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# Are PEG Molecules a Universal Protein Repellent?

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***Abstract*—**Poly (ethylene glycol) (PEG) molecules attached to surfaces have shown high potential as a protein repellent due to their flexibility and highly water solubility. A quartz crystal microbalance recording frequency and dissipation changes (QCM-D) has been used to study the adsorption from aqueous solutions, of lysozyme and α-lactalbumin proteins (the last with and without calcium) onto modified stainless steel surfaces. Surfaces were coated with poly(ethylene imine) (PEI) and silicate before grafting on PEG molecules. Protein adsorption was also performed on the bare stainless steel surface as a control. All adsorptions were conducted at 23°C and pH 7.2. The results showed that the presence of PEG molecules significantly reduced the adsorption of lysozyme and α-lactalbumin (with calcium) onto the stainless steel surface. By contrast, and unexpected, PEG molecules *enhanced* the adsorption of α-lactalbumin (without calcium). It is suggested that the PEG -α-lactalbumin hydrophobic interaction plays a dominant role which leads to protein aggregation at the surface for this latter observation. The findings also lead to the general conclusion that PEG molecules are not a universal protein repellent. PEG-on-PEI surfaces were better at inhibiting the adsorption of lysozyme and α-lactalbumin (with calcium) than with PEG-on-silicate surfaces.

***Keywords*—**Stainless steel; PEG; QCM-D; protein; PEI layer; silicate layer.

## I. Introduction

PROTEIN adsorption on solid surfaces is a complex phenomenon and appears to involve many dynamic steps such as bond formation between proteins and surfaces, lateral diffusion on the surface and conformational changes or rearrangements of adsorbed proteins. Driving forces for protein adsorption have demonstrated the importance of hydrophobic interaction, electrostatic attraction, van der Waals and hydrogen bonding [1]. Elimination of protein adsorption requires the suppression of all these attractive forces between proteins and surface. A common approach for blocking the adsorption of proteins is to immobilize polymers in the form of well-solvated brushes on the surface. The polymer layer shields the surface, introducing a high activation barrier for the proteins to adsorb.

Poly (ethylene glycol) (PEG) modified surfaces have attracted much attention due to their excellent protein repellent properties [2-5]. It has been suggested that PEG chain length, conformation and number density on the surface are important factors for resisting protein adsorption [3,5-7]. Surfaces forming a large number of hydrogen bonds with water molecules produce large repulsive forces on the protein, leading to lower protein adsorption [8]. Yet there is evidence, which indicates that in some cases PEG does bind to proteins [9,10].

A range of methods have been employed for the immobilization of PEG onto surfaces and these can be broadly classified as either physisorptive or chemisorptive [2-4]. In this study, a careful choice was made of a technique which could be practically used in process equipments.

A number of techniques have been utilized in the study of protein adsorption, including ellipsometry [11], optical waveguide lightmode spectroscopy (OWLS) [12], surface plasmon resonance (SPR) [13] and quartz crystal microbalance-dissipation (QCM-D) [14-15].

The objective of this study is to inhibit protein adsorption onto a stainless steel surface. Stainless steel surface has been chosen as a substrate as it is a commonly used material in many relevant applications such as in the dairy industry, food processing and clinical uses.

Three types of proteins were used in this study; lysozyme and α-lactalbumin (the last with and without calcium). α-lactalbumin is the second most abundant protein in whey and has been chosen to represent proteins in the dairy products. Lysozyme was chosen as it was widely used in many studies and was well characterized. α-lactalbumin is a compact globular protein with the dimensions of 3.7 nm x 3.2 nm x 2.5 nm. Its molar mass is about 14,200 Da and is an acidic protein with an isoelectric point (pI) value of 4.3. The calcium enriched α-lactalbumin is also known as holo α-lactalbumin whereas that with is apo α-lactalbumin. Lysozyme also is a globular protein of slightly ellipsoidal shape with the dimensions of 4.5 nm x 3.0 nm x 3.0 nm. Its molar mass is approximately 14,600 Da. Lysozyme is relatively a stable protein (that is, 'hard' protein) compared to α-lactalbumin. It is a basic protein with a pI value of 11.1.

In this study, adsorption of proteins was performed on an AT-cut quartz crystal coated with gold and then a stainless steel surface. The adsorption and desorptions were done in situ and monitored in real time using a quartz crystal microbalance with the interpretation allowing also for dissipation (QCM-D). For modification of the surface, PEI or sodium silicate solutions were first adsorbed by physisorption

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onto bare stainless steel surfaces. Then PEG molecules were grafted physically onto the resulting PEI or silicate layers.

