Ambient Surface Analysis of Organic Monolayers using Direct Analysis in Real Time Orbitrap Mass Spectrometry

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Chemicals and materials

All chemicals and solvents (analytical grade) were obtained from Sigma Aldrich and used without purification, unless stated otherwise. Dichloromethane and 1,2-epoxydecene (Alfa Aesar, Karlsruhe, Germany) were distilled before use. Tris(2-aminoethyl)amine, perfluorononanoyl chloride, 2-[[methoxypoly(ethyleneoxy)$_{6-9}$]-propyl]trimethoxysilane were purchased from ABCR (Karlsruhe, Germany); 10-undecynylphosphonic acid was obtained from SiKÉMIA (Clapiers, France). Substrates to be investigated by DART-HRMS were typically 1 × 1 cm, unless noted otherwise. The gold substrates (200 nm of Au sputtered on glass) were purchased from SSens (Enschede, The Netherlands). Silicon nitride (Si_3N_4, 120 nm on Si) and glass (SiO_2) substrates were obtained from Lionix B.V. (Enschede, The Netherlands); the SiO_2 substrates were obtained from a 95.1 nm thick tetraethylorthosilicate (TEOS) layer, deposited on a Si surface and annealed at 1150 °C. Porous aluminum oxide (PAO) substrates (3.6 × 0.8 cm; average pore size 200 nm) were purchased from MicroDish BV (Utrecht, Netherlands). Single-side polished Si(111) (n-type, 475 - 550 μm thick, resistivity 1.0 - 5.0 Ω•cm) was purchased from Siltronix (Archamps, France). Microarray glass slides with 3D-NHS (N-hydroxy-succinimide ester groups), and PEGs were purchased from PolyAn GmbH (Berlin, Germany). Surface plasmon resonance (SPR) chips with carboxymethyl-polyethylene-glycol coating were ordered from XanTec bioanalytics (Duesseldorf, Germany). Sonication steps were performed in an Elmasonic P 30 H ultrasonic unit at a frequency of 80 kHz. Optical rotation was measured at 589 nm on a Perkin-Elmer 241 polarimeter.

Synthesis of azidoundecanyl carbohydrates (1, 4 and 7)

Synthesis of β-D-galactopyranosyl-(1→4)-1-(11-azido-undecanyl)-α-D-glucopyranoside (1)

The β-D-galactopyranosyl-(1→4)-1-(11-azidoundecanyl)-α-D-glucopyranoside (**1**) was synthesized as previously reported.[1,2]

Synthesis of 1-(11-azidoundecanyl)-α-d-mannopyranoside (4).

A solution of peracetylated mannose iodide[3] (**2**) (0.86 g, 1.9 mmol), 11-azido-1-undecanol (0.80 g, 3.75 mmol), and activated molecular sieves (1.15 g, 4 Å) in acetonitrile (12 mL) was cooled to 0 ºC under an argon atmosphere. Iodine (0.95 g, 3.75 mmol) was added and the mixture was stirred for 4 h and allowed to warm up to room temperature. The dark brown suspension was diluted with ethyl acetate (90 mL) and filtered. The filtrate was washed with 1 M $Na_2S_2O_3$ (2x 70 mL) and saturated NaCl solution (30 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent petroleum ether/ethyl acetate, 80:20) to yield 2,3,4,6-tetra-*O*-acetyl-1-(11-azidoundecanyl)-α-D-mannopyranoside (**3**) as a yellowish syrup. Intermediate **3** (0.30 g, 0.55 mmol) was then dissolved in dry methanol (3 mL) and a solution of sodium methoxide in methanol (0.5 M, 60 µL) was added. The mixture was stirred for 16 h and after complete conversion (monitored by TLC, eluent: ethyl acetate), additional methanol (6 mL) was added to fully dissolve the product. Afterwards, Dowex 50 (H^+ form) was added to the solution until the pH was 7 and the mixture was filtered over a Celite layer. The solution was concentrated under reduced pressure to yield 1-(11-azidoundecanyl)-α-D-mannopyranoside (**4**) as a cream-colored solid (0.15 g, 0.4 mmol, 22%). $[\alpha]_D^{20}$ +40.7 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 5.54 (1H, s, OH), 4.78 (1H,

d, H-1, J = 1.8 Hz), 3.86 (1H, dd, H-6a, J = 2.1 Hz, 11.8 Hz), 3.80 (1H, m, –OCHa–), 3.76 – 3.70 (3H, m, H-2, H-3, H-6b), 3.66 (1H, t, H-4, J = 9.4 Hz), 3.57 (1H, m, H-5), 3.45 (1H, m, –OCHb–), 3.32 (2H, t, $–CH_2–N_3$, J = 6.8 Hz), 1.68 – 1.59 (4H, m, $–C\underline{H_2}CH_2N_3$, $–OCH_2C\underline{H_2}–$), 1.49 – 1.32 (14H, m, $–(CH_2)_7–$). ^{13}C NMR (100 MHz, MeOD): δ 101.6 (CH, C-1), 74.6 (CH, C-5), 72.7, 72.3 (2 x CH, C-2, C-3), 68.7 (CH, C-4), 68.6 (CH_2, $–CH_2O–$), 62.9 (CH_2, C-6), 52.5 (CH_2, $–CH_2N_3$), 30.7, 30.6, 30.6, 30.6, 30.5, 30.2, 29.9, 27.8, 27.3 (9 x CH_2, $–(CH_2)_9–$, overlap of three peaks). HRMS: m/z 374.2291; calcd for $C_{17}H_{32}N_3O_6$ ($[M – H]^–$), 374.2286.

Synthesis of 1-(11-azidoundecanyl)-β-D-fucopyranoside (7).

Peracetylated fucose iodide (**5**) was prepared according to a method previously described in the literature.[3] A solution of **5** (2.19 g, 5.5 mmol), 11-azido-1-undecanol (2.33 g, 10.9 mmol), and activated molecular sieves (1.05 g, 4 Å) in acetonitrile (50 mL) was cooled to 0 ºC under an argon atmosphere. Iodine (2.77 g, 10.9 mmol) was added and the mixture was stirred for 16 h, slowly warming up to room temperature. The dark brown suspension was diluted with ethyl acetate (60 mL) and filtered. The filtrate was washed with 1 M $Na_2S_2O_3$ (2x 35 mL) and saturated NaCl solution (35 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent petroleum ether/ethyl acetate, 10:90 to 80:20) to yield 2,3,4-tri-*O*-acetyl-1-(11-azidoundecanyl)-β-D-fucopyranoside (**6**) as a yellowish syrup. Intermediate **6** (0.50 g, 1 mmol) was then dissolved in dry methanol (5 mL) and a solution of sodium methoxide in methanol (0.5 M, 100 µL) was added. The mixture was stirred for 16 h and after complete conversion (monitored by TLC, eluent: ethyl acetate), additional methanol (10 mL) was added to fully dissolve the product. Afterwards, Dowex 50 (H^+ form) was added to the solution until the pH was 7 and the mixture was filtered over a Celite layer. The solution was concentrated under reduced pressure to yield 1-

(11-azidoundecanyl)-β-D-fucopyranoside (**7**) as a colorless syrup (0.36 g, 0.9 mmol, 46%). $[\alpha]_D^{20}$ +12.2 (*c* 1.0, MeOH). ^{1}H NMR (400 MHz, MeOD): δ 4.80 (1H, s, OH), 4.17 (1H, d, H-1, J = 7.6 Hz), 3.83 (1H, dt, –OCHa–, J = 6.8, 9.4 Hz), 3.62 (1H, m, H-5), 3.59 (1H, m, H-4), 3.53 (1H, ddd, –OCHb–, J = 3.5, 6.7, 9.5 Hz), 3.46 (1H, m, H-3), 3.45 (1H, m, H-2), 3.27 (2H, t, $-CH_2-N_3$, J = 6.9 Hz), 1.64 – 1.55 (4H, m, $-C\underline{H_2}CH_2N_3$, $-OCH_2C\underline{H_2}-$), 1.42 – 1.29 (14H, m, $-(CH_2)_7-$), 1.26 (3H, d, H-6, J = 6.4 Hz). ^{13}C NMR (100 MHz, MeOD): δ 104.8 (CH, C-1), 75.2, 73.0, 72.3, 71.8 (4x CH, C-2, C-3, C-4, C-5), 70.8 (CH_2, $-CH_2O-$), 63.0 (CH_2, $-CH_2N_3$), 30.8, 30.7, 30.6, 30.6, 30.5, 30.2, 29.9, 27.8, 27.1 (9 x CH_2, $-(CH_2)_9-$, overlap of two peaks). HRMS: *m*/*z* 358.2343; calcd for $C_{17}H_{32}N_3O_5$ ($[M - H]^-$), 358.2336.

Synthesis of methoxy-tri(ethylene oxide) undec-1-ene, methoxy-hexa(ethylene oxide) undec-1-ene, and methoxy-nona(ethylene oxide) undec-1-ene.

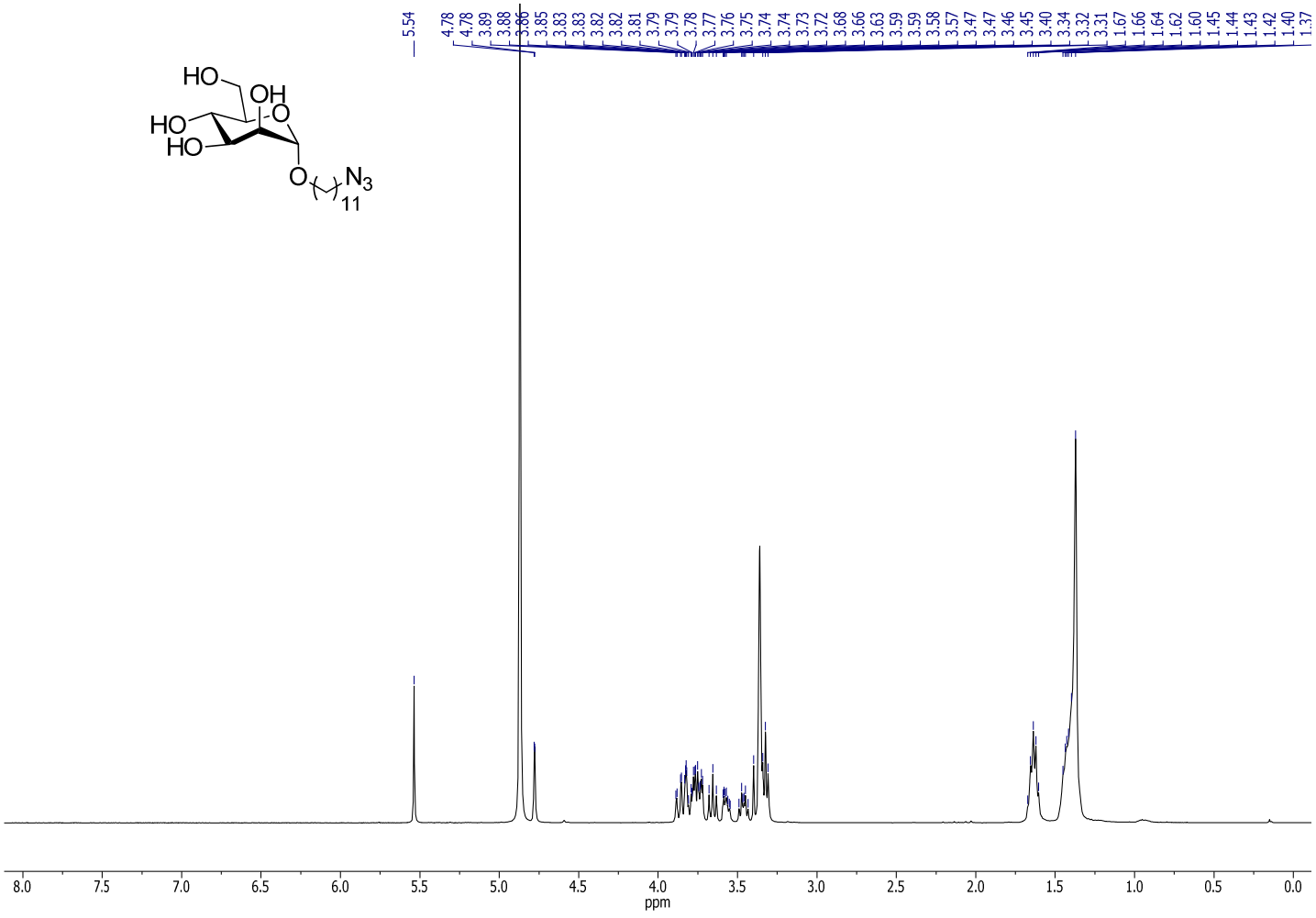
Methoxy-tri(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_3(CH_2)_9CH=CH_2$; **EO3**), methoxy-hexa(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_6(CH_2)_9CH=CH_2$; **EO6**), and methoxy-nona(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_9(CH_2)_9CH=CH_2$; **EO9**) were synthesized according to the literature.[4] The compounds **EO3**, **EO6** and **EO9** were purified before use with preparative HPLC (column C18; isocratic separation in MeCN/H_2O 90:10, UV (195 nm and 220 nm) and ELSD detection).

