Relationship between Redox State and Dimerization of animal-like Cryptochrome

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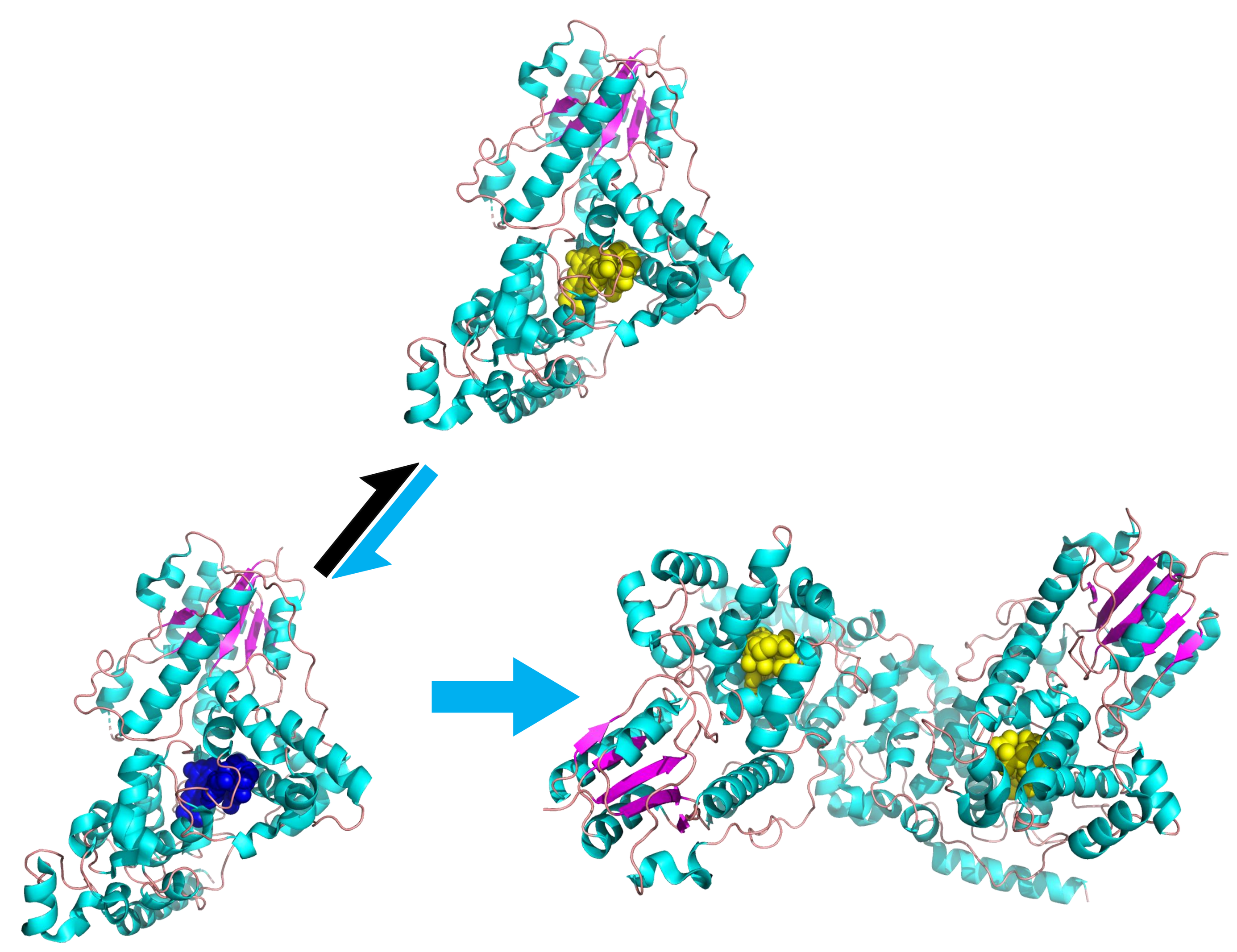
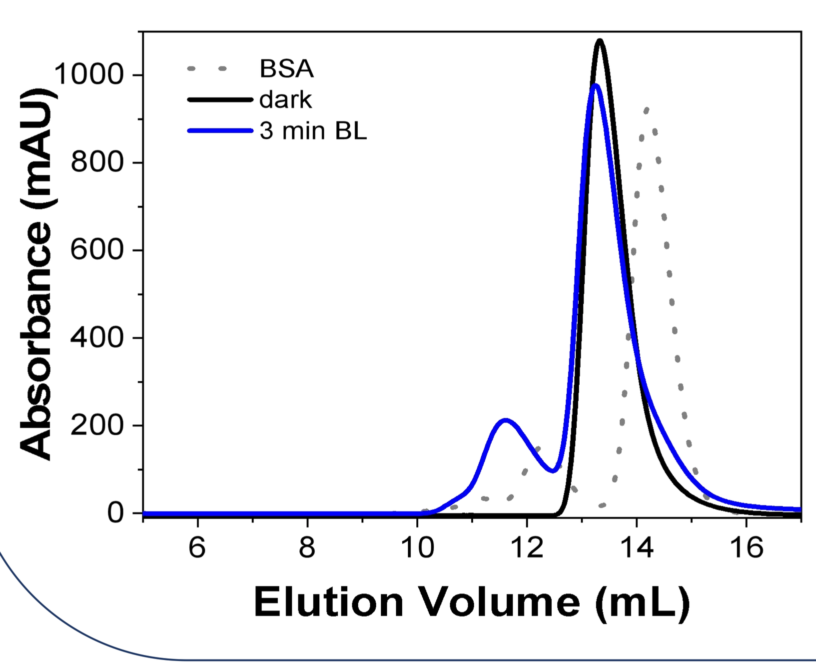


