Single-molecule observation of the induced fit of k-turn RNA structure on binding L7Ae protein

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Supplementary Information.

1. Supplementary Data

Additional examples of time traces obtained in the presence of 2 mM Mg^{2+} ions

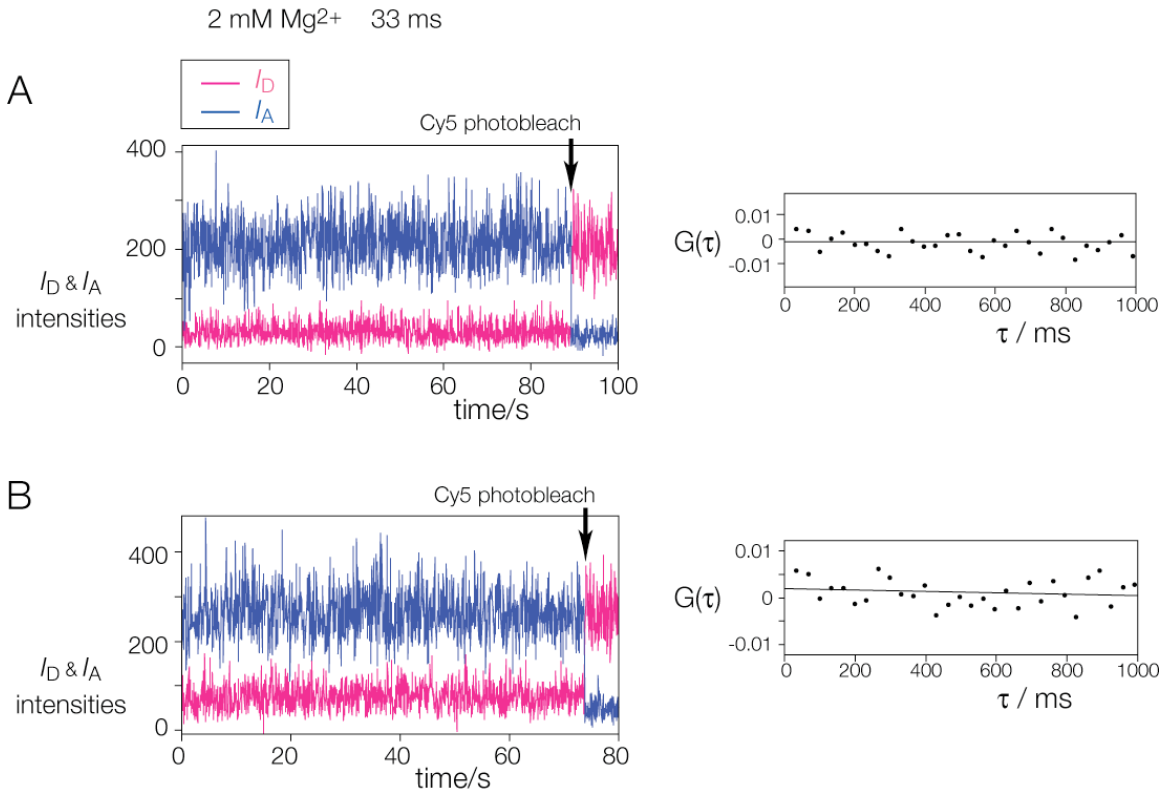

Figure S1. Two further examples of plots of Cy3-donor intensity (I_D) and Cy5-acceptor intensity (I_A) from Kt-7 RNA bound to immobilized U1A-L7Ae fusion as a function of time at 33 ms frame rate. These data were collected in the presence of 2 mM Mg^{2+} ions. Note that in both cases the Cy5 underwent photobleaching after > 1 min. (arrowed), with a corresponding increase in the Cy3 intensity (since it is no longer deactivated by FRET beyond this point). Thus before the photobleaching occurs we can be sure that the RNA has single active Cy3 and Cy5 fluorophores. These regions have been subjected to cross correlation analysis (Equation 1, main text), shown as plots of $G(\tau)$ as a function of

the interval τ, found to the right of its parent time trace In both cases these are horizontal and pass through G(τ) = 0, showing an absence of anticorrelation between I_D and I_A, i.e. no transitions are detectable in the RNA down to the frame rate of the camera.

