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Differential Sensing Using Proteins: Exploiting the Cross-Reactivity of Serum Albumin To Pattern Individual Terpenes and Terpenes in Perfume

Michelle M. Adams and Eric V. Anslyn*

Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas 78712

Received September 30, 2009; E-mail: anslyn@austin.utexas.edu

The use of differential sensing methods in a wide variety of applications continues to grow.[1,2] These methods are inspired by the senses of taste and smell, where an array of cross-reactive receptors creates patterns in order to identify flavors and odors.[2a−c] These biological senses use protein-based receptors targeted to classes of chemical structures.[2d] However, proteins are not commonly used in cross-reactive arrays.[3] For example, enzymes and antibodies are not ideal because of their inherently high selectivity. In contrast, the proteins known as serum albumins (SAs) bind to a wide range of hydrophobic molecules, including fatty acids, steroids, bilirubin, hormones, fluorescent indicators, bile acids, and several pharmaceutical compounds.[4] The amino acid sequences in bovine and human SA differ by 24%, and similar differences exist for other species.[4a] Therefore, we postulated that SAs would be particularly suitable for differential sensing purposes. As a proof-of-principle for differential sensing, we targeted chemical entities for which SAs have never even been reported to bind: terpenes.

Terpenes are commonly used as fragrances in perfumes, flavor additives in foods and drinks, and starting materials in the production of medicinally important natural products.[5a,b] Their alkene and alcohol functional groups are not easily targeted in aqueous media with synthetic receptors, and hence, there are few reports of terpene molecular recognition.[5c,d]

Our approach to terpene sensing and differentiation employs an ensemble of bovine serum albumin (BSA), human serum albumin (HSA), and rabbit serum albumin (RSA) along with an optical indicator and an additional hydrophobic small molecule that binds to serum albumins (Figure 1).

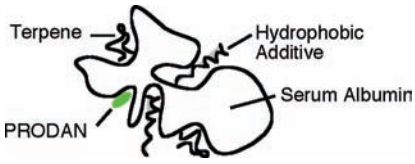

Figure 1. Schematic illustration of the proposed sensing ensemble, composed of the indicator PRODAN, terpene, and a hydrophobic additive, all of which are added to the serum albumin protein.[4d]

To signal terpene binding to the SAs, 6-propionyl-2-dimethylaminonaphthalene (PRODAN) was chosen because, like terpenes, it is hydrophobic and neutrally charged. It also has visible fluorescence and a large Stokes shift and is known to associate with SA.[6] Upon addition of SAs to PRODAN, a hypsochromic shift in emission occurred (Figure 2A). On the basis of a 1:1 stoichiometry isotherm, affinity constants of $(5.0 \pm 0.3) \times 10^5$, $(9.6 \pm 0.5) \times 10^4$, and $(8.3 \pm 0.5) \times 10^4$ M^{-1} for binding to BSA, HSA, and RSA, respectively, were obtained.[6]

The binding of five terpenes (linalool, α-terpineol, nerol, geraniol, and citronellol) were then monitored by fluorescence. The addition of concentrated terpene solutions in ethanol to SAs and PRODAN

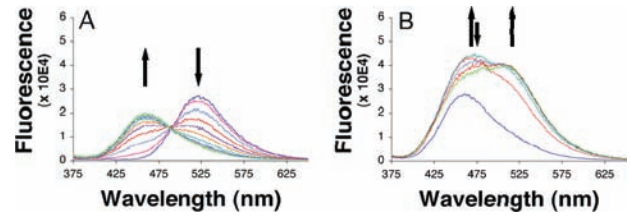

Figure 2. (A) Addition of BSA (0−40 μM) to PRODAN (2 μM) in 10 mM phosphate buffer (H_2O, pH 7.00, 0.02% NaN_3), $\lambda_{ex} = 365$ nm. (B) Addition of geraniol in EtOH (0.0−2.5 mM) to BSA (20 μM) and PRODAN (2 μM) in 10 mM phosphate buffer (H_2O, pH 7.00, 0.02% NaN_3), $\lambda_{ex} = 365$ nm, final EtOH concentration = 0.25% (v/v).

in phosphate buffer led to an intensity increase at the λ_{max} for the free indicator. The fluorescence at the λ_{max} of the bound indicator increased for HSA and RSA and increased then decreased for BSA (Figure 2B). The data suggest that the mechanism for fluorescence modulation primarily involves allosteric changes in the binding site of the indicator, but depending upon the SA species, modulation may also be due to some degree of indicator displacement. When the terpenes were titrated into PRODAN without any SA, little to no fluorescence modulation was observed, providing evidence for the role of SA in this system. Similarly, the addition of ethanol, at the level used in this study, did not significantly change the environment around PRODAN.

There were several noticeable trends in the fluorescence responses. First, all five terpenes showed a change in fluorescence at the λ_{max} of the free indicator (522 nm) in the following order: RSA < HSA < BSA. Second, the tertiary alcohols linalool and α-terpineol showed the lowest modulation of fluorescence. Nerol and geraniol, which are configurational isomers, showed similar but measurably different responses. Lastly, citronellol, the only monoolefin and primary alcohol studied, showed the greatest change of fluorescence. It is likely that a greater change in emission indicates a greater affinity of the terpene.

