Langmuir-Blodgett films of unidirectionally aligned α-helical diblock copolypeptides
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Chapter 3

Double-brush Langmuir-Blodgett monolayers of α-helical diblock copolypeptides

Abstract

The monolayer behavior of amphiphilic diblock copolypeptides consisting of poly(α-L-glutamic acid) (PLGA) and poly(γ-methyl-L-glutamate-ran-γ-stearyl-L-glutamate) with 30 mol% of stearyl substituents (PMLGSLG) at the air-water interface has been investigated. The study of their Langmuir and Langmuir-Blodgett monolayers demonstrated the formation of a stable α-helical double-brush structure, with the helices tilted away from the substrate surface. These double-brush monolayers combine the unique properties arising from the unidirectionally aligned helix macrodipole and the liquid-like features of the side chain mantle of the PMLGSLG block. Such systems are promising for thin film applications requiring incorporation and orientation of bio- and optical molecules.
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3.1 Introduction

In the past, Langmuir-Blodgett layers of polymers mostly involved the situation where the water surface forced the polymeric chains into a two-dimensional (2-D) random coil conformation, either condensed or expanded, depending on the balance of the interactions between neighboring polymeric segments and water-polymer interactions.\(^1\)\(^2\) In some cases, these monolayers can be transferred to substrates, but the 2-D conformations of the polymer molecules are intrinsically unstable in the three-dimensional (3-D) situation on a substrate. However, if the glass transition temperature of the polymer is high enough, this unfavorable situation might persist for longer times. Polymers with a rigid-rod conformation are particularly interesting in this respect as they have no other choice but to orient themselves parallel to the water surface, at least in cases at the water surface, where the adhesive forces exceed the cohesive forces.\(^3\)\(^4\) Representatives of this class of molecules are polypeptides and synthetic polypeptides in the \(\alpha\)-helical conformation in particular. Many studies have been published revealing a range of interesting properties derived from the specific behavior in the Langmuir-Blodgett process and derived from their specific chemical structure.\(^5\)\(^6\) Special effects in the multilayers have been introduced by flow-induced orientation during the transfer process.\(^9\)\(^10\)

These polypeptides have also been used in surface grafting of polymer brushes.\(^11\)\(^14\) Many studies on the properties of these ultra-thin layers have been published revealing the unique properties of these systems.\(^15\)\(^16\) However, the disadvantage of the surface grafting process is the difficulty in analyzing the grafted polymers.

One possible way to prepare functional ultra-thin polymer films with controlled directional properties is by combining the Langmuir-Blodgett (LB) technique with the unique properties of polymer brushes.

Several polypeptide-based amphiphiles have been explored to achieve a perpendicular and unidirectional orientation of \(\alpha\)-helices in polypeptide Langmuir monolayers\(^17\)\(^24\) as reviewed in Chapter 1 and not further discussed here.

The purpose of the present study is to fabricate monolayers with unidirectionally aligned \(\alpha\)-helices of poly(\(\gamma\)-methyl-L-glutamate-ran-\(\gamma\)-stearyl-L-glutamate) with 30 mol\% of stearyl substituents (PMLGSLG). The hydrophobic PMLGSLG with its hairy-rod structure provides stable LB films with a highly ordered array of \(\alpha\)-helices aligned parallel to the substrate, with the flexible stearyl side chains acting as an oily mantle for the rigid rods.\(^9\) Hairy-rod polyglutamates show lyotropic and thermotropic behavior\(^25\)\(^26\) and have received considerable attention in the past years.\(^10\)\(^27\)\(^40\) Besides the rigid-rod feature, the liquid-like properties of the surrounding amorphous side chains make PMLGSLG attractive as
Double-brush Langmuir-Blodgett monolayers for embedding and orienting dyes, transporting charge carriers in photoconductive devices as well as optical recording and storage materials. Therefore, monolayers with unidirectional helix orientation of PMLGSLG are potential as functional polar ordered thin films for incorporating and orienting optical molecules, and for stabilizing biomolecules. To induce the helix orientation of the PMLGSLG rods at the interface we studied diblock copolymers of PMLGSLG and poly(γ-benzyl-L-glutamate) (PLGA), since PLGA as the hydrophilic block has been demonstrated for the poly(γ-benzyl-L-glutamate)-poly(L-glutamic acid) diblock copolymer by Higashi et al. to be well-oriented in the water sub-phase with a stable secondary structure. This chapter reports on the monolayer behavior and double-brush formation of the PLGA-b-PMLGSLG α-helical amphiphilic diblock copolymers in Langmuir and LB monolayers.

