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Computer Simulation of the Light Absorption band of the Jumping Spider Isorhodopsin

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Honors Project

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Abstract:

In order to simulate the photoisomerization of the 9-cis Jumping Spider Isorhodopsin (JSiR-1) it is necessary to first simulate its light-absorption band. Here we report on the absorption band simulated using protein models constructed using the advanced Automatic Rhodopsin Modeling (a-ARM) program. A population of S_0 models was created and the corresponding S_0 to S_1 transitions were determined for each member of the resulting population. The calculation resulted in a Gaussian plot showing that the wavelength of the absorption maximum of 560 nm (a violet color) that is consistent, but red-shifted, with respect the experimentally observed value.

Literature Review:

Nagata provided information and extermination which suggests that the Jumping Spider, *Hasarius adansoni*, use "defocusing" exclusively as their way of perceiving depth and have ruled other possibilities such as motion parallax, making the spider's eyes of particular interest to study[1]. Varma explained the importance of studying the 9-cis JSiR-1 molecule due to the difference in ground (9-cis) and activated (all-trans) state absorbance compared to the 11-cis JSR-1 molecule which has the same absorbance in both ground (11-cis) and activated (all-trans) states. The difference in absorbance of the JSiR-1 molecule allows for the determination of the photoisomerization quantum yield in a lab setting opposed to the JSR-1 molecule where the two states are indiscernible from each other[2].

Eherenberg successfully isolated the 9-cis JSiR-1 molecule and crystalized the molecule which was a necessary step before the molecule could be simulated[3]. Gozem provided the majority of the theory behind the dynamic simulations of the molecule which is based on a model generated by using the X-ray structure found on the RCSB protein databank[4,5]. The steric hinderance of the 9-cis molecule opposed to the wild type 11-cis is also noted which could potentially play a role in any difference in results between the 11-cis and 9-cis quantum yields or absorbances. To convert the crystalized JSiR-1 molecule structure into a model to run dynamic simulations on, Pedraza- González described the computational protocol used for building the protein model using a technique called advanced Automatic Rhodopsin Modeling (a-ARM). The resulting model uses classical and quantum mechanical calculations, depending on the environment or reactive/light-absorbing part of the molecule)[6]. The method used to generate a sample size of JSiR-1 molecules was described by Manathunga, which is important for quantum yield and absorption calculations[7]. The 12 π-electron conjugated system of the retinal, which is called the active space, is described by Andruniów which explained the importance of using quantum mechanical calculations on the retinal, as the electron movement is crucial in the mechanism of the cis to trans isomerization[8]. Yang provided more insight into the speed of the reaction, along with explanation of the relaxation from the excited state back to the ground state in which the retinal either stayed in the cis conformation or isomerized into the trans conformation[10].

Introduction:

The jumping spider rhodopsin was studied due to their unique way of visually focusing on objects. Jumping spiders (*Hasarius adansoni*) exclusively use a method called defocusing to determine its distance from an object, meaning that the spider uses how blurry something looks in order to determine its distance[1]. Jumping Spider Isorhodopsin JSiR-1 consists of a 9-cis retinal chromophore encapsulated in the cavity formed by a 7 *a*-helices structure as reported by Varma at al and shown in Figure 1[2]. Previous experiments have been done to crystalize the rhodopsin with the 9-cis retinal as described by

Ehrenberg[3]. This isorhodopsin serves as a comparable representation of the wild type JSR-1 that has an 11-cis retinal chromophore and studied to gain insight into the relationship between photosensitive and non-photosensitive G Protein Coupled Receptors (GPCRs)[2].

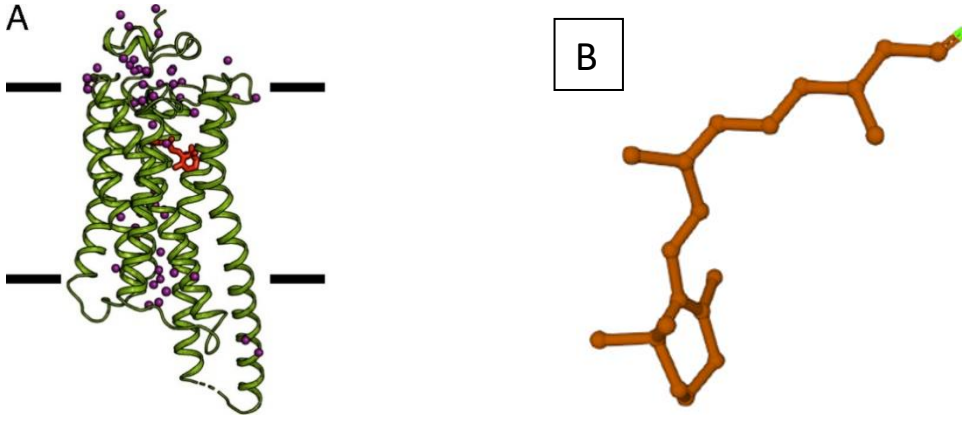

Figure 1[2]: (A) JSiR-1 Rhodopsin molecule with 9-cis retinal encapsulated[5] *Varma et al. 2019, Figure 2A,* (B) 9-cis retinal chromophore

