Submission Date: 05/08/2023

2022 Academic Year Bio-SPMs Collaborative Research
Research Report Summary

Title of the research project		Mechanosensitive behavior of recombinant myosin 1b and myosin 5b motor domain constructs in the presence or absence of mechanical loads studied by HS-AFM	
PI (Person in charge of the research project)	Name	Dr. Oleg Matusovsky	
	Affiliated Institution and Department/Division/etc.	McGill University, Department of Kinesiology and Physical Education	
	Position	Staff Research Associate	
Bio-SPMs that you used (Check the boxes)		☐	Super-resolution AFM (FM-AFM/3D-AFM)
		☑	High-speed AFM
		☐	SICM
Collaborative NanoLSI Faculty Members		Prof. Noriyuki Kodera	

The protein constructs of myosin-1b (Myo1b) and myosin-5b (Myo5b) produced in *Dictyostelium discoideum* were visualized by HS-AFM. Each monomer contains a long rod-shaped domain composed of two (2R) or three (3R) *Dictyostelium* alpha-actinin repeats used as an artificial lever arm. In both constructs, the actin-binding loop 2 (amino acids 647–683) in the motor domain was engineered to contain six consecutive GKK motifs, which form a flexible cluster of positive charges. Since a dimeric structure is essential for processivity, the protein monomers were fused by a Leucine Zipper domain (LZ) from the transcriptional activator GCN4 in the C terminus of the constructs. The dimerization of the Myo1b or Myo5b is strengthened by the introduction of the mutation Arg238Asp with the creation of an additional interchain interaction in the LZ. The Myo1b or Myo5b constructs were placed on mica surface in solution, containing 100 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 25 mM imidazole, pH 7.0. According to the molecular structure, the dimers of Myo1b or Myo5b with two motor domains and two artificial lever arms were observed. The size of the lever arm in the 2R and 3R constructs were calculated as ~13 nm ± 2.4 nm (SD) and ~18 nm ± 2.1 nm (SD). The variability in the measurements is caused by the flexibility of the lever arm and its motion during scanning, as well as the challenges in determining the location of the LZ domain in the dimers. The different length of the artificial lever arms allows us to test a lever arm hypothesis (i.e., the step size of molecular motor depends on the length of its lever arm) more precisely. Our next goal will be to study the processivity of dimers in the F-actin-Myo1b (first time for the non-processive myosin) or F-actin-Myo5b complexes at high spatiotemporal resolution in the presence of ATP. It might be useful to use such molecular cargos in medicine to cure different types of disease by delivering the treatment to a specific location or in lab-on-a-chip applications.

*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.