Coating of poly(dimethylsiloxane) with *n*-dodecyl-β-D-maltoside to minimize nonspecific protein adsorption

Bo Huang, Hongkai Wu, Samuel Kim and Richard N. Zare*

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Poly(dimethylsiloxane) (PDMS) surface is coated with *n*-dodecyl-β-D-maltoside, which reduces the nonspecifically adsorbed protein on the PDMS surface to the single molecule level.

Recently, PDMS has become a highly attractive material for microfluidic chips because of its ease of fabrication.[1] Unfortunately, for protein analysis, the hydrophobicity of the PDMS surface causes strong adsorption of proteins. Current methods to reduce protein binding include polymer grafting,[2] biopassivation using an antibody–neutravidin–dextran coating,[3] lipid bilayer formation on a plasma-oxidized PDMS surface,[4] and coating with poly(vinyl alcohol).[5] These methods have the drawback that either the treatment procedure is complex or the adsorption reduction (100–1000 fold) is not sufficient for some high sensitivity applications.

n-Dodecyl-β-D-maltoside (DDM) is an alkyl polyglucoside, which belongs to a family of very mild nonionic surfactants. It adsorbs strongly on hydrophobic surfaces, such as graphite, and forms a monolayer.[6] This monolayer coverage causes the surface to become hydrophilic and nonionic, thus reducing the interaction between the protein and the surface. Because alkyl polyglucosides do not affect the functionality of many proteins, which has been demonstrated by solublizing proteins without denaturation,[7] we can keep a certain concentration of DDM in the solution so that it equilibrates with the adsorbed surfactant molecules. Dynamic coating with ionic reagents has been used to change the electroosmotic flow (EOF) in PDMS channels.[8,9] For nonionic surfactants, Towns and Regnier[10] have demonstrated that the dynamic coating of alkylsilane-modified silica capillaries with poly(ethylene glycol) (PEG)-based surfactant reduces the surface adsorption of analyte proteins in capillary electrophoresis. This result suggested to us that a DDM coating could be efficient in reducing protein nonspecific binding on the hydrophobic PDMS surface.

To monitor the protein adsorption, we use fluorescence imaging either with epifluorescence, wide-field excitation, or with total internal reflection (TIR) excitation. We use 1 μM Alexa Fluor 647 labeled streptavidin or bovine serum albumin (BSA) in Hepes buffered saline (HBS) containing 20 mM Hepes and 100 mM NaCl at pH 7.5. For achieving a dynamic DDM coating, we use HBS with 0.1% DDM, which we refer to as DHBS. The protein solution in HBS or DHBS is injected into a 100 μm high × 1 mm wide × 25 mm long rectangular PDMS channel and then removed with HBS or DHBS after an incubation time of 5 min. The buffer washing is performed either (1) by flushing 50 μL buffer in about 2.5 s with a pipet or (2) by flowing buffer at 1 mL h^{-1} for 3 min (50 μL), which results in almost identical surface fluorescence (Table 1).

Without DDM in the buffer, both streptavidin and BSA adsorb strongly to the PDMS surface, yielding bright, homogeneous fluorescence emission at low excitation power (0.15 W cm^{-2} at the sample) (Figs. 1A and D). In contrast, with DHBS, only scattered fluorescent spots can be observed under much higher excitation power (8.9 W cm^{-2}) and TIR excitation

Table 1 Surface fluorescence intensities of Alexa 647 labeled streptavidin with different methods to wash the surface coating[a]

	Washing method for DDM coating			
Surface coating	Pipet[b]	Pipet[c]	One hour in HBS after pipet wash	Constant flow for one hour
None	170	159	—	—
DDM (dynamic)	10^{-4}[d]	10^{-4}[d]	—	—
DDM (permanent)	2.3	2.3	0.77	10^{-3}[d]

[a] Normalized by the excitation power. [b] Unbound streptavidin is washed by flushing 50 μL HBS with a pipet. [c] Unbound streptavidin is washed by flowing HBS at 1 mL h^{-1} for 3 min. [d] Estimation.

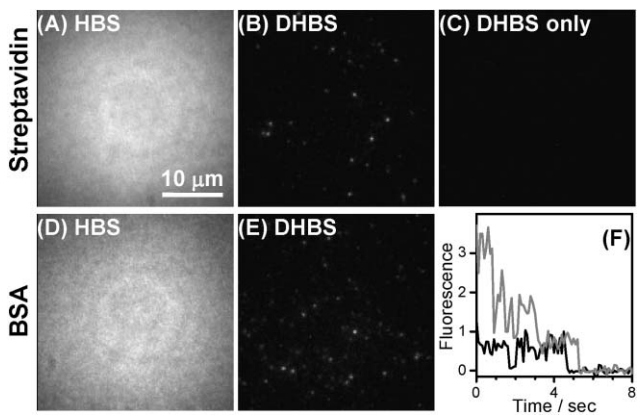

Fig. 1 Fluorescence images of PDMS surfaces washed by HBS or DHBS after incubating with: (A) streptavidin/HBS; (B) streptavidin/DHBS; (C) DHBS buffer only; (D) BSA/HBS and (E) BSA/DHBS. (F) is the time behaviour of two fluorescent spots in (B). The circular-shaped structures in (A) and (D) are caused by the diffraction of the excitation laser, and the brightness difference between the center and the edges reflects the laser profile. Images (B), (C), and (E) are adjusted to have the same contrast. All images have the same scale as indicated in (A).