NMR spectra

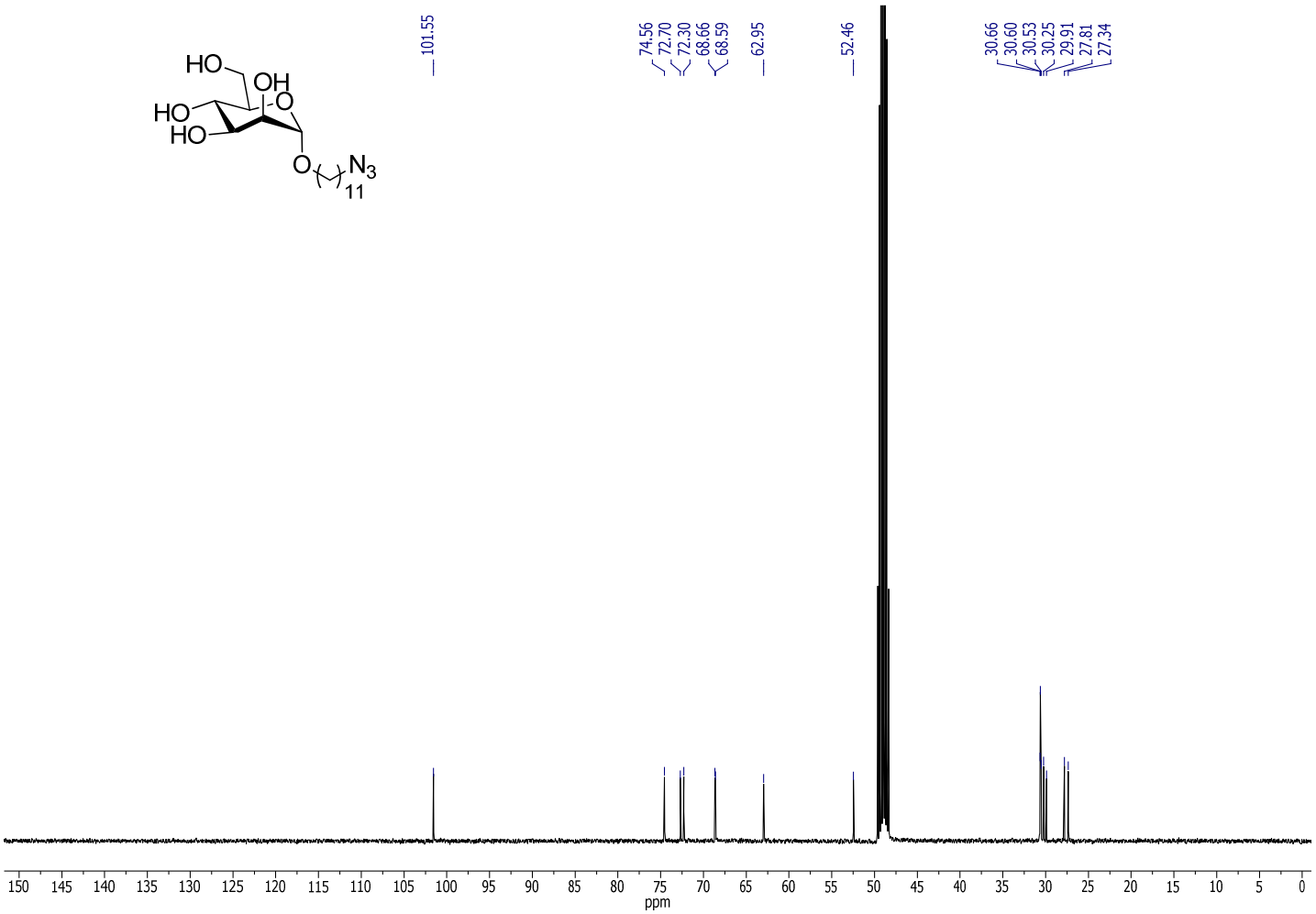
Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 400 MHz spectrometer. NMR peak assignments were made based on COSY and HSQC experiments.

NMR spectra of 1-(11-azidoundecanyl)-α-D-mannopyranoside (4)

^{1}H NMR spectrum of **4**

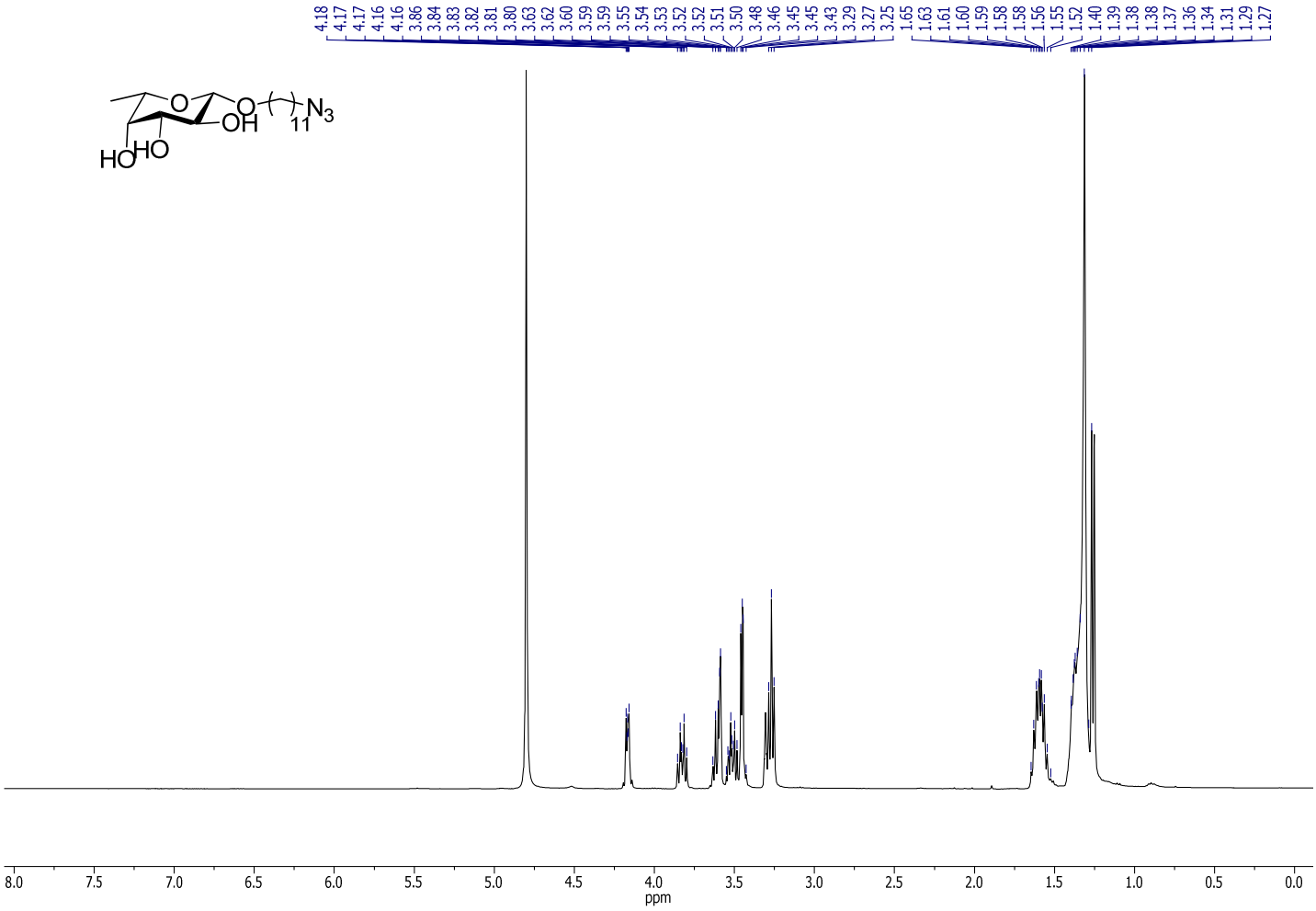


^{13}C NMR spectrum of **4**

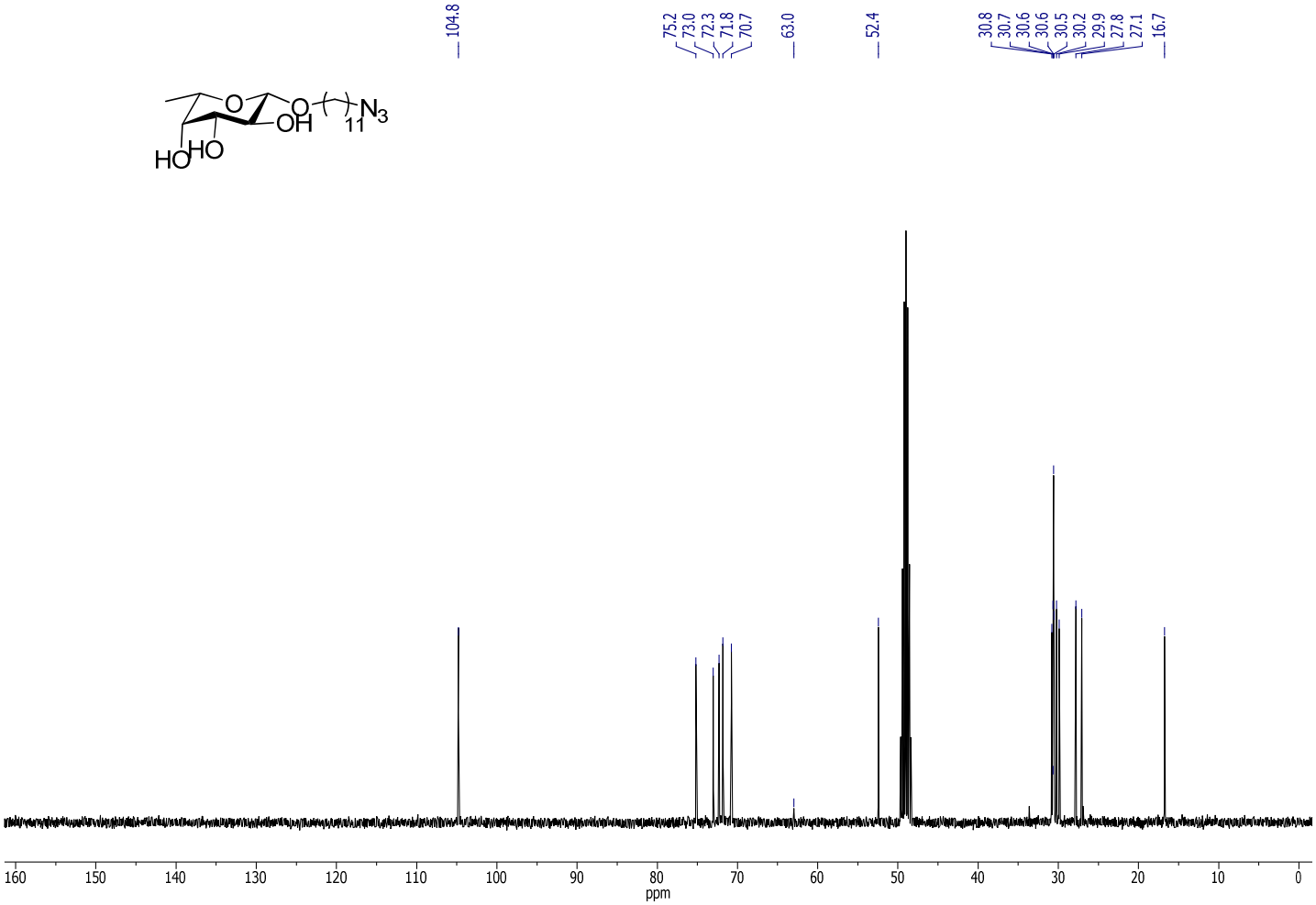


NMR spectra of 1-(11-azidoundecanyl)-β-D-fucopyranoside (7)

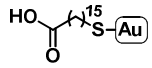
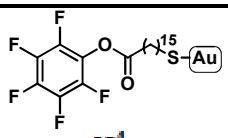
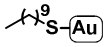
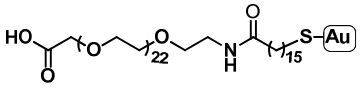
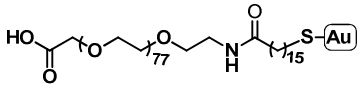
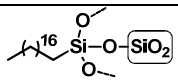
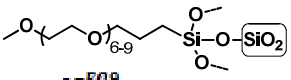
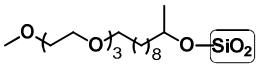
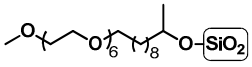
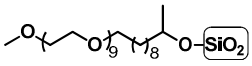
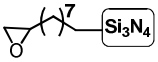
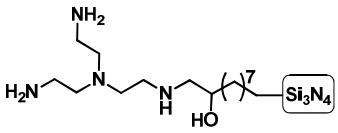
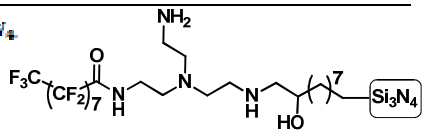
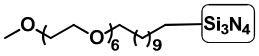
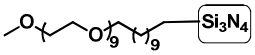
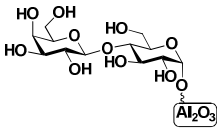
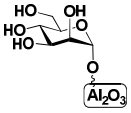
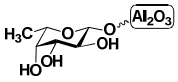
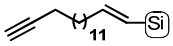
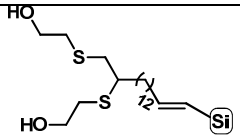
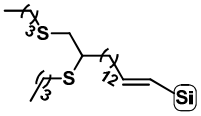
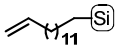
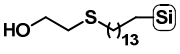
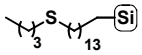
^{1}H NMR spectrum of **7**



^{13}C NMR spectrum of **7**



Preparation of monolayers with different head and tail groups.

