PI: **Dewhurst, Stephen**	Title: The Semen Enhancer of HIV Infection as a Novel Microbicide Target	
Received: 07/09/2010	FOA: AI10-011	Council: 01/2011
Competition ID: ADOBE-FORMS-B	FOA Title: MICROBICIDE INNOVATION PROGRAM (MIP VI) (R21/R33)	
1 R21 AI094511-01	Dual: MH,OD	Accession Number: 3315565
IPF: 7047101	Organization: UNIVERSITY OF ROCHESTER	
Former Number:	Department: Microbiology & Immunology	
IRG/SRG: ZAI1 RB-A (J1)	AIDS: Y	Expedited: Y
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted] Year 2: [redacted] Year 3: [redacted] Year 4: [redacted] Year 5:	Animals: N Humans: Y Clinical Trial: N Current HS Code: 30 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Stephen Dewhurst Ph.D.	University of Rochester	PD/PI
Changyong Feng	University of Rochester	Faculty
Jerry Yang Ph.D	University of California, San Diego	Other (Specify)-Co-Investigator Subcontractor
[redacted]	[redacted]	Other (Specify)-Co-Investigator subcontractor
Charlene Dezzutti Ph.D	University of Pittsburgh	Consultant

OMB Number: 4040-0001
Expiration Date: 06/30/2011

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE: | **State Application Identifier:**

1. * TYPE OF SUBMISSION
☐ Pre-application ☐ Application ☒ Changed/Corrected Application

4. a. Federal Identifier: [redacted]

b. Agency Routing Identifier:

2. DATE SUBMITTED:

Applicant Identifier: NIHR21DewhurstMicrobicide

5. APPLICANT INFORMATION

*** Organizational DUNS:** [redacted]

* Legal Name: University of Rochester

Department: ORPA | Division:

* Street1: Office of Research and Project Administration

Street2: 518 Hylan Building

* City: Rochester | County / Parish:

* State: NY: New York | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 146270140

Person to be contacted on matters involving this application

Prefix: | * First Name: Brenda | Middle Name:

* Last Name: Kavanaugh | Suffix:

* Phone Number: [redacted] | Fax Number: [redacted]

Email: [redacted]

6. * EMPLOYER IDENTIFICATION *(EIN) or (TIN):* [redacted]

7. * TYPE OF APPLICANT: O: Private Institution of Higher Education

Other (Specify):

Small Business Organization Type ☐ Women Owned ☐ Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
☒ New ☐ Resubmission
☐ Renewal ☐ Continuation ☐ Revision

If Revision, mark appropriate box(es).
☐ A. Increase Award ☐ B. Decrease Award ☐ C. Increase Duration ☐ D. Decrease Duration
☐ E. Other (specify):

* Is this application being submitted to other agencies? Yes ☐ No ☒ What other Agencies?

9. * NAME OF FEDERAL AGENCY: National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

TITLE:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
The Semen Enhancer of HIV Infection as a Novel Microbicide Target

12. PROPOSED PROJECT:
* Start Date: 03/01/2011 | * Ending Date: 02/29/2016

*** 13. CONGRESSIONAL DISTRICT OF APPLICANT:** NY-028

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: | * First Name: Stephen | Middle Name:

* Last Name: Dewhurst | Suffix: Ph.D.

Position/Title: Professor and Chair

* Organization Name: University of Rochester

Department: Microbiology & Immunology | Division: School of Medicine & Dentistry

* Street1: 601 Elmwood Avenue, Box 672

Street2:

* City: Rochester | County / Parish:

* State: NY: New York | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 14642-0672

* Phone Number: [redacted] | Fax Number:

* Email: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested	[redacted]
b. Total Non-Federal Funds	0.00
c. Total Federal & Non-Federal Funds	[redacted]
d. Estimated Program Income	0.00

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

a. YES ☐ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ☒ PROGRAM IS NOT COVERED BY E.O. 12372; OR

☐ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious. or fraudulent statements or claims may subject me to criminal, civil, or administrative penalities. (U.S. Code, Title 18, Section 1001)

☒ *** I agree**

**** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.***

18. SFLLL or other Explanatory Documentation

Add Attachment | Delete Attachment | View Attachment

19. Authorized Representative

Prefix: | * First Name: Tammie | Middle Name:

* Last Name: Martin | Suffix:

* Position/Title: Research Administrator

* Organization: University of Rochester

Department: ORPA | Division:

* Street1: Office of Research and Project Administration

Street2: 518 Hylan Building

* City: Rochester | County / Parish:

* State: NY: New York | Province:

* Country: USA: UNITED STATES | * ZIP / Postal Code: 14627-0140

* Phone Number: [redacted] | Fax Number: [redacted]

* Email: [redacted]

*** Signature of Authorized Representative**: Tammie Martin

*** Date Signed**: 07/09/2010

20. Pre-application Add Attachment | Delete Attachment | View Attachment

424 R&R and PHS-398 Specific Table Of Contents

OMB Number: 4040-0010
Expiration Date: 08/31/2011

Project/Performance Site Location(s)

Project/Performance Site Primary Location ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of Rochester
DUNS Number: [redacted]
* Street1: UR Medical Center
Street2: 601 Elmwood Avenue
* City: Rochester County:
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 14642-0672 * Project/ Performance Site Congressional District: NY-028

Project/Performance Site Location 1 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: University of California, San Diego
DUNS Number: [redacted]
* Street1: Office of Contract and Grant Administration
Street2: 9500 Gilman Drive #093
* City: La Jolla County:
* State: CA: California
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 92093-0934 * Project/ Performance Site Congressional District: CA-053

Project/Performance Site Location 2 ☐ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: [redacted]
DUNS Number: [redacted]
* Street1: [redacted]
Street2:
* City: [redacted] County:
* State: [redacted]
Province:
* Country: [redacted]
* ZIP / Postal Code: [redacted] * Project/ Performance Site Congressional District: [redacted]

Performance Sites

Additional Location(s) Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Other Project Information

1. * Are Human Subjects Involved? ☒ Yes ☐ No

1.a If YES to Human Subjects

Is the Project Exempt from Federal regulations? ☐ Yes ☒ No

If yes, check appropriate exemption number. ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6

If no, is the IRB review Pending? ☐ Yes ☒ No

IRB Approval Date: 06/28/2010

Human Subject Assurance Number: [redacted]

2. * Are Vertebrate Animals Used? ☒ Yes ☐ No

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ☒ Yes ☐ No

IACUC Approval Date:

Animal Welfare Assurance Number [redacted]

3. * Is proprietary/privileged information included in the application? ☐ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? ☐ Yes ☒ No

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ☐ Yes ☐ No

4.d. If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? ☐ Yes ☒ No

5.a. If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? ☐ Yes ☒ No

6.a. If yes, identify countries:

6.b. Optional Explanation:

7. * Project Summary/Abstract 1234-PROJSUMM-MIP-070410.pdf Add Attachment Delete Attachment View Attachment

8. * Project Narrative 1235-PROJNARR-MIP-070410.pdf Add Attachment Delete Attachment View Attachment

9. Bibliography & References Cited 1236-BIBLIO-MIP.pdf Add Attachment Delete Attachment View Attachment

10. Facilities & Other Resources 1237-RESENV-MIP-0710.pdf Add Attachment Delete Attachment View Attachment

11. Equipment 1238-MAJEXPT-MIP-0710.pdf Add Attachment Delete Attachment View Attachment

12. Other Attachments Add Attachments Delete Attachments View Attachments ☐

PROJECT SUMMARY/ABSTRACT

Human semen contains cationic amyloid fibrils, termed the "Semen Enhancer of Virus Infection" (SEVI), which strongly enhance HIV-1 infection and may play an important role in viral transmission. Our preliminary data show that amyloid-binding molecules bind to SEVI, and block semen-mediated enhancement of HIV-1 infection. This suggests that (i) SEVI is responsible for semen-mediated enhancement of HIV infection and (ii) SEVI represents a novel microbicide target. We therefore propose to explore a ***novel, innovative approach to HIV-1 microbicide development, using agents that selectively target SEVI***. This high-risk/high-reward approach is fundamentally different from traditional microbicidal strategies that target the virus itself, and is expected to be highly complementary with direct antiviral approaches. Indeed, our long-term goal is to use SEVI-targeting agents in combination with traditional microbicides, to achieve optimal antiviral effectiveness.

In the R21 phase, we will test whether novel amyloid-binding small molecules inhibit semen-mediated enhancement of HIV infection. The feasibility of this approach has been established using two amyloid-binding small molecules which contain “shielding” oligo-ethylene glycol (EG) moieities: BTA-EG_4 and –EG_6. These agents efficiently inhibit SEVI- and semen-mediated enhancement of HIV infection. In Aim 1, we will generate and test novel derivatives of these and other amyloid-binding molecules, including oligovalent molecules that are expected to possess increased SEVI binding affinity. We will then test their ability to inhibit SEVI- and semen- mediated enhancement of HIV infection using a panel of R5 virus strains (including different clades and transmitted strains). In Aim 2, we will examine the interaction between novel amyloid-binding small molecules and cells from the female reproductive tract. We will evaluate whether our compounds are toxic to human cervicovaginal epithelial cells (HCEC), and we will test whether they inhibit SEVI-enhanced binding of HIV-1 to HCEC and/or SEVI-enhanced *trans*-infection of PBMC by HCEC exposed to HIV-1 virions.

The R33 phase will be undertaken only if well-defined milestones are achieved. In Aim 3, we will use structure-activity relationship (SAR) data to refine our chemical compositions. We will also test whether our lead molecules have efficacy in a cervical explant model for HIV-1 infection, and whether they have a synergistic or additive effect on the ability of other candidate microbicides to inhibit HIV-1 infection in the presence of semen. In the final Aim, we will assess the toxicity and inflammatory effects of the most promising candidate molecules, using beneficial *Lactobacilllus* strains and cervical explants. The R33 phase will culminate with an evaluation of the safety and tolerability of the most promising compound in the rabbit vaginal irritation (RVI) model.

The overall goal of these studies is to carefully determine whether small molecules that target SEVI have potential utility as a novel class of microbicides.

PROJECT NARRATIVE

New approaches to prevent the transmission of human immunodeficiency virus type-1 (HIV-1) are urgently needed. This application seeks to develop a new class of microbicidal agents that are targeted not to the virus itself, but to a host protein found in semen that strongly enhances HIV-1 infection. This high risk, high reward approach is fundamentally different from traditional microbicidal strategies that target the virus, and is expected to be highly complementary with direct antiviral approaches.

RESOURCES AND ENVIRONMENT

Dewhurst Lab: University of Rochester

LABORATORY: 1200 sq. ft. of new research space is available to Dr. Dewhurst, with a fully-equiped adjoining BL2+ tissue culture facility (for work with SIV and HIV-1), a cubicle for PCR set-up and dedicated incubator/shaker facilities for bacterial growth. This space is located on the 3rd floor of the University's newly constructed Kornberg Medical Research Building. Additional facilities include extra tissue culture facilities (3 laminar flow hoods), as well as shared electroporation apparatus, luminometer, ELISA plate reader, liquid nitrogen storage, -80 freezers, UV/digital photography suite, two fluorescence microsopes (with digital cameras) and cell harvester. Equipment shared with immediately adjacent labs includes (ultra)centrifuges, two FACS analyzers (FACScan and FACScalibur, owned as a consortium between 8 investigators), a 2D proteomics analyzer (shared with one other lab), a real-time PCR instrument (BioRad iCycler, shared with one other lab), an Alpha-Innotech gel analyzer and a Nucleofector (Amaxa; shared with one other lab).

CLINICAL: The University of Rochester Medical Center (URMC) owns Strong Memorial Hospital, which serves as its teaching hospital. It has well over 600 beds, and is located under the same roof as the URMC. It has a GCRC (General Clinical Research Center), Clinical Research Institute (CRI), Vaccine Testing and Evaluation Unit (VTEU), Cancer Center (URCC) & more.

ANIMAL: Animals are housed and cared for by the facilities and programs of the Vivarium and Division of Laboratory Animal Medicine (DLAM). These facilities are fully accredited by AAALAC and are in compliance with state law, federal statute and NIH policy. DLAM consists of one board certified laboratory animal veterinarian, two clinical laboratory animal veterinarians, a veterinary pathologist & a staff of trained and licensed technicians.

COMPUTER: Dr. Dewhurst has a desktop Macintosh computer and a laser printer available for his personal use. In addition, ten Macintosh personal computers (G5, G4, iMac, iBook), five PCs (Dell) and two networked laser printers are available in Dr. Dewhurst's laboratory; all are connected via T1 line to the internet. Each pre- and post- doctoral fellow has their own computer. In addition, there are portable machines which are used for shared dongled software (Vector NTI, CellQuest, FlowJo). A scanner and densitometric imaging software are available, and a computer/network support staff is available via a central campus facility.

OFFICE: Dr. Dewhurst occupies a 200 sq. ft. office and additional office space is available for fellows and students. The office of the Dept. of Microbiology and Immunology has secretarial and administrative support personnel, available to Dr. Dewhurst.

OTHER: Additional shared facilities include additional light/fluorescent microscope facilities with image capture (MCID camera) and analysis capabilities, as well as image reconstruction software, and a microinjection device for introduction of DNA or proteins into individual cells.

Yang Lab: University of California, San Diego

All laboratory and office space for Prof. Yang are located in Pacific Hall at the University of California, San Diego.

LABORATORY
>4000 sq. ft. total laboratory space is available to Dr. Yang, including the following:

- Wet chemical Laboratories: 6112, 6114, 6115, 6117, 6213 Pacific Hall (3025 sq. ft.)
- Shared analytical Laboratory: 6015 Pacific Hall (603 sq. ft.)
- Tissue Culture Facilities: 6019 Pacific Hall (382 sq. ft.)
- Shared hazardous chemicals laboratory: 6020A Pacific Hall (150 sq. ft.)

CLINICAL:
Not applicable to the UCSD component of this research program

ANIMAL:
Not applicable to the UCSD component of this research program

COMPUTER:
Three PC to control chromatography equipment, one PC to control a bilayer setup, two PCs to control UV-Vis and Fluorescence microplate readers, two PC's to control UV-Vis and Fluorescence spectrophotometers, one PC to control an FTIR spectrometer, one PC to run molecular modeling software, two PCs for general student use, one PC in the office of the PI.

OFFICE

- Office for PI: 6100C Pacific Hall (129 sq. ft.)
- Shared student office/lounge: 6100 Pacific Hall (386 sq. ft.)
- Secretarial services are also available, including a Fiscal administrator and a Personal administrator

OTHER
Other departmental and University resources (access through recharge):

- NMR (300 MHz, two 400 MHz, two 500 MHz)
- Small molecule and biomolecular mass spec facility (APCI, EI, ESI, Hires-FAB, MALDI-TOF, GC-MS, LC-MS)
- Transmission Electron and Scanning Probe, Deconvolution and Confocal Microscopy facilities (NIH-funded National Center for Microscopy and Imaging Research, UCSD School of Medicine)
- Electronics and Machine shops
- Experts for computers and networking

[redacted]

LABORATORY: [redacted] has over 42,000 sq ft of dedicated BSL-2+ laboratory animal space and over 8000 sq ft of laboratory space. Currently, the laboratories are involved with HIV/SIV and other viral disease of humans. [redacted] OSHA and BMBL regulations. All laboratories are equipped with typical equipment needed for tissue culture and molecular biology, such as SterilGARD, The Baker Company, 6 ft. biological safety cabinets class II, Forma scientific water-jacketed incubators, MWG Biotech Primus 96 Plus thermocyclers, Ultraspec 2000 spectrophotometer, Mettler Toledo Fine Scale XS104, Corning pH meter 440, Hoefer DNA Fluorometer Model TKO100, Sorvall Legend RT+ centrifuges, standard waterbath at 37ºC or 56ºC, minicentrifuges from Fisher Scientific, Hereaus, and Eppendorf, Fininstruments plate washer, Inotech cell harvester, Nikon TMS inverted microscope, Olympus CH microscope, Manitowec Ziegro ice maker, chemical fume hood, Multiskan Ascent ELISA reader, Beckman Avant 30 centrifuge, and Lab-Line incubator shaker model no. 3525. Within the lab area also located are -80ºC and -20ºC freezers, refrigerators, a BD FACS Canto II Flow cytometer and two TAQMAN real-time ABI instruments (7700 and 7500), a Perkin Elmer MicroBeta scintillation counter, a Syngene's G-box.

CLINICAL: N/A

ANIMAL: Animals will be housed at [redacted] Care and husbandry of all non-human primates will be provided in compliance with federal laws and guidelines as well as in accordance with recommendations provided in the NIH guide and other accepted standards of laboratory animal care and use. [redacted] is fully accredited by the Association for Assessment and Accreditation for Laboratory Animal Care International (AAALAC File Number [redacted]). [redacted] Assurance with the Office of Laboratory Animal Welfare (OLAW) is current (Assurance Number [redacted]). [redacted] facilities are registered with and annually inspected by the USDA-APHIS (Registration Number [redacted]). Research at [redacted] is currently conducted in over 70,000 sq. ft. of facilities. Nearly 50,000 sq. ft. of this space is dedicated to facilities that directly or indirectly support animal research in BSL2 and BSL3 containment. The proposed studies will be conducted in the [redacted]. One Board Eligible (American College of Laboratory Animal Medicine) Veterinarian and four certified (AALAS) Veterinary Technicians are on staff at this facility.

COMPUTER: [redacted] is a fully networked organization utilizing Microsoft Windows 2007 Server and Windows XP/Vista client software to operate over an Ethernet backbone (10/100/1000 BaseT; Cat 5). At an Institute-wide level, the Microsoft Office software suite is utilized to generate reports (MS Word), graph results (MS Excel, GraphPad Prism), manage data (MS Access), and email final reports/results to clients (MS Outlook or Outlook Express). Although the [redacted] exists primarily on a PC platform, multiple Macintosh computers are also connected to the network via TCP/IP; this beneficial configuration permits utilization of unique, Macintosh-specific, scientific software and permits sharing of their data files and peripheral resources across the PC network. Specific internet and email access to the server is provided via a firewall system. Daily incremental backups and weekly full backups of experimental data and results files are utilized to provide protection against accidental or catastrophic loss of data. At all facilities, password administration is utilized to restrict/regulate access and provide an extra level of security for designated files and data.

OFFICE: Every employee has its own computer and desk at the [redacted] facility. Sufficient office space is available for all investigators and technical staff to perform the proposed work.

OTHER: [redacted] has three building, all located in [redacted]. The building on [redacted] is the corporate headquarters, and has office space, and BSL2 *in vitro* labs and small animal labs. [redacted] three facilities have security cameras installed at strategic locations and 24/7 physical security.

MAJOR EQUIPMENT

UNIVERSITY OF ROCHESTER/DEWHURST LAB

Dr. Dewhurst occupies laboratory space within the Kornberg Medical Research Building (KMRB) at the University of Rochester (UR)'s School of Medicine and Dentistry (URSMD). Dr. Dewhurst has access to shared equipment including scintillation counters, several ultracentrifuges, rotors, and 2 Sorvall RC5B centrifuges. Dark room facilities, as well as cold and warm (37^{o}C) rooms are shared with three other labs. The Dewhurst lab also has a dedicated ELISA plate reader, and shares dedicated access to a FACScalibur flow cytometer with a small consortium of other laboratories that are also located on the 3rd floor of the KMRB.

URSMD CORE FACILITIES AND SHARED RESOURCES SUMMARY

Overview and environment: The University of Rochester School of Medicine and Dentistry (URSMD) is highly committed to providing shared instrumentation and core facilities in support of basic, translational and clinical research across departments and centers. Indeed, core facilities and shared instrumentation play a central role in URMC's 2007-2012 Strategic Plan. This is evidenced in part by the major increase in resources that URMC has allocated for operational support (a 46% increase in core subsidies from FY 06 to FY 09). It is also reflected by a major commitment to acquistion of new core instrumentation (see below).

New equipment and core enhancements: The University supported significant enhancement of multiple cores in the past 2 years with addition of state of the art instrumentation. An ABI SOLiD high throughput DNA sequencer (HTDS) was added to the Functional Genomics core in early 2010, and a second high throughpput sequencing instrument is in the process of being added to the core (a funded NIH S10 award will support the purchase cost of this instrument). An Olympus FV-1000 Confocal Microscope with SIM scanner capable of 2-laser synchronized scanning was added to the Confocal and Conventional Microscopy Core in mid-2008. A Fourier Transform Mass Spectrometer (FTMS) and a Triple Quad Mass Spectrometers capable of high sensitivity and resolution were purchased for the Proteomics Core in 2008. Two new cores were formed in the past two years including (1) the Multiphoton Imaging Core, which was established with the purchase of a multiphoton confocal microscope capable of live imaging, and (2) the High Throughput Screening (HTS) core, which was established to permit the screening of libraries of small molecules, for desired biological activity. A Gammacell 40 Exactor Low Dose-rate Research Irradiator was purchased and installed for animal work within the vivarium. In addition, the UR Clinical Translational Research Institute (CTSI) provided incremental support to multiple cores including an 18-color BD LSRII special order flow cytometer and additional equipment for the flow cytometry core.

Major core facilities: The UR Medical Center has a number of successful core research facilities which provide services to all researchers at the University. These facilities are listed below.

- **Biomolecular Interaction Core Laboratory.** The Biomolecular Interaction core facility seeks to give access to custom affinity reagents to researchers in the University. We utilize phage display to generate recombinant antibodies to user provided target molecules and produce the single-chain Fv proteins for the user. We also can perform affinity measurements using surface plasmon resonance or provide access to trained users to this equipment. This facility is of special value to UR researchers with interests in immunology and/or flow cytometry.
- **Biosafety Level 3 (BSL-3) Core.** The Biosafety level three facility (BSL-3) is available for the use of any researcher at the university whose work requires manipulation of biological agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route (such as TB).
- **Cold Storage Core (CSC).** The CSC provides a discrete area where investigators can keep freezers for long term storage of research materials. The entire facility is alarmed and power protected.
- **Confocal and Conventional Microscopy Core (CCMC).** The Confocal and Conventional Microscopy Core—formerly called the Pathology/Morphology Imaging Core—provides a new Olympus FV100 laser scanning confocal microscope, an SP1 Leica confocal microscope, an Olympus fluorescence microscope, and capabilities for large specimen imaging. All users are provided the ability and

expertise to characterize biologic specimens using sophisticated fluorescent and/or brightfield microscopy.

- **Electron Microscope Research Core.** The principal mission of this Electron Microscope Research Core (EM Core) is to provide University of Rochester researchers support in high magnification image analysis of cells and tissue in the fields of Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and combined Scanning/Transmission EM (STEM). The STEM microscope was newly added to the facility in 2007, and permits both image and physical analysis of samples; this instrument is presently in high demand for studies on nanotoxicity.
- **Flow Cytometry and Immunologic Analysis Core.** Flow cytometry and immunologic assessment resources within the medical center are available in several venues. Flow cytometry resources are currently in the process of being restructured and integrated into a single new flow cytometry core structure. The overall mission of the core is to provide researchers with access to and training to operate state-of-the art flow cytometry, as well as increasing the outreach to casual users. Additionally, the core will continue to serve as an expert resource to push the limits of current technology to support cutting edge research. The Core currently has analytical tools including a FACSCanto (8-colors) and 3 LSR-II's (one 11-color, one 12-color, and an 18-color instrument) from BD Biosciences. The Core also has a BD 13-color FACSAria cell sorter. The Core's most recent (2009) acquisition is an next generation Amnis ImageStream Imaging Cytometer. This represents cutting-edge technology that is not presently available on the commercial market and was brought to UR through a collaboration between the Core and the instrument manufacturer.
- **Functional Genomics Center.** This core facility consolidates the Microarray Core, & the Nucleic Acid Core Facilites into one entity. The center provides DNA sequencing; DNA and RNA extraction, purification, and measurement; microarray services (Affymetrix platform), quantitative RT-PCR, genotyping, DNA fragment analysis; SNP analysis; assistance with interpretation of results. A new ABI SOLiD high throughput DNA sequencer (HTDS) was added to the core in early 2010, and a second high throughout sequencing instrument will be added later this year.
- **Gene Targeting and Transgenic Core.** At this time, this core provides expertise and assistance in the production of transgenic mouse models by either DNA microinjection or gene targeting in embryonic stem (ES) cells.
- **High Throughput Screening (HTS) Core.** This Core provides low cost access to screening chemical libraries for identification of novel molecules that can be used to perturb biological systems. The Core offers the capacity to develop assays for high throughput screening, the ability to screen relatively small libraries to obtain preliminary data for grant proposals as well as to screen much larger libraries for identification of leads for therapeutics. Core instrumentation includes an Envision High Throughput Plate Reader, a Janus dual robot arm for liquid handling, a Flexdrop Plate Filler and a plate washer. Available chemical libraries include the Spectrum Collection that contains natural products and marketed and experimental drugs (2000 compounds) as well as the Chembridge Diversity set, which contains 20,000 randomly selected molecules from Chembridge's DIVERset library, and is representative of the chemical diversity in the 450,000 compound parent library.
- **Human Immunology Center Laboratory.** The Human Immunology Center (HIC) and its core laboratory were established to acquire, refine and develop expertise in cutting-edge techniques and to support applications in Human Immunology research. The HIC is directed by Dr. Mosmann, and serves to enhance multi-disciplinary research initiatives catalyzing key clinical and basic immunology research in vaccines, HIV/AIDS, autoimmunity, allergy/asthma, transplantation as well as cancer immunology. The Center's core lab provides assistance and expertise in immunological method development, standardization and validation through individual and group training programs. Rochester Human Immunology Center (RHIC) services include: (1) flow cytometry analysis instrumentation that is integrated into the URSMD Flow Cytometry Core Facility providing quality control, maintenance, and calibration services for flow users (instruments include an 11-color LSRII and an 18-color LSRII); (2) Design, development, and validation of cell sorting strategies using a 7-color FACSAria located in the CVBI or a 13-color FACSAria located in the Flow Core facilit; (3) Luminex Xmap Multiplexed microbead array technology; the RHIC maintains a Luminex 200 core service with a BioRad BioPlex instrument, and provides seminars and hands on training for multiplex bead technology; (4) A CTL Immunospot® S2A Core Analyzer; this is an automated ELISPOT reader for ELISPOT work in 96 well format.

