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Serum Albumins as Differential Receptors for the Discrimination of Fatty Acids and Oils

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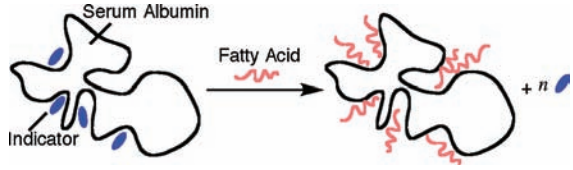
ABSTRACT



Using fluorescent indicators and several serum albumins, a sensing system for fatty acids was developed. Fatty acids were able to be differentiated based on their carbon chain length and degree of unsaturation. A sensing ensemble was then applied to study complex mixtures of triglycerides, namely, edible oils. The oils, with different fatty acid compositions, were successfully differentiated using principal component analysis.

Fatty acids and their associated triglycerides are biologically important molecules, most specifically in their role with common chronic diseases such as cardiovascular disease and type 2 diabetes. However, few molecular recognition examples of fatty acid or triglyceride detection are found in the literature.[1] Herein, we report a sensing ensemble able to detect subtle differences in fatty acid structure, which we have applied to differentiate complex mixtures of fatty acids in the form of triglycerides in edible oils.

In recent years, the use of differential sensing has gained popularity in the area of molecular recognition.[2] In contrast to the highly specific receptors generally required by the classic "lock and key" principle, differential sensing uses an array of receptors with varying binding affinities and characteristics.[3] These arrays, inspired by the mammalian senses of taste and smell, are generally cross-reactive. In cross-reactive arrays, the analytes of interest interact with each receptor differently, resulting in a composite signal that can be interpreted by pattern recognition.

Recent work in our laboratories demonstrated that the low-selective proteins, serum albumins, could be used as receptors for the differential sensing of hydrophobic terpene molecules.[4] Serum albumins are plasma proteins which have many binding sites and are known to bind a large selection of both endogenous and exogenous compounds.[5] The most important of these endogenous compounds are long chain fatty acids which are transported in blood by serum albumins and are otherwise insoluble without the presence of this protein.[5] Human serum albumin (HSA) has been confirmed to have seven binding sites for fatty acids,[6] with fatty acids

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having an association constant of around 10^7-10^8 M^{-1} to the primary binding site.[1a] Different species of serum albumin are also known to have significant differences in amino acid sequence; for example, bovine serum albumin and human serum albumin have a 24% difference in sequence homology.[5] For these reasons, we hypothesized that serum albumin would be an ideal differential receptor to pattern fatty acids and possibly their corresponding triglycerides.

After screening a variety of fluorophores for fatty acid recognition with bovine serum albumin (BSA), we found three suitable indicators for our purposes which had been previously shown to bind to BSA: 2-anthracene carboxylate (2-AC), 1-anilino-8-naphthalene sulfonic acid (1,8-ANS), and fluorescein (Figure 1).[1b,7] As described below, these fluorophores showed an appreciable change in optical signal from their serum albumin bound state in comparison to their free, unbound state. Additionally, these fluorophores displayed reasonable chromophoric resistance to small changes in the solvent system, which was necessary because fatty acids and oils thereof are extremely hydrophobic and consequently required small volumes of ethanol to remain dissolved.

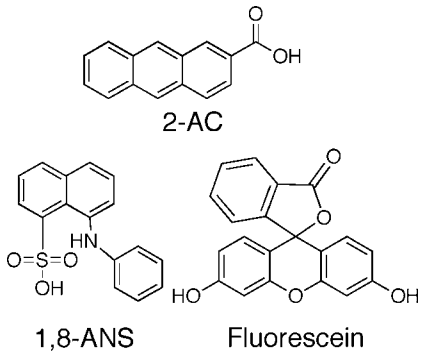

Figure 1. Fluorophores chosen for sensing ensemble.

