Fluorescence Protein for Long-term Live-cell Super-resolution Imaging of Various Cellular Structures

Jiwoong Kwon,[1,2] Jong-Seok Park,[3] Min Su Kang,[1,2] Gyeong Tae Kim,[4] Soohyun Jang,[1,2] Hyun-Woo Rhee[3] and Sang-Hee Shim[1,2]*

[1] *Center for Molecular Spectroscopy and Dynamics, Institute for Basic Science (IBS), Korea University, Seoul 02841, Korea*
[2] *Department of Chemistry, Korea University, Seoul 02841, Korea*
[3] *Department of Chemistry, School of Natural Sciences, UNIST, Ulsan 44919, Korea*
[4] *Department of Biomedical Engineering, School of Life Sciences, UNIST, Ulsan 44919, Korea *E-mail: sangheeshim@korea.ac.kr*

ABSTRACT UnaG is a fluorescent protein discovered form Japanese Eel, whose fluorescence arise only when the protein binds to bilirubin (BR), a nonfluorescent metabolite. We investigated UnaG's photoswitchable nature caused by repetitive binding and unbinding of BR. Mainly the photooxidation of BR induces the off-switching of UnaG fluorescence. When the damaged BR is exchanged to fresh one, the protein recovers the fluorescence capability. Since more than 50% of UnaG molecules can survive after >300 switching cycles, UnaG should be an attractive candidate for a fluorescent probe for single-molecule localization based super-resolution imaging, especially for the long-term live-cell imaging. We imaged various UnaG labeled subcellular structures with sub-diffraction limit resolution in live Cos7 cells. As a result, we can increase the number of super-resolution snapshots more than 10 times with UnaG than conventional fluorescent probes (except lipophilic dyes that have limited applications).

Introduction

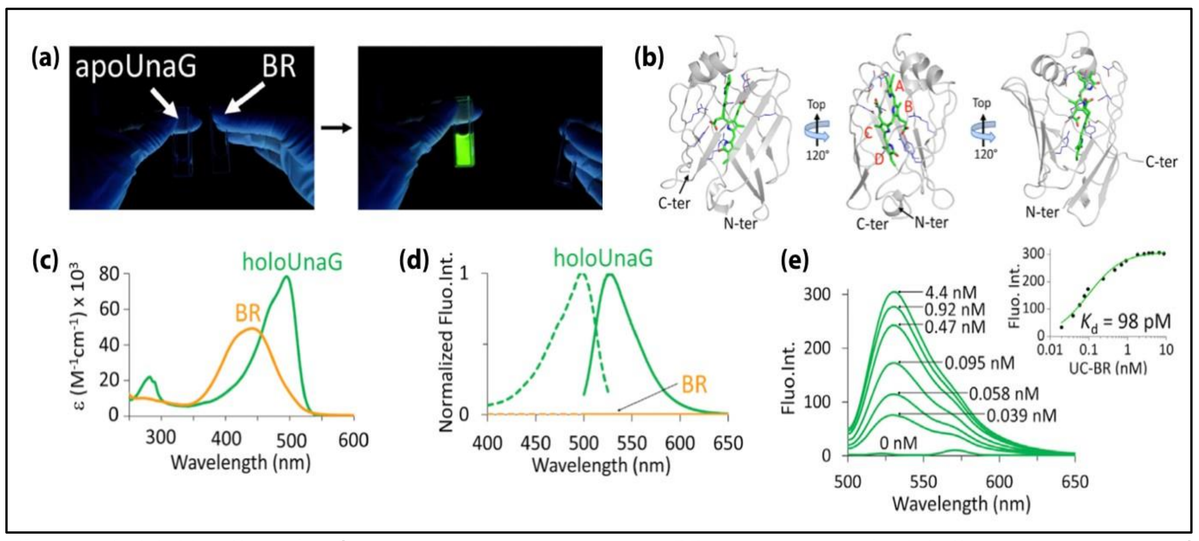

Figure 1 (a) UnaG emits fluorescence only when a BR bounds on it. (b) Overall structure of holoUnaG represented in three different angles. (c) Absorption spectrum of holoUnaG (green) and BR (orange). (d) Excitation (dashed lines) and emission (solid lines) spectrum of holoUnaG (green) and BR (orange). (e) Titration of apoUnaG (5 nM) with unconjugated BR.

