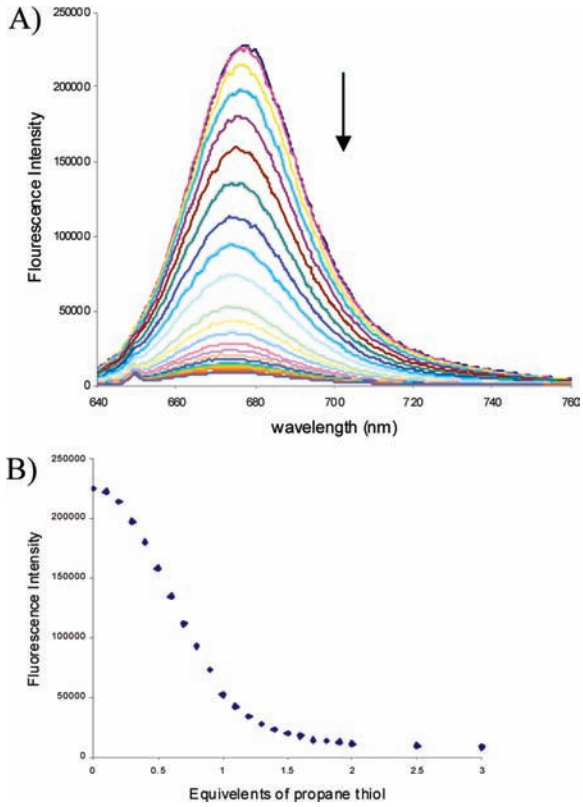


Figure 2. Titration of a solution of propanethiol and Hunig's base (4×10^{-5} M) in DMSO into a solution of **SQ** at 2×10^{-6}M in DMSO. A) Spectra. B) Isotherm showing decrease of fluorescent intensity of **SQ** with added propane thiol.

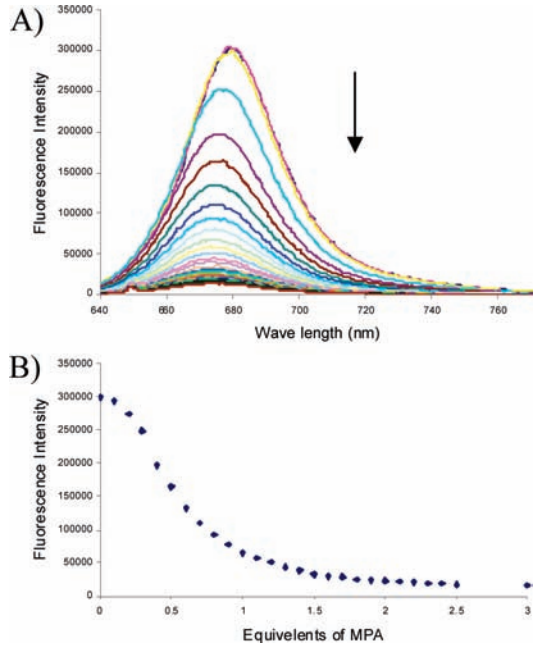


Figure 3. Titration of a solution of mercaptopropionic acid and Hunig's base (4×10^{-5} M) in DMSO into a solution of **SQ** at 2×10^{-6} M in DMSO. (A) Spectra. (B) Isotherm showing decrease of fluorescent intensity of **SQ** with added mercaptopropionic acid.

this process influences the equilibrium for the **SQ/PT** complex, causing the complex to break down. The overall resulting effect is the removal of the thiol from the **SQ/PT** complex and switching on of the fluorescence signal.

