Published in final edited form as:

J Mol Biol. 2007 December 14; 374(5): 1251–1269.

THE CONTROL OF TRANSMEMBRANE HELIX TRANSVERSE POSITION IN MEMBRANES BY HYDROPHILIC RESIDUES

Shyam S. Krishnakumar and Erwin London*

Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215

SUMMARY

The ability of hydrophilic residues to shift the transverse position of transmembrane (TM) helices within lipid bilayers was studied in model membrane vesicles. Transverse shifts were detected by fluorescence measurements of the membrane depth of a Trp residue at the center of a hydrophobic sequence. They were also detected by the change in the effective length of the TM-spanning sequence, derived from the stability of the TM configuration under conditions of negative hydrophobic mismatch. Hydrophilic residues (placed at the fifth position in a 21-residue hydrophobic sequence composed of alternating Leu and Ala residues and flanked on both ends by two Lys) induced transverse shifts such that the hydrophilic residue located closer to the membrane surface. At pH 7, the dependence of the extent of shift upon the identity of the hydrophilic residue increased in the order L<G~Y~T<R~H<S<P<K<E~Q<N<D. By varying pH, shifts with ionizable residues fully charged or uncharged could be measured, and the combined shift data increased in the order: L<G~Y~H⁰~T<E⁰~R<S<P<K⁺<Q~D⁰~H⁺~N~E⁻<D⁻. The dependence of transverse shifts upon residue identity was consistent with the hypothesis that shift is largely controlled by the combination of amino acid hydrophilicity, ionization state, and the ability of side chains to position polar groups near the bilayer surface (snorkeling). Additional experiments showed that the magnitude of transverse shifting was also modulated by the position of the hydrophilic residue in the sequence and the hydrophobicity of the sequence moving out of the bilayer core upon shifting. Combined, these studies show that the insertion boundaries of TM helices are very sensitive to sequence, and can be altered even by weakly hydrophilic residues. Thus, many TM helices may have the capacity to exist in more than one transverse position. Knowledge of the magnitudes of transverse shifts induced by different hydrophilic residues should be useful for design of mutagenesis studies measuring the effect of transverse TM helix position upon function.

Keywords

membrane proteins; transmembrane helices; hydrophilic residues; transverse helix shifts; transmembrane helix boundary

INTRODUCTION

Transmembrane (TM) α -helices are the primary membrane-inserted segments of membrane proteins. Because isolated TM helices are independently folding units, the dependence of their behavior upon sequence can yield fundamental insights into the membrane protein structure/

* Corresponding author: Erwin London, Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215, Tel: (631) 632-8564, FAX: (631) 632-8575, E-mail: erwin.london@stonybrook.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

function relationship. Indeed, studies of individual TM helices have led to important advances in understanding membrane protein folding, topography, interactions with lipids, and interactions with other helices^{1–11}

TM helices are predominantly composed of hydrophobic residues that are buried in the membrane. Polar and ionizable (charged) residues are less abundant in TM segments because the energetic cost involved in burying a hydrophilic residue in the nonpolar environment of the bilayer. Nevertheless hydrophilic residues within a TM sequence are very important in determining helix behavior. In many cases, hydrophilic residues have been shown to promote TM helix association^{12–17}. The ability of the hydrophilic residues to form hydrogen bonds^{18,19} or salt bridges²⁰ with other membrane inserted hydrophilic residue drive these TM helix-helix interactions. Hydrophilic residues can also alter the topography of a membrane-inserted hydrophobic sequence. The introduction of hydrophilic residues in a hydrophobic sequence destabilizes the TM state. This can result in change from a TM topography to a membrane-bound non-TM state, i.e. one in which the helix moves close to the bilayer surface^{8,9,21}. In some cases, helices with intermediate hydrophobicity can equilibrate between TM and non-TM states^{8,9,22–24}.

However, even when the TM state is maintained in presence of highly hydrophilic residues, hydrophilic residues can still affect the position of a helix within the bilayer such that there is a transverse shift (i.e. a change in the distance of amino acid residues from the center of the bilayer) so that the hydrophilic residues locate at (or close to) the bilayer surface^{20,25–29}. This shift can shorten the effective length of the hydrophobic segment that spans the lipid bilayer^{25,27,28}. Transverse shifts are important because membrane proteins, unlike soluble proteins, are positionally-constrained in a fixed plane relative to the plane of the membrane. Two separate sequences (e.g. on two different helices or two different membrane proteins) may only interact properly if they are located in the same plane. Thus, TM helix position can control membrane protein function via a mechanism that has no analog for soluble proteins.

The possibility that changes in the TM position of proteins might be functionally important was pointed out long ago³⁰, and there already have been several examples in which transverse shifts in TM helix position appear to have an important role in function. In the case of bacterial chemoreceptors it has been well-documented that shifts in TM helix position control signal transduction by modulating kinase activity inside the cell^{31–33}. Similarly, in integrins, the activation of the receptor by ligand binding results in movement of the part of a TM helix out of the lipid bilayer, effectively shortening the TM helix by 4–5 residues³⁴. Based on the difference in the transverse position of TM hydrophobic helices containing charged and uncharged Asp we have suggested that Asp ionization state could alter TM helix transverse positions as a protein cycles between acidic vacuoles and the plasma membrane in a functionally important manner²⁵.

In pioneering studies, differences in the ability of several types of hydrophilic residues to shift TM helix position in the translocon was measured by von Heijne and colleagues using glycosylation mapping^{20,27,28,35,36}. In the present report, transverse shifts in TM hydrophobic helices were measured for the complete series of hydrophilic residues within lipid bilayers. To do this a series of membrane-inserted peptides suitable for detecting shifts in bilayers with physiologically-relevant bilayer widths was designed. The fluorescence of a Trp residue located at the center of the hydrophobic sequence was used to monitor the behavior of the peptides. We found that the extent of shift depends on the identity of the hydrophilic residue, and that even a weakly polar residue, such as Gly or Ser, can induce significant shifts in helix position. These results imply that the identity of the TM insertion boundaries of a TM sequences can be a much more ambiguous and even dynamic parameter than generally realized. In many

cases, membrane protein TM helices are likely to be able to undergo facile switching between TM states that differ in their transverse position and function.

RESULTS

Using Fluorescence Emission λ_{max} and Fluorescence Quenching to Define the Topography of the Membrane-Inserted Hydrophobic Helices

The fluorescence properties of a Trp residue located at the center of a hydrophobic sequence was used to define the topography of membrane-inserted helical peptides^{8,9,22,25}. There are several topographies that helical peptides can form in membranes. A full TM orientation in which the Trp locates at or near the bilayer center (Figure 1, center), results in blue-shifted Trp emission λ_{max} (320–325 nm for the peptides in this study)^{8,9}. If the peptide adopts a non-TM membrane-bound topography, in which it resides close to the bilayer surface, then the Trp locates close to the surface (Figure 1, left) and the λ_{max} red shifts strongly (to as high as 345–350 nm)^{8,9}. The exact λ_{max} value in the non-TM state value depends upon how deeply the peptide is buried, and upon whether the Trp faces aqueous solution or the bilayer interior when the peptide is in the non-TM state^{22,25}. Values of λ_{max} between those for the TM and non-TM states are observed for mixtures of TM and non-TM topographies^{8,9}, and for a shifted TM state wherein only a part of the hydrophobic sequence forms the membrane-spanning segment (e.g. to allow a polar residue to locate close to the bilayer surface), and so the Trp is shifted to an intermediate depth^{22,25} (Figure 1, right). These two alternatives can be distinguished (see below).

Interpretation of λ_{max} results is aided by combining them with more direct measurements of Trp depth. To do this, a dual fluorescence quenching method was used³⁷. This method utilizes two quenchers of Trp fluorescence, acrylamide and 10-DN. Acrylamide, although membrane permeable³⁷, resides primarily in aqueous solution and preferentially quenches the fluorescence of Trp residues near the membrane surface, while 10-DN, a membrane-inserted quencher, preferentially quenches the fluorescence of Trp buried in the membrane bilayer³⁷. The ratio of acrylamide quenching to 10-DN quenching (quenching ratio or Q-ratio) responds nearly linearly to Trp depth in the bilayer, with a low Q-ratio (as low as 0.05–0.1) indicating a deeply located Trp near the center of the bilayer, while a high Q-ratio (1.0–3.0) usually indicates a Trp near the bilayer surface³⁷. As in the case of λ_{max} , intermediate Q-ratios can arise either from peptides adopting a mixture of conformations with shallow and deep Trp depths, or from peptides adopting a shifted TM conformation with an intermediate Trp depth.

Cases in which a Trp is at an intermediate depth can be distinguished from cases in which mixtures of shallow and deep Trp are present by measurement of quencher-induced shifts in Trp λ_{max} ^{22,25,37}. If a membrane-inserted peptide forms co-existing deep and shallow conformations, then its Trp emission spectrum will be a composite of blue- and red-shifted emission spectra arising from the deep and shallow conformations, respectively. In such samples, acrylamide preferentially quenches the shallow Trp, inducing a blue shift in λ_{max} . Conversely, 10-DN preferentially quenches the deep Trp, inducing a red shift. In such cases, the difference in λ_{max} in the presence of acrylamide and 10-DN can be up to 15 nm^{22,25,37}. On the other hand, for a homogenous population with a single intermediate Trp depth, the difference in λ_{max} in the presence of acrylamide and 10-DN is very small (0–2 nm)^{22,25,37}. It should be noted that due to incomplete quenching, the λ_{max} values in the presence of a quencher often are not the exact values of the pure shallow state or pure deep state.

Topography of Membrane-Inserted p(LeuAla) Peptides With a Hydrophilic Substitution: Effect Upon Trp Depth in DOPC Vesicles

The peptides studied were of the pLA-type ^{8,9,13,17,22,25}, consisting of 25-residues with a helix-forming 21-residue hydrophobic core composed of alternating L and A residues, a Trp at the center of the hydrophobic sequence, a single variable (X) residue located five residues from the N-terminal end of the hydrophobic sequence, and flanking di-Lys sequences at both its N and C-termini (general sequence: acetyl-KKLALAXALALAWLALALALAKK-amide). This sequence was chosen because it is long enough form a stable TM structure even if the TM segment begins after residue X (i.e. if the TM sequence is formed by residues 8–23⁸). In addition, a shift between the topography in which the entire hydrophobic sequence (residues 3–23) forms the TM segment and one in which residues 8–23 form the TM segment results in an easily-detected change in Trp depth. Sequences with X = L (parental peptide), or a “hydrophilic” residue: S, T, G, Y, P, Q, N, H, R, K, E or D, were compared.

