

Faculty of Science Research Fellow Society Symposium – 8 July 2022

This will primarily be an in person event with the option of zoom:

<https://auckland.zoom.us/j/92173269797>

Time	Event	Location
10:30	Opening of the Symposium by Prof. Jan Lindsay	302-G20
10:40	Introduction to the Faculty of Science Research Fellow Society	
10:50	<u>Yuliana Yosaatmadja</u> <i>Biochemical, structural characterisation and fragment screening of SARS-CoV-2 ExoN (nsp14^{ExoN}-nsp10): a starting point for antiviral drug development</i>	
11:15	<u>Matthew Conder</u> <i>Isometries of the Bruhat-Tits tree</i>	
11:30	<u>Arie Spyksma</u> <i>Diver-generated photomosaics as a tool for monitoring temperate rocky reef ecosystems</i>	
11:45	<u>Tayla Rees</u> <i>CGRP and the AMY1 receptor: Identifying targets for migraine and craniofacial pain</i>	
12:00	<u>Stefano Schenone</u> <i>Advances in benthic ecosystems assessment – biogenic features and structure as surrogates of ecosystem functioning</i>	
12:15	Lunch	302-G60
13:00	Annual General Meeting (AGM)	302-G20
13:35	<u>Michael Barnett</u> <i>Developments in a novel approach to dynamically visualise protein data using Unreal Engine</i>	
14:00	<u>Sandesh Deshpande</u> <i>A fluorescence-based assay to screen inhibitors against flavivirus RNA polymerases</i>	
14:15	<u>Annie West</u> <i>Influence of management practice on the microbiota of a critically endangered species: a longitudinal study of kākāpō chick faeces and associated nest litter</i>	
14:30	<u>Alexandra Palmer</u> <i>Social and ethical challenges to Predator Free 2050</i>	
14:45	<u>Scott Claessens</u> <i>The non-independence of nations and why it matters</i>	
15:00	Afternoon Tea	302-G60
15:30	<u>Shaun Hotchkiss</u> <i>Bringing Science Communication into the 21st Century</i>	302-G20
15:55	<u>Danny McDougall</u> <i>Trace metals in New Zealand green-lipped mussels and the effect of water treatment on trace metal bioavailability and mussel survival</i>	
16:10	<u>Whitney Whitford</u> <i>Multiplex amplicon sequencing for mutation identification using the MinION nanopore sequencer</i>	
16:25	<u>Taniela Lolohea</u> <i>Diving into the depths of academia – young, naive and out of place</i>	
16:50	Closing remarks	
17:00	Networking and Prizegiving	302-L10

Biochemical, structural characterisation and fragment screening of SARS-CoV-2 ExoN (nsp14^{ExoN}–nsp10): a starting point for antiviral drug development

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The current global COVID-19 pandemic caused by the SARS-CoV-2 virus has infected over 500 million people and over 6.3 million fatalities worldwide. Despite the successes with vaccines, there are currently a lack of effective drugs to treat people infected with SARS-CoV-2, and identification of such agents is a global priority. SARS-CoV-2 belongs to an order of Nidovirales with very large RNA genomes¹. Several studies proposed that the fidelity of coronavirus (CoV) genome replication is aided by an RNA nuclease complex, comprising the non-structural proteins 14 and 10 (nsp14–nsp10)^{2,3}. Nsp14 is a dual function enzyme, containing an N-terminal exonuclease domain (ExoN) and C-terminal Guanine-N7-methyltransferase (N7-MTase) domain^{3,4}. Both activities are essential for the viral life cycle and, therefore, attractive targets for anti-viral therapeutics.

We have determined the crystal structure of SARS-CoV-2 nsp14, in the absence of nsp10, to 1.7 Å resolution⁵. Comparisons with nsp14/10 complexes reveal significant conformational changes that occur within the nsp14 ExoN domain, which provide a structural basis for the stimulation of its nuclease activity by nsp10. We performed an X-ray fragment screening on nsp14, revealing 72 hits bound to both the ExoN and MTase domains⁵. We also identified several inhibitors with potential therapeutic efficacy, including the known SARS-CoV-2 major protease (Mpro) inhibitor, ebselen, and the HIV integrase inhibitor, raltegravir³. These fragments and small molecule inhibitors serve as excellent starting point for structure guided development of nsp14 inhibitors that may be used to treat COVID-19 and potentially other future viral threats.