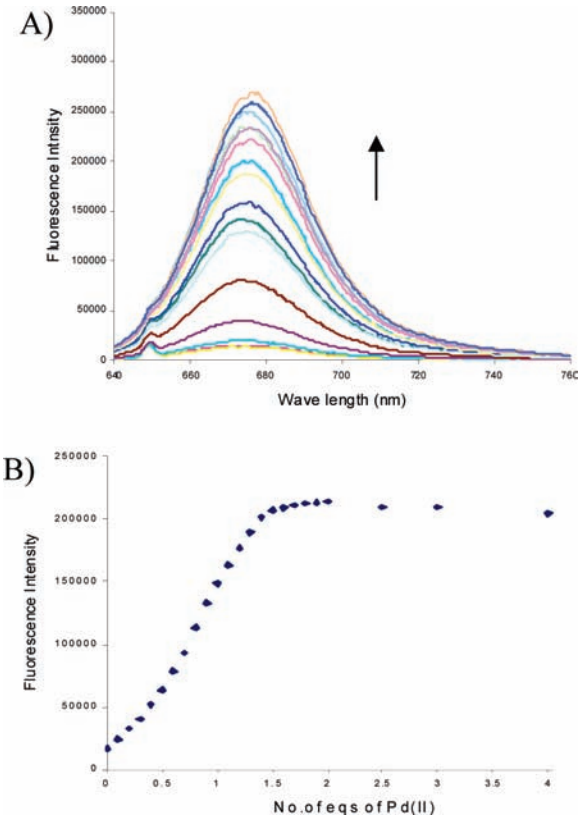


Figure 4. Titration of a solution of $Pd(NO_3)_2$ 4×10^{-5} M in DMSO into a solution of **SQ/PT** complex 2×10^{-6} M in DMSO. (A) Spectra. (B) Isotherm showing increase of fluorescence intensity of **SQ/PT** complex with added Pd(II).

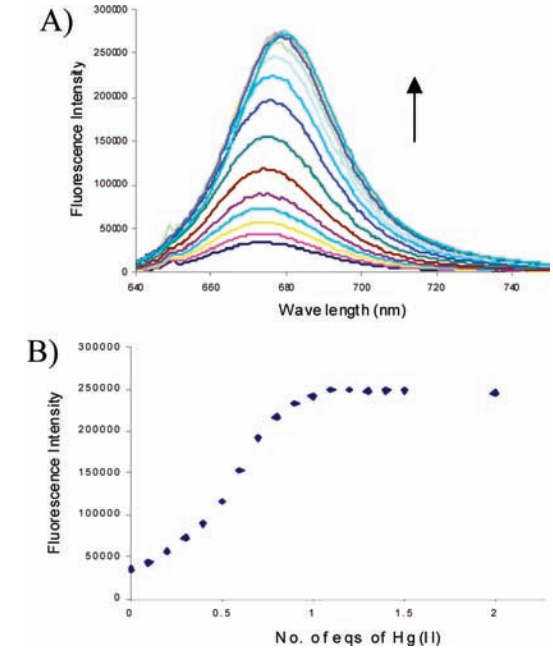


Figure 5. Titration of a solution of $Hg(OAc)_2$ 4×10^{-5} M in DMSO into a solution of **SQ/PT** complex 2×10^{-6} M in DMSO. (A) Spectra. (B) Isotherm showing increase of fluorescence intensity of **SQ/PT** complex with added Hg(II).

According to the isotherm in Figure 4, the observed saturation behavior indicates that approximately 1.5 equiv of Pd(II) added to **SQ/PT** are required to completely turn on the fluorescence by scavenging the propanethiol. In the case of the mercury(II)

