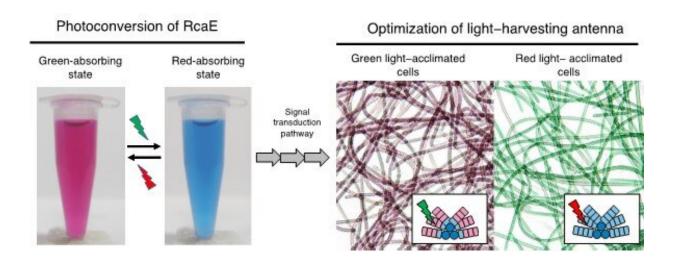


## Structural uniqueness of the green- and redlight sensing photosensor in cyanobacteria

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RcaE senses green and red lights and regulates the absorptive maxima of lightharvesting antenna supercomplex (phycobilisome, inset figure) in cyanobacteria. Credit: Toyohashi University Of Technology

Certain cyanobacteria can change the absorbing light colors for photosynthesis using a green- and red-light sensing photosensor protein. A Japanese research group elucidated the molecular structure of RcaE, a representative member of the photosensors. They revealed the unique conformation of the bilin chromophore and the unique protein structure that potentially functions as a proton transfer route to bilin. They also demonstrated that RcaE undergoes protonation and deprotonation of the bilin chromophore during the green and red photoconversion. These

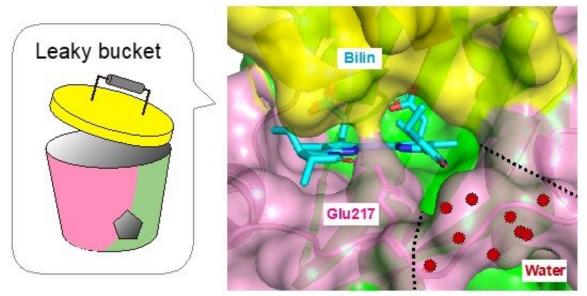


results provide insights into how cyanobacteria evolved photosensors with diverse spectral sensitivities and contribute to the development of new photoswitches of gene expression.

Certain cyanobacteria can utilize both green and red lights for photosynthesis by using their light-harvesting antenna supercomplex called phycobilisome. They can control the absorptive maxima of phycobilisome, which results in remarkable changes in cell color. This phenomenon is regulated by RcaE that belongs to cyanobacteriochrome family of photosensors. RcaE harbors a bilin chromophore and photoconverts green- and red-absorbing states to sense ambient light colors. Although the green and red photoconversion is involved in bilin photoisomerization and subsequent change in bilin protonation state, the structural basis of this photoconversion remains unknown.

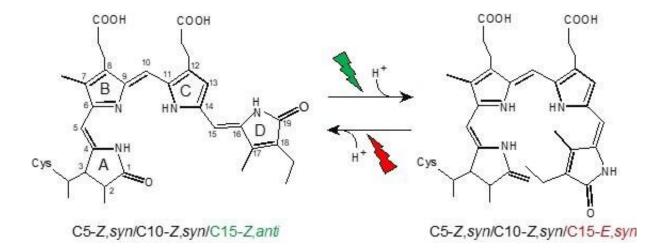
The research group comprised Takayuki Nagae (Nagoya University), Masaki Mishima (Tokyo University of Pharmacy and Life Science), Yuu Hirose (Toyohashi University of Technology), Masashi Unno (Saga University), Kei Wada (Miyazaki University), and Yutaka Itoh (Tokyo City University). They determined the high-resolution <u>structure</u> of RcaE in its red-absorbing state via X-ray crystallography. The bilin chromophore showed a conformation with co-planar A-C rings, wherein the <u>nitrogen atoms</u> were facing inward; the nitrogen of the tilted D-ring was facing outward (classified as C15-E,syn structure). Additionally, they identified a porous cavity containing <u>water molecules</u> in the bilinbinding pocket of RcaE. The clustered water molecules were connected with the nitrogen atoms of bilin A-C rings by a hydrogen bond network through the conserved acidic residue, Glu217.





Porous cavity filled with water molecules

Unique leaky bucket structure of RcaE. Clustered water molecules behind the pick loop are shown as red balls. Credit: Toyohashi University Of Technology.



A proposed model of the green and red photoconversion of RcaE. Credit: Toyohashi University Of Technology.



The research group demonstrated by molecular dynamic simulations that the water molecules in the cavity were exchanged with the solvent water. They also demonstrated by 15N NMR spectroscopy that four pyrrole nitrogen atoms of bilin are fully protonated in the red-absorbing state, whereas one nitrogen atom is deprotonated in the green-absorbing state. They assume that the unique porous cavity functioned as a proton exit or inlet pathway during the green and red photoconversion. Considering previous study reports on Raman spectroscopy of RcaE, they proposed that bilin deprotonation occurred in the B-ring nitrogen with the C15-Z,anti structure. They are currently working on the crystallization of the green-absorbing state of RcaE to confirm this model.

Elucidating the structure and spectral tuning mechanisms of RcaE provides insights into how cyanobacteria have evolved diverse cyanobacterial subfamilies to acclimate to different light environments. Green and red light-sensing cyanobacteriochromes have been utilized in <u>synthetic biology</u> as sophisticated photoswitches that control gene expression. Amino acid residue modification based on RcaE structure will contribute to the development of new photoswitches with desirable photosensitivities.

**More information:** Takayuki Nagae et al, Structural basis of the protochromic green/red photocycle of the chromatic acclimation sensor RcaE, *Proceedings of the National Academy of Sciences* (2021). DOI: 10.1073/pnas.2024583118

Provided by Toyohashi University of Technology

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