Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

A DNA rotary nanodevice operated by enzyme-initiated strand resetting

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MATERIALS AND METHODS

Preparation of DNA complexes

All oligonucleotides were purchased from Integrated DNA Technologies (IDT). Full sequences are listed in Table 1. Strand routing for the structures is shown in Figure S1. To form the different nucleic acid complexes, the specific component strands were mixed in equimolar ratios to a final concentration of 250 nM in Tris-Acetic-EDTA-Mg^{2+} (TAE/Mg^{2+}) buffer containing 40 mM Tris base (pH 8.0), 20 mM acetic acid, 2 mM EDTA, and 12.5 mM magnesium acetate. The solution was slowly cooled from 95 °C to 20 °C by placing the tubes in 2 liters of hot water in a styrofoam box for 48 hours to facilitate hybridization.

RNase treatment

Annealed samples (at ~250 nM) were mixed with RNase H buffer (at 1× final concentration) and then incubated with the desired amount of RNase H (New England Biolabs) at room temperature (20 °C). After incubation (time duration is specified in main text for different experiments), JX_2 set strands were added and samples were incubated at room temperature (20 °C) for 20 min to 1 h.

Polyacrylamide gel electrophoresis (PAGE)

Non-denaturing gels containing 10-12% polyacrylamide (19:1 acrylamide/bisacrylamide, National Diagnostics) were run at 4 °C (125 V, constant voltage) in 1× TAE/Mg^{2+} running buffer. DNA samples were mixed with 1 µl of 10× loading dye containing bromophenol blue and glycerol before loading in the gels. Gels were stained with 0.5× GelRed (Biotium) in water and imaged on a Bio-Rad Gel Doc XR+ imager using the default settings for GelRed with UV illumination and analyzed using Image J.

Table S1. Sequences used in this study. Strand combinations for different structures are shown in **Figure S1**.

Name	Sequence (5′-3′)	Length
Frame 1	GCCAAGCCTAGCCACCTTTTGGTGGCTAGGACCGATCGGCGCGA GTGTAGGTCCGAGCACATCATGCCTTTTGGCATGATGTATCCGC	88
Frame 2	ATCGGTTCAGCACGTCTTTTGACGTGCTGACTTGGCACGGTTGT GGTACGACGCGGATCTGGAATCACTTTTGTGATTCCAGGCTCGG	88
PX set strand 1	ACCTAACCACACGCCG	16
PX set strand 2	GTCGTCACTCGACCGT	16
JX set strand 1	GTCGTACCACAACCGT	16
JX set strand 2	ACCTACACTCGCGCCG	16
PX set strand 1 RNA	ACCUAACCACACGCCG	16
PX set strand 2 RNA	GUCGUCACUCGACCGU	16

