Light activation mechanism of channelrhodopsin 2

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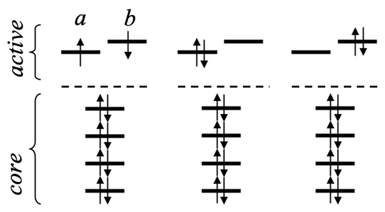
Abstract

The channelrhodopsin 2 (ChR2) is a light-gated ion channel and a widely used tool in optogenetics. The photoisomerization of the retinal protonated Schiff base (RPSB) in ChR2 triggers the channel opening and firing of neuronal signals. Despite the importance of the ChR2, its light activation mechanism is still not fully understood in atomistic detail. In this work, we combine quantum dynamics, classical dynamics, electronic structure, and free energy calculations to comprehensively characterize the light activation mechanism of ChR2. Nonadiabatic dynamics simulations of both the wild type (WT) ChR2 and its E123T mutant are carried out using the *ab initio* multiple spawning (AIMS) method in a QM/MM setting, where spin-restricted ensemble-referenced Kohn-Sham (REKS) method is used to describe the QM region. Our simulations agree well with the experiments, and highlight the interplay between the photochemical reaction and the surrounding protein environment. (1) The E123T mutation changes the protein's electrostatic environment around the RPSB, and significantly slows down its photoisomerization. (2) The photoisomerization facilitates its subsequent deprotonation and the hydration of the ion channel. This work presents the first simulation of the photodynamics of ChR2 with a correlated first-principles electronic structure method, and provides design principles for new optogenetic tools.

Method

- System setup: crystal structures of ChR2 (PDB ID: 6EID) and the E123T mutant embedded in POPC lipid bilayer and solvated by water molecules.
- Classical molecular dynamics (MD) equilibration for ~200ns using the Amber14 force field
- Quantum mechanics/molecular mechanics (QM/MM) ground state MD equilibration for ~2 ps:
  - QM method: ωPBEh/6-31G for retinal and counterions
  - MM method: Amber14 for rest of protein, lipid and water
- Non-adiabatic dynamics simulation using *ab initio* multiple spawning (AIMS)
  - Accurate on-the-fly calculation of energies, gradients and nonadiabatic couplings by REKS(2,2)/Amber14, with GPU acceleration in TeraChem
- Umbrella sampling simulations of ~5 ps with QM/MM on both the excited and ground state.

REKS(2,2)



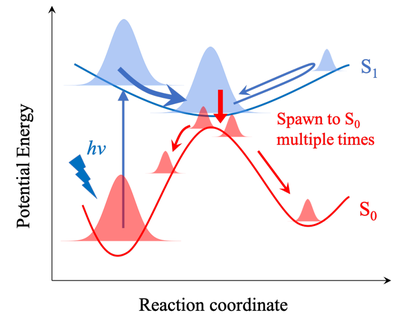
$$\rho_s = \sum_{L=1}^{M} C_L \rho_L$$

$$E[\rho_s(\vec{r})] = \sum_{L=1}^{M} C_L E[\rho_L(\vec{r})], 0 \le C_L \le 1, \sum_{L=1}^{M} C_L = 1$$

GPU acceleration

TeraChem

AIMS



Results

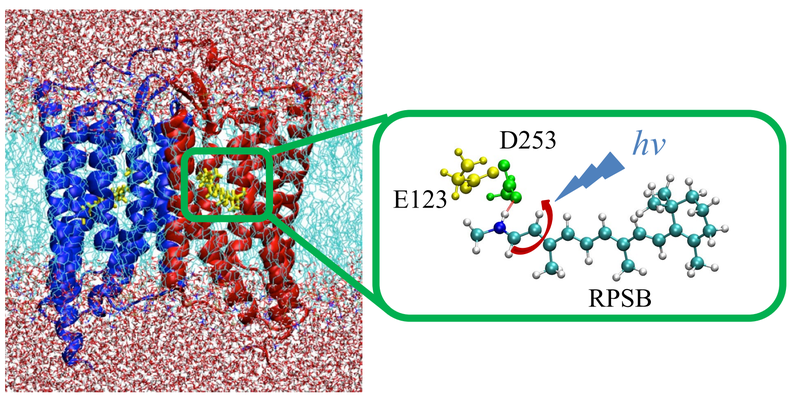


Figure 1. The ChR2 embedded in a lipid bilayer and solvated by water. The QM region (green box) contains the RPSB and the two nearby counterions.

