Biophysical Journal, Volume 97

Supporting Material

Tilt and Rotation Angles of a Transmembrane Model Peptide as Studied by Fluorescence Spectroscopy

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Supplementary material

S1 Calculation of the tilt and rotation angles

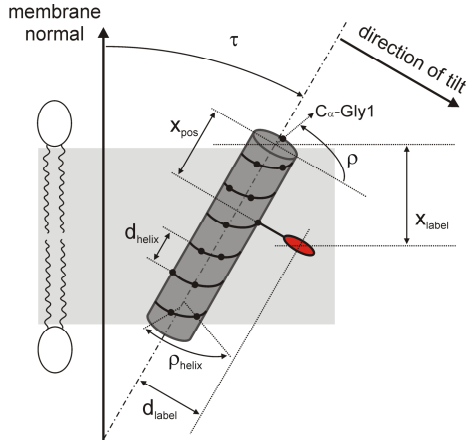


Figure S1: Schematic drawing of the angles describing the orientation of a tilted transmembrane peptide in the membrane. The tilt angle τ describes the angle between the membrane normal and the helix axis and the rotation angle ρ describes the angle between the direction of tilt and the C_α-atom of Gly1 in WALP23 with respect to the helix axis.

The geometry of a regular α-helix is defined by an increment along the helix axis of d_{helix} = 1.5 Å per amino acid and a turn around the helix axis of ρ_{helix} = 100° per amino acid. The distance x_{pos} of the labeled cysteine at position n_{label} with respect to the reference residue (Gly1) at position n_{ref} along the helix axis can then be calculated by

$$x_{pos} = d_{helix}\left(n_{ref} - n_{label}\right).$$

The orientation of a tilted transmembrane peptide is described by the angle τ between the membrane normal and the helix axis and the rotation angle ρ describes the angle between the direction of tilt and the C_α-atom of the reference residue with respect to the helix axis along the helix axis (for definitions see Fig. S1). The position of the label in a tilted transmembrane peptide is then calculated by

$$x_{label} = x_{pos}\cos(\tau) - d_{label}\sin(\tau)\cos\left(\left(n_{label} - n_{ref}\right)\rho_{helix} - \rho\right),$$

where d_{label} is the distance between the label and the helix axis.

The peak positions of the $HICT_m$ state and the total hydrogen-bonded fractions were fitted using a Gaussian function

$$y = y_0 + a * \exp\left\{-\frac{1}{2}\left(\frac{\left(x_{label} - x\right)}{b}\right)^2\right\},$$

where y_0 is the emission peak position of $HICT_m$ state of a cysteine-bound BADAN label in aqueous environment (y_0 = 19200 cm^{-1} [1]), a is a scaling factor representing the shift in emission peak position between the membrane center and the aqueous environment, x denotes the distance parallel to the membrane normal between membrane center and the reference residue Gly1 (n_{ref} = 1), and b represents the width of the Gaussian distribution.

S2 Analysis of the total fractions of hydrogen-bonded labels

The total fractions of hydrogen-bonded labels f_{HB} obtained from the spectral decomposition for WALP23 peptides inserted into bilayers of different thickness (A) and bilayers containing different cholesterol concentration (B) are depicted in Fig. S2. Similar to the peak position P_m of the mobile hydrogen-bonded labels f_{HB} not only depends on the position of the label in the α-helix but also on the thickness of the bilayer. For all bilayer thicknesses used, f_{HB} is largest (and close to 100%) for labels positioned close to the ends of the α-helix, and decreases for positions in the interior of the bilayer. As can be expected, this decrease of f_{HB} in the bilayer interior is more pronounced for thicker bilayers. Compared to the changes for f_{HB} in bilayers of lipids with different thickness, only small decreases in f_{HB} were observed when increasing the cholesterol concentration in bilayers of 18:1PC.

Thus, similar effects as observed for the polarity data, described in the main article. In principle, like the polarity data also the total fraction of hydrogen-bonded labels f_{HB} can be used to retrieve tilt and rotation angle of a peptide inserted in a bilayer. The results are qualitatively similar. However, the accuracy is lower due to lower sensitivity of this parameter. In our study, we used the f_{HB} data sets to get a better estimate of the distance between label and the helix axis, which has great influence on the estimated tilt angle.

