SUMMARY STATEMENT

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(Privileged Communication)

Release Date: **02/01/2021**
Revised Date:

Application Number: **1 R01 AI161245-01**

Principal Investigator

CHODERA, JOHN DAMON

Applicant Organization: SLOAN-KETTERING INST CAN RESEARCH

Review Group: **ZAI1 AMC-W (M1)**
National Institute of Allergy and Infectious Diseases Special Emphasis Panel
Emergency Awards: Rapid Investigation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Coronavirus Disease 2019 (COVID-19)

Meeting Date: **01/14/2021**
Council: **MAY 2021**
Requested Start: **09/01/2020**

RFA/PA: **PAR20-178**
PCC: **M51C S**

Project Title: **Development of small molecule SARS-CoV-2 Mpro inhibitors via large-scale crystallography and computation**
SRG Action: **++**
Next Steps: **Visit https://grants.nih.gov/grants/next_steps.htm**
Human Subjects: **10-No human subjects involved**
Animal Subjects: **30-Vertebrate animals involved - no SRG concerns noted**

Project Year	Direct Costs Requested
1	486,630
2	487,060
3	487,499
TOTAL	1,461,189

++NOTE TO APPLICANT: Members of the Scientific Review Group (SRG) were asked to identify those applications with the highest scientific merit, generally the top half. Written comments, criterion scores, and preliminary impact scores were submitted by the assigned reviewers prior to the SRG meeting. At the meeting, the more meritorious applications were discussed and given final impact scores; by concurrence of the full SRG, the remaining applications, including this application, were not discussed or scored. The reviewers' comments (largely unedited by NIH staff) and criterion scores for this application are provided below. Because applications deemed by the SRG to have the highest scientific merit generally are considered for funding first, it is highly unlikely that an application with an ND recommendation will be funded. Each applicant should read the written critiques carefully and, if there are questions about the review or future options for the project, discuss them with the Program Contact listed above.

1R01AI161245-01 Chodera, J

DESCRIPTION (provided by applicant): Therapies for COVID-19which has claimed over 700K lives and infected more than 19M just nine months after the ?rst casesare urgently needed. While multiple therapeutic modalities are currently being developed, only non-covalent small molecule inhibitors have the potential to be produced rapidly and inexpensively at scale. The SARS-CoV-2 main viral protease (Mpro, also known as 3C-like, 3CL, or nsp5) is an attractive target for the development of new therapies given its distinctiveness from host proteases, essentiality in the viral life cycle, and high sequence conservation to SARS-CoV-1 Mpro. Following a high-throughput fragment screen conducted at the DiamondMX/XChem automated beamline which revealed over 70 small molecule interactions that densely cover the protease active site, the COVID Moonshot collaboration was formed to rapidly develop novel hits and existing scaffolds into potent and safe patent-free inhibitors that can be taken into clinical trials. Since its inception four months ago, the Moonshot has synthesized and assayed over 900 compounds, solved over 200 X-ray structures of new compounds bound to Mpro, identi?ed numerous sub-micromolar inhibitor lead series with viable paths to rapid therapeutic development, and demonstrated antiviral activity for multiple lead series. In this project, Moonshot will rapidly progress three distinct noncovalent chemical series to develop a complete preclinical package by integrating high-throughput crystallography (DiamondMX/XChem) and biochem- ical assays (Weizmann) with rapid alchemical free energy calculations exploiting the world's largest computing resource (Folding@home/MSKCC), automated synthetic route planning (PostEra), medicinal chemistry expertise (MedChemica), and synthesis and in vivo assay contract research organizations operating at near cost (Enamine, Sai Life Sciences, WuXi, UCSD, and others).

PUBLIC HEALTH RELEVANCE: Therapies for COVID-19—which has claimed over 700K lives and infected more than 19M just nine months after the ?rst cases—are urgently needed. This open science project—the COVID Moonshot—seeks to rapidly develop a new patent-free inexpensive small molecule therapeutic targeting the main viral protease of SARS-CoV-2 (the virus that causes COVID-19 disease) with the help of the world's only high-throughput X-ray crystallography beamline (DiamondMX\/XChem) with the largest distributing computing resource in the world (Folding@home) to accelerate design-make-test cycles.

