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for

An albumin fluorescent sensor array discriminates ochratoxins

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Experimental section

1. Chemicals and Reagents. The albumins, HSA (A9731) and BSA (V900933), used in this work were purchased from Sigma-Aldrich without further purification. Ochratoxins were purchased from Aladdin without further purification. The 0.1 mM PBS buffer was purchased from Aladdin and used in the whole experimental process.

2. Procedures of Albumins for Discriminating Different Ochratoxins. 10 μL of 0.5 mM albumin stock solution were mixed with the three Ochratoxins with different concentrations in PBS buffer with total volume at 1 mL. the mixture was shaken for 30 sec. and then was measured by Thermo Lumina Fluorescent spectrometer. We processed the raw data matrix using classical linear discriminant analysis (LDA) and hierarchical cluster analysis (HCA) in SPSS (version 27).

3. Determination of binding constant. The values of equilibrium binding constant (K_b) of the albumin-ochratoxin complexes were determined by intercept and slope of the modified Stern-Volmer equation:

$$Log\left[\frac{F_0 - F}{F}\right] = Log\ K_b + n\ Log[Q]$$

Here, n is the number of binding stoichiometries of the albumin-ochratoxin interaction, [Q] is the molar concentration of quencher (ochratoxin).

4. Molecular Docking. The 3D geometry of Ochratoxins was constructed using the Gaussian viewer, then optimized at the level of B3LYP/6-31g* with the PCM implicit water solvent model. The ligand-free crystal structure of HSA (PDB id: 4k2c) and BSA (PDB id: 4f5s) were taken from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb). The R-value and the resolution of the files were 0.213 and 3.23 Å, 0.197 and 2.47 Å respectively. Flexible ligand docking was performed by AutoDock 4.2 molecular docking program using the implemented empirical free energy function and the Lamarckian Genetic Algorithm. The Autogrid was used to calculate force field grids. The grid spacing was 0.375 Å as default. 20 docking runs with 25,000,000 energy evaluations were performed. The output from AutoDock was rendered with PyMol and the ligand site analysis was assisted with LigPlus.

5. Discrimination of Blind Samples. Ten samples containing a concentration of 2 μM ochratoxins were prepared by adding to three ochratoxins randomly and given the numbers 1-10. The operational procedures and data processing methods followed the established protocol for discriminating ochratoxins at the same concentration.

6. Discrimination of the Mixture of Ochratoxins in Varying Ratios. The binary mixture and ternary mixture with a total concentration of 2 μM were prepared in different molar ratios. Operation

procedures and the data processing method were in accordance with the discrimination of ochratoxins with the same concentration. The data were analyzed by using LDA and HCA in SPSS (version 27).

7. Discrimination of Ochratoxins and Its Mixtures in Real Food Samples. Millet sample was purchased from a local market. Sample treatment involved taking 5 g of millet and immersing it in a mixture of 50 mL PBS buffer and methanol (v : v = 4 : 1). Then, the mixture was subjected to 10 min of vertexing, following by 10 min of ultrasonic treatment. The supernatant of mixture was extracted and filtered through a 0.22 μm organic membrane for subsequent analysis. To evaluate the potential practical application in real samples, ochratoxins and their mixtures with an initial concentration of 2 μM were spiked into the processed food samples. The analysis of these spiked samples followed the established procedure for discriminating the target ochratoxins.

