Supplementary Materials

Monitoring SARS-CoV-2 Nsp13 Helicase Binding Activity Using Expanded Genetic Code Techniques

Eryn Lundrigan,[a] Christine Hum,[a] Nadine Ahmed [a] and John Paul Pezacki [a]

[a]*Department of Chemistry and Biomolecular Sciences, University of Ottawa, Ottawa, Ontario, K1N 6N5, Canada.*

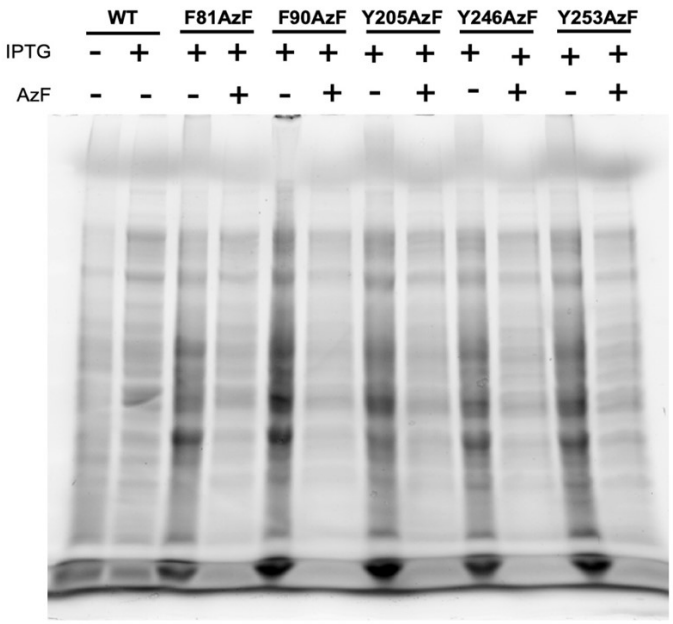

Figure S1. Stain-free gel showing protein expression of site-specific Nsp13-AzF constructs in bacterial lysates in presence and absence of AzF in the growth media.

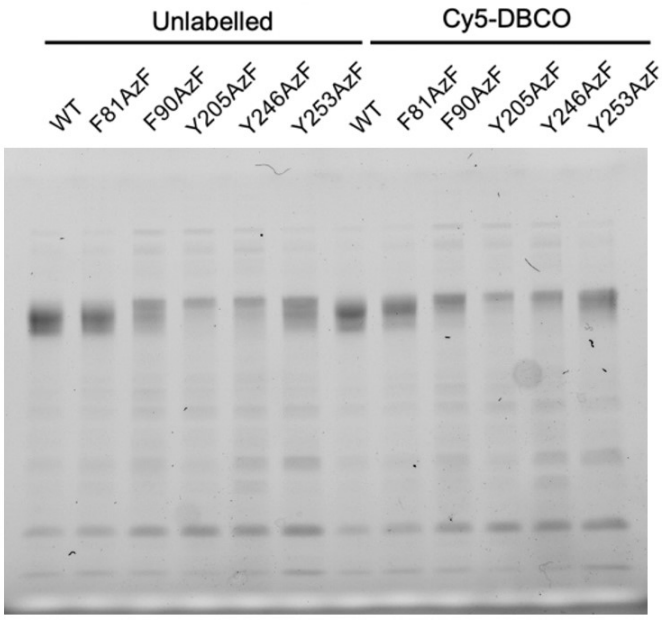

Figure S2. Stain-free gel of purified WT Nsp13 and Nsp13-AzF constructs incubated with Cy5-DBCO at a molar ratio of 1:2 protein to dye.