CRITIQUE: The comments in the CRITIQUE section were prepared by the reviewers assigned to this application and are provided without significant modification or editing by staff.

CRITIQUE 1

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

DESCRIPTION (provided by reviewer): This application aims at exploring small non-covalent inhibitors of the SARS-CoV-2 main protease (Mpro) derived from aminopyridine, quinolone and benzotriazoles. The application combines expertise in the use and development of computational tools for drug design, X-ray crystallography, biochemical and cell functional assays, and live pharmacokinetics studies in animal models. The PIs propose to collaborate with Enamine, a contract research organization for the chemical synthesis of the selected potential drug candidates. The application is a multi-collaborative effort resulting from the Moonshot project, an international, crowdsourced initiative to produce COVID antivirals.

Overall Impact: The overall goal of the project is to produce oral SARS-CoV-2 Mpro inhibitors as antiviral treatment. The resources and expertise combined in the research team are impressive and indicate high probability of success. Three different categories of compounds will be investigated, which also increases chances of success. The approach is thorough in considering aspects to prepare a preclinical evaluation of the identified compounds.

1. Significance:

Strengths

- The project addresses the important problem of identifying patent-free and low-cost, non-covalent inhibitors of SARS-CoV-2 protease as potential oral antiviral drugs.
- The premises are rigorous as preliminary data already show compounds with low micromolar EC50 values in viral replication assays.

Weaknesses

- The importance of non-covalent inhibitors is not properly addressed as compared to covalent inhibitors, and data on the benefits of exploring non-covalent versus covalent inhibitors is not adequately presented.

2. Investigator(s):

Strengths

- The application includes the necessary expertise: computational for drug design (3 experts), structural biology applied to drug design, in vivo pharmacokinetics, animal models, biochemistry.
- The coordination of the research team as described is thorough and relies on an already working platform.

Weaknesses

- None noted

3. Innovation:

Strengths

- The crowdsourcing strategy, multi-partner collaboration and combined expertise are significant innovative aspects in the application.

Weaknesses

- Concepts, theories, or interventions as described in the INNOVATION page are not novel.

4. Approach:

Strengths

- The approach states two different logical and adequate criteria for compound advancement and a third group of criteria for selecting preclinical candidates.
- The logistics for decision making, selection, compound synthesis and data management are properly described and rigorous.
- The combined intervention of each partner involved in the project to achieve the desired goals is adequate.
- The assay cascade is thorough and well-conceived.

Weaknesses

- The three aims of the project might seem redundant by dealing with the exploration of three different types of compounds in analogous ways.

5. Environment:

Strengths

- The facilities, equipment, collaborations and logistics are perfectly adjusted to achieve the proposed goals.

Weaknesses

- None noted

CRITIQUE 2

Significance:	3
Investigator(s):	3
Innovation:	6
Approach:	7
Environment:	4

Overall Impact: This project focuses on the design and development of non-covalent inhibitors of Mpro using a combination of molecular modeling/FEP calculations, protein crystallography, bioassays, and contracted chemical synthesis. A significant portion of the application is devoted to explaining the attributes of the approach and preliminary work that led to the discovery of the current lead molecules. Most of the sections focus on how the investigators selected and refined the current leads which include 3 scaffolds: aminopyridines, quinolones, and benzotriazoles. One of the primary innovations offered by the team of investigators is the concept of HT X-ray crystallography and crowdsourcing (of chemical structures) in the identification leads. The description of how these components work together, however, is not clearly described (or published). In the development of the lead scaffolds, the approach only appears to be used in the design the case of the aminopyridines. Furthermore, from the description of how the lead was assembled it does not appear that crowdsourcing was part of the final design (that mated two fragments identified using X-ray data). The remaining two scaffolds were taken from prior work on CoV-1. It is important to point out that the project under evaluation is Phase 3 of the overall plan. Phase 3 (seen in Fig 1) is refinement and optimization of the scaffolds that are selected

