Graduate Research Plan Statement

Introduction: Electrophysiology is a branch of biomedical sciences which characterizes the role of electricity in biology and develops methods to **influence biological systems through various electric field (EF) sensitivities. Electroporation is a ubiquitous electrophysiological method** utilized throughout biomedical science for various applications - **primarily in drug/gene therapy delivery and tissue/tumor ablation** [1]. Generally, this is a technique where **EFs induce pore formation in cell membranes**, leading to various effects depending on the field strength. **Reversible electroporation (RE) occurs when the EF strength is below a threshold of approximately 1 kV/cm where the induced pores can heal** [2]. **RE is typically used to introduce molecules into target cells**, such as in electrochemotherapy, **by both increasing cell membrane permeability and inducing electrostatic transport, thereby significantly increasing uptake efficiency** [1-2]. While the permeability effects have been relatively well studied, **the effects of intense EFs on molecules transferred into the target cell are not fully understood.** This is due to the difficulty in experimentally measuring the properties of complex biomolecules (BMs) while applying strong enough EFs to be relevant to biomedical applications. Nonetheless, computational studies suggest EFs could affect BM structural dynamics, subsequently modifying their biological properties [3].

To begin exploring the effects of EFs on BMs, I will first outline standard characterization methods and then build upon them to include EFs. **The structural dynamics of a BM can be largely characterized by its free energy landscape** [4]. This is given as the Gibbs free energy of the BM as a function of a characteristic reaction coordinate, $G(x)$:

$$G(x) = U(x) + PV(x) - TS(x) = H(x) - TS(x) \quad (1)$$

where $U(x), V(x), H(x), S(x)$ are the internal energy, free volume, enthalpy, and entropy of the BM and P, T are the ambient pressure and temperature, respectively. Studies of structural dynamics typically focus on variations in P, T due to their biological relevance and relative ease of experimental implementation [4]. However, EFs primarily couple into $G(x)$ by modifying $U(x)$ and $S(x)$ via electrostatic interactions, making their effects difficult to predict [3].

Single-molecule Fluorescence Resonance Energy Transfer (smFRET) is a method of measuring structural dynamics under external perturbations. As depicted in Fig. 1, this is a biophysical technique where an excited donor molecule transfers an amount of its energy to a nearby acceptor molecule depending on their relative proximity, allowing one to infer BM structure based on the relative emission intensity of the donor-acceptor pair [4]. Single BMs are studied by attaching these donor-acceptor pairs to different locations on the BM and restricting their motion with a biomolecular tether, allowing for single-molecule laser excitation and emission measurement [4]. **The Nesbitt Group at the University of Colorado Boulder and JILA are at the forefront of single-molecule biophysics and the development of smFRET technology.** I have been **regularly meeting with Dr. David Nesbitt over the past few months shaping this research plan.** Moreover, JILA has state-of-the-art experimental equipment, manufacturing capabilities, and world-class researchers which will be vital resources to the success of this project. For instance, one can **deposit micron-scale electrodes onto a microscope slide using standard lithography techniques available at JILA, enabling smFRET experiments with EFs** [5]. Using an **interdigitated electrode geometry** as shown in Fig. 2, with $d = 10\ \mu m$ and $w = 1\ \mu m$, **a potential difference of 10 V reaches the typical upper bound for RE of 1 kV/cm** at the center of the electrode gaps**.**

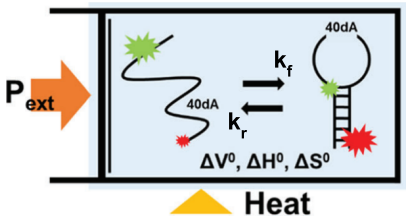

Fig. 1: smFRET experiment diagram using a hairpin construct. Adapted from [4]

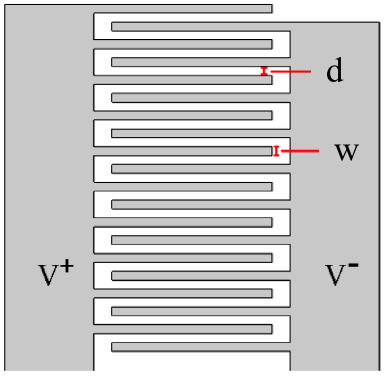

Fig. 2: Interdigitated electrode geometry. Adapted from [5]

Intellectual Merit: As described in my personal statement, **I have considerable experience in experimental physics, mathematical modeling, and computational simulation from many years of collaborative work in academic and industrial R&D.** While the majority of this experience has been in solid-state physics and quantum systems, I believe much of my expertise is directly transferable to biophysics. In the following, **I develop an analytic model of the DNA hairpin shown in Fig. 1 to determine the feasibility of measuring EF effects with smFRET**. For the sake of brevity, I leave out many of the finer details in this analysis. **Please visit my GitHub page for more information** [6].

