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Critical role of surface hydration on the dynamics of serum adsorption studied with monoethylene glycol adlayers on gold†

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The dynamics of serum adsorption on bare and monoethylene glycol adlayer-modified gold surfaces is investigated using acoustic wave physics. Hydration experiments support the pivotal role ascribed to water in the antifouling of surfaces. Behavioural discrepancy is interpreted in terms of difference in water structuring properties (surface kosmotropicity).

Upon contact with bodily fluids/tissues, exogenous materials spontaneously develop a layer of proteins on their surface.[1–6] With respect to biosensing technology, such a process leads to an overwhelming background signal that prevents the detection, and ability to quantify, target analytes present at considerably lower concentrations.[7,8] In the case of biomedical implants and equipment, biological processes with deleterious effects may ensue.[1–4,9–11] To address this ubiquitous problematic phenomenon, tremendous efforts have been dedicated over the years to engineer protein-resistant coatings.[12–16] There is now substantial literature available on stealth organic adlayers able to reduce fouling down to technologically relevant levels (*i.e.* a few ng cm^{-2} or less) when exposed to highly complex proteinaceous, real-world media such as blood serum and plasma.[17]

Key issues with fouling by these biological milieux are not only the nature, strength and amount but also the dynamics of adsorption between the protein multicomponents of blood and man-made surfaces. In this connection, we recently studied and qualitatively compared the antifouling behaviour, against full goat serum, of a series of ultrathin organosilane adlayers constructed on piezoelectric quartz substrates.[18] In that work, we used the electromagnetic piezoelectric acoustic sensor (EMPAS),[18–24] an ultra-high frequency (GHz)[22,24] bulk acoustic wave (BAW) device able to monitor molecular interactions occurring at the sensor–liquid interface in a real-time and label-free manner. The measured resonant frequency is both governed by addition/loss of mass on the sensor and also non-gravimetric phenomena such as interfacial slip and alteration of viscoelasticity *via* macromolecular conformational changes. Among the silane films investigated, the monoethylene glycol (MEG) variety bearing distal hydroxyl groups (MEG-OH, Fig. 1 – top) is responsible for the most dramatic alteration of the dynamics of serum adsorption, as characterized with the EMPAS.[18]

Given the evident success with the hydroxylated quartz substrate, we next set out to adapt the MEG-OH surface chemistry to another typical substrate, gold, which is widely employed for detection in conventional BAW and surface plasmon resonance (SPR) biosensor devices.[25] The latter technology is available in many highly sensitive commercial systems.[26] It is the object of the present communication to report on the surface modification of gold with short MEGylated thiol molecules and the antifouling behaviour of

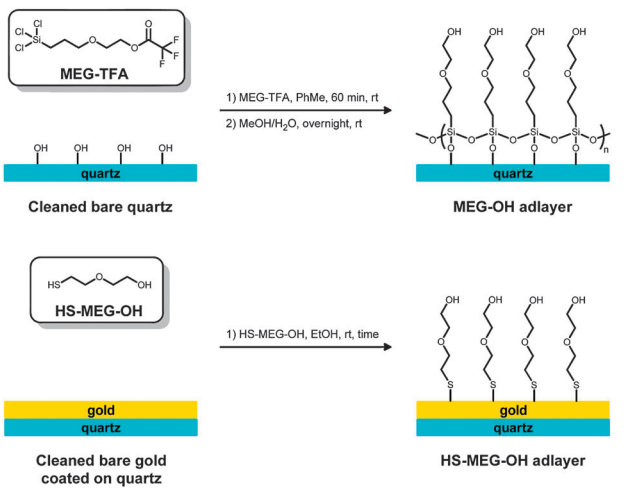

Fig. 1 Surface modification of (top) hydroxylated quartz and (bottom) gold metal with MEG-OH adlayers.

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the resulting imposed adlayers against 10% goat serum, as probed using a more conventional BAW thickness shear mode (TSM) sensor device.[27] Also presented is the significant effect of surface hydration on the dynamics of serum adsorption.

