

# Increasing IgG Permeability in the Blood Brain Barrier Through the Isolation and Removal of Glycans to Successfully Treat Alzheimer's Disease

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## INTRODUCTION

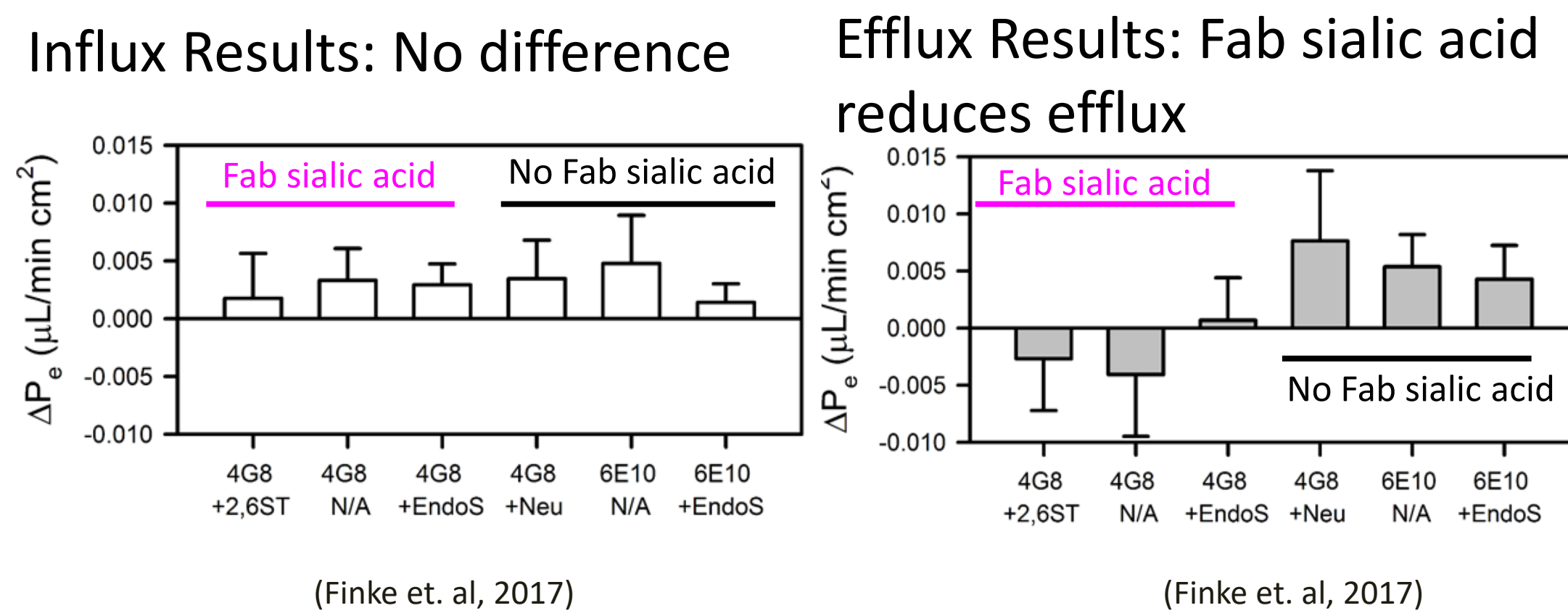
Alzheimer's disease, similar to dementia, is a neurological disorder that affects thinking, memory, and behavior that can progressively become worse. Alzheimer's disease affects millions of families and can be very labor-intensive and expensive to treat. There have been attempts to find a cure, but unfortunately, most have been unsuccessful due to various drug treatments not penetrating the blood-brain barrier (BBB). Fortunately, some progress has been made on researching the permeability of modified human antibodies through the BBB. 4G8 is a type of IgG antibody with a sialylated Fab glycan, meaning it has a sialic acid on the tip of the molecule. This sialic acid directly recognizes and binds the amyloid plaque that causes neuronal decay. Previous research has shown that specific glycosylation may improve the possibility of antibody delivery to the brain. We hypothesized that replacing the human IgG sialic acid group with that of 4G8 would be an effective way to get these antibodies to cross the BBB as a possible treatment for Alzheimer's disease. We were able to successfully replace the sialic acid groups on IgG antibodies, but we were unable to determine whether these antibodies can pass through the blood-brain barrier.