❖ **UnaG is a protein derived from Japanese eel, and can bind to a fluorogen, bilirubin, to emit fluorescence.**

*A. Kumagai et al., Cell, **153**, 1602 (2013)*

Background

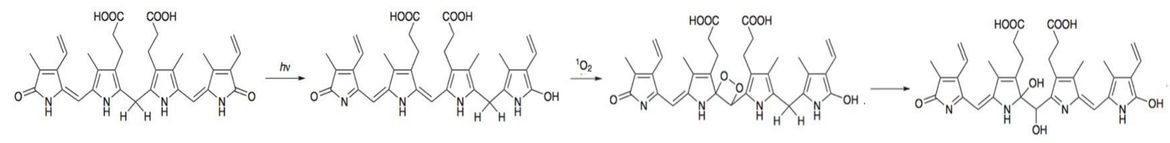

Figure 2 Photochemical reaction of BR under light illumination. Singlet oxygen oxidize BR to biliverdin (BV), which proceeds further internal molecular transition.

❖ **BR is an endogenous metabolite that can bind to the UnaG to form holoUnaG. Although free BR do not fluoresce, the holoUnaG emit strong fluorescence by absorbing blue light. Singlet oxygen can interact with the BR to form BV, and it results the photobleaching of holoUnaG.** *C. S. Berry et al., Biochem. Biophys. Res. Comm., **49**, 1366 (1972)*

