and Applied Genetics **1998**, *97* (4), 617-625.

16. Wang, L.; Mu, M.; Li, X.; Lin, P.; Wang, W., Differentiation between true mangroves and mangrove associates based on leaf traits and salt contents. *Journal of Plant Ecology* **2010**, *4* (4), 292-301.
17. Ratnaweera, P.; de Silva, E. D.; Wijesundera, R. L.; Andersen, R. J., Antimicrobial constituents of Hypocrea virens, an endophyte of the mangrove-associate plant Premna serratifolia L. *Journal of the National Science Foundation of Sri Lanka* **2016**, *44* (1).
18. Dissanayake, R. K.; Ratnaweera, P. B.; Williams, D. E.; Wijayarathne, C. D.; Wijesundera, R.; Andersen, R.; de Silva, E. D., Antimicrobial activities of mycoleptodiscin B isolated from endophytic fungus Mycoleptodiscus sp. of Calamus thwaitesii Becc. *J Appl Pharm Sci* **2016**, *6*, 1-6.
19. Ortega, H. E.; Graupner, P. R.; Asai, Y.; TenDyke, K.; Qiu, D.; Shen, Y. Y.; Rios, N.; Arnold, A. E.; Coley, P. D.; Kursar, T. A., Mycoleptodiscins A and B, cytotoxic alkaloids from the endophytic fungus Mycoleptodiscus sp. F0194. *Journal of natural products* **2013**, **76** (4), 741-744.
20. Dissanayake, R. K.; Ratnaweera, P. B.; Williams, D. E.; Wijayarathne, C. D.; Wijesundera, R. L.; Andersen, R. J.; de Silva, E. D., Antimicrobial activities of endophytic fungi of the Sri Lankan aquatic plant Nymphaea nouchali and chaetoglobosin A and C, produced by the endophytic fungus Chaetomium globosum. *Mycology* **2016**, *7* (1), 1-8.

Guest Articles

Nanopore: An Ostensibly Simple Sensor Stamping Single Molecule-Level Ohmic Readouts

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Single-molecule/particle level analysis has surpassed the era of ensemble average studies and now faces grander challenges unique to each molecular class, for example, genomic sequencing with minimal financial foot-print been the most notable. The eventual goal of most of these efforts is to create a hand-held device that can read data on-site, upload it to a cloud, perform analysis and provide feedback in minutes while having an accuracy comparable or surpassing that of conventional instrumentation. Nanopore sensors have emerged at a time of demand with the promise to tackle a wide spectrum of biomolecules to cater fields such as biomedicine, mechanical engineering, pharmaceutical chemistry, physics, *etc*. A nanopore in its simplest definition is a nanoscale aperture spanning an impervious natural or solid-state membrane which separates two electrolyte reservoirs. The analyte is added to one chamber (cis) and driven across the nanopore in response to a voltage bias applied to the other chamber (trans). The transiting analyte perturbs the ionic-current of the open-pore generally causing a drop in the current (exceptions exist)—more formally termed an event—which bares molecular information characterized by the duration (Δt), depth (ΔI) and inter-event duration (Δf). Charged molecules travel by electrophoresis (EP) and depending on the pH, the nanopore surface might have a net charge, generating an electroosmotic force (EO) that opposes or reinforces the electrophoretic force. Uncharged molecules may travel solely by electroosmosis. Manipulation and optimization of these forces (EP and EO) enable successful sensing.

The first demonstration of a nanopore to profile DNA was merely two decades ago using α-Hemolysin—a natural nanopore excreated by a bacteria—and since then, it has evolved into characterizing a plethora of biological and non-biological analytes—proteins, glycans, viruses, liposomes, exosomes, polymers. The focal point of nanopores since its inception has been on DNA sequencing. Natural pores were chosen because of their immaculate size, reproducibility and comparative dimensions of the sensing zone with nucleotide spacing being amongst other beneficial factors. Nanopore sensing is nondestructive, label-free and usually operate at a nM to pM concentration range at a bias of $\leq$ 1V, requiring only few microliters of the sample. While the technology associated with biological nanopores has expanded

into some commercial setups, sequencing the human genome still remains a challenge. With time, inevitably, many realized that confining nanopores to merely DNA sequencing limits its potential. Even though the natural nanopores are size reproducible, and the size of the sensing constriction is well suited for DNA sequencing, the inability to tune the size was a major drawback to profile other natural analytes. For example, an AAV virus is about ~25 nm in diameter, HIV is about ~100 nm in diameter, proteins are few nanometers in diameter whereas α-hemolysin has an aperture that is too small even for double-stranded DNA to translocate—thus limiting itself to a single-stranded DNA. The approach to profile proteins and branched molecules such as glycans were to denature and digest them respectively—the natural nanopore is unable to profile the pristine state of these molecules. So what is the solution?

To overcome the limitations of natural nanopores while preserving the best of it, solid-state nanopores (**SSN**) came to the picture in the early 2000s. These are nanopores fabricated through impervious solid membranes such as silicon nitride (**SiN_x**), glass and polymers like polycarbonate. The more abundantly used material is SiN_x due to its robust, microfabrication compatible nature. The pores were initially milled using microscopy methods such as TEM (transmission electron microscope), FIB (focused ion beam microscope), SEM (scanning electron microscope) and HIM (helium ion microscope). The choice of microscope depends on the desired size of the nanopore: for smaller nanopores (<10 nm), TEM and HIM are preferred and for larger pore diameters, FIB is preferred. While TEM can be used to make pores with a larger diameter, it is time-consuming. Even though the nanopore is a simple sensor, the fabrication of it seemed complicated and requiring state-of-the-art instruments. This hampered the development of SSN greatly.