### Testing BBB permeability:

Comparing influx and efflux results of the Fab sialic acid presence and absence by testing BBB in vitro.

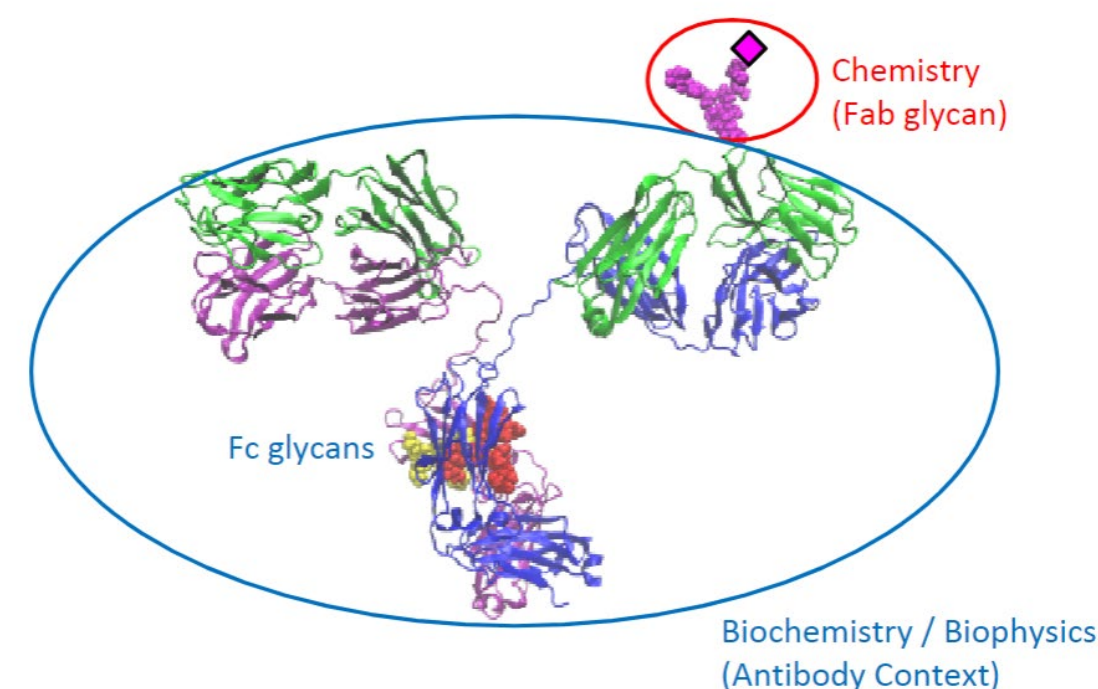
**Influx (into the brain):** Altering antibody drugs to increase passage through the BBB.

