B6

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Structural snapshot of a bacterial phytochrome in functional intermediate states

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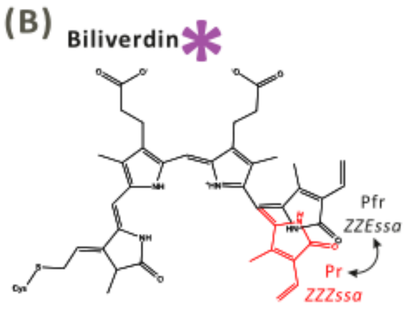
INTRODUCTION

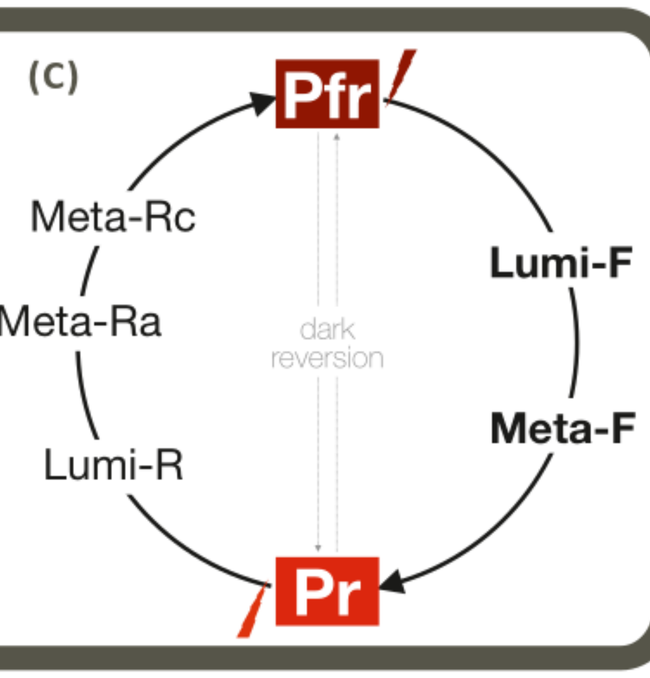
(A)

Phytochromes are primary photoreceptors which were found in plants, bacteria and fungi[1]. They enable the organism to adapt on their light environment, regulating essential processes as *e.g.* photosynthesis[1,2].
Because of their advantageous red-shifted absorption maxima, they allow *in vivo* deep tissue imaging[1], therefore they are interessting as optogenetic tools.
Bacteriophytochromes consist of a highly conserved photosensory core module (PCM) builded up by PAS-, GAF- and PHY-domain and a variable output module which is usually a histidine-kinase **(A)**. The chromophore, biliverdin **(B)**, is covalently attached to a cysteine within the PAS-domain[3].

(B)



(C)



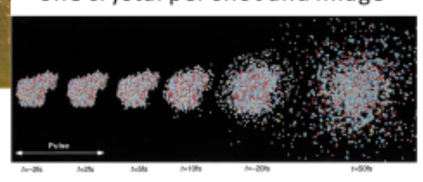
Induced by red/far-red light illumination phytochromes undergo a complex photocycle **(C)** with two stable parent states **Pfr** and **Pr**. For activation/deactivation of downstreaming signal cascades the protein has to translate light energy into structural information in terms of a chromophore isomerization **(B)** and a conformational change of the protein, by passing several intermediate states (Lumi/Meta). Structural information of the intermediates (especially Meta[4]) are still lacking, therefore, pump-freeze experiments to trap these states are required.
The fluorescence optimzed mutant Agp2-PAiRFP2 (truncated to PCM and including 24 subsitutions based on Agp2 from *Agrobacterium fabrum*) has a slowed photoconversion[1,5] and for this reason is appropiate for such investigations.
The free electron laser setup is ideal for room temperature pump-probe experiments. Here we present dark adapted and light triggered and time resolved intermediate state crystal structures of Agp2-PAiRFP2 gained at the free electron laser (XFEL) using pump-probe time-resolved protein X-ray Crystallograpy.

