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The Effect of Hydrophilic Substitutions and Anionic Lipids Upon the Transverse Positioning of the Transmembrane Helix of the Erb b2 (*neu*) Protein Incorporated into Model Membrane Vesicles

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Abstract

The sequence of the transmembrane (TM) helix of Erb b2, a member of the epidermal growth factor receptor family, can influence its activity. In this report, the sequence and lipid dependence of the transverse position of a model membrane-inserted peptide containing the Erb b2 TM helix and juxtamembrane (JM) residues was studied. For the Erb b2 TM helix inserted into phosphatidylcholine vesicles, the activating V664E mutation was found to induce a transverse shift involving the movement of the E residue towards the membrane surface. This shortened the effective length of the TM spanning portion of the sequence. The transverse shift was observed with both the E664 residue in the uncharged and charged state, but the extent of the shift was larger when the E residue was charged. When a series of hydrophilic residues was substituted for V664 the resulting transverse shifts at pH 7 decreased in the order D,H>E>Q>K>G>V. Except for His, this order is strongly correlated to that reported for the degree to which these substitutions induce cellular transformation when introduced into full length Erb b2. To examine the effect of lipid on transverse shift, the uncharged V664Q mutation was studied. The presence of 20% of the anionic lipid dioleoylphosphatidylserine (DOPS) in the model membrane vesicles, which introduces a physiologically relevant level of anionic lipid, did not affect the degree of transverse shift. However, in the case of a peptide containing a V674Q substitution, in which the Q is closer to the C-terminal of the Erb b2 TM helix than the N-terminal, transverse shift was suppressed in vesicles containing 20% DOPS. This suggests that the interaction of the cationic JM residues flanking the C-terminus of the Erb b2 TM helix interact with anionic lipids to anchor the C-terminal end of the TM helix. This anchoring site may act as a pivot which amplifies transverse movements of the Erb b2 TM segment to induce a large swinging-type motion in the extracellular domain of the protein, affecting Erb b2 activity. Interactions interrupting C-terminal JM residue association with anionic lipid might partly impact Erb b2 activity by disrupting this pivoting.

INTRODUCTION

The epidermal growth factor receptor (Erb b) family is one of the most well studied subgroups of the receptor tyrosine kinase (RTK) superfamily. Like other RTKs, the Erb b family contains a single transmembrane (TM) domain, an N-terminal extracellular ligand binding domain, and a C-terminal catalytic kinase domain^{1–2}. When monomeric, the catalytic domains of RTK

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proteins are inactive. Dimerization, which can be promoted by ligand binding to the N-terminal extracellular domains, can bring the two cytoplasmic domains in close contact, which stimulates catalytic activity and result in phosphorylation of a tyrosine residue 3. This dimerization can involve lateral association of TM domains in response to the ligand binding 1^{–4}. The activation of the receptor then sets off an intracellular signaling cascade that can alter cell growth, survival and/or differentiation 4. Erb b receptors are an attractive target for the treatment of malignancy. Several anti-Erb b monoclonal antibodies and small molecule tyrosine kinase inhibitors have been approved for cancer treatment and more are in development 5–6.

Erb b2, is an important member of the Erb b family, and it is overexpressed in 20–30% of metastatic breast cancer cells 3, 7–8. Mutation of a single valine to glutamine at position 664 in the TM domain of *neu*, a version of the Erb b2 receptor found in rats, has been shown to overactivate the receptor turning the *protooncogene* into an oncogene 9–10. Studies have shown that the glutamic acid mutation alters some aspects of TM helix association 10–15. Erb b2 receptor activation may be a result of enhanced dimerization due to inter-receptor hydrogen bond formation by the protonated carboxyl group of glutamic acid 14–17. However, the glutamic acid may also influence additional aspects of Erb b2 behavior. Analysis of *neu* proteins with mutations in TM the domain revealed that dimerization of the oncogenic form or Erb b2 receptor through its TM domain is important, but not sufficient for transformation 12. In addition, the Glu mutation had very little effect on dimerization of human Erb b2 as reported by the TOXCAT assay 18. Furthermore, engineered mutations expected to increase dimer strength do not cause *neu*/Erb b2 receptor overactivation 12.

It is possible that altered TM topography due to Glu mutation in the Erb b2 sequence results in rearrangement of pre-existing dimers in a fashion that leads to receptor activation 13, 19. In addition, NMR studies by Smith et al 14 suggested that a charged Glu664 residue causes a local unfolding of the ErbB2 TM sequence N-terminal the Glu to residue. However, it was also proposed that the Glu residue in its protonated state, which did not show this unfolding, might be more relevant for function. It was also found that the Glu664 shifted the boundary of the TM segment so that the Glu was close to the membrane surface. This could shift the transverse position of the TM helix in the membrane might and well as shorten the length of the membrane spanning sequence. If so, some parameter associated with a change in the location of a TM helix within the bilayer or its length may be linked to receptor activation.

In this study, we examined the effect of various “mutations” in the TM segment of Erb b2 upon the transverse position of the TM helix and its effective TM length in membranes. How lipid composition impacts transverse position and the length of the helix spanning sequence was also examined. The results indicate that various hydrophilic substitutions at V664 can indeed induce large transverse shifts that shorten the effective transverse length. The pattern of shifts was similar to that observed previously for a TM helix with a simplified hydrophobic sequence²⁰. In addition, there was a correlation between the extent of transverse shift induced by hydrophilic residues and the previously reported activating effects of these residues upon transformation due to Erb b2 expression 9. Other experiments indicated that anionic lipid could suppress transverse shifts when the juxtamembrane residues flanking the TM sequence were cationic, but not when they had no net charge 21. How such transverse movements and their regulation might influence Erb b2 function is discussed.

RESULTS

Defining the Topography of Membrane-Inserted Erb B2 WT and V664E Peptides Using Fluorescence Spectroscopy

The peptides studied had the sequence of the TM helix (underlined residues) and surrounding juxtamembrane (JM) residues of the neu (rat homologue of Erb b2) receptor [Acetyl-⁶⁴⁹AEQRASPVTFIATVVGVLFLILVVVGILIKRRRYK⁶⁸⁶-Amide]. The TM sequence also contained a single Trp substitution near the center of the hydrophobic sequence, and in most cases a single additional hydrophilic substitution (Table 1).

