Supporting Information

Electrochemical Activation of the Natural Catalytic Cycle of Cytochrome P450s in Human Liver Microsomes

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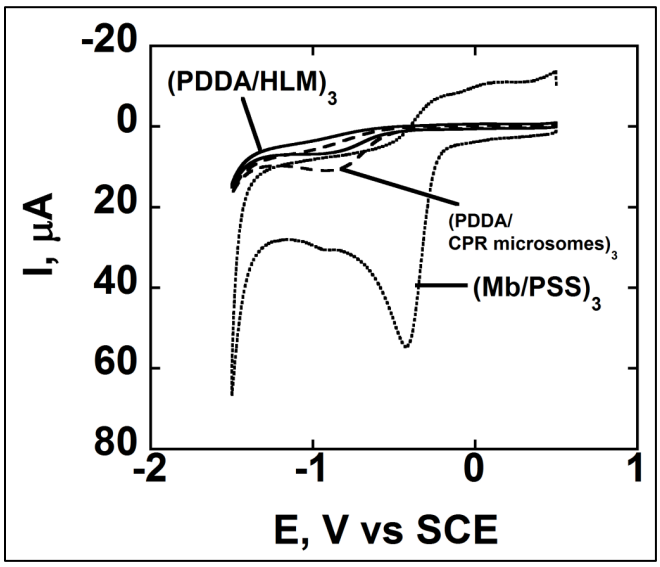

Fig. S1. Influence of 2 mM H_2O_2 on background subtracted cyclic voltammograms of films of HLM, CPR microsomes, and positive control heme protein myoglobin (Mb) on PG electrodes at 1 V s^{-1} in 50 mM phosphate + 0.1 M NaCl buffer, pH 7.4.

The Fe^{III} center of cyt P450s reduces H_2O_2 to H_2O forming the active ferryloxy radical cation that is reduced in a complex process producing ferric cyt P450 and oxygen [1]. The voltammetric signature shows an increased reduction peak current upon addition of H_2O_2 and disappearance of the oxidation peak of Fe^{II}, due to competition of ferric reduction with ferryloxy radical formation and reaction of any Fe^{II} protein formed with oxygen [2,3]. However, enzyme sources containing HLM or CPR microsomes did not show this behavior in 2 mM H_2O_2 of pH 7.4 phosphate buffer (Fig. S1), instead, broad reduction peaks were observed attributing to the overlap of direct reduction of the redox protein (Fig. 1) in the microsomal films and the direct reduction of peroxide. In contrast, films containing positive control heme protein myoglobin (Mb) gave a large catalytic peak at the potential of the Fe^{III} heme reduction (Fig. 2). These findings further confirm that the redox peaks of HLM and CPR microsomes (Fig. 1) originate from CPR not cyt P450s. Although cyt P450s alone have less negative formal potentials, this occurs due to

complexation of cyt P450s with CPR that we previously elaborated for cyt P450s added to CPR microsomes [4].

1. Chemicals and materials

Pooled male human liver microsomes (HLM, 20 mg mL^{-1} in 250 mM sucrose), baculovirus-insect cell expressed human cyt P450 1B1 supersomes (cyt P450 1B1) and baculovirus-insect cell expressed human CPR + b_5 supersomes (CPR microsomes) were from BD Gentest. NNK was from Toronto Research Chemicals. Horse heart myoglobin (Mb, MW 17400), 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) and all the other chemicals were from Sigma unless otherwise specified.

1.1 Film fabrication, characterization and voltammetry

The nominal thickness and mass of each layer were measured by using quartz crystal microbalance (QCM, USI Japan) [5]. Film formation for QCM analysis involved initial deposition of a negatively charged self-assembled monolayer of 3-mercaptopropionic acid on gold-coated 9 MHz QCM resonators (AT-cut, International Crystal Mfg.) followed by monitoring frequency shift after deposition and drying of each subsequently adsorbed layer.

1.2 *Microfluidic array fabrication and metabolite detection*

LbL assembly of HLM, DNA and RuPVP with a film architecture of PDDA/PSS/$(RuPVP/DNA)_2$/RuPVP/HLM/PDDA/DNA was constructed on screen printed carbon 8-electrode array (single electrode A=0.0038 cm^2, Kanichi Research Inc.). RuPVP was used as an electrochemical catalyst to detect DNA damage caused by reactive metabolites generated from enzyme reactions [1,6]. Metabolite generation was accomplished by constant potential electrolysis at -0.65 V vs. Ag/AgCl (-0.7V/SCE) while flowing oxygenated 50 μM NNK solution at 50 μL/min. After washing 5 min with anaerobic phosphate buffer of pH 7.4 at 50 μL/min, square wave voltammograms (SWV) were acquired at stopped flow.

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