SELF-ASSEMBLED GOLD NANOPARTICLE FILM FOR NANOSTRUCTURE-INITIATOR MASS SPECTROMETRY WITH PASSIVE ON-LINE SALT FRACTIONATION

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ABSTRACT

We present the self-assembly of fluorinated-Au nanoparticle films as a mass-producible fabrication methodology for generating nanostructure-initiator mass spectrometry substrates. The nanostructured surfaces enable the direct high sensitivity detection of peptides (20 fmol) and other small molecules using laser desorption ionization. Further, through a photolithographic liftoff technique we can realize micropatterned fluorinated-Au nanoparticle films. These micropatterns create a discrete wettability patterns, allowing us to passively fractionate hydrophobic molecules of interest from high-salt background environments for robust and predictable MS.

INTRODUCTION

Surface Assisted Laser Desorption Ionization (SALDI) is a method of ionization for mass spectrometry that uses a nanostructured medium to absorb energy from an incident laser and transfer that energy onto a target sample. The transferred energy ionizes and desorbs the target sample such that it can be injected into a mass analyzer for the charge to mass ratio of the molecules to be detected. In contrast to Matrix Assisted Laser Desorption Ionization (MALDI), SALDI does not require the addition of matrix ions to facilitate the LDI process. Thus eliminating deleterious 'matrix effects' such as ionization suppression of the molecules of interest, high background, or matrix ions with a similar mass to charge ratio obscuring the detection. SALDI platforms, as a practical example, have allowed forensic scientists to detect polymers associated with the use of contraceptive products that were undetectable with MALDI previously [1]. Common SALDI nanostructures include porous silicon, carbon nanotubes, graphene, and various nanoparticles [2]. In the last decade, it was shown that SALDI sensitivity could be further increased when the nanostructures were either functionalized or coated with hydrophobic perfluoroalkyl molecules [3]. The coating enhances the assay sensitivity by two means. First, due to the nature of a hydrophobic coating, sample containing droplets dry into a small spot on the surface, effectively concentrating their contents. And second, the perfluoroalkyl

