Reversible and controllable nano-locomotion of an RNA-processing machinery

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Supporting Information

SI Text

SI Methods and Materials:

RNA/DNA hybrid-construct preparation.

An 18-nt DNA strand was purchased from Integrated DNA Technologies and RNA strands from Dharmacon Inc. RNA and DNA strands were annealed by mixing at the ratio of 1 to 1.2, heating up to 80C, and cooling down slowly to room temperature. The sequence and modifications are shown below.

1. RNA/DNA construct with an overhanging tail of (rA)$_{15}$ used for polymerization assays.

3'- poly 15rA degradation RNA strand:
5'- rUrGrG rCrGrA rCrGrG rCrArG rCrGrA rGrGrC rArArA rArArA rArArA rArArA rArArA-3'
DNA complementary strand:
5'-/Cy5/- GCC TCG CTG CCG TCG CCA /Biotin/ -3'

2. RNA/DNA construct with an overhanging tail of (rA)$_{30}$ used for degradation and polymerization assays.

3'-poly 30rA degradation RNA strand:

5’-rUrGrG rCrGrA rCrGrG rCrArG rCrGrA rGrGrC rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA-3’

DNA complementary strand:

5’-/Cy5/- GCC TCG CTG CCG TCG CCA /Biotin/ -3’

3. RNA strand for gel degradation assays.

5’-/Cy3/- rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA rArArA -3’

