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Desorption/Ionization on Silicon (DIOS)

1. Desorption/Ionization on Silicon (DIOS) Background

Since the 1970s, direct laser desorption/ionization (without a matrix) has been extensively studied on a variety of surfaces, yet it has not been widely implemented due to the rapid molecular degradation usually observed upon direct exposure to laser radiation. The utility of direct laser desorption/ionization for biomolecular analysis could be highly beneficial owing to the dramatically simplified sample preparation, the elimination of matrix background ions, and the potential for rapid analyses.

Given that the matrix used in matrix-assisted laser desorption/ionization (MALDI) (see this chapter (this volume): *UV Matrix-Assisted Laser Desorption Ionization: Principles, Instrumentation, and Applications*) serves to trap analyte molecules and to absorb UV radiation, desorption from nanoporous materials, such as polystyrene beads, can be considered as an alternative to desorption from a molecular matrix because the nanoporous materials can potentially mimic the features of the MALDI matrix. Although porous polystyrene does not exhibit the required properties, porous silicon is an effective medium for desorbing compounds and generating intact ions in the gas phase (*1*). The observed phenomenon is named DIOS, and a typical DIOS-MS setup is illustrated in Fig. 1.

The mechanism underlying the DIOS activity is not fully understood, but the nanostructured surface is of importance for trapping the analyte molecules (2–4). Furthermore, silicon surfaces effectively absorb UV light (*5*). The energy absorbed can subsequently be released through vibrational pathways (heat), causing analyte desorption from the surface. The porous surface might also lose energy through photoluminescent radiation, which potentially could result in reduced desorption efficiency. Rapid heating/vaporization of solvent molecules may further enhance the vaporization and ionization if analyte molecules are trapped in the porous silicon (*1*).

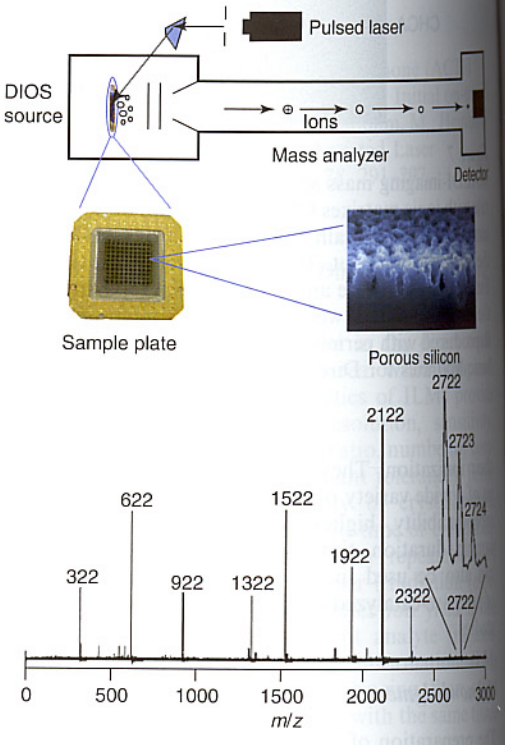


Figure 1
The DIOS surface is attached to a modified MALDI target and inserted in a standard MALDI-TOF mass spectrometer. The scanning electron microscopy picture shows typical morphology of the porous silicon. The mass spectrum is that of an Agilent calibration mixture for ESI-MS illustrating mass range and typical low background ion interference.

Optimal DIOS performance is typically obtained in the 0–3000 Da mass range, and a typical DIOS spectrum is shown in Fig. 1. DIOS demonstrates characteristics similar to MALDI (i.e., intact molecules are observed at the femtomole to attomole level with little or no fragmentation). More important is the absence of matrix material that allows the technique to be applied to small molecules without any interference from matrix ions. Although small-molecule analysis using MALDI is also possible, a matrix-free approach such as DIOS is simpler and more versatile for this purpose. In addition, in the absence of matrix dilution, samples of interest may be concentrated in spot sizes of less than 1 mm in diameter to improve the detection limit significantly.

