BCHM 421/422 Project **2022-23**

Project #3: 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 over 500 different mutations in the human calpain-3 gene that cause a specific muscular dystrophy (limb girdle muscular dystrophy type 2A). These mutations are distributed all over the enzyme's four domains and two insertion sequences one of which (IS2) is thought to be the binding site for titin in the sarcomere. We will study the muscular dystrophy mutations in IS2 to see if some of them block the binding of calpain-3 to titin as an explanation for how they cause muscular dystrophy and how some of these might be countered.

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Project Title: Limb girdle muscular dystrophy mutations and calpain-3 protease binding to titin

Keywords (3-5):

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

Project Goals: Investigate which limb girdle muscular dystrophy mutations that occur in IS2 of calpain-3 affect titin binding. Determine the severity of these mutations in terms of how much they decrease the affinity of calpain-3 for its titin binding partner. Once the crystal structure of calpain-3 bound to titin has been solved these binding data will help validate the protein-protein interaction.

Experimental Approaches: Production and purification of recombinant calpain-3 in *E. coli*. Introduce the IS2 limb girdle muscular dystrophy type 2A mutations into wild-type calpain-3 to study the mutant enzyme's binding to titin in pull-down experiments and size exclusion chromatography. Introduce these mutations into the 44-residue IS2 sequence made by solid phase peptide synthesis and measure the affinity for titin by isothermal calorimetry and fluorescence polarization.

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.