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ARTICLE

Dimerization of Neu/Erb2 transmembrane domain is controlled by membrane curvature

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Abstract Secondary structures of the proto-oncogenic Neu/ErbB2 transmembrane segment and its mutant analogue have been determined in phospholipids. It is found that the mutated peptide possesses less helical character possibly due to the valine/glutamic acid point mutation. Embedding peptides in lipid systems whose topology can change from small (100–200 Å) tumbling objects to bilayer discs of 450 Å diameter leads to the finding that coiled-coil interactions are only observed in the presence of a bilayer membrane of low curvature, independent of mutation. This strongly suggests that any event that may change membrane topology can therefore perturb the dimerization/ologomerization and subsequent phosphorylation cascade leading to cell growth or cancer processes.

Keywords Circular dichroism · Oncogenic peptides · Bicelle membranes · ^{31}P-NMR · Coiled-coil interactions

Abbreviations

CD	Circular dichroism
DMPC	1,2-Dimyristoylphophatidylcholine
DCPC	1,2-Dicaproylphosphatidylcholine
DPC	Dodecylphosphocholine
SDS	Sodium dodecylsulfate
Neu_{TM35}	^{1}EQRASPVTFIIATVVGVLLFLILV VVVGILIKRRR^{35}
Neu^*_{TM35}	^{1}EQRASPVTFIIATV**E**GVLLFLILVV VVGILIKRRR^{35}
TFE	Trifluoroethanol
L_iK_j	Leucine-lysine alternated synthetic peptides of *i* and *j* number of residues

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Introduction

Activation of growth factor receptors of the tyrosine kinase family is initiated by ligand binding to their extracellular part, which triggers their dimerization. This enables the trans-phosphorylation of the intracellular domain of the partner and recruitment of signaling proteins, leading to the appropriate downstream cellular response. In the rat version, Neu, or in the human version, ErbB2, a single point mutation of a valine residue for glutamic acid at position 664 within the transmembrane domain enhances receptor dimerization, leading to constitutive activation (Bargmann et al. 1986a, b; Bargmann and Weinberg 1988; Weiner et al. 1989). Recent studies suggest that wild type Neu/ErbB2 forms an inactive dimer even in the absence of ligand. The dimer would be preformed on the cell surface and a structural change of the transmembrane domain would be necessary for the trans-phosphorylation of the intracellular domain (Moriki et al. 2001; Schlessinger 2000).

The structure of proto-oncogenic Neu_{TM35} and mutated (V15E) Neu^*_{TM35} has been already studied by CD in TFE and in SDS or DPC micelles (Goetz et al.

2001; Houliston et al. 2004). Helical content estimation using various methods (Bohm et al. 1992) led to 62–80% for Neu_{TM35} and 65–81% for Neu^*_{TM35}. Resolution of the Neu_{TM35} 3D structure in TFE by ^{1}H-NMR led to 74%. CD results, which can be obtained very rapidly, unfortunately show a large disparity. This is due, we believe, to the differences in the analysis methods relying in databases that are made of large proteins of which a majority are soluble and may not be suitably adapted to membranous peptides. Because we developed an algorithm based on standard curves obtained from small peptides being entirely in one of the canonical structures α-helix, β-sheet, helix typeII and random coil, we decided to apply this procedure to wild type and mutant transmembrane Neu/ErbB2 in various media, including lipid membranes.

CD can also be used to follow possible coiled-coil association. The CD trace of helix monomer is well described by one intense maximum at 190 nm and two characteristic minima at 208 and 220 nm. Helix ologomerization will enhance the CD trace and induce variations in the intensity of the two minima, a criterion that has been used by several authors to follow coiled-coil association (Cooper and Woody 1990; Lau et al. 1984; Sourgen et al. 1996); typically an equal intensity of the two minima is a signature of a coiled-coil association. Potential dimerization of Neu_{TM35} and Neu^*_{TM35} was also studied in TFE and micelles (Goetz et al. 2001; Houliston et al. 2004) by following the variation of the CD bands intensity ratio $\theta_{220}/\theta_{208}$ (Sourgen et al. 1996) as function of temperature and concentration. No dimerization/oligomerization was reported nor studies for peptides embedded in lipid membranes. However, it was recently demonstrated that bicelle membranes are well suited for studying by CD the state of aggregation of helical peptides in membranes (Loudet et al. 2005). Bicelles are mixtures of long (DMPC) and short (DCPC) chain that form under controlled conditions of concentration, hydration and temperature well defined bilayer discoid structures of ca. 450 Å diameter and 40 Å thickness. Their composition-temperature-hydration diagram interestingly shows transitions from isotropic highly curved phases (micelles) at 10°C to rather flat discs at 35°C (Raffard et al. 2000) that are oriented by magnetic fields (80% DMPC, 90% hydration). Using such lipid systems we therefore studied the influence of surface topology on dimerization of wild type and mutated peptides.

