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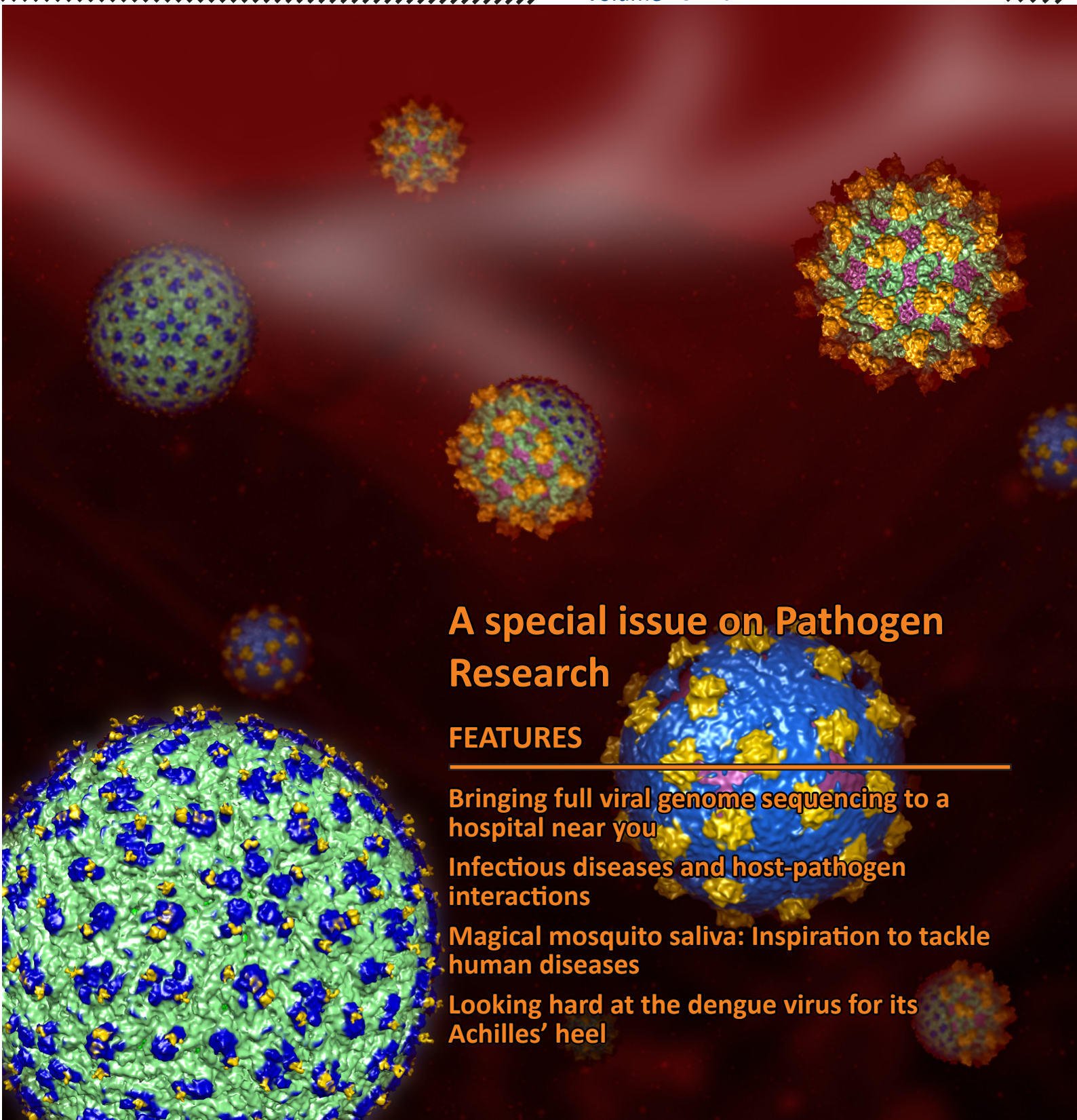

A special issue on Pathogen Research

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Advances in Science

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Advances in Science is published online twice a year. It is written for a broad scientific audience interested to keep up with some of the key areas of science pioneered by researchers at the Faculty of Science.

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On the cover: Computational methods can be used to reconstruct three-dimensional maps to reveal the atomic details of the virus structures. [Credit: Dr Guntur FIBRIANSAH]

Bringing full viral genome sequencing to a hospital near you

Viral genome sequencing capabilities in the fight against COVID-19

Introduction

The year 2020 has given us all a number of new terms to ponder on while we continue to largely work from home and avoid social gatherings: stay home notices, serological tests, molecular tests and contact tracing, among others. At the time of writing, there have been over 60 million cases of COVID-19 in 191 countries causing 1.4 million deaths - these numbers are rapidly increasing as the Northern Hemisphere enters its winter months with the virus raging nearly unchecked in populous countries such as the United states and India. Other countries such as South Korea, China and Singapore have had more success at curbing the spread of the virus by aggressively employing 'test and trace' programs.

Traditionally, these contact tracing programs have involved interviewing infected people about their recent interactions, and advising those exposed that they should get tested. Singapore has also implemented a robust electronic tracking system, known as TraceTogether, that employs a downloadable application for citizens' smartphones or a digital token for those who do not have access to a mobile phone. This electronic solution relies on bluetooth information that can tell the authorities when someone had a close brush with an actual COVID carrier (around 10 m). While this kind of approach has strong implications on individual privacy, the government has stressed that TraceTogether does not collect geolocation data for its function. The drawback of this technological alternative to traditional contact tracing programs is that it is only as effective as the number of people who adopt it. Current figures suggest that only about half the population has signed on, but new regulations being implemented soon seek to fill this gap.

Figure 1: The Oxford Nanopore sequencing device. This device enables direct, real-time analysis of DNA or RNA fragments. It detects the changes to an electrical current when biological molecules are passed through a nano-scale hole or near it. This information is then decoded to identify the specific DNA or RNA sequence. The genomic data created through this effort will be added to a central database to assist in the national contact tracing effort.

Tracking the virus genetic code

Another cutting-edge technology in the armamentarium for contact tracing is one that can map the spread of a virus by tracking tiny changes in its genetic code. This type of genetic analysis can reveal shared mutations that link cases in Singapore and other affected countries. In addition to its capacity to dramatically improve existing contact tracing programs, implementation of such a tool can be used to determine whether the virus is evolving to escape the protection afforded by a vaccine as they start to become available. Such genetic testing programs have been implemented in other countries to great effect. In Austria, researchers were able to use traditional epidemiological data combined with genome sequencing of 750 samples to uncover a cryptic link between two epidemiological clusters and track the transmission of a novel mutation within the country. In New Zealand, researchers have developed a mathematical model that harnesses genomic and epidemiological data to predict down to the neighbourhood where the virus might spread. In the United Kingdom, the government has partnered with the Wellcome trust to establish a real-time genomic surveillance system that will leverage on the more than 100,000 viral genomes that have already been sequenced in

the country to enable the public health authorities to more expeditiously respond to local outbreaks and monitor for the emergence of viral mutations associated with escape from vaccines once they are available.

