Coupling of X-ray crystallography and spectroscopy in crystals to characterize a new photochromic fluorescent protein

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Photoactivatable fluorescent proteins (FPs) are powerful fluorescent markers. They are homologous to GFP (Green Fluorescent Protein) but their photochromic properties offer new perspectives for tracking molecules in live cell, for the development of super-resolution optical imaging and the for the engineering of biophotonic devices. Two types of photoactivation are currently being distinguished, reversible photoswitching between fluorescent and non-fluorescent forms (REF) and irreversible photoconversion (REF). Here we have combined crystallography and *in crystallo* spectroscopy to characterize the fluorescent protein IrisFP, which incorporates both types of phototransformations (3).

We have studied these phototransformations by coupling x-ray crystallography with both off-line spectroscopy at the Cryobench laboratory (4), and on-line spectroscopy at the ESRF MX beamlines (5). The near-atomic global structural data brought by X-ray diffraction, along with the complementary UV/visible absorption, fluorescence and Raman data brought by spectroscopy allowed us to characterize the phototransformation mechanisms of IrisFP and to identify early structural modifications induced by photo-bleaching, a general problem with fluorescent molecules.

In its green fluorescent state, IrisFP displays photoswitching: its fluorescence can be reversibly turned off, due to a light-induced cis-trans isomerization of the chromophore. IrisFP also photo-converts irreversibly to a red-emitting state under violet light due to an extension of the conjugated □-electron system of the chromophore. This red form of IrisFP exhibits a second reversible photoswitching process, which again results from cis-trans isomerization. Finally, irreversible photobleaching appears to arise from a light-induced loss of planarity of the chromophore, possibly associated with a photo-oxidation process.

Beyond the specific conclusions of IrisFP, this work demonstrates the importance of combining techniques to gain mechanistic insight into the functioning of biomolecules.

References

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