Influence of membrane-fusogen distance on the secondary structure of coiled coil peptides

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Supporting Information

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**LANGMUIR FILM BALANCE**

For compression-expansion cycles of lipid monolayers containing monolayer tethered peptides CP,E and/or CP,K, monolayers on PBS were prepared. After equilibration, compression-expansion cycles of the monolayer were performed with a compression/expansion speed of $3 \text{Å}^2 / (\text{molecule min})$, and experiments were repeated twice to ensure reproducibility of the isotherm shape. For determination of lateral compressibility, compression isotherms over a surface pressure range of 0 to 30 mN/m were smoothed by means of an adjacent average method and the film compressibility was calculated as:

$$ C_S = \frac{-1}{A} \left( \frac{\delta A}{\delta \pi} \right)_T $$

with molecular area $A$ and surface pressure $\pi$.

![Graph showing results of monolayer experiments with standard lipid composition and functionalized with 4% lipopeptide.](image)

Figure S1. Results of monolayer experiments with standard lipid composition (DOPC : DOPE : Cholesterol 2:1:1) and functionalized with 4% lipopeptide. 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.

**DETAILS OF IRRAS 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 $25^\circ$ to $70^\circ$ with an increment of $3^\circ$, while spectra close to the Brewster angle ($52^\circ$, $55^\circ$ and $58^\circ$) were discarded, due to the low reflectivity close to the Brewster angle. 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 $(R_0)$ were...
recorded at the same experimental conditions at an identical subphase without monolayer. The reflectance absorbance spectra were calculated as $RA = -\log(R / R_0)$.

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 procedure 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 was extended to calculate and fit the reflectance absorbance bands consisting of multiple overlapping spectral components as reported by Schwieger et al. Simulations and fitting of IRRAS 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 $\nu_0$, the full width at half height $fwhh$ and the absorption coefficient $k$. Depending on the orientation of the transition dipole moment within the molecule ($\alpha$), the tilt angle of the molecule with respect to the layer normal ($\theta$), the intensity varies with experimental parameters as the 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 $d$ 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. 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 $\nu_1 = 1631$ cm$^{-1}$ and $\nu_2 = 1651$ cm$^{-1}$, accounting for amide bonds in hydrophilic and hydrophobic environment, respectively. For the simulation / spectral fitting of both components $\alpha$ was set to 38°.

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 at 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 $fwhh_1$ and $fwhh_2$ 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 the helix 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 S2. Experimental data of amide I absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_6$K functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O PBS pH 7.4.
Figure S3. Experimental data of amide I' absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_{12}$K functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O PBS pD 7.4.
Figure S4. Experimental data of amide I' absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_0$E functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O PBS pH 7.4.
Figure S5. Experimental data of amide I’ absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_4$E functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O PBS pH 7.4.
Figure S6. Experimental data of amide I' absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_2$E functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O 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 a CP$_{12}$E functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O 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 a CP$_{16}$E functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 4 mol% lipopeptide, D$_2$O 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 a CP$_0$E and CP$_0$K functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 2 + 2 mol% lipopeptides, D$_2$O PBS pH 7.4.
Figure S10. Experimental data of amide I' absorption bands (1600-1800 cm$^{-1}$, lines), and best fits obtained with Matlab (dots) of a CP$_{13}$E and CP$_{13}$K functionalized monolayer 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. Incident angles of the IR beam are given in the legend. The monolayer contains 2 + 2 mol% lipopeptides, D$_2$O 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 1672 (± 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 area of the Gaussians was used to calculate the surface-ratio and hydrophilic fraction of the absorption bands at 1631 and 1651 cm⁻¹, for each lipopeptide 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 S11.** Averaged s-polarized spectra over all AoI of a CP,K functionalized 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. The monolayer contains 4 mol% lipopeptide, D₂O PBS pH 7.4.
Figure S12. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_{12}\)K functionalized 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\(^{-1}\). The monolayer contains 4 mol% lipopeptide, D2O PBS pD 7.4.

Figure S13. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_{0}\)E functionalized 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\(^{-1}\). The monolayer contains 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S14. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_4\)E functionalized 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\(^{-1}\). The monolayer contains mol% lipopeptide, D2O PBS pD 7.4.

Figure S15. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_8\)E functionalized 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\(^{-1}\). The monolayer contains 4 mol% lipopeptide, D2O PBS pD 7.4.
Figure S16. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm$^{-1}$ of a CP$_{12}$E functionalized 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$^{-1}$. The monolayer contains 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$_{16}$E functionalized 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$^{-1}$. The monolayer contains 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\(_0\)E and CP\(_0\)K functionalized 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\(^{-1}\). The monolayer contains 2 + 2 mol\% lipopeptides, D2O PBS pD 7.4.

Figure S19. Averaged s-polarized spectra over all AoI in the range of 1600–1680 cm\(^{-1}\) of a CP\(_{12}\)E and CP\(_{12}\)K functionalized 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\(^{-1}\). The monolayer contains 2 + 2 mol\% lipopeptides, D2O PBS pD 7.4.
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