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Surfactant Activated Dip-Pen Nanolithography

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ABSTRACT

Direct nanoscale patterning of maleimide-linked biotin on mercaptosilane-functionalized glass substrates using dip-pen nanolithography (DPN) was facilitated by the addition of a small amount of the biocompatible nonionic surfactant Tween-20. A correlation was found between activated biotin transfer from the AFM tip with surfactant included in the ink and an increase in the wettability of the partially hydrophobic silanized substrate. Surfactant concentration represents a new control variable for DPN that complements relative humidity, tip–substrate contact force, scan speed, and temperature. Using surfactants systematically as ink additives may expand the possible ink–substrate combinations that can be used for patterning biotin and other biomolecules, including proteins.

Dip-pen nanolithography (DPN) is a direct-write printing technique that uses chemically modified AFM tips to pattern materials on a variety of substrates at the nanoscale level.[1] DPN was first demonstrated by patterning self-assembled monolayers (SAMs) of alkylthiols on gold substrates.[2] Since then, the technique has evolved to include many new ink−substrate combinations. Examples of inks include solid-state materials,[3] small molecules,[4] polymers,[5] dendrimers,[6] DNA[7] and proteins.[8] Metals,[2] semiconductors,[9] insulators,[4] and organic thin films[5,7,8] adsorbed on these surfaces have served as substrates. Although the details of the various ink−substrate chemistries employed differ from one system to the next, the ink deposition process in general depends on the solubility of the ink in the water meniscus that forms at the point of contact between the AFM tip and the substrate, the efficiency of activated transfer of the ink from the tip and its stability within the meniscus, and adsorption of the ink to the substrate surface.[1,9−11]

The majority of DPN experiments have used the thiol-on-gold system, where chemisorption of the thiol molecules on the gold surface is the driving force for ink transfer from the tip. Thiol molecules are not reactive with each other, but freely diffuse across previously chemisorbed regions until they become bound at the periphery to reactive gold sites, resulting in isotropic growth.[11] The driving force for deposition is thus strongly dependent on molecular diffusion along concentration gradients extending outward from the AFM tip to bare gold. This surface is highly polarizable and is completely wetting at all temperatures, due to the strong affinity the water/ink fluid has for gold. Under ambient conditions, water adsorbs on the gold surface to form a continuous liquid film of monolayer thickness with an equilibrium contact angle $\theta_E = 0°$.[12] Molecular diffusion of the ink from the tip in this case is not impeded by pinning of the fluid at the boundary separating the liquid, solid, and vapor phases, as it would for a substrate surface that is not totally wetting ($\theta_E > 0°$).[13] A nonwetting or partially wetting substrate surface presents an additional activation barrier to ink transport.

Direct patterning of biological materials such as DNA, peptides, and proteins at the nanoscale without loss of activity requires the ability to immobilize these biomolecules through specific recognition chemistries that minimize nonspecific binding. Patterning biologically active proteins directly onto gold[14] and nickel oxide[15,16] surfaces using DPN in this manner has been demonstrated. Immobilization of biomolecules on oxidized silicon or glass substrates requires the incorporation of an adsorbed functional film that can act as a specific binding template. Recently, Mirkin's group described the direct writing of acrylamide-modified oligonucleotides onto oxidized silicon surfaces functionalized with 3′-mercaptopropyltrimethoxysilane (MPTMS) monolayers.[7] The use of organofunctional silane chemistry such as that of MPTMS is a successful strategy for immobilizing biomolecules on glass and oxidized substrates that has been used extensively for fabricating DNA, small-molecule, and protein microarray chips.[17]

Organic thin films such as MPTMS monolayers are less wettable than "high energy" surfaces, such as ionic, covalent, or metallic materials. MPTMS-coated glass is partially hydrophobic; pure water forms a droplet on this surface with

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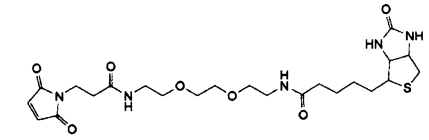
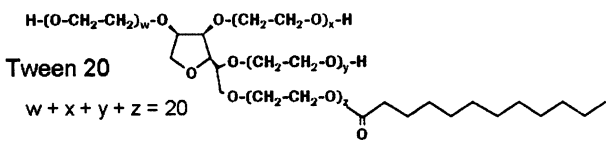
an equilibrium contact angle of 58°. While this may have minimal consequences at the micron length scale, it can have important ramifications for DPN. However, it is also reasonable that the charged and highly hydrophilic oligonucleotide inks used by Mirkin and co-workers actually increase the wettability of the substrate as it binds to the mercaptosilane surface, which facilitates the ink deposition process. It is well-known that wetting of surfaces can be strongly influenced by molecular adsorption,[18] a fact that has been discussed previously in the context of DPN.[19]