Supporting Figures and Tables

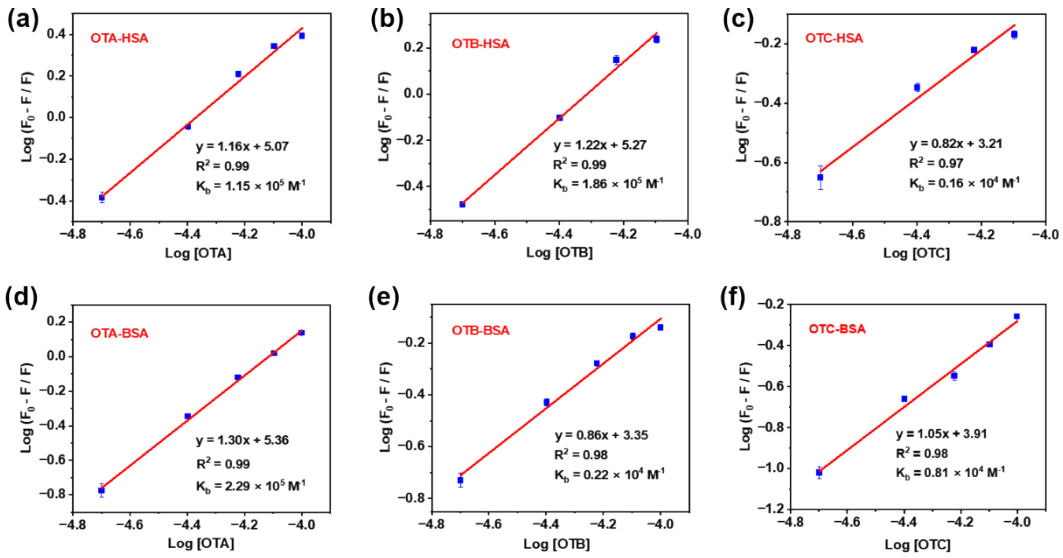

Fig. S1. Double-logarithm plot for the quenching of (a-c) HSA and (d-f) BSA by ochratoxins at different concentrations.

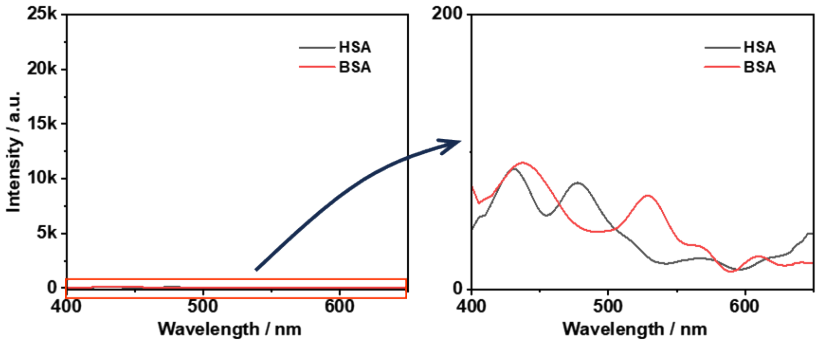

Fig. S2. The fluorescent spectra of HSA (5 μM) and BSA (5 μM) under an excitation wavelength of 380 nm.

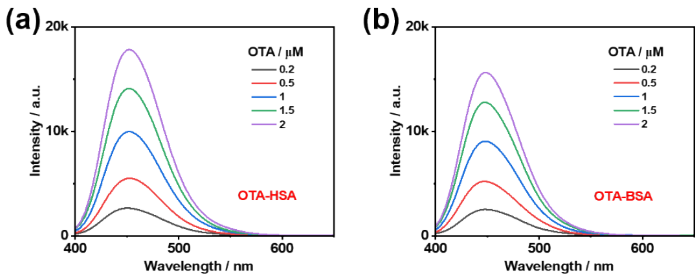

Fig. S3. The fluorescent spectra of OTA with different concentrations binding with (a) HSA and (b) BSA. [HSA] = [BSA] = 5 μM. λ_{ex} = 380 nm.

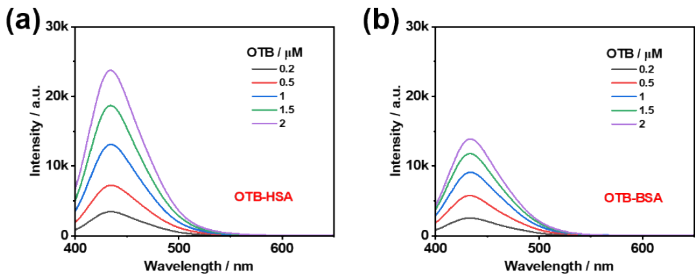

Fig. S4. The fluorescent spectra of OTB with different concentrations binding with (a) HSA and (b) BSA. [HSA] = [BSA] = 5 μM. λ_{ex} = 380 nm.

