In-situ Neutron Reflectometry Study on Adsorption of Glucose Oxidase at Mesoporous Aluminum Oxide Film

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Abstract

In the present study, the adsorption of glucose oxidase (GOD) to a mesoporous aluminum oxide (MAO) film was examined with in-situ neutron reflectometry (NR) measurements. The MAO film was deposited on a cover glass slip and a Si disc, and its pore structure was characterized by X-ray reflectometry (XRR) and NR. The Si disc with MAO film was applied for in-situ NR experiment, and its NR profiles before/after adsorption GOD was continuously measured with a flow cell. The results indicated that almost exclusive outer surface adsorption with the negatively-charged GOD molecules unable to penetrate into the narrow pore channel (pore diameter = ca. 10 nm) with opposite surface charge.
Introduction

Protein adsorption at an inorganic nanoporous material is a fundamental process in the chromatographic separation of proteins and synthesis of an artificial biocatalytic system. The nanoporous material possesses both an outer surface and internal pore surfaces, and both can be regarded as adsorption sites for protein molecules. The distribution of protein molecules at both adsorption sites affects the chromatographic separation and performance of the biocatalytic system. The protein distribution on and in nanoporous materials is thus an important subject to be clarified, and in-situ spectroscopic techniques for the protein adsorption have been developed. The distribution of adsorbate at a single nanoporous particle has been often observed by microspectroscopy techniques. For a nanoporous film on a solid substrate, spectroscopic methods utilizing optical interference and waveguide mode are available for the in-situ observation of the molecular adsorption.

In-situ Neutron reflectometry (NR) is one useful technique for structural characterization of an adsorption layer at a solid/liquid interface. The Scattering length density (SLD) of D$_2$O and H$_2$O is quite different (Table 1), and SLD contrast between materials within the nanoporous film and solvent can be easily tuned by adjusting solvent composition of a D$_2$O/H$_2$O mixture. When NR measurement is performed under a series of D$_2$O/H$_2$O mixture, simultaneous analysis of reflectivity data obtained for the D$_2$O/H$_2$O mixtures can reduce ambiguities while modeling, and facilitates determination of structural properties of adsorption layer. The in-situ NR has been applied to characterize lipid membranes assembled at a surfactant-templated mesoporous silica film and adsorption of polyelectrolyte at an anodized nanoporous aluminum oxide film.

The purpose of this study is application of in-situ NR to clarify whether
negatively-charged glucose oxidase (GOD) can penetrate into pores in mesoporous aluminum oxide (MAO) film or not. The MAO film used in this study was prepared by a surfactant-templated method, and contained ordered pores with diameter of ca. 10 nm. This pore size is much smaller than that of a conventional nanoporous aluminum oxide film prepared by anodization (≥ 20 nm). We previously reported penetration of negatively-charged bovine serum albumin (BSA, 67 kDa, $14 \times 4 \times 4$ nm) into large pores (diameter: 33 nm) of anodic aluminum oxide film. Since the pore diameter of the MAO film is close to molecular dimensions of GOD (160 kDa, $6 \times 5.2 \times 7.7$ nm), it is expected that the size-matching between GOD and pore affects the adsorption behavior of GOD.

In the present study, we deposited the MAO film on a conventional cover glass slip for structural characterization of the MAO film by X-ray reflectometry (XRR), NR, and scanning electron microscopy (SEM). For the in-situ NR experiment, we deposited the MAO film on a Si disc. The structure of MAO film on the Si disc was characterized by XRR prior to the in-situ NR experiment. In-situ NR experiments were performed in D$_2$O/H$_2$O mixtures to characterize the amount and distribution of GOD adsorbed at the MAO film.

**Experimental**

**Materials and chemicals**

A square cover glass slip ($24 \times 24 \times 0.1$ mm) was purchased from Matsunami Glass Ind., Ltd., Osaka, Japan. Pluronic® F127 (PEO$_{106}$ PPO$_{70}$ PEO$_{106}$) and glucose oxidase (GOD) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Aluminum nitrate was purchased from Fujifilm Wako Pure Chemical Corp. (Osaka, Japan). Deionized-water was used for preparation of MAO film and for in-situ NR measurement. Polished silicon
disc (100 mm in diameter and 10 mm in height) and D$_2$O used for the in-situ NR measurement were supplied from Australian Nuclear Science and Technology Organisation (ANSTO, Sydney, Australia).