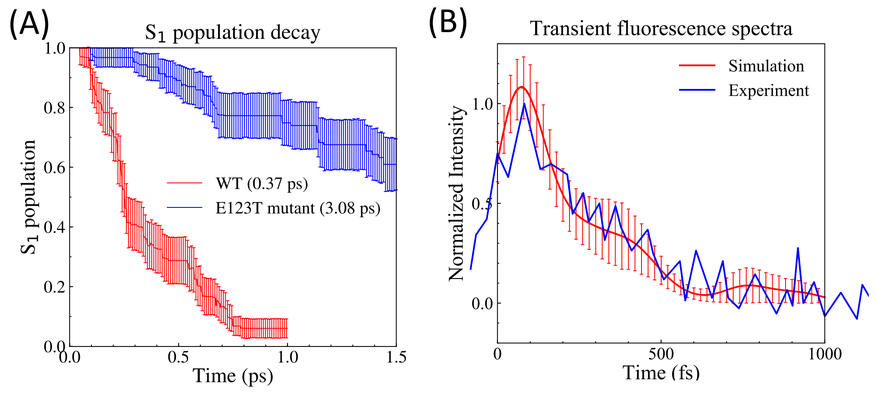
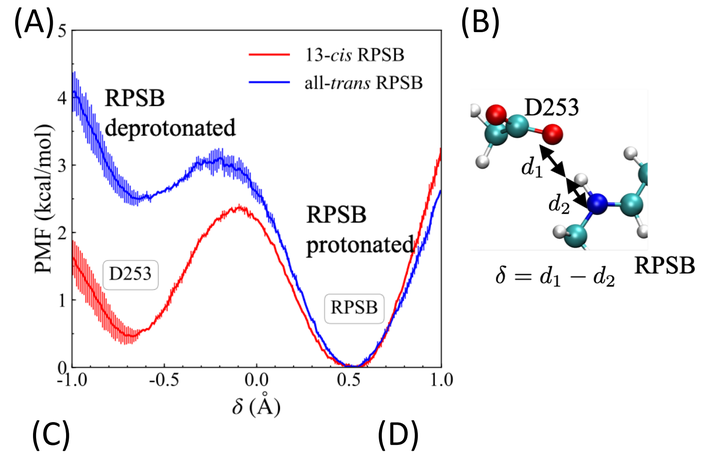
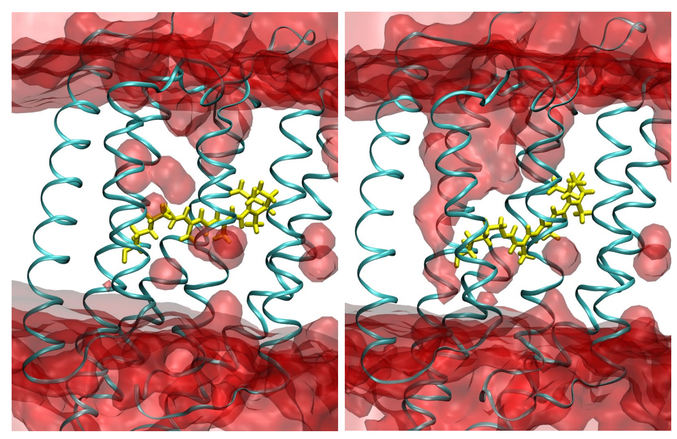


Figure 2. The E123T mutation slows down the decay of S_1 state population. (A) Decay of S_1 state population over time for WT ChR2 and E123T mutant. The calculated decay time constants (in the parenthesis) agree well with experiments. (B) Simulated transient fluorescence spectra of WT (red) agrees well with experiment (blue).