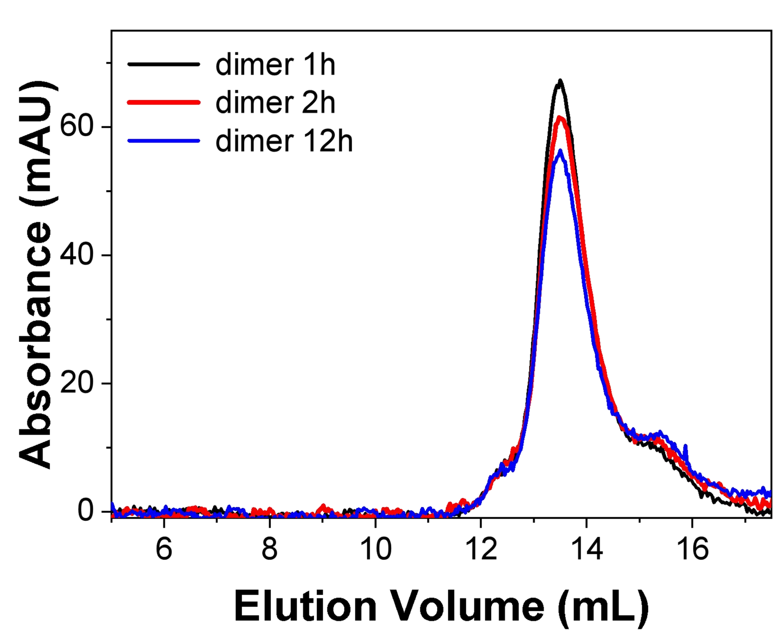
Introduction

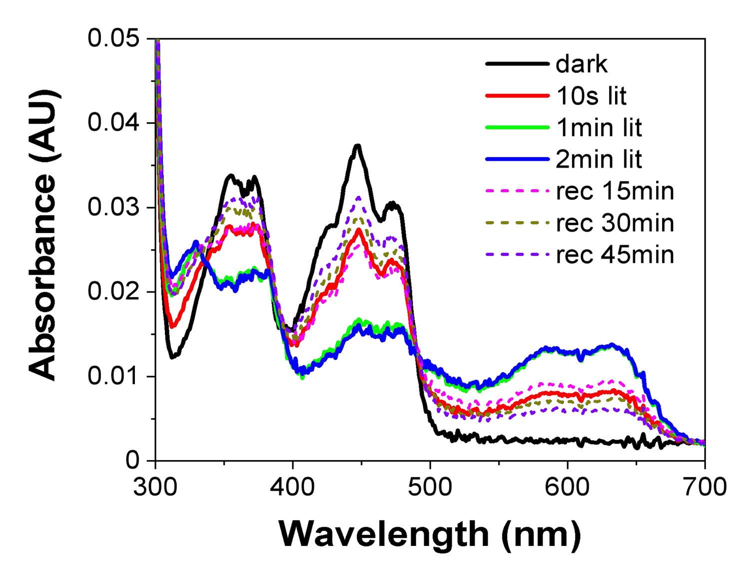
Cryptochromes (CRYs) are blue-light receptors involving in mediating circadian rhythms and magnetic sensing in diverse organisms. And cofactor FAD as an antenna of CRYs has four different redox states regulated by light. Functions of almost all CRYs are induced by blue light (BL), whereas the specific function is dependent on the redox state of CRY. Many CRYs can dimerize induced by BL like *Arabidopsis* CRY2 and CRY1, *Columba livia* Cry4, and animal-like Cryptochrome (aCRY) in *Chlamydomonas reinhardtii*. But the dimerization mechanisms are not elucidated clearly yet. For aCRY, Dimerization occurss only in a proportion of protein.So we want to know the relationship between redox state and dimerization of aCRY.

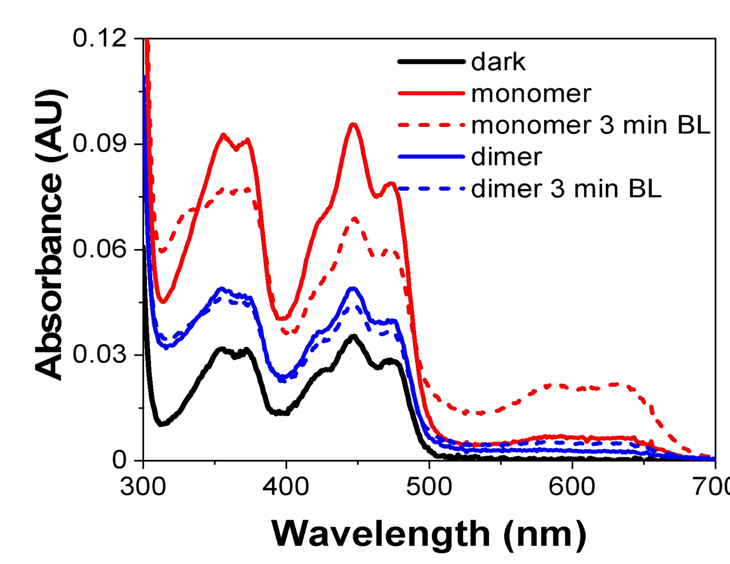
Redox State of Dimer

The state of aCRY can change from oxidation to reduction state in BL, and then recover to oxidation state in dark. **That means a redox cycling.**



