# Conformational study of small peptides and their circular dichroism spectra

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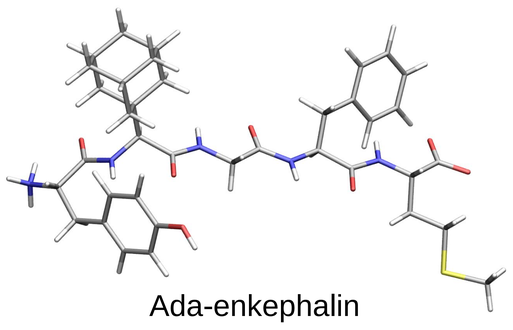
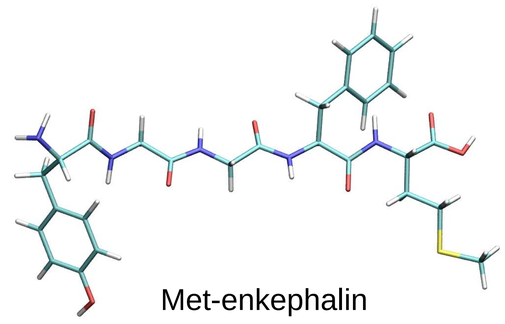
## Introduction



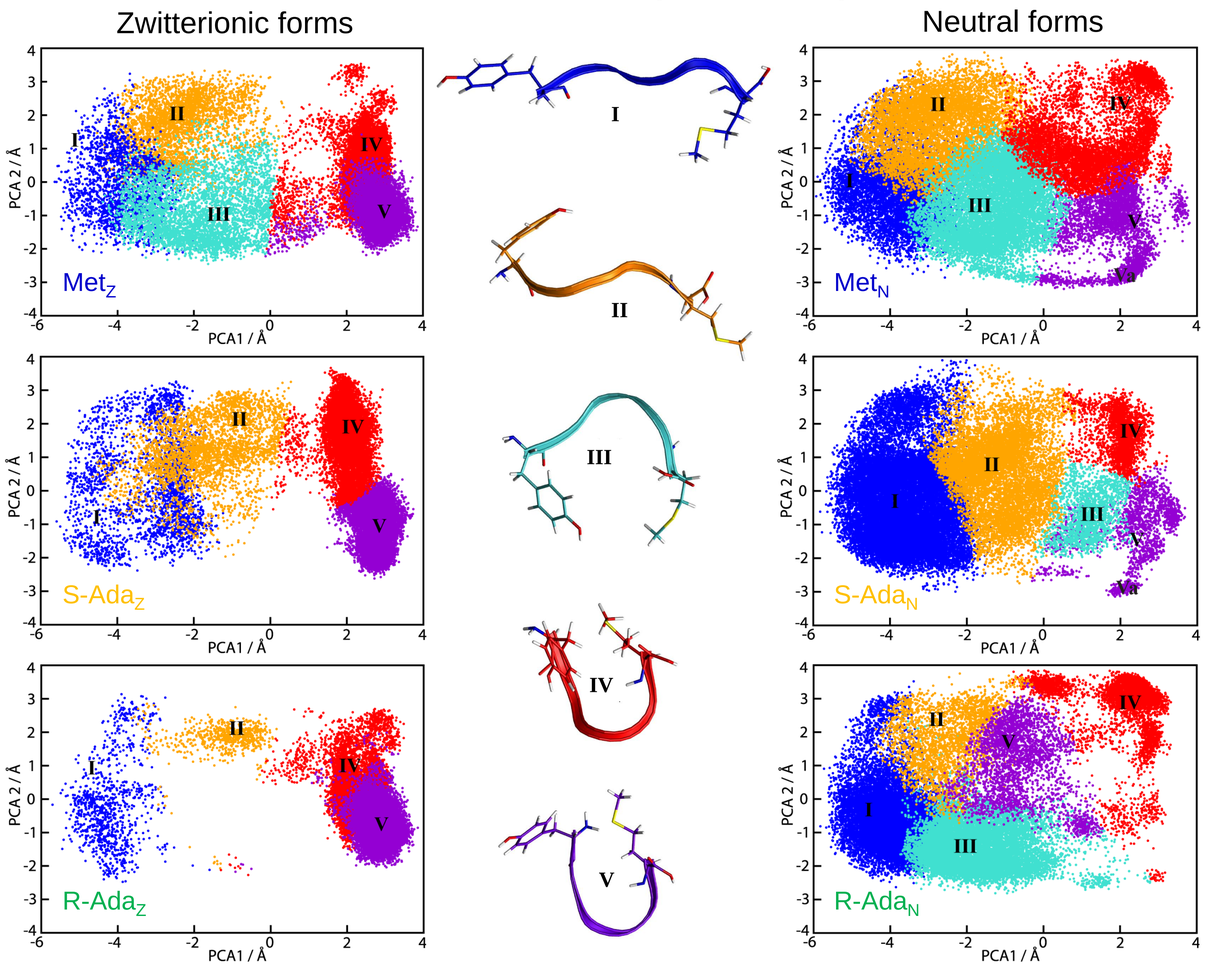
Ada-enkephalin

**Opioid peptides** are known to be associated with many physiological features such as pain mediation,opiate dependence, and euphoria [1]. **Met-enkephalin** (Tyr-Gly-Gly-Phe-Met) is a pentapeptide that belongs to the group of opioid peptides, however, unlike other opioid peptides, it acts as an **inhibitor of tumor-cells** in a receptor-mediated fashion. This intriguing effect is even more pronounced when an unnatural analogue of Met-enkephalin, **Ada-enkephalin**, which contains an adamantane group, is employed [2]. Previous studies indicate that the solution conformation of the peptides affects their biological activity [3]. Thus, to investigate the structural preferences of these peptides, we used **replica exchange molecular dynamics** [1]. The simulations were performed in explicit **trifluoroethanol**, which is a prototype for the membrane enviroment. To provide a direct link with experiment, **circular dichroism spectra** of the peptides were calculated using a **QM/MM approach** in which **the peptide was treated quantum mechanically**, while the effect of **the solvent was included classically**.

