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CourseSource

Lesson

Investigating the Function of a Transport Protein: Where is ABCB6 Located in Human Cells?

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Abstract

One of the challenges of teaching Cell Biology is helping students understand important research methods used to study cells. The goal of understanding cell biology methods is especially challenging in courses without a laboratory component. When studying cells, determining the location of a protein is important for understanding the protein's cellular function. In this lesson, upper-division Cell Biology students will learn about two commonly used methods for protein localization: 1) immunoblotting after differential centrifugation, and 2) immunofluorescence microscopy. They will work in small groups to answer questions in a problem set written in the spirit of Process-Oriented Guided Inquiry Learning (POGIL). Students will explore key points of the two methods and then apply their knowledge to the analysis of protein localization data from the primary literature. Analyzing protein localization data will help students develop the ability to "apply the process of science", which is one of the Vision and Change core competencies.

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Supporting Materials: S1. Problem Set: Investigating the Location of a Transport Protein: Where is ABCB6 Functioning in Mammalian Cells, S2. Description of Individual Roles, S3. Figures and Data in PowerPoint Format, and S4. Matched-pair exam questions. Available for instructors only upon request: (S5) Protein Localization in Cells: Answer Key to Problem Set.

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Learning Goal(s)

How do protein localization methods enable and limit our understanding of the cell?

Learning Objective(s)

At the end of this activity students will be able to:

- describe the use of two common research techniques for studying proteins: SDS-PAGE and immunoblot analysis.
- determine a protein's subcellular location based on results from: 1) immunoblotting after differential centrifugation, and 2) immunofluorescence microscopy.
- analyze protein localization data based on the limitations of differential centrifugation and immunofluorescence microscopy.

INTRODUCTION

Students of Cell Biology need to understand how new knowledge is obtained in the field. For example, proteins carry out critical functions for cells. How do cell biologists begin to characterize a protein's function in a cellular context? Through genomics and bioinformatics efforts, scientists have identified an impressive number of protein-coding genes, but our understanding of the functions of their products is less advanced. A first step towards understanding a protein's function is to identify the cellular location of the protein. In our 300-level Cell Biology course, we wanted to help students understand two major methods for localizing proteins within cells: 1) immunoblotting after differential centrifugation, and 2) immunofluorescence microscopy. Then, we wanted them to be able to interpret data from these methods. We realized that to do so, we first had to help them review common research techniques covered in their pre-requisite courses, such as SDS-PAGE and immunoblot analysis (or Western blotting). To meet these goals, we created a student-centered lesson in the style of Process Oriented Guided Inquiry Learning (POGIL) (1, 2).

Background on protein localization methods

Protein localization is an important biological process and its study spans research across the life sciences including fundamental biology research and medical research. It even has application in therapeutic treatments of human disease (3). One way to determine a protein's cellular location is by using differential centrifugation followed by immunoblot analysis (4, 5). In differential centrifugation, cell components are separated based on size and density. Hypotonic solutions and sonication or homogenization are used to break open or "lyse" cells, resulting in a "whole cell lysate". The lysate is centrifuged at a low speed to pellet nuclei, which are the largest cell component. The supernatant of the low-speed spin contains all other cell components besides nuclei. The low-speed spin supernatant is then centrifuged at a higher speed to pellet other cell components. This process is repeated with progressively higher centrifuge spins. The higher the centrifuge speed, the smaller the cell component that can be obtained in a pellet. At the end of differential centrifugation, the pellets from the different centrifuge steps represent different "fractions" of the cells. Proteins from each pellet can be separated based on size and charge using SDS-PAGE. Separated proteins are then transferred to a membrane for immunoblot analysis. Primary antibodies that recognize marker proteins are used to identify fractions that contain a particular organelle. For example, an immunoblot probed for the mitochondrial marker protein, porin, will indicate which pellets contain mitochondria. If a protein of interest is present in the same fractions as porin, the protein of interest is likely to be located in mitochondria. If a protein is localized to the mitochondria, this suggests that the protein may have a role in mitochondrial structure or function.