Observing the isomerization mechanism was desirable to better understand the function of the jumping spider rhodopsin however, wild type the 11-cis and all-trans retinal in ground and activated states respectively are indiscernible from one another in the UV-vis spectra, as seen in Figure 2. Figure 2B shows the 11-cis absorption overlap of the activated and ground states at 535 nm, whereas Figure 2A shows the 9-cis has a ground state absorbance at 505 nm, but an activated state absorbance at 535 nm. This made the 9-cis molecule more desirable to study as cis and trans conformations were easily disernable from one another based on absorbances and therefore easier to determine if the trans isomer was created in a lab setting.

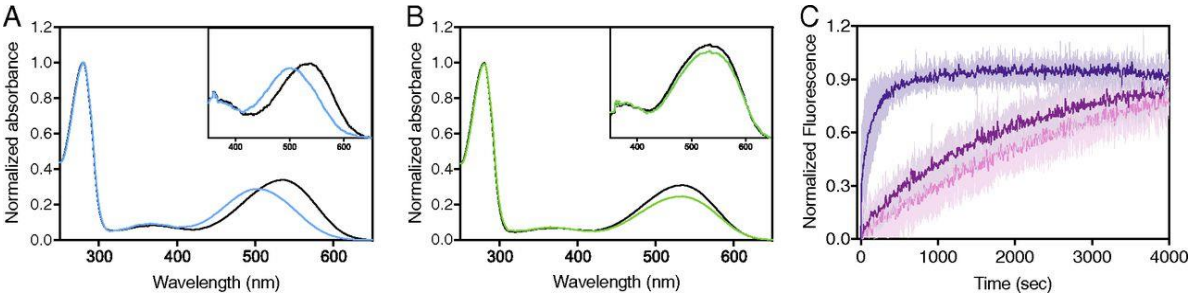

Figure 2[2]: *Varma et al. 2019,* (A) 9-cis activated absorbance (535nm, black line) and ground state absorbance (505nm, blue line), (B) 11-cis activated (black line) and ground state (green line) absorbance (535nm), (C) fluorescence of the JSiR-1 GPCR which shows light absorbance and resulting fluorescence of G protein

The calculations of the photoisomerization quantum yield have not been done for JSiR-1. In fact, the limited availability of this rhodopsin, being only available from harvesting tiny spider eyes, coupled with the speed at which the isomerization takes place has limited the experimentation done on it. Due to the lack of experimental data to compare with, there has been a general lack of interest in simulating the photoisomerization process by calculating the quantum yield for both rhodopsin (11-cis) and isorhodopsin (9-cis) of the JSiR-1. Furthermore, the interest has been decreased even further by the hypothesis that the quantum yield of JSiR-1 isorhodopsin may not be close to the one of rhodospin due to the steric hinderance in the 9-cis chromophore[4]. This resulted in the present lack of knowledge that

we are trying to acquire with the present project in which we prepare the model of an initial ground state JSiR-1 isorhodopsin to be successively used for quantum yield computations by running dynamic calculations in a realistic JSiR-1 isorhodopsin model.

Method:

The PBD file containing the structural parameters of JSiR-1 isorhodopsin was obtained from the RSCB Protein Data Bank[5] and then optimized for the most realistic geometric structure using the a-ARM method, which also gives hydrogens to the structure. The model was then used to generate room temperature motion and snapshots were taken at regular intervals to record the velocity and geometry and used to construct an equilibrium population of molecules. The molecular population was then used to generate, for all members, the corresponding QM/MM models where a quantum mechanical (QM) part corresponded to the retinal chromophore and where a molecular mechanical (MM) part described the rest of the protein with a technique described by Pedraza-González[6]. Accordingly, to have a sample size from which to calculate quantum yield, many copies of the structure were generated using the method described by Manathunga[7]. In total, 100 copies of the structure were created with this procedure.