Titration curves are shown in Figure 2 for the association of these three fluorophores to BSA, HSA, and rabbit serum albumin (RSA). We observed that both 2-AC and fluorescein resulted in a fluorescence intensity decrease upon binding to serum albumin, whereas 1,8-ANS showed an increase in intensity when bound to serum albumin. Many of the titration curves obtained show a distinct inflection before 1 equiv of SA is added, confirming that several indicator molecules bind to one SA, either in the same binding site or in different binding sites.

Using 2-AC and fluorescein, we conducted preliminary tests to see if our system responded to the addition of fatty acids. For our primary studies, palmitic acid, stearic acid, and linoleic acid were chosen as fatty acid analytes due to their structural shape and degree of unsaturation. Palmitic acid (C16) and stearic acid (C18) are saturated, linear fatty acids and differ only in carbon chain length, while linoleic acid (C18) contains two double bonds (Figure 3). Oleic acid (C18) containing one double bond (Figure 3) was incorporated into our assay once we transitioned our array to a well plate.

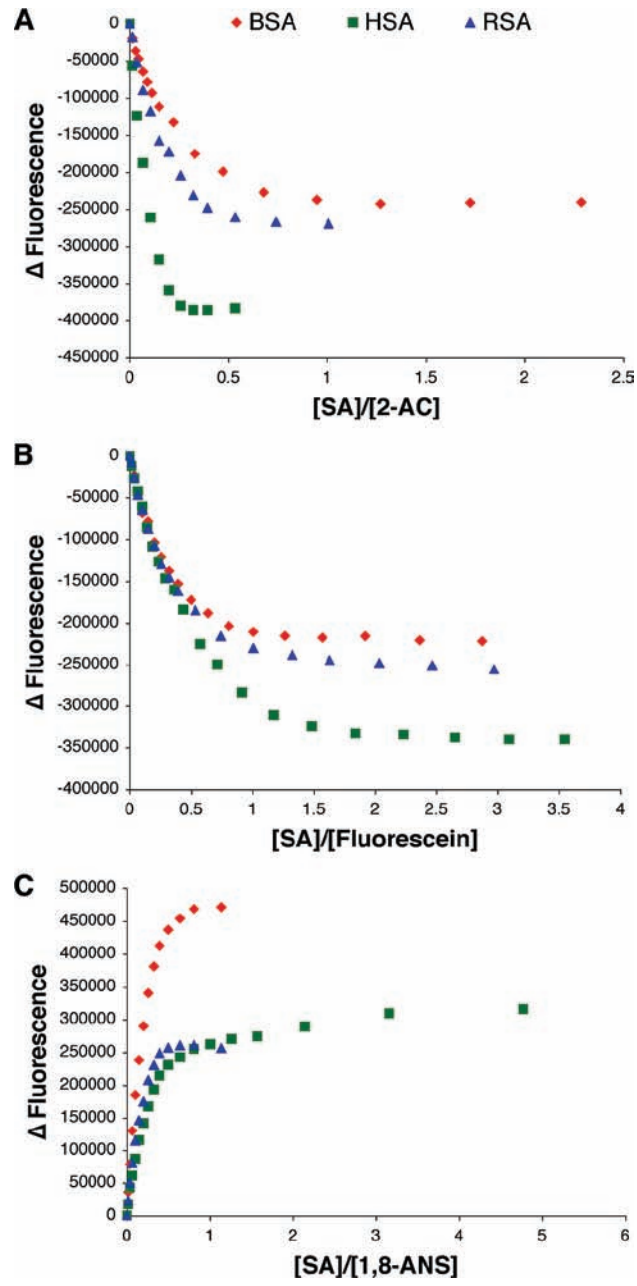

Figure 2. (A) Addition of BSA (0−22 μM), HSA (0−5 μM), and RSA (0−10 μM) to 2-AC (10 μM) in 50 mM HEPES buffer (H_2O, pH 7.00), λ_{ex} = 386 nm, λ_{em} = 423 nm. (B) Addition of BSA (0−30 μM), HSA (0−35 μM), and RSA (0−30 μM) to fluorescein (10 μM) in 50 mM HEPES buffer (H_2O, pH 7.00), λ_{ex} = 490 nm, λ_{em} = 521 nm. (C) Addition of BSA (0−12 μM), HSA (0−20 μM), and RSA (0−12 μM) to 1,8-ANS (10 μM) in 50 mM HEPES buffer (H_2O, pH 7.00), λ_{ex} = 350 nm, λ_{em} = 470 nm.

