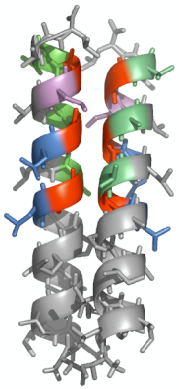

# FRET studies of transmembrane domains association : specificity vs promiscuity revisited.

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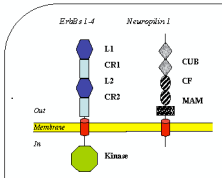
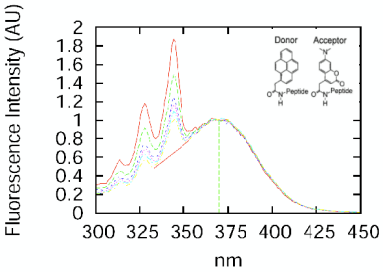
***Introduction :*** Membrane proteins frequently exist as oligomeric complexes. The formation of these complexes can transform a simple membrane *anchor* into an integral functional part of a biologically *active* complex, which requires the existence of specific interactions between the individual components. Thus, knowledge of protein/protein-interactions in this group of proteins is of paramount importance for understanding functional networks within the cell membrane, and in particular their role in cell signaling. Fluorescent methods such as FRET are very powerful for the quantitative studies of such interactions. We have undertaken the characterization of homo- and hetero-interactions between transmembrane domains of receptors of importance in cell proliferation and differenciation : the erbB receptors and neuropilins.




| | |
|---|---|
| Glycophorin A | SEPEITLIIFGVMAGVIGTILLISYGIRRLIKK |
| EGFR | KIPSIATGMVGALLLLLVVALGIGLFMRRRH |
| ERB2 | ASPLTSIISAVVGILLVVVLGVVFGILIKRRQ |
| ERB3 | KTHLTMALTVIAGLVVIFMMLGGTFLYHRGRR |
| ERB4 | RTPLIAAGVIGGLFILVIVGLTFAVYVRRKS |
| Neuropilin 1 | ILITIIAMSALGVLLGAVCGVVLYRKR |
| Neuropilin 1 (3G>V) | ILITIIAMSALVVLLVAVCVVVLYRKR |

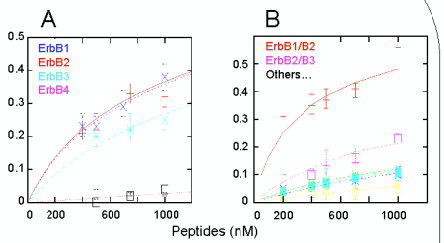
***Material & methods 1 :*** The ErbB receptors and Neuropilins are single-pass transmembrane proteins which functions imply homo- and hetero-associations. Their transmembrane domains contain putative interactions motifs analogous to that of Glycophorin A (the so-called "GxxxG motif"), and have been shown to participate in receptor activation (1, 2).

***Material & methods 2 :*** Peptides corresponding to the TM domains of Glycophorin A, the 4 erbB receptors, and Neuropilin 1 as well as a triple G>V mutant were synthetized, labelled with pyrene (donor) and coumarin (acceptor). The peptides were purified by RP-HPLC and used for FRET assays in LDAO micelles as described by Fisher et al. (3).

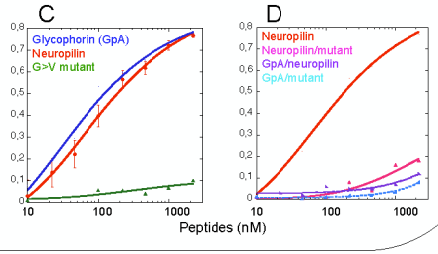



***Results :***

Panels A and B display results obtained with the four erbB peptides for their homo- (A) and hetero-dimerization (B) in 1 mM detergent. Apparent Kds were calculated as 2.3, 2.2, 4.0 and 70 µM for erbB 1, 2, 3, and 4 respectively. Knowledge of this Kd allowed for calculation of the apparent Kd for heterodimerization, as 0.2 µM for erbB1/2, 0.85 µM for erbB2/3, 2.0 µM for erbB1/3, and so on (4).




Panels C and D display results for the glycophorin and neuropilin peptides for their homo- (C) and hetero-dimerization (D) in 4 mM detergent. Neuropilin 1 peptide homodimerizes with an apparent Kd (0.1 µM) slightly higher than that of Glycophorin (0.04 µM), whereas the triple mutant does not show any tendency to associate. No heterodimerization could be evidenced between these three peptides, nor for neuropilin with erbB1 or erbB2 peptides.




***Conclusions and future directions :***

- The FRET method allows for quantitative analysis of transmembrane peptides homo- and hetero-interactions.
- Results with the ErbB peptides show that they display a hierarchy of interactions, in good agreement with genetic results for the homodimers (5).
- This hierarchy for heterodimers corresponds to what is observed for whole receptors, i.e. erbB2 and erbB1 are the preferred partners.
- The results with the neuropilin peptides confirm the role of the GxxxG motif, and are in excellent agreement with biological results obtained with the same synthetic peptides (2).
- *Most importantly, the different functionnally unrelated peptides we studied so far display no tendency for significant hetero-associations, thus their interactions are more specific than promiscuous.*

*To do list includes :*

✓ extension of these results in more biologically relevant environments (lipid vesicles, cell membranes), and peptide interactions with whole receptors;
✓ mutational analysis of the structural determinants of neuropilin dimerization;
✓ studies of interactions between the transmembrane domain of neuropilin with those of its partners in signaling complexes.

***References :***

1. Bennasroune A. et al. *Mol. Biol. Cell* (2004) **15**, 3464.
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3. Fisher LE., Engelman DM. and Sturgis JN. (2003) *Biophys J*, **85**, 3097.
4. Vegh A., Sturgis JN. and Duneau JP. *submitted*.
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