DOI: 10.1002/anie.201107671

Pattern Formation

Unmasking Photolithography: A Versatile Way to Site-Selectively Pattern Gold Substrates**

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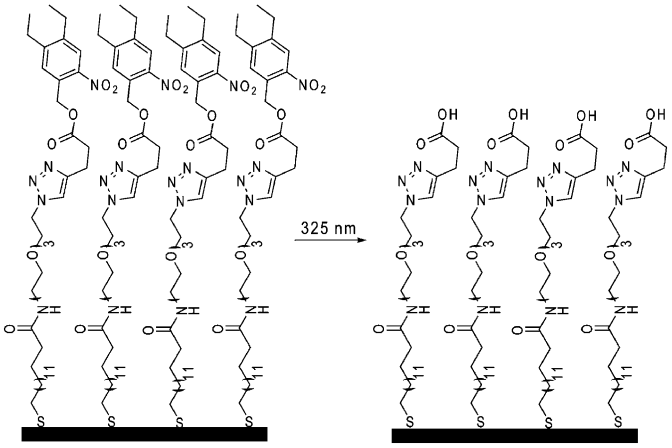
Patterned substrates with well-defined micro- and nanoscale features are central to the development of a broad range of applications and fields, including, but not limited to, microelectronics,[1] solar cell development,[2] and biotechnology.[3] Typically, these applications require the functionalization of inorganic substrates to meet the specific demands of an application. While this is classically achieved using photoresist, lift-off techniques, and chemical etching, one of the methods that has emerged for direct conjugation of active molecules to substrates is thiol-terminated self-assembled monolayers (SAMs) on gold, silver, copper, palladium, and platinum substrates.[4] These substrates are especially useful for biological applications and have been employed in a wide variety of studies ranging from basic cell biology[5] to biosensing.[6] SAMs are an ideal platform for direct functionalization because the monomers bind covalently to substrates through the thiol "head" group and self-assemble through van der Waals packing interactions between adjacent long-chain alkane "tail" groups. This packing orients the terminal functional group to create a new interface with defined chemistry. As a result, many techniques have been developed to pattern SAMs, including soft photolithography,[7–10] photooxidation,[9,11] and dip-pen nanolithography.[12] However, the development of a single technique to create smooth gradients of functional groups and for patterning multiple molecules on a single substrate remains a major challenge in pattern generation. For example, functional group gradients have been generated by diffusing two molecules across a substrate[13] or through photolithographic methods, including gradient photomasks[7] and controlling light exposure.[8,14] While molecular diffusion produces defined gradients, in its most basic form, it does not allow for pattern generation. Patterned gradients can be prepared using microfluidic devices.[15] However, traditional polydimethylsiloxane devices are susceptible to monomer leeching and solvent swelling that can lead to pattern distortion and limits precise molecular control. While gradient photomasks have previously been used to produce functional groups on a surface,[16] the fabrication of high-quality gradient masks is expensive. Moreover, controlling the overall light exposure to a surface has produced regions of varying functional group densities;[7,8,17] however these methods have failed to produce a continuous gradient. Another major shortfall of all these methods is the inability to provide a simple method for patterning multiple molecules on a single substrate. By utilizing a commercial direct-write grayscale photolithography system, we have removed the need for the tradition photomask which provides us with two distinct advantages. First, we can produce smooth, complex functional group gradients on a surface and, second, we are able to pattern multiple molecules sequentially on the same substrate.

To produce continuous gradients using direct-write photolithography, a glycol-terminated photoprotected carboxylic acid monomer was synthesized, shown in Scheme 1 attached to a gold substrate. The nitroveratryl photoprotecting group was employed for our monomer, because it has sufficient absorption and reactivity at 325 nm[18] to allow for rapid photodeprotection by the He–Cd laser in our commercial direct-write photolithography system. Gradient patterns were created from 8-bit gray scale bitmap images with black representing 100% exposure and white representing 0% exposure (Figure 1 A,C). These images were directly read by the photolithography system and transferred to the photoprotected SAM using beam scan direct-write photolithography. In this mode, the laser power is tightly controlled using a mirror mounted on a piezoelectric actuator and the surface is scanned by a beam in one dimension.[18] The second writing dimension is achieved with a high-resolution linear encoded motorized stage. After gradient patterns were generated with the direct-write system, they were imaged using scanning probe microscopy (SPM).



