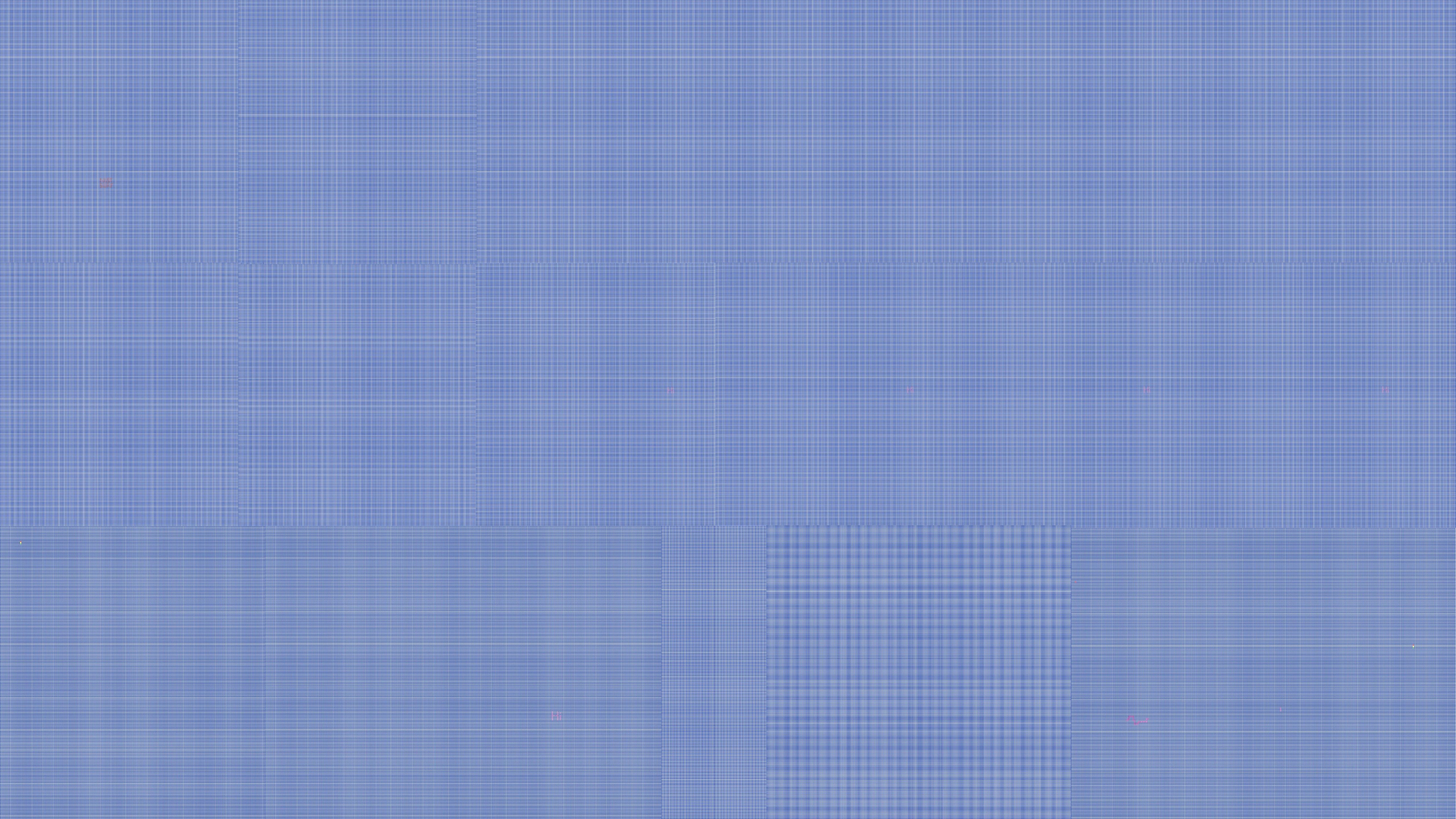
i won’t tell you until i tell you

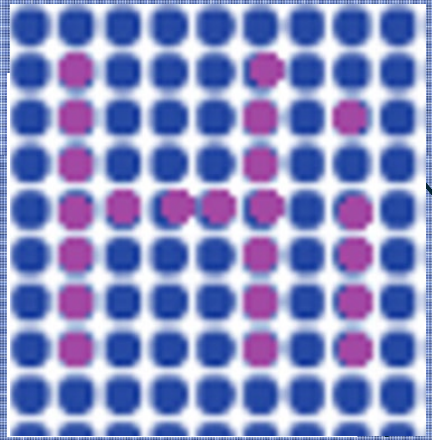
Let's Play a Game!

How would you describe the next slide?

Also I apologize to anyone who is color blind.

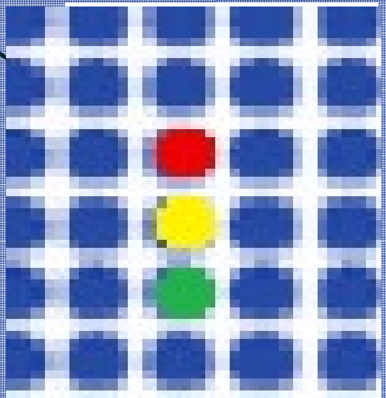

NIST
SURF
Hi
Hi
Hi
Hi
Hi

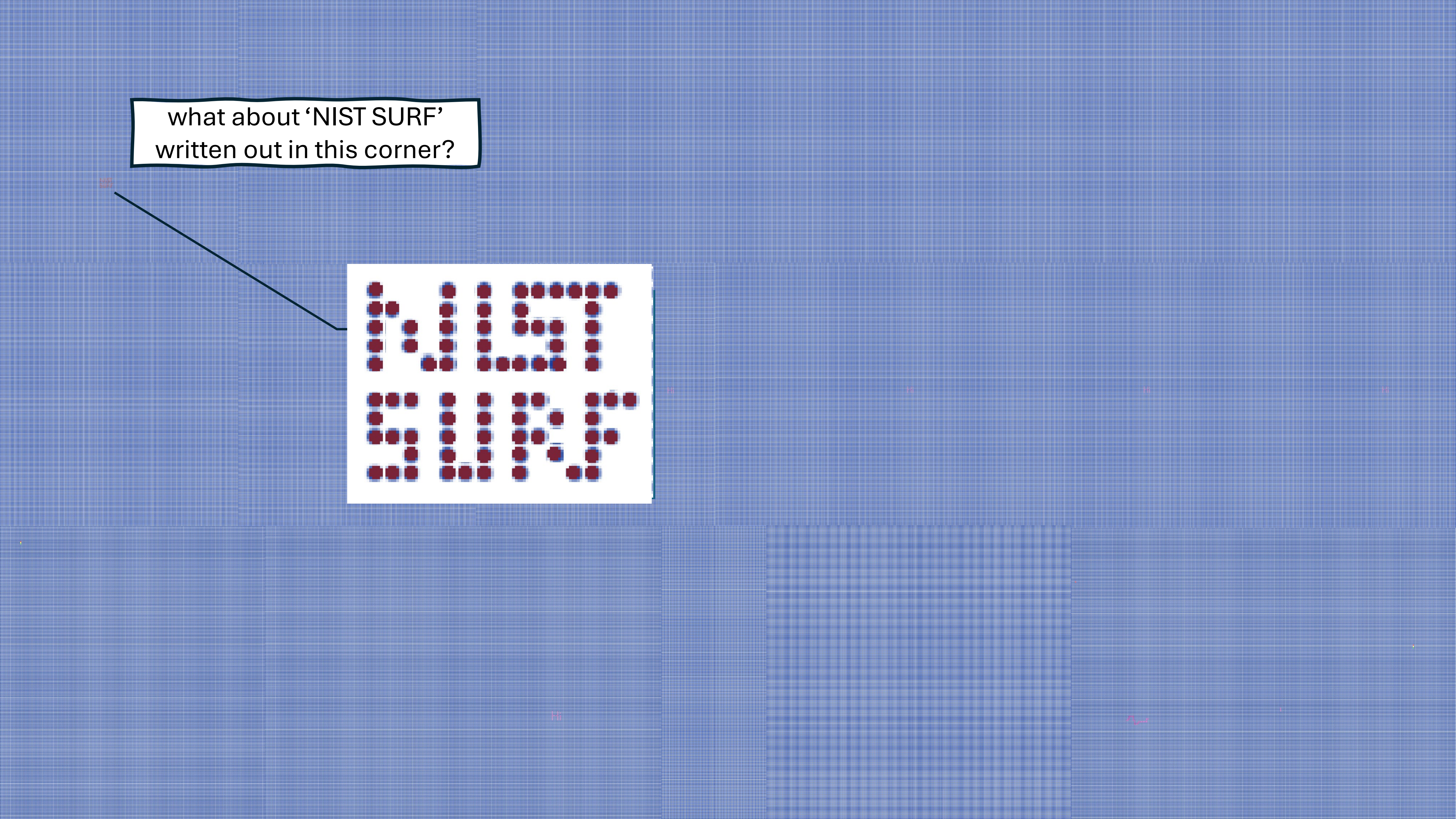
blue....plaid...slightly uniformed
dotted boringness right?



did you notice I said ‘Hi?’

or the stoplight?





