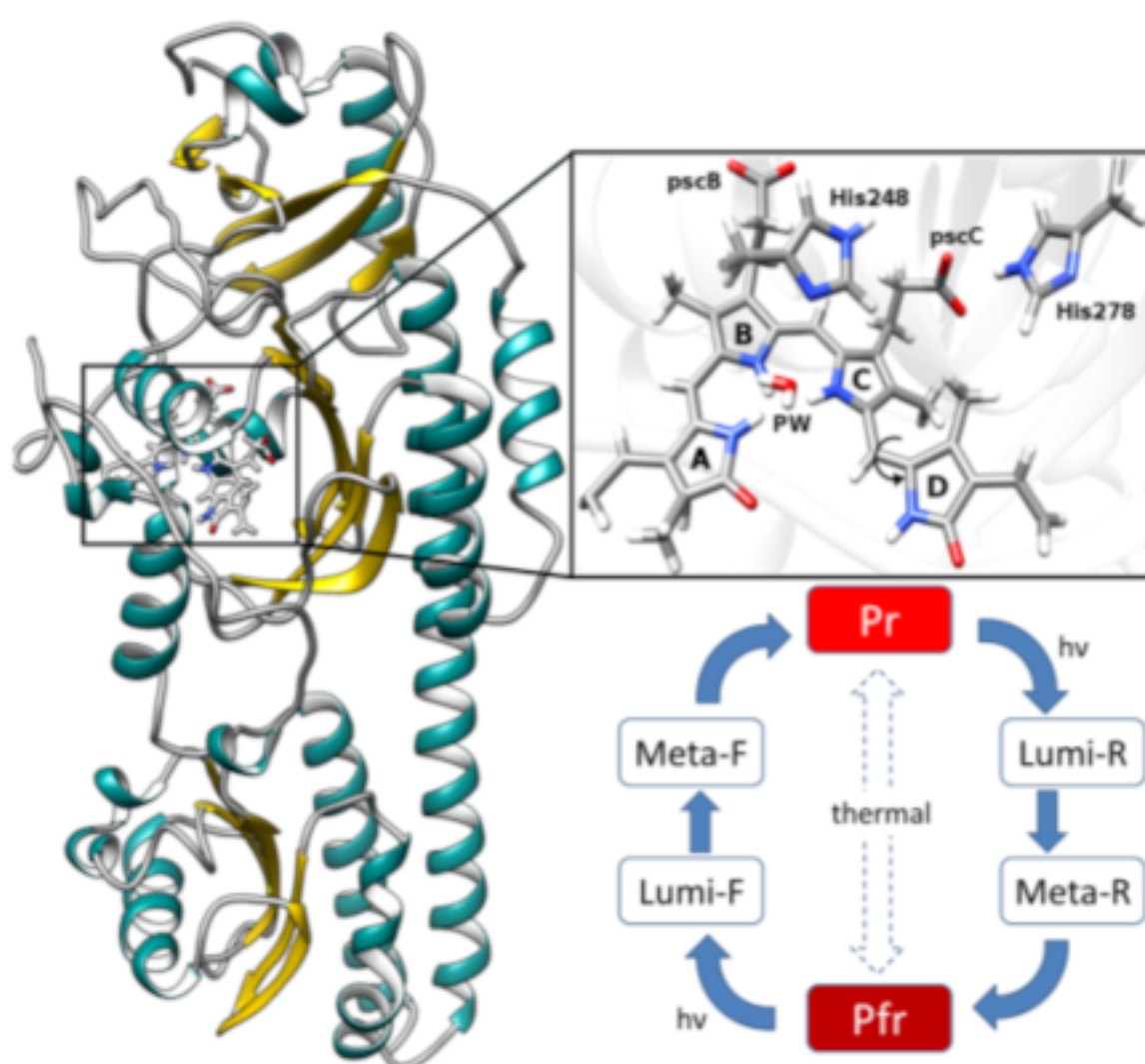


## Introduction

The photoactivation processes of photoreceptor proteins are achieved in part by protonation changes of the chromophore and key residues of the protein matrix. These protonation changes, in turn, facilitate the proton transfer reactions involved in the photoactivation. Thus, the precise determination of the protonation states of chromophores and titratable amino acids constitutes a major step in computational modeling of photoreceptor proteins. In this work, the protonation states of Agp2 phytochrome structure in the Pfr and Meta-F states will be investigated by using classical and constant pH-MD simulations.