The fluorescence properties of the Trp residue was used to define the topography of the membrane-inserted peptides ^{22–23}. To do this the effect of bilayer width (varied by preparing vesicles composed of lipids with various length monounsaturated acyl chains) upon Trp fluorescence was measured. Figure 1A schematically illustrates the dependence of Trp emission upon bilayer width for peptides with different numbers of TM residues ^{20, 24}. When Trp is near the center of the hydrophobic sequence, its fluorescence is most blue-shifted at the bilayer width where there is match between the length of the hydrophobic TM segment and the width of the hydrophobic core of the bilayer ^{22–25}, in which case the Trp locates close to the center of the bilayer.

Red shifts in Trp fluorescence are generally observed in both thinner and wider bilayers, i.e. when there is hydrophobic mismatch. The red shift in wider bilayers (Figure 1A) occurs because TM state is destabilized due to unfavorable burial of hydrophilic JM residues within the bilayer. This destabilization results in formation of appreciable fractions of a non-TM membrane surface-bound state, and in wide enough bilayers full formation of the non-TM state ^{22–23}. The non-TM state has red shifted fluorescence because the Trp is located in a polar environment. Fluorescence also generally red-shifts, although to a lesser degree, in thinner bilayers. This is partly due to the fact that the distance from a Trp near the center of a bilayer to the membrane surface automatically decreases as bilayer width decreases ²³.

As a result, peptides with different length TM segments can be distinguished by measurement of Trp emission λ_{max} vs. lipid acyl chain length. The effective TM spanning length of a peptide can be defined as being equivalent to the number of residues necessary to span the width (in angstroms) of the hydrophobic part of the bilayer at the point at which Trp emission λ_{max} is most highly blue-shifted (dashed line in Figure 1A) ²⁰.

Figure 1B shows experimental data for the effect of bilayer width upon Trp fluorescence at pH 7.0 for the Erb b2 “WT” peptide (which we define here as a peptide having a wild-type sequence except for the Trp substitution), and for the V664E “mutant” peptide (i.e. with a V664E substitution). V664E peptide behavior was also measured at both pH 4.0 and pH 9.0 to investigate the consequence of Glu664 ionization state upon TM helix behavior. (A pK_a near 7 was measured for the Glu664 in both DOPC and DEuPC vesicles (see Supplemental Figure 1).

For the WT sequence, the pattern of λ_{max} as a function of bilayer width shows a minimum with a very blue-shifted emission in vesicles composed of the 22 carbon acyl-chain lipid DEuPC (Figure 1B). This bilayer width has a hydrophobic core of about 35 Å ²², and can be spanned by a 23–24 residue helical sequence (i.e. based on the calculation 35Å/1.5Å helix rise per residue). This is consistent with membrane spanning segment being composed of hydrophobic residues 656–680.

The V664E peptide exhibited a λ_{max} minimum in thinner bilayers than the WT sequence (Figure 1B), indicating the V664E peptide contained a shorter membrane spanning segment.

At high pH, at which the Glu residue is charged, the λ_{max} minimum corresponds to an effective TM length equivalent to the number of residues spanning the hydrophobic core of a bilayer composed of lipids with 16–17 carbon acyl chains (which would have a hydrophobic core of 25–26 Å 22) or about 17 residues (i.e. from the Glu664 residue to residue 680). This decrease in TM length reflects a shift in TM helix position such that the highly hydrophilic charged Glu moves close to the surface of the bilayer (see below), and is similar to what we observed previously with artificial hydrophobic sequences containing a Glu residue 20.

At pH 4.0, where the Glu is protonated and thus uncharged, the λ_{max} minimum indicated an TM spanning length sufficient to span a bilayer composed of lipids with 19.5 carbon acyl chain or about 20 residues. This shows the uncharged Glu does not shift TM helix position to as great a degree as a charged Glu, consistent with its lesser hydrophilicity in the uncharged state, and thus lesser tendency to locate near the membrane surface. This result is also in agreement with our previous observations of the difference between the behavior of charged and uncharged ionizable residues in an artificial hydrophobic sequence ²⁰.

To confirm that a shift in TM helix position occurs in the presence of the Glu residue, Trp depth was measured directly using a dual quenching method ²⁶. This method utilizes two quenchers of Trp fluorescence, acrylamide and 10-doxylnonadecane (10-DN), to assess Trp depth. Acrylamide, an aqueous quencher, preferentially quenches Trp residues near the membrane surface, while 10-DN, a membrane-inserted quencher, preferentially quenches Trp buried in the membrane bilayer. The ratio of acrylamide quenching to 10-DN quenching (Q-ratio) responds nearly linearly to Trp depth in the bilayer ²⁶, such that a low quenching ratio indicates a deeply located Trp, while a high Q-ratio is indicative of a Trp near the bilayer surface. Previous studies show a Trp at the bilayer center generally gives a Q-ratio of < 0.15 while Trp close to the bilayer surface gives a Q-ratio ≥ 1 26. In previous experiments, the Trp was located in the middle of the hydrophobic segment being studied, so that it was at the bilayer center in the TM state. However, for the Erb b2 peptides, we found it was easiest to distinguish TM and non-TM topographies when we substituted the Trp about three residues closer to the C-terminus than to the N-terminus of the hydrophobic sequence.

Figure 2 shows the result of the quenching experiments for the WT and V664E peptides. In DOPC (di C18:1PC) vesicles (Figure 2A), the Q-ratio for the WT peptide is indicative of a residue that is located deeply, but not quite at the center of the bilayer, as expected. (Raw quenching data for the individual quenchers is shown in Supplemental Table 1). In the presence of the V664E substitution at pH 4.0, the Trp is located more deeply than in the WT peptide. As shown in Figure 3 (top) this is consistent with a moderate shift of the Glu664 residue towards the membrane surface close to the N-terminus of the peptide. At high pH, the Trp in the V664E mutation is almost as shallow as in the WT protein. This is consistent with a further shift of residue 664 towards the membrane surface (Figure 3, (top)). Of course, it is also consistent with no shift of residue 664 relative to its position in WT peptide, but this is unlikely given the above described pattern of λ_{max} vs. bilayer width for this peptide.