As mentioned above the simulation treated the retinal at the QM level and the rest of the molecule, called the opsin, at the classical MM level using the method described by Pedraza-González[6]. The entire molecule was not treated at the QM level due to the high amount of computing power and time that would be required not currently being possible. In fact, quantum mechanical calculations have to describe the demanding electronic motion in the system, whereas molecular mechanical calculations only consider nuclei and charge. Quantum mechanical calculations for the retinal are desirable because of the movement of the 12 π-electrons in the retinal that takes place when the rhodopsin isomerizes from the cis conformation to the trans[8].

Technically, the QM method uses a CASSCF, complete active space self-consistent field, along with CASPT2, which uses second-order perturbation theory, for ensuring a more accurate potential energy of the rhodopsin. CASPT2 is a method that takes more time, however, is more accurate and prevents some of the issues with blue shifting that the CASSCF calculations cause. CASPT2 was used for all energy calculations for the vertical excitation from the S_0 to the S_1 state, while the rest, including all MM calculations such as geometry optimization, only used CASSCF. CASPT2 is not used for all calculations because it requires increased computing power and time.

Once the population was generated, the energy difference between the S_0 and S_1 of all 100 models was calculated to find the probability to transfer from the S_0 to S_1 state and therefore mimic the absorption of a quantum of light (i.e. the photon) of specific wavelengths. The population was generated using Equation 1 to create a gaussian plot from the excited state data. The standard deviation used was 0.4 eV = σ = 1/3099.6 nm^{-1} = 10^{-7}/3099.6 cm^{-1}. The resulting gaussian curve showed the wavelength at which portions of the population transfer from the S_0 state to the S_1 state vertically.

$$\varepsilon_1(\lambda) = 1.3062974 \times 10^8 \frac{0.0002}{10^7/3099.6} \exp\left[-\left(\frac{1/\lambda - 1/284.45}{1/3099.6}\right)^2\right] \quad (1)[9]$$

Results:

In Table 1, the data for the Gaussian curve was calculated from the excited S_1 and the ground S_0 state. After running the caclulations in the range of 400nm to 600nm, the range where we hoped to find the peak in the Gaussian curve, it was determined to have a maximum at 560 nm (energy gaps are expressed in nm, i.e. with the wavelength of the photon carrying the corresponding amount of energy). More specifically, out of all of the geometry samples, the largest portion of them had the highest probability of going from the S_0 to the S_1 state at 560nm. An absorbance at a given wavelength indicates that the transition from S_0 to the S_1 occurred at that wavelenght. This result was normalized in Table 1 with the majority the population, the peak wavelength, being 1.00 and every other data point being a fraction of the highest value, all the way to 0.00.

Table 1: Gaussian curve and normalization

Wavelenght (nm)	Population Fraction
440	0.001945
460	0.023184
480	0.059504
500	0.261973
520	0.617894
540	0.960556
560	1
580	0.819091
600	0.604151
620	0.397558
640	0.224874
660	0.124966
680	0.072685
700	0.03988

The results from Table 1 were then used to create the graph in Figure 3, where the population fraction is more clearly shown. The majority of the population absorbed at 560nm shown by the peak of the Gaussian curve. As shown in Figure 4, the ground state absorbance of the 9-cis retinal has a max absorbance at 505nm, a 55nm difference between the simulation of the 9-cis retinal with a ground state maximum absorbance of 560nm.

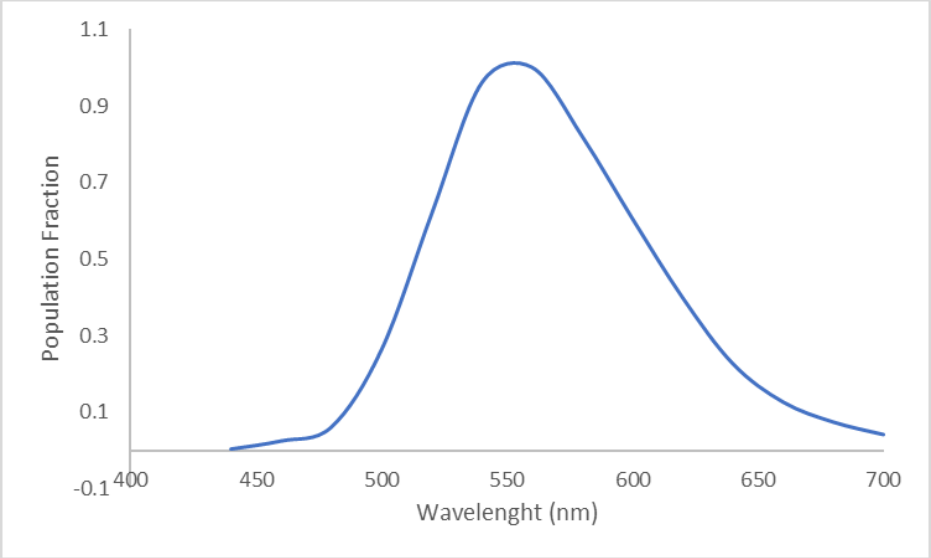

Figure 3: Normalized UV/Vis Gaussian plot for absorption wavelength of JSiR-1 population

