SUMMARY STATEMENT

PROGRAM CONTACT: [redacted] **(Privileged Communication)** ***Release Date:*** **10/20/2016**
Revised Date:

Application Number: **1 R43 AI132075-01**

Principal Investigators (Listed Alphabetically):

BAILEY-KELLOGG, CHRIS
BROOKS, BENJAMIN DELBERT (Contact)
COHEN, GARY H
EISENBERG, ROSELYN J

Applicant Organization: WASATCH MICROFLUIDICS

Review Group:	**ZRG1 IMST-K (14)** **Center for Scientific Review Special Emphasis Panel** **Small Business: Computational, Modeling, and Biodata Management**		
Meeting Date:	**10/13/2016**	***RFA/PA:***	**PA16-302**
Council:	**JAN 2017**	***PCC:***	**M34**
Requested Start:	**07/01/2017**	***Dual PCC:***	**P146SS**
		Dual IC(s):	**GM**

Project Title: **High-throughput, multiplexed characterization and modeling of antibody:antigen binding, with application to HSV**
SRG Action: **Impact Score:** [redacted]
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.

1R43AI132075-01 BROOKS, BENJAMIN

COMMITTEE BUDGET RECOMMENDATIONS

SCIENTIFIC REVIEW OFFICER'S NOTES

RESUME AND SUMMARY OF DISCUSSION: Investigators at Wasatch Microfluidics, Dartmouth College, and the University of Pennsylvania seek to develop an integrated solution combining surface plasmon resonance (SPR) with computational modeling to identify specific residues involved in antigen:antibody binding. Reviewers noted that if successful, the availability of tools that provide structural insight into the residues involved in epitope binding potentially would have a significant impact on the development of novel therapeutic antibodies. The assigned reviewers initially expressed a high level of enthusiasm for this application. Strengths that were identified included the significance of the topic to be addressed, the strong premise that was supported by preliminary data, the exceptional investigative team, and the clear Aims that were presented with alternative strategies. Minor concerns were noted that included the lack of details around the metrics to be used to assess the accuracy of clustering antibodies and the lack of a more detailed description of the measures for assessing the performance of epitope identification. At the conclusion of the discussion however, enthusiasm for the significance of the topic to be addressed, the superior investigative team, and the clearly presented approach was not dampened by the minor concerns raised around details in the approach. As a consequence, the panel's final evaluation of the application's potential overall impact was noted to be exceptional.

DESCRIPTION (provided by applicant): All therapeutic antibodies and most vaccines critically depend on the ability of antibodies to specifically recognize particular antigens; consequently, detailed characterization of antibody:antigen binding can provide invaluable information to understand and guide development. Unfortunately, due to the time and expense required, atomic resolution structure determination is typically used sparingly, late in a development process or for a small number of different antibodies or antigen variants. We seek to enable earlier and larger-scale, but still detailed, characterization and modeling of antibody:antigen binding, applicable to panels of antibodies that could result from screening polyclonal samples or engineered libraries, along with panels of antigens that could result from attempts to understand and account for diversity across populations. While not at atomic resolution, our approach will still allow residue-level localization of specific epitopes for specific antibodies, as well as group-level identification of functionally similar antibodies and their associated binding regions on the antigen. The approach will be enabled by a unique integration of a powerful experimental platform, the high-throughput multiplexed Wasatch Surface Plasmon Resonance (SPR), with powerful computational methods to design and analyze binding experiments. Studies of glycoprotein D (gD) of herpes simplex virus (HSV) will provide a solid foundation for developing, testing, and applying the technology to better understand critical differences across antibodies and antigenic variation. Ultimately, the approaches developed here will allow researchers to leverage extensive epitope characterization data generated with Wasatch's SPR instrument in order to broadly and deeply characterize the basis for antibody:antigen recognition in wide-ranging vaccine and therapeutic antibody discovery and development programs.

