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Dear Sir,

Desorption/ionization on self-assembled monolayer surfaces (DIAMS)

In mass spectrometry, the laser desorption/ionization (LDI) method refers to an overall process by which the energy absorption of a laser beam by a localized region of an irradiated surface leads to the emission of gaseous charged particles. The most important steps involve the rapid dissipation of energy followed by the vaporization of the analyte, which acquires translational energy. Therefore, the direct irradiation of a sample, which induces a very rapid local heating and the absorption in the UV or IR region by the analyte, reduces the ionization efficiency and activates the dissociation of weak bonds during the energy transfer. This constitutes a limitation of the LDI technique, since only fragmented ions are detected even in the case of small-molecule analysis.

To circumvent this problem, Karas and Hillenkamp[1] and Tanaka[2] have developed the matrix assisted laser desorption/ionization (MALDI) technique. This method uses a photon-absorbing mediator, i.e. an aromatic matrix molecule, which is co-crystallized with the analyte. The analyte dispersion into the matrix crystals and the structure of the irradiated matrix crystal surface play a key role in the energy dissipation process.[3,4] That leads to the vaporization of the matrix and the release of the analyte molecules as ions in the gas phase. In the MALDI mass spectra, the detected charged species are either preformed ions that are directly desorbed, or vaporized neutral analytes undergoing gas-phase ionization by the matrix–analyte reaction.[5] The use of such 'softened' ablation/ionization processes has allowed MALDI to be considered as a powerful mass spectrometric method for biochemical analysis because of the detection of ions from high-molecular-weight molecules (M.W. > 5000 u).[6]

The UV irradiation of the matrix produces a wide range of ions, which lead to high-intensity background signals in the low-mass range. In addition, the sample preparation involving co-crystallization of the guest and a matrix (with a molar ratio of 1000–10 000) is a matter of trial and error since a good matrix for a particular sample must be found.

In order to overcome these limitations, free-matrix laser ablation/ionization methods have been proposed in the literature. The LDI on porous silicon (DIOS) technique was developed because of the structure of the surface, which in this case provides a scaffold for retaining solvent and analyte molecules, and the UV absorptivity of the substrate, which affords a conversion mode for the transfer of the photon energy to the translational energy, leads to the sample vaporization process.[7] The 'surface-assisted laser desorption/ionization' (SALDI) method uses a thin layer of activated carbon particles immobilized on an aluminum support.[8] In this case, the sample can be directly deposited on the surface plate. An alternative technique uses a suspension of a fine graphite powder or functionalized nanoparticles[9] in a solution of the analyte in an organic solvent. Peptides and organic compounds were then detected in DIOS and SALDI mass spectrometry as protonated molecules and/or as alkali metal adducts.[10,11] The functionalization concept of the mass spectrometric probe surface has been previously proposed through the pioneering work of Hutchen and Yip, and has promoted the development of the surface-enhanced laser desorption/ionization (SELDI) method.[12] In this case, the chemical and biochemical protein chips array surfaces play a role, namely, for the extraction and/or concentration of the sample prior to depositing a matrix solution on the derivatized surface.[13] An alternative method consists also in attaching directly a matrix molecule, such as an aromatic thiol derivative, on a gold surface without any chemical modification between the metal surface and the energy-absorbing compounds.[14] The latter was named *surface-enhanced neat desorption* (*SEND*) and has been mainly compared to the DIOS method in terms of the photon-absorbing surface.

