SUMMARY STATEMENT

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(Privileged Communication)

Release Date: **10/25/2016**
Revised Date:

Application Number: **1 R15 AI124044-01A1**

Principal Investigator

DOMASHEVSKIY, ARTEM

Applicant Organization: JOHN JAY COLLEGE OF CRIMINAL JUSTICE

Review Group: **MSFB**
Macromolecular Structure and Function B Study Section

Meeting Date: **10/13/2016**
Council: **JAN 2017**
Requested Start: **04/01/2017**

RFA/PA: **PA16-200**
PCC: **M64A B**

Project Title: **Development of a Novel Inhibitor of Ricin: A Potential Therapeutic Lead against Deadly Shiga and Related Toxins**
SRG Action: **Impact Score:15**
Next Steps: **Visit http://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **10-No live vertebrate animals involved for competing appl.**

Project Year	Direct Costs Requested	Estimated Total Cost
1	[redacted]	[redacted]
TOTAL	[redacted]	[redacted]

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1R15AI124044-01A1 Domashevskiy, Artem

RESUME AND SUMMARY OF DISCUSSION: This application proposes to develop new inhibitors for ribosome inactivating proteins such as ricin and Shiga toxin using a combination of structural (Crystallography and NMR), biophysical (fluorescence and calorimetry) and computational approaches. Given the heightened interests and importance of measures to counter bioterrorism, this project has a solid premise. The diverse techniques involved in this program offer an excellent training opportunity for undergraduate students. Particularly, the proposed research is likely to be highly impactful in the setting of a city university. In this resubmitted application, the applicant has done a great job addressing the previous critiques by providing high-quality preliminary data, thus mitigating the major concerns. The planned experiments are feasible, meritorious and rigorously designed, from which valuable outcomes can be expected. The excellent investigative team that was assembled by the applicant consists of nearby laboratories with all needed expertise. The host institution is also very supportive. Although there are a few weaknesses such as that the description of Aim 2 is a little vague, the validation procedures of the structures by modeling may not be justified, and that some Biosketches are missing, these were all considered as relatively minor concerns. In general, the panel was extremely positive and enthusiastic about the program. The overall impact of this application was deemed to be very high.

DESCRIPTION (provided by applicant): The PI proposes a high-impact collaborative research project to develop new inhibitors for ribosome inactivating proteins (RIPs), such as ricin and Shiga toxin. Ricin toxin, produced by the castor bean plant, has a nefarious past. Ricin is a well-known homicidal poison and has been used in several bioterrorist attacks. Shiga toxin is a deadly product of enterobacterial Escherichia coli. RIPs are RNA N-glycosidases that enzymatically remove specific purine residues from the universally conserved sarcin/ricin loop (S/R loop) of large ribosomal RNA, causing cellular death. There are no effective pharmaceuticals for either ricin or Shiga toxin poisoning. The PI proposes using a viral protein (VPg) from turnip mosaic virus that we have shown to inhibit ricin activity in vitro. The goals of this proposal are: 1) Establish conditions for VPg-ricin complex formation and the rates of VPg-ricin interactions; 2) Determine minimum VPg peptide that effectively inhibits ricin and Shiga toxins; and 3) Optimize conditions to enhance inhibition of these toxic proteins. This is an innovative activity. Based on the structural and mechanistic similarities of these toxins and previously acquired data showing that VPg peptides inhibit ricin activity in vitro, we believe the outcomes of this activity will provide leads for the synthesis of therapeutic peptides. In turn, this will serve as a catalyst for the development of constructively applied solutions for the inhibition of these deadly toxins. Furthermore, this proposal will enhance the infrastructure of research and education at John Jay College, introducing biochemical and biomedical research experiences to underrepresented minority and female students, who would otherwise lack such opportunities. This would allow them to experience a broad spectrum of techniques, and acquire skills such as data analysis used in modern scientific investigations, while developing a vast network of partnerships among scientists from national and international institutions.

