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

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Alkyl monolayer modified porous silicon functional surfaces are employed for selective binding of proteins from complex mixtures (through washing of the deposited mixture spot using appropriate buffer) and MALDI-MS is used to detect the components retained on the surface.

Porous silicon (PSi) is a high surface area semiconducting material suitable for the development of biosensors.[1] Due to its properties of photoluminescence it has also potential applications for the design of optoelectronics devices and displays.[1,2] In addition, it has the properties of high thermal conductivity and ultraviolet absorptivity, and it is interesting for applications as sample platform in matrix-free desorption/ionization mass spectrometry (DIOS-MS).[3] PSi substrates are also ideal substrates for designing surfaces with controlled properties that could be used in the identification and possible quantification of biomolecular materials such as peptides and proteins. Functionalized porous silicon surfaces could serve as platforms to selectively capture and sequester such large molecules from complex mixtures for identification by techniques such as matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). PSi substrates with controlled morphology, porosity, porous layer thickness and chemical properties can be fabricated from crystalline silicon using known experimental methods for a particular application.[4,5] For example, surfaces with tailored chemical properties can be produced through thermal hydrosilylation reactions between H-terminated PSi (formed through galvanostatic etching of crystalline silicon in HF–ethanol mixture) and ω-functionalized 1-alkene (or 1-alkyne).[5] Such reactions lead to the formation of thermally and chemically stable Si–C bond linked alkyl monolayers containing terminal functional groups.

This paper discusses the use of functionalized PSi surfaces prepared by employing the strategy of thermal hydrosilylation for investigating the interaction of proteins with surfaces. For the preparation of functionalized PSi substrates, Si(100) samples were at first etched in hydrofluoric acid (49%) and anhydrous ethanol mixture (1:1, volume:volume) galvanostatically (with a current density of 5 mA cm^{-2}) for 20 s. The freshly etched H-terminated porous silicon substrates were then reacted thermally (115–150 °C) with neat 1-alkene reagents in Schlenk tubes under argon. Two types of surfaces, namely a purely hydrophobic surface (–Si–$(CH_2)_9CH_3$) that was prepared by using neat 1-decene, and a carboxyl-terminated surface (–Si–$(CH_2)_{10}COOH$) that was produced from methyl-ester-terminated surface (–Si–$(CH_2)_{10}$-$COOCH_3$) through acid catalyzed hydrolysis of the ester functional group,[6]‡ were employed in these studies. The methyl-ester-terminated surface itself was synthesized using neat methyl 10-undecenoate. FTIR spectroscopy was used to confirm the surface functionalization. To demonstrate the performance of the different surfaces, we investigated a model sample prepared by mixing eight randomly selected proteins with an average molecular weight of ~6–66 kDa. The components of the mixture were 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). As depicted schematically in Fig. 1, a droplet of the protein mixture (2.5 μl) is deposited on the functionalized silicon substrates and left to interact with the surface for 5 min. And then the excess solution was removed and the active spot rinsed four times with each 5 μl buffer solution using a pipettor. We employed pH 3.0 (formate) or pH 9.0 (tris) buffer solutions of 0.15 M ionic strength for this purpose. After the final rinsing step, 2.0 μl of a matrix solution (saturated sinapinic acid in 60% acetonitrile with 0.3% trifluoroacetic acid) was applied onto the active spot, and the surface retained components are then detected using MALDI-MS in a subsequent step. With the types of surfaces employed in these studies, adsorption of proteins involves only non-specific interactions. The strength of surface interactions is obviously determined by both the properties of protein molecules and the nature of surfaces. The size and shape of molecules, as well as their polarity (as determined by both effective and local molecular charges) are important in this respect. In addition, the chemical properties and other characteristics of porous silicon surfaces (*e.g.* morphology, pore sizes and surface functional groups) could also influence the

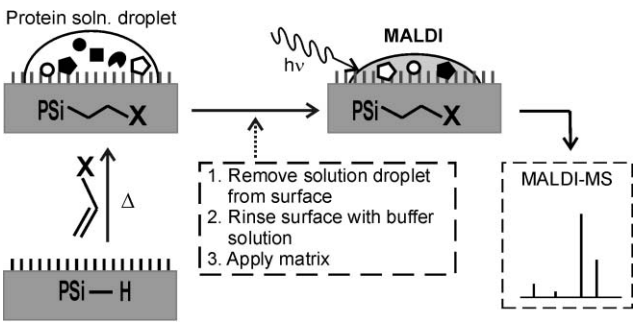

Fig. 1 Sample preparation steps for protein analysis on functionalized porous silicon by MALDI-MS.

