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[11] Based on the comparison of the C–O bond lengths (C6–O4 1.34(2), C16–O10 1.24(2), C66–O35 1.33(3), C76–O39 1.23(2) Å), the Re2 unit containing O10 and the Re8 unit containing O39 are assigned to have a deprotonated form (see Figure 3).

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[13] As indicated by the referees, the solid-state structures of **1** and **2** would not be retained in solution. The electrospray-ionization (ESI) mass spectrum of **2** in water mainly showed four clusters of peaks centered at m/z 605.1 (relative intensity: 100%), 623.2 (19%), 1103.1 (83%), and 1209.0 (21%). The calculated molecular mass and the isotopic distribution of these clusters of peaks match very well with those for $[Ag\{ReO(D\text{-}Hpen)(D\text{-}pen)\}]^+$, $[Ag\{ReO(D\text{-}Hpen)(D\text{-}pen)\}(H_2O)]^+$, $[Ag\{ReO(D\text{-}Hpen)(D\text{-}pen)\}_2]^+$, and $[Ag_2\{ReO(D\text{-}Hpen)(D\text{-}pen)\}\{ReO(D\text{-}pen)_2\}]^+$ ions, respectively. This result suggests that in water the $Re^V_8Ag^I_6$ 14-nuclear structure in **2** breaks down to $Re^V_2Ag^I$ trinuclear and $Re^V_2Ag^I_2$ tetranuclear species, followed by the dissociation of $Re^V_2Ag^I_2$ tetranuclear species into Re^VAg^I dinuclear species. Attempts to measure the ESI mass spectrum of **1** were unsuccessful, because of its extremely poor solubility in water.

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Using Mass Spectrometry to Characterize Self-Assembled Monolayers Presenting Peptides, Proteins, and Carbohydrates**

Jing Su and Milan Mrksich*

Mass spectrometry (MS) is an important technique for characterizing the structures of surfaces and has several characteristics that are especially valuable in bioanalytical applications. In biochip applications, for example, MS offers the significant advantage that it does not require analytes to be labeled—either by direct attachment of fluorescent and radioactive labels or by binding of antibodies—and therefore offers greater flexibility in experiments.[1–4] Further, the use of immobilized ligands to isolate active proteins from the

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background in a complex sample greatly simplifies the analysis of MS spectra.[5–7] Yet MS remains a secondary option to the use of fluorescence and radioactivity for characterizing biochips, in part because many programs still use home-built instrumentation and often require sophisticated protocols for analyzing mass data.[8–10] Here we demonstrate that matrix-assisted laser desorption/ionization and time-of-flight mass spectrometry (MALDI-TOF MS), when combined with self-assembled monolayers (SAMs) that are engineered to give specific interactions with biomolecules, is well suited for characterizing biological activities. We describe examples that use MS to characterize the immobilization of ligands, the selective binding of proteins, and the enzymatic modification of immobilized molecules.

Our approach relies on SAMs that present oligo(ethylene glycol) groups and peptide or carbohydrate ligands in low densities. The glycol groups are critical to this design, because they are highly effective at preventing nonspecific interactions with proteins and thereby ensure that the interactions of soluble proteins occur only by way of the immobilized ligands.[11–14] First, we used MALDI to characterize the immobilization of a peptide on a monolayer. Figure 1 a shows the mass spectrum of a SAM presenting maleimide and pentakis(ethylene glycol) groups in the ratio 1:4.[15–18] The spectrum shows the peaks for the sodium adducts of the symmetric glycol-substituted disulfide ($C_{42}H_{86}O_{12}S_2Na$, m/z 870) and for the mixed disulfide containing one maleimide group ($C_{52}H_{98}N_2O_{16}S_2Na$, m/z 1094).[19] The monolayer was treated with an aqueous solution containing the cysteine-terminated peptide Ac-IYAAPKKKC-NH_2 (2 mM) for 2 h, rinsed, and then analyzed by MS. The absence of the peak at m/z 1094 showed that the maleimide group had reacted under these conditions and gave rise to two new peaks (Figure 1 b), which represent the peptide-terminated alkanethiol ($C_{80}H_{139}N_{15}O_{21}S_2Na$, m/z 1733) and the mixed disulfide resulting from Michael addition of the cysteine-terminated peptide with the maleimide group ($C_{101}H_{181}N_{15}O_{27}S_3Na$, m/z 2155). We found that MALDI-TOF MS can identify substituted alkanethiolates with excellent sensitivity, with an adequate signal for the maleimide group even when presented at a density of 1 %.[20]

