Electronic Supplementary Material (ESI) for Chemical Communications.

Rapid and ultrasensitive detection of endocrine disrupting chemicals using a nanosensor-enabled cell-based platform

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1. Materials: All chemicals and solvents for synthesis were purchased from Fisher Scientific, except $HAuCl_4$ that was purchased from Strem Chemicals Inc., and used without further purification, unless otherwise stated.

Synthesis of BenzNP. The organic ligand and the particle core were synthesized and characterized following the previous report.[1]

Cloning and expression of fluorescent proteins. Genetic manipulations and bacterial culture were performed according to standard protocols.[2] In brief, Green Fluorescence Protein (GFP), was expressed in BL21 (DE3) strain of *E. coli*, and purified using Cobalt His-Pur columns. The purity of the proteins was checked by SDS-PAGE gel electrophoresis.

2. Nanosensor (BenzNP and GFP) fabrication: To determine the appropriate ratio of BenzNP and GFP for the assay, different concentrations of BenzNP were first titrated with 150 nM GFP to find out at which concentration of BenzNP, GFP intensity can be quenched. Final concentrations of 100 nM BenzNP and 150 nM GFP were used for all assays. The appropriate ratio of BenzNP and GFP was mixed with 5mM phosphate buffer (5mM PB) for 30 minutes to form nanosensor complexes and quench the fluorescence intensity of GFP. This mixture was then added to cell microplate for estrogenic activity detection.

3. Cell culture: For routine cell culture, MCF-7 cells were maintained in Dulbecco's Minimum Essential Medium (DMEM) with phenol red as pH indicator, supplemented with 10% Fetal Bovine Serum and 1% antibiotics. Cells were grown in a humidified atmosphere containing 5% CO2 at 37 °C. For estrogenic activity experiments, MCF-7 cells were cultured in low steroid conditions with charcoal dextran treated fetal bovine serum (CDFBS) in order to minimize estrogenic activity of serum and arrest all cells at G_0/G_1 phase. More specifically, cells were transferred to DMEM-F12 (phenol red free due to its known estrogenic activity) media, supplemented with 5% CDFBS and 1% antibiotics for 3 days. After that, cells were seeded on 96-well plate for experiments using only 2.5% CDFBS DMEM-F12 media to avoid cell overgrown and enhance estrogenic effect in a serum deprived environment.

4. Cell number titration: A series of cell number from 2,000 to 10,000 cells were plated on 96-well plate overnight. BenzNP-GFP complex solution with co-incubation of Hoechst 33342 was added to the 96-well plate after one time washing with phosphate buffer saline (PBS). Alamar blue/cell media solution was prepared in the ratio of 1/10. This mixture was incubated with cells for approximately 3 hours before being read out at EX560/EM590.

5. Cell-based assay for estrogenic activity detection: 10,000 cells were seeded on 96-well plate in 2.5% CDFBS DMEM-F12 media overnight. A series of E2 or BPA concentrations were prepared in 2.5% CBFBS DMEM-F12 media with 0.1% EtOH. Cells were washed with PBS and treated with these concentrations for 24 hours. After that, cells were washed one more time with PBS. Appropriate ratio of BenzNP-GFP nanosensor was mixed with 10 µg/ml of Hoechst 33342 and added into 96-well plate for 15-minute incubation time. The GFP and Hoechst fluorescence were measured with EX/EM wavelengths of 475/495 nm and 355/460 nm on a Molecular Devices SpectraMax M3 microplate reader at 25 °C. Normalization of fluorescence intensity was done by I/I_0, where I is the final fluorescence intensity and I_0 is the initial fluorescence intensity of the sensor.

6. Flow cytometry and cell cycle analysis: Serum deprived cells were seeded on 6-well plate at a density of 180,000cells/well in CDFBS DMEM-F12. Six concentrations of E2 and six concentrations of BPA (three replicates each treatment) were incubated with cells for 24 hours. The highest concentration of E2 (1×10^{-10} M) and BPA (5×10^{-7} M) were mixed with 10 nM ER inhibitor (ICI 182,780) to co-incubate with cells, also for 24 hours. Cells were washed with PBS once before being trypsinized and transferred into 1.5 ml eppendorf tubes. 1000 µL of culturing media was added into each tube to stop the trypsin activity. Samples were centrifuged at 3000 rpm for 5 minutes and the supernatant was discarded. 150 µL of PBS was added into each tube to resuspend cells into solution. 350 µL of EtOH was added to stabilize cells at 4°C for two hours. Cells were then centrifuged again to discard the supernatant before staining with PI/RNAse mixed with PBS at 1:1. Flow cytometric measurements for cell samples were performed using a LSRFortessa 3 Laser, HTS flow cytometer (BD Biosciences). The data was then analyzed using FlowJo 7.6 using Watson Pragmatic model for cell cycle analysis.

