Electronic Supplementary Information (ESI)

High-Throughput Metabolic Genotoxicity Screening with a Fluidic Microwell Chip and Electrochemiluminescence

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Chemicals

Benzo[a]pyrene (B[a]P, M_W= 252.31), poly(diallyldimethylammonium chloride) (PDDA, average M_w= 100,000-200,000), poly(sodium 4-styrenesulfonate) (PSS, average M_W= 70000), calf thymus DNA (Type I), horse heart myoglobin (Mb, MW= 17400) and all other chemicals were from Sigma. Pooled male human liver microsomes (HLM, 20 mg mL^{-1} in 250 mM sucrose) contained (a) 20 mg mL^{-1} total protein content , (b) total cyt P450 content of 340 pmol mg^{-1} of protein using the method of Omura and Sato,[1] pooled male rat liver microsomes (RLM, 20 mg mL^{-1} in 250 mM sucrose) contained (a) 20 mg mL^{-1} total protein content , (b) total cyt P450 content of 590 pmol mg^{-1} of protein using the method of Omura and Sato, human microsomal epoxide hydrolase (EH), 10 mg/mL in 100 mM potassium phosphate buffer of pH 7.4; pooled human liver s9 (Hs9) 20.0 mg/mL in 150 mM KCl, 50 mM Tris-HCl +2.0 mM EDTA; baculovirus-insect cell expressed cyt P450 1B1 supersomes (cyt P450 1B1), 4.5 mg/ml in 100mM potassium phosphate buffer of pH 7.4 with representative total cyt P450 content of 220 pmol mg^{-1} of protein; baculovirus-insect cell expressed cyt P450 1A1 supersomes (cyt P450 1A1), 5.0 mg/ml in 100mM potassium phosphate buffer of pH 7.4 with representative total cyt P450 content of 120 pmol mg^{-1} of protein; baculovirus-insect cell expressed cyt P450 1A2 supersomes (cyt P450 1A2), 5.0 mg/ml in 100mM potassium phosphate buffer of pH 7.4 with representative total cyt P450 content of 200 pmol mg^{-1} of protein; and control supersomes were from BD Gentest (Woburn, MA). Furafylline (M_w=260.25).

Microfluidic set up fabrication; Metabolite generation and measurements

The silicone rubber microfluidic channel was placed on the micro-well printed electrode array chip, where Ru^{II}PVP/DNA/enzyme films were already constructed. This assembly was supported by PMMA plates machined to fit on either side of the silicone gasket, and bolted together tightly to provide a sealed microfluidic channel. The device connected to a dual syringe pump (55-3333, Harvard Inc.) via a 4-way switching valve (v101D, IDEX Inc.) was then placed inside G:Box

Chemi imaging work station (Syngene Inc.) equipped for ECL signal acquisition. The switching valve was used to direct wash buffer and B[a]P substrate solution in to the device as necessary (Fig. S1, SI†).