- **Molecular Imaging Facility.** The Molecular Imaging Facility provides researchers at the UR with access to state of the art instruments capable of detecting and quantifying the levels and positions of radio- and fluorescently labeled molecules in a variety of formats including gels, blots and microtiter plates. Core instrumentation includes a Storm 820 Phosphorimager and a Typhoon 9410 (purchased in 2008).
- **Multiphoton Imaging Core.** The Multiphoton Core Facility is available to all investigators at the University of Rochester to acquire and analyze high-quality images using cutting-edge multi-photon technology. The facility is equipped with a brand new Mai Tai HP Deep Sea Laser from Spectra Physics and an Olympus Fluoview 1000 AOM-MPM Microscope.
- **Pathology/Morphology Imaging Facility.** This core facility provides a Leica Confocal Microscope, an Olympus Flourescent Microscope, and an Arcturus PixCell Laser Capture Microscope for use by UR faculty. All users are provided the ability and expertise to characterize biologic specimens using sophisticated fluorescent and/or brightfield microscopy.
- **Proteomics Center.** The UR Proteomics Center provides protein characterization support, predominantly in the form of proteomic technology. The analytical strengths of the core are mass spectrometry and separation sciences. In a classical proteomic application, a complex sample matrix, such as serum, is separated into individual protein fractions, the identities of which are then determined with mass spectrometry. The core processes a myriad of sample types, from complex clinical matrices to simple purified proteins, for identification of unknowns or verification of identity. Instrumentation includes MALDI Mass Spectrometers (AutoflexIII TOF/TOF with smartbeam laser), Liquid Chromatography Mass Spectrometers (LC/MS) (LTQ LC/MS Linear Ion Trap, LCQ DECA XP MAX LC/MS 3D Ion Trap, Apex Ultra 9.4T FT-ICRMS with Apollo II dual source and micrOTOF-Q) as well as Separation Devices (ProteomeLab PF 2D, ProteomeLab PPS, ProteomeLab PA 800 Capillary, and HPLC-Robot-Spotter in-line with MALDI) and additional instrumentation (Ciphergen SELDI PBSII Mass Spectrometer). A new $1.5M Fourier Transform (FT) Mass Spectrometer was added to the facility in 2008.
- **RCBI (Rochester Center for Brain Imaging).** The Rochester Center for Brain Imaging (RCBI) provides researchers at the UR, as well as neighboring institutions, with access to a state-of-the-art 3T magnet for research using magnetic resonance imaging (MRI). This facility was recently (2009) enhanced by a $500,000 NIH small instrumentation grant award to upgrade the Siemens 3T magnet system that is the heart of this Center.
- **Vivarium.** The Vivarium is a centralized resource facility with staff and programs that support the research and educational uses of laboratory animals. These facilities are fully accredited by AAALAC and are in compliance with state law, federal statute and NIH policy. The Division of Laboratory Animal Medicine (DLAM) consists of one board certified laboratory animal veterinarian, two clinical laboratory animal veterinarians, a veterinary pathologist & a staff of trained and licensed technicians.
- **Xenogen IVIS In Vivo Imaging Core.** The mission of the in vivo bioluminescence imaging core is to allow the detection of bioluminescent tracer molecules in living small animals.

Other Facilities

High performance computing (HPC): The University of Rochester recognizes the key role of high-performance computing (HPC) in the advancement in research both within and across disciplines spanning all areas of academic scholarship. As a consequence, the University created the **Center for Computational Arts, Sciences, and Engineering (CASE)**. This University-funded center provides researchers across the university with the both the resources (i.e. CPU, storage, software tools, etc) and the assistance (training, operational support, software development support, etc) necessary to fully utilize high-performance computation in their research activities. CASE maintains two high-performance computing (HPC) clusters: the 7 teraflop BlueHive cluster, consisting of 84 compute nodes totaling 672 CPU cores, 672GB RAM, and 24 TB storage; and the Nova cluster, consisting of 144 CPU cores, 144GB RAM, 1.2 TB storage. CASE provides supporting software applications and tools, including Intel, GNU, and IBM compilers, parallel communications libraries, math libraries, and domain-specific applications, and cross-domain scientific applications including R and MATLAB. All of these resources are supported and maintained by a dedicated support team. In addition to computing resources and tools, CASE staff assists researchers to apply these tools to their own research by providing expertise and training, including: technical assistance applying computing to research; training in general HPC use, application use, and software development; and assistance in porting and tuning applications. The UR has further expanded its research facilities through a recent joint HPC research alliance with IBM. This activity provides researchers across the University with the resources and the assistance necessary to fully leverage high-performance computation in support of their research activities. The acquisition of a 13.9 teraflop BlueGene supercomputer, with 2048 processors, 2TB RAM, Storage Server with 90 TB storage, Front End Server, and 8 File Servers, complements the existing IBM Linux cluster in the CASE (see above). These high performance resources are professionally managed in the University's new state-of-the-art data center and offer researchers the power and the complete suite of software tools, including Intel optimizing compilers, Math and MPI libraries, IBM compilers, IMSL, ESSL, GPFS, the R statistical computing system, Matlab, Mathematica, System S, and SolidDB. In addition to this extensive computational research environment, University staff assists researchers to apply these tools to their own research by providing expertise and training, including: technical assistance applying computing to research; training in general HPC use, application use, and software development; and assistance in porting and tuning applications.

More recently, the University has also created the **Health Sciences Center for Computational Innovation (HSCCI).** Dr. David Topham (Associate Professor of Microbiology and Immunology in the David H. Smith Center for Vaccine Biology and Immunology) serves as Vice Provost and Executive Director of the HSCCI, a partnership between the University and IBM. The goal of the HSCCI is to support collaboration in biomedical research using High Performance Computational Resources, by bringing together academic biomedical and health-related Research Investigators, High Performance Computational Biologists, and HP Research Computing resources.

YANG LAB / University of California, San Diego

Personal (Yang Lab):
17 chemical fume hoods (4-ft wide)
Two tissue culture hoods (1 6-ft wide, 1 3-ft wide)
Two tissue culture incubators
Three Analytical HPLC
Preparative HPLC
Preparative FPLC
UV-Vis spectrophotometer
Upright optical microscope
Inverted tissue culture microscope
FT-IR spectrophotometer
Isothermal titration calorimeter
Inverted epi-fluorescence microscope
Circular dichroism spectrophotometer
Titration fluorimeter
UV-Vis plate reader
Fluorescence plate reader
Microwave synthesizer

Available through recharge within Dr. Yang's home department:
Dynamic Light Scattering Instrument
Typhoon Gel Imager
Confocal, deconvolution, transmission electron, and scanning probe microscopes
Maldi, ESI, LC-MS Mass Spectrometers
NMR Spectrometers

[redacted]

Major equipment at [redacted] that is available for this project includes the following:

SterilGARD, The Baker Company, 6 ft. biological safety cabinets class II (5), Forma scientific water-jacketed incubators (3), MWG Biotech Primus 96 Plus thermocyclers (3), Ultraspec 2000 spectrophotometer (1), Mettler Toledo Fine Scale XS104 (1), Corning pH meter 440 (1), Hoefer DNA Fluorometer Model TKO100 (1), Sorvall Legend RT+ centrifuges (3), standard waterbath at 37ºC or 56ºC (2), minicentrifuges from Fisher Scientific, Hereaus, and Eppendorf (4), Fininstruments plate washer (1), Inotech cell harvester (1), Nikon TMS inverted microscope (1), Olympus CH microscope (1), Manitowec Ziegro ice maker (1), chemical fume hood (1), Multiskan Ascent ELISA reader (1), Beckman Avant 30 centrifuge (1), Lab-Line incubator shaker model no. 3525 (1), -80ºC (1) and -20ºC freezers (2), refrigerators (3), BD FACS Canto II Flow cytometer (1), TAQMAN real-time ABI instruments (7700 and 7500, 1 each), Perkin Elmer MicroBeta scintillation counter (1), Syngene's G-box (1)

Vivarium Equipment

Rabbit cages/racks, portable surgery table (3), portable surgery tray table (3), downdraft table, ultrasomic cleaner, bead sterilizer (2), scavinging ventilator system, autoclave, balances (3), heavy duty scale, analytical balance, microbalance, Brinkman polytron, heat lamp, surgery lamp(3), cautery (3), -20C freezer (3), -80C freezer (3), refrigerator/freezer (3), walk-in cold room, flammable liquids storage cabinet, acid storage cabinet, pipetter (assorted), stirrer (assorted), vortexer (assorted), homogenizer, water bath (assorted), cage washer, rack washer

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: | * First Name: Stephen | Middle Name:
* Last Name: Dewhurst | Suffix: Ph.D.
Position/Title: Professor and Chair | Department: Microbiology & Immunology
Organization Name: University of Rochester | Division: School of Medicine & Dentistry
* Street1: 601 Elmwood Avenue, Box 672
Street2:
* City: Rochester | County/ Parish:
* State: NY: New York | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 14642-0672
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** PD/PI | **Other Project Role Category:**

Degree Type: PhD
Degree Year: 1987

***Attach Biographical Sketch** 1245-Dewhurst-NIHBio-NoHDR-06 | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 1

Prefix: | * First Name: Changyong | Middle Name:
* Last Name: Feng | Suffix:
Position/Title: Assistant Professor | Department: Biostatistics & Comp. Biology
Organization Name: University of Rochester | Division: School of Medicine & Dentistry
* Street1: UR Medical Center
Street2: 601 Elmwood Avenue, Box 630
* City: Rochester | County/ Parish:
* State: NY: New York | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 14642-0630
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Faculty | **Other Project Role Category:**

Degree Type: PhD
Degree Year: 2002

***Attach Biographical Sketch** 1246-Feng-NIHBio-NoHDR-0610.p | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 2

Prefix: | * First Name: Jerry | Middle Name:
* Last Name: Yang | Suffix: Ph.D
Position/Title: Associate Professor | Department: Biochemistry & Chemistry
Organization Name: University of California, San Diego | Division: School of Medicine
* Street1: 9500 Gilman Drive, MC 0358
Street2:
* City: La Jolla | County/ Parish:
* State: CA: California | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: 92093-0358
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Other (Specify) | **Other Project Role Category:** Co-Investigator Subcontractor

Degree Type: Ph.D.
Degree Year: 2001

***Attach Biographical Sketch** 1247-Yang NIH Bio noHDR 0610x | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

PROFILE - Senior/Key Person 3

Prefix: | * First Name: [redacted] | Middle Name:
* Last Name: [redacted] | Suffix:
Position/Title: Senior Scientist | Department:
Organization Name: [redacted] | Division:
* Street1: [redacted]
Street2:
* City: [redacted] | County/ Parish:
* State: [redacted] | Province:
* Country: USA: UNITED STATES | * Zip / Postal Code: [redacted]
* Phone Number: [redacted] | Fax Number:
* E-Mail: [redacted]

Credential, e.g., agency login: [redacted]

*** Project Role:** Other (Specify) | **Other Project Role Category:** Co-Investigator subcontractor

Degree Type: Ph.D., D.V.M.
Degree Year: [redacted]

***Attach Biographical Sketch** [redacted] | Add Attachment | Delete Attachment | View Attachment
Attach Current & Pending Support | Add Attachment | Delete Attachment | View Attachment

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Senior/Key Person 4

Prefix:		* First Name: Charlene	Middle Name:
* Last Name: Dezzutti		Suffix: Ph.D	
Position/Title: Associate Professor		Department: Obstetrics, Gynecology	
Organization Name: University of Pittsburgh		Division:	
* Street1: Magee-Womens Research Institute			
Street2: 204 Craft Ave., Room 542			
* City: Pittsburgh		County/ Parish:	
* State: PA: Pennsylvania		Province:	
* Country: USA: UNITED STATES		* Zip / Postal Code: 15213-0000	
* Phone Number: [redacted]		Fax Number:	
* E-Mail: [redacted]			

Credential, e.g., agency login: [redacted]

*** Project Role:** Consultant **Other Project Role Category:**

Degree Type: Ph.D.

Degree Year: 1989

***Attach Biographical Sketch**	1249-Dezzutti NIH Bio noHDR 0	Add Attachment	Delete Attachment	View Attachment
Attach Current & Pending Support		Add Attachment	Delete Attachment	View Attachment

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Stephen Dewhurst	Professor and Chair of Microbiology and Immunology
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Bristol University, Bristol, UK	B.S.	1984	Cellular Pathology
Univ of Nebraska Med Ctr, Omaha, NE	Ph.D.	1987	Path & Microbiology
Columbia University, New York, NY	Postdoc.	1988	Virology (HIV)
Harvard School of Public Health, Boston, MA	Postdoc.	1988-90	Virology (SIV)

A. Personal Statement

The goal of the proposed research is to understand the novel inhibitors of the semen enhancer of virus infection (SEVI) with a goal to preventing HIV-1 transmission. My role on the project is to oversee all of the experiments, reporting and administrative/financial responsibilities, and to interface with the other participating investigators. I already have strong collaborative relationships with these individuals. I also have over 20 years' experience as a molecular virologist, working on both RNA and DNA viruses, and am expert in the areas of HIV-1 virology, HIV-1 vaccine development and virally-mediated gene transfer. In addition, I am knowledgeable in the area of neuroscience, which is important in light of the similarities (and differences) between SEVI and other amyloid fibrils. The present project has emerged unexpectedly out of ongoing collaborative studies with Dr. Yang, and fits well with our mutual interests in SEVI and in the development of inhibitors of SEVI's biological activities. Of relevance to this application, and its focus on an innovative scientific concept, I have been named as an inventor on 20 patent applications or invention disclosures, and have a strong history of translational scientific research contributions. This is exemplified by my work in the area of neuroAIDS. With my colleagues, Drs. Handy Gelbard and Sanjay Maggirwar, I showed that HIV-1 mediated neurotoxicity is dependent on activation of the cellular kinase, glycogen synthase kinase 3ß (GSK3ß). The work was progressed from in vitro studies with cultured neurons, to studies in a preclinical animal model for HIV-associated neurologic disease (HAND) and ultimately into human clinical trials – with the aim of exploring the therapeutic potential of GSK3ß inhibitors such as lithium and valproate. These studies were paradigm-shifting because they identified a previously unknown therapeutic target in HAND, and resulted in bench-to-bedside translation of basic research findings.

B. Positions and Honors

Positions and Employment

1984-1987: **Graduate Researcher**, Dept. of Path. & Microbio., U. of Nebraska Med. Cntr, Omaha
1987-1988: **Research Assistant**, Dept. of Medicine & Peds, Columbia Univ., New York
1988-1990: **Post-Doctoral Fellow**, Dept. of Cancer Biology, Harvard Schl. Public Health, Boston.
1990-2009: **Assistant Professor** (1990-95), **Associate Professor** (1995-2001), **Professor** (2001-pres), **Dean's Professor** (2002-pres), **Associate Chair** (2005-2009), Department of Microbiology & Immunology, Univ. of Rochester (UR), Rochester NY.
2007-2009: **Senior Associate Dean of Basic Research**, UR School of Medicine & Dentistry
2009-: **Chair,** Dept of Microbiology and Immunology, UR

Other Experience and Professional Memberships

1994-pres: *Ad hoc* member of NIH SEPs: AIDS vaccine grants and vaccine-related programs (R21, P01, U19) (1994, 1997, 1998-2000, 2002, 2005, 2006, 2008, 2009, 2010 x3), Loan Repayment Program (LRP) (2002, 2004); F31 fellowships (2002, 2003); Bioterrorism-related research (2002); drug abuse/CEBRA (2010); drug abuse/systems science (2010); malignancy (1996, 2004); PREP training grants (2006); predoctoral T32 awards (2006-2010); virology and immunology (2009 x2, 2010)
1995-96: *Ad hoc* member, Experimental Virology Study Section, NIH (NIAID)
1999-04: Member & Chair (2003-2004), AIDS Research Review Committee (AIDSRRC), NIAID
2000-06: Director, NSF BIO REU Site Program in Cellular and Molecular Biology, UR
2001-pres: Director, NIH Predoctoral Training Program in HIV Replication and Pathogenesis, UR
2002-07: Director, PREP Program in Microbiology & Immunology, UR
2004-08: Member, NIH Recombinant DNA Advisory Committee (RAC)
2007-09: Co-Chair, CTSA Public-Private Partnership (PPP) Steering Committee, NIH
2008-pres: Director, University of Rochester Developmental Center for AIDS Research
2008-pres: Member, NIH OBA Working Group on Biosafety (advisory to the RAC)

Honors and Awards:

1988-91: AIDS Research Scholar, American Foundation for AIDS Research.
1995: Friend of Education Award, Rochester City School District, Rochester NY
1996: Graduate Student Society (GSS) Faculty Teaching Award, URSMD
1994-99: Research Career Development Awardee, NIAID, NIH
2002-pres.: Dean's Professor of Microbiology and Immunology, URSMD
2002: Graduate Alumni Award for Excellence in Graduate Education, URSMD
2003: University Dean's Award for Meritorious Service in Ph.D. Defenses, UR
2008: William H. Riker University Award for Excellence in Graduate Education, UR

C. Selected Peer-reviewed Publications (Selected from 115 peer-reviewed publications)

1. Renda MJ, Bradel-Tretheway B, Planelles V, Bambara RA, Dewhurst S. Inhibition of HIV type 1 replication using lentiviral-mediated delivery of mutant tRNA(Lys3)A58U. AIDS Res Hum Retroviruses. 2004;20(12):1324-34.
2. Dou H, Ellison B, Bradley J, Kasiyanov A, Poluektova LY, Xiong H, Maggirwar S, Dewhurst S, Gelbard HA, Gendelman HE. Neuroprotective mechanisms of lithium in murine human immunodeficiency virus-1 encephalitis. J Neurosci. 2005;25(37):8375-85.
3. Zeng L, Planelles V, Sui Z, Gartner S, Maggirwar SB, Dewhurst S, Ye L, Nerurkar VR, Yanagihara R, Lu Y. HIV-1-based defective lentiviral vectors efficiently transduce human monocytes-derived macrophages and suppress replication of wild-type HIV-1. J Gene Med. 2006;8(1):18-28. PMCID: 2825118.
4. Duke CM, Maguire CA, Keefer MC, Federoff HJ, Bowers WJ, Dewhurst S. HSV-1 amplicon vectors elicit polyfunctional T cell responses to HIV-1 Env, and strongly boost responses to an adenovirus prime. Vaccine. 2007;25(42):7410-21. PMCID: 2092414.
5. Santos K, Duke CM, Rodriguez-Colon SM, Dakwar A, Fan S, Keefer MC, Federoff HJ, Frelinger JG, Bowers WJ, Dewhurst S. Effect of promoter strength on protein expression and immunogenicity of an HSV-1 amplicon vector encoding HIV-1 Gag. Vaccine. 2007;25(9):1634-46. PMCID: 1851942.
6. Santos K, Sanfilippo CM, Narrow WC, Casey AE, Rodriguez-Colon SM, McDermott MP, Federoff HJ, Bowers WJ, Dewhurst S. Infectivity of herpes simplex virus type-1 (HSV-1) amplicon vectors in dendritic cells is determined by the helper virus strain used for packaging. J Virol Methods. 2007;145(1):37-46. PMCID: 2080840.
7. Sapinoro R, Maguire CA, Burgess A, Dewhurst S. Enhanced transduction of dendritic cells by FcgammaRI-targeted adenovirus vectors. J Gene Med. 2007;9(12):1033-45.
8. Sui Z, Sniderhan LF, Schifitto G, Phipps RP, Gelbard HA, Dewhurst S, Maggirwar SB. Functional synergy between CD40 ligand and HIV-1 Tat contributes to inflammation: implications in HIV type 1 dementia. J Immunol. 2007;178(5):3226-36.

9. Chugh P, Bradel-Tretheway B, Monteiro-Filho CM, Planelles V, Maggirwar SB, Dewhurst S, Kim B. Akt inhibitors as an HIV-1 infected macrophage-specific anti-viral therapy. Retrovirology. 2008;5:11. PMCID: 2265748.
10. Bimber BN, Chugh P, Giorgi EE, Kim B, Almudevar AL, Dewhurst S, O'Connor DH, Lee HY. Nef gene evolution from a single transmitted strain in acute SIV infection. Retrovirology. 2009;6:57. PMCID: 2701916.
11. Schifitto G, Zhong J, Gill D, Peterson DR, Gaugh MD, Zhu T, Tivarus M, Cruttenden K, Maggirwar SB, Gendelman HE, Dewhurst S, Gelbard HA. Lithium therapy for human immunodeficiency virus type 1-associated neurocognitive impairment. J Neurovirol. 2009;15(2):176-86. PMCID: 2747099.
12. Volcy K, Dewhurst S. Proteasome inhibitors enhance bacteriophage lambda (lambda) mediated gene transfer in mammalian cells. Virology. 2009;384(1):77-87. PMCID: 2654414.
13. Eggert D, Dash PK, Gorantla S, Dou H, Schifitto G, Maggirwar SB, Dewhurst S, Poluektova L, Gelbard HA, Gendelman HE. Neuroprotective activities of CEP-1347 in models of neuroAIDS. J Immunol. 2010;184(2):746-56. PMCID: 2805820.
14. Gorantla S, Makarov E, Finke-Dwyer J, Gebhart CL, Domm W, Dewhurst S, Gendelman HE, Poluektova LY. CD8+ cell depletion accelerates HIV-1 immunopathology in humanized mice. J Immunol. 2010;184(12):7082-91.
15. Love TM, Thurston SW, Keefer MC, Dewhurst S, Lee HY. Mathematical modeling of ultradeep sequencing data reveals that acute CD8+ T-lymphocyte responses exert strong selective pressure in simian immunodeficiency virus-infected macaques but still fail to clear founder epitope sequences. J Virol. 2010;84(11):5802-14. PMCID: 2876615.

D. Research Support (Selected)

Ongoing Research Support

R01 AI084111-01 Dewhurst & Rose (MPI) 09/01/2009 – 06/30/2011
Transmission blocking vaccine for HIV-1
Major goal: To develop a novel transmission-blocking vaccine for HIV-1, that targets the native form of the virus as present in semen
Role: PI (Communicating PI)

R21 AI087149-01A1 Dewhurst (PI) 03/01/2010 – 02/28/2012
Use of Discontinuous Peptide Display for Generation of HIV Antigen Mimics
Major goal: To develop a novel antigenic mimic for the broadly neutralizing antibody epitope recognized by the b12 monoclonal antibody, using a discontinuous peptide display approach
Role: PI

R01 DA026325-01 Dewhurst (PI) 09/30/2008 – 05/31/2013
Cerebrovascular mechanisms in methamphetamine mediated exacerbation of neuroAIDS
Major goal: To examine the effect of methamphetamine on cerebrovascular aspects of neuroAIDS.
Role: PI

[redacted]

Completed Research Support (Selected)

PO1 AI056356 Keefer (PI) 07/01/2003 – 12/31/2007

HSV-1 Amplicon Vectors for HIV Vaccine Delivery
(Project 1 Title: Immunogenicity of HSV amplicon vectors in small animals)
Major goal: This HIVRAD program was intended to explore the development and use of helper-free HSV-1 amplicon vectors as a HIV/AIDS vaccine delivery modality – leveraging the large gene insert capacity and mucosal tropism of HSV-1
Role: PI, Project 1

NIH R21 AI058791 Dewhurst (PI) 01/01/2005 – 08/31/2007
Bacteriophage gene transfer for HIV vaccine development
Major goal: To develop bacteriophage lambda as a gene transfer vector for mammalian cells, with a view to potential future use for HIV/AIDS vaccine delivery
Role: PI

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Changyong Feng	Assistant Professor of Biostatistics
eRA COMMONS USER NAME (credential, e.g., agency login) [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Science and Technology of China	BS	1991	Operations Research
University of Rochester, Rochester, NY	MA	1999	Statistics
University of Rochester, Rochester, NY	PhD	2002	Statistics

A. Personal Statement

The goal of the proposed research is to develop a new microbicide for prevention of HIV transmission. My role on the project is to provide biostatistical support for experimental design and analysis of experimental data. I received my PhD in Statistics from the University of Rochester in 2002. From 2002 to 2003 I was an Assistant Professor of Biostatistics in the Department of Preventive Medicine and Kansas Cancer Institute at the University of Kansas Medical Center. I have been an Assistant Professor of Biostatistics in the University of Rochester Medical Center since 2006. My research interests include multivariate survival analysis, empirical processes theory, longitudinal data analysis and statistical methods in epidemiology and clinical trials. I have a pre-existing collaboration with the PI, Dr. Dewhurst (supported by two current NIH awards), and considerable experience with the analysis of infectious disease-related datasets, as reflected by numerous coauthored publications in this and related areas.

