# Experimental report

09/02/2016

**Proposal:** **9-13-608**

**Council:** 4/2015

**Title:** Adsorption of undeuterated and deuterated myoglobin on a block copolymer surface with tailored nanopores size and hydrophilicity

**Research area:** Chemistry

**This proposal is a new proposal**

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**Samples:**
Myoglobin
polystyrene based block copolymer

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| D17 | 4 | 3 | 02/11/2015 | 05/11/2015 |
| FIGARO | 4 | 0 | | |

## Abstract:

The aim of the proposal is the study of the physical adsorption of myoglobin in the nanopores of block copolymer (BCPs) thin films of tailored size. The small amount of studies on the effect of constrained environment on the proteins adsorption in nanopores is partly due to the lack of information relative to the exact morphology and physical-chemical features of the used porous media. Exploiting the BCPs self-assembly, we have set up a procedure that allows building nanostructured thin films with well-defined architecture containing pores delimited by hydrophilic walls. Very promising preliminary results have been obtained in neutron reflectivity measurements performed during the test experiment 2428 on FIGARO. For these reason, we intend to study more in depth the protein adsorption at the solid/liquid interface by using a fully deuterated myoglobin. Also, we intend to perform time-resolving experiments at a number of myoglobin concentrations, in order to better understand how the nanoscale morphology of our substrate influences the protein adsorption/desorption mechanism.

## INTRODUCTION

We have studied the adsorption of two hemoproteins, myoglobin (Mb) and *peroxidase from horseradish* (HRP), on different polymeric thin films. In particular, the adsorption of these biomolecules on porous and non porous thin films based on block copolymers (BCPs) has been studied.

The non porous BCP based material (blend) is a nanostructured thin films (thickness ~ 50 nm) with lamellar morphology, obtained by spin coating dilute solutions of polystyrene-*b*-poly(L-lactide) (PS-PLLA) and polystyrene-*b*-poly(ethylene oxide) (PS-PEO). The material is characterized by PS lamellar domains alternating to layers of mixed PLLA/PEO blocks.

The BCP based nanoporous surface (etched blend) has been fabricated by selective removal of PLLA blocks from the non porous material. The so obtained thin film exhibits a well-defined architecture containing nanochannels (width ~ 20 nm) delimited by walls of PS lamellar domains with pendant PEO hydrophilic chains.

Adsorption of myoglobin on thin films of hydrophobic polystyrene (PS) homopolymer has been also studied in order to underline differences between the layers and mechanisms of biomolecule adsorption to the interface due to the different proprieties of the surfaces.

## EXPERIMENTAL SECTION

All the polymeric thin films (blend, etched blend and PS) have been directly prepared on crystalline and polished silicon (111) wafers, covered with a thin amorphous silicon oxide ($SiO_x$) layer at the surface.

The protocol for the neutron reflection measurements has been designed to measure initially the polymeric thin films in 3 or 4 contrast configurations ($D_2O$, 4MW, SiMW and $H_2O$). This has been followed by an in-situ injection of Mb or HRP solutions in $D_2O$. The so obtained systems have been characterized after 3-6 hours equilibration (depending on the sample), and after rinsing in different contrast configurations.

Data have been analyzed by using the fitting program Aurore [1], that uses the Parratt's formalism to analyze the neutron reflectivity data [2]. In Parratt's formalism the scattering length density (SLD) profile is modeled by the concatenation of finite size layers; each layers is characterized by three parameters, namely thickness $t$, total SLD and interfacial roughness $\sigma$. The total SLD of each layer is re-parameterized as

$$SLD = f^{w} SLD_{s} + f^{dry} SLD_{dry}$$

where $f^{w}$ is the volume fraction occupied by the solvent in the layer, $SLD_s$ is the scattering length density of the solvent, and $f^{dry}$ is the volume fraction of dry constituents in the same layer. By definition $f^{w} + f^{dry} = 1$. The SLD profile obtained from the concatenation of the different layers is then converted into the corresponding reflectivity curve and compared with the experimental data.

