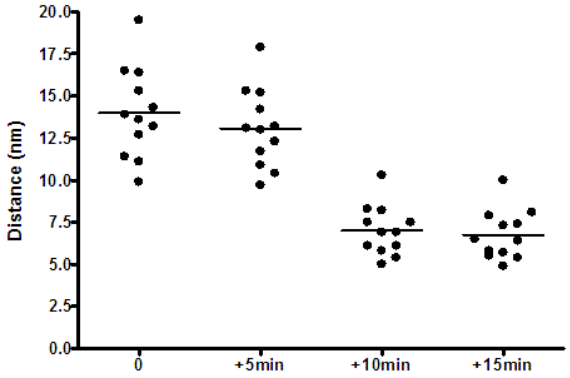
## Data Set:

I created this data set just for PHCO250-homework purpose. Each row stands for a measure for one cell. The cell value is the Cell-Peak-Distance (nanometer) of the measured cell. (See Appendix for more detail.)

| | A | B | C | D |
|---|---|---|---|---|
| | 0 | +5min | +10min | +15min |
| | Y | Y | Y | Y |
| 1 | 13.9 | 13.1 | 7.5 | 7.3 |
| 2 | 13.2 | 13.0 | 8.3 | 8.1 |
| 3 | 15.3 | 14.2 | 6.9 | 6.5 |
| 4 | 16.4 | 15.2 | 7.5 | 7.4 |
| 5 | 11.4 | 10.9 | 6.1 | 5.8 |
| 6 | 12.7 | 11.7 | 5.8 | 5.5 |
| 7 | 19.5 | 17.9 | 10.3 | 10.0 |
| 8 | 13.6 | 12.3 | 6.9 | 6.4 |
| 9 | 9.9 | 9.7 | 5.4 | 5.4 |
| 10 | 11.1 | 10.4 | 6.1 | 5.7 |
| 11 | 16.5 | 15.3 | 8.2 | 7.9 |
| 12 | 14.3 | 13.2 | 5.0 | 4.9 |

Set alpha=0.05. Use *Prism*® *4* to do one-way repeated-measures ANOVA:




| | Parameter | A<br>Value | B<br>Data Set-B | C<br>Data Set-C | D<br>Data Set-D |
|---|---|---|---|---|---|
| | | Y | Y | Y | Y |
| 1 | Table Analyzed | | | | |
| 2 | Data 1 | | | | |
| 3 | Repeated Measures ANOVA | | | | |
| 4 | P value | P<0.0001 | | | |
| 5 | P value summary | *** | | | |
| 6 | Are means signif. different? (P < 0.05) | Yes | | | |
| 7 | Number of groups | 4 | | | |
| 8 | F | 199.737 | | | |
| 9 | R squared | 0.947802 | | | |
| 10 | | | | | |
| 11 | Was the pairing significantly effective? | | | | |
| 12 | R squared | 0.216126 | | | |
| 13 | F | 15.8463 | | | |
| 14 | P value | P<0.0001 | | | |
| 15 | P value summary | *** | | | |
| 16 | Is there significant matching? (P < 0.05) | Yes | | | |
| 17 | | | | | |
| 18 | ANOVA Table | SS | df | MS | |
| 19 | Treatment (between columns) | 537.352 | 3 | 179.117 | |
| 20 | Individual (between rows) | 156.315 | 11 | 14.2105 | |
| 21 | Residual (random) | 29.5933 | 33 | 0.896767 | |
| 22 | Total | 723.260 | 47 | | |

a)

Rat2 is a fibroblast cell line suitable for single cell tracking. Our lab's interest is protein X's function in cell migration. Stable Rat2 cell line with EGFP-X expressed was constructed by retrovirus infection and FACS. In this experiment, I want know exactly when protein X is recruited to the leading edge during lamellipodia formation, which may indicate protein X's function during lamellipodia formation. (See Appendix for more detail.)

b)

In this experiment, there is only one dependent variable: protein X's Cell-Peak-Distance (CPD for short); one independent variable: protein X's sub-cellular localization during different time points after sodium azide was washed off and regular media was put back. (See Appendix for more detail.)

c)

The required assumptions for one-way RMANOVA are:

Matched subjects / Repeated measures -- Protein X's CPDs were measured from different cells after sodium azide was washed off and regular media was put back. CPDs from the same cell were placed in a block. (See Appendix for more detail.) Prism's calculation also points out that the pairing was significantly effective.

Random sampling -- Cells were picked up randomly from the movie.

Independent observations between blocks – As cells for analysis were picked up randomly, there was no relationship between blocks. And Protein X's sub-cellular localization is independent during the experiment.

Continuous interval -- The measurement of CPD is continuous.

Normal population distribution -- I check the values in each group by leaf-and-stem method, which shows that the distribution is normal presumably.

