Supporting Information

An array-based nanosensor for detecting cellular responses in macrophages induced by femtomolar levels of pesticides

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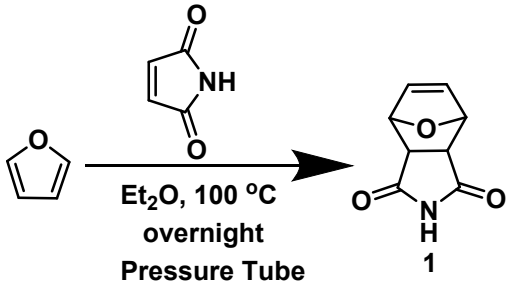
1. Materials

All chemicals and solvents were purchased from Fisher Scientific and Sigma-Aldrich unless otherwise stated.

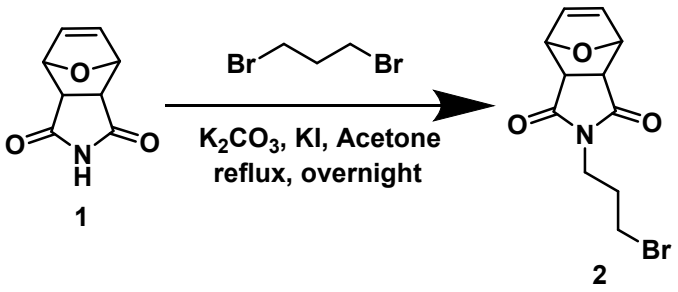
2. Synthesis of PONI-Benzyl-Pyrene polymer (PONI-C_3-Bz-Py)

The PONI-C_3-Bz-Pyrene polymer was synthesized according to a previous report with some modification.[1]



Synthesis of **1**

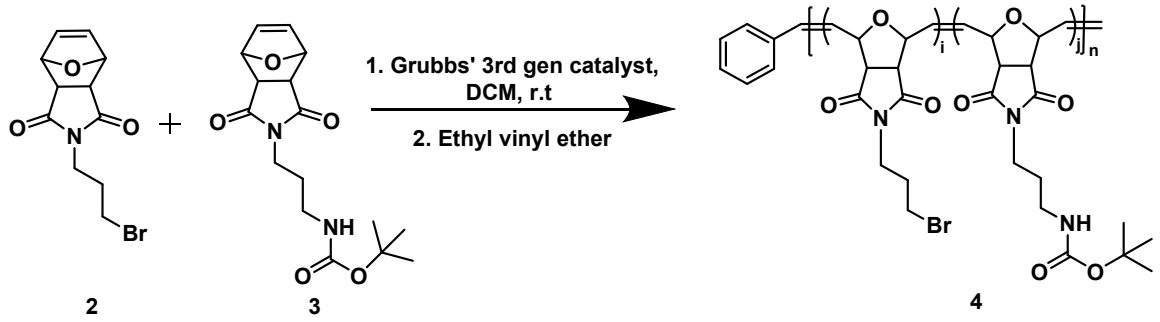
4.5 mL of furan (61.7 mmol, 1.5 eq) and 4.0 g of maleimide (41.1 mmol. 1.0 eq) were mixed in a pressure tube and added 5 mL of diethyl ether. The tube was heated overnight at 100 °C and then cooled down to room temperature. Then, the formed solid was filtered and washed with copious amounts of diethyl ether to isolate **1** as a white solid and was used without further purification. ^{1}H NMR (400 MHz, MeOD) 11.14 (s, 1H), 6.54 (s, 2H), 5.34 (s, 2H), 2.91 (s, 2H).