Materials and methods

Lipids were purchased from avanti polar lipids (Birmingham, AL, USA). The chemical syntheses and purifications of Neu_{TM35} and Neu^*_{TM35} have been already described (Loudet et al. 2005). Neu^*_{TM35} and Neu_{TM35} were incorporated into DCPC/DMPC bicelles (80% DMPC, 90% hydration, 1/900 and 1/300 peptide–lipid molar ratio) as reported in Arnold et al. (2002), Aussenac (2002), Loudet et al. (2005) and Raffard et al. (2000). Control of bicelle formation was performed by ^{31}P-NMR, as reported in Loudet et al. (2005) and Raffard et al. (2000).

Circular dichroism spectra were run on a Mark VI Jobin Yvon dichrograph at 0.2 nm intervals over the 180–270 nm range and using 0.1 mm path length cells; four scans were accumulated. Dichroic signals of pure DMPC/DCPC bicelles and of TFE give little background (Loudet et al. 2005) but they were nonetheless recorded for subtraction. Samples were allowed to equilibrate 30 min at a given temperature, regulated to ±2°C, before the CD signal was acquired. Spectra are smoothed with a 5-point FFT Filter before deconvolution with the CDfriend program, developed in the laboratory, which uses standard curves of α-helix, β-sheet, helix-II and random coil obtained from L_iK_j peptides of known length, secondary structure (Castano et al. 2000; Castano et al. 1999) and CD spectrum. The procedure will be described elsewhere (Buchoux and Dufourc, in preparation).

Results

CD of peptides in TFE

CD spectra of the proto-oncogenic Neu_{TM35} and of the mutant Neu^*_{TM35} peptides in TFE are presented in Fig. 1. For comparison, our reference α-helix is also included. All show pronounced positive (190 nm) and negative (208 and 220 nm) absorption bands characteristic of α-helix. We can nonetheless notice that intensities of maxima are different: that of the reference α-helix is greater than those of Neu/ErbB2 peptides; the maximum at 190 nm for Neu_{TM35} being in turn more intense than that of Neu^*_{TM35}. This suggests that the former has larger α-helix content than the later. This is confirmed by deconvolution of CD traces, Table 1, where 76% α-helical contribution is obtained for Neu_{TM35} and only 63% for Neu^*_{TM35}.

CD spectra were also recorded for peptide amounts ranging from 100 μM to 2 mM (data not shown). Very little spectral changes were detected suggesting that peptides retain the same structural state in TFE, i.e., there is no peptide dimerization in the studied range of concentrations.

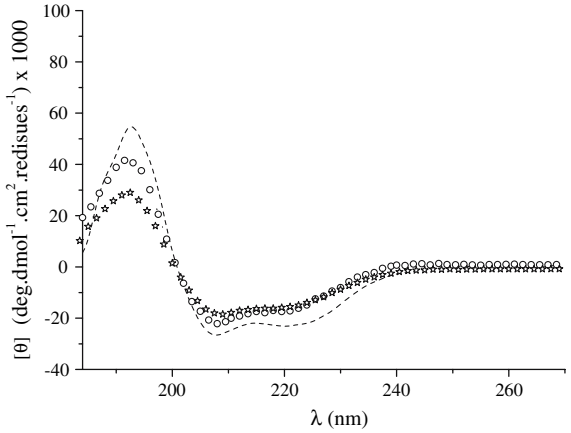

Fig. 1 CD spectra of Neu_{TM35} and Neu^*_{TM35} in TFE (500 μM), at room temperature. Neu_{TM35} *empty circle*, Neu^*_{TM35} *empty star*, standard α-helix *dash*