B. Positions and Honors

Professional Experience

08/2002-08/2003	Assistant Professor, Department of Preventive Medicine and Public Health, University of Kansas Medical Center
09/2003-Present	Biostatistician, Eastman Dental Center, University of Rochester Medical Center
09/2003-08/2006	Research Assistant Professor, Department of Biostatistics and Computational Biology, University of Rochester Medical Center
08/2006-Present	Assistant Professor, Department of Biostatistics and Computational Biology, University of Rochester Medical Center

Honors

2002	International Biometric Society (ENAR) Student Award

C. Selected Peer-reviewed Publications

1. Vacca Smith, A. M., Scott-Anne, K. M., Whelehan, M.T., Berkowitz, R. J., Feng, C., and Bowen, W. H. (2007). Salivary Glucosyltransferase B as a Possible Marker for Caries Activity. Caries Research, 41, 445-450. PMCID: PMC2820324
2. Tu, X.M., Zhang, J., Kowalski, J., Shults, J., Feng, C., Sun, W. and Tan, W. (2007). Power analyses for longitudinal study designs with missing data. Statistics in Medicine, 26: 2958-1981.

3. Tu, X. M., Feng, C., Kowalski, J., Tang, W., Wang, H., Wan, C., and Ma, Y. (2007). Correlation Analysis for Longitudinal Data: Applications to HIV and Psychosocial. Research. Statistics in Medicine, 26: 4116-413.
4. Anandarajah, A., Schwarz, E.M., Totterman, S., Monu, J. Feng, C., Shao, T., Haas-Smith, S.A., and Ritchlin, C. T. (2008). The effects of etanercept therapy on clinical outcome, osteoclast precursor frequency and bone marrow edema in patients with psoriatic arthritis. Ann Rheum Dis. 67:296-301.
5. Winterborn AN, Bates WA, Feng C, Wyatt JD. (2008) The efficacy of orally dosed ketamine and ketamine/medetomidine compared with intramuscular ketamine in rhesus macaques (Macaca mulatta) and the effects of dosing route on haematological stress markers. J Med Primatol. 37:116-27.
6. Ma, Y., Tang, W., Feng, C., and Tu, X. M. (2008). Inference of Kappas for Longitudinal Study Data: Applications to HIV Prevention and Sexual Abuse. Biometrics, 64: 781-789.
7. Chen, J., Li, K-H, and Feng, C. (2008). Bayesian computation and adaptive control for dynamic linear system. International Journal of Contemporary Mathematical Sciences, 3: 269-284.
8. Morrison-Beedy D, Carey MP, Feng C, Tu XM. (2008) Predicting sexual risk behaviors among adolescent and young women using a prospective diary method. Res Nurs Health. 31: 329-40. PMCID: PMC2562714
9. Scosyrev E, Noyes K, Feng C, Messing E. (2009) Sex and racial differences in bladder cancer presentation and mortality in the US. Cancer 115:68-74.
10. Messing EM, Madeb R, Feng C, Stephenson L, Gilchrist KW, Young T, Gee J. (2009) Grade and Stage at Presentation Do Not Predict Mortality in Patients With Bladder Cancer Who Survive Their Disease. J Clin Oncol. 27:2443-9.
11. O'Connor AB, Zwemer FL, Hays DP, Feng C. (2009) Outcomes after intravenous opioids in emergency patients: a prospective cohort analysis. Acad Emerg Med. 16:477-87.
12. [redacted]
13. [redacted]
14. [redacted]
15. Chiu YG, Shao T, Feng C, Mensah KA, Thullen M, Schwarz EM, Ritchlin CT. (2010) CD16 (FcRgammaIII) as a potential marker of osteoclast precursors in psoriatic arthritis. Arthritis Res Ther. 12:R14. PMCID: PMC2875642

D. Research Support (Selected)

<u>Ongoing Research Support</u>

R01 AI084111-01 Dewhurst & Rose (MPI) 09/01/2009 – 06/30/2011
Transmission blocking vaccine for HIV-1
Major goal: To develop a novel transmission-blocking vaccine for HIV-1, that targets the native form of the virus as present in semen
Role: Biostatistician

R21 AI087149-01A1 Dewhurst (PI) 03/01/2010 – 02/28/2012
Use of Discontinuous Peptide Display for Generation of HIV Antigen Mimics
Major goal: To develop a novel antigenic mimic for the broadly neutralizing antibody epitope recognized by the b12 monoclonal antibody, using a discontinuous peptide display approach
Role: Biostatistician

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Yang, Jerry Curtis	Associate Professor of Chemistry and Biochemistry
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of California, Berkeley, CA	B.S.	1995	Chemistry
Columbia University, New York, NY	M.A.	1998	Chemistry
	M. Phil.	2000	Chemistry
	Ph.D.	2001	Chemistry
Harvard University, Cambridge, MA	Postdoc	2001-2003	Chemistry

A. Personal Statement

The goal of the proposed research is to examine the effect of amyloid-targeting synthetic molecules for attenuating the infection of sexually-transmitted microbes, including HIV. Specifically, this research will test the effect of monomeric and oligomeric analogs of benzothiazole anilines (BTAs)—a well known class of amyloid-targeting molecules—for their ability to abrogate SEVI-mediated transmission of HIV in cells. The proposed studies build from extensive results from *in vitro* experiments carried out in my laboratory, which seeks to use amyloid-targeting molecules to neutralize the cell-damaging effects of amyloid peptides and proteins in neurological disorders such as Alzheimer's disease (AD). My group was the first to introduce the idea of generating molecular assemblies on aggregated amyloid peptides that could function as bio-resistive surface coatings on these disease-related materials (in analogy to generation of a "non-stick" coating on a surface). We have already published several proof-of-principle studies that demonstrate that small molecules that bind with high density and uniformity to aggregated peptides associated with AD (called β-amyloid (Aβ) peptides) are capable of forming surface coatings that inhibit the interaction of engineered and natural, cellular proteins with these peptides. We have subsequently demonstrated in cell culture experiments that bio-resistive coatings on aggregated Aβ peptides can protect cultured neuroblastoma cells from the Aβ-induced injury and can neutralize the toxicity of aggregated Aβ in cells. From a novel synthetic library of Aβ-targeting molecules, my lab has developed oligoethylene glycol derivatives of BTA as candidates that exhibit excellent protective properties against Aβ-induced cellular injury. We also showed that one member of this class of molecules (BTA-EG_4) exhibited good *in vivo* tolerance and pharmacokinetic properties in wt mice. The goal of this proposal is to evaluate several members of this novel class of molecules (as well as other examples of amyloid-targeting molecules developed in my lab) for their capability to attenuate interactions between amyloid proteins and other biologics that may play an important role in sexually-transmitted microbial infection. I have a broad background in organic and biophysical chemistry, biochemistry, and materials science, with specific expertise in amyloid and biomembrane research. My students and postdocs routinely synthesize structurally and functionally diverse organic molecules and small molecule libraries, develop biochemical and cellular screening assays, and develop biophysical techniques to study the structure and function of biomolecules and biomolecular aggregates in solution. The common goal of our research is the design, synthesis, and investigation of functional molecular assemblies for biomedical applications. To achieve our research goals, I have collaborated, and jointly secured funding and published papers, with biomedical researchers within UCSD and at other institutions across the country. I am, thus, aware of the importance of frequent communication with collaborators and of constructing a realistic research plan, timeline, and budget. The current application will evaluate novel synthetic molecules from my lab in cellular and *in vivo* studies examining SEVI-mediated

transmission of HIV. Professor Stephen Dewhurst has excellent qualifications and expertise to lead the proposed research. I have been selected by the PI (Professor Dewhurst) due to my expertise in the synthesis and the chemical and biochemical activity of the molecules to be evaluated in the proposed research. I will provide additional expertise, effort, and any other resources available to me to achieve the specific aims of this very promising and exciting grant application.

B. Positions and Honors.

Positions and Employment

1993-1995 Research in Nuclear Science, LBL Nuclear Science Division, Berkeley, CA
1994-1995 Consulting in Nuclear Chemistry, LBL Division of Ion Beam Technology, Berkeley, CA
2001-2003 Postdoctoral Research in Biochemistry, Bionanotechnology, and Materials Science, Harvard University, Department of Chemistry and Chemical Biology, Cambridge, MA
2003-2009 Assistant Professor of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA
2009- Associate Professor of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA

Professional Memberships

1997- Member, the American Chemical Society
2001- Member, the American Physical Society
2001- Member, the Biophysical Society
2007- Member, the American Association for Cancer Research
2007- UCSD CFAR Affiliate

Honors and Awards

1995 B.S. with *Honors*, U.C. Berkeley
1995-1996 Outstanding Teaching Award, U.C. Berkeley
1998 Sylvia and Victor G. Fourman Graduate Fellowship
1998 Jack Miller Award for excellence in teaching, Columbia University
1999 Bristol-Myers Squibb Graduate Fellowship
1999-2001 EPA NCERQA STAR Graduate Fellowship
2001 Hammett Award for most outstanding graduate research, Columbia University
2001 Ph.D. with *Distinction*, Columbia University
2004 Thieme Journal Award, *Synthesis* and *Synlett*
2004 Faculty Career Development Award, UCSD
2006 Hellman Fellow
2006 Wallace H. Coulter Foundation Translational Research Partnership Award
2007 American Cancer Society Research Scholar
2008 Alzheimer's Association New Investigator Award
2009 National Science Foundation CAREER Award

C. Selected Peer-reviewed publications (Selected from 38 total publications)

Five most relevant to the current application

1. P. Inbar and **J. Yang*** Inhibiting Protein-Amyloid Interactions Using Small Molecules: A Surface Chemistry Approach *Bioorg. Med. Chem. Lett.,* **2006**, *16*, 1076-1079.
2. P. Inbar, C.Q. Li, S.A. Takayama, M.R. Bautista, and **J. Yang*** Oligo(ethylene glycol) Derivatives of Thioflavin T as Inhibitors of Protein-Amyloid Interactions *ChemBioChem*, **2006**, *17*, 1563-1566
3. P. Inbar, M.R. Bautista, S.A. Takayama, and **J. Yang*** A Simple Assay to Screen for Molecules that Associate with Alzheimer's-Related β-Amyloid Fibrils *Anal. Chem.*, **2008**, *80*, 3502-3506
4. J. Sutharsan, M. Dakanali, C.C. Capule, M.A. Haidekker, **J. Yang,*** and E. Theodorakis* Rational Design of Amyloid Binding Agents Based on the Molecular Rotor Motif *ChemMedChem* **2010**, *5*, 56-60.
5. R. Capone, P. Prangkio, A. Sauer, M.R. Bautista, R.S. Turner, **J. Yang,*** and M. Mayer* Amyloid-β Ion Channels in Artificial Lipid Bilayers and Neuronal Cells: Resolving a Controversy *Neurotoxicity Res.* **2009**,

16, 1-13.

Additional publications within the last 3 years

1. S. Blake, T. Mayer, M. Mayer, and **J. Yang*** Monitoring Chemical Reactions Using Ion-Channel-Forming Peptides *ChemBioChem*, **2006**, *7*, 433-435.
2. S.D. Kong, A. Luong, G. Manorek, S.B. Howell, and **J. Yang*** Acidic Hydrolysis of N-Ethoxybenzylimidazoles (NEBIs): Potential Applications as pH-Sensitive Linkers for Drug Delivery *Bioconj. Chem.*, **2007**, *18*, 293-296
3. R. Capone, S. Blake, M.R. Restrepo, **J. Yang,*** and M. Mayer* Designing Nanosensors Based on Charged Derivatives of gramicidin A, *J. Am. Chem. Soc.*, **2007**, *129*, 9737-9745.
4. M. Mayer, V. Semetey, I. Gitlin, **J. Yang**, and G.M. Whitesides* Quantifying Protein-Ligand Interactions Using Ion Channel-Forming Peptides *J. Am. Chem. Soc.*, **2008**, *130*, 1453-1465.
5. S. Blake, R. Capone, M. Mayer,* and **J. Yang*** Chemically Reactive Derivatives of Gramicidin A for Developing Ion Channel-Based Nanosensors *Bioconj. Chem.* **2008**, *19*, 1614-1624.
6. B. Bilgiçer, S.W. Thomas, B.F. Shaw, G.K. Kaufman, V.M. Krishnamurthy, L.A. Estroff, **J. Yang**, and G.M. Whitesides* A Non-Chromatographic Method for the Purification of a Bivalently Active Monoclonal IgG Antibody from Biological Fluids *J. Am. Chem. Soc.* **2009**, *131*, 9361-9367.
7. M.X. Macrae, S. Blake, M. Mayer,* and **J. Yang*** Reactive Derivatives of Gramicidin Enable Light- and Ion Modulated Ion Channels *Proc. SPIE* **2009**, *7397*, 739709.
8. S. Majd, E.C. Yusko, A.D. MacBriar, **J. Yang,*** and M. Mayer* Gramicidin Pores Report the Activity of Membrane-Active Enzymes *J. Am Chem. Soc.* **2009**, *131*, 16119-16126.
9. M.X. Macrae, S. Blake, X. Jiang, R. Capone, D.J. Estes, M. Mayer,* and **J. Yang*** A Semi-Synthetic Ion Channel Platform for Detection of Phosphatase and Protease Activity *ACS Nano* **2009**, *3*, 3567-3580.
10. M.X. Macrae, S. Blake, M. Mayer,* and **J. Yang*** Nanoscale Ionic Diodes with Tunable and Switchable Rectifying Behavior *J. Am. Chem. Soc.* **2010**, *132*, 1766-1777.

D. Research Support

Ongoing Research Support

[redacted]

Recently Completed Research Support (Selected)

[redacted]

Alzheimer's Disease Research Center (NIH 3P50 AG005131) Glasko (PI) 04/01/08-03/31/09
"Exploring methods to chemically degrade aggregated Aβ peptides"
Role: PI of pilot grant

Center for AIDS Research (NIH 5P30 AI36214) Richman (PI) 0/01/07-12/31/07
"Development of New Methods for the Ultrasensitive Quantification of HIV-1 Protease Activity"
Role: PI of developmental grant

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
[redacted]	Senior Scientist
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Ludwig-Maximilians-Universität, Munich, Germany	D.V.M	1984-1990	Veterinary Medicine
Ludwig-Maximilians-Universität, Munich, Germany	Ph.D.	1989-1992	Virology
Hood College, Frederick, Maryland	Certificate	2003-2008	Regulatory Compliance of Biologics and Pharmaceutics
Regulatory Affairs Board Certification	RAC	2008	Regulatory Affairs Professional Society (RAPS)

A. Personal Statement

My role on this application is two-fold: (1) During the R21 phase, I will serve as a consultant and advisor to the research program, to review annual progress reports, participate in data evaluations, and to provide technical assistance and guidance on assessments of candidate microbicides; (2) During the R33 phase, I will serve as a collaborator and coinvestigator on this project and as PI of the [redacted] subaward.

As PI/Co-PI on many contracts and grants involving microbicides, my research interests have focused on HIV microbicide development for many years. I have expertise in pre-clinical microbicide development and safety and efficacy testing of novel microbicides. I also have extensive experience on studies using nonhuman primate (NHP) models for HIV/AIDS, including the simian (human) immunodeficiency virus (SIV-SHIV)/macaque model. I received my Ph.D. in 1992 for studies on FeLV and FIV, and – in addition to my experience in microbicide research – have expertise in HIV vaccine development, analysis of immune responses in SIV-infected macaques, and evaluation of novel candidate antiviral drugs. Thus, the present proposal is well matched to my expertise and interests.

B. Positions and Honors.

Positions and Employment

[redacted]

Awards

2002 National Institutes of Health Fellows Award for Research Excellence

C. Selected Peer-reviewed publications (Selected from 52 total publications)

[redacted]

[redacted]

D. Research Support

Ongoing Research Support (Selected)

[redacted]

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Dezzutti, Charlene Susan	Associate Professor – Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine, University of Pittsburgh
eRA COMMONS USER NAME [redacted]	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Pittsburgh, Pittsburgh PA	B.Sc.	1986	Microbiol./Biochem.
The Ohio State University, Columbus, OH	M.Sc.	1987	Veterinary Pathology
The Ohio State University, Columbus, OH	Ph.D.	1989	Viral Immunology
The Ohio State University, Columbus, OH	Postdoctoral	1991-1992	Molecular Virology

A. Personal Statement

My role on this application is to serve as a consultant and advisor to the research program, to review annual progress reports, participate in data evaluations, and to provide technical assistance and guidance on assessments of the potential toxicity of candidate microbicides, using the cervical explant models that we have developed (including MTT, histology assessments of toxicity and assessment of cytokine release).

I am well qualified for this role because I am the principal investigator of the Network Laboratory for the Microbicide Trials Network (MTN), an HIV/AIDS clinical trials network established by the National Institute of Allergy and Infectious Diseases (NIAID). In this capacity, I conduct side-by-side comparative assessments of different microbicide candidates that will be used in clinical trials. Of relevance to this proposal are the following research questions that my laboratory is presently studying:

1) The mechanism(s) of transmission. We have developed cervical and colorectal tissue explant systems to study HIV transmission ex vivo. Using these systems we are attempting to determine the first HIV infected cells.
2) The factors that influence transmission. We have an active program studying the interaction(s) between sexually transmitted infections and HIV in acute in vitro model systems as well as our tissue explant systems.
3) The ways to prevent transmission of HIV. For prevention of sexually acquired HIV, we are evaluating microbicides that may be utilized in human clinical trials. We are examining innate immune factors found in genital secretions that could be augmented or exploited for microbicide development. We have also developed in vitro and ex vivo model systems that can be used to evaluate the potential toxicity of candidate microbicides.

The objectives of this proposal therefore align closely with the interests of my research.

B. Positions and Honors.

Positions and Employment

1989-1990 Supervisor, Viral Oncology/Immunopathology Laboratories, Department of Veterinary Pathobiology, Ohio State University, Columbus, OH
1992-1993 Staff Fellow, Retrovirus Diseases Branch, Centers for Disease Control and Prevention, Atlanta, GA
1993-2005 Research Microbiologist, Laboratory Branch, Centers for Disease Control and Prevention, Atlanta, GA
2001-2005 CDC, NCID, Science Education Committee - Distinguished Lecturer's Series Chairperson
2005-date Associate Professor, Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine and Department of Infectious Diseases and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh PA
2006-date Associate Investigator, Magee-Womens Research Institute, Pittsburgh, PA

Other Experience and Professional Memberships

1989 – Date American Association for the Advancement of Science
1989 – Date American Society for Microbiologists
2000 – Date Society for Mucosal Immunology
2008 – Date Infectious Diseases in Obstetrics and Gynecology (Editorial board)
1998/2002 Emory CFAR grant review committee
2004 NIH, DAIDS, grant reviewer (RFP NIH-NIAID-DAIDS-04-04) December 2004
2007 NIH, NIAID, special emphasis panel (ZAI1-RB-A-J1) for RFA-IA-07-009

Honors

1985 Beta Beta Beta Biological Honors Society (Alpha Gamma Chapter)
1997 NCID Branch Recognition Award

C. Selected Peer-reviewed publications (Selected from 52 total publications)

1. **Dezzutti, C.S.**, Guenthner, P.C., Cummins, J.E., Cabrera, T., Marshall, J.H., Dillberger, A., and Lal, R.B. Cervical and prostate primary epithelial cells are not productively infected with HIV-1, but sequester the virus. J. Infect. Dis. 183:1204-1213, 2001. PMID: 1126220
2. Zunt, J.R., **Dezzutti, C.S.**, Montano, S.M., Thomas, K., Alarcón, J.O.V., Quijano, E., Courtois, B., Sánchez, J., Campos, P., Gotuzzo, E., Guenthner, P.C., Lal, R.B., and Holmes, K.K. Cervical shedding of human T-cell lymphotropic virus type-I is associated with cervicitis. J. Infect. Dis. 186:1669-1672, 2002. PMID: 12447745
3. Cummins, Jr., J.E., Villanueva, J.M., Evans-Strickfaden, T., Abner, S.A., Shekou, S., Bush, T.J., Green, T.A., Lennox, J.L., Wright, T., Folks, T.M., Hart, C.E., and **Dezzutti, C.S.** Detection of infectious HIV-1 in female genital secretions using a short-term culture method. J. Clin. Microbiol.41:4081-4088, 2003. PMID: 12958229
4. **Dezzutti, C.S.**, James, V.N., Ramos, A., Sullivan, S.T., Siddig, A., Bush, T.A., Grohskopf, L.A., Paxton, L., Subbarao, S., Hart, C.E. In Vitro Comparison of Topical Microbicides for the Prevention of HIV Transmission. Antimicrob. Agents Chemother. 48:3834-3844, 2004. PMID: 15388443
5. Guenthner, P.C., Secor, W.E., and **Dezzutti, C.S.** Trichomonas vaginalis-induced epithelial monolayer destruction and HIV-1 replication: implications for the sexual transmission of HIV-1. Infect. Immun. 73:4155-4160, 2005. PMID: 15972505
6. Abner, S.R., Guenthner, P.C., Guarner, J., Hancock, K.A., Cummins, Jr., J.E., Fink, A., Gilmore, G.T., Staley, C., Ward, A., Ali, O., Binderow, S., Cohen, S., Mayer, R., Grohskopf, L.A., Paxton, L., Hart, C.E., and **Dezzutti, C.S.** A human colorectal explant model to evaluate topical microbicides for the prevention of HIV infection. J. Infect. Dis. 192:1545-1556, 2005. PMID: 16206069
7. Cummins, Jr., J.E, Christensen, L.L., Switzer, W.M., Boneva, R.S., Heneine, W., Folks, T.M., Chapman, L.E., Sandstrom, P.A., **Dezzutti, C.S.** Mucosal Antibody Responses in Humans Occupationally Infected with SFVcpz. J. Virol. 79:13186-13189, 2005. PMID: 16189020
8. Bower, W., Culver, D.H., Castor, D., Wu, Y., James, V.N., Hammer, S., Kuhnert, W.L., Williams, I.T., Bell, B.P., Vlahov, D., and **Dezzutti, C.S.** Suppression of HIV RNA titers with HAART results in elevated HCV RNA titers in HIV/HCV coinfected patients. J. AIDS 42:293-297, 2006. PMID: 16763522
9. Cummins, Jr., J.E., Christensen, L., Lennox, J.L., Bush, T.J., Wu, Z., Malamud, D., Evans-Strickfaden, T., Siddig, A., Caliendo, A.M., Hart, C.E., and **Dezzutti, C.S.** Mucosal innate immune factors in the female genital tract are associated with vaginal HIV-1 shedding independent of plasma viral load. AIDS Res. Hum. Retroviruses 22:788-795, 2006. PMID: 16910835
10. Cummins, Jr., J.E., Denniston, M., Christensen, L.L., Mayer, K., Pickard, R., Novak, R.M., Graham, P., Gurwith, M., Orelind, K., Ackers, M.L., and **Dezzutti, C.S.** Mucosal Innate Immune Factors in Secretions from High Risk Individuals Immunized with a Bivalent gp120 Vaccine. AIDS Res. Hum. Retroviruses 23:748-754, 2007. PMID: 17531002
11. Cummins, Jr., J.E., Guarner, J., Flowers, L., Guenthner, P.C., Bartlett, J., Morken, T., Grohskopf, L.A., Paxton, L., and **Dezzutti, C.S.** Preclinical Testing of Candidate Topical Microbicides for Anti-HIV-1 Activity and Tissue Toxicity in a Human Cervical Explant Culture. Antimicrob. Agents Chemother. 51:1770-1779, 2007. PMID: 17353237
12. Roth, S., Monsour, M., Dowland, A., Guenthner, P.C., Hancock, K., Ou, C.-Y., and **Dezzutti, C.S.** Effect of topical microbicides on infectious HIV-1 binding to epithelial cells. Antimicrob. Agents Chemother. 51:748-

754, 2007. PMID: 17404008
13. Ham, A., Cost, M., Sassi, A., **Dezzutti, C.S.**, and Rohan, L.C. Targeted Delivery of PSC-RANTES for HIV-1 Prevention using Biodegradable Nanoparticles. Pharmaceutical Res. 26:502-511, 2009. PMID: 19002569
14. Richardson-Harman, N., Lackman-Smith, C., Fletcher, P.S., Anton, P.A., Bremer, J.W., **Dezzutti, C.S.**, Elliot, J., Grivel, J.C., Guenthner, P.C., Gupta, P., Jones, M., Lurain, N.C., Margolis, L.B., Mohan, S., Ratner, D., Reichelderfer, P., Roberts, P., Shattock, R.J., and Cummins Jr. J.E. Multi-site comparison of anti-HIV microbicide activity in explant assays using a novel assay. J. Clin. Microbiol. 47:3530-9, 2009. PMID: 19726602
15. Rohan, L.C., Moncla, B.J. Kunjara Na Ayudhya, P.K., Cost, M., Huang, Y., Fang, G., Billitto, N., Lynam, JD, Pryke, K., Graebing, P., Hopkins, N., Rooney, J.F., Friend, D., **Dezzutti, C.S.** In vitro and Ex vivo Testing of Tenofovir shows it is Effective as an HIV-1 Microbicide. PLoS ONE 5(2): e9310. PMID: 20174579

D. Research Support

Ongoing Research Support

5 U01 AI068633-02 (Hillier, S.) 6/29/06 – 5/31/13
NIH/NIAID
Microbicide Trials Network
Major goal: The mission of the MTN is to reduce the sexual transmission of HIV through the development and evaluation of products, which reduce the transmission of HIV when applied topically to mucosal surfaces. The goal is to conduct scientifically rigorous and ethically sound clinical trials of microbicide safety and effectiveness, which will support licensure of these products.
Role: Network Laboratory Principle Investigator

U19 AI077289-01 (Buckheit, R.) 03/08 – 02/13
NIH/NIAID
Long acting acceptable microbicides: novel delivery, activity and pharmacodynamic
Major goals: Our role is to determine the safety and efficacy of new microbicide products/formulations using our explant culture system. We have contracted with ImQuest Biosciences, Inc. to perform this work.
Role: Core D Principle Investigator

RFA-AI-08-006 (McGowan, I.) 7/1/09 – 6/30/14
NIH/NIAID
Combination HIV Anteviral Rectal Microbicide Program
Major goal: The specific aims of this project are: 1) to determine the potential protective effect of UC781 to prevent rectal HIV-1 infection; 2) to determine the efficacy of topically administered 9-R-2-phosphonylmethoxypropyl-adenine (PMPA) to prevent rectal HIV-1 transmission; and 3) to determine the protective effect of a combination of PMPA and UC781 to prevent rectal HIV-1 transmission.
Role: Project 1 Principle Investigator

[redacted]

[redacted]

Completed Research Support

3 U19 AI060614-03S1 (Anton, P.) 9/25/06 – 7/31/09
NIH/NIAID
Compartment-Specific Topical Microbicide Formulations
Major goal: The subcontract site will be responsible for directing the pre-formulation, formulation development, and formulation assessment components of the project for rectal formulation of UC781.
Role: Investigator

[redacted]

OMB Number: 4040-0001
Expiration Date: 06/30/2011

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2011 *** End Date:** 02/29/2012 **Budget Period** 1

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		Stephen		Dewhurst	Ph.D.	PD/PI	[redacted]	1.20			[redacted]	[redacted]	[redacted]
2.		Changyong		Feng	PhD	Faculty	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

	* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
	1	Post Doctoral Associates	12.00			[redacted]	[redacted]	[redacted]
		Graduate Students						
		Undergraduate Students						
		Secretarial/Clerical						
	1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2011 *** End Date:** 02/29/2012 **Budget Period 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 1

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry * Start Date: 03/01/2011 * End Date: 02/29/2012 Budget Period 1

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	53.00	[redacted]	[redacted]
2. MTDC	54.50	[redacted]	[redacted]
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency DHHS, Robert Aaronson, [redacted]
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-FINALBUDG-JUST-ROCHESTER.pdf Add Attachment Delete Attachment View Attachment
(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2012 *** End Date:** 02/28/2013 **Budget Period** 2

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		Stephen		Dewhurst	Ph.D.	PD/PI	[redacted]	1.20			[redacted]	[redacted]	[redacted]
2.		Changyong		Feng	PhD	Faculty	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												

Total Senior/Key Person [redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1	Post Doctoral Associates	12.00			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	6.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2012 *** End Date:** 02/28/2013 **Budget Period 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 2

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry * Start Date: 03/01/2012 * End Date: 02/28/2013 Budget Period 2

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	5[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	54.50	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency: DHHS, Robert Aaronson, 212-264-2069
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification: 1240-FINALBUDG-JUST-ROCHESTER.pdf (Only attach one file.)

