Differential Receptors Create Patterns Diagnostic for ATP and GTP

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Historically, chemical sensing has required the creation of a highly selective receptor for each component to be detected within a complex mixture. A more recent approach has been to use devices that rely on a series of chemo- or biosensors where analysis of complex mixtures arises from patterns produced by the combined response of all the sensors in the array.[1] This approach has been particularly successful for vapor-phase analysis.[2]

Patterns can also be created that are diagnostic for single analytes.[3] We previously postulated that a series of random combinatorial receptors biased toward a class of analytes would be effective in an array setting.[4] To this end, we describe a sensing method utilizing a combinatorial library of receptors that can differentiate between highly structurally similar analytes, such as nucleotide phosphates, in water.

The receptors consist of a rationally designed core with a binding cleft possessing guanidinium groups (Figure 1A). These guanidinium groups impart an affinity for nucleotide triphosphates and are appended with tripeptides to incur differential binding properties. A previously reported screening of this same 4913-member library led to the identification a sensor (tripeptide Ser-Try-Ser) that was highly selective for adenosine 5′-triphosphate (ATP) over guanosine 5′-triphosphate (GTP).[5] The objective of this work is to determine whether the patterns generated by an unscreened library of receptors in an array can discriminate between structurally similar compounds, using ATP and GTP as test cases.

Thirty beads from the library were randomly chosen, given an index number, and placed in a micromachined chip-based array platform that has been previously reported.[6] An electron micrograph of a representative 3 × 4 array platform is shown in Figure 1C. Sample delivery to the chemosensors occurs using a previously described flow cell.[6] A schematic of the flow cell is shown in Figure 1D. The sample is introduced over the array and passes around and through the beads to exit the bottom of the wells. Red, green, and blue (RGB) transmitted light intensity values were recorded for each bead in the array over the period of the assay via a charge-coupled device (CCD).

The signaling protocol used with the array platform was an indicator-displacement assay similar to those exploited in many of our single-analyte sensing schemes.[7] A schematic of the indicator-displacement scheme for this system is shown in Figure 1B. To impart color to the library members, an anionic chromophore, fluorescein (2), was introduced into the array containing different members from the combinatorial library of receptors. The cationic receptors (1) associate with the indicator, bringing about a distinct orange color to each bead. Blank beads show no orange color, indicating little indicator uptake. Upon exposure to solutions of nucleotide phosphates, the analyte displaces the indicator, fluorescein, at different rates (Figure 2A and 2B) and each bead loses color.

The RGB intensity values for the 30 library beads in the array are recorded over time after a 2-mL injection of a 20 mM sample
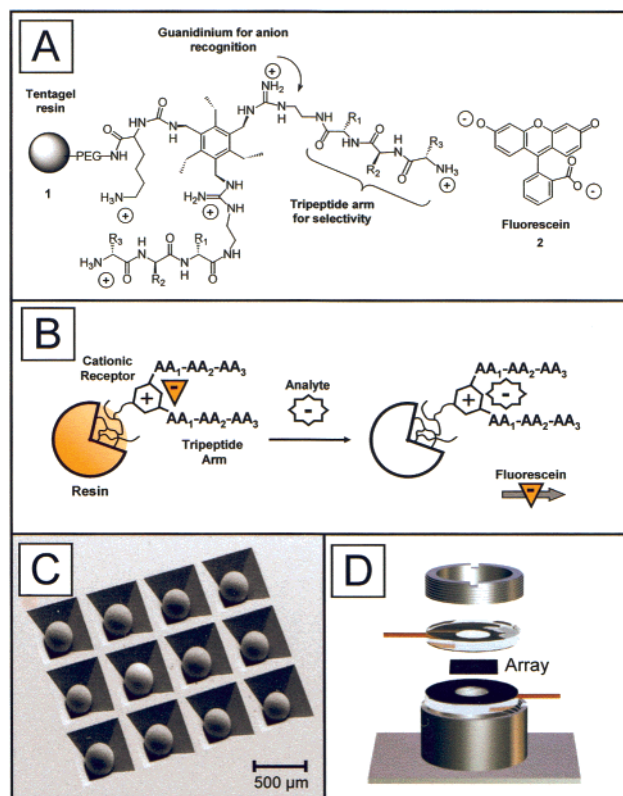
Figure 1. A microscopic to macroscopic representation of the sensing protocol. (A) General molecular structure of resin bound library of receptors (**1**) and fluorescein (**2**). (B) Signal transduction scheme used to detect nucleotide phosphates within the resin bound sensor. AA_n = amino acid. (C) Scanning electron micrograph of a representative 3 × 4 micro-array containing glass beads. (D) Design of flow cell used in experiments.