Table 1 Secondary structure elements for peptides in TFE and in DMPC/DCPC isotropic phases

	TFE (%)		DMPC/DCPC micelles (%)	
	Neu_{TM35}	Neu^*_{TM35}	Neu_{TM35}	Neu^*_{TM35}
α-helix	76	63	77	61
Helix II	5	10	2	14
β-sheet	0	5	0	0
Random coil	19	22	21	25

Deconvolution of CD spectra was accomplished using the CD-friend software (see text), accuracy ≤5%

Peptides in phospholipid bicelles at 35°C

CD spectra of the Neu_{TM35} and Neu^*_{TM35} in DMPC/DCPC bicelles (peptide–lipid molar ratio of 1/900) at 35°C are presented in Fig. 2a, b. For comparison, spectra in TFE and of the standard 100% α-helix are also shown. Although spectra have the same shape, marked changes in intensity are detected when peptides are embedded in membrane bilayers. For Neu_{TM35} (Fig. 2a), the maximum at 190 nm is much more intense than that in TFE; it is even higher than that of the 100% pure α-helix. The abrupt decrease of the curve below 192 nm indicates a saturation of the detector. Please note that the intensity of the minima at 208 and 220 nm are roughly identical. Similar observations are seen for Neu^*_{TM35} (Fig. 2b).

CD of peptides in DMPC/DCPC mixtures as a function of temperature

Because DMPC/DCPC mixtures show a morphological change on going from 10–30°C to 30–40°C, we looked at possible peptide dimerization/oligomerization by

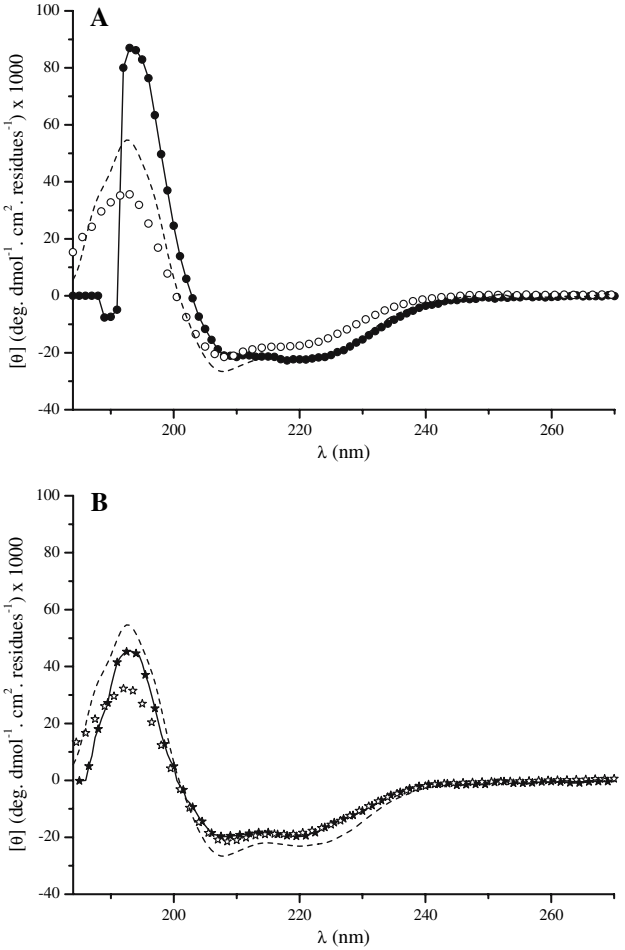

Fig. 2 CD spectra of Neu_{TM35} and Neu^*_{TM35} in DMPC/DCPC at 35°C (peptide/total lipids molar ratio 1/900). **a** Neu_{TM35} *filled circles*, Neu_{TM35} in TFE *empty circle* for comparison. **b** Neu^*_{TM35} *filled stars*, Neu^*_{TM35} in TFE *empty star* for comparison. The standard α-helix *dash* is shown in both (**a**, **b**)