and advanced using a classic decision tree (Assay Cascade, Fig. 6) to preclinical studies. It is unfortunate that the bulk of the proposal is dedicated to a description of how the project moved through Phase 0 and Phase 1 instead of focusing on the work to be completed in Aims 1-3 (which is very brief and not detailed). Part of the problem is that none of the work is published and many references are made to data in websites that have not been peer reviewed. There are also very few references to support the work provided. The preliminary data provided is mainly descriptive in the logistics of the approach applied to arrive at the 3 scaffolds. Experimental data is given that indicates the compounds were initially low μM inhibitors of Mpro and μM inhibitors of CoV-2 replications. Updated data was also submitted showing the activity was improved for the aminopyridines (sub-μM at Mpro and low μM in antiviral assays). Insufficient in vitro biological data was given for the other two scaffolds. In completing the 3 Aims of the project the approach applies a combination of molecular modeling/FEP calculations, X-ray crystallography, and bioassays to refine the current lead compound for each scaffold (where Aim 1 is aminopyridine, Aim 2 is quinolones, and Aim 3 is benzotriazoles). The aims are repetitive and lack a clear plan of how the steps of the cascade tree will be performed. The approach involves the use of SAR generated from X-ray data and biological activity of the synthesized analogs. However, no SAR analysis of the 264 aminopyridines already assayed is presented to guide the synthesis of additional analogs. The plan for synthesis (in Phase 2) is also not clear since this work will be contacted it is critical to clearly define how this strategy will be implemented. The work performed by PostEra in vetting the synthesis of routes and prioritization is also not relevant at this stage in the “phases” as outline in Fig. 1). The team has set clear criteria for biological activity, ADME/PK and progression through the decision tree. Compounds will move forward from basic Mpro and CPE-type antiviral assays to ADME and more advanced PK and antiviral screening based on fairly traditional set values for thresholds. Very little detail is provided, however, on the strategy for implementing these later studies. Instead, logistic are discussed which do very little to support the scientific basis to the work. The cascade details that are provided are “canned” methods and not written to account for the unique properties of the scaffolds or approach taken in the design of the compounds and research plan. More details are also needed in the use of FEP modeling, X-ray crystallography, synthetic prioritization, and SAR planning. These are critical issues that define the potential success of Phase 2 (as described in Fig. 1) in completing the project as proposed.

1. Significance:

Strengths

- Mpro is a well-established target for antiviral drug development.
- The design non-covalent inhibitors of Mpro would represent a significant advance in the design of treatments for COVID-19 and may offer alternate strategies to defeating resistance mechanisms.

Weaknesses

- While this project focuses seeks to advance one of 3 molecular scaffolds to a therapeutic outcome, the plan lacks detail and depth, greatly limiting the potential significance of the work.

2. Investigator(s):

Strengths

- The PI is trained in computational biophysics and has outstanding qualifications to conduct the FEP computational experiments described in the application.

- The PI has assembled an excellent team of investigators to complete the bioassays and X-ray work.

Weaknesses

- A major weakness to the project lies in the lack of experience of the PI in drug discovery, translational studies (ADME/PK), synthetic medicinal chemistry, and antiviral bioassays.
- There is also no evidence in prior publications that the PI can lead a scientific effort in the field of drug discovery and has no principle author papers to support the work.
- The use of CRO's to complete the synthesis is a major weakness to the team.
- There is one citation to the PI's work in the application which refers to a non-peer reviewed deposit to the bioRxiv archives (which is unrelated to CoV-2).
- The use of multiple sites/collaborators (including a pass through arrangement by Northeastern to AstraZeneca) present significant hurdles to maintain fidelity and integrity to the project and the PI has no experience in managing these efforts (that include animals).