**Efflux (out of the brain):** Inhibition of efflux prolongs the presence of antibodies and drugs in the brain.



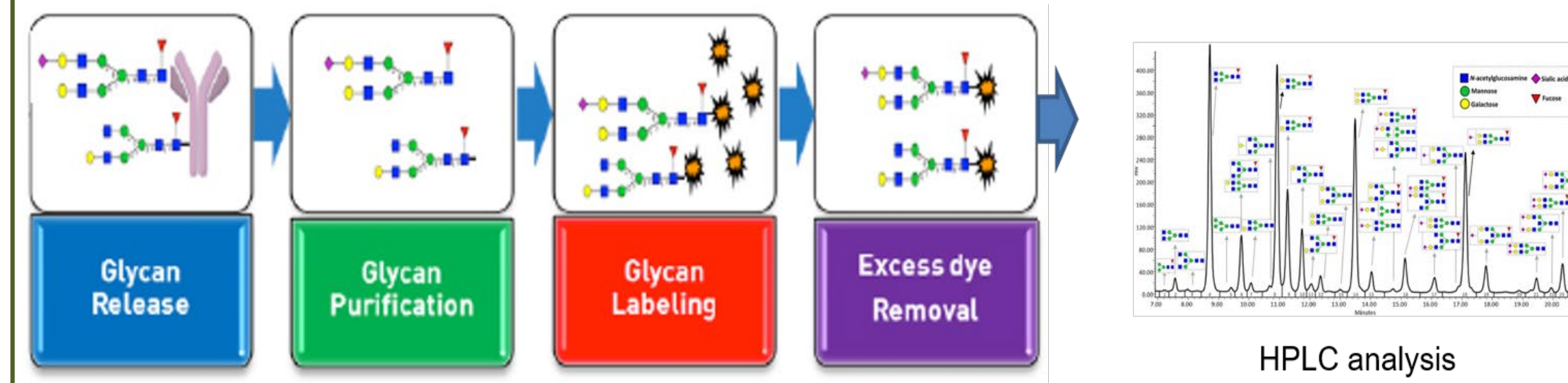
## OBJECTIVE

Why does sialylation lower human IgG influx but not 4G8 ?



Make 4G8 homogeneous.  
- Take sialic acid off the tips of 4G8 antibody and swap with the sialic acid in IgG antibody

