Supplementary Information

Live imaging of mRNA using RNA-stabilized fluorogenic proteins

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Supplementary Table 1. ssDNA oligo probes used in Supplementary Figure 10a.

Probe-1	GTTGAGTGATTAGCGATTGATTCCGGCC
Probe-2	GTCGGATGATTTTCGTAATAGATTGCGCTG
Probe-3	TTGACGTGATTTTGTGAGATTTTCCGCAG
Probe-4	TGCCTGATTGTAAGTATGTGGATTATCGG
Probe-5	GGATAGGTATGGAGGAAGTAGCTTGGA
Probe-6	ACAATATCTTGCGCCGTTCGATCTTG
Probe-7	GGCCGCCAAGAAGAACGACCAA
Probe-8	CCTAAGAACCTAACATATCTAGCGAGG
Probe-9	TGTGCACCTTGAAGCGCATGAA
Probe-10	CCTGGGTCACGGTCACCACG
Probe-11	GCCCATGGTCTTCTTCTGC
Probe-12	GGGTGCTTCACGTAGGCCTT
Probe-13	GTCACCTTCAGCTTGGCGGTC
Probe-14	GCCTCTGCTTGATCTCGCCCTTC
Probe-15	GTCTTGACCTCAGCGTCGTAGTG
Probe-16	CGGCGCGTTCGTACTGTTCC
Probe-17	GCCGATAATCCACATACTTACAATCAGG

Supplementary Table 2. ssDNA oligo probes used in RT-qPCR

EYFP fw	ACGTAAACGGCCACAAGTTC
EYFP rv	CTTCATGTGGTCGGGGTAGC
mCherry fw	CACGAGTTCGAGATCGAGGG
mCherry rv	CAAGTAGTCGGGGATGTCGG

Supplementary Video 1. $(F30\text{-}2xPepper)_{10}$ tag enables visualization of mRNAs in live cells.

U2OS cells were transiently expressing a reporter mRNA tagged with $(F30\text{-}2xPepper)_{10}$. Coexpression of a fluorogenic protein, $(mNeonGreen)_2$-tDeg, enables the visualization of this Pepper-tagged mRNA as green fluorescent puncta. These green fluorescent puncta are photostable, which enables tracking during our imaging duration. Exposure time of each frame: 200 msec. This experiment was performed three times with similar results. Scale bar, 20 μm.

Supplementary Video 2. Puromycin treatment liberated the Pepper-tagged reporter mRNA from the ER, and increased its mobility.

U2OS cells were transiently expressing a reporter ER-targeting reporter mRNA and the $(mNeonGreen)_4$-tDeg fluorogenic protein. This ER-targeting reporter mRNA encodes the first 29 amino acids of cytochrome p450, CytERM, and the encoding sequence of mCherry followed by $(F30\text{-}2xPepper)_{10}$ in the 3'UTR. Prior to puromycin treatment, the reporter mRNAs seen as green fluorescent puncta were tethered to the outer ER membrane, and showed low mobility. Upon puromycin (100 μg/mL) treatment, these reporter mRNAs were liberated from the ER to the cytosol, and showed a significant mobility increase. Exposure time of each frame: 50 msec. This experiment was performed twice with similar results. Scale bar, 10 μm.