Langmuir-Blodgett films of unidirectionally aligned α-helical diblock copolypeptides
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Chapter 7

Annealing-induced changes in double-brush Langmuir-Blodgett films of α-helical diblock copolypeptides

Abstract

The effect of annealing on the structure and the helix orientation in Langmuir-Blodgett (LB) monolayers of diblock copolymers (PLGA-b-PMLGSLGs) 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) with unidirectional helix orientation was characterized by means of small angle X-ray reflectivity, transmission Fourier transform infrared spectroscopy and atomic force microscopy. Upon annealing at 100 °C for 24 h, the α-helices became less tilted toward the substrate surface normal. Surface area shrinkage accompanied the change in tilt, indicated by an increase in both film thickness and electron density, resulting in more compact and uniform films. The enhancement of the helix orientation by thermal annealing was greater for the PMLGSLG block and for the diblock copolymers with the shorter block lengths. For these diblock copolymers, annealing resulted in post-orientation of the PMLGSLG block helices almost perpendicular to the substrate surface. This effect originates from a considerable increase in intermolecular packing of the PLGA block caused by hydrogen bonds between the carboxylic groups upon annealing, as well as the high mobility of the PMLGSLG block helices for rearrangement favored by the melted side chain mantle at elevated temperatures.
7.1 Introduction

Thin films of oriented α-helical polypeptides have attracted considerable attention due to their remarkable electro-optical properties generated by unidirectionally aligned helix macrodipoles.\textsuperscript{1,2} Studies of self-assembled polypeptide systems have shown potential applications in chemical biology, optoelectronics and biosensors.\textsuperscript{3-6}

Among synthetic polypeptides, hairy-rod polyglutamates have been widely studied because of their surface activity and liquid crystalline behavior.\textsuperscript{7-10} Their structures are useful for many optical and photoconductive applications.\textsuperscript{11-13} Therefore, highly ordered monolayers of hairy-rod polyglutamates with unidirectional helix orientation show promising properties for thin film applications requiring incorporation and orientation of bio- and optical molecules. The surface-grafting-from technique has been used to orient the chains of hairy-rod polyglutamates at solid surfaces.\textsuperscript{14,15} We have studied 4-5 nm thick double-brush monolayer films containing segments of poly(γ-methyl-L-glutamate-ran-γ-stearyl-L-glutamate) with 30 mol% of stearyl substituents (PMLGSLG), unidirectionally oriented by the Langmuir-Blodgett (LB) technique, using poly(α-L-glutamic acid)-b-poly(γ-methyl-L-glutamate-ran-γ-stearyl-L-glutamate) (PLGA-b-PMLGSLG) diblock copolymers (Chapter 5). The use of the LB technique in film fabrication overcomes the disadvantage of the difficulty in analyzing grafted polymers and has the advantage to readily incorporate and orient guest molecules into the films.

Notably, for the PLGA-b-PMLGSLG diblock copolymer system, we found that the helix length strongly affects the helix tilt order of the polypeptide brush (Chapter 5). This effect has been tentatively attributed to the dipolar interactions of the off-axis components of the unscreened peptide dipoles between parallel aligned α-helices. As a result, the average helix tilt angle between the helix axis and the surface normal increases with increasing the helix length in order to lower the interaction energy of the oriented dipoles in a smectic C-like phase (Chapter 5). For example, the average tilt angle has been reported to be 48-66° for surface-grafted PMLGSLG films.\textsuperscript{14,15} For LB films of the PLGA-b-PMLGSLG diblock copolymers, where the alkyl side chains surround the PMLGSLG block segments, the smallest average helix tilt angle obtained for the PMLGSLG block was 29°.

Several film treatment methods to induce permanent perpendicular helix orientation in surface-grafted polypeptide films have been reported, such as film swelling in n-hexadecane,\textsuperscript{14} by means of solvent-quenching\textsuperscript{16} or interhelical cross-linking.\textsuperscript{15} In order to further manipulate the helix orientation in the LB monolayers of PLGA-b-PMLGSLGs, we combined advantages of the PLGA block containing a side chain carboxylic acid group capable of forming hydrogen bonds and the mobility of the side chain mantle of the PMLGSLG block. By means of annealing
interchain hydrogen bonding interaction is enhanced between the carboxylic groups of the PLGA blocks and changes in the helix packing of one block is expected to alter the orientation of the other.