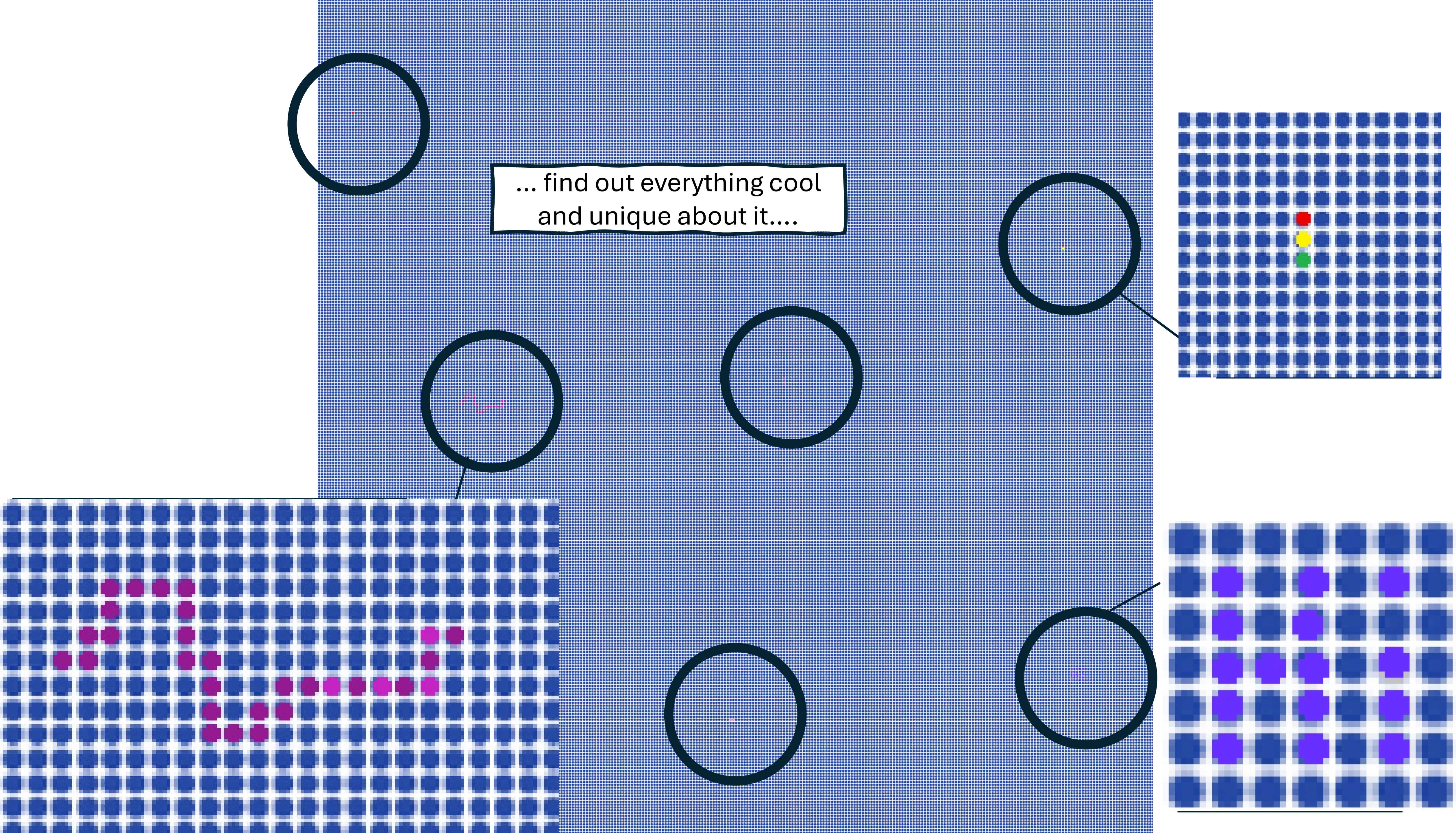
what about 'NIST SURF' written out in this corner?

Why does this matter?

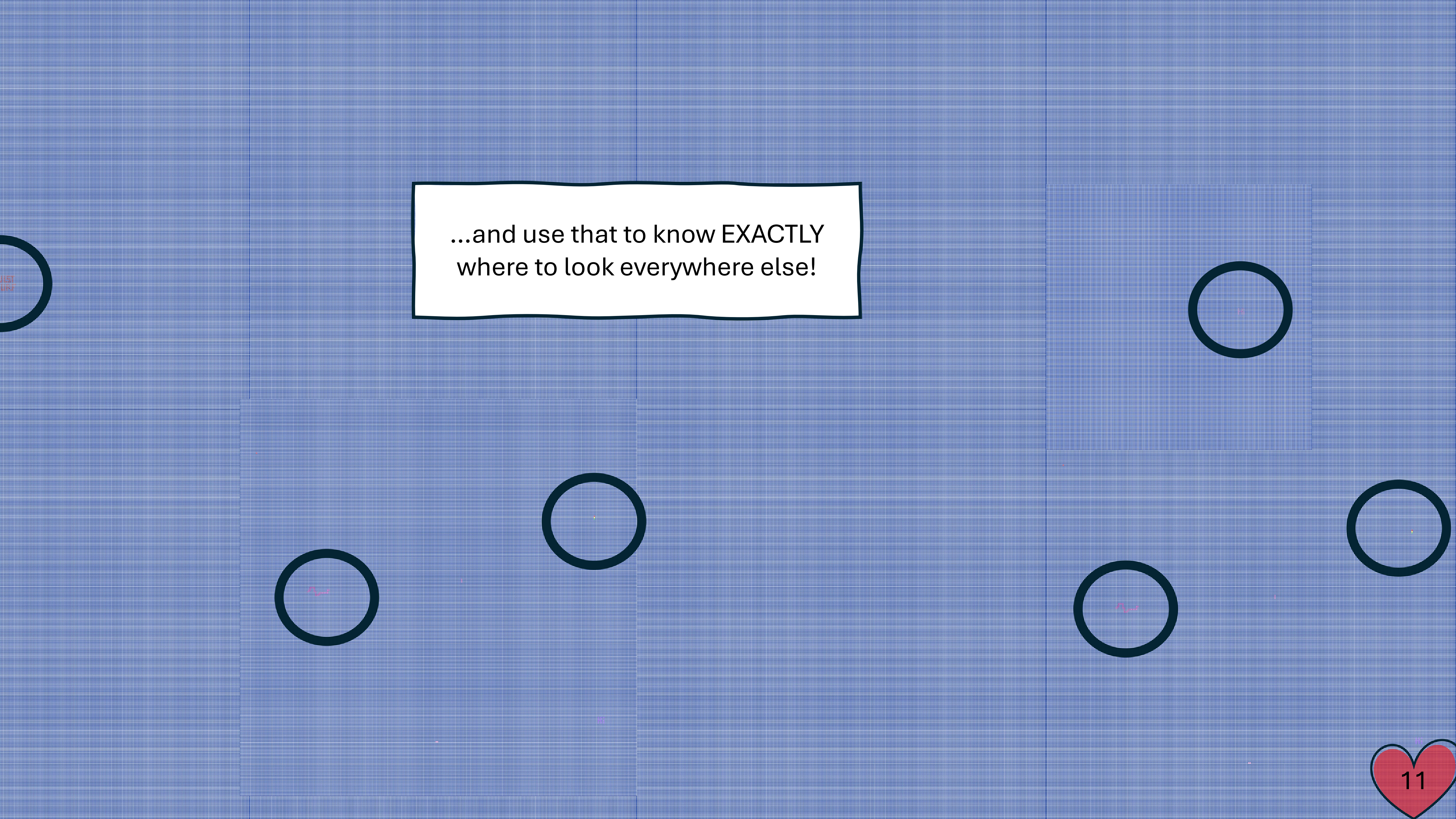
I want you to understand. There are 1.6 million dots on this page. If each was an hour long experiment it would take us 182 YEARS to test every dot!

