

# Real-time tracking of phytochrome's ring D orientational changes during Pr photoisomerization: Two Pr isoforms with different photoisomerization yields

Y. Yang<sup>1</sup>, M. Linke<sup>1</sup>, T. von Haimberger<sup>1</sup>, J. Hahn<sup>2</sup>, R. Matute<sup>3</sup>, L. González<sup>3</sup>, P. Schmieder<sup>2</sup>, K. Heyne<sup>1</sup>

<sup>1</sup>Freie Universität Berlin, Department of Physics, Arnimallee 14, 14195 Berlin, Germany

<sup>2</sup>Leibniz-Institut für Molekulare Pharmakologie, Robert-Rössle Str. 10, 13125 Berlin, Germany

<sup>3</sup>Friedrich-Schiller Universität Jena, Institut für Physikalische Chemie, Helmholtzweg 4, 07743 Jena, Germany

**Abstract.** Phytochromes' ring D orientational changes are tracked during Z-to-E photoisomerization by polarization resolved femtosecond visible pump-infrared probe spectroscopy. Two distinct Pr isoforms Pr-I and Pr-II exhibit photoisomerization yields of 3% and 29%, respectively.

## 1 Introduction

Photoisomerization of the phycocyanobilin (PCB) chromophore is the basis of the light sensing and signaling response of Cph1 phytochrome. Phytochromes are a family of dimeric chromoproteins that absorb light and regulate numerous photoresponses in plants, bacteria, and fungi.[1]

Two thermally stable ground states conformers sensing red (Pr form) and far-red light (Pfr form) can be interconverted by photoexcitation. Excitation of the Pr form with ZZZssa (C<sub>5</sub>-Z, C<sub>10</sub>-Z, C<sub>15</sub>-Z, C<sub>5</sub>-syn, C<sub>10</sub>-syn, C<sub>15</sub>-anti)[2] PCB chromophore geometry initiates an ultrafast Z-to-E photoisomerization of phytochromes' ring D around C<sub>15</sub>=C<sub>16</sub> methine bridge resulting in the Lumi-R photoproduct.

Two time constants of about 3 ps to 16 ps and 25 ps to 40 ps are reported for the initial processes.[3] The low photoproduct quantum yield of 7% to 16% in phytochromes was proposed to result from photoreceptor heterogeneities.[4] Recently, two Pr isoforms were identified by NMR spectroscopy.[5]

## 2 Materials and Methods

We used polarization resolved femtosecond visible pump-infrared probe spectroscopy to determine the orientation of the  $\nu(\text{C}=\text{O})$  vibrational modes of the PCB chromophore on a femtosecond time scale in the Pr\* and Pr state.[6]

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We investigated  $^{13}\text{C}/^{15}\text{N}$  labelled Cph1 $\Delta$ 2 protein with non-labeled PCB chromophore, shifting all  $\nu(\text{C}=\text{O})$ ,  $\nu(\text{C}=\text{C})$ , and  $\nu(\text{C}=\text{N})$  stretching vibrations of the protein out of our spectral window of 1670 – 1745  $\text{cm}^{-1}$ .

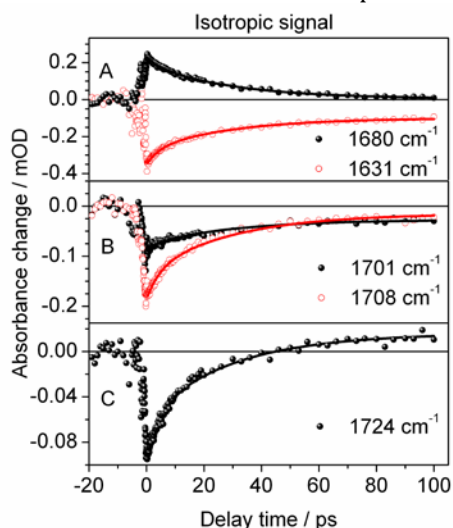
The data were acquired by excitation of less than 3% of the Cph1 $\Delta$ 2 sample at 660 nm ( $S_0 \rightarrow S_1$  transition). The signals were recorded with a 2x32 element MCT array detector at a spectral resolution of 1.5  $\text{cm}^{-1}$ . Parallel and perpendicular polarization signals with respect to pump pulse polarization were recorded at the same time.