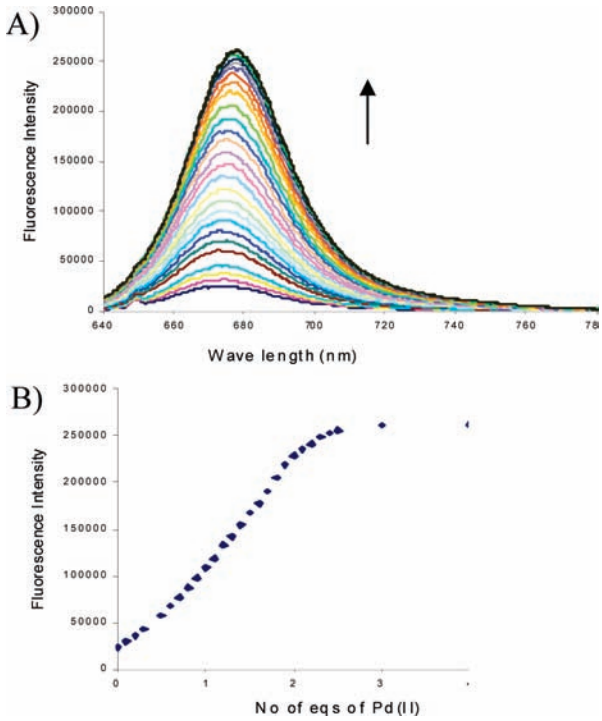


Figure 6. Titration of a solution of $Pd(NO_3)_2$ 4×10^{-5} M in DMSO into a solution of **SQ/MPA** complex 2×10^{-6} M in DMSO. (A) Spectra. (B) Isotherm showing increase of fluorescence intensity of **SQ/MPA** complex with added Pd(II).

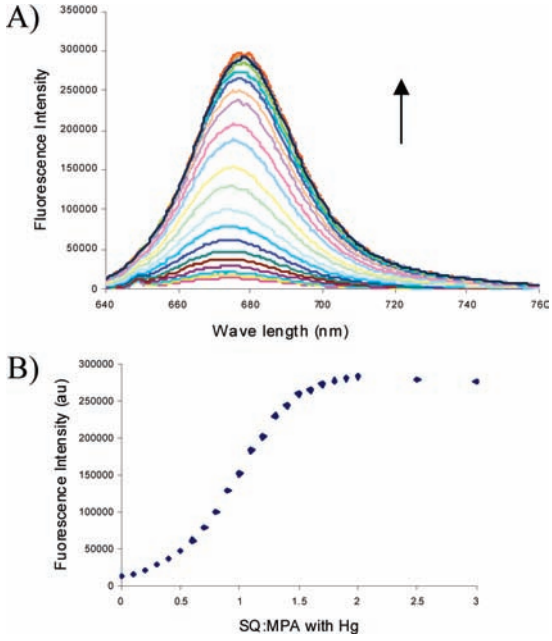


Figure 7. Titration of a solution of $Hg(OAc)_2$ 4×10^{-5} M in DMSO into a solution of **SQ/MPA** complex 2×10^{-6} M in DMSO. (A) Spectra. (B) Isotherm showing increase of fluorescent intensity of **SQ/MPA** with added Hg(II).

titration, addition of only 1 equiv of metal ion is sufficient to completely scavenge the thiol, as indicated by saturation of the fluorescence signal (Figure 5). Taking into consideration the concentrations of metal ions necessary to reach saturation suggests that mercury(II) has a better affinity for propanethiol than palladium(II). Most important for our goals, the saturation and shape of the isotherms are different for the two metals.

In analogy to the titrations of the **SQ/PT** complex with Hg(II) and Pd(II) salts shown above, the similar **SQ/MPA** complex was titrated with the same two metals. Similar, but different, behavior was observed. For example, the fluorescence signal was switched on upon addition of mercury(II) and palladium(II) salts (Figures 6 and 7). In addition, both isotherms were sigmoidal in shape. However, the isotherm in Figure 6 shows that approximately 2.5 equiv of Pd(II) are required to get complete reviving of the fluorescence signal in the titration of **SQ/MPA**. On the other hand, approximately 1.5 equiv of Hg(II) are required to completely scavenge the thiol and liberate the squaraine from the **SQ/MPA** complex (Figure 7). Again, mercury(II) was found to have a better affinity toward the thiol than Pd(II). Importantly, the isotherms, when using **MPA**, are different for the two metals than those formed when using **PT**. Therefore, these preliminary studies showed that patterns of metals and thiols may be obtainable in an array setting.

The isotherms reveal that both metals show higher affinity to **PT** than **MPA**. As discussed earlier the affinities of **PT** and **MPA** to **SQ** are similar, and therefore the increased metal affinity to **PT** is not due to its weaker affinity to **SQ**. Although **MPA** could chelate the metals, this does not increase its affinity over **PT**. Hence, it is possible that some fraction of added metal coordinates the carboxylate of **MPA**, leading to the requirement of more metals to scavenge all the thiol. Alternatively, the weakly electron-withdrawing nature of the carboxylate makes **MPA** a weaker ligand in a monodentate fashion. This is our preferred postulate for reasons given below.

