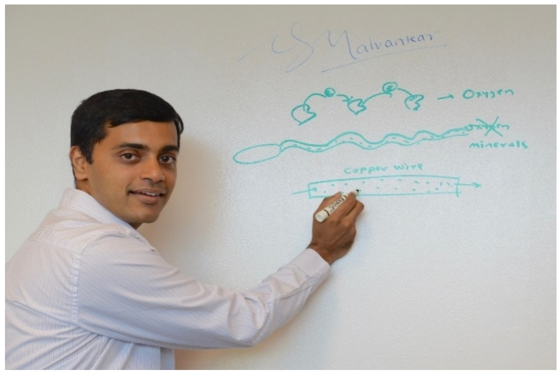
NATURAL AND SYNTHETIC MICROBIAL PROTEIN NANOWIRES FOR BIOELECTRONIC INTERFACES

Nikhil S. Malvankar, Ph.D.

Associate Professor of Molecular Biophysics and Biochemistry, Microbial Sciences Institute
Yale University



Abstract: Deep in the ocean or underground, where there is no oxygen, *Geobacter* “breathe” by projecting tiny protein filaments called "nanowires" into the soil, to dispose of excess electrons resulting from the conversion of nutrients to energy. These nanowires enable the bacteria to perform environmentally important functions such as cleaning up radioactive sites, generating electricity (*Nature Nano*. 2011, or sharing electrons with other bacteria (*Science* 2010). Although it is long known that *Geobacter* make nanowires, it was not clear what they are actually made of and why they are conductive. I will present recent discoveries that resolve two decades of confounding observations in thousands of publications that thought these nanowires as pili filaments (*Current Opinion in Chemical Biology* 2020). Our studies have revealed a surprise: the protein nanowires have a core of metal-containing molecules called hemes Using high-resolution cryo-electron microscopy, we found that hemes line up to create a continuous path along which electrons travel. Using multimodal functional imaging (*Physical Biology* 2020) and a suite of electrical, biochemical and physiological studies, we find that rather than pili, nanowires are composed of cytochromes OmcS and OmcZ that transport electrons via seamless stacking of hemes over micrometers (*Cell* 2019, *Nature Chem.Bio.* 2020). I will discuss the physiological need for two different nanowires and their potential applications for sensing, synthesis, and energy production. I will also present our recent experimental and computational studies to identify the mechanism of electron transfer that occurs at unprecedented ultrafast (~200 fs) rates and over centimeter distances, 10,000-times the size of a bacterium, through measurements of DC conductivity as a function of nanowire length, temperature (*Science Adv.* 2022) and photoexcitation *(Nature Comm.* 2022). We have further found that the nanowires are translocated to the bacterial surface via a novel secretion pathway using unique heterodimeric pili remain hidden inside the cell and serve as a piston to secrete cytochromes rather than functioning as a nanowire as previously thought (*Nature* 2021).
These studies solve a longstanding mystery of how nanowires move electrons to minerals in the soil or help generate electricity. Our work has thus resolved decades of confounding work to explain our previous findings that these bacteria transport electrons via nanowires (*Nature Nano.* 2014) over 100-times their size to electron acceptors (*Nature Nano*. 2011) and partner cells (*Science* 2010) and store electrons when acceptors are absent akin to how humans use their lungs (*ChemPhysChem*

2012) . I will also present a new technique for contact-free measurements of intrinsic electron conductivity in individual protein nanowires with atomic-resolution structures to determine true voltage and temperature dependence of conductivity that reveals how energetics and proximity of proton acceptors modulate protein conductivity by 100-fold (*PNAS* 2021, *Biochem. Journal* 2021). Our methodology will help to set standards for reporting protein conductivity for accurate comparison of different protein systems. Using these advances, we have developed synthetic protein nanowires with tunable conductivity and programmable self-assembly using non-natural click chemistry functionality (*Nature Comm.* 2022). Our studies are helping to understand, predict and ultimately control extracellular electron transfer by protein nanowires used by diverse environmentally-important microbes to capture, convert and store energy

Bio: Nikhil has received the Blavatnik Award for Innovation and Camille Dreyfus Teacher-Scholar Award in 2021, NSF CAREER Award in 2018, NIH Director's New Innovator Award and Hartwell Foundation Individual Biomedical Research Award in 2017, Charles H. Hood Foundation Child Health Research Award in 2016 and the Burroughs Wellcome Fund Career Award at the Scientific Interface in 2014.