UTRIP Working Paper

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I have been in Tokyo for more than 25 days. Since I came here 10 days later than other UTRIP participants, I had only 30 days to finish my project. Actually, when I first got to the lab, there were many problems, the biggest of which is time. As for other participants, they had already done 10 days' work and their projects here were highly related to their major at university. Therefore they did not have to spend too much time learning background knowledge before getting started for the projects. For me, however, things were a little different. Although I always have a great interest in Biology and had done some research work on Bioinformatics at Peking university, my major, after all, is statistics and I had little knowledge of System Biology. That is why I had to spend longer time than others to learn background knowledge before I actually started my research. This really worsened the already difficult situation caused by the limited time. Frankly speaking, I had no confidence to complete the project at that time.

Anyway, after the first day of my orienting myself in Todai and Kuroda lab, my project officially started on July 7th. The first few days in the lab was reading time. Professor Kuroda lent me some books including *Molecular Biology of the Cell* by Alberts et al. Honestly speaking, for a student who spent last three years learning only Mathematics and Statistics, reading a Biology textbook was not an easy task. The first day was the most difficult one, because I needed to look up a pile of Biology technicalities on the Internet to support my reading. Sometimes, I had to look up plenty of other words first so as to understand the definition of the technicality that I originally wanted to know. Rather than saying "learn Biology", it is better to use the word "discover" in my case, for everything seemed brand new and fascinating to me. Anyway, after 4 days of reading, I was finally clear about my research background.

The goal of my project is to statistically analyze a cell signaling pathway called "Akt-S6 pathway", of which the signaling dynamics and regulations remained to be elucidated. Before analysis, I need to do experiments to get data, which was quite a challenge to me. I had never done biological experiments at university before I came here and this was a new land for me again. The next 10 days was for experiments. I had two experiments to learn but before that, I needed to learn some basic operations such as preparing and cleaning a bench, using pipette, how to do cell culture and so on. I practiced these operations on some PC12 cells sample, which was not just some training but also a preparation for the following experiments. I was glad that I did a great job at this part and mastered almost all the basic operations.

After that, the main part of my experiment learning began. Every day after the lecture in the morning, my TA, Saito Kun, would give me a paper with all the procedures of that day's experiment written on it. What I did was first reading the whole procedure and then watched Saito Kun demonstrating the whole process. After that, I did the whole experiment myself. During my DIY, Saito Kun would check every procedure I finished in

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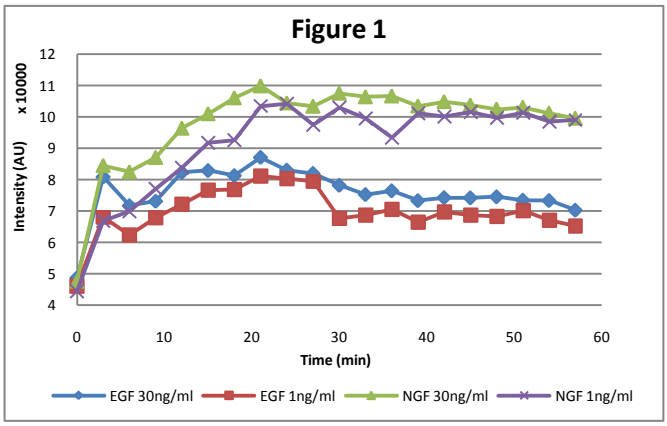
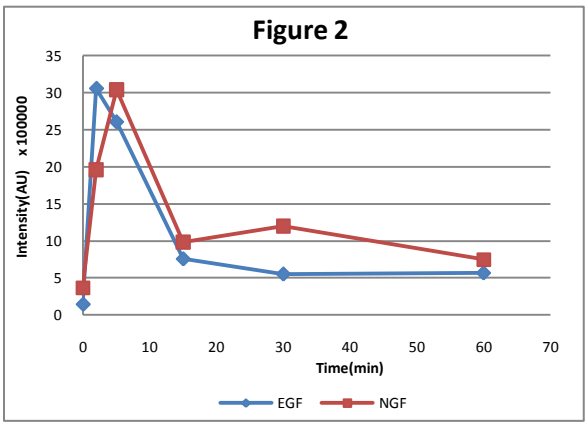
case that I made some mistakes and ruined the whole experiment.

One of the two experiments I need to learn is western blotting, a traditional but quite useful method of detecting protein signal. I treated the PC12 sample with two different growth factors, epidermal and nerve growth factor (EGF & NGF) for different time length: 0, 2, 5, 10, 15 and 30 minutes, labeled the protein (phosphorylated Akt and S6) and detected the intensity of protein signal. The only disadvantage of WB, in my opinion, is that it takes too much time. I spent more than a week to finish this experiment. Saito Kun said many students in Kuroda lab made mistakes when they first learned to do this experiment and the most common consequence of the mistake was inability to detect signals. Fortunately, I did not make any big mistake and managed to detect protein signals. Although the result turned out to be a little different from our expectation, yet the pattern of the curves is acceptable. Saito Kun said that considering that it was my first time doing this experiment, this result was quite favorable.