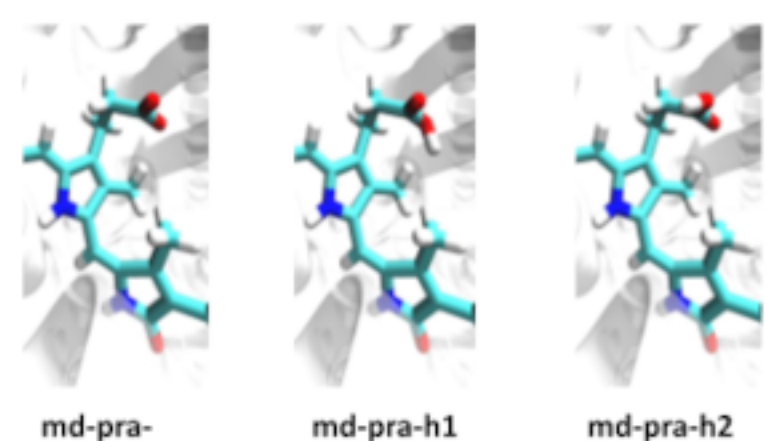
## MD+Karlsberg2+

- According to the experimental Raman spectra, the protonation state of propionic side chain C of ring C (pscC) can change during the photocycle of Agp2 phytochrome.
- pscC is protonated in the Pfr and Meta-F state.
- The proton release event takes place in the transition from Meta-F to Pr state. Ref.1,2.

