

## MS08 Serial crystallography, obtaining structures from many crystals

MS8-05

Photoactivation in a Fluorescent Protein Proceeds via the Hula-Twist Mechanism : Revealed through TR-SFX

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### Abstract

The light-induced cis/trans isomerization of a chromophore double bond is a fundamental reaction in photochemistry and it has been shown to be the primary activation event for a variety of protein photoreceptors. A detailed understanding of the reaction pathway and of how it is steered by protein environment is key for the rational design of more effective photosystems.

In the 1980s, Liu & Asato coined and proposed a mechanism for chromophore photoisomerization in proteins: the hula twist. This was supposed to be volume conserving and therefore favorable inside a sterically-crowded binding pocket. By exploiting the time resolution available at XFELs and the insertion of a heavy atom to break the symmetry of the chromophore ring, we capture the ultrafast photoproduct of the trans-to-cis reaction in a fluorescent protein. This, to our knowledge, is the first direct experimental structural evidence that unequivocally supports hula-twist chromophore photoisomerization in a protein, more than 30 years after it was first hypothesized. By analyzing data from time points that span from 300 fs to 1  $\mu$ s, we also resolve how photoisomerization leads to the sequential movement and relaxation of secondary structure elements. This data demonstrates the power of time-resolved crystallography for elucidating the ultrafast dynamical response in photoactivated systems.

### References

Work so far unpublished.

Key relevant references :

Liu, R. S. H. & Asato, A. E. The primary process of vision and the structure of bathorhodopsin: A mechanism for photoisomerization of polyenes\* (lumirhodopsin/hypsorhodopsin/binding site/concerted twist). Proc. Natl. Acad. Sci. USA 82, 259–263 (1985)

Chang, J., Romei, M. G. & Boxer, S. G. Structural Evidence of Photoisomerization Pathways in Fluorescent Proteins. J. Am. Chem. Soc. 141, 15504–15508 (2019)

Colletier, JP., Schirò, G., Weik, M. (2018). Time-Resolved Serial Femtosecond Crystallography, Towards Molecular Movies of Biomolecules in Action. In: Boutet, S., Fromme, P., Hunter, M. (eds) X-ray Free Electron Lasers. Springer, Cham. [https://doi.org/10.1007/978-3-030-00551-1\\_11](https://doi.org/10.1007/978-3-030-00551-1_11)

