Translational Research Scholars Program
Personal Statement

- How will the Translational Research Scholars Program benefit your career development?

The benefits that the Translational Research Scholars (TRS) program provides for my career development are multi-faceted:

1. The TRS program provides essential seed money to jump start my pilot project in a new translational research direction;

2. The TRS program allows me to get 15% release time for the pilot project;

3. The TRS program enables research mentorship and collaborations with productive researchers;

4. The TRS program provides important peer-to-peer feedback through monthly conference calls and face-to-face interactions;

5. The TRS program provides expert grant development assistance through mock reviews and training workshops.

- How will the program facilitate submission of your grant proposal?

I have a clear goal to submit a R-type NIH grant proposal upon successful completion of the TRS program. The program is designed to assist and monitor my grant proposal development on a monthly basis. This approach will help me tremendously because junior faculty like myself can easily get lost in heavy teaching and service assignments. The peer environment that the TRS program creates is also important as it will motivate me when I interact with other awardees with shared goals. Finally, grant expert reviews will provide valuable feedback and increase the odds of success for my grant proposal.

- What are your longer-term (next 5 years) translational and/or clinical research goals?

My goals for the next 5 years are:

Year 1: Complete the TRS project; Submit at least one manuscript based on the TRS project.

Year 2: Submit the R15 proposal. (Note: Idaho State Univ is eligible for the R15 grant mechanism).

Years 3-5: If funded, start the 3-year R15 grant project. If not, revise and re-submit the R15 proposal.

SPECIFIC AIMS

Clostridium sordellii infections are highly lethal and difficult to diagnose.[1] While some antibiotics can be used to prevent *C. sordellii* infections, few therapeutic options are available to mitigate the actions of already circulating exotoxins in infected patients. Thus, *there is a critical need to design and develop novel anti-toxins to neutralize the exotoxins released during C. sordellii infections*. In the absence of such intervention, the prospect of improving patient survivability will likely remain bleak. Our overarching goal is to develop effective small molecule anti-toxins for post-infection intervention. Our objective in this application is to screen FDA-approved drugs for the new indication of inhibiting the key *C. sordellii* exotoxin Mcs1. We recently reported the discovery and characterization of Mcs1 (*RSC Advances*, 2017, 7, 13928-13938).[2] We showed that Mcs1 is a plausible drug target for anti-toxin design because it is directly responsible for the extreme leukemoid reactions and fatality of *C. sordellii* infection.[3] Our specific aims are:

1. Screen and identify FDA-approved small molecule drugs, and evaluate their repurposability against Mcs1 using advanced bioinformatics and computer-aided drug design strategies.
2. Validate the identified lead molecules using *in vitro* assays.

SIGNIFICANCE

C. sordellii infections exacerbate rapidly within days. By the time the infected patients receive adequate antibiotic regimens, there is nothing they can do to limit the damages of exotoxins already in circulation as there is no anti-toxin available, Therefore, the significance of this proposed project is that it directly addresses the need to improve clinical outcomes by mitigating exotoxins at the critical stage of *C. sordellii* infections. Particularly, FDA-approved drug repurposing will greatly facilitate the development of deployment of the anti-toxins. This project is an important part of the collaboration between Dr. Xu (PI) and Dr. Aldape (Collaborator) to synergize their complementary research strengths, and the pivotal data generated from this project will enable them to pursue an NIH R15 grant application within a much shorter time frame.

INNOVATION

Antibiotic regiments are the only available treatment option for *C. sordellii* infections. Antibiotics, however, cannot mitigate the circulating exotoxins. This proposal is potentially innovative because the design of adjunct anti-toxins will address the unmet medical need and lead to substantial improvement in patient survivability. It will also advance the development of novel small-molecule anti-toxins against other microbial infections. In contrast to traditional antibody-based anti-toxins, this project will provide new interdisciplinary strategies for small-molecule based anti-toxin development and accelerate “bench to bedside” translation through repurposing FDA-approved drugs.

