LOCATION AND CONFORMATION OF THE LKα14 PEPTIDE IN WATER/ETHANOL MIXTURES

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S1. SFG spectrum for LKα14 in D$_2$O in the CH-stretching region

![SFG spectrum](image)

**Figure S1.** SFG spectrum in the CH-stretching region acquired after equilibration (~30 minutes) following the peptide injection in D$_2$O.

The acquired SFG spectrum for LKα14 peptide in pure D$_2$O in the CH-stretching region (Figure S1) is similar to that obtained for leucine molecules at the air-water interface.$^1$
S2. SFG spectra fitting for the amide I spectral region

The SFG spectra were fitted according to the following formula:

$$\chi^{(2)}(\omega) = A_{NR}e^{i\phi_{NR}} + \sum_j \frac{A_j}{\omega - \omega_{IR,j} + i\Gamma_j},$$

where $A_{NR}$ and $\phi_{NR}$ are the amplitude and the phase of the non-resonant contribution and $A_j$, $\omega_{IR,j}$, and $\Gamma_j$ are amplitude, center frequency, and half-width at half maximum (HWHM) of the j-th resonant contribution, respectively.

The fits of the amide I region SFG spectra for LK\(\alpha\)14 in $f_{\text{EtOH}}=0$ (pure H\(_2\)O) and $f_{\text{EtOH}}=0.1$ solutions are shown in Figure S2 and the corresponding fitting parameters are listed in Table S1.

As shown in the Figure S2, on the high frequency side of the spectrum there is a very broad feature extending above 1800 cm\(^{-1}\). Its nature is still a debated point in the SFG community and, to the best of our knowledge, this contribution has not yet been assigned, even though it has been observed in previous SFG studies, for example, for LK\(\alpha\)14 peptides at the air/water interface.\(^{S2-S4}\) In our fit it appears as a very broad peak centered at \(~1655\) cm\(^{-1}\).

![Figure S2. SFG spectra in the amide I region (empty circles) acquired after equilibration (~30 minutes) following the peptide injection in H\(_2\)O/EtOH mixtures with ethanol volume fraction $f_{\text{EtOH}}=0$ (pure H\(_2\)O, red) and $f_{\text{EtOH}}=0.1$ (orange). The solid lines present the corresponding fitting curves.](image-url)
Table S1. Results of the fitting of the SFG spectra in amide I region for LKα14 in H₂O/EtOH mixtures with \( f_{\text{EtOH}} = 0 \) (pure H₂O) and \( f_{\text{EtOH}} = 0.1 \).

|                  | LKα14 in solution with \( f_{\text{EtOH}} = 0 \) | LKα14 in solution with \( f_{\text{EtOH}} = 0.1 \) |
|------------------|-----------------------------------------------|-----------------------------------------------|
| \( A_{NR} \)    | 0.2                                           | 0.1                                           |
| \( \phi_{NR} \) | 1.0                                           | 0.2                                           |
| \( A(\text{amide I}) \) | 3.6                                           | 2.1                                           |
| \( \omega(\text{amide I}) \) | 1650                                          | 1648                                          |
| \( 2\Gamma(\text{amide I}) \) | 28                                            | 28                                            |
| \( A(\text{higher-frequency shoulder}) \) | 14.1                                          | 13.4                                          |
| \( \omega(\text{higher-frequency shoulder}) \) | 1655                                          | 1657                                          |
| \( 2\Gamma(\text{higher-frequency shoulder}) \) | 108                                           | 180                                           |

Based on the fitting results we can conclude that \(~5 \text{ cm}^{-1}\) center frequency shift which can be observed in the SFG spectra (Figure S2) actually originates from the difference in the contribution of the higher-frequency shoulder.
S3. N-terminus orientation of the interfacial LKα14 molecules from interfacial MD simulations

Figure S3. The probability of the N-terminus pointing away from the interface and towards the bulk phase for the interfacial LKα14 molecules in H₂O/EtOH mixtures with various EtOH volume fractions $f_{\text{EtOH}}$.

Whether the N-terminus of the peptides points away or towards the bulk was determined by comparing the center of mass of the whole backbone with the center of mass of the backbone of the seven residues closest to the N-terminus. If the center of mass of the backbone of the seven residues was closer to the center of the simulation box than the center of mass of the whole peptide was, the N-terminus was considered to be pointing away from the interface towards the bulk.
The probability that the N-terminus of the peptides at the interface points away from the interface is 0.77 in pure H₂O, which matches previously reported results.⁵² For \( f_{\text{EtOH}} \) in the range from 0.10 to 0.68 the probability is lower, between 0.55 and 0.60. For higher EtOH volume fraction the probability shows less of a clear pattern, likely in part due to less prevalence of the peptide at the interface. Still, the probability of the N-terminus pointing towards the bulk is always higher than the probability of it pointing towards the interface, except in pure EtOH where the probability of the N-terminus pointing towards the bulk is 0.47.
References

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