Redox mechanism of bifunctional animal-like cryptochrome from *Chlamydomonas reinhardtii* induced by blue light

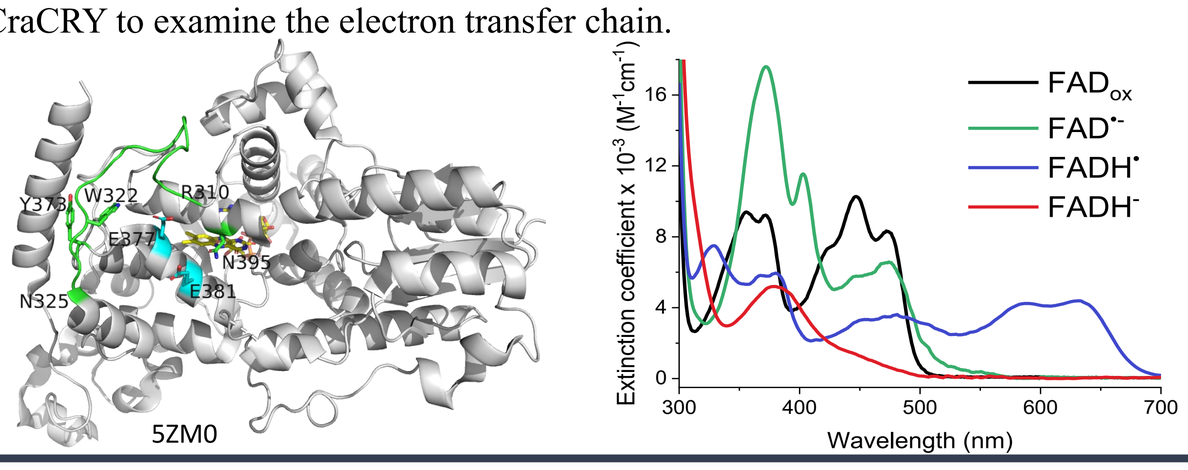
Huaqiang Cheng[1], Yan-Wen Tan[1,*]

[1]State Key Laboratory of Surface Physics, Multiscale Research Institute of Complex Systems, Department of Physics, Fudan University, Shanghai 200433, China

Abstract: Cryptochromes (CRYs) are blue light receptors that mediate circadian rhythm and magnetic sensing in various organisms. The functions of CRYs depend on the redox reaction of FAD, which is a cofactor bound in the core of protein. During photoreduction, electron transfer is found between FAD and tryptophan triad conserved in CRYs. The animal-like cryptochrome from the green algae *Chlamydomonas reinhardtii* (CraCRY) is unusual because it is capable of not only regulating circadian clock including control of transcript levels but also repairing DNA damage. In this study, we found that it is electron transfer rather than FAD state that determined the function the CraCRY. Reducing environment and Y373 play key roles in balancing of the two distinct functions through charge, structure change, dimerization, and interaction.