Together, the following factors render DIOS a technique of particular interest:

- Small molecules and peptides can be analyzed directly with high sensitivity from the surface without any addition of matrix.
- Analyte signal is observed from both porous silicon hydride surfaces and porous silicon surfaces capped with covalently bound organic monolayers, especially fluorocarbon groups (*6*), suggesting that significant improvements can be made to DIOS-MS through further investigation of surface modifications. Significant potential also exists for the construction of chemical devices that employ combined sample preparation and direct desorption/ionization mass spectrometry for rapid and informative readout.
- Most existing MALDI mass spectrometers can be used to perform DIOS simply by changing the sample plate; no spectrometer modification is necessary.
- Silicon wafers are inexpensive and their conversion into porous silicon is relatively simple (Fig. 2), which provides the opportunity for disposable MS chips and reduces sample preparation efforts.

In the following sections, several aspects of chip preparation and practical utility of DIOS will be illustrated.

2. *Chip Preparation/Sample Application*

Micron-thick porous silicon is a photoluminescent semiconducting material with a high surface area (up to hundreds of m^2/cm^3), produced from crystalline silicon by a straightforward electrochemical etching process (Fig. 2) (*1,7–10*). Typically, *n*-type (phosphorus doped) silicon is etched under white-light illumination. Etching is accomplished by passing current in an electrochemical cell using an electrolyte with 25% aqueous HF in ethanol, in which the silicon wafer is the anode (anodization). This process produces a network of nanometer-scale

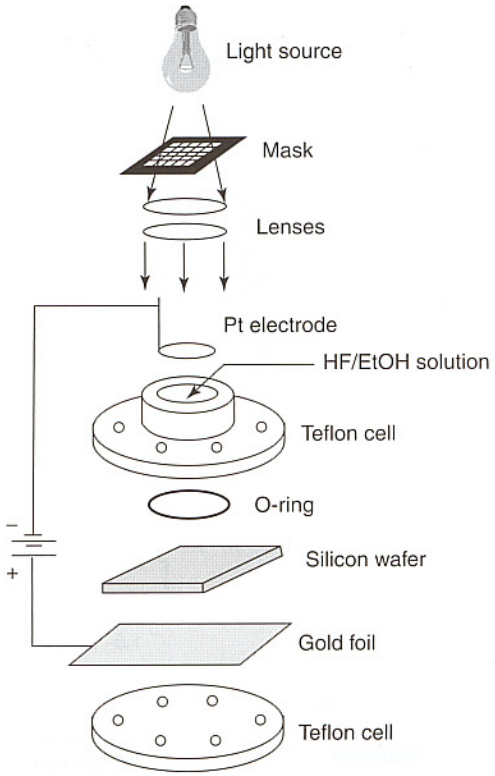


Figure 2
Design of a cell used for making porous silicon.

Si structures, principally cylindrical pores (*11,12*) in which surface silicon groups are terminated by Si–H bonds. Wafer properties such as crystalline structure, dopant type, and resistivity as well as etching conditions, HF concentration, etching duration, current density, and light exposure, are critical for the final porosity and subsequent desorption performance (*3,10,13*). Investigations of several of the variables in the etching procedure reveal the following results:

1. Porous silicon generated from heavily doped n-type silicon wafers (resistivity 0.008–0.05 Ω cm) at low etching current densities ($4\,mA\,cm^{-2}$) for short times (1–2 min) under moderate white-light intensity gives the best DIOS-MS results thus far. Scanning electron microscope (SEM) investigations of these surfaces reveal macrospaced pores, spaced ~ 100 nm apart. The pores have a diameter of 70–120 nm and a depth of up to 200 nm. The porous silicon samples appear to be fairly robust toward fracturing or peeling of the porous layer, perhaps because their porosities appear to be well below 50%. A material giving very similar DIOS results is also obtained without irradiation, using

