

# Biochemically Responsive Smart Surface

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**ABSTRACT** A design of smart surfaces responsive to biochemical analytes is demonstrated in the example of mixed monolayers of biotin/fluorocarbon. The contact angle of aqueous solutions on such surfaces decreases upon streptavidin binding and can be used in detecting this protein. The specificity of the effect is confirmed by the lack of a contact angle change by streptavidin blocked with biotin and by bovine serum albumin.

**KEYWORDS:** hydrophobicity switching • biotin • streptavidin • biochemically responsive surface

## INTRODUCTION

Materials that can respond selectively to their environment or to a certain stimuli by switching one or more of their critical properties are called “smart” (1). One of the properties that have received special attention is wettability. A number of groups have demonstrated the fabrication of smart surfaces that display wettability changes induced by temperature (2), light (3–6), electrical potential (7), fumes of solvents (8), and pH (9). A contact angle (CA) of a liquid drop on such surfaces can be altered by the above-mentioned stimuli and, for example, switch it from hydrophobic to hydrophilic.

The CA is the most common measure of wettability that describes the angle,  $\theta$ , at the three-phase contact line formed by a drop of liquid resting on a surface. The Young's equation

$$\cos \theta = \frac{\gamma_{sv} - \gamma_{sl}}{\gamma_{lv}} \quad (1)$$

relates it to surface energies at the solid/vapor,  $\gamma_{sv}$ , the solid/liquid,  $\gamma_{sl}$ , and the liquid/vapor,  $\gamma_{lv}$ , interfaces.

The CA can vary between two values: the maximum, called advancing CA, and the lowest, called receding CA. The former can be measured using the Sessile drop method by increasing the size of the drop until no variations on the CA are observed. Alternative method relates it to the maximum CA achieved in front of a droplet on a tilted surface. The receding CA is measured as a minimum angle while gradually removing liquid from the drop until the contact line begins to move backward. It also corresponds to the minimum CA for a droplet on the tilted surface. If manipulation with the droplet is problematic, the receding angle can be measured by observing droplet evaporation. When the volume of a droplet shrinks, its shape changes while the contact line stays initially the same but eventually, upon reaching the minimum possible receding angle, the contact

line detaches and the droplet continues shrinking without further changes in the shape.

The hysteresis between advancing and receding CA can vary significantly and is due to metastable states at the solid/liquid/vapor interface (10). The surface roughness, chemical heterogeneity, molecular reorientation, and penetration of the small-sized liquid molecules into the voids of the solid surface have been identified among the numerous causes for metastable states on surfaces modified with organic molecules (11). Notably, even small amounts of impurities on the surface (chemical heterogeneities) can lead to a large hysteresis (10).

Amino acid residues in the polypeptide chain of a protein vary in their hydrophobicity, and the hydrophobic interactions between them as well as with the surrounding water are the driving force for proteins folding into their native state (1). Except for the membrane proteins, the outer surface of a typical protein is usually enriched with hydrophilic residues, which make the protein water-soluble. Hydrophobic surfaces can be rendered hydrophilic by covering them with proteins. Such coverage can be achieved by relying on the amphipathic properties of proteins: after a prolonged contact with a hydrophobic surface, they can change conformation to “bind” in a nonnative form via exposure of their hydrophobic residues to the surface. Such a binding is weak and is efficient at a very high protein concentration; it is nondiscriminative and forms a protein film that switches a hydrophobic surface into a hydrophilic one (12). Different proteins have varying tendencies of binding to hydrophobic surfaces, with bovine serum albumin (BSA) being one example with a strong conformationally induced adsorption (13) that is often used for hydrophobic surface modification (14).

