Applicability of the extended Derjaguin–Landau–Verwey–Overbeek theory on the adsorption of bovine serum albumin on solid surfaces

Hua Wang and Bi-min Zhang Newby^{a)}

Department of Chemical and Biomolecular Engineering, The University of Akron, Akron, Ohio 44325-3906

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Protein adsorption is the prerequisite for bacterial attachment and cellular adhesion, which are critical for many biomedical applications. To understand protein adsorption onto substrates, predictive models are generally informative prior to experimental studies. In this study, the extended Derjaguin–Landau–Verwey–Overbeek (XDLVO) theory was employed to determine whether or not it could interpret the protein adsorption behaviors. The experimental results of fluorescein isothiocyanate labeled bovine serum albumin (BSA) adsorbed on six different surfaces: glass, octadecyltrichlorosilane modified glass, 2-[methoxypoly(ethyleneoxy)propyl]trimethoxy-silane (PEG)-modified glass, polystyrene, poly(dimethylsiloxane), and poly(methyl methacrylate) were utilized. The XDLVO interaction energy curves, especially from the contribution of acid–base interactions, obtained using the surface properties of substrates and BSA molecules qualitatively predict/interpret the protein adsorption behaviors on these surfaces. Some derivation of the experimental results from the prediction was noticed for the glass and the PEG-modified glass. When including a hydration layer to the PEG-modified glass surface, the nonfouling result of such surface by proteins was also elucidated by the XDLVO theory. © 2014 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4904074>]

I. INTRODUCTION

Interactions between proteins and different solid substrates are very important in many technological applications and scientific fields. Bacteria are believed to normally adhere to substrates that carry an adsorbed protein layer.¹ The adsorption of protein will affect bacterial attachment by building polymer bridging and changing the interaction energies between bacterial cells and substrates,² and it will also influence the wettability of substrate surface to affect cell adhesion.³ Once a substrate is introduced into a biological environment, the surface will be covered by proteins within seconds due to the protein–surface interactions. This is followed by the bacterial or cell attachment, reproduction, and propagation.⁴ Consequently, protein adsorption is critical in various biomedical, environmental, and maritime systems.^{5,6}

To study protein adsorption onto substrates, predictive models of protein–substrate interactions are useful. One of the models is the classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, which was originally developed to describe the forces between two charged surfaces in a liquid medium.^{7,8} In the theory, two main interactions, the Lifshitz–van der Waals (LW) and the electrostatic (EL) interactions are included. In order to consider polar (electron acceptor/donor) interactions, van Oss and co-workers^{9,10} added the short-range Lewis acid–base (AB) interactions, and the new model is termed the extended DLVO (XDLVO) theory. For protein adsorption studies, some researchers pointed out that AB interactions were the dominating interactions over LW and EL interactions in the short-range region.^{11–14} Therefore, AB

interactions should be considered into the model to predict/interpret protein adsorption. The LW and AB interactions between a particle (1) and a flat substrate surface (2) in a medium (3) with a minimum equilibrium cut-off distance d_0 (~ 0.158 nm, the critical distance below which the outer electron shells of adjoining noncovalently interacting molecules would overlap^{15,16}) can be expressed as¹⁷

$$\Delta G_{d_0}^{LW} = -2 \left(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_2^{LW}} \right) \left(\sqrt{\gamma_3^{LW}} - \sqrt{\gamma_1^{LW}} \right), \quad (1)$$

$$\Delta G_{d_0}^{AB} = 2 \left(\sqrt{\gamma_1^+ \gamma_3^-} + \sqrt{\gamma_2^+ \gamma_3^-} + \sqrt{\gamma_1^- \gamma_3^+} + \sqrt{\gamma_2^- \gamma_3^+} - \sqrt{\gamma_1^+ \gamma_2^-} - \sqrt{\gamma_1^- \gamma_2^+} - 2\sqrt{\gamma_3^+ \gamma_3^-} \right), \quad (2)$$

where γ_i^{LW} , γ_i^+ , and γ_i^- denote the Lifshitz–van der Waals component, electron-acceptor (acid) and electron-donor (base) of the Lewis acid–base component of the surface energy (i.e., $\gamma_i = \gamma_i^{LW} + \gamma_i^{AB}$ and $\gamma_i^{AB} = 2(\gamma_i^+ \gamma_i^-)^{1/2}$).

