Probing the hydration of ultra-thin antifouling silane adlayers using neutron reflectometry

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Neutron reflectometry data supports the existence of a relatively thick (~22 Å), physically distinct phase of water contiguous to an antifouling monoethylene glycol silane adlayer prepared on quartz. In contrast, the less effective adlayer lacking internal ether oxygen atoms exhibits a much thinner (~4 Å), less organized interphase. These findings support the link between antifouling and surface hydration.

Contact of artificial materials with biological fluids (*e.g.* blood, serum, urine) is very common in many aspects of modern medicine. Whether applied *in vitro* or *in vivo*, the range of equipment/devices is immense and includes, for instance, the circuitry used in renal haemodialysis and coronary bypass, catheters, stents and pacemakers as well as biosensors for clinical diagnostics and, more recently, lab-on-a-chip technology. Medical applications – which involve surfaces made of titanium, stainless steel, ceramics or polymers – have spawned considerable research into the molecular and cellular interactions that occur at the material/biological fluid interface.[1] There are two key related factors that are responsible for this activity: first and foremost are the surface-induced deleterious effects that may be instigated as a result of material bioincompatibility; and second, biosensor platform fouling which radically interferes with the detection process.[2] The common ground that connects these issues is the spontaneous surface adsorption of proteins.[2] Accordingly, it is not surprising that extensive literature documenting the development of antifouling coatings has appeared over the years.[2]

One of the most studied antifouling surface chemistries is the family of poly- and oligoethylene glycol-based constructs (PEG and OEG).[3] Although the origin of the 'PEG/OEG-effect' has long been debated, there is now a general consensus that hydration plays a key role, especially for coatings made of short EG building block molecules where the role of entropy linked to chain flexibility/compression is less significant.[3] One explanation for surface antifouling connected to hydration is the concept of a 'water barrier', where embedded and interfacial water molecules are tightly bound and organized into permeated structures that have an energy cost in terms of disturbance.[3] Such barriers have been shown to be physically distinct, solute-free exclusion zones projecting up to several hundred microns into the contiguous aqueous phase.[4] An alternative suggestion presents the notion that there is simply no energy gain provided through adsorption of highly hydrated proteins to a surface already significantly hydrated.[5]

Recently, we showed that surface hydration is intimately involved in the mechanism of antifouling, for thiol and silane monoethylene glycol (MEG) adlayers prepared on gold[6] or quartz.[7] In the latter work, another key feature was the observation that the internal ether oxygen atom is necessary to dramatically alter the dynamics of full serum adsorption, the synergy being strongest for silane adlayers possessing terminal hydroxyl moieties (MEG-OH, Fig. 1 – top).[7] In contrast, the effect was considerably less pronounced for OTS-OH adlayers lacking internal oxygen atoms (Fig. 1 – bottom).[7] It was then hypothesized that the antifouling properties of the MEG-OH system are rooted in a special intrafilm zone of hydration, from which a physically distinct interfacial phase of water stems.[6,7]

Herein, we examine this theory and present the results of a follow-up study investigating the hydration of both MEG-OH and OTS-OH silane adlayers using neutron reflectometry (NR). For the antifouling MEG-OH system, NR data are compatible with the presence of a ~22 Å-thick, physically distinct interfacial phase of water. Conversely, this interphase is much thinner (~4 Å) for the less effective OTS-OH system. These observations confirm the critical role played by the internal ether oxygen atom with respect to surface hydration hence antifouling.

Neutron reflectometry is one of various techniques[8] available to investigate the properties of water at surfaces. In practice, NR probes the specular reflection of neutrons off atomic nuclei and, being sensitive to scattering by light isotopes (*i.e.* H and D), indeed allows for the assessment of interfacial water density[9] as well as film water absorption.[10,11] Reflectivity is collected as a function of the neutron scattering vector (q), which depends on the neutron wavelength and scattering angle. Most commonly, Parratt's recursion algorithm is used for calculating a reflectivity curve (based on a model of the system under study) which is then compared to measured reflectivity data.[12] Data fitting for a selected layered model is then achieved by adjusting the thickness (d), interfacial roughness (σ) and scattering length density (SLD) of the various layers. In the process, substrate (*e.g.* quartz) and bulk liquid (*e.g.* water) are assigned fixed and known SLD values with an infinite thickness. More details on NR can be found elsewhere.[13,14] In the present work, NR measurements were performed on the D3 neutron reflectometer at the NRU reactor in Chalk River, Canada.

