Interfacing Porous Silicon with Biomolecules

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Abstract

The control of protein binding into nanostructured porous surfaces is highly relevant to the development of advanced biosensors and other biodevices. Here, an investigation of the covalent immobilisation of a model protein (albumin) onto porous silicon (pSi) films was conducted using a new alkene linker, the synthesis of which was developed. This alkene linker contained both hydrophobic and hydrophilic (oligoethylene glycol) sections and terminated in a protected thiol. The alkene was attached to freshly etched porous silicon via thermal hydrosilylation, where further surface reactions resulted in the attachment of a maleimido N-hydroxysuccinimidyl (NHS) heterobifunctional crosslinker. Albumin was then covalently immobilised on the porous silicon layer through reaction of the protein’s amine groups and the NHS functional group of the crosslinker. Surface modification reactions were monitored by infrared spectroscopy and interferometric reflectance spectroscopy. Protein binding was monitored by infrared spectroscopy, fluorescence imaging and atomic force microscopy.

Keywords: Porous silicon, biointerfaces, hydrosilylation, oligoethylene glycol, protein immobilisation, biosensors

1. INTRODUCTION

The ability to manipulate and control the binding of biomolecules into porous silicon (pSi) structures is highly desirable for the design of biosensors, biomaterials and other biodevices such as MEMS based on this nanostructured material. Porous silicon is a highly versatile material, suited for these types of applications. Pore characteristics, including pore depth and diameter are highly tunable, from micropores (<5nm), mesopores (5 – 50nm) and macropores (>50nm)[1]. The high surface area of porous silicon is also a desirable trait, which allows the structure to be loaded with various biomolecules[2]. Porous silicon surfaces can be functionalised with a range of different organic compounds. This functionalisation is commonly performed via silanisation or hydrosilylation[3]. These functionalisation techniques are attached via Si-O-Si linkages, in the case of silanisation and via Si-C-R linkages, for hydrosilylations. Various chemical functionalities have been incorporated into pSi using these techniques, including aliphatic and aromatic organic compounds as well as alcohols, esters and amines[4]. The ability to control these functionalisations and perform surface reactions in a stepwise fashion to incorporate biomolecules into the pSi structure will be investigated here. Step by step functionalisation of pSi structures provides a high degree of control over biomolecule conjugation, a highly desirable trait for pSi based biosensors and biodevices.

The stability of pSi structures functionalised by these techniques has previously been investigated, and the literature results indicate that pSi surface functionalised to incorporate Si-C bonds are more stable in aqueous media than those with Si-O functionality[5, 6]. Stability in aqueous media is an essential requirement for pSi based biosensors. Furthermore, biomolecule conjugation and biosensing reactions are generally carried out in aqueous medium[7]. It is anticipated that by assembling a monolayer of oligoethylene glycol appended alkenes, capped with protected nucleophiles on the pSi surface, a stable interface suitable for surface modification and biomolecule conjugation can be produced. Oligoethylene glycol moieties have been shown to minimise the effect of non-specific protein adsorption to surfaces, allowing low-fouling surfaces to be generated, thus affording more control over surface functionalisation[8, 9].

The alkene compound described here is terminated in a protected thiol, allowing the generation of a reactive nucleophile on demand, once the alkene has been attached to the pSi surface. The use of a protecting group also avoids side reactions like direct reaction of the thiol with the pSi surface and oxidation reactions, like intermolecular disulfide bond formation[10]. A thioacetate protected thiol is chosen over other bulky protecting groups in an attempt to minimise the effect of steric hindrance in the formation of uniform self assembled monolayer on the pSi surface. Deprotection of this thioacetate by LiAlH₄ affords a reactive thiol group, allowing the conjugation of a thiol reactive compound such as a
maleimide group. Here, a maleimide N-hydroxysuccinimidyl (NHS) heterobifunctional crosslinker reacted with the thiol giving an active ester terminated monolayer that can undergo further reactions with proteins.