Scheme 1. Photodeprotection of glycol-terminated photoprotected carboxylic acid monomer at 325 nm.

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[**] This work was supported by the National Institute of Mental Health (grant number 1R01MH085495).

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107671.

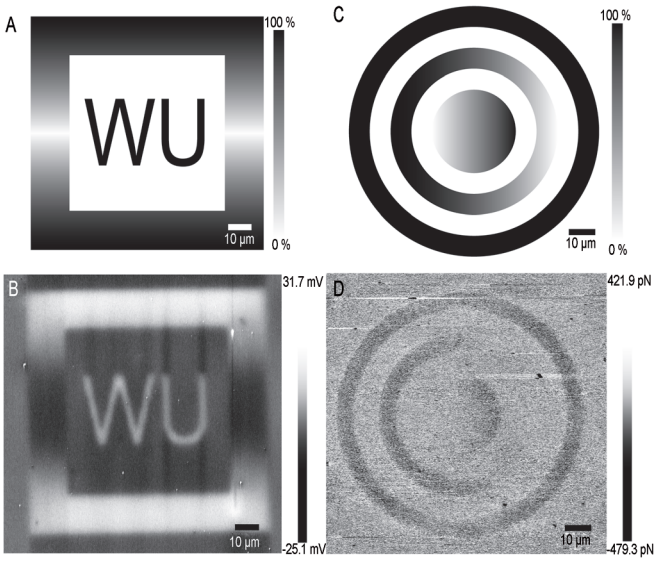
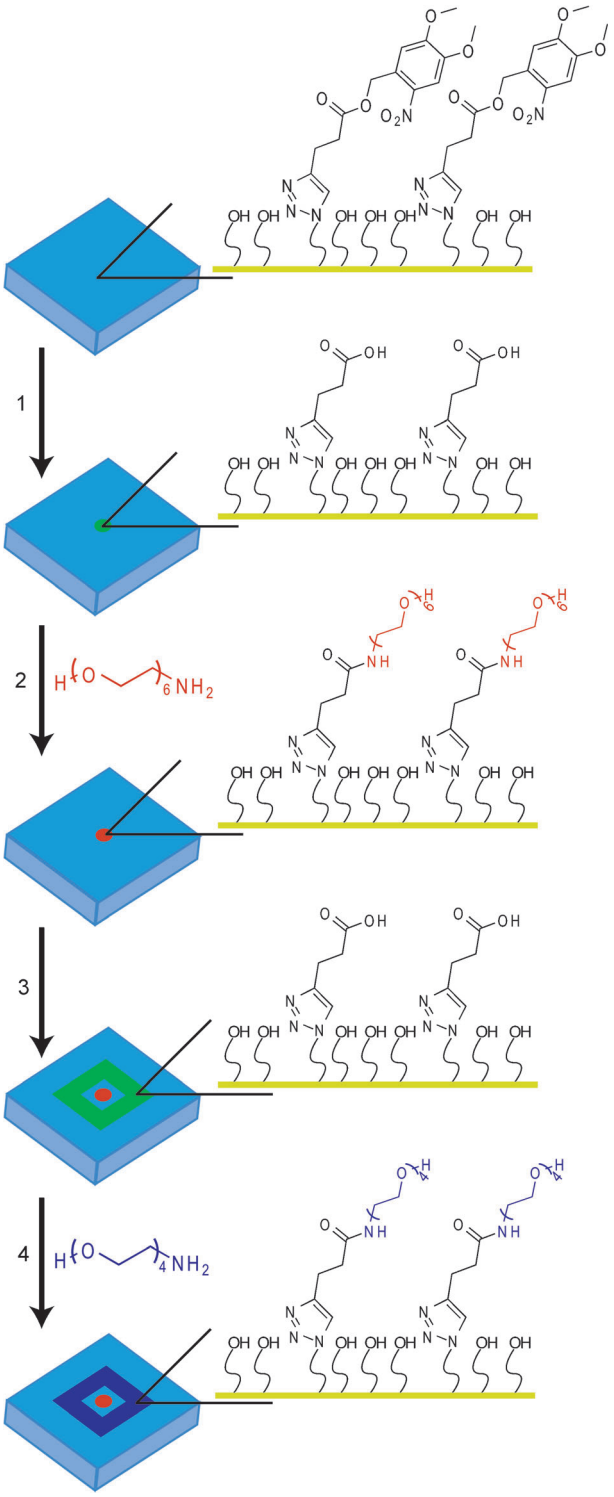


