SUPPORTING INFORMATION

Critical role of surface hydration on the dynamics of serum adsorption studied with monoethylene glycol adlayers on gold

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I. Materials and methods

The following includes procedures for HS-MEG-OH surface modifier synthesis, gold substrate cleaning, adlayer formation (including the surface hydration step) and TSM experiments as well as surface characterization using contact angle goniometry and angle-resolved X-ray photoelectron spectroscopy. Piezoelectric quartz discs (AT-cut, 13.4 mm in diameter, 9.0 MHz fundamental frequency) with symmetric gold electrodes (4.9 mm in diameter) were purchased from Laptech Precision Inc. (Bowmanville, Ontario, Canada). Anhydrous ethanol (Commercial Alcohols Inc., Brampton, Ontario, Canada) was systematically used for adlayer formation and rinsing steps. Goat serum (45-75 mg protein/mL) and Dulbecco's phosphate buffered saline (PBS, $CaCl_2$ and $MgCl_2$ free - pH 7.4) were purchased from Sigma-Aldrich®. Other chemicals were also purchased from Sigma-Aldrich® and used as received unless otherwise noted. ^1H and ^{13}C NMR spectra were recorded at room temperature on Varian 300 and 400 MHz spectrometers using $CDCl_3$ as the NMR solvent. ^1H and ^{13}C NMR spectra are referenced to the residual solvent peak: δ = 7.27 ppm (^1H) and 77.23 ppm (^{13}C).

II. HS-MEG-OH surface modifier synthesis

HS-MEG-OH II was synthesized in two steps from 2-(2-chloroethoxy)-ethanol **I** in 95% overall yield, as follows (**Fig. 1**):

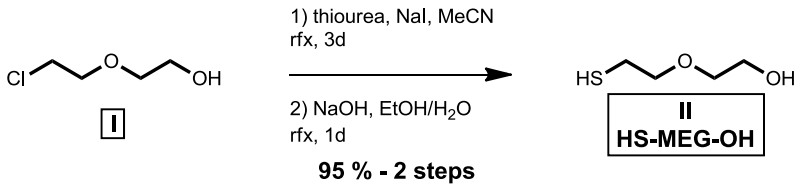


Fig. 1 HS-MEG-OH surface modifier synthesis.

2-(2-mercaptoethoxy)-ethanol (HS-MEG-OH) II. To a stirred solution of 2-(2-chloroethoxy)-ethanol **I** (4.28 mL, 40.0 mmol, 1.0 equiv.) in MeCN (120 mL) were successively added thiourea (15.4 g, 200.0 mmol, 5.0 equiv.) and NaI (6.0 g, 40.0 mmol, 1.0 equiv.) at room temperature. The reaction was then refluxed for three days, after which volatiles were evaporated under reduced pressure. The residue was dissolved in a 1/2 (v/v) mixture of EtOH (50 mL) and H_2O (100 mL) to which was added, carefully portionwise, powered NaOH (40.0 g, 1.0 mol, 25 equiv.) at room temperature. The reaction was then refluxed for one day. The resulting solution was carefully acidified (at 0ºC) to pH ~ 2 by addition of concentrated (37%) HCl then repeatedly extracted with copious amounts of CH_2Cl_2. The combined organic layers were dried over anhydrous Na_2SO_4, filtered then evaporated under reduced pressure.

Distillation under vacuum finally provided **HS-MEG-OH II** as a colorless oil (4.64 g, 95% yield – 2 steps); bp ~ 150ºC (~ 20 Torr). Spectroscopic data were consistent with those reported in the literature:[1] ^{1}H NMR (400 MHz, $CDCl_3$) 3.75 (t, *J* = 4.6 Hz, 2H), 3.64 (t, *J* = 6.2 Hz, 2H), 3.59 (t, *J* = 4.6 Hz, 2H), 2.72 (td, *J* = 8.2, 6.2 Hz, 2H), 2.4-2.0 (brs, 1H), 1.57 (t, *J* = 8.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) 72.6, 72.0, 61.5, 24.2.

III. Gold substrate cleaning

TSM discs were first soaked in a 1% sodium dodecyl sulfate (SDS) aqueous solution, gently wiped with a clean cotton swab while in solution then placed on a spinning plate for 20 min. Next, the discs were copiously rinsed with hot tap water followed by distilled water before being washed in acetone on a spinning plate for 10 min. The previous washing procedure was repeated with absolute ethanol then methanol, both steps including cotton swabbing. The discs were next dried under a gentle stream of nitrogen then nitrogen plasma-cleaned for 20 min. To avoid contamination by air pollutants, cleaned discs were immediately employed in the procedure of adlayer formation using freshly prepared solutions of HS-MEG-OH surface modifier (*vide infra*).