XFEL

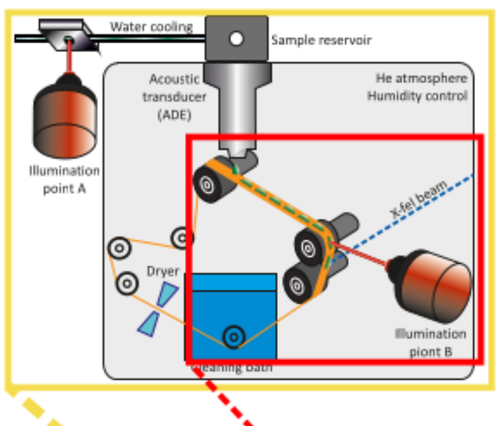
X-ray Free Electron Laser (XFEL)

[6]

- Linac Coherent Light Source (LCLS) at SLAC National Accelerator Laboratory, Stanford, USA
- high intense femtosecond X-ray pulses
- diffraction before destruction[7]
- one crystal per shot and image

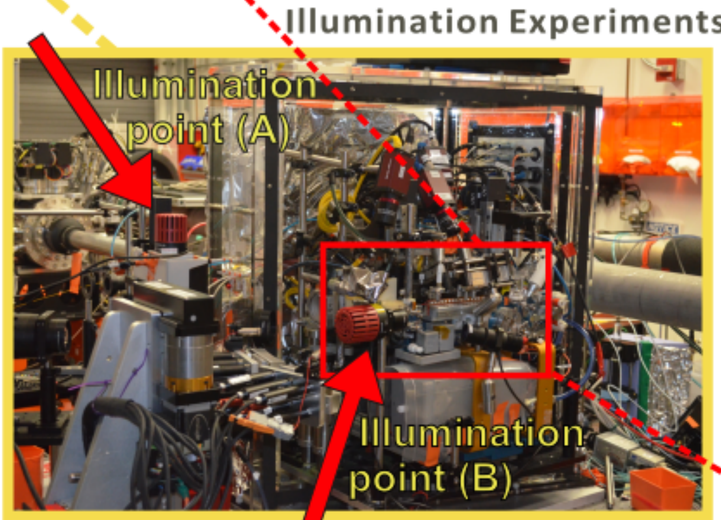


Sample Delivery System



Drop-on-demand sample delivery sytem[8]:
- huge amount of high dens crystal slurry needed (millions of crystals)
- crystal slurry injected via syringe and capillary to the delivery system
- acoustic droplet ejection (ADE) transfers drops on the belt (orange)
- conveyor belt system transports drops to the X-ray intersection point

Illumination Experiments



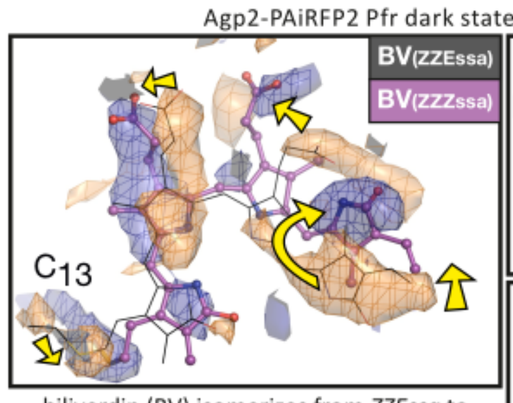
- experiments were performed based on illumination experiments at synchrotrons and spectroscopic data
- to reach the Meta-F intermediate state a few seconds are needed
- Lumi-F intermediate state needs a few nanoseconds

- for different illumination times two LEDs were installed
- illumination point (A): outside the chamber for longer illumination times
- illumination point (B): inside the chamber right above the belt for shorter illumination times
- both were used to trap different states between the Pfr and photoactivated state

