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Hydrolysis of Acetylcholinesterase Inhibitors–Organophosphorous Acid Anhydrolase Enzyme Immobilization on Photoluminescent Porous Silicon Platforms

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SUPPORTING INFORMATION

Porous silicon formation

Porous silicon (PSi) samples were electrochemically etched from monocrystalline n-type silicon substrates (phosphorus doped, 1-5 Ω cm, (100) oriented, from Silicon Quest Inc.), for 5 min, at a current density of 90 mA/cm^2, in a 1:1 (v/v) mixture of aqueous HF (49% hydrofluoric acid, Fisher Chemicals, Inc.) and ethanol (99.5% absolute ACS reagent, Sigma-Aldrich Inc.). The counter-electrode was a platinum ring, immersed in the electrolyte solution and the working electrode was contacted to the back of the Si wafer with In-Ga eutectic paste. All the samples used in this study were illuminated with the unfiltered light from a 150 W halogen bulb during the electrochemical etching procedure in order to photo-generate the positive charges required to dissolve silicon in the presence of HF. After etching, the eutectic back contact was removed, the samples were rinsed in three 20-ml portions of absolute ethanol and immediately placed under vacuum to avoid air-borne contamination.

Enzyme preparation

The recombinant cells carrying *Alteromonas sp.*JD6.5- OPAA gene were grown at 30°C in flasks using Luria-Bertani (LB) complex media supplemented with 0.1 mM manganese chloride ($MnCl_2$). The cells were induced for 5 h with 0.6 mM isopropyl-β-D-thiogalactoside (IPTG) at early-mid-log phase (A_{600nm}= ~0.5). After induction, cells were harvested, re-suspended in 10 BM buffer (10 mM bis-tris propane, pH 7.2, containing 0.1 mM $MnCl_2$ and 0.1 mM dithiothreitol (DTT)), and lysed by passing through a French Pressure cell. Cellular debris was removed by centrifugation and the cell-free lysate was subjected to ammonium sulfate ($(NH_4)_2SO_4$) fractionation, and Q-sepharose chromatography.

Buffer composition

The buffer used in all the experiments was composed of 50 mM bis-tris propane, 50 μM $MnCl_2$ and 1 mM DTT in deionized water. The pH was adjusted to 7.5 with hydrochloric acid (HCl).

Enzyme immobilization

N-BOC-amino-3-butene. Amino-3-butene was prepared from 4-penteneoyl chloride using the methods described by Pfister and Wymann, *Synthesis* **1983**, *1*, 38. The previous step results in the free amine being partitioned in a basic aqueous solution. To this solution was added Di-*tert*-butyl dicarbonate (5.8 g, 27 mMol) in dichloromethane (CH_2Cl_2, Sigma-Aldrich Inc., 100 mL). The reaction mixture was stirred vigorously

overnight at room temperature. The aqueous phase was extracted twice with 50 mL CH_2Cl_2. The organic fractions were combined, washed twice with 100 mL 1N HCl, (Sigma-Aldrich Inc.) and concentrated. The resulting oil was purified by flash column chromatography using 5% ethyl acetate in hexanes.

Surface modification.

Light promoted hydrosilylation. Hydrosilylation of PSi samples with an area of 0.95 cm^2 were carried out in a Teflon flow cell equipped with a quartz window and inlet and outlet ports for inert gas. *N*-BOC-amino-3-butene (10 μL) was placed on the PSi, which was then placed in the flow cell under nitrogen. Light from a 30 W halogen bulb was focused on the sample at a distance of approximately 5 cm from the silicon surface. After 2 h illumination time, the sample was removed from the flow cell and rinsed with CH_2Cl_2 and dried under nitrogen flow followed by vacuum.

Removal of BOC (t-butyloxycarbonyl) protecting group. Following surface modification, the sample was placed in a vial and exposed to 20% trifluoroacetic acid (TFA) by volume in CH_2Cl_2 for 2 h. The sample was removed from the vial and rinsed with CH_2Cl_2 and dried under nitrogen flow followed by vacuum.

SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate) attachment. The amino functionalized PSi wafer was immersed in a solution of SPDP (2 mg, 6.4 x 10^{-3} mMol) in dimethyl formamide (DMF, Sigma-Aldrich Inc.) under nitrogen. The wafer was left to react for 3 h with occasional agitation. The remaining SPDP solution was removed and the silicon wafer was rinsed with DMF (3 × 5 mL) followed by EtOH (Sigma-Aldrich Inc., 3 x 5 mL) then dried under a nitrogen stream followed by vacuum.