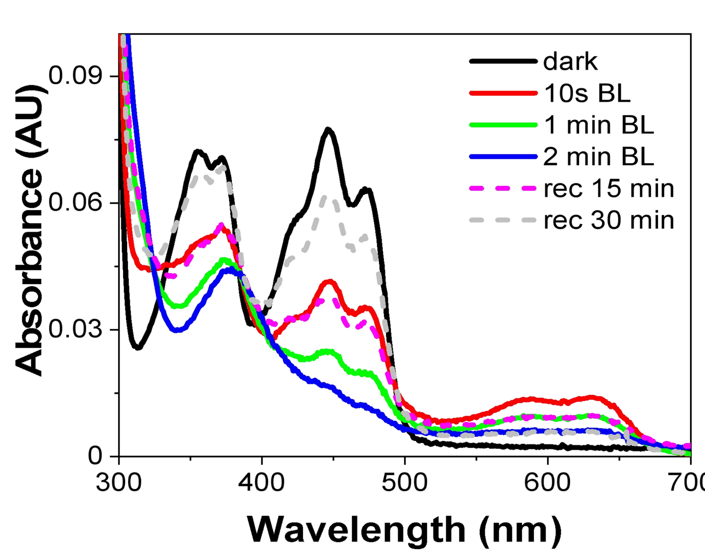


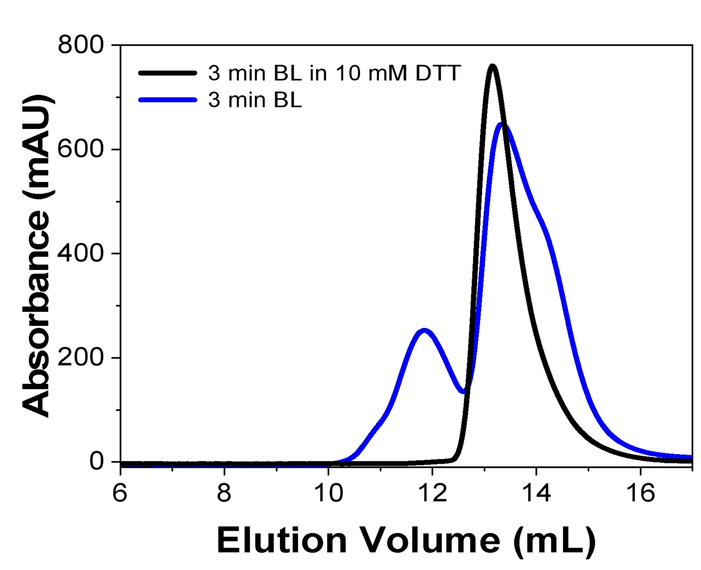




After aCRY was illuminated by BL and separated with SEC, dimer recovered to oxidation state and can not be excited by BL again. The isolated dimer was assayed with SEC 3 times again, which shows that the dimer would not dissociate to monomer in dark. **It was not a cycle.**

