The Global COE Program

"The Next Generation of Physics, Spun from Universality and Emergence"

Bilateral International Exchange Program (BIEP, invite) report

Send report to: Your responsible Professor in Kyoto University

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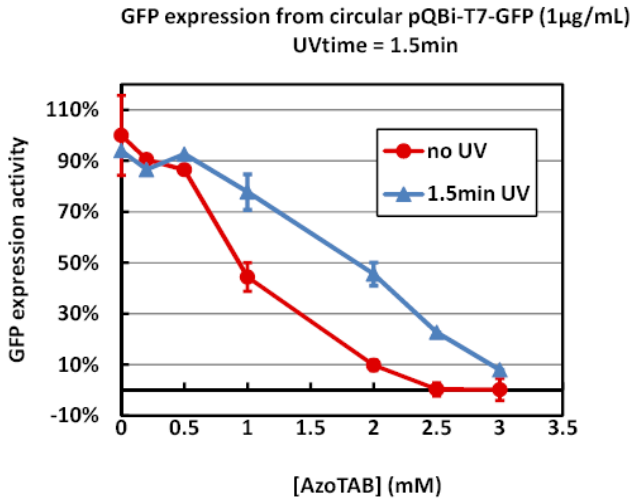
Research Project

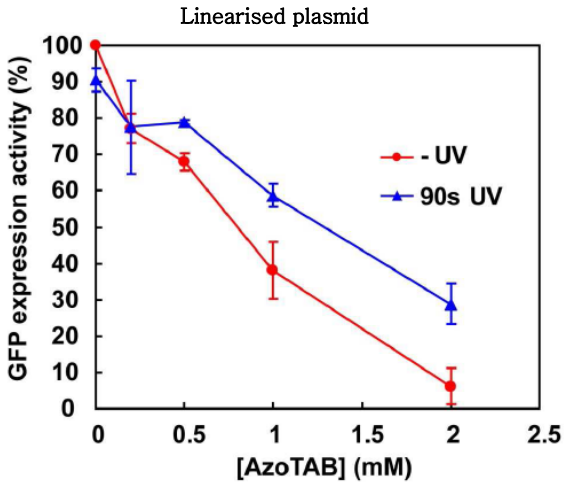
Title	Photocontrol of RNA and protein synthesis. Motion of droplet induced by partial illumination.
Duration	2 months

Please summarize your activities and results during your stay in Kyoto University .
You can add a sheet, if you need more space. You can also write any comments and requests to the GCOE program. We will appreciate them.

I came here to continue the work done by Dr. André Estevez Torres last year. He shown that the transcriptional activity of different DNA template could be switch on and off by UV light, in presence of a photosensitive surfactant, named AzoTAB.

The work is now to apply this system to the protein synthesis. I collaborated with Dr. Cécile crozatier (post-doc in Prof. Yoshikawa's lab) and Dr. Hirohide Saito, from the biology department. We performed different experiments in a cell free protein synthesis system (PURE System). The synthesized protein was the GFP, easily quantifiable by fluorescence. Different factors have been investigated: AzoTAB concentrations, UV illumination time, template concentration, conformation of the template (circular or linearised plasmid of 5 kbp). Finally, after optimization of these parameters, the following curves have been obtained, and are satisfying!





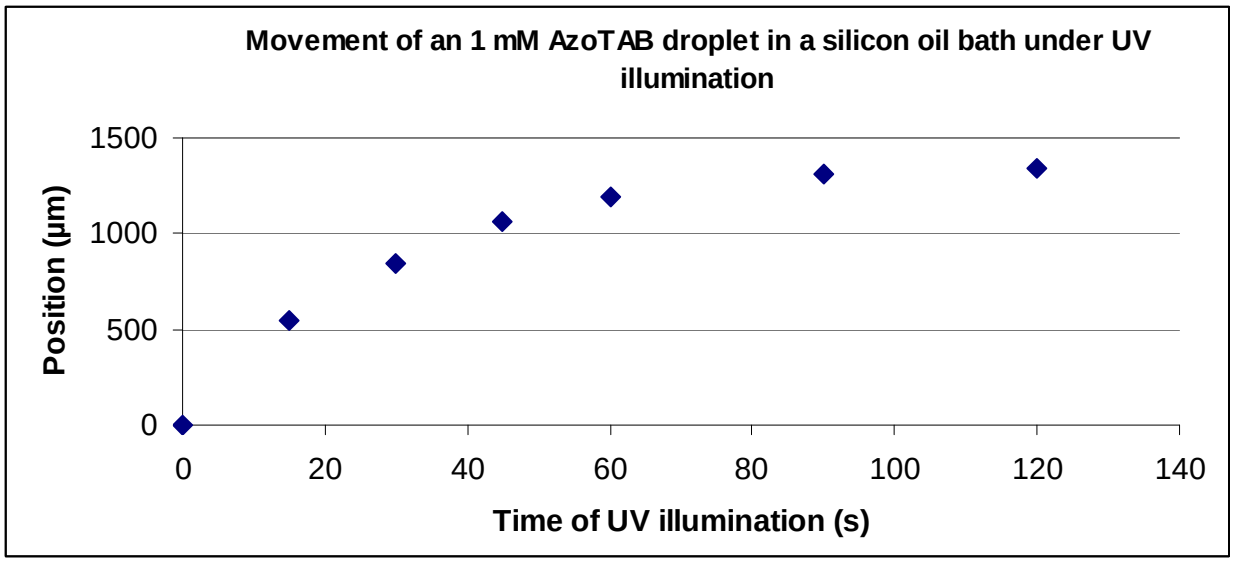
They show that it is possible to stop GFP expression with an AzoTAB concentration of about 2 mM (template concentration = 1 µg.mL^{-1}), for the two plasmid ; and at the same time, to get a recovery of GFP expression after 1,5 min of UV illumination of the starting tubes (about 30% for the linearised, 50% for the circular).

