A computational method to estimate the stability of protein mutants

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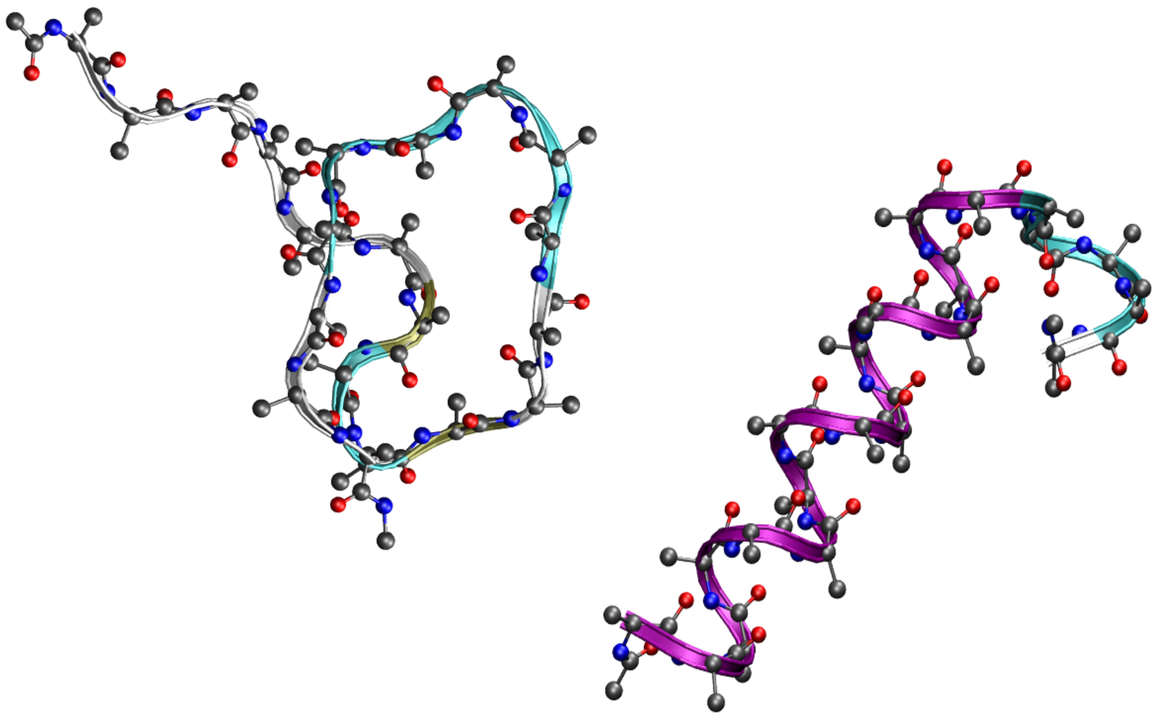
ABSTRACT

