Electronic Supplementary Material (ESI) for Chemical Science
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Exploration of Plasticizer and Plastic Explosive Detection and Differentiation with Serum Albumin Cross-Reactive Arrays

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Supporting Information

Materials and Methods

All reagents and solvents were purchased from Sigma Aldrich and/or Fisher and used as is without purification. BSA and HSA are fatty acid free and are purchased from Sigma Aldrich. Cuvette assays were completed with a PTI fluorimeter with an 814 photomultiplier detection system using a 75W xenon short arc lamp. The 96-well plates were analyzed using the Biotek Synergy 2 Multi-Mode Microplate Reader. XLSTAT2011 was used for linear discriminant analysis.

Experimental:

A solution of 10mM phosphate buffer in H_2O at pH 7.00 was prepared and filtered using a 2:1 molar ratio of Na_2HPO_4:NaH_2PO_4 in degassed deionized water. 0.02% (m/v) NaN_3 was added to the phosphate buffer to limit the growth of microbial agents and prevent contamination in the prepared stock solutions.

To determine the binding ratios of dansyl amide to serum albumins, cuvette assays were done by titrating serum albumin (800 µM BSA or 800 µM HSA in 10 mM phosphate buffer) into 30 µM dansyl amide in phosphate buffer. To study plasticizer interaction with the sensing ensemble, cuvette assays were completed by adding plasticizer (250 mM tri-n-butyl citrate, 250 mM dioctyl sebacate, 250 mM dioctyl adipate, 250 mM phthalate, or 250 mM di-n-octyl phthalate in ethanol) into 30 µM dansyl amide

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and 150 µM serum albumin (BSA or HSA) in phosphate buffer. Cuvette assays were completed with λ_{ex} = 350 nm and λ_{em} = 360 – 690 nm.

Cuvette assays to study the binding interaction of dansyl cadaverine were completed by the titration of serum albumin (1500 µM BSA in 10 mM phosphate buffer) into 30 µM dansyl cadaverine in phosphate buffer. Plasticizer interaction with the sensing ensemble was studied by adding plasticizer (250 mM tri-n-butyl citrate, 250 mM dioctyl sebacate, 250 mM dioctyl adipate, 250 mM phthalate, or 250 mM di-n-octylphthalate in ethanol) into 30 µM dansyl cadaverine and 150 µM bovine serum albumin in phosphate buffer. Cuvette assays were done using λ_{ex} = 335 nm and λ_{em} = 345 – 660 nm.

Well plate assays were completed using 96-well plates that were flat-bottom, non-treated, black polystyrene plates. To test the sensing ensemble's ability to differentiate between plasticizers, a comparison of the previously conducted cuvette assays with the five plasticizers was made and it was determined that 375 µM was the optimal plasticizer concentration for differentiation purposes. A well plate assay was prepared to have final concentrations of: 30 µM dansyl amide, 150 µM serum albumin (BSA or HSA) and 275 µM plasticizer (tri-n-butyl citrate, dioctyl sebacate, dioctyl adipate, phthalate, or di-n-octylphthalate) in phosphate buffer. The final ethanol concentration in each well was 4.99% (v/v). The 96-well plates were read with λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm, using a top 400 mirror.

To further increase the LDA F2 axis differentiation, a variety of additives all of which are known to bind to SA were studied. To explore the affects of these additives on the fluorescence signal of the assay, 150 µM stearic acid, 750 µM stearic acid, 1500 µM deocycholate, 1500 µM cholate, 1500 µM ascorbic acid, and 2250 µM ascorbic acid were each individually added to the assay. Although ascorbic

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acid did not give the greatest change in fluorescence signal, it was found that it best further discriminates the plasticizer analytes in comparison to the other additives studied.

Addition of ascorbic acid to the array was carried out using a well plate assay prepared to have final concentrations of: 30 µM dansyl amide, 150 µM serum albumin (BSA or HSA) in phosphate buffer, and 375 µM plasticizer (tri-n-butyl citrate, dioctyl sebacate, dioctyl adipate, phthalate or di-n-octylphthalate) in phosphate buffer, with half of the wells containing 2250 µM ascorbic acid in phosphate buffer. The final ethanol concentration in each well was 9.99% (v/v). The 96-well plates were read with λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm, using a top 400 mirror.

Plastic explosives were prepared with reagents purchased from Sigma Aldrich. Composition of the explosives was modified by using surrogates for RDX and PETN. Semtex 1A was prepared by combining 4.6% 2,4-diamino-1,3,5-triazine in ethanol, 76% pentaerythritol tetraacetate in acetone, 9.4% styrene-butadiene in tetrahydrofuran, 0.5% N-phenyl-2-napthylamine in methanol and 0.5% sudan IV in methanol. The solution was sonicated until all components dissolved into solution and a homogenous pink solution remained. The solution was rotovapped and ethanol was added. This solution was then used to make 8 homogenous solutions: (1) with no plasticizer, (2) with no plasticizer and 4527 µM ascorbic acid, (3) 1.125% tri-n-butyl citrate, (4) 1.125% tri-n-butyl citrate and 4527 µM ascorbic acid, (5) 1.125% phthalate, (6) 1.125% phthalate and 4527 µM ascorbic acid, (7) 1.125% di-n-octylphthalate, and (8) 1.125% di-n-octylphthalate and 4527 µM ascorbic acid. Ethanol was added to each solution to help solubilize the plasticizer in solution and resulted in an ethanol concentration of 27.8% (v/v). Semtex H was prepared by combining 40.9% pentaerythritol tetraacetate in acetone, 41.2% 2,4-diamino-1,3,5-triazine in ethanol, 9% styrene-butadiene in tetrahydrofuran and 0.5% sudan I in methanol. The solution was sonicated until all components dissolved into solution and a homogenous pink solution remained. The solution was rotovapped and ethanol was added. This solution was then used to make 8