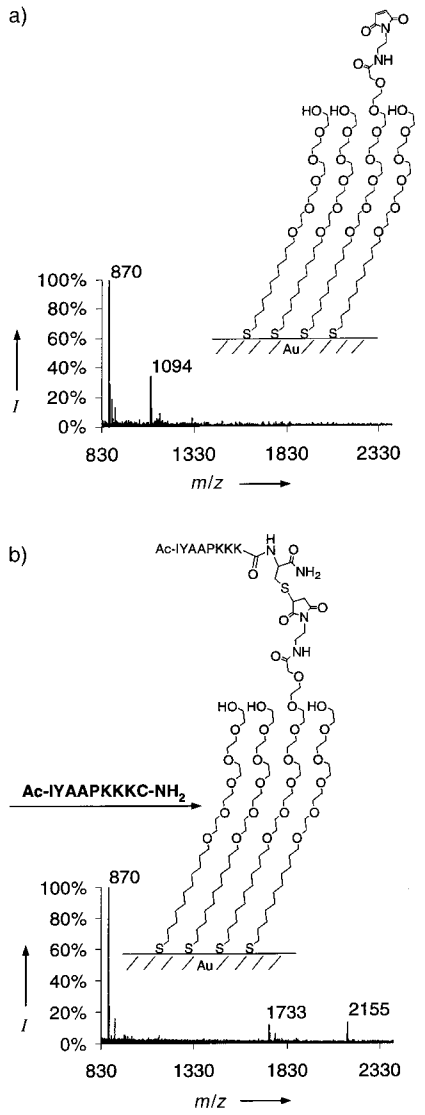

Figure 1. a) The MALDI spectrum of a mixed monolayer presenting pentakis(ethylene glycol) groups and maleimide groups shows a peak for the mixed disulfide (m/z 1094). b) The MALDI spectrum of the monolayer after treatment with the cysteine-terminated peptide Ac-IYAAPKKKC-NH_2 shows mass peaks resulting from immobilization of the peptide. The structure of each monolayer is shown above the spectra.

In an example relevant to biochips, a monolayer presenting the carbohydrate α-mannose and tris(ethylene glycol) groups in the ratio 1:4 was treated with a solution of Concanavalin A ($M \approx 102$ kD, 1 mg mL^{-1} in phosphate buffer, pH 7.4, containing 1 mM Ca^{2+} and 1 mM Mn^{2+}) for 30 min and then rinsed with distilled water (Figure 2 a). 3,5-Dimethoxy-4-hydroxycinnamic acid in 50 % aqueous acetonitrile solution containing 0.1 % trifluoroacetic acid (10 mg mL^{-1}) was applied to the monolayer and allowed to evaporate prior to MALDI analysis. The spectrum in Figure 2 b reveals peaks corresponding to the doubly ionized tetramer, trimer, and monomer of Concanavalin A, and to the singly charged dimer and monomer; this demonstrates that MALDI can directly observe proteins bound to ligands immobilized on monolayers.[21] Identical experiments with monolayers presenting only glycol groups gave no peaks in this mass range, and incubation of the mannose-presenting SAMs with lectin from *E. Cristagalli* ($M = 56$ kD), which does not bind to mannose, also gave no protein ion peaks. This example demonstrates that MALDI can identify proteins that are bound to SAMs presenting ligands.

In a third example, we used MALDI to characterize the enzymatic modification of an immobilized ligand (Figure 3 a). The MALDI spectrum of a monolayer presenting the carbohydrate *N*-acetylglucosamine (β-GlcNAc) and tris(ethylene glycol) groups in the ratio 1:4 showed a single intense peak at m/z 1211 that corresponds to the mixed disulfide with a single GlcNAc group ($C_{58}H_{113}N_3O_{17}S_2Na$, m/z 1211; Figure 3 b).[13] This monolayer was then treated with a HEPES buffer (50 mM, pH 7.5) containing β-1,4-galactosyltransferase (GalTase, 250 mU mL^{-1}), $MnCl_2$ (10 mM), and uridine diphosphogalactose (UDP-Gal, 20 μM) for 1 h at 20 °C and then rinsed. Analysis by MALDI showed a single intense peak at m/z 1373 that corresponds to the mixed disulfide containing *N*-acetyllactosamine (LacNAc, $C_{64}H_{123}N_3O_{22}S_2Na$, m/z 1373),

