SUPPLEMENTAL MATERIAL FOR:

Steric Exclusion and Wrapping of the Excluded DNA Strand Occurs Along Discrete External Binding Paths During MCM Helicase Unwinding

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SUPPLEMENTAL TABLES:

Table S1: DNA substrates

DNA[1]	Total Length	Duplex	Sequence[2,3]
3'-tail-30 nt	66	36	5′-CACCTCTCCCTACGCTTCCCACCCACCCCGACCGGCATCTGCTATGGTACGCTGAGCGAGAGTAGC
5'-tail-0 nt	36	36	5′-GCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
5'-tail-20 nt	56	36	5′-GAGTCGCATGGTATCGTCTAGCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
5'-tail-30 nt	66	36	5′-CGATGAGAGCGAGTCGCATGGTATCGTCTAGCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
5'-tail-40 nt	76	36	5′-CGATGAGAGCCGATGAGAGCGAGTCGCATGGTATCGTCTAGCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
5'-tail-50 nt	86	36	5′-CGATGAGAGCCGATGAGAGCCGATGAGAGCGAGTCGCATGGTATCGTCTAGCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
5'-tail-80 nt	100	20	5'-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATGAGAGCGAGTCGCATGGTATCGTCTAGCCGGTCGGGGTGGGTGGGA
3'-tail-30 nt (IntB)	66	36	5′-CACCTCTCCCTACGCTTCCCACCCACCCCGACCGGCABCTGCTATGGTACGCTGAGCGAGAGTAGC
5'-tail-30 nt (IntB)	66	36	5′-CGATGAGAGCGAGTCGCATGGTATCGTCBAGCCGGTCGGGGTGGGTGGGAAGCGTAGGGAGAGGTG
3'-tail-30 nt (5'Cy3)	66	36	5′-3CACCTCTCCCTACGCTTCCCACCCACCCCGACCGGCATCTGCTATGGTACGCTGAGCGAGAGTAGC
3'-tail-31 nt (5'-B)(3'Cy3)	49	18	5′-BTGGCGACGGCAGCGAGGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT3T
5'-tail-31 nt (5'Cy5)	49	18	5′-5TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGCTCGCTGCCGTCGCCA
5'-tail-51 nt (5'Cy5)	69	18	5′-5TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCTCGCTGCCGTCGCCA
5'-tail-71 nt (5'Cy5)	89	18	5′-5TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGCCTCGCTGCCGTCGCCA

[1]nt – nucleotides; [2]Modifications are underlined; [3]B – Biotin, 3 – Cy3, 5 - Cy5

Table S2: Quantification Nuclease Protection of 5'-Tails, related to Figure 3[1]

	*Sso*MCM Variant - Fraction Protected[2]			
DNA Type[3]	WT	K323A	R440A	K323A/R440A
5'-tail - 30 nt	0.906 ± 0.030	0.689 ± 0.037	0.563 ± 0.035	0.590 ± 0.030
5'-tail - 50 nt	0.896 ± 0.008	0.619 ± 0.010	0.512 ± 0.027	0.506 ± 0.036
5'-tail-- 80 nt	0.828 ± 0.023	0.747 ± 0.013	0.627 ± 0.012	0.604 ± 0.036

[1]Data is taken from Figure 3. [2]Fraction of DNA (90 nM) protected in the presence of 10 units of mung bean nuclease where *Sso*MCM concentration is 540 nM. Averaged from at least three separate mung bean nuclease protection assays. [3]Using a 30mer 3' tail.

Table S3: Individual spFRET peak values.

*Sso*MCM Variant	5'-Tail Length (nt)	E_{app}[1]	Percent of Total Population (%)
WT	30	0.95 ± 0.14	67
		0.84 ± 0.06	33
	50	0.96 ± 0.06	83
		0.89 ± 0.10	17
	70	0.95 ± 0.09	18
		0.75 ± 0.12	61
		0.45 ± 0.14	21
K323A/R440A	30	0.82 ± 0.12	67
		0.72 ± 0.28	33
	50	0.88 ± 0.08	6
		0.69 ± 0.28	94
	70	0.82 ± 0.11	66
		0.43 ± 0.18	34

[1] Mean ± STDEV of individual deconvoluted peaks.

