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Hydroxylamine-functionalised silica surfaces for biochip applications

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Abstract—The syntheses of triethoxy and trimethoxy silanes possessing an unprotected hydroxylamine group are described. The grafting of these coupling agents at the surface of oxidised silicon wafers was studied. Accessibility of the hydroxylamine group at the surface was demonstrated with chemical reagents, and the surface proved efficient for covalent immobilisation of peptides possessing the COCHO function.

A breakthrough in biological research and diagnostics has recently been achieved by means of microarray-based technologies.[1] These microarrays usually necessitate immobilisation of biomolecules (DNA,[2a] peptide,[2b] antibody,[2c] sugar,[2d]...) using a highly reproducible specific reaction in very mild conditions. One of the most developed method consists of reacting supported amine groups with aldehyde functions, but this ligation through the imine bond is readily cleaved in aqueous media. Further reduction in secondary amine is necessary to stabilise the supported biomolecule,[2c] which is not compatible with sensitive bioorganic functions such as disulfide bridges. In order to avoid this subsequent reduction, we turned to the oxime ligation. As the oxime bond is readily formed by reacting hydroxylamine and aldehyde functions, and is very stable in aqueous media, it has been extensively used for the modification sensitive biomolecules.[3] Lam[4] has applied the oxime ligation with α-oxo aldehyde-supported glass slides, which were reacted with hydroxylamine-functionalised peptides. The slide functionalisation needed several steps and the organic film was not organised as a monolayer. The chemical steps were not characterised by spectroscopy and chemoselectivity was not quantified. We have also studied the limitations of α-oxo aldehyde-modified silicas.[5] We believe that the reverse approach (functionalisation of silicas with hydroxylamine groups) on silicon wafers would be more efficient in terms of ease of preparation, characterisation and stability of the surface. Furthermore, trialkoxysilanes and silica surface monolayers possessing a free hydroxylamine group have not been described yet to our knowledge. We present here the synthesis of unprotected hydroxylamine coupling agents possessing the trialkoxysilane function, the reactivity of these agents with silica surfaces, and the selectivity of the functionalised surfaces for the ligation of rhodaminated COCHO peptides.

For surface reproducibility and terminal function accessibility, the organoalkoxysilane should be grafted in a two dimension polycondensation manner at the surface of the silica, to obtain a monolayer of well-organised molecules. With alkyltrialkoxysilanes, (C_nH_{2n+1}-$Si(OAlk)_3$ Alk = Me, Et) the number of carbons should be $30 > n > 8$ to achieve this purpose.[6] We thus undertook the synthesis of C_{11} dicarboxyimide-protected hydroxylamine derivatives (Scheme 1). We used a Williamson's type reaction between *N*-hydroxy-5-norbornene-2,3-dicarboxyimide[3b] and 11-bromoundecene. Diimide **1** was classically removed by Gabriel's-type deprotection using hydrazine in refluxing EtOH. Karsterdt's hydrosilylation of ethylenic derivative **2** with $HSi(OAlk)_3$ provided the trialkoxysilylated hydroxylamine derivatives **3** (**3a**: 68%, **3b**: 65%). No reduction

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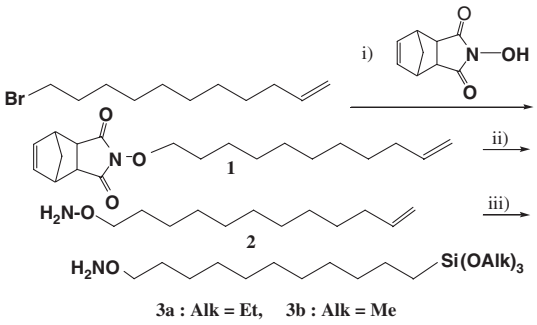
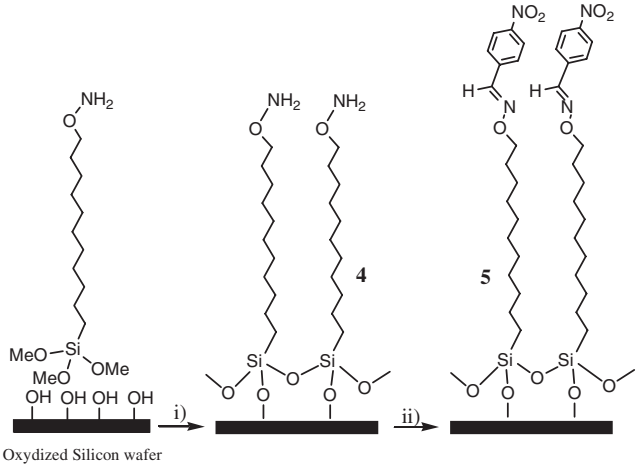


Scheme 1. Synthesis of trialkoxysilylated hydroxylamine derivatives. Reagents and conditions: (i) K_2CO_3, DMF, 50 °C, 90%. (ii) H_2N–NH_2, EtOH, reflux, 96%. (iii) **3a**: Alk = Et, $HSi(OEt)_3$, 2% Karstedt's catalyst, 68%; **3b**: Alk = Me, $HSi(OMe)_3$, 2% Karstedt's catalyst, 65%.

of the hydroxylamine bond was observed and the trialkoxysilanes were stable.