Additional example of a time trace obtained without addition of Mg^{2+} ions

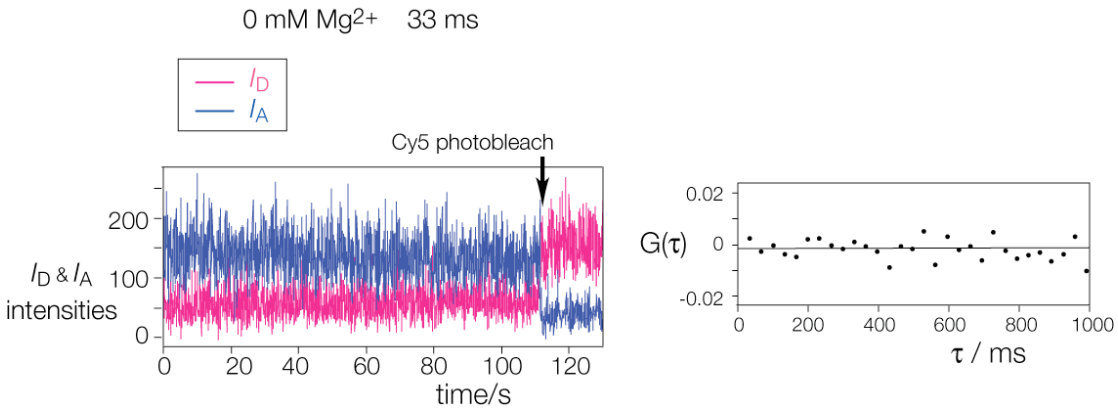

Figure S2. A further example of a plot of donor and acceptor intensity from Kt-7 RNA bound to immobilized U1A-L7Ae fusion as a function of time at 33 ms frame rate in the presence of 0 mM Mg^{2+} ions. The region prior to photobleaching (arrowed) was subjected to cross correlation analysis, shown as a plot of G(τ) as a function of the interval τ, found to the right of the parent time trace. This is horizontal and passes through $G(\tau) = 0$, showing an absence of anticorrelation between I_D and I_A, i.e. no transitions are detectable in the RNA down to the frame rate of the camera.

Additional examples of time traces showing the binding of Kt-7 RNA to U1A-L7Ae fusion protein

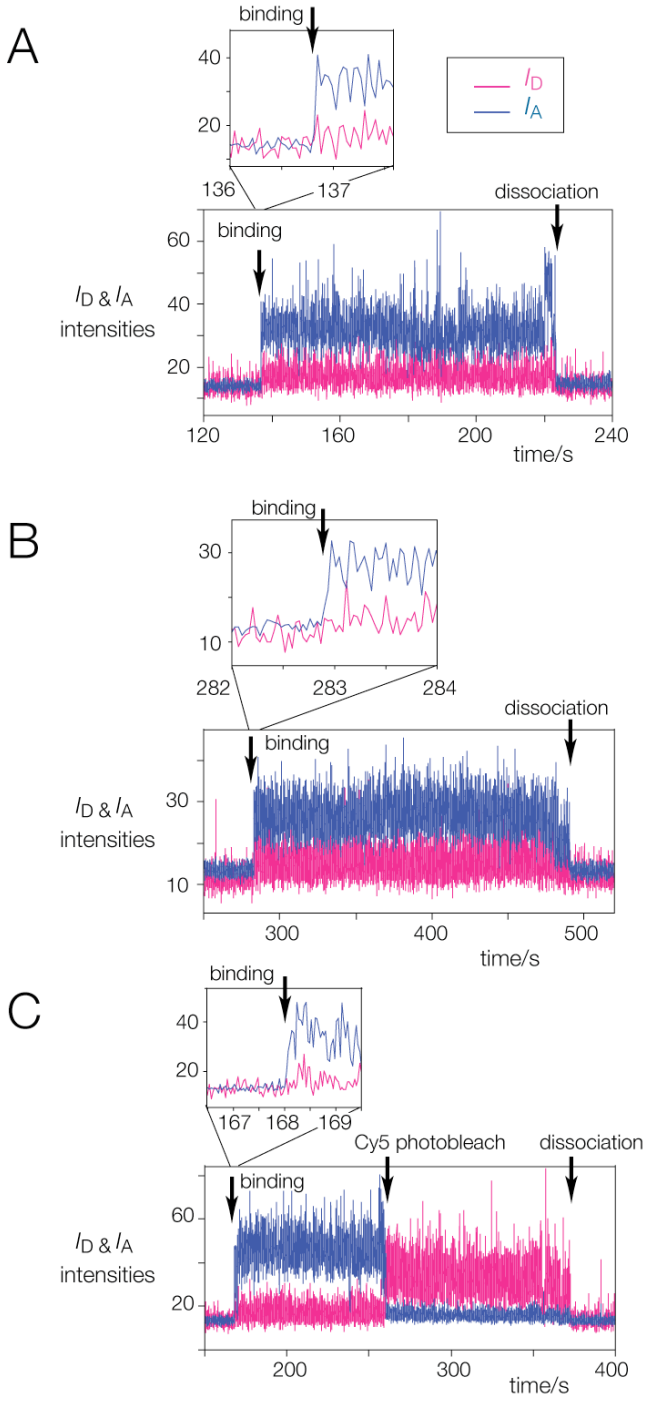

Figure S3. Three further examples of traces showing the process of Kt-7 binding to immobilized U1A-L7Ae fusion protein in the presence of 0 mM Mg^{2+} ions, with data collected at 16 ms frame rate. In these experiments we collect donor and acceptor fluorescent emision before and during the period in which the RNA is added onto the slide and allowed to bind to the U1A-L7Ae protein. At this point (shown arrowed) the acceptor intensity rises. Expansion of this region (shown above each trace)