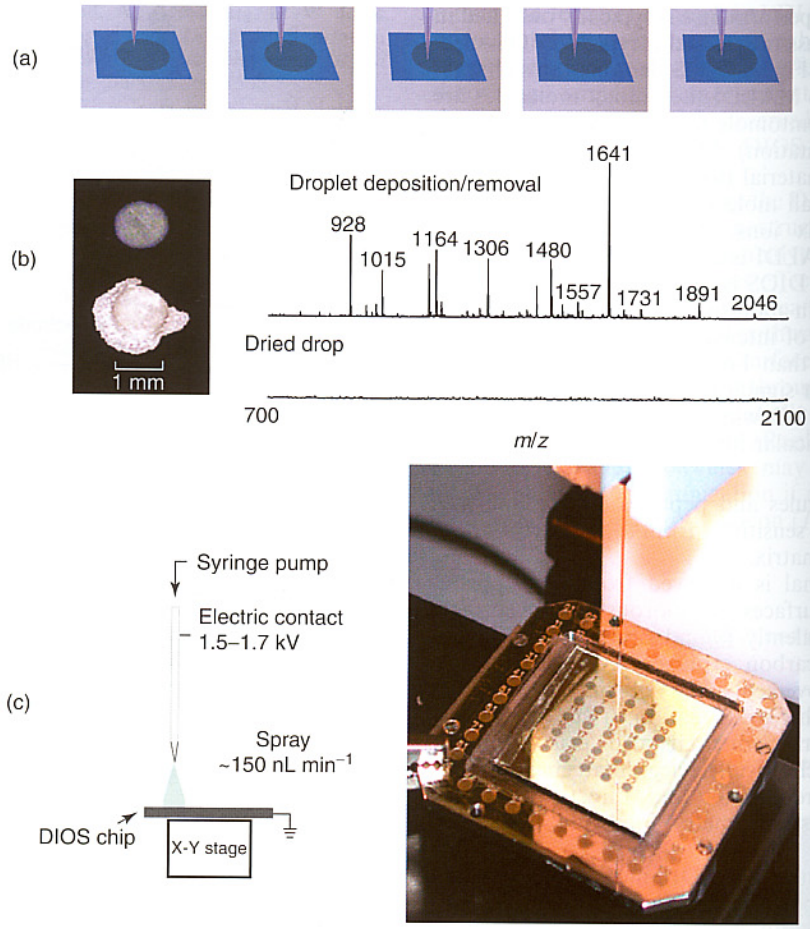


Figure 3
(a) Illustration of droplet deposition/removal (differential adsorption technique). (b) Picture and spectra of sample applied with differential adsorption (top) and dried droplet (bottom). (c) Electrospray deposition setup for DIOS sample application.

less heavily doped silicon wafers (0.5–2 Ω cm) etched at larger current densities (20 mA cm^{-2}) for slightly longer times (5 min).

2. Storage in air for extended periods of time, or brief exposure to ozone or aqueous hydrogen peroxide, results in oxidation of surface groups to oxide (Si–O–Si) and hydroxide (Si–OH) functions, which are characterized by IR spectroscopy and surface contact angles. In general, DIOS performance degrades with increasing surface oxidation, yet some hydrophilic compounds can be detected at higher sensitivity on lightly oxidized surfaces or surfaces derivatized with polar functional groups.
3. Although pore depth generally increases with increasing HF concentration in the etching solution, there is little effect on DIOS performance when the HF concentration is lowered (to 15%) or raised (to 35%) from the standard mixture (25%).
4. Similar performance in DIOS-MS analysis of standard peptide samples pertains to porous silicon prepared from both ⟨100⟩ and ⟨111⟩ silicon wafers using the same standard etching conditions (4 mA cm^{-2}, 1 min, with irradiation), suggesting that Si crystal orientation does not play an important role in DIOS performance.
5. DIOS surfaces may be photo-patterned by etching-n^+-type silicon with illumination through a mask. Note that the low current densities employed here facilitate the creation of sharp boundaries because hydrogen-bubble formation is minimized; such bubbles can stick to the surface and induce heterogeneity in both the lateral and vertical dimensions. A complementary type of photo-patterning is available (*14*) in the covalent derivatization of porous silicon following the etching step.

Several other fabrication methods can also be used for DIOS chip preparation. For example, wet-chemical etching with H_2O_2–metal–HF (*15*) or dry-chemical etching with reactive atom/ion plasma (*16*) can generate porous Si substrates. Porous structures have also been made into ordered arrays (*4*) or channels (*17*).

After etching, exposure to ozone through the use of an ozone generator, swiftly converts Si–H terminations into Si–OH, which subsequently can be modified using a variety of differently functionalized chlorosilane derivatization reagents. These modifications are done simply by applying 50–100 µL of the reagent in question onto the oxidized DIOS chip and incubating in an oven for 15–60 min.