## II. Experiment

### A. Materials

Branched polyethylenimine (PEI) with MW 25000 Da, sodium silicate solution (reagent grade) with molecular weight 180 Da, lysozyme (MW 14,600 Da) from hen white egg and alpha lactalbumin proteins (the last with and without calcium) with MW 14,200 Da, from bovine milk were purchased from Sigma-Aldrich (St.Louis,Mo,USA). Polyethylene glycol monomethyl ether (OH-PEG-$CH_3$, MW 350, 2000 and 5000 Da) was purchased from Fluka (Darmstadt, Germany). All chemicals were used as received without further purification. Phosphate buffer (pH 7.2) was prepared in our laboratory with appropriate proportions of ultra high purity MilliQ water, $Na_2HPO_4$ and $NaH_2PO_4$ (from Sigma Aldrich, St.Louis,Mo,USA).The buffer solutions were degassed with helium prior to use to avoid bubble formation during QCM experiments. PEG and protein solutions were prepared in phosphate buffer solution. The concentrations of protein and PEG solution were 0.1 and 1.0 g/L, respectively for all runs. Stocks of protein solution were kept in the freezer at 4 °C. Protein solutions not used within 48 hours of thawing were discarded. The PEI and sodium silicate solution were prepared in milliQ water at concentration of 30 mg/mL and 5 %w/v, respectively. All experiments were conducted at 23 °C and pH 7.2.

### B. Quartz Crystal Microbalance-Dissipation (QCM-D) experiments

A Q-4 model QCM was used (Q-Sense, Goteborg, Sweden) with frequency and dissipation monitoring (QCM-D) and AT-cut quartz crystals with a fundamental resonant frequency of 5 MHz and a diameter of 14 mm. One side of each diaphragm crystal was coated by the manufacturer with 100 nm of gold and then 50 nm of stainless steel (SS2343). The mass composition of the stainless steel (SS) was carbon (0.03%), chromium (16.5-18.5%), nickel (11-14.5%), molybdenum (2.5-3%) and iron (64-70%). The quartz crystal was mounted in a flow cell with the SS surface exposed to the solution. For adsorption of protein, the protein sample solutions were pumped through the flow cell by a peristaltic pump at a flow rate of 100 μL/min. Desorption was performed immediately after the adsorption reach steady state, by replacing the protein solution with a pure buffer flow. If the surface was modified before protein adsorption, it was done in situ by pumping the PEI or sodium silicate solution first, followed by PEG solution. The kinetics of sample adsorption and desorption were followed by changes in the resonant frequency of the crystal and dissipation of the crystal vibrations. All measurements reported in this paper were done with the system temperature stabilized at 23±0.5 °C. The crystals were cleaned by immersion in a 5:1:1 mixture of milliQ water, ammonia (25% v/v) and hydrogen peroxide (30% v/v) for 5 minutes at 75°C, followed by thorough rinsing with milliQ water and drying with a moisture-free nitrogen gas stream. To finish the cleaning, the crystals were treated with UV light and ozone for 5-10 minutes to remove organic contamination. The general procedure for using this model of QCM has been reported elsewhere [14].

## III. Results

### A. Surface Density of PEI and Silicate Layers on Bare Stainless

Fig. 1 shows the mass density of PEI and silicate layers with time on a stainless steel surface interpreted using the Voigt model. Adsorption of PEI onto the surface was very fast and reached a plateau in less than 2 minutes. The mass density adsorbed at steady state was about 20 mg/m$^2$ (that is, about 0.5 chains/nm$^2$). When the PEI layers were rinsed with phosphate buffer, mass decreased and presumably weakly bound PEI molecules were desorbed. Almost 85 % of the PEI mass was desorbed, leaving approximately 3 mg/m$^2$ (that is, 0.07 chains/nm$^2$), corresponding to around 4 nm spacing between chains and 2 nm average layer thickness.

