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ABHD5 Induced Morphological Changes on Model Membrane Systems

Nasser S. Junedi
Wayne State University, njunedi@gmail.com

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Nasser Junedi

Dr. Christopher Kelly

Wayne State University

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Abstract:

Proper regulation of neutral lipid storage (lipogenesis) and release (lipolysis) are critical molecular processes localized to an organelle called the Lipid Droplet (LD). The LD consists of a core with neutral lipids such as triacylglycerols (TAGs) and sterol esters surrounded by a phospholipid monolayer.[1] Dysregulation of the processes localized to the LD are involved in the pathology of various diseases such as Neutral Lipid Storage Disease, diabetes, stroke and cancer.[2,3,4] The non-enzymatic protein ABHD5 (α-β Hydrolase Domain-Containing Protein 5), is thought to play a key role in the process of lipolysis by forming homo-oligomers on the surface of the LD that create packing defects on phospholipids and allowing the TAGs to be liberated by another protein called PNPLA2 (Patatin-Like Phospholipase Domain-Containing Protein 2). The goal of my research is to understand how ABHD5 changes the morphology of membranes by using various model membrane systems. Localization of the protein to the membrane and morphological changes of the lipids are visualized with epifluorescence microscopy. Results from this study will enable us to understand how lipolysis is regulated via protein interactions with lipid membranes to induce functional lipase activation perhaps through inducing membrane shape change.

Introduction:

Lipid membranes play a very important role in human health. They are the substrates which regulate many different processes inside cells such as the firing of a neuron, signal transduction through G-Protein coupled receptors, and the inter/intracellular export and import of different substances. The system I studied in this project is the membrane interface of the Lipid Droplet and the Endoplasmic Reticulum

(ER). The ER is a branched organelle found inside eukaryotic and prokaryotic cells that is responsible for the production of proteins and lipids. It spans a large part of the cell and is continuous with the nuclear envelope.[5] It is of particular interest in this project because the ER is the site of biogenesis of the LD.[1] Neutral lipids are synthesized in between the leaflets of the bilayer by proteins through an esterification reaction followed by an acyltransferase reaction. When sufficient neutral lipid has been synthesized, a lens like protrusion will form. As more neutral lipid aggregates, a bud like structure will start to take shape. It is thought that the neutral lipid wanting to minimize contact with aqueous solution helps to drive the formation of the bud.[1] With the help of the FIT protein, the bud detaches from the ER.[1]

Lipolysis is the reaction in which fat is broken down and released. The main protein behind this activity is PNPLA2, an enzymatic protein that hydrolyzes TAG to DAG leading to the release of a fatty acid. However, it is not the only protein in the mechanism. ABHD5 is a non-enzymatic protein that plays a vital role in this process. Previous work from our collaborator, Dr. James Granneman, showed that even though ABHD5 lacks a nucleophilic serine group required for hydrolase activity, it plays a very important role in lipolysis.[6] It is thought that ABHD5 may play a role in lipolysis by oligomerizing on the surface of the membrane and creating sites of curvature (Figure 1). These sites of curvature create packing defects in the membrane allowing PNPLA2 to enter and release fatty acids. The goal of my project was to test this hypothesis by adding ABHD5 to three different model membrane systems, Supported Lipid Bilayers (SLB), Artificial Lipid Droplets (aLDs), and Droplet Embedded Vesicles (DEVs), and looking for morphological changes in the shape of the membrane.

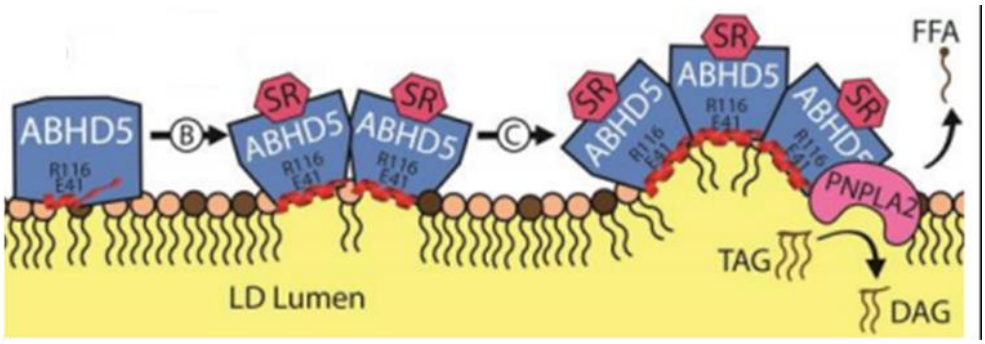

Figure 1: Cartoon that demonstrates ABHD5 oligomerizing on the surface of the LD inducing curvature. This curvature creates packing defects in the phospholipid monolayer exposing TAG to the enzymatic protein PNPLA2 which hydrolyzes and releases a fatty acid.

Project 1: Supported Lipid Bilayers

Introduction:

Model membrane systems are very useful in trying to understand the key underlying phenomenon in biological membranes without the complexity involved with cells. An SLB consists of the fusion of a bilayer to a substrate, typically glass. To learn more about how ABHD5 interacts with sites of curvature, I aimed to induce the formation of tubules and buds from the SLB following a published protocol.[7] Tubules and buds are protrusions of the membrane up away from the glass simulating curvature found in the real Lipid Droplet and Endoplasmic Reticulum. After the formation of tubules and buds, ABHD5 was added to see types of morphological changes that are occurring and if the protein is preferentially binding to the tubules and buds.

Methods:

There are two main components in the process of forming buds and tubules from SLBs. The first step is to form spherical membrane structures called Large Unilamellar Vesicles (LUVs). The LUVs are to be added to the glass of a 96 well plate, where they

fuse and form an SLB as (Figure 1). The second step involves changing the buffer to induce the formation of buds and tubules (Figure 2).

A mixture of 55% DOPC (w/w) ,25% DOPE (w/w), 20% DOPS(w/w) , and 1% (w/w) of the fluorophore Cy5-PE were mixed together in a vial; all lipids were bought from Avanti Polar Lipids®. The mixture was then dried off with nitrogen gas and resuspended in 1 milliliter of 1 M NaCl for a final lipid concentration of 0.2 mg/ml. The lipid mixture was then passed through a liposome extruder with a 50nm filter. This process formed the LUVs, each with a diameter of 50nm. A 1M NaOH solution was deposited on the glass followed by a rinse with mQ water to remove any impurities on the glass. To ensure binding of the LUVs with the substrate, 1 µl of $CaCl_2$ was added to the glass. To form the SLB, 40 µl of the extruded LUV solution was added to the well and left to incubate for 20 minutes. During this time the LUVs are making impact and bursting onto the glass. In this process often excess bilayers and lipid chunks are deposited on the glass; to remove them, a micropipette aspirator was used to rinse the sample 8-10 times with 1M NaCl in conjunction with a suction vacuum to collect said waste. The SLB will look quite uniform with some bright spots indicating the lipid chunks that were not able to be rinsed away (Figure 2). After the formation of the SLB, the 1M NaCl solution was switched out for mQ water and left to sit in the well for 1-2 minutes. This was followed by an exchange of the water for a HEPES buffer; this exchange of buffer led to the formation of lipid buds and tubules which can be seen as the large spherical structures and elongated structures that are partially out of the plane of focus respectively (Figure 2). The ABHD5 protein was diluted from a 39mM stock solution suspended in Intracellular Buffer (IB) to 2mM with HEPES buffer. The protein was

added into the well with a micropipette; proper protein mixing was ensured by pulling and releasing liquid multiple times in the sample. The control experiment consisted of the addition of HEPES buffer only to the tubules and buds acting a vehicular control.