PUBLIC HEALTH RELEVANCE: Detailed characterization of antibody:antigen binding is fundamental to understanding and potentially improving mechanisms of action of biotherapeutics and vaccines. Here, in order to support such characterization for large panels of related antibodies and antigen variants, computational design and analysis methods will be integrated with a high-throughput multiplexed experimental platform, enabling the overall grouping of antibodies by binding preferences as well as the detailed localization of particular antibody epitopes. By enabling a rich analysis at much

higher throughput than traditional structural studies, this approach promises to better drive discovery and development of vaccines and therapeutic antibodies.

CRITIQUE 1:

Significance: 2

Investigator(s): 1

Innovation: 2

Approach: 1

Environment: 2

Overall Impact: Antibodies are a major class of therapeutic agents, and a critical determinant of the functional effect of binding of an antibody to its target antigen is mediated by the specific residues involved in the binding epitope. Currently, antibodies are typically developed with little structural insight into the residues involved in the binding epitope. The investigators propose to address this limitation of current technologies by developing a faster and more efficient approach to identify specific residues involved in antigen:antibody binding through an integrated solution combining multiplex surface plasmon resonance (SPR) with computational modeling. The investigators will implement and leveraged two elegantly complementary approaches: antibody vs. antibody binning to identify clusters of similar antibodies, and antibody vs. antigen binding to identify candidate residues at the binding epitope.

The proposal builds upon current independent approaches for SPR and modeling, and will validate the platform using the herpes simplex virus (HSV) glycoprotein D (gD) antigen bound with a collection of diverse antibodies with a variety of epitopes. This outstanding proposal has a strong scientific premise with convincing preliminary evidence supporting the approach, excellent scientific rigor with a clear and logical set of well-defined aims, an extraordinary team of scientists working together to deliver this innovative proposal, and the potential to significantly illuminate the structural details of antibody:antigen interactions.

1. Significance:

Strengths

- Improved structural insight into antibody:antigen binding epitopes will have significant benefits in the selection and optimization of antibodies for therapeutic assessment.
- The proposal has a strong scientific premise with preliminary data demonstrating that multiplex SPF can successfully cluster antibodies with similar antigen binding patterns, as well as initial evidence demonstrating that the approach can localize binding epitopes.
- The increased speed by which this proposal will provide structural insight regarding binding epitopes will enable this determination to be made much earlier in the drug discovery process, and will enable a more diverse set of candidate mAbs to be investigated.

Weaknesses

- None that are apparent to this reviewer

2. Investigator(s):

Strengths

- The investigators are leaders in the development and application of multiplex SPR to antibody:antigen structure determination, the basic biology of HSV and in particular characterization of glycoprotein D, and computational modeling of antibody:antigen interfaces.
- The investigators represent a unique collection of expertise and are well-positioned to successfully implement this multidisciplinary proposal.
- This proposal will require an extraordinary level of collaboration and cooperation across the three labs and institutions. The investigators have established productive interactions such as the Wasatch employee who is embedded at U Penn in the laboratory of Gary Cohen and Roselyn Eisenberg.

Weaknesses

- None that are apparent to this reviewer

3. Innovation:

Strengths

- Combining the multiplexed experimental technique (SPR) with computational modeling of antibody:antigen epitopes is novel.
- By including biologists who have significant gold-standard insight into the structure and function of HSV gD and antibodies, the antibody binning and computational model predictions can be assessed and the predictions refined and optimized.

Weaknesses

- None that are apparent to this reviewer

4. Approach:

Strengths

- This fit-for-purpose approach does not attempt to generate atomic-resolution structural insight: rather it focuses on the critical need for residue-level information at significantly higher throughput than is currently available.
- The two aims of the proposal are clearly articulated and build on each other: first the investigators will define clusters of antibodies with similar antigen binding patterns, and then seek to map the epitopes onto specific hot-spot residues.
- The two techniques proposed by the investigators (antibody vs. antibody binning, and antibody vs. antigen binding) are designed to leverage the advantage of the multiplex SPR approach, specifically the ability to measure relative binding affinities between pairs of antibodies for a common antigen, and the binding affinity for a single antibody towards two related antigens.
- The investigators clearly articulate potential risks and pitfalls associated with each aim, and provide alternatives to deliver on these aims in the event of challenges.
- While not required for this phase I proposal, the investigators presented a phase II plan that builds upon the intended results from phase I and extends and amplifies in new directions: development of an integrated software package for the analytic pipeline, validation of the predicted epitope residues via X-ray structure determination by Felix Rey (letter of support included in proposal), and extension to larger-scale antibody libraries.