2.4. Metal Discrimination. Having these preliminary results in hand we turned to an array analysis. Squaraine, the five different thiols, and five metal ions were arranged in an array format to identify any patterns generated using a 96-well plate reader. The use of a single receptor to analyze two different sets of analytes is quite unique to this recognition process.

The first 5 rows of 96-well plates were loaded with 200 μL of 2×10^{-6} M solution of **SQ** in DMSO. We define a row along the 8-well side of the plate. Then to each individual row 50 μL of 8×10^{-6} M solution of a different thiol in DMSO were loaded to make **SQ/T1**, **SQ/T2**, **SQ/T3**, **SQ/T4**, and **SQ/T5**. The fluorescence was recorded. Next, the first 6 columns of the plate containing **SQ**/thiol complexes were loaded with 50 μL of 8×10^{-6} M solution of a single metal in DMSO, while the second 6 columns were treated with 50 μL of 8×10^{-6} M solution of a different metal. This fills all 12 columns in the plate. The total volume of each well is 300 μL, with a final analyte concentration of 1.33 μM. The 96-well plate was then submitted to measurements of fluorescence intensity. The fluorophore solutions were excited using a tungsten light source with a 645/15 bandwidth filter. The emission radiation was taken by passing through a 680/30 bandwidth filter. This process was repeated with the other metals. The ratio of equivalents of reagents for this particular data set is 1**SQ**:1**T**:1**M** (**SQ**, squaraine; **T**, thiol; **M**, metal).

Principal component analysis was used to reduce the dimensionality of the data set. The first PC axis lies along the line of maximum variance; subsequent PC axes define diminishing levels of variance. Separation between data points on a PC plot describes how different they are from one another. Ideally multiple trials of the same analyte will cluster very closely and a different analyte will generate a different cluster of data points, well separated in space from the first.

Figure 8 shows how the complex between propanethiol and **SQ** is able to discriminate the five different metals. The

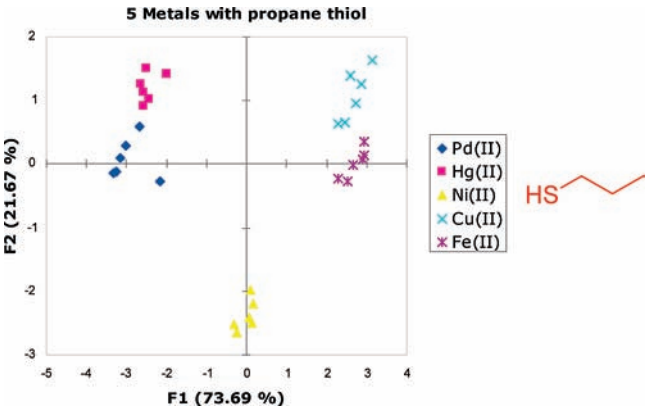


Figure 8. Pattern-based recognition of five metals with propanethiol.

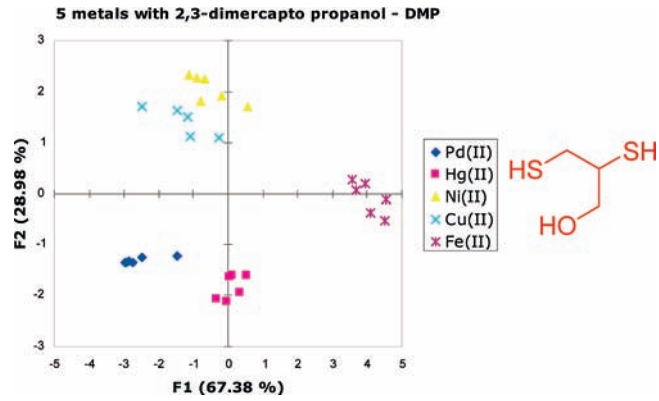


Figure 9. Pattern-based recognition of five metals with 2,3-dimercaptopropanol.