Full genome sequencing capacity

In Singapore, the Sessions Lab (NUS) has partnered with researchers at Tan Tock Seng Hospital (TTSH), the Genome Institute of Singapore (A*STAR) and the Bioinformatics Institute (A*STAR) to establish full viral genome sequencing capability directly within the TTSH Microbiology laboratory. Establishment of full genome sequencing capacity via an Oxford Nanopore device (see Figure 1) directly within the hospital has enabled us to sequence over 400 SARS-CoV-2 genomes collected as part of the normal diagnostic responsibilities of the laboratory. The technology we have utilised in this collaborative effort is highly portable and the methodology requires little in the way of additional specialised equipment. This portability and simplicity make the solution we have implemented in TTSH easy to replicate in other tertiary settings around the country.

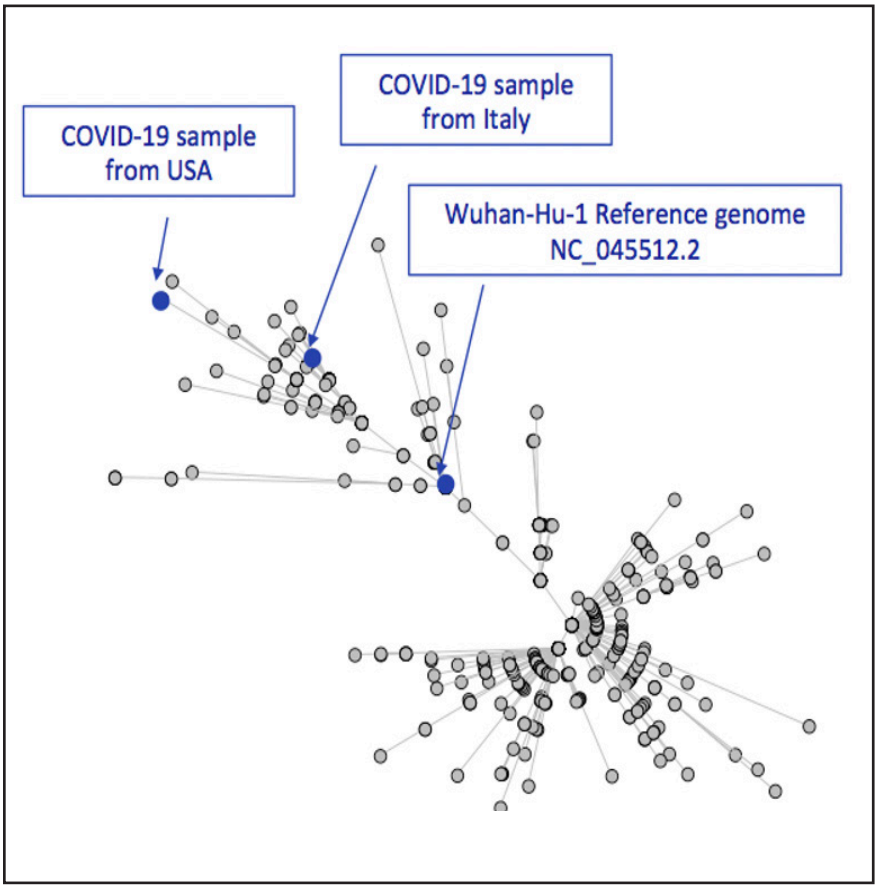


Figure 2: Phylogenetic tree of 409 COVID-19 patients in Singapore (grey nodes). Contemporary strains from China, Italy and the USA are highlighted in blue to show likely sources of introduction into the country. A phylogenetic tree is a diagram that shows the evolutionary relationships among organisms.

Although the analysis of the data we have collected from our study is ongoing, the data we have generated to date shows that Singapore has had virus importation from many different parts of the world since the outbreak started locally (Figure 2). Working closely with the National Centre for Infectious Diseases (NCID), we are in the process of obtaining basic epidemiological information that will allow us to retrospectively model the transmission of the virus over the course of the epidemic in Singapore. Continuation of these efforts will also enable us to monitor for the presence of escape mutants once a vaccine is made available to the general public.

Return to normal

Even with several of the front-runner vaccines nearing the end of trials, the current situation is likely to persist well into 2021. With global economies suffering and people's patience stretched to the breaking point, there is an urgent need for a return to normal. Despite this desire, it is clear from other countries' experiences, notably the United States, that a rush to open can be counter-productive and lead to new, uncontrolled outbreaks of the virus. Hence, a carefully measured reopening plan with a robust contact tracing program that includes viral genome sequencing will allow us to navigate this purgatory without a return to the nightmarish situation we have experienced over the last year.

October SESSIONS is an Assistant Professor in the Department of Pharmacy and the Saw Swee Hock School of Public Health, NUS where his laboratory has a particular interest in the molecular mechanisms of pathogenesis. He earned his Bachelor of Science degree from the University of Arkansas and his Ph.D. in Molecular Genetics and Microbiology from Duke University. His post-doctoral studies at the Duke-NUS Graduate Medical School in Singapore involved the application of high-throughput sequencing for the characterisation of the interactions between dengue and its human and mosquito hosts.

For more information about his research work, please visit:
https://sph.nus.edu.sg/faculty-directory/sessions-october-michael