By analogy with the surface properties of a UV-absorbing semiconductor (such as porous silicon), and with regard to the analytical flexibility offered by the possibility of functionalization of activated surfaces, we propose here the use of some organic surfaces in the LDI techniques. This new free-matrix laser desorption ionization method developed is termed *desorption/ionization on self-assembled monolayer surfaces* (*DIAMS*) and uses self-assembled monolayers (SAMs). SAMs are defined as two-dimensional films, one molecule thick, covalently assembled at an interface. These organic assemblies result, in most cases, from the reaction in solution between the headgroup function of a molecular constituent and a metal, oxide or semiconductor surface.[15] For the present work, the adsorbates fixed onto a gold surface by a headgroup had to be designed to organize themselves spontaneously on a crystalline or semicrystalline structure, namely, by van der Waals interaction, hydrogen bonds, etc. We have focused our attention on alkanethiols covalently bonded to a redox chromophore. The latter has to absorb at the laser wavelength (i.e. 337 nm) and has to maintain the conductivity of the sample–metal interface of the MALDI plates of the mass spectrometer. The redox chromophore used in this work is the 5,5′-disubstitued-2,2′-bithiophene. The redox properties of the chromophore allow for a simple and quick characterization of the SAMs by electrochemistry. An alkane-thiol of ten carbons has been used as a linker to the gold surface. This number gives a better thermodynamic stability to the organosulfur adsorbates on the gold surface and better organization than a shorter linker.[15] The introduction of the methyl group as terminal function allows the formation of a hydrophobic surface and avoids the possibility of polymerization of the bithiophene unit.

The synthesis of the SAMs precursor compounds starts from the 2-bromothiophene and is described in Scheme 1. Because of the propensity of the thiol function to oxidation, all characterizations in solution were carried out on the protected alkane-thiol (**1**). As expected from previous results reported in the literature, the characteristic optical band of **1** is observed at 340 nm.[16] The redox behavior of **1** was investigated by cyclic voltammetry (CV) in a glove box containing dry, oxygen-free (<1 ppm) argon at room temperature. CV of **1** in 0.2 M TBAHP/CH_3CN exhibits a reversible one-electron oxidation wave at 0.49 V (*vs* Fc^+/Fc).[17]

The SAMs were obtained by immersing freshly prepared Au electrodes (typically, an adhesion layer of chrome of *ca* 2 nm, followed by a layer of gold of *ca* 35 nm, deposited by physical vapor deposition on a glass support) for 48–96 h in a solution of alkane-thiol (**2**) (dichloromethane, 1×10^{-3} M). All experiments were

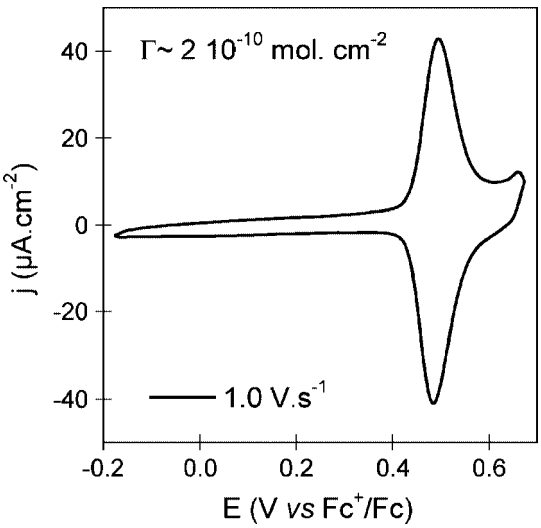
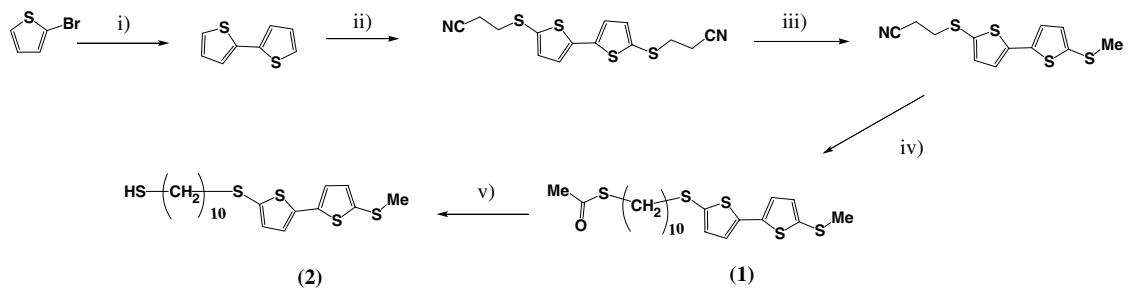


Figure 1. Cyclic voltammetry of the SAMs of **2** in 0.2 M TBAHFP/CH_2Cl_2.

