Electronic Supplementary Material (ESI) for Analyst

Supplementary informations

Affinity surface-assisted laser desorption/ionization mass spectrometry for peptide enrichment.

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Experimental procedures

1) Materials and Reagents

Crystalline silicon wafers (Siltronix, Archamps, France) were used as substrates for the preparation of NanoSi and porous silicon (pSi) interfaces. All cleaning and etching reagents were VLSI grade. Sulphuric acid 96%, hydrofluoric acid 50%, and hydrogen peroxide 30% were purchased from Carlo Erba. Nitric acid 65% and hydrochloric acid 37% were purchased from Merck. All other chemicals were reagent grade or higher and were used as received unless otherwise specified. Nickel chloride ($NiCl_2$), octadecyltrichlorosilane (OTS), sodium hydroxide (NaOH), acetone, isopropyl alcohol (*i*PrOH), dichloromethane (CH_2Cl_2), undecylenic acid, N-hydroxysuccinimide, dicyclohexylcarbodiimide, triethylamine, dimethylsulfoxide (DMSO) and dimethylformamide (DMF) were purchased from Sigma-Aldrich. N-(5-Amino-1-carboxylpentyl) iminotriacetic acid was purchased from Interchim.

2) Preparation of surfaces

Nanostructured silicon (NanoSi)

For the fabrication of the NanoSi interface, a single side polished (100) silicon wafers (p-type, 0.009-0.01 Ohm/cm) were used as substrates. They were first degreased in acetone and isopropanol, rinsed with Milli-Q water and cleaned in a piranha solution (3:1 concentrated H_2SO_4/30% H_2O_2) for 15 min at 80 °C followed by copious rinsing with Milli-Q water.

Then, the clean crystalline silicon substrate is dipped into a solution of $HF/AgNO_3$ (2.6M/0.005M) at 50 °C for 10 min. The resulting surface was rinsed copiously with deionized water and immersed in an aqueous solution of $HCl/HNO_3/H_2O$ (1/1/1) at room temperature overnight to remove the silver nanoparticles and dendrites deposited on the silicon nanowires during the chemical etching.

Porous silicon (pSi)

For the fabrication of the Porous silicon interfaces, a double-side polished Si (100) oriented p-type silicon wafers (boron-doped, 5–10 Ohm/cm resistivity) were first degreased in acetone and isopropanol, rinsed with Milli-Q water and cleaned in a piranha solution (3:1 concentrated H_2SO_4/30% H_2O_2) for 15 min at 80 °C followed by copious rinsing with Milli-Q water. The clean wafers were immersed in 50% HF aqueous solution for 1 min at room temperature to remove the native oxide. The hydrogen-terminated surfaces were electrochemically etched in a 1:1 (v/v) solution of pure ethanol and 50% HF for 5 min at a

current density of 10 mA/cm^2. After etching, the samples were rinsed with pure ethanol and dried under a stream of dry nitrogen prior to use.

Safety Considerations

The mixture H_2SO_4/H_2O_2 (piranha) solution is a strong oxidant. It reacts violently with organic materials. It can cause severe skin burns. It must be handled with extreme care in a well-ventilated fume hood while wearing appropriate chemical safety protection. HF is a hazardous acid, which can result in serious tissue damage if burns were not appropriately treated. Etching of silicon should be performed in a well-ventilated fume hood with appropriate safety considerations: face shield and double layered nitrile gloves.

3) Surface Characterizations

Contact Angle Measurements

Water contact angles were measured using deionized water. We used a remote-computer controlled goniometer system (DIGIDROP by GBX, France) for measuring the contact angles. The accuracy is ± 2°. All measurements were made in ambient atmosphere at room temperature.

Fourier Transform Infrared (FTIR) Spectroscopy

Transmission FTIR spectra were recorded using a Perkin-Elmer Spectrum 2000 single-beam spectrophotometer equipped with a tungsten-halogen lamp and a liquid nitrogen cooled MCT detector. All measurements were made after purging the sample chamber for 30 min with dry N_2. Spectra were recorded at 4 cm^{-1} resolution and averaged over 200 scans. Background spectra were obtained using a flat untreated Si (100) surface.

X-ray Photoelectron Spectroscopy (XPS)

XPS measurements were performed with an ESCALAB 220 XL spectrometer from vacuum Generators. A monochromatic Al Kα X-ray source (1486.6 eV) was operated in the constant analyzer energy (CAE) mode (CAE= 100 eV for survey spectra and CAE = 40 eV for high resolution spectra), using the electromagnetic lens mode. The angle between the incident X-rays and the analyzer is 58°. The detection angle of the photoelectrons is 90°, as referenced to the sample surface and the C_{1s} at 285 eV was used for binding energy correction.

