Coiled-coil mediated liposomal fusion: Asymmetric behaving peptide fusogens
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CHAPTER III

INFLUENCE OF MEMBRANE-FUSOGEN DISTANCE ON THE SECONDARY STRUCTURE OF COILED-COIL PEPTIDES
ABSTRACT

Liposomal membrane fusion is an important tool to study complex biological fusion mechanisms. We use lipidated derivatives of the specific heterodimeric coiled-coil pair E: (EIAALEK)_3 and K: (KIAALKE)_3 to study and control the fusion of liposomes. In this model system peptides are tethered to their liposomes via a poly(ethylene glycol) (PEG) spacer and a lipid anchor. The efficiency of the fusion mechanism and function of the peptides is highly affected by the PEG-spacer length and the lipid anchor type. Here, the influence of membrane-fusogen distance on the peptide-membrane interactions and the peptide secondary structures is studied with Langmuir film balance and infrared reflection absorption spectroscopy (IRRAS). We found that the introduction of a spacer to monolayer tethered peptide E changes its conformation from solvated random coils to homo-oligomers. In contrast, the described peptide-monolayer interaction of peptide K is not affected by the PEG-spacer length. Furthermore, the co-existence of different conformations when both CPE and CPK are present at the membrane surface is demonstrated empirically, which has many implications for the design of effective fusogenic recognition units and the field of artificial membrane fusion.
INTRODUCTION

Natural membrane fusion is a key process for cellular logistics and signaling. Thus, it has been extensively studied for decades. Whilst the overall process of membrane fusion is well understood, mechanisms at the molecular level still are a matter of debate due to the intrinsic complexity of biological systems. To promote the understanding of the naturally occurring fusion machinery and to identify important parameters in such mechanisms, a variety of functional mimics were developed recently. In our group, we employed the lipidated heterodimeric coiled-coil pair E (EIAALEK) and K (KIAALKE) as a molecular recognition motif and fusogen. The peptides are held in lipid membranes by a lipid anchor, either cholesterol (C) or 1,2-dioleoyl-sn-glycero-3-phosphoethanol-amine (DOPE), and this is conjugated to the peptide via a PEG-spacer of variable length to yield C/LPE and C/LPK. The ability of these lipopeptides to effectively mediate liposomal fusion both in vitro and in vivo has been shown in several papers.

In these studies, coiled-coil formation between E and K was thought to initiate the fusion of the bilayers. However, it was demonstrated recently that peptide K can insert into the lipid membrane because of the amphipathic nature of its α-helix. Peptide K orients its helical axis parallel to the monolayer surface with the hydrophobic moment of the α-helix pointing towards the hydrophobic part of the monolayer. It is anticipated that K interacts with the negatively charged phosphate moieties of the phospholipids via ‘snorkeling’ its positively charged Lys residues towards the polar region of the bilayer. The membrane insertion of peptide K may locally disrupt the uniformity of the membrane in its proximity, promoting the formation of protrusions. It is hypothesized that such protrusions are a fundamental step in the merging of the opposing membranes in the fusion process.

Until now, the peptide conformation of peptides E and K on membrane surfaces was studied using LP12 functionalized derivatives, because significant fusion efficiency was obtained when liposomes were functionalized with these peptides. However, liposome fusion mediated by cholesterol anchored peptides CP12E / CP12K, is twice as efficient compared to DOPE anchored derivatives, which raised interest in the influence of the anchor on the peptide dynamics. Furthermore, it was found recently that the membrane-
fusogen distance as defined by PEG spacer length is crucial for efficient fusion, and highest fusion efficiency was obtained with CP_{12}E and CP_{8}K. Measuring the dependency of the CP_{n}E and CP_{n}K fusion efficiency on \( n \) revealed the distinct asymmetric roles of both peptides in the fusion process (Figure 10). Liposome tethered peptide E is thought to act as a handle to enable docking of peptide K decorated liposomes, with the effectiveness of this handle being enhanced by elongated spacers. After docking, fusion process is dictated by peptide K via incorporation into both opposing liposomal membranes, which destabilizes both lipid membranes and promotes the formation of fusion intermediates. The results suggested that equilibria between these multiple peptide interactions in the docking state are affected by anchor type and PEG-spacer length of the utilized lipopeptides. Furthermore, it is anticipated that the incorporation of CP_{n}K in the opposing liposomal membrane is crucial for efficient fusion. It is hypothesized that this highly dynamic docking state is rate-limiting the fusion cascade.

Here we study the influence of the membrane-fusogen distance on the peptide structure and the peptide-membrane interaction. A systematic variation of CP_{n}E and CP_{n}K was used by varying the length of the PEG-spacer from 0 to 16 monomers in steps of 4 monomers. The ability of the peptide to immerse into a lipid monolayer was measured by Langmuir film balance, while IRRA spectroscopy was used to determine the peptide orientation with respect to the monolayer, and the solvent exposure of the peptide bonds. The results found for CP_{12}E/CP_{12}K are compared with the results reported for LP_{12}E/LP_{12}K derivatives to assess the influence of the anchor on the peptide structure.