In this paper, we demonstrate direct DPN writing of the small-molecule ligand biotin on mercaptosilanized glass employing the functionalized linker molecule (+)-biotinyl-3-maleimidopropionamidyl-3,6-dioxaoctanediamine (EZ-Link maleimide PEO_2−biotin, Pierce) as the ink. The fabrication of streptavidin or avidin nanostructures built upon DPN patterning of biotin represents a general route toward molecular recognition-mediated protein immobilization at the nanoscale due to the prevalence of biotin-tagged biomolecules. A stepwise procedure for functionalizing DPN-written SAMs of 16-mercaptohexadecanoic acid on gold with biotin has been published,[20] and we have shown that analogous molecular recognition chemistry can be applied to patterning glass substrates as well, by writing MPTMS ink onto clean glass followed by covalent attachment of biotin.[4] However, successful patterning of the reactive MPTMS ink with DPN was limited to narrow operating conditions (low RH), due to polymerization of the alkoxysilane ink in the hydrated environment of the scanning AFM tip on the glass surface. Recently, the direct DPN writing of *N*-hydroxysuccinimide functionalized biotin onto polyethyleneimine-coated silicon oxide surfaces has been reported.[21]

The coupling chemistry for maleimide is similar to that for acrylamide-modified DNA:[7] both react with the pendant thiol groups of MPTMS by conjugate addition to form stable thioether bonds. In addition, the maleimide PEO_2-biotin molecule is soluble in aqueous solutions (≥ 25 mg/mL) due to the poly(ethylene oxide) (PEO_2) spacer arm. Therefore, we expected that the biotin-maleimide linker molecule would perform comparably to acrylamide-DNA as an ink for DPN. However, we were not able to obtain consistent patterning of the maleimide−PEO_2−biotin ink, even at relative humidity (RH) values above 90%.



Maleimide PEO_2-biotin



The addition of small amounts of the nonionic surfactant Tween-20 (Sigma) to the ink (as little as 50 ppm by volume) activated the transfer of the biotin linker molecule from the AFM tip to the MPTMS-coated substrate, enabling us to directly pattern biotin routinely with sub-100 nm resolution and at moderate relative humidity values (RH = 50−65%). Tween-20 (polyoxyethylene sorbitan monolaurate) is a nondenaturing, nonionic detergent that is commonly used to suppress nonspecific reactions between antibodies, antigens, and other biomolecules.[22,23] It has also been used as a solubilizer in membrane chemistry.[24] Tween-20 has proven effective in blocking nonspecific binding of biomolecules to hydrophobic surfaces, such as carbon nanotubes.[25] The degree to which detergents such as Tween-20 can perform these functions is based on their ability to bind to hydrophobic surfaces in aqueous solution.[26]

We believe the inclusion of Tween-20 in the ink for DPN activates biotin writing primarily by increasing the wettability of the ink on the MPTMS substrate. This in turn increases the driving force for ink transport from the AFM tip due to increased accessibility of maleimide PEO_2-biotin to the thiol groups of MPTMS. The biotin written areas were functional for subsequent immobilization of fluorescently labeled streptavidin with minimal nonspecific binding. We also immobilized avidin-linked horseradish peroxidase enzymes to biotin-written areas on silanized glass and directly characterized enzymatic activity from the sites with a fluorescence-based assay involving conversion of fluorogenic substrate molecules to fluorescent products (see Supporting Information).

Glass substrates (VWR #1 coverslips) were cleaned by standard methods[27] and coated with MPTMS monolayers by a two-step silanization procedure which involved an intermediate water treatment.[28] Clean glass coverslips were silanized via evaporation from a 2 μL drop of neat liquid MPTMS (Aldrich) at 120 °C for 10 min in a covered 150 mL glass jar, followed by extensive washing with deionized water (Millipore Gradient). After drying with nitrogen gas, the glass coverslips were further silanized for an additional 10 min with a fresh 2 μL drop of MPTMS under the same conditions. Silanized glass coverslips were cured at 100 °C for 16 h and used as substrates for DPN writing.