To the best of our knowledge, there are no reports of surfaces that could be switched from hydrophobic to hydrophilic by specific interactions between the analyte proteins and their ligands on the surface. In the present work, we illustrate a design of such smart surfaces using the well-known couple biotin–streptavidin (SA), which is commonly used for protein micropatterning. We show here that the

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Received for review November 6, 2008 and accepted February 24, 2009

DOI: 10.1021/am800166t

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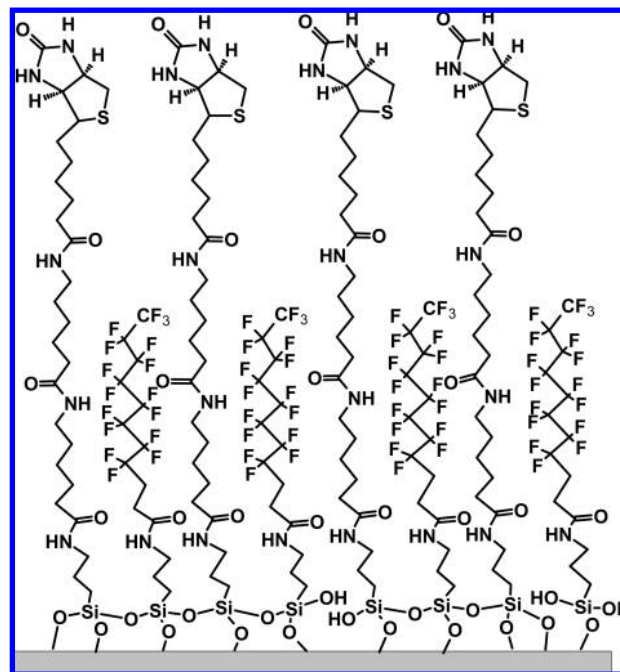
mixed hydrophobic–biotin surfaces respond specifically to the presence of the SA analyte by lowering the CA at the surface.

## EXPERIMENTAL SECTION

**Materials.** (3-Aminopropyl)trimethoxysilane (APTS) was obtained from Aldrich. 2*H*,2*H*,3*H*,3*H*-Perfluoroundecanoic acid was obtained from Fluorous Technologies. Biotin–LC–LC–COOH and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide (EDC) were obtained from Anaspec. Streptavidin (SA), a 53 kDa protein, was obtained from Invitrogen. Bovine serum albumin (BSA), a 69 kDa protein, was received from Sigma. They have similar  $pI = 5$  and 4.7, respectively. Methanol and ethanol, both of absolute grade from Aldrich, were used as received. Glass slides were cleaned with Piranha solution (30 %  $H_2O_2$  and 70 %  $H_2SO_4$ ) for 20 min at 70 °C, washed with copious amounts of distilled water, and dried in an oven for 30 min at 115 °C. **Caution!** *Piranha solution is explosive.*

**Preparation of Aminated Surfaces.** The first step for surface modification is silanization of cleaned glass slides with an ethanol solution of APTS (15, 16) for 12 h at room temperature and with constant shaking. This reaction produces amino groups for further steps and is prone to multilayer growth in low-polarity solvents, but if a proper solvent is used, this problem can be minimized and practically avoided. In our experience, silanization using a 2 % solution of APTS in ethanol results in monolayer coverage, as judged by the surface density of amines. Evaluation of the amino group surface density was performed using the method of Moon et al. (16), and it was established that at least 6 h was necessary to attain monolayer coverage, ca.  $3 \times 10^{14}/cm^2$  (15, 17). No further increase beyond monolayer coverage was detected for up to 24 h of treatment (in contrast with nonpolar solvents). Silanization for all surfaces reported in this paper was performed using 12 h of treatment in ethanol, ensuring close to monolayer coverage. Afterward, slides were washed with ethanol and methanol and finally cured for over 1 h at 115 °C.