It is also noteworthy that Figure 2A also shows that the λ_{max} and Q-ratio patterns for the WT and V664E peptides parallel each other closely. This confirms the close relationship between λ_{max} and membrane depth, in agreement with our previous studies ²⁰.

The pattern of Trp depths is different in bilayers composed of DEuPC (di C22:1 PC). In DEuPC, the Trp is located most deeply for the WT protein, less deeply for the V664E peptide at pH 4.0 and most shallowly for the V664E peptide at high pH (Figure 2B). A very analogous pattern is seen in λ_{max} values. This quenching and λ_{max} pattern can be explained by the effects of hydrophobic mismatch upon topography. In the thick DEuPC bilayers, the effective TM length of the V664E peptide is too short to span the bilayer, i.e. there is negative mismatch. Under

such conditions, several previous studies^{20, 22–23, 25} have shown that the TM topography will be in equilibrium with a non-TM topography in which the Trp is close to the membrane surface, so that average Trp depth is shallower than in the fully TM state (Figure 3 (bottom)). The fraction of the V664E peptide in the non-TM state should be highest at pH 9.0, and thus Trp depth shallowest, because at pH 9.0 effective TM length is shortest. This is exactly what is observed.

It should be noted that circular dichroism experiments show that the WT and V664E peptide have a similar fraction of residues forming a helical structure (65–72%). This is true in both DOPC and DEuPC bilayers, and at various pH (Supplemental data Table 2). This % helical content is consistent with the helix formation by the entire hydrophobic segment of the peptide.

Effect of Other Hydrophilic Mutations at Position 664 upon Membrane Positioning of Erb b2

The effect of peptides with other hydrophilic substitutions at position 664 (Table 1) was also studied. It has been reported that while expression of Erb b2 with hydrophilic mutations like V664E, V664Q and V664D can transform cells, mutations such as V664G, V664H and V664K do not^{9, 27}. We wanted to examine whether there was a correlation between the transverse position of the TM helices containing these substitutions and their transforming ability.

Figure 4 shows the effect of bilayer width upon Trp λ_{max} at pH 7.0 for peptides having an D, Q, H, K, G, or E (included for comparison) substitution, or a WT V at position 664. The V664D and V664H mutants exhibited a λ_{max} profile similar to V664E, in which there is a short effective TM spanning length, corresponding to a large transverse shift due to the movement of the residue at 664 towards the membrane surface. In contrast, the V664G peptide gave a similar to that of the WT sequence, with a large effective TM spanning length, corresponding to very little transverse shift. The V664Q and V664K peptides showed an intermediate pattern, indicative of a somewhat shortened TM spanning length resulting from a transverse shift that is somewhat less than that for the V664E peptide. The overall order of effective TM spanning lengths was D,H<E<Q<K<G<V. (This parameter is usually the inverse of the order for the degree of transmembrane shift²⁰.) This order is very similar to that we observed previously when a single hydrophilic residues was introduced into a peptide with an artificial hydrophobic sequence composed primarily of alternating Leu and Ala residues²⁰.

Again, the results for TM spanning length were consistent with Trp depths derived from fluorescence quenching. Q-ratio values observed for peptides incorporated into DOPC vesicles were those expected for a moderate transverse shift for the V664K and V664Q substitutions and no shift for the V664G substitution (Table 2). As in the case of the V664E substitution, the V664D and V664H peptides Q-ratio gave values consistent with either a large shift or no shift. However, the latter interpretation is neither consistent with the effective TM length data nor the Q-ratio and λ_{max} values for these peptides in DEuPC. In DEuPC vesicles Q-ratios increase in the order: V<G<Q~E<H,D<K, and λ_{max} ratio in a similar order: V<G<Q~K~E<H,D. This order is as predicted if there are transverse shifts reducing TM helix membrane spanning length to the extent given above, so that the increased negative mismatch in DEuPC vesicles is accompanied by partial formation of the non-TM state, in which Trp is shallow. The only unusual result is that the Q-ratio for the K peptide in DEuPC was anomalously high relative to that predicted from either effective TM length or λ_{max} in DEuPC. We have no explanation for this anomaly.

It is also noteworthy that the degree of transverse shift induced by residues at position 664 is in most cases well correlated with the effect of the corresponding mutations in the Erb b2 protein upon transforming activity^{9, 27}. The implications of this are considered in the Discussion.

Effect of Varying Trp Position

As noted above, in the above experiments the Trp was not positioned exactly at the center of the Erb b2 hydrophobic sequence. For some experiments described below, a peptide with a Trp closer to the center of the hydrophobic sequence was desirable. For this purpose, a Trp substitution was made at residue 667 (Table 1). The overall hydrophobicity of this sequence was should be nearly identical to that with Trp at position 671 because the substituted residues (Leu at 671 and Ile at 667) have nearly equal hydrophobicities^{28–29}.

Figure 5 compares Trp emission λ_{max} vs. bilayer width profile of the WT W671 and W667 peptides at pH 7.0. The λ_{max} pattern of the W667 peptide was blue-shifted relative to that of the W671 peptide, but was otherwise similar, with a λ_{max} minimum in wide bilayers, indicative of a long membrane spanning segment about equivalent in length to that of the W671 peptide. The more highly blue-shifted fluorescence of the W667 peptides is as expected because a Trp at position 667 should be closer to the bilayer center than one at position 671. Quenching also showed that Trp 667 locates more deeply than Trp 671 (see below).

Comparison of ErbB2 TM Topography in Zwitterionic and (20mol%) Anionic Lipid Vesicles

We previously found that anionic lipids strongly stabilize the formation of a TM topography when a hydrophobic sequence is flanked by cationic JM residues^{21, 30}. This appears to involve Coulombic electrostatic interactions between the cationic JM residues and anionic lipids²¹. Such interactions might also have an important modulatory influence on the tendency of a hydrophobic sequence to shift transverse position in membranes. To test this hypothesis the behavior of the Erb b2 peptides in zwitterionic and anionic lipid vesicles was compared. In particular, whether Erb b2 TM helix transverse movement would be suppressed by anionic lipid was investigated using WT sequences and ones with a Gln substitution. The non-ionizable Gln residue was chosen rather than Glu to avoid complications due to effects of anionic lipid effects on anionic residue pKa, and thus anionic residue charge²¹.