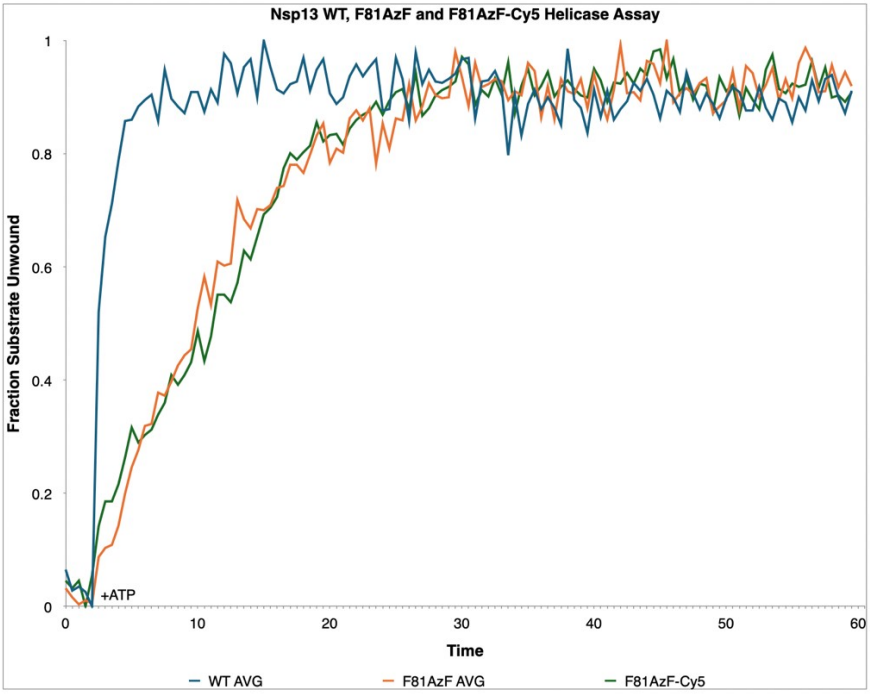

Figure S3. The unwinding activity of purified WT Nsp13, Nsp13-F81AzF and Nsp13-F81AzF-Cy5 constructs was monitored. 5 nM of purified protein was incubated with 50 nM of dsDNA substrate and the reaction was initiated with the addition of 0.1 mM ATP. The graph is presented as the fraction of substrate unwound over time.

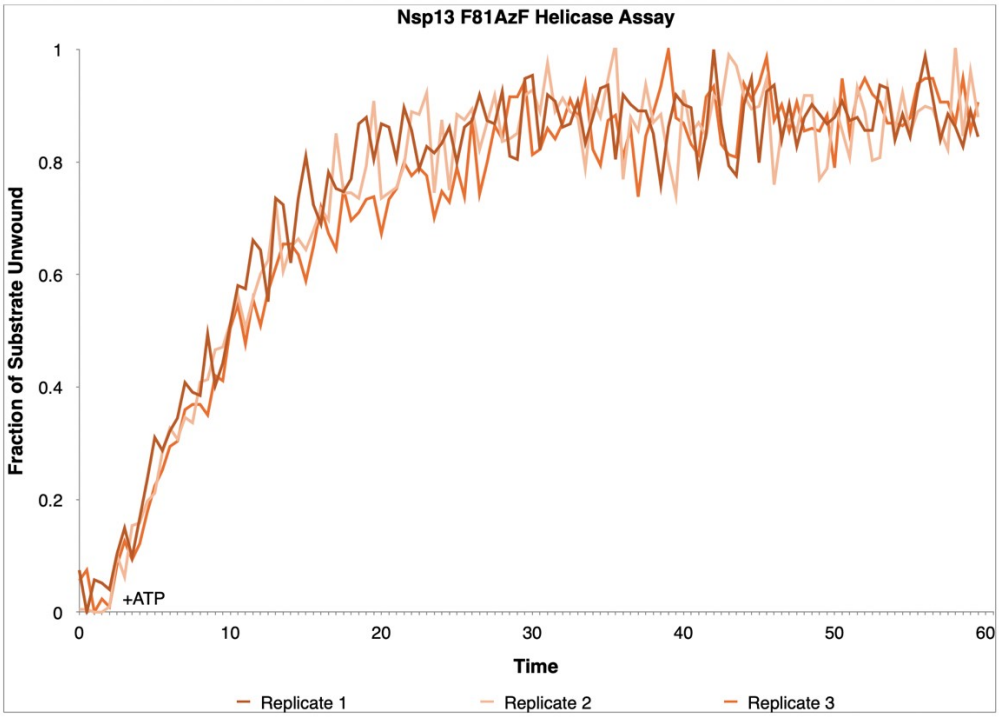

Figure S4. The unwinding activity of purified Nsp13-F81AzF technical triplicates was monitored. 5 nM of purified protein was incubated with 50 nM of dsDNA substrate and the reaction was initiated with the addition of 0.1 mM ATP. The graph is presented as the fraction of substrate unwound over time