following the thermal evolution of lipid–peptide systems. Changes in bilayer curvature were followed in parallel by ^{31}P-NMR. The Fig. 3 reports both CD and ^{31}P-NMR spectra of Neu_{TM35} and Neu^*_{TM35} embedded in DMPC/DCPC. Fig. 3d shows a single NMR line centered at ca. 0 ppm characteristic of a membrane system (DMPC/DCPC/Neu_{TM35}) of high curvature (isotropic phase), at 15°C. Mixed "micelles" or small size (100–200 Å) bicelles undergoing fast isotropic tumbling would give such a line. This line is converted into two sharp lines at ca. −3 and −10 ppm at 35°C (Fig. 3c), which characterize large discs of ca. 400–500 Å that are oriented by the magnetic field such that their plane is parallel to the magnetic field direction (Arnold et al. 2002; Raffard et al. 2000). It is noteworthy that peptides can be well inserted into DMPC/DCPC systems that stay with their initial morphology (micelles or discs). Same NMR features are observed with the mutated peptide (not shown). Fig. 3a reports corre-

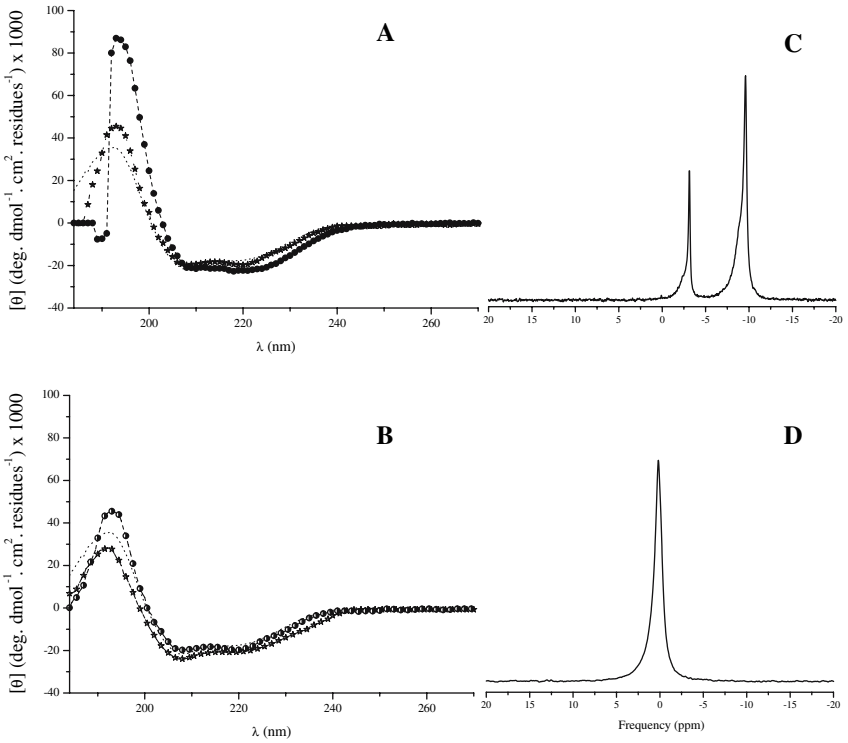

Fig. 3 CD and ^{31}P-NMR spectra of Neu_{TM35} and Neu^*_{TM35} incorporated in DMPC/DCPC at 35°C (**a**, **c**) and 15°C (**b**, **d**), peptide/total lipids molar ratio is 1/900. **a** Neu_{TM35} *filled circles* and Neu^*_{TM35} *filled stars*, Neu_{TM35} in TFE *dots* for comparison. **b** Neu_{TM35} *half-filled circles* and Neu^*_{TM35} *half-filled stars*, Neu_{TM35} in TFE *dots* for comparison. **c** NMR *sharp lines* (ca. −2 and −10 ppm) characteristic of discoid bicelles oriented with their plane parallel to the magnetic field, DMPC/DCPC/Neu_{TM35} system. **d** Single NMR line (0 ppm) characteristic of isotropic phase *mixed micelles*, DMPC/DCPC/Neu_{TM35} system

