FRET study of M13 major coat protein: simulation-based fitting approach

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The geometry determination of membrane proteins is still at the frontier of the structural biology. The complexity and delicacy of membrane-protein systems substantially impede the application of standard methods of protein study, such as X-ray crystallography and NMR [1]. As a successful alternative to named techniques, the Förster resonance energy transfer (FRET) spectroscopy was proposed [1, 2]. However, the complexity of protein-lipid systems hampers and limits the analytical interpretation of FRET data. On the other hand, the simulation modeling of photophysical processes in the experimental system during a fluorescence measurement was proved to be a powerful alternative to analytical modeling, not restricted to special conditions [3].

The goal of the current work is developing and testing the methodology for fluorescent data analysis and simultaneous determination of membrane protein geometry and protein-protein aggregation by the means of steady-state FRET experiments. To perform this goal the model of the energy transfer in a protein lipid system was built. It includes the simplified structural model of a protein-lipid system and steady-state FRET simulation. The developed model is applied to experimental data analysis via the simulation-based fitting approach, which intend approximation of the experimental FRET data by their simulated analogues.

The developed methodology was tested on a well-known bacteriophage M13 major coat protein [4]. Different Cys mutants of this protein were produced and specifically labeled with AEDANS. By observing the energy transfer from natural Trp26 to AEDANS and application the simulation-based fitting approach the structural parameters of the protein were determined and the protein-protein aggregation characterized. The found values of structural parameters are in a good accordance with previously reported ones, obtained from ESR experiments and fluorescence polarity probing [4]. The main advantage of the proposed technique is a simultaneous determination of parameters describing protein state in a bilayer by only one series of experiment.

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