Why does this matter?

It would be helpful if we could look at this corner...



... find out everything cool and unique about it....



...and use that to know EXACTLY where to look everywhere else!

Now…think to yourself

If every dot represented a handful of different lipids comprised in a membrane. And this lipid membrane interacted with a peptide sequence of 20 amino acids.

How many dots would be needed to account for EVERY possible combination?

Why does this matter?

If each dot is a specific lipid composition and 20 amino acid sequence there are > 10^{40} dots

Which would look like this!

Or actually this, times a trillion times another trillion times itself 10,000 times because your computer screen has a 2 million dot maximum.

UM | NIST
INSTITUTE FOR
BIOSCIENCE &
BIOTECHNOLOGY
RESEARCH

enter ROADMAP

Reflectometry-driven

Optimization

And

Discovery of

Membrane

Active

Peptides

ROADMAP

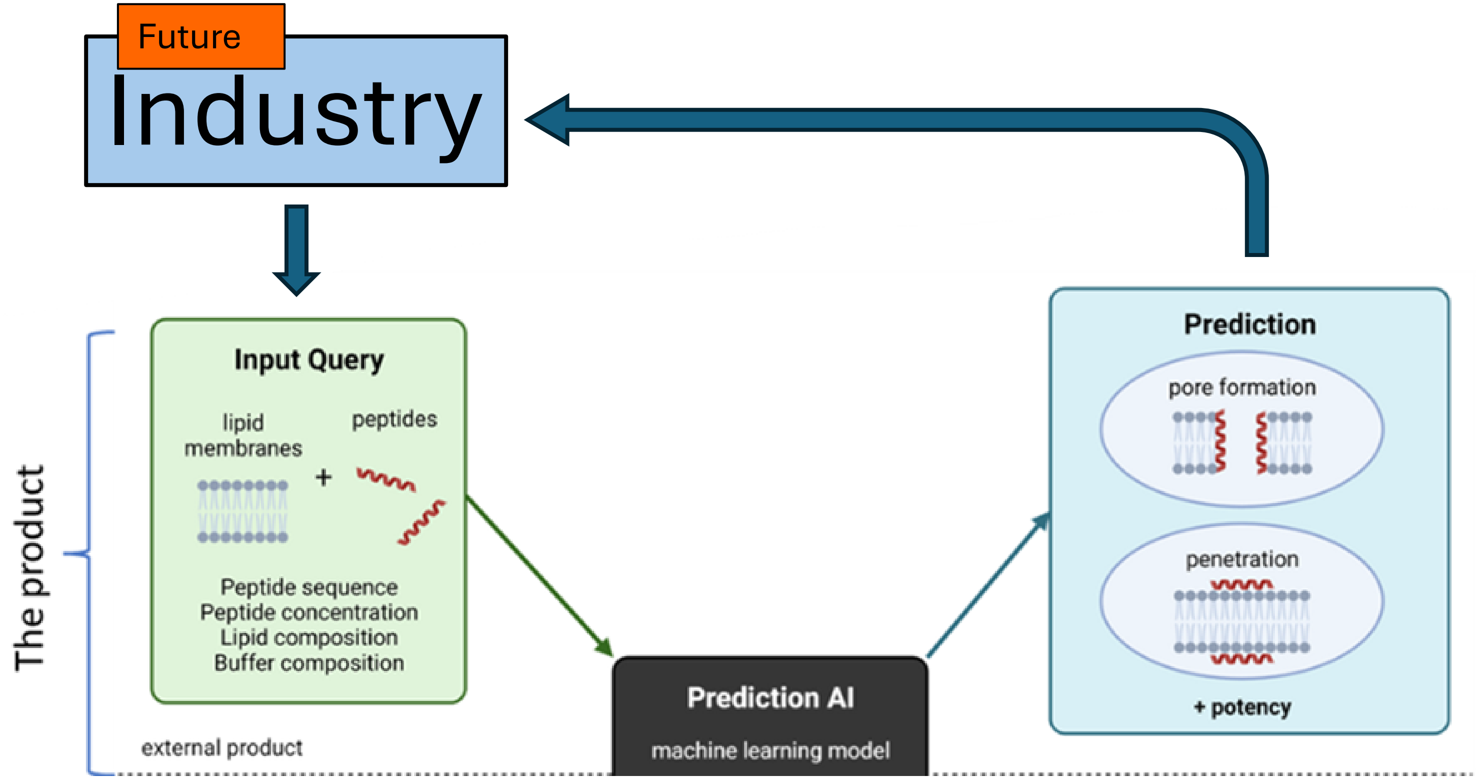
Big Idea

Build a new AI to predict lipid-peptide interactions using **Neutron Reflectometry** data

ROADMAP

Big Idea

Build a new AI to predict lipid-peptide interactions using **Neutron Reflectometry** data



The goal is for NIST to assist industry partners with the pure-science-trial-and-error-discovery with this AI.

So that American companies can focus on the development and manufacture of pharmaceuticals and the next generation of antibiotic classes.

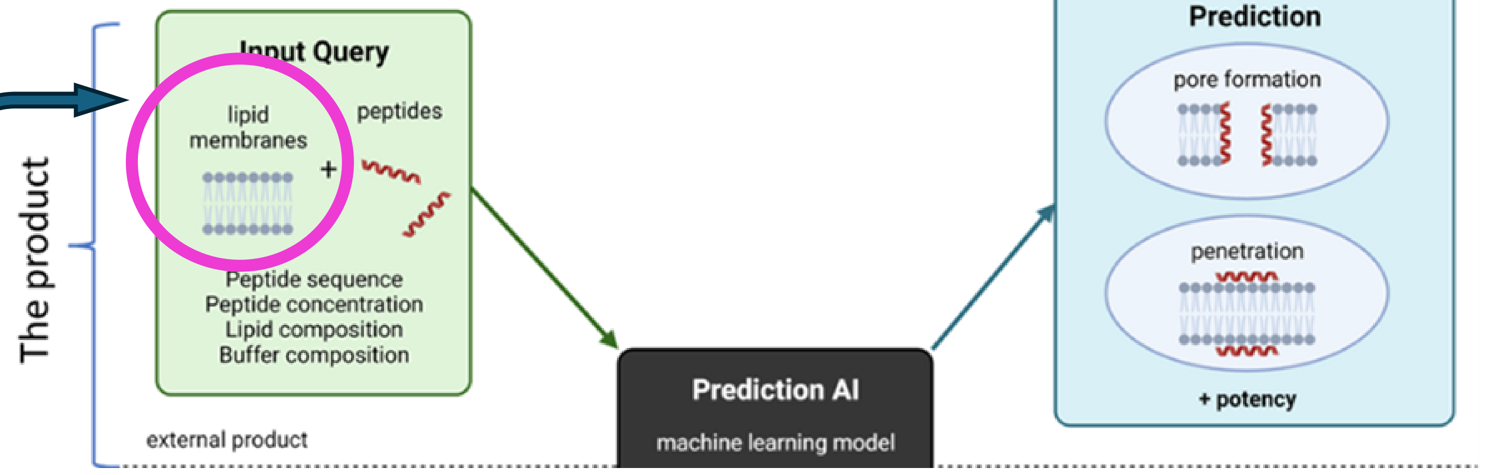
ROADMAP

Big Idea

Build a new AI to predict lipid-peptide interactions using **Neutron Reflectometry** data