**Mixed Biotinylated and Fluorinated Surfaces.** To prepare mixed monolayers, mixtures of two carboxylic acids in different proportions were reacted with the amino groups of the aminosilane layer using an EDC coupling reagent. Perfluoric acid was chosen to minimize the passive adsorption of proteins (7, 18). The biotinylated acid consisted of D-biotin attached to a long linker (LC-LC-COOH). The purpose of the long linker is to extend the biotin moiety above the fluorinated monolayer to ensure its interaction with SA. Solutions of biotinylated and fluorinated carboxylic acids, both of 50 mM concentration, were mixed in a desired proportion to make 100  $\mu L$  and diluted by ethanol to the final volume of 2 mL. The cleaned glass slides were deposited inside, and after the addition of 30 mg of EDC, the entire solution was agitated at room temperature for at least 6 h. The slides were finally washed with ethanol and methanol and dried by purging with  $N_2$ . We will refer to these mixed monolayers in accordance with the mole fraction of the carboxylated biotin in the carboxylic acid mixture used



**FIGURE 1.** Representation of the surface modified with a mixed monolayer of biotin–LC–LC and perfluoric acid. The biotin moiety must extend at least three bond lengths above the top of the fluorinated monolayer to ensure SA binding.

during the preparation; i.e., B20 was prepared using a mixture of 0.2 mole fraction of biotin and 0.8 of the fluorinated acid. Note that the actual fraction of the biotin moiety in the surface monolayer can differ from that because of the possible reactivity difference for different carboxylic acids. The surface F100 (B0) corresponds to a solely fluorinated monolayer. Figure 1 shows a schematic of the expected monolayer configuration.

**CA Measurements.** The CA measurements were carried out using a home-made apparatus consisting of a microscope connected to a digital camera, a horizontal beam holding the microscope parallel to the surface, a light-dispersive plate, and a three-axis moving platform. Most experiments were conducted without control of the humidity or temperature; the latter was typically within the range between 20 and 25 °C. Each series of experiments represented by a graph was conducted on the same day to ensure the same humidity. The water-vapor-saturated environment used in some experiments was achieved by placing a modified glass substrate on a support inside a glass rectangular cuvette, the bottom of which was filled with deionized water. When used, this condition almost eliminated droplet evaporation (a very slow Kelvin evaporation due to a small droplet size still took place).

Drops of approximately 1  $\mu L$  were deposited on the surface using a Hamilton microsyringe. Movies and pictures of the drops' profiles were recorded every 2 min in most of the experiments. The images were analyzed using the CA plug-in (written by Brugnara (19)) in the *ImageJ* software. All measurements were done in triplicate. Cleaning of fouled surfaces by sonication (see the text) was performed in a Branson 1200 ultrasonic cleaner.

## RESULTS AND DISCUSSION

Our motivation was to investigate whether mixed hydrophobic surfaces can be triggered into hydrophilic ones by interaction with biochemical analytes. There are various possible applications for such a phenomenon, including electrical biosensors. We have demonstrated before (20–25) that nanoporous membranes, the surface of which is modified by organic monolayers, can be made responsive to physical and (bio)chemical stimuli. When the surface is modified by a mixed layer of hydrophobic molecules and hydrophobicity switching triggering elements, not only the ionic conductance (22, 23) but also the whole solution flow through the membrane can be switched by the stimulus (24, 25). Whether or not it is possible to realize such a switching with biochemical analytes is the driving force behind this investigation, where SA is used as a representative protein.

SA is a tetrameric protein that has four sites for binding biotin (26). The binding of biotin to SA is among the strongest noncovalent interactions known. It has a very small dissociation constant, estimated between  $10^{-15}$  (27) and  $4 \times 10^{-14}$  M (28), and a long dissociation time, up to 3 days, making it an essentially irreversible reaction. The biotin binding site is buried quite deep in SA (29, 30). Thus, an effective coupling between the two can be achieved only when the linker, by which biotin is attached to the surface, extends sufficiently enough, at least by 8 Å as measured from the carboxylate carbon of biotin (29, 30). To ensure this and, at the same time, provide enough hydrophobicity to the surface, the above-described procedure for mixed-monolayer formation was chosen, where the surface was first aminated by APTS and then linked to carboxyl-terminated molecules using the EDC coupling reagent. The LC-LC linker on biotin–LC-LC-COOH is sufficiently long to bind SA effectively (30).