## SOME RESULTS

The reflectivity curves of a PS thin film are shown in Figure 1. The symbols represent the experimental data and the lines show the fits. The contrast variation method has been employed by characterizing the film in three different contrast liquids of different scattering length densities (SLD): $D_2O$, 4MW and SiMW with SLDs 6.35 x $10^{-6}$ Å$^{-2}$, 2.07 x$10^{-6}$ Å$^{-2}$ and 4 x $10^{-6}$ Å$^{-2}$, respectively. The three contrasts have been fitted simultaneously to reduce the number of possible models describing the layer and so increase the resolution and confidence in the results. The SLDs have been fixed at 2.07 x $10^{-6}$ Å$^{-2}$ for silicon and 3.41 x $10^{-6}$ Å$^{-2}$ for silicon oxide. The fitted parameters show that the silicon oxide layer has a thickness $t_{SiOx}$ = 11 Å, a roughness $\sigma_{SiOx}$ = 5 Å and contains no solvent. The obtained values are in good agreement with literature [3]. The PS layer exhibits a thickness $t_{PS}$ = (430 ± 15) Å, a negligible roughness ($\sigma_{PS} \leq 3$ Å) and a SLD$_{dry}$ = 1.5 ± 0.1 × $10^{-6}$ Å$^{-2}$, consistent with the literature value of 1.4 × $10^{-6}$ Å$^{-2}$ [3]. Within the detection limits the PS contains no water. No significant changes are detected in the reflectivity curves of the PS thin film after Mb adsorption (data not shown), so indicating a negligible protein adsorption on this surface (with the used experimental set up).

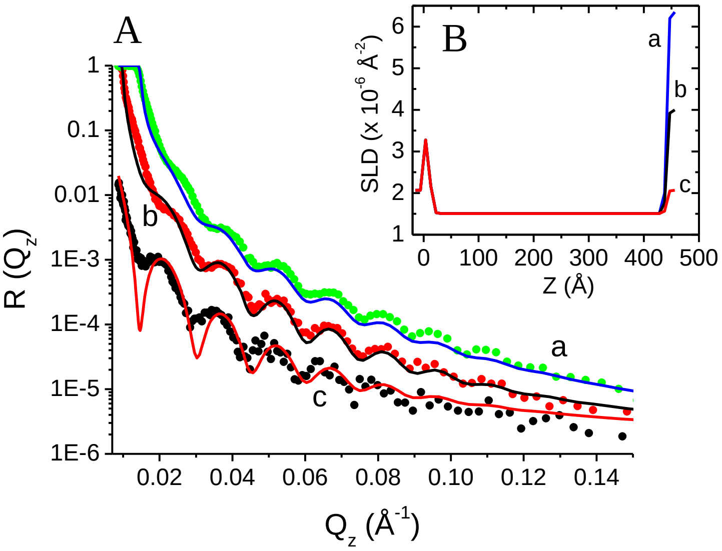

***Figure 1. A)*** *Experimental (symbols) and simulated (lines) reflectivity profiles of a PS thin film in different contrast configurations: $D_2O$ (a), 4MW (b) and SiMW (c). Error bars have been removed for clarity. Fits have been obtained according to the parameters listed in the text.* ***B)*** *Scattering length density (SLD) profiles corresponding to the fits reported in A.*

Selected reflectivity curves of block copolymers (BCP) based thin films collected at the Si/$D_2O$ interface before (blend) and after (etched blend) pores formation and before and after Mb adsorption are reported in Figure 2.

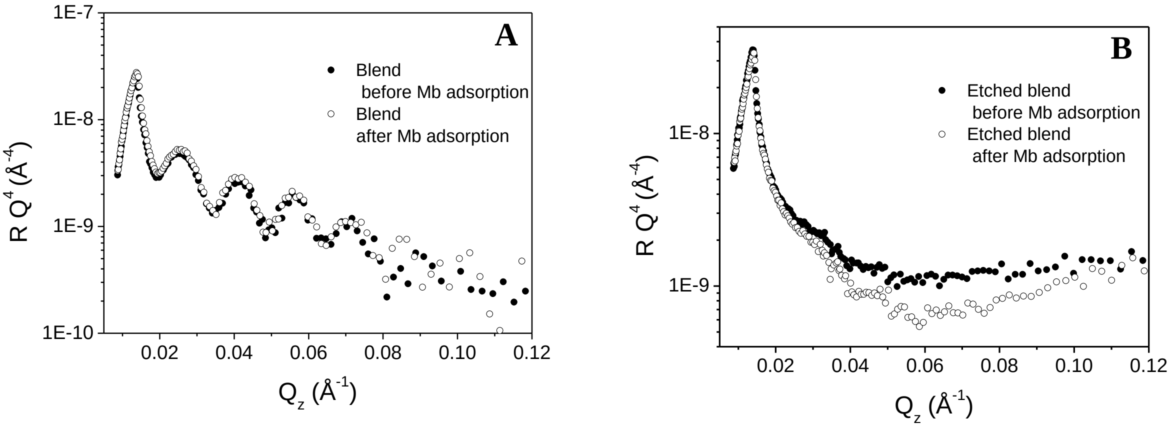

***Figure 2.*** *Reflectivity curves of the blend* ***(A)*** *and etched blend* ***(B)*** *support before (full circles) and after (empty circles) adsorption of hydrogenated Mb. The reported curves (empty circles) have been acquired after 2 hours equilibration between the supports and 0.4 µM Mb solutions, before rinsing. The curves have been collected at the Si/$D_2O$ interface. Error bars have been removed for clarity.*

