with MS# LA990685K

Supplementary Materials

1. Detailed Experimental Section

Chemicals. Lyophilized horse heart myoglobin was from Sigma. Cytochrome P450cam (MW 46500) from *Pseudomonas Putida* expressed in *E. coli* DH52α containing P450cam cDNA was isolated and purified as described previously.[3] Camphor-free cytochrome P450 was prepared immediately before use by passing a solution of the enzyme through a Sephadex C-15 column equilibrated with 50 mM TRIS•HCl buffer (pH 7.4) containing 100 mM KCl at 4 ^{0}C. After this procedure, the electronic absorption peak (Soret band) shifted from 392 nm for camphor-bound cytochrome P450cam to 416 nm for substrate-free cytochrome P450cam. Didodecyldimethylammonium bromide (DDAB) was 99+% from Eastman. Polyions were sodium poly(styrenesulfonate) (PSS, MW 70,000, Aldrich, poly(dimethyldiallylammonium chloride) (PDDA, Aldrich). Styrene, styrene oxide, benzaldehyde and phenylpropylene oxide (methylstyrene oxide) were from Aldrich. Cis-β-methylstyrene was from Farchan Laboratories. Hydrogen peroxide (30%) was from J. T. Baker. All other chemicals were reagent grade. The buffer was 50 mM TRIS•HCl buffer (pH 7.4) containing 100 mM KCl.

Apparatus and Procedures. Cyclic voltammetry was done as described previously.[3,4] A vapor deposited gold disk on a quartz crystal resonator[4] or a basal plane pyrolytic graphite (PG) disk (Union Carbide HPG-99, A = 0.2 cm^2) served as working electrode. PG electrodes were polished on a metallographic polishing wheel on billiard cloth with a 0.3 μm alumina dispersion for 2 min, followed by 1 min ultrasonication in pure water. The electrode was cooled with water while polishing with alumina. Then the electrode was ultrasonicated for 0.5-1.0 min in pure

water. The resulting mirror-like surface was wiped with a Kimwipe before use, either for coating or as a bare electrode.

Controlled potential electrolysis was done using a PARC 273 electrochemical analyzer or a BAS 100B electrochemical analyzer. Water jacketed, separated three-electrode cells were equipped with a Vycor tipped saturated calomel reference electrode (SCE), a Pt foil (1 × 5 cm) counter electrode, and a carbon cloth (1.5 × 6 cm, National Electrical Carbon Corp.) working electrode or a Au (1 × 5 cm) working electrode. The counter electrode was separated from reaction solutions by a saturated KCl-agar bridge. Au electrodes were coated with protein-polyion films and carbon cloth electrodes were coated with protein-surfactant films.

Protein-polyion films were prepared as described previously.[4] Au electrodes were first cleaned in $HNO_3/HCl/H_2O$ (1/3/4) for 20 seconds, then ultrasonicated in ethanol/KOH/H_2O (40/1/59) for 1 min. The cleaned Au electrode was immersed in 1 mM MPS in ethanol for 16 hours. It was then exposed to appropriate protein or polyion solutions (3 mg mL^{-1}) for 20 min. to obtain the desired layers.[4] Electrodes were rinsed then dried with N_2 between formation of any two layers.

Protein-surfactant films were prepared by soaking carbon cloth electrodes in a 5 mM DDAB aqueous vesicle dispersion containing 0.25 mM Mb or a 2 mM DDAB dispersion containing 30 μM cytochrome P450cam. Water was evaporated in air overnight.

Electrochemical oxidations of styrene and cis-β-methylstyrene were mediated by myoglobin or cyt P450cam in films in 4 mL of 0.05 mM TRIS•HCl buffer containing 0.1 M KCl (pH 7.4) saturated with styrene or cis-β-methylstyrene (ca. 10 mM). Control reactions were

done on bare carbon cloth and gold electrodes with 80 μM myoglobin or 7 μM cytochrome P450 in solutions. The working electrode potential was -0.60 V vs. SCE. Chemical oxidations of styrene and cis-β-methylstyrene were done by adding hydrogen peroxide to solutions identical to those used for electrolysis. For experiments under oxygen, pure oxygen was bubbled through the reaction mixture for the first 1/3 of the reaction time, then shut off. Vigorous or prolonged bubbling with oxygen decreased styrene oxide yields.

Product mixtures were analyzed using a Hewlett-Packard Model 6890 gas chromatograph with a flame ionization detector and an HP-1 fused silica capillary column (0.53 mm i.d. × 10 m). Column temperature was programmed to hold at 50 ^{0}C for 2 minutes and then rise 15 ^{0}C/min to 250 ^{0}C. Average retention times were 6.0 min for styrene, 6.8 min for benzaldehyde, 8.3 min for styrene oxide, 7.5 min for cis-β-methylstyrene, 7.9 min for trans-β-methylstyrene, 8.9 min for cis-β-methylstyrene oxide, and 9.0 min for trans-β-methylstyrene oxide.