To make the switching specific to the analyte (SA in our case), one needs to minimize the effect of a well-known phenomenon of passive adsorption of proteins, which occurs even on hydrophobic surfaces (7, 12, 18). Among the various options for handling this effect (31), fluorination, i.e., surface modification using fluorinated molecules, was chosen for its relative simplicity. When compared to aliphatic surfaces, fluorinated surfaces show lower fouling by proteins, but even they eventually succumb to fouling at high concentrations of proteins, especially after prolonged exposures.

The effect of passive adsorption (physisorption) can be evaluated by measuring the CA of the droplets with different concentrations of a protein (e.g., SA) on the fully fluorinated surface B0 (F100). The simplest approach is to monitor the free-standing sessile droplet shapes in time upon their slow evaporation rather than to measure the advancing and receding angles. Besides providing more reproducible data for the receding angle, this approach also allows identification whether there is any delayed spreading of the droplets due to SA binding to biotin, similar to what happens with solutions of small amphiphile molecules (32).

Figure 2A demonstrates that for SA concentrations of 100 mg/L ( $\sim 2 \mu\text{M}$ ) or higher there is significant nonspecific

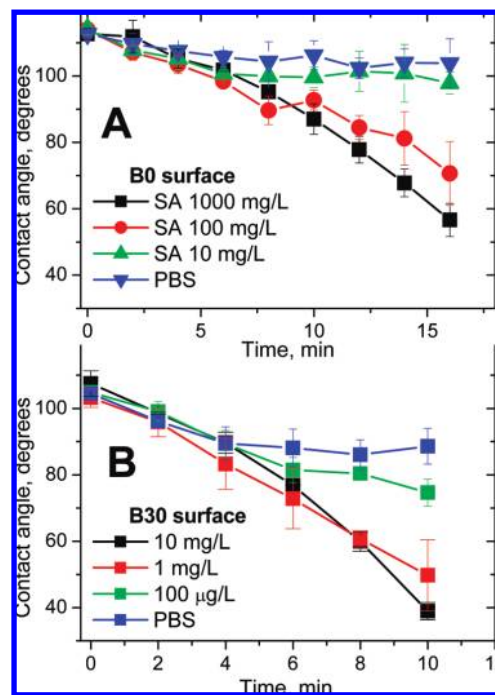


FIGURE 2. CA variations for droplets with SA solutions of different concentrations on B0 (A) and B30 (B) surfaces.

adsorption of SA to the fluorinated surface while the solution of a lower concentration, e.g., 10 mg/L or lower, presents very minimal adsorption and almost matches the behavior of a plain phosphate-buffered saline (PBS) buffer. At the same time, mixed-monolayer modification, B30, has visibly changed the receding CA down to SA concentrations of 100  $\mu\text{g/L}$ , as shown in Figure 2B.

Figure 3 provides the time snapshots of PBS- and SA-containing droplets slowly evaporating on the surfaces modified with different solitary and mixed monolayers, B0 (F100), B25, B50, B75, and B100. The analysis of their CAs, given in Figure 4, illustrates that all surface modifications (except for fully fluorinated F100) have dramatically different evolution of the PBS- and SA-containing droplets. The surfaces with a higher content of biotin demonstrate lower initial CAs, as was expected because of the more hydrophilic character of biotin. The CA values on each surface are almost identical for the two droplets at first, but with time, the PBS droplet starts to shrink upon reaching the corresponding receding CA. The SA-containing solutions, on the other hand, show a continuous decrease of the CA within this time frame, which correlates well with the receding angles measured at different times after placement of the droplet on the surface. Obviously, this behavior is due to the specific interaction between SA in solution and the surface-bound biotin.

As Figure 5 illustrates, upon evaporation of the droplet, biotin moieties act as “anchors” for SA binding and thus pin the contact line to its original position. When the volume of the droplet shrinks, the resulting CA decreases. With no biotin on the surface, the droplet decreases in size while maintaining the same shape as soon the CA reaches the receding angle value.