Scanning electron microscopy (SEM)

SEM images were obtained using an electron microscope ULTRA 55 (Zeiss) equipped with a thermal field emission emitter and a high-efficiency In-lens SE detector.

4) Surface functionalizations

Introduction of the NTA-Ni^{2+} complex on NanoSi and pSi surfaces.

Carboxylic acid termination

Concerning the carboxylic acid termination, NanoSi and pSi interfaces were first immersed in 50% hydrofluoric acid for 1 min, resulting in hydrogen-terminated surfaces (NanoSi-H and pSi-H, respectively). The hydrogenated interfaces were placed in a Schlenk tube containing previously deoxygenated neat undecylenic acid and heated to 150 °C. This thermal reaction takes place under nitrogen gas bubbling, avoiding the oxidation of the Si-H bonds. After 6h, the reaction was stopped and the surfaces were washed thoroughly twice in dichloromethane and twice in ethanol (5 min each) under stirring. However, the formation of multilayer of undecylenic acid can occur, because of the weak interactions between the molecules such as the van der Waals forces and hydrogen bonding. Thus, the surfaces have been immersed in hot acetic acid for 5 min in order to obtain a carboxylic acid monolayer on the surface. Then, the surfaces were rinsed twice with deionized water to remove the traces of acetic acid followed by an ethanol bath under stirring and finally dried under a gentle stream of nitrogen.

NHS-ester activation

Then, NHS-ester activation was performed prior the NTA grafting. For that, freshly prepared carboxylic acid interfaces were activated by *N*-hydroxysuccinimide (NHS) leading to the formation of reactive NHS-ester terminated interfaces (NanoSi-NHS or pSi-NHS). The COOH-interfaces were dipped in a solution of 0.2 M NHS and 0.4 M dicyclocarbodiimide (DCCI) in dimethylformamide (DMF) for 3 h at room temperature. The excess of unreacted reagents were removed using DMF and ethanol. Finally, the interfaces were dried under a gentle stream of nitrogen.

NTA immobilization

Then, a freshly prepared NHS-activated interfaces were immersed in a solution of 0.01 M N-(5-amino-1-carboxylpentyl) iminodiacetic acid (NH_2-NTA) in dimethylsulfoxide solution containing 0.07 M of triethylamine for 20h at room temperature under stirring. The resulting

interfaces were washed three times with DMSO and twice with ethanol with stirring to remove the excess of unreacted reagents. Finally, the NTA-terminated surfaces were dried under a gentle stream of nitrogen.

Ni^{2+} loading

To form NTA-Ni^{2+} complex, a Ni^{2+} loading step is necessary. The NTA-terminated interfaces were dipped in a 50 mM NaOH during 5 min and thoroughly rinsed with ultrapure water to generate carboxylate groups. The deprotonated interfaces were dipped in a $NiCl_2$ aqueous solution during 1 h and then rinsed with water and ammonium citrate buffer (1 mM).

Preparation of OTS-terminated NanoSi

The NanoSi interface was UV/ozone-treated (UV O Cleaner, Jelight Company, Inc., 4 mW/cm^2 at 220 nm) for 20 min to remove any organic contaminants on the surface and to generate surface hydroxyl groups. The resulting surface was then reacted with a 10^{-3} M of OTS solution in hexane for 16 h at room temperature in a dry nitrogen purged glovebox. The resulting surface was rinsed with CH_2Cl_2, *i*-PrOH and dried under a gentle stream of nitrogen. The OTS-terminated NanoSi surface displays a superhydrophobic character with a water contact angle higher than 150°. A water droplet on the surface has a tendency to roll off. To allow analyte deposition, the contact angle of the OTS-terminated interface was decreased to 120° after its exposure to UV ozone irradiation for 5 min.

5) Peptide solution preparation

Two peptides were investigated in this study:

- Des-Arg^1-Bradykinin-6*His-Tag (**DAB-His**): (Ac)-*HHHHHH*RPPGFSPF-OH (MW=1768 Da, pI=9.84, 1 positive charge @ pH 7.4, polar) purchased from Innobiochips, France.
- Des-Arg^1-Bradykinin (**DAB**): NH_2-RPPGFSPF-OH (MW=904 Da, pI=10, 1 positive charge @ pH 7.4, relatively nonpolar) purchased from Sigma-Aldrich. Then, an equimolar solution of DAB/DAB-His (1/1) was prepared in a 1 mM adjusted ammonium citrate buffer (1 mM, pH 7.3) solution at 50 fmol/µL concentration.