SUPPLEMENTAL FIGURES AND LEGENDS

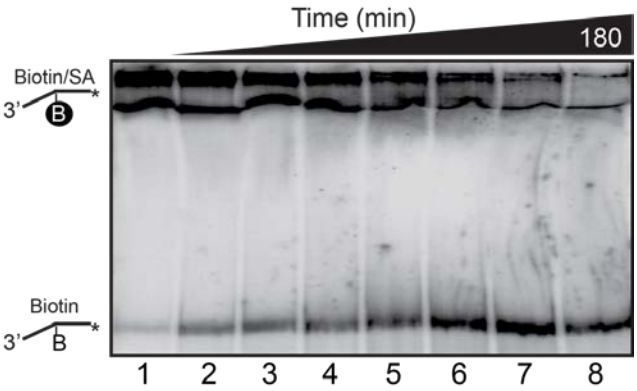

Figure S1: Streptavidin displacement by *Sso*MCM related to Figure 1. 3'-tail - 30nt (IntB) (Supplementary Table S1) was 5' radiolabeled as described in Materials and Methods. ^{32}P-DNA (15 nM) was preincubated for five minutes at 60 ^{o}C with helicase buffer, ATP and 750 nM streptavidin. *Sso*MCM followed immediately by 20-fold excess biotin was added to initiate the reaction. Time points were taken from 0-180 minutes. A linear rate of 0.0067 ± 0.0002 min^{-1} fraction of streptavidin displaced was calculated over 60 minutes. This background value was subtracted and used to create Figure 1C and D.

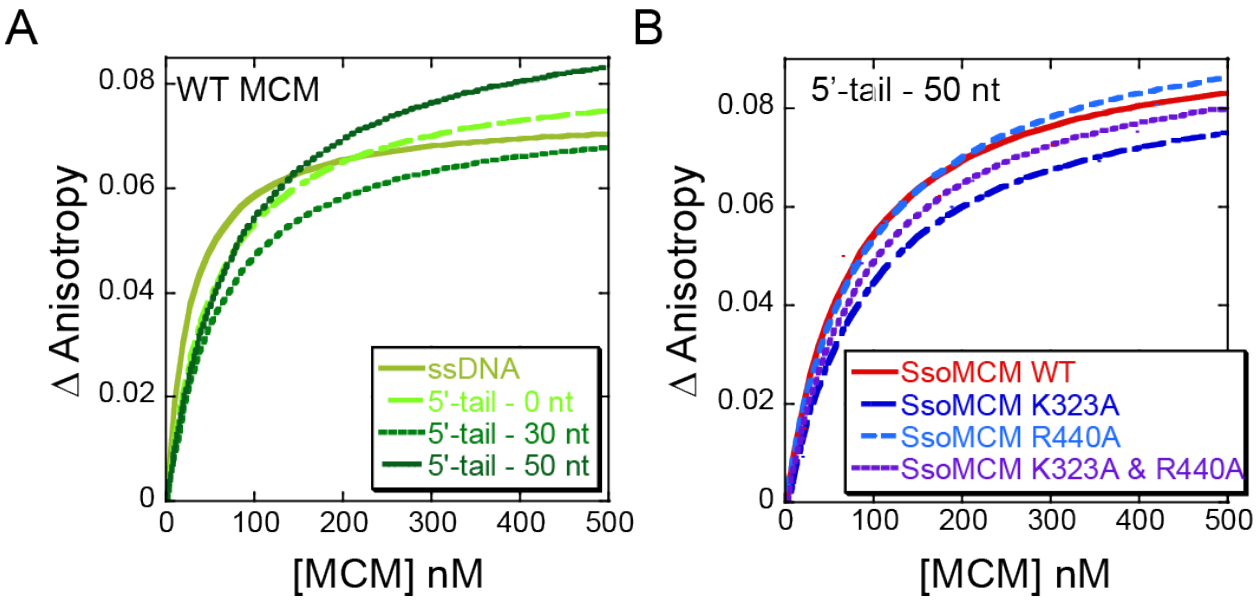

Figure S2: *Sso*MCM variants binding to various DNA substrates monitored by fluorescence anisotropy. (A) Change in fluorescence anisotropy for *Sso*MCM WT binding to four different DNA substrates: ssDNA, 5'-tail – 0 nt, 5'-tail – 30 nt, and 5'-tail – 50 nt, as described in the Materials and Methods. The K_d values were calculated from the average of at least three sets of data using Equation 1. The values are shown in Table 1. (B) Change in fluorescence anisotropy for *Sso*MCM WT, K323A, R440A, and K323A/R440A when binding to fluorescently labeled forked DNA (3'-tail – 30 nt and 5'-tail - 50 nt with duplex region of 36 bp). K_d values were calculated from the average of at least three sets of data using Equation 1 and reported in Table 2.