3.2 Experimental section

Materials

The synthesis and molecular weight characterization of the α-helical PLGA-b-PMLGSLG diblock copolymers are described in Chapter 2. PLGA-b-PMLGSLGs are abbreviated as (LGA)_m-b-(MLGSLG)_n, where m and n denote the degrees of polymerization (DPs) of the PLGA and PMLGSLG blocks, respectively.

PLGA (DP = 63) was obtained by hydrolysis of PtBuLG using trifluoroacetic acid. PMLGSLG (DP = 118) was prepared by random copolymerization of MLG-NCA and SLG-NCA (70:30 mole ratio) in chloroform at 0 °C using triethylamine as initiator. A detailed description of the synthesis of these polymers can be found in Chapter 2.

HCl (2N) and NaOH (1N) standard aqueous solutions were purchased from Aldrich. H_2O_2 (30%), NH_3 (25%) and HCl (37%) were purchased from Merck. N-methylpyrrolidone (NMP) (99.5%), acetic acid (99.5%) and hexamethyldisilazane (98%) were purchased from Acros. Methanol (99.8%) and chloroform (99.5%) were purchased from Lab-Scan.

Surface pressure-area (π-A) isotherms

π-A isotherms were measured using a home-modified computer-controlled Lauda Filmbalance (FW2), with an accuracy of 0.05 mN/m. The water used for the sub-phase was purified by reverse osmosis and subsequently through a Milli-Q filtration system. The pH of the sub-phase was adjusted by adding HCl (2N) or NaOH (1N) standard aqueous solutions. PLGA-b-PMLGSLGs were spread from NMP/chloroform (3/7, v/v) solutions with 1-3% of acetic acid added, at concentrations of 0.4-0.6 mg/mL. PLGA was spread from a NMP/chloroform (3/7,
v/v) solution at a concentration of 0.5 mg/mL. PMLGSLG was spread from a
chloroform solution at a concentration of 0.5 mg/mL.

The Langmuir trough used was 600 mm long and 200 mm wide. Polymer
solutions were spread dropwise on the water surface using a microsyringe. The
monolayer was allowed for 15 to 30 min to equilibrate, during which the spreading
solvent could either evaporate (chloroform) or mix with the sub-phase (NMP,
acetic acid). A movable Teflon barrier was used to decrease the available surface
area. During compression the surface pressure was continuously recorded by a
floating barrier connected to a highly sensitive film balance.

**Substrate preparation**

The double-sided polished silicon wafers (Topsil Semiconductor Materials A/S,
Frederikssund, Denmark, 1000 ± 25 μm thick) used as substrates were cleaned by
treating them with a mixture of H2O2 (30%)/NH3 (25%)/H2O (1:1:5, v/v/v) for 30
min at 60 °C, followed by extensive rinsing with Milli-Q water, ultrasonication in a
mixture of HCl (37%)/H2O (1:6, v/v) for 25 min, rinsing with Milli-Q water, and
finally ultrasonication in methanol, methanol/chloroform (1:1, v/v) and chloroform
for 15 min. The cleaned silicon wafers were hydrophobized by treating them with a
mixture of hexamethyldisilazane/chloroform (1:4, v/v) at 50 °C, and rinsed with
chloroform before use.

**Langmuir-Blodgett (LB) films**

Deposition of LB films was carried out in the vertical mode. The sub-phase
temperature was maintained at 20 ± 0.1 °C. Transfer of a monolayer of (LGA)₆₃-b-
(MLGSLG)₃₉ onto a hydrophilic substrate was done at 40 mN/m and at down- and
up-stroke speeds of 100 and 1 mm min⁻¹, respectively. Transfer of a monolayer of
(LGA)₅₀-b-(MLGSLG)₈₂ onto a hydrophilic substrate was done at 35 mN/m and at
down- and up-stroke speeds of 100 and 10 mm min⁻¹, respectively. Monolayers
were deposited onto both sides of the hydrophilic substrates during the upward
stroke. Transfer ratios were around unity (± 10%). Additional layers were
transferred on both down- and up-stroke at the transfer speed of the first upstroke
(Y-type). A multilayer film (4 layers on each side of the substrate) of PMLGSLG
was deposited onto a hydrophobic silicon substrate by Y-type transfer, at 20
mN/m and at down- and up-stroke speeds of 3 mm min⁻¹.