Add Attachment | Delete Attachment | View Attachment

Tracking Number:GRANT10650670 Funding Opportunity Number:RFA-AI-10-011 Received Date:2010-07-09T11:07:08-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2013 *** End Date:** 02/28/2014 **Budget Period** 3

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		Stephen		Dewhurst	Ph.D.	PD/PI	[redacted]	1.80			[redacted]	[redacted]	[redacted]
2.		Changyong		Feng	PhD	Faculty	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1	Post Doctoral Associates	12.00			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2013 *** End Date:** 02/28/2014 **Budget Period 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 3

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry * Start Date: 03/01/2013 * End Date: 02/28/2014 Budget Period 3

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	54.50	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency DHHS, Robert Aaronson, [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-FINALBUDG-JUST-ROCHESTER.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2014 *** End Date:** 02/28/2015 **Budget Period** 4

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		Stephen		Dewhurst	Ph.D.	PD/PI		1.80			[redacted]	[redacted]	[redacted]
2.		Changyong		Feng	PhD	Faculty		0.60			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
												Total Senior/Key Person	[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1	Post Doctoral Associates	12.00			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2014 *** End Date:** 02/28/2015 **Budget Period 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 4

Next Period

* ORGANIZATIONAL DUNS: [redacted]

* Budget Type: ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry * Start Date: 03/01/2014 * End Date: 02/28/2015 Budget Period 4

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	54.50	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency DHHS, Robert Aaronson, [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-FINALBUDG-JUST-ROCHESTER.pdf Add Attachment Delete Attachment View Attachment

(Only attach one file.)

OMB Number: 4040-0001
Expiration Date: 06/30/2011

Previous Period

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2015 *** End Date:** 02/29/2016 **Budget Period** 5

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.		Stephen		Dewhurst	Ph.D.	PD/PI	[redacted]	1.80			[redacted]	[redacted]	[redacted]
2.		Changyong		Feng	PhD	Faculty	[redacted]	0.60			[redacted]	[redacted]	[redacted]
3.													
4.													
5.													
6.													
7.													
8.													
9.	**Total Funds requested for all Senior Key Persons in the attached file**												
											Total Senior/Key Person		[redacted]

Additional Senior Key Persons: Add Attachment Delete Attachment View Attachment

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1	Post Doctoral Associates	12.00			[redacted]	[redacted]	[redacted]
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Technician	12.00			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]

Total Salary, Wages and Fringe Benefits (A+B) [redacted]

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2015 *** End Date:** 02/29/2016 **Budget Period 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

	Equipment item	* Funds Requested ($)
1.		
2.		
3.		
4.		
5.		
6.		
7.		
8.		
9.		
10.		
11.	**Total funds requested for all equipment listed in the attached file**	
	Total Equipment	

Additional Equipment: Add Attachment Delete Attachment View Attachment

D. Travel **Funds Requested ($)**

1.	Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)	[redacted]
2.	Foreign Travel Costs	
	Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs **Funds Requested ($)**

1.	Tuition/Fees/Health Insurance	
2.	Stipends	
3.	Travel	
4.	Subsistence	
5.	Other	
	Number of Participants/Trainees **Total Participant/Trainee Support Costs**	

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ☒ Project ☐ Subaward/Consortium

Enter name of Organization: University of Rochester

Delete Entry *** Start Date:** 03/01/2015 *** End Date:** 02/29/2016 **Budget Period 5**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	[redacted]
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8.	
9.	
10.	
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. MTDC	54.50	[redacted]	[redacted]
2.			
3.			
4.			
Total Indirect Costs			[redacted]

Cognizant Federal Agency DHHS, Robert Aaronson, 212-264-2069
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification 1240-FINALBUDG-JUST-ROCHESTER.pdf Add Attachment Delete Attachment View Attachment
(Only attach one file.)

PERSONNEL JUSTIFICATION: R21 PHASE (Years 1 and 2)

UNIVERSITY OF ROCHESTER

Principle Investigator

Dr. Steve Dewhurst (PI, 1.2 person months) is Dean's Professor and Chair of Microbiology and Immunology at the UR.

ROLE: His role on the project is to oversee all of the experiments, reporting and administrative/financial responsibilities, and to interface with the other participating investigators. He already has strong collaborative relationships with these individuals. He also has over 20 years' experience as a molecular virologist, working on both RNA and DNA viruses, and is expert in the areas of HIV-1 virology, HIV-1 vaccine development and virally-mediated gene transfer. In addition, he is knowledgeable in the area of neuroscience, which is important in light of the similarities (and differences) between SEVI and other amyloid fibrils. The present project has emerged unexpectedly out of ongoing collaborative studies with Dr. Yang, and fits well with our mutual interests in SEVI and in the development of inhibitors of SEVI's biological activities.

Faculty

Dr. Chengyong Feng (Coinvestigator, 0.6 person months) is Assistant Professor of Biostatistics and Computational Biology at the UR.
ROLE: He is an established collaborator of Dr. Dewhurst, and will perform all statistical data analysis for this project. He will also provide advice on experimental design. Dr. Feng contributed the statistical analysis of all data included in this application.

Other Personnel

TBN (Postdoctoral Fellow, 12 person months; Years 1 and 2). This individual will be responsible for efficacy assessments of the ability of novel amyloid-binding compounds to inhibit SEVI- and semen-mediated enhancement of HIV-1 infection, as well as assessments of the toxicity of these novel compounds. This person will also interface with the Yang lab to assure effective exchange of reagents and data.

TBN (Laboratory Technician, 6 person months; Year 2 ONLY). A 50% FTE technician will be added to the project in year 2, to deal with the expected increase in work load as the project matures and we progress to more time-intensive secondary assays of compound efficacy and toxicity. This individual will assume responsibility for production and QC analyses of SEVI in year 2, and will work closely with the postdoctoral fellow.

Other Costs

Travel

We are requesting a small amount of travel funds ($2,000/year) to support attendance of the PI at one national meeting, and to allow Dr. Yang (UCSD subcontract PI) to attend an annual program team meeting in Rochester.

Overhead calculations

UR's approved F&A Rate is 53% for the period 03/01/11 - 06/30/11, and 54.5% thereafter

UR's cognizant agency contact is: DHHS, Robert Aaronson, Tel: [redacted]

The date of its most recent indirect cost rate negotiation agreement is: 01/21/2009

CONSULTANTS

Consultant for both R21 and R33 phases:

Dr. Charlene S. Dezzutti (Consultant) is Associate Professor in the Department of Obstetrics, Gynecology, and Reproductive Sciences, and an Associate Investigator in the Magee-Womens Research Institute at the University of Pittsburgh. Her role on this application is to serve as a consultant and advisor to the research program, to review annual progress reports, participate in data evaluations, and to provide technical assistance and guidance on assessments of the potential toxicity of candidate microbicides, using the cervical explant models that she has developed (including MTT, histology assessments of toxicity and assessment of cytokine release). She is well qualified for this role because she is the principal investigator of the Network Laboratory for the Microbicide Trials Network (MTN), an HIV/AIDS clinical trials network established by the National Institute of Allergy and Infectious Diseases (NIAID). In this capacity, she conducts side-by-side comparative assessments of different microbicide candidates that will be used in clinical trials. Her laboratory is presently studying:

1) The mechanism(s) of transmission. Dr. Dezzutti has developed cervical and colorectal tissue explant systems to study HIV transmission ex vivo.
2) The factors that influence transmission. Dr. Dezzutti has an active program studying the interaction(s) between sexually transmitted infections and HIV in acute in vitro model systems as well as tissue explant systems.
3) The ways to prevent transmission of HIV. For prevention of sexually acquired HIV, Dr. Dezzutti is evaluating microbicides that may be utilized in human clinical trials. She is examining innate immune factors found in genital secretions that could be augmented or exploited for microbicide development. She has also developed in vitro and ex vivo model systems that can be used to evaluate the potential toxicity of candidate microbicides.

Consultant for R21 phase only (and coinvestigator for R33 phase):

PERSONNEL JUSTIFICATION: R33 PHASE (Years 3 thru 5)

UNIVERSITY OF ROCHESTER

Principle Investigator

Dr. Steve Dewhurst (PI, 1.8 person months) is Dean's Professor and Chair of Microbiology and Immunology at the UR. His effort will increase to 1.8 calendar months in the R33 phase, from 1.2 calendar months in the R21 phase – reflecting the increased volume of work in the R33 phase.

ROLE: See justification for R21 phase. Added responsibilities in the R33 phase will include interfacing with, and oversight of, the [redacted] site (which will be added in the R33 phase).

Faculty

Dr. Chengyong Feng (Coinvestigator, 0.6 person months) is Assistant Professor of Biostatistics and Computational Biology at the UR.
ROLE: See justification for R21 phase.

Other Personnel

TBN (Postdoctoral Fellow, 12 person months). ROLE: See justification for R21 phase. In addition to interfacing with the UCSD site, this individual will also interface with the [redacted] (to provide materials/compounds and review data).

TBN (Laboratory Technician, 12 person months). This individual's effort will increase to 12 calendar months in the R33 phase, from 6 calendar months in the R21 phase – reflecting the increased volume of work in the R33 phase, and the fact that the postdoctoral fellow will have the additional responsibility of interfacing with the [redacted] site. ROLE: See justification for R21 phase.

Other Costs

Travel

We are requesting a small amount of travel funds ($3,000/year) to support attendance of the PI at one national meeting, and to allow Dr. Yang (UCSD subcontract PI) and [redacted] to attend an annual program team meeting in Rochester.

Overhead calculations

UR's approved F&A Rate is 53% for the period 03/01/11 - 06/30/11, and 54.5% thereafter

UR's cognizant agency contact is: DHHS, Robert Aaronson, Tel: [redacted]

The date of its most recent indirect cost rate negotiation agreement is: 01/21/2009

RESEARCH & RELATED BUDGET - Cumulative Budget

		Totals ($)
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	9	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs		
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1		
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		[redacted]
Section H, Indirect Costs		[redacted]
Section I, Total Direct and Indirect Costs (G + H)		[redacted]
Section J, Fee		

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2011 *** End Date:** 02-29-2012 **Budget Period: 1**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jerry		Yang		PD/PI	[redacted]			1.00	[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates Graduate Students Undergraduate Students Secretarial/Clerical			4.50	1.50	[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**						**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2011 *** End Date:** 02-29-2012 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2011 *** End Date:** 02-29-2012 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission Fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Cost	54.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS Office of Inspector General, Region IX, Office of Audit Services, 50 United Nations Plaza, Room 304, San Francisco, California 94102, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-FINALBUDG-JUST-UCSD[1].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2012 *** End Date:** 02-28-2013 **Budget Period: 2**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jerry		Yang		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates Graduate Students Undergraduate Students Secretarial/Clerical			4.50	1.50	[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**						**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2012 *** End Date:** 02-28-2013 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2012 *** End Date:** 02-28-2013 **Budget Period: 2**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission Fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Cost	54.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS Office of Inspector General, Region IX, Office of Audit Services, 50 United Nations Plaza, Room 304, San Francisco, California 94102, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-FINALBUDG-JUST-UCSD[1].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 3**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jerry		Yang		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates Graduate Students Undergraduate Students Secretarial/Clerical			9.00	3.00	[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**						**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 3**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission Fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Cost	54.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS Office of Inspector General, Region IX, Office of Audit Services, 50 United Nations Plaza, Room 304, San Francisco, California 94102, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-FINALBUDG-JUST-UCSD[1].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 4**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jerry		Yang		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates Graduate Students Undergraduate Students Secretarial/Clerical			9.00	3.00	[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**						**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 4**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 4

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 4**

F. Other Direct Costs	Funds Requested ($)
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Tuition Remission Fees	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	Funds Requested ($)
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	* Funds Requested ($)
1. Modified Total Direct Cost	54.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS Office of Inspector General, Region IX, Office of Audit Services, 50 United Nations Plaza, Room 304, San Francisco, California 94102, 415-437-7820

I. Total Direct and Indirect Costs	Funds Requested ($)
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	Funds Requested ($)

K. * Budget Justification File Name: 1243-FINALBUDG-JUST-UCSD[1].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 5**

A. Senior/Key Person

	Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	Dr.	Jerry		Yang		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel		* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
1	Post Doctoral Associates Graduate Students Undergraduate Students Secretarial/Clerical			2.40	0.80	[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**						**Total Other Personnel**	[redacted]
							Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 5**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	* Funds Requested ($)

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	Funds Requested ($)
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	Funds Requested ($)
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	

Number of Participants/Trainees **Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 5

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: University of California, San Diego

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 5**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. Modified Total Direct Cost	54.50	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS Office of Inspector General, Region IX, Office of Audit Services, 50 United Nations Plaza, Room 304, San Francisco, California 94102, [redacted]

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: 1243-FINALBUDG-JUST-UCSD[1].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	5
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	
10. Other 3	
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

PERSONNEL JUSTIFICATION: R21 PHASE (Years 1 and 2)

UNIVERSITY OF CALIFORNIA, SAN DIEGO (Subcontract)

CoInvestigator (Subcontract PI)

Dr. Jerry Yang (Coinvestigator, 1.0 person months; PI, UCSD subcontract) is Associate Professor of Chemistry and Biochemistry at the University of California, San Diego (UCSD).
ROLE: In collaboration with Dr. Dewhurst, Dr. Yang helped to generate the preliminary data in this application, which builds from extensive in vitro experiments carried out in the Yang laboratory, with the goal of generating molecular assemblies on aggregated amyloid peptides that can function as bio-resistive surface coatings (in analogy to generation of a “non-stick” coating on a surface). Dr. Yang has a broad background in organic and biophysical chemistry, biochemistry, and materials science, with specific expertise in amyloid and biomembrane research. His students and postdocs routinely synthesize structurally and functionally diverse organic molecules and small molecule libraries, develop biochemical and cellular screening assays, and develop biophysical techniques to study the structure and function of biomolecules and biomolecular aggregates in solution. He is an established collaborator of Dr. Dewhurst, and will be responsible for directing all aspects of the synthesis and in vitro evaluation of the amyloid-targeting agents described in the research proposal.

Other Personnel

TBN (Graduate Researcher Assistant, 6 person months, Yang Lab, UCSD). This individual will be responsible for carrying out the synthesis, purification, and characterization of all amyloid-binding agents.

Other Costs

Travel

We are requesting a small amount of travel funds ($1,000/year) to support attendance of Dr. Yang at one national meeting.

Overhead calculations

UCSD’s approved F&A Rate is 54.5%

UCSD’s cognizant agency contact is: DHHS Office of Inspector General, Region IX, Office of Audit; Tel: [redacted]

The date of its most recent indirect cost rate negotiation agreement is: 05/28/2010

PERSONNEL JUSTIFICATION: R33 PHASE (Years 3 thru 5)

UNIVERSITY OF CALIFORNIA, SAN DIEGO (Subcontract)

CoInvestigator (Subcontract PI)

Dr. Jerry Yang (Coinvestigator, 1.0 person months; PI, UCSD subcontract) is Associate Professor of Chemistry and Biochemistry at the University of California, San Diego (UCSD).
ROLE: See justification for R21 phase

Other Personnel

R33 phase: years 3 and 4:

TBN: Graduate Researcher Assistant (12 person months). This individual will be responsible for carrying out the synthesis, purification, and characterization of all amyloid-binding agents and analogs. S/he will assess new analogs for biocompatibility (i.e., drug-like properties) and toxicity. Candidates deemed suitable for further analysis for bioactivity will be prepared on a large scale for additional testing in the proposed studies.

R33 phase: year 5:

TBN: Graduate Researcher Assistant (3.2 person months). This individual will be responsible for developing the synthetic methodology for preparing large quantities of the most promising compounds for late stage testing. S/he will responsible for supplying and assuring the high quality of compounds throughout this final year of the project. Effort is reduced for the final year, because of the greater emphasis on efficacy and toxicity testing of lead candidate(s) in this final year.

Other Costs

Travel

We are requesting a small amount of travel funds ($1,000/year) to support attendance of Dr. Yang at one national meeting.

Overhead calculations

UCSD's approved F&A Rate is 54.5%

UCSD's cognizant agency contact is: DHHS Office of Inspector General, Region IX, Office of Audit; Tel: [redacted]

The date of its most recent indirect cost rate negotiation agreement is: 05/28/2010

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 1**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]		[redacted]		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	5.00			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 1**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	*** Funds Requested ($)**

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 1

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2013 *** End Date:** 02-28-2014 **Budget Period: 1**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. OVERHEAD	107.50	[redacted]	[redacted]
2. G&A	18.70	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency NIH Div. Fin. Services, Lorraine Trexler, 301-496-2444

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: [redacted] Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 2**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]		[redacted]		PD/PI	[redacted]	1.00			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	5.00			[redacted]	[redacted]	[redacted]
1	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 2**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	*** Funds Requested ($)**

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 2

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2014 *** End Date:** 02-28-2015 **Budget Period: 2**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. OVERHEAD	107.50	[redacted]	[redacted]
2. G&A	18.70	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency NIH Div. Fin. Services, Lorraine Trexler, 301-496-2444

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: [redacted] Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION A & B, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 3**

A. Senior/Key Person

Prefix	* First Name	Middle Name	* Last Name	Suffix	* Project Role	Base Salary ($)	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits ($)	* Funds Requested ($)
1.	[redacted]		[redacted]		PD/PI	[redacted]	1.20			[redacted]	[redacted]	[redacted]

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name: Mime Type: **Total Senior/Key Person** [redacted]

B. Other Personnel

* Number of Personnel	* Project Role	Cal. Months	Acad. Months	Sum. Months	* Requested Salary ($)	* Fringe Benefits	* Funds Requested ($)
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Laboratory Technician	3.60			[redacted]	[redacted]	[redacted]
1	Study Director	1.20			[redacted]	[redacted]	[redacted]
1	Animal Technician	1.20			[redacted]	[redacted]	[redacted]
1	Animal CT	1.00			[redacted]	[redacted]	[redacted]
4	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Tracking Number: GRANT10650670

OMB Number: 4040-0001
Expiration Date: 04/30/2008

RESEARCH & RELATED BUDGET - SECTION C, D, & E, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 3**

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	*** Funds Requested ($)**

Total funds requested for all equipment listed in the attached file

Total Equipment

Additional Equipment: File Name: Mime Type:

D. Travel	**Funds Requested ($)**
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	[redacted]
2. Foreign Travel Costs	
Total Travel Cost	[redacted]

E. Participant/Trainee Support Costs	**Funds Requested ($)**
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant/Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, BUDGET PERIOD 3

*** ORGANIZATIONAL DUNS:** [redacted]

*** Budget Type:** ❍ Project ● Subaward/Consortium

Enter name of Organization: [redacted]

*** Start Date:** 03-01-2015 *** End Date:** 02-29-2016 **Budget Period: 3**

F. Other Direct Costs	**Funds Requested ($)**
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Animal purchase, supplies and disposal	[redacted]
9. Consultant costs and overtime premium	[redacted]
10. Histology/Pathology Costs	[redacted]
Total Other Direct Costs	[redacted]

G. Direct Costs	**Funds Requested ($)**
Total Direct Costs (A thru F)	[redacted]

H. Indirect Costs

Indirect Cost Type	**Indirect Cost Rate (%)**	**Indirect Cost Base ($)**	*** Funds Requested ($)**
1. OVERHEAD	107.50	[redacted]	[redacted]
2. G&A	18.70	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency NIH Div. Fin. Services, Lorraine Trexler, [redacted]

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	**Funds Requested ($)**
Total Direct and Indirect Institutional Costs (G + H)	[redacted]

J. Fee	**Funds Requested ($)**

K. * Budget Justification File Name: 1244-FINALBUDG-JUST-[redacted].pdf Mime Type: application/pdf

(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)
Section A, Senior/Key Person	[redacted]
Section B, Other Personnel	[redacted]
Total Number Other Personnel	6
Total Salary, Wages and Fringe Benefits (A+B)	[redacted]
Section C, Equipment	
Section D, Travel	[redacted]
1. Domestic	[redacted]
2. Foreign	
Section E, Participant/Trainee Support Costs	
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other	
6. Number of Participants/Trainees	
Section F, Other Direct Costs	[redacted]
1. Materials and Supplies	[redacted]
2. Publication Costs	
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8. Other 1	[redacted]
9. Other 2	[redacted]
10. Other 3	[redacted]
Section G, Direct Costs (A thru F)	[redacted]
Section H, Indirect Costs	[redacted]
Section I, Total Direct and Indirect Costs (G + H)	[redacted]
Section J, Fee	

PERSONNEL JUSTIFICATION: R21 PHASE (Years 1 and 2)

[redacted]

[redacted] will not participate in the R21 phase.

PERSONNEL JUSTIFICATION: R33 PHASE (Years 3 thru 5)

[redacted] (Subcontract)

CoInvestigator (Subcontract PI)

[redacted] (Coinvestigator, 1.0 person months; PI, [redacted] subcontract) is a Senior Scientist at [redacted] effort will increase to 1.2 person months in Year 5 of the proposal, coincident with performance of the RVI experiments.
ROLE: She will be responsible for the overall management of the subaward and coordination of tasks with Dr. Dewhurst. She will provide scientific input for the *in vitro* and *in vivo* testing of the SEVI-targeting small molecule inhibitors. Specifically, she will:

- Supervise the efficacy assessment in the cervical explant model under the consultancy of Dr. C. Dezzutti (Years 3-5)
- Supervise and design the *Lactobacillus* toxicity assays (Years 3-5)
- Supervise the cytotoxicity assays (Years 3-5)
- Collaborate with [redacted] on the Rabbit Vaginal Irritation Model (Year 5)

Other Personnel

R33 phase: years 3 and 4:
[redacted] (Lab Technician, 5 person months).
ROLE: [redacted] is a lab tech trained in all of the proposed *in vitro* assays. He will conduct all aspects of tissue culture including the cervical explant model. He also will perform the lactobacillus assays. As assay endpoints for virus replication, he will use HIV-1 p24 assays, HIV-1 TaqMan RT-PCR, and reverse transcriptase assays; assays endpoints for cytotoxicity will include MTT assay as well as CytoTox-Glo and resazurin assays.

