

# Understanding Liposome Flux Assays in the context of a Bacterial Sodium Voltage Gated Channels (NavAb)

Biological data analysis in R

---

Tingwei Adeck

July 23, 2022

AlphaPrime University

# Table of contents

1. Introduction
2. Diagnostic results in Excel
3. Normalized Results (Makes all the sense in the world)
4. Noise vs Signal
5. Conclusion

# Introduction

---

## Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na <sup>+</sup>	1.0

## Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na <sup>+</sup>	1.0
K <sup>+</sup>	0.14

## Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na <sup>+</sup>	1.0
K <sup>+</sup>	0.14
Rb <sup>+</sup>	0.02

## Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na <sup>+</sup>	1.0
K <sup>+</sup>	0.14
Rb <sup>+</sup>	0.02
Cs <sup>+</sup>	0.005

# Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na <sup>+</sup>	1.0
K <sup>+</sup>	0.14
Rb <sup>+</sup>	0.02
Cs <sup>+</sup>	0.005
H <sup>+</sup>	??

**Table 1:** NavAb affinity constants



# Characteristics of liposome flux assays (LFAs)

- Channel-insert liposomes

# Characteristics of liposome flux assays (LFAs)

- Channel-insert liposomes
- A fluorophore is present within the channel-insert liposome

# Characteristics of liposome flux assays (LFAs)

- Channel-insert liposomes
- A fluorophore is present within the channel-insert liposome
- liposomes are a form of lipid bilayers

# Characteristics of liposome flux assays (LFAs)

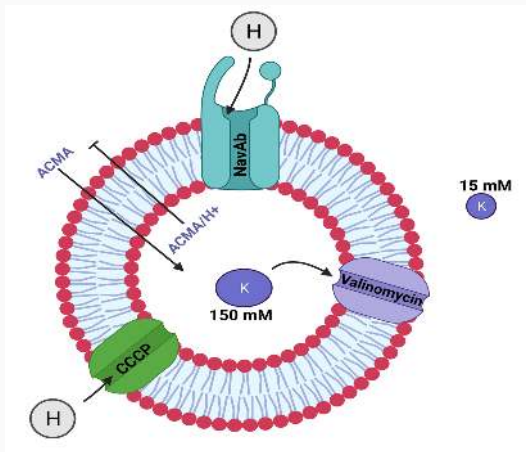
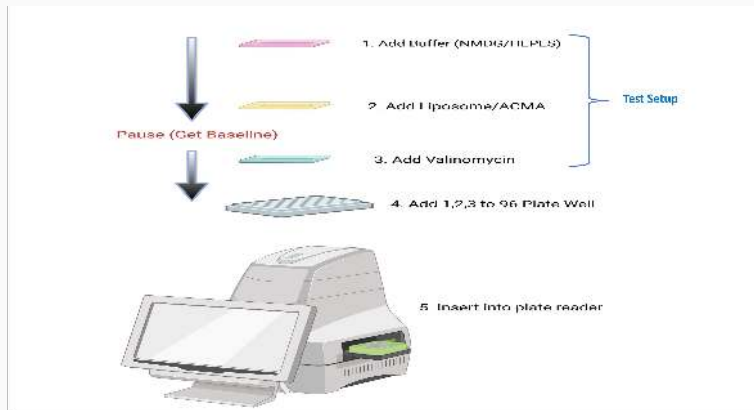


Figure 1: Na<sup>+</sup>-insert liposome

# Practical image of experiment



Created with BioRender.com

Figure 2: experiment

## Diagnostic results in Excel

---

# Characteristics of liposome flux assays (LFAs)

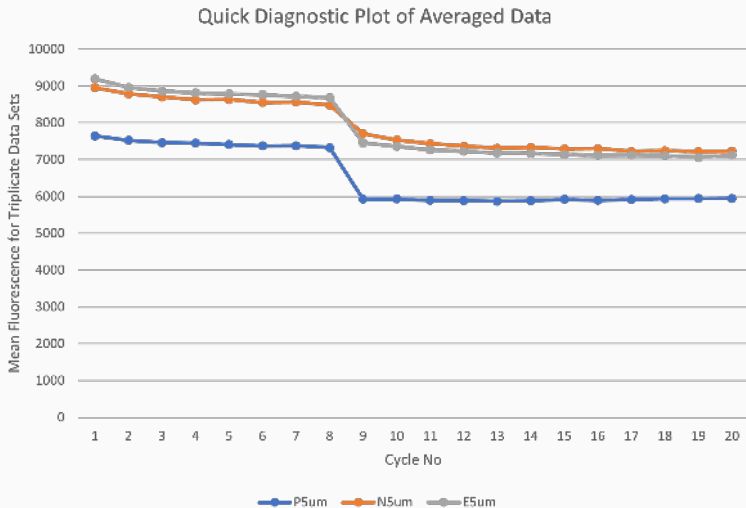


Figure 3: Na<sup>+</sup>-insert liposome

Normalized Results (Makes all the sense in the world)

