Supporting Information

Controlled Silanization and Biomolecular Conjugation via Ultra-Stable Carboxyl Silatrane for Neurofilament Light Chain Detection

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Hydrolysis assessment

The ability of COOHSiT and COOHSi to maintain their structure in an environment with water was assessed using a 600 MHz ^{1}H NMR (AVANCE III HD 600MHz NMR, Bruker, UK). A solution of D-methanol with 10% (v/v) deionized water was utilized as the solvent for the NMR analysis, and the ethoxy group signals (chemical shift δ = 3.8 ppm and 1.2 ppm) along with the silatranyl ring signals (δ = 3.0 and 3.8 ppm) were tracked over time. The signals of a fixed CH_2 group in the unchanging part of the COOHSiT and COOHSi molecules were selected as standards and the signal changes of the Silatrane/Silane groups were calculated based on the standard signals.

Cytotoxicity test

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay was chosen as a colorimetrical indicator for the cell viability of L-929 (NCTC clone 929), a mouse connective tissue cell line, after exposed to COOHSiT or COOHSi. To each single slot of a 24-well plate, 10^4 cells contained in 1 mL α-MEM (α-modified Eagle's minimum essential medium) with 10% horse serum were seeded and cultured at maintained condition of 37°C, 5% CO_2, 95% air in 100% humidity. After 16 h, the culture medium was replaced by 250 µL of α-MEM including serially diluted COOHSiT or COOHSi (0, 0.5, 1, 5, 20 mM), following with additional incubation for 24 h. The cultured cells exposed to only α-MEM (0 mM of COOHSiT or COOHSi) were later recorded as control. Thereafter, MTT solution prepared at 5 mg/mL in PBS was added to each well until reaching a concentration of 0.5 mg/mL. The wells were then incubated for 3 h before removing the medium. Finally, 500 µL of DMSO was dispensed to each well for dissolving the formazan crystals and forming purple solutions with different intensities. The absorbance values of the final solutions were determined by a BioTek Synergy 2 Microplate Reader under a 540 nm light wavelength. The mean value was calculated from three experimental replicates and reported as percentages in reference to the control values.

Surface characterizations

Surface hydrophilicity: Wetting behavior of modified surfaces were examined via water contact angles measured by the sessile drop method. Briefly, a 5 µL water droplet was placed randomly on at least three spots of the tested surface using a micro-syringe. The angle formed between the tangent to the droplet at the three-phase boundary (solid-liquid-air interface) and the solid surface was captured by an optical contact angle goniometer (Phoenix mini, Surface Electro Optics, Seoul, Korea).

Thin film thickness: An ellipsometer (alpha−SE, J.A. Woollam Co., US) was applied to record the thickness of COOHSiT and COOHSi thin films. The He-Ne laser, emitting light at a wavelength of 632.8 nm, was incident to the surface at three distinct angles: 65°, 70°, and 75°. Measurements were conducted on three randomly selected areas on each substrate before and after each modification.

Surface morphology: The surface profile of bare and coated samples was captivated by an AFM SPI 3800N (Hitachi, Japan) using the AFM cantilevers (Olympus Corporation, Japan) with parameters as: spring frequency = 70 kHz, force constant = 2.0 N/m, tip radius = 7 nm. Surface topography and statistical roughness were acquired at three random locations of each substrate.

Chemical composition of coatings: The elemental composition and chemical state of coatings were determined by an XPS (Sigma Probe, Thermo VG Scientific) with a narrowly focused and single-wavelength X-ray source (1486.6 eV and 25 W) using aluminum K-alpha radiation. Atomic detection limit is 0.1% and X-ray spot size exhibit a range of 15 − 400 µm. The experiment was conducted within an ultra-high vacuum condition with a pressure of 10^{-8} Pascal, and the take-off angle of photoelectron source was fixed at 45°. The photoelectron spectra were acquired using an analyzer with a pass energy of 23.5 eV, ensuring a resolution precisely maintained within 0.2 eV and calibrated against the $Si2p_{3/2}$ peak at 98.5 eV. The high-resolution Si2p spectra were deconvoluted by Voigt function in the Origin software. Information regarding atomic percentage of each substrate was quantified on the spectra of Si2p, C1s and N1s.