R33 phase: year 5 (includes the RVI experiment):
[redacted] (Lab Technician, 3.6 person months).
ROLE: See above. [redacted] effort is reduced because of a reduction in emphasis on *in vitro* experiments at [redacted] during the final project year, and the need to perform and analyze the RVI study.

[redacted] (Study Director, RVI; 1.2 person months).
ROLE: [redacted] is [redacted] will be the study director for the Rabbit Vaginal Irritation Model, which will be performed under 21 CFR Part 58. She will perform her duties as required in the CFR regulations, e.g., sign the study protocol, be the single point of study control and sign the final report. She also will assure that the study is carried out in compliance with 21 CFR Part 58, she will interact with management and QAU and assure that the personnel engaged in the study is trained by education and/or experience. *The goal of the RVI study will be to identify a non-irritating (maximum tolerated) dose and to "de-risk" this novel inhibitory strategy prior to future studies (planned after the end of this R21/R33). The study will be performed under 21 CFR Part 58 as a mechanism to ensure high quality data checks and QC of the analysis.*

[redacted] (Consultant)
ROLE: [redacted] is [redacted] subcontractor for the QAU unit. [redacted] provides Qau services for [redacted] since 1986, and she is employed in the field of regulatory compliance since 1967. [redacted] also regularly provides GLP training to [redacted] employees and consults [redacted] on GLP compliance issues.

TBN (Lab Animal Tech, 1.2 person months).
ROLE: An animal tech will be assigned to perform the rabbit procedures. Since the study will be conducted more than 5 years from now, we refrain from naming a specific individual. [redacted] has many animal techs on the ALAT, LAT, and LATG level available and will assure that a tech with at least an ALAT certification will be assigned to the study.

TBN (Animal Care Taker, 1 person month).
ROLE: One animal care taker will be assigned to provide food and water to the animals and ensure cleanliness of the animal room and caging. Rather than naming a specific individual, [redacted] has provided a typical animal care salary for the period of performance. [redacted] has many animal care takers on staff and will assign an appropriate individual during the spring of 2015.

Note: [redacted] person year is 1,880 hours, except the caretaker levels are bid at 2,000 hours per year. The time commitments listed above are based on these numbers.

Other Costs

Travel

In years 3-5, we are requesting a small amount of travel funds ($1,000/year) to support attendance of [redacted] at one national meeting.

Other Notable Costs

In year 5 (only), we are requesting funds ($240 per rabbit) to purchase 27 NZW rabbits for the RVI study, as well as for animal supplies and reagent costs associated with this study and its analysis.

Overhead calculations

[redacted] DFAS approved overhead rate small animal and laboratory projects is [redacted]. This has been applied to the funds requested for lab personnel (including [redacted]

[redacted] G&A (General & Administrative) rate is 18.7%. The rate is applied to all contract costs (Labor, Fringe. Overhead, Other-Direct-Costs).

[redacted] cognizant agency contact is: DFAS, Andrew Sandburg, [redacted]

The date of its most recent indirect cost rate negotiation agreement is: 09/29/2008

PHS 398 Cover Page Supplement

OMB Number: 0925-0001

1. Project Director / Principal Investigator (PD/PI)

Prefix:
* First Name: Stephen
Middle Name:
* Last Name: Dewhurst
Suffix: Ph.D.

2. Human Subjects

Clinical Trial? ☒ No ☐ Yes

* Agency-Defined Phase III Clinical Trial? ☐ No ☐ Yes

3. Applicant Organization Contact

Person to be contacted on matters involving this application

Prefix:
* First Name: Brenda
Middle Name:
* Last Name: Kavanaugh
Suffix:

* Phone Number: [redacted]
Fax Number: [redacted]
Email: [redacted]

* Title: Senior Research Administrator

* Street1: Office of Research & Project Administration
Street2: 518 Hylan Building
* City: Rochester
County/Parish:
* State: NY: New York
Province:
* Country: USA: UNITED STATES
* Zip / Postal Code: 14627-0140

PHS 398 Cover Page Supplement

4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells? ☒ No ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s): ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Tracking Number:GRANT10650670 Funding Opportunity Number:RFA-AI-10-011 Received Date:2010-07-09T11:07:08-04:00

OMB Number: 0925-0001

PHS 398 Research Plan

1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

1. Introduction to Application (for RESUBMISSION or REVISION only)		Add Attachment	Delete Attachment	View Attachment
2. Specific Aims	1241-AIMS-MIP.pdf	Add Attachment	Delete Attachment	View Attachment
3. *Research Strategy	1242-FINALRESSTRAT-MIPrv[1]	Add Attachment	Delete Attachment	View Attachment
4. Inclusion Enrollment Report		Add Attachment	Delete Attachment	View Attachment
5. Progress Report Publication List		Add Attachment	Delete Attachment	View Attachment

Human Subjects Sections

6. Protection of Human Subjects	1250-HUMSUB-PROTXN-MIP.pdf	Add Attachment	Delete Attachment	View Attachment
7. Inclusion of Women and Minorities	1251-HUMSUBJ-WomMinor-MIP.p	Add Attachment	Delete Attachment	View Attachment
8. Targeted/Planned Enrollment Table	1252-HUBSUBJ-ENROLTAB-MIP.p	Add Attachment	Delete Attachment	View Attachment
9. Inclusion of Children	1253-HUMSUBJ-KIDS-MIP.pdf	Add Attachment	Delete Attachment	View Attachment

Other Research Plan Sections

10. Vertebrate Animals	1254-VERTANIM-MIP.pdf	Add Attachment	Delete Attachment	View Attachment
11. Select Agent Research	1255-SELECTAG-MIP.pdf	Add Attachment	Delete Attachment	View Attachment
12. Multiple PD/PI Leadership Plan		Add Attachment	Delete Attachment	View Attachment
13. Consortium/Contractual Arrangements	1256-CONSORTIA-MIP.pdf	Add Attachment	Delete Attachment	View Attachment
14. Letters of Support	1257-LOS-Setof3-070710.pdf	Add Attachment	Delete Attachment	View Attachment
15. Resource Sharing Plan(s)	1258-RESSHARE-MIPrv.pdf	Add Attachment	Delete Attachment	View Attachment

16. Appendix Add Attachments | Remove Attachments | View Attachments

SPECIFIC AIMS: The Semen Enhancer of HIV Infection as a Novel Microbicide Target

Human semen contains a proteolytic cleavage product of prostatic acidic phosphatase (PAP), designated PAP(248-286), that spontaneously forms cationic amyloid fibrils which strongly enhance HIV-1 infection. These fibrils, termed the "Semen Enhancer of Virus Infection" (SEVI), have been postulated to play an important role in HIV-1 transmission.

Our preliminary data show that amyloid-binding molecules bind to SEVI, and block semen-mediated enhancement of HIV-1 infection. This suggests that (i) SEVI is responsible for semen-mediated enhancement of HIV infection and (ii) SEVI represents a novel microbicide target. ***We therefore propose to explore a novel, innovative approach to HIV-1 microbicide development, using agents that selectively target SEVI.*** This high-risk/high-reward approach is fundamentally different from traditional microbicidal strategies that target the virus itself, and is expected to be highly complementary with direct antiviral approaches. Indeed, our long-term goal is to use SEVI-targeted agents in combination with traditional microbicides. R21 phase aims are:

- **Aim 1: To test whether novel amyloid-binding small molecules inhibit semen-mediated enhancement of HIV infection.** The feasibility of this approach has been established using two amyloid-binding small molecules which contain “shielding” oligo-ethylene glycol (EG) moieities: BTA-EG_4 and –EG_6. These agents efficiently inhibit SEVI- and semen-mediated enhancement of HIV infection. We will generate novel derivatives of these and other amyloid-binding molecules, including oligovalent molecules that are expected to possess increased SEVI binding affinity. We will then measure their affinity for SEVI, and we will assess their ability to inhibit SEVI- and semen- mediated enhancement of HIV infection using a panel of R5 virus strains, including variants associated with heterosexual virus transmission and multidrug resistant virus.
- **Aim 2: To examine the interaction between novel amyloid-binding small molecules and cells from the female reproductive tract.** Toxicity of candidate microbicides to cells of the female reproductive tract has been associated with an increased risk of HIV-1 transmission. In Aim 2A, we will assess the toxicity of our small molecules for human cervicovaginal epithelial cells (HCEC). In Aim 2B, we will perform efficacy assessments using HCEC. First, we will test whether our small molecules can inhibit (i) SEVI-enhanced binding of HIV-1 virus particles to HCEC, and (ii) SEVI-enhanced *trans*-infection of PBMC by HCEC exposed to HIV-1 virions. Finally, since binding of HIV-1 virions to HCEC elicits the release of pro-inflammatory chemokines that may recruit CD4+ target cells to the initial site of virus infection, we will test whether our small molecules inhibit SEVI-mediated enhancement of HIV-1 induced chemokine release by HCEC.

The R33 phase will further develop the candidate agents identified in the R21 phase. R33 phase aims are:

- **Aim 3: To improve the efficacy of first generation compounds identified in the R21 phase.** In Aim 3A, we will synthesize improved compounds by using structure-activity relationship (SAR) data to refine chemical composition. We will then test these molecules using the efficacy and toxicity testing assays delineated in the R21 phase, prior to evaluating their activity in a pH transition assay and their physicochemical stability in seminal plasma, artificial vaginal fluid and the Universal Placebo gel, hydroxyethyl cellulose. In Aim 3B, we will assess the efficacy of the most promising compounds in a cervical explant model for HIV-1 infection. Finally, in Aim 3C, we will test whether our lead molecules have a synergistic or additive effect on the ability of other microbicides to inhibit HIV-1 infection in the presence of semen.
- **Aim 4: To assess the toxicity and inflammatory effects of lead molecules.** We will assess the toxicity and inflammatory effects of the most promising molecules, both in PBS and as a simple admixture in HEC. The following parameters will be measured: (i) toxicity to beneficial *Lactobacillus* species; (ii) effect on epithelial monolayer integrity; (iii) toxicity and inflammatory effects on human cervical explant tissue. Finally, the R33 phase will culminate with an evaluation of the safety and tolerability of the most promising compound in the rabbit vaginal irritation (RVI) model. The goal of these studies is not to obtain definitive IND-enabling data, but rather to rigorously assess the potential toxicity of our lead molecules so as determine whether they merit progression to future studies at the end of the R33 phase.

RESEARCH STRATEGY

SIGNIFICANCE

Problem to be addressed: The urgent need for fresh approaches to microbicide development

NIH RFA-AI-10-011 notes that a "*topical microbicide that prevents the sexual transmission of HIV could play a major role in world-wide reduction of the over 7,000 new HIV infections per day*." To date, however, no such product has been identified – and several promising candidates have failed in phase III trials.

The nonionic surfactant, nonoxynol-9 (N9) showed robust antiviral activity in preclinical analyses, but failed to protect against virus transmission in clinical trials and increased the risk of HIV-1 infection in women who used the agent repeatedly (1, 2) due to toxic effects on female reproductive tissue (3). The anionic polymer PRO-2000 also showed robust activity in preclinical studies (4-6), but failed to show efficacy in MDP 301 trial (7, 8), possibly becauses its antiviral activity is inhibited by seminal plasma (9, 10).

Collectively, these results establish the urgent need for fresh, new approaches to microbicide development.

The Semen Enhancer of Virus Infection (SEVI): Potential contributions to virus transmission

Human semen contains cationic amyloid fibrils that strongly enhance HIV-1 infection (11), and have been termed "Semen Enhancer of Virus Infection" (SEVI). Multiple laboratories (including those of Drs. Greene, Hahn, Kirchhoff & Shaw), as well as our own, have shown that: both purified SEVI and whole semen enhance HIV-1 infection in cultured cells - including primary cells exposed to R5 viruses (11-15) (**Fig. 2-5**). Moreover, the ability of individual semen samples to enhance HIV-1 infection is correlated with levels of SEVI (15).

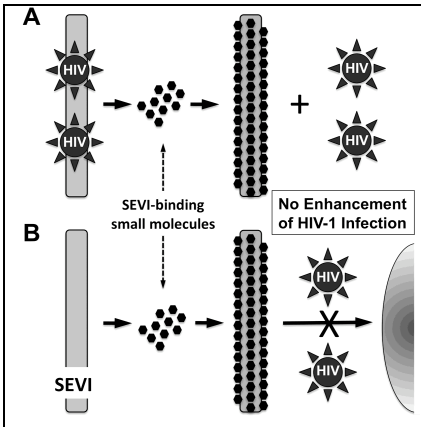
SEVI may also exert effects through human cervicovaginal epithelial cells (HCEC). Binding of HIV-1 virions to HCEC results in release of chemokines (including IL-8, CCL20/MIP-3α) which may accelerate CD4+ T cell recruitment to the initial site of virus infection (16) - thereby contributing to the sexual transmission of HIV-1. Consistent with this, our preliminary data show that SEVI enhances virus binding to HCEC and that it also increases virally-mediated chemokine release from these cells (**Fig. 12**).

INNOVATION

Novel concept and innovation: Development of a novel class of microbicidal agents

Our preliminary data show that amyloid-binding molecules bind to SEVI, and block semen-mediated enhancement of HIV-1 infection. This suggests that (i) SEVI is responsible for semen-mediated enhancement of HIV infection and (ii) SEVI represents a novel microbicide target. ***We will therefore test a novel, innovative approach to HIV-1 microbicide development, by testing agents that target SEVI.***

Figure 1: Inhibition of SEVI-mediated infection enhancement using amyloid-binding small molecules



LEGEND: *SEVI-binding compounds that provide extensive surface coverage willl prevent binding of SEVI to HIV virions* ***(A)*** *and host cell membranes* ***(B)****. Effective small molecules include amyloid-binding compounds conjugated to oligo(ethylene glycol) that are capable of sequestering a large surface area. Blockade of virion binding* ***(A)*** *will interfere with enhancement of virus infection. Blockade of cell binding* ***(B)*** *may also interfere with virally-induced immune activation of genital epithelial cells (16).*

This high-risk/high-reward approach is fundamentally different from traditional microbicidal strategies that target the virus itself. The feasibility of this approach has been established using two amyloid-binding small molecules which contain "shielding" oligo-ethylene glycol (EG) moieities: BTA-EG_4 and $-EG_6$ (**Fig. 1**). These agents inhibit both SEVI- and semen-mediated enhancement of HIV infection.

Impact statement: How completion of the project will change concepts that drive this field

If this high-risk/high-reward project is successful it will result in the identification of a novel class of microbidical agents. Our approach is fundamentally different from traditional microbicidal strategies that target

the virus itself, and is expected to be highly complementary with direct antiviral approaches. Indeed, our long-term goal is to use SEVI-targeting agents in combination with traditional microbicides, for optimal effectiveness.

Finally, our approach is also different from previously described SEVI inhibitors, such as polyanionic compounds (17), and the heparin antagonist Surfen (18). These agents block the actions of SEVI on a simple electrostatic basis. Given recent negative clinical results with the polyanionic agent PRO-2000 (8), and the presence of small cationic peptides in both semen and cervicovaginal fluid (19, 20), this raises concerns. In contrast, amyloid binding small molecules do not rely on electrostatic properties to bind to SEVI, and are expected to offer a new class of SEVI inhibitors with reduced off-target effects and enhanced effectiveness in an *in vivo* setting.

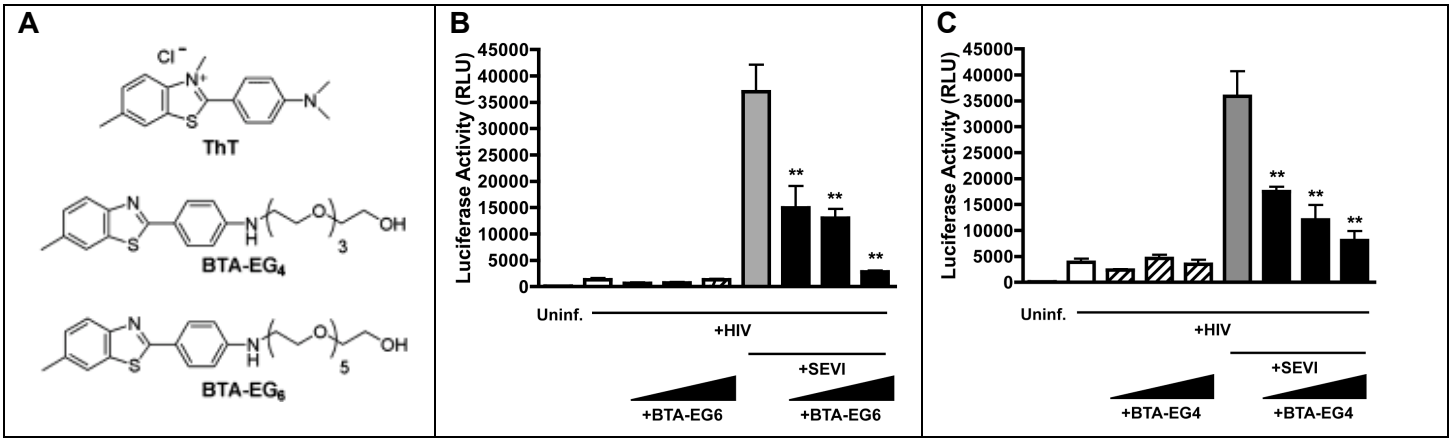
Data supporting this novel concept

Molecules derived from ThioT intercalate into generic cross-ß fibril structures derived from a large variety of amyloid peptides, and have been used image fibrils *in vivo* (21, 22) and to inhibit protein interactions with amyloid fibrils (23). Initial studies have been conducted using lead compounds encompassing a benzothiazole aniline derivative carrying a oligo(ethylene glycol) moiety (BTA-EG_4 and BTA-EG_6, **Fig. 2A;** (23)) which:

(1) Obey Lipinski's Rules of five and are, hence, drug-like. The Mw of BTA-EG_4 is 418 g/mol, it has an octanol-water partition coefficient (log P) of 1.05, and a topological polar surface area (PSA) of 91 $Å^2$ (23). The Mw of BTA-EG_6 is 504 g/mol, with a log P of 1.43, and a PSA of 73 $Å^2$ (23).
(2) Bind efficiently to aggregated forms of amyloid peptides and to SEVI. The K_d of BTA-EG_6 is 8 µM for binding to Aβ fibrils, and 44 µM for binding to SEVI.
(3) Can be readily prepared in high purity and quantity.

Fig 2 shows that BTA-EG_6 and BTA-EG_4 both exert a dose-dependent, statistically significant ($p<0.001$) inhibitory effect on SEVI-mediated enhancement of HIV-1 infectivity (see dark filled bars, **Fig. 2B, 2C**). BTA-EG_6 and BTA-EG_4 had no effect on HIV-1 infection in the absence of SEVI (see hatched bars, **Fig. 2B, 2C**).

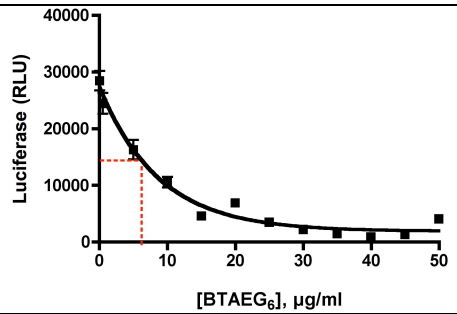
Figure 2: BTA-EG_6 and BTA-EG_4 inhibit SEVI mediated enhancement of HIV infection



LEGEND: ***(A)*** *Shown are ThioT and 2 analogs (BTA-EG_4, BTA-EG_6) (23).* ***(B, C)*** *CEM-M7 cells were exposed to HIV-1 (IIIB) virions for 2 hr in the absence or presence (25 µg/mL) of SEVI; BTA-EG_6* ***(B)*** *or BTA-EG_4* ***(C)*** *were added at concentrations of 5.5, 11 and 16.5 µg/mL. At 2 hr cells were washed in PBS and resuspended in RPMI-1640 media with 20% FBS. Cells were collected by centrifugation after 72 hrs, and luciferase activity was measured in cell lysates (Promega). CEM-M7 cells are stably transduced with a HIV-1 long terminal repeat (LTR)-luciferase and LTR-green fluorescent protein (GFP) cassette. The HIV-1 LTR has weak constitutive activity but becomes strongly activated upon intracellular expression of the HIV-1 transactivator, Tat. As a result, luciferase expression provides a measure for HIV-1 infection in CEM-M7 cells. Data represent mean values from triplicate infections; bars denote standard deviation. **: indicates $p<0.001$ (one way ANOVA), when compared to cells treated with HIV-1 plus SEVI alone (gray shaded bar).*

Since most sexually transmitted HIV-1 infections are the result of R5 viruses (24), we examined whether the effect of BTA-EG_6 extended to a well-characterized R5 strain. CEM-M7 cells were infected with HIV-1_{ADA} and SEVI, with and without increasing concentrations of BTA-EG_6. BTA-EG_6 showed a significant dose dependent inhibition of SEVI mediated enhancement of HIV-1_{ADA} infection (**Fig. 3**). The IC_{50} of the BTA-EG_6 for inhibition of SEVI-mediated enhancement of HIV-1_{ADA} infection was calculated from this experiment and determined to be 6.6 µg/mL (13 µM).

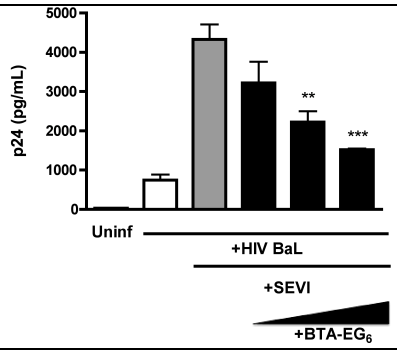
Fig 3: BTA-EG_6 inhibits SEVI-mediated enhancement of R5 HIV-1_{ADA} infection with an IC_{50} of 13 µM



LEGEND: *Infectious R5 HIV-1 (ADA; 33 ng of virus, as determined by p24 ELISA assay) was incubated in the presence or absence of 15 µg/mL of SEVI with or without increasing concentrations of BTA-EG_6 (0.4 to 50 µg/mL) as indicated. The samples were then added to CEM M7 cells. Cells were washed at 2 hours, and infection was assayed at 48 hours. Results shown are average values +/- SD of triplicate measurements. An exponential decay curve was then fit to the data, and used to calculate the IC_{50} of BTA-EG_6's inhibitory effect on SEVI-mediated enhancement of HIV-1_{ADA} infection.*

We tested whether the effects of SEVI and BTA-EG_6 extended to infection in primary cells. To do this, PBMCs were exposed to HIV-1_{BAL} plus SEVI, in the presence or absence of BTA-EG_6. BTA-EG_6 efficiently inhibited SEVI-mediated enhancement of HIV-1 infection in PBMCs, in a dose-dependent manner (**Fig. 4**).

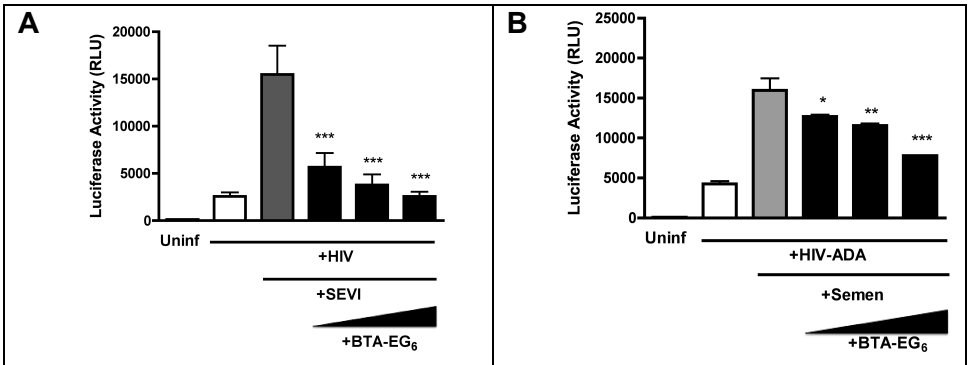
Fig 4: BTA-EG_6 inhibits SEVI-mediated enhancement of R5 HIV-1 infection in primary PBMC



LEGEND: *Human PBMCs were stimulated with IL-2/PHA and infected with HIV-1_{BAL} and 15 µg/mL SEVI with or without increasing concentrations of BTA-EG_6 (5.5, 11 and 22.5 µg/mL). Cells were washed at 3 hours and infection was assayed at 4 days by measuring HIV-1 p24 antigen in cell lysates using an ELISA assay. Results shown are average values +/- SD of triplicate measurements. **: p<0.01 or ***: p< 0.001, compared to control PBMCs exposed to HIV-1_{ADA} + SEVI alone (ANOVA with Tukey's post-test).*

We also examined the effect of BTA-EG_6 and BTA-EG_4 on semen-mediated enhancement of HIV-1 infection (**Fig. 5**). Since human semen can be toxic if added directly at high concentration to cultured cells (25), we followed an established protocol to minimize this toxicity (11, 15) (see **Fig 5** legend).