Department of Chemistry, Stanford University, Stanford, CA 94305-5080, USA. E-mail: zare@stanford.edu; Fax: +1-650-7239262; Tel: +1-650-7239262

(Figs. 1B and E). Stepwise photobleaching of these spots can be observed (Fig. 1F). As a control, a PDMS surface treated with DHBS does not show significant fluorescence under the same excitation conditions (Fig. 1C). According to the manufacturer, the sample proteins are labeled with an average of about three dye molecules per protein. We conclude that these fluorescent spots are actually individual protein molecules. Therefore, with DDM dynamic coating, the nonspecific binding of streptavidin and BSA on PDMS is almost completely eliminated (an estimated 10^6 fold reduction) without any separate surface treatment.

We have also tested the effect of 0.1% DDM on reducing the surface adsorption of cytochrome *c*, an antibody (mouse anti-FLAG M2 antibody), and a transmembrane protein (β_2 adrenergic receptor), all of which show scattered molecules on the surface even with high protein concentration, while maintaining the activity of each protein. Thus, DDM dynamic coating can be applied to a wide variety of systems in which the presence of a small percentage of DDM can be tolerated.

In a few situations, DDM in the buffer solution could still affect adversely the experimental system, suggesting the use of a permanent coating. Geffroy *et al.*[11] have shown that PEG-based nonionic surfactants remain adsorbed onto the hydrophobic polystyrene surface after removing surfactants in the solution, which is attributed to the strong hydrophobic interaction between the alkyl chain and the surface. Because of the structural similarity, we expected that DDM should also irreversibly adsorb to PDMS.

To test the permanent coating of the PDMS surface with DDM, the PDMS channel is incubated with DHBS for 5 min and then washed either with pipet flushing (50 μL HBS) or with constant flow (1 mL h^{-1}). The channel is subsequently incubated with protein/HBS solution for 5 min and then washed with HBS. In the case of pipet flushing, the amount of streptavidin bound to the PDMS surface is reduced by a factor of 10^2 (Table 1). With the other method, we have found that longer washing times favor the reduction of protein adsorption. As can be seen in Fig. 2, extending the washing time from 2.5 min to 10 min reduces the nonspecifically bound streptavidin to the single molecule level. Further increase in washing time up to 1 h does not significantly change the binding of streptavidin or BSA, indicating that the DDM coating is stable.

We find continuous washing is necessary for efficient DDM coating. For example, after pipet washing, if we incubate the channel with HBS for 1 h without flowing, the streptavidin adsorption is only slightly lower than the case without such incubation (Table 1). This phenomenon might be caused by the same mechanism as the desorption behavior of PEG surfactants observed by Geffroy *et al.*,[11] in which it takes about 5 min for the surface-bound surfactants to desorb and equilibrate with the buffer. Thus, continuous washing would be able to remove the DDM molecules that are desorbed from the surface and allows DDM on the surface to rearrange its configuration. The exact mechanism is under investigation.

In addition to its ability to suppress protein adsorption, the DDM coating also reduces the EOF by covering the charges on the PDMS surface.[10] Our measurements using current monitoring method[9] show that the electroosmotic mobility of 20 mM Hepes buffer (pH 8) in a PDMS channel is reduced from

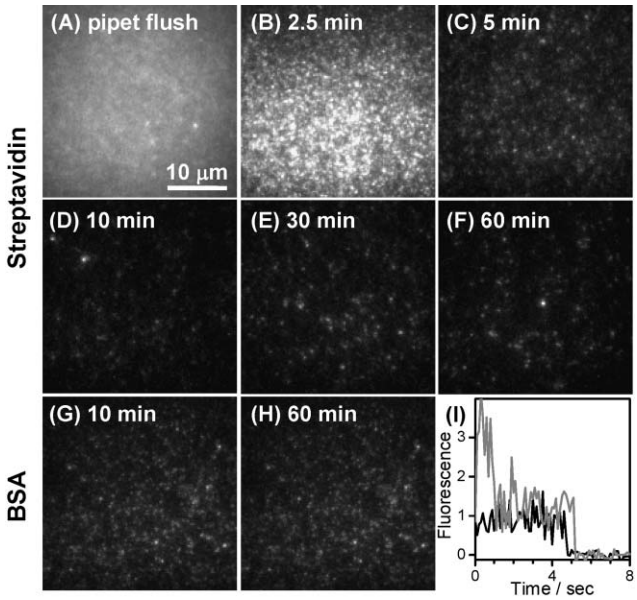

Fig. 2 (A)–(H): Streptavidin and BSA adsorption on PDMS surfaces with pipet flush or continuous flow wash to prepare a permanent DDM coating. (I): Time traces of two fluorescent spots in image (F). Image (A) is obtained with an excitation laser power of 8.9 W cm^{-2} at the sample and epifluorescence, wide-field excitation; and (B)–(H) are acquired and displayed with the same parameters as in Fig. 2B. All images are at the same scale as indicated in (A).

5.6×10^{-4} cm^2 V^{-1} s^{-1} to 2.8×10^{-4} cm^2 V^{-1} s^{-1} (dynamic coating) or 4.0×10^{-4} cm^2 V^{-1} s^{-1} (permanent coating). Such EOF reduction is beneficial in many cases.

In conclusion, DDM coating, dynamic or permanent, is a simple, but highly efficient method to minimize protein adsorption to a PDMS surface. The hydrophobic interaction between the alkyl chain of DDM and the PDMS surface promotes binding and causes the resulting surface to be passivated. We propose that DDM coating might become a general procedure for reducing nonspecific protein adsorption on other hydrophobic surfaces, such as alkylsilane-modified silica surfaces and alkylthiol-modified gold surfaces.

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