**Name:** (Chris) SHAM Lok To
**Title:** Assistant Professor
**Office Mailing Address:** 5 Science Drive 2, Level 3, Block MD4, Singapore 117545
**Email**: miclts@nus.edu.sg
**Contact No:** 6601-6356

**Academic Qualifications**

| | |
|---|---|
| 2012 | Ph.D. in Microbiology, Indiana University Bloomington |
| 2006 | M. Phil. in Biology, the Chinese University of Hong Kong |
| 2004 | B.Sc. in Molecular Biotechnology, the Chinese University of Hong Kong |
| 2004 | BBA minor in Integrated BBA program, the Chinese University of Hong Kong |

**Positions and Appointments**

| | |
|---|---|
| 2017- | Assistant Professor, Infectious Diseases Translational Research Programme and Department of Microbiology and Immunology, National University of Singapore |
| 2024- | Research Director, Infectious Diseases Translational Research Programme, National University of Singapore |
| 2023- | Executive Board Member, Asian Federation of Biotechnology |
| 2020- | Vice President, Singapore Society of Microbiology and Biotechnology |
| 2022-2024 | Deputy Research Director, Infectious Diseases Translational Research Programme, National University of Singapore |
| 2019-2020 | Visiting Assistant Professor, Department of Microbiology, Harvard Medical School |
| 2012-2017 | Postdoctoral fellow, Department of Microbiology and Immunobiology, Harvard Medical School |

**Awards and Prizes**

| | |
|---|---|
| 2024 | The Graduate Mentor of the Year Award, Yong Loo Lin School of Medicine, NUS |
| 2024 | The 2024 Rising Star in Biological, Medicinal, and Pharmaceutical Chemistry, American Chemical Society (ACS) |
| 2019-2023 | Singapore National Research Foundation Fellowship Class 2019 |
| 2019-2021 | National Medical Research Council Open Fund Young Individual Research Grant (withdrawn in favor of the NRF fellowship) |
| 2014-2016 | American Heart Association Postdoctoral Fellowship |
| 2012 | Floyd Award for Outstanding Publication in Microbiology, Indiana University Bloomington |
| 2011 | NSF Travel Award to Molecular Genetics of Bacteria and Phages meeting, Madison, Wisconsin |
| 2011 | Floyd Travel Award, Indiana University Bloomington |
| 2008-2011 | Floyd Microbiology Fellowship, Indiana University Bloomington |
| 2006-2007 | Muller Fellowship, Indiana University Bloomington |
| 2005-2006 | Oversea Travel Fellowship, The Chinese University of Hong Kong |
| 2004-2006 | Graduate Student Fellowship, The Chinese University of Hong Kong |

**Personal Statement:** I am a bacterial geneticist and molecular biologist. My passion for *Streptococcus pneumoniae* starts with my training with Dr. Malcolm Winkler at Indiana University Bloomington, a leading expert on *S. pneumoniae* and bacterial pathogenesis. In his laboratory, I identified the FtsEX complex as a regulator that controls cell wall hydrolase activity during cell division. This line of research continued after I established my laboratory (**section A**). After completing my Ph.D., I joined Dr. Thomas Bernhardt at Harvard Medical School. In his team, I established MurJ as the peptidoglycan precursor transporter (or 'flippase') and elucidated the determinants for the specificity of polysaccharide flippases (**section B**). Since I started my laboratory in 2017, my research focused on the synthesis and regulation of capsular polysaccharides (CPS) in *S. pneumoniae* (**section C**). We also seek to understand how the CPS interacts with the host. This research program will inform vaccine development against CPS-producing pathogens, such as *Klebsiella pneumoniae*, *Neisseria meningitidis*, and Group B Streptococci.

**Contribution to Science**

**A. Elucidate the coordination between cell wall remodeling and cell division.**

To split the daughter cells, the bacterial cell envelope undergoes coordinated remodeling. The cell wall hydrolases involved must be carefully controlled. Otherwise, the integrity of the cell wall will be compromised, leading to cell lysis. The essential cell wall hydrolase PcsB interacts with the cell division protein FtsX. This interaction ensures cell wall hydrolysis coordinates with cell expansion. The FtsEX regulatory mechanism is widely conserved and is identified in

many prokaryotes like *Escherichia coli*, *Bacillus subtilis*, *Mycobacterium tuberculosis*, and *Caulobacter crescentus*. In collaboration with Drs. Luo Min (NUS) and Juan Hermoso (the Instituto de Química-Física "Rocasolano"), we solved the structures of FtsEX in three species with their cognate peptidoglycan hydrolases and regulators. These structures support a universal "inside-out" mechano-transmission mechanism that involves the displacement of multiple regulatory helices, which ultimately activates the peptidoglycan hydrolase on the other side of the membrane.