Here, the biomolecule albumin is conjugated to the NHS-functionalised pSi surface through reaction of the NHS group and primary amine groups of the protein. This protein was chosen, as it is known to bind to surfaces through non-specific adsorption reactions. By using this ‘sticky’ protein, the effectiveness of the oligoethylene glycol moiety in the alkene compound to minimise non-specific protein adsorption can be effectively tested upon quenching of the NHS group.

2. EXPERIMENTAL METHODS

2.1 Materials:
All solvents for organic synthesis were purified following standard laboratory procedures. Anhydrous sodium metal and calcium hydride were used to dry the organic solvents used. Silicon wafers used were p-type, \(3 – 6\ \Omega\) resistivity, boron doped, \(<100>\) orientation, 381 ± 25 \(\mu\)m, supplied by Silicon Quest International. Other chemical, including \(\omega\)-undecylenyl alcohol, LiAlH\(_4\) and albumin were supplied by Sigma-Aldrich.

2.2 Analytical Methods:
All NMR spectra were recorded on Varian 200MHz NMR spectrometer using deuterochloroform (CDCl\(_3\)) as the solvent. All proton (\(H^1\)) and carbon (\(C^{13}\)) spectra were recorded using standard parameters, with chemical shifts (\(\delta\)) recorded in parts per million (ppm), referenced to deuterochloroform (CDCl\(_3\)), and coupling constants in hertz (Hz). The following abbreviations are used in proton NMR analysis: s = singlet, d = doublet, t = triplet, q = quartet, qui = quintet, m = multiplet, br = broad, J = coupling constant (Hz).

IR spectra were recorded on a Nicolet Avater 370MCT spectrometer (Thermo Electron Corporation). The spectrometer was fitted with a transmission accessory and all spectra were recorded and analysed using OMNIC version 7 software. Spectra were recorded over a range of \(500 – 4000\text{cm}^{-1}\), at a resolution of 2 \(\text{cm}^{-1}\) and taken as an average of 64 scans. All samples were blanked to a clean unetched/non-functionalised silicon wafer, of the same type as the samples.

All High Resolution Mass spectrometry (HRMS) samples were recorded by Sally Duck at Monash University, using a BioApex II 47e FTMS (Bruker) fitted with an Analytica Electrospray Source, run at a capillary voltage of approximately 100V.

Atomic force microscopy (AFM) images of porous silicon were acquired on a Nanoscope IV Multimode microscope (Veeco Corp.) operating in tapping mode using silicon tips (FESP, Digital instruments) with a resonance frequency of 50-70 kHz. Image processing was done using Nanoscope v5.32r3 software.

Fluorescence images were obtained using a Laborlux fluorescence microscope (Leitz), with a Nikon CCD camera and capture software. Images were obtained at 4X zoom, with a 15 second exposure time.

Interferometric reflectance spectroscopy investigations were performed on a custom built interferometer, with an Ocean Optics S2000 detector. Spectra were recorded and analysed using Igor Pro software version 4.

2.3 Porous Silicon Preparation
P-type (\(3 – 6\ \Omega\ \text{cm}\)) silicon wafers were cut into small pieces (1.5 – 2 cm\(^2\)) with a diamond cutter. Each piece was washed with ethanol, acetone and dichloromethane (DCM) and dried under a stream of nitrogen prior to etching. A clean piece of silicon was clamped into a Teflon etching cell and washed with ethanol, acetone and DCM and dried under a stream of nitrogen. The silicon wafer was etched, using a Keithley Source Meter as the current source. The following etching parameters were used: etching current 66mA, electrolyte 25% HF in EtOH, etching time 120 seconds.
2.4 Organic Synthesis