What is happening during the protein expression remains difficult to explain completely. Even if the interaction of AzoTAB with DNA is well known, its interaction with RNA has to be more precisely described. The buffer solution of the PURE System is also something to better know. We are now trying to observe RNA synthesis in this buffer to compare the effect of AzoTAB between the first step of the GFP expression and the whole process.

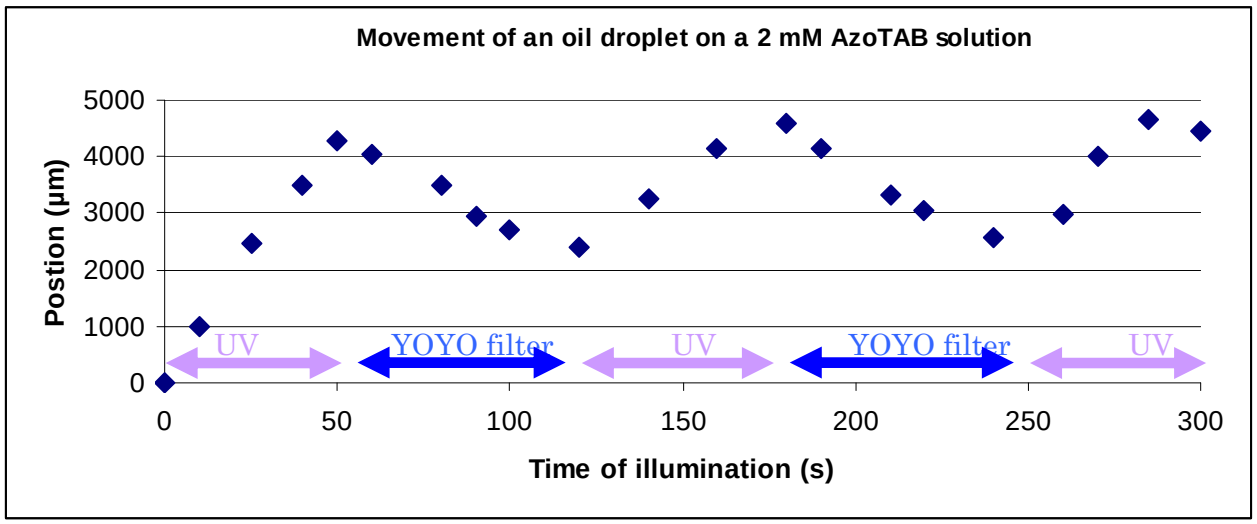
On the other hand, following an idea of Prof. yoshikawa, I tried to use the photosensitive surfactant AzoTAB, in order to move a droplet by light. Indeed, a partial UV illumination of an AzoTAB droplet can induce a surface tension gradient on the droplet. If it is strong enough, the droplet can move. For these experiments, I collaborated with Dr. Nobuyuki Magome of the lab.

Unfortunately, illuminating the half of the droplet, deposited on a glass surfarce, on a PDMS surface, or on a super hydrophobic surface (made of modified wax), doesn't make any motion. Friction forces are certainly too high between the liquid and the substrate.

Then, a 1 mM AzoTAB droplet of 5 µL in suspension in a silicon oil was partially illuminated with UV, focused with a microscope. A movement was here visible. Its position was recorded and reported on the following figure. The droplet can be moved outside UV, on a length of about 1,5 mm, with a maximum speed of about 100 µm.s^{-1}, but a saturation of the movement is visible. Some talc particles have been added to the droplet, in order to observe the internal convection during the movement. Particles are immediately moved at the back of the droplet, but it wasn't possible to describe their entire trajectory. This saturation has been explained by the progressive internal mixing of the two types of surfactants, decreasing the initial surface tension gradient.



In a last experiment, a 3 µL oleic acid droplet was deposited on a 2 mM AzoTAB solution. Its illumination by UV makes it moving on length of more than 4 mm, with a constant speed of about 70 $\mu m.s^{-1}$. And by illuminating it with a light of 480 nm (YOYO excitation filter) it was possible to make it move toward illumination area! Its speed is relatively constant and is about 30 $\mu m.s^{-1}$. Modifications of the direction of the movement of the droplet have been observed by successively changing light wavelength, even if the movement is not perfectly reversible (cf. next figure). The precise mechanism of the movement is now under investigation.



Comments:

This collaboration within the GCOE program was a unique and great experience for me! I could discover science in another lab, exchange and discuss with a lot of different people, and better know Japan. The planning and accommodations (Seifu-Kaikan and Kitashirakawa Kagusha) were perfectly organized. And I got all the necessary things (informations, money etc…) immediately after my arriving in Japan, to enjoy and work in very good conditions. Thank you very much!!