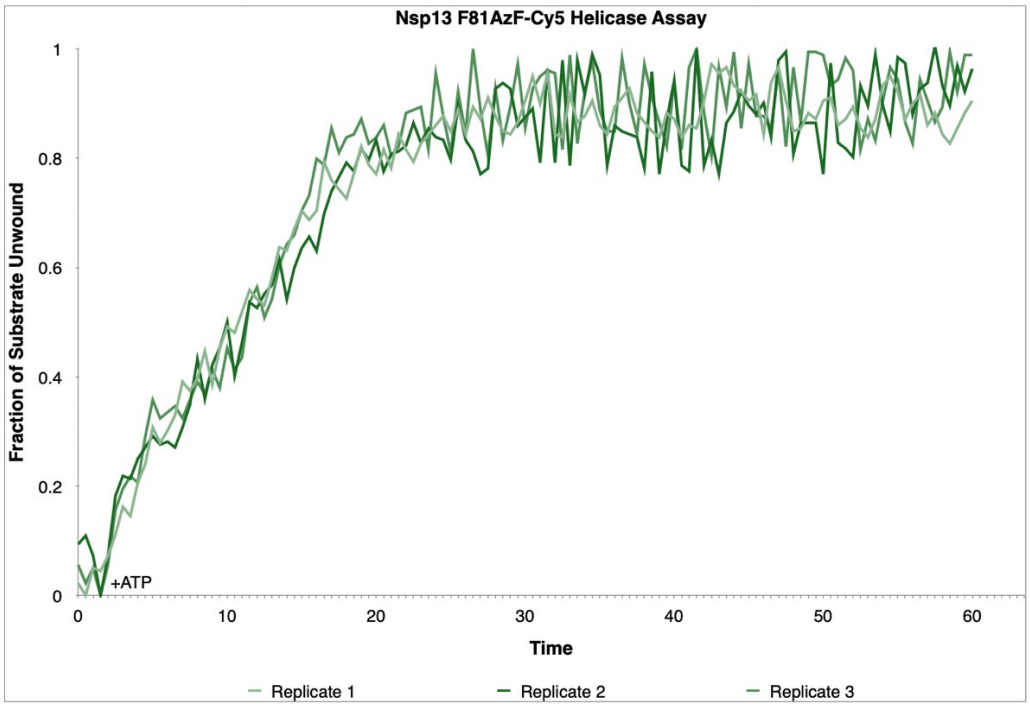

Figure S5. The unwinding activity of purified Nsp13-F81AzF-Cy5 technical triplicates was monitored. 5 nM of purified protein was incubated with 50 nM of dsDNA substrate and the reaction was initiated with the addition of 0.1 mM ATP. The graph is presented as the fraction of substrate unwound over time