The details of the preparation procedures of MAO film are described in a previous report.\textsuperscript{19} In brief, Pluronic\textsuperscript{®} F127 (0.1 g) was dissolved in 2 mL of water containing aluminum nitrate (0.3 – 0.7 M), and the mixture was stirred at room temperature for 4 hours. This precursor solution was spin-coated on the clean glass substrate at 600 rpm for 20 s, acceleration of 4000 rpm for 60 s. The glass substrate was dried overnight at room temperature and then calcined at 500 \degree C for 6 hours in ambient atmosphere. Hereinafter, we designated the glass substrate with MAO film as Al$_{xx}$, where $xx$ indicates the concentration of aluminum nitrate (Table 2). For the deposition of MAO film on the Si disc, the dip-coating was performed at 600 rpm for 20 s, acceleration of 3500 rpm for 60 s. The concentration of aluminum nitrate in the precursor solution was 0.7 M. The rotation speed was reduced due to heaviness of the thick and large Si disc. The calcination of the MAO film on the Si disc was performed at 350 \degree C to avoid formation of thick and inhomogeneous SiO$_2$ layer by the thermal oxidation of the Si surface. The formation of ordered pores in the MAO film calcined at 350 \degree C were confirmed by SEM observation. We designated the Si disc with MAO film as Al07/Si (Table 2).

**Measurements**

The MAO film deposited on the cover glass slip was characterized by XRR, NR, and SEM measurements. The SEM measurements were performed with field-emission scanning electron microscopy (FE-SEM, Hitachi S-4800). The XRR profiles were measured in air on a PANalytical X’pert Pro reflectometer at ANSTO. The XRR measurement was performed with an accelerating voltage of 45 kV, current of 40 mA and
with Cu Kα radiation (1.54 Å). NR measurements were performed at the PLATYPUS time-of-flight reflectometer at the 20 MW OPAL reactor of the ANSTO nuclear facility\textsuperscript{23} with an incident cold neutron wavelength range from 2 to 20 Å. The NR measurements were performed at two different angles of incidence; 0.7° and 2.5° in air condition, and 0.9° and 4.0° in solution conditions. The scattering vectors for XRR and NR were calculated by \( Q = \left( \frac{4\pi}{\lambda} \right) \sin \theta \), where \( \lambda \) is the wavelength and \( \theta \) is the angle of incidence.

The XRR and NR experiments in air were performed by irradiating X-ray or neutron beam from the upper side of the MAO film. For in-situ NR experiment, the Si disc with MAO film was fitted in a solid-liquid cell (Figure 1).\textsuperscript{21} After introduction of a solution into the cell, the NR measurement was performed at room temperature (ca. 25°C). The neutron beam impinged onto the MAO film by penetrating through the silicon disc. By changing the solution in the cell, NR profiles in D\textsubscript{2}O, 90% D\textsubscript{2}O (D\textsubscript{2}O/H\textsubscript{2}O 90:10, v/v), and H\textsubscript{2}O were recorded. Then, we introduced 10 wt% GOD solution (90% D\textsubscript{2}O) and incubated for 1 h. After incubation, NR measurement was performed in the GOD solution. We rinsed the sample surface by successive flow of D\textsubscript{2}O into the measurement cell. Finally, NR profiles after the rinsing were recorded in D\textsubscript{2}O and 90% D\textsubscript{2}O. Data reduction and modelling of the NR data were conducted using the IgorPro based macro MOTOFIT.\textsuperscript{24} The \( Q \) resolutions for the analysis of XRR and NR data were fixed at 1 and 8 %, respectively. SLD values used for the analysis are summarized in Table 1.

**Results and Discussion**

*XRR and NR experiments for MAO film on glass substrate*

Figure 2 (A) to (C) shows typical SEM top views of the MAO films on cover glass slips. The ordered pore structures are recognized for Al05 and Al07, while Al03 shows less ordered pore structure. The XRR and NR data recorded in air with glass substrates
are shown in Figure 3. Al03 and Al05 exhibited clear Kiessig fringes from the MAO film, while the Kiessig fringes were not seen for Al07. According to XRR study on a mesoporous silica film prepared by a surfactant-templated method, all reflectivity data were analyzed by three-layer (top, main, and bottom layers) model as schematically shown in inset of Figure 3. As listed in Table 2, large SLD contrasts between the MAO film and glass substrate enable the observation of Kiessig fringes, due to the total film thickness, for Al03 and Al05. On the other hand, the lower SLD contrasts for Al07 results in disappearance of Kiessig fringes. Herein, porosity, \( P_{\text{por}} \), of each MAO layer is defined as