6) LDI-MS experiments

The LDI-Mass spectrometry analysis were performed using a Voyager-DE-STR time-of-flight (ToF) mass spectrometer (Applied Biosystem) with delayed extraction, operating with a pulsed N_2 laser at 337 nm (3 ns pulse). NanoSi substrates were attached to the usual MALDI

target using conductive double-side carbon tape. Positive ion mass spectra were acquired with a reflector mode of operation, an accelerating potential of 20 KV, and a grid voltage at 73%. The extraction delay time was 200 ns and each spectrum is the result of ~50 averaged laser pulses.

1 µL of the peptide solution was deposited either on the NTA-Ni^{2+}-terminated or on OTS-terminated NanoSi surface at room temperature. After incubation on NTA-Ni^{2+}-terminated NanoSi surface, the surface was washed thoroughly several times with ammonium citrate (1 mM) buffer solution before LDI-MS analysis whereas spot of peptides, dried on OTS-terminated NanoSi surface, was analyzed without any further rinsing step.

Additional figures

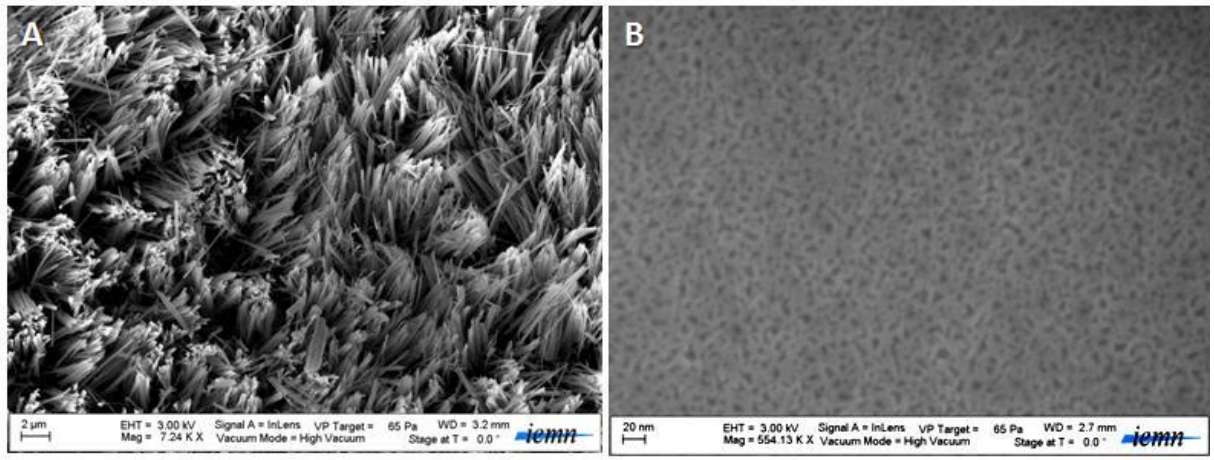

Figure S1: Top view SEM images of NanoSi (A) and pSi (B) substrates.

The figure S1A and B shows SEM images of nanostructured silicon (NanoSi) and porous silicon (pSi) interfaces.