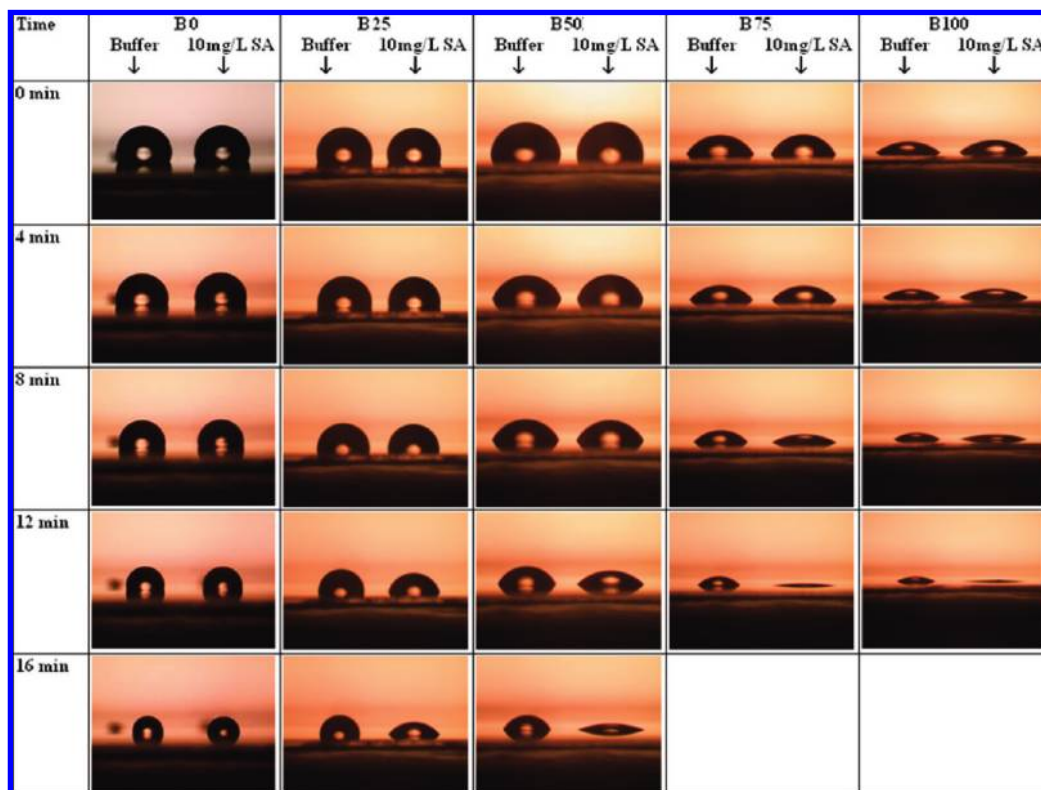


FIGURE 3. Time evolution of the slowly evaporating droplets without (left) and with 10 mg/L SA solutions on the surfaces with different percentages of biotin (fluorocarbon is the remaining component of the surface modification).

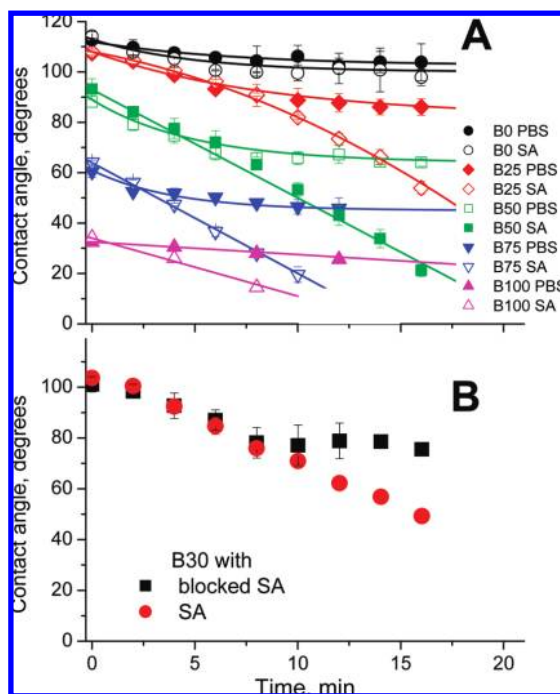


FIGURE 4. Variation of the CA with time for sessile drops with PBS and 10 mg/L SA solutions on surfaces with different amounts of biotin moieties (A), solutions with 10 mg/L free SA and 10 mg/L SA capped with biotin, both on the B30 surface (B). The capped SA variation is practically indistinguishable from that of the PBS buffer.