Figure 1. A and C) 8-bit images patterned by direct-write lithography (the scale bar is relative to the laser intensity). B) The resulting KFM image after deprotection. D) The adhesion channel for our patterned surface using PeakForce QNM.

To image our gradient patterns, we have taken advantage of the chemical differences that result upon photodeprotection. One of the most pronounced changes that we would expect to occur upon photodeprotection is a change in surface potential. Upon deprotection, we generate highly polar carboxylic acids in a relatively hydrophobic monolayer background. As a result, we would expect regions with exposed carboxylic acids to have a larger surface potential than the background monolayer. Moreover, the observed surface potential should be related to the number of free carboxylate groups. Kelvin probe microscopy (KFM), an SPM technique, allowed us to directly measure the surface potential. As shown in Figure 1B, our gradient pattern is clearly visible using KFM with white representing the relative amount of carboxylic acid in a particular region, which is consistent with the image patterned on the surface, Figure 1A. As expected, the regions of high carboxylate concentration gave a larger surface potential than the nonpatterned region.

While KFM allows us to clearly visualize our molecular gradients, it requires the use of a relatively large SPM probes (20 nm) compared to high-resolution probes (1–2 nm). However, by utilizing quantitative nanomechanical mapping (QNM), we can image changes in the mechanical properties that result upon photodeprotection using high-resolution SPM probes. Upon photodeprotection, we remove a hydrophobic portion of the molecule that alters the mechanical properties of the underlying structure with the magnitude of the change being proportional to the amount of carboxylic acids revealed. Using QNM SPM, we observe changes in adhesion, dissipation, and deformation (Figure 1D and Figure S1 in the Supporting Information). In Figure 1D, the patterned regions show a lower adhesion signal (darker) than the nonpatterned regions due to a greater adhesion force between the probe and the nitroveratryl monomer compared to a free carboxylic acid. The observed images are a result of both changes in the bulk nanomechanical properties and in tip-sample interactions that result from protecting group cleavage.



Scheme 2. Two-molecule patterning scheme. 1) Pattern circle, 2) activate carboxylic acid with EDC/HOAt and couple hexaethylene glycol amine, 3) pattern frame around the circle, and 4) activate carboxylic acid with EDC/HOAt and couple tetraethylene glycol amine.

Another major advantage of our methodology is the ability to pattern two molecules on the same surface. To accomplish this, photolithography was carried out using two distinct overlaid patterns with each pattern encoding the spatial distribution of a different amine molecule as shown in Scheme 2. Briefly, a circle was patterned on the substrate producing free carboxylic acids, which were subsequently activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimid/1-hydroxy-7-azabenzotriazole (EDC/HOAt). Hexaethylene glycol amine was then added to the solution, which resulted in the molecule being coupled to the surface. After coupling the first amine, a frame was patterned around the circle. The newly formed carboxylic acids were activated with EDC/HOAt and tetraethylene glycol amine was coupled to the substrate. This multi-molecule pattern was designed to highlight the flexibility of maskless photolithography, because production of this image by traditional methods would require at least two masks and the alignment would be extremely difficult.

