RESEARCH NOTE

A voltage-dependent fluorescent indicator for optogenetic applications, archaerhodopsin-3: Structure and optical properties from *in silico* modeling [version 1; peer review: awaiting peer review]

Dmitrii M. Nikolaev¹, Anton Emelyanov¹, Vitaly M. Boitsov¹, Maxim S Panov², Mikhail N. Ryazantsev^{id}2,3

¹Saint-Petersburg National Research Academic University of the Russian Academy of Science, St. Petersburg, Russian Federation

²Saint-Petersburg State University, St. Petersburg, Russian Federation

³SPbSC RAS, Saint-Petersburg Scientific Center of the Russian Academy of Sciences, St. Petersburg, Russian Federation

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Abstract

It was demonstrated in recent studies that some rhodopsins can be used in optogenetics as fluorescent indicators of membrane voltage. One of the promising candidates for these applications is archaerhodopsin-3. However, the fluorescent signal for wild-type archaerhodopsin-3 is not strong enough for real applications. Rational design of mutants with an improved signal is an important task, which requires both experimental and theoretical studies. Herein, we used a homology-based computational approach to predict the three-dimensional structure of archaerhodopsin-3, and a Quantum Mechanics/Molecular Mechanics (QM/MM) hybrid approach with high-level multireference ab initio methodology (SORCI+Q/AMBER) to model optical properties of this protein. We demonstrated that this methodology allows for reliable prediction of structure and spectral properties of archaerhodopsin-3. The results of this study can be utilized for computational molecular design of efficient fluorescent indicators of membrane voltage for modern optogenetics on the basis of archaerhodopsin-3.

Keywords

optogenetics, archaerhodopsin, protein structure prediction, QM/MM, spectral tuning in rhodopsins

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1. **Kirill A. Afonin**, The University of North Carolina at Charlotte, Charlotte, USA
2. **Adam E. Cohen**, Harvard University, Cambridge, USA
Samouil Farhi, Harvard University, Cambridge, USA
3. **Jeremy N. Harvey**, KU Leuven, Leuven, Belgium

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Mikhail N. Ryazantsev (mikhail.n.ryazantsev@gmail.com)

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Introduction

Precise and quick control of physiological processes using integrated optical and genetic methods is a vast area with a high number of important applications¹. One possible approach in this field is using fluorescent voltage-dependent indicators for detecting the activity of mammalian neurons, which allows achievement of the precision of a single neuron without perceptible time delays. It was recently shown that some rhodopsins, especially archaerhodopsin-3, can be potential candidates for such a task^{2–5}. However, the fluorescent signal for these rhodopsins is not strong enough for real applications, but insertion of specific mutations into these proteins can dramatically improve the signal quality^{2–5}.

Unfortunately, the fundamental mechanisms underlying the processes that determine fluorescence are not well understood. This lack of knowledge leads to difficulties with design of desired rhodopsin mutants. While rational design is not based on a solid foundation and, for this reason, is not very effective, another experimental approach, random mutagenesis, is very time consuming. Computational studies can provide additional insights into the problem. One of the main obstacles for computational modeling of proteins is the absence of three-dimensional structures of high quality, especially for membrane proteins, which are a challenge for crystallization. On the other hand, computational prediction of three-dimensional structures is not trivial. The goal of this study was to obtain a good-quality structure for archaerhodopsin-3, one of the most used voltage-dependent fluorescent sensors^{4,5}, and based on this structure to predict the optical properties of this protein.

To achieve this goal, we used a homology-based computational approach for structure prediction. As the choice of a structure prediction algorithm is not straightforward, we tested several methods. To evaluate the quality of obtained structures we performed subsequent *Quantum Mechanics/Molecular Mechanics* (QM/MM) calculations of absorption maxima and compared the results with available experimental data.

Methods

The structure of archaerhodopsin-3 was built using a homology modeling approach. Primary structures for all rhodopsins with crystallographic data are available in the Protein Data Bank library⁶ (24 structures as of September 2016) were compared with the primary structure of archaerhodopsin-3. Archaerhodopsin-1, which has the highest sequence identity to the target protein, was chosen as a template (RCSB code, 1UAZ). Three algorithms of homology-based model building were tested: Medeller⁷, I-TASSER⁸ and RosettaCM⁹. All methods of homology modeling heavily rely on externally made target-template alignment of primary sequences, which serves as the main instruction for model building. For this reason, we tested three algorithms of pairwise alignment using their results as an input for each method of model building. Two

of the alignment methods are specifically constructed for membrane proteins, MP-T¹⁰ and AlignMe¹¹, and the third one, MUSTER¹², gains its quality from evolutionary predictions. The latter algorithm is a built-in algorithm of I-TASSER suite and its results were used only for this method of structure prediction.

