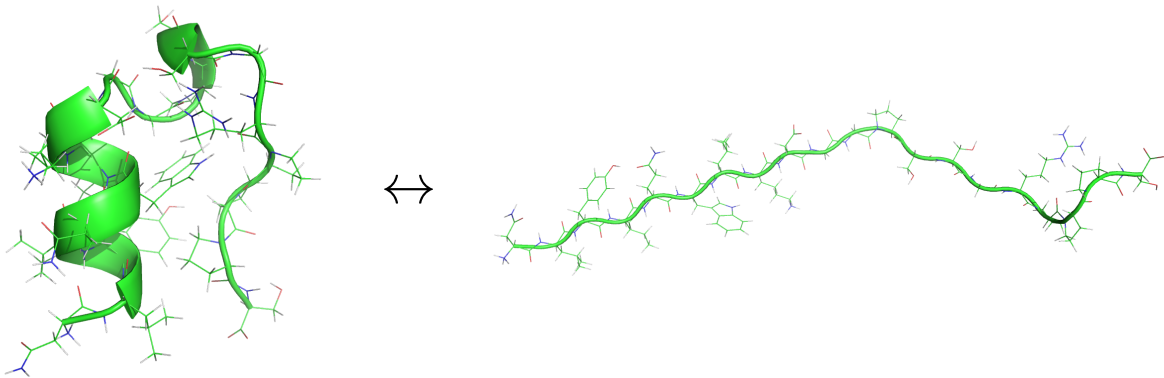# Structure and stability of Trp-cage

The objective of the TP is to study the structure and stability of a small protein, the Trp-cage.

Introduction

Trp-cage is a small artificial protein of 20 amino acids, which has been designed to fold easily. Its amino acid sequence is NLYIQWLKDGGPSSGRPPPS. The protein folding problem is one of the most important challenges of structural bioinformatics. It consists in predicting the three-dimensional structure of a protein from the sequence information alone.

We will employ the methods of molecular mechanics to model the Trp-cage.

— The dynamics of the folded protein at equilibrium will be studied first.
— Then the unfolding of the Trp-cage will be studied by simulating the denaturation of the folded protein.
— At last, the most difficult part of the TP will consist, starting from a linear unfolded conformation of the Trp-cage, to try to fold the protein by calculation without any experimental information.

Protocol

A   Dynamics at equilibrium of the folded Trp-cage

1. Inspect the files available :

folded.pdb	experimental (NMR) structure of the folded Trp-cage
unfolded.pdb	linear unfolded structure of the Trp-cage
amber.rtf	topology file for XPLOR
amber.prm	parameter file for XPLOR
build.inp	model building and energy minimization
md.inp	molecular dynamics at 300K
traj2mpdb.inp	conversion of the trajectory to the multiple PDB format
analyze.inp	analysis of the trajectory
run.sh	script to drive the calculations

2. Model building

   `xplor < build.inp > build.out`

   This script builds a model of the Trp-cage with XPLOR and performs an energy minimization to improve the geometry.

   Inspect the output file and visualize the structures produced.

3. Molecular dynamics

   `xplor < md.inp > md.out`

   This script performs a molecular dynamics of the Trp-cage during 20ps, assigning random initial velocities and then maintaining the temperature at 300K.

   Inspect the output file and track the energy and temperature as a function of time.

4. Visualization of the trajectory

   `xplor < traj2mpdb.inp > traj2mpdb.out`

   This script converts the format of the trajectory produced from DCD to multiple PDB.

   We can then visualize the trajectory with PyMOL by loading it as follows :

   `load md.multi.pdb, multiplex=0`

5. Analysis of the trajectory

   `xplor < analyze.inp > analyze.out`

   This script reads the produced trajectory (**md.dcd**) and performs structural or energetic calculations at each step. The results are written in a text file (**md.dat**). Represent them graphically.

   The analyses included in the script are only examples, it is your task to add more relevant ones with the help of the XPLOR documentation.

   — Which descriptors of the structure, dynamics and stability of the Trp-cage have you studied ?
   — Is the simulation time sufficient to obtain converged results ? Extend the simulation if necessary.
   — Does the Trp-cage simulation show a stable structure ?
   — Indicate possible deformations with respect to the experimental structure.

B   Unfolding the Trp-cage

— Design a protocol to denature the Trp-cage by molecular dynamics and implement it by adapting the XPLOR scripts. Describe the procedure followed.
— Can we define a limit between the folded and unfolded states ?
— Can we observe different stages in the unfolding ?

C   Folding the Trp-cage

— Adapt the XPLOR scripts and repeat the first steps of A) to build a model of the unfolded Trp-cage (replace "folded" by "unfolded" in scripts) and simulate it by molecular dynamics.
— Develop a protocol to fold the Trp-cage. Do not hesitate to draw inspiration from the literature. Describe the strategy followed.
— To what extent have you succeeded in folding the protein ?
— Can we observe intermediate states during folding ?
— What improvements could be made to the model ?