Infectious diseases and host-pathogen interactions

Strategies to fight bacterial and viral infections

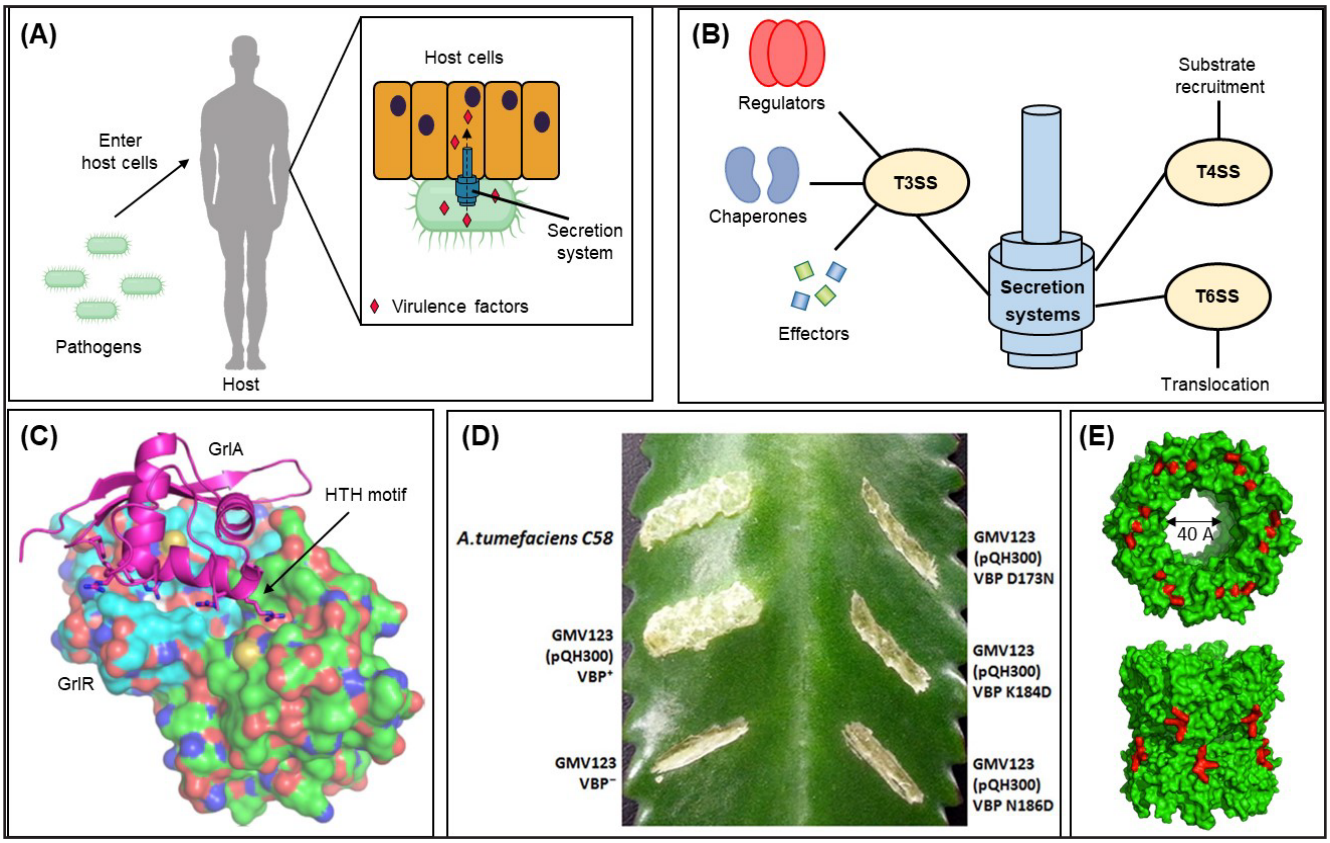


Figure 1: (a) Host-pathogen interaction and injecting the virulence proteins into the host system using secretion systems to initiate infection. (b) Schematic representation of effectors, chaperons and regulators; substrate recruitment and translocation component with respect to the secretion systems for successful host cell contact to cause infection. (c) Structure of the GrlR–GrlAΔ complex. The GrlR dimer is shown in surface representation and GrlAΔ is shown as a cartoon. (d) The wounds on the leaves of *Kalanchoe* plants were inoculated with *A.tumefaciens* WT strain C58 or GMV123 strain complemented with plasmid expressing VBP (protein) WT, VBP N186D, VBP D173N or K184D. Tumours were photographed at 25 days. Only wounds inoculated with *A.tumefaciens* WT strain or GMV123 strain and the plasmid expressing VBP WT showed tumour formation. Thus, only *Agrobacterium* harbouring full length dimeric VBP can induce tumours. (e) Surface representation of EvpC hexameric ring (translocation pore) in top view and side view. The residues targeted for the mutational studies are shown in red.

Introduction

Infectious diseases are responsible for over one-third of deaths world-wide. With this in mind, our long-term goal is to gain a better understanding of the biological processes associated with pathogen infection and to develop strategies to prevent host-pathogen interactions. These studies will require an understanding of the molecular mechanisms of bacterial and viral pathogenesis in the context of diseases. Our approach centres on neutralising the mode of infection (1) by targeting the secretion systems and (2) through the use of neutralising antibodies.

Unique ways to combat bacterial infections

Gram-negative pathogens can cause mild to deadly diseases (e.g. cholera, plague, pneumonia, whooping cough, etc.) by utilising their secretion systems to inject virulence proteins (effectors) into host cells (Figure 1a). These injected effectors divert host cell functions for the survival of the pathogen and thus initiate infection. Traditional antibiotics that cause cell death or inhibit bacterial growth invariably lead to drug resistance when they are over-used. By targeting the secretion system, we can develop broad-spectrum antibacterial compounds that pose no direct damage to the bacterium but prevent the delivery of effectors, thereby rendering the bacterium harmless. Such drugs would help prevent the selection pressure that results in the development of mutations, which

invariably leads to drug resistance and antibody ineffectuality. Posing no immediate threat, the bacteria would be rapidly cleared by the host immune system, causing minimal damage to the host.

Thirteen types of secretion systems (SS) have been identified to date. We seek to design novel ways to target the action(s) of 3 of the most commonly harnessed types (Types 3SS, T4SS, and T6SS) for therapeutic intervention. To this end, we have examined ways to inactivate and destabilise the regulatory processes, substrate recruiting machinery, and translocation apparatus employed by bacteria in the delivery of virulence proteins to host cells (Figure 1b).

We previously discovered how one major regulatory protein complex (GrlR–GrlA) modulates virulence in the T3SS (Figure 1c). This was demonstrated using a pathogenic strain of *E. Coli* (Enteropathogenic *E. Coli* - EPEC) that closely resembles Enterohemorrhagic *E. Coli* (EHEC). We show that GrlR (positive regulator; green/cyan surface) interacts with GrlA (negative regulator; pink ribbon) at its helix-turn-helix (HTH) structural motif, preventing GrlA from binding to its target promoter(s). This work has greatly advanced our understanding of how GrlR-GrlA regulates virulence operons in EHEC/EPEC strains, and has provided a target for therapeutic intervention. We have also studied substrate recruitment in the T4SS, and found that tumours in plants can be prevented by destabilising key recruiting proteins (Figure 1d). This mode of action can be extended to other pathogenic bacteria using similar secretion systems. In T6SS, we explored

