Fluorescence Fluctuation Spectroscopy Enables Quantitative Imaging of Single mRNAs in Living Cells

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Supporting Materials

Movie S1: Time-lapse movie of MBS MEF cells stably expressing NLS-MCP-EGFP. The excitation is 488 nm and the exposure time is 50ms.

Movie S2: Time-lapse movie of MBS MEF cells stably expressing tdMCP-EGFP. The excitation is 488 nm and the exposure time is 50ms.

Figure S1 A U2OS cell is transiently transfected with CFP-24xPBS, tdPCP-EGFP and mCherry. The fluorescence intensity traces of green (green curve) and red (red curve) channels are normalized and plotted in (A). There is larger fluctuation in the green channel than red channel since the mRNA brightness is larger. (B) A two-species TIFCA model fits the data and yield the mRNA normalized brightness $b = 26.1$, diffusion constant 0.35 $\mu m^2/s$ and the concentration 13 nM. The four panels in the figure represent the first four factorial cumulants of photon counts (symbols) and the theoretical fit to Eq. (2).

Figure S1

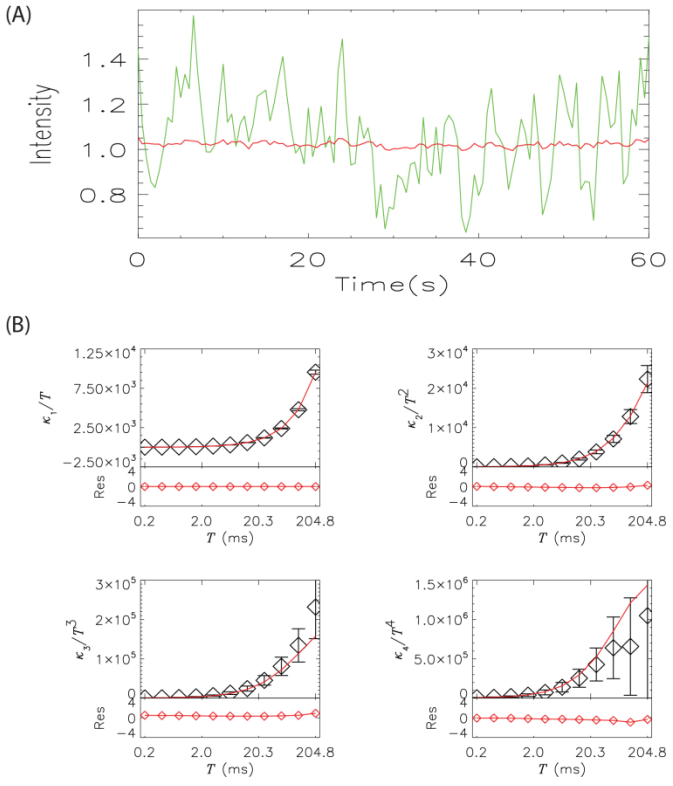


Figure S2 A MBS MEF cell stably expressing tdMCP-EGFP is measured for 3 minutes in the perinuclear region. The fluorescence intensity trace is plotted in (A). (B) The autocorrelation function (symbol) is fit to a two species model (Eq. 3) (red curve). From the fit, the diffusion constant of mRNA is determined as 0.4 $\mu m^2/s$.

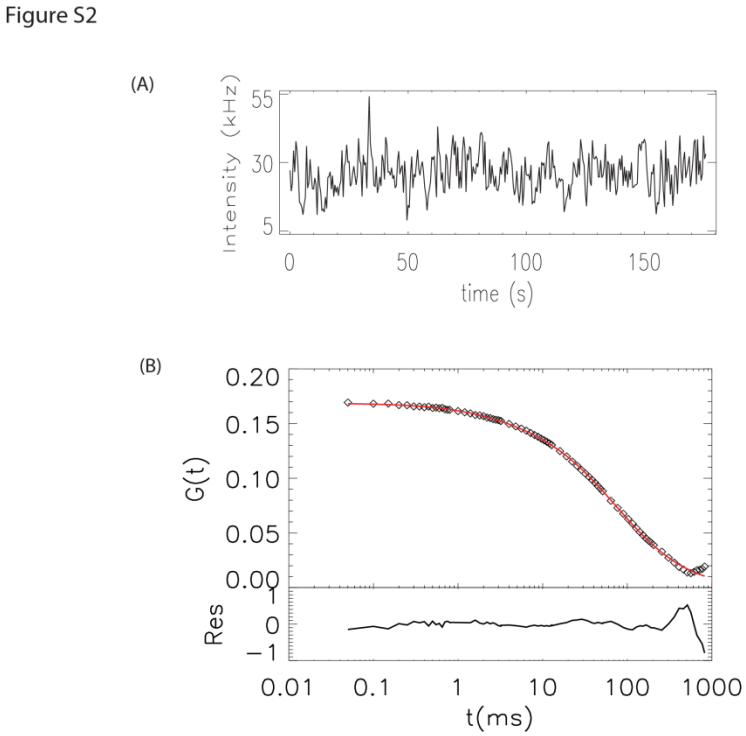
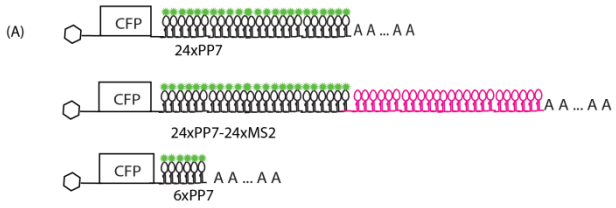
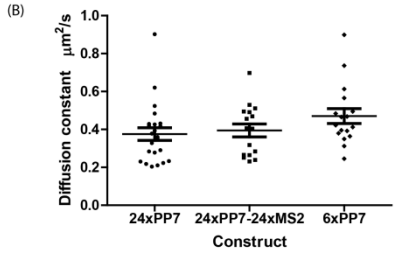


Figure S3 Diffusion constant of mRNA with different length. (A) The schematics of the mRNA constructs used in the experiment. All mRNAs have coding region of cyan fluorescent protein. In the 3′UTR, 24xPP7, 6xPP7 or 24xPP7-24xMS2 were inserted right after the stop codons respectively. (B) U2OS cells were transfected with tdPCP-EGFP and each of the mRNA constructs listed in (A). The cells were measured for 3 minutes in perinuclear region 24 hours after transfection. The autocorrelation function of the data was fit to a two-species model and the diffusion constant of mRNA is recovered. The scatter plot of the diffusion constant of mRNAs is shown and compared. The mean diffusion constants are CFP-24xPP7: 0.38 ± 0.04 $\mu m^2/s$; CFP-24xPP7-24xMS2: 0.39 ± 0.04 $\mu m^2/s$; CFP-6xPP7: 0.47 ± 0.04 $\mu m^2/s$. Although, CFP-24xPP7-24xMS2 has one thousand nucleotides more than CFP-24xPP7, their diffusion constants are the same within experimental error. CFP-6xPP7 has slightly larger diffusion constant than CFP-24xPP7.

Figure S3


(A)
CFP
A A ... A A
24xPP7
CFP
A A ... A A
24xPP7-24xMS2
CFP
A A ... A A
6xPP7


(B)
Diffusion constant μm²/s
1.0
0.8
0.6
0.4
0.2
0.0
24xPP7
24xPP7-24xMS2
6xPP7
Construct