All imaging was done with an inverted IX71 Olympus Fluorescence Microscope. The basic principle behind fluorescence microscopy is that certain molecules absorb one wavelength of light and emit a different wavelength of light. When a specific wavelength of light is incident on a sample, the electrons in the fluorescent molecule are excited to a higher energy level: in that energy level there are many vibrational states the electron can be in. Upon excitation, the electron recedes to a lower vibrational state in the excited energy level (Figure 3). From this lower vibrational state, the electron falls back down to ground state, emitting a longer wavelength of light; this can be explained by the Planck-Einstein Relation, $\Delta E=hc/\Delta\lambda$, where the change in energy is inversely proportional to the change in wavelength. The lipids and ABHD5 were tagged with the fluorescent molecules Cy5 and mCherry respectively. Lipids and proteins were imaged through LED with excitation cubes of 647nm and 561nm respectively (Figure 4). After data acquisition images were analyzed using image analysis software ImageJ.

Results and Discussion:

The formation of just the SLB sample was a feat that proved to be very difficult. In the early stages of the process, either too much lipid was being incorporated into the sample, leading to large chunks of lipid being concentrated on the membrane or too little lipid was being incorporated due to the harsh washing technique. This process was quite iterative and was a matter of trial and error till the most appropriate settings on the micropipette aspirator were found which controlled the flow of liquid just right removing

the large chunks of lipid without ruining the membrane. To induce the membrane buds and tubules, a protocol from a paper by *Knight et al.* was followed. The authors state that to induce the formation of buds and tubules, excess lipid needs to be incorporated into the sample followed by a salt exchange leading to the protrusion of the membrane.[7]

To properly incorporate excess lipid in the sample, the rate at which the liposomes adsorb to the substrate needs to be larger than the rate at which they are rupturing.[7,8] This is because as the liposomes adsorb to the surface faster than they rupture, more lipid can be incorporated on substrate as there is available surface area. A study done using SUPER template assays found that an increased concentration of NaCl leads to a larger amount of membrane being incorporated into the template.[8] A sample with a concentration of 1mM NaCl compared to 1M NaCl had 2.4-fold less lipid incorporated. The mechanism behind this is not yet understood, however, the authors argue that the salts play a large role in the kinetics of the liposome adsorption and SLB formation. The authors also demonstrate that samples with large amounts of DOPS leads to more robust SLB formation. They explain this phenomenon as the silica beads which the membrane is wrapped around has repulsive effects with negatively charged DOPS encouraging the excess membrane to spill out and form an SLB. The DOPS preferentially binding to the glass substrate in my SLB system makes sense as the surface is intentionally made hydrophilic to encourage this behavior. To complete the process and induce tubule and bud formation the buffer had to be switched out from NaCl to mQ water followed by another buffer exchange to HEPES. This is because the switch from water to a physiological buffer causes the membrane to expand laterally and fill in microscopic defects in the SLB.[7] Once these have been filled, the excess

membrane with nowhere to go protrudes upwards in the z-plane forming buds and tubules. This process is favorable because introduction of HEPES into the solution (i.e. salts back into the sample) leads to a decrease in the Van der Waal forces between the leaflets of the bilayer. To minimize this “energetic cost”, a “new equilibrium” is reached by the swelling of the membrane; this helps with the formation of buds and tubules in the SLB.[8]

The control experiment shows that both the bud and the tubule maintain their shape after having the HEPES buffer added and mixed into the sample (Figure 5). This indicates that the flow of the liquid from the pipette into the sample is not causing shape changes. However, when the ABHD5 is added to the buds and tubules drastic morphological changes appear. The addition of ABHD5 to a tubule structure leads to the breakdown and formation smaller vesicles; the original structure of the tubule is completely altered (Figure 6). Furthermore, looking at any one of the smaller vesicles, they are getting brighter over time indicating that the protein is sorting to these sites. Similar results are seen, when the protein is added to the sample with a bud (Figure 7). The bud immediately fractures into many small pieces, resembling a pearl necklace. These morphological changes on sites of curvature indicate that the ABHD5 is interacting with the membrane and supports the hypothesis of the non-enzymatic protein causing physical remodeling of the membranes. Observing the protein channel shows that the protein is aggregating to the sites of curvature preferentially indicated by the increase in fluorescence (Figures 6 and 7). The idea that this increase in brightness could be due to the protein mixing and settling into the sample over the period of imaging is negated by the fact that the protein is thoroughly mixed into well prior to

imaging. Furthermore, all images were acquired following control experiments for bleedthrough and imaging parameters both during imaging and in analysis afterwards were kept constant.

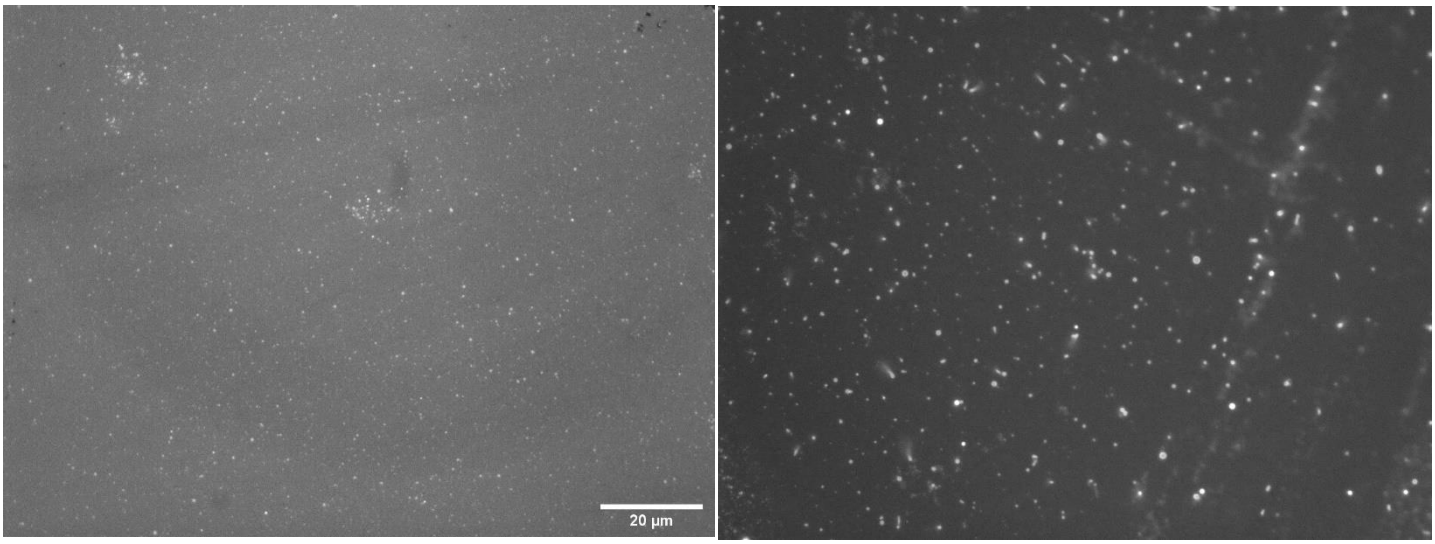

Figure 2: (Left) SLB (55% DOPC, 25% DOPE, 20% DOPS, 1% CY5) made by LUV fusion on glass substrate after excess lipids have been rinsed away. (Right) The SLB after a solvent exchange from mQ water HEPES buffer leading to the formation of buds and tubules. Both images were taken with $\lambda_{exc} = 647nm$ epifluorescence.

