

Affinity mass spectrometry from a tailored porous silicon surface†

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The development of chemically stable porous silicon (pSi) materials for DIOS (Desorption/Ionization on Silicon) mass spectrometry, covalent linkers cleaved in the DIOS laser pulse, and efficient methods for bond formation to immobilized species, allows for on-chip affinity purification and mass detection.

The identification of chemical interactions in complex mixtures is an analytical challenge at the heart of mechanistic chemistry and biology. Mass spectrometry (MS) is a powerful tool for such purposes,¹ especially when the attachment of optical tags is inconvenient or overly perturbing to the system being studied. For MS analysis, a purification step is often required in which the desired interacting species, present at very low concentration, are separated from the other components of the mixture. A popular approach is to preconcentrate samples with cleanup devices, some of them commercially available.² An alternative is to “fish” for interactions of interest with probe molecules immobilized on a platform from which MS can be performed directly. A number of affinity enrichment methods of this type have been described for matrix-assisted laser desorption/ionization (MALDI) MS,³ and one for the desorption/ionization on silicon (DIOS) technique.⁴ We describe here a variation of the affinity mass spectrometry theme, enabled by the use of cleavable covalent linkages and highly active chemical and biochemical attachment reactions to an MS-supportive surface.

DIOS-MS employs MALDI instrumentation from a porous silicon (pSi) platform to analyze small molecules without the use of a matrix material.⁵ Porous silicon is a hydride-covered surface that is unstable in aqueous solutions with concomitant loss of DIOS activity. We have recently discovered that the lost DIOS function of oxidized pSi surfaces can be recovered by silylation of surface hydroxyl residues, giving materials impervious to oxidation and highly resistant toward hydrolysis.⁶ Glass, non-porous silicon, and related materials are not suitable, as both the pore morphology and light-absorbing properties of pSi are important to achieving DIOS-MS activity. It has further been observed that furan-maleimide Diels–Alder adducts undergo clean [4+2] cycloreversion during DIOS,⁷ and preliminary experiments confirmed that such linkers work on silylated oxidized pSi.⁸

Fig. 1 shows a sequence of transformations which highlights the ability of DIOS chips made with these features to support small-molecule MS in the presence of proteins.⁹ Silylated surface **1** was deprotected and treated with biotin-dithiopyridyl compound **2** to provide biotinylated chip **3**. Incubation in 10 μ M avidin followed by extensive washing with phosphate buffer (PB, pH 7.4) introduced a layer of protein to give **4**. Treatment with a mixture of biotinylated triazine **5** and two non-biotinylated peptides (ASTTTNYT, MW 858; MKRSRGPSRR, MW 1328) was expected to give selective adsorption of **5**. DIOS-MS after deposition of a mixture of the three species showed strong signals for each, but washing (3 : 1 PB : MeCN, then water) left only the biotinylated compound adsorbed as **6** and detected by retro-Diels–Alder (rDA)

detachment. DIOS chips lacking attached biotin showed none of these species after identical analyte deposition and washing procedures.

Triazine **5** has several functions. In addition to the cleavable Diels–Alder adduct unit linking the biotin arm to the rest of the structure, it bears a terminal alkyne for connection to any organic azide by the copper-catalyzed 1,3-dipolar cycloaddition reaction.¹⁰ Given that azides and alkynes are largely unreactive toward proteins, lipids, nucleic acids, and cofactors, the azide–alkyne linkage process is especially well suited to the highly reliable and selective formation of bonds in an environment rich in biomolecules.^{10b,11b,12} In addition, triazines are very well ionized in DIOS-MS, and so the unit imparts good sensitivity to whatever is attached to it. The last triazine arm in **5** can be used for a mass tag or an additional probe unit. The use of the well-ionized triazine spacer and cycloreversion cleavage gives rise to strong small-molecule signals in spite of the presence of much larger amounts of protein.

We established that the azide–alkyne cycloaddition process was able to capture sample azides with a biotinylated alkyne (each reagent at low micromolar concentration), with product detection by DIOS-MS from avidin-coated pSi surface **4**.⁸ Furthermore, MS signal intensity correlated with the relative concentration of the starting azides, suggesting that the triazole-forming reaction, avidin–biotin binding, and rDA cleavage all proceeded with equivalent (and presumably high) efficiencies. The importance of rDA detachment was examined by comparing signal intensities of **5** and a biotinylated analogue lacking the oxanorbornene (retro-Diels–Alder) linker, when deposited together on **4**.⁸ Although both compounds bore the same highly ionizable functional groups (triazine and pyridine), rDA-**5** was detected with approximately ten times the signal strength, revealing that the rDA process provides a much more efficient way than dissociation of the avidin–biotin complex to release biotinylated compounds from the protein-coated surface.