**Brewster angle microscopy (BAM)**

A small Teflon trough (24.0 x 2.5 cm) was equipped with a quartz window
flush in the middle bottom of the trough. Two hydrophobic barriers were moved
symmetrically to change the surface area of the monolayer. BAM was performed
using p-polarized light from a 10 mW argon laser beam set to an angle of 53.2°
to the air-water interface. To magnify the reflected light from the monolayer a 50 mm
planoconvex lens was used. An analyzer was used to enhance the contrast and a 
CCD camera was used for image recording.

Circular dichroism (CD) and linear dichroism (LD) spectroscopy

CD spectra of LB films on quartz plates were recorded on a Jasco J-815 
spectropolarimeter. The quartz plate supporting the film was placed in the standard 

table holder of the instrument. Different orientations of the quartz plate were 
analyzed to check for contributions from linear dichroism. The operating 
conditions were set as follows: bandwidth, 2 nm; scanning speed, 50 nm/min; 
D.I.T., 1 sec; data pitch, 1 nm. Each spectrum was an accumulation of 5 scans. 

LD measurements of LB films on quartz plates were performed using a Jasco J- 
815 spectropolarimeter. The operating conditions were set as follows: bandwidth, 2 
nm; scanning speed, 200 nm/min; D.I.T., 1 sec; data pitch, 1 nm.

Infrared measurements

Transmission Fourier Transform Infrared (Transmission FT-IR) measurements 
of LB films on double-sided polished silicon substrates were performed at a 
resolution of 3 cm$^{-1}$, under vacuum on a Bruker IFS66 V/S FT-IR spectrometer 
equipped with a MIR DTGS detector. A sample shuttle accessory was used for 
interleaved sample and background scanning. A clean silicon substrate was used as 
the reference. Each spectrum is an average of 40 cycles of 120 scans.

Small angle X-ray reflectivity

Small angle X-ray reflectivity measurements of LB films on silicon substrates 
were performed in θ/2θ geometry on a Philips X’pert materials research 
diffactometer (MRD) instrument, employing copper K$_\alpha$ radiation of 1.541 Å and 
with a divergence slit of 1/8 degrees, an anti-scatter slit of 1/4 degrees and a 
progressive receiving slit of 0.3 nm. The X-ray tube was operated at V = 40 kV and 
I=40 mA. For analysis, the measured reflectivity, R, was normalized by the Fresnel 
reflectivity, R$_F$. To model the electron density distribution along the z-direction, the 
film was divided into slabs (boxes) with thicknesses $d_i$, electron densities $\rho_i$ and 
interface roughnesses $\sigma_i$ between slabs $i$ and $i+1$, using a home-made computer 
program written in IDL 6.0 by Hibma. A separate SiO$_2$ layer was not taken into 
account in the simulation, as this layer contributes insignificantly to the reflectivity 
of the wafer ($\rho_{SiO2}/\rho_{Si} = 0.95$) and could not be resolved from the reflectivity curves 
of bare silicon wafers.
3.3 Results and discussion

3.3.1 Surface pressure-area (π-A) isotherm

Figure 3.1 shows the π-A isotherms for (LGA)_{63}-b-(MLGSLG)_{39} and (LGA)_{39}-b-(MLGSLG)_{82} spread on a pure water surface at 20 °C, in comparison with those for PLGA (DP = 63) and PMLGSLG (DP = 118). The isotherm of PLGA, spread on pure water, was similar to that reported for sodium salt of PLGA (DP = 405) at a water sub-phase pH of 3.47 PLGA is surface active to some extent, exhibiting a transition at a low surface pressure of ca. 5-7 mN/m. The isotherm of PMLGSLG has been reported in literature,9 showing a steep rise in pressure upon compression due to the packing of the α-helices lying flat on the water surface, followed by a liquid-condensed phase. Upon further compression, the monolayer of PMLGSLG collapses, indicated by a plateau transition at about 33 mN/m. The isotherms of the PLGA-b-PMLGSLG diblock copolymers displayed a completely different behavior. Upon compression, first a transition analogous to the isotherm of PLGA spread under the same condition is detected, and then there is a linear increment in surface pressure. For (LGA)_{39}-b-(MLGSLG)_{82} with the longer hydrophobic block, another transition above 40 mN/m appears.

