Supporting Information

A Versatile Method for Functionalizing Surfaces with Bioactive Glycans

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Trifluoroethyl derivation of DVS-activated surface. Trifluoroethyl derivation was carried on in three fluoride reagents bearing hydroxyl, amine, and thiol group respectively. The DVS-modified surfaces on silicon wafer substrates were immersed in aqueous solutions of the trifluoroethyl nucleophile (trifluoroethanol 20% w/v in pH 10 carbonate buffer, trifluoroethanethiol 10mM in pH 7.4 PBS buffer, trifluoroethylamine 10mM in pH 10 carbonate buffer) at ambient temperature for 16 h unless specified. The surfaces were then thoroughly rinsed with ~10 mL water and ~5 mL ethanol, dried under a stream of argon for 1 min and stored in dark at 4^{o}C.

Synthesis of ethylvinyl sulfone mannosides. Ethyl vinyl sulfone (0.67 g, 5.5 mmole) was added to mannose (1 g, 5.5 mmole) in 10 mL buffer (0.5 M sodium carbonate, pH 10), and stirred at room temperature for 16 h. The reaction was concentrated by rotary evaporation and a portion of the resulting solid (containing product and buffer salts) was extracted in a minimal volume of methanol and applied to a silica flash column (methanol: dicholormethane = 2:8). The anomeric mixture of product was separated by preparative thin layer chromatography (isopropanal: methanol: water = 8:2:0.5) to isolate the anomers. The separated fractions were analyzed by ESI-MS and were found to have the same mass ($[M+Na]^+$ m/z 323.2). α-Anomer ^{1}H NMR (500 MHz, D_2O): δ 4.83 (d, J = 1.5 Hz, 1H; H_1), 4.18-4.14 (m, 1H; $-O-CH_aH_b-CH_2SO_2CH_2CH_3$), 3.90-3.83 (m, 3H; H_2, H_{6a}, $-O-CH_aH_b-CH_2SO_2CH_2CH_3$), 3.74-3.71 (m, 1H, H_5), 3.68-3.62 (m, 2H, H_3, H_{6b}), 3.58-3.54 (m, 1H, H_4), 3.41-3.37 (m, 2H, $-O-CH_aH_b-CH_2SO_2CH_2CH_3$), 3.18-3.13 (m, 2H, $-O-CH_aH_b-CH_2SO_2CH_2CH_3$), 1.39-1.36 (t, 3H, $-O-CH_aH_b-CH_2SO_2CH_2CH_3$). ^{13}C NMR (500 MHz, D_2O): δ 100.56 (C_1), 73.76 (C_5), 71.21 (C_3), 70.48 (C_2), 67.01 (C_4), 61.48 (C_6), 60.88 ($-O-CH_2-CH_2SO_2CH_2CH_3$), 51.50 ($-O-CH_2-CH_2SO_2CH_2CH_3$), 48.59 ($-O-CH_b-CH_2SO_2CH_2CH_3$), 5.21 ($-O-CH_2-CH_2SO_2CH_2CH_3$).

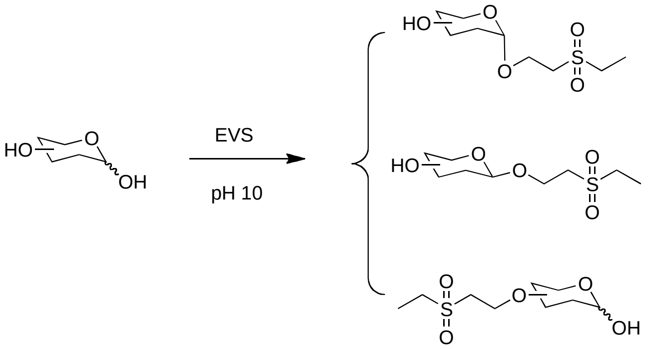
Table S1. Comparison of theoretical versus observed XPS composition of modified surfaces.

	SAM (11-mercaptoundecanol)		DVS-modified SAM		Mannose-functionalized SAM via DVS	
	Theoretical	Observed	Theoretical	Observed	Theoretical	Observed
C	84.6	88.2±0.6	75.0	84.3±1.2	65.6	84.9±0.7
O	7.7	8.3±0.6	15.0	12.0±1.0	28.1	10.7±0.5
S	7.7	3.5±0.2	10.0	3.7±0.2	6.3	4.4±0.2

a. DVS/hydroxyl reaction yield is assumed to be 100%;

b. The sequential mannose coupling yield is assumed to be100%;

c. XPS values represent the averages and standard deviations from at least three spots on two replicates or more.