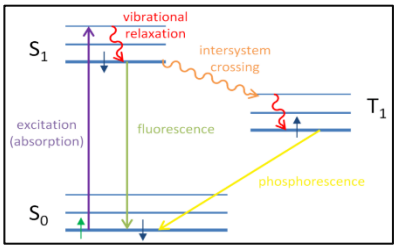

Figure 3: Jablonski Diagram demonstrating how an electron is excited to a higher energy level and then relaxes vibrationally followed by a return to ground state emitting a longer wavelength of light.[9]

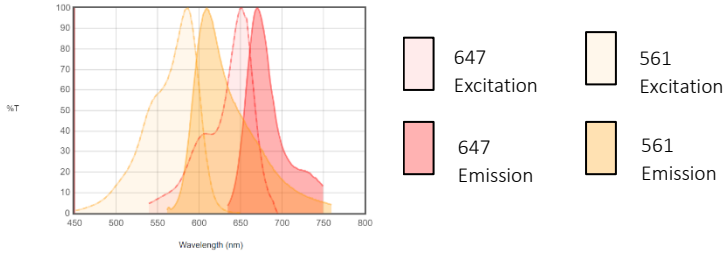

Figure 4: Excitation/Emission spectrum showing how light is absorbed at one wavelength and emitted at a slightly longer wavelength.[10]

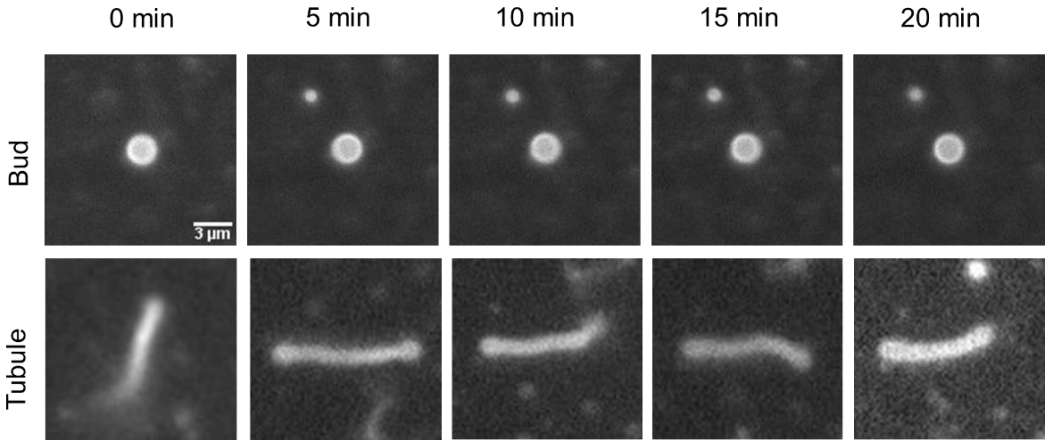

Figure 5: Time lapse vehicular control experiment, via the addition and mixing of HEPES buffer to buds and tubules, shows stability in the shape of bud and tubule after addition of buffer. Both sets of images taken with $\lambda_{exc} = 647nm$ epifluorescence.

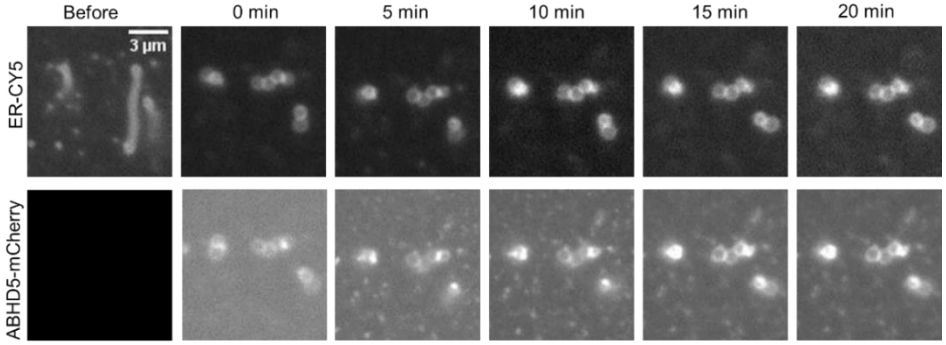

Figure 6: Addition of ABHD5 to tubule shows vesiculation and drastic membrane remodeling. Time lapse shows that vesicles get brighter due to protein sorting. (Top) CY5 imaged with $\lambda_{exc} = 647nm$ epifluorescence. (Bottom) ABHD5 imaged with $\lambda_{exc} = 561nm$ epifluorescence.

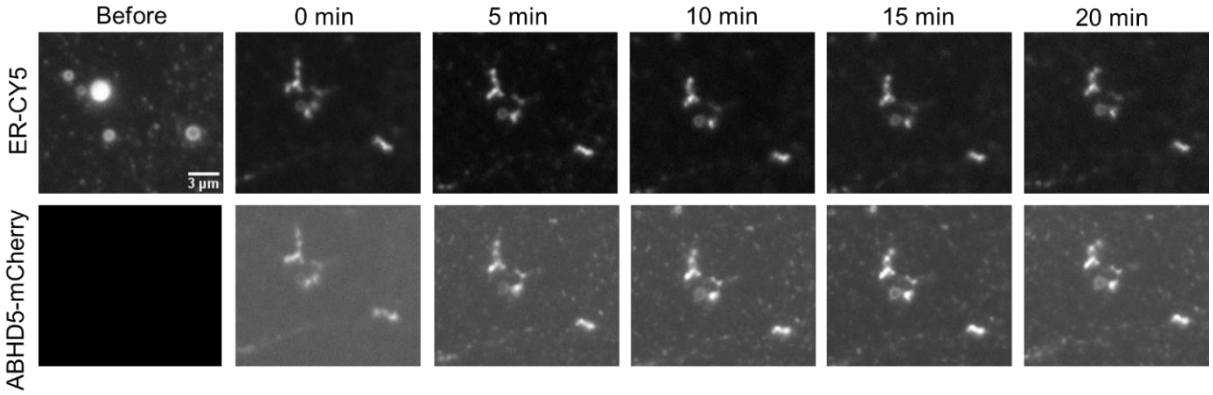

Figure 7: Addition of ABHD5 to a bud shows vesiculation and drastic membrane remodeling. (Top) CY5 imaged with $\lambda_{exc} = 647nm$ epifluorescence.(Bottom) ABHD5 imaged with $\lambda_{exc} = 561nm$ epifluorescence.

