Grafting Organic and Biomolecules on H-Terminated Porous Silicon from a Diazirine

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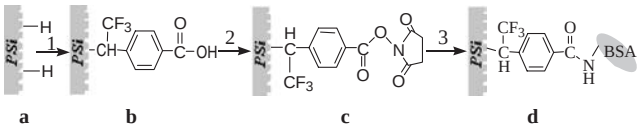
A diazirine compound, 1,4-(1-azi-2,2,2-trifluoroethyl)benzoic acid, was used as a stable carbene precursor to react with Si–H terminated porous silicon (PSi) under microwave irradiation. After formation of a molecular monolayer, the end carboxyl group was converted to an amine-reactive crosslinker species, NHS ester, and finally to a Bovine Serum Albumin (BSA) monolayer on PSi.

The assembly of organic monolayers on semiconductor surfaces by chemisorptive strategies remains an active area of research.[1] Many organic species bearing functionalities such as alkenes, alkynes,[2] alkyl halides,[3] alcohols,[4] aldehydes,[5] carboxylic acids,[6] acid chloride,[7] Grignard reagents,[8] and alkyllithium reagents[9] had been used to assemble organic monolayers on silicon surfaces through the robust Si–C or Si–O–C bonds by heating, photo irradiation,[10] microwave irradiation,[11] electrochemical reduction, catalysis, or other methods. The reaction mechanism is commonly accepted as a surface-propagated chain reaction in which an alkyl radical formed by the addition of organic functionalities to a surface silicon radical abstracts a hydrogen atom from an adjacent silicon hydride. To our knowledge, the typical carbene intermediates from diazirines have been employed for grafting biomolecules on silicon oxide and diamond substrates,[12,13] but not on hydrogen-terminated silicon surfaces. Similar examples include: 1) diazirine diazomethane[14] under a 365-nm light irradiation forms a linear alkane monolayer on silicon through a singlet methylene intermediate and 2) benzenediazonium salts[15] under an electrochemical reduction generate aryl radicals to react with Si–H for coupling organic monolayers.

Herein, we report the assembly of a carboxyl-terminated organic molecule on porous silicon (PSi) from a diazirine species in order to graft biomolecules (Scheme 1). In view that the carboxyl terminal monolayers possess versatile chemical possibility to immobilize biomolecules, in particular proteins, a bifunctional species, diazirine- and carboxyl-functionalized crosslinker, 1,4-(1-azi-2,2,2-trifluoroethyl)benzoic acid (labeled as H in Supporting Information Scheme S1), was synthesized and self-assembled on PSi under microwave irradiation. The resulted carboxyl group (b) was further converted to an amino-reactive linker, NHS (N-succinimidyl) ester (c), under the presence of NHS and an activator DCC (dicyclohexylcarbodiimide) in 1,4-dioxane, and finally to a protein monolayer (d) of BSA in a slightly alkaline PBS (phosphate buffered saline) solution (pH = 9.0). In the final step, the side-chain amino groups of lysine residues or N-terminals on the protein surface displace NHS groups, resulting in covalent attachment of the protein. The slightly alkaline solution was used to deprotonate amines to enable an easy reaction with the NHS ester.



Scheme 1. Surface modification of porous silicon. 1,4-(1-Azi-2,2,2-trifluoroethyl)benzoic acid (H) in *p*-xylene under microwave irradiation; 2, NHS/DCC in 1,4-dioxane; 3, BSA in PBS at pH 9.0; a, H-terminated porous silicon; b, carboxyl-terminated PSi; c, NHS ester-terminated PSi; d, protein BSA pendant PSi.

The grafting procedure in Scheme 1 was monitored by FTIR and XPS analyses. The IR spectra of each surface after stepwise reactions were recorded in Figure 1. The spectrum of a fresh hydride-terminated PSi (**a**) exhibits typical tripartite bands around 2100 cm^{-1}, contributed from Si–Hx (x = 1–3) stretching modes (2084 from νSi–H_1, 2109 from νSi–H_2, and 2130 cm^{-1} from νSi–H_3). The Si–Hx bending modes exhibit the absorption peaks at 906, 660, and 623 cm^{-1} respectively. After reaction with a diazirine compound H, 4-(1-azi-2,2,2-trifluoroethyl)benzoic acid, under the microwave irradiation for 20 min, surface b was obtained, and its spectrum in Figure 1b exhibits distinctive difference from a with the appearance of a broad IR band of oxidized silicon at 1087 cm^{-1} and organic groups. The typical stretching band of the carboxylic acid at 1696 cm^{-1} indicates the existence of free benzoic acid functionalities. The skeleton vibration of benzene also appears at 1610, 1510, and 1415 cm^{-1}. The peak at 1255 cm^{-1} can be attributed to the CF_3 stretching vibration. However, the weak band at 2923 cm^{-1} from alkyl CH_2 should be attributed to the surface contamination of hydrocarbons from the lab atmosphere. The characteristic tripartite bands of Si–Hx from a evolve into two broad bands at 2098 and 2248 cm^{-1}. According to the calculation of Lucovsky,[16] the peak 2248 cm^{-1} is assigned to the oxygen back-bonded Si–H species, $(O_2)SiH_2$ and $(O_3)SiH$. These oxygen back-bonded Si–H species also show broad bending modes at 867 and 626 cm^{-1}. The peak 2111 cm^{-1} is assigned to the unreacted residues of Si–H species. Surface c was obtained by activation of b with NHS/DCC, its IR