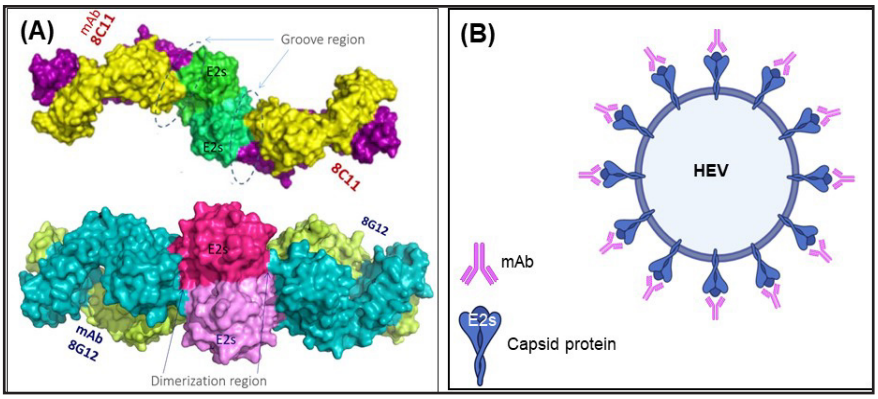


Figure 2: (a) HEV neutralising mAbs 8C11 and 8G12 binds at the groove and dimerisation region respectively on capsid protein (E2-domain) of HEV. (b) Model of the proposed neutralisation mechanism of HEV by mAb. The mAb binds at the protruding region of the virus (capsid) and prevents the viral-host interaction.

the structure-based function of EvpC and its homologue Hcp1. EvpC is a major secreted protein that forms the molecular syringe apparatus required for inserting effectors into the host (Figure 1e). It is also a marker protein for T6SS targeted for the production of monoclonal antibodies for diagnostic applications.

Vaccine for Hepatitis E virus

Besides bacterial-host interactions, we have also investigated viral-host interactions, particularly infectious hepatitis viral diseases, which pose a major burden to health worldwide. Hepatitis E virus (HEV) in particular is an important cause of severe hepatitis in humans, characterised by jaundice, fever, liver enlargement and abdominal pain. The mortality rate is ~5% in the general population but a staggering ~25% in pregnant women.

As part of a collaborative project with Prof Ningshao XIA from Xiamen University in China, we have shown that the HEV protein shell (capsid) is partially enclosed, comprising homodimers of the capsid protein, E2. These dimers protrude from the viral surface and engage with the host cell to initiate infection. To target this, we raised several monoclonal antibodies (mAbs) against all four genotypes of HEV, and found that the various genotypes are preferentially targeted by specific neutralising antibodies at different regions, e.g. the groove region (mAb 8C11 for genotype-1 HEV) or the dimeric interface (mAb 8G12 for all genotype) (Figure 2a). Of note, mAb 8G12 has a protective, neutralising capacity that significantly blocks virus infection in host cells, and this has been confirmed in animal studies. Using structure-based mutations and cell model assays with virus-like-particles (VLP), we identified the conserved residues in all four genotypes that are required for virus-host interactions, and, consequently, 8G12-mediated virus neutralisation (Figure 2b). This VLP consists of HEV capsid protein (E2 domain). The neutralisation mechanism identified with this domain has resulted in the development of an HEV vaccine.

J SIVARAMAN is a Professor with the Department of Biological Sciences, NUS. He obtained his Ph.D. from Anna University, India and did his post-doctoral fellowship at the Biotechnology Research Institute, Montreal, National Research Council Canada. His research interest is to study the structure and function of proteins related to infectious diseases (host-pathogen interactions) and cancers.

For more information about his research work, please visit:
https://www.dbs.nus.edu.sg/staffs/j-sivaraman

Magical mosquito saliva: Inspiration to tackle human diseases

Understanding the interactions with disease-causing pathogens

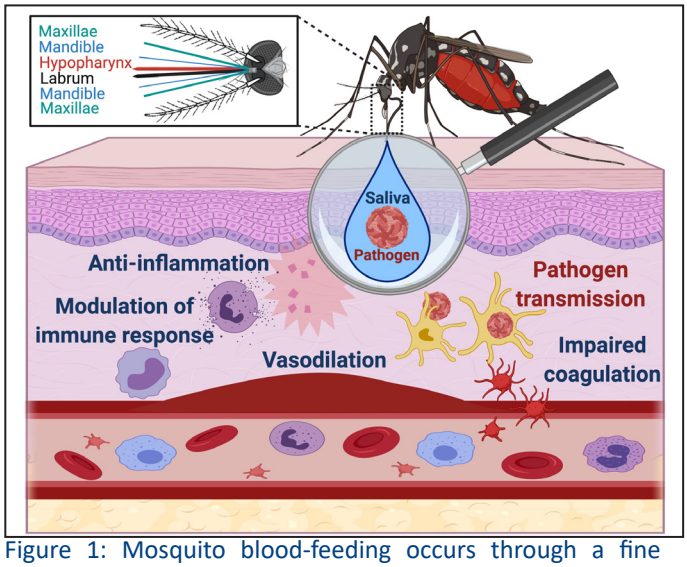


Figure 1: Mosquito blood-feeding occurs through a fine proboscis where both pathogens and bioactive salivary proteins are delivered into the host. Salivary proteins counteract the host's inflammatory and immune response, as well as cause vasodilation and inhibit coagulation to aid mosquito feeding though one of six fine needles (subset image; also see https://www.youtube.com/watch?v=xHhnGGN0KF4). Pathogens take advantage of salivary protein activity to enhance transmission.

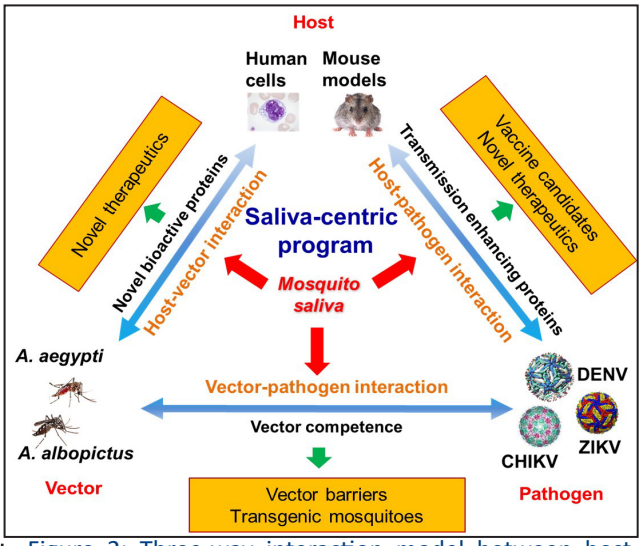


Figure 2: Three-way interaction model between host, vector and pathogen.

Introduction

Mosquito bites can kill. They transmit deadly infections like malaria, dengue, West Nile virus, chikungunya, yellow fever, various encephalitis and Zika fever. About 700 million people are affected each year, resulting in over one million deaths. Female mosquitoes rely on blood meals for proper development of their eggs, and their promiscuity results in the spread of disease-causing pathogens. In 2020, Singapore experienced an unprecedented 27,280 dengue cases and 20 deaths until August 2020. We also experienced chikungunya and Zika in 2008 and 2016, respectively. Our tropical climate provides a conducive mosquito-breeding environment, exposing us to mosquito-borne diseases.