Project 2: Artificial Lipid Droplets

Introduction:

Artificial Lipid Droplets (aLDs) are another synthetic model membrane system used to study the properties of membranes. They differ structurally from SLBs in that they consist of an oil filled center, typically triacylglycerol, surrounded by a monolayer of phospholipids (Figure 7). I conducted a very similar experiment with this apparatus, where I added in ABHD5 to the aLDs and looked for morphological changes and preferential sorting of ABHD5 to the sites of curvature. The reason this system was chosen is because the structure of an aLD, as the name implies, is very representative of real LDs.

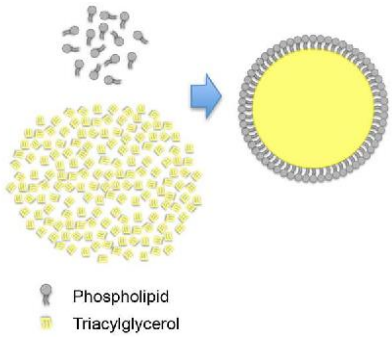

Figure 7: Structure of an Artificial Lipid Droplet.[11]

Methods:

To prepare the artificial lipid droplets, I mixed DOPC and the neutral lipid Triolein, purchased from Avanti Polar Lipids®, in a small Eppendorf tube at a mass ratio of 500:1 respectively. This tube was then placed in a bath sonicator for 30 minutes; this process helped to mix the phospholipids and triolein to form the aLDs. To view the aLDs, I used a microscope glass slide and cover slip. The glass slide was rinsed with ethanol and was passivated with a casein solution for at least 20 minutes then rinsed thoroughly by mQ water. The glass passivation helps to ensure the aLDs stick to the glass and do not fuse to each other. After the glass was passivated 5 µl would be place on the slide followed by 1 µl of 20 µm 1:100 diluted silica beads. The beads ensure there is proper

spacing between the cover slip and the glass slide. ABHD5 was added to the slide at a 1µl volume at a concentration of 50nM: this concentration is more physiologically relevant as shown in a lipolysis assay done by our collaborator (Figure 8), Dr. Matthew Sanders. A cover slip was then placed on the glass slide and sealed with vacuum grease to ensure that it does not detach from the slide. Images were taken with the IX71 fluorescence microscope both with LED and white light transmission. The DOPC was tagged with DOPE-CY5 and the ABHD5 was tagged with the fluorophore CFP. The ABHD5 was allowed five minutes to bind prior to imaging.

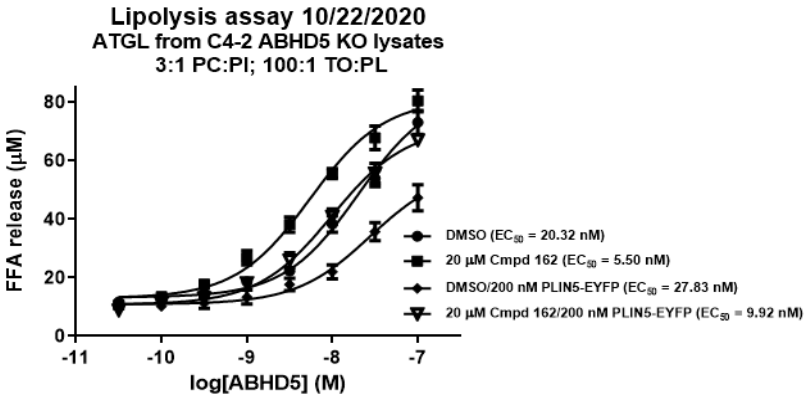

Figure 8: A binding isotherm curve created by our collaborator, Dr. Matthew Sanders, describing the various concentrations of ABHD5 at which fatty acids are released in a lipolysis assay.

Results and Discussion:

The results of the experiment where ABHD5 was added to the sample was a bit shocking. After seeing the drastic changes induced by in the SLB sample, I anticipated a similar result in this system. However, after comparing images of the aLDs with protein to the images of the aLDs without protein, no distinguishing physical changes are visible (Figure 9). Specifically, I was looking for the circularity of the aLDs to change because if ABHD5 was oligomerizing on the surface and exposing packing defects, we would expect some deformities in the shape of the membrane to be present.

One explanation for this phenomenon could be the lack of excess phospholipids surrounding an aLD. The neutral core of lipids is surrounded by phospholipids, and I am

looking for deformations in the shape of monolayer. However, if there are only enough phospholipids to just surround the aLD, then it is possible that membrane protrusions or physical changes will not be present. Recall that in the SLB sample, for membrane tubules and buds to form, there had to be some excess lipid present on the substrate that could protrude up. In this situation, any excess phospholipids that are incorporated in the process of aLD formation could be leaving the aLD as the phospholipids prefer to be in water by themselves rather than sequestered at the aLD. Another factor that could be explaining the lack of visible physical changes could be the time given to incubate the ABHD5. In this experiment I gave the ABHD5 only 5 min after being plated with the aLDs before imaging. A longer period of incubation could allow for more aggregation and oligomerization of the ABHD5 on the surface of the aLD. To improve this experiment, I would like to first try increasing the incubation time of ABHD5. Another step to potentially improve the experiment is changing the TAG to Phospholipid ratio. I used a ratio of 500:1 and got aLDs on the size of a few microns in diameter. It is possible that increasing the TAG:PL ratio could lead to larger aLDs and more surface area for the ABHD5 to bind, oligomerize, and induce membrane shape changes; however, with larger aLDs an even longer incubation time could be required.

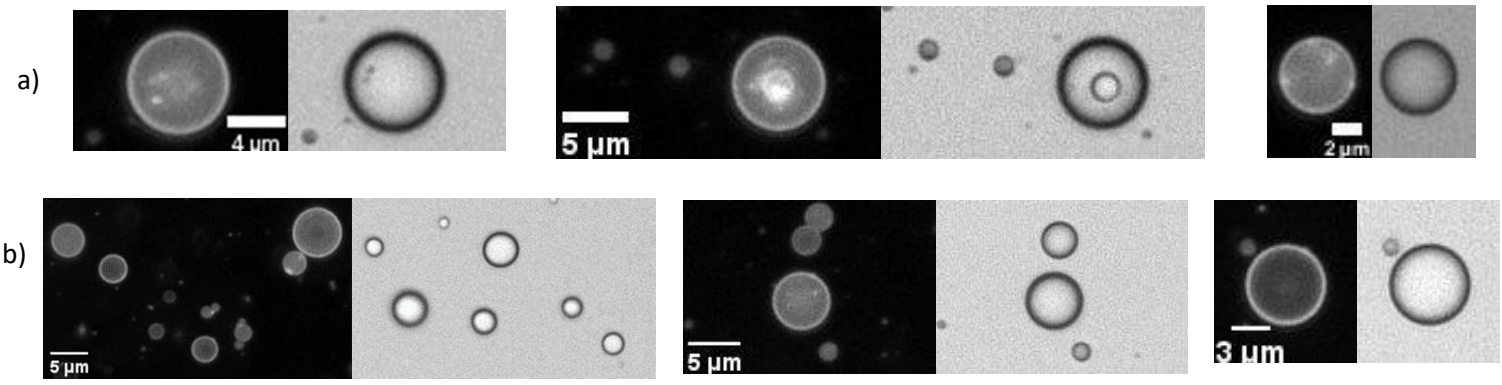

Figure 10: a) aLDs without protein **b)** aLDs with the addition of ABHD5. aLDs in both sets of images demonstrate similar circularity and shape indicating ABHD5 has not caused a morphological change in the sample. Images on the left were taken with $\lambda_{exc} = 647nm$ epifluorescence, and images on the right were taken with white-light transmission.