This paper reports on the effect of annealing on the structure and helix orientation of the LB monolayers of the PLGA-b-PMLGSLG diblock copolymers, studied by small angle X-ray reflectivity (XRR), transmission Fourier transform-infrared (FT-IR) spectroscopy and atomic force microscopy (AFM).

7.2 Experimental section

Materials

PLGA-b-PMLGSLG diblock copolymers are abbreviated as CoPo_m_n, where m and n denote the degrees of polymerization (DPs) of the PLGA and PMLGSLG blocks, respectively. A detailed description of the synthesis of PLGA-b-PMLGSLGs can be found in Chapter 2. In short, α-helical PLGA-b-PMLGSLG was synthesized via a diblock copolymer precursor consisting of poly(γ-tert-butyl-L-glutamate) (PtBuLG) and PMLGSLG (with 30 mol% of stearyl substituents), with the tert-butyl group as a mild acid-labile, protecting group for the carboxylic acid. PtBuLG-b-PMLGSLG was synthesized employing the primary amine-initiated stepwise ring-opening polymerization of α-amino acid N-carboxyanhydrides (NCAs) in chloroform at 0 °C. The molecular weights of the PtBuLG-b-PMLGSLGs were characterized by 1H-NMR (CDCl3) and gel permeation chromatography (tetrahydrofuran eluent, polystyrene standard, universal calibration). PMLGSLG with 30 mol% of stearyl substituents (DP = 118) was prepared by NCA random copolymerization in chloroform at 0 °C using triethylamine as initiator.

Film preparation and annealing

The substrate cleaning procedure was described in Chapter 3. The LB film transfer was performed 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. PLGA-b-PMLGSLGs were spread from N-methylpyrrolidone (NMP)/chloroform, (3/7, v/v) solutions with 1-3 % (v/v) of acetic acid added, at a concentration of 0.4-0.6 mg/mL. PMLGSLG was spread from a chloroform solution at a concentration of 0.6 mg/mL. Vertical deposition of a PLGA-b-PMLGSLG monolayer onto a chemically cleaned silicon substrate was done at a sub-phase temperature of 20 ± 0.1 °C, at down- and up-stroke speeds of 100 and 10 mm min⁻¹, respectively, for CoPo_59_82 and at down- and up-stroke speeds of 100 and 1 mm min⁻¹ for the other diblock copolymers. Monolayers of PLGA-b-
PMLGSLGs were deposited onto both sides of hydrophilic substrates during the upward stroke. Transfer ratios were around unity (± 10%). A multilayer film of PMLGSLG can be deposited onto a hydrophobic substrate by Y-type transfer, at down- and up-stroke speeds of 3 mm min\(^{-1}\).

The LB monolayers of three diblock copolymers with different block lengths, CoPo\(_{60\_19}\), CoPo\(_{37\_24}\) and CoPo\(_{59\_82}\), deposited on both sides of double-sided polished silicon substrates at transfer pressures of 40, 45 and 35 mN/m, respectively (Chapter 5), were used for investigation of the annealing effect. A multilayer LB film of PMLGSLG (DP = 118) was deposited at 20 mN/m on a hydrophobized double-sided polished silicon substrate (6 layers on each side of the substrate). In annealing experiments, the films were placed in an oven at 100 °C for a period of time, cooled down to room temperature and characterized by AFM, transmission FT-IR and small angle XRR measurements.

**Small angle X-ray reflectivity (XRR)**

Small angle XRR measurements of LB films on silicon substrates were performed in \(\theta/2\theta\) geometry on a Philips X’pert materials research diffractometer (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 for the reflectivity curves of bare silicon wafers. A two-slab model composing of consecutive layers of the PLGA and PMLGSLG blocks with three surface roughnesses were employed to simulate the XRR curves (Chapter 5).

**Atomic force microscopy (AFM)**

AFM measurements were carried out on a Digital Instruments MultiMode AFM equipped with a Nanoscope IIIa controller in tapping mode using silicon cantilevers (Veeco, model RTESPW). For each sample, AFM scans were performed on several surface positions to check the surface uniformity.

**Fourier transform infrared (FT-IR)**

Transmission FT-IR measurements of 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.

Attenuated total reflection (ATR) FT-IR measurements were carried out on a Bruker IFS88 FT-IR spectrometer equipped with a MCT-A detector, at a resolution of 4 cm$^{-1}$ and with an average of 50 scans.