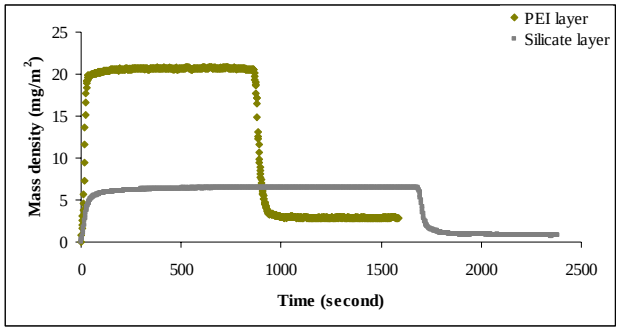


Fig. 1 Mass density of PEI and silicate layers on a stainless steel surface as a function of time interpreted using the Voigt model

Silicate molecules adsorbed onto the stainless steel surface relatively slowly compared to that of PEI (shown by a less steep initial slope) and reached steady state after approximately 5 minutes. The mass density adsorbed at steady state was about 6.5 mg/m$^2$ (that is, about 21 chains/nm$^2$). When the silicate layers were rinsed with phosphate buffer, about 87 % of the silicate mass was desorbed, leaving approximately 0.8 mg/m$^2$ (that is, 2.5 chains/nm$^2$), corresponding to around 0.6 nm spacing between chains and 0.66 nm average layer thickness.

### B. Surface Density of PEG layer on Modified Stainless Steel

Fig. 2 shows the number density of tightly bound PEG molecules on the stainless steel surface coated with

PEI/silicate layers (those PEG molecules remaining after desorption with fresh buffer solution).

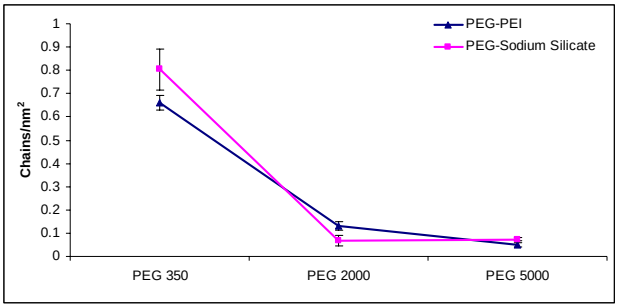


Fig. 2 Number density of tightly–bound PEG molecules adsorbed on a PEI/silicate layer

Either coated with PEI or silicate, the trend of PEG density with change in PEG molecular weight was almost similar (the chain density decreased as PEG molecular weight increased). There was about 80 % decrement in the PEG density attached on a PEI layer as molecular weight increased from 350 to 2000 Da and about 60 % decrement with further increase in PEG molecular weight (5000 Da). Meanwhile, PEG density attached on a silicate layer showed more than 90 % reduction as molecular weight increased from 350 and 2000 Da and almost no change with further increase. The density of PEG molecules was about 20 to 30 % higher on a stainless steel surface coated with silicate than that those with a PEI layer. Overall, the grafting density on PEI and silicate coated surfaces was ranged from about 0.66 to 0.05 and 0.8 to 0.07 chains/nm$^2$, respectively, corresponding to about 1 to 4 nm spacing between molecule to molecule.

*C. Surface density of protein layer on modified stainless steel*

Fig. 3 shows the number density of various protein molecules adsorbed on a bare stainless steel surface and on PEI and PEI-PEG layers after exposure each surface to the solution until steady state was reached, followed by desorption with buffer. The lines were drawn to make easy comparisons between modified and unmodified surfaces. As can be seen, the number of molecules of holo α-lactalbumin and lysozyme adsorbed on a PEG modified stainless steel was lower than that on a bare stainless steel surface, up to 80% reduction. It is interesting to note, almost no adsorption of lysozyme occurred on the PEI layer (less than 5 % adsorption compared to that on the bare surface). By contrast, the number of molecules of apo α-lactalbumin was about 25 % *higher* on the PEI layer and all PEI layers modified with PEG.

Fig. 4 compares protein adsorbed on a bare stainless steel surface, silicate and silicate-PEG layers. Again, presence of PEG molecules *enhanced* the adsorption of apo α-lactalbumin (≅30 %) and reduced adsorption of lysozyme (≅70 %). However, the number of holo α-lactalbumin molecules on the modified stainless steel was almost identical to that on the bare surface. Increasing grafting density or chain length of PEG either on silicate or PEI layers appeared to not affect the protein adsorption.

