Paper

Analysis for Cross-Section of Squid Rhodopsin

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Synopsis: We consider the pore of rhodopsin that transport protons through the inner/outer of the cell lipid bilayer depending on the change the pH of water solutions. The movement of hydrogen atoms forms chemical bonds with different amino acids. Thus the hydrogen atoms can move through the rhodopsin. Here we look at the amino acids that form the cross-section through the rhodopsin helices. We consider the pores of rhodopsin are possible through the cross-section where enough difference can be found in the amino acids' distribution. In this paper we show the cross-sections we made and the amino acid distribution as they appeared in the cross-section. The number of selected amino acids depends on how many amino acids we picked up to form the aimed cross-section. We summarize these data on the pores of rhodopsin and discuss our summary.

1. Introduction

Squid rhodopsin is a kind of channel for the proton transfer as everyone knows. The crystallization data of squid rhodopsin assessed by X-ray diffraction have been used to visualize the pore of rhodopsin. We can easily find the position of every atom in the PDB data for squid rhodopsin. We have studied various different viewpoints of rhodopsin as can be read from our previous work [1–7]. Here we use the software RasWin to explore the PDB data for squid rhodopsin. RasWin is the name of the windows version of RasMol. Thus, we considered that rhodopsin should have a pore to transfer proton so that specific cut section visualize the pore as a hole. Using ribbon viewing of PDB data, PDB identification 2Z73 provides helix area. We concentrated on the chain A of 2Z73 to analyze the pore hole of rhodopsin. The actual residue number and component amino acids can be known from the RanMol command line when we selected the ribbon sites. We made cut-sections to search for pores formed by known residues. In this paper we made two sections in the chain A of crystallized data 2Z73 of PDB identification.

General features of rhodopsin shown the utility of squid rhodopsin as a study object. Our studies characterize many features of squid rhodopsin as alluded to in the Introduction. The rhodopsin exists on the Disc membrane of rod cells [8, 9, 10]. Photoreception is a familiar material for people so ''Encyclopedia'' have defined this word [9, 10]. The function of rhodopsin seems to be under pH control, in the other words, the rhodopsin is a kind of channel to move hydrogen atoms [8, 9, 10]. As for studies concerned with squid rhodopsin we found many papers [11–18]. The absorption of light against wavelength of light have been studied [11, 13]. The purification has been carried out [12]. The dynamics internal water have been considered [16]. Recently, the crystal structure have been studied and have provided the crystallography data for PDB of squid rhodopsin [14, 15, 17, 18]. Thus we can analyze cross-sections and discover the pore from the distances between

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amino acids. We only analyze the chain A of 2Z73 of PDB. We show the amino acid distribution on the cross-sections. From these distributions we can find the pore in the rhodopsin cross-sections.

In the next section (Section 2), the viewing pictures using RasWin are depicted and we make comments about how to look them. Actually rhodopsin is a monomer under pH control since we can find embedded rhodopsin in in the lipid bilayer as the rhodopsin monomer. In section 3, we looked at the cross-sections where we can see the pore of rhodopsin. We make the cross-sections at the near end of both sides of the squid rhodopsin. The production manner of the cross-sections are explained in the Section 2. Section 4 is the summary of this paper, namely how we decided on the pore in the cross-sections. We looked at amino acids on the cross-section so that pore may be the area around that amino acids exist. We should consider whether this visualization is appropriate or not. Thus we discuss this point in Section 4.

2. RanWin Picture for Chain A of PDB 2Z73

Secondary structure can be simply understood using the ribbon viewing facility of RasWin. There are many viewing methods to visualize the PDB data. Firstly we show the ''ball and stick'' representation. As seen figure 1, it is very difficult to see the helix in this method. The viewing settings are related to what things we want know. Here we want know which residues form the helix.