The basic outline of the hairpin model is depicted in Fig. 3a. It consists of an ideal chain representing the long linker with charged hydrogen bonding ends representing the short complementary strands. The ideal chain is a polymer model which consists of freely-jointed links following a random walk from one end to the other. As the ends of the ideal chain are moved apart, the entropy decreases, leading to an increase in free energy [7]. A Lennard-Jones potential is used to represent the pair potential of the ends. Implementing the above properties, $G(x)$ for the harpin model is calculated using Eq. 1 and plotted in Fig. 3(b). **In aggregate, these properties exhibit a canonical double-well potential, representing the folded (F) and unfolded (UF) states** [6].

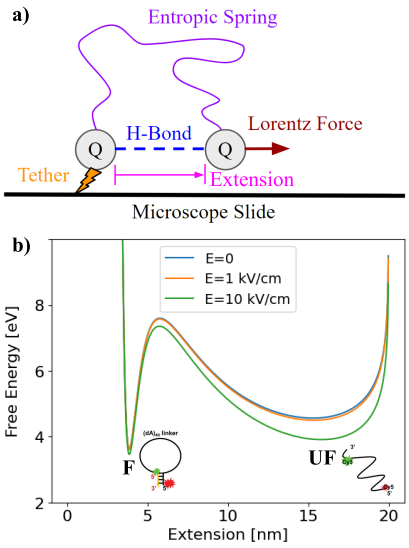

Fig. 3: Simple DNA hairpin model diagram (a) and free energy w/wo EFs [6] (b)

In practice, the EF emitted by the electrodes is complicated since they are immersed in a mildly cationic solution, as required by the BM [4]. Nevertheless, **one can apply a >1 kHz oscillating voltage to the electrodes to mitigate both electrolyte shielding and electrolysis** [6,8]. It can also be assumed the EF is spatially uniform within the expected nanoscale BM extensions. As depicted in Fig. 3b, **a uniform EF superimposes a linear function onto the initial free energy of the BM, leading to a reduction in UF energy** [6].

Finally, the effect of EFs on the structural dynamics of the hairpin model can be determined from transition state theory. Assuming the local extrema of $G(x)$ are stationary in x, the relative change in equilibrium constant as a function of EF magnitude, $K(E) = [UF](E)/[F](E)$, is given by:

$$\Delta K(E) = K(E)/K(E = 0) \approx kT/h \exp[QE\Delta x/kT] \quad (2)$$

where $[F](E)$, $[UF](E)$ are the EF dependent F and UF concentrations, $Q, \Delta x$ are the charges on the chain ends and the extension difference between F and UF, and k, h are Boltzmann and Planck's constants, respectively. Using Eq. 2, one can calculate $\Delta K(E) =$ **6.4 with an EF of 1 kV/cm**, implying an **increase in unfolded hairpin concentration** well within the sensitivity of standard smFRET methods [4,6]. While this **result is for a simple model, it suggests a measurable effect and motivates further investigation.**

Broader Impacts: Experimentally measuring the effects of EFs on individual BMs is a next step towards thoroughly understanding the effect of electromagnetic fields on biological systems. It will allow researchers to experimentally test computational models, validate current treatment methods, and inform future applications. I believe the study of primary importance is to **ensure the functionality and safety of current electrophysiological treatment methods**. **BMs can be ineffective or even harmful if structurally deformed** [9]. **Moreover, the above analysis suggests EFs may induce unfolding in some cases** [6]. With the increasing complexity of drugs and gene therapy BMs, the EFs utilized for delivery may lead to unexpected structural deformations. **Therefore, this work could more broadly define best practices in electrophysiological treatment design to minimize EF susceptibilities.**

References: [1] Tieleman *BMC Biochem* 5, 10 (2004). [2] Tellado, et al. *Front. Vet. Sci*. 9, 868989 (2022) [3] Wu, et al. *Journal of Phys. Chem. B*, 126, 2 [4] Sung, et al. *Journal of Phys.l Chem. B*, 124, 1 (2019) [5] Santos-Neto, et al. *Sensors* 21, 7288 (2021) [6] Bimstefer, "BioProposal_NSFGRFP." *GitHub,* https://github.com/gbimGit/BioProposal_NSFGRFP [7] Rubinstein, et al. *Polymer physics*. Oxford Univ. Press (2003) [8] Cohen, Ph.D. dissertation, Stanford (2006) [9] Herczenik, et al. *FASEB J.* 22, 7 (2008)