Supplementary Information

Dual DNA rulers reveal an “mRNA looping” intermediate state during ribosome translocation

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Fig. S1. Magnetic signal measurement of the samples. (a) Scheme of scanning magnetic detection with an atomic magnetometer. The sample is scanned along the x-axis, driven by a linear motor. Its magnetic field along the d-axis is measured by an atomic magnetometer (Yao, L. & Xu, S.-J. Long-range, high-resolution magnetic imaging of nanoparticles. \textit{Angew. Chem. Int. Ed.} 48, 5679-5682 (2009)). (b) Representative magnetic measurement of a sample. The typical signal amplitude is 50 pT, corresponding to approximately $1.1 \times 10^5$ magnetic particles (Hu, Q., Yang, H., Wang, Y. & Xu, S.-J. Controlled formation and precise resolution of multiple noncovalent bonds. \textit{Chem. Commun.} 52, 3705-3708 (2016)). The noise level is approximately $\pm 3.5$ pT. Prior to magnetic measurement, the sample was subjected to 1000 rpm (revolution per minute) centrifugal force to remove nonspecifically absorbed particles on the surface.
Fig. S2. Scheme of probing the hindered mRNA at the 5′-terminus. The arc AB is a quarter of the circumference of the ribosome, which is approximated as a ~20-nm diameter circle (Schuwirth, B. S., Borovinskaya, M. A., Hau, C. W., Zhang, W., Vila-Sanjurjo, A., Holton, J. M. & Cate, J. H. D. Structures of the bacterial ribosome at 3.5 Å resolution. Science 310, 827-834 (2005).). The length of arc AB is therefore 16.5 nm. For the probing DNA labeled with a magnetic bead, it should have a linker that overcomes this distance to reach the mRNA.
Fig. S3. Plot of the dissociation force vs. bp for the DNA-mRNA duplexes. The duplexes include both formed by Ruler-Ins (probing 5′-mRNA with 50T linker) and Ruler-Outs (probing 3′-mRNA). The Ruler-In 15bp with 70T linker gives the same force as that measured by probe with 50T linker.
Fig. S4. FIRMS results of MF-Pre and MF-Post using different antibiotics (the number of nt indicated the FIRMS measured mRNA movements). (a, b) With neomycin only. (c, d) With hygromycin B only. Left panels: Ruler-In from the 5′ side; right panels: Ruler-Out from the 3′ side.