---



# Fluorescence measured at 2,3,5 $\mu\text{M}$

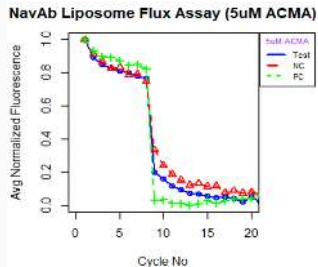
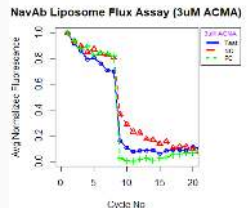
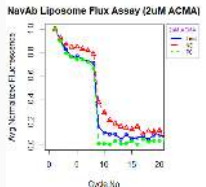
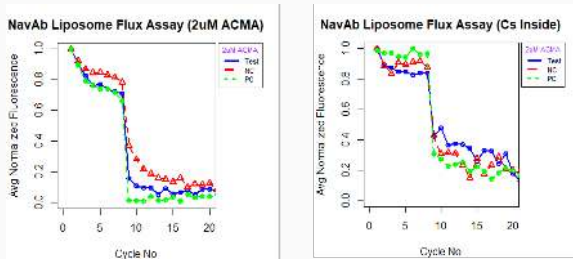


Figure 4: Legend: Normalized fluorescence in R within the signal zone

# Fluorescence measured with $K^+$ vs $Cs^+$ inside the liposome $\mu M$



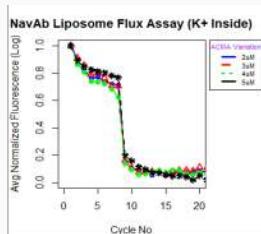
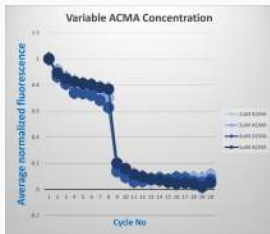
**Figure 5:** NavAb K-conductivity is stronger than Cs-conductivity as indicated by the initial affinity table

# Fluorescence measured with $K^+$ vs $Cs^+$ inside the liposome $\mu M$



**Figure 6:** NavAb K-conductivity is stronger than Cs-conductivity as indicated by the initial affinity table

# ACMA dosage at 2,3,4,5 $\mu\text{M}$



**Figure 7:** Fluorophore dosage is not a factor in its quenching, rather system properties (Ion conductivity etc.) determine quenching

## Noise vs Signal

---

# Fluorescence measured at 0.2 and 20 $\mu\text{M}$

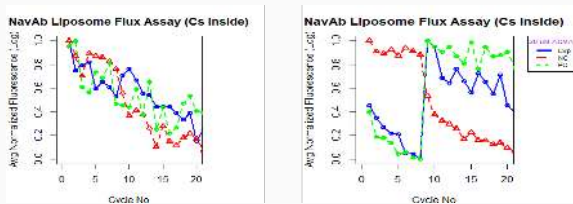


Figure 8: Legend: Normalized fluorescence in R within the signal zone

## Conclusion

---

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.



# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

## Noise vs Signal

We showed that we could use noise to interpret our flux assays.

# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

## Noise vs Signal

We showed that we could use noise to interpret our flux assays.

# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

## Noise vs Signal

We showed that we could use noise to interpret our flux assays.

# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

## Noise vs Signal

We showed that we could use noise to interpret our flux assays.

# Conclusion

## Quenching drivers

We showed that the Nernst Potential (Valinomycin) and proton flux (CCCP) should be investigated as potential factors in ACMA quenching behavior.

## ACMA concentration

We showed that there is a concentration for ACMA to make it useful as a signaling molecule in flux assays.

## Noise vs Signal

We showed that we could use noise to interpret our flux assays.

Thanks and Questions?

Thanks and Questions?



Thanks and Questions?

Thanks and Questions?

Thanks and Questions?

Thanks and Questions?

Thanks and Questions?

## References

---

- [1] Zhenwi. Su. *Novel cell-free high-throughput screening method for pharmacological tools targeting K<sup>+</sup> channels*. PNAS, 113(5744-5788), 2016.
- [2] Joshua V. *A Single Molecule Study on The Structural Basis of Ion Selective Permeation in Voltage-Gated Sodium Channels*. , 2021.