Electronic Supplementary Material (ESI) for Dalton Transactions

Supporting information

Structural and biological implications of the binding of Leu-enkephalin and its metal derivatives to opioid receptors

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1 Synthesis of [η^5-Cp*Rh(III)Tyr^1]-Leu-enkephalin

The synthethis of [Cp*Rh(III) $(H_2O)_3$]$(OTf)_2$ and its reaction with the tyrosine residue of various peptides including Leu-enkephalin have been described [1].

2 NMR spectroscopy

For NMR spectroscopy both Leu-enkephalin and [η^5-Cp*Rh(III)Tyr^1]-Leu-enkephalin were prepared in water and the concentration of peptide was adjusted to 12.5 mg/ml. NMR spectra with WATERGATE solvent suppression of peptides were recorded at 600.13 MHz proton frequency and at 293 K on a Bruker DRX 600 spectrometer equipped with a pulsed field gradient and a triple resonance probe-head. Details on ^{1}H- and ^{13}C-NMR data as well as TOCSY and ROESY spectra of [η^5-Cp*Rh(III)Tyr^1]-Leu-enkephalin have been published previously [1]. All spectra were processed with NMR-Pipe [2]. Assignment and data handling were performed using CcpNmr Analysis 2.1.5 [3].

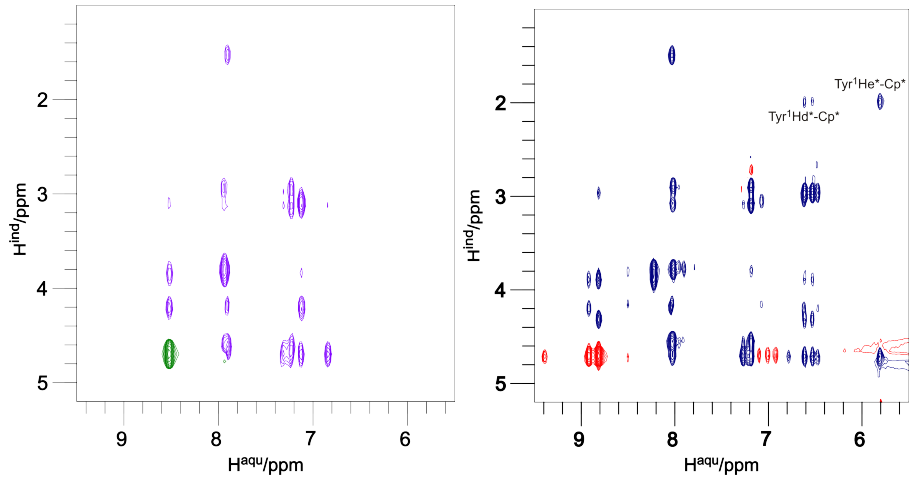

Figure 1: 2D ^{1}H-^{1}H-ROESY spectra of Leu-enkephalin (a) and [η^5-Cp*Rh(III)Tyr^1]-Leu-enkephalin (b)

3 Structure calculation

NOE assignment and structure calculations was performed using ARIA 2.3 [4] with CNS [5] for calibration and final ROE assignments. XPLOR-NIH 2.29 [6] was used for the final calculation including the Cp* moiety. The intensities of the ROEs between the tyrosine residue and Cp* were similar to those reported for the [η^5-Cp*Rh(III)Tyr^3]-octreotide. However, distances and

angle definition of the [η^5-Cp*Rh(III)Tyr]-complexes were derived from a YASARA[7] model. Structural visualization was carried out using PyMol [8].

