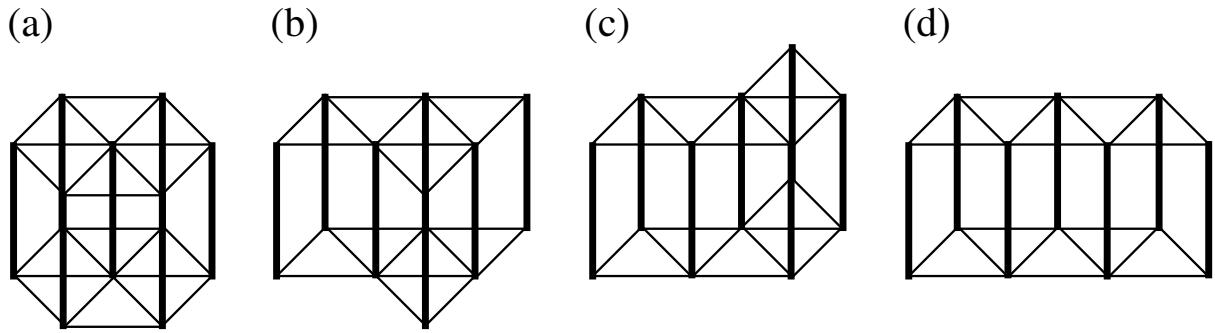

Figure 1: Four kinds of helix configurations in triangular lattice, having twelve pairs of the nearest neighbor (a) and eleven nearest pairs (b), (c) and (d). The first step of modeling is to correlate seven partial sequences of transmembrane helices to the lattice points of these configurations and to calculate energetically favorable arrangements.

$E_{bind}(\Theta_{ij}, \Theta_{ji})$, shows a normalized angular distribution of interaction energy which is a single function of the polar interaction energy. The angle Θ_{ij} represents the orientation of i-th helix with respect to the j-th helix. The factor A is a normalization factor with tension of energy.

L_{ij} is the length of loop segments between i-th and the next helix, and C is the elasticity constant. Each helix was rotated by 30 degrees and the interactions energy of 12^7 structures was calculated for each arrangements. The best structure was quite similar to the experimentally determined native one of bacteriorhodopsin.

Analysis of tilt angles of transmembrane helix: The final step of the determination of helix configuration is tilting with respect to the membrane normal. We have analyzed this problem by giving different arrangements of helices to the upper and the lower halves. When four helices, A, E, F and G, of bacteriorhodopsin were tilted, assuming one of native-like structures in triangular lattice, the most stable structure had same tilt as the native structure.

In conclusion, the continuum theory of the structure prediction was employed and the positioning, the orientation and the tilt angle were predicted without any assumption of structure template.

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Theoretical Prediction of Positioning, Orientation and Tilt Angles of Helices of a Small Membrane Protein Without Structural Template.

Takatsugu Hirokawa hirokawa@cc.tuat.ac.jp
Makiko Suwa suwa@cc.tuat.ac.jp
Shigeki Mitaku mitaku@cc.tuat.ac.jp

Department of Biotechnology, Tokyo University of Agriculture and Technology
Nakacho 2-24-16, Koganei, Tokyo 184, Japan

Three kinds of analysis are necessary for the theoretical prediction of membrane protein structure: (1) determination of transmembrane helical regions, (2) prediction of their configuration, and (3) energy minimization of fine structure including the docking of substrate. The present work deals with the second problem, that is the prediction of the positioning, the orientation and the tilt angle of transmembrane helices without any assumption of structural template.

The assumptions for the prediction are the followings: First, polar interactions, electrostatic interaction and hydrogen bond are a dominant factor in the process of positioning and orientation of transmembrane helices[1]. Second, true positioning and orientation of helices are determined into the stage in which all members of helices bundle are perpendicular to the membrane plane. Third, the tilt angles of helices are determined by the different distribution of polar arcs of upper and lower halves of helices.

Energy calculation of polar interaction of transmembrane helices: Once amino acid sequence of transmembrane segments are given, the are folded into a-helices in a graphic workstation. Then, 48 indices, that correspond to 24 angles for upper and lower halves, are calculated for the characterization of polar interaction of each helix by probe helix method[2] using software, QUANTA and CHARMm, on a workstation, IRIS 4D/35.

Conformational search of lateral positioning on some lattice fields: The first step for modelling is the search of preferable positioning in triangular unit. Because it is apparent that the structures with smaller numbers of pairs of the nearest neighbor have to be energetically unfavorable (see Fig.1.). Therefore, we have selected four types of configurations of seven helices for bacteriorhodopsin. For each configuration, all arrangements of 5040(7!) structure are examined and the most favorable 20 selected for further analysis of helix orientation. When the best 20 arrangements for each configuration of bacteriorhodopsin was examined, we found several native-like structures.

Energy calculation of rotational positioning to stable arrangements: To determine a rotational positioning of each helix, the following energy function was used.

$$E = \sum_{n=1}^{N} A \left(\frac{r^0}{r_{ij}} \right)^2 E_{bind}(\Theta_{ij}, \Theta_{ji}) + \sum_{n=1}^{N} \left(\frac{C}{L_{ij}} \right) r_{ij}^2 \qquad (1)$$

The first term, sum of E_{bind}, indicates binding energy of between each helices based on polar interaction. The second term represent geometrical restrictions of rubber elasticity due to loop segments between helices. Distance of between i-th helix and j-th helix is denoted by r_{ij}. The function,

広川貴次, 諏訪牧子, 美宅成樹 : 東京農工大学工学部生命工学科, 〒 184 東京都小金井市中町 2-24-16