IMPORTANT: SUBJECT TO EMBARGO UNTIL SEPTEMBER, THE 11th, AT 5:00 PM (Paris time)

Paris, 11.09.2017

Ultra-fast molecular movie: watching proteins absorbing light

Using a revolutionary method, scientists have been able to film ultra-rapid processes at work in fluorescent proteins, which are extensively used as markers in *in vivo* imaging. This new method, which uses enormous X-ray lasers, permits the analysis of processes such as vision, bioluminescence and other phenomena which have not been observable to date. These results are to be published in *Nature Chemistry* on September, the 11th, as part of an international collaboration involving scientists from the CEA, the CNRS (French national centre for scientific research), the Universities of Grenoble-Alpes[1], Lille, Rennes 1 and Paris-Sud, and the Max Planck Institute for Medical Research at Heidelberg in Germany.

Super-resolution optical microscopy, or "nanoscopy", has revolutionized *in vivo* imaging, by the mark-up of biological cells to be imaged using fluorescent proteins, which are described as "photo-switchable". These tiny molecular switches can be reversibly toggled from a fluorescent state (*on*) to an extinguished state (*off*) further to excitation by a light flash (this is described as photo-switching). For the purposes of rational design, it is necessary to understand the mechanism of photo-switching, which involves ultra-rapid transient states.

For the first time, scientists have been able to film the switching of a fluorescent protein in real time, using an entirely new generation of X-ray sources, so-called X-ray free electron lasers (or "XFEL"). These devices generate X-ray pulses of very short duration, of the order of a femtosecond (one millionth of a billionth of a second), with a very high intensity, at the core of an installation several kilometers in length.

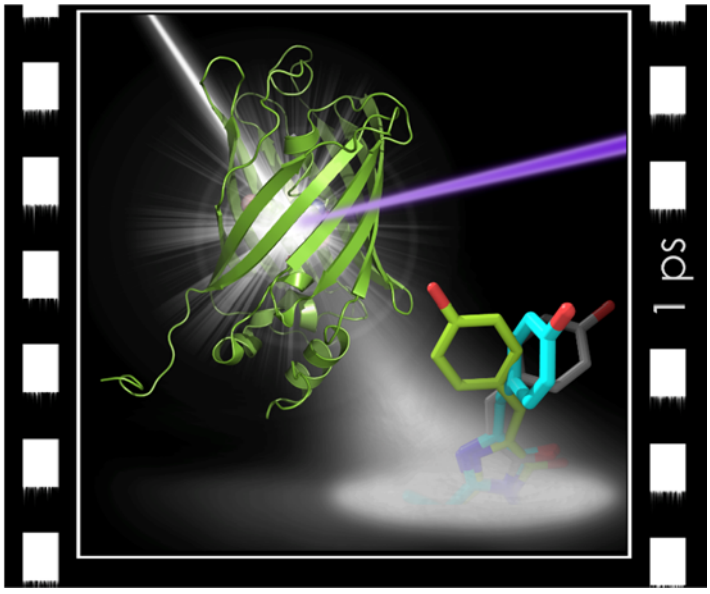

Figure: Ultra-fast movie of a light-sensitive protein in action. The fluorescent protein (green) is excited by a visible light laser (violet ray) and impacted by an X-ray pulse (white ray) generated by an X-ray free electron laser. One picosecond after excitation, the (blue) chromophore at the core of the protein can be observed midway between its stable forms (grey for 'off', green for 'on'); its speed of movement (4.6 Angströms in 1 picosecond) is equivalent to that of a supersonic aircraft (460 m/s). © Virgile Adam / IBS

[1] These three organizations collaborate in the Institute of Structural Biology.

At the Stanford Linear Accelerator Centre (or "SLAC"), the authors of the study have been able to match a jet of tiny fluorescent protein crystals with X-ray pulses, thus permitting the recording of a myriad of diffraction patterns. As a result of the extreme intensity of the X-ray beam, each impacted crystal explodes immediately after the diffraction information has been recorded – hence the necessity for the constant renewal of the sample in this technique, described as "serial femtosecond crystallography". For the observation of intermediate states between the two static *on* and *off* states, a photochemical reaction is initiated in the protein by a laser flash, which is triggered barely one picosecond (one thousandth of a billionth of a second) before the impact of an X-ray pulse.

The authors have thus been able to identify the transient structure of the fluorescent protein in its excited state, and to observe the chromophore – the part of the protein which absorbs light – in a twisted state, midway between the stable configurations of the *on* and *off* states (see figure). This observation, which has been confirmed by simulations, has allowed researchers to formulate hypotheses on the photo-switching mechanism of the protein. These hypotheses have subsequently been confirmed using site-directed mutagenesis.

So far, only two X-ray free electron lasers are operational throughout the world: the one used for the present study, located in Stanford in the USA (the LCLS instrument at the SLAC National Accelerator Laboratory), and the other in Japan, in the province of Osaka (SACLA). The first European X-ray free electron laser, to the construction of which France has contributed, has been inaugurated September, the 1st, in Hamburg (Germany), and one of the first scheduled experiments will be conducted by the consortium of the present study. A brigth future for ultra-fast molecular movies is anticipated.

Installation of the SLAC National Accelerator Laboratory*, based in the USA. Researchers have used this X-ray laser, which is several kilometers long, to "film" internal conformational changes in a protein, involving movements of several Angströms (10^{-10} meters) which occur in a few thousandths of billionths of a second. © SLAC National Accelerator Laboratory*

References: "*Chromophore twisting in the excited state of a fluorescent protein captured by time-resolved serial femtosecond crystallography*", Coquelle N. *et al.*, *Nature Chemistry*, September 2017

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