**Design and Synthesis**

For peptide detection and quantitation, peptide E was functionalized at the C-terminus with a glycine and a tyrosine residue, while peptide K was equipped with a C-terminal glycine and a tryptophan residue. A PEG series P_{n} was used with 0, 4, 8, 12, and 16 units of ethylene glycol, and cholesterol was used as the anchor moiety. The molecular structures and used peptide sequences are shown in Figure 10. Peptides were synthesized with automated solid phase peptide synthesis (SPPS) using Fmoc chemistry,
and after coupling of the final amino acid, the PEG component and the anchor moiety were coupled manually. The lipopeptides were cleaved from the resin and subsequently purified using reverse phase chromatography yielding purities >95%. Details of the synthetic procedures are described in Chapter II.¹⁶

RESULTS AND DISCUSSION

PROTEIN-MEMBRANE INTERACTION BY LANGMUIR FILM BALANCE

The interactions of the various CPₙE and CPₙK derivatives with membranes were investigated by compression isotherms of lipid monolayers functionalized with peptides. (Figure 11, Figure 12, Figure 13) Monolayers with 4% lipopeptide were prepared on PBS pH 7.4 and the surface pressure (σ) was measured as a function of molecular area (A) during compression and expansion of the monolayer. The σ/A isotherm of the pure lipid mixture (DOPC : DOPE : Cholesterol (2 : 1 : 1)) showed no irregularities such as plateaus or break points, which is consistent with monolayers in a liquid expanded state.
without phase transitions. With lipopeptides tethered to the monolayer, all \( \pi/A \) isotherms showed higher molecular areas at low pressures (0 – 15 mN/m) compared to the isotherm of the pure lipid film.

The absorption of peptides to lipid monolayers can be analyzed by calculation of lateral compressibility of the lipid film, \( C_S = -1/A \left( \delta A/\delta \pi \right)_T \). The observed plateaus in \( \pi/A \) isotherms of CP\(_n\)E are converted to peaks in \( C_S/\pi \) isotherms. We interpret these plateaus or peaks to be caused by a squeeze-out of peptide material from the monolayer into the subphase, as was found previously with untethered peptides E and K.\(^{17}\)

**MONOLAYERS FUNCTIONALIZED WITH CP\(_n\)K**

Upon compression of CP\(_n\)K functionalized monolayers a uniform surface pressure increase was observed until the membrane collapsed around \( \sim 35 \) mN/m. (Figure 11). The isotherms were shifted to higher molecular areas with respect to the pure lipid mixture, indicating the incorporation of K into the monolayer. Similar results were found for LP\(_{12}\)K functionalized monolayers.\(^{17}\)

These results showed that the anchor and various spacers did not inhibit the peptide-membrane interaction, regardless of their type and length. The compressibility of the lipopeptide decorated monolayers were found to be slightly increased compared to the pure monolayer. The increase in molecular area is similar for all spacer variants and no squeeze out was observed for any variant. Although, slightly higher values for A were observed for CP\(_{16}\)K at low surface pressures, and slightly lower values for A were observed for CP\(_0\)K above a surface pressure of 20 mN/m.

**MONOLAYERS FUNCTIONALIZED WITH CP\(_n\)E**

Upon compression of CP\(_n\)E functionalized monolayers, plateaus were observed at intermediate surface pressures (15-20 mN/m). Further compression above these plateaus led to isotherms comparable to those of the pure lipid film, as shown in Figure 12. The observed plateaus indicate the squeeze out of CP\(_n\)E upon increasing surface pressure. The reversibility of the squeeze out of CP\(_n\)E is demonstrated by the occurrence of plateaus at pressures around \( \pi_{SO} \) during both stages of compression/expansion cycles. (Figure S5) \( \Delta A \) calculation of the \( \pi/A \) graphs at 10 mN/m reveal that with increasing
PEG-INFLUENCE ON PEPTIDE SECONDARY STRUCTURE

Figure 11. Results of compression experiments with monolayers (lipid composition DOPC : DOPE : Cholesterol 2 : 1 : 1), functionalized with 4 mol% CP₄K. a) surface pressure (π) against molecular area (A), b) lateral compressibility $C_s = -1/A (\delta A/\delta \pi)$ of the lipid film against π. PBS, pH 7.4, 20 °C.

Table 4. Characteristics of monolayer experiments, with standard lipid composition and 4 mol% CP₄E.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>$\pi_{SO}^{a}$ (mN/m)</th>
<th>$A_{SO}^{b}$ (Å²)</th>
<th>$A_{SO}-A_{E}^{c}$ (Å²)</th>
<th>$A_{PEG}^{c}$ (Å²)</th>
<th>%E incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP₄E</td>
<td>18.8</td>
<td>8.1</td>
<td>-5.7</td>
<td>0.6</td>
<td>59</td>
</tr>
<tr>
<td>CP₄E</td>
<td>17.1</td>
<td>10.7</td>
<td>-3.1</td>
<td>3.0</td>
<td>78</td>
</tr>
<tr>
<td>CP₆E</td>
<td>16.1</td>
<td>14.1</td>
<td>0.3</td>
<td>5.4</td>
<td>100</td>
</tr>
<tr>
<td>CP₁₂E</td>
<td>16.0</td>
<td>20.9</td>
<td>7.1</td>
<td>7.8</td>
<td>100</td>
</tr>
<tr>
<td>CP₁₆E</td>
<td>16.0</td>
<td>24.3</td>
<td>10.5</td>
<td>10.1</td>
<td>100</td>
</tr>
</tbody>
</table>

$^{a}$ Squeeze-out pressures ($\pi_{SO}$) are determined from peak position in $C_s/\pi$ graph. $^{b}$ Experimental squeeze-out surfaces ($A_{SO}$) were obtained via $\Delta A$ calculation of $\pi/A$ graphs between 10 mN/m and 23 mN/m with pure lipid isotherm as baseline. $^{c}$ Theoretical occupied surface areas of lipopeptides ($A_{E}$, 13.8 Å²) and PEG-spacers ($A_{PEG}$, 0.598(n+1) Å²) were obtained via assumption of stiff rod models with appropriate molecular dimensions.
PEG-spacer length \( n \) an increasing amount of molecular area is lost. (Table 4) To estimate the occupied surface area of peptide E we assumed a simple model for its helix of a stiff rod (diameter 10 Å, length 34.5 Å, see SI), and the expected loss of molecular area if all helices are squeezed out is 13.8 Å\(^2\) for 4 mol% of lipopeptide. For CP\(_0\) and CP\(_4\) derivatives, ∆A is less than expected, implying respectively 59% and 78% or less of the helices are incorporated. Due to limited degrees of freedom for peptides equipped with a short spacer, a partial incorporation of all helices in the lipid monolayer is also reasonable.