Photo-oxidation and Fluorescence Recovery

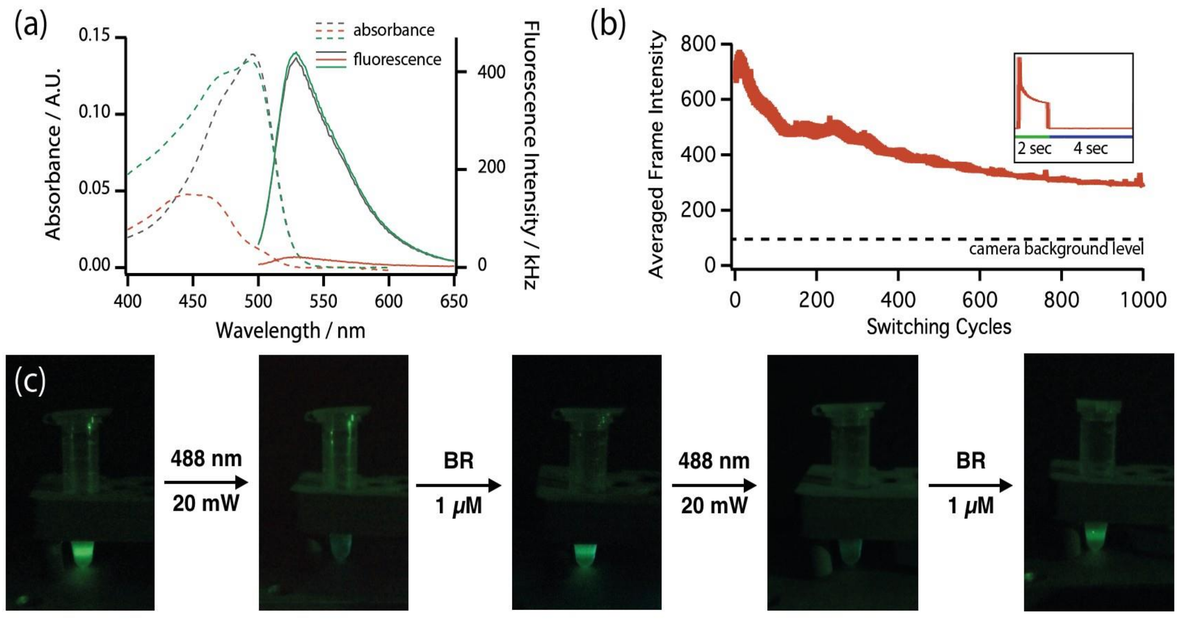

Figure 3 (a) Absorption (dotted lines) and fluorescence emission (solid lines) spectrums of normal holoUnaG (black), photo-oxidized holoUnag (red) and recovered holoUnaG (green). Abnormal large absorbance at <460 nm of recovered holoUnaG came from the 1 μM of additional BR for the recovery. (b) Repetitive photo- oxidation and recovery cycles of surface - immobilized holoUnaG. 3 kW/cm2 of 488-nm and 600 nM BR were used to deplete and restoring the fluorescence. More than 50 % of molecules remain in the non-bleached state after ~300 switching cycles. inset: single representative photo-oxidation and recovery event. (c) Photobleaching and fluorescence recovery of holoUnaG. Each picture was captured with 200 μW of 488-nm excitation light, after incubating for 60 minutes under indicated conditions.

❖ **Photobleaching of holoUnaG induces the photo-oxidation of BR rather than the photodamage to the protein itself. Damaged BR can be replaced to fresh one to make UnaG fluoresce again.**

