Submission Date: 05/08/2020

# 2019 Academic Year Bio-SPMs Collaborative Research
# Research Report Summary

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| Title of the research project | | Elucidating molecular basis of kinetochore-microtubule interaction: probing dynamic interaction of kinetochore protein complexes with microtubules | |
| PI (Person in charge of the research project) | Name | TAPAS KUMAR MANNA | |
| | Affiliated Institution and Department/Division/etc. | School of Biology, Indian Institute of Science Education and Research (IISER) Thiruvananthapuram, India | |
| | Position | Professor | |
| Bio-SPMs that you used (Check the boxes) | | ☐ | Super-resolution AFM (FM-AFM/3D-AFM) |
| | | ✓ | High-speed AFM |
| | | ☐ | SICM |
| Collaborative NanoLSI Faculty Members | | NORIYUKI KODERA | |

The microtubule-binding proteins, EB1 and Ska1, both individually and in mixture were imaged by HS AFM. Images of EB1 clearly showed the dynamics of its globular N-terminal domain and the coiled-coil C-terminus. While the two coiled-coiled C-terminal domains of two EB1 molecules being tightly associated, the N-terminal heads of two EB1 molecules exhibit dynamic movements of expansion and contraction through the connecting central interlinking regions of two EB1 molecules. The Imaging of Ska1 showed its existence as a monomer and further, showed its N- and C-terminal domains connected by a flexible linker region. The two domains also exhibited dynamic movement on both sides of the linker, but the dynamicity was less vigorous. HS AFM images of the mixture of EB1 and Ska1 revealed formation of extended organized structures, which were distinct than the individual EB1 and Ska1. Our biochemical results indicated that the flexible loop region of Ska1 is the main interacting domain of Ska1 with EB1. Therefore, by HS AFM, we sought to visualize how EB1-Ska1 structures are affected in the absence of Ska1 loop region. HS AFM images of Ska1-Δloop clearly showed the absence of the loop and the close localization of the N-and C-terminal domains. The images of the mixture of EB1 and Ska1-Δloop showed no apparent association of the Ska1-Δloop molecule with EB1 dimer, even though the two proteins appeared to be localized in close proximity. Next, we acquired microtubules dynamics images in control and in the presence of DAM1 ring-bound microtubules and observed the DAM1 structure to stabilize the microtubules more time in the pause state. We next imaged the outer kinetochore protein complex NDC80 and its association with microtubules. NDC80 complex components Hec1 and Nuf2 were resolved clearly and stabilization of microtubule dynamics by the complex was observed.