**Differential scanning calorimetry (DSC)**

DSC measurements were performed on a DSC 2920 (TA instruments) at a heating rate of 10 °C/min under nitrogen atmosphere.

**Thermogravimetric analysis (TGA).**

TGA measurements were performed on a Perkin-Elmer thermogravimetric analyzer at a heating rate of 10 °C/min under nitrogen atmosphere.

### 7.3 Results and discussion

In our previous study (Chapter 5 and 6), the LB monolayers of the PLGA-$b$-PMLGSLG diblock copolymers after deposition have been determined to be uniform and exhibit the α-helical double-brush structure (Scheme 6.1) with an average helix tilt angle ($θ$, Scheme 7.1) and a preferential azimuthal helix orientation ($φ$, Scheme 7.1). The average helix tilt angle between the helix axis and the surface normal ($θ$) can be detected both by transmission FT-IR and from the brush layer thickness determined by XRR (Chapter 5). Estimation of the average helix tilt angle ($θ$) from the brush layer thickness ($L$) and the degree of polymerization (DP) follows the relation:

\[
\cos \theta = \frac{L}{0.15 \text{DP}}, \quad \theta < 90^\circ
\]  \hspace{1cm} (7.1)

θ values of 67-82° and 29-79°, estimated from the layer thicknesses, were obtained for the PLGA block and PMLGSLG block in the PLGA-$b$-PMLGSLG LB monolayers, respectively, depending on the block lengths (see Table 7.1). The PLGA-$b$-PMLGSLG diblock copolymers were found to be thermally stable up to 225 °C, with the α-helix structure preserved up to 150 °C (see Appendix 7.5).
The XRR curves of the PLGA-\textit{b}-PMLGSLG LB monolayers after annealing at 100 °C show an increase in both layer thicknesses and electron densities (Figure 7.1-3 and Table 7.1). The two-slab models simulating the PLGA-\textit{b}-PMLGSLG double-brush structure give good fits of all the X-ray diffractograms. AFM analysis of the films before and after annealing show a homogeneous and uniform structure (representative images shown in Figure 7.4). Together with the results from XRR, this suggests that upon annealing the films shrink from the edge to the center. As a result, the structure of the films becomes more packed. The average surface roughnesses determined by AFM and XRR measurements also show a slightly smoother surface after annealing. The increase in thickness and electron density associated with film shrinkage occurs gradually, indicated by a gradual change in the XRR pattern with annealing time up to 24 h (demonstrated for CoPo\textsubscript{60_19} in Figure 7.1 and Table 7.1). The increase in thickness observed for both the PLGA and PMLGSLG slabs of the double-brush monolayers suggests a decrease in the average helix tilt angles. The estimated $\theta$ values for the two blocks in the PLGA-\textit{b}-PMLGSLG LB films are shown in Table 7.1. The change in the helix orientation is dominant for the PMLGSLG block layer and for the two diblock copolymers with the shorter block lengths (CoPo\textsubscript{60_19} and CoPo\textsubscript{37_24}). For these diblock copolymers, annealing resulted in an orientation of the PMLGSLG block segments almost perpendicular to the substrate surface.

\textbf{Scheme 7.1. Helix coordinate system.}
Figure 7.1. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo 60.19 transferred onto silicon substrates at 40 mN/m before and after annealing; the dots represent the experimental data and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) 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).
Figure 7.2. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo\textsubscript{37} 24 transferred onto silicon substrates at 45 mN/m before and after annealing; the dots represent the experimental data and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) 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).
Figure 7.3. (a) Two-slab fits to the XRR curves of the LB monolayers of CoPo$_{59}$$_{82}$ transferred onto silicon substrates at 35 mN/m before and after annealing; the dots represent the experimental data and the full lines represent the fitted curves; the individual curves are shifted vertically for clarity. (b) 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).
Table 7.1. Best two-slab fit parameters for the XRR curves of the PLGA-b-PMLGSLG LB monolayers transferred onto silicon substrates before and after annealing

