abstracts submitted). While a diverse group of pharmacophores have shown viral family selectivity, several chemical classes have shown activity across multiple viral families. Conversely, some hits identified early in the screening process as active against multiple viral families have progressed into subseries with greater selectivity and increased potency. We hypothesized that key recognition features necessary for antiviral activity in cell culture live virus assays were present in a good number of our early hits and likely targeted host factors. Thus it might be possible to bypass the need for plate screening to identify initial active small molecules for additional viral families. We tested this hypothesis on members of two unrelated viral families: rabies virus (RABV), a member of the Rhabodviridae (a single strand negative polarity RNA virus with a bullet-shaped capsid assembled in the cytoplasm) and Monkey pox (MPXV), a member of the Poxviridae (a double stranded DNA virus whose capsid forms in the cytoplasm). A subset of Prosetta's active antiviral pharmacophores were screened for effects on capsid assembly as measured by velocity sedimentation. Several hits were achieved for each of these viral families, and a significant number were found to be active against live virus in cell culture in each case. ELISA-based plate screens were established for RABV and MPXV and successfully validated the same hits. These findings demonstrate the versatility of CFPS-based drug screening, extend its reach to two more viral families, and suggest a novel strategy for management of emerging or engineered (bioweapon) viral threats.

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### Cell-Free Protein Synthesizing Systems As Tools for Discovery of Drugs Inhibiting Viral Capsid Assembly

Vishwanath R. Lingappa[1,*], Emma Harrell[1], Kiel Copeland[1], M. Dharma Prasad[2], Vinod Asundi[1], Nick Hahner[1], Jean Francis[1], Debendranath Dey[1], Jennifer Welsh[1], Amanda Macieik[1], Jaisri R. Lingappa[3], Colm Kelleher[1], Clarence R. Hurt[1], William Hansen[1]

[1] *Prosetta Bioconformatics, Inc, San Francisco, USA;* [2] *Prosetta Bioconformatics Pvt Ltd, Mysore, India;* [3] *Department of Global Health, University of Washington, Seattle, USA*

Cell-free protein synthesis (CFPS) successfully reconstitutes transient protein–protein interactions difficult to detect in the crowded environment of living cells further complicated by downstream events (e.g. post-translational modifications and metabolism). Thus, CFPS allows a select subset of events to be studied in a more biochemically tractable manner. Upon CFPS programmed by mRNA encoding capsid protein(s), structures resembling viral capsids are formed as corroborated by biochemical, biophysical and electron microscopic analysis, first for Hepatitis B virus, and subsequently for HIV and Hepatitis C virus. We have extended the approach to 17 of the 23 families of viruses causing human disease. In each case, the capsid protein(s) assemble into high molecular weight structures via viral family-distinctive pathways. In some cases, subcellular fractions derived from mammalian tissues drive capsid protein-associated assembly events. These features can be accentuated by manipulation of conditions under which synthesis vs. assembly are conducted, for some viral families. Eight of these viral family-specific pathways have been converted into moderate throughput drug screens in a 384 well ELISA format, and have been used to screen all or part of a library of over 80,000 compounds conforming to Lipinski's rules. Multiple distinct chemical classes or pharmacophores MW < 500 Da have been identified that block putative steps of host-catalyzed capsid-related complex formation. Activity of many of these compounds have been demonstrated against live virus in cell culture for 10 viral families (see other abstracts being presented). In several cases hits have been successfully advanced to pre-lead status with enhanced potency and moderation of toxicity, and are being used for target identification and dissection of mechanism. In conclusion, an important new anti-viral drug discovery platform has been established and validated.

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### A Chemoenzymatic Synthesis of Carbocyclic Nucleosides and Nucleotides

Miriam Mahler[1,*], Bastian Reichardt[1], Jan Balzarini[2], Chris Meier[1]

[1] *Organic Chemistry, Department of Chemistry, Faculty of Sciences, University of Hamburg, Hamburg, Germany;* [2] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Leuven, Belgium*

In the last decades carbocyclic nucleosides attracted much attention due to their interesting antiviral activity. For example abacavir and carbovir were found to be potent inhibitors of the HIV reverse transcriptase. Entecavir was shown to be an anti-HBV agent, but also possesses activity against HIV, HSV-1, VZV and Influenza. To be antivirally active the nucleosides need to be phosphorylated intracellularly into their triphosphate metabolites in order to be incorporated into the growing DNA strand. Advantages of the carbocyclic compounds are higher stability against enzymatic degradation due to the replaced oxygen atom. Therefore, they are more flexible which should allow a higher rate of phosphorylation in contrast to natural nucleosides.

Here, we present a new and efficient chemoenzymatic synthetic strategy to carbocyclic nucleosides. Starting from cyclopentadiene it is possible to synthesize racemic 3-benzyloxymethylcyclopent-3-enol which can be used in a chemoenzymatic resolution using Pancreatin. The enantiomerically pure cyclopentenol precursors were converted into different 3′,4′-cyclopentenyl nucleosides by a modified Mitsunobu reaction (Ludek and Meier, 2005). In addition, the modification of the 3′,4′-double bond of the carbocyclic moiety can lead to further promising carbocyclic nucleosides (Reichardt et al., 2006). Furthermore, several carbocyclic analogues were converted into their mono-, di- and triphosphates. This was achieved by a nucleophilic attack of water or phosphate salts to the corresponding *cyclo*Sal-phosphate triester (Warnecke and Meier, 2009). Antiviral data of nucleoside analogues and phosphate triesters will be presented.

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