Helix position was estimated from Trp depth, using both the Trp λ_{max} and fluorescence quenching assays described above. Figure 2 shows λ_{max} and Q-ratio (raw quenching data is in Supplemental Table 1) data for the peptides incorporated into vesicles composed of DOPC (which has C18:1 acyl chains) at pH 7. For almost all X residues, λ_{max} (325–330nm) and Q-ratio (0.05–0.25) values (Figures 2A and 2B, respectively) indicate a relatively deep Trp location, consistent with a primarily TM topography. The λ_{max} and Q-ratio values are very closely correlated with each other, confirming that both parameters measure Trp depth (Figure 3). Averaging the results from λ_{max} and Q-ratio indicates that Trp depth becomes progressively less deep in the order X= L<G~Y<T<R~H<S<P<K<Q~E<N<D. In almost all cases there are no significant quencher-induced shifts in λ_{max} ($\Delta\lambda_{\text{max}}$) (Table 1), indicating that the decrease in Trp depth when X is hydrophilic is associated with a shift in the TM position of the helix to locate the X residue closer to the surface of the bilayer, rather than formation of a population in a non-TM state. An exception to this is X=D, which forms a significant amount of the non-TM state in DOPC vesicles as judged by quencher-induced λ_{max} shifts. This suggests that the D residue induces such a large shift in the TM position that the residual TM hydrophobic segment is too short to be *fully* stable in the TM state in DOPC bilayers. This interpretation is supported by measurements of the effective TM length for this peptide (see below). There are also some peptides (X=S, K, and perhaps R) in which there are modest quencher-induced shifts in DOPC vesicles. These cases involve two blue-shifted states as judged by λ_{max} values in the presence of quencher, suggesting that they are cases in which un-shifted and shifted TM states co-exist.

The absolute change in Trp depth in the presence of a hydrophilic X residue in Å can be roughly estimated by using standard curves derived in previous studies for the dependence of Trp depth on λ_{max} (a 2 nm red shift equals slightly less than a ~ 1 Å decrease in Trp depth) and Q-ratio (an increase of 0.1 in Q-ratio is equivalent to slightly more than a ~ 1 Å decrease in Trp depth)^{9,37}. This reveals that the near-maximal change in depth (i.e. for X=N relative to X=L) corresponds to a movement of Trp of ~ 3 –4 Å closer to the bilayer surface. This movement is close to the 3.4 Å predicted when a hydrophilic residue moves to the membrane interface and so the effective length of the TM segment decreases from 21 to 16 residues (see Discussion).

It is important to note that the dependence of λ_{max} and Q-ratio upon X residue identity do not exactly parallel X residue hydrophilicity. Other variables, such as the ionization state of a side chain and side chain ‘snorkeling’ (i.e. formation of a conformation in which long side chains orient such that the polar group at the end of the side chain locates as close as possible to the bilayer surface³⁸) affect the extent of shift (see below).

Topography of Membrane-Inserted p(LeuAla) Peptides With a Hydrophilic Substitution: Effect Upon Trp Depth in DEuPC and DEiPC Vesicles

The pattern of the change in λ_{max} and Q-ratio as a function of X residue identity was different when the peptides were incorporated into vesicles composed of DEuPC (which has C22:1 acyl chains), a lipid forming much wider bilayers than DOPC⁸ (Figure 4 and Table 1, raw quenching data in Supplemental Table 2). In DEuPC vesicles, there is negative hydrophobic mismatch, because the width of the hydrophobic core of the bilayer ($\sim 35\text{\AA}$) exceeds the length of the full hydrophobic sequence (21 residues, $\sim 31.5\text{\AA}$). Several studies have shown that negative mismatch destabilizes TM states relative to non-TM states^{8,9,17,22,25}. Consistent with this, the peptides exhibit much more red-shifted λ_{max} and much higher Q-ratios than observed in DOPC vesicles. The red shift and Q-ratio are again affected by the identity of the X residue, and increase in the order $X=L<G<T<Y<S<P<H<R<K<Q<E<N<D$. This order is somewhat different than that in DOPC vesicles (the reasons for this are discussed below). The differences between peptides with $X=H, R, K, Q, E, N$ and D are very small, with the values of λ_{max} (345–348 nm) and Q-ratio (3–3.5) being indicative of a Trp location very close to the bilayer surface. This indicates that these seven sequences are fully forming the non-TM topography in DEuPC vesicles. However, these peptides remain bound to the DEuPC vesicles, as their fluorescence is still accessible to 10-DN quenching and their Trp emission λ_{max} (346–348 nm) is not consistent with being dissolved in aqueous solution (on our instrument the λ_{max} for Trp in water is about 355 nm). Nor does there seem to be a sub-population of these peptides in solution, as there are no significant quencher induced- λ_{max} shifts when $\lambda_{\text{max}} = 345\text{--}348$ nm (Table 1).

The peptides that exhibit intermediate λ_{max} values in DEuPC vesicles (with $X=G, Y, T$ or S) form mixtures of the non-TM and TM states as judged by large quencher-induced λ_{max} shifts ($\Delta\lambda_{\text{max}}$) (Table 1). As noted above, such shifts are characteristic of co-existing shallow and deep Trp depth populations. This conclusion is confirmed by the behavior of the peptides in vesicles composed of DEiPC (which has C20:1 acyl chains), a lipid forming bilayers with a width intermediate between that of DOPC and DEuPC⁸. It would be expected that the TM state would have an intermediate stability in DEiPC vesicles, and consistent with this Table 1 shows that almost all of the peptides incorporated into DEiPC vesicles tended to give λ_{max} and Q-ratio values intermediate between those in DOPC vesicles and DEuPC vesicles, and very large quencher-induced large shifts in λ_{max} , indicative of TM/non-TM mixtures.

Analysis of $\Delta\lambda_{\text{max}}$ as a function of the λ_{max} in the absence of quencher illustrates the correlation of quencher-induced λ_{max} shifts with the relative amounts of TM and non-TM topographies present (Figure 5). The largest quencher-induced λ_{max} shifts should occur when the populations of TM and non-TM states are nearly equal, which corresponds to $\lambda_{\text{max}} \sim 335$ nm (in absence of quencher), half-way between λ_{max} in the TM state and non-TM state (345–348 nm). This pattern is observed in Figure 5 for peptides incorporated into DEiPC and DEuPC vesicles. In contrast, when the peptides are incorporated into DOPC vesicles, peptides with intermediate λ_{max} values tend to exhibit small $\Delta\lambda_{\text{max}}$ values (Table 1), consistent with the presence of a single shifted TM population.

Using the Effect of Bilayer Width Upon Helix Behavior to Investigate the Topography of Membrane-Inserted Helices

Shifts in helix position were also detected by the decrease in the number of residues spanning the lipid bilayer when the X residue moves closer to the bilayer surface (Figure 1, right). This decrease in length can be detected via the decrease in the stability of the TM state under conditions of negative mismatch. The degree of negative mismatch is controlled by the length of the membrane spanning sequence and the width of the bilayer. The TM state of a short hydrophobic helix is destabilized in much thinner bilayers than is the TM state of a long

hydrophobic helix^{8,9,22,25}. Previous studies have shown that Trp fluorescence is most blue-shifted at about the maximum bilayer width at which the TM state is not significantly destabilized by negative mismatch (see Figure 6A)^{8,9}. Trp fluorescence becomes red-shifted in wider bilayers due to formation of the non-TM state, in which Trp locates shallowly. Fluorescence also generally red shifts, although to a lesser degree, in thinner bilayers (i.e., in conditions of *positive* mismatch between helix length and bilayer width)^{8,9}. This is due to the fact that the distance of a Trp at the center of a bilayer to the membrane surface automatically decreases as bilayer width decreases. In addition, there can be a modest red shift due to (weak) oligomerization under conditions of positive mismatch in thin bilayers^{8,9,17}.

The bilayer width at which Trp fluorescence is most blue shifted (dashed lines in Figure 6A) is defined as the “effective TM length” ($L_{TM\text{ eff}}$). This is the bilayer width (in units of acyl chain length) at which there is a near-match between the width of the hydrophobic core of the bilayer and the length of the hydrophobic sequence. $L_{TM\text{ eff}}$ can also be expressed in Å or as the number of residues that span this bilayer width (see Materials and Methods).

It should be noted that the decrease in the length of a TM segment in the presence of a hydrophilic X residue can exceed both the decrease in X residue depth and the change in Trp depth, by simple geometrical factors described in the Discussion. In addition, $L_{TM\text{ eff}}$ sometimes differs from the actual length of the membrane spanning sequence (L_{TM}), especially in the presence of snorkeling X residues. This difference can contain valuable information (see Discussion.)

Effective TM Length of Membrane-Inserted p(LeuAla) Peptides With a Hydrophilic Substitution

Figure 6B shows the effect of bilayer width on the Trp emission λ_{max} for the various pLA peptides at pH 7.0. The parental peptide (X=L) has a profile with a λ_{max} minimum in DEiPC bilayers, indicative of an $L_{TM\text{ eff}} \sim 31.5\text{ Å}$ ⁸. This corresponds to the predicted $L_{TM\text{ eff}}$ for the 21-residue (residues 3–23) hydrophobic core sequence of this peptide (21 residues $\times$ 1.5 Å rise per residue = 31.5 Å). The presence of hydrophilic X substitutions resulted in the λ_{max} minimum occurring in thinner bilayer widths. This shows that the hydrophilic substitutions decrease the effective length of the TM segment. $L_{TM\text{ eff}}$ values for pLA peptides with various X residues are given in Table 1. In the most extreme case, when X=D, $L_{TM\text{ eff}}$ decreases to 16.6 residues (25 Å), close to the value (16 residues, 24 Å) expected when the X residue shifts to the bilayer surface and the TM segment extends from residue 8 to 23. Intermediate $L_{TM\text{ eff}}$ values, indicative of an intermediate extent of helix shift, are observed for the other X residues. The overall order in which $L_{TM\text{ eff}}$ decreases is: X= L>G>T>Y~S>P>K~E~H~R~Q~N>D. This is very similar, but not identical, to the order of the dependence of Trp depth in DOPC vesicles upon X residue type. In particular, residues having long side chains (e.g. K and R) tend to have a relatively larger effect on $L_{TM\text{ eff}}$ than on Trp depth. This behavior is likely to be related to snorkeling, and this subject is explored in detail in the Discussion section.

