THE UNIVERSITY OF ARIZONA
COLLEGE OF ENGINEERING
Biomedical Engineering

DEPARTMENT OF BIOMEDICAL ENGINEERING SEMINAR SERIES

PRESENTS

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"Structurally Defining the Flexible cTnT Linker and Determining Mutational Effects"

ABSTRACT:

Mutations throughout cardiac troponin T (cTnT), a component of the cardiac thin filament (CTF) cause changes in protein structure and dynamics leading to pathologic cardiac remodeling observed in patients with hypertrophic (HCM) and dilated (DCM) cardiomyopathies. Two mutational hotspots within the cTnT-linker region cause severe and highly penetrant cardiomyopathies. Our understanding of the molecular mechanisms involved has been limited by the lack of a high-resolution structure in this domain. We employed time-resolved fluorescence resonance energy transfer (TR-FRET) utilizing fully reconstituted CTFs with donor-labeled (IAEDANS) cTnT on one of four Cys-substituted residues (A168/177/192/198C) and acceptor-labeled (5-IAF) actin on Cys-374 to gain high-resolution insight into cTnT-linker's positioning across the actin filament. Our WT data suggest the cTnT-linker is proximal to 2-3 adjacent actin monomers in both +/- Ca^{2+} conditions. To determine how cTnT-linker mutations alter the native linker structure, we investigated three cardiomyopathy-linked mutations: DCM-associated R173W and R173Q, and HCM-associated Δ160E. We hypothesize that R173Q/W and Δ160E cause differential repositioning of the cTnT-linker in relationship to actin. Investigation of the mutations vs. WT cTnT-linker position showed a significant increase in the distance between cTnT-168 and the distal actin in the -Ca^{2+} condition. Additionally, R173W's proximal actin showed a significant distance increase. Upon addition of Ca^{2+}, there was no significant difference between WT vs. mutations' cTnT-linker positions. No other cTnT-linker sites exhibited a difference in both +/- Ca^{2+}. This suggests that while the cTnT-linker is unstructured, it is inherently flexible thus providing a "buffer" for structural changes and resulting movements. Furthermore, we are utilizing *in-vitro*-motility-assays to determine potential functional changes associated with these mutations. Through this approach, we can craft a cTnT-linker high-resolution structure and gain an understanding of region mutation-specific structural alterations.

Please join us on

Monday, April 19th, 2021

12:00-12:50 pm, https://arizona.zoom.us/j/85468611706

Hosts: Dr. DK Kang and Dr. Russ Witte
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