The sample can be applied to the DIOS plate either with the standard "dried droplet" (Fig. 3a) technique or by a solid–liquid extraction (differential adsorption technique) (*6*). Using the latter technique, an aliquot of the sample is applied to the spot using a pipette. The solution is subsequently aspirated back to the pipette after "sitting" on the spot for a few seconds. This procedure can be repeated up to five times to maximize the loading (Fig. 3a). Analyte molecules are adsorbed onto the hydrophobic surface whereas salt or buffer molecules remain in solution and are drawn back into the pipette tip. The advantage with this method of applying sample is that the analysis becomes independent of buffer or salts used for sample preparation (Fig. 3b). Yet another strategy for sample application is electrospray deposition (*18*) (Fig. 3c). This creates a very homogeneous sample deposition, which is important for quantitative DIOS work and also provides a convenient way of coupling LC effluent to offline DIOS (*19*).

3. *DIOS for High Sensitivity and Fast Small Molecule Analysis*

A wide variety of compounds are readily detected by DIOS-MS with little or no fragmentation (Fig. 4).

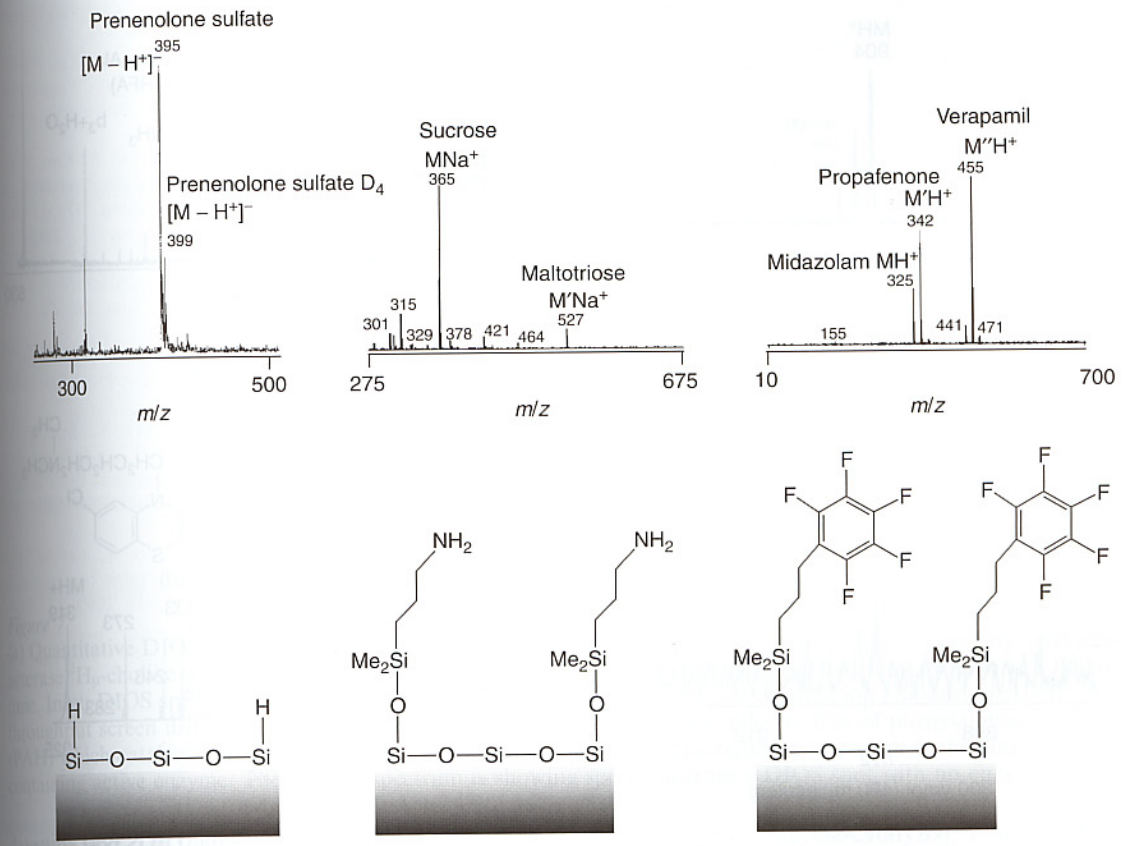


Figure 4
Example of different molecules analyzed using DIOS-MS (top) and the different surface modifications used for respective set of compounds (bottom).

Analysis can be performed in either the positive- or negative-ion mode for compounds that differ greatly in polarity and range in mass from 100 to 3000 Da. In general, it appears that those molecules with ionizable functionalities are effectively detected with both electrospray and DIOS. Recently a sensitivity record was set with the analysis of 800 yoctomol (800×10^{-24} mol), corresponding to 480 molecules detected using DIOS (Fig. 5) (*6*).