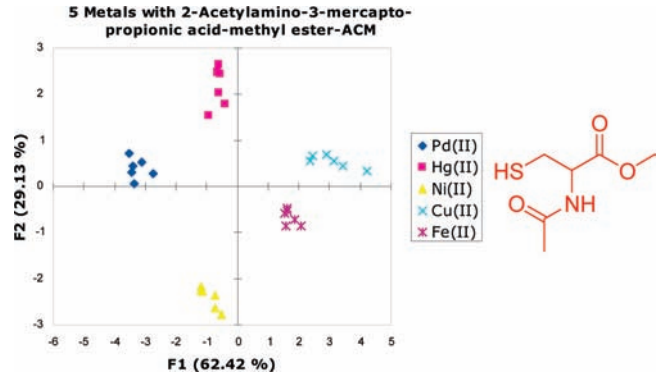


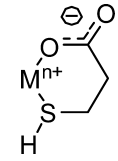
Figure 10. Pattern-based recognition of five metals with 2-acetylamino-3-mercaptopropionic acid methyl ester.

horizontal axis has 73.69% weight, whereas the vertical PC2 axis has 21.67% weight. This two-dimensional PCA plot effectively separates Hg(II), Pd(II), Cu(II), and Fe(II) from Ni(II) along the PC2 axis. Pd(II) and Hg(II), Cu(II) and Fe(II), and Ni(II) separate into three groups along PC1. It is also clear that Ni(II) has its own place in the PC plot and is clearly different from the other four metal ions considered.

Hg(II) and Pd(II), as well as Cu(II) and Fe(II), are clustered together along the PC1 axis. It was shown above in the calibration curves that Hg(II) and Pd(II) have comparable affinity toward propanethiol, and therefore they cluster close to each other in the PCA. However, although Hg and Pd cluster in close proximity, there is still acceptable spatial separation between the two. Our postulate is that separation arises from the slightly different ratios of Hg(II) and Pd(II) that are required to completely switch on the squaraine fluorescence (Figures 6 and 7). Cu(II) and Fe(II) are less thiophilic than Hg(II) and Pd(II) and cluster in close proximity to each other, well separated from Hg(II) and Pd(II).

A comparison of the behavior of propanethiol (Figure 8) with that of naphthalenethiol (Supporting Information) when complexed to **SQ** does not show any significant difference in the relative importance of the first principle components, with the five metals in the same relative positions in PCA space. This observation indicates that the variance between binding the metals with the two simplest thiols is essentially the same. However, it is known that an aromatic thiol is significantly more acidic than an aliphatic one: for instance the pK_a of benzenethiol[28] in DMSO has been measured to be 10.3, whereas *n*BuSH[29] has a pK_a of ~17.0 in the same solvent. This makes an aliphatic thiol a stronger Lewis base. A comparable difference in acidity is likely to be found between propanethiol and naphthalenethiol in our solvent system. However, because the PCA plots are similar, the differences between the binding of the metals to the two thiols must be proportional, even if the thiol pK_a's and metal coordination affinities are different.

Complexes of mercaptopropionic acid also show similar PCA plots to those for **PT** and naphthalene thiol (**NT**) with **SQ** for the five metals (see Supporting Information). The fact that **MPA** makes a pattern similar to those for the monodentate ligands **PT** and **NT** again supports it acting as a monodentate ligand. This leads us to tentatively exclude a bidentate coordination mode as dominant (shown below) or indeed present at all, with the considered transition metal ions. Or, if the coordination is present it does not change the variance between the metal affinities for the thiols significantly.