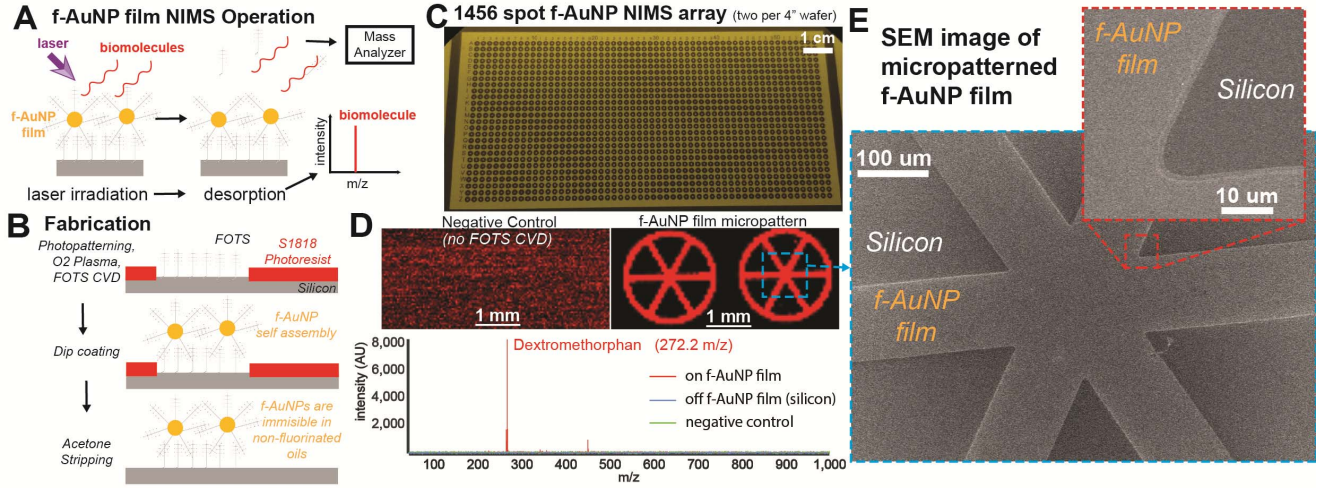

Figure 1: Fluorinated Au nanoparticle (f-AuNP) films are nanostructure-initiator mass spectrometry (NIMS) substrates for direct and high-sensitivity laser-based MS analysis of adsorbed molecules (A). (B) Fabrication of the f-AuNP film is mediated by a photo-patterned, covalently bound perfluorooctyltrichlorosilane (FOTS) monolayer attached to a silicon wafer [6]. The FOTS-coated substrate is dipped into a 0.3 g/L solution of f-AuNP (2-4 nm diameter) in HFE 7500 for 10 min then dried. The S1818 photoresist is stripped with acetone, then washed in IPA, DI water and dried. The f-AuNPs are immiscible in any non-fluorinated oils. Therefore, the films are stable in stripping and washing. (C) The simplicity of fabrication enables scale up to batch wafer processing. The aluminum traces serve as a visual cue - f-AuNP patterns are not visible by eye. (D) Substrates fabricated with FOTS and without were soaked in DI water containing 250 nM dextromethorphan for 1 hour and then imaged using mass spectrometry. The micro-patterned wheel shaped f-AuNP film is apparent in the mass spectra while no ionization occurred off-pattern or in the negative control. (E) Scanning Electron Microscopy (SEM) corroborated MS results – revealing the micro-patterned f-AuNP film.

molecules acted as an initiator for desorption – enabling a more efficient process.

Nanostructure-initiator mass spectrometry (NIMS) is a SALDI-MS platform that has, in the past, leveraged laser-resonant wet etched-silicon nanostructures and initiator molecules for high sensitivity detection of adsorbed small molecules, lipids, and peptides in LDI-MS [3]. Fabricating the nanostructured-silicon wafer in NIMS required hydrofluoric acid etching followed by the manual coating of an initiator molecule. Due to the low-repeatability of both steps – a wide degree variability is observed from one NIMS surface to the next. Black silicon fabricated with plasma etching eliminated the wet hydrofluoric acid step for a safer and easier method of fabricating NIMS [4]. Unfortunately black silicon NIMS saw a sharp reduction in sensitivity and no improvement in repeatability. Recently, Kurczy et. al. [5] demonstrated that spotting perfluorodecanethiol functionalized gold nanoparticles (f-AuNPs) could be used to directly analyze tissue sections. In their work they sprayed or spotted f-AuNPs atop tissue slices to directly assess the samples.

In this work, we sought to create a NIMS platform utilizing f-AuNPs assembled on a surface for the analysis of spotted samples. We developed a facile and mass producible method for fabricating NIMS surfaces. Fluorine-functionalized nanoparticles are allowed to directly self-assemble on a surface that has been covalent modified with a perfluoroalkyl moiety. Due to the highly fluorophilic nature of the functionalized nanoparticles they are stably adsorbed to the surface and are immiscible (i.e. do not to desorb) in the aqueous or a non-fluorinated organic solvents. We can perform photolithography lift-off to create micro-patterned nanoparticle films to add additional functionality. Adsorbed molecules, such as peptides, lipids, and small molecules, can be directly detected from the nanoparticle film using laser desorption ionization, thus functioning similarly to silicon-based NIMS substrate.

METHODS

Materials and Chemicals

We purchased octanethiol functionalized gold nanoparticles (2-4 nm diameter), perfluorodecanethiol, acetone, ethanol, methanol, sodium chloride, dextromethorphan, and 2,5-Dihydroxybenzoic acid from Sigma Aldrich (St. Louis, MO, USA). The fluorinated oil HFE 7500 (Nova Chemicals, Calgary, Alberta, Canada) was used to solubilize the modified gold nanoparticles. STAL-2 peptide was purchased from AnaSpec (Fremont, CA, USA).

Device Operation

The f-AuNP NIMS film is interfaced with by simply pipetting a sample of interest and letting it dry atop the surface. Positive mode LDI with a standard MALDI-MS, we used an AB Sciex 4800, enables direct interrogation of adsorbed lipids, small molecules, and peptides atop the surface (Figure 1A).

Gold Nanoparticle Functionalization

This protocol was adopted from a previously published protocol [5]. 500 μL of octanethiol-functionalized AuNPs in toluene (20 mg/ml) was added to 10 ml of ethanol to precipitate the AuNPs with centrifugation at 4,000 r.p.m. for 15 min. AuNPs were washed with acetone and spun down three times. AuNPs were mixed with 1 ml of hexane and 400 ml of perfluorodecanethiol and purged with nitrogen gas to remove air. The solution was stirred for one to three days until f-AuNPs precipitated. The solvent is evaporated with nitrogen gas flow and nanoparticles are washed three times with hexane, three times with acetone and two times with 1:1:1 methanol/acetone/water. The f-AuNPs were then dried in an oven. Before use, the AuNP were dissolved in HFE 7500 at a concentration of 0.3 mg/ml.