The silanes **3** were then grafted at the surface of silicon wafers (1,0,0), which were first treated to obtain a 1.8–2 nm layer of silica at the surface.[7] The grafting reactions were carried out at 0 °C in trichlorethylene for 24 h (Scheme 2). The kinetics of the reaction were followed in situ by ATRFTIR spectroscopy. Spectra for silane **3b** are presented in Figure 1.

At the beginning of the reaction, silane **3b** was observed in solution. The kinetic showed an increase of the aliphatic bands of the carbon chain and a decrease of the bands of the methoxy groups. After 24 h, no more variation of absorbance was detected with the IR spectra, indicating the end of the grafting procedure. The wafer was then reacted with *p*-nitrobenzaldehyde in order to determine the accessibility of the hydroxylamine function at the surface. The reaction was performed at rt. A new band appeared at 1524 cm^{-1}, which is characteristic of the nitro group. The monolayers **4b** and **5b** were characterised by contact angle measurements, AFM, ellipsometry, respectively. Results are summarised in Table 1.



Scheme 2. Grafting reaction of trimethoxysilylated hydroxylamine derivative. Reagents and conditions: (i) $ClHC{=}CCl_2$, 0 °C; (ii) *p*-nitrobenzaldehyde, $ClHC{=}CCl_2$, rt.

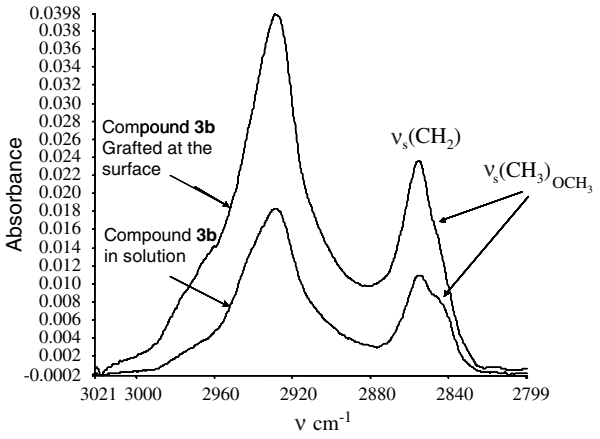
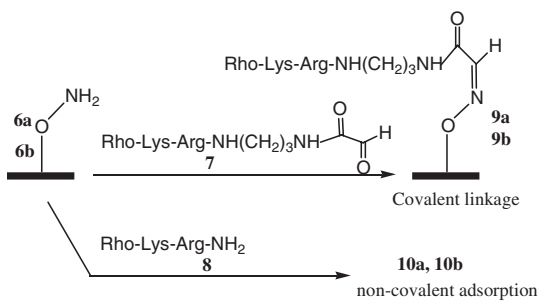


Figure 1. ATRFTIR of the grafting reaction with **3b**.

Table 1. Analyses of the monolayers **4b** and **5b**

Wafer	IR (cm^{-1})	θ	Roughness (nm)	Thickness (nm)
4b	2928, 2855, 2842	60°	0.15	1.9
5b	2928, 2855, 2842, 1524	64°	0.20	2.9

AFM studies indicated a low roughness of the layer, which confirmed the homogeneity of the grafting process as a monolayer.[4] Contact angle measurements were in agreement with the presence of hydrophilic groups at the surface of the monolayer (for a monolayer of primary amine[8] contact angle is known to be 63°). Ellipsometry confirmed the grafting of **3b** as a high-density monolayer. The length of *p*-nitrobenzaldehyde oxime is 0.85 nm, thus the thickness of the monolayer after reaction with *p*-nitrobenzaldehyde was measured with less than 7% error. Fluorescence spectroscopy was then used to study the selectivity of peptide immobilisation at the surface of the functionalised silica (Scheme 3). But the fluorescence emission depends on the distance of the dye from the silicon. Indeed at low distances, interferences between the exciting light reflected at the surface of the silicon and the emitted light occur, which modulate the intensity of fluorescence.[9] Thus, the conditions of the grafting procedures were applied to thermically oxidised silicon wafers.[9] These wafers have a 300 nm silica layer coverage, which allows fluorescence studies of chromophores at the surface. COCHO peptide **7** and amide peptide **8** both possessing (5)-6-carb-



Scheme 3. Reactivity of hydroxylamine-supported silicon wafers.