Substrate	Symbol and the corresponding monolayer structure
Au	M^1_{Au}  M^2_{Au}  M^5_{Au}  M^3_{Au}  M^4_{Au} 
SiO_2	$M^1_{SiO_2}$  $M^2_{SiO_2}$  $M^{EO3}_{SiO_2}$  $M^{EO6}_{SiO_2}$  $M^{EO9}_{SiO_2}$ 
Si_3N_4	$M^1_{Si_3N_4}$  $M^2_{Si_3N_4}$  $M^3_{Si_3N_4}$  $M^{EO6}_{Si_3N_4}$  $M^{EO9}_{Si_3N_4}$ 
Al_2O_3	$M^{Lac}_{Al_2O_3}$  $M^{man}_{Al_2O_3}$  $M^{fuc}_{Al_2O_3}$ 
Si	M^1_{Si}  M^2_{Si}  M^3_{Si}  M^4_{Si}  M^5_{Si}  M^6_{Si} 

a) Preparation of monolayers on gold

Prior to modification, the gold substrates were rinsed with ethanol and water followed by drying under nitrogen. The specimens were immersed in a 1 mM solution of the 16-mercaptohexadecanoic acid (**MHDA**) in ethanol for 24 h. The MHDA-modified surfaces ($\boldsymbol{M^1_{Au}}$, Table 1) were then removed and rinsed with ethanol and water, sonicated in ethanol, and dried under nitrogen. The acid terminated substrates ($\boldsymbol{M^1_{Au}}$) were activated using 2,3,4,5,6-pentafluorophenol (**PFP**) and *N,N′*-dicyclohexylcarbodiimide (**DCC)** as reported earlier.[2] The PFP-terminated substrates ($\boldsymbol{M^2_{Au}}$) were then immersed in a 1 mg/ml solution of either **NH_2-$(CH_2CH_2O)_{22}$-COOH** (1 kDa) or **NH_2-$(CH_2CH_2O)_{77}$-COOH** (3.5 kDa) in dichloromethane for 24 h, resulting in layers $\boldsymbol{M^3_{Au}}$ and $\boldsymbol{M^4_{Au}}$, respectively. The PEG-modified surfaces were removed and rinsed with dichloromethane, sonicated in the same solvent, and dried under nitrogen. The monolayer $\boldsymbol{M^5_{Au}}$ was prepared as described above using **1-decanethiol** instead of **MHDA**.

b) Preparation of monolayers on silicon nitride (Si_3N_4)

Preparation of the epoxy- terminated monolayer $\boldsymbol{M^1_{Si_3N_4}}$ (primary modification).

Silicon nitride substrates were cleaned by rinsing and sonication in acetone for 3 min and dried in a stream of argon. Thereafter, the substrates were exposed to an O_2-plasma for 5 min. The resulting oxidized surfaces were etched in 2.5% HF solution for 2 min, properly rinsed with water and finally dried with argon. The prepared samples were immediately used for modification. For preparation of the epoxy- terminated monolayer $\boldsymbol{M^1_{Si_3N_4}}$ an 1,2-epoxy-9-

decene was transferred into a clean, dry, and degassed, flat bottom quartz flask, followed by three consecutive freeze-pump-thaw cycles to remove trace amounts of oxygen and water. Finally, the flask was backfilled with argon and the alkene was frozen again and freshly cleaned samples were transferred in the flask. The grafting of Si_3N_4 samples was carried out following the UV-assisted method described elsewhere[5]. Two UV pen lamps (254 nm, 9 mW•cm^{-2}, low pressure mercury vapor, double bore lamps from Jelight Company Inc., California) were aligned at a distance of ~5 mm from the flat bottom of the flask and the samples were irradiated for 10 h in argon atmosphere at ambient temperature. After the formation of $\boldsymbol{M^{1}_{Si_3N_4}}$, the samples were removed from the flask and cleaned by rinsing and sonication in DCM for 3 min to remove physisorbed molecules and dried under argon.

Preparation of amine-terminated monolayer $\boldsymbol{M^{2}_{Si_3N_4}}$ (secondary modification). The epoxide-terminated silicon nitride surfaces $\boldsymbol{M^{1}_{Si_3N_4}}$ were transferred into neat, argon saturated tris(2-aminoethyl)amine in a flat-bottom flask. The flask was closed under argon and the reaction mixture was kept for 16 h at 40 °C. Subsequently, the samples were rinsed several times with ethanol, sonicated in ethanol for 3 min rinsed with DCM and finally dried with an argon flow.

Preparation of reversed amide monolayer $\boldsymbol{M^{3}_{Si_3N_4}}$ (tertiary modification).

Amine-terminated silicon nitride surfaces were transferred into 5 ml degassed, freshly prepared 0.1 M solution of perfluorononanoyl chloride in $C_2H_2Cl_2$ in a flat bottom flask. Thereafter, 70 µl triethylamine (one equivalent pertaining to the perfluorononanoyl chloride) were added to the reaction mixture under argon. The flask was closed under argon and kept for 16 h at 60 °C.

Subsequently, the samples were rinsed several times with ethanol and DCM, sonicated in DCM for 3 min and finally dried under argon.

Preparation of oligo(ethylene glycol)-terminated monolayers.

Preceding the modification, Si_3N_4 samples were cleaned and etched (2.5 % HF, 2.5 min) as described elsewhere.[2] Afterwards, the UV-induced formation of oligoethylene glycol monolayers on Si_3N_4 was carried out following a procedure as reported.[4] The methoxy-hexa(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_6(CH_2)_9CH=CH_2$; **EO6**) and methoxy-nona(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_9(CH_2)_9CH=CH_2$; **EO9**) were used for the preparation of monolayers $M^{EO6}_{Si_3N_4}$ and $M^{EO9}_{Si_3N_4}$, respectively.

c) Preparation of monolayers on glass (SiO_2)

Formation of octadecyl-monolayer and mixed oligo(ethylene glycol)-terminated monolayer

Silicon oxide samples were cleaned by rinsing and sonication in acetone for 3 min, dried with argon and treated with O_2-plasma for 5 min. Hereafter, the substrates were activated in a freshly made 1:1 (v:v) mixture of hydrochloric acid (37%) and methanol for at least 30 min, rinsed with water and methanol and dried with argon. The prepared samples were immediately used for modification as follows. Dry toluene (3 ml) and 30 µl of the silane were transferred into a dry, argon filled, flat bottom flask which was backfilled with argon. The clean SiO_2 surfaces were transferred into this solution and left overnight at ambient temperature. The samples were removed from the flask, rinsed and sonicated in toluene to remove physisorbed molecules and dried with argon. Subsequently, the samples were cured at 130 °C at reduced pressure for at least 4 hours. Afterwards, the samples were again rinsed and sonicated in toluene and dried under

argon. Trichloro(octadecyl)silane was used to obtain octadecyl monolayer on SiO_2 ($M^1_{SiO_2}$), while 2-[[methoxypoly(ethyleneoxy)$_{6-9}$]-propyl]trimethoxysilane was employed to obtain oligo(ethylene glycol)-terminated monolayer ($M^2_{SiO_2}$), containing 6 to 9 ethylene oxide units.

Preparation of oligo(ethylene glycol)-terminated monolayers on SiO_2

Preceding the modification, SiO_2 substrates were cleaned and etched in a freshly prepared 1:1 (v:v) mixture of hydrochloric acid (37%) and methanol as described above. The substrates were, then, rinsed with water and methanol, dried with argon and transferred in a quartz reactor to carry out UV-induced monolayer formation as reported.[5] The samples were irradiated for 10 h in the presence of methoxy-poly(ethylene oxide) undec-1-ene. For instance, methoxy-tri(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_3(CH_2)_9CH=CH_2$; **EO3**), methoxy-hexa(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_6(CH_2)_9CH=CH_2$; **EO6**), and methoxy-nona(ethylene oxide) undec-1-ene ($CH_3O(CH_2CH_2O)_9(CH_2)_9CH=CH_2$; **EO9**) were utilized for the formation of monolayers $M^{EO3}_{SiO_2}$, $M^{EO6}_{SiO_2}$ and $M^{EO9}_{SiO_2}$, respectively.

d) Preparation of monolayers on alumina (Al_2O_3)

The monolayer preparation was done according to the literature.[1] Briefly, PAO substrates were rinsed and sonicated in acetone (5 min) and ultrapure water (5 min). After this the PAO surfaces were immersed in a fresh mixture of 37% hydrochloric acid and methanol (1:1, v/v) for 30 min. Then, the substrates were rinsed and sonicated with ultrapure water and absolute ethanol and finally were immersed in a 1 mM solution of 10-undecynylphosphonic acid in absolute ethanol (room temperature, 16 h). Hereafter, the substrates were washed with absolute ethanol, heated at 140 ºC under vacuum for 6 h. After the curing step, the samples were rinsed and sonicated in

ethanol and dichloromethane (5 min each), and dried in air. Afterwards, a copper-catalyzed azide–alkyne cycloaddition (CuAAC) reaction on the alkyne-terminated PAO surfaces was done in a reaction tube using a solution of azidoundecanyl carbohydrate (0.1 mM), copper (I) sulfate (0.2 mM), and sodium ascorbate (0.2 mM). The reaction tube was equipped with a stirring bar and a platform to protect the fragile surface from the stirring bar. The alkyne-terminated PAO surfaces were immersed in the solution and heated in a microwave oven (CEM Discover) for 30 min at 70 ºC under stirring. After the reaction, the substrate was thoroughly washed and sonicated in water, ethanol, and dichloromethane (5 min per solvent) and dried in air. In this way, utilizing **1**, **4**, and **7,** three types of sugar-terminated monolayers were prepared on PAO substrates: lactose-, mannose- and fucose-terminated monolayers ($M^{lac}_{Al_2O_3}$, $M^{man}_{Al_2O_3}$, $M^{fuc}_{Al_2O_3}$, accordingly).

e) Preparation of monolayers on silicon Si(111)

The modification of Si (111) was performed according to a procedure described in the literature.[6] Briefly, an alkyne-terminated monolayer M^{1}_{Si} was formed on Si(111) using 1,15-hexadecadiyne and heating at 80 °C, under oxygen free and water free conditions for 16 h. The resultant alkyne-terminated monolayers were further modified via a thiol-yne click reaction with freshly prepared mixture of a thiol and 2,2-dimethoxy-2-phenylacetophenone (DMPA) as photoinitiator in a 5 : 1 molar ratio. A few drops of freshly prepared reaction mixture (thiol and initiator) were transferred onto an alkyne-terminated monolayer on Si(111) substrates. The thiol-yne click reaction was initiated by irradiating with 365 nm light (800 μW/cm^2, Spectroline, Westbury, NY) for 1.5 h. Afterwards, the modified Si substrates were rinsed with THF and

dichloromethane and sonicated for 10 min in these solvents. The reactions were performed either with 2-mercaptoethanol or butanethiol to obtain monolayers M^{2}_{Si} and M^{3}_{Si}, respectively.

Similarly, alkene-terminated monolayers M^{4}_{Si} were prepared on Si(111) wafers using 1,13-tetradecadiene.[6] The alkene-terminated monolayers were further modified via a thiol-ene click reaction with freshly prepared mixture of a thiol and DMPA as described above. A few drops of it were transferred onto an alkene-terminated monolayer on Si(111) substrates and subsequently the substrates were irradiated with the same source of UV light (365 nm, 800 μW/cm^2, Spectroline, Westbury, NY) for 1.5 h. Afterwards, the modified Si substrates were rinsed with THF and dichloromethane, and sonicated for 10 min in the same solvents. The a thiol-ene click reactions were done either with 2-mercaptoethanol or butanethiol to obtain monolayers M^{5}_{Si} and M^{6}_{Si}, correspondingly.

X-ray Photoelectron Spectroscopy (XPS).

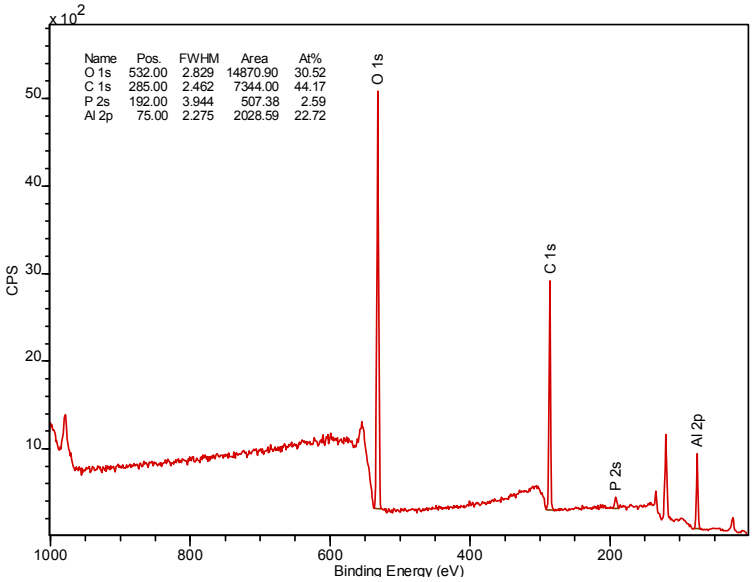
Prior to DART-HRMS analysis, the monolayer formation and quality were first assessed by XPS. Before the analysis with XPS, all substrates were extensively cleaned by sonication in appropriate solvents, dried under a stream of argon and stored under oxygen-free and water-free conditions (in a glovebox MBraun MB200G, under an argon atmosphere and a content of H_2O and O_2 each below 0.1 ppm) till the XPS measurement. XPS analyses were performed using a JPS-9200 photoelectron spectrometer (JEOL). The spectra were obtained under ultrahigh

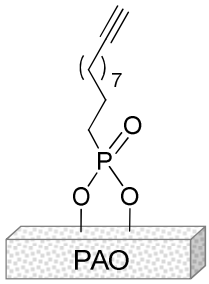
vacuum (UHV) conditions using monochromatic Al Kα X-ray radiation at 12 kV and 20 mA, and an analyzer pass energy of 10 eV. The X-ray incidence angle and the electron acceptance angle were 80° and 10° to the surface normal, respectively. In case of electrostatic charging in the positive direction on the surface, a charge compensation was used during the XPS scans with an accelerating voltage of 2.8 eV and a filament current of 5.00 A. Afterwards, the spectra were reprocessed with the CASA XPS peak fit program (version 2.3.15) using the alkyl C 1s component calibrated at 285.0 eV. For the curve fitting of C 1s spectra, linear background subtraction and a Gaussian/Lorentzian peak shape model GL(30) were used. The full widths at half maximum (FWHM) were constrained to be equal for all peaks within one spectrum, resulting in FWHM values ranging from 0.9 to 1.4 eV. After successful confirmation of the resultant monolayers, the samples were subjected to DART-HRMS analysis.

