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Unraveling Pathological Mechanisms Of Muscular Dystrophy

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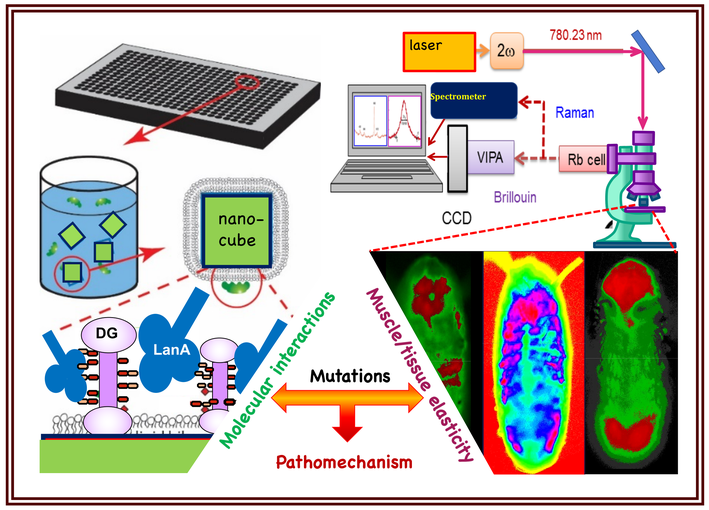

Background

Protein O-mannosylation (POM) is a posttranslational modification that has a profound effect on the development and physiology. Important target of POM is Dystroglycan (DG) that plays a crucial role in muscle physiology[1]. POM is particularly important for DG functions[2], and defects in POM modifications of DG cause severe muscular dystrophies called dystroglycanopathies[3]. Uncovering molecular mechanisms POM-mediated effect on DG activity is essential for understanding pathogenesis of dystroglycanopathies. However, the molecular mechanisms of POM function remain poorly understood due to complexity of glycosylation and limitations of in vivo approaches in mammalian organisms, which provides a major roadblock for mechanistic studies in this important biomedical area. To address these challenges, here we applied an interdisciplinary approach that combines the power of Drosophila genetics with innovative biophysical and biochemical strategies. Drosophila provides important advantages of simplified model, while offering a large arsenal of genetic tools, superb amenability, and efficient time-saving experimental protocols. Our previous results demonstrated that the POM pathway and its functions are conserved between mammals and Drosophila[4,5].

Summary

We developed a *Drosophila* model of muscular dystrophy and discovered that POM mutants have defect in muscle elasticity. In this project, we demonstrated that Brillouin spectroscopy-based approaches can be used for *in vivo* analyses of muscle elasticity (Fig.1). We also applied a unique nanotechnology-based approach to analyze binding of mammalian laminin, a DG extracellular ligand, to *Drosophila* DG purified from normal and pathological conditions (POM mutants) (Fig.2). Taken together, the results of the project contribute to our long-term goal to develop a comprehensive platform to study interconnected genetic, physiological, biochemical and biophysical mechanisms that underlie the function of POM in normal and pathological conditions. Our project provided conceptual foundation and initial data for further research into molecular pathological mechanisms of muscular dystrophies.

References

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Brillouin spectroscopy measurements of intact muscle elasticity in POM mutants

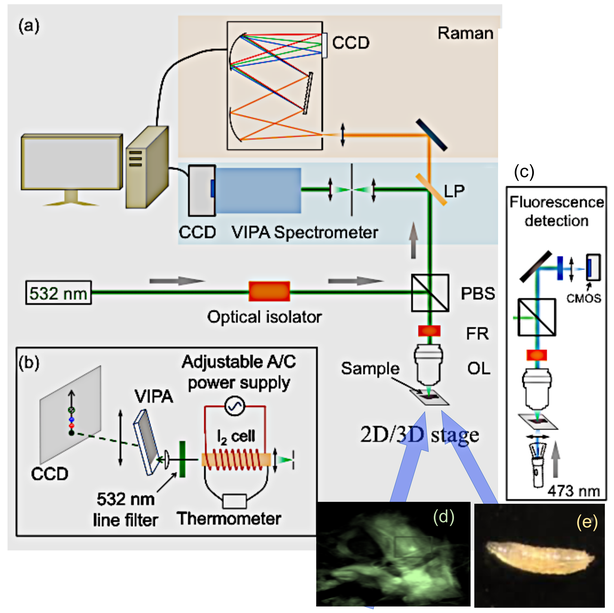

Figure 1A. Experimental setup of Brillouin microspectroscopy

Detection Tool:

Experimental setup (a-b): collimated beam is sent into a confocal microscope, while the back-scattered light is collected by the same objective and passed through a Rb cell to filter inelastically scattered light. Then the light is analyzed by a single-stage virtual image phase array (VIPA) spectrometer and CCD camera (b). In parallel, the sample is also monitored by fluorescent microscopy that visualized GFP-expressing muscles. Both types of samples, including dissected tissues (d) and intact live larvae (e) were analyzed by the technique.