4. Oligonucleotides used to make the Rrp41 mutant for the protein labeling. The mutations introduced are marked in bold.

afRrp41_C43A_for CTGACGGGTCT **GCG** TACCTTGAAATG

afRrp41_C43A_rev CATTTCAAGGTA **CGC** AGACCCGTCAG

afRrp41_C140S_for GCAGGACAGCC **AGT** CTGAATGCTGC

afRrp41_C140S_rev GCAGCATTCAG **ACT** GGCTGTCCTGC

afRrp41_Cys_STOP_Hind_rev TTTTT **AAGCTT** TCA **GCA** GGCATCTTCACC

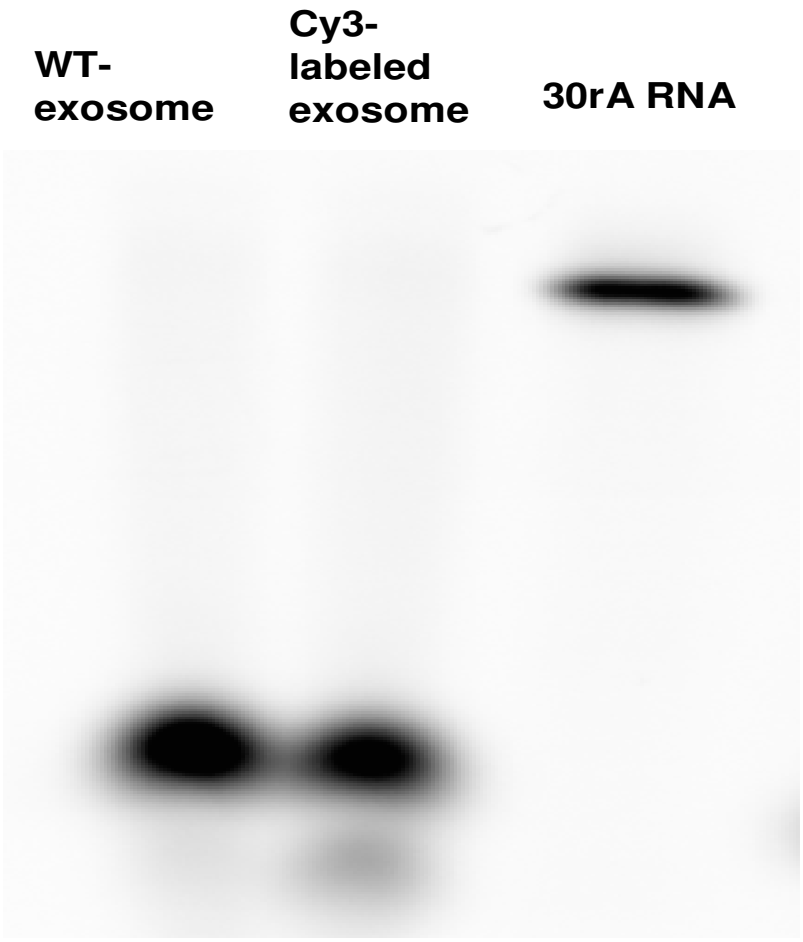

Figure S1. A comparison of RNA degradation activity between the wild type (WT) exosome and the Cy3-labeled exosome mutant. Cysteine engineering and fluorescent labeling do not significantly influence the activity of exosome because both produced the same degradation product. In the degradation assay, 20 pmol protein was incubated with 85 pmol of a 30rA RNA strand labeled with Cy3 at the 5' end in a 50μl buffer containing 20 mM Tris (pH 7.8), 60mM KCl, 10 mM $MgCl_2$, 2mM DTT, 50 μg/ml BSA and 10mM P_i for 2 min at 22°C.

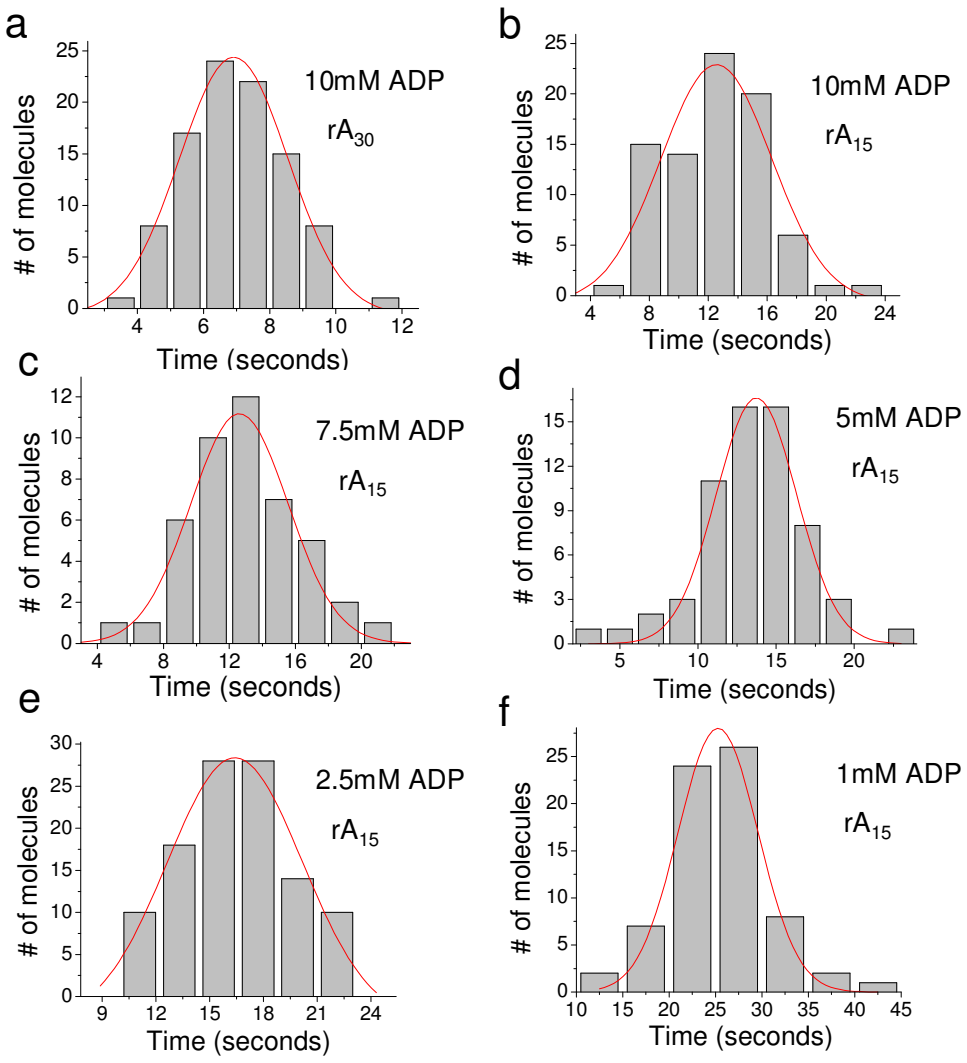

Figure S2. Histograms of polymerization times obtained at various ADP concentrations. (**a**) 30 nt overhang RNA at 10 mM ADP. (**b-f**) 15nt overhang RNA at the indicated ADP concentrations.