\[
P_{\text{por},i} = \frac{\rho_i}{\rho_{\text{AO}}}
\]

where \( \rho_i \) is SLD of \( i \)-th MAO layer, and \( \rho_{\text{AO}} \) is SLD of pure aluminum oxide (Table 1). The average porosity, \( \langle P_{\text{por}} \rangle \), is defined by

\[
\langle P_{\text{por}} \rangle = \frac{\sum_i t_i \times P_{\text{por},i}}{t_{\text{tot}}}
\]

where \( t_i \) is thickness of \( i \)-th MAO layer, and \( t_{\text{tot}} \) is total thickness of MAO film. The calculated \( \langle P_{\text{por}} \rangle \) tends to be larger with increasing the concentration of aluminum nitrate in the precursor solution (Table 2). This tendency indicates that the density of aluminum oxide framework in MAO increases with increasing the aluminum nitrate in the precursor solution. The aluminum oxide framework in Al03 and Al05 would possess a large number of micropores, and the number of micropores would be relatively lower for the framework in Al07. This difference in the aluminum oxide framework was also supported by the fact that better structural stability of the MAO film in Al07. When SEM observation was performed after immersion of Al05 and Al07 in an aqueous solution for a few days, Al07 exhibited longer time stability of the pore structure.

The \( t_{\text{tot}} \) and \( \langle P_{\text{por}} \rangle \) values derived from XRR and NR are almost same for Al05 and
Al07 (Table 2). On the other hand, those for Al03 are somewhat different. We consider that this difference is due to structural ununiformity of MAO film for Al03 as shown in SEM image (Figure 2 (A)).

Our previous study reported that MAO film in Al05 has perpendicularly oriented cylindrical mesopore channels. This pore structure was confirmed by the absence of Bragg peaks due to out-of-plane mesoscale periodicity in NR and GI-SAXS data. Similarly, the present Al05 and Al07 exhibited no Bragg peaks in NR and XRR profiles (Figure 3). It hence can be considered that the pore structure of the MAO film in Al07 is same with that in Al05. This pore structure for Al07 is supported by the SEM cross-sectional view (Figure 2 (D)). The pore channels appear to be oriented perpendicular to the substrate surface.

In-situ NR for MAO film on Si disc.

The characterization of MAO film on the glass substrate confirmed that the stable MAO film could be formed by using a precursor solution containing 0.7 M aluminum nitrate. This precursor solution was hence used for deposition of MAO film on a Si disc. The resulting sample (Al07/Si) shows clear Kiessig fringes in XRR profile obtained in air (Figure 4 (A)). The XRR for Al07/Si shows no Bragg peaks due to out-of-plane mesoscale periodicity. The appearance of Kiessig fringes is due to the SLD contrasts between MAO film and Si disc (Table 1 and 2). Since thermal oxidation of the silicon surface was expected during the calcination of Al07/Si, we added SiO2 layer on the silicon disc for the analysis of XRR data as schematically shown in inset of Figure 4.

After the XRR measurement in air, Al07/Si was applied for the in-situ NR experiment. Figure 4 (B) shows NR profiles for Al07/Si in D2O, 90% D2O, and H2O. Kiessig fringes shown in D2O and H2O disappeared for 90% D2O. In addition, the NR
profile for Al07/Si in 90% D$_2$O was essentially same with that for Si disc in 90% D$_2$O. These results indicate that the contrast of the aluminum oxide matrix in the MAO film is matched to that of 90% D$_2$O (Table 1). The SLD of the aluminum oxide matrix in the MAO film was therefore fixed to be $5.67 \times 10^{-6}$ Å$^{-2}$ for the analysis of the in-situ NR profiles.

The three NR data sets were simultaneously refined with thickness, volume fraction of solvent in each MAO layers, and surface roughness parameters linked in a single model. In the co-refinement, structural parameters of the SiO$_2$ layer were fixed to those derived from XRR. The structural parameters obtained by the co-refinement are listed in Table 3. Herein, the volume fraction of solvent in the MAO layer is regarded to be equal to porosity. The thicknesses of each MAO layers were almost same with those derived from XRR, but porosity values were different between NR and XRR. Plausible reason for this discrepancy is contribution of water molecules to the XRR profile. Since the XRR measurement was performed in air, it can be considered that water molecules adsorb within the hydrophilic pore surfaces of MAO film. The difference of X-ray SLD values between H$_2$O and Al$_2$O$_3$ is not so large; The X-ray SLD values are $9.5 \times 10^{-6}$ Å$^{-2}$ for H$_2$O and $33.3 \times 10^{-6}$ Å$^{-2}$ for Al$_2$O$_3$, respectively. The water molecules within the pores would increase the SLD of each MAO layers.