The specificity of biotin–SA interaction as being responsible for the observed CA hysteresis can be confirmed by the lack of such CA changes with solutions of other proteins.

To minimize the number of variables and to have a better comparison, we performed experiments with SA whose active sites were “capped” with biotin. Capping was achieved by the addition of a slightly above the stoichiometric amount (5:1) of D-Biotin to the SA solution, thus rendering the protein inactive to binding with biotin on the surface. Figure 4B confirms that the variation of CA for the capped SA on B30 is practically indistinguishable from that of the PBS buffer in a dramatic distinction from the uncapped SA. Again, the distinction emerges as soon as the shrinking due to evaporation droplets reached their corresponding minimum receding CAs. The solution with capped SA does not demonstrate hydrophobicity switching and the droplet starts shrinking early with a large CA, while the uncapped SA binds specifically to the biotin on the surface, lowers its surface tension, and pins the contact line until a much lower CA is realized.

Alternatively, the CA with a dull PBS buffer (free of any protein) can be used to study binding of proteins to the same surfaces after their prolonged exposure to the protein solutions. This approach allows a convenient way of discriminating between specific binding and passive adsorption (physisorption). The fully fluorinated surface (B0) gets fouled after 30 min of exposure to either SA or BSA solutions. Because of a longer exposure time, the CA drops from  $108 \pm 2^\circ$  before to  $78 \pm 6^\circ$  and  $78 \pm 7^\circ$ , respectively, for the two proteins (see Figure 6) due to their physisorption. The hydrophobic property fully recovers after 1 min of sonication in 50 % (v/v) methanol. This treatment is mild enough not to denature the protein, at least not SA. As seen in Figure 6, a partially biotinylated B30 surface experiences a significant

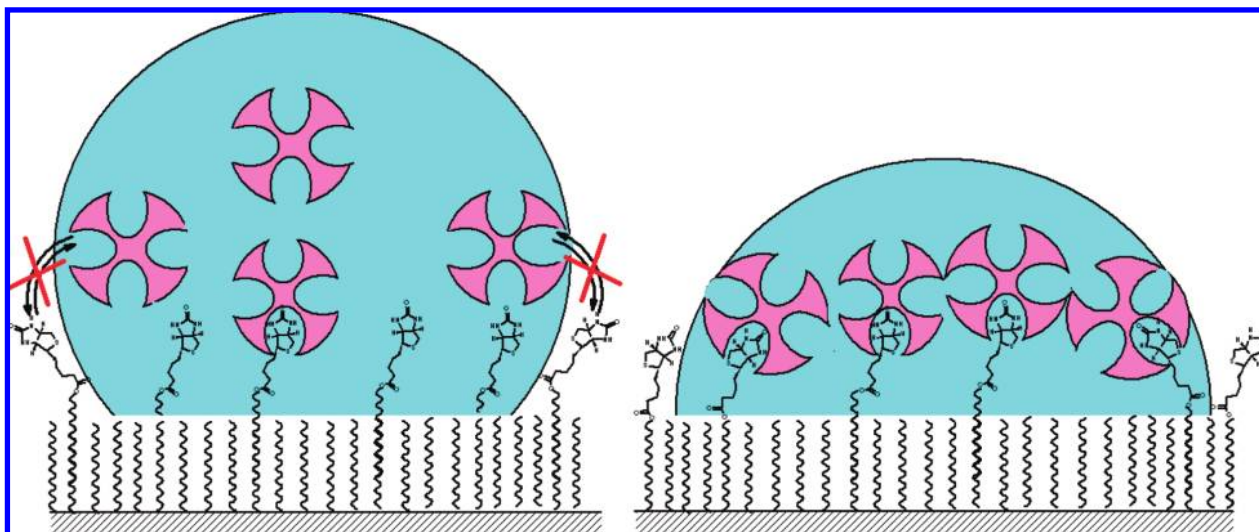


FIGURE 5. Illustration of the mechanism of CA variation. The advancing CA is not affected by SA binding onto biotin because neither biotin nor SA can sway across the contact line and the thermal oscillations of the contact line are not sufficient either.

