The Use of Alpha-Synuclein in Artificial Cell Division

Alexander Orebic, Andrew Rudd, Neal Devaraj

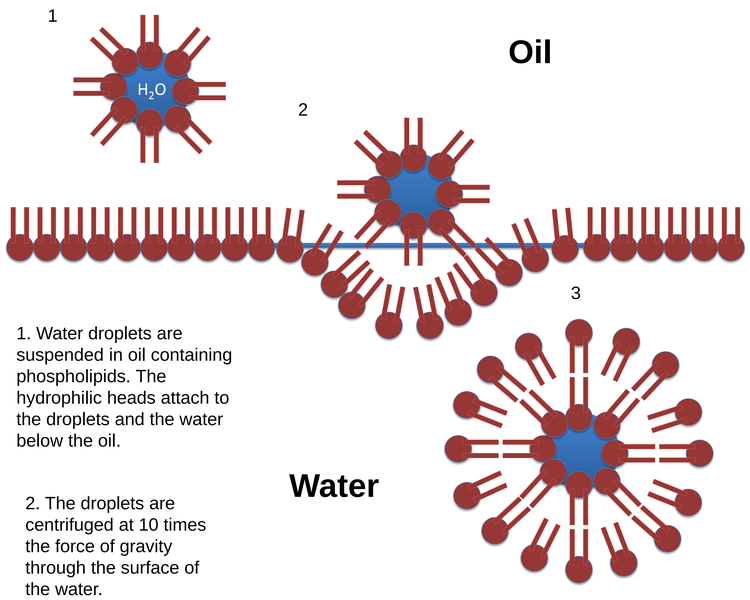
The Department of Chemistry & Biochemistry, University of California San Diego, 9500 Gilman drive, La Jolla, California

ABSTRACT

The protein a-synuclein is thought to be partially responsible for certain neurodegenerative diseases such as Parkinson's and Huntington's. In those afflicted, a-synuclein is found in aggregations known as lewy bodies. The reason for this is hypothesized to be a rare mutation causing misfolds of α-synuclein leading to a problem with the nervous system. Normally α-synuclein is found in the axon terminals of neurons and is theorized to contribute to later development of the nervous system.

Up until recently α-synuclein was researched with a biomedical goal in mind, however, it shows potential as a tool for membrane division. In vivo α-synuclein has been shown to cause turbulation and destruction of vesicles, whereas in vitro it causes division of vesicles. After encapsulation of a-synuclein vesicle divide in an organized way without any other necessary cellular machinery. This ability makes it a primary candidate for use in artificial cells.

The Pull Down Method

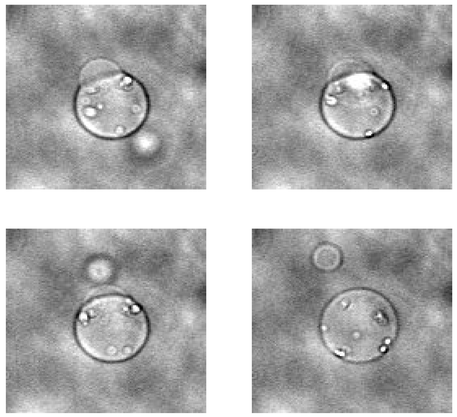


1. Water droplets are suspended in oil containing phospholipids. The hydrophilic heads attach to the droplets and the water below the oil.

2. The droplets are centrifuged at 10 times the force of gravity through the surface of the water.

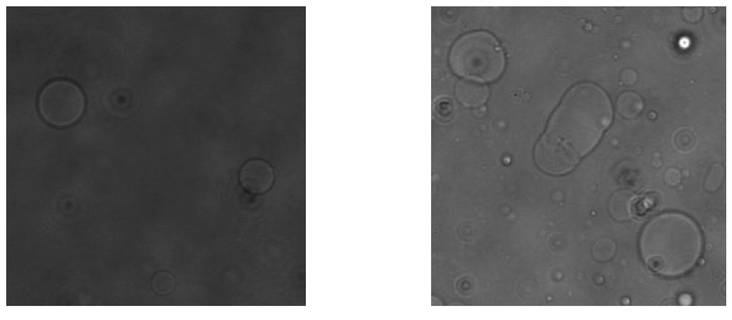
3. The hydrophilic heads remain facing outward but the hydrophilic tails face inward creating a vesicle.

Vesicle Division Overtime



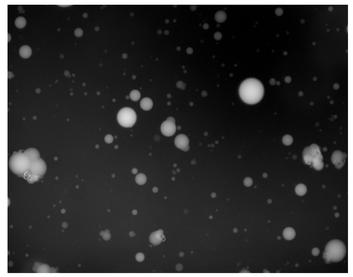
Encapsulation of a 10μM solution consisting of α-synuclein and sucrose results in vesicle budding and division. Material on the inside of these vesicles is aggregation.

Control Analysis

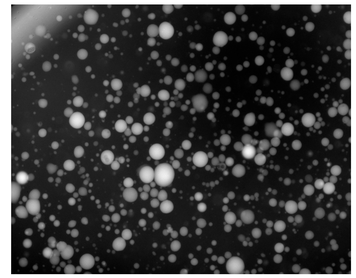


The control on the left contains BSA (Bovine Serum Albumin) at a concentration of 10 μM. There was no division of vesicles within the control. The BSA vesicles were made with the pull down method which produces unpredictable numbers of vesicles which is why there are more in the example on the right. On the right α-synuclein was encapsulated in the same way with the same concentration of protein. The center vesicle is budding off due to the presence of the a-synuclein.

Pull Down Variability



An 10μM a-synuclein solution encapsulated in vesicles.



An 100μM a-synuclein solution encasulated in vesicles.

Although these images aren't the best examples* there was no clear increase in vesicle division with the addition of a higher concentration of a-synuclein. In fact it seemed that the most consistent results were obtained when the concentration of protein was at 10μM.

*the number of vesicles created in the 100μM concentration sample are greater in number this is because of the unpredictability of the pull down method.

CONCLUSIONS

- We demonstrated the division of vesicles using encapsulated a-synuclein. In an artificial cell this is a simpler and therefore preferable method of division rather than use of conventional cellular machinery.

FUTURE RESEARCH

The overall goal of my lab was the creation of an artificial cell. Unlike many the methods of my lab have been from the bottom up. My lab is creating the cell structures and tools to build such a cell. This is just one of the potential tools created in order to make this idea a reality.