

Electronic Supplementary Information for

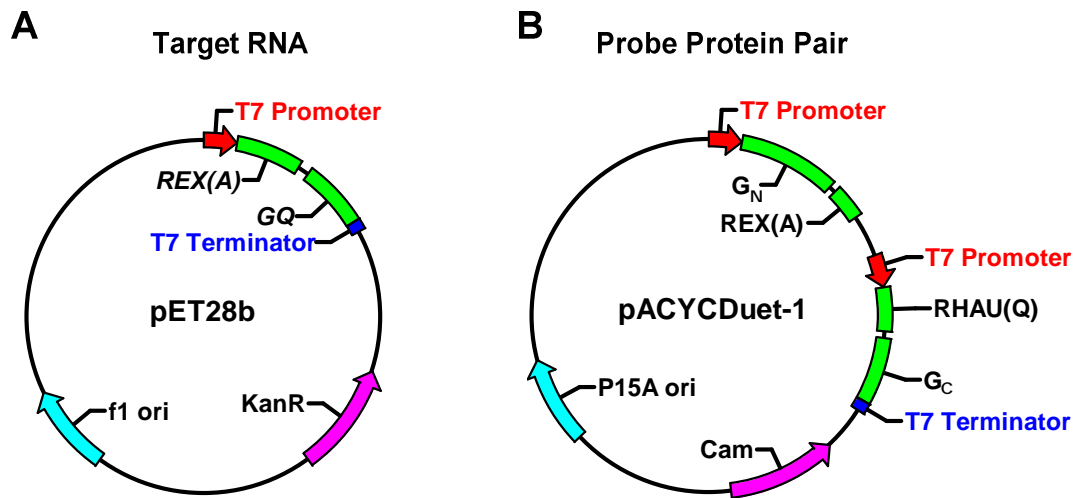
**RNA G-Quadruplex Formation in Defined Sequence in Living Cells Detected by  
Bimolecular Fluorescence Complementation**

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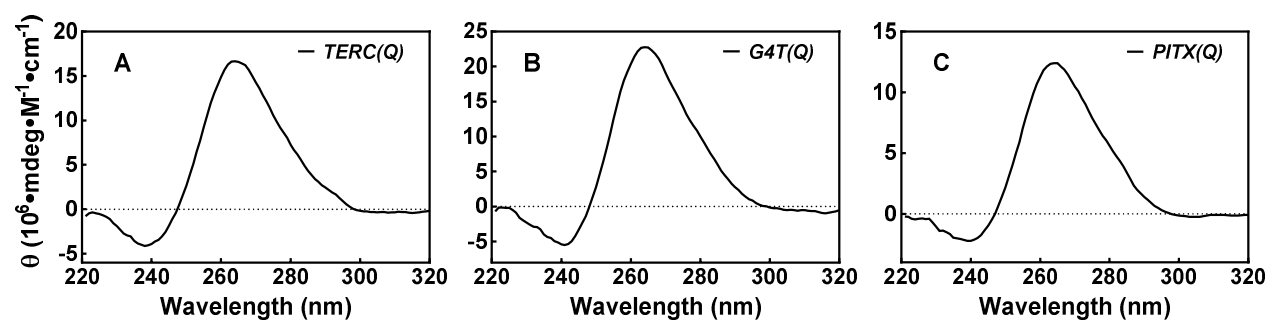
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**Figure S1**



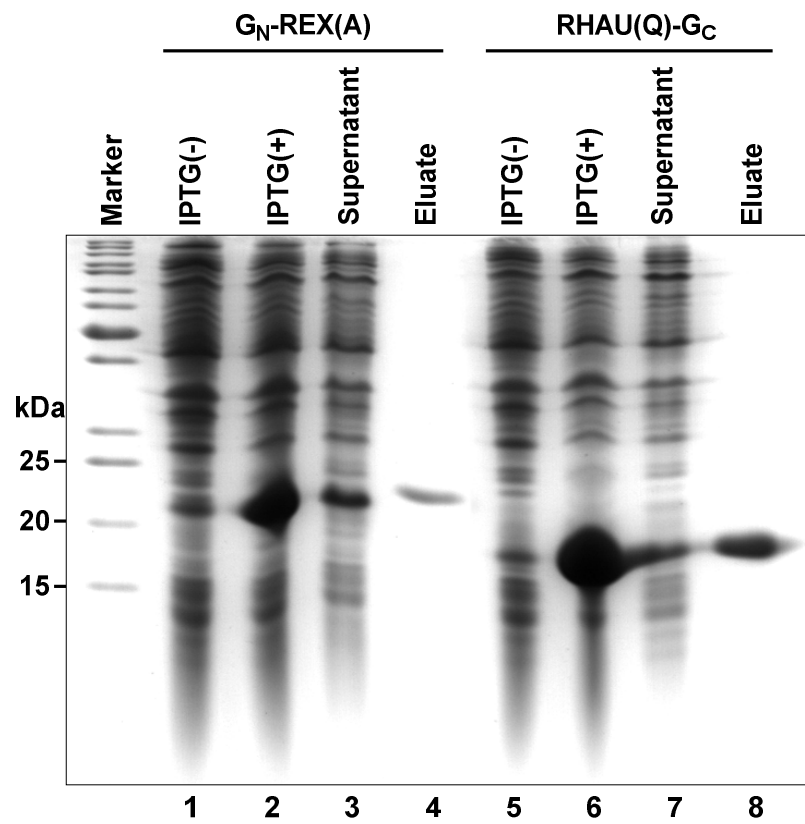
**Figure S1.** Plasmids used to produce (A) target RNA or (B) probe proteins (right). (A) The plasmid produces RNA containing a REX aptamer *REX(A)* and a G-quadruplex *GQ*. (B) The plasmid expresses two proteins: an  $G_N$ -*REX(A)* that binds to aptamer *REX(A)* and an *RHAU(Q)*- $G_C$  that binds to G-quadruplex.