Commercially available AFM tips (silicon nitride cantilever, 0.58 N/m, Digital Instruments) were cleaned with "piranha" solution (3:7 (v/v) mixture of 30% H_2O_2 and H_2SO_4) (*Caution: this mixture reacts violently with organic materials*) for 30 min at room temperature, rinsed copiously with deionized water (Millipore Gradient), and dried at 100 °C. MPTMS was evaporated onto the clean tips at 120 °C for 30 min to facilitate ink adsorption on the AFM tip surface. The tips were then dipped for 10 min into ink solutions consisting of maleimide PEO_2−biotin (25 mg/mL) and varying concentrations of Tween-20 (from 0 to 0.1% v/v) dissolved in phosphate-buffered saline (PBS, pH = 7.2−7.4), blow-dried with compressed nitrogen gas, and used immediately for DPN.

DPN experiments were performed using a Multimode AFM (Nanoscope IV controller) from Digital Instruments in a large glovebag purged with nitrogen gas which was either bubbled through water or passed through a desiccant. The RH, measured with a digital hygrometer, was controlled

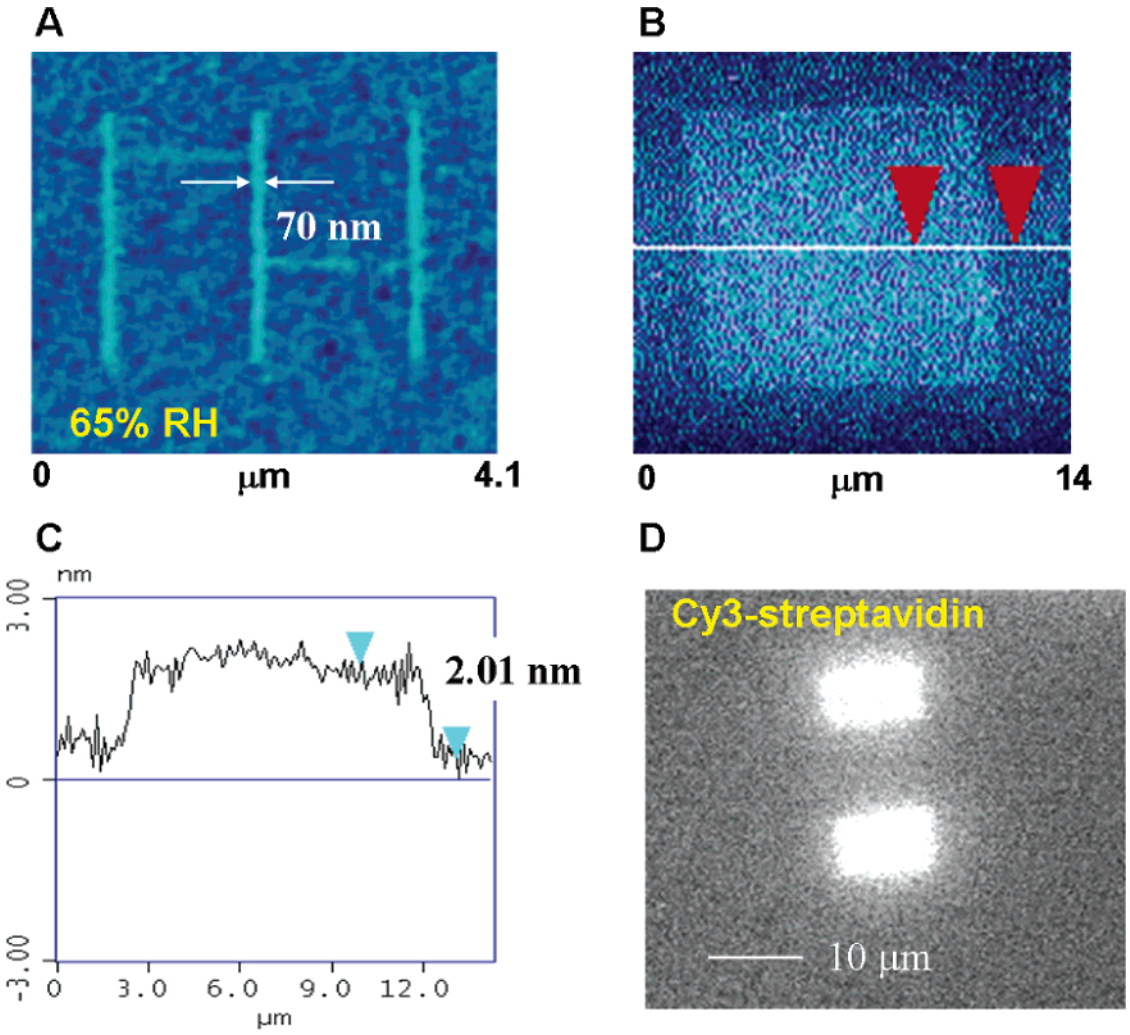


Figure 1. (a) Lateral force microscopy (LFM) image of 2-μm long lines of maleimide PEO_2-biotin written with DPN on MPTMS silanized glass at 65% RH and with 0.1% Tween-20 included in the ink. (b) AFM tapping mode image and (c) corresponding averaged cross-sectional height trace of 10 μm square pattern of biotin. (d) Fluorescence image of two 5 $\times$ 10 μm regions of biotin conjugated with Cy3-streptavidin.

in this way from 22% to 92%. All experiments were performed in contact mode at room temperature between 23 and 24 °C. Patterns were imaged by lateral force microscopy (LFM) or tapping mode AFM immediately after writing.

Without Tween-20 included in the DPN ink, biotin patterns were not observed on MPTMS-coated substrates below 80% RH. Biotin patterns could be imaged by LFM after increasing the RH up to 92%, but the patterns were not reproducible and the spatial resolution was poor. Systematic variation of other DPN parameters, such as tip speed and tip−surface contact force, did not improve ink transport.

The addition of Tween-20 to the ink did not diminish the maleimide−thiol reactivity for the range of concentrations used[29] and facilitated direct biotin patterning with DPN. Figure 1a shows an LFM image of three 2 μm-long lines of maleimide PEO_2-biotin written with a tip speed of 0.4 μm/s at 65% RH, with 0.1% Tween-20 included in the ink. In the LFM images, written areas are brighter than the more hydrophobic silanized glass background, indicating an increase in friction between the tip and these areas. The average width of the patterns was 70 nm. Patterns could be written for several hours with no noticeable changes in feature appearance or size. Figure 1b shows the tapping mode