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homogenous solutions: (1) with no plasticizer, (2) with no plasticizer and 4527 µM ascorbic acid, (3) 1.125% tri-n-butyl citrate, (4) 1.125% tri-n-butyl citrate and 4527 µM ascorbic acid, (5) 1.125% phthalate, (6) 1.125% phthalate and 4527 µM ascorbic acid, (7) 1.125% di-n-octylphthalate, and (8) 1.125% di-n-octylphthalate and 4527 µM ascorbic acid. Ethanol was added to each solution to help solubilize the plasticizer in solution and resulted in an ethanol concentration of 25% (v/v). Semtex 2P was prepared by combining 58.45% pentaerythritol tetraacetate in acetone, 22.9% 2,4-diamino-1,3,5-triazine in ethanol, 9.7% styrene-butadiene in tetrahydrofuran, 0.5% sudan III in methanol. The solution was sonicated until all components dissolved into solution and a homogenous pink solution remained. The solution was rotovapped and ethanol was added. This solution was then used to make 8 homogenous solutions: (1) with no plasticizer, (2) with no plasticizer and 4527 µM ascorbic acid, (3) 1.125% tri-n-butyl citrate, (4) 1.125% tri-n-butyl citrate and 4527 µM ascorbic acid, (5) 1.125% phthalate, (6) 1.125% phthalate and 4527 µM ascorbic acid, (7) 1.125% di-n-octylphthalate, and (8) 1.125% di-n-octylphthalate and 4527 µM ascorbic acid. Ethanol was added to each solution to help solubilize the plasticizer in solution and resulted in an ethanol concentration of 22.61% (v/v). C-4 was prepared by combining 84.5% 2,4-diamino-1,3,5-triazine in ethanol, 2.7% polyisobutylene in tetrahydrofuran and 3.6% SAE 30 non-detergent motor oil purchased from Autozone in pentane. The solution was sonicated until all components dissolved into solution and a homogenous pink solution remained. The solution was rotovapped and ethanol was added. This solution was then used to make 6 homogenous solutions: (1) with no plasticizer, (2) with no plasticizer and 4527 µM ascorbic acid, (3) 1.15% dioctyl sebacate, (4) 1.15% dioctyl sebacate and 4527 µM ascorbic acid, (5) 1.15% dioctyl adipate, (6) 1.15% dioctyl adipate and 4527 µM ascorbic acid. Ethanol was added to each solution to help solubilize the plasticizer in solution and resulted in an ethanol concentration of 21.25% (v/v).

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To determine the equivalents to be used in the well plate assays, a range of concentrations of the plastic explosive mixtures (C-4 with no plasticizer [0-5700 µM], C-4 with dioctyl sebacate [0-5700 µM], C-4 with dioctyl adipate [0-4000 µM], Semtex 1A with no plasticizer [0-450 µM], Semtex 1A with tri-n-butyl citrate [0-450 µM],and Semtex 1A with di-n-octylphthalate [0-450 µM]) were each added to wells with 30 µM dansyl amide and 150 µM serum albumin (BSA) in phosphate buffer. The 96-well plates were read with λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm, using a top 400 mirror. From this experiment, we determined that 100 µM of Semtex mixtures and 600 µM of C4 mixtures would give good differentiation.

Well plate assays of the plastic explosive mixtures were completed by the use of several stock solutions: (**A**) 90 µM indicator (dansyl amide or dansyl cadaverine), 450 µM serum albumin (BSA or HSA), (**B**) 161 µM Semtex 1A (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), (**C**) 625 µM Semtex 2P (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), (**D**) 2120 µM Semtex H (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), and (**E**) 1935 µM C-4 (no plasticizer, no plasticizer with ascorbic acid, dioctyl sebacate, dioctyl sebacate with ascorbic acid, dioctyl adipate, or dioctyl adipate with ascorbic acid); the Semtex 1A wells were prepared by pipetting 100 µL of **A**, 186 µL of **B** and 14 µL of phosphate buffer, the Semtex 2P wells were prepared by pipetting 100 µL of **A**, 48 µL of **C** and 152 µL of phosphate buffer, the Semtex H wells were prepared by pipetting 100 µL of **A**, 21.2 µL of **D** and 178.8 µL of phosphate buffer, and the C4 wells were prepared by pipetting 100 µL of **A**, 93 µL of **E** and 107 µL of phosphate buffer. The final ethanol concentration was

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10%. The 96-well plates were read with either λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm or λ_{ex} filter of 340 ± 11 nm and λ_{em} filter of 528 ± 20 nm, using a top 400 mirror.