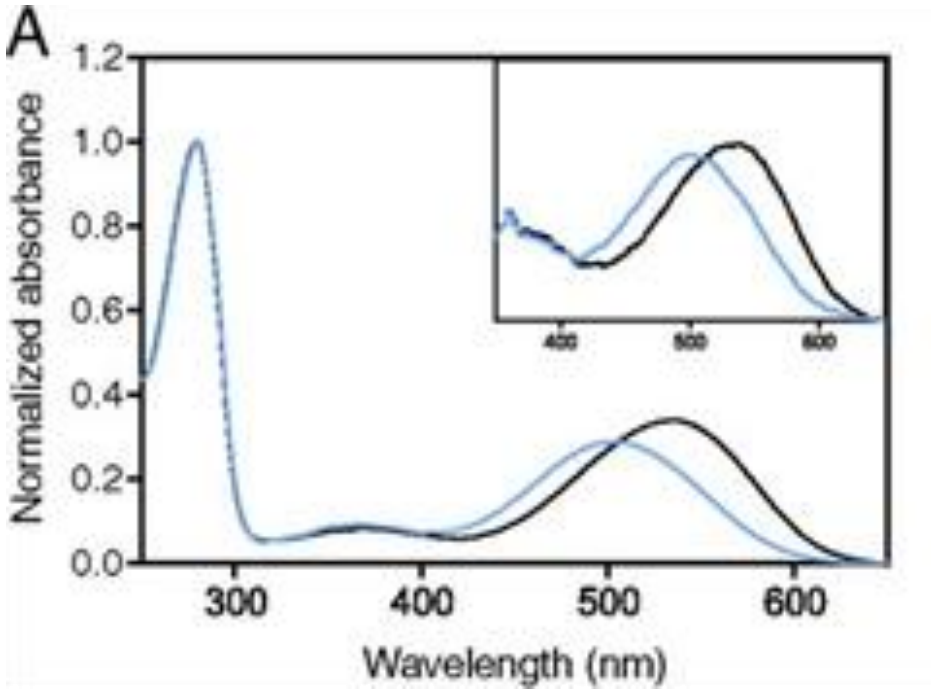

Figure 4[2]: Varma et al. 2019, (A) 9-cis activated and ground state absorbance

Discussion and Conclusion and Perspective:

Based on the results of the Gaussian curve, the study was paused with the resesult of a maximum of absorbance being at 560 nm opposed to the expected 505nm reported in Varma[2]. This redshifting of the predicted/computed absorbance could possibiliy be due to the code used initially being designed for the use with the 11-cis retinal, while the actual molecule being tested was the 9-cis JSiR-1. Though almost entirely identical, the 9-cis opposed to 11-cis may have caused some sort of error in calculation during the process since the classical mechanics (MM) parameters were based off the 11-cis model. Further

work could be done to adjust the code to create a more accurate representiation of the 9-cis absorption at the 505nm wavelength, however those adjustments are beyond the scope of this research.

As a follow up of the above research effort, the time evolution of the geometry of the 100 molecular structures composing the light-absorbing population, will be recorded over the time of the isomerization, allowing for insight into photomechanical isomerization and its quantum yield. The isomerization of the rhodopsin from the cis to the trans conformation is incredibly fast, making the photoisomerization difficult to observe in a lab setting[4]. Using the method described in by Manathunga, we will be able to measure the dihedral geometry of the simulated retinal across femtoseconds to get a smoother look at the mechanism[7]. After the samples are excited and relax to the ground state, some of the sample will stay in the cis conformation, while others will have isomerized to the trans conformation. This effect, and the speed at which it happens is further described by Yang and illustrated by Figure 5[10]. The percentage of the sample that isomerizes to the trans conformation is the quantum yield, which is one of the main targets of this research.

