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Supporting Information

Cofactor-assisted three-way junction-driven strand displacement

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S1: DNA sequences

Name	Sequence (5′→3′)	Length (n.t.)
HM	FAM-CATATTCCCTCATTCAATACCCTACG	26
HA	GGGCCTGATTGAGGTATTGAATGAGGGAATATG- BHQ	33
HI1	GCTTGTCGTAGTCAATCAGGCCC	23
HI3	CATATTCCCTCATTCAATACCCTACGTCTAGC	32
AM	FAM-AACTAATCCTCAGATCCAGCTAGTGTC- BHQ	27
AA	ATTAGGCCGAATCTAGCTGGATCTGAGGATTAGT	34
AI1	CGCTATATACCTGGGGGGAGTATGACACATTCGG CCTAAT	39
AI3	ACTAATCCTCAGATCCAGCTAGTGTCTGCGGAG GAAGGTATAT	43
AI3-N	ACTAATCCTCAGATCCAGCTAGTGTCAGCGGAG GAAGGTATAT	43
PM	AACTAATCCTCAGATCCAGCTAGTGTC	27
PA	AGTCAGATTACGATATCGGCACGCGC	36
PI1	TCTCCAATTCTACCCCTAACCCCTGACACATTCG GCCTAAT	41
PI3	ACTAATCCTCAGATCCAGCTAGTGTCTCCCCTAA CCCCTAGAATTGGA	48

Table S1. Sequences of the Oligonucleotides (5' to 3')

S2: ATP-assisted three-way junction-driven strand displacement



Figure S1. Native 15% polyacrylamide gel analysis of the formation of the three-way junction structure. DNA strands added in every lane are indicated above the image. The concentration of each DNA strand in PAGE was 2µM.



Figure S2. Native 15% polyacrylamide gel analysis of the strand displacement reaction with different inputs. DNA strands added in every lane are indicated in the table. Concentrations for each DNA strand in PAGE are all 2µM.

S3: Hg²⁺-assisted three-way junction-driven strand displacement



Figure S3. Native 15% polyacrylamide gel analysis of the formation of the three-way junction structure. DNA strands added in every lane are indicated above the image. The concentration of each DNA strand in PAGE was 2µM.



Figure S4. (a). Native 15% polyacrylamide gel analysis of Hg²⁺-assisted three-way junction-driven strand displacement at 25°C for 20 hours. DNA strands added in every lane are indicated above the image. The concentration of three-way complex HM/HA/HI1 in each lane was 2µM. The concentration of HI3 in lane 2-5 was 2µM and in lane 6-9 was 3µM. (b). Native 15% polyacrylamide analysis of strand displacement without HI1 at 25°C for 20 hours. DNA strands added in every lane are indicated above the image. The concentration of DNA complex HM/HA in each lane was 2µM. The concentration of HI3 in lane 2-5 was 2µM and in lane 6-9 was 3µM.



Figure S5. Fluorescence intensity analysis of the complex HM/HA/HI1 upon addition of H13 and different concentrations of Hg²⁺. **Curve 1: 0μM; Curve 2: 0.5μM; Curve 3: 1μM; Curve 4: 2μM.** [HM/HA/HI1] =100nM, [HI3] =150nM.



Figure S6. Native 15% polyacrylamide gel analysis of the formation of the three-way junction structure in **pH=7.5**. DNA strands added in every lane are indicated above the image. The concentration of each DNA strand in PAGE was 2µM.

S4: pH-assisted three-way junction-driven strand displacement



Figure S7. Native 15% polyacrylamide comparison of strand displacement reaction at 25°C for **half an hour**, **pH=5.5 and pH=7.5 respectively.** DNA strands added in every lane are indicated above the image. The concentration of DNA complex in each lane was 2µM. The concentration of PI3 was 2µM, 4µM and 8µM respectively.