Submission Date: 03/29/2025

2024 Academic Year Bio-SPMs Collaborative Research
Research Report Summary

Title of the research project		High-speed atomic force microscopy accelerates designs and development of protein-based vaccines and drugs for controlling pathogenic *Staphylococcus aureus*	
PI (Person in charge of the research project)	Name	Dr. Le Thi Phuong Ngan	
	Affiliated Institution and Department/Division/etc.	University of Science, Ho Chi Minh City, Vietnam (Vietnam National University, Ho Chi Minh City)	
	Position	Researcher	
Bio-SPMs that you used (Check the boxes)		☐	Atomic resolution/3D-AFM
		☑	High-speed AFM
		☐	SICM
		☐	AFM for Cell Measurement
Collaborative NanoLSI Faculty Members		Dr. Ngo Xuan Kien	

Describe the summary of the research project

Staphylococcus aureus is one of six highly virulent and antibiotic-resistant bacterial pathogens (ESKAPE). Among its exotoxins, alpha-hemolysin (Hla) is a key cytotoxin that forms pores in host cell membranes, contributing to pathogenicity. Despite extensive research, no approved vaccine exists. This study aims to develop a protein-based vaccine using a non-cytolytic Hla mutant and explore therapeutic inhibitors targeting Hla toxicity.

High-Speed Atomic Force Microscopy (HS-AFM) was employed to analyze the structural properties of Hla variants, focusing on:

(i) Designing and visualizing HlaW179AR200A pores immobilized on silica nanoparticles to develop a vaccine against Hla toxicity.

(ii) Screening and elucidating novel inhibitory compounds that prevent Hla pore formation in lipid membranes, leading to new therapeutic strategies.

Key Findings:

- Hla variant visualization: HS-AFM imaging confirmed that HlaH35A and HlaH35L formed pore-like structures, while HlaH35LH48L remained monomeric, indicating disrupted oligomerization.
- Effect of β-Cyclodextrin (CD): CD destabilized HlaWT pores, showing potential as an inhibitor.
- Silica nanoparticle-based vaccine: HS-AFM confirmed that HlaW179AR200A forms stable pre-pore structures on silica nanoparticles, supporting its vaccine potential.

This study highlights the structural dynamics of Hla variants and provides insights into vaccine development and novel therapeutic interventions against *S. aureus* infections.

*This form (Form 3) will be open on the NanoLSI website in the following academic year.

*Note that this form should be prepared in one A4-size paper.

*Submission Deadline: May 9, 2025 (Friday). Submit it as a PDF file.

*Submission Destination: the person in charge of Bio-SPMs collaborative research at WPI-NanoLSI, Kanazawa University

Email: **nanolsi_openf01@ml.kanazawa-u.ac.jp**