How pH Affects the Topography of the p(LA) Peptides with Ionizable Residues

In order to investigate the effect of charge on an ionizable X residue upon TM helix behavior, the first step was to measure their pK_a s. To do this, samples containing peptides inserted into vesicles prepared at low pH were titrated with base as described previously^{17,25}. Changes in ionization were detected by pH-induced changes on Trp emission²⁵ (Supplemental Figure 1). The pK_a values in DOPC vesicles are 5.5, 6.7 and 7.0 for X= Asp, Glu and His residues, respectively, and are no more than 0.1 units higher in DEuPC vesicles (Supplemental Table 3). The pK_a values for X= Lys and Arg residues could not be determined, presumably because they occur at too high a pH and/or are masked by deprotonation of Lys residues flanking the hydrophobic sequence.

Next, λ_{max} and Q-ratio were measured for samples containing X=D, E, and H peptides when inserted into DOPC vesicles, under conditions in which the X residues would be fully protonated (pH 4.0), or fully deprotonated (pH 9.0) based on their pK_{a} values (Figure 7, raw quenching data in Supplemental Table 4). Trp depth is shallowest when the X residues were charged (pH 4 for the X = H peptide and at pH 9 for the X = D and X = E peptides), and thus most hydrophilic. This indicates that in the charged state H, D, and E residues resist burial within the lipid bilayer and thus induce large shifts in TM helix position. For X = H⁺, the change in Trp depth relative to X = L was $\sim 2.5\text{--}3$ Å. It is difficult to specify Trp depth for the TM population formed by the X = D[−] and E[−] peptides because they form a mixture of populations in which it is likely that some of the non-TM form is present as judged by quencher-induced shifts in λ_{max} (Table 2). However, an estimate of the extent of TM helix shifts when Asp and Glu are charged can be obtained from $L_{\text{TM eff}}$ values derived from λ_{max} vs. bilayer width curves (Figure 8). For X = H⁺, E[−], and D[−] $L_{\text{TM eff}}$ values (Table 2) are 3.6, 3.8, and 4.1 residues, respectively, shorter than that for the X=L peptide (Table 1). It should be noted that the value of $L_{\text{TM eff}}$ is in reasonable agreement with Trp depth for the H⁺ residue ($L_{\text{TM eff}}$ predicting a shift of Trp depth by 2.4 Å relative to the X=L peptide, see Discussion).

Although locating more shallowly than the X = L peptide, peptides with uncharged ionizable X residues (at pH 9 for X=H and at pH 4 for X=D and X=E) affect Trp depth in DOPC vesicles to a much lesser degree than when charged (Figure 7). The effects of H⁰ and E⁰ residues on Trp depth are modest, giving a 0.5–1 Å shallower Trp location than when X=L, while that of D⁰ is substantial, with a 2–3 Å shallower Trp than when X=L. $L_{\text{TM eff}}$ for peptides with uncharged ionizable residues are also shorter than for the X=L peptide, confirming the shift in TM helix position. The decreases in $L_{\text{TM eff}}$ values for X= E⁰, H⁰ and, D⁰ are ~ 2.2 , 2.9 and 3.4 residues, respectively, relative to that for the X=L peptide (Table 2). The value of $L_{\text{TM eff}}$ are in reasonable agreement with Trp depth for the D⁰ residue ($L_{\text{TM eff}}$ predicting a shift of Trp depth by 2.3 Å relative to the X=L peptide) but larger than expected for E⁰, and more strikingly so for H⁰ ($L_{\text{TM eff}}$ predicting a shift of Trp depth of 1.5 and 2 Å further from the bilayer center, respectively, see Discussion). The likely origin of this difference is described in the Discussion.

Effect of a Change in the Position of the Hydrophilic Residue In the Hydrophobic Sequence Upon Hydrophilic Residue-Induced Transverse Shifts

We also examined whether a change in the position of a hydrophilic residue within the hydrophobic sequence would affect the extent to which it would shift TM helix position. To do this, pLA peptides with the hydrophilic (X) residue moved from position 7 to position 8 were used (general sequence: Acetyl-KKLALALXLALAWLALALALALAKK-amide). Figure 9 shows a comparison of Trp emission λ_{max} (Figure 9A) and Q-ratio (Figure 9B, raw quenching data in Supplemental Table 5) for pLA peptides with hydrophilic substitutions at position 8 (= X8 peptides) to that for peptides having hydrophilic substitutions at position 7 (=X7 peptides), when incorporated into DOPC vesicles. The order of the dependence of Trp depth upon the identity of the X residue (X = L<S<E<N) is the same for X8 and X7 peptides. This shows that the relative effects of different hydrophilic residues are not altered by the small change in X residue position in the sequence. However, the Trp residue is deeper for the X8 peptides (filled bars) than for X7 peptides (striped bars). This indicates that the TM segment shifts less in the X8 sequences than in the X7 sequences. This is confirmed by measurements of $L_{\text{TM eff}}$, derived from the effect of bilayer width upon λ_{max} (Figure 10), which shows that the X8 peptides exhibit a larger $L_{\text{TM eff}}$ than the corresponding X7 peptides (Table 3).

This pattern is the opposite of that predicted if the X residues were moving to the same location at the bilayer surface for the X7 and X8 peptides. In that case, the Trp depth would have been shallower and the shift greater in the case of the X8 peptides, in which the Trp is closer to the

X residue. Two factors are likely to contribute to the tendency of hydrophilic X8 residues to shift TM helix position less hydrophilic X7 residues. First, the length of the hydrophobic sequence shifted out the bilayer core if the X residue moves to the bilayer surface is longer for the X8 peptides (5 residues) than for the X7 peptides (4 residues). Furthermore, X replaces L in the X7 peptides and replaces A in the X8 peptides. As a result, the sequence shifted out of the bilayer is more hydrophobic (having one extra L) for the X8 peptides than for the X7 peptides. Thus, the hydrophobic sequence at the N-terminal end would resist being shifted out of the bilayer to a greater extent for the X8 peptides. Second, for the X8 peptides the length of the truncated TM sequence formed if the X residue moves to the bilayer surface (15 residues) is shorter than that formed by the X7 series (16 residues). Thus, the negative mismatch forming upon helix shifting should result in a greater degree of energetically unfavorable strain on the bilayer for the X8 peptides if the X residue shifted to the same location for the X7 and X8 peptides.

Effect of the Hydrophobicity of the Non-Polar Residues Shifted Out of the Hydrophobic Core of the Bilayer Upon Transverse Shifts

The experiments above suggested that the hydrophobicity of a sequence pushed out of the bilayer upon a transverse shift could affect the extent of the shift induced by hydrophilic X residues. To test this hypothesis, we measured the transverse positions of peptides varying the sequence (residues 3–6) that would be shifted out of the core of the lipid bilayer by the movement of an X residue at position 7 to the bilayer surface (general sequence: Acetyl-K₂Z₄EALALAWLALALALAKK-amide). In the sequences compared the X residue was E and the Z residues (residues 3–6) were (LA)₂, A₄ or G₄. The pK_a of Glu for the Z = (LA)₂, A₄ and G₄ peptides, determined as described above, is 6.7, 7.1 and 7.3, respectively (data not shown). Thus, to compare sequences with both protonated and ionized Glu, experiments were carried out at pH 4.0 and pH 9.0, respectively.

When these peptides are incorporated in DOPC vesicles at pH 4.0 (Glu protonated) Trp depth decreases in the order Z = (LA)₂ < A₄ < G₄ as judged by both by $\lambda_{\max}$ (filled bars, Figure 11A) and Q-ratio (filled bars, Figure 11B, raw quenching data in Supplemental Table 6), suggesting that the E residue shifts increasingly towards the bilayer surface in that order. In the case of the peptide with the Z=G₄ sequence, there appears to be some non-TM state formed in DOPC vesicles at pH 4 as judged by the observation that it (unlike the peptides with Z = (LA)₂ or Z=A₄ sequences) exhibits significant quencher-induced shifts of $\lambda_{\max}$ (Table 4). The conclusion that the shift increases in the order Z = (LA)₂ < A₄ < G₄ is supported by L_{TM eff} values derived from the effect of lipid acyl chain length on Trp $\lambda_{\max}$ (Figure 12A). At pH 4.0, L_{TM eff} for the Z = (LA)₂, A₄ and G₄ peptides decrease relative to the parental [Z=(LA)₂, X=L] peptide by 2.2, 3.3, and 3.8 residues, respectively (Table 4).

When these peptides are incorporated in DOPC vesicles at pH 9.0 (Glu ionized) Trp depth decreases in the same order as at pH 4, Z = (LA)₂ < A₄ < G₄, as judged by both by $\lambda_{\max}$ (striped bars, Figure 11A) and Q-ratio (striped bars, Figure 11B). This indicates that the dependence of helix shift on Z sequence follows the same order when Glu is protonated or ionized. However, Trp depth at pH 9.0 is shallower than at pH 4.0 for all three peptides. In addition, quencher-induced $\lambda_{\max}$ shifts suggest that when incorporated into DOPC vesicles at pH 9 there is a mixture of a shifted TM and non-TM topography formed by all three peptides (Table 4). The conclusion that at pH 9.0 the degree of shift increased in the order Z = (LA)₂ < A₄ < G₄ and that the shift is greater than at pH 4 is supported by L_{TM eff} values (Figure 12B). At pH 9.0, L_{TM eff} decreased relative to the parental [Z = (LA)₂, X=L] peptide by about 3.9, 4.8 and 4.9 residues for the Z=(LA)₂, A₄ and G₄ peptides, respectively (Table 4).

Secondary Structure of the pLA peptides: Circular Dichroism (CD) Measurements

In order to evaluate the secondary structure of the various pLA peptides used in this study, CD measurements were carried out (Supplemental Tables 7–9). For almost all peptides and conditions studied, the α -helix content is estimated to be 80–85 %, which corresponds to helix formation by the entire hydrophobic sequence. This is true both for peptides incorporated into DOPC vesicles and incorporated into DEuPC vesicles, with only about 1% loss in helix content in DEuPC vesicles. Many of the peptides form the non-TM state in DEuPC vesicles, so these results indicate that, at least for those peptides, the non-TM state is as helical as the TM state. There is a very small sequence dependence of helix content: more polar the X residue or Z residues, the lower the helix content (maximum 5–10 % decrease). This can be explained if residues extruded from the bilayer have a lower tendency to form a helix. Since the more highly polar residues induce a larger shift, they would extrude more residues and thus induce the largest changes in secondary structure. The lowest helix values are observed with X = Pro, consistent with its helix-breaking properties.

