Electronic Supplementary Information

Single-molecule observations of RNA-RNA kissing interactions in a DNA nanostructure

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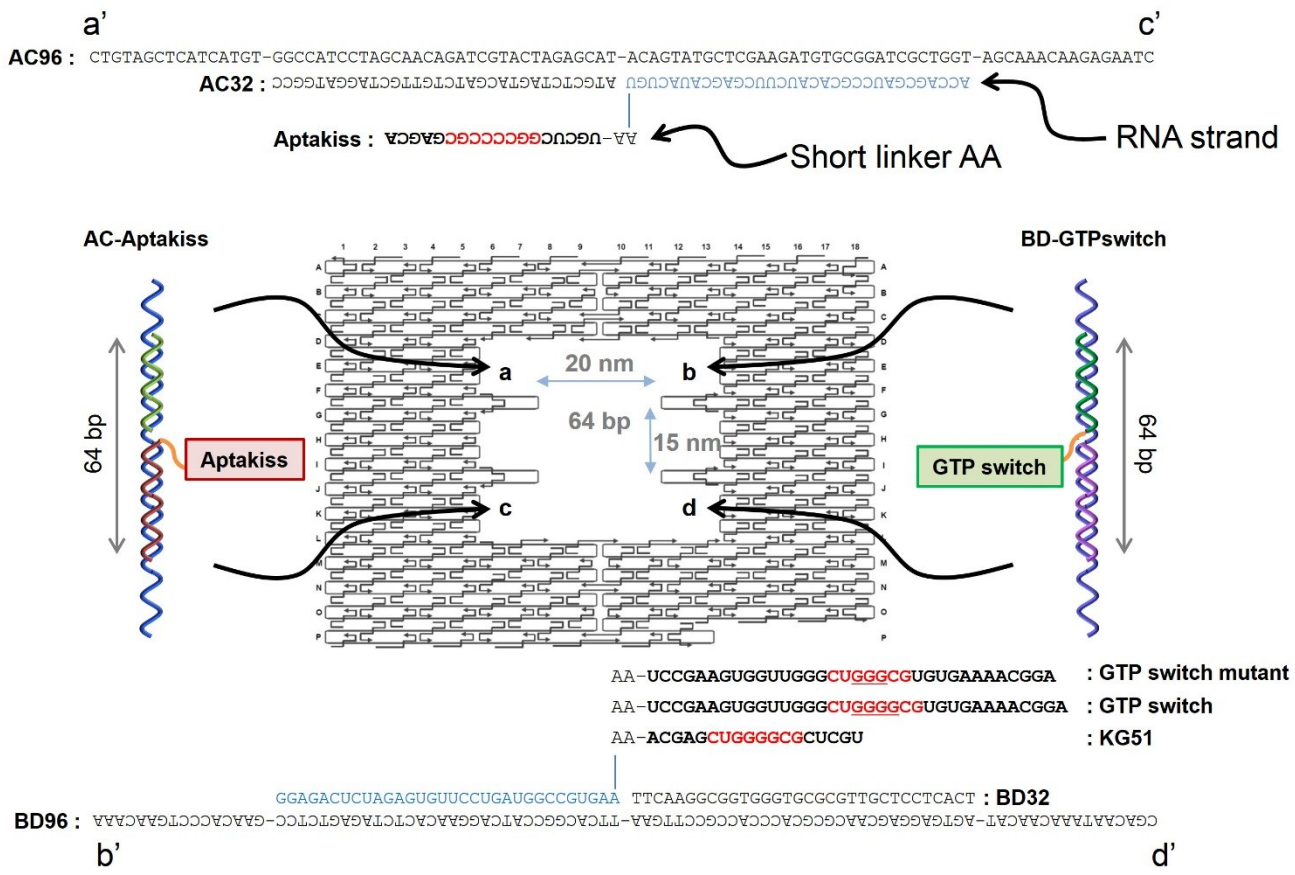

Fig. S1. RNA molecules and supporting DNA strands incorporated to the DNA frame for single-molecule observation.

Table S1. Sequence of kissing RNA molecules and supporting DNA strands.