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strength of surface interactions. In Fig. 2 are shown, for example, mass spectral patterns for standard protein mixture spots analyzed on porous and flat§ functionalized silicon substrates containing carboxyl functional groups. Under otherwise similar experimental conditions, we observed significantly higher protein mass signal-to-noise ratios for porous than for flat functionalized substrates. For example, the intensity of the signal corresponding to ubiquitin is more than six times as large on porous silicon than on flat silicon substrate. These observations indicate that there is a higher amount of proteins adsorbed on the porous functionalized silicon substrate as a result of the increased surface area. It should be noted, that the same acquisition conditions were used for all the mass spectra shown in this work. Additional results by which other experimental parameters (such as the pH of the rinsing buffer and the polarity of the surface) are varied are presented in Fig. 3. The

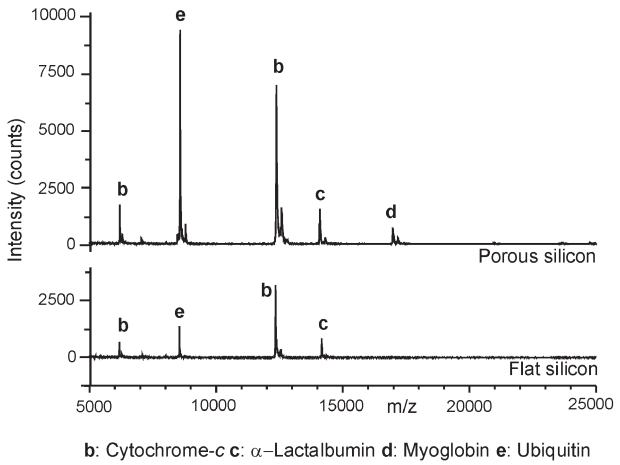

Fig. 2 Mass spectral patterns of a protein mixture analyzed on carboxyl-terminated (–Si–$(CH_2)_{10}$COOH) flat or porous silicon after rinsing with 4 × 5 μl pH 9.0 buffer.

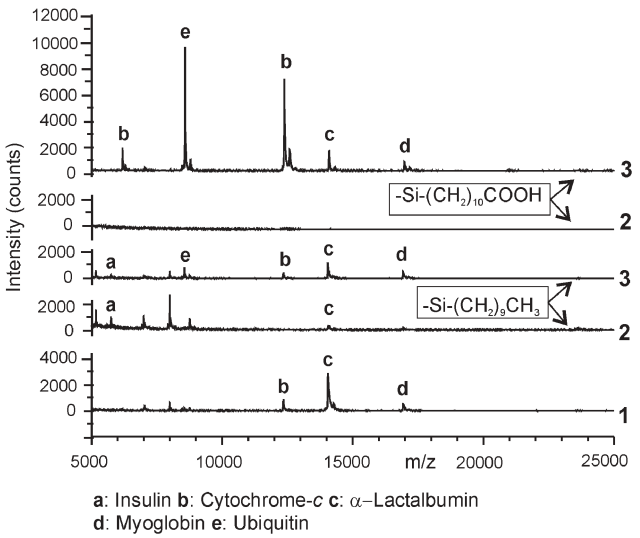

Fig. 3 Mass spectral patterns of a protein mixture analyzed on functionalized porous silicon after rinsing with 4 × 5 μl pH 3.0 (2) or 4 × 5 μl pH 9.0 (3) buffer. Spectrum 1 is for a sample spot analyzed without rinsing (pattern independent of surface modification, result shown here is obtained using unmodified flat silicon surface).

