Supporting Information

Antifouling Coatings Generated from Unsymmetrical Partially Fluorinated Spiroalkanedithiols

Lydia R. St. Hill,[1] John W Craft, Jr.,[2] Pawilai Chinwangso,[1] Hung-Vu Tran,[1] Maria D. Marquez,[1] and T. Randall Lee[1,]*

[1]Department of Chemistry and the Texas Center for Superconductivity, University of Houston, 4800 Calhoun Road, Houston, Texas 77204-5003, United States

[2]Department of Biology and Biochemistry, University of Houston, 4800 Calhoun Road, Houston, Texas 77204-5001, United States

*Corresponding author: trlee@uh.edu

Materials and Methods

Absolute Ethanol (200 proof) from Decon Labs, Inc., Tetrahydrofuran (THF), sulfuric acid (H_2SO_4) both from Avantor Performance Materials, and hydrogen peroxide (30% H_2O_2) from Mallinckrodt Chemicals were all used as received. The adsorbate 1-hexadecanethiol (**C16SH**) was purchased from Sigma-Aldrich. The other adsorbates, 9,9,10,10,11,11,12,12,13,13,14,14,15,15,16,16,16-heptadecafluorohexadecane-1-thiol (**F8H8SH**) 2-decyl-2-(9,9,10,10,11,11,12,12,13,13,14,14,15,15,-16,16,16-heptadecafluorohexa-decyl)propane-1,3-dithiol (**F8H10-C12**), and 2-(9,9,10,10,11,11,12,12,-13,13,14,14,15,15,16,16,16-heptadecafluorohexadecyl)-2-hexadecylpropane-1,3-dithiol (**F8H10-C18**) were synthesized according to procedures found in the literature.[1,2] Fibrinogen, lysozyme, bovine serum albumin (BSA), protamine were all obtained from Sigma Aldrich and used as received. Phosphate buffer solution (PBS) 10x from GenDepot was diluted to 1x using water (H_2O) generated from a Milli-Q water system with resistance of 18.2 MΩ.

Gold shot (99.999%) was purchased from Kamis, Inc. Chromium rods (99.9%) were purchased from Kurt J. Lesker Company. Polished single-crystal Si(100) wafers were purchased from University Wafer and rinsed with absolute ethanol before use. Surface plasmon resonance spectroscopy (SPR) sensors composed of 500 Å of Au plated onto Ti-coated silica substrates were purchased from Sofchip. Quartz crystal microbalance (QCM) sensors composed of polished AT-cut quartz crystals oscillating at a 10 MHz frequency coated with Ti and plated with 1000 Å of Au were purchased from International Crystal Manufacturing Company, Inc.

Preparation of Substrates. Gold substrates used in ellipsometric thickness measurements were prepared by thermal evaporation onto Si(100) wafers under high vacuum at a pressure ≤ 6 x 10^{-5} torr. A chromium adhesion layer (100 Å) was initially deposited, followed by a gold layer (1000 Å) at a rate of 0.5 Å/s. Prior to being used, QCM and SPR sensors were cleaned with piranha solution, rinsed with copious amounts of Milli-Q water, then absolute ethanol, and finally dried in a stream of ultra-pure nitrogen gas. *Caution: Piranha solution reacts violently with organic materials and should be handled carefully!*

Preparation of SAMs. All vials were cleaned with piranha solution prior to preparation of the adsorbate solutions. SAMs were prepared by incubating the respective gold samples (evaporated gold, QCM, and SPR sensors) in 1 mM solutions of thiol adsorbates, prepared in absolute ethanol. The SAMs were allowed to equilibrate for 48 h at rt in the dark. Prior to any measurements, all films were rinsed with THF, followed by absolute ethanol, and dried with ultra-pure nitrogen gas.

Protein Preparation/Exposure. Protein solutions were prepared by dissolving 1 mg of the protein in 1 mL of the PBS solution. All buffers and protein solutions were filtered through 0.2 µm filters. Following preparation, all solutions were sonicated for 15 min. After SAM formation, the thermally evaporated Au samples and QCM sensors were immersed in the protein solutions for 1 h to allow protein adsorption on the SAMs to reach surface saturation. Subsequently, samples

were rinsed first with PBS buffer followed with Milli-Q water, and then dried with a stream of ultra-pure nitrogen gas. For the SPR sensors, the protein solution was loaded into a Biacore instrument (details below), where the protein solution was injected and then flowed over the surface of the SAM.

