Electronic Supplementary Material (ESI) for Analyst.

A Poly(arylene ethynylene)-Based Microfluidic Fluorescence Sensor Array for Discrimination of Polycyclic Aromatic Hydrocarbons

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General Information

Materials and apparatus

Whatman filter paper grade 2 were purchased from Whatman GE Healthcare (U.S.). PAHs including chrysene, anthracene, fluorene, phenanthrene, acenaphthene, naphthalene, acetone and chloroform were obtained from Merck Chemical Company. Benzo[a]anthracene, benzo[a]pyrene, pyrene, and fluoranthene were bought from Sigma Aldrich. All the chemicals were of analytical reagent grade and used without further purification. Stock solution of the PAHs were prepared in acetone, Solutions with concentrations of 5, 10, 50, and 100 mg L^{-1} were prepared.

Unless otherwise stated, solvents were purchased from the chemical store of the Organisch-Chemisches Institut der Universität Heidelberg and distilled prior to use. Absolute solvents were prepared according to standard procedures and stored under argon over appropriately sized molecular sieve.3Absolute THF, Et_2O, toluene, CH_2Cl_2 and MeCN were purchased from Sigma-Aldrich and purified by a solvent purification system (MBraun, MB SPS-800, filter material: MB-KOL-A, MB-KOL-M; catalyst: MB-KOL-C). Chemicals other than solvents were either purchasedfrom the chemical store of the Organisch-Chemisches Institut Universität Heidelberg or from commercial laboratory suppliers unless reported otherwise. Tetraethyl-2,2',2",2"'-((2,2'-((2,5-diethynyl-1,4phenyl-ene)bis(oxy))bis(acetyl))bis(aza-netriyl))tetraacetate and Tetraethyl-2,2',2",2"'-((2,2'-((2,5-diethynyl-1,4phenyl-ene)bis(oxy))bis(acetyl))bis(aza-netriyl))tetraacetate: [1].

PAE 1 and 3-6: Synthesize of these compounds were previously reported by Bunz' research group; PAE 1: [2], PAE 3:[3], PAE 4:[4], PAE 5:[5], PAE 6:[6]

All fluorescence spectra were recorded on a Perkin-Elmer LS50B fluorescence spectrophotometer with excitation and emission slits of 5 nm. UV-Vis absorption were studied by Shimadzu 1601 UV-Vis spectrometer, using quartz analytical cells of 1 cm path length. For image analysis, images were captured by a smart phone camera, iPhone 7.

For experiments conducted under an atmosphere of nitrogen, standard Schlenk and Glovebox (Unilab-2000, MBRAUN) techniques were used. The addition of solvents or reagents was carried out using nitrogen flushed stainless steel cannulas and plastic syringes. For device preparation, spectroscopic and analytic characterizations, the following devices were used:

^{1}H NMR spectra were recorded in $CDCl_3$ at room temperature on Bruker Avance III 300 (300 MHz), Bruker Avance III 400 (400 MHz), Bruker Avance III 500 (500 MHz) or Bruker Avance III 600 (600 MHz) spectrometer. The data were interpreted in first order spectra. All spectra were recorded in $CDCl_3$, D_2O or DMSO-d_6. Chemical shifts are reported in δ units relative to the solvent residual peak ($CHCl_3$ in $CDCl_3$ at δ_H = 7.27 ppm, HDO in D_2O at δ_H = 4.75 ppm) or TMS (δ_H = 0.00 ppm). The following abbreviations are used to indicate the signal multiplicity: m (multiplet). All NMR spectra were integrated and processed using ACD/Spectrus Processor.

^{13}C NMR spectra were recorded at room temperature on the following spectrometers: Bruker Avance III 300 (75 MHz), Bruker Avance III 400 (100 MHz), Bruker Avance III 500 (125 MHz) or Bruker Avance III 600 (150 MHz). The spectra were recorded in $CDCl_3$. Chemical shifts are reported in δ units relative to the solvent signal: $CDCl_3$ [δ_C = 77.00 ppm] or TMS (δ_c = 0.00 ppm).

IR spectra were recorded on a JASCO FT/IR-4100. Substances were applied as a solid or in solution. Processing of data was done using the software JASCO Spectra manager™.

Gel Permeation Chromatography (GPC) was performed using a JASCO PU 2080 HPLC equipped with three commercially available **P**olymer **S**tandards **S**ervice (PSS) cross-linked poly(stryrene) columns. The consecutive exclusion sizes were 1000 Å, 10000 Å, and 100000 Å respectively. For detection, a JASCO UV-2075 Plus detector were used. The column was operated with chloroform as an eluent and a flow rate of 1.0 mL/s.

Synthesis

Poly[tetraethyl2,2',2'',2'''-((2,2'-((2-ethynyl1,4phenylene)bis(oxy))bis(acetyl))bis(aza netriyl))tetraacetate] (**PAE Pre2**): Tetraethyl 2,2',2'',2'''-((2,2'-((2,5-diethynyl-1,4phenyl-ene)bis(oxy))bis(acetyl))bis(azanetriyl))tetraacetate (220 mg, 357 µmol) and tetraethyl 2,2',2'',2'''-((2,2'-((2,5-diiodo1,4phenylene)bis(oxy))bis(acetyl))-bis(azanetriyl))tetra-acetate (293 mg, 357 µmol) were dissolved in degassed THF/CHCl3/NEt3 (1:1:0.5, 5 mL/5 mL/2.5 mL). $Pd(PPh_3)_2Cl_2$ (1.9 mg, 2.7 µmol) and CuI (1.0 mg, 5.4 µmol) was added and the mixture was stirred under nitrogen at 60 °C for 5 d. The solvent was evaporated *in vacuo*. Two times, the crude product was dissolved in a small amount of CHCl3 and slowly added to an excess of *n*-pentane. Filtration afforded the title compound **PAE Pre2** (339 mg, 80%) as orange solid. The *M*n was estimated to be 4.3 x 10^3 g/mol with a PDI of 1.3 (CHCl3). ^{1}H NMR (600 MHz, CDCl3): δ = 7.20-7.12 (m, 1 H), 7.09-7.03 (m, 1H), 4.93-4.86 (m, 4 H), 4.47-4.32 (m, 4 H), 4.25-4.07 (m, 12 H), 1.28-1.23 (m, 12 H) ppm. due to low solubility, ^{13}C NMR spectrum could not be obtained. IR (cm^{-1}): ν 3240, 2982, 1742, 1659, 1469, 1404, 1372, 1351, 1310, 1263, 1189, 1094, 1069, 1023, 970, 872, 822, 765, 650, 561, 495, 466, 442, 421, 409.