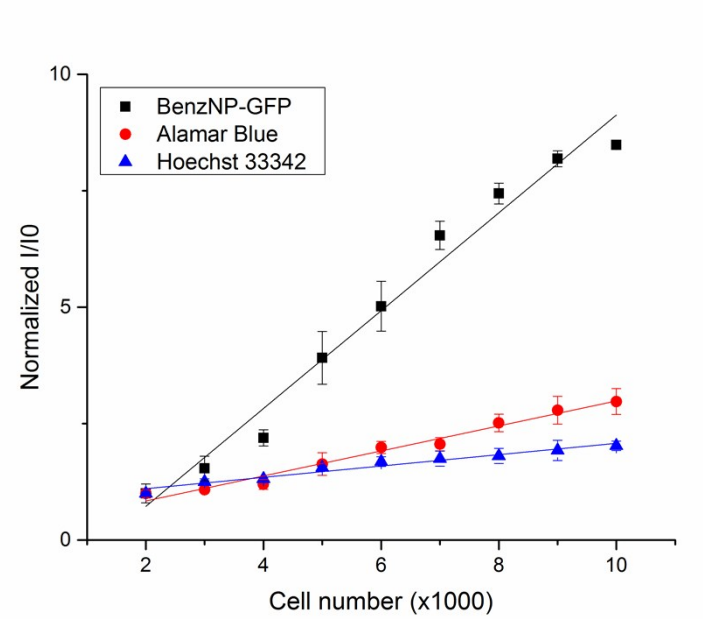

Figure S1: Response of different sensing systems (BenzNP-GFP sensor, Alamar Blue, and Hoechst 33342) with different cell numbers from 2,000 to 10,000 MCF-7 cells. BenzNP-GFP complex is more sensitive to cell numbers than Alamar Blue and Hoechst 33342.

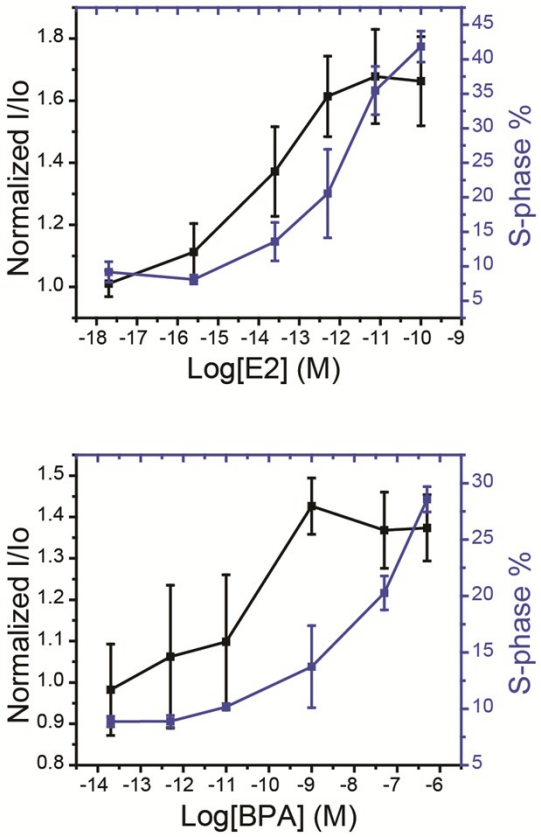

Figure S2: Overlay of normalized sensor response with percentage of S-phase for E2 and BAP-treated cells at selected concentrations.

Table S1: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for E2-treated cells at different concentrations.

0.1 % EtOH	[E2] (M)											
	2.0×10^{-18}	5.0×10^{-17}	2.5×10^{-16}	1.0×10^{-15}	5.0×10^{-15}	2.5×10^{-14}	1.0×10^{-13}	5.0×10^{-13}	1.0×10^{-12}	7.5×10^{-12}	5.0×10^{-11}	1.0×10^{-10}
1.016	1.020	1.175	1.223	1.162	1.218	1.477	1.558	1.794	1.603	1.718	1.713	1.855
0.996	1.066	1.092	1.133	1.067	1.256	1.365	1.696	1.620	1.868	1.742	1.813	1.675
1.037	0.971	1.022	1.093	1.081	1.093	1.170	1.342	1.508	1.634	1.796	1.933	1.603
0.951	0.987	0.954	1.002	0.992	1.228	1.476	1.586	1.534	1.561	1.455	1.520	1.518

Table S2: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for BPA-treated cells at different concentrations.

0.1 % EtOH	[BPA] (M)									
	2.0×10^{-14}	1.0×10^{-13}	5.0×10^{-13}	1.0×10^{-11}	5.0×10^{-11}	1.0×10^{-9}	5.0×10^{-9}	5.0×10^{-8}	2.5×10^{-7}	5.0×10^{-7}
1.082	1.072	0.896	1.102	1.007	1.382	1.344	1.460	1.383	1.419	1.316
0.996	0.865	1.087	1.278	1.280	1.330	1.498	1.504	1.473	1.318	1.469

0.911	0.912	1.134	0.869	1.182	1.281	1.462	1.411	1.367	1.497	1.411
1.011	1.081	0.876	1.000	0.924	1.156	1.401	1.323	1.249	1.317	1.299

Table S3: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for DCHP-treated cells at different concentrations.