Synthesis of methanesulfonic acid undec-10-enyl ester (1)
Triethylamine (94.7mmol, 13.2mL) was added to a solution of undecylenyl alcohol (59.6mmol, 12mL) in DCM (250mL) under inert atmosphere (N2), with stirring at 0°C. Methanesulfonyl chloride (66.3mmol, 6.1mL) was then added dropwise over a period of five minutes with stirring. Stirring for an additional 24 hours allowed for completion of the reaction. The reaction mixture was transferred to a separating funnel and washed with ice water (2 × 100mL), followed by 10% HCl solution (2 × 100mL), sodium bicarbonate solution (2 × 100mL) and brine (2 × 100mL). The organic phase was subsequently dried over MgSO4 and concentrated by rotary evaporation to give a yellow oil, methanesulfonic acid undec-10-enyl ester, designated compound 1 (14.8g, 99%). 1H NMR (CDCl3) δ 5.909 – 5.705 (m, 1H), 5.032 – 4.889 (m, 2H), 3.745 – 3.550 (m, 12H), 3.445 (t, J = 7Hz, 2H), 2.650 (br, 1H), 2.040 (q, J = 7Hz, 2H) 1.574 (qui, J = 6.6Hz 2H), 1.271 (m, 12H); 13C NMR (CDCl3) δ 139.1, 114.0, 72.5, 71.5, 70.51, 70.49, 70.2, 69.9, 61.6, 33.7, 29.5, 29.4, 29.35, 29.3, 29.0, 28.8, 26.0.

Synthesis of 2-[2-(2-undec-10-enyloxy-ethoxy)-ethoxy]-ethanol (2)
To a stirred solution of NaH (66.54mmol, 2.665g of 60% suspension in mineral oil) in THF (200mL) under inert atmosphere (N2), triethylene glycol (333.57mmol, 44.52mL) was slowly added using a pressure equalising funnel. The mixture was stirred on ice for 30 minutes before heating to 80°C and stirring continually for a further two hours. After this time, the mesylate, compound 1 was slowly added over 30 minutes and the reaction was stirred at 80°C for an additional 12 hours. The reaction was cooled to room temperature and extracted with hexane (2 × 250mL). The organic solution was then washed with brine (2 × 50mL) and deionised water (2 × 50mL), dried over MgSO4 and concentrated by rotary evaporation to give the crude product (15.16g, 75.1%). The crude was purified by column chromatography (elution with 2:1 X4:ethyl acetate) and evaporation of the solvent gave the crude product thioacetic acid S-[2-[2-(2-undec-10-enyloxy-ethoxy)-ethyl] ester (compound 4), (5.97g, 94.5%). 1H NMR (CDCl3) δ 5.909 – 5.705 (m, 1H), 5.032 – 4.889 (m, 2H), 3.788 – 3.743 (m, 2H), 3.681 – 3.468 (m, 9H), 3.435 (t, J = 7Hz, 2H), 3.075 (s, 3H), 2.976 (t, J = 7Hz, 2H), 2.650 (br, 1H), 2.040 (q, J = 7Hz, 2H) 1.574 (qui, J = 6.6Hz 2H), 1.271 (m, 12H); 13C NMR (CDCl3) δ 139.1, 114.0, 72.5, 71.5, 70.51, 70.49, 70.2, 69.9, 61.6, 33.7, 29.5, 29.4, 29.35, 29.3, 29.0, 28.8, 26.0.

Synthesis of methanesulfonic acid 2-[2-(2-undec-10-enyloxy-ethoxy)-ethoxy]-ethyl ester (3)
Triethylamine (26.4mmol, 3.68mL) was added to a solution of compound 2 (16.5mmol, 5g) in DCM (100mL) under inert atmosphere, with stirring at 0°C. Methanesulfonyl chloride (18.3mmol, 1.42mL) was then added over 30 minutes before heating to 80°C and stirring continually for a further two hours. After this time, the mesylate, compound 1 was slowly added over 30 minutes and the reaction was stirred at 80°C for an additional 12 hours. The reaction was cooled to room temperature and extracted with hexane (2 × 250mL). The organic solution was then washed with brine (2 × 50mL) and deionised water (2 × 50mL), dried over MgSO4 and concentrated by rotary evaporation to give the crude product (15.6g, 77.1%). The crude was purified by column chromatography (elution with hexane/ethyl acetate 2:1) and evaporation of the solvent gave the product thioacetic acid S-[2-[2-(2-undec-10-enyloxy-ethoxy)-ethyl] ester (compound 4), (4.09g, 72.8%). 1H NMR (CDCl3) δ 5.903 – 5.701 (m, 1H), 5.015 – 4.895 (m, 2H), 3.642 – 3.562 (m, 12H), 3.440 (t, J = 6.6Hz, 2H), 3.085 (t, J = 6.6Hz, 2H), 2.327 (s, 3H), 2.036 (q, J = 6.6Hz, 2H), 1.566 (qui, J = 6.6Hz 2H), 1.269 (m, 13H); 13C NMR (CDCl3) δ 195.5, 139.2, 114.0, 71.5, 70.6, 70.5, 70.3, 70.0, 69.7, 33.7, 30.5, 30.4, 29.6, 29.5, 29.4, 29.37, 29.1, 28.9, 28.8. HRMS (ESI) calculated for C19H36O4S: 360.2329, found: 360.2328.
2.4 Surface Modification Procedures

