Supporting Information

RNA scanning of a molecular machine with a built-in ruler

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Table S1. Sequences of TRBP mutants

name	sequences
D12-1 (17aa)	MLAANPGKTPISLLQEYGTRIGKTPVYDLLKAEGQAHQPNFTFRVTVGDTSCTGQG PSKKAAKHKAAEVALKHLKGGSMLEPALPVSPQQSECNPVGALQELVVQKGWRLP EYTVTQESGPAHRKEFTMTCRVERFIEIGSGTSKKLAKRNAAAKMLLRVHTVPLDAR D
D12-3 (37aa)	MLAANPGKTPISLLQEYGTRIGKTPVYDLLKAEGQAHQPNFTFRVTVGDTSCTGQG PSKKAAKHKAAEVALKHLKGGSMLEPALAATPVPSVVLTRSPPMELQPPVSPQQSE CNPVGALQELVVQKGWRLPEYTVTQESGPAHRKEFTMTCRVERFIEIGSGTSKKLA KRNAAAKMLLRVHTVPLDARD
D12-5 (61aa)	MLAANPGKTPISLLQEYGTRIGKTPVYDLLKAEGQAHQPNFTFRVTVGDTSCTGQG PSKKAAKHKAAEVALKHLKGGSMLEPALEDSSSFSPLDSSLPEDIPVFTAAAAATPV PSVVLTRSPPMELQPPVSPQQSECNPVGALQELVVQKGWRLPEYTVTQESGPAHR KEFTMTCRVERFIEIGSGTSKKLAKRNAAAKMLLRVHTVPLDARD
D12-1C	MSEEEQGSGTTTGSGLPSIEQMLAANPCKTPISLLQEYGTRIGKTPVYDLLKAEGQA HQPNFTFRVTVGDTSSTGQGPSKKAAKHKAAEVALKHLKGGSMLEPALEDSSSFSP LDSSLPEDIPVFTAAAAATPVPSVVLTRSPPMELQPPVSPQQSESNPVGALQELVVQ KGWRLPEYTVTQESGPAHRKEFTMTSRVERFIEIGSGTSKKLAKRNAAAKMLLRVHT VPLDARD
D12-2C	MSEEEQGSGTTTGSGLPSIEQMLAANPGKTPISLLQEYGTRIGKTPVYDLLKAEGQA HQPNFTFRVTVGDTSSTGQGPSKKAAKHKAAEVALKHLKGGSMLEPALEDSSSFSP LDSSLPEDIPVFTAAAAATPVPSVVLTRSPPMELQPPVSPQQSECNPVGALQELVVQ KGWRLPEYTVTQESGPAHRKEFTMTSRVERFIEIGSGTSKKLAKRNAAAKMLLRVHT VPLDARD
D12-DC	MSEEEQGSGTTTGSGLPSIEQMLAANPCKTPISLLQEYGTRIGKTPVYDLLKAEGQA HQPNFTFRVTVGDTSSTGQGPSKKAAKHKAAEVALKHLKGGSMLEPALEDSSSFSP LDSSLPEDIPVFTAAAAATPVPSVVLTRSPPMELQPPVSPQQSECNPVGALQELVVQ KGWRLPEYTVTQESGPAHRKEFTMTSRVERFIEIGSGTSKKLAKRNAAAKMLLRVHT VPLDARD

Yellow-shaded: dsRBD1 Cyan-shaded: dsRBD2

Table S2. Sequences of RNA substrates

name	sequences
siRNA (19RNA)	5'-rUrAdTrArCrArArUrCrUrArCrUrGrUrCrUrUrArCrC-biotin-3' (up) 5'-rUrArArGrArCrArGrUrArGrArUrUrGrUrAdTrArUrU-3' (down)
15RNA	5'-rUrAdTrArCrArArUrCrUrArCrUrGrUrCrUrUrArCrC-biotin-3' (up) 5'rArCrArGrUrArGrArUrUrGrUrAdTrArUrU-3' (down)
pre-siRNA (38RNA)	5'- rGrCdTrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrUrCrUrUrUrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-DY547-3' (down)
40RNA	5'- rArCrGrCrUrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrUrCrUrUrUrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-DY547-3' (down)
55RNA	5'- rArCrGrCrUrUrArArCrArArCrCrArGrArUrCrArArArGrArArArArArArCrArGrArCrArUrUrGrUrCrArArArUrUrGrCrArArArGrCrArArArA-biotin-3' (up) 5'- rUrUrUrUrUrGrCrUrUrUrUrGrCrArArUrUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrUrCrUrUrUrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-DY547-3' (down)
DI3	5'- rGrCdTrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrUrUrCTTTrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-3' (down)
DI6	5'- rGrCdTrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUrUrTTCTTTrGrArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-3' (down)
DI9	5'- rGrCdTrUrArArCrArArCrCrArGrArUrCrArArArGAAArArArArCrArGrArCrArUrUrGrUrCrA-biotin-3' (up) 5'- rUrGrArCrArArUrGrUrCrUrGrUrUrUTTTCTTTGArUrCrUrGrGrUrUrGrUrUrArArGrCrGrU-3' (down)
	dT is a DNA nucleotide T with an amino C6 linker, which is conjugated with a dye NHS ester at the labeling reaction and red fonts are DNA.

