SAINT LOUIS UNIVERSITY

PRESIDENT'S RESEARCH FUND (PRF)
GRANT APPLICATION PACKAGE

TYPE OF APPLICATION: New

PRINCIPAL INVESTIGATOR (administrative responsibility):

LAST NAME: Tavis **FIRST NAME: John**

DEPARTMENT: Molecular Microbiology & Immunology SCHOOL/COLLEGE: School of Medicine

CROSS-COLLEGE/SCHOOL COLLABORATIVE PROPOSALS

- to be considered for a $25,001 - $50,000 funding range:

CO-PRINCIPAL INVESTIGATOR:

LAST NAME: Click here to enter text. **FIRST NAME:** Click here to enter text.

DEPARTMENT: Click here to enter text. **SCHOOL/COLLEGE:** Click here to enter text.

CO-PRINCIPAL INVESTIGATOR:

LAST NAME: Click here to enter text. **FIRST NAME:** Click here to enter text.

DEPARTMENT: Click here to enter text. **SCHOOL/COLLEGE:** Click here to enter text.

TITLE OF PROJECT: Biochemical characterization of the HBV RNAseH as a novel drug target

IF APPLICABLE:

This is resubmission # ☐ **of a previous PRF application.**

☐ **Yes** ☒ **No** **This proposal includes expenses for work to be done internationally.**

Section I: Abstract – Provide a lay abstract of no more than 350 Words (with spaces).

Hepatitis B virus (HBV) chronically infects >350 million people and causes >600,000 deaths annually. Antiviral therapy employs nucleoside analogs that inhibit the viral DNA polymerase during reverse transcription of the viral genome. Therapy profoundly suppresses HBV, but only a few percent of patients are cured because the drugs cannot completely eliminate viral replication. The ability of these drugs to actually cure a few patients indicates that they push the virus to the brink of clearance, and this presents an opportunity to cure patients by suppressing HBV just a bit more. Improving therapeutic efficacy will require novel antivirals that act on targets other than the DNA polymerase. This application will lay the groundwork for developing antiviral drugs against HBV's only other enzyme, the RNAseH.

The HBV RNAseH destroys RNA in RNA:DNA heterodulexes, and it is essential for viral reverse trasncription. Anti-RNAseH drugs against the HIV reverse transcriptase have been developed, but screening for HBV RNAseH inhibitors has been impossible because no active purified form of the HBV RNAseH exists. *Our lab just surmounted this obstacle by purifying active recombinant HBV RNAseH from E. coli.* Therefore, we will perform basic biochemical and genetic characterizations of the HBV RNAseH as the first step toward developing an antiviral drug screen.

Aim 1: Characterize the biochemical activity of the HBV RNAseH. We will systematically vary the salt type/concentration, pH, divalent cation type/concentration, temperature, and substrate in RNAseH reactions to identify optimal conditions for drug screening.

Aim 2: Determine how HBV's high genetic diversity affects its RNAseH activity. We will characterize RNAseH variants from each of HBV's 8 genotypes to determine if functional variation among the genotypes must be accommodated during drug development.

Aim3: Evaluate anti-HIV RNAseH drug candidates for anti-HBV activity. We will test 7 anti-HIV RNAseH drug candidates against the HBV RNAseH to determine if drugs developed against HIV may work against HBV, in analogy to the cross-inhibition of the HIV and HBV DNA polymerases by some nucleoside analogs.

These studies will directly support 4 external funding strategies to develop an antiviral screen and explore the structure/function relationships within the HBV RNAseH.

Section II: Introduction (FOR RESUBMISSIONS ONLY) – 1-page limit.

Section III: Specific Aims and Research Plan – Type or copy and paste a description of your specific aims and research plan. You may include graphs and tables, but they must fit within the 3-page limit. For cross-college applications, please explain why and how the particular disciplines contribute synergistically to the project

Specific Aims

Hepatitis B virus (HBV) is a DNA virus that replicates by reverse transcription (1). It chronically infects >350 million people and causes >600,000 deaths annually. Antiviral therapy primarily employs nucleoside/ nucleotide analog drugs. These drugs profoundly suppress HBV titres in most patients (often 10^5-fold), but only a few percent of patients are cured because the drugs cannot completely suppress viral replication (1-3).