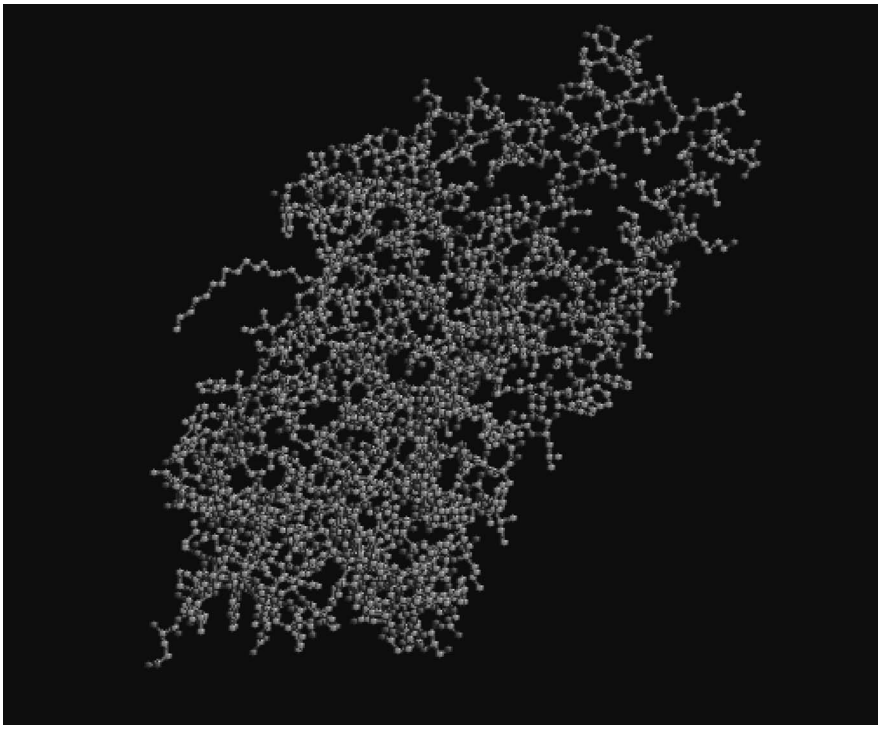

Fig. 1 Stick and ball view for 2Z73 as used for PDB identification.
Anyone easily see that it is difficult to find where helix part is in this representation. So we use ribbon view (Fig. 2) to better visualize the helix conformation.