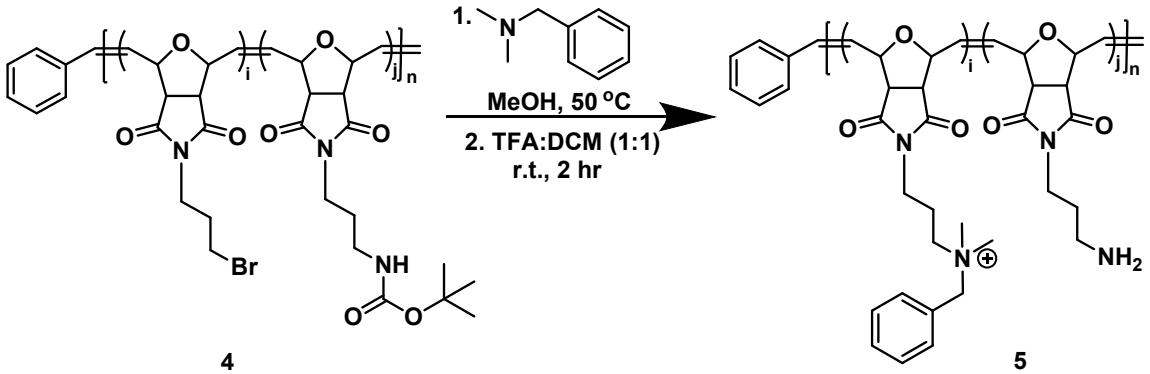
Synthesis of **2**

To a 250 mL round bottom flask equipped with a stir bar was added 70 mL of acetone. Next, **1** (3.75 g, 22.7 mmol, 1.0 eq) was added along with potassium carbonate (12.60 g, 91.2 mmol, 4.0 eq). The reaction mixture was connected to a reflux condenser and heated at 65 °C for 5 minutes. Finally, potassium iodide (0.70 g, 4.5 mmol, 0.2 eq) and 1,4-dibromopropane (6.28 g, 25.0 mmol, 1.1 eq) were added and stirred at 65 °C overnight. Afterwards, the reaction mixture was cooled down to room temperature, diluted with 150 mL of ethyl acetate and washed with water (7x, 50 mL) and brine (1x, 50 mL). The organic layer was dried with sodium sulfate, filtered and rotavaped. Column chromatography was performed to yield **2** as a white solid (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 6.52 (s, 2H), 5.29 (s, 2H), 3.63 (t, 2H), 3.32 (t, 2H), 2.87 (s, 2H), 2.18 (q, 2H).

Polymer synthesis scheme:

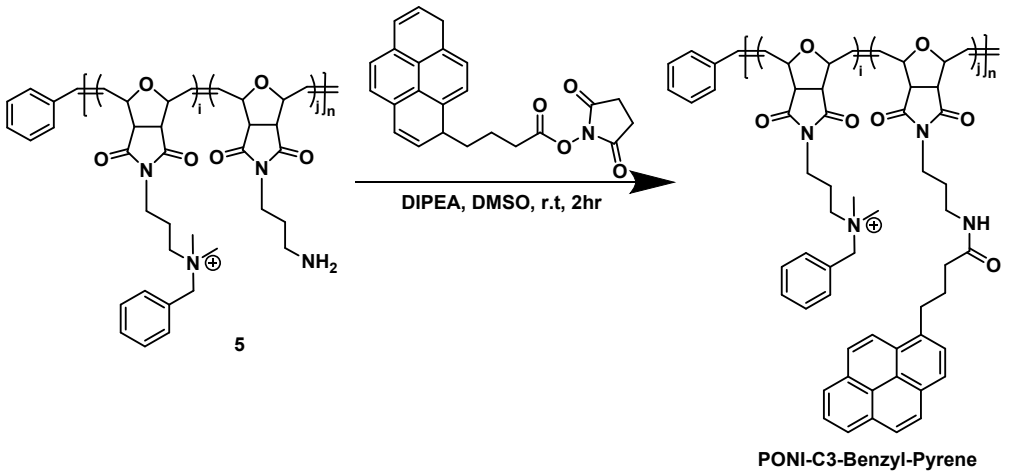


To a 15 mL pear-shaped air-free flask equipped with a stir bar was added **2** (0.5 g, 1.076 mmol, 0.9eq), **3** (39 mg, 0.1196 mmol, 0.1eq) and 5 mL of DCM. In a separate 10 ml pear-shaped air-free flask was added Grubbs' 3rd generation catalyst (13.6 mg, 0.015 mmol) and 1 mL DCM. Both flasks were sealed with septa and attached to a Schlenk nitrogen/vacuum line. Both flasks were freeze-pump-thawed three times. After thawing, Grubbs' 3rd generation catalyst was removed via syringe and quickly added to the flask containing **2** & **3** and allowed to react for 15 min. After the allotted time, ethyl vinyl ether (300 μL) was added and allowed to stir for 20 mins. Afterwards, the reaction was diluted to two times the volume and precipitated into a heavily stirred solution of a 1:1 mixture of ethyl ether and hexane. The precipitated polymer was filtered and dissolved into tetrahydrofuran (THF). The polymer was precipitated again into the same mixture solvent and filtered to yield **PONI**. ^{1}H NMR (400MHz, CDCl3) 11.49 (s, 1H), 8.48 (m, 1H), 6.53 (m, 1H), 6.1 (br, 1H), 5.79 (br 1H), 5.3 (m, 1H), 5.1 (br 1H), 4.5 (br, 1H), 4.5 (br, 1H), 3.58 (t, 3H), 3.41 (m, 4H), 2.88 (m, 1H), 1.9 (m, 2H), 1.59 (s, 9H), 1.51 (m, 24 H), 1.45 (m, 2H). The polymer was also characterized by GPC (gel permeation chromatography) in tetrahydrofuran. The M_W was ~13,000 and the PDI (polydispersity index) was 1.04.



To generate the quaternary ammonium poly(oxanorborneneimide), 4 (200 mg) was added to 20 ml vials equipped with a stir bar. 4 mL of trifluoroacetic acid was added to dissolve the polymer; the mixture was transferred to a 50 mL round bottom flask equipped with a stir bar and 4 mL of dichloromethane. This mixture was allowed to react at room temperature for two hours, followed by solvent removal via rotary evaporation with dichloromethane 3 times (5 mL each time). Next, excess of N, N-dimethylbenzylamine was added (1 ml) to the vial and purged with nitrogen. First stage of the reactions involved stirring for 30 minutes at 80 °C. The polymers precipitated during this time. Half of the THF was evaporated and replaced with

methanol which re-dissolved the polymers. The reaction was allowed to proceed overnight at 50 °C. Afterwards, the solvent was completely evaporated and washed with hexane 2 times and dissolved into a minimal amount of water. The polymers were added to 10,000 MWCO dialysis membranes and allowed to stir for 3 days in Milli Q water, changing the water periodically. The polymers were filtered through PES syringe filters and freeze-dried to yield **PONI-C_3-Benzyl-NH_2.** NMR indicated conversion into the desired quaternary ammonium salt.



