Modeling of FRET-experiments on protein folding

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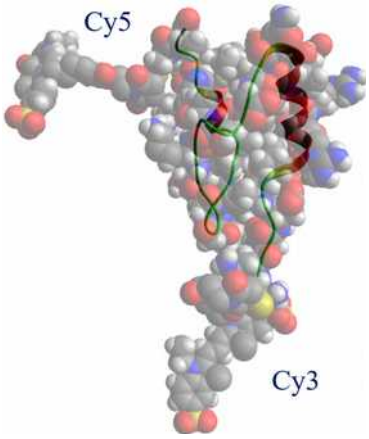
A key question in the application of the Förster resonance energy transfer (FRET) to study protein folding is how the dyes can affect the process of folding. Understanding of these effects is particularly important for small proteins, for which the dyes can be comparable in size with the protein. Moreover, the FRET-experiments require a support from simulations in order to interpret the results of the measurements.



In this work, we modeled FRET-experiments on folding of BBL domain, the protein that consists of 45 amino acid residues [1,2]. The FRET protein construct is a system that is presented by the protein and two dyes Cy3- and Cy-5 maleimides at the protein termini, as it is in the experiment and as shown in figure. This study was conduct by the molecular dynamics simulation (MD) – the protein and dyes were described at atomic level of resolution, an explicit solvent was used, and a brute-force modeling of folding trajectories from an extended state of the protein was performed. The protein and 25000 water molecules (TIP3P) modeling the solvent were placed in a cube with a 95 A edge and periodic boundary conditions. The simulations were performed by the MD method in the framework of the CHARMM program [3].

It was found that at T = 280 K, T = 300 K and T = 350 K, the native states of the protein and the FRET-construct are stable. Fifteen MD folding trajectories for both the protein and FRET-construct were generated and for each temperature. It has been found that the presence of dyes does not change the overall picture of folding except that the FRET-construct folds considerably slower than the original protein. It also means that the folding kinetics of the FRET-construct remain to be two-state single-exponential kinetics which are observed for the original protein. The FRET-efficiency histograms constructed on the basis of simulated MD-trajectories have a tendency to converge and are found in agreement the experimental histograms. In addition, the influence of dyes on the structure of solvent molecules near the protein, as well as on the corresponding charge distribution, was studied.

[1] F. Huang et al., *Proc. Natl. Acad. Sci.* **2009**, *106* pp. 16239-16244.

[2] J. Liu et al., *Proc. Natl. Acad. Sci.* **2012**, *109* pp. 179-184.

[3] B.R. Brooks et al., *J. Comput. Chem.* **2009**, *30* pp. 1545-1614.