Another approach commonly used by cell biologists to determine protein localization is immunofluorescence microscopy (6, 7). Similar to immunoblotting, immunofluorescence microscopy relies on the use of primary antibodies specific for a protein of interest and marker proteins. Cells are "fixed" to slides by chemically treating the cells to kill and preserve them. Cell fixation is also important for permeabilizing cell membranes to allow antibodies to enter and bind to their target proteins. Then cells are viewed using a fluorescence microscope with filters for each fluorophore, compounds that emit light of a specific wavelength when excited by another wavelength of light. If the signal for a lysosomal marker protein, such as Lamp1, overlaps with the signal for a protein of interest, the protein of interest is likely to be in the lysosomes. Thus, it can be hypothesized that the protein has a role in the structure or function of lysosomes. For a more detailed explanation of these techniques we recommend instructors consult Alberts' Molecular Biology of the Cell (8), or Lodish's Molecular Cell Biology (9).

Background on Process-Oriented Guided Inquiry Learning (POGIL)

POGIL is an active-learning approach centered on small-group work. One of the principles behind the POGIL approach is the idea that a learner must construct knowledge in their own mind in order to learn (10). In addition, POGIL acknowledges that students working in groups can learn more, understand more, and remember more than when they work alone (11, 12). POGIL encourages students to explore content, outline concepts, and apply concepts (1), while focusing on the use of process skills such as teamwork, communication, and problem solving (13). We use POGIL in our Cell Biology class because it allows our students to actively learn and use cell biology concepts while developing process skills that they can transfer to a variety of settings.

POGIL involves small groups of three to six students with each student playing a defined role. Because we teach our Cell Biology class in a SCALE-UP room with tables designed for groups of three (14), we limit our groups to three students playing the following roles: Manager, Recorder, or Presenter (1). The Manager keeps the group focused and makes sure all members contribute. The Recorder writes down the group's answers on the official copy of the problem set, which is the only one from the group that will be graded. The Presenter speaks on behalf of the group in class discussion and shares answers on the white boards. All students contribute their ideas to help complete the problem set. The problems sets are designed to guide students from knowledge-based questions to analysis and evaluation questions (1). We do not call our problem sets "worksheets" because our students say this reminds them of being in grade school.

Our protein localization lesson

An education in the life sciences involves both the acquisition of knowledge and the development of skills to apply that knowledge. In some circumstances, it is not possible to provide students with hands-on experience in the laboratory. We know from cognitive learning theory that providing our students with the opportunity to construct their own knowledge during an engaging activity leads to improved learning (15, 16). Using primary literature to provide students with data to analyze is one way to involve them in investigating commonly used research methods. Applying the process of science in this way gives students the opportunity to "discover" the way a method works and then to analyze real data without the constraints of having to successfully perform the method in the laboratory.

We provide a lesson in the spirit of POGIL to allow students to explore protein localization methods. This activity is completed in a single class period and is completely student -driven, meaning that it involves no lecture time. When students encounter unfamiliar terms or concepts they work together to develop their understanding. Students work in small groups on a problem set designed to guide them from knowledge-based questions to analysis and evaluation questions. At the end of the lesson, students engage in a class discussion. Students volunteer to share their answers to the problem set and the instructors address misunderstandings that may have emerged during the activity. For example, some students may imagine that the four panels shown in question 3 are from one immunoblot. To address this, we remind students that four different immunoblots were performed and the researchers cropped the immunoblot images so readers can compare the results from each fraction more easily.

We centered our POGIL activity around a paper by Kiss et al. because the paper's results provided students with the opportunity to analyze cell biology localization data (17). The authors of the paper studied a type of transport protein from the adenosine triphosphate–binding cassette (ABC) family called ABCB6. ABCB6 was predicted to be responsible for porphyrin uptake into mitochondria. The authors wanted to characterize the function of ABCB6, and they started by investigating the protein's location. One of the major research questions they

asked was: "Where is ABCB6 found in mammalian cells?" The authors used multiple cell biology methods to answer this question, and they were surprised to conclude that ABCB6 does not localize to mitochondria. One reason we chose this paper is because it underscores the importance of documenting the location of a protein rather than just predicting its location. Another reason we chose this paper is because the data are not as clear as they are in textbook cartoons. We wanted our students to appreciate that real data can sometimes be challenging to interpret and that using additional methods can help strengthen conclusions.