*Correspondence to: E. Levillain and D. Rondeau, Laboratoire de Spectrométrie de Masse – Service Commun d'Analyses Spectroscopiques – Université d'Angers, 2 Bd Lavoisier, 49045 Angers, France. E-mail: David.rondeau@univ-angers.fr



Scheme 1. Synthesis of alkane-thiol **(2)**. Reagents and conditions: (i) Magnesium, $NidpppCl_2$, Et_2O, reflux; (ii) nBuLi, sulphur and 2-bromopropionitrile, THF, room temperature; (iii) Cesium hydroxide (1 equiv.) and methyl iodide, DMF/MeOH, room temperature, 2 h;[18] (iv) Cesium hydroxide (2 equiv.) and 10-thioacetate-bromodecane, DMF/MeOH, room temperature, overnight; (v) Cesium hydroxide, THF, room temperature, 3 h.

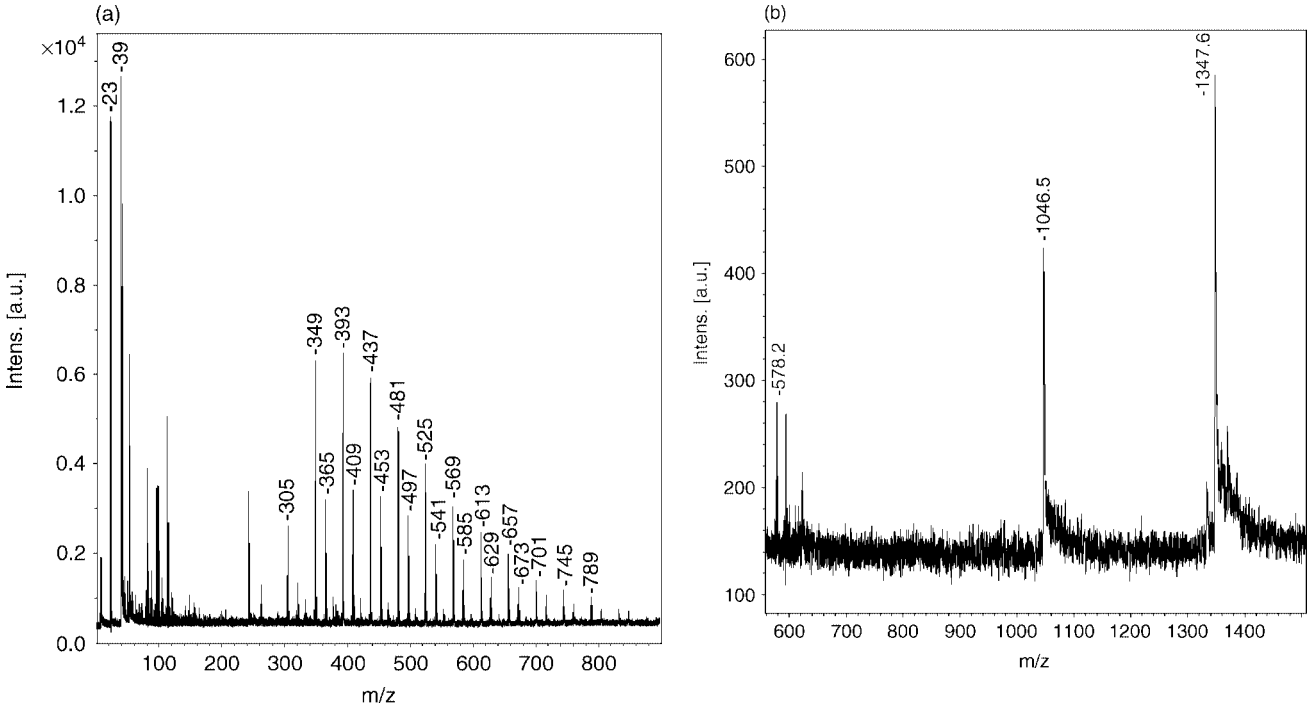


Figure 2. (a) Positive ion DIAMS mass spectrum of two poly(ethyleneglycol) mixtures with nominal molecular weights centered around 400 and 600. The peaks of Na^+ (m/z 23) and K^+ m/z 39 are depicted in the mass spectrum with the alkali metal adducts of PEG, i.e. $[M + Na]^+$ and $[M + K]^+$. (b) Expansion of the positive ion DIAMS mass spectrum of a peptide mixture. Signals at m/z 578.2, m/z 1046.5 and m/z 1347.6 correspond to the $[M + Na]^+$ of the leucine enkephaline (2×10^{-10} mol deposited on the plate), the $[M + H]^+$ of the angiotensine II (8×10^{-11} mol deposited on the plate) and the $[M + H]^+$ of the substance P (8×10^{-11} mol deposited on the plate), respectively.

performed in a glove box at room temperature. The characteristics of the SAMs were as expected. Firstly, as shown Fig. 1, the electrochemical response of the SAMs of (**2**) is consistent with a reversible redox system (+0.49 V (*vs* Fc^+/Fc)) confined at the Au electrode, as shown by the linearity of peak currents with the scan rate (up to 50 V s^{-1}). Secondly, the surface coverage (Γ) reaches a value of 2 (±0.4) $\times 10^{-10}$ mol cm^{-2}. This reproducible value shows a good organization.[15] Finally, the SAMs show excellent stability over several weeks.

