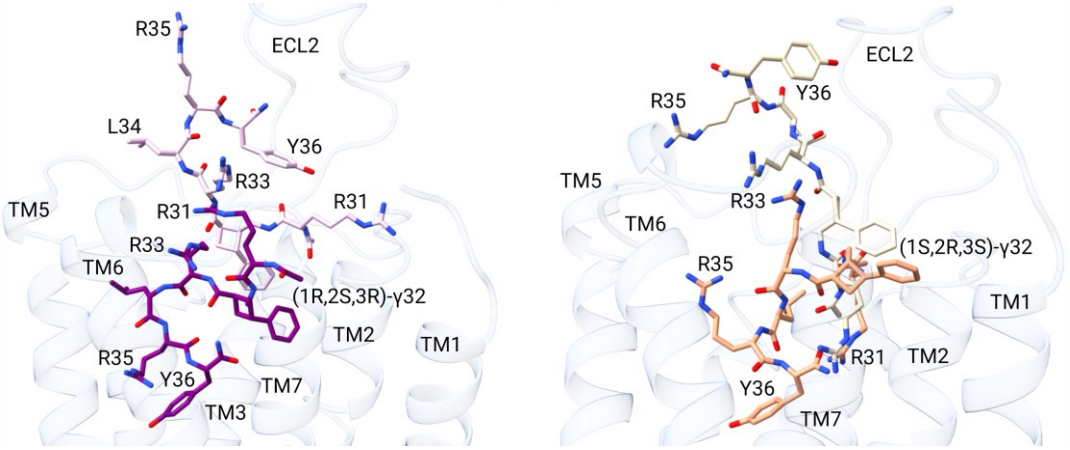
Understanding α,γ-peptide efficacy and binding selectivity in the neuropeptide Y Y_4-receptor

Jacqueline C. Calderón,[1] Eva Plut,[2] Max Keller,[3] Chiara Cabrele,[2] Oliver Reiser,[2] Francesco L. Gervasio[4], Timothy Clark[1]

[1]Computer-Chemistry-Center, Department of Chemistry and Pharmacy, Friedrich-Alexander-University Erlangen-Nürnberg, Nägelsbachstraße 25, 91052 Erlangen, Germany, [2]Institute of Organic Chemistry, Faculty of Chemistry and Pharmacy, University of Regensburg, 93040 Regensburg, Germany, [3]Institute of Pharmacy, Faculty of Chemistry and Pharmacy, University of Regensburg, D-93040 Regensburg, Germany, [4]Institute of Pharmaceutical Sciences of Western Switzerland, CH1206 Geneva, Switzerland.



The neuropeptide Y (NPY) receptor family comprises four physiologically relevant class A GPCRs, Y_1R, Y_2R, Y_4R, and Y_5R. The endogenous ligands of NPY receptors are the homologous 36-residue linear peptides NPY, peptide YY (PYY), and pancreatic polypeptide (PP). Because of its role in appetite suppression, the Y_4-receptor is an attractive therapeutic target against obesity. Unlike small molecules, peptides exhibit high conformational flexibility in their unbound states due to the large number of rotatable bonds along the backbone and in the side chains. In contrast, the receptor binding pocket imposes a stringent constraint on the conformation of these peptides. To date, few studies that allow us to gain insight into the molecular basis of ligand recognition on Y_4R-peptide systems have been reported. Computational methods are essential tools for investigating protein-ligand interactions and subsequent characterization of binding pockets. Providing details at an atomistic level of the main features related to the binding process will facilitate the rational development of Y_4R-selective ligands. We have studied two C-terminally amidated α,γ-hexapeptides (RSR/SRS) with sequence *Ac*-R31-γ-CBAA32-R33-L34-R35-Y36-*NH_2*, where γ-CBAA is the (1*R*,2*S*,3*R*)-configured 2-(aminomethyl)-3-phenylcyclobutanecarboxyl moiety (RSR) or its mirror image (SRS). Both peptides bind to Y_4R (K_i of RSR/SRS: 0.66/12 nM) and act as partial agonists (intrinsic activity of RSR/SRS: 50/39%). [1] To investigate the binding mode of the α,γ-hexapeptides, induced-fit docking, molecular dynamics and metadynamics simulations were performed. We found that the di-arginine motif R33-X-R35 of the peptide plays a prominent role in the interaction of the ligands with the Y_4R. A more stable network of H-bond and salt-bridge interactions between peptide RSR and Y_4R is suggested to be responsible for its observed higher binding affinity and potency, in comparison to peptide SRS. In addition, we applied a metadynamics-based protocol [2] to characterize the peptides' binding free-energy profiles. Comparison of the binding poses for global (orthosteric) and secondary (vestibule) minima indicates a significant role of the extracellular vestibule in driving the binding process. In the global minimum, peptide ligands show a binding pose in excellent agreement with that of the equilibrated starting structure. Most importantly, in agreement with previous studies, [3,4] the secondary minimum (vestibule binding pose) found for the α,γ-peptide SRS is proposed to play a role in its suggested antagonistic-like effect.

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