BIOGRAPHICAL SKETCH

NAME: Malvankar, Nikhil

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

POSITION TITLE: Associate Professor

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	END DATE	FIELD OF STUDY
Indian Institute of Technology, Mumbai	MS	05/2003	Physics
University of Massachusetts, Amherst	PhD	08/2010	Biophysics
University of Massachusetts, Amherst	Postdoctoral Fellow	06/2015	Microbiology

A. Personal Statement

My research is focused on developing single-cell imaging and control of cellular metabolism via protein nanowires. Nanowires are hair-like filaments that eliminate excess electrons produced during metabolism by diverse environmentally- and clinically important bacteria and archaea via extracellular electron transfer (EET) to other cells or materials. EET allows cells to grow & survive in harsh environments such as extremely high or low pH, temperatures, pressures, or radiations. Therefore, controlling cell pathophysiology and ecology via EET can mitigate climate change (via lowering methane), pollution (via bioremediation), dysbiosis & diseases.

Despite its importance, the mechanism of EET is unknown despite ~50 years of research. EET via surface-displayed Type-IV pili (T4P) serving as "nanowires" has been hypothesized since 2002, based primarily on genetic studies. My measurements found that surface-displayed filaments confer electrical conductivity to living biofilms rather than monomeric cytochromes (*Nature Nano*'11 & '14, *Science*'10). But the bioenergetic need for nanowires, their exact composition, and the mechanism of electron flow that could explain EET was unclear.

By developing *In situ* structural and functional imaging of cellular metabolism, my lab has discovered how microbes build & use nanowires for EET by addressing the following: **(1)** How do nanowires enable cell growth? **(2)** What are nanowires made of? and **(3)** How do cells move electrons through nanowires from the cytoplasm to extracellular electron acceptors? **(4)** How can we modulate nanowire conductivity to control cell growth?

Our **long-term vision** is to monitor and control cells and enzymes via protein nanowires through the following:

- **Fundamental studies** to elucidate how diverse cells assemble and use various types of nanowires.
- **Repair environmental health** using cellular communities through nanowire-mediated electron exchange.
- **Restore human health** by controlling the growth of cells & neurons by targeting nanowire-mediated EET.
- **Control enzyme catalysis** by electronically imaging & controlling the enzymes bound to protein nanowires.

- **B. Positions, Scientific Appointments, and Honors**

Asst. Professor (7/15-6/22) Molecular Biophysics & Biochemistry, Microbial Sciences Institute, Yale University
Assoc. Professor on Term (7/22-)

2024 Burroughs Wellcome Fund – Climate Change & Human Health Award
2023 Human Frontier Science Program Award
2021-2026 Camille Dreyfus Teacher-Scholar Award
2021-2023 Blavatnik Innovation Award. Highlighted in Yale Magazine (Cover, Inside Cover)
2018-2023 NSF CAREER Award
2017-2022 NIH Director's New Innovator Award
2017-2020 Hartwell Foundation Individual Biomedical Research Award
2017-2019 Charles H. Hood Foundation Child Health Research Award
2016-2017 Helmsley Interdisciplinary Research Fellowships, Cold Spring Harbor Laboratory
2014-2019 Burroughs Wellcome Fund Career Award at Scientific Interfaces
2009 Rao Biophysics Scholarship, UMass, Amherst
2008 First Prize, Innovation Challenge Elevator Pitch and Business Plan Competition, UMass, Amherst
2008 Entrepreneurship Fellowship, University of Massachusetts, Amherst
2004 Graduate Fellowship American Alumni Association, India

C. Contributions to Science Google Scholar Citations >12,500 (>7000 in last 5 years), H-index: 44.

1. **Electrogenetic control of microbial growth by stimulating nanowire expression using E-fields.**

Ability to monitor and control the growth and colonization of microbes deep inside the Earth and in human cells is central to understanding primary microbial function in their native environment with various applications. To achieve this, **we are studying proteins that allow microbial growth and colonization to be imaged and controlled with electrons**. This work is enabled by our discovery that diverse microbes use chains of heme or aromatic moieties to export electrons to partner or host cells. This allows microbes to switch metabolism to respiration and promote growth and colonization without oxygen-like soluble electron acceptors. We found that microbes use intracellular pili as a switch to secrete nanowires and overexpress nanowire genes by sensing natural electric fields, allowing electronic control of gene expression.