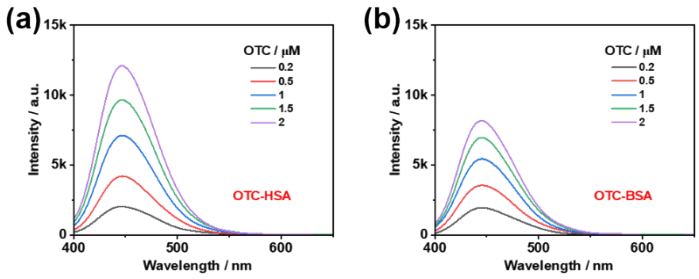

Fig. S5. The fluorescent spectra of OTC with different concentrations binding with (a) HSA and (b) BSA. [HSA] = [BSA] = 5 μM. λ_{ex} = 380 nm.

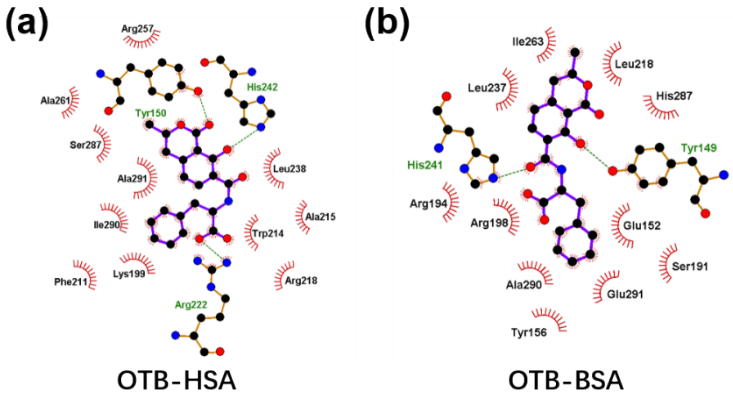

Fig. S6 The binding model of OTB with surrounding amino acid residues in the DS1 site of (a) HSA and (b) BSA.

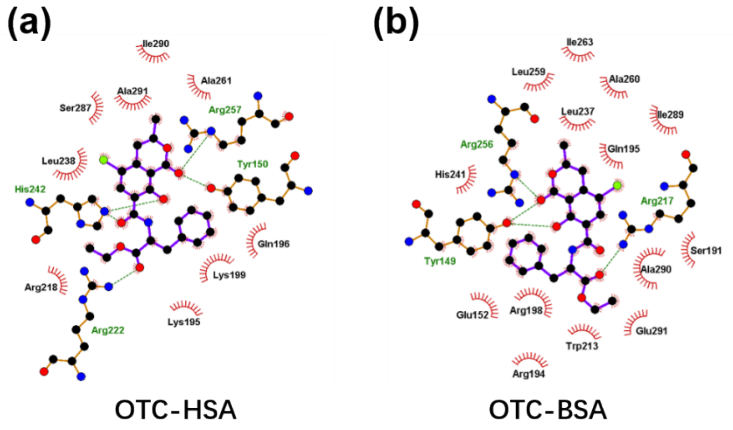

Fig. S7 The binding model of OTC with surrounding amino acid residues in the DS1 site of (a) HSA and (b) BSA.