Scheme S1. Model reaction of free mannose with ethylvinyl sulfone (EVS).

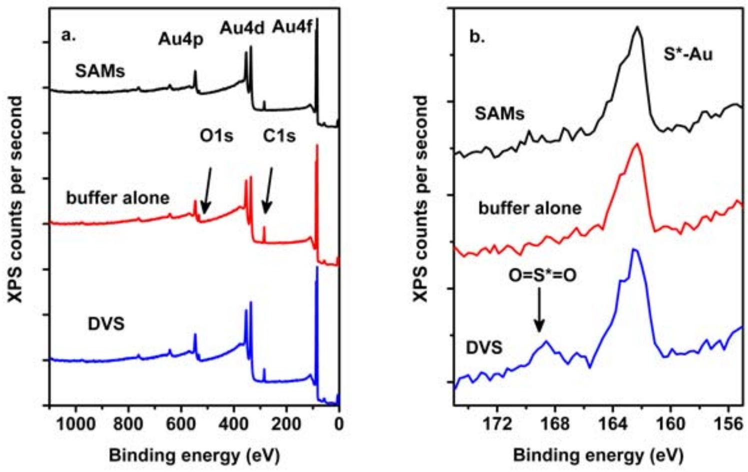


Figure S1. XPS spectra (a) wide scan and (b) sulfur 2p scan for hydoxyl-terminated alkane thiol (SAMs), control SAMs treated with buffer (buffer alone), and DVS-modified surface (DVS). The sulfur species at high binding energy (168 eV for oxidized sulfur) (*1*, *2*) indicates that DVS is conjugated to the SAM.

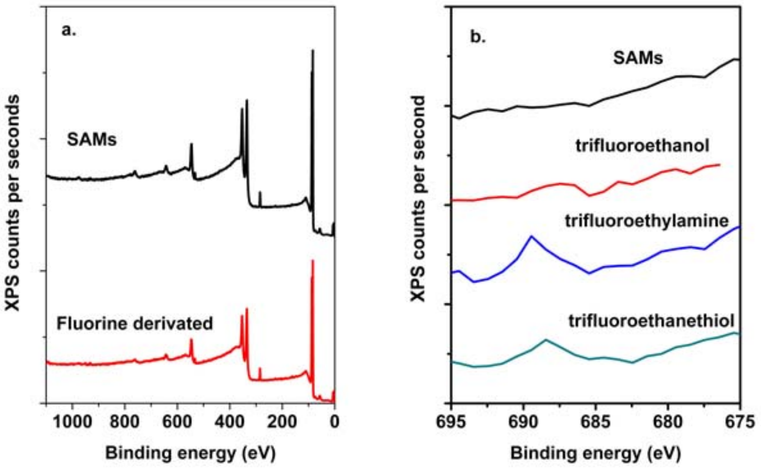


Figure S2. XPS (a)wide survey scan and (b) F 1s scan for fluorine-derivated SAMs by trifluoroethanol, trifluoroethylamine, and trifluoroethanethiol.

