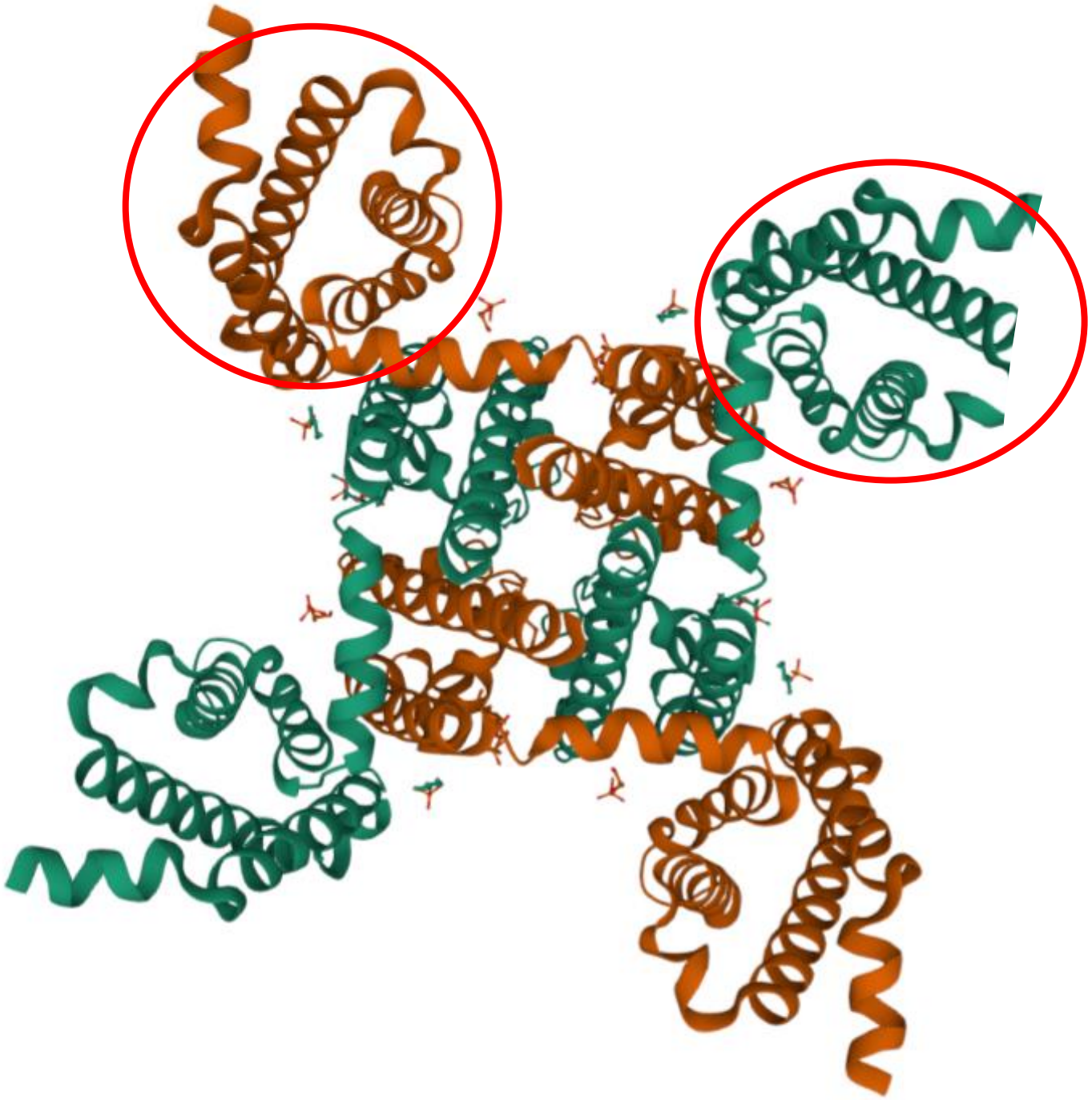
Understanding LFAs in the context of NavAb

By Tingwei Adeck

Wang Lab



NavAb Crystal Structure

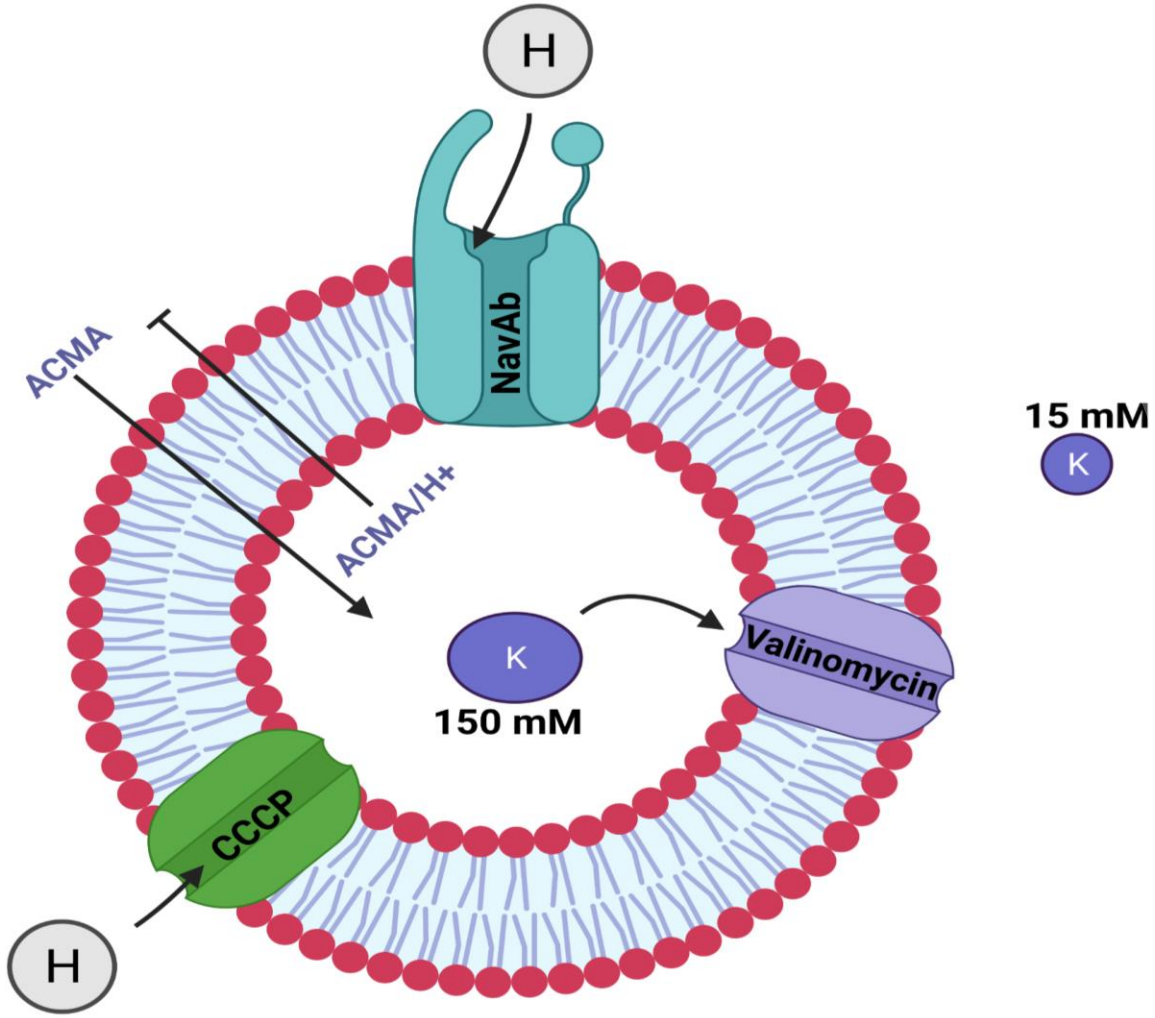
The Red circles indicate regions of proton conductivity.

Chart of NavAb Ion Conductivity and Affinity Constants

Ion(s)	K_a (Affinity Constant)
Na^+	1
K^+	0.14
Rb^+	0.02
Cs^+	0.005
H^+	??

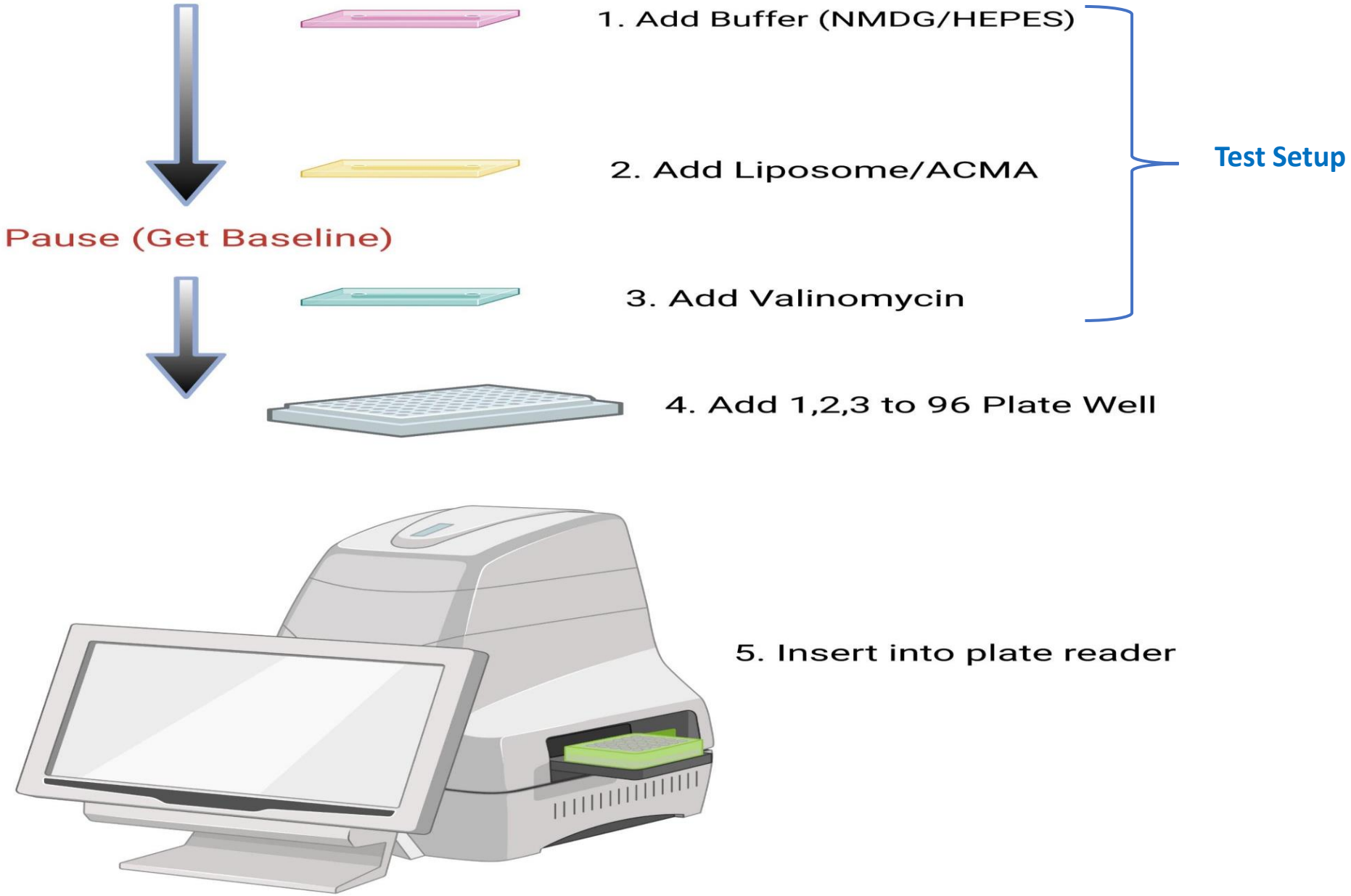
What are LFAs?

- Assays that use channel-insert Liposomes coupled with a fluorophore (ACMA in this case) to understand the properties or behaviors of these channels in an out of cell context.



