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# Influence of surface charge on the rate, extent, and structure of adsorbed Bovine Serum Albumin to gold electrodes

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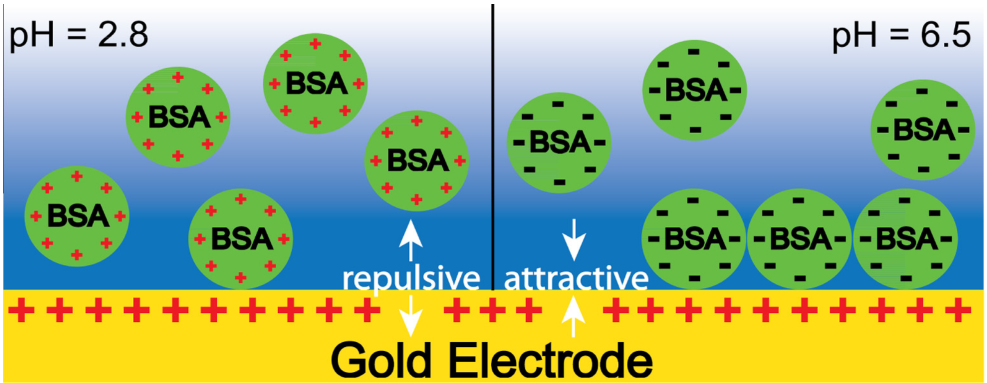
## GRAPHICAL ABSTRACT



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## ABSTRACT

The objective of this work is to investigate the rate, extent, and structure of amphoteric proteins with charged solid surfaces over a range of applied potentials and surface charges. We use Electrochemical Quartz Crystal Microbalance with Dissipation Monitoring (E-QCM-D) to investigate the adsorption of amphoteric Bovine Serum Albumin (BSA) to a gold electrode while systematically varying the surface charge on the adsorbate and adsorbent by manipulating pH and applied potential, respectively. We also perform cyclic voltammetry-E-QCM-D on an adsorbed layer of BSA to elucidate conformational changes in response to varied applied potentials. We confirm previous results demonstrating that increasing magnitude of applied potential on the gold electrode is positively correlated with increasing mass adsorption when the protein and the surface are oppositely charged. On the other hand, we find that the rate of BSA adsorption is not governed by simple electrostatics, but instead depends on solution pH, an observation not well documented in the literature. Cyclic voltammetry with simultaneous E-QCM-D measurements suggest that BSA protein undergoes a conformational change as the surface potential varies.

## 1. Introduction

Understanding and manipulating interactions between colloidal particles and solid substrates is of critical importance to engineered systems for coatings, biomedicine, food processing, and

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environmental science. Extensive prior research has investigated the role of colloidal properties [1–6], solution characteristics [7–11], surface materials and patterning [12–15], as well as external fields [16–20] in controlling the rate, extent, and structure of the adsorbed layer.

While protein adsorption to non-polarized surfaces has been thoroughly examined experimentally and theoretically [21–28], considerably less effort has been dedicated to understanding the influence of electric fields on the electrostatic interactions of proteins, or other amphoteric macromolecules, with a charged substrate. In the presence of an electric field, the rate of attachment, the total mass deposition, and the conformation of colloids and macromolecules may be altered due to enhanced electrostatic interactions with the electrode surface [29–34].

To probe these effects, Ying et al. [20] investigated the adsorption of Human Serum Albumin (HSA) on gold electrodes for four different applied potentials via electrochemical and ellipsometric methods. They report enhanced protein adsorption on both positive and negatively charged surfaces. Moulton et al. [19] used radiolabeling to determine the total mass adsorbed on the gold electrode for HSA and immunoglobulin G (IgG) proteins at 3 different applied potentials, while also investigating the electrochemical behavior of the adsorbed layer via cyclic voltammetry. In contrast with the results reported by Ying et al., Moulton et al. reported that negatively charged electrodes hindered the adsorption of negatively charged HSA. Brusatori et al. [35] employ optical waveguide lightmode spectroscopy (OWLS) to study Bovine Serum Albumin (BSA) and horse heart cytochrome C protein adsorption kinetics to charged surfaces, finding that both positively and negatively charged proteins demonstrated increased adsorption with positive applied potential, though only the rate of BSA adsorption increased. And, finally, Benavidez and Garcia [36] demonstrated that under applied voltages in excess of 0.5 V, high protein concentrations, certain pH values, and select ionic strengths, the polarizability of pre-adsorbed BSA leads to enhanced electrostatic interactions with dissolved proteins and ultimately to multilayer adsorption. Their experimental work is limited, however, in addressing the effect of applied potential during the initial stages of protein-electrode interaction.