DIOS is also a good platform for addressing structural elucidation, employing both postsource decay (PSD) (*1,6*) and time-of-flight/time-of-flight (TOF-TOF) MS–MS techniques (*20*). Examples of DIOS-MS–MS data are illustrated in Fig. 6.

DIOS is also used for quantitative analysis using stable isotope-labeled standards (*18*). The dried-droplet method yielded satisfactory results, and by employing electrospray deposition even better reproducibility and linearity was achieved (*18*).

The use of DIOS in the detection of metabolites in both plant samples (*4*) and animal models has also been demonstrated (*21*). The applications of DIOS-MS are further expanded by the coupling of IR radiation (*22*) and atmosphere-pressure interface (*23*).

4. DIOS for Protein Identification and Functional Characterization

Optimal performance of DIOS-MS is typically obtained for molecules with a mass of less than 3000 Da. Consequently, analysis of large intact proteins is not feasible. However, DIOS-MS is suitable for protein identification through peptide mass fingerprinting. Very high-sequence coverages, up to 100%, can be obtained by combining LC with offline DIOS-MS

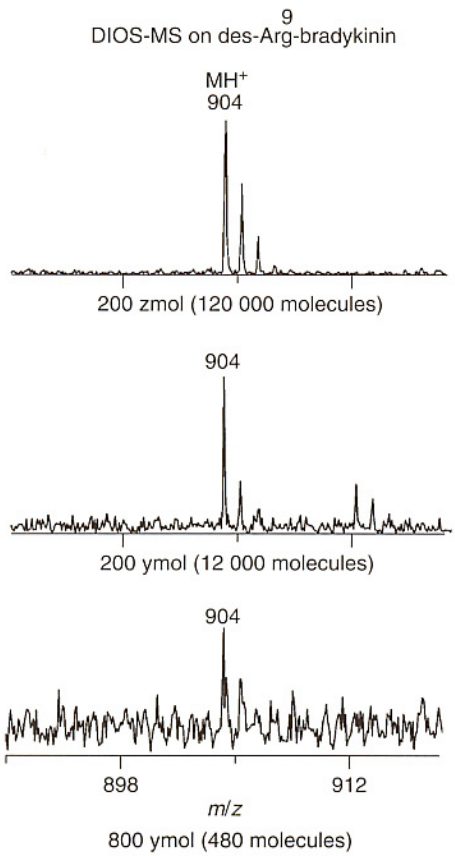


Figure 5
DIOS mass spectra obtained for 200 zmol, 20 zmol and 800 ymol for des-Arg^9-bradykinin using a perfluorophenyl silylated DIOS chip.

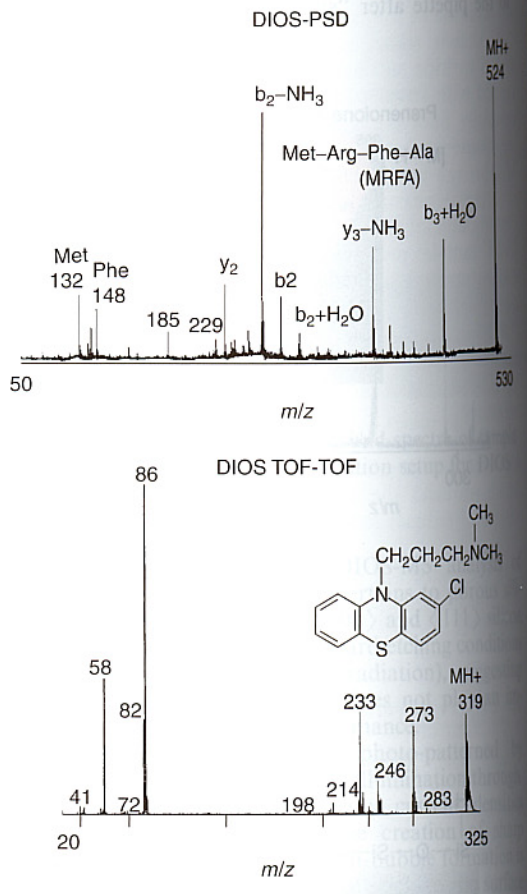
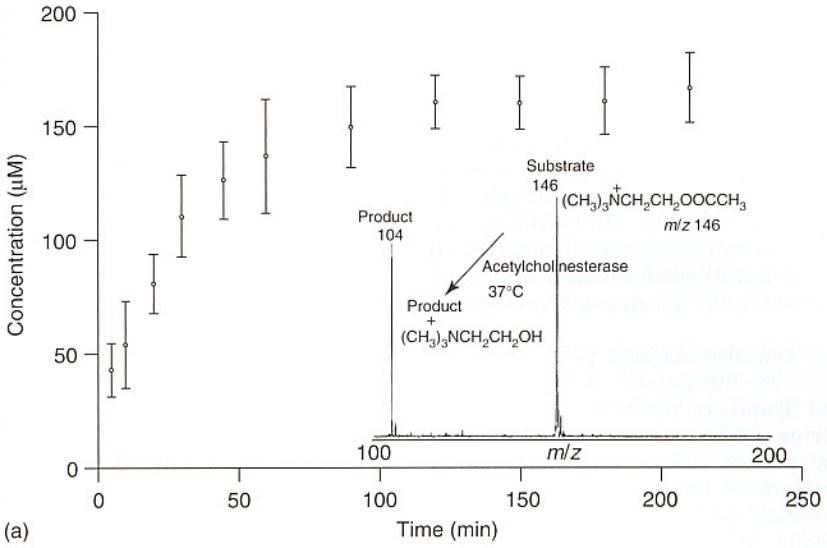