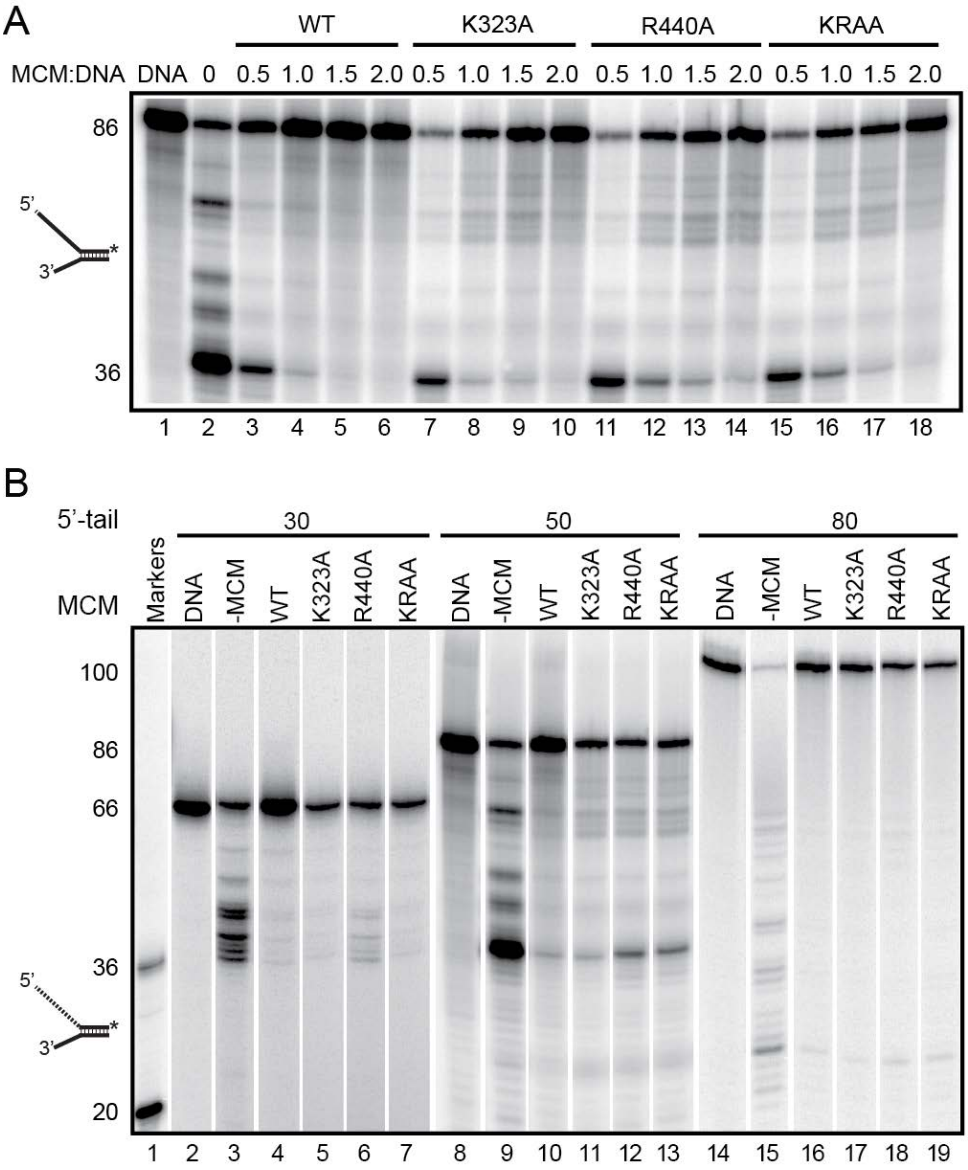

Figure S3: Mung bean nuclease 5’-tail protection assay gels for *Sso*MCM variants. (A) Nuclease assays were performed in the absence and presence of increasing *Sso*MCM (WT, K323A, R440A and KRAA) concentration to titrate stoichiometry. 90 nM of fork 50mer 5’-tail and 30mer 3’-tail DNA was utilized with MCM hexamer:DNA 0.5 (45 nM), 1.0 (90 nM), 1.5 (135 nM), and 2.0 (180 nM). (B) Nuclease assays were performed in the presence and absence of 540 nM *Sso*MCM WT, K323A, R440A and K323A/R440A (KRAA) with different length 5’-tails as described in Materials and Methods. The 5’-tail strand was labeled at the 3’-end with ^{32}P. DNA markers (Markers) are shown in lane 1. DNA alone is shown in lanes 2, 8, and 14. The length of the 5’-tail was varied from 30, 50, and 80 bases. The duplex region (36 bases) and 3’-tail (30 bases) were identical for lanes 2-13. The duplex region for lanes 14-19 is 20 bases and 3’-tail is 30 bases. Quantification of this data is shown in Figure 3E and Supplementary Table S2.