sponding CD spectra of both peptides in lipids, at 35°C. The spectrum of Neu_{TM35} in TFE is also shown (dash) for comparison. As in Fig. 2, features characteristic of coiled-coil association are seen for both peptides in membranes. At low temperature, Fig. 3b, CD spectra for both peptides resemble to those in TFE, with no indication of dimerization/oligomerization. Similar spectra were observed when performing the same temperature variation with higher peptide content (peptide/lipid molar ratio of 1/300, data not shown (Aussenac 2002)). The intensity of the minima at 208 and 220 nm is directly measured on spectra and the thermal variation of the $\theta_{220}/\theta_{208}$ ratio, as it has been proposed as a coiled-coil indicator by several authors (Cooper and Woody 1990; Lau et al. 1984; Sourgen et al. 1996), is reported in Fig. 4. This ratio stays in the range 0.8–0.9 for temperatures below 30°C and reaches ca. 1.0 at 35 and 40°C. According to Cooper and Woody (1990), Lau et al. (1984) and Sourgen et al. (1996) this suggests monomer state in the isotropic membrane phase and helix–helix association when the DMPC/DCPC system is under the form of bilayer discs.

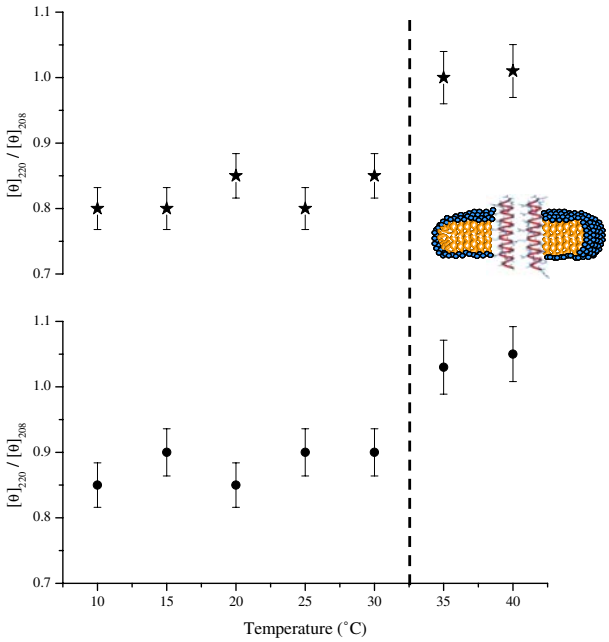

Fig. 4 Temperature dependence of dimer indicator, $\theta_{220}/\theta_{208}$ ratio. **a** Peptide Neu_{TM35}, **b** Peptide Neu^*_{TM35}, in DMPC/DCPC at peptide/total lipids molar ratio of 1/900. The *vertical dashed line* delineates the boundary between an isotropic system and magnetically orientable discs according to (Raffard et al. 2000). Inserted drawing is a tentative representation of the state of association of helical peptides in bilayered discs above 30°C

Discussion

Two main results appear: i) the retention of helical secondary structure in lipids, with a higher percentage for the pro-oncogenic form, and ii) evidence for coiled-coil association in bilayer discs of DMPC/DCPC only, independently of the mutation. These points are discussed below.

α-Helical structure in TFE and in highly curved membranes

Secondary structures in TFE and DMPC/DCPC membranes, in their isotropic form, show that Neu_{TM35} has a greater helical content than Neu^*_{TM35} (76–77% vs. 63–61%, respectively). Before discussing the implications of this finding, a comment must be made on the deconvolution procedure. Some of the above peptides have already been studied in TFE or micelles of SDS or DPC and secondary structure analysis performed using various methods (Bohm et al. 1992; Manavalan and Johnson 1987) showed large variability (*vide supra*). This appears to originate from the very different methods used, having all in common the fact that protein secondary structure databases are used. Unfortunately none of the bases have data from membrane proteins and secondary structures such as turn and loops that are in the bases may not be well suited for peptides. We decided to choose a much simpler deconvolution approach based on CD spectra of a reduced number of canonical CD secondary structures (α-helix, β-sheet, helix-II and random coil) that does not imply a choice between several databases. The procedure will be detailed elsewhere (Buchoux & Dufourc, in preparation). Our algorithm leads to 76–77% α-helix content for Neu_{TM35}, which agrees very well with the 74% obtained from the atomic structure determined by NMR (Goetz et al. 2001). Although the atomic structure of a peptide very close to Neu^*_{TM35} has been resolved in TFE and in detergent micelles (Houliston et al. 2003, 2004), no data is available for comparison. Our deconvolution procedure gives ca. 10% more helical content, which is outside the experimental error, for Neu_{TM35} than for its mutated analogue, both in TFE and in DMPC/DCPC micelles. The lesser content in α-helix in Neu^*_{TM35} could be related to the presence of glutamic acid in the middle of the hydrophobic sequence that could disturb or break the helicity. Interestingly, it has be shown by several techniques that an increase in the number of glutamic acid residues per chain from 0 to 10 in 35-residue peptides results in a gradual decrease in the amount of helical content from approximately 91 to 66% in 50% TFE (Kohn et al. 1995).