IV. Adlayer formation

HS-MEG-OH surface modifier (6.1 mg, 0.05 mmol) was first dissolved in 10 mL of anhydrous ethanol (*Note: this solution should be prepared fresh while TSM discs are being plasma-cleaned*). The resulting 5 mM solution was then portioned (1000 μL) in test tubes into which freshly cleaned TSM discs (*vide supra*) were then individually soaked. The test tubes were sealed with rubber stoppers then placed on a spinning plate for increasing periods of time (5, 15, 20, 25, **30**, 40, 50, 60 and 1080 min). Finally, TSM discs were thoroughly rinsed with anhydrous ethanol (x3) then dried under a gentle stream of nitrogen.

For the surface hydration experiments (performed with HS-MEG-OH adlayers prepared in anhydrous ethanol for 30 min), TSM discs were individually soaked in deionized water (~ 2 mL) immediately following adlayer formation, overnight at room temperature with gentle stirring on a spinning plate. Finally, the discs were dried under a gentle stream of nitrogen.

For the surface hydration experiments omitting the final drying step of the top gold electrode, TSM discs were immediately transferred, wet, into the TSM chamber (the bottom face of the disc was however carefully dried under a gentle stream of nitrogen).

[1] G. H. Woehrle, M. G. Warner and J. E. Hutchison, *Langmuir*, 2004, **20**, 5982.

V. TSM measurements

TSM measurements were performed at 9.0 MHz (1st harmonic). After the standard set-up of the TSM device, including systematic calibration of the network analyzer (Hewlett Packard, model 4395A) prior to each run, the TSM discs were individually inserted into the flow-through chamber and the resonant signal stabilized in air at room temperature. PBS buffer was then flown using a syringe-pump at a rate of 300 µL/min, which was decreased to 100 µL/min once the chamber was filled and free of air bubbles. Once the resonant frequency stabilized again (~ 60 min overall, on average), a 10% goat serum solution in PBS was then injected at the same rate for exactly 1 min before final re-introduction of the PBS buffer flow. Measurements were stopped once the resonant frequency finally stabilized again.

VI. Surface analyses

VI. A. Contact angle goniometry

Static contact angle measurements (CAM) were performed using a KSV goniometer (KSV Instruments Ltd.) and Milli-Q water as the test liquid. Once the sessile water droplets were gently deposited on the surfaces, 5 frames were recorded with 1s intervals. Contact angle values (**Table 1**), calculated for both left and right sides of the droplets, were generated using the CAM101 software provided with the instrument. CAMs were measured for bare and HS-MEG-OH adlayer-modified gold surfaces, both before and after overnight surface hydration.

	Contact angle			
	Before hydration		After hydration	
	θ_L (°)	θ_R (°)	θ_L (°)	θ_R (°)
As-received gold	92	91	–	–
Cleaned bare gold	16	16	30	30
HS-MEG-OH adlayer	33	37	22	20

Table 1 Static contact angle measurements recorded with Milli-Q water for bare and HS-MEG-OH adlayer-modified gold surfaces, both before and after overnight surface hydration.

VI. B. Angle-resolved X-ray photoelectron spectroscopy (ARXPS)

ARXPS was performed with a Theta probe ThermoFisher Scientific Instrument (East Grinstead, UK) located at *Surface Interface Ontario* (University of Toronto, Toronto, Ontario, Canada). The samples were analyzed with monochromated Al Kα X-rays with take-off angles of 27.5°, 42.5°, 57.5° and 72.5° relative to the normal. The binding energy scale was calibrated to the C_{1s} signal at 285 eV. Peak fitting and data analysis were performed using the *Avantage* software. Relative atomic percentages and narrow XPS scans for the characteristic elements of HS-MEG-OH surface modifier and Au substrate are available below (**Table 1** and **Fig. 2**). The formation of HS-MEG-OH adlayers on bare gold was determined following the appearance of a peak for sulfur at ~ 164 eV (sulfide S_{2p}), the one element unambiguously attributable to HS-MEG-OH surface modifier. This peak is not present for bare gold. In the latter case however, there is one peak at ~ 169 eV which is due to contamination by adventitious sulfur oxide species (this signal has substantially decreased for the HS-MEG-OH adlayer). The presence of carbon and oxygen on bare gold is also caused by unavoidable contamination by adventitious species.