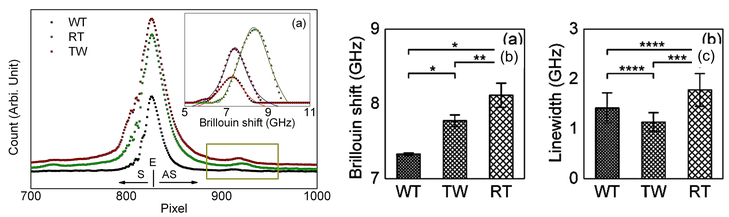

Figure 1A. Muscle elasticity of POM measured by Brillouin microspectroscopy. Brillouin spectra (a) of *Drosophila* muscles measured in wildtype (WT) and POM mutants (RT, TW) revealed significant differences in muscle elasticity (b) and organic composition (b)

Results 1:

- ❖ POM mutants revealed significant defects in muscle elasticity
- ❖ More prominent decrease in elasticity was detected in mutants with more severe disruption of protein O-mannosylation (RT vs TW)
- ❖ Next step: Analyses of DG^{-} mutant and DG^{-}/$POMT^{-}$ double mutant genotypes are expected to uncover functional relationships between muscle elasticity and POM modifications of DG.

Measurements of Laminin – Dystroglycan Interaction

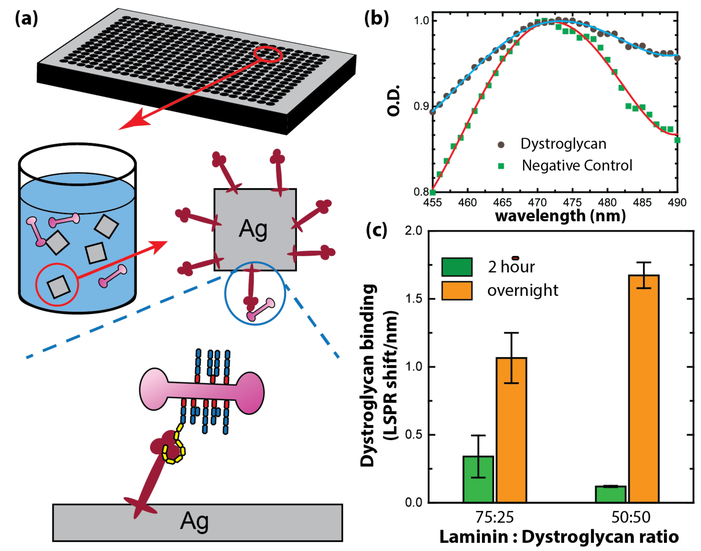

Figure 2. Analysis of binding between purified Drosophila DG and mouse laminin immobilized on nanocubes

Detection Tool:

- ❖ Laminin-coated silver nanocube sensor
- ❖ Detection Principle: Localized Surface Plasmon Resonance

Experiment:

- ❖ Dystroglycan were mixed with laminin-coated nanocube sensors with different mass ratios at 37 deg C in a 384 well plate.
- ❖ Dystroglycan bindings to laminin were monitored by UV-vis spectrometer. (Figure 2 (b)). The red shift of the spectra indicates Dystroglycan-laminin bindings.

Results 2:

- ❖ Laminin-Dystroglycan binding could be measured by nanocube sensor platform.
- ❖ Increase of Dystroglycan concentration enhance the bindings.
- ❖ Drosophila DG binding to mammalian laminin revealed remarkable conservation of DG-ligand interactions
- ❖ Mammalian laminin binding may be initiated by non-elongated O-mannosyl glycans (initial 'sliding' interactions), which is followed by strong binding to elongated glycans (matriglycan) (strong 'locking' interactions)
- ❖ Next step: Measuring interactions between laminin and mutated Dystroglycan.

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