References:

1. Chan, J. F.-W. *et al.* Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerging Microbes & Infections* **9**, 221–236 (2020).
2. Gao, Y. *et al.* Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science* (1979) **368**, 779–782 (2020).
3. Baddock, H. T. *et al.* Characterization of the SARS-CoV-2 ExoN (nsp14ExoN–nsp10) complex: Implications for its role in viral genome stability and inhibitor identification. *Nucleic Acids Research* **50**, 1484–1500 (2022).
4. Ma, Y. *et al.* Structural basis and functional analysis of the SARS coronavirus nsp14–nsp10 complex. *Proc Natl Acad Sci U S A* **112**, 9436–9441 (2015).
5. Imprachim, N., Yosaatmadja, Y. & Newman, J. A. Crystal structures and fragment screening of SARS-CoV-2 NSP14 reveal details of exoribonuclease activation and mRNA capping and provide starting points for antiviral drug development. doi:10.1101/2022.03.11.483836.

Isometries of the Bruhat-Tits tree

Matthew Conder

Department of Mathematics, University of Auckland

A *group* is an abstract algebraic structure used to model and analyse the symmetries of an object. The fundamental idea of *geometric group theory* is that certain properties of a group can be determined by investigating the geometry associated with this object.

A *tree* is a collection of vertices and edges such that any two vertices are connected by a unique sequence of edges, and a *Bruhat-Tits tree* is a special type of tree with key applications in cryptography and computer science. My research focuses on a class of *linear groups* (that is, groups consisting of matrices) which 'act' by distance-preserving *isometries* on a Bruhat-Tits tree. I will discuss the geometry of this action and outline how this can be used to determine key properties of these linear groups.

