BCHM 421/422 Calpain and Muscular Dystrophy 2023-24

Project #2: Calpains are a family of intracellular proteases involved in calcium signaling. Calpain-3 is the isoform abundant in muscle thought to be involved in the repair of damaged myofibrils. There are ~500 different mutations in the human calpain-3 gene that cause a specific muscular dystrophy (Limb Girdle Muscular Dystrophy R1). These mutations are distributed all over the enzyme's four domains and three short unique sequences (NS, IS1 and IS2) one of which (IS2) is thought to be the binding site for titin in the sarcomere. We will use these three sequences in pulldown experiments with muscle cell extracts to identify their putative binding partners. By incorporating the muscular dystrophy mutations that occur in these three sequences we can see how their binding partners are affected and how this might result in Limb Girdle Muscular Dystrophy R1.

Supervisor: Peter L. Davies **TA:** Mathias Bell

Project Title: Limb girdle muscular dystrophy mutations in the calpain-3 protease

Keywords (3-5):

1. Recombinant protein
2. Protein purification
3. Pull-down assays
4. Protein identification
5. Protein-protein interactions

Project Goals: To isolate and identify muscle proteins that bind to the three unique sequences of muscle-specific calpain-3. Use this information to understand how this protease is regulated to cut only damaged muscle proteins.

Experimental Approaches: The NS, IS1, and IS2 unique sequences will be made by solid phase peptide synthesis with the addition of affinity tags. Using pull-down experiments with muscle cell extracts we will examine the proteins that bind to the three polypeptides and identify them by tandem mass spectrometry sequencing. Recombinant versions of these binding proteins will be produced for co-crystallography experiments with NS, IS1, and IS2. Introduction of LGMD R1 mutations into the polypeptides will done to see how they affect binding affinity.

References:

Campbell, R.L., Davies, P.L. (2012) Structure-function relationships in calpains. Biochem. J. 447(3), 335-351. PubMed: 23035980

Beckmann J.S., Spencer, M. (2008) Calpain 3, the "gatekeeper" of proper sarcomere assembly, turnover and maintenance Neuromuscul. Disord. 18(12):913-21.