

# Supporting Information

## Flashbody: a next generation Fluobody with fluorescence intensity enhanced by antigen binding

Devina Wongso<sup>1</sup>, Jinhua Dong<sup>2</sup>, Hiroshi Ueda<sup>2,\*</sup>, and Tetsuya Kitaguchi<sup>1,3,\*</sup>

<sup>1</sup>Cell Signaling Group, Waseda Bioscience Research Institute in Singapore (WABIOS), 11 Biopolis Way #05-02 Helios, Singapore 138667, Singapore

<sup>2</sup>Laboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259-R1-18, Nagatsuta-cho, Midori-ku, Yokohama, Kanagawa 226-8503, Japan

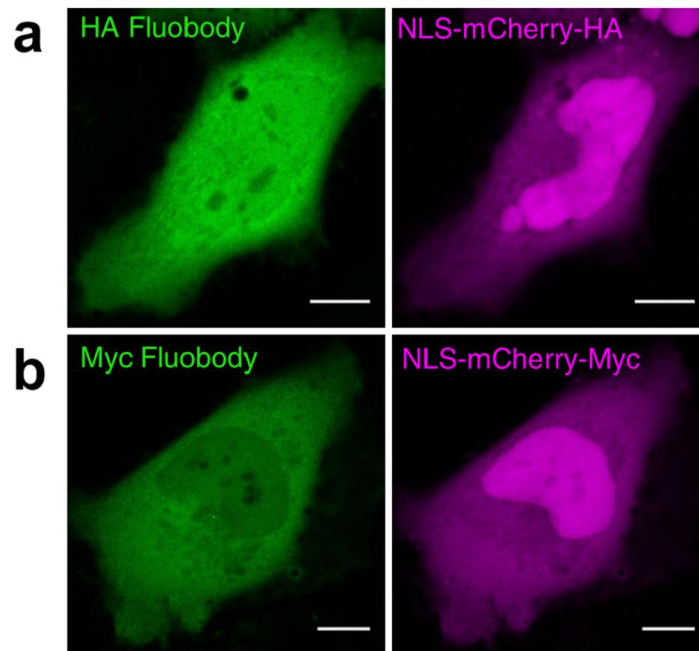
<sup>3</sup>Comprehensive Research Organization, Waseda University, #304, Block 120-4, 513 Wasedaturumaki-cho, Shinjuku, Tokyo 162-0041, Japan

\*Corresponding authors Tel.: +65 6478 9721, E-mail: ueda@res.titech.ac.jp; kitaguct-gfp@umin.ac.jp

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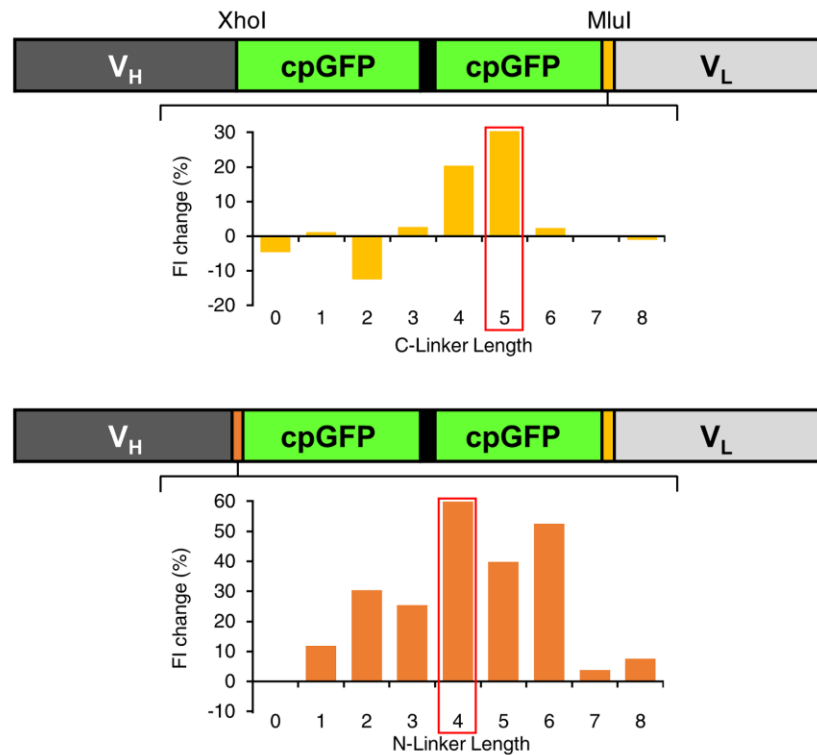
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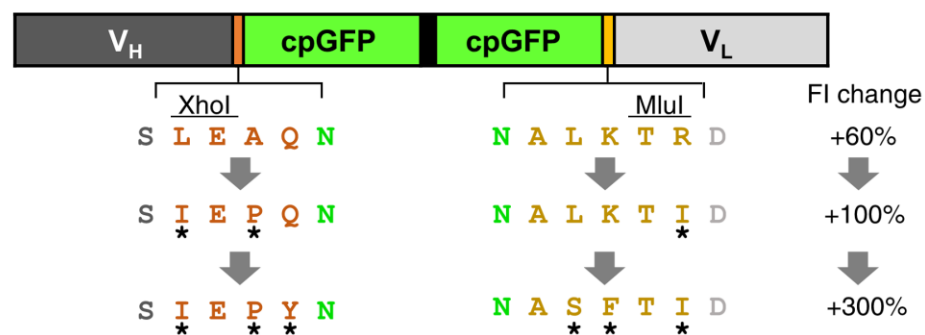