f-AuNP Film Fabrication

Four inch silicon wafers coated with 30 nm of aluminum were purchased from Polishing Corporation of America (Santa Clara, CA, USA). S1818 photoresist and MF-321 developer (Microchem, Westborough, MA, USA) was used to create an etching mask. Aluminum traces were fabricated with an aluminum etchant (Transene, Danvers, MA, USA) at 60˚C for 5 minutes. After etching, residual photoresist was stripped in acetone and a new layer of S1818 resist was patterned to define the f-AuNP film's micropattern. After development, the wafer was O_2 plasma treated and then immediately placed into a chemical vapor deposition chamber (under house vacuum) to self-assemble perfluorooctyltrichlorosilane (FOTS) atop the exposed silicon surface. The wafer is immersed in a solution of 0.3 mg/mL f-AnNP for 1 minute and then N_2 dried. The remaining photoresist is then stripped off in acetone, leaving only the aluminum traces and self-assembled fAuNPs behind, as shown in Figure 1C.

RESULTS

We evaluated the sensitivity of our f-AuNP films against a common matrix-assisted laser desorption ionization (MALDI) matrix in Figure 2 (2,5-dihydroxybenzoic acid, 20 g/L, 50% ethanol). Figure 2A displays the MS image of a MALDI plate and a micro-patterned f-AuNP film spotted with samples containing between 200,000 to 20 fmols of the peptide STAL-2. As expected for NIMS, we observed enhanced peptide sensitivity with a limit of detection of less than 20 fmols (Figure 2B). Further, as shown in Figure 2C, the f-AuNP had considerably lower background ions than the MALDI matrix.

In Figure 3 we dried samples of 1 M NaCl and 500 fmol dextromethorphan on a MALDI plate and a micro-patterned f-AuNP film. The f-AuNP film fractionates

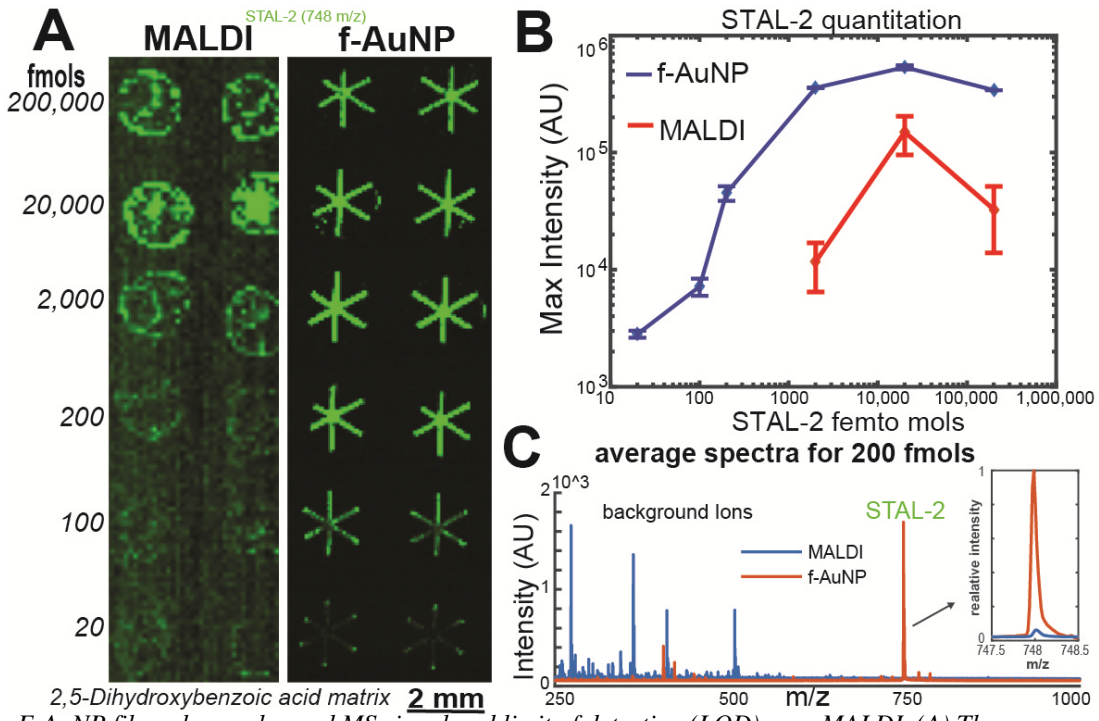

Figure 2: F-AuNP films show enhanced MS signal and limit of detection (LOD) over MALDI. (A) The common MALDI matrix 2,5-dihydroxybenzoic acid (20 g/L, 50% EtOH) was compared to f-AuNP films for the detection of the peptide STAL-2 from 200,000 to 20 fmols. (B) A log-log plot of mols and max intensity with error bars (n=3) highlights a robust quantitative region between 20 and 2000 fmols. As expected for NIMS [3] the f-AuNP film had a STAL-2 LOD of ~20 fmols - while MALDI required 200 fmols. (C) Average spot spectra are displayed for the 200 fmol samples for both conditions. In addition to the 15-times higher STAL-2 greater signal, the f-AuNP had a clean spectra devoid of dominant matrix ions.

passively. Salt dries atop the bare-silicon regions while dextromethorphan is adsorbed onto the f-AuNP regions. Predictable on-line fractionation could be applied for the analysis of complex samples such as cell lysate or solutions that contain MS-suppressing surfactants.