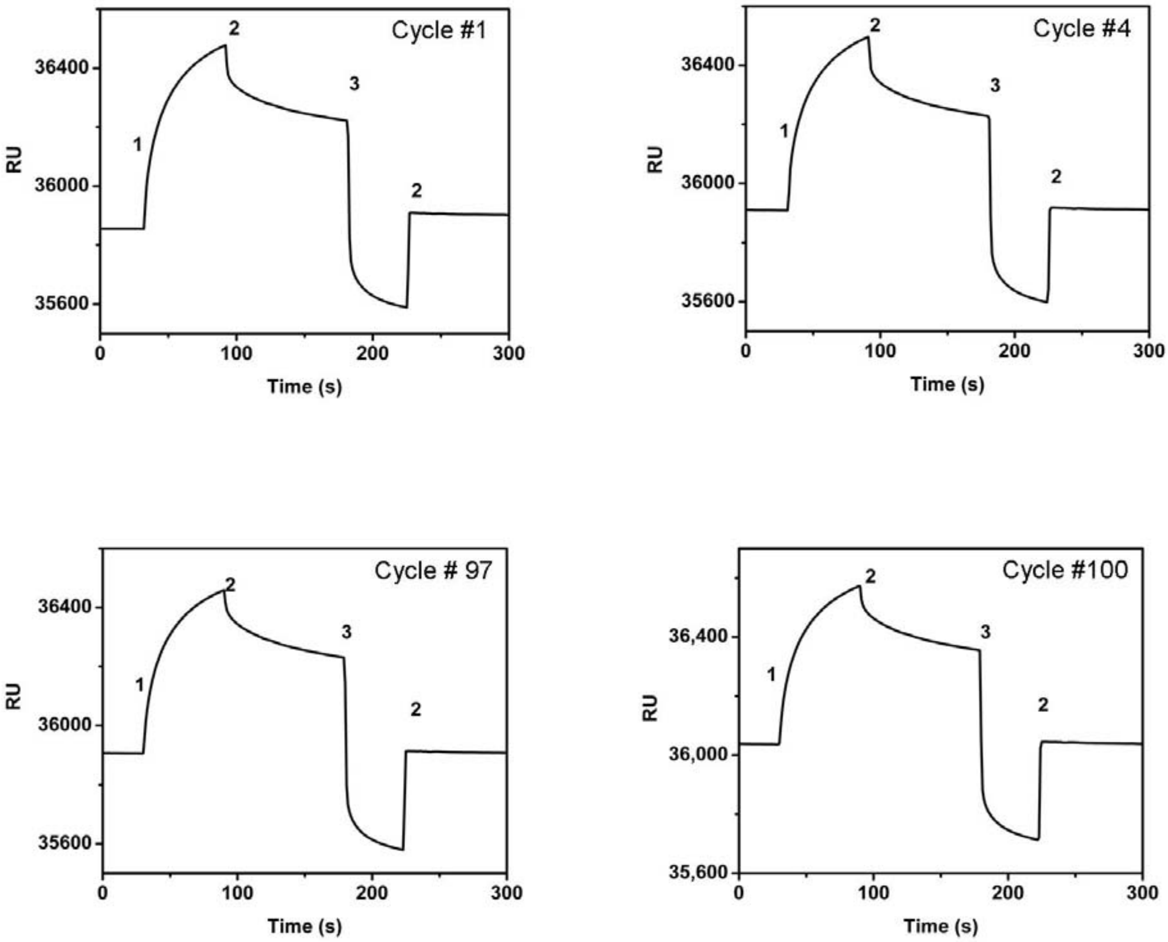


Figure S3. Glycan modified surfaces conjugated via DVS chemistries are highly stable to multiple rounds of protein capture and regeneration. Bioacore SPR sensorgrams for 100-cycle lectin binding-regeneration on a mannose-modified Biacore chip. (1) 500 nM ConA, (2) HEPES buffer, and (3) Glycine (10mM, pH 2) for surface regeneration.

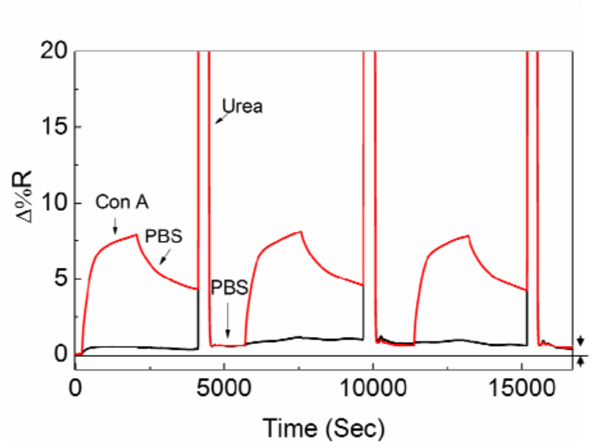


Figure S4. SPR sensorgrams (without background subtraction) of Con A binding to an OEG-SH inactivated DVS surface without blocking by BSA. — Mannose, — Background (non-glycan functionalized region). During the first Con A binding cycle, the baseline rose ~0.5 Δ%R (indicated by the arrows), which is attributed to Con A nonspecific uptake to the inactivated DVS surface. However, the baseline returned to the initial level in the second and third cycle of Con A binding. These results indicate that there is some nonspecific Con A uptake to the inactivated DVS surface, but after the first binding cycle, only the specific Con A binding is observed on the SPR sensorgram.