Palmitic acid, stearic acid, and linoleic acid each gave a different response with all of the SA−2-AC and SA−fluorescein complexes (Figure 4 for 2-AC and Figure S13, Supporting

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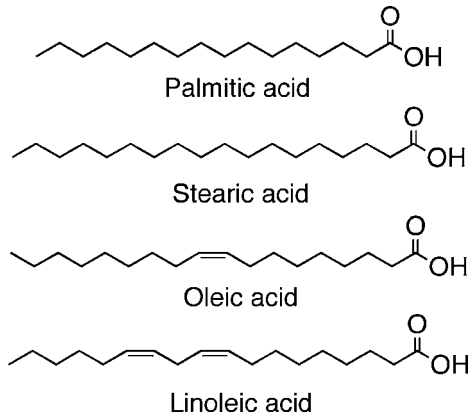

Figure 3. Chemical structure of four fatty acid analytes.

Information, for fluorescein). We found with the SA−2-AC and SA−fluorescein complexes that fatty acid addition resulted in a signal opposite from that observed upon indicator binding to SA. Therefore, we propose that the fluorescence modulation after fatty acid addition is a result of indicator displacement. One trend seen for the titration with the indicator 2-AC is that a fluorescence modulation is not observed until several equivalents of fatty acid have been added. We hypothesize that this observation resulted from the indicator 2-AC residing in areas other than the primary fatty acid binding sites. Once these primary binding sites have been saturated with fatty acid, the additional fatty acids bind to other binding sites, leading to displacement of 2-AC.

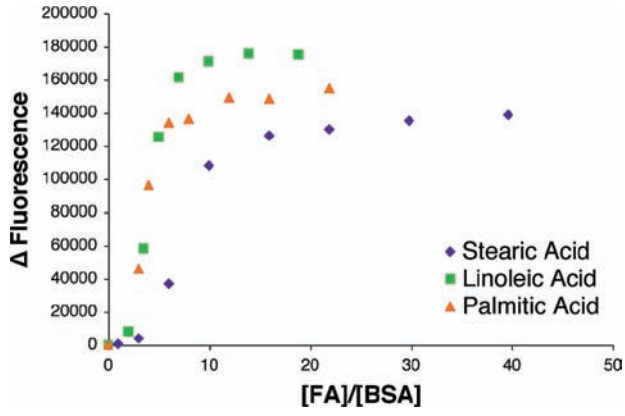

Figure 4. Addition of stearic acid (0−400 μM), linoleic acid (0−200 μM), and palmitic acid (0−215 μM) to a solution of BSA (10 μM) and 2-AC (10 μM) in 50 mM HEPES buffer (H_2O, pH 7.00), λ_{ex} = 386 nm, λ_{em} = 423 nm.

Having successfully found a sensing ensemble capable of responding to fatty acid analytes, we wanted to transition our method from a cuvette to a 96-well plate array, to easily collect the data repetitions needed for pattern recognition. The data which were collected were statistically analyzed with principal component analysis (PCA). PCA reduces the