APPROACH

Aim 1 *Overall Strategy*: The molecular mechanisms and the 3D structure of Mcs1 have been characterized by the PI in a recent publication.[2] These new protein structures opened the door for computer-aided drug design (CADD). The PI has completed a pilot CADD screening campaign of 2,000 National Cancer Institute diversity set compounds. 7 compounds showed inhibitory activities on Mcs1. Figure 1 shows the most potent compound NSC71881 bound to the Mcs1 catalytic site with an IC50 of 19nM. These results demonstrated the feasibility and predictability of our interdisciplinary approach. In this aim, we will use NSC71881 as the lead compound to perform a ligand-based virtual screening (VS) of all FDA-approved drugs using a combination of 2D molecular fingerprints and 3D ROCS search algorithms.[4] Molecular structures of all FDA-approved

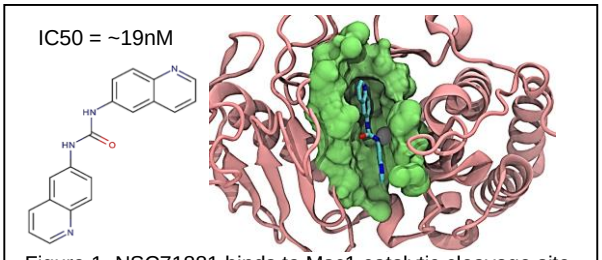

Figure 1. NSC71881 binds to Msc1 catalytic cleavage site.

drugs will be extracted from DrugBank.[5] The ligand-based VS will rank the FDA-approved drugs by their structural and chemical similarity to NSC71881 in a consensus fashion. The top 500 ranked drugs will be retained and subject to the receptor-based VS. The widely used molecular docking programs such as Schrodinger Glide, Moldock, and Autodock Vina will be used to perform the receptor-based VS and examine the molecular recognition between the 500 FDA-approved drugs and the Msc1 catalytic binding site characterized in the PI's published work.[2] Multiple Mcs1 structures that represent the open, close, and average states of the binding site cleft will be used in the receptor-based VS to account for protein flexibility. The final repurposability ranking of the 500 FDA-approved drugs will be evaluated from the consensus docking scores reported by all 3 docking programs. The top 50 ranked drugs will be purchased for experimental validation in Aim 2. Figure 2 shows the proposed project work flow.

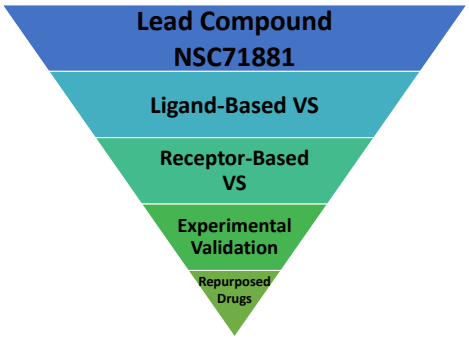

Figure 2. Project work flow.

Potential pitfalls and alternative strategy: Few difficulties are anticipated as the same CADD approaches have been successfully employed by the PI in other drug discovery projects.

