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Functional dynamics of a single tryptophan residue in a BLUF protein revealed by fluorescence spectroscopy

Blue Light Using Flavin (BLUF) domains are increasingly being adopted for use in optogenetic constructs. Despite this, much remains to be resolved on the mechanism of their activation. Trp104 is a key player in the photocycle of the BLUF photoreceptor, AppA (Activation of Photopigment and PUC A) in communicating the electronic excitation of the flavin ring to the protein backbone1. The exact conformation of W104 during the photoactivation process in AppA and other BLUF domains has been a controversial topic in the field. The first crystal structure of AppA (pdb:1yrx)2 showed that W104 in the dark-adapted state is located close to flavin in the so called Trpin conformation, whereas subsequent crystal structures (pdb: 2iyg, 2iyi)3 presented a different picture where the tryptophan is pointing away from the flavin both in the dark- and light-adapted state (Trpout conformation).

In order to investigate the functional dynamics of the crucial Trp104 residue during photoactivation of the protein we have incorporated the tryptophan analogue, 7-aza-Trp (7AW) in the BLUF domain. The 7-aza modification to Trp makes selective excitation possible using 310 nm excitation and 380 nm emission, separating the signals of interest from other Trp and Tyr residues. We used Förster energy transfer (FRET) between 7AW and the flavin to estimate the distance between Trp and flavin in both the light- and dark-adapted states in solution. Nanosecond fluorescence anisotropy decay and picosecond fluorescence lifetime measurements for the flavin revealed a rather dynamic picture for the tryptophan residue. In the dark-adapted state, the major population of the tryptophan 104 is pointing away from the flavin and can move freely, in contrast to previous results reported in the literature. Upon blue-light excitation, the tryptophan population is reorganized, the dominant population moves closer to the flavin occupying a rigidly bound state participating in the hydrogen-bond network around the flavin molecule4. Overall, our study addresses previous discrepancies on the position of Trp104 during the photocycle of AppA and supports a conformation of Trp104 close to the flavin in the light-adapted state whereas in the dark-adapted state Trp104 is present in a less restricted environment pointing away from the flavin.

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