Research Highlight #2007

Dr. Rengasayee "Sai" Veeraraghavan
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Nanoscale imaging of cell-to-cell communication in the heart with super-resolution microscopy

Despite recent advances in cardiac healthcare, there are still about a half million deaths every year resulting from cardiac arrhythmia in the United States alone. A critical component of heart health is proper cell-to-cell communication via electrical signaling, a phenomenon that Dr. Veeraraghavan has been committed to researching long before his current role at The Ohio State University. During his Ph.D. studies, Dr. Veeraraghavan conducted high-speed imaging of whole hearts, labeling cardiac cells with voltage sensitive dyes and visualizing the propagation of electrical currents. This work resulted in both answers and more questions about how heart cells communicate, which led him to his first postdoc in mathematical modeling of heart signaling. It was during this experience that he realized the phenomenon occurred on a range of scales, from ~10 nanometers to one micron, and that data was lacking for many of the nanoscale structural properties that needed to be incorporated into the model.

> "This led me to abruptly conclude my modeling postdoc and run off to a microscopy lab saying, 'if nobody else will get me this data, I will get it myself.' STORM ideally lent itself to understanding the molecular map of the cell-cell contact structures that my lab is most interested in. The goal now is still the same goal I had naively set for myself during my math postdoc, which was to generate realistic models of those structures, and put them into physiological models to ask how does structure impact function."

Sai was excited by the prospects of STORM, a super-resolution imaging technique that enables the nanoscale imaging—down to ~10-20 nanometers—of fluorescently labeled signaling molecules and structures to acquire the data he had been missing. When first coming into this technique for his research, he faced a couple of challenges. First, the heart is more optically dense than other samples typically used with super-resolution microscopy, such as model cells or neurons, which makes light microscopy challenging. Second, performing quantitative analysis of clusters of molecules and their relative organization in tissue was an under-explored area, so there were not many analysis tools already developed for this purpose. With the help of Bruker application specialists, and a lot of trial and error, he developed protocols that enabled the acquisition of reliable 3D STORM images from cardiac muscle sections.

> "One of the things we love about the Vutara is the fact that it's in a wide field configuration, and therefore it is usable for our problem space. Cardiac muscle tissue, even in 5-micron sections, is so optically dense that if you're doing TIRF or sub-critical angle illumination, you simply do not get enough photons into the sample to do the job. And I know this from painful experience, having tried out those types of systems. The biplane and the widefield configuration of Vutara are absolutely mission critical for what we do."

ABOUT THE RESEARCHER

Dr. Rengasayee "Sai" Veeraraghavan is an Associate Professor in the Department of Biomedical Engineering at The Ohio State University. He earned his Bachelor of Technology in chemical engineering from Anna University in Chennai, India and his Ph.D. from University of Utah in Salt Lake City, Utah. Before his current position, he held two postdoctoral positions studying Math Biology at University of Utah and Connexin Biology at Virginia Tech Carilion Research Institute.

Website: Visit Dr. Rengasayee website

Recent Publications

Bogdanov V*, Soltisz AM*, Moise N, Ivanova M, Andreev I, Skuta G, Weinberg SH, Davis JP, Veeraraghavan R†, Györke S†. Distributed synthesis of sarcolemmal and sarcoplasmic reticulum membrane proteins in cardiac myocytes. *Basic Res Cardiol.* 2021 Oct 28;116(1):63. *Co-first authors. †Co-corresponding authors.

Moise N, Struckman HL, Dagher C, Veeraraghavan R, Weinberg SH. Intercalated disk nanoscale structure regulates cardiac conduction. *J Gen Physiol.* 2021 Aug 2;153(8):e202112897.

Mezache L, Struckman HL, Greer-Short A, Baine S, Györke S, Radwański PB, Hund TJ, Veeraraghavan R. Vascular Endothelial Growth Factor Promotes Atrial Arrhythmias by Inducing Acute Intercalated Disk Remodeling. *Sci Rep.* 2020 Nov 24. 10 (1), 1-14.

He also spent many months developing his own code to do cluster analysis for studying tissue-scale physiology in the heart, some of which is now included in Bruker's SRX software. Once he overcame these initial challenges, he was ready to tackle applied problems in cardiology.

Visualizing protein dysfunction with STORM and impacts in drug discovery

One current protein of interest in Dr. Veeraraghavan's research is calmodulin, a small cytosolic protein that regulates various ion channels and other proteins. Calmodulin is critical for life; in the heart there is triple redundancy of this protein, and it is highly conserved, with not a single amino acid variation existing across the mammalian class. Unfortunately, people can have mutations in this protein and develop diseases as a result, termed calmodulinopathies, which can lead to sudden death or defects in the brain that cause learning difficulties. On this topic, Dr. Veeraraghavan partnered with collaborators studying a phenomenon called sudden unexplained death in epilepsy and associated calmodulin properties. They wanted to know what was happening at the tissue scale if there is a mutation in calmodulin, so they created a mouse model with a single amino acid mutation in the protein. The result was that this one neuronal sodium channel isoform doubled its cluster density only in very specific niches where another protein sodium calcium exchanger was also upregulated in the hearts, and the niches were places where calcium movements are happening during every heartbeat. This caused altered sodium regulation and altered sodium-calcium exchange, ultimately causing extreme calcium mishandling in the mouse.

> "The whole heart was remodeled and rebuilt in terrible ways that are conducive to arrhythmia. The Vutara was instrumental in getting those data, particularly on the structural side.On that project, we went from looking at isolated proteins interacting with peptides using isothermal titration calorimetry, all the way to in vivo ECGs studies on intact animals and every possible scale along the way. What proved especially informative was how the Vutara showed us all the sub-cellular structural remodeling."