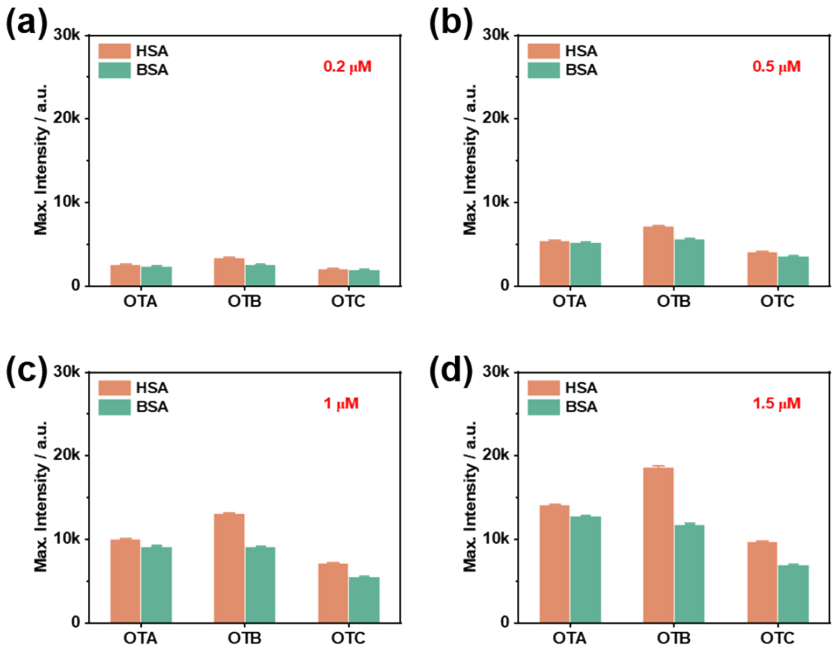

Fig. S8 Fluorescence response patterns of the array for ochratoxins with the concentration of (a) 0.2 μM, (b) 0.5 μM, (c) 1 μM, (d) 1.5 μM.

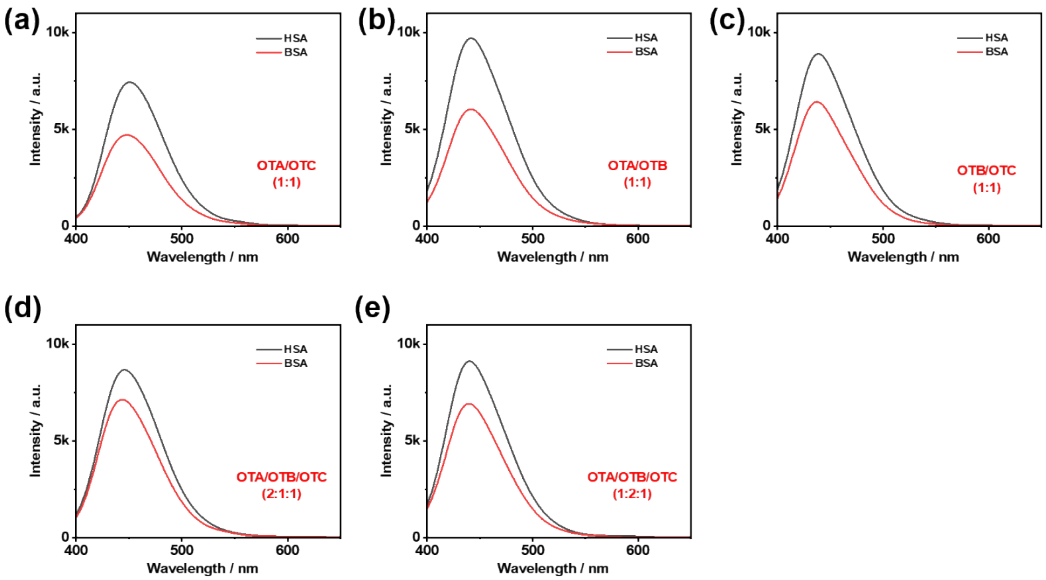

Fig. S9 The fluorescent spectra of (a-c) binary and (d-e) ternary mixtures with different ratios binding with HSA and BSA. [HSA] = [BSA] = 5 μM. Total concentration of mixtures is 1 μM. λ_{ex} = 380 nm.