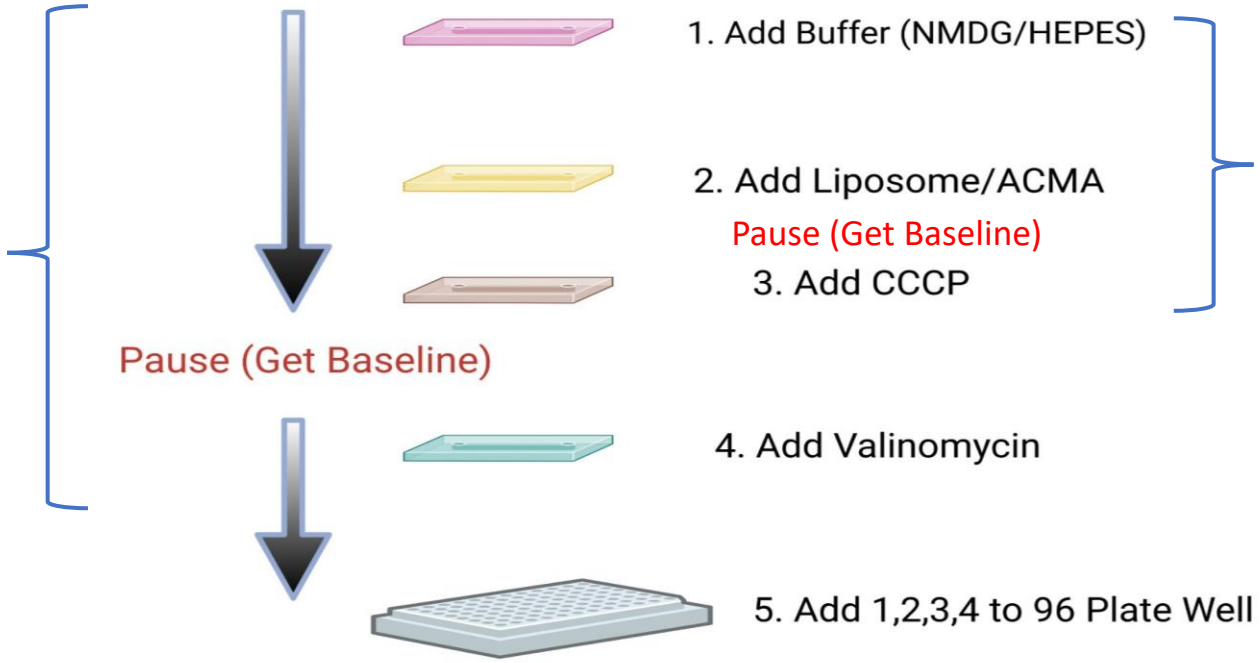
Rough Schematic of Our Concept

Rendered by BioRender



Created with BioRender.com

Positive Control



Negative Control

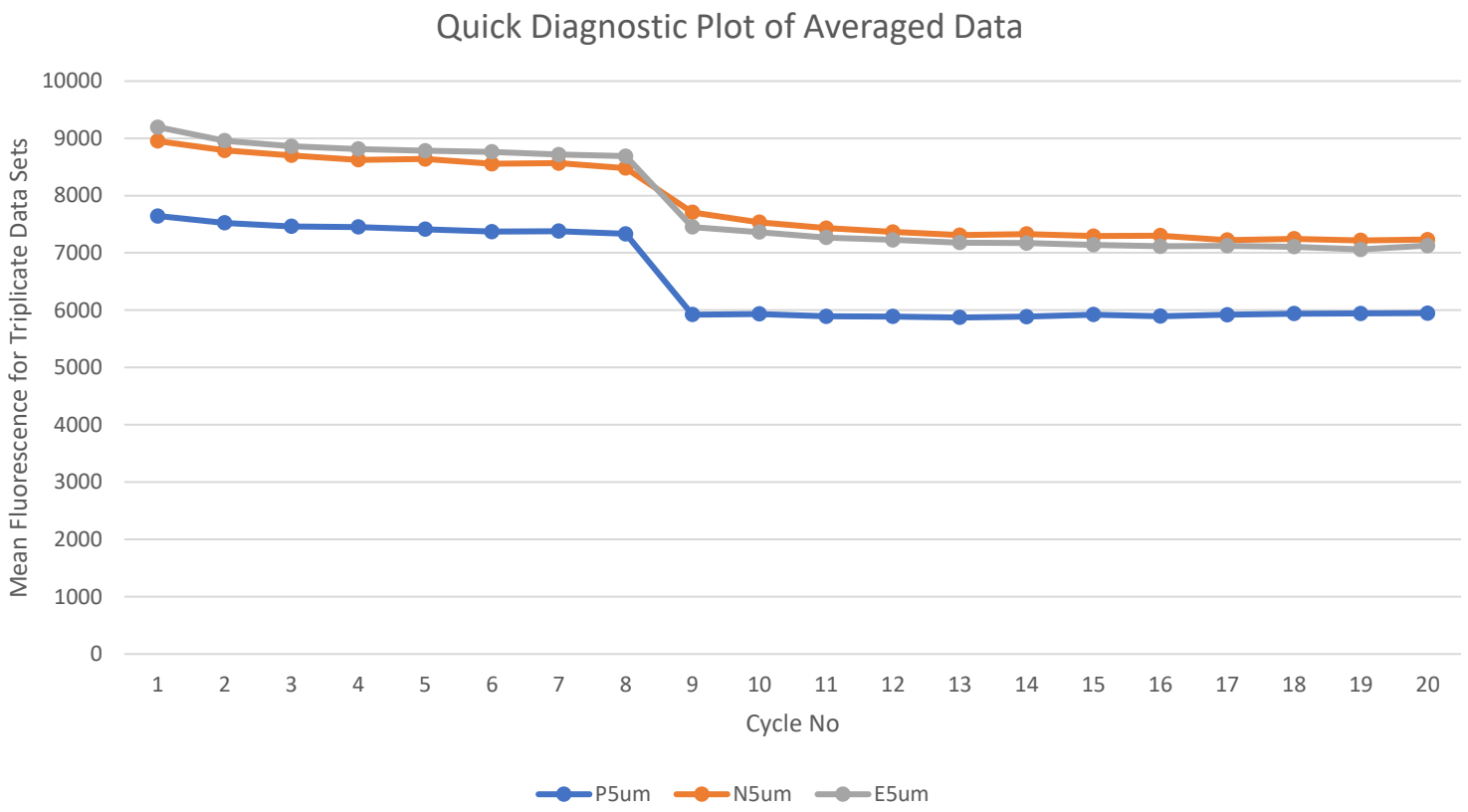
6. Insert into plate reader

Add 1,2,3 for our negative control.

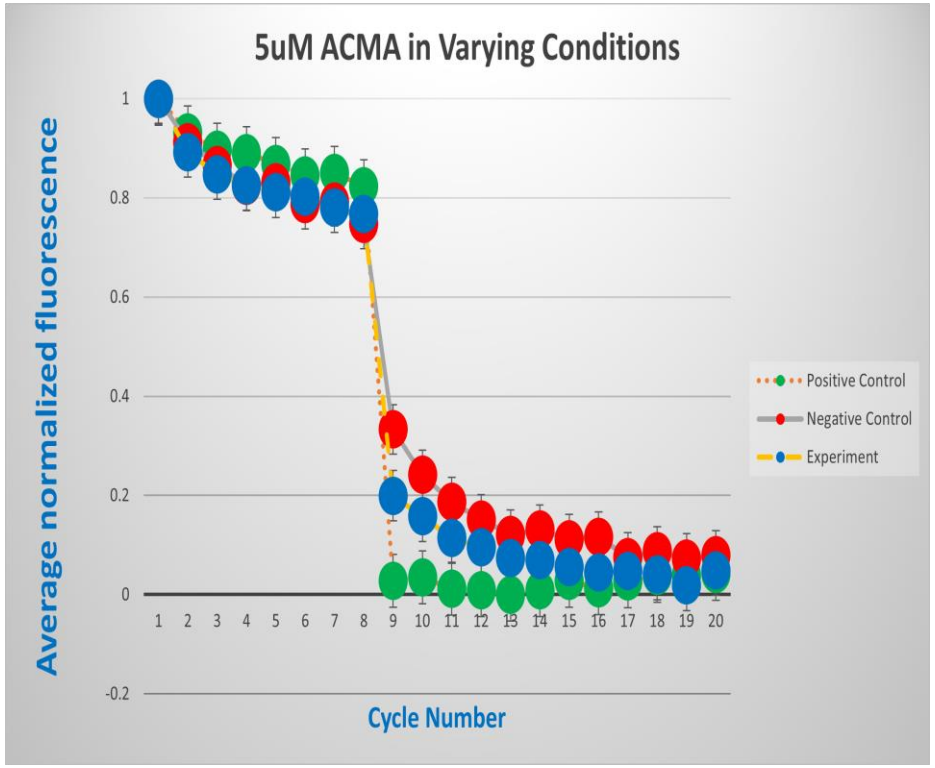
Add 1,2,3,4 for our Positive Control.

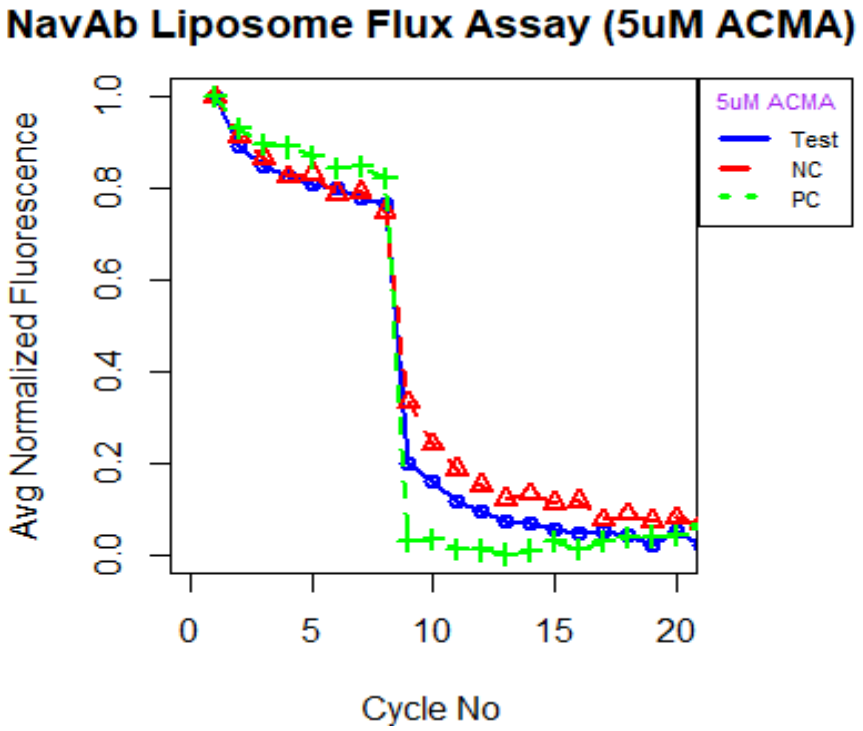
Created with BioRender.com

Mean Fluorescence of Raw Data on a Sliding Scale at 5uM ACMA-K inside



Constant ACMA (5uM) vs Varying Conditions-K inside

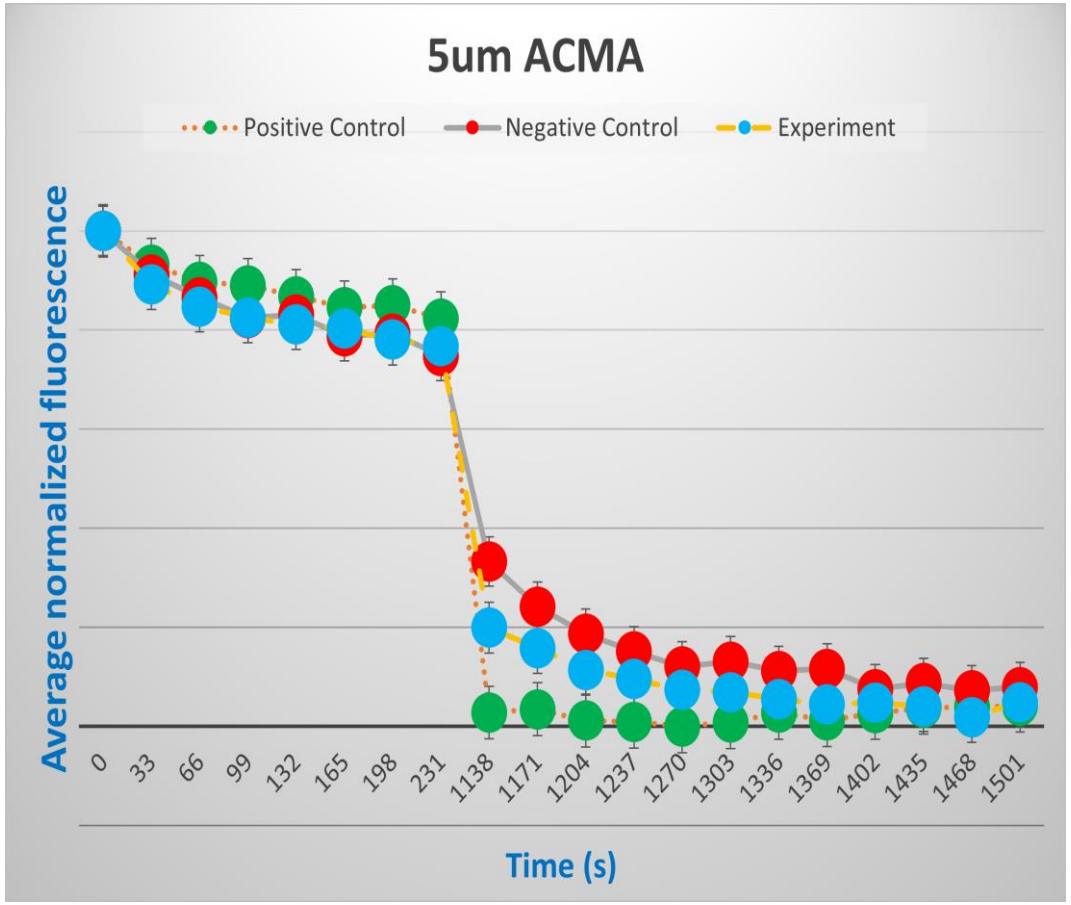




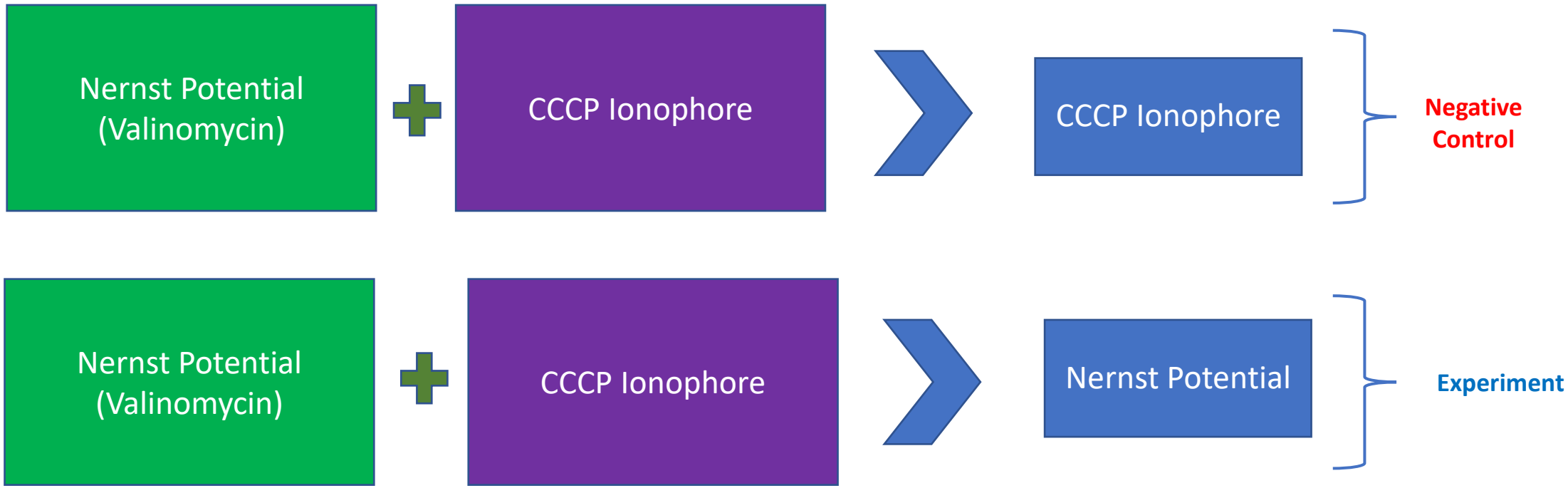
Legend: Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for three groups (Experiment, Negative and positive control); ***SEM values- Exp (0.05091), NC (0.049876), PC (0.05325).*** *N =40 based on averaged data from triplicate experiments. We conclude that ACMA quenching in the PC group is more significant relative to the Exp (Test) and NC groups. The cumulative effect of Nernst potential and CCCP presence should outweigh the presence of a Nernst potential only (Exp) OR CCCP only (NC).*

