PI: **Brooks, Benjamin Delbert**	Title: High-throughput, multiplexed characterization and modeling of antibody:antigen binding, with application to HSV	
Received: 09/06/2016	FOA: PA16-302	Council: 01/2017
Competition ID: FORMS-D	FOA Title: PHS 2016-02 OMNIBUS SOLICITATION OF THE NIH, CDC, FDA AND ACF FOR SMALL BUSINESS INNOVATION RESEARCH GRANT APPLICATIONS (PARENT SBIR [R43/R44])	
1 R43 AI132075-01	Dual: GM	Accession Number: 3968518
IPF: 10005562	Organization: WASATCH MICROFLUIDICS	
Former Number:	Department:	
IRG/SRG: ZRG1 IMST-K (14)B	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: [redacted]	Animals: N Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
Benjamin Brooks Ph.D	WASATCH MICROFLUIDICS	PD/PI
Chris Bailey-Kellog	Dartmouth College	MPI
GARY COHEN	University of Pennsylvania	MPI
Roselyn Eisenberg	University of Pennsylvania	MPI

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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

b. Agency Routing Number

c. Previous Grants.gov Tracking Number

2. DATE SUBMITTED 2016-09-01

Application Identifier

5. APPLICANT INFORMATION

Organizational DUNS*: [redacted]

Legal Name*: WASATCH MICROFLUIDICS
Department:
Division: Wasatch Microfluidics
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: [redacted]
ZIP / Postal Code*:

Person to be contacted on matters involving this application
Prefix: Prof. First Name*: Benjamin Middle Name: Last Name*: Brooks Suffix:
Position/Title:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*:
Province:
Country*: [redacted]
ZIP / Postal Code*:
Phone Number*: [redacted] Fax Number: Email: [redacted]

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

7. TYPE OF APPLICANT* R: Small Business

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*
High-throughput, multiplexed characterization and modeling of antibody:antigen binding, with application to HSV

12. PROPOSED PROJECT
Start Date* 07/01/2017
Ending Date* 06/30/2018

13. CONGRESSIONAL DISTRICTS OF APPLICANT
UT-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: Benjamin Middle Name: Delbert Last Name*: Brooks Suffix: Ph.D
Position/Title: Vice President of Research
Organization Name*: WASATCH MICROFLUIDICS
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
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 penalties. (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 File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: Josh Middle Name: Last Name*: Eckman Suffix:
Position/Title*: President
Organization Name*: Wasatch Microfluidics
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative*
Josh Eckman

Date Signed*
09/06/2016

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:Cover_Letter_PA-16-302.pdf

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

OMB Number: 4040-0010
Expiration Date: 06/30/2016

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: Wasatch Microfluidics
Duns Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County: [redacted]
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: UT-002

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: Dartmouth College
DUNS Number: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

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: Trustees of the University of Pennsylvania
DUNS Number: [redacted]
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: PA-002

Funding Opportunity Number: PA-16-302. Received Date: 2016-09-06T17:27:06.000-04:00

File Name

Additional Location(s)

OMB Number: 4040-0001
Expiration Date: 06/30/2016

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:

Human Subject Assurance Number

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

3. Is proprietary/privileged information included in the application?* ❍ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - 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 the United States or partnership with international collaborators?* ❍ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	Abstract_PA-16-302.pdf
8. Project Narrative*	Narrative_PA-16-302.pdf
9. Bibliography & References Cited	References_v2_PA-16-302.pdf
10.Facilities & Other Resources	Facilities_PA-16-302.pdf
11.Equipment	Equipment_PA-16-302.pdf
12. Other Attachments	CBK_-_Personnel_Justification_PA-16-302.pdf SBC_000348985.pdf

Funding Opportunity Number: PA-16-302. Received Date: 2016-09-06T17:27:06.000-04:00

Abstract. 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.

Project Narrative

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.

Facilities,
Wasatch Microfluidics, University of Pennsylvania, and Dartmouth College have the necessary facilities to complete this research and development project. A brief summary of their equipment and facilities follows:

Wasatch Microfluidics Facilities

General: The Wasatch facility has 5,000 sq.ft. of modern laboratory, manufacturing and office space in Salt Lake City, UT with room to grow up 2,000 sq. ft. if necessary.

Laboratory: Wasatch Microfluidics has 2,000 sq.ft. of research and development labs with lab benches, fume hoods, purified water, desk space and network connections. Our manufacturing and engineering labs are equipped with electro-optical test, signal processing tools, and ample power and workspace for the proposed work.

Computer: Wasatch has personal computers for all of the personnel. Lab computers are also used to run the detection systems and for data analysis. Wasatch also has Visual Studio and a cloud-based, Team Foundation Server (TFS) for all software projects to handle versioning, feature, and bug tracking.

Office: Our new facility has 3,000 sq.ft. of office space including a reception area, break room and conference room. Individual offices are equipped with standard office furniture as well as phone and internet jacks.

Machine Shop: Wasatch has access an agreement to a small machine shop for rapid prototyping.

Libraries: The Library at the University of Utah is 15 minutes from the Company's facility.

Wasatch also has an embedded employee at the University of Pennsylvania Dental school in the labs of Gary Cohen and Roselyn Eisenberg.

Dartmouth College, Bailey-Kellogg Facilities

Laboratory:
N/A

Computer:
The Bailey-Kellogg group has an extensive array of individual and shared computing resources. Each group member has a laptop and a workstation. For computationally demanding applications, the group uses the Computer Science department's GridIron compute cluster, which thanks to a recent NSF infrastructure grant currently includes over 100 high-performance nodes totaling over 1200 cores, with over 4000GB of distributed RAM and a high-throughput filesystem with over 200TB of storage. We also have shared access to the Discovery cluster maintained by the university; Discovery is a world-class Linux supercomputer with over 2400 cores (constantly expanding), supported by staff dedicated to research computing. Dartmouth provides a campus-wide wired network with a gigabit backbone and minimum 100 megabit service to researchers. Dartmouth also covers the entire campus with wireless access points.

Office:
The new wing of the Computer Science building houses an office for the PI, shared (two-person) offices for his postdocs and graduate students, and a 900 ft^2 computer lab / meeting space for his group.

Clinical:
N/A

University of Pennsylvania, Cohen/Eisenberg Facilities

Laboratory: The labs are in the Levy Building, rooms 212, 215, 218, 225 & 233.The labs are 400 to 600 sq.ft each, close to each other and adequate to house the investigators and the support staff. The Department of Microbiology provides secretarial services, microbiology kitchen and dishwashing facilities as well as incubation and cold rooms, common equipment rooms and a darkroom. The library is located in the adjacent Evans building.

Clinical: N/A

Office

There are four offices available. Rooms 213 and 216 are for Drs. Cohen and Eisenberg respectively. Rooms 217 and 233 have desks available for students, postdocs and Research Specialists. Room 214 is equipped

with a PC and Macintosh computer for common use. It also has a color printer and scanner for common use. Multiple Macintosh and PC computers are available in each of our labs and offices. All are equipped with programs for routine and scientific applications. We have black and white and color printers, scanners and LCD projectors.

Scientific Environment

Our laboratory space is in adjoining rooms near the offices within approximately 2500 square feet of space. Our confocal microscope is housed in a specially equipped darkroom. Room 233 contains clean air hoods and C02 incubators and microscopes, etc., for cell and virus culture. We maintain a baculovirus facility within the Microbiology department of the Dental School in Room 225. Equipment includes 2 New Brunswick Celligen Bioreactors for insect culture under precise conditions of pH, gases and temperature. These are equipped with 5L vessels and are used routinely for preparing larger amounts of protein. We also have multiple specially designed spinners for preparing smaller batches of insect cells; There are 2 low temperature incubators dedicated to insect cell-baculovirus incubation, a cell culture hood, tangential flow equipment, centrifuge, microscope and FPLC for protein purification. There are multiple CORE facilities in the Levy Building including FACS, EM, Confocal microscope etc. The University of Pennsylvania and Wistar provide access to multiple CORE facilities. We have all of the equipment and facilities needed to carry out the research in this grant. The Wasatch Continuous Flow Microspotter (CFM) coupled to the Ibis MX96 imager is now in our laboratory on long term loan.

Equipment

Wasatch Microfluidics, Dartmouth College, and University of Pennsylvania have the necessary equipment to complete this research and development project.

Wasatch Microfluidics Equipment.
Lab equipment: Wasatch is equipped with centrifuges, biosafety cabinet, CO2 incubators, vacuum pumps, and an Leica, inverted microscope with phase contrast, brightfield, and fluorescent capabilities (expandable if necessary). For storage, Wasatch has access to a cryogenic dewar, refrigerators, and a –80 ºC freezer. Wasatch has access to three CFMs for cell or biomolecule printing. Wasatch owns two IBIS MX96 SPR imagers (one collocated at UPenn in Cohen/Eisenberg lab) and prototypes of our current SPR sensors.
Chemistry equipment: Chemical/fume hoods are available. A water bath, digital thermometers, pH meters, centrifuges, water purification, and other basic lab equipment are also located in the facility.
Microfabrication equipment: Two microscopes with attached cameras/video are available in the lab. The lab also has a small manufacturing area for producing the PDMS printheads which includes: injection molding setup, ovens, vacuum chambers, vacuum pumps, and mold making tools. Specialized chemistry facilities required to test the MFCA / SPR platform application testing is available. Wasatch has an established cleanroom manufacturing facility for the CFM printheads, which can also be used for some R&D efforts when a clean area is necessary. Wasatch also has a small milling machine.
Office, computer, and general business space are also available to all participants in this project.

University of Pennsylvania, Cohen and Eisenberg Equipment.
Within the Dental School Microbiology department (housed in the Leon Levy Building) we have: two ultracentrifuges: a Beckman L8M70 and an Optima L90K ultracentrifuge, a Sorvall RC26+ high speed centrifuge, two Beckman GPR centrifuges, several microcentrifuges, ultra-low temperature freezers, liguid nitrogen apparatus, FPLC equipment, including a variety of columns, monitors and fraction collectors, Pharmacia spectrophotometer, SDSPAGE gel equipment, Biotek Synergy Bioassay plate reader, for ELISA, Fluorescence& Luminescence and a new Li-Cor Odyssey Infrared imaging system. We have a full tissue culture laboratory, including three biological hoods, and 4 incubators. We have developed a full state-of-the-art Baculovirus laboratory to grow insect cells for recombinant baculovirus infection that enables us to prepare and purify large amounts proteins. The equipment includes two Celligen bioreactors, 2 low temperature incubators, tangential flow apparatus, etc. We have a full SPR laboratory that includes a BiaCore T100 Biosensor & BIACore 3000, and a MicroCal ITC200. In our laboratory, we have the use of the WM Continuous Flow Microspotter (CFM) coupled to the Ibis MX96 imager for doing biosensor studies of larger numbers of samples to augment the BIAcore 3000.The Levy building has a state of the art FACS facility, a JOEL EM and a Leica histochemistry facility. All are available to UPENN members. We have access, within the Levy Bldg, and on campus to most other equipment needed to perform the proposed studies.

Personnel Justification

Prof. Chris Bailey-Kellogg (.25 Summer Months) will oversee all computational components of the project, advising the Dartmouth PhD student and working together with the Wasatch programmer. We will research, design, develop, and implement methods to analyze Wasatch SPR data in order to define antibody communities and localize antibody epitopes. We will work with the other investigators to apply the methods to the study of antibody:antigen binding in HSV.

Dartmouth PhD student (TBD, 9 Calendar Months) will lead the research effort into new methods for analyzing Wasatch SPR data for antibody:antigen binding analysis.

U.S. Small Business Administration

SBIR.gov SBC Registration Control ID Form

SBC CONTROL ID	SBC_000348985

FIRM INFORMATION					
Company	Wasatch Microfluidics, LLC				
Address	4909 BROWN VILLA COVE				
City	SALT LAKE CITY	State	UT	Zip	84123-
TIN/EIN	[redacted]	DUNS	[redacted]		
Company URL					
Number of Employees:	5				
Is this SBC majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?					No
What percentage (%) of the SBC is majority-owned by multiple venture capital operating companies, hedge funds, or private equity firms?					0%

SBC CONTROL ID	SBC_000348985

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: First Name*: Benjamin Middle Name Delbert Last Name*: Brooks Suffix: Ph.D

Position/Title*: Vice President of Research
Organization Name*: WASATCH MICROFLUIDICS
Department:
Division:
Street1*: [redacted]
Street2:
City*:
County:
State*: [redacted]
Province:
Country*:
Zip / Postal Code*:

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: B_BrooksBioSketch_v2.pdf

Attach Current & Pending Support: File Name: Pending_SUpport_BB.pdf

Tracking Number: GRANT12244575 Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

PROFILE - Senior/Key Person

Prefix: First Name*: Chris Middle Name Last Name*: Bailey-Kellog Suffix:

Position/Title*: Professor
Organization Name*: Dartmouth College
Department:
Division:
Street1*: [redacted]
Street2:
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]

Phone Number*: [redacted] Fax Number:

E-Mail*: [redacted]

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

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

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: cbk-biosketch.pdf

Attach Current & Pending Support: File Name: cbk-support.pdf

PROFILE - Senior/Key Person

Prefix: First Name*: GARY Middle Name H Last Name*: COHEN Suffix:

Position/Title*: Professor
Organization Name*: University of Pennsylvania
Department:
Division:
Street1*: UNIVERSITY OF PENNSYLVANIA
Street2: DEPT OF MICROBIOLOGY
City*: PHILADELPHIA
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 191046030

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

E-Mail*: [redacted]

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

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

Degree Type: PHD Degree Year:

Attach Biographical Sketch*: File Name: GHC_Biosketch_April_SBIR.pdf

Attach Current & Pending Support: File Name: GC_Support_PA-16-302.pdf

Tracking Number: GRANT12244575 Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

PROFILE - Senior/Key Person

Prefix:	First Name*: Roselyn	Middle Name J	Last Name*: Eisenberg	Suffix:

Position/Title*:	PROFESSOR OF MICROBIOLOGY
Organization Name*:	University of Pennsylvania
Department:	
Division:	
Street1*:	Department of Pathobiology
Street2:	School of Veterinary Medicine
City*:	Philadelphia
County:	
State*:	PA: Pennsylvania
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	191046002

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

E-Mail*: [redacted]

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

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

Degree Type: PHD,BA Degree Year:

Attach Biographical Sketch*: File Name: RJE_HSV_Biosketch_SBIR.pdf

Attach Current & Pending Support: File Name:

OMB No. 0925-0001/0002 (Rev. 08/12 Approved Through 8/31/2015)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Benjamin D. Brooks

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Vice President of Research & Development

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
University of Wyoming, Laramie, WY	B.S.	08/1999	Mathematics
University of Wyoming, Laramie, WY	MBA	08/2002	Business Administration
University of Wyoming, Laramie, WY	PhD	12/2008	Molecular Biology
Scripps Research Institute, San Diego, CA	Visit. Grad Student	12/2007	Immunology
University of Utah, Salt Lake City, UT	Postdoc	10/2009	Pharmaceutics

A. Personal Statement

Wasatch Microfluidics' is in the product development stage for engineering a label-free, Surface Plasmon Resonance based biosensor with integrated, dual-switching, next generation, flow cells for bioanalytical characterization of drug candidates. In 2011, while working on a microarray project at Grainger/Brooks lab at the University of Utah in Salt Lake City, Utah, using traditional pin printing, I began to work on Wasatch's Continuous Flow Microspotter (CFM) for printing biomolecules. After the completion of my postdoctoral fellowship, I joined Wasatch to head their R&D efforts to facilitate both the label-free and the cell printing product development. My scientific expertise in developing immunoassays, surface functionalization, and surface property measurement will be invaluable for these products. In addition to my scientific skills and knowledge, this project will also draw on my MBA and practical business experience in driving technology towards solving real world clinical problems, a challenge I welcome. Lastly, I have extensive experience managing software project both academically (see reference #5 in the **SPR, Protein Microarrays, and Immunoassays section)** and commercially.The team driving this project forward involves world class academicians, clinicians, and business professionals within Wasatch Microfluidics as well as in Gary Cohen's group at UPenn and Biosensor Tools, all well prepared to create the foundation for a quantum leap in biosensor design and cell printing for biochemical screening applications through the development of a high-throughput, 3D printer with integrated imaging systems.

B. Positions and Honors

Positions

2012 – Present	VP, Research and Development, Wasatch Microfluidics, Inc., Salt Lake City, UT
2009 – 2012	Postdoctoral Fellow, Dept. of Pharmaceutical Chemistry, University of Utah, Salt Lake City, UT
2006 – 2008	Visiting Graduate Student, The Scripps Research Institute, Sand Diego, CA
2000 – 2003	Instructor, Dept. of Computer Science and Business Technology, Casper College, Casper, WY

Honors

2003	Gamma Sigma Delta Honor Society of Agriculture
1995	Mortar Board and Golden Key Honor Societies

C. Contribution to Science

My contributions to science fall into four categories; immunoassays, nanoparticles, cell printing, and antibiotic release. During my graduate work, I had the privilege of working on multiple immunoassays formats including, ELISAs, both traditional and nanoparticle based, immunoblotting, and protein microarrays, in an attempt to develop an immunoassay to prion protein. During my postdoc, I had the privilege to work for Dr. David Grainger, an expert in surface chemistry and Dr. Amanda Brooks, an expert in drug delivery. In the Grainger/Brooks lab, I managed multiple graduate student projects relating to nanoparticles and immunoassays. As a new investigator at Wasatch, I work currently on SPR microarrays and cell printing.