PUBLIC HEALTH RELEVANCE: Ricin and related Shiga toxins are potentially lethal poisons, which as of yet, have no effective pharmaceutical treatments or preventions. The global problem of these poisons is an increasing threat to public health, directly relating to the mission of the National Institutes of Allergy and Infectious Diseases. Ricin, astoundingly easily acquired, is an ideal toxin for bioterrorism, while the widespread transmission of E. coli poisoning is responsible for many severe human illnesses and several fatalities. In the future, it may be possible to utilize these toxins for their public health benefit, by repurposing them to attack specific human and animal diseases.

CRITIQUE 1

Significance: 2
Investigator(s): 2

Innovation: 2
Approach: 2
Environment: 2

Overall Impact: Ribosome inactivating proteins (RIPs) are an important class of cytotoxin. RIPs, such as Ricin A-chain (RTA) and Shigella toxin, are N-glycosidases that modify rRNA thereby deactivating ribosomes. RIPs are extremely toxic and there is significant interest in strategies to inhibit their action. To date there is neither an effective vaccine nor a therapeutic agent available to reverse the effects if Ricin once exposed. The PI has previously discovered a peptide inhibitor of RTA, called VPg, that binds to RTA with nM affinity. Here the PI plans to further characterize the parameters of VPg binding by RTA and determine the minimum VPg peptide that binds and inhibits RTA. Resulting VPg constructs will be tested in vivo by a collaborator, Dr. O'Brien. Structural information on the complex will be obtained by either X-ray crystallography or NMR, for which good collaborations are established. Residues that participate in RTA-VPg binding will be probed by in silco analysis to find mutants of VPg that bind RTA more tightly. These mutations will then be validated in vitro (and in cell-based assays?). On the whole this is a strong proposal with only a few weaknesses. The undergraduate environment is excellent, very supportive of the PI, and serves a large population of URMs.

1. Significance:

Strengths

- Strong scientific premise.
- RIPs are an important class of toxins for which there are no current treatments or vaccines.

Weaknesses

- None stated.

2. Investigator(s):

Strengths

- The PI discovered that VPg inhibits PAP and has all the necessary expertise (along with the collaborators) to complete the proposed studies.
- The PI has a solid publication record relevant to the proposed studies.

Weaknesses

- None stated.

3. Innovation:

Strengths

- Employing peptide inhibitors of RIP is innovative.

Weaknesses

- Not really a weakness but all approaches, including site-directed mutagenesis, are standard.

4. Approach:

Strengths

- Strong preliminary data showing that VPg binds RTA with high affinity.

- A rigorous and well thought out approach is provided, with good alternative strategies outlined should problems arise.
- Proposed experiments are accessible to undergraduates and the PI provides a strong mentoring statement.
- Use of ITC and fluorescence anisotropy is a plus.

Weaknesses

- Lack of RTA-VPg crystals (or preliminary NMR data).
- It is unclear how successful the creation of VPg constructs with higher affinity for RTA will be, given that the interaction is already quite strong (~50 nM). The proposed mutagenesis strategy will more likely reveal residues that do not contribute to affinity significantly. Modification of these residues may help in generating a peptide with better properties for use as a drug.

5. Environment:

Strengths

- The PI or collaborator labs have access to all the necessary equipment.
- A strong list if collaborators.
- Institute is very supportive and has a high URM representation.

Weaknesses

- Many of the experiments proposed will be performed outside of the PI lab.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

Resubmission:

- The PI was receptive to previous comments/suggestions.