To a 20 ml scintillation vial equipped with a stirbar was added the deprotected polymer (100 mg), dissolved in 2 ml of DMSO followed by the addition of ~100 µl of DIPEA. Meanwhile, the solvatochromic dye pyrene (3 mg) was weighed and dissolved in DMSO. The dye dissolved in DMSO was added to the stirred reaction mixture and allowed to react for two hours at room temperature. Afterwards the progress of the reaction was monitored with TLC (9.5/0.5 –ethyl acetate/methanol) to ensure the dye was conjugated (Free dye moves while polymer stays on the baseline). After the completion of the reaction, the mixture was transferred to 10,000MW cutoff dialysis membrane bags and allowed to dialyze in Milli Q water for three days to remove free dye. After dialysis, the polymer was collected and lyophilized to yield **PONI-C_3-Benzyl-Pyrene.**

3. Expression of EGFP

The synthesis and characterization of EGFP were carried out according to previous reports.[2] Briefly, starter cultures from a glycerol stock of GFP in BL21(DE3) *E. coli* host were grown in 50 mL of 2× YT media with 50 µL of 1000x ampicillin overnight. After growth, 10 mL of the culture were added into 1 L of 2x YT media with 1 mL of 1000x ampicillin and shaken until OD600 reaching to 0.7. And then, IPTG with the final concentration of 1 mM was added for inducing the culture and shaken at 28 °C. After incubation for 3 h, the cells were centrifugated at 4 °C for 15 min with the speed of 5000 *rpm*. The pellet was then resuspended in lysis buffer and the cells were lyzed using a microfluidizer. After centrifugation for 45 min at 15000 *rpm*, the supernatant was further purified by HisPur Cobalt columns. After that, the imidazole was removed by dialyzing in 5 mM sodium phosphate buffer (pH=7.4). The protein was characterized by gel electrophoresis, absorption and emission spectra.

4. Characterization of C_3-Bz-Py polymer and polymer-GFP assembly

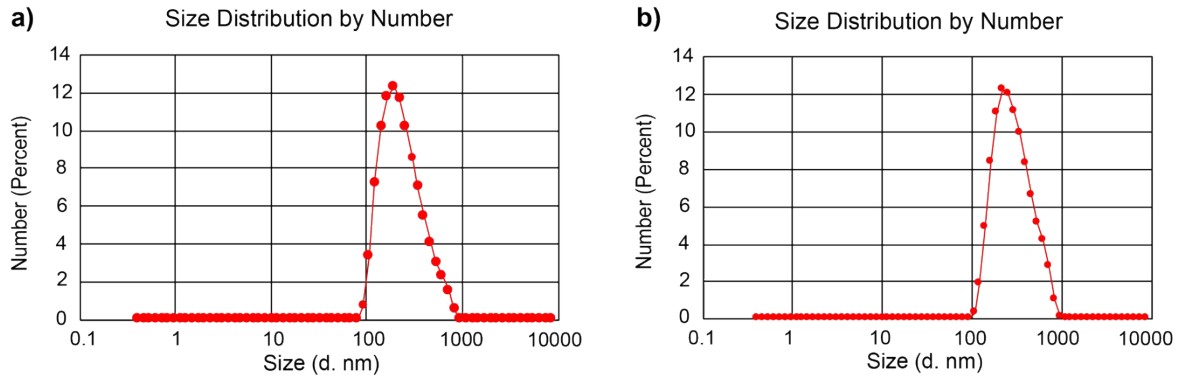


Fig. S1 Hydrodynamic size of PONI-C_3-Bz-Py polymer a) and polymer-GFP assembly b) in PBS buffer. C_3-Bz-Py polymer formed a complex with an average diameter of 257.7 ± 139.3 nm. With the addition of EGFP, the size of polymer-EGFP assembly is approximately 290.9 ± 153.0 nm in diameter.

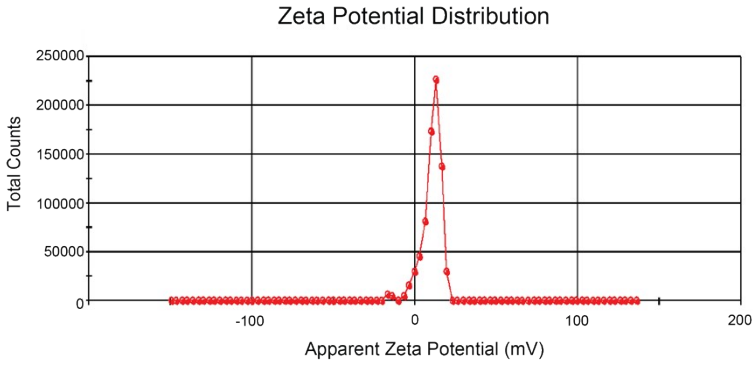


Fig. S2 Zeta potential of C_3-Bz-Py polymer in water. The value is 10.9 ± 5.2 mV.

5. Fluorescence titration

40 µg/mL of C_3-Bz-Py was titrated with various concentration of EGFP ranging from 0 to 300 nM. The solution was prepared in 10 mM HEPES buffer. After 30 min incubation, the fluorescence spectrum was measured using a Molecular Devices SpectraMax M2 microplate reader at the excitation of 344 nm.