Intended Audience

We teach this lesson in a 300-level Cell Biology course at a public research university. The lesson we share has been taught in more than ten sections of Cell Biology, including by the authors and other instructors. Our Cell Biology students are typically junior or senior life science majors.

Required Learning Time

We teach this lesson in a single 75-minute class period. We have also taught it in a 50-minute class period (see Adaptations in Teaching Discussion).

Pre-requisite Student Knowledge

Our Cell Biology students learn about SDS-PAGE and immunoblotting in their pre-requisite courses, which include Genetics and Biochemistry. If your students are not familiar with SDS-PAGE and immunoblotting, you can introduce them to these techniques in class or through a homework assignment.

Pre-requisite Teacher Knowledge

You should read the Kiss, et al., paper on the ABCB6 transporter, which contains the original data used in the lesson (17). You should be familiar with all of the methods covered in the problem set: SDS-PAGE, immunoblotting, differential centrifugation, and immunofluorescence assay. Alberts' Molecular Biology of the Cell (8) and Lodish's Molecular Cell Biology (9) have excellent coverage of these topics. Older versions of both books are available at no cost via the National Center for Biotechnology Information Bookshelf (www.ncbi.nlm.nih.gov/books).

SCIENTIFIC TEACHING THEMES

Active learning

Active learning occurs when instructors step away from the traditional lecturer role and allow students to take control of developing their own conceptual understanding (18). This student-driven lesson involves three major activities.

- Students work collaboratively in groups to answer the problem set questions. These peer-to-peer interactions allow the students to share their prior knowledge and discover new understanding together.
- Students work together to interpret data from the primary literature. Working together to decipher the data models the collaborative nature of the scientific process.
- Students present their findings during class discussion. These brief presentations help students build their communication skills while also keeping the responsibility for learning focused on the students rather than on the instructor.

Assessment

Formative Assessment: Each group of three students turns in one official copy of the problem set for grading. This is the Recorder's problem set, which will be returned to the Recorder after grading. The instructor or a teaching assistant provides detailed written feedback. We have taught this lesson in class sizes ranging from 40 to over 200 students. We cut grading down to a third by only providing feedback on the Recorder's official copy. We often have teaching assistant (TA) support for grading, but we have also given feedback ourselves when TA support was not available. When the official copies of the problem set are returned we encourage group members to photograph the written feedback if they did not serve as the Recorder. We discuss common points of confusion at the beginning of the next class session.

Summative Assessment: We include "matched-pair" questions on our unit exam and/or final exam that are related to those in the problem set (Supporting Materials File S4). The exam questions are similar to, but not the same as, the ones on the problem set. Matched-pair exam questions require students to apply the knowledge and skills they gained in the lesson to new situations (19).

Inclusive teaching

This lesson uses groups of three with roles that were defined by the POGIL method. Each group member has a specific role: Manager, Recorder, and Presenter; explained below. The roles rotate weekly to ensure that students have the opportunity to experience each role. This structure encourages students with different backgrounds to share their knowledge. For example, students from underrepresented groups will have the chance to play each type of role, rather than only the roles that students from the predominant group might unconsciously restrict them to.

LESSON PLAN

We designed this lesson for a single 75-minute breakout period, but we have also adjusted it for a 50-minute class period. We teach the lesson in a SCALE-UP classroom equipped with round tables and computers that students can use in groups of three. The lesson can also be taught in classrooms with moveable chairs or even lecture halls with fixed seats (see Adaptations). Students will need internet access and an electronic device to answer some of the questions. A suggested timeline is provided in Table 1.

Pre-class Preparation

Before class you will need to assign students to groups (if you choose to) and print copies of the problem sets (see Supporting Materials File S1). The problem set is five pages long and requires color printing. If you are unable to print in color you can project the figures that require color for interpretation using an LCD projector (see Supporting Materials File S3).

Beginning of Class

We use this as the first activity of the semester; if your students are already used to working in groups you may be able to spend less time preparing them for the activity. You will begin by explaining and assigning roles: Manager,

Recorder and Presenter (see Supporting Materials File S2). The Manager keeps the group on task, the Recorder writes the group's answers on the official copy of the problem set, and the Presenter presents the groups answers during the class discussion at the end of class. The group roles are randomly assigned. The student with the birthday closest to that day is the Manager, the student with the birthday next closest is the Recorder, and so on. Give the students a few minutes to meet each other and determine who has what role. Once the roles are determined, the groups answer the questions provided in the problem set in the order in which they appear. We ask our students to use just one computer per group so that all three members of a group work together rather than working on their own in parallel.