SPR, Protein Microarrays, and Immunoassays

The development of a high-throughput, SPR sensor drives a majority of my current research and will represent a major advancement in label-free characterization of protein binding. At Wasatch we are combining our advanced microfluidics with industry standard SPR technologies to improve throughput over current technologies. Prototypes of this technology predict a major advancement in the drug discovery and development field.

1. "Surface Plasmon Resonance Arrays for Antibody Characterization." Label-free biosensor methods in drug discovery. Label-Free Biosensor Methods in Drug Discovery (Book Chapter in Springer Protocols Book Series.) [2015: 35-76]. SN Davidoff, NT Ditto, AE Brooks, JW Eckman, **BD Brooks**.
2. [redacted]
3. "The Importance of Epitope Binning for Biological Drug Discovery." Current Drug Discovery Technologies. [2014/06, 11(2):109]. **BD Brooks.**
4. "High-throughput epitope binning of therapeutic monoclonal antibodies: why you need to bin the fridge!" Drug Discovery Today. [2014/08, 19(8): 1040–1044] **BD Brooks**, AR Miles, YN Abdiche.
5. "Identification of problems developing an ultrasensitive immunoassay for the ante mortem detection of the infectious isoform of the CWD-associated prion protein." Journal of Immunoassay and Immunochemistry. 30 (2), 135-149. **BD Brooks**, A Brooks, SS Wulff, RV Lewis

Cell Printing

Cell printing represents an emerging field in drug discovery and development. Controlled delivery of cells would represent a major advancement for the screening and toxicity characterization of drug development. Wasatch's 3D flow cells allows scientist the ability to print cells in a submerged environment and in a microarray format. Numerous advances in this area are forthcoming.

1. "Maximizing fibroblast adhesion on protein-coated surfaces using microfluidic cell printing." RSC Advances 5 (126), 104101-104109. SN Davidoff, D Au, BK Gale, AE Brooks, **BD Brooks.**

2. "The Submerged Printing of Cells onto a Functionalized Surface Using a Continuous Flow Microspotter." Journal of Visualized Experiments. [2014, 86: e51273-e51273]. SN Davidoff, **BD Brooks.**

3. "Comparison of Submerged and Unsubmerged Printing of Ovarian Cancer Cells." Biomedical sciences instrumentation. [2015, 51: 24-30]. SN Davidoff, D Au, S Smith, AE Brooks, **BD Brooks.**

Antimicrobial Controlled Release

Antibiotics remain one of the greatest advances in modern medicine. With the emergent threats of antibiotic resistance, the field has been reinvigorated. Controlled and local release will become an important tenant of future delivery of the drug. I have been on the forefront of pushing for localized, surface delivery of drugs with an emphasis on bone applications.

1. “A Resorbable Antibiotic-Eluting Polymer Composite Bone Void Filler for Perioperative Infection Prevention in a Rabbit Radial Defect Model.” PLOS One. [In Press]. **BD Brooks**, KD Sinclair, DW Grainger, AE Brooks.
2. “Therapeutic Strategies to Combat Antibiotic Resistance.” Advanced Drug Discovery Reviews. Volume 78, 30 November 2014, Pages 14–27. **BD Brooks**, AE Brooks.
3. "Antimicrobial medical devices in preclinical development and clinical use." 2012 Springer Book Chapter in Biomaterial Associated Infection. **BD Brooks**, AE Brooks, DW Grainger, et al.
4. "Polymer-Controlled Release of Tobramycin from Bone Graft Void Filler.” Drug Delivery and Translational Research. [2013/02, 3(6): 518-530]. **BD Brooks**, SN Davidoff, DW Grainger, AE Brooks.

Nanoparticles

Nanoparticle research represents one of the fastest growing areas of translational research; however, major limitations exist in the understanding of the toxicity of these nanoparticles. As a postdoctoral fellow, I managed several projects that facilitated our understanding in the field of nanoparticle toxicity.

1. Nanoparticle toxicity assessment using an in vitro 3-D kidney organoid culture model.” Biomaterials. [2014]. AI Astashkina, CF Jones, G Thiagarajan, K Kurtzeborn, H Ghandehari, **BD Brooks**, DW Grainger.
2. “Cationic PAMAM dendrimers aggressively initiate blood clot formation.” ACS-Nano. [2012, 6 (11): 9900–9910]. CF Jones, AS Weyrich, H Ghandahari, **BD Brooks**, AS Weyrich, DW Grainger, et al.
3. "Cationic dendritic nanoparticles disrupt key platelet functions." Molecular Pharmaceutics. [2012/06 4;9(6):1599-611]. CF Jones, R Campbell, **BD Brooks**, H Ghandahari, AS Weyrich, DW Grainger.
4. “A Critical Comparison of Protein Microarray Fabrication Technologies.” Analyst. [2014, 139:303-1326]. V Romanov, SN Davidoff, AR Miles, DW Grainger, BK Gale, **BD Brooks**.

D. Research Support

Ongoing Research Support

- Fasttrack SBIR; NIH; NIGMS R44 4R44GM109738-02 Date 05/01/2014 – 04/30/2015

Title: “HT Label-Free Screening and Kinetic Analysis of Small Molecules and Biologics
Purpose: To develop an innovative SPR instrument with throughput and sensitivity for screening new drug candidates. Role: PI

- Innovations Grant/Convertible Debt. Pfizer-Rinat; Date 09/25/2014 – 09/25/2016

Title: “HT Label-Free Screening using Epitope Binning for Biologics”
Purpose: To develop a HT SPR instrument with throughput and sensitivity for epitope binning new drug candidates. Role: PI

Completed Research Support

- **Phase I SBIR;** NIH; NCI R43 1R43CA177146-01A1 Date 07/01/2014 – 06/30/2015

Title: “Multiplexed Ovarian Cancer Microfluidic Tissue Microarray”
Purpose: The purpose is to develop an instrument system for automated, multiplexed cell- and tissue-based experiments (i.e. tissue microarrays).The proposed instrument will be used to novel cell- and tissue-based assays in a highly parallel manner that are otherwise difficult to perform. Role: PI

- **Phase I SBIR;** NIH; NIGMS R43 1R43GM101859-01 (MPI) GRANT10940803

Date: 9/01/2012 – 8/31/2014
Title: “Submerged Printing of Lipid and Membrane Protein Arrays”
Purpose: This SBIR project developed a device for automated array printing of lipids and membrane proteins onto submerged microtiter plate surfaces in a way that maintains their activity and function. **Role:** PI

- **Phase II STTR;** NIH; NIMH R43 5R42MH084372-03 (MPI)

Date: 9/01/2011 – 8/31/2014

Title: “Multiplexed GPCR Characterization Using SPR”

Purpose: This Phase II STTR project developed a real-time label-free biosensor that can analyze 96 samples at a time, compared to the 6 samples possible with current technologies. **Role:** PI

Patents

Drug Release from a Polymer-Controlled Local Antibiotic Delivery System Using a Degradable Bone Graft
Inventors: Amanda Elaine Brooks, Benjamin Delbert Brooks, David W Grainger
Publication date: 2013/2/5 Application number: 13/759,904

OMB No. 0925-0001 and 0925-0002 (Rev. 10/15 Approved Through 10/31/2018)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Bailey-Kellogg, Chris

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: 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
Massachusetts Institute of Technology, Cambridge, MA	S.B., S.M.	05/1993	Electrical Engineering & Computer Science
Ohio State University, Columbus, OH	Ph.D.	3/1999	Computer & Information Science
Dartmouth, Hanover, NH	Postdoc	06/2001	Computational Biology

A. Personal Statement

Research in my laboratory focuses on the development and application of computational methods for data analysis and experiment optimization, in the context of protein sequence/structure/function studies. By tightly integrating computation with experiment, we seek to maximize information gain while minimizing experimental complexity, and to rigorously evaluate the resulting confidence in the data interpretation. Recently my primary focus has been computational modeling and computationally-driven engineering of the immune response to foreign proteins, in the contexts of both desired responses in infection/vaccination and undesired responses to therapeutics.

Of particular relevance to the proposed project, we have developed methods to computationally model and design protein:protein interactions. Our methods integrate both biophysical modeling and experimental data in order to capture the amino acid determinants of specific recognition in a manner that enables prediction of binding as well as optimization of variants for improved or disrupted binding. We have recently begun putting this to use in the context of antibody:antigen recognition, and have demonstrated the utility of computationally-directed engineering in helping identify antibody epitopes. The proposed project will enable us to scale up our methods to the high-throughput, multiplexed context enabled by the Wasatch Microfluidics SPR, analyzing entire panels of antibodies and antigenic variants in parallel. It will also provide a compelling context for studying antibody:antigen recognition, in order to understand the impact of antibody and antigen variability in HSV.

B. Positions and Honors

Positions and Employment

1998 Research Intern, Xerox PARC.
1999-2000 Postdoctoral Research Associate, Computer Science, Dartmouth College.
2001 Research Assistant Professor, Computer Science, Dartmouth College.
2001-2004 Assistant Professor, Computer Sciences, Purdue University.
2004-2007 Assistant Professor, Computer Science, Dartmouth College.
2007-2013 Associate Professor (with tenure), Computer Science, Dartmouth College.
2013-present Professor, Computer Science, Dartmouth College.

Honors

1993 MIT chapters of Eta Kappa Nu, Sigma Xi, and Tau Beta Pi honor societies
1998 OSU chapter of Phi Kappa Phi honor society
2003 NSF CAREER award (awarded at Purdue University)
2004 Alfred P. Sloan Research Fellowship (awarded at Dartmouth College)
2007 Dartmouth Friedman Family Fellowship
2007 Dartmouth Karen E. Wetterhahn Award for Distinguished Creative or Scholarly Achievement
2014 Dartmouth Melville and Leila Straus 1960 Faculty Fellowship

C. Contributions to Science

1. **Protein design.** We have developed a portfolio of complementary computational methods to optimize the selection of mutations so as to reduce immunogenicity of foreign proteins (e.g., bacterial enzymes) while maintaining their therapeutic activity.
    a. A.S. Parker, W. Zheng, K.E. Griswold, and C. Bailey-Kellogg, “Optimization algorithms for functional deimmunization of therapeutic proteins”, *BMC Bioinf*., 2010, 11:180. PMCID: PMC2873530.
    b. L. He, A.M. Friedman, and C. Bailey-Kellogg, “A divide-and-conquer approach to determine the Pareto frontier for optimization of protein engineering experiments”, *Proteins*, 2012, 80:790-806. PMCID: PMC4939273.
    c. R.S. Salvat, A.S. Parker, Y. Choi, C. Bailey-Kellogg, and K.E. Griswold, “Mapping the Pareto optimal design space for a functionally deimmunized biotherapeutic candidate”, *PLoS Comput. Biol.*, 2015, 11:e1003988. PMCID: PMC4288714.
    d. Y. Choi, C. Hua, C. Sentman, M.E. Ackerman, and C. Bailey-Kellogg, “Antibody humanization by structure-based computational protein design”, *MAbs*, 2015, 7:1-13. PMID: 26252731.

2. **Computationally-driven protein engineering.** We have applied computationally-driven protein engineering methods to successfully reengineer a number of therapeutic candidates for improved properties.
    a. R.S. Salvat, Y. Choi, A. Bishop, C. Bailey-Kellogg, and K.E. Griswold, “Protein deimmunization via structure-based design enables efficient epitope deletion at high mutational loads”, *Biotechnol. Bioeng.,* 2015, 112:1306-1318. PMCID: PMC4452428.
    b. K. Blazanovic, H. Zhao, Y. Choi, W. Li, R.S. Salvat, D.C. Osipovitch, J. Fields, L. Moise, B.L. Berwin, S.N. Fiering, C. Bailey-Kellogg, and K.E. Griswold, “Structure-based redesign of lysostaphin yields potent anti-staphylococcal enzymes that evade immune cell surveillance”, *Mol. Ther. Methods Clin. Dev.,* 2015, 2:15021. PMCID: PMC4470366.
    c. H. Zhao, D. Verma, W. Li, Y. Choi, S.N. Fiering, C. Bailey-Kellogg, and K.E. Griswold, “Depleting T cell epitopes in lysostaphin mitigates anti-drug antibody response and enhances antibacterial efficacy *in vivo*”, *Chem. Biol*., 2015, 22:629-639. PMCID: PMC4441767.
    d. Y. Choi, C. Ndong, K.E. Griswold, and C. Bailey-Kellogg, “Computationally driven antibody engineering enables simultaneous humanization and thermostabilization”, *Protein Eng. Des. Sel*., 2016, epub. PMID: 27334453.

3. **Protein interaction modeling.** We have developed data- and physics-driven approaches to model, in an interpretable and predictive fashion, the amino acid level determinants of protein interaction specificity.
    a. J. Thomas, N. Ramakrishnan, and C. Bailey-Kellogg, “Graphical models of protein-protein interaction specificity from correlated mutations and interaction data”, *Proteins*, 2009, 76:911-929. PMID: 19306342.
    b. A.N. Kettenbach, T. Wang, B.K. Faherty, D.R. Madden, S. Knapp, C. Bailey-Kellogg, and S.A. Gerber, “Rapid determination of multiple linear kinase substrate motifs by mass spectrometry”, *Chem. Biol.*, 2012, 19:608-618. PMCID: PMC3366114.
    c. H. Kamisetty, A. Ramanathan, C. Bailey-Kellogg, and C.J. Langmead, “Accounting for conformational entropy in predicting binding free energies of protein-protein interactions”, *Proteins*, 2011, 79:444-462. PMID: 21120864.
    d. H. Kamisetty, B. Ghosh, C.J. Langmead, and C. Bailey-Kellogg, “Learning sequence determinants of protein:protein interaction specificity with sparse graphical models”, *J. Comput. Biol.*, 2015, 22:476-486. PMCID: PMC4449715.

4. **High-dimensional immune profiling.** We have developed a suite of data analysis methods to predictively model the antibody response to infection and vaccination, based on multiplexed data regarding antigen specificity, Fc properties, and effector functions, across subjects from different clinical classes and outcomes.
    a. E.P. Brown, A.F. Licht, A.-S. Dugast, C. Bailey-Kellogg, G. Alter, and M.E. Ackerman, "High-throughput, multiplexed IgG subclassing of antigen-specific antibodies from clinical samples", *J. Immunol. Methods*, 2012, 386:117-123. PMCID: PMC3475184.
    b. A.W. Chung, M. Ghebremichael, H. Robinson, E. Brown, I. Choi, et al., and C. Bailey-Kellogg, M.E. Ackerman, and G. Alter, "Polyfunctional Fc-Effector Profiles Mediated by IgG Subclass Selection Distinguish RV144 and VAX003 Vaccines", *Sci. Transl. Med.*, 2014, 6:228ra38. PMID: 24648341.
    c. I. Choi, A.W. Chung, T.J. Suscovich, et al., and G. Alter, M.E. Ackerman, and C. Bailey-Kellogg, "Machine learning methods enable predictive modeling of antibody feature:function relationships in RV144 vaccinees", *PLoS Comput. Biol*., 2015, 11:e1004185. PMCID: PMC4395155.
    d. M.E. Ackerman, A. Mikhailova, E.P. Brown, K.G. Dowell, B.D. Walker, C. Bailey-Kellogg, T.J. Suscovich, and G. Alter, "Polyfunctional HIV-Specific Antibody Responses Are Associated with Spontaneous HIV Control", *PLOS Path*., 2016, 2:e1005315. PMCID: PMC4706315.

5. **Immunoinformatics.** We have developed methods to predict the binding of peptides to specific MHC proteins as well as more generally characterize the potential for and implications of cross-reactive binding of T cell receptors to peptide-MHC complexes.
    a. C. Bailey-Kellogg, A.H. Gutierrez, L. Moise, F. Terry, W.D. Martin, and A.S. De Groot, "CHOPPI: a web tool for the analysis of immunogenicity risk from host cell proteins in CHO-based protein production", Biotechnol Bioeng. 2014, 111:2170-2182. PMCID: PMC4282101.
    b. P.T. Losikoff, S. Mishra, F. Terry, A. Gutierrez, M.T. Ardito, L. Fast, M. Nevola, W.D. Martin, C. Bailey-Kellogg, A.S. De Groot, and S.H. Gregory, "HCV epitope, homologous to multiple human protein sequences, induces a regulatory T cell response in infected patients", *J. Hepatol*., 2015, 62:48-55. PMID: 25157982.
    c. A.H. Gutierrez, W.D., Martin, C. Bailey-Kellogg, F. Terry, L. Moise, and A.S. De Groot, "Development and validation of an epitope prediction tool for swine (PigMatrix) based on the pocket profile method", *BMC Bioinf*., 2015, 6:290. PMCID: PMC4570239
    d. L. He, A.S. De Groot, and C. Bailey-Kellogg, "Hit-and-run, hit-and-stay, and commensal bacteria present different peptide content when viewed from the perspective of the T cell", *Vaccine*, 2015, 33:6922-6929. PMID: 26428457.