The nucleos(t)ide analogs are all chemically similar prodrugs that share import and activation mechanisms. Their failure to clear HBV is due to limitations to their maximal inhibitory potential (they must compete with natural dNTPs), plus limitations to their import/activation rates and halflives. The net effect is that nucleos(t)ide therapy is life-long, with high ongoing costs and unpredictable long-term side effects for the patients. Importantly, the profound suppression of HBV induced by the nucleos(t)ide analogs and their ability to actually cure a few patients indicate that these drugs routinely push the virus to the brink of elimination. *This presents a huge opportunity to cure patients by developing inhibitors that can suppress HBV just a bit more and clear it from infected patients.* This will require novel antivirals that act on targets other than the DNA polymerase active site and that are under a different set of pharmacological constraints than the nucleos(t)ide analogs. *This application seeks to take advantage of a recent breakthrough our lab to lay the groundwork for developing antiviral drugs against the HBV RNAseH activity*.

RNAseH enzymes destroy RNA when it is in a heteroduplex with DNA. This activity is essential for HBV replication because it exposes the minus-strand DNA to permit second-strand DNA synthesis during reverse transcription (1). The RNAseH is located at the C-terminus of the multi-domain HBV reverse transcriptase protein. This protein also contains HBV's only other known enzymatic activity, the DNA polymerase activity that is targeted by the nucleos(t)ide analogs. The RNAseH activity of the HIV reverse transcriptase has attracted significant attention as a drug target (4-12). However, screening for HBV RNAseH inhibitors will be complicated because our work has revealed that the native HBV enzyme cannot engage exogenous RNAseH substrates (13), and no reproducible recombinant form of the HBV RNAseH enzyme has been developed. As a result, the HBV RNAseH is uncharacterized and there is no recombinant enzyme suitable for antiviral drug screening. **Importantly, our lab has just surmounted this obstacle by purifying active recombinant HBV RNAseH from *E. coli*.** Therefore, this applications seeks to perform the primary biochemical and genetic characterizations of the HBV RNAseH in preparation for antiviral drug screening.

Aim 1: Characterize the biochemical activity of the HBV RNAseH. We will systematically vary the salt type/concentration, pH, divalent cation type/concentration, temperature, and substrate in RNAseH reactions to identify the proper conditions for subsequent drug screening.

Aim 2: Determine how HBV's high genetic diversity affects its RNAseH activity. We will express RNAseH variants from each of HBV's 8 genotypes and evaluate their biochemical profiles to determine if biochemical variation among the genotypes must be accommodated during drug development.

Aim3: Evaluate anti-HIV RNAseH drug candidates for anti-HBV activity. We have 7 anti-RNAseH drug candidates developed against HIV. We will test these drugs against the HBV RNAseHs to determine if they work against HBV, in analogy to the cross-inhibition of the HIV and HBV DNA polymerase activities by some nucleos(t)ide analogs.

Research Plan

Preliminary Data

In vivo RNAseH assay. All known RNAseH enzymes need a carboxylate tetrad called the "DEDD" motif to coordinate essential divalent cation(s) (5, 14-17). These residues are predicted to be D702, E731, D750, and D790 in HBV. We mutated these residues in the HBV genome to test if they were essential. The mutant genomes were transfected into Huh7 cells, viral particles were purified, and the HBV DNAs were detected by Southern analysis. The signature of an RNAseH-deficient enzyme is production of RNA:DNA heteroduplexes

that migrate as double-stranded DNAs on native gels but as single-stranded DNAs of multiple lengths following digestion of the RNA. Wild-type DNAs were unaffected by RNAse treatments, but all 4 mutations led to accumulation of RNA:DNA heteroduplexes whose mobility increased upon RNAse treatment, indicating that the mutations impaired the RNAseH activity (Fig. 1). *This assay will not be used here, but it is presented to demonstrate that we have an in vivo assay available to permit coordinated in vitro/in vivo drug screening*.