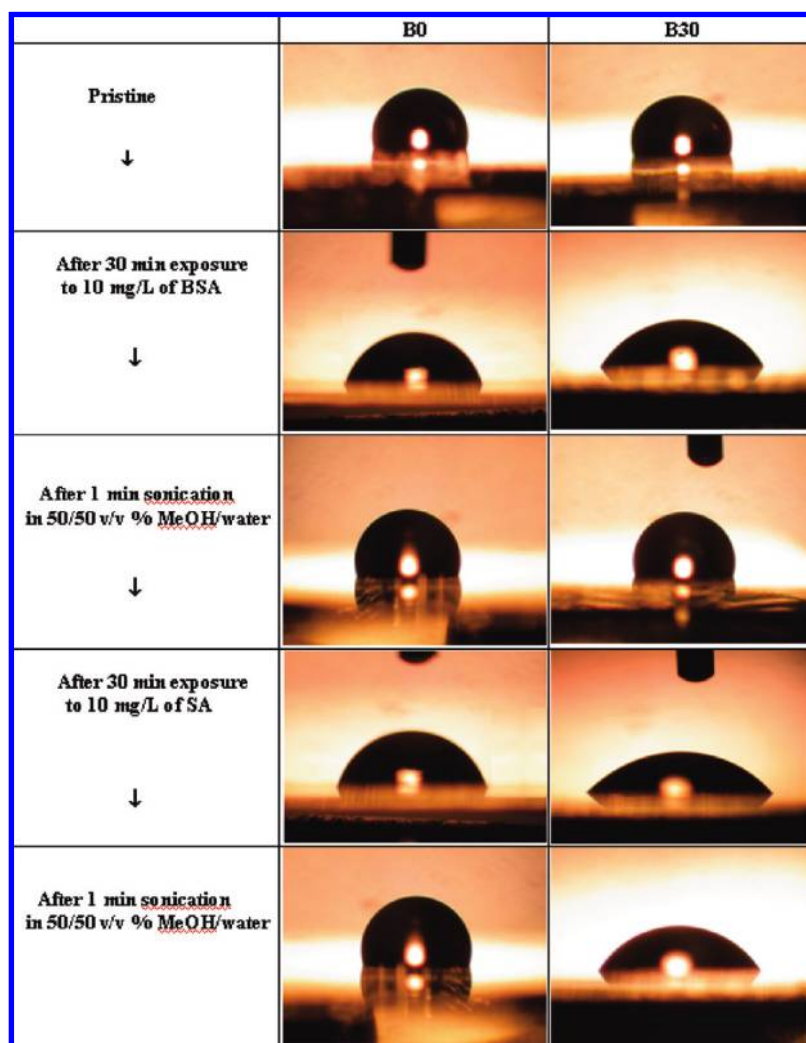


FIGURE 6. Variation of the drop with the PBS buffer on B0 (F100) and B30 surfaces after different treatments.

drop of the CA after exposure to either SA or BSA solution. The CA decreases from  $100 \pm 9^\circ$  to  $54 \pm 6^\circ$  and  $71 \pm 10^\circ$  after SA and BSA, respectively. Remarkably, sonication of

the BSA-fouled B30 surface for 1 min in 50% methanol completely recovers, while it has an insignificant change, to  $56 \pm 7^\circ$ , for the SA-treated B30 surface. More harsh condi-

tions of 30 min of sonication in pure methanol are likely to denature proteins more significantly, as is observed in the recovery of the CA for the SA-treated B30 surface back to the original value of greater than 100°. Multiple uses of this procedure eventually deteriorate the surface properties, which is first revealed in a lowering of the receding angle.

