19a-D4-4

Superstrong luminescent protein for high speed imaging at single cell and whole body level

Takeharu Nagai[1,2]

[1] ISIR, Osaka Univ, [2] PRESTO, JST
E-mail: ng1@sanken.osaka-u.ac.jp

Fluorescence imaging is widely used, but the dependence on external illumination prevents its universal application. For example it cannot be used to study light dependent biological processes such as photosynthesis. Moreover it is incompatible with the non-invasive imaging of whole organisms, or other applications where the cellular substrate is autofluorescent, saturated with photopigments or extremely photosensitive. Experimental conflicts arise when external illumination is essential in other biological technologies, such as optogenetics, chromophore-assisted light inactivation, or photolysis of caged compounds, preventing simultaneous use of fluorescence imaging. Finally sub W/cm^2 external illumination, which is general power density for fluorescence excitation in live cell imaging under the wide field microscopic observation, sometimes causes phototoxic effects in visualized substrates which alter cellular behavior and ultimately cause cell death. By contrast chemiluminescence generates a visible light signal by a localized chemical reaction without the need for external illumination. The k_{cat} values of conventional luciferases are ranging from 0.1 for Fluc (quantum yield=0.5) and aprox. 4.4 for RLuc8 (quantum yield=0.053). Provided that cells express several μM Rluc8, a typical concentration of transiently expressed protein, the power density of light emitted from the Rluc8-expressing cells could be calculated as approximately 0.1 μW/cm^2 which is 1/10^3 of general power density for fluorescence excitation in live cell imaging. It is therefore theoretically independent of the associated restrictions which limit biological application of fluorescence. However the 0.1 μW/cm^2 power density is not bright enough to image events on a biological scale with subsecond/micrometer accuracy. Therefore although the chemiluminescent protein including aequorin and luciferases have been used to image living cells, and organisms, the temporal and spatial resolution of this strategy is unable to match that of fluorescence.

In luminous organisms such as the sea pansy *Renilla reniformis*, nature has solved this problem using BRET (bioluminescence resonance energy transfer), in which the excited energy of a luminescent substrate, coelenterazine, bound to *Renilla* luciferase (RLuc) (quantum yield=0.053) is efficiently transferred inter-molecularly to the acceptor *Renilla* green fluorescent protein (RGFP) (quantum yield=0.3) by a Förster resonance energy transfer (FRET) mechanism, thereby increasing the emitted photon number approximately 5 fold. Based on this natural inter-molecular BRET, intra-molecular BRET probes such as aequorin-GFP and BAF-Y have been developed. Although these allow live-cell imaging with improved resolution in space and time, they still underperform compared with FP-based probes because of low brightness.

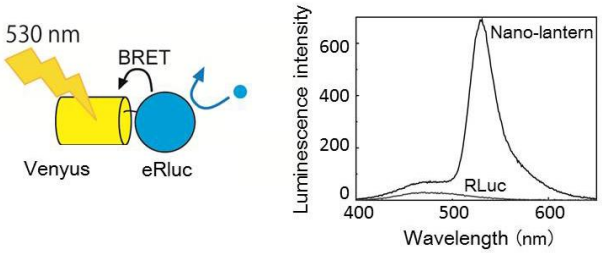

Fig.1. Schematic structure and luminescence spectra of Nano-lantern.

To overcome this drawback, *Renilla reniformis* luciferase (Rluc) was conducted on random mutagenesis to improve the intensity. Then, the luminescence intensity was further increased by fusion of the enhanced Rluc (eRluc) to a yellow fluorescent protein, Venus with high BRET efficiency. The chimeric protein, Nano-lantern, showed much brighter luminescence than the commercially available Rluc (Fig. 1), enabling not only real-time imaging of intracellular structures in living cells with spatial resolution equivalent to fluorescence but also sensitive tumor detection in freely moving mice which has never been possible before (Fig.2) [1]. We then applied the intense luminescent protein to design Ca^{2+}, cAMP, and ATP indicators, thereby we succeeded imaging these bioactive molecules in environments where fluorescent indicators have failed. These super-duper luminescent proteins will revolutionize conventional bioimaging by allowing visualization of biological phenomena not seen before at the single-cell, organ, and whole-body level, in animals and plants.

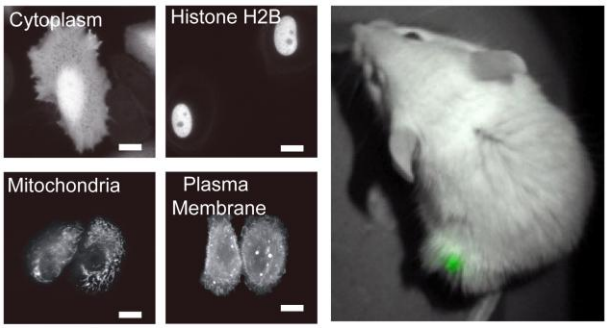

Fig 2. Luminescence imaging of HeLa cells and mouse carrying Nano-lantern-expressing tumors.

References

[1] Saito K et al., *Nat. Commun.* **3**, 1262, 2012