Figure S1. Representative time trace of homo-labeling of two dsRBDs. Two-step sequential quenching of Cy3 dye as marked by blue arrows shows that two dsRBDs were labeled with the same Cy3 dye, but homo-quenching was not observed even with the conformational change between two dsRBDs in Figure 3.

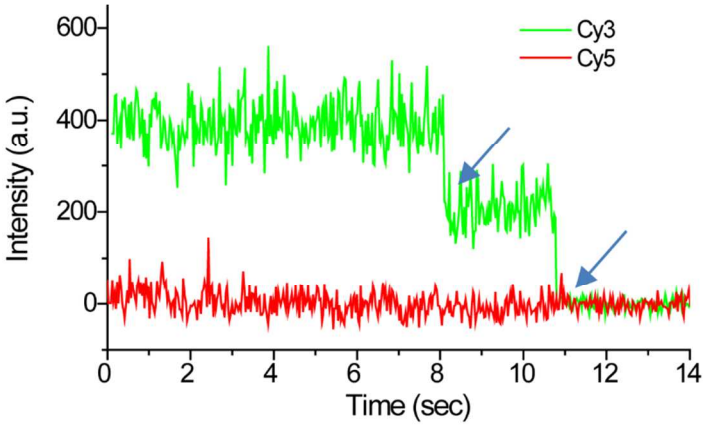

Figure S2. Representative PIFE time trace for two linker length mutants of TRBP (L17 and L61) looks similar because the distance sensitivity range of PIFE is much shorter than FRET, where the difference in the diffusion range between two linker length mutants was hidden unlike the FRET traces (Figure 4C).

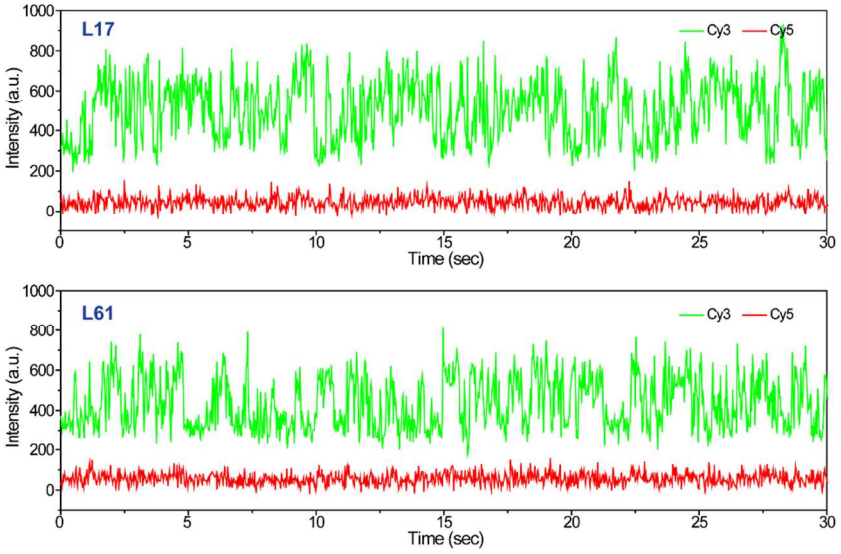

Figure S3. PACT diffuses the shorter range of dsRNA than TRBP due to its shorter linker. (A) FRET histograms showing the diffusion of D12 possessing various linker length (a, 17 aa, b, 37 aa, c, 61 aa) on 40-mer dsRNA. (B) Relative diffusion range of D12 on 40-mer dsRNA calculated from the FRET peak (red circle) and the one of PACT and TRBP (blue square). The length of dsRNA also restricts the diffusion range unlike the linear relationship in Figure 4D.

