

Local electric field effects in the chromophore binding pocket of Agp2

Introduction

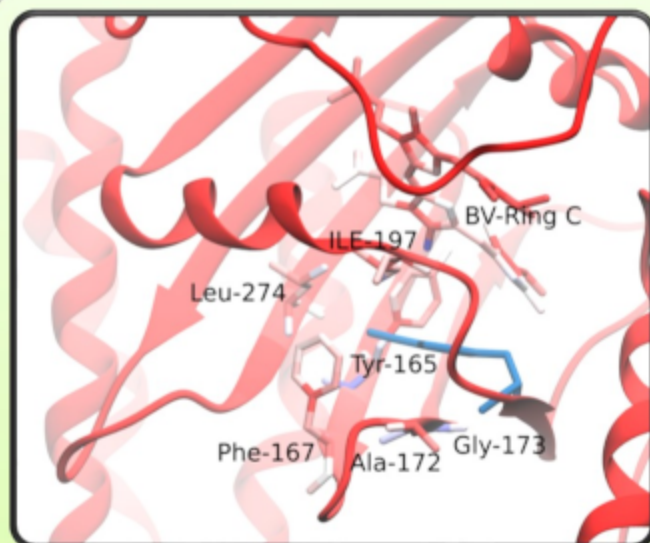
The bacteriophytochrome Agp2 belongs to the group of biliverdin IX α (BV) binding photoreceptors that control the switch between the physiologically active state, which for the bathyphytochrome Agp2 is the Pfr state and the inactive Pr state. The Pfr state is characterized by a ZZssa conformation of the chromophore and a protonated propionic side chain on ring C (PsC). After illumination the methine bridge between ring D and ring C isomerizes resulting in a change from a ZZssa to a ZZZssa conformation. This event triggers the formation of the Lumi-F and Meta-F intermediaries ultimately leading to the formation of the Pr state. There is strong experimental evidence that the trigger that leads to the secondary structure change of the tongue is of electrostatic nature [4]. To investigate the electrostatic effects a combination of vibrational stark spectroscopy and computational methods using wild type Agp2 protein as well as PairFP2 structure in the Meta-F state as templates for the model systems

Methods

Energy minimization, heating, equilibrating and production of the model systems were performed on molecular mechanical level using CHARMM36 force field [1] and the NAMD-V2.10 software [2]. After thermal equilibration a simulation run of twenty nanoseconds was performed. Fifty snapshots were extracted at the end of the simulation for the subsequent QM/MM geometry optimization.

Geometry optimization was performed at the QM/MM level. In all models the QM region included the chromophore, the side chains of Cys20, the Asp196 as well as the pyrrole water described and depending on the model F192pCNF or Y165pCNF at the B3LYP/6-31G* level of theory. The protein matrix was described molecular mechanically using CHARMM36 force field [1]. The mobile part of the system was chosen to be 20Å around atom N22 of the BV cofactor. The charge-shifted scheme in combination with the electrostatic embedding approach was used to couple the QM and the MM region [3].

Pfr model



No hydrogen bonds in stark reporter vicinity.

Linear correlation of CN stretching frequency and E-field

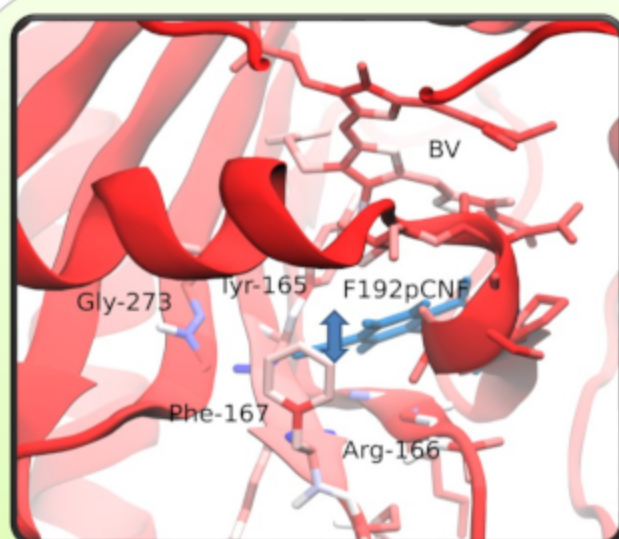
Pr model



In Pr crystal water is better stabilized -> no interaction with CN.