4 Docking studies

Docking was performed using Haddock 2.1 [9, 10] and CNS 1.21 [5]. For δ-OR (PDB:4EJ4) residues D128, Y129, M132, W273, I277, H278, V281, W284, L300 and Y308 and for μ-OR (PDB:4DKL) residues D147, Y148, M151, K233, W293, I296, H297, V300 and Y326 were selected as active binding residues. These were reported to be involved in naltrindole and β-funaltrexamine binding [11, 12]. Furthermore, all five Leu-enkephalin residues were set as active binding sites. Docking interfaces were defined by ambiguous interaction restraints (AIR). The Leu-enkephalin starting structure was generated randomly followed by molecular dynamics and energy minimization performed using a simulated annealing protocol to ensure correct covalent geometry using XPLOR-NIH [6]. For [η^5-Cp*Rh(III)Tyr^1]Leu-enkephalin a new residue was defined in the parallhdg5.3.pro and topallhdg5.3.pro of CNS 1.21 [5] and the Cp*Rh(III)-tyrosine-complex was treated as rigid body. For the angle and bond definition the solution structure of [η^5-Cp*Rh(III)Tyr^1]Leu-enkephalin was used as a template. The distance measurement was performed with VMD [13] and Antechamber 1.27 [14]. 1000 structures were calculated during the first iteration, then 100 were selected for further structural refinement. As a control, constraints were set between the NH3 of TYR1 and OD1 and OD2 of Asp128 (δ-OR) and Asp147 (μ-OR). This charge-charge interaction site has been described for Met-enkephalin [15]. No significant changes were observed in comparison to the unbiased docking without these additional constraints.

Further docking results

During the course of docking studies we have also observed alternative conformations. Leu-enkephalin when docked to the δ-OR can adopt a structure as proposed for the μ-OR (2, A). Surprisingly, the phenylalanine and the leucine residue are also oriented similarly with the C-terminus facing away from Trp284. In the δ-OR, the orientation of the phenylalanine to Trp284 is more favorable, which is also supported by the energy values. This indicates that the orientation of these two residues strongly depends on the orientation of the tyrosine side chain. As enkephalin is more selective for the δ-receptor that has the characteristic tryptophan residue it is likely that the orientation with the tyrosine between Tyr308 and Trp274 is the one activating the receptor. N-terminal tyrosine is a conserved residue among all opioid peptides [16]. For both of the suggested conformations there is no residue in close proximity that may help to stabilize the tyrosine and thus might explain the conservation of this residue. In the

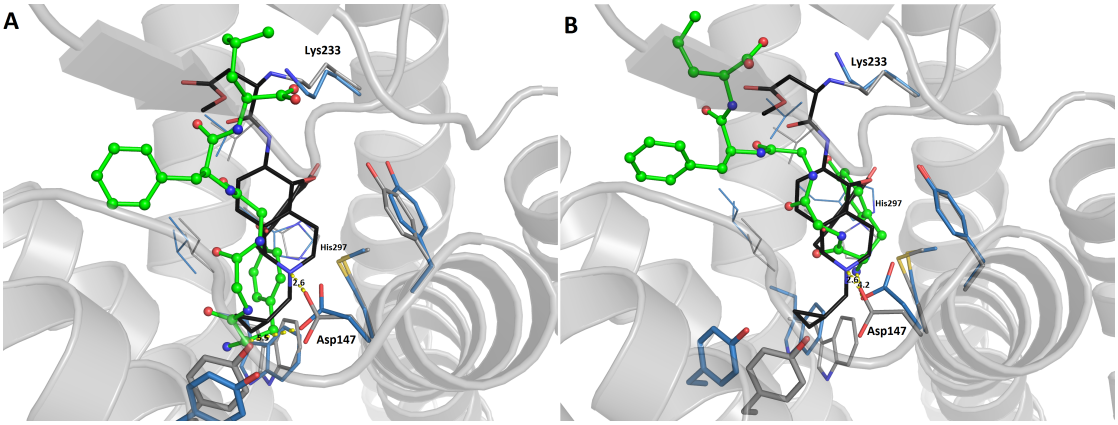

Figure 2: Docking of Leu-enkephalin (green) to the μ-OR (PDB:4DKL) reveals two similar topologically conformations. The distance between N-terminus and Asp147 is larger than for the co-crystallized β-FNA (B, black). Flexible residues are emphasized (crystal structure:grey; docking:blue).

proposed hydrophobic environment it could as well be a phenylalanine as in nociceptin, an 'orphan' receptor (ORL) selective peptide. In the δ-OR there are some asparagine residues in the region close to Trp274 and Tyr308, which might be able to form a hydrogen bridge to the hydroxyl-group of the tyrosine upon activation leading to a structural change.

As for the μ-receptor the distance between the N-terminus and the aspartate residue is rather long. There is also an alternative structure with a shorter distance (2, B), but here the tyrosine side chain is pushed towards the backbone of the peptide leading to an energetically unfavorable structure.