Solutions of contaminated explosives were made from the original stock solutions of the plastic explosives. Soil obtained from Michelle Ivy's garden was weighed out (10g) onto a petri dish. The explosives were added to individual petri dishes by pipetting 4830 µL of the Semtex 1A solution (**B**), 2492 µL of the Semtex 2P solution (**C**), 1150 µL of the Semtex H solution (**D**)**,** or 2741 µL of the C4 solution (**E**) directly onto the soil. Ethanol (20 mL) was added to soil directly after explosive addition and was allowed to sit for 5 minutes. The soil was then filtered using filter paper and funnel and rotovapped. The solvent system was then added to mixture (75% buffer/25%Ethanol) and it was sonicated. All dirty explosives were a dark yellow color.

Well plate assays of the dirty plastic explosive mixtures were completed by the use of several stock solutions: (**F**) 3125 µM Dirty Semtex 1A (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), (**G**) 3125 µM Dirty Semtex 2P (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), (**H**) 3260 µM Dirty Semtex H (no plasticizer, no plasticizer with ascorbic acid, tri-n-butyl citrate, tri-n-butyl citrate with ascorbic acid, phthalate, phthalate with ascorbic acid, di-n-octylphthalate or di-n-octylphthalate with ascorbic acid), and (**I**) 1765 µM Dirty C-4 (no plasticizer, no plasticizer with ascorbic acid, dioctyl sebacate, dioctyl sebacate with ascorbic acid, dioctyl adipate, or dioctyl adipate with ascorbic acid); the Dirty Semtex 1A wells were prepared by pipetting 100 µL of **A**, 9.6 µL of **F** and 190.4 µL of phosphate buffer, the Dirty Semtex 2P wells were prepared by pipetting 100 µL of **A**, 9.6 µL of **G** and 190.4 µL of phosphate buffer, the Dirty Semtex H wells were prepared by pipetting 100 µL of **A**, 9.2 µL of **H** and 190.8 µL of phosphate

buffer, and the Dirty C4 wells were prepared by pipetting 100 µL of **A**, 17 µL of **I** and 183 µL of phosphate buffer. The final ethanol concentration was 10%. The 96-well plates were read with either λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm or λ_{ex} filter of 340 ± 11 nm and λ_{em} filter of 528 ± 20 nm, using a top 400 mirror.

Supplementary Figures

(A) **(B)**

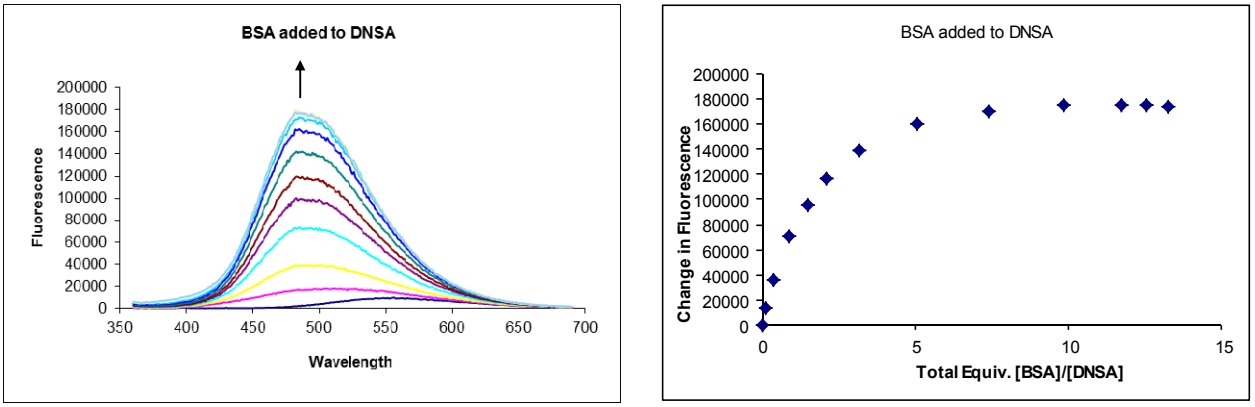


Figure S1. (A) Addition of BSA (0.0 – 396.98 µM) to DNSA (30 µM) in 10 mM phosphate buffer, H_2O, pH 7.00, 0.02% NaN_3, λ_{ex}= 350 nm. (B) Binding curve for the addition of BSA to DNSA from the data in Figure S1A at 495 nm.