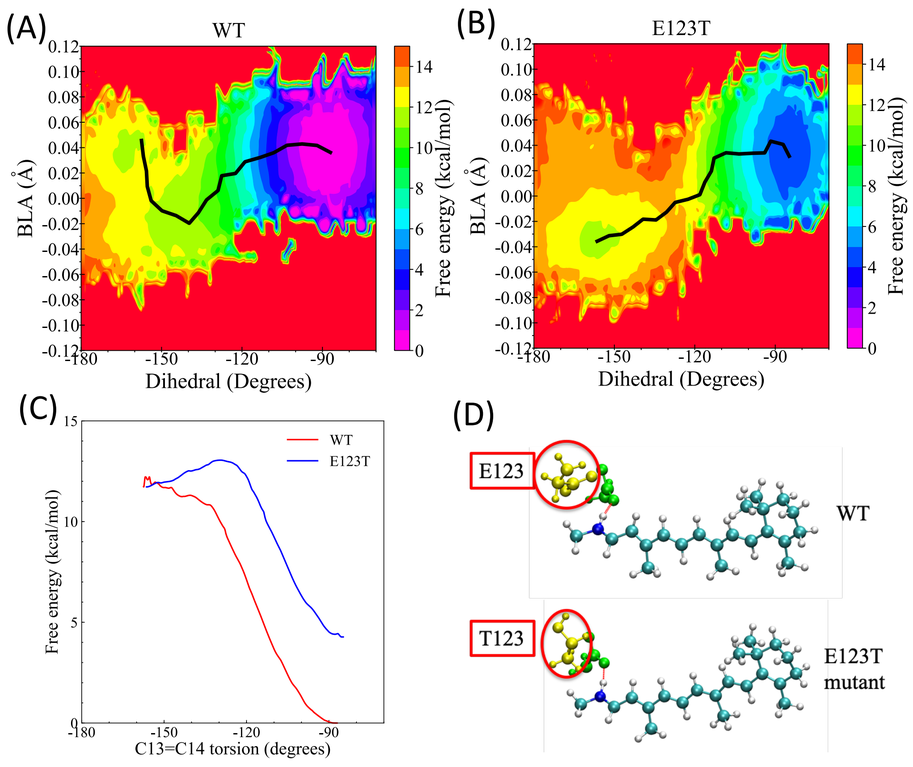


Figure 3. The E123T mutation alters the pathway and barrier of the RPSB photoisomerization. (A-B) 2D free energy surface of RPSB photoisomerization on the S1 state. (C) Free energy profile along the minimum free energy pathway in (A) and (B). (D) The amino acids nearby the RPSB in WT ChR2 and the E123T mutant.

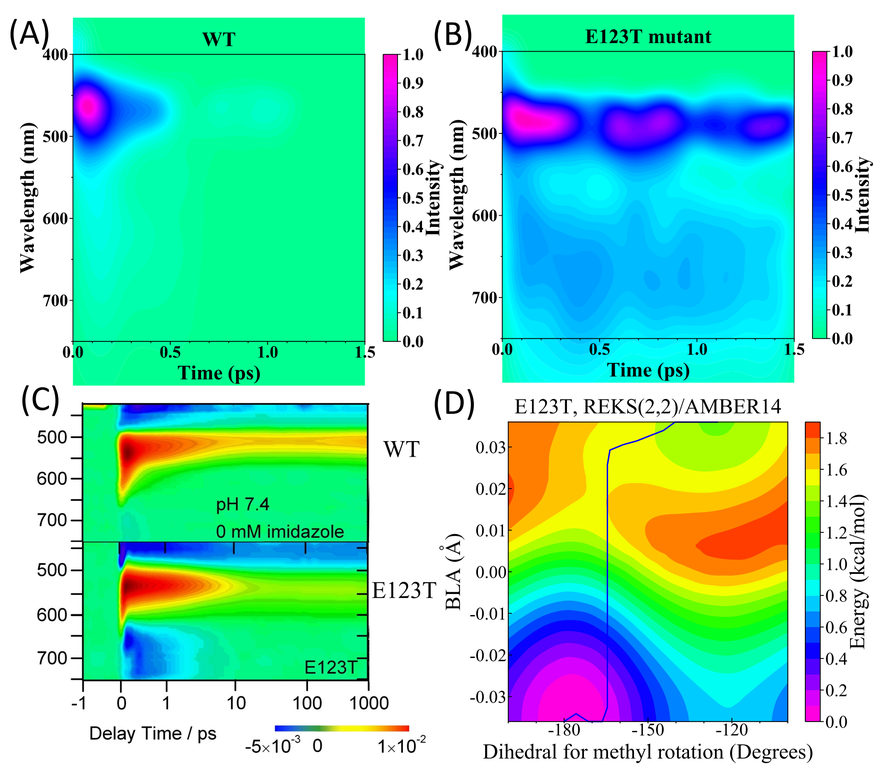


Figure 4. The E123T mutation elongates the lifetime of the fluorescent states. (A-B) The simulated transient 2D fluorescence spectra for WT ChR2 and the E123T mutant, respectively. (C) The experimental transient absorption difference spectra for WT ChR2 and the E123T mutant.[4] (D) The two S_1 state minima with different BLA values corresponding to the two fluorescent states in (B).

Figure 5. The RPSB photoisomerization facilitates its deprotonation and increases channel hydration. (A) The QM/MM free energy profile of RPSB deprotonation along the reaction coordinate (defined in (B)) on the ground state before (blue) and after (red) isomerization. (C-D) density of water molecules (red) calculated from 1μs classical MD simulation for (C) all-*trans* RPSB (before isomerization) and (D) 13-*cis* RPSB (after isomerization).

Conclusions

- Protein electrostatic environment around the RPSB changes the pathway and barrier of the photoisomerization reaction.
- RPSB photoisomerization triggers its deprotonation and increases the hydration level in the channel, which leads to channel opening.

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1. **Liang R**, Liu F, & Martínez TJ (2019) *J. Phys. Chem. Lett.* 10(11):2862-2868.
2. **Liang R**, Yu JK, Meisner J, Liu F, & Martinez TJ (In preparation)
3. Verhoefen M-K, et al. (2010) *ChemPhysChem* 11(14):3113-3122.
4. Scholz F, Bamberg E, Bamann C, & Wachtveitl J (2012) *Biophys. J.* 102(11):2649-2657.