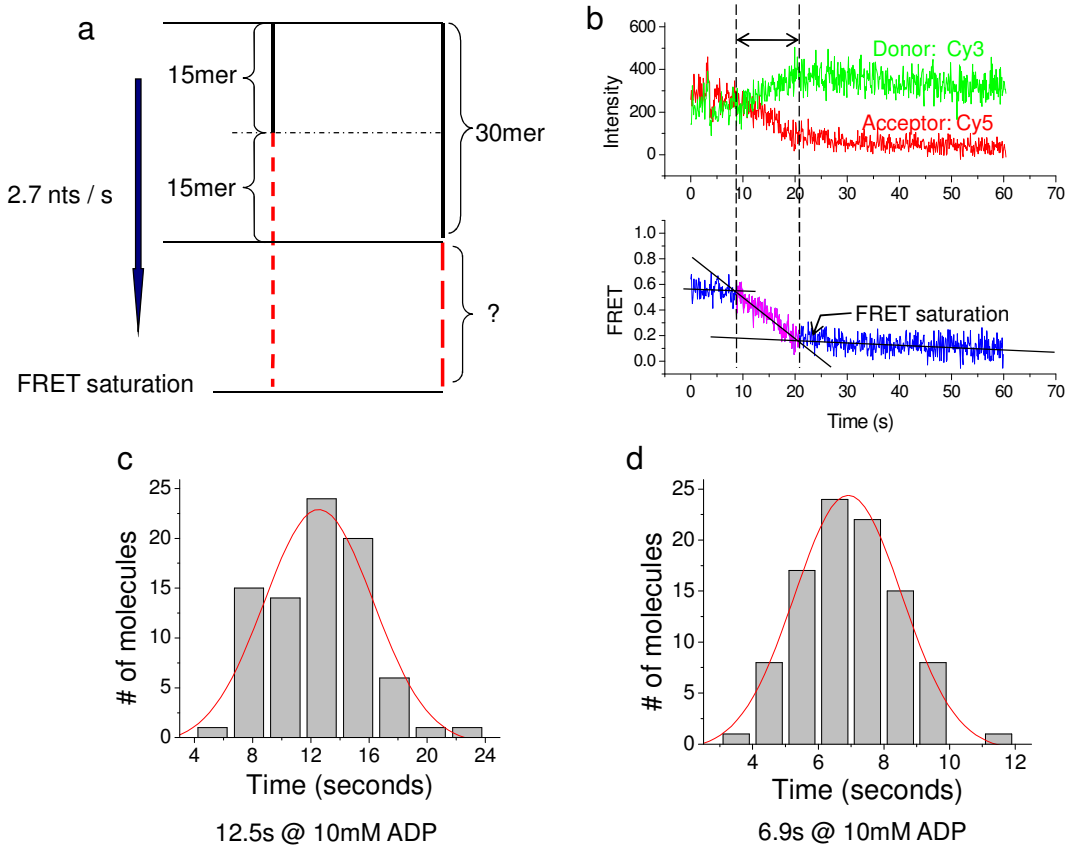

Figure S3. Determination of polymerization rate. (**a**) The reaction time was measured from the time period of FRET decrease as shown in **b** for two RNA constructs (15 and 30 nt 3' ssRNA tails). The difference in time between the two RNA constructs should correspond to the synthesis time of 15 nt (length difference: 30 nt – 15 nt). (**b**) An exemplary single molecule record shows the period of FRET decrease which corresponds to the reaction time (black arrow). (**c**) Histogram of reactions times obtained from the 15 nt overhang RNA. (**d**) Histogram of reaction times obtained from the 30 nt overhang RNA. Gaussian fits were used to calculate the mean values of the reaction time.