Weaknesses

- None that are apparent to this reviewer

5. Environment:

Strengths

- The proposal represents a joint cross-disciplinary and cross-institution effort composed of recognized leaders in SPR and 3D printing (Wasatch), biophysical analysis and characterization (U Penn), and computational modeling (Dartmouth).
- The combined resources of Wasatch (microfluidics and SPR imagers), the University of Pennsylvania (cell and tissue culture, FACS, SPR and biophysics), and Dartmouth College (computation) have the necessary capability to deliver this proposal.

Weaknesses

- None that are apparent to this reviewer

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: Not Applicable (No Vertebrate Animals)

Biohazards: Not Applicable (No Biohazards)

Authentication of Key Biological and/or Chemical Resources: Unacceptable

- The investigators will use a collection of HSV reagents including gD variants. This reviewer was unable to find a discussion of the authentication of these reagents.

Budget and Period of Support: Recommend as Requested

- This reviewer did not see any section where the investigators explicitly discussed the reasons that their proposal exceeds the hard cap of $[redacted] The investigators did delineate the activities that will be performed and appropriately justify their cost: the investigators should also discuss why this Phase I proposal requires such a significant investment.

CRITIQUE 2:

Significance: 2

Investigator(s): 2

Innovation: 3

Approach: 3

Environment: 2

Overall Impact: The project aims to develop a powerful experimental platform – the high-throughput multiplexed Wasatch Surface Plasmon Resonance (SPR) coupled with computational methods to model antibody-antigen binding and apply it to study glycoprotein D (gD) of herpes simplex virus (HSV). The application is put forward by a team of experts that have complementary expertise in computational

and experimental study of antibody-antigen interactions. If successful, the project will develop a technique that can generate rich information about antibody-antigen binding at large scale, which is not possible with existing methods and is useful for the design of biotherapeutics and vaccine. The idea of integrating a multiplexed antibody-antigen binding technique with computational analysis is innovative. The preliminary data on both computational and experimental work is solid. The approaches for identifying antibody clusters and localizing epitopes are generally well reasoned. However, some aspects of the approach such as metrics for clustering antibodies and measures for assessing the performance of epitope identification lack details. Overall, the project will likely have a relatively high impact on the field.

1. Significance:

Strengths

- If successful, the project may produce a technique that can generate detailed information about antibody-antigen binding at a large scale, which is not possible with existing techniques such as X-ray crystallography, NMR and alanine scanning.
- The proposed technique, if it works, will be useful for designing of biotherapeutics.
- The application of the technique to herpes simplex viruses can help understand the impacts of antigenic variability on antibody recognition.
- Since Wasatch SPR platform was reported to improve some drug discovery process, the scientific premise of the project appears to be strong.

Weaknesses

- None identified

2. Investigator(s):

Strengths

- The PI has expertise in developing immunoassays, surface functionalization, surface property measurements, business experience, and software management, which is relevant to this project.
- The co-PI Dr. Bailey-Kellogg has expertise in protein-protein interaction, immunoinfomatics and bioinformatics that is relevant to modeling protein recognition and interaction in this project.
- The co-PIs Drs. Cohen and Eisenberg are experts of studying the infection process of herpes simplex virus (HSV), who can lead the application of the proposed technology to HSV infection.

Weaknesses

- None identified

3. Innovation:

Strengths

- Integration of computational docking with experimental characterization of antibody:antigen binding is new.
- Designing mutations based on prior knowledge and modeling to evaluate putative binding modes is interesting.
- The analysis of antibody competition assays across multiple antigens is different from traditional antibody competition with respect to a single antigen.

Weaknesses

- It is unclear that how new the proposed method of analyzing the relationship between sequence difference and binding difference is since existing methods in this area is not surveyed in detail.