Figure 5: BTA-EG_6 inhibits semen-mediated enhancement of HIV infection



LEGEND: (A) *HIV-1_{IIIB} virions were preincubated with 50% pooled human semen, +/- increasing concentrations of BTA-EG_6 (5.5, 11 and 22.5 µg/mL). After 10 minutes these stocks were diluted 15 fold into CEM M7 cells. Cells were washed after 1 hr and luciferase expression was measured at 48 hrs to quantify the extent of infection. Results shown are average values +/- SD of triplicate measurements from one of 3 independent experiments that yielded equivalent results. ***: indicates p<0.001 compared to control cells exposed to HIV-1_{IIIB} + semen alone (gray shaded bar), by ANOVA with Tukey's post-test.* ***(B)*** *Cells were treated as above, but with HIV-1_{ADA} and a 50% concentration of an individual semen sample. *: p<0.05, **: p<0.01, ***: p<0.001 compared to control cells exposed to HIV-1_{ADA} + semen alone; ANOVA w. Tukey's post-test.*

As shown in **Fig. 5A**, BTA-EG_6 efficiently inhibited the semen mediated enhancement of HIV-1 infection, at similar concentrations to those active against SEVI alone. **Fig 5B** shows that this effect extended to infection with an R5 virus, HIV_{ADA}, as well. Similar results were obtained with BTA-EG_4 (not shown, for reasons of space).

Collectively, our data show that the novel amyloid-binding small molecules BTA-EG_6 and BTA-EG_4:

(1) Inhibit SEVI- (**Fig 2-4**) and semen- (**Fig 5**) mediated enhancement of HIV-1 infection by X4 and R5 viruses, in both cell lines and primary PBMC.

(2) Inhibit SEVI-mediated enhancement of HIV-1 infection with an IC_{50} of 13 μM for BTA-EG_6 (**Fig. 3**) and of 9 μM for BTA-EG_4 (not shown).

(3) Are non-toxic and non-inflammatory to human cervicovaginal epithelial cells - including A2En cells, which are an immortalized line derived from primary endocervical tissue (26, 27), as well as SiHa cervical carcinoma cells (28). Data are not shown for reasons of space, but BTA-EG_6 was non-toxic at concentrations up to at least 66 μM (i.e., 10x the IC_{50}). This therapeutic index ($\geq$10) is above the threshold necessary to warrant in-depth followup analysis of candidate topical microbicides (29).

APPROACH: R21 PHASE

AIM 1: To test whether novel amyloid-binding small molecules inhibit semen-mediated enhancement of HIV infection.

Overview: We propose to explore a novel, innovative approach to HIV-1 microbicide development, by using novel amyloid binding small molecules to selectively target SEVI (**Fig. 1**). The feasibility of this approach has been established using two amyloid-binding small molecules which contain "shielding" oligo-ethylene glycol (EG) moieities: BTA-EG_4 and –EG_6. These agents potently inhibit SEVI- and semen-mediated enhancement of HIV infection. In Aim 1, we will generate novel derivatives of these and other amyloid-binding molecules, including oligovalent molecules that are expected to possess increased SEVI binding affinity. We will then measure their binding affinity for SEVI, and we will assess their ability to inhibit SEVI- and semen- mediated enhancement of HIV infection using a panel of R5 virus strains, including variants associated with heterosexual virus transmission and multidrug-resistant virus.

Aim 1A: Generation of novel amyloid-binding molecules, including derivatives of BTA-EG_6 & BTA-EG_4.

Overview: Dr. Yang has developed additional, biocompatible amyloid-binding molecules that will be evaluated for that activity against SEVI. He has also developed methods for the synthesis of oligomeric analogs of BTA-EG_4 and BTA-EG_6, which we will also test. We anticipate that these oligovalent analogs of BTA-EG_x will show improved efficacy for inhibiting SEVI-mediated enhancement of HIV infection due to their increased affinity for SEVI and increased size compared to monovalent BTA analogs.

Approach: The Yang lab will supply BTA-EG_4 and BTA-EG_6 over the course of the project period. The Yang lab has already been able to synthesize BTA-EG_4 on the multi gram-scale and will perform a large-scale preparation of BTA-EG_6 using an analogous procedure. BTA-EG_6 exhibits *in vitro* all of the promising beneficial properties as BTA-EG_4 as a drug candidate (see preliminary studies; (23)), but has an additional potential advantage in that it is significantly more soluble in aqueous solutions than BTA-EG_4.

To assess whether other classes of amyloid-targeting molecules affect SEVI-mediated infection of HIV, we will also evaluate two additional amyloid-targeting candidates (ANCA-11 and CA-140) recently developed in the Yang lab (**Fig. 6**). These molecules were developed through rational design based on a molecular rotor motif (30) or through structure-binding optimization using a screening assay developed in the Yang lab (31). Briefly, both molecules exhibit: 1) drug-like properties, 2) solubility in aqueous solutions at > 1 mg/mL, 3) no significant toxicity to SH-SY5Y cells at up to 100 μM, and 4) low micromolar affinity for aggregated Aβ peptides (30). ANCA-11 and CA-140 associate with aggregated amyloid deposits in brain sections and also stain PrP (prion) deposits in brain sections (not shown), suggesting that they may bind effectively to many different types of amyloid fibrils - including SEVI. The Yang lab has synthesized both of these molecules in > 400 mg quantities.

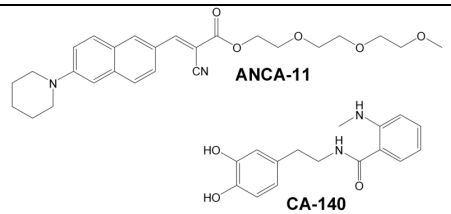


Figure 6. Structures of novel amyloid-binding molecules

LEGEND. *Structures of proprietary amyloid-targeting agents ANCA-11 and CA-140 developed in the Yang lab.*

Oligomeric derivatives of BTA-EG_6 and BTA-EG_4 will also be produced and tested in the R21 phase. **Fig. 7** outlines the structures and syntheses of a monomer (CC-1), dimer (CC-2), and trimer (CC-3) of BTA-EG_4.

Figure 7. Scheme for the synthesis of oligomeric analogs of BTA-EG_4.

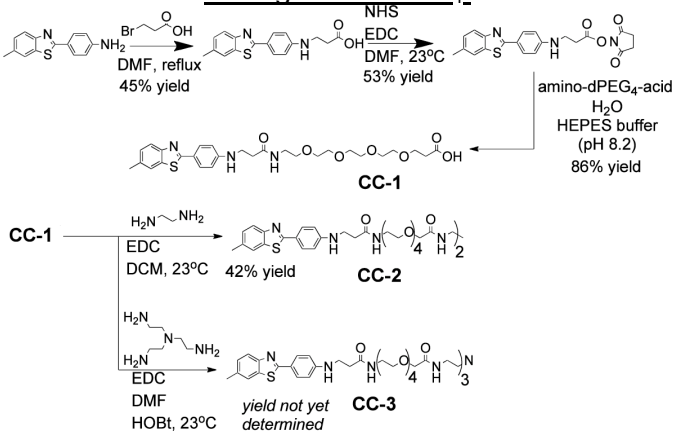
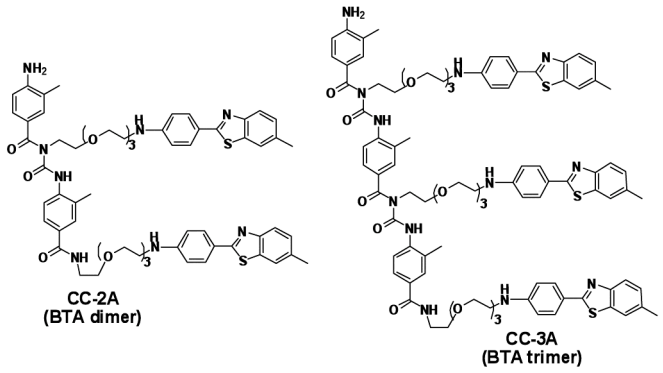


Figure 8. Proposed oligomeric analogs of BTA with a rigid scaffold.



LEGEND: FIG 7: *6-methyl benzothiazole aniline (6-Me-BTA) was purchased from City Chemical, West Haven, CT. Amino-dPEG-acid was from Quanta Biodesign, Powell, OH. All other chemicals were from Sigma-Aldrich. All final compounds were characterized by NMR and MS and determined to be > 95% pure. All yields shown are isolated yield.*

The binding constant (K_d) of CC-1, CC-2, and CC-3 to aggregated Alzheimer's Aβ(1-42) peptides was measured using a fluorescence binding assay (31) and found to be 26, 18 and 2 nM, respectively. These results show that (i) **identification of small molecules with low nanomolar binding affinity for amyloid fibrils is feasible** (which has implications for new SEVI-binding molecules) and (ii) that **simple trimerization can result in a >10-fold improvement in amyloid binding-affinity.** Since larger oligomers may exhibit even greater affinity, Dr. Yang will also synthesize a second class of multivalent agents, where the BTA moieties will extend from a rigid scaffold. Here, a synthetic strategy first reported by Hamilton (32) will be used to synthesize discrete oligomers of BTA-EG_x (examples of dimers & trimers are shown in **Fig. 8**).

Expected results, potential pitfalls and solutions: We do not anticipate any significant difficulties with compound synthesis. Should some of our novel compounds be colored, we will employ a differential standard for advancing active compounds on the basis of color, as previously explained by [redacted] (29) (**Fig. 11**).

Aim 1B: Measurement of SEVI-binding by novel amyloid-binding compounds.

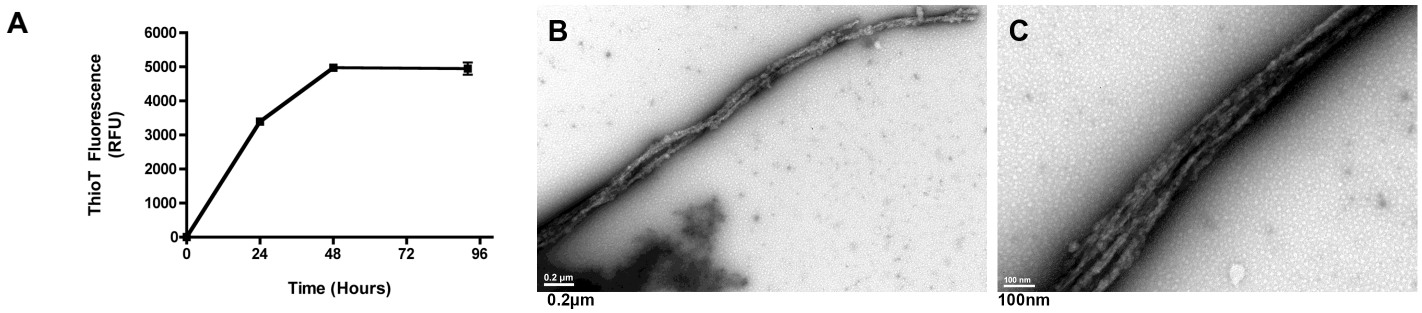
Overview: We will assess whether our novel compounds bind SEVI using a fluorescence polarization (FP) assay, and then measure the binding affinity for SEVI using a fluorescence binding assay (31).

QC of SEVI fibrils (applies to all Aims): A single large batch of PAP[248-286] peptide will be purchased and fibrillized as described (**Fig. 9**). Fibril formation will be confirmed by ThioT staining, and verified a physical method (TEM or AFM; UR Core Facilities). All peptides and fibrils will also be tested for LPS by the Limulus amebocyte lysate (LAL) assay, and discarded if positive. *Finally, SEVI will be generated from disaggregated peptide stocks to remove preformed aggregates or seeds that can alter self-assembly (33).*

Approach: To assess SEVI-binding by our novel compounds, we will use a fluorescence polarization (FP) assay. To do this, test compounds will be added to SEVI fibrils that have been preincubated with FITC-heparin, a known SEVI binder (13). This FP assay has already been used to validate that BTA-EG_6 binds SEVI in a dose dependent fashion (**Fig. 10**), and can be readily adapted to a high-throughput format. After initial FP assays, we will determine the SEVI binding affinity for our compounds using a fluorescence binding assay (31).

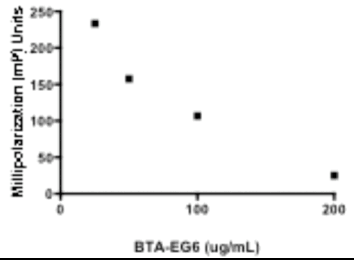
Expected results, potential pitfalls & solutions: We do not expect major problems, since the assays are developed and validated. Based on the binding affinities of oligomeric derivatives of BTA-EG_x for Aß aggregates, we expect that their affinity for SEVI will be 1 μM or less (i.e., at least 10-fold better than monomeric compounds).

Figure 9: Kinetics of SEVI formation and EM visualization of fibril morphology



LEGEND: (A) *HPLC purified PAP[248-286] peptide (10 mg/ml in PBS) was agitated at 1400RPM while incubating at 37°C. Samples were collected at 0, 24, 48, 72 hrs and subjected to Thioflavin T (ThioT) analysis. RFU values reported represent measured RFU values minus background (RFU for PBS alone with no added peptide).* ***(B, C)*** *EM images of SEVI fibrils produced in (A) are shown. Numbers represent the length of the scale bars (shown in white).*

Figure 10: Fluorescence polarization analysis reveals binding of BTA-EG_6 to SEVI fibrils



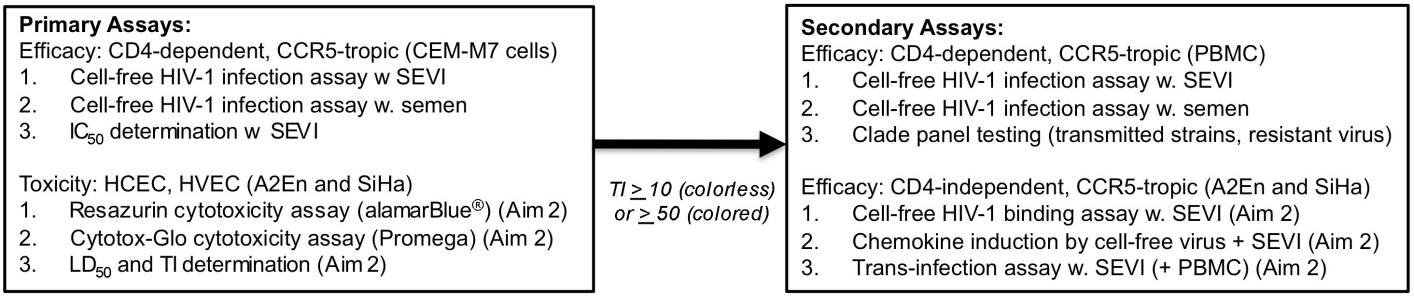
LEGEND: *100 µg/mL of SEVI was mixed with 16 µg/mL FITC-Heparin in varying concentrations of BTA-EG_6 ranging from 0 to 200 µg/mL. Samples were incubated 1 hr at RT and polarized fluorescence intensities were measured. Decreased mP units indicate a displacement of FITC-heparin from SEVI fibrils due to BTA-EG_6 binding.*

Aim 1C: Assessment of the ability of novel amyloid-binding compounds to inhibit SEVI- and semen-mediated enhancement of HIV-1 infection.

Overview: We will assess the ability of our novel compounds to inhibit SEVI- and semen- mediated enhancement of HIV infection using X4 and R5 virus strains, including variants associated with heterosexual virus transmission and multidrug resistant virus. We will also determine the IC_{50} for our compounds.

Approach: Microbicides that target SEVI require a different screening algorithm than "conventional" microbicides, because they do not target the virus itself, and also because SEVI has no documented activity in cell-associated HIV-1 transmission assays. Our screening algorithm for the R21 phase is shown in **Fig. 11**; it is adapted from [redacted] *in vitro* screening algorithm for "conventional" microbicide development (29).

Figure 11: Efficacy and toxicity screening algorithm for novel amyloid-binding compounds (R21 phase)



Primary efficacy assays (cell infection/CEM-M7):

1. Cell-free HIV-1 infection with SEVI (CEM-M7 target cells). Assessment of the ability of novel compounds to inhibit SEVI-mediated enhancement of R5 HIV-1 (HIV-1_{ADA}) infection in CEM-M7 cells. See **Fig. 3** for methods. *Because of its simplicity and low cost, this assay will be used as the principle initial screen for compound efficacy. Only a subset of compounds will be progressed from this assay to followup assays, since otherwise the potential burden of work may become overwhelming.*
2. Cell-free HIV-1 infection with pooled human semen (CEM-M7 target cells). Assessment of the ability of novel compounds to inhibit semen-mediated enhancement of HIV-1_{ADA} infection in CEM-M7 cells. See

Fig. 5 for methods. *Since several candidate microbicides have shown reduced activity in the presence of semen (9, 34), this assay will be used as the second major triage point for test molecules.*

3. IC_{50} determination. Serial 2-fold compound dilutions spanning the active concentration will be used to determine the IC_{50} for blockade of SEVI-mediated enhancement of HIV-1 infection in CEM-M7 cells. For compounds with promising initial TI values (≥ 10), we will also determine the IC_{50} for blockade of semen-mediated enhancement of HIV-1 infection in CEM-M7 cells. *This will not, however, be done on a routine basis since supplies of semen are limiting, and insufficient for routine IC_{50} assays.*

- *Note: For assays #1, 2, at least 6 half log_{10} compound dilutions will be tested, in triplicate (29).*

Secondary efficacy assays (cell infection/PBMC): Compounds will be progressed to secondary efficacy assays only if they have a therapeutic index (TI) of ≥10 (for non-colored compounds) or ≥50 (for colored compounds) (29). PBMC assays will be performed using cells from at least 2 donors to ensure reproducibility:

1. Cell-free HIV-1 infection with SEVI (PBMC target cells). Assessment of the ability of novel compounds to inhibit SEVI-mediated enhancement of R5 HIV-1 (HIV-1_{BaL}) infection in PBMC. Method: see **Fig. 4**.
2. Cell-free HIV-1 infection with pooled human semen (PBMC targets). Assessment of the ability of novel compounds to inhibit semen-mediated enhancement of HIV-1_{BaL} infection in PBMC. Method: **Fig. 5**.
3. Clade panel testing (transmitted strains, resistant virus). Assessment of the ability of compounds to inhibit SEVI-mediated enhancement of HIV-1 infection by: (A) 2 transmitted HIV-1 infectious molecular clones (AIDS Reagent Repository [ARR] #11919) (35, 36), (B) 2 R5-using clade A and C viruses (ARR), and (C) a multi-drug resistant virus (ARR; HIV-1 AD.MDR01 #11700) (37, 38). Method: **Fig. 4**.

- *Note: For assays #1, 2, at least 6 half log_{10} compound dilutions will be tested, in triplicate (29).*

Expected results, potential pitfalls and solutions: BTA-EG_6 has a TI ≥10 and possesses activity in both primary and secondary efficacy assays, as well as in the presence of semen. We anticipate that oligomeric derivatives of this and related molecules will have considerably improved potency (by at least 10-fold or more, based on expected gains in SEVI-binding affinity). Thus, we anticipate that multiple compounds will show significant activity in secondary efficacy assays. The fact that BTA-EG_6 inhibits SEVI-mediated enhancement of infection by multiple HIV-1 strains (**Fig. 2-5**) suggests that it is likely to be active against multiple clades.

AIM 2: To examine the interaction between novel amyloid-binding small molecules and cells from the female reproductive tract.

Overview: Toxicity of candidate microbicides to cells of the female reproductive tract has been associated with an increased risk of HIV-1 transmission (1, 4, 39). In Aim 2A, we will assess the toxicity of our small molecules for human cervicovaginal epithelial cells (HCEC). In Aim 2B, we will perform efficacy assessments using HCEC. First, we will test whether our small molecules can inhibit (i) SEVI-enhanced binding of HIV-1 virus particles to HCEC, and (ii) SEVI-enhanced *trans*-infection of PBMC by HCEC exposed to HIV-1 virions. Finally, since binding of HIV-1 virions to HCEC elicits the release of pro-inflammatory chemokines that may recruit CD4+ target cells to the initial site of virus infection, we will test whether our small molecules inhibit SEVI-mediated enhancement of HIV-1 induced chemokine release by HCEC.

Aim 2A: Assessment of toxicity of novel amyloid-binding small molecules to cells from the female reproductive tract: We will assess the toxicity of our small molecules in A2En cells, which are an immortalized line derived from primary endocervical tissue (26, 27), as well as SiHa cervical carcinoma cells (28).

Primary toxicity assays (HCEC): Nonoxynol-9 (0.1% final) will be used as a positive toxicity control (40).

1. Resazurin cytotoxicity assay (alamarBlue®) in A2En and SiHa cells.
2. Cytotox-Glo cytotoxicity assay (Promega) in A2En and SiHa cells. *For assays #1 & #2, at least 6 half log_{10} compound dilutions will be tested, in triplicate (29), and the TC_{50} will be calculated.*
3. Determination of therapeutic index (TI). *Compounds will be progressed to secondary efficacy assays (Aim 2B) only if TI ≥10 (for non-colored compounds) or ≥50 (for colored compounds) (29) (**Fig. 11**).*

Expected results, potential pitfalls & solutions: BTA-EG_6 has a TC_{50} of > 66 μM and a TI of ≥10. Oligomeric derivatives of BTA-EG_x are expected to have improved efficacy but similar toxicity – leading to improved TIs.

Aim 2B: Assessment of efficacy of novel amyloid-binding small molecules in assays using cells from the female reproductive tract: We will perform efficacy assessments using A2En and SiHa cells. First, we will test whether our small molecules can inhibit (i) SEVI-enhanced binding of HIV-1 virus particles to A2En and SiHA cells, and (ii) SEVI-enhanced *trans*-infection of PBMC by A2En cells exposed to HIV-1 virions. Finally, since binding of HIV-1 virions to vaginal epithelial cells elicits the release of pro-inflammatory chemokines that may recruit CD4+ target cells to the site of virus infection (16), we will test whether our small molecules inhibit SEVI-mediated enhancement of HIV-1 induced chemokine release by A2En and SiHa cells.

Secondary efficacy assays (HCEC): See **Fig. 11**. Assays will be as follows:

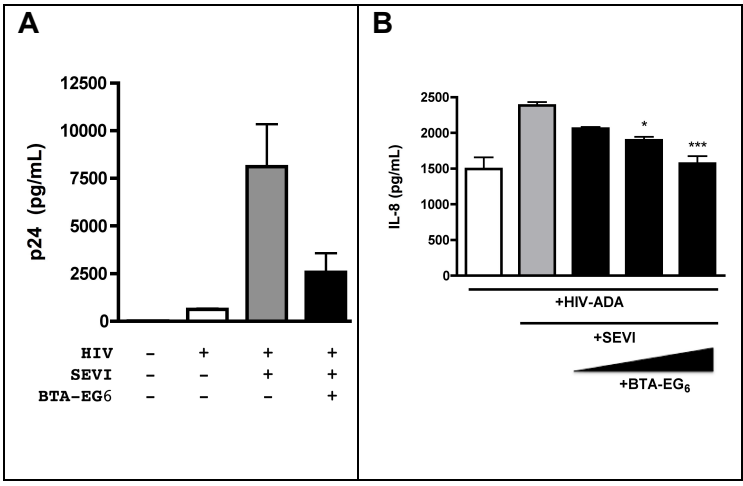
(1) Cell-free HIV-1 binding with SEVI (A2En, SiHa target cells). Assessment of the ability of novel compounds to inhibit SEVI-mediated enhancement of R5 HIV-1 (HIV-1_{ADA}) binding to A2En and SiHa cells. See **Fig. 12A** for methods and preliminary results using BTA-EG_6.

(2) *Trans*-infection assay with SEVI and PBMC. Assessment of the ability of novel compounds to inhibit SEVI-mediated enhancement of R5 HIV-1 (HIV-1_{ADA}) *trans*-infection of PBMC by virus-exposed A2En cells. A2En cells will be exposed to cell-free HIV-1_{ADA} plus SEVI, in the presence or absence of test compounds, as outlined in **Fig. 12A**. After extensive washing, the cells will be cocultured with PHA/IL-2 stimulated PBMC, and virus production will be measured using p24 Gag ELISA assay 4 days later (**Fig. 5**). This experiment will be repeated using PBMC from at least 2 different donors.

(3) Chemokine induction by cell-free HIV-1 with SEVI (A2En, SiHa target cells). Assessment of the ability of novel compounds to inhibit SEVI-mediated enhancement of R5 HIV-1 (HIV-1_{ADA}) induced chemokine release (IL-8 & CCL20/MIP3α; (16)). See **Fig. 12B** for methods and preliminary results using BTA-EG_6.

