Chemiluminescence imaging had shed light on not only bioscience but also medical and pharmaceutical research fields in situations when fluorescence cannot be used, for example non-invasive deep tissue imaging of whole animals in the anesthetized condition. In spite of their potential utility, the universal applications had been precluded by its low brightness. To overcome this problem, we previously developed a bright chemiluminescent protein, Nano-lantern (Saito K. et al. Nat. Commun. 3, 1262, 2012), which enabled us real-time imaging of tumor tissue in freely locomoting mouse. However, the brightness of Nano-lantern is not enough to trace faster biological phenomena such as protein dynamics without compromising the spatial resolution. Moreover, the luminescent color of Nano-lantern has been limited to green. Here we report further development of a superduper chemiluminescent protein, the enhanced Nano-lantern (eNano-lantern) and its color variant (red-eNano-lantern). The eNano-lantern emits approximately 8 times brighter signal than that of Nano-lantern mainly due to the fast substrate turn-over rate. The emission peak of red-eNano-lantern is 585 nm which is relatively transparent in living tissue than green color. With the use of eNano-lantern, we could perform sub-video rate imaging of representative intracellular structures in living cells with high spatial resolution. Furthermore, by engineering the eNano-lantern, we could make a novel Ca^{2+} indicator which gets brighter up to 170% upon Ca^{2+} binding. To make maximal use of newly developed palette of these superduper chemiluminescent probes, we would like to demonstrate in the conference not only multiple malignant tumors imaging in an awake mouse but also multifunctional video rate imaging of protein dynamics in conjunction with optogenetic manipulations at single cell level.

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