Supporting Information

Development of a Split SNAP-CLIP Double Labeling System for Tracking Proteins Following Dissociation from Protein−Protein Complexes in Living Cells

Masayasu Mie, Tatsuhiko Naoki, Eiry Kobatake

Department of Life Science and Technology,

School of Life Science and Technology,

Tokyo Institute of Technology

4259 Nagatsuta, Midori-ku, Yokohama 226-8502, Japan

*mie.m.aa@m.titech.ac.jp

Materials and Methods

Construction of Plasmids

The pcDNA-FKBP12-nCLIP(1−91)-$(GGGS)_3$-cSNAP(109−182) plasmid was constructed as follows. Using pcDNA-F_M-nCLIP(1−91)-$(GGGS)_3$-cSNAP(109−182) as a template, point mutagenesis was performed with the primer set FMtoFKBP12S and FMtoFKBP12AS, followed by sequence confirmation.

The pcDNA-FRB-cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122) plasmid was constructed as follows. The cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122) fragment derived from pcDNA-F_M-cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122) by digestion with *Kpn* I and *Not* I was inserted into the suitably digested pcDNA-NLS-FRB-cSNAP(109−182) plasmid. Using the resulting plasmid, (pcDNA-NLS-FRB-cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122)), as a template, deletion mutagenesis was performed with the primer set FRB signal deletion S and FRB signal deletion AS to remove the NLS sequences, followed by sequence confirmation.

The pcDNA-F_M-nSNAP(1−122) and pPKCα-F_M-nSNAP(1−122) plasmids were constructed as follows. The nSNAP(1−122) fragment derived from pUC-nSNAP(1−122) by digestion with *Eco*R I and *Kpn* I was cloned into the suitably digested pcDNA-F_M-cCLIP and pPKCα-F_M-cSNAP plasmids, respectively. The

resulting plasmids were termed pcDNA-F_M-nSNAP(1−122) and pPKCα-F_M-nSNAP(1−122), respectively. The pcDNA-F_M-cSNAP(109−182) and pPKCα-F_M-nSNAP(109−182) plasmids were constructed according to the same procedure using pUC-cSNAP(109−182) instead of pUC-nSNAP(1−122).

SNAP-tag Fragment Complementation Study

For SDS-PAGE analysis, HeLa cells were coexpressed with all combinations of nSNAP-FKBP12 and NLS-FRB-cSNAP. After transfection, transfected cells were incubated with cell culture medium containing rapamycin (final concentration: 1 μM) for 60 min. After washing, cells were incubated with cell culture medium in the presence of the SNAP-tag substrate SNAP-Cell Oregon Green (final concentration: 500 nM) for 20 min. Then, cells were harvested using a cell scraper with 30 μL of cold phosphate-buffered saline (PBS) containing 0.1% Nonident P-40. Collected cells were sonicated and centrifuged to remove insoluble debris. The supernatant (10 μL) was separated by SDS-PAGE, and labeled proteins were detected by a fluorescence scanner.

Split SNAP-tag Double Labeling System Study

Cells expressing FKBP12-nCLIP(1−91)-$(GGGS)_3$-cSNAP(109−182) and

FRB-cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122) were incubated in cell culture medium in the presence of SNAP-Cell Oregon Green and CLIP-Cell TMR-Star (final concentration: 250 nM each) for 60 min with or without rapamycin during labeling. After washing, cells were incubated with cell medium for 2 h to remove unreacted substrate, and subsequently observed by confocal laser scanning microscopy.