Fourier-Transform Infrared Spectrometry: FTIR spectrometer (Bruker, Vertex 80v, Germany), specifically designed for high-resolution infrared spectroscopy, was deployed to identify the spectra of modified Si wafers with a resolution of 1 cm^{-1}. Modified

wafers were analyzed under an infrared beam with incident angle of 80° using Attenuated Total Reflectance (ATR) mode to explore the nanoscale-analyzed surface.

Statistical analysis

Student's t-test was applied for analyzing the experimental results and the statistical data were presented as means ± standard deviation (SD) or standard error of the mean (SEM). The probabilities of $p \leq 0.05$ were considered as significant difference. All the data processing and presentation were operated in Origin 9.0 software (OriginLab Corporation, MA, USA).

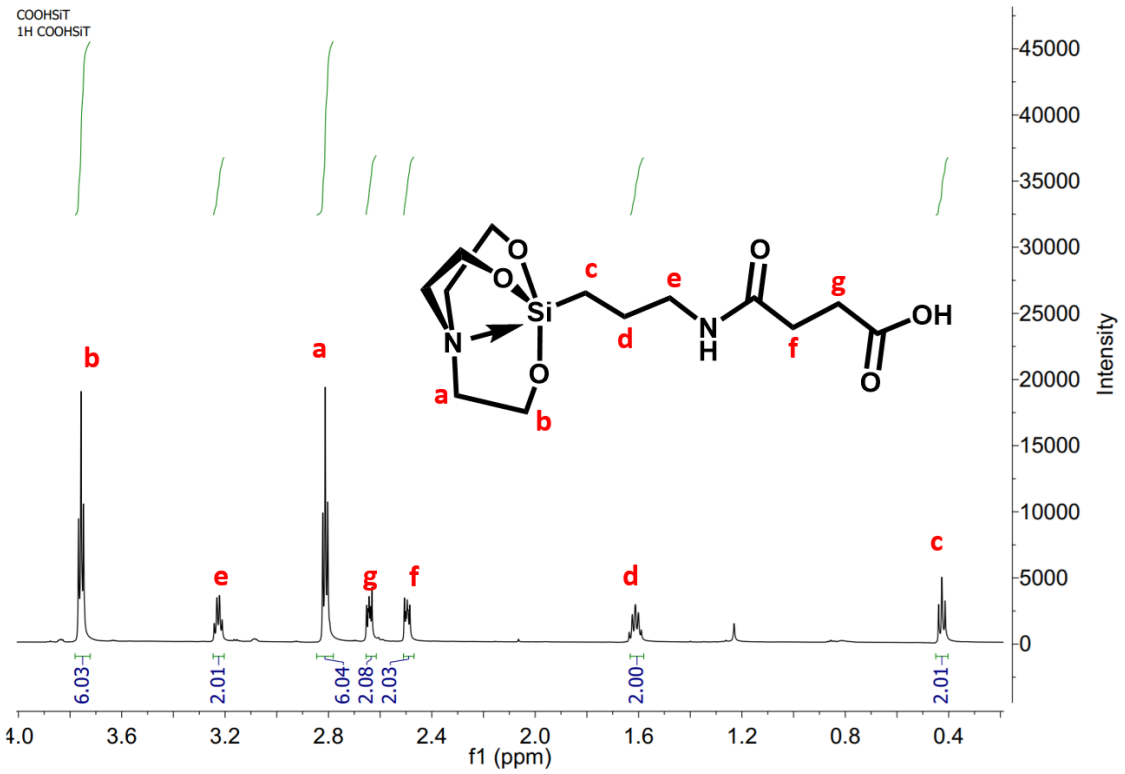

Figure S1. NMR characterization for COOHSiT. ^{1}H NMR (600 MHz, $CDCl_3$) δ: (ppm) = 0.38−0.44 ($SiC\underline{H}_2CH_2CH_2$, 2H), 1.58−1.64 ($SiCH_2C\underline{H}_2CH_2$, 2H), 3.20−3.26 ($SiCH_2CH_2C\underline{H}_2NH$, 2H), 2.78−2.84 ($OCH_2C\underline{H}_2$, 6H), 3.72−3.78 ($OC\underline{H}_2CH_2$, 6H), 2.48−2.52 ($COC\underline{H}_2CH_2$, 2H), 2.66−2.70 ($COCH_2C\underline{H}_2$, 2H).