Reductive cleavage of pyridyl disulfide protecting group. The SPDP functionalized porous silicon wafer was immersed in a solution of DTT (15.4 mg, 0.1 mMol) in 10% EtOH/H_2O (10 mL). The wafer was left to react for 1 h with occasional agitation. The remaining DTT solution was removed and the wafer was rinsed with fresh 10% EtOH/H_2O (3 × 5 mL) then EtOH (5 × 5 mL) then dried under a nitrogen stream followed by vacuum.

GMBS (N-(γ-maleimidobutyryloxy)succinimide ester) attachment. The sulfhydryl functionalized PSi wafer was immersed in a solution of GMBS (2.0 mg, 7.1 x 10^{-3} mMol) in DMF under nitrogen. The wafer was left to react for 3 h with occasional agitation. The remaining GMBS solution was removed and the silicon wafer was rinsed with DMF (3 × 5 mL) followed by EtOH (3 x 5 mL) then dried under a nitrogen stream followed by vacuum.

OPAA enzyme immobilization. The PSi sample was then incubated for 4 h at room temperature in 1 mL of buffer solution containing 1 mg/mL OPAA (95% pure). The sample was removed and rinsed with two 1 mL aliquots of buffer followed by 1 mL of 1M buffer solution of sodium chloride and finally rinsed again with two 1 mL aliquots of buffer. Wash solutions were stored at –20°C prior to protein analysis.

Enzyme activity assay

The functionalized PSi samples were mounted in the flow cell shown in Scheme 2 of the manuscript. The flow cell is constructed of Teflon and is fitted with a quartz window that allows for fiber optic interrogation of fluorescence, and is also connected by tubing to a UV-vis quartz cell in a closed loop. A total volume of 2.5 mL was re-

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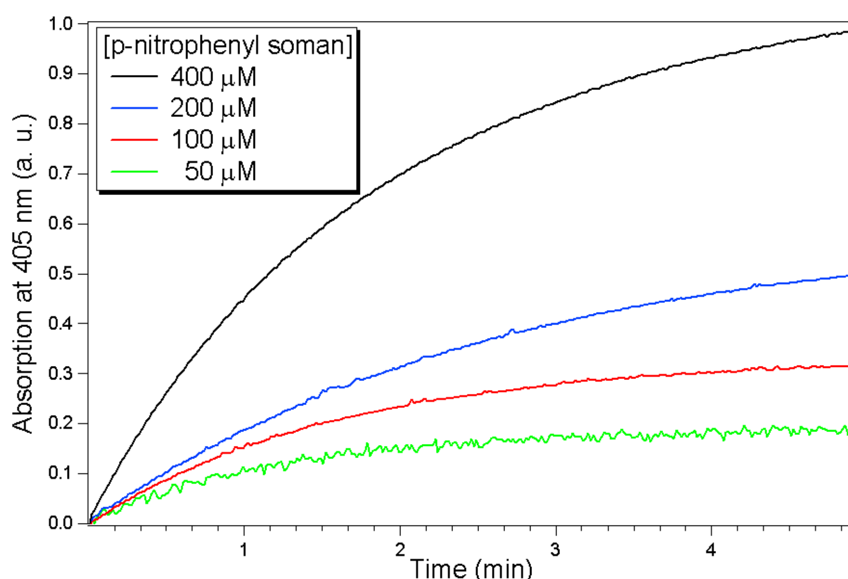
circulated through the flow cell and the UV-vis cell with a peristaltic pump at a flow rate of 8 mL/min. The rinses were performed with buffer and the activity assays were performed with various concentrations of the substrate *p*-nitro-phenyl-soman (NP-GD) in buffer. OPAA activity was detected as an increase of *p*-nitrophenol concentration measured *in situ* and in real time in the UV-vis cell with a Cary 300 spectrophotometer (Varian Inc.) by using the kinetics-recording mode at 405 nm.

Photoluminescence spectra measurements

The photoluminescence (PL) of the functionalized PSi samples was measured *in situ* in real time through the quartz window of the flow cell with a Cary Eclipse fluorescence spectrophotometer fitted with a fiber optic coupler and a remote read fiber optic probe. The PL was excited at 290 nm and recorded in the full spectrum mode.