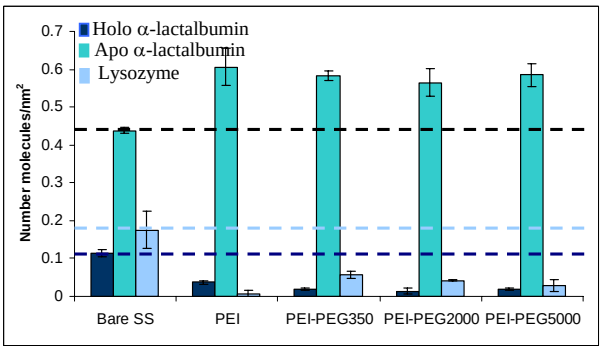


Fig. 3 Comparison of number density of three proteins tightly adsorbed on a bare, PEI and PEI-PEG surfaces for PEG of various molecular weights

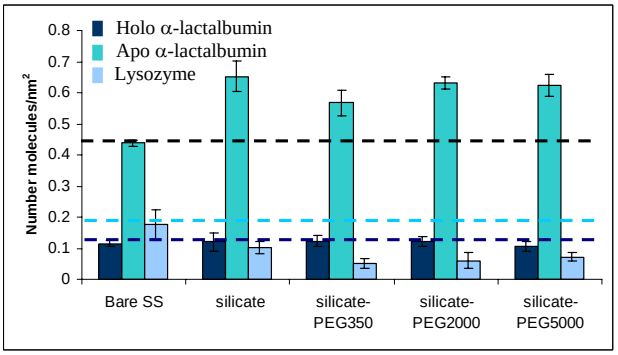


Fig. 4 Comparison of number density of three proteins tightly adsorbed on a bare, silicate and silicate-PEG surfaces for PEG of various molecular weights

## IV. Discussion

All the results presented in this study have been obtained using the Voigt model [16] as the molecules formed a viscoelastic layer (data was not shown). It is important to note that the mass estimated by the Voigt model of QCM-D is the mass of the whole layer, which includes both protein (or PEG or PEI/ silicate) molecules plus water that is bound or trapped in the layer. In other words, all the data presented here is too high by the mass proportion of water in the adsorbed layer. In this study, it was assumed that the effective protein (or PEG or PEI/silicate) layer density was 1200 kg/m$^3$. The effective density of the layer should lie approximately between that of protein (or PEG or PEI/silicate) layer and that of water. Changing the effective density from 1000 to 1400 kg/m$^3$ (the density of protein is 1400 kg/m$^3$) to cover this range gave less than 20 % difference in the estimated mass of the layer, indicating that the assumption of 1200 kg/m$^3$ in this study was quite reasonable. Thus, the number of protein molecules presented here would then be halved to get a better estimate of protein molecules on the surface. The density of sodium silicate solution (wt ratio $SiO_2/Na_2O$ = 1) is around 1530 kg/m$^3$. However, changing the effective density of silicate

from 1200 to 1530 kg/m$^3$ only gave about 22 % difference. Therefore, assumption of 1200 kg/m$^3$ for silicate solution was also reasonable.

Attachment of PEI onto the stainless steel surface was expected to be based on electrostatic interaction. PEI is a polycationic molecule containing a high concentration of primary, secondary and tertiary amino groups in the ratio of 25:50:25 while a stainless steel surface has a strong negative potential due to the presence of metal oxides and hydroxides. There may also be hydrogen bonding between amine groups of PEI and the surface OH groups [17]. These interaction forces were relatively weak and therefore the PEI coatings were somewhat unstable. A study done by [17] found out that crosslinking the PEI with glutaraldehyde does indeed prevent any desorption or transfer of the PEI from a silica surface.

There was a huge decrease in PEG number density as PEG molecular weight increased from 350 to 2000 Da. This observation was expected since higher chain lengths result in low number density [5-7] compared to short chain lengths. The PEG grafting density achieved in this study can be considered as high and fairly stable even though grafting was achieved using a physisorption method (desorption was about 30 %). The grafting density of chemically grafted PEG-succinimidyl propionate (PEG-SPA) (MW 5000 Da) on a PEI-silica surface was reported to be 0.02 chains/nm$^2$ [17]. In another study, the grafting density of PEO with molecular weights of 750, 2000 and 5000 Da on a gold coated silica surface was 0.4, 0.33 and 0.12 chains/nm$^2$, respectively [18]. However, it should be noted that comparison between results from different laboratories are generally difficult.

PEI and PEI-PEG surfaces generally resisted adsorption of lysozyme and holo α-lactalbumin. Adsorption of holo α-lactalbumin on a PEI-PEG surface was about 65 % lower than that on a PEI surface. It was believed that PEG molecules shielded some of the positively charge of PEI. At pH 7.2, holo α-lactalbumin has net negative charges. However, it is still unexplained why the adsorption of holo α-lactalbumin on a PEI surface was lower than that on the bare surface. The adsorption of holo α-lactalbumin on a PEI surface should be either the same as on the bare or higher considering two main driving forces; hydrophobic and electrostatic interactions. In other cases, PEI surfaces seemed superior for inhibiting lysozyme adsorption than PEG-PEI surfaces. It was presumably because of electrostatic repulsion between lysozyme and PEI molecules since both of them have net positive charges at pH 7.2. More proteins adsorbed on the PEI-PEG layer than that on the PEI layer, indicating a possibility of *secondary adsorption* occurred [19].