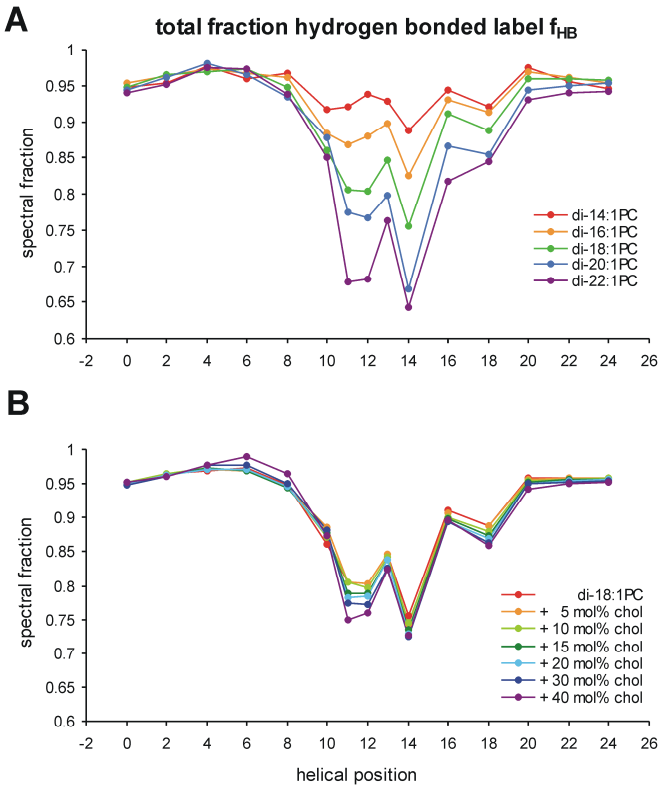


Figure S2: Total fraction f_{HB} of hydrogen-bonded labels obtained from spectral decomposition of emission spectra of BADAN labeled WALP23 peptides incorporated in bilayers of unsaturated phospholipids with different thickness (A) and bilayers of di-C18:1c-PC containing different cholesterol concentrations (B). The lines are drawn to guide the eyes, as only a selection of positions in the WALP23 peptide was labeled with BADAN.

S3 Accuracy of the estimated tilt angle

To fit the polarity data sets, we used an estimated distance of 7.5 Å for the distance of the label to the α-helix. A similar study employing a related fluorescent label indicated a direct relationship between the tilt angles obtained and this distance (R2). The BADAN label is connected to the α-helix via a linker chain including five single bonds (see Fig. 6, main article), allowing for a large ensemble of spatial conformations. This leads to a distribution of distances for the label connected to the peptide helix. To get insight into the relationship between tilt angle and the distance of the label to the helix axis, we performed fits to the polarity data sets for a range of distances. Fig. S3 A shows that the tilt angles strongly depend on the distance of the label to the helix axis and have smaller values the larger the distance used for the fit.