Complete List of Published Work in MyBibliography:
http://www.ncbi.nlm.nih.gov/myncbi/browse/collection/43195993/?sort=date&direction=ascending

D. Research Support

Ongoing Research Support

2R01GM098977 (Bailey-Kellogg) 07/01/11 – 06/30/20
NIH
Functional Deimmunization of Therapeutic Proteins
The goals of this project are the development of protein deimmunization algorithms and the application of these methods in the design and evaluation of functionally-deimmunized beta-lactamase enzyme variants.
Role: PI

[redacted]

[redacted]

STTR 1R41AI118133 (Bailey-Kellogg, Stealth Biologics, LLC) 02/15/15 – 01/31/17
NIH
Design and Development of Immunotolerant S. aureus Biotherapies
The goal of this project is advanced pre-clinical development of anti-*S. aureus* enzyme therapies that have been engineered for reduced immunogenicity.
Role: PI

1R21AI119741 (Griswold) 07/01/16 – 06/30/18
NIH
Engineer bifunctional antibacterial enzymes for treatment of S. aureus infections
The goal of this project is to develop a modular platform for construction of bifunctional anti-*Staphylococcal* lysin therapies designed to possess intra-molecular synergy between complementary catalytic domains.
Role: co-investigator

1P01AI120756 (Tomaras, Duke) 04/01/16 – 03/31/21
Analytical Core (Ackerman, Dartmouth)
NIH
Bridging Antibody Fc-mediated Antiviral Functions Across Humans and Non-human Primates
The program seeks to rigorously evaluate and improve the use of the non-human primate model for testing antibody-based interventions and vaccines.
Role: co-investigator

Recently Completed Research Support

NSF CCF-0915388 (Bailey-Kellogg) 09/01/09 – 08/31/12
Algorithmic Problems in Protein Structure Studies
The project entails designing and analyzing efficient algorithms for fundamental problems that arise in studies of the three-dimensional structures of proteins, with a particular focus on graph-theoretic approaches.
Role: PI

IIS-0905206 (Bailey-Kellogg, Friedman, Langmead, Ramakrishnan) 08/15/09 – 07/31/13
NSF
III-Medium: Collaborative Research: Integration, Prediction, and Generation of Mixed Mode Information using Graphical Models, with Applications to Protein-Protein Interactions
The project seeks to develop new approaches to integrating heterogeneous data, in order to produce models that are predictive and diagnostic of key physical properties, and can be used for design purposes.
Role: PI

IIS-1017231 (Bailey-Kellogg, Friedman) 09/15/10 – 08/31/14
NSF
III: Small: Collaborative Research: Analysis of Multi-dimensional Protein Design Spaces with Pareto Optimization of Experimental Designs
The project aims to develop new methods to characterize complex protein design spaces and optimize high-quality designs for experimental evaluation, balancing multiple complementary criteria assessing design quality in terms of sequence-structure-function relationships.
Role: PI

[redacted]

[redacted]

1R21AI098122-01 (Bailey-Kellogg, Griswold, De Groot) 02/01/12 – 01/31/15
NIH
Computationally optimized anti-staphylococcal biotherapeutics
The goal of this project is to engineer lysostaphin enzyme variants that efficiently kill drug-resistant *Staphylococcus aureus* while simultaneously exhibiting low level immunogenicity.
Role: PI

R01 AI102691 (Ackerman) 07/15/14 – 07/14/15
NIH
One year supplement to *Applying High-Performance Protein Engineering Tools to HIV Immunogen Design*
The goal of this supplement is to develop epitope probes that capture the variable loop topology and sequence diversity of the HIV envelope trimer, but in the context of a monomeric scaffold.
Role: co-investigator

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Gary H. Cohen, Ph.D.	POSITION TITLE Professor of Microbiology
eRA COMMONS USER NAME ([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
Brooklyn College, Brooklyn, NY	B.S.	1956	Biology
University of Vermont, Burlington, VT	Ph.D.	1964	Microbiology
University of Pennsylvania, Philadelphia, PA	PDF	1964-67	Virology

A. Personal Statement of research interests.

The overall goal of my research is to understand the molecular events that mediate herpes simplex virus (HSV) entry into mammalian cells and promotes its pathogenesis in the human. Recently, I have broadened my efforts directed at understanding how the human responds to HSV entry glycoproteins after natural infection or vaccination. HSV entry requires binding of gD to a cell receptor, either HVEM or nectin-1. This interaction triggers virus-cell fusion involving three additional virion glycoproteins, gB, and gH/gL. Our approach has been to relate the essential viral glycoproteins' functional properties to their 3-D structures (all of which we solved in collaborations with crystallographers). Our working model for fusion posits that in a sequential fashion, binding of gD to receptor causes gD to undergo radical conformational changes that allow it to interact with gH/gL thereby activating it into a form that causes up-regulation of the prefusion form of gB into a functional fusion protein. Over the years we have developed extensive panels of monoclonal antibodies to gD, gH/gL, gB as well as to HVEM and nectin-1. We have expressed each protein in a baculovirus expression system and have become expert in preparing and studying purified proteins. We have also prepared various forms of each glycoprotein to understand more about how each protein functions. In addition we have also developed monoclonal antibodies to two other important glycoproteins, gC and gE, both of which have been implicated in evading the innate responses of the host to virus infection, and we have developed methods to purify each of these proteins as well. Our proteins have become valuable candidates for human vaccines and are being studied by multiple labs as potential vaccine candidates: gD2306t is considered a "Gold Standard" to compare efficacy of vaccine candidates. We have learned how to dissect the humoral response to HSV in naturally infected or vaccinated humans. These were small scale studies in which we employed prototypes of our extensive panels of monoclonal antibodies to gain an epitope profile for each specimen. We carried out competition studies between our monoclonals and IgGs purified from human sera on the BIAcore biosensor and these small scale studies will be expanded upon in this grant by taking advantage of the Wasatch Microfluidics (WM) technology for doing competition studies between sera and our panels of monoclonal antibodies. This will able us to examine a much larger cohort of naturally infected people (over 3000 samples). We will study the responses to sera from guinea pigs vaccinated with gD, or with gD in combination with gB or with a trivalent gD/gC/gE combination. Ultimately, we believe our studies will reveal the mechanism(s) of an effective host response to virus infection, thereby providing a solid intellectual basis for generating a unique "second" generation vaccine.

B. Positions and Honors

Positions and Employment

1964 - 1967 Postdoctoral Fellow (NIAID), University of Pennsylvania
1967 - 1973 Assistant Professor of Microbiology, School of Dental Medicine, U. of PA
1973 - 1980 Associate Professor of Microbiology, School of Dental Medicine, U. of PA
1976 - 1977 Visiting Scientist, Swiss Institute for Experimental Cancer Research, Lausanne
1980 -Present Professor of Microbiology, School of Dental Medicine, U. of PA

1985 -2012 Chair, Department of Microbiology, School of Dental Medicine, U. of PA

Other Experience and Professional Memberships

1990 Senior International Fellow, Fogarty International Center, NIH
1990 Invited Professor, Institute of Biochemistry of the Faculty of Medicine, University of Lausanne, Lausanne, Switzerland
1993-2003 Member, Cancer Center, U. of PA
1969 - 1974 NIH Career Development Award (AI)
2003 Elected, Fellow of the American Academy of Microbiology
2004 Elected, Fellow of the American Association for the Advancement of Science
2005-2015 Merit Award Recipient National Institute of Allergy and Infectious Diseases

C. Selected Publications (Selected from over 200)

Selected Publications on HSV

Hannah, BP, Cairns, TM, Bender FC, Whitbeck JC, Lou H, Eisenberg RJ and Cohen GH. 2009. Glycoprotein B of herpes simplex virus associates with target membranes via its fusion loops. J. Virol. 83(13):6825-6836. PMCID: PMC2698560

Wright CC, Wisner TW, Hannah BP, Eisenberg RJ, Cohen GH and Johnson DC. 2009. Fusion between perinuclear virions and the outer nuclear membrane requires the fusogenic activity of herpes simplex virus gB. J. Virol. 83(22):11847-11856. PMCID: PMC2772685

Atanasiu D, Whitbeck JC, Ponce de Leon M, Lou H, Hannah BP, Cohen GH and Eisenberg RJ. 2010. Bimolecular complementation defines functional regions of HSV gB that are involved with gH/gL as necessary steps leading to cell fusion. J. Virol. 84:3825-34. PMCID: PMC2849501

Atanasiu, D., W.T. Saw, G.H.Cohen and R.J. Eisenberg. 2010. The cascade of events governing cell-cell fusion induced by HSV glycoproteins gD, gH/gL and gB. J.Virol. 84: 12292-12299. PMCID: PMC2976417

Chowdary TL, Cairns TM, Atanasiu D, Cohen GH, Eisenberg RJ, and Heldwein EE. 2010. Crystal structure of the gH/gL complex – a conserved herpesvirus fusion regulator. Nat Struct Mol Biol. 2010:882-888. PMCID: PMC2849501

Stampfer SD, Lou H, Cohen GH, Eisenberg RJ and Heldwein EE. 2010. Structural basis of the local pH-dependent conformational changes in fusion glycoprotein B from Herpes Simplex virus 1. J. Virol. 84: 12924-12933. PMCID: PMC3004323

Stiles KM, Whitbeck, JC, Lou H, Gary H. Cohen GH, Eisenberg RJ and Krummenacher C. 2010. Herpes simplex virus glycoprotein D interferes with HVEM binding to its ligands through down-regulation and direct competition. J. Virol. 84:11646-11660. PMCID: PMC2977867

Bernstein DI, Earwood JD, Bravo FJ, Cohen GH, Eisenberg RJ, Clark JR, Fairman J, Cardin RD. 2011. Effects of herpes simplex virus type 2 glycoprotein vaccines and CLDC adjuvant on genital herpes infection in the guinea pig. Vaccine. 29:2071-8. PMCID: PMC3082315.

Cairns, TM, Whitbeck JC, Lou H, Heldwein EE, Chowdary TK, Eisenberg RJ, Cohen GH. 2011. Capturing the herpes simplex virus core fusion complex (gB-gH/gL) in an acidic environment. J.Virol. 85:6175-6184. PMCID: PMC3126480

Di Giovine P, Settembre EC, Bhargava AK, Luftig MA, Lou H, Cohen GH, Eisenberg RJ, Krummenacher C, Carfi A. Structure of HSV gD bound to the human receptor nectin- 1. PLoS Pathog. 2011 Sep;7(9):e1002277. Epub 2011 Sep 29. PMCID: PMC3182920

Lazear E, Whitbeck JC, Ponce-de-Leon M, Cairns TM, Willis SH, Zuo Y, Krummenacher C, Cohen GH, and Eisenberg RJ. 2012. Antibody-induced conformational changes in HSV gD reveal new targets for virus neutralization. J Virol. 86:1563-76. PMCID: PMC3264331

[redacted]

Atanasiu, D, Cairns TM, Whitbeck JC, Saw WT, Rao S, Eisenberg RJ, and Cohen GH: Regulation of Herpes Simplex Virus gB-Induced Cell-Cell Fusion by Mutant Forms of gH/gL in the Absence of gD and Cellular Receptors. mBio 4(2):(e00046-13), February 2013. PMCID: PMC3585445

Maurer UE, Zeev-Ben-Bordehai T, Pandurangan AP, Cairns TM, Hannah BP, Whitbeck JC, Eisenberg RJ, Cohen, GH, Topf M, Huiskonen JT and Gruenewald K. 2013. The Structure of Herpesvirus Fusion Glycoprotein B-Bilayer Complex Reveals the Protein-Membrane and Lateral Protein-Protein Interaction. Structure. 2013:1396-13405. PMCID: PMC3737472

Atanasiu D, Saw, WT, Gallagher JR, Hannah BP, Matsuda Z, Whitbecke JC, Cohen GH and Eisenberg, RJ. 2013. A dual split protein-based fusion assay reveals that mutations to HSV glycoprotein gB alter the kinetics of cell-cell fusion induced by HSV entry glycoproteins. J. Virol.87:11332-11345. PMCID: PMC3807322

Gallagher JR, Saw WT, Atanasiu D, Lou H, Eisenberg RJ, Cohen GH. 2013. Displacement of the C terminus of herpes simplex virus gD is sufficient to expose the fusion-activating interfaces on gD. J Virol. 2013 87(23):12656-12666. PMCID: PMC3838169

Lazear E, Whitbeck JC, Zuo Y, Carfi A, Cohen GH, Eisenberg RJ, Krummenacher, C. 2014 Virology. 448:185-195. PMCID: PMC2224591

Cairns TM, Fontana J, Huang ZY, Whitbeck JC, Atanasiu D, Rao S, Shelly, SS, Lou H, de Leon, MP, Steven, AC, Eisenberg RJ, and Cohen GH. 2014. Mechanism of neturalization of HSV by antibodies directed at the fusion domain of glycoprotein B. J. Virol. 88(5): 52677-2689. PMCID: PMC3958082.

Whitbeck JC, Huang ZY, Cairns TM, Gallagher JR, Lou H, Ponce-de-Leon M, Belshe RB, Eisenberg RJ, Cohen GH. 2014. Repertoire of epitopes recognized by serum IgG from humans vaccinated with herpes simplex virus 2 glycoprotein d. J Virol. 2014 88:7786-95. PMCID: PMC4097771

Gallagher JR, Atanasiu D, Saw WT, Paradisgarten MJ, Whitbeck JC, Eisenberg RJ, Cohen GH. 2014. Functional Fluorescent Protein Insertions in Herpes Simplex Virus gB Report on gB Conformation before and after Execution of Membrane Fusion. PLoS Pathogens. 2014 Sep 18;10(9):e1004373. doi: 10.1371/journal.ppat.1004373. eCollection 2014. PMCID: PMC4169481

Cairns TM, Huang ZY, Whitbeck JC, de Leon MP, Lou H, Wald A, Krummenacher C, Eisenberg RJ and Cohen GH. 2014. Dissection of the antibody response against herpes simplex virus glycoproteins in naturally infected humans. J. VIrol. 88(21):12612-12622. PMCID: PMC4248929

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D. Research Support
Ongoing

R37-AI-18289, Gary H. Cohen (PI); RJ Eisenberg, Co-Investigator **Dates:** 6/1/10-5/31/15
NIH/NIAID
Studies of Herpes Simplex Virus glycoproteins

The major goal of this project is to understand the two roles of glycoprotein gD in binding to its receptors and activating the fusion machinery of HSV. Because it is the receptor binding proteins, we are developing new ways to identify small molecules that inhibit receptor binding. We are also dissecting the composition of polyclonal antibodies to gD using Biacore and our panel of monoclonal antibodies. This dissection of the immune response has direct bearing on development of subunit vaccines.

2R01- AI-076231, Roselyn J. Eisenberg (PI) **Dates:** 7/1/97-7/31/14
NIH/NIAID
Early events in herpes simplex virus entry into cells
Title of renewal changed: Studies of the structure, function and regulation of gB, the fusion protein of HSV. This is the grant under review.
The major focus of this grant is to define functional regions of gB using mutants and antibodies and develop new gB constructs that could be used to define pre-and post-fusion forms of this protein. We will explore new methods to study how gB functions as a fusion protein. No overlap (or less than 10% in science, none in budget)

R01-AI-056045, Roselyn J. Eisenberg (PI) **Dates:** 09/15/03-11/30/15
NIH/NIAID
Uncovering the regulatory role of gH/gL in HSV fusion
The major focus of this project is to understand the functional role of different regions of gH/gL in regulating cell fusion based on its newly solved structure. No overlap (or less than 10% in science, none in budget).

Completed Research Support (past 3 years)

NIH 1 UC1 AI-062486. Richard Welch, P.I., G.H. Cohen (PI of Subproject) Dates: 09/01/05 – 8/31/08
NIH/NIAID Gary H. Cohen (PI) and Roselyn J. Eisenberg (Co-Investigator)
Goal: To evaluate recombinant proteins of vaccine and smallpox developed by C-PERL using baculoviruses grown in insect larvae to produce proteins for a multiprotein subunit vaccine against poxvirus infections and to develop antisera to them.

Evaluation of Control Measures Against Infectious Diseases Other Than AIDS. Dates: 12/1/11-10/31/13
This is a contract that originated at St. Louis University.
Our subcontract has G.H. Cohen as PI and RJE as Co-Investigator.
The purpose of our subcontract is to evaluate the composition of human sera from patients that had been immunized with a truncated form of gD by Glaxo Smith Kline as a test for an anti-HSV-2 vaccine. Because of the failure of the trial, one question posed to us was what constituted a neutralizing response in terms of the epitopes of gD in the vaccine. Could we dissect how people responded to immunization with gD?