**Figure S2**



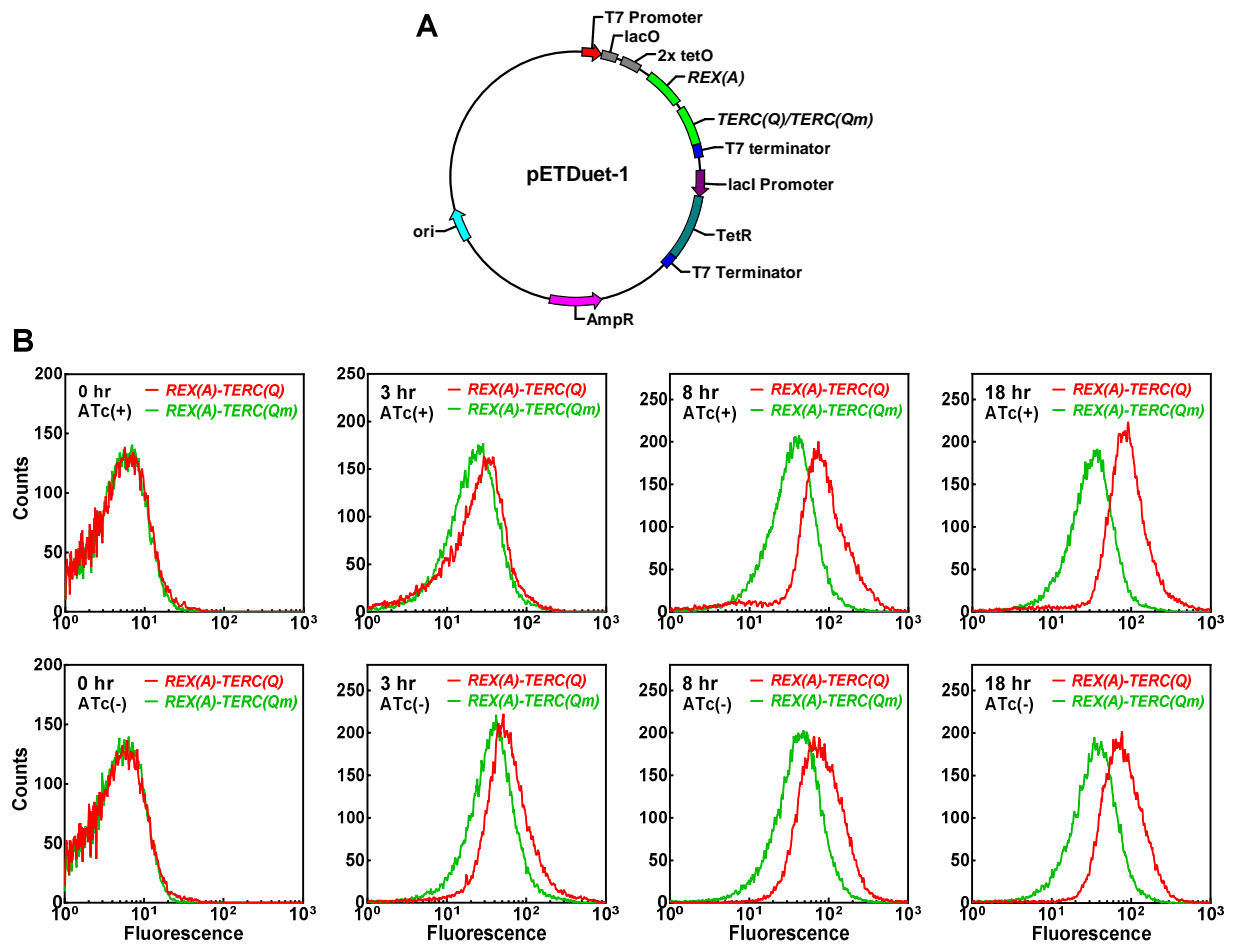
**Figure S2.** CD spectra of RNA oligonucleotides of *TERC(Q)*, *G4T(Q)*, and *PITX(Q)*.