The sensing ensemble was then transitioned from a cuvette assay to a 96-well-plate assay. An array was generated by adding solutions of SAs, PRODAN, and terpene analytes in 24:1 aqueous phosphate buffer/ethanol. The emission data were analyzed using linear discriminant analysis (LDA). LDA is a pattern recognition technique used for the classification of data and the assignment of new analytes to their appropriate classes.[7a] The LDA plot (Figure 3A) shows discrimination almost completely along the F1 axis, indicating that a single discriminant function suffices to describe the majority of the differences among the analytes. The terpenes are organized along F1 by emission modulation to the sensing ensemble, with citronellol to the far right and linalool to the far left. The accuracy of the LDA classification was examined using a jackknife analysis,[7b] which afforded an accuracy of 100%.

Although discrimination of the five terpenes can be seen along the F1 axis, we sought to probe whether the addition of other

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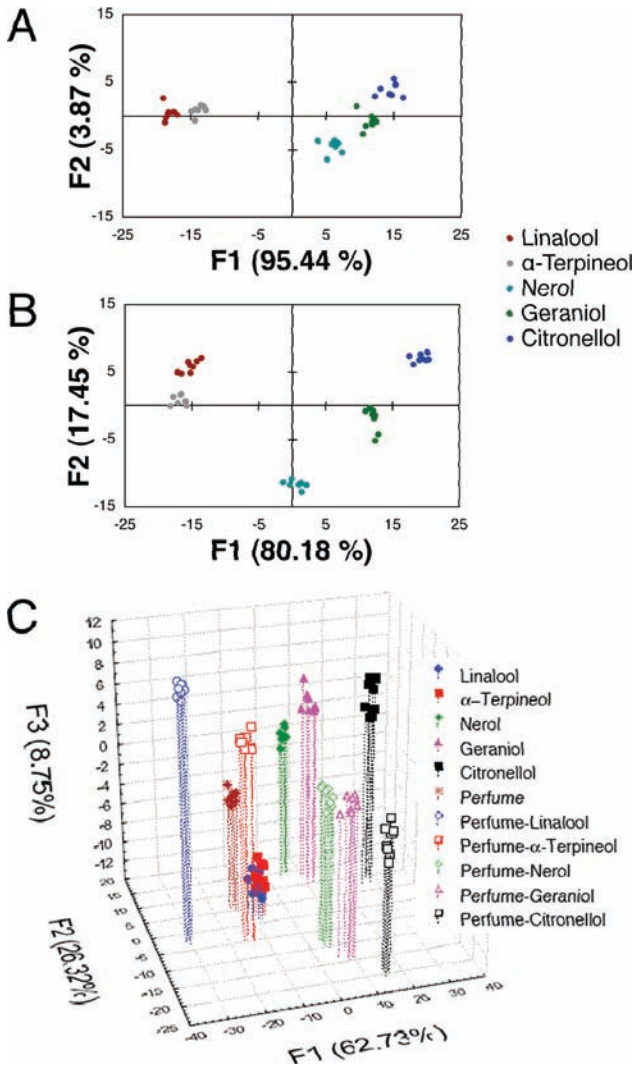

Figure 3. (A) LDA response patterns for the five terpenes. (B) LDA response patterns with deoxycholate additions. (C) LDA response patterns for the terpenes with deoxycholate and Masakï perfume additions.

molecules would improve cross-reactivity. After screening a series of ions and small molecules that are known to bind SAs, we hit upon the bile acid deoxycholate. Deoxycholate is known to have two binding sites to SAs with K_a values of 4×10^4 M^{-1} for HSA.[4a,c] Upon addition of deoxycholate to the SAs, PRODAN, and terpene solutions, the fluorescence signal was modulated to a varying degree for each terpene. Emission data from the arrays with and without deoxycholate were used to construct a second LDA plot (Figure 3B). The F2 axis variance was increased from 4 to 17% while also retaining excellent discrimination (jack-knife analysis gave 100% classification).

We next sought to test whether the sensing ensemble could discriminate terpenes in a complex mixture. Perfumes are composed of essential oils in an ethanol−water solution containing 300 or more compounds.[8] Terpenes (primarily linalool, geraniol, and citronellol) are common components of these essential oils that impart floral and citrus smells.[8] Electronic noses have been used for discrimination of the gaseous components from perfume.[9] As now described, our approach monitors terpenes in perfume solutions.

A third LDA plot (Figure 3C) shows good discrimination of the terpenes alone and in the presence of the perfume "Masakï", with a jack-knife analysis of 99%.[10] A distinctive placement was obtained for each terpene in the presence of perfume. Generally, the new placement for the terpene-doped perfume moved toward the position of the original terpene. The LDA shows that the added terpene content in this perfume can be correlated to individual terpene identity.

In summary, this study has introduced the use of natural proteins, serum albumins, as low-selectivity receptors for use in differential sensing. We applied the concept to terpene analysis and found identity discrimination in both pure solutions and complex mixtures. We also found additives that can be used to increase the differences between signaling changes for proteins in an array. We are currently expanding the use of SAs to detect additional analyte classes that these proteins do not naturally bind.

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Supporting Information Available: Experimental procedures and supporting figures from cuvette assays and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) All of the perfume data collected for the LDA plot in Figure 3C contained 18% (v/v) terpene added to the perfume.

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