Image(s) Source: Microsoft Excel and R

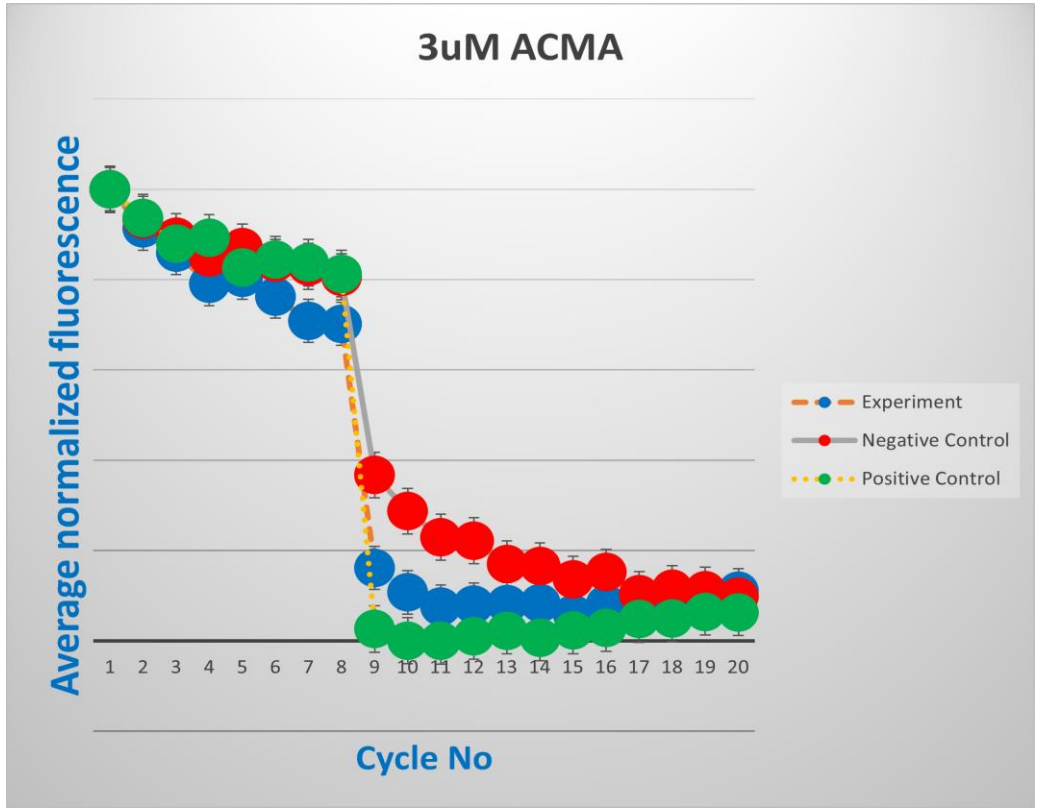
Same graph as above on a Time Scale-K inside

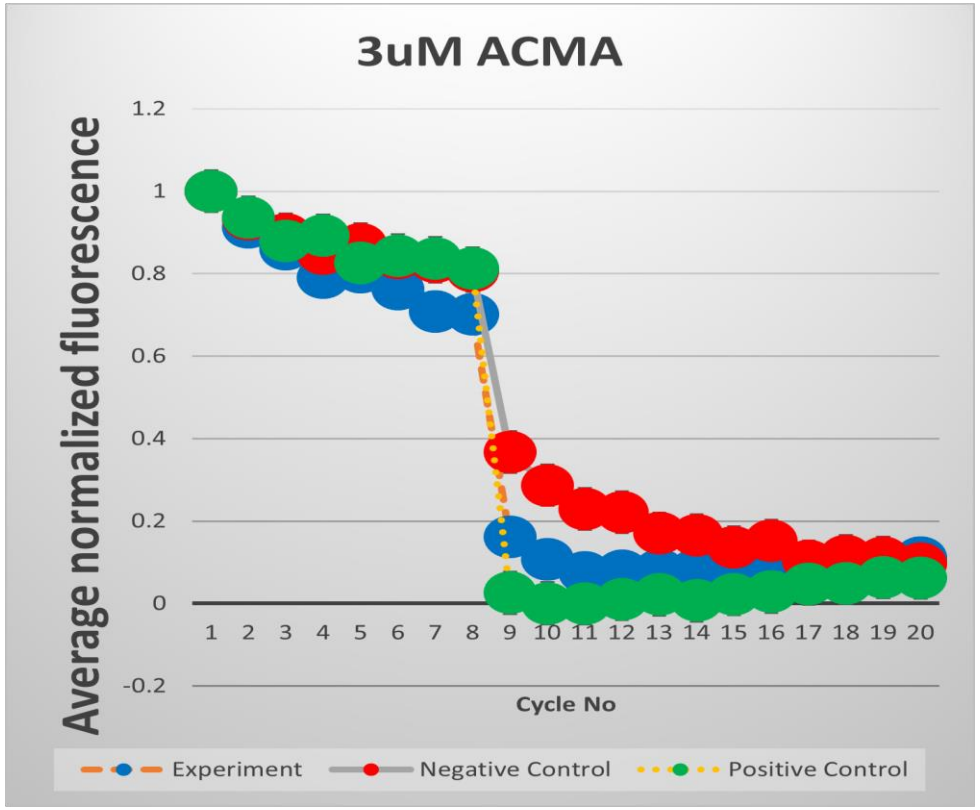


Quick Model to Understand ACMA Quenching



Constant ACMA (3uM) vs Varying Conditions- K inside

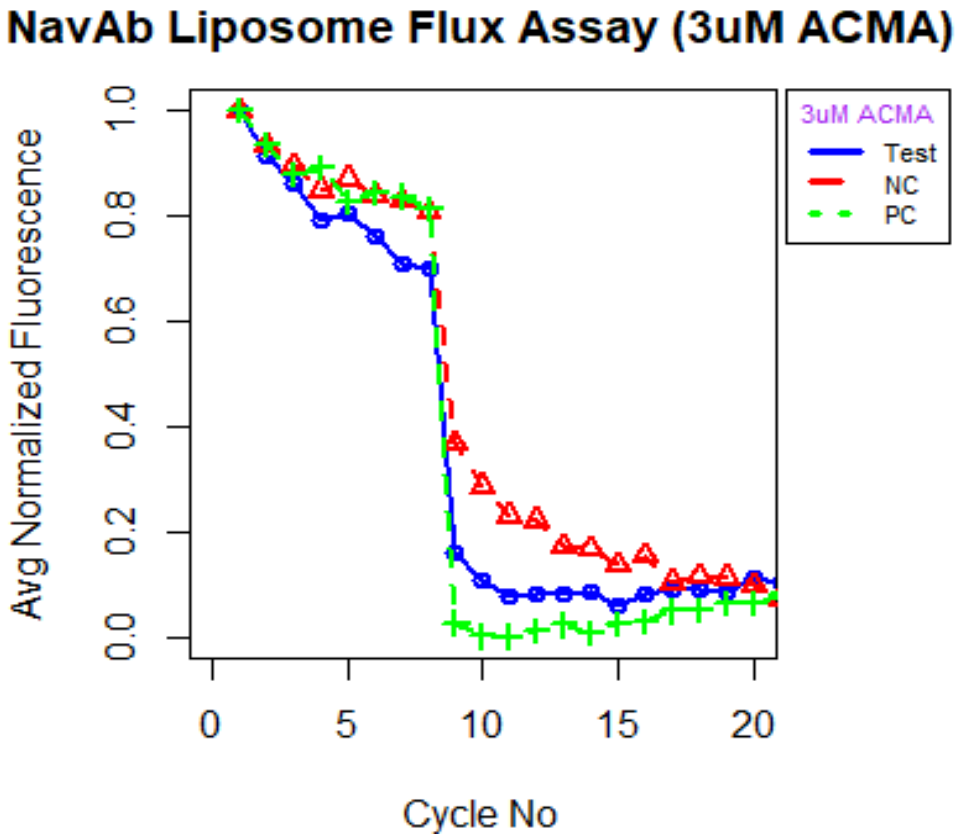




***Legend:** Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for three groups (Experiment, Negative and positive control); **SEM values- Exp (0.047958), NC (0.050838), PC (0.05148).** N =40 based on averaged data from triplicate experiments. We conclude that ACMA quenching in the PC group is more significant relative to the Exp (Test) and NC groups. The cumulative effect of Nernst potential and CCCP presence should outweigh the presence of a Nernst potential only (Exp) OR CCCP only (NC).*

Image(s) Source: Microsoft Excel and R

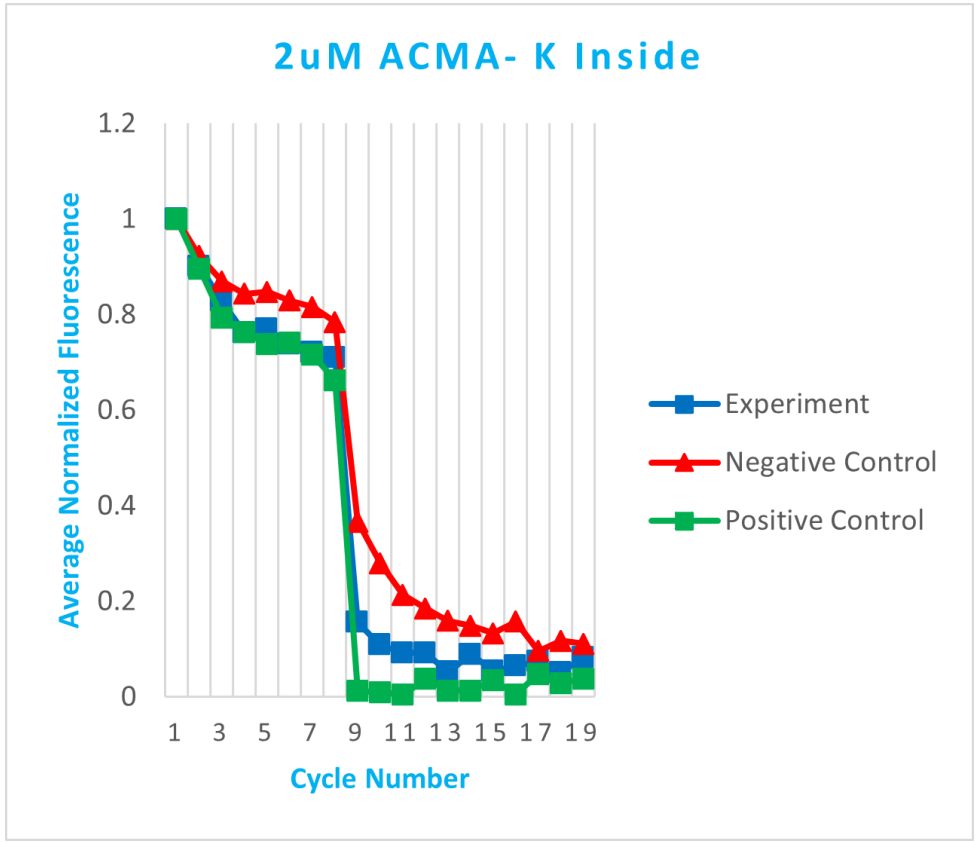
Constant ACMA (3uM) vs Varying Conditions- K inside

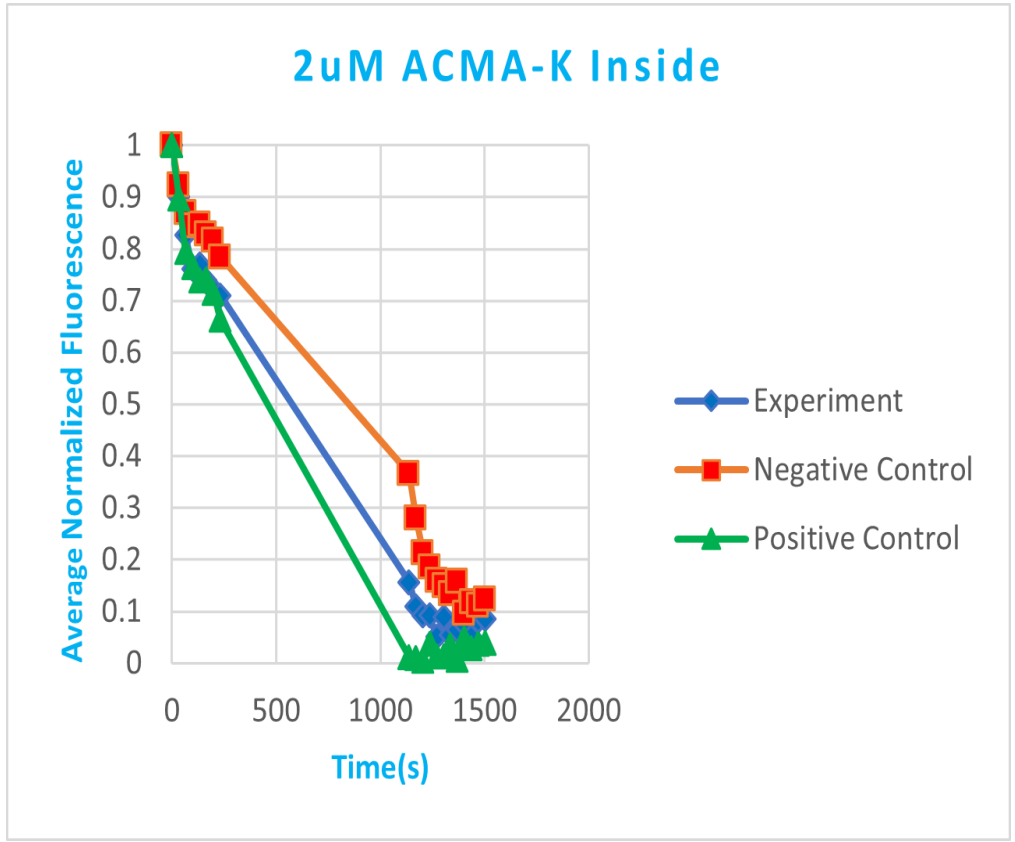


Legend: Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for three groups (Experiment, Negative and positive control). N =40 based on averaged data from triplicate experiments. We conclude that ACMA quenching in the PC group is more significant relative to the Exp (Test) and NC groups. The cumulative effect of Nernst potential and CCCP presence should outweigh the presence of a Nernst potential only (Exp) OR CCCP only (NC).