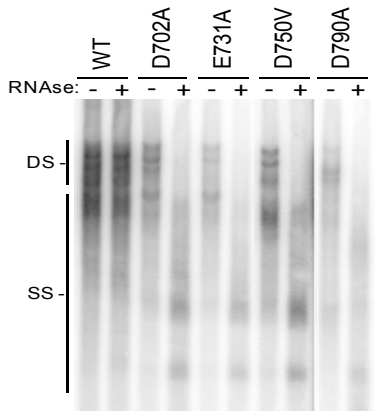

Fig. 1. Identification of the HBV RNAseH active site residues. HBV DNAs synthesized by DEDD-mutant enzymes were analyzed by Southern blot. RNAseH-deficiency is indicated by an increase in DNA mobility when treated with RNAse prior to electrophoresis.

Expression and purification of the HBV RNAseH. Five reports of *in vitro* HBV RNAseH activity have been published (13, 18-21). These reports are unconvincing because the reported activity was weak, controls excluding contaminating RNAseHs were inadequate, and none of them were ever confirmed. Based on the construct employed in the most plausible report (21) and molecular modeling by our collaborator Dr. Rajeev Aurora, we designed a hexa-histidine tagged version of the HBV RNAseH and its D750V active-site knockout for expression in *E. coli*. Surprisingly given our many previous failures since 1992, these enzymes were soluble and could be purified by nickel-affinity chromatography under native conditions (Fig. 2). Further purification by collaborators in Dr. Enrico Di Cera's lab revealed that the RNAseH could be highly purified with only minimal processing beyond nickel-affinity chromatography (Fig. 2 right lane).

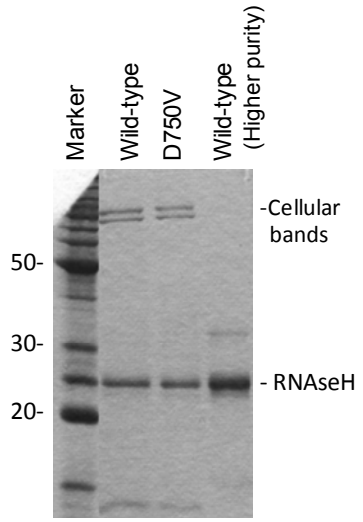

Fig. 2. Purification of recombinant HBV RNAseH. Proteins were purified by nickel-affinity chromatography; lane 3 shows enzyme further purified by ion-exchange chromatography in the Di Cera laboratory.

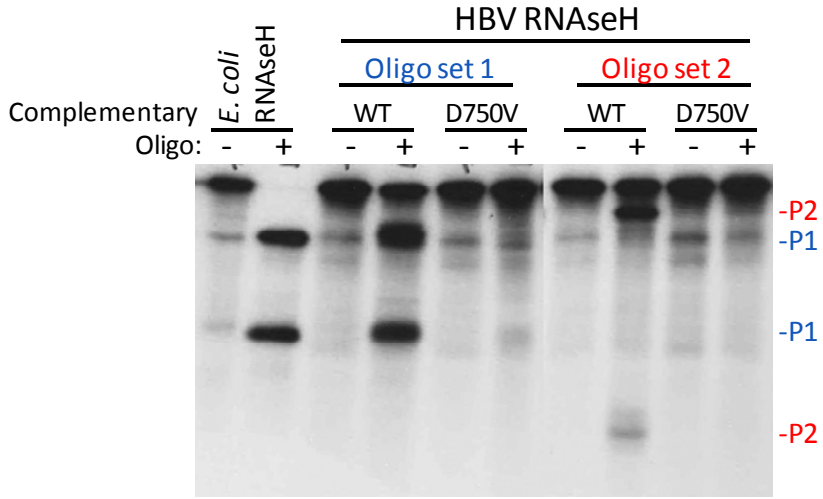

Fig. 3. *In vitro* RNAseH assays. The assay shows cleavage of a uniformly-[^{32}P]labeled RNA at the site where it is annealed to a DNA oligonucleotide. *E. coli* RNAseH was a positive control. Products from oligonucleotides that annealed at different spots on the RNA are shown. P1, products from oligo set 1; P2, products from oligo set 2.