topographic image of a 10 μm square pattern of maleimide PEO_2-biotin, while Figure 1c shows an average of cross-sectional traces of the height taken perpendicularly to the top of the pattern. Although the measured height of the deposited maleimide PEO_2-biotin (2.05 nm) was less than the fully extended spacer arm length of the maleimide PEO_2−biotin molecule (2.91 nm, Pierce), it indicated that maleimide PEO_2-biotin was most likely deposited as a monolayer.

Figure 1d is a fluorescence image of Cy3-streptavidin conjugated to two 2.5 μm $\times$ 10 μm patterned regions of maleimide PEO_2-biotin. The patterns were written with a scanning speed of 10 μm/s and at 70% RH. Before the Cy3-streptavidin was introduced, unpatterned regions of the mercaptosilanized glass surface were passivated with a 1 mM solution of PEG-maleimide in PBS buffer (O−(2-maleimidoethyl)-O′-methyl-poly(ethylene glycol), MW 5000, purity >90%, Fluka) to prevent nonspecific binding of proteins. The sample was then incubated for 10 min using 2 μg/mL of Cy3-streptavidin in PBS buffer with 0.02% (v/v) Tween

20. After exhaustively washing the patterned samples in PBS buffer containing 0.1% Tween 20, the sample was rinsed with Millipore water and dried under N_2 gas. Fluorescence was observed using an inverted epi-fluorescence microscope with a Hg/Xe arc lamp (Eclipse TE 300, Nikon). Images of fluorescent patterns were captured with a high-resolution, Peltier-cooled CCD camera (CoolSnap-HQ, Roper Scientific). The degree of nonspecific binding of Cy3-streptavidin was minimal compared to previous results reported by us[4] and others,[21] as indicated by the fluorescence intensity from the patterned regions relative to the background. Finally, Cy3-streptavidin conjugated patterns were imaged with tapping mode AFM *after* fluorescence had been observed from the written areas (Supporting Information). The height of the Cy3-streptavidin patterned regions was roughly 0.4 nm less than that of the surrounding PEG film, resulting in negative contrast against the background in the topographic images. The patterns were also imaged in phase contrast mode.

Systematic variation of the RH and the Tween-20 concentration in the ink was performed to find the optimum conditions for patterning the biotin linker molecules on MPTMS. We found that concentrations of surfactant as low as 0.005% and at RH values as low as 39% would result in transfer of biotin to the surface, after a time delay of about 30 s. Included in the Supporting Information are data that show the minimum RH needed to observe biotin deposition onto MPTMS for Tween-20 concentrations ranging from 0.005% to 0.1%. The minimum RH needed for biotin writing scaled inversely with surfactant concentration; with 0.1% surfactant in the ink, the minimum RH was 22%.

