Alzheimer's is a neurodegenerative disease that has unfortunately impacted more than 6 million families in the United States, alone. Alzheimer's is involved in the disruption of neurons that are vital for communication, the repair of the body, as well as metabolism. These neurons are disrupted by the formation of amyloid plaques which are formed by breaking down the amyloid precursor protein. Previous studies have shown that IgG is an effective means of trying to target these proteins, but the blood-brain barrier (BBB) poses a problem. Due to the low permeability of the BBB, this presents an obstacle in trying to get a sufficient amount of IgG through in order to target the proteins before they become plaques. It is through this project that we attempt to find a solution that will allow IgG to be able to travel through the BBB more effectively.

**BBB Permeability Experiments**
- Wanted to test BBB permeability in vitro
- Tested whether Fab sialylation impacted this permeability (efflux vs. influx)
- Fab sialylation is when you introduce a Fab group to a molecule.
- This is being done in hopes of it influencing the permeability of antibodies through the BBB
- We compared the influx and efflux differences both with the presence of Fab sialic acid and the absence of Fab sialic acid. The results are shown here to the right:

**Further Implications:**
- Why does this matter?
- Correlation to antibodies (and the IgG mentioned in the Introduction)
- So, what now?

**Ultimate Goal:** Take the sialic acid group off the 4G8 antibody and swap it with the sialic acid group that is originally on the IgG antibody.

**ABSTRACT**
Alzheimer’s is a neurodegenerative disease that has unfortunately impacted more than 6 million families. Although there have been many attempts to find a cure for the disease, none have been successful in either the prevention or curing of Alzheimer’s. The biggest obstacle that makes Alzheimer’s so hard to treat is the inability to transport medicine through the blood-brain barrier (BBB). Prior research indicates that human antibodies, IgG, are not able to cross this blood-brain barrier successfully. 4G8, however, has been reported to have a reduced efflux, meaning decreased ‘traffic out of the brain’ with a non-impacted influx, referring to the ‘traffic’ into the brain, with the treatment of neuraminidase. 4G8 is an IgG antibody that has a sialylated Fab Glycan that specifically binds to and recognizes amyloid plaques in the brain; these plaques are the driving factor in causing Alzheimer’s. In order to increase the permeability of IgG through the BBB, we will use glycosylation, the addition of a sugar molecule, to make these antibodies homogenous to 4G8 by replacing it with that of 4G8. We are hoping that making the Fab Glycan of IgG homogeneous to 4G8 should allow for a more effective means of treating Alzheimer’s. Although we were able to glycosylate the antibodies successfully, we were not yet able to determine if this would be an effective method to move the crossing of the blood-brain barrier.

**INTRODUCTION:**
Alzheimer’s is a neurodegenerative disease that has unfortunately impacted more than 6 million families in the United States, alone. Alzheimer’s is involved in the disruption of neurons that are vital for communication, the repair of the body, as well as metabolism. These neurons are disrupted by the formation of amyloid plaques which are formed by breaking down the amyloid precursor protein. Previous studies have shown that IgG is an effective means of trying to target these proteins, but the blood-brain barrier (BBB) poses a problem. Due to the low permeability of the BBB, this presents an obstacle in trying to get a sufficient amount of IgG through in order to target the proteins before they become plaques. It is through this project that we attempt to find a solution that will allow IgG to be able to travel through the BBB more effectively.

**METHODS AND MATERIALS**

**Glycan Analysis-General Process**

**1. Selective Fc glycan cleavage (native conditions):**
   - 4C and Fab glycans are lyophilized and labeled via reductive amination using 2-aminobenzamide and CNBH reductant

**2. Isolation of cleaved Fc glycans:**
   - Glycans were purified with ABZ dye (blue).

**3. Cleavage of remaining Fab glycans (in SDS):**
   - Labeled glycans were purified with size exclusion chromatography. Glycans eluted between 20-40% ammonium formate pH 4.5 in acetonitrile.

**4. Fc and Fab glycans are Sialylated and labeled via reductive amination using 2-aminobenzamide and CNBH reductant:**
   - Glycans were eluted earlier than free ABZ dye (blue).

**5. Efflux results:**
   - Efflux reduced with Fab sialic acid

**6. HPLC profile of glycans with polar glycan column:**
   - Glycans eluted between 20-40% ammonium formate pH 4.5 in acetonitrile.
   - Measured with ABZ fluorescence.

**REFERENCES**