“Firefly” Rat Connects Researchers on Global Scale through the Contribution to the Visualization of Invisibility!

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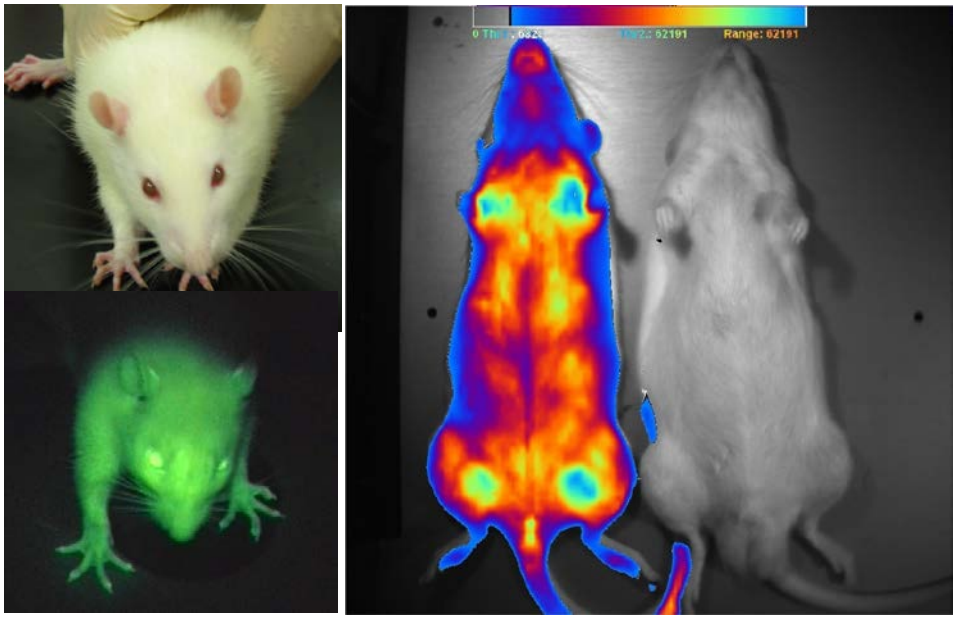
In vivo luminescent method is the optimization of luminescent protein “Luciferase” which merges into the light-emitting substrate “Luciferin” ending up with acquiring light-emitting function by itself. On the other hand, the florescence imaging method is to observe the long-wave visible light on live tissues as a reflected light by emitting a visible short-wave excitation light.



GFP Tg rat **Luciferase Tg rat**

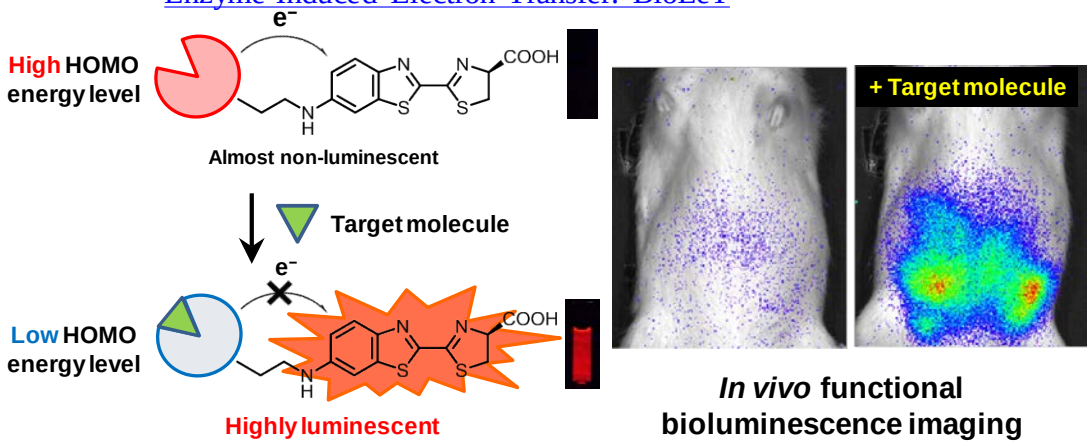
We had been created GFP Tg (2001) and Luciferase Tg rat (2006) and worked for organ transplantation /stem cell research. There are pros and cons that it emits sharp light and has no energy consumption, on the contrary, it requires decrease in the signal sensitivity to avoid halation and auto-fluorescence because it creates the light intake halation inside the tissue.