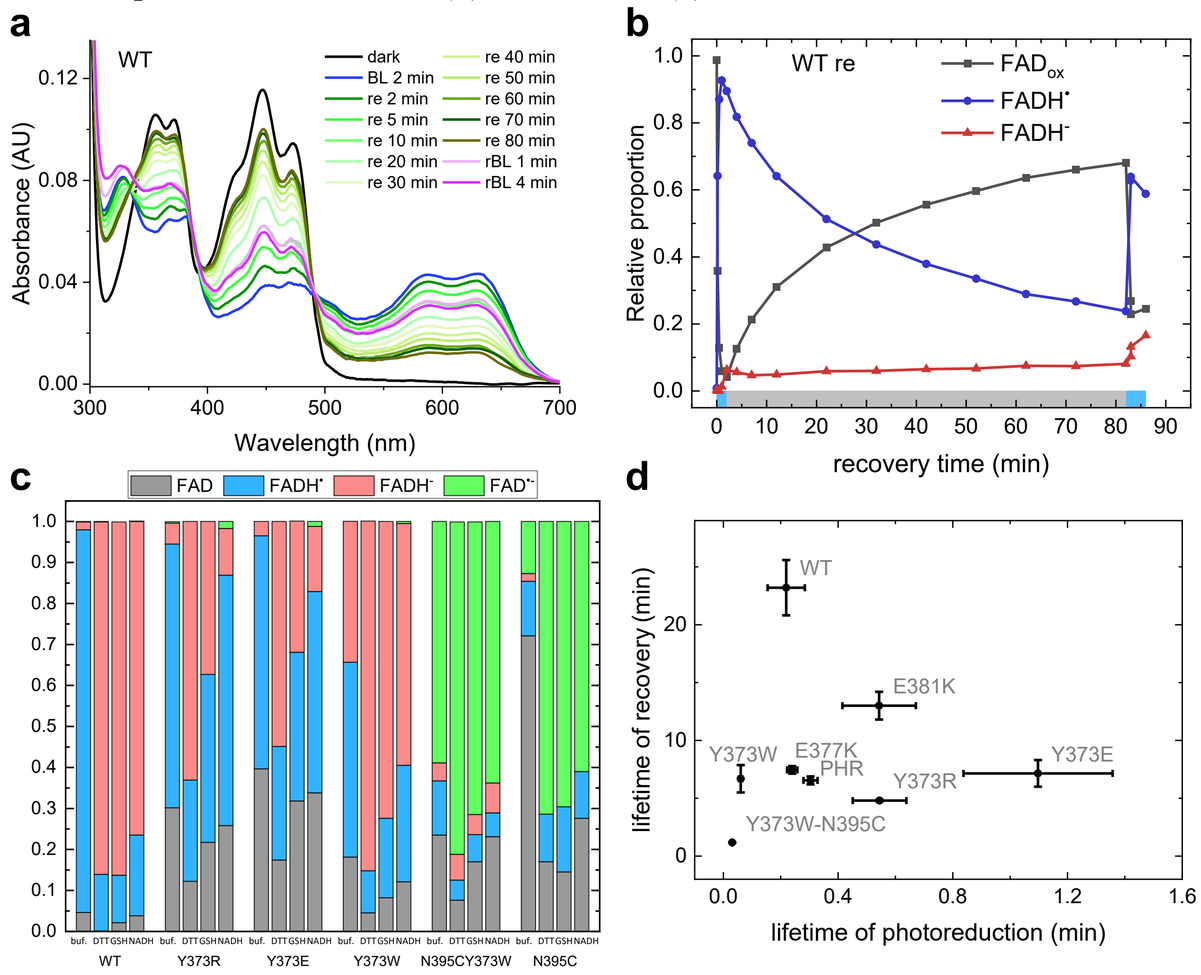
1. Electron transfer chain and redox states of FAD in CraCRY

Y373 is the electron donor[1] of photoreduction and N395 is proton donor[2]. But electron donor is Trp-triad in AtCRY and Trp-tetrad in DmCRY and ClCRY4. N395 is Cys in DmCRY. So we mutated Y373 to Trp, Arg or Glu and N395 to Cys in CraCRY to examine the electron transfer chain.



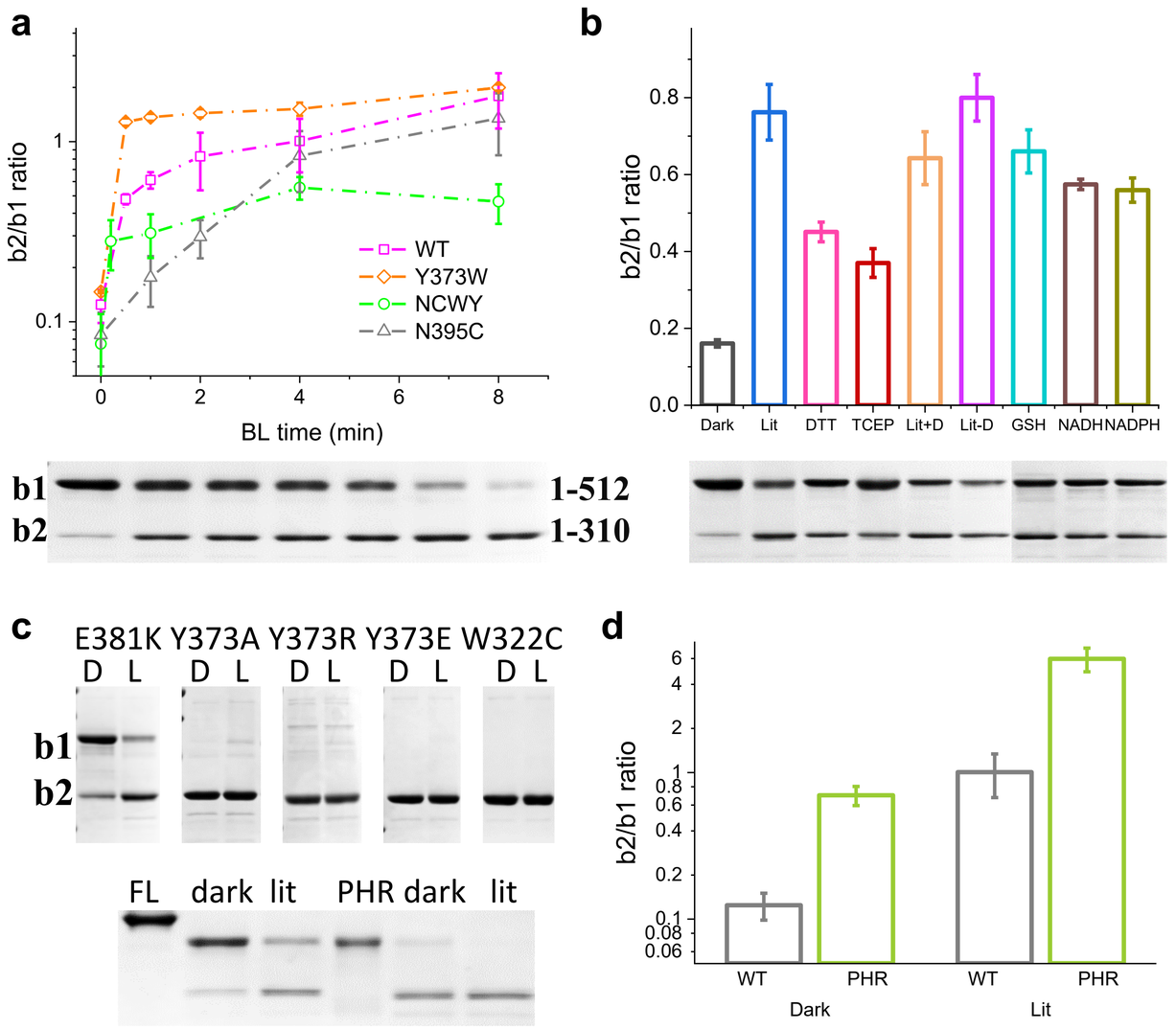
2. Photoreduction rates and photoreduction extents of CraCRY