1. M. Alcorlo, S. Martínez-Caballero, J. Li, **L.-T. Sham**, M. Luo, J. A. Hermoso, Modulation of the lytic apparatus by the FtsEX complex within the bacterial division machinery. FEBS Lett, doi: 10.1002/1873-3468.14953 (2024).

2. J. Li, Y. He, X. Xu, M. Alcorlo, J. Shi, D. I. Roper, J. A. Hermoso, **L.-T. Sham,** M. Luo, Structural insights into peptidoglycan hydrolysis by the FtsEX system in *Escherichia coli* during cell division. Elife. *Reviewed pre-printed https://doi.org/10.7554/eLife.94336.1* (2024).

3. J. Li, X. Xu, J. Shi, J. A. Hermoso*, **L.-T. Sham*,** M. Luo*, Regulation of the Cell Division Hydrolase RipC by the FtsEX system in *Mycobacterium Tuberculosis*. Nat Commun. 14, 7999 (2023). *co-corresponding authors.

4. X. Xu, J. Li, W.-Z. Chua, M. A. Pages, J. Shi, J. A. Hermoso*, T. Bernhardt*, **L.-T. Sham***, M. Luo*, Mechanistic insights into the regulation of cell wall hydrolysis by FtsEX and EnvC at the bacterial division site. Proc Natl Acad Sci U S A. 120, e2301897120 (2023). *co-corresponding authors

5. **L.-T. Sham**, K. R. Jensen, K. E. Bruce, M. E. Winkler, Involvement of FtsE ATPase and FtsX extracellular loops 1 and 2 in FtsEX-PcsB complex function in cell division of *Streptococcus pneumoniae* D39. mBio. 4, e00431-13 (2013).

6. J. Meisner, P. Montero Llopis, **L.-T. Sham**, E. Garner, T. G. Bernhardt, D. Z. Rudner, FtsEX is required for CwlO peptidoglycan hydrolase activity during cell wall elongation in *Bacillus subtilis*. Mol Microbiol. 89, 1069–1083 (2013).

7. **L.-T. Sham**, S. M. Barendt, K. E. Kopecky, M. E. Winkler, Essential PcsB putative peptidoglycan hydrolase interacts with the essential FtsXSpn cell division protein in *Streptococcus pneumoniae* D39. Proc Natl Acad Sci U S A. 108, E1061-1069 (2011).

8. S. M. Barendt, A. D. Land, **L.-T. Sham**, W.-L. Ng, H.-C. T. Tsui, R. J. Arnold, M. E. Winkler, Influences of capsule on cell shape and chain formation of wild-type and *pcsB* mutants of serotype 2 *Streptococcus pneumoniae*. J Bacteriol. 191, 3024–3040 (2009).

**B. Identify the transporter for precursors of cell envelope synthesis in bacteria.**
The identity of the flippase that translocates cell wall precursors across the cytoplasmic membrane has been elusive for decades. My work established the MOP-family protein MurJ is the transporter of lipid-linked peptidoglycan precursor in *Escherichia coli*. In collaboration with Drs. Ry Young, Andrew Kruse, and David Rudner's laboratories, we solved the structure of MurJ, identified a protein antibiotic that inhibits it, and discovered an alternative pathway that bypasses its function in *Bacillus subtilis*. After establishing my independent laboratory, we switched focus to the pneumococcal capsule flippases. This model enabled us to address the long-standing question of how MOP-family flippases select their substrates.

1. W.-Z. Chua, R. L. E. Wong, Y.-Y. Chun, N. C. S. Ng, T. Su, M. Maiwald, K. L. Chew, R. T.-P. Lin, A. M. Hockenberry, M. Luo, **L.-T. Sham**, Massively parallel barcode sequencing revealed the interchangeability of capsule transporters in *Streptococcus pneumoniae*. Sci Adv. in review (2024).

2. L. Yu[#], X. Xu[#], W.-Z. Chua[#], H. Feng[#], Z. Ser, K. Shao, J. Shi, Y. Wang, Z. Li, R. M. Sobata, **L.-T. Sham***, M. Luo*, Structural basis of peptide secretion for quorum sensing by ComA. Nat Commun. 14, 7178 (2023). [#]contributed equally. *co-corresponding authors.

3. W.-Z. Chua, M. Maiwald, K. L. Chew, R. T.-P. Lin, S. Zheng, **L.-T. Sham**, High-throughput mutagenesis and cross-complementation experiments reveal substrate preference and critical residues of the capsule transporters in *Streptococcus pneumoniae*. mBio. 12, e0261521 (2021).

4. S. Zheng, **L.-T. Sham**, F. A. Rubino, K. P. Brock, W. P. Robins, J. J. Mekalanos, D. S. Marks, T. G. Bernhardt, A. C. Kruse, Structure and mutagenic analysis of the lipid II flippase MurJ from *Escherichia coli*. Proc Natl Acad Sci U S A. 115, 6709–6714 (2018).

