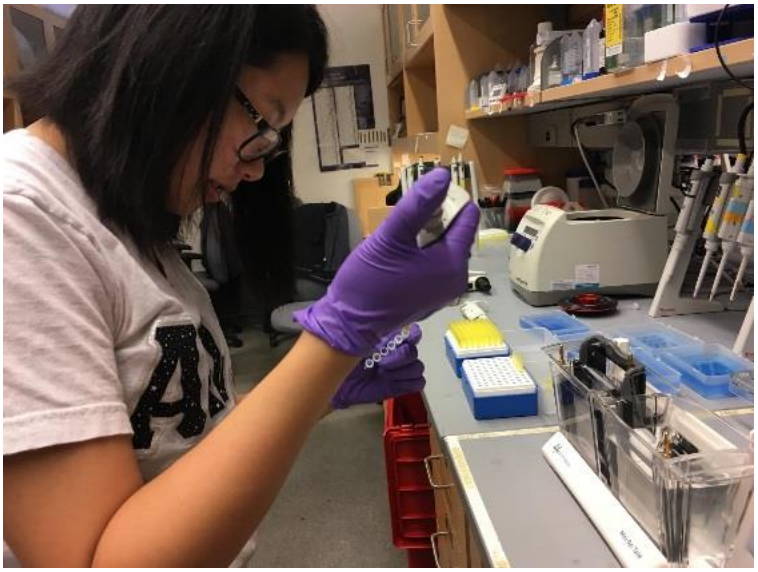

Construction of Protein and Nano Structures as Methods of Drug Delivery
Sunnia Chen (EAS 2018)
Advisor: Andrew Tsourkas

Over the summer, I worked with the Tsourkas lab of the bioengineering department at Penn. This lab focuses on developing novel methods of drug delivery using antibodies, protein structures, and nanotechnology. I was assigned to two major projects: the expression of anti-transferrin single-chain variable fragments (anti-TfR scFv's) and the expression of ferritin-3-helica (FTN3H).

Anti-TfR scFv is a protein that targets and binds to transferrin, a receptor on the blood brain barrier capable of moving bound molecules across to the central nervous system. Anti-TfR is also able to be crosslinked to the heavy chain of an IgG antibody through Protein G (PG), an antibody-binding domain. This PG-anti-TfR-scFv-IgG structure is known as a bispecific antibody, since the unbound part of IgG is able to hold a drug package that can be used for monoclonal antibody therapy. The bispecific antibody would allow for monoclonal antibody therapy to pass the blood brain barrier and target diseases of the central nervous system. As the first piece of this project, my job was to express anti-TfR scFv and crosslink it to IgG. This was a challenge due to scFv's being difficult for bacteria to express as well as the formation of aggregate structures – proteins that did not fold correctly – despite expression of the amino acid sequence. Ultimately, we decided to move to a yeast cell line.

FTN3H is another proposed method of engineering a drug delivery system. FTN3H is a protein structure composed of ferritin (FTN) and a 3-helical bundle (3H). FTN acts as a nano-platform upon which to attach 3H, a protein scaffold that has been proven to be able to hold a large payload of drugs. Though we initially tried to express FTN3H in a variety of cell lines, none worked, so we moved on to expressing FTN and 3H separately with greater success. In the future, the two proteins will be linked together.

Being involved in these two projects, I learned and enhanced many wet lab, molecular biology techniques: bacterial transformation, plasmid cloning, protein extraction and purification, UV

radiation crosslinking, BCA assays, and high performance liquid chromatography, to name a few. I also learned how to read and analyze DNA sequences and design primers for cloning. But most importantly, this research experience gave me the insight to building a scientific process in order to work to an end goal. When a protein did not express properly, we had to develop hypotheses as to why and change our procedure to fix the problem. This taught me the perseverance, patience, and attention to detail one needs to achieve an ambitious research goal, as well as the responsibility of having my own research project. Problem-solving, revising, optimizing – this was to think like an engineer. I believe that my work this summer has built the base and contributed valuable information these projects need to continue forward.