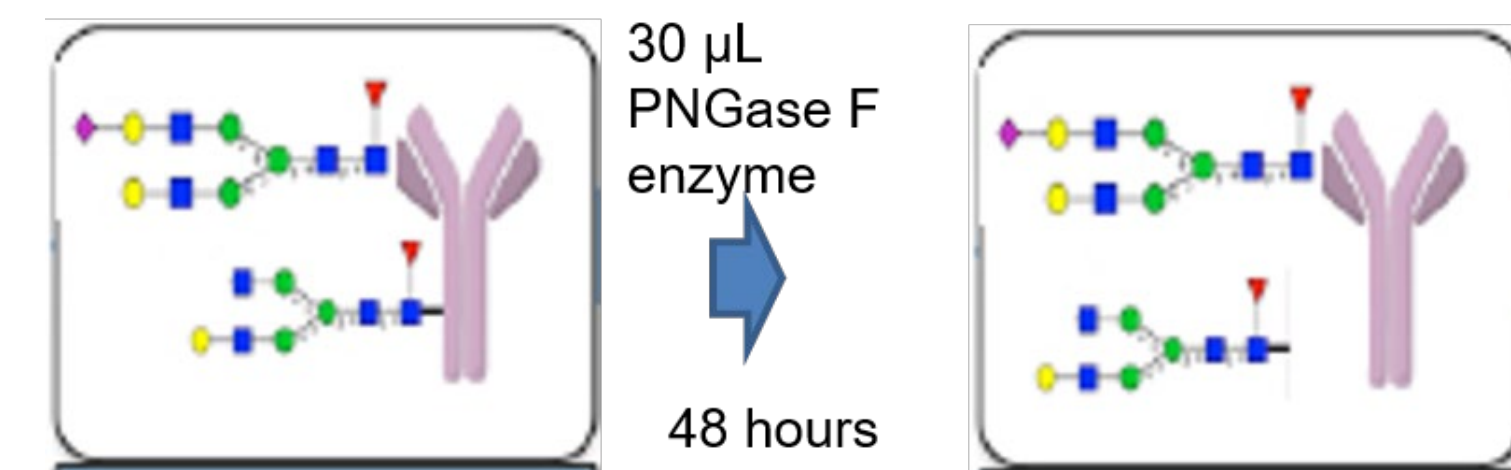
## Methods and Materials



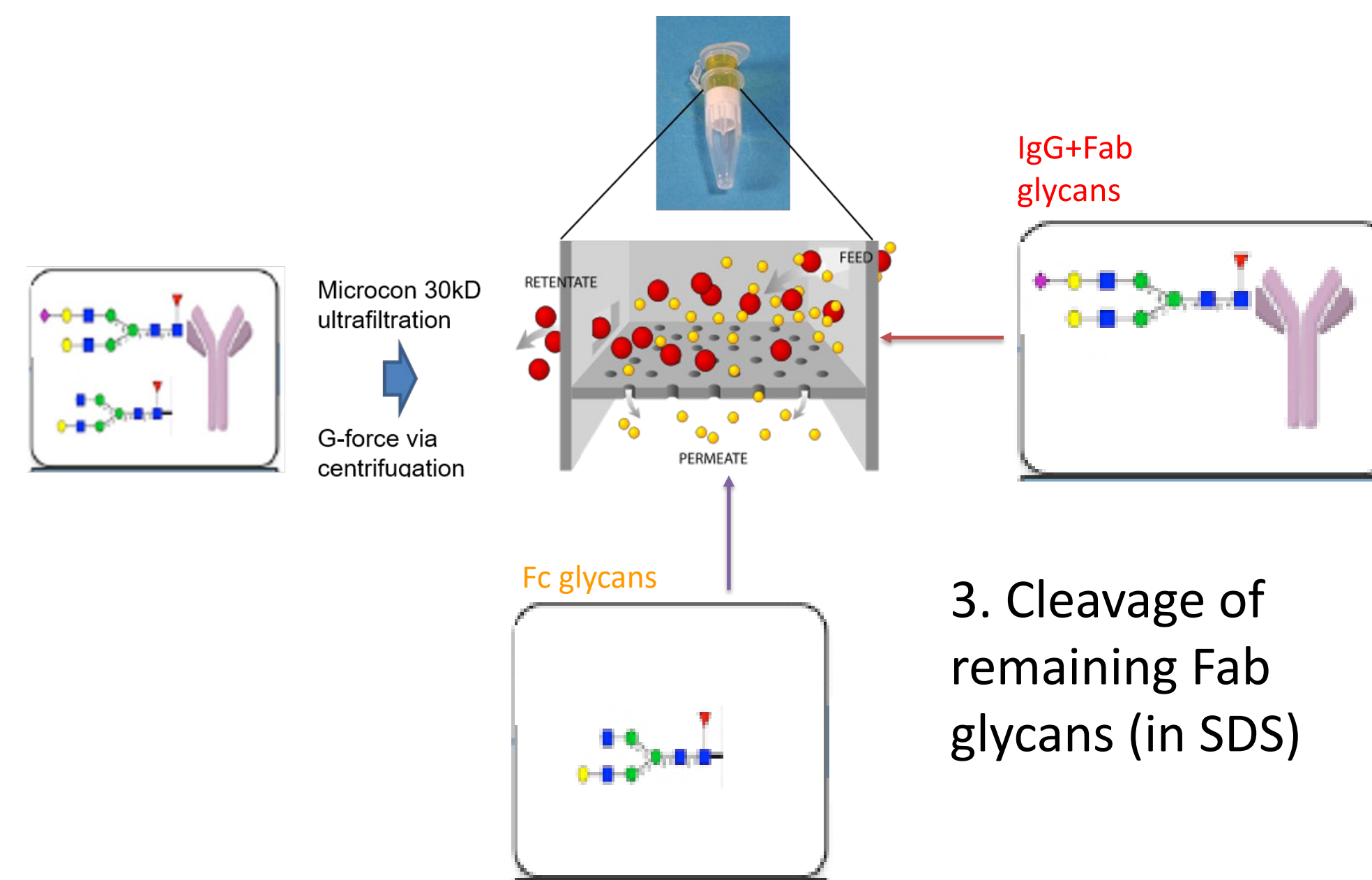
### Fab/Fc glycan Analysis

#### Separating Fab/Fc Glycans

1. Selective Fc glycan cleavage (native conditions):