On the other hand, monolayer tethered CP\(_{12}\)E and CP\(_{16}\)E show an extra contribution in molecular area ∆A. To estimate the theoretical molecular area occupied by a PEG-spacer of length \( n \) we assumed a simple molecular model of a stiff rod (diameter 4.15 Å, length 3.6(n+1) Å, see SI). The estimated surface area is in good agreement with the found extra contribution in lost molecular area. Although PEG is usually regarded as inert, it is known to interact with lipid monolayers at low \( \pi \) especially when conjugated to a membrane constituent.\(^{18-21}\) The observed single peaks in the \( C_S/\pi \) graph show the simultaneous squeeze-out of both spacer and peptide, which demonstrates the interdependent behavior of both peptide and PEG spacer during the squeeze-out process.

The loss in molecular area for CP\(_8\)E containing monolayers is 14.1 Å. This could be explained in two ways: Peptides and spacers both insert partially in the monolayer, or the peptide is completely incorporated in the monolayer leaving no area for the PEG-spacer. Since there is an energy penalty on solvent exposed hydrophobic peptide residues, it is more likely that the peptide fully incorporates in the monolayer, with a solvent exposed PEG-spacer. Thus, we conclude that for CP\(_n\)E peptides, elongated PEG-spacers incorporate in the membrane at low surface pressures, while the PEG-spacer of peptide CP\(_n\)K stays solvated over the whole pressure-range as shown above.

Increasing the spacer length causes a decrease in squeeze-out pressure \( \pi_{SO} \) to 16 mN/m implying an easier squeeze-out for those lipopeptides. This indicates a smaller energy-gap between low and high pressure monolayer states for E with elongated spacers, compared to lipopeptides with shorter spacers. A decreased energy-gap with increasing spacer length could be caused by a stabilization of the peptide conformation upon exclusion from the monolayer. Detailed study of the water accessibility of the peptide
bonds with IRRAS measurements supports the hypothesis that peptides with elongated PEG-spacers can form stabilized homo-oligomers after squeeze-out. \textit{(vide infra)}

**Monolayers functionalized with CP$_n$E and CP$_n$K**

As shown in Figure 13, monolayer films decorated with a mixture of 2 mol\% CP$_n$E and 2 mol\% CP$_n$K show a peak in compressibility at a pressure around 19 mN/m. $\Delta A$ calculation showed that a molecular area of 5.2 Å$^2$ is still occupied by peptides at a surface pressure of 25 mN/m. It was demonstrated previously that when both peptide E and peptide K are present in the proximity of vesicles, peptide K is in an equilibrium between coiled-coil formation with peptide E and membrane insertion.$^{14}$

![Figure 13](image)

**Figure 13.** Results of compression experiments with monolayers (lipid composition DOPC : DOPE : Cholesterol 2 : 1 : 1), functionalized with 4 mol\% CP$_n$E : CP$_n$K (1:1, $n = 0, 12$). a) surface pressure ($\pi$) against molecular area (A), b) lateral compressibility ($C_L$) against $\pi$. PBS, pH 7.4, 20 °C.

Because E-K coiled-coils lack the amphipathic nature needed for strong membrane interaction and E was found to squeeze out at moderate surface pressures, it can be assumed that the increased molecular area compared to pure lipid above the squeeze out pressure was solely caused by membrane-immersed peptide K.

Since peptides E and K were incorporated in equimolar amount in the monolayer, the dynamic equilibrium between the 3 hypothetical peptide conformations can be deduced. Assuming the occupied molecular area at high surface pressure, 5.2 Å$^2$, corresponds to 1.5 mol\% of membrane embedded peptide K, 1.5 mol\% of membrane tethered E is left in a solvated state. The remaining 1 mol\% peptides can be assigned to the E-K coiled-coil complex. The dynamic equilibrium $[EK] : ([E]+[K]) = 1 : 3$, which supports the
previous finding that membrane immersion of K happens concomitant to E/K coiled-coil formation.\textsuperscript{16-17}

**IRRAS Measurements**

To further study the conformation and the alignment of the peptides with respect to the supporting monolayer, we performed angle and polarization dependent infrared reflection absorption spectroscopy (IRRAS) measurements of the described monolayers at the air-water interface. Lipid monolayers containing 4 mol\% CP\textsubscript{n}E and/or CP\textsubscript{n}K were prepared on \textit{d}-PBS buffer pD 7.4 (prepared with pure D\textsubscript{2}O). D\textsubscript{2}O was used to avoid overlapping water vapor contributions of H\textsubscript{2}O in the amide I spectral region. An important spectral region to study lipid and peptide properties is between 1600 and 1800 cm\textsuperscript{-1}. In this region the peptide amide I’ band and the lipid C=O stretching vibrational band can be found, centered around \textasciitilde1640 and \textasciitilde1735 cm\textsuperscript{-1}, respectively. In all experiments the intensity of the C=O band increases upon monolayer compression due to the increasing lipid density in the IR-spot, and the relative intensity of the amide I’ band, $A_{\text{Amide I'}}/A_{C=O}$ (short: AI/CO) is determined to quantify the amount of peptide in the IR-spot.