Mosquitoes use six needles to cut into our skin and suck blood through a very fine 'straw' (Figure 1). To prevent blood clots that could block this narrow straw, mosquitoes infuse a cocktail of over 100 pharmacologically active salivary proteins. Mosquito saliva initiates a cascade of events when it enters into the host, and pathogens responsible for vector-borne diseases appear to utilise the saliva proteins to enhance their transmission and infectivity (Figure 1). With this background, we developed a saliva-centric program to understand the role of saliva proteins in the three-way interactions between the host, vector and pathogen (Figure 2). I had the pleasant privilege of leading an energetic team of collaborators from NUS, Duke-NUS Medical School, Nanyang Technological University, Agency for Science, Technology and Research (SIgN and BioTrans) and Singapore-MIT Alliance for Research and Technology, and their highly talented, enthusiastic and world-class graduate students and postdoctoral fellows. Our team was aptly supported by scientists from the United Kingdom (Oxford University and Wellcome Trust Centre) and United States (National Institutes of Health, Massachusetts Institute of Technology, University of Texas Medical Branch, Baylor College of Medicine and Johns Hopkins Bloomberg School of Public Health), and by the Ministry of Education, Singapore Tier 3 funding.

Role of saliva proteins

The main objectives of the research are to identify and characterise (a) transmission-enhancing proteins (TEPs) in vector saliva (Host-pathogen interactions). Such TEPs provide opportunities to develop vaccine candidates and novel therapeutic strategies to fight vector-borne diseases. As these interventions target the vectors and not the pathogens, they are not easily susceptible towards the development of pathogen resistance; (b) saliva factors that contribute to vector competence (Vector-pathogen interactions). Such factors contribute to our understanding of vector barriers that prevent pathogen transmission. They provide opportunities to develop transgenic mosquitoes that cannot transmit pathogens and hence, the

disease; and (c) novel bioactive proteins and peptides (Host-vector interactions). These molecules provide the prototype for the design of novel therapeutic agents to treat other human diseases. They provide ligands or targets that are amenable for developing new strategies.

In this proof-of-concept program, we had to establish solid foundations defining the changes in profiles of salivary proteins in mosquitoes (*Aedes aegypti* and *A. albopictus*) infected with pathogens (dengue, Zika and chikungunya viruses). The miniscule amounts of saliva and dissection of tiny salivary glands in mosquitoes pose significant and distinct challenges. We overcame these hurdles through hard work and cutting-edge technologies. Studies on structure, function and mechanism of saliva proteins mandated a steady source of significant amounts of proteins and peptides. We optimised solid phase peptide synthesis and recombinant expression in *E. coli*, yeast, and insect and mammalian cell lines to obtain the required proteins. We established several new assays, procedures and processes to evaluate the structure, function and mechanism of action of mosquito saliva proteins. Through our integrative, multi-disciplinary approach, we made several significant contributions to the field (see box for details).

Our accomplishments

(i) Vector-pathogen interactions

- *Profiled the differentially expressed proteins in A. aegypti and A. albopictus salivary glands in response to DENV, ZIKV and CHIKV infections. They include proteins related to immunity, blood feeding, metabolism and digestion.*
- *Identified the antiviral function of JNK pathway against all three infections in mosquito salivary glands. It is mediated through complement system and apoptosis.*
- *Identified several antiviral genes in mosquitoes and currently engineering transgenic mosquitoes resistant to arboviral infections.*
- *Identified the mutation in a live-attenuated dengue vaccine strain responsible for engendering refractoriness to the mosquito vector.*

(ii) Host-vector interactions

- *Studied structure-function relationships of Sialokinin, a neurokinin receptor agonist found in A. aegypti saliva and developed highly selective agonists for three distinct receptors (NK1R, NK2R or NK3R). These agonists are useful in the treatment of cancer, incontinence and infertility, respectively. Animal experiments are being initiated to validate these novel therapeutic leads.*
- *Identified mosquito salivary gland proteins that interact with human receptors responsible for viral invasion.*
- *Studied the cryoEM structures of flaviviruses and alphaviruses and their complexes to define the molecular determinants in their pathology and life cycle.*

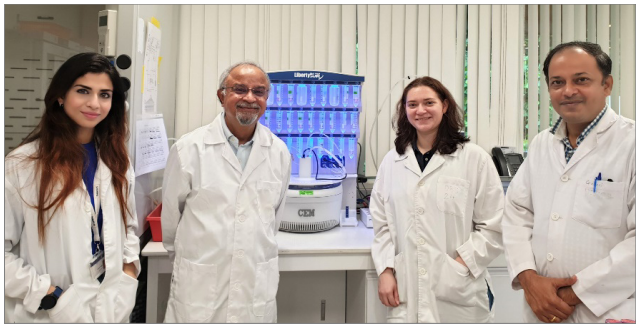
(iii) Host-pathogen interactions

- *Found several synthetic and recombinant mosquito salivary proteins that suppress arboviral infections in mouse models.*
- *Discovered that DENV subgenomic flaviviral RNA (sfRNA) is transmitted to host through mosquito saliva inside detergent sensitive vesicles. These sfRNA increase infection in keratinocytes at the bite site by inhibiting host innate immune response.*
- *Developed ZIKV inhibitors that could be used as antiviral therapeutics.*
- *Reprogrammed embryonic lung fibroblast cell strains into induced pluripotent stem cells (iPSCs) to readily grow live attenuated virus for vaccine purposes.*

Future prospects

Our integrative, multi-disciplinary and comprehensive approach has helped to delineate various aspects of the three-way interactions between the host, vector and pathogen. Research findings from the team have laid a strong foundation for developing novel strategies to combat the arboviral infections. The promising antiviral genes identified from our research could potentially lead to the development of transgenic refractory mosquitoes, whereas the antiviral salivary proteins and viral inhibitors could be used as transmission blocking therapeutics or vaccine candidates. The proof-of-concept saliva-centric platform developed is expected to facilitate the study and development of strategies to tackle other vector-borne diseases.

R. Manjunatha KINI is a Professor with the Department of Biological Sciences, NUS. He is a global leader in snake venom chemistry and blood coagulation. He works on structure, function and mechanism of toxins from animal venoms and saliva of blood-feeding animals. He published 270 original articles, reviews and book chapters and has 50 patent applications. He edited two monographs and six special issues.



Photograph shows the research team comprising of (from left) Dr Avisha CHOWDHURY, Prof R. Manjunatha KINI, Dr Cassandra MODAHL and Dr Ganesh Dharma PATIL.