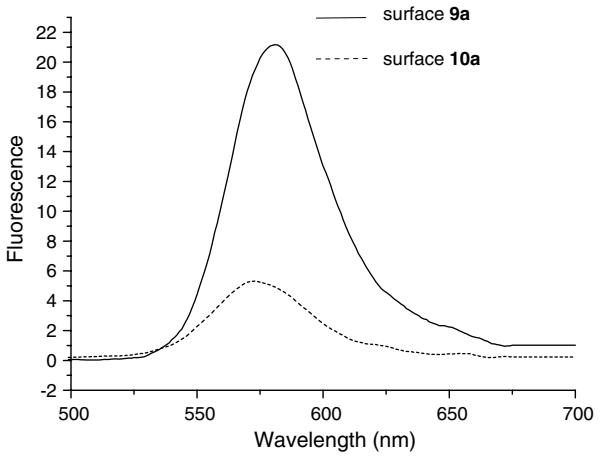


Figure 2. Fluorescence analysis of surface **9a** and **10a** ($\lambda_{ex} = 400$ nm).

oxytetramethylrhodamine were synthesised to investigate the reactivity of semicarbazide glass slide.[2b] These peptides were used in our study to determine the accessibility and reactivity of hydroxylamine surfaces towards biomolecules (Scheme 3).

Peptide **7** should bind covalently to the hydroxylamine-supported wafers through the α-oxo oxime linkage whereas peptide **8** should be adsorbed at the surface through non-covalent interactions. Thermically oxidised silicon wafers were functionalised as above with compounds **3a** and **3b** as for **4** to give surfaces **6a** and **6b**. Both surfaces were reacted with peptides **7** and **8**, washed following the method described with semicarbazide glass slide.[2b] The results of the fluorescence were similar for surfaces **9a** and **9b** and for surfaces **10a** and **10b**. The fluorescence of surfaces **9a** and **10a** is reported in Figure 2. The intensity of fluorescence with peptide **7** was 5-fold higher than with peptide **8**. These experiments demonstrate the covalent attachment of COCHO peptide whereas peptide **8** was only adsorbed at the surface through non-covalent links.

In conclusion, trialkoxysilanes possessing an unprotected hydroxylamine group were successfully synthesised at a preparative scale. These coupling agents were efficient for the functionalisation of oxidised silicon wafers with a high-density monolayer. The accessibility of the hydroxylamine group at the surface was demonstrated and the surface proved useful for the covalent immobilisation of peptides. Work is in progress to develop this methodology for biochips applications.

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References and notes

1. New focus: (a) Service, Robert F. *Science* **2001**, *294*, 2080–2082; (b) Zhu, H.; Bilgin, M.; Bangham, R.; Hall, D.; Casamyor, A.; Bertone, P.; Lan, N.; Jansen, R.; Bidlingmaier, S.; Houfek, T.; Mitchell, T.; Miller, P.; Dean, R. A.; Gerstein, M.; Snyder, M. *Science* **2001**, *293*, 2101–2105.
2. (a) Nonglaton, G.; Benitez, I. O.; Guisle, I.; Pipelier, M.; Léger, J.; Dubreuil, D.; Tellier, C.; Talham, D. R.; Bujoli, B. *J. Am. Chem. Soc.* **2004**, *126*, 1497–1502; (b) Olivier, C.; Hot, D.; Huot, L.; Ollivier, N.; El-Mahdi, O.; Gouyette, C.; Huynh-Dinh, T.; Gras-Masse, H.; Lemoine, Y.; Melnyk, O. *Bioconjugate Chem.* **2003**, *14*, 430–439; (c) Qian, W.; Yao, D.; Xu, B.; Yu, F.; Lu, Z.; Knoll, W. *Chem. Mater.* **1999**, *11*, 1399–1401; (d) Houseman, B. T.; Gavalt, S. E.; Mrksich, M. *Langmuir* **2003**, *19*, 1522–1531.
3. (a) Shao, J.; Tam, J. P. *J. Am. Chem. Soc.* **1995**, *117*, 3893–3899; (b) Forget, D.; Boturyn, D.; Defrancq, E.; Lhomme, J.; Dumy, P. *Chem. Eur. J.* **2001**, *7*, 3976–3984; (c) Boturyn, D.; Coll, J. L.; Garanger, E.; Favrot, M. C.; Dumy, P. *J. Am. Chem. Soc.* **2004**, *126*, 5730–5739.
4. (a) Falsey, J. R.; Renil, M.; Park, S.; Li, S.; Lam, K. S. *Bioconjugate Chem.* **2001**, *12*, 346–353; (b) Xu, Q.; Lam, K. S. *Tetrahedron Lett.* **2002**, *43*, 4435–4437.
5. (a) Kar, S.; Joly, P.; Granier, M.; Melnyk, O.; Durand, J. O. *Eur. J. Org. Chem.* **2003**, 4132–4139; (b) Joly, P.; Ardès-Guisot, N.; Kar, S.; Granier, M.; Durand, J.-O.; Melnyk, O. *Eur. J. Org. Chem.* **2005**, 2473–2480.
6. (a) Ulman, A. *Chem. Rev.* **1996**, *96*, 1533–1554; (b) *Organic Thin Films and Surfaces:Directions for the Nineties*; Ulman, A., Ed.; Academic Press: San Diego, CA, 1995.
7. Granier, M.; Lanneau, G. F.; Moineau, J.; Girard, P.; Ramonda, M. *Langmuir* **2003**, *19*, 2691–2695.
8. Balachander, N.; Sukenik, C. N. *Langmuir* **1990**, *11*, 1621–1627.
9. Lambacher, A.; Fromherz, P. *Appl. Phys. A* **1996**, *63*, 207–216.