<table>
<thead>
<tr>
<th></th>
<th>CoPo_63_19 before annealing</th>
<th>CoPo_63_19 after annealing for 4 h</th>
<th>CoPo_63_19 after annealing for 24 h</th>
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<tbody>
<tr>
<td>Layer thickness (nm)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>L1 (PLGA)</td>
<td>2.04</td>
<td>2.27</td>
<td>2.30</td>
</tr>
<tr>
<td>L2 (PMLGSLG)</td>
<td>2.27</td>
<td>2.41</td>
<td>2.71</td>
</tr>
<tr>
<td>Total thickness</td>
<td>4.31</td>
<td>4.68</td>
<td>5.01</td>
</tr>
<tr>
<td>Electron density $\rho_i$ ($10^3$ e-/nm$^3$)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\rho_0$ (silicon)</td>
<td>0.705$^a$</td>
<td>0.705$^a$</td>
<td>0.705$^a$</td>
</tr>
<tr>
<td>$\rho_1$ (PLGA)</td>
<td>0.478</td>
<td>0.482</td>
<td>0.543</td>
</tr>
<tr>
<td>$\rho_2$ (PMLGSLG)</td>
<td>0.350</td>
<td>0.365</td>
<td>0.378</td>
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<tr>
<td>Interface roughness (nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma_{01}$ (silicon/PLGA)</td>
<td>0.63$^b$</td>
<td>0.76$^b$</td>
<td>0.56$^b$</td>
</tr>
<tr>
<td>$\sigma_{12}$ (PLGA/PMLGSLG)</td>
<td>0.76</td>
<td>0.81</td>
<td>1.32</td>
</tr>
<tr>
<td>$\sigma_{23}$ (PMLGSLG/air)</td>
<td>0.31</td>
<td>0.15</td>
<td>0.03</td>
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<td>Average tilt angles estimated from layer thicknesses (°)</td>
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<td></td>
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<tr>
<td>$\theta_1$ (PLGA)</td>
<td>77.5</td>
<td>76.1</td>
<td>75.9</td>
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<tr>
<td>$\theta_2$ (PMLGSLG)</td>
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<td>32.3</td>
<td>18.0</td>
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<th>CoPo_37_24 before annealing</th>
<th>CoPo_37_24 after annealing for 24 h</th>
<th>CoPo_59_82 before annealing</th>
<th>CoPo_59_82 after annealing for 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layer thickness (nm)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 (PLGA)</td>
<td>2.20</td>
<td>2.35</td>
<td>1.30</td>
<td>1.30</td>
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<tr>
<td>L2 (PMLGSLG)</td>
<td>3.14</td>
<td>3.51</td>
<td>2.26</td>
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<td>Total thickness</td>
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<td>5.86</td>
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<td>Electron density $\rho_i$ ($10^3$ e-/nm$^3$)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>$\rho_0$ (silicon)</td>
<td>0.705$^a$</td>
<td>0.705$^a$</td>
<td>0.705$^a$</td>
<td>0.705$^a$</td>
</tr>
<tr>
<td>$\rho_1$ (PLGA)</td>
<td>0.480</td>
<td>0.546</td>
<td>0.474</td>
<td>0.549</td>
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<td>$\rho_2$ (PMLGSLG)</td>
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<td>0.428</td>
<td>0.368</td>
<td>0.391</td>
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<tr>
<td>Interface roughness (nm)</td>
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<td></td>
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<td></td>
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<tr>
<td>$\sigma_{01}$ (silicon/PLGA)</td>
<td>0.74$^b$</td>
<td>0.88$^b$</td>
<td>0.48 $\pm$ 0.04$^a$</td>
<td>0.61$^b$</td>
</tr>
<tr>
<td>$\sigma_{12}$ (PLGA/PMLGSLG)</td>
<td>0.98</td>
<td>1.70</td>
<td>0.35</td>
<td>0.78</td>
</tr>
<tr>
<td>$\sigma_{23}$ (PMLGSLG/air)</td>
<td>0.16</td>
<td>0.34</td>
<td>0.18</td>
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</tr>
<tr>
<td>Average tilt angles estimated from layer thicknesses (°)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\theta_1$ (PLGA)</td>
<td>66.6</td>
<td>64.9</td>
<td>81.6</td>
<td>81.6</td>
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<tr>
<td>$\theta_2$ (PMLGSLG)</td>
<td>29.3</td>
<td>12.8</td>
<td>79.4</td>
<td>78.7</td>
</tr>
</tbody>
</table>

$a$ Values kept fixed for the curve fits.