It is worthy to note that, holo α-lactalbumin and apo α-lactalbumin behaved oppositely towards tethered PEG molecules; with adsorption of apo α-lactalbumin *enhanced* by the presence of PEG molecules. This indicates the presence of direct protein-PEG attraction. The interaction between proteins and PEG-coated surfaces was believed to arise from combinations between *primary*, *secondary* and *tertiary* adsorptions. Removal of calcium induced a conformational change of α-lactalbumin and increased the hydrophobicity. Thus, it is suggested that the adsorption of apo α-lactalbumin onto the PEG layer was dominantly driven by hydrophobic interaction. Research done by [9], using surface force measurements revealed the evidence of attraction between streptavidin and PEO. [9] explained the attraction between PEO and streptavidin with a change in conformation of the PEO segments from a protein repellent polar conformation to a protein attractive apolar conformation. The change from polar to apolar conformation may be induced by compressing the PEO layer. Meanwhile, the adsorption of apo α-lactalbumin on a PEI surface was higher than that on the bare surface and was not expected.

Coating the stainless steel surface with silicate or silicate-PEG layers reduced adsorption of only lysozyme whereas the adsorption of holo α-lactalbumin was almost the same as on the bare surface. PEG molecules attached on a silicate or PEI layer have almost similar in grafting density, thickness and distance between molecules but behaved differently towards the proteins. Adsorption of lysozyme for example, was about 95 and 60 % higher on silicate and PEG-silicate surfaces compared to that on PEI and PEG-PEI surfaces, respectively. Meanwhile, adsorption of holo α-lactalbumin was about 70 and 90 % higher on silicate and PEG-silicate surfaces compared to that on PEI and PEG-PEI surfaces, respectively. It has been demonstrated that the surface of silica gel predominantly exhibits electron-acceptor properties due to the presence of Si–OH groups, whereas the surface of the sample modified with a complete monolayer of PEG exhibits electron-donor properties due to the presence of oxygen atoms belonging to PEG ether groups [20].

Increasing either grafting density or chain length did not affect much to the adsorption of protein. This observation was quite contradicted with the others; protein resistance has improved as the length of the PEG chains and grafting density increased [19,21]. The results obtained also showed that short PEG chains (350 Da) could resist protein adsorption as effectively as long chains (5000 Da). This was presumably because the density of short chain was sufficiently high to prevent adsorption of protein (the distance between PEG molecules was smaller than that of the size of the proteins). In other hand, other experimental data found out that short oligomers do not resist protein as effectively as long chains [22]. There is, however, no reason that proteins should interact with both short and long chains by the same mechanism, especially when the phase behaviour of PEG/water solution varies with the molecular weight.

From the results obtained, it is suggested that grafted PEG chains could exist in two different states; a protein-repulsive state and a protein-attractive state. It was reported that ethylene oxide segments can adopt multiple configurations and different conformers interact differently with water molecules. The rational conformers can be divided into a large group of trans and a small group of gauche conformers.

Trans conformers had non polar characters and were favored at high temperature, whereas the gauche conformers had polar characters [23]. The structures and interfacial properties of OEG chains suggested that the interconversion between protein-attractive and protein-resistance PEG was due to segment rearrangements in the polymer chains with non polar segments concentrated near the surface and polar segments at the outer edge. [9] revealed that compressing the PEO layer may induce the change from a polar to a non polar conformation. Increasing the temperature or altering the polymer molecular weight could also induce to an attractive state [10].

Therefore, it is suggested that protein conformation properties were more dominant than PEG properties for adsorption.

## V. Conclusion

From this work, it can be concluded that:

- PEG number density was almost the same either on PEI or silicate layer and relatively high and stable even though the PEG was grafted using physisorption method.
- Modification of stainless steel with PEI-PEG provided lysozyme and holo α-lac resistance up to 85 % whereas silicate-PEG surfaces reduced adsorption of lysozyme only by about 70 %.
- Either PEI-PEG or sodium silicate-PEG coated surface *enhanced* the adsorption of apo-α-lactalbumin.
- PEG molecules are not a universal protein repellent.
- Protein conformation properties were more dominant that PEG properties for adsorption.

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