IRRAS spectra with varying angle of incidence and polarization were recorded at constant surface pressures below and above the observed pressures $\pi_{SO}$, at 10 mN/m and 30 mN/m respectively. The peptide secondary structure was assumed to be on average in an $\alpha$-helical conformation, as shown with circular dichroism.\textsuperscript{16} Since peptide bonds in $\alpha$-helices have comparable orientations, the band intensities of s-polarized spectra are sensitive to surface concentration provided that the helix orientation does not change. Thus, the recorded spectra were averaged over all angles of incidence to compare the results qualitatively. Also, fitting of angle and polarization dependent spectra yields the order parameter $S$ of the peptide bonds. The comparable orientations of amide bonds in an $\alpha$-helical conformation allows the determination of the angle of the helix axis with respect to the membrane normal, i.e. the peptide angle $\theta$. $S$ can have values between -0.5 and 1, parallel or perpendicular to the monolayer plane respectively, and when $S$ is close to 0 it is most likely that the peptides have a random orientation or no defined secondary structure. Detailed procedures and resulting spectra are available in Experimental section, Figure S6 -Figure S14.
Previous studies demonstrated that squeezed-out peptides readily leave the measurement spot by dissociation from the air-water interface into the subphase. However, monolayer tethered lipopeptides are confined close to the air-water interface and are still (partially) detected by the IR-beam, even after a peptide squeeze-out.\(^1\) The obtained \(\theta \approx 90^\circ\) at low surface pressure (10 mN/m), show that all peptides are aligned parallel to the monolayer plane. (Table 5, Table 6) Taken together with the results of the compression isotherms it is evident that the peptides are located in between the phospholipids at the air-water interface, most likely with the hydrophobic moment pointing to the hydrophobic region of the monolayer.

For monolayers functionalized with CP\(_0\)K and CP\(_{12}\)K, AI/CO is more or less equal at both low and high pressure, supporting the incorporation of K in the monolayer at all pressures. Peptide incorporation at higher surface pressure (30 mN/m) is further demonstrated by a parallel orientation of CP\(_0\)K and CP\(_{12}\)K with respect to the monolayer (\(S = -0.5\), Table 5). These results are in agreement with the characteristics of LP\(_{12}\)K decorated monolayers, and no anchor induced effect on the peptide structure or the peptide membrane interaction was found.

### Table 5. Results of IRRAS measurements of monolayers with unbound peptide and monolayer tethered peptide K.

<table>
<thead>
<tr>
<th>Monolayer +</th>
<th>(\pi_{SO})</th>
<th>(\pi)</th>
<th>AI/CO (b)</th>
<th>(S^c)</th>
<th>(\theta^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcK(e)</td>
<td>20.5</td>
<td>5</td>
<td>1.5</td>
<td>-0.5</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.46</td>
<td>-0.45</td>
<td></td>
<td>80</td>
</tr>
<tr>
<td>LP(_{12})K(e)</td>
<td>29.5</td>
<td>15</td>
<td>0.77</td>
<td>-0.47</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.76</td>
<td>-0.49</td>
<td></td>
<td>85</td>
</tr>
<tr>
<td>CP(_0)K</td>
<td>n.a.</td>
<td>10</td>
<td>0.53</td>
<td>-0.5</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.46</td>
<td>-0.5</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>CP(_{12})K</td>
<td>n.a.</td>
<td>10</td>
<td>0.44</td>
<td>-0.5</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.48</td>
<td>-0.5</td>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>

\(a\) Squeeze-out pressure obtained by monolayer compression cycles.  
\(b\) Ratio between observed peptide Amide I’ band and lipid carbonyl band.  
\(c\) Order parameter \(S\), calculated by fitting of angle and polarization dependent IR spectra.  
\(d\) Angle of peptide helix with respect to the membrane normal, derived from \(S\).  
\(e\) Experiments conducted with monolayers and AcK/LP\(_{12}\)K are taken from Rabe et al, 2014.
For CP₃E, Al/CO decreases significantly upon pressure increase, while it stays rather constant for CP₃K (Table 6). This observation supports once more the hypothesis that the plateaus in the π-A isotherms come along with a significant change in the peptide structure and/or orientation with respect to the incident IR-beam. The change in \(S\) to values around 0 at \(\pi = 30\) mN/m supports the squeeze-out of all CP₃E derivatives and indicates that excluded peptides probably have a random orientation of their helix or have no defined structure at all.

The equimolar mixture of CP₃E and CP₃K (\(m = n\)) shows ordered peptide structures below the squeeze-out pressure. Thus both peptides E and K independently incorporate into the monolayer, since the coiled-coil complex lacks an amphipathic nature. The exclusion of peptide E upon monolayer compression is supported here, by a decrease in Al/CO and an increase in \(S\). Due to the occurrence of multiple different peptide conformations at high surface pressures, \(S\) is an average value that is difficult to interpret.

### Table 6. Results of IRRAS measurements of monolayers with unbound peptide and monolayer tethered peptide E and \(E + K\).