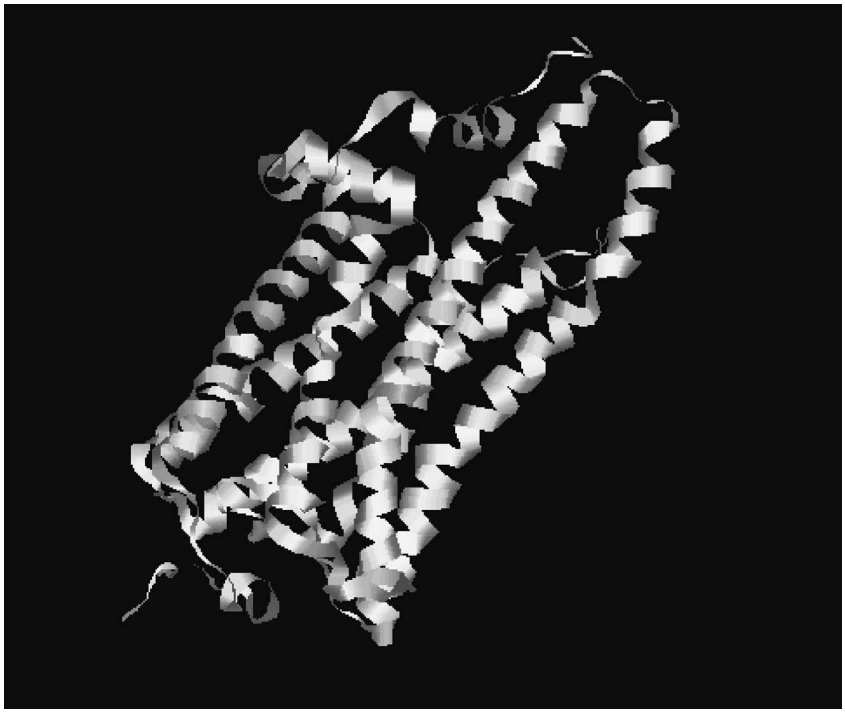

Fig. 2 Ribbon viewing for PDB id 2Z73
Helices are easily seen in ribbon feature of RasWin, while it is very difficult to recognize helices in the fulfilled picture of RasWin (Fig. 1). Therefore we used ribbon picture to form the cross-section across the helices.

The rhodopsin molecules have 7 helices in their structure. Better methods exist to look the helices using other representations. Thus we changed the viewing method of the RasWin software.

We would like to know where the helix parts are. So we used the *ribbon viewing* feature of RasWin. The result is shown at figure 2. As we can see from figure 2, there exist many helical areas including: two long helices, short helices, curved helices, and so on. We analyzed chain A of 2Z73. The rhodopsin forms ion channel for proton transport. This implies that inner area forms a channel or ion pore. We therefore make the cross-sections to examine the pore structure (namely the ion channel) of rhodopsin. We did not form a thin square, somewhat thicker cross-section were required to visualize the amino acid molecules. The appropriate thickness will be discussed below, to set thresholds at which the amino acids are well shown. The cross-sections particularly examined the helices. So we made cross sections on the data of figure 2 which are ribbons. The helix is the curled part of the ribbon representation. We made two cross-sections near both ends of rhodopsin. We should recognize the helix part of rhodopsin to make the cross-sections.

The ribbon structure does not denote the real protein. One of the authors, professor Wako, suggested to use the fulfilled representation. It is better to look the protein. The fulfilled picture means that the van der Waals size of each atom is presented so that the protein in the lipid membrane covered with atoms to organize the protein. We find the cross-section on the ribbon representation, which is not representing the van der Waals size of the atoms, means that it is difficult to find the true structure of the protein. Two cross-sections are made to look at the ribbon viewing of

Fig. 3 Full picture of 2Z73 chain A
Chain A of crystalized rhodopsin PDB data 2Z73 is used and is shown as a fulfilled picture view that is almost the real outside appearance of rhodopsin. The rhodopsin is embedded in the lipid bilayer.

rhodopsin protein (chain A of 2Z73). We find the residue groups in cross-section. The actual manner to make the cross-section is described after figure 3. The rhodopsin is embedded on the lipid bilayer so that there is no gap between rhodopsin and lipid bilayer. As seen figure 3, we could not see any gap in the rhodopsin directly. Now we know the full view of rhodopsin is an embedded protein in the lipid bilayer. Rhodopsin and lipid bilayers are independent of each other from the viewing of rhodopsin using the fulfilled view.

We find the cut-section using ribbon viewing and to see real protein structure using the fulfilled view shown in figure 3. These two figures are important in that rhodopsin protein is directly embedded in the lipid bilayer. Thus it is clear that the proton movement can performed through the rhodopsin ion channel only.

3. Cross-section of Rhodopsin

Every amino acid interacts with other amino acids. The actual amino acid size is almost fulfilled shape using van der Waals scaling. In this sense, we use the fulfilled viewing of RasWin. The fulfilled picture for chain A of 2Z73 is shown in the above (Fig. 3). The rhodopsin protein using the expression of fulfilled viewing has no space from it outward appearance, while the ribbon view exposes the helix structure. Thus we know it is better to make cross-sections of 2Z73 chain A. We made two cross-sections cutting helices in the outer side of rhodopsin protein. We checked the number of amino acid residue by using RasMol command line. Mouse click on the helix allowed us to quantify: ''Atom: what atom, sequential number of atoms, Group: amino acid name number, chain letter''. We could find the group to discover the amino acid name and number. Thus we made