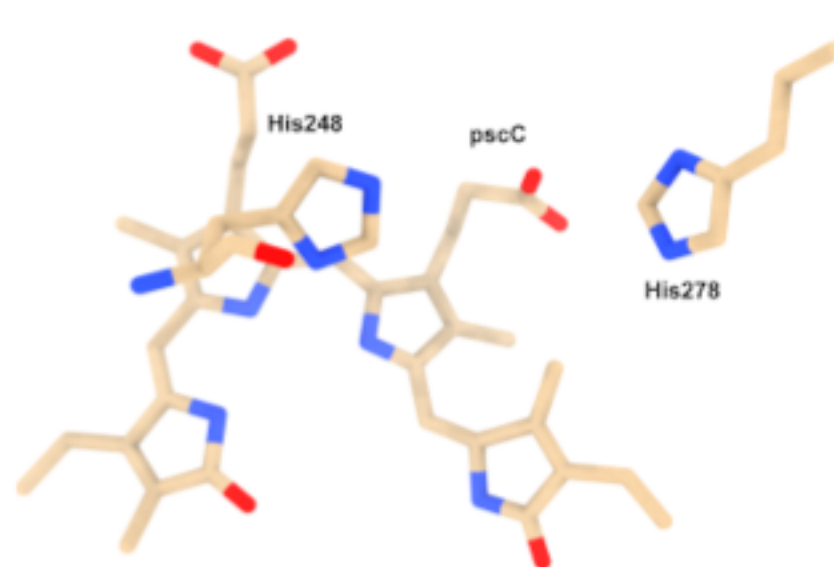


## Methods / procedure

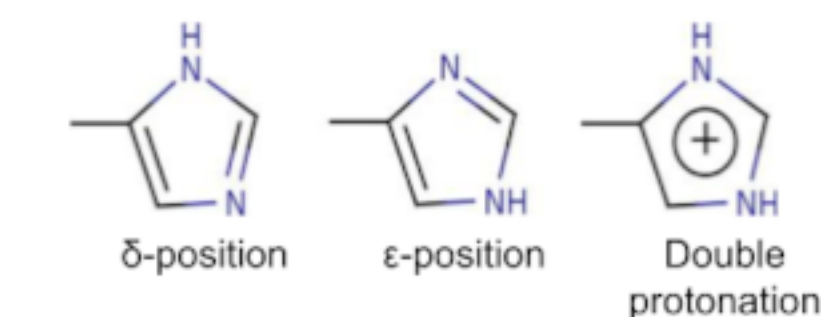
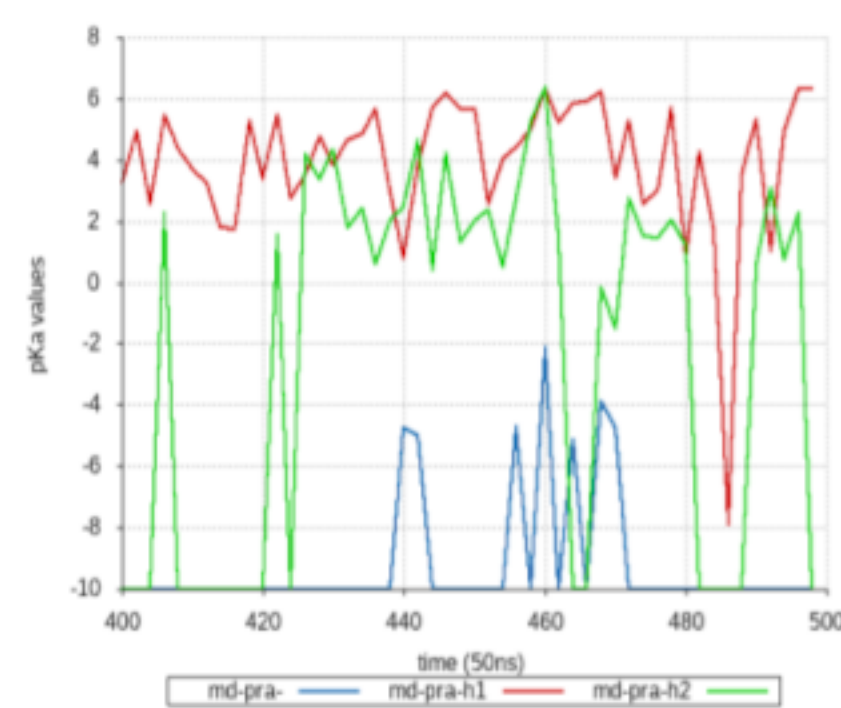
### Classical MD simulations of Agp2 phytochrome structure