Project 3: Droplet Embedded Vesicles:

Introduction:

Droplet Embedded Vesicles (DEVs) are a model membrane system in which Giant Unilamellar Vesicles (GUVs), large spherical membrane structures typically larger than 1 micron in diameter, are mixed with neutral lipids to form a structure that has neutral lipid sequestered in between the leaflets of the bilayer (Figure 11a).[12] When sufficient neutral lipid is between the leaflets, a protrusion forms which looks like a lens or a bud (Figure 11b).[13] This system is representative of LD formation that occurs at the ER. I wanted to learn if upon the addition of ABHD5, there were physical membrane shape changes or enhanced sorting of ABHD5 at the site of the curvature where the neutral lipid is present. This would further support the role that ABHD5 alters membrane shape to allow PNPLA2 to carry out lipolysis.

Methods:

DEVs were constructed by mixing a neutral lipid nano emulsification with Giant Unilamellar Vesicles.[12] Lipid stock solution used to make GUVS consisted of 74.4% DOPC (w/w), 24.4%(DOPE), and 0.2% DOPE-CY5(w/w) all purchased from Avanti Polar Lipids®. To prepare GUVs, two ITO plates were acquired and tested with a multimeter to determine conducting and nonconducting sides. The plates were cleaned with ethanol, dried off with nitrogen gas, and placed in a vacuum for five minutes. After being cleaned, a square shaped silicone spacer, with a slit at one of the edges, was placed on the conducting side of one of the plates. 30 µl of the lipid stock solution was placed on the plate followed by spin coating in increments of two to three drops to have the lipids stick to the glass while removing the solvent. Vacuum grease was applied to the edges of the spacer and the other ITO plate was attached. A 200 mM solution of sucrose was injected quickly into the chamber through the slit in the spacer till the chamber was full of the solution: it is important in this process to ensure that no air bubbles are present as this will hinder GUV formation. The chamber was then connected to a waveform generator with a frequency of 10Hz and an amplitude of 4Vpp for two hours. The chamber was then disconnected and the solution of GUVs was extracted, placed in an Eppendorf tube, and left on a rotator in 4-degree fridge.

The nano-emulsification was made by mixing 70ul of intracellular buffer (IB), filtered with a 0.45µm filter, with 5µl of triolein; the volume of triolein was increased to 150ul in later experiments to improve DEV formation. In a second tube, 50µl of the GUV solution was placed. The tube with the IB and triolein, was then vortexed and sonicated for 10s two times until the nano-emulsification had a creamy, cloudy texture. With a

truncated 200µl micropipette tip, 50µl was rapidly collected and gently poured into the tube with the GUVs. The solution was mixed for about 2-3 minutes, during which the volume of the nano-emulsification was sucked and then released in the solution while moving the micropipette tip back and forth. This mixing process is what helps to incorporate the triolein into the GUVs to form DEVs. The solution was then placed on a mixer for at least five minutes to allow for further mixing. DEV samples were placed on a 96 well plate that had been passivated with a 10% (w/w) BSA solution. The images were collected on the Olympus IX71 epifluorescence microscope and an Olympus spinning disk confocal microscope. To ensure that DEVs were being seen, white light imaging was used because the index of refraction of oil is much higher and provides good contrast. Epifluorescence images were analyzed using ImageJ and the confocal images were analyzed with QuPath and ImageJ.

Results and Discussion:

In the first experiment, the DEVs were plated and ABHD5 was flowed into the well similar to the SLB experiment. Drastic changes in the sample started to take place, even at low concentrations of ABHD5, such as 0.4nM. The spot in the sample where the protein was being injected into the well displaced/destroyed DEVs, meanwhile spots further away from the point of injection were more intact (Figure 13). To confirm this was due to flow, a control experiment in which only IB was injected, in which ABHD5 is suspended, confirmed the heterogeneity in the sample based on where the liquid was injected. Another issue with the sample was the size of the DEVs (Figure 14). The portion of the DEV where the TO is concentrated is only 1µm in diameter compared to the GUV which is 4µm (Figure 12). Having a DEV on this size scale can make it difficult

to see the ABHD5 sorting. Furthermore, few DEVs were being formed when including only 5ul of triolein. To remedy the first issue, ABHD5 was added to the DEVs in an Eppendorf tube and placed on a spinner for thirty minutes to allow for binding. To increase the size of the triolein droplet attached to the GUV, the volume of triolein injected to make the nano-emulsification was increased to 100µl. This did lead to greater triolein incorporation in the GUV and consequently larger DEVs (Figure 15).

When ABHD5 was added to the DEVs, no apparent physical changes were occurring similar to the aLD project. However, to get a better understanding of whether the protein was sorting to the site of curvature, the sample was imaged on a confocal microscope. Images were acquired in white-light transmission, $\lambda_{exc} = 647nm$ for the Cy5 fluorescent lipids, and $\lambda_{exc} = 440nm$ for ABHD5 CFP. Preliminary results indicate that ABHD5 is binding to the portion of the DEV where triolein is concentrated. When ABHD5 was added to the DEV sample at a concentration of 50nM, in the CFP channel there was some preferential sorting of ABHD5 to the neutral lipid bud in the DEV (Figure 16); this is quantified by the large spike in fluorescence intensity shown in the line scan histogram in the CFP color channel (Figure 16). This result was compared to the line scan histogram of a DEV with the addition of the protein buffer as a control. The control does not show any increased fluorescence signal. The signal the histogram from the control recorded matches the background signal present in areas of the sample where the DEV is not present in the (Figures 17,18). Although no large-scale membrane changes were observed with the addition of ABHD5, the preferential sorting to the site of the neutral lipid bud is an encouraging preliminary result that supports the hypothesis

that ABHD5 oligomerizes on the surface of an LD and creates packing defects to liberate fatty acids.