For more information about their research work, please visit: https://www.dbs.nus.edu.sg/staffs/kini-r-manjunatha

Looking hard at the dengue virus for its Achilles' heel

Developing an effective vaccine against the dengue virus

Degue virus

Dengue, a mosquito transmitted disease, infects hundreds of millions of people each year in the tropical and sub-tropical countries. It is also endemic in Singapore. Dengue can cause diseases ranging from mild dengue fever to severe dengue haemorrhagic fever which can lead to death. Thus far, there is no specific treatment for it. Therefore, the development of an effective vaccine or a cure is needed to control the disease. There is a licensed vaccine (Sanofi Pasteur) available but it has shown poor efficacy especially for individuals who have never been exposed to the dengue virus before. It is also possible that this vaccine could even prime some vaccine recipients to develop the more severe form of the disease (dengue haemorrhagic fever) if recovered dengue patients get infected later on. Part of the problem associated with the development of drugs and vaccines is the lack of thorough understanding of how the virus infects cells and how the virus can escape detection from our immune system.

CryoEM

Cryogenic electron microscopy (CryoEM) is a very powerful technique to visualise native protein/virus structures under very high magnification at liquid nitrogen temperature (-196°C). After image requisition, we use computational methods to reconstruct three-dimensional maps revealing the atomic details of the proteins/virus structures. This technique has been hailed as the future of structural biology, as evident by the recent 2017 Nobel prize for Chemistry which was awarded to the three cryoEM pioneers (Richard HENDERSON, Jacques DUBOCHET and Joachim FRANK). My laboratory uses cryoEM to conduct research work to visualise the structures of the virus components that are important for (1) infecting cells, (2) causing the disease symptoms such as bleeding, and (3) affecting how the virus changes shapes to escape detection from our immune response. These studies will lead to the design and development of more effective therapeutic drugs/antibodies and vaccines against all four dengue serotypes.

The trickiness of making effective vaccines against dengue virus

There are four dengue serotypes (DENV1-4). If an individual gets infected with one serotype and when he or she is later infected with a second serotype, he or she may have an increased chance of developing the severe form of the disease (dengue haemorrhagic fever). Therefore for vaccine design, we need to include all four serotypes and the vaccine recipient will have to produce equally robust immune responses against all serotypes. This has been tough, as individually each serotype can stimulate good responses from recipient but when the four serotypes are combined, immune responses may not be even. Further complicating this, our laboratory has shown that the different strains within a serotype can also mutate to disguise themselves, by adopting different shapes to evade recognition by our immune system. As the purpose of a vaccine is to train the human body to recognise the pathogen, a good dengue vaccine will have to take into account not just the different serotypes, but also all possible virus shapes and forms.

How to combat dengue virus

Vaccine development

We need to know what shapes the dengue viruses can morph into. For this, we can use cryoEM to look at the different strains of each dengue serotype that are naturally circulating in the world and to also determine their atomic structures computationally. By including these different shapes of the virus into the vaccine cocktail, we can train our body to recognise all of them and take into account all the different virus shapes and serotypes.

When new viruses are initially made inside an infected cell, the virus exists in an immature virus state, characterised by its spiky appearance. The immature virus state will then undergo maturation when it moves through the trans-Golgi network in the cell. During this process, an extra protein prM has to be cleaved by the host protease (furin) to become the M protein. As a result, the spiky immature virus acquires a smooth surface. Our laboratory has shown that the dengue virus maturation process is not efficient and therefore a number of viruses at different maturation states (highly immature, partially immature, highly mature) can be released outside the cell. In addition, the fully mature virus can also morph into different shapes - the smooth or bumpy surfaced and the golf-club shape particles (Figure 1). These viruses, regardless of maturation states or shapes, are all infectious - although they might employ different ways of infecting cells, and thus, should not be neglected. We have also observed that in a single outbreak, a virus that used to have a smooth surface can morph into a particle with a bumpy surface when humans develop immunity against it. In this way, the virus can escape detection from the human immunity system and this ensures its continued survival. Thus, the ability to change into various shapes allows the virus to trick and evade our immune system via multiple ways.

Drug development

To help with the drug design aspects, my research laboratory uses cryoEM

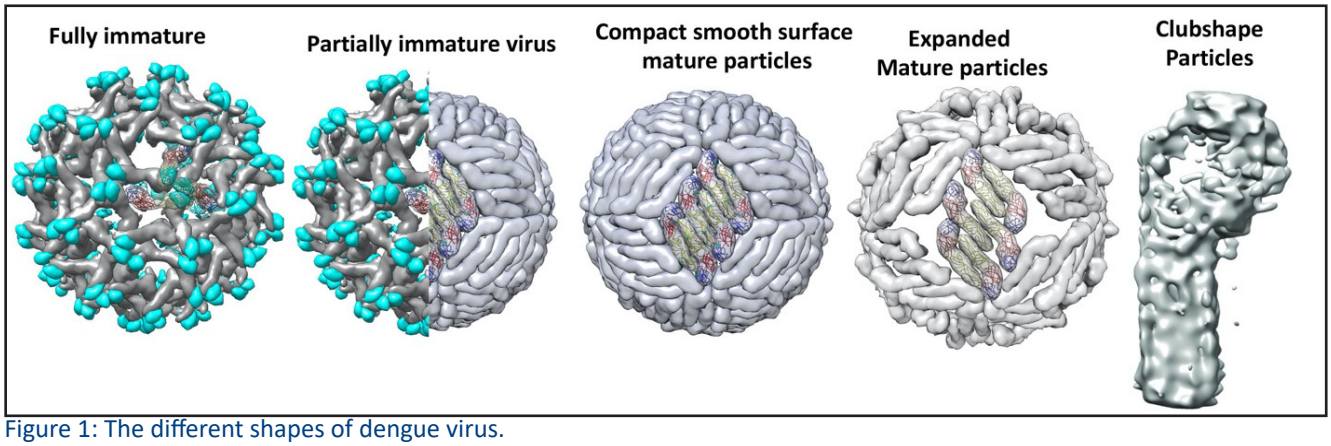


Figure 1: The different shapes of dengue virus.

to examine the structural changes of the virus when it infects the cells – how the virus (1) attaches to cells, (2) releases its viral genome into the cell, (3) makes new virus particles, and (4) processes these new immature virus particles into mature virus. We produce the virus particles and then expose them to conditions similar to what they would encounter while infecting cells and then we look at their atomic structures by using the cryoEM. The results will show various important virus structural changes in great detail and allow us to design drugs that can either block these structural changes or completely disrupt them. These drugs can potentially stop the virus from infecting and multiplying in the cell, thus providing a cure for the dengue disease.

The development of the severe form of dengue - dengue hemorrhagic fever/shock syndrome, usually occurs after the virus has been cleared from the host. Therefore, depending on when the patients are admitted to the hospital – whether the virus is present or not, the treatment can be different. Our laboratory is examining some proteins made by the dengue virus that can cause excessive bleeding in patients. By visualising how these proteins interact with human cell proteins, we can also design drugs that will prevent the development of the more severe form of the disease.