For the purpose of our study, the simplest approach to assess the presence of an interfacial phase of water physically distinct from bulk was to fit our NR data with a 2-layer model (Fig. 2). For the fitting procedure, the roughness at both quartz and adlayer surfaces, $\sigma_{Q\text{-}A}$ and $\sigma_{A\text{-}I}$, was fixed (at 2 Å) as were the SLD values for quartz ($SLD_Q = 4.18 \times 10^{-6}$ Å^{-2}) and bulk water[15]

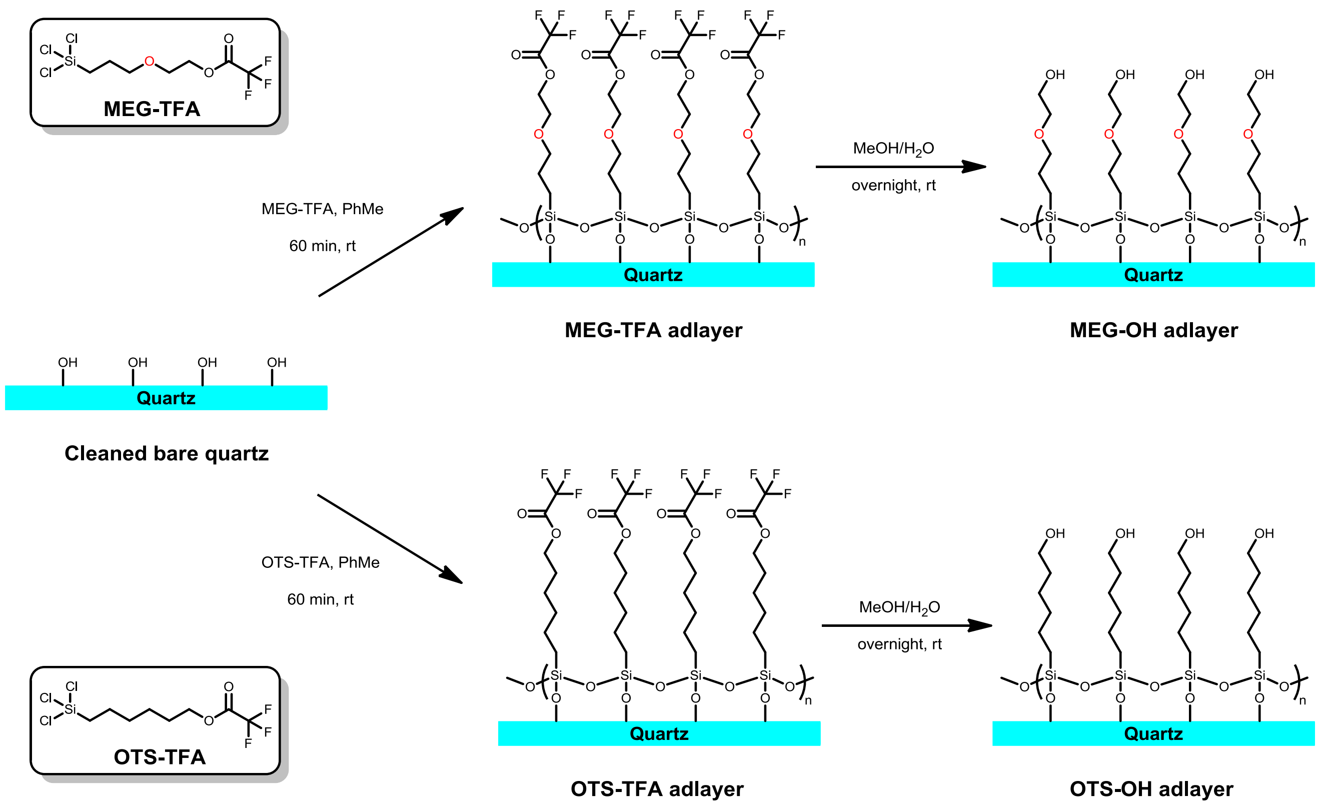

Fig. 1. Two-step formation of the MEG-OH adlayer on quartz (top) and of its OTS-OH alkylated homolog lacking internal ether oxygen atoms (bottom).[7]

(SLD_B = 4.50 x 10^{-6} $Å^{-2}$). Reflectivity and resulting SLD profiles are gathered in Fig. 3a and 3b, respectively.

With respect to the MEG-OH system, data fitting resulted in an adlayer thickness of 6.1 Å. Interestingly, NR data thus suggested that, despite the removal of the labile trifluoroacetyl groups (TFA) upon solvolysis (Fig. 1), MEG-OH adlayers may be slightly thicker than their MEG-TFA precursors, for which previous ellipsometric measurements gave a dry thickness of 5.1 Å.[7] Importantly, this swelling implies that the MEG-OH film absorbed water, a first hint that a special intrafilm zone of hydration involving the internal atoms of oxygen may indeed exist.[16] A recent account of water penetrating into EG films supports this hypothesis.