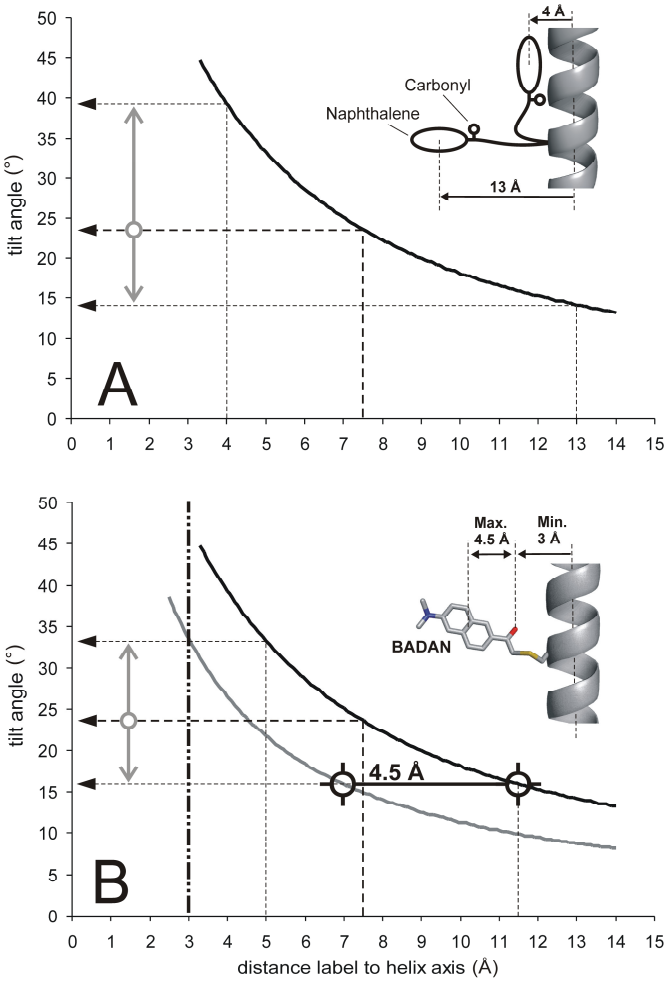


Figure S3: Dependence of the tilt angles obtained for WALP23 peptides in di-C18:1PC on the distance of the label to the helix axis. (A) Fit to the polarity data. The distance between naphthalene and helix axis can be minimal 4 Å and maximal 13 Å, corresponding to tilt angles of 13° and 39° (follow thin dashed lines). The grey arrows indicate the uncertainty in tilt angle. (B) Fit to the polarity data (solid black line) and to the total fraction of hydrogen-bonded labels (solid grey line). The possible distances can be further narrowed down by consideration of steric hindrances imposed from the peptide helix, which limits the carbonyl to minimal 3 Å distance, corresponding to a tilt angle of maximal 33° and of the geometry of the label, which allows the carbonyl and naphthalene maximal 4.5 Å apart, corresponding to a tilt angle of minimal 16° (for further explanations see text).

Let us now consider some extreme conformational situations for a BADAN label attached to a cysteine in the peptide helix (inset in Fig. S3 A). The largest distance can be found for a label with a completely extended linker chain (ca. 13 Å), and the shortest distance is estimated by the limitations due to steric hindrance near the α-helix (ca. 4 Å), leading to tilt angles of 13° and 39° from the polarity data, respectively (see Fig. S3 A, dashed arrows). Thus, the tilt angle of 23.6° obtained using 7.5 Å has a large uncertainty, and it is evident that to determine the correct tilt angle, we need a good estimate of the distance of the label to the helix axis.

It is possible to somewhat narrow down the uncertainty by including fits to the total fraction of hydrogen-bonded labels. This reflects the hydrogen-bonding capacity in the local vicinity of the BADAN label, which, based on the results depicted in Fig. S2, can be expected to follow a more or less similar profile as the polarity. At first sight surprisingly, the fits to the hydrogen bonding data yielded much smaller tilt angles (see Fig. S3 B, grey line). However, this difference can be explained by the fact that the local polarity is sensed from the naphthalene unit while the carbonyl in the linker chain senses the hydrogen bonding capacity. Looking at the chemical structure of the BADAN label linked to a peptide helix, for most conformations in the spatial ensemble the hydrogen bond accepting carbonyl in the linker will be located much closer to the helix axis than the naphthalene unit (inset in Fig. S3 B). Based on the chemical structure of the label, these two moieties have a fixed distance of 4.5 Å to each other. Therefore, in the most extended conformation of the label the carbonyl can be located up to 4.5 Å closer to the helix axis, allowing the determination of a lower limit for the tilt angle.

Inspection of the curves in Fig. S3 B shows that when the tilt angle gets smaller, the distance between carbonyl and naphthalene unit increases. At a tilt angle of ca. 16°, the distances used to fit the different data sets have a difference of 4.5 Å (indicated in Fig. S3 B), which corresponds to the largest distance for the naphthalene unit to the helix axis of ca. 11.5 Å. Similarly, the upper limit for the tilt angle is confined by the geometry of the α-helix. The C_α-atoms have an average distance of 2.7 Å to the helix axis. This implies a distance of at least 3 Å for the carbonyl of the BADAN to the helix axis, which yields an upper limit of 33° for the tilt angle.

Thus, we were able to determine a reliable upper and lower limit for the tilt angle by simple consideration of the geometry of a regular α-helix and the chemical structure of the BADAN label. For a spatial ensemble, the mean value of the distance between naphthalene unit and the helix axis can be found between these extreme values and was fixed to 7.5 Å in our analysis. The values for all tilt angles obtained using this distance are estimated to be correct within 5°. If the actual average distance of the label to the helix axis is smaller, this will lead to larger tilt angles, and vice versa. However, in case that the actual distance differs from the one chosen, a systematic error is introduced in the tilt angles obtained for peptides inserted in different types of bilayers. Hence, the conclusions drawn with respect to effects of mismatch situations on the peptide orientation still remain valid. The range of possible values for the distances of the sensing locations of the BADAN label with respect to the helix axis could be further narrowed down by molecular modeling or MD simulations of a WALP23 peptide labeled with BADAN. Another possibility is the use of ALADAN, a fluorescent amino acid including a DAN moiety with an even shorter linker chain, requiring specialized synthesis work (R3).

