Supplementary Material (ESI) for Chemical Communications

Functionalized porous silicon surfaces as MALDI-MS substrates for protein identification studies

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Experimental details and FTIR results

Preparation of functionalized silicon surfaces and FTIR characterization. Shards of Si(100) p-type single side polished wafer (doped with boron, 1-8 Ω cm resistivity), or Si(111) n-type single side polished wafer (doped with phosphorous, 0.02-0.5 Ω cm resistivity), were cleaned in a mixture of H_2O_2 (30 %) and H_2SO_4 (96 %) (1:1, volume:volume) heated to near boiling. H-terminated porous silicon substrates were produced through galvanostatic etching (current density of 5 mA cm^{-2}) of cleaned Si(100) samples in hydrofluoric acid (49 %) and anhydrous ethanol mixture (1:1, volume:volume) for 20 seconds (0.6 cm^2 circular etched area). H-terminated flat silicon substrates were produced by etching cleaned Si(111) samples in deoxygenated 40 % NH_4F (aq.) for 30 minutes in Teflon vials. The freshly etched substrates were then reacted with neat 1-decene (at 150 ºC) or methyl 10-undecenoate (at 115 ºC) for 23 hrs. in a Schlenk tube while purging the reagent continuously with argon (*Note*: porous silicon substrates were rinsed with ethanol and dried under a stream of argon to remove traces of water from the pores immediately after etching, rinsing was not required in the case of flat silicon substrates). After functionalization the samples were thoroughly rinsed with the appropriate organic solvents (ethanol and tetrahydrofuran (THF) for 1-decene, and THF and water for methyl 10-undecenoate functionalized samples). Carboxyl-terminated surfaces were prepared from methyl-ester-terminated samples through acid catalyzed hydrolysis of the ester functional group (see also reference 1 below). For this purpose the ester-terminated samples were treated with 2.4 M HCl at 70 ºC for 2 ½ hours and rinsed with water and THF. Functionalized surfaces were analyzed by FTIR spectroscopy (Nexus 870 Spectrometer, ThermoNicolet). As shown in Figure S1 (a-c) for

porous silicon samples, all functionalized surfaces produced absorption peaks corresponding to alkyl C-H stretch (~2852 cm^{-1} and ~2925 cm^{-1}). A peak due to carbonyl stretch (~1742 cm^{-1}) was observed when surfaces terminated with methyl ester group were analyzed (Figure S1 (b)). This peak disappeared after hydrolysis of the ester functional group, and a new peak corresponding to carbonyl stretch of carboxyl group appeared at ~1715 cm^{-1} (Figure S1 (c)). Absorption peaks corresponding to the stretching of C-H (alkyl group) and C=O (carboxyl group) were also observed for ammonium fluoride etched flat silicon samples after functionalization with methyl 10-undecenoate and acid hydrolysis. Analysis was performed on samples prepared using Si(111) n-type ATR elements in such cases.