[11] However, a simple straightening of the MEG chains (without intervention of water) upon loss of the bulky TFA groups is also likely responsible for such increase in thickness. Regardless, in closest proximity to the adlayer ($SLD_I \approx$ 3.91 x 10^{-6} $Å^{-2}$) starts a physically distinct, lower-density interphase of water which spans over ~22 Å until bulk water resumes (SLD_B = 4.50 x 10^{-6} $Å^{-2}$), as can be seen in Fig. 3b.

To conclusively determine whether the internal atoms of oxygen are involved with the formation of this interphase of water, we next performed NR measurements with an OTS-OH adlayer that lacks such feature (Fig. 1). When comparing both reflectivity profiles (Fig. 3a), it is immediately evident that the absence of internal oxygen atoms in OTS-OH measurably altered the interaction of water with the adlayer.

The effect is more dramatic on the SLD profiles (Fig. 3b). For the OTS-OH system, data fitting showed that an interphase of water also exists but is much thinner (only ~4 Å-thick) and comparatively denser ($SLD_I \approx$ 4.29 x 10^{-6} $Å^{-2}$, which corresponds to ~95% of bulk water density assuming unchanged D_2O/H_2O ratio[17]). In other words, this interphase of water is barely discernable from the bulk. With respect to adlayer thickness,

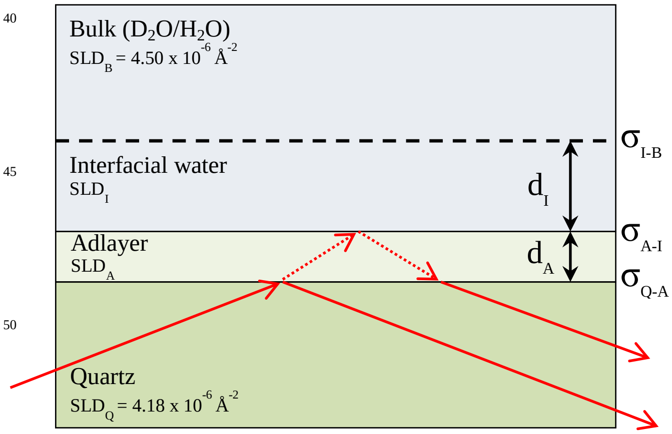

Fig. 2. Two-layer model showing (from bottom to top) the quartz substrate, the silane adlayer as well as interfacial and bulk water. Also shown are the various layer thickness (d), roughness (σ) and scattering length density (SLD). Arrows indicate the direction of the incident and reflected neutron beams.