Our laboratory in collaboration with Dr Peter BOND (Agency for Science, Technology and Research) and Prof Ganesh ANAND (NUS) have shown how the newly formed immature dengue virus particles are assembled inside the cell, and how their genome is packaged to form the new immature particle. We have also observed the new immature particle undergoing very dramatic structural changes on its virus particle surface to become the mature virus.

Therapeutic antibody development

We observed under cryoEM how different potent antibodies interact with the dengue virus of different serotypes and shapes. From this, we can then understand how they kill the viruses and select specific ones to make a cocktail of antibodies that are effective against all serotypes and shapes.

Our laboratory in collaboration with Prof James CROWE (Vanderbilt University), whose laboratory has isolated hundreds of human monoclonal antibodies against the dengue virus, showed how some antibodies can specifically kill certain virus shapes and how others can kill the virus regardless of whether they are smooth round particles or have changed into bumpy surfaced particles. We also showed that for mature DENV particles with smooth compact surface, antibodies that can bind across viral surface proteins are highly potent as they locked these proteins together, thus preventing further essential structural changes required for cell entry.

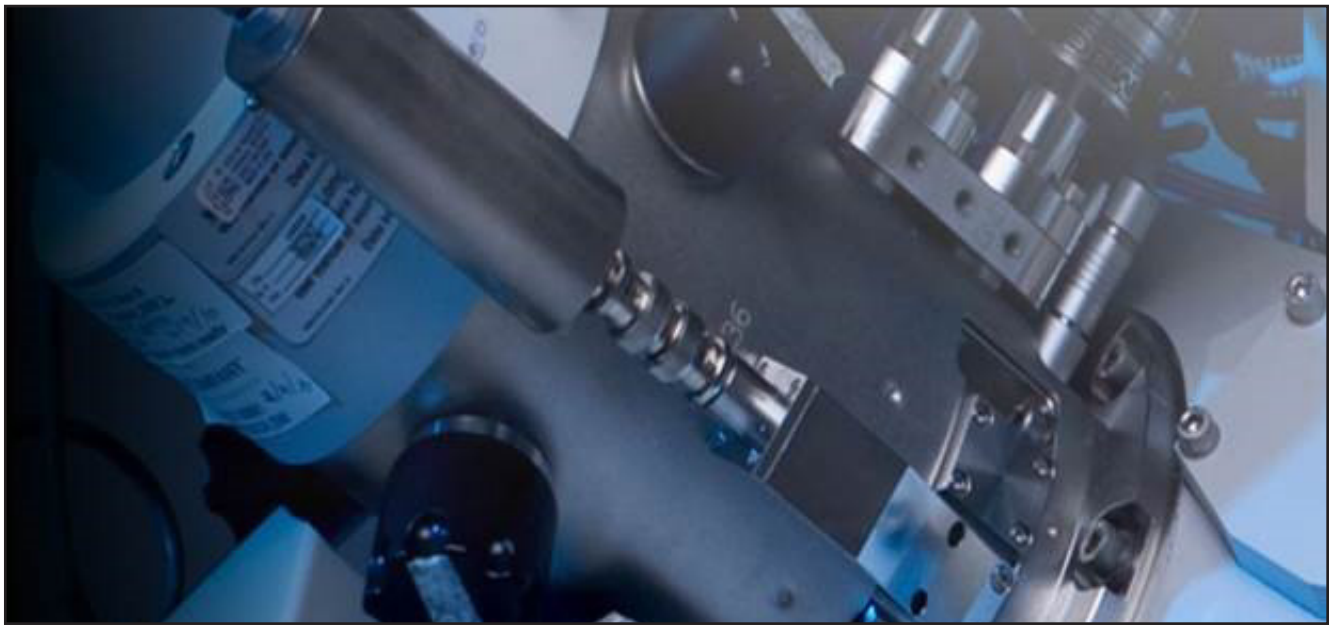
In addition to developing therapeutics, we also use these antibodies to understand the transitional structures of the dengue virus which are important for the cell entry and maturation process. The binding of these antibodies to the virus can trap them in different stages of these processes, allowing us to study them.

In summary, our laboratory mainly focuses on understanding the infection and disease development of the dengue virus and contributes to the development of therapeutics and vaccines.

Shee-Mei LOK is a Professor in the Department of Biological Science, NUS and also in the Emerging Infectious Disease Programme, Duke-NUS Medical School. She is also a Principal Investigator at the NUS Centre for BioImaging Sciences. She is awarded the National Research Foundation (NRF) Fellowship (class of 2009) and NRF Investigatorship (class of 2016). Her research studies major human pathogens such as dengue, zika chikungunya, and hepatitis B viruses.

For more information about her research work, please visit:
http://www.loksheemeilab.com

Centre for BioImaging Sciences (CBIS)



The Centre for BioImaging Sciences (CBIS) research is focused on the science and application of biological imaging by light and electron microscopy and the development of computational and microscopy-based methods and technologies. The visual was taken from the FEI Helios NanoLab 660 Dual Beam Focused Ion Beam (FIB) instrument from the Electron Microscopy (EM) facility.

As one of the leading technological resources in Singapore, the Centre for BioImaging Sciences (CBIS) was founded by faculties from the Departments of Biological Sciences, Chemistry and Physics in December 2010.

Recent advances in biology, chemistry, computer sciences, and physics – and the remarkable evolution of microscopes and computation – enable us to watch single molecules in action, create three-dimensional images of molecules over time, and observe them in live cells and tissues. The biologists, chemists, computer scientists, physicists and engineers from CBIS develop and apply novel computational and microscopy-based methods and technologies to address key problems in biology, with a focus on biological imaging using light and electron microscopy. Interdisciplinary interactions and scientific collaborations among different groups in CBIS are extremely beneficial to both the research groups and the bioimaging community.