![Figure 3.1](image)

**Figure 3.1.** π-A isotherms of (LGA)_{63}-b-(MLGSLG)_{39} (solid line), (LGA)_{39}-b-(MLGSLG)_{82} (dashed line), (MLGSLG)_{118} (dotted line), and (LGA)_{63} (open circles) on a water surface at 20 °C. The compression speeds were ca. 38 cm² min⁻¹ for (MLGSLG)_{118} and ca. 19 cm² min⁻¹ for the others. The open squares and triangles indicate stabilization points of (LGA)_{63}-b-(MLGSLG)_{39} and (LGA)_{39}-b-(MLGSLG)_{82}, respectively.

The first transition at 5-10 mN/m can be assigned to the water immersion of the PLGA block. Hence, varying the sub-phase pH can influence the transition. Figure 3.2 demonstrates the effect of sub-phase pH on this transition. At high pH, upon spreading the helix-to-coil conformational change of the PLGA block results in
surface area constraints caused by coil expansion, indicated by a low surface pressure recorded even at a very large area. Upon compression, a large decrease in surface area with a very small increase in surface pressure implies a facile watersolubilization of the PLGA block at high pH. At pH 3.2, the isotherm is identical to that measured on pure water. At low pH, up to 2.0, the first transition is shifted to a slightly higher pressure due to the diminished solubility of the PLGA block.

**Figure 3.2.** $\pi$-A isotherms of (LGA)$_{63}$-b-(MLGSLG)$_{39}$ on a water surface at 20°C at various pH.

**Figure 3.3.** $\pi$-A isotherms of (LGA)$_{59}$-b-(MLGSLG)$_{82}$ on a water surface at different temperatures.
The second transition above 40 mN/m, only observed for (LGA)_{59}-b-(MLGSLG)_{82}, was affected by changing temperature. As shown in Figure 3.3, increasing the temperature from 5 to 35 °C shifts the transition to lower surface pressures and the isotherm beyond that to smaller surface areas. This suggests that the transition reflects reorganization, favored at elevated temperatures, of the PMLGSLG segments (see Chapter 5).

The high collapse surface pressures, at 50-55 mN/m, and high compressibilities of the PLGA-b-PMLGSLG monolayers compared with PMLGSLG suggest that the diblock copolymer molecules are oriented out of the water surface to form a double-brush. The molecular surface areas of PLGA-b-PMLGSLGs at high surface pressures, of 35-45 mN/m, where the monolayers are still stable and transferable, lies in the range of the helix cross-sectional area of PMLGSLG, of 2.4-9.6 nm^2, depending on the molecular packing and the side chain configuration. This suggests that upon high compression, the helices are oriented with a tilt away from the water surface.

3.3.2 Stabilization

Figure 3.4 displays the stabilization curves of (LGA)_{63}-b-(MLGSLG)_{39} and (LGA)_{59}-b-(MLGSLG)_{82} performed at different surface pressures. Stable, transferable monolayers were obtained with transfer ratios of around unity over a range of surface pressures. Indicated by stabilization points in Figure 3.1, the molecular areas at which the monolayers stabilize are in good agreement with the π-A isotherms, indicating the absence of relaxation effects. Only above the second transition in the isotherm recorded for (LGA)_{63}-b-(MLGSLG)_{82}, e.g. at 45 mN/m, does the stabilization point shift to a smaller area. This implies that this transition, which might be related to rearrangement of the chains, is a rather slow process. Though the monolayer is stabilized at 45 mN/m, film deposition resulted in an incomplete transfer, probably because at this state the monolayer does not maintain its molecular mobility.
3.3.3 Surface imaging by BAM

