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Computational studies for the structure and function of *m*RPE65

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Abstract

The *m*RPE65 protein is one form of the RPE65 protein and plays a very important role in the visual cycle. However, its 3D structure and detailed mechanism of function are still unclear because of difficulties with isolation and crystallization. This computational study reports a model for the *m*RPE65 protein structure derived from a model for *s*RPE65. The natural substrate for RPE65 has been shown to be a retinyl ester and, by utilizing the Autodock and the Ligplot programs, the interactions between the ester and the protein as well as the effects of several mutations on these interactions are studied. Finally, the position of the binding site is proposed based on an iterative process and the effects of the mutations on the binding site are also discussed.
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Keywords: *m*RPE65; Protein structure and function

1. Introduction

The RPE65 protein is located in retinal pigment epithelial cells (Bavik et al., 1992; Gollapalli et al., 2003; Jahng et al., 2003; Ma et al., 2001; Mata et al., 2004; Redmond et al., 1998; Tsilou et al., 1997; West et al., 2003; Wolf, 2005; Xue et al., 2004; Znoiko et al., 2002) and plays a very important role in the visual cycle (Rando, 2001). Its dysfunction can lead to various diseases, such as autosomal recessive *retinitis pigmentosa* (Hamei et al., 2001; Yzer et al., 2003) and Lebers congenital *amaurosis* (Gouras et al., 2002; Hamei et al., 2001; Seeliger et al., 2001). It exists in two forms: *s*RPE65 and *m*RPE65. The *s*RPE65 protein is the cytosolic form and has a very high binding affinity to all-*trans* retinol whereas *m*RPE65 is membrane associated and has a very high binding affinity to all-*trans* retinyl ester (Wolf, 2005). They have the same amino acid sequence but different post-translation modifications; *s*RPE65 undergoes no post-translation modification in contrast to *m*RPE65 which has three covalently attached palmitic acids at CYS231, CYS329 and CYS330 (Xue et al., 2004). The function of *m*RPE65 in the visual cycle has not been unambiguously determined. Until recently it was believed that *m*RPE65 can extract the ester from the membrane and deliver it to an isomerahydrolase (IMH) but by itself has no catalytic ability (Gollapalli et al., 2003; Jahng et al., 2003; Mata et al., 2004; Redmond et al., 1998; Tsilou et al., 1997). Dysfunction of *m*RPE65 will then cause the accumulation of all-*trans* retinyl ester and, concomitantly, a deficiency of 11-*cis* retinol. However recently, several studies suggest that *m*RPE65 not only binds with all-*trans* retinyl ester but can also isomerize and hydrolyse it to 11-*cis* retinol, indicating that *m*RPE65 is an IMH (Jin et al., 2005; Moiseyev et al., 2005; Redmond et al., 2005). The *s*RPE65 protein is a chaperone for all-*trans* retinol when it is esterified to all-*trans* retinyl ester. The conversion between these two forms of the RPE65 protein can modulate the visual cycle (Wolf, 2005; Xue et al., 2004). Experimental observations show that the LEU450-MET or TYR368HIS mutations in *m*RPE65 will cause dysfunction of this protein (Kim et al., 2004; Wenzel et al., 2001; Yzer et al., 2003) and are associated with diseases such as early onset retinal dystrophy.

In this paper, a computational model for *m*RPE65, including its binding site, is constructed and studied. The interactions with all-*trans* retinyl ester and the effects of several specific mutations on the binding site are also investigated through computational modeling methods. The *m*RPE65 protein model is developed on the basis of

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the *s*RPE65 model since the only structural differences between them are their different post-translation modifications. Three palmitic acids are added onto the *s*RPE65 model at CYS231, CYS329 and CYS330 residues by using the Autodock program and the VMD programs. By utilizing the Autodock (Goodsell and Olson, 1990; Morris et al., 1996, 1998) and Ligplot programs (Wallace et al., 1995), the binding site of *m*RPE65 for all-*trans* retinyl ester was found and the interactions between the ester and the protein as well as the effects of LEU450MET and TYR368HIS mutations on the binding site were studied.

