WAGENINGEN UNIVERSITY
BIOMOLECULAR SCIENCES

FRET study of membrane proteins: simulation based fitting for analysis of protein structure, embedment and association

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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.

The 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.

Models

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

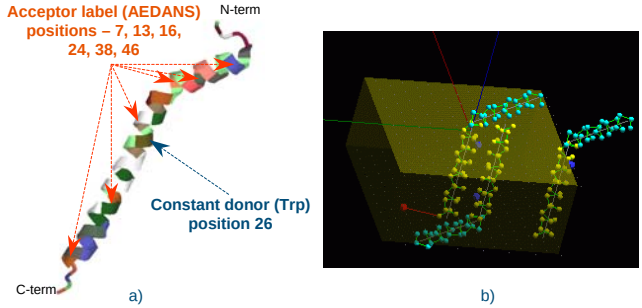

Fig. 1. One of the possible structures of M13 major coat protein with fluorescent labels (a) and the spatial model the membrane with proteins incorporated (b)

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 (*). In the equation r_i are the distances to all acceptors.

Main input parameters

- Lipid/protein ratio, r_{LP}
- Location of acceptor probe
- Labelled/unlabelled protein ratio, r_{ul}
- Förster distance, R_0
- Experimental lipid loss
- Protein structure (upper helix tilt angle and direction)
- Protein embedment (protein tilt angle, depth in a membrane)
- Coefficient of protein-protein association

Simulated output values

- Energy transfer efficiency

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

Goal

To get simultaneously in one series of fluorescence experiments the information about the protein structure, membrane embedment and protein-protein association.

Experimental method

Experimentally energy transfer efficiencies can be obtained from excitation spectra of acceptor. The emission was detected at 470 nm while excitation scans from 260 to 400 nm. The equation for experimental E is given below.

In (**) 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.

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

Results

Experimental data

The experiments were performed by titration the sample with proteins containing only donor label and calculation of the energy transfer intensity. In fig. 2 the black points correspond to experimental data, the red line to simulated values. The FRET data were obtained for the the acceptor label (AEDANS) at positions 7, 13, 16, 24, 38 and 46.

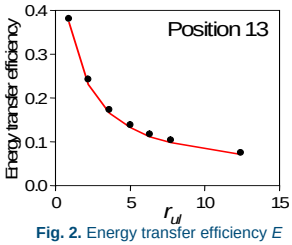

Fig. 2. Energy transfer efficiency *E* in a titration experiment for pos. 13

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. The fitting procedure was performed by means of Nelder-Mead simplex method.

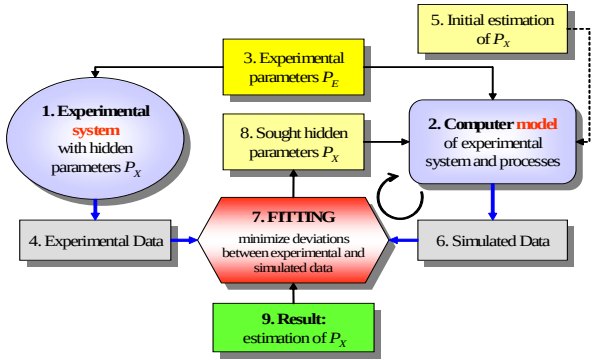

Fig. 3. General scheme for the simulation-based fitting

Resulting protein configuration

The final protein structure and membrane embedment obtained via fitting is shown in fig. 4.

- Protein upper helix tilt = **56 ± 4°**
- Protein tilt angle **= 16 ± 4°**
- Protein bent at position **21 ± 1**
- Trp(26) depth = **8.2 ± 0.5 Å**
- Protein-protein association ~ **7%**
- Experimental lipid loss ~ **28 %**

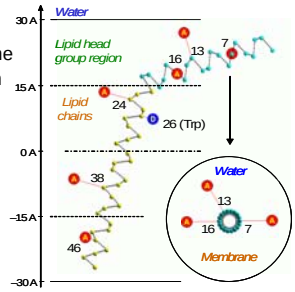

Fig. 4. Resulting protein configuration

Conclusion

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

Literature

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