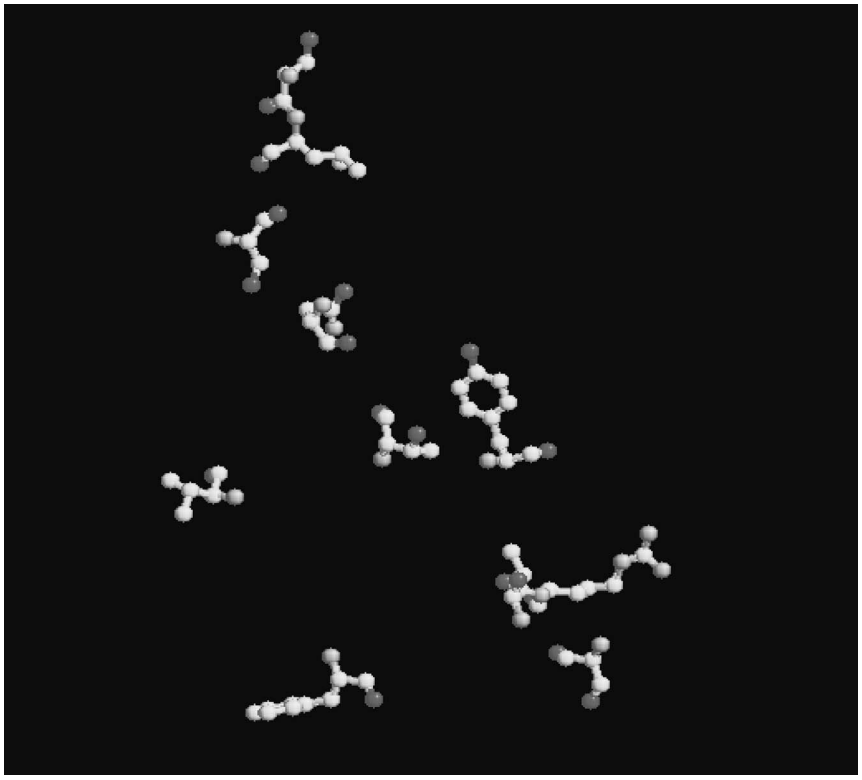

Fig. 4 Amino acids appearing in cross-section1
The amino acids Ser 36, Leu 37, Ser 95, Val 110, Phe 172, Thr178, Asn 185, Tyr 190, Ser 195, Thr 197, Arg 198 are located in the figure manner. The picture used the ball and stick representation.

a file for each of the amino acids to from each cross section.

The amino acids contributing to cross-section1 are Ser 36, Leu 37, Ser 95, Val 110, Phe 172, Thr178, Asn 185, Tyr 190, Ser 195, Thr 197, Arg 198. Note that the three letter abbreviations identify the amino acid name, and the numbers are the sequential residue number. For cross-section two, the amino acid names and residue numbers are Lys 61, Gln 66, Met 71, Phe 72, Met 128, Ileu 129, Tyr 134, Asn 135, Ala 152, Phe 153, Met 155, Met 225, Ser 228, Ala 260, Ileu 262, Ser 263, Ileu 314, Ser316, His 319, Ser327, Phe 339, Ala 325. The corresponding ball and stick representations are shown at Fig. 4 and Fig. 7. The helical turn of the amino acid sequence is about 1 nm. This value is nearly equal to the thickness of cross-section considered here. 1 nm is 10 A. The diameter of the hydrogen atom is about 100 pm ($pm = 10^{-12}\ m = 0.001$ nm). This denotes that the hydrogen diameter is about 0.1 nm. The thickness of cross-section is therefore about one 10th of the diameter of a hydrogen atom. The membrane thickness is between 2.5 nm and 3.5 nm. The 1 nm cross-section can therefore be said to be a little bit large. Also the cross-section is shorter than one-turn length of a helix. If we imagine that the thickness of cross-section is less than 0.5 nm, then the thickness of cross-section can say to be a cross-section. We show the cross-sections below using amino acids distributions. Two kinds of amino acid representation are shown. One is a ball and stick representation (Fig. 4 and Fig. 7), the other is the fulfilled one for the actual amino acids on