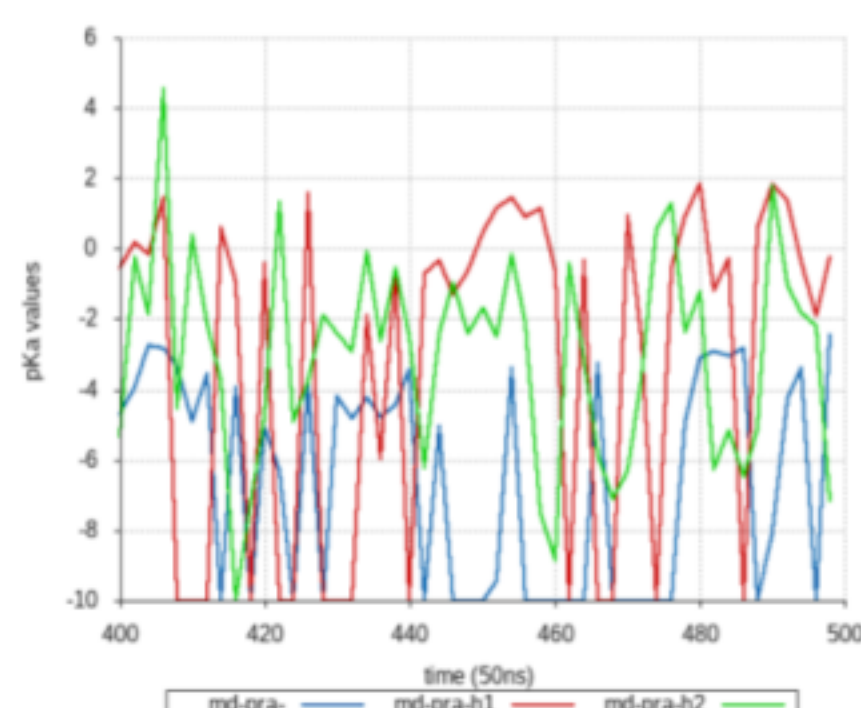
- Choosing a set of protonation patterns.
- Electrostatic energy computations for each frame of the MD trajectories.
- Averaging the results of the electrostatic energy computations within each MD trajectory and combining the averaged results to obtain titration curves and pKa values using Karlsberg program Ref.3.