Problem Set Page One

Question 1

Question 1 of the problem set guides students through a review of SDS-PAGE and immunoblotting. It includes a cartoon depiction of both methods (S1-Figure 1). Students explain the purpose of SDS-PAGE and how it works (S1-Question 1A). This information is prior knowledge for our Cell Biology students, but we encourage them to use an online search engine to look up any information they cannot remember. The process of looking up forgotten information keeps the responsibility for learning focused on the students rather than on the instructor. Students then explain the purpose of an immunoblot and how it works (S1-Question 1B). Finally, students explain why a scientist would choose immunoblotting instead of an SDS-PAGE gel alone (S1-Question 1C). These questions set students up for the next section of the problem set where SDS-PAGE and immunoblotting are both used for protein localization.

Problem Set Page Two

Question 2

Question 2 introduces students to the use of differential centrifugation to purify cell components. The students are asked to read about differential centrifugation and examine Figure 2. Then they are asked about how a hypotonic solution can help break open or lyse cells. Differential centrifugation is used to obtain the data in Question 3.

Question 3

Question 3 introduces students to data from Kiss, et al., 2012 (17), where the authors report their investigation of the cellular location of ABCB6, a member of the adenosine triphosphate-binding cassette (ABC) transporter family. The researchers used differential centrifugation, SDS-PAGE, and four immunoblots. Figure 3 is the data from immunoblots of organelle pellets obtained from human K562 leukemia cells.

Question 3A asks students to find out what an ABC transporter is, using an online search engine if necessary, and to explain where they might find such a transporter. Question 3B asks about the suitability of the organelle marker proteins. Their answer prepares them for Question 3C, which asks them to describe the data they see in Figure 3. They are asked to determine where ABCB6 is located based on these data. They are then asked to provide a justification for their analysis.

Question 3D asks students whether or not the researchers were able to achieve a high level of organelle purity based on the data in Figure 3. Students are also asked to provide a rationale for their conclusion. This question helps students learn to analyze data critically.

Problem Set Page Three

Question 4

Question 4 introduces students to immunofluorescence assay, an additional method for identifying the localization of a protein. The description explains how immunofluorescence labeling of proteins uses similar principles as immunoblotting. The description then introduces the use of fluorescent probe-conjugated antibodies to visualize protein locations.

Question 4A asks students to identify the "blue" organelle in the micrographs. Most students will correctly identify the blue organelle as the nucleus, especially with the provided hint to consider the shape and size of the blue organelle. If some students say "plasma membrane", encourage them to think about where the lysosomes and mitochondria are located. These organelles are shown outside the "blue" organelle. Thus, the "blue" organelle cannot be the plasma membrane.

Question 4B asks students to interpret the data in the figure and make a conclusion about where the ABCB6 transporter is located in the cell. The three-dimensional nature of the cell versus the two-dimensional nature of a micrograph may confuse students. Some will say the mitochondria should not be inside the nucleus. Encourage them to consider how a micrograph is different from an actual cell, and whether or not the image indicates that one organelle is inside the other.

Question 4C asks students whether co-localization data can definitively tell you the location of an organelle. Similar to Question 3D, students must consider the limitations of using immunofluorescence assay for determining protein location.

Question 4D asks students to explain why the researchers used two approaches to test the location of ABCB6. This question will prompt them to consider the limitations of the methods.

Question 5

Question 5 asks students to think about why it is useful to know the location of a protein in a cell. This brings a greater context to the lesson.

Completion of Problem Set/End of Class

When about 15 minutes of class remain, ask students to stop working. Then ask for volunteers to come to the front of the room to share their answers. A document camera can be used to project students' answers. Begin the discussion with Question 3, unless students have questions about the earlier problem set questions. Ask for one of the Presenters to volunteer to answer question 3C. In discussing Question 3C, students should mention that the patterns of Lamp1 (lysosomes) and the ABC transporter are similar, but that the results are not conclusive since there are no samples with pure lysosomes.