These techniques were combined to probe the omega class glutathione transferase (GST ω or GSTO1-1),¹³ as shown in Fig. 2.

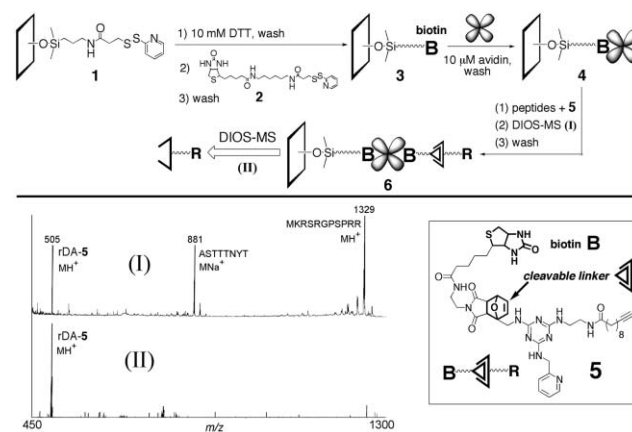


Fig. 1 (Top) Steps in the assembly of an avidin-coated DIOS chip and the selective binding and release of probe molecule **5**. (Bottom) Structure of **5** and DIOS-MS spectra before and after washing.

† Electronic supplementary information (ESI) available: synthesis and characterization of new compounds, experimental details. See <http://www.rsc.org/suppdata/cc/b4/b408200a/>

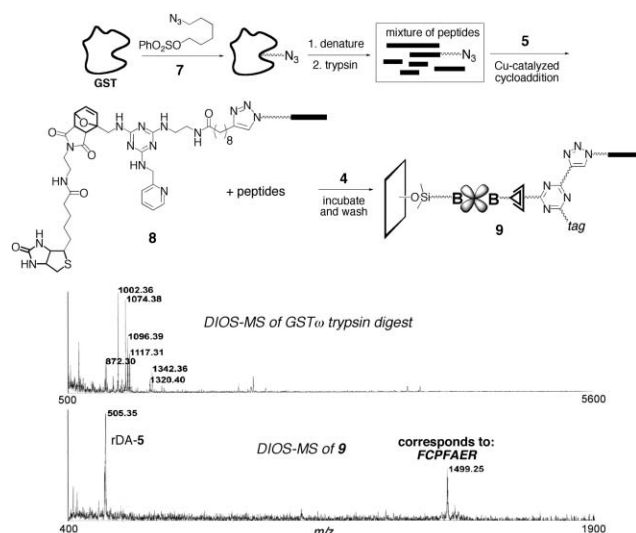


Fig. 2 Top: Scheme used for covalent labeling and identification of the active site sequence of GST ω . Bottom: Representative DIOS spectra.

The active site of GST ω contains a functionally important cysteine residue (Cys32), which suggests a mechanism of action distinct from that of other mammalian GST enzymes. Activity-based profiling of GST ω has been described by Cravatt and coworkers,¹¹ employing a phenylsulfonate ester electrophilic probe connected by a tether to a remote aliphatic azide (compound 7).

Reaction of the enzyme with 7 was followed by denaturation, trypsin digestion, and treatment with 50 μ M 5 in the presence of Cu^I. When the crude reaction mixture was deposited on avidin-coated 4 and then washed, DIOS-MS gave a dominant signal at m/z 1499.3, attributed to the rDA fragmentation product of 8 containing the peptide of interest. The only part of the GST ω sequence that fits this mass is F31-R37, which contains the active site Cys32 residue, presumably the site of reaction of 7. DIOS-MS and MALDI analysis of the trypsin digest of underivatized GST ω showed at best only a very small peak corresponding to this peptide fragment, which was difficult to distinguish from noise. The biotinylation, binding to surface avidin, washing, and rDA release process therefore accomplished an estimated enhancement in sensitivity of at least 100-fold by affinity purification on the DIOS chip. As a control, initial heat denaturation of the GST ω sample, which is known to abolish reactivity with 7,¹¹ gave a DIOS spectrum showing only the rDA-5 peak (m/z 505.4) after an otherwise identical set of manipulations.

We have shown here that silylated oxidized pSi supports the presence of protein without compromising DIOS activity¹⁴ and retro-Diels–Alder detachment of covalently linked units. Its chemical flexibility allows chemical (Cu-catalyzed azide–alkyne cycloaddition) and biochemical (avidin–biotin binding) interactions of high selectivity to be used for affinity purification on the DIOS chip. These techniques can provide great flexibility in designing affinity-based MS analyses for such applications as proteomic protein profiling¹⁵ and other chemically demanding assays.

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