RCE-U54-AI57168, **Gary H. Cohen (PI of Subproject)** **Dates:** 03/01/09-02/28/14
NIH/NIAID
Defense Against Biowarfare and Emerging Infectious Agents
The major goal of this project was to develop a multiprotein subunit vaccine against smallpox and monkey pox viruses (consisting of the membrane proteins L1, A27, A33 and B5) and to better understand the role each protein in poxvirus infection. Current studies focus principally on L1 as the principal receptor binding protein of the MV form of vaccinia virus and a major contributor to fusion by the membrane fusion complex. No overlap with any herpes projects listed above.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Roselyn J. Eisenberg	POSITION TITLE Professor of Microbiology
eRA COMMONS USER NAME (credential, e.g., agency login)	

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
Bryn Mawr College	A.B.	1960	Biology
University of Pennsylvania, Philadelphia, PA	Ph.D.	1965	Microbiology
Princeton University, Princeton NJ	PDF	1966-68	Virology

A. Personal Statement of research interests specific to this proposal).

The goal of my research is to understand the process by which the four essential glycoproteins of HSV cooperate to carry out membrane fusion, a process essential for productive virus infection and for cell-cell fusion. Together with my colleague, Dr. Gary H. Cohen, I have been interested in the basic biology of this process and in the humoral responses to those glycoproteins in cohorts of vaccinated people and in the course of natural infection and recurrences caused by HSV-1 and HSV-2. Over the course of many years, we have amassed a large collections of reagents, including antibodies (both monoclonal and polyclonal) to each of the proteins involved in the HSV entry process. In collaboration with several crystallographers, we have solved the structures of the four essential glycoproteins (gD. gH/gL and gB) as well as the two gD receptors, HVEM and nectin-1. Glycoproteins gB and gH/gL together comprise the core fusion machinery of all herpesviruses, so that what we learn about how they function is broadly applicable. Although we have learned a great deal about the cascade of events leading to fusion, many details are still lacking. Our current understanding is that this process involves key conformational changes in each protein in a well-regulated and coordinated fashion. Monoclonal antibodies in our collection are capable of blocking key steps. A hallmark of our research is to study the properties of purified glycoproteins and receptors using techniques such as Biosensor analysis, electron microscopy and other biochemical and immunological approaches to characterize wild type and mutant forms of each. Recently, we have used our knowledge of key epitopes of the glycoproteins to dissect the humoral responses of people who have been vaccinated with gD and of people who have naturally been infected by HSV-1 and/or HSV-2. These were small scale atudies in which we employed prototypes of our extensive panesl of monoclonal antibodies to gain an epitope profile for each specimen. We cararied out competition studies between our monoclonals and IgGs purified from human sera on the BIAcore biosensor and we also dissected the IgGs into gD and gB specific fractions. We found that the human antibodies competed with several of our mouse monoclonals while other epitopes we had characterized were not well represented. These initial studies did not examine epitope profiles of other important HSV glycoproteins. It is now our goal to expand these initial studies to different and larger cohorts and use all of our mouse antibodies to gain a more completed profile to match with such variables as shedding, recurrence and protection (the latter in the case of vaccinated animals and humans). We have panels of monoclonal antibodies to other key glycoproteins that we would like to incorporate into these new studies. We have >3000 samples of human sera from naturally infected people who have had genital HSV infections and we also have sera from people in two different vaccine trials as well as guinea pig sera from ongoing new vaccine trials to test. The Wasatch Microfluidics (WM) technology for doing SPR (in place of the BIAcore) will enable us to scale up our studies to larger sample numbers, take adavantage of our full panels of monocloanal antibodies and using sera rather than IgG for the competition studies. Ultimataly, our findings may impaact the design of future HSV vaccines.

B. Positions and Employment

1966 - 1968 Postdoctoral Fellow (NIGMS), Princeton University
1968 - 1969 Research Assoc. Dept. of Microbiology, Sch. of Medicine, U of Pennsylvania (UPenn)

1969 - 1978 Assistant Professor of Microbiology, School of Dental Medicine, UPenn
1978 - 1982 Assistant Professor of Microbiology, School of Veterinary Medicine, UPenn
1982 - 1985 Associate Professor of Microbiology, School of Veterinary Medicine, UPenn
1989 - 1993 Chair, Program in Microbiology, Molecular Biology Graduate Group, UPenn
1989 - Head, Laboratory of Microbiology, School of Veterinary Medicine, UPenn
1985 - Professor of Microbiology, School of Veterinary Medicine, UPenn

Current Editorial Board Memberships: Journal of Virology, Virology
A-hoc reviews: PNAS, Nature, Vaccine, J.Gen. Virol. PLoS Pathogens and a number of others

NIH Service

1994 - 1998 Member, Experimental Virology Study Section, NIH
1998 - Member, Center for AIDS Research, U. of PA.
2003 - 2006 Member, Virology A Study Section, NIH
2006 - present: Ad Hoc service on many special emphasis panels in Virology

Honors

1972 - 1977 NIH Career Development Award (NIDR), University of Pennsylvania
2003 Elected, Fellow of the American Academy of Microbiology
2004 Elected, Fellow of the American Association for the Advancement of Science
2007 to present ISI highly cited researcher

C. Selected peer–reviewed publications most relevant to the current application (from >170)

Hannah, BP, Cairns, TM, Bender FC, Whitbeck JC, Lou H, Eisenberg RJ and Cohen GH. 2009. Glycoprotein B of herpes simplex virus associates with target membranes via its fusion loops. J. Virol. 83(13):6825-6836. PMCID: PMC2698560

Wright CC, Wisner TW, Hannah BP, Eisenberg RJ, Cohen GH and Johnson DC. 2009. Fusion between Perinuclear Virions and the Outer Nuclear Membrane Requires the Fusogenic Activity of Herpes Simplex Virus gB. J. Virol. 83(22):11847-11856. PMCID: PMC2772685

Atanasiu D, Whitbeck JC, Ponce de Leon M, Lou H, Hannah BP, Cohen GH and Eisenberg RJ. 2010. Bimolecular complementation defines functional regions of HSV gB that are involved with gH/gL as necessary steps leading to cell fusion. J. Virol. 84:3825-34. PMCID: PMC2849501

Atanasiu, D., W.T. Saw, G.H.Cohen and R.J. Eisenberg. 2010. The cascade of events governing cell-cell fusion induced by HSV glycoproteins gD, gH/gL and gB. J.Virol. 84: 12292-12299. PMCID: PMC2976417

Chowdary TL, Cairns TM, Atanasiu D, Cohen GH, Eisenberg RJ, and Heldwein EE. 2010. Crystal structure of the gH/gL complex – a conserved herpesvirus fusion regulator. Nat Struct Mol Biol. 2010:882-888. PMCID: PMC2849501

Stampfer SD, Lou H, Cohen GH, Eisenberg RJ and Heldwein EE. 2010. Structural basis of the local pH-dependent conformational changes in fusion glycoprotein B from Herpes Simplex virus 1. J. Virol. 84: 12924-12933. PMCID: PMC3004323

Stiles KM, Whitbeck, JC, Lou H, Gary H. Cohen GH, Eisenberg RJ and Krummenacher C. 2010. Herpes simplex virus glycoprotein D interferes with HVEM binding to its ligands through down-regulation and direct competition. J. Virol. 84:11646-11660. PMCID: PMC2977867

Bernstein DI, Earwood JD, Bravo FJ, Cohen GH, Eisenberg RJ, Clark JR, Fairman J, Cardin RD. 2011. Effects of herpes simplex virus type 2 glycoprotein vaccines and CLDC adjuvant on genital herpes infection in the guinea pig. Vaccine. 29:2071-8. PMCID: PMC3082315.

Cairns, TM, Whitbeck JC, Lou H, Heldwein EE, Chowdary TK, Eisenberg RJ, Cohen GH. 2011. Capturing the herpes simplex virus core fusion complex (gB-gH/gL) in an acidic environment. J.Virol. 85:6175-6184. PMCID:

PMC3126480

Di Giovine P, Settembre EC, Bhargava AK, Luftig MA, Lou H, Cohen GH, Eisenberg RJ, Krummenacher C, Carfi A. Structure of HSV gD bound to the human receptor nectin- 1. PLoS Pathog. 2011 Sep;7(9):e1002277. Epub 2011 Sep 29. PMCID: PMC3182920

Lazear E, Whitbeck JC, Ponce-de-Leon M, Cairns TM, Willis SH, Zuo Y, Krummenacher C, Cohen GH, and Eisenberg RJ. 2012. Antibody-induced conformational changes in HSV gD reveal new targets for virus neutralization. J Virol. 86:1563-76. PMCID: PMC3264331

[redacted]

Shelly SS, Cairns, TM, Whitbeck JC , Lou H, Gallagher J, Cohen GH and Eisenberg RJ. 2012. The membrane proximal region (MPR) of herpes simplex virus gB regulates association of the fusion loops with lipid membranes. mBio. 2012 Nov 20;3(6). doi:pii: e00429-12. 10.1128/mBio.00429-12. PMCID: PMC3585445

Atanasiu, D, Cairns TM, Whitbeck JC, Saw WT, Rao S, Eisenberg RJ, and Cohen GH: Regulation of Herpes Simplex Virus gB-Induced Cell-Cell Fusion by Mutant Forms of gH/gL in the Absence of gD and Cellular Receptors. mBio 4(2):(e00046-13), February 2013. PMCID: PMC3585445

Maurer UE, Zeev-Ben-Bordehai T, Pandurangan AP, Cairns TM, Hannah BP, Whitbeck JC, Eisenberg RJ, Cohen, GH, Topf M, Huiskonen JT and Gruenewald K. 2013. The Structure of Herpesvirus Fusion Glycoprotein B-Bilayer Complex Reveals the Protein-Membrane and Lateral Protein-Protein Interaction. Structure. 2013:1396-13405. PMCID: PMC3737472

Atanasiu D, Saw, WT, Gallagher JR, Hannah BP, Matsuda Z, Whitbecke JC, Cohen GH and Eisenberg, RJ. 2013. A dual split protein-based fusion assay reveals that mutations to HSV glycoprotein gB alter the kinetics of cell-cell fusion induced by HSV entry glycoproteins. J. Virol.87:11332-11345. PMCID: PMC3807322

Gallagher JR, Saw WT, Atanasiu D, Lou H, Eisenberg RJ, Cohen GH. 2013. Displacement of the C terminus of herpes simplex virus gD is sufficient to expose the fusion-activating interfaces on gD. J Virol. 2013 87(23):12656-12666. PMCID: PMC3838169

Lazear E, Whitbeck JC, Zuo Y, Carfi A, Cohen GH, Eisenberg RJ, Krummenacher, C. 2014 Virology. 448:185-195. PMCID: PMC2224591

Cairns TM, Fontana J, Huang ZY, Whitbeck JC, Atanasiu D, Rao S, Shelly, SS, Lou H, de Leon, MP, Steven, AC, Eisenberg RJ, and Cohen GH. 2014. Mechanism of neturalization of HSV by antibodies directed at the fusion domain of glycoprotein B. J. Virol. 88(5): 52677-2689. PMCID: PMC3958082.

Whitbeck JC, Huang ZY, Cairns TM, Gallagher JR, Lou H, Ponce-de-Leon M, Belshe RB, Eisenberg RJ, Cohen GH. 2014. Repertoire of epitopes recognized by serum IgG from humans vaccinated with herpes simplex virus 2 glycoprotein d. J Virol. 2014 88:7786-95. PMCID: PMC4097771

Gallagher JR, Atanasiui D, Saw WT, Paradisgarten MJ, Whitbeck JC, Eisenberg RJ, Cohen GH. 2014. Functional Fluorescent Protein Insertions in Herpes Simplex Virus gB Report on gB Conformation before and after Execution of Membrane Fusion. PLoS Pathogens. 2014 Sep 18;10(9):e1004373. doi: 10.1371/journal.ppat.1004373. eCollection 2014. PMCID: PMC4169481

Cairns TM, Huang ZY, Whitbeck JC, de Leon MP, Lou H, Wald A, Krummenacher C, Eisenberg RJ and Cohen GH. 2014. Dissection of the antibody response against herpes simplex virus glycoproteins in naturally infected humans. J. VIrol. 88(21):12612-12622. PMCID: PMC4248929

D. Research Support
Ongoing

R01-AI-056045, Roselyn J. Eisenberg (PI) **Dates:** 09/15/03-11/31/15
NIH/NIAID
Uncovering the regulatory role of gH/gL in HSV fusion
The major focus of this project is to understand the functional role of different regions of gH/gL in regulating cell fusion based on its newly solved structure. No overlap (or less than 10% in science, non in budget)

R37-AI-18289, Gary H. Cohen (PI); RJ Eisenberg, Co-Investigator **Dates:** 8/1/00-5/31/15
NIH/NIAID
Studies of Herpes Simplex Virus glycoproteins
The major goal of this project is to understand the two roles of glycoprotein gD in binding to its receptors and activating the fusion machinery of HSV. Because it is the receptor binding proteins, we are developing new ways to identify small molecules that inhibit receptor binding. We are also dissecting the composition of polyclonal antibodies to gD using Biacore and our panel of monoclonal antibodies. This dissection of the immune response has direct bearing on development of subunit vaccines.

Completed Research Support (past 3 years)

NIH/NIAID. Evaluation of Control Measures Against Infectious Diseases Other Than AIDS. This is a contract that originated at St. Louis University. Our subcontract has G.H. Cohen as PI and RJE as Co-Investigator. **Dates:** 12/111-6/30/12

The purpose of our subcontract is to evaluate the composition of human sera from patients that had been immunized with a truncated form of gD by Glaxo Smith Kline as a test for an anti-HSV-2 vaccine. Because of the failure of the trial, one question posed to us was what constituted a neutralizing response in terms of the epitopes of gD in the vaccine. Could we dissect how people responded to immunization with gD?

RCE-U54-AI57168, **Gary H. Cohen (PI of Subproject)** **Dates:** 03/01/09-02/28/14
NIH/NIAID
Defense Against Biowarfare and Emerging Infectious Agents
The major goal of this project was to develop a multiprotein subunit vaccine against smallpox and monkey pox viruses (consisting of the membrane proteins L1, A27, A33 and B5) and to better understand the role each protein in poxvirus infection. Current studies focus principally on L1 as the principal receptor binding protein of the MV form of vaccinia virus and a major contributor to fusion by the membrane fusion complex. No overlap with any herpes projects listed above.

2R01- AI-076231 (formerly NS-36731), Roselyn J. Eisenberg (PI) **Dates:** 7/1/97-7/31/14
NIH/NIAID
Early events in herpes simplex virus entry into cells
Title of renewal changed: Studies of the structure, function and regulation of gB, the fusion protein of HSV.
The major focus of this grant is to define functional regions of gB using mutants and antibodies and develop new gB constructs that could be used to define pre-and post-fusion forms of this protein. We will explore new methods to study how gB functions as a fusion protein. No overlap (or less than 10% in science, non in budget)

Principal Investigator: Brooks, Benjamin D
For New and Renewal Applications (PHS 398) – DO NOT SUBMIT UNLESS REQUESTED For Non-competing Progress Reports (PHS 2590) – Submit only Active Support for Key Personnel PHS 398/2590

OTHER SUPPORT
Brooks, Benjamin

Active Support:
1R44GM109738-01 (Brooks) "HT Label-Free Screening and Kinetic Analysis of Small Molecules and Biologics." 4/1/2014 – 3/31/2017 ($[redacted] 3.0 Calendar

Completed Support:
1R43CA177146-01A1 (Brooks) "Multiplexed Ovarian Cancer Microfluidic Tissue Microarray."
8/1/2014 – 7/31/2015 ($[redacted] 1.0 Calendar

1R43GM101859-01 (Brooks) "Submerged Printing of Lipid and Membrane Protein Arrays "
9/1/2012 – 8/31/2014 ($[redacted] 2.0 Calendar

5R42MH084372-03 (Brooks) "Multiplexed GPCR Characterization Using SPR "
9/1/2012 – 8/31/2014 ($[redacted] 2.0 Calendar

Pending:
NSF SBIR Phase I. "High-throughput SPR for Screening and Characterizing Vaccines "
10/1/2016 – 9/30/2017 ($[redacted] 1.0 Calendar

NIH SBIR Direct to Phase II. "High-throughput SPR for Screening and Characterizing Vaccines "
10/1/2016 – 9/30/2018 ($[redacted] 2.0 Calendar

NIH SBIR Phase II (Brooks) "Multiplexed Ovarian Cancer Microfluidic Tissue Microarray."
8/1/2016 – 7/31/2018 ($[redacted] 2.0 Calendar

Overlap:
Not relating to this proposal. Overlap related to NIH and NSF proposals ""High-throughput SPR for Screening and Characterizing Vaccines."

Dartmouth

Department of Computer Science

August 31, 2016

Dr. Ben Brooks
Wasatch Microfluidics

Dear Ben,

I'm writing to offer you my enthusiastic support for your NIH SBIR proposal, "High-throughput, multiplexed characterization and modeling of antibody:antigen binding, with application to HSV". As you know, my lab has long been developing and applying computational methods for modeling and designing protein:protein interactions. Recently, we have been focusing on antibody:antigen interactions, and have developed new, integrated computational-experimental techniques to identify the amino acids driving a particular interaction. This proposal provides a chance to scale these methods to the high-throughput, multiplexed context enabled by your technology, and to apply them to the interesting HSV system brought by Dr. Cohen. If the proposal is funded, I will work with both the Wasatch and Penn teams to research, design, develop, and implement methods to analyze Wasatch SPR data in order to define antibody communities and localize antibody epitopes, and apply these methods to the study of antibody:antigen binding in HSV. In short, I offer you my full support for this proposal, and I look forward to building a strong collaboration, developing exciting new technology, and gaining interesting new scientific insights.

