Submission Date: 05/27/2024

## 2023 Academic Year Bio-SPMs Collaborative Research Research Report Summary

Title of the research project		Pore formation of alpha-toxin from Staphylococcus aureus and its	
		toxoids investigated by Atomic Force Microscopy	
PI	Name	Assoc. Prof. Dr. Nguyen Duc Hoang	
(Person in	Affiliated Institution and	University of Science, Ho Chi Minh City, Vietnam	
charge of the	Department/Division/etc.	(Vietnam National University, Ho Chi Minh City) / Center for	
research		Bioscience and Biotechnology	
project)	Position	Director	
			Atomic resolution/3D-AFM
Bio-SPMs that you used		$\overline{\mathbf{A}}$	High-speed AFM
(Check the boxes)			SICM
			AFM for Cell Measurement
Collaborative NanoLSI Faculty Members			Dr. Ngo Xuan Kien

Describe the summary of the research project

Staphylococcus aureus is a dangerous pathogen with many virulence factors, the most prominent of which is alpha-toxin (also known as alpha-hemolysin, Hla). Hla is the first bacterial exotoxin identified as pore-forming in lipid membranes. The research project aims to elucidate the pore formation of Hla on bilayer lipids. This project used biochemical experiments and High-speed AFM. Hla and its mutants (W179A/R200A) were successfully constructed, purified, and available for all experiments. We made liposomes composed of different lipid compositions to investigate the pore formation of Hla in lipid membranes. The mechanism of Hla pore formation includes three steps: (i) association of monomers to a lipid bilayer; (ii) oligomerization into pre-pore on the lipid bilayer; and (iii) transition from the pre-pore to the pore state. We have elucidated the pore formation process of Hla in lipid membranes and shown that the pre-pore structure forms with beta-barrel before insertion into the membrane to form the pore structure. Additionally, the dependence of pore formation by Hla on cholesterol was demonstrated. We are also developing a data analysis method to analyze big data acquired by HS-AFM.

<sup>\*</sup>This form (Form 3) will be open on the NanoLSI website in the following academic year.

<sup>\*</sup>Note that this form should be prepared in one A4-size paper.

<sup>\*</sup>Submission Deadline: May 10, 2024 (Friday). Submit it as a PDF file.

<sup>\*</sup>Submission Destination: the person in charge of Bio-SPMs collaborative research at WPI-NanoLSI, Kanazawa University Email: <a href="mailto:nanolsi">nanolsi</a> openf01@ml.kanazawa-u.ac.ip