Supplementary Material for Chemical Communications
This journal is © The Royal Society of Chemistry 2003

New Method for Attachment of Biomolecules to Porous Silicon

Bradley R. Hart, Sonia E. Létant, Staci R. Kane, Masood Z. Hadi,
Sharon J. Shields, and John G. Reynolds*

SUPPORTING INFORMATION

Etching of Silicon

Porous silicon 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 grade, 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 150W 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 born contamination.

Hydrosilylation of Porous Silicon

Hydrosilylation of porous silicon samples with an area of 0.95 cm^2 were carried out in a round-bottom Schlenk type flask equipped with a single 24/40 taper joint and glass stopcock controlled side-arm. The silicon sample was placed in the flask and which was then sealed with a septum and purged with dry, oxygen-free nitrogen gas. 5-Hexynenitrile (0.10 □L, 0.95 mM) was added to the porous silicon surface followed by the addition of a 1.0 M hexane solution of $EtAlCl_2$ (150 μL, 0.15 mmol). After 2 h at room temperature, the silicon surface was washed under a nitrogen atmosphere with THF, followed by CH_2Cl_2 and then EtOH. The wafer was then dried under vacuum.

Reduction of Surface Bound Nitrile

Reduction of the surface bound nitrile to a 1° amine was achieved by adding a 1.0 M diethyl ether solution of $LiAlH_4$ (150 μL, 0.15 mmol) to the surface of the porous silicon. After 30 min at room temperature, the silicon surface was washed under a nitrogen atmosphere with THF (3 x 5 mL), followed by CH_2Cl_2 (3 x 5 mL) then ethanol (3 x 5 mL) then dried under a nitrogen stream followed by vacuum.

Attachment of SPDP

The amino functionalized porous silicon wafer was immersed in a solution of SPDP (2 mg, 6.4x10^{-3} mmol) in 3:1 DMF/PBS (pH 7.2) 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 ethanol (3 x 5 mL) then dried under a nitrogen stream followed by vacuum.

This journal is © The Royal Society of Chemistry 2003

Reductive Cleavage of Pyridyl Disulfide Protecting Group

The SPDP functionalized porous silicon wafer was immersed in a solution of dithiothreitol (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.

Attachment of GMBS

The sulfhydryl functionalized porous silicon wafer was immersed in a solution of GMBS (2.0 mg, $7.1x10^{-3}$ mmol) in 3:1 DMF/PBS (pH 7.2) 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 ethanol (3 x 5 mL) then dried under a nitrogen stream followed by vacuum.

Attachment of Biotin or Dansyl Fluorescent Probe

The NHS-ester functionalized porous silicon wafer was immersed in a solution of Dansyl cadaverine (5.0 mg, 0.015 mmol) or Biotin cadaverine (5.0 mg, 0.011 mmol) in 1:1 DMF/PBS (pH 7.2) under nitrogen. The wafer was left to react for 3 h with occasional agitation. The remaining solution was removed and the silicon wafer was rinsed with DMF (5 × 5 mL) followed by ethanol (5 x 5 mL) then dried under a nitrogen stream followed by vacuum. Supplementary Material Spectrum 4 shows the FTIR of the biotin functionalized PSi.

Attachment of Streptavidin

To the surface of a Biotin functionalized PSi was added a solution of Streptavidin (0.3 mL, 1 mg/mL protein) in PBS (pH 7.2). After 1 h the excess protein solution was removed and the surface was washed extensively with PBS (pH 7.2).

Photoluminescence Measurements

Photoluminescence measurements were performed using the 325 nm line of a 10 mW He-Cd laser. The spot size on the PSi sample was 1 mm^2. The signal was focused on the 10 μm x 2 mm slit of an Oriel monochromator equipped with a 1200 mm^{-1} grating and was detected with a CCD analyzer. The spectral resolution of the set up is 1.5 nm. All the spectra were corrected from the instrumental response.

Trypsin Digestion of Streptavidin

The modified surface containing streptavidin was washed with 50mM NH_4HCO_3 (pH 7.8) prior to addition of trypsin (20 μL of 0.01 μg/μL). The porous silicon was then held at 37°C for 12 hours to maintain protease activity. To extract the streptavidin peptide fragments resulting from the digest 1:1 CH_3CN/H_2O containing 0.1% TFA (100 μL) was applied to the surface followed by extraction with a pipette. The extracted volume was reduced by speed-vac to 10 μL to increase the peptide concentration. 1 μL was mixed with 3 μL of 7 mg/mL α-cyano-4-hydroxycinnamic acid and 6 μL of H_2O and deposited on a stainless steel target for MALDI MS.

Supplementary Material for Chemical Communications
This journal is © The Royal Society of Chemistry 2003

Effect of Surface Modification on Photoluminescence

Supplementary Materials Spectrum 3 shows the photoluminescence of the silicon surface before functionalization and after functionalization with biotin attached (structure 7). The reduction in photoluminescence is about 50%. The first functionalization step (the nitrile attachment), the photoluminescence is reduced this amount and remains unchanged after ensuing steps.

Spectrum 1. IR spectrum of nitrile functionalized PSi surface **1**.

Spectrum 2. MALDI-TOF MS spectrum of surface-bound Streptavidin Trypsin digest solution.

Spectrum 3. Porous silicon photoluminescence spectrum of freshly etched unfunctionalized surface and biotin link (structure 7)

Spectrum 4. FTIR of biotin functionalized PSi.