4. Approach:

Strengths

- The preliminary work of Aim 1 clearly demonstrates that communities of antibodies that have similar binding patterns can be identified, and the structural difference between the communities may be recognized.
- The experimental plan for Aim 1 is feasible. The approach for clustering antibodies in Aim 1 is well designed.
- The preliminary work of Aim 2 on experimentally mapping epitopes and computationally designing disruptive mutations is solid.
- The plan of Aim 2 for localizing antibody epitopes is well designed.
- The potential for Phase II development is well defined.
- The integration of experimental work and computational analysis strengthens the scientific rigor of the approach.

Weaknesses

- The custom metrics for clustering antibodies are not described in detail.
- The statistical metrics for assessing of the accuracy of recognizing epitopes is not clearly defined. No baseline method is used to compare with the proposed method. This weakens the scientific rigor.

5. Environment:

Strengths

- The research team has sufficient experimental equipment and lab/office space to carry out the project.
- The research team has necessary computing equipment for the project.

Weaknesses

- None.

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: Not Applicable (No Vertebrate Animals)

Biohazards: Not Applicable (No Biohazards)

Authentication of Key Biological and/or Chemical Resources: Unacceptable

- Some experimental assays are proposed. But there is no plan for authentication of key biological and chemical resources.

Budget and Period of Support: Recommend as Requested

CRITIQUE 3:

Significance: 1

Investigator(s): 2

Innovation: 3

Approach: 4

Environment: 2

Overall Impact: Antibodies are central to modern biomedicine. Understanding how antibodies interact with their antigens is critical for Lead Identification (LI) and Lead Optimization (affinity, function, epitope, freedom to operate). Traditional computational and experimental techniques are quite expense, and not amenable to high throughput techniques, so not typically run early in a project. Here the proposal is to integrate two complementary high-throughput approaches: the experimental measurement of binding via multiplexed Wasatch Microfluidics Surface Plasmon Resonance (SPR) and the computational modeling and design of interactions. Glycoprotein D (gD) from herpes simplex virus (HSV) us an ideal system for development, testing, and application of the new approaches. This is a good scientific question and project. This approach can certainly speed up and reduce costs for LI and LO for biologics.

1. Significance:

Strengths

- The computational approach combined with high throughput (and high dimensional data) investigating antibody epitope and affinities
- This could reduce screening times significantly

Weaknesses

- None to note

2. Investigator(s):

Strengths

- The PIs have a strong history of working well together, and completing grants
- Excellent cross disciplinary teams, with the right experience
- Good test / benchmarking with HSV

Weaknesses

- None to note

3. Innovation:

Strengths

- Novel territory in analyzing data across large panels of antibodies and antigens for general binding patterns and localizing binding regions
- Integration of computational and experimental methods to rationally design antigenic variants (beyond simple alanine scans and natural variants) so as to improve resulting experimental information

Weaknesses

- None to note

4. Approach:

Strengths

- Model system (HSV) with high throughput data generation with computational approach

Weaknesses

- A better description of the computational approach is needed

5. Environment:

Strengths

- Wasatch Microfluidics has strong experience in the space with needed computing power
- Bailey-Kellogg group is one of the right computational labs in the world
- Eisenberg very strong in model system

Weaknesses

- None to note

Protections for Human Subjects: Not Applicable (No Human Subjects)

Vertebrate Animals: Not Applicable (No Vertebrate Animals)

Authentication of Key Biological and/or Chemical Resources: Not Applicable (No Relevant Resources)

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:

SCIENTIFIC REVIEW OFFICER'S NOTES: During the review of this application, reviewers and/or NIH staff noted that one or more biosketches did not comply with the recent change to the required format (NOT-OD-15-032). Please review the new policy and check that all of the biosketches associated with your applications comply with the new format. NIH has the authority to withdraw noncompliant applications from review or consideration for funding.

COMMITTEE BUDGET RECOMMENDATIONS:

It was noted during the discussion that while the amount requested is reasonable, the budget is over the mandatory cap and that this was not justified as required.

Footnotes for 1 R43 AI132075-01; PI Name: Brooks, Benjamin Delbert

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.