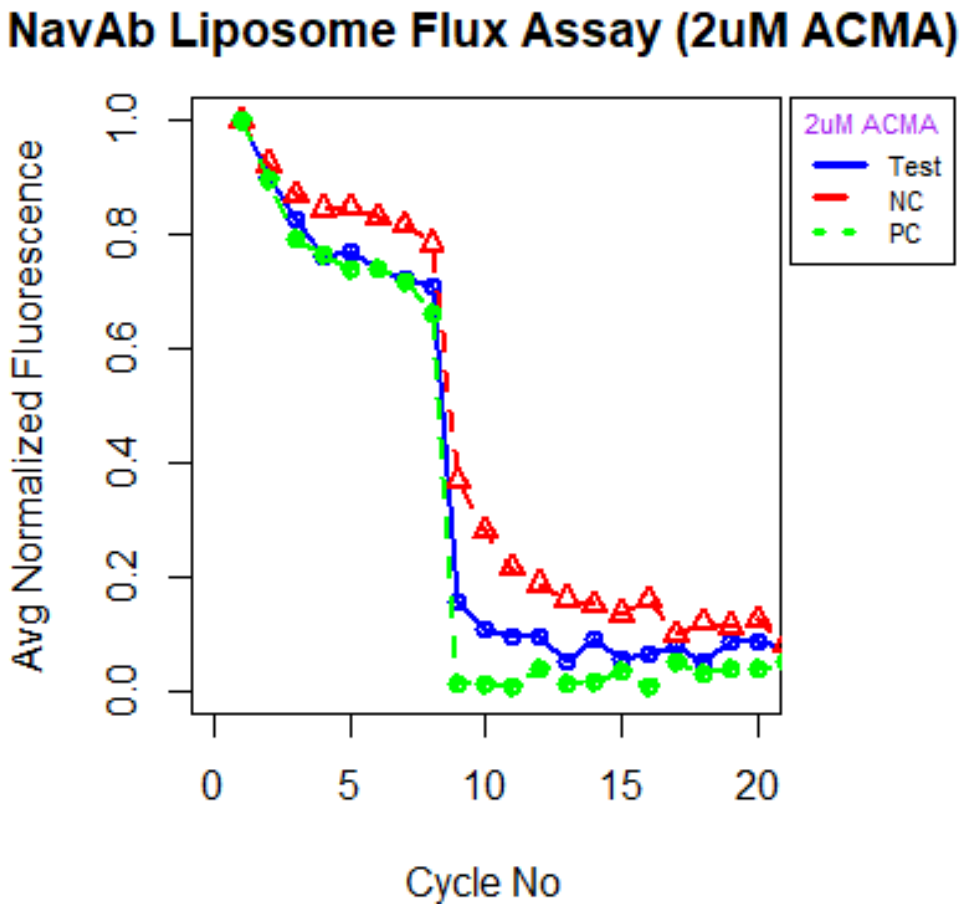
Image(s) Source: Microsoft Excel and R

Constant ACMA (2uM) vs Varying Conditions-K inside

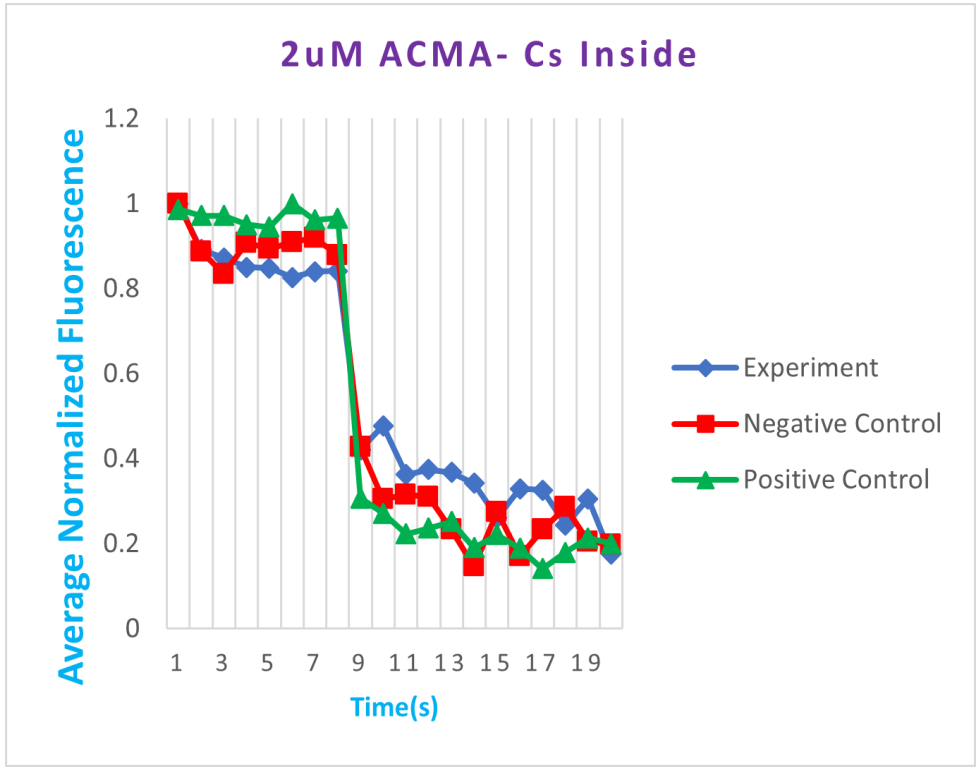


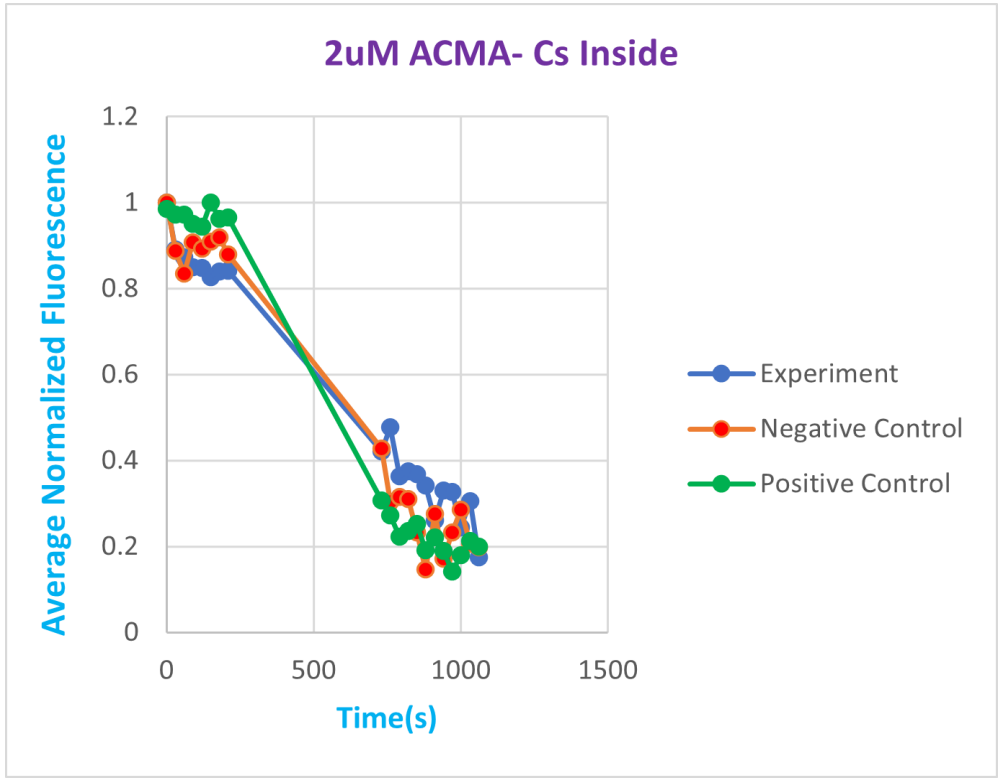


Constant ACMA (2uM) vs Varying Conditions- K inside

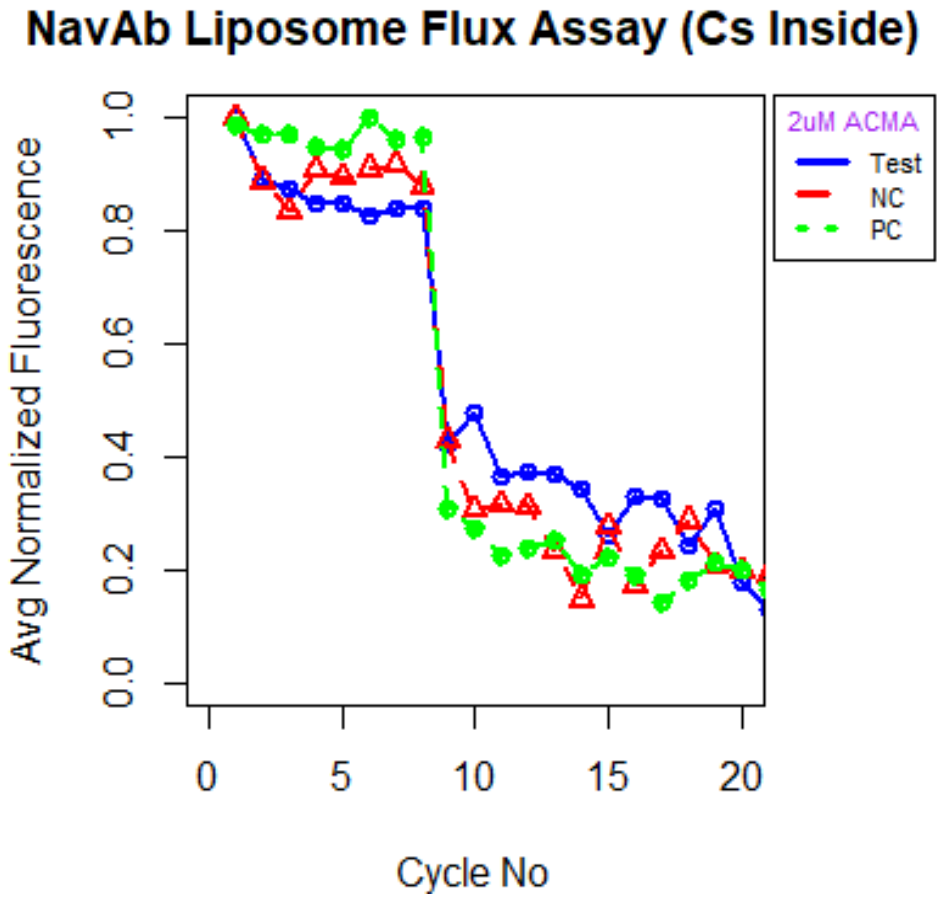


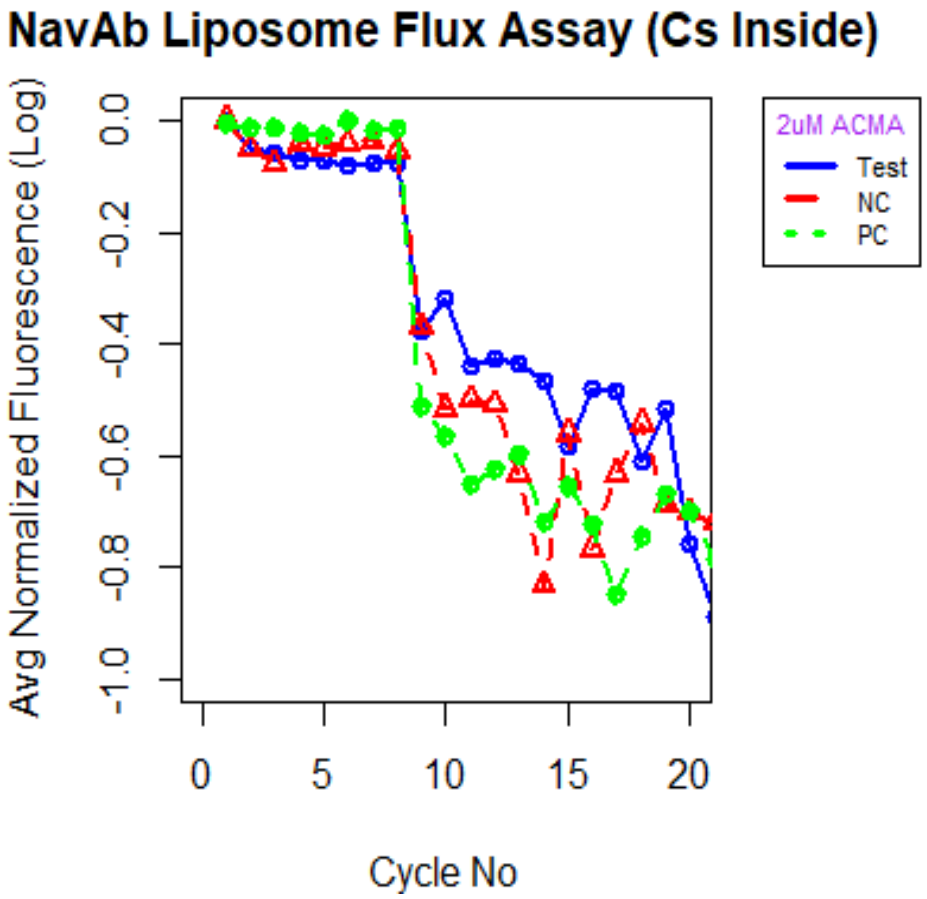
Constant ACMA (2uM) vs Varying Conditions- Cs inside