Future

Industry



An autonomous **Solvent Assisted Lipid Bilayer** sample creator and **QCM-D measurement** device incorporating a predictive **Gaussian process model**

Present

ROADbot

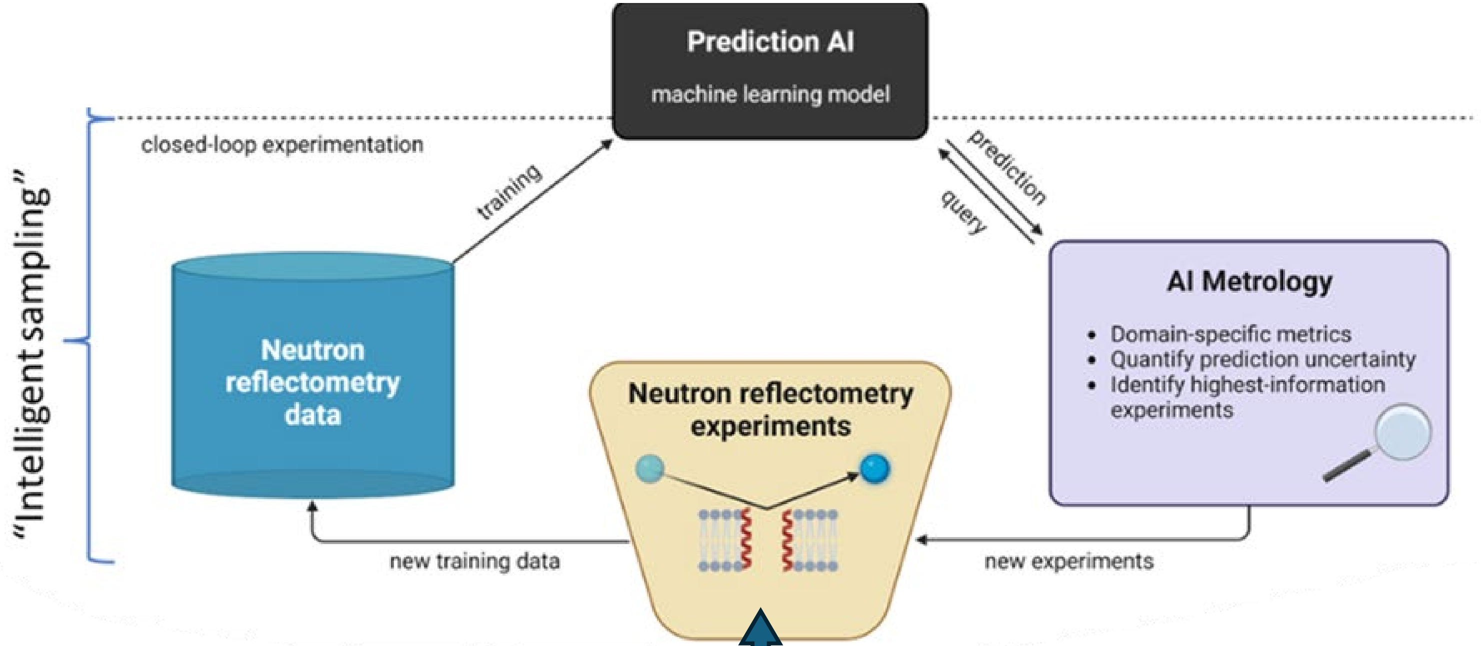
Currently the ROADMAP project is developing and using ROADbot to figure out the best lipid membrane compositions and how to make them.

ROADMAP

Big Idea

Build a new AI to predict lipid-peptide interactions using **Neutron Reflectometry** data

And in 2026 when the NIST Center for Neutron Research reactor restarts. ROADbot will make samples for neutron reflectometry experiments to build the AI using the Angstrom-scale structural data that only neutrons provide!



ROADbot

planned 2026

ROADbot

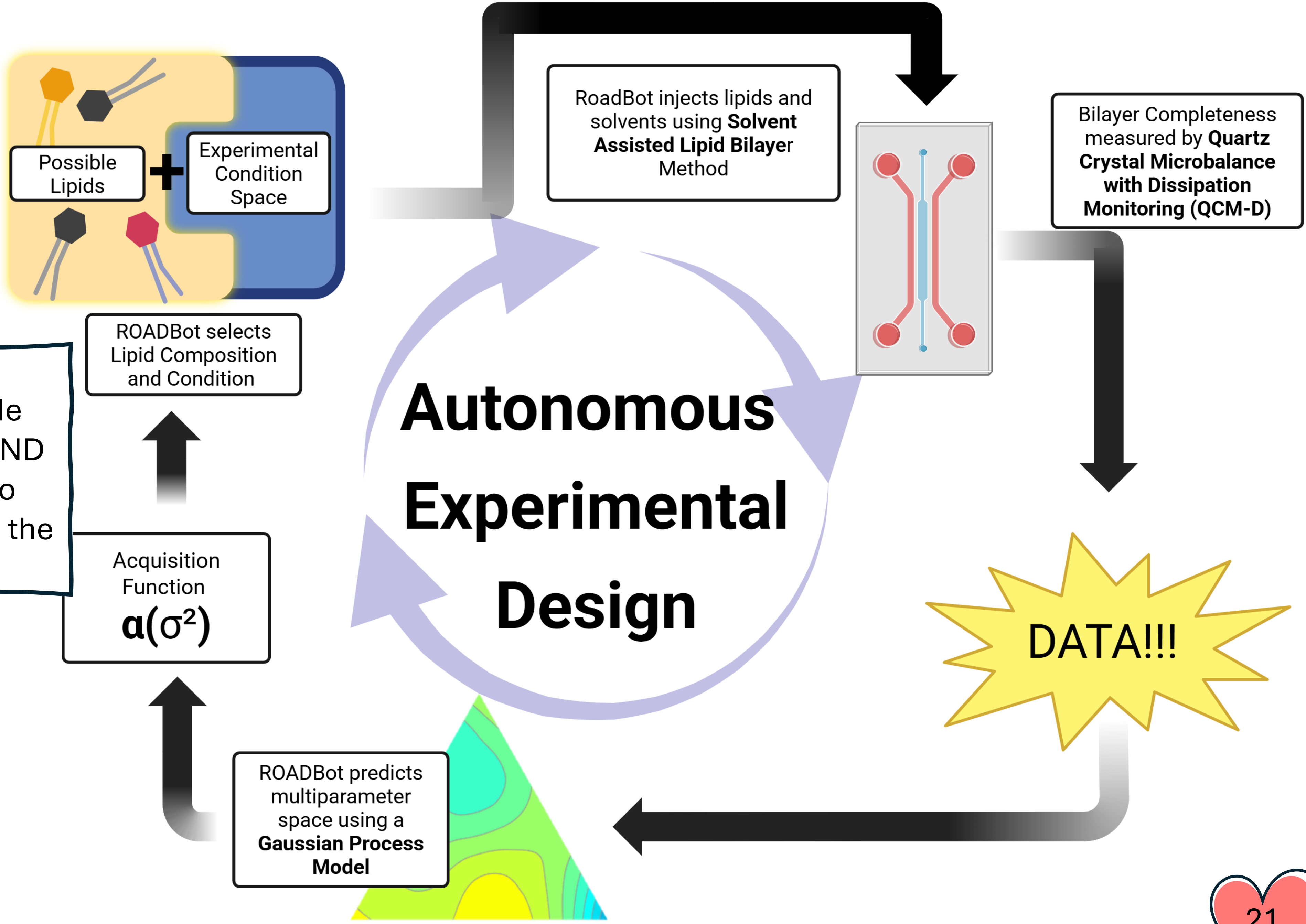
Autonomous optimization of compositionally diverse lipid bilayers on solid supports

Xuliana O; Virginia Commonwealth University
David Hoogerheide; Mentor
Megan Mitchell; Post-Doc

CHRNS
CENTER FOR HIGH RESOLUTION NEUTRON SCATTERING
NIST

Good Info

Because ROADBot is autonomous. I would decide which lipids could be used AND what conditions I wanted to explore. Then ROADBot does the rest!