**Figure S3**



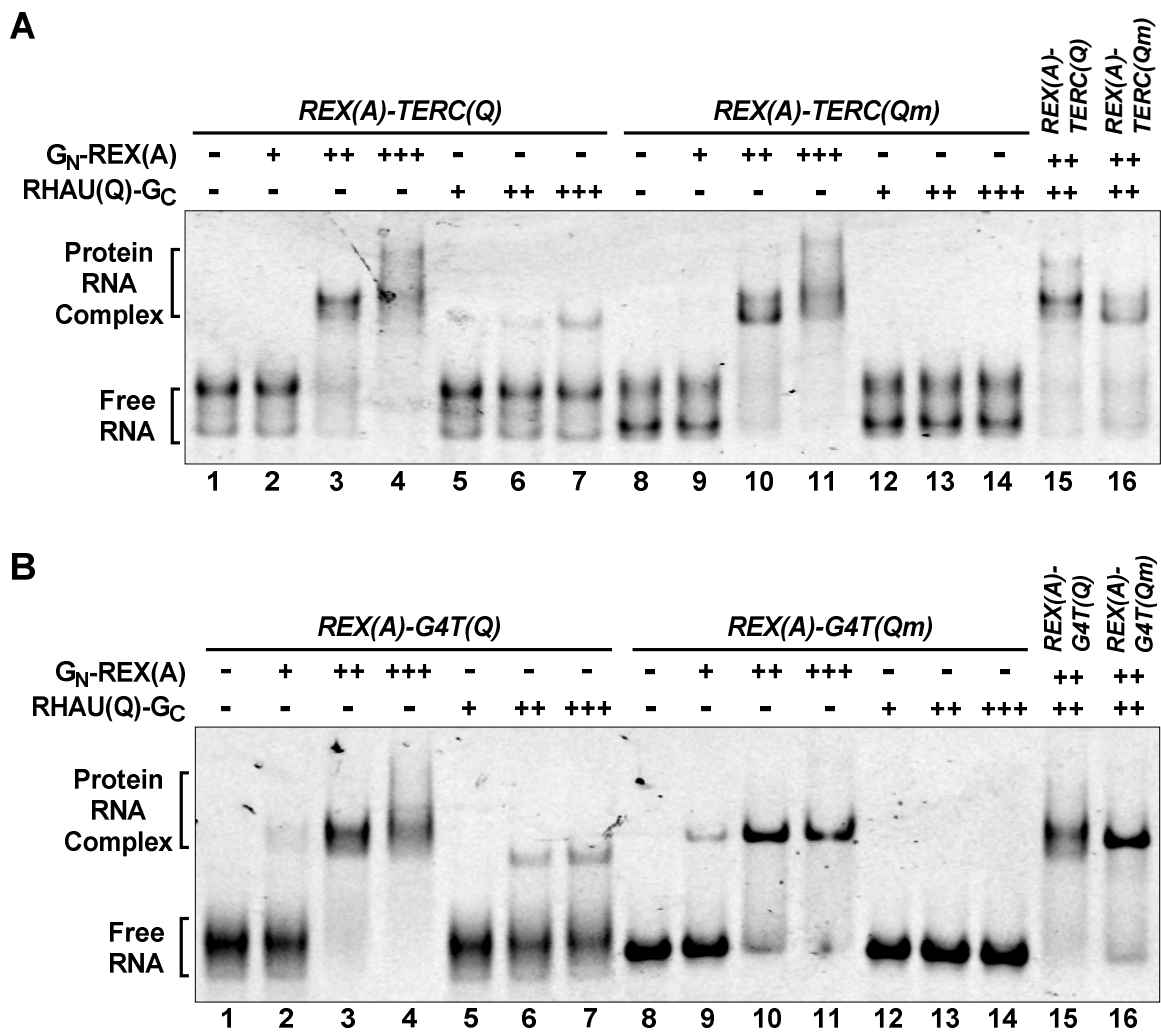
**Figure S3.** Expression and purification of probe fusion proteins. Lanes 1-4 and 5-8 show protein in whole cell lysate of BL21 (DE3) *E. coli* without and with induction by IPTG, in lysate supernatant and in elute of His-affinity column, respectively.  $G_N$ -REX(A) and RHAU(Q)- $G_C$  has an molecule weight of 23.0 and 18.2 kDa, respectively.