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Acceptable

Resource Sharing Plans:

Unacceptable

- None provided

Authentication of Key Biological and/or Chemical Resources:

Acceptable

Budget and Period of Support:

Recommend as Requested

CRITIQUE 2

Significance: 2
Investigator(s): 4
Innovation: 2
Approach: 2
Environment: 3

Overall Impact: This is a resubmission of a R15 application that seeks to develop new antitoxins for ricin and Shiga toxin using viral protein VPg, making the significance of this proposal very high. The previous proposal was criticized for lack of key preliminary binding data, no discussion of other biophysical methods such as NMR and X-ray, and poorly outlined molecular dynamics simulation. All of these weaknesses have been adequately addressed. Strong preliminary data show actual binding of VPg to and RTA and inhibition of RTA making this project entirely feasible. Prior postdoctoral experience, publications and preliminary data indicate the PI is capable of carrying out the project. The judicious choice of experts to round out areas of weakness (NMR with Dr. Greenbaum; X-ray with Dr. Jeruzalmi; and molecular modeling, docking and scoring algorithms with Drs. Shaneen Singh and Emillio Gallicchio) is also a big plus. The proposed research is clearly written, rationales are outlined and the methodology clearly laid out for easy participation by undergraduate students, with many opportunities for the students to interact with these collaborators. Given the large population of underrepresented groups potentially being exposed to a strong undergraduate educational environment, given that conditions for forming a stable RTA-VPg complex have been worked out, and given the physiological relevance of RTA-VPg complex, I am very enthusiastic for the R15 proposal.

1. Significance:

Strengths

- Premise is sound: ~50nM high affinity binding between VPg and RTA and ability to inhibit RTA enzymatic activity does provide an avenue to develop novel inhibitors.
- No currently known inhibitors of ricin makes this work significant.

Weaknesses

- None stated.

2. Investigator(s):

Strengths

- Has extensive training and expertise in area proposed.

- Areas of weakness are supplemented nicely with expertise in X-ray crystallography (Dr. Jeruzalmi); NMR (Dr. Greenbaum); molecular modeling, docking and scoring algorithms (Drs. Shaneen Singh and Emillio Gallicchio).

Weaknesses

- None stated.

3. Innovation:

Strengths

- Work to use peptides derived from VPg as inhibitors is novel.

Weaknesses

- None stated.

4. Approach:

Strengths

- Rigorous application of sound biophysical tools are a strength: outlined NMR experiments to do assignments and monitor and calculate changes in chemical shift perturbation (with Greenbaum), stopped flow kinetics (in Goss lab) and ITC (in Latham lab).
- Has relevant clones in hand.
- Will be able to test developed antitoxins (with O'Brien).
- Uses knowledge gained to expand to small molecule discovery using virtual screening and molecular docking.

Weaknesses

- Some details in the approaches missing. Minor.

5. Environment:

Strengths

- Environment at JJC is very supportive and a large population of URMs stand to greatly benefit from this proposal's success and therefore potentially will have a high impact on exposure of students to state of art research.

Weaknesses

- None stated.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Not Applicable (No Biohazards)

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Authentication of Key Biological and/or Chemical Resources:

Not Applicable (No Relevant Resources)

Budget and Period of Support:

Recommended budget modifications or possible overlap identified:

CRITIQUE 3

Significance: 3
Investigator(s): 2
Innovation: 5
Approach: 2
Environment: 1

Overall Impact: This is a well-written proposal from a new investigator who is an Assistant Professor at John Jay College. The PI proposes to explore the interaction of the VPg protein from the turnip mosaic virus with the A chain of the ricin toxin (RTA). The long term goal is to define the molecular details of VPg binding and inhibition of ricin and Shiga toxin so that novel peptide-based inhibitors might be developed for these toxins. The experimental plan, especially in Aim 1, is extremely well described as are areas of involvement for student researchers at John Jay College. The PI has previously described the interaction of VPg with another ribosome inactivating protein (PAP) and is clearly capable of conducting the binding and in vitro activity studies with RTA. In this amended application the PI has now included clear preliminary data demonstrating that VPg binds and inhibits RTA. He has also made other changes in response to the previous review that have strengthened the study. One area of concern is that the studies in Aim 2 are a bit outside of the PI's direct expertise and are not described in sufficient detail. Nevertheless, the project as a whole is meritorious, will expose students to research, and will strengthen the research environment at John Jay College – in short – it meets the goals of the R15 program quite well.