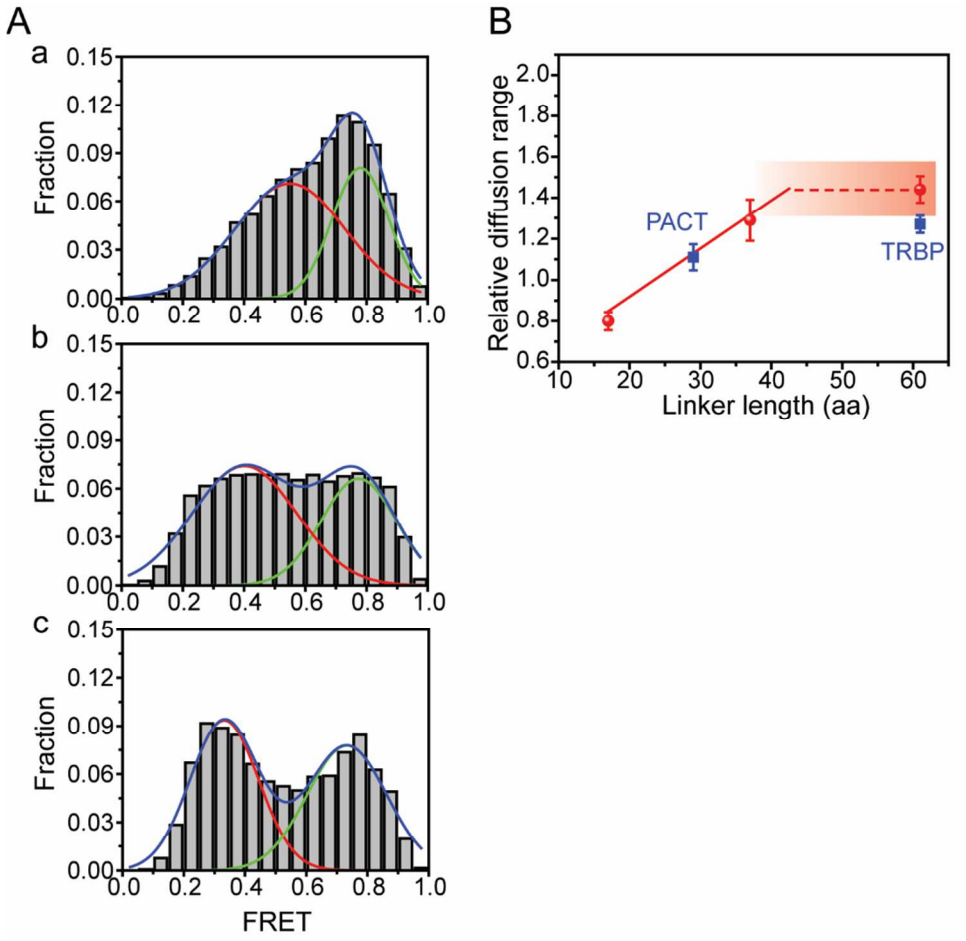

Figure S4. TRBP can hardly bypass the barrier of DNA insertion on dsRNA if the length is longer than one turn of helical structure of dsRNA.

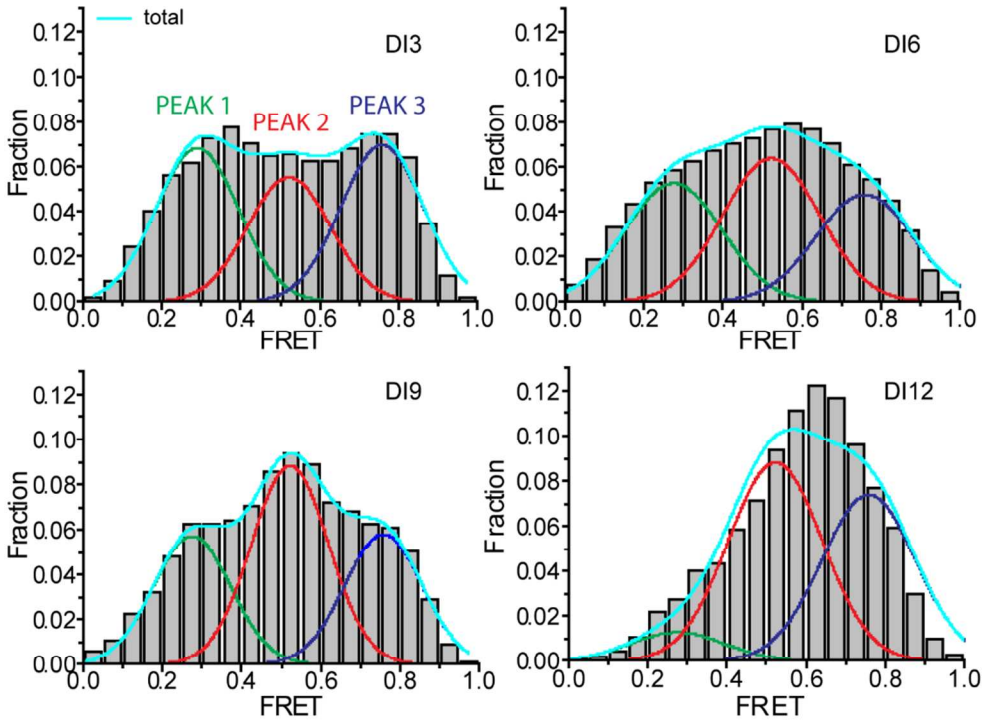

Figure S5. TRBP diffuses through the various barriers such as mismatches and bulges with stalling at the barriers. Stalling at the barrier elongates the diffusion time, showing that dsRNA with four bulges (B4) induces more frequent stalling than the one with four mismatches (M4).