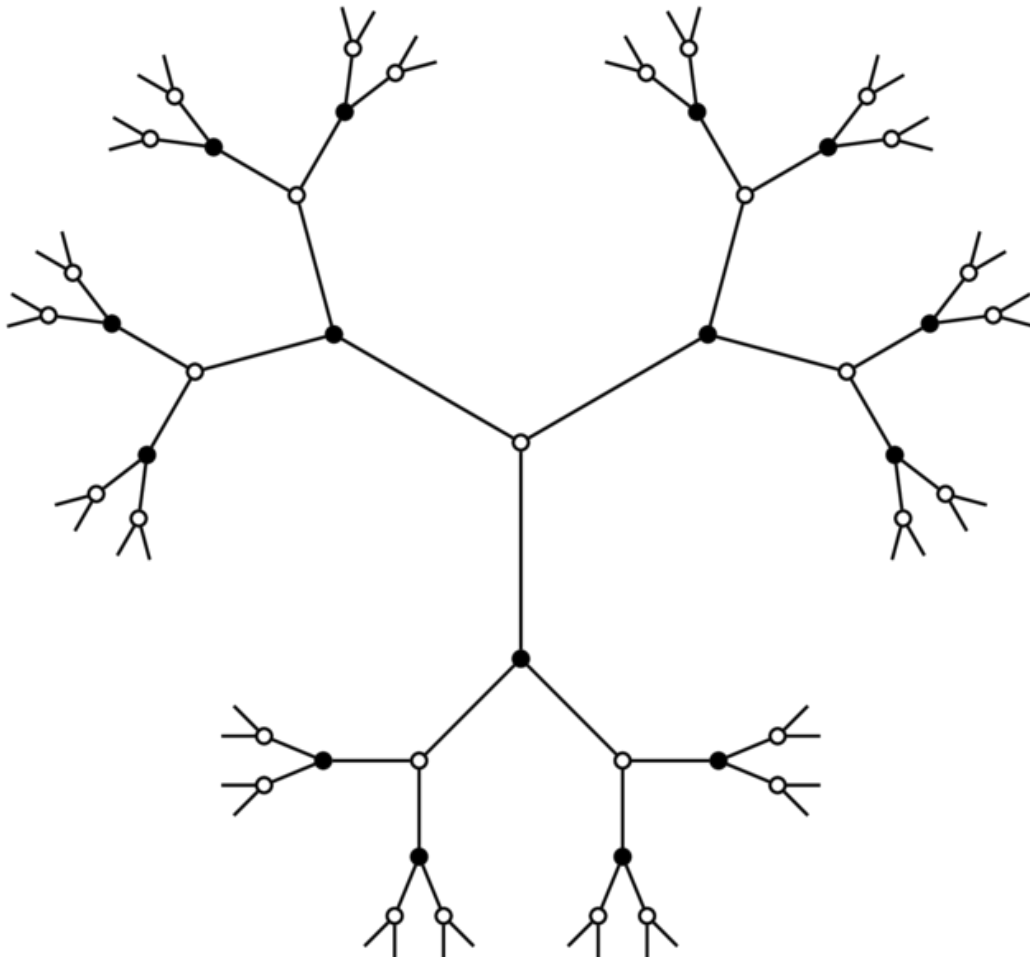


Figure: A Bruhat-Tits tree

Diver-generated photomosaics as a tool for monitoring temperate rocky reef ecosystems

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Robust monitoring data provides important information on ecosystem responses to anthropogenic stressors however, traditional monitoring methodologies, which rely heavily on field time, are resource intensive. Consequently, operating within realistic monitoring budgets and timeframes leads to trade-offs between data metrics captured and spatial/temporal coverage. Advances in remote sensing technology have reduced the severity of these trade-offs by providing cost-effective, high-quality data at increased spatial and temporal scales. Structure-from-motion (SfM) photogrammetry, a form of remote sensing, is well established in terrestrial applications and can be a key tool for monitoring marine benthic ecosystems, which are particularly vulnerable to anthropogenic stressors. Diver-generated photomosaics, an SfM photogrammetry output, are now routinely used for benthic monitoring within tropical ecosystems. Their use within temperate rocky reef ecosystems has received less attention. Here, benthic monitoring data collected from diver-generated photomosaics is compared with traditional field quadrat data to understand the utility of photomosaics for monitoring temperate rocky reef ecosystems. We evaluated these methods at sites where sea urchin barrens were prevalent finding key metrics (sea urchin densities, macroalgae canopy cover and benthic community composition) were similar between the two methods, but data collected via photogrammetry required less field time and resources and allowed greater spatial coverage than field quadrats. However, the use of photomosaics was limited by high macroalgal cover and shallow water reducing stitching success and obscuring understory species. The results demonstrate that photomosaics can be used as a robust and resource efficient method for effectively assessing and monitoring temperate rocky reef ecosystems.

CGRP and the AMY₁ receptor: Identifying targets for migraine and craniofacial pain

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The trigeminal ganglia (TG) are important for the transmission and modulation of head pain and migraine. Calcitonin gene-related peptide (CGRP), a potent pain neuropeptide, is highly expressed in the TG and is proposed to act in an auto-regulatory manner. The mechanism underlying this behaviour is unknown as CGRP and the canonical CGRP receptor are not co-expressed in the TG. However, another CGRP-responsive receptor, the AMY₁ receptor, is also reported to be present in the TG, but its distribution relative to CGRP is currently undefined. Therefore, the relative distribution of CGRP and the AMY₁ receptor component, the calcitonin receptor (CTR), was compared. First, anti-CGRP and CTR antibodies were validated for specificity and cross-reactivity using immunoblotting and immunocytochemistry. Mouse, rat, and human TG sections were then triple-immunostained with the validated antibodies and neuronal markers. The potential contribution of β CGRP was also explored in pilot studies using α CGRP knockout mice. The anti-CGRP and anti-CTR antibodies generally displayed good specificity. In the TG, CGRP and CTR-like immunoreactivity colocalised in small to medium β -tubulin III expressing neurons of all three species. Interestingly, CGRP and CTR immunoreactivity was not typically observed in NF200 expressing neurons suggesting that co-expression primarily occurs in C-fibre neurons, and infrequently in A-fibre neurons. Initial investigations using α CGRP knockout mice indicate that β CGRP and CTR immunoreactivity are also colocalised in small to medium neurons which do not express NF200. In conclusion, CGRP could be acting via the AMY₁ receptor in an autocrine manner in TG C fibre neurons.