$^b$ $\sigma$ was first fixed at 0.48 $\pm$ 0.04 nm, the average roughness of bare silicon wafers, and the other parameters were varied. Finally $\sigma$ was varied to obtain the best curve fit.
Annealing-induced changes in double-brush Langmuir-Blodget films

More evidence for the change in the helix orientation in the annealed films was observed by transmission FT-IR. The transition dipole moments of the amide I (1653 cm\(^{-1}\)) and amide A (3290 cm\(^{-1}\)) vibrations are oriented nearly parallel to the helix axis, while that of the amide II (1546 cm\(^{-1}\)) is nearly perpendicular to the helix axis.\(^{14}\) Thus, the decrease in the amide I/amide II and amide A/amide II band area ratios shown for the annealed films in Figure 7.5 clearly indicates a decrease in the average helix tilt angles. The results of deconvolution of the spectra and calculation of the average helix tilt angle are in agreement with those from the XRR measurements.

Figure 7.4. AFM height images (\(\Delta z = 10 \text{ nm}\)) of an LB monolayer of CoPo\(_{60.19}\) transferred onto a silicon substrate at 40 mN/m before annealing (a) and after (b) annealing at 100 °C for 24 h.
Figure 7.5. Transmission FT-IR spectra of the LB monolayers of CoPo\textsubscript{60.19} (a), CoPo\textsubscript{37.24} (b) and CoPo\textsubscript{59.82} (c) transferred onto silicon substrates before annealing (solid lines) and after (dashed lines) annealing at 100 °C for 24 h.
The change in the LB films of the PLGA-b-PMLGSLG diblock copolymers might likely originate from the removal of a considerable amount of water molecules bound to the PLGA block upon drying at 100 °C. This leads to stronger intermolecular interactions between the PLGA block segments arising from hydrogen bond formation between the carboxylic groups. The FT-IR C=O stretching vibration of the carboxylic groups in the PLGA-b-PMLGSLG LB films exhibits two bands at 1736 and 1711 cm⁻¹, corresponding to non-hydrogen bonded and dimer hydrogen-bonded carboxylic groups, respectively (Chapter 5). After annealing, the intensity of the band at 1736 cm⁻¹ slightly decreases while that of the band at 1711 cm⁻¹ slightly increases (Figure 7.5), indicating more dimerization of carboxylic groups in the annealed films. A small increase in intensity of the FT-IR band at around 1670 cm⁻¹ for the annealed films, visible as a shoulder, also indicates a larger transition dipole coupling between parallel oriented helices (Chapter 5). Due to water removal, accompanied with a molecular rearrangement, the substrate/PLGA and PLGA/PMLGSLG interface roughnesses increased considerably. However, compensating this, the top surface became smoother.

It has been found that due to the transfer-induced orientation effect, in monolayers of PLGA-b-PMLGSLGs the α-helices are oriented azimuthally, forming a smectic C-like phase (Chapter 6). On the other hand, the melting point of the partly crystalline stearyl side chain has been reported to be around 20 °C for cast films of PMLGSLG. The stearyl side chain of star-shaped poly(γ-stearyl-L-glutamate)s has been found to start to melt at 65 °C and to freely move as a liquid at 107 °C. Therefore, upon annealing the PLGA-b-PMLGSLG films at 100 °C, the stearyl side chain is fully flexible, acting as a liquid mantle for the PMLGSLG helix cores. Along with the surface area shrinkage during the annealing process, both the smectic C-like order of the helices and the mobility of the side chain mantle seem to favor the increased packing and decreased tilting towards the substrate-normal of the PMLGSLG block helices.

When there is no desorption of the polymer from the substrate surface and no change in the polymer chemical structure upon annealing, the change in surface area can be estimated from the changes in electron density and thickness of the layer by the following relation:

\[ \rho_i \cdot L_i \cdot S_i = \rho_i^{\text{ann}} \cdot L_i^{\text{ann}} \cdot S_i^{\text{ann}} \] (7.2)

where \( \rho_i \), \( L_i \) and \( S_i \) are the electron density, layer thickness and surface area of slab \( i \), respectively, and the superscript “\text{ann}” indicates the annealed sample. Indeed, the XRR fit parameters (Table 7.1) show that the changes in surface areas, derived from the changes in electron density and thickness, of the PLGA (\( i = 1 \)) and PMLGSLG (\( i = 2 \)) slabs are in strong accordance with each other:
Depending on the electron density of the PLGA slab in the original films, the decrease in surface area due to film shrinkage was ca. 14-22%, reaching a maximum electron density of $0.546 \pm 0.003 \times 10^3$ e/\(\text{nm}^3\) for the PLGA block. This value is about 9% higher than that calculated from the core density of esterified polyglutamates in the solid state ($0.503 \times 10^3$ e/\(\text{nm}^3\))\(^{22}\), probably due to the high molecular orientation and a very dense rod packing. The electron density of the PMLGSLG slab increased by 6-10%, suggesting a large increase in side chain interdigitation.