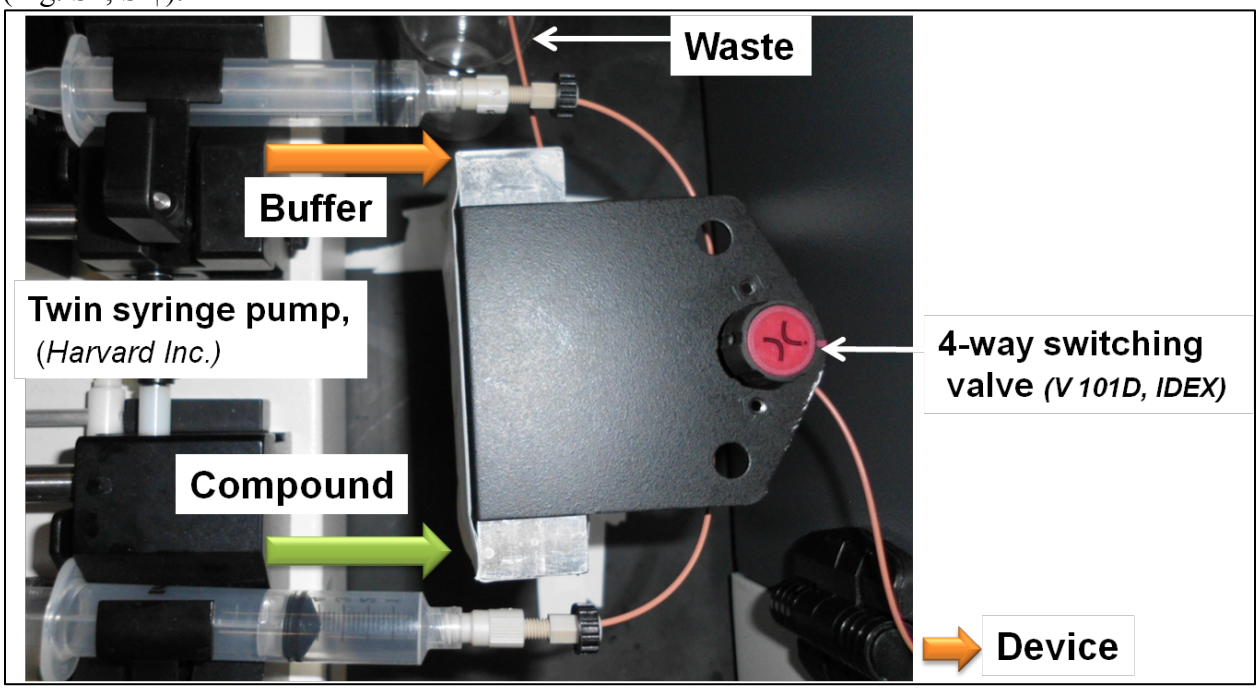

Fig. S1 Fluidic pumping system equipped with twin syringe pump and 4-way switching valve, which was used to direct wash buffer and B[a]P substrate solution into the device as necessary.

DNA-metabolite structure elucidation by CapLC-MS/MS

20µL of magnetic particles (20 mg mL^{-1}) was dispersed in 80 µL of 5 mM Tris buffer (pH 7.0, 5 mM NaCl). 100 µL PDDA was added dropwisely followed by a 20-min incubation to coat the negative charged surface with positively charged polyions. After incubation PDDA coated magnetic particles were pulled towards the side of the tube by placing a magnet and the supernatant was discarded. The particles were washed twice with Tris buffer to remove loosely bound polyions and redispersed in 100 µL of Tris buffer. In a similar fashion 40 µL of cyt P450 1B1 supersomes and DNA were incorporated with 30 min incubation for each to yield the final film architecture of PDDA/cyt P450 1B1/PDDA/DNA on magnetic beads. These magnetic particle bioreactors were dispersed in 10 mM phosphate buffer (pH 7.4) to a final volume of 200 µL and stored at ~0 ºC till use. 200 µL of magnetic bead bio colloidal suspension in 10 mM phosphate buffer (pH 7.4) was incubated with 25 µM B[a]P for 4 hours in the presence of NADPH regeneration system (10 mM glucose 6-phosphate, 4 units of glucose-6-phosphate dehydrogenase, 10 mM $MgCl_2$, 0.80 mM $NADP^+$) at 37 °C for metabolite generation and DNA adduct formation. After the reaction, particles were washed three times in Tris buffer to remove any unreacted B[a]P and redispersed in 100 µLTris buffer. DNA in the reactors was enzymatically hydrolyzed by incubating with an enzyme system consists of deoxyribonuclease I (400 unit mg^{-1} of DNA), phosphodiesterase I from snake venom (0.2 unit mg^{-1} of DNA), phosphodiesterase II (0.01 unit mg^{-1} of DNA), nuclease P1 (5 units mg^{-1} DNA), 10 µL of 10 mM $MgCl_2$, and phosphatase alkaline (1.2 unit mg^{-1} of DNA), for 12 h at 37 °C. Supernatant which contains DNA-metabolite adducts was separated from magnetic particle bioreactors and vacuum