BAM images taken during the compression-decompression process on a pure water surface and at different sub-phase pH are shown for PLGA-b-PMLGSLGs in Figure 3.5-7. On spreading on a pure water surface, there exists monolayer islands with gaps of water surface in between. Compression reduces the water gaps and as soon as the surface pressure is built up, a homogeneous monolayer is formed. For both PLGA-b-PMLGSLGs, no monolayer collapse was detected upon compression to 45-50 mN/m. Upon decompression the monolayer first ruptures and then reverts back to the initial state. It has been reported for poly(γ-benzyl-L-glutamate) (PBLG) that the molecules self-aggregate without external pressure into 2D islands of single molecule thickness on the water surface.\textsuperscript{48} Compared with PBLG, on a pure water surface, (LGA)\textsubscript{63}-b-(MLGSLG)\textsubscript{39} forms much smaller aggregates (Figure 3.5). This is most likely due to the interaction between the long PLGA hydrophilic block and the water sub-phase, limiting mutual antiparallel attractions between molecules. In agreement with this explanation, when increasing the surface activity of the diblock copolymers by either decreasing the sub-phase pH or increasing DP\textsubscript{PMLGSLG}, more monolayer self-aggregate formation is found to occur, as shown in Figure 3.6a, b and Figure 3.7. On the contrary, at a high pH of 8.6, where the PLGA block is fully charged, no aggregate was observed (Figure 3.6c, d). The water-solubilization of the PLGA block at this pH leads to molecular reorientation in a manner favoring a double-brush formation, preventing antiparallel orientations of the helices.

*Figure 3.4. A: stabilization curves of (LGA)\textsubscript{63}-b-(MLGSLG)\textsubscript{39} at 6 (a), 9 (b), 20 (c), 40 (d) and 45 (e) mN/m; B: those of (LGA)\textsubscript{39}-b-(MLGSLG)\textsubscript{82} at 20 (a), 35 (b), and 45 (c) mN/m.*
Figure 3.5. BAM images (420 x 522 μm) of (LGA)₆₃-b-(MLGSLG)₃₉ during compression to 45 mN/m on a pure water surface at 20 °C, taken at A = 32 (a) and 17 (b) nm²/molecule. On subsequent decompression BAM images were taken at A = 17 (c) and 32 (d) nm²/molecule. The darkness in the images is the water; the regular inclined stripes are from the background.
Figure 3.6. BAM images (420 x 522 μm) of (LGA)_{63}-b-(MLGLG)_{39} during compression to 45 mN/m, at 20 °C and a sub-phase pH of 2, taken at A = 32 (a) and 25 (b) nm²/molecule. At a sub-phase pH of 8.6, BAM images of the same monolayer were taken at A = 30 (c) and 12 (d) nm²/molecule during compression. The darkness in the images is the water; the regular inclined stripes are from the background.
Figure 3.7. BAM images (420 x 522 μm) of (LGA)_{39-b-}(MLGSLG)_{82} on a pure water surface during compression to 50 mN/m, taken at A = 39 (a), 27 (b) and 23 (c) nm^2/molecule. On subsequent decompression BAM images were taken at A = 29 nm^2/molecule (d). The darkness in the images is the water; the regular inclined stripes are from the background.
3.3.4 CD spectra of LB films

LB films of the PLGA-b-PMLGSLG diblock copolymers were transferred onto both sides of hydrophilic substrates. Homogeneous and uniform films were obtained, as demonstrated by atomic force microscopy (see Figure 7.4a). Figure 3.8 shows typical CD spectra corrected for linear dichroism (LD) contributions, analyzed in different directions in the spectropolarimeter, of a multilayer LB film of PLGA-b-PMLGSLG. The LD effect was caused by preferential orientation of the α-helices along the dipping direction. The corrected spectra analyzed in different directions are in agreement with each other, showing one maximum at 191 nm and two minima at 208 and 222 nm. This indicates that in transferred films, PLGA-b-PMLGSLG has a right-handed α-helical structure.

