Supplementary Material (ESI) for Molecular BioSystems

Human Cyt P450 Mediated Metabolic Toxicity of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) evaluated using Electrochemiluminescent Arrays

Supplemental Information

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Quartz Crystal Microbalance (QCM). Assembly was monitored at each step with a quartz crystal microbalance (QCM, USI Japan) by making films on 9 MHz QCM gold coated resonators (AT-cut, International Crystal Mfg., Oklahoma City, OK) as described elsewhere[1] and summarized in Table 1. Briefly, resonators were treated with 3-mercaptopropanoic acid before applying layers as for arrays. Adsorbed mass/area (M/A) of each layer for dried films was obtained from the frequency change (ΔF) using the Sauerbrey equation.[2]

$$\text{M/A (g cm}^{-2}) = -\Delta F \text{ (Hz)} / 1.83 \times 10^{8} \quad (1)$$

Nominal thickness (d) was estimated using an expression confirmed by high-resolution electron microscopy:[3]

$$d \text{ (nm)} = (-0.016 \pm 0.002)\, \Delta F \text{ (Hz)} \quad (2)$$

Table S1. Surface coverage (Γ) for each enzyme used in this study obtained using QCM.

Enzyme	Γ (nmol cm^{-2})
Cyt P450 1A2	0.064 (±0.010)
Cyt P450 2E1	0.054 (±0.007)
Cyt P450 1B1	0.052 (±0.006)
CPO	0.022 (±0.002)
Mb	0.220 (±0.020)

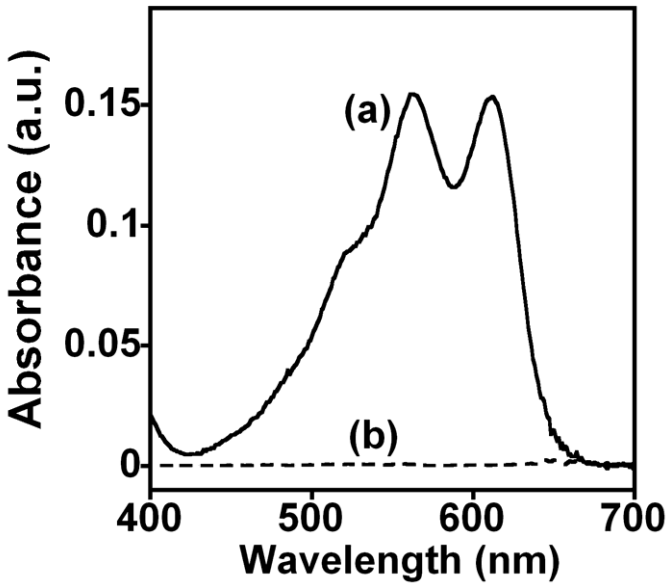

Figure S1. Colorimetric assay for immobilized chloroperoxidase (CPO) activity. Peroxide dependent oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD, Sigma) by CPO immobilized on silica nanoparticles. UV-Vis spectra of (a) PDDA/PSS/CPO film on silica nanoparticles reacted with 10 mM TMPD, and 2 mM H_2O_2 for 10 min in 10 mM acetate buffer plus 50 mM NaCl, pH 5.5 at 37^{o}C; (b) Control PDDA/PSS film on the particles reacted with TMPD under the same conditions. The reaction was stopped by quickly centrifuging the reaction mixture to remove the particles and the supernatant was diluted 30 times in acetate buffer to measure the absorbance maxima at 563 and 610 nm as reported previously.[4]

