

**Quantitative analyses of cargo trafficking and compartmental integrity in Lowe Syndrome
(September 1, 2015 to August 31, 2016)**

Participants:

Student	Faculty Advisor	Department	University
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Details of dates and attendance of team interactions/meetings

S.No	Dates	Venue	Participants
1	December 8-9 2015	NSF Site Visit - Purdue	Swetha, Ilan

Details of money spent

Grant awarded: \$5000

Money spent: \$0

Money remaining: \$5000

Posters:

1. Swetha Ramadesikan, Ilan Shomorony, Prashant Thapa, Priya Venkatasubramani, Yucong Zhang, Mark Ward, Walid Sharabati, R. Claudio Aguilar. "Quantitative analysis of protein intracellular localization in Lowe Syndrome". NSF Site Visit 2015 – Purdue University

Presentations:

Same title, presented to CSol through virtual brown bag forum

Introduction

In order to develop novel and more efficient therapeutic approaches we need to understand disease mechanisms, which in turn requires the collection and analysis of biologically-relevant information. Unfortunately, the complex nature and high-information content of biological data, together with the lack of proper analysis methods make the information gathering and interpretation process very slow and imprecise. For example, following microscopy, image analysis is typically performed by visual examination of hundreds of cells or structures per experiment. This method, in addition to limiting the amount of samples that are to be analyzed, is not accurate and potentially poorly reproducible as it introduces human error and bias. Although

some algorithms are currently available, they rely on subjective assumptions and are not suitable for widespread applications. We are interested in studying the distribution of mutated protein Ocr11, mutations in whose gene results in the lethal developmental disease called Lowe Syndrome. Our preliminary semi-quantitative results suggests that mutations in the *OCRL1* gene results in differential distribution of the mutated protein product. We predict that this has consequences on organelle function and morphology. We rationalize that depending on the nature of mutation, the distribution of mutant protein varies, thereby giving us the ability to determine possible cellular changes the patient might have. The objective of this student-initiated proposal is to develop tools to extract and analyze quantitative biological information related to organelle content and localization of target proteins. In particular, we will focus on an organelle – the Golgi apparatus – which is the major compartment in which Ocr11 localizes.

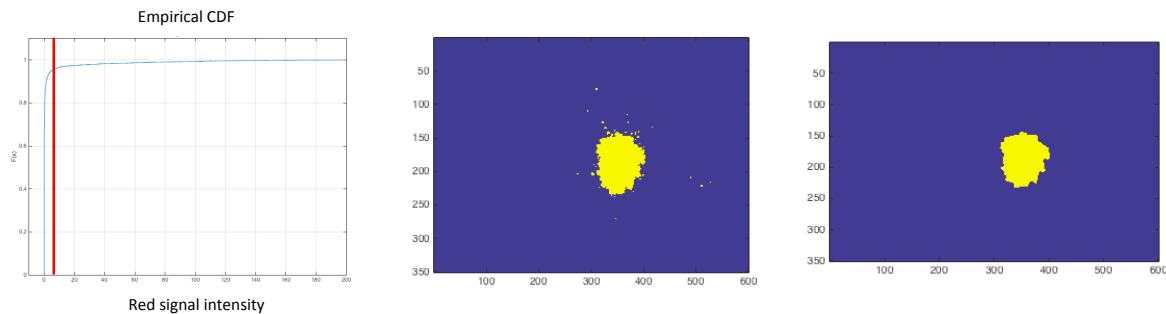
Quantification of Colocalization between Golgi Apparatus and OCRL1 protein in Lowe Syndrome (LS) patient cells

The data used for this project was in the form of fluorescent microscopic images of cells both from Lowe syndrome patients and healthy individuals. As illustrated in Figure 1, the red and green channels are used to store images for different fluorescent markers, associated with the Golgi apparatus, and the OCRL1 protein respectively.



Figure 1: Sample input: Cell expressing WT Ocr11 and stained with trans-Golgi marker TGN46

Our pipeline utilizes these two images in order to obtain a visual and quantitative characterization of the level of colocalization between the Golgi apparatus and OCRL1. The first step is to use the TGN46 image (red image) to identify the position of the Golgi. In order to do we first remove the leakage between the channels of the microscope (see Figure 2a). Then we utilize a threshold operation in order to identify the image pixels that correspond to the Golgi apparatus (Figure 2b) and we remove some of the remaining noise by utilizing a low pass filter and a second threshold operation on the image (Figure 2c)