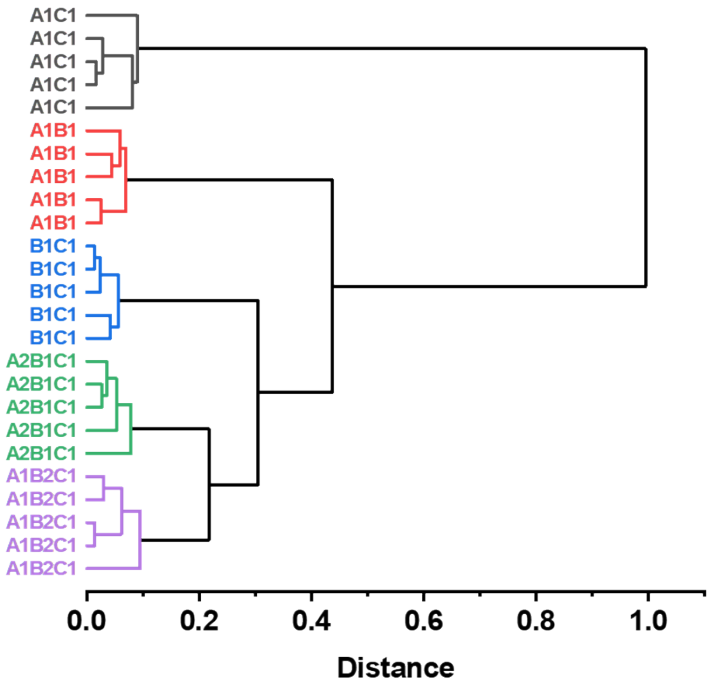

Fig. S10 HCA plot of the array for the identification of binary and ternary mixtures with different ratios. A1C1 represents a mixture of OTA and OTC with a molar ratio of 1:1. A1B1 represents a mixture of OTA and OTB with a molar ratio of 1:1. B1C1 represents a mixture of OTB and OTC with a molar ratio of 1:1. A2B1C1 represents a mixture of OTA, OTB and OTC with a molar ratio of 2:1:1. A1B2C1 represents a mixture of OTA, OTB and OTC with a molar ratio of 1:2:1.