<table>
<thead>
<tr>
<th>Monolayer +</th>
<th>(\pi_{SO}^a)</th>
<th>(\pi)</th>
<th>Al/CO(^b)</th>
<th>(Se)</th>
<th>(\theta^d)</th>
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<tbody>
<tr>
<td>AcE(^c)</td>
<td>7</td>
<td>5</td>
<td>1.4</td>
<td>-0.5</td>
<td>89</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LP₁₂E(^c)</td>
<td>17.9</td>
<td>15</td>
<td>0.7</td>
<td>-0.47</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.52</td>
<td>0.05</td>
<td>53</td>
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<td>CP₀E</td>
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<td>0.74</td>
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<td>84</td>
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<td>0.24</td>
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<td>CP₄E</td>
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<td>80</td>
</tr>
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<td>0.26</td>
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<tr>
<td>CP₇E</td>
<td>16.1</td>
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<td>0.52</td>
<td>-0.50</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.39</td>
<td>0.12</td>
<td>50</td>
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<td>CP₁₀E</td>
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<td>0.67</td>
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<td>90</td>
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<td></td>
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<tr>
<td>CP₁₀E</td>
<td>16.0</td>
<td>10</td>
<td>0.84</td>
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<td>30</td>
<td>0.7</td>
<td>-0.08</td>
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<td></td>
</tr>
<tr>
<td>E + K(^e)</td>
<td>9.7</td>
<td>5</td>
<td>2.68</td>
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<tr>
<td></td>
<td>22.5</td>
<td>15</td>
<td>1.71</td>
<td>-0.5</td>
<td>89</td>
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<td>30</td>
<td>0.96</td>
<td>-0.45</td>
<td>80</td>
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<td>LP₁₂E + LP₁₂K(^e)</td>
<td>18.4</td>
<td>15</td>
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<td>-0.23</td>
<td>65</td>
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<td>0.57</td>
<td>0.02</td>
<td>54</td>
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</table>

\(^a\) Squeeze-out pressure obtained by monolayer compression cycles.  
\(^b\) Ratio between observed peptide amide I’ band and lipid carbonyl band.  
\(^c\) Order parameter \(S\), calculated by fitting of angle and polarization dependent IR spectra.  
\(^d\) Angle of amide I’ moiety with respect to the membrane normal, derived from \(S\).  
\(^e\) Experiments conducted with monolayers and AcE/LP₁₂K/LP₁₀E + LP₁₀K are taken from Rabe et al, 2014.
**AMIDE I’ BAND FITTING**

The position of the amide I’ band is highly dependent on the peptide secondary structure and the solvent accessibility of its amide bonds, and is thus an important parameter for structure elucidation.\(^{22\text{-}24}\) For E-K coiled-coil peptides, it is known that the amide I’ bands observed by IRRAS at intermediate surface pressures (15 mN/m) consist of a maximum around 1634 cm\(^{-1}\) and a shoulder around 1651 cm\(^{-1}\).\(^{17}\) Band shape analysis revealed two underlying main contributions that are often found in coiled-coil forming peptides. The two different band positions are caused by differences in hydrogen bonding of carbonyls buried in the hydrophobic core and solvent accessible carbonyls.\(^{23\text{-}29}\)

Water inaccessible amide bonds absorb at higher wavenumbers (1651 cm\(^{-1}\)) than the water accessible ones (1631 cm\(^{-1}\)). For membrane incorporated peptides, these differences in water-accessibility of the carbonyls is caused by the alignment of the peptides at the hydrophobic/hydrophilic interface and a partial insertion of hydrophobic side-chains into the hydrophobic part of the monolayer.\(^{14}\)

Normalized S-polarized IRRAS spectra, averaged over all angles of incidence, were fitted with 3 gauss peaks at positions around 1631 cm\(^{-1}\), 1651 cm\(^{-1}\), and 1672 cm\(^{-1}\) (Figure 14, Figure S15 - Figure S23). The nature of the third minor contribution around 1672 cm\(^{-1}\) is unclear; it was assumed previously to be caused by residual TFA from HPLC purification of the lipopeptides,\(^{30}\) but can also be assigned to C-terminal CONH\(_2\) since the absorption occurs consistently in all spectra. However, most useful information is conserved in the intensities of the peaks at 1631 cm\(^{-1}\) and 1651 cm\(^{-1}\). The relative intensity of the peak at 1631 cm\(^{-1}\) (hydrophilic fraction, as percentage of total amide I’ band intensity) indicates the relative hydrophilicity of the environment of monolayer-tethered peptides, provided that the secondary structures are similar.

Monolayer incorporated peptides at low pressure show a hydrophilic fraction of 40 - 50%, which was found for all peptides consistently throughout the compression experiments. (Figure 15, Figure 16) At high pressure however, spacer and peptide-dependent trends are visible in the relative hydrophilicity of peptide helices.

For CP\(_n\)K the hydrophilic fraction is still around 50% at high pressure, suggesting only minor differences in water accessibility. Also, the overall peptide orientations remain parallel to the membrane upon compression.
Figure 14. Observed peptide amide I’ bands of lipopeptide decorated monolayers at surface pressure of 10 mN/m and 30 mN/m. Peak positions at 1631 and 1651 cm$^{-1}$ are marked with dotted lines. Monolayers consists of 4 mol% lipopeptide, D$_2$O PBS pH 7.4, with angle-averaged spectra of s-polarized light.

Figure 15. Left: Shape-analysis of amide I’ band (1610-1680 cm$^{-1}$) of lipopeptide decorated monolayers at surface pressure of 10 mN/m (black) and 30 mN/m (red). The amide I’ band is fitted with three Gaussian peaks (1631, 1651 and 1671 cm$^{-1}$) and the hydrophilic fraction is defined as: (surface area of Gaussian peak at 1631 cm$^{-1}$)/(total surface area of amide I’ band). Right: Calculated peptide orientation $\theta$ of monolayer tethered peptides at surface pressure of 10 mN/m (black) and 30 mN/m (red). Monolayers consists of 4 mol% lipopeptide, D$_2$O PBS pH 7.4.
Figure 16. Conformations of lipidated peptides in monolayers. CPₖₖ is immersed in the monolayer at both low and high surface pressure while peptide CPₖₑ is expelled upon compression of the lipid monolayer. Compressed monolayers containing a mixture of CPₑₑ and CPₖₖ suggest the simultaneous presence of hetero dimeric coiled-coil (EK), individual solvated (E), and membrane-bound (K) peptides. Lipid composition is DOPC : DOPE : Cholesterol 2 : 1 : 1.

