Assaying protein-protein interaction by the complementation of firefly luciferase catalytic steps

Protein - protein interaction (PPI) assay is a key technology for various fields from basic molecular biology to diagnosis and drug discovery. Previously, PPI assays based on fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET) and protein-fragment complementation (PCA in short) are generally used. FRET- and BRET-based assays are established methods and their reproducibility is high. To conduct these assays, fluorescent proteins and luciferases are generally used, however, the interaction between large proteins is difficult to detect, because the signal intensity decreases inversely correlated with the 6^{th} power of the distance between the probes. In addition, the attainable signal/background (S/B) ratios are rather low. On the other hand, PCA is a user-friendly assay and shows high S/B ratio, and many enzymes and fluorescent proteins can be used. However, the signal intensity and the stability of the probes are low due to the split form of the probes.

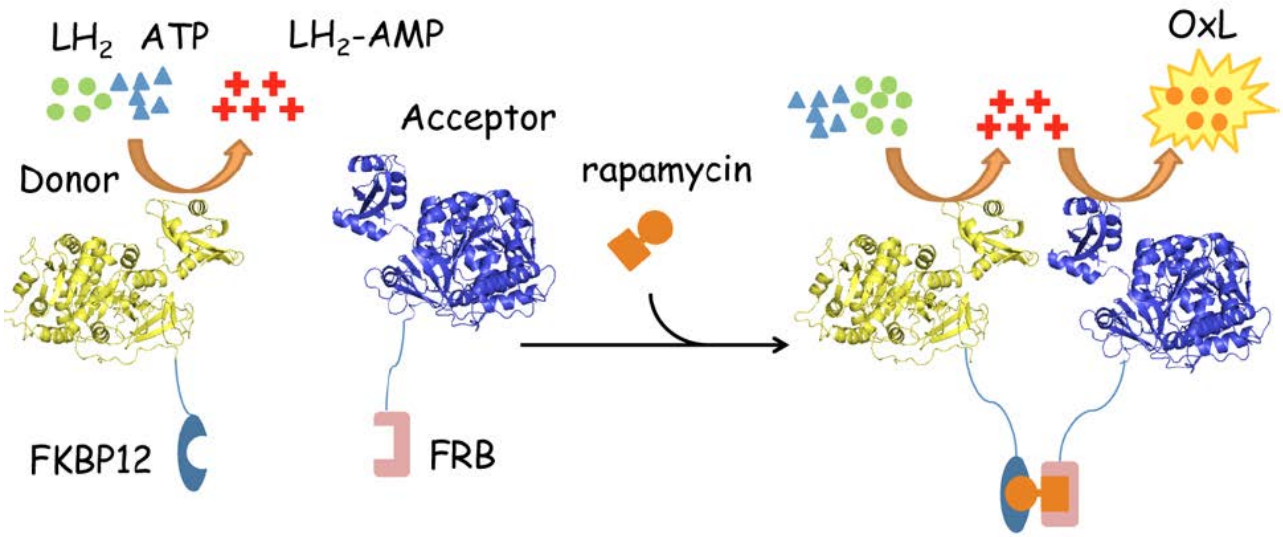

Fig. 1.

To overcome the problems of these conventional methods, we developed a new PPI assay utilizing a unique property of firefly luciferase (Fluc) reaction. Namely, the light-emitting reactions of Fluc can be divided into two half-reactions, the adenylation step and the following oxidative luminescent steps. In the adenylation step, D-luciferin is reacted with ATP to produce a reaction intermediate luciferyl-adenylate (LH_2-AMP), while in the following steps LH_2-AMP is added with oxygen, decarboxylated, and converted to light-emitting oxyluciferin (OxL). To detect PPI, we made two mutant Flucs without luminescent activity on its own. One mutant called Donor is defective in

converting LH_2-AMP to OxL. On the other hand, another mutant called Acceptor is defective in converting D-luciferin and ATP to LH_2-AMP. When the Donor and the Acceptor are each fused with an interacting protein, and are brought into close proximity due to the interaction, the Donor provides LH_2-AMP to the Acceptor, and the Acceptor produces OxL to emit light. We named this PPI assay as *f*irefly *l*uminescent *i*nter*m*ediate-based *p*rotein *i*nteraction *a*ssay, FlimPIA.

FlimPIA already manifests several merits as an *in vitro* PPI assay. First, the interaction between larger proteins can be detected compared with FP-FRET assay and Fluc PCA, because the signal intensity of FlimPIA decreases more slowly according to the distance between the probes. Second, the S/B ratio attained was higher than that of those other assays. Third, the detection limit of PPI was the lowest in these assays. Fourth, the stability of probes is higher than that of Fluc PCA, probably due to the use of full-length enzyme as a probe. Fifth, the signal intensity was higher than that of Fluc PCA. Although FlimPIA performed *in cellulo* provides fewer benefits at this moment, we believe it will be improved soon.

FlimPIA is based on the unique principle of compensating the catalytic steps of an enzyme. Fluc belongs to an acyl/adenylate-forming enzyme superfamily. The chemical reactions catalyzed by the enzyme in this superfamily all consist of two half-reactions including an adenylation step, to which the principle of FlimPIA will be applied. Hopefully, by employing other enzymes in this family of enzymes, more useful PPI assays and other applications will be developed.

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