Patterned samples were characterized by imaging matrix-assisted laser desorption–ionization time of flight mass spectrometry (MALDI-TOF MS),[19] to ensure that only site-selective deprotection and coupling had occurred. Imaging was carried out using 100 μm spots spaced 250 μm apart (center to center) with each spectrum consisting of 20 averaged spectra containing 50 shots. The resulting spectra were then analyzed for the molecular weights of the coupled products (**1** and **2**), the disulfide of the glycol (**3**), and the photoprotected monomer, and the corresponding heat maps were generated (Figure 2). As shown in Figure 2A, a bright circle was produced when analyzing for **1** which is consistent with our patterning scheme. A bright frame was produced when analyzing for **2**, Figure 2B. However there is a small amount of **2** observed in the circle region, which is a result of steric packing of the acids limiting activated ester formation. In addition, we observed a loss in signal when analyzing for the photoprotected monomer as shown in Figure 2C. This result is expected because the photoprotected monomer is converted to a coupled product in the patterned regions. The versatility of this method is shown in Figure 2D, which is an overlay of the two heat maps generated from the coupled molecules **1** and **2** showing that two distinct molecules were coupled to the same substrate in a site-specific manner using our patterning methodology.

Here we have developed a versatile method for patterning multiple molecules on a single substrate at defined molecular densities using direct-write photolithography. Smooth molecular gradients were straightforward to generate using gray-scale photolithography and could be characterized using SPM in both KFM and QNM modes. The alignment of two molecules on a single substrate was implemented using multilayer photolithography. In conclusion, the methodology developed here is broadly applicable to the development of patterned molecular substrates for materials applications and is especially pertinent to the development of biosensors and cell-based assays.