3. Innovation:

Strengths

- The primary innovation to the project lies in the integration of FEP calculations with HT X-ray crystallography and synthesis to rapidly identify analogs with improved Mpro activity.

Weaknesses

- Although significant claims to innovation are made in the application which refer to the use of crowdsourcing and HT X-ray structure determination and large scale FEP screening in generating preliminary data, this project is focused on the final phase of this work and is based on traditional iterative drug discovery approaches that combine molecular modeling, X-ray crystallography, bioassays, and chemical synthesis to refine "hits" to generate pre-clinical leads.

4. Approach:

Strengths

- The primary strength of the project lies in the application of FEP modeling of free energies, HT X-ray crystallography validation of the models, and SAR-based refinement of hits with ADME prioritization.

Weaknesses

- The primary weakness of the project lies in lack of detail and support given to the plan for optimization of the hits in this phase of the project (as detailed below).
- The approach follows a traditional decision tree involving compounds design, synthesis, bioassay, and refinement with criteria for advancing to CPE and intro profiling (Criteria A) and more advanced PK and CoV-2 animal studies (Criteria B) with a final goal of clinical handoff. While the criteria is given for advancing through the cascade it is not clear how revision/refinement to the scaffold will be performed.
- In the 3 Aims (which focus on the three scaffolds - 1 aim per scaffold), a discussion is presented that suggests the team has generated SAR from the initial set of 264 compounds (aminopyridines) but a SAR analysis is not given and a plan is not shown.
- Figure 7 shows some general trends and basic interactions but this is not distilled into a model and plan of attack.

- The shortcomings of a clear plan is in part due to the fact that the team is outsourcing the chemistry.
- In addition, the PI is not trained in the field of synthetic medicinal chemistry and may be unaware of the basic methods and techniques used in the field. No prior work by the PI is cited to support the chemical synthesis or fidelity of preliminary work. (Overall there are 32 refs to support the R01. One shows Chodera as a middle author and it is in BioRxiv.)
- Much of the approach reads as a logistics plan and does not clearly define the research plan. Team interactions are discussed and a plan for meetings and oversight are reported in the Approach section. It is possible, however, to get insight to the Approach through the Innovation section.
- Based on the preliminary results reported, it is possible to piece together the approaches taken and general methods applied in the “assay cascade” shown in Fig. 6. Initially, it appears that the at least one lead compound (the aminopyridines) were identified using a fragment-based X-tal screening technique and constructed from 2 fragments. The other two compounds are taken from prior HTS screens using SARS-CoV-1.
- The details of how the FEP and modeling worked in this process are not clearly explained. While it is not entirely clear how the “design cycle(s)” are started or advanced, there appear to be 3 key steps: FEP/molecular modeling of binding free energies, HTS bioassays (Mpro activity) and HTS X-ray crystallography. Given the CPU intensive nature of FEP methods, it is unclear how many analogs were evaluated and exactly what was learned from this data.
- There is one BioRxiv (non-peer reviewed) manuscript that indicates Chodera has some experience with FEP algorithms used in machine learning but no CoV work is described.
- Given the potential diversity of ligands structures, the utility of FEP is limited and would need to be selectively applied.
- Inadequate consideration is given to the use of structure-based design techniques that are much simpler and effective in developing SAR profiles at this point is the drug development process (Phase 2 in their fig.1.) The synthesis of compounds is then determined using tools developed by Postera.
- Based on the description given in the application, the tools allow the most efficiently accessible compounds to be identified for contracting out to CRO’s. Once synthesized these compounds are subject to Mpro screening and HT X-ray analysis using DiamondMX. The role of PostEra is uncertain given the project has advanced to Phase 2 (and the scaffolds and synthetic routes are established). This issue must be addressed. It is not clear how many analogs will be co-crystalized or how this data will be used in the refinement cycles. It is implied that this data will be used to develop the SAR plan and to refine the analogs but no details are given.
- Also, no publications are cited to support the work (although you can access the X-tal structure online at a website given in the text).
- The compounds synthesized will also be subject to Mpro screening in the first pass (or Tier 1), as well as cell-based target engagement (unclear) and early ADME screens. Compounds meeting specific criteria (<1μM Mpro) will move to Tier 2 screen. No mention is made of the EC50 cell-based data so it is unclear what this us referring to.
- A plan is not presented to validate Mpro as the primary mechanism of action of the compounds in viral titer reduction/replication inhibition. No preliminary data is given that validates the antiviral effect is due to Mpro inhibition.
- No validation of enzymatic mechanism using kinetic measurements is given to show if the compounds are competitive, non-comp, or un-comp inhibitors.