Poly[2,2',2'',2'''-((2,2',2'',2'''-((2,2'-((2-ethynyl-1,4-phenylene)bis(oxy))bis(acetyl))bis-(aza-netriyl))tetrakis(acetyl))tetrakis(azanediyl))tetrakis(*N*,*N*,*N*-trimethylethan-1-aminium)iodide] (**PAE 3**): The neutral precursor polymer **PAE Pre3** (100 mg, 169 μmol) was dissolved in *N*,*N*'-dimethylethylenediamine (10 mL) and stirred at 70 °C for 12 h. The reaction mixture was evaporated to dryness and washed with copious amounts of *n*-pentane. The crude product was dissolved in CH_2Cl_2 (20 mL). After addition of MeI (10 mL) the reaction mixture was stirred for 3 d at ambient temperature. All volatiles were removed under reduced pressure. The residue was dissolved in H2O and dialyzed against DI H2O for 3 d. Filtration and freeze-drying afforded **PAE 3** as yellow fluffy solid (62 mg, 51%). The *M*n and PDI result from **PAE Pre3**. ^{1}H NMR (500 MHz, D2O): δ = 7.36-6.95 (m, 2 H), 5.15-4.84 (m, 4 H), 4.42-4.08 (m, 8 H), 3.68-3.48 (m, 16 H), 3.14-3.08 (m, 36 H) ppm. due to low solubility, ^{13}C NMR spectrum could not be obtained. IR (cm^{-1}): ν 3431, 3248, 3015, 2948, 1651, 1538, 1474, 1414, 1336, 1296, 1254, 1200, 1144, 1075, 1034, 955, 917, 554, 502, 487, 454, 419.

Detection and data analysis

In order to obtain high quality and reproducible images, a dark cabinet, which was fabricated previously in Hemmateenejad's research group was used [7,8]. The box was equipped with two UV lamps as the excitation source (254 and 366 nm). The lamps were fixed at a height of approximately 10 cm, on the top of the plate on which the device was placed Images were taken before and after exposing the immobilized sensor to the analyte's solutions (Fig. S1).

In this work, PCA-DA analysis was performed using a classification toolbox written by Milano chemometrics (http://michem.disat.unimib.it/chm/). In this toolbox, the graphical user interface

(GUI) was used that enables the user to perform all the analysis steps (data loading, setting preparation, model calculation, sample prediction, analysis of results, cross validation) [9,10].

Table S1. The structures of 10 PAHs identified by the PAE as sensing materials.

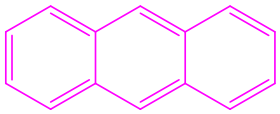
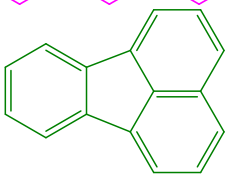
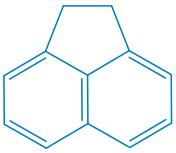
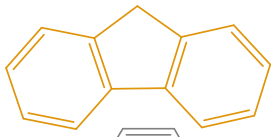
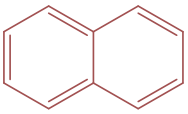
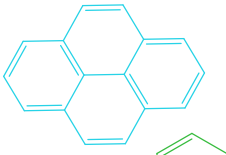
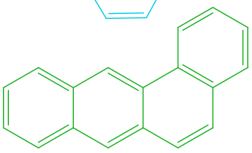
Name	Abbreviation	Structure
Anthracene	Ant	
Fluoranthene	Fla	
Acenaphthene	Ace	
Fluorene	Fl	
Phenanthrene	Phe	
Benzo[a]pyrene	BaP	
Chrysene	Chry	
Naphthalene	Nap	
Pyrene	Py	
Benzo[a]anthracene	BaA	

Table S2. Classification accuracies of calibration and cross-validation for three different volume of reagents' solutions.

Volume of the reagents' solutions (µL)	**Accuracy (%)**	
	Calibration	Cross-validation
0.4	100	97
0.8	100	90
1.2	100	75

Table S3. Statistical parameters for evaluating the classification performance of the different concentrations of PAHs.

Analyte	Sensitivity for different concentrations (mg L^{-1})					Specificity for different concentrations (mg L^{-1})					Precision for different concentrations (mg L^{-1})				
	1	5	10	50	100	1	5	10	50	100	1	5	10	50	100
Acenaphthene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Anthracene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	0.97	1.00	1.00	0.80	1.00	0.80
Benzo[a]anthracene	0.50	1.00	1.00	1.00	1.00	0.92	0.97	1.00	1.00	1.00	0.40	1.00	1.00	1.00	1.00
Benzo[a]pyren	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chrysene	1.00	1.00	0.75	0.75	1.00	0.92	1.00	0.97	1.00	1.00	0.57	1.00	0.75	1.00	1.00
Fluorene	0.75	1.00	0.75	1.00	1.00	0.97	1.00	1.00	0.94	0.94	0.75	1.00	1.00	0.67	0.67
Fluoranthene	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00	1.00	1.00	0.80	1.00	1.00
Phenanthrene	0.50	1.00	0.75	1.00	0.75	0.94	0.94	1.00	1.00	1.00	0.50	0.67	1.00	1.00	1.00
Naphthalene	0.50	0.25	1.00	0.75	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Pyrene	0.25	1.00	1.00	1.00	1.00	0.92	1.00	1.00	1.00	1.00	0.25	1.00	1.00	1.00	1.00

Table S4. Classification accuracies of calibration and cross-validation for four different concentration of PAHs in the presence and absence of sensing zone #7.

Concentration (mg L^{-1})	**Accuracy (%) in the presence of sensing zone #7**		**Accuracy (%) in the absence of sensing zone #7**	
	Calibration	Cross-validation	Calibration	Cross-validation
5	100	93	100	90
10	100	93	100	75
50	100	95	100	87
100	100	95	100	90

Fig. S1. The image of handmade dark box with the UV lamp and sample holder that fixed inside of platform.

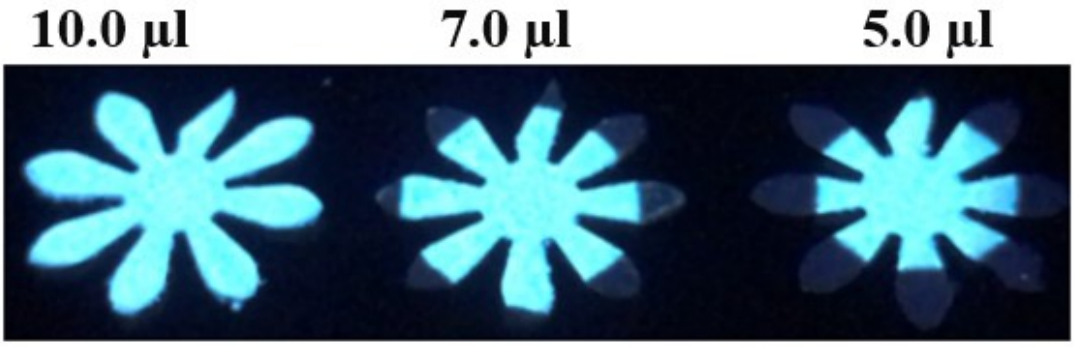


Fig. S2. Investigating the loading capacity of µPADs by introducing three volumes of 100 mg L^{-1} pyrene (5, 7 and 10 µL) into the sample zone.