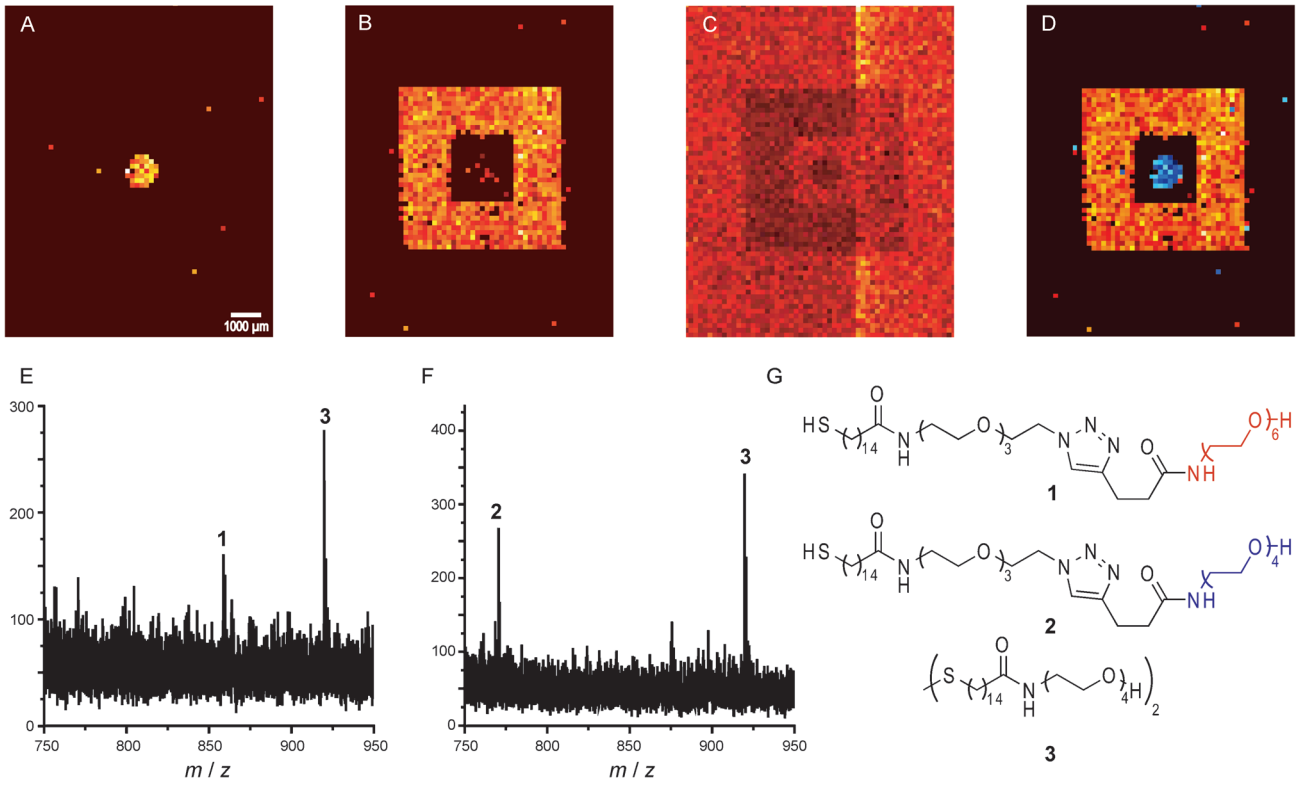


Figure 2. A,B,C) Heat maps generated after analysis for molecules **1**, **2**, and glycol-terminated photoprotected carboxylic acid monomer, respectively. D) Overlay of A and B. E,F) Representative MALDI-TOF spectrums for the circle and frame region, respectively. G) Molecules analyzed in the MALDI-TOF spectrum.

Experimental Section

For detailed synthetic methods and procedures for the surface preparation, analysis, and characterization see the Supporting Information.

Two molecule attachment: Photoreactive SAMs for surface coupling studies were prepared by soaking a gold slide (50 Å Ti, 100 Å Au) in an ethanolic solution of a 0.25 mM glycol-terminated photoprotected carboxylic acid monomer and a 0.75 mM hydroxy-terminated glycol monomer. The substrates were then photodeprotected using our direct-write photolithography system according to the uploaded 8-bit file in beam scan mode. After photodeprotection, slides were rinsed with ethanol, water, and ethanol, and dried under a stream of nitrogen. The freshly exposed carboxylate groups were then activated with 1 mL of 5 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC·HCl) in anhydrous CH_2Cl_2 for 1 min followed by the addition of 1 mL of 2.5 mM 1-hydroxy-7-aza-benzotriazole (HOAt) in anhydrous dimethylformamide/dichloromethane (DMF/CH_2Cl_2) at a volume ratio of 1:1. The reaction proceeded with shaking at 200 rpm for 15 min before 1 mL of 1.5 mM hexaethylene glycol amine in anhydrous DMF was added. The reaction proceeded for an additional hour. Slides were then removed from the reaction mixture and rinsed with ethanol, water, and ethanol, and dried under nitrogen. Three rounds of activation and coupling were carried out before the process was repeated for tetraethylene glycol.

Received: October 31, 2011
Revised: January 3, 2012
Published online: January 23, 2012

Keywords: monolayers · photochemistry · scanning probe microscopy · surface chemistry

[1] H. Yamada, H. Imahori, Y. Nishimura, I. Yamazaki, T. K. Ahn, S. K. Kim, D. Kim, S. Fukuzumi, *J. Am. Chem. Soc.* **2003**, *125*, 9129 – 9139; C.-H. Kuo, C.-P. Liu, S.-H. Lee, H.-Y. Chang, W.-C. Lin, Y.-W. You, H.-Y. Liao, J.-J. Shyue, *Phys. Chem. Chem. Phys.* **2011**, *13*, 15122 – 15126; D. Mandler, S. Kraus-Ophir, *J. Solid State Electrochem.* **2011**, *15*, 1535 – 1558.

[2] S. Khodabakhsh, B. M. Sanderson, J. Nelson, T. S. Jones, *Adv. Funct. Mater.* **2006**, *16*, 95 – 100.

[3] D. Falconnet, G. Csucs, H. Michelle Grandin, M. Textor, *Biomaterials* **2006**, *27*, 3044 – 3063.

[4] J. C. Love, L. A. Estroff, J. K. Kriebel, R. G. Nuzzo, G. M. Whitesides, *Chem. Rev.* **2005**, *105*, 1103 – 1170.