Coiled-coil association in bilayer discs of DMPC/DCPC

Neu_{TM35} and Neu^*_{TM35} embedded in phospholipids change from a state where there are no coiled-coil association in the isotropic phase ("micelles" or small bicelles) below 30°C and dimeric/oligomeric at 35°C, when the system is under the form of large bilayers discs. This clearly indicates that an extended bilayer membrane is a requirement for helix–helix association and that the complex may be dissociated if the membrane curvature is elevated. It is noteworthy that rough calculations using bicelles sizes and an average lipid surface area of 60 $Å^2$ leads to 2–4 and 5–14 peptides in small isotropic objects and in large bicelles, respectively, i.e., conditions for possible dimerization/oligomerization are fulfilled especially for small tumbling objects observed at low temperature. Our results are in agreement with the work of Grant et al. (Jones et al. 1997) who showed by ^{2}H-NMR that peptides of the Neu family aggregate in POPC liposomal membranes (with and without cholesterol). Interestingly, we find that both proto-oncogenic and mutated peptides are associated in large membrane discs independent of the mutation. Our results are in agreement with the work of Schlessinger (2000), who proposes the existence of dimers (inactive) even in the absence of ligand fixation. Our findings agree also with the model proposed by Moriki and Smith for receptor activation in which dimers are preformed in the membrane in the absence of ligand (Moriki et al. 2001; Smith et al. 2002) and whereby activation by ligand or by a mutation would be due to a change in transmembrane helix orientations. Of great interest is the fact that membrane curvature appears to control the dimer formation: as found by others (Goetz et al. 2001; Houliston et al. 2003, 2004) only a monomeric state is found in micelles of high curvature. This strongly suggests that any event that may change membrane topology can therefore perturb the dimerization and subsequent phosphorilation cascade leading to cell growth or cancer processes.

A molecular dynamics study of preformed dimers of Neu^*_{TM35} in a fully hydrated DMPC membrane has been recently reported by Aller et al. (2005). They found that the best energetic balance was found for helices in symmetrical left-handed interactions. Our findings are in clear agreement with the molecular dynamics work. i) In the associated state the CD spectrum does not change its overall shape (maximum at 190 nm and minima at 208 and 220 nm), this is a proof that the helices are left-handed. Right-handed helices would give an inversed CD trace. ii) The increase in overall CD spectra intensity on going from monomers to coiled-coil interactions indicates that the helices associate in a symmetrical fashion (see schematics in Fig. 4). Antisymmetric arrangement would decrease of cancel the overall chirality of the complex and therefore decrease or cancel the CD signal. Unfortunately our study does not allow identification of dimerization motifs as has been proposed by some authors (Aller et al. 2005; Bechinger 2000).

References

Aller P, Voiry L, Garnier N, Genest M (2005) Molecular dynamics (MD) investigations of preformed structures of the transmembrane domain of oncogenic Neu receptor dimer in a DMPC bilayer. Biopolymers 77:184–197

Arnold A, Labrot T, Oda R, Dufourc EJ (2002) Cation modulation of "bicelle" size and magnetic alignment as revealed by solid state NMR and electron microscopy. Biophys J 83:2667–2680

Aussenac F (2002) Les "bicelles" et les "rafts" : de nouvelles membranes biomimétiques pour l'étude de protéines membranaires par RMN des solides. Ph.D, Thesis Université Bordeaux 1, Bordeaux, France, pp 212

Bargmann CI, Hung MC, Weinberg RA (1986a) Multiple independent activations of the neu oncogene by point mutation altering the transmembrane domain of p185. Cell 45:649–657

Bargmann CI, Hung MC, Weinberg RA (1986b) The neu oncogene encodes an epidermal growth factor receptor-related protein. Nature 319:226–230