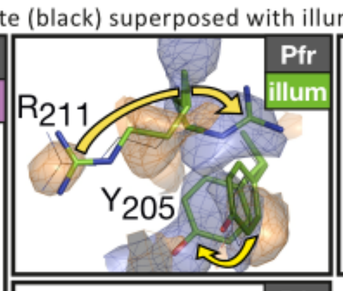
PUMP-PROBE TIME-RESOLVED X-RAY CRYSTALLOGRAPHY ON XFELs

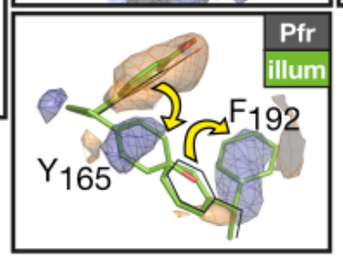
Details of XFEL Data

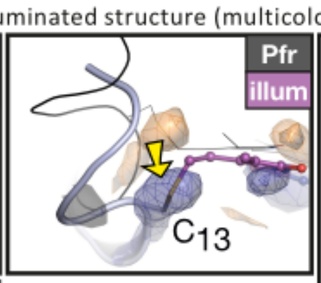
Agp2-PAiRFP2 Pfr dark state (black) superposed with illuminated structure (multicolored)



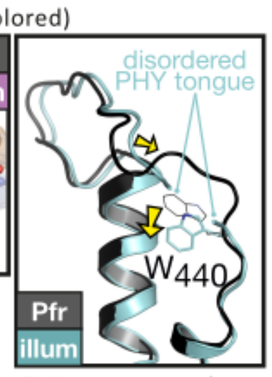
biliverdin (BV) isomerizes from *ZZEssa* to *ZZZssa* and relaxes
ΔFo (i7 state - dark state) electron density map





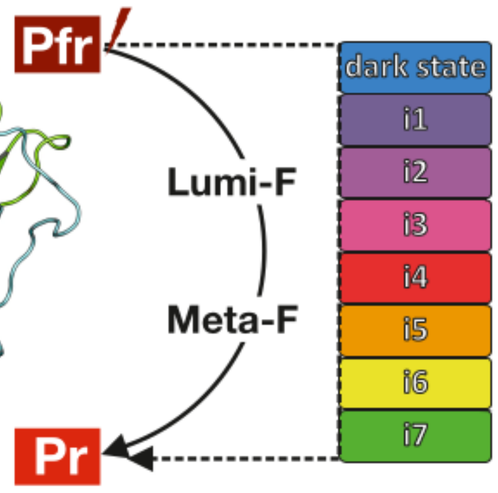


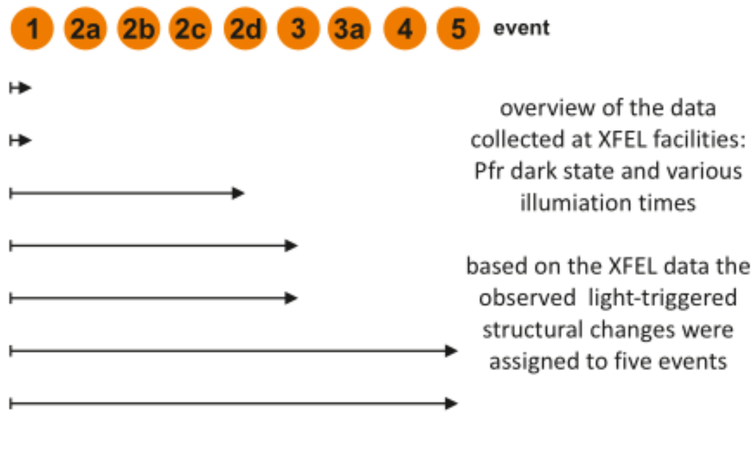
$Fo_{illum} - Fo_{dark}$ maps are contoured at 3 σ and show differences at the chromophore and in the chromophore binding pocket; selected amino acids are shown



PHY tongue reveals an alternative backbone trace in the illuminated state

Sequence of Events - Proposed Mechanism[9]