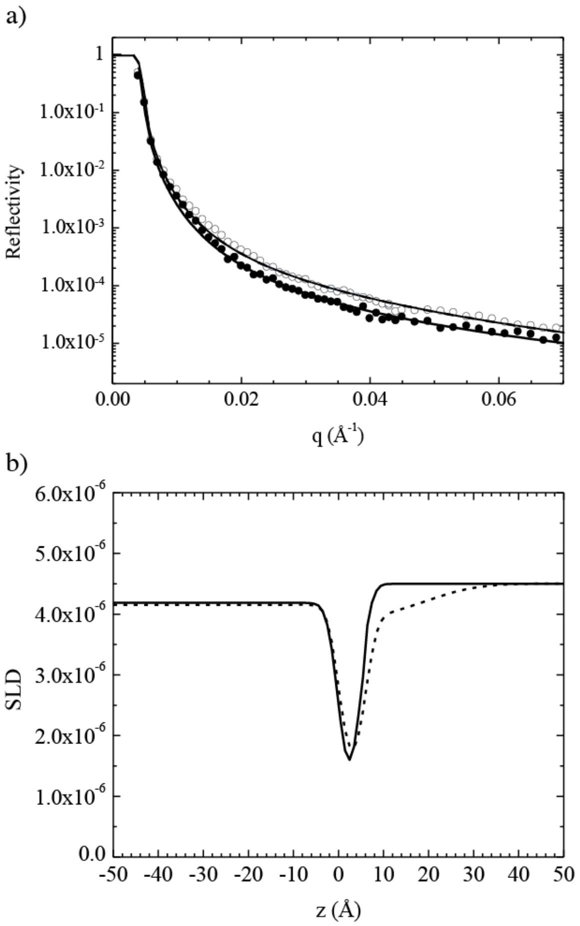

Fig. 3. (a) Reflectivity data and corresponding fits for MEG-OH (white circles) and OTS-OH (black circles) adlayers on quartz, measured with near contrast-matched water.[15] (b) Corresponding SLD profiles for MEG-OH (dashed line) and OTS-OH (solid line) films using a 2-layer model.

fitting resulted in a value of 4.8 Å, which is substantially thinner than MEG-OH (6.1 Å). Assuming MEG-OH and OTS-OH films present comparable surface coverage, this observation corroborates the water absorption properties ascribed earlier to the MEG-OH adlayer (*vide supra*). Collectively, our NR data are consistent with the existence, for the MEG-OH system, of a special intrafilm zone of hydration that involves the key participation of the internal atoms of oxygen and provides a strong foundation onto which water molecules can gradually organize into a quite extensive, lower-density interphase.[18] The (long-range) water structuring properties of the MEG-OH system are confirmed.[6,7] The antifouling properties previously reported for the MEG-OH adlayer correlates well with the presence of this physically distinct interfacial network of water.[7] The opposite is true for the OTS-OH system.

Conclusion

Using neutron reflectometry (NR), we have showed that a relatively thick (~22 Å), physically distinct interfacial phase of water may indeed exist at the surface of an ultra-thin (< 1 nm) monoethylene glycol silane adlayer prepared on quartz (MEG-OH). Conversely, the adlayer lacking internal ether oxygen atoms (OTS-OH) exhibits a much thinner (~4 Å), less organized interphase barely distinguishable from bulk water. NR data support the hypothesis according to which the antifouling properties of the MEG-OH system stem from a special intrafilm zone of hydration that involves the key participation of the internal atoms of oxygen and from which the interphase of water originates. To our knowledge, this work constitutes one of the first examples studying the hydration of subnanometric EG coatings. NR experiments with adlayers prepared on Si/SiO$_2$ substrates, for enhanced scattering contrast and improved resolution, are underway.

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16 Whether water reached the quartz substrate and the latter participated in the instigation of the intrafilm zone of hydration would also be an interesting question to answer.

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