Higher RH values at a given concentration of Tween-20 resulted in increased diffusion rates, as did increased surfactant concentrations in the ink for a given value of the RH. The optimal conditions for surfactant-mediated biotin writing were 0.1% Tween-20 at 50−65% RH. There was zero time delay between the moment of initial AFM tip contact and actual material deposition under these conditions. The growth of feature size with contact time with these conditions had the characteristic square root time dependence indicative of growth by isotropic molecular diffusion.[30] Figure 2a is a tapping mode AFM image of biotin dot patterns on MPTMS-functionalized glass for increasing tip−substrate contact times at 65% RH. The dependence on contact time is shown in Figure 2b. These results suggest that the relative concentration of Tween-20 can be systematically varied in addition to other parameters, such as RH, temperature, scan speed, and force, to control the deposition of ink in DPN. Increased Tween-20 concentration in the ink beyond 0.1% did not result in improved performance.

Control experiments were performed with DPN using AFM tips coated with either maleimide PEO_2-biotin or Tween-20, but not both. LFM data from these experiments are included in the Supporting Information. The biotin ink could be transferred from the coated AFM tip and patterned on hydrophilic clean glass (not silanized) at 45% RH, which is consistent with the high solubility of the molecule and suggests that dissolution of the ink from the AFM tip into

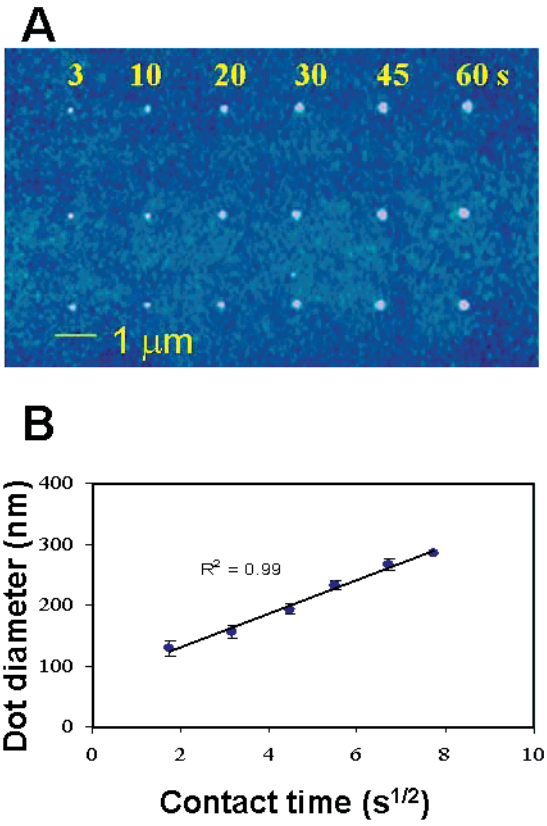


Figure 2. (a) Tapping mode AFM topographical image of maleimide PEO_2-biotin dot patterns on MPTMS-functionalized glass for indicated tip−substrate contact times (in seconds) at 65% RH. The ink included 0.1% Tween-20. (b) Dependence of dot diameter with the square root of contact time from (a).

the water meniscus is not the limiting barrier for DPN writing. In this case, the biotin was weakly physisorbed to the glass surface, and the patterns blurred with repeated LFM scans. In a separate experiment, Tween-20 (0.1%) without biotin was patterned with DPN onto MPTMS-coated glass. LFM images of the patterns were similar in appearance to those of maleimide PEO_2-biotin with surfactant, except that the rate of deposition of Tween-20 alone was considerably faster than when biotin was also included in the ink. This indicates that adsorption of surfactant to the substrate plays an important role in activating biotin writing with DPN. PEO-based detergents similar to Tween-20 adsorb readily to silanized silica surfaces of comparable wettability (same contact angle) to our MPTMS-coated substrate, as determined by an in-situ ellipsometry study.[31]

To learn more about how surfactant activates DPN writing of biotin, we measured the surface tension and contact angles of maleimide PEO_2-biotin inks with varying amounts of Tween-20 on MPTMS-coated glass surfaces. We also measured the contact angles of pure water droplets on MPTMS-glass surfaces that were "inked" with maleimide PEO_2-biotin and different amounts of Tween-20 in PBS buffer and dried to simulate the wetting of an inked AFM tip by the water meniscus.