filtered before CapLC-MS/MS analysis. A 10μL of sample was loaded to on a C18 trap column (Atlantis dC18 110A C18, 20.0 mm, 0.5 mm i.d., 5µm particle size, Phenomenex, Torrance, CA) and flushed at a flow rate of 10 μL min^{-1} with ammonium acetate buffer (10 mM, pH 4.5 with 0.1% formic acid) to eliminate the residual salt and most of the unmodified bases. After 2 min, the adducts were back-flushed to the analytical column (Atlantis dC18, 150 mm, 0.3 mm i.d., 5µm particle size, Waters) and separated using a binary separation gradient composed of ammonium acetate buffer (A) (10 mM, pH 4.5 with 0.1% formic acid) and acetonitrile with 0.05% formic acid (B), with the following acetonitrile composition.

Time, min	A %	B%	Flow rate, µL min-1
0	80	20	5
20	80	20	5
30	35	65	5
40	35	65	5
50	00	100	5
60	00	100	5
65	80	20	5
70	80	20	5

A 4000 QTRAP (AB Sciex, Foster City, CA) mass spectrometer with Analyst 1.4 software was operated in the positive ion mode. Multiple reactions monitoring (MRM) and enhanced product ion (EPI) modes were conducted at 5500 V ion spray voltage, auxiliary gas flow rate setting 30, sweep gas flow rate setting 20, capillary temperature 200 °C and declustering potential was 60 V with collision energy of 35 eV.

Results

Film fabrication and characterization

Film characterization.

A quartz crystal microbalance (QCM,USI Japan) was used to monitor the LbL assembly of the film on 9 MHz QCM resonators (AT-cut, International Crystal Mfg.), where a negatively charged monolayer was constructed on gold (0.16(±0.01)cm^2) by incubating the resonators in 0.5 mM 3-Mercaptopropionic acid in ethanol overnight. The adsorption conditions and stability of each layer was optimized and frequency change (ΔF) was measured after washing with deionized water (D.I. water) and drying over a stream of nitrogen. The mass per unit area M/A (g cm^{-2}) in each layer and the nominal thickness are directly related to ΔF, and mass and nominal thckness were determined from ΔF by using establihsed equations from the literature.[2]

