Metrology and Elastometry of Nanoscale Objects

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Rapid, sensitive sterility testing of cell and gene therapies is a challenge in the timely release of safe medicinal therapies to critically ill patients. In particular, the state-of-the-art technologies for adventitious virus detection take 2-4 weeks and are destructive. The absence of a label-free, high-throughput assessment method for microbial safety has impeded the manufacturing upscaling and widespread adoption of cell therapy products.

Nanopore sensors have the potential to be the ultimate, multimodal detectors of charged biological species by electrophoretically driving them through a nano-scale pore submerged in an electrolytic solution. The physical detection principle of this technology is like that of Coulter counter used to detect charged, micron-sized particles. While nanopores have proven to be a versatile single-molecule tool for nucleic acid sequencing applications, their use for screening molecules larger than a few nanometers has been limited, presenting several opportunities for innovation.

The overarching goal of this thesis is the design and development of nanopores as a platform technology to detect and fingerprint biomolecules. We develop a toolkit including analytical models, multi-scale simulations, and machine learning methods to push the boundaries of nanopore physics and the force-deformation behavior of soft biomolecules. A combination of theory, simulations, and experiments informs the rational design of the nanopore system and DNA nanostructures.

First, we attempt to understand how different biomolecules such as viruses and double-stranded DNA chains interact with and influence the output signatures of solid-state nanopores. Using a combination of continuum modeling approaches and experiments, we design a sensitive tool for viral detection and develop an accurate analytical model to predict the amplitude of the nanopore signal, also known as the conductance blockade. We probe the sensitivity of various physicochemical properties on virus 'metrology', *i.e.,* virus size prediction, and inform the optimization of the experiments to obtain robust virus size measurements. The analytical conductance blockade model surpasses the state-of-the-art Kowalczyk model for a broad parameter range and its versatility enables accurate analyte size inference.

Next, DNA nanostructures or nucleic acid nanoparticles (NANPs) are proposed as surrogates for viruses to better understand the mass transport across a nanopore membrane. Given the tunability and precise control over their size and shape as well as biosafety, nanopores are then used for fingerprinting and discrimination of NANPs using machine learning methods. A novel ML framework which includes the use of continuous wavelet transform to process the current-time nanopore data, a convolutional neural network for classification, and explainability of the decision-making process can discriminate between two NANPs with similar sizes but different shapes with over 95% accuracy. The approach is a new way to treat complex physical phenomena supported with a proof-of-concept.

Furthermore, as NANPs are being proposed as vehicles for drug delivery and vaccines, their mechanical deformation needs to be better understood. While microbial detection is critical for quality control and sterility testing of medicinal therapies, the mechanical integrity of synthetic NANPs is important for their proposed applications. As a result, nanopores are used as a tool for 'elastometry' of squishy biomolecules. In particular, different mechanisms associated with the deformation of these particles and their influence on the nanopore pulse signals are understood. Two distinctive regimes where the particles undergo creep deformation and then collapse at higher driving forces are discovered.

Finally, polymer physics principles are used to probe the structural transitions of linear DNA, the basic building block of NANPs, with coarse-grained molecular dynamics simulations. Overstretching transition and associated hysteresis due to the disruption of nucleotide base pairing are reported at higher stretching forces which can potentially lead to the failure of these materials. The insights into the non-linear elastic response can be used to better engineer these motifs.

This thesis and the associated toolkits will enable the development of more sensitive, rapid nanopore-based analytical tools and guide the design of DNA-based nanostructures.

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