5. **L.-T. Sham**, S. Zheng, A. A. Yakhnina, A. C. Kruse, T. G. Bernhardt, Loss of specificity variants of WzxC suggest that substrate recognition is coupled with transporter opening in MOP-family flippases. Mol Microbiol. 109, 633–641 (2018).

6. K. R. Chamakura, **L.-T. Sham**, R. M. Davis, L. Min, H. Cho, N. Ruiz, T. G. Bernhardt, R. Young, A viral protein antibiotic inhibits lipid II flippase activity. Nat Microbiol. 2, 1480–1484 (2017).

7. A. J. Meeske, **L.-T. Sham**, H. Kimsey, B.-M. Koo, C. A. Gross, T. G. Bernhardt, D. Z. Rudner, MurJ and a novel lipid II flippase are required for cell wall biogenesis in *Bacillus subtilis*. Proc Natl Acad Sci U S A. 112, 6437–6442 (2015).

8. **L.-T. Sham**, E. K. Butler, M. D. Lebar, D. Kahne, T. G. Bernhardt, N. Ruiz, Bacterial cell wall. MurJ is the flippase of lipid-linked precursors for peptidoglycan biogenesis. Science. 345, 220–222 (2014).

**C. Investigate the mechanism and regulation of capsule synthesis.**
The capsular polysaccharide (CPS) is one of the most important virulence factors in *S. pneumoniae.* It is the target of all clinically relevant pneumococcal vaccines. Our research program focuses on understanding the molecular mechanisms behind the synthesis and regulation of CPSs. For example, we elucidated how CPS synthesis is coordinated with cell division, uncovered the role of thermosensors and quality control proteins in regulating CPS synthesis, and identified the glycan motifs that promote binding to epithelial cells. Our research is supported by an international team of experts, including the laboratories of Birgitta Henrique-Normark, Edmund Loh, and Staffan Normark from Karolinska Institutet, Xueli Guan from NTU, and Gan Yunn Hwen, Vincent Chow, Wang De Yun, and Yang Daiwen from NUS. The CPS can also be found in nearly half of the bacterial species. Thus, our research is broadly relevant and promises to inform new avenues for vaccines and therapeutics.

1. T. Su, W.-Z. Chua, Y. Liu, J. Fan, S.-Y. Tan, D.-W. Yang, **L.-T. Sham**, Rewiring the pneumococcal capsule pathway for investigating glycosyltransferase specificity and genetic glycoengineering. Sci Adv. eadi8157 (2023).

2. R. Nakamoto, S. Bamyaci, K. Blomqvist, S. Normark, B. Henriques-Normark, **L.-T. Sham**, The divisome but not the elongasome organizes capsule synthesis in *Streptococcus pneumoniae*. Nat Commun. 14, 3170 (2023).

3. Y.-Y. Chun, K. S. Tan, L. Yu, M. Pang, M. H. M. Wong, R. Nakamoto, W.-Z. Chua, A. Huee-Ping Wong, Z. Z. R. Lew, H. H. Ong, V. T. Chow, T. Tran, D. Yun Wang, **L.-T. Sham**, Influence of glycan structure on the colonization of *Streptococcus pneumoniae* on human respiratory epithelial cells. Proc Natl Acad Sci U S A. 120, e2213584120 (2023).

4. T. Su, R. Nakamoto, Y.-Y. Chun, W.-Z. Chua, J.-H. Chen, J. J. Zik, **L.-T. Sham**, Decoding capsule synthesis in *Streptococcus pneumoniae*. FEMS Microbiol Rev. 45, fuaa067 (2021).

5. R. Nakamoto, J. M. C. Kwan, J. F. L. Chin, H. T. Ong, J. Flores-Kim, C. Midonet, M. S. VanNieuwenhze, X. L. Guan, **L.-T. Sham**, The bacterial tyrosine kinase system CpsBCD governs the length of capsule polymers. Proc Natl Acad Sci U S A. 118, e2103377118 (2021).

6. H. Eichner, J. Karlsson, L. Spelmink, A. Pathak, **L.-T. Sham**, B. Henriques-Normark, E. Loh, RNA thermosensors facilitate *Streptococcus pneumoniae* and Haemophilus influenzae immune evasion. PLoS Pathog. 17, e1009513 (2021).

7. Y. H. Tan, Y. Chen, W. H. W. Chu**, L.-T. Sham**, Y.-H. Gan, Cell envelope defects of different capsule-null mutants in K1 hypervirulent *Klebsiella pneumoniae* can affect bacterial pathogenesis. Mol Microbiol. 113, 889–905 (2020).