In vitro RNAseH assays. Purified HBV RNAseHs were incubated with uniformly-[^{32}P]labeled RNA and either a complementary DNA oligonucleotide to create a defined RNA:DNA heteroduplex, or with a non-complementary oligonucleotide as a control. The reaction products were resolved by electrophoresis and detected by autoradiography. The wild-type RNAseH gave cleavage products of the predicted size for each oligo set,

whereas reactions with the D750V enzyme yielded only background products (Fig. 3). *This demonstrates robust, bona fide HBV RNAseH activity in the wild-type recombinant HBV RNAseH.*

Aim 1. Characterize the biochemical activity of the HBV RNAseH. We will systematically characterize the reaction conditions for the HBV RNAseH to identify optimal conditions for subsequent drug screening. We will vary the monovalent cation (Na vs. K), monovalent cation concentration (0-500 mM), pH (5.5-8.5), divalent cation type (Mg, Mn, Ca, Zn), divalent cation concentration (0-15 mM), temperature (20-50^{o}C), and the length of the DNA:RNA heteroduplex (10-100 bp). Once single-variable studies have been completed, combinations of conditions will be evaluated to ensure we have identified the optimum. No difficulties are expected because the assay is functioning in the lab (Fig. 3). We anticipate identifying optimal conditions within a typical physiological range that would be suitable for high through-put screening.

Aim 2: Determine how HBV's high genetic diversity affects its RNAseH activity. HBV has 8 genotypes (A through H) that differ genetically by >8%. Therefore, we will employ gene synthesis to create analogs of our current RNAseH expression vector for all 8 genotypes. The biochemical activities of these enzymes will be evaluated based on the patterns found in Aim 1 to determine if there is biochemical variation among the genotypes that will need to be accommodated during drug development. We cannot predict the outcome of these experiments because HBV's diversity is easily large enough to affect its enzymatic activity.

Aim3: Evaluate anti-HIV RNAseH drug candidates for anti-HBV activity. HBV and HIV both depend on virally-encoded RNAseHs during reverse transcription. Many candidate RNAseH inhibitors have been developed for HIV (4-12). If these drugs also work against HBV, it would greatly accelerate antiviral drug development. There is precedence for such cross-inhibition because anti-HBV nucleos(t)ide drugs were originally developed against HIV and then screened against HBV. We have 7 anti-HIV RNAseH drug candidates in the lab that were obtained through a collaboration with Dr. Stefan Sarafianos at the University of Missouri. One of these drugs inhibits HBV in the *in vivo* assay shown in Fig. 1. Therefore, we will determine the IC_{50} *in vitro* for each of these drugs against the HBV RNAseHs from all genotypes displaying different activity profiles in Aim 1. This will reveal if drugs developed against HIV may work against HBV, if there are differences in enzymatic sensitivity *in vivo* and *in vitro,* and if the drugs could be effective against all HBV strains in circulation. We anticipate that the drug that works *in vivo* will also work *in vitro*, and that some of the others may also work *in vitro* because the additional constraints imposed by the cells are absent in a test tube.

Significance & Impact of Research

HBV is a hepatotropic virus that chronically infects over 350 million people world-wide. It causes chronic hepatitis, cirrhosis, and liver failure, with male HBV carriers having a ~40% lifetime risk of dying from the infection (2, 22). HBV is also the largest cause of liver cancer globally (23), and about 20% of the 600,000-1,000,000 people who die from HBV each year succumb to virally-induced hepatocellular carcinoma.

The nucleos(t)ide analogs used against HBV are wonderful drugs, but they cannot quite eradiate the virus. This presents an enormous opportunity to use a second drug against a different viral target in combination with the nucleos(t)ide analogs in order to eliminate the virus from infected patients. The obvious target for a second drug is HBV's only other enzymatic activity, the RNAseH. However, inability to produce active recombinant HBV RNAseH has prevented high through-put drug screening. Our recent breakthrough in expressing recombinant HBV RNAseH with robust *in vitro* activity has removed this roadblock. Therefore, we are ready to begin developing a screen for HBV RNAseH drugs, and in this PRF application we will perform the basic biochemical characterization needed for this process. Importantly, we have preliminary data indicating that some of the anti-RNAseH drugs developed for HIV may also work against HBV. Many RNAseH inhibitors have been developed for HIV that could be screened against HBV (4-12), so much of the complex medicinal chemistry needed to target an RNAseH enzyme has already been done. Therefore, the time is ripe to attack the second essential enzymatic activity of the HBV reverse transcriptase. Successful identification of an anti-HBV RNAseH drug may finally provide a cure to the hundreds of millions of people whose lives are being destroyed by this devastating virus.