Table S1

Primers used in this study

Name	Sequence
nSNAP(s)	GCTAGGTACCGCTAGCGCCATGGACAAAGACTGCGAA
nSNAP(1−72 as)	CCCGAATTCAAAGCTTCTGGTGAAAGTAGGCGTTGA
nSNAP(1−91 as)	CCCGAATTCAAAGCTTCTGCTGGAACACTGGGTGGT
nSNAP(1−108 as)	CCCGAATTCAAAGCTTGAACTTCACCACTTTCAGCA
nSNAP(1−122 as)	CCCGAATTCAAAGCTTGCCGGCCAGGGCGGCCAGCT
cSNAP-1(as)	GCCGAATTCAAGCTTTCACTCGAGGGATCCTGGC
cSNAP(73−182 s)	GCTAGGTACCATGGTCGACCCTGAGGCCATCGAGGAGTT
cSNAP(92−182 s)	GCTAGGTACCATGGTCGACGAGAGCTTTACCCGCCAGGT
cSNAP(109−182 s)	GCTAGGTACCATGGTCGACGGAGAGGTCATCAGCTACCA
cSNAP(123−182 s)	GCTAGGTACCATGGTCGACAATCCCGCCGCCACCGCCGC
FRB(s)	GCTAGCCTCGAGATGGCTTCTAGAATCCTC
FRB(as)	GAATTCTTAGGTACCCTTTGAGATTCGTCGGAACAC
nCLIP-Linker(as)	GGGGAATTCGCTAGCTGATCCTCCTCCTGATCCTCCTCCT GATCCTCCTCCACCCAGCCCAGGCTTGCCCAGTCTGTGG CCCTCGTGGGCCAGCAGCCACTCTTTC
FM to FKBP12 S	AAGAAATTTGATTCCTCCCGGGACAGA
FM to FKBP12 AS	GGAATCAAATTTCTTTCCATCTTCAAG
FRB signal deletion S	GGCTAGCCTCGAGATGGCTTCTAGA
FRB signal deletion AS	ATCTCGAGGCTAGCCAGCTTGGGTCT

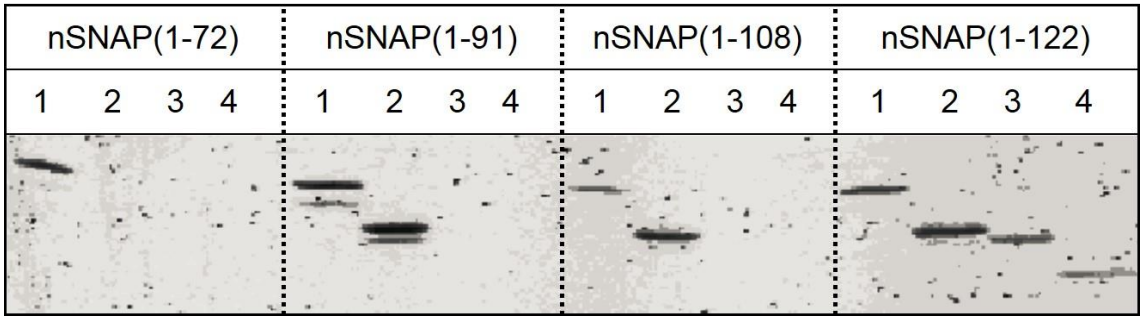

Figure S1. SDS-PAGE analysis of SNAP-tag fragment complementation. All combinations of nSNAP-FKBP12 and NLS-FRB-cSNAP were coexpressed in HeLa cells. After exposure to rapamycin, cells were incubated with SNAP-Cell Oregon Green. The labeled cSNAP fragments were detected by a fluorescence scanner. 1:cSNAP(73−182); 2:cSNAP(92−182); 3:cSNAP(109−182); 4:cSNAP(123−182).