Aim 3 *Overall Strategy*: The *C. sordellii* (ATCC 9714) strain will be used to generate exotoxins for the molecular inhibition validation assays. A Bactron II anaerobic chamber (atmosphere of 90% N2, 5% H2, and 5% CO2) will be utilized to maintain the anaerobic. *C. sordellii* will be cultured in brain heart infusion broth (BHI) and exotoxins will be collected from 10 mL BHI stationary phase cultures overnight (~12 hrs). Supernatants collected will be filter sterilized. All inhibitors identified in Aims 1 and 2 will be dissolved in DMSO to final concentrations of 0.005µg/ml – 50µg/mL. 5µL of each reconstituted inhibitor will be added to separate tubes containing 100µL of filter-sterilized supernatant from the toxin preparations. Drug/toxin preparations will be incubated in a 37ºC water bath for 2 hours prior to the inhibition validation assays. Mcs1 inhibitory activity will be determined by a drug's ability to block the cleavage of human vascular cell adhesion molecule (VCAM-1).[2] 30 µg/mL VCAM-1 will be incubated with an equal volume *C. sordellii* exotoxins and placed in a 37°C water bath for 30 minutes. The reaction will be terminated by a SDS-PAGE sample buffer containing no reducing agents, and samples will then be run on a 12% SDS-PAGE gel and transferred to a polyvinylidene difluoride membrane. Western blots will be developed using a monoclonal mouse anti-human VCAM-1 antibody followed by detection with an ECL2 Western Blotting substrate. Comparisons will be made between the VCAM-1 molecule degraded by the untreated Mcs1 preparation to VCAM-1 species exposed to toxin preparations pre-incubated with the inhibitors. Figure 3 shows the experimental validation of the best lead compound NSC71881 identified in the preliminary screen.

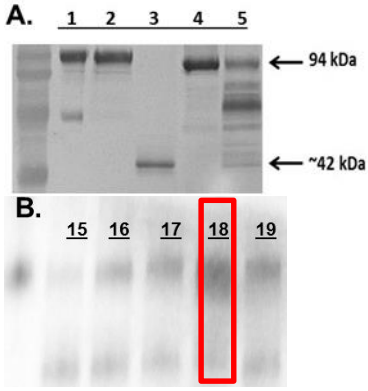

Figure 3. Inhibition validation assay. (A) Mcs1 cleaves human VCAM-1. VCAM-1 incubated with nothing (Lane 1), uninoculated culture media (Lane 2), *C. sordellii* culture supernatant (Lane 3), *C. sordellii* culture supernatant pre-treated with EDTA (Lane 4), or heat inactivated *C. sordellii* culture supernatant (Lane 5). (B) Western blot depicting the preliminary screen of NCI compounds. #18 is the best lead compound NSC71881.

Potential pitfalls and alternative strategy: Few difficulties are anticipated as the same experimental approach has been used in the PI's published work.[2] Fluorescence resonance energy transfer (FRET)-based assays will also be used to improve sensitivity and characterize drug inhibitory kinetics including inhibition constants (Ki).

References Cited

1. Aldape M, Bryant A, Stevens D. Clostridium sordellii infection: epidemiology, clinical findings, and current perspectives on diagnosis and treatment. Clinical Infectious Diseases 2006;43(11):1436-46.
2. Aldape MJ, Tao A, Heeney DD, McIndoo ER, French JM, Xu D. Experimental identification and computational characterization of a novel extracellular metalloproteinase produced by Clostridium sordellii. RSC advances 2017;7(23):13928-38.
3. Aldape MJ, Bryant AE, Stevens DL. Clostridium sordellii Infection: Epidemiology, Clinical Findings, and Current Perspectives on Diagnosis and Treatment. Clinical Infectious Diseases 2006;43(11):1436-46. 10.1086/508866
4. Schnecke V, Boström J. Computational chemistry-driven decision making in lead generation. Drug discovery today 2006;11(1):43-50.
5. Wishart DS, Knox C, Guo AC, et al. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic acids research 2006;34(suppl_1):D668-D72.

Applicant Name (Last, First, Middle):

ITHS | Institute of Translational Health Sciences
Accelerating Research. Improving Health.

