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# New method for attachment of biomolecules to porous silicon†

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**Biomolecules have been attached to porous silicon by a new linking method that forms a direct Si–C bond on the surface and retains the photoluminescence of the porous silicon.**

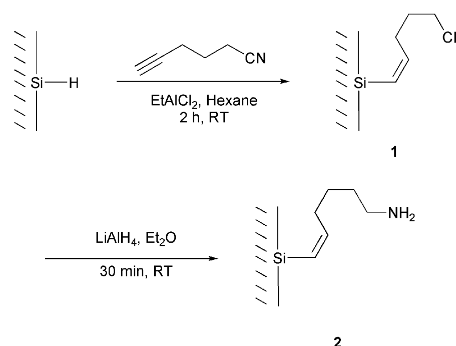
Porous silicon (PSi) has been employed for detection of chemical substances.[1] Devices made from this material have utilized changes in photoluminescence (PL)[2] or refractive index[3] caused by the presence of target analytes as transduction modes. The specificity of the interaction that causes these changes can depend upon the surface of the silicon, which can be modified through the Si–H surface sites. Our objective has been to enhance this selectivity by modifying the surface with biomolecules such as enzymes.[4] Our approach involves covalently modifying the hydride-terminated surface of porous silicon with an organic linker. This linker can then be used to covalently attach various molecules to the surface including small molecules such as fluorescent probes and large biomolecules such as proteins and enzymes.

Because of the heightened interest in sensor development for anti-terrorist activities, a particularly active area is the utilization of porous silicon as a platform for detection because of its photoluminescence properties. In the development of the site for molecular recognition, a critical step is connecting the receptor site to the porous silicon without destroying the photoluminescence. This work shows an alternate method to accomplishing this linking, replacing more laborious methods that generally cause more degradation of the photoluminescence.

Surface modification of PSi is achieved by Lewis acid catalyzed hydrosilylation with nitrile terminated alkynes, followed by reduction with $LiAlH_4$, thereby providing reactive functionality.[5–7] Conventional protein cross-linker chemistry is then used to extend the linker and provides for the attachment of various molecules. Steptavidin has been chosen to demonstrate the effectiveness of this ligand system for biomolecule attachment. Characterization of these surfaces has been performed using IR and mass spectrometry techniques. In addition, examination of the PL spectra of the modified surfaces indicates that a high degree of photoluminescence is retained.

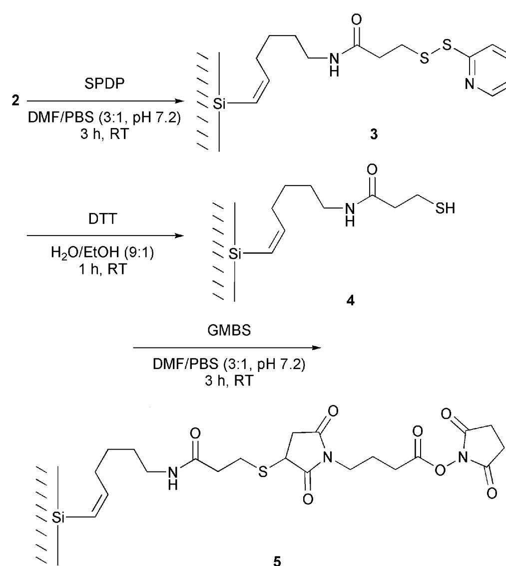
N-type bulk monocrystalline silicon was etched by standard techniques yielding photoluminescent PSi samples suitable for functionalization.[8] The Lewis acid catalyzed hydrosilylation of hydrogen terminated porous silicon was used to connect hex-5-ynenitrile to the surface (Scheme 1).[6] The surface-bound nitrile was then reduced to the 1° amine **2** using $LiAlH_4$.[7]

Heterobifunctional protein cross-linking reagents *N*-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) and *N*-(γ-maleimidobutyryloxy)succinimide (GMBS) were then used to bridge between the silicon surface and the molecules of interest (Scheme 2).[9] Amide formation between the NHS activated ester of SPDP and the surface amine in **2** was accomplished by exposing the surface to a solution of SPDP in 3 : 1 DMF–PBS. Reductive cleavage of the 2-pyridyldithio group in **3** was accomplished using dithiothreitol (DTT) to reveal a terminal thiol, **4**. This reaction releases 2-thiopyridone, which is then quantified by UV/Vis spectroscopy ($\lambda_{max}$ = 343 nm) to give the number of free thiols present on the surface (2.8 × $10^{10}$ mol $mm^{-2}$). These thiols were then reacted with the maleimide group of GMBS to provide the linker containing a terminal NHS activated ester, **5**.