6. Cell culture

Raw 264.7 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) high glucose media with 10% fetal bovine serum (FBS) and 1% antibiotics under a 37 °C humidified atmosphere containing 5% CO_2. The cells were sub-cultured approximately once every two days.

7. Pesticide solution preparation

A series of pesticides were prepared to 10 µM stock solution by dissolving first in EtOH and then diluted in MilliQ water. The final ratio of EtOH was 0.05%. At the day of treatment, the pesticides were diluted to 10^{-14} M by DMEM with gradient dilution method.

8. Array-based sensing procedures

The sensor assembly was prepared by mixing 40 μg/mL of C_3-Bz-Py with 200 nM of EGFP in 10 mM HEPES buffer for 30 min in dark. Cells were seeded at 10^4 on 96-well plate in DMEM high glucose media for attachment. After 12 h, media were removed and then the cells were treated with 150 μL of pesticides at the concentration of 10^{-14} M for 24 h (Cells without treatment as a control). Subsequently, cells were washed once with phosphate buffered saline (PBS), and then 150 μL of the fluorescent polymer-EGFP assembly was added to the cells with or without pesticide treatment. After 30 min, fluorescence intensities were recorded using the microplate reader at 25 °C. The fluorescence channels of the sensor were 344/390 nm (Pyrene monomer 1), 344/420 nm (Pyrene monomer 2), 344/470 nm (Pyrene excimer), 475/510 nm (EGFP), and 344/510 nm (FRET).

9. Linear discriminant analysis (LDA)

The fluorescence change (I/I_0) patterns were subjected to linear discriminant analysis (LDA) using SYSTAT (version 13, SystatSoftware, Richmond, CA, U.S.A.) to classify different pesticides-treated cells. LDA is a revised multivariate method used to find a linear combination of features that characterizes or separates two or more classes of objects.[3] All variables were used in the complete mode and the tolerance was set as 0.001. The raw fluorescence response patterns were transformed to canonical patterns where the between-class variance was maximized while the within-class variance was minimized.

10. Unknown identification

The identity of unknown samples was predicted by computing their Mahalanobis distance to the center of each of the training groups, followed by determining the probability of cells belonging to its closest cluster using an appropriate F-distribution for the minimum distance.[4]

11. Sensing data

11.1. Sensing data for RAW 264.7 cells treated by chlorpyrifos and methoxychlor

Table S1. Normalized fluorescence responses and LDA output for RAW 264.7 cells treated by chlorpyrifos (Chlor) and methoxychlor (Metho). Score (1) and score (2) correspond to Figure 3 in the main text.

Sample name	I/I_0					LDA output	
	Pyrene monomer 1	Pyrene excimer	GFP	FRET	Pyrene monomer 2	Score (1)	Score (2)
Control	1.283	1.021	1.255	0.784	1.123	0.573	-1.145
Control	1.303	1.050	1.274	0.789	1.133	-0.780	-1.122
Control	1.324	1.066	1.279	0.792	1.169	-0.756	-0.699
Control	1.321	1.101	1.355	0.790	1.357	-1.552	-4.318
Control	1.349	1.087	1.315	0.845	1.195	-1.280	-2.736
Control	1.346	1.087	1.330	0.790	1.355	0.500	-2.801
Control	1.377	1.102	1.311	0.842	1.220	-1.032	-1.520
Control	1.383	1.111	1.310	0.851	1.249	-1.207	-1.296
Chlor	1.358	0.997	1.287	0.700	1.147	-4.360	2.172
Chlor	1.459	1.077	1.323	0.670	1.272	-4.606	1.559
Chlor	1.356	1.019	1.257	0.796	1.172	-6.946	0.889
Chlor	1.393	1.038	1.255	0.806	1.186	-4.714	2.042

Chlor	1.413	1.043	1.282	0.778	1.214	-5.225	0.701
Chlor	1.424	1.038	1.253	0.789	1.234	-4.173	0.985
Chlor	1.418	1.029	1.267	0.822	1.223	-3.881	1.206
Chlor	1.414	1.045	1.252	0.808	1.239	-2.281	-0.068
Metho	1.276	1.076	1.212	0.815	1.095	5.873	-0.661
Metho	1.274	1.080	1.219	0.827	1.108	5.027	1.848
Metho	1.254	1.096	1.229	0.837	1.081	4.121	-0.176
Metho	1.300	1.097	1.227	0.829	1.129	4.259	0.969
Metho	1.326	1.119	1.262	0.849	1.146	5.012	0.409
Metho	1.342	1.117	1.267	0.832	1.165	6.020	1.956
Metho	1.365	1.127	1.268	0.853	1.187	6.319	0.182
Metho	1.341	1.089	1.274	0.827	1.142	5.091	1.626

Table S2. Percentage of accurate classification of chlorpyrifos or methoxychlor treated RAW 264.7 cells from Jackknifed analysis. The results show an overall 96% correct classification.

	Control	Chlorpyrifos	Methoxychlor	% Correct
Control	8	0	0	100
Chlorpyrifos	0	8	0	100
Methoxychlor	1	0	7	88
Total	9	8	7	**96**

Table S3. Prediction of RAW 264.7 cells treated by different pesticides using training set from Figure 3 and Table S1. The results show an overall 96% correct unknown identification.