2. Methods

The *m*RPE65 model is constructed by adding three palmitic acids onto the *s*RPE65 model obtained previously (Guo et al., 2006). This was carried out using the Autodock program and the VMD program. The model allows for a qualitative discussion of the role of these three palmitic acids. Because the palmitic acids are covalently attached to the protein, the bond lengths and angles are fixed. Initially, in the model of *s*RPE65, the position for the carbonyl carbon atom of a palmitic acid was calculated according to known bond lengths and angles. Then, the Autodock program was used to add the palmitic acids to the *s*RPE65 protein model and to fix the specific carbon atom at the calculated position. The deviation for this process is about 0.01 Å due to finite grid size. Finally, the VMD program was used to adjust the position of the long carbon chains of the palmitic acids to reduce the energetically unfavorable contacts between the palmitic acids and the protein. After obtaining this model of the *m*RPE65 protein, the interactions between the all-*trans* retinyl ester and the protein were evaluated and the binding site of *m*RPE65 for the ester was also modeled. The Autodock program and the Ligplot program are used to study the interactions between the *m*RPE65 protein and the all-*trans* retinyl ester. The Autodock program can calculate the binding energy of a ligand when it binds with a protein (Goodsell and Olson, 1990; Morris et al., 1996, 1998). Recently this program is used to screen the drug candidates against severe acute respiratory syndrome, a disease that has spread mainly throughout eastern Asia (Wei et al., 2006). Since the docking process in the Autodock program begins with a population of random ligand conformations in random orientations at random translations, it gives different docking results at different times even if all initial conditions are the same. Thus, in each docking study, the average value of twenty trials was used to represent the final docking energy results. Because the final docking energy results are a statistically averaged value, they are only used for a qualitative analysis. It is anticipated that the binding site of *m*RPE65 for the all-*trans* retinyl ester is in one of the three hydrophobic domains of *m*RPE65. Therefore, initially the Autodock program was used to study the docking energies of the all-*trans* retinyl ester in the three domains of *m*RPE65 by docking the all-*trans* retinyl ester near the center of each domain. Because the LEU450MET and TYR368HIS mutations can cause dysfunction of the protein (Kim et al., 2004; Wenzel et al., 2001; Yzer et al., 2003), these two residues may be related to the binding of the ester. Therefore, the all-*trans* retinyl ester is also docked near these two residues and the effects of the LEU450MET and TYR368HIS mutations on the binding energy of the ester were examined by using the Autodock program. The mutated protein is obtained from the Jackal software package. To find out the binding site of *m*RPE65 for all-*trans* retinyl ester, an iterative method was used to approach the binding site. At first, the Ligplot program was used to analyse the docking results of all-*trans* retinyl ester near the center of domain two and LEU450 by generating the schematic diagrams for the protein–ester interactions. These diagrams indicate which residues in the *m*RPE65 protein model interact with the all-*trans* retinyl ester. After obtaining these diagrams, those common binding residues showing up in the diagrams more than eighteen times among the total twenty trials were selected and the ester was docked near the center of these residues to obtain the docking results for the first iteration. Then the Ligplot program was used again to analyse the docking results of the first iteration and select the binding residues, which appear in the binding site more than 18 times among the total 20 trials. Finally, the ester was docked near the center of these residues and the docking results for the second iteration were obtained. By using the same strategy, the docking results for the third iteration can be obtained. To study the effects of the LEU450MET and TYR368HIS mutations on the ester binding, the Autodock program is used to study the docking energies of the all-*trans* retinyl ester around the center of the binding site with these two mutations. The VMD program is also used to study the effects of these two mutations on the structure of the binding site.