The PCA plot obtained from 2,3-dimercaptopropanol (**DMP**) and the five metals (Figure 9) also shows good spatial separation but a different pattern. The five metal ions have the same general PCA positioning observed as for propanethiol with the notable exception of Cu(II), whose data cluster ends up in a significantly different relative position in the principal component space. Besides the positive implication for an envisaged application of our detection method, the observation suggests that Cu(II) displays a different coordination behavior with the present hydroxo-dithia ligand and also that it is the only metal ion to do so. Because 2,3-dimercaptopropanol possesses two thiol groups and an alcohol functionality, it has differing degrees of interaction with the transition metal ions. In this case, the horizontal PC1 axis has 67.38% weighting, whereas the vertical PC2 axis represents 28.98% of the overall weighting. Again, Hg(II) and Pd(II) ions cluster in close proximity along the PC1 axis, whereas Ni(II) and Cu(II) show similar binding characteristics. Fe(II) is now found isolated. There is a possibility that once the thiol is liberated from the **SQ**/thiol complex, the metal ion forms coordination complexes with the thiol as shown

(28) Bordwell, F. G.; Hughes, D. L. *J. Org. Chem.* **1982**, *47*, 3224–3232.
(29) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456–463.

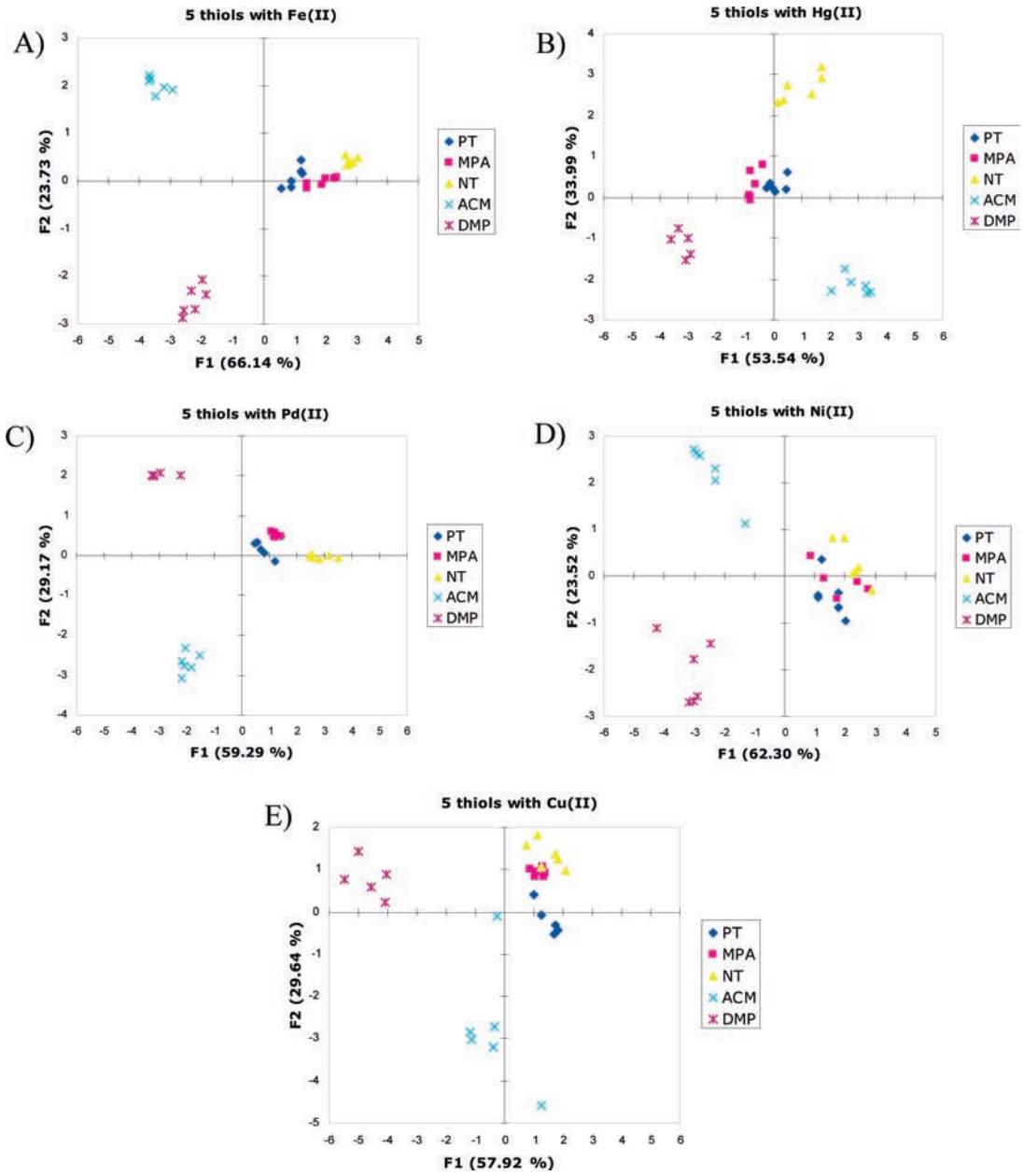
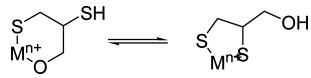
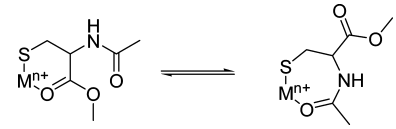