(A) **(B)**

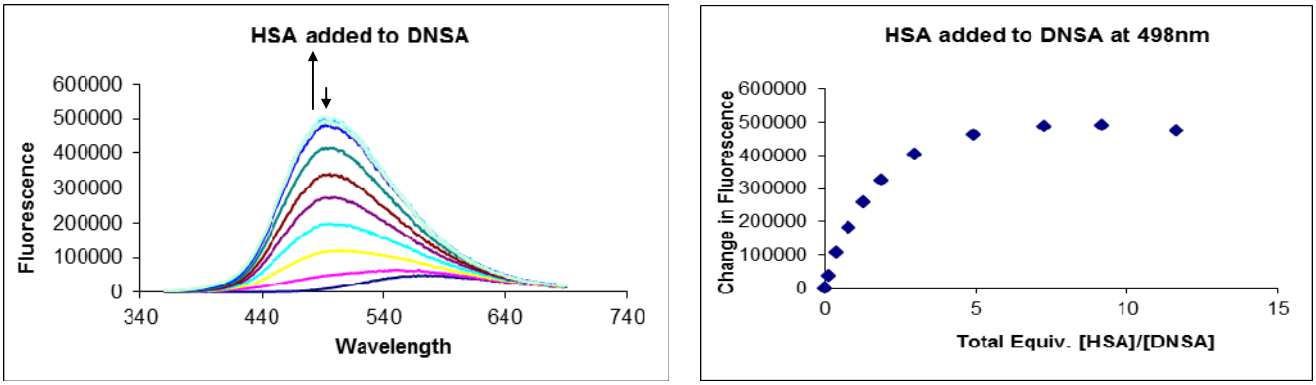


Figure S2. (A) Addition of HSA (0.0 – 349 µM) to DNSA (30 µM) in 10 mM phosphate buffer, H_2O, pH 7.00, 0.02% NaN_3, λ_{ex}= 350 nm. (B) Binding curve for the addition of HSA to DNSA from the data in Figure S2A.

(A) **(B)**

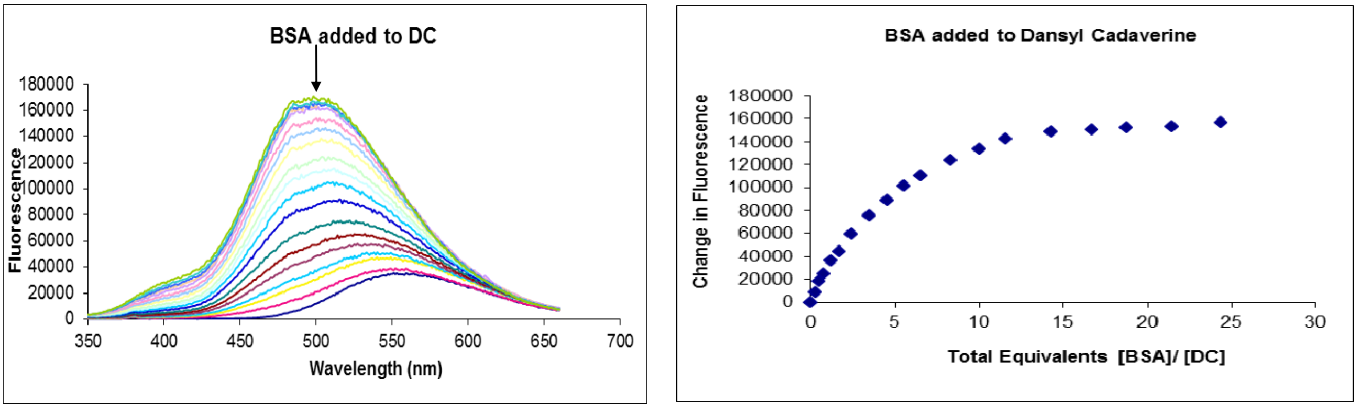
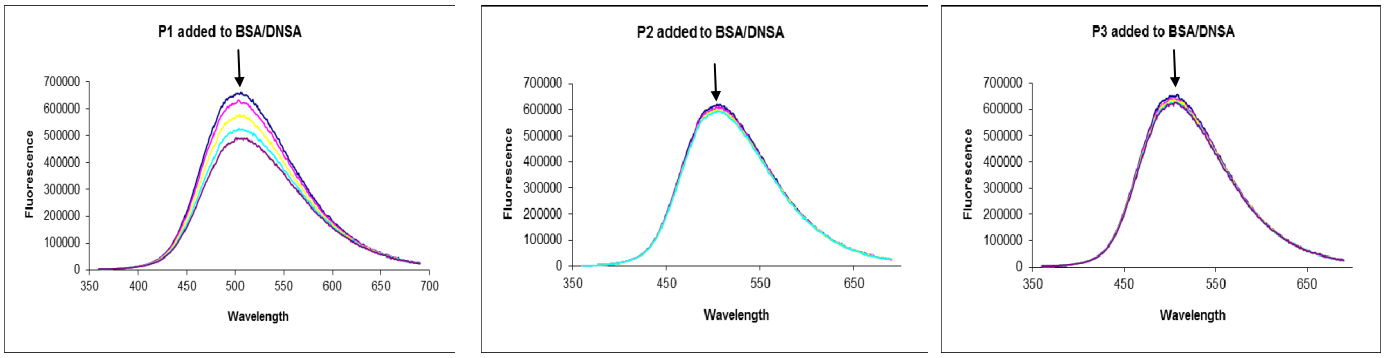
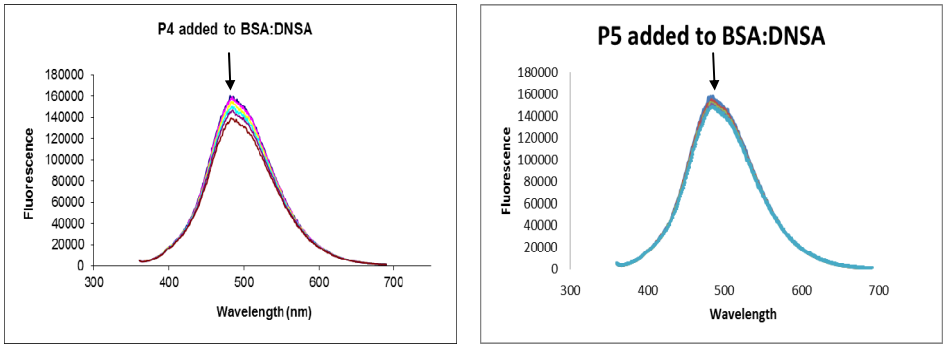