All assays: at least 6 half log_{10} compound dilutions will be tested, in triplicate (29)

Fig 12: BTA-EG_6 inhibits SEVI-enhanced binding of R5 HIV-1 to A2En cells and IL-8 release



LEGEND: (A) *HIV-1 ADA virions (60 ng/mL HIV-1 p24) were pretreated with 10 µg/mL SEVI and added to A2En cells +/- 22.5 µg/mL BTA-EG_6. After 90 min., cells were washed to remove unbound virus and bound virions were detected using a p24 ELISA. Results shown are average values +/- SD of triplicate measurements (p = <0.05 for cells treated with SEVI plus 22.5 µg/mL of BTA-EG_6 versus cells treated with SEVI alone; ANOVA with Tukey's post-test).*
(B) *A2En cells were treated with HIV-1_{BaL} and 15 µg/mL SEVI +/- BTA-EG_6 (5.5, 11 and 22.5 µg/mL). At 24 hrs supernatants were analyzed by ELISA for levels of IL-8. (*: p <0.05 or ***: p <0.001 for cells treated with SEVI plus 11 or 22.5 µg/mL of BTA-EG_6 versus cells treated with SEVI alone; ANOVA with Tukey's post-test).*

Expected results, potential pitfalls and solutions: BTA-EG_6 has generated positive preliminary data in 2 of the 3 proposed secondary efficacy assays in A2En cells (**Fig. 12**). We anticipate that it will also inhibit SEVI-enhanced *trans*-infection of PBMC by virus-exposed A2En cells, since it strongly enhances HIV-1 binding to these cells (**Fig. 12A**). Other expectations are outlined under Aim 2A.

APPROACH: R33 PHASE

Overview: If specific milestones are met, the R33 phase will further develop the candidate microbicides identified in the R21 phase. To do this, we will employ a new development paradigm, distinct from that applied to traditional microbicides that directly target the virus. The focus of the R33 phase will be on generating efficacy data using translationally relevant *in vitro* and *ex vivo* models, and on "de-risking" our novel inhibitors using a panel of rigorous toxicity assessments – culminating in the evaluation of the most promising compound in the rabbit vaginal irritation (RVI) model. The goal of these studies is not to obtain definitive IND-enabling data, but rather to rigorously assess the potential toxicity of our lead molecules in order to determine whether they merit progression to future studies at the end of the R33 phase.

AIM 3: To improve the efficacy of first generation compounds identified in the R21 phase.

Overview: In Aim 3A, we will synthesize improved 2nd generation compounds by using structure-activity relationship (SAR) data to refine chemical compositions. We will then assess the efficacy and toxicity of these molecules using assays delineated in the R21 phase. The most promising molecules will be selected for progression to specialized R33 assays (**Fig 14**). First, we will test their activity in a pH transition assay as well as their physicochemical stability in seminal plasma, artificial vaginal fluid (42) and the Universal Placebo gel, hydroxyethyl cellulose (43). In Aim 3B, we will assess the efficacy of the most promising compounds in a cervical explant model for HIV-1 infection. Finally, in Aim 3C, we will test whether our lead molecules have a synergistic or additive effect on the ability of other microbicides to inhibit HIV-1 infection in the presence of semen.

Figure 14: Specialized R33 Assays

Specialized Assays:
Efficacy & Stability
1. Activity in a pH transition assay
2. Stability in biological fluids
3. Combination drug assays
4. Human cervical explant model

Toxicity & Safety
1. *Lactobacillus* growth inhibition
2. Epithelial monolayer integrity assay
3. Human cervical explant model
4. Rabbit vaginal irritation model

Aim 3A: Generation and evaluation of improved, second-generation SEVI inhibitors: We will synthesize improved 2nd generation compounds by using structure-activity relationship (SAR) data to refine chemical compositions. We will then evaluate their efficacy and toxicity using the R21 phase testing algorithm (**Fig. 11**), prior to assessing their physicochemical stability in key fluids/materials (SP, SVF, HEC).

3A1: Synthesis of proposed analogs of BTA-EG$_x$: We will synthesize novel analogs of BTA-EG$_x$ (**Fig. 13**) so as to evaluate whether small structural variations (by themselves or in combination) in three different sections of the BTA-EG$_x$ chemical framework can improve solubility, efficacy, and biocompatibility compared to BTA-EG$_4$ or BTA-EG$_6$. Specifically, these analogs will probe whether steric, electronic, or hydrophobic effects of substituents incorporated on the perimeter of the core chemical framework of the BTA structure results in improved chemical properties as a microbicide candidate. **Fig. 13** outlines a proposed general scheme for the synthesis of analogs of BTA-EG$_4$ based on commercially available precursors and on previously reported chemical transformations (44-46). ***This synthetic strategy can provide access to ~900 analogs of BTA-EG$_x$***. These analogs will initially be tested and compared to BTA-EG$_4$ or BTA-EG$_6$ for their solubility in aqueous solutions, their toxicity in cells, their biocompatibility with respect to Lipinski's rule of five, and their binding to SEVI. Novel analogs that show improved chemical/bioactive properties compared to BTA-EG$_4$ or BTA-EG$_6$ will be selected for further testing. Potential pitfalls and solutions: [redacted]

3A2: Initial efficacy and toxicity analysis of novel BTA-EG$_x$ analogs. We will evaluate the efficacy and toxicity of our 2nd generation BTA-EG$_x$ analogs using the algorithm in **Fig. 11** (see Aim 1, 2 for methods).

[redacted]

Progression criteria: Compounds will be selected for progression to specialized assays (Aims 3A3 and beyond) if their TI is ≥ 100 (non-colored compounds) or ≥ 500 (colored compounds) and if they show sub-micromolar activity against semen-mediated enhancement of virus infection in CEM-M7 cells and PBMC. Since the specialized assays are time- and resource- intensive, no more than 3-4 compounds will be progressed per year based on relative ranking of TI values and IC_{50} in primary cell-based assays.

3A3: Activity in a pH transition assay. The pH transition assay mimics the pH change that occurs during sexual intercourse when semen (pH ~7.7) mixes with vaginal fluid (pH ~4), and will be adapted from a method described by Lackman-Smith *et al* (29). Compounds will be briefly incubated in assay medium at pH 4 with target cells (A2En, representing the vaginal tract). Cell-free HIV-1 (R5 virus) will be incubated with 50% semen (pH ~7.7, representing the ejaculate), and diluted 1:15 in target cell medium - resulting in neutral pH. After incubation for 1 hr, cells will be extensively washed and PBMC added to perform a *trans*-infection assay (see Aim 2B); an aliquot of A2En cells will also be collected for direct analysis of cell bound HIV-1 p24 antigen (**Fig. 12A**). *Note: the effect of low pH on cell viability is minimal due to the short duration of the exposure (29).*

3A4: Analysis of the physicochemical stability of novel BTA-EG_x analogs in relevant fluids. The physicochemical stability of the most promising compounds will be assessed by HPLC and/or MS (URMC Proteomics Core) in (i) seminal plasma, (ii) simulated vaginal fluid at pH 4 (42, 49, 50), and (iii) HEC (43). Compounds will be incubated in these fluids for 0-24 hrs, at 37°C, with sampling every 2-4 hrs.

Expected results, potential pitfalls & solutions (3A3, 3A4): Compounds are expected to be stable and active in the pH transition assay. If not, we will modify the BTA-EG_x chemical framework (see Aim 3A1).

Aim 3B: Efficacy assessment in a cervical explant model: We will evaluate the efficacy of the most promising compounds using a human cervical explant model, with advice from Dr. Dezzutti (39, 51-53). Recognizing that the field may advance before the R33 phase starts, we plan to use an unstimulated cervical explant (CE) model described by Lackman-Smith *et al* (29). A recent comparison of explant assays revealed that this and similar CE models yielded more consistent IC_{50} values for PRO 2000 than other models (52).

The assay is published (29). Briefly, normal ectocervical tissue will be obtained from the National Disease Research Interchange (NDRI; Philadelphia, PA). Tissue punches will then be generated, and used to test each compound's efficacy at 4 concentrations with 4 replicates. Infection will be performed with HIV-1_{BaL} in the presence or absence of SEVI (15 µg/ml) and test compounds. Virus replication will be measured by HIV-1 Gag p24 ELISA over 14-18 days and the IC_{50} determined at the assay soft endpoint (SOFT) (52); all compounds will be tested in tissue from 2 donors and tenofovir will serve as a positive control (29).

Expected results, potential pitfalls and solutions: We do not yet know whether SEVI enhances HIV-1 infection in a CE model, although this seems very likely in light of its strong enhancing effect on HIV-1_{BaL} infection of PBMC (**Fig. 4**). If problems are encountered, we will test polarized, stimulated cervical explants. If problems persist, we will focus on other efficacy assessments (Aims 1, 2) and on toxicity evaluations (**Fig. 14**).

Aim 3C: Combination drug assays: We will assess whether our lead molecules have a synergistic or additive effect on the ability of other candidate microbicides to inhibit HIV-1 infection in the presence of semen. These studies will focus on examples of different classes of candidate microbicides – notably, glycerol monolaurate (Monomuls 90-L12; Cognis (16)), griffithsin (ARR #11610; (54, 55)) and tenofovir (ARR #10199). Combination drug assays will be performed in CEM-M7 and PBMC-based assays of semen-enhanced virus infection (Aim 1), and results evaluated as described by Lackman-Smith *et al.* (29). Two-drug combination assays will be set up in which 8 concentrations of the test BTA-EG_x compound will be evaluated in all possible combinations with 5 concentrations of the microbicides identified above. Each drug will also be tested alone. Data analysis will be conducted using MacSynergy II \(56, 57), to test if the combination is antagonistic, additive, or synergistic; stavudine + ribavirin will be used as an antagonism control (29).

Expected results, potential pitfalls and solutions: SEVI inhibitors have a different mechanism of action than conventional microbicides and are thus expected to have an additive or synergistic effect when used in combination with such agents. If this is not observed, we will test whether modifications of the BTA-EG_x chemical framework improve drug-drug interactions, and we will also test derivatives of ANCA-11 and CA-140.

AIM 4: To assess the toxicity and inflammatory effects of lead molecules.

Overview: We will assess the toxicity and inflammatory effects of the most promising candidate molecules, both in PBS and as a simple admixture in HEC. The following parameters will be measured: (i) toxicity to

beneficial *Lactobacillus* species; (ii) effect on epithelial monolayer integrity; (iii) toxicity and inflammatory effects on human cervical explant tissue. Finally, the R33 phase will culminate with an evaluation of the most promising compound in the rabbit vaginal irritation (RVI) model.

Aim 4A: Toxicity to beneficial *Lactobacillus* species: The effect of candidate compounds on *Lactobacillus crispatus* and *L. jensenii* will be assessed by incubation of organisms in *Lactobacillus* MRS broth in the presence of test compounds for 24 hrs (6 half $\log_{10}$ dilutions will be used, in triplicate, and penicillin-streptomycin will be used as a positive control for toxicity) (29). Expected results, potential pitfalls & solutions: We do not anticipate toxicity to *Lactobacilli*. If toxicity occurs, we will test modifications of the BTA-EG_x chemical framework and/or derivatives of ANCA-11, CA-140.

Aim 4B: Effect on epithelial monolayer integrity: Effects on the integrity of HEC-1-A and Caco-2 cell monolayers will be assessed using methods described by our advisor, Dr. Dezzutti (51). Briefly, cells will be grown on transwell supports until confluent, and a polarized monolayer has developed. A non-toxic dose of the test compounds will then be added to the apical surface of the monolayer, and electrical resistance will be measured using a MilliCell-ERS resistance system (Millipore, Billerica, MA). N9 will be used as a positive control for disruption of monolayer integrity (51), and the isomolar Universal Placebo (HEC) will be used as a negative control. Expected results, potential pitfalls & solutions: See Aim 4A.

Aim 4C: Toxicity and inflammatory effects on human cervical explants: Toxicity to human cervical explants (see **Aim 3**; (29)) will be assessed by MTT assay after incubation with test compounds for 18-24 hrs. Two to 4 half $\log_{10}$ dilutions of each compound will be used, in triplicate, and N9 will be used as a positive toxicity control; results will repeated using tissue from at least 2 donors (29). We will also assess production of a limited set of pro-inflammatory cytokines and chemokines, including IL-1ß, IL-6, TNFα, MCP-1/CCL2, MIP3α/CCL20 and IL-8 (58). To do this, culture supernatants will be analyzed using the Luminex assay platform (URMC Human Immunology Center). Expected results, potential pitfalls & solutions: See Aim 4A.

Aim 4D: Evaluation in the rabbit vaginal irritation (RVI) model: The most promising lead molecule will be tested in the RVI model (59). We will select this compound on the basis of (i) the relative ranking of TI and IC_{50} values, (ii) stability in HEC and biofluids (**Aim 3A3**), (iii) efficacy in the explant infection model (**Aim 3B**) and (iv) a lack of toxicity in **Aims 4A thru 4C**. For the RVI toxicity assessment, the following groups will be used: [1] HEC alone (control), [2-4] HEC plus test compound at 3 escalating dose levels, intended to represent 5x, 25x and 100x the IC_{50} in the explant infection model (Aim 3B), [5] N9 gel (4%; Conceptrol, Ortho-McNeil). 27 animals will be used for this study (n=6 for all groups, except for the N9 group [n=3]; see **Vertebrate Animals** for a discussion of group sizes). Outcomes will be measured as described (59, 60) after completion of the 14-day intravaginal delivery; very briefly, tissue sections will be assessed for epithelial cell damage, immune cell infiltration, edema and vascular congestion on a semiquantitative scoring system (59). Expected results, potential pitfalls & solutions: Our selection criteria (above) should ensure that our lead compound is non-toxic in **Aim 4D**. The goal of the RVI study will be to identify a non-irritating (maximum tolerated) dose and to "de-risk" our novel strategy prior to future studies (after the end of this R21/R33).

Statistical Considerations, Timeline & Interactions Between Participating Sites

Statistics: Comparisons between treatment groups are generally performed by one-way analysis of variation (ANOVA), followed by Tukey's post-test, using GraphPad PRISM software. Single statistical comparisons of a treatment group versus its control group (when necessary) are generally performed using a two-tailed Student's *t* test. Comparison of more than two factors (e.g. different treatments and time courses or dose variations) will be made using 2-way ANOVA followed by Tukey's post-test. If data are not normally distributed, non-parametric tests (Kruskal-Wallis or Mann-Whitney *U* test) will be used. All data will be analyzed in consultation with Dr. Feng, our Biostatistical coinvestigator. Significance will be taken at $p<0.05$. Group sizes for *in vivo* studies are discussed in the *Vertebrate Animals* section.

Timeline:

- **R21 Phase:** Year 1: Aim 1 and Aim 2 (initiation); Year 2: Completion of Aims 1 & 2
- **R33 Phase:** Year 3: Aim 3 (initiation); Year 4: Aim 3 (continuation; cervical explant and drug combination studies), Aim 4 (initiation); Year 5: completion of all Aims 3 & 4 (including RVI testing)

Interactions between participating sites:

- **R21 Phase:** Weekly internal project meetings will be held at the UR and UCSD sites, with weekly exchange of data via the secure UR SharePoint site. We will also hold monthly meetings between the UR and UCSD research teams by tele/video conference, with an annual full day program team meeting to be held once a year in Rochester.
- **R33 Phase:** As for the R21 phase, except that we will also include the [redacted] site. Monthly meetings will include all 3 participating sites, as will the annual full day program team meeting.

MILESTONES FOR THE R21 AND R33 PHASES

R21 milestones: Milestone decisions will be made following discussion with NIH Program Staff (Dr. Turpin) and with our R21 phase consultants (Drs. Dezzutti and [redacted]). Proposed milestones are:

Milestone #1: Chemical synthesis of ≥1 molecule(s) with submicromolar affinity for SEVI.

- Rationale: The binding affinity of BTA-EG_6 for SEVI is 8 μM. While the compound has a TI of ≥10, this affinity is suboptimal and must be improved if we are to enter the R33 phase with a high likelihood of achieving clinically useful and commercially viable levels of efficacy.

Milestone #2: Identification of ≥1 molecule(s) with a therapeutic index (TI) ≥ 50.
The TI will be based on: (i) the IC_{50} for blockade of semen-mediated enhancement of HIV-1 infection in CEM-M7 cells & (ii) the TC_{50} for A2En cervicovaginal epithelial cells. For colored compounds, TI will be ≥ 250.

- Rationale: High potency is required for eventual development of a clinically useful product. We have selected a TI of ≥ 50 to balance the need for high potency at the end of the R33 phase versus the need to avoid prematurely discarding our approach at an early stage of development.

Milestone #3: Identification of ≥1 molecule(s) with submicromolar activity in primary cell-based assays.

- Rationale: Activity in primary cell based assays is essential. Thus, the ability to inhibit semen-mediated enhancement of HIV-1 infection at submicromolar levels in either PBMC cultures or in A2En/PBMC cocultures (*trans*-infection model) will be required.

Milestone #4: Identification of ≥1 molecule(s) with submicromolar activity in clade panel testing.

- Rationale: Activity against diverse strains is essential. The ability to inhibit SEVI-mediated enhancement of infection by diverse HIV-1 strains in PBMC (clades A, B, C, transmitted virus) will be required.

R33 milestones: The following milestones are proposed for determining success of the R33 phase:

Milestone #5: Identification of ≥1 molecule(s) with additive or synergistic activity in drug combination testing.

- Rationale: SEVI-binding molecules should be well suited for use in combination with "conventional" microbicides that possess a different mechanism of action. Additive or synergistic activity with "conventional" microbicides will therefore be required.

Milestone #6: Identification of ≥1 molecule(s) with minimal or undetectable toxicity in specialized assays.

- Rationale: Lack of toxicity to beneficial *Lactobacillus* species, epithelial monolayer integrity and human cervical explants will be required. In addition, toxicity in the RVI model should be below the cutoff score (8) for acceptability in clinical trials.

Milestone #7: Identification of ≥1 molecule(s) with TI > 1,000 and activity in the nanomolar range

- Rationale: A viable lead candidate microbicide must have robust activity in order to be clinically useful.

Future Directions: If the R33 phase is successful, compounds will be developed further via the NIH RAID program and SBIR or STTR mechanisms (in partnership with a small business company). Followup studies will include formulation studies, efficacy analysis in an animal model, production of a GMP batch of material and FDA-enabling safety/toxicity studies. *Note that we have already filed intellectual property on the concepts outlined in this proposal, and that the PI has experience as President of a startup vaccine company (Codevax) which was recently awarded its first SBIR.*

8-11. HUMAN SUBJECTS

8. Protection of Human Subjects

UNIVERSITY OF ROCHESTER SITE
See extended description/discussion below.
This site WILL conduct Human Subjects Research

UNIVERSITY OF CALIFORNIA, SAN DIEGO
No human subjects. No human materials.

[redacted]
Studies at the [redacted] site will involve the collection of cervical tissue. **This does NOT constitute human subjects research** (45 CFR 46.102). This is because we will use deidentified, pre-existing samples. These materials will be as follows:

- Cervical tissue obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) or from MatTek Corporation (Ashland, MA) with no patient identifiers.

Explanation: The human materials will be obtained from 3rd party suppliers without identifying information on the subjects/source. As noted in the Federal regulation, the use of materials that have been collected for some other purpose by someone other than the investigator (e.g., a third party vendor), and which are not linked to the individual by any personal identifiers, does NOT meet the definition of research that involves human subjects (45 CFR 46.102).

UNIVERSITY OF ROCHESTER (UR) SITE

Studies at the UR site will involve the collection of semen from human subjects. This constitutes human subjects research. Semen will be needed to perform many of the assays outlined in the proposal. See below.

Additional materials that do not constitute human subjects research.
Additional human materials will also be used in our experiments**,** but these other materials do not qualify as human subjects research (45 CFR 46.102). This is because we will use deidentified, pre-existing samples. These materials will include:

- Cervical tissue obtained from the National Disease Research Interchange (NDRI, Philadelphia, PA) or from MatTek Corporation (Ashland, MA)
- Human blood obtained from the American Red Cross or from Tennessee Blood Services (Memphis, TN)
- Pre-existing human semen samples obtained from Fairfax CryoBank (Fairfax, VA) or from Tennessee Blood Services (Memphis, TN)

Explanation: In all cases, the human materials will be obtained from 3rd party suppliers without identifying information on the subjects/source. As noted in the Federal regulation, the use of materials that have been collected for some other purpose by someone other than the investigator (e.g., a third party vendor), and which are not linked to the individual by any personal identifiers, ***does NOT meet the definition of research that involves human subjects*** (http://www.hhs.gov/ohrp/humansubjects/guidance/45cfr46.htm#46.102).

Human Subjects Research: Semen samples

In addition to obtaining semen samples from pre-existing sources (see above), we will also obtain semen samples on a prospective basis. There are several reasons why this is necessary. First, the prospective collection of semen samples will allow us control over sample provenance and storage, which is not possible with pre-existing samples. This will be important in ensuring the quality control of the semen used in this study. Second, our proposed assays require reasonably large amounts of semen – which will likely exceed the supply of pre-existing samples that are available to us for purchase through the Fairfax CryoBank.

Protection of Human Subjects

1. Risks to Human Subjects

a. Human Subjects Involvement, Characteristics and Design

As described in the research plan, one of the important aspects of the proposed project is to explore how candidate microbicides interact with semen and seminal plasma, and whether they can inhibit semen-mediated enhancement of HIV-1 infection.

A total of 60 healthy volunteers will be recruited. Subjects enrolled will be male only (since we will be collecting sperm) over the age of 18 and below the age of 55. Samples from patients with known HIV infection will not be collected. Vulnerable populations such as children below the age of 18, prisoners and institutionalized individuals will also not be enrolled.

Catherine Bunce, RN, of the Infectious Diseases Division (IDD) of the University of Rochester's Department of Medicine will enroll and consent the volunteers. A nominal fee will be provided to the volunteers to reimburse them for their time and effort. The cost of Ms. Bunce's effort in this subject enrollment capacity will be covered by the Core services of University of Rochester Developmental Center for AIDS Research (DCFAR; NIH P30AI078498).

b. Sources of Materials

Subjects will be asked to donate semen samples for research purposes; it is expected that 0.5 to 5 ml of ejaculate will be collected per donation. Ejaculate will be collected following manual stimulation in a private room at the UR Vaccine Trials Unit clinic, located onsite at the UR School of Medicine and Dentistry. Immediately after collection of the samples, we will add a protease inhibitor cocktail to the semen to prevent proteolytic degradation of the sample by proteases present in the semen. Samples may then either be used directly in experiments or frozen for future use. In some cases, samples may be collected without addition of the protease inhibitor cocktail (to confirm that addition of the protease inhibitor cocktail does not adversely affect downstream assay results using semen samples).

Ms. Bunce will code each sample with a unique subject identifier prior to being sent to the Dewhurst laboratory. With the exception of Race and Ethnicity (in order to complete the annual IRB and NIH progress reports) no other medical information or data will be provided with the samples.

HIPAA Authorization will be obtained during the consent process, as Protected Health Information (PHI) will be collected in this study. To enhance subject protection, all samples will be deidentified and assigned a unique code known only to Ms. Bunce, prior to being sent to the Dewhurst laboratory. Ms. Bunce will be the only individual who will have access to the study records and PHI, which will be secured in a locked filing cabinet in her locked office. Samples will be stored until the end of the study, unless a subject agrees in writing that their their sample(s) may be retained and used for future research. No samples will be sold nor used to develop a commercial product.

c. Potential Risks

This study constitutes very minimal risks to the subjects. Breach of confidentiality may accidentally occur if Ms. Bunce's office and file cabinet are breached. There is also some risk of personal embarassment associated with sample provision.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Recruitment will be done by means of local advertisements and flyers. Although not specifically targeted, volunteers who respond will most likely be employees of the University of Rochester.

The study, risks and benefits of participation will be presented to the donors by Ms. Bunce. Consent forms will be presented at the time of their evaluation visit. All subjects will be encouraged to read over the information and ask questions before signing the consent form.

b. Protections Against Risk

As noted above, no PHI will be provided with the samples, considerably reducing the risk to subjects' privacy. While every effort will be made to protect the study records and any PHI they may contain, an unforeseen breach in confidentiality may occur if Ms. Bunce's office and file cabinet are breached. Consequently, while the likelihood of such event occurring is extremely remote, complete confidentiality cannot be guaranteed to the subjects.

3. Potential Benefits

The immediate potential benefits to the subjects are nil, however most volunteers take pride in knowing that their involvement has a direct impact in increasing disease-related knowledge.

4. Importance of Knowledge to be Gained

The ultimate objective of this research relates to the development of a new topical microbicide for prevention of HIV-1 transmission. To do this, it is necessary to understand how candidate microbicides interact with human semen, and whether they inhibit semen-induced enhancement of HIV-1 infection.

Risks to subjects are minimal. The most likely risk is breach of confidentiality, which will be mitigated by not providing any PHI with the samples. The importance of the knowledge to be gained is substantial. By studying how semen and seminal plasma affect the activity of candidate microbicides, we expect to be able to gain insights that improve the effectiveness of these agents. This will bring us closer to our ultimate of developing an effective topical microbicide for HIV/AIDS.

