

Submission Date: 05/26/2020

2019 Academic Year Bio-SPMs Collaborative Research Research Report Summary

Title of the research project		Single molecule imaging of DNA gyrase activity on DNA origami	
PI (Person in charge of the research project)	Name	Yusuke Sakai	
	Affiliated Institution and Department/Division/etc.	Jagiellonian University/Malopolska Centre of Biotechnology	
	Position	Postdoc researcher	
Bio-SPMs that you used (Check the boxes)		<input type="checkbox"/>	Super-resolution AFM (FM-AFM/3D-AFM)
		<input checked="" type="checkbox"/>	High-speed AFM
		<input type="checkbox"/>	SICM
Collaborative NanoLSI Faculty Members		Dr. Ayumi Sumino	
<p>DNA gyrase is a prokaryotic type II topoisomerase which controls gene expression by introducing negative supercoil to bacterial genome. As DNA gyrase catalyses double strand break of genome during the process, its inhibitors kill bacteria efficiently and specifically and thus it is an excellent drug discovery target. Direct observation of DNA gyrase turnover will bring profound insight of its mechanism. We designed a novel DNA nanostructure by DNA origami method to scaffold DNA gyrase for high-speed AFM (HS-AFM) observation. The DNA origami scaffold offers well-balanced liberty for stable HS-AFM imaging of active gyrase, thanks to tight interaction of DNA origami scaffold to mica stage. Here, we set the major goal to visualise the catalytic cycle of DNA gyrase directly using HS-AFM and DNA origami scaffold.</p> <p>We optimised of the protocols and the conditions for the HS-AFM imaging of DNA gyrase using DNA origami scaffold. The optimal protocol successfully spread the DNA origami scaffold over mica stage in nearly 100% coverage to accommodate DNA gyrase in higher than 65% of occupancy yield. DNA gyrase was observed as multidomain particles consisting of three to four domains connected to each other. Though the characterisation was limited yet, substantial transition of domains of DNA gyrase was observed. Further research is required to determine domains of gyrase in AFM image and to observe the enzymatic turnover of DNA gyrase in real-time.</p>			

*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 8, 2020 (Friday). **Submit it as a PDF file.**

*Submission Destination: the person in charge of Bio-SPMs collaborative research at WPI-NanoLSI, Kanazawa University (Bio-spmscr_nano@ml.kanazawa-u.ac.jp)