In control experiments, the λ_{max} vs. bilayer width curves for both the WT W667 and W671 peptides were found to be unaffected by the inclusion of 20mol% of the anionic lipid DOPS in the vesicles (Figure 5). There was also no effect of DOPS on Trp depth for these peptides as measured by quenching (Figure 6). These results show that these two peptides have similar effective TM spanning lengths, and thus similar transverse positions, in zwitterionic and anionic vesicles.

The absence or presence of 20 mol% DOPS in vesicles also had little effect on λ_{max} vs. bilayer width curves (Figure 7A) and quenching (Figure 6) for the V664Q peptide (in which Trp is at position 671). In both cases, the V664Q peptide formed a TM segment with an effective TM length (~ 18 residues) much shorter than the WT peptide, i.e. indicative of a transverse shift in which the Q residue moves closer to the surface. Thus, in this case DOPS does not affect the transverse shift induced by the Q residue. This is not surprising, because the Q664 residue is closest to the N-terminal JM residues, which have no net charge (1 Glu and 1 Arg) and so the presence of anionic lipids should not strongly affect the tendency of N-terminal JM residues to move away from the bilayer surface when the Q residue locates close to the bilayer surface.

To determine whether different behavior would be observed for transverse shifts involving movement of the highly cationic C-terminal JM sequence, the behavior of a peptide with a V674Q mutation (and Trp at position 667) was studied. Figure 7B shows the Trp emission λ_{max} profile for the V674Q peptide in PC vesicles and those containing 20% DOPS. As shown by the position of the arrows, the V674Q peptide also formed a shorter TM structure than the WT sequence in the zwitterionic PC vesicles indicating the formation of a shifted TM helix. However, in vesicles containing 20mol% DOPS the V674Q peptide exhibited a profile with a

λ_{max} minimum increased to a value close to those observed with the WT sequence, indicating a much smaller transverse shift than in the absence of DOPS.

Q-ratio values provide more details concerning the behavior of the V674Q peptide. The quenching data show that both in DOPC and 80:20 DOPC:DOPS vesicles the V674Q peptide forms a topography with a shallower Trp location than does the WT (W667) peptide (Figure 6). This is indicative of a transverse shift in which the Q residue moves somewhat closer to the membrane surface. [Since the W667 residue is at the bilayer center in the absence of a transverse shift, it moves to a shallower location when a transverse shift occurs.] Figure 6 also shows there is a large difference between Trp depth for the V674Q peptide in vesicles composed of 80:20 DEuPC:DOPS and this in vesicles composed of 80:20 DEuPC:DOPC. In the latter case the average Trp depth is almost as shallow as in the V664Q peptide, while in the former case (i.e. in the presence of DOPS) the Trp is very close to the bilayer center. This indicates that the transverse shift in V674Q position due to the Q residue is suppressed in the 80:20 DEuPC:DOPS vesicles.

DISCUSSION

Hydrophilic Substitutions at Position 664 in the Transmembrane Domain of Erb b2 Induce Transverse Shifts in Helix Position With Membranes

Using a peptide containing the TM domain of neu/Erb b2 receptor we found that the V664E substitution changed the membrane boundary of the TM helix in such a way as to shift its transverse position in a lipid bilayer and shorten the effective length of the membrane spanning sequence. Although the transverse shift was greatest when the Glu residue was charged, an uncharged Glu also induced a significant transverse shift. Transverse helix shifts were also observed for several other hydrophilic substitutions at position 664. Interestingly, the relative extent of shift induced by different hydrophilic residues (D,H>E>Q~K>G>V) at pH 7 has an order similar to that (D>H⁺>E~Q>K>H^o>G>L) we previously observed in a hydrophilic helix with an alternating LeuAla sequence²⁰. The only ambiguity involves His, which behaves in the Erb b2 peptide as if it were charged. It is possible that in cells, the His exists in the uncharged form due to its having a different pKa than in our model membranes. In that case, there would be a smaller transverse shift induced by the His in cellular membranes.

The similarity between transverse shifts in the Erb b2 and alternating LeuAla sequences suggests that the relative extent of transverse shifts are not strongly affected by the nature of the hydrophobic sequence. This is important because it suggests that the “transverse shift scale” we established using the alternating LeuAla peptide may be widely applicable to various TM segments, and help in investigation of the role of TM helix transverse position in function (see below).

In these experiments, peptides with a Trp substitution were used. The Trp located at the expected depths in the membrane for the “wild-type” sequences, indicating that Trp perturbation of peptide behavior was not significant. Furthermore, these studies compared peptides that all had a Trp, so that the differences in the effects observed for different hydrophilic substitutions cannot be attributed to the Trp, especially as the Trp was placed seven residues away from the hydrophilic substitutions, which prevented direct interactions between Trp and the hydrophilic substitutions.

Correlation Between TM Helix Transverse Position and Transforming Activity of Erb b2 Protein: How Transverse Shift Might Affect Erb b2 Function

When the transverse position of a TM helix is crucial for function, there should be a strong correlation between function and the extent of transverse shifts induced by hydrophilic

substitutions in the TM helix. We tested whether this relationship would exist for Erb b2 by comparing the extent of helix shift induced by hydrophilic substitutions at position 664 to their previously measured transforming activity when introduced into the TM helix of intact Erb b2. Mutations with the strongest transforming ability (Glu, Asp, Gln) do induce a significant shift in TM helix transverse position, while a Gly mutation, which does not enhance transformation, had no effect upon transverse position^{9, 27}. On the other hand, Lys and His mutations, do not result in enhanced transforming ability⁹, but did induce transverse shifts. One possible explanation for the behavior of His is that it is charged in our experiments but uncharged in cell membranes. Transverse shifts induced by an uncharged His are smaller than those induced by a charged His²⁰, and thus might not affect activity. Similarly, Lys, is not one of the most strongly shift-inducing residues, under some conditions induces shifts that are even smaller than those measured here²⁰.