Unknown sample #	**I/I_0**							**Correct prediction**
	Pyrene monomer 1	**Pyrene excimer**	**GFP**	**FRET**	**Pyrene monomer 2**	**True ID**	**Identified as**	
1	1.249	0.982	1.246	0.750	1.075	Control	Control	Yes
2	1.287	1.026	1.266	0.770	1.115	Control	Control	Yes
3	1.332	1.045	1.259	0.825	1.157	Control	Control	Yes
4	1.370	1.071	1.288	0.817	1.217	Control	Control	Yes
5	1.373	1.065	1.270	0.801	1.189	Control	Control	Yes
6	1.404	1.096	1.285	0.760	1.190	Control	Control	Yes
7	1.399	1.066	1.285	0.785	1.191	Control	Chlor	No
8	1.371	1.079	1.288	0.804	1.175	Control	Control	Yes
9	1.252	1.064	1.207	0.820	1.057	Chlor	Chlor	Yes
10	1.264	1.132	1.161	0.838	1.096	Chlor	Chlor	Yes
11	1.314	1.099	1.255	0.808	1.143	Chlor	Chlor	Yes
12	1.301	1.107	1.262	0.835	1.151	Chlor	Chlor	Yes
13	1.305	1.133	1.227	0.873	1.153	Chlor	Chlor	Yes

14	1.315	1.146	1.283	0.887	1.132	Chlor	Chlor	Yes
15	1.327	1.150	1.290	0.887	1.177	Chlor	Chlor	Yes
16	1.325	1.139	1.285	0.871	1.153	Chlor	Chlor	Yes
17	1.480	1.095	1.319	0.646	1.256	Metho	Metho	Yes
18	1.437	1.079	1.315	0.731	1.239	Metho	Metho	Yes
19	1.490	1.094	1.350	0.712	1.298	Metho	Metho	Yes
20	1.535	1.137	1.369	0.656	1.347	Metho	Metho	Yes
21	1.526	1.129	1.382	0.718	1.314	Metho	Metho	Yes
22	1.501	1.137	1.361	0.757	1.344	Metho	Metho	Yes
23	1.556	1.141	1.374	0.667	1.355	Metho	Metho	Yes
24	1.536	1.140	1.366	0.748	1.347	Metho	Metho	Yes

11.2. Sensing data for RAW 264.7 cells treated by methoxychlor at different concentrations

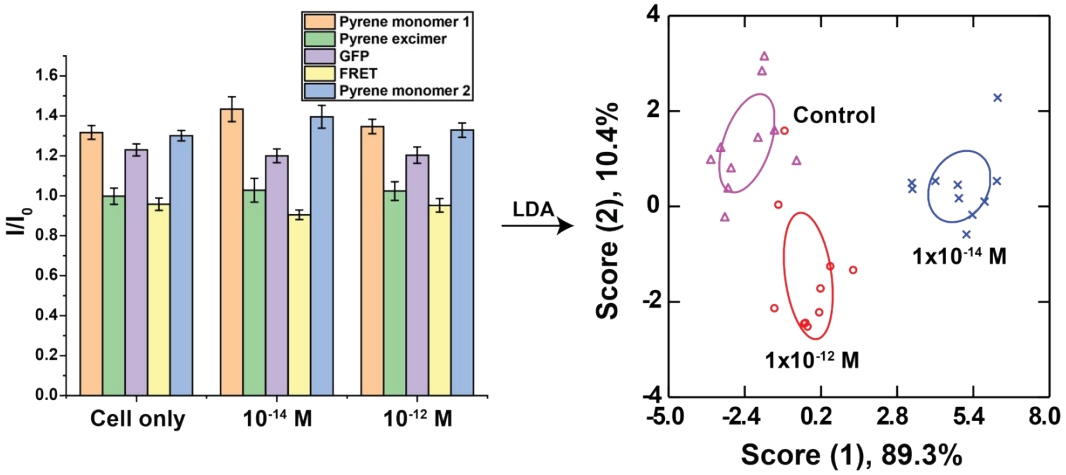


Fig. S3 Fluorescence responses of PONI-C_3-Bz-Py incubated with Raw 264.7 cells under different concentrations of methoxychlor exposure. Each value is the average of ten parallel measurements. LDA plot of the first two canonical scores were plotted.

Table S4. Normalized fluorescence responses and LDA output for RAW 264.7 cells treated by methoxychlor at different concentrations. Score (1) and score (2) correspond to Figure 3S above.

Sample name	I/I_0					LDA output	
	Pyrene monomer 1	Pyrene excimer	GFP	FRET	Pyrene monomer 2	Score (1)	Score (2)
Control	1.233	0.913	1.177	0.903	1.261	-2.448	0.040
Control	1.310	0.962	1.176	0.907	1.290	0.272	-0.300
Control	1.310	0.966	1.231	0.962	1.274	-2.702	-0.197
Control	1.341	1.024	1.259	0.988	1.293	-3.316	-0.230
Control	1.315	1.000	1.255	0.964	1.303	-3.362	0.660
Control	1.355	1.049	1.226	0.974	1.344	-1.119	-1.487
Control	1.340	1.039	1.242	0.994	1.343	-2.471	-1.717
Control	1.300	1.000	1.226	0.955	1.296	-2.979	-0.316
Control	1.329	1.001	1.251	0.958	1.301	-2.589	0.764