Together, electron-conducting proteins and electrogenetics are the **electronic analogs of GFP and optogenetics. We recently reported the first electrogenetic control of biofilm growth by demonstrating that it is possible to stimulate the expression of nanowire genes deep inside biofilms using an electric field** (*Nature Chem._Bio*). This work followed our earlier paper (*Cell*), which showed that bacteria overexpress nanowires when grown in an external electric field. These bacterial nanowires conduct electrons at unprecedented, ultrafast (<200 fs) rates and over large distances of 10,000 times the size of a microbe.

Using multimodal functional imaging at the nanoscale my lab developed, we discovered these nanowires' identity and structure. Remarkably, we showed that despite being made of proteins, nanowires can withstand and function in highly acidic environments where most proteins break down. This discovery provides a **unique opportunity to develop novel sensors and highly resilient materials**. The strength and conductivity of these nanowires and the ability of bacteria to repair themselves will help create durable, self-healing electronics composed of living cells.

As this area of research is in its infancy, many of our advancements are made possible by the new methods we develop. For example, the studies described above (*Nature Chem._Bio*) also established a **new atomic force microscopy-based multimodal imaging platform** to correlate protein structures with electrical and mechanical properties in response to environmental changes, such as pH and redox potential. Using this platform, we found that the electron transfer rate in protein nanowires depends on the environment. Thus, we could make electron transfer 100 times faster by changing environmental conditions, suggesting a possible path to engineering more conductive protein nanowires.

Furthermore, methodological innovation has been a cornerstone of my lab, as the availability of new methods has enabled us to make unexpected discoveries and challenge thinking in the field. For example, our process for **growing biofilms in electric fields** (*Current Protocols*) allowed not only the first electrogenetic control of biofilm growth (*Nature Chem._Bio*) but also enabled us to make the initial discovery that bacterial biofilms conduct electricity (before our work, bacterial biofilms had been thought to be electronically non-conducting).

2. Discovery of nanowires, identification of their structures, functions and assembly mechanisms.

My lab led the effort to solve the first structure of protein nanowires and discovered an unprecedented polymer of cytochrome OmcS (*Cell*). My lab showed that these are the same filaments that had been thought to be pili since 2002. This study also reported the **first example of cryo-EM protein 'sequencing,'** whereby difficult-to-purify proteins can be identified using a combination of cryo-EM and mass spectrometry. Now, many labs use this bottom-up structural proteomics method, which has garnered more than **450 citations in five years**.

This paper has greatly impacted the field. It turned Electromicrobiology from a field focused for ~20 years on Type-IV pili or monomeric cytochromes to one focused on polymeric cytochromes that mediate the transfer of electrons from the bacterial periplasm to extracellular acceptors. This insight has guided subsequent work from our lab and many others to understand the molecular mechanism of microbial respiration via extracellular electron transfer by diverse species in various environments. Furthermore, this shift in focus led to discoveries of other cytochromes polymerizing into conductive filaments, thus establishing a new class of nanowires. The Malvankar lab subsequently found that cytochromes OmcZ, ExtA, and MacA polymerize into conductive filaments under different growth conditions and carry out distinct functions. Three other labs have together found OmcE filaments when mutated *omcS* that couldn't form filaments (Nature Micro'22). Based on the structures of

two archaeal cytochrome filaments that show heme arrangement akin to OmcE, it has been hypothesized that diverse microbial species could be transferring electrons via cytochrome nanowires (Cell'23).

Despite our discovery of high conductivity in *G. sulfurreducens* OmcZ nanowires (Nature Chem Bio'20), the molecular mechanism of conductivity was unclear. This work established the **first method to obtain highly purified nanowires** using OmcZ, as a model system. Here, we achieved the **first synthetic assembly of nanowires *in vitro***, at high yield and purity. These studies explained how bacteria make nanowires on demand using an environmentally controlled, protease-mediated switch. We reported the highest resolution cryo-EM structure of OmcZ. It revealed the presence of highly linear and closely stacked hemes in contrast to OmcS nanowires. We showed that this unique arrangement of the hemes causes **strong excitonic coupling** and could explain why the OmcZ nanowires show the **highest conductivity** known to date in natural biomolecules.