After injection of 10 wt% GOD solution (90% D$_2$O) into the measurement cell, the sample cell was stored for 60 min. Then, the NR measurement was performed in the GOD solution. As shown in Figure 5, slight difference was recognized at $Q$ around 0.6 Å$^{-1}$ for NR profiles obtained in 90% D$_2$O and in the GOD solution. When we fitted the NR profile in the GOD solution by assuming GOD adsorption at only external surface of MAO film, the overall reflectivity profile could be described. However, Kiesssig fringes at $Q$ around 0.2 Å$^{-1}$ appeared in the refined profile were not seen in the experimental profile. On the
other hand, when the $Q$ resolution was considered as variable parameter, good agreement between the experimental and refined profiles was obtained with 14% of $Q$ resolution, which was larger than that derived from instrumental setup (8 %). These results suggest large inhomogeneities of the GOD layer. Since the NR profile was observed in concentrated GOD solution (10 wt % GOD solution), stacking or aggregation of GOD molecules at the MAO film surface could be expected. The molecular stacking or aggregation results in large vertical inhomogeneity of the thickness of the GOD layer (surface roughness). In addition, large-scale lateral inhomogeneity of the GOD layer is also expected, because domain structure of protein molecules at an interface is sometimes more than micrometers. These inhomogeneities would disturb the resolution of NR, resulting in disappearance of the Kiessig fringes.

The GOD solution in the measurement cell was replaced by flow of D$_2$O solution, and NR measurement was performed in the D$_2$O and 90% D$_2$O solutions (Figure 6). In the NR profiles after removal of the GOD solution clear Kiessig fringes were recognized (Figure 6). The appearance of the Kiessig fringes indicates that the homogeneity of the GOD layer becomes improved during the removal of the GOD solution, that is, excess amount of GOD adsorbed was removed. Co-refinement of both NR data was hence performed by fixing the $Q$-resolution to be 8%. The multilayer model composed of one GOD layer on the MAO film could be used to describe the experimental data (Figure 6). It can therefore be concluded that majority of the GOD molecules adsorb at the external surface of the MAO film, and penetration of GOD molecules hardly occur in the concentrated (10 wt%) GOD solution.

SLD profiles obtained by the co-refinement of NR data are shown in Figure 7, together with that obtained for the GOD solution with 14% of $Q$ resolution. Figure 8 shows the schematic illustrations of the distribution of GOD molecules at the MAO films.
Since the SLD of GOD is smaller than those for other matrices (Table 1), the GOD layer is recognized as dip at the top surface of the MAO film. The dip obtained after the rinsing process is shallower than that in the GOD solution, indicating removal of the excess amount of GOD adsorbed. On the assumption that SLD of GOD was $3.5 \times 10^{-6}$ Å$^{-2}$, we calculated the amount of GOD, $A_{\text{GOD}}$, at the MAO surface by using Eq. (3).

$$A_{\text{GOD}} = \frac{M_w(1 - f_{\text{sol}})t_{\text{GOD}}}{V_{\text{GOD}}N_A} \quad (3)$$

Where $M_w$, $V_{\text{GOD}}$, and $N_A$ are molecular weight of GOD (160 kDa), volume of single GOD molecule (145000 Å$^3$), and Avogadro’s number, respectively. $t_{\text{GOD}}$ and $f_{\text{sol}}$ are thickness of GOD layer and volume fraction of solvent in the GOD layer, respectively (Table 3). The amount of GOD at the MAO surface was estimated to be $8.0 \times 10^{-13}$ mol cm$^{-2}$. By assuming that the area occupied by a single GOD is square of the GOD diameter (65 Å), the surface coverage of GOD was calculated to be 0.2 from the amount of GOD at the surface of MAO film. This coverage value suggests that the GOD molecules located at the MAO film surface without significant aggregation as schematically shown in Figure 8.