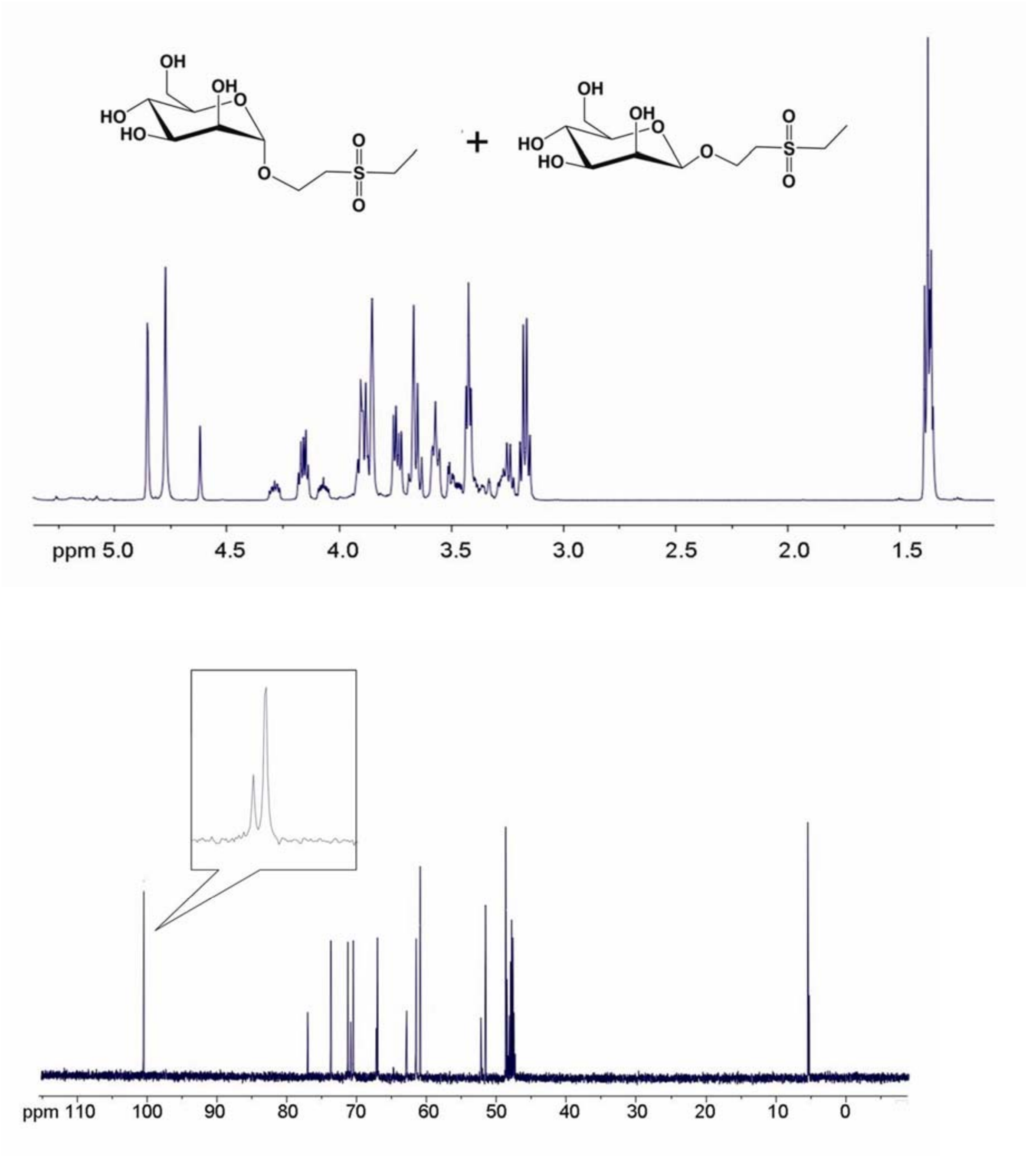


Figure S5. ^{1}H and ^{13}C NMR of mannose/EVS reaction products (major fraction), indicating the conjugate contains a (3:1 α:β) mixture of the alpha and beta anomer.

Reference:

(1) Castner, D., Hinds, K., and Grainger, D. (1996) X-ray photoelectron spectroscopy sulfur 2p study of organic thiol and disulfide binding interactions with gold surfaces. *Langmuir 12*, 5083-5086.

(2) Cheng, F., Gamble, L. J., Grainger, D. W., and Castner, D. G. (2007) X-ray photoelectron spectroscopy, time-of-flight secondary ion mass spectrometry, and principal component analysis of the hydrolysis, regeneration, and reactivity of N-hydroxysuccinimide-containing organic thin films. *Anal. Chem. 79*, 8781-8.