Figure 6
Examples of DIOS MS–MS data. DIOS post source decay (PSD) spectrum of peptide MRFA (MW 523.65) (top) and DIOS TOF-TOF spectrum of chlorpromazine (MW 318.1) (bottom).



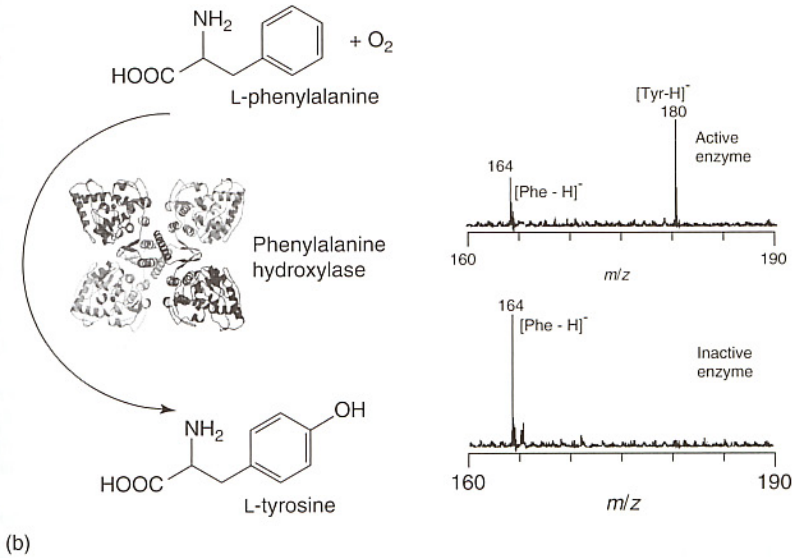


Figure 7
(a) Quantitative DIOS-MS analysis of the conversion of acetylcholine to choline at 25°C catalyzed by acetylcholine acterase $^2H_9$-choline was added as internal standard. The data follow the increase of product (choline) as a function of time. Inset: DIOS spectra showing substrate ion (acetylcholine, *m/z* 146) and product ion (choline, *m/z* 104). (b) High throughput screen using DIOS-MS for finding enzymes with properties similar to that of phenylalanine hydroxylase (PAH) which catalyzes the conversion of phenylalanine to tyrosine. The right top spectrum is showing a DIOS spot containing active enzyme. The bottom spectrum is showing spectrum from a DIOS spot with no enzyme activity.

detection (*19*). The plate format of a DIOS chip offers advantages in terms of integrated sample incubation/analysis and for protein characterization where many data points are necessary (e.g., kinetic analysis). For example, enzyme-catalyzed reactions can be monitored by incubating the catalyst and substrate directly on the porous silicon chip for a desired period, after which the mixtures are allowed to dry, and

the residues analyzed directly by DIOS-MS (*24*). The result of a kinetic investigation is illustrated in Fig. 7a. In a similar fashion, DIOS can be used for enzyme discovery in a high-throughput screen for finding enzymes with properties similar to those of phenylalanine hydroxylase (PAH) (*25*). The purpose of this work is to find a potential therapeutic drug for treatment of the inherited metabolic disorder phenylketouria (PKU). By employing DIOS-MS in the negative-ion mode in substrate/enzyme incubation on the chip, the conversion of phenylalanine into tyrosine by enzymes tested in the screen can be monitored (Fig. 7b).