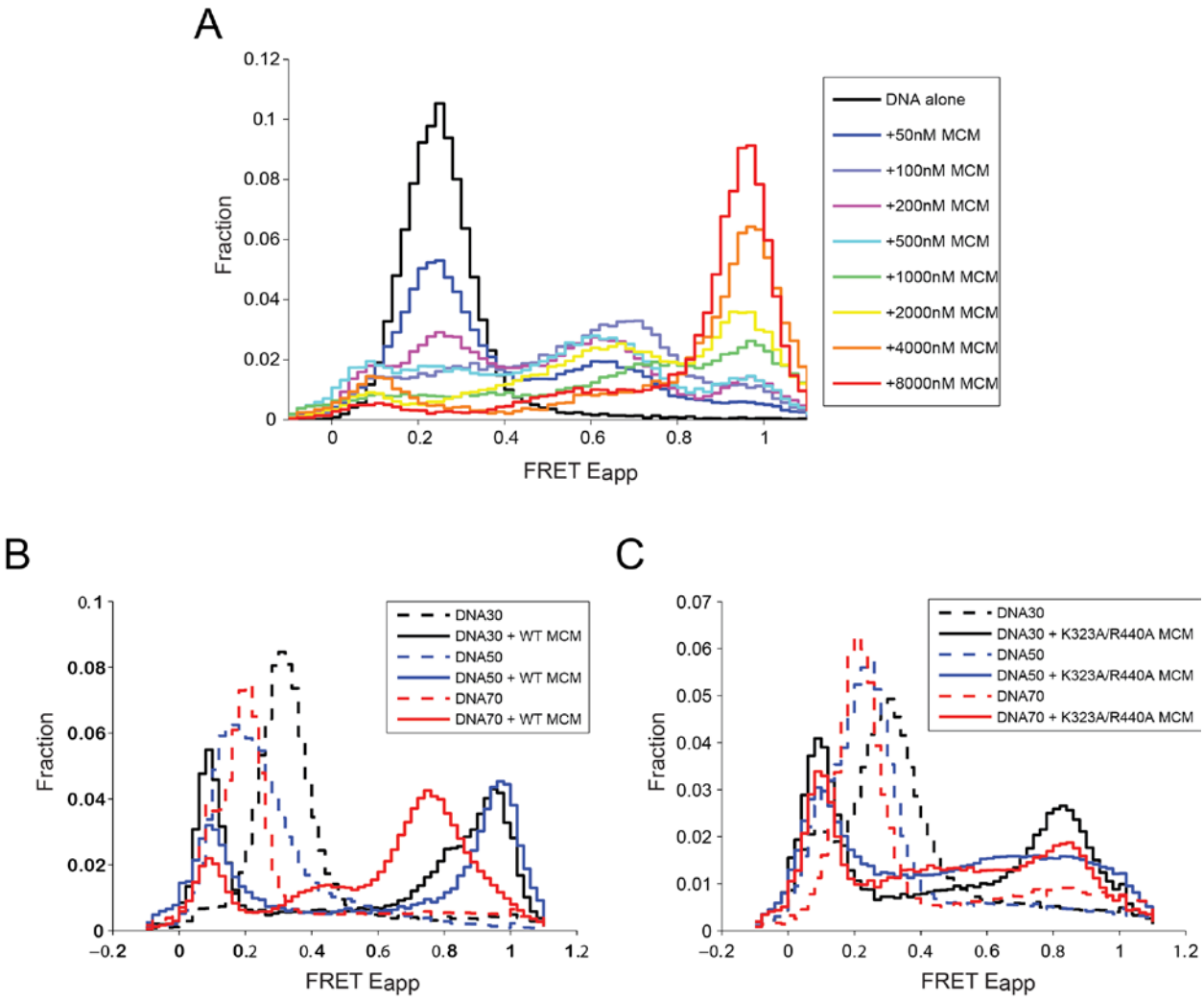

Figure S4: spFRET titration of *Sso*MCM to visualize hexamer formation. (A) DNA forked substrates with 50mer 5'tails were tethered to a flow cell, followed by incubation with WT *Sso*MCM helicase at the concentration indicated in the legend. Histograms represent data from all time points of every identified spFRET trace (i.e., regions of traces were not hand selected to eliminate signal from photobleached species, etc.). Example unfiltered datasets for (B) WT or (C) K323A/R440A *Sso*MCM. Raw spFRET histograms derived from all time points of every identified spFRET trace are indicated. Dataset represents the data displayed in Figure 4 before hand-picking regions in order to eliminate