Conference

Biological Dynamics; *from molecules to cells*

7 – 9 November 2005
Amsterdam, The Netherlands

Committee for Biochemistry and Biophysics
Royal Netherlands Academy of Arts and Sciences

ALW

Van ‘t Hoff-Fonds, KNAW

FRET Study of Membrane Proteins: Simulation-Based Fitting for Analysis of Protein Structure, Embedment and Association

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A new formalism for the simultaneous determination of the structure, membrane embedment, and aggregation of membrane proteins was developed. This method is based on steady-state Fluorescence resonance energy transfer (FRET) experiments on site-directed labelled proteins in combination with global data analysis utilizing simulation-based fitting (SBF). The simulation of FRET was validated by a comparison with a known analytical solution for energy transfer in idealized membrane systems. The applicability of the SBF approach was verified on simulated FRET data and then applied to determine the structural properties of the well-known major coat protein from bacteriophage M13 reconstituted into unilamellar DOPC:DOPG (4:1 mol/mol) vesicles. For our purpose, the cysteine mutants A7C, S13C, A16C, Y24C, G38C, and T46C of this protein were produced and specifically labeled with fluorescence probe AEDANS. The energy transfer data from the natural Trp-26 to AEDANS were analyzed assuming a two-helix protein model. As a result of the FRET data analysis the low-resolution structure of the protein, and its bilayer embedment were quantitatively characterized. The resulting structure of the protein is a stretched L-shape with the angle between the transmembrane and N-terminal helix axes about 56°. Trp-26 is located at a distance of 8.2 ± 0.5 Å from the membrane centre, and the tilt angle of the entire protein is 16 ± 4°. No specific aggregation of the protein was found. The methodology developed here is not limited to the M13 major coat protein and can be used in principle to study the bilayer embedment and structure of any protein for which a one or two-helix model can be applied.