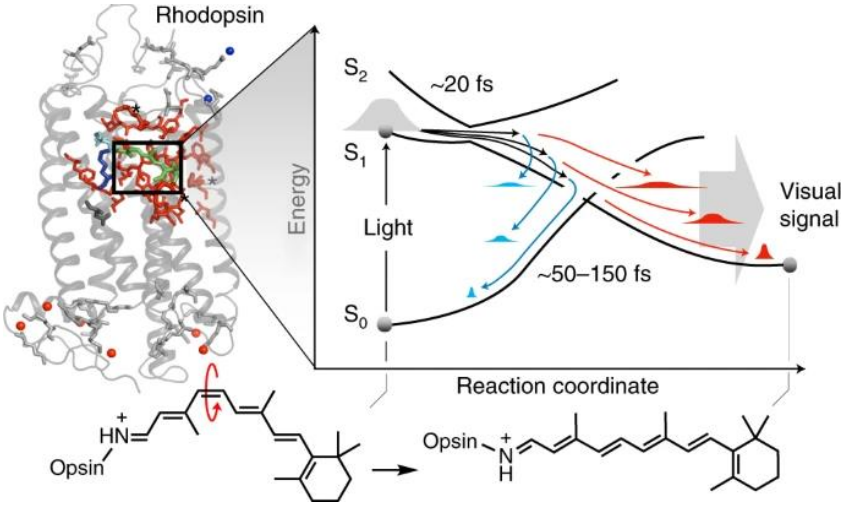

Figure 5[10]: *Yang et al. 2022*

References

1. Nagata, T., Koyanagi, M., Tsukamoto, H., Saeki, S., Isono, K., Shichida, Y., Tokunaga, F., Kinoshita, M., Arikawa, K., & Terakita, A. (2012). Depth Perception from Image Defocus in a Jumping Spider. Science, 335(6067), 469–471. https://doi.org/10.1126/science.1211667

2. Varma, N., Mutt, E., Mühle, J., Panneels, V., Terakita, A., Deupi, X., Nogly, P., Schertler, G. F. X., & Lesca, E. (2019). Crystal structure of jumping spider rhodopsin-1 as a light sensitive GPCR. Proceedings of the National Academy of Sciences, 116(29), 14547–14556. https://doi.org/10.1073/pnas.1902192116

3. Ehrenberg, D., Varma, N., Deupi, X., Koyanagi, M., Terakita, A., Schertler, G. F. X., Heberle, J., & Lesca, E. (2019). The Two-Photon Reversible Reaction of the Bistable Jumping Spider Rhodopsin-1. Biophysical Journal, 116(7), 1248–1258. https://doi.org/10.1016/j.bpj.2019.02.025

4. Gozem, S., Luk, H. L., Schapiro, I., & Olivucci, M. (2017). Theory and Simulation of the Ultrafast Double-Bond Isomerization of Biological Chromophores. Chemical Reviews, 117(22), 13502–13565. https://doi.org/10.1021/acs.chemrev.7b00177

5. *RCSB Protein Data Bank 6I9K*. https://www.rcsb.org/structure/6I9K (accessed 2022-11-7)

6. Pedraza-González, L., de Vico, L., Marín, M. del C., Fanelli, F., & Olivucci, M. (2019). a-ARM: Automatic Rhodopsin Modeling with Chromophore Cavity Generation, Ionization State Selection, and External Counterion Placement. Journal of Chemical Theory and Computation, 15(5), 3134–3152. https://doi.org/10.1021/acs.jctc.9b00061

7. Manathunga, M., Yang, X., Luk, H. L., Gozem, S., Frutos, L. M., Valentini, A., Ferrè, N., & Olivucci, M. (2016). Probing the Photodynamics of Rhodopsins with Reduced Retinal Chromophores. Journal of Chemical Theory and Computation, 12(2), 839–850. https://doi.org/10.1021/acs.jctc.5b00945

8. Andruniów, T., Ferré, N., & Olivucci, M. (2004). Structure, initial excited-state relaxation, and energy storage of rhodopsin resolved at the multiconfigurational perturbation theory level. Proceedings of the National Academy of Sciences, 101(52), 17908–17913. https://doi.org/10.1073/pnas.0407997101

9. *Creating UV/Visible Plots from the Results of Excited States Calculations.* https://gaussian.com/uvvisplot/ (accessed 2022-10-02).

10. Yang, X., Manathunga, M., Gozem, S., Léonard, J., Andruniów, T., Olivucci, M. (2022). Quantum–classical simulations of rhodopsin reveal excited-state population splitting and its effects on quantum efficiency. Nature Chemistry, 441-449, 14(4). https://doi.org/10.1038/s41557-022-00892-6