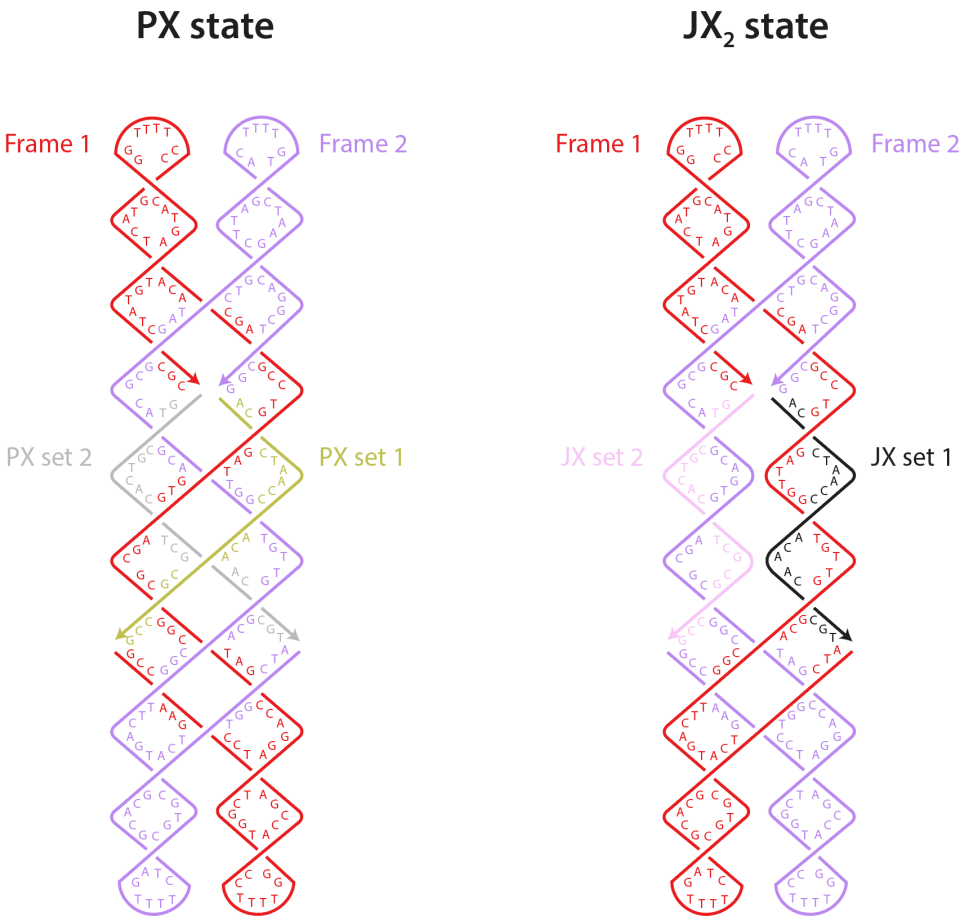

Figure S1. Sequences of strands used in this study.

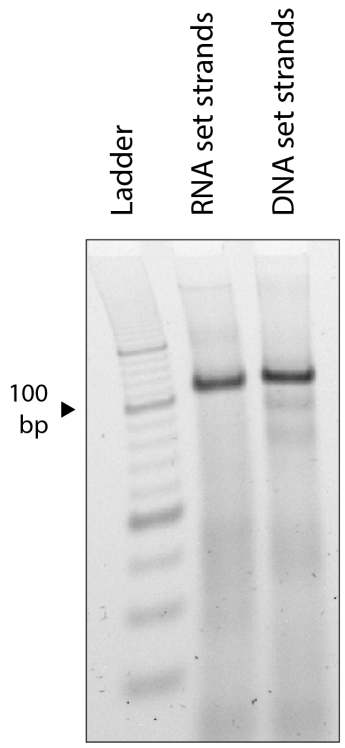

Figure S2. Assembly of the PX state with RNA and DNA set strands.

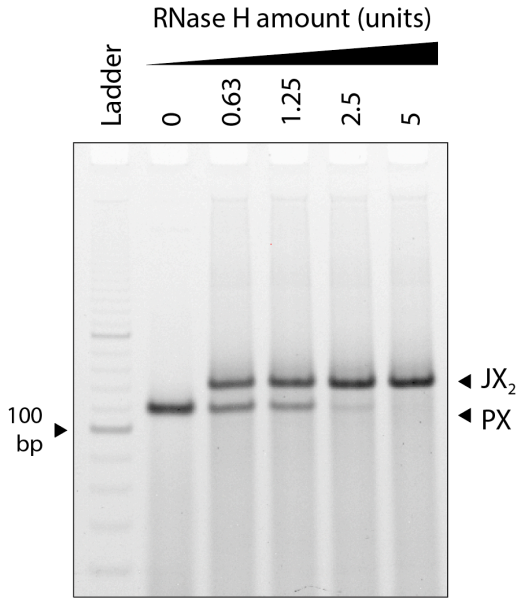

Figure S3. RNase H concentration series for resetting the PX device. Full image of gel shown in Figure 3c.