CraCRY is FAD$_{ox}$ state in dark and can convert to FADH$^{\bullet}$ state upon blue light (BL) or FADH^{-} state upon blue light in reducing environment. The reduced CraCRY recovered (denoted as 're') to FAD$_{ox}$ state in dark (a, b). We used the 4 FAD states to decompose the absorption spectra and found the mutants of Y373 showed different photoreduction rates (d) and extents (c).



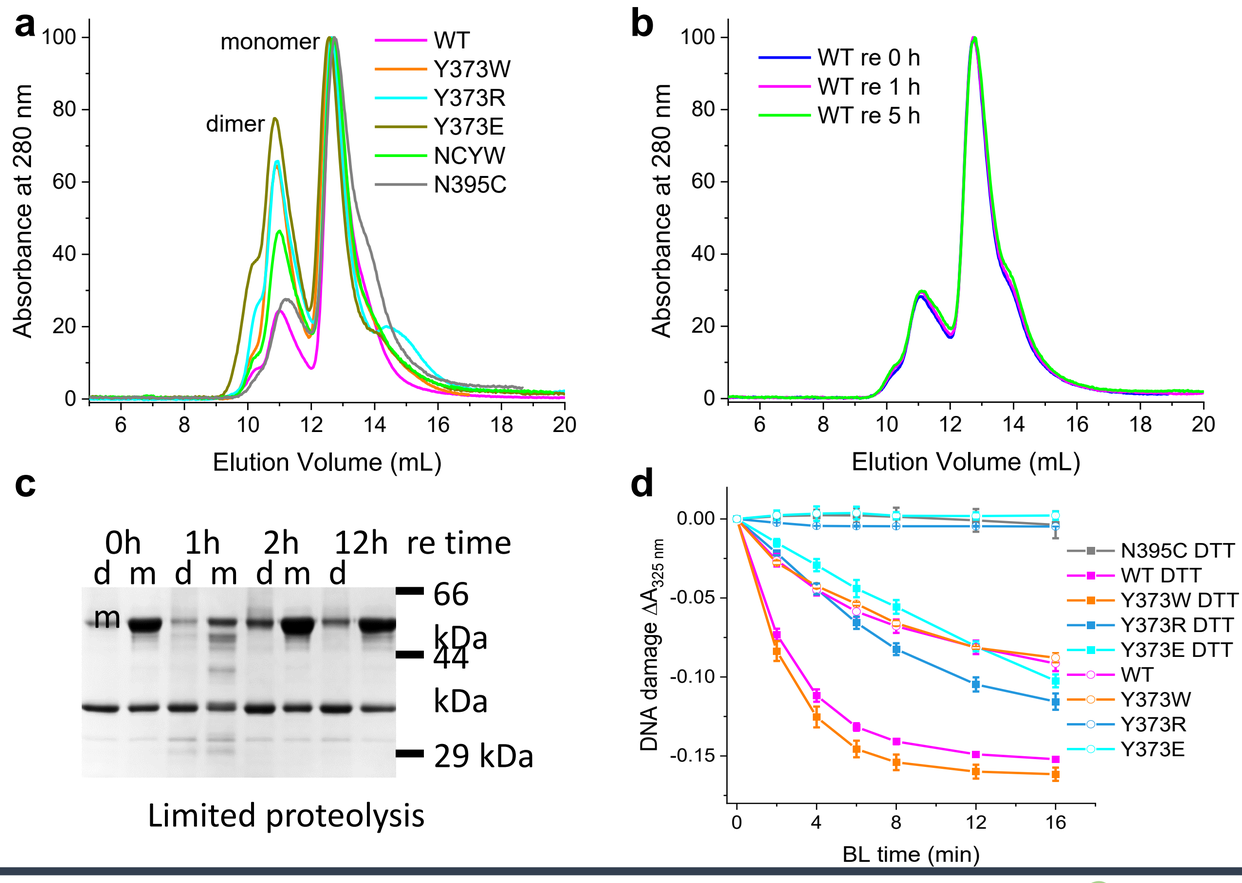
3. Conformational change of CraCRY by limited proteolysis

