The mouse MrgC11 (mMrgC11) and MrgA1 (mMrgA1) mas-related gene G Protein-Coupled receptors are expressed in a specific subset of sensory neurons known to detect painful stimuli, suggesting that they could be involved in pain sensation or modulation. Although the endogenous ligand for these receptors is not known, they have been shown experimentally to bind to NPFF and BAM22. A particularly interesting result is that FMRFa tetrapeptide along with dFMRFa and FdMRFa activate MrgC11 at ~100 nM concentration while FMdRFa and FMRdFa and do not activate (>30 µM) while the dipeptide RFa activates at ~500 nM.

To aid the development of small molecule selective agonists and antagonists to these receptors, we have predicted the 3D structures for mMrgC11 and mMrgA1, using the MembStruk computational method and further used the HierDock method to predict the binding sites of six di- and tetra-peptide ligands.

In all six cases the RFa part of the di- or tetrapeptide protrudes into the protein between transmembrane (TM) domains 3, 4, 5 and 6. The Phe (Fa) of the peptide interacts favorably with Tyr110 (TM3), while the Arg makes a salt bridge to Asp161 (TM4) and Asp179 (TM5). We find that the Met of the tetrapeptide is oriented towards the extracellular loops and the terminal Phe is located inside the TM domains in an aromatic/hydrophobic site flanked by Tyr237, Leu238, Leu240, and Tyr256, all in TM6 and W162 on TM4. This structure provides conformational constraints so that the calculated binding energy for the 3 strong binders: FMRFa, dFMRFa, and FdMRFa are strong and similar while the 2 nonbinders FMdRFa and FMRdFa are predicted to bind much weaker. This indicates that these agonists bind preferentially to the TM3, TM4, TM5, and TM6 regions, similar to those of biogenic amine receptors (although the sites bear no resemblance).

Subsequent to the predictions we carried out site directed mutagenesis experiments followed with intracellular calcium release assay for the six peptides with mutant mMrgC11 receptors to validate the predicted binding site. Indeed for six peptide agonists showing EC50 values ranging from 113 to 398 nM in the wild type, we found that the Y110A mutants showed no activity (at 33 µM of agonist concentration). For D161A mutant we found no activity for 4 of the peptides while the other two showed 100 times weaker activity than for wild type. For D179A mutant we found no activity for 3 of the peptides while the other 3 showed activity10 times weaker than the wild type.

This validation by experiment of the dramatic decrease in activity of the peptides for the residues predicted to be critical in binding, and the correct prediction of the 3 strong

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agonists and 2 nonbinders among the various chiral isomers of the FMRFα tetrapeptide provides a strong indication that our predicted 3D structure is useful to predict binding site for selective ligands.