We also showed that OmcZ nanowires allow bacteria to produce the highest electrical power reported to date. We explained the physiological **role** of nanowires in bacterial survival against extreme environments and their **ecological role** in forming microbial communities in biofilms. This work also demonstrated the **ultrastability of nanowires in response to highly acidic and protein-denaturing environments** by showing that bacteria respond to extreme environments by changing nanowire organization.These studies also present **design principles** for rationally engineering the assembly and conductivity of cytochrome nanowires.

We also showed that OmcZ nanowires allow bacteria to produce the highest electrical power reported to date. We explained the physiological **role** of nanowires in bacterial survival against extreme environments and their **ecological role** in forming microbial communities in biofilms. This work also demonstrated the **ultrastability of nanowires in response to highly acidic and protein-denaturing environments** by showing that bacteria respond to extreme environments by changing nanowire organization. These studies also present **design principles** for rationally engineering the assembly and conductivity of cytochrome nanowires.

Furthermore, we found that both OmcS (*Cell Chem Bio*'25) and OmcZ (*Nature Micro.*'23) **nanowire assembly machinery is widespread** in both methane-producing and methane-consuming microbes. Remarkably, methanogens release half of the atmospheric methane, whereas methane-consuming microbes that harbor nanowire genes consume 80% of the methane flux from the ocean sediments. With the Banfield lab (UC Berkeley), we found the OmcZ gene cluster in *Methanoperedens* and their giant (>1 Mb) extrachromosomal elements called Borgs that were hypothesized to increase methane-consuming capacity (Nature Commun,'24). In this work, our lab discovered the *omcZ* gene cluster in these samples and showed its potential to form nanowires. The Welte group confirmed our finding that electricity-producing archaea overexpress OmcZ homologs (Nature Commun,'24). This finding of OmcZ-like nanowires in archaea suggests an intriguing possibility that nanowires play a role in regulating atmospheric methane levels and, thus, global climate. With samples from Welte (Netherlands) and Wagner (Germany), we analyzed filaments made by these microbes. These filaments show structural features akin to cytochrome nanowires (manuscript).

3. Discovery of intracellular pili involved in cytochrome secretion, and engineering pili as nanowires.

The filaments' involvement in microbial electron export was first reported over two decades ago. These structures were referred to as type 4 pili in thousands of papers. My lab combined structural, functional, and localization studies to establish that these pili cannot function as nanowires and instead function as **pseudopili-like pistons to secrete polymerized cytochromes (***Nature***)**. Furthermore, we found that instead of being extracellular as widely believed, they remain intracellular and display **unique heterodimeric pili**.

Our follow-up studies with *pilA* mutants further established this discovery (in revision). These studies showed a direct relationship between *pilA* mutants and the secretion of OmcS and OmcZ nanowires. Furthermore, electron imaging confirmed that cells transfer electrons via cytochrome nanowires, not pili. We also combined genetic studies with cryo-electron tomography in collaboration with Liu's lab (Yale). This further confirmed that *G. sulfurreducens* pili are not surface-displayed like Type-IV and cannot function as nanowires. We discovered that these pili show a novel secretion system comprised partly of Type-II secretion system components.

The discovery of *Geobacter* pili showing low conductivity and functioning akin to secretion pseudopili to translocate cytochrome nanowires has received pushback from Derek Lovley (UMass, Amherst), who proposed the pili hypothesis and Malvankar lab's former postdoc, Matthew Guberman-Pfeffer who is now working with Lovley lab. (Nature Reviews'24). Their critiques have been addressed in prior reviews (Current Opinion '20) and

commentary articles under revision in Nature Commun. (Manuscript-1 and Manuscript-2). Several other labs have also supported our work through multiple articles (e.g. *Trends Microbiol.* 2023, 31, P384, P548 & P550)

Over the past seven decades, many publications on protein conductivity have been made, and contact resistances dominate the measurements in all these studies. Therefore, measuring the intrinsic conductivity of proteins by isolating the contribution of contact resistance has long been a major challenge in the field.