DISCUSSION

Defining the TM Helix-Shifting Propensities of Hydrophilic Residues Using Fluorescence

This study used TM helices incorporated into model membrane vesicles to examine how features of hydrophobic helix sequence influence TM helix position within membranes. In seminal studies using a glycosylation-mapping technique, von Heijne and colleagues defined the effects of several polar and ionizable residues upon the transverse position of TM helices at the time that they are likely to be associated with the translocon^{20,27,28,35,36}. Their technique probes the boundary of a TM segment via the fact that an exofacial Asn residue in an extra-membranous segment of a membrane protein has to be 12 residues away from the membrane surface to be accessible to glycosylation by oligosaccharyl transferase. Our experiments have extended studies of transverse shift to lipid bilayers and to all types of hydrophilic residues, measuring the position and length of the TM helix itself. Several previous studies show that in the model membrane system TM helices form their equilibrium structures^{9,17,22} and this equilibrium behavior within a lipid bilayer is particularly relevant to post-translational shifts in TM helix position. In addition, in our system we could vary pH to look at the behavior of ionizable residues in both the charged and uncharged states. Thus, this report yields a more detailed picture of the sequence-dependence of TM helix topography.

It is important to understand the mathematical relationship between the distance that a hydrophilic X residue shifts towards the bilayer surface (the surface defined here as the boundary of the hydrophobic core of the bilayer), the resulting changes in the length of the TM helix spanning the bilayer, and Trp depth. Under conditions in which there is initially no hydrophobic mismatch (Figure 13A) the movement of the X residue to the surface (by n Å) should equal the length by which TM-spanning sequence decreases, and a Trp at the center of the hydrophobic sequence should shift $\frac{1}{2}n$ Å away from the bilayer center, assuming that the negative mismatch induced by the shortening of the TM sequence is accommodated by a symmetric positioning within the lipid bilayer, as inferred previously by Monné and von Heijne²⁸. At the other extreme is the case in which there is initially positive mismatch and a tilted TM helix (Figure 13B). If positive mismatch is lost after the shift of the X residue to the surface (by n Å), the decrease in the length of the TM spanning sequence will be $n/\cos\theta$ Å, where θ is the tilt angle prior to helix shift. The Trp residue will again shift $\sim \frac{1}{2}n$ Å away from the bilayer center.

In our experiments, peptide lengths were such that they should switch from slight positive mismatch to moderate negative mismatch upon movement of the X residue to the surface (Figure 13C). Based on the considerations described above this movement would decrease the

length of the TM sequence (L_{TM}) from 21 to 16 residues ($7.5\text{\AA}$), move the X residue $6.8\text{\AA}$ closer to the bilayer surface (assuming that 19 hydrophobic residues span a DOPC bilayer under conditions of hydrophobic match^{8,9}, and shift the Trp residue $\sim 3.4\text{\AA}$ away from the bilayer center. When X was a highly hydrophilic residue with a short side chain or when the hydrophobicity of the Z residues was low, observed shifts approached these values (see Results).

TM Helix Transverse Shift Propensities of Different Hydrophilic Amino Acid Residues

The extent of helix shift was strongly dependent upon the identity of the X residue, with most residues inducing intermediate degrees of shift such that the X residue did not fully move to the membrane surface. Figure 14 summarizes λ_{max} (Figure 14A) and Q-ratio (Figure 14B) for all of the X residues tested, and Figure 15 shows the relationship between $L_{TM\text{ eff}}$ and Trp depth in DOPC vesicles as measured by Q-ratio (a very similar pattern is observed for a graph of $L_{TM\text{ eff}}$ vs. λ_{max} in DOPC vesicles, not shown). Based on these graphs, the transverse shifting properties of different X residues can be compared. This reveals a number of novel and even surprising findings.

Asparagine and Glutamine—Asn and Gln induced very large shifts, as large or larger than most of the charged residues. In fact, at pH 7 Asn induced larger transverse shifts than any residue other than Asp. This probably reflects the strong hydrophilicity of the amide group, and is consistent with the observation that Asn and Gln are the two least abundant uncharged polar residues in TM segments^{39,40}. The lesser shifts induced by Gln relative to Asn may be due to its extra methylene, which increases its hydrophobicity and ability to snorkel (see below). Substitution of hydrophobic residues with Asn and Gln may be particularly useful for probing the functional effects of large changes in TM helix position (see below).

Lysine and Arginine—Lys and Arg shifted helix position to a much lesser degree than the Asn or Gln or even other charged residues (see below). The main reason for this is likely to be snorkeling. Arg and Lys with long side chain can snorkel to positions their charged groups as close as possible to the membrane surface^{38,41}. This explains the small change in Trp depth in the presence of Lys and Arg because the alignment of the Lys or Arg side chains towards the surface would minimize the shift of the entire TM helix necessary to locate charged groups near the membrane surface. Charge delocalization in the Arg side chain may also aid its burial within the lipid bilayer.

It is interesting that even though snorkeling should significantly increase the length of the TM segment (L_{TM}) relative to a non-snorkeling charged residue, $L_{TM\text{ eff}}$ for Arg and Lys was smaller than predicted from Trp depth, as shown by comparison of $L_{TM\text{ eff}}$ and Trp depth for different X residues (Figure 15). The likely explanation for this behavior is that snorkeling is costly in terms of lost entropy due to the restriction of the side chain orientation required to position the charged group near the surface. As a result, negative mismatch destabilizes the TM state relative to the non-TM state even when TM helix transverse position and length is not much different from that of the parental peptide (when X=L). Thus, when X = Arg or Lys the stability of the TM state and its sensitivity to bilayer width is similar to that of a hydrophobic helix that has a highly hydrophilic X residue that cannot snorkel and so forms a short TM segment.

Histidine, Aspartate and Glutamate—The other ionizable residues had a very strong tendency to shift helix position when charged. This is expected as they are very hydrophilic when charged and have shorter side chains than Lys and Arg. The order of the extent of transverse shifting was X=Asp>Glu>His. The smaller shifts induced by charged Glu and His may reflect their side chains being longer than that of Asp. Thus, they are a bit more

hydrophobic and can snorkel to a greater degree than Asp, although not nearly as much as Lys or Arg. The fact that His⁺ induces smaller shifts than Glu⁻ might partly reflect the delocalization of the positive charge on His⁺.

Uncharged Glu, Asp and His induced much smaller transverse shifts than when charged, consistent with their lower hydrophilicity in the uncharged state. The extent of shift induced in the uncharged state followed the same order as in the charged state: X=Asp>Glu>His. The uncharged Asp retained the ability to induce very large transverse shifts, while the weaker helix shifting propensity of an uncharged Glu was, at most, no greater than that of a Ser residue. This agrees with hydrophobicity results of Wimley and White⁴², who found that an uncharged Glu was slightly more hydrophobic than an uncharged Ser as judged from their relative effects upon binding of a peptide to the non-polar/polar boundary of a bilayer. The weak extent of shift induced by His⁰ as measured by Trp depth may partly reflect an ability to snorkel, as formation of the His⁰ tautomer with a proton on the N3 atom would have a hydrophobic side chain three carbon atoms long. An ability of His⁰ to snorkel is consistent with the disproportionately large decrease in $L_{TM\text{ eff}}$ relative to the shift in Trp depth (Figure 15), a pattern similar to that observed for Lys and Arg residues.

The effects of ionization state upon the transverse position of TM helices containing Asp, Glu and His residues may be biologically important because when incorporated into TM helices their pKa values fall in a physiologically significant pH range. At pH 7, the overall order of transverse shifting remains X = Asp>Glu>His, but the relative amounts of shift are affected by whether these residues were charged or not. (It should be noted that the difference between Glu and Asp at neutral pH is in agreement with the glycosylation mapping data of Monné *et al*²⁷.) As we previously observed for Asp residues^{17,25}, these ionizable residues have pKa values such that their protonation, and thus effect upon transverse position, could be altered in acidic organelles, and this might have important biological effects, e.g. upon signaling in acidic organelles.

Proline—Pro had a strong tendency to induce transverse shifts at pH 7, but not as much as Lys, Gln, Asn, Glu and Asp. The observation that Pro did not induce shifts larger than Lys, Glu and Asp at neutral pH appears to contrast with previous glycosylation mapping studies which found that Pro residues induced the largest transverse shifts (Asn and Gln were not tested) in the position of the extra-membranous glycosylated sequences³⁶. However, as noted by Nilsson *et al.*³⁶, the large effect of Pro in those studies partly reflects the helix-breaking properties of Pro, which probably results in formation of an extended structure by the sequence extruded from the bilayer (the length of which is what glycosylation mapping measures), and thus a larger shift of sequences outside of the membrane than that occurring within the bilayer.

Local destabilization of helical structure could also explain why Pro was one of the residues that exhibited a disproportionately larger effect on $L_{TM\text{ eff}}$ relative to that it had upon Trp depth in DOPC vesicles (Figure 15). The energetic cost of burial of Pro could have an unusually strong dependence on its depth of burial in the hydrophobic core of the membrane because it destabilizes the helical structure of neighboring residues³⁶, so that their burial is also energetically unfavorable. This would show up as a decrease in $L_{TM\text{ eff}}$ because the TM state would be destabilized more severely under conditions of negative mismatch, which would tend to bury both the Pro and neighboring residues.

Serine—Ser, a modestly hydrophilic residue, induced an intermediate, but still substantial, extent of transverse shift. There was evidence that this intermediate shift was an average arising from co-existing of shifted and un-shifted populations. The significant shifting ability of Ser is important because it is very common in TM sequences^{39,40}. TM helices with Ser not too

far from one end of the hydrophobic sequence may have an appreciable ability to switch between TM topographies with different TM segment boundaries.

Tyrosine—Tyr induced a rather small shift. This may partly reflect the fact that its lowest free energy location is not in a polar environment, but rather near the polar/non-polar interface^{39,43,44}. Consistent with this explanation it has been found that Trp, the other heteroaromatic residue that prefers to locate at the polar/non-polar interface, has little tendency to induce transverse shifts when located within five residues from the end of a hydrophobic sequence³⁵. In addition, there may be snorkeling by the large phenyl side chain to place the Tyr OH group close to the bilayer surface. This is consistent with the observation that $L_{TM\text{ eff}}$ for Tyr was smaller than Ser (a residue that cannot snorkel to a significant degree), even though Tyr did not shift helix position as much as Ser.

