BIOGRAPHICAL SKETCH

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NAME: Saveez Saffarian

eRA COMMONS USER NAME (credential, e.g., agency login): SAFFARIAN

POSITION TITLE: Associate Professor of Physics and Astronomy, Adjunct Associate Professor of Biology

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/YY	FIELD OF STUDY
Sharif University of Technology	B.Sc.	09/1997	Physics
Washington University in St. Louis	PhD	12/2002	Physics
Harvard Medical School/IDI	Post Doc	08/2009	Cell Biology

A. Personal Statement

I established my lab in Jan 2010 to focus on two fundamental questions, HIV assembly and negative strand RNA virus replication. My journey to virology has been long and involved passing through multiple disciplines of science. I was trained for my Physics PhD in the lab of Elliot Elson and during this period, I built a state of the art single molecule imaging and fluorescence correlation spectroscopy instrument. Using this instrument we discovered the first extra cellular Brownian ratchet enzyme (1) and we also performed pioneering studies on EGF receptor oligomerization in live cells (2). In Elliot's lab, I was also trained in computational methods especially fluctuation based theoretical biophysics. After my PhD, I decided to focus on a biological problem instead of technique development. Given this desire, I moved to Harvard Medical School and started a 5 year postdoctoral study on endocytosis with Tom Kirchhausen where I was also introduced to Steve Harrison and structural biology of large protein assemblies. In Tom's lab, I became a cell biologist/virologist. I learned how to avoid biological artifacts and design experiments that capture the most biologically relevant information. I also developed a new imaging method named Differential Evanescence Nanometry (3) which we used to distinguish between Clathirn coated pits and plaques (4). My interactions with Steve Harrison and Sean Whelan however lured me into virology. I found viruses fascinating. Viral interactions with cells can uncover many interesting new biological phenomena as is evident by the progress in the past decades and therefore as Arthur Kornberg put it, I let the viruses open the doors and study virus assembly and replication in my lab. I chose to study HIV budding because of pioneering work on HIV budding which had mapped out the extensive cellular interactions of the virus during assembly. I knew I could apply many of my skills developed in my work in endocytosis to the problem of HIV budding. Because of my work with Sean Whelan at Harvard medical school, I was also interested in the replication mechanics of VSV which is the prototype virus in negative sense non segmented RNA viruses which include Ebola, measles and Rabies among other major human pathogens. The summary of my lab's contribution would follow in section C.

1. Saffarian S, Collier IE, Marmer BL, Elson EL, Goldberg G. Interstitial Collagenase Is a Brownian Ratchet Driven by Proteolysis of Collagen. Science. 2004;306(5693):108-11. PMCID: PMID: 15459390.
2. Saffarian S, Li Y, Elson EL, Pike LJ. Oligomerization of the EGF Receptor Investigated by Live Cell Fluorescence Intensity Distribution Analysis. Biophysical Journal. 2007;93(3):1021-31. PMCID: PMID: 17496034.
3. Saffarian S, Kirchhausen T. Differential evanescence nanometry: Live-cell fluorescence measurements with 10-nm axial resolution on the plasma membrane. Biophysical Journal. 2008;94(6):2333-42. PMCID: PMID: 17993495.
4. Saffarian S, Cocucci E, Kirchhausen T. Distinct Dynamics of Endocytic Clathrin-Coated Pits and Coated Plaques. PLoS Biol. 2009;7(9):e1000191. PMCID: PMID: 19809571.