Before QM/MM calculations, several preparation steps were performed: hydrogen atoms were added using pdb2pqr package¹³ version 2.1.1 using CHARMM force field version 27¹⁴, pH=7; hydrogen atoms were equilibrated by energy minimization in NAMD package¹⁵, version 2.11. The retinal chromophore was bound to the lysine residue Lys226, whole lysine + retinal system was parameterized in CHARMM force field¹⁶. The protein was inserted in the POPC membrane¹⁷, the whole system was inserted in a water solvent box, the TIP3P water model¹⁸ was used, the size of water box was selected so that there were at least 10 Å from any atom of protein to the edge of the system, and the system was neutralized by addition of Na⁺ and Cl⁻ ions.

Relaxation of the system was performed in several steps: relaxation of retinal + lysine complex with all other atoms fixed, relaxation of all atoms that were within 6 Å of the chromophore system, relaxation of whole protein and water box. During all these steps the following parameters were used: a 10 Å cutoff with switching starting at 8.5 Å was applied to the electrostatics and van der Waals interactions; Particle Mesh Ewald method¹⁹ was used for dealing with electrostatics interactions, grid spacing 1 Å. Equilibrated protein structure was extracted; internal waters were added into protein cavities using WaterDock program²⁰.

To calculate absorption maxima, we used the methodology that has been proven as efficient in a number of our previous studies for different kind of rhodopsins and rhodopsin mimics^{21–27}. The structures of the archaerhodopsin-3 obtained at the previous step were optimized using two-layer ONIOM (QM:MM-EE) scheme. (QM=B3LYP/6-31G*; MM= AMBER for aminoacids and TIP3P for water, EE=electronic embedding) was implemented in the Gaussian09 package²⁸. To calculate the spectral properties of the chromophore in the presence of the protein environment (described as AMBER point charges) SORCI+Q/6-31G* level of the theory was used, as it is implemented in ORCA6.0 package²⁹. For details of previously performed QM/MM methodology see Altun *et al.*^{30,31}.

Results

We obtained seven structures of archaerhodopsin-3 and chose the best one by comparison of experimental spectral data with the calculated one. The results of the calculation of absorption maximum wavelength are all in the range of 31 nm from the experimental value (556 nm). They are presented in Table 1. From these results we can conclude that the best matching result was obtained using I-TASSER suite with AlignMe²⁴ alignment (Figure 1).

Table 1. Absorption spectrum maximum of archaerhodopsin-3 for different models.

Alignment method	Model building method	λ_{max} , nm
--	Experimental wild-type structure	556
AlignMe	I-TASSER	578
MP-T	I-TASSER	581
MUSTER	I-TASSER	581
AlignMe	RosettaCM	587
MP-T	RosettaCM	585
AlignMe	Medeller	580
MP-T	Medeller	586

Figure 1. Predicted structure of archaerhodopsin-3 at the B3LYP/6-31G*: AMBER96 (I-TASSER) level of the theory.

Conclusions

In this study, we predicted the structure of fluorescent voltage-dependent sensor archaerhodopsin-3 and evaluated its quality with subsequent QM/MM high level *ab initio* calculations of spectral properties. The calculated absorption maximum is within 31 nm from the experimental value. Several methods of model building were tested and spectral characteristics were calculated for all resulting models. We showed that our methodology allowed for reliable prediction of optical properties of archaerhodopsin-3. The results of this study can be utilized for high-level QM/MM investigation of different aspects of photochemistry of this voltage-dependent fluorescent sensor and, therefore, to contribute in development of the efficient molecular tools for modern optogenetics.

Data availability

The sequence of archaerhodopsin-3 was taken from Uniprot database: <http://www.uniprot.org/uniprot/P96787>

The template for homology modeling was taken from PDB database (rcsb code 1UAZ): <http://www.rcsb.org/pdb/explore/explore.do?structureId=1UAZ>

The input files of I-TASSER suite, RosettaCM, Medeller algorithms with corresponding README files, zipped output of I-TASSER suite, scripts for processing structure after homology modeling stage (with instructions in README file), input files for spectra calculations are available: doi, [10.5281/zenodo.229168](https://zenodo.org/record/229168#.WG5jyFWLTcs)³² (<https://zenodo.org/record/229168#.WG5jyFWLTcs>)

Author contributions

MNR proposed and designed the research, DMN, MSP and MNR made the calculations. AE and VMB provided valuable consultation during research. MNR and DMN wrote the paper

Competing interests

No competing interests were disclosed.

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