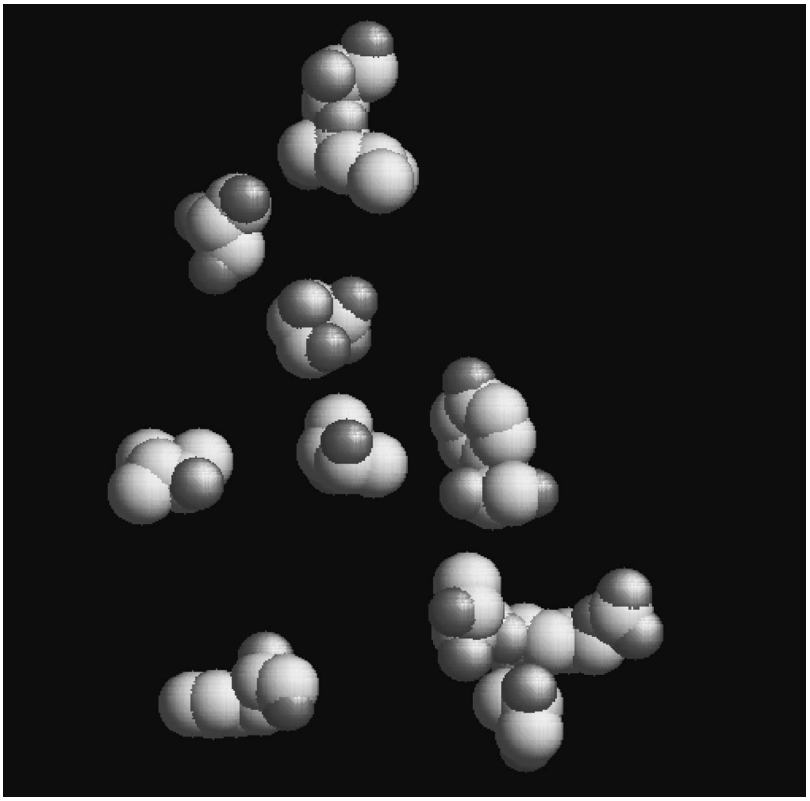

Fig. 5 Fulfilled picture of RasWin for amino acids appeared in the cross-section1
The size of amino acids for the van der Waals diameters of the atoms. The picture realized the actual amino acids.

Table 1 Distances between alpha-carbons of pair amino acids

	Ser^{36}	Leu^{37}	Ser^{95}	Val^{110}	Phe^{172}	Thr^{178}	Asn^{185}	Tyr^{190}	Ser^{195}	Thr^{197}	Arg^{198}
Ser^{36}	*	3.79	12.06	23.84	33.88	21.77	14.00	24.92	36.86	31.74	32.63
Leu^{37}	3.79	*	9.87	20.35	30.15	18.20	10.39	21.44	33.31	28.10	29.07
Ser^{95}	12.06	9.87	*	15.06	25.38	14.00	7.49	21.43	31.60	26.22	28.40
Val^{110}	23.84	20.35	15.06	*	12.41	13.37	11.09	17.25	23.48	18.08	20.76
Phe^{172}	33.88	30.15	25.38	12.41	*	13.50	20.01	16.24	14.91	11.48	14.63
Thr^{178}	21.77	18.20	14.00	13.37	13.50	*	8.27	9.81	17.77	12.48	15.19
Asn^{185}	14.00	10.39	7.94	11.09	20.01	8.27	*	14.38	24.95	19.44	21.30
Tyr^{190}	24.92	21.44	21.43	17.25	16.24	9.81	14.38	*	17.77	12.48	15.19
Ser^{195}	36.86	33.31	31.60	23.48	14.91	17.77	24.95	17.77	*	5.69	5.30
Thr^{197}	31.74	28.10	26.22	18.08	11.48	12.48	19.44	12.48	5.69	*	3.80
Arg^{198}	32.63	29.07	28.40	20.76	14.63	15.29	21.30	15.29	5.30	3.80	*