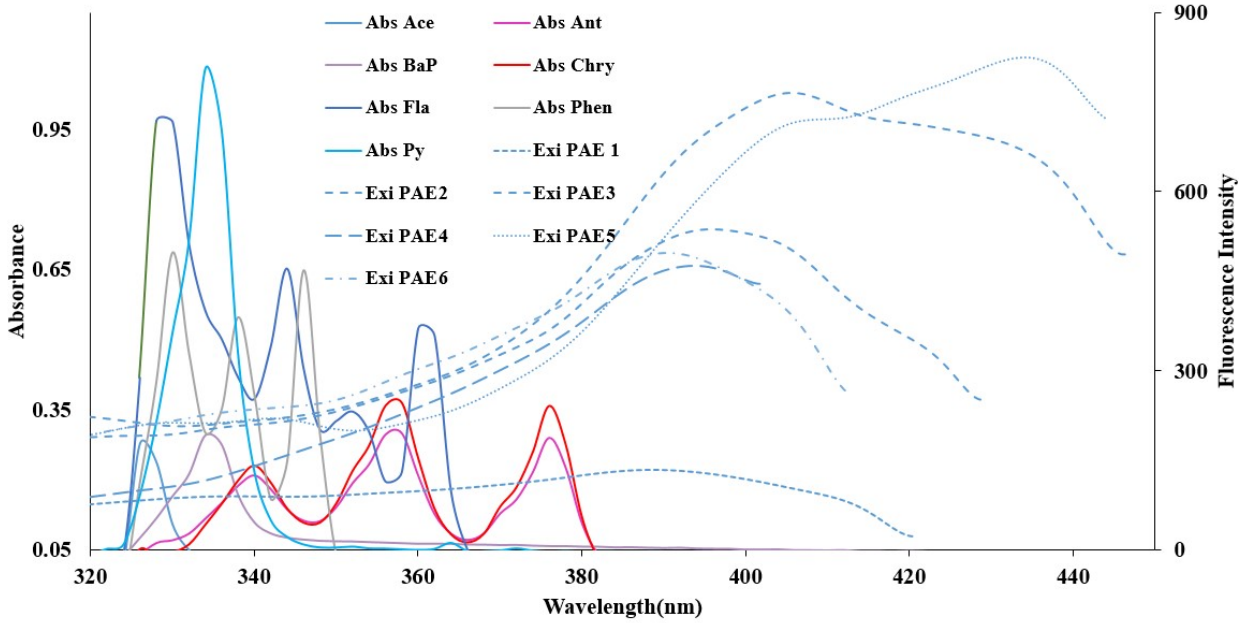


Fig. S3. Overlapping the absorption spectra of PAHs with the excitation spectra of PAEs.

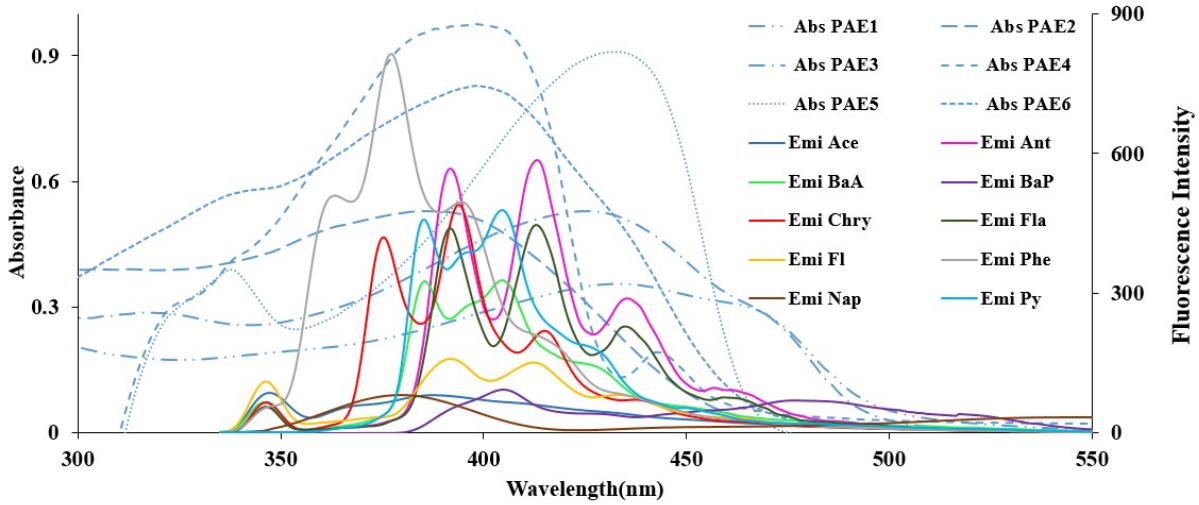


Fig. S4. Overlapping the emission spectra of PAHs with the absorption spectra of PAEs.

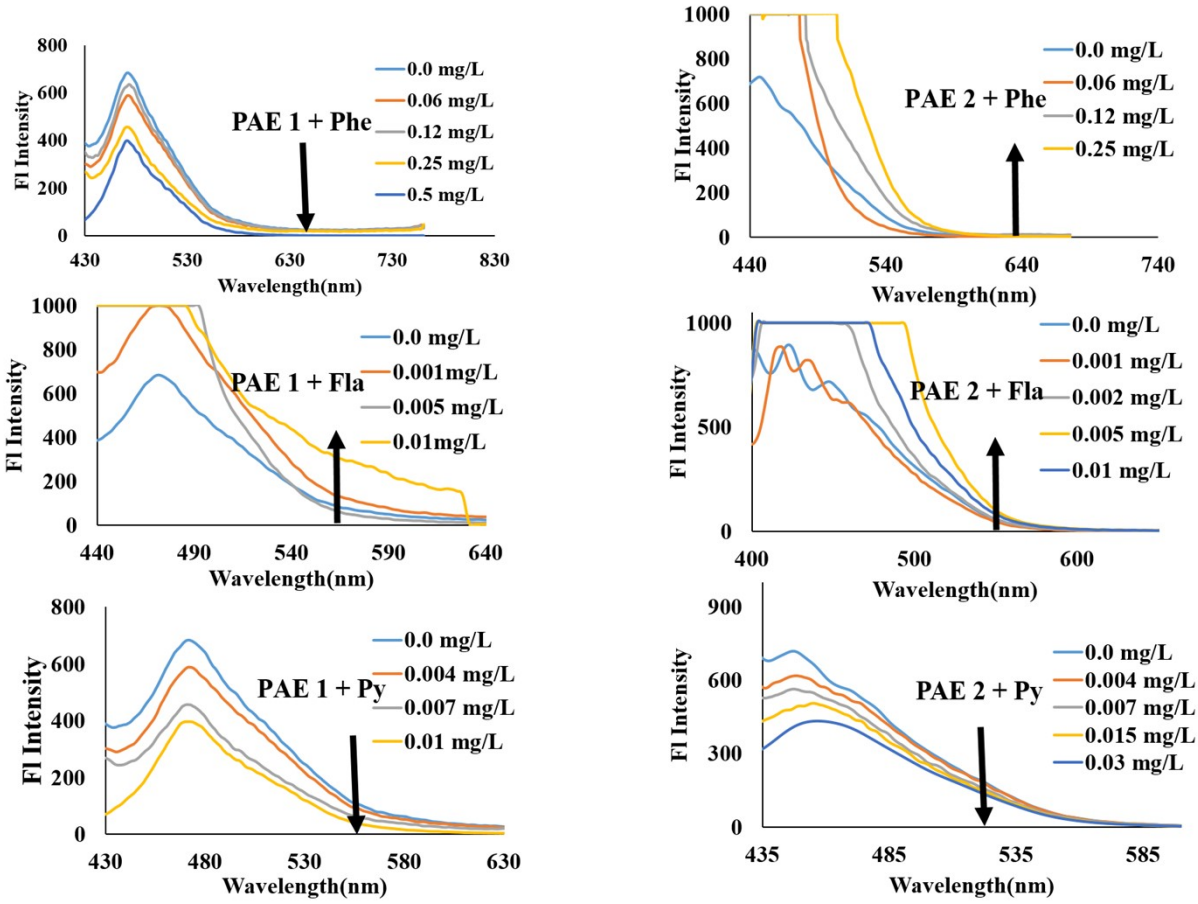


Fig. S5. Fluorescence titration profiles of PAE1 (5.0 mg L^{-1}) and PAE2 (1.0 mg L^{-1}) upon the addition of Phenanthrene, Fluoranthene, and pyrene in Acetone.