**Scheme 1**

Dansyl cadaverine was coupled to the NHS activated ester of the linker (Scheme 3), producing the dansyl terminated linked surface, **6**. The fluorescence of the surface-bound fluorescent probe as well as the photoluminescence of the PSi was measured (Fig. 1). The fluorescence emission from the dansyl group was observed as well as the broad signal corresponding to the PSi photoluminescence indicating the dansyl probe was successfully attached to the surface of the PSi. This and the other surface modifications reduce photoluminescence to about one half the unfunctionalized PSi surface (see ESI†). After the first synthesis step, there is virtually no more change in the photoluminescence.




**Scheme 2**

† Electronic supplementary information available. Experimental section. See http://www.rsc.org/suppdata/cc/b2/b209453c/

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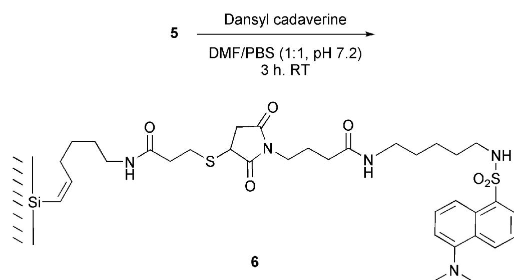



**Scheme 3**

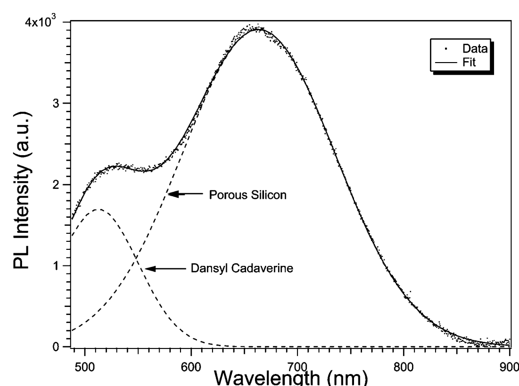
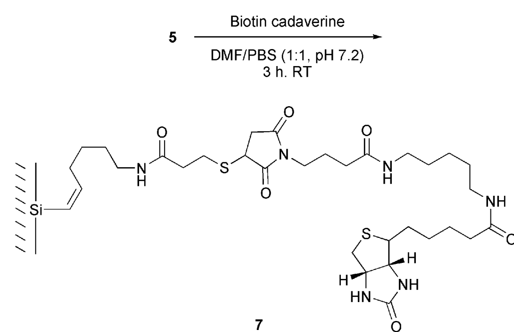



**Fig. 1** Fluorescence spectrum of dansyl terminated linkage, **6**. Circles represent the experimental data and the bold line represents the sum of two gaussians, one being the emission from dansyl cadaverine and the other one being the emission from PSi.

Biotin was also attached to the surface of the silicon through the NHS activated ester in **5** using an amino functionalized biotin derivative (Scheme 4), producing the biotin terminated linked surface **7**. The biotin was in turn used to bind Streptavidin to the PSi surface. The presence of Streptavidin was confirmed by performing a Trypsin digest of the protein directly on the surface of the modified PSi. The digest was then analyzed by MALDI-TOF MS to identify the peptide fragments.[10]

The presence of a primary amine on the surface of the PSi opens the door to a variety of options for linker design and construction. In the present work, we have focused on the use of the heterobifunctional protein cross-linking reagents SPDP and GMBS to bridge between the silicon surface and the molecules of interest. The presence of the activated ester provided by GMBS at the end of the linker provides an electrophilic platform, which is available to react with a variety of nucleophilic species. In the present study these attached species include small molecules such as Dansyl and Biotin derivatives as well as large biomolecules such as Streptavidin. However, potentially any molecule containing an appropriate nucleophilic moiety (*e.g.* 1° amine) could be attached to the PSi surface through this type of linker.




**Scheme 4**

The luminescence spectrum of the PSi with the dansyl group attached, and the MS verification of the Streptavidin attachment to a biotin modified surface, confirm the linkage can be used to attach a variety of species and the silicon retains relatively high levels of photoluminescence throughout the functionalization process.

Methods have been developed to link molecules to silicon surfaces through direct Si–C bonds (ref. 5, for example). In addition, approaches have been used in the literature to link biomolecules to silicon surfaces (ref. 11,12, for examples). This new linking system is less synthetically laborious and less deleterious to the photoluminescence of the PSi, capitalizing on an attachment method that forms the direct Si–C bond through Lewis acid catalyzed hydrosilylation. Traditional protein cross-linking chemistry is then used to form covalently bound systems available for binding a variety of molecules.

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