DETAILED BUDGET

FROM	THROUGH
03/01/18	02/28/19

List PERSONNEL *(* Applicant Organization Only*)*
Enter Dollar Amounts Requested (omit cents) for Salary Requested and Fringe Benefits

Use Cal, Acad, or Summer to Enter Months Devoted to Project

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
TBD	Part-time Research Assistant	7.00			$15/hr	9,000	801	9,801
SUBTOTALS →						9,000	801	9,801

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)* Project data backup and storage	199
TRAVEL	
OTHER EXPENSES (Itemize by category)	

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR BUDGET PERIOD		**$** 10,000
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATION COSTS	
TOTAL DIRECT COSTS FOR BUDGET PERIOD		$ 10,000
TOTAL INDIRECT COSTS FOR BUDGET PERIOD		$ 0
TOTAL COSTS FOR BUDGET PERIOD		**$ 10,000**

BUDGET JUSTIFICATION

PERSONNEL

Dong Xu, Ph.D., Principal Investigator (15% effort, 1.8 months). Dr. Xu will supervise and conduct all aspects of the project, including project execution, personnel and data management, preparing abstracts and manuscripts, presenting findings at scientific meetings.

Michael Aldape, Ph.D., Collaborator (No salary requested, but will commit 2-3 person months effort). Dr. Aldape will be responsible for the experimental validation of the identified drug leads from the *in silico* screening campaign. He will also assist in manuscript preparation.

OTHER PERSONNEL

Part-Time Research Assistant (TBD, 7 months). The research assistant will assist the PI with project execution, including data analysis and collection, preparing abstracts and manuscripts.

SUPPLY

$199 is request to acquire project data backup and storage devices.

BIOGRAPHICAL SKETCH

NAME: Xu, Dong

eRA COMMONS USER NAME (credential, e.g., agency login): dannyxu

POSITION TITLE: Assistant Professor

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Nan Kai University, China	B.S	06/1996	Chemistry
San Diego State University	Ph.D.	05/2008	Computational Science
University of California, San Diego	Postdoctoral	08/2010	Computational Biochemistry/Biophysics

A. Personal Statement

My training, expertise, motivation, and project management experiences are well-suited for the successful completion of the proposed project. I have a broad background in advanced molecular modeling and biomedical informatics, with specific training and expertise in molecular dynamics (JMB, 2009; RSC Adv, 2017), protein-ligand binding free energy calculations (JACS, 2009), software development (JCTC, 2012), computer-aided drug design, and drug-induced toxicity (TADS, 2017). As NSF XSEDE campus champion, I am heading a research laboratory that focuses on computer-based biomedical discovery at Idaho State University. As PI or co-Investigator on several university- and state-funded grants, I have been developing novel computational approaches for predicting protein-ligand interactions and identifying new drug leads from *in silic*o screening. These efforts have led to a US patent (US20150093391 A1, 2015) issued on new anticancer agents and numerous publications. This proposed project builds logically on the prior *in silico* drug screening techniques that I have developed for small-molecules, as well as my recent structural characterization of Mcs1 exotoxin in *C. sordellii* (RSC Adv, 2017).

My research productivity was severely impacted during 2010-2015 when I was a teaching/visiting faculty member with substantially heavy teaching load. However, upon returning to the tenure-track position, I immediately resumed my research activities, and successfully competed for several university and state grants.

1. Xu D, Newhouse EI, Amaro RE, Pao HC, Cheng LS, Markwick PRL, McCammon JA, Li WW, Arzberger PW. (2009) Glycan topology for avian and human sialo-pentasaccharide receptor analogues upon binding different hemagglutinins: A molecular dynamics perspective. *J. Mol. Bio*. 387, 465-491.
2. Newhouse EI, Xu D, Amaro RE, Pao HC, Markwick PRL, Wu KJ, Alam M, McCammon JA, Li WW, Arzberger PW. (2009) Mechanism of glycan receptor recognition and specificity switch for avian, swine and human adapted influenza virus hemagglutinins: A molecular dynamics perspective. *J. Am. Chem. Soc*., 131 (47), 17430–17442.
3. Götz AW, Williamson MJ, Xu D, Poole D, Le Grand S, Walker RC. (2012) Routine microsecond molecular dynamics simulations with AMBER on GPUs – Part I: Generalized Born. *J. Chem. Theory Comput*. 8(5): 1542–1555.
4. Aldape MJ, Tao A, Heeney DD, McIndoo ER, French JM, Xu D. (2017) Experimental identification and computational characterization of a novel extracellular metalloproteinase produced by *Clostridium sordellii RSC Advances*, 7, 13928-13938.
5. Xu D, Anderson HD, Tao A, Hannah KL, Linnebur SA, Valuck RJ, Culbertson VL. (2017) Assessing and predicting drug-induced anticholinergic risks: an integrated computational approach. *Therapeutic Advances in Drug Safety*.
6. U.S. Patent US20150093391 A1. (2015) Oncostatin M (OSM) Antagonists for preventing cancer metastasis and IL-6 related disorders. Jorcyk, C, Xu D. Issued Apr 2, 2015.