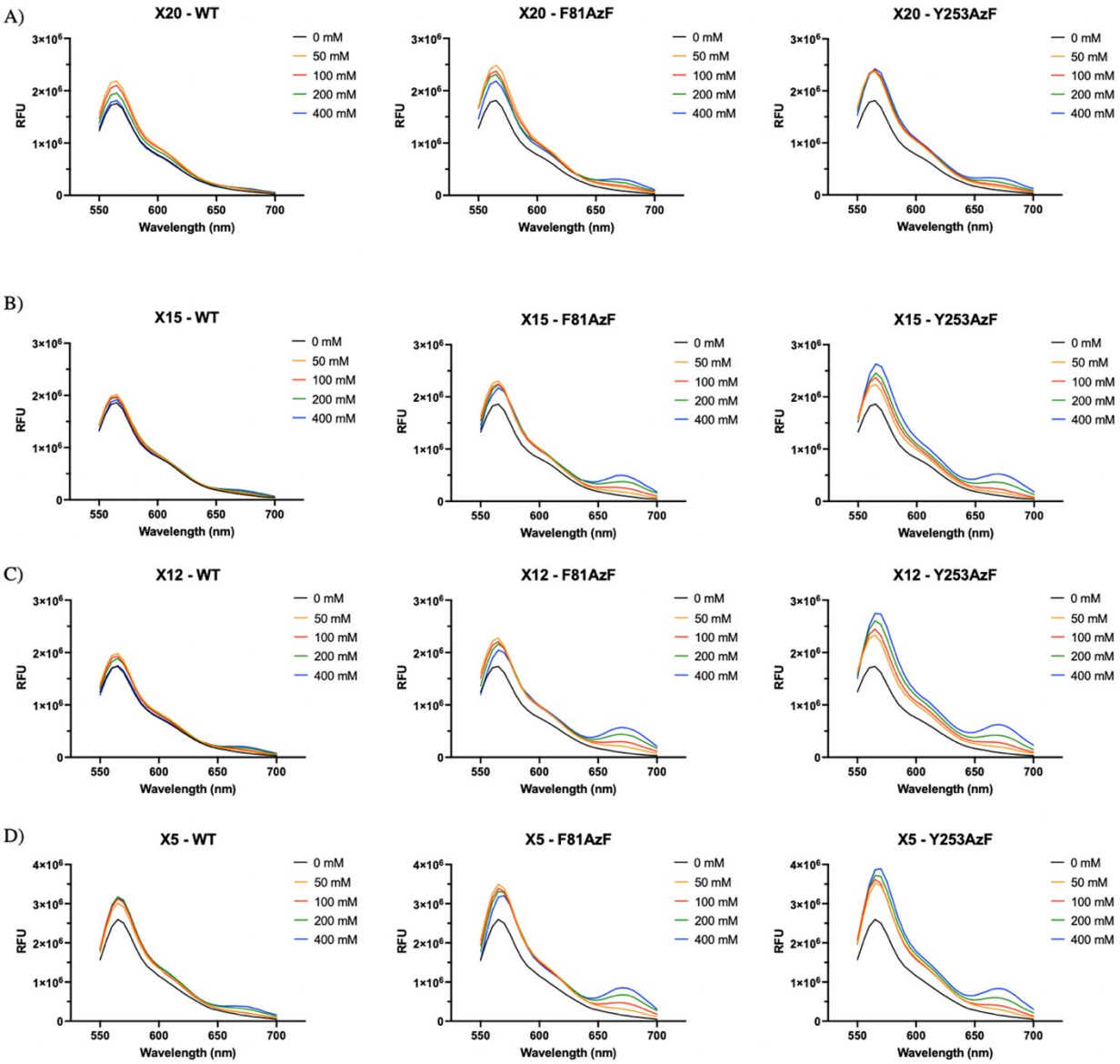

Figure S6. Binding of Cy5-labelled Nsp13-AzF to Cy3-labelled dsDNA measured by FRET. The emission spectra for the binding of Cy5-labelled WT Nsp13, F81AzF, and Y253AzF to (A) S20-Cy3/X20, (B) S20-Cy3/X15, (C) S20-Cy3/X12, and (D) S20-Cy3/X5 with a Cy3 peak at 565 nm and a Cy5 peak at 670 nm.

Supplemental Table 1: List of Primers used for Subcloning and Site-directed Mutagenesis.

Oligonucleotide	**Sequence (5′ – 3′)**
Nsp13 Fwd primer with BamHI	GTGTATGGATCCATGGCTGTTGGTGCATGCGTTTTG
Nsp13 Rev primer with HindIII	ATTTATAAGCTTTTGAAGGGTTGCCACGTTACGGCG
F81AzF Fwd	CATTCGCGCAGAGAGGCTATGAAATCGGCGGCTTATG
F81AzF Rev	CATAAGCCGCCGATTTCATAGCCTCTCTGCGCGAATG
F90AzF Fwd	GGTGTTTTTATACAAACCCTACACCTGGCCATTCGCGCAGA
F90AzF Rev	TCTGCGCGAATGGCCAGGTGTAGGGTTTGTATAAAAACACC
Y205AzF Fwd	ATACTACGGCATCACCCTAGTCGCCTTTCTCAAAG
Y205AzF Rev	CTTTGAGAAAGGCGACTAGGGTGATGCCGTAGTAT
Y246AzF Fwd	AAACCCGTGATACGTACCTAGTGTTCCTGCGGTAC
Y246AzF Rev	GTACCGCAGGAACACTAGGTACGTATCACGGGTTT
Y253AzF Fwd	GTTCAGCGTCGGCTATAAACCCGTGATACGTACATA
Y253AzF Rev	TATGTACGTATCACGGGTTTATAGCCGACGCTGAAC

Supplemental Table 2: List of Oligonucleotides used for the Helicase Unwinding Assay.

Helicase Assay Substrates	**Sequence (5′ – 3′)**
Cy3 DNA strand	(Cy3)-GGTAGTAATCCGCTC
BHQ-2 DNA strand	TTTTTTTTTTTTTTTTTTTTGAGCGGATTACTACC-(BHQ-2)
Competitor strand	GAGCGGATTACTACC

Supplemental Table 3: List of Oligonucleotides used for the FRET Binding Assay

FRET Assay Substrates	**Sequence (5′ – 3′)**
S20-Cy3	GAGCGGATTACTATACTACC-(Cy3)
X20	GGTAGTATAGTAATCCGCTC
X17	GGTAGTATAGTAATCCG
X15	GGTAGTATAGTAATC
X12	GGTAGTATAGTA
X10	GGTAGTATAG
X8	GGTAGTAT
X5	GGTAG