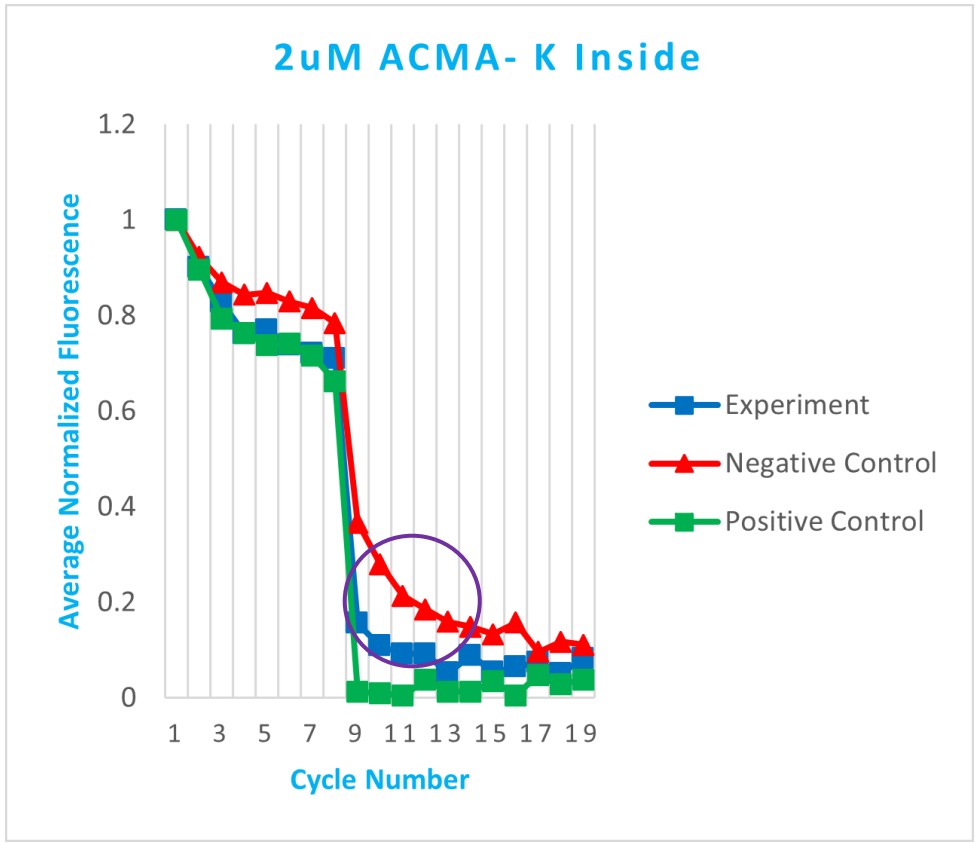


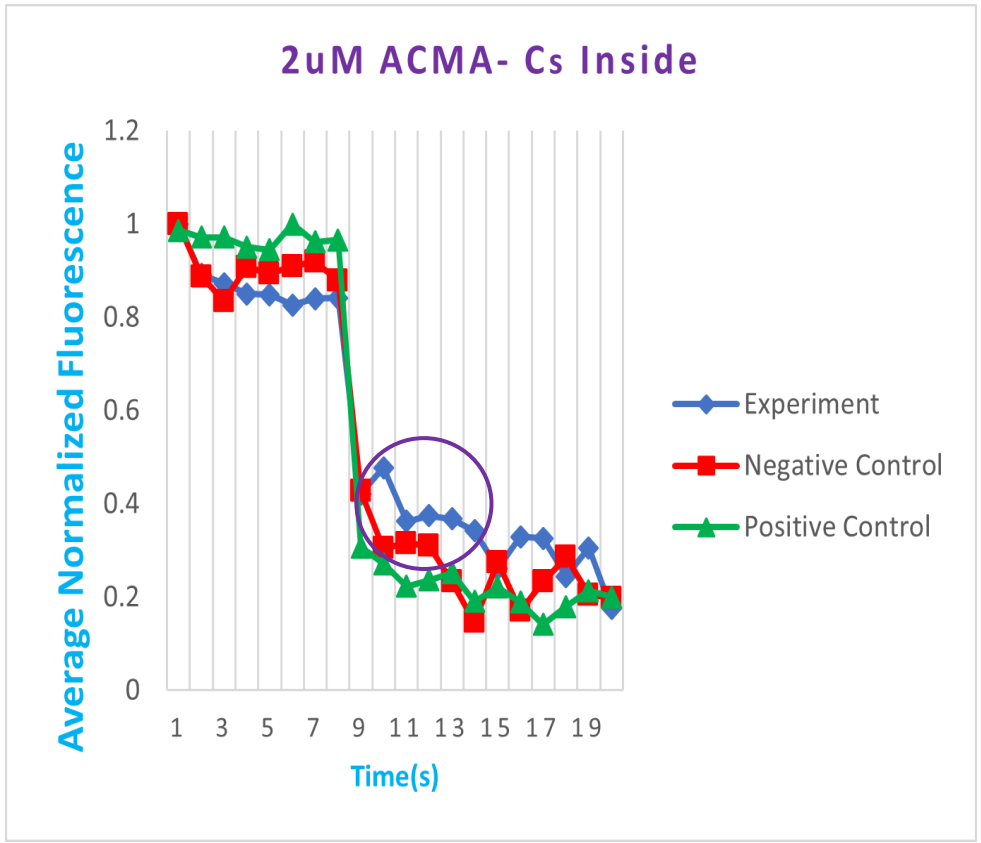
Constant ACMA (2uM) vs Varying Conditions- Cs inside





Comparing Data with K inside vs Cs inside Fascinating (2uM ACMA)

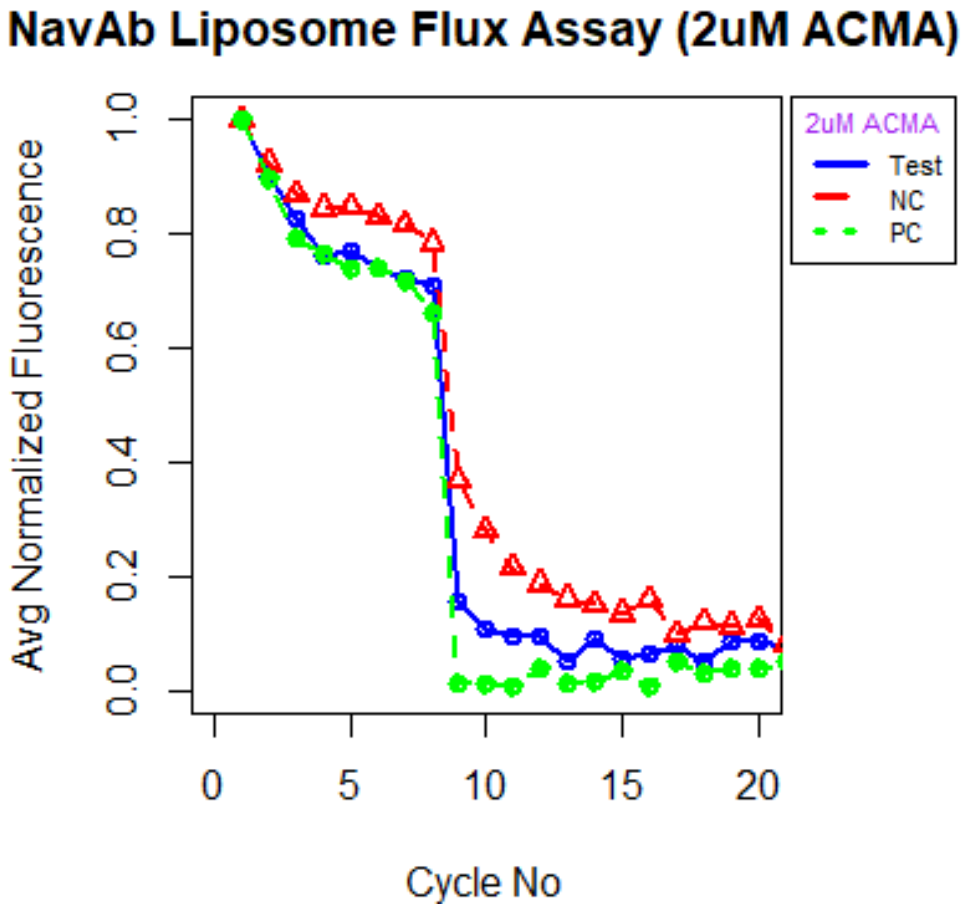


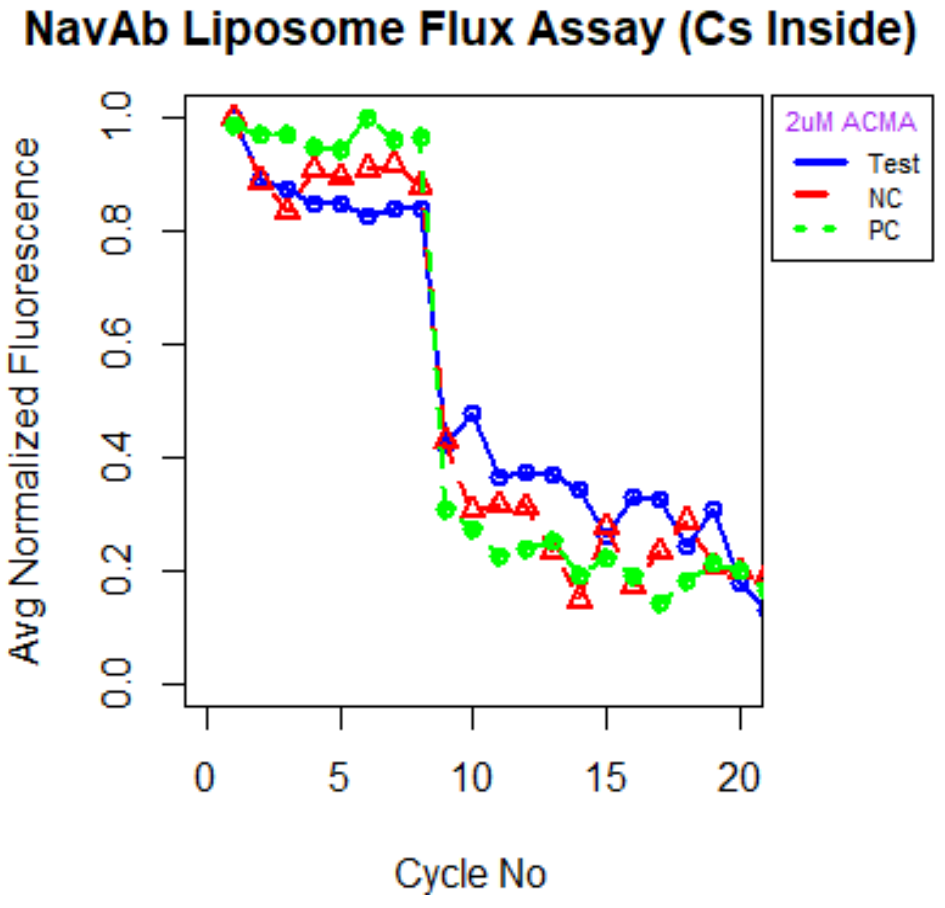


Legend: Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for three groups (Experiment, Negative and positive control). N =40 based on averaged data from triplicate experiments. We conclude that ACMA quenching in the PC group is more significant relative to the Exp (Test) and NC groups. Purple circles indicate the difference in quenching behaviors between NC and Exp groups. Our expected result is found with Cs inside and so we ponder over this difference when we use different ions inside. However, we conclude that with Cs inside we will see higher quenching of ACMA in the NC group relative to the Exp group and vice versa with K inside.