SPR Procedures. The SPR sensorgrams were obtained using a Biacore 2000. Sensors were initially exposed to PBS buffer at a rate of 30 µL/min, followed by a 300 µL injection of a protein solution at a concentration of 1 mg/mL. After the injection of protein solutions, measurements were recorded with the buffer continuing to flow over the sensor surface for 2 h. The reported values are an average of 3 separate sets of experiments using freshly prepared SAMs. The response signal arises from changes in the resonance angle of light reflected from the Au surface. As the protein solution flows through the cell, protein interacts with the surface through binding and disassociation, leading to a change in the angle of reflection of light from the Au surface. The SPR technique is based on the Kretschmann theory.[3] A sensorgram is then generated as a response to changes in the refractive index at the interface of the surface. After exposing the surface to the protein solution, PBS was allowed to flow again to wash away any unbound or weakly attached protein on the surface. The complete removal of protein solution from the cell was reflected as a decrease in signal intensity in the curve. The difference between the initial baseline response and the final response is represented as ΔRU.

QCM Measurements. Frequency measurements (*f*) of functionalized QCM sensors were performed using a Gamry eQCM with a continuous driving voltage to the piezoelectric-crystal oscillator, which simultaneously collects parallel and in series measurements on the crystal. Each QCM crystal was allowed to reach equilibrium after the driving voltage was applied from the oscillator. The QCM sensor is a thin piezoelectric crystal sandwiched between two Au plates acting as electrodes. This arrangement allows for voltage to be applied to the electrodes, which generates an alternating electric field on each side of the crystal that causes the crystal to vibrate at a characteristic resonant frequency. Any change in mass (addition or loss) gives rise to a change in this resonant frequency, which can be measured. In our studies, the frequency change was normalized by taking readings of the bare gold sensor, the sensor after SAM formation, and then again after protein exposure. The reported values are an average of the resonant frequency after stabilization. A decrease in the resonant frequency (Δf) was observed when proteins adhered to the surface of the sensor. The value of Δf is proportional to the change in mass (Δm) following the Sauerbrey equation (Equation 1).[4-6]

$$\Delta m = -C_f \Delta f \qquad (1)$$

The quantification of the mass loading on the surface of the QCM crystal was determined based on Equation 1, where C_f (the mass sensitivity constant) is -226 µg/ (Hz cm^2) for the 10 MHz AT-cut QCM crystal used in our studies.[6]

Ellipsometry Measurements. Monolayer thicknesses were measured using a Rudolph Auto EL III ellipsometer equipped with a He-Ne laser (632.8 nm) at an incident angle of 70°. The refractive index was set to 1.45. The reported thickness values of the SAMs formed on the evaporated gold substrate are an average of 24 measurements (2 measurements per slide with 4 slides for each adsorbate with 3 independent trials). The thickness values obtained for the untreated SAMs are provided in Table S1. After SAM formation, the thin films were exposed to protein solutions for 1 h to allow the surface to become saturated, the ellipsometric thickness was measured again to determine changes in thickness due to protein exposure. The change in thickness was obtained by subtracting the initial SAM thickness from the total thickness measurement of the SAM after the protein exposure, which yields the thickness value of the absorbed protein layer. The reported thickness measurements of the SAMs after protein exposure are an average of 6 measurements from 3 individual trials. No distinguishable change in thickness between the evaporated gold, QCM, as well as SPR sensor surfaces before and after exposure to protein solutions were observed.

Surface Energy Calculations. The estimated values for the surface energy of the SAMs can be obtained by using the contact angle values found in Table S2 below and Young's equation, which describes the energy balance at the intersection of the three phases (solid-vapor, liquid-vapor, and solid-liquid).[7-10] Equation 2 shows Young's equation, which relates the interfacial tensions and the contact angle θ of a droplet of probe liquid deposited on the surface.[7-10]

$$\gamma_{SV} = \gamma_{LV} \cos \theta + \gamma_{SL} \tag{2}$$

One difficulty that arises when using Young's equation is the inability to directly measure the tension of the solid-liquid interface, γ_{SL}. To solve the equation, some assumptions with regard to the relationship between γ_{SV}, γ_{LV}, and γ_{SL} must be made. Using the Owens-Wendt method, γ_{SL} can be determined according to Equation 3.

$$\gamma_{SL} = \gamma_{SV} + \gamma_{LV} - 2 \left(\sqrt{\gamma_{SV}^D \gamma_{LV}^D} + \sqrt{\gamma_{SV}^P \gamma_{LV}^P} \right) \tag{3}$$

In Equation 3, γ_{SV}^D and γ_{LV}^D are the dispersive components and γ_{SV}^P and γ_{LV}^P are the polar components of the surface tensions. Combining Equation 1 with Equation 3 yields Equation 4.

$$\sqrt{\gamma_{SV}^D \gamma_{LV}^D} + \sqrt{\gamma_{SV}^P \gamma_{LV}^P} = \frac{1}{2} [\gamma_{LV}(1 + \cos \theta)] \tag{4}$$

The Owens-Wendt method requires the use of two probe liquids with known dispersive and polar components along with the contact angle of the probe liquids on the substrate and the surface tensions of the probe liquids. For our study, the surface energies of the SAMs were estimated using the contact angles of H_2O and HD, and are provided in Table S2.[2,5,6,11] The surface tensions and the polar and dispersive components of the contacting liquids are listed in Table S3.[8,9] The surface energies of the SAMs, listed in Table S4, were calculated using Equation 4 and the numerical values provided in Tables S2 and S3.