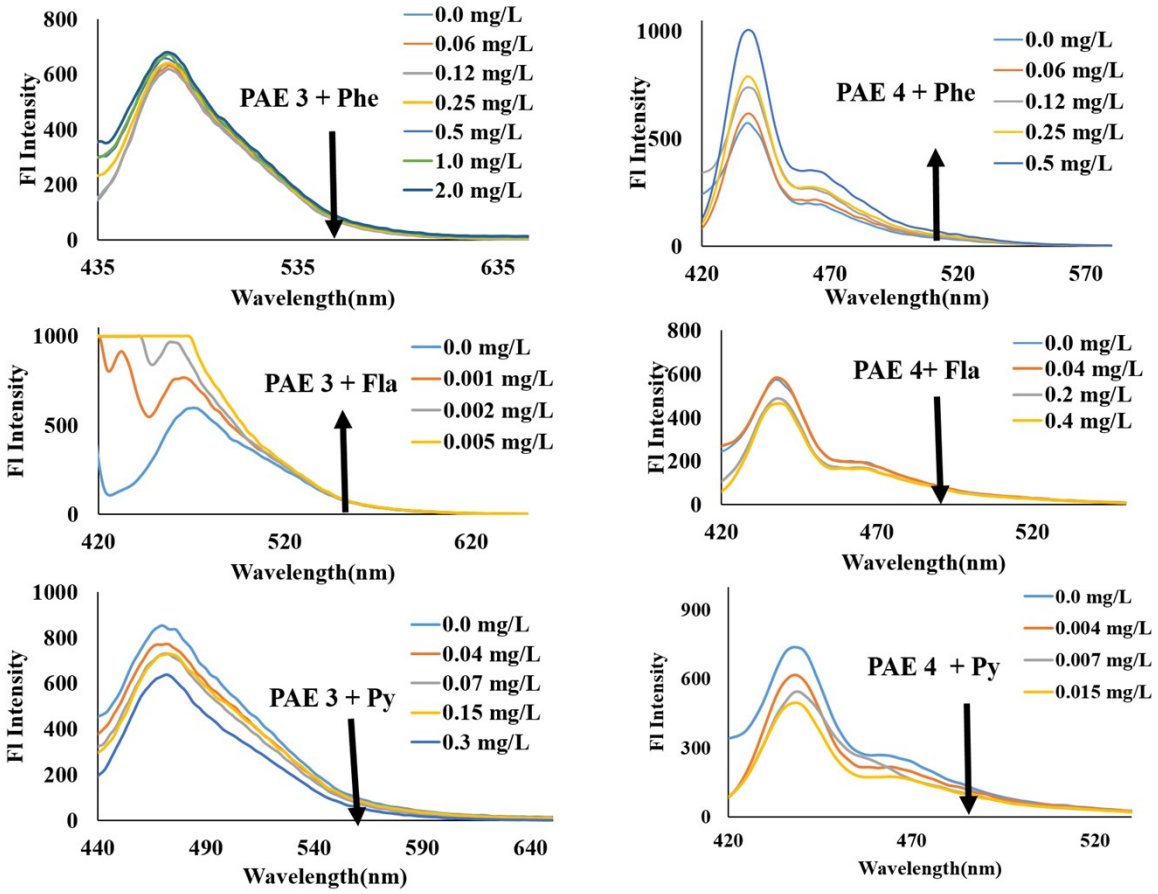


Fig. S6. Fluorescence titration profiles of PAE 3 (0.4 mg L^{-1}) and PAE 4 (0.22 mg L^{-1}) upon the addition of Phenanthrene, Fluoranthene, and pyrene in Acetone.

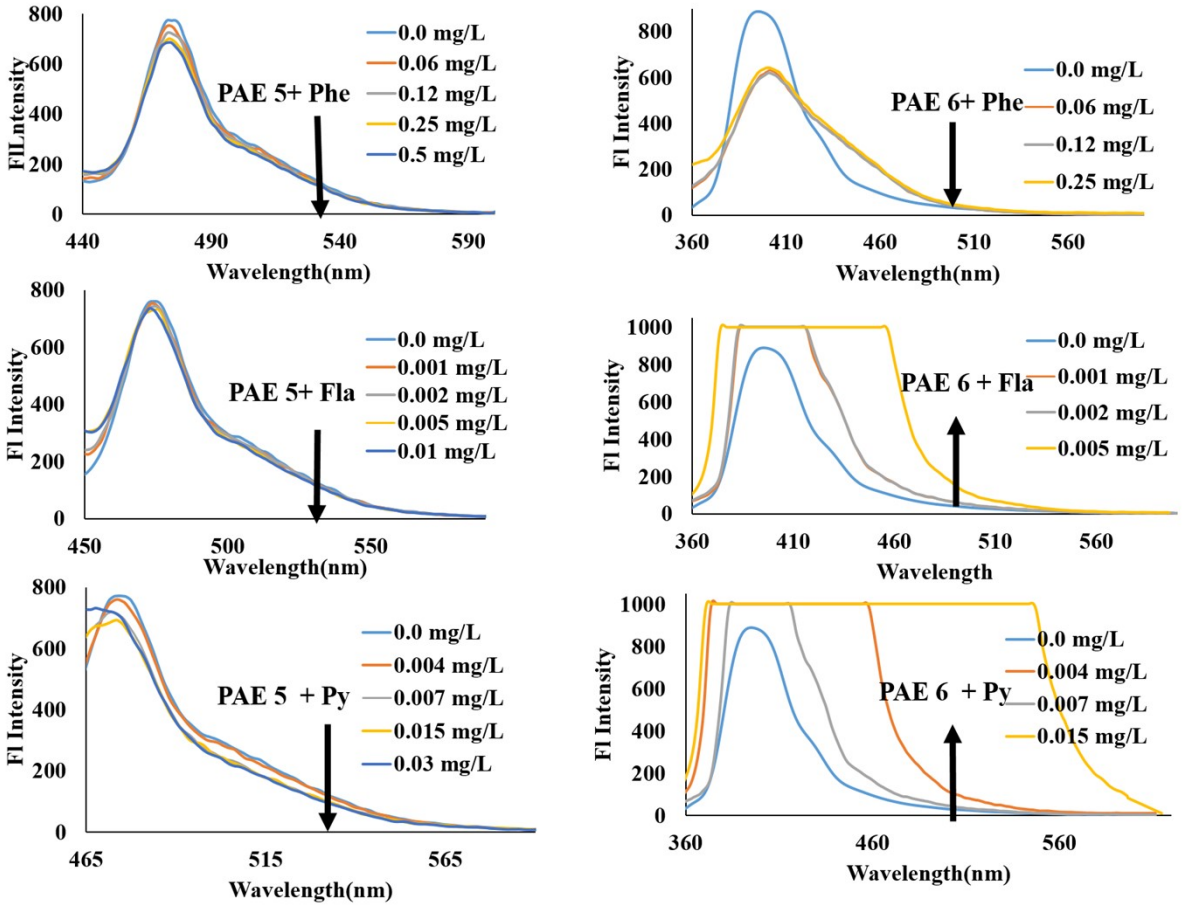


Fig. S7. Fluorescence titration profiles of PAE 5 (0. 2 mg L^{-1}) and PAE 6 (4.0 mg L^{-1}) upon the addition of Phenanthrene, Fluoranthene, and pyrene in Acetone.