Changes in transverse position are unlikely to fully explain the effect of hydrophilic mutations upon Erb b2 activation because hydrophilic residues also directly affect helix-helix and helix-lipid interactions via Coulombic, H-bonding and van der Waals interactions. Nevertheless, there are several mechanisms by which transverse shifts might influence Erb b2 structure and function. First, they could directly alter the distance of extracellular and cytosolic domains from the membrane surface (Figure 8). This is important because a change in the position of solution-exposed domains could alter their ability to interact with solution-exposed domains on partner proteins (e.g. a second Erb b protein molecule) by shifting an interaction site into a position in which it can interact (because it is the same distance from the membrane as its complementary partner site) or cannot interact (because it is at a different distances from the membrane than its complementary site). Such motions could be greatly amplified if transverse shifts involve a swinging motion around a fixed pivot (Figure 8 (right)), rather than a simple piston-type motion (Figure 8 (left)). The potential role of JM residues in forming a pivot are considered in a following section.

Transverse shifts could also influence structure and function via an effect upon the extent or nature of TM helix dimerization. Using computational analysis Ben-Tal and colleagues proposed that activation of the human Erb b2 (HER2) receptor involves a switch from a C-terminal mediated dimerization motif to an N-terminal mediated motif¹⁹. Similar dimerization modes may be possible in neu, and a 5-residue consensus sequence in the N-terminal of the TM segment (AXXXG in neu and SXXXG in human erb b2) is thought to be involved in TM helix dimerization^{15-17, 19}. Studies by Sharpe et al reported that Glu664-induced structural rearrangements in the Erb b2 TM domain extend over four helical turns downstream of the mutation, and could alter the strength of the lateral association of the TM helix with other helices¹³. A shift in the transverse position of these motifs might affect the strength of motif-motif interactions by altering the hydrophilicity of their local environment or by altering motif-lipid headgroup interactions. It is also possible that the identity or side chain configuration of residues involved in dimerization surfaces could be altered by transverse shifts in TM helix positions. This could alter TM helix orientation within the dimer.

Another way altered TM helix transverse position could affect function is by altering Erb b2 dimerization partners. Since Erb b2 has no physiological ligand it is believed that for signaling function it forms heterodimers with other Erb b receptors³¹⁻³². It is possible that by altering helix transverse position, activating mutations favor formation of specific functional heterodimers.

It will be important for future studies of Erb b2 peptides to investigate the effect of hydrophilic substitutions upon dimerization and dimer structure. It will be of particular interest to determine if the transverse position of a helix in a membrane is closely linked to dimerization and/or dimer structure.

The Effect of JM Segment Lipid Interaction upon TM Helix Behavior

Although the peptides we used did not contain the complete cytosolic juxtamembrane sequence from Erb b2, those JM residues included in the peptides studied did affect TM helix behavior, which may have implications for function. The cytoplasmic JM domain, located between the TM domain and the kinase domain, is rich in cationic residues, and may play a key role in receptor dimerization and activation^{33–37}. Studies have suggested that binding of the positively charged JM region to the negatively charged membrane surface can modulate Erb b kinase domain dimerization, and that molecules or modifications that disrupt the interaction between lipid and JM residues alter activity, and least in the case of Erb b1^{38–39}.

The influence of JM residues upon the TM helix may be important for function. As noted above, a transverse movement of a TM segment can be piston-like or involve a swinging motion⁴⁰ (Figure 8). A swinging motion would be favored when one end of the TM segment acts as a pivot that is relatively fixed in position, which our data suggests will occur when cationic JM residues are involved in tight interactions with anionic lipid headgroups. Cytosolic molecules that prevent JM residues from binding to the membrane surface^{38–39} might control the balance between piston-like and swinging motions, favoring the former. In addition, the interactions between TM and JM sequences may be reciprocal, such that shifts in TM helix position affect the position of JM residues relative to the membrane, and thus their functioning.

MATERIALS AND METHODS

Materials

ErbB2 peptides were purchased from Keck Facility at Yale University (New Haven, CT) and purified by an analytical reverse phase microsb C4 HPLC column (Varian Inc.) using a (60:40 v:v 2-propanol:acetonitrile)/water gradient containing 0.5% (v/v) trifluoroacetic acid. Peptides were dissolved in (60:40 2-propanol:acetonitrile)/water (at close to the lowest ratio of organic solvent to water sufficient to dissolve the peptide), and then injected onto the column bathed in the same solvent. Purified peptides were dried under N₂, redissolved in 1:1 (v/v) 2-propanol/water, and stored at 4°C. Peptide purity was confirmed by MALDI-TOF (Proteomics Center, Stony Brook University) and generally was ~90% pure. The concentrations of purified peptides were measured by absorbance spectroscopy using a Beckman DU-650 spectrophotometer, using an ϵ for Trp of 5560 M⁻¹ cm⁻¹ at 280 nm. Phosphatidylcholines (1,2-diacyl-*sn*-glycero-3-phosphocholines), [diC14:1Δ9cPC (dimyristoleoylphosphatidylcholine); diC16:1Δ9cPC; diC18:1Δ9cPC, (dioleoylphosphatidylcholine, DOPC); diC20:1Δ11cPC; diC22:1Δ13cPC, (dierucoylphosphatidylcholine); and diC24:1Δ15cPC]; 1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine] sodium salt (DOPS) were purchased from Avanti Polar Lipids (Alabaster, AL). Lipids were stored in chloroform at -20° C. Lipid concentrations were determined by dry weight. Acrylamide was purchased from Sigma-Aldrich Chemical Co.(St. Louis, MO). 10-doxylnonadecane (10-DN) was custom-synthesized (contact authors for availability) by Molecular Probes (Eugene, OR). It was stored as a 6 mM stock solution [determined using ϵ = 12 M⁻¹ cm⁻¹ at 422 nm²⁵ in ethanol at -20°C.

Model Membrane Vesicle Preparation

Model unilamellar membranes were prepared using the ethanol-dilution method as in previous studies^{22–23}. Peptides dissolved in 1:1 (v/v) 2-propanol/water and lipids dissolved in chloroform were mixed and then dried under a stream of N₂. Samples were then further dried under high vacuum for 1h. Then, 10 μL of 100% ethanol was added to dissolve the dried peptide/lipid film. To disperse the lipid-peptide mixtures 790 of PBS (10 mM sodium phosphate and 150 mM NaCl, pH 7), was added to the samples while vortexing. For samples prepared at low or high pH, the pH of the PBS was first adjusted using glacial acetic acid, for

low pH, or 2 M NaOH, for pH 9.0. The final concentrations were 2 μ M peptide and 500 μ M lipid.

