BIOGRAPHICAL SKETCH

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NAME: **Fera, Daniela**

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POSITION TITLE: Associate 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)*	END DATE MM/YYYY	FIELD OF STUDY
New York University	B.A.	05/2005	Chemistry (honors), Mathematics
University of Pennsylvania	Ph.D.	08/2012	Biological Chemistry
Boston Children's Hospital (postdoc)	n/a	07/2017	Structural Biology

A. Personal Statement

The goal of my research is to understand the development of broadly neutralizing antibodies (bnAbs) against rapidly evolving pathogens, with a particular focus on HIV. My work is driven by the need to understand the molecular mechanisms underlying immune responses and to translate this knowledge into improved vaccine strategies. Thus, my research program integrates two main areas: (1) detailed characterization of antigen-antibody interactions to inform immunogen design, and (2) investigation of kinase signaling pathways that regulate B cell responses.

I bring to this project a strong foundation in structural biology, developed during my postdoctoral training in the laboratory of Stephen C. Harrison at Boston Children's Hospital/Harvard Medical School. There, I developed expertise in X-ray crystallography and negative stain electron microscopy while determining and analyzing 3D structures of antibodies and their complexes with the HIV Env, a target for vaccine development. I also trained in preparing cryo-EM grids and performing data collection. With the increased accessibility to the Titan Krios TEM microscopes since beginning my faculty position, I further honed these skills through the Embedded Cross-Training Program in cryo-EM at the NIH National Center for CryoEM Access and Training.

The first project area is a direct extension of the structural characterizations of antibody-antigen interactions that I conducted both during my postdoctoral training and as an independent faculty member. By determining structures, together with biochemical analyses of antibody and Env mutants we generated, I identified key structural and mutational changes that lead to antibody breadth in two infected individuals who were studied longitudinally from the onset of infection. I also analyzed structures of antibodies produced from immunization studies in complex with HIV Env to highlight improvements that could be incorporated into future sequential immunogen designs. Recognizing parallels between HIV and influenza, I extended this work to influenza antibodies derived from mouse immunizations. Our structural analysis of these antibodies showed how hyper-glycosylating the viral spike can direct the immune response toward a highly conserved site—a strategy that holds promise for the design of a universal influenza vaccine and has direct implications for HIV vaccine design.

My more recent investigations also focus on signaling via the B-Raf, MEK, ERK, and Lyn kinases. Using biochemical approaches, we identified critical regions mediating kinase-kinase interactions and downstream phosphorylation activity. For example, our analysis of the B-Raf-MEK complex structure already available, together with our studies of B-Raf mutants, have revealed an α-helix on B-Raf essential for MEK binding. These results provide insights into potential allosteric drug targets. Similarly, we have mapped key interaction sites on MEK and ERK. Once we trap their transient interaction, we will seek a high-resolution structure of the MEK-ERK complex.

I am equipped to perform the proposed project because of my expertise in structural biology and my access to EM grid preparation instrumentation at the University of Pennsylvania. Additionally, my expertise on biochemical studies (biolayer interferometry, isothermal titration calorimetry, etc.) could complement our structural studies and highlight specific sites that are critical for interaction between antibodies and HIV Env, and between kinases. In turn, this would highlight key components of immunogens and identify new drug targets.