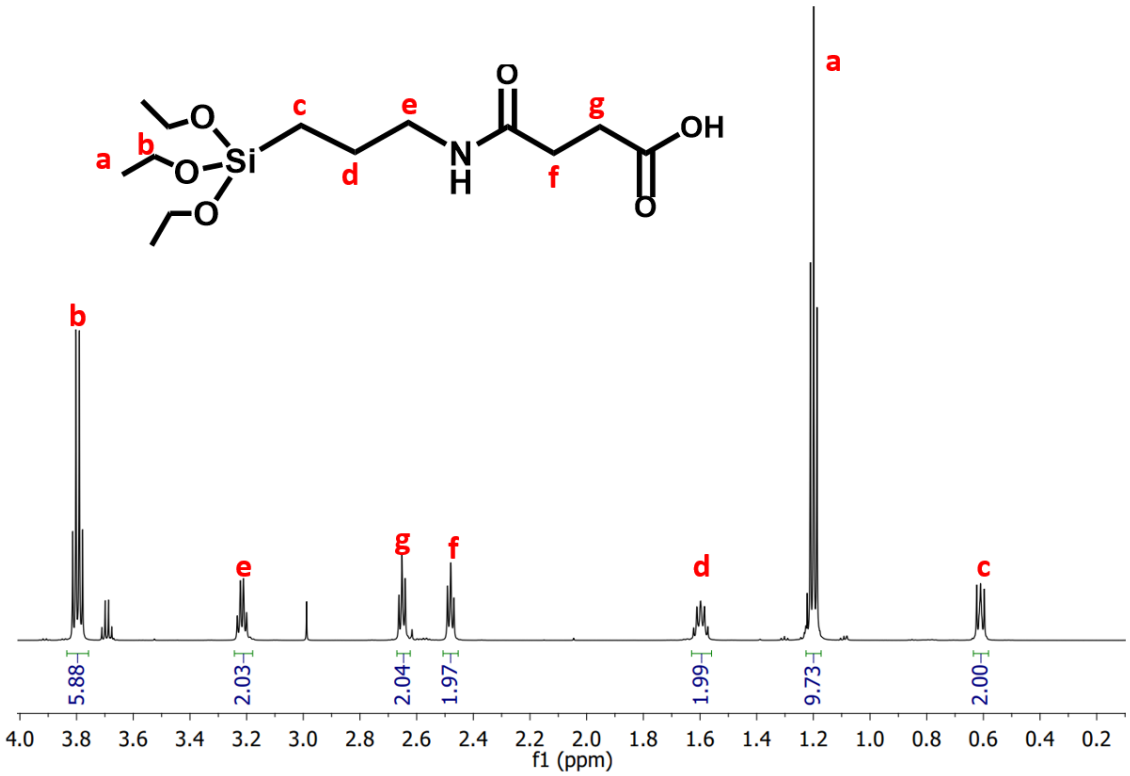

Figure S2. NMR characterization for COOHSi. ^{1}H NMR (600 MHz, $CDCl_3$) δ: (ppm) = 0.58−0.62 ($SiC\underline{H}_2CH_2CH_2$, 2H), 1.58−1.64 ($SiCH_2C\underline{H}_2CH_2$, 2H), 3.20−3.26 ($SiCH_2CH_2C\underline{H}_2NH$, 2H), 3.78−3.84 ($OC\underline{H}_2CH_3$, 6H), 1.18−1.22 ($OCH_2C\underline{H}_3$, 9H), 2.48−2.52 ($COC\underline{H}_2CH_2$, 2H), 2.66−2.70 ($COCH_2C\underline{H}_2$, 2H).