Unlike the electrode-free EMPAS configuration described elsewhere,[19,20] the TSM system employed herein relies on the use of gold contact electrodes plated on both sides of the piezoelectric quartz substrate to excite acoustic resonance.[19,20] Obviously, the trichlorosilane chemistry developed for the hydroxylated surface of quartz[18] (Fig. 1 – top) is not suitable for the metal surface of gold. As a result, its surface modification required the appropriate sulfhydryl-based MEG-OH molecule (HS-MEG-OH, Fig. 1 – bottom) to be first synthesized.[28] Next, a series of HS-MEG-OH adlayers were then prepared *via* immersion of cleaned gold-coated quartz resonators into a 5 mM solution of HS-MEG-OH surface modifier in anhydrous ethanol, for increasing periods of time (5 to 1080 min) at room temperature, as illustrated at the bottom of Fig. 1.†

The dynamics of serum adsorption on the resulting adlayers was next investigated with the flow-through TSM sensor. After stabilization of the resonance signal in air, phosphate buffered saline (PBS) was flown at a rate of 100 μL min^{-1} until equilibration of the system (~60 min overall, on average). Goat serum (10% in PBS) was then injected for exactly 1 min before final re-introduction of the PBS buffer flow. This time trial experiment revealed that resonant frequency was least affected upon serum injection for adlayers prepared in anhydrous ethanol for 30 min ($\Delta F = -121 \pm 16$ Hz, $n = 3$). This represented a modest but clear decrease in resonant frequency shift compared to unmodified gold for which $\Delta F = -147 \pm 23$ Hz ($n = 3$). Typical TSM responses to serum injection are gathered on Fig. 2. At this point, it is worth noting that adlayer formation was solely optimized with respect to time but not temperature, another important parameter which also directly affects surface coverage thereby adlayer stability.

There is in the literature a growing body of experimental and theoretical evidence to suggest that surface hydration is intimately involved in the mechanism of protein repellence.[12–15] We have also referred to this concept and recently shown the dramatic effect surface hydration can have on serum adsorption in our previous work with MEG organosilane adlayers on quartz substrates.[18] One argument proposes the concept of 'water barrier', wherein embedded and interfacial water molecules are tightly bound and organized into permeated structures that have an energy cost in terms of disturbance.[12,15] Such barriers have been shown to be physically distinct, solute-free exclusion zones projecting up to several hundred microns into the contiguous aqueous phase.[29] Another argument puts forth the notion of 'interfacial energy matching', in which there is no net energy gain for native proteins, that are fully solvated in the aqueous medium where they reside, to adsorb on stably hydrated surfaces.[14]

To test this surface hydration theory, we immersed the HS-MEG-OH films in deionized water overnight.† In this case, the experiment showed no improvement in resonant frequency shift. Quite to the contrary, and for a reason which still remains unclear, ΔF increased to -145 ± 7 Hz ($n = 3$). Comparative analysis of the shifts in motional resistance (ΔR_m), a parameter relating to the dissipation of acoustic energy and simultaneously recorded to glean information about the viscoelastic properties of a system,[30] did not provide further answers. In fact, ΔR_m values (1.7 ± 0.5 and 2.2 ± 1.4 Ω for hydrated and non-hydrated adlayers, respectively) were not statistically different. At this point, we questioned the stability of surface hydration vis à vis the final drying step (a gentle stream of nitrogen) samples systematically undergo prior to TSM analysis.† In fact, we suspected the latter to have irretrievably altered 'fragile' water networks. Accordingly, we repeated the experiment omitting the drying of the top electrode (*i.e.* the surface for analysis) and directly encased wet samples into the TSM chamber. We observed that, under these conditions, the resonant frequency shift was substantially reduced to -87 ± 8 Hz ($n = 3$) from -121 ± 16 Hz ($n = 3$) for the non-hydrated adlayers. These results are in line with those obtained during our study with the EMPAS system.[18] In comparison, bare gold surfaces that had undergone the same hydration treatment displayed a much higher frequency shift of -150 ± 12 Hz ($n = 3$).