These findings are further evidence for membrane immersed helices of peptide K, with no apparent influence of spacer length n. For E-peptides the hydrophilic fraction increases which points to a more hydrophilic environment at high pressure. High solvent exposure of peptides is suggested by a significant decrease of the 1651 cm⁻¹ absorption band, and this is most pronounced for CP₀ₑ containing monolayers.

Remarkably, the hydrophilic fraction at high surface pressure decreases with increasing spacer length, and for CP₁₀ₑ, the value is similar at both high and low pressure. Since all CPₙₑ derivatives are excluded from the monolayer and show order parameters close to 0 at high pressure, we attribute the increasing 1651 cm⁻¹ absorption in the Amide I’ band to the formation of less water accessible hydrophobic pockets of CPₙₑ homo-oligomers,²⁸, ³¹ which thus becomes more pronounced for elongated spacers. So for this lipopeptide, two trends are observed with increasing PEG-spacer length. A decrease in squeeze-out pressure was measured with increasing spacer length. Secondly, at high surface pressure, peptide bonds were found to experience a partial hydrophobic environment, and the effect became more pronounced with increasing spacer length. These two observations suggest the increasing presence of homo-oligomer formation of monolayer tethered peptide E with increasing PEG-spacer length, at room temperature. When these results are compared with the reported characteristics of LP₁₂ₑ, similar values of πSO and AI/CO are found for CP₀ₑ rather than for CP₁₂ₑ.
For mixtures of CP₀E + CP₀K and CP₁₂E + CP₁₂K in the lipid monolayer, the hydrophilic fraction at low and high pressures are close to 50%. This demonstrates that peptide bonds in monolayer-excluded peptides still experience a combined hydrophilic/hydrophobic environment, which is in agreement with the formation of solvated hetero-oligomers. At high surface pressure, orientation changes were found for CP₀E/K and CP₁₂E/K, which supports a random orientation of solvated heterodimeric coiled-coils of E/K peptides.

**CONCLUSIONS**

In this study, we showed that the peptide-monolayer interaction of the E/K fusion model system is affected by the lipid anchor and the length of the conjugated PEG-spacer. Monolayer-tethered peptide K incorporates in the monolayer up to surface pressures of 30 mN/m. These peptides are known to incorporate in phospholipid bilayers, due to their amphipathic α-helical structure. Slight differences were found between the derivatives; the absence of a spacer hinders full monolayer incorporation, as CP₀K endured a partial squeeze-out at high surface pressure, although the peptide orientation remained parallel to the monolayer. Remarkably, PEG-spacers did not incorporate in the membrane at any given surface pressure, although a slightly increased molecular area was found for CP₁₀K at π < 10 mN/m. No squeeze out was measured for CPₙK up to surface pressures of 30 mN/m, in contrast to LP₁₂K, which partially squeezed out at this pressure.

All monolayers functionalized with CPₙE showed a plateau during compression-expansion cycles, which evidenced the squeeze-out of peptide material at high surface pressure. Furthermore, elongated PEG-spacers were found to immerse together with the conjugated peptide in the phospholipid monolayer at low surface pressures and were found to promote the formation of homo-oligomers at high surface pressure.

Compression experiments on monolayers consisting a mixture of CPₙE and CPₙK supports the simultaneous existence of coiled-coil structures and membrane bound peptides.

The length of the PEG spacer of peptide CPₙK was found to be the major factor influencing the fusion efficiency of CPₙE/CPₙK decorated liposomes. We hypothesized
that the PEG-spacer length \( n \) of peptide K is crucial for efficient fusion by defining the distance between opposing, coiled-coil connected liposomes. Another possible explanation – the membrane interaction of peptide K is affected by \( n \), thereby influencing the fusion efficiency – could not be supported here. Peptide CP\(_{n}E\) exhibits a minor enhancement of the fusion process with increasing spacer length. This can be attributed to an increased accessibility of peptides with elongated spacers and/or to the increased tendency of homo-oligomer formation for peptides with elongated spacers.
EXPERIMENTAL SECTION

MATERIALS

DOPC, DOPE and cholesterol were purchased from Avanti Polar Lipids. Chloroform, methanol (both HPLC grade), D_{2}O (99.9 % deuterium content), buffer salts and HCl and DCl (99% deuterium content) for pH/pD adjustment, were purchased from Sigma-Aldrich. All water was ultrapure with resistance ≥ 18 MΩ and TOC ≤ 2 ppm produced from a MilliQ Reference A+ purification system. All monolayer and IR experiments were carried out in phosphate buffered saline of the following composition: 150 mM NaCl, 20 mM PO_{4}^{3−} in H_{2}O (PBS) or D_{2}O (d-PBS) at pH / pD 7.4, respectively.

METHODS

CALCULATION OF LIPOPEPTIDE DIMENSIONS

To estimate the dimensions of the used lipopeptides we assumed stiff rod models for both peptide and PEG components. Since the movements of lipopeptides at monolayers is regarded predominantly 2-dimensional, bending of the linear molecule will not alter occupied surface area significantly.

Peptide helices have a diameter of 10 Å, 3.6 residues per turn and a pitch of 5.1 Å. This gives a peptide length of 34.5 Å for 23 residues.