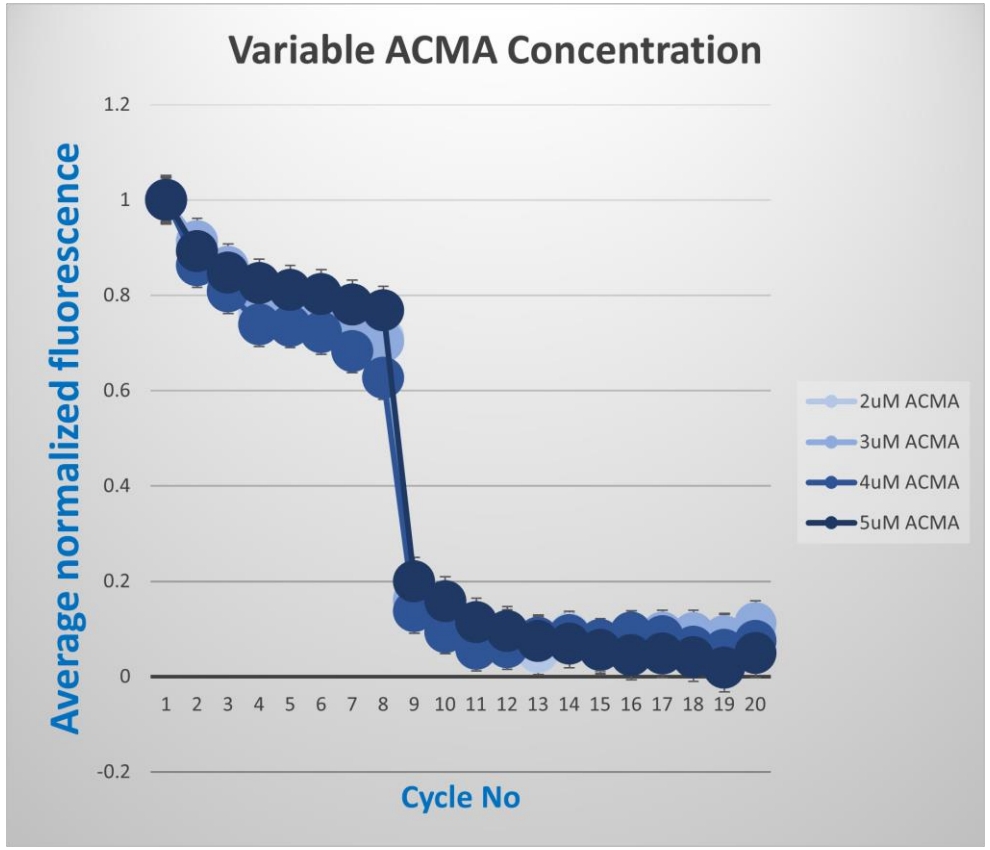
Image(s) Source: Microsoft Excel and R

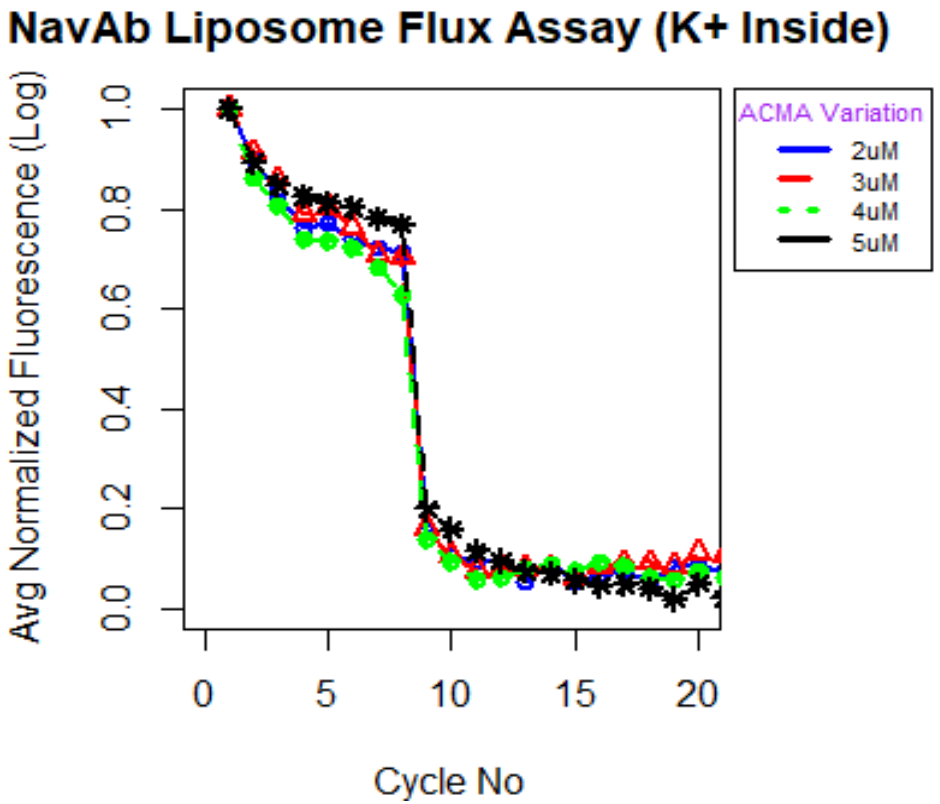
Comparing Data with K inside vs Cs inside Fascinating (2uM ACMA)





Variable ACMA in Constant Conditions (Experiment-K inside)

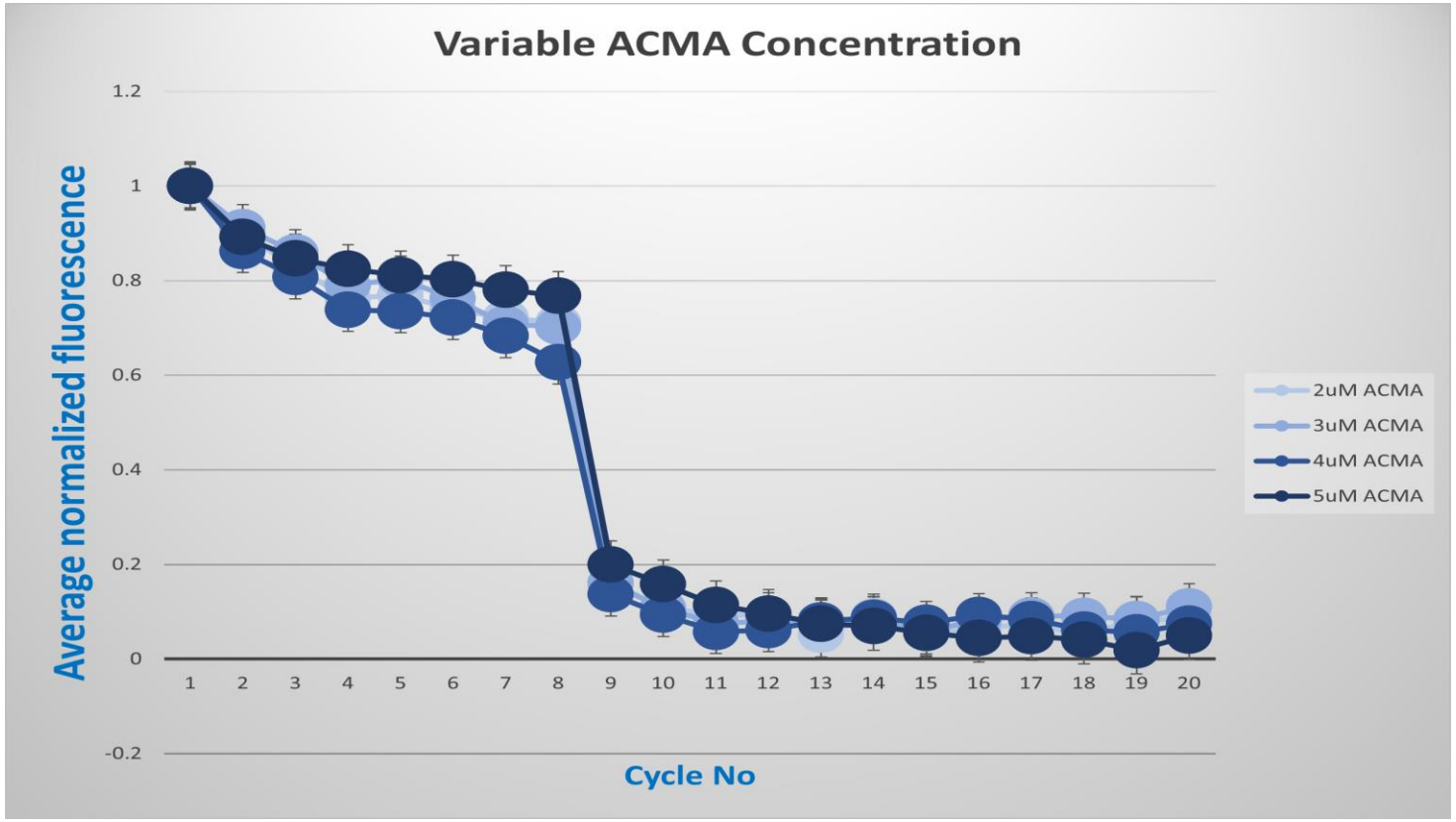




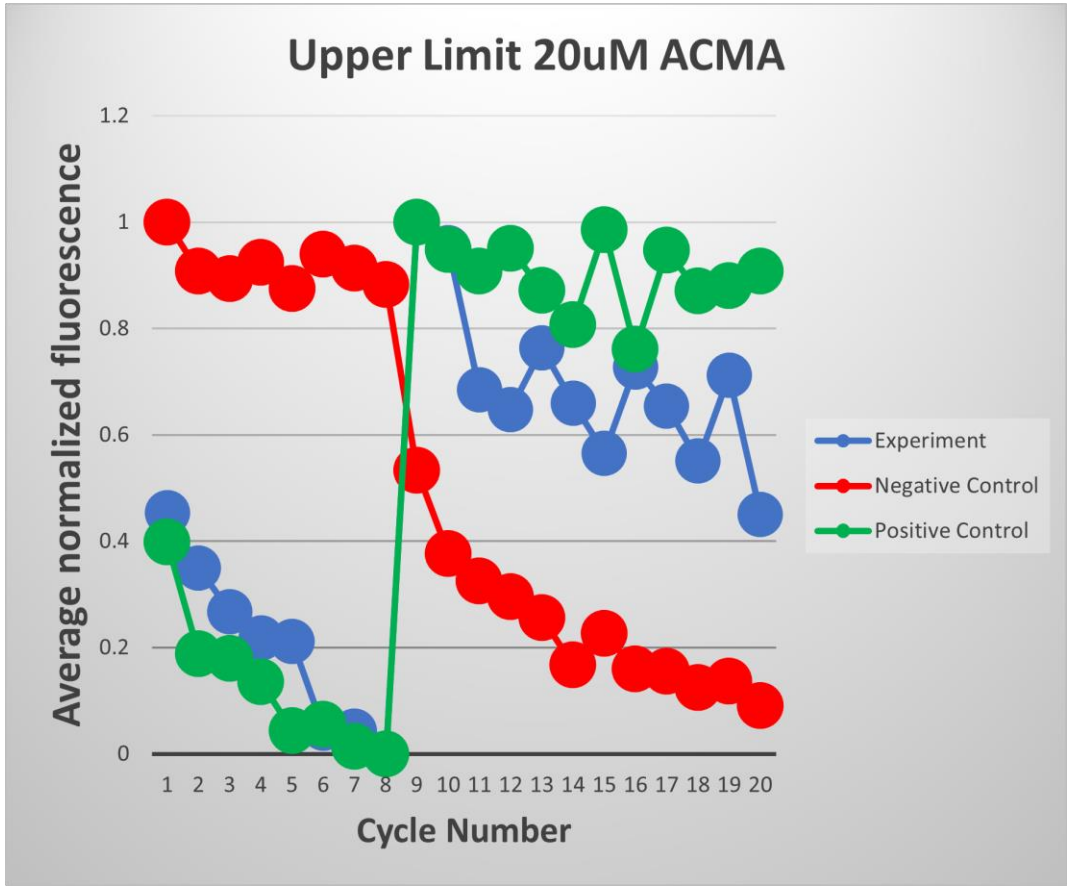
Legend: Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for four groups (2uM ACMA, 3uM ACMA, 4uM ACMA and 5uM ACMA). N =40 based on averaged data from triplicate experiments. We conclude that ACMA concentration within the acceptable range (between ??0.2uM ACMA and 20uM ACMA) has no effect on quenching behavior (magnitude). We also reaffirm here that Nernst potential (K+ and Valinomycin) and CCCP (proton flux) will be principal determinants of ACMA quenching.

