Submission Date: 07/29/2025

2024 Academic Year Bio-SPMs Collaborative Research
Research Report Summary

Title of the research project		Dynamic and functional analysis of vinculin oligomerization induced by *Shigella* IpaA	
PI (Person in charge of the research project)	Name	Guy TRAN VAN NHIEU	
	Affiliated Institution and Department/Division/etc.	Cell Biology Department, I2BC-CNRS UMR9198-Inserm U1280	
	Position	Head of team, Principal Investigator	
Bio-SPMs that you used (Check the boxes)		☐	Atomic resolution/3D-AFM
		☒	High-speed AFM
		☐	SICM
		☐	AFM for Cell Measurement
Collaborative NanoLSI Faculty Members		Noriyuki KODERA	

Shigella, the causative agent of bacillary dysentery, invades epithelial cells by injecting type III effectors that locally reorganize the actin cytoskeleton. The *Shigella* type III invasion effector IpaA targets vinculin (HV). HV is a component of cell-matrix and cell-cell adhesion classically described as a three-domain protein containing a head (Vh) and carboxyterminal tail (Vt) domain connected by a flexible linker. Activating VBSs disrupt the Vh-Vt intramolecular interactions to free the F-actin binding region in Vt. We found that the cooperative action of IpaA, vinculin undergoes Vh-mediated vinculin trimerization. The aim of the collaborative works is to characterize the dynamics and mechanism of IpaA-induced formation of vinculin oligomers, as well as their actin bundling and talin binding functions.

Using High-Speed AFM (HS-AFM), we found that vinculin (HV) spontaneously unfold of its tail domain Vt on mica. We confirmed that constructs containing the Shigella IpaA VBS1,2 and VBS1-3 induce the formation of HV dimers and trimers, respectively. HS-AFM allowed to visualize the domain organization of HV oligomers, with a domain stably interacting with mica connected to Vt-containing mobile domains by a linker region. In HV alone, this linker region was determined to span 14 ± 9 nm (mean ± SD), consistent with the prediction of its 43 amino acid length during canonical activation. Unexpectedly, our measurements indicated that the linker region between the stable and mobile domains in HV trimers could extend twice this length. The HS-AFM simulated image of the HV trimer stable amino terminal domain docks an atomic model of a trimer of the HV D1D2 subdomains.

*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

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