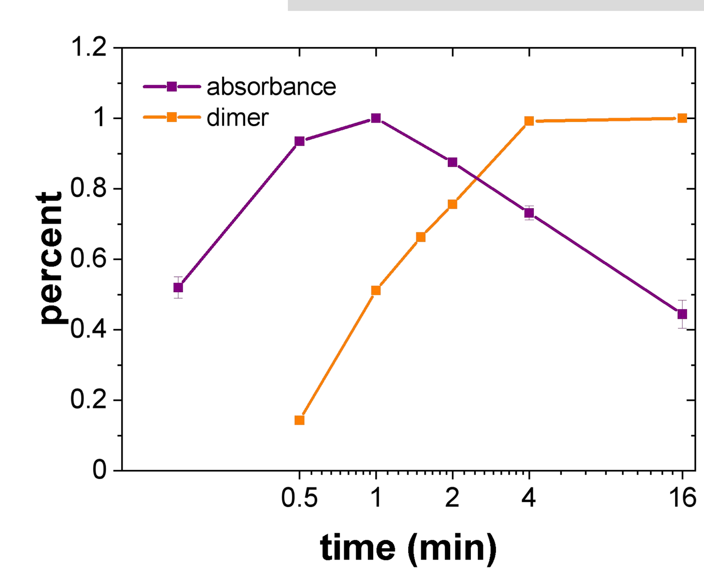
Effect of DTT

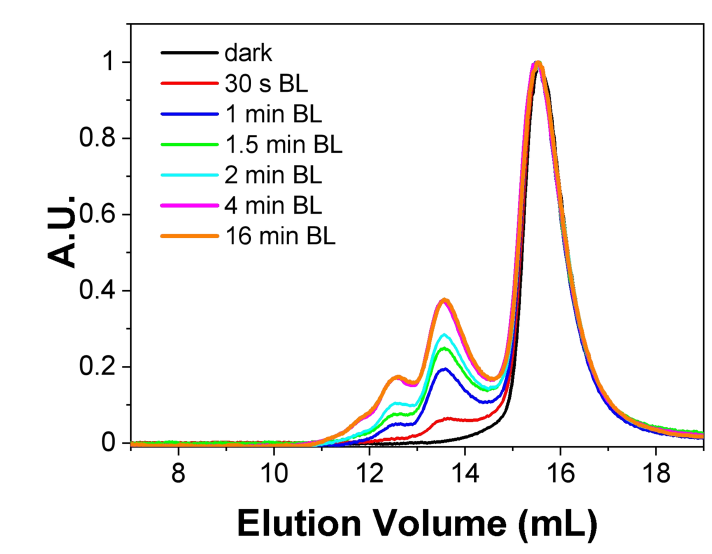




DTT as a reductant can help the aCRY to be reduced further by BL but abolish dimerization.
$FADH^-$ is necessary for dimerization.

Time Dependent change

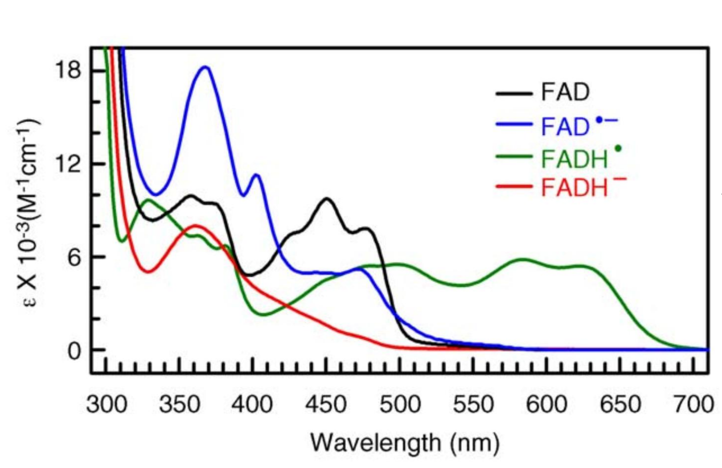




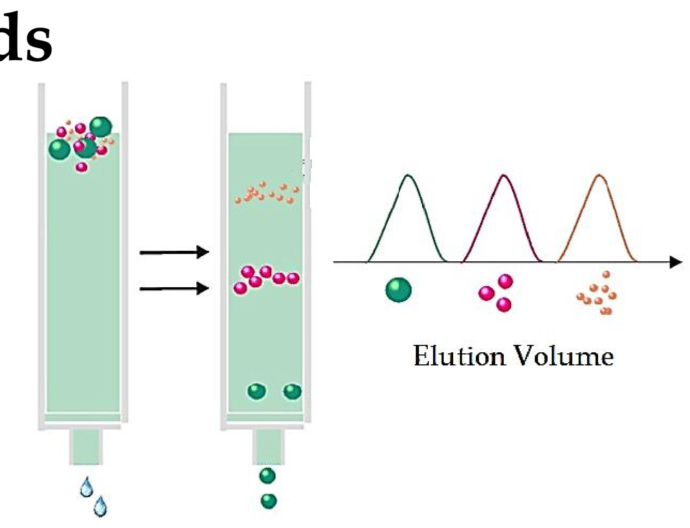
Dimerization and photoinduced reduction are illumination-time dependent. The percentage curve of dimer lagged behind that of reduction state monitored at 633 nm.

Conclusion: Although the redox state of aCRY is a cycle, the monomer-dimer form is not. And dimerization lags behind the reduction process. These imply that dimerization maybe a protection against too bright light.

Methods



The four redox states can be identified by absorption spectra. FAD is the oxidation state.



The dimer and monomer were separated with size exclusion chromatography (SEC).

Reference:1. S. Franz *et al.*, *Nucleic Acids Res.* 46(15), 8010-8022 (2018).