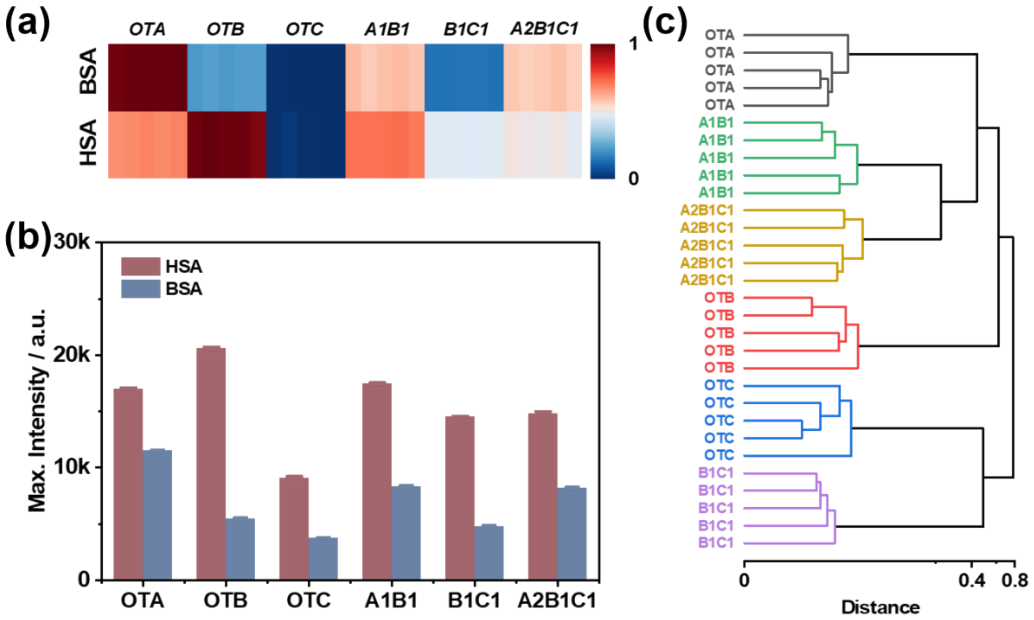

Fig. S11. (a) Heat map and (b) fluorescent response pattern of the array for ochratoxin and their mixture in real sample. Error bars indicate the standard deviation of five replicates. (c) HCA plot of the array for the identification of ochratoxins and their mixtures in real sample. B1C1 represents a mixture of OTB and OTC with a molar ratio of 1:1. A1B1 represents a mixture of OTA and OTB with a molar ratio of 1:1. B1C1 represents a mixture of OTB and OTC with a molar ratio of 1:1. A2B1C1 represents a mixture of OTA, OTB and OTC with a molar ratio of 2:1:1.

Table S1 The fluorescence wavelength information of the albumin-ochratoxin complexes.

Complex	**OTA**		**OTB**		**OTC**	
	HSA	BSA	HSA	BSA	HSA	BSA
Wavelength / nm	451	447	434	433	445	445

Table S2 Training matrix of fluorescence response pattern from the array for ochratoxins (2 μM). LDA was carried out and results in two factors of the canonical scores.

		Fluorescence response		Results LDA	
Ochratoxins	Repetition	HSA	BSA	LD1	LD2
OTA	1	17792.1	15604.4	-3.01	26.37
	2	17929.7	15677.9	-1.39	26.41
	3	17936	15572	-1.25	25.47
	4	17795.7	15727.6	-3.03	27.42
	5	18023.1	15855.1	-0.35	27.53
OTB	1	23737	13885.8	69.64	-14.22
	2	23819.1	14229.6	70.45	-11.60
	3	23787.6	14095.3	70.14	-12.63
	4	23632.7	14031.2	68.30	-12.51
	5	23731.4	14022.6	69.50	-13.01
OTC	1	12073.3	8171.9	-68.14	-13.07
	2	12203.8	8107.5	-66.53	-14.19
	3	12147.2	7999.9	-67.16	-14.88
	4	11988.5	7922.1	-69.03	-14.86
	5	12079.6	8274.7	-68.12	-12.21

Table S3 LDA jackknifed classification matrix table obtained from the array for ochrantoxins. The jackknifed classification matrix with cross-validation reveals a 100% accuracy.

			Predicted group			Total
			OTA	OTB	OTC	
Original value	Count	OTA	5	0	0	5
		OTB	0	5	0	5
		OTC	0	0	5	5
	%	OTA	100	0	0	100
		OTB	0	100	0	100
		OTC	0	0	100	100
Cross validation	Count	OTA	5	0	0	5
		OTB	0	5	0	5

		OTC	0	0	5	5
		OTA	100	0	0	100
	%	OTB	0	100	0	100
		OTC	0	0	100	100

Table S4 Detection and identification of unknown samples using LDA from the array.