The above distances between amino acids on the cross-section1 are tabulated in the scale of A unit between two alpha-carbons of the residues. Upper suffices imply residue number and we use the three-character notation of amino acid.

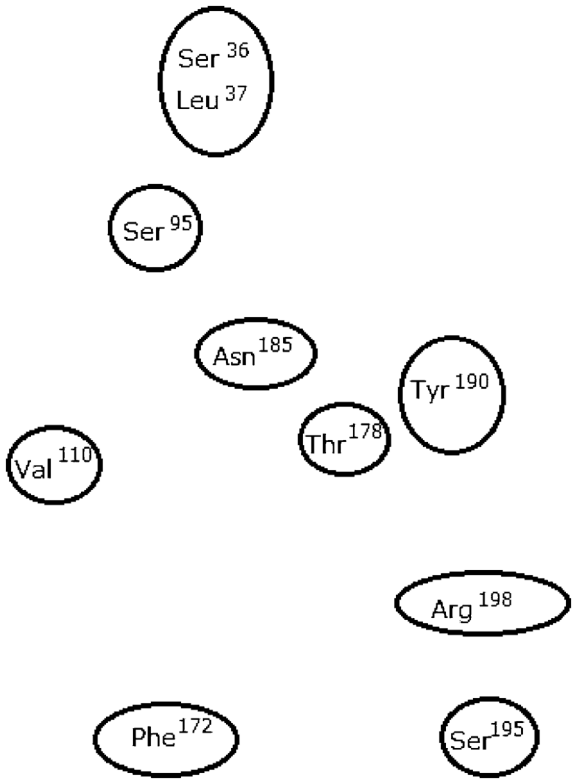

Fig. 6 Schematic picture of amino acids appeared at the cross-section1
Table 1 makes it difficult to know how the different amino acids in the cross-section1. We therefore draw this picture schematically that appeared amino acids on the cross-section1.

the considered cross-sections (Fig. 5 and Fig. 8).

We know the distances among amino acids. The number of possible combination becomes large if we use every atom of each amino acids. To reduce the calculation, we only pick the alpha-carbons of each amino acid. The calculated distances between alpha-carbons are tabulated in Table 1. The distances are presented in the A (angstroaem) order. Large values indicate far different amino acids, while small values indicate near amino acids. To provide easier understanding we present a rough picture of the amino acid residues (Fig. 6). Each amino acid residue has its sequential number. The distribution of amino acid residues is represented using amino acid residues with residue number.

4. Summary and Discussion

From Figure 5, we can image one pore through the cross-section plane. From figure 8 one pore can be seen, but it is difficult to know if a pore exists, or not, around Met 225. We rotated the cross-section to provide a clearer view of amino acid residues. Thus, we cannot image the whether a pore appeared at in the cross-section as a straight manner or pore translating along a zig-zag line. We can conclude that one pore of rhodopsin exists the cross-section such that there is an ion channel in