Advances in benthic ecosystems assessment – biogenic features and structure as surrogates of ecosystem functioning

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Assessing and mapping ecosystem functions and services at scales relevant to society is crucial for ecosystem management and conservation. While practices for terrestrial ecosystems are well established and mainly rely on land cover, marine ecosystems face challenges that hinder our ability to quantify and understand the distribution of ecosystem services. In particular, soft-sediment marine habitats are complex and heterogeneous but data on the distribution of communities and habitats in these systems is scarce. Habitat characterization relies primarily on the sampling of abiotic variables and physical attributes, easier to obtain at broad scale, and therefore underestimates the role of the underlying biodiversity. Our interdisciplinary research aims at developing procedures for both intertidal and subtidal environments that can infer and up-scale meaningful ecological data on biodiversity and ecosystem functioning from the microtopographic features and 3-dimensional structures created by benthic organisms. To do so, we combined field measurements of benthic communities and fluxes, imagery, artificial intelligence and 3D modelling. Our results demonstrate the importance and value of characterizing soft sediments heterogeneity and their diversity. Intertidal and subtidal benthic ecosystem assessment applications will be presented.

Developments in a novel approach to dynamically visualise protein data using Unreal Engine.

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Proteins are the biological machines that enable life through cellular work, enzymatic function, and signal recognition. Proteins consist of a single polypeptide chain of many amino acids, that fold into unique 3-Dimensional shapes, dictating each proteins function. Protein structure determination is an important area of teaching and research, driving understanding of function and increasingly drug development. Consequently for both teaching and research purposes dynamic visualisation of protein structural data is increasingly important. This is further reinforced by the dynamic nature of proteins in solution that are often studied or examined in isolation outside their biological context providing a single snapshot of their function. Furthermore, proteins rarely function in isolation, often forming large macro-molecular complexes.

Current visualisation software solutions are fit for purpose for experienced users, often focusing on a single protein, in a single state. I present a novel visualisation tool created in the Unreal Engine, a powerful tool typically used in the gaming, architecture, and movie industries. The software uses a GPU shader and method for storing protein data in a standardised format within UV texture space, allowing for real time visualisation of large amounts of dynamic protein data. Classical cartoon and surface and ball and stick representations can be generated in real time and dynamically. Furthermore, animation of proteins between conformational states becomes trivial with multiple textures as frames. The method can be expanded to molecular dynamic simulation data, allowing real time play back up to hundreds of thousands of atoms, or coarse grain beads.



Figure 1: Example of the Proteasome, Vault complex, ATP Synthase, and TRIM5 protein rendered in the default Unreal 5 scene.

A fluorescence-based assay to screen inhibitors against flavivirus RNA polymerases

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Mosquito borne flaviviruses, such as Dengue virus (DENV) and Zika virus (ZIKV), are globally distributed, and are now endemic in our nearest Pacific neighbours. These viruses infect millions of people annually, causing serious and sometimes fatal disease. Currently there are no antiviral drugs available to treat flavivirus infections. Nucleoside analogues, which can interfere with viral RNA synthesis, are an attractive and well-proven class of small molecule therapeutics. These analogues exploit the general lack of error checking by viral RNA polymerases, and often act by inducing premature chain termination. The recently developed antiviral Galidesivir™, is one such compound. Galidesivir™ has been shown to suppress replication of flaviviruses in cell-based assays, and afford protection against infection in some animal models. This study describes the development of a fluorescence-based inhibition assay for screening of Galidesivir™ against flavivirus RNA polymerases. The assay can be used as a high-throughput inhibition assay to screen for antiviral activity of nucleoside analogues against viral RNA-dependent RNA polymerases.

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Influence of management practice on the microbiota of a critically endangered species: a longitudinal study of kākāpō chick faeces and associated nest litter

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² Kākāpō Recovery, Ta Papa Atawhai | Department of Conservation, Waihōpai | Invercargill, Aotearoa NZ.