![Figure 3.8](image)

3.3.5 Transmission FT-IR spectra of LB films

Figure 3.9 shows representative transmission FT-IR spectra of the LB monolayers on silicon substrates of PLGA-b-PMLGSLG prepared on pure water and at different sub-phase pH. At sub-phase pH 8.6, the C=O stretching band of the hydrogen bonded carboxyl group at 1711 cm\(^{-1}\) disappears and the ionized carboxyl band appears at 1560 cm\(^{-1}\), indicating the helix-to-coil conformational change of the PLGA block. The spectra of the monolayers prepared on pure water and at sub-phase pH 2 are identical to each other, showing the characteristic α-helix amide I and amide II bands at 1653 and 1548 cm\(^{-1}\). This indicates that even when PLGA-b-PMLGSLG was spread on a pure water surface, the α-helix structure was predominant. PLGA has been reported to form a completely α-helix structure in
aqueous solution of pH below 5. Its transition between the \( \alpha \)-helical and random coil conformations occurs in the region of pH 5 to 6, where the \( \alpha \)-helix percentage decreases to 80-70\%.\(^{50}\) The predominant \( \alpha \)-helix structure of the PLGA-\( b \)-PMLGSLG monolayer on a pure water surface is in part due to the surface water being rather acidic (pH about 5.5), arising from a large amount of atmospheric CO\(_2\) dissociating in the sub-phase. Besides, the degree of charging of the PLGA block depends on the local pH inside the PLGA brush, which can be slightly lower than the pH in the sub-phase at a high brush density.\(^{51}\) Studies of the helix-coil transition of PLGA in aqueous solutions containing osmolytes have shown that increasing the osmotic pressure favors more \( \alpha \)-helix structure over hydrated coils.\(^{52,53}\) So, the osmotic pressure inside the PLGA brush may enhance the stability of the \( \alpha \)-helix structure. As a result, for a PLGA brush, the helix-coil transition pH could be raised to a higher value than observed for PLGA in solution. In fact, a pH range for the helix-coil transition of a surface-grafted PLGA film has been reported to be 5.75-7.\(^{54}\)

![Figure 3.9. Transmission FT-IR spectra of the LB monolayers (on both sides of the silicon substrate) of (LGA)\(_{63}\)-b-(MLGSLG)\(_{39}\) deposited at 40 mN/m. Prior to LB deposition, the diblock copolymer was spread either on pure water or at different sub-phase pH.](image)

Figure 3.10 compares the transmission FT-IR spectra of the LB monolayer films of (LGA)\(_{63}\)-b-(MLGSLG)\(_{39}\) and (LGA)\(_{59}\)-b-(MLGSLG)\(_{82}\), transferred onto silicon substrates at 40 and 35 mN/m, respectively, with that of an LB multilayer film of PMLGSLG. From the absorption intensity ratio of the amide I and amide II bands the average helix tilt angle in the monolayers can be determined.\(^{13}\) A higher amide I/amide II band area ratio (AI/AII) corresponds to a larger tilt angle between the helices and the surface normal.\(^{13}\) Since the PMLGSLG helices in an LB film are oriented parallel to the substrate surface,\(^9\) the helix tilt angle is 90° and AI/AII has its maximum value for this system. As seen in Figure 3.10, for the PLGA-\( b \)-
PMLGSLG monolayers, the AI/AII ratios are much lower than that for the PMLGSLG LB film, suggesting average helix tilt angles much smaller than 90°. This is a clear indication of double-brush monolayer formation, in which the diblock copolymer helices are tilted away from the substrate surface.

![Graph](image_url)

**Figure 3.10.** Transmission FT-IR spectra of the LB films on double-sided polished silicon substrates of (MLGSLG)$_{118}$ (96% of $\alpha$-helix structure, 4 layers on each side of the substrate, transferred at 20 mN/m) (a), (LGA)$_{63}$-b-(MLGSLG)$_{39}$ (monolayer on each side of the substrate, transferred at 40 mN/m) (b), and (LGA)$_{59}$-b-(MLGSLG)$_{82}$ (monolayer on each side of the substrate, transferred at 35 mN/m) (c). For comparison, the spectral absorption intensities are normalized relative to the height of the amide II band.

### 3.3.6 Small angle X-ray reflectivity

Specular X-ray reflectivity can be used to characterize the layer structure of thin films along the substrate-normal direction ($z$-direction). For a sufficient electron density contrast between layers (slabs), the layer thicknesses, electron densities and interface roughnesses can be determined.

