Project Description (Yong Wang, Department of Physics, University of Arkansas)

Project 1. Study the motion of bacteria in fluids and porous media using fluorescence microscopy.

Significance: Many types of bacteria inhabit naturally in aqueous solutions, complex fluid, or fluid-filled porous media [1]. Migration and mobility of bacteria in these media play essential role for bacterial health, growth and survival [2–4]. Therefore, it is of great interest to investigate the motion and growth of bacteria in various environments [5]. Although extensive studies have been performed on the motion of bacteria in fluids, much less is known about how bacteria move in porous media [6]. This project will investigate the motion of bacteria, with *E. coli* as a model system, and address this knowledge gap.

Approach: In this project, REU students will use fluorescence microscopy to track the motion of individual *E. coli* bacteria in both aqueous solutions and porous media. The *E. coli* strain for this project will be K12 transformed with a plasmid expression enhanced green fluorescent proteins (EGFP) [7]. The aqueous solutions that REU students will use include LB broth medium, defined minimal M9 medium, and M9 medium supplemented with various carbon sources such as glucose, glycerol, and lactose. Porous media will be made of agar, agarose, or hydrogel. REU students will observe and record movies for the Levy-walk-like motions of bacteria. From the recorded movies, students will extract the trajectories of bacteria, quantify their displacements, and infer the diffusive behaviors of bacteria. The students will also perform statistical analysis among large number of bacteria, and compare the differences between bacteria in fluids and in porous media.

Learning Outcome: REU students in this project will have hands-on experience on basic microbiology and fluorescence microscopy. They will learn to make buffers and media, grow bacteria, and operate standard fluorescence microscopy. In addition, the students will gain computational skills, including learning to use MATLAB/Python and write MATLAB/Python programs for data analysis. Furthermore, the students will gain and/or reinforce their knowledge and skills on statistical analysis. Overall, this project will provide the REU students hands-on training on both experimental and computational/analytical skills.

Project 2. Investigate bacterial response to low electric voltage/current at the molecular level using super-resolution fluorescence microscopy

Significance: As antibiotic resistance of bacteria has become one of the biggest threats to public health [8], alternatives to antibiotics have been attracting broad interest and attention [9, 10]. It has been known for a long time that high electric voltages damage bacteria; more recently, low electric voltages/currents (< 5V) have been found to significantly suppress the growth of bacteria, possibly providing a new safe way to fight against antibiotic resistant bacteria [11, 12]. While most studies have reported the antibiotic phenomena of low electric voltage/current, much less progresses have been made towards understanding the mechanism at the molecular level, partly due to the lack of right tools. Lack of such basic knowledge hinders our understanding on the fundamental mechanism of antibiotic activities of low electric voltage/current and will make the promise of further applications to be stagnant.

Approach: The long-term goal of this project is to understand the molecular mechanism of low electric voltage/current's antibiotic effects against bacteria. In this project, REU students will use super-resolution fluorescence microscopy [13–16] to investigate the spatial reorganization of DNA and proteins in *E. coli* bacteria after subjecting the bacteria to low electric voltages (0 – 5 V). The candidate proteins include the histone-like nucleoid structuring protein (H-NS) [17] and mechanosensitive channels of large conductance (MscL) [18, 19]. Both proteins will be fused to fluorescent protein mEos3.2 [20] to allow super-resolution fluorescence imaging. The DNA will be labeled by DAPI, which is also suitable for super-resolution fluorescence microscopy [21]. From the microscopic data, the students

will reconstruct the super-resolved images of the DNA and proteins with a spatial resolution of ~20 nm. In addition, the REU students will perform quantitative analysis to characterize the spatial reorganization of the DNA and proteins in bacteria due to the application of low electric voltage/current. Furthermore, the students will measure the dependence of the spatial reorganization on the magnitude of voltage/current.

Learning Outcome: REU students in this project will have a chance to hands-on learn super-resolution fluorescence microscopy, an advanced optical technique that won the Nobel Prize in 2014. In addition to learn to make buffers and media, grow bacteria, and operate a super-resolution fluorescence microscope, the students will gain skills on processing and analyzing super-resolution data. Furthermore, the students will learn MATLAB/Python and write MATLAB/Python programs for data analysis. Overall, this project will provide the REU students hands-on training on both experimental and computational/analytical skills.

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