B. Positions and Honors

Positions and Employment

2003 – 2004	Research Instructor, Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO
2004 – 2009	Research Fellow, Harvard Medical School/Immune Disease Institute, Boston, MA
2010 – 2015	Assistant Professor of Physics/Biology University of Utah, Salt Lake City, UT
2015 – Present	Associate Professor of Physics/Biology University of Utah, Salt Lake City, UT
2015 – Present	Director of University of Utah Biological Physics Cluster

Other Experience and Professional Memberships

2002-present	Member Biophysical Society
2010-present	Member of American Society of Virology

Honors

1993	Honorable Mention XXIV International Physics Olympiad, The College of William & Mary
1994-1997	IPM (Institute of Physics and Mathematics) Bachelor Fellowship
2001-2002	Jill Abrams Scholarship in Physics
2002	Biophysical Society Student Research Achievement Award
2005	Finalist in Gregorio Weber International Prize in Biological Fluorescence
2006	Young Fluorescence Investigator Award, Biophysical Society

C. Contribution to Science

Methods:

1. Interferometric fluorescence cross correlation spectroscopy

   Measuring transport properties like diffusion and directional flow is essential for understanding dynamics within heterogeneous systems including living cells and novel materials. Fluorescent molecules traveling within these inhomogeneous environments under the forces of Brownian motion and flow exhibit fluctuations in their concentration, which are directly linked to the transport properties. We present a method utilizing single photon interference and fluorescence correlation spectroscopy (FCS) to simultaneously measure transport of fluorescent molecules within aqueous samples. Our method, within seconds, measures transport in thousands of homogenous voxels (100 nm)3 and under certain conditions, eliminates photo-physical artifacts associated with blinking of fluorescent molecules. A comprehensive theoretical framework is presented and validated by measuring transport of quantum dots, associated with VSV-G receptor along cellular membranes as well as within viscous gels.

   Saha I and Saffarian S. Interferometric fluorescence cross correlation spectroscopy. PLoS One . 2019;14(12):e0225797.

2. Correlative iPALM and SEM resolves virus cavity and Gag lattice defects in HIV virions

   Interferometric Photo-Activation-Localization-Microscopy (iPALM) localizes single fluorescent molecules with 20 nm lateral and 10 nm axial resolution. We present a method utilizing glass coverslip lithography for correlative imaging between iPALM and scanning electron microscopy (SEM). Using iPALM on HIV Gag-Dendra virus-like particles (VLPs) we localized the position of HIV Gag proteins. Based on these localizations we reconstructed the central cavity of the VLPs along with imperfections within the HIV Gag lattice. The SEM images and iPALM images overlap and show imaging from single VLPs immobilized on glass coverslips. The localization of many HIV proteins including accessory proteins and Gag-Pol remains unknown, we discuss how the specificity of iPALM coupled with SEM has the potential for resolving more of HIV proteins.

Pedersen M, Jamali S, Saha S, Daum R, Bendjennat M and Saffarian S. 2. Correlative iPALM and SEM resolves virus cavity and Gag lattice defects in HIV virions. Eur. Biophys J . 2019;48(1):15-23.

HIV assembly:

1. Dynamics of the HIV Gag Lattice Detected by Localization Correlation Analysis and Time-Lapse iPALM
   Immature human immunodeficiency virus (HIV) virions have a lattice of Gag and Gag-Pol proteins anchored to the lumen of their envelope. We performed iPALM imaging on immobilized HIV Gag-Dendra2 VLPs and demonstrated that we can localize and count 900-1600 Dendra2 molecules within each immobilized VLP with a single-molecule localization precision better than (10 nm)3. We further calculate temporal correlation functions of localization data, which we presented as localization correlation analysis, and showed dynamics within the lattice of immobilized VLPs in the timescale of 10-100 s. The iPALM time-lapse images showed significant lattice dynamics within the lumen of VLPs. In a complementary approach, we utilized HaXS8 cross-linking reactions between Halo and SNAP proteins and verified lattice dynamics in purified VLPs incorporating 10% Gag-SNAP, 10% Gag-Halo, and 80% Gag proteins. The HIV Gag lattice, along with the structural lattice of other enveloped viruses, has been mostly considered static. Our study provides an important tool to investigate the dynamics within these enveloped viruses.
   Saha I and Saffarian S. Dynamics of the HIV Gag Lattice Detected by Localization Correlation Analysis and Time-Lapse iPALM. Biophys J. 2020;119(3):581-592.