Sincerely,

Chris Bailey-Kellogg
Professor

cbk@cs.dartmouth.edu ■ http://www.cs.dartmouth.edu/∼cbk/
6211 Sudikoff Laboratory ■ Hanover, NH 03755, USA
(603) 646-3385 ■ Fax: (603) 646-1672

OTHER SUPPORT –COHEN, GH

ACTIVE

R01-AI-18289-34 (Cohen) 07/01/15-12/31/19 3.6 calendar months
NIH/NIAID $[redacted]
Title: Functions of HSV glycoproteins in virus entry and the humoral immune response

The major goals of this project are: 1) to use biochemical and immunological approaches to explore how conformational changes to each of the entry glycoproteins impact function, using monoclonal antibodies and protein structure; and 2) to use these monoclonal antibodies and purified glycoproteins to dissect the humoral response to vaccination against HSV as well as to natural HSV infections. This analysis will impact the design of future vaccine.
OVERLAP: None

1R44AI127039-01 (B. Brooks) 07/01/16-06/30/18 2.4 calendar months
NIH/NIAID $[redacted]
Title: Improving vaccine development through high-throughput immunogenicity screening

The major goals of this project are: 1) Develop the user interface and HT data analysissoftware. 2) Characterize the epitope profile of sera obtained from vaccinated guinea pigs and from naturally infected humans.

PENDING

None.

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Enter name of Organization: WASATCH MICROFLUIDICS

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 1**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .		Benjamin	Delbert	Brooks		PD/PI	[redacted]	2.0			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Computer Programmer	1.0			[redacted]	[redacted]	[redacted]
1	Application Sciences	1.0			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: WASATCH MICROFLUIDICS

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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	**0.00**

Additional Equipment: File Name:

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**	**0.00**

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

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ● Project ❍ Subaward/Consortium

Organization: WASATCH MICROFLUIDICS

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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	
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 . MTA	40.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(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 ($)*
	[redacted]

K. Budget Justification*	File Name: WasatchBudgetJustification-PA-16-302.pdf (Only attach one file.)

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

Tracking Number: GRANT12244575 Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

Budget Justification Wasatch Microfluidics

Senior Personnel

Two months support is requested for the PI, Ben Brooks. Ben will supervise all grant and research efforts and reagent/antibody generation and immunogenicity efforts through contract research organizations.

Engineers and Technicians

Funding for a senior programmer and application scientist are requested for 1 month to write updates for our epitope binning software and to perform experiments with Cohen/Eisenberg groups.

Fringe Benefits

Fringe benefits are estimated at ~25% of salaries. These benefits include FICA for all personnel.

Travel Funds are requested ($5,000) to allow the PI and/or application scientists to travel to or from UPenn and Dartmouth.

Supplies- We request funds for supplies for work on the project ($[redacted] $[redacted] is requested for lab supplies and materials Including assorted chemicals, glassware, proteins and reagents, pumps, electrical supplies, fluid flow connections, and other test supplies. Most of these will only be used if necessary as wet lab experiments have already been performed. $[redacted] will be to conduct the X-Ray crystallography at the Pasteur and $[redacted] will be to generate mutants with Integral Molecular. $[redacted] is requested to clone and sequence mAbs used in computer algorithm

Indirect Costs

40% is the indirect cost rate applied by the Wasatch Microfluidics to NIH Awards, which is charged on all items except equipment and subcontracts.

Fee A fee of less than 7% is requested to cover other business costs, such as intellectual property costs.

Budget Justification: Cohen and Eisenberg Lab

Personnel:

Gary H. Cohen, Ph.D., P.I., (1.8 calendar months) will be responsible for all scientific and budgetary aspects of this grant.

Roselyn J. Eisenberg, Ph.D., Co-Investigator, (1.8 calendar months) will collaborate with Dr. Cohen on all scientific aspects of the grant.

Tina Cairns, Ph.D., Research Specialist, (1.8 calendar months) She will carry out the monoclonal antibody blocking experiments using the ursing She is developing a high throughput method of dissecting human sera from vaccines (both human and animal) and naturally infected people to gain an epitope profile of large numbers of specimens using the WM Continuous Flow Microspotter (CFM) coupled to the Ibis MX96 imager. She also assembles and catalogues all of our monoclonal antibodies into Groups depending on their properties. As part of this, she will do antibody binning on known and thus far uncharacterized monoclonal antibodies using the WM Continuous Flow Microspotter (CFM) plus Ibis MX96 imager. She will collaborate with Dr. Atanasiu in this effort.

Doina Atanasiu, Ph.D., Research Specialist, (1.8 calendar months) is a mainstay in the running of the laboratory, in manuscript preparation and formatting. She will help to characterizd specific monoclonals to the entry glycoproteins in terms of their functions in virus entry and cell-cell fusion. She is responsible for maintaining all stocks of cells and viruses used for this purpose. She will purify antibodies to specific glycoproteins from serum specimens using streptavidin coated magnetic beads to capture biotinylated proteins. She will also assist Dr. Cairns in carrying out biosensor studies. A number of MAbs have not been fully characterized, particularly ones to gE and gC and she will be in charge of fully elucidating their properties. She will assist Dr. Cairns in grouping of Mabs in terms of their ability to neutralize virus and to block fusion.

Ms. Huan Lou, Research Specialist, (1.8 calendar months) is an accomplished insect culturist who grows large volumes of baculovirus and insect cells using the Celligen Bioreactor. She is a mainstay of our laboratory in its efforts to purify the large quantities of protein needed for all experiments involving purified proteins. She is skilled in the use of this equipment as well as equipment designed for smaller scale purification. She routinely handles 3-5 liters of cell supernatant fluids and she purifies the proteins by various chromatography techniques and characterizes them by SDSPAGE, silver staining, Western blotting, and ELISA.

Ms. Wan Ting Saw, Research Specialist, (1.8 calendar months) works closely with Dr. Atanasiu. She is highly skilled in all of the techniques used including cell culture molecular biology and virology. Her work is exceptional in quality.

Supplies. We are requesting funds on an annual basis to cover the costs of reagents, hybridoma production, preparation of immunosorbent columns for additional protein purification, magnetic beads, tissue culture supplies, media serum, plasticware, etc for cell culture and virus production and neutralization assays. We are

also requesting funds for supplies related to the WM biosensor. We are also requesting funds to cover page charges on publications, service for the ultracentrifuges and gel filtration equipment.

Travel. Funds are requested to offset travel to relevant scientific meetings such as the International Herpesvirus Workshop and others as appropriate.

Budget Justification Biosensor Tools
David Myszka, PhD.
Pi Myszka will test the new CFM/E-SPR at the end of Aim 2 for one calendar month per year. Rebecca Rich of Biosensor Tools will be employed as an application scientist for two weeks each year.
Materials and Supplies.
Funds are requested to test the next-generation CFM/SPR at $4500 per year.

Indirect Costs 25% is the indirect cost rate applied by Biosensor Tools for this grant.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	[redacted]	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
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		[redacted]

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 . Dr.	Gary	H	Cohen		PD/PI	[redacted]	0.6			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Scientist	0.6			[redacted]	[redacted]	[redacted]
1	Research Specialist	1.2			[redacted]	[redacted]	[redacted]
2	**Total Number Other Personnel**					**Total Other Personnel**	[redacted]
						Total Salary, Wages and Fringe Benefits (A+B)	[redacted]

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

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The Trustees of the University of Pennsylvania

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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	**0.00**

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	
2. Foreign Travel Costs	
Total Travel Cost	**0.00**

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**	**0.00**

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

Tracking Number: GRANT12244575 Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: The Trustees of the University of Pennsylvania

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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 . Research	61.0	[redacted]	[redacted]
		Total Indirect Costs	[redacted]

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

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: Cohen_Lab_Budget_Justification_PA-16-302.pdf (Only attach one file.)

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

Budget Justification: Cohen Lab

Personnel:

Gary H. Cohen, Ph.D., P.I., (0.6 calendar months) will be responsible for all scientific and budgetary aspects of this grant.

Tina Cairns, Ph.D., Research Specialist, (0.6 calendar months) Dr. Cairns will carry out high throughput epitope dissection of different herpes proteins and antibodies using the WM Continuous Flow Microspotter (CFM) coupled to the Ibis MX96 imager. She will assemble, analyze and catalogue the novel proteins and antibodies generated in this study. She will collaborate with Dr. Atanasiu in this effort.

Doina Atanasiu, Ph.D., Research Specialist, (0.6 calendar months) is a mainstay in the laboratory. She will be involved in the day-to-day effort to characterize the novel glycoproteins in terms of their altered function in virus entry and cell-cell fusion. She will assist Dr. Cairns in carrying out biosensor studies

Ms. Huan Lou, Research Specialist, (0.6 calendar months) grows baculovirus and insect cells to produce and purify protein needed for experimentation. She purifies proteins by various chromatography techniques and characterizes them by SDS-PAGE, silver staining, Western blotting, and ELISA.

Supplies:

We are requesting funds to cover the costs of reagents, DNA sequencing, preparation of reagents, protein purification, tissue culture supplies; media, serum, plastic ware, etc.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	2	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		0.00
1. Domestic	0.00	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		[redacted]
1. Materials and Supplies	[redacted]	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
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		0.00

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

OMB Number: 4040-0001
Expiration Date: 06/30/2016

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Enter name of Organization: Dartmouth College

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 1**

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	Christopher		Bailey-Kellogg		PD/PI	[redacted]	0.5			[redacted]	[redacted]	[redacted]

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

Additional Senior Key Persons: File Name:

Total Senior/Key Person [redacted]

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
1	Graduate Students	12.0			[redacted]	[redacted]	[redacted]
	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 {A-B} (Funds Requested)

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Dartmouth College

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **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	**0.00**

Additional Equipment: File Name:

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**	**0.00**

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

Tracking Number: GRANT12244575 Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: [redacted]

Budget Type*: ❍ Project ● Subaward/Consortium

Organization: Dartmouth College

Start Date*: 07-01-2017 **End Date*:** 06-30-2018 **Budget Period: 1**

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	
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	**0.00**

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 . 62% of Dartmouth total direct - equip			[redacted]
		Total Indirect Costs	[redacted]

Cognizant Federal Agency
(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: Dartmouth_Personnel_Justification.pdf (Only attach one file.)

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

Personnel Justification

Prof. Chris Bailey-Kellogg (.25 Summer Months) will oversee all computational components of the project, advising the Dartmouth PhD student and working together with the Wasatch programmer. We will research, design, develop, and implement methods to analyze Wasatch SPR data in order to define antibody communities and localize antibody epitopes. We will work with the other investigators to apply the methods to the study of antibody:antigen binding in HSV.

Dartmouth PhD student (TBD, 9 Calendar Months) will lead the research effort into new methods for analyzing Wasatch SPR data for antibody:antigen binding analysis.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		[redacted]
Section B, Other Personnel		[redacted]
Total Number Other Personnel	1	
Total Salary, Wages and Fringe Benefits (A+B)		[redacted]
Section C, Equipment		0.00
Section D, Travel		[redacted]
1. Domestic	[redacted]	
2. Foreign	0.00	
Section E, Participant/Trainee Support Costs		0.00
1. Tuition/Fees/Health Insurance	0.00	
2. Stipends	0.00	
3. Travel	0.00	
4. Subsistence	0.00	
5. Other	0.00	
6. Number of Participants/Trainees	0	
Section F, Other Direct Costs		0.00
1. Materials and Supplies	0.00	
2. Publication Costs	0.00	
3. Consultant Services	0.00	
4. ADP/Computer Services	0.00	
5. Subawards/Consortium/Contractual Costs	0.00	
6. Equipment or Facility Rental/User Fees	0.00	
7. Alterations and Renovations	0.00	
8. Other 1	0.00	
9. Other 2	0.00	
10. Other 3	0.00	
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		0.00

Funding Opportunity Number: PA-16-302 . Received Date: 2016-09-06T17:27:06.000-04:00

Total Direct Costs less Consortium F&A

NIH policy (NOT-OD-05-004) allows applicants to exclude consortium/contractual F&A costs when determining if an application falls at or beneath any applicable direct cost limit. When a direct cost limit is specified in an FOA, the following table can be used to determine if your application falls within that limit.

Category	Budget Period 1	Budget Period 2	Budget Period 3	Budget Period 4	Budget Period 5	TOTALS
Total Direct Costs less Consortium F&A	[redacted]	0	0	0	0	[redacted]

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

Program Type (select only one)*

● SBIR ❍ STTR ❍ Both (See agency-specific instructions to determine whether a particular agency allows a single submission for both SBIR and STTR)

SBIR/STTR Type (select only one)*

● Phase I ❍ Phase II ❍ Fast-Track (See agency-specific instructions to determine whether a particular agency participates in Fast-Track)

Questions 1-7 must be completed by all SBIR and STTR Applicants:

1a. Do you certify that at the time of award your organization will meet the eligibility criteria for a small business as defined in the funding opportunity announcement?* ● Yes ❍ No

1b. Anticipated Number of personnel to be employed at your organization at the time of award.* 15

2. Does this application include subcontracts with Federal laboratories or any other Federal Government agencies?* ❍ Yes ● No

If yes, insert the names of the Federal laboratories/agencies:*

3. Are you located in a HUBZone? To find out if your business is in a HUBZone, use the mapping utility provided by the Small Business Administration at its web site: http://www.sba.gov * ❍ Yes ● No

4. Will all research and development on the project be performed in its entirety in the United States?* ❍ Yes ● No

If no, provide an explanation in an attached file. Explanation:* Pasteur_Letter_-_PA-16-302.pdf

5. Has the applicant and/or Program Director/Principal Investigator submitted proposals for essentially equivalent work under other Federal program solicitations or received other Federal awards for essentially equivalent work?* ❍ Yes ● No

If yes, insert the names of the other Federal agencies:*

6. 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

7. Commercialization Plan: If you are submitting a Phase II or Phase I/Phase II Fast-Track Application, include a Commercialization Plan in accordance with the agency announcement and/or agency-specific instructions.*

Attach File:*

OMB Number: 4040-0001
Expiration date: 06/30/2016

SBIR/STTR Information

SBIR-Specific Questions:

Questions 8 and 9 apply only to SBIR applications. If you are submitting ONLY an STTR application, leave questions 8 and 9 blank and proceed to question 10.

8. Have you received SBIR Phase II awards from the Federal Government? If yes, provide a company commercialization history in accordance with agency-specific instructions using this attachment.* ● Yes ❍ No

Attach File:* Wasatch_Microfluidics_Commercialization_History.pdf

9. Will the Project Director/Principal Investigator have his/her primary employment with the small business at the time of award?* ● Yes ❍ No

STTR-Specific Questions:

Questions 10 and 11 apply only to STTR applications. If you are submitting ONLY an SBIR application, leave questions 10 and 11 blank.

10. Please indicate whether the answer to BOTH of the following questions is TRUE:* ❍ Yes ❍ No

(1) Does the Project Director/Principal Investigator have a formal appointment or commitment either with the small business directly (as an employee or a contractor) OR as an employee of the Research Institution, which in turn has made a commitment to the small business through the STTR application process; AND

(2) Will the Project Director/Principal Investigator devote at least 10% effort to the proposed project?

11. In the joint research and development proposed in this project, does the small business perform at least 40% of the work and the research institution named in the application perform at least 30% of the work?* ❍ Yes ❍ No

Professor Felix A. Rey
Unité de Virologie Structurale
Institut Pasteur - CNRS UMR 3965
25 rue du Docteur Roux
Paris 75015 France

Tel : +33 1 45 68 85 63
e-mail rey@pasteur.fr

Paris, August 18th 2016

OBJECT: Collaboration letter for NIH small business grant "Predicting Epitopes in Vaccine and Therapeutic Antibody Research"

To: Gary Cohen and Roselyn Eisenberg, Benjamin Brooks, Chris Bailey-Kellogg

The *Structural Virology Unit* of Institute Pasteur in Paris studies viruses of global public health and/or of veterinary concern by using structural biology techniques, primarily X-ray crystallography. The knowledge gained can be used for translational structure-based design of preventive or curative antiviral agents. One of our scientific goals is to provide a structural basis for understanding the molecular mechanisms of membrane fusion used by enveloped viruses to enter a target cell. Herpesviruses are important human pathogens and they present an interesting and challenging model for studying this process. We have been using an animal herpesvirus called pseudorabies, as well as human herpesviruses such as cytomegalovirus and human herpesvirus-8 as model systems in our lab.

Herpes simplex viruses (HSV) have been the most examined and best described of all human herpesviruses, and it is our pleasure to have established a collaboration with Gary Cohen and Roz Eisenberg on co-crystallization of HSV envelope glycoproteins in complex with key Fab molecules, such as DL11 Fab bound to HSV-2 gD. We think that solving the structures of these complexes will yield important data regarding the interface between virus–neutralizing antibodies and their epitopes. The extensive studies done in the Cohen and Eisenberg labs, on epitope mapping using biochemical techniques and cryo-EM, will augment what we will discover from the structures, and of course what we learn will be of value to understanding the roles of these glycoproteins in HSV entry. As our experiments develop, a deeper insight into how antibodies block important glycoprotein functions involved in viral entry will be gained.

