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Chain length dependence of the helix orientation in Langmuir–Blodgett monolayers of α-helical diblock copolypeptides†

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The effect of chain length on the helix orientation of α-helical diblock copolypeptides in Langmuir and Langmuir–Blodgett monolayers is reported for the first time. Amphiphilic diblock copolypeptides (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) of various block lengths were studied. The tilt angle between the helices and the substrate-normal decreases upon increasing the transfer pressure coincident with “double brush” formation. The hydrophobic block length strongly affects the maximum surface chain density and thereby the helix orientation of the diblock copolypeptides. Increasing the degree of polymerization of the hydrophobic block (DP_{PMLGSLG}) results in an increase in the helix tilt angle tentatively attributed to the off-axis interactions of the unscreened peptide dipoles between the parallel aligned α-helices. In those cases where the alkyl side chains surround the PMLGSLG helices, the smallest helix tilt angle of 29° for the PMLGSLG block and 67° for the PLGA block were obtained for the diblock copolymer of DP_{PLGA} 37 and DP_{PMLGSLG} 24. For smaller DP values of the hydrophobic block, in particular the diblock copolymer having DP_{PMLGSLG} 11, the long alkyl side chains are partially expelled from the brush layer and the α-helices of the PMLGSLG block are oriented nearly perpendicular to the interface.

Introduction

Polymer brushes provide a powerful tool for surface modification as well as for fabrication of highly oriented ultrathin films with controlled surface chemistry and functionality. They have attracted great interest for numerous applications in colloid stabilization, actuation and sensing, and nano- and biotechnology.1–3 Polymer brushes of α-helical synthetic polypeptides are particularly intriguing in this respect due to a large electric field generated by the accumulation of unidirectionally aligned helix macrodipoles.4,5

The surface-grafting-from technique of α-helical polypeptides has been published revealing the interesting electrical properties of these systems.6,7

An alternative way to prepare ultra-thin layers of unidirectionally aligned α-helical polypeptide brushes is via the use of the Langmuir–Blodgett (LB) technique and amphiphilic block copolymers as spreading materials. The latter method overcomes the disadvantage of difficulty in analyzing the grafted polymers in the former approach. Several polypeptide-based amphiphilic systems have been explored by fabrication of Langmuir and LB monolayers with unidirectional helix orientation.8–15 For these layers, the α-helices are usually oriented with a certain tilt angle with respect to the surface normal.

Fourier transform infrared reflection absorption spectroscopy (FT-IR/RAS) has been used to measure the average tilt angle between the helix axis and the surface normal in these studies and depending on different polypeptide systems, different values of the helix tilt angle have been published.11–15

The molecular orientation in Langmuir monolayers can be affected by various factors, including the mutual interactions of polymeric segments and the interactions of the segments with the tethering surface.16 In addition to the specific chemical structure of the amphiphilic polymer system, the chain length of the hydrophobic and hydrophilic segments is expected to be an important factor in controlling the tilting of the helices. However, so far there has been no systematic demonstration of the influence of chain length on the helix orientation in the monolayers of polypeptide amphiphiles.

To estimate the average helix tilt angle, FT-IR/RAS on gold surfaces has been widely used applying the “surface selection rule”, of which only transition dipole moment components perpendicular to the surface can be detected.17 Using a film of the same polypeptide type with a known helix orientation as a reference for calculating a proportionality constant K, which relates to the intrinsic oscillator strengths of the amide I and amide II vibrations specific to that polypeptide, the orientation of the α-helices can be revealed. The “surface selection rule” of specular reflection FT-IR spectroscopy for metal substrate-supporting thin layers was first described by Greenler.18 However, dispersion effects leading to band shifts and distortions in specular reflection FT-IR spectra have been highlighted.19,20 In particular for α-helical diblock copolypeptides, the amide absorption bands, characteristic of the α-helix structure for the two blocks, are usually closely positioned and even overlapping.

† Electronic supplementary information (ESI) available: FT-IR spectra of PtBuLG-b-PMLGSLGs, and X-ray reflectivity electron density profiles and fit parameters. See DOI: 10.1039/c001163k
Evaluation of the helix orientation for such a system has been reported for an amphiphilic diblock copolymer of poly(γ-β-1,6-glutamate) and poly(β-1,6-glutamic acid). The authors assumed the same average helix tilt angle for both blocks by considering the β-helical amide I and amide II absorptions as single bands. However, this might lead to erroneous tilt angle values, because the two blocks, with different cross-sectional helix areas and chain lengths, can be tilted at different angles to the surface normal and the proportionality constant $K$ can be different for different polyamino acids.

The present work examines for the first time the effect of chain length on the helix orientation in the Langmuir and LB monolayers of unidirectionally aligned β-helices. For this, we studied amphiphilic diblock copolyptides of poly(β-1,6-glutamic acid) and poly(γ-methyl-1,6-glutamate–ran-γ-stearyl-1,6-glutamate) with 30 mol% of stearyl substituents (PLGA-b-PMLGSLG), which have been found to form stable double-brush monolayers with the β-helices tilted away from the interface. Here, we performed the helix orientation analysis method reported by Wieringa et al. using transmission FT-IR spectroscopy for thin films on silicon substrates, which was developed from the approach described by Enriquez et al. In this method, the incoming IR light beam is normal to the substrate surface and only transition dipole moment components parallel to the surface can be observed. For the LB monolayers of the PLGA-b-PMLGSLG diblock copolyptides studied, the helix tilt angle was calculated separately for the two blocks by careful band identification and deconvolution. Moreover, as the average helix tilt angle relates to the chain length and the layer thickness, small angle X-ray reflectivity for determining the layer thickness was used as an alternative method to confirm the transmission FT-IR results and to deduce the layer properties.

