Fluoppi (Fluorescence based technology visualizing protein-protein interactions) is a novel technology to monitor protein-protein interactions (PPIs) in living cells in reversible manner. The most advantage of Fluoppi is establishing assays are very easy and simple, which do not require fine optimizations such as peptide linker sequence or length.

Fluoppi detects PPIs as fluorescent foci.

**Fluorescent proteins | Code. | Product**

| Monti-Red (Red) | AM-8002M | Fluoppi : Ash-Red (Ash-MNL/MCL + Monti-Red-MNL/MCL) |
| AM-VS0802M | Monti-Red for Fluoppi (pMonti-Red-MNL/MCL) |
| hAG (Green) | AM-8001M | Fluoppi : Ash-hAG (Ash-MNL/MCL + hAG-MNL/MCL) |
| AM-VS0801M | humanized Azami-Green for Fluoppi (pHAG-MNL/MCL) |
| AM-8201M | Fluoppi : Ash-hAG [p53-MDM2] |
| AM-8202M | Fluoppi : Ash-hAG [mTOR-FKBP12] |

* Fluoppi does not guarantee detection of all Protein-Protein Interactions.

* Any other uses by non-profit research organizations, or any uses by for-profit organizations regardless of commercial or non-commercial purpose, require a license.

* The fluorescent proteins used in product, hAzami-Green and Monti-Red, differ from each other in fluorescence and other properties.
**Key components and Mechanism of action**

Fluoppi is a tag technology. Tetramer fluorescent protein (FP-tag) and Assembly helper tag (Ash-tag) are genetically fused to Protein X and Y, respectively. For the FP-tag, tetramer Fluorescent protein, Azami Green (hAG) and Monti-Red can be used.

By way of example, genetic fusion of protein X with FP-tag, and Y with Ash-tag creates a tetrameric fluorescent fusion protein X-FP and an oligomeric fusion protein Y-Ash respectively. Because each fusion protein has multiple Xs or Ys, interaction between X and Y causes large lattice like complexes where the fluorescence by X-FP is concentrated and detectable as fluorescent foci.

**Work Flow of Fluoppi**

At first, proteins X & Y of your interest are fused to FP-tag and Ash-tag respectively. We recommend to prepare all the eight possible constructs to identify the best workable combinations.

Because fluorescence signal of Fluoppi is very high, conventional fluorescence microscopy can be used to image the cells. If the proteins interact with each other upon expression, fluorescent foci will be detected. Formation of foci is reversible so that they can be dissociated and the fluorescent signal will spread over the cell by PPI inhibitors, and vice versa by PPI inducer.

Fluoppi Kit (code: AM-8001M, AM-8002M) includes 4 expression plasmids as pAsh-MNL/MCL, pFP-MNL/MCL. For the FP-tag, tetramer Fluorescent protein, Azami Green (hAG) (AM-8001M) or Monti-Red (AM-8002M) can be used.
Localization

Because location of foci is not restricted to specific site inside the cell, Fluoppi can visualize PPIs at several subcellular localizations such as cytosol, nucleus, and juxtamembrane. The upper pictures represent foci at several subcellular localizations, and the lower pictures are negative controls which express hAG tagged protein and Ash-tag without fusing any proteins. The images of juxtamembrane are taken by Total Internal Reflection Fluorescence Microscopy (TIRFM).

Visualization of protein complex

p21 (CIP/WAF) interacts to and inhibits the activity of cyclin-CDKs complex and thus functions as a regulator of cell cycle progression at G1 and S phase. In this experiment, HeLa cells expressing both AG-p21 and CDK4-Ash displayed disseminated pattern of green fluorescence (upper panels). By contrast, additional cotransfection of cyclin D1 resulted in formation of clear foci in the nucleus (lower panels). These results indicate CDK4 alone do not interact to p21 and requires additional factor in live cells, which is consistent with previous studies.
An example of quantitative analysis for PPI and Screening of PPI modulator

**< p53-DM2 >**

p53-DM2 is a famous target of PPI modulators in the field of anti cancer drug development. We applied this PPI to Fluoppi for demonstration. First, hAG-DM2 and Ash-p53 was selected from the 8 pairs as represented above, then stable CHO-K1 cell line was established by using two selection marker; G418 and Hygromycin. Fluoppi plasmids including Hygromycin resistance gene is under released at present.

**< Quantitative analysis >**

Stable cell lines were seeded on black-wall 96-well plates. PPI modulator was treated for 15 minutes in a room temperature followed by fixation with 4 % PFA. Cells were then stained with Hoechst33342. Imaging was performed with IN Cell Analyzer 1000 (GE Healthcare) using x10 objective lens. Green channel (AG) and blue channel (Hoechst33342) images were used to define foci and nuclear regions respectively. Foci or nucleus are segmented with red or blue enclosed lines respectively using IN Cell Investigator software. (If you don't have an In Cell Analyzer and similar equipment, you can use the open sources such as, Spot Detector, provided by Icy: an open community platform for bioimage informatics. http://icy.bioimageanalysis.org/)

Foci Intensity was calculated as fluorescence intensities inside the region of foci divided by the number of nucleus.

**< Data analysis >**

In the case of p53-DM2 experiment, IC$_{50}$ value was determined to be 6.3 μM. Enough Z'-factor could be obtained, suggesting high reliability of this technology.
A result of pilot screening using commercially available reference library (630 compounds x 2 conc.). High robustness of this screening system is verified.

In this screening, compound X was calculated as a HIT compound, however the image represented it was just a quenching compound. Many library contains that kind of compound, therefore Fluoppi can easily exclude pseudo-positive sample without 2nd screening.

Visualizing dual PPIs in single cell.

mTOR (FRB domain) and FKBP12 is a well known PPI whose interaction is induced by Rapamycin. On the other hand, mTOR (FRB domain) and mCAB (the BBH region of Calcineurin A (residues 340-394) fused to Calcineurin B*) interaction is induced by FK506. In this experiment, The mCAB-hAG, mTOR-Monti-Red and Ash-FKBP12 were cotransfected into the cells at the same times. When the cells were exposed to Rapamycin, only the red foci were formed as shown in the left 4 pictures. On the other hand, in the case of FK506, only the green foci were detected. The results demonstrate the possibility that multi-color Fluoppi could be applied to visualize branched signaling pathway.


Publications

Koyano F et al. Ubiquitin is phosphorylated by PINK1 to activate parkin. Nature 510, 162-6 (2014)

References