Figure 1 shows an overview of the surface modification scheme, resulting in the attachment of the model biomolecule albumin to the pSi surface.

Neat hydrosilylation procedure
A freshly etched pSi surface was placed into a reaction vessel and the porous surface was covered with 60mg of neat alkene. The alkene was spread over the porous surface, taking care not to damage the porous structure. The reaction vessel was then sealed and nitrogen (that had been passed through a Dry Rite™ drying column) was constantly flushed through the system. The reaction vessel was lowered into an oil bath that was maintained at a constant temperature of 125°C and the hydrosilylation reaction was left for 3 hours. The wafer was removed and washed with DCM and dried under a stream of nitrogen. The functionalised wafer was stored in a desiccator (not under vacuum), for further use.

Solution hydrosilylation procedure
A freshly etched pSi surface was placed into a 0.2M solution of alkene in xylene, which had been degassed with nitrogen for 15 minutes. The reaction vessel was then sealed and flushed with nitrogen that had been passed through a Dry Rite™ drying column. The reaction vessel was lowered into an oil bath that was maintained at a constant temperature of 125°C and the hydrosilylation reaction was left for 3 hours. The wafer was removed and washed with xylene and DCM and dried under a stream of nitrogen. The functionalised wafer was stored in a desiccator (not under vacuum), for further use.

Deprotection of thioacetate
A porous silicon wafer, functionalised with the alkene compound 4, was immersed in a 0.1M solution of LiAlH₄ in THF. The reaction vessel was sealed and nitrogen was constantly flushed through the system. The reaction was left for 30 minutes at room temperature with occasional agitation, after which the wafer was removed and washed with THF, 1M HCl(aq), ethanol and DCM, then dried under a stream of nitrogen. The wafer was immediately used for further functionalisation procedures.

Conjugation of β-maleimido propionic acid N-hydroxy succinimide ester crosslinker to pSi surface
A porous silicon wafer, functionalised with the alkene compound 4 and deprotected to afford reactive thiol (-SH) functionality, was clamped into a Teflon reaction cell. 1mL of a 3.76mM solution of crosslinker in a 1:1 DMF:H₂O mixture (1mg.mL⁻¹), was added into the reaction cell and left to react with the thiol groups for 3 hours with constant agitation at room temperature. The wafer was then washed with MilliQ water, ethanol and DCM and dried under a stream of nitrogen.

Albumin attachment to crosslinker
A porous silicon wafer functionalised with the alkene compound 4 and β-maleimido propionic acid NHS ester crosslinker, was clamped into a Teflon reaction cell. 1mL of a solution of 4mg.mL⁻¹ of albumin in phosphate buffer
saline (PBS) was added to the reaction cell and allowed to react for two hours with constant agitation at room temperature. After protein conjugation, the wafer was washed with MilliQ water and dried under a stream of nitrogen.

**Fluorescein-5-isothiocyanate (FITC) labelled albumin attachment to crosslinker**

A porous silicon wafer functionalised with compound 4 and β-maleimido propionic acid NHS crosslinker, was clamped into a Teflon reaction cell. 1mL of a solution of 250µg mL⁻¹ of FITC-albumin in PBS was added to the reaction cell and allowed to react for two hours with constant agitation at room temperature. After protein bioconjugation, the wafer was washed with MilliQ water and dried under a stream of nitrogen.