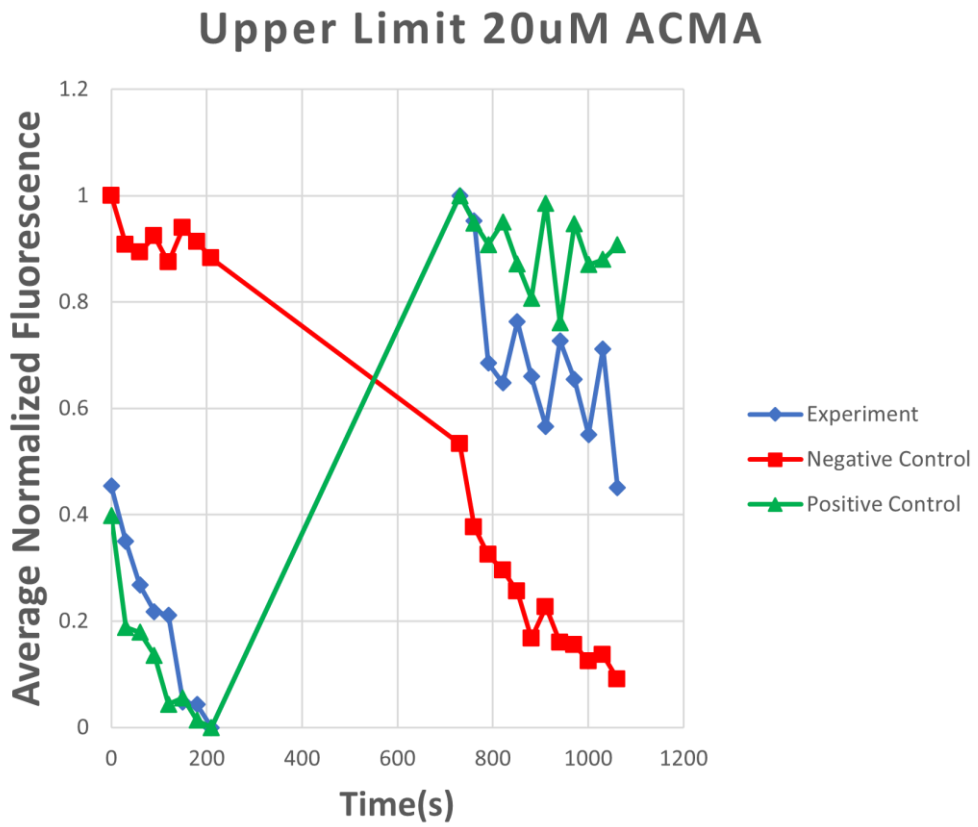
Image(s) Source: Microsoft Excel and R

Variable ACMA in Constant Conditions (Experiment)-For Clarity

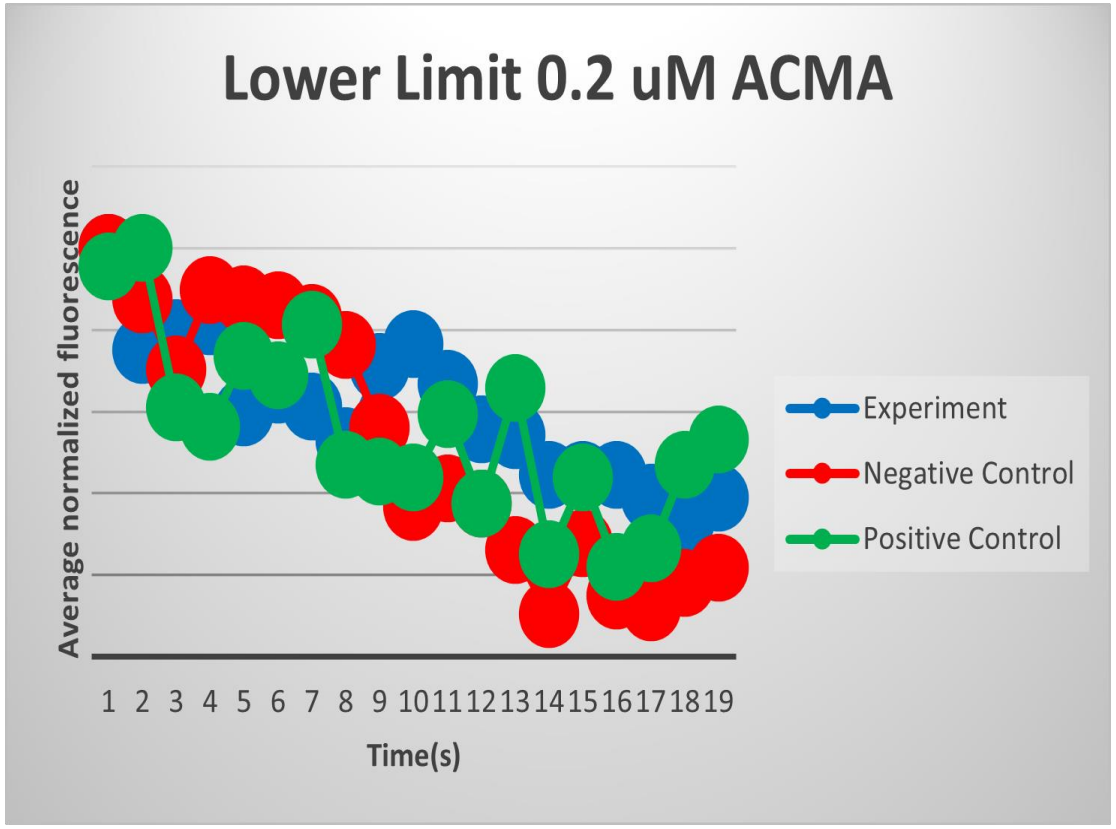


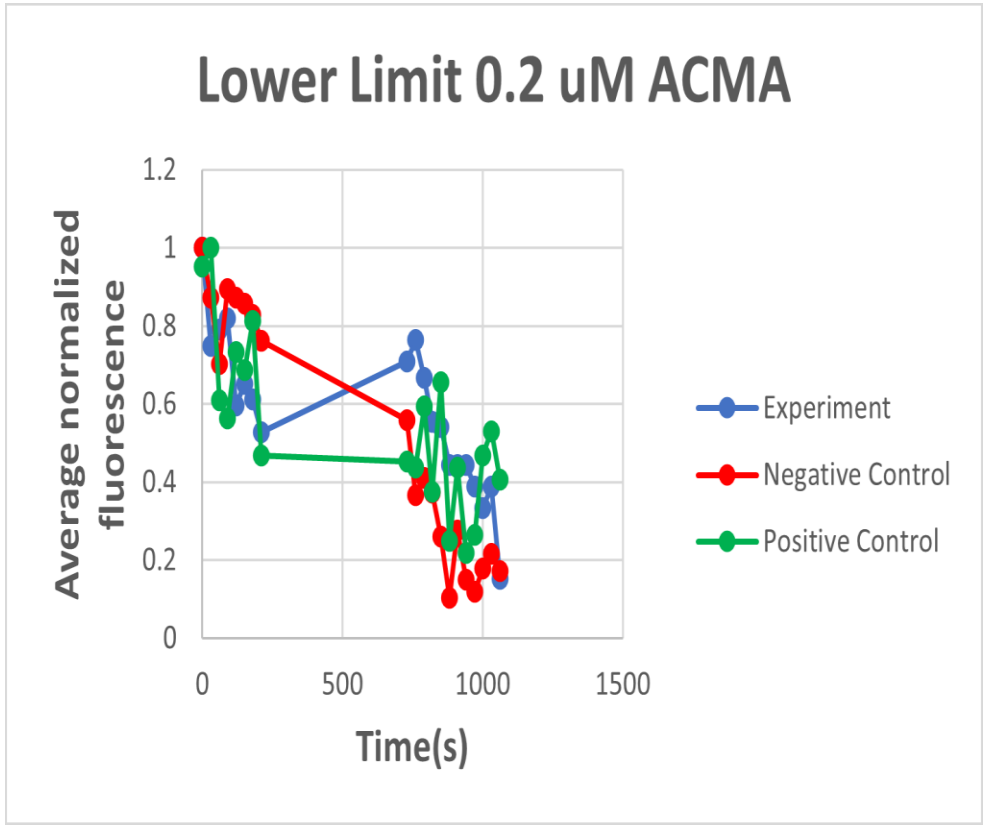
Upper Limit ACMA Concentration-Cs Inside Gain=5





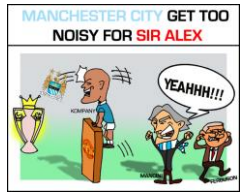
Lower Limit ACMA Concentration-Cs Inside Gain=5

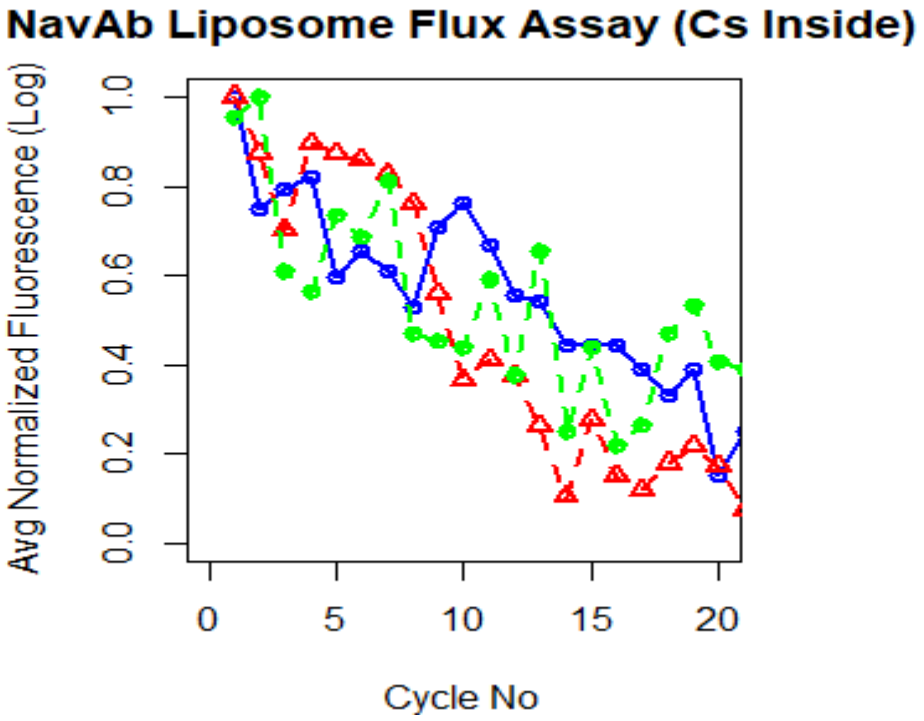


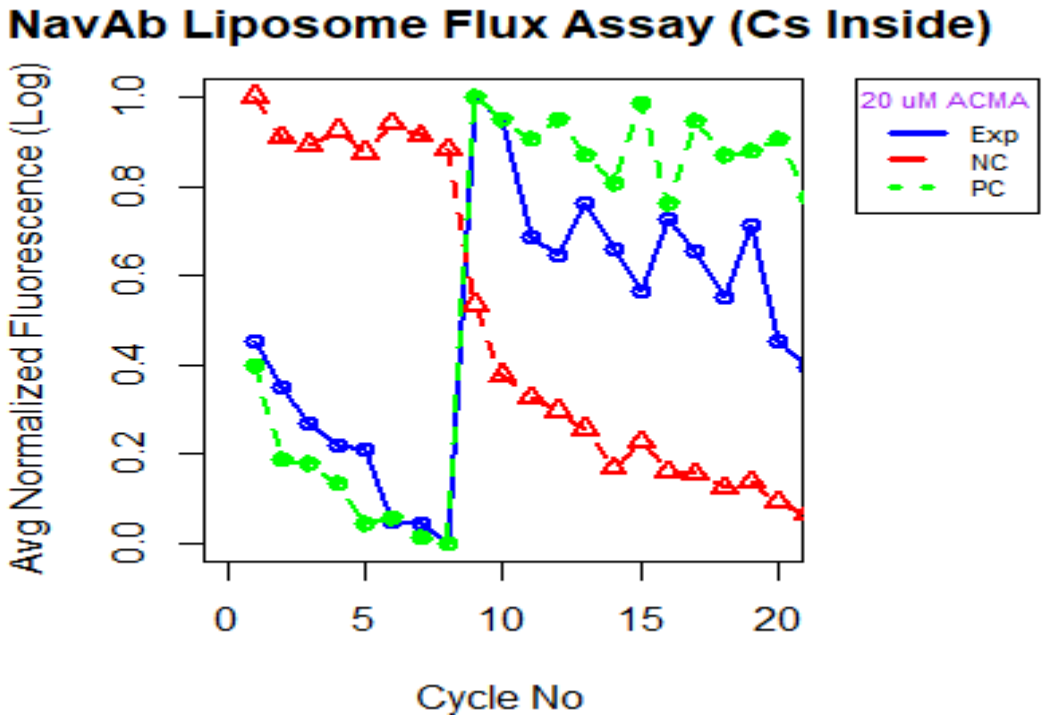


Its so noisy I am frightened to imagine this is possible or is it just pipetting errors on my part.

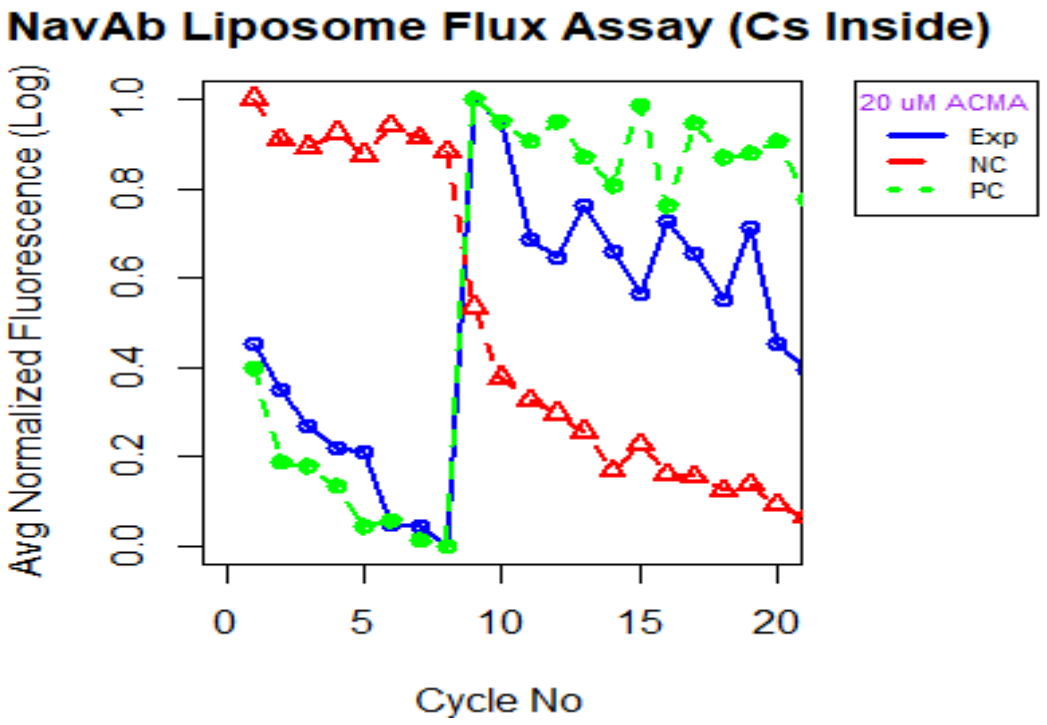
Comparing 0.2uM vs 20uM ACMA Data







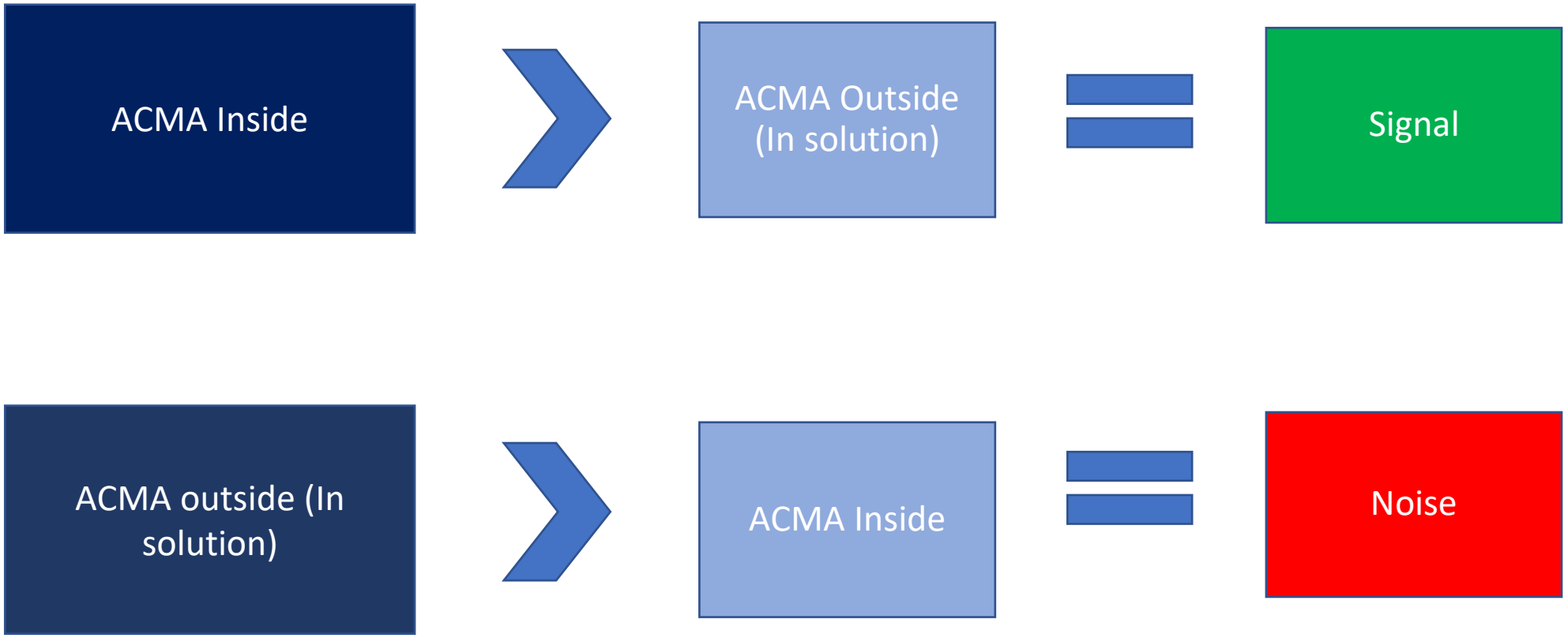
Upper Limit ACMA Concentration-Cs Inside Gain=5