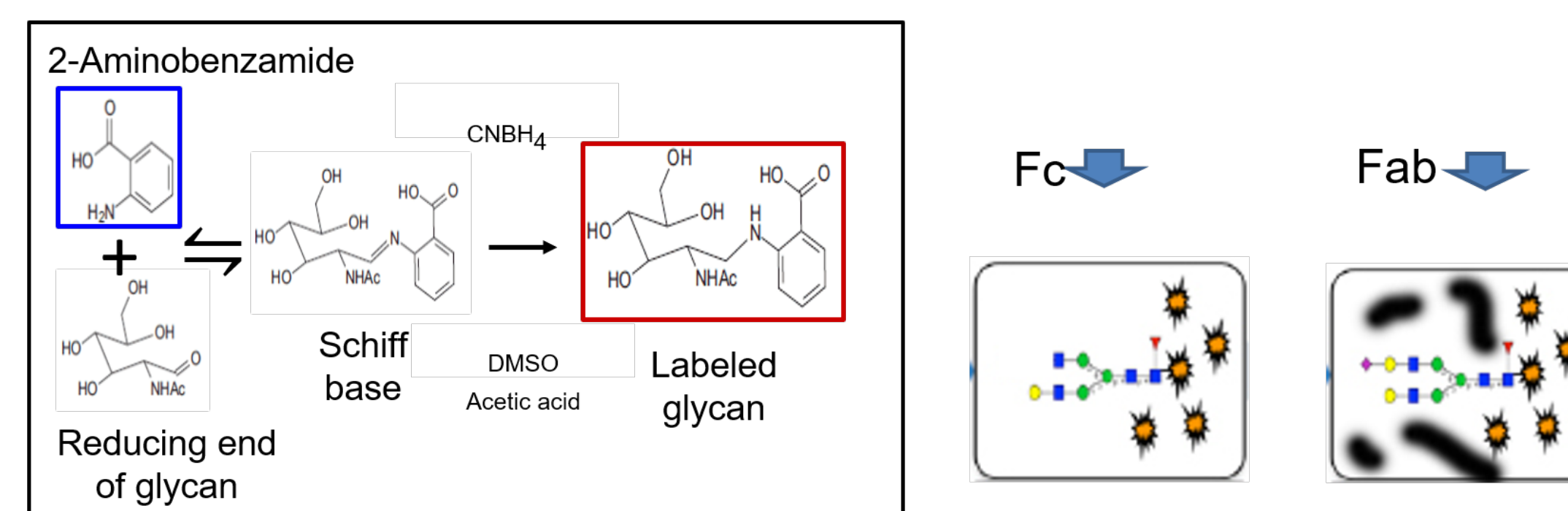


2. Isolation of cleaved Fc glycans:



3. Cleavage of remaining Fab glycans (in SDS)

4. Fc and Fab glycans are glycosylated and labeled via reduction amination using 2-aminobenzamide and CHBH4 reductant:



5. HPLC analysis to verify intended modifications.

## RESULTS

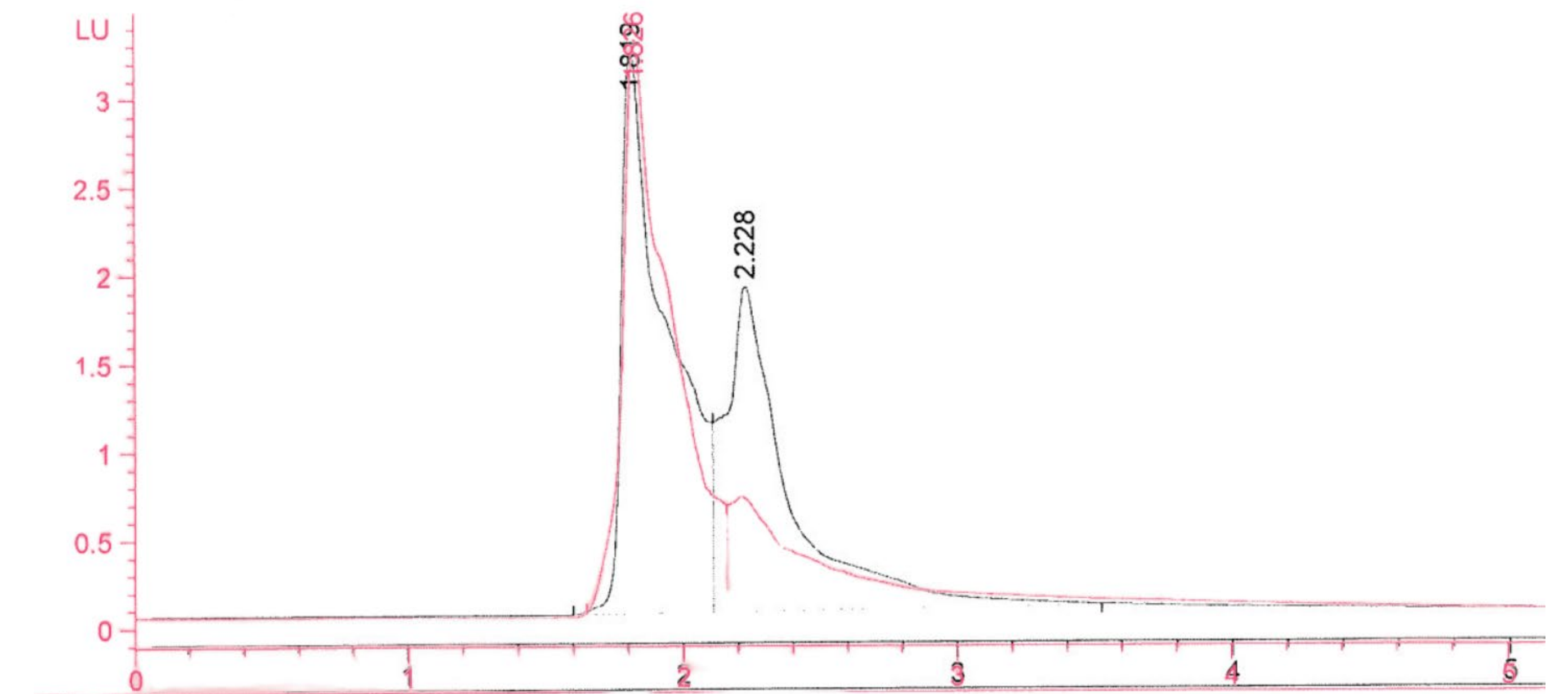


Figure 1. HPLC data shows sialylation of Fc (red) along with Fab glycans (black) with the successful removal from the antibody