When a single protein molecule, assuming it is rigid and perfectly spherical, is involved, the LW, AB, and EL interaction energies in a medium can be modified to^{17,18}

$$\Delta G_{pms(d)}^{LW} = -\frac{AR}{6d} = 2\pi R d_0 \Delta G_{d_0}^{LW} \frac{d_0}{d}, \quad (3)$$

$$\Delta G_{pms(d)}^{AB} = 2\pi R \lambda \Delta G_{d_0}^{AB} \exp\left(\frac{d_0 - d}{\lambda}\right), \quad (4)$$

$$\Delta G_{pms(d)}^{EL} = \pi R \epsilon \epsilon_0 \left(2\psi_{01} \psi_{02} \ln\left(\frac{1 + e^{-\kappa d}}{1 - e^{-\kappa d}}\right) + (\psi_{01}^2 + \psi_{02}^2) \ln(1 - e^{-2\kappa d}) \right). \quad (5)$$

^{a)} Author to whom correspondence should be addressed; electronic mail: bimin@uakron.edu

The subscript p, m, and s represent a single protein, the medium, and the flat substrate, respectively. A is the unretarded sphere-substrate Hamaker constant in water. R is the protein molecule mean radius. d is the separation distance between the protein molecule and the flat substrate. λ is the characteristic decay-length of AB interactions in water [~ 0.6 nm (Ref. 17)]. ϵ and ϵ_0 are the relative dielectric permittivity of medium and the permittivity in a vacuum, respectively. $1/\kappa$ is the Debye–Hückel length and κ (m^{-1}) is related to the ionic strength, I (M), of the medium by $\kappa = 3.28 \times 10^9 I^{1/2}$. ψ_{01} and ψ_{02} are the surface potentials of the protein molecule and the flat substrate, respectively, which are correlated to their zeta potentials (ζ_1 and ζ_2). Based on the Gouy–Chapman theory:¹⁹ $\zeta = (2kT/ze) \ln((1 + \tanh(ze\psi_0/4kT) \exp(-\kappa ds))/(1 - \tanh(ze\psi_0/4kT) \exp(-\kappa ds)))$, where ζ is the zeta potential and ψ_0 is the surface potential, z is the valency including the sign of the charge, k is the Boltzmann constant, T is the temperature, e is the elementary charge, and ds is the distance to the slip plane and has a value of ~ 0.3 nm.²⁰ If $|\zeta| < 25$ mV, then the expression can be simplified to: $\psi_{01} = \zeta_1(1 + (ds/R)) \exp(\kappa ds)$ for proteins and $\psi_{02} = \zeta_2 \exp(\kappa ds)$ for the flat substrate.

Since established, the DLVO and, especially, the XDLVO theory have been found to adequately interpret/predict the adhesion/attachment behaviors in many fields, especially in colloid adhesions^{13,21,22} and bacterial attachments.^{23–26} Compared to the micron sized colloid particles and bacterial cells, nanometer sized protein molecules are far more complicated in structures and surface charges, which can dramatically influence the interactions during the adsorption process between the protein molecules and the substrates. For example, “soft” protein structures could be rearranged by extra driving force (nonXDLVO), leading to an enhanced adsorption, while the actual mechanism/process is still unclear.² Nevertheless, researchers have utilized the DLVO theory, by assuming protein molecule as a sphere with uniform composition and uniform potential, to interpret protein adsorption onto surfaces.^{10,27} For example, the XDLVO theory has been employed to determine the kinetic constants of protein adsorption following the approach of von Smoluchowski’s flocculation kinetics of suspended particles.^{11–13} Just recently, a couple of studies have utilized the XDLVO theory to interpret their experimental results of protein adsorption, but they did not explicitly point out the specific contribution of AB interactions.^{28,29}

Since the XDLVO theory is based on the LW, AB, and EL interactions, in a short-range region (separation distance < 5 nm), we hypothesized that all three types of interactions, especially AB interactions, are critical in governing the protein adsorption process. In order to verify the hypothesis, adsorption of a model protein—fluorescein isothiocyanate labeled bovine serum albumin (FITC-BSA) on six types of surfaces: poly(ethylene glycol) (PEG) modified glass, octadecyltrichlorosilane (OTS) modified glass, polystyrene (PS), poly(dimethylsiloxane) (PDMS), poly(methyl methacrylate) (PMMA), and glass surfaces was utilized. The results indicated that the interaction energies estimated using the XDLVO theory did roughly agree with the experimental

FITC-BSA adsorption on these surfaces, and the AB interactions were found to play a dominant role for these six cases.