**Figure S-1. Intracellular binding of anti-HA and anti-Myc antibody in living cells.** (a) HA Fluobody co-expressed with mCherry localized to the nucleus with the HA tag. (b) Myc Fluobody co-expressed with mCherry localized to the nucleus with the Myc tag. The scale bars represent 10  $\mu\text{m}$ .

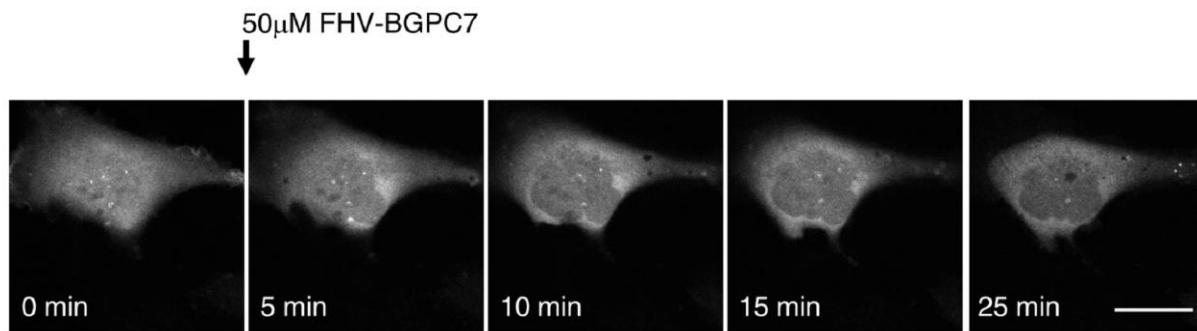
## Stage 1: Linker Length Optimization



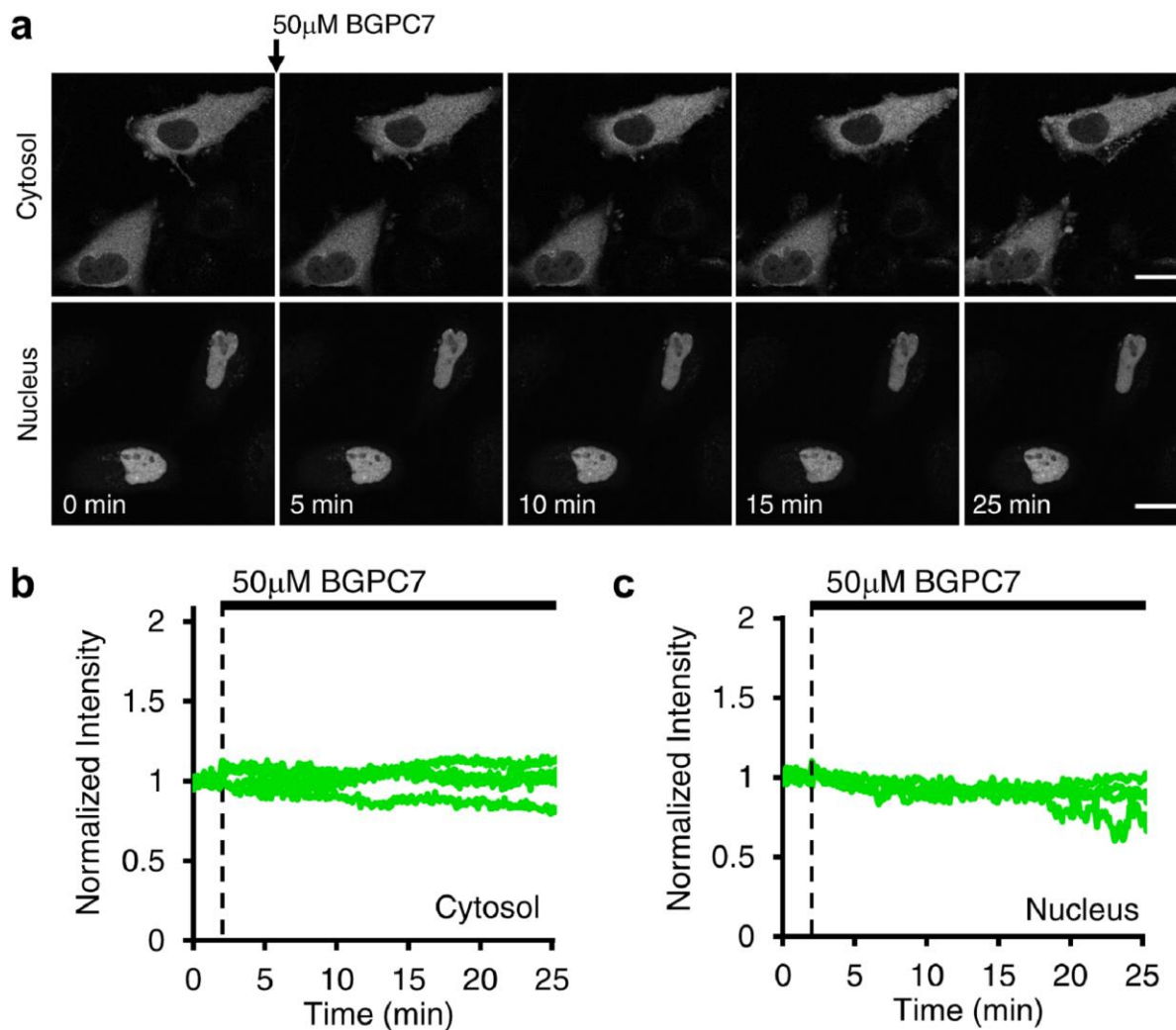
## Stage 2: Amino Acid Optimization



**Figure S-2. Systematic screening strategy for BGP Flashbody.** The length of the linkers between antibody fragments and the fluorescent protein was optimized in the first stage of the screening process. The amino acid sequence composition of the linkers was then optimized in the second stage of the screening process. The stars represent the location of amino acid substitutions. Abbreviation: cpGFP, circularly permuted green fluorescent protein; FI, fluorescence intensity; V<sub>H</sub>, variable region heavy chain; V<sub>L</sub>, variable region light chain.



**Figure S-3. Visualization of translocation of cell penetrating peptide by BGP Fluobody in living cells.** Sequential images of BGPC7 fused to Flock House Virus (FHV) cell penetrating peptide sequence entry to HeLa cell, visualized by BGP Fluobody. The scale bar represents 20  $\mu$ m.



**Figure S-4. Time-lapse imaging of cytosolic and nuclear BGP Flashbody treated with BGPC7.** (a) Sequential images of HeLa cells expressing BGP Flashbody localized to the cytosol (upper) and nucleus (lower). BGPC7 peptide was added at 2 min after the start of image acquisition. The scale bars represent 20  $\mu\text{m}$ . (b-c) Changes in fluorescence intensity of the BGP Flashbody localized to cytosol (b) and nucleus (c) treated with BGPC7. Green traces are from at least three independent experiments.

**Supporting Movie 1. Movie of BGP Flashbody expressed in HeLa cells cytosol treated with 50  $\mu$ M FHV-BGPC7.**

**Supporting Movie 2. Movie of BGP Fluobody expressed in HeLa cells cytosol treated with 50  $\mu$ M FHV-BGPC7.**

**Supporting Movie 3. Movie of BGP Flashbody expressed in HeLa cell nucleus treated with 50  $\mu$ M FHV-BGPC7.**