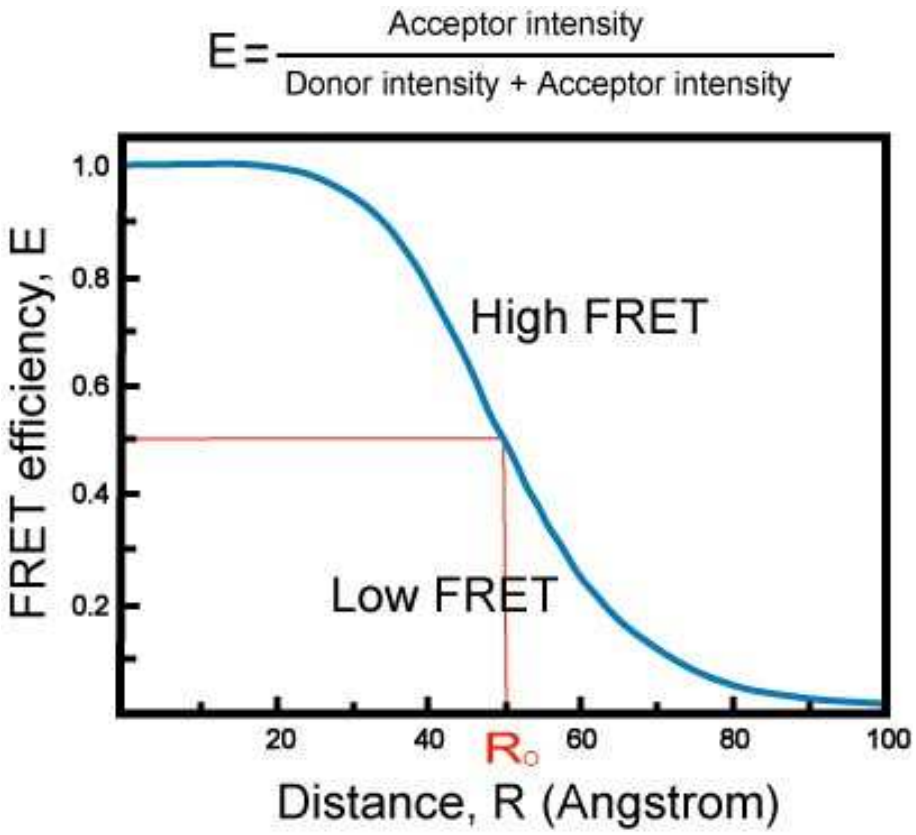

Figure S4. FRET efficiency as a function of distance (R) between the donor and the acceptor, where E=0.5 at R=R_0 assuming R_0= 50Å. FRET efficiency is sensitive only between 2 and 8 nanometers.