UR RSRB APPROVAL INFORMATION

RSRB: RSRB00032146 **Principal Investigator**: Stephen Dewhurst
Study Title: Characterization of Amyloid Fibrils in Semen

- **Initial Approval:** 6/28/2010 ; **Study Approval Expires**: 6/27/2011 ; **Length of Review:** 1 year
- **Risk Level:** - Minimal Risk - Adults only
- **Review Level:** Expedited
- **Expedited Category:**- 3 - non-invasive, prospective collection of biological specimens

From: "irb@urmc.rochester.edu" [redacted]
Subject: **RSRB: Application Approval Notification**
Date: June 29, 2010 8:10:16 AM EDT
To: "Dewhurst, Stephen" [redacted]
Reply-To: IRB <IRB@URMC.Rochester.edu>

Letter of Approval

RSRB: RSRB00032146 **Principal Investigator**: Stephen Dewhurst

Study Title: Characterization of Amyloid Fibrils in Semen

Initial Approval: 6/28/2010

Study Approval Expires: 6/27/2011

Length of Review: 1 year

Risk Level:
- Minimal Risk - Adults only

Review Level: Expedited

Expedited Category:

- 3 - non-invasive, prospective collection of biological specimens

This approval is contingent upon the investigation being conducted in compliance with the approved study protocol including all requirements and/or determinations of the RSRB. Unless a Waiver of Consent is specified above, consent must be obtained and documented in the manner approved by the RSRB. Please note all remarks and/or attachments. Only consent forms bearing a current 'RSRB Approved' Watermark may be used. Only the most recently approved version of any consent or recruitment document may be used when obtaining consent. **Consent forms/recruitment letters must be printed on department letterhead.**

As the Principal Investigator, you are responsible for the following activities:

- Timely submission of continuing review progress reports apply to RSRB at least 8 weeks before expiration. Federal Regulations require that the RSRB conduct continuing review of research. You will receive an email notification when the expiration date is approaching.
- Requesting any proposed changes in the above research activity. All subject recruitment materials must be approved prior to use. Changes may not be initiated without RSRB approval except when necessary to eliminate apparent immediate hazards to the subject(s) and then a report must be submitted along with the amendment request
- Maintaining all approved study documents in your study file
- Maintaining signed consent forms for at least three years after the research is completed or for a longer term if required by FDA regulations
- Reporting any unexpected serious problems involving risks to subjects or others (including unexpected deaths, hospitalizations or serious injuries) in accordance with the RSRB Adverse Event guidelines
- Submitting a final progress report to the RSRB upon completion of this study

Jeanne Grace, RSRB Chair June 28, 2010

The Department of Health and Human Services has approved a Federalwide Assurance (FWA) with the University of Rochester (FWA9386), which is in effect through September 27, 2010.

601 Elmwood Avenue, Box 315
Rochester, New York 14642
(585) 275-2398

Inclusion of Women and Minorities

Women will not be included in this study because it seeks to collect semen.

Due to the small number of volunteers needed, there will be no selected, targeted recruitment. We anticipate that the subject distribution will mirror the demographics of Monroe County, New York (where UR is located), which are listed below. See also the **Targeted/Planned Enrollment Table**

Demographics of Monroe County, New York

[redacted]	Females: 51.5%; Males: 48.5%
Race	White 80.5% Black 14.8% American Indian/Alaska Native 0.3% Asian 2.8% Native Hawaiian and other Pacific Islander 0.1%
Ethnicity	Persons of Hispanic or Latino origin: 6%

Targeted/Planned Enrollment Table

Study Title: Characterization of Amyloid Fibrils in Semen

Total Planned Enrollment: 60

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/[redacted]**		
	Females	**Males**	**Total**
Hispanic or Latino		10	10
Not Hispanic or Latino		50	50
Ethnic Category: Total of All Subjects *		60	60
Racial Categories			
American Indian/Alaska Native			
Asian		2	2
Native Hawaiian or Other Pacific Islander		1	1
Black or African American		15	15
White		42	42
Racial Categories: Total of All Subjects *		60	60

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

Note: The study involves collection of semen, so includes only males

Inclusion of Children

Individuals over the age of 18 are eligible to participate. Children below 18 have been excluded, along with individuals over the age of 55. Subjects above 55 years of age, or below 18 years of age will not be enrolled because the quality and composition of sperm varies with age, and also because the principle target population for our HIV prevention efforts will be adults in their peak years of sexual activity.

VERTEBRATE ANIMALS

BIOHAZARDS

Biohazards will be used at the UR and [redacted] sites (ONLY) and will include the following:

- Recombinant DNA: Plasmids containing HIV-1 gene sequences or proviral DNA will be propagated under BSL1 containment in *E. coli*.
- Bacteria: Beneficial *Lactobacillus* species (*Lactobacillus crispatus* and *L. jensenii*) will be handled under BSL1 containment.
- Tissue culture: Experiments that involve the use of infectious HIV-1 virus will be performed under BSL2 containment with BSL3 practises and procedures (i.e., so called "BSL2+" containment). Human biological fluids and primary cells will be handled under "universal precautions". All personnel who work with biohazards will be required to undergo hands-on training by experienced laboratory members, prior to being permitted to handle infectious virus. The laboratories (Dewhurst at UR, [redacted] will be registered with, and inspected by, the relevant Institutional Biosafety Committee, and all personnel working with primary human cells will be offered the Hepatitis B virus (HBV) vaccine at no charge

No biohazards will be used at the UCSD site.

F. VERTEBRATE ANIMALS

A response to the "5 points" required by the PHS398 application (http://grants.nih.gov/grants/funding/phs398/instructions2/phs398instructions.htm) follows:

Animals will not be used in the R21 phase of this project, but will be used at [redacted] (only) during the R33 phase (if funded).

No animals will be used at the UR or UCSD sites, at any point in this project.

1. *PHS398: Provide a detailed description of the proposed use of the animals in the work outlined in the Research Design and Methods section. Identify the species, strains, ages, sex, and numbers of animals to be used in the proposed work:*

Response: Below. Also, see Experimental Design for additional information.

(1) Description of Use	Species/strain	Age	Sex	N/Yr
Vaginal irritation analysis	Rabbits (NZW)	10-20 wk	F	27 total (Yr 5 only)

2. *PHS398: Justify the use of animals, the choice of species, and the numbers to be used. If animals are in short supply, costly, or to be used in large numbers, provide an additional rationale for their selection and numbers.*

Response: The FDA's current guidance document for the development of novel topical microbicides stipulates that one must test all such candidates for their propensity to cause irritation to rabbit vaginal tissue *(FDA document entitled: "Nonclinical Pharmacology/Toxicology Development of Topical Drugs Intended to Prevent the Transmission of Sexually Transmitted Diseases (STD) and/or for the Development of Drugs Intended to Act as Vaginal Contraceptives").* The reason for this is that the rabbit vaginal irritation (RVI) assay is considered the "gold standard" in evaluation of the tolerability and likely safety of candidate microbicides. While there has been considerable effort to develop *in vitro* replacements for this assays, none has taken its place – due in large part to the complex interplay

between sensitive vaginal epithelial tissue, circulating immune cells, and resident microbial flora. It is not possible to adequately model this assay using an *in vitro* system.

Our experiments will use a small group size (n=6) that is standard in RVI studies (59, 60). The following groups will be used: [1] HEC alone (control), [2-4] HEC plus test compound at 3 escalating dose levels, intended to represent 5x, 25x and 100x the IC_{50}, [5] N9 gel (4%; Conceptrol, Ortho-McNeil). 27 animals will be used for this study (n=6 for all groups, except for the N9 group [n=3]). Outcomes will be measured as described (59, 60) after completion of the 14-day intravaginal delivery; very briefly, tissue sections will assessed for epithelial cell damage, immune cell infiltration, edema and vascular congestion on a semiquantitative scoring system (59).

It is important to emphasize that our novel microbicide candidates have not previously been evaluated in rabbits. Thus, it is not possible to derive a more robust sample/group size calculation at the present time. However, the initial rabbit experiments will allow us to gather key data that will inform the design of followup or repeat experiments, should such experiments become necessary. Should our initial data reveal a high degree of variation in outcome measures in individual animals, we will work with **Dr. Feng** (our biostatistical coinvestigator) to re-evaluate the sample size for the rabbit studies, and to arrive at a statistically robust group size.

3. PHS398: Provide information on the veterinary care of the animals involved.

Response: [redacted] is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) and has a long history of providing state-of-the-art facilities and trained, experienced animal care and research personnel for clients involved in infectious disease investigations, cancer research, and a wide variety of other studies including those requiring Good Laboratory Practice (GLP) documentation and conditions. Three veterinarians, who are certified or certification eligible as American College of Laboratory Animal Medicine diplomates, are available to provide health care for [redacted] animals. [redacted], [redacted] ([redacted] subcontract PI) is the project manager for the Rabbit Vaginal Irritation Model at [redacted] holds an Animal Welfare Assurance with the Office of Laboratory Animal Welfare, NIH, PHS; the assurance number is [redacted]. The USDA registration number is [redacted] and the AAALAC file number is [redacted] provided quotations and detailed descriptions of how the animal studies are performed and importantly, how the animals are cared for and handled in strict compliance with the Animal Welfare Act and the PHS Policy on Humane Care and Use of Laboratory Animals.

4. *PHS398: Describe the procedures for ensuring that discomfort, distress, pain, and injury will be limited to that which is unavoidable in the conduct of scientifically sound research. Describe the use of analgesic, anesthetic, and tranquilizing drugs and/or comfortable restraining devices, where appropriate, to minimize discomfort, distress, pain, and injury.*

Response: Sedation will be performed prior to vaginal administration of test agents to rabbits. This is necessary to prevent material for being dislodged prematurely from the vaginal cavity, and also because the animal must be held in a supine position for vaginal delivery of test agents. Sedation will be achieved either with a combination of droperidol/fentanyl solution (0.4 mg/ml + 20 mg/ml solution, delivered IM at a dosage of 0.125 - 0.3 ml/kg) or with acepromazine (a dose of 0.25 mg/kg - 0.75 mg/kg will be used, delivered IM).

Detailed methods:

Vaginal delivery of test agents: Animals will be sedated and held in a supine position. One ml of test agent will then be delivered intravaginally using a sterilized stainless steel needle specifically designed for rabbits to depth of 6-8 cm for 14 consecutive days. On the 15th day, the animals will be

humanely sacrificed and vaginal tissue samples collected for gross pathological and histopathological analysis of irritation.

5. *PHS398: Describe any method of euthanasia to be used and the reasons for its selection. State whether this method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. If not, present a justification for not following the recommendations.*

Response: All methods of euthanasia are performed in accordance with the 2000 Report of the AVMA Panel on Euthanasia and 2007 AVMA guidelines on euthanasia. The routine euthanasia method for rabbits is by intravenous injection of a sodium pentobarbital overdose and subsequent auscultation of the heart. Euthanasia may be performed: 1) as a result of study design (i.e. at a certain time point in the study), 2) as a result of the animal exhibiting study end-point criteria (as described in the ACUP), or 3) as a result of unexpected complications for which the veterinarian in consultation with the study director determines there is no cure or chance of recovery without the animal experiencing undue discomfort, pain, or distress. Additionally, if treatment/therapy is warranted, and it is determined that such treatment/therapy cannot be provided without adversely affecting the study, a decision to euthanize the animal will be made in consultation with the veterinarian and project manager.

13. SELECT AGENT RESEARCH

Select Agent Program: The UR has been and continues to be a registered entity with the CDC's Select Agent Program since 2003. In addition to requiring Institutional Biosafety Committee (IBC) approval for the use of non-toxin select agents (i.e. regulated genetic material and pathogens), Principal Investigators are required to annually declare select agent toxin possession. IBC registration and the select agent toxin declaration serve to educate and remind Principal Investigators of these regulations and to ensure due diligence on the part of the University in maintaining regulatory compliance. Facilities compatible with use of select agents include a BSL3 containment laboratory with keycard-only and alarmed access (restricted to specified users), and containment equipment suitable for use with BSL3 respiratory pathogens. This laboratory has been inspected several times by the federal government including the CDC and is in compliance with the BSL3 requirements as outlined in the CDC/NIH *"Biosafety in Microbiological and Biomedical Laboratories*" and with the select agent requirements. Storage of select agents is very tightly controlled, using physical security measures. Agent inventories are maintained and are physically reconciled with stock on hand. The Select Agent Responsible Official/ Biosafety Officer reviews all security and biocontainment controls periodically. Laboratory personnel training records and select agent use approval (select agent registration information) are also carefully monitored and updated regularly.

All faculty who work with select agents are registered with the IBC, and all scientists, technicians, students and others who propose to conduct research with select agents will be required to register with pertinent federal authorities and to prove evidence of approval to use the requested agent(s). Trainees will also receive necessary hands-on training (and training in appropriate record keeping) prior to initiating any experiments with select agents.

The studies in this proposal do NOT involve select agents. Should this change in the future, information will be provided on the identity of the select agent to be used. Other information (institutional registration status and facilities descriptions are outlined above).

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15: CONSORTIA/CONTRACTUAL ARRANGEMENTS

CONSORTIUM/CONTRACTUAL AGREEMENTS

Subcontract with the University of California, San Diego (for both the R21 and R33 phases):

In all years of the project (years 1-5, both R21 and R33 phases), a subcontract is included with University of California, San Diego (UCSD). This subcontract will support the development of novel amyloid-binding small molecules that will bind to SEVI and interfere with SEVI-mediated enhancement of HIV-1 infection.

A detailed budget is included with the application, and the two parties (UR, UCSD) are fully committed to the necessary contractual relationship. The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the NIH consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

The scientist in charge of this project at UCSD will be **Dr. Jerry Yang**. Dr. Yang is Associate Professor of Chemistry and Biochemistry at the University of California, San Diego (UCSD). In collaboration with Dr. Dewhurst, Dr. Yang helped to generate the preliminary data in this application, which builds from extensive *in vitro* experiments carried out in the Yang laboratory, with the goal of generating molecular assemblies on aggregated amyloid peptides that can function as bio-resistive surface coatings (in analogy to generation of a “non-stick” coating on a surface). Dr. Yang has a broad background in organic and biophysical chemistry, biochemistry, and materials science, with specific expertise in amyloid and biomembrane research. His students and postdocs routinely synthesize structurally and functionally diverse organic molecules and small molecule libraries, develop biochemical and cellular screening assays, and develop biophysical techniques to study the structure and function of biomolecules and biomolecular aggregates in solution. He is an established collaborator of Dr. Dewhurst (as noted above), and will be responsible for directing all aspects of the synthesis and *in vitro* evaluation of the amyloid-targeting agents described in the research proposal.

Subcontract [redacted] (for R33 phase only):

In the R33 phase of the project (years 3-5), a subcontract is included with [redacted] This subcontract will support the performance of *in vitro* and *in vivo* testing of the SEVI-targeting small molecule inhibitors. Studies at [redacted] will be directed by [redacted] who will:

- Supervise the efficacy and toxicity assessment in the cervical explant model *(under the consultancy of Dr. C. Dezzutti)*
- Supervise and design the *Lactobacillus* toxicity assays
- Supervise the cytotoxicity assays
- Perform the Rabbit Vaginal Irritation Model

A detailed budget is included with the application, and the two parties (UR, [redacted] are fully committed to the necessary contractual relationship. The appropriate programmatic and administrative personnel of each organization involved in this grant application are aware of the NIH consortium agreement policy and are prepared to establish the necessary inter-organizational agreement(s) consistent with that policy.

The scientist in charge of this project at [redacted] will be [redacted]. [redacted] is a Senior Scientist at [redacted] Inc. During the R33 phase, she will serve as a collaborator and coinvestigator on this project and as PI of the [redacted] Subcontract. She is a member of the Microbicide Trials Network, and her research interests have focused on HIV microbicide development for many years. She has expertise in microbicide development and preclinical safety and efficacy testing of novel microbicides. She also has

extensive experience on studies using nonhuman primate (NHP) models for HIV/AIDS, including the simian immunodeficiency virus (SIV)/macaque model. She received her Ph.D. in 1992 for studies on SIV, and – in addition to her experience in microbicide research – has expertise in HIV vaccine development, analysis of immune responses in SIV-infected macaques, and evaluation of novel candidate antiviral drugs. [redacted] collaborated closely with Dr. Dewhurst during the preparation of this proposal.

CONSULTANTS/OTHER SIGNIFICANT CONTRIBUTORS

See letters of support for additional documentation

Consultant for both R21 and R33 phases:

Dr. Charlene S. Dezzutti (Consultant) is Associate Professor in the Department of Obstetrics, Gynecology, and Reproductive Sciences, and an Associate Investigator in the Magee-Womens Research Institute at the University of Pittsburgh. Her role on this application is to serve as a consultant and advisor to the research program, to review annual progress reports, participate in data evaluations, and to provide technical assistance and guidance on assessments of the potential toxicity of candidate microbicides, using the cervical explant models that she has developed (including MTT, histology assessments of toxicity and assessment of cytokine release). She is well qualified for this role because she is the principal investigator of the Network Laboratory for the Microbicide Trials Network (MTN), an HIV/AIDS clinical trials network established by the National Institute of Allergy and Infectious Diseases (NIAID). In this capacity, she conducts side-by-side comparative assessments of different microbicide candidates that will be used in clinical trials. Her laboratory is presently studying:

1) The mechanism(s) of transmission. Dr. Dezzutti has developed cervical and colorectal tissue explant systems to study HIV transmission ex vivo. Using these systems she is attempting to determine the first HIV infected cells.
2) The factors that influence transmission. Dr. Dezzutti has an active program studying the interaction(s) between sexually transmitted infections and HIV in acute *in vitro* model systems as well as tissue explant systems.
3) The ways to prevent transmission of HIV. For prevention of sexually acquired HIV, Dr. Dezzutti is evaluating microbicides that may be utilized in human clinical trials. She is examining innate immune factors found in genital secretions that could be augmented or exploited for microbicide development. She has also developed *in vitro* and *ex vivo* model systems that can be used to evaluate the potential toxicity of candidate microbicides.

Consultant for R21 phase only (and coinvestigator for R33 phase):

[redacted] (Consultant/R21 phase, Coinvestigator/R33 phase) is a Senior Scientist at [redacted] Her role on this application is two-fold: (1) During the R21 phase, she will serve as a consultant and advisor to the research program, to review annual progress reports, participate in data evaluations, and to provide technical assistance and guidance on assessments of candidate microbicides; (2) During the R33 phase, she will serve as a collaborator and coinvestigator on this project and as PI of the [redacted] Subcontract. [redacted] is a member of the Microbicide Trials Network, and her research interests have focused on HIV microbicide development for many years. She has expertise in microbicide development and preclinical safety and efficacy testing of novel microbicides. She also has extensive experience on studies using nonhuman primate (NHP) models for HIV/AIDS, including the simian immunodeficiency virus (SIV)/macaque model. She received my Ph.D. in 1992 for studies on SIV, and – in addition to her experience in microbicide research – has expertise in HIV vaccine development, analysis of immune responses in SIV-infected macaques, and evaluation of novel candidate antiviral drugs.

[redacted]

Steve Dewhurst, Ph.D.
Professor and Chair, Dept. of Microbiology and Immunology
University of Rochester Medical Center
575 Elmwood Avenue, Box 672
Rochester, NY 14642
Tel.: [redacted]
Email: [redacted]

June 3, 2010

Dear Steve,

I would be very pleased to serve as a consultant and a collaborator on your R21/R33 application to the Microbicide Innovation Program (MIP VI) (RFA-AI-10-011).

As you know, I am working in the microbicide field since 2002, and I have published many abstracts and papers on microbicides. After reading *Munch J., et al. Semen-derived amyloid fibrils drastically enhance HIV infection. Cell 131:1059-71, 2007*I and related papers, I am intrigued by your idea of targeting semen-derived fibrils that enhance HIV-1 infection and therefore may play an important role in HIV-1 transmission. Your proposed approach of targeting these fibrils is both innovative and logical.

I would be pleased to serve as a consultant on your application during its R21 phase, and its initial R33 years. I will also serve as a coinvestigator and collaborator during the final year of the R33 phase of your proposal, and will perform studies of lead candidate molecules in the rabbit vaginal irritation (RVI) model.

I believe that my expertise in microbicide development and preclinical safety and efficacy testing of novel microbicides will prove useful to you as your work progresses. I would also be happy to serve as an overall advisor to your project, providing guidance and input over the entire period of your project – not just in the late stages of the R33 phase. I understand that this commitment would include reviewing an annual progress report and participating in an annual evaluation of progress on the project. I wish you best of luck with your application!

[redacted]

University of Pittsburgh

School of Medicine
Department of Obstetrics, Gynecology and Reproductive Sciences

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July 5, 2010

Stephen Dewhurst, Ph.D.
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Re: "The Semen Enhancer of HIV Infection as a Novel Microbicide Target."

Dear Steve,

I'm writing to confirm my enthusiastic willingness to serve as a consultant and a collaborator on your above-referenced R21/R33 application to the Microbicide Innovation Program (MIP VI) (RFA-AI-10-011).

As you know, I am the principal investigator of the Network Laboratory for the Microbicide Trials Network (MTN), an HIV/AIDS clinical trials network established by the National Institute of Allergy and Infectious Diseases (NIAID). In this capacity, I conduct side-by-side comparative assessments of different microbicide candidates that will be used in clinical trials.

My laboratory has pioneered the use of polarized ectocervical and colorectal explant cultures to evaluate topical microbicides (Abner, S.R., et al. J. Infect. Dis. 192:1545-1556, 2005, Cummins, J.E., et al. Antimicrob. Agents Chemother. 51:1770-1779, 2007 and Rohan et al., PLoS ONE 5(2): e9310, 2010). Using these models we are attempting to understand mechanisms of HIV transmission and ways to prevent it. Semen is an integral component in HIV transmission yet one of the least studied. The objectives of your proposal align closely with the interests of my research and I am intrigued by your idea of targeting SEVI as a means to reduce the transmission of HIV.

I would be pleased to serve as a consultant and advisor to your research program for all 5 years of the proposal (assuming that the proposal is selected for progression to the R33 phase). I understand that this commitment will include reviewing an annual progress report, and participating in an annual evaluation of progress on the project.

I will provide you and [redacted] with technical assistance and guidance on toxicity experiments using the ectocervical explant models that we have developed (including MTT assay, histology assessments of toxicity and assessment of cytokine release) and epithelial monolayer integrity assays (including assessments of electrical resistance of confluent polarized monolayers).

Best regards,

Charlene S. Dezzutti, PhD
Associate Professor

[redacted]

UNIVERSITY OF CALIFORNIA, SAN DIEGO UCSD

BERKELEY • DAVIS • IRVINE • LOS ANGELES • MERCED • RIVERSIDE • SAN DIEGO • SAN FRANCISCO SANTA BARBARA • SANTA CRUZ

Jerry Yang
Associate Professor
[redacted]

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May 24th, 2010

Dear Steve,

I'm writing to express my enthusiastic support for your NIH R21/R33 grant application entitled "The Semen Enhancer of HIV Infection as a Novel Microbicide Target." We are excited to continue our research on developing drug candidates for the modification of the deleterious function of amyloids. We are very excited to continue our collaboration and to test whether the compounds identified by our lab can have an effect on SEVI-mediated HIV infection. If successful, our collaborative efforts will result in the identification and optimization of new microbicide candidates that exhibit protective properties against sexually transmitted infection of microbes such as HIV through the attenuation of interactions between aggregated SEVI proteins and viral particles and cells.

Based on the very promising preliminary data over the past 6 months, we have been focusing on developing novel multivalent versions of the amyloid-targeting agents and related molecules to afford multi-grams scale quantities for further *in vitro* and *in vivo* testing. We have also developed two additional molecular frameworks for molecules that target amyloids and have chemical properties that are suitable for examination in the proposed experiments.

The funds we request from the sub-award will be used for partial salary support of a graduate student in my lab so we can continue our efforts to synthesize analogs and to characterize them for their suitability in the proposed experiments. We will, for instance, examine all analogs for their toxicity, log P values, and amyloid-binding properties. All of the most promising compound will be synthesized in large scales for further testing throughout the project period. I expect this project will require 10% of my effort during the first two years of project period, increasing to 15% during the third and fourth years, and reducing to 5% during the last year of the project period. I have found this to be a stimulating and exciting collaboration and I look forward to many thought-provoking conversations. I wish you success with your proposal.

Sincerely,

Jerry Yang

17. RESOURCE SHARING PLAN

Sharing of data and reagents generated by this project will be carried out in several different ways. Our plan includes:

Presentation at national scientific meetings: We expect to have at least one presentation per year at national meetings on microbicides and/or HIV research (e.g., CROI, Keystone Symposium on Protection from HIV). It is expected that the investigators from this project will be active participants in these meetings and share the data with scientists interested in microbicide development.

Publications: Publication in Open Access journals, in which the scientist retains copyright on the published material, will greatly expand general access to our findings and allow us to post articles to our web server as they appear in print. For this reason, we will consider such journals for our own publications, as well as journals such as the *Journal of Virology*, which provide free-online access to all published content with a 6-month lag period following publication.

Reagents: Immediately following publication of experiments that describe novel reagents or materials (virus vectors), we will make all such clones and constructs available upon request to all researchers who request them, after completion of a Biological Materials Transfer Agreement. For most non-profit institutions, when the transfer involves a biological material, we will use a simple AUTM implementing letter to document acceptance of the terms of the Uniform Biological Material Transfer Agreement (UBMTA). In those instances where the transfer involves a non-profit institution that is not a signatory to the UBMTA, the pertinent institution's standard Biological Materials Transfer Agreement will be used or (if no intellectual property is involved) the AUTM Simple Letter Agreement.

PHS 398 Checklist

OMB Number: 0925-0001

1. Application Type:

From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer the questions that are specific to the PHS398.

* Type of Application:

☒ New ☐ Resubmission ☐ Renewal ☐ Continuation ☐ Revision

Federal Identifier: [redacted]

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

Prefix:
* First Name:
Middle Name:
* Last Name:
Suffix:

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents (For renewal applications only)

* Inventions and Patents: Yes ☐ No ☐

If the answer is "Yes" then please answer the following:

* Previously Reported: Yes ☐ No ☐

4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☒ Yes ☐ No