A little over 5 years ago, an overhead, time and cost-efficient method came into the picture called the controlled dielectric breakdown (CDB) to fabricate SSN. In simple, a high voltage (<1 V/nm) is applied across the membrane (both cis and trans contain 1 M KCl typically) until an abrupt increase in current is observed which is indicative of pore formation. Then smaller voltage pulses are applied until the pore size of interest is reached. A pore can be fabricated under 10 minutes and the circuitry needed for the CDB setup is cheap (<50$)—there are other costs associated with nanopore science that we have discussed at the end of this article. We note that there was enormous resistance in the nanopore field when CDB was introduced—such an invention should typically end up in a top-tier journal such as Nature, yet, the authors who demonstrated it first had to settle for a medium impact journal. However, as heavy users of CDB, we are delighted (even though we are not the inventors) to see that the use of CDB in the nanopore community is continuosly increasing. We introduced a method similar to CDB in 2018 where a Tesla coil lighter was used to fabricate nanopores—the slogan been don't smoke, make nanopores!

While nanopore fabrication protocols became simpler and faster, it attracted more attention for single-molecule sensing beyond genomics. The SSN was soon explored for other molecules and particles. Rigid nanoparticles could be discriminated by size and demonstrated significant size resolution as compared to classical methods like Dynamic Light Scattering (DLS) and TEM. Soon SSN was used for single-molecular analysis of proteins (without resorting to denaturing agents) which enabled the evaluation of a myriad of molecular information that natural nanopores could not harness, including, but not limited to, the folding-unfolding (e.g. voltage and temperature-induced), protein-receptor interactions and protein response to change of media (pH, salt, cations, etc.). In recent years SSN has been used for mechanical characterization of bio-nano particles like liposomes and viruses; i.e: rigidity, viscoelasticity, stiffness, etc. The big question is, why is it important? For starters, liposomes are widely used drug carriers and it has been shown that they can be eletro-deformed as they transit through a nanopore if a sufficiently high voltage is applied. The degree of deformation is speculated to be a function of the cargo content, thus in the future, we would see SSN discerning liposomes based on their packaging and playing a leading role in dose-related assaying. The membrane properties of the HIV virus change as it matures which can be used as a marker for its infectivity. This was successfully demonstrated by the group of Prof. Kim. The SSN continues to improve over the years with different protocols having been introduced to improve and custom tune the surface chemistry of the SSN. This

was done to minimize clogging, enhance current stability, and to improve the signal-to-noise. Protocols such as PDMS coating, silane thin films, gold thin films with thiol chemistry alongside the direct surface functionalization of organic moiteies by using hydrosilylation is also in use.

Recently, nanopores have been further challenged by a rather complicated class of biomolecules—polysaccharides—that have higher diversity in monomer composition, charge, and isomeric forms as compared to DNA or proteins.2 The sensing platform demonstrated astounding promise in profiling polysaccharides by discriminating heparin (a commonly used anticoagulant drug) from the adulterant over sulfated chondroitin sulfate (OSCS). In 2008, there were over 100 deaths in the US due to a heparin batch being contaminated by OSCS and gone undetected by classical techniques.

A typical setup to perform nanopore experiments includes a low current amplifier, a digitizer (e.g. Digidata 1440A), asoftware (custom written or purchased) and a computer. The total cost would therefore be ~70,000$. There is only a handful of countries in the whole world that have nanopore expertise. In addition to genomics, both proteomics and glycomics have been tackled by this tiny sensor and currently presents great promise in -omics. Despite the robust nature and ability to be integrated into a handheld device, there are no successful SSN based devices in use just yet. Thus, there is a great opportunity to fill this void. Can Sri-Lanka afford this? The answer is a simple yes. Does Sri-Lanka have the expertise for this? Again the answer is a simple yes. The more pressing question, however, is, would Sri-Lanka be proactive and have a foothold in this evergrowing portion of molecular sensing anytime soon—we hope the answer will soon be a grand yes!

References

1. Kasianowicz, J. J.; Brandin, E.; Branton, D.; Deamer, D. W., Characterization of individual polynucleotide molecules using a membrane channel. P*roceedings of the National Academy of Sciences* **1996**, *93* (24), 13770-13773.
2. Karawdeniya, B. I.; Bandara, Y. N. D.; Nichols, J. W.; Chevalier, R. B.; Dwyer, J. R., Surveying silicon nitride nanopores for glycomics and heparin quality assurance. *Nature Communications* **2018**, *9* (1), 3278.
3. Saharia, J.; Bandara, Y. N. D.; Goyal, G.; Lee, J. S.; Karawdeniya, B. I.; Kim, M. J., Molecular-Level Profiling of Human Serum Transferrin Protein through Assessment of Nanopore-Based Electrical and Chemical Responsiveness. *ACS Nano* **2019**, *13* (4), 4246-4254.
4. McMullen, A.; De Haan, H. W.; Tang, J. X.; Stein, D., Stiff filamentous virus translocations through solid-state nanopores. *Nature Communications* **2014**, *5*, 4171.
5. D.Y. Bandara, Y. M. N.; Tang, J.; Saharia, J.; Rogowski, L. W.; Ahn, C. W.; Kim, M. J., Characterization of Flagellar Filaments and Flagellin through Optical Microscopy and Label-Free Nanopore Responsiveness. Analytical Chemistry **2019**.
6. Darvish, A.; Lee, J. S.; Peng, B.; Saharia, J.; VenkatKalyana Sundaram, R.; Goyal, G.; Bandara, N.; Ahn, C. W.; Kim, J.; Dutta, P., Mechanical characterization of HIV-1 with a solid-state nanopore sensor. *Electrophoresis* **2019**, *40* (5), 776-783.