reveals that this occurs within a single frame, i.e. 16 ms. In traces A and B, intensity I_A falls to background levels after a period (arrowed and marked as dissociation). This could result from dissociation of the complex (arising either from loss of the Kt-7-L7Ae interaction, or the U1A RNA-protein interaction), or from photobleaching of the Cy3 donor (although this fluorophore is significantly more photostable than Cy5). Trace C exhibits a Cy5 photobleaching at 255 s, followed by dissociation/Cy3 photobleaching at 340 s (both events arrowed). The noise level in these data is relatively high because of the fast frame rate used; in these experiments the dynamic information is more important than measurement of the absolute level of FRET efficiency.

2. Supplementary experimental methods

Synthesis of gene to encode a U1A - L7Ae protein fusion.

An in-frame fusion of the genes for U1A and L7Ae was constructed, starting from plasmids containing the genes for *Homo sapiens* U1A (originally supplied by Dr K. Nagai) and *Archeoglobus fulgidus* L7Ae (originally supplied by Dr A. Hüttenhofer) (shown schematically in Figure S5A).

Each plasmid was separately subjected to PCR, using the primers GGAATTCCATATGGCAGTTCCCGAGAC and CCGGAATTCACCGCCACCGCCACCGCCCACGAAGGTGCCTTTCATCTTG (the forward and reverse primers respectively for U1A, all sequences written 5' to 3') and CCGGAATTCTACGTGAGATTTGAGGTTCCTGAG and CGCGGATCCTTACTTCTGAAGGCCTTTAATCTTC (forward and reverse primers for L7Ae). PCR reactions were performed using 50 ng of template DNA, 0.5 μM of primers, 2 mM of $MgCl_2$, 0.2 mM NTPs, 5% DMSO and 10 units of KOD enzyme in 0.12 M Tris.HCl (pH 8), 10 mM KCl, 6 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 0.001% BSA. 30 cycles (1 min at 94°C; 30 s at 55°C; 30 s at 68°C) were performed after an initial 2 min at 94°C. The products of PCR were purified by electrophoresis in 1.5% agarose gels followed by use of a QIAquick gel extraction kit (Qiagen). The U1A DNA was cleaved with the restriction enzymes *Nde*I and *Eco*RI, and the L7Ae DNA with *Eco*RI and *Bam*HI, and the products purified from a 1.5% agarose gel as before. The two fragments were then mixed and ligated via their common *Eco*RI cohesive termini using T4 DNA ligase (BioRad) for 12 h at 16°C in 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM dithiothreitol, 1 mM ATP. The ligated product was then subjected to a further round of PCR, using the U1A-forward and L7Ae reverse primers, and the product once again purified from 1.5% agarose gel. The DNA was then cleaved with *Nde*I and *Bam*HI, purified once more as before and ligated into the vector pET15b that had been similarly cleaved with *Nde*I and *Bam*HI using T4 DNA ligase (BioRad) for 12 h at 16°C. The ligated plasmid was transformed into competent XL1-Blue *Escherichia coli* cells (Stratagene), and plated onto Luria broth agar containing 0.1 mg/ml ampicillin. Individual colonies were checked by growing in LB medium containing 0.1 mg/ml ampicillin for 12 h, extraction of DNA using a mini-kit (Qiagen), PCR using the above primers and electrophoresis of the product in 1.5 % agarose. Plasmid containing a full-length fusion gene was then fully sequenced.