The DIOS chip can also act as a probe with immobilized proteins on the porous surface. In this manner, potential ligands or molecules that interact with certain proteins can be screened (*26,27*), by simply exposing the porous silicon with immobilized proteins to a mixture of molecules. After washing with solvent to rinse off molecules that do not bind to the capture proteins, the plate is submitted to MS analysis to reveal any molecules that are retained on the plate.

5. *Perspectives*

DIOS is an effective MS tool with unique capabilities for chemical, biochemical, and protein characterization. In a chip-based format, it is capable of combined activity/analysis screenings and/or enrichment/analysis procedures. The well-documented silane chemistry opens the door to many options in targeted surface tailoring to increase sensitivity toward specific analytes. Recent improvements in DIOS surface modifications resulting in very low ($\sim 800 \times 10^{-24}$ mol) detection limits illustrate the capabilities of porous silicon as a versatile tool for MS analysis. Furthermore, DIOS offers a platform on which multiple experiments can be performed on a wide variety of molecules.

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Solvent-Free Matrix-Assisted Laser Desorption Ionization

1. *Introduction and Scope of Method*

"Solvent-free matrix-assisted laser desorption ionization (MALDI) mass spectrometry" was developed for the analysis of synthetic polymers (*1*) and organic macromolecules (*2*) with the aim of overcoming solubility restrictions in MALDI analysis. Thus far, only a few reports describe solvent-free MALDI-MS for the analysis of peptides and proteins (*3–6*). The technique generally consists of two steps: dry homogenization through mechanical mixing of sample and matrix, and transfer of the resulting powder to the MALDI plate. Parallel homogenization and transfer of the sample directly on-target, however, is also possible (*6*). In any case, at no point is solvent employed to mix the analyte and matrix or to transfer the sample onto the MALDI plate. If a sample in solution is to be analyzed, it is first dried to completeness prior to mixing with the matrix. Analysis by MALDI-MS is, therefore, simplified because fewer combinations and issues of compatibility or solubility need be considered.

2. *Application to Large Organic Compounds and Synthetic Polymers*

Generally, the solvent-free method allows for more homogeneous analyte/matrix mixtures, as well as higher shot-to-shot and sample-to-sample reproducibility than the solvent-based method (*4,7*). As a result, lower laser power may be used (*3,4,7*), affording milder MALDI conditions, less fragmentation (*7,8*), reduced background signals (*7,8*), and better mass resolution of the analyte signals (*4,7*). The quality of analytical results for simple polymer standards is generally improved when the solvent-free method is used (*4,7*). Most importantly, difficult samples (i.e., those that do not dissolve in common solvents or that readily oxidize or degrade) can often be prepared for MALDI analysis by the solvent-free methods and analyzed with good results (*9,10*). Even insoluble compounds such as large polycyclic aromatic hydrocarbons (PAHs) (*2*) (the largest at present being $C_{474}H_{132}$, 5826 Da, a nanosized molecular propeller (*11*)), poly(9,9-diphenyl-2,7-fluorene) (*8*), and carbonaceous pitches (*12*) are reliably characterized by using the solvent-free approach. Solvent-free MALDI-MS even expanded the capabilities to analyze molecules by atomic force microscopy (AFM) and scanning transmission microscopy (STM) (*10*), studies which were inaccessible earlier owing to the limited solubility and sublimation properties of the molecules. Briefly, these insoluble molecules can be successfully desorbed and ionized, fragmentation-free, employing solvent-free MALDI-MS. The ions are decelerated in the gas-phase, soft-landed on a plate producing a thin layer of self-organized molecules, and then analyzed by STM and AFM methods.

3. *Application to Biological Samples*

The solvent-free MALDI method can even be an improvement over the solvent-based MALDI procedure for analyzing some biological samples. This is especially true in the analysis of proteins and peptides in cases where the use of solvents creates difficulties as, for example, with hydrophobic and solubility-limited peptides (*13*) and membrane proteins (*14*) or where suppression effects (*5*) may be a problem. Significant advantages of solvent-free MALDI analysis are gained in comparison to traditional solvent-based