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Wetting and Protein Adsorption of Self-Assmebled Monolayers of Alkanethiolates Supported on Transparent Films of Gold[1]

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We have formed self-assembled monolayers (SAMs) of alkanethiolates on 5-nm- and 10-nm-thick films of gold supported on titanium-primed glass. These electrically-conductive films transmit visible light,[5] support SAMs that have wetting properties superior to those of SAMs supported on opaque 200-nm-thick films commonly used in this area of surface chemistry,[6] and have surfaces that are smoother than those of thicker films. We present two examples demonstrating the combination of flexibility in control of surface chemistry offered by SAMs of alkanethiols and optical transparency provided by these thin films: measurements of the adsorption of RNase A [EC 3.1.27.5] labeled with eosin-5-isothiocyanate (EITC) on methyl- and hexaethylene glycol-terminated SAMs using transmission spectroscopy, and observations of the differential attachment and growth of living MG63 osteosarcoma cells on patterned SAMs of hydroxyl- and methyl-terminated alkanethiolates using conventional phase-contrast microscopy.

To prepare 5-nm- and 10-nm-thick films of gold on titanium-primed glass, we sequentially deposited 1 nm of titanium at 0.1 nm/s and gold at 0.1 nm/s; thick films of gold (200 nm) were deposited at 0.5 nm/s on glass primed with 5 nm of titanium at 0.2 nm/s.[7,8] For studies of protein adsorption, 0.5 nm of titanium and 5 nm of gold were deposited on each side of glass substrates. The continuity of these films of gold was verified using X-ray photoelectron spectroscopy (XPS).[9] They also were electrically conductive, with the resistivities $(7.1 \pm 0.3) \times 10^{-6}$ Ω-cm (5-nm-thick Au), $(4.8 \pm 0.4) \times 10^{-6}$ Ω-cm (10-nm-thick Au), and $(3.7 \pm 1.5) \times 10^{-6}$ Ω-cm (200-nm-thick Au).[10] SAMs were formed by chemisorption from 1 mM ethanolic solutions of ω-functionalized alkanethiols for 2 h at room temperature.

The transparency of SAMs on thin films of gold increased with decreasing thickness of gold (Figure 1). Films became more blue and gray as the thickness of gold decreased (200-nm-thick gold had the same color as bulk gold). Formation of SAMs on these films did not affect their transparency or color.[11] Transparency could be increased using a (3-mercaptopropyl)trimethoxysiloxy layer rather than titanium as an adhesion promoter between gold and glass, but evaporation of titanium was a more reliable and convenient technique than silanization.[12] Thin gold films (5 and 10 nm) deposited in the absence of either adhesion promoter were significantly grayer and less conductive than gold supported on titanium-primed glass and did not support well-ordered SAMs.

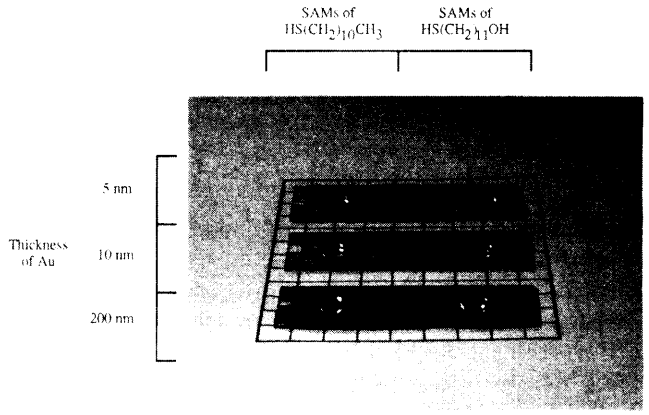

Figure 1. Photograph of 100-μL drops of water on methyl- and hydroxyl-terminated SAMs on 5-nm-, 10-nm-, and 200-nm-thick films of gold supported on titanium-primed glass slides.