Figure S3. (A) Addition of BSA (0.0 – 730.8 µM) to DC (30 µM) in 10 mM phosphate buffer, H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm. (B) Binding curve for the addition of BSA to DC from the data in Figure S3A at 515 nm.

(A) **(B)** **(C)**



(D) **(E)**



(F)

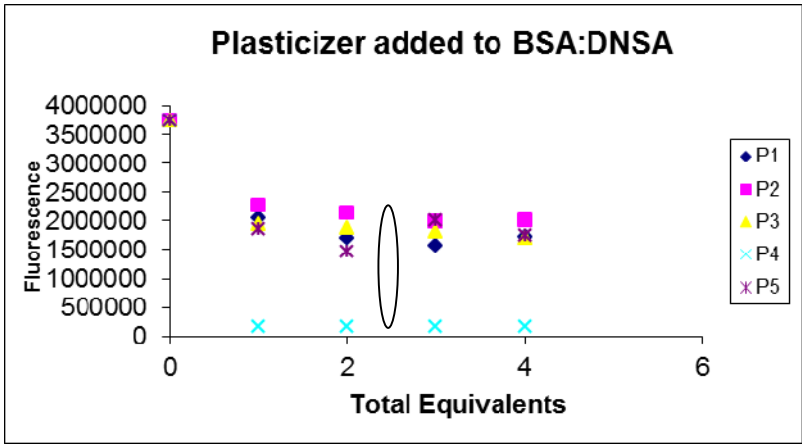
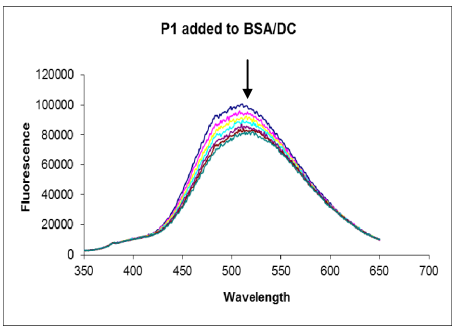
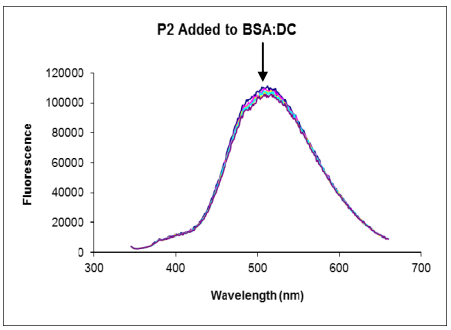
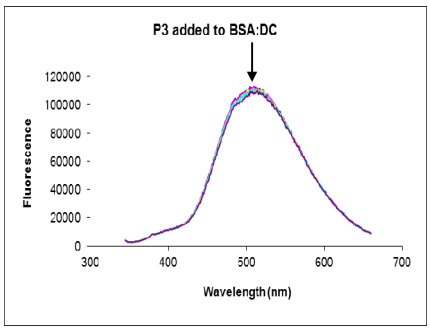


Figure S4. (A) Addition of tri-n-butyl citrate in ethanol (0.0 –623.44 µM) to BSA (150 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (B) Addition of dioctyl sebacate in ethanol (0.0 – 374.44 µM) to BSA (150 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (C) Addition of dioctyl adipate in ethanol (0.0 – 623.44 µM) to BSA (150 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (D) Addition of phthalate in phosphate buffer (0.0 –748.37 µM) to BSA (150 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (E) Addition of di-n-octylphthalate in ethanol (0.0 – 623.44 µM) to BSA (150 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm. (F) Binding curve for the addition of plasticizer to BSA and DNSA from the data in Figures S4(A-E). The circle indicates the total equivalents chosen for the plasticizers.