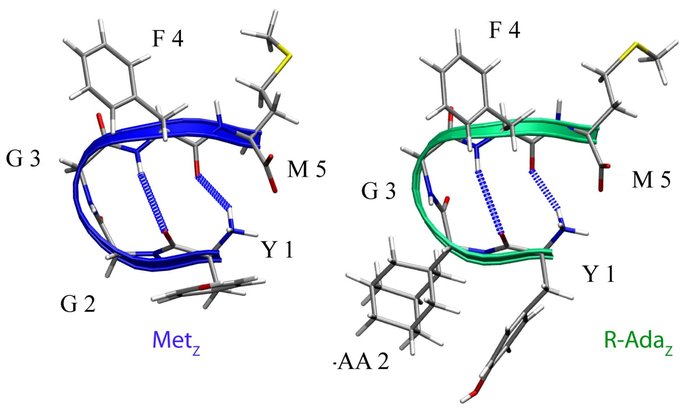


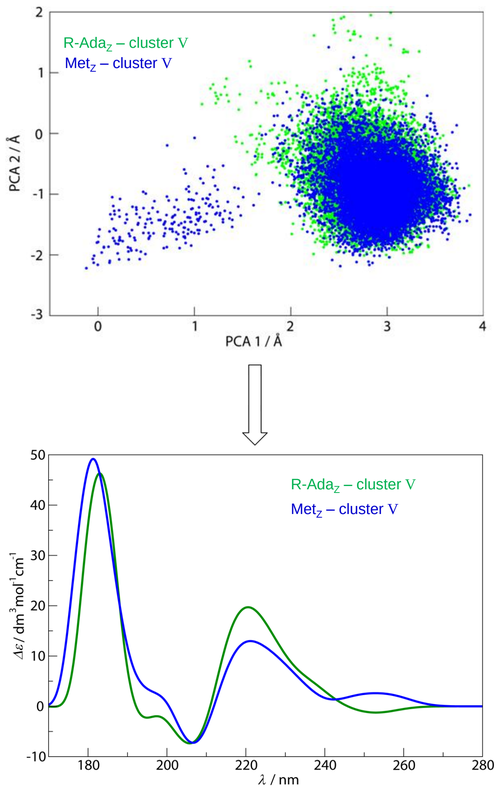
Met-enkephalin

## Conformational phase space



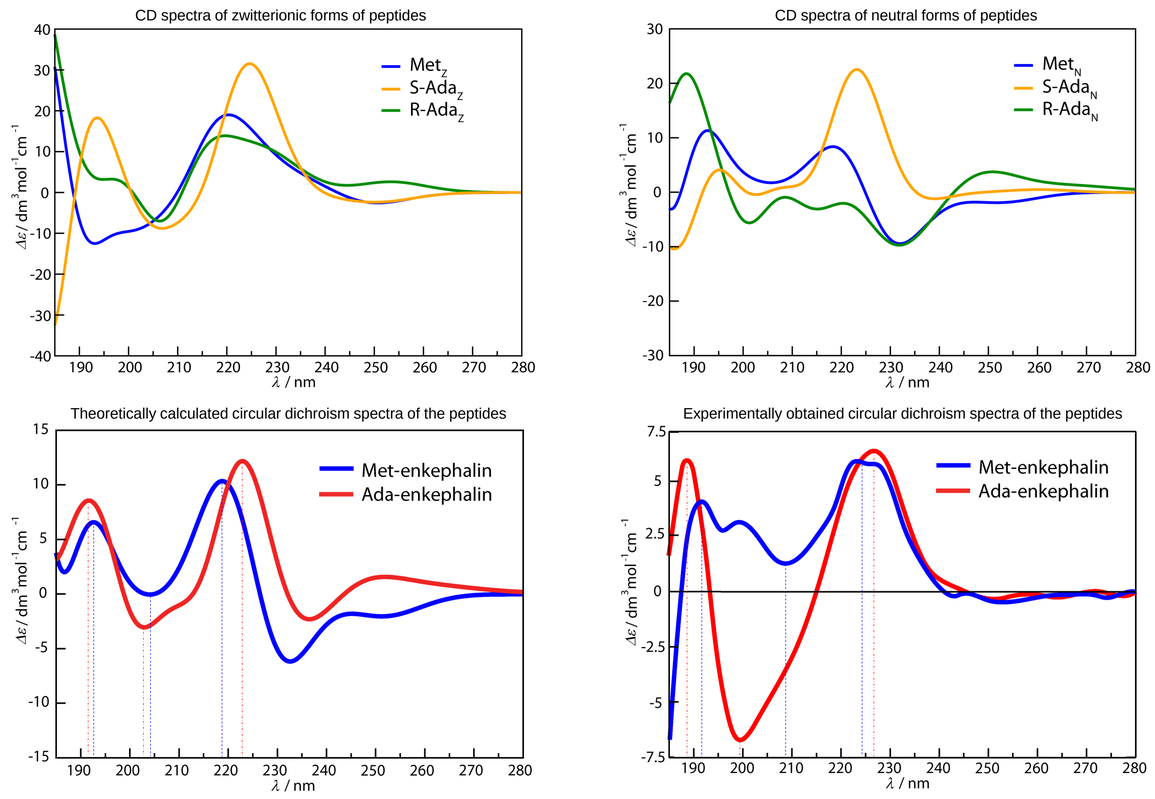

The conformational spaces of the peptides were generated by the **replica exchange molecular dynamics** (**REMD**) technique. Both *S*- and *R*-enantiomers of Ada-enkephlin were simulated because experimental CD spectra was measured using racemic mixture of two enantiomers. 16 replicas were prepared and simulated for a total of 320 ns for each peptide. The trifluoroethanol (TFE) was included explicitly. The target temperatures of the replicas ranged from 275 K to 420 K. All simulations were performed using the AMBER 10 suite of programs [4] **. Principal component analysis** (**PCA**) and **clustering** (K-mean algorithm) [4] were used in order to compare the data obtained from the REMD simulations. α-C-atoms of all peptides were used in the PCA method, thus the peptides are plotted on the common principal components.