Control	1.333	1.024	1.252	0.976	1.308	-3.014	-0.084
10^{-14} M	1.313	0.919	1.138	0.852	1.304	3.301	0.497
10^{-14} M	1.351	0.938	1.163	0.881	1.307	3.322	0.367
10^{-14} M	1.428	1.015	1.183	0.911	1.383	5.166	-0.586
10^{-14} M	1.437	1.022	1.209	0.913	1.405	4.868	0.453
10^{-14} M	1.457	1.067	1.212	0.922	1.446	5.368	-0.173
10^{-14} M	1.473	1.073	1.230	0.935	1.430	4.907	0.171
10^{-14} M	1.474	1.076	1.214	0.920	1.440	5.785	0.102
10^{-14} M	1.522	1.097	1.262	0.920	1.468	6.232	2.282
10^{-14} M	1.466	1.057	1.196	0.898	1.406	6.205	0.535
10^{-14} M	1.412	1.012	1.194	0.901	1.362	4.106	0.536
10^{-12} M	1.261	0.939	1.127	0.889	1.266	0.203	-1.732
10^{-12} M	1.313	0.968	1.161	0.913	1.278	0.536	-1.267
10^{-12} M	1.352	0.998	1.179	0.929	1.314	1.319	-1.344
10^{-12} M	1.341	1.026	1.186	0.950	1.331	0.160	-2.233
10^{-12} M	1.370	1.069	1.206	0.975	1.340	-0.238	-2.533
10^{-12} M	1.365	1.071	1.208	0.974	1.350	-0.352	-2.470
10^{-12} M	1.391	1.070	1.241	1.003	1.392	-0.313	-2.456
10^{-12} M	1.351	1.066	1.220	0.979	1.346	-1.372	-2.147
10^{-12} M	1.362	1.031	1.248	0.966	1.339	-1.233	0.024
10^{-12} M	1.358	1.003	1.256	0.944	1.333	-1.025	1.577

Table S5. Percentage of accurate classification of RAW 264.7 cells treated by methoxychlor at different concentrations from Jackknifed analysis. The results show an overall 90% correct classification.

	Control	10^{-14} M	10^{-12} M	% correct
Control	8	0	2	80
10^{-14} M	0	10	0	100
10^{-12} M	1	0	9	90
Total	9	10	11	**90**

11.3. Sensing data for RAW 264.7 cells treated by different classes of pesticides

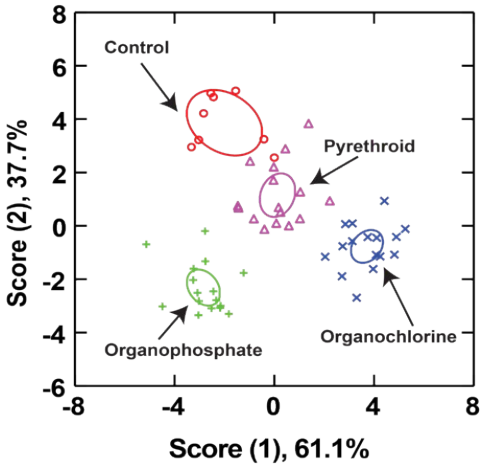


Fig. S4 FRET-based sensor array was incubated with Raw 264.7 cells under different pesticide exposure. LDA plot of the first two canonical scores were plotted after combining the same class of pesticide.

Table S6. Normalized fluorescence responses and LDA output for RAW 264.7 cells treated by six pesticides from different classes of pesticides. Score (1) and score (2) correspond to Figure 4 in the main text.

Sample name	Pesticide family	I/I_0					LDA output	
		Pyrene monomer 1	Pyrene excimer	GFP	FRET	Pyrene monomer 2	Score (1)	Score (2)
Control	Control	1.452	1.088	1.198	0.551	1.218	0.018	2.542
Control	Control	1.391	1.148	1.170	0.727	1.252	-3.306	2.938
Control	Control	1.390	1.144	1.172	0.740	1.248	-3.015	3.202
Control	Control	1.481	1.147	1.185	0.558	1.252	-0.400	3.230
Control	Control	1.433	1.165	1.168	0.724	1.240	-1.532	5.044
Control	Control	1.408	1.161	1.163	0.721	1.227	-2.816	4.199
Control	Control	1.421	1.145	1.153	0.693	1.237	-2.529	4.955
Control	Control	1.408	1.139	1.160	0.710	1.212	-2.421	4.809
Bifen	Pyrethroid	1.448	1.130	1.236	0.659	1.251	1.042	1.272
Bifen	Pyrethroid	1.424	1.180	1.209	0.748	1.257	-0.972	2.420
Bifen	Pyrethroid	1.442	1.192	1.224	0.774	1.253	0.442	2.889
Bifen	Pyrethroid	1.498	1.245	1.220	0.748	1.297	1.373	3.833
Bifen	Pyrethroid	1.439	1.202	1.225	0.775	1.274	-0.023	2.209
Bifen	Pyrethroid	1.473	1.239	1.252	0.715	1.310	0.584	0.004
Bifen	Pyrethroid	1.451	1.237	1.245	0.763	1.284	0.172	0.690
Bifen	Pyrethroid	1.479	1.273	1.274	0.816	1.292	2.224	0.938
Cyper	Pyrethroid	1.430	1.140	1.222	0.704	1.260	-0.034	1.712
Cyper	Pyrethroid	1.426	1.173	1.215	0.684	1.270	-1.464	0.757
Cyper	Pyrethroid	1.445	1.187	1.241	0.714	1.282	0.245	0.512
Cyper	Pyrethroid	1.415	1.184	1.222	0.731	1.264	-1.440	0.673
Cyper	Pyrethroid	1.416	1.173	1.233	0.731	1.268	-0.821	0.266
Cyper	Pyrethroid	1.449	1.214	1.246	0.712	1.267	0.081	0.096

