

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

Biological data analysis in R

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Introduction

Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na^+	1.0

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Na ⁺	1.0
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Rb ⁺	0.02

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Cs ⁺	0.005

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H ⁺	??

Table 1: NavAb affinity constants

Characteristics of liposome flux assays (LFAs)

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- liposomes are a form of lipid bilayers

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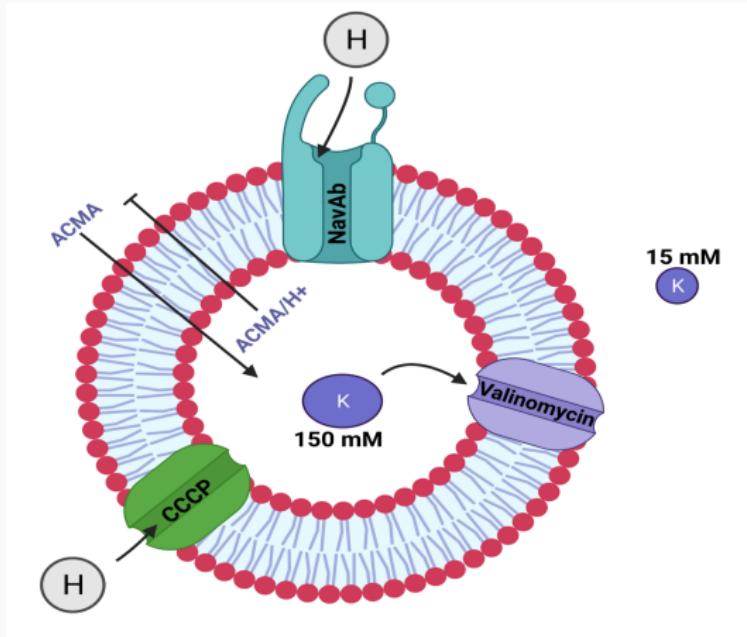
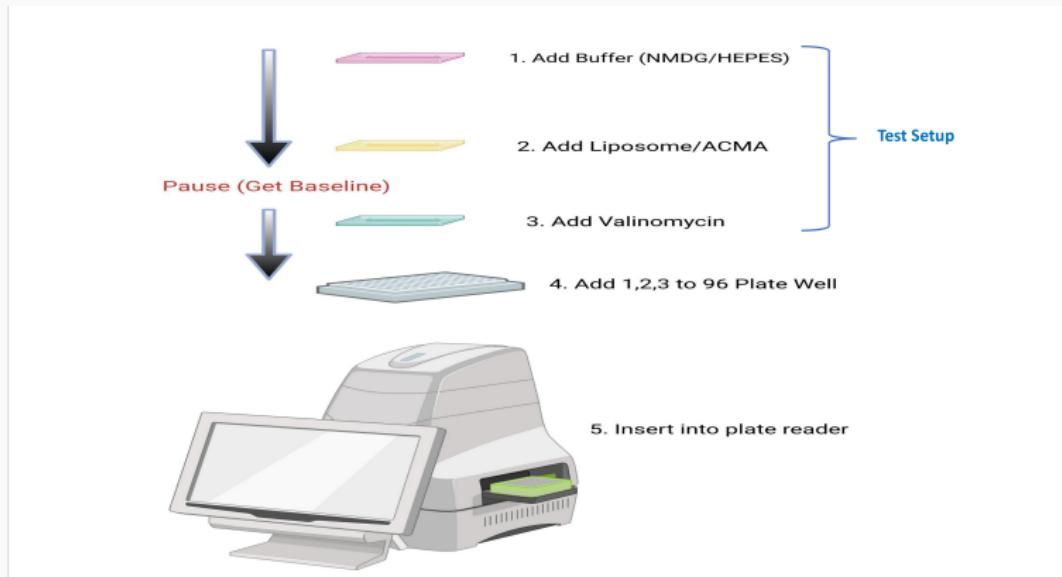


Figure 1: Na^+ -insert liposome

Practical image of experiment



Created with BioRender.com

Figure 2: experiment

Diagnostic results in Excel

Characteristics of liposome flux assays (LFAs)