**Quenching of NHS ester functionalised pSi with ethanolamine**

A porous silicon wafer functionalised with compound 4 and β-maleimido propionic acid NHS crosslinker, was immersed in a 0.1M solution of ethanolamine in water for thirty minutes with occasional agitation at room temperature. After reaction, the wafer was washed with water, ethanol and DCM and dried under a stream of nitrogen. For the fluorescence investigation, this control sample was allowed to react with the FITC-ALBUMIN under the same conditions as for FITC-ALBUMIN conjugation to the crosslinker functionalised surface.

### 3. RESULTS AND DISCUSSION

#### 3.1 Characterisation of the Porous Silicon Surface

Porous silicon samples were prepared according to the etching conditions described in the experimental methods, with figure 1 displaying the IR spectrum for a freshly etched sample. Three different Si-H stretching vibrations as well as the vibration corresponding to SiH₂ scissoring appear in the spectrum in figure 1, with SiH₃ stretching at 2140cm⁻¹, SiH₂ at 2120cm⁻¹ and SiH at 2091cm⁻¹[11]. The spectrum also shows a small amount of oxidation, by the peaks at 1075cm⁻¹ that corresponds to Si-O stretching vibrations[12]. The spectrum in figure 1 can be used as a reference to estimate the extent of surface coverage by alkenes, in the hydrosilylation reactions.

![IR spectrum of freshly etched, hydride terminated porous silicon.](image)

Fig. 1. IR spectrum of freshly etched, hydride terminated porous silicon.

The freshly etched porous silicon surfaces were also characterised by atomic force microscopy (AFM). Figure 2 shows the AFM image of the freshly etched porous silicon sample etched at 66mA for 2 minutes in 25% HF/EtOH solution. Image analysis revealed pore sizes of 10 – 20nm. Pores of this size (termed meso pores) are frequently used for biosensor and biomaterial applications[13]. The same etching conditions were hence used to prepare porous silicon samples for the subsequent surface functionalisation investigations.
3.2 Organic Synthesis

The oligoethylene glycol appended, thioacetate capped alkene was synthesised from the starting material \(\omega\)-undecylenyl alcohol. This alcohol was first converted to a mesylate (compound 2) following a procedure modified from that used by Crossland et al.\cite{14}. This mesylate group was incorporated to act as a good leaving group for the following substitution reaction, where triethylene glycol was conjugated to the alkene. The procedure from Lee et al.\cite{15} was adapted for this oligoethylene glycol conjugation. The resulting alcohol, compound 2 was again converted to a mesylate and used in the conjugation of thioacetic acid, to generate the protected thiol functional group.

3.3 Surface Modification Reactions

The first step in the surface functionalisation process was to attach the thioacetate terminated alkene (compound 4) to the pSi surface via thermal hydrosilylation. A number of different reaction methods were trialled in an attempt to optimise the alkene coverage on the surface, while minimising the amount of surface oxidation. Hydrosilylation in solution was tested, in an attempt to minimise alkene usage during the reaction. A 0.2M solution of the alkene in xylene was tested, with the reaction heated to 125°C. This solution hydrosilylation however showed signs of significant oxidation to the porous surface, an unwanted side reaction. Figure 3 shows the IR spectra for such a hydrosilylation reaction, where a large peak at 1100cm\(^{-1}\) is observed. This peak is attributed to Si-O stretching vibrations, a common sign of oxidation to the pSi surface\cite{16}. Admittedly, C-O stretching of the oligoethylene glycol section of the alkene might also contribute to this peak.
In an attempt to minimise this surface oxidation, neat hydrosilylation reactions were carried out using the thioacetate-terminated alkene. The procedure for these neat hydrosilylations is described in the methods section and the IR spectra after surface functionalisation by this technique is shown in figure 4. It can be seen that the amount of surface oxidation has decreased for this technique, with a much smaller Si-O stretching peak at 1100 cm\(^{-1}\). This peak at 1100 cm\(^{-1}\) also corresponds to the C-O stretching of the oligoethylene glycol section in the alkene molecule, suggesting that the amount of surface oxidation was minimal. The aliphatic C-H stretching peaks at 2900 cm\(^{-1}\) and the carbonyl stretching peak at 1700 cm\(^{-1}\) demonstrate that the alkene has been successfully attached to the pSi surface. There is an intense Si-H stretching peak in the IR spectra in figure 4, which indicates that the surface reaction did not go to completion. Some amount of Si-H stretching is expected and is commonly observed in hydrosilylation reactions. Due to the small amount of surface oxidation, this method of hydrosilylation was chosen over solution hydrosilylation for all further surface modifications and protein bioconjugation investigations.