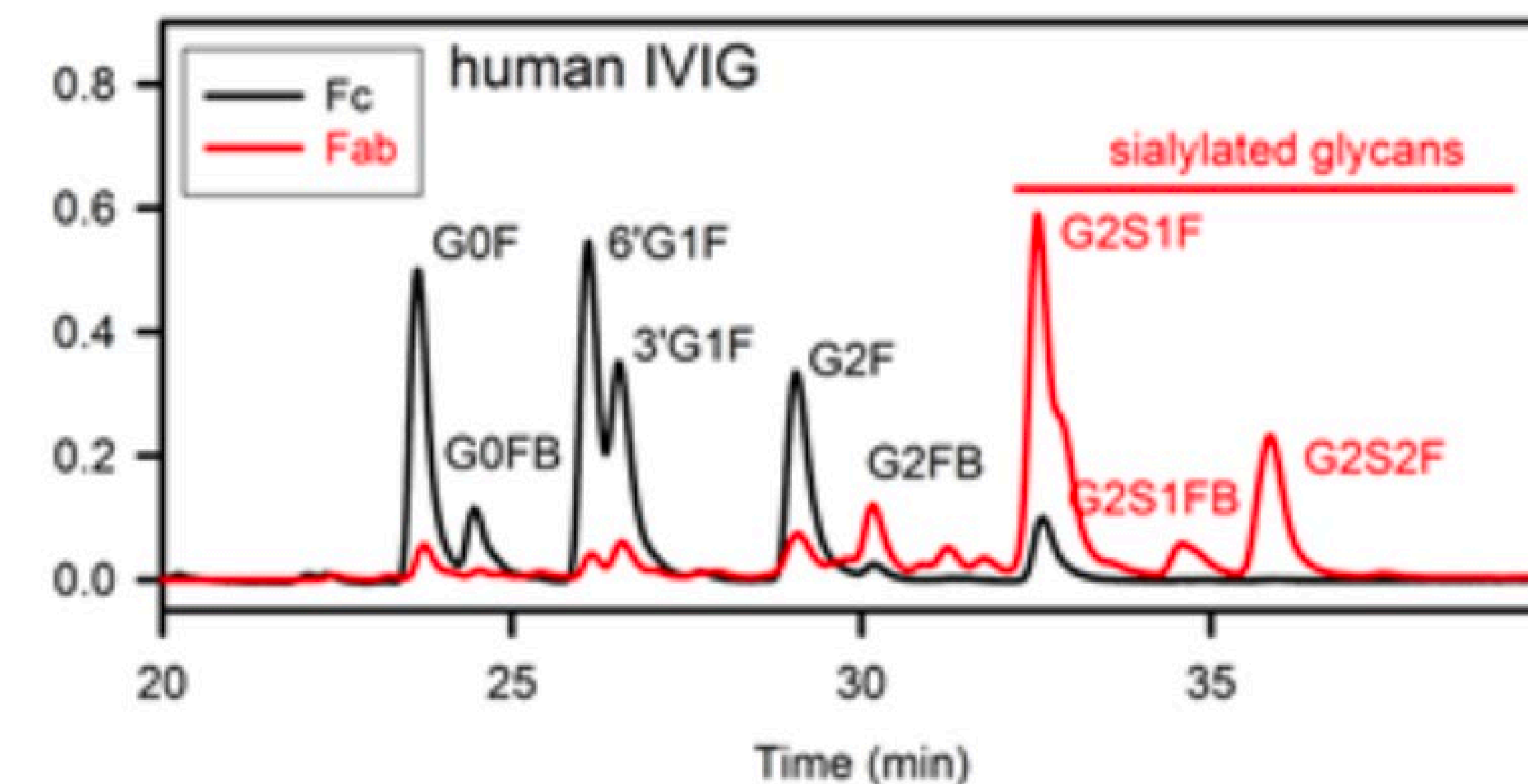


Figure 2: Dr. Finke's research group from last year (Finke et. al 2022) was not able to successfully sialylate the glycans, as seen in the red peaks above, differing than the black peaks to the right. The peaks in Figure 1 match the red peaks seen in Figure 2, indicating successful sialylation.

## REFERENCES

- CDC. 2021 Apr 7. What is Alzheimer's Disease? | CDC. www.cdc.gov. <https://www.cdc.gov/aging/aginginfo/alzheimers.htm#:~:text=Alzheimer%27s%20disease%20is%20the%20most>.
- Finke JM, Ayres KR, Brisbin RP, Hill HA, Wing EE, Banks WA. 2017. Antibody blood-brain barrier efflux is modulated by glycan modification. *Biochimica et Biophysica Acta (BBA) - General Subjects*. 1861(9):2228–2239. doi: <https://doi.org/10.1016/j.bbagen.2017.06.008>.

## ACKNOWLEDGEMENTS

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