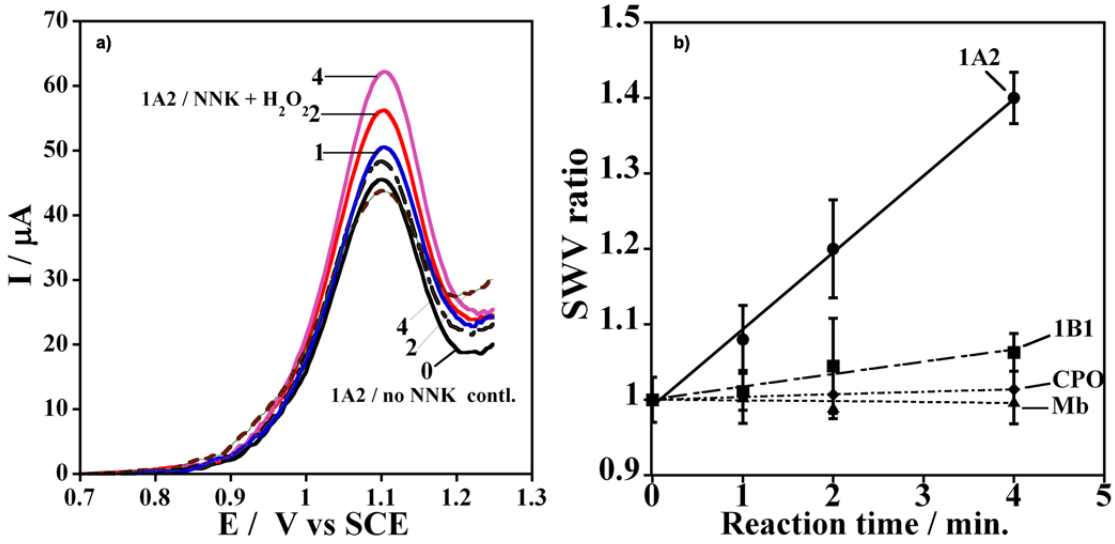

Fig. S2. a) Square wave voltammograms (SWVs) for the DNA/RuPVP/cyt P450 1A2 films on PG electrodes exposed to 1mM each NNK and H_2O_2 for the denoted times in 50mM acetate buffer, pH 5.5 at 37^{o}C. Control is DNA/RuPVP/cyt P450 1A2 films exposed to only 1 mM H_2O_2 under the same conditions. The increase in peak currents for DNA/RuPVP/cyt P450 1A2 films in the SWVs as the reaction proceeds results from increasing damage to the DNA caused by the reactive NNK metabolites metabolized by cyt P450 1A2 present in the assembly. Control electrodes did not show any significant increase in peak currents in the SWVs under the same conditions (lower plots labeled no NNK contl). Fig.4 shows the increase in SWV signal as peak current ratio (SWV ratio) versus the enzyme reaction time of the sensor similar to that described for the ECL sensor (Fig.1, % increase, main document). DNA/RuPVP/cyt P450 1A2 films showed significant increase in the SWV ratio with incubation time. On the other hand, cyt P450 1B1 films showed a small SWV ratio increase whereas DNA/RuPVP/CPO or Mb sensors on PG electrodes gave negligible SWV ratio increases with incubation time in NNK and H_2O_2.

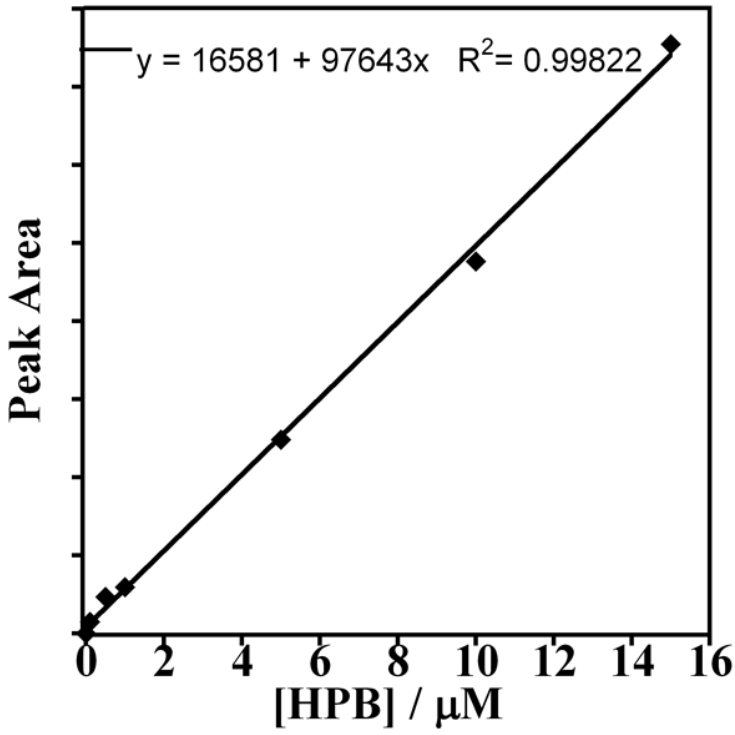

Fig. S3. Calibration plot showing the LC-UV peak area response to injected HPB standards.

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1. (a) Zhou, L.; Rusling, J. F. *Anal. Chem*. **2001**, *73*, 4780-4786; (b) Munge, B.; Estavillo, C.; Schenkman, J. B.; Rusling, J. F. *ChemBiochem.* **2003**, *4*, 82-89.

2 . a) Zhou, L.; Yang, J.; Estavillo, C.; Stuart, J. D.; Schenkman, J. B.; Rusling, J. F. *J. Am. Chem. Soc.* **2003**, *125*, 1431-1436; b) Lvov, Y. M.: Lu, Z.: Schenkman, J. B.; Zu X.: Rusling, J. F. *J. Am. Chem. Soc*., **1998**, *120*, 4073-4080.

3. Lvov, Y.; Aviga, K.; Ichinose, I.; Kunitake, T. *J. Am. Chem. Soc.* **1995**, *22*, 6117-6123.

4 . (a) T.A.Kadima, and M.A. Pickard, *Can. J. Microbiol.* **1990**, *36*, 302-304; (b) T.A.Kadima, and M.A. Pickard, *Appl.Environ.Microbiol.* **1990**, *56*, 3473-3477.