Results

I. Imaging of various subcellular structures

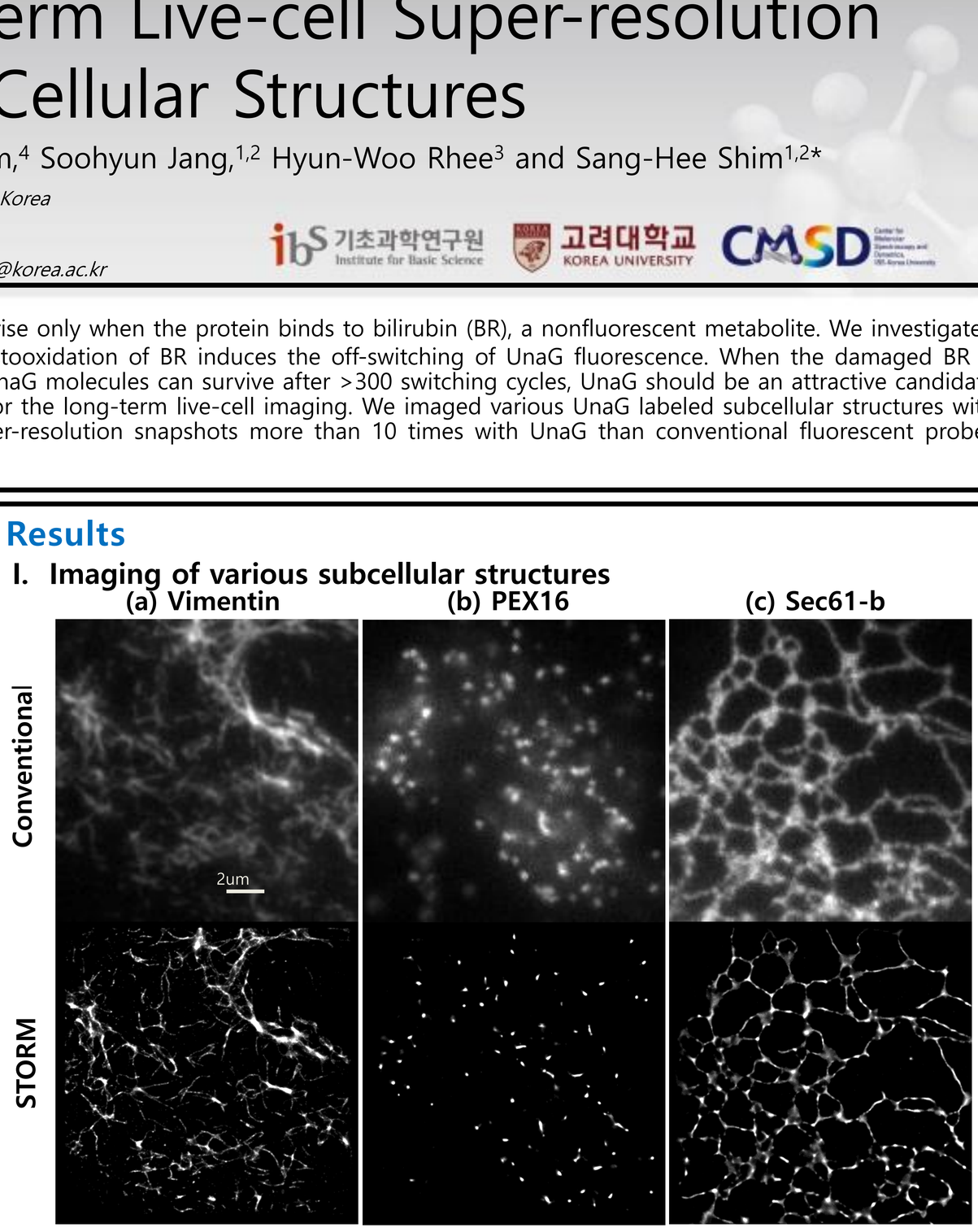

Figure 4 Super-resoultion imaging of fixed Cos7 cells expressing vimentin-UnaG (a), PEX16-UnaG (b), Sec61b-UnaG (c). (d) Change of the endoplasmic reticulum (ER) morphology at various transient expression level of HaloTag-RTN4.

❖ **UnaG enable to various subcellular structures with sub-diffraction limit resolution in fixed Cos7 cells.**

II. Live-cell super-resolution imaging

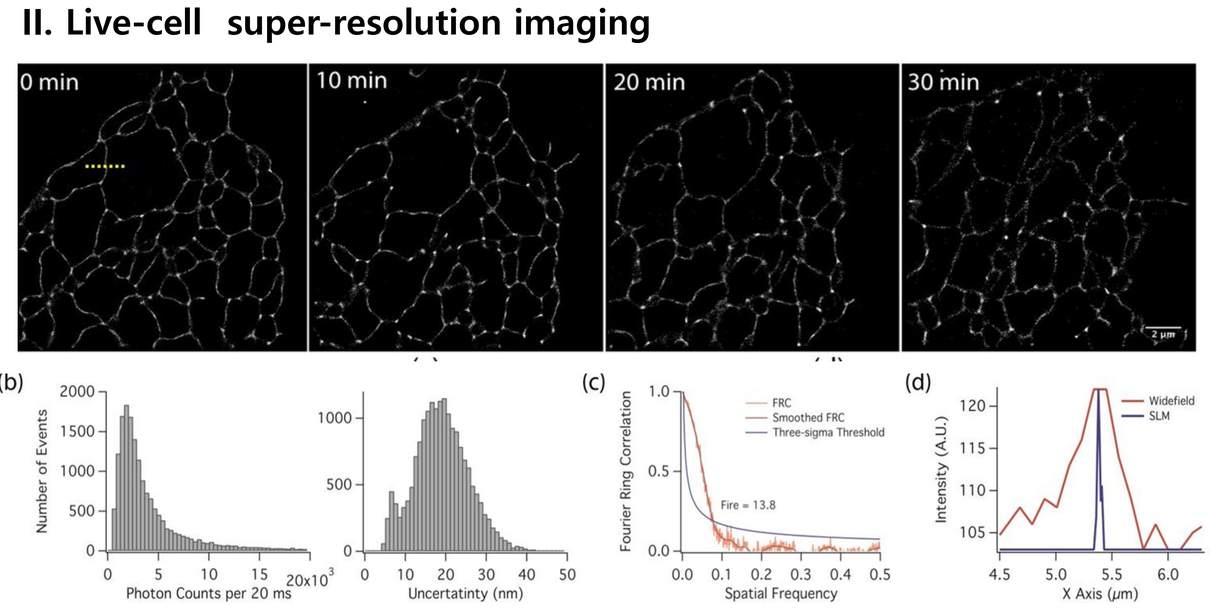

Figure 5 Long-term live-cell super-resolution imaging of a Cos7 cell expressing Sec61b-UnaG. (a) Conventional (far left) and super-resolution images at 0, 10, 20 and 30 mins. (b) Photon counts and uncertainty distribution from the first super-resolution image. (c) Fourier ring correlation analysis with three- sigma threshold gave 13.8 nm of spatial resolution. (d) Intensity profile along single ER tubule marked by yellow-dotted lines in (a).

❖ **UnaG enables imaging a living cell with 10 times enhanced spatial resolution more than 30 minutes.**