Ask for another Presenter to answer 3D. Students should note that all of the fractions have more than one cell component, indicating a lack of purity. You may want to ask them how they could improve the purity (e.g., purification with antibody-

conjugated beads or additional gradients). Ask them to predict the immunoblotting results from a fraction of pure lysosomes as a hypothetical 6th fraction.

Request a new Presenter to answer 4A and 4B. For 4A, students will use size and shape to determine that the organelle in question is the nucleus. For 4B, students should discuss the three-dimensional nature of the cell versus the two-dimensional format of a micrograph. If time allows, ask for another Presenter to discuss question 4C and 4D. Students should note that the apparent overlap in a 2D image might represent structure in front of or behind one another, rather than inside one or the other. They should also point out that the use of two methods helps strengthen the authors' conclusion.

When discussion is complete be sure to thank the students for their participation as you collect the Recorder's completed copy of the problem set.

TEACHING DISCUSSION

Activity's effectiveness at achieving the stated learning goals and objectives

This activity is effective in helping students understand two protein localization methods. The lesson also helps students interpret data generated by these two methods. The constructive nature of the activity gives the students ownership of the knowledge. Their level of ownership is evident in their confidence while presenting their answers during class discussion. Groups who do not complete the activity have the opportunity to hear how their classmates answered those questions. During the discussion, volunteers also explain and justify their answers. This explanation ensures that those who came to incorrect conclusions or did not complete the questions are exposed to both the correct answer as well the reasoning behind the correct answer. After completing this lesson, our students do well on matched-pair exam questions designed to assess the extent to which they have met the problem set's learning objectives (see Supporting Materials File S4 for example exam questions).

Reactions to the activity

We have used this activity in at least ten sections of Cell Biology in the past three years and the response from students has been very positive. The discovery nature of the activity often leads to lively discussion and even excitement from the students. Students appreciate the opportunity to learn new methods and apply their understanding to problems.

For students who have little or no experience with small-group work, it is important to allot enough time at the beginning of the lesson to allow them to settle into their groups. Once the students have gotten through this brief "getting to know each other" period, we have found that they will work through the assignment with little or no resistance. Groups that are working very quietly or do not seem to be working together may need encouragement to engage with their classmates.

Adaptations

If you do not have access to a SCALE-UP classroom, this activity can be done in a regular classroom with movable chairs. If necessary, it is possible to do the activity in a room with fixed seating because there are only three people per group. In general, if you are unable to achieve an even distribution of three-member groups, we have found that groups of two stay on task with less social loafing than groups of four.

We have done this activity in both 75-and 50-minute class periods. If you only have a 50-minute class period, you may want to give the first question of the problem set as homework for students to complete before class. Students would then begin collaborating with their groups to answer questions on page 2. Alternately, you can pace the students using a displayed countdown clock (http://timer.onlineclock.net/) to help them progress through the questions.

SUPPORTING MATERIALS

- S1. Problem Set: Investigating the Location of a Transport Protein: Where is ABCB6 Functioning in Mammalian Cells?
- S2. Description of Individual Roles
- S3. Figures and Data in PowerPoint Format
- S4. Matched-Pair Exam Questions
- Available for instructors only upon request: (S5) Protein Localization in Cells: Answer Key to Problem Set

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Table 1. Investigating the Function of a Transport Protein: Teaching Timeline