The tendency of the side chains to interdigitate more strongly upon annealing has been reported for multilayer LB films of PMLGSLG with the helices oriented parallel to the substrate.\(^{23,24}\) This was indicated by a decrease in film thickness and increase in electron density. To compare with the LB films of PLGA-\(b\)-PMLGSLGs, we studied a multilayer LB film of PMLGSLG by transmission FT-IR. After annealing, no change in the amide bands was found for the PMLGSLG film, indicating a preserved parallel helix orientation with respect to the substrate. Nevertheless, the same trend for change in the methylene stretching vibration bands was observed for both LB films of PLGA-\(b\)-PMLGSLGs and PMLGSLG: the bands were shifted to higher frequencies and the band absorptions slightly decreased (Figure 7.6 and Figure 7.7). The frequencies of the asymmetric and symmetric methylene stretching vibrations are sensitive to the trans/gauche conformer ratio of the alkyl chains, shifting to higher wavenumbers with increasing number of gauche conformers.\(^{25,26}\) Both pressure and temperature affect trans-gauche populations.\(^{27}\) For the LB films of PLGA-\(b\)-PMLGSLGs and PMLGSLG, the \(\nu_a(\text{CH}_2)\) and \(\nu_s(\text{CH}_2)\) bands are around 2924-2925 cm\(^{-1}\) and 2854-2855 cm\(^{-1}\), respectively, indicating a substantial number of gauche conformers.\(^{25,26}\) As shown in Figure 7.7, these frequencies correlate decreasingly with the electron density of the PMLGSLG slab. This shows that the gauche population decreases, due to enhanced side chain order, with increasing molecular packing of the layer. Upon annealing, the shift in \(\nu_a(\text{CH}_2)\) and \(\nu_s(\text{CH}_2)\) to higher frequencies indicates an increase in the gauche population, in agreement with the occurrence of a liquid-like phase. This allows a considerable increase in side chain interdigitation. Besides, the terminal bonds of alkyl chains in the gauche conformation afford a more compact molecular shape.\(^{28}\) The slight decrease in the \(\nu_a(\text{CH}_2)\) and \(\nu_s(\text{CH}_2)\) absorptions for the films after annealing suggests reorientation of the side chains towards the surface plane.\(^{29}\) Under compression on the water surface, the side chains are stretched away from the water surface and this non-equilibrium arrangement is retained in the transferred films. Hence, the orientation change of the side chains

\[
\frac{S_1^{\text{ann}}}{S_1} = \frac{\rho_1 \cdot L_1}{\rho_1^{\text{ann}} \cdot L_1^{\text{ann}}} \approx \frac{\rho_2 \cdot L_2}{\rho_2^{\text{ann}} \cdot L_2^{\text{ann}}} = \frac{S_2^{\text{ann}}}{S_2} \tag{7.3}
\]
Annealing-induced changes in double-brush Langmuir-Blodget films upon annealing can be related to their relaxation towards a more uniform distribution in the layer.

From a comparison of the annealing effects on the LB films of the PLGA-b-PMLGSLG diblock copolymers, it follows that the surface area shrinkage and thereby the change in helix orientation are greater for the diblock copolymers with small block lengths, i.e. CoPo_{60\_19} and CoPo_{37\_24}. Though the trend for an increase in electron density of the slabs are similar for all the diblock copolymers, for CoPo_{59\_82} there was only a slight increase in thickness and so an insignificant change in helix tilt angle for both blocks. This might be partly due to the pre-orientation with a large average helix tilt angle in the original film. It might also be that the change in the films upon annealing is dependent on an interplay between the mutual dipolar interactions of the PMLGSLG block helices and the surface constraint caused by the intermolecular interactions between the side chains of the PLGA block segments. For the large hydrophobic block length of CoPo_{59\_82}, the interactions of the off-axis dipole components between the parallel PMLGSLG \( \alpha \)-helices might tentatively hinder the orientation of the \( \alpha \)-helices towards the substrate-normal (Chapter 5).