- The two HTS-based scaffolds are not active.
- The optimization of the aminopyridine shown in Fig. 7 failed. This is due to the lack of an SAR plan.

5. Environment:

Strengths

- The PI has exceptional facilities to perform the computational work involved in the project.
- All collaborators have the appropriate facilities to perform the biological work and biophysical studies.

Weaknesses

- It is not possible to evaluate the out-sourced facilities to ensure rigor or fidelity and safety of this work.
- In addition, significant concerns are raised over the use of multiple sites with little to no history of collaboration (as evidenced by publications).

CRITIQUE 3

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

Overall Impact: The Mpro is possibly the most clear-cut target for small molecule drug discovery against SARS-CoV-2. This team has developed lead inhibitors, along three chemical scaffolds, which need further potency increases. They have in place activity assays, modeling expertise, and high-throughput x-ray crystallography, along with structures of various bound chemotypes - so they should be able to make progress. They understand that optimization is a multi-parameter problem involving not just activity but also drug-likeness, and have laid out a proper list of criteria for a clinical candidate. The goal is a non-covalent inhibitor, which will make potency harder to achieve, but will eliminate toxicity worries that come with a reactive warhead. The synthetic chemistry will be outsourced. An AI approach to devising syntheses and selecting CROs hopes to avoid the need for chemists to do troubleshooting, but this seems risky. An experienced ex-AstraZeneca medicinal chemist will advise, but on a volunteer time basis. Many of the ADME assays will also be outsourced - the text said "see letter from Northeastern", but it wasn't included.

Overall, the goal of arriving at a clinical candidate to hand off for animal studies is possible, but would be less risky with an in-house synthetic lab under an experienced medicinal chemist as a full co-investigator.

1. Significance:

Strengths

- None were noted.

Weaknesses

- None were noted.

2. Investigator(s):

Strengths

- None were noted.

Weaknesses

- None were noted.

3. Innovation:

Strengths

- None were noted.

Weaknesses

- None were noted.

4. Approach:

Strengths

- None were noted.

Weaknesses

- None were noted.

5. Environment:

Strengths

- None were noted.

Weaknesses

- None were noted.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW OFFICER BASED ON INDIVIDUAL REVIEWER COMMENTS OR ADMINISTRATIVE REVIEW BY NIH STAFF:

PROTECTION OF HUMAN SUBJECTS: NOT APPLICABLE (CODE 10)

VERTEBRATE ANIMAL: ACCEPTABLE (CODE 30)
The protection of Vertebrate Animal welfare is adequately described.

NIH has modified its policy regarding the receipt of resubmissions (amended applications).See Guide Notice NOT-OD-18-197 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-18-197.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

National Institute of Allergy and Infectious Diseases Special Emphasis Panel
NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES
Emergency Awards: Rapid Investigation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and Coronavirus Disease 2019 (COVID-19)
ZAI1 AMC-W (M1)
01/14/2021 - 01/15/2021

Notice of NIH Policy to All Applicants: Meeting rosters are provided for information purposes only. Applicant investigators and institutional officials must not communicate directly with study section members about an application before or after the review. Failure to observe this policy will create a serious breach of integrity in the peer review process, and may lead to actions outlined in NOT-OD-14-073 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-14-073.html and NOT-OD-15-106 at https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-106.html, including removal of the application from immediate review.

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