In unbuffered aqueous solution, the surface of aluminum oxide framework is positively-charged. It hence can be considered that the negatively-charged GOD (pI = 4.2) adsorb at the external surface of MAO film by strong electrostatic interaction. This strong interaction would prevent the penetration of GOD (60 × 50 × 77 Å$^3$) molecules into the aluminum oxide pore channel (ca. 10 nm). We previously reported that negatively-charged BSA (140 × 38 × 38 Å$^3$) could penetrate into aluminum oxide pore channel with large pore diameter (33 nm). It hence can be considered that no penetration of GOD for present MAO film is due to the almost matching of size and curvature between GOD and pore channel. Multiple electrostatic interaction would limit the
diffusion of GOD along the narrow pore channel.

In inorganic nanoporous films, penetration of protein molecules into pore channels with 2.6 to 50 nm in diameters has been studied by utilizing optical interferometry\textsuperscript{7-9} and waveguide mode\textsuperscript{15-17}. These studies reported that the charged protein molecules could penetrate into silica and aluminum oxide pores with opposite surface charges when the pore diameter was larger than the protein dimensions.\textsuperscript{7,8,15,17} No significant penetration of cytochrome c (12 kDa, 3.2 nm in diameter) was reported for mesoporous silica film with 2.6 nm in pore diameter.\textsuperscript{9} These results imply that the relationship between pore size and protein size is predominant factor for the penetration of a protein into a small pore. Similar relationship was also predicted for microscopic study of a single inorganic nanoporous particle.\textsuperscript{30} The present result derived from in-situ NR investigations is the first example that a charged protein cannot penetrate into relatively a large aluminum oxide pore channel with opposite charge. This result suggests that the electrostatic interaction is also critical factor for the penetration of protein molecules into narrow inorganic pores.

**Conclusions** (optional)

In the present study, distribution of GOD molecules adsorbed at MAO film was examined by in-situ NR experiment. The results provided information on amount and the vertical distribution of GOD molecules adsorbed by the MAO film; there was no evidence of penetration within the nanoporous film with the GOD molecules adsorbed exclusively at the external surface of MAO film. We consider that multiple electrostatic interaction in the narrow pore channel limits the diffusion of GOD along the narrow pore channel.

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Table 1  SLD values used for the analysis of XRR and NR data.

|                  | neutron / $10^{-6}$ Å$^{-2}$ | X-ray / $10^{-6}$ Å$^{-2}$ |
|------------------|-------------------------------|-----------------------------|
| D$_2$O           | 6.34                          | -                           |
| H$_2$O           | -0.56                         | -                           |
| Si               | 2.07                          | 20.7                        |
| SiO$_2$          | 3.47                          | 18.9                        |
| Al$_2$O$_3$      | 5.67                          | 33.3                        |
| glass$^a$        | 3.6                           | 21                          |
| GOD              | 3.5$^b$                       |                             |

a. cover glass slip.

b. Ref [27].
Table 2  Structural parameters obtained from fitting the NR and XRR profiles for MAO films on glass substrates.

|      | Al03 |       | Al05 |       | Al07 |       |
|------|------|-------|------|-------|------|-------|
|      | NR   | XRR   | NR   | XRR   | NR   | XRR   |
| top  |      |       |      |       |      |       |
| $t^a$| 63±3 | 76±1  | 30±8 | 33±2  | 39±1 | 30±1  |
| SLD$^b$| 0.32±0.03 | 2.3±0.1 | 1.8±0.4 | 8.3±0.3 | 2.6±0.1 | 17±1  |
| $\sigma^c$| 11±6  | 17±1  | 55±2 | 24±1  | 10±1 | 6.5±0.1 |
| $t$  | 594±2 | 646±2 | 854±3 | 827±1 | 877±3 | 893±6 |
| main |      |       |      |       |      |       |
| SLD  | 0.95±0.02 | 2.8±0.1 | 1.2±0.1 | 5.8±0.1 | 3.3±0.1 | 21±1  |
| $\sigma$ | 26±2 | 23±1  | 42±4 | 18±1  | 15±1 | 13±1  |
| $t$  | 20±3 | 15±10 | 58±8 | 61±1  | 25±40 | 23±20 |
| botto m|      |       |      |       |      |       |
| SLD  | 2.5±0.2 | 19±5  | 3.8±0.3 | 22±1  | 3.6±0.2 | 21±1  |
| $\sigma$ | 7.7±2.8 | 9.9±0.7 | 16±5 | 9.8±0.1 | 10±16 | 14±1  |
| $t_{tot}$ | 677±5 | 737±10 | 942±12 | 920±2 | 940±40 | 946±21 |
| Total |      |       |      |       |      |       |
| $<P_{por}>^d$ | 0.83 | 0.91  | 0.76 | 0.79  | 0.41 | 0.38  |

a. thickness (Å).