spectra shown in the same figure are for protein sample spots analyzed on the two types of surfaces investigated after rinsing with pH 3.0 or pH 9.0 buffer. The spectra pattern for a protein sample spot analyzed on a flat unmodified surface without applying rinsing is also shown for comparison (see spectrum 1).¶ The results of the experiments with rinsing depict that the selectivity of the substrates toward binding proteins is also dependent on the pH of the rinsing buffer and the types of surface functional groups. There is a significant difference between the spectra pattern obtained using carboxyl-terminated surfaces with pH 3.0 and pH 9.0 buffer rinsing in particular (see the corresponding spectra in Fig. 3). While no signals were detected when rinsing is performed with pH 3.0 buffer on carboxyl-terminated surface, the same surface shows relatively strong affinities toward ubiquitin, cytochrome-*c*, α-lactalbumin and myoglobin when rinsing is performed using pH 9.0 buffer. These differences could be due to the influence of pH on the three-dimensional structure of protein molecules (and also on the charge of such molecules) as discussed above. In addition, the charge on ionizable functional surfaces containing acidic or basic terminal groups (such as carboxyl as in the present case) is also pH dependent. Thus, the protein binding properties of such surfaces could be influenced by the pH of the rinsing buffer. The relatively strong cytochrome-*c* signal obtained when analysis is performed on carboxyl-terminated surface using pH 9.0 rinsing buffer (see the corresponding spectra in Fig. 3) is possibly the result of strong electrostatic interactions between the protein and the surface in the same pH region. The pI value of cytochrome-*c* is between pH 9 and 10 (see ref. 7). Thus, at pH 9.0 cytochrome-*c* molecule would have a net positive charge, while at the same pH almost all of the surface COOH groups of a carboxyl-terminated surface exist in the deprotonated form as COO^- (as confirmed by chemical force titration experiments performed on carboxyl-terminated surfaces, which yielded a pK_a value of *ca.* 4.5).[8] Unlike with carboxyl-terminated surfaces, the influence of rinsing buffer pH on the responses of methyl-terminated hydrophobic surface was relatively low for the protein mixture analyzed in our experiments (see the corresponding spectra in Fig. 3).

The above presented result is also interesting when considering application of functionalized PSi substrates as MALDI-MS sample platform for direct analysis of complex biological samples. Commercially available products (such as ProteinChip Arrays from Ciphergen) have been shown to be useful tools for protein identification studies such as in the areas of biomedical research.[9] The use of Si–C bond linked alkyl monolayer functional surfaces for similar applications is rather promising because of their advantages of high chemical and thermal stability. These properties of Si–C bond linked alkyl monolayers are important when one considers the options of regenerating surfaces for repeated use (for example, through cleaning with organic solvents, or with strong acids or bases). The above described surface modification procedure could also be employed in the fabrication of PSi surfaces onto which more complex organic or biological molecules (*e.g.* DNA, proteins, antibodies) are attached. This may be accomplished, for example, by further derivatization of reactive terminal groups (such as NH_2 or COOH, see for example refs. 6 and 10*a–c*). Such biomolecular surfaces could have potential applications in the selective binding and enrichment of low abundant biological molecules (such as disease marker proteins)

from complex mixtures, and their identification by MALDI-MS. This technique may also be potentially applied to quantify proteins in solutions (see also ref. 11). As demonstrated in Fig. 2 above, the amount of proteins that can be adsorbed on surfaces under given experimental conditions is dependent on the effective area of surfaces. Analogously, the amount of proteins that can be adsorbed on a substrate of controlled surface area may also be related to the concentration of proteins in solutions. For example, we may obtain information on the progress of diseases by comparing the MS profiles of disease and control samples acquired under the same conditions (components for which the relative signal intensities do not appear to change significantly between samples could be used as references to normalize the MALDI-MS spectra). Our ongoing research focuses on the design of surfaces for applications related to both relative quantification and identification of in particular less abundant proteins in relevant biological samples.