Click here to enter text.

References

1. Seeger C, Zoulim F, Mason WS. Hepadnaviruses. In: Knipe DM, Howley P, Griffin DE, Lamb RA, Martin MA, Roizman B, et al., eds. Fields Virology. 5 ed. Philadelphia: Lippincott Williams & Wilkins, 2007. 2977-3029.
2. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. N Engl J Med 2004 Mar 11;350(11):1118-1129.
3. Zoulim F. Antiviral therapy of chronic hepatitis B. Antiviral Res 2006 Sep;71(2-3):206-215.
4. Klarmann GJ, Hawkins ME, Le Grice SF. Uncovering the complexities of retroviral ribonuclease H reveals its potential as a therapeutic target. AIDS Rev 2002 Oct;4(4):183-194.
5. Klumpp K, Hang JQ, Rajendran S, Yang Y, Derosier A, Wong K, I, et al. Two-metal ion mechanism of RNA cleavage by HIV RNase H and mechanism-based design of selective HIV RNase H inhibitors. Nucleic Acids Res 2003 Dec 1;31(23):6852-6859.
6. Klumpp K, Mirzadegan T. Recent progress in the design of small molecule inhibitors of HIV RNase H. Curr Pharm Des 2006;12(15):1909-1922.
7. Takada K, Bermingham A, O'Keefe BR, Wamiru A, Beutler JA, Le Grice SF, et al. An HIV RNase H inhibitory 1,3,4,5-tetragalloylapiitol from the African plant Hylodendron gabunensis. J Nat Prod 2007 Oct;70(10):1647-1649.
8. Bokesch HR, Wamiru A, Le Grice SF, Beutler JA, McKee TC, McMahon JB. HIV-1 ribonuclease H inhibitory phenolic glycosides from Eugenia hyemalis. J Nat Prod 2008 Sep;71(9):1634-1636.
9. Wendeler M, Lee HF, Bermingham A, Miller JT, Chertov O, Bona MK, et al. Vinylogous ureas as a novel class of inhibitors of reverse transcriptase-associated ribonuclease H activity. ACS Chem Biol 2008 Oct 17;3(10):635-644.
10. Fuji H, Urano E, Futahashi Y, Hamatake M, Tatsumi J, Hoshino T, et al. Derivatives of 5-nitro-furan-2-carboxylic acid carbamoylmethyl ester inhibit RNase H activity associated with HIV-1 reverse transcriptase. J Med Chem 2009 Mar 12;52(5):1380-1387.
11. Su HP, Yan Y, Prasad GS, Smith RF, Daniels CL, Abeywickrema PD, et al. Structural basis for the inhibition of RNase H activity of HIV-1 reverse transcriptase by RNase H active site-directed inhibitors. J Virol 2010 Aug;84(15):7625-7633.
12. Di GM, Olson M, Prashad AS, Bebernitz G, Luckay A, Mullen S, et al. Small molecule inhibitors of HIV RT Ribonuclease H. Bioorg Med Chem Lett 2010 Jan 1;20(1):398-402.
13. Gong Y, Yao E, Tavis JE. Evidence that the RNAseH activity of the duck hepatitis B virus is unable to act on exogenous substrates. BMC Microbiology 2001;1:12.
14. Nowotny M, Gaidamakov SA, Crouch RJ, Yang W. Crystal structures of RNase H bound to an RNA/DNA hybrid: substrate specificity and metal-dependent catalysis. Cell 2005 Jul 1;121(7):1005-1016.
15. Yang W, Steitz TA. Recombining the structures of HIV integrase, RuvC and RNase H. Structure 1995 Feb 15;3(2):131-134.
16. Keck JL, Goedken ER, Marqusee S. Activation/attenuation model for RNase H. A one-metal mechanism with second-metal inhibition. J Biol Chem 1998 Dec 18;273(51):34128-34133.