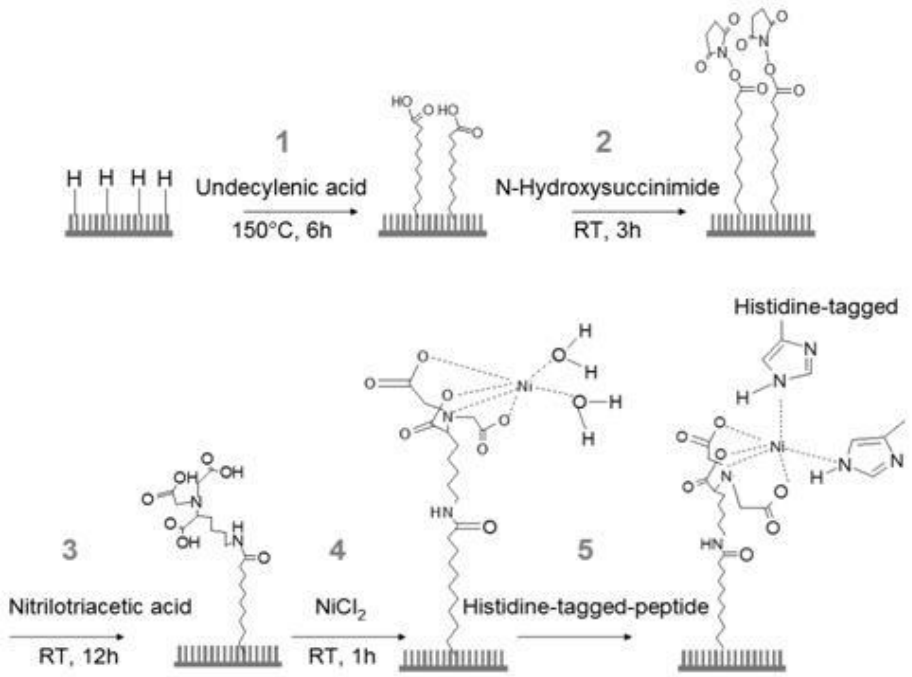

Figure S2: Schematic illustration of the functionalization steps for the formation of NTA-terminated silicon nanostructured surfaces: 1) hydrosilylation of undecylenic acid, 2) NHS-activation, 3) amino-NTA immobilization, 4) Ni^{2+} loading and 5) interaction with His-tag peptide.

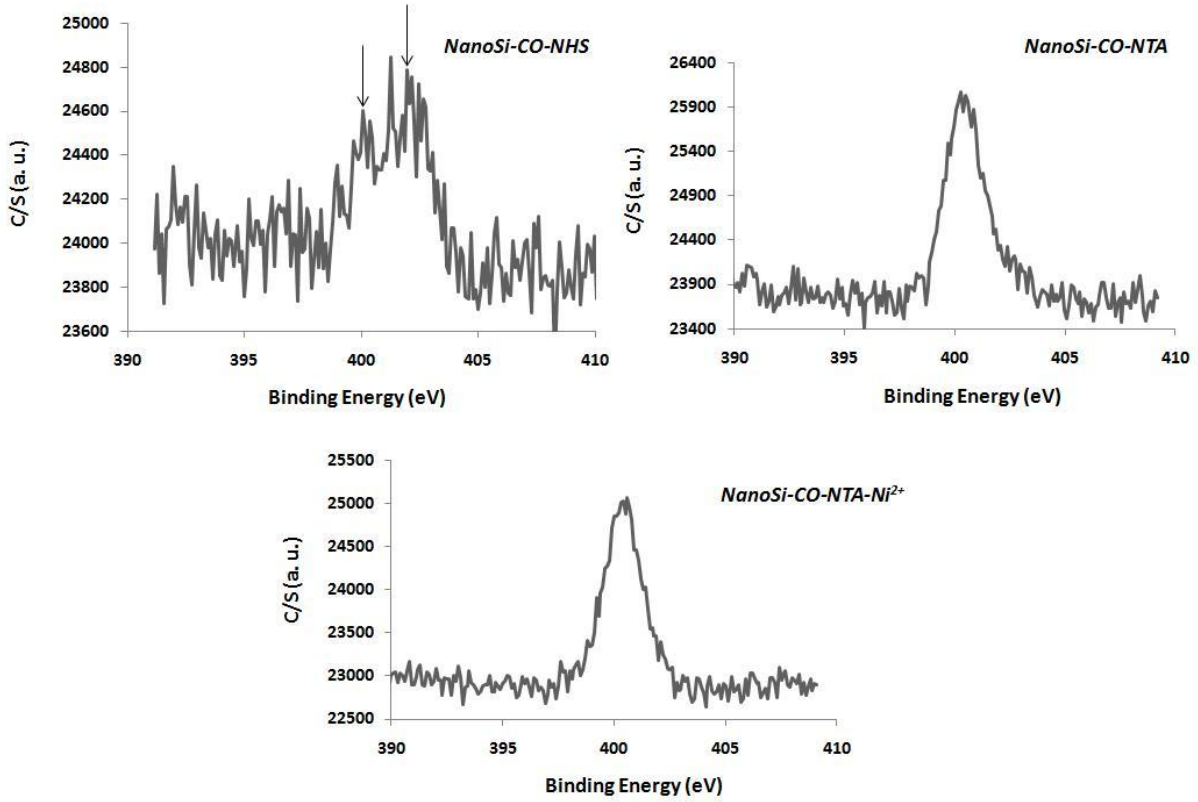

Figure S3: High resolution XPS spectra of N1s for NanoSi-NHS, NanoSi-NTA and NanoSi-NTA-Ni^{2+}.