2. The race against protease activation defines the role of ESCRTs in HIV budding
   As part of our efforts to understand HIV budding and maturation, we created a minimal functional system for the study of HIV budding and protease activation which produces virus like particles incorporating both Gag and Gag-Pol proteins. Using this system, we showed that the HIV protease is activated immediately after the virion assembly and delays in virion release result in the loss of Pol enzymes. Specifically, alterations of Gag p6 which reduce interactions with the endosomal sorting complexes required for transport result in delayed virion release and proportional degrees of Pol enzyme loss. In addition, we showed that budding of HIV Gag is sensitive to ESCRT involvement when additional protein cargos are fused to the Gag C-terminus. Interestingly, while for large cargos both Tsg101 and ALIX recruitments are essential for budding, small cargos are substantially more sensitive to Tsg101. Our results highlight the sensitivity of HIV to budding “on-time” and suggest “budding delay” as inhibitory for infectious virion release (a).

   Bendjennat M, Saffarian S. The race against protease activation defines the role of ESCRTs in HIV budding. PLoS Pathogens. 2016;12(6):e1005657..

3. ALIX is recruited temporarily into HIV-1 budding sites at the end of Gag assembly
   Endosomal sorting complexes required for transport (ESCRTs) are required for fission of the membrane during budding HIV virions. In my lab we set out to investigate the dynamics of ESCRT engagement during HIV budding. ALIX is an ESCRT associated protein whose biochemical interactions with both HIV Gag and the later ESCRT components (CHMP4 proteins) had been well characterized. Previous live imaging experimnets as well as the afinity of ALIX for HIV Gag p6 domain which is present from the intial stages of HIV assembly strongly suggested that ALIX is recruited into the forming HIV virions from the beginning of assembly. We used a novel linker design strategy and screened for functional GFP tagged ALIX protein fusions. We identified a GFP fused ALIX which retained the rescue of PTAP mutated virions with similar efficiency as wild type ALIX. Using these fusion proteins, we created cell lines and visualized the recruitment of ALIX into forming HIV virions. We observed that contrary to our expectations ALIX was recruited at the end stages of the HIV Gag VLP assembly and most of the recruited ALIX dissociated from the forming virion during membrane fission (b). Our lab is currently investigating the dynamics of ALIX recruitment at the end of assembly to find the essential interactions required for the late stage ALIX recruitment and release. This study is funded through an R21 grant from the NIH.

Ku P-I, Bendjennat M, Ballew J, Landesman MB, Saffarian S. ALIX Is Recruited Temporarily into HIV-1 Budding Sites at the End of Gag Assembly. PLoS ONE. 2014;9(5):e96950. PMCID: PMC4023924

Negative strand non segmented RNA virus replication

4. Vesicular stomatitis virus polymerase's strong affinity to its template suggests exotic transcription models

Negative strand non segmented RNA viruses include potent human and animal pathogens e.g. Ebola, measles, Rabies and VSV. The genome template in these viruses is consistent of the single strand negative sense RNA fully encapsidated with thousands of copies of ~40kD nucleocapsid protein N. These viruses package multiple copies of a special RNA dependent RNA polymerase (RdRP) which is composed of a catalytically active 250kD protein L which does not bind the template directly with phosphoprotein P which binds both the template and L. Transcription is the first step in viral replication after delivery of the genome template to the cytosole of the infected cell. Transcription can only initiate at or near the 3' end of this genome template. We have measured the dissociation constant of VSV L proteins from the virion extracted genome templates and found them to be bound with a 20nM dissociation constant. Given this high afinity, we constructed many possible models of transcription and showed that transcription likely requires a mechanism for long range sliding of polymerases on the genome templates (d). Recently we have observed this long range sliding of VSV L proteins using PALM imaging on genome templates purified from recombinant VSV virions in which L protein is fused to the photoswichable protein Dendra2 (e). This work has been supported by the NSF.