The presence of fringes in the reflectivity profile of the non porous support (Fig. 2A, full circles) indicates the presence of a homogeneous thin film (thickness ~ 45 nm). The profile significantly changes after pore formation (Fig. 2B, full circles), confirming the presence of pores in which the solvent can easily access. Furthermore, while no significant changes are detected in the reflectivity curves after Mb adsorption in the case of non porous support (Fig. 2A), more evident changes are visible after Mb adsorption onto the porous supports (Fig. 2B), confirming the higher capability of the etched blend to absorb the biomolecules. It is worth noting that no significant changes are detected in the reflectivity curves of the etched blend containing adsorbed Mb after rinsing (data not shown).

We have also studied the adsorption of *peroxidase from horseradish* (HRP) on the etched blend (Figure 3). HRP is an enzyme largely used in electrochemical and optical sensing devices [4]. Previous UV-visible spectroscopy experiments demonstrated that our BCP based nanoporous surfaces (etched blend) constitute the ideal support for the physical absorption of this biomolecule, since the confinement in the nanopores results in a noticeable improvement of the catalytic performance and long term stability of the enzyme. The data acquired during the experiment on D17 (Figure 3) confirm that the adsorption of HRP on our porous support can be studied by neutron reflectometry techniques.

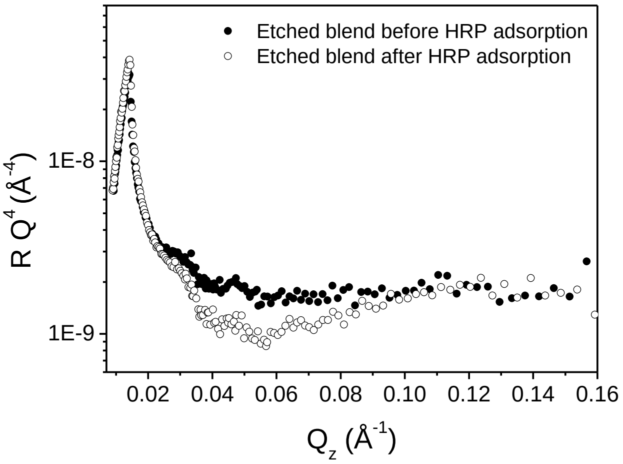

***Figure 3.*** *Reflectivity curves of the etched blend support before (full circles) and after (empty circles) adsorption of hydrogenated HRP. The reported curve (empty circles) has been acquired after 6 hours equilibration between the porous support and 4 µM HRP solution, after rinsing. The curves have been collected at the Si/$D_2O$ interface. Error bars have been removed for clarity.*

Detailed analysis of all the reflectivity curves collected in different contrast is in progress. These data clearly show a difference in the layer composition before and after proteins adsorption. However, because of the low contrast between polymer and proteins it is difficult to exactly quantify the amount of adsorbed proteins and they location (in the pores or on the surface). The use of deuterated Mb (d-Mb) would help in clarifying these aspects **[3]**. The use of d-Mb was foreseen for this experiment, but because of unexpected problems of protein aggregation, occurred one day before the experiment, these measurements have not been performed.

**[1]** Y. Gerelli, *J. Appl. Cryst.* **2016**, *49*, 330. **[2]** L. G. Parratt, *Phys. Rev.* **1954**, *95*, 359. **[3]** N. Brouette, G. Fragneto, F. Cousin, M. Moulin, M. Haertlein, M. Sferrazza, *J. Colloid Interface Sci.* **2013**, *390*, 114. **[4]** H. Y. Lu, Z. Li, N. F. Hu, *Biophys. Chem.* **2003**, *104*, 623. S. Q. Liu, Z. H. Dai, H. Y. Chen, H. X. Ju, *Biosens. Bioelectron.* **2004**, *19*, 963. M. L. Ferrer, F. Del Monte, C. R. Mateo, J. Gomez, D. J. Levy, *Sol-Gel Sci. Technol.* **2003**, *26*, 1169.