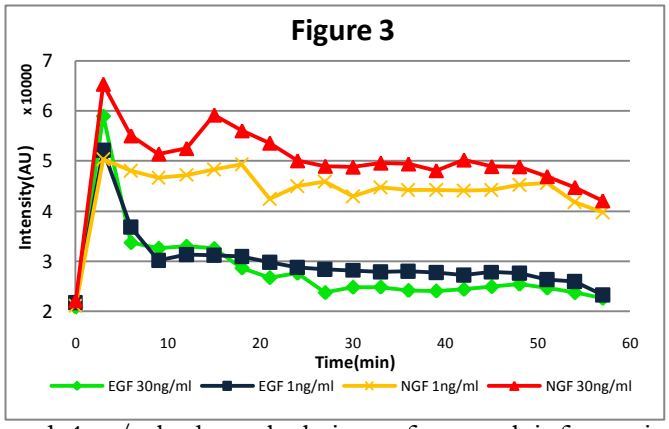
After western blotting, I began to learn Quantitative Immunostaining. It is another method to get data and more time-saving but expensive than western blotting. It only took me a couple of hours to finish the whole process and part of the experiment was completed by a robot. I only need to prepare the cells and the robot would do the stimulation for me. The stimulation lasts for different time length: 0, 3, 6, 9, … , 54 and 57 minutes. If this stimulation operation was done manually, it would take much time and quite difficult to control the time accurately. Fortunately, Kuroda lab develops the way of using robot to do the job, which makes this experiment much easier. After several hours, I got my first result which, beyond my expectation, was a little strange. There was supposed to be a peak in the time course curves of pAkt but none in my curves (Figure 1). At first we thought it was caused by the interval of time. The immunostaining machine would stimulate the cells every 3 minutes and the WB results (Figure 2) showed that there was a peak happening around 2 minutes after stimulation. Therefore, there was the possibility that QI did not catch the peak. After a discussion with Professor Kuroda and Saito Kun, I decided to redo the experiment with a 2-minute stimulation interval.



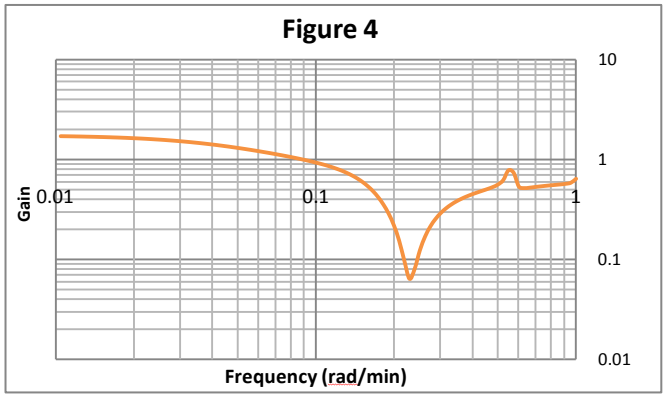


The next day, before I started doing QI again, Saito Kun showed me his result of QI. It was part of another project but also involved pAkt and pS6 activation detection. There was clearly a peak in his figures. The only difference between his QI and mine was that he

detected the protein signals immediately after the stimulation was over and I, however, did the detection 10 hours after the stimulation. Considering this, I still decided to redo the experiment but used a 3-minute stimulation interval instead of 2 and detected the signal immediately after the stimulation. This time, the result was quite satisfactory. I got a clear peak! (Figure 2) These data were very important because the later analysis would be based on them and if the data were wrong, the whole analysis would be meaningless.



It was already July 26th when I finished all the experiments and got the data. The data analysis was supposed to have two parts. One is frequency response analysis based on ARX Model and the other is mutual information analysis. Since we only treated the cells with two doses of growth factor: 30ng/ml and 1ng/ml, the calculation of mutual information may not be convincing enough. Therefore, we decided to do frequency response analysis first. Compared with experiments, ARX Model is relatively easy. Using Matlab, I got the result of frequency response analysis (Figure 4). From the figure, we can see that Akt-S6 pathway serves as low-pass filter and the cutoff frequency is 0.058 rad/min. Previous result obtained by other researchers using a different method was 1/33.3 rad/min, which is quite similar with mine. Note that there is an ascending part of the curve when frequency is high. This is caused by an exceptional data point from the experiment, and after we remove the exceptional data point and redid the simulation, this ascending part disappeared.



Although the mutual information analysis might not be reliable with only two doses, we still did the calculation for learning purpose. Just as we expected, no meaningful result was obtained. But it is OK. As Uda Kun, another TA of mine, mentioned, if I had 10 more days like others, I could do the experiments with more doses and get a convincing MI result.

Now the internship is almost over. To make a summary, I did learn a lot in Kuroda lab, such as cell signaling, basic experiment operations, western blotting, quantitative immunostain, frequency response analysis, mutual information, and even how to make good presentation slides. These things are really valuable to me. Like I wrote in my research plan for my application for the internship, I hoped I could learn things which would help my research in the future. And now I can write in this working paper, as a reply, that I have learned far more than I expected.