In order to test the ability of DIAMS in the field of application of free-matrix laser ablation/ionization mass spectrometry, samples such as poly(ethylene glycols) (PEGs), peptides, fatty acids and glyceride mixtures were directly spotted on the organic surface. MS analyses were performed on a Bruker Biflex III time-of-flight (TOF) mass spectrometer (Bruker-Daltonic, Bremen Germany) equipped with a Scout 384 probe ion source. Samples were directly deposited on the gold–SAMs interface and desorbed/ionized from the probe tip with a 337-nm pulsed nitrogen laser (model VSL-337i, Laser Science Inc., Boston, MA) having a repetition rate of 3 Hz and a pulse width of 2 ns. All analyzed samples were purchased from Aldrich. Positive-ion mode mass spectrum of PEGs (Fig. 2(a)) shows Na^+ and K^+ abundant ions together with the alkali adducts of the PEGs. Although some relatively low-intensity ions between m/z 39 and the first PEG ions (m/z 305) appear on the DIAMS mass spectrum of Fig. 2, no signals are observed from the SAMs themselves. Peptide analysis shows that DIAMS can produce quasi-molecular $[M + H]^+$ ions or even $[M + Na]^+$ ions if the peptide does not contain basic amino acids such as leucine enkephaline (Fig. 2(b)). DIAMS can be mainly suitable for lipids analysis since glyceride can be detected in the positive-ion mode through the $[M + Na]^+$ ions (Fig. 3(a)), whereas the fatty acids are observed in the negative-ion mode as deprotonated species (Fig. 3(b)), and this, without prior sample treatment. Note that in the negative-ion mode no gold or gold-thiolates emission is observed in the DIAMS mass spectrum of Fig. 3(b).[14,19]

In conclusion, the results show that DIAMS can be considered as a novel free-matrix LDI method for generating gas-phase ions directly from samples directly deposited onto a self-assembled