In this work, we utilize fluorine-mediated self-assembly of f-AuNPs to create micropatterned NIMS films. Importantly, our new approach enables wafer-level batch-fabrication and enhances reproducibility of traditional NIMS substrates which require HF etching [3] and are plagued with inconsistency from the initiator application step. Unlike previous salt fractionation protocols that require manual interventions (e.g. washing [7] or vortexing [8]), our designed micropatterned f-AuNP film allows us to directly extract undesired contaminants during passive sample drying.

DISCUSSION

In this work, we have developed a simple fabrication method for self-assembling NIMS surfaces. In addition to making NIMS easier to mass produce, this approach is amenable to alternative substrates (such as indium tin oxide coated glass) and nanoparticle. Going forward we will explore alternative nanoparticle materials (e.g. silver and titanium dioxide) and shapes (e.g. rods, nanourchins) that could provide enhanced SALDI-MS properties.

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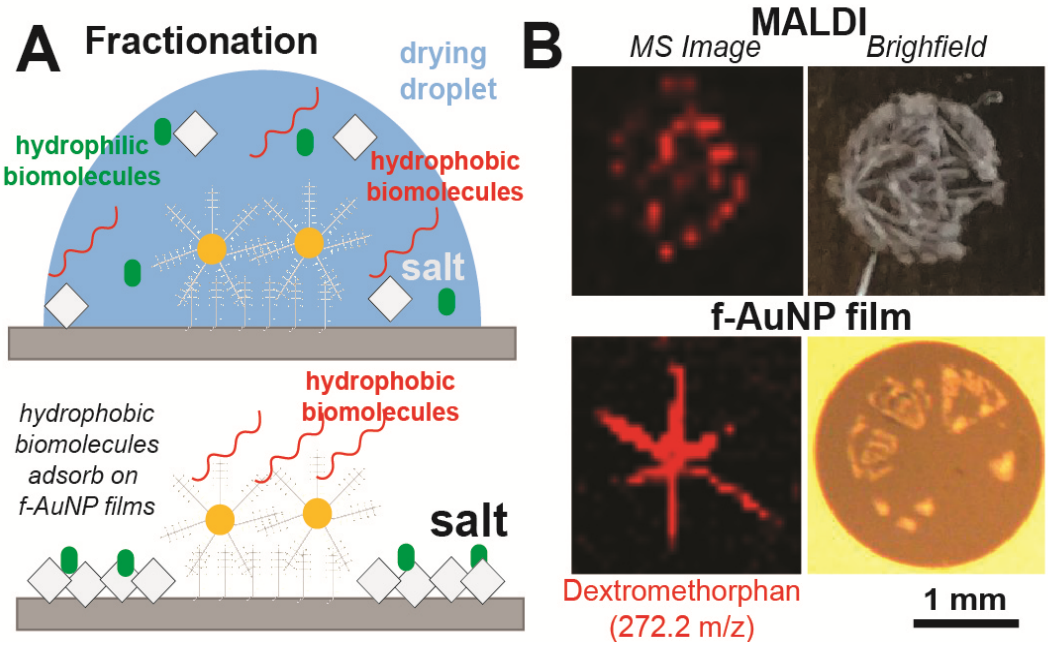

Figure 3: Micro-patterned f-AuNP films enable passive and on-line fractionation of salt from complex samples. (A) A sample is spotted directly atop the f-AuNP film and allowed to dry. During this process, hydrophobic molecules adhere to the f-AuNPs while salts and hydrophilic components dry atop the bare silicon region. (B) Droplets containing 500 fmols of dextromethorphan and 1 M NaCl were analyzed with MALDI (same conditions as Fig 2) f-AuNP. For MALDI, a web of salt and matrix conceals ions of interest (top panels). For the micro-patterned f-AuNP film, dextromethorphan adhered to the f-AuNP film whereas salts were preferentially excluded from those regions (bottom panels). This predictable fractionation will enable high-throughput analysis of complex samples.

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