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BIOMOLECULAR SCIENCES

FRET study of membrane proteins: determination of the tilt and orientation of the N-terminal domain of M13 major coat protein

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Introduction

Computer simulation and simulation-based fitting are helpful and powerful methods to study complex systems. We apply such techniques to the analysis of Förster resonance energy transfer (FRET) in membrane protein systems.

Bacteriophage M13 major coat protein in DOPC/DOPG vesicles was selected as a model system. This transmembrane protein consists of 50 amino acid residues. The protein was specifically labelled with AEDANS.

Goal of the study is to retrieve from one series of fluorescence experiments information about the protein structure, membrane embedment and protein-protein association.

Models

In our model a membrane protein is represented by a two-domain helical structure connected by a flexible link. The fluorescent labels are attached to specific amino acid positions (Fig. 1). A membrane is considered as a two-dimensional structure with a random packing of molecules (both proteins and lipids).

Modelling of FRET is performed by a semi-analytical approach. For each donor j its efficiency of energy transfer E_j is calculated. The total average of E_j gives the energy transfer efficiency E for the entire system (Eq. 1). In the equation r_i are the distances to all acceptors.

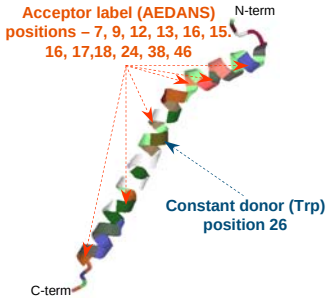

Fig. 1. One of the possible structures of M13 major coat protein with fluorescent labels

$$E = \langle E_j \rangle = \left\langle \frac{\sum_i (R_0/r_{ji})^6}{1+\sum_i (R_0/r_{ji})^6} \right\rangle \quad (1)$$

Experimental method

Experimentally energy transfer efficiencies are obtained from excitation spectra of the acceptor. Emission was detected at 490 nm in scans from 260 to 400 nm. The equation for the experimental efficiency E is given in Eq. 2.

In Eq. 2: F^{290}, F^{340} – emission under excitation at 290 and 340 nm; ε_A, ε_D – molar extinction coefficients of AEDANS and Trp at corresponding excitation wavelengths.

$$E = \frac{1}{1+r_{ul}} \left(\frac{F^{290}}{F^{340}} - \frac{\varepsilon_A^{290}}{\varepsilon_A^{340}} \right) \frac{\varepsilon_A^{340}}{\varepsilon_D^{290}} \quad (2)$$

Results

Experimental data

The experiments were performed by titration of the sample with proteins containing only donor label and calculation of the energy transfer intensity. In Fig. 2 the points correspond to experimental data, the line to interpolated values. The FRET data were obtained for the acceptor label at positions 7, 9, 12, 13, 15, 16, 17, 18, 24, 38 and 46.

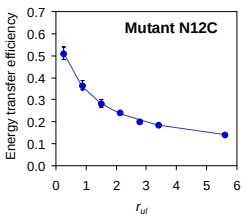

Fig. 2. Results of FRET in titration experiment

Analysis via simulation-based fitting

The experimental data were fitted by simulation modelling (Fig. 3). For this, a global analysis approach was applied – all experimental data points, obtained for different acceptor label positions, were fitted simultaneously. Fitting was performed by means of the Nelder-Mead simplex method.

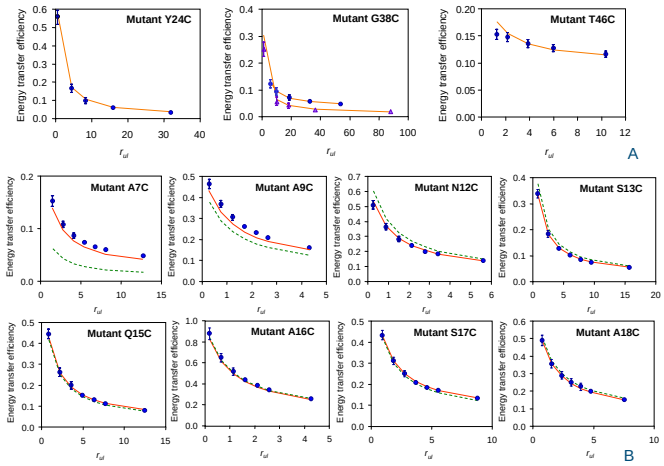

Fig. 3. Result of simulation-based fittings for the transmembrane part (orange line in A) and the N-terminal domain (red and green lines in B). Blue and violet marks present the experimental data. In plots (B) green line shows the results of fitting without an unstructured domain; the red lines are for the case of an unstructured domain presence at positions 1-9

In the analysis we aimed first at the transmembrane domain (positions 24, 28, 46) to determine the protein position in the bilayer. Then the structure of the N-terminal domain was taken into account (positions 7-18). The impossibility of fitting the FRET data (Fig. 3B, green) was solved by considering an unstructured region for positions 1-9. The results of this improvement of the model are given in Fig. 3B (red).

Resulting protein configuration

The final protein structure and membrane embedment are shown in Fig. 4.

Embedment:

- Protein-protein association ~ **3%**
- Protein tilt angle = **18 ± 2°**
- Protein tilted towards position **29**
- Trp(26) position = **8.5 ± 0.5 Å**

Structure:

- Protein is unstructured at positions **<10**
- Protein upper helix tilt = **5 ± 4°**
- Protein bent at position **20 ± 2**
- Mismatch with continuous α-helix ~ **40°**

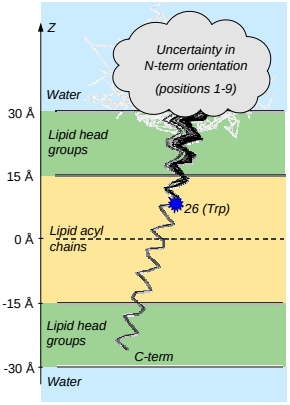

Fig. 4. Resulting protein configuration

Conclusion

FRET in combination with simulation-based fitting can be used for simultaneous determination of structure, membrane embedment and associations of membrane proteins[1,2]. The method can be easily used for any 1-2 helical membrane protein [3]

Literature

1. Nazarov P.V., et al. (2006) . FRET study of membrane proteins: simulation-based fitting for analysis of membrane protein embedment and association. *Biophysical Journal*, 91, p. 454-466.
2. Nazarov P.V., et al. (2007) . FRET study of membrane proteins: determination of the tilt and orientation of the N-terminal domain of M13 major coat protein. *Biophysical Journal*, 92:4, in press.
3. Sparr E., et al. (2005) Self-association of transmembrane a-helices in model membranes: importance of helix orientation and role of hydrophobic mismatch. *Journal of Biological Chemistry*, 280:47, p. 39324-39331

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