3. Results and discussions

The *m*RPE65 protein model is shown in Fig. 1 as drawn by the VMD program. In this model, the *m*RPE65 protein has three domains and each domain consists of a right-handed twisted anti-parallel β-barrel, which is consistent with other reports in the literature (Chou et al., 1990a–c; Chou and Carlacci, 1991). The hydrophobic interiors of these β-barrels are likely places to bind with the hydrophobic all-*trans* retinyl ester. Three palmitic acids bind with CYS231, CYS329 and CYS330 and their long carbon chains are all extended to the exterior of the protein. These long carbon chains are highly hydrophobic and prefer to reside in a hydrophobic environment, so it is expected that they will be located in the hydrophobic interior of the membrane and act as an anchor to immobilize the *m*RPE65 protein on the surface of the membrane. The model is shown in Fig. 2 as drawn by the VMD program. The *s*RPE65 protein is treated as a rigid body when the palmitic acid residues are added onto it because the binding site is

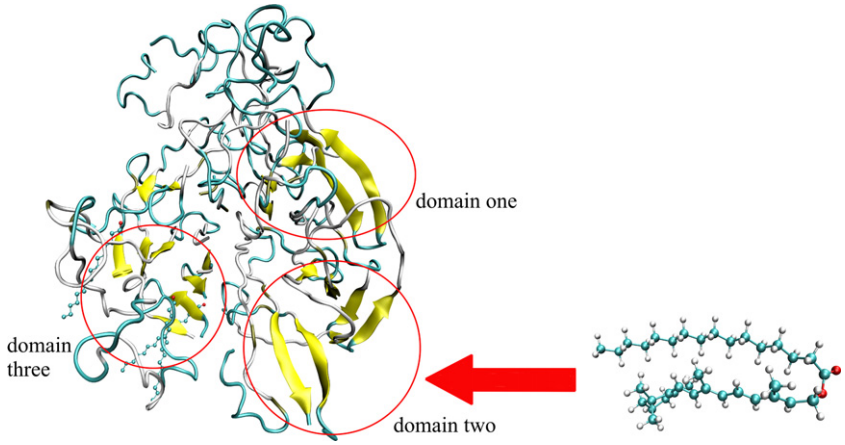

Fig. 1. The *m*RPE65 protein and the all-*trans* retinyl ester model (not to scale for clarity).

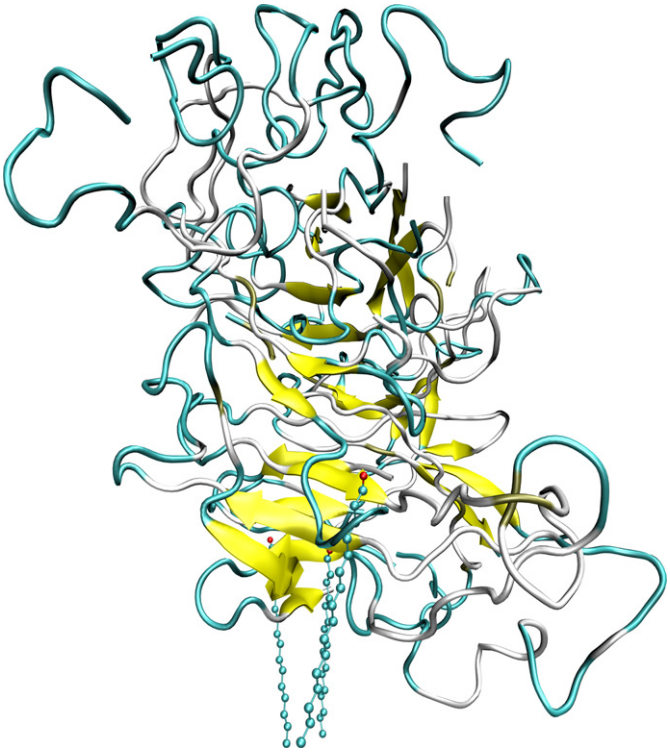

Fig. 2. The role of the palmitic acid molecules in the *m*RPE65 model.