**Experimental section**

**Materials**

β-Helical PLGA-b-PMLGSLG was synthesized via a diblock copolymer precursor consisting of poly(γ-tert-butyl-1-glutamate) (PtBuLG) and PMLGSLG, with the tert-butyl group as a mild acid-labile protecting group for the carboxylic acid (Scheme 1). A detailed description of the synthesis can be found elsewhere. The molecular weights of PtBuLG-b-PMLGSLGs were characterized by $^1$H NMR (CDCl$_3$) and gel permeation chromatography (tetrahydrofuran eluent, polystyrene standard, universal calibration). The polydispersity indexes (PDIs) of the diblock copolymers studied are slightly smaller for copolymers of larger degrees of polymerization (DPs), and are in the ranges of 1.16–1.34 for the PtBuLG block and 1.15–1.37 for the corresponding PtBuLG-b-PMLGSLG, respectively. The tert-butyl group was removed using trifluoroacetic acid (TFA). The PLGA-b-PMLGSLG diblock copolymers (PLGA-b-PMLGSLGs) are abbreviated as CoPo$_m$n, where $m$ and $n$ denote the DPs of the PLGA and PMLGSLG blocks, respectively. PLGA (DP = 60) was obtained by hydrolysis of PtBuLG using TFA. PMLGSLG (DP = 118) was prepared by random copolymerization of γ-methyl and γ-stearyl 1,6-glutamate N-carboxyanhydrides (70:30 mole ratio) in chloroform at 0 °C using triethylamine as initiator.

**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$^{-1}$. The water used for the sub-phase was purified by reverse osmosis and subsequently through a Milli-Q filtration system. The acidic pH of the sub-phase was adjusted by adding a HCl (2 N) standard aqueous solution (Aldrich), PLGA-b-PMLGSLGs were spread from N-methylpyrrolidone (NMP) (Acros, 99.5%)/chloroform (Lab-Scan, 99.5%), (3/7, v/v) solutions with 1–3% (v/v) of acetic acid (Acros, 99.5%) added, at a concentration of 0.4–0.6 mg mL$^{-1}$. PLGA was spread from an NMP/chloroform (3/7, v/v) solution at a concentration of 0.5 mg mL$^{-1}$. PMLGSLG was spread from a chloroform solution at a concentration of 0.6 mg mL$^{-1}$. The compression speeds were 38.5 cm$^2$ min$^{-1}$ for PMLGSLG and ca. 19 cm$^2$ min$^{-1}$ for the others.

**Stabilization of monolayers**

The surface pressure was maintained at a constant value while the surface area was monitored as a function of time. The monolayer stabilized when the surface area remained constant with time and this surface area is defined as the stabilization point at that pressure.

**Substrate preparation**

The double-sided polished silicon wafers (Topsil Semiconductor Materials A/S, Frederikssund, Denmark, 1000 ± 25 µm thick) and quartz slides used as substrates were cleaned by treating them with a mixture of H$_2$O$_2$ (Merck, 30%)/NH$_3$ (Merck, 25%)/H$_2$O (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 (Merck, 37%)/H$_2$O (1:6, v/v) for 25 min, rinsing with Milli-Q water, and finally ultrasonication in methanol (Lab-Scan, 99.8%), methanol/chloroform (Lab-Scan, 99.5%, 1:1, v/v) and chloroform for 15 min. The cleaned silicon wafers were hydrophilized by treating them with a mixture of hexamethyldisilazane (Acros, 98%)/chloroform (1:4, v/v) at 50 °C, and rinsed with chloroform before use.

**Langmuir–Blodgett films**

Deposition of LB films was carried out in the vertical mode. The sub-phase temperature was maintained at 20 ± 0.1 °C. Transfer

[Image 57x70 to 275x183]
of a monolayer of PLGA-$b$-PMLGSLGs onto a hydrophilic silicon substrate was done 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 PLGA-$b$-PMLGSLG diblock copolymers. Monolayers were deposited onto both sides of the hydrophilic substrates during the upward stroke. Transfer ratios were around unity (±10%). Multilayer films of PMLGSLG and PtBuLG-$b$-PMLGSLGs were deposited onto hydrophobized silicon substrates by Y-type transfer, at 20 mN m$^{-1}$ and at down- and up-stroke speeds of 3 mm min$^{-1}$.

Transmission Fourier transform infrared (transmission 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.

Curve-fittings of the side chain ester (C=O stretching), acid (C=O stretching), amide I (mainly C=O stretching) and amide II (mainly C-N stretching) regions of transmission FT-IR spectra were performed using Bruker OPUS software (version 4.2). The parameters were optimized using a Levenberg–Marquardt algorithm.

Small angle X-ray reflectivity

Small angle X-ray reflectivity 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 ($p_{SiO2}/p_{Si} = 0.95$) and could not be resolved for the reflectivity curves of bare silicon wafers.

Results and discussion

Surface pressure-area ($\pi$–$A$) isotherms

Fig. 1 shows the $\pi$–$A$ isotherms for the PLGA-$b$-PMLGSLG diblock copolymers with different block lengths spread on pure water at 20 °C, in comparison with those for the two separate polymers, PLGA and PMLGSLG. Please note that the x-axis is expressed as area per molecule, which produces a large dependency of the isotherms on the degree of polymerization. The isotherms of PLGA and PMLGSLG have been reported in literature. PLGA is surface active to some extent, exhibiting a transition at a low surface pressure of below 15 mN m$^{-1}$.