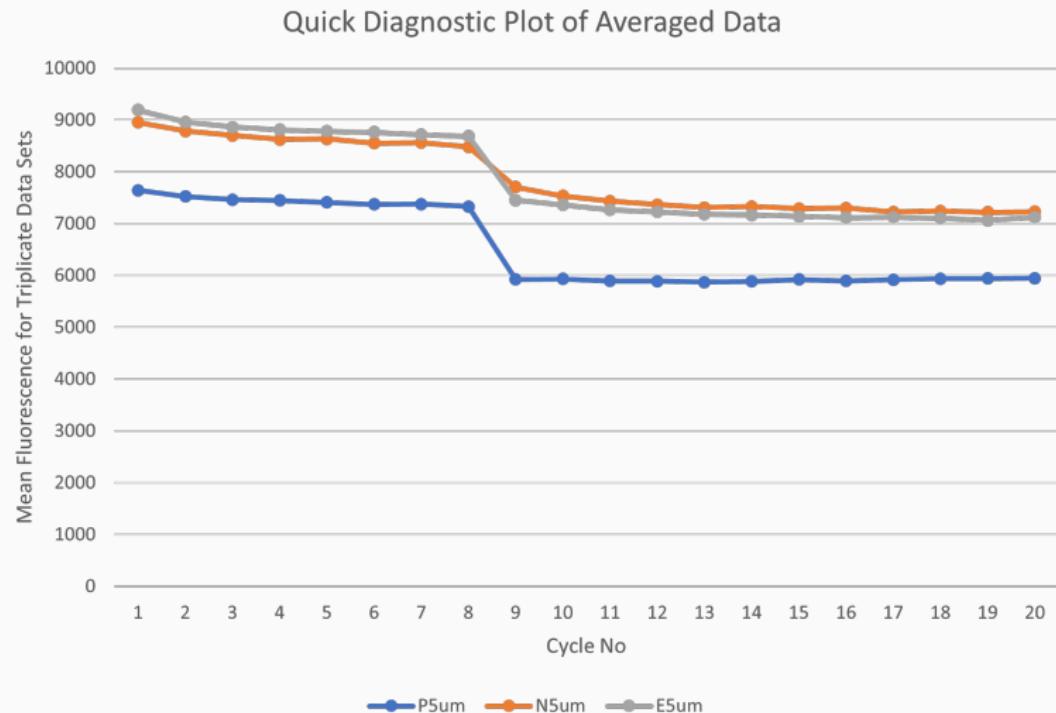
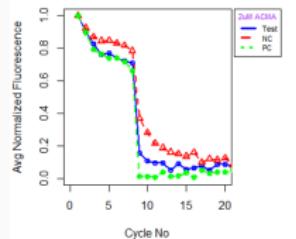


Figure 3: Na^+ -insert liposome

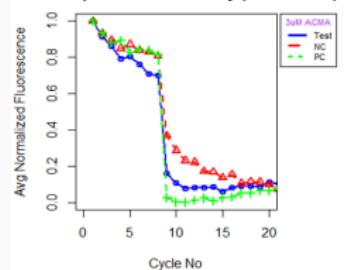
Normalized Results (Makes all the
sense in the world)

Fluorescence measured at 2,3,5 μ M

NavAb Liposome Flux Assay (2 μ M ACMA)



NavAb Liposome Flux Assay (3 μ M ACMA)



NavAb Liposome Flux Assay (5 μ M ACMA)