The critically endangered kākāpō is a flightless, nocturnal parrot endemic to Aotearoa New Zealand. Recent efforts to describe the gastrointestinal microbial community of this threatened herbivore revealed a low-diversity microbiota that is often dominated by *Escherichia-Shigella* bacteria. Given the importance of associated microbial communities to animal health, and increasing appreciation of their potential relevance to threatened species conservation, we sought to better understand the development of this unusual gut microbiota profile. To this end, we conducted a longitudinal analysis of faecal material collected from kākāpō chicks during the 2019 breeding season, in addition to associated nest litter material. Using an experimental approach rarely seen in studies of threatened species microbiota, we evaluated the impact of a regular conservation practice on the developing kākāpō microbiota, namely the removal of faecal material from nests. Artificially removing chick faeces from nests had negligible impact on bacterial community diversity for either chicks or nests ($p > 0.05$). However, the gut microbiota did change significantly over time as chick age increased ($p < 0.01$), with an increasing relative abundance of *Escherichia-Shigella coli* over the study period and similar observations for the associated nest litter microbiota ($p < 0.01$). Supplementary feeding substantially altered gut bacterial diversity of kākāpō chicks ($p < 0.01$), characterised by a significant increase in *Lactobacillus* bacteria. Overall, chick age and hand rearing conditions had the most marked impact on faecal bacterial communities. Similarly, the surrounding nest litter microbiota changed significantly over time since a kākāpō chick was first placed in the nest, though we found no evidence that removal of faecal material influenced the bacterial communities of either litter or faecal samples. Taken together, these observations will inform ongoing conservation and management of this most enigmatic of bird species.

Social and ethical challenges to Predator Free 2050

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Aotearoa New Zealand ambitiously aims to eradicate all rats, possums, and mustelids (e.g. stoats) within the country by 2050. Predator Free 2050 (PF2050) is a fundamentally social project, relying on 'social licence' and voluntary work from New Zealanders to be achieved. Yet little work to date has explored what potential social and ethical challenges might lie on the horizon for PF2050. Drawing on in-depth qualitative research with key stakeholders such as predator control project managers, critics, and Māori representatives, I look to the future to predict what some of these challenges might be. These include: controversy around the use of poisons – currently exemplified by opposition to 1080, but potentially set to expand in light of regulatory and social changes; perceived risk of scope creep into valued pets and hunted species; the necessity (but impossibility) of securing permission from 100% of landowners to control predators on their property; challenges around teaching children about non-native species and getting them involved in trapping; varying interpretations of the ethical implications of failure, if PF2050 does not succeed; how to work towards genuine co-governance with Māori, rather than consultation after the fact; and questions around who will do the work, and who will pay for it, which are tied up with issues around governance, expertise, and respect for the skills of professional and volunteer trappers. In sum, I emphasise the centrality of the social and the ethical for PF2050, highlighting the importance of researching these issues rather than focusing purely on PF2050's technical feasibility.

The non-independence of nations and why it matters

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Cross-national analyses test hypotheses about the drivers of global variation in national outcomes. However, since nations are connected in various ways, such as via spatial proximity and shared cultural ancestry, cross-national analyses often violate assumptions of non-independence, inflating false positive rates. Here, we show that, despite being recognised as an important statistical pitfall for over 200 years, cross-national research in economics and psychology still does not sufficiently account for non-independence. In a review of the 100 highest-cited cross-national studies of economic development and values, we find that controls for non-independence are rare. When studies do include controls for non-independence, our simulations suggest that commonly used methods continue to produce false positives. In reanalyses of twelve cross-national relationships, we show that half are no longer significant after controlling for non-independence using global proximity matrices. We urge social scientists to sufficiently control for non-independence in cross-national research.

Bringing Science Communication into the 21st Century

Shaun Hotchkiss

I love to experiment and in late 2019 I grew tired of thinking that various aspects of how we communicate our research (to each other and to the rest of the community) “could be better” and decided to try some ways to do it better. I am a cosmologist, so most of the experiments so far have been in cosmology, but they’re all easily transferred to other disciplines.

So far there is *Cosmology Talks*¹ (a YouTube channel with technical talks on cosmology papers); *Cosmology from Home*² (an annual online conference); *The Hunt for Dark Matter*³ app (an interactive web-app giving users an intuitive understanding of weak gravitational lensing) and a few other less developed projects.

I will talk about these experiments and try to convince non-cosmologists in the audience to collaborate on various non-cosmology versions of the above!