Figure 11. PCA plots showing clustering of the analytes illustrating the ability of differential array to discriminate various thiols. (A) Discrimination of thiols with Fe(II). (B) Discrimination of thiols with Hg(II). (C) Discrimination of thiols with Pd(II). (D) Discrimination of thiols with Ni(II). (E) Discrimination of thiols with Cu(II).

below. The degree of formation of these complexes varies according to the type of metal, and as a result we see a distinct separation in the PCA plot.



By far, the best pattern based discrimination for the five metals was obtained with the complex of 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**) with **SQ**, as seen in Figure 10. In this plot the horizontal PC1 axis has 62.42% weighting, whereas the vertical PC2 axis has a 29.13% weighting. This greater distribution of the weighting results in better spatial distribution of the analytes in all four quadrants of the plot. In analogy to the previous case, the free 2-acetylamino-3-mercaptopropionic acid methyl ester is also capable of chelating the metal ion as shown below. The different stabilities of such complexes with different metal ions provide a further means of differentiation, resulting in better separation in the PCA plot. The PCA plot suggests that the versatility of this ligand and the different coordination tendencies toward the investigated metal ions result in a highly differential response, key to the observed excellent discrimination capabilities of this system.



2.5. Thiol Discrimination. Thiol discrimination was carried out using the same data collected for the analysis discussed above. PCA plots were created to distinguish the five thiols by pairing the different metals, Fe(II), Hg(II), and Pd(II)

(Figure 11A−C), with **SQ**. Ni and Cu show some overlap of thiols in their PCA plots (Figure 11D and E). One similarity that we see in all five plots is that propanethiol, mercaptopropionic acid, and naphthalene thiol cluster in close proximity showing a similar reactivity with each metal (Figure 11). This behavior is complementary to the fact that these three thiols showed similar patterns in the recognition of the five different metals.

As would be expected from the discussion of the metal discrimination patterns presented above, the two simple aliphatic thiols (propanethiol, **PT**; 3-mercaptopropionic acid, **MPA**) could not be well discriminated by any of the considered metal ions. On the other hand, all the metal ions used in this study are able to discriminate 2,3-dimercaptopropanol (**DMP**) and 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**) very clearly from one another and from the other three thiols (**PT**, **NT**, **MPA**). In addition to this, mercury(II) is the best metal ion to effect the discrimination of naphthalenethiol (**NT**) from **PT** and **MPA** and, thus, gives the best discrimination overall (Figure 11B).

3. Summary and Conclusion

In summary, the squaraine system in combination with 2-acetylamino-3-mercaptopropionic acid methyl ester (**ACM**) afforded very good differentiation of five metal ions, as evidenced by the clearly separated clusters in Figure 10. Differentiation between different thiols has also proven successful, with the system being able to discriminate 2,3-dimercaptopropanol (**DMP**) and methyl 2-acetamido-3-mercaptopropanoate (**AMP**), both from one another and from the other three thiols (**PT**, **NT**, **MPA**) considered in this study. Further, the results presented show how powerful a single receptor/sensor system can be when used in combination with data-reducing chemometric tools.

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Supporting Information Available: Experimental details, calibration curves, PCA plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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