Name	Sequence(5'-3')
AC96	CTGTAGCTCATCATGT-GGCCATCCTAGCAACAGATCGTACTAGAGCAT-ACAGTATGCTCGAAGATGTGCGGATCGCTGGT-AGCAAACAAGAGAATC
AC32	ATGCTCTAGTACGATCTGTTGCTAGGATGGCC
Aptakiss	ACCAGCGAUCCGCACAUCUUCGAGCAUACUGU-AA-UGCUCGGCCCCGCGAGCA
BD96	CGACAATAAACAACAT-AGTGAGGAGCAACGCGCACCCACCGCCTTGAA-TTCACGGCCATCAGGAACACTCTAGAGTCTCC-GAACACCCTGAACAAA
BD32	TTCAAGGCGGTGGGTGCGCGTTGCTCCTCACT
KG51	GGAGACUCUAGAGUGUUCCUGAUGGCCGUGAA-AA-ACGAGCUGGGGCGCUCGU
GTPswitch	GGAGACUCUAGAGUGUUCCUGAUGGCCGUGAA-AA-UCCGAAGUGGUUGGGCUGGGGCGUGUGAAAACGGA
GTPswitch mutant	GGAGACUCUAGAGUGUUCCUGAUGGCCGUGAA-AA-UCCGAAGUGGUUGGGCUGGGCGUGUGAAAACGGA
Cover strand for GTPswitch	TTCACCCCAG

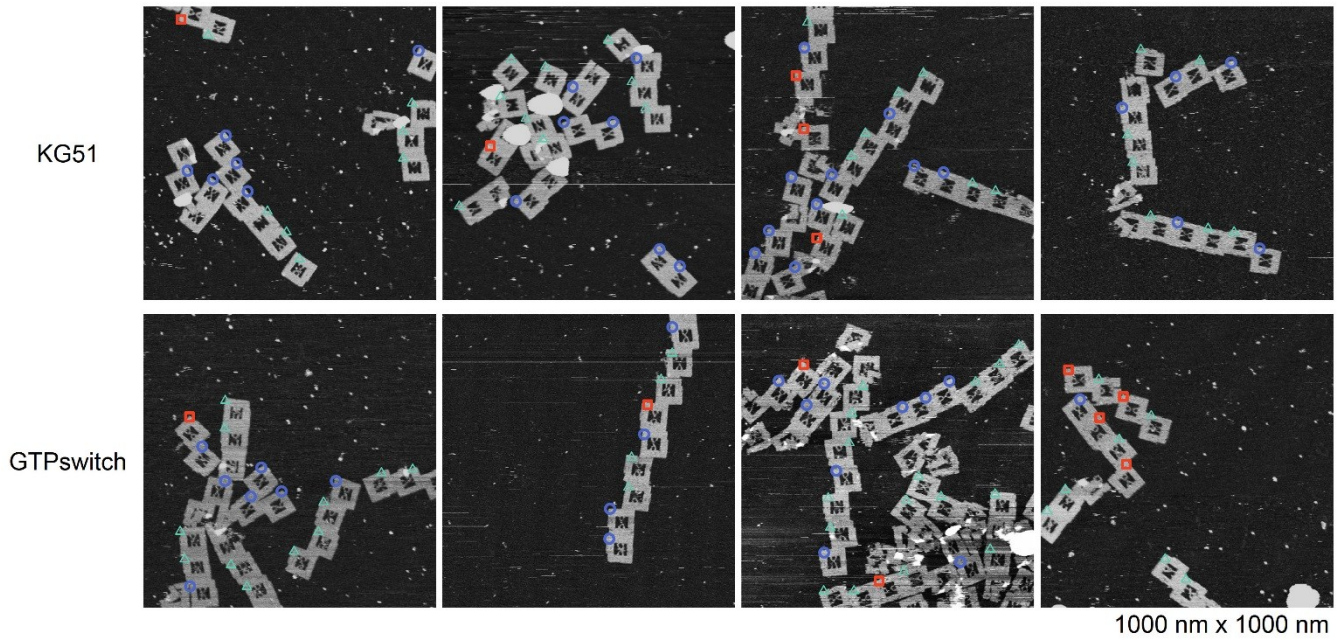

Fig. S2. AFM images of interaction of KG51 or aptakiss and GTPswitch incorporated to the DNA frame. Top and bottom images are KG51 and GTPswitch, respectively. Blue circle and red rectangle indicate the DNA frame containing X-shape and Double-loop shape, respectively. Green triangle means unclear DNA frame.

Table S2. Summary of the X-shape formation using Aptakiss and GTPswitch in the absence and presence of ligands.

	X-shape (%)	Double-loop shape (%)	±S.D.	Counted numbers
KG51	84.9	15.	3.3	280
GTPswitch	75.1	24.9	4.3	285
GTPswitch + GTP	83.9	16.1	2.5	248
GTPswitch + ATP	74.4	25.6	3.1	203

The data are represented as the mean ± S.D of triplicate experiments (n = 3).