spurious data such as data without clear anticorrelated donor/acceptor signal as well as the "zero-peak" arising from photobleached species or species without acceptor. Forked substrate is indicated by DNAXX, where XX represents the length of the 5'tail in bases.

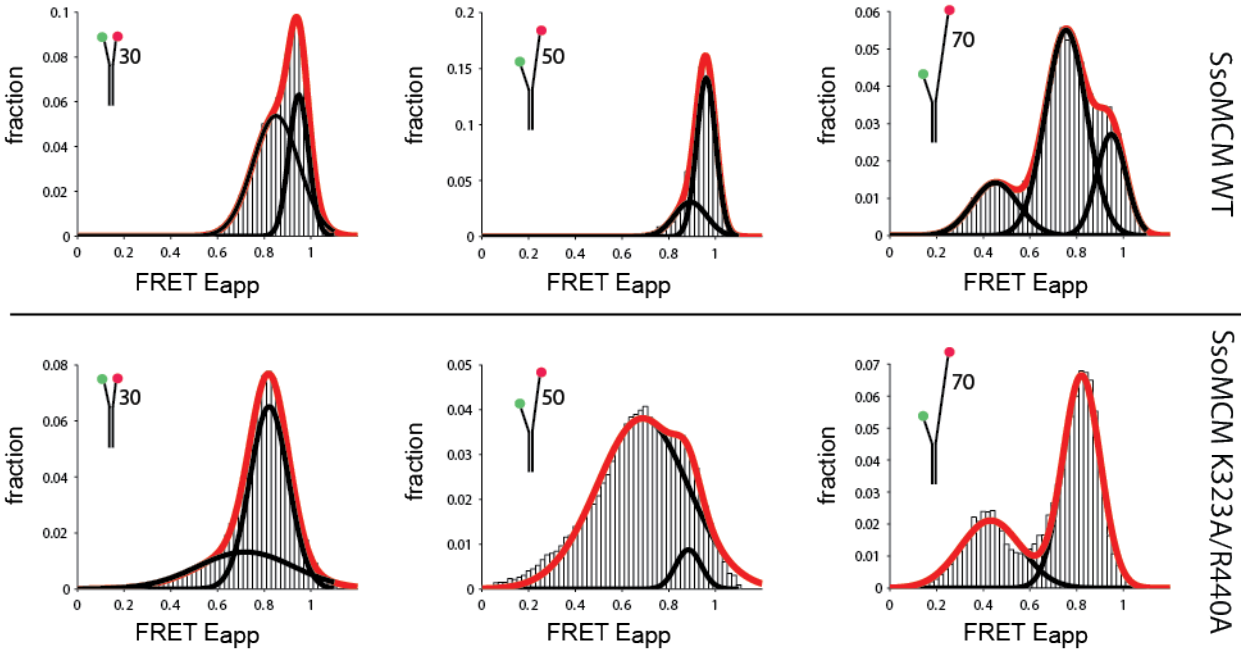

Figure S5: Deconvolution of composite spFRET histograms from Figure 4. For each experiment indicated with 30, 50 or 70 base 5’-tail and either WT or K323A/R440A *Sso*MCM, the histogram is shown as a bar graph. The composite curve fit is shown in red, and the individually identified peaks are shown in black. Also see Supplementary Table S3, where individual curves were numerically integrated and expressed as a percentage of total.