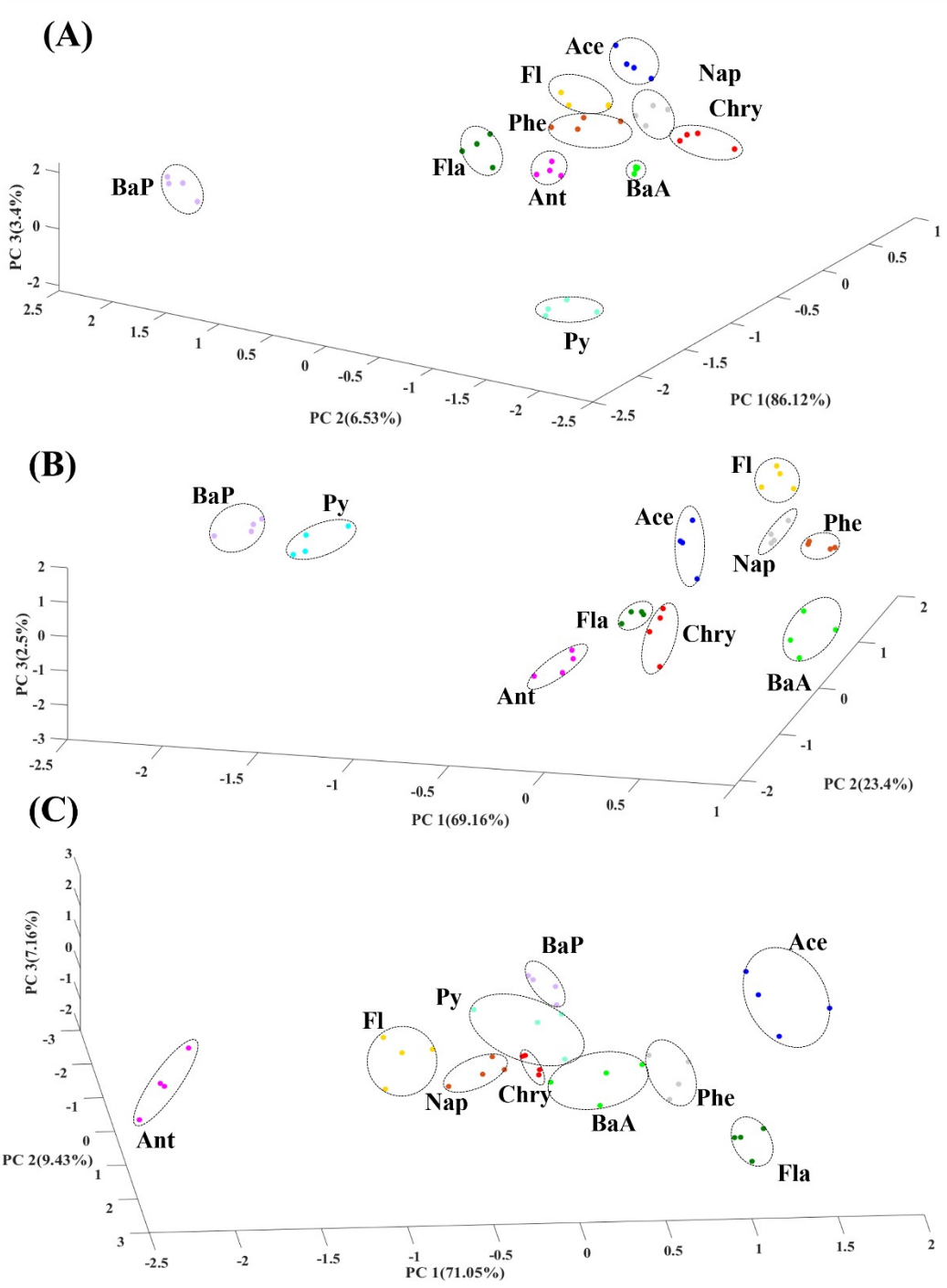


Fig. S8. 3D PCA-DA scores for different volumes (A) 0.4, (B) 0.8 and (C) 1.2 µL of the prepared reagents.

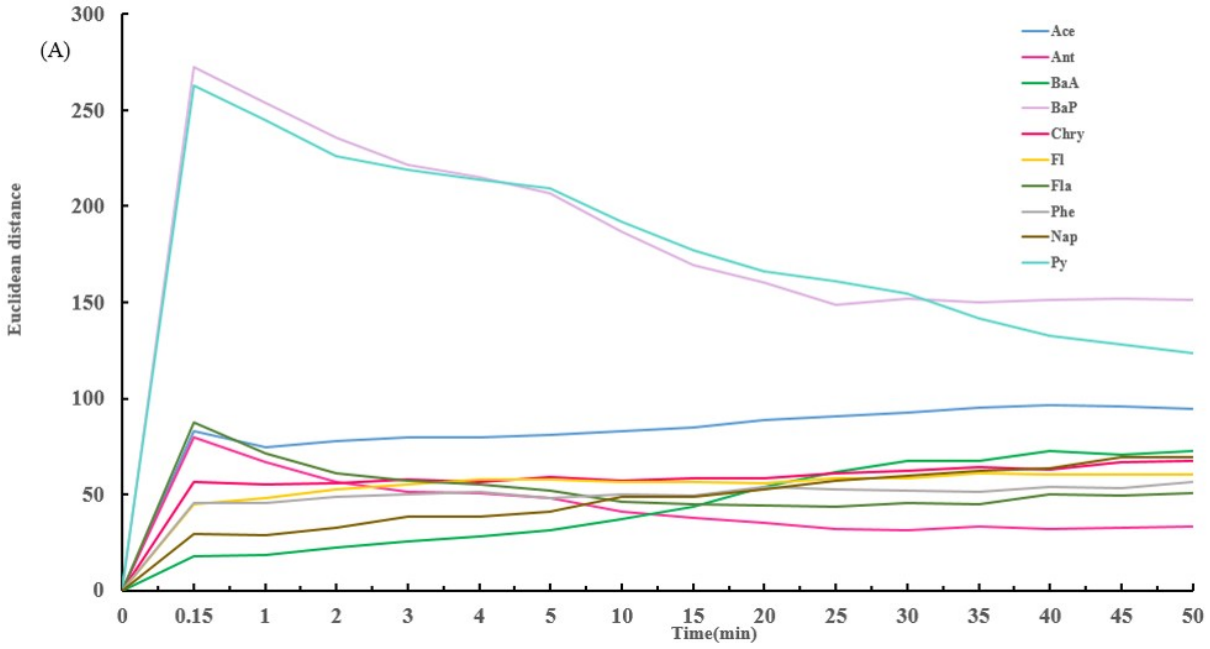


Fig. S9. Euclidean distance between 10 PAHs plotted versus reaction time at room temperature (100.0 mg L^{-1} concentration).