XPS spectra

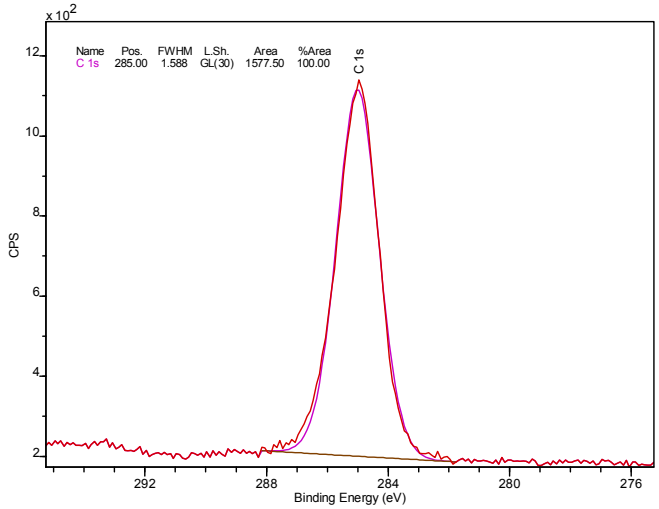
XPS spectra of monolayers on Al_2O_3

a)





b)



c)

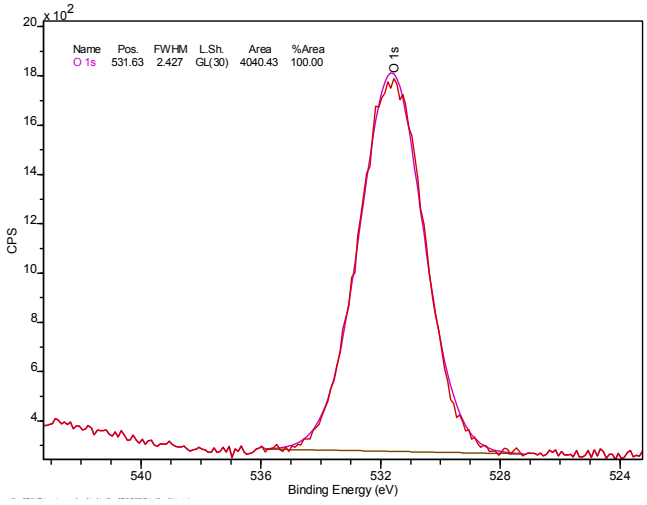


Figure SS1. XPS spectra of alkyne-terminated monolayer on alumina before "click reactions" with **1**, **4** or **7** (before formation of lactose-, mannose- or fucose-terminated monolayers). a) survey spectrum lacking a N 1s peak; b) C 1s narrow scan c) O 1s narrow scan.

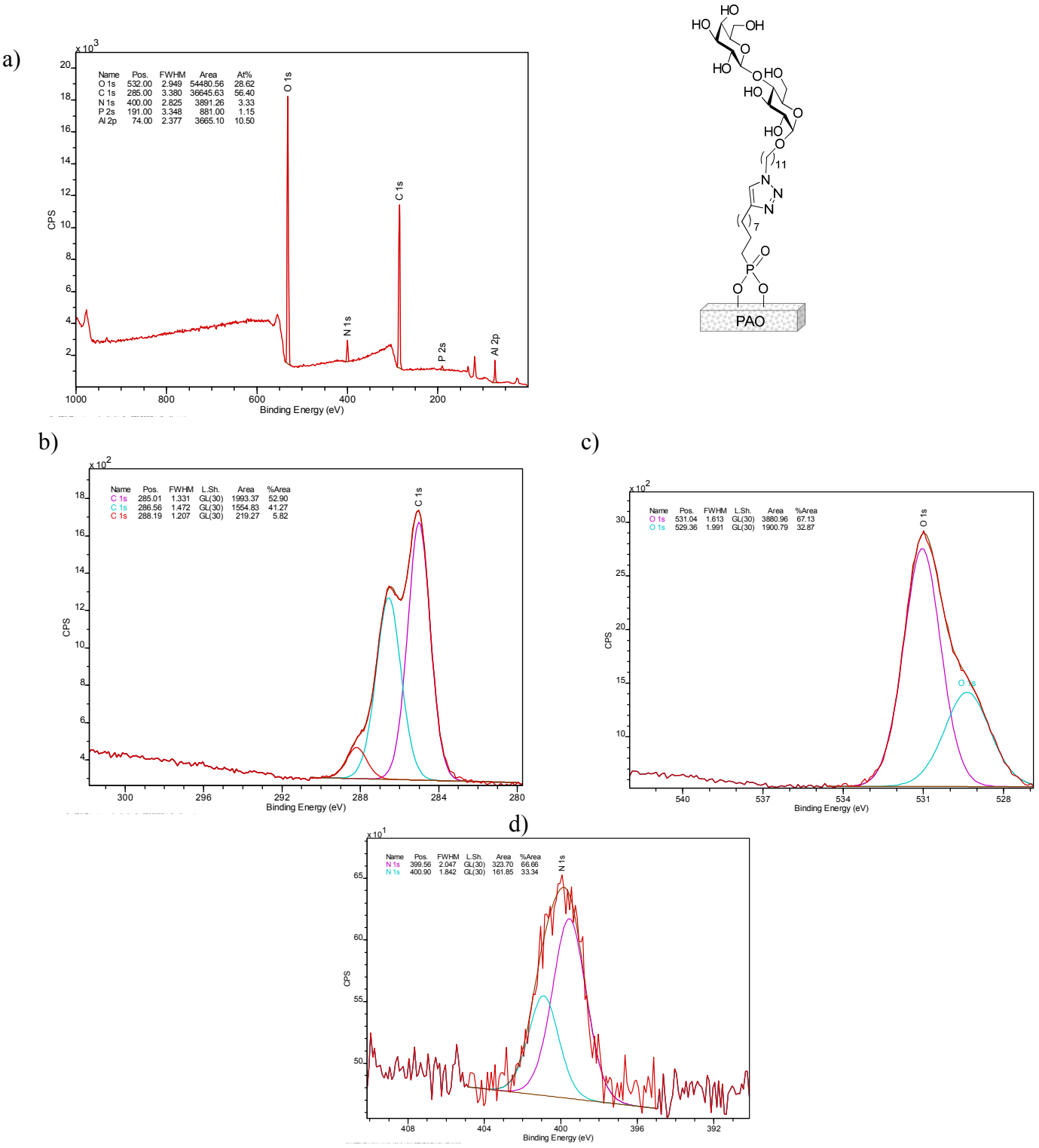
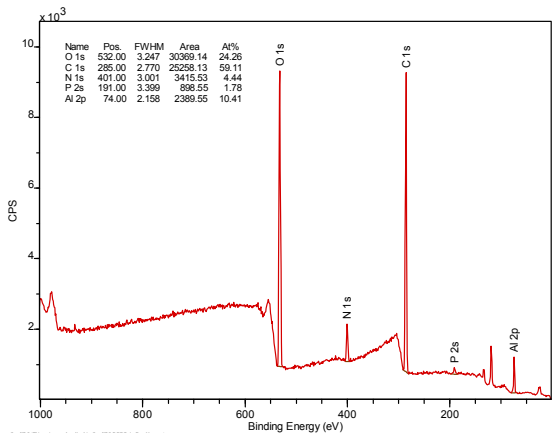
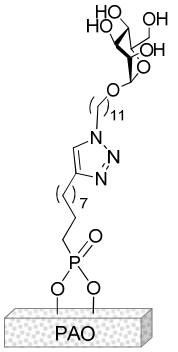


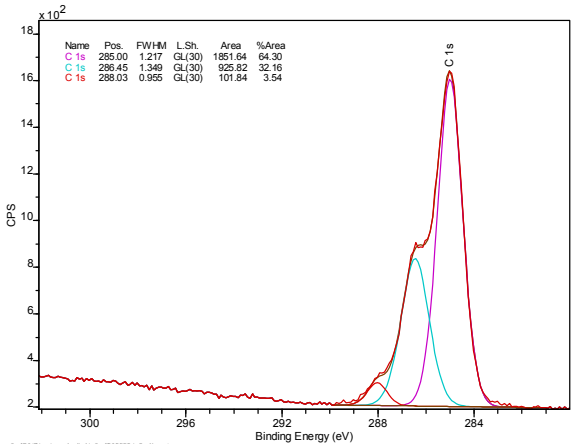
Figure SS2. XPS spectra of lactose-terminated monolayer $M^{lac}_{Al_2O_3}$ (after "click reaction" with **1**). a) survey spectrum with presence of N 1s peak; b) C 1s narrow scan and a clear contribution of C-O bonds introduced by the attachment of **1** on the surface c) O 1s narrow scan d) N 1s narrow scan indicating formation of triazole ring and lack of azide group at 404 eV Binding Energy (BE).

a)

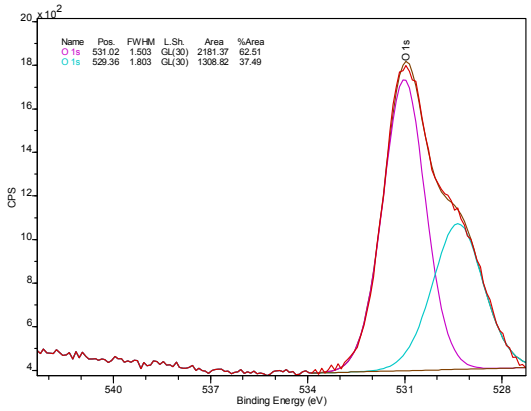




b)



c)



d)

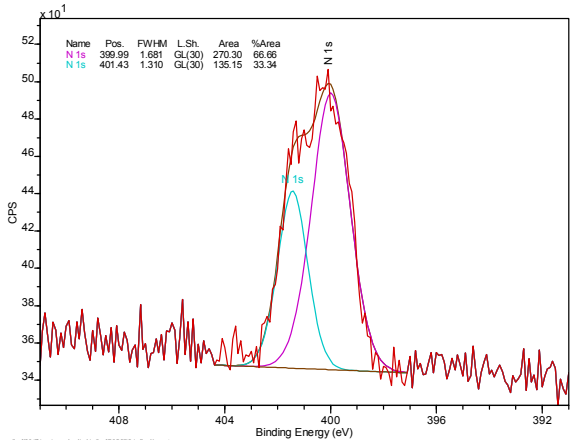


Figure SS3. XPS spectra of manose-terminated monolayer $M^{mon}_{Al_2O_3}$ (after "click reaction" with **4**). a) survey spectra with N 1s peak; b) C 1s narrow scan with a clear contribution of C-O bonds introduced with the attachment of the sugar on the surface c) O 1s narrow scan d) N 1s narrow scan indicating formation of triazole ring and lacking peak for an azide group at 404 eV (BE).

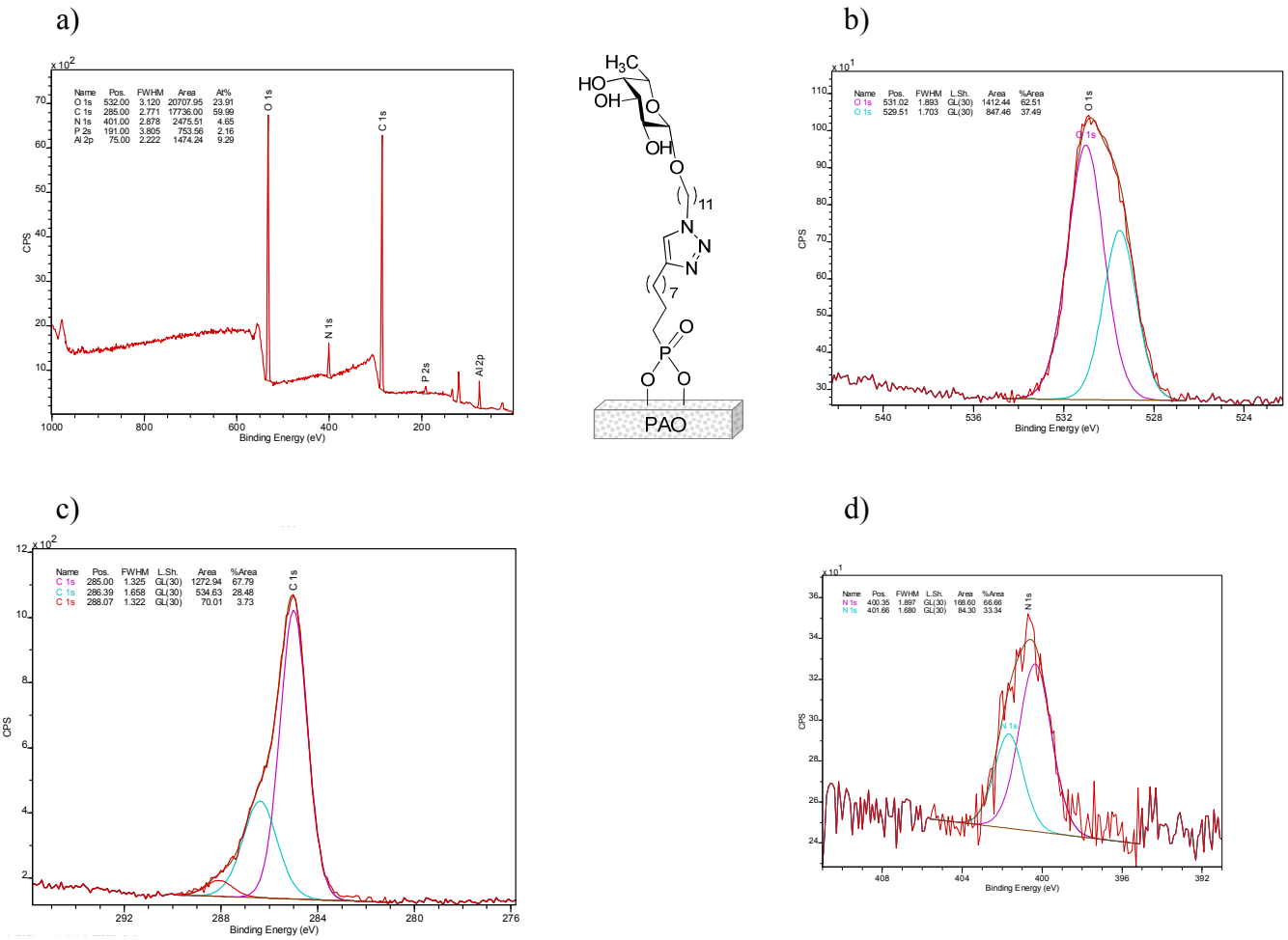
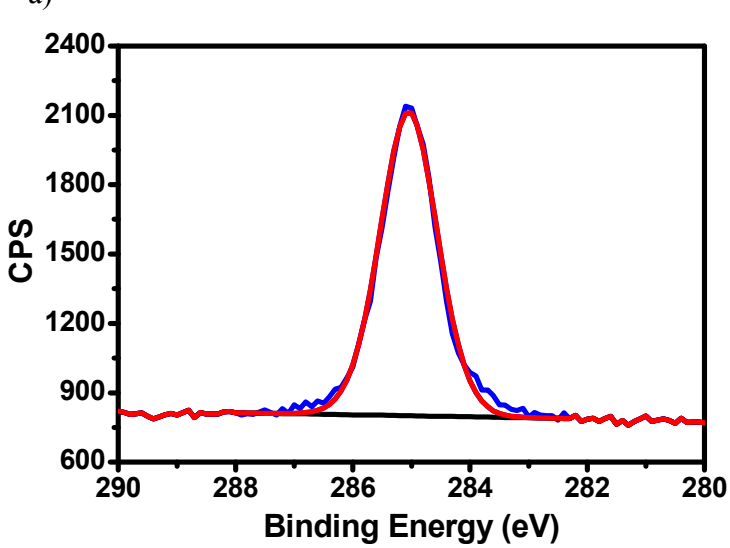
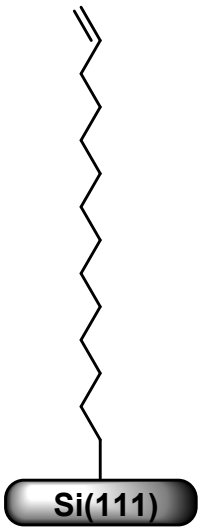


Figure SS4. XPS spectra of mannose-terminated monolayer $M^{fuc}_{Al_2O_3}$ (after "click reaction" with **7**). a) survey spectra with N 1s peak; b) C 1s narrow scan with a clear contribution of C-O bonds introduced with the attachment of the sugar on the surface c) O 1s narrow scan d) N 1s narrow scan indicating formation of triazole ring and the lack of an azide group at 404 eV (BE).