The both methods are widely used in the biotechnology research field for enabling the detection of signals *in vivo* luminescent imaging which enables analyzing in detail through bioluminescence probe which turns on only when there exists physiologically active molecule in order to locate and fix timing for light emission. Nevertheless, there have been enormous limitations to the types of currently detectable bioluminescence probe which was designed and developed to recover luminescence in time for being released from protecting substrate.

The team of Prof. Yasuteru Urano, Laboratory of Chemical Biology and Molecular Imaging, Graduate School of Medicine, Tokyo University first time has initiated the methodology to logically control ON/OFF of bioluminescent substrate through the induction of controllable chemical switch of bioluminescent enzyme-induced electron transfer to firefly luminescent substrate named "Aminoluciferin" analogue. In addition, by optimizing the firefly transgenic rat filled with Luciferase gene, Prof. Eiji Kobayashi, Department of Organ Fabrication, Keio University School of Medicine has succeeded in *in vivo* imaging of nitrogen monoxide which is one of the important physiological molecule. The achievement was published to the world on 「**Journal of the American Chemical Society**」**(Manuscript title : New class of bioluminogenic probe based on bioluminescent enzyme-induced electron transfer: BioLeT**).

Luminescent and florescent molecules emit light when the electron in molecules in active status called "excitation state" returns to inactive status called "ground state". The research group up to now has found out the fact that the transfer of electron can logically control ON/OFF of florescent molecule (it emits photon from excitation state or not) through electron transfer from the binding electron donor around florescent molecule to luminophore (electron acceptor) in excitation state (See the below figure).

New Class of Bioluminogenic Probe Based on Bioluminescent Enzyme-Induced Electron Transfer: BioLeT



(Takakura H, et al.J Am Chem Soc. 2015)

The research has started based on the assumption that we can control ON/OFF of bioluminescent substrate due to the fact that it turns into excitation state same as florescent molecules by combining it with precisely-designed chemical switch around luminophore.

In the first place the research group has checked the correlation between electron-donating ability and emission intensity of the substrate by combining molecules with various electron-donating abilities. In the consequence, we have discovered the fact that we can suppress light emission to OFF state by the transfer of electron from the molecule to the luminophore in case it combines with highly electron-donating molecules because there exists good correlation between emission intensity and electron-donating ability of combined molecules. The research group named the newly found phenomenon as "**Bioluminescent enzyme-induced electron transfer (BioLeT)**".

This new ON/OFF control principle of bioluminescence has made it possible to develop bioluminescent probes which are considered to be extremely difficult to develop. It has been impossible for conventional bioluminescent probe to detect bioactive molecules such as nitrogen monoxide with no activity for releasing substrate protection structure up to now. The research group first time ever has developed a bioluminescent probe "Diaminophenylpropyl-aminoluciferin (DAL)" which detects nitrogen monoxide through the precise structural design with multiple functions such as electron-donating ability to turn the chemical switch for light emitting to OFF in case there exists no nitrogen monoxide and to turn the switch to ON when electron-donating ability drastically decreases when nitrogen monoxide reacts against the chemical switch. In addition the group has succeeded in detecting highly sensitive nitrogen monoxide inside live animals through the optimization of "**Firefly Ra**t" with Luciferase emerged in entire body, which has been difficult to detect so far.

The above bioluminescent ON/OFF control principle is considered to be applicable to various detection principles for bioactive molecules, which is expected to act as a key to unravel the life phenomenon thanks to its function to observe varieties of *in vivo* bioactive molecules in animal bodies.