One of the interesting aspects of the system are the different tensions that exist in the DEV: bilayer tension and monolayer tension (Figure 19)**.**[15] The literature shows that lower bilayer and monolayer tensions in the system lead to more budding activity (i.e. neutral lipid protrusion in the bilayer is more spherical). Experimentally this was confirmed by varying the phospholipid PC:PE ratio in a variety of systems. For both monolayer and bilayer tensions, reduced tensions via reduced PC:PE ratios led to increased egression of the neutral lipids from the bilayer indicating that at lower tensions, the budding activity occurs.[15] In the computational effort, a PC bilayer with a triolein lens was simulated with molecular dynamics. The results showed as the bilayer tension was increased, neutral lipid molecules left the lens.[15] The conclusions from these experiments are very important to keep in mind as membrane tension plays a role in the size of the lipid droplet formation and hence how well the ABHD5 membrane shape changes are resolved.

To improve the results of the experiment, it would be very useful to increase the size of the lipid droplet portion of the DEV. Attempts at this were made in this project by increasing the volume of triolein, however it may be pertinent to add even more triolein to improve incorporation into the DEV. Furthermore, lowering the tension in the DEV may increase activity of ABHD5, as less energy may be required to remodel the membrane. This can be done by lowering the ratio of PC:PE in the sample.[15]

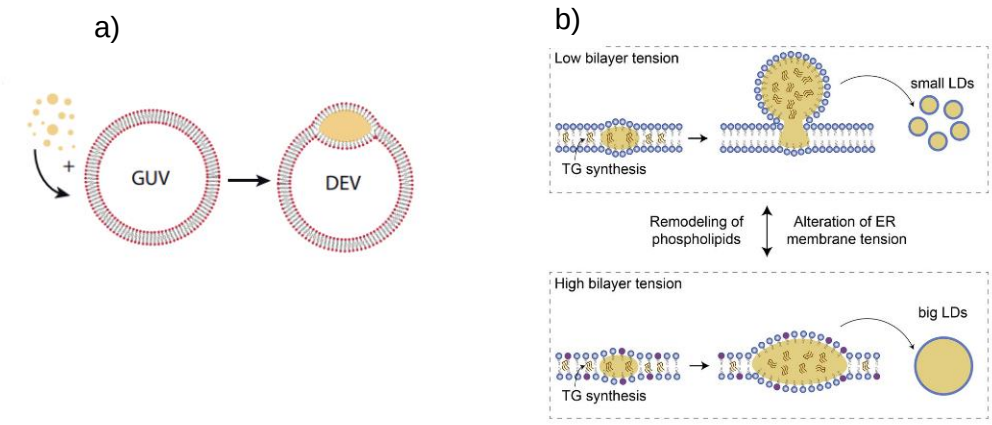

Figure 11: a) Incorporation of triolein into the GUV to form a DEV.[12] **b)** Low bilayer tension leads to the formation of spherical bud. High bilayer tension leads to a lens like shape. These are structural variants of DEVs.[13]

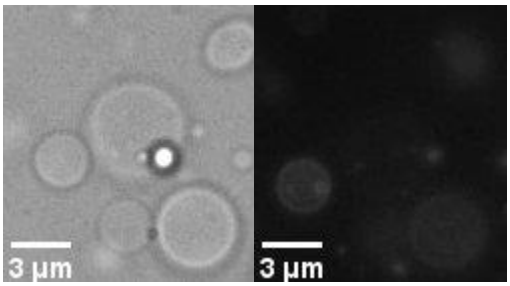

Figure 12: White-light transmission(left) and $\lambda_{exc} = 647nm$ epifluorescence image of a DEV (right). The bright white circle present in the left panel is the lipid droplet attached to the DEV. It appears this way because the index of refraction of oil.

a)

b)

c)

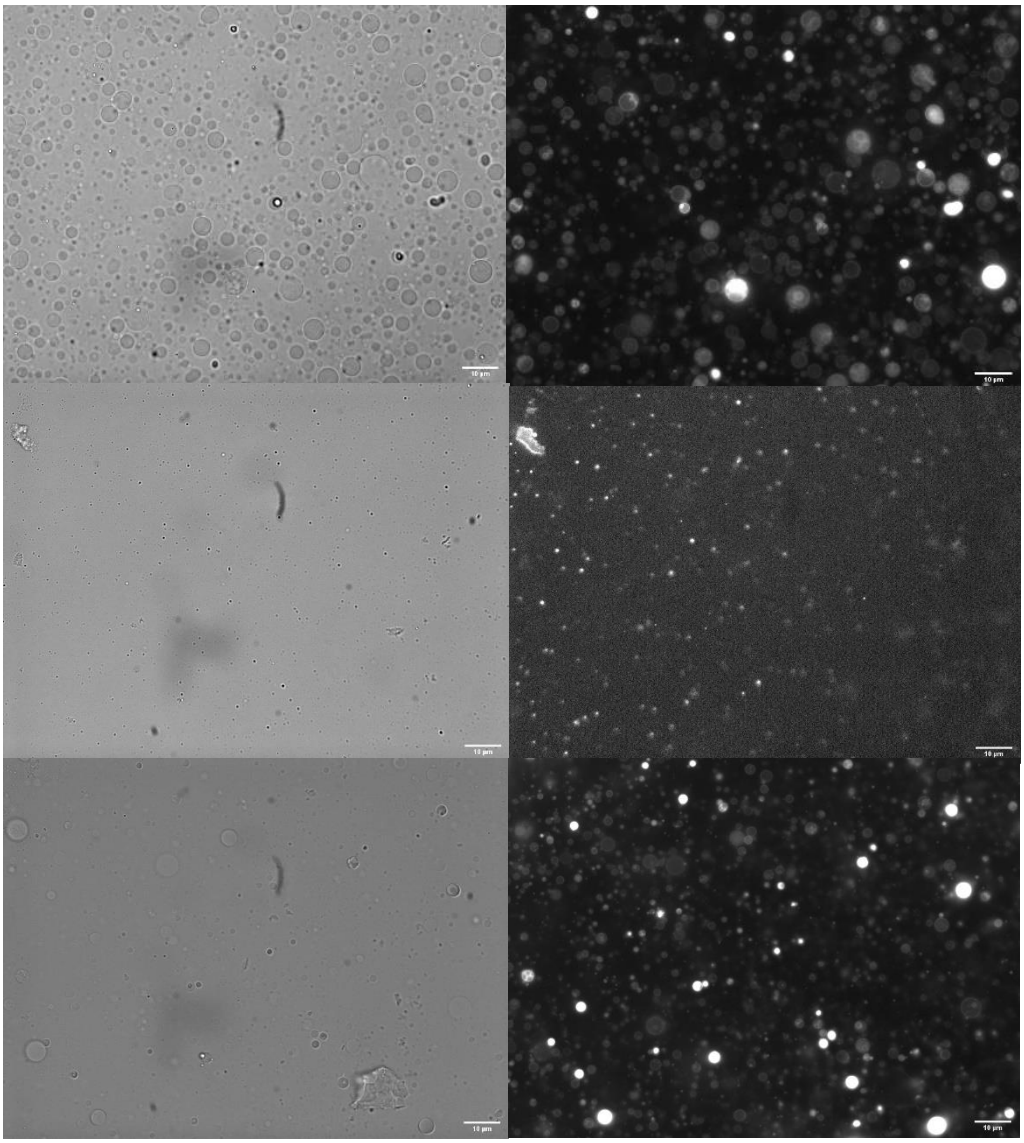

Figure 13:) White-light transmission images of DEVs (left) and $\lambda_{exc} = 647nm$ epifluorescence images (right). **a)** Before ABHD5 was flowed into the sample**. b)** Spot in the well where ABHD5 was injected. The DEVs and GUVs have been displaced and/or destroyed due to the perturbation from the flow.**c)** In the same well as (b) but imaged away from the point of injection. Density of GUVs and DEVs visible in the right image confirm the flow of the liquid is disturbing the sample making observing protein induced changes very difficult.

