

# Subsurface ferroelectric water provokes a controlled protein adsorption

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**Abstract:** The region of the outermost 3 layers of atoms or molecules commonly defines the surface properties such as chemistry, wettability and charges. Hence, this top most surface layer is a key factor for controlling of molecular adsorption onto a surface. As a distinctive approach from this well-studied prominent parameter, we have explored the contribution of deeper sub-surface layers, the region several nanometres below the surface, to the protein adsorption via generating long-range interaction forces (e.g. > 10 nm).

**Keywords:** plasma polymerization, vertical chemical gradient, film hydration, BSA adsorption, subsurface structuring, ferroelectric water

## 1. Introduction

An elaborate understanding of how proteins interact with various surfaces is of continued interest in surface and biological sciences. In the view of any bio-response cascade, the controlling of initial protein adsorption and the suppression of non-specific protein adsorption are particularly sought for the prevention of the initiation of undesired biological reactions. Bovine serum albumin (BSA) is a well-studied model protein for adsorption studies. It is one of the most abundant globular proteins having a 66.5 kDa molecular weight and has an enormous dipole moment with  $\mu_{BSA} = 384$  D which causes a possible alteration in its orientation and conformation upon interaction with a surface dipolar field [1]. The recent conducted researches to shed light on the BSA-surface interaction have been only limited to surface modifications, the effect of water molecules at the surface and the properties of BSA solution [2-5]. In this study, we bring a new perspective to the adsorption of proteins on a material by revealing the generation of long-range interaction forces by oriented water molecules at the hydrophilic/hydrophobic sub-surface of a vertical gradient plasma polymer film buried few nanometres below the surface [6, 7].

Within the above framework, various sub-surface structured crosslinked vertical gradient thin films as well as reference layers are designed by plasma polymerization. For this purpose, HMDSO (hexamethyldisiloxane) was utilized as precursor due to its tuneable wetting properties which can be adjusted through the control of the applied plasma conditions. Based on this monomer, a hydrophilic, siloxane-based SiO<sub>2</sub>-like layer (ppSiOx) and a hydrophobic, PDMS like plasma polymerized HMDSO (ppHMDSO) were deposited as homogeneous reference coatings. The selected vertical gradient matrix (ppVGRAD) consists of a relatively thin ppHMDSO terminating layer on a thicker nanoporous ppSiOx base layer. Furthermore, a novel enhanced gradient architecture (enhanced ppVGRAD)

was obtained by post-oxidizing the hydrophilic SiOx base layer reducing the amount of residual hydrocarbon groups yet maintaining nano-porosity prior to depositing the terminating hydrophobic HMDSO layer. Therefore, a distinctive hydrophobic/hydrophilic interface was generated leading to variation in the hydrophilicity and also in the nanoporosity of the vertical gradient plasma polymer. The plasma polymerized thin films were characterized for water wettability, chemical composition and topography of the film surfaces by contact angle measurement, angle resolved x-ray photoelectron spectroscopy (ARXPS), time of flight secondary ion microscopy (ToF-SIMS) and atomic force microscopy (AFM).

Furthermore, neutron reflectivity (NR) was utilized, which facilitates the determination of scattering length density as well as the degree of water content inside the vertical gradient films.

With the acquired knowledge about the resulting film chemistry and hydration abilities of the films, the adsorption of bovine serum albumin (BSA) on plasma polymerized gradients as well as reference layers were investigated using Transmission interferometric adsorption sensor (TInAS) in order to figure out how the adsorbed mass of BSA is modified upon the change not only in surface but also in sub-surface chemistry. Moreover, in order to investigate the role of water at the surface or in the near-surface region in molecular adsorption, dry and hydrated films with varying hydration time were compared.

## 2. Experimental Methods

In this study, plasma polymer films were deposited using a capacitively coupled, asymmetric reactor. The plasma chamber had a RF driven electrode ( $f = 13.56$  MHz) and was operated at low pressure (7 Pa). Four different films were produced according to the well-defined protocol given in literature [6]; i) a 50 nm hydrophobic film

(ppHMDSO) deposited from the vaporized HMDSO using a power input of 50 W, ii) a 50 nm inorganic glass – like hydrophilic film (ppSiOx) deposited from the additional admixture of oxygen with the HMDSO plasma using a power input of 100 W, iii) a vertical gradient film (ppVGRAD) consisting of a 50 nm thick ppSiOx base layer followed by the deposition of 4 nm ppHMDSO terminating layer and - as a novel approach - iv) an enhanced vertical gradient film (enhanced ppVGRAD) produced by shortly oxidizing the hydrophilic 50 nm ppSiOx bottom layer prior to the deposition of the 4 nm ppHMDSO top layer.

### 3. Results and discussions

Initially, the wetting properties of samples were measured at different positions on the film surfaces. The films terminating with ppHMDSO layer demonstrated the same hydrophobicity, whereas the ppSiOx film surface possesses hydrophilic character. Due to the homogenous and flat surface topography of the films, as proved by AFM, the effect of roughness on water contact angle can be neglected.

