Optimizing and Evaluating Signal Peptide Peptidase Crystallization Conditions Using Electron Crystallography

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According to the Alzheimer's Foundation of America, it is estimated that about 5.1 million Americans have Alzheimer's disease. Signal Peptide Peptidase (SPP) is a membrane protein that is located in the ER, lysosome, and cell membrane. It's involved in immune surveillance, Hepatitis C Virus maturation, and works in a similar way as gamma secretase. Gamma secretase is involved in the development of Alzheimer's. The overall focus of this study is to determine the structure of SPP using 2D Electron Crystallography. The structural information can be used by pharmaceutical companies to discover medicine for Alzheimer's disease and Hepatitis C Virus maturation. The structure of SPP is determined by optimizing different crystallization conditions. This is achieved by obtaining very pure SPP samples and reconstituting it into a lipid bilayer using dialysis. After dialysis, samples are prepared and imaged using the electron microscope. After the images are collected, they are evaluated for the presence and size of crystals. Current experiments are testing if the volume of dialysis buffer has any effect on the size and quality of crystal produced. I hypothesized that a larger volume of dialysis buffer would positively affect the crystals. Analyzing samples from 100mL, 200mL, 300mL, and 400mL of dialysis buffer, my hypothesis was accepted. The images from 400mL displayed elevated levels of crystals. In conclusion, the results indicate an increase in the amount of dialysis buffer gives better order. However, further experiments must be conducted to ensure reproducibility of data. This data can be used with other optimal conditions to obtain the structure of SPP and gamma secretase. Using this information, pharmaceutical companies can create medicines to treat Alzheimer's disease.

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