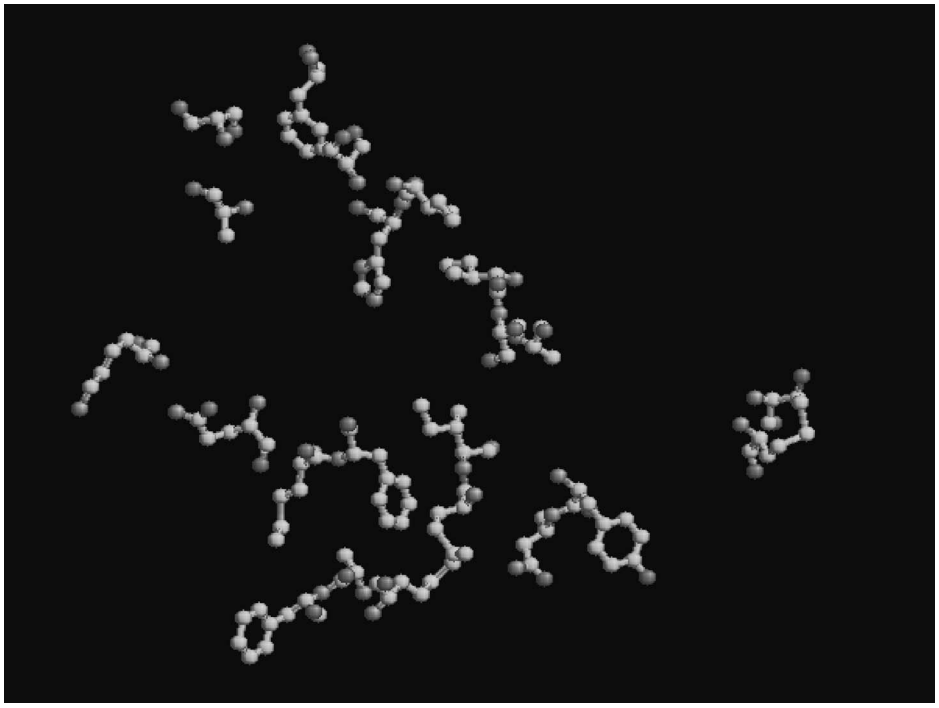

Fig. 7 Amino acids appeared in the cross-section2
The amino acids as they appeared in cross-section2 are following ones, namely: Lys 61, Gln 66, Met 71, Phe 72, Met 128, Ileu 129, Tyr 134, Asn 135, Ala 152, Phe 153, Met 155, Met 225, Ser 228, Ala 260, Ileu 262, Ser 263, Ileu 314, Ser316, His 319, Ser327, Phe 339, Ala 325. The ball and stick representation is used for the picture.

the rhodopsin that transports protons from one-side the other. The size of ion channel is small at the amino acid level pore, but it is plausible that protons are smaller than small amino acids. So rhodopsin may provide an ion channel to pass protons. The channel in the rhodopsin protein can control the pH of photoreceptor cells.

As seen Figure 5 and 8, it is difficult to find the pore of rhodopsin in the cross-section because we viewed the fulfilled picture that denotes the real protein at the real scale. The pore is relatively the same size of the somewhat separated amino acid residues. Probably, the helix part has many amino acid residues that form the helix as an amino acid line. It is difficult to image the cross-section from the fulfilled image of rhodopsin (Fig. 3). We should consider from the fulfilled picture as professor Wako says. He know many things about protein so that his saying is important signal for us.