Threonine—Thr is less hydrophilic than Ser due to its extra methyl group, and had a weaker ability to shift helix position than Ser as judged by Trp depth. However, it had a more significant effect upon $L_{TM\text{ eff}}$, decreasing $L_{TM\text{ eff}}$ slightly more than Ser. We do not understand the origin of this behavior, which cannot be explained by snorkeling.

Glycine—Gly induced the smallest shift of all residues tested, but it was still significant relative to X=Leu. The observation that such a modestly hydrophilic residue as Gly can influence TM helix transverse position emphasizes the strong sensitivity of transverse position to sequence.

The Effect of the Sequence Pushed Out of the Bilayer Core Upon Transverse Helix Shift

It was also found that modest changes in the hydrophobicity of the sequence extruded from the bilayer core when a hydrophilic residue shifts towards the bilayer surface strongly influences TM helix position. This indicates that there is a considerable change in free energy when a hydrophobic sequence moves from a position slightly below, to slightly above, the formal non-polar/polar boundary, and thus that the actual polarity gradient in the direction perpendicular to the plane of the membrane must be reasonably steep in the interfacial region. This conclusion is consistent with the predictions of recently proposed models for the membrane polarity gradient^{45,46}.

Effect Upon Transverse Shift of the Position of the Hydrophilic Residue Within the Hydrophobic Sequence and the Length of the Truncated TM Sequence Formed After a Transverse Shift

The position of a hydrophilic residue within a hydrophobic sequence also was found to have a significant effect upon the extent of transverse shift, in agreement with glycosylation mapping studies^{20,27,28}. The closer a hydrophilic residue is to the center of a hydrophobic sequence, the more energetically unfavorable its location, which should increase transverse shift. However, if a hydrophilic residue is too close to the center of a hydrophobic sequence then its movement to the surface would be resisted by two factors: having to shift too long a segment of hydrophobic sequence out of the bilayer core, and forming too short a truncated TM sequence upon shifting. Assuming bilayer distortion has a significant energetic cost, strong negative mismatch resulting from the formation of a very short truncated TM helix (Figure 13A) would reduce the degree the movement of a hydrophilic residue to the surface is energetically favorable, and thus reduce the extent of the helix shift relative to a case of a longer hydrophobic sequence in which the shift of the hydrophilic residue to the surface would not result in negative mismatch (Figure 13B).

Additional Factors Likely to Affect Transverse Helix Shifts

Additional variables may affect transverse shifts of TM helix position. One is interactions between a hydrophilic residue and neighboring residues. Polar interactions between two residues separated by one helical turn away can reduce the unfavorable free energy of burial and thus the tendency to shift helix position²⁰. Steric effects in which large neighboring residues reduce the exposure of the X residue to its environment could also influence its propensity to shift position⁴². Lipid composition may also be an important variable. The positioning of cationic residues might be stabilized by interaction with anionic lipids, and a lipid composition with an especially limited ability to distort to accommodate hydrophobic mismatch could limit the extent of transverse shifts that increase hydrophobic mismatch.

Finally, helix-helix interactions should modulate TM helix positioning. If only one of two transverse positions formed by a TM helix can interact with a second helix, that position would be stabilized by helix-helix interaction. An important reciprocal concept is that helix positioning could control helix-helix interaction if there is an energetic cost of shifting a TM helix into a transverse position in which it can interact with another helix.

It should be noted that several of our studies show that electrostatic repulsions between the multiple flanking Lys sequences in our peptides generally prevent helix oligomerization within model membrane vesicles, and even in the absence of electrostatic repulsions, only very weak oligomerization is observed^{13,17,22}. This weak oligomerization can be detected by a dependence of Trp fluorescence properties, or fluorescence resonance energy transfer between labeled peptides, upon peptide concentration within the bilayer^{13,17}. With the exception of the X = Lys peptide no effects of peptide concentration upon Trp fluorescence in DOPC vesicles were observed for the peptides used in this report (data not shown). The X = Lys peptide exhibited a blue shift (4 nm) in Trp λ_{max} and a small decrease in Q-ratio when the peptide/DOPC ratio was diluted from 1:100 to 1:500 mol:mol. This indicates that the X = Lys peptide forms weakly-associating oligomers at higher peptide/lipid ratio, and that Lys snorkeling may limit the extent of transverse shift even more strongly in the monomeric state.

Functional Role of Transverse Helix Shifts and Its Investigation Using Mutagenesis to Control Helix Position

This study shows that TM helix transverse position is very sensitive to sequence. This implies that transverse shifts in TM helix position may be common, and that the actual boundaries of TM segments may often be different than the values assigned by simple analyses of sequence hydrophobicity. As pointed out above, the constraint peculiar to membrane proteins, that interactions between two domains can require that the domains be properly aligned in a specific plane, implies that shifts in TM helix position represent a unique mechanism for control of membrane protein function. In addition to control of TM helix-TM helix interactions, this mechanism may control function by shifting the position of extra-membranous domains. The behavior of juxtamembrane sequences can affect the degree to which TM sequence shifts are transmitted to domains outside of the membrane (Figure 13 and 28).

In the most well studied example of such behavior, bacterial TM chemoreceptors, there are very long helices that extend through and far beyond the membrane, and so can directly transmit shifts in TM helix position to domains outside of the membrane³². Similar transmission of TM helix shifts to non-TM domains has been proposed for integrins³⁴.

Shifts in TM domain position by polar residues introduced by mutations may be molecular basis for disease states. A hydrophilic (G380R) mutation in the TM domain of the FGFR3 receptor results in abnormal bone development (dysplasias)⁴⁷. This mutation leads to slow downregulation of the activated mutant receptors, and is likely to prolong signaling^{48,49}. It

has recently been shown that this mutation alters the TM helix boundary of FGFR3 receptor. It has been proposed that the altered membrane position of the TM helix might affect downregulation by altering the ubiquitination of FGFR3^{47,49}.

Similarly, for the ErbB2 receptor, which is involved in cell growth and development, a Val to Glu mutation (V664E) in the TM domain can induce a TM helix shift²⁹. This mutation has been linked to the constitutive activation of the tyrosine kinase activity of the receptor, resulting in oncogenicity^{29,50}. Although it has been proposed that oligomerization induced by this mutation may be critical for oncogenicity^{29,51,52}, further experimentation is needed to see if the shift in helix position is also functionally important.

In the case of amyloid precursor protein (APP) processing, the membrane position of APP is a major determinant for the cleavage site of γ -secretase⁵³. Hydrophilic/polar mutations in and around the TM domain has been shown to affect proteolytic processing of APP by altering the boundary of the TM segment of APP,^{53,54}.

Functions of membrane proteins that are strongly dependent on TM helix transverse position should be affected by hydrophilic substitutions in a manner that reflects the degree of transverse shift induced more closely than other properties of the substituting residue. Thus, knowledge of how transverse helix shifts depend upon hydrophilic residue structure could be useful in mutagenesis-based studies in which amino acid substitutions are used to probe the functional significance of transverse helix position.

MATERIALS AND METHODS

Materials

Peptides with blocked N and C-termini, Acetyl-K₂(LA)₂X(A)(LA)₂W(LA)₅K₂-NH₂ [pLA(X7)], where X = L, G, Y, T, S, P, H, R, K, Q, N, E and D; Acetyl-K₂(LA)₂LX(A)(LA)₂W(LA)₅K₂-NH₂ [pLA(X8)], where X = S, E and N and Acetyl-K₂Z₄EA(LA)₂W(LA)₅K₂-NH₂ [pLAZ(3–6)E(7)], where Z(3–6) = G or A, were purchased from Anaspec Inc. (San Jose, CA). Peptides were purified via reverse-phase-HPLC using a C18 column with 2-propanol/water/0.5% v/v trifluoroacetic acid as the mobile phase as described previously⁵⁵. Peptide purity was confirmed using MALDI-TOF mass spectrometry (Proteomics Center, Stony Brook University). We estimated that final purity was on the order of 90% or better. After drying the HPLC fractions the peptides were stored in 1:1 (v/v) 2-propanol/water at 4 °C. Peptide concentrations were measured by absorbance spectroscopy on a Beckman DU-650 spectrophotometer, using ϵ for Trp of 5560 M⁻¹cm⁻¹ at 280 nm. A series of 1,2-diacyl-*sn*-glycero-3-phosphocholines (phosphatidylcholines, PC): diC14:1 Δ 9cPC (dimyristoleoyl-PC, DMOPC); diC16:1 Δ 9cPC (dipalmitoleoyl-PC, DPOPC); diC18:1 Δ 9cPC (dioleoyl-PC, DOPC); diC20:1 Δ 11cPC (dieicosenoyl-PC, DEiPC); diC22:1 Δ 13cPC (dierucoyl-PC, DEuPC) and diC24:1 Δ 15cPC (dinervonoyl-PC, DNPC) were purchased from Avanti Polar Lipids (Alabaster, AL). Concentrations of lipids purchased as liquid solutions were confirmed by dry weight. The lipids were stored in chloroform at –20°C. 10-doxylnonadecane (10-DN) was custom synthesized by Molecular Probes (Eugene, OR). It was stored as a 5 mM stock solution in ethanol at –20 °C.

Model Membrane Vesicle Preparation

Model membrane vesicles were prepared using the ethanol dilution method as previously described^{8,9}. Peptides dissolved in 1:1 (v/v) 2-propanol/water and lipids dissolved in chloroform were mixed and dried under a stream of N₂ gas. Samples were then dried under high vacuum for 1h. The dried peptide/lipid samples were then dissolved in a minimum volume (10 μ l) of ethanol and diluted to the desired final volume (typically 800 μ l) using PBS (10mM

sodium phosphate and 150mM NaCl), pH 7.0 unless otherwise noted, with constant vortexing. Low and high pH samples were prepared using PBS adjusted to the required pH using glacial acetic acid (pH 4.0) or NaOH (pH 9.0), respectively. Unless otherwise noted, for model membrane samples the final concentrations were 2 μ M peptide and 200 μ M lipid.

Fluorescence Measurements

Fluorescence data was obtained on SPEX τ 2 Fluorolog spectrofluorometer operating in steady-state fluorescence mode at room temperature. Excitation slits of 2.5 nm (4.5 nm band-pass) and emission slits of 5 nm (9 nm band-pass) were used for all measurements. The fluorescence emission spectra were measured over the range 300–375 nm. Fluorescence from background samples containing lipid and buffer with no peptide were subtracted to calculate reported fluorescence values. All measurements were made at room temperature.