II. EXPERIMENT

A. Materials and equipment

n-Octadecyltrichlorosilane [$\text{CH}_3(\text{CH}_2)_{17}\text{SiCl}_3$, OTS], 2-[methoxy-poly(ethyleneoxy)propyl]trimethoxysilane [$\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_{6-9}(\text{CH}_2)_3\text{Si}(\text{OCH}_3)_3$, PEG-silane, $M_w = 460$ –590 g/mol] were purchased from Gelest. PDMS Sylgard[®] 184 was from DowCorning. PMMA was from McMaster-Carr. FITC-BSA (Product No. A9771, one of the most commonly used proteins in adsorption studies; its basic physical properties were provided in the supplementary material⁴⁰), methylene iodide (MI) (99%), and ethylene glycol (EG) (99.8%) were purchased from Sigma-Aldrich. Glass slides ($25 \times 75 \times 1$ mm) were purchased from VWR. High performance liquid chromatography (HPLC)-grade hexane and toluene were products of Fisher Scientific and used without further purification. PS, sulfuric acid (98% H_2SO_4), H_2O_2 (30% technical grade), and hydrochloric acid (38% HCl) were from Fisher Scientific. Deionized (DI) water was purified in-house and had a conductivity of ~ 0.1 μS or less. Atomic force microscope (AFM) (MultiMode SPM) was from Digital Instruments, and optical microscope (IX70) and digital camera (E-420) were from Olympus.

B. Surface preparation

Glass slides were cut into $1 \times 1 \text{ cm}^2$ pieces and cleaned by immersing them in a freshly prepared piranha solution (70/30 v/v 98% H_2SO_4 /30% H_2O_2) heated at 80°C for 1 h and copious rinsing with DI water, and then dried with compressed nitrogen (N_2).

The PEG-silane-modified surface (PEG surface) was prepared according to the previous method.³⁰ Briefly, cleaned glass slides ($1 \times 1 \text{ cm}^2$) were oxidized with UV/ozone oxidation for 6 min. Modification was done by immersing the samples in a solution of PEG-silane in HPLC-grade toluene (3 mM with 0.8 ml of $\text{HCl}_{\text{conc}}/\text{L}$) for 18 h at room temperature. Afterward, the samples were washed once in toluene, twice in ethanol, and twice in DI water and sonicated in DI water for 2 min to remove the nongrafted PEG molecules. Then the samples were dried with compressed N_2 . The OTS-silane-modified surface (OTS surface) was also prepared according to the previous method.²³ Briefly, followed by the cleaning and oxidation procedures described above, glass slides ($1 \times 1 \text{ cm}^2$) modification was done by immersing them in a solution of 0.168 wt. % of OTS in HPLC-grade hexane for 30 min at room temperature. After removing the OTS/hexane solution, the samples were sonicated in hexane for 5 min to remove the nongrafted OTS molecules, then rinsed thoroughly with ethanol and DI water, and finally dried with compressed N_2 .

PS thin film was prepared by hot-melt ($\sim 190^\circ\text{C}$) pressing in between a glass slide and an OTS-modified glass slide. The thickness of the surface (1 mm, similar to that of glass slide) was controlled by the amount of PS utilized. The PDMS surface was prepared by curing a mixture of the silicone

prepolymer and its curing agent (in a 10:1 mass ratio) inside a polystyrene petri dish onto an OTS-modified glass slide at 70 °C for 4 h. The thickness of the surface (1 mm, similar to that of the glass slide) was controlled by the amount of silicone mixture poured into the petri dish. The PMMA surface was prepared by hot-melt (~120 °C, between the glass transition temperature and melting temperature) pressing in between a glass slide and an OTS-modified glass slide. The thickness of the surface (1 mm, similar to that of glass slide) was controlled by the amount of PMMA utilized. All six substrates in this study were fully transparent, which allowed the fluorescent images be taken using the transmission mode of the optical microscope, and the fluorescent intensity, in term of mean-gray-value, of the images was quantified using ImageJ (Version 1.43, National Institutes of Health).