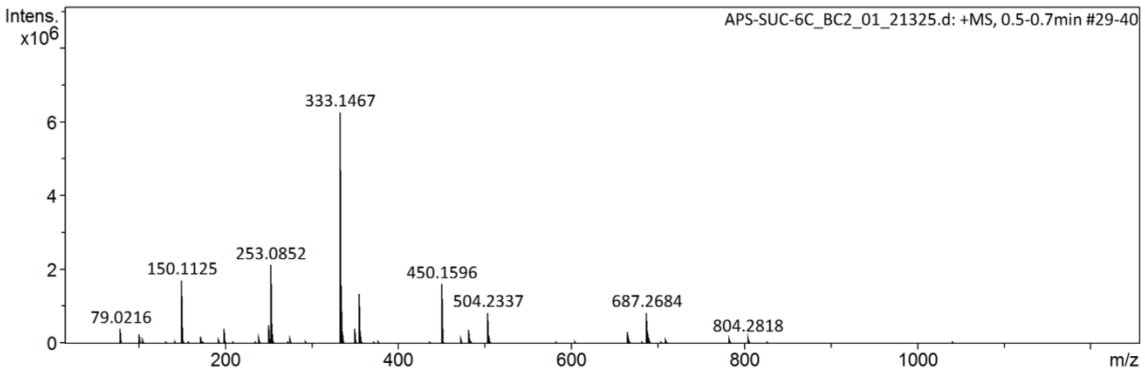

Figure S3. Mass spectrum of COOHSiT. Molecular weight of COOHSiT is 332.43 g/mol.

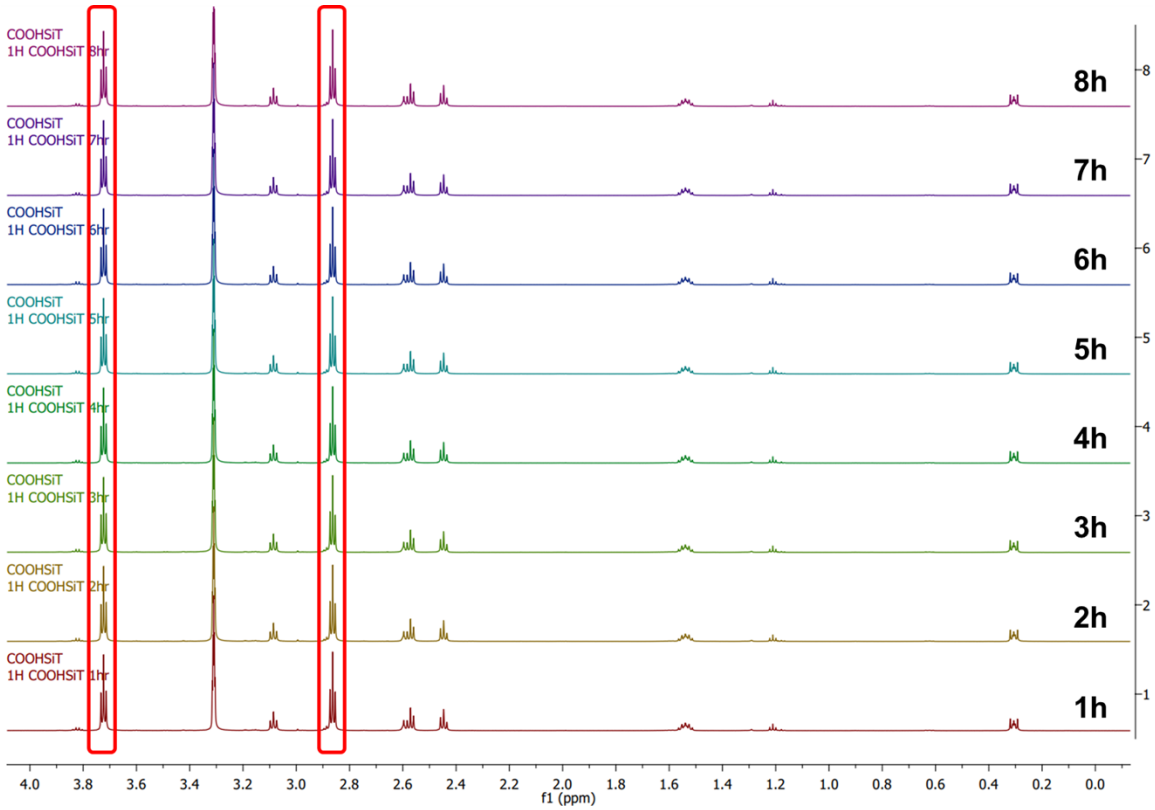

Figure S4. Time-course NMR spectra of COOHSiT in MeOD containing 10% diH_2O.

