B6

SFB 1078

Structural Characterization of bacterial and fungal phytochromes in dark and illuminated state

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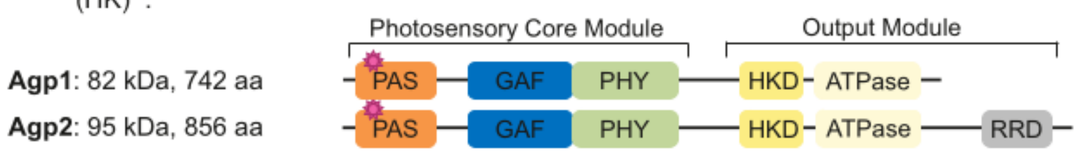
INTRODUCTION

Phytochromes are modular photoreceptors that use red light as source of information to mediate various reactions in different organisms such as plants, cyanobacteria, fungi and heterotrophic bacteria[1].

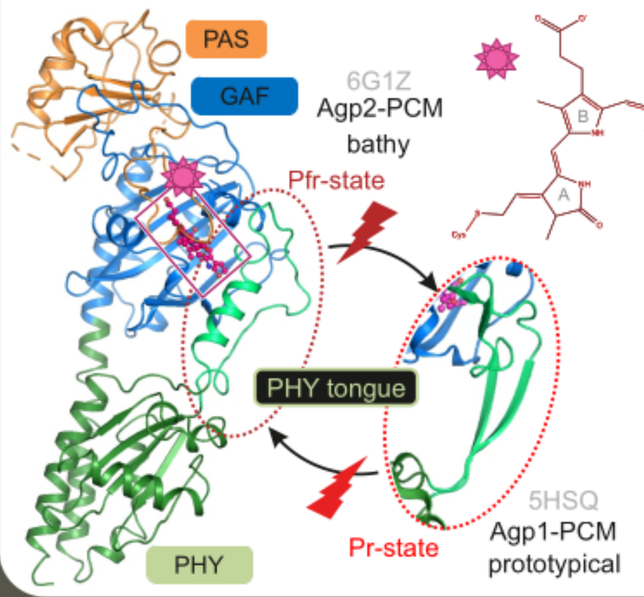
The bacterial pytochromes Agp1 and Agp2 of *Agrobacterium fabrum* are involved in infection of plant stems as well as roots causing plant tumors[3].

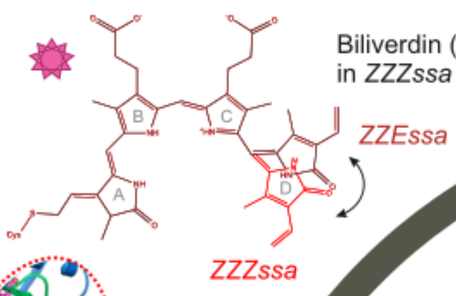
Based on Agp2-PCM, Pietkevich et al. engineered the fluorescence-optimized Agp2-PAiRFP2. Due to its photoswitchability and its photoactivation in NIR light, it is a very promising candidate for optogenetic applications[4].

The photoswitchable protein usually consists of two conserved parts: a N-terminal photosensory core module (PCM) and a C-terminal output module. The PCM includes PAS, GAF and PHY domains. The variable output module is mostly a histidine kinase (HK)[1].



PAiRFP2-FL = Agp2-FL + 24 mutations (fluorescent optimized)





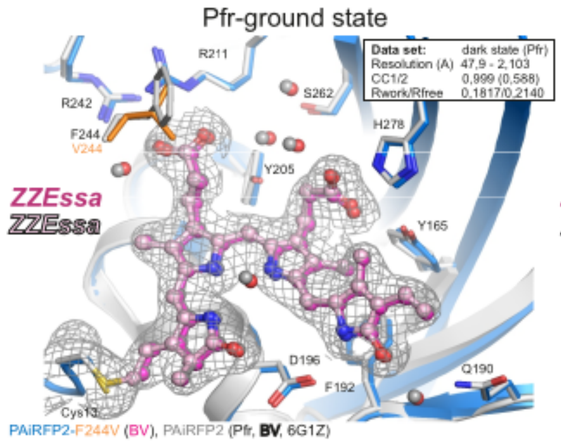
Biliverdin (BV) is incorperated in *ZZZssa* stereochemistry.