The isotherm of PMLGSLG shows a steep rise in pressure upon compression due to the packing of the $\alpha$-helices lying flat on the water surface, followed by a liquid-condensed phase and subsequently a plateau transition indicating monolayer collapse at about 33 mN m$^{-1}$.

The PLGA-$b$-PMLGSLG diblock copolymers give rise to completely different $\pi$–$A$ isotherms. Upon compression, the $\pi$–$A$ isotherms first show a transition at a low surface pressure which is analogous to the isotherm of PLGA, spread under the same condition, and might be attributed to the immersion of the PLGA block into the sub-phase. Another transition in the isotherms occurring above 40 mN m$^{-1}$, only clearly observed for CoPo$_{59.82}$ and CoPo$_{53.63}$, might be related to rearrangement of the PMLGSLG chains into a denser and more ordered packing. At surface pressures beyond this transition, film deposition resulted in an incomplete transfer, probably because at this state the monolayers do not maintain their molecular mobility. All the diblock copolymers show high collapse surface pressures of 50–55 mN m$^{-1}$.

Brewster angle microscopy images of the monolayers of PLGA-$b$-PMLGSLGs upon compression from the surface...
pressure buildup to 45 mN m\(^{-1}\) showed homogenous monolayers.\(^{21}\) The compression–decompression of these monolayers is a reversible process with a large hysteresis upon monolayer compression to a pressure above the second transition and no hysteresis before this transition.\(^{30}\)

The linear increment in surface pressure following the first transition in the isotherms of PLGA-\(b\)-PMLGSLGs corresponds to the packing and tilting of the PMLGSLG block segments away from the water surface after the PLGA block is immersed. Assuming the same maximum compression of the PMLGSLG blocks for all of the PLGA-\(b\)-PMLGSLGs (i.e. their ultimate position is perpendicular to the water surface), the isotherms are expected to coincide at the point of monolayer collapse. However, some differences, in terms of molecular surface area, can be observed in the \(\pi-A\) isotherms of PLGA-\(b\)-PMLGSLGs.

The behavior of the diblock copolymers in the 3 regimes in the isotherms is shown schematically in Scheme 2. The first regime (Scheme 2a) is at the onset of the surface pressure buildup, where the diblock copolymer molecules lying flat on the water surface upon spreading just come into contact. This is supported by the agreement of the surface area measured with that calculated supposing that both blocks are lying parallel to the water surface as shown in Fig. 2. The second regime, the start of double-brush formation, occurs just after the PLGA block is pushed into the water sub-phase (Scheme 2b). The surface area per molecule corresponding to this regime can be indicated by extrapolation of the steep rise in pressure after the first transition to zero pressure. As shown in Fig. 3, this extrapolated area increases linearly with DP\(_{\text{PMLGSLG}}\). In the third regime, upon further compression to reduce the surface area, the \(\alpha\)-helices become less tilted to the surface normal due to an increase in chain density at the interface (Scheme 2c). The difference in molecular surface area in this regime corresponds to the different surface chain density and hence reflects the different tilt order of the PMLGSLG block.

Generally, the relation between the average helix tilt angle (\(\theta\)) and the layer thickness (\(h\)) of an \(\alpha\)-helical polymer brush film is described in Scheme 3 and can be estimated as follows:

\[
\cos \theta = \frac{h}{L} = \frac{0.15 \text{DP}}{L}, \quad \theta < 90^\circ
\]

where \(L\) is the helix length, DP is the degree of polymerization, and 0.15 (nm) is the \(\alpha\)-helix pitch per monomer unit along the.

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**Scheme 2** Simple schematic representation of the arrangement of the PLGA-\(b\)-PMLGSLG diblock copolymers with a long (A) and short (B) hydrophobic block at the air–water interface.

**Fig. 2** Molecular surface area at the onset of surface pressure buildup of the PLGA-\(b\)-PMLGSLG monolayers. The calculated values were obtained by \(A_{\text{cal}} = 0.185 \text{DP}_{\text{PLGA}} + 0.265 \text{DP}_{\text{PMLGSLG}}\), assuming that both blocks are lying flat on the water surface; 0.185 and 0.265 (nm\(^2\)) are the monomer unit areas of PLGA and PMLGSLG, respectively, as deduced from the areas at onset pressures in their isotherms.

**Scheme 3** Schematic representation of the relation between the layer thickness (\(h\)), the helix length (\(L\)) and the average helix tilt angle (\(\theta\)) in an \(\alpha\)-helical polymer brush film.

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helix axis. The volume of a helix can be estimated from the relation:

\[ V = A \times h = \frac{DP \times M_0}{\rho \times L_{AV}} \quad (2) \]

where \( V \) is the molecular volume, \( A \) is the molecular surface area, \( M_0 \) is the repeating unit molecular weight, \( \rho \) is the polymer density and \( L_{AV} \) is the Avogadro’s number. From eqn (1) and (2), the average helix tilt angle can be estimated from the molecular surface area using the relation:

\[ \cos \theta = \frac{M_0}{0.15 \times \rho \times A \times L_{AV}} \quad (3) \]

In the double-brush formation regime (the third regime), the molecular area in the \( \pi-A \) isotherms of PLGA-\( b \)-PMLGSLGs corresponds to the average surface area per helix of the PMLGSLG block. With the density of PMLGSLG at about 1.1 \( \text{g cm}^{-3} \),\(^{22} \) the relation between the average helix tilt angle of the PMLGSLG block and the molecular surface area can be derived as shown in Fig. 4. Obviously, the helix tilt angle decreases with decrease in molecular surface area, i.e. with increase in surface pressure. A comparison of the minimum helix tilt angles calculated from the minimum molecular areas (in the transferable region) for PLGA-\( b \)-PMLGSLGs is shown in Fig. 5. The minimum surface area and the corresponding helix tilt angle are observed to increase with increasing DP\( \text{PMLGSLG} \).