Table 1
Docking energy results of all-*trans* retinyl ester near the center of domains

	Domain one	Domain two	Domain three
Docking energy (kcal/mol)	265.71	105.35	952.08

remote from these residues and their main role is to anchor *m*RPE65 onto the surface of the membrane. Thus, it is very likely that the local conformational changes around the three palmitic acids will have little effect on the binding site of the protein. This strategy is similar to other investigations of pharmaceutically important proteins (Chou et al., 1992, 2000; Chou, 1993, 1994, 2004d). Interestingly, the *s*RPE65 protein does not bind with any palmitic acids therefore its hydrophilic surface cannot be immobilized on the surface of the membrane and this may explain why *m*RPE65 is membrane associated but *s*RPE65 is cytosolic. The membrane association of *m*RPE65 may also give it an opportunity to interact with the all-*trans* retinyl ester buried in the membrane and extract it from the interior of the membrane to the hydrophobic interior of the protein. The three palmitic acids may also act to guide the all-*trans* retinyl ester into the protein. A similar mechanism for guiding a ligand into an active site and the channel of membrane protein is reported for the GABAA receptor (Chou, 2004a) and human potassium and sodium channels (Chou, 2004b), respectively. This may explain the different binding affinities of *m*RPE65 and *s*RPE65 to all-*trans* retinyl ester. All-*trans* retinyl ester is highly hydrophobic and, therefore, it should be buried in a hydrophobic environment. In the model of the *m*RPE65 protein, the most favorable binding site for the hydrophobic all-*trans* retinyl ester is the hydrophobic interior of the β-barrel in each domain. Therefore, our model suggests that the binding site of the *m*RPE65 protein for all-*trans* retinyl ester should be located in the hydrophobic interior of the β-barrel in each domain. According to the docking energy results summarized in Table 1, the binding site should be located in the β-barrel of domain two because the all-*trans* retinyl ester exhibits the lowest docking energy there. This conclusion is also supported by the observation that LEU450, whose mutation can cause dysfunction of *m*RPE65 (Kim et al., 2004; Wenzel et al., 2001), is located at the β-barrel of this domain. LEU450 and TYR368 are very important to the binding of the all-*trans* retinyl ester because it has been observed experimentally that their mutations can cause dysfunction of *m*RPE65 (Kim et al., 2004; Wenzel et al., 2001; Yzer et al., 2003). According to the docking energy results summarized in Table 2, when the all-*trans* retinyl ester is docked near LEU450, its

Table 2
Docking energy results of all-*trans* retinyl ester near LEU450 and TYR368

	Docking energy (kcal/mol)
LEU450	63.12
Domain two	105.35
TYR368	316.06
LEU450MET	90.72
TYR368HIS	209.72
TYR368HIS*	76.39

TYR368HIS*: the docking energy of the ester near LEU450 with TYR368HIS mutation.

Table 3
The docking energy of all-*trans* retinyl ester in the binding site

	Docking energy (kcal/mol)
Domain two	105.35
LEU450	63.12
First iteration	0.756
Second iteration	−7.87
Third iteration	−3.73

docking energy is lower than when it is docked near the center of domain two. The LEU450MET mutation also causes an increase in the docking energy when the all-*trans* retinyl ester is docked near residue 450. These results indicate that LEU450 is closer to the binding site than to the center of domain two and that LEU450 may be an intrinsic part of binding site. In contrast, when all-*trans* retinyl ester is docked near TYR368, its docking energy is higher than when it is docked near the center of domain two. The TYR368HIS mutation also causes a decrease of the docking energy when the all-*trans* retinyl ester is docked near residue 368. These results indicate that TYR368 is farther away from the binding site than the center of domain two and cannot be a part of the binding site. However the TYR368HIS mutation does increase the docking energy of all-*trans* retinyl ester when it is docked near LEU450 indicating this mutation can affect the binding site and the binding of all-*trans* retinyl ester. The binding site of *m*RPE65 for the all-*trans* retinyl ester was mapped by using the Autodock program and the Ligplot program. The docking energy results from this procedure are summarized in Table 3. According to these results, the docking energy of the first iteration is substantially lower than those when the all-*trans* retinyl ester is docked near the center of domain two and LEU450 indicating this docking center is much closer to the binding site. The docking energy of the second iteration is also lower than the docking energy of the first iteration but it increases in the third iteration, therefore, the docking results of the second iteration were chosen as the binding site of *m*RPE65 for all-*trans* retinyl ester. The model of the binding site with the bound all-*trans* retinyl ester is shown in Fig. 3 as drawn by the VMD program. The complex of *m*RPE65 with