Bargmann CI, Weinberg RA (1988) Oncogenic activation of the neu-encoded receptor protein by point mutation and deletion. EMBO J 7:2043–2052

Bechinger B (2000) Understanding peptide interactions with the lipid bilayer: A guide to membrane protein engineering. Curr Opin Chem Biol 4:639–644

Bohm G, Muhr R, Jaenicke R (1992) Quantitative analysis of protein far UV circular dichroism spectra by neural networks. Protein Eng 5:191–195

Castano S, Desbat B, Dufourcq J (2000) Ideally amphipathic b-sheeted peptides at interfaces: structure, orientation, affinities for lipids and hemolytic activity of $(KL)_mK$ peptides. Biochim Biophys Acta 1463:65–80

Castano S, Desbat B, Laguerre M, Dufourcq J (1999) Structure, orientation and affinity for interfaces and lipids of ideally amphipathic lytic $L_iK_j(i = 2j)$ peptides. Biochim Biophys Acta 1416:176–194

Cooper TM, Woody RW (1990) The effect of conformation on the CD of interacting helices: a theoretical study of tropomyosin. Biopolymers 30:657–676

Goetz M, Carlotti C, Bontems F, Dufourc EJ (2001) Evidence for an α-helix -> π-bulge helicity modulation for the *neu/erbB-2* membrane-spanning segment. A ^{1}H NMR and circular dichroism study. Biochemistry 40:6534–6540

Houliston RS, Hodges RS, Sharom FJ, Davis JH (2003) Comparison of proto-oncogenic and mutant forms of the transmembrane region of the neu receptor in TFE. FEBS Lett 535:39–43

Houliston RS, Hodges RS, Sharom FJ, Davis JH (2004) Characterization of the proto-oncogenic and mutant forms of the transmembrane region of neu in micelles. J Biol Chem 279:24073–24080

Jones DH, Rigby AC, Barber KR, Grant CWM (1997) Oligomerization of the EGF receptor transmembrane domain: a ^{2}H NMR study in lipid bilayers. Biochemistry 36:12616–12624

Kohn WD, Monera OD, Kay CM, Hodges RS (1995) The effect of interhelical electrostatic repulsions between glutamic acid residues in controlling the dimerization and stability of two-stranded a helical coiled-coils. . J Biol Chem 270:25495–25506

Lau SY, Taneja AK, Hodges RS (1984) Synthesis of a model protein of defined secondary and quaternary structure. Effect of chain length on the stabilization and formation of two-stranded alpha-helical coiled-coils. J Biol Chem 259:13253–13261

Loudet C, Khemtemourian L, Aussenac F, Gineste S, Achard MF, Dufourc EJ (2005) Bicelle membranes and their use for hydrophobic peptide studies by circular dichroism and solid state NMR. Biochim Biophys Acta 1724:315–323

Manavalan P, Johnson WCJ (1987) Variable selection method improves the prediction of protein secondary structure from circular dichroism spectra. Anal Biochem 167:76–85

Moriki T, Maruyama H, Maruyama IN (2001) Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain. J Mol Biol 311:1011–1026

Raffard G, Steinbruckner S, Arnold A, Davis JH, Dufourc EJ (2000) Temperature-composition diagram of dimyristoyldicaproyl phosphatidylcholine "Bicelles" self-orienting in the magnetic field. A solid state 2H and 31P-NMR study. Langmuir 16:7655–7662

Schlessinger J (2000) Cell signaling by receptor tyrosine kinase. Cell 103:211–225

Smith SO, Smith C, Shekar S, Peersen O, Ziliox M, Aimoto S (2002) Transmembrane interactions in the activation of the neu receptor tyrosine kinase. Biochemistry 41:9321–9332

Sourgen F, Maroun RG, Frère V, Bouziane M, Auclair C, Troalen F, Fermandjian S (1996) A synthetic peptide from the human immunodeficiency virus type-1 integrase exibits coiled-coil properties and interferes with the in vitro integration activity of the enzyme. Correlated biochemical and spectroscopic results. Eur J Biochem 240:765–773

Weiner DB, Liu J, Cohen JA, Williams WV, Greene MI (1989) A point mutation in the neu oncogene mimics ligand induction of receptor aggregation. Nature 339:230–231