### 3 Results

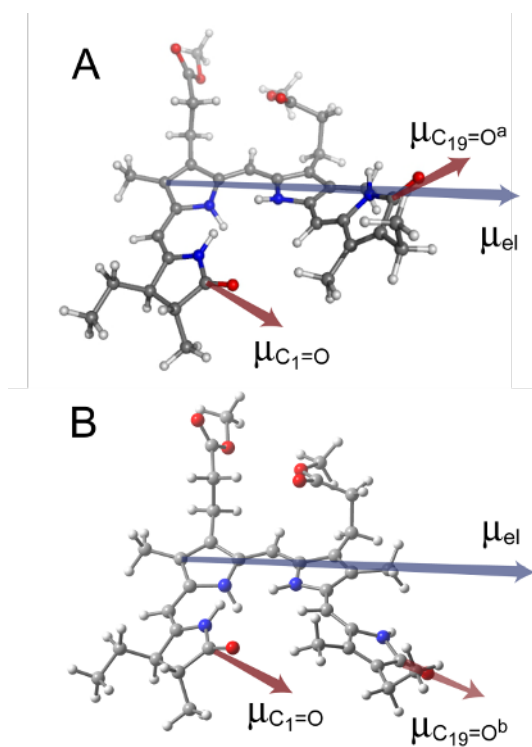
Isotropic transients are presented in Figure 1. Transients are fitted with two time constants of 30 ps and 4.7 ps. Two distinct bleaching signals at 1701  $\text{cm}^{-1}$  and 1708  $\text{cm}^{-1}$  display two Pr isoforms with different photoreaction quantum yields (Figure 1B).

By analysis of the relative angles between electronic transition dipole moment (tdm) and vibrational tdm's measured at 1701  $\text{cm}^{-1}$  and 1708  $\text{cm}^{-1}$ , we were able to assign the two orientations to the Pr-II and Pr-I isoform, respectively. The isoforms exhibit different ring D orientations presented in Figure 2 allowing for assignment of the bleaching signals.

In addition, rotation of ring D was observed in the electronic excited state of Pr (Pr\*). The measured polarization resolved signals indicate 16% of the excited chromophores reach a 90° twisted transition state after 21 ps.



**Fig. 1.** Transient dynamics at frequency positions of marker bands calculated for isotropic conditions. A) Bleaching signal of  $\nu(\text{C}_{15}=\text{C}_{16})$  and  $\nu(\text{C}_{17}=\text{C}_{18})$  stretching vibrations (open red circles), absorption of  $\nu(\text{C}_{19}=\text{O})^*$  stretching vibration in the electronically excited state. B) Bleaching signals of  $\nu(\text{C}_{19}=\text{O}^a)$  and  $\nu(\text{C}_{19}=\text{O}^b)$  stretching vibrations in the Pr state (solid and open red circles, respectively). C) Bleaching signal of  $\nu(\text{C}_1=\text{O})$  stretching vibration and  $\nu(\text{C}_{19}=\text{O})$  absorption of lumi-R.



**Fig. 2** . PCB chromophore geometries with Pr  $S_0 \rightarrow S_1$  transition dipole moment  $\mu_{el}$  (black arrow) and vibrational tdm's of  $\nu(C_{19}=O)^a$  of Pr-II and  $\nu(C_1=O)$  (red arrows). A: PCB<sup>a</sup> geometry of Pr-II, B: PCB<sup>b</sup> geometry of Pr-I, Note, tdm arrows can point in either direction.

The overall photoisomerization is best explained by a single rotation around  $C_{15}=C_{16}$  methine bridge in the Pr\*. The Pr-I isoform shows a quantum yield of 3%, while the Pr-II isoform exhibits a much higher quantum yield of 29%. The Pr-II isoform has a hydrogen bond between His290 and ring D and is more twisted than the Pr-I isoform. These data suggest an efficacy increase of the photoreaction by side chain assisted pre-twisting.

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