**Figure 7.6.** High-frequency transmission FT-IR spectra of an LB monolayer of CoPo_{60\_19} (a) and a multilayer of PMLGSLG (b) transferred onto silicon substrates before (solid lines) and after (dashed lines) annealing at 100°C for 24 h.
Figure 7.7. Peak max. frequencies of the methylene symmetric (circles) and asymmetric (squares) stretching vibrations for the LB monolayers of PLGA-b-PMLGSLGs and a multilayer LB film of PMLGSLG. Inset: Electron densities of the PMLGSLG slab in the corresponding PLGA-b-PMLGSLG LB films. The open and filled points represent the samples before annealing and after annealing at 100 °C for 24 h, respectively.

Taken together, the results from the XRR, AFM and transmission FT-IR demonstrate clearly the structural change in the LB monolayers of PLGA-b-PMLGSLGs upon annealing. Due to the increased interactions between the carboxylic groups of the PLGA segments upon annealing, the surface shrinkage of the films occurred with an increase in both film thickness and density. As a result of the side chain melting and surface constraint, the PMLGSLG block helices, in a smectic C-like phase, became less tilted towards the substrate-normal and the side chains were oriented towards the substrate surface with an increase in the gauche population.
7.4 Conclusions

Annealing, at 100 °C for 24 h, enhanced the helix orientation in double-brush LB monolayers of PLGA-b-PMLGSLG diblock copolymers. The annealing process removed the bound water, giving rise to significant interchain interactions between the carboxylic groups of the PLGA segments. In accordance with the surface constraint caused by the shrinkage of the PLGA slab, the melting of the side chain mantle into a liquid-like state of the PMLGSLG block during annealing favored the helices to be further oriented toward the substrate-normal. This resulted in a more compact uniform film with a decrease in the average helix tilt angle of the PMLGSLG block. For the diblock copolymer with the shorter block lengths, CoPo_60_19 and CoPo_37_24, the post-orientation effect was more pronounced and a nearly perpendicular orientation of the PMLGSLG block helices with respect to the substrate was obtained.

7.5 Appendix

Thermal stability of PLGA-b-PMLGSLG

As shown by the thermal gravimetric analysis (TGA) result in Figure 7.8, the PLGA-b-PMLGSLG diblock copolymer is thermally stable up to 225 °C. Above this temperature, a two-step decomposition process occurs, which is in good agreement with the appearance of two distinct endothermic peaks in the differential scanning calorimetry (DSC) curve at the same temperature range (Figure 7.9). From the comparison of the DSC curve of PLGA-b-PMLGSLG with those of PLGA and PMLGSLG, the composition process can be assigned to the successive degradation of the PLGA and PMLGSLG blocks, occurring at about 225 and 300 °C, respectively.

In addition, the ATR FT-IR spectra of PLGA-b-PMLGSLG as a function of heating temperature show the conformational change from the α-helix to β-sheet structure starting at ca. 150 °C (Figure 7.10). Above 220 °C, the decrease in intensity of the C=O absorption bands and the appearance of new absorption bands at 1775 and 1734 cm⁻¹, which might be ascribed to the vibration frequencies of an imide group, indicate the thermolysis of the diblock copolymer. Further heating to above 300 °C results in carbonization of the polymer.
Figure 7.8. TGA thermogram of PLGA-b-PMLGSLG (solid line) and its derivative (dashed line), recorded at a heating rate of 10 °C/min under nitrogen. Just before the measurement, the specimen was dried at 40 °C under vacuum for 24 h to remove the moisture.

Figure 7.9. DSC thermograms of PLGA-b-PMLGSLG, in comparison with those of PLGA and PMLGSLG, recorded at a heating rate of 10 °C/min under nitrogen. For clarity the curves are shifted vertically. In order to remove the moisture, the specimens were first scanned up to 120 °C and thereafter cooled to room temperature at 10 °C/min. The results of the subsequent scanning runs are shown in the figure.
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Figure 7.10. ATR-FTIR spectra of PLGA-b-PMLGSLG as a function of heating temperature. The samples were heated to the desired temperature at a heating rate of 10 °C/min under nitrogen. For clarity the curves are shifted vertically.

7.6 References

(10) Watanabe, J.; Fukuda, Y.; Gehani, R.; Uematsu, I. Macromolecules 1984,