There are two questions worthy of further discussion. First, nonspecific binding of proteins (physisorption) occurs on all surfaces, with and without the biotin ligand, but it is a much slower process. When the kinetics of the CA variation with evaporation are measured after allowing a SA-containing droplet to soak onto that surface in saturated vapor, changes in the receding angle are observed with 10 mg/L SA as well (32). It requires at least an additional 10 min to observe a significant effect for that concentration on the F100 surface. During this time, biotinylated surfaces demonstrate a strong binding even with lower concentrations. In applying this method for sensing SA, one can eliminate the nonspecifically bound proteins by sonication, as explained above. Whether or not physisorption is a cooperative effect would require additional studies and probably a more appropriate technique.

The second question that motivated this work is about the contact-line movement as a result of specific protein binding. We observe no such movement for either specific or nonspecific protein interaction with the surface. Despite the large CA hysteresis upon SA binding, which exceeds 70° for B50, we do not observe the contact-line movement outward; i.e., there is no delayed droplet spreading. Even on the B100 surface, i.e., when only biotin is present on the surface, no movement of the contact line is observed in a 100% humid atmosphere over a 12 h period with SA concentrations of 10 mg/L or lower. This behavior is different from that of small amphiphile molecules. For the latter, it has been established that the process, which primarily determines the spreading of surfactant solutions over hydrophobic substrates, is the transfer of surfactant molecules onto a bare hydrophobic substrate in front of the moving three-phase contact line (33). This process results in a partial hydrophilization of the hydrophobic surface in front of the drop and determines the delayed spontaneous spreading. Indeed, it is easy to see from eq 1 that the decrease of only  $\gamma_{sl}$  and  $\gamma_{lv}$  resulting from the relatively fast adsorption of amphiphiles on solid/liquid and liquid/vapor interfaces cannot explain the switch from hydrophobic ( $\theta > 90^\circ$ ) to hydrophilic ( $\theta < 90^\circ$ ) behavior. Obviously, it can be realized only when  $\gamma_{sv}$  becomes greater than  $\gamma_{sl}$ , i.e., when  $\gamma_{sv}$  increases in the vicinity of the three-phase contact line as well.

Transfer of surfactants from the solution onto the solid/vapor interface just in front of the drop increases the local free energy, but the total free energy of the system decreases. The process goes via a relatively high potential barrier and hence is considerably slower than the adsorption at liquid/solid and liquid/vapor interfaces; i.e., the time scale for the droplet spreading is defined by the characteristic time of surfactant transfer from the drop onto the solid/vapor

interface. If the latter is slow, the system can “get stuck” in the metastable state for a very long time. Large proteins such as SA or BSA cannot follow this route directly because of the size; the only option left for them to affect the advancing CA is if the contact line can fluctuate itself, thus exposing SA to the possibility of binding with biotin on the surface (see Figure 5). The alternative version would be with biotin ahead of the contact line fluctuating in and out of the droplet and occasionally “fishing-out” SA from an aqueous solution into the dry region. Both of these options are apparently too much of an uphill process and are not realized to a sufficient degree.

## CONCLUSIONS

Mixed fluorinated surfaces with covalently bound biotin demonstrate smart active hydrophobicity switching, where specific binding of SA from a low-concentration solution can decrease the CA with water from being greater than 90° down to less than 60°. The effect is clearly visible in the receding CA for solutions of SA, while the advancing angle remains identical for the buffer and SA solutions, thus proving that advancement of large protein molecules onto hydrophobic surfaces ahead of the contact line even via the help of specific interactions with ligands is a highly unfavorable process.

SA with blocked biotin binding sites and BSA lack active hydrophobicity switching but do show nonspecific binding by physisorption that can be eliminated by sonication of the exposed surface in a 50% methanol solution for 1 min. A harsher treatment of 30 min of sonication in pure methanol denatures these proteins and recovers the hydrophobicity of these surface.

**Acknowledgment.** This work was partially supported by a grant from the National Institutes of Health (NIH SCORE GM08136).

**Supporting Information Available:** Effect of the exposure time to a SA solution on the variation of the CAs for Sessile drops on B0 (F100). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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AM800166T