- 27 MD trajectories, too many combinations with the current implementation of Karlsberg!



- His248 and His278 are in direct contact with the pscC.
- Both His248 and His278 have to be sampled in order to get a more realistic description.
- Sampling of both conformational and protonation states.



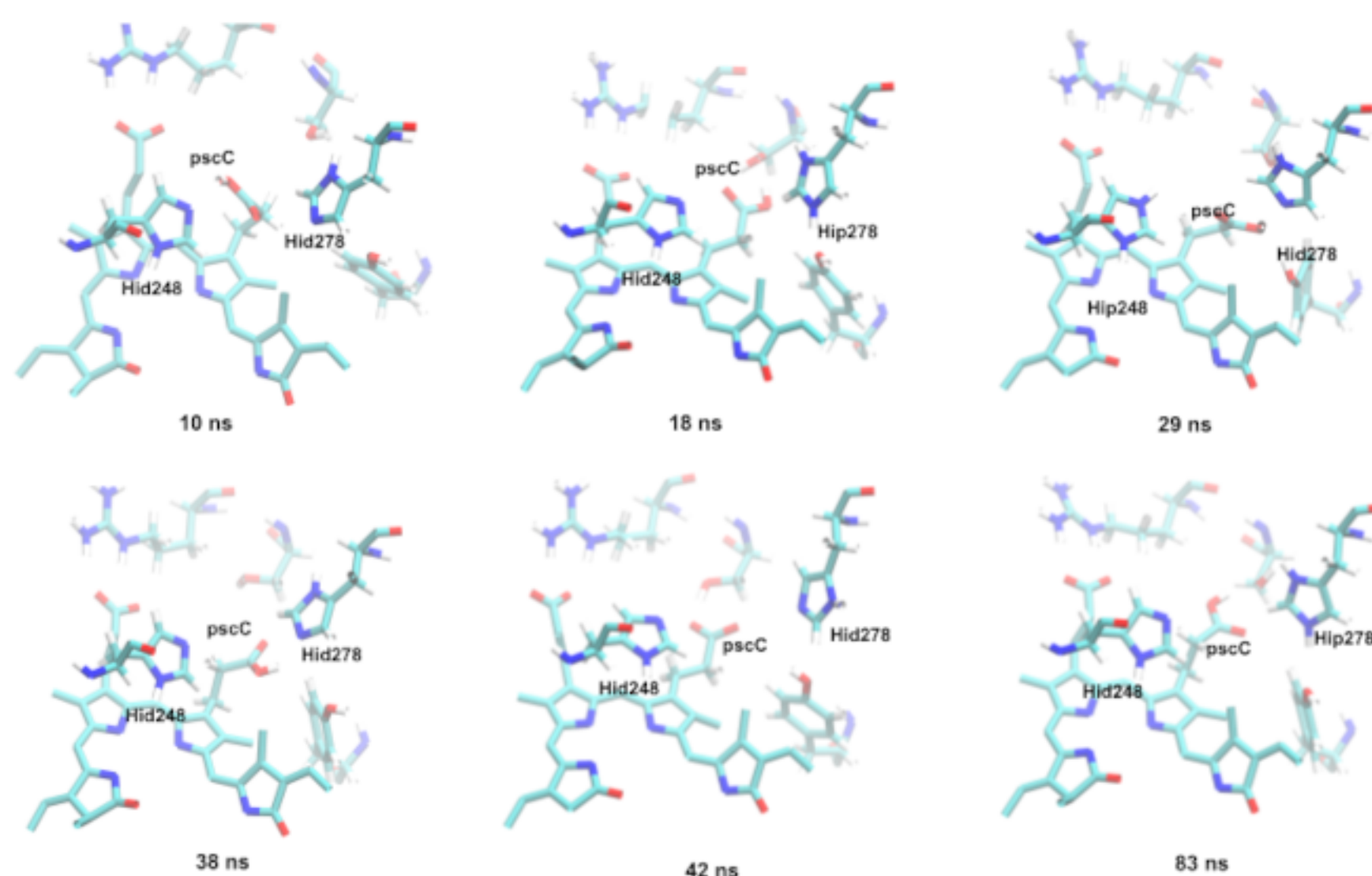
	Agp2 Pfr	Agp2 Meta-F
pKa (pscC)	1.10	0.10