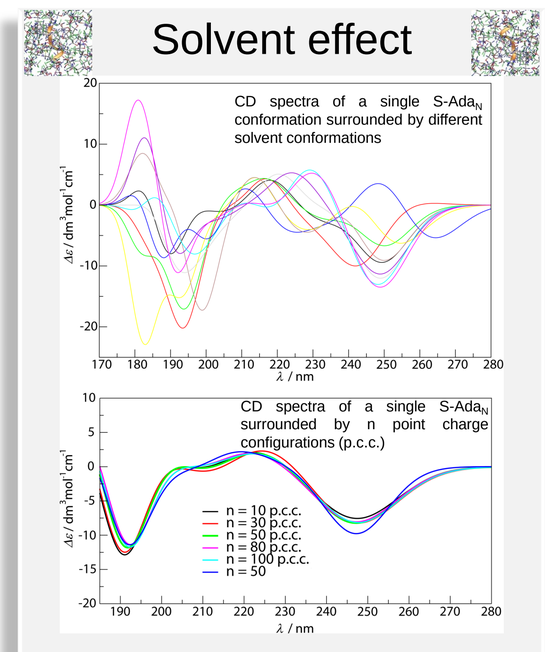

Representative conformations of clusters V of Met$_Z$ and R-Ada$_Z$ show similarities in respect with their backbone structures and their respective clusters lie in the same part of the conformational phase space. Most interestingly, the average CD spectra of these clusters are also very similar, implying that the **backbone structure of investigated peptides plays dominant role in the calculated CD spectra**.

## CD spectra calculations



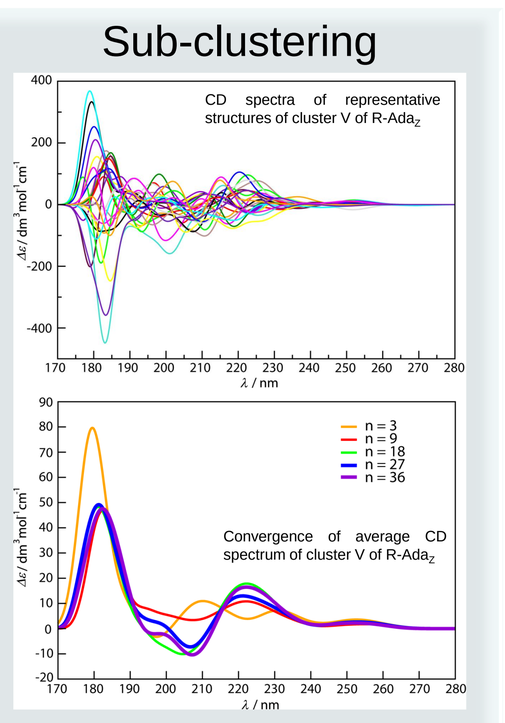

CD spectra of the peptides were calculated using a QM/MM approach, in which the peptides were treated with TD-DFT (B3LYP/6-31G(d)) and where the solvent effect was introduced via an **average point charge field**. The CD spectra of individual peptides were obtained as a weighted average of the individual spectra of **50 representative structures** that were found by **sub-clustering** 5 initial clusters. The single CD spectra were convoluted with Gaussian functions of width 0.32 eV. The final CD spectra of Met-enkephalin and Ada-enkephalin were obtained by combining neutral and zwitterionic CD spectra with the 4:1 ratio. All CD spectra calculations were performed using Gaussian software package [5].

### Solvent effect




CD spectra of a particular peptide conformation greatly depends on the positions of the solvent molecules (TFE) that sorrounds it. To account for this effect in the CD calculations, we employed an average point charge field around each conformation of the peptide, which is obtained by superimposing coordinates of *n* different solvent configurations. When **n=50** p.c.c**.** the CD spectra of particular peptide conformation **converges**, as shown on the graph above.

### Sub-clustering




To obtain representative structures via which the average CD spectra of peptides are calculated, clusters of each peptide were further sub-clustered. To show that the average CD spectra of peptides converges, cluster V of R-Ada$_Z$ was sub-clusterd into n different sub-clusters. The convergence occurs when 27 conformations are used in making of the average CD spectrum.

## References

**[1]** K. Y. Sanbonmatsu, A. E. Garcia, *Proteins* **46** (2002) 225
**[2]** M. Gredičak, F. Supek, M. Kralj, Z. Majer, M. Hollosi, T. Šmuc, K. Mlinarić-Majerski, Š.Horvat, *Amino Acids* **38** (2009) 1185
**[3]** G.T. Knipp et al., *Phar. Res.*, **14** (1997) 1332
**[4]** D. A. Case et al., (2008), AMBER 10, University of California, San Francisco
**[5]** M. J. Frisch et al., (2009) Gaussian 09; Gaussian, Inc., Wallingford CT