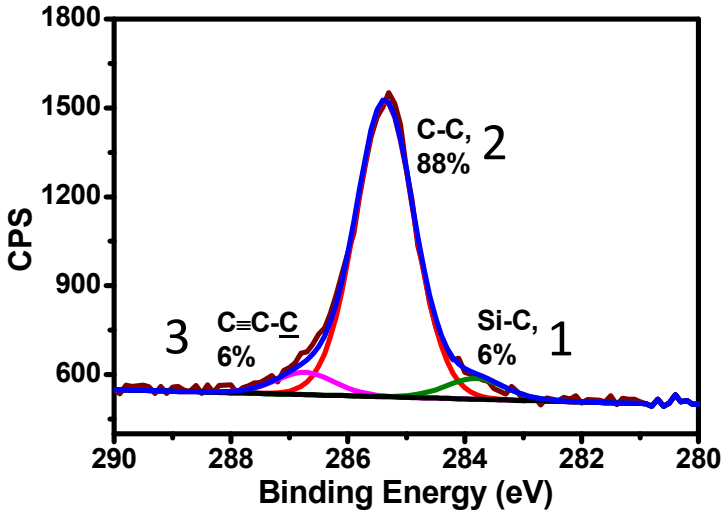
XPS spectra of monolayers on Si(111)

a)





b)



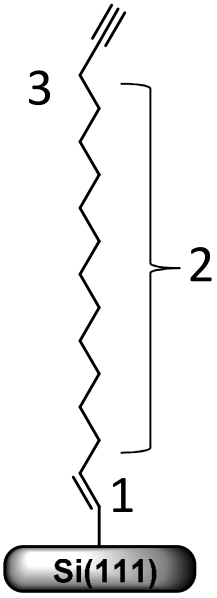
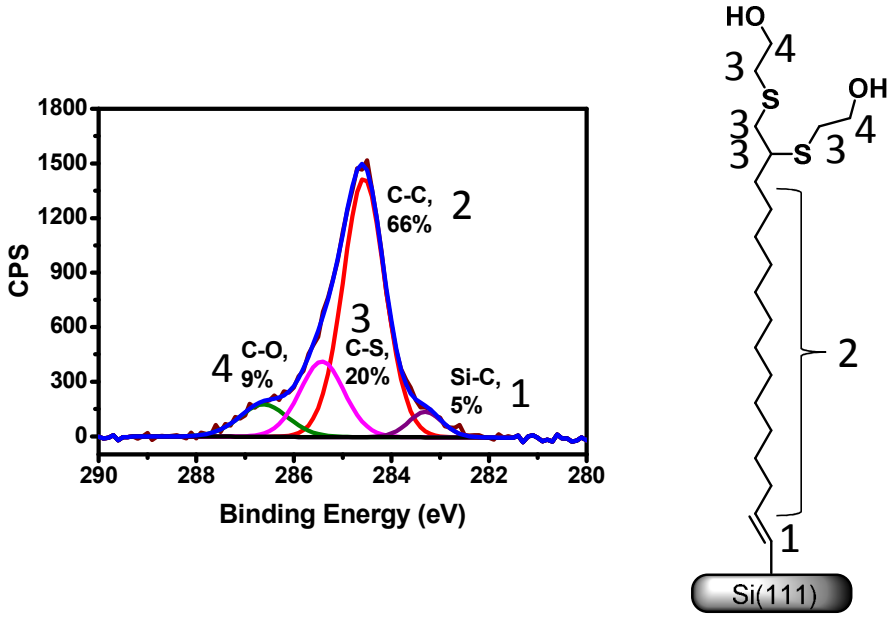


Figure SS5. XPS spectra[6] of monolayer a) M_{Si}^{1} and b) M_{Si}^{1}.

a)



b)

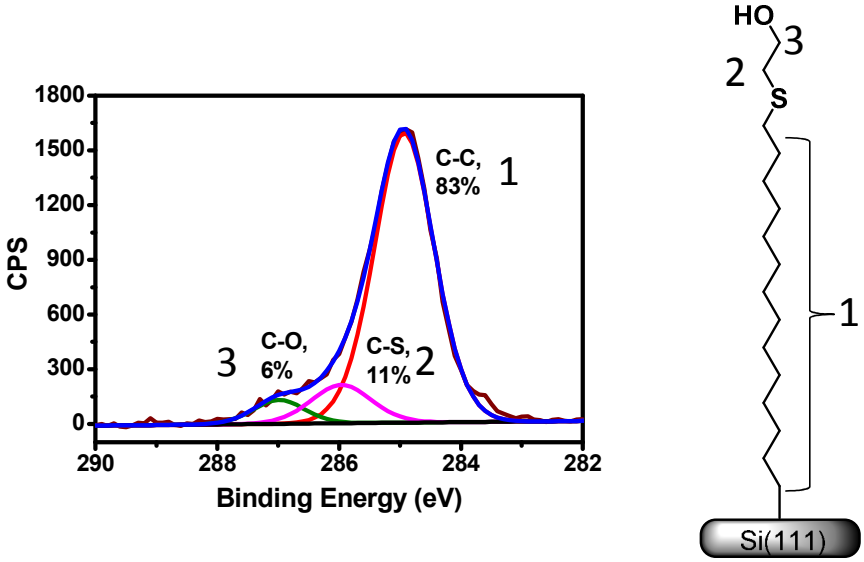
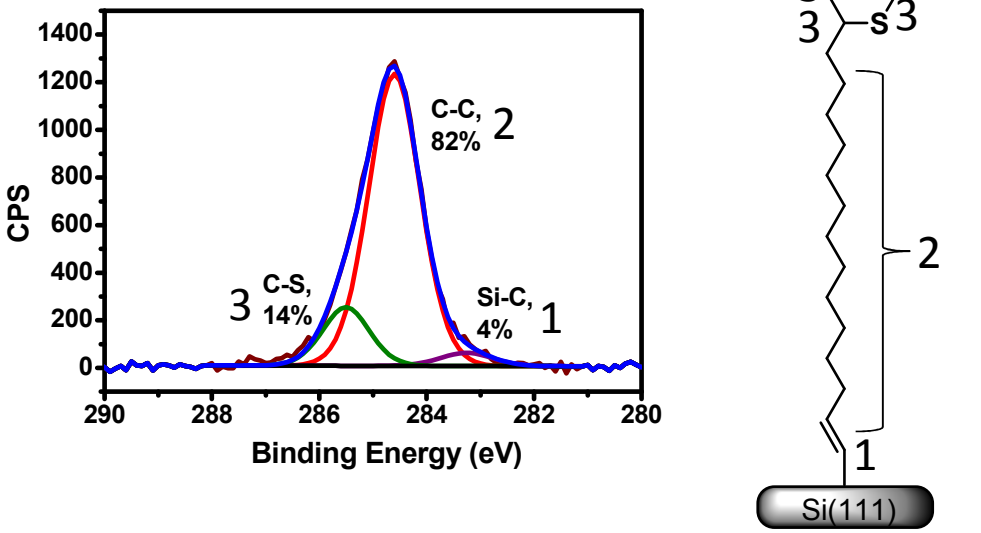


Figure SS6. XPS spectra[6] of monolayer a) M_{Si}^{2} and b) M_{Si}^{3}

a)



b)

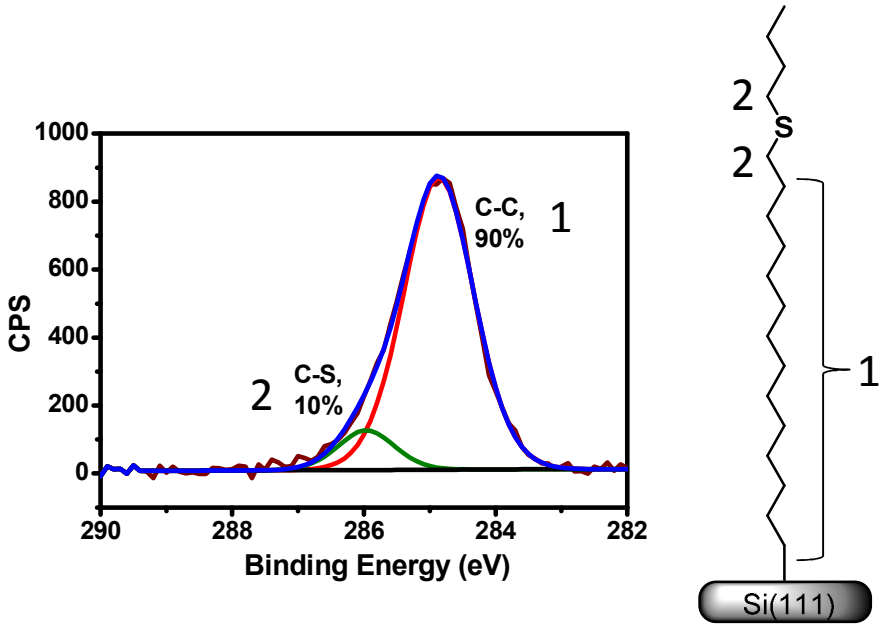
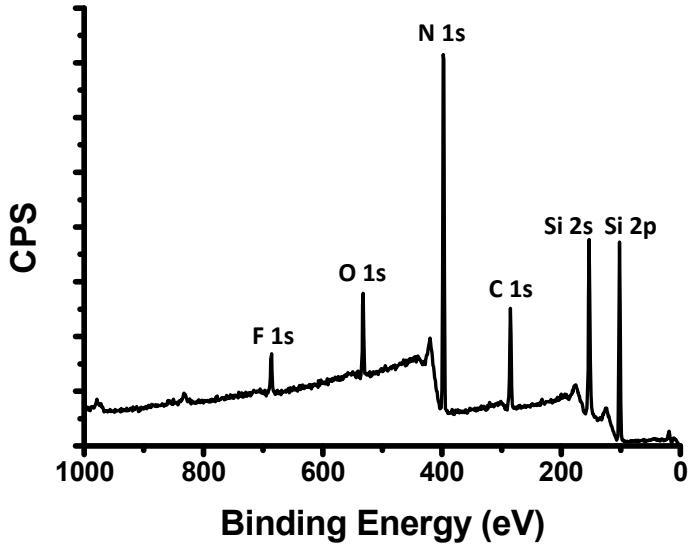


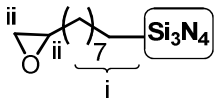
Figure SS7. XPS spectra of monolayer a) M_{Si}^{3} and b) M_{Si}^{6}

XPS spectra of monolayers on Si_3N_4

a)



b)



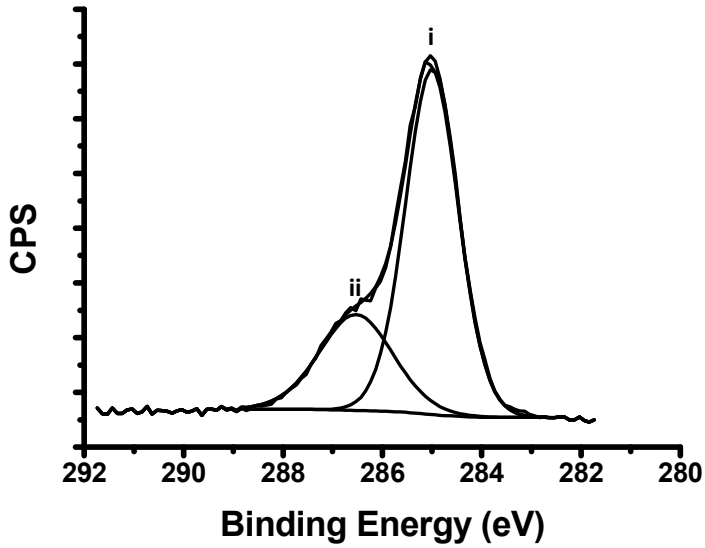


Figure SS8. XPS spectra of monolayer $M^1_{Si_3N_4}$ a) survey spectra and b) C 1s narrow scan