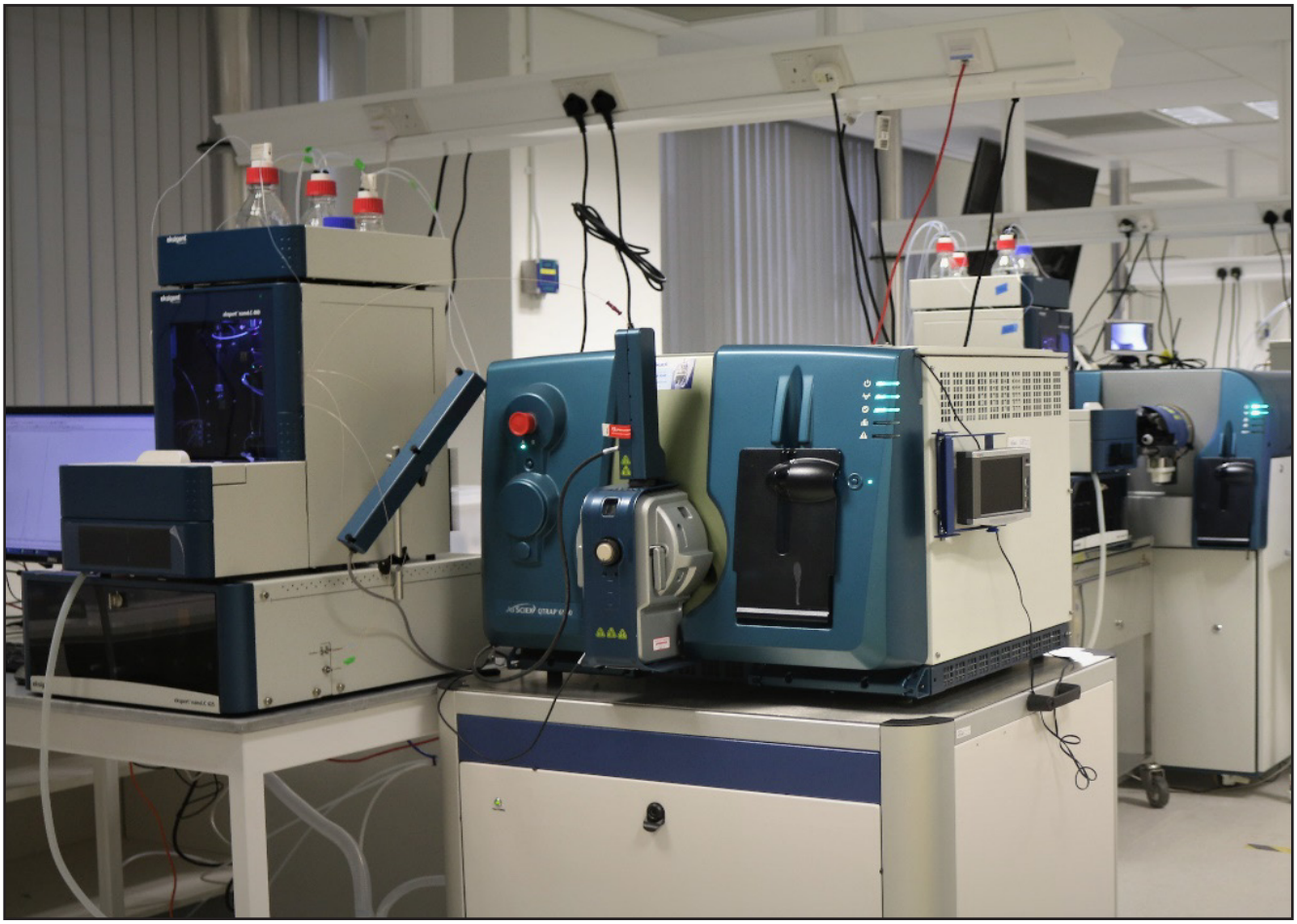
CBIS maintains and continuously upgrades its shared facilities with state-of-the-art instruments. This includes the Electron Microscopy (EM), Light Microscopy (LM), Computational (IT) facilities and Laboratory Cores. The CBIS EM facility is one of the most advanced TEM facilities in Singapore. It is also a prominent member of a select group of high-end EM facilities in the world. Microscopes from the CBIS EM core (e.g. FEI Titan Krios Cryo-TEM, JEOL JEM 20210F TEM, FEI HELIOS Dual Beam, etc.), consistently achieve high performance standards when assessed by external parties. The CBIS LM facility is part of SingaScope, an island-wide light microscopy national facility, and is connected internationally to various national bioimaging facilities and the European network of bioimaging analysts. It offers advanced confocal and live imaging equipment to meet research requirements for a large variety of biological samples, from bacteria and yeast, to cells, tissues and organisms. The LM Facility also provides image processing software to enable researchers to extract useful information from image data to answer biological questions. The CBIS IT Core provides high performance HPC computational resources, GPU server and PetaBytes space to serve the light and electron microscopy facilities in their image data collection, analysis, computational modelling and storage needs. The CBIS Laboratory Core is equipped with shared laboratory instruments and sample preparation space in close proximity to designated optical instrument rooms equipped with a wide range of advanced and/or custom-built microscopy systems from different research groups.

CBIS offers a full workflow, from pre-experimental consultation and discussion, to user training and advice during measurements, to post-experiment data evaluation. It also offers microscopy services.

We invite you to explore the CBIS, to take part in our graduate BioImaging Training Programme (BIT), and to join us in applying our tools, resources, and expertise to the biological problems of your interest.

Website: https://www.dbs.nus.edu.sg/cbis

Protein and Proteomics Centre (PPC)



Housing state-of-the-art equipment, Protein and Proteomics Centre (PPC)'s expertise areas include quantitative and structural analyses of proteins as well as biomarker discovery.

While nucleic acids are the blueprint for life, proteins are the workhorses. Proteins catalyse and regulate virtually all the processes that occur in living cells, and are collectively known as the proteome. The elucidation of the structure, function and dynamics of proteins and the proteome is one of the key aspects in understanding biology.

To address this, the Protein and Proteomics Centre (PPC) was established in the Department of Biological Sciences in the year 2000, by then Head of Department, Emeritus Professor HEW Choy Leong. The aim of PPC was to provide researchers with powerful analytical protein research technologies, focused around mass spectrometry. Through the years, PPC has evolved to become among the leading mass spectrometry core facilities in Singapore and the region, providing cutting-edge technologies and solutions to resolve diverse research needs and questions in protein biology.

PPC offers core and collaborative services in several areas of expertise, including biomarker discovery, qualitative and quantitative proteomics, phosphoproteomics, targeted mass spectrometry and structural mass spectrometry. Adopting a cooperative and consultative approach for all customers, PPC forges close working relationships with both academia and industry, locally and beyond.

PPC's expertise and capabilities are recognised beyond Singapore, with accolades such as the Waters World Centre of Innovation and SCIEX Regional Centre of Distinction. PPC is also the leading unit and headquarters of SingMass, the Singapore National Laboratory for Mass Spectrometry. SingMass is a national platform for mass spectrometry solutions, supported under the shared infrastructure support grant from the National Research Foundation, offering complementary expertise from three established core facilities.

PPC continues to push the boundaries of mass spectrometry applications in addressing current real-life challenges. At present, PPC is focusing on expanding capabilities into food research, with the aim of developing innovative mass spectrometry solutions to address the needs of Singapore's recent focus on food sciences.

Website: https://www.dbs.nus.edu.sg/research/ppc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