Mass spectra were obtained with a Hewlett-Packard GC-MS which has an HP 5790 quadrupole mass detector attached to an HP 5890 GC with an HP-1 column, with same conditions described above. A 0.5-mL aliquot was removed from reaction mixture after each reaction. The aliquot was extracted with 0.5 mL of CH_2Cl_2. The extraction mixture was centrifuged for about 20 minutes to make two clear layers. The CH_2Cl_2 layer was analyzed by gas chromatography. This procedure gave 98% recovery.

Styrene oxide, *cis*-β-methylstyrene oxide and *trans*-β-methylstyrene oxide were identified by comparing retention times with authentic standards. Identities were confirmed by GC-MS. Quantitative determinations were carried out with standard curves using phenylpropylene oxide

as internal standard for styrene oxide and using styrene oxide as internal standard for *cis*- and *trans*-β-methylstyrene oxides.

UV-VIS spectra were measured with a Perkin Elmer Lamda 6 UV-Visible spectrophotometer. Hydrogen peroxide was estimated by using Quantofix Peroxide 100 test sticks (Macherey-Nagel GmbH & Co., Germany).

2. Supplementary Figure

Figure 1S. Cyclic voltammograms at 0.2 V s^{-1} of Au-MPS-(PDDA-cyt P450)$_2$ in pH 7.4 TRIS buffer all anaerobic under Argon showing decreases in cyt P450 reduction-oxidation peaks after electrolysis: (a) before using for electrolysis; (b) after 1 hr electrolysis at 4 °C and -0.6 V vs SCE in saturated styrene buffer open to air; and (c) after 1 hr at 4 °C and -0.6 V vs. SCE in saturated styrene buffer under oxygen.

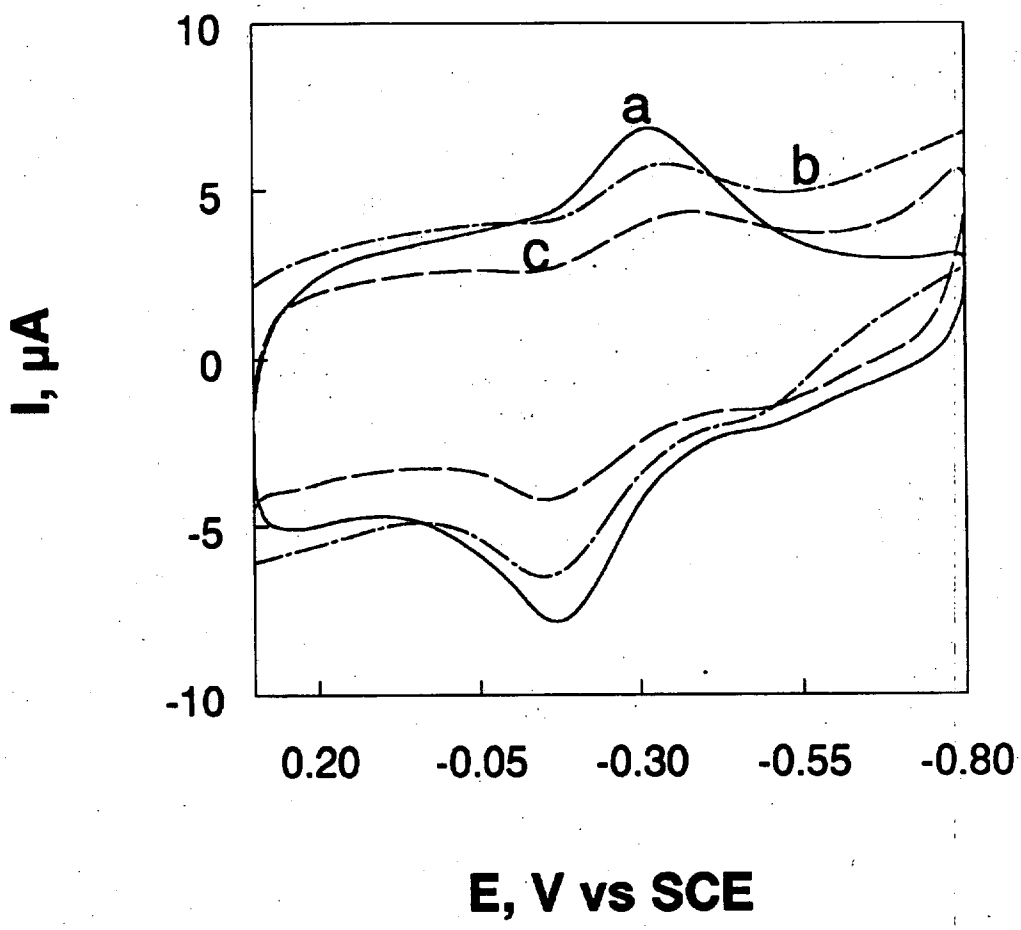

Fig. 15