Equal variances and covariances -- According to the experimental design, the same subjects is measured at different time points, which fits to the compound symmetry. The stand deviations of the four groups are 2.67, 2.33, 1.48, 1.46; F=2.67/1.46=1.83 < 5.67, which means this data set meets ***"homogeneity of variance"*** (http://helios.bto.ed.ac.uk/bto/statistics/tress6.html).

d)

Null hypothesis: Protein X's CPDs do not change after sodium azide was washed off and regular media was put back.

Alternative hypothesis: After sodium azide was washed off and regular media was put back, protein X's CPDs change after certain time point.

d)

Alpha=0.05.

f)

Numerator degree of freedom for F is 3; denominator degree of freedom is 33.

g)

Value of F-critical is 2.892.

h)

p value of observed result is <0.0001.

i)

Reject the null hypothesis.

j)

| | | | | | |
|---|---|---|---|---|---|
| 24 | Dunnett's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff |
| 25 | 0 vs +5min | 0.908334 | 2.34953 | P > 0.05 | -0.0430940 to 1.85976 |
| 26 | 0 vs +10min | 6.98333 | 18.0634 | P < 0.01 | 6.03191 to 7.93476 |
| 27 | 0 vs +15min | 7.24167 | 18.7316 | P < 0.01 | 6.29024 to 8.19309 |

Dunnett's test was conducted and result shows that 10mins after sodium azide was washed off and regular media was put back protein X's sub-cellular localization changes significantly.

k)

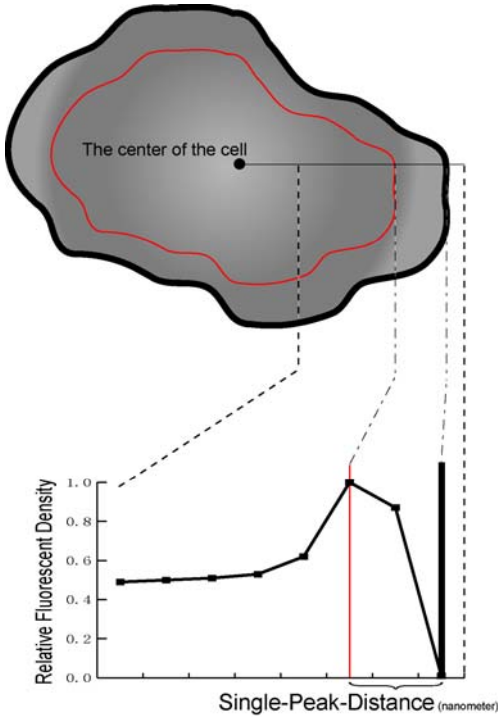
Protein X's sub-cellular localization after sodium azide was washed off and regular media was put back, which was represented by CPDs, was analyzed with one-way RMANOVA, performed with the PC-based program PRISM (GraphPad, San Diego, CA). Following the significant omnibus ANOVA, further Dunnett's multiple comparison was conducted. Alpha was set at 0.05 for all comparisons. Results of the omnibus ANOVA indicated the presence of a significant change of protein X's sub-cellular localization after the restore [$F(3,33)=199.737$, $p<0.0001$]. Additional Dunnett's multiple comparison indicated that the protein X's sub-cellular localization changed significantly after 10mins restore.

# Appendix:

## Study protein dynamics by measuring Cell-Peak-Distance

**EXPERIMENTAL DESIGN**: Living cells are adapted to the Petri dish. Treat cells with PBS (20mM sodium azide) for 1hr. *Sodium azide depletes ATP and synchronizes cells' activity.* Mount the dish to the confocal microscope. Focus and take photos from either GFP or RFP channel as control "0min". Different fields are set to collect more data from one dish. Wash off sodium azide and put back regular media. Take photos at different time points after the restore. *To avoid the effect of photo-bleaching, I prefer capture at different time points rather than real-time movie.*

**MEASUREMENT AND ANALYSIS**: Series of confocal sections from one cell are placed in a block and analyzed at the same time. The center of the cell is calculated based on the outline of the cell shape. Single line is drawn from the center to the periphery of the cell. The fluorescent density on the line is measured and the position of the highest value is marked as "Peak". The pixel from the "Peak" to the periphery of the cell on the line was calculated and defined as Single-Peak-Distance. According to the object used in the experiment, the Single-Peak-Distance is translated from pixel to nanometer. "*A large number of*" lines from the center of the cell are generated automatically by the program. *The definition of "A large number of" is based on statistics.* The average of all these Single-Peak-Distances is called Cell-Peak-Distance, which gives information about the protein's sub-cellular localization at certain time point. As there is a difference between different cells, repeated-measures ANOVA is employed to analysis the data.




*Cell-Peak-Distance*
= Average(*Single-Peak-Distance*)