1. Finkelstein*, M.T, Parker Miller*, E., Erdman, M.C., and **Fera, D.** Analysis of two cooperating antibodies unveils immune pressure imposed on HIV Env to elicit a V3-glycan supersite broadly neutralizing antibody lineage. *Front Immunol.* 2022;13:962939. PubMed Central PMCID: PMC9548623. (*equal contribution)
2. Bajic, G., Maron, M., Caradonna, T., Tian, M., Mermelstein, A., **Fera, D.**, Kelsoe, G., Kuraoka, M, Schmidt, A. Structure-Guided Molecular Grafting of a Complex Broadly Neutralizing Viral Epitope. *ACS Infect Dis.* 2020;6(5):1182-91. PubMed Central PMCID: PMC7291361.
3. **Fera, D.**, Lee, M.S., Wiehe, K., Meyerhoff, R.R., Piai, A., Bonsignori, M., Aussedat, B.,Walkowicz, W.E., Ton, T., Zhou, J.O., Danishefsky, S., Haynes, B.F., and Harrison, S.C. HIV envelope V3 region mimic embodies key features of a broadly neutralizing antibody lineage epitope. *Nat Commun.* 2018;9(1):1111. PubMed Central PMCID: PMC5856820.
4. Williams*, W.B., Zhang*, J., Jiang*, C., Nicely*, N.I., **Fera*, D.**, Luo, K., Moody, M.A., Liao, H.X., Alam, S.M., Kepler, T.B., Ramesh, A., Wiehe, K., Holland, J.A., Bradley, T., Vandergrift, N., Saunders, K.O., Parks, R., Foulger, A., Xia, S.M., Bonsignori, M., Montefiori, D.C., Louder, M., Eaton, A., Santra, S., Scearce, R., Sutherland, L., Newman, A., Bouton-Verville, H., Bowman, C., Bomze, H., Gao, F., Marshall, D.J., Whitesides, J.F., Nie, X., Kelsoe, G., Reed, S.G., Fox, C.B., Clary, K., Koutsoukos, M., Franco, D., Mascola, J.R., Harrison, S.C., Haynes, B.F., Verkoczy, L. Initiation of HIV neutralizing B cell lineages with sequential envelope immunizations. *Nat Commun.* 2017;8(1):1732. PubMed Central PMCID: PMC5701043 (*equal contribution)

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

10/24 – present	Member, Institute of Structural Biology, University of Pennsylvania
02/23 – present	Associate Professor of Biochemistry Department of Chemistry and Biochemistry, Swarthmore College
08/18 – present	Adjunct Assistant Professor of Biochemistry and Biophysics, University of Pennsylvania
08/17 – 02/23	Assistant Professor of Biochemistry Department of Chemistry and Biochemistry, Swarthmore College
11/12 – 07/17	Postdoctoral Researcher, Boston Children's Hospital / Harvard Medical School
09/15 – 12/15, and 09/16 – 12/16	Adjunct Professor of Chemistry, School of Arts and Sciences, Massachusetts College of Pharmacy and Health Sciences
09/14 – 05/15	Adjunct Faculty, Department of Chemistry and Physics, Emmanuel College
09/14 – 12/14	Adjunct Faculty, Department of the Sciences, Wentworth Institute of Technology
05/07 – 05/08	CTL Graduate Fellow, Center for Teaching and Learning, University of Pennsylvania
07/06 – 08/06	Teaching Assistant, New York University Department of Chemistry
11/05 – 05/06	Math Teacher (after-school program), York College/Far Rockaway HS
09/05 – 06/06	Math Teacher, Frederick Douglass Academy VI High School
09/02 – 05/05	Teaching Assistant, New York University Department of Chemistry

Honors

08/18	Scientific Teaching Fellow, 2018 Summer Institute on Scientific Teaching, led by Yale Center for Teaching & Learning
08/17	Kiehl's LifeRide for amfAR Grant Recipient
02/17	Travel Award, Boston Children's Hospital Postdoctoral Association
11/16 – present	Mathilde Krim Fellowship in Basic Biomedical Research
09/15	Poster Prize, CHAVI-ID Annual Retreat
09/15	Postdoctoral Award, CHAVI-ID Annual Retreat

12/14 – 10/16	NRSA NIH Ruth L. Kirschtein National Research Service Award, F32
05/12	Second Place Prize for Poster Presentation, Wistar Institute Cancer Retreat
09/09 – 08/10	BMB Structural Biology Training Grant, University of Pennsylvania
09/07 – 08/09	NIH Chemistry-Biology Interface Pre-doctoral Training Grant (GM071339)
05/07 – 05/08	Center for Teaching and Learning Fellowship, University of Pennsylvania
04/07	Penn Prize for Excellence in Teaching by Graduate Students, University of Pennsylvania
04/07	Chemistry Department Teaching Award, University of Pennsylvania
04/05	Founder's Day Award, New York University
04/05	Merck & Company Award, New York University
09/02 – 05/05	College of Arts and Science Presidential Scholar, New York University
06/03 – 08/03	Department of Chemistry Research Fellowship, New York University
01/03 – 05/03	Dean's Undergraduate Research Fund Recipient, New York University