Autonomous Experimental Design

Possible Lipids + Experimental Condition Space

ROADBot selects Lipid Composition and Condition

RoadBot injects lipids and solvents using **Solvent Assisted Lipid Bilaye**r Method

Bilayer Completeness measured by **Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D)**

DATA!!!

ROADBot predicts multiparameter space using a **Gaussian Process Model**

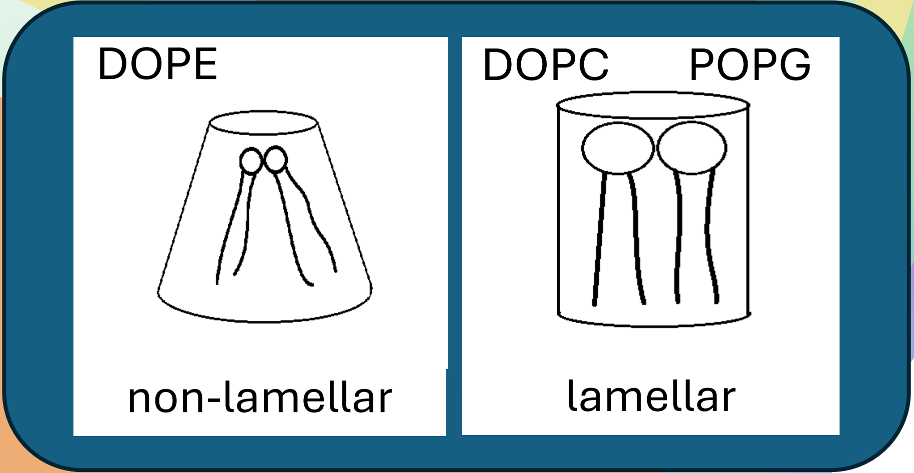
Acquisition Function

$\alpha(\sigma^2)$

intro

Good Info

Lipids Used

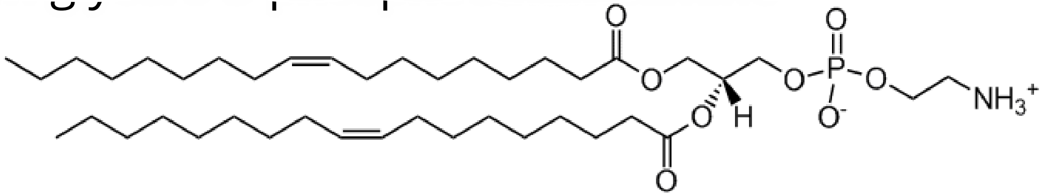


DOPE

1,2-dioleoyl-sn-glycero-3-phosphoethanolamine

non-lamellar

zwitterionic

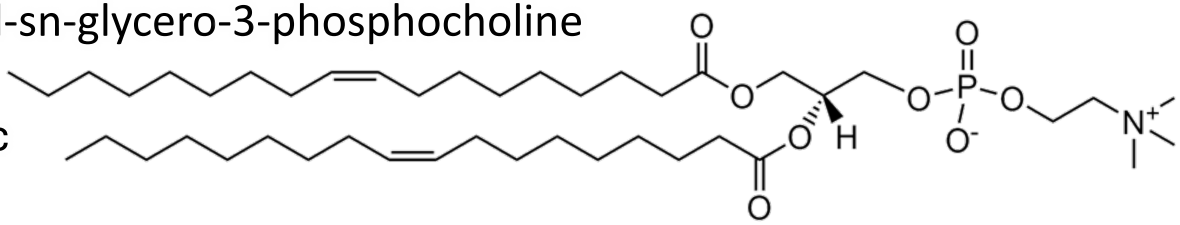


DOPC

1,2-Dioleoyl-sn-glycero-3-phosphocholine

lamellar

zwitterionic

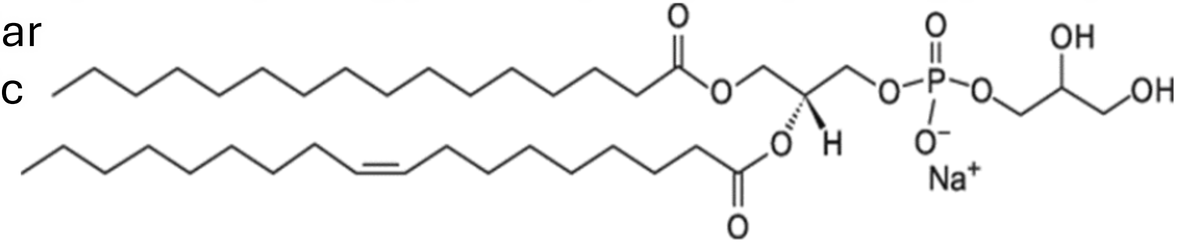


POPG

1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol)

lamellar

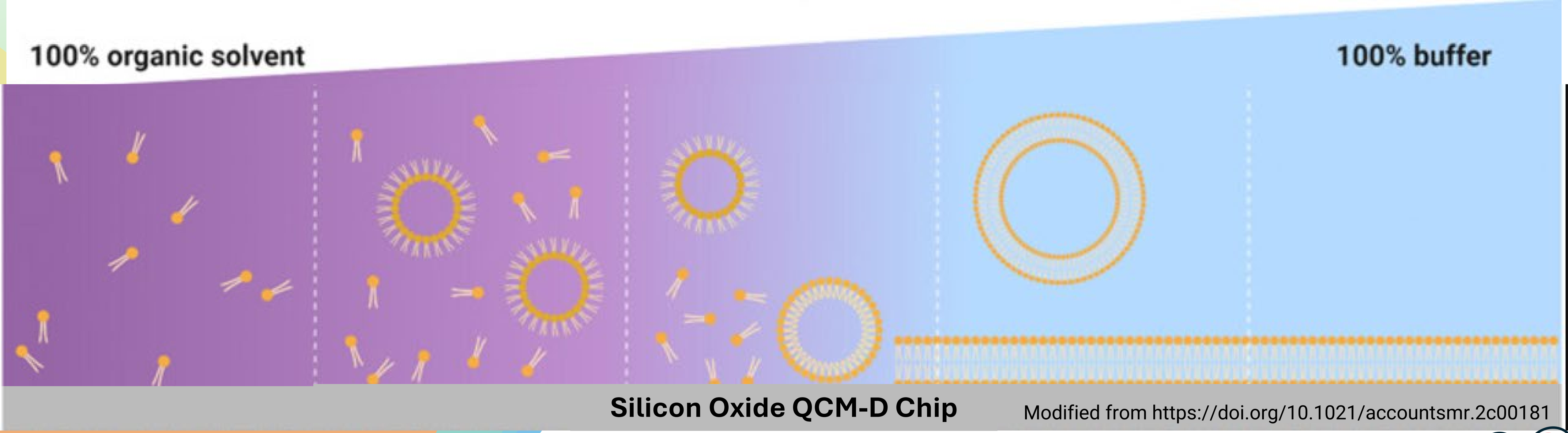
anionic



Good Info

Solvent Assisted Lipid Bilayer (SALB) Method

Dissolved lipid species in alcohol can self-assemble onto a surface in a bilayer as the alcohol is diluted by a biological mimic buffer.