Acrylamide Quenching Measurements

To measure acrylamide quenching, fluorescence intensity and emission spectra were first measured in samples containing peptides incorporated into model membranes or background samples, prepared as described above. Then a 50 μ l aliquot of acrylamide from a 4M stock solution dissolved in water was added. After a brief incubation (5 min), the fluorescence was remeasured. In these experiments, fluorescence intensity was measured using an excitation wavelength of 295 nm and emission wavelength of 340 nm. This excitation wavelength was chosen to reduce acrylamide absorbance (and the resulting inner-filter effect). Fluorescence intensity was corrected both for dilution from addition of acrylamide and due to the inner-filter effect³⁷. To determine emission λ_{max} , Trp emission spectra in the presence of acrylamide were measured with an excitation wavelength of 280 nm, which gave stronger fluorescence intensity than with excitation at 295 nm despite the increased inner-filter effect due to acrylamide absorbance at 280 nm. Previous controls show that in these experiments the emission spectra λ_{max} is not affected by this choice of excitation wavelength³⁷.

10-Doxylnonadecane Quenching Measurements

To measure quenching by 10-DN, the fluorescence of samples containing model membrane-incorporated peptide without 10-DN was compared to that in samples containing 10-DN. To prepare the later, samples were prepared as described above except that either 10% mol (for DOPC) or 12% mol (for DEuPC) of the lipid was replaced with an equivalent mol % of 10-DN³⁷. Fluorescence intensity was measured using an excitation wavelength of 280 nm and emission wavelength of 330 nm. Emission spectra were measured with excitation wavelength of 280 nm.

Calculation of Acrylamide/10-DN Quenching Ratio

The acrylamide/10-DN quenching ratio (Q-ratio) was used to estimate Trp depth in the bilayer. The ratio was calculated from the formula $Q\text{-ratio} = [(F_o/F_{\text{acrylamide}})-1]/[(F_o/F_{10\text{-DN}})-1]$, where F_o is the fluorescence of a sample with no quencher present and $F_{\text{acrylamide}}$ and $F_{10\text{-DN}}$ are the fluorescence intensities in presence of acrylamide or 10-DN respectively. The Q-ratio varies inversely with depth of the Trp in the membrane³⁷.

Circular Dichroism Measurements

Circular dichroism (CD) spectra were recorded at room temperature using a JASCO J-715 CD spectrometer and 1 mm path length quartz cuvettes. Typically, final spectra were the average of 50 scans taken at a rate of 50 nm/min. All CD measurements were made with 2 μ M peptide and 200 μ M lipid. Estimation of α -helical content was done using DICHROWEB, a online server for secondary structure analyses from CD data⁵⁶. Intensities in background samples (lipid without peptides) were subtracted before analysis of secondary structure.

Calculation of Effective TM Length ($L_{TM\text{ eff}}$)

Bilayer width at which λ_{max} was a minimum was identified for a polynomial fit of λ_{max} vs. bilayer width curves using the SlideWrite program (Advanced Graphics Solution, Encinitas, CA). Correlation coefficients (r^2) were greater than 0.99 for all fits. $L_{TM\text{ eff}}$ (in units of numbers of residues) was calculated using the formula: $L_{TM\text{ eff}} = \text{bilayer core width at } \lambda_{\text{max}} \text{ minimum} / 1.5 \text{ \AA per residue}$; where bilayer width = $1.8 \text{ \AA} \times \text{number of acyl chain carbon atoms} - 4.5 \text{ \AA}$.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

† This work was supported by NIH grant HL63768.

References

1. Bechinger B. Towards membrane protein design: pH-sensitive topology of histidine-containing polypeptides. *J Mol Biol* 1996;263:768–75. [PubMed: 8947574]
2. Bechinger B. Membrane insertion and orientation of polyalanine peptides: a (15)N solid-state NMR spectroscopy investigation. *Biophys J* 2001;81:2251–6. [PubMed: 11566795]
3. Hunt JF, Rath P, Rothschild KJ, Engelman DM. Spontaneous, pH-dependent membrane insertion of a transbilayer alpha-helix. *Biochemistry* 1997;36:15177–92. [PubMed: 9398245]
4. Killian JA, Nyholm TK. Peptides in lipid bilayers: the power of simple models. *Curr Opin Struct Biol* 2006;16:473–9. [PubMed: 16828281]
5. Liu F, Lewis RN, Hodges RS, McElhaney RN. Effect of variations in the structure of a polyleucine-based alpha-helical transmembrane peptide on its interaction with phosphatidylethanolamine Bilayers. *Biophys J* 2004;87:2470–82. [PubMed: 15454444]
6. Park SH, Opella SJ. Tilt angle of a trans-membrane helix is determined by hydrophobic mismatch. *J Mol Biol* 2005;350:310–8. [PubMed: 15936031]
7. Popot JL, Engelman DM. Helical membrane protein folding, stability, and evolution. *Annu Rev Biochem* 2000;69:881–922. [PubMed: 10966478]
8. Ren J, Lew S, Wang J, London E. Control of the transmembrane orientation and interhelical interactions within membranes by hydrophobic helix length. *Biochemistry* 1999;38:5905–12. [PubMed: 10231543]
9. Ren J, Lew S, Wang Z, London E. Transmembrane orientation of hydrophobic alpha-helices is regulated both by the relationship of helix length to bilayer thickness and by the cholesterol concentration. *Biochemistry* 1997;36:10213–20. [PubMed: 9254619]
10. van Duyl BY, Meeldijk H, Verkleij AJ, Rijkers DT, Chupin V, de Kruijff B, Killian JA. A synergistic effect between cholesterol and tryptophan-flanked transmembrane helices modulates membrane curvature. *Biochemistry* 2005;44:4526–32. [PubMed: 15766283]
11. Zhang YP, Lewis RN, Henry GD, Sykes BD, Hodges RS, McElhaney RN. Peptide models of helical hydrophobic transmembrane segments of membrane proteins. 1. Studies of the conformation, intrabilayer orientation, and amide hydrogen exchangeability of Ac-K2-(LA)12-K2-amide. *Biochemistry* 1995;34:2348–61. [PubMed: 7857945]
12. Gratkowski H, Lear JD, DeGrado WF. Polar side chains drive the association of model transmembrane peptides. *Proc Natl Acad Sci U S A* 2001;98:880–5. [PubMed: 11158564]
13. Lew S, Caputo GA, London E. The effect of interactions involving ionizable residues flanking membrane-inserted hydrophobic helices upon helix-helix interaction. *Biochemistry* 2003;42:10833–42. [PubMed: 12962508]
14. Russ WP, Engelman DM. The GxxxG motif: a framework for transmembrane helix-helix association. *J Mol Biol* 2000;296:911–9. [PubMed: 10677291]

15. Zhou FX, Merianos HJ, Brunger AT, Engelman DM. Polar residues drive association of poly-leucine transmembrane helices. *Proc Natl Acad Sci U S A* 2001;98:2250–5. [PubMed: 11226225]
16. Fleming KG, Engelman DM. Specificity in transmembrane helix-helix interactions can define a hierarchy of stability for sequence variants. *Proc Natl Acad Sci U S A* 2001;98:14340–4. [PubMed: 11724930]
17. Lew S, Ren J, London E. The effects of polar and/or ionizable residues in the core and flanking regions of hydrophobic helices on transmembrane conformation and oligomerization. *Biochemistry* 2000;39:9632–40. [PubMed: 10933779]
18. Hermansson M, von Heijne G. Inter-helical hydrogen bond formation during membrane protein integration into the ER membrane. *J Mol Biol* 2003;334:803–9. [PubMed: 14636604]
19. Zhou FX, Cocco MJ, Russ WP, Brunger AT, Engelman DM. Interhelical hydrogen bonding drives strong interactions in membrane proteins. *Nat Struct Biol* 2000;7:154–60. [PubMed: 10655619]
20. Chin CN, von Heijne G. Charge pair interactions in a model transmembrane helix in the ER membrane. *J Mol Biol* 2000;303:1–5. [PubMed: 11021965]
21. Hessa T, Kim H, Bihlmaier K, Lundin C, Boekel J, Andersson H, Nilsson I, White SH, von Heijne G. Recognition of transmembrane helices by the endoplasmic reticulum translocon. *Nature* 2005;433:377–81. [PubMed: 15674282]
22. Caputo GA, London E. Cumulative effects of amino acid substitutions and hydrophobic mismatch upon the transmembrane stability and conformation of hydrophobic alpha-helices. *Biochemistry* 2003;42:3275–85. [PubMed: 12641459]
23. Harzer U, Bechinger B. Alignment of lysine-anchored membrane peptides under conditions of hydrophobic mismatch: a CD, ¹⁵N and ³¹P solid-state NMR spectroscopy investigation. *Biochemistry* 2000;39:13106–14. [PubMed: 11052662]
24. Vogt B, Ducarme P, Schinzel S, Brasseur R, Bechinger B. The topology of lysine-containing amphipathic peptides in bilayers by circular dichroism, solid-state NMR, and molecular modeling. *Biophys J* 2000;79:2644–56. [PubMed: 11053137]
25. Caputo GA, London E. Position and ionization state of Asp in the core of membrane-inserted alpha helices control both the equilibrium between transmembrane and nontransmembrane helix topography and transmembrane helix positioning. *Biochemistry* 2004;43:8794–806. [PubMed: 15236588]
26. Fujita K, Krishnakumar SS, Franco D, Paul AV, London E, Wimmer E. Membrane topography of the hydrophobic anchor sequence of poliovirus 3A and 3AB proteins and the functional effect of 3A/3AB membrane association upon RNA replication. *Biochemistry* 2007;46:5185–99. [PubMed: 17417822]
27. Monne M, Nilsson I, Johansson M, Elmhed N, von Heijne G. Positively and negatively charged residues have different effects on the position in the membrane of a model transmembrane helix. *J Mol Biol* 1998;284:1177–83. [PubMed: 9837735]
28. Monne M, von Heijne G. Effects of 'hydrophobic mismatch' on the location of transmembrane helices in the ER membrane. *FEBS Lett* 2001;496:96–100. [PubMed: 11356190]
29. Smith SO, Smith CS, Bormann BJ. Strong hydrogen bonding interactions involving a buried glutamic acid in the transmembrane sequence of the neu/erbB-2 receptor. *Nat Struct Biol* 1996;3:252–8. [PubMed: 8605627]
30. Borochoy H, Shinitzky M. Vertical displacement of membrane proteins mediated by changes in microviscosity. *Proc Natl Acad Sci U S A* 1976;73:4526–30. [PubMed: 1070002]
31. Boldog T, Hazelbauer GL. Accessibility of introduced cysteines in chemoreceptor transmembrane helices reveals boundaries interior to bracketing charged residues. *Protein Sci* 2004;13:1466–75. [PubMed: 15133159]
32. Falke JJ, Hazelbauer GL. Transmembrane signaling in bacterial chemoreceptors. *Trends Biochem Sci* 2001;26:257–65. [PubMed: 11295559]
33. Miller AS, Falke JJ. Side chains at the membrane-water interface modulate the signaling state of a transmembrane receptor. *Biochemistry* 2004;43:1763–70. [PubMed: 14967017]
34. Armulik A, Nilsson I, von Heijne G, Johansson S. Determination of the border between the transmembrane and cytoplasmic domains of human integrin subunits. *J Biol Chem* 1999;274:37030–4. [PubMed: 10601259]