The dynamics governing molecular transport in DPN are complex and not well understood, but they will depend on balances between adhesive and cohesive intermolecular

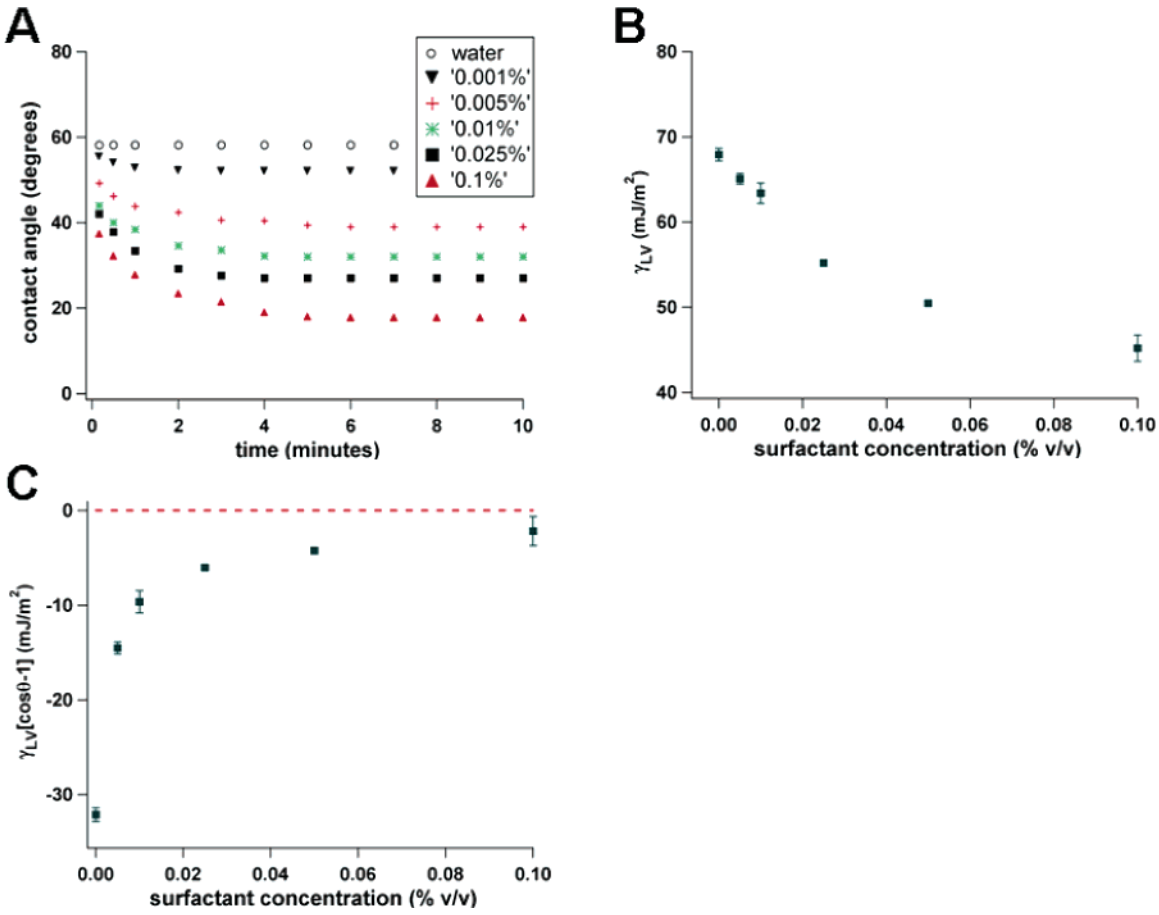


Figure 3. (a) Variation of the contact angle, θ, with time for several aqueous solutions containing increasing amounts of Tween-20 and 25 mg/mL maleimide PEO_2-biotin on MPTMS-coated glass substrates. Solutions without biotin gave identical results. The relative humidity was kept at 100%. (b) Variation of liquid−vapor surface tension (γ_{LV}) of buffer solutions containing 25 mg/mL biotin and increasing Tween-20 concentration. (c) Variation of the spreading parameter $S = \gamma_{LV}$ [cosθ_E − 1] as a function of surfactant concentration. The dashed line, at $S = 0$, corresponds to complete wetting of the liquid on MPTMS.

forces at the coated AFM tip, the water meniscus, and the substrate. The strength of attraction of a fluid to a solid depends on the relative energies at the solid−vapor, the liquid−vapor, and the solid−liquid interfaces, which can be determined with high sensitivity by measuring contact angles and interfacial tensions.[18] Although these measurements are typically carried out at the macroscopic scale, they are extremely sensitive to intermolecular forces that operate at the nanoscale,[32] and so are useful for understanding how Tween-20 activates DPN writing, even though the dynamics occurring at the AFM tip cannot be directly measured.