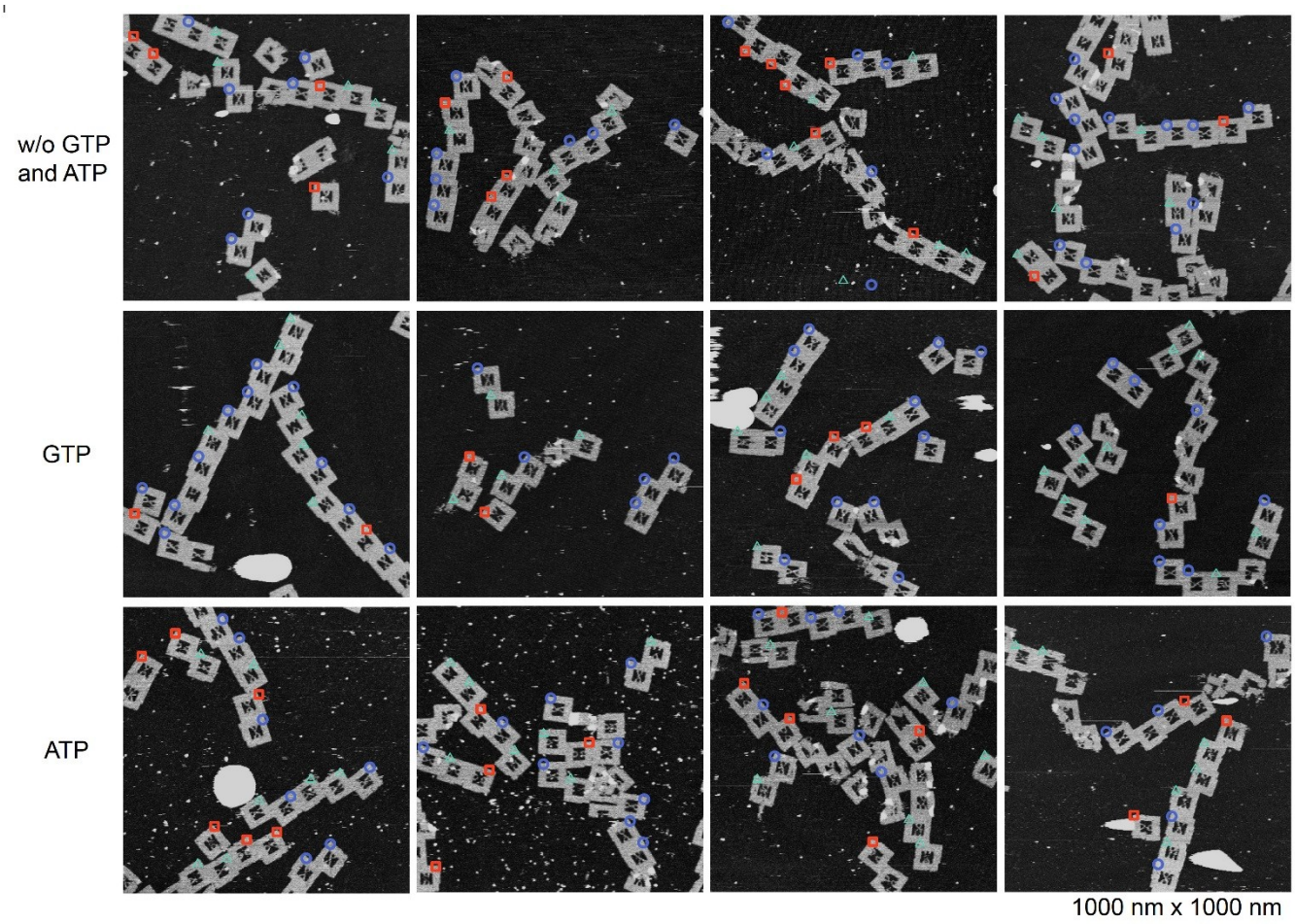

Fig. S3. AFM images of interaction of aptakiss and GTPswitch with cover strand incorporated to the DNA frame. Top, middle, and bottom images are no addition of ligand, addition of GTP and ATP, respectively. Blue circle and red rectangle indicate the DNA frame containing X-shape and Double-loop shape, respectively. Green triangle means unclear DNA frame.

Table S3. Summary of the X-shape formation using Aptakiss and GTPswitch with a cover strand in the absence and presence of ligands.

	X-shape (%)	Double-loop shape (%)	±S.D.	Counted numbers
GTPswitch/cover strand	64.4	35.6	4.4	381
GTPswitch/cover strand + GTP	82.5	17.5	2.1	324
GTPswitch/cover strand +ATP	66.5	33.5	2.3	281

The data are represented as the mean ± S.D of triplicate experiments (n = 3).