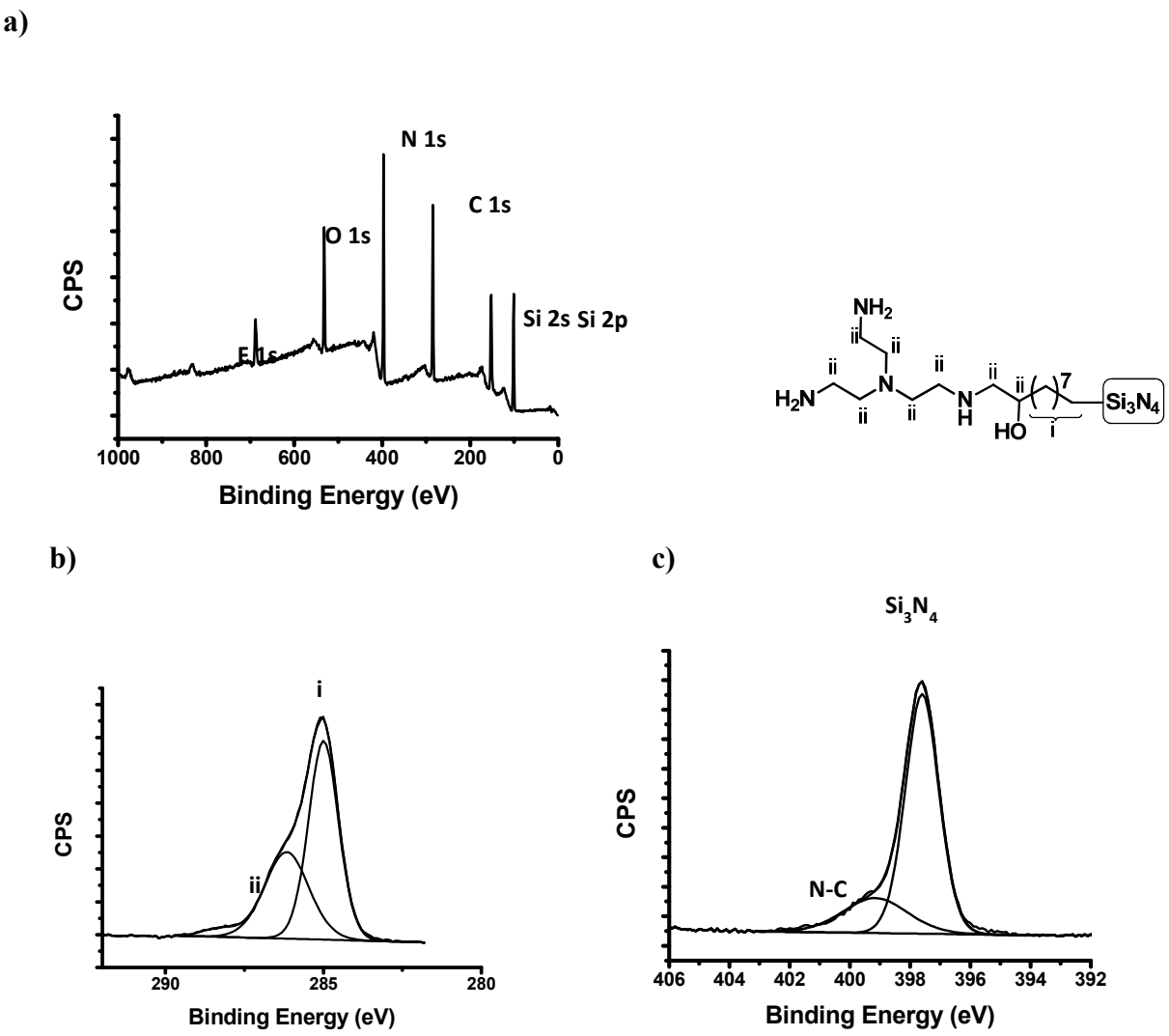
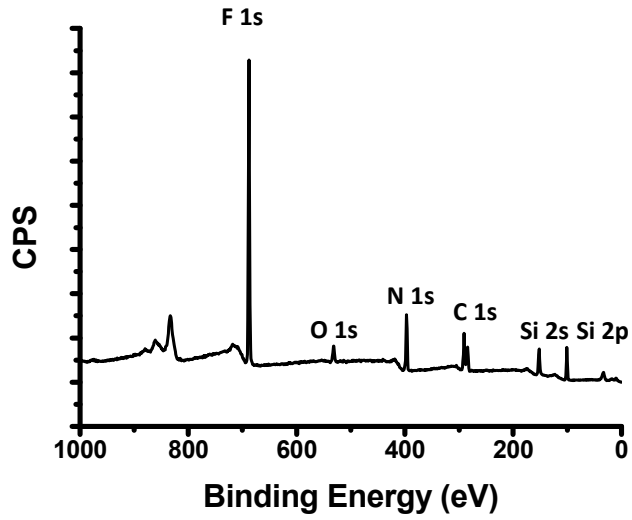
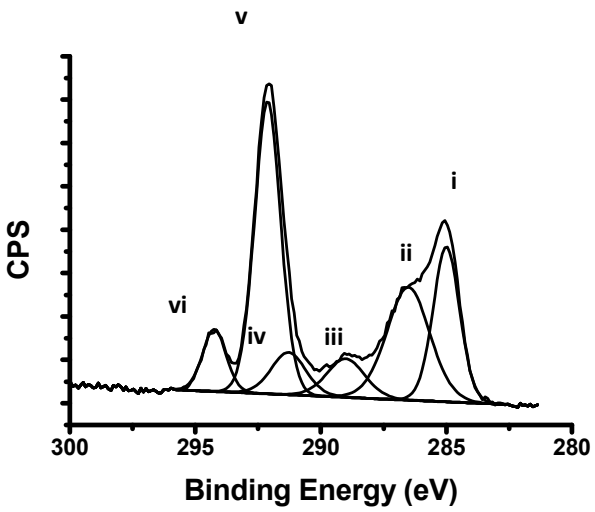


Figure SS9. XPS spectra of monolayer $M^2_{Si_3N_4}$ a) survey spectra and b) C 1s narrow scan c) N 1s spectra with a new contribution indicating formation of N-C bonds

a)



b)



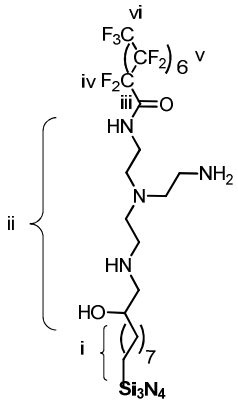


Figure SS10. XPS spectra of monolayer $M^3_{Si_3N_4}$ a) survey spectra and b) C 1s narrow scan

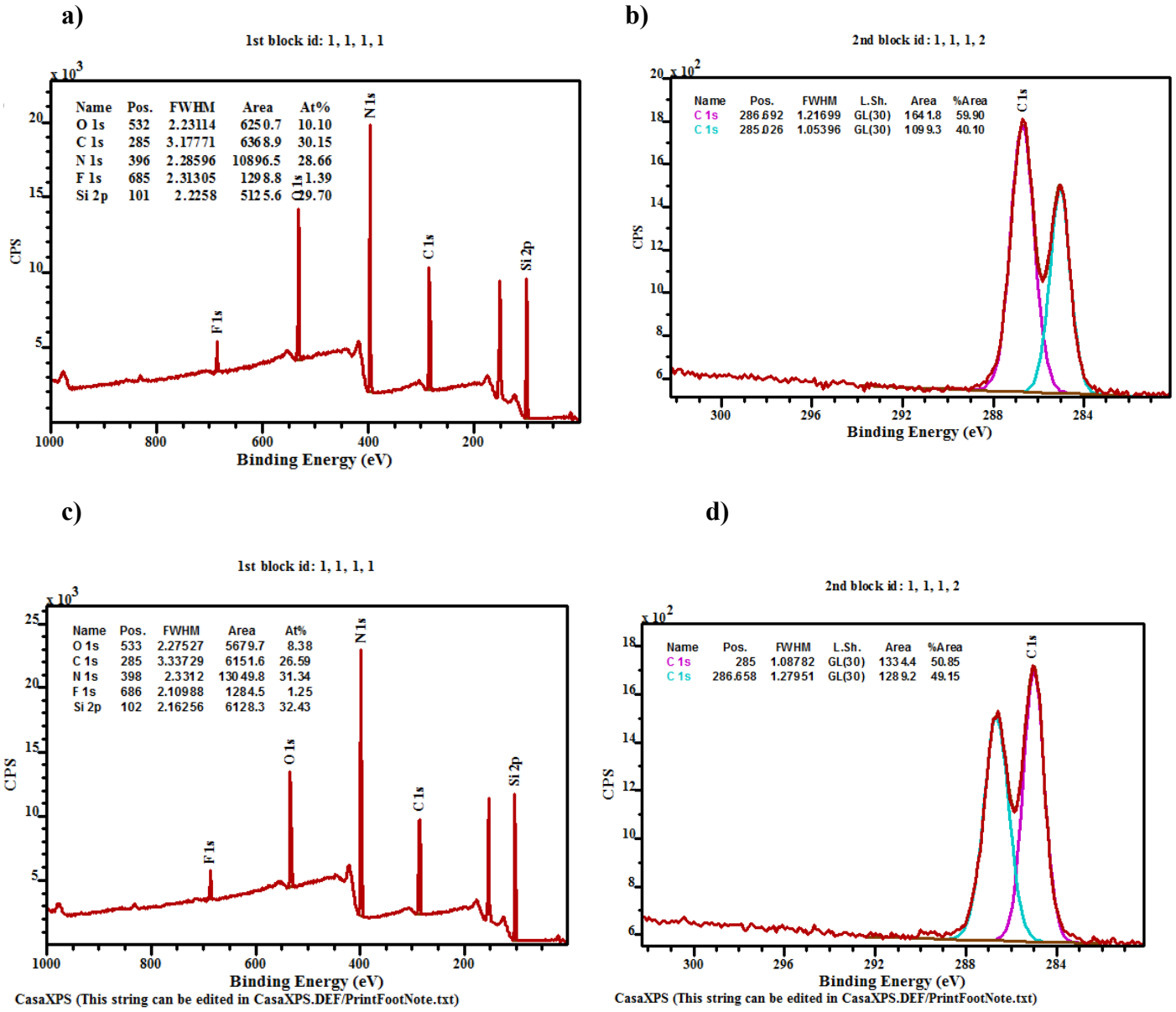
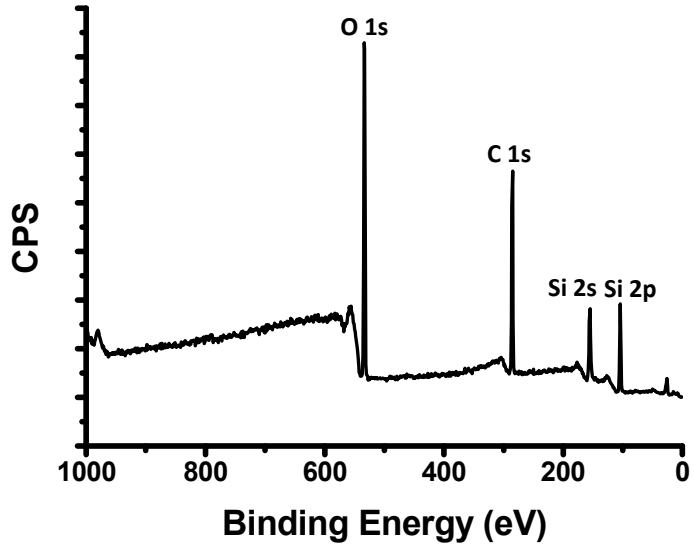


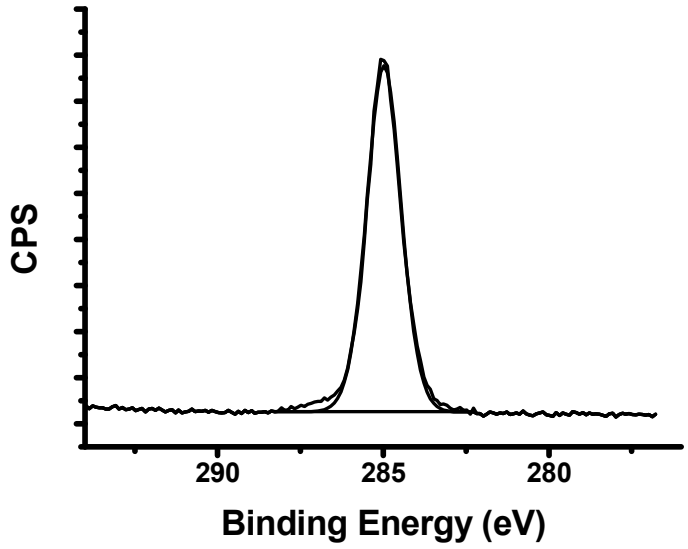
Figure SS11. XPS spectra of monolayers $M_{Si_3N_4}^{EO6}$ and $M_{Si_3N_4}^{EO9}$ a) survey spectra of $M_{Si_3N_4}^{EO6}$; b) C narrow scan of $M_{Si_3N_4}^{EO6}$ c) survey spectra of $M_{Si_3N_4}^{EO9}$ d) C narrow scan of $M_{Si_3N_4}^{EO9}$

XPS spectra of monolayers on SiO_2

a)



b)



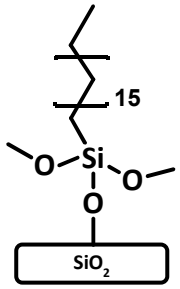
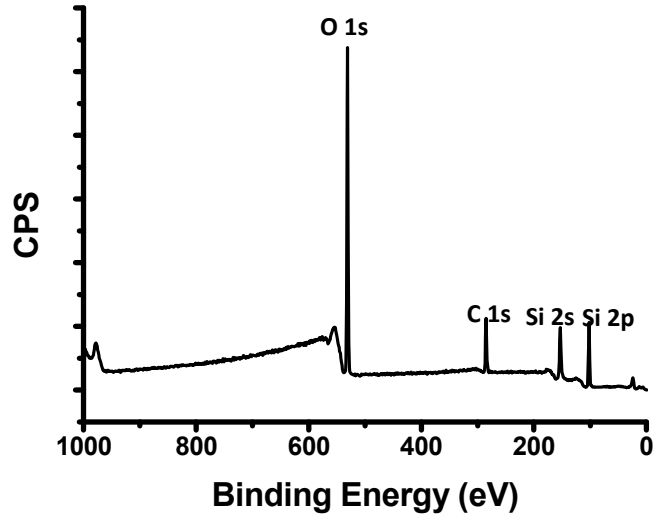
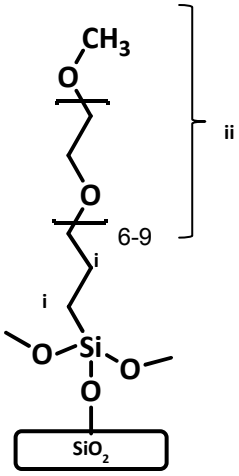