[5] C. B. Herbert, T. L. McLernon, C. L. Hypolite, D. N. Adams, L. Pikus, C. C. Huang, G. B. Fields, P. C. Letourneau, M. D. Distefano, W.-S. Hu, *Chem. Biol.* **1997**, *4*, 731 – 737; D. M. Johnson, J. A. Maurer, *Chem. Commun.* **2011**, *47*, 520 – 522; D. M. Yanker, J. A. Maurer, *Mol. BioSyst.* **2008**, *4*, 502 – 504.

[6] D. Samanta, A. Sarkar, *Chem. Soc. Rev.* **2011**, *40*, 2567 – 2592.

[7] W. S. Dillmore, M. N. Yousaf, M. Mrksich, *Langmuir* **2004**, *20*, 7223 – 7231.

[8] M. Álvarez, J. M. a. Alonso, O. Filevich, M. Bhagawati, R. Etchenique, J. Piehler, A. n. del Campo, *Langmuir* **2011**, *27*, 2789 – 2795.

[9] J. Huang, J. C. Hemminger, *J. Am. Chem. Soc.* **1993**, *115*, 3342 – 3343.

[10] X. Han, S. N. D. Pradeep, K. Critchley, K. Sheikh, R. J. Bushby, S. D. Evans, *Chem. Eur. J.* **2007**, *13*, 7957 – 7964; J. M. Alonso, A. Reichel, J. Piehler, A. del Campo, *Langmuir* **2008**, *24*, 448 – 457; S. A. Alang Ahmad, L. S. Wong, E. ul-Haq, J. K. Hobbs, G. J. Leggett, J. Micklefield, *J. Am. Chem. Soc.* **2011**, *133*, 2749 – 2759.

[11] R. E. Ducker, S. Janusz, S. Sun, G. J. Leggett, *J. Am. Chem. Soc.* **2007**, *129*, 14842 – 14843.

[12] R. D. Piner, J. Zhu, F. Xu, S. Hong, C. A. Mirkin, *Science* **1999**, *283*, 661 – 663.

[13] B. Liedberg, P. Tengvall, *Langmuir* **1995**, *11*, 3821 – 3827.

[14] P. Burgos, M. Geoghegan, G. J. Leggett, *Nano Lett.* **2007**, *7*, 3747 – 3752.

[15] N. L. Jeon, S. K. W. Dertinger, D. T. Chiu, I. S. Choi, A. D. Stroock, G. M. Whitesides, *Langmuir* **2000**, *16*, 8311 – 8316; S. K. W. Dertinger, X. Jiang, Z. Li, V. N. Murthy, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 12542 – 12547; X. Jiang, Q. Xu, S. K. W. Dertinger, A. D. Stroock, T.-m. Fu, G. M. Whitesides, *Anal. Chem.* **2005**, *77*, 2338 – 2347; N. P. Westcott, B. M. Lamb, M. N. Yousaf, *Anal. Chem.* **2009**, *81*, 3297 – 3303; B. M. Lamb, S. Park, M. N. Yousaf, *Langmuir* **2010**, *26*, 12817 – 12823.

[16] D. Ryan, B. A. Parviz, V. Linder, V. Semetey, S. K. Sia, J. Su, M. Mrksich, G. M. Whitesides, *Langmuir* **2004**, *20*, 9080 – 9088.

[17] A. del Campo, D. Boos, H. W. Spiess, U. Jonas, *Angew. Chem.* **2005**, *117*, 4785 – 4791; *Angew. Chem. Int. Ed.* **2005**, *44*, 4707 – 4712; M. Bhagawati, S. Lata, R. Tampé, J. Piehler, *J. Am. Chem. Soc.* **2010**, *132*, 5932 – 5933.

[18] G. Lullo, R. Leto, M. Oliva, C. Arnone, *Proc. SPIE-Int. Soc. Opt. Eng.* **2006**, *6290*, 62900A.

[19] C. Henke, C. Steinem, A. Janshoff, G. Steffan, H. Luftmann, M. Sieber, H.-J. Galla, *Anal. Chem.* **1996**, *68*, 3158 – 3165.