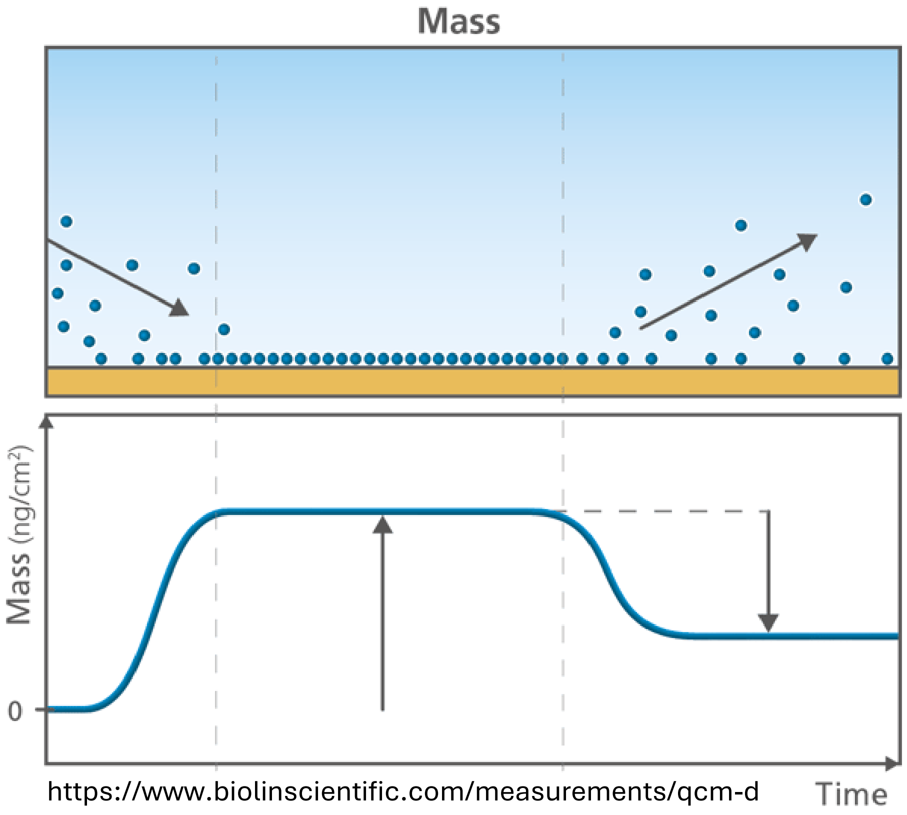


Modified from https://doi.org/10.1021/accountsmr.2c00181

Good Info

What is a QCM-D?

Quartz **C**rystal
Microbalance with
Dissipation monitoring



https://www.biolinscientific.com/measurements/qcm-d

Resonant frequencies across a quartz crystal can be analyzed electronically (current required to maintain a constant voltage)

Essentially, it is a tiny scale that has nanogram (ng/cm^2) sensitivity!

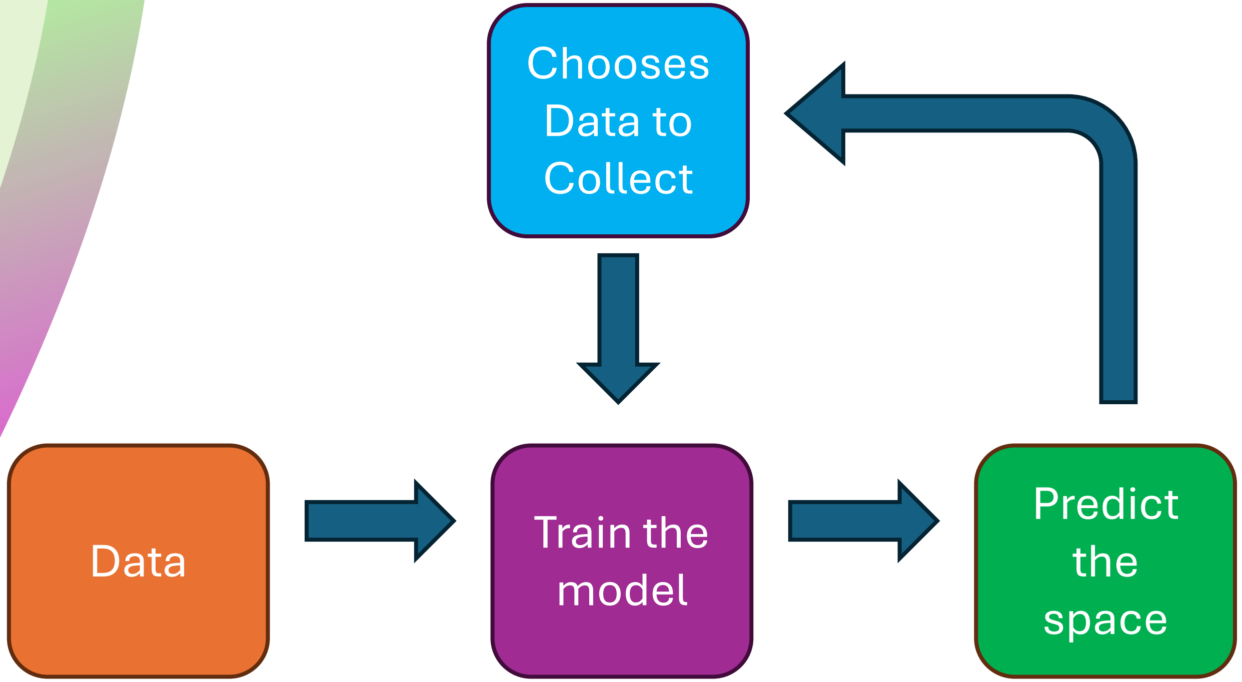
Dissipation monitoring is the tracking of that vibration through the sample and flow cell

This makes it ideal for biosystems that require fluidic environments!

Good Info

What is a Gaussian Process Model?

Predictive algorithm to map a multiparameter space



Chooses Data to Collect

Data

Train the model

Predict the space

Topics of this Presentations

How does flow rate of solvent exchange effect lipid bilayer self-assembly?

DOPC
DOPE

How does NaCl concentration affect lipid self-assembly?

DOPC
DOPE
POPG

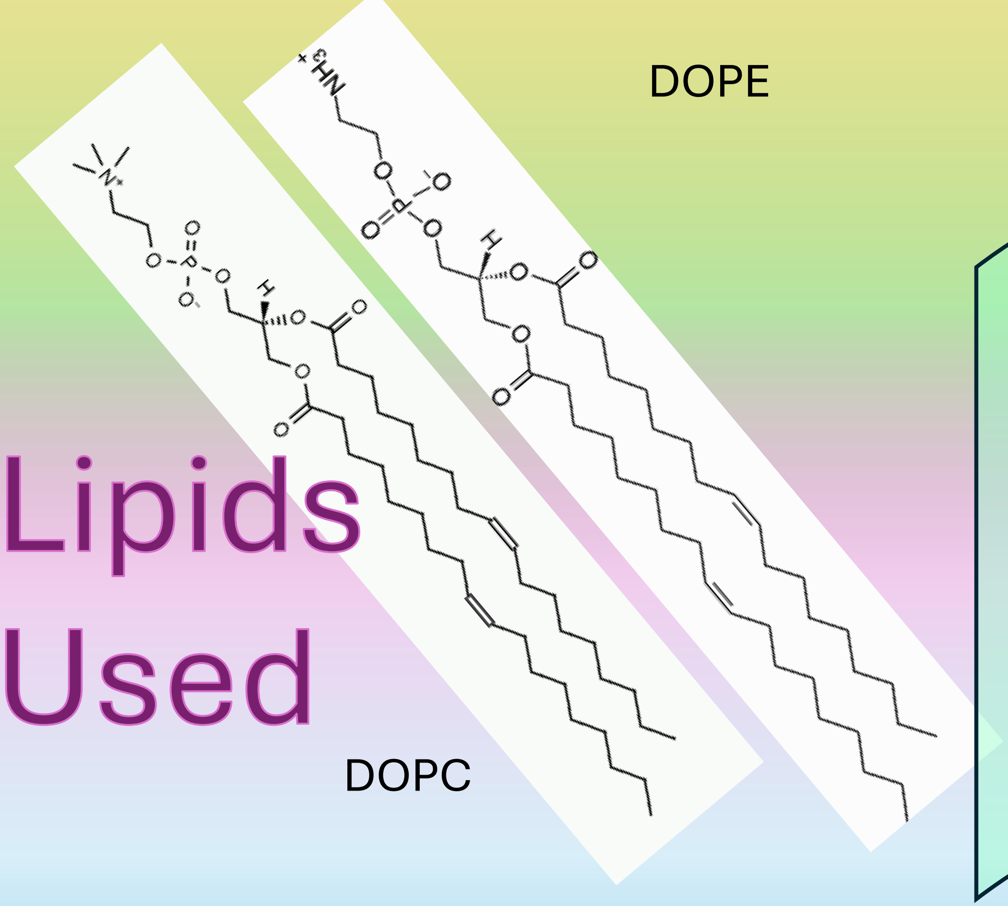
How sure are we of predicted data?