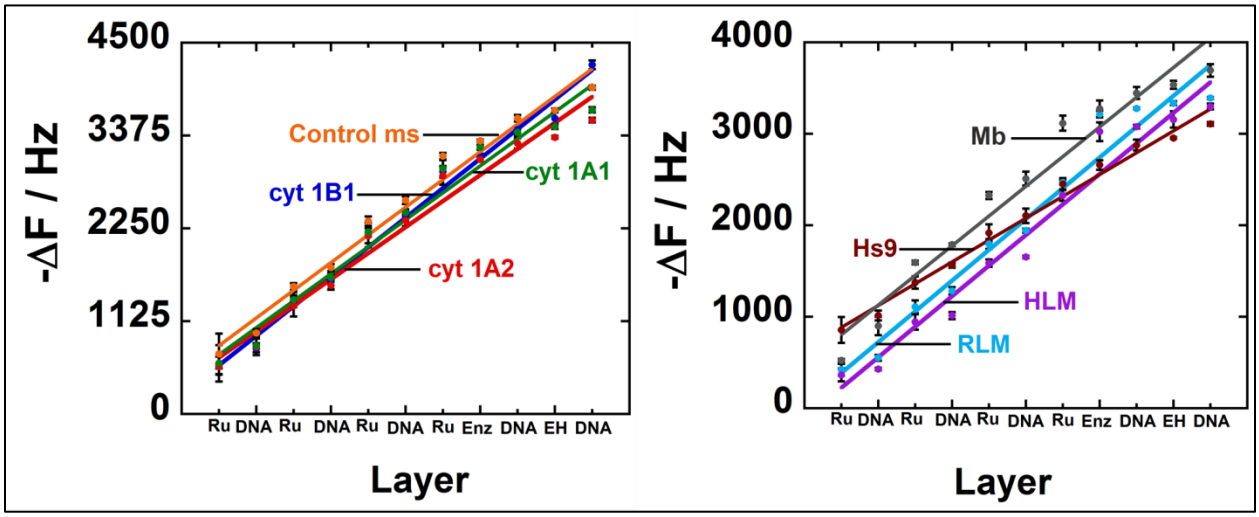

Fig. S2 QCM frequency changes as a function of the number of adsorbed layers during the film growth. Error bars reflect SD for 3 resonators. Ru - $Ru^{II}PVP$, Enz- cyt P450 enzyme source, and EH- epoxide hydrolase.

Table S1 Characteristics of the films; film assembly denoted by cyt P450 enzyme source.

Film assembly	Cyt P450 1B1	Cyt P450 1A2	Cyt P450 1A1	Control supersomes	Mb	HLM	Hs9	RLM
Nominal thickness / nm	55	54	56	59	56	53	47	54
Mass density of RuPVP / μg cm^{-2}	10.3±0.9	9.1±0.7	9.3±0.9	9.8±0.6	9.7±0.3	11.6±0.6	10.5±0.5	11.1±0.3
Mass density of DNA / μg cm^{-2}	4.4±0.2	4.4±0.8	4.5±0.4	5.2±0.7	4.5±0.6	2.2±0.4	4.9±0.2	3.1±0.6
Mass density of cyt P450 source / μg cm^{-2}	0.7±0.5	0.8±0.6	1.0±0.7	0.8±0.5	0.6±0.5	3.8±0.5	1.2±0.5	4.1±0.2
Mass density of EH / μgcm^{-2}	0.5±0.2	0.3±0.1	0.3±0.1	0.4±0.2	0.4±0.3	0.4±0.4	0.4±0.1	0.3±0.1
Concentration of total cyt P450s[a] / pmol $μg^{-1}$	0.22	0.20	0.12	n.d.	n.d.	0.34	0.31	0.59
a – source, BD biosciences Inc., n.d. – not detectable.								

Reproducibility

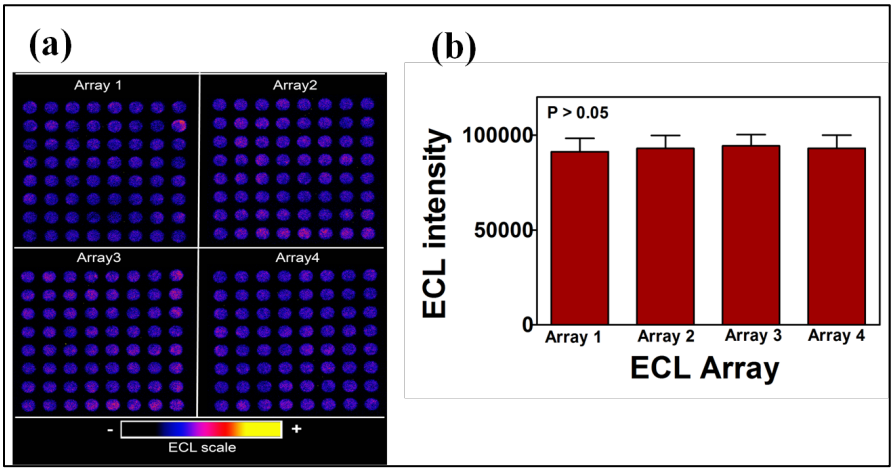

Fig. S3 Chip-chip reproducibility of $(Ru^{II}PVP/DNA)_3$ films in microwells of four different PG chips; (a) reconstructed ECL images. (b) variation of average ECL intensity of four chips. One way analysis of variance showed that ECL intensity did not differ at $p>0.05$.