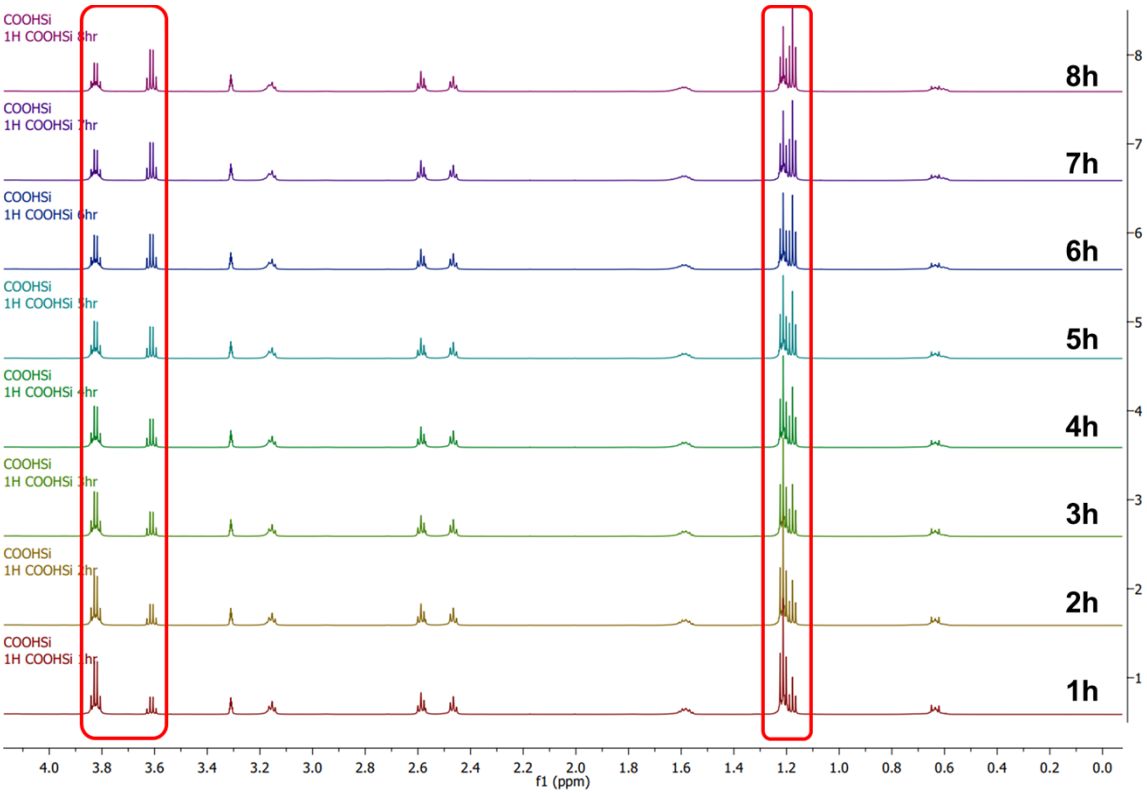

Figure S5. Time-course NMR spectra of COOHSi in MeOD containing 10% diH_2O.