ARXPS yielded the information about the in-depth film composition of gradient films as well as of reference layers. Depending on the different photoemission take-off angles, the sampling depth varies allowing to distinguish sub-surface from the surface. From the fitting of the ARXPS data with chemical composition profiles, we were able to detect the transition from hydrophobic terminating layer to hydrophilic base layer which corresponds to a thickness of 2-3 nm below the surface. As a complementary technique to ARXPS, ToF-SIMS measurement also provides distinctive information between O-rich bottom part and C-rich top part. As shown in Fig. 1, the C-rich reference layer ppHMDSO yields the highest intensity in carbon containing fragment, CH<sup>+</sup>, while the ppSiOx reference, which is O-rich, revealed the lowest intensity in CH. As one would expect, the intensity of this fragment for the vertical gradient films lies in a range between the two references. In a good agreement with ARXPS results, gradient films demonstrated an increase in the density of carbon rich fragments towards the surface. By excluding the bombarding effect of ToF-SIMS for the outermost few atomic layers, it is also confirmed that the vertical gradient film surfaces show a terminating closed layer which supports the WCA and AFM results.

In a next step, using neutron reflectometry it was scrutinized that regardless of the hydrophobicity of the terminating layer, diffusion of water molecules through the gradient structure is energetically favourable which results in enrichment of water molecules underneath the surface after a certain hydration time (Fig. 2). In more detail, the hydrolysis of Si-O-Si bonds, which is present in a limited amount in hydrophobic terminating layer, and therefore the formation of silanol groups is one of the well-studied mechanisms to describe diffusion pathway of

water molecules through the vertical gradient film. In a preceding project, the water uptake capabilities of reference layers were already confirmed [8].

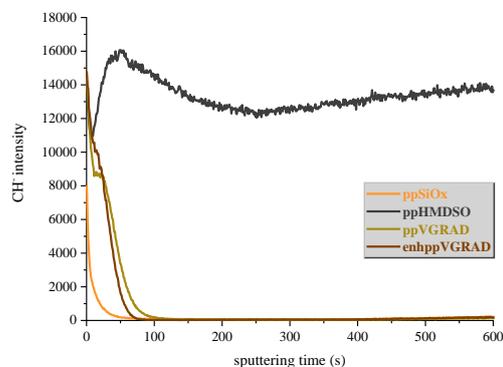


Fig. 1. ToF-SIMS profiles of the two vertical gradient films comparing to references

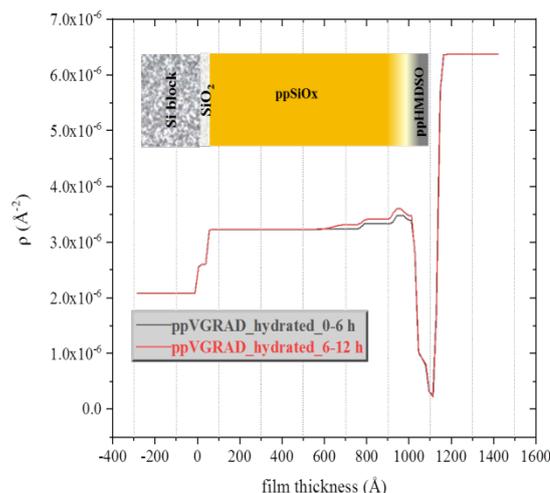


Fig. 2. Scattering length density profile of ppVGRAD differing in hydration time as a function of film thickness

The impact of this penetrated water molecules in the gradient on BSA adsorption was observed via a unique instrument, TInAS. The adsorption of BSA molecules on the hydrophilic ppSiOx surface reaches the highest amount compared with the hydrophobic ppHMDSO surface. The adsorbed amount of BSA was found to be decreased on the gradient-containing surfaces, where it is remarkably even more diminished on the enhanced gradient film. Furthermore, the adsorption of BSA was studied on the investigated layers with respect to different

hydration states. Hydration, on the order of hours was found to affect protein adsorption as observed for all investigated siloxane coatings including the reference layers.

#### 4. Conclusion

Well-defined vertical gradient thin films have been generated by plasma polymerization and systematically examined for chemical composition and water diffusion. The impact of this water mediated gradient on protein adsorption was observed. It was notably perceived that the BSA adsorption is substantially reduced on the hydrated gradient showing a distinctive change in wettability below the surface compared to reference surfaces.

It is hypothesized that the designed enhanced gradient architecture supports the orientation of nanoconfined water molecules in the sub-surface hydrophilic/hydrophobic gradient. Hence, the formation of a dipole moment from more ferro-electric water molecules creates strong long range interaction forces modulating protein adsorption.

To go beyond this hypothesis, the electrostatic surface potential of highly crosslinked plasma polymer films has recently been characterized by Kelvin probe force microscopy and electrochemical measurement. Moreover, those above mentioned experimental findings are going to be supported by molecular dynamic (MD) simulations.

#### 5. Acknowledgement

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