Table S1. Ellipsometric Thicknesses of SAMs without Exposure to Proteins

Adsorbate	Thickness (Å)
C16SH	20 ± 1
F8H8SH	17 ± 1
F8H10-C12	16 ± 1
F8H10-C18	21 ± 1

Table S2. Advancing contact angles (θa, °) for water and hexadecane as probing liquids on SAMs surface.[2,5,6,11]

	Water (H_2O)	**Hexadecane** (HD)
	θa, °	θa, °
C16SH	108	51
F8H8SH	120	83
F8H10-C12	125	73
F8H10-C18	121	59

Table S3. Surface Tension, Polar, and Dispersive Components for Water and Hexadecane

Liquid	γ_{LV} (mN·cm^{-1})	γ_{LV}^{P} (mN·cm^{-1})	γ_{LV}^{D} (mN·cm^{-1})
Water	72.1	52.2	19.9
Hexadecane	27.5	0	27.5

Table S4. Surface Energies of the SAMs

Adsorbate	Surface Energy (mJ/m^2)
C16SH	18.6
F8H8SH	9.5
F8H10-C18	15.8
F8H10-C12	11.5

References

1. Graupe, M.; Koini, T.; Wang, V. Y.; Nassif, G. M.; Colorado, R.; Villazana, R. J.; Dong, H.; Miura, Y. F.; Shmakova, O. E.; Lee, T. R. Terminally Perfluorinated Long-Chain Alkanethiols. *J. Fluor. Chem.* **1999**, *93*, 107–115.

2. Chinwangso, P.; St. Hill, L. R.; Marquez, M. D.; Randall Lee, T. Unsymmetrical Spiroalkanedithiols Having Mixed Fluorinated and Alkyl Tailgroups of Varying Length: Film Structure and Interfacial Properties. *Molecules* **2018**, *23*, 2632.

3. Akimov, Y.; Pam, M. E.; Sun, S. Kretschmann-Raether Configuration: Revision of the Theory of Resonant Interaction. *Phys. Rev. B* **2017**, *96*, 155433–155440.

4. Gamry Instruments. Basics of a Quartz Crystal Microbalance. *Spectrum* **2012**, *1*, 1–7.

5. Lee, H. J.; Jamison, A. J.; Lee, T. R. Two Are Better than One: Bidentate Adsorbates Offer Precise Control of Interfacial Composition and Properties. *Chem. Mater.* **2016**, *28*, 5356–5364.

6. Weinstein, R. D.; Moriarty, J.; Cushnie, E.; Colorado, R., Jr.; Lee, T. R.; Patel, M.; Alesi, W. R.; Jennings, G. K. Structure, Wettability, and Electrochemical Barrier Properties of Self- Assembled Monolayers Prepared from Partially Fluorinated Hexadecanethiols. *J.Phys. Chem. B.* **2003**, *107*, 11626–11632.

7. Chen, J.; Wang, Z.; Oyola-Reynoso, S.; Thuo, M. M. Properties of Self-Assembled Monolayers Revealed via Inverse Tensiometry. *Langmuir* **2017**, *33*, 13451–13467.

8. Shon, Y.-S.; Lee, S.; Colorado, R.; Perry, S. S.; Lee, T. R. Spiroalkanedithiol-Based SAMs Reveal Unique Insight into the Wettabilities and Frictional Properties of Organic Thin Films. *J. Am. Chem. Soc.* **2000**, *122*, 7556–7563.

9. Colorado, R.; Lee, T. R. Wettabilities of Self-Assembled Monolayers on Gold Generated from Progressively Fluorinated Alkanethiols. *Langmuir* **2003**, *19*, 3288–3296.

10. Takenaga, M.; Jo, S.; Graupe, M.; Lee, T. R. Effective van der Waals Surface Energy of Self-Assembled Monolayer Films having Systematically Varying Degrees of Molecular Fluorination. *J. Colloid Interface Sci.* **2008**, *320*, 264–267.

11. Drelich, J.; Wilber, J. L.; Miller, J. D.; Whitesides, G. M. Contact Angles for Liquid Drops at a Model Heterogeneous Surface Consisting of Alternating and Parallel Hydrophobic/Hydrophilic Strips. *Langmuir* **1996**, *12*, 1913–1922.