Apparent, for a diblock copolymer with a larger DP\( \text{PMLGSLG} \), the monolayer cannot be compressed further to a surface chain density as high as for one with a smaller DP\( \text{PMLGSLG} \). We have measured the surface potentials of the PLGA-\( b \)-PMLGSLG monolayers during compression at the air–water interface.\(^{19} \) The results showed that the polypeptide macrodipole moment immersed in water was effectively screened out. However, the effective dipoles of the \( \alpha \)-helices of the hydrophobic block tilted away from the water surface were largely maintained and related increasingly to DP\( \text{PMLGSLG} \). To some extent, the parallel helix–helix dipole interactions seem tentatively to account for the increase in the helix tilt angle with increasing DP\( \text{PMLGSLG} \).

When DP\( \text{PMLGSLG} \) is sufficiently large (CoPo\( _{53,63} \) and CoPo\( _{59,82} \)), the second transition at high surface pressures, accompanied by a considerable decrease in the molecular surface area, appears. We found that this transition is dependent on temperature (demonstrated for CoPo\( _{59,82} \) in Fig. 1),\(^{21} \) indicating that the transition is related to reorganization, favored by the flexibility of the long alkyl side chains, of the PMLGSLG segments. Moreover, monolayers, though not transferable, are stable above this transition (see inserted points in Fig. 1). Stabilization curves are shown for CoPo\( _{53,63} \) and CoPo\( _{59,82} \) in Fig. 6. As indicated by stabilization points in Fig. 1, the molecular areas at which the monolayers stabilize before the transition (e.g. at 35 mN m\(^{-1} \)) are consistent with the isotherms. However, above the transition, the stabilization points shift to smaller areas coinciding with the areas recorded for the other diblock copolymers with a small DP\( \text{PMLGSLG} \) (≤39). It is therefore tempting to speculate that at high surface pressures the monolayers undergo a slow transition from an isotropic phase, where the PMLGSLG block segments are oriented isotropically around the water surface normal with an average tilt angle, to a liquid-crystalline-like phase as depicted in Scheme 2Ac. Along the transition, the average helix tilt angle decreases considerably. As shown in Fig. 6, upon stabilization of the monolayer of CoPo\( _{59,82} \) at 5 °C at 48 mN m\(^{-1} \), the molecular surface area decreases very slowly, likely toward the surface areas stabilized at 20 °C and ≤45 mN m\(^{-1} \). This might indicate that this transition to a smectic C-like phase is a slow process which is dependent on the compression speed and that increasing temperature accelerates the phase transition. In addition, from the isotherms (Fig. 1),

**Fig. 4** Calculated average helix tilt angle of the PMLGSLG block (\( \theta_{\text{PMLGSLG}} \)) as a function of molecular surface area for the PLGA-\( b \)-PMLGSLG monolayers (solid line). A \( \rho_{\text{PMLGSLG}} \) of 1.1 g cm\(^{-3} \) is used for the calculation.

**Fig. 5** Measured molecular surface areas (squares) (from the isotherms in Fig. 1) and the corresponding calculated helix tilt angles (circles) of the PMLGSLG block at the highest surface pressures (in the transferable region) as a function of DP\( \text{PMLGSLG} \). The highest surface pressure is at just before the second transition for CoPo\( _{53,63} \) and CoPo\( _{59,82} \), and is just before the monolayer collapse for the others.

**Fig. 6** Stabilization curves of CoPo\( _{59,82} \) at 20 °C and 35, 45, 48 and 50 mN m\(^{-1} \) (a, b, c and d, respectively); CoPo\( _{59,82} \) at 5 °C and 35, 45 and 48 mN m\(^{-1} \) (a‘, b’ and c’, respectively); and CoPo\( _{53,63} \) at 20 °C and 47.5 mN m\(^{-1} \) (a”).
it seems that this transition also occurs for CoPo$_{63.39}$, although the monolayer collapses soon after that. Hence, the DP$^{\text{PMLGSLG}}$ of 39 is likely to be a critical value below which monolayers of PLGA-$b$-PMLGSLG$_{\text{Co}}$ exhibit a smectic C-like phase already at the beginning of double-brush formation regime (Scheme 2Bc).

In such a liquid-crystalline-like phase where the PMLGSLG block helices are oriented parallel to one another, besides the axial dipole components of the individual peptide units, their off-axis dipole components are distributed around, along the helix and can interact strongly with an off-axis dipole component in the neighboring $\alpha$-helix. It has been reported that rod-like molecules with lateral permanent dipole moments can exhibit the smectic C mesophase. Using a mean-field theory, Govind and Madhusudana have shown that the off-axis character of the lateral polar groups is responsible for a molecular tilt in the smectic C phase of liquid crystals. Depending on the number, positions and strengths of the dipoles, the tilt angle can be varied to minimize the interaction energy of the oriented dipoles. It has also been indicated that molecules with stronger dipoles lead to smectic C crystals with larger tilt angles. To some extent, the off-axis dipole components of the peptide units may account for the formation of a tilted smectic layer of the helices in the monolayers of PLGA-$b$-PMLGSLG$_{\text{Co}}$. Increasing DP$^{\text{PMLGSLG}}$ results in an increased number of the peptide dipoles, which may tentatively change the tilting potential and give rise to an increase in the average helix tilt angle.