Our novel method enabled the **first measurement of intrinsic electron conductivity**, revealing high conductivity at physiologically relevant potentials without metal cofactors. Using these newly discovered conductivity principles, we introduced natural and non-natural aromatic residues in *E. coli* pili to confer high electron conductivity (*Nature_Comm.*). These studies thus provide design principles in engineering electron conductivity in bacterial filaments and developing novel chassis to secrete nanowires on bacterial surfaces.

Although *G. sulfurreducens* pili are intracellular, the Malvankar lab has found that diverse pathogens use surface-displayed Type 4 pili for extracellular electron transfer for switching metabolism to fast-growing respiration and host-cell colonization despite their low conductivity (in revision). This is owing to highly conserved aromatic amino acids that serve as a novel electron escape route to avoid oxidative damage (*PNAS*'20). With the Eisenberg lab (UCLA)'s short peptides and structures and the Batista lab (Yale)'s computations, the Malvankar lab showed micrometer-long electron transfer through tyrosines due to a proton-rocking mechanism, whereby the energetics and proximity of the proton acceptor control electron transfer. We also introduced natural and non-natural aromatic residues in *E. coli* Type I pili with click-chemistry functionality to enhance conductivity 100-fold. (*Nature Commun.*'22). The Malvankar lab members purified, imaged, modeled pili, and measured their conductivity. The Isaacs and Batista labs (Yale) guided genetics and modeling studies, respectively.

4. Mechanism of electron transfer in nanowires - Light and cooling accelerates electron export.

We combined experiments with computations to establish that quantum effects confer extremely high conductivity to nanowires and living biofilms at ultrafast (~200 fs) rates and over micrometer distances not previously reported for natural biomolecules. This quantum control could be important because it may explain light- and temperature-activated (Science_Adv.) control of bacterial growth and biofilm formation. To our knowledge, this is the first time such ultrafast electron transfer has been observed in non-photosynthetic proteins.

This work elucidated the structural features and conditions that could increase conductivity. Transient absorption (TA) spectroscopy revealed ultrafast (~200 fs) electron transfer between nanowire hemes upon photoexcitation, enhancing electron density and mobility. Photoconductive atomic force microscopy showed a 100-fold increase in photocurrents in nanowires. Photocurrents respond rapidly (<100 ms) to the excitation and persist reversibly for hours. Furthermore, nanowires and biofilms showed that cooling accelerates electron transport 300-fold (*Science_Adv*.) These behaviors contrast the commonly known hopping model for electron transfer.

Efforts led by the Malvankar lab's postdoc Guberman-Pfeffer to model the heme redox potential and conductivity of nanowires computationally yielded up to a million-fold lower conductivity than experiments (*JPCB'21*, *JPCB'22*). This discrepancy is not due to variations in conductivity measurements because multiple researchers in different labs have measured nanowire conductivity in various buffers and hydrations and found similar values. Measurements of fully-hydrated (*Nature*'21 and *Cell*'19) or air-dried OmcS nanowires (*Nature Chem. Bio*'20, *Science_Adv*'22) showed similar structural features and conductivity to that measured in the Lovley lab (RSC Adv., 2016), which we showed to be OmcS (Cell'19). Similarly, the Malvankar lab's conductivity measurements of air-dried OmcZ nanowires (*Nature Chem. Bio*'20) showed similar structural features and conductivity to that measured previously in the Lovley lab (Small, 2016), which we showed to be OmcZ.

This discrepancy between measurement and computations could be due to nanowires' conformation changing upon binding to electron acceptors. Our simulations showed a large structural change in OmcS nanowires during binding to the gold electrode, which altered the heme redox potentials and enhanced conductivity 100-fold.

High nanowire conductivity also raises the possibility of a fundamentally different mechanism. Our and other groups' computations suggest the role of quantum effects in OmcS/Z nanowires (JPCL'25, Nanotech.'20 and ACS Nano'23). We are measuring these effects directly in individual OmcS nanowires (manuscript). In this work, we found that nanowires show 30,000-fold higher magnetoconductance with (>99%) spin polarization at physiological B-fields (<0.5 Tesla) and potentials (<0.1 V), with the metal-like temperature dependence of conductivity. Surprisingly, we found in this work that substituting iron heme with zinc did not change nanowire

conductivity. Therefore, the classical iron-to-iron hopping model does not apply to these nanowires. Quantum computations by the Anantram group (UW Seattle) could partly explain the high nanowire magnetoconductance spin polarization. The substitution of Fe with Zn in nanowires could enable efficient exciton transport over micrometer distances, which is critical for efficient solar energy conversion and energy storage technologies.