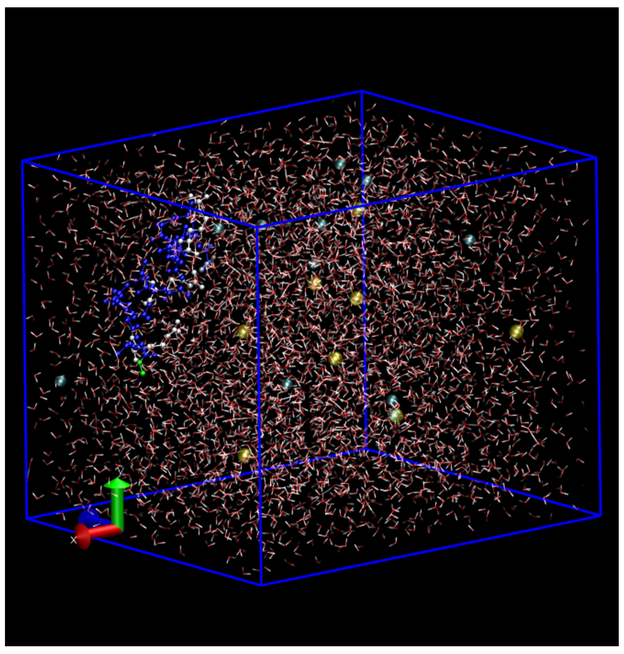
The study of statistical mechanics of polymers such as proteins and nucleic acids provides indeed valuable information about these molecules, for example estimating the variation of stability associated to single point mutations. In order calculate the free energy variation in protein mutants it is necessary to get an atomistic description of the protein and to properly sample the phase-space. To this purpose, starting from **molecular dynamics (MD)** and **Monte Carlo (MC)** method, many algorithms have been invented to explore the phase space in a guided way. Metadynamics samples a **collective variable (CV)** that represents a feature of the system, such as the distance between two atoms, the number of H-bonds in a molecule or the number of residues in a given secondary motif. The method described here involves the application of several computational strategies: **MC** [1][2], **metadynamics** [3][4] and **principle of maximum constrained entropy (MCE)[**1]. The complementary use of these three methods allows us to build a system, to sample the phase space and finally to calculate free energy variations upon introducing a certain information (knowledge) into meta-statistics. These computational methods have been applied to two systems: a peptide chain (a short protein fragment) composed by 21 alanine residues (Ala_{21} peptide) and the same chain in which 3 residues have been mutated and replaced with 3 arginine residues (*folded short* peptide). All the simulations were performed in explicit water and ions, representing a physiological solution. The **Marconi HPC** architecture at CINECA was used.

COMPUTATIONAL METHOD

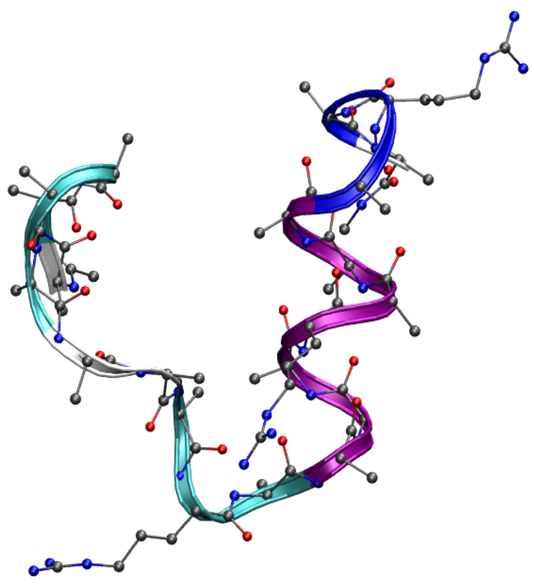
1. **Metropolis MC** to generate several replicas of the same system that represent different initial condition;
2. **Altruistic metadinamics** to explore the **CV (number of residues in alpha-helix)** relevant phase-space and collect a statistical sample;



Low ($CV = 0$) and high ($CV = 19$) helicity in Ala_{21} peptide.