35. Braun P, von Heijne G. The aromatic residues Trp and Phe have different effects on the positioning of a transmembrane helix in the microsomal membrane. *Biochemistry* 1999;38:9778–82. [PubMed: 10423258]
36. Nilsson I, Saaf A, Whitley P, Gafvelin G, Waller C, von Heijne G. Proline-induced disruption of a transmembrane alpha-helix in its natural environment. *J Mol Biol* 1998;284:1165–75. [PubMed: 9837734]
37. Caputo GA, London E. Using a novel dual fluorescence quenching assay for measurement of tryptophan depth within lipid bilayers to determine hydrophobic alpha-helix locations within membranes. *Biochemistry* 2003;42:3265–74. [PubMed: 12641458]
38. Killian JA, von Heijne G. How proteins adapt to a membrane-water interface. *Trends Biochem Sci* 2000;25:429–34. [PubMed: 10973056]
39. Landolt-Marticorena C, Williams KA, Deber CM, Reithmeier RA. Non-random distribution of amino acids in the transmembrane segments of human type I single span membrane proteins. *J Mol Biol* 1993;229:602–8. [PubMed: 8433362]
40. Zhao G, London E. An amino acid “transmembrane tendency” scale that approaches the theoretical limit to accuracy for prediction of transmembrane helices: relationship to biological hydrophobicity. *Protein Sci* 2006;15:1987–2001. [PubMed: 16877712]
41. Chamberlain AK, Lee Y, Kim S, Bowie JU. Snorkeling preferences foster an amino acid composition bias in transmembrane helices. *J Mol Biol* 2004;339:471–9. [PubMed: 15136048]
42. Wimley WC, White SH. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. *Nat Struct Biol* 1996;3:842–8. [PubMed: 8836100]
43. Kachel K, Asuncion-Punzalan E, London E. Anchoring of tryptophan and tyrosine analogs at the hydrocarbon-polar boundary in model membrane vesicles: parallax analysis of fluorescence quenching induced by nitroxide-labeled phospholipids. *Biochemistry* 1995;34:15475–9. [PubMed: 7492549]
44. Mall S, Broadbridge R, Sharma RP, Lee AG, East JM. Effects of aromatic residues at the ends of transmembrane alpha-helices on helix interactions with lipid bilayers. *Biochemistry* 2000;39:2071–8. [PubMed: 10684657]
45. Senes A, Chadi DC, Law PB, Walters RF, Nanda V, Degradó WF. E(z), a depth-dependent potential for assessing the energies of insertion of amino acid side-chains into membranes: derivation and applications to determining the orientation of transmembrane and interfacial helices. *J Mol Biol* 2007;366:436–48. [PubMed: 17174324]
46. Ulmschneider MB, Sansom MS, Di Nola A. Properties of integral membrane protein structures: derivation of an implicit membrane potential. *Proteins* 2005;59:252–65. [PubMed: 15723347]
47. Han X, Mihailescu M, Hristova K. Neutron diffraction studies of fluid bilayers with transmembrane proteins: structural consequences of the achondroplasia mutation. *Biophys J* 2006;91:3736–47. [PubMed: 16950849]
48. Monsonego-Ornan E, Adar R, Feferman T, Segev O, Yayon A. The transmembrane mutation G380R in fibroblast growth factor receptor 3 uncouples ligand-mediated receptor activation from down-regulation. *Mol Cell Biol* 2000;20:516–22. [PubMed: 10611230]
49. Monsonego-Ornan E, Adar R, Rom E, Yayon A. FGF receptors ubiquitylation: dependence on tyrosine kinase activity and role in downregulation. *FEBS Lett* 2002;528:83–9. [PubMed: 12297284]
50. Li E, Hristova K. Role of receptor tyrosine kinase transmembrane domains in cell signaling and human pathologies. *Biochemistry* 2006;45:6241–51. [PubMed: 16700535]
51. Sharpe S, Barber KR, Grant CW. Val(659)-->Glu mutation within the transmembrane domain of ErbB-2: effects measured by (2)H NMR in fluid phospholipid bilayers. *Biochemistry* 2000;39:6572–80. [PubMed: 10828974]
52. Weiner DB, Liu J, Cohen JA, Williams WV, Greene MI. A point mutation in the neu oncogene mimics ligand induction of receptor aggregation. *Nature* 1989;339:230–1. [PubMed: 2654648]
53. Tischer E, Cordell B. Beta-amyloid precursor protein. Location of transmembrane domain and specificity of gamma-secretase cleavage. *J Biol Chem* 1996;271:21914–9. [PubMed: 8702994]
54. Lichtenthaler SF, Behr D, Grimm HS, Wang R, Shearman MS, Masters CL, Beyreuther K. The intramembrane cleavage site of the amyloid precursor protein depends on the length of its transmembrane domain. *Proc Natl Acad Sci U S A* 2002;99:1365–70. [PubMed: 11805291]

55. Lew S, London E. Simple procedure for reversed-phase high-performance liquid chromatographic purification of long hydrophobic peptides that form transmembrane helices. *Anal Biochem* 1997;251:113–6. [PubMed: 9300091]
56. Whitmore L, Wallace BA. DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res* 2004;32:W668–73. [PubMed: 15215473]

Figure 1.

Schematic comparison of three distinct topographies of hydrophobic helices (rectangles) in lipid bilayers. The position of a Trp (W) residue located in the center of the hydrophobic sequence is shown. The dashed line represents the plane of the center of the bilayer. Left: Non-TM state in which the helix and W residue lie close to the bilayer surface. Center: Normal TM state in which there is near-match between helix length and bilayer width and W residue is at the bilayer center. Right: Shifted-TM state in which there is a near match between the total hydrophobic helix length and bilayer width, but in which part of the helix has moved out of the core of the bilayer in order for a hydrophilic residue (X) to locate nearer the bilayer surface. There is negative mismatch when the X residue shifts to the bilayer surface, and a resulting symmetric distortion of the bilayer²⁸. Notice that in this case the W residue is at an intermediate depth.

Figure 2.

Trp λ_{max} and quenching ratio for pLA peptides with single substitutions at position 7 when incorporated into DOPC vesicles at pH 7.0. (A) Trp emission λ_{max} . (B) Quenching ratio. Samples contained 2 μ M peptide incorporated into 200 μ M lipid dispersed in PBS. Average values from three to six samples and standard deviations are shown.

Figure 3. Correlation between λ_{max} and quenching ratio for peptides incorporated into DOPC vesicles at pH 7.0. Data is from Figure 2.

Figure 4.

Trp λ_{max} and quenching ratio for pLA peptides with single substitutions at position 7 when incorporated into DEuPC vesicles at pH 7.0. (A) Trp emission λ_{max} . (B) Quenching ratio. Samples contained 2 μ M peptide incorporated into 200 μ M lipid dispersed in PBS. Average value from three to six samples and standard deviations are shown.

Figure 5. Dependence of the shift induced by quenchers upon λ_{max} in the absence of quencher for peptides incorporated into DEuPC (Δ) or DEiPC (+) vesicles. Data from Table 1.

Figure 6.

Effect of lipid acyl chain length on Trp emission λ_{max} . (A) Schematic illustration of the effect of hydrophobic helix length on λ_{max} for peptides incorporated into lipid vesicles with different acyl chain lengths. Vertical dashed lines indicate the bilayer width equivalent to the effective TM length ($L_{TM\text{ eff}}$). (B) Effect of lipid acyl chain length upon Trp emission λ_{max} for pLA peptides with single substitutions at position 7 at pH 7. The substitutions are Leu (X); Gly (+); Tyr ($\square$); Ser ($\triangle$); Thr ($\blacklozenge$); Pro ($\circ$); His (∇); Lys (+); Arg ($\blacktriangledown$); Glu ($\blacktriangle$); Asn ($\bullet$); Gln ($\blacksquare$) and Asp ($\circ$). The lipids used were a series of di n:1 phosphatidylcholines, in which n is the number of carbon atoms per acyl chain and the number 1 indicates that the acyl chains were

monounsaturated. Samples contained 2 μM peptide incorporated into 200 μM lipid dispersed in PBS. The λ_{max} values shown are averages from three to six samples. λ_{max} values were reproducible to within $\pm 1\text{nm}$.

Figure 7.

Effect of pH on the Trp emission λ_{max} and quenching ratio for pLA peptides containing Asp, His or Glu residue at position 7 when incorporated into DOPC vesicles at different pH. (A) Trp emission λ_{max} of Asp, His and Glu peptides in DOPC vesicles at pH 4.0 (filled bars), pH 7.0 (striped bars) and pH 9.0 (white bars). (B) Quenching ratio for peptides incorporated into DOPC vesicles at pH 4.0 (filled bars), pH 7.0 (striped bars) and pH 9.0 (white bars). The samples contained 2 μ M peptide incorporated into 200 μ M lipid dispersed in pH-adjusted PBS. Average values from three samples and for Q-ratios standard deviations are shown. λ_{max} values were reproducible to within ± 1 nm.

Figure 8.

Effect of lipid acyl chain length on Trp emission λ_{max} of pLA peptides with Asp, His or Glu at position 7 at different pH. (A) Peptide with Asp substitution. (B) Peptide with Glu substitution. (C) Peptide with His substitution. Symbols: pH 4 ($\circ$), pH 7 (Δ), pH 9 (+). The curve for the parental pLA peptide with Leu at position 7 ($\ast$, dashed line) is shown for comparison. Experimental conditions are as in Figure 6. Average values from three samples are shown. λ_{max} values were reproducible to within ± 1 nm.