We thank our collaborators for continuing to send purified recombinant gD and and Fab proteins. As you know, Marija Backovic in the lab has obtained crystals of HSV-2 gD bound to Fab DL11, an important neutralizing antibody against HSV-2. Efforts to improve those crystals are underway. We have open access to the robotic facilities for protein crystallization at the crystallogenesis core facility of the Institute, together with fluent synchrotron X-ray beam time allocation. We thus

believe that we have all the elements required for the execution of our collaborative project of co-crystallization and successful structure determination of HSV-2 gD-DL11 Fab complex.

We look forward to a fruitful collaboration.

Professor Felix A. Rey

[redacted]

Wasatch Microfluidics Commercialization History.

Wasatch Microfluidics has received 4 Phase II award (less than the 15 required for documentation).

The NSF Phase II grant had a start date of September 1, 2009. The grant was for the Continuous Flow Microspotter which has been successfully commercialized and is currently being sold.

Wasatch is currently in Phase III for the NIH Phase II that was completed in June of 2013 entitled "Multiplexed GPCR Characterization Using SPR Multiplexed GPCR Characterization Using SPR" (5R42MH084372-03). Release of this product is scheduled for Q3 of 2017.

Wasatch is in Phase II of a Fasttrack for the third awarded Phase II grant entitled "HT Label-Free Screening and Kinetic Analysis of Small Molecules and Biologics" (1R44GM109738-01).

Wasatch just began working on a Phase II of a Direct to Phase II award entitled "Improving vaccine development through high-throughput immunogenicity screening" . 1R44AI127039-01 (GRANT12062711).

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OMB Number: 0925-0001

Expiration Date: 10/31/2018

1. Human Subjects Section

Clinical Trial? ○ Yes ● No

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

2. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and proved scientific justification

3. *Program Income Section

*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)

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4. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ❍ Yes ● No

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://grants.nih.gov/stem_cells/registry/current.htm. 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:

❑ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

5. Inventions and Patents Section (RENEWAL)

*Inventions and Patents: ❍ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ❍ Yes ❍ No

6. Change of Investigator / Change of Institution Section

❑ Change of Project Director / Principal Investigator

Name of former Project Director / Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 10/31/2018

Introduction	
1. Introduction to Application (Resubmission and Revision)	
Research Plan Section	
2. Specific Aims	aims_PA-16-302.pdf
3. Research Strategy*	Research_Plan-v4.5_PA-16-302.pdf
4. Progress Report Publication List	
Human Subjects Section	
5. Protection of Human Subjects	
6. Data Safety Monitoring Plan	
7. Inclusion of Women and Minorities	
8. Inclusion of Children	
Other Research Plan Section	
9. Vertebrate Animals	
10. Select Agent Research	
11. Multiple PD/PI Leadership Plan	MultiPI_Leadership_Plan_PA-16-302.pdf
12. Consortium/Contractual Arrangements	
13. Letters of Support	Letters_of_Support_2016_NIH_-_PA-16-302.pdf
14. Resource Sharing Plan(s)	
15. Authentication of Key Biological and/or Chemical Resources	
Appendix	
16. Appendix	

Specific Aims

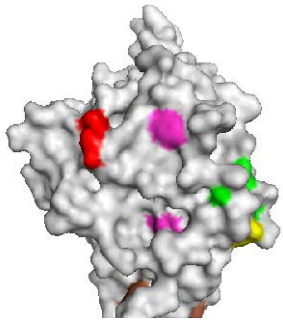
Antibodies are central to modern biomedicine, with their discovery, characterization, and engineering experiencing explosive growth, yielding powerful new treatments, and enabling breakthroughs in both biotherapeutic and vaccine development. Understanding how antibodies interact with their antigens is critical to defining and distinguishing mechanisms of action and even developing improved versions of therapeutic antibodies as well as the antigen components of vaccines. While structure determination by x-ray crystallography or cryo-EM can define antibody:antigen interactions at atomic resolution, these techniques (and other related and even less detailed methodologies) are too expensive and time consuming to support studies with large sets of antibodies from polyclonal samples or engineered libraries, or likewise large sets of antigen variants from diverse populations. At the same time, more experimentally tractable methods, such as alanine scanning and pairwise antibody blocking, do not provide nearly as rich or robust information.

In order to scale detailed characterization of antibody:antigen binding to handle entire panels of antibody and antigen variants, we seek here 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) provides an ideal focus for development, testing, and application of the new approaches, due to the availability of a wide variety of antibody and antigen variants and extensive prior low-throughput data for assessing results from the new methods. GD also still poses interesting biological questions suitable for study with the new methods, regarding variation in two HSV serotypes that resulted in failure of a vaccine trial.

The proposed methodologies will address two distinct levels of characterization:

Aim 1. **Define communities of antibodies with similar antigen binding patterns**. Here, we seek broad strokes across a wide range of antibodies, not being too sensitive to small differences, and requiring limited experimental effort. By analyzing patterns of antibody blocking with a set of antigen variants, our approach will identify functionally-related antibodies to infer the general binding regions on the antigen.

Aim 2. **Localize antibody epitopes**. Here, we seek to tease apart key contributors that can explain and predict subtle but significant impacts on interaction, requiring relatively more experimental effort to gain this level of detail. By analyzing binding between a panel of antibodies and a panel of natural and computationally designed antigen variants, our approach will identify hot-spot residues mediating binding.



Positions on HSV gD targeted by a few different antibodies.

The methods will be tested retrospectively against existing low-throughput data, and applied prospectively to predict binding of new antibodies and binding modes to be confirmed by x-ray crystallography.

Strength of the Premise: Other experimental techniques either do not scale or do not robustly provide the desired richness of information required to address these aims. Computational techniques are improving but are not yet by themselves able to reliably map interactions. The Wasatch SPR instrument provides a wealth of data and scales to large panels, but the panels need to be appropriately defined and analyzed. By combining computational modeling with Wasatch multiplexed SPR experimental measurement, this proposal thus builds on solid technologies and promises to hurdle limitations of existing techniques.

Proposed Innovation: The project will chart as-yet unexplored territory in analyzing data across large panels of antibodies and antigens, both carefully defining general binding patterns and specifically localizing binding regions. It will integrate computational and experimental methods to rationally design antigenic variants (beyond simple alanine scans and natural variants) so as to improve resulting experimental information.

Unmet Clinical Need and Potential Health Impact: The methods will be broadly applicable in the development of vaccines and antibody therapeutics. The specific application to HSV will provide deeper insights into vaccine studies and neutralizing antibodies that may be effective against different serotypes.

Team and Outlook: The project brings together investigators with the necessary complementary expertise in the instrument (Brooks), the experimental system (Cohen), and the computational methods (Bailey-Kellogg), along with collaborators to generate variants (Integral Molecular) and to structurally validate models (Felix Ray, Pasteur Institute), see Letters of Support. The successful completion of Phase I will lay the foundation for application to additional antigens from HSV and other targets, scale up and engineering of the analysis platform for commercial distribution, and incorporation of both more detailed kinetics data and even broader antibody and antigen sequence data from next-generation sequencing.

Research Strategy

A. Significance

Due to their inherent capacity to be developed against wide-ranging antigens at high affinity and specificity, antibodies are driving much of the therapeutic market[1] and provide correlates of protection for most vaccines[2]. Characterizing antibody:antigen binding, including the localization of epitopes on an antigen that are central to recognition by different antibodies, provides important insights into functionality and mechanisms of action[3–5]. Such information is particularly valuable when faced with significant antigenic diversity (e.g., from rapidly evolving pathogens) or a large set of lead candidates (e.g., from a polyclonal sample or engineered library)[6]. Thus the characterization would ideally be performed early in the development of a drug or vaccine, so as to fill the pipeline with epitopically-diverse candidates, increasing the number of different shots on goal and reducing the risk of exceedingly costly late-stage failure [7]. Unfortunately, while techniques such as x-ray crystallography, NMR spectroscopy, and cryo-EM characterize antibody:antigen recognition at atomic resolution, these techniques are laborious, expensive, and time-consuming [5,6,8,9], and thus not suitable for studying large sets of antibodies and antigenic variants. They are typically employed too late in a development pipeline, and on too limited a sample, to identify and help overcome a lack of epitope diversity [7,10–12].

In order to enable large-scale but still detailed characterization of antibody:antigen recognition, we pursue here a novel approach based on surface plasmon resonance (SPR), a mature biosensing technology that provides high-resolution data regarding the kinetics and affinity of two (or more) binding partners[7]. Wasatch Microfluidics has developed commercial products with previous SBIR funding, including the commercially-available Continuous Flow Microspotter (CFM) and the soon-to-be-released, high-throughput SPR instrument for screening antibody candidates. Plans also exist to improve the SPR instrument to possess small-molecule sensitivity and 384 spots that allows for >80X increase in throughput versus current SPR systems.

Of course, kinetics and affinity data for an individual antibody:antigen pair characterize *how well* they bind, not *how* they bind. However, we hypothesize that the high-throughput multiplexed binding studies supported by the Wasatch SPR instrument, over entire arrays of antibodies and antigens, will provide sufficiently rich information to enable computational analysis methods to infer and model details of recognition[6,7]. In particular, by characterizing differences in binding across antibody and antigen variants, the methods will group functionally similar antibodies and associated antigenic binding regions, as well as more finely localize epitopes of particular antibodies[7]. Thus this integrated computational-experimental analysis will enable the epitope profile of an antigen to be mapped much earlier and in more detail than previously possible, supporting better and faster prioritization, reducing failure rates, lowering drug/vaccine costs, and saving lives[6,7]. *The opportunity to develop this approach is unique as the extent and quality of binding data is only available with a high-throughput SPR instrument such as the Wasatch SPR instrument.*

The herpes simplex viruses (HSVs) serve as an excellent system for developing and applying our new methodology, with extensive availability of suitable antibodies and antigenic variants for experimental evaluation, sufficient “ground truth” data by which to evaluate the new methods, and compelling biological questions to be answered with the methods[13]. As enveloped viruses, HSVs must fuse their membranes with a host membrane for replication to occur. Entry is a coordinated process requiring four glycoproteins: gD, gB, a heterodimer of gH/gL, and either of two cell receptors—herpes virus entry mediator (HVEM) or nectin-1 [14]. Absence of any single protein abolishes entry and cell-cell

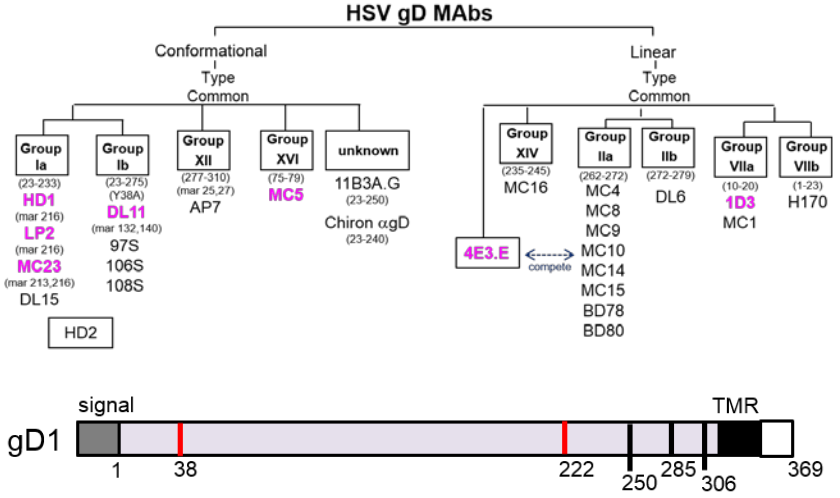


Figure A.1. (A) Previously established relationships of the gD mAbs used in these studies. ( ) = regions of gD involved in binding. Magenta = neutralizing mAbs. (B) Representation of extracellular domain of gD used in these studies. Nectin-1 disrupting mutations are in red.

fusion. Each step in this cascade of events leading to fusion is subject to blocking by epitope-specific virus-neutralizing mAbs. The Cohen-Eisenberg labs have crystallized and solved the structures of each of the entry proteins and the cell receptors, so that epitopes may be mapped onto the 3-D structures of the proteins. In this phase I project, we will focus on the gD glycoprotein, for which the Cohen-Eisenberg labs have developed a large library of antibodies against both type I and type II HSV (Figure A.1). In addition to generally gaining a broader and deeper understanding of the binding patterns in these antibodies and other variants as much still remains uncharacterized in the library (especially specific epitopes), we will study an intriguing question about antigen diversity raised by a recent vaccine trial. GlaxoSmithKline developed a vaccine using gD from type II HSV; unfortunately the vaccine only provided protection against Type II HSV for ~35% of the study participants, but surprisingly it provided over 80% protection against Type I HSV[15]. Studying the large-scale impacts of antigenic variability on antibody recognition will be made possible by the proposed approaches. Moreover, this work stands uniquely at the intersection between basic studies of recognition and immune responses to glycoproteins responsible for virus entry.

Strength of the premise. The importance of characterizing antibody:antigen binding, particularly localizing epitopes, has been well established [5,7,11]. Due to the time and expense of structure determination discussed above[6,12], a variety of other methods have been developed, but they do not provide both the throughput and resolution of the proposed approach. For example H-D exchange mass spectrometry is still relatively low throughput[6,12], while peptide arrays do not use native antigenic structures[6,12]. In addition, alanine scanning is laborious and of a low hit rate[16], while at the other extreme shotgun mutagenesis provides a lot of data but not of the same resolution and quality as SPR and due to random variation so literally hit-or-miss while not capturing impacts due to natural variation[17]. Purely computational approaches for antibody epitope prediction are not yet of sufficient accuracy to be generally useful[18,19], though the steady improvement in antibody modeling and docking[20–27] provides some hope particularly for antibody-specific approaches, especially when integrated with experimental data [28] as we propose to do here. In particular, the proposed approach builds upon the well-established Wasatch SPR platform, for multiplexed evaluation of a matrix of either antibody:antigen binding or antibody:antibody competition for a single antigen. The latter approach is commonly termed “binning”, as antibodies possessing similar blocking profiles are placed in the same “bin” [7]. It is now commonly being conducted at the earliest possible stages in drug discovery [7,10–12]; e.g., Yasmina Abidche recently detailed how epitope binning using instrument packages from Wasatch and ForteBio is improving Pfizer-Rinat’s drug discovery processes[29]. Thus our proposed method builds upon this strong technological platform in order to address a significant gap in large-scale, rapid, detailed characterization of antibody:antigen binding.

B. Innovation

Researchers from both the vaccine community and the antibody therapeutic community need better, faster, and more scalable techniques to characterize antibody epitopes, across entire arrays of antibodies and antigenic variants. Since neither purely computational nor purely experimental techniques meet these goals, our innovative integrated methodology promises to combine the best characteristics of each side. The high-throughput, multiplexed analysis of antibody:antigen binding promises to fill pipelines with epitopically diverse candidates, inherently leading to more functional diversity and increased odds of success at the clinical stage.[7]

Specific innovations of our proposed approach include the following:

- **Integration of computational docking with large-scale antibody:antigen binding studies**. Computational methods alone are not yet sufficient to localize epitopes and binding mechanisms, and neither are binding studies alone, but the integration of these techniques promises to provide complementary insights enabling modeling of the binding.
- **Computational design of sets of mutations to test specific binding hypotheses**. In contrast to alanine scanning, single point mutation, and site saturation, our methods will design mutations based on prior knowledge and modeling, so as to best (most efficiently and confidently) evaluate putative binding modes.
- **Exploitation of natural and artificial antigenic variability**. In order to localize epitopes, our methods will analyze the relationship between sequence differences and binding differences, inferring which sets of residues are likely to play key roles.

- **Systematic analysis of antibody competition assays across multiple antigens**. While antibody competition with respect to a single antigen has become a standard approach, our approach will combine competition results across multiple variants to better characterize communities.
- **Development of reference panels**. Our computational methods will select among natural variants and design new ones, so as to best support both coarse and fine grained epitope localization.
- **Computational analysis methods for large-scale antibody competition and binding studies**. Our platform breaks new ground in characterizing antibody:antigen binding by integrated analysis of the large-scale data provided by the Wasatch SPR.