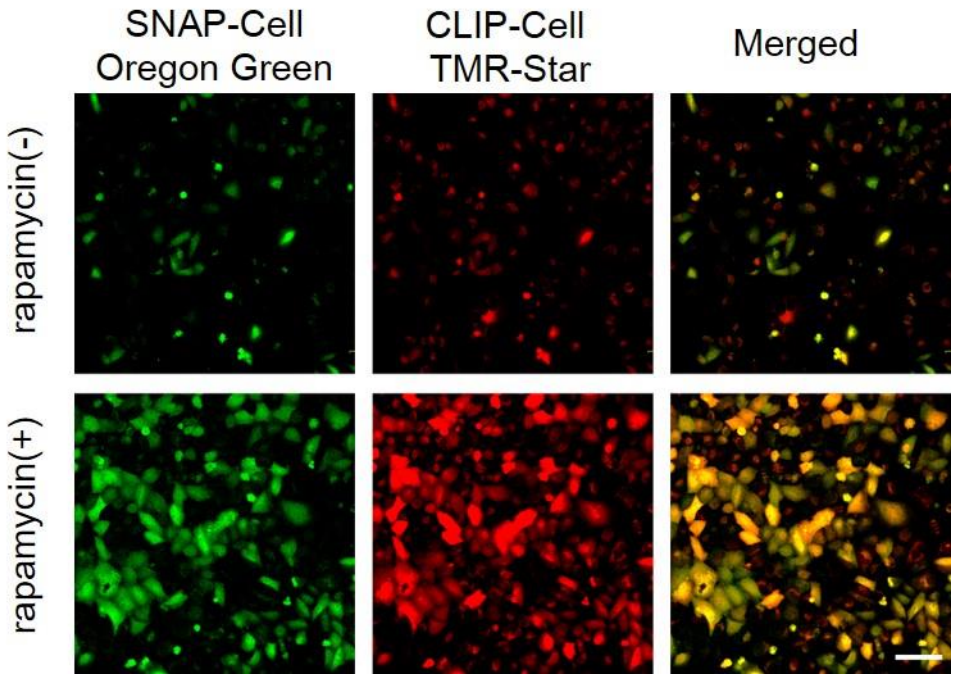

Figure S2. Split SNAP-CLIP double labeling system with FKBP12-rapamycin-FRB interaction. Cells coexpressing FKBP12-nCLIP(1−91)-$(GGGS)_3$-cSNAP(109−182) and FRB-cCLIP(92−182)-$(GGGS)_3$-nSNAP(1−122) were cultured with or without rapamycin, before addition of SNAP-Cell Oregon Green and CLIP-Cell TMR-Star. The scale bar represents 100 μm.

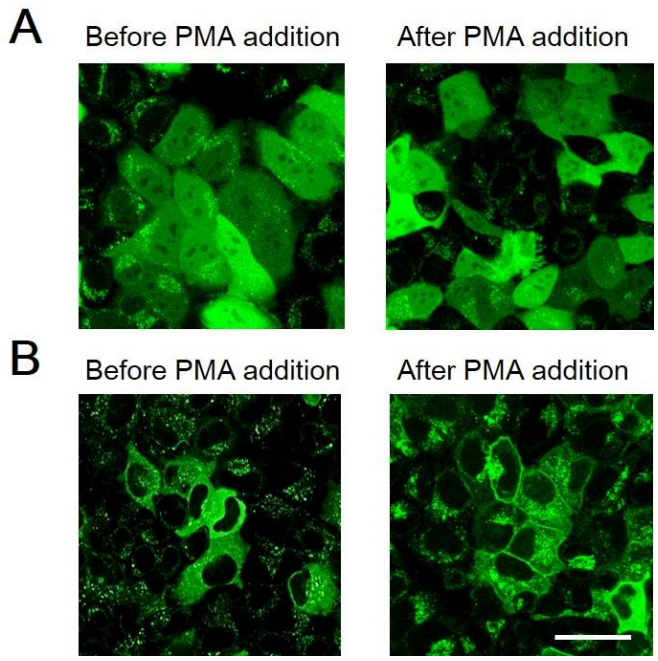

Figure S3. Tracking proteins following F_M homodimer dissociation induced by addition of FK506. Cells expressing (A) PKCα-F_M-nSNAP(1−122) and F_M-cSNAP(109−182), and (B) F_M-nSNAP(1−122) and PKCα-F_M-cSNAP(109−182). Fluorescently labeled PKCα-F_M-cSNAP(109−182) was observed at different places before and after addition of PMA. The scale bar represents 50 μm.