C. Contributions to Science

1. Structural Analysis of Antibody Affinity Maturation and Interactions with HIV Env.

My current laboratory and postdoctoral work has focused on developing an understanding of the antibody response to HIV to provide insights for vaccine design. I focused on understanding antibody affinity maturation in two HIV-infected patients who developed antibodies of significant breadth. One of these was the first patient studied from the onset of infection through the development of broadly neutralizing antibodies against the receptor binding site, making it an important case study for understanding immunogen design. The hypothesis was that studying longitudinal data would help highlight key changes in both the virus and antibodies throughout the co-evolutionary process and therefore help design vaccine components to replicate the breadth of the antibodies produced in infection more quickly by vaccination. I used X-ray crystallography and biochemical approaches to show that sites outside the antigen-binding surface of the antibodies may be critical during affinity maturation and thus should be considered in addition to the commonly mutated complementarity determining regions. I have extended my structural toolkit in analyzing antibody affinity maturation against a glycan site on the HIV envelope from the second donor. I developed a 3D model of a mature antibody in complex with Env using negative-stain electron microscopy. I also trained undergraduate students in this structural technique and we determined a 3D model of a cooperating antibody that exerted immune pressure on Env to mutate and become sensitive to the broadly neutralizing antibodies. Together with kinetic analyses and other structures, we identified the binding epitopes and revealed that improbable mutations were critical in the development of breadth in this donor.

a. **Fera, D.**, Schmidt, A.G, Haynes, B.F., Gao, F., Liao, H.X., Kepler, T.B., and Harrison, S.C. Affinity maturation in an HIV broadly neutralizing B-cell lineage through reorientation of variable domains. *Proc Natl Acad Sci U S A*. 2014;111(28):10275-80. PubMed Central PMCID: PMC4104889.

b. Bonsignori*, M., Kreider*, E.F., **Fera*, D.**, Meyerhoff*, R.R., Bradley*, T., Wiehe, K., Alam, S. A., Aussedat, B., Walkowicz, W.E., Hwang, K.K., Saunders, K.O., Zhang, R., Gladden, M.A., Monroe, A., Kumar, A., Xia, S.M., Cooper, M., Louder, M.K., McKee, K., Bailer, R.T., Pier, B.W., Jette, C.A., Kelsoe, G., Williams, W.B., Morris, L., Kappes, J., Wagh, K., Kamanga, G., Cohen, M.S., Hraber, P.T., Montefiori, D.C., Trama, A., Liao, H.X., Kepler, T.B., Moody, M.A., Gao, F., Danishefsky, S.J., Mascola, J.R., Shaw, G.M., Hahn, B.H., Harrison, S.C., Korber, B.T., Haynes, B.F. Staged induction of HIV-1 glycan-dependent broadly neutralizing antibodies. *Sci Transl Med*. 2017;9(381). PubMed Central PMCID: PMC5562350. (*equal contribution)

c. **Fera, D.**, Lee, M.S., Wiehe, K., Meyerhoff, R.R., Piai, A., Bonsignori, M., Aussedat, B.,Walkowicz, W.E., Ton, T., Zhou, J.O., Danishefsky, S., Haynes, B.F., and Harrison, S.C. HIV envelope V3 region mimic embodies key features of a broadly neutralizing antibody lineage epitope. *Nat Commun*. 2018;9(1):1111. PubMed Central PMCID: PMC5856820.

d. Finkelstein*, M.T, Parker Miller*, E., Erdman, M.C., and **Fera, D.** Analysis of two cooperating antibodies unveils immune pressure imposed on HIV Env to elicit a V3-glycan supersite broadly neutralizing antibody lineage. *Front Immunol*. 2022;13:962939. PubMed Central PMCID: PMC9548623. (*equal contribution)

2. Structural Analysis of HIV Env – Antibody Complexes from Immunization Trials.

To determine the immune response from vaccination against HIV, I am currently collaborating with Dr. Amelia Escolano at the Wistar Institute to analyze antibodies produced in humanized mice through vaccination. During my postdoctoral studies I worked with collaborators at the Duke Human Vaccine Institute (DHVI) to identify the binding modes of anti-HIV antibodies elicited from rhesus macaque immunizations and human vaccination trials. I used X-ray crystallography and negative stain electron microscopy in these studies, which revealed that the elicited antibodies could either only bind open HIV envelope trimers or only glycans on the viral spike. Such antibodies could not develop the breadth one would desire to eradicate this rapidly evolving pathogen. These results suggested that immunizations should be performed with HIV envelope trimers that are stable in a closed conformation, which mask non-neutralizing antibody epitopes, and by exposing key protein elements on the spike by modifying the glycan shield.

