Supporting Information

A Genetically Encoded Molecular Tension Probe for Tracing Protein—Protein Interactions in Mammalian Cells

Sung Bae Kim,*[,1] Ryo Nishihara,[2] Daniel Citterio,[2] Koji Suzuki[2]

1. Research Institute for Environmental Management Technology, National Institute of Advanced Industrial Science and Technology (AIST), 16-1 Onogawa, Tsukuba 305-8569, Japan,
2. Department of Applied Chemistry, Faculty of Science and Technology, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan

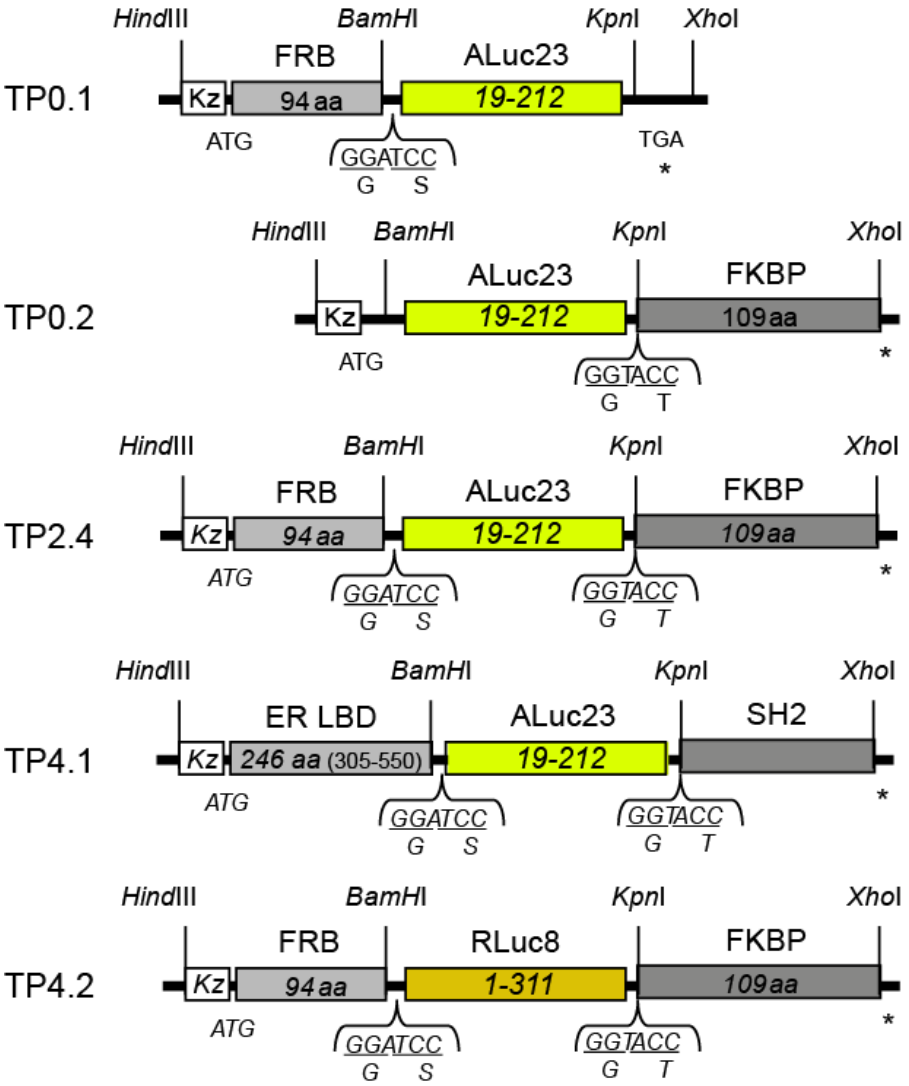
*** Corresponding author:** kimu-sb@aist.go.jp