C. Surface characterization

Contact angles of DI water, MI, and EG on the surfaces were measured to determine the surface energy of the substrate. They were measured by the sessile drop technique using a Rame-Hart contact angle goniometer under ambient conditions (1 atm, 24 ± 2 °C). Both advancing and receding angles were measured on two randomly chosen spots on each of the triplicate samples. One-Touch Video Capture was used to record the drop shapes, and ImageJ was used to measure the contact angles.

D. FITC-BSA protein adsorption and surface coverage analysis

A 100 µl of 20 µg/ml FITC-BSA in phosphate buffered saline (PBS, pH 7.4) was deposited on each sample surface and covered the entire surface. Adsorption was carried out for 20 min at room temperature. Upon removal from the FITC-BSA solution, the samples were washed thoroughly

with PBS and DI water to remove nonadsorbed FITC-BSA molecules and residual salt from the buffer.

The fluorescence of FITC-BSA was imaged using a microscope fitted with appropriate filters (Axiovert 200, Carl Zeiss) and a digital camera. To determine the FITC-BSA adsorption, the mean-gray-values of adsorption images were quantified by ImageJ. The mean-gray-values were obtained from eight images.

III. RESULTS AND DISCUSSION

A. Surface properties evaluations

The surface energies and their components of all the interested surfaces were estimated using measured contact angle values. Three probe liquids—DI water, MI, and EG—were employed. The surface energy of substrates were estimated based on the contact angle of probe liquids and the approach of van Oss and co-workers⁹

$$(1 + \cos \theta) \gamma_L = 2(\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+}), \quad (6)$$

where L denotes probe liquid and S denotes substrate surface.

Table I summarizes the obtained values of water, MI, and EG contact angles on glass, OTS-modified glass, PEG modified glass, PS, PDMS, and PMMA surfaces. According to the results, the freshly cleaned glass surface was the most hydrophilic (a water contact angle of ~7.9°), and PEG and PMMA surfaces were also hydrophilic, while PS, PDMS, and OTS-modified surface were hydrophobic with the OTS-modified glass being the most hydrophobic (a water contact angle of ~102.9°).

The estimated surface energies and their (LW and AB) components for the surfaces are also summarized in Table I. Glass showed the highest AB component, with a value of ~8 mJ/m², leading to its surface energy of ~45 mJ/m². The

TABLE I. Contact angles of the three probe liquids on the six surfaces, along with surface energy and its components, as well as zeta potential of BSA and the six substrate surfaces, are summarized. Each of the standard deviation is obtained from at least 12 sets of measurements.

	Glass	OTS	PEG	PS	PDMS	PMMA	BSA ^a
θ_W (deg)	7.9 ± 0.3	102.9 ± 0.8	39.8 ± 0.4	83.1 ± 0.5	98.0 ± 0.4	52.1 ± 0.5	54
θ_{MI} (deg)	45.6 ± 0.8	69.3 ± 1.4	27.3 ± 1.6	35.2 ± 1.2	69.9 ± 0.7	29.5 ± 0.9	38
θ_{EG} (deg)	12.9 ± 0.6	80.3 ± 1.9	21.0 ± 0.4	66.7 ± 0.6	79.3 ± 0.8	31.5 ± 0.6	5
ζ (mV)	−20.3	−18.9	−16.1	−25 ^b	−30 ^c	−31 ^d	−13
γ	44.7	23.5	47.9	41.9	23.1	46.4	50.3
γ^{LW}	36.7	23.3	45.3	41.9	22.9	44.4	40.6
γ^{AB}	8.02	0.19	2.59	0.22	0.12	1.92	9.65
γ^+	0.24	0.01	0.04	0.08	0	0.03	1.16
γ^-	68.27	1.10	39.92	0.15	3.05	27.90	20.03
Energy barrier ^e (kT)	61.2	—	11.8	—	—	—	—

^aValues from Ref. 34 and some of the values obtained from the literature were at 18 or 20 °C, while our experiments were carried out at 24 ± 2 °C. The temperature difference could lead to ~1% variation in γ , 0.8% variation in γ^{LW} , 7% variation in γ^{AB} (13% variation in γ^+ due to the small γ^+ value, and 1% variation in γ^-). These variations were used to elucidate the potential experimental uncertainties, but no noticeable change was detected in the XDLVO energy curves. The unit of all γ values is mJ/m².

