BCHM 421/422 – 2019/2020

Project 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 human calpain-3 to understand how alterations in the enzyme cause muscular dystrophy and how some of these defects might be countered.

Supervisor: Peter L. Davies

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

Keywords:

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. Solve the structure of whole calpain-3 using individual domains solved by our lab for molecular replacement. Design and test calpain-3 inhibitors. Develop a pull-down method to identify protein binding partners in muscle.

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, 335-351. PubMed: 23035980

Ye, Q., Campbell, R.L., Davies, P.L. (2018) Structures of human calpain-3 protease core with and without bound inhibitor reveal mechanisms of calpain activation. J. Biol. Chem. 293, 4056-407 PubMed: 29382717.