(A) **(B)** **(C)**







(D) **(E)**

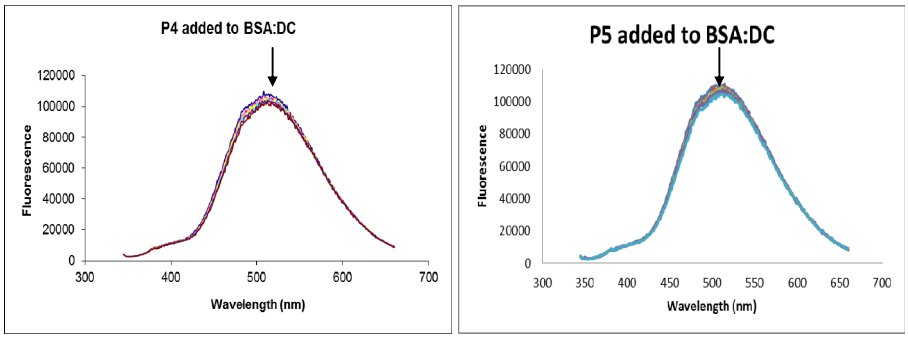


Figure S5. (A) Addition of tri-n-butyl citrate in ethanol (0.0 – 747.76 µM) to BSA (150 µM), DC (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm (B) Addition of dioctyl sebacate in ethanol (0.0 – 623.44 µM) to BSA (150 µM), DC (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm (C) Addition of dioctyl adipate in ethanol (0.0 – 623.44 µM) to BSA (150 µM), DC (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm. (D) Addition of phthalate in phosphate buffer (0.0 – 748.37 µM) to BSA (150 µM), DC (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm (E) Addition of di-n-octyl phthalate in ethanol (0.0 – 623.44 µM) to BSA (150 µM), DC (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 335 nm.

(A) **(B)** **(C)**

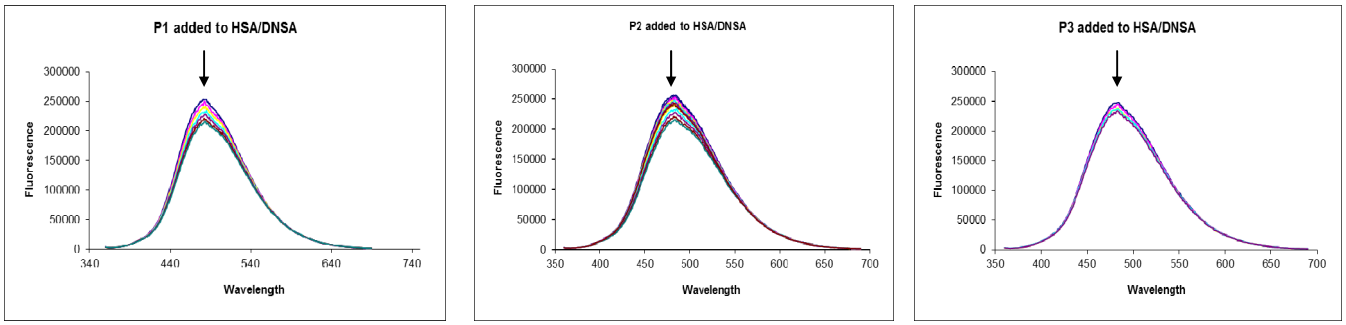


Figure S6. (A) Addition of tri-n-butyl citrate in ethanol (0.0 – 747.6 µM) to HSA (210 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (B) Addition of dioctyl sebacate in ethanol (0.0 – 623.7 µM) to HSA (210 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm (C) Addition of dioctyl adipate in ethanol (0.0 – 499.8 µM) to HSA (210 µM), DNSA (30 µM) in 10 mM phosphate buffer,H_2O, pH 7.00, 0.02% NaN_3, λ_{ex} 350 nm

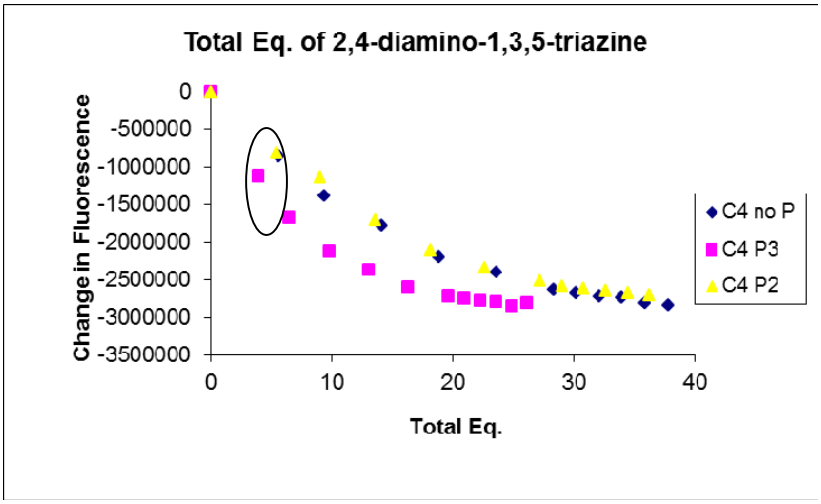


Figure S7. Addition of 2,4-Diamino-1,3,5-triazine (0-5.67 mM) in a "C-4" mixture without plasticizer in 10% ethanol to a solution of BSA (150 µM), DNSA (30 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00), 2,4-Diamino-1,3,5-triazine (0-3.93 mM) in a "C4" mixture with dioctyl sebacate in 10% ethanol to a solution of BSA (150 µM), DNSA (30 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00), 2,4-Diamino-1,3,5-triazine (0-3.93 mM) in a "C4" mixture with dioctyl adipate in 10% ethanol to a solution of BSA (150 µM), DNSA (30 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00 λ_{ex} filter of 320-400 nm and λ_{em} filter of 465-505 nm, top 400 nm mirror. The circle indicates the total equivalents chosen for C-4.