of ATP, GTP, or adenosine 5′-monophosphate (AMP) in 25 mM HEPES buffer (pH 7.5). Three trials were performed for ATP, GTP, and AMP for a total of nine trials, and absorbance values were calculated by taking the negative log of the ratio of the blue channel intensity over the red channel intensity for each bead.[6] In Figure 2, A and B show a representative normalized absorbance trace for two of the beads in the array after an injection of GTP and AMP, respectively. These traces reveal that each chemosensor responds differently to various nucleotide phosphate samples. The slope of the absorbance values from 210 to 435 s was calculated for each bead in each sample, and these values were used for analysis because the indicator displacement rates were found to be most reproducible in this region. The slope of bead 28 differs by 40% between the AMP and GTP trials, whereas the slope value for bead 23 in each plot differs by only 26%. Although these slopes are easily differentiated by qualitative visual inspection, the rates of displacement for several trials can be compared more quantitatively by using pattern recognition algorithms.
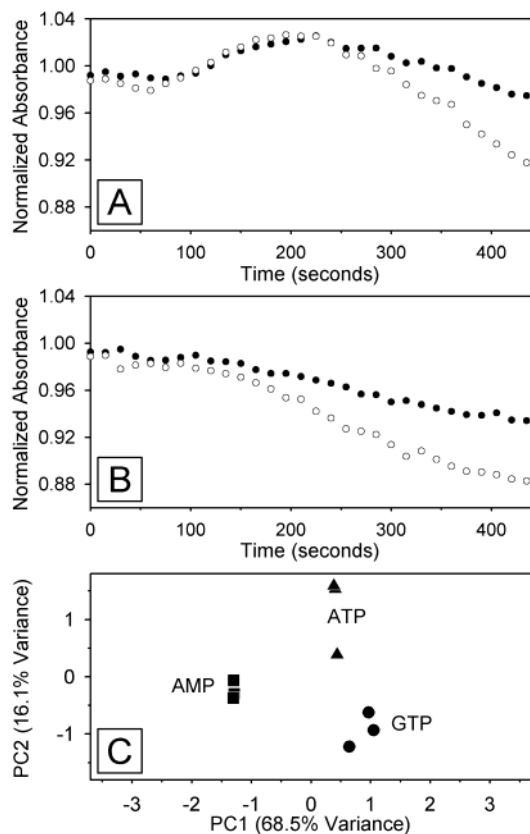
Figure 2. Absorbance values over time and principal component plot. To aid slope comparison, the plots were shifted so that the average of the initial 30 data points prior to sample injection (not shown) had an absorbance of 1.00. (A) 20 mM GTP sample in 25 mM HEPES buffered aqueous solution (pH 7.5) while eluting with 25 mM HEPES buffer (pH 7.5) ● Bead 23 and ○ Bead 28. (B) 20 mM AMP sample in 25 mM HEPES buffered aqueous solution (pH 7.5) while eluting with 25 mM HEPES buffer (pH 7.5) ● Bead 23 and ○ Bead 28. (C) PCA score plot of the three trials for each nucleotide phosphate samples (▲ ATP, ■ AMP, and ● GTP).

Table 1. Sequencing Results and Factor Loading Values for the First Two Principal Components (PC1 and PC2)

tripeptide sequence	factor loading (PC1)	factor loading (PC2)	bead index number
Asp-Asn-Ser	−0.98	0.14	28
Phe-Trp-Phe	−0.88	−0.16	23
Thr-Thr-Ser	−0.88	0.39	33
Val-Asn-Tyr	−0.84	−0.20	24
Val-Pro-Ala	0.01	0.21	14
Ala-Met-Thr	0.17	−0.91	34
Val-Gly-Ile	0.74	−0.46	6
Ser-His-Tyr	0.75	−0.47	25
Thr-Thr-Ile	0.89	−0.36	2

chosen to be sequenced on the basis of their large and nearly overlapping factor loading values (Table 1). Bead 14, which possessed the lowest values, was sequenced to elucidate which receptors elicit minimal discrimination. It is interesting to note that the beads with high loading values possess serine (or the structurally similar amino acid threonine) and other aromatic amino acids, as found in our earlier screening of this library.[5] The role of these amino acids in differentiating nucleotide phosphates is still under investigation.

In conclusion, the use of a combinatorial library of sensors and indicator-displacement assays in the context of a chip-based array allows for the differentiation of structurally similar analytes in aqueous solutions. The coupling of supramolecular chemistry principles with pattern recognition leads to this protocol for sensing. We are currently analyzing mixtures of analytes and other phosphate derivatives as a means to explore cross-reactivity between individual library members.

Supporting Information Available: Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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Principal component analysis[8] (PCA) was utilized to reduce the dimensionality of the data set. For each of the nine trials, a slope value was recorded for each of the 30 beads. To identify patterns in the data, a principal component (PC) axis is calculated to lie along the line of maximum variance in the original data. Subsequent PC axes lie along lines describing diminishing levels of variance. The coordinates of the sample relative to the PC axes are termed scores and can be used as an indicator of correlation between analytes. Proximity in space on a score plot directly correlates to similarities in indicator displacement rates. Figure 2C shows a two-dimensional score plot for the first two principal components (PC1 and PC2) with clustering of the AMP, GTP and ATP samples with one possible ATP outlier. PCA demonstrates that the array of library sensors can differentiate between structurally similar molecules such as AMP, GTP, and ATP.

Factor loading values can be used to evaluate the individual sensors in the array. The values correspond to the cosine of the angle between a principal component axis and the original variable axis. Therefore, similar loading values correspond to similar sensor responses. Furthermore, loading values approaching 1 or −1 indicate that the individual sensor played a significant role in the formation of a particular component axis. Eight beads were