Table of Contents

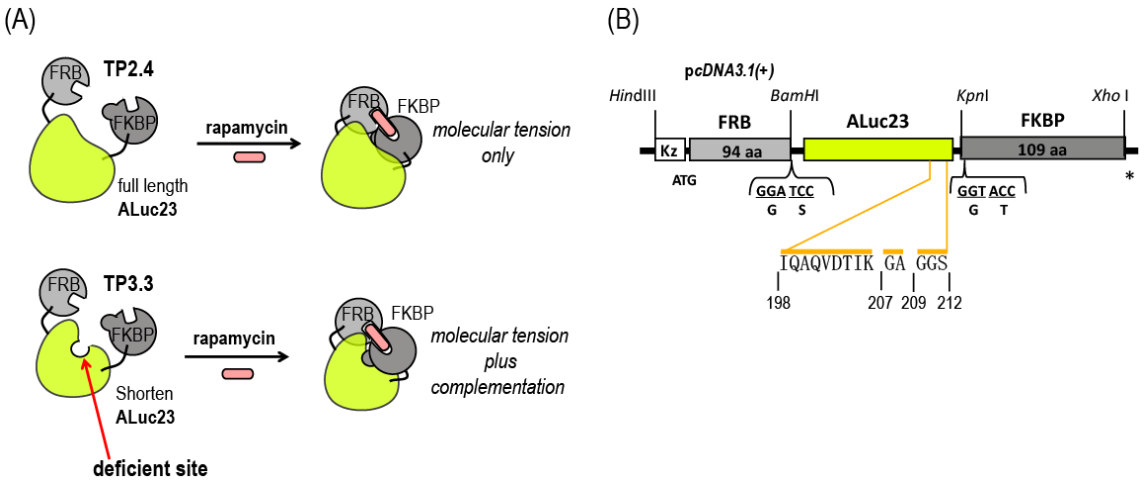
Suppl. Figure 1. Schematic structures of cDNA constructs encoding the molecular tension probes.

Suppl. Figure 2. Illustration showing the working mechanism and cDNA constructs of combinational bioluminescent probes

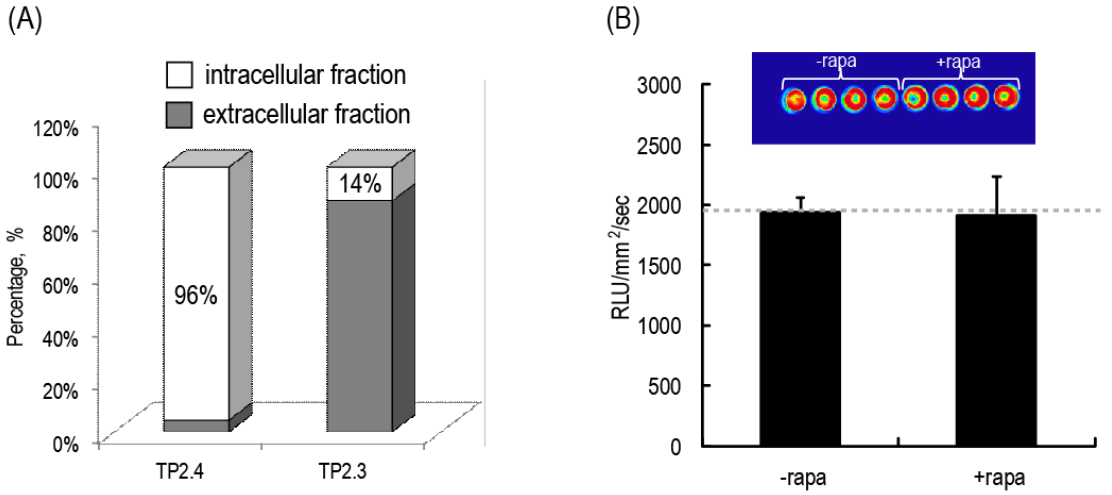
Suppl. Figure 3. The intra- and extracellular fractions of the molecular tension probes, TP2.3 and TP2.4.



Suppl. Figure 1. Schematic structures of cDNA constructs encoding the molecular tension probes. TPv0.1 and 0.2 are FKBP- and FRB-deficient forms of TPv2.4, respectively. Abbreviations: Kz, kozak sequence; FRB, the FKBP-rapamycin-binding domain of mTOR; FKBP, the FK506-binding protein; ER LBD, the ligand binding domain of human estrogen receptor (205-550 aa); SH2, the SH2 domain of *v*-Src; RLuc8, a 8-mutation-bearing variant of *Renilla reniformis* luciferase (RLuc).



Suppl. Figure 2. (A) Illustration showing the working mechanism of combinational bioluminescent probes. The FRB-FKBP binding induces intramolecular tension on both upper and lower probes. Furthermore, the shortened ALuc23 activity in the upper probe is compensated by the N-terminal end of FKBP. (B) Schematic diagram of the cDNA construct of a combinational bioluminescent probe, highlighting the eliminated amino acids of ALuc23. Several bases at the 3'-terminal end of ALuc23 were removed to reduce the background intensity and constitute a compensation scheme in the probe. The amino acid sequence highlights the eliminated C-terminal end of ALuc23.



Suppl. Figure 3. (A) The intra- and extracellular fractions of the probes, TP2.3 and TP2.4. The majority of TP2.4 is retained into the intracellular compartment, whereas TP2.3 is secreted into the extracellular compartment. (B) The rapamycin sensitivity of TP2.3 secreted into the supernatant. TP2.3 in the supernatant has no rapamycin sensitivity. Inset shows the optical image in bioluminescence. Abbreviations: “+rapa” and “-rapa” mean the presence and obsence of rapamycin, respectively.