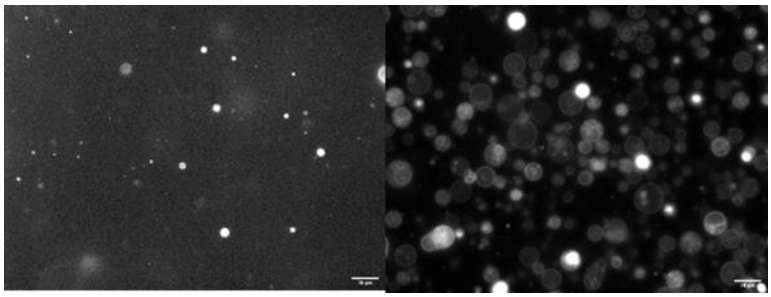

Figure 14: Injection of IB into the well of DEVS as a control experiment. Left panel shows point of injection. Right panel shows a spot further from injection. Difference in the density of GUVs and DEVs confirms flow-based perturbations were disturbing the sample. Both images were taken with $\lambda_{exc} = 647nm$ epifluorescence.

a) b)

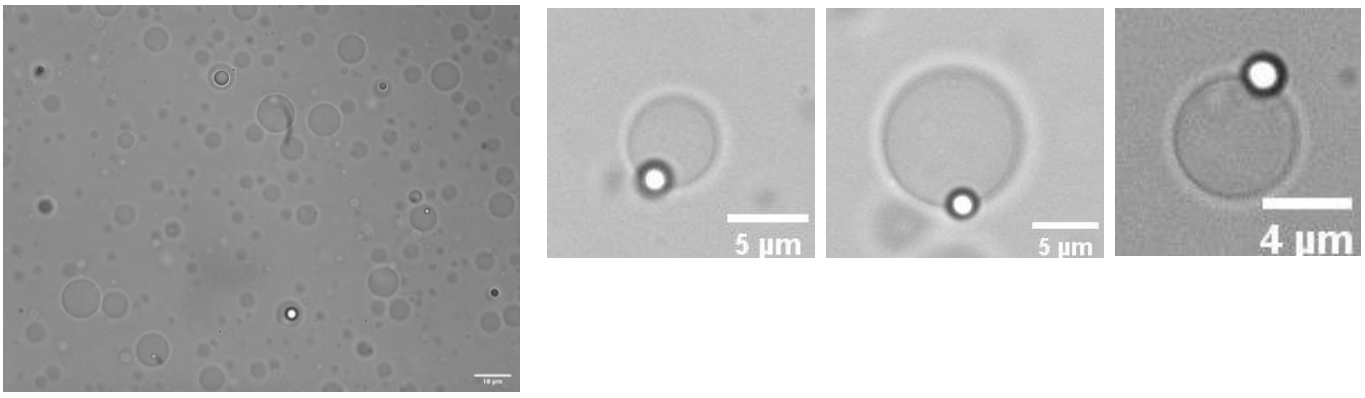

Figure 15: a) Image of larger DEVs present after the increase of triolein in the nano-emulsification. **b)** Various images of aLDs with the increased triolein incorporation. All images were taken with the white-light transmission.

a) b) c)

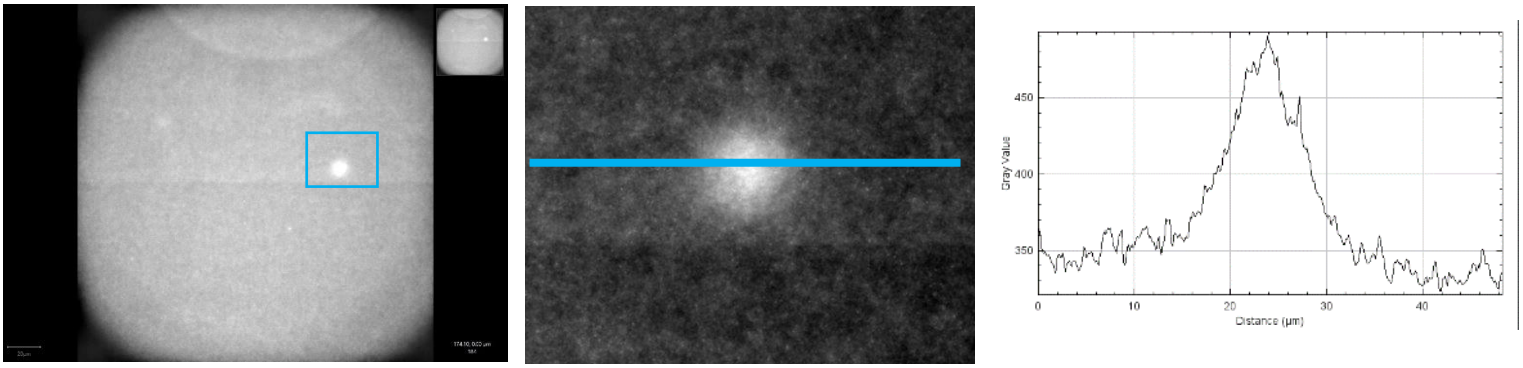

Figure 16: a) Image of a DEV with $\lambda_{exc} = 440nm$ epifluorescence**. b)** Zoomed in section of the blue box in (a). The blue line are the locations used to create the histogram. Images were acquired with a spinning disk confocal microscope. **c)** Line scan histogram of (b). There is a very large spike in fluorescence intensity in the middle. This indicates that ABHD5 is sorting to lipid droplet in the DEV.

a) b)