Protein nanowires could provide non-photosynthetic protein analogs for flexible testbeds for studies of quantum processes in which the monomer structure and nanowire assembly can be controlled at the atomic and mesoscales. In contrast to most synthetic quantum materials that require very low temperatures to function, these proteins could have evolved to optimize ultrafast electron transfer in aqueous environments at physiological temperatures. Understanding how to optimize systems electron transfer may have implications for designing protein nanowire-based quantum sensors and developing new quantum computing applications.

Our efforts to computationally model the heme redox potential and conductivity of nanowires yielded up to a billion-fold lower conductivity than experiments, illustrating that the existing computational models based on electron hopping assumption fail to capture electron transfer in biological nanowires. This raises the possibility that biological nanowires employ a fundamentally different, currently unknown mechanism. Thus, existing models predict the same conductivity for all nanowires with computed timescales for heme-to-heme electron transfers (100 ns), million-fold lower than that measured using transient absorption spectroscopy (*Nature_Commun.*) and conductivity for fully hydrated (*Nature* and *Cell*) or air-dried nanowires (*Science_Adv.*).

We are now directly measuring the role of electron delocalization and spin polarization in nanowire conductivity. Our work will advance the basic biological and physical understanding of nanowires and have a major impact, eventually enabling us to predict and control the microbial electron export to transport and store energy.

5. Quantitative imaging of electron export correlated with protein structure and mechanical properties

Beyond conceptual innovations of protein nanowires and novel mechanism of electron transfer, my lab has also contributed to technological innovations in bottom-up proteomics using cryo-EM and mass-spectrometry (*Cell*), and in imaging of electron export in individual nanowires (*Nature_Nanotechnology*).

Recently, we focused on developing nanoscopic imaging techniques to address major bottlenecks for the field by quantitatively visualizing the electron export via protein nanowires by living diverse environmentally and clinically important bacteria at single-cell-level. For example, a major challenge in studying how the metabolic switch from slow-growing fermentation to fast-growing respiration is the lack of methods to follow individual bacteria in their environmental context. We have found that niche dictates metabolic switch by tracking bacterial electron export and niche behaviors at a single-cell resolution over extended periods in real-time. Further findings indicate that electron transfer via nanowires modulates microbe-to-microbe direct interspecies electron transfer that promotes methane production, a critical step in global warming. We have reduced bacterial growth and adhesion by diverting metabolic electrons using small molecule electron acceptors. Our expertise in the bacterial metabolic niche, therefore, uniquely positions us to address causes of infection as well as rising temperatures due to elevated methane levels.

Our nanoscopic imaging methods are **widely applicable to diverse biological systems**. For example, my lab has applied these imaging methods for nanoscopic visualization of water binding to minerals (*Sciences_Adv.*). We revealed that, instead of growing layer by layer as previously thought, water growth starts near edges before surface tension takes over to engulf the surface and forms droplets. My lab's methodology is allowing to visualize directly how bacteria export electrons via nanowires to minerals and electrodes (*in revision*).

Many labs are contacting us to apply these methods to systems diverse as pathogenic biofilms, brain, and diabetic cells. For example, with Yaldiz lab (UCSZ), we have applied these nanoscale imaging methods to help discover how pathogenic bacteria form biofilms using vesicle packaging proteins (*mBio*). With Kahle lab (Harvard), we also found how fluid-filled brain cavities (hydrocephalus) arise due to reduced stiffness of brain tissue (*Nature_Neuro.*). Moreover, with Kyriakides lab (Yale Biomedical Engineering), we showed how diabetes increases the stiffness of fibroblasts, which is critical for wound healing (*Sci. Reports*). The Malvankar lab members performed all the nanoscale imaging biomechanical measurements and data analysis in these studies. Our lab is also collaborating with several other labs at Yale and beyond for similar electrical and mechanical measurements on diverse systems ranging from zebrafish to neuronal cells. This paper established multimodal imaging of biosystems for the simultaneous analysis of structural, electrical, mechanical and optical properties. For example, we later found that OmcS nanowire photoconductivity increases 100-fold (Nature Commun.'22).