The next step is to consider the green image,
 Figure 2: (a) Empirical cdf of signal intensity in the TGN46 image. The signal leakage from the other channel is removed by identifying the point where the cdf becomes approximately flat and removing pixels with lower intensities. (b) A simple thresholding on the resulting image is used to identify the location of the Golgi apparatus (c) A lowpass filter is applied on the image, followed by another threshold operation, to remove additional noise.

which indicates where the OCRL1 protein is present. We utilize the location of the Golgi obtained from the TGN46 image (Figure 2c) and consider the intensity of the corresponding pixels in the green image to quantify the presence of OCRL1 in the Golgi (Figure 3a). In addition, as shown in Figure 3b, we identify a “moat” around the Golgi apparatus (the set of pixels that are within a fixed distance from any pixel inside the Golgi) and also quantify the presence of OCRL1 in the moat (Figure 3c). This gives us two measurements of average OCRL1 intensity: inside and outside the Golgi. If the former is greater than the latter, we declare that OCRL1 is colocalized with the Golgi apparatus.

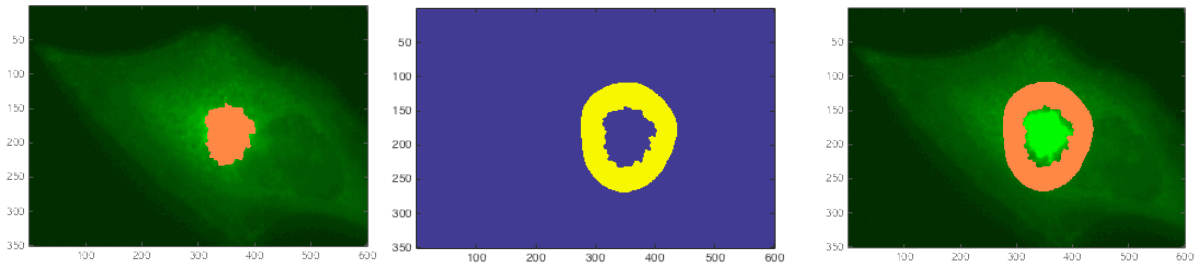


Figure 3: (a) The pictures identified in the TGN46 image are translated to the green image to determine the presence of OCRL1 in the Golgi. (b) The moat around the Golgi region corresponds to the set of pixels that are within a fixed distance d of any pixel inside the Golgi. (c) The moat is used to determine the presence of OCRL1 in the near vicinity of the Golgi apparatus.

In addition to this binary classification (OCRL1 colocalized with Golgi or not), our approach provides a numerical quantification of colocalization. More precisely, we can compute (1) the fraction of the total OCRL1 that is in the Golgi (measured via the intensity of the pixels in the green image), and (2) the fraction of the Golgi apparatus that contains OCRL1. These two quantities can be intuitively visualized via Venn diagrams, as illustrated in Figure 4.

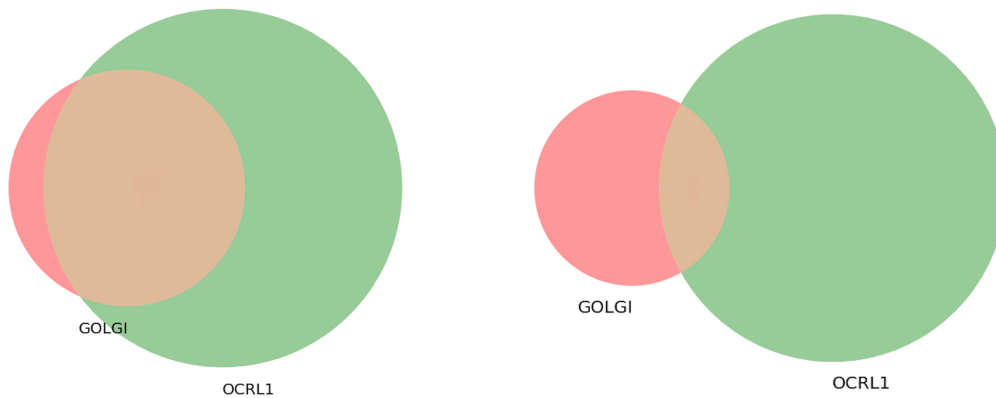


Figure 4: (a) For a Wild Type (WT) cell (no Lowe Syndrome), we notice that most of the Golgi (87%) contains a significant amount of OCRL1. Moreover, 38% of the total OCRL1 in the cell is located in the Golgi. (b) For a Lowe Syndrome patient cell (mutation on Q215X), only 23% of the Golgi contains a significant amount of OCRL1, and only 16% of the total cell OCRL1 is located in the Golgi apparatus

Future goals: Extend this pipeline of steps to other organelles such as early endosomes, lysosomes and centriole.