		Fluorescence response		Results LDA		Analyte	
Ochratoxins	Repetition	HSA	BSA	LD1	LD2	Identification	Verification
S1	1	12429	7128.7	-63.30	-23.62	OTC	OTC
	2	12263.1	7135.7	-65.31	-22.85		
	3	12176.4	7198.5	-66.39	-21.93		
	4	12430	7083.4	-63.27	-24.02		
	5	12209	7155	-65.97	-22.44		
S2	1	18516.1	14952.2	6.07	17.60	OTA	OTA
	2	18440.3	15101.8	5.08	19.22		
	3	18358.8	15114.4	4.09	19.68		
	4	18496.9	15315.3	5.65	20.82		
	5	18571.3	15225.8	6.59	19.72		
S3	1	12226.1	7703.1	-66.05	-17.78	OTC	OTC
	2	12256	7812.3	-65.75	-16.97		
	3	12197.8	7790.9	-66.44	-16.90		
	4	12195.1	7945.9	-66.55	-15.55		
	5	12054.9	7777.6	-68.16	-16.40		
S4	1	23429.6	12384.3	66.71	-25.86	OTB	OTB
	2	23383.6	12715.1	65.98	-22.80		
	3	23473.6	12749.5	67.05	-22.89		
	4	23432.9	12758.7	66.55	-22.64		
	5	23380.1	12975.7	65.80	-20.53		
S5	1	17530.9	14382.3	-5.52	16.94	OTA	OTA
	2	17546.1	14494.8	-5.40	17.85		
	3	17597.9	14444.3	-4.75	17.19		
	4	17437.1	14525.2	-6.73	18.58		
	5	17535.3	14539.2	-5.55	18.28		
S6	1	12065.5	7358.8	-67.81	-20.06	OTC	OTC
	2	12032.9	7403.5	-68.23	-19.54		
	3	12003.2	7283	-68.52	-20.45		
	4	12066.4	7252.1	-67.74	-20.99		
	5	12086.4	7318.7	-67.54	-20.50		
S7	1	17340.1	14376.7	-7.82	17.72	OTA	OTA
	2	17219	14365	-9.28	18.14		
	3	17321.8	14434.2	-8.07	18.30		
	4	17262.8	14400.8	-8.77	18.26		
	5	17261.5	14434.2	-8.80	18.56		

S8	1	22887.9	12149.8	60.30	-25.54	OTB	OTB
	2	22940.7	12363.1	60.82	-23.92		
	3	23000.4	12502.4	61.47	-22.98		
	4	22746.2	12567.4	58.37	-21.32		
	5	22696.6	12411.1	57.85	-22.45		
S9	1	22634.3	11956.6	57.34	-26.11	OTB	OTB
	2	22517.9	12302.9	55.75	-22.61		
	3	22455.8	12076.9	55.12	-24.30		
	4	22456.5	12466.6	54.92	-20.93		
	5	22478.7	12303.4	55.28	-22.44		
S10	1	16611	14105.4	-16.48	18.53	OTA	OTA
	2	16715.3	14397.9	-15.37	20.61		
	3	16571.3	14315.6	-17.07	20.52		
	4	16353.9	14432.6	-19.75	22.47		
	5	16466.5	14321.1	-18.34	21.02		

Table S5 LDA jackknifed classification matrix table obtained from the array for mixtures. The jackknifed classification matrix with cross-validation reveals a 100% accuracy.

			Predicted group					Total
			OTA-OTC (1:1)	OTA-OTB (1:1)	OTB-OTC (1:1)	OTA-OTB-OTC (2:1:1)	OTA-OTB-OTC (1:2:1)	
Original value	Count	OTA-OTC (1:1)	5	0	0	0	0	5
		OTA-OTB (1:1)	0	5	0	0	0	5
		OTB-OTC (1:1)	0	0	5	0	0	5
		OTA-OTB-OTC (2:1:1)	0	0	0	5	0	5
		OTA-OTB-OTC (1:2:1)	0	0	0	0	5	5
	%	OTA-OTC (1:1)	100	0	0	0	0	100
		OTA-OTB (1:1)	0	100	0	0	0	100
		OTB-OTC (1:1)	0	0	100	0	0	100
		OTA-OTB-OTC (2:1:1)	0	0	0	100	0	100
		OTA-OTB-OTC (1:2:1)	0	0	0	0	100	100
Cross validation	Count	OTA-OTC (1:1)	5	0	0	0	0	5
		OTA-OTB (1:1)	0	5	0	0	0	5
		OTB-OTC (1:1)	0	0	5	0	0	5
		OTA-OTB-OTC (2:1:1)	0	0	0	5	0	5
		OTA-OTB-OTC (1:2:1)	0	0	0	0	5	5
	%	OTA-OTC (1:1)	100	0	0	0	0	100
		OTA-OTB (1:1)	0	100	0	0	0	100
		OTB-OTC (1:1)	0	0	100	0	0	100
		OTA-OTB-OTC (2:1:1)	0	0	0	100	0	100
		OTA-OTB-OTC (1:2:1)	0	0	0	0	100	100