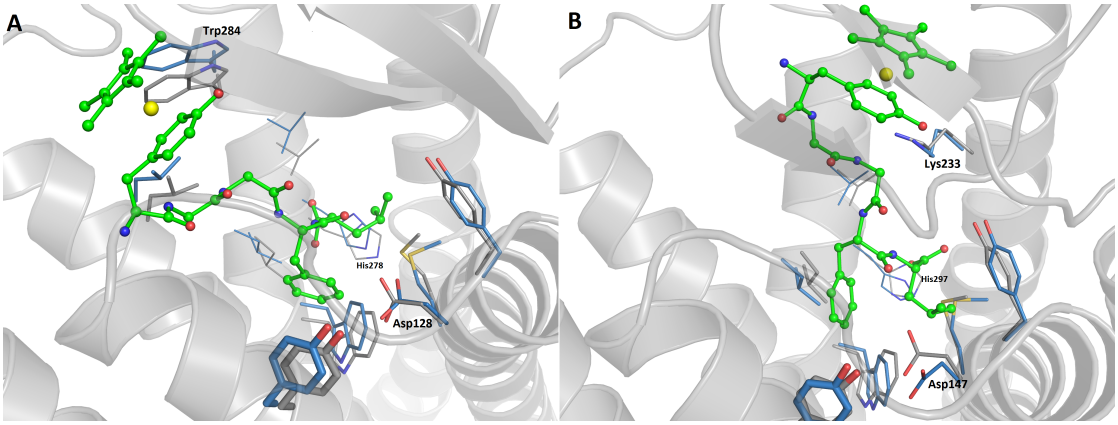

Figure 3: [η^5-Cp*Rh(III)]Leu-enkephalin (green) docked to the δ-OR (A; PDB:4EJ4) and μ-OR (B; PDB:4DKL). Flexible residues are emphasized (crystal structure:grey; docking:blue).

[η^5-Cp*Rh(III)]Leu-enkephalin can also bind in an 'upside-down' fashion. Here, the tyrosine

residue with the Cp* moiety is located near Trp284 and Lys233, respectively. The phenylalanine and the leucine residue are heading into the pocket. In this manner the aspartate residue of the receptor cannot interact with the N-terminus.

Hydrogen bonds stabilize the docked structures in the binding pocket

In the structures presented here some stabilizing hydrogen bounds can be identified.

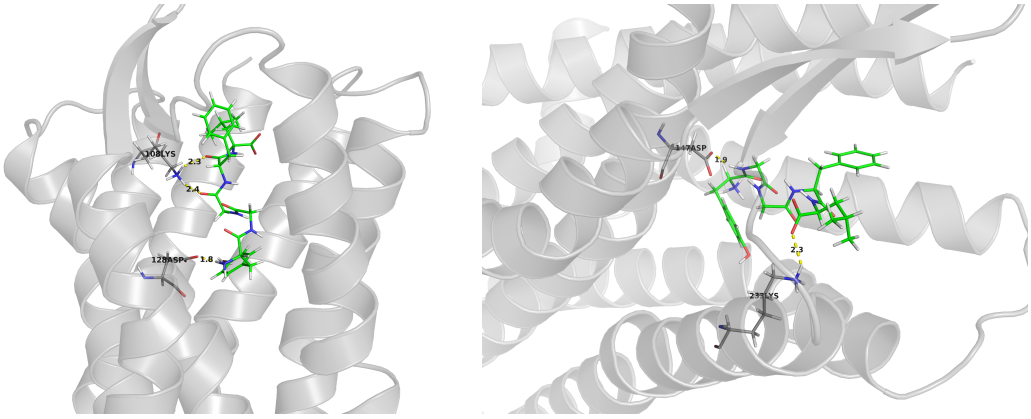

Figure 4: Hydrogen bonds found in the docking results of enkephalin and the δ-OR (left) or μ-OR (right).