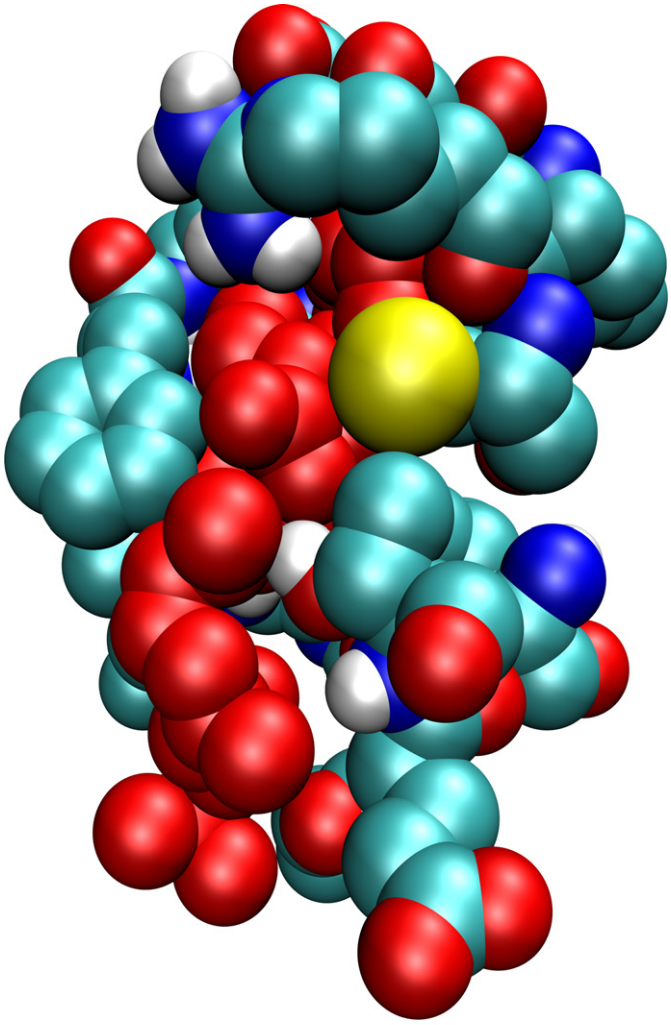

Fig. 3. The binding site of *m*RPE65 for the all-*trans* retinyl ester.

bound all-*trans* retinyl ester is shown in Figs. 4a and b. The models are drawn in van der Waals style and the bound all-*trans* retinyl ester is colored in red. Additionally, Fig. 4b is rendered to display the relative hydrophobicities of the residues. In this model, the long carbon chain of the all-*trans* retinyl ester is buried in the hydrophobic interior of the β-barrel in domain two. The effects of the LEU450-MET and TYR368HIS mutations on the binding site are shown in Figs. 5 and 6. The LEU450MET mutation causes dramatic changes in the binding site. It changes the side chain of residue 450, the position of the sulfur atom in CYS448 and the positions of polar hydrogen atoms in other residues. The TYR368HIS mutation has a small effect on the structure of the binding site; it only changes the positions of polar hydrogen atoms in the binding site. According to the docking energy results summarized in Table 4, both of these mutations cause the binding energy of all-*trans* retinyl ester to increase. These results may explain why these two mutations are observed to cause dysfunction of *m*RPE65. The van der Waals interactions are mainly responsible for favoring domain two as the