The advancing contact angles of water and hexadecane (HD) for SAMs of undecanethiol and 11-(hexaethylene glycol)-undecane-1-thiol were relatively independent of the thickness of the supporting gold (Table 1).[13] Hysteresis, however, was smaller for the SAMs on 5-nm- and 10-nm-thick gold than for these SAMs on 200-nm thick gold because the receding contact angles for these liquids *increased* with decreasing thickness of gold.[14] The thickness of the gold on hysteresis also correlates with the morphology of the surface: atomic force microscopy (AFM) demonstrated that the mean absolute roughness of films of gold decreased from 1.2 nm for 100-nm-thick gold to 0.2 nm for 10-nm-thick gold and 0.09 nm for 5-nm-thick gold.[15]

We measured the adsorption of RNase A on methyl- and hexaethylene glycol-terminated SAMs by observing the change in optical absorbance of SAMs after incubation with 1 mg/mL solutions of protein covalently labeled with EITC (Figure 2).[16,17] The visible spectra for both types of SAMs before and after exposure to unlabeled RNase A were comparable, but the spectrum for EITC-RNase A on a methyl-terminated SAM showed a distinct peak at 532 nm.[18,19] This peak corresponded to 0.35 μg/cm² of EITC-RNase A;[20] we estimate that a close-packed monolayer of RNase A would contain 0.21–0.37 μg/cm².[21] In contrast, no peak was observed for a hexaethylene glycol-terminated SAM exposed to EITC-RNase A; the demonstration that these SAMs resist protein adsorption agrees with previous results.[16,18]

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(2) NIH postdoctoral fellow, 1992–1993.
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(4) Ford Foundation postdoctoral fellow, 1992–1993.
(5) For a review of transparent metallic films, see: Vossen, J. L. In *Physics of Thin Films, Vol 9*; Haas, G., Francombe, M. H., Hoffman, R. W., Eds.; Academic Press: New York, 1977; pp 1–71. For other examples of transparent gold, see: Johnson, P. B.; Christy, R. W. *Phys. Rev. B* **1972**, *6*, 4370–4379. Smith, G. B.; Niklasson, G. A.; Svensson, J. S. E. M.; Granqvist, C. G. *J. Appl. Phys.* **1986**, *59*, 571–581.
(6) For reviews see: Whitesides, G. M.; Laibinis, P. E. *Langmuir* **1990**, *6*, 87–96. Ulman, A. *An Introduction to Ultrathin Organic Films from Langmuir–Blodgett to Self-Assembly*; Academic Press, Inc.: San Diego, CA, 1991.
(7) It is also possible to prepare discontinuous transparent metallic films by photoelectrodeposition (*e.g.*, Porter, J. D.; Heller, A.; Aspnes, D. E. *Nature* **1985**, *313*, 664–666).
(8) Glass slides (1-in. × 3-in., 1.2 μg thick, VWR Scientific, catalog no. 48300-036) were cleaned with 70/30 v/v concentrated H_2SO_4/30% H_2O_2.
(9) The intensities of the Au(4f), Ti(2p), and Si(2p) peaks by XPS were more consistent with the intensities predicted by a model in which continuous films of gold were present on titanium than with a model in which gold formed islands on titanium.[12]
(10) Resistivities were determined by measuring the voltage drop at constant applied current using a four-point probe with osmium tips (tip spacing of 0.0625 in., tip radius of 0.005 in. Alessi, Irvine, CA) and correcting for the finite size of the film. The resistivity of bulk gold is 2.4×10^{-6} Ω-cm (Kittel, C. *Introduction to Solid-State Physics*; John Wiley and Sons: New York, 1976).
(11) Allara, D. L.; Hebard, A. F.; Padden, F. J.; Nuzzo, R. G.; Falcone, D. R. *J. Vac. Sci. Technol. A* **1983**, *1*, 376–382.
(12) DiMilla, P. A.; Folkers, J. P.; Biebuyck, H. A.; Whitesides, G M., unpublished data.
(13) Bain, C. D.; Troughton, E. B.; Tao, Y.-T.; Evall, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 321–335. Troughton, E. B.; Bain, C. D.; Whitesides, G. M.; Nuzzo, R. G.; Allara, D. L.; Porter, M. D. *Langmuir* **1988**, *4*, 365–385.
(14) We define hysteresis in the contact angle as the difference between the cosines of the receding and advancing contact angles.
(15) Values for mean absolute roughness were measured using a TMX-2000 AFM (Topometrix, Sunnyvale, CA) with a electrodeposited carbon tip and silicon cantilever. AFM rather than STM was used because the total conductance of our films was limited by the total amount of metal present.[12] The roughness of bare glass and 5 nm of gold on glass primed with 1 nm of titanium was indistinguishable; we expect that 200-nm-thick gold is at least as rough as 100-nm-thick gold.