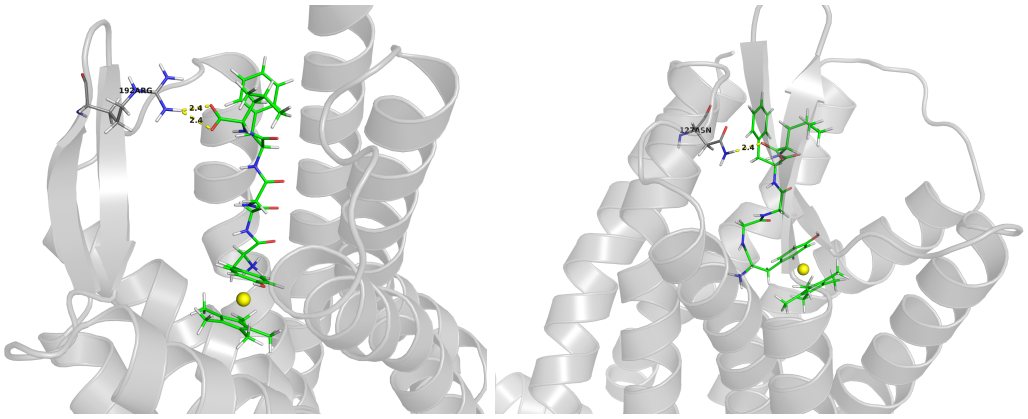

Figure 5: Hydrogen bonds found in the docking results of [η^5-Cp*Rh(III)]Leu-enkephalin and the δ-OR (left) or μ-OR (right).

Comparison of the energetically most favorable structures

The four energetically most favorable structures are compared. Whereas Leu-enkephalin shows only slight changes in orientation, structures of [η^5-Cp*Rh(III)]Leu-enkephalin are diverse with the Cp* moiety heading both out of and into the pocket.

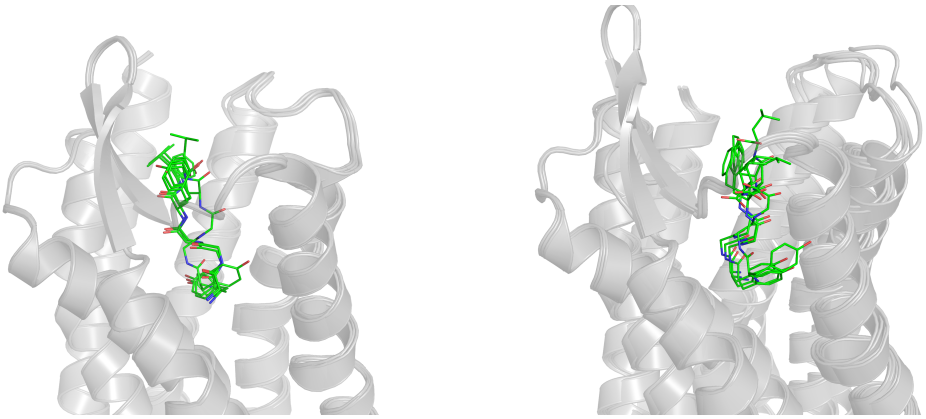

Figure 6: Energetically favorable structures of Leu-enkephalin docked to the δ-OR (left) or μ-OR (right).

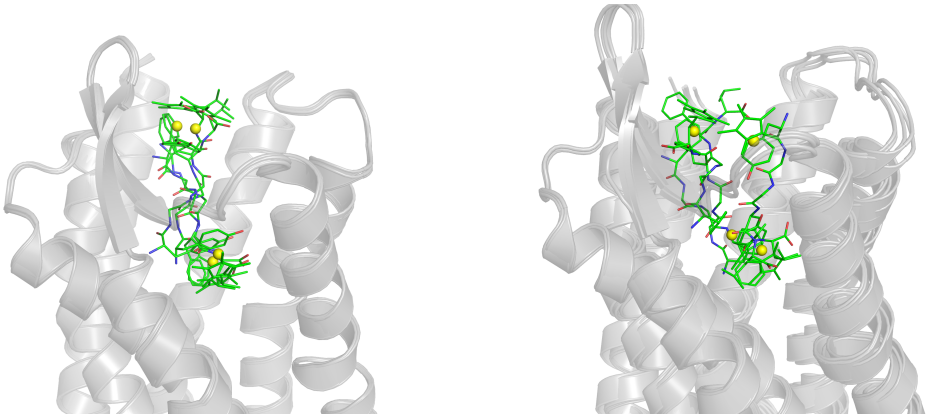

Figure 7: Energetically favorable structures of [η^5-Cp*Rh(III)]Leu-enkephalin docked to the δ-OR (left) or μ-OR (right).

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