The very small length of the PMLGSLG block in CoPo$_{50.11}$ led to a completely different situation. The surface area of 1.50 nm$^2$ at 40 mN m$^{-1}$, the highest pressure where the monolayer was transferable, is smaller than the minimum cross-sectional area of a PMLGSLG helix with surrounding side chains. The helix length of the PMLGSLG block (1.65 nm corresponding to 11 monomer units, each 0.15 nm in length) is smaller than the length of the fully extended (all-trans) stearyl side chain (2.3 nm). Therefore, upon high compression the long side chain might be aligned along the helix with part of it going on top of the monolayer. Indeed, the observed surface area is in accordance with the helix cross-sectional area of poly($\gamma$-methyl-L-glutamate), of 1.54 nm$^2$, including the ester and methyl groups.

**Helix orientation by transmission FT-IR**

The LB monolayers of the PLGA-$b$-PMLGSLG diblock copolymers were transferred onto both sides of hydrophilic silicon substrates and quartz plates. The monolayers consist of molecules that are in a predominantly $\alpha$-helix structure, as demonstrated by circular dichroism (CD) and transmission FT-IR. From the position and the absorption intensity ratio of the amide I and amide II bands in the transmission FT-IR spectrum of a polypeptide film, the polypeptide conformation and the average helix tilt angle can be determined. Due to the different orientations of the transition dipole moments of these vibrations, a higher amide I to amide II band area ratio (AI/AlII) corresponds to a larger tilt angle between the helices and the surface normal. Fig. 7 shows representative spectra of the LB monolayers of PLGA-$b$-PMLGSLG$_{\text{Co}}$ deposited onto silicon substrates at different surface pressures. For all the diblock copolymers, AI/AlII decreases, indicating a decrease in the average helix tilt angle, upon increasing the transfer pressure.

Fig. 8 shows a set of representative curve fits of the ester and acid C=O stretching vibrations at 1738 and 1711 cm$^{-1}$, respectively. The determination method of the average helix tilt angle ($\theta$) is described in ref. 22. The $\theta$ values for the PMLGSLG and PLGA
blocks were estimated from the α-helix AI/AII ratios corresponding to each block, using the following relation:

\[
\frac{\text{AI}}{\text{AII}} = K \frac{\left( \frac{1}{2} \sin^2 \theta \sin^2 \alpha_I + \cos^2 \theta \cos^2 \alpha_I \right)}{\left( \frac{1}{2} \sin^2 \theta \sin^2 \alpha_{II} + \cos^2 \theta \cos^2 \alpha_{II} \right)} \tag{4}
\]

where \(\alpha_I\) and \(\alpha_{II}\) are the transition dipole moment angles of the amide I (38°) and amide II band (73°) with respect to the helix axis.\(^7\) To determine the proportionality constant \(K\), a reference sample with a known helix tilt angle is needed for each block. Because the PMLGSLG helices in an LB film are oriented parallel to the substrate surface,\(^28\) the helix tilt angle is 90° and AI/AII has its maximum value for this system. PLGA was spin-coated onto silicon substrates at different speeds, giving films with different thicknesses, indicated by the difference in intensity in the transmission FT-IR spectra (Fig. 9a). As shown in Fig. 9b, the film with the lowest thickness results in the highest AI/AII ratio, corresponding to the helices oriented largely parallel to the substrate. The AI/AII ratio of 3.92 obtained for this sample is very close to the AI/AII value of 3.88 obtained for a film prepared by transfer of the PLGA monolayer spread on the water surface at sub-phase pH 2.\(^38\) Thus, the AI/AII ratio of 3.92 was used as a reference corresponding to a helix tilt angle of nearly 90° for PLGA. The helix tilt angles estimated for the two blocks are summarized in Table 2 for the PLGA-b-PMLGSLG monolayers deposited at the maximum transfer pressures. Comparison of the FT-IR spectra of some of these LB monolayers is given in Fig. 10.

Assuming a random orientation of the side chain ester and acid groups, the chain density in an LB monolayer of PLGA-b-PMLGSLG can be correlated with the integral area of the C=O stretching vibration bands of these groups by the following relation:

\[
I_{\text{C}=\text{O}} = \frac{1}{A} (\text{DP}_{\text{PLGA}} + \text{DP}_{\text{PMLGSLG}}) \tag{5}
\]

with \(I_{\text{C}=\text{O}}\) is the integral area of the spectral region of the C=O stretching vibrations, \(A\) is the molecular surface area and DP is the degree of polymerization. On this basis, the chain densities in the monolayers are roughly compared in Fig. 11. A higher chain density corresponds to a smaller tilt angle. Apparently, the change in the chain density agrees well with the estimated helix tilt angles shown in Table 2.

The transmission FT-IR results (Table 2, Fig. 10 and 11) show the same trend in the dependence of the helix tilt angle on the hydrophobic block length as observed from the isotherms (Fig. 5). The average helix tilt angle of the PMLGSLG block in the transferred monolayers considerably increases with increasing \(\text{DP}_{\text{PMLGSLG}}\). The helix tilt angle of the PLGA block is slightly larger than that of the PMLGSLG block and changes with the same trend as the PMLGSLG block.

Helix orientation by small angle X-ray reflectivity

The average helix tilt angle can also be calculated from the layer thickness, as determined from small angle X-ray reflectivity measurements (Scheme 3 using eqn (1)). The X-ray reflectivity simulation results for monolayers transferred at different pressures are depicted for CoPo\(_{63}\) as an example in Fig. 12 and 13. At first, curve fits were performed with one-slab models. The best one-slab fits to the reflectivity curves for the LB monolayers of CoPo\(_{63}\) transferred onto silicon substrates at 6 and 20 mN m\(^{-1}\) are shown in Fig. 12. At 6 mN m\(^{-1}\) in the π-\(\cal{A}\) isotherm, the monolayer is at the beginning of the transition corresponding to the immersion of the PLGA block in water.