Figure 9.

Effect of the position of the hydrophilic residue within the hydrophobic core of pLA peptide on the Trp emission λ_{max} and quenching ratio when incorporated into DOPC vesicles at pH 7.0. (A) Trp λ_{max} for peptides with single substitution at position 8 (filled bars) or position 7 (striped bars). (B) Quenching ratio for peptides with single substitutions at position 8 (filled bars) and position 7 (striped bars). The samples contained 2 μ M peptide incorporated into 200 μ M lipid dispersed in PBS. Average values from three samples and for Q-ratios standard deviation are shown. λ_{max} values were reproducible to within ± 1 nm.

Figure 10.

Effect of lipid acyl chain length on Trp emission $\lambda_{\max}$ of pLA peptides with single hydrophilic residues at position 7 or 8 when incorporated into vesicles at pH 7.0. Substitutions: Ser (Δ); Glu ($\circ$), and Asn (∇). Substitutions at position 8 are shown as open symbols and dashed lines, and substitutions at position 7 are shown as filled symbols and solid lines. The curve for the parental (X=L) peptide (+, dashed line) is also shown for comparison. Experimental conditions are as in Figure 6. Average from three samples is shown. $\lambda_{\max}$ values were reproducible to within ± 1 nm.

Figure 11.

Trp λ_{max} and quenching ratio of Glu-containing pLA peptides with varying 'Z' residues (residues 3–6) when incorporated into DOPC vesicles. (A) Trp emission λ_{max} in DOPC vesicles at pH 4.0 (filled bars), and pH 9.0 (striped bars). (B) Quenching ratio for peptides incorporated into DOPC vesicles at: pH 4.0 (filled bars) and pH 9.0 (striped bars). The samples contained 2 μ M peptide incorporated into 200 μ M lipid dispersed in pH-adjusted PBS. Average obtained from three samples and for Q-ratios standard deviations are shown. λ_{max} values were reproducible to within ± 1 nm.

Figure 12.

Effect of lipid acyl chain length on Trp emission λ_{max} of X=Glu pLA peptides with varying Z residues (residues 3–6) incorporated into vesicles. (A) pH 4.0 (B) pH 9.0. Z residues: $(LA)_2$ (+), A_4 (Δ) and G_4 ($\circ$). The curve for the parental pLA peptide with X = L and Z = $(LA)_2$ (dashed line) is shown for comparison. Experimental conditions are as in Figure 10. Average value of three samples is shown. λ_{max} values were reproducible to within ± 1 nm.

Figure 13.

Schematic figure summarizing the consequences of helix length and transverse shifts upon the position of TM helices in membranes. (A) Case in which there is match between the length of the entire hydrophobic sequence (rectangle) and bilayer width. There is negative mismatch when the X residue shifts to the bilayer surface, and a resulting symmetric distortion of the bilayer²⁸. Notice that in this case the extent of the shift of the X residue is directly transmitted to that of the juxtamembrane sequences (points a and b), unless the extruded sequence tends to lie along the bilayer surface (right)²⁸. This would decrease the transmission of the shift to point b. (B) Case in which there is positive mismatch between the length of the entire hydrophobic sequence (rectangle) and bilayer width, and in which a shift sufficient to relieve the positive mismatch occurs when the X residue shifts to the bilayer surface. The (maximum) tilt angle in the presence positive mismatch is θ . Notice that in this case the extent of the shift of the X residue is not identical to the shift of the juxtamembrane residues. Instead, the sequence

should to pivot around the distal boundary of the TM sequence (point a) reducing the amount of shift of distal juxtamembrane residues. (C) Likely behavior of the pLA peptides. They should show slight positive mismatch in DOPC vesicles if the entire hydrophobic sequence spans the lipid bilayer, but when the X residue shifts to the surface, there should be slight negative mismatch.

Figure 14. Summary of λ_{max} and quenching ratio for pLA peptides with different residues at position 7. Combined data from Figures 2 and 7 is shown.

Figure 15.

Correlation between Trp depth in DOPC vesicles in the TM state (as reported by the quenching ratio) and $L_{TM\text{eff}}$. Filled triangles represent residues that define a line with the theoretical slope for the relationship between $L_{TM\text{eff}}$ and Trp depth for the pLA peptides used in this study ($\sim 1\text{\AA}$ shift in Trp depth per $2\text{\AA}$ decrease in $L_{TM\text{eff}}$). Open circles represent hydrophilic residues that show a smaller change in Trp depth than predicted from the decrease in $L_{TM\text{eff}}$ relative to X=L. Note: Values corresponding to E⁻ and D⁻ are not shown because the pLA peptides containing these residues form co-existing shifted TM and non-TM states in DOPC vesicles.

Quencher-induced shifts in Trp emission λ_{max} and L_{TM} eff values for pLA peptides with single substitutions at position 7 when incorporated into vesicles at pH 7.0. Samples contained 2 μM peptide incorporated into 200 μM lipid vesicles dispersed in PBS.

Table 1

X Residue	Acyl Chain Length ^a	λ_{max} (nm) ^b	λ_{max} (nm) Acrylamide	λ_{max} (nm) 10-DN	$\Delta\lambda_{\text{max}}$ (nm) ^c	$L_{\text{TM,eff}}$ (number of residues) ^d
Leu	18	324	325	325	0	20.9
	20	323	322	323	1	
	22	327	327	331	4	
Gly	18	325	324	325	1	19.8
	20	326	325	329	4	
	22	332	325	339	14	
Tyr	18	324	322	324	2	18.4
	20	328	324	330	6	
	22	339	335	344	9	
Thr	18	325	324	326	2	18.5
	20	330	325	330	5	
	22	338	326	342	16	
His	18	326	326	327	1	18.0
	20	340	335	343	8	
	22	345	343	346	3	
Arg	18	326	325	328	3	17.7
	20	340	330	347	16	
	22	347	347	348	1	
Ser	18	327	326	330	4	18.9
	20	331	326	331	5	
	22	340	337	342	5	
Pro	18	327	327	328	1	17.8
	20	337	337	346	9	
	22	344	343	345	2	
Lys	18	328	326	330	4	17.6
	20	338	335	345	10	
	22	347	347	347	0	
Glu	18	329	329	331	2	17.3
	20	340	336	344	9	
	22	345	342	347	5	
Gln	18	330	329	330	1	17.1
	20	341	336	347	12	
	22	347	346	348	2	
Asn	18	332	332	333	1	17.2
	20	341	337	343	6	
	22	347	347	347	0	
Asp	18	336	333	340	7	16.6
	20	344	345	348	3	
	22	348	346	348	2	

^a18 = DOPC vesicles, 20 = DEIPC vesicles and 22 = DEuPC vesicles.

^b λ_{max} values are average from 3–6 samples and the λ_{max} values were reproducible to within ± 1 nm.

^c $\Delta\lambda_{\text{max}}$ is the difference between λ_{max} in the presence of 10-DN and that in the presence of acrylamide.

^dNote that the L_{TM} eff does not apply to a specific acyl chain length given on the left.

Quencher induced shifts in Trp emission λ_{max} and $L_{\text{TM eff}}$ values for pLA peptides with Asp, His or Glu substitutions at position 7 when incorporated into DOPC vesicles at different pH. Samples contained 2 μM peptide and 200 μM lipid dispersed in pH-adjusted PBS.

Table 2

X Residue	pH	λ_{max} (nm) ^d	λ_{max} (nm) Acrylamide	λ_{max} (nm) 10-DN	$\Delta\lambda_{\text{max}}$ (nm) ^b	$L_{\text{TM eff}}$ (number of residues)
Glu	4.0	325	324	325	1	18.7
	7.0	329	329	331	2	17.3
	9.0	330	329	333	4	17.1
Asp	4.0	330	329	331	2	17.5
	7.0	336	333	340	7	16.6
	9.0	337	334	340	6	16.8
His	4.0	330	329	331	2	17.3
	7.0	326	326	327	1	18.0
	9.0	324	324	325	1	18.0

^a Reported λ_{max} values are average from three samples. The λ_{max} values were reproducible to within ± 1 nm.

^b $\Delta\lambda_{\text{max}}$ is the absolute value of difference in Trp λ_{max} in presence of 10-DN and Trp λ_{max} in presence of acrylamide.

Quencher induced shifts in Trp emission λ_{max} and $L_{\text{TM eff}}$ values for pLA peptides with Asn, Glu or Ser substitutions at position 8 when incorporated into DOPC vesicles at pH 7. Samples contained 2 μM peptide and 200 μM lipid dispersed in PBS.

Table 3

X Residue	λ_{max} (nm) ^a	λ_{max} (nm)/Acrylamide	λ_{max} (nm) 10-DN	$\Delta\lambda_{\text{max}}$ (nm) ^b	$L_{\text{TM eff}}$ (number of residues)
Asn	328	327	328	1	17.6
Glu	327	326	328	2	17.5
Ser	325	325	326	1	18.6

^a Reported λ_{max} values are average from three samples. The λ_{max} values were reproducible to within $\pm 1\text{ nm}$.
^b $\Delta\lambda_{\text{max}}$ is the absolute value of difference in Trp λ_{max} in presence of 10-DN and Trp λ_{max} in presence of acrylamide.

Quencher induced shift in Trp emission λ_{max} and $L_{\text{TM}}^{\text{eff}}$ values for pLA peptides with Glu at position 7 and different Z residues (at positions 3–6) when incorporated into DOPC vesicles at pH 4.0 or pH 9.0. Samples contained 2 μM peptide and 200 μM lipid dispersed in pH-adjusted PBS.

Table 4

Z residues	pH	λ_{max} (nm) ^a	λ_{max} (nm)/Acrylamide	λ_{max} (nm) 10-DN	$\Delta\lambda_{\text{max}}$ (nm) ^b	$L_{\text{TM}}^{\text{eff}}$ (number of residues)
(LA) ₂	4.0	325	324	325	1	18.7
	9.0	330	329	333	4	17.1
A ₄	4.0	327	327	328	1	17.7
	9.0	336	335	338	3	16.1
G ₄	4.0	331	329	335	6	17.1
	9.0	337	335	340	5	15.9

^a Reported λ_{max} values are average from three samples. The λ_{max} values were reproducible to within ± 1 nm.

^b $\Delta\lambda_{\text{max}}$ is the absolute value of difference in Trp λ_{max} in presence of 10-DN and Trp λ_{max} in presence of acrylamide.