a. Bradley*, T., **Fera*, D.**, Bhiman, J., Eslamizar, L., Lu, X., Anasti, K., Zhang, R., Sutherland, L.L., Scearce, R.M., Stolarchuk, C., Lloyd, K.E., Parks, R., Martelli, A., Foulger, A., Abdool-Karim, S.S., Barnett, S., Kepler, T.B., Alam, S.M., Montefiori, D.C., Moody, M.A., Liao, H.X., Morris, L., Santra, S., Harrison, S.C., and Haynes, B.F. *Cell Rep.* 2016;14(1):43-54. PubMed Central PMCID: PMC4706810. (*equal contribution)
b. Easterhoff, R., Moody, M. A., **Fera, D.,** Cheng, H., Ackerman, M., Wiehe, K., Saunders, K.O., Vandergrift, N., Parks, R., Kim, J., Michael, N.L., O'Connell, R.J., Excler, J.L., Robb, M.L., Vasan, S., Rerks-Ngarm, S., Kaewkungwal, J., Pitisuttithum, P., Nitayaphan, S., Sinangil, F., Tartaglia, J., Phogat, S., Kepler, T.B., Alam, S.M., Liao, H.X., Ferrari, G., Seaman, M.S., Montefiori, D.C., Tomaras, G.D., Harrison, S.C. and Haynes, B.F. Boosting of HIV envelope CD4 binding site antibodies with long variable heavy third complementarity determining region in the randomized double blind RV305 HIV-1 vaccine trial. *PLoS Pathog.* 2017;13(2):e1006182. PubMed Central PMCID: PMC5342261
c. Williams*, W.B., Zhang*, J., Jiang*, C., Nicely*, N.I., **Fera*, D.**, Luo, K., Moody, M.A., Liao, H.X., Alam, S.M., Kepler, T.B., Ramesh, A., Wiehe, K., Holland, J.A., Bradley, T., Vandergrift, N., Saunders, K.O., Parks, R., Foulger, A., Xia, S.M., Bonsignori, M., Montefiori, D.C., Louder, M., Eaton, A., Santra, S., Scearce, R., Sutherland, L., Newman, A., Bouton-Verville, H., Bowman, C., Bomze, H., Gao, F., Marshall, D.J., Whitesides, J.F., Nie, X., Kelsoe, G., Reed, S.G., Fox, C.B., Clary, K., Koutsoukos, M., Franco, D., Mascola, J.R., Harrison, S.C., Haynes, B.F., Verkoczy, L. Initiation of HIV neutralizing B cell lineages with sequential envelope immunizations. *Nat Commun.* 2017;8(1):1732. PubMed Central PMCID: PMC5701043 (*equal contribution)
d. Williams, W.B, Meyerhoff, R.R., Edwards R.J., Li, H., Manne, K., Nicely, N., Henderson, R., Zhou, Y., Janowska, K., Mansouri, K., Gobeil, S., Evangelous, T., Hora, B., Madison, B., Abuahmad, A.Y., Sprenz, J., Deyton, M., Stalls, V., Kopp, M., Hsu, A., Borgnia, M., Stewart-Jones, G., Lee, M., Bronkema, N., Moody, M.A., Wiehe, K., Bradley, T., Alam, S.M., Parks, R.J., Foulger, A., Oguin, T., Bonsignori, M., LaBranche, C.C., Montefiori, D.C., Seaman, M., Santra, S., Perfect, J., Francica, J., Lynn, G., Aussedet, B., Walkowicz, W.E., Laga, R., Kelsoe, G., Saunders, K.O, **Fera, D.**, Kwong P.D., Seder, R., Bartesaghi, A., Shaw, G.M., Acharya, P., and Haynes, B.F., Fab-dimerized glycan-reactive antibodies are a structural category of natural antibodies. *Cell.* 2021;184(11):2955-72 e25. PubMed Central PMCID: PMC8135257.

