BCHM 421/422 – 2018/2019

Project #4 Outline: Calpains are a family intracellular proteases involved in calcium signaling. Calpain-3 is the isoform abundant in muscle, where it is thought to be involved in the repair of damaged myofibrils. There are about 500 different mutations in the human gene that cause a specific muscular dystrophy. We have characterized three of the four calpain-3 domains, and would now like to solve the structure of the whole enzyme, which is a dimer of 94-kDa subunits. We are also investigating its binding partners and developing inhibitors that will be specific for this isoform. We are studying this enzyme to understand how defects in the enzyme cause muscular dystrophy and how some of these might be countered.

Supervisor: Peter L. Davies

Project Title: Structure, function and inhibition of the calcium-activated calpain-3 protease

Keywords (3-5):

1. **Recombinant protein**
2. **Protein purification**
3. **Enzyme inhibitors**
4. **X-ray crystallography**
5. **Protein-protein interactions**

Project Goals: Produce and purify full-length calpain-3 for crystallization trials. Develop a pull-down method to identify protein binding partners in muscle. Design and test calpain-3 inhibitors. Solve the structure of whole calpain-3 using individual domains solved by our lab for molecular replacement.

Experimental Approaches: Production of recombinant enzyme in bacteria. Purification of recombinant proteins for crystallization. 3-D structure determination by X-ray crystallography. Design and testing of peptide-based enzyme inhibitors. Identification of binding partners by fluorescence tagging and pull-down experiments.

References:

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