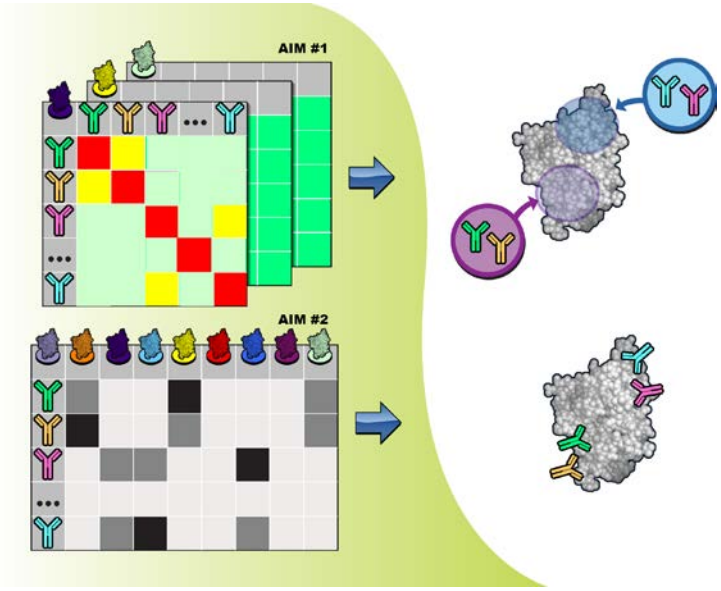


Figure C.1. Cartoon intuition for the approaches.

(*top, aim 1*) Binning-based inference of antibody communities and general epitope localization. Each matrix captures binning results between each pair of antibodies (rows and columns) competing to bind a single antigen; red indicates high competition, yellow medium, green low, and gray indeterminate. The data enable the inference of antibody communities from mutual competition, as well as general regions on the antigen recognized by each community from competition differences with respect to antigenic sequence variation.

(*bottom, aim 2*) Binding-based inference of fine epitope localization. The single matrix represents binding between each antibody:antigen pair (antibodies as rows, antigenic variants as columns), with a quantitative binding measurement indicated in grayscale. Once again, the impact of antigenic sequence variation on the experimental results enables localization of antibody binding, but here at a finer resolution due to the larger extent of antigenic variability and the incorporation of additional antibody and antigen structural modeling.

C. Approach

We will pursue two complementary approaches for the systematic characterization of antibody:antigen binding, requiring different experimental effort and yielding representations of different generality and specificity (Fig. C.1).

An antibody vs. antibody binning experiment (Fig. C.1, top) evaluates competition between antibodies in binding an antigen, and can thereby reveal which antibodies are likely to be recognizing overlapping regions on the antigen. Thus competition results from a large binning experiment, as Wasatch SPR can produce, are naturally represented in terms of "communities" of antibodies that tend to compete with each other. In our preliminary results below, we showed that binning identified communities consistent with previous low-throughput experiments conducted over many years, and that binning across antigenic variants resulted provided important information about the epitopic landscape of the protein, has been traditionally been much more difficult to obtain. In our proposed work, we will extend from using binning only to identify antibody communities to also simultaneously characterize their rough "footprints" on the antigen. Consider a hypothetical scenario where two antigenic variants differ by only one amino acid, and the antibodies in a community bind one variant but not the other. Intuitively, we can infer that the variable amino acid is likely to be important for the binding of those antibodies. This intuition also drives the popular but limited alanine scanning approach to epitope[16], more focused site-directed mutagenesis methods[17,30] as well literally hit-or-miss approaches like 'shotgun mutagenesis'[16]. Our proposed approach will exploit this insight in the more natural scenario, which is also generally more helpful, where there are multiple antigen variants and each pair of antigens differs at multiple positions (Fig. C.2).

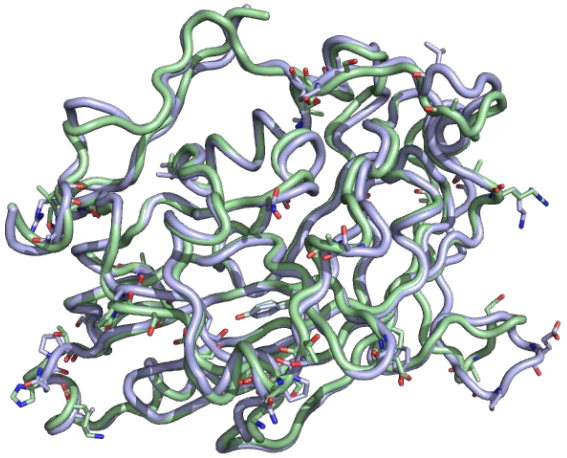


Figure C.2. Superposition of gD1 structure and gD2 homology model, with backbones in ribbon mode and side chains shown only for positions with different amino acids.

An antibody vs. antigen binding experiment (Fig. C.1, bottom)

evaluates the strength of a particular interaction; Wasatch SPR can do this in parallel for a whole matrix of pairs of antibodies and antigens. In our preliminary results below, we show that the SPR instrument readily enables identification of linear epitopes via antibody:peptide binding. In our proposed work, we will more precisely localize the "hotspots" mediating antibody:antigen recognition, with respect to native antigen conformations rather than individual peptides, and we will scale up to whole panels of antibodies. As described intuitively above, impacts of antigenic variation on antibody binding enables inference of residues important for recognition. Here, in characterizing the epitope of a particular antibody, we will have a richer set of variants enabling finer grained localization. Our proposed approach will leverage this data with and without reference to predictions of putative binding modes by computational docking methods. Furthermore, we will assess the augmentation of preexisting natural antigenic variants with new ones that have been computationally designed to be informative in revealing epitopes.

Overall, the contrast in the two approaches is that our binning method will generalize across antibodies, providing coarse localization information such that, assuming we have a sufficiently comprehensive panel, new antibodies are likely to fall into the same communities with the same interaction patterns. On the other hand, our mapping method will finely localize a specific interaction in a way that captures details of particular antibody:antigen pairs. In both cases, we leverage and assess the impact of antigenic variability, though using more extensive variability (at consequently more expense) for binding than for binning. In the binding case, we also consider use of antibody sequence (again, at more expense) and computationally hypothesized binding modes, and target the design of artificial antigenic variants to provide further information.

Aim 1. Define communities of antibodies with similar antigen binding patterns.

Preliminary Data. A preliminary epitope binning study was performed by Wasatch and the Cohen/Eisenberg teams to evaluate the effectiveness of the high-throughput system for characterizing epitopes of a library of mAbs with multiple variants of an antigen. The results set the stage for this Phase I SBIR project. The study assessed four different gD constructs (truncations of length 285 and 306, for both type I and type 2

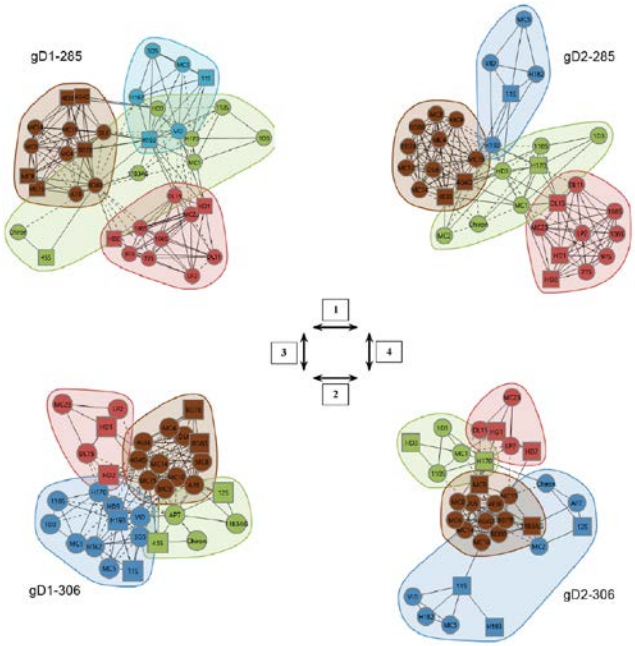


Figure C.3. Antibody community maps from four gD variants. A community plot is a network plot (e.g. social networks) generated from the epitope binning heat maps. The different colors represent 4 Wasatch derived unique communities formed by the binning characteristics of 40 different gD monoclonal antibodies obtained from 6 different laboratories. Circles represent binding in both directions (surface attached and in solution) whereas squares represent binding only in one direct. Chord length indicates degree of relatedness s defined by relative completion in the binning assay. Dashed chord indicate that there is asymmetry in the relationship (i.e. blocking does not reciprocate).

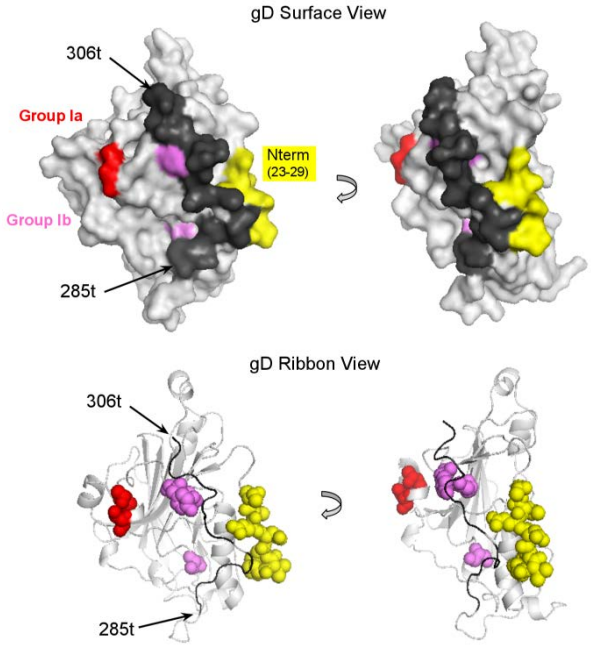


Figure C.4. Structural localization of antibody epitopes for antibodies of different specificities. The colors on the 3-D structures represent the presumed location of two major epitopes IA and IB (pink and red) derived by genetic and mutagenic analysis. The C-terminus shown in black, lies along the intersection of IA and IB and also intersects the Nectin-1 receptor binding site. The N-terminus (shown in yellow) is presented to show the complexity of the Nectin-1 binding face.

HSV). The classical sandwich binning assay format was used, with antibody competition used to identify pairs of antibodies with overlapping HSV epitopes via Wasatch's CFM and Ibis' MX96 surface plasmon resonance imager (SPRi) biosensor. Communities of co-competing antibodies were then identified, separately for each antigen (Figure C.3) which shows how the four general binding communities are related for each mutant. While the communities are generally the same between type 1 and 2 for gD 285, important structural differences in the proteins are exposed in gD 306 between type 1 and 2 two as shown in the differences between the community maps. Importantly, these differences in communities relate to important structural and epitope details that had been identified by previous low-throughput methods (Figure C.4).

Proposed Work: Experimental. The Cohen/Eisenberg Labs at the University of Pennsylvania has available multiple gD variants and multiple antibodies (Fig. A,1). These constructs as well as constructs obtained from Integral Molecular (see letters of support) will be used to conduct a set of epitope binning experiments for up to 60 selected antibodies across at least 10 gD variants, to feed into the computational analysis.

Proposed Work: Computational. Our goal is to find communities of related antibodies and corresponding regions on the antigen that mediate their binding. We will focus on the case where antibody communities are already defined by binning as in our preliminary results, since we have shown that that method yields communities consistent with external characterizations of related antibodies. But now we will have communities identified for multiple different antigenic variants, with amino acid differences in the antigen leading to perturbations in binding (see again Fig. C.3) and thereby the competition results and identified antibody clusters. Under the assumption that antibodies in a community recognize nearby residues in the antigen's structure (since they compete for binding to the antigen), those residues should be spatially proximal on the antigenic surface. Thus the approach is to cluster, with respect to the antigenic surface, the variable residues that perturb a community. We will implement and assess standard clustering techniques (hierarchical, k-means, etc.), with custom metrics that account for a combination of residue proximity on the surface and expected vs. observed perturbation due to mutation. Robustness of the identified epitope clusters will be assessed by bootstrapping-like techniques, where subsets of antibodies and/or antigens are selected for clustering, and consistency of the results characterized (e.g., by region overlap). Accuracy will be evaluated in retrospective tests by comparison to known epitope localization obtained from other experimental studies.

Pitfalls and Alternatives. Given our extensive experience with analysis of binning experiments, and our preliminary analysis of gD antigenic variants, we see no significant hurdles to successfully analyzing perturbations across multiple antigens and localizing binding regions. If we do not find sufficient local variation in the antigens, we will leverage artificial alanine variants, or even computationally designed variants (see aim 2). On the other hand, if it is necessary to reduce the set of antigens to test, we will employ computational sequence analysis to select a relatively diverse pool. Finally, if we find too little consistency of perturbation effects within a community, we will move from staged clustering (first antibodies then epitopes) to co-clustering (simultaneously).

Aim 2. Localize antibody epitopes.

Preliminary work: Experimental. A preliminary epitope mapping study was performed by Wasatch and the Cohen/Eisenberg teams to evaluate the effectiveness of a high-throughput system for characterizing epitopes of a library of mAbs. The 306 truncation of gD1 was represented by an overlapping library of 33 biotinylated peptides . All of the known linear epitopes (mainly the brown community in Fig. C.4) were identified. The logical extension of this study is to create a larger panel with conformational mutated epitopes to identify conformational epitopes.

Preliminary work: Computationally-driven design of disruptive mutations. Building upon their extensive track record in developing and applying computational protein design methods to redesign therapeutic proteins so as to remove T cell epitopes, the Bailey-Kellogg lab has recently begun developing methods to optimize variants so as to disrupt protein:protein interactions (e.g., to eliminate allosteric hotspots, antibody binding, or aggregation). In the context of disrupting antibody:antigen interactions, an initial cross-validation assessment found that a statistical sequence potential like that of Pons et al., trained on a dataset of 250 mutations from 11 Ab:Ag pairs, yielded ~70% accuracy in predicting disruptive mutations[31].

Preliminary work: Computationally-driven epitope mapping. While computational docking methods are not yet sufficient to always correctly identify how an antibody binds an antigen, recent studies have shown that at least one near-native conformation is usually found among the sampled poses[20]. Thus we treat the docking poses as hypotheses to be experimentally tested, so as to ferret out the correct one. Building upon the

disruption design method described in the preceding paragraph, the Bailey-Kellogg lab has developed a computational protein design method to optimize, for each such docking hypothesis, a variant whose antibody binding should be ablated if the pose is correct. Thus experimental assessment of binding would confirm/reject the various docking poses. In order to gauge the feasibility of this approach, we performed an initial retrospective analysis of 20 diverse antibody:antigen pairs from SAbDab [32], with server-generated models for both antigen[33] and antibody[26] based on moderate identity templates, combined into server-generated docking models [20]. Sets of triple mutants were then optimized for each docking model, predicted to disrupt binding. Strikingly, at least one of the known epitope residues was targeted for mutation within the top 3 designs for 80% of the targets, and within the top 5 designs for all targets.

Proposed Work: Computational. Our core approach uses natural antigenic variants and arbitrary antibodies, exploiting the same intuition underlying our binning analysis regarding impacts of sequence variation on possible binding, but now on a per-antibody basis and with a much larger set of antigenic variants. (The scale up is due to the fact that a single binding experiment generates a matrix of antibodies vs. antigens, whereas a single binning experiment generates a matrix of antibodies vs. antibodies for a single antigen.) Again, clustering methods and metrics will be tested retrospectively with respect to known epitope localization. We will then evaluate the utility of computational docking in further refining the analysis. Note that this requires an antibody sequence from which to model the antibody structure[25–27] and generate antibody:antigen docking poses[20,22]. These poses can then either be integrated with the clustering of perturbed residues (i.e., preferring clusters supported by docking) or potentially even supplant them (i.e., directly assessing clusters of docking poses for enrichment in perturbed residues). After localizing epitopes as much as possible with natural variants, we will use our computational antibody:antigen disruption design to design additional informative variants. The antibody and antigen will be re-docked to focus on the epitope regions hypothesized from the initial analysis, and mutational variants designed to confirm/reject the docking hypotheses as described in preliminary results.

Proposed Work: Experimental. Seven to ten mAbs, including DL11[34–36], and at least ten gD variants will be selected based on an initial computational analysis of existing data. Antibodies will be sequenced to enable computational modeling. An SPR binding experiment will then be conducted across all these antibody:antigen pairs, to feed into the computational analysis approach described. Subsequent to this analysis, a follow-up set of at least ten antigenic variants will be selected or computationally designed to further disambiguate and localize epitopes

To verify the model, the structure of DL11 will be characterized using X-Ray crystallography in the Felix Rey group at the Pasteur Institute as a subcontractor. In addition, virus neutralizations and functional binding assays will also be performed to evaluate with the goal of the functionality of the epitopes. In past studies, the Cohen/Eisenberg labs have studied these gD mutants against a panel of characterized murine monoclonal antibodies (selected from amongst those tested for this grant) possessing virus-neutralizing activity. Cohen/Eisenberg groups identified multiple epitopes that elicit strong humoral responses that provided new insight into the human response to known neutralizing gD2 epitopes [14,15].

Pitfalls and Alternatives. Again, given our extensive experience with the computational and experimental methods, and our extensive set of available antibodies and antigens, we are confident that this aim will be successfully accomplished. As in aim 1, if necessary, computational methods can be used to preselect antigens, and alanine variants can also be incorporated.