S4 Relationship between emission peak position and polarity

In this study, we used the emission peak position of the most red-shifted spectral component as a ruler to determine the insertion depth of the probe in the bilayer, because it reports on the polarity of the environment. To investigate the exact relationship between the emission peak position and the polarity of the environment we used PRODAN, an inert member of the group of DAN containing fluorophores (see Figure S4 for the chemical structure) to mimic BADAN coupled to a peptide.

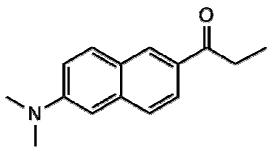
PRODAN 

Figure S4: Structure of PRODAN (6-propionyl-2-dimethylaminonaphthalene), an inert analog of BADAN.

This compound was dissolved in alcohols with increasing alkyl chain lengths, i.e. methanol to decanol to study the effect of polarity on the position of the emission maximum. The polarity of these alcohols decreases with increasing chain length. Unlike in lipid systems, all emission spectra recorded in these homogeneous solvents could be fitted using a single Gaussian and the emission peak position as a function of the rotational polarizability parameter Δf are shown in Figure 5S. From these results we also conclude that the steady state fluorescence of PRODAN in alcohols is dominated by that of a $HICT_m$-like state, obviously because hydrogen bonding, conformational and solvent relaxation is relatively fast in these alcohols compared to within motionally restricted lipid systems. The range of emission peak positions of PRODAN dissolved in the different alcohols i.e. 20900 cm^{-1} for decanol to 19700 cm^{-1} for methanol, corresponds approximately to the range of the emission peak positions observed for the different BADAN-WALP peptides inserted in bilayers i.e. 21200 cm^{-1} to 19300 cm^{-1}. The results for PRODAN in alcohols agree with the general view that - concerning polarity - alcohols can be used for mimicking local environments in lipid systems (R4).

The data points plotted in Figure S5 may suggest a quadratic relationship between the emission peak position of the $HICT_m$ state and the polarizability i.e. polarity if also the data point for PRODAN in water is fully taken into account. Therefore, we reanalyzed the emission peak positions obtained from BADAN-labeled peptides using a quadratic relationship. However, we observed only minor changes in tilt and rotation angles with respect to the analysis using a linear relationship (compare tilt and rotation angles listed in Table S1 and in Table 2 of the main article), suggesting that a linear relationship can be used in good approximation.

Table S1: Tilt and rotation angles estimated for WALP23 peptides in bilayers of different thickness using a quadratic relationship between emission peak position and polarity for analysis.

Bilayer	τ (°)	ρ(°)
16:1PC	25.1	96
18:1PC	23.9	108
20:1PC	19.8	108

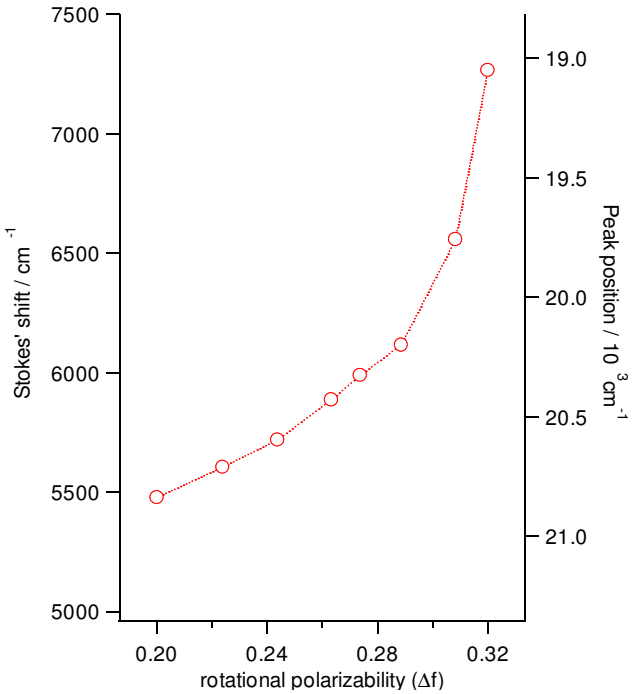


Figure S5: Stokes' shift / emission peak position of PRODAN dissolved in alcohols with different alkyl chain lengths and water as a function of the rotational polarizability Δf of the solvents used (from left: decanol, octanol, hexanol, butanol, propanol, ethanol, methanol and water).

References

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