Figure SS12. XPS spectra of monolayer $M^{1}_{SiO_2}$ a) survey spectra and b) C 1s narrow scan

a)





b)

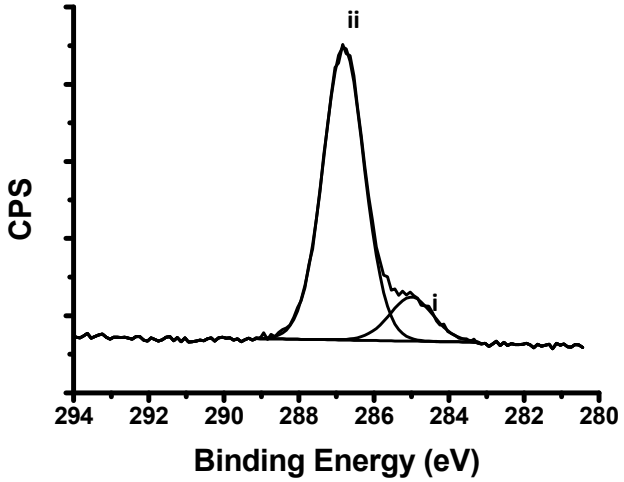


Figure SS13. XPS spectra of monolayer $M^2_{SiO_2}$ a) survey spectra and b) C 1s narrow scan

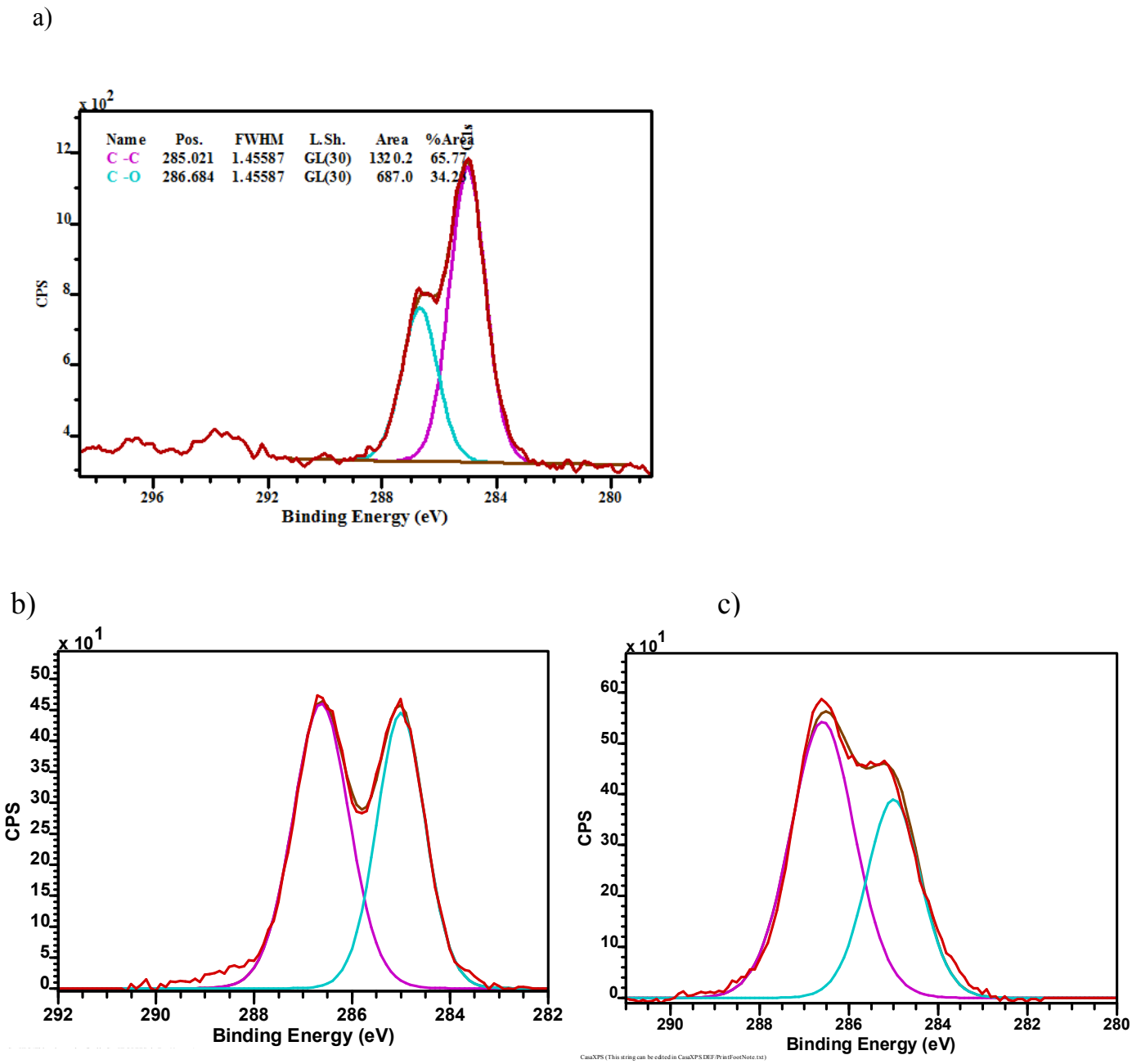
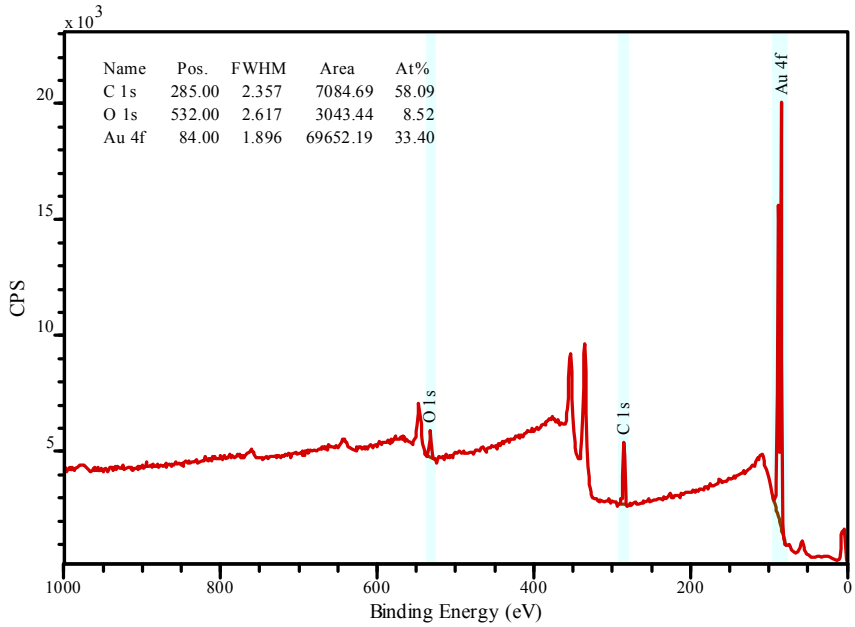


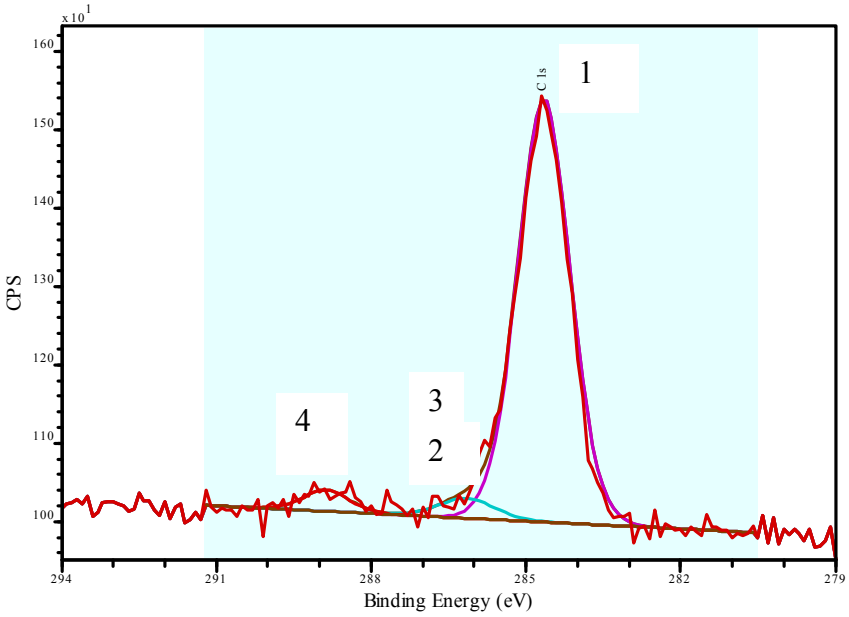
Figure SS14. XPS spectra C 1s narrow scan of monolayers a) $M_{SiO_2}^{EO3}$ b) $M_{SiO_2}^{EO6}$ and c) $M_{SiO_2}^{EO9}$

XPS spectra of monolayers on Au

a)



b)



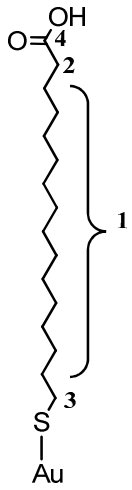
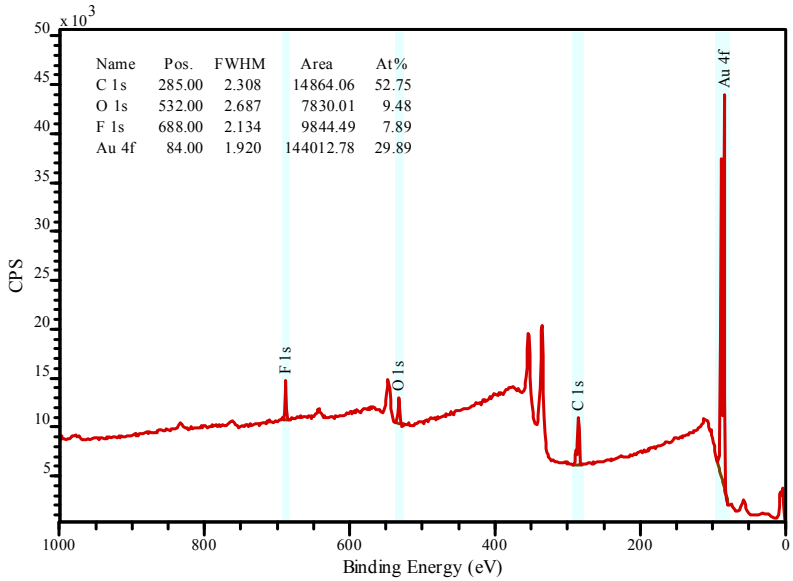
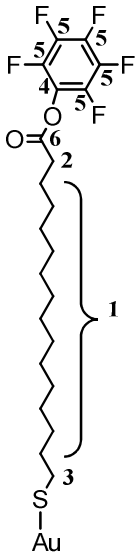


Figure SS15. XPS spectra of monolayer M^{1}_{Au} a) survey spectra b) C 1s narrow scan

a)





b)