17. Goedken ER, Marqusee S. Co-crystal of Escherichia coli RNase HI with Mn2+ ions reveals two divalent metals bound in the active site. J Biol Chem 2001 Mar 9;276(10):7266-7271.

18. Wei X, Peterson DL. Expression, purification, and characterization of an active RNase H domain of the hepatitis B viral polymerase. J Biol Chem 1996 Dec 20;271(51):32617-32622.

19. Lee YI, Hong YB, Kim Y, Rho HM, Jung G. RNase H activity of human hepatitis B virus polymerase expressed in Escherichia coli. Biochem Biophys Res Commun 1997 Apr 17;233(2):401-407.

20. Choi J, Kim EE, Park YI, Han YS. Expression of the active human and duck hepatitis B virus polymerases in heterologous system of Pichia methanolica. Antiviral Res 2002 Aug;55(2):279-290.

21. Potenza N, Salvatore V, Raimondo D, Falanga D, Nobile V, Peterson DL, et al. Optimized expression from a synthetic gene of an untagged RNase H domain of human hepatitis B virus polymerase which is enzymatically active. Protein Expr Purif 2007 Sep;55(1):93-99.

22. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. Epidemiol Rev 2006;28:112-125.

23. Nguyen VT, Law MG, Dore GJ. Hepatitis B-related hepatocellular carcinoma: epidemiological characteristics and disease burden. J Viral Hepat 2009 Jul;16(7):453-463.

Section V: Future Extramural Funding: How will data from the PRF award be used in a subsequent extramural application? Identify potential sponsors and a timeline if possible. 1-page limit.

Future Extramural Funding

Four funding avenues will be pursued once we have the data to be generated through this PRF project.

1. Develop an antiviral screen for the HBV RNAseH. The immediate goal of this project is to support development of a screen for anti-HBV RNAseH drugs. This PRF study will identify the correct reaction conditions, the range of HBV genotypes against RNAseH screening must be done, and whether we can take advantage of the previously conducted screens against the HIV RNAseH to guide selection of the chemical library to be employed. The screen design efforts will be supported by an NIH R01 project under PA-10-213 *Development of Assays for High-Throughput screening for use in Probe and Pre-therapeutic Discovery (R01)*. The key steps are:

1. Adopt the fluorescence-based RNAseH assay developed for HIV as described in (21) to permit rapid and sensitive detection during robotic screening.
2. Miniaturize the assay to a 96-well or 384-well plate format, and establish the reproducibility parameters for the assay to enable definition of a cutoff for a "hit" in the assay.
3. Conduct a proof-of-principle screen using a small chemical library.

Collaborators with expertise in drug screening will be involved in this process. The leading candidates are scientists within SLU's Center for World Health and Dr. Stefan Sarafianos at the University of Missouri (Dr. Sarafianos is an established collaborator who provided the anti-HIV RNAseH drugs).

2. Determine the 3-dimensional structure of the HBV RNAseH domain. There is no structural information available for the HBV reverse transcriptase, including the RNAseH domain. These PRF studies lay the groundwork for structure-based analyses of the RNAseH enzymatic mechanism and the mechanism by which the RNAseH is inhibited by anti-RNAseH compounds. Therefore, we have started a collaboration Dr. Enrico Di Cera's structural biology group in the SLU Biochemistry department. They have purified the HBV RNAseH protein to adequate purity and quantity for crystallization trials (Fig. 2). Once a crystal that diffracts to adequate resolution is obtained, we will submit an R01 application to determine the crystal structure of the enzyme and to conduct *in vitro* and *in vivo* mechanistic studies on the enzyme. This collaborative project will take advantage of Dr. Di Cera's deep understanding of structural biology and Dr. Tavis' 20-years experience with the HBV reverse transcription mechanism and biochemistry of the reverse transcriptase.

