Submission Date: 05/06/2024

2023 Academic Year Bio-SPMs Collaborative Research Research Report Summary

Title of the research project		High	resolution imaging of lactose permease (LacY) from	
		Escherichia coli reconstituted in different lipidic environments		
PI	Name	Oscar Domenech Cabrera		
(Person in	Affiliated Institution and	University of Barcelona (Spain)		
charge of the	Department/Division/etc.	Department of Pharmacy, Pharmaceutical Technology and		
research		Physical Chemistry		
project)	Position	Professor		
			Atomic resolution/3D-AFM	
Bio-SPMs that you used		X	High-speed AFM	
(Check the boxes)			SICM	
			AFM for Cell Measurement	
Collaborative NanoLSI Faculty Members		Assit. Prof. Kien Xuan Ngo and Professor Toshio Ando		

Lactose permease protein (LacY) serves as a fundamental model for secondary transporters, with its structure and function intricately tied to the lipidic environment in which it operates. Acting as a symporter for lactose and H⁺, LacY undergoes a conformational shift in response to changes in pH or potassium concentration, facilitating the passage of lactose through the membrane.

In this preliminary investigation we focused on visualizing and characterizing LacY embedded in four different lipid membrane compositions: POPC, POPC:POPG (3:1, mol/mol), POPC:POPE:POPG (1.5,1.5,1.0, mol/mol/mol) and the polar lipid extract from *E. coli* bacterium. The experiments were designed to evaluate the conformational changes of LacY when transporting its substrate using a gradient of potassium ions between the proximal (close to mica surface) and the distal leaflets of the lipid bilayer. The study of lipid bilayers containing LacY in the different lipid compositions revealed significant lateral diffusion of the individual protein molecules. We evaluate LacY diffusion rate obtaining values indicating that the protein can diffuse several times its own size in the timeframe of substrate transport across the lipid membrane.

This preliminary study has brought light on how LacY interact with lipid matrices evidencing that the main issue to obtain a high-resolution image of the protein is its diffusion throughout the lipid matrix.