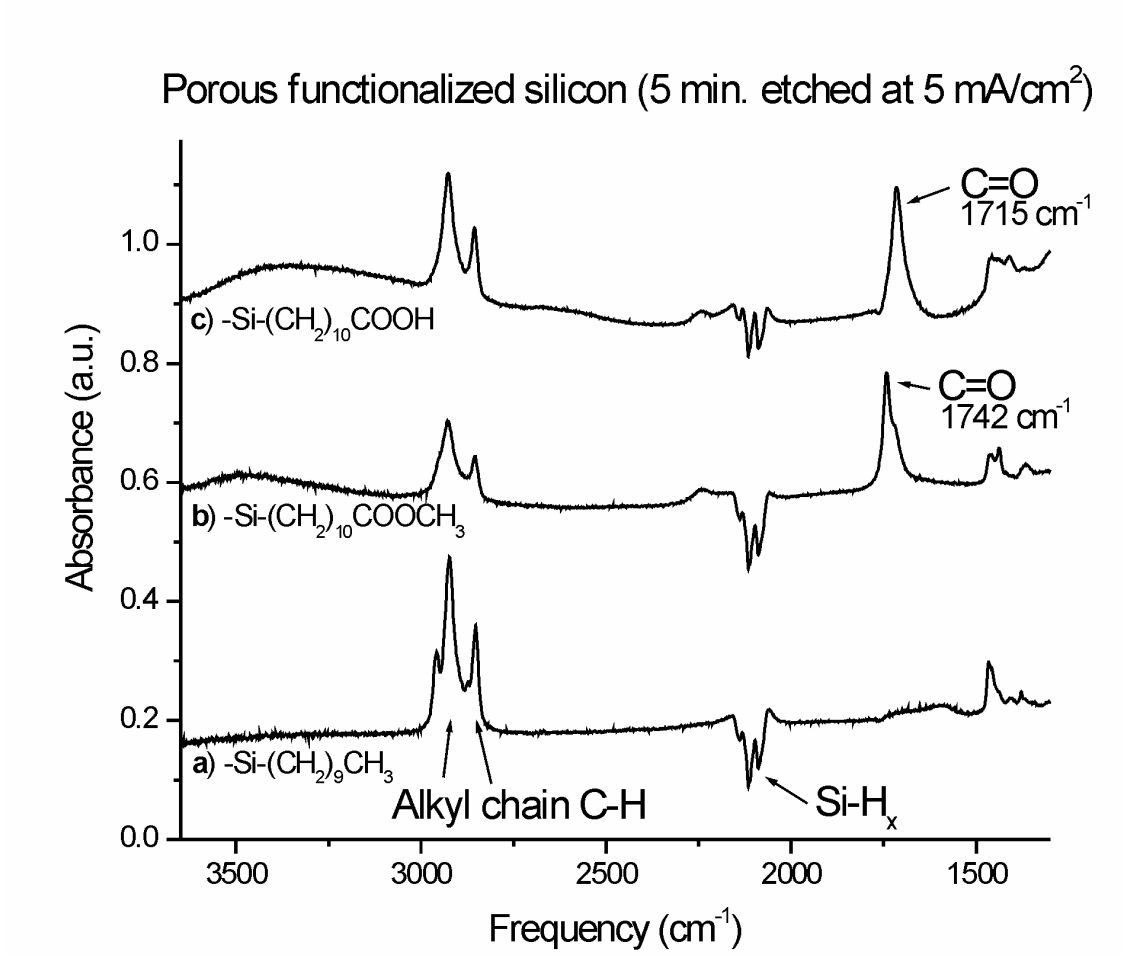

Fig. S1 FTIR spectra of Si-H-terminated surfaces functionalized with a) 1-decene, b) methyl 10-undecenoate and c) same surface as (b) after treatment with 2.4 M HCl at 70 ^{o}C for 2 ½ hours to yield a carboxyl-terminated surface. Porous Si-H terminated surface was used as reference background. For samples analyzed by FTIR, etching of Si(100) p-type wafer was performed for 5 min. in HF/ethanol/water mixture (with a current density of 5 mA cm^{-2}).

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Adsorption of proteins on functionalized silicon surfaces and analysis by MALDI-MS. The composition of the standard protein sample used in the investigations is as follows: α-casein, carbonic anhydrase, α-lactalbumin, bovine serum albumin, ubiquitin and cytochrome-*c* (1 mg ml^{-1} each); insulin (10 % saturated) and myoglobin (10 % saturated). The buffer solutions used for rinsing protein sample spots were prepared from formate (pH = 3.0) and Tris (pH = 9.0). The ionic strength of these solutions was 0.15 M. All solutions were prepared using ultrapure water (Millipore, 18 MΩ cm, 1 ppb TOC). The functionalized porous silicon samples were attached to a stainless steel PAGE gel plate (Applied Biosystems) side by side using double-sided tape. 2.5 μl of standard protein mixture was deposited on each substrate. After 5 minutes the excess sample was removed from the surface using a pipettor. Then the active surface was rinsed with buffer (pH 3.0 or 9.0) as follows: 5.0 μl rinsing solution was applied onto the protein spot and pipetted up and down 5 times and removed. This procedure was repeated 3 times. After that 2.0 μl of a matrix solution (saturated sinapinic acid in 60 % acetonitrile with 0.3 % trifluoroacetic acid) was applied and allowed to dry. The sample spots prepared as described above were analyzed by MALDI-MS using Voyager DE-STR TOF Mass Spectrometer equipped with nitrogen laser (337 nm wavelength) in positive ion mode. The mass spectral data shown are averages of 150 accumulated spectra (*not*e: the same laser power was used in all experiments).

Reference:

1. R. Boukherroub and D. D. M. Wayner, *J. Am. Chem. Soc.* 1999, **121**, 11513.