

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
K ⁺	0.14

Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(K_a)
Na^+	1.0
K^+	0.14
Rb^+	0.02

Chart of NavAb affinity constants for different ions

Ion(s)	Affinity Constant(Ka)
Na ⁺	1.0
K ⁺	0.14
Rb ⁺	0.02
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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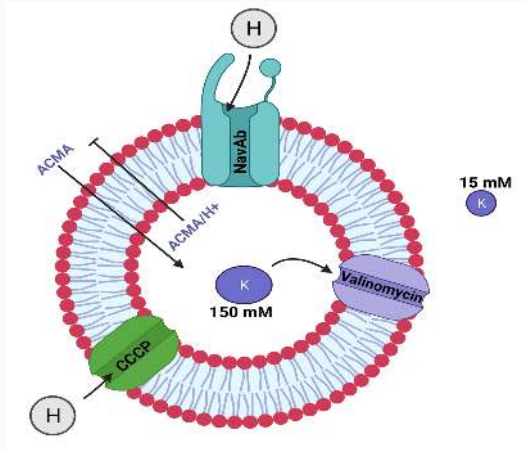
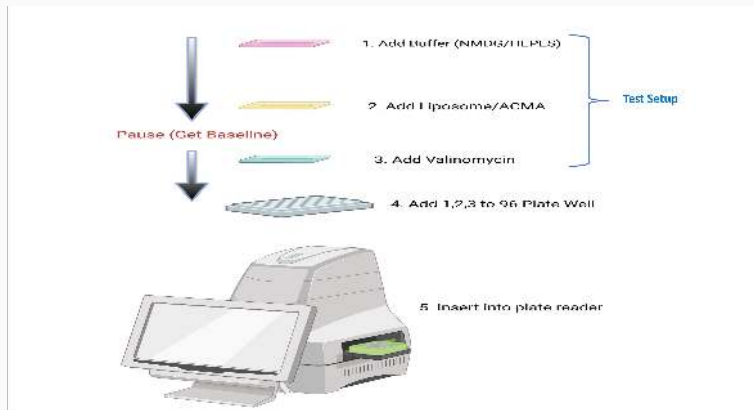


Figure 1: Na⁺-insert liposome

Practical image of experiment



Created with BioRender.com

Figure 2: experiment

Diagnostic results in Excel

Characteristics of liposome flux assays (LFAs)

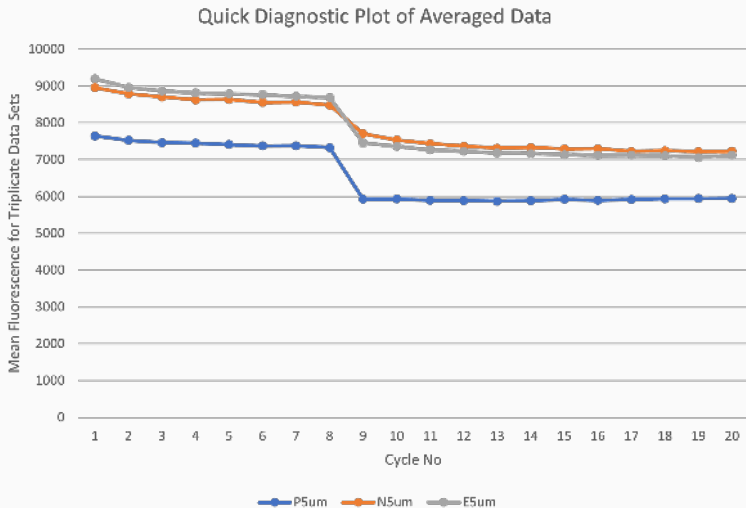


Figure 3: Na⁺-insert liposome

Normalized Results (Makes all the sense in the world)

Fluorescence measured at 2,3,5 μM

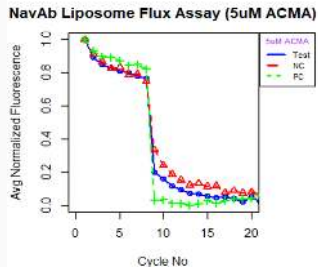
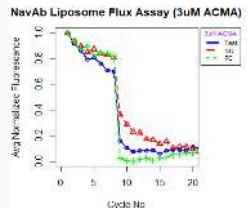
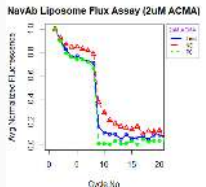


Figure 4: Legend: Normalized fluorescence in R within the signal zone

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

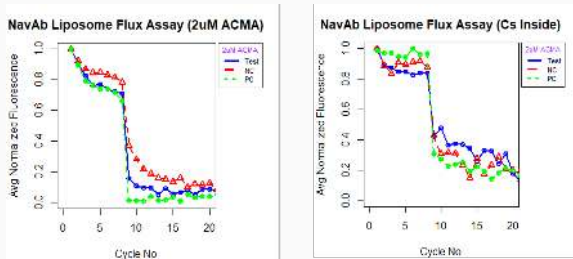


Figure 5: NavAb K-conductivity is stronger than Cs-conductivity as indicated by the initial affinity table

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



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

ACMA dosage at 2,3,4,5 μM

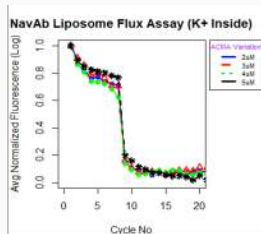
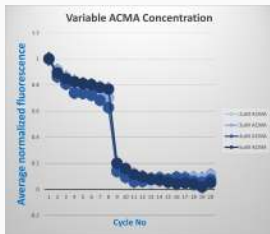


Figure 7: Fluorophore dosage is not a factor in its quenching, rather system properties (Ion conductivity etc.) determine quenching

Noise vs Signal

Fluorescence measured at 0.2 and 20 μM

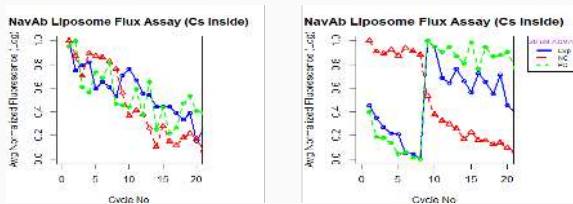


Figure 8: Legend: Normalized fluorescence in R within the signal zone

Conclusion

Quenching drivers

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

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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.