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Fig. 8  Curve fits of the transmission FT-IR spectra of the multilayer LB film of PMLGSLG (18 layers on each side of the silicon substrate) deposited at 20 mN m\(^{-1}\) (a), the film of PLGA spin-coated from a 1 wt% solution in dimethylformamide onto a silicon substrate at a speed of 5000 rpm (b) and the LB monolayer of CoPo\(_{63}\) deposited onto a silicon substrate at 40 mN m\(^{-1}\) (c). The solid lines represent the fitted curves, while the dotted lines represent the measured spectra.
Table 1  Band assignment and curve fit parameters for the transmission FT-IR spectra of PMLGSLG, PLGA and PLGA-b-PMLGSLG

<table>
<thead>
<tr>
<th>Vibration</th>
<th>Observed</th>
<th>PLGA-b-PMLGSLG diblock Copolymers</th>
<th>In literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency (FWHH, contour shape/cm⁻¹, f_C)/cm⁻¹</td>
<td>Frequency (FWHH, contour shape/cm⁻¹, f_C)/cm⁻¹</td>
<td>Frequency (FWHH, contour shape/cm⁻¹, f_C)/cm⁻¹</td>
</tr>
<tr>
<td>PMLGSLG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C=O (ester)</td>
<td>LB film</td>
<td>LB film</td>
<td></td>
</tr>
<tr>
<td>Amide I (transition dipole interactions between helices)</td>
<td>1738.5 (20, 0.65)</td>
<td>1738–1740 (21, 0.65)</td>
<td>1737–173822,28 (2648)</td>
</tr>
<tr>
<td>Amide I (z-helix, r_I)</td>
<td>1672</td>
<td>1669–1672 (for both blocks)</td>
<td>1670–168222,44</td>
</tr>
<tr>
<td>Amide II (z-helix, r_I)</td>
<td>1551 (14.5, 0.45)</td>
<td>1550–1551 (15–16, 0.45)</td>
<td>155022,28 (14.4 0.44)</td>
</tr>
<tr>
<td>Overtone of Amide V</td>
<td>1540</td>
<td>1537–1539 (for both blocks)</td>
<td>1536–153845</td>
</tr>
<tr>
<td>Amide II (z-helix, r_I)</td>
<td>1520 (35, 1)</td>
<td>1513–1518</td>
<td>1510–151743,46</td>
</tr>
<tr>
<td>PLGA</td>
<td>Spin-coated film from DMF/LB film</td>
<td>LB film</td>
<td></td>
</tr>
<tr>
<td>C=O (acid, non-hydrogen bonded)</td>
<td>1734 (35, 1)</td>
<td>1735–1737 (26–35, 1)</td>
<td>1725–173843,47 (50 0, 49)</td>
</tr>
<tr>
<td>Amide I (anti-parallel β-sheet, r_I)</td>
<td>1690 (19, 0.4)</td>
<td>1685–1691</td>
<td>1685–169245</td>
</tr>
<tr>
<td>Amide II (transition dipole interactions between helices)</td>
<td>1549 (19, 0.45)</td>
<td>1546–1548 (20, 0.45)</td>
<td>1540–155143,46,40 (17 for stable z-helix or 33 for unstable z-helix,4,41,44,45,48)</td>
</tr>
<tr>
<td>Overtone of Amide V</td>
<td>1538</td>
<td>1537–1539 (for both blocks)</td>
<td>1536–153843</td>
</tr>
<tr>
<td>Amide II (anti-parallel β-sheet, r_I)</td>
<td>1526 (20, 0.4)</td>
<td>1526–1530</td>
<td>1520–153045</td>
</tr>
<tr>
<td>Amide II (z-helix, r_I)</td>
<td>1514 (20, 0.4)</td>
<td>1513–1518</td>
<td>1510–151743,46</td>
</tr>
</tbody>
</table>

The one-slab fit gives a film thickness of 1.75 nm, a film electron density of 0.370 × 10⁻¹ e⁻ nm⁻³ and a roughness of 0.69 nm (Fig. 12a). These values are similar to those reported for a monolayer of PMLGSLG with the helices parallel to the substrate.²⁸ This confirms that at 6 mN m⁻¹ the PLGA-b-PMLGSLG monolayer is in the first regime as described in Scheme 2a. At a transfer pressure of 20 mN m⁻¹, the best one-slab fit gives an unreasonably high electron density of 0.505 × 10⁻¹ e⁻ nm⁻³ and the curve fit is insufficient at high scattering angles (Fig. 12b).

Instead, as shown in Fig. 13, the two-slab models resulted in good fits of the reflectivity curves for the films transferred at 20 mN m⁻¹ and at higher pressures. The first slab is assumed for the PLGA layer and the second slab for the PMLGSLG layer. The good fits using the two-slab models confirm the third regime of double-brush formation observed in the π–A isothersms. The increase in monolayer thickness, corresponding to a decrease in the helix tilt angle with increasing transfer pressure as demonstrated for CoPo_63_39 at the range of 6–40 mN m⁻¹, agrees well with the isotherm and transmission FT-IR results.