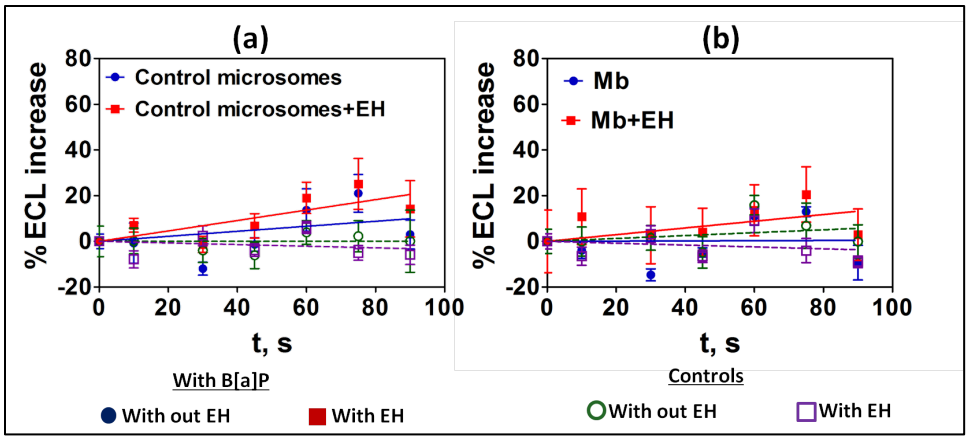

Fig. S4 Influence of enzyme reaction time on ECL increase for fluidic sensor chips exposed to 25 μM B[a]P at pH 7.4, (a) control supersomes (cyt P450 free), (b) Mb. Error bars represent standard deviations for n=4.