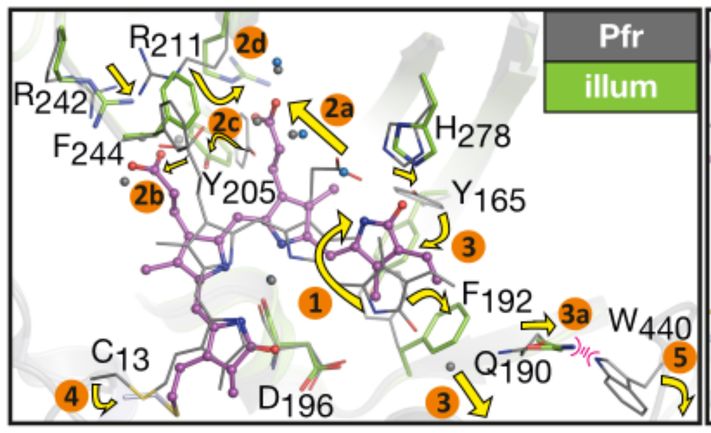


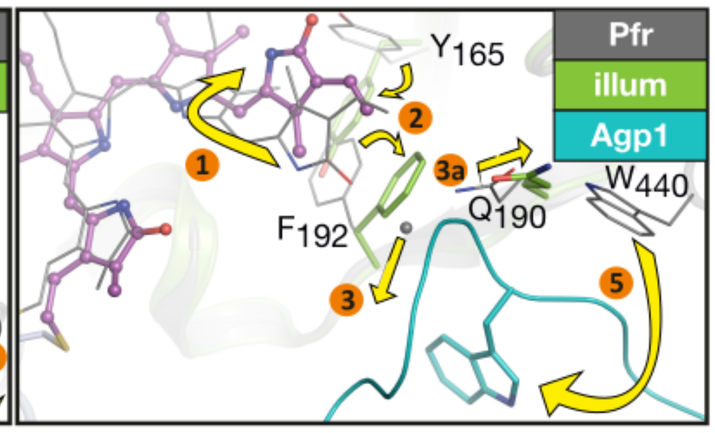


overview of the data collected at XFEL facilities: Pfr dark state and various illumiation times

based on the XFEL data the observed light-triggered structural changes were assigned to five events

superposition of Pfr dark state in black (biliverdin in *ZZEssa* conformation) and the i7 state multicolored (BV in *ZZZssa* conformation) **Event 1**: isomerization of BV; **Event 2**: shift of propionic side chains, rotation of Y205 and R211; **Event 3**: rotation of Y165 and F192, thus a water molecule is replaced and shift of Q190; **Event 4**: switch of thioether linkage; **Event 5**: switch of W440 and changed backbone trace within the PHY-tongue





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SUMMARY & FUTURE PLANS

Summary

- time-resolved measurement of Agp2-PAiRFP2 crystals at the free electron laser (LCLS, USA and SACLA, Japan)
- collection of datasets (2.1-2.8 Å) with different illumination times: dark state, i1 - i7 state
- sequence of changes within the protein observable
- **long range effects** (up to the PHY-tongue)

Sequence

- dark state: typical Pfr BV pocket and overall fold (α-helix)
- Event 1: isomerization of BV
- Event 2: chromophore relaxation, shift of Y205 and R211
- Event 3: switch of Y165 and F192, displacement of a water molecule and shift of Q190
- Event 4: rearrangement of thioether linkage
- Event 5: rotation of W440 and newly ordered backbone of a region within PHY-tongue

Future Plans

- pump-probe time-resolved X-ray Crystallography on XFELs and on conventional (on-chip approaches) synchrotrons for the prototypical wild-type Agp1 (Pr-Pfr conversion)
- protein X-ray Crystallography on single site mutations of Agp2-PCM and Agp2-PAiRFP2-PCM
- Cryo-EM on bacterial and fungal **full-length** phytochromes