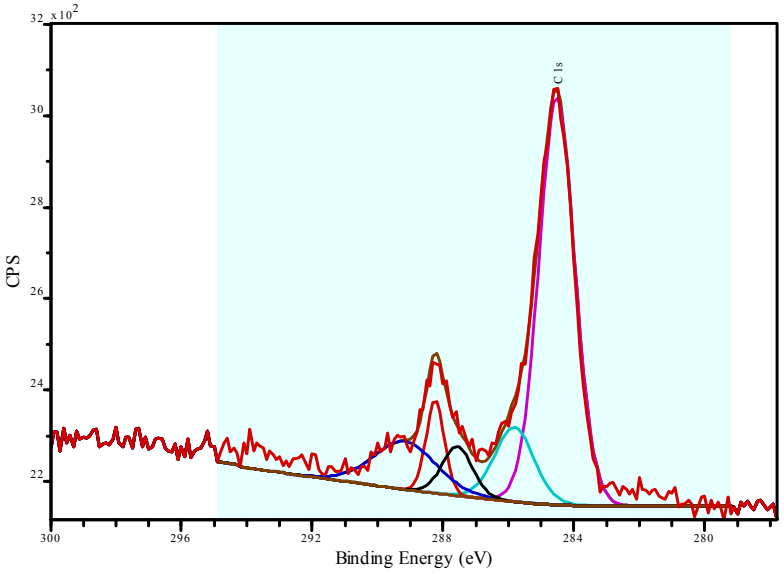
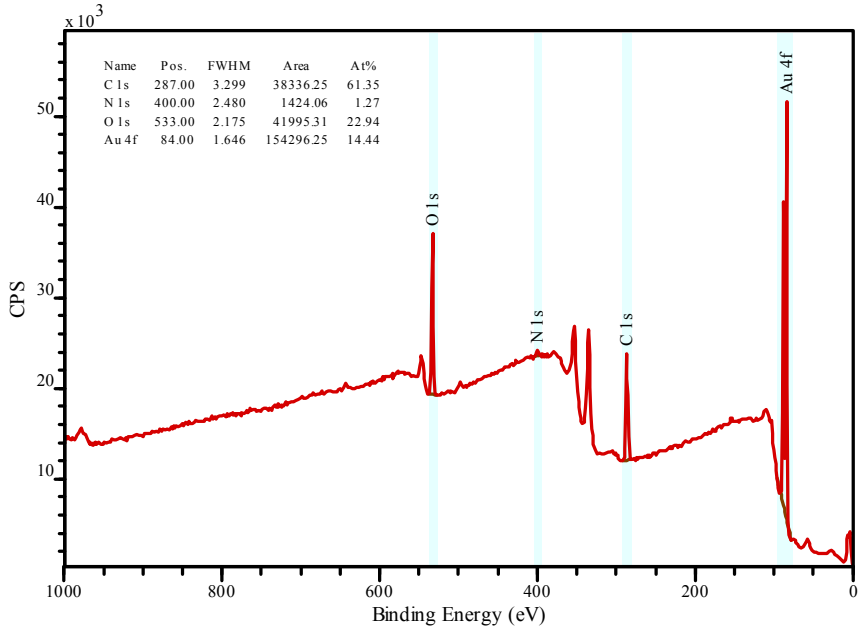
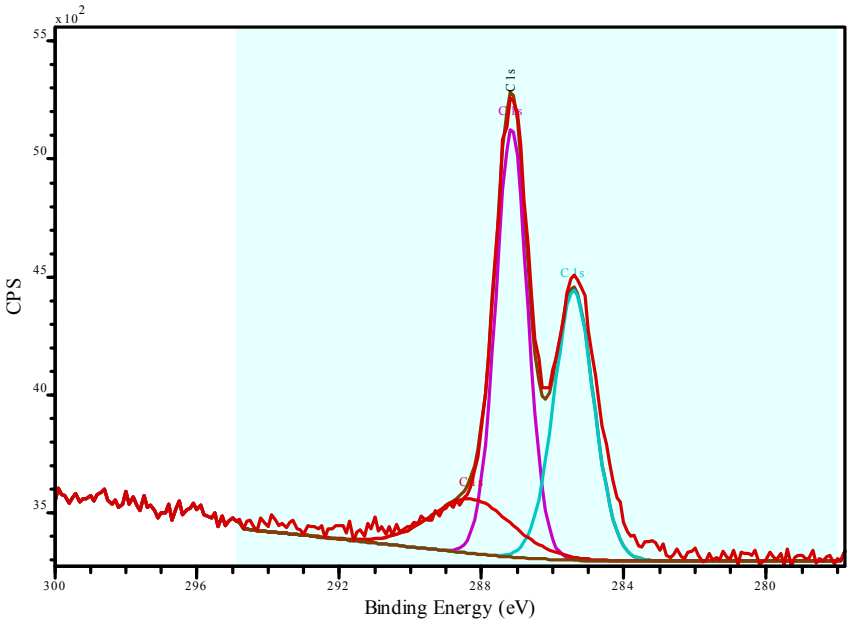


Figure SS16. XPS spectra of monolayer M^2_{Au} a) survey spectra b) C 1s narrow scan

a)



b)



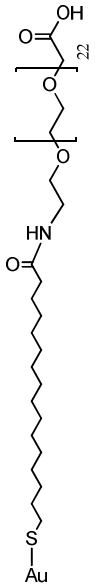
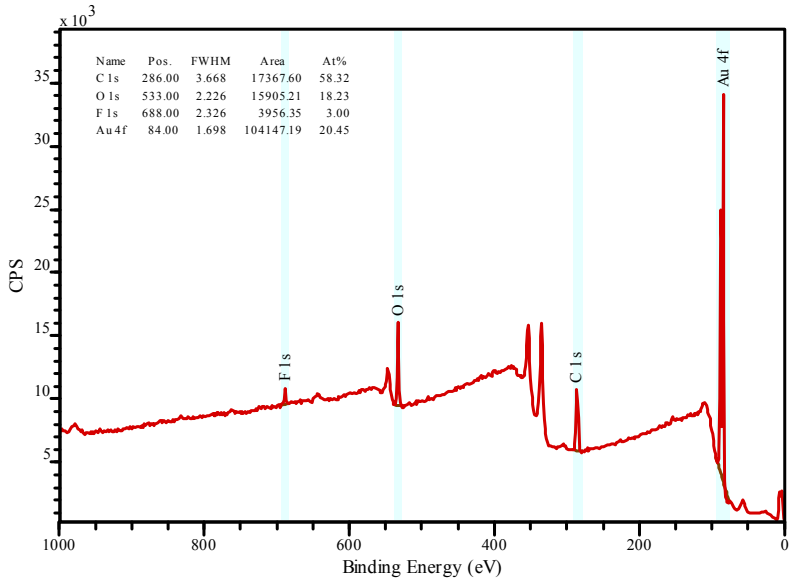
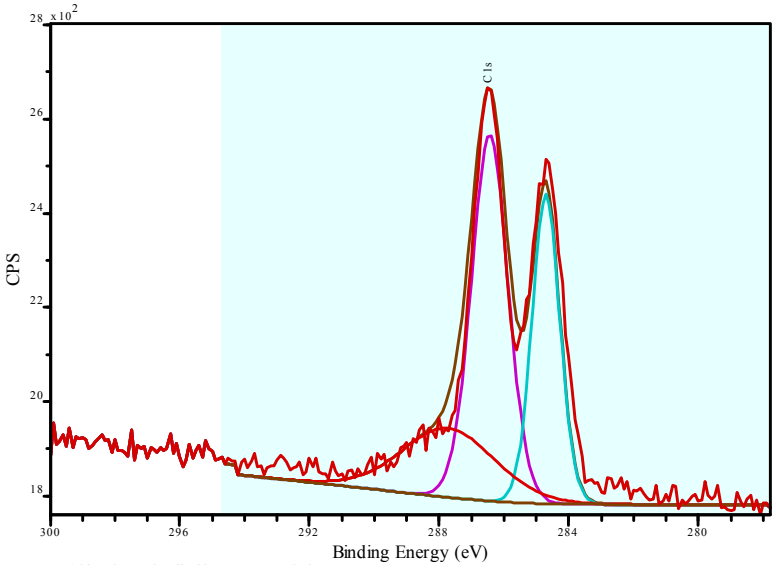


Figure SS17. XPS spectra of monolayer M^{3}_{AuH} a) survey spectra b) C 1s narrow scan

a)



b)



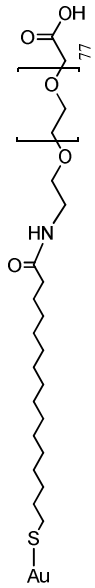
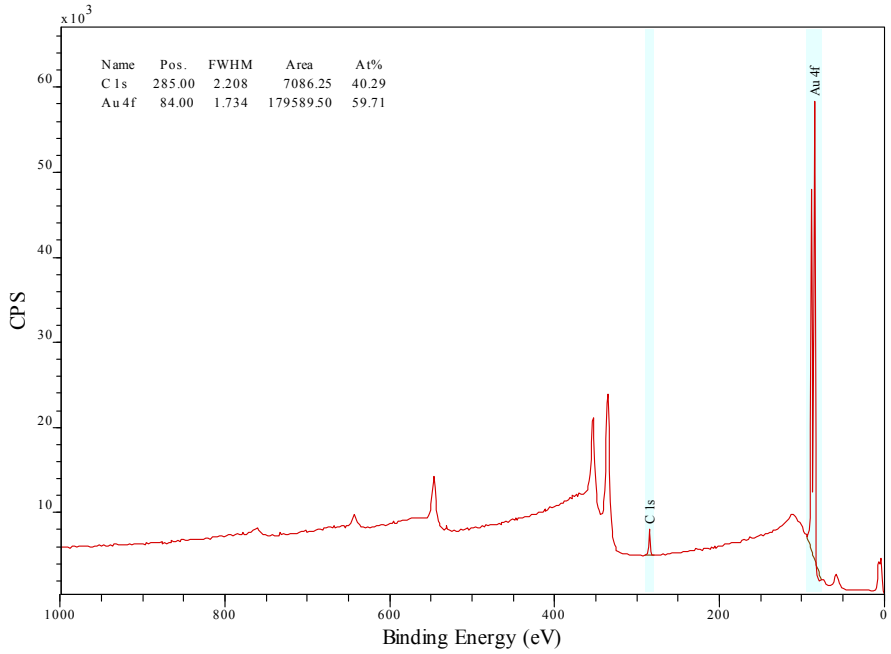


Figure SS18. XPS spectra of monolayer M^1_{Au} a) survey spectra b) C 1s narrow scan

a)



b)

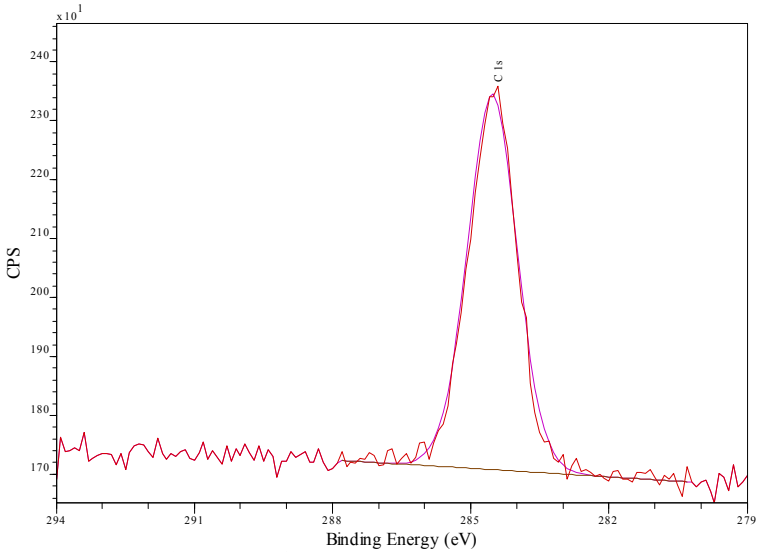


Figure SS19. XPS spectra of monolayer M^{5}_{Au} a) survey spectra b) C 1s narrow scan

DART-HRMS of SPR chip

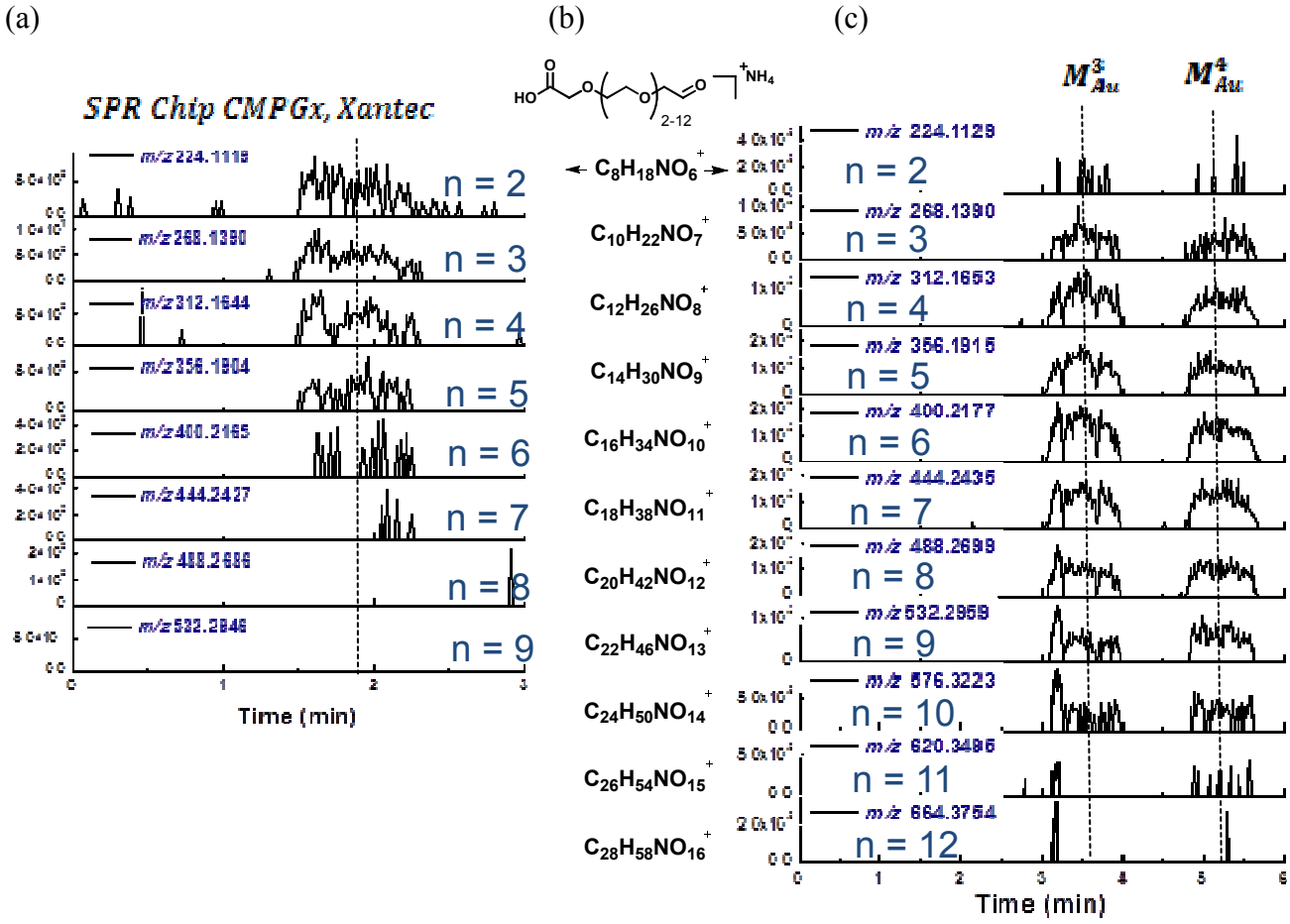


Figure SS20. DART-HRMS of monolayers with carboxymethyl-PEG coatings: (a) commercially available gold SPR chip CMPGx with attached carboxymethyl-PEG (6 kDa) analyzed between 1.5 - 2.7 min; (b) the most intense ions observed for carboxymethyl-PEG, and (c) in-house prepared monolayers M^3_{Au} - carboxymethyl-PEG (1 kDa), measured between 3.0 - 4.0 min, and M^4_{Au} - carboxymethyl-PEG (3.5 kDa), measured between 5.0 - 6.0 min.

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