b. scattering length density (10$^{-6}$ Å$^{-2}$)

c. surface roughness (Å).

d. average porosity calculated by Eq. (2).
Table 3  Structural parameters for Al07/Si obtained from fitting the NR and XRR profiles.

|          | XRR     | NR     |
|----------|---------|--------|
| top      | $t^a$   | 60±1   | 60±3   |
|          | $P_{\text{por}}^b$ | 0.44±0.01 | 0.56±0.01 |
|          | $\sigma$ | 6.5±0.1   | 2.0±0.1 |
|          | $t$     | 978±1   | 989±5  |
| main     | $P_{\text{por}}$ | 0.47±0.02 | 0.63±0.01 |
|          | $\sigma$ | 6.2±0.1   | 9.5±4.3 |
|          | $t$     | 27±1    | 26±7   |
| bottom   | $P_{\text{por}}$ | 0.31±0.01 | 0.55±0.02 |
|          | $\sigma$ | 25±1     | 10±6   |
|          | $t$     | 15±1    | 15     |
| SiO$_2$  | $\sigma$ | 12±1     | 1.3±0.6 |
|          | $t_{\text{tot}}$ | 1065±3  | 1075±9 |
| Total    | $<P_{\text{por}}^d$ | 0.46     | 0.62   |

a. thickness (Å).

b. porosity for XRR was calculated by Eq. (2), and porosity for NR was equal to volume fraction of solvent at each MAO layer.

c. surface roughness (Å).

d. average porosity calculated by sum of $(t_i \times P_{\text{por}})/t_{\text{tot}}$. 
Table 4  Structural parameters for GOD layer onto MAO film

|                | GOD\(^a\) | rinse\(^b\) |
|----------------|-----------|-------------|
| \(t\)^c        | 38±2      | 10±5        |
| \(f_{\text{sol}}\)^d | 0.34±0.05 | 0.31±1.0    |
| \(\sigma\)^e   | 8.2±3     | 15±4        |
| \(\sigma\)^f   | 5.2±4.4   | 19±7        |

a. in 10 wt% GOD solution  
b. after rinse by the solution flow  
c. thickness (Å).  
d. solvent fraction at GOD layer.  
e. roughness at GOD/solvent interface (Å).  
r. roughness at GOD/MAO interface (Å).
Fig. 1  Schematic illustration of solid-liquid cell used for in-situ NR measurement.

Fig. 2  SEM top views of (A) Al03, (B) Al05, and (C) Al07. (D) cross-sectional SEM view of Al07.
Fig. 3  (A) XRR and (B) NR profiles for Al03, Al05, and Al07. All measurements were performed in air. The solid lines show the fit of the reflectivity data. Inset of (A) shows a model used for the analysis of reflectivity data. The arrow in inset represents incidences of neutron and X-ray beam.
Fig. 4  (A) XRR profile for Al07/Si in air, and (B) NR profiles for Al07/Si in D₂O, 90 % D₂O, and H₂O. The solid lines show the fit of the reflectivity data. Inset shows a model used for the analysis of XRR and NR data. The arrow in inset represents incidences of neutron and X-ray beam.
Fig. 5  Comparison of NR profiles obtained in 90 % D$_2$O solution (green squares) and in 90 % D$_2$O solution containing 10 wt% GOD (black circles). The green and black solid lines show the fit of the NR data. Inset shows expanded NR profile in 90 % D$_2$O solution containing GOD. Blue solid line in inset shows the fit of the NR data by fixing $Q$ resolution derived from instrumental setup (8 %). Red solid line in inset shows the fit by treating $Q$ resolution as variable parameter (14 %).
Fig. 6  NR profiles for Al07/Si after rinsing with D$_2$O. Measurements were performed in D$_2$O and 90 % D$_2$O. The solid lines show the fit of the reflectivity data.
Fig. 7  SLD profiles of the models used for fitting the respective NR data for (top) before GOD adsorption, (middle) for GOD solution, and (bottom) after rinse. The black and red solid lines are respectively for D$_2$O and 90% D$_2$O.
Fig. 8  Schematic illustrations of the distribution of GOD molecules at the MAO film in 10 wt% GOD solution and in water after removal of excess amount of GOD adsorbed.
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