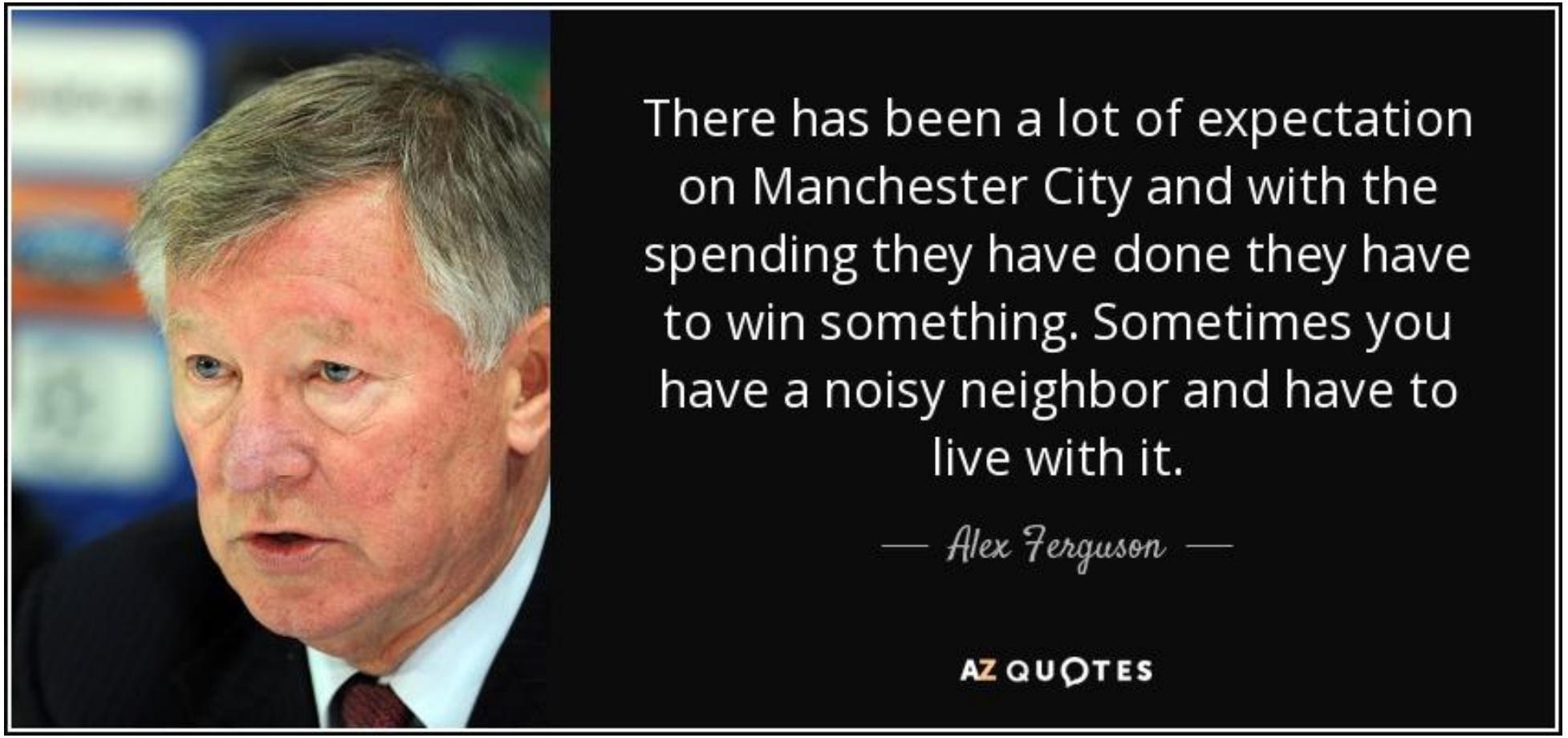
Legend: Averaged Normalized Fluorescence (+- SEM) vs Time (s) or Cycle Number, following NavAb flux assay experiments for three groups (Experiment, Negative and positive control). N =40 based on averaged data from triplicate experiments. We conclude that 20uM ACMA is our upper limit of the acceptable concentration range. We conclude here that our data is indicative of noise but fortunately this noise is useful in interpreting the system. For the P and Exp, proton influx leads to increased fluorescence outside liposome; In the negative control, ACMA affinity for H+ is enough to overcome low Nernst potential generated by NavAb leading to H+ efflux.

Image(s) Source: Microsoft Excel and R

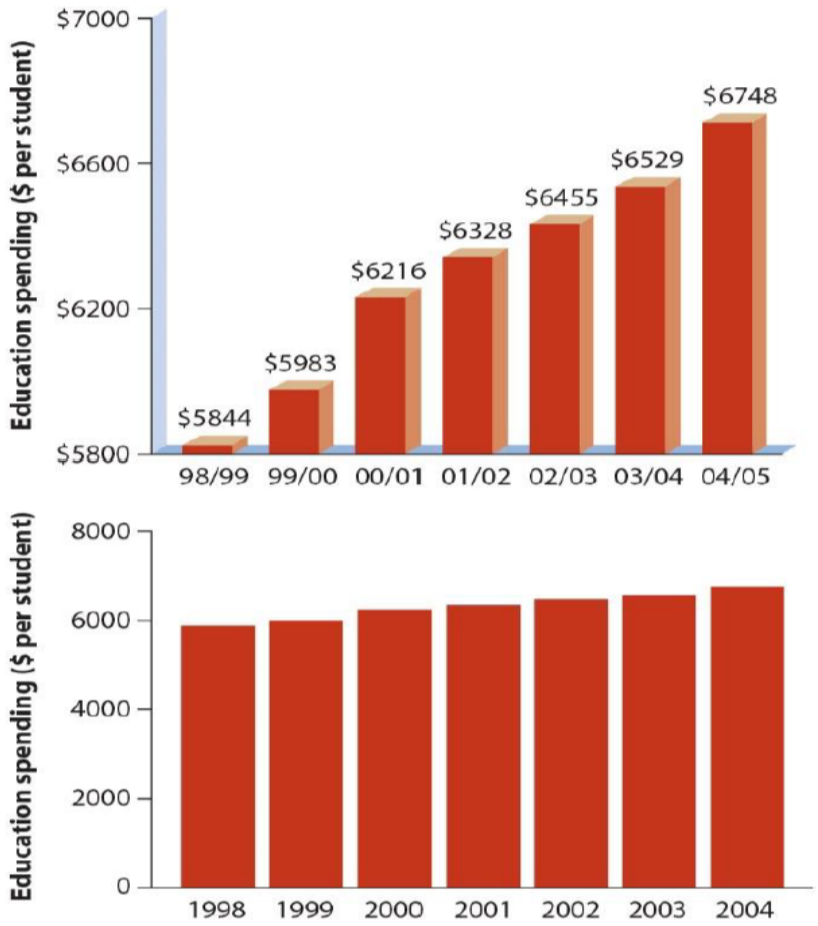
What do we consider Noise?



Funny Moment in history towards the Derby Final about 10 Years ago when I was in college in Cameroon



Representation of Magnitudes Problem

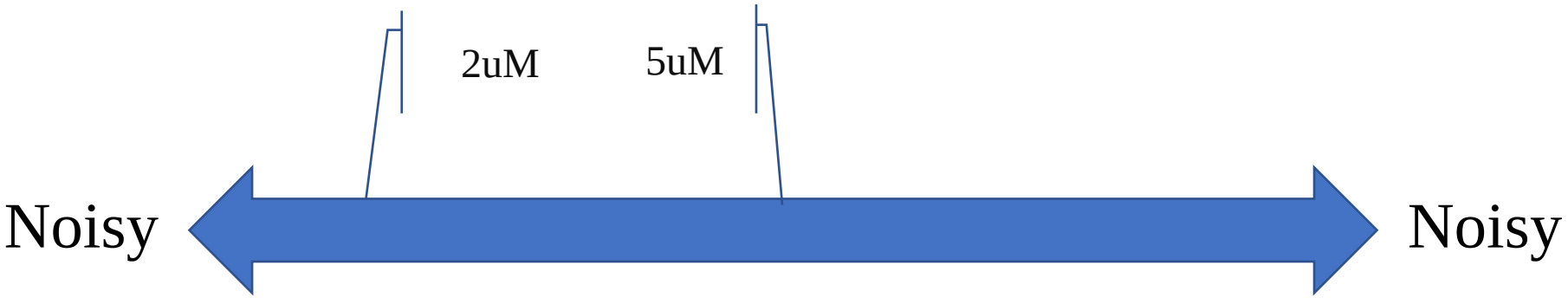


- Connecting the dots looking back. A snippet from my Biometry Class (Prof Johnson) on the importance of representing magnitudes correctly.
- Misrepresentation of magnitudes could be misleading.
- Image reference from Dr. Johnson's lectures.

Why all the fuss about ACMA?

- As our signal provider we need to know what concentration of ACMA makes it an adequate signaling substance for our data collector (flux machine) in the context of LFAs.
- We can see that too much could scramble the system or force us to interpret the data differently (reverse) in some instances and in other instances find a completely different explanation for the data obtained. 
- Knowing what concentration range of ACMA is good for obtaining useful flux assay data is important and even more important is nailing down the concentration of ACMA that provides the best possible signal

ACMA Concentration Scale Rationale Schematic



ACMA Concentration Range

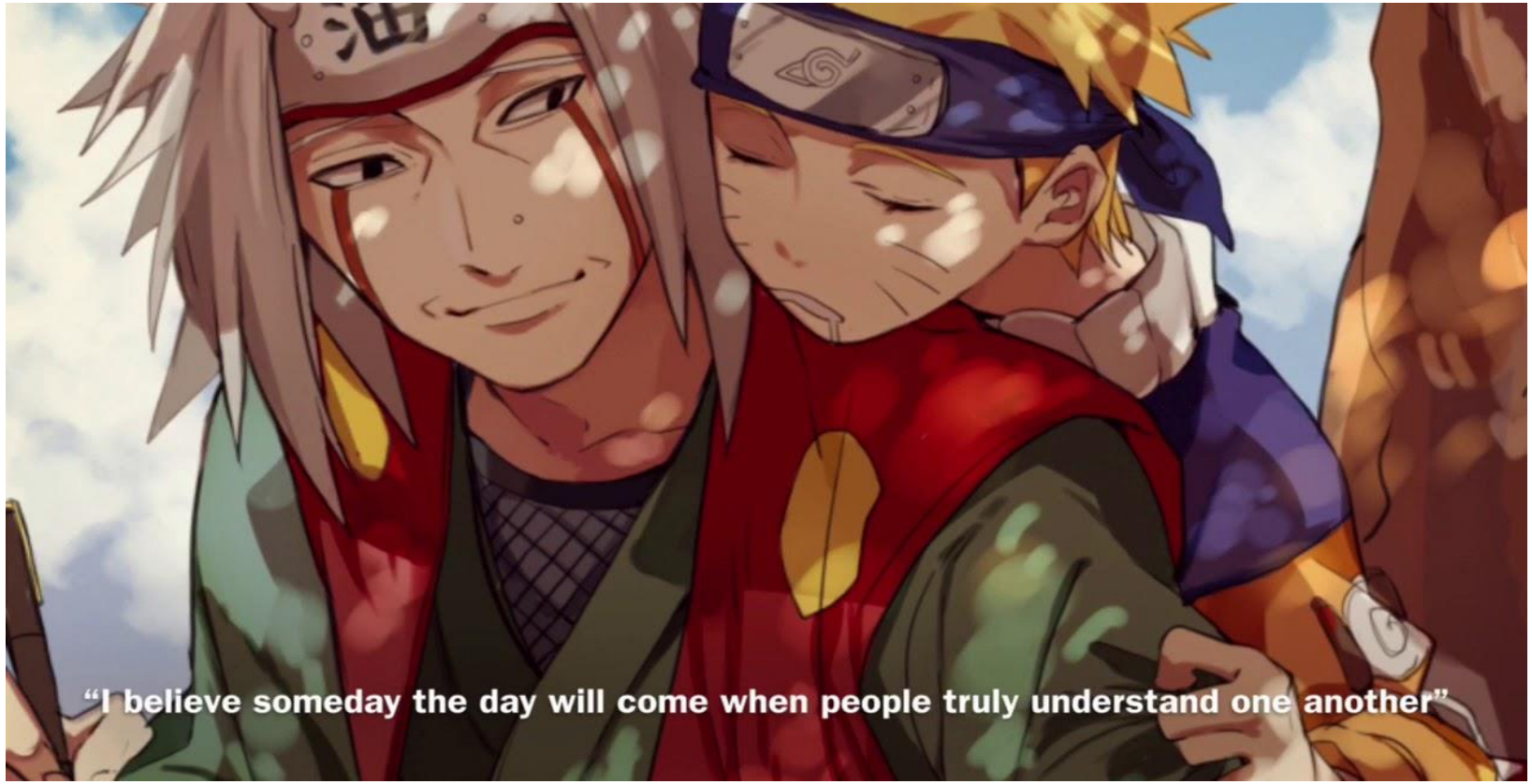
Future Experiments

- NavAb LFA in the context of Na conductivity.
- Other projects still brewing up in the Noggin. There are other channels of interest that could benefit from flux assays and provide opportunities for future collaborations.

Conclusions

- We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.
- We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.
- We showed that we could use noise to interpret our flux assays.

THANKS DR. WANG



Great Thanks to Dr. Wang and Dr. Shuo & Sir Ryan on teaching me a lot of stuff and most importantly providing me with the space to retain sophistication in my thinking as I looked through our data.

Questions