Contact angles of sessile drops on a MPTMS-coated glass substrate of aqueous solutions having the same composition as the inks used were estimated visually with a contact angle goniometer (Ramé-Hart). For these measurements, the goniometer stage was enclosed in a large glovebag and the RH was kept saturated at 100% to prevent evaporation from the drops over time.[33] Contact angle measurements of pure water on dry coated surfaces emulating an inked AFM tip quickly equilibrated within a few seconds and could be performed at ambient RH. The liquid−vapor surface tensions (γ_{LV}) of pure water (Millipore) and PBS buffer solutions containing increasing amounts of Tween-20 (0−0.1% v/v) and 25 mg/mL biotin were determined using the pendant drop technique, since this could be carried out with ∼50 μL volumes and consumed small amounts of the expensive biotin reagent. Images of pendant drops were captured with a digital camera and fit with an axisymmetric drop shape analysis program[34] to give the surface tension (see Supporting Information). All drops were allowed to age in air at ambient RH for 30 s before being photographed. The surface tensions of pure (HPLC-grade) water (72.95 mJ/m^2) and hexadecane (27.60 mJ/m^2) determined by this method agreed to within 1 mJ/m^2 with literature values.[35]

Figure 3a is a plot of contact angles (θ) taken over 10 min of several aqueous solutions that served as models for biotin and Tween-20 DPN inks on MPTMS-glass substrates.[36] The entry labeled "water" in the legend was for pure Millipore water (with or without biotin), while all of the other solutions were composed of PBS buffer with the indicated concentration (%v/v) of Tween-20. Each data point for all curves was taken from the average of 6 trials taken at different locations on the substrate. The maximum standard deviation in θ was 1.6° but the measured uncertainty was typically less than 1.0°. Importantly, the data were exactly identical for all the solutions whether or not 25 mg/mL biotin was included, which indicates that the biotin molecule is not surface active. The contact angles relaxed over the course

of several minutes to lower values, which did not change further with time. The change in contact angle increased with increasing surfactant concentration. There was no change in the contact angle for water ($\theta = 58°$) over 10 min, with or without 25 mg/mL biotin, while the contact angle decreased by 20 degrees for the solution containing 0.1% surfactant.

While the contact angles in Figure 3a took several minutes to equilibrate and never dropped below approximately 20°, the contact angles of drops of pure water on dry surfaces emulating inked AFM tips quickly equilibrated (within seconds) to near-zero values (for 0.1% Tween-20), suggesting that the water meniscus, once formed, completely wetted the tip (see Supporting Information).

Figure 3b shows the change in liquid−vapor surface tension (γ_{LV}) of buffer solutions containing 25 mg/mL biotin and various amounts of Tween-20. Each data point is the average of four to six trials taken from separate identically prepared solutions with ± one standard deviation indicated by error bars. A transition in the slope of γ_{LV} versus surfactant concentration occurs in the region corresponding to the critical micelle concentration (cmc) for Tween-20 (~0.007% v/v).[26]

Figure 3c shows the dependence of the surfactant concentration on the spreading parameter, S. The spreading parameter is the energy difference per unit area of a substrate when dry versus wet and is given by the expression $S = \gamma_{SV} - [\gamma_{SL} + \gamma_{LV}]$, where the surface tensions in the expression are at the solid−vapor, solid−liquid, and liquid−vapor interfaces, respectively.[13] The sign of S determines whether a liquid will completely wet a surface. When $S \geq 0$, the liquid completely spreads over the substrate as a nanometer-thick film ($\theta_E = 0°$), while $S < 0$ corresponds to the liquid partially wetting the surface and forming a droplet at equilibrium with a nonzero contact angle. The three coefficients in the expression for S are related by Young's equation: $\gamma_{LV} \cos\theta_E = \gamma_{SV} - \gamma_{SL}$.[37] By substitution, the spreading parameter can be determined experimentally from $S = \gamma_{LV} (\cos\theta_E - 1)$, where θ_E values are the contact angles versus Tween-20 concentration measured at 10 min in Figure 3a, and γ_{LV} are the corresponding surface tension values measured from aqueous solutions of the same compositions, shown in Figure 3b.