With the exception of CoPo_50_11, good two-slab fits were also obtained for the reflectivity curves of the LB monolayers of the other PLGA-b-PMLGSLG diblock copolymers transferred at 35–45 mN m⁻¹. The resulting curve fits are shown in Fig. 14 (see ESI) for electron density profiles and fit parameters. The electron densities of the first and second slab corresponding to the PLGA and PMLGSLG layer were determined to be 0.474 × 10⁻¹ to 0.496 × 10⁻¹ and 0.350 × 10⁻¹ to 0.389 × 10⁻¹ e⁻ nm⁻³, respectively. From the reported density and specific volume, the core density of esterified polyglutamates ρ = 1.58 g cm⁻³.⁴⁰ The electron density of PMLGSLG derived from multilayer studies was reported to be 0.360 × 10⁻¹–0.381 × 10⁻¹ e⁻ nm⁻³ (from ρ = 1.1–1.154 g cm⁻³).⁴¹ Therefore, the electron density values obtained by the two-slab fits are reasonable. The ±2% difference in electron density from the reported limit values of the PMLGSLG slab for some PLGA-b-PMLGSLG LB monolayers probably arises from the different order of interchain interactions between the PMLGSLG block segments.
Table 2  Average helix tilt angles calculated from the transmission FT-IR and X-ray reflectivity results for the LB monolayers of PLGA-b-PMLGSLGs

<table>
<thead>
<tr>
<th>Entry</th>
<th>Maximum transfer pressure/mN m⁻¹</th>
<th>Transmission FT-IR</th>
<th>X-Ray reflectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PLGA block</td>
<td>PMLGSLG block</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AI/AII</td>
<td>θ/°</td>
</tr>
<tr>
<td>1</td>
<td>PMLGSLG</td>
<td>6.69</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>PLGA</td>
<td>3.92</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>CoPo_63_39</td>
<td>40</td>
<td>77</td>
</tr>
<tr>
<td>4</td>
<td>CoPo_37_24</td>
<td>45</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>CoPo_60_19</td>
<td>40</td>
<td>69</td>
</tr>
<tr>
<td>6</td>
<td>CoPo_59_25</td>
<td>45</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>CoPo_45_38</td>
<td>40</td>
<td>68</td>
</tr>
<tr>
<td>8</td>
<td>CoPo_53_63</td>
<td>40</td>
<td>76</td>
</tr>
<tr>
<td>9</td>
<td>CoPo_59_82</td>
<td>35</td>
<td>77</td>
</tr>
<tr>
<td>10</td>
<td>CoPo_50_11</td>
<td>40</td>
<td>36</td>
</tr>
</tbody>
</table>

Fig. 10  Comparison of the transmission FT-IR spectra for the region of 1800–1475 cm⁻¹ of the LB monolayers of PLGA-b-PMLGSLGs (transfer pressures listed in Table 2) and an LB multilayer of PMLGSLG on silicon substrates. For ease of comparison, the spectral absorption intensities are normalized relative to the height of the amide II band.

Fig. 11  Correlation of the chain density from the absorption intensities of the side chain C=O stretching vibrations at 1738 and 1711 cm⁻¹ (circles) and the average helix tilt angles of the PMLGSLG block (triangles) and the PLGA block (squares) determined by transmission FT-IR for the LB monolayers of PLGA-b-PMLGSLGs (transfer pressures shown in Table 2).

Fig. 12  Left-hand side: best one-slab fits to the X-ray reflectivity curves of the LB monolayers of CoPo_63_39 transferred onto silicon substrates at 6 (a) and 20 mN m⁻¹ (b); the dots represent the experimental data and the full lines represent the fitted curves; curve a 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).

Fig. 13  Left-hand side: two-slab fits to the X-ray reflectivity curves of the LB monolayers of CoPo_63_39 transferred onto silicon substrates at 20 (a) and 40 mN m⁻¹ (b); the dots represent the experimental data and the full lines represent the fitted curves; curve a 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).
Surprisingly, for the LB monolayer of CoPo\textsubscript{50\_11}, transferred at 40 mN m\textsuperscript{-1}, only a three-slab model gives a good fit of the X-ray reflectivity curve. The fit result is shown in Fig. 15 (see ESI\textsuperscript{†} for fit parameters). As discussed above from the \(\pi–A\) isotherm, it is indicated that part of the stearyl side chains is pushed out off the helix layer. In this case, the electron densities determined for the first, second and third slab are 0.460 \(\times\) 10\textsuperscript{-1}, 0.370 \(\times\) 10\textsuperscript{-1} and 0.284 \(\times\) 10\textsuperscript{-1} e\textsuperscript{-}\ nm\textsuperscript{3}, respectively, attributed to the electron densities of PLGA, PMLGSLG and fluid alkyl chains,\textsuperscript{25} respectively. A thickness of 1.37 nm was derived for the alkyl top layer, corresponding to about 60\% of the full stearyl chain length.

The average helix tilt angles (\(\theta\text{PLGA}\) and \(\theta\text{PMLGSLG}\)) calculated from the layer thicknesses are listed for each block for the LB films of PLGA-\(b\)-PMLGSLGs in Table 2. Fig. 16 compares the values of \(\theta\text{PLGA}\) and \(\theta\text{PMLGSLG}\) determined by the different methods. The results obtained by transmission FT-IR and X-ray reflectivity are in good agreement. A similar tendency for change in \(\theta\text{PMLGSLG}\) observed in the monolayers at the air–water interface is found in the transferred films, indicating that the helix orientation is preserved upon transfer. \(\theta\text{PLGA}\) is larger than \(\theta\text{PMLGSLG}\) and is influenced by the tilt order of the PMLGSLG block. A smaller value of \(\theta\text{PMLGSLG}\) results in a slightly smaller \(\theta\text{PLGA}\). The difference in tilt angle between the two blocks arises from the very large helix cross-sectional area of the PMLGSLG helix compared with PLGA. For most of the diblock copolymers, \(\theta\text{PMLGSLG}\) in the transferred films agrees with that estimated from the \(\pi–A\) isotherms, indicating the absence of relaxation effects in the stabilized monolayers. For the diblock copolymers with a small \(DP\text{PMLGSLG}\), CoPo\textsubscript{60\_19} and CoPo\textsubscript{37\_24}, the helix tilt in the LB films is smaller than that obtained from the \(\pi–A\) isotherms. This indicates that these monolayers relax during stabilization and stabilize to a smaller surface area at the equilibrium state. At equilibrium, the monolayer of CoPo\textsubscript{37\_34} can be compressed to a much smaller area resulting in smaller \(\theta\text{PLGA}\) and \(\theta\text{PMLGSLG}\) compared with CoPo\textsubscript{60\_19}. However, when \(DP\text{PMLGSLG}\) is very small (i.e.11), the \(\alpha\)-helices of the PMLGSLG block are oriented nearly perpendicular to the interface and the long alkyl side chains are partially expelled from the brush layer.