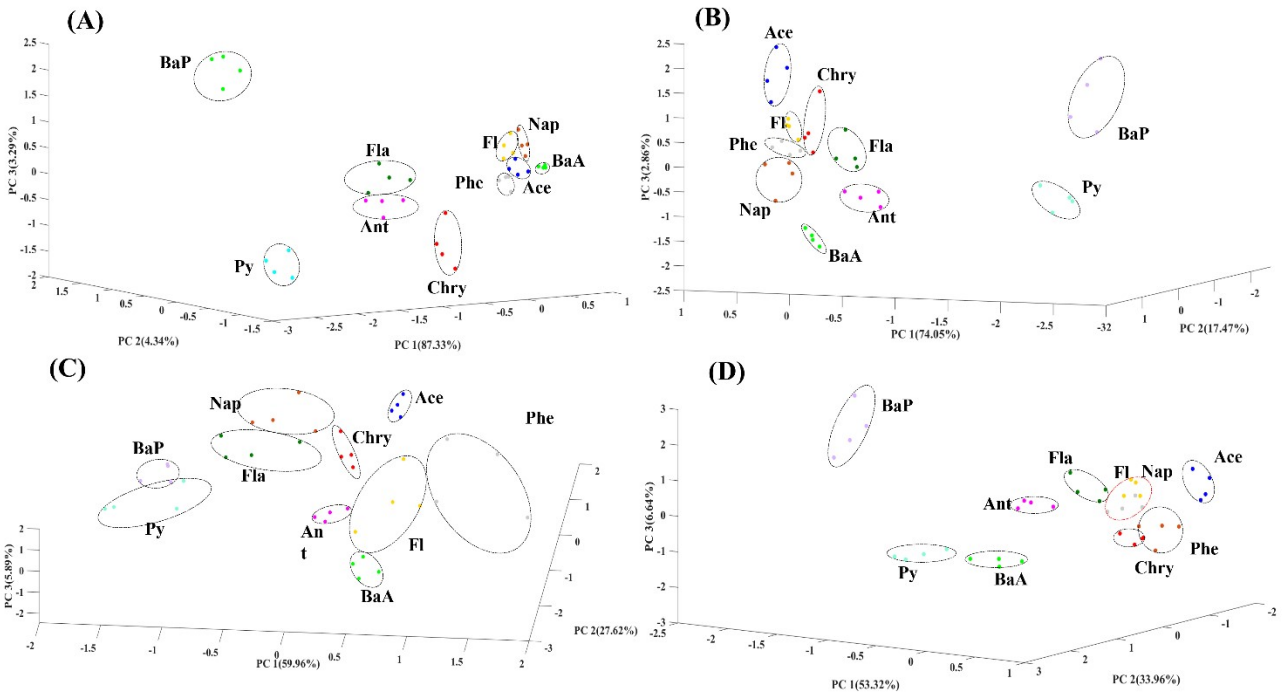


Fig. S10. The PCA-DA response of the sensor array at four different times (A) 10 s, (B) 15 min, (C) 30 min, and (D) 50 min.

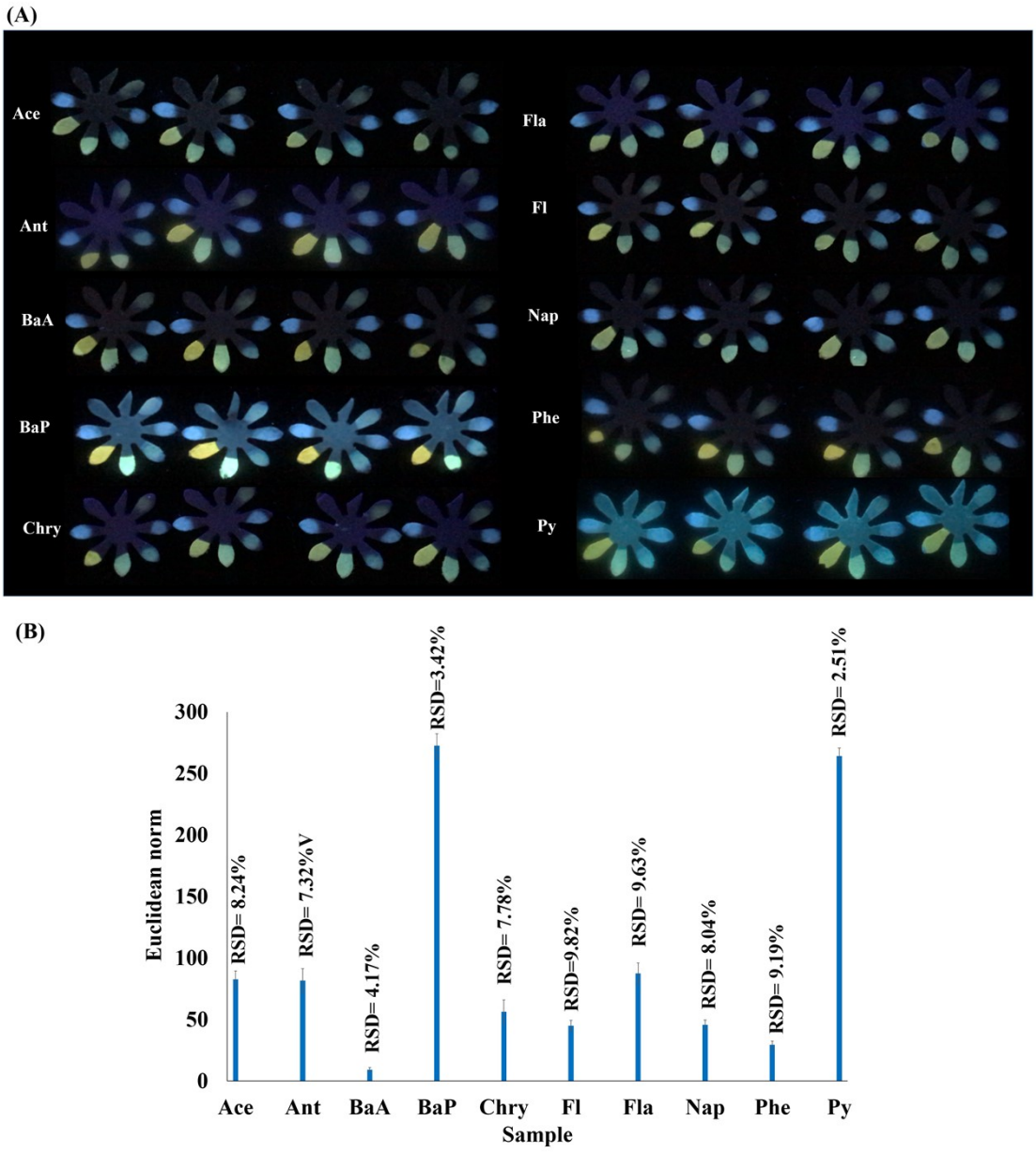


Fig. S11. The fluorescence response of μPAD (A) and the reproducibility of the response of sensor array (B) for discrimination of 100 mgL^{-1} of 10 PAHs. Error bars refer to standard deviation (n=4)