For spreading (wetting) to occur, $[\gamma_{SL} + \gamma_{LV}]$ must be made small relative to γ_{SV}. For highly hydrophilic substrates commonly used in DPN, such as gold, NiO or SiO_2, γ_{SV} exceeds $[\gamma_{SL} + \gamma_{LV}]$. Water-based inks will completely wet these substrates ($\theta_E = 0°$). Tween-20 promotes spreading of the ink on the partially hydrophobic MPTMS substrate ($\theta_{water} = 58°$) by adsorption at both the solid−liquid (γ_{SL}) and liquid−vapor (γ_{LV}) interfaces, which lowers these surface tensions. This is exactly how a surfactant functions as a detergent (an agent that can remove or prevent binding of foreign material by surface chemistry). The mechanism by which Tween-20 minimizes nonspecific binding of proteins to hydrophobic surfaces is fundamentally the same as how it promotes adhesion of water molecules onto MPTMS, through preferential adsorption at the solid−aqueous and aqueous−hydrophobic phase interfaces (either air or an "oily phase" such as the hydrophobic domains on the surface of a protein).[18]

In summary, we have demonstrated that the inclusion of a small amount of the detergent Tween-20 lowers an activation barrier for ink adsorption to MPTMS surfaces by lowering the surface tension at both the liquid−vapor and solid−liquid interfaces, thereby increasing the wettability of the substrate. This in turn increases the driving force for ink transport from the AFM tip due to increased chemisorption of maleimide-biotin to the thiol groups of MPTMS. The increased wettability of the substrate also likely stabilizes the water meniscus,[30] which would act to further facilitate ink transport, although we cannot test this directly. It is also possible that the detergency of Tween-20 aids in the dissolution of biotin from the AFM tip into the water meniscus, although the evidence suggests this is not as important an effect.

Biotin deposition from the coated AFM tip to MPTMS can be activated by as little as 0.005% v/v (50 ppm) of Tween-20 in the ink, although reliable patterning under ambient conditions occurs at higher surfactant concentrations in the range 0.05−0.1%, which is about an order of magnitude greater than the cmc. Without Tween-20 in the ink, reliable patterning of maleimide-biotin on MPTMS with DPN was impossible. The actual local fluxes and concentrations of Tween-20 and maleimide PEO_2-biotin in the water meniscus in DPN are unknown and may be very different from the composition of the as-prepared ink solutions. We have shown unequivocally, however, that increasing the relative amount of Tween-20 leads to improved wettability of the MPTMS substrate, and that this correlates with the activation of direct biotin writing with DPN. On one level, this is a useful result since the direct patterning of biotin by DPN provides a universal platform for molecular recognition-mediated protein immobilization on glass and silicon oxide surfaces. However, the significance of including detergent additives to DPN inks extends beyond biotin conjugation chemistry.

Surfactants are extensively used in macroscopic coating applications, for example, in optimizing how paints and inkjet inks wet and spread on various media.[18] This paper describes an analogous application at the nanoscale. The systematic exploration of surfactant additives to inks for DPN may represent a useful strategy for controlling the writing of biological molecules on previously inaccessible or difficult to pattern substrates. Organic solvents that have been used to ink AFM tips (such as acetonitrile for alkylthiols and dimethylformamide for DNA) may serve to facilitate ink transport in certain cases by leaving behind residues (even after the tips have been dried) that can dissolve in the nascent water meniscus. These volatile compounds can form surface tension gradients in water that can drive ink transport from the tip due to convection. However, these effects are not likely to be consistent or reproducible as the cosolvent evaporates, and we do not know of any work that has sought to systematically control them for DPN. Although the local concentration of Tween-20 in the meniscus is not known, Tween-20 is nonvolatile. A large number of surfactants and

detergents have been developed as tools for biological research, resulting in a wide range of chemical and physical properties that can be selected and optimized for a particular application, such as crystallization of membrane-bound proteins.[38] We believe that these compounds can be used in a new role: as additives to facilitate the direct patterning of biological macromolecules on select substrates with scanning probe nanolithography.

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Supporting Information Available: Fluorescence based assay for catalytic activity from HRP-avidin enzymes conjugated to DPN biotin patterns, correlations between minimum RH and Tween-20 concentrations needed for activation of biotin writing, additional LFM images of biotin and Tween-20 DPN patterns on clean glass and MPTMS, tapping mode AFM image of Cy3-streptavidin conjugated to biotin patterned with DPN, and surface tension and additional contact angle data. This material is available free of charge via the Internet at http://pubs.acs.org.

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