Curve fits to the X-ray diffractograms measured for the PLGA-$b$-PMLGSLG monolayer LB films were at first performed with one-slab models. In this case, it is assumed that the $\alpha$-helices lie flat on the substrate and hence the film consists of a layer of a constant electron density. The best one-slab fit to the reflectivity curve of the monolayer LB film of (LGA)$_{63}$-$b$-(MLGSLG)$_{39}$ is shown in Figure 3.11. However, the curve fit is insufficient at high scattering angles.
Figure 3.11. Left-hand side: best one-slab fit to the X-ray reflectivity curve of the monolayer LB film of (LGA)_{63}-b-(MLGSLG)_{39} transferred onto a silicon substrate at 40 mN/m; the dots represent the experimental data and the full line represents the fitted curve. Right-hand side: electron density profile corresponding to the curve fit (smooth curve); for clarity the same electron density profile is shown assuming all interface roughnesses to be equal to zero (step-like curve).

Figure 3.12. Left-hand side: two-slab fits to the X-ray reflectivity curves of the monolayer LB film of (LGA)_{63}-b-(MLGSLG)_{39} transferred onto a silicon substrate at 40 mN/m (a) and the monolayer LB film of (LGA)_{59}-b-(MLGSLG)_{82} transferred onto a silicon substrate at 35 mN/m (b); the dots represent the experimental data and the full lines represent the fitted curves; curve b is shifted vertically for clarity. Right-hand side: electron density profiles corresponding to the curve fits (smooth curves); for clarity the same electron density profiles are shown assuming all interface roughnesses to be equal to zero (step-like curves).
Instead, as shown in Figure 3.12, the two-slab models result in good fits of the reflectivity curves for the monolayer films of PLGA-b-PMLGSLGs. In this case, the film is assumed to consist of two layers of different electron densities due to different layer chemical structures and bulk densities. The first slab is assumed for the PLGA layer and the second slab for the PMLGSLG layer. Table 3.1 shows the two-slab fit parameters for the PLGA-b-PMLGSLG monolayer films. From the density values reported for PLGA and PMLGSLG, the electron densities of PLGA and PMLGSLG lie in the ranges of $0.473 \times 10^3 - 0.503 \times 10^3$ e/\text{nm}^3 and $0.360 \times 10^3 - 0.381 \times 10^3$ e/\text{nm}^3, respectively. Thus, the determined values in the two-slab fits shown in Table 3.1 are reasonable. The good fits using the two-slab models give ample evidence for the double-brush structure of the monolayer films.

### Table 3.1. Two-slab fit parameters for the curve fits shown in Figure 3.12

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<th>(LGA)_{37}b-</th>
<th>(LGA)_{38}b-</th>
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<td>(MLGSLG)_{32}</td>
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<tr>
<td>$\rho_2$ (PMLGSLG)</td>
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<td>0.368</td>
</tr>
<tr>
<td>Interface roughness (nm)</td>
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<td></td>
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<tr>
<td>$\sigma_{01}$ (silicon/PLGA)</td>
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<td>$0.48 \pm 0.04$</td>
</tr>
<tr>
<td>$\sigma_{12}$ (PLGA/PMLGSLG)</td>
<td>0.82</td>
<td>0.35</td>
</tr>
<tr>
<td>$\sigma_{23}$ (PMLGSLG/air)</td>
<td>0.29</td>
<td>0.18</td>
</tr>
</tbody>
</table>

$^a$ Values kept fixed for the curve fits.

$^b$ The average roughness of bare silicon wafers.

Taken together, the above results clearly demonstrate that the PLGA-b-PMLGSLG diblock copolymers form stable monolayers with the $\alpha$-helical chains tilted to the surface normal. Moreover, the resulting monolayers are well-transferred onto solid substrates with the double-brush structure retained. However, factors such as the chain lengths, which might influence the molecular orientation and the double-brush structure, as well as other effects such as the introduction of anisotropy during the transfer process still need to be investigated in order to fully understand and control the film properties.
3.4 Conclusions

The amphiphilic PLGA-b-PMLGSLG diblock copolymers formed a stable α-helical double-brush structure in monolayers at the air-water interface and in LB films, as evidenced by the π-A isotherm, transmission FT-IR and X-ray reflectivity results. The 3-5 nm thick monolayer films obtained with unidirectionally aligned helix macrodipoles can provide an extremely large electric field across the peptide monolayers. Such directional properties and the liquid-like matrix provided by the amorphous stearyl side chain make these films suitable for functionalization with various bio- and optical molecules with potential applications as functional materials.

3.5 References

Double-brush Langmuir-Blodgett monolayers

122, 2850.