

## System

### Agp2 Phytochrome

- Dimer photosensory domain in Pfr and Meta-F state
- Monomer photosensory domain WT and mutants:
- Hip278, H278A, Y165F, R211A

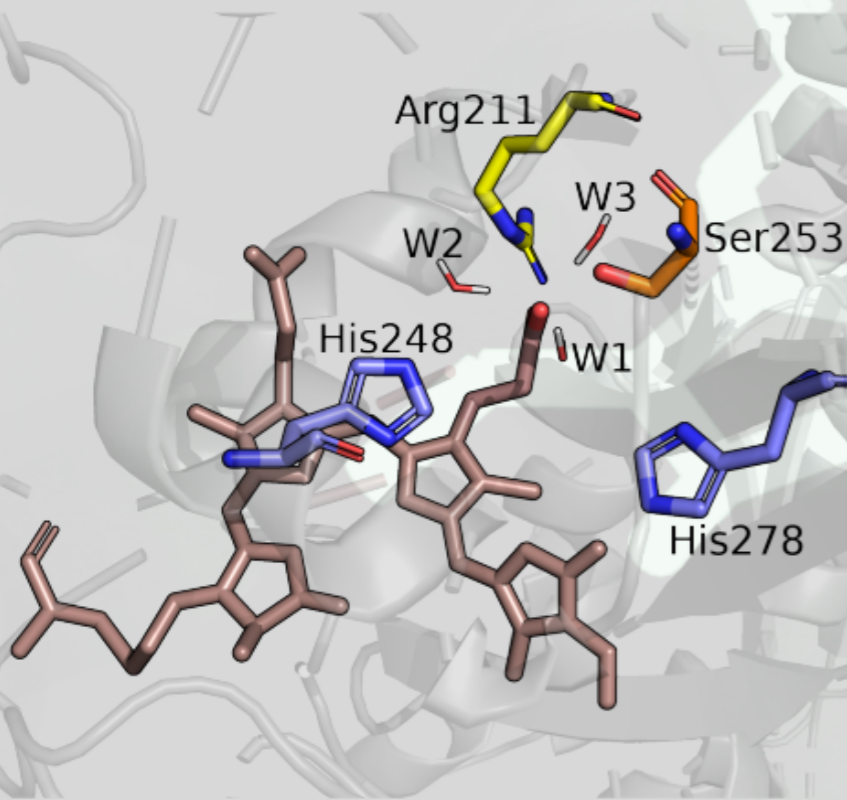
## Method

### Constant pH

- PH value set (7.0)
- Sampling concurrent with the MD
- MD done in explicit solvent, proton assignment in implicit solvent
- Monte Carlo sampling of the Boltzmann distribution of discrete protonation states
- New protonation attempted every n frames (100 fs)
- Library of titratable AA: Asp, Glu, His, Lys, Tyr, Cys and Propionate

## Results

Chromophore binding pocket  
Including Biliverdin and the two  
key Histidine residues



### Dimer

Chain A	Pfr pKa	Pfr pop	Meta-F pKa	Meta-F pop
Prop-C	8.9	D 4% P 96%	7.6	D 21% P 79%
HIS 248	4.5	P 0% D/E 100%	5.3	P 2% D/E 98%
HIS 278	5.9	P 7% D/E 93%	7.1	P 56% D/E 44%
Chain B				
Prop-C	8.7	D 1% P 99%	6.8	D 61% P 39%
HIS 248	4.9	P 1% D/E 99%	6.2	P 23% D/E 77%
HIS 278	6.4	P 22% D/E 78%	7.9	P 89% D/E 11%

### Monomer

WT	Pfr pKa	Pfr Pop	Meta-F pKa	Meta-F Pop
Prop C	7.9	D 30%, P 70%	7.0	D 50%, P 50%
His248	5.0	P 1%, D/E 99%	6.1	P 29%, D/E 71%
His278	6.1	P 14%, D/E 86%	7.3	P 67%, D/E 33%
HIP278				
Prop C	8.0	D 21%, P 79%	7.9	D 25%, P 75%
His248	4.7	P 1%, D/E 99%	4.8	P 1%, D/E 99%
H278A				
Prop C	9.2	D 1%, P 99%	7.1	D 46%, P 54%
His248	4.3	P 0%, D/E 100%	4.9	P 1%, D/E 99%
Y165F				
Prop C	9.6	D 0%, P 100%	7.3	D 38%, P 62%
His248	5.0	P 1%, D/E 99%	4.6	P 0%, D/E 100%
His278	6.0	P 12%, D/E 88%	7.7	P 71%, D/E 29%
R211A				
Prop C	7.6	D 22%, P 78%	6.8	D 62%, P 38%
His248	5.8	P 15%, D/E 85%	4.7	P 1%, D/E 99%
His278	6.8	P 39%, D/E 61%	7.5	P 77%, D/E 23%

## Conclusions

- Constant pH method provides good pKa values, in agreement with experimental results
- Strong correlation between Prop-C and His278 pKa values
- His278 is the most likely protonation partner of Prop-C