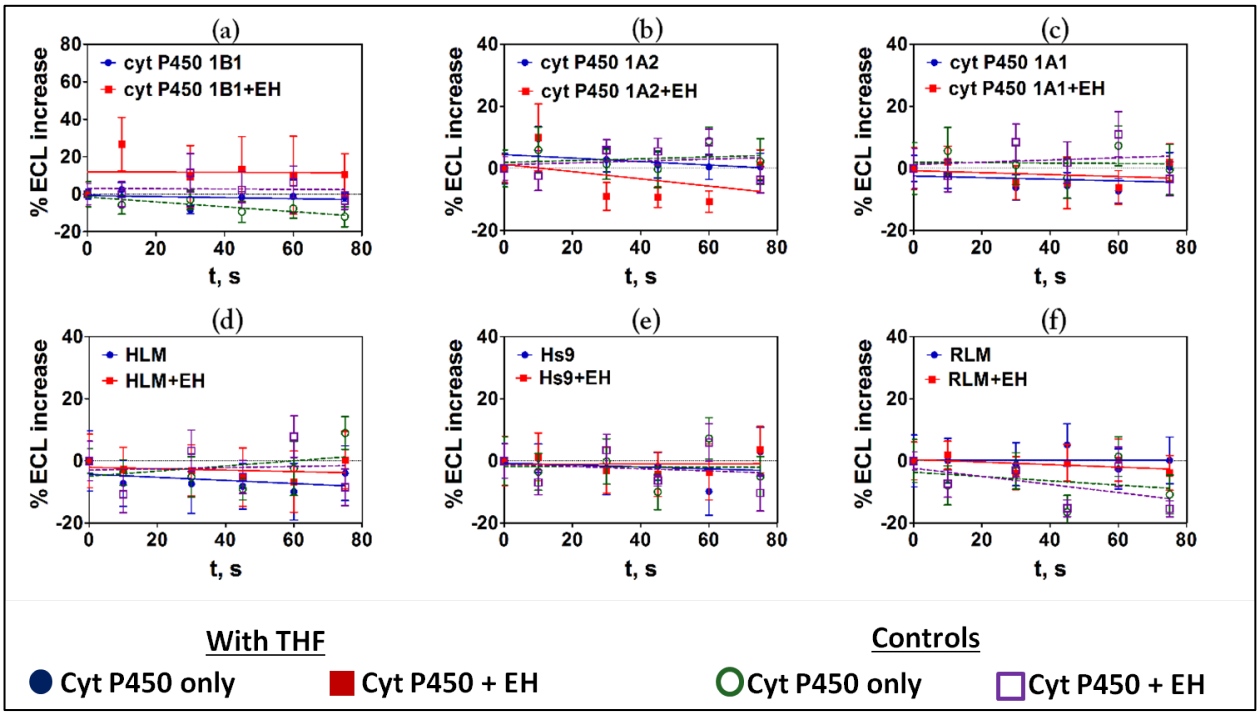

Fig. S5 Influence of enzyme reaction time on ECL increase for fluidic sensor chips exposed to 25 μM tetrahydrofuran (THF) at pH 7.4 with electronic activation of cyt P450s, (a) cyt P450 1B1, (b) cyt P450 1A2, (c) cyt P450 1A1, (d) HLM, (e) Hs9, (f) RLM. Controls are without substrate or with substrate but without electronic activation of cyt P450s, which gave equivalent results. Error bars represent standard deviations for n=4.

Inhibition

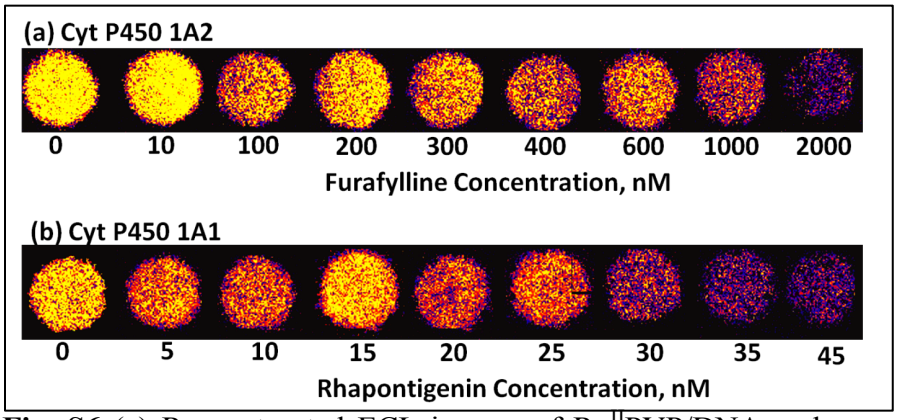

Fig. S6 (a) Reconstructed ECL images of Ru^{II}PVP/DNA and enzyme arrays allowed to react with oxygenated 25 μM of B[a]P+1% DMSO in pH = 7.4 phosphate buffer for 30 sec with different concentrations of (a)furafylline (b) rhapontigenin.

Voltammetry of cyt P450s

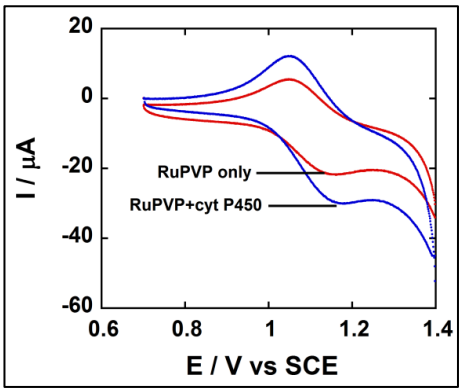

Figure S7 Cyclic voltammograms of film containing only Ru^{II}PVP and Ru^{II}PVP+cyt P450 1A2 on PG electrodes acquired in pH = 7.4 buffer at a scan rate of 0.2 V/s.

References

1 R. Sato and T. Omura, *J. Biol. Chem.*,1964, **239**, 2370-2378.
2 Y. Lvov, in *Handbook of Surfaces and Interfaces of Materials*, ed. R. W. Nalwa, Academic Press, San Diego, CA, 2001, Vol. 3, pp 170-189.