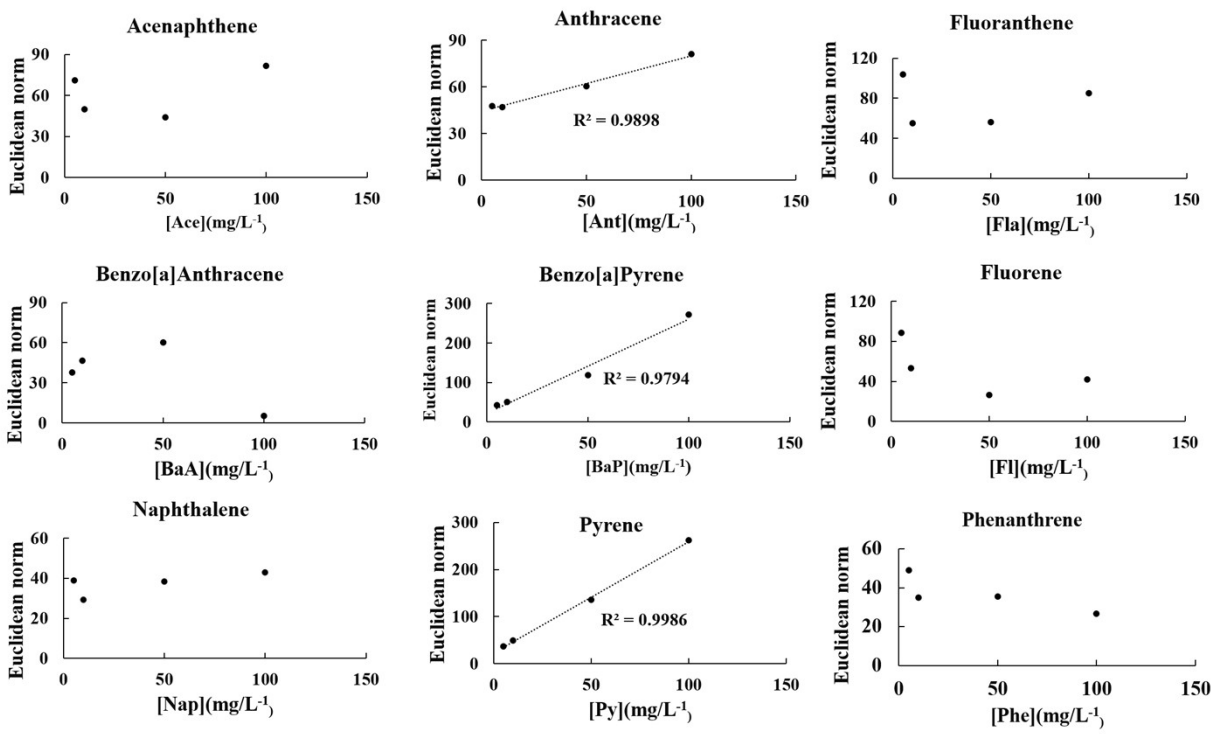


Fig. S12. Changes in the overall response of the sensor array (reported as Euclidean norm) as function of concentration for semiquantitative analysis of different PAHs

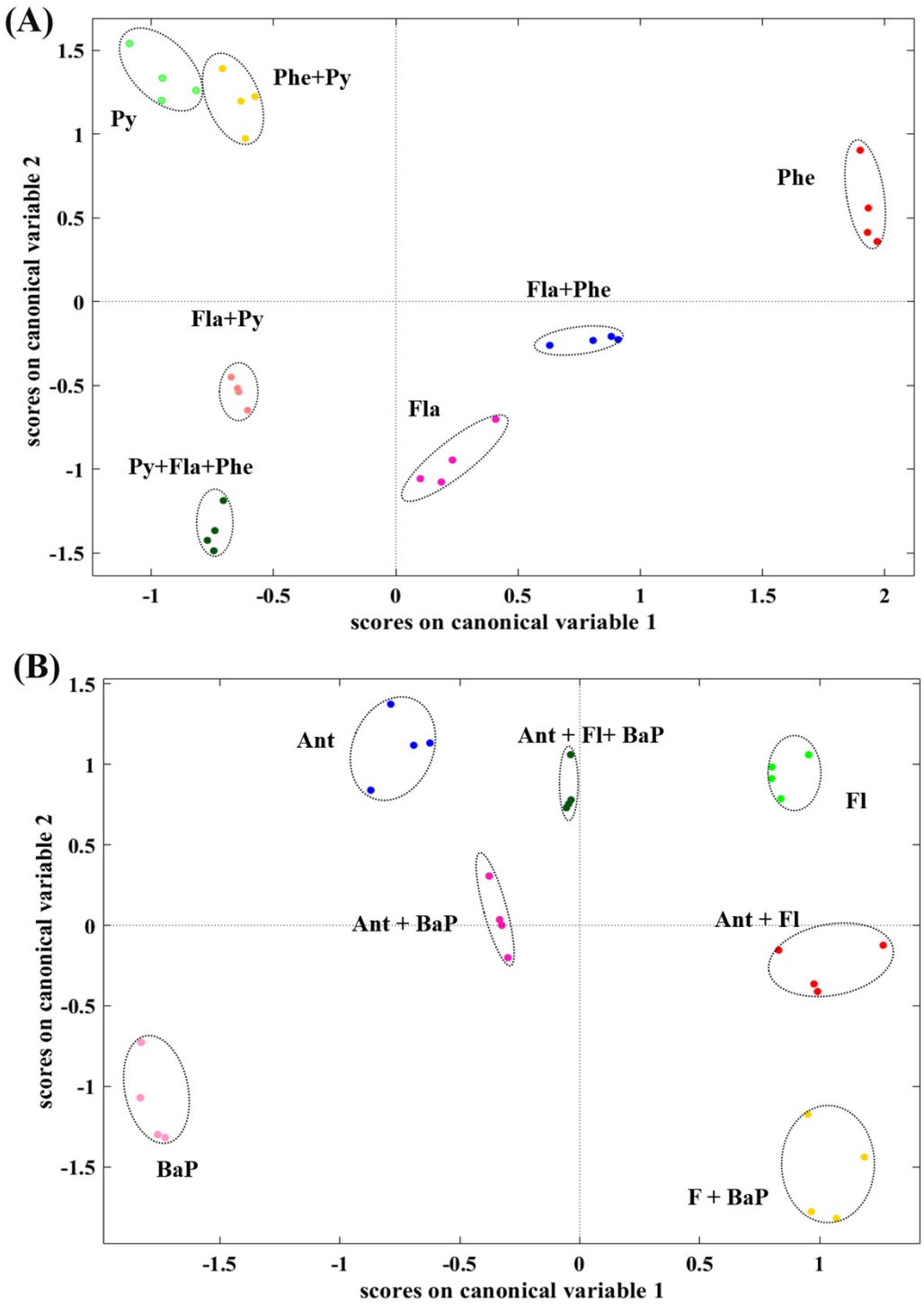


Fig. S13. PCA-DA score plot for the binary and ternary mixtures of (A) Py with Fla and Phe and (B) Ant with BaP and Fl.