	Analysis angle (°)	**% S2p (162-166 eV)**	**% C1s (283-291 eV)**	**% O1s (528-536 eV)**	**% Au4f (82-92 eV)**
Cleaned bare gold	27.5	0.0	28.4*	36.4*	35.2
	42.5	0.3	32.9*	38.9*	27.9
	57.5	0.0	40.1*	40.0*	19.9
	72.5	0.0	47.5*	40.9*	11.6
HS-MEG-OH adlayer	27.5	1.4	12.9	4.0	81.7
	42.5	0.9	19.4	4.9	74.8
	57.5	1.9	24.5	7.2	66.4
	72.5	1.7	42.5	9.1	46.7

Table 1 Angle-resolved XPS analysis (relative atomic percentages) for bare and HS-MEG-OH adlayer-modified gold surfaces. * These signals are due to unavoidable contamination by adventitious species.

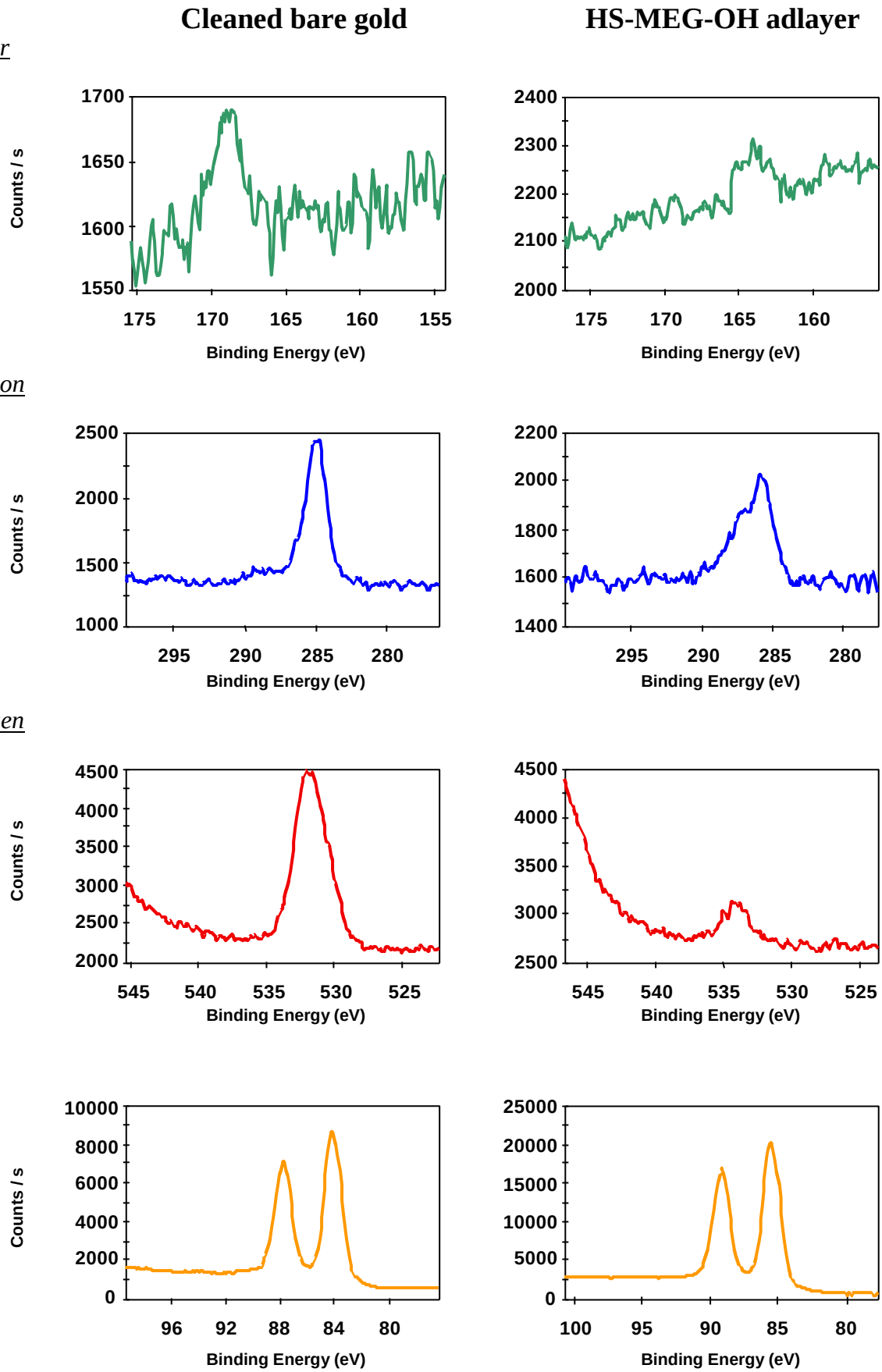
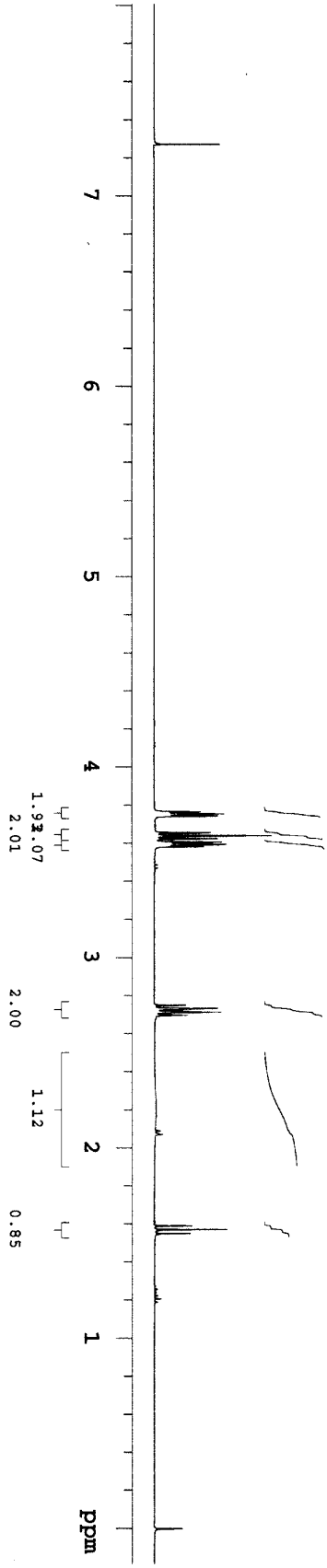
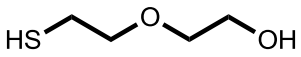