Future Work

Common Errors and Where to Find Them

How does flow rate of solvent exchange effect lipid bilayer self-assembly?

Lipids Used

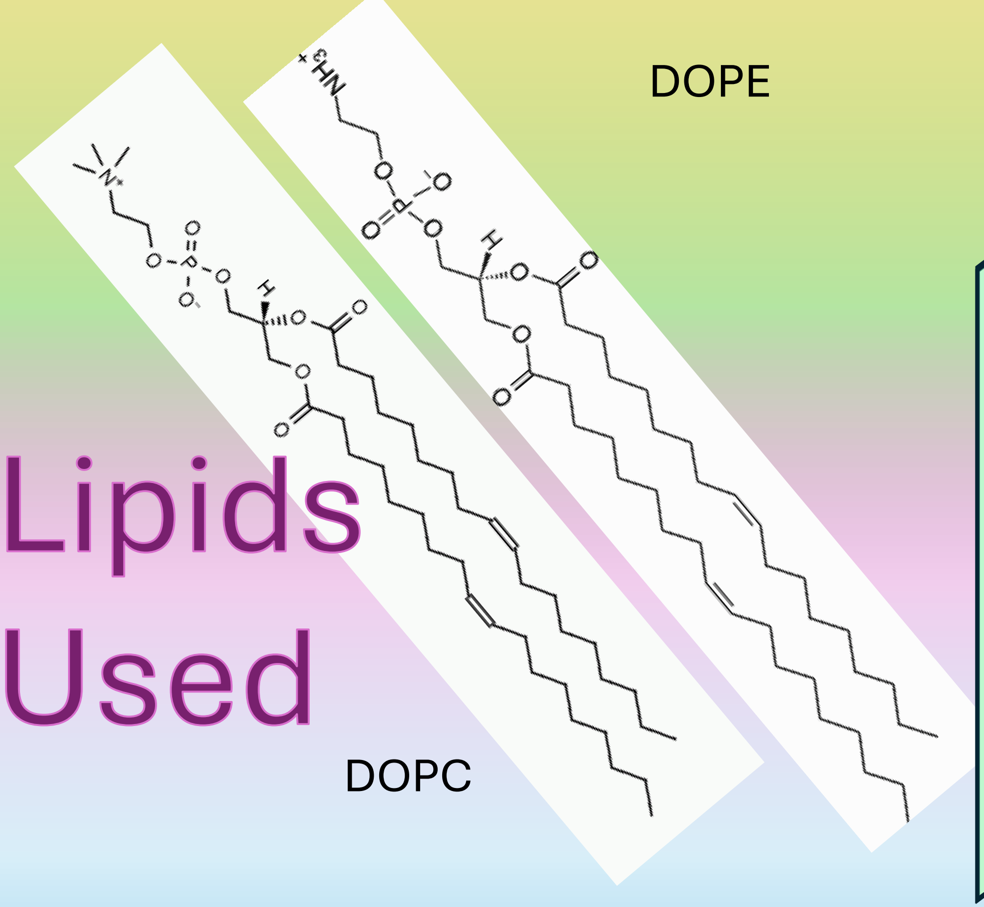


Current Knowledge:

A literature review suggests exchanging solvent at 0.1 ml/min to allow lipids to self-assemble.

How does flow rate of solvent exchange effect lipid bilayer self-assembly?

Lipids Used



DOPE

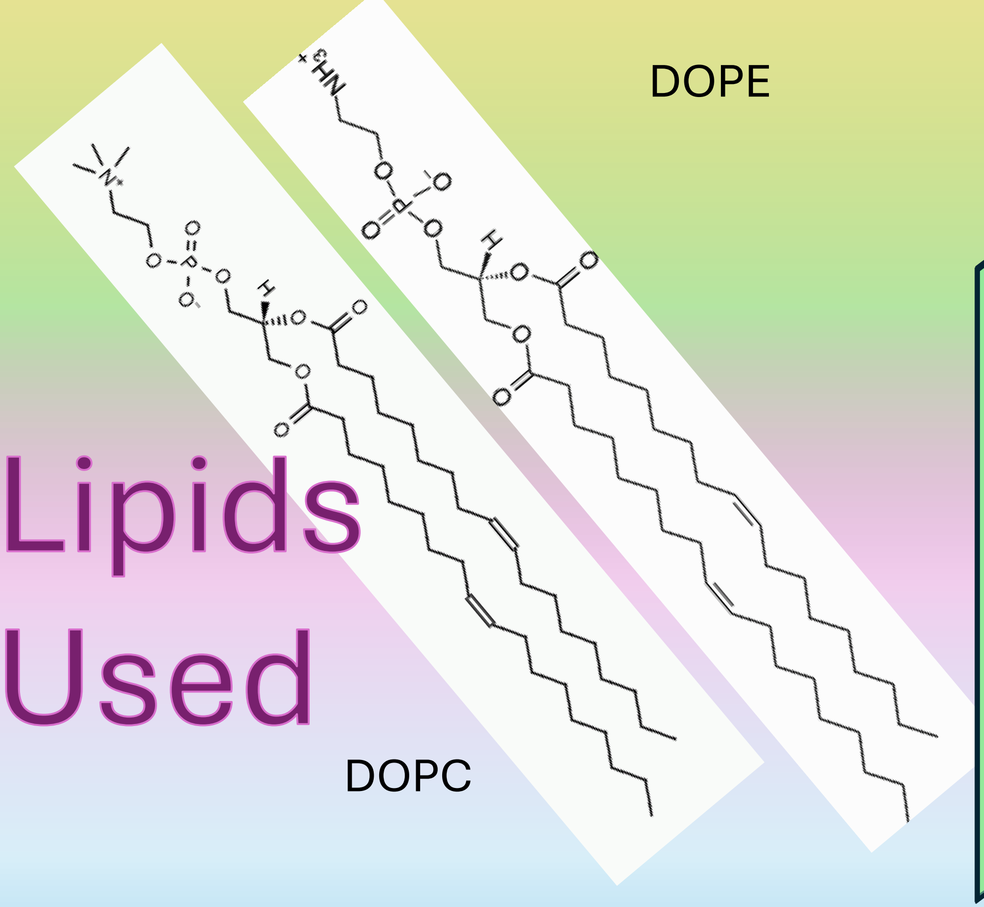
DOPC

Problem:

That is the biggest time-taker of ROADbot’s function, increasing flow-rate increases speed!

How does flow rate of solvent exchange effect lipid bilayer self-assembly?

Lipids Used


DOPE

DOPC

Experiment:
Observe the effect of solvent exchange rates of 0 to 4 ml/min on lipid bilayer formation.

Lipid Concentration

means total concentration of DOPC/DOPE

X-axis

0 = pure DOPE, 1 = pure DOPC

Y-axis

Flow Rate (ml/min)

Color scale

Value of 1 signifies a complete bilayer (green or 🖤)

The next graphs are predicted space based on 25 measurements taken by ROADBot.