Tang X, Bendjennat M, Saffarian S. Vesicular Stomatitis Virus Polymerase's Strong Affinity to Its Template Suggests Exotic Transcription Models. PLoS Comput Biol. 2014;10(12):e1004004. PMCID: PMC4263359

5. Assymetric localization of polymerases in vessicular stomatitis virus

VSV is a bullet shaped negative sense non segmented RNA virus. The N-RNA genome template is tightly wound in a left handed helix bound to the envelope of the virus through a layer of the M protein. Inside VSV there is a cavity with a diameter of 30nm and length of 150nm where all 50 VSV polymerases are located. Sinve VSV polymerases are found distributed randomly along purified genome templates and CryoEM reconstruction of VSV virions did not show any localization of proteins within the central cavity of the virus, it was believed that VSV polymerases are also randomly distributed within this cavity. We used Photoactivation Localization Microscopy and Atomic Force Microscopy (f) to show that the VSV polymerases are localized at the blunt end of the VSV central cavity. This assymetric localization positions the 50 polymerases close to the 5' end of the genome template (g). VSV polymerases, however, initiate transcription at or near the 3' end of the genome template after delivery of the templates to the cytosole of the infected cells. How do VSV polymerases redistribute along the template to reach the 3' end and start transcription is an active area of research in our lab. This work is supported through an NSF award.

Hodges JA, Saffarian S. Sample Preparation for Single Virion Atomic Force Microscopy and Super-resolution Fluorescence Imaging. JoVE. 2014(83):e51366. PMCID: PMC4047664

Hodges J, Tang X, Landesman MB, Ruedas JB, Ghimire A, Gudheti MV, et al. Asymmetric packaging of polymerases within vesicular stomatitis virus. Biochemical and Biophysical Research Communications. 2013;440(2):271-6. PMCID: PMC4350925

Complete List of Published Work in PubMed:
http://www.ncbi.nlm.nih.gov/pubmed/?term=saffarian+saveez

D. Research Support
Ongoing Research Support

NSF 2026657 Saffarian (Co-PI) 03/09/2020-03/09/2021
RAPID: physics of coronavirus sars-cov-2 survival outside a host and implications for seasonal dependence of covid-19 outbreaks.

Goals: To generate SARS-CoV2 Virus Like Particels and test their structural stability under varied environmental conditions.

Role: Co-PI

NIH R01GM125444 Saffarian (PI) 09/01/2017-09/01/2022
Dynamics of Gag-Pol auto-processing and ESCRT recruitment during HIV budding

Goals: To visualize HIV budding and Gag-Pol auto-processing in single virions *in vivo* to: 1) Establish the dynamics and regulation of early ESCRT recruitment during full HIV virion assembly, 2) Establish the dynamics of Gag-Pol incorporation and auto-processing during HIV budding and resolve the localization of Gag-Pol within immature HIV virions using correlative light and electron microscopy.

Role: PI

Completed Research Support

NSF 1615076 Saffarian (PI) 08/15/2016-08/15/2020
Collaborative Research: Dynamics of RNA dependent RNA polymerases
Goals: To characterize the sliding dynamics of RNA dependent RNA polymerases of negative strand RNA viruses on their respective genome templates.
Role: PI

R21 AI114432 Saffarian (PI) 07/01/2014 - 6/30/2016
Dynamics of early ESCRT recruitment during HIV budding
Goals: Establish the sequence of events that underlie ALIX recruitment to HIV virus like particles (VLP) and create tools and methodologies that can be used to define how other ESCRT components are recruited during HIV budding.
Role: PI

NSF 1121972 Saffarian (PI) 08/01/2011-08/31/2015
Developing Methods for High-resolution Measurements of Enveloped Virus Budding in Live Cells
Goals: expand the reach of ultra-high precision quantitative measurements into a new frontier in biological sciences, namely the interactions between viruses and cells, resulting in a detailed molecular understanding of the budding process. Increase the participation of women and underrepresented groups in the pursuit of basic science in general and in the proposed activities in particular.
Role: PI