## "Discovery of inhibitors of a novel host activity required for human rhinovirus replication"

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**Background:** The experiments outlined in this application will attempt to discover inhibitors of a novel target for anti-viral therapy against human rhinovirus (HRV), a member of the picornavirus family of positive strand RNA viruses. Respiratory infections by human rhinoviruses are major exacerbating factors in disease morbidity for individuals suffering from asthma and other pulmonary dysfunctions. Anti-viral therapies against HRV are particularly desirable as they would lessen both the severity and duration of upper and lower respiratory distress in asthmatic individuals. To date, the available anti-viral treatments targeting specific steps in the human rhinovirus replication cycle have not been shown to be efficacious. Thus, there is an important need to develop new targets for anti-rhinovirus chemotherapy. One such target is the cellular activity (termed "VPg unlinkase") that removes a small viral peptide (VPg) from the 5' end of human rhinovirus genomic RNA (and the genomic RNAs of all picornaviruses) by cleaving a protein-nucleotidyl bond prior to the onset of viral protein synthesis. The resulting RNAs lacking VPg are translated and replicated, producing negative-strand RNA intermediates as well as progeny positive-strand RNAs that both contain the VPg-RNA linkage. These RNAs are putative substrates for VPg unlinkase activity; however, they are not uniformly cleaved during the course of infection, suggesting that there is regulation of VPg unlinkase activity by picornaviruses. In 2012, we identified VPg unlinkase from human cells as tyrosyl-DNA phosphodiesterase 2 (TDP2), a host enzyme involved in DNA repair, cell signaling, and transcriptional regulation. This proposal aims to implement a high throughput assay for VPg unlinkase/TDP2 to screen for small molecule inhibitors of this activity and, ultimately, human rhinovirus replication.

**Specific Aims:**

**Aim 1** Screen small molecule libraries to identify putative VPg unlinkase/TDP2 inhibitors

**Aim 2** Test candidate VPg unlinkase/TDP2 inhibitors on human rhinovirus and enterovirus replication in cell culture and in cell free assays

**Research Plan:**

We will implement a previously described assay for human TDP2. This approach uses a synthetic substrate in a colorimetric assay that is readily adaptable to a 96 well plate format. We will use this assay in an initial screen of compounds provided by our Co-investigator at UCI, Professor Richard Chamberlin, via UCI's Bioactive Fragment small molecule library. This small molecule library is comprised of more than 3,000 compounds generated by research groups in the Department of Chemistry over the past decade or more. Most of the molecules in this library are intermediates that have been produced in the synthesis of complex, biologically active natural products, and simpler sub-structures have proven to be a rich source of lead compounds in random screening against many different targets using a wide range of protocols here at UCI.

Once candidate inhibitors have been identified though our screening of small molecule libraries, we will test them for their ability to inhibit the growth of HRV during infections of cultured cells. We will also use an array of *in vitro* assays for different steps in HRV replication to determine the precise step(s) inhibited by candidate compounds. Knowing that any putative inhibitor affects steps potentially related to VPg unlinkase function (viral mRNA association with polysomes, RNA replication, RNA encapsidation/virion assembly) will aid in deciding how to modify specific compounds to improve their efficacy and/or therapeutic index and in the design of additional small molecules that will disrupt the interaction of VPg unlinkase/TDP2 with the tyrosyl-RNA linkage in genomic RNA.

**Plans for extramural funding:**

If we are successful in identifying lead compounds that inhibit TDP2 activity as well as human rhinovirus replication, we will submit an R01 application to NIH for the "Development of Assays for High-Throughput Screening for Use in Probe and Pre-therapeutic Discovery" program (PAR-13-364). We will also submit an exploratory R21 application to NIH to investigate if any of the identified compounds have inhibitory effects on other picornaviruses (e.g., coxsackievirus or enterovirus D68).