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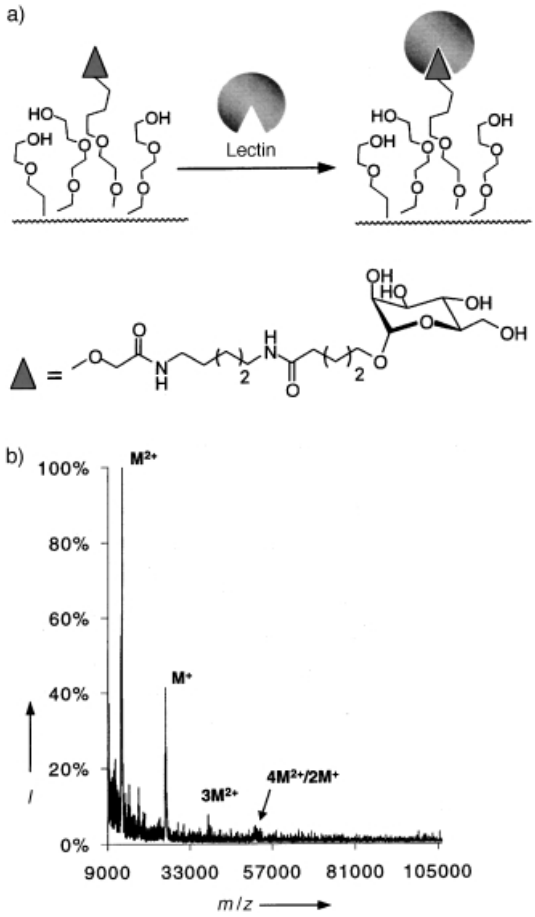

Figure 2. MALDI was used to characterize the selective binding of protein to ligands immobilized on a monolayer. a) A monolayer presenting the carbohydrate α-mannose was treated with an aqueous solution containing the lectin Concanavalin A (1 mg mL^{-1} in phosphate buffer, pH 7.4, 1 mM Ca^{2+} and 1 mM Mn^{2+}), rinsed, and then analyzed by MALDI. b) The peaks at *m*/*z* 25.6 kD and 12.8 kD correspond to the singly and doubly ionized monomer of Concanavalin A, respectively, and the small peaks at *m*/*z* 51 kD and 38 kD correspond to doubly ionized tetramer and trimer, respectively. This demonstrates that MALDI can be used to identify selective protein-binding interactions.

