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2019 Academic Year Bio-SPMs Collaborative Research
Research Report Summary

Title of the research project		Polymerization dynamics and filament architecture of the bacterial tubulin FtsZ at high spatiotemporal resolution	
PI (Person in charge of the research project)	Name	Dr. Batirtze Prats Mateu, MSc.	
	Affiliated Institution and Department/Division/etc.	Institute of Science and Technology (IST) Martin Loose Laboratory	
	Position	Postdoc researcher	
Bio-SPMs that you used (Check the boxes)		☐	Super-resolution AFM (FM-AFM/3D-AFM)
		☒	High-speed AFM (HS-AFM)
		☐	SICM
Collaborative NanoLSI Faculty Members		Prof. Richard Wong	

FtsZ, a bacterial homologue of tubulin, is the main organizer of bacterial cell division. In the living cell, FtsZ filaments organize into the so-called Z-ring, a cytoskeletal structure (the future division site) that then recruits the other components of the cell division machinery. FtsZ shows treadmilling behavior *in vitro on* supported lipid bilayers and can form large-scale patterns through self-organization.

The *in vitro* reconstitution mimics the bacterial inner membrane and thus confers near-native conditions. We were able to visualize for the first time the polymerization *in situ* of FtsZ (and two mutants conferring stronger and weaker lateral interactions) using HS-AFM in the presence of the membrane anchor FtsA and the additional FtsZ filament-crosslinker ZapA.

The dynamic behavior of this protein “orchestra” self-organization was monitored at the very beginning with one FtsZ-filament (~4 nm ∅) resolution. The three FtsZ proteins showed different dynamics and assembly patterns, which contribute to their also distinct large-scale organization. The presence of the crosslinker ZapA also affected their architecture further. The interfilament distance of the pattern calculated from HS-AFM movies about 2-fold for the FtsZ WT when compared to the mutant with strong lateral interactions. Upon addition of ZapA, for FtsZ WT, dynamic comet-like structures between the filaments were visible for the first time and the interfilament distance increased about 20% whereas for the mutant thicker bundles (clusters of lateral bound filaments) were observed. This indicates a different ZapA effect in the WT and mutant version of FtsZ, probably due to a different binding strategy between them. For the mutant with weaker lateral interactions, no quality images were achieved, even at very high concentrations. Altogether, the results show the important role of lateral interactions for mechanical stability of the FtsZ pattern on the membrane. In addition, we observed different filament persistence length (a measure of rigidity) between WT, mutants and the presence or absence of ZapA.