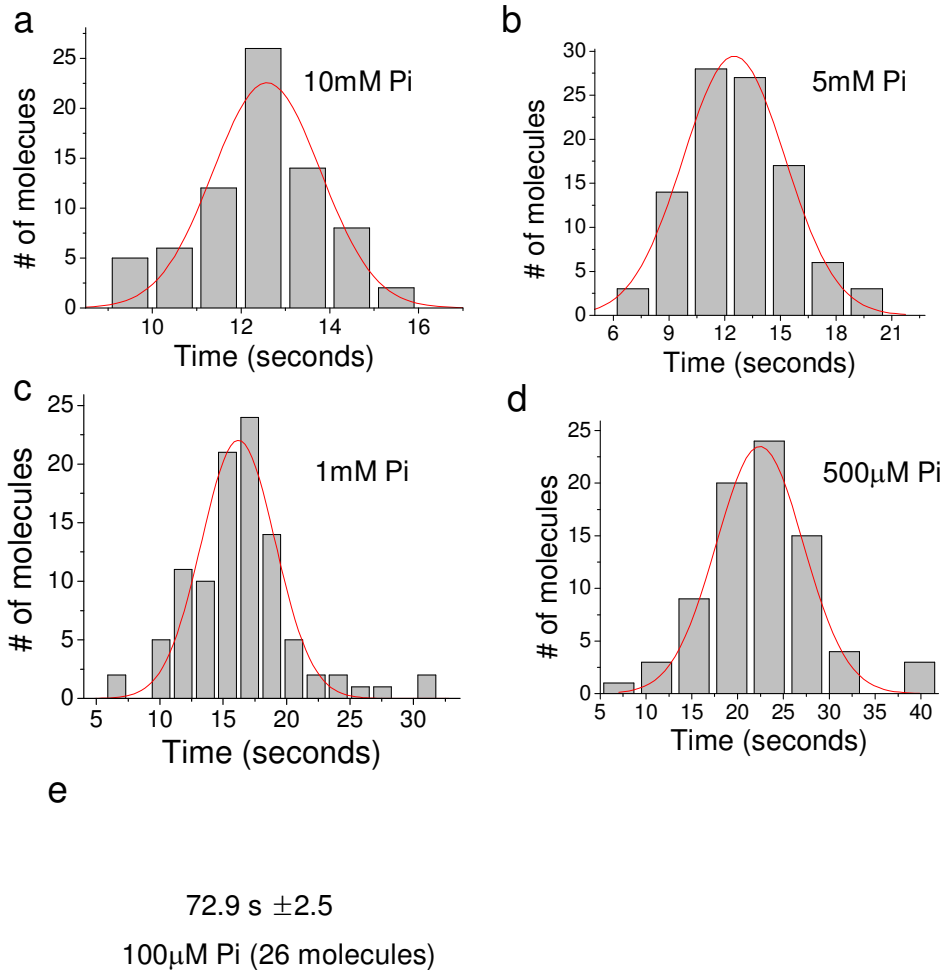

Figure S5. Histograms of degradation times obtained at the indicated various P_i concentrations (**a-d**). Gaussian fits were used to calculate the mean values of the reaction time. (**e**) The yield at 100 μM P_i was too low for histogram-based analysis. Therefore, we simply calculated the mean degradation time was determined from 26 molecules with a standard deviation of 2.5 s.

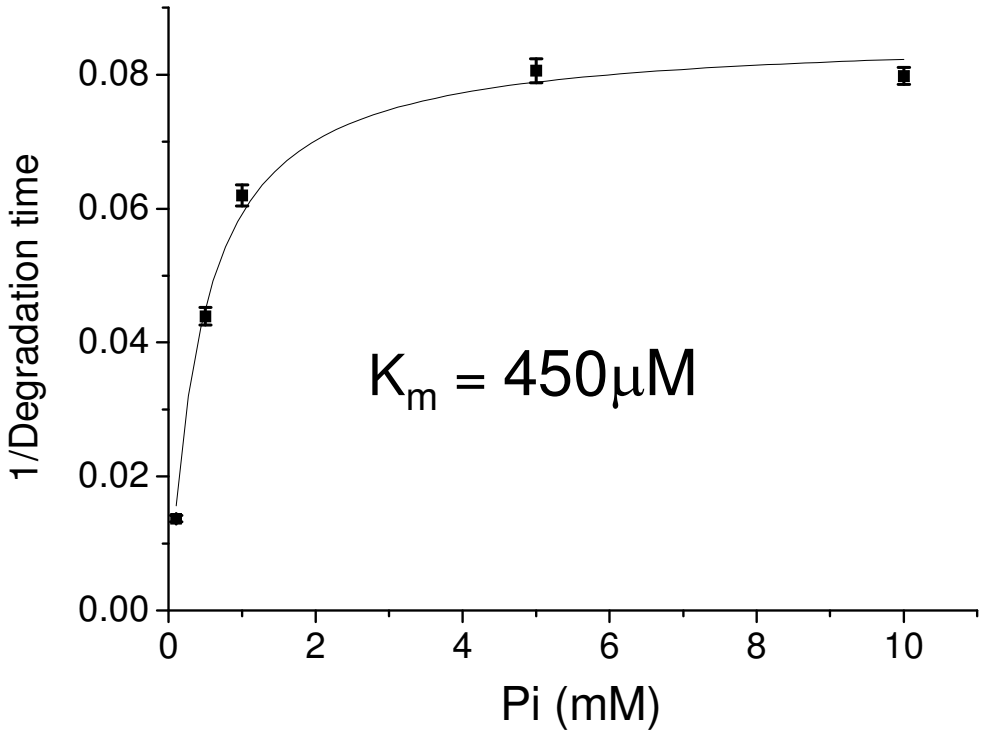

Figure S6. The inverse of average Δt, $(t_{avg})^{-1}$, of degradation reaction was plotted against P_i concentration and then fitted to the Michaelis-Menten equation. The P_i concentration of half maximum velocity (K_m) was 450 μM.