References:

1. <https://www.youtube.com/CosmologyTalks>
2. <https://www.cosmologyfromhome.com/>
3. not-public-yet (I’ll show gameplay during the talk though)

Trace metals in New Zealand green-lipped mussels and the effect of water treatment on trace metal bioavailability and mussel survival

Danny McDougall

School of Chemical Sciences

The green-lipped mussel (*Perna canaliculus*) is New Zealand's most valuable aquaculture export and is of significant value to New Zealand's economy. Trace metals such as Cr, Cu, Zn, As, Cd, Hg, and Pb present in the seawater can be toxic to New Zealand green-lipped mussels as well as most other aquaculture shellfish grown worldwide. Common practice to alleviate the toxicity of trace metals is to add the metal chelating agent, EDTA, to the seawater used for incubation of the shellfish in their rearing tanks. This works extremely well, improving survival of shellfish as they develop from a fertilised egg to a 2 day old larvae from as low as 0% to up to 100%. This PhD research analysed the concentrations and distributions of trace metals in *P. canaliculus* larvae using ICPMS at the University of Auckland and XFM at the Australian Synchrotron. One experiment incubated *P. canaliculus* larvae for 2 days with EDTA or a more readily biodegradable alternative (EDDS) at a mussel hatchery near Nelson, and the effect of these treatments on concentrations and distributions of trace metals were compared with 2 day old larvae reared without chelating agent added. Survival was significantly improved with the addition of EDTA or EDDS, and significant differences in the concentrations and distribution of several trace metals with the mussels were observed.

Multiplex amplicon sequencing for mutation identification using the MinION nanopore sequencer

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² Centre for Brain Research, The University of Auckland, New Zealand

The rapid evolution of the study of genetic variation, and the discovery of disease susceptibility and causal variants has led to a demand for methods for rapid variant discovery or accurate verification. Trio sequencing (where proband and parents are sequenced) aids in variant analysis by determining the inheritance of putative causative variants. However, even Sanger sequencing for a large number of individuals can be a slow and costly exercise. As an alternative to single amplicon, sample by sample, Sanger sequencing, we considered that there may be efficiency gains to be made by multiplex sequencing using the Oxford Nanopore Technologies MinION sequencer.

We developed a multiplexing assay which pools PCR amplicons for MinION sequencing to enable sequencing of multiple templates from multiple individuals. A combined strategy of barcoding and sample pooling allowed for simultaneous multiplex MinION sequencing of 100 PCR amplicons, spanning 30 loci in DNA isolated from 82 neurodevelopmental cases and family members. Our multiplexing approach produced interpretable and expected sequence from 29 of the 30 targeted genetic loci. The sequence variant which was not correctly resolved in the MinION sequence was adjacent to a five nucleotide homopolymer, a known issue with MinION sequencing^{1,2}.

The results act as a proof-of-concept for the use of multiplexed amplicon MinION sequencing for the investigation of varied target regions. This method could be applied to targeted genetic analyses for any variant from any organism of interest, with the potential to reduce the cost of sequencing to as little as 5 cents per amplicon^{3–5}.

References:

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2. Delahaye, C. & Nicolas, J. Sequencing DNA with nanopores: Troubles and biases. *PLoS One* **16**, e0257521 (2021).
3. Nanopore Store. (2021). Available at: <https://store.nanoporetech.com/native-barcoding-expansion-1-12.html>. (Accessed: 1st June 2021)
4. Product comparison | Oxford Nanopore Technologies. (2021). Available at: <https://nanoporetech.com/products/comparison>. (Accessed: 1st June 2021)
5. Liou, C. H. *et al.* Nanomlst: Accurate multilocus sequence typing using oxford nanopore technologies minion with a dual-barcode approach to multiplex large numbers of samples. *Microb. Genomics* **6**, 1–8 (2020).

Diving into the depths of academia – young, naïve and out of place

Lolohea, T.F.P

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Academia can often feel separated from the “real world”, with little to no work-life balance, where academic social norms being social don'ts in the real world. In this conversational talk towards the journey into academia so far, Taniela look's to share his experiences, and perhaps share some of the little wisdom he has. An introduction into his research, motivations and experiences will be covered, in an effort to share his trials and tribulations to date. The motivation behind this talk is to share the very new experience of becoming a lecturer, from the lens of a young, naïve and out of place wanderer.