Activity	Description	Time	Notes
Preparation for Class			
Print problem sets	You will need one copy of the problem set for each student in the class. The Recorder will turn in his or her copy and may wish to take a picture of it before submitting it. He or she will have this copy returned to them after grading.	Depends on number of students	Print problem sets in color or be prepared to project Supporting File S3 PowerPoint file with figures and data in color.
Assign groups	We allow students to choose their own groups for the first four weeks of this type of activity. After the first four weeks, we randomly assign new groups by having students count off "1, 2, 3, and so on" so that all the "1"s are in a group, all "2"s are in a group, and all the "3"s are in a group and so on. This ensures that no one is in a new group with the same people.	Depends on assignment method	You can assign students to groups or you can allow them to choose their own groups in class.
Beginning of Class			
Instructor orients students to the activity	1. Share the student group assignments or allow students to choose their own groups. 2. Pass out the "Description of Individual Roles" handout. Explain the roles: manager, recorder, and presenter. 3. Randomly assign roles: whoever has a birthday closest to today is the Manager, whoever has a birthday after that is the Recorder, and so on. 4. Pass out copies of the problem set "Investigating the Function of a Transport Protein: Where is ABCB6 Located in Human Cells?"	5-10 minutes	• The problem set and description of individual roles handout are provided as Supporting Files S1 & S2. • We have found that groups of two are better than groups of four. For a group of two, one person plays both the Manager and Recorder roles.
Problem Set - Question 1			
Students answer Question 1	• Question 1 helps students review the basic methods they will be using throughout the lesson. Students may use the internet if necessary to answer these questions. • Question 1A asks the students to explain what SDS-PAGE is used for and how it works. • Question 1B asks the students to explain what an immunoblot is used for and how it works. • Question 1C asks the students to explain why a scientist would choose an immunoblot over a SDS-PAGE gel alone.	5-10 minutes	• This material is expected to be a review for upper-division cell biology students. • We recommend that each group use just one computer (or just one electronic device) to ensure they work together. • The room will become quite noisy. If any groups are quiet, check in with them.

continued on next page...

Table 1. Investigating the Function of a Transport Protein: Teaching Timeline continued

Activity	Description	Time	Notes
Problem Set - Questions 2 and 3			
Students answer Question 2	• Question 2 introduces students to differential centrifugation, a method commonly used to separate cell components. This method can be used to determine a protein's location in the cell. • Question 2 asks students to explain how a hypotonic solution could help break open (or lyse) cells.	5-10 minutes	If a group seems stuck they can look up "hypotonic" on the Internet.
Students answer Question 3	• Question 3 presents students with original data from Kiss et al 2012 (1). The research group wanted to identify the localization of an ABC transporter, ABCB6. • Question 3A asks students to use the internet to find out what an ABC transporter is, and then to explain where they might find such a transporter. • Question 3B asks students to consider the appropriateness of the organelle marker proteins. • Question 3C asks students to describe what they see in a figure from the paper, which is immunoblot analysis of cell fractions after differential centrifugation. They are then asked to determine in which cellular compartment ABCB6 is located based on these data. They must explain the reason for this determination. • Question 3D asks students to consider the quality of the organelle purification and to explain their answer.	10-15 minutes	• Question 3D helps students realize the limitations of this method. • Students may be reluctant to state that the purification is not good. Ask them if any of the samples (lanes) contains a pure fraction of lysosomes and how they know this.
Problem Set - Questions 4 and 5			
Students answer Question 4	• Question 4 introduces students to the use of immunofluorescence microscopy to determine protein localization. Question 4 includes original data from the Kiss et al 2012 paper (1). • Question 4A ask students to identify the blue organelle in the figure. • Question 4B asks students to analyze the data presented in the figure and to make a conclusion about the location of the ABCB6 transporter. • Question 4C asks students to consider the limitations of immunofluorescence microscopy data. • Question 4D asks students why the authors of the paper used two different methods for determining the location of ABCB6.	10-15 minutes	• Some groups may not complete this page. • Students find the hints for Questions 4A and 4C to be helpful.
Students answer Question 5	Question 5 asks students to consider why the researchers wanted to know the location of ABCB6 and why protein location matters.		
End of Class Discussion			
Students present their answers to Problem Set questions	• Ask students if they have questions about Questions 1, 2, or 3A before beginning class discussion. • Ask for volunteer presenters to share their answers. Focus the discussion on students' answers to Questions 3 and 4. • Ask for a new volunteer for each question.	15 minutes	You can also number the groups at the start of the lesson and use a random-number generator to call on groups.
Groups Turn in Handout			
Groups turn in their handout	At the end of the class period the Recorder turns in the official copy of the problem set with their group's answers.		Recommend that the students take a photo of the handout before turning it in, that way everyone has a copy of what was turned in.

Reference:

Kiss K, Brozik A, Kucsma N, Toth A, Gera M, Berry L, Vallentin A, Vial H, Vidal M, Szakacs G. 2012. Shifting the Paradigm: The Putative Mitochondrial Protein ABCB6 Resides in the Lysosomes of Cells and in the Plasma Membrane of Erythrocytes. PLoS One. 7 (5): e37378.