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Table 1. Wetting Properties of Water and Hexadecane (HD) on SAMs Prepared by Adsorption of Methyl- and Hexaethylene Glycol-Terminated Alkanethiols on Thin Films of Gold Supported on Titanium-Primed Glass

thickness of gold (nm)	contact angles (advancing, θ_a, receding, θ_r, in deg)[a] and hysteresis ($-\Delta \cos\theta = \cos\theta_r - \cos\theta_a$) in wetting of H_2O and HD on SAMs							
	$HS(CH_2)_{10}CH_3$				$HS(CH_2)_{11}(OCH_2CH_2)_6OH$			
	H_2O		HD		H_2O		HD[b]	
	θ_a, θ_r	$-\Delta \cos\theta$	θ_a, θ_r	$-\Delta \cos\theta$	θ_a, θ_r	$-\Delta \cos\theta$	θ_a, θ_r	$-\Delta \cos\theta$
5	112, 104	0.133	44, 39	0.058	36, 31	0.048	<15, –	<0.034
10	110, 98	0.203	44, 39	0.058	34, 27	0.062	<15, –	<0.034
200	113, 98	0.252	45, 30	0.159	37, 20	0.141	<15, –	<0.034

[a] Values for contact angles were slightly smaller than those reported previously but were internally consistent within this study.[13,16] [b] A dash indicates a receding contact angle for a contacting liquid that completely wet the surface. For these systems, $\theta_r \approx \theta°$, and an upper bound on the hysteresis is given.

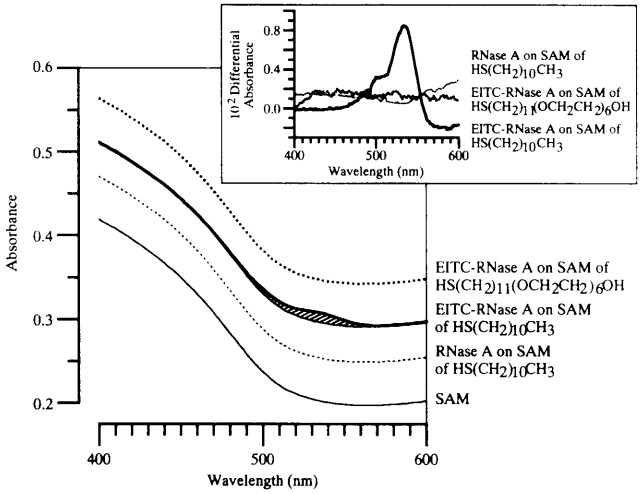

Figure 2. Visible absorbance spectra of SAMs supported on 5-nm-thick films of gold before and after adsorption of unlabeled RNase A or EITC-RNase A. Spectra for methyl- and hexaethylene glycol-terminated SAMs before protein adsorption and after adsorption of unlabeled RNase A were comparable. The spectrum for SAM is plotted as absolute absorbance; absorbances for other spectra are offset for clarity by 0.05 (RNase A on SAM of $HS(CH_2)_{10}CH_3$), 0.10 (EITC-RNase A on SAM of $HS(CH_2)_{10}CH_3$), and 0.015 (EITC-RNase A on SAM of $HS(CH_2)_{11}(OCH_2CH_2)_6OH$). The hatched region shows a peak for adsorbed EITC-RNase A. Inset: spectra for adsorbed RNase A and EITC-RNase A after subtraction of spectra for supporting SAMs; this peak corresponds well with that for the labeled protein in solution.

SAMs on 5-nm- and 10-nm-thick gold supported on glass are an attractive system for investigating interfacial phenomena on chemically well-defined surfaces; they combine the susceptibility to chemical functionalization of SAMs on thick, opaque gold with optical transparency and increased flatness.[22] For example, using conventional phase-contrast optics, we could observe live MG63 osteosarcoma cells on SAMs patterned on 10-nm-thick gold supported on titanium-primed glass (Figure 3): cells preferentially attached and grew on hydroxyl-terminated SAMs but not on adjacent methyl-terminated SAMs.[23] SAMs on thin films of gold also provide the basis for a new system of transparent electrodes whose surfaces can be functionalized readily.[12,24] These surfaces can be patterned with micron-scale features using techniques—micromachining, microwriting, and microstamping—developed for thicker films.[25]