Cyper	Pyrethroid	1.443	1.217	1.243	0.681	1.227	-0.397	-0.128
Cyper	Pyrethroid	1.462	1.213	1.256	0.691	1.240	1.028	0.272
Dime	OP	1.370	1.156	1.250	0.699	1.225	-2.778	-0.198
Dime	OP	1.350	1.112	1.234	0.682	1.225	-2.763	-1.328
Dime	OP	1.394	1.182	1.252	0.688	1.269	-2.519	-3.091
Dime	OP	1.384	1.171	1.262	0.700	1.250	-4.483	-3.020
Dime	OP	1.373	1.170	1.245	0.683	1.250	-3.236	-1.605
Dime	OP	1.399	1.189	1.252	0.683	1.277	-2.446	-2.455
Dime	OP	1.400	1.204	1.237	0.661	1.263	-3.256	-2.033
Dime	OP	1.425	1.198	1.248	0.661	1.251	-5.136	-0.691
Chlor	OP	1.350	1.096	1.215	0.709	1.198	-2.331	-2.790
Chlor	OP	1.357	1.094	1.223	0.652	1.192	-3.082	-2.511
Chlor	OP	1.372	1.133	1.246	0.670	1.257	-2.155	-2.996
Chlor	OP	1.358	1.122	1.215	0.634	1.274	-1.827	-3.299
Chlor	OP	1.380	1.129	1.216	0.624	1.232	-3.043	-3.346
Chlor	OP	1.439	1.184	1.227	0.560	1.294	-2.173	-3.075
Chlor	OP	1.487	1.231	1.201	0.464	1.326	-3.020	-2.817
Chlor	OP	1.385	1.156	1.178	0.582	1.227	-1.231	-1.764
Metho	OC	1.530	1.224	1.284	0.680	1.301	4.416	0.934
Metho	OC	1.529	1.284	1.303	0.715	1.296	4.106	-0.445
Metho	OC	1.472	1.200	1.316	0.687	1.267	3.301	-2.695
Metho	OC	1.522	1.286	1.324	0.768	1.314	4.816	-1.079
Metho	OC	1.485	1.285	1.312	0.849	1.301	3.751	-0.419
Metho	OC	1.545	1.314	1.319	0.758	1.299	5.253	-0.115
Metho	OC	1.515	1.281	1.320	0.783	1.288	4.896	-0.414
Metho	OC	1.511	1.328	1.326	0.796	1.289	3.967	-1.617
Endo	OC	1.465	1.145	1.281	0.610	1.199	2.741	-0.765
Endo	OC	1.491	1.164	1.269	0.639	1.295	2.850	0.064
Endo	OC	1.497	1.211	1.292	0.626	1.285	2.717	-1.890
Endo	OC	1.506	1.207	1.276	0.659	1.296	3.131	0.092
Endo	OC	1.525	1.270	1.311	0.681	1.270	4.246	-1.145
Endo	OC	1.505	1.238	1.288	0.688	1.296	3.124	-0.572
Endo	OC	1.484	1.267	1.288	0.705	1.248	2.039	-1.157
Endo	OC	1.496	1.236	1.312	0.728	1.272	4.082	-1.086

Table S7. Percentage of accurate classification of RAW 264.7 cells treated by different types of pesticides from Jackknifed analysis. The results show an overall 77% correct classification.

	Control	Bifen	Cyper	Dime	Chlor	Meth	Endo	% correct
Control	7	0	1	0	0	0	0	88
Bifen	0	4	3	0	0	1	0	50
Cyper	0	1	7	0	0	0	0	88
Dime	0	0	0	8	0	0	0	100

Chlor	0	0	0	3	5	0	0	63
Metho	0	0	0	0	0	6	2	75
Endo	0	0	0	0	0	2	6	75
Total	7	5	11	11	5	9	8	**77**

Table S8. Percentage of accurate classification of RAW 264.7 cells treated by three classes of pesticides from Jackknifed analysis. The results show an overall 98% correct classification.

	Control	Pyrethroid	OP	OC	% correct
Control	7	1	0	0	88
Pyren	0	16	0	0	100
OP	0	0	16	0	100
OC	0	0	0	16	100
Total	7	17	16	16	**98**

Table S9. Prediction of RAW 264.7 cells treated by different classes of pesticides using training set from Figure 4and Table S6. The results show an overall 97% correct unknown identification.