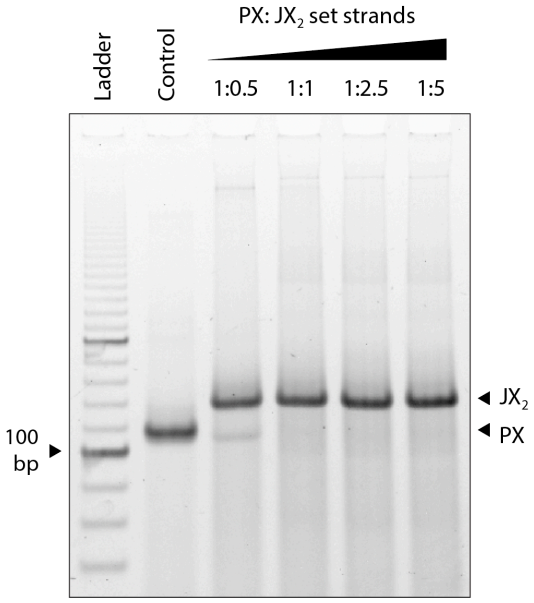

Figure S4. Different ratios of JX_2 set strands used to reconfigure the intermediate frame to the JX_2 state. Full image of gel shown in Figure 3d.

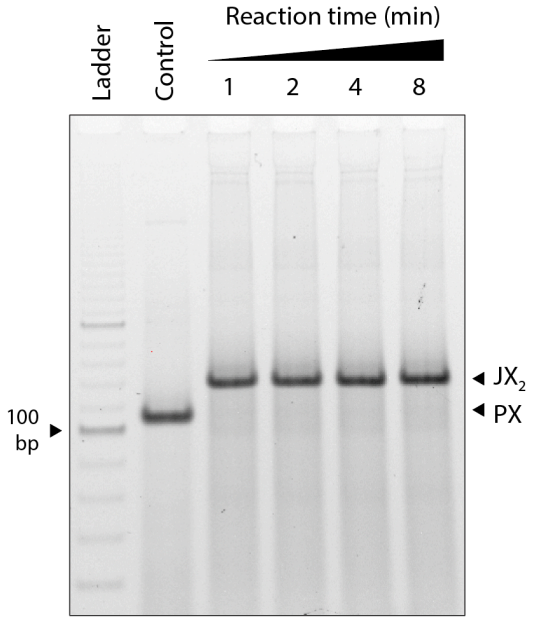

Figure S5. Time series for reconfiguration of the intermediate frame to the JX_2 state on addition of JX_2 set strands. Full image of gel shown in Figure 3e.

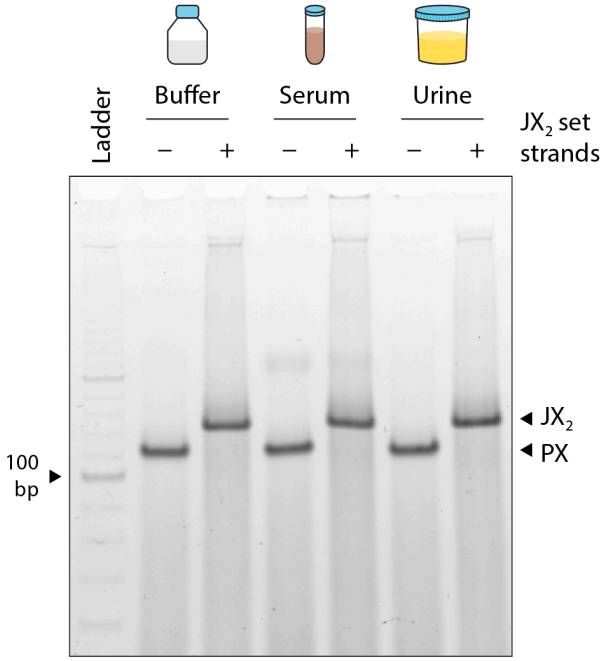

Figure S6. Operation of the device in biofluids. Full image of gel shown in Figure 3f.