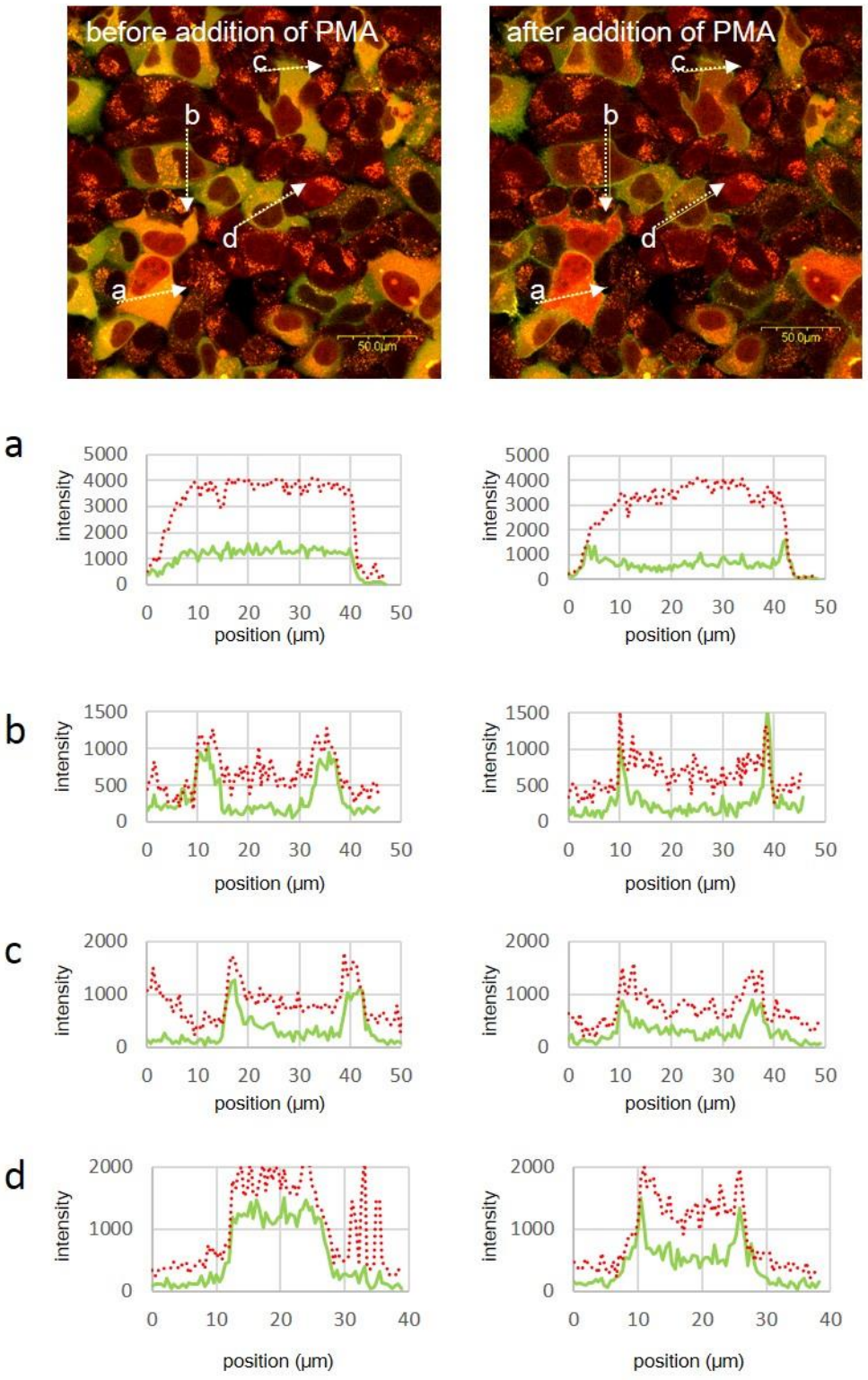

Figure S4. The differences of fluorescence signal intensity across the cells before and after addition of PMA analyzed by FLUOVIEW software (Olympus).