The PEG length is estimated via calculation of stretched end-to-end distance of the PEG-chain in ChemDraw, which yielded an average length of 3.6 Å per ethyleneglycol unit. The length of the succinimide moiety is comparable to one unit of ethyleneglycol and covered by factor n+1. A diameter of 4.15 Å is found for any primary alkylchain and a good approximation of the diameter of a polyethyleneglycol chain.

MONOLAYER PREPARATION

Lipid solutions of DOPC : DOPE : Cholesterol 2 : 1 : 1 ([total lipid] = 1 mM) with 0 or 4 mol% lipopeptides were prepared in CHCl_{3}/CH_{3}OH 3:1 vol%. For the preparation of a lipid monolayer a distinct volume of these solutions was spread on PBS buffer at 25 °C by means of a microliter syringe, the solvent was allowed to evaporate and the film to equilibrate for at least 15 minutes. A microbalance was used for surface pressure measurements using a filter paper as pressure probe.
Initial cleaning of the troughs was achieved by means of diluted detergent solution (2% Hellmanex (Hellma Analytics, Müllheim, Germany) in H2O), followed by extensive rinsing with ~50°C Milli-Q water, wiping with CHCl3/MeOH and final multiple rinsing with Milli-Q water. All Langmuir trough setups were held constant at 25 °C by means of a circulating water bath and were isolated from the environment by plastic covers.

**COMPRESSION-EXPANSION CYCLES**

For compression-expansion cycles of lipid monolayers containing monolayer tethered peptides CPnE and/or CPnK, monolayers on PBS were prepared. After equilibration, compression-expansion cycles of the monolayer were performed with a compression/expansion speed of 3 Å² / (molecule min), and experiments were repeated twice to ensure reproducibility of the isotherm shape. For determination of lateral compressibility, compression isotherms over a surface pressure of 0 to 30 mN/m were smoothed by means of a 60 point adjacent average method and the film compressibility was calculated as:

\[ C_s = \frac{-1}{A} \times \left( \frac{\delta A}{\delta \pi} \right)_T \]

with molecular area \( A \) and surface pressure \( \pi \).
**Figure S5.** Results of compression-expansion experiments with monolayers (lipid composition DOPC : DOPE : Cholesterol 2 : 1 : 1) functionalized with 4% lipopeptide CP₁₂K or CP₁₂E. Surface pressure (\(\pi\)) against molecular area (\(A\)) of compression-expansion cycles show reversible squeeze-out of \(E\) peptides, and a loss of monolayer material over the course of the experiment. PBS, pH 7.4, 20 °C.

**IRRAS MEASUREMENTS**

IRRAS measurements were performed on a BRUKER Vector 70 FT–IR spectrometer equipped with a nitrogen cooled MCT detector and an A511 reflection unit (Bruker Optics, Germany), placed over the Langmuir trough setup (Riegler & Kierstein, Germany). The sample trough (30 x 6 cm²) was equipped with a Wilhelmy balance using a filter paper as pressure probe. A circular reference trough (\(r = 3\) cm) placed next to the sample trough can be brought into the focus of the IR beam by means of a shuttle. The filling levels of both troughs were kept equal and constant by means of an automated, laser reflection controlled, pumping system connected to reservoirs of D₂O.

Lipid monolayers containing the lipopeptides were prepared as described above, on a subphase of d-PBS. The pD was set to 7.4 by adding 0.4 to the read-out of a standard pH-meter. IRRA spectra were recorded at constant surface pressures below (10 mN/m) and above (30 mN/m) the expected squeeze out pressure of the peptides.
**DETAILS OF IRRA SPECTRA MEASUREMENTS**

At each pressure at least 2 cycles of angle dependent IRRA spectra were recorded. For each spectral cycle s- and p-polarized spectra were recorded at incident angles varying from 25° to 70° with an increment of 3°, while spectra close to the Brewster angle (52°, 55° and 58°) were discarded. For each sample reflectance spectrum (R) a total number of 2000 scans at a resolution of 8 cm\(^{-1}\) and a zero-filling factor of 2 were averaged. Reference reflectance spectra (R\(\text{0}\)) were recorded at the same experimental conditions at an identical subphase without monolayer. The reflectance absorbance spectra were calculated as \(R\!A = -\log (R / R\!0)\).

RA spectra recorded at identical polarization and angle of incidence were averaged and corrected with a \(d\!\)-PBS background spectrum prior to data evaluation to minimize spectral contributions of water vapor absorptions and to improve the signal to noise (S/N) ratio. Additional mathematical baseline corrections were avoided as much as possible. All spectra obtained in s-polarization were averaged for obtaining spectra with good S/N ratio being interpretable in terms of secondary structure and for quantification of the amount of peptide present.

**FITTING OF ANGLE DEPENDENT AMIDE I’ IRRAS BANDS**

The intensities of IRRAS bands strongly depend on the angle of incidence and the polarization (parallel (p) or perpendicular (s) to the plane of incidence) of the incident IR beam. These dependencies contain detailed information about the orientation of the transition dipole moment of the absorbing moieties with respect to the monolayer normal. Fitting of the angle dependent absorbance can be achieved and yields molecular order parameter \(S\), and additionally the angle \(\alpha\) between the molecular axis and the transition dipole moment can be estimated. The optical model of Kuzmin and Mikhailov\(^{33,34}\) was extended to calculate and fit the reflectance absorbance bands consisting of multiple overlapping spectral components as reported by Schwieger \(\text{et al.}\)\(^{35}\). Simulations and fitting of IRRA spectra was performed using MATLAB software (The MathWorks Inc., USA).