The group's collaborators, led by Dr. Przemysław 'Prez' Radwański (@NanoEPlab), then validated these nanoscale structural changes with scanning ion conductance microscopy and smart patch clamp. Through functional experiments, they validated that sodium channel cluster densities were indeed doubled. They went on to conduct studies at tissue scales, and based on the findings, designed further experiments to understand the arrhythmia mechanism.

> "The happy conclusion to that story is that we identified ways of treating those arrhythmias based on this insight that it was specifically this NaV1.6 isoform of the sodium channel, which is a neuronal sodium channel isoform—which means there are already drugs that are in use to treat neurological disorders mediated by this drug channel. And we're now finding that they can effectively suppress these arrhythmias. In fact, our collaborator, Przemysław (Prez) Radwański, has just received approval to start a prospective, double blinded clinical trial to evaluate a drug used to treat Lou Gehrig's disease as a potential treatment for atrial fibrillation."

There are generally only two types of therapy for cardiac arrhythmia disorders: (1) canonical ion channel modulates that open or block ion channels, which make up the vast majority of treatments; and (2) treatments that more indirectly reduce risks, such as beta blockers. Even with these treatments, it is not always understood why and how they work when they do.

> "What we are understanding using techniques like STORM is that there's fundamentally a missing piece in how we think of disorder. We typically think there must be a protein somewhere that got modified and is now malfunctioning, and therefore disorder. What we realized is there's a whole other recipe for a disorder, which is a perfectly normal protein that either moves dynamically through an abnormal location or its structural environment changes dynamically, and therefore results in dysfunction."

Now, new drugs are being designed to keep these structural niches intact. For instance, Dr. Veeraraghavan is finding that in the heart there are nano-pockets next to gap junctions that can swell up, and they are particularly swelled in patients with atrial fibrillation. With this knowledge, he is designing peptides that can purposely grab the adhesion molecules that already exist in the system and hold them together, preventing them from coming apart under provocation. This sparks the development of not just new therapeutic strategies using current types of therapy, but whole new classes of therapy.

Beyond cardiology: STORM implications in cancer therapeutic research

These applications of STORM for understanding nanoscale tissue organization and subsequent drug development extend beyond the field of cardiology into fields such as oncology. Dr. Veeraraghavan has dedicated much of his time and effort in cardiac research to studying connexin 43, a gap junction protein expressed in all 40 tissue types in the human body (Figure 1). As this protein is ubiquitous in the human body and serves critical functions, research applications extend beyond just the heart. Dysfunction in this protein has implications in skin wound healing, brain function, and even macular degeneration in the eye.

It was found by Dr. Veeraraghavan's postdoc mentor, Dr. Robert Gourdie at Virginia Tech, that connexin 43 is involved in glioblastoma tumor resistance to current chemotherapy agents. They discovered that if connexin43 hemichannels are removed from these cancer cells the treatment is more effective against them because removing the channels prevents the release of the chemotherapy agent out of the cell. Dr. Veeraraghavan plans to extend this work further by testing the efficacy of drug delivery devices using STORM. He can fluorescently tag peptide drugs and image them with STORM to visualize if the drug is reaching its molecular target or not, which would not be possible with diffraction-limited fluorescence

An intercalated disk from the mouse heart.

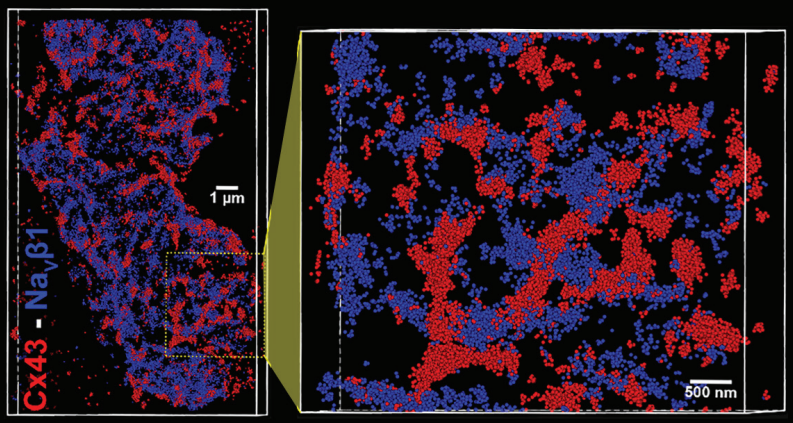

FIGURE 1

En face (end-on) view of an intercalated disk specialized structures that provide electrical, chemical, and mechanical coupling between adjacent heart muscle cells from a healthy mouse heart. The image shows the gap junction protein connexin43 (Cx43; red) and a non-pore-forming sodium channel subunit (NaVβ1; blue).

microscopy techniques, or electron microscopy, which prevents the user from specifically labeling molecules. Also with STORM, he can understand which cell types express connexin 43 the most, and where they are located within the cells.

Super-resolution microscopy techniques, such as single-molecule localization with STORM, have applications transcending life science fields, including cardiology, cancer biology, neuroscience, cell and developmental biology, and more. This technique enables research that requires imaging specifically labeled structures below the diffraction limit of light. STORM and other super-resolution techniques can be utilized independently or in conjunction with other methods in a correlative manner. Discoveries with this technique and the Vutaraimaging platform can support the advancement of drug development and therapeutic research.

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Further resources

To learn more about super-resolution microscopy and Bruker's Vutara super-resolution microscope, visit: https://www.bruker.com/vutara-vxl

To watch Dr. Veeraraghavan's webinar with Bruker titled, Linking Cardiac Nanostructure and Molecular Organization to Cardiac Function, visit: https://www.bruker.com/ja/news-and-events/webinars/2021/linking-cardiac-nanostructure-and-molecular-organization-to-cardiac-function.html

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