^bValues from Ref. 31.

^cValues from Ref. 32 and the value measured in a pH 7.0 HEPES buffer.

^dValues from Ref. 33.

^eEnergy barrier estimated based on the XDLVO theory.

FIG. 1. AFM topography scans of the six substrate surfaces: (a) glass, (b) OTS-modified glass, (c) PEG-modified glass, (d) PS, (e) PMMA, and (f) PDMS are presented. Each of the scan is 500×500 nm, and the Z-scale is from -5 to 5 nm.

PEG-modified glass and PMMA had similar surface energies, ~ 47 mJ/m², and their LW (44 – 45 mJ/m²) and AB (2 – 3 mJ/m²) components. The OTS-modified glass and PDMS had the lowest surface energy of ~ 24 mJ/m², primarily contributed by their LW component. Also contributed by its LW component was the surface energy of PS, which had a value of ~ 42 mJ/m².

Surface roughness of the six substrate surfaces was estimated from the AFM topography scans (Fig. 1). The average surface roughness (Ra) and root mean square (Rq) are summarized in Table II. All six substrate surfaces had no statistical difference in roughness ($p = 0.09$ for Ras, and 0.34 for Rqs). Therefore, it was anticipated that surface roughness would

TABLE II. Average surface roughness (Ra) and root mean square (rms) (Rq) of the six substrates obtained from AFM topographic scans, over an area of 500×500 nm, are presented. The standard deviation is obtained from at least four scans.

	Ra (nm)	Rq (nm)
Glass	0.30 ± 0.01	0.42 ± 0.03
OTS	0.31 ± 0.01	0.41 ± 0.02
PEG	0.30 ± 0.01	0.41 ± 0.02
PS	0.37 ± 0.10	0.49 ± 0.15
PMMA	0.37 ± 0.07	0.48 ± 0.10
PDMS	0.30 ± 0.04	0.39 ± 0.08

only have minimal effects on BSA adsorption on these surfaces.

B. FITC-BSA protein adsorption

The FITC-BSA adsorption amount was estimated using the mean-gray-values from the fluorescent microscopic images [Figs. 2(a)–2(g)]. According to the images, the PS

surface [Fig. 2(d)] had the most proteins adsorbed, followed by the PMMA surface [Fig. 2(f)]. The OTS [Fig. 2(b)] and the PDMS surfaces [Fig. 2(e)] had similar but less proteins adsorbed than on PMMA, and glass [Fig. 2(a)] had even less proteins adsorbed. The surface with the least proteins adsorbed, as expected, was the PEG surface [Fig. 2(c)], whose mean-gray-value (Fig. 3) was similar to the surface [Fig. 2(g)] prior to protein adsorption. According to the

FIG. 2. Fluorescent microscope images of FITC-BSA adsorbed on (a) glass, (b) OTS-modified glass, (c) PEG-modified glass, (d) PS, (e) PDMS, and (f) PMMA are shown. (g) The fluorescent image for glass prior to adsorption. The pictures were taken under exactly the same conditions (e.g., light intensity and exposure time). Scale bars represent 50 μm .

FIG. 3. Normalized relative mean-gray-values of FITC-BSA on the six surfaces are shown. Each of the errors is the standard deviation obtained from at least eight sets of measurements.

estimation based on the assumption that the PS surface was fully covered with a layer of randomly oriented side-on BSA molecules and the size of BSA molecule [$4 \times 4 \times 14$ nm (Refs. 35–37) or $9.5 \times 5 \times 5$ nm;³⁸ no significant difference in area coverage, 56 or $48 \text{ nm}^2/\text{molecule}$, was resulted from either of the dimensions. More details can be found in the supplemental material⁴⁰], the PS surface had a protein coverage of $\sim 0.20 \mu\text{g}/\text{cm}^2$, followed by PMMA ($\sim 0.15 \mu\text{g}/\text{cm}^2$), OTS ($\sim 0.08 \mu\text{g}/\text{cm}^2$), PDMS ($\sim 0.08 \mu\text{g}/\text{cm}^2$), and then glass ($\sim 0.04 \mu\text{g}/\text{cm}^2$), while the PEG surface had the lowest protein coverage of $\sim 4 \text{ ng}/\text{cm}^2$. Similar results on PEG and silicon wafer (surface properties similar to that of glass) were obtained by Sharma *et al.*,³⁹ who also used fluorescence intensity from fluorescence images, and their adsorption results were confirmed by ellipsometry measurements.