1. Significance:

Strengths

- There are few effective therapies for ribosome inactivating toxins.
- Ricin toxin has been identified as a possible bioterrorism agent.
- This work could lead to the development of therapeutic inhibitors for RIPs including Shiga toxin, which is a contributor to food-borne illness.

Weaknesses

- None stated.

2. Investigator(s):

Strengths

- The PI has previously described VPg binding and inhibition of PAP and is well qualified to conduct the studies described in Aim 1.

- The PI has been engaged in training students in research.
- The array of collaborators should provide the necessary expertise to contribute to the areas where the PI has less experience (Aim 2).

Weaknesses

- While the collaborators appear to have the necessary experience, it was hard to discern this without having their biosketches included.

3. Innovation:

Strengths

- The use of peptides to inhibit RIPs is novel.

Weaknesses

- The research proposed uses standard approaches and is not very innovative.

4. Approach:

Strengths

- The premise of the study is very clear and solid.
- The experiments described in Aim 1 are very detailed and rigorous.
- Considerable attention is given to describe expected outcomes and alternative approaches.
- The new preliminary data demonstrating interaction of RTA and VPg indicates that these studies aimed at defining this interaction will yield useful information.
- The PI has included additional biophysical methods (NMR, X-ray crystallography) to more completely define the RTA-VPg interaction, and has identified collaborators to assist with this work.

Weaknesses

- The molecular docking experiments do not seem to be very well justified. They are proposed to “confirm” the X-ray and NMR studies which seems to be somewhat odd. If the PI is successful in generating a high-resolution co-crystal structure of RTA and VPg, then I do not see the need to conduct docking after the fact.
- Unlike in Aim 1, the studies in Aim 2 using NMR and X-ray crystallography are not well described. For instance, attention is given to some issues relating to generating crystals but little information in provided on how the structures will be determined.

5. Environment:

Strengths

- The PI and his collaborators have access to all the necessary equipment to do this work.
- The institution is well positioned to benefit from this R15 project.
- The high representation of URM students and the plan to provide support for these students to participate in the research.

Weaknesses

- None stated.

Protections for Human Subjects:

Not Applicable (No Human Subjects)

Vertebrate Animals:

Not Applicable (No Vertebrate Animals)

Biohazards:

Acceptable

- The work proposed with full ricin and Shiga toxin will be conducted in an approved facility with the appropriate controls in place.

Resubmission:

- The PI has done an excellent job in responding to the previous critiques. In particular the inclusion of new preliminary data has alleviated the major concern.

Applications from Foreign Organizations:

Not Applicable (No Foreign Organizations)

Select Agents:

Not Applicable (No Select Agents)

Resource Sharing Plans:

Unacceptable

- No resource sharing plan was included.

Authentication of Key Biological and/or Chemical Resources:

Unacceptable

- No mention of how the various peptides or proteins will be authenticated was included in this section.

Budget and Period of Support:

Recommend as Requested

THE FOLLOWING SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE, OR REVIEWERS' WRITTEN CRITIQUES, ON THE FOLLOWING ISSUES:

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.

NIH has modified its policy regarding the receipt of resubmissions (amended applications). See Guide Notice NOT-OD-14-074 at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-074.html. The impact/priority score is calculated after discussion of an application by averaging the overall scores (1-9) given by all voting reviewers on the committee and multiplying by 10. The criterion scores are submitted prior to the meeting by the individual reviewers assigned to an application, and are not discussed specifically at the review meeting or calculated into the overall impact score. Some applications also receive a percentile ranking. For details on the review process, see http://grants.nih.gov/grants/peer_review_process.htm#scoring.

MEETING ROSTER
Macromolecular Structure and Function B Study Section
Biological Chemistry and Macromolecular Biophysics Integrated Review Group
CENTER FOR SCIENTIFIC REVIEW

MSFB
10/13/2016 - 10/14/2016

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* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.