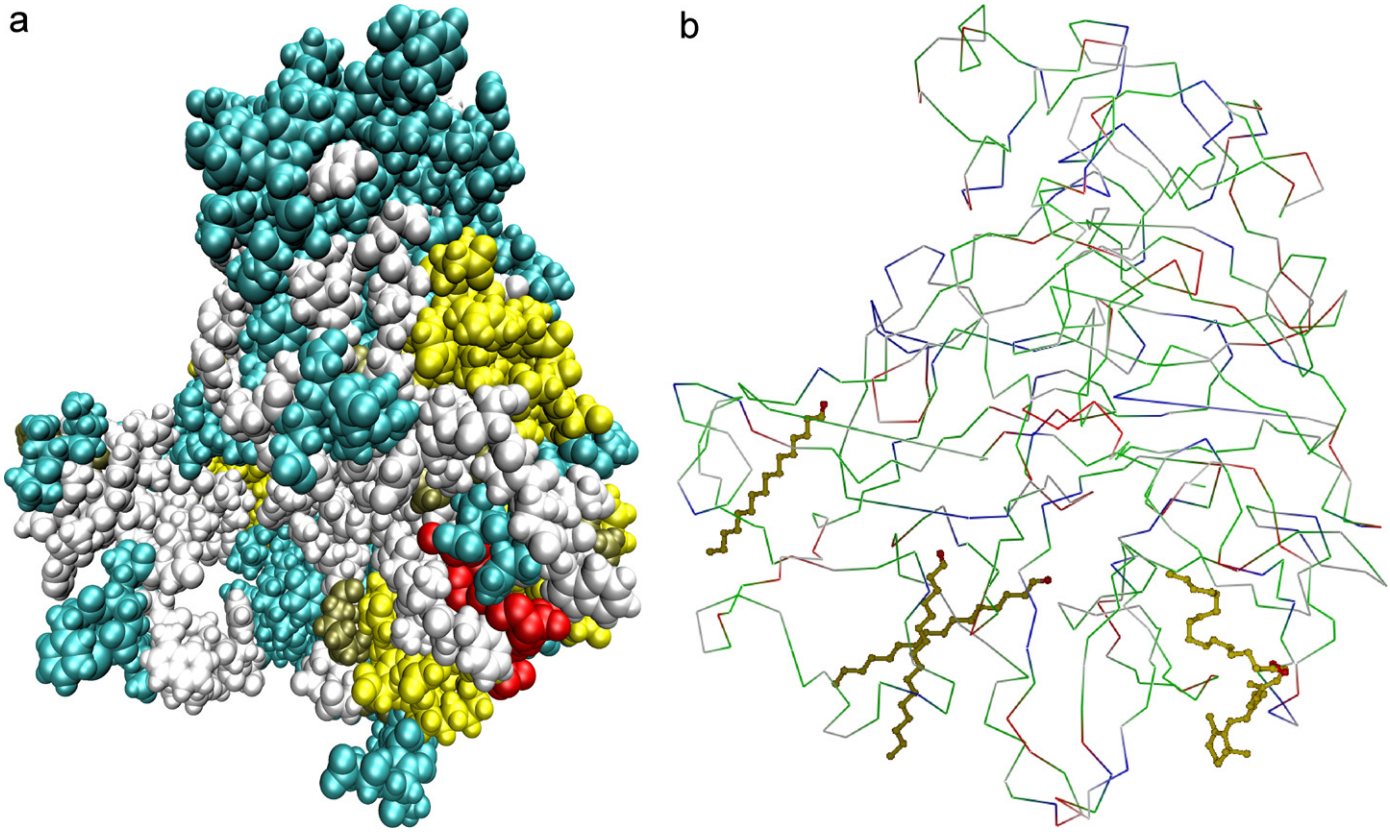

Fig. 4. (a) The model of *m*RPE65 with bound all-*trans* retinyl ester (as space-filling diagrams), (b) the model of *m*RPE65 with bound all-*trans* retinyl ester that has been rendered to show residue hydrophobicity. Highly hydrophobic residues are in green, neutral residues are in grey, acidic residues are in red, basic residues are in blue.

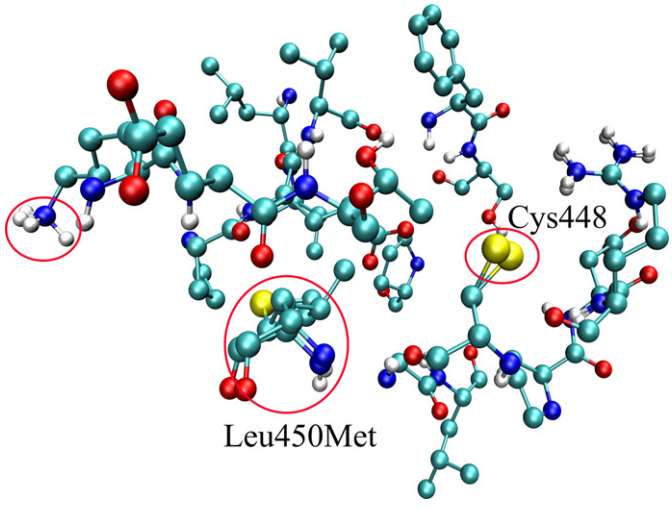

Fig. 5. The effects of LEU450MET mutation on the binding site. Circles indicate the changes on mutation.