0.1 %	[DCHP] (M)									
EtOH	1.0×10^{-12}	1.0×10^{-11}	1.0×10^{-10}	1.0×10^{-9}	1.0×10^{-8}	1.0×10^{-7}	5.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}
0.977	0.918	1.199	1.342	1.447	1.401	1.435	1.422	1.468	1.521	1.420
0.979	1.120	1.282	1.546	1.336	1.362	1.715	1.515	1.610	1.337	1.293
0.991	0.999	1.218	1.360	1.550	1.675	1.513	1.594	1.590	1.414	1.448
1.053	1.168	1.447	1.372	1.303	1.557	1.704	1.499	1.485	1.517	1.353

Table S4: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for BaP-treated cells at different concentrations.

0.1 %	[BaP] (M)									
EtOH	1.0×10^{-12}	1.0×10^{-11}	1.0×10^{-10}	1.0×10^{-9}	1.0×10^{-8}	1.0×10^{-7}	5.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}
1.050	1.239	1.101	1.037	1.213	1.338	1.384	1.234	1.363	1.457	1.440
1.032	1.197	1.153	1.114	1.290	1.371	1.375	1.224	1.325	1.468	1.550
0.948	1.049	1.129	1.169	1.072	1.280	1.544	1.194	1.425	1.511	1.344
0.970	1.090	1.060	1.068	1.154	1.329	1.529	1.541	1.403	1.512	1.292

Table S5: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for BPA-E2 mixture-treated cells at different concentrations.

0.1 %	[BPA] (M) + 1 fM E2									
EtOH	2.0×10^{-14}	1.0×10^{-13}	5.0×10^{-13}	1.0×10^{-11}	5.0×10^{-11}	1.0×10^{-9}	5.0×10^{-9}	5.0×10^{-8}	2.5×10^{-7}	5.0×10^{-7}
1.051	1.122	1.375	1.663	1.955	1.994	1.849	1.913	1.889	1.887	1.587
1.008	1.021	1.591	1.728	1.895	1.879	1.616	1.831	2.064	1.893	2.147
0.764	1.343	1.592	1.729	1.781	1.764	1.718	1.719	1.802	1.863	1.913
1.177	1.183	1.557	1.687	1.801	1.766	1.628	1.850	1.776	1.777	1.836

Table S6: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for DCHP-E2 mixture-treated cells at different concentrations.

0.1 %	[DCHP] (M) + 1 fM E2									
EtOH	1.0×10^{-12}	1.0×10^{-11}	1.0×10^{-10}	1.0×10^{-9}	1.0×10^{-8}	1.0×10^{-7}	5.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}
1.044	0.984	1.088	1.053	1.136	1.113	1.243	1.221	1.135	1.130	1.345
1.006	1.005	1.142	1.074	1.108	1.335	1.170	1.255	1.221	1.132	1.208
0.947	0.993	1.221	1.228	1.531	1.234	1.213	1.461	1.255	1.201	1.242
1.003	1.188	1.324	1.181	1.199	1.289	1.194	1.282	1.241	1.325	1.171

Table S7: Normalized I/I_0 fluorescence intensity of BenzNP-GFP nanosensor for BaP-E2 mixture-treated cells at different concentrations.

0.1 % EtOH	[BaP] (M) + 1 fM E2									
	1.0×10^{-12}	1.0×10^{-11}	1.0×10^{-10}	1.0×10^{-9}	1.0×10^{-8}	1.0×10^{-7}	5.0×10^{-7}	1.0×10^{-6}	5.0×10^{-6}	1.0×10^{-5}
0.892	1.142	1.200	0.961	0.997	1.239	1.238	1.342	1.152	1.291	1.373
1.007	0.807	0.993	0.945	0.878	1.171	1.198	1.161	1.135	1.157	1.197
1.022	1.098	0.840	1.050	0.946	1.212	1.142	1.118	1.258	1.639	1.345
1.079	0.888	1.049	0.958	0.993	1.217	1.144	1.198	1.224	1.278	1.147

1 A. Bajaj, O. R. Miranda, I.-B. Kim, R. L. Phillips, D. J. Jerry, U. H. F. Bunz, V. M. Rotello, *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 10912-10916.
2 a) H.-w. Ai, N. C. Shaner, Z. Cheng, R. Y. Tsien, R. E. Campbell, *Biochemistry* **2007**, *46*, 5904-5910; b) S. Rana, N.D. B. Le, R. Mout, K. Saha, G. Y. Tonga, R. E. S. Bain, O. R. Miranda, C. M. Rotello, V. M. Rotello, *Nat. Nanotechnol.* **2015**, *10*, 65–69.