Unknown sample #	I/I_0							Correct prediction
	Pyrene monomer	Pyrene excimer	EGFP	FRET	Pyrene minor peak	True ID	Identified as	
1	1.418	1.080	1.151	0.500	1.175	Control	Control	Yes
2	1.425	1.082	1.141	0.482	1.156	Control	Control	Yes
3	1.446	1.105	1.141	0.483	1.244	Control	Control	Yes
4	1.440	1.122	1.137	0.467	1.223	Control	Control	Yes
5	1.449	1.117	1.132	0.466	1.235	Control	Control	Yes
6	1.474	1.143	1.144	0.473	1.225	Control	Control	Yes
7	1.481	1.171	1.160	0.469	1.259	Control	Control	Yes
8	1.452	1.145	1.147	0.469	1.227	Control	Control	Yes
9	1.412	1.110	1.219	0.641	1.221	Pyrethroid	Pyrethroid	Yes
10	1.443	1.167	1.213	0.649	1.263	Pyrethroid	Pyrethroid	Yes
11	1.447	1.161	1.206	0.658	1.270	Pyrethroid	Pyrethroid	Yes
12	1.465	1.201	1.224	0.698	1.278	Pyrethroid	Pyrethroid	Yes
13	1.463	1.220	1.226	0.709	1.288	Pyrethroid	Pyrethroid	Yes
14	1.429	1.187	1.232	0.761	1.224	Pyrethroid	Pyrethroid	Yes
15	1.453	1.223	1.267	0.762	1.292	Pyrethroid	Pyrethroid	Yes
16	1.441	1.227	1.267	0.745	1.223	Pyrethroid	Pyrethroid	Yes
17	1.289	1.020	1.252	0.781	1.104	OP	Pyrethroid	No
18	1.275	1.016	1.238	0.772	1.107	OP	OP	Yes
19	1.284	1.080	1.276	0.801	1.170	OP	OP	Yes
20	1.318	1.090	1.270	0.794	1.183	OP	OP	Yes
21	1.321	1.127	1.282	0.793	1.204	OP	OP	Yes
22	1.355	1.129	1.284	0.772	1.194	OP	OP	Yes
23	1.342	1.138	1.292	0.778	1.194	OP	OP	Yes
24	1.331	1.162	1.294	0.780	1.191	OP	OP	Yes
25	1.493	1.222	1.281	0.756	1.255	OC	OC	Yes
26	1.509	1.230	1.274	0.768	1.275	OC	OC	Yes
27	1.533	1.297	1.294	0.754	1.267	OC	OC	Yes
28	1.500	1.256	1.313	0.792	1.291	OC	OC	Yes
29	1.477	1.267	1.322	0.729	1.297	OC	OC	Yes
30	1.512	1.261	1.342	0.764	1.292	OC	OC	Yes
31	1.550	1.293	1.327	0.727	1.314	OC	OC	Yes
32	1.539	1.267	1.316	0.696	1.317	OC	OC	Yes

12. Mitochondrial activity-based viability assay

AlamarBlue is an important redox indicator for examining mitochondrial function.[5] 10^4 cells were plated on 96-well plates with DMEM high glucose media overnight for attachment. After

removing media, 150 µL of pesticides was added to cells for 24 h. At the end of the treatment, cells were washed once with PBS to remove excess pesticides and then incubated with 10% of AlamarBlue (130 µL) in DMEM high glucose media for 3 h at 37 °C. Subsequently, 110 µL of supernatant was transferred to a black 96-well plate and the fluorescence was recorded with excitation and emission at 560 nm and 590 nm, respectively.

13. Live-dead membrane integrity assay

Trypan blue exclusion assay is a direct identification method of live and dead cells, which is based on the cell membrane impermeable property.[6] Following exposure to pesticides for 24 h, cells were washed once with PBS and then treated with 50 µL of trypsin for 10 min for trypsinization. Cells were transferred to 600 µL microcentrifuge tube and centrifuged at 3000 *rpm* for 5 min. Next, the supernatant was removed, and the cells were resuspended in base DMEM medium without FBS. Then, 15 µL of cell suspensions were mixed with 15 µL of 0.4% trypan blue, and 10 µL of the mixture was pipetted into a disposable Countess chamber slide and counted using a Countess Automated Cell Counter.

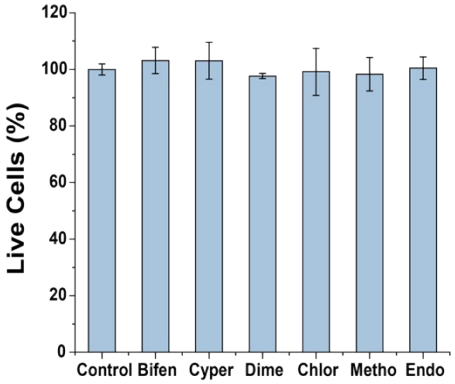


Fig. S5 Membrane integrity detection of Raw 264.7 cells with trypan blue exclusion assay after treatment with 10^{-14} M pesticides.

14. ROS detection assay

ROS production in macrophages under pesticide exposure was determined by measuring the fluorescence intensity of dichlorofluorescein (DCF), which is the oxidized form of the non-fluorescent dichlorofluorescein diacetate (DCFDA).[7] At the end of 24 h pesticide treatment, cells were washed once with PBS and incubated with 10 µM DCFDA at 37 °C in dark. DCFDA fluorescence was quantified every 15 min using the plate reader with the excitation wavelength of 490 nm and the emission wavelength of 525 nm.

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