Fluorescence Measurements

Fluorescence data were obtained on a SPEX τ 2 Fluorolog spectrofluorometer operating in steady-state mode. Measurements were taken on samples in semimicro quartz cuvettes (1-cm excitation path length and 4-mm emission path length). A 2.5-mm excitation slit width and 5-mm emission slit width (band pass of 4.5 and 9 nm, respectively) were used for all experiments. Trp fluorescence emission spectra were measured over the range of 300–375 nm. Fluorescence from background samples containing lipid but lacking peptide was subtracted from the reported values. All measurements were made at room temperature.

Acrylamide Quenching Measurements

Fluorescence intensity and emission spectra were first measured for model membrane-incorporated peptides or background samples. After addition of a 50 μ L aliquot of acrylamide from a 4 M stock solution in water and a 5min incubation period, fluorescence was remeasured. (This is sufficient time for acrylamide to equilibrate across the bilayer²⁶. Fluorescence intensity was measured at an excitation wavelength of 295 nm and an emission wavelength of 340 nm. This excitation wavelength was chosen to reduce the resulting inner filter effect due to acrylamide absorbance. Corrections to intensity were made both for dilution by the addition of acrylamide and for the residual inner filter effects²⁶. Fluorescence emission spectra were recorded using an excitation wavelength of 280 nm, despite the stronger inner-filter effect arising from acrylamide at 280 nm, because the overall intensity was greater than when an excitation wavelength of 295 nm was used. Controls show that emission spectra have very similar wavelength maxima using either excitation wavelength²⁶.

10-DN-Quenching Measurements

To measure quenching in samples containing 10mol% 10-DN, samples containing model membrane-incorporated peptides or background samples without peptide were prepared in which 10 mol % of each of the lipids present was replaced by an equivalent mole fraction of 10-DN. Fluorescence intensity and emission spectra were measured for samples with and without 10-DN. Fluorescence intensity was measured using an excitation wavelength of 280 nm and an emission wavelength of 330 nm. Emission spectra were recorded using an excitation wavelength of 280 nm.

Calculation of the Acrylamide/10-DN Quenching Ratio (Q Ratio)

The ratio of quenching by acrylamide to quenching by 10-DN was used to estimate Trp depth in the bilayer. This 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 the sample with no quencher present and $F_{\text{acrylamide}}$ and $F_{10\text{-DN}}$ are the fluorescence intensities in the presence of acrylamide or 10-DN, respectively²⁶.

Circular Dichroism (CD) Measurements

Circular dichroism spectra were recorded from 295nm to 340nm on a Jasco J-715 CD spectrophotometer at room temperature using a 1-mm path-length quartz cuvette. Unless otherwise noted, samples were prepared using 5 μ M or 10 μ M peptide and 500 μ M lipid. The spectra were obtained from at least 50 scans. Backgrounds from samples lacking peptide were subtracted. Estimation of helical content was done using DICROWEB, an online server (<http://dichroweb.cryst.bbk.ac.uk>) for secondary structure analysis from CD data⁴¹.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. van der Geer P, Hunter T, Lindberg RA. Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu Rev Cell Biol* 1994;10:251–337. [PubMed: 7888178]
2. Fantl WJ, Johnson DE, Williams LT. Signalling by receptor tyrosine kinases. *Annu Rev Biochem* 1993;62:453–481. [PubMed: 7688944]
3. Bublil EM, Yarden Y. The EGF receptor family: spearheading a merger of signaling and therapeutics. *Curr Opin Cell Biol* 2007;19:124–134. [PubMed: 17314037]
4. Normanno N, De Luca A, Bianco C, Strizzi L, Mancino M, Maiello MR, Carotenuto A, De Feo G, Caponigro F, Salomon DS. Epidermal growth factor receptor (EGFR) signaling in cancer. *Gene* 2006;366:2–16. [PubMed: 16377102]
5. Yu D, Hung MC. Overexpression of ErbB2 in cancer and ErbB2-targeting strategies. *Oncogene* 2000;19:6115–6121. [PubMed: 11156524]
6. Yu D, Hung MC. Role of erbB2 in breast cancer chemosensitivity. *Bioessays* 2000;22:673–680. [PubMed: 10878580]
7. Blume-Jensen P, Hunter T. Oncogenic kinase signalling. *Nature* 2001;411:355–365. [PubMed: 11357143]
8. Robertson SC, Tynan JA, Donoghue DJ. RTK mutations and human syndromes when good receptors turn bad. *Trends Genet* 2000;16:265–271. [PubMed: 10827454]
9. Bargmann CI, Weinberg RA. Oncogenic activation of the neu-encoded receptor protein by point mutation and deletion. *Embo J* 1988;7:2043–2052. [PubMed: 2901345]
10. Gullick WJ, Bottomley AC, Lofts FJ, Doak DG, Mulvey D, Newman R, Crumpton MJ, Sternberg MJ, Campbell ID. Three dimensional structure of the transmembrane region of the proto-oncogenic and oncogenic forms of the neu protein. *Embo J* 1992;11:43–48. [PubMed: 1346763]
11. 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;339:230–231. [PubMed: 2654648]
12. Burke CL, Lemmon MA, Coren BA, Engelman DM, Stern DF. Dimerization of the p185neu transmembrane domain is necessary but not sufficient for transformation. *Oncogene* 1997;14:687–696. [PubMed: 9038376]
13. 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;39:6572–6580. [PubMed: 10828974]
14. 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;3:252–258. [PubMed: 8605627]
15. Sternberg MJ, Gullick WJ. Neu receptor dimerization. *Nature* 1989;339:587. [PubMed: 2567498]
16. Bocharov EV, Mineev KS, Volynsky PE, Ermolyuk YS, Tkach EN, Sobol AG, Chupin VV, Kirpichnikov MP, Efremov RG, Arseniev AS. Spatial structure of the dimeric transmembrane domain of the growth factor receptor ErbB2 presumably corresponding to the receptor active state. *J Biol Chem* 2008;283:6950–6956. [PubMed: 18178548]
17. Sternberg MJ, Gullick WJ. A sequence motif in the transmembrane region of growth factor receptors with tyrosine kinase activity mediates dimerization. *Protein Eng* 1990;3:245–248. [PubMed: 2160658]
18. Mendrola JM, Berger MB, King MC, Lemmon MA. The single transmembrane domains of ErbB receptors self-associate in cell membranes. *J Biol Chem* 2002;277:4704–4712. [PubMed: 11741943]