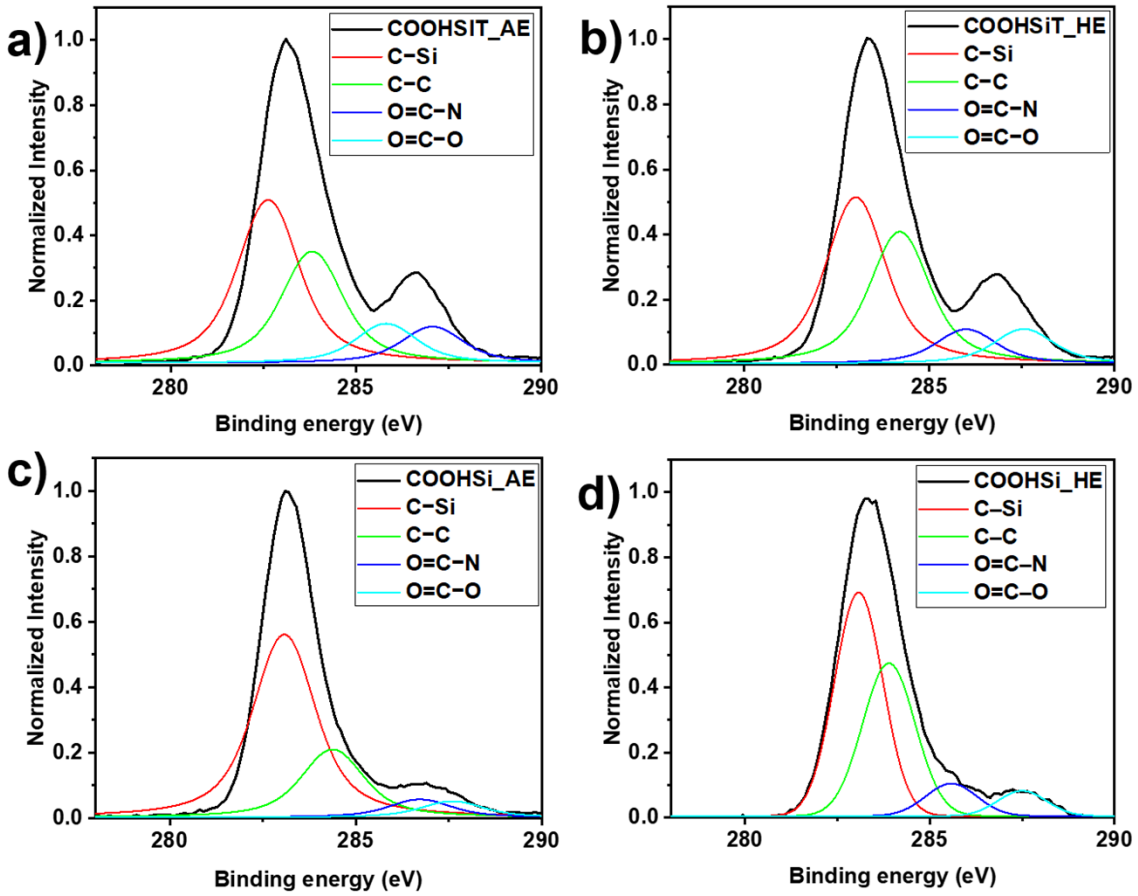

Figure S6. Deconvolution of XPS C1s peaks of wafers modified with (a) COOHSiT in anhydrous ethanol, (b) COOHSiT in hydrated ethanol, (c) COOHSi in anhydrous ethanol and (d) COOHSi in hydrated ethanol.