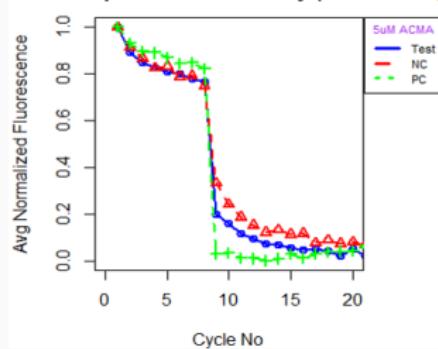


Figure 4: Legend: Noramlized fluorescence in R within the signal zone

Fluorescence measured with K⁺ vs Cs⁺ inside the liposome μM

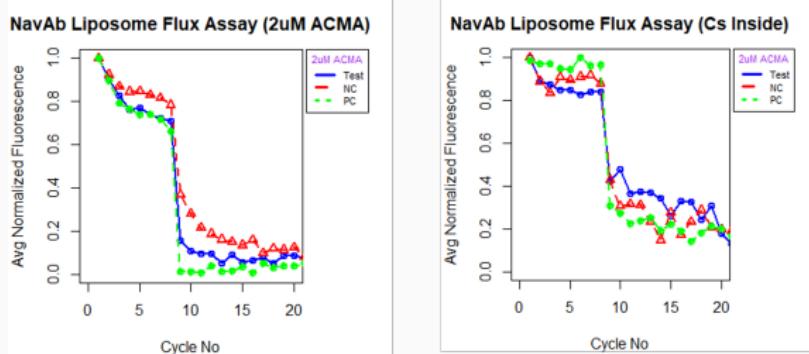


Figure 5: NavAb K-conductivity is stronger than Cs-conductivity as indicated by the initial affinity table

Fluorescence measured with K⁺ vs Cs⁺ inside the liposome μ M

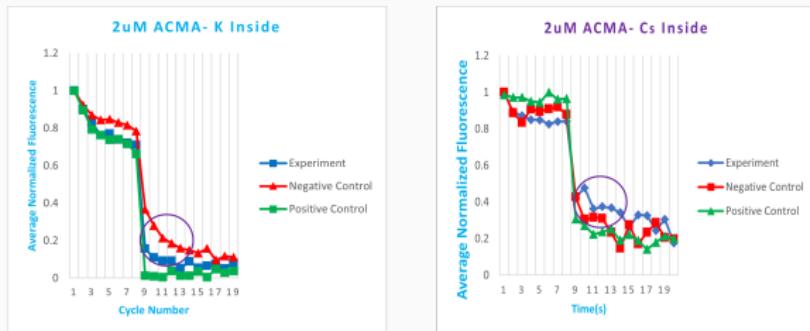


Figure 6: NavAb K-conductivity is stronger than Cs-conductivity as indicated by the initial affinity table

ACMA dosage at 2,3,4,5 μ M

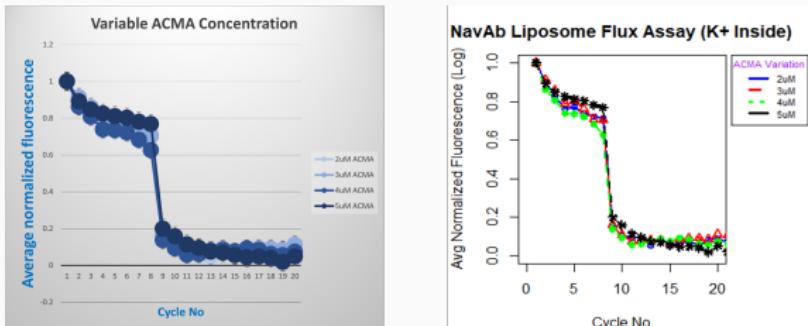


Figure 7: Fluorophore dosage is not a factor in its quenching, rather system properties (Ion conductivity etc.) determine quenching

Noise vs Signal

Fluorescence measured at 0.2 and 20 μ M

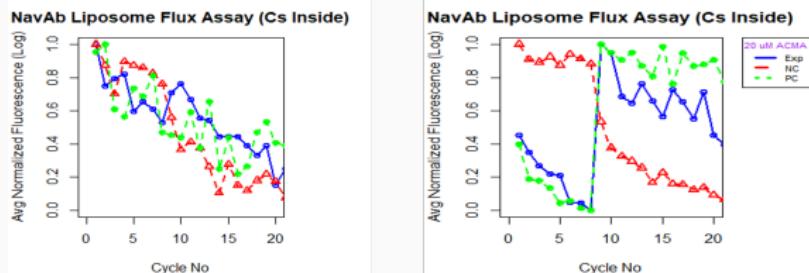


Figure 8: Legend: Noramlized fluorescence in R within the signal zone

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Quenching drivers

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

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Thanks and Questions?

References

- [1] Zhenwi. Su. *Novel cell-free high-throughput screening method for pharmacological tools targeting K⁺ 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.