**pKa < 7.0**  
**In contradiction with the experiment!**  
**Sampling of both conformational and protonation states is needed!**

## FMO calculations

### Constant pH-MD simulations of Agp2 phytochrome structure

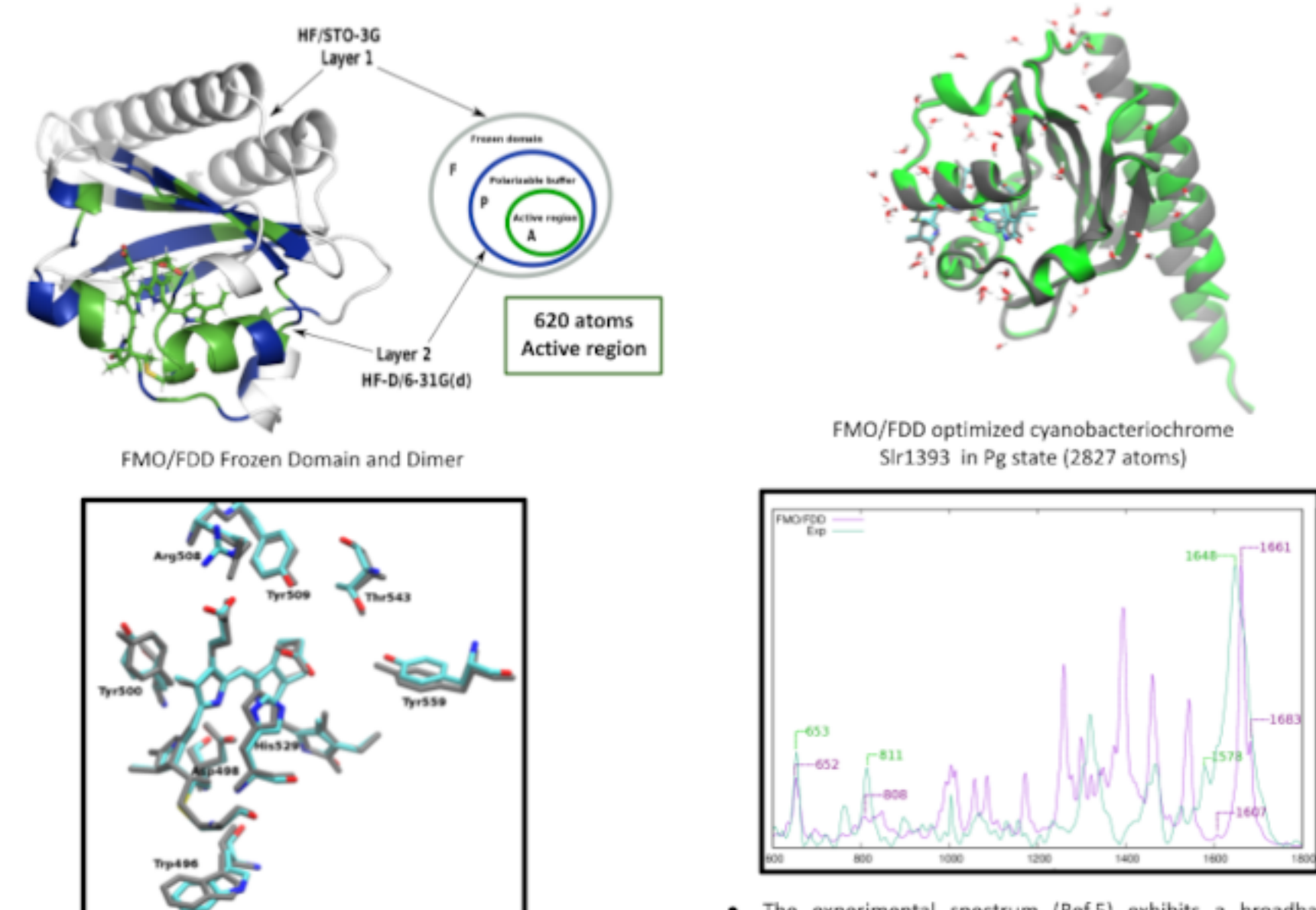
The constant-pH molecular dynamics (pHMD) based-methods have been used for studying many important biological processes caused by changes in solution pH. One of the main advantages of the constant pHMD approaches is that the protonation states may change in the course of conformational dynamics. Thus, the dynamical processes coupled to a change in protonation states can be directly studied. In this work, we used the constant-pH MD algorithm implemented in the AMBER program Ref.4.



	pKa-Agp2 Pfr	pKa-Agp2 Meta-F	Population-Pfr	Population-Meta-F
pscC	7.1	7.0	Deprot 44%, Prot 46%	Deprot 47%, Prot 43%
His248	4.9	4.0	Double-P 1%, $\delta/\epsilon$ 99%	Double-P 0%, $\delta/\epsilon$ 100%
His278	6.4	7.5	Double-P 20%, $\delta/\epsilon$ 80%	Double-P 77%, $\delta/\epsilon$ 33%

- pKa calculations based on constant-pH MD simulations predict a protonated pscC in both Pfr and Meta-F states, which is in good agreement with the experimental Raman spectra.
- The protonation states of both His278 and pscC are highly correlated. This suggests that the His278 is the most likely proton acceptor site.

### Simulations of Raman spectra using the FMO method



- The root-mean-square-deviation (RMSD) value obtained for the heavy atoms of the PCB chromophore (optimized structure) with respect to the crystal structure is relatively small (0.34 Å).
- The most significant changes observed correspond to the reorientation of the side chains of the Trp496, His259, Arg508, and Tyr559 residues.
- The experimental spectrum (Ref.5) exhibits a broadband corresponding to the C=C stretching mode (1648 cm<sup>-1</sup>) this band is reflected in the FMO/FDD Raman spectrum located at 1661 cm<sup>-1</sup> with a very similar intensity.
- The band at 1578 cm<sup>-1</sup> (experimental) is characteristic of a protonated chromophore in the Pg state. This band is located at 1607 cm<sup>-1</sup> in the FMO/FDD spectrum.
- In general, the FMO/FDD spectrum shows a reasonable agreement with the experimental Raman spectrum.