Solution enzymatic activity assay



ESI Figure 1: OPAA enzymatic activity assay in solution. 8 µg/mL of OPAA enzyme was placed in buffer solutions of the substrate *p*-nitrophenyl-soman ranging from 50 to 400 µM. The absorption of *p*-nitrophenol was measured at 405 nm in a 1-cm path length quartz cell fitted with a Starna Spinette magnetic stirring system.

Breakdown product concentration dependence on substrate concentration.

As seen in ESI Figure 1 above, the amount of *p*-nitrophenol produced from enzymatic action is dependent on both time of reaction and initial substrate concentration. At a given time, the higher the initial substrate concentration, the more *p*-nitrophenol produced. This behavior is seen for the surface immobilized enzyme also, as shown in Figure 1 of the manuscript. The inverse of this is seen in the behavior of the PL during the enzymatic action, as seen in Figure 2 in the manuscript.

ESI Figure 2 shows the linear relationship of the initial substrate concentration with the enzymatic breakdown product and effect on the PL at various reaction times.

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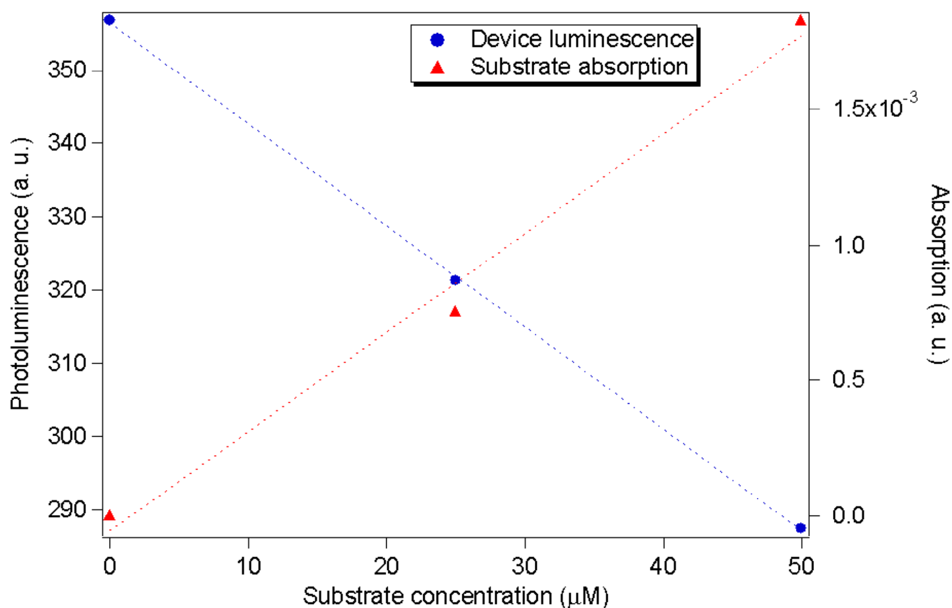

ESI Figure 2. Relationship of initial substrate concentration to PL and p-nitrophenol production at 0, 25 µM, and 50 µM concentrations at 5 min.

Safety measures

To the best of our knowledge, there is no published human toxicity data for p-nitrophenyl-soman. This substrate therefore had to be handled assuming that it was as toxic as soman, for which the LD_{50} values for a 70 Kg person are 100-900 mg for skin exposure and 30 mg for ingestion (see National Academy of Sciences, N. R. C., Committee on Toxicology Possible long-term health effects of short-term exposure to chemical agents; National Academy Press: Washington, D.C., 1982; Vol. 1: Anticholinesterases and Anticholinergics).

All concentrated solutions were handled in a fume hood only and all the vials had secondary containment. The room in which the *p*-nitrophenyl-soman was handled was under negative pressure and protective equipment constituted of a lab-coat, safety goggles and butyl rubber gloves, was worn at all times. A decontamination solution of sodium hydroxide or sodium hypochlorite was also stored in the laboratory in the event of a spill.

The solutions used to test the PSi sensor had a maximal concentration of 400 µM and the volume of the flow cell was 2.5 mL. The amount of substrate used in an experiment was therefore well below the lethal dose. The dilute solutions were injected into the sealed flow cell via a septum. All the components of the cell were made of Teflon except the window, which was made out of quartz.