Fig. 2 Narrow scans for (top to bottom) sulfur, carbon, oxygen and gold for (left to right) bare and HS-MEG-OH adlayer-modified gold surfaces. The presence of sulfur, carbon and oxygen for bare gold is due to unavoidable contamination by adventitious species.

VII. ^{1}H and ^{13}C NMR spectra





HS-MEG-OH

^{1}H NMR (400 MHz, $CDCl_3$)

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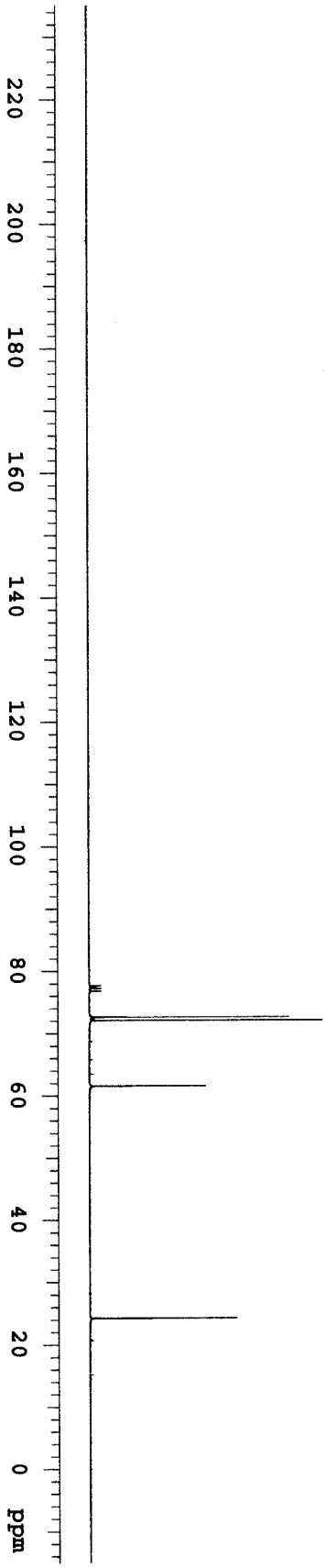

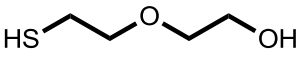

HS-MEG-OH

^{13}C NMR (300 MHz, $CDCl_3$)

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