Lipid Concentration

means total concentration of DOPC/DOPE

X-axis

0 = pure DOPE, 1 = pure DOPC

Y-axis

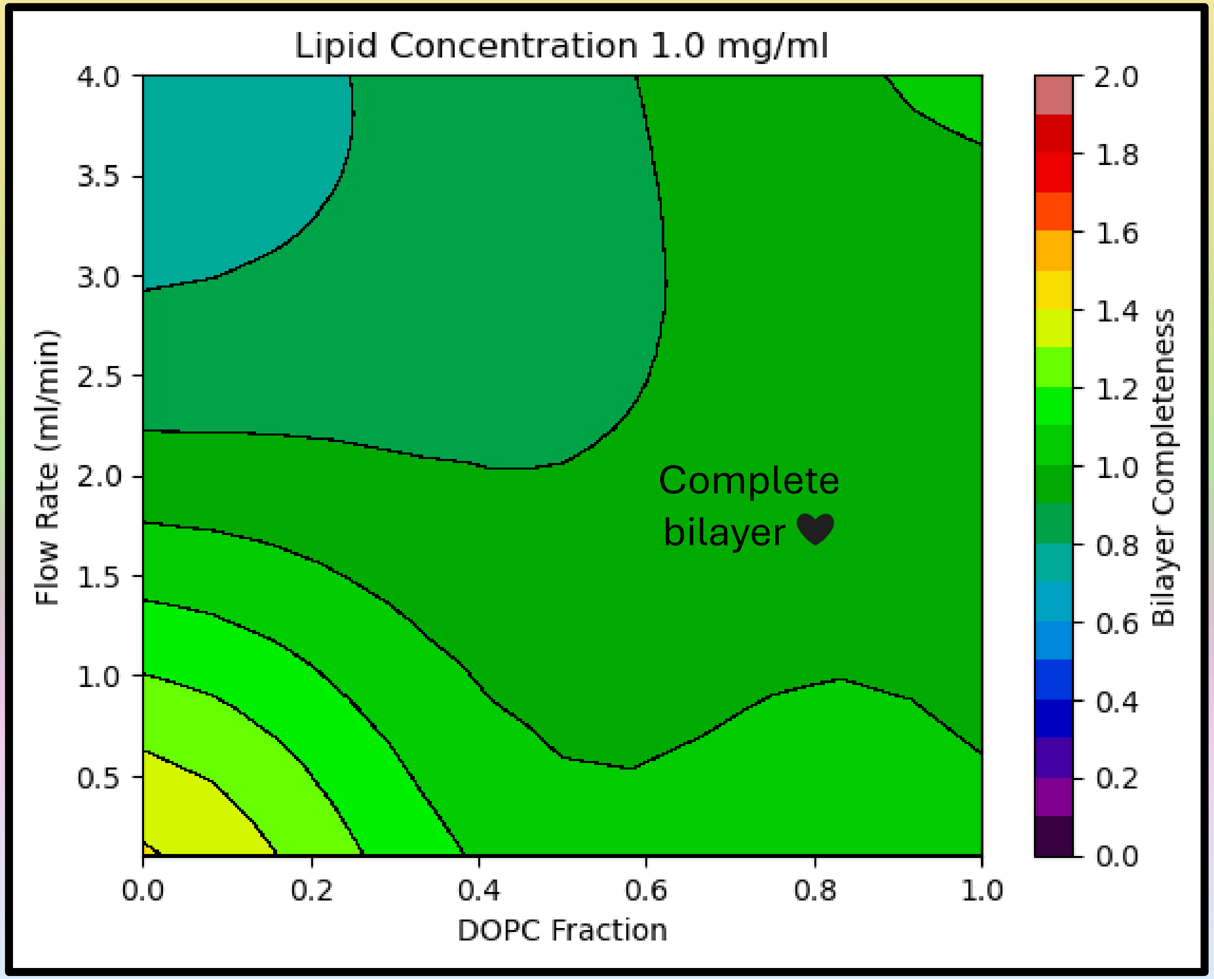
Flow Rate (ml/min)

Color scale

Value of 1 signifies a complete bilayer (green or 🖤)

On the right side of the graph, DOPC is unaffected by increasing flow rate of the solvent exchange!

The same is not true for DOPE....



Lipid Concentration

means total concentration of DOPC/DOPE

X-axis

0 = pure DOPE, 1 = pure DOPC

Y-axis

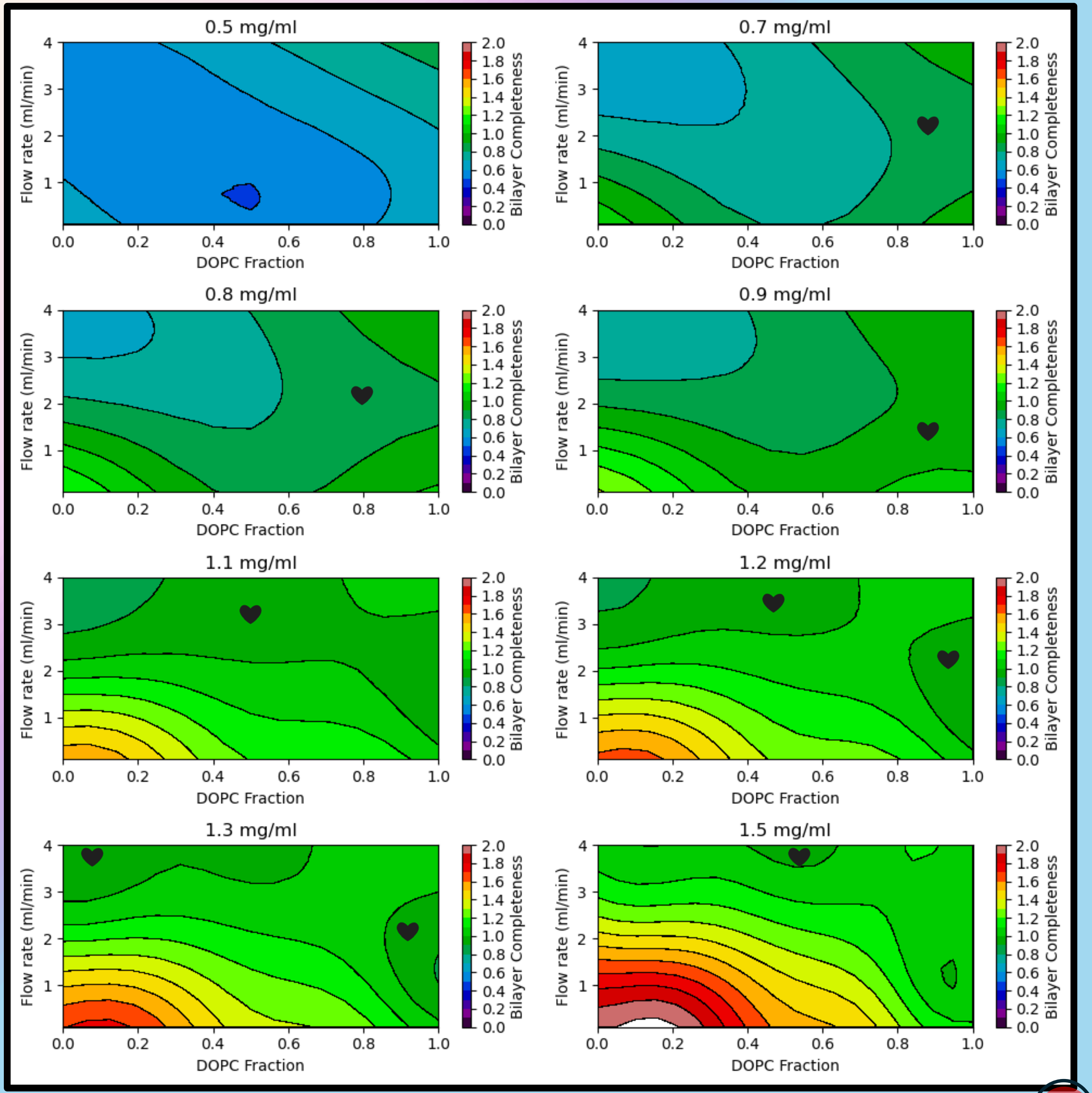
Flow Rate (ml/min)

Color scale

Value of 1 signifies a complete bilayer (green or ♥)

If we predict greater total lipid concentration DOPE, at low flow rate, aggregates even more.

It would be great to use neutrons to find out what is going on!



Lipid Concentration

means total concentration of DOPC/DOPE

X-axis