domain containing the binding site for the all-*trans* retinyl ester. In other words, the size and shape of the binding site fit the ligand better than the center of domain two or the other domains. Hydrogen bonds also play an important role in the ligand binding as has been reported by others (Chou et al., 2000, 1999, 2003, 1997; Chou, 2004c, 2005, 1992). In our docking and Ligplot trials, THR457 in the binding site often forms hydrogen bonds with the binding ester to stabilize the complex. Furthermore, the mutations noted earlier also cause the internal energy of the all-*trans* retinyl ester to increase, implying that the ligand no longer fits well in the binding site of the mutants without added strain to its own structure.

4. Summary

This study constructs a model for *m*RPE65 based on the *s*RPE65 protein model with the addition of three palmitic acids. These three palmitic acids can act as anchors to immobilize the *m*RPE65 on the surface of the membrane and may play a role in guiding the ester into the interior of the protein. The *m*RPE65 model has three domains and each domain consists of a right-handed twisted anti-parallel β-barrel. The binding site is most likely located in domain two and close to LEU450 based on the docking results. By using the Autodock program and the Ligplot program, the binding site of *m*RPE65 for all-*trans* retinyl ester was mapped and the effects of LEU450MET and TYR368HIS mutations on the binding of the ester were calculated.

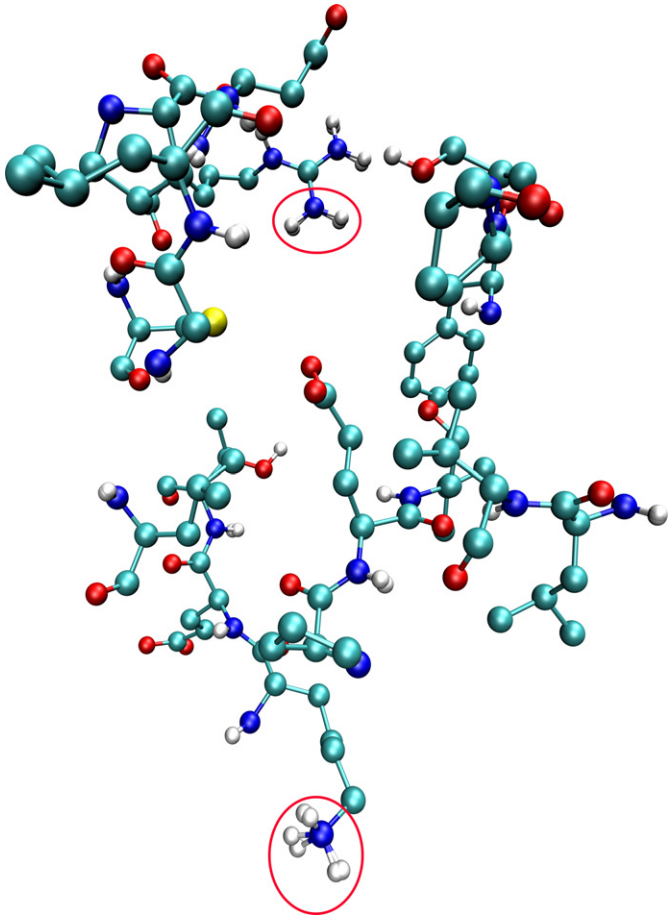

Fig. 6. The effects of TYR368HIS mutation on the binding site. Circles indicate the changes on mutation.

Table 4
Effects of LEU450MET and TYR368HIS mutations on the binding of the all-*trans* retinyl ester

	No mutation	LEU450MET	TYR368HIS
Docking energy of ester on binding site (kcal/mol)	−7.87	−4.87	−6.05

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