PHOTOCONVERSION OF AGP2-PAiRFP2-F244V

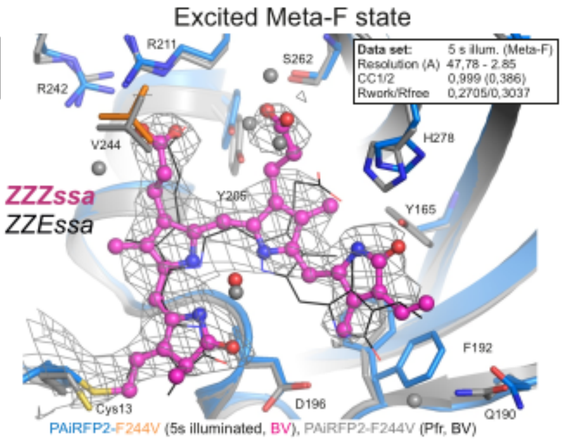
The back-mutation of phenylalanine 244 from Agp2-PAiRFP2 to the natural valine residue, which occurs in wild-type Agp2-PCM, leads to a one-third decrease in fluorescence. How this mutation affects the photocycle is shown below:

grey mesh: 2*Fo-Fc* electron density map contoured at 1σ

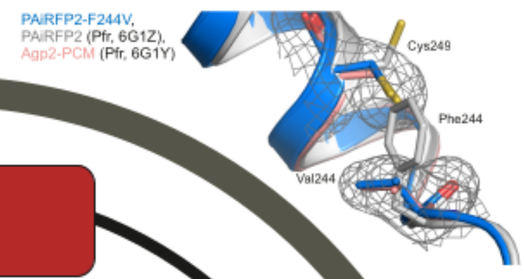
Pfr-ground state



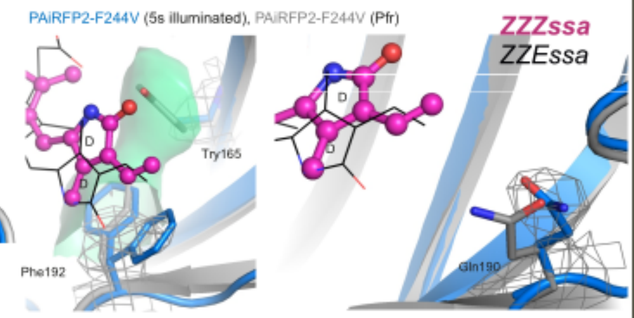
Excited Meta-F state



Conformation of PAiRFP2-F244V mutant matchs with PAiRFP2 except for Cys249:



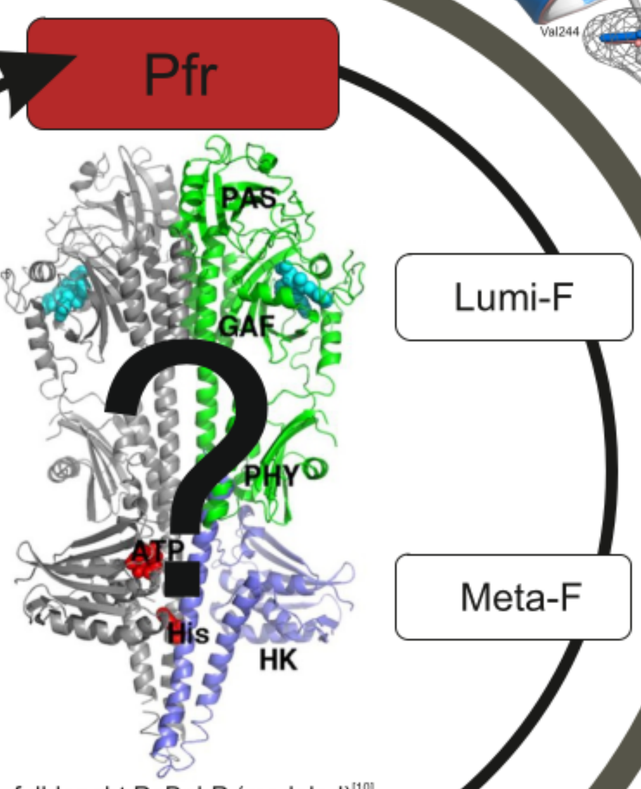
After excitation PAiRFP2-F244V undergoes a similar photocycle as PAiRFP2. Both stuck in a Meta-F or Meta-F like state.



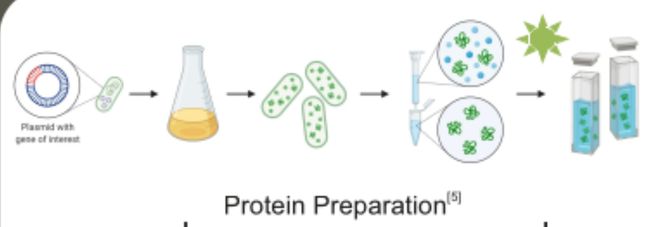
grey mesh: 2*Fo-Fc* electron density map contoured at 1σ
green: Δ Fo Polder Map contoured at 3σ

Conjugated π-electrons of Phe244 extend the π-electron system of BV via π-stacking.