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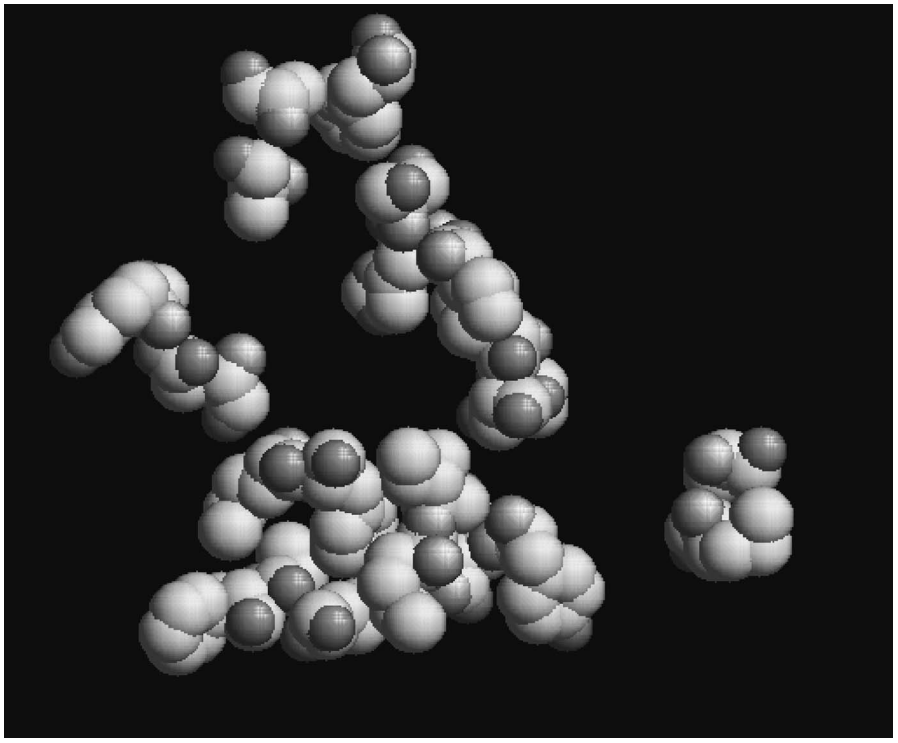

Fig. 8 Fulfilled picture of RasWin for amino acids as they appeared in cross-section2
In the cross-section2, we find the block of amino acids group that are parts of helices.

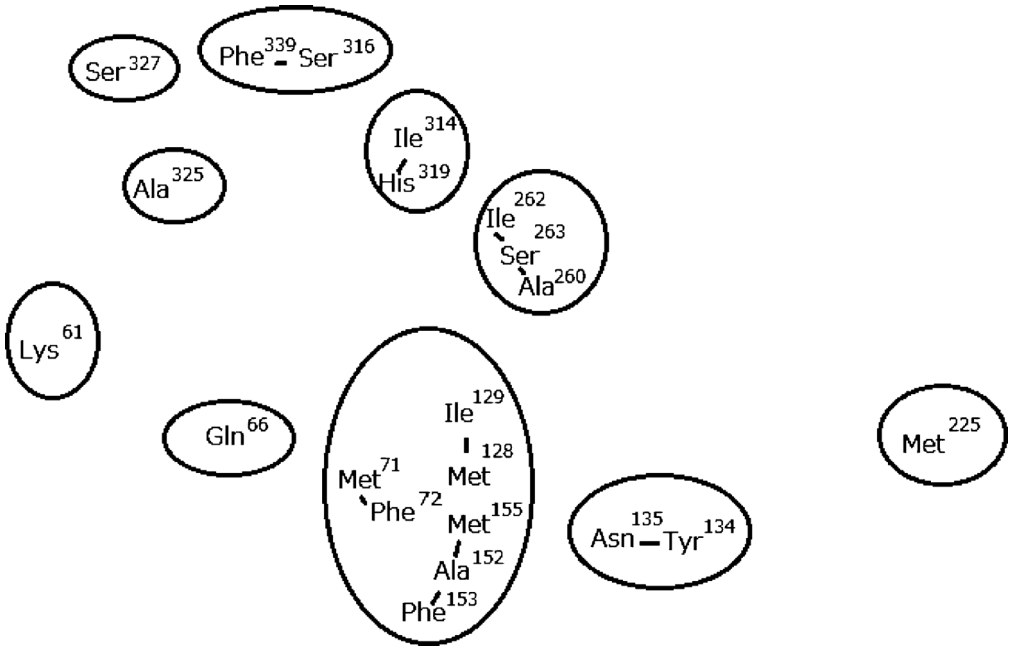

Fig. 9 Schematic picture of amino acids appeared at cross-section2
We show the amino acids schematically. We easily understand what amino acids forms the cross-section2 from the schematic picture of residues.

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