8. V. Sender, K. Hentrich, A. Pathak, A. Tan Qian Ler, B. T. Embaie, S. L. Lundström, M. Gaetani, J. Bergstrand, R. Nakamoto, **L.-T. Sham**, J. Widengren, S. Normark, B. Henriques-Normark, Capillary leakage provides nutrients and antioxidants for rapid pneumococcal proliferation in influenza-infected lower airways. Proc Natl Acad Sci U S A. 117, 31386–31397 (2020).

**Ongoing research projects as the primary PI (for grant SGD>100,000 only):**

- Exploiting capsule synthesis pathway in *Streptococcus pneumoniae* to identify new antimicrobial compounds. NRF fellowship, class of 2019; **SGD$2,903,484**; 1/4/2019 to 31/03/2024
    - The overall goal of this project is to investigate the mechanism of capsule synthesis in pneumococcus and identify inhibitors that block this process. This project focuses on studying the specificity determinants of the capsule flippases and glycosyltransferases using the conditional essentiality of these enzymes.

- Investigate the innate and adaptive immune response against capsular polysaccharides in *Streptococcus pneumoniae*. MoE Tier-2 grant; **SGD$783,252**; 1/11/2021-31/10/2024.
    - This project aims to elucidate innate and adaptive immunity against various capsular polysaccharides (CPS) from *S. pneumoniae*. The response from epithelial cells will be measured after exposure to different CPS. We will also conduct animal experiments to understand the antigenicity of structurally diverse glycans.

- Identify novel antimicrobial targets by high-throughput profiling of genetic interactions in human respiratory pathogens. NMRC OF-IRG grant; **SGD$1,455,053**; 1/9/2023-31/8/2026.

- With the rapid advancements in sequencing technologies, there is an explosion of genome information in the public database. Yet, about one-third of bacterial genes have no known function. To address this issue, we developed two new approaches called dual RB Tn-seq and E-seq for identifying genetic interactions in a bacterial genome. The genetic interactions identified will be used to design pathway-directed screens.

**Completed research projects as the primary PI:**

- Capsular polysaccharide biosynthesis in *Streptococcus pneumoniae*. NUHS start-up grant; SGD**$300,000**; 1/11/2017 to 31/10/2021
  - The University start-up grant aims to help my research group build infrastructure and generate preliminary results on capsule synthesis in pneumococcus.

**Patent**: T. Su*, **L.-T. Sham*** Modified Bacterial Glycans and Conjugates thereof; PCT/SG2023/050010. *co-inventor; all contributors are from my laboratory.

**Service**

| Year | Events |
|---|---|
| July 2024 | Reviewer, Swiss National Science Foundation Grant, Switzerland. |
| May 2024-present | Editorial Board Member, Journal of Microbiology. |
| Mar 2024 | Reviewer, Programmes Transversaux de Recherche (PTR grant) n°1775, Institut Pasteur, France. |
| Feb 2024-present | Head, Department Microbial Culture Collection (DMCC), Department of Microbiology and Immunology, National University of Singapore. |
| Feb 2024-present | Editorial Board Member, Infection and Immunity. |
| Dec 2023 | Reviewer, MRC Infections and Immunity Programme, United Kingdom Research and Innovation, United Kingdom. |
| Dec 2023 | Reviewer, Discovery Grants Program, Natural Sciences and Engineering Research Council of Canada (NSERC), Canada. |
| Oct 2023 | Reviewer and Committee Member, University Research Committee Expert Panel (Biomedical Engineering and Life Sciences), National University of Singapore. |
| Mar 2023-present | Academic committee member and the Capstone Project coordinator of the Master of Science in Applied Biomedicine, Yong Loo Lin School of Medicine, National University of Singapore. |
| July 2022 to present | Co-organizer of the Bacterial UltraGoup (BUG) Singapore with Dr. Qiao Yuan at NTU. BUG consists of nine research labs in Singapore who congregate to share ideas, unpublished work, and latest technologies. The group has been established for about 15 years and is gaining traction regionally and internationally. |
| Aug 2022 | Organizer of the Amgen-SSMB Connect with the Industry seminar. We hosted Dr. Feras Hatahet, Principal Investigator at Amgen. Dr. Hatahet is the founder of Genophore and has extensive experience in drug discovery. |
| Sep 2021 | Organizer of the Pfizer-SSMB Connect with the Industry seminar. We invited several speakers from Pfizer to introduce the company and link students who are interested in an industry job with Pfizer. |
| Feb 2021-Feb 2024 | Organizer of the NCID IDTRP joint seminar series. The National Centre for Infectious Diseases is co-hosting a seminar series with IDTRP, which typically attracts ~100 to ~200 clinicians, scientists, postdocs, and students to attend. |
| Sep 2019-present | Consultant panelist for the finalists of the NRF fellowship. |
| Jan 2018-present | Department co-coordinator for UROPS projects with A/P Justin Chu. |