Notes and references

‡ Carboxyl-terminated surfaces were prepared by treating ester-terminated samples with 2.4 M HCl at 70 °C for 150 min (C=O absorption bands: $\sim$1742 cm^{-1} (ester), $\sim$1715 cm^{-1} (carboxyl)), see also ref. 6.
§ For the preparation of flat functionalized silicon samples, Si(111) substrates (etched in deoxygenated 40% NH_4F (aq.) for 30 min) were derivatized under the same conditions as with porous silicon substrates.
¶ The mass spectra pattern for a sample spot analyzed without applying rinsing is independent of surface modification.

1 (*a*) A. G. Cullis, L. T. Canham and P. D. J. Calcott, *J. Appl. Phys.*, 1997, **82**, 909; (*b*) M. P. Stewart and J. M. Buriak, *Adv. Mater.*, 2000, **12**, 859.
2 Y. Kanemitsu, *J. Lumin.*, 1999, **83**, 283.
3 (*a*) J. Wei, J. M. Buriak and G. Siuzdak, *Nature*, 1999, **399**, 243; (*b*) Z. Shen, J. J. Thomas, C. Averbuj, K. M. Broo, M. Engelhard, J. E. Crowell, M. G. Finn and G. Siuzdak, *Anal. Chem.*, 2001, **73**, 612; (*c*) R. A. Krusse, X. Li, P. W. Bohn and J. W. Sweedler, *Anal. Chem.*, 2001, **73**, 3639; (*d*) H. Zou, Q. Zhang, Z. Guo, B. Guo, Q. Zhang and X. Chen, *Angew. Chem., Int. Ed.*, 2002, **41**, 646; (*e*) S. A. Trauger, E. P. Go, Z. Shen, J. V. Apon, B. J. Compton, E. S. P. Bouvier, M. G. Finn and G. Siuzdak, *Anal. Chem.*, 2004, **76**, 4484; (*f*) J.-C. Meng, G. Siuzdak and M. G. Finn, *Chem. Commun.*, 2004, **18**, 2108.
4 (*a*) H.-C. Choi and J. M. Buriak, *Chem. Mater.*, 2000, **12**, 2151; (*b*) J. M. Buriak, *Chem. Rev.*, 2002, **102**, 1271.
5 (*a*) R. Boukherroub, S. Morin, D. D. M. Wayner and D. J. Lockwood, *Phys. Status Solidi A*, 2000, **182**, 117; (*b*) R. Boukherroub, S. Morin, D. D. M. Wayner, S. Bensebaa, G. I. Sproule, J.-M. Baribeau and D. J. Lockwood, *Chem. Mater.*, 2001, **13**, 2002.
6 R. Boukherroub and D. D. M. Wayner, *J. Am. Chem. Soc.*, 1999, **121**, 11513.
7 S. D. Moré, J. Hudecek and T. Urisu, *Surf. Sci.*, 2003, **532–535**, 993.
8 T. Z. Mengistu, V. Goel, J. H. Horton and S. Morin, unpublished work.
9 (*a*) M. Merchant and S. R. Weinberger, *Electrophoresis*, 2000, **21**, 1164; (*b*) S. R. Weinberger, E. Boschetti, P. Santambien and V. Brenac, *J. Chromatogr., B*, 2002, **782**, 307; (*c*) H. J. Issaq, T. D. Veenstra, T. P. Conrads and D. Fleschow, *Biochem. Biophys. Res. Commun.*, 2002, **292**, 587; (*d*) F. von Eggeling, K. Junker, W. Fiedler, V. Wollscheid, M. Dürst, U. Claussen and G. Ernst, *Electrophoresis*, 2001, **22**, 2898.
10 (*a*) N. Patel, M. C. Davies, M. Hartshorne, R. J. Heaton, C. J. Roberts, S. J. B. Tendler and P. M. Williams, *Langmuir*, 1997, **13**, 6485; (*b*) T. Strother, R. J. Hamers and L. M. Smith, *Nucleic Acids Res.*, 2000, **28**, 3535; (*c*) T. Strother, W. Cai, X. Zhao, R. J. Hamers and L. M. Smith, *J. Am. Chem. Soc.*, 2000, **122**, 1205.
11 S. Vorderwülbecke, S. Cleverley, S. R. Weinberger and A. Wiesner, *Nat. Methods*, 2005, **2**, 393.