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Cloning, expression, purification, and characterization of Dengue virus proteins

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The Dengue virus is a tropical positive-stranded RNA virus responsible for 25,000 deaths annually. There are four Dengue virus serotypes. The RNA-dependent-RNA-polymerase (RdRp, or ns5) polypeptide among all four types is conserved with 67% amino acid sequence identity. Because of this conservation, and because ns5 is vital to Dengue virus replication, it is an attractive target for drug design. For this reason, this study aims to clone, express, purify, and characterize Dengue ns5 and its interactions with other Dengue proteins. Furthermore, this study aims to screen a library of compounds to identify inhibitors for Dengue ns5, and initiate crystallographic studies of inhibitor-ns5 complexes. A plasmid containing the Dengue serotype 2 cDNA was provided by Dr. Barry Falgout. Ns5, ns2a, and ns4a were cloned in a pet15b plasmid, and then transformed into overexpressing bacterial strains. The ns5 was purified to homogeneity by successive chromatographic separations: immobilized metal affinity chromatography, FPLC, cation exchange and FPLC gel filtration. Preliminary results show that the ns5 prepared in this study is enzymatically active. Furthermore, its activity is suppressed by a polyoxometalate known to inhibit other viral polymerases. We are currently developing high throughput screening assays to identify other ns5 inhibitors.