Along with IR characterisation, the hydrosilylation reactions were analysed by interferometric reflectance spectroscopy. This technique involves calculating the effective optical thickness (EOT) of the pSi surface before and after the surface functionalisation. Figure 5 shows the Fabry-Perot fringe pattern produced by the pSi surface before and after reaction with compound 4 along with the EOT values. The EOT increased after the alkene was bound into the pSi structure, which is the expected result due to the fact that the refractive index of the surface will increase as air in the pores is
replaced by the organic compound[17]. This analysis confirms and backs up the IR results for the neat hydrosilylations, proving that the alkene has been incorporated into the porous silicon structure.

![Graph showing Fabry-Perot fringe pattern and EOT values for pSi before and after hydrosilylation of compound 4.]

3.3 Surface Modification Reactions

After hydrosilylation of the thioacetate alkene to the pSi surface, further surface modification reactions were performed, first to incorporate the crosslinker β-maleimido propionic acid NHS ester and second the covalent attachment of the biomolecule albumin. The initial step in these surface modification reactions was to remove the acetyl protecting group of the thioacetate to generate a free thiol group. LiAlH₄ had successfully been used previously for the deprotection of thioacetate groups in solution, however deprotection of a thioacetate conjugated to a pSi surface had not been attempted to the best of our knowledge[16]. LiAlH₄ deprotection was performed under a nitrogen atmosphere using a 0.1M solution of LiAlH₄ in THF. IR analysis confirmed the complete removal of the acetyl group, by the complete absence of the carbonyl vibrations in the spectrum. After the washing steps, a pristine and homogenous porous silicon surface was obtained. Figure 6 shows the IR spectra of the pSi surface before and after deprotection of the thioacetate, with the only difference between the two being the disappearance of the carbonyl stretching peak at 1700cm⁻¹. The disappearance of this peak confirms that the deprotection reaction had gone to completion.

Following the deprotection of the thioacetate and the generation of a reactive thiol group, the heterobifunctional crosslinker β-maleimido propionic acid NHS ester was conjugated to the alkene linker. This conjugation followed a simple Michael Addition mechanism, where the thiol reacted with the maleimide functional end of the crosslinker. This conjugation procedure was carried out following a modified procedure of that used by Reynolds et. al.[17].

The final step in the surface functionalisation process was to attach albumin (in this case bovine serum albumin), to the functionalised porous silicon. Albumin was chosen as a model protein for this system, because it is a notoriously “sticky” protein that readily adsorbs to surfaces. The main reason for incorporating the triethylene glycol section into the alkene molecules was to minimise this non-specific binding of albumin, whilst the terminal NHS ester on the linker would provide a site for bioconjugation to proteins like albumin. Albumin attachment occurs through the formation of an amide bond, via reaction of the NHS ester of the crosslinker and primary amine groups of the protein.

The albumin functionalised porous silicon was analysed by IR and the results are shown in figure 6. The difference in the spectra between the crosslinker and albumin functionalised porous silicon suggests that protein attachment has occurred. The main peaks of interest in the overlayed spectra in figure 6 are the carbonyl peaks at approximately 1700cm⁻¹. These peaks can be used to follow the surface reactions, from the thioacetate to the thiol and the crosslinker conjugated surface.
The group of peaks between 1700cm\(^{-1}\) and 1800cm\(^{-1}\) in the crosslinker conjugated surface’s IR spectrum arise from the symmetric and asymmetric stretching of the succinimidyl (1710 – 1740cm\(^{-1}\)) and maleimide’s (1790 – 1830cm\(^{-1}\)) carbonyl groups[18]. The appearance of this group of peaks changes for the protein coated surface, and only two carbonyl stretching peaks are seen. The larger one of these peaks (1707cm\(^{-1}\)), corresponds to the carbonyl stretching of the amide bond that is formed after protein conjugation, with the shoulder of this peak corresponding to the N-H bending vibration. The other peak at (1785cm\(^{-1}\)) can be assigned to the carbonyls of the maleimide group, which are still present in the structure after protein conjugation. This result indicates that protein bioconjugation had been successful, however further analysis was required to confirm this fact.