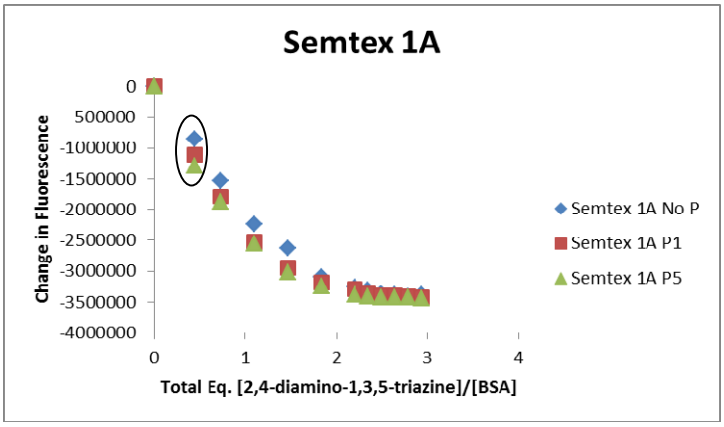


Figure S8. Addition of 2,4-Diamino-1,3,5-triazine (0-3.93 mM) in a "Semtex 1A" mixture consisting of no plasticizer, 9% tri-n-butyl citrate or 9% di-n-octyl phthalate with 10% ethanol to a solution of BSA (150 µM), DNSA (30 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00), λ_{ex} filter of 320-400 nm and λ_{em} filter of 465-505 nm, top 400 nm mirror. The circle indicates the total equivalents used for Semtex 1A.

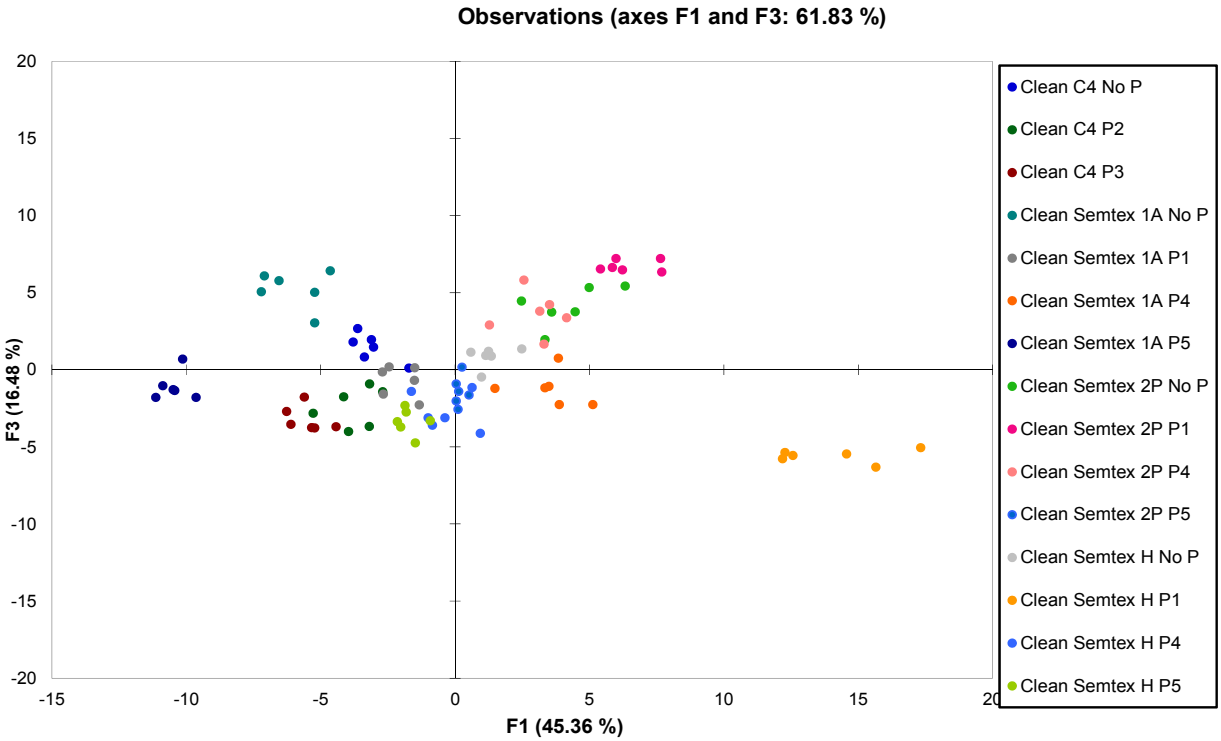


Figure S9. The differentiation of C4 without plasticizer (600 µM), C4 with dioctyl sebacate (600 µM). C4 with dioctyl adipate (600 µM), Semtex 1A without plasticizer (100 µM), Semtex 1A with tri-n-butyl citrate (100 µM), Semtex 1A with phthalate (100 µM) and Semtex 1A with di-n-octyl phthalate (100 µM), Semtex 2P without plasticizer (100 µM), Semtex 2P with tri-n-butyl citrate (100 µM), Semtex 2P with phthalate (100 µM) and Semtex 2P with di-n-octyl phthalate (100 µM), Semtex H without plasticizer (100 µM), Semtex H with tri-n-butyl citrate (100 µM), Semtex H with phthalate (100 µM) and Semtex H with di-n-octyl phthalate (100 µM) was examined in a 96 well plate of BSA (150 µM), HSA (150 µM), dansyl amide (30 µM) and dansyl cadaverine (30 µM) with ascorbic acid (2250 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00), λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm or λ_{ex} filter of 340 ± 11 nm and λ_{em} filter of 528 ± 20 nm, using a top 400 mirror.