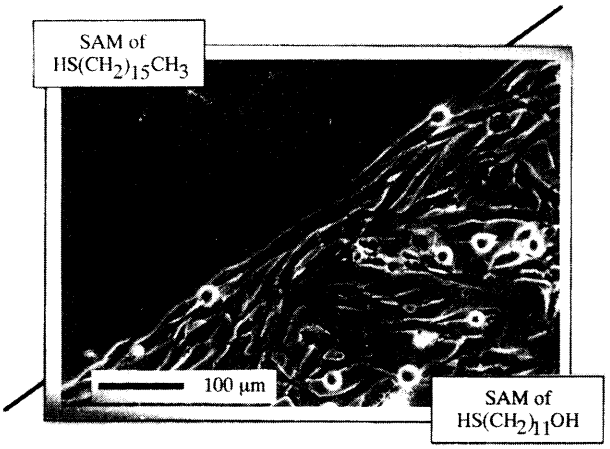

Figure 3. Photograph taken with phase-contrast optics of live MG63 osteosarcoma cells (after incubation for 2 days at 37 °C) on a patterned SAM formed by the adsorption of methyl- and hydroxyl-terminated alkanethiols on a 10-nm-thick film of gold supported on a titanium-primed glass slide.

Supplementary Material Available: A detailed description of the methods used to prepare and characterize SAMs (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(16) Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 10714–10721. Lopez, G. P.; Biebuyck, H. A.; Harter, R.; Kumar, A.; Whitesides, G. M. *Ibid.*, **1993**, *115*, 10774–10781.

(17) Eosin-labeled RNase A was prepared by reacting EITC with RNase A in a borate buffer (Tilton, R. D.; Robertson, C. R.; Gast, A. P. *J. Colloid Interface Sci.* **1990**, *137*, 192–203). Using UV–visible spectrophotometry, the average number of labels per protein was determined to be 0.7.

(18) We confirmed protein adsorption using XPS: N(1s) for unlabeled RNase A and N(1s) and Br(1s) for EITC-RNase A were detected only on methyl-terminated SAMs after exposure to protein. The protein and fluorophore were the only nitrogen-containing species; the fluorophore was the only bromine-containing species.

(19) The thicknesses of RNase and EITC-RNase on methyl-terminated SAMs on 200-nm-thick gold, measured by ellipsometry, were comparable with previous results.[16]

(20) The amount of adsorbed EITC-RNase was calculated from differential absorbance based on an extinction coefficient of 10^5 cm^{-1} M^{-1} for EITC at 522 nm (Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*, 5th ed.; Molecular Probes: Eugene, OR, 1992) and a molecular weight of 13 700 per EITC-RNase conjugate (Wlodawer, A.; Svensson, L. A.; Sjoelin, L.; Gilliland, G. L. *Biochemistry* **1988**, *27*, 2705–2717).

(21) We estimated the range of densities for a close-packed monolayer of RNase by assuming a cross sectional area per molecule of 620–780 $Å^2$, based on crystallographic dimensions of 22 × 28 × 38 Å (Wlodawer, A.; Sjölin, L. *Biochemistry* **1983**, *22*. 2720–2728).

(22) Transmission optics with thin films of gold have been applied to study the adsorption of biomolecules (Wahl, M. C.; Kim, H. S.; Wood, T. D.; Gaun, S.; Marshall, A. G. *Anal. Chem.*, in press) and to fabricate novel lasers (Hasnam, G.; Tai, K.; Wynn, J. D.; Wang, Y. H.; Fischer, R. J.; Hang, M.; Weir, B. E.; Zydzik, G. J.; Mannaerto, J. P.; Ganelin, J.; Cho, A. Y. *Electronics Lett.* **1990**, *26*, 1590–1592).

(23) Cells were plated in Minimal Essential Media (GIBCO, BRL) containing 10% heat-inactivated fetal bovine serum (GIBCO, BRL), and unattached cells were removed after 2 h by rinsing three times with media. Patterned SAMs were prepared as described previously (López, G. P.; Albers, M. W.; Schreiber, S. L.; Carroll, R.; Peralta, E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*. 5877–5878).

(24) Frisbie, C. D.; Fritsch-Faules, I.; Wollman, E. W.; Wrighton, M. S. *Thin Solid Films* **1992**, *210*, 341–347.

(25) Abbott, N. L.; Folkers, J. P.; Whitesides, G. M. *Science* **1992**, *257*, 1380–1382. López, G. P.; Biebuyck, H. A.; Frisbie, D. C.; Whitesides, G. M. *Science* **1993**, *260*, 647–649. Kumar, A.; Whitesides, G. M. *Appl. Phys. Lett.* **1993**, *63*, 2002–2004.