These contrasting and sometimes counterintuitive results suggest the need for additional investigation into the factors affecting protein adsorption in the presence of an electric field, particularly during the initial stages of interaction. In particular, optical and radiolabeling methods would benefit from complimentary mass-based adsorption measurements via the Electrochemical Quartz-Crystal Microbalance with Dissipation Monitoring (E-QCM-D) method. E-QCM-D offers the additional benefit of capturing the viscoelasticity of the adsorbed layer on an electrode, which is of critical importance in understanding the structure of the adsorbed layer and the conformational changes of the protein during cyclic voltammetry. Xie et al. [37] studied the QCM–EIS system for simultaneous measurement of electrochemical and acoustic impedance and showed that this novel method can generate accurate and interference-free results. Thus, we adopt a similar system where we use the E-QCM-D to quantify adsorption to charged surfaces.

In this work, we seek to isolate the contributions of electrostatic forces originating at both the protein and the electrode and to investigate their relative importance in electroadsorption and protein conformation. We investigate the rate and extent of adsorption of amphoteric BSA, a model soft protein, to a gold electrode by systematically varying the surface charge on the adsorbate and adsorbent by manipulating pH and applied potential, respectively. We also perform cyclic voltammetry-E-QCM-D on an adsorbed layer of BSA to elucidate conformational changes in response to varied applied potentials.

## 2. Materials and methods

### 2.1. Measuring the zeta potential to determine the charge of Bovine Serum Albumin as a function of pH

The charge on Bovine Serum Albumin (BSA) molecules are tracked by measuring the zeta potential of 12 samples of 10 g/L BSA (Amresco) suspended in 10 mM NaCl solution at different pH values using Malvern ZetaSizer Nanoseries ZSP. The pH of each sample is adjusted by titrating either with 0.1 M HCl (Fisher Scientific, Certified ACS Plus, 36.5–38.0% w/w) or 0.1 M NaOH (Fisher Scientific, Certified ACS).

### 2.2. Measuring adsorption to charged surfaces

We measure the adsorption of BSA to a gold surface using a Quartz-Crystal Microbalance with Dissipation Monitoring (QCM-D; Q-Sense E4, Biolin Scientific). QCM-D is a nanogram sensitive instrument for assessing macromolecule–surface interactions. A piezoelectric quartz crystal is oscillated at its resonance frequency as mass is adsorbed to the surface of the crystal. Resulting changes in the frequency and dissipation of the crystal provide real-time insight into adsorbed mass and the viscoelastic and inertial characteristics of the deposited layer in real-time [38–41].

The frequency and the dissipation data can be converted to units of adsorbed mass per unit area by using the Sauerbrey or viscoelastic models. Sauerbrey equation relates the change in frequency to change in mass, as in Eq. (1),

$$\Delta m = -\frac{C}{n}\Delta f \tag{1}$$

where $n$ is the overtone number ($n = 1, 3, 5, 7, \ldots$) and $C$ is the mass sensitivity constant which has the value of $-17.7$ Hz ng/cm$^2$ for a 5 MHz crystal [25,38]. This relation holds under three major assumptions [38]. First, the adsorbed mass follows the motion of the sensor, thus the adsorbed film is rigid. Second, the adsorbed mass is small relative to the mass of the sensor. And third, the adsorbed mass is evenly distributed on the crystal.

If the adsorbed film layer has viscous and elastic contributions to the frequency change, the Sauerbrey relation no longer holds. Instead, the Voigt based viscoelastic film model can be incorporated in the calculation of the adsorbed mass. Voigt based model assumes a homogenous viscoelastic layer with uniform thickness covering the surface of the piezoelectric quartz crystal [41–44]. As described by Höök et al. [42], the adsorbed film is in contact between the gold coated electrode and a semi-infinite Newtonian liquid. Using this model, Voinova et al. [44] derived the relationship between the viscoelastic properties of the adsorbed mass and the QCM-D response as in Eqs. (2) and (3),

$$\Delta f \approx -\frac{1}{2\pi\rho_o h_o}\left\{\frac{\eta_3}{\delta_3} + h_1\rho_1\omega - 2h_1\left(\frac{\eta_3}{\delta_3}\right)^2 \frac{\eta_1\omega^2}{\mu_1^2 + \omega^2\eta_1^2}\right\} \tag{2}$$

$$\Delta D \approx \frac{1}{\pi f \rho_o h_o}\left\{\frac{\eta_3}{\delta_3} + 2h_1\left(\frac{\eta_3}{\delta_3}\right)^2 \frac{\eta_1\omega}{\mu_1^2 + \omega^2\eta_1^2}\right\} \tag{3}$$

where $\rho_o$ and $h_o$ is the density and the thickness of the crystal, respectively, $\eta_3$ is the viscosity of the bulk liquid, $\rho_3$ is the density of liquid, $\delta_3$ is the viscous penetration depth of the shear wave on bulk liquid, and $\omega$ is the angular frequency of oscillation. $\rho_{1,}$ $\eta_1$, $\mu_1$, and $\delta_1$ represents the density, viscosity, shear elasticity and the thickness of the adsorbed layer, respectively. In the present work, we use the Q-tools (Q-sense) software to model the total mass adsorption.

QCM-D allows users to monitor the changes in frequency and dissipation as a function of surface charge when coupled with a