Pro-459(tongue) and Asn-443(coil) can modulate E-Field via crystal water -> possible way to trigger secondary structure change

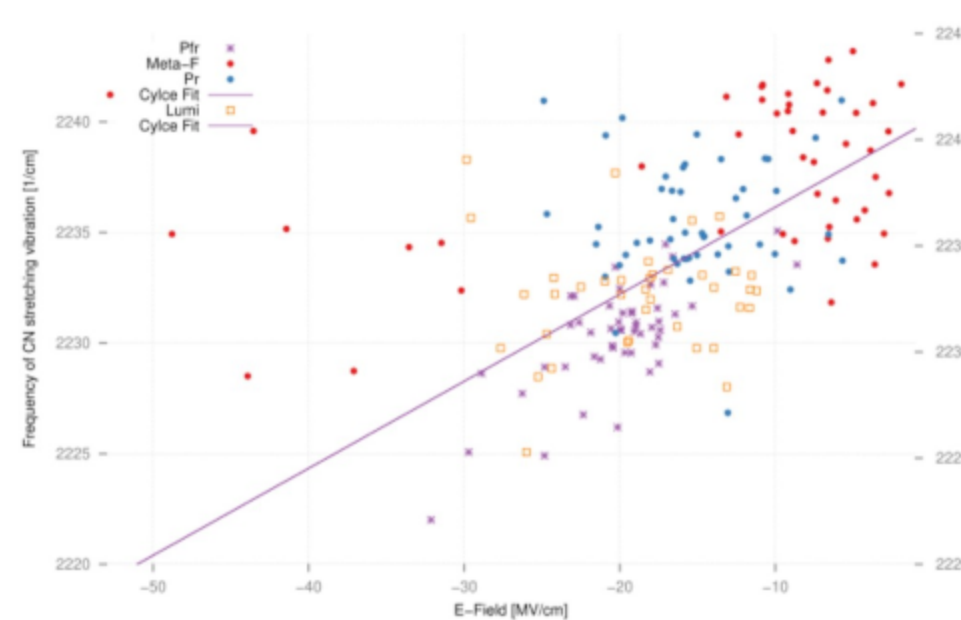
Lumi-F model



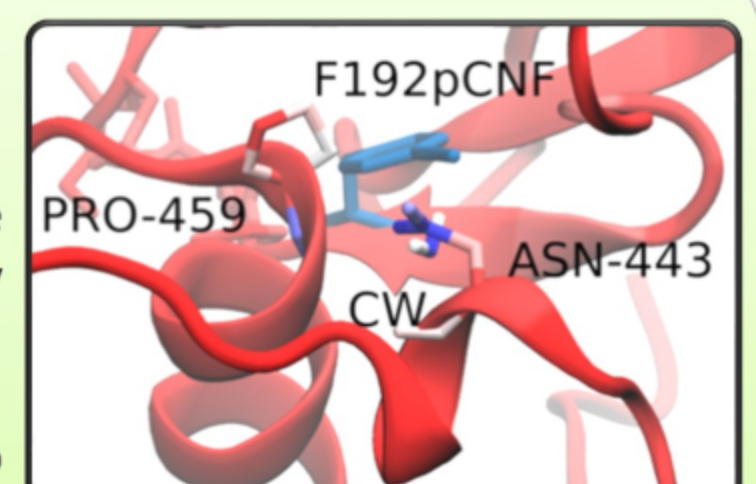
Two conformation of PCN

Flipping of Ring D has significant influence on protein structure (RMSD 2,4 -> 3.1)

Frequency vs E-Field during Photocycle



Meta-F model



Only model where the E-field significantly exceeds 30MV/cm.

Crystal water able to form hydrogen bond with CN -> increasing the E-Field

References

- [1] MacKerell Jr, Alex D., et al. "All-atom empirical potential for molecular modeling and dynamics studies of proteins." *The Journal of physical chemistry B* 102.18 (1998): 3586-3616.
- [2] Phillips, James C., et al. "Scalable molecular dynamics with NAMD." *Journal of computational chemistry* 26.16 (2005): 1781-1802.
- [3] Billeter, Salomon R., Alexander J. Turner, and Walter Thiel. "Linear scaling geometry optimisation and transition state search in hybrid delocalized internal coordinates." *Physical Chemistry Chemical Physics* 2.10 (2000): 2177-2186.
- [4] Fernandez Lopez M, Nguyen AD, Velazquez Escobar F, González R, Michael N, Nogacz Z, Piwowarski P, Bartl F, Siebert F, Heise I, Scheerer P, Gärtner W, Mroginski MA, Hildebrandt P. Role of the Propionic Side Chains for the Photoconversion of Bacterial Phytochromes. *Biochemistry*. 2019 Aug 20;58(33):3504-3519. doi: 10.1021/acs.biochem.9b00526. Epub 2019 Aug 6. PMID: 31348653.