Figure S4



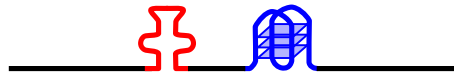
**Figure S4.** BiFC fluorescence in *E. coli* cells before and after induction of RNA expression detected by flow cytometry. Cells transformed with the RNA- (A) and probe protein-expressing (Fig. S1B) plasmids were supplied with IPTG together with (B, top panels) or without (B, bottom panels) ATc to induce the expression of probe proteins and target RNA *REX(A)-TERC(Q)* or *REX(A)-TERC(Qm)*, respectively at 0 hr. Distribution of eGFP fluorescence was collected by flow cytometry and processed as in Fig. 4. Forward scattering intensity showed little difference between cells of each panel as in the other figures.

Figure S5



**Figure S5.** Protein-RNA interaction in  $Li^+$  solution evaluated by electrophoretic mobility shift assay (EMSA). (A) *REX(A)-TERC(Q)*, (B) *REX(A)-G4T(Q)* and their G-quadruplex mutants, *REX(A)-TERC(Qm)*, *REX(A)-G4T(Qm)* were processed in the same way as in Fig. 2, except that  $K^+$  was replaced by  $Li^+$ .

Figure S6



TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATCCCCTCTA  
GATTTAGGCGACGGTACGCAAGTACTTTGCGCCGGCCTATTCTCGAG  
TTT-[G-Core]-TTTGCTGAGCAATAACTAGCATAACCCCTTGGGGCCT  
CTAAACGGGTCTTGAGGGGTTTTTT

Green: T7 Promoter

Red: REX Aptamer

Magenta: T7 Terminator

Blue: [G-Core]

*TERC(Q)*: [GGGTTGCGGAGGGTGGGCCTGGGAGGGGTGG]

*TERC(Q<sub>m</sub>)*: [GAGTTGCGGAGAGTGAGCCTGGGAGGGGTGG]

*G4T(Q)*: [GGGGTGGGGTGGGGTGGGG]

*G4T(Q<sub>m</sub>)*: [GGAGTGAGATGGGGTGGGG]

*PITX(Q)*: [GTTGGGGCGGGCGTTGGGTTTGGGGGACG]

*PITX(Q<sub>m</sub>)*: [GTTGGGGCGAGCGTTGAGTTTGGGGGACG]

*4G3(Q)*: [GGGTGGGTGGGTGGG]

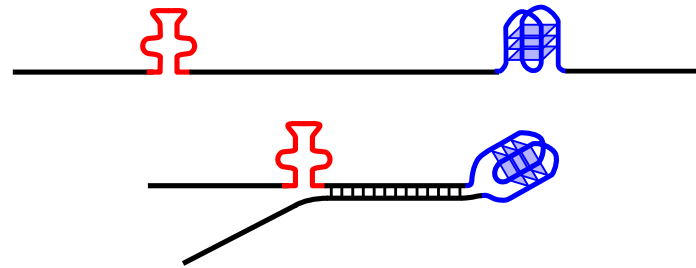
*3G3*: [GGGTGGGTGGGTGAG]

*2G3*: [GGGTGGGTGAGTGAG]

*1G3*: [GGGTGAGTGAGTGAG]

**Figure S6.** Plasmid DNA sequence of corresponding RNAs, G-quadruplexes and their mutants. Scheme at the top illustrates the structure of the RNAs.

**Figure S7**



TAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTA  
 GATTTAGGCGACGGTACGCAAGTACTCTTGGCGCGGCTA Ttctcgag  
 tgactgagagctT- [G-Core]- AACAAGCagctctcagtcactcgagaa  
 GCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAG  
 GGGTTTTT

Green: T7 Promoter  
 Red: REX Aptamer  
 Magenta: T7 Terminator

[G-Core]  
 TERC(Q): [GGGTTCCGGAGGGTGGGCCTGGGAGGGGTGG]  
 TERC(Qm): [GAGTTCCGGAGAGTGAGCCTGGGAGGGGTGG]

G4T(Q): [GGGTTGGGGTGGGGTGGGG]  
 G4T(Qm): [GGAGTGAGATGGGGTGGGG]

**Figure S7.** Plasmid DNA sequence of corresponding RNAs, G-quadruplexes and their mutants. Schemes at the top illustrate the structures of the RNAs in which the aptamer and G-quadruplex/mutant were separated by a longer spacer that can fold into a double-stranded stem (lowercase in sequence).