Phase II Look Ahead. Upon Phase I completion, the team will demonstrate the ability to integrate multiplexed experimental binding studies with sophisticated computational analysis methods to characterize antibody:antigen binding more broadly and more deeply than previously possible at this throughput.. In Phase II, Wasatch will integrate the analysis and visualization tools into its custom epitope binning and mapping software that is sold currently with our Wasatch SPR instrument packages, enabling facile analysis of the results by scientists. In addition, additional targets within the HSV system will be used to further apply the algorithms, with selective validation (as prioritized by the analysis) through our collaboration with the X-Ray crystallography group at the Pasteur Institute directed by Felix Rey (see letters of support). Also, the algorithms will be expanded to leverage kinetics and quantitative affinity data. Lastly, the approach will be applied at an even larger scale in the context of next-generation sequencing (NGS) of antibody libraries and natural repertoires. Wasatch can then commercialize this approach as a structural characterization tool to can fundamentally shift the focus of vaccine and drug development to leverage this information more extensively and earlier in the process.

Multiple PI Leadership plan:

Benjamin Brooks, Gary Cohen, Roselyn Eisenberg and Chris Bailey-Kellogg: Multiple PD/PI leadership plan

This application has a Primary Investigators (B. Brooks with Wasatch Microfluidics) with three additional consulting investigators, (Chris Bailey-Kellogg with Dartmouth College and Dr. Gary Cohen/Dr. Roselyn Eisenberg for the University of Pennsylvania). PI Brooks will provide the overall management of the project. Dr. Bailey-Kellogg will provide the management and computer programming. Drs. Cohen and Eisenberg will provide HSV glycoproteins, monoclonal antibodies to those proteins and human as well as guinea pig sera obtained from collaborations with Drs. Friedman, Bernstein and Cardin (see letters).

PI Ben Brooks provides expertise in assay development, microfluidic printing, and SPR Imaging design. PI Gary Cohen and his colleague Roselyn Eisenberg provide vaccine development management expertise. PI Bailey Kellogg provides extensive expertise in computer algorithm for predicting protein-protein interaction. Each will be responsible for corresponding components of the project.

PIs Brooks, Bailey-Kellogg and Cohen will converse regularly including monthly group meetings, while they each meet with employees and students assigned to their portions of projects weekly, or more often as needed. In cases of disagreement or discussion, we will defer to the PI with the most expertise in the particular area. The entire team will meet monthly as noted, and overarching project aims will be discussed and progress assessed. Adjustments will be made as needed by forming a consensus within the group. Meeting organization and reports will be the responsibility of PI B. Brooks.

As needed, either PI Brooks will fly to the other's facility for face to face management meeting, for troubleshooting, and for cross-training. ***Currently, a senior application scientist from Wasatch (Noah Ditto) and Wasatch equipment (CFM and SPRi) are collocated within the Cohen lab to facilitate communication and workflow.***

References

1. (Rob) Aggarwal, S. What's fueling the biotech engine—2012 to 2013. *Nat. Biotechnol.* **32,** 32–39 (2014).
2. Plotkin, S. A. Correlates of Protection Induced by Vaccination. *Clin. Vaccine Immunol.* **17,** 1055–1065 (2010).
3. Ladner, R. C. Mapping the epitopes of antibodies. *Biotechnol. Genet. Eng. Rev.* **24,** 1–30 (2007).
4. Liljeroos, L., Malito, E., Ferlenghi, I. & Bottomley, M. J. Structural and Computational Biology in the Design of Immunogenic Vaccine Antigens. *J. Immunol. Res.* **2015,** (2015).
5. Abbott, W. M., Damschroder, M. M. & Lowe, D. C. Current approaches to fine mapping of antigen-antibody interactions. *Immunology* **142,** 526–535 (2014).
6. Noah Ditto & Benjamin Brooks. The emerging role of biosensor-based epitope binning and mapping in antibody-based drug discovery. *Expert Opin. Drug Deliv.* (2016). doi:http://dx.doi.org/10.1080/17460441.2016.1229295
7. Abdiche, Y. N. *et al.* High-Throughput Epitope Binning Assays on Label-Free Array-Based Biosensors Can Yield Exquisite Epitope Discrimination That Facilitates the Selection of Monoclonal Antibodies with Functional Activity. *PLoS ONE* **9,** e92451 (2014).
8. Garman, E. F. Developments in x-ray crystallographic structure determination of biological macromolecules. *Science* **343,** 1102–1108 (2014).
9. Zheng, H. *et al.* X-ray crystallography over the past decade for novel drug discovery–where are we heading next? *Expert Opin. Drug Discov.* **10,** 975–989 (2015).
10. Benjamin D. Brooks. The Importance of Epitope Binning for Biological Drug Discovery. *Curr. Drug Discov. Technol.* **14,** (2014).
11. Brooks, B. D., Miles, A. & Abdiche, Y. High-throughput epitope binning of therapeutic monoclonal antibodies: why you need to bin the fridge. *Drug Discov. Today* doi:10.1016/j.drudis.2014.05.011
12. Davidoff, S. N., Ditto, N. T., Brooks, A. E., Eckman, J. & Brooks, B. D. Surface Plasmon Resonance for Therapeutic Antibody Characterization. *Label-Free Biosens. Methods Drug Discov.* 35–76 (2015).
13. Eisenberg, R. J. *et al.* Herpes Virus Fusion and Entry: A Story with Many Characters. *Viruses* **4,** 800–832 (2012).
14. Eisenberg, R. J. *et al.* Herpes virus fusion and entry: a story with many characters. *Viruses* **4,** 800–832 (2012).
15. Awasthi, S., Shaw, C. & Friedman, H. Improving immunogenicity and efficacy of vaccines for genital herpes containing herpes simplex virus glycoprotein D. *Expert Rev. Vaccines* **13,** 1475–1488 (2014).
16. Cunningham, B. C. & Wells, J. A. High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis. *Science* **244,** 1081–1085 (1989).
17. Davidson, E. & Doranz, B. J. A high-throughput shotgun mutagenesis approach to mapping B-cell antibody epitopes. *Immunology* **143,** 13–20 (2014).
18. Kringelum, J. V., Lundegaard, C., Lund, O. & Nielsen, M. Reliable B Cell Epitope Predictions: Impacts of Method Development and Improved Benchmarking. *PLOS Comput Biol* **8,** e1002829 (2012).
19. Ponomarenko, J. V. & Bourne, P. E. Antibody-protein interactions: benchmark datasets and prediction tools evaluation. *BMC Struct. Biol.* **7,** 64 (2007).
20. Brenke, R. *et al.* Application of asymmetric statistical potentials to antibody–protein docking. *Bioinformatics* **28,** 2608–2614 (2012).
21. Krawczyk, K., Liu, X., Baker, T., Shi, J. & Deane, C. M. Improving B-cell epitope prediction and its application to global antibody-antigen docking. *Bioinforma. Oxf. Engl.* **30,** 2288–2294 (2014).
22. Sircar, A. & Gray, J. J. SnugDock: paratope structural optimization during antibody-antigen docking compensates for errors in antibody homology models. *PLoS Comput. Biol.* **6,** e1000644 (2010).
23. Lensink, M. F. & Wodak, S. J. Docking, scoring, and affinity prediction in CAPRI. *Proteins* **81,** 2082–2095 (2013).
24. Kozakov, D. *et al.* How good is automated protein docking? *Proteins* **81,** 2159–2166 (2013).
25. Sivasubramanian, A., Sircar, A., Chaudhury, S. & Gray, J. J. Toward high-resolution homology modeling of antibody Fv regions and application to antibody-antigen docking. *Proteins* **74,** 497–514 (2009).
26. Marcatili, P., Rosi, A. & Tramontano, A. PIGS: automatic prediction of antibody structures. *Bioinformatics* **24,** 1953–1954 (2008).
27. Marze, N. A., Lyskov, S. & Gray, J. J. Improved prediction of antibody VL-VH orientation. *Protein Eng. Des. Sel. PEDS* (2016). doi:10.1093/protein/gzw013

28. Sela-Culang, I., Ofran, Y. & Peters, B. Antibody specific epitope prediction-emergence of a new paradigm. *Curr. Opin. Virol.* **11,** 98–102 (2015).
29. Miles, Adam & Abdiche, Yasmina. High-throughput epitope binning assays on label-free array-based biosensors can yield exquisite epitope discrimination that facilitates the selection of clones with functional activity. *PLoS ONE*
30. Benjamin, null & Perdue, null. Site-Directed Mutagenesis in Epitope Mapping. *Methods San Diego Calif* **9,** 508–515 (1996).
31. Pons, C., Talavera, D., de la Cruz, X., Orozco, M. & Fernandez-Recio, J. Scoring by Intermolecular Pairwise Propensities of Exposed Residues (SIPPER): A New Efficient Potential for Protein−Protein Docking. *J. Chem. Inf. Model.* **51,** 370–377 (2011).
32. Dunbar, J. *et al.* SAbDab: the structural antibody database. *Nucleic Acids Res.* **42,** D1140–D1146 (2014).
33. Arnold, K., Bordoli, L., Kopp, J. & Schwede, T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. *Bioinformatics* **22,** 195–201 (2006).
34. Whitbeck, J. C. *et al.* Repertoire of epitopes recognized by serum IgG from humans vaccinated with herpes simplex virus 2 glycoprotein D. *J. Virol.* **88,** 7786–7795 (2014).
35. Chen, K.-J. A thermoresponsive bubble-generating liposomal system for triggering localized extracellular drug delivery. *ACS Nano* **7,** 438–446 (2013).
36. Whitbeck, J. C. *et al.* The major neutralizing antigenic site on herpes simplex virus glycoprotein D overlaps a receptor-binding domain. *J. Virol.* **73,** 9879–9890 (1999).

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September 1, 2016

LETTER OF COMMITMENT

DARTMOUTH – WASATCH MICROFLUIDICS

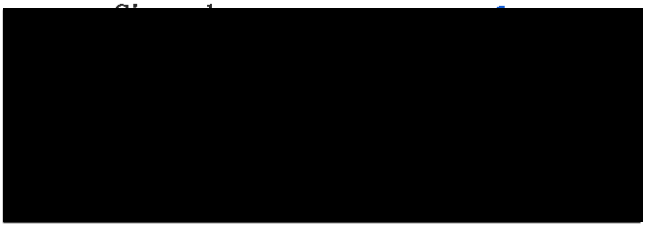
PROGRAM ANNOUNCEMENT: Sub-Contracting on National Institute of Health proposal

This letter confirms that the appropriate program and administrative personnel of the Trustees of Dartmouth College have reviewed the proposal entitled "High-throughput, multiplexed characterization and modeling of antibody: antigen binding, with applications to HSV" being submitted in support of the above–referenced program. The Dartmouth Principal Investigator on this proposal is Christopher Bailey-Kellogg. The performance period is 07/01/2017 to 06/30/2018 and the estimated cost is $50,000 which includes appropriate direct and indirect costs.

The current negotiated F&A rate agreement can be found on our website at: http://www.dartmouth.edu/~osp/resources/profile-fa.html.

Dartmouth's support of the application assumes that the negotiated federal flow–down requirements and organizational regulations will be appropriate for an educational and research institution.

Please contact our office directly at (603) 646–3007 or sponsored.projects@dartmouth.edu with any questions. Thank you for your time and consideration.



Enclosures

Perelman School of Medicine
University of Pennsylvania

Director, Botswana-UPenn Partnership
Harvey M. Friedman, M.D.
Professor of Medicine

August 4, 2016
Gary H. Cohen
School of Dental Medicine
University of Pennsylvania
Philadelphia PA 19104

Dear Gary:

I am pleased to join you in collaborating with Wasatch Microfluidics who have existing technology that improves the throughput over more traditional biosensor instruments such as the BIAcore. The Wasatch approach allows for analysis of many more samples in much less time. I am pleased to be included as a collaborator with you and Wasatch in the submission of an SBIR application to evaluate the performance of a second-generation instrument that combines two current technologies for printing and imaging into a single unit. Thus, I anticipate that we will be in a position to evaluate the array of antibody epitopes present in the sera of immunized animals that we have available and currently are in the process of obtaining. These samples are from guinea pigs immunized with HSV-2 gD2 subunit glycoproteins. The new instrument that you are evaluating will be an invaluable addition to a full understanding of the immune responses in our animal experiments. We look forward to a more thorough epitope analysis and will be pleased to collaborate with you on this project.

Sincerely,

[redacted]

Harvey Friedman

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Botswana-UPenn Partnership
Building a Healthy Future Together

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August 30, 2016

Ben Brooks, Ph.D.
Wasatch Microfluidics
825 N 300 W Ste C309
Salt Lake City, UT 84103

Dear Ben,

I am very pleased to work with you on your upcoming project on the high throughput multiplexed characterization of anti-herpesvirus antibodies. I have had a longstanding interest in optical biosensor characterization of virus glycoprotein interactions since characterizing the kinetics of HSV gD binding to HSV entry mediators during my postdoctoral fellowship at the University of Pennsylvania.

Shotgun Mutagenesis is Integral Molecular's proprietary comprehensive protein structure mapping and engineering platform. Using comprehensive mutation libraries, the binding site of an antibody, drug, or other protein can be mapped to amino acid resolution, even on conformationally-complex proteins such as GPCRs and viral envelope proteins. In the last 10 years Integral Molecular scientists have mapped the epitopes of over 300 monoclonal antibodies against viral envelope proteins including Chikungunya, Ebola, HCV, Zika, and all four serotypes of Dengue virus. Integral Molecular's Shotgun Mutagenesis should be a strong companion technology to your high throughput multiplexing platform to increase the depth of characterizing antibody-antigen binding.

Sincerely,

[redacted]

Sharon Willis, Ph.D.
Vice-President, Sales and Customer Relations

Pfizer Inc
235 East 42nd Street
New York, NY 10017

Worldwide Research & Development

Tuesday, September 6, 2016
825 North 300 West, C309
Salt Lake City, UT 84103

Reference: Non-Binding Letter of Support for Wasatch Microfluidics

Dear Dr. Brooks

Thank you for contacting us on a non-confidential basis seeking a letter from us to give non-binding and general support of the grant that you are seeking from the SBIR. Within my role as leader of the bioanalytical group at Rinat-Pfizer, I have been collaborating with the Wasatch team for the past three years and have acquired first-hand, in-depth working knowledge of their platform in the context of monoclonal antibody characterization. The Wasatch system is helping to fill a gap between the capacity to generate antibodies and our industry's ability to characterize them.

This area of research may be of potential interest to Pfizer in the future and as you continue to develop your technology related to this field, we would be interested in maintaining an open dialogue with you on a non-confidential basis (unless we agree otherwise in writing) regarding such technology and any potential related emerging needs at Pfizer. Any such potential future interest by Pfizer will be predicated on several factors, including Pfizer's strategic needs, agreement upon business terms, and Pfizer's completion of an appropriate due diligence process. However, for clarity, neither Pfizer nor you is in any way obliged to negotiate or enter into any agreement in relation to this work or any research performed in relation to this work or otherwise.

We wish you every success in attempting to address this important area of research through your pursuit of the grant from the SBIR.

Sincerely,

Yasmina Abdiche

Professor Felix A. Rey
Unité de Virologie Structurale
Institut Pasteur - CNRS UMR 3965
25 rue du Docteur Roux
Paris 75015 France

Tel : +33 1 45 68 85 63
e-mail rey@pasteur.fr

Paris, August 18th 2016

OBJECT: Collaboration letter for NIH small business grant "Predicting Epitopes in Vaccine and Therapeutic Antibody Research"

To: Gary Cohen and Roselyn Eisenberg, Benjamin Brooks, Chris Bailey-Kellogg

The *Structural Virology Unit* of Institute Pasteur in Paris studies viruses of global public health and/or of veterinary concern by using structural biology techniques, primarily X-ray crystallography. The knowledge gained can be used for translational structure-based design of preventive or curative antiviral agents. One of our scientific goals is to provide a structural basis for understanding the molecular mechanisms of membrane fusion used by enveloped viruses to enter a target cell. Herpesviruses are important human pathogens and they present an interesting and challenging model for studying this process. We have been using an animal herpesvirus called pseudorabies, as well as human herpesviruses such as cytomegalovirus and human herpesvirus-8 as model systems in our lab.

Herpes simplex viruses (HSV) have been the most examined and best described of all human herpesviruses, and it is our pleasure to have established a collaboration with Gary Cohen and Roz Eisenberg on co-crystallization of HSV envelope glycoproteins in complex with key Fab molecules, such as DL11 Fab bound to HSV-2 gD. We think that solving the structures of these complexes will yield important data regarding the interface between virus–neutralizing antibodies and their epitopes. The extensive studies done in the Cohen and Eisenberg labs, on epitope mapping using biochemical techniques and cryo-EM, will augment what we will discover from the structures, and of course what we learn will be of value to understanding the roles of these glycoproteins in HSV entry. As our experiments develop, a deeper insight into how antibodies block important glycoprotein functions involved in viral entry will be gained.

We thank our collaborators for continuing to send purified recombinant gD and and Fab proteins. As you know, Marija Backovic in the lab has obtained crystals of HSV-2 gD bound to Fab DL11, an important neutralizing antibody against HSV-2. Efforts to improve those crystals are underway. We have open access to the robotic facilities for protein crystallization at the crystallogenesis core facility of the Institute, together with fluent synchrotron X-ray beam time allocation. We thus

believe that we have all the elements required for the execution of our collaborative project of co-crystallization and successful structure determination of HSV-2 gD-DL11 Fab complex.

We look forward to a fruitful collaboration.

Professor Felix A. Rey
[redacted]