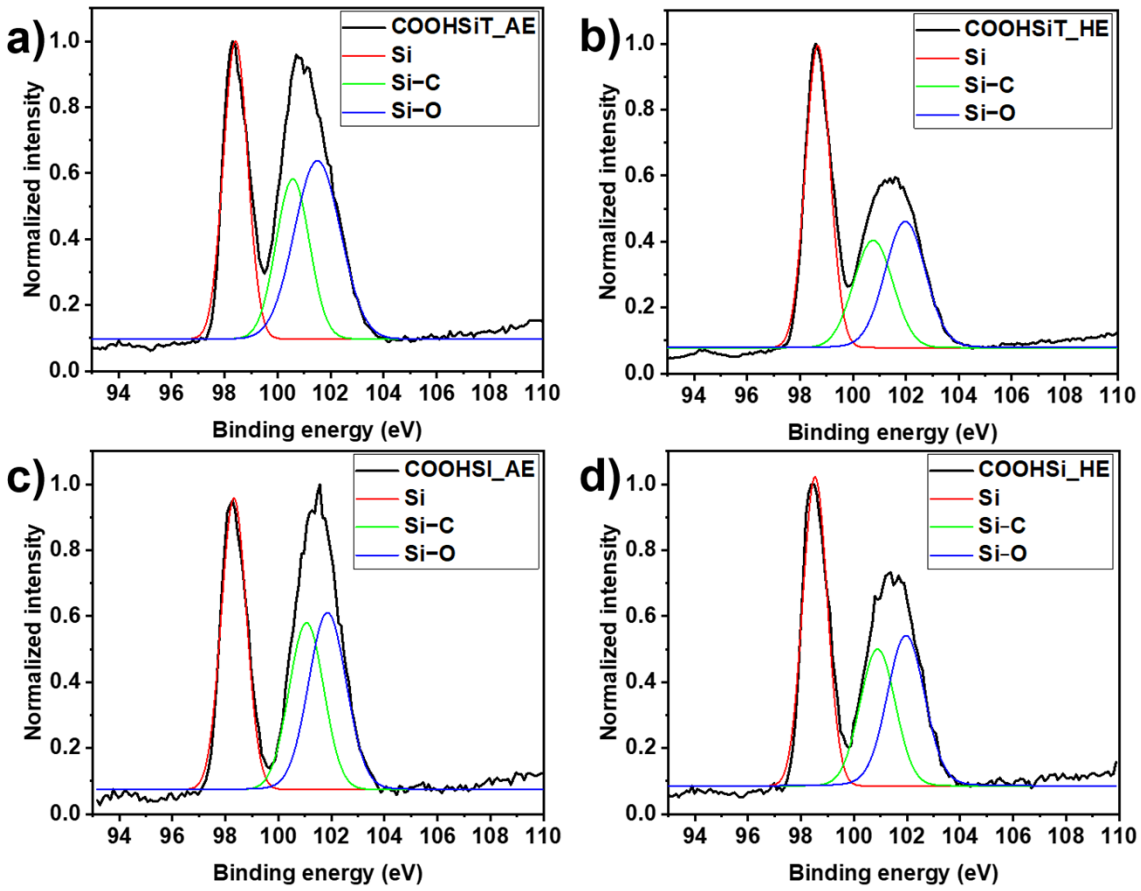

Figure S7. Deconvolution of XPS Si2p peaks of wafers modified with (a) COOHSiT in anhydrous ethanol, (b) COOHSiT in hydrated ethanol, (c) COOHSi in anhydrous ethanol and (d) COOHSi in hydrated ethanol.

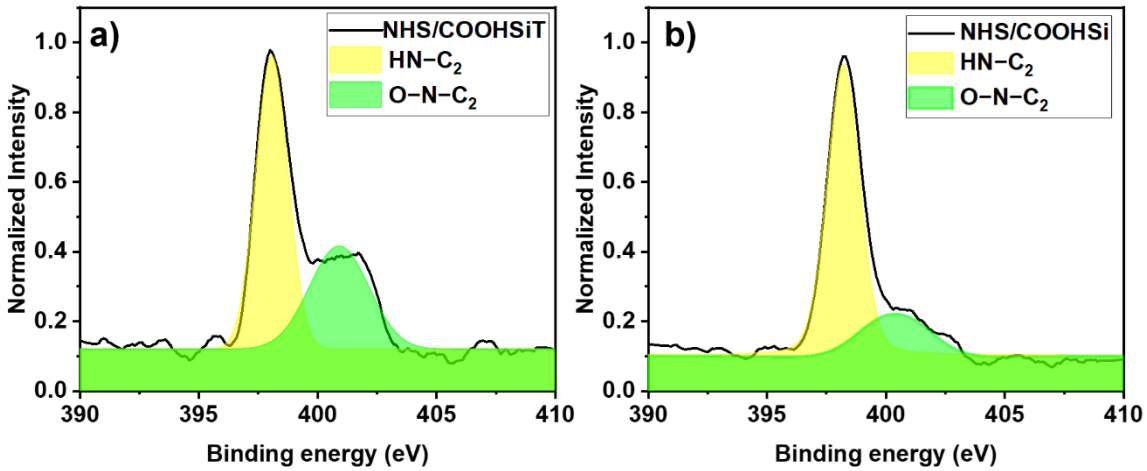

Figure S8. Deconvolution of XPS N1s peaks of (a) COOHSiT- and (b) COOHSi-coated wafers after EDC/NHS activation.

Table S1. Solubility profile of COOHSiT and COOHSi across different organic solvents.

Solvents	COOHSiT	COOHSi
Water	Soluble	Soluble
DMF	Soluble	Soluble
DMSO	Soluble	Soluble
Methanol	Soluble	Slightly soluble
Ethanol	Slightly soluble	Slightly soluble
Acetonitrile	Slightly soluble	Slightly soluble
Chloroform	Slightly soluble	Slightly soluble
Acetone	Slightly soluble	Insoluble
DCM	Insoluble	Insoluble
Diethyl ether	Insoluble	Insoluble
Toluene	Insoluble	Insoluble
n-pentane	Insoluble	Insoluble
n-hexane	Insoluble	Insoluble

Table S2. Atomic% of COOHSiT- and COOHSi-modified wafers calculated by XPS.

Atomic%	COOHSiT (anhydrous)	COOHSi (anhydrous)	COOHSiT (aqueous)	COOHSi (aqueous)
C1s	43.7	38.87	41.02	41.64
Si2p	17.46	21.54	20.69	16.85
N1s	5.4	5.49	5.23	7.14
O1s	33.14	34.1	33.06	34.36

Table S3. Atomic% of COOH-terminated wafers after NHS activation calculated by XPS.

Atomic%	NHS/COOHSiT	NHS/COOHSi
C1s	27.37	17.38
Si2p	25.06	39.83
N1s	1.75	1.74
O1s	45.82	41.05