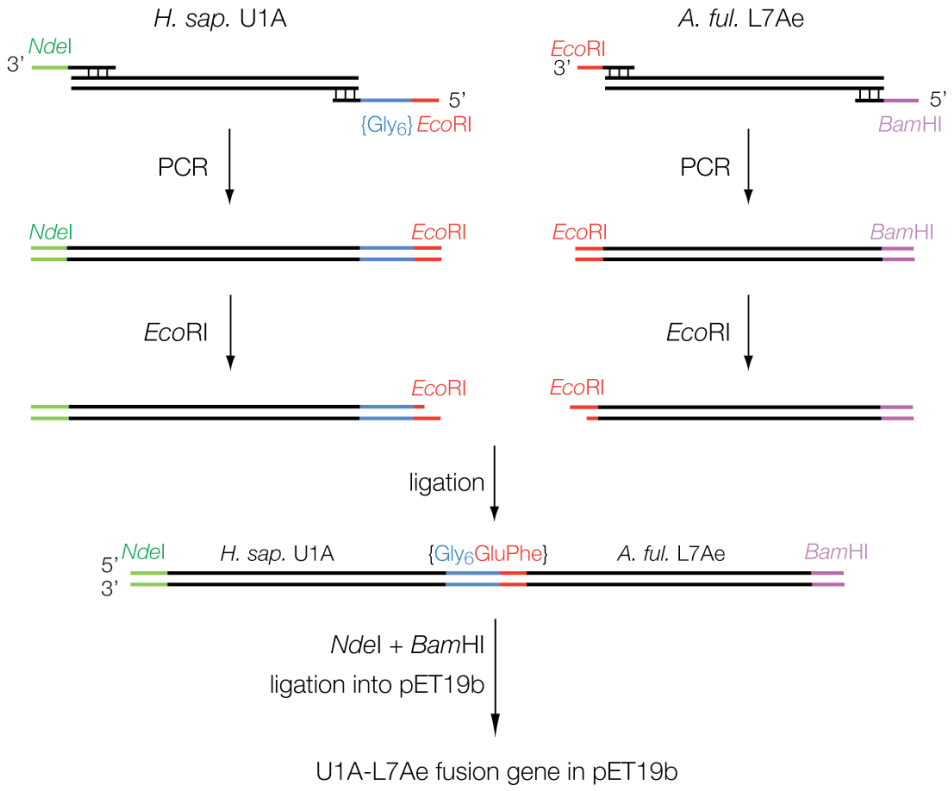

Figure S4. Schematic showing the construction of a gene encoding a U1A-L7Ae fusion protein.

Expression and purification of a U1A - L7Ae protein fusion.

pET15b containing the complete gene for the U1A-L7Ae fusion protein with a hexahistidine tag was transformed into *E. coli* BL21-Gold (DE3) pLysS cells (Stratagene), and protein expression was induced by the addition of IPTG to 1 mM. The cells were shaken for 4 h at 37 °C and harvested by centrifugation at 4,000 rpm for 30 min at 4°C. The cells were immediately resuspended in 20 mM phosphate (pH 9.0), 0.5 M NaCl and Complete Protease Cocktail (Roche). The protein suspension was sonicated nine times for 5 s on ice, each separated by 5 s on ice. Denatured endogenous protein was removed by centrifugation at 40,000 g for 40 min at 4 °C. The U1A-L7Ae fusion protein was purified from the cleared supernatant by application to a Ni^{2+}-chelated HiTrap column (GE Healthcare) installed on a ÄKTApurifier (GE Healthcare). Buffer A was 20 mM phosphate (pH 9.0), 0.5 M NaCl, and buffer B was buffer A plus 0.5 M imidazole. With the protein bound the column was washed with 5 volumes of 20 mM phosphate (pH 9.0), 2M NaCl. The column was washed with 5 volumes of 3% buffer B (i.e. 15 mM imidazole), removing most contaminating proteins, and the fusion protein was eluted in buffer B,

giving a final volume of ~ 10 ml.

The protein was diluted 5 times with 20 mM Tris-HCl (pH 8.0) and applied to a HiTrap heparin HP column (GE Healthcare) and eluted using a NaCl gradient in 20 Tris-HCl (pH 8.0); the protein eluted at ~500 mM. This step was included to remove any RNA from the protein preparation. The protein was concentrated to 0.5 ml and applied to a Superdex 75 10/300 GL column (GE Health care), eluted with 10 mM Tris-HCl (pH 8.0), 5 mM NaCl. The purified U1A-L7Ae fusion protein was analyzed by electrophoresis in polyacrylamide in the presence of SDS, alongside a mixture of proteins as size standards (Figure S5B). The solution containing the U1A-L7Ae fusion protein was confirmed by mass spectrometry to contain both *H. sapiens* U1A and *A. fulgidus* L7Ae proteins.

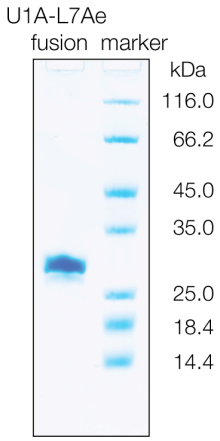

Figure S5. Gel electrophoresis of purified U1A-L7Ae fusion protein. 2.5 μg of purified fusion protein was loaded onto a 15% polyacrylamide gel adjacent to a mixture of proteins (Thermo Scientific) to provide an indication of molecular mass - their sizes are indicated on the right side. The proteins were electrophoresed under denaturing conditions in the presence of 0.1% SDS.