B. Positions and Honors

Positions and Employment

2010-2012 Visiting Assistant Professor, Boise State University, Boise, ID
2012-2015 Visiting Assistant Professor, Idaho State University, Meridian, ID
2015- Assistant Professor, Department of Biomedical and Pharmaceutical Sciences, Idaho State University, Meridian, ID

Other Experience and Professional Memberships

2007- Member, American Chemical Society (ACS)
2008-2011 Member, Biophysical Society
2012-2014 Member, Society of Neuroscience (SofN)
2012- Member, American Association of Colleges of Pharmacy (AACP)
2012- Member, American Association of Pharmaceutical Scientists (AAPS)
2008- Invited Reviewer for 25 High-Impact Scientific Journals
2010- Editorial Board Member, Journal of Proteome Science & Computational Biology
2010- Editorial Board Member, Journal of Molecular Engineering & Systems Biology

Honors

2016 NSF XSEDE Campus Champion
2015 NIH IDeA Mountain West Clinical and Translational Research Best Research Poster Award
2014 Elsevier Excellence Award in Peer-Review

C. Contribution to Science

1. Molecules are inherently flexible and many molecular motions that are extremely anharmonic. Accurate prediction of molecular vibrational states and energies requires new approaches to go beyond harmonic approximation and take into account of couplings among two or more distinct vibrational coordinates. I sought to develop a novel *ab initio* method that addresses these challenges. My work resulted in the FEMVib software package, a vibrational Schrodinger equation solver that works on any arbitrary multi-dimensional potential energy surface. FEMVib has been extensively validated to resolve the eigenvalues and wave functions of hundreds of vibrational energy states at high accuracy. It computes the complete vibrational spectra of small to large molecular systems and allows complete coupling of vibrational coordinates. I undertook this research as a graduate student.
    a. Xu D, Cooksy AL. Ab initio study of the torsional motion in tolane. (2007) *J. Mol. Struct*. THEOCHEM, 815, 1, 119-125.
    b. Xu D, Stare J, Cooksy AL. (2009) Solving the vibrational schrodinger equation on an arbitrary multidimensional potential energy surface by the finite element method. *Comp. Phys. Comm*., 180, 2079-2094.
    c. Zou W, Xu D, Zajac P, Cooksy AL, Bersuker IB, Liu Y, Boggs JE. (2010) Symmetry breaking in linear ZnCl2+: A theoretical study, *J. Mol. Struct*., 978, 263-268.

2. Influenza viruses have been a major and persistent threat to human health. Recent global pandemics highlighted the critical need of developing effective antiviral medicines. My postdoctoral work involved advancing the fundamental biological knowledge of influenza viruses and designing new antivirals using novel computational methods. My publications reported that that influenza viral protein hemagglutinin preferentially binds to host cell glycan receptors that exhibit unique conformational topologies and the topologies are distinct between human and avian host cells. I further revealed the molecular mechanisms of glycan receptor recognition by free energy calculations. These mechanistic insights led to the discovery of multiple anti-influenza inhibitors using the molecular dynamics ensemble-based *in silico* approach.
    a. Xu D, Newhouse EI, Amaro RE, Pao HC, Cheng LS, Markwick PRL, McCammon JA, Li WW, Arzberger PW. (2009) Glycan topology for avian and human sialo-pentasaccharide receptor analogues upon binding different hemagglutinins: A molecular dynamics perspective. J. Mol. Bio. 387, 465-491.
    b. Newhouse EI, Xu D, Amaro RE, Pao HC, Markwick PRL, Wu KJ, Alam M, McCammon JA, Li WW, Arzberger PW. (2009) Mechanism of glycan receptor recognition and specificity switch for avian,