C. Interpretation of the FITC-BSA adsorption by the XDLVO theory

To interpret the FITC-BSA adsorption behaviors, we first assumed that the properties of FITC-BSA were the same as those of BSA (see supplementary material⁴⁰). Using surface energies and their components of BSA and substrates, the profiles of the DLVO and XDLVO interaction energies

between BSA molecules and all six surfaces were generated. The energy curves for all six combinations: BSA-glass and BSA-PEG are presented in Fig. 4; and BSA-OTS, BSA-PS, BSA-PDMS, and BSA-PMMA are presented in Fig. 5.

For the glass surface [Fig. 4(a)], both DLVO and XDLVO interaction energy curves showed negative secondary energy minima, and energy barriers between the primary minima and secondary minima. For the BSA-PEG surface combination [Fig. 4(b)], the DLVO interaction energy curve only exhibited a primary minimum without a secondary minimum or an energy barrier; while the XDLVO interaction energy curve showed a secondary minimum and a relatively high energy barrier in addition to the primary minimum. The shapes of interaction energy curves for four combinations: BSA-OTS [Fig. 5(a)], BSA-PS [Fig. 5(b)], BSA-PDMS [Fig. 5(c)], and BSA-PMMA [Fig. 5(d)] were similar. For each of these four combinations, with the DLVO theory, an energy barrier existed between the primary minimum and the secondary minimum; whereas for the XDLVO theory, all energy curves went directly down to the primary minimum without a secondary minimum or an energy barrier.

With an energy barrier, the protein molecules may not be able to overcome it to adsorb at the primary minimum; instead, they adsorb reversibly at the secondary minimum (i.e., weaker adsorption). Without an energy barrier, the proteins would probably be able to adsorb at the primary minimum (e.g., stronger adsorption) and likely more proteins could be adsorbed. As shown in Figs. 4 and 5, the DLVO interaction energy curves of all BSA-substrate combinations had energy barriers except the BSA-PEG combination, which only showed a primary minimum. Also, very similar DLVO energy curves were obtained for BSA-glass [Fig. 4(a)], BSA-PMMA [Fig. 5(d)], and BSA-PS [Fig. 5(b)] combinations, while the FITC-BSA adsorption behaviors were obviously different (Fig. 2). Furthermore, higher energy barriers were obtained for BSA-OTS and BSA-PDMS [Fig. 5(c)] combinations, while the amount of FITC-BSA adsorbed on OTS and PDMS surfaces was much higher than that on the nonenergy barrier PEG surface. Therefore, the energy curves obtained using the DLVO theory, which does not include the acid-base interactions, could not interpret BSA adsorption on most surfaces.

FIG. 4. Estimated DLVO and XDLVO interaction energy profiles between BSA and (a) glass and (b) PEG are presented.

FIG. 5. Estimated DLVO and XDLVO interaction energy profiles between BSA and (a) OTS, (b) PS, (c) PDMS, and (d) PMMA are presented.

The XDLVO interaction energy curves of these combinations, on the other hand, appeared to be able to explain the observed protein adsorption. The energy profiles of the BSA-glass and BSA-PEG combinations showed energy barriers. This indicated that kinetically, if BSA molecules could not overcome the energy barrier for irreversible adsorption, then less adsorption would occur. The energy curves of the BSA-OTS, BSA-PS, BSA-PDMS, and BSA-PMMA combinations all only have the primary minimum, suggesting that FITC-BSA molecules could adsorb irreversibly on OTS, PS, PDMS, and PMMA surfaces, and the amount of adsorption would likely be higher than those on glass or on the PEG surface over a fixed amount of time (e.g., 20 min).

