

POSTER 3**KINETICS OF NUCLEIC ACID UNWINDING AND NUCLEOTIDE HYDROLYSIS BY SARS-COV HELICASE**Adeyemi Adedeji¹, Susan R. Weiss², Kamlendra Singh¹, and Stefan G. Sarafianos¹

¹Christopher Bond Life Sciences Center and Department of Molecular Microbiology and Immunology, University of Missouri School of Medicine, Columbia, MO; ²University of Pennsylvania School of Medicine, Philadelphia, PA

Helicases are motor proteins that unwind double stranded DNA and/or RNA by utilizing the energy of nucleotide hydrolysis. This group of enzymes participates in multiple processes including nucleic acid replication, repair, translation, and transcription. The helicase of severe acute respiratory syndrome coronavirus (SARS-CoV) is known as non-structural protein 13 or nsp13. It is a superfamily 1 helicase containing seven conserved motifs. It has been reported to unwind DNA and RNA in the 5' → 3' direction. We have cloned and purified a GST-fused recombinant nsp13 SARS-CoV helicase. Initial characterization of its biochemical properties showed that it unwound DNA and RNA similarly to a previously reported MBP-fused nsp13. Here we present a detailed kinetic characterization of the GST-nsp13 SARS-CoV helicase using steady- and transient-state kinetics. To efficiently unwind double-stranded nucleic acid substrates, GST-nsp13 requires a 5'-end overhang that is at least 10 nucleotides long. Nsp13 can utilize all dNTPs and NTPs as the energy sources. The optimal rate of ATP hydrolysis was 45 s⁻¹ with apparent binding affinity ($K_{d,ATP}$) of 54 μM. The unwinding of nucleic acid follows burst kinetics. Once bound to nucleic acid substrate, nsp13 unwinds it with a rate of 30 bp/s, which is greater than that of T7 helicase (15 bp/s). Based on our kinetic analysis under single-turnover conditions, we estimated that the averaged energetic cost of SARS-CoV nsp13 translocation is 3 molecules of ATP for translocation through 2 bp. These results provide insights that enhance our understanding of nsp13 function during SARS-CoV replication.