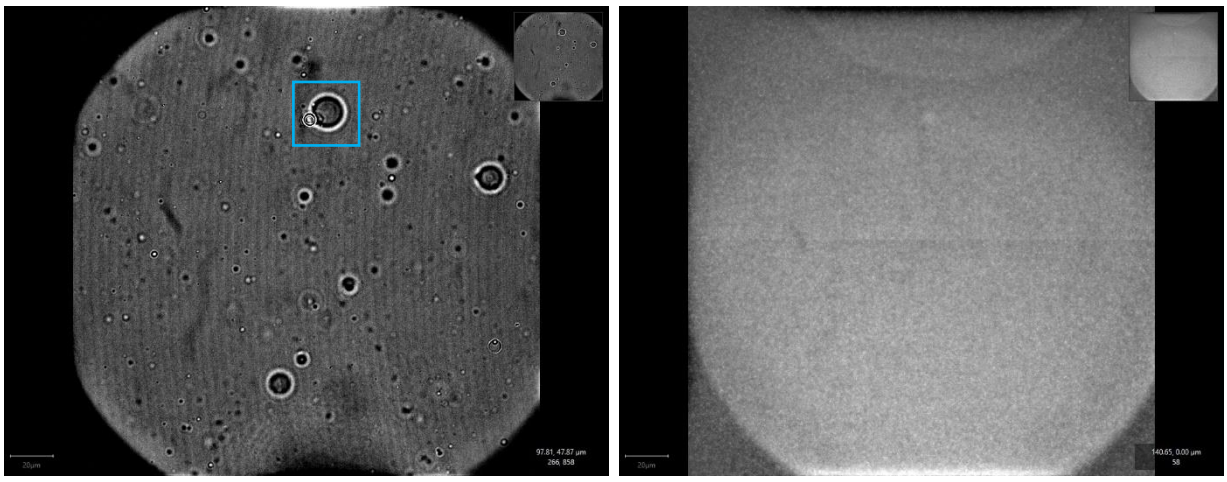

Figure 17: a) White-light transmission image of a DEV. Only the protein buffer was added to this sample as a control. **b)** $\lambda_{exc} = 440nm$ confocal image of (a). Both images were acquired with a spinning disk confocal microscope.

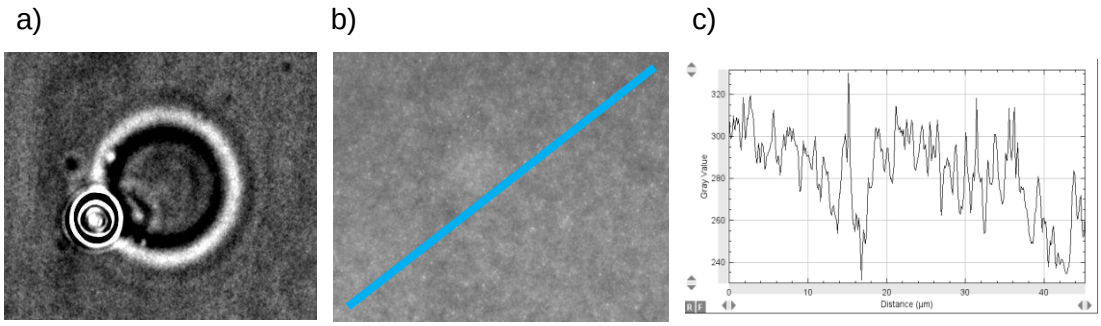

Figure 18: a) Zoomed in white-light transmission image of a DEV from the figure 16a as indicated by the blue box. Oil in the lipid droplet provides the high contrast in the image due to its index of refraction. **b)** $\lambda_{exc} = 440nm$ confocal image of (a). Both images were acquired with a spinning disk confocal microscope**. c)** Line scan histogram of (b) as indicated by the blue line. The histogram shows fluctuations in fluorescence intensity in the range of 250-300 a.u. This is the same level of fluorescence intensity seen in figure 16c in the areas away from the DEV indicating sorting of ABHD to the DEV.

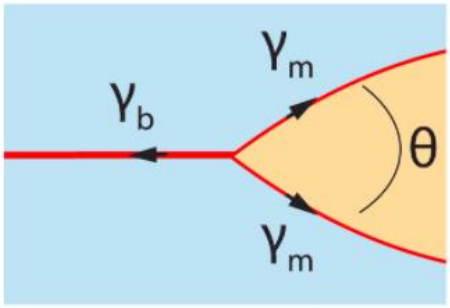

Figure 19: The various tensions that exist in a droplet embedded vesicle. γ_b shows the tension due to the bilayer and γ_m shows the tension due to the monolayer. Lower tensions lead to more spherical protrusions mimicking the LD.[15]

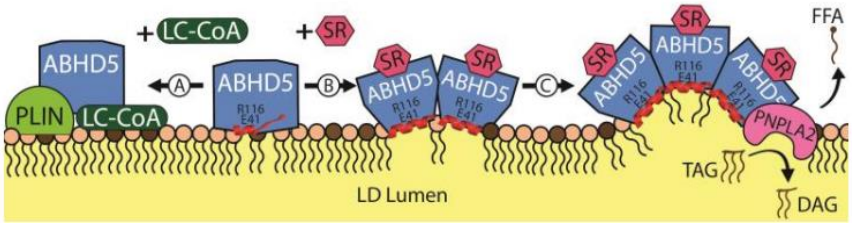

Figure 20: The various compounds proteins and compounds involved in lipolysis. ABHD5 is in its repressed state when bound to PLIN-1. Upon the addition of the SR compound, it begins to oligomerize and remodel the monolayer exposing packing defects. This then allows the enzymatically active protein PNPLA2 to hydrolyze the release of a fatty acid. LC-CoA is a compound that stabilizes the repressed state of ABHD5, which binds to PLIN-1.

Future Work:

ABHD5 is not the only protein/molecule involved in lipolysis. PLIN-1 is a perilipin protein that binds to ABHD5 and represses its function. Our collaborator, Dr. Granneman, discovered that there are synthetic ligands (SR compounds) which can modulate the activity of ABHD5 by increasing its dissociation from PLIN1 (Figure 20).[16] The next step in my research would be to confirm these findings. An experiment to confirm these findings would be to compare how ABHD5 binds and remodels the membrane in the presence of these compounds on a DEV. I expect to see the activity and sorting of ABHD5 to the site of curvature to decrease upon the addition of PLIN-1. When SR compound is added to the sample in this state, activity of ABHD5 should increase again. Confirming these findings on the DEV would further support the hypothesis that ABHD5 has membrane remodeling activity.

An interesting direction for the work is trying to understand the energies at the protein-protein interaction between oligomers of ABHD5. What kinds of bonds are being formed between the proteins? How much energy is released in the formation of the bonds? Do different phospholipid compositions enhance or hinder oligomer formation of ABHD5? What membrane tensions do the proteins operate at best? Knowing that ABHD5 has non-enzymatic activity illustrates the need to understand the protein-protein interactions between the oligomers. There may be pertinent information in this part of the problem that can help to regulate the process of lipolysis.

Conclusion:

The mechanism by which lipolysis functions is an important process that has important health implications. The complexity of live cells makes understanding the

relevant membrane shape change phenomenon difficult. However, with model membrane systems employed in this paper, these changes can be resolved much more easily. The idea that ABHD5 creates packing defects in the membrane exposing the neutral lipids to the enzymatically active PNPLA2 was tested in this project by adding ABHD5 to various model membrane systems. The SLB system showed drastic morphology changes by the way of vesiculation and fracturing of lipid buds and tubules. Furthermore, increased fluorescence intensity of the protein where buds and tubules were in the sample is encouraging evidence that ABHD5 remodels membranes. Preliminary results from the confocal images of the addition of ABHD5 to DEVs, indicate that ABHD5 is sorting the protrusion in the GUV where neutral lipid has aggregated. These results help to paint the picture that lipolysis may be mediated by a membrane remodeling process.

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