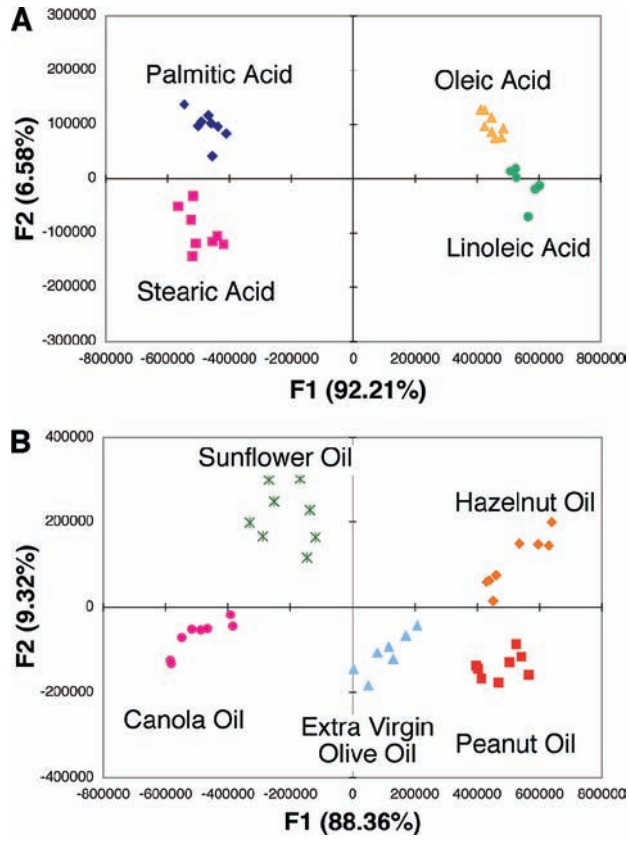

Figure 5. PCA response patterns for (A) four fatty acids and (B) five edible oils.

dimensionality of a data set to display those components which contain the greatest extent of variation in the data.[8] Our PCA plot result, shown in Figure 5A, was obtained by analyzing data from well plates consisting of BSA, HSA, and RSA as receptors, along with 2-AC, 1,8-ANS, and fluorescein as indicators, and plamitic acid, linoleic acid, stearic acid, and oleic acid as fatty acid analytes. We found the optimal working range for fatty acid concentration to be 0.5−10 mM for this array, although a measurable signal can still be observed at fatty acid concentrations as low as 50 μM. The PCA result for this array gave a plot which shows 92% of the variance along the F1 axis, while 7% of the variance is along the F2 axis. Two trends which can be observed are that the F1 axis discriminates the fatty acids by saturation level, with the saturated fatty acids on the left side of the plot (palmitic and stearic acids) and the unsaturated fatty acids on the right side (oleic and linoleic acids), while the F2 axis differentiates some of the fatty acids by carbon chain length with the C16 fatty acid, palmitic acid, projected above the *x*-axis and the C18 fatty acid, stearic acid, projected below the *x*-axis. This PCA plot shows that the sensing system is able to differentiate subtle differences between all four fatty acid analytes with the largest separation of analytes occurring between the saturated and unsaturated fatty acids.

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After successfully discriminating four fatty acids, we wanted to see if we could extend the scope of our system to differentiate complex mixtures of fatty acids in the form of triglycerides, such as edible oils. Edible oils each contain a different fatty acid profile or lipid fingerprint,[9] and our goal was to apply our sensing ensemble to differentiate between various oils. For our study, hazelnut oil, extra virgin olive oil, peanut oil, sunflower oil, and canola oil were chosen. Since these oils contain approximately 98% fatty acids and 2% nonglyceride substances (such as phospholipids, tocopherols, and sterols), we reasoned that we could apply our sensing ensemble to discriminate the edible oils based on their fatty acid compositions. Our results show that we were effectively able to discriminate all five oil solutions, giving us the PCA plot in Figure 5B, with 88% of the variance along the F1 axis and 9% of the variance along the F2 axis. We note that the oils are organized along the F1 axis by their ratio of unsaturated fatty acids to saturated fatty acids, with canola oil having a large ratio and peanut oil having a small ratio.[10]

One interesting point in the plot is that we are clearly able to distinguish hazelnut oil and extra virgin olive oil. This is important, as hazelnut oil is often fraudulently used to adulterate extra virgin olive oil as a cost cutting technique.[10]

In summary, we have shown that by using a sensing ensemble consisting of three species of serum albumin and three fluorophores, fatty acid composition and identity can be classified from patterns in fluorescence spectroscopy. PCA successfully discriminated four fatty acid analytes and associated edible oils. We are currently investigating other uses of serum albumins as differential receptors.

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Supporting Information Available: Experimental conditions and full spectroscopic data for all cuvette titrations. This material is available free of charge via the Internet at http://pubs.acs.org.

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