The function of CRYs is correlated to their conformational changes[3,4]. By limited proteolysis, we found conformation changes induced by blue light in Y373W, N395C and Y373W-N395C (NCYW) which are similar to wild type CraCRY (a). Besides, external electron donor inhibited this conformational change (b), and mutants of Y373 made it happen in dark (c). C-terminal protected CraCRY in dark according to the larger ratio of proteolysis bands of N-terminal (d).



4. Dimerization of CraCRY by SEC and DNA repair

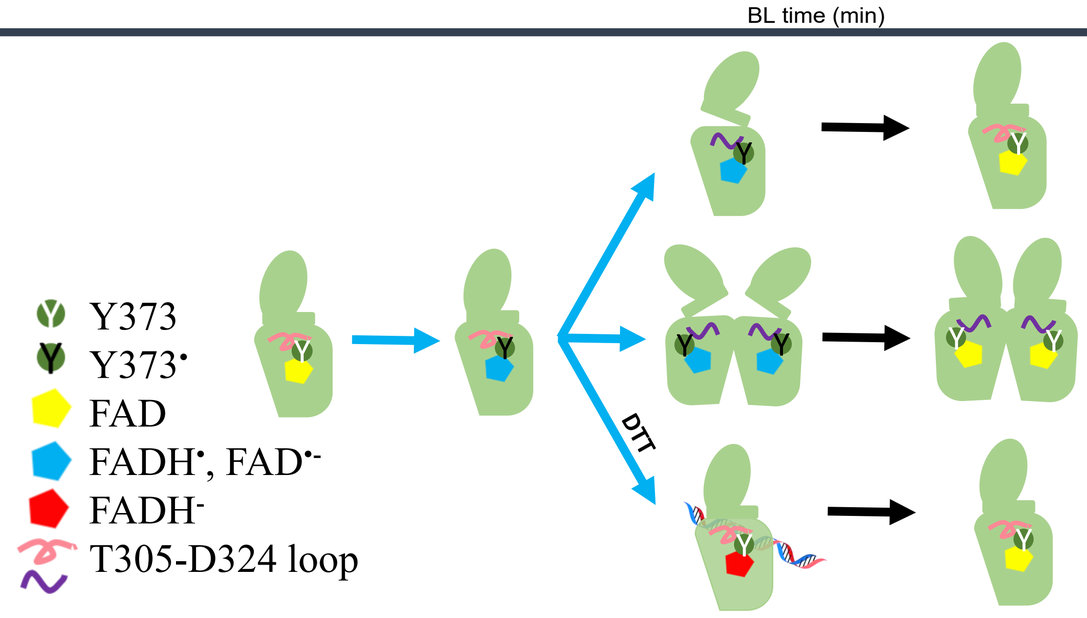
Dimerization is also induced by blue light. And most mutants of Y373 had a trend favoring dimer than monomer (a). The dimer was stable in the solution (b), and kept the changed structure of T305-D324 loop (c). DNA repair activity was correlated to the photoreduction rate. DTT enhanced it (d).



Conclusions

I. WT, Y373W, and N395C had different photoreduction states (2.c), but all of them showed conformational change (3.a) and dimerization (4.a). This implied the FAD state is not the key factor for CraCRY to regulate the circadian rhythm, but photoreduction is necessary. So, electron transfer from Y373 is crucial.

II. The reducing environment inhibited the conformational change (3.b) and dimerization but improved the DNA repair (4.d). Mutants of Y373 in dark had similar proteolysis bands with WT in light (3.c) and increased dimerization (4.a). That means DTT and Y373 are regulators of bifunctional CraCRY.



[1] F. Lacombat, et al. *J. Am. Chem. Soc.* **2019**, *141*, 13394. [2] H. Cheng, unpublished. [3] H. Cheng, P. Li. *Comms. Bio.* [4] Vaidya, A. T, et al. *Proc Natl Acad Sci U S A.* **2013**, *110*, 20455.