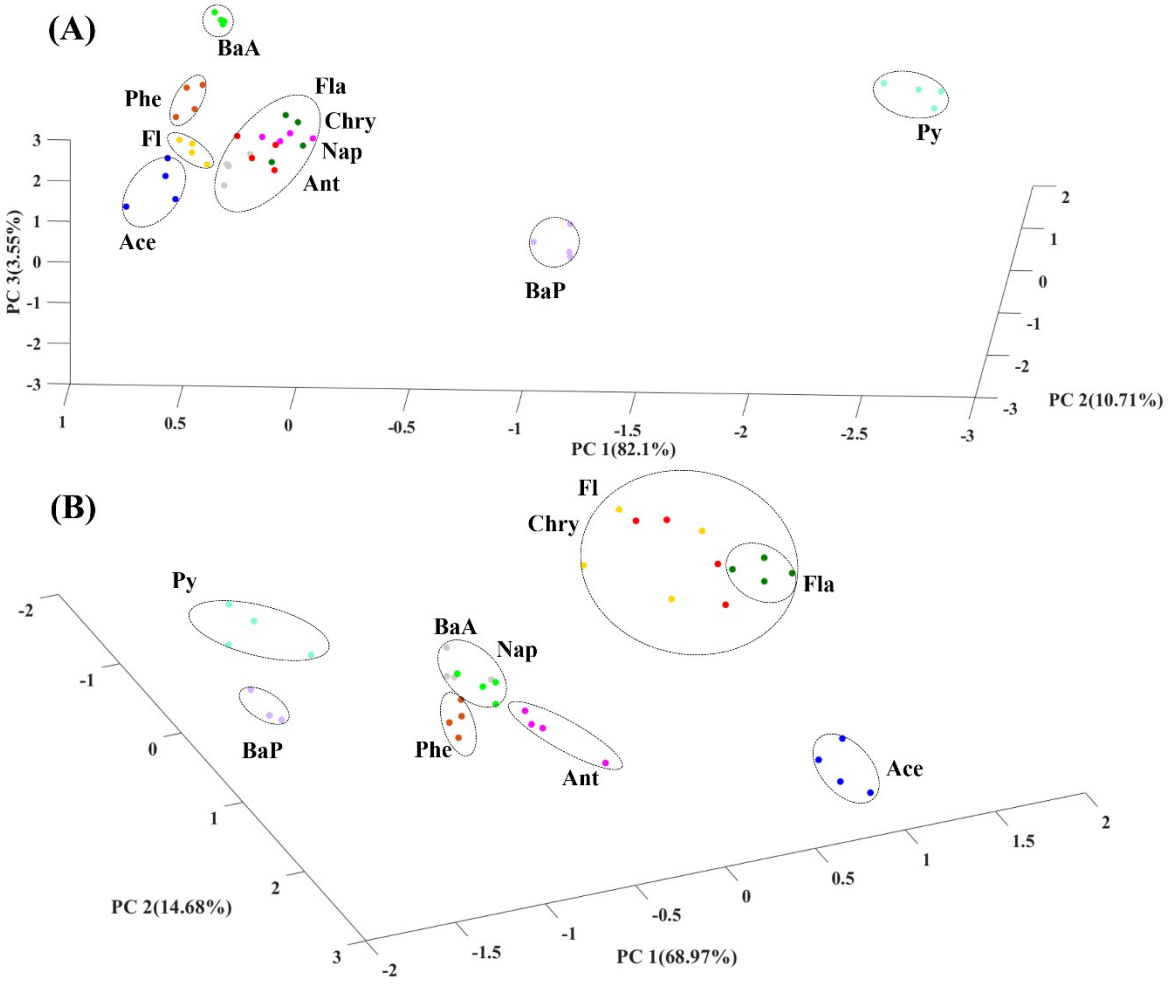


Fig. S14. The PCA-DA score plots for classification of ten PAHs at concentration of (A) 100.0 and (B) 10.0 mg L^{-1} after excluding the data of sensing zone #7.

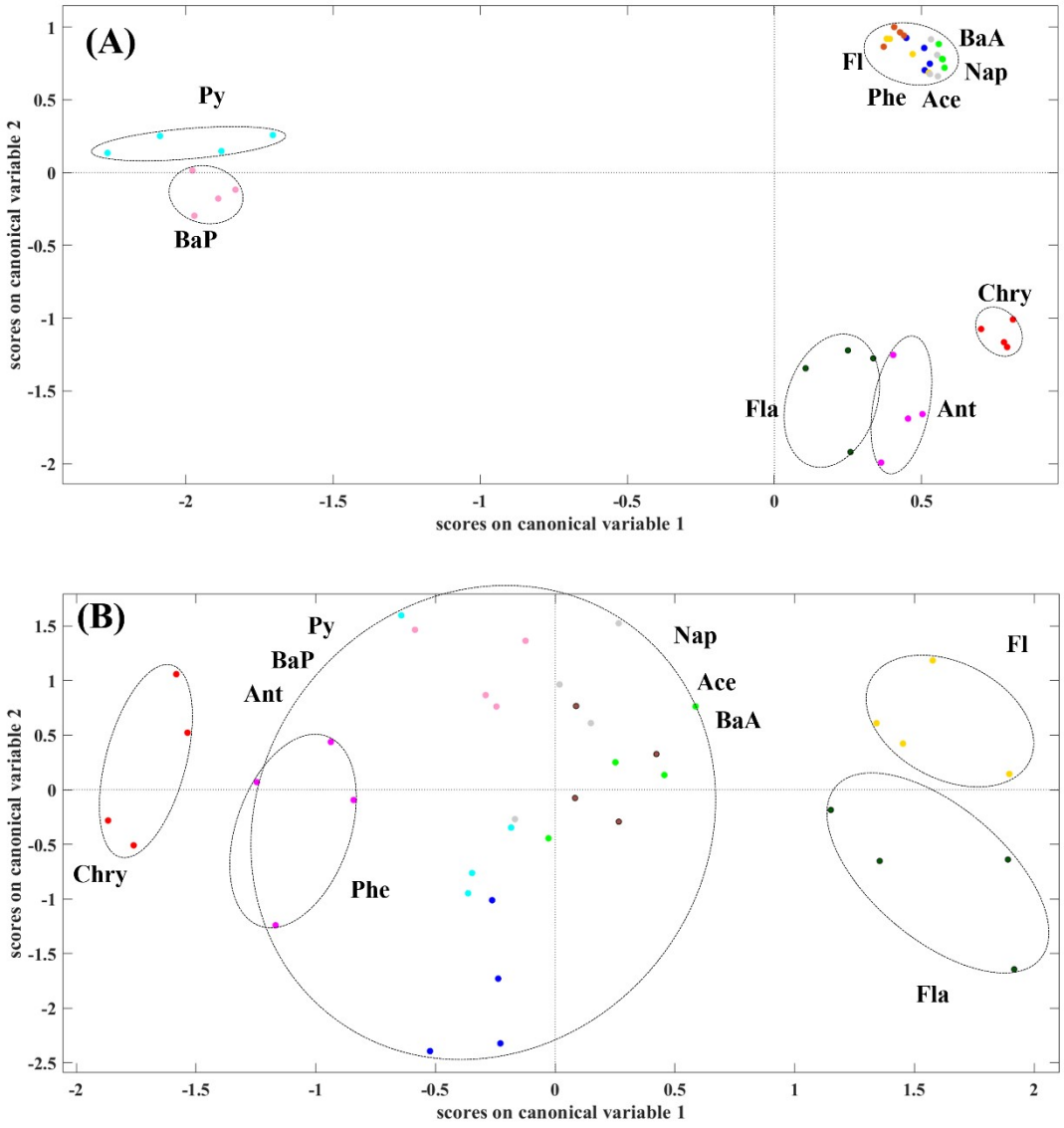


Fig. S15. 2D PCA-DA score plots for the R, G and B color elements of just sensing zone #7, (A) 100.0 and (B), 5.0 mg L^{-1}.

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