19. Fleishman SJ, Schlessinger J, Ben-Tal N. A putative molecular-activation switch in the transmembrane domain of erbB2. *Proc Natl Acad Sci U S A* 2002;99:15937–15940. [PubMed: 12461170]
20. Krishnakumar SS, London E. The control of transmembrane helix transverse position in membranes by hydrophilic residues. *J Mol Biol* 2007;374:1251–1269. [PubMed: 17997412]
21. Shahidullah K, London E. Effect of lipid composition on the topography of membrane-associated hydrophobic helices: stabilization of transmembrane topography by anionic lipids. *J Mol Biol* 2008;379:704–718. [PubMed: 18479706]
22. Ren J, Lew S, Wang J, London E. Control of the transmembrane orientation and interhelical interactions within membranes by hydrophobic helix length. *Biochemistry* 1999;38:5905–5912. [PubMed: 10231543]
23. 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;36:10213–10220. [PubMed: 9254619]
24. 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;43:8794–8806. [PubMed: 15236588]
25. 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;42:3275–3285. [PubMed: 12641459]
26. 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;42:3265–3274. [PubMed: 12641458]
27. Segatto O, King CR, Pierce JH, Di Fiore PP, Aaronson SA. Different structural alterations upregulate in vitro tyrosine kinase activity and transforming potency of the erbB-2 gene. *Mol Cell Biol* 1988;8:5570–5574. [PubMed: 2907606]
28. 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;433:377–381. [PubMed: 15674282]
29. 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]
30. Cheng HT, Megha&London E. Preparation and properties of asymmetric vesicles that mimic cell membranes: effect upon lipid raft formation and transmembrane helix orientation. *J Biol Chem* 2009;284:6079–6092. [PubMed: 19129198]
31. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. *Nat Rev Mol Cell Biol* 2006;7:505–516. [PubMed: 16829981]
32. Marmor MD, Skaria KB, Yarden Y. Signal transduction and oncogenesis by ErbB/HER receptors. *Int J Radiat Oncol Biol Phys* 2004;58:903–913. [PubMed: 14967450]
33. Hunter T, Ling N, Cooper JA. Protein kinase C phosphorylation of the EGF receptor at a threonine residue close to the cytoplasmic face of the plasma membrane. *Nature* 1984;311:480–483. [PubMed: 6090944]
34. Segatto O, Lonardo F, Wexler D, Fazioli F, Pierce JH, Bottaro DP, White MF, Di Fiore PP. The juxtamembrane regions of the epidermal growth factor receptor and gp185erbB-2 determine the specificity of signal transduction. *Mol Cell Biol* 1991;11:3191–3202. [PubMed: 1674818]
35. Macdonald-Obermann JL, Pike LJ. The intracellular juxtamembrane domain of the epidermal growth factor (EGF) receptor is responsible for the allosteric regulation of EGF binding. *J Biol Chem* 2009;284:13570–13576. [PubMed: 19336395]
36. Aifa S, Aydin J, Nordvall G, Lundstrom I, Svensson SP, Hermanson O. A basic peptide within the juxtamembrane region is required for EGF receptor dimerization. *Exp Cell Res* 2005;302:108–114. [PubMed: 15541730]
37. Thiel KW, Carpenter G. Epidermal growth factor receptor juxtamembrane region regulates allosteric tyrosine kinase activation. *Proc Natl Acad Sci U S A* 2007;104:19238–19243. [PubMed: 18042729]

38. McLaughlin S, Smith SO, Hayman MJ, Murray D. An electrostatic engine model for autoinhibition and activation of the epidermal growth factor receptor (EGFR/ErbB) family. *J Gen Physiol* 2005;126:41–53. [PubMed: 15955874]
39. Sato T, Pallavi P, Golebiewska U, McLaughlin S, Smith SO. Structure of the membrane reconstituted transmembrane-juxtamembrane peptide EGFR(622–660) and its interaction with Ca²⁺/calmodulin. *Biochemistry* 2006;45:12704–12714. [PubMed: 17042488]
40. Stefansson A, Armulik A, Nilsson I, von Heijne G, Johansson S. Determination of N- and C-terminal borders of the transmembrane domain of integrin subunits. *J Biol Chem* 2004;279:21200–21205. [PubMed: 15016834]
41. Whitmore L, Wallace BA. DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res* 2004;32:W668–W673. [PubMed: 15215473]

Figure 1.

Effect of lipid acyl chain length on Trp emission λ_{max} of peptides incorporated into lipid vesicle. (A) Schematic representation of Trp λ_{max} dependence upon lipid acyl chain length for hydrophobic peptides with different lengths and tendency to form TM topography. Short, medium and long refer to length of hydrophobic sequence sufficiently hydrophobic enough to form TM sequence in the absence of any mismatch. Dotted lines represent the lipid acyl chain lengths where the TM sequence is most stable. Non-TM shows the behavior of a sequence not hydrophobic enough to form a TM state at any bilayer width. (B) Effect of lipid acyl chain length upon the Trp emission λ_{max} of ErbB2 WT (W671) and ErbB2V664E peptides incorporated into monounsaturated phosphatidylcholine vesicles. WT at pH 7.0 (▲), V664E

at pH 4.0 (○), pH 7.0(Δ) and pH 9.0(+). The samples contained 2μM peptide incorporated into 500μM lipid dispersed in pH-adjusted PBS buffer. Average values of three to six samples are shown. The λ_{max} values are reproducible within $\pm 1-2$ nm.

Figure 2.

(A) Trp λ_{max} (striped bar) and quenching ratio (filled bar) for *neu/ErbB2* WT(W671) at pH 7.0 and V664E mutant peptide at different pH incorporated into DOPC vesicles. (B) Trp λ_{max} (striped bar) and quenching ratio (filled bar) for *neuErbB2* WT at pH 7.0 and V664E mutant peptide at different pH incorporated into DEuPC vesicles. Samples contained 2 μ M peptide incorporated into 500 μ M lipid dispersed in pH-adjusted PBS buffer. Average values from three to six samples and standard deviations for quenching ratios are shown. The λ_{max} values are reproducible within ± 1 –2 nm.