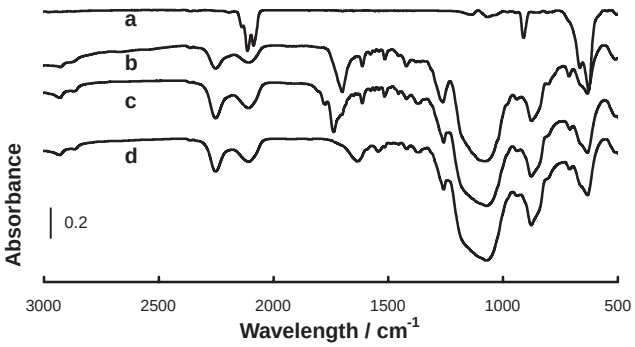


Figure 1. Transmittance FTIR spectra of surfaces a, b, c, and d.

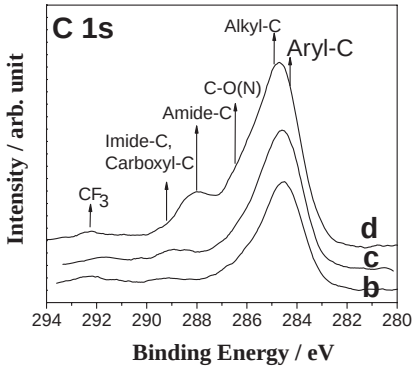


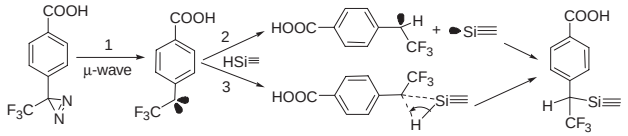
Figure 2. Evolution of XPS high-resolution spectra of C 1s of surfaces b, c, and d.

spectrum exhibits three characteristic vibration bands of NHS ester, 1733, 1770, and 1799 cm^{-1}.[17] However, a complete conversion of the carboxylic acid to NHS is not always the result. A shoulder of the carboxylic acid band at 1696 cm^{-1} in Figure 1c points to the unreacted residues of acid. Our experience indicated that the conversion efficiency relates not only to the incubation solution but also to the wafer batches. After incubation of c in a 50 mg/mL BSA solution for 1 h, the protein BSA was immobilized on surface d. Instead of the NHS-ester bands, the characteristic vibrations of amide I and II from BSA occur at 1631 and 1535 cm^{-1}, respectively.

The evolution of the XPS high-resolution spectra of C 1s is shown in Figure 2 from b to c and finally to d. Their survey and N 1s spectra, atomic concentrations, and the fitting results of C 1s are listed in Supporting Information.[18] On surface b, the appearance of fluorine F 1s at 687.0 eV, of trifluoromethyl-C 1s at 292.1 eV, of carboxyl-C 1s at 289.0 eV, and of aryl-C 1s at 284.1 eV firmly confirms the formation of organic monolayers. The distinctive difference of c from b is the appearance of a new element, N, and the signal increase of C 1s at 289.0 eV, due to the substitution of COOH by NHS ester. Finally from c to d, the concentration of nitrogen dramatically increases from 1.7 to 7.4%, and the protein-rich amide-C 1s at 288.0 eV contributes 12% to the whole carbon. All XPS data support the surface reaction evolution in Scheme 1 very well.

As it is well known that trifluoromethyldiaziridine is a typical carbene precursor, the reaction mechanism is supposed to go through a carbene intermediate. Microwave radiation mainly yields the thermal effect on silicon and solution. The reaction solution temperature was 80 °C, and the temperature of the PSi wafer measured by a contacted thermometer reached 180 °C. Such a thermal source can dissociate diazirine into carbene intermediates. And then both a singlet carbene insertion into Si–H and a triplet carbene mechanism involving hydrogen extraction and recombination to form the Si–C linkage are possible (Scheme 2).

As indicated above, the carbene intermediate resulted from a diazirine species is neat to generate molecular monolayers on porous silicon surfaces. The end carboxyl group of monolayers



Scheme 2. The reaction mechanism of diazirine on PSi is suggested as: 1, formation of a carbene intermediate; 2, a triplet carbene process involving hydrogen extraction and recombination of organic radical and silicon radical; 3, an insertion of a singlet carbene into Si–H; final formation of carboxyl-terminated monolayers.

can be converted to an amine-reactive crosslinker species, NHS ester, and finally to a protein BSA monolayer. Protein-grafted PSi is of growing interest for applications in biosensor, bioreactor, affinity chromatography, and many diagnostic techniques.

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18 Supporting Information is available electronically at the CSJ website, http://www.csj.jp/journals/chem-lett/.

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