The IRRAS band intensities and shapes are simulated assuming lorentzian absorption bands with the vibrational frequency \(n_0\), the full width at half height \(fwhb\) and the
absorption coefficient $k$. Depending on the orientation of the transition dipole moment within the molecule ($a$), the tilt angle of the molecule with respect to the layer normal ($\theta$), the intensity varies with experimental parameters angle of incidence (AoI) and polarization (p or s) of the IR beam. Furthermore, the refractive index of the film $n$ and the film thickness as well as the polarizer quality $\Gamma$ have to be known to simulate complete reflectance-absorbance spectra. $\Gamma$ was determined from independent measurements and set to 0.007 for all simulations. The frequency dependent refractive indices and absorption coefficients of the D$_2$O subphase are taken from Bertie et al.$^{36}$ Simulations were fitted to the experimental spectra by means of a non-linear least-square fit in the amide I’ region (1620-1670 cm$^{-1}$).

The parameters $n$ and $d$ were determined for each measurement by a fit of the D$_2$O absorption in the spectral range of 2300 - 2800 cm$^{-1}$. The spectra were then simulated with three bands: one C=O stretching vibrational band in the range of 1710 - 1770 cm$^{-1}$ and two amide I’ components at $\nu1 = 1631$ and $\nu2 = 1651$ cm$^{-1}$, accounting for amide bonds in hydrophilic and hydrophobic environment, respectively. For both components $\alpha$ was set to 38°.$^{37}$

The lipid C=O band intensity was fitted via adjustment of the respective $k$ value. Finally, the amide I’ band of s- and p-polarized spectra over all measured AoI were fitted by means of a global non-linear least square method using a Levenberg-Marquardt algorithm, in the wavenumber range of 1620 – 1670 cm$^{-1}$. The helix tilt angle $\theta$, as well as the absorption coefficients $k_1$ and $k_2$, and fwhh1 and fwhh2 of both amide I’ components were free fitting parameters. To yield reliable and reproducible fits different starting values for the fitting parameters were tested.

Within the applied model$^{34,35}$ the tilt angle $\theta$ is calculated from the order parameter $S$ under the assumption of a unimodal and small tilt angle distribution, according to:

$$S = \frac{3(cos^2 \theta) - 1}{2}$$
Figure S6. Experimental data of Amide I’ absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of CP$_0$K decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S7. Experimental data of Amide I’ absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of CP$_{12}$K decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S8. Experimental data of Amide I' absorption bands (1600-1800 cm\(^{-1}\), lines), and best fits obtained with Matlab (dots) of CP\(_0\)E decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S9. Experimental data of Amide I’ absorption bands (1600-1800 cm⁻¹, lines), and best fits obtained with Matlab (dots) of CP₂E decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consist of 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S10. Experimental data of Amide I’ absorption bands (1600-1800 cm⁻¹, lines), and best fits obtained with Matlab (dots) of CP₈E decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S11. Experimental data of Amide I’ absorption bands (1600-1800 cm⁻¹, lines), and best fits obtained with Matlab (dots) of CP₁₂E decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S12. Experimental data of Amide I' absorption bands (1600-1800 cm⁻¹, lines), and best fits obtained with Matlab (dots) of CP₁₆E decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consist of 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S13. Experimental data of Amide I absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of CP$_0$E and CP$_0$K decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipoepitope, D2O PBS pH 7.4.
Figure S14. Experimental data of Amide I’ absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of CP$_{12}$E and CP$_{12}$K decorated monolayers at a surface pressure of 10 mN/m and 30 mN/m. Left graphs are angle dependent P-polarized spectra, right graphs are angle dependent S-polarized spectra. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
FITTING PROCEDURE OF S-POLARIZED AMIDE I’ IRRAS BANDS

Amide I’ absorption bands were evaluated by a Gaussian band fitting procedure by OriginPro 9.1 (OriginLab Corp., USA). S-polarized spectra in the range of 1600–1680 cm\(^{-1}\) were averaged over all AoI. Peak positions were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Free fitting parameters were restricted to sensible boundaries and in some cases fixed to obtain reasonable and comparable results for all spectra. The integrated surface areas of the Gaussian peaks were used to calculate the surface-ratio and hydrophilic fraction of the absorption bands at 1631 and 1651 cm\(^{-1}\), for each peptide derivative at both applied surface pressures. The relative change in peak shape as a function of surface pressure may indicate the relative hydrophilicity of the environment of monolayer-tethered peptides, upon assumption of similar secondary structures (α-helices) at both surface pressures.

Figure S15. Averaged S-polarized spectra over all AoI of a CP\(_3\)K decorated monolayer at a surface pressure of 10 mN/m (left, range of 1600–1680 cm\(^{-1}\)) and 30 mN/m (right, range of 1600–1780 cm\(^{-1}\)). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S16. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP:K decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.

Figure S17. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP:E decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
**Figure S18.** Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_4\)E decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.

**Figure S19.** Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_8\)E decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm\(^{-1}\). Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S20. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm$^{-1}$ of a CP$_{12}$E decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm$^{-1}$. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.

Figure S21. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm$^{-1}$ of a CP$_{16}$E decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm$^{-1}$. Monolayers consists of 4 mol% lipopeptide, D2O PBS pH 7.4.
Figure S22. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm⁻¹ of a CP₀E and CP₀K decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm⁻¹. Monolayers consists of 4 mol% lipopeptide, D2O PBS pD 7.4.

Figure S23. Averaged S-polarized spectra over all AoI in the range of 1600–1680 cm⁻¹ of a CP₁₂E and CP₁₂K decorated monolayer at a surface pressure of 10 mN/m (left) and 30 mN/m (right). Peak positions for the gaussian fitting procedure were fixed at 1631, 1651 and 1671 (± 4) cm⁻¹. Monolayers consists of 4 mol% lipopeptide, D2O PBS pD 7.4.
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