What is the impact of Phe244 in Agp2-V244F?

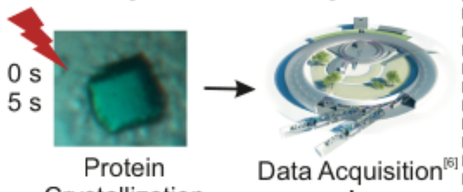


X-RAY VS. CRYO EM



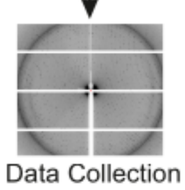
Protein Preparation[5]

X-Ray Structure Analysis



Protein Crystallization

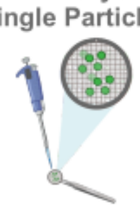
Data Acquisition[6]



Diffraction Data Processing[5]

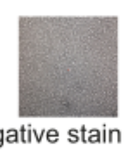
Data Collection

Cryo-EM Single Particle Analysis



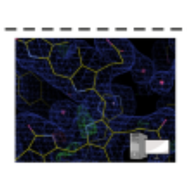
Grid Preparation[5]

Data Acquisition[5]



negative stain + cryo data

Image Processing[5]



Structure Determination

Refinement and Validation of structure model

+ high atomar resolution[7]
+ not limited by molecular weight[7]
- static snapshot[7]
- sample must be crystallizable
→FL-constructs so far not crystallizable

+ closer to native state[8]
+ heterogenity possible[9]
- relatively low resolution[8]
- limited to large complex[8]
BUT: Cryo-EM techniques are continually improving
→applicable for FL-constructs

FULL-LENGTH PHYTOCHROME

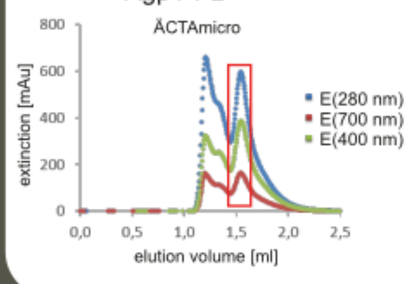
The structural conformation of phytochrome-PCM leads to a new arrangement of the PHY tongue either from an α-helix, loop and coiled conformation to a β-sheet consisting of two β-stands and a β-hairpin or vice versa[1]. The conformational reorganization of the PHY tongue influences the activity of the output module[1].

Since it has not yet been possible to obtain a structure of full-lenght phytocrome, there are still open questions remaining:

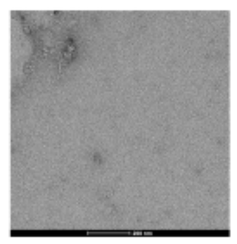
What is the protein structure of the Histidine Kinase?? How does the PCM interacts with the output module after excitation??

Method: Observation of full-lenght protein structure by Cryo-EM

Agp1-FL

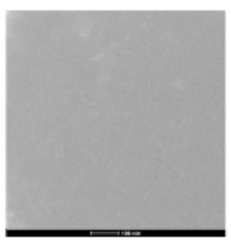


Negative stain



25 ng/µL

Cryo grid



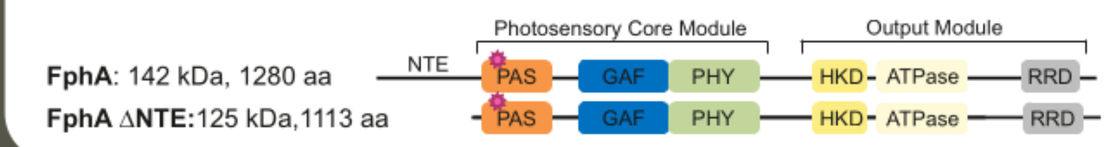
1 mg/mL

→ needs further optimization

FUNGAL PHYTOCHROMES

In fungi, phytochromes contribute to germination, sporulation, orientation of hyphal growth and the transition from the vegetative to the generative growth phase[2].
N-terminal extension (NTE) is involved in the control of thermal relaxation.

How does the NTE influences the photoconversion?



GOALS

Fluorescence properties:
- Crystal structure of Agp2-V244F
- role of Phe244 towards fluorescence

Full-lenght protein structure using Cryo-EM:
- structure of full-lenght phytochromes in their ground states and excited states
- investigation of coupling of PHY tongue and output module
- comparison of fungal and bacterial full-lenght phytochromes

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