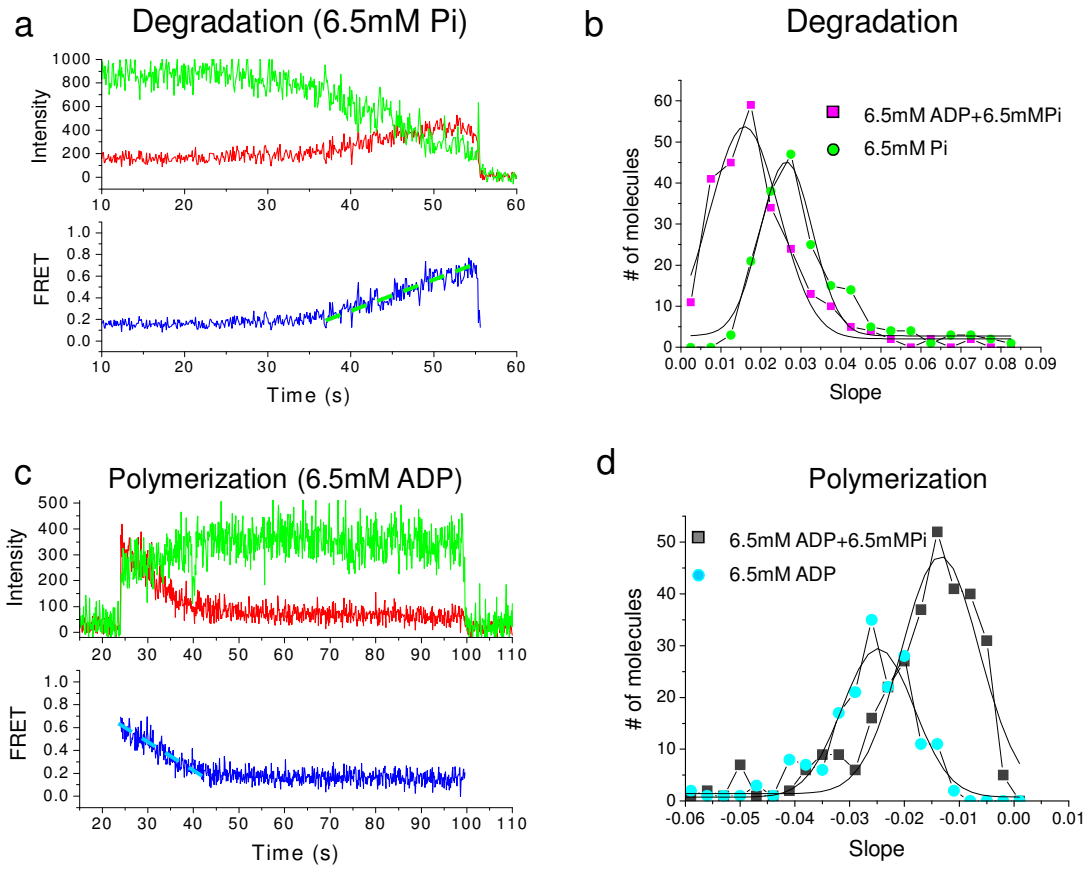

Figure S7. Speed of reaction at 6.5mM P_i only, 6.5mM ADP only and their mixture. (**a**) A representative FRET time trace at 6.5 mM P_i concentration. (**b**) A comparison of degradation speed for 6.5 mM P_i only and 6.5 mM of the mixture of both P_i and ADP. (**c**) A representative FRET time trace at 6.5 mM ADP. (**d**) A comparison of polymerization speed for 6.5 mM ADP only and 6.5 mM of the mixture of both P_i and ADP.