One might wonder why the PEG and PMMA surfaces, which have similar surface energy and its components (Table I), had such different XDLVO energy curves with BSA. As shown in Table I, their zeta potentials and γ^- s were quite different. Zeta potential and γ^- influenced ΔG^{EL} and ΔG^{AB} , respectively. Using γ^- of $\sim 40 \text{ mJ/m}^2$ for PEG and of $\sim 28 \text{ mJ/m}^2$ for PMMA in Eq. (2) yielded a positive $\Delta G_{d_0}^{\text{AB}}$ for PEG (15.6 kJ) and a negative $\Delta G_{d_0}^{\text{AB}}$ for PMMA (-12.7 kJ). When the BSA molecule was very close (e.g., 1 nm) to the substrate, ΔG^{AB} was about three-fold to one-order magnitudes greater than ΔG^{LW} and ΔG^{EL} , respectively. The differences caused by zeta potential for these two cases were not significantly different, $\Delta G_{d_0}^{\text{EL}}$ was 1.1 kJ for PEG and 3.1 kJ for PMMA. Consequently, the difference in γ^- of PMMA and PEG, which led to the acid-base interactions, caused the dramatic difference in the interaction energy curves.

While a negative energy minimum estimated by the XDLVO analysis can suggest the thermodynamic tendency of protein adsorption, the correlation between the minimum energy and the actual adsorbed amount, which related to adsorption kinetic rate constant, was difficult to draw. Protein kinetic adsorption parameters (e.g., rate constant) were influenced by the types of protein and substrate surfaces as well as protein solution concentration and buffered solution composition.^{11,13,14} For a specific type of protein (e.g., FITC-BSA in our case) with the same initial concentration, interaction energy, which reflects the adsorption rate constant,¹¹ could relatively determine the adsorption amount (i.e., higher rate constant leads to greater adsorption amount with a certain fixed period).⁴¹ In our case, the energy barrier between the primary energy minimum and the secondary energy minimum was found to be more important to elucidate whether or not proteins could adsorb on a surface, as well as the amount of protein molecules overcame the energy barrier to adsorb on the surface over a fixed period of time. For the three polymer surfaces and OTS-modified glass, no energy barrier from the XDLVO analysis was noticed, and the protein molecules were easily adsorbed to these four surfaces and a greater adsorption amount was noticed. For glass and PEG-modified glass, less FITC-BSA adsorption, as compared to the other four surfaces, was noticed, which appeared to correlate to the energy barrier of the XDLVO energy curve for these two combinations (BSA-glass and BSA-PEG). One might even expect fewer proteins to be adsorbed on glass, since the energy barrier was higher for the BSA-glass combination as compared to that of the

BSA-PEG combination, but the opposite experimental results were observed. One of the possible reasons was the role of the hydration layer on the PEG surface, which was one of the important factors for the nonfouling characteristics of the PEG surface. This factor was not taken into account in our initial analysis based on the XDLVO theory.⁴² If this hydration layer were being considered, the PEG surface would behave more like a water surface. When surface energy and its components of water ($\gamma = 72.8 \text{ mJ/m}^2$, $\gamma^{\text{LW}} = 21.8 \text{ mJ/m}^2$, $\gamma^{\text{AB}} = 51 \text{ mJ/m}^2$ with $\gamma^+ = \gamma^- = 25.5 \text{ mJ/m}^2$) were used to replace the PEG surface in the XDLVO analysis, the energy barrier between the primary and the secondary energy minimum was greatly increased, as shown in Fig. 4(b). Another possible reason was the steric repulsion due to the elastic force caused by PEG molecule long chain compression,⁴³ which could make it even harder for protein molecules to adsorb on the PEG surface.

IV. CONCLUSIONS

In this study, we evaluated FITC-BSA adsorption on glass, OTS-modified glass, PEG-modified glass, PS, PDMS, and PMMA. Greater adsorption amounts were observed on PS, PMMA, OTS, and PDMS, which all showed no energy barrier between the primary and secondary minimum energy of the XDLVO interaction energy curves with BSA. In contrast, for BSA-glass and BSA-PEG, lower BSA adsorption and an energy barrier in the interaction energy curve were noticed. In order to interpret the nonfouling characteristics of the PEG surface, an inclusion of the hydration layer on the PEG surface for the XDLVO analysis was necessary. This study indicated that the XDLVO theory could be a thermodynamic model to be employed for a rough and qualitative prediction of protein adsorption behaviors on surfaces. The energy barrier, which was primarily resulted from the AB interactions, was found to be more useful for interpreting FITC-BSA adsorption on the six surfaces. For more quantitative analysis, other factors, such as molecular conformation of proteins, hydration layer of the PEG, etc., should be included into the analysis.

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