Figure 3.

Schematic illustration of transverse positions of “wild type” and V664E Erb b2 TM sequences (shown as rectangles) as a function of pH and bilayer width. In the thinner DOPC bilayers (top), the presence of the E residue results in a transverse shift which results in a shorter segment spanning the bilayer. Notice that the Trp residue first moves to a deeper, and then a shallower position as the degree of transverse shift increases. In the wider DEuPC bilayers (bottom) the E residues destabilizes the TM state, as the shorter membrane spanning segment in the presence of the E residue results in destabilization of the TM state by negative mismatch. In this case Trp depth in the membrane largely reflects the degree to which the non-TM state forms^{20, 25}.

Figure 4.

Effect of lipid acyl chain length upon Trp emission $\lambda_{\max}$ for *neu*/ErbB2 mutant peptides V664D ($\diamond$), V664Q ($\blacktriangledown$), V664H ($\square$), V664K ($\blacksquare$), and V664G ($\circ$) at pH 7.0. The WT (+), and V664E ($\triangle$) at pH 7.0 from Figure 1 are shown here for comparison. Samples contained 2 μ M peptide incorporated into 500 μ M lipid dispersed in pH-adjusted PBS buffer. Average values from three to six samples are shown. The $\lambda_{\max}$ values are generally reproducible to ± 1 nm.

Figure 5.

Effect of 20mol% anionic lipid upon the conformation of *neu*/ErbB2 WT sequence with different Trp location. (A) Trp λ_{max} dependence of W667 and W671 peptides upon the lipid bilayer width at pH 7.0. Dashed lines shows (80:20 mol:mol) mixtures of different length PC and DOPC. Filled lines refer to (80:20 mol:mol) mix of different length PC and DOPS. (B) Quenching ratio of W671 and W667 peptides incorporated into DOPC vesicles (filled bar) and (80:20 mol:mol) DOPC/DOPS vesicles. Samples contained 2 μ M peptide incorporated into 500 μ M lipid dispersed in pH-adjusted PBS buffer. Average values of three samples are shown here. The λ_{max} values are generally reproducible to ± 1 nm.

Figure 6.

Q ratio of Erb b2 peptides in PC and (80:20) PC:DOPS vesicles at pH 7.0. (A) Q ratio of Erb b2 WT and V664Q sequence with W at 671 position. (B) Q ratio of Erb b2 WT and V664Q sequence with W at 667 position. Filled light grey and dark grey bar represent DOPC and (80:20) DEuPC:DOPC respectively. Striped bar and clear bar represent (80:20) DOPC:DOPS and DEuPC:DOPS respectively. Samples contained 2 μ M peptide incorporated into 500 μ M lipid dispersed in pH-adjusted PBS buffer. Average values of two to three samples are shown here.

Figure 7. Effect of lipid acyl length upon Trp emission λ_{max} of (A) *neu*/ErbB2 W671V664Q and (B) W667V674Q peptide at pH 7.0. Dashed lines shows peptides incorporated into (80:20 mol:mol) mixtures of different length PC and DOPC. Filled lines refer to peptide incorporation into (80:20 mol:mol) mix of different length PC and DOPS. Samples contained 2 μM peptide incorporated into 500 μM lipid dispersed in pH-adjusted PBS buffer. Average values of two to three samples are shown here. The λ_{max} values are generally reproducible to $\pm 1\text{nm}$.

Figure 8.

Schematic illustration of the differential consequences of transverse shift. On the left, a piston-type movement is shown. On the right, a swinging-type movement. The latter is favored by a TM segment (shown in black) that is longer than the minimum necessary to span the hydrophobic part of the bilayer, and by pivoting around a fixed point (point b). Notice that a swinging motion can amplify the transverse movement of residues distant from the pivot via a lever action, and can result in both lateral and transverse displacements. The interaction of anionic lipids and cationic cytosolic juxtamembrane residues may form a pivot. The piston-type motion only results in a transverse displacement. Interaction of anionic lipids and cationic juxtamembrane residues would resist transverse movements.

Table 1

Peptides used in this study. Hydrophobic core residues are underlined. Hydrophilic substitutions shown in bold.

Peptide name	Sequence
“WT” (W671)	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATVVGVL</u> FLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
“WT” (W667)	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATVVGVL</u> FLIL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664E	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> E GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664H	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> H GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664D	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> D GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664G	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> G GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664K	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> K GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V664Q	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATV</u> Q GVLLFLWL VVVVGILIKRRRYK ⁶⁸⁶ -amide
V674Q	Acetyl- ⁶⁴⁹ AEQRASPVT <u>FIIATVVGVL</u> FLIL VQ VVGILIKRRRYK ⁶⁸⁶ -amide

Table 2

Quenching ratio (Q ratio) and Trp λ_{max} values for *neu*/ErbB2 peptides incorporated into DOPC and DEuPC vesicles at pH 7.0. The samples contained 2 μ M peptide incorporated into 500 μ M lipid dispersed in pH-adjusted PBS buffer. Samples were prepared in triplicate. Average and standard deviation of Q-ratios are shown. λ_{max} values were reproducible to within ± 1 –2 nm.

Peptide (W671)	DOPC		DEuPC	
	Q-Ratio	λ_{max} (nm)	Q-Ratio	λ_{max} (nm)
WT	0.266 $\pm$ 0.03	325	0.088 $\pm$.006	319
V664G	0.276 $\pm$ 0.1	324	0.15 $\pm$ 0.04	319
V664K	0.101 $\pm$ 0.03	320	1.3 $\pm$ 0.2	326
V664Q	0.09 $\pm$ 0.02	319	0.31 $\pm$ 0.03	326
V664E	0.131 $\pm$ 0.004	322	0.305 $\pm$ 0.02	327
V664D	0.187 $\pm$ 0.02	324	0.59 $\pm$ 0.02	330
V664H	0.294 $\pm$ 0.05	324	0.69 $\pm$ 0.07	331