

## ***Properties of Peptide-Anchored Lactoferricins in Neutral and Anionic Lipids***

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**Background and Objective:** Electrostatic interactions between charged amino acids and membrane lipids are important for membrane protein structure and function. We have investigated amphipathic, cationic lactoferricin (**LfB**), of sequence **RRWXWR**, either alone or anchored to a model  $\alpha$ -helical transmembrane (TM) peptide having a (Leu-Ala)<sub>7</sub>  $\alpha$ -helical core. The **LfB** and TM domains are separated by an Ala-Ala spacer or by a longer, helix-breaking Gly-Pro-Gly-Gly spacer to give sequences **RRWAWR-AA-(LA)<sub>7</sub>KKA-NH<sub>2</sub>** and **RRWQWR-GGPG-(LA)<sub>7</sub>KKA-NH<sub>2</sub>**, respectively. Residue X was either glutamine or deuterated alanine (**A**) for solid-state <sup>2</sup>H-NMR. The main objective has been to characterize the influence of the TM and spacer domains upon the **LfB** interactions with lipid membranes.

**Methods:** The peptides were examined in neutral and anionic lipid mixtures by solid state <sup>2</sup>H NMR, <sup>31</sup>P NMR, circular dichroism (CD) and fluorescence spectroscopy.

**Results:** Within oriented peptide/lipid mixtures, the lipids retain a predominantly bilayer organization, and the (anchored) **LfB** domain is oriented with respect to the membrane normal, suggesting a motionally restricted (interfacial) environment. The GGPG linker reduces the  $\alpha$ -helical content compared to the AA linker in all environments. Tryptophan fluorescence spectra of the TM-anchored peptides in lipids are blue-shifted, with decreased intensity compared to TFE:water, confirming a more hydrophobic environment for the tryptophans.

**Discussion and Conclusions:** These peptides offer opportunities for investigating the importance of juxtamembrane domains of transmembrane proteins.

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