3. Explore the structural and enzymatic parallels between the HBV and HIV RNAseHs. Dr. Aurora designed 2 HBV:HIV chimeric RNAseH enzymes as part of his molecular design efforts that contributed to our successful production of active recombinant HBV RNAseH. Both of these chimeras retain at least some RNAseH activity despite the lack of amino acid homology between HBV and HIV in the sequences that were inserted into the HBV enzyme. This indicates that the sequences probably form similar secondary structures that are functionally interchangeable. This presents a good opportunity to study the mechanism, structure, and protein-folding parameters leading to production of a functional enzyme. These parameters will be explored in collaboration with Drs. Aurora and Di Cera through the NIH R21 mechanism.

4. Seek a commercial partner for drug development. The commercial potential of a potent anti-HBV RNAseH drug would be enormous given that there are >350,000,000 chronically-infected people in the world. Therefore, as soon as we have established data supporting feasibility of a screening assay and solidified our intellectual property protections, we will seek a commercial partner with the expertise and resources to develop a drug. The immediate commercial potential stemming from this project would be the licensing and royalty fees to be obtained upon transfer of the screening technology to a pharmaceutical company for high-throughput compound library screening. However, if our preliminary studies identify potential drugs or promising chemical leads for subsequent chemical modification, the royalties would extend to the drugs themselves, with commiserate financial potential for the University.

Section VI: Budget – Enter funds requested for each category. Do not enter special characters (e.g. $#, decimal points). Requests cannot exceed $25,000, unless they are for collaborative applications, as described on page 1 of the PRF guidelines.

Salaries	**$ 7,500**
Fringe Benefits	**$ 2,500**
Supplies	**$ 13,000**
Minor Equipment	**$ 500**
Travel	**$ 1,500**
Consultants	**$ 0**
Other	**$**
GRAND TOTAL:	**$ 25,000**

Section VII: Budget Justification – For each budget category above, itemize all expenses, using Banner account codes on page 4 of the PRF Guidelines. Explain in your narrative how the calculations were derived. 1-page limit.

Budget Justification

John Tavis, Ph.D. (P.I.), 10% effort; no salary support. Dr. Tavis will direct all aspects of this study and will directly supervise Ms Cheng.

NAME B.S. (Research Assistant), ##% effort. (641000 and 690000) NAME is a research technician in the Tavis lab who will perform all of the laboratory work under Dr. Tavis' supervision.

Supplies. (752000) Funds are sought for standard lab supplies (tips, tubes, gloves, chemicals, etc.; all at RNAse-free grade), gene synthesis of 7 HBV RNAseH genes (7 genes X 600 nt X $0.35/nt = $1470), [$\alpha^{32}$P]UTP and [$\gamma^{32}$P]ATP for labeling RNA and RNA oligonucleotides (14 X $111 = $1554), Ambion Megascript RNA synthesis kits, DNA and RNA oligonucleotides (for substrate generation/exploration), electrophoresis reagents, autoradiography film, etc.

Minor Equipment. (757300) Funds are sought to purchase a controlled-temperature water bath because preliminary studies have indicated the optimal temperature for the RNAseH reactions is near 42^{o}C. We do not have a spare precision waterbath that could be routinely set to this unusual temperature, and we will need an additional unit permanently set to the optimal reaction temperature for reproducibility and efficiency of these studies.

Travel. (731000) Funds are sought to support travel for Dr. Tavis to the annual Molecular Biology of the Hepatitis B Viruses meeting to report results from this study. This is the most important HBV meeting each year, and Dr. Tavis sits on its governance committee.

Consultants. None.

Other. (741002) Funds are sought to support publication of the data to be generated in this study.

Section VIII: Reviewers – Please provide the names and email addresses of at least 5 internal reviewers who have the expertise to deliver a quality review of this proposal. To avoid conflicts of interest, please do not provide the name of your department chair, or anyone who is a co-investigator on your current, active grant award (s).

Reviewer 1:

Reviewer 2:

Reviewer 3:

Reviewer 4:

Reviewer 5:

- **Please save this document and combine it with a two (2) page biosketch as described in the PRF guidelines, with a summary statement (for resubmissions), letter(s) of support (required for all co-principal investigators on collaborative proposals).**
- **Combine all documents into one PDF file, named as follows: PILastName_PIFirstName_DeadlineMonthDeadlineYear (Example: Tait_Raymond_May2011).**
- **Upload to eRS using the Internal Proposal function.**