For \(DP\text{PMLGSLG} > 39\) in the transferable region (\(\leq 40\) mN m\textsuperscript{-1}), the \(\alpha\)-helices are oriented isotropically around the surface normal, probably to minimize the unfavorable dipole-dipole interactions between neighboring segments since in such a phase the dipole moments are partly antiparallel to some extent. However, upon compression to high surface pressures, they exhibit a transition to a liquid-crystalline-like phase. For these diblock copolymers at high surface pressures (>40 mN m\textsuperscript{-1}) and those with \(DP\text{PMLGSLG} \leq 39\), the PMLGSLG block segments are
preferentially oriented parallel to one another in a liquid-crystalline-like phase. The interactions of the off-axis dipole components between parallel helices may tentatively account for the formation of this smectic C-like phase as well as the increase in the helix tilt angle with increasing DP_{PMLGSLG}.

Conclusions

We have demonstrated the chain length dependence of the helix orientation in double-brush Langmuir and LB monolayers of PLGA-b-PMLGSLG amphiphilic diblock copolypeptides. The average tilt angle between the helices and the surface normal decreased upon compression as a result of the increase in chain density. In particular, the hydrophobic block length was found to significantly affect the maximum surface chain density and hence the helix orientation of the diblock copolypeptides. Monolayers were transferred at 6–10 mN m⁻¹ below the highest surface pressures (in the transferable region) in the isotherms. Decreasing DP_{PMLGSLG} resulted in a decrease in the helix tilt angle. For the diblock copolymers with DP_{PMLGSLG} < 25, relaxation during stabilization was observed and for a smaller DP_{PLGA}, at equilibrium the monolayer can be compressed to relax during stabilization was observed and for a smaller chain density. For this system and when the side chains of the diblock copolypeptides. Monolayers of DPPMLGSLG they seem to orient isotropically around the water surface normal. Upon compression to a high surface pressure, the isotherms of the diblock copolymers with DP_{PMLGSLG} > 39 showed a transition to a smectic C-like phase. The origin of the decrease in helix packing ability in monolayers as a function of increasing hydrophobic block length is not fully understood. However, from the surface potential measurements, we found that the effective dipoles of the α-helices of the PLGA block in Langmuir monolayers were significantly suppressed due to water screening effects, whereas those of the PMLGSLG block were largely maintained and increased with DP_{PMLGSLG}.

Therefore, the helix tilt in the monolayers most likely originates from the off-α-axis interactions of the unscreened peptide dipoles between parallel α-helices. The correlation of the helix tilt angle and the PMLGSLG block length tentatively arises from a change in the tilting potential as a function of the number of dipoles.

Acknowledgements

This research is supported by NanoNed, a nanotechnology program of the Dutch Ministry of Economic Affairs.

Notes and references

33 H. Block, Poly(γ-Benzyl-L-Glutamate) and Other Glutamic Acid Containing Polymers, Gordon and Breach Publishers, New York, 1983.
34 The amide I band at 1653 cm⁻¹ in the spectra of the PtBuLG-b-PMLGSLG precursors, even for a very small DP_{PMLGSLG} of 11, is narrow, characteristic of a completely α-helix structure (see ES1† for FT-IR spectra). The β-sheet amide I band at 1626 cm⁻¹ appears as a small shoulder only after removing the tert-butyl group. In addition, the β-sheet amide I band at 1626 cm⁻¹ disappears when the PLGA block is ionized at a subphase pH 8.6, suggesting that the β-sheets belong to the PLGA block (see ref. 21).
35 Both the antiparallel β-sheet amide I (1626 cm⁻¹, perpendicular to the sheet fiber) and amide II (1526 cm⁻¹, parallel to the sheet fiber) vibrations are in the plane of the sheet. From the spectra of the PLGA-b-PMLGSLG monolayers, the decreasing β-sheet amide I to amide II band absorption intensity ratio correlates with the helix tilt angle of the PLGA block, suggesting that the β-sheet fiber is tilted from the substrate. Thus, the dipole moment of the β-sheet amide I vibration is parallel to the substrate and its absorption intensity does not change irrespective of the β-sheet tilt order. The β-sheet content, arising mainly from the PLGA block, was roughly estimated by comparing the absorption intensities of the α-helix and β-sheet amide I bands, taking into account the average helix tilt angles.
38 The film was prepared by spreading PLGA on the water surface at subphase pH 2, followed by surface compression to 4 mN m$^{-1}$, vertical dip and subsequent withdrawal of the substrate through the polymer layer.


43 Y. N. Chirgadze and E. V. Brazhnikov, Biopolymers, 1974, 13, 1701.


46 J. L. Koenig and B. Frushour, Biopolymers, 1972, 11, 1871.