3. Probing Allosteric Interactions between and within kinases

My current laboratory has also recently begun investigating kinases with the goal of understanding how they regulate B cell biology and antibody development. We have specifically focused on the molecular mechanisms governing the MAPK and Src-family kinase pathways. Our initial work has provided key insights into substrate recognition within the MAPK cascade, driven largely by undergraduate students at Swarthmore College. In our published study, we identified and characterized a critical alpha helix in B-Raf kinase that is essential for MEK activation, highlighting that kinase activity can be disrupted by

targeting allosteric sites. To accomplish this, we used a variety of approaches, including biolayer interferometry, *in vitro* phosphorylation assays, and cell-based studies to study binding and activity. More recently, my research group identified a binding patch on MEK that is crucial for ERK binding, further refining our understanding of kinase-substrate interactions in this pathway (to be published). Expanding our work beyond MAPK signaling, we have initiated studies on the domain interactions within Lyn kinase, a Src-family kinase, to better understand its regulatory mechanisms. Collectively, these studies will advance our knowledge of kinase signaling dynamics and may inform the development of targeted therapeutics for diseases driven by dysregulated kinase activity.

a. Nguyen D, Lin LY, Zhou JO, Kibby E, Sia TW, Tillis TD, Vapuryan N, Xu MR, Potluri R, Shin Y, Erler EA, Bronkema N, Boehlmer DJ, Chung CD, Burkhard C, Zeng SH, Grasso M, Acevedo LA, Marmorstein R, **Fera D.** Identification and Characterization of a B-Raf Kinase α-Helix Critical for the Activity of MEK Kinase in MAPK Signaling. Biochemistry. 2020 Dec 22;59(50):4755-4765. doi: 10.1021/acs.biochem.0c00598. Epub 2020 Dec 3. PMID: 33272017; PMCID: PMC8407401.

4. Interactions of Viral Oncoproteins with Host Proteins and Their Inhibition.

As a graduate student in the laboratory of Ronen Marmorstein at Penn, I identified and characterized small molecule inhibitors against the HPV oncoproteins, E6 and E7. Identifying potential therapeutics was important even though HPV vaccines have already been developed, since they only target a subset of available HPV strains and only act prophylactically. The E6 and E7 oncoproteins deregulate the cell cycle and cause cancer by targeting the p53 tumor suppressor and pRb retinoblastoma protein, respectively. I also analyzed and compared the interactions of adenovirus E1A and HPV E7 to the transcriptional co-activator p300. Notably, I was able to identify small molecules that could inhibit critical host-viral interactions to prevent tumor suppressor degradation, or induce apoptosis in HPV positive cells and in mouse tumors. I also showed that the different DNA tumor viruses could evolve to bind differently to a host protein, whose deregulation is implicated in cancer. These studies identified potential early-stage small molecule therapies against HPV-mediated pathologies, as well as an understanding of virus evolution, which would provide insight into potential therapeutic routes against HPV. Through this work, I gained expertise in numerous techniques, including isothermal titration calorimetry, analytical ultracentrifugation, thermal melts, ELISA assays, western blotting, etc.

a. **Fera, D.**, Schultz, D.C., Hodawadekar, S., Reichman, M., Donover, P.S., Melvin, J., Huryn, D.M., and Marmorstein, R. Identification and characterization of small molecule antagonists of pRb inactivation by viral oncoproteins. *Chem Biol.* 2012;19(4):518-28. PubMed Central PMCID: PMC3334872.
b. **Fera, D.** and Marmorstein R. Different regions of the HPV-E7 and Ad-E1A viral oncoproteins bind competitively but through distinct mechanisms to the CH1 transactivation domain of p300. *Biochemistry.* 2012;51(47):9524-34. PubMed Central PMCID: PMC3521592.
c. Malecka*, K.A., **Fera*, D**., Schultz, D.C., Hodawadekar, S., Reichman, M., Donover, P.S., Murphy, M., and Marmorstein, R. Identification and characterization of small molecule human papillomavirus E6 inhibitors. *ACS Chem Biol.* 2014;9(7):1603-12. PubMed Central PMCID: PMC4145632.

Complete List of Published Work in MyBibliography:
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