swine and human adapted influenza virus hemagglutinins: A molecular dynamics perspective. *J. Am. Chem. Soc*., 131 (47), 17430–17442.

c. Amaro RE, Cheng X, Ivanov I, Xu D, Mccammon JA. Characterizing loop dynamics and ligand recognition in human- and avian-type influenza neuraminidases via generalized born molecular dynamics and end-point free energy calculations. (2009) *J. Am. Chem. Soc*., 131(13), 4702-4709.

d. Cheng LS, Amaro RE, Xu D, Li WW, Arzberger PW, McCammon JA. (2008) Ensemble-based virtual screening reveals novel antiviral compounds for avian influenza neuraminidase. *J. Med. Chem*., 51 (13), 3878–3894.

3. Molecular dynamics simulation studies have to confront two challenges: (1) achieving adequate conformational sampling; (2) capturing dynamics over long timescales. My collaboration with AMBER software developers addressed these challenges by implementing new GPU-accelerated molecular dynamics algorithms. Enhanced conformational sampling was achieved over long-timescale dynamics running on powerful GPUs, providing new atomic insights into many important biological processes.
    a. Xu D, Williamson MJ, Walker RC. (2010) Advancements in molecular dynamics simulations of biomolecules on graphical processing units, *Annu. Rep. Comp. Chem*., 6, 2-19.
    b. Götz AW, Williamson MJ, Xu D, Poole D, Le Grand S, Walker RC. (2012) Routine microsecond molecular dynamics simulations with AMBER on GPUs – Part I: Generalized Born. *J. Chem. Theory Comput*. 8(5): 1542–1555.

4. In addition to the contributions described above, with an experimental collaborator, I discovered several anticancer agents that disrupt Oncostatin M induced signaling pathways and effectively prevent breast cancer metastases. These compounds are protein-protein interaction blockers, identified using the molecular dynamics ensemble-based *in silico* approach. A US patent has been issued and further development is underway with the goal to transform the compounds into new cancer treatment.
    a. U.S. Patent US20150093391 A1. (2015) Oncostatin M (OSM) Antagonists for preventing cancer metastasis and IL-6 related disorders. Jorcyk, C, Xu D. Issued Apr 2, 2015.

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Idaho NASA Research Initiation Grant — Xu (PI) — 5/1/2017-4/30/2018
Computational nanoreactor reveals how organic compounds are formed in space.
The goal of this study is to gain atomic insights into the astrochemical process of organic compounds formation in space, in collaboration with experimental researchers at the NASA Ames Research Center.
Role: PI

Completed Research Support

Idaho State University Collaborative Partnership — Xu (PI) — 12/15/15-11/30/16
Graduate preparation for the biomedical and behavioral sciences at Idaho State University
The goal of this study is to develop a competitive NIH R25 grant proposal which will improve graduate student education, recruitment, and diversity in biomedical and behavioral sciences at ISU.
Role: PI

NIH IDeA Mountain West CTR — Xu (PI) — 7/1/15-6/30/16
Predicting the risks of drug-induced anticholinergic toxicity
The goal of this project was to develop a novel computational toxicity scoring system to assess and predict the risks of clinical anticholinergic toxicity in general population.
Role: PI

Idaho Beef Council Research Grant — Xu (PI) — 7/1/14-6/30/16
Computer aided design of novel antibiotics to treat bacterial infections in cattle
The goal of this project was to develop novel antibacterial agents to mitigate *E. coli* colonization in cattle.
Role: PI