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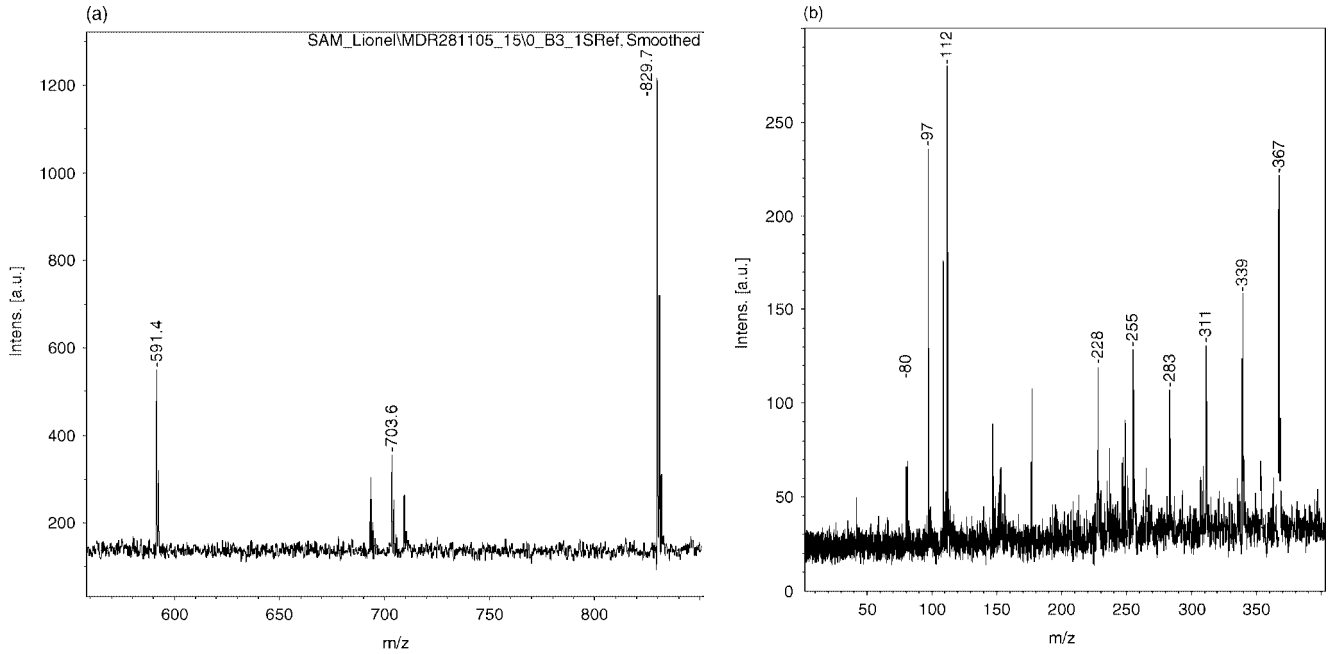

Figure 3. (a) Expansion of the positive-ion DIAMS mass spectrum of a mixture of α,γ-dihexadecanoin $[M + Na]^+$ m/z 591.4, tritridecanoin $[M + Na]^+$ m/z 703.6 and trihexadecanoin $[M + Na]^+$ m/z 829.7. (b) Negative-ion DIAMS mass spectrum of a fatty acid mixture. Signals at m/z 228, m/z 255, m/z 283, m/z 311, m/z 339 and m/z 367 correspond to the $[M - H]^-$ of the tetradecanoic, hexadecanoic, octadecanoic, eicosanoic, docosanoic and tetracosanoic acids, respectively (5×10^{-10} mol of each analyte was deposited on the plate).

monolayer surface. The analytical versatility and the ease of use of this technique are demonstrated through the studied samples, which encompass small peptides, natural organic compounds and synthetic polymers. In particular, the potential of this DIAMS-MS method appears in the case of lipids analysis. Therefore, one can consider that the chemical modifications of SAMs may induce other surface properties and can lead to numerous applications. For example, in this paper, the nature of the synthetized SAMs has allowed a hydrophobic target suitable for analyses in a lipidomic context to be obtained, even though the detection of peptides was also possible. We envisage the design of new SAMs that may induce different surface properties with more hydrophilic spacers and a specific terminal functional group that should improve the ionization yield and allow molecular recognition processes for specific analyses. A convenient engineering could also increase the spot concentration and therefore the sensitivity of detection.

Yours,

L. SANGUINET,[1] O. ALÉVÊQUE,[1] P. BLANCHARD,[1] M. DIAS,[1] E. LEVILLAIN[1]* and D. RONDEAU[2]*

[1] Chimie, Ingénierie Moléculaire et Matériaux d'Angers, UMR CNRS 6200 – Université d'Angers, 2 Bd Lavoisier, 49045 Angers, France.

[2] Laboratoire de Spectrométrie de Masse – Service Commun d'Analyses Spectroscopiques – Université d'Angers, 2 Bd Lavoisier, 49045 Angers, France.

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