Fig. 6. Overlayed IR spectra for the different surface modifications. Insert, enlarged carbonyl region of spectra.

### 3.4 Fluorescence Measurements

To corroborate the results of the IR spectroscopy and confirm the attachment of the protein, fluorescently labelled albumin was conjugated to the functionalised surface using the same protocol to the one used in the last section. Fluorescein-5-isothiocyanate (FITC) labelled albumin was used as the fluorescently labelled protein, which was allowed to react with a crosslinker functionalised porous silicon surface along with a control surface. The control surface was prepared from a crosslinker functionalised surface, to which ethanolamine was added to quench all of the crosslinker’s active NHS groups and which was then exposed to FITC-albumin using the same conditions as for the non-quenched surface. By using this control sample, the effect of non-specific binding of the albumin could be investigated.

Figure 7 shows the relative fluorescence intensity of each sample along with the relative fluorescence for a freshly etched non-functionalised pSi surface. The protein functionalised surface has a significantly greater fluorescence then the pSi surface without protein (freshly etched pSi was used here) confirming the attachment of the FITC-albumin.

The fluorescence intensity of the control surface is higher than for a pSi surface not incubated with protein but is low compared to that of the sample. This result shows that there is barely any protein attached to the control surface. We attribute this result to the oligoethylene glycol section in the linker and the ability of the oligoethylene glycol layer to minimise non-specific protein adsorption. These results demonstrate both the effectiveness of the alkene/crosslinker
approach to facilitate protein conjugation and the ability of the oligoethylene to minimise non-specific protein adsorption.

![Graph showing relative fluorescence intensities.](image.png)

Fig. 7. Relative fluorescence intensities of FITC-albumin conjugated surface control surface, ethanolamine quenched crosslinker after FITC-albumin incubation and a freshly etched pSi surface (relative fluorescence value of 0.1).

### 3.5 AFM Imaging of Functionalised pSi Surfaces

Atomic force microscopy imaging of the functionalised pSi structure confirmed the presence of albumin on the surface. The white/lighter areas of the image in figure 8 correspond to individual protein structures on top of the pSi layer. Height analysis gave an average protein height of 7.30nm. From this image, it is unclear how much of the protein is immobilised in the pores. This is difficult to achieve by AFM because AFM is only sensitive to the very surface of a sample and because of AFM tip convolution effects which limit the lateral resolution.

![AFM image of protein functionalised pSi.](image.png)

Fig.8. Tapping mode AFM image of protein functionalised pSi (dimensions 1µm × 1µm). Arrow indicating a single protein molecule.
4. CONCLUSIONS

An alkene linker containing both hydrophobic (alkane) and hydrophilic (oligoethylene glycol) sections, capped by a protected thiol has been successfully synthesised and characterised. The attachment of this alkene to pSi surfaces by thermal hydrosilylation has been carried out and thoroughly characterised. The covalently bound monolayer was further modified by thiol deprotection and conjugation of a heterobifunctional crosslinker which enabled attachment of albumin. These surface modification steps were followed by IR spectroscopy, fluorescence and atomic force microscopy analysis. This investigation has shown the effectiveness of oligoethylene glycol moieties to minimise non-specific protein adsorption and how a protected thiol can be used as a starting point for biomolecule incorporation into pSi structures.

The alkene/crosslinker setup described in this investigation has potential application for use in highly specific biosensors or biodevices where greater controls over surface reactions are required. The low-fouling properties of this biomolecule interface setup may allow for more selective and interference-resistant biosensors to be developed.

REFERENCES