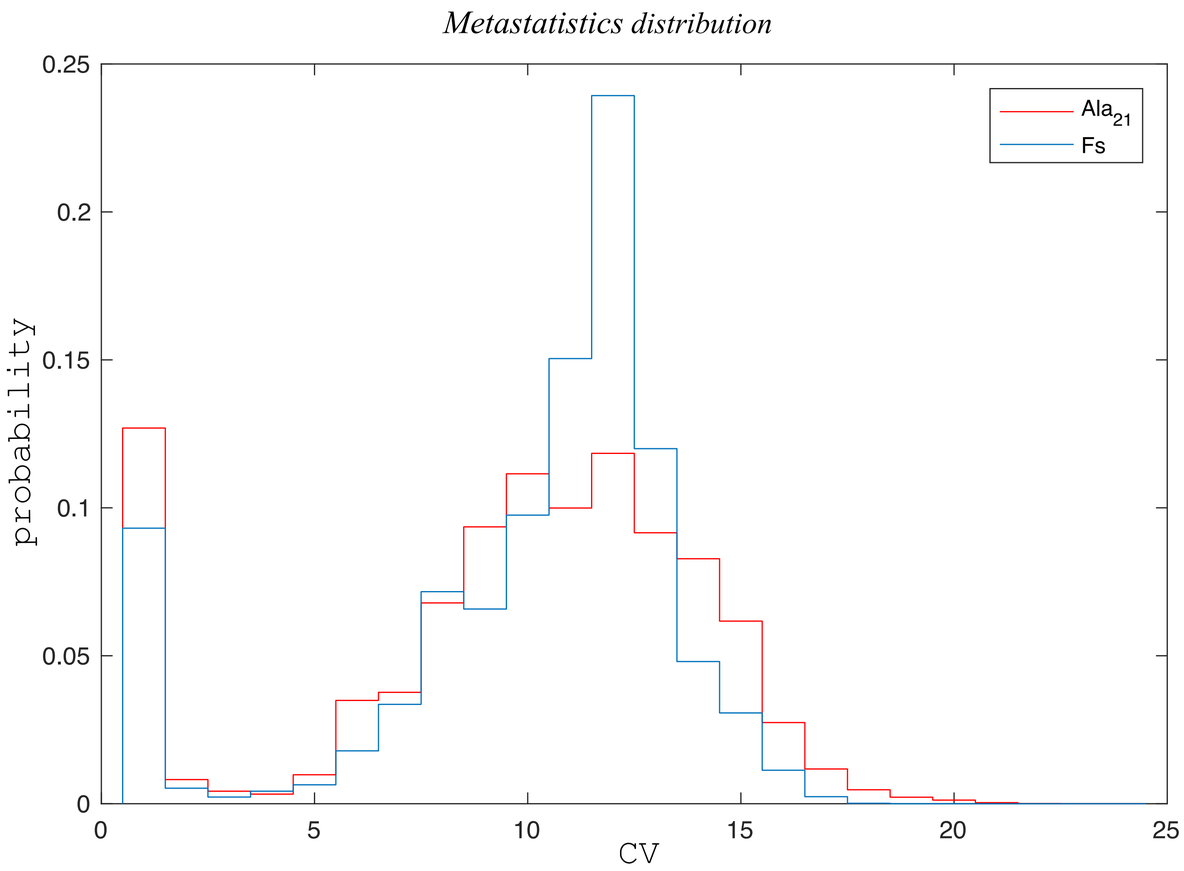


Fs peptide inside the simulation box with water and ions.



Configuration with CV=11 in Fs.

3. **MEC** to correct metastatistical distribution of **CV** and calculate the free energy variations associated to mutations.



RESULTS and CONCLUSIONS

$$\Delta A = \langle U(\lambda_h)\rangle - \langle U(\lambda_l)\rangle - [T_h S(U(\lambda_h)) - T_l S(U(\lambda_l))] = \Delta A_U + \Delta A_S$$

$T_h = 298\ K, h(s = 16) \rightarrow high\ alpha\text{-}helicity$
$T_l = 400\ K, l(s = 0{,}1) \rightarrow$ low *alpha–helicity*

$\Delta A_U \left(^{kJ}/_{mol}\right)$	$\Delta A_S \left(^{kJ}/_{mol}\right)$
-20934	$5 \div 6$
-20896	$1 \div 1.3$

Ala_{21}

$\Delta A_U \left(^{kJ}/_{mol}\right)$	$\Delta A_S \left(^{kJ}/_{mol}\right)$
-22991	$10 \div 13$
-23096	$5 \div 7$

Fs

For the two systems at hand, the signs of the calculated free energy variations indicate that the **alpha-helix structure is the most stable in both the wild-type and the mutant peptides**. Furthermore, the mutant peptide shows a higher tendency to form alpha-helix content, consistently with experiments.

Generally this method allow us to:

- **parallel computing with large number of processors (low frequency of data exchange);**
- **dynamical configuration exchanges to allow modeling explicit solvent;**
- **introduction of known information that significantly characterizes the system;**
- **study the phases transistion and calculate the configurational means;**
- **application range: medium/large systems (~ 50000 atoms) such as complex biomolecules.**

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