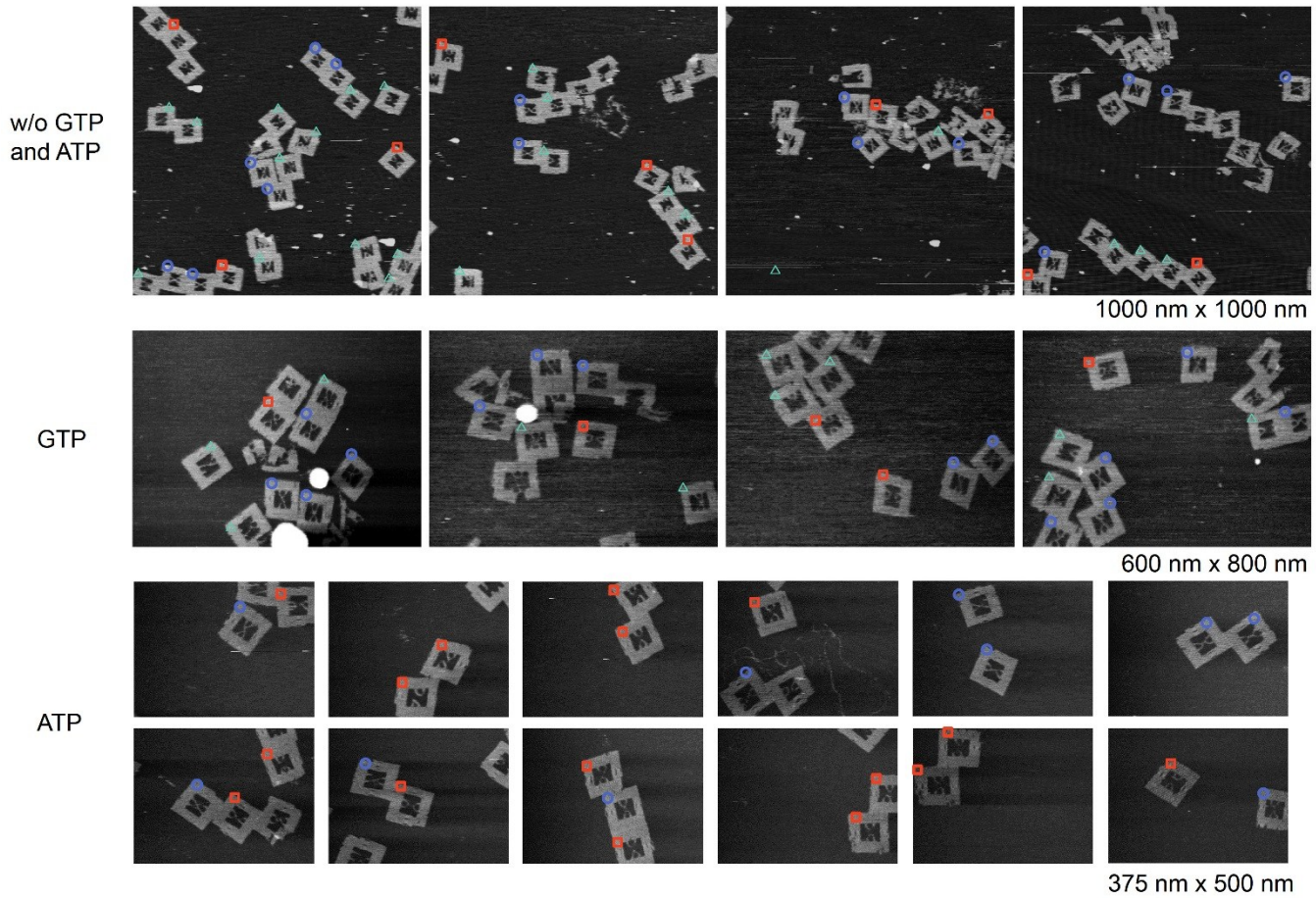

Fig. S4. AFM images of interaction of aptakiss and GTPswitch mutant incorporated to the DNA frame. Top, middle, and bottom images are no addition of ligand, addition of GTP and ATP, respectively. Blue circle and red rectangle indicate the DNA frame containing X-shape and Double-loop shape, respectively. Green triangle means unclear DNA frame.

Table S4. Summary of the X-shape formation using Aptakiss and GTPswitch mutant in the absence and presence of ligands.

	X-shape (%)	Double-loop shape (%)	±S.D.	Counted numbers
GTPswitch mutant	44.0	56.0	2.0	202
GTPswitch mutant + GTP	65.2	34.8	0.5	230
GTPswitch mutant + ATP	46.4	53.6	2.5	214

The data are represented as the mean ± S.D of triplicate experiments (n = 3).