For both types of surface, the resonant frequency profile is characterized by a sharp initial drop (~−170 Hz in ~2 min) followed by a rinse-off phase (Fig. 3). However, while the latter is limited (~+30 Hz) and essentially over in approximately 10 min for bare gold, HS-MEG-OH surfaces exhibit a radically different behaviour with a progressive and comparatively more

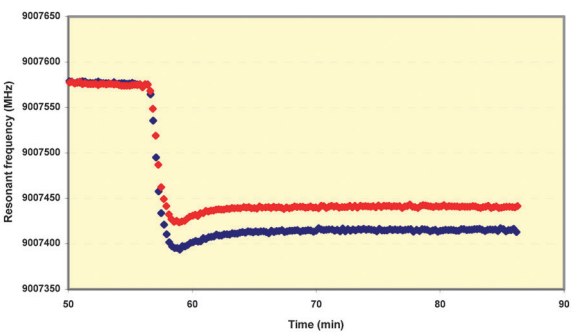

Fig. 2 TSM resonant frequency responses to 10% goat serum injection for (bottom – blue) bare and (top – red) HS-MEG-OH adlayer-derivatized gold surfaces. For comparison purposes, injection times ($t \sim 56$ min) and initial resonant frequencies have been normalized.

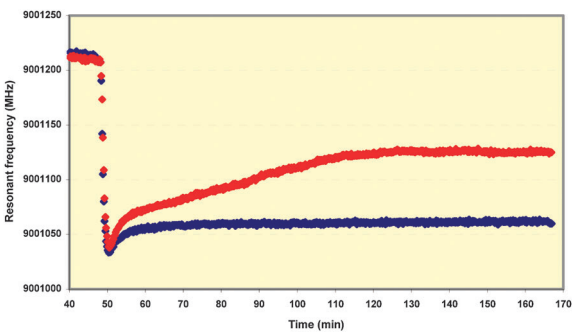

Fig. 3 TSM resonant frequency responses to 10% goat serum injection for (bottom – blue) bare and (top – red) HS-MEG-OH-derivatized gold surfaces, immersed overnight in deionized water.

extensive rinse-off (~+80 Hz in ~60 min). With respect to bare gold, a limited rinse-off phase likely indicates that species responsible for fouling accumulate readily and quasi-irreversibly. The fact that bare gold surfaces are heavily fouled ($\Gamma \gg 100$ ng cm^{-2}) upon contact with serum, even diluted, has already been documented on several occasions.[31–35] In contrast, it appears for the HS-MEG-OH case that the majority of serum species adsorb in a reversible fashion involving transient interaction with the surface. The occurrence of a sequential 'Vroman-like' process or/and stiffening events within the fouling layer also constitute reasonable explanations.[18]

We note finally that whether or not bare gold surfaces are submitted to the hydration treatment, ΔF values are not statistically different (-150 ± 12 *vs.* -147 ± 23 Hz) and TSM profiles actually almost perfectly overlap (compare Fig. 2 and 3). This observation may reflect an inherent inability for bare gold to structure water molecules on its surface in a robust and thick enough manner.[36] This likely explains why gold presents a surface that is fouled with facility by serum species in a manner akin to quartz[18] and other bare metal(loid) oxide surfaces.[37–39] Conversely, we attribute the behaviour observed for the hydrated HS-MEG-OH adlayers to be the consequence of stronger and longer-range water structuring properties. These are likely deeply rooted in a special intrachain zone of hydration involving, synergistically, the internal ether oxygen atoms and distal hydroxyl moieties.

In summary, we have herein elaborated on the antifouling behaviour against serum of monoethylene glycol (MEG) adlayers bearing distal hydroxyl groups. This MEG-OH surface chemistry was successfully adapted from the hydroxylated surface of quartz to the metal surface of gold using sulfhydryl-based building block molecules. Dynamics of serum adsorption was probed in a real-time and label-free manner using acoustic wave physics and shown to be significantly altered when compared to the results obtained for unmodified gold. Through surface hydration experiments, empirical evidence was provided to support the widely proposed hypothesis that water plays an important, if not pivotal, role in the antifouling of surfaces constructed from short EG molecules. Integration of this MEG-OH-type surface chemistry in biosensing platforms for the serological detection of various disease-related biomarkers in clinical diagnostics is currently underway. We also aim to generate biocompatible coatings on stainless steel and plastic substrates for medical implants and equipment.

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