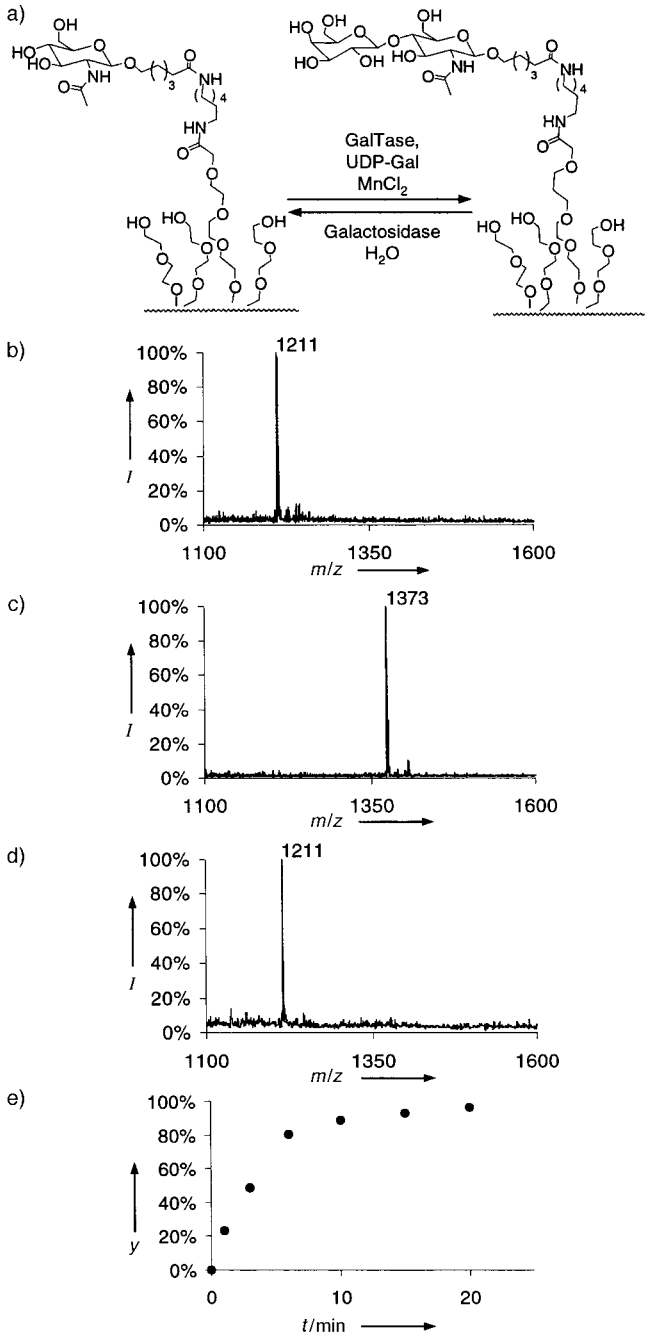

Figure 3. MALDI was used to characterize the enzymatic modification of an immobilized substrate. A monolayer presenting the carbohydrate β-GlcNAc (a) was analyzed by MALDI to reveal a peak at *m*/*z* 1211 for the mixed disulfide (b). c) After the monolayer was treated with the enzyme GalTase to introduce a terminal galactose residue, analysis by MALDI revealed a new peak at *m*/*z* 1373 corresponding to the disaccharide product. d) Treatment of this monolayer with the enzyme galactosidase removed the terminal galactose residue and regenerated the GlcNAc group. e) MALDI was used to determine the time-dependence of the enzymatic galactosylation which demonstrates that this technique can provide kinetic information on biological activities.

the disaccharide product that results from enzymatic galactosylation of GlcNAc (Figure 3c). The absence of a peak at *m*/*z* 1211 demonstrates that the enzymatic reaction was essentially complete. We next treated this chip with a solution containing the enzyme galactosidase (25 U mL^{-1}), $MgCl_2$ (1 mM), KCl (10 mM), and β-mercaptoethanol (50 mM) in phosphate buffer (pH 7.0) for 8 h at 37 °C. MALDI revealed that the LacNAc was enzymatically converted to GlcNAc in quantitative yield (Figure 3d). Control experiments showed that treatment of monolayers presenting α-mannose with either GalTase or galactosidase had no effect which again demonstrates the specificity intrinsic to this class of SAMs.

In a final example, we show that the combination of MALDI-TOF MS and SAMs can provide kinetic data for biological interactions on chips. We investigated the time-dependence of the GalTase-mediated galactosylation by treating identical SAMs presenting β-GlcNAc with the enzyme as described above for periods of time ranging from 0 to 20 min. The monolayers were each rinsed, dried, and analyzed by MALDI. We calculated the yield of the enzymatic conversion on each chip by taking a ratio of the peak integration area for LacNAc relative to the sum of the peak areas for LacNAc and GlcNAc (percentage yield $= A_L/[A_L + A_G] \times 100$).[22] Figure 3e demonstrates that the yield

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increased smoothly with time and reached a maximum at complete conversion. The same experiment was repeated several times, and the variability for each time point was less than 5%. This result indicates that MALDI, when combined with SAMs, may find wider use for kinetic analysis of interfacial reactions.

The most significant result of this work is that a commercial instrument for MALDI-TOF MS, when combined with self-assembled monolayers engineered for bioanalytical applications, is a very effective technique for characterizing biological activities at interfaces. We believe that this finding will be exploited for a range of purposes, but in particular for examining biochips. The recent development of strategies that use self-assembled monolayers for the preparation of peptide, protein, and carbohydrate arrays makes this technique immediately applicable.[14,23,24] The use of MALDI in these applications is significant because it can identify unexpected biological activities, while current methods for characterizing biochips require preliminary knowledge of the activity to be identified. Fluorescence detection of antibodies that bind to arrays, for example, will only identify activities that introduce or delete an antigen. Mass spectrometry, by contrast, will identify any change in mass at the interface—whether resulting from binding of a protein or modification by an enzyme—and hence can discover unanticipated activities. We believe that these properties, together with the widespread availability of commercial instruments, will make MALDI a popular and effective technique for applications in bioanalytical and surface chemistry.

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[18] When reporting the ratio of alkanethiolates present in a mixed SAM, we give the ratio of alkanethiols in the solutions used for the formation of the monolayer.
[19] The majority of the SAMs in this work gave mainly sodium adducts of the molecular ions. Throughout the text we provide empirical formulas and the expected monoisotopic masses for each observed disulfide.
[20] We found that application of a matrix was necessary for the observation of molecular ions from the monolayers, but we have not optimized the formulation of the matrix for these applications.
[21] Concanavalin A is a tetramer with four identical subunits. Under acidic conditions, this noncovalent protein polymer dissociates easily. For a reference spectrum, see: M. Monatte, C. Lesieur, B. Vécsey-Semjén, J. T. Buckley, F. Pattus, F. G. van der Goot, A. Van Dorsselaer, *Int. J. Mass Spectrom. Ion Processes* **1997**, *169*/170, 179 – 199.
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A Method for Determining the Difference in Relative Apicophilicity of Carbon-Containing Substituents of 10-P-5 Phosphoranes**

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Apicophilicity is the relative preference of substituents to occupy the apical positions as opposed to the equatorial positions in trigonal bipyramidal (TBP) structures and is one of the fundamental properties of pentacoordinate compounds.

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