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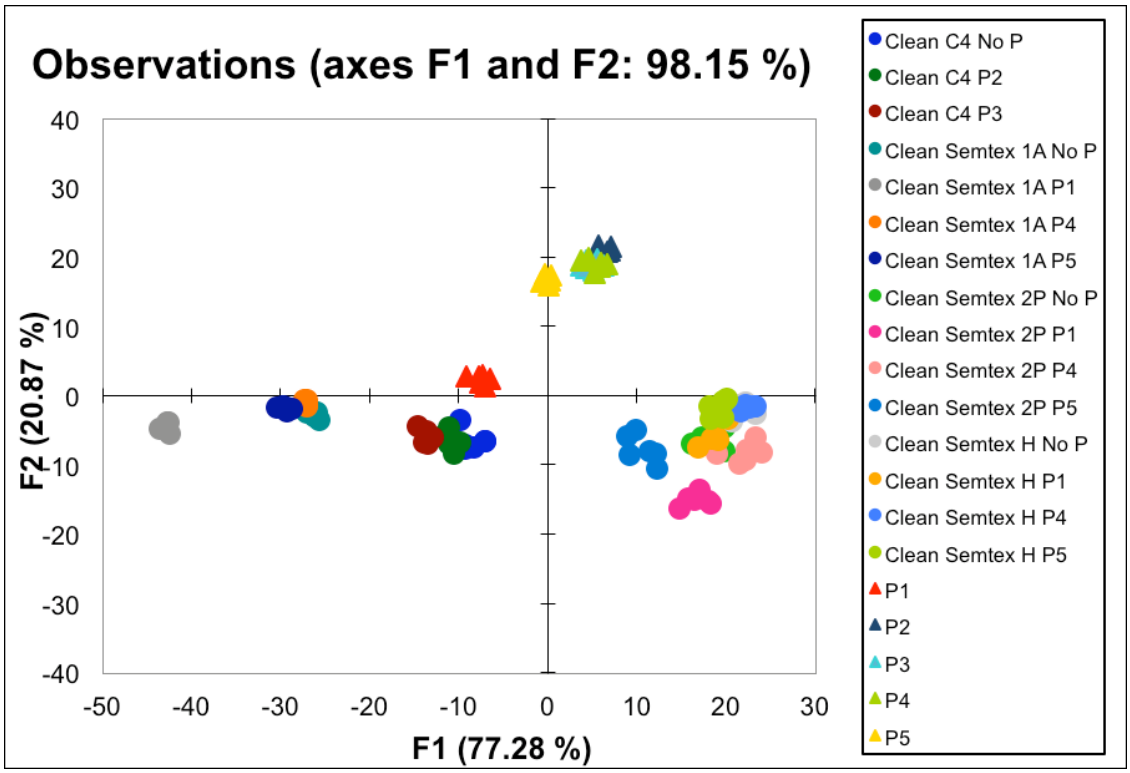

Figure S10. Differentiation of plasticizers done with bovine and human serum albumins (150 µM), dansyl amide (30 µM), plasticizers (375 µM), and ascorbic acid (2250 µM) in phosphate buffer with 10% ethanol with 0.02% NaN_3 (H_2O, pH 7.00), λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm or λ_{ex} filter of 340 ± 11 nm and λ_{em} filter of 528 ± 20 nm, using a top 400 mirror. Differentiation of C4 without plasticizer (600 µM), C4 with dioctyl sebacate (600 µM). C4 with dioctyl adipate (600 µM), Semtex 1A without plasticizer (100 µM), Semtex 1A with tri-n-butyl citrate (100 µM), Semtex 1A with phthalate (100 µM), Semtex 1A with di-n-octyl phthalate (100 µM), Semtex 2P without plasticizer (100 µM), Semtex 2P with tri-n-butyl citrate (100 µM), Semtex 2P with phthalate (100 µM), Semtex 2P with di-n-octyl phthalate (100 µM), Semtex H without plasticizer (100 µM), Semtex H with tri-n-butyl citrate (100 µM), Semtex H with phthalate (100 µM), and Semtex H with di-n-octyl phthalate (100 µM). Differentiation of explosive mixtures done with a 96 well plate of BSA (150 µM), HSA (150 µM), dansyl amide (30 µM) and dansyl cadaverine (30 µM) with ascorbic acid (2250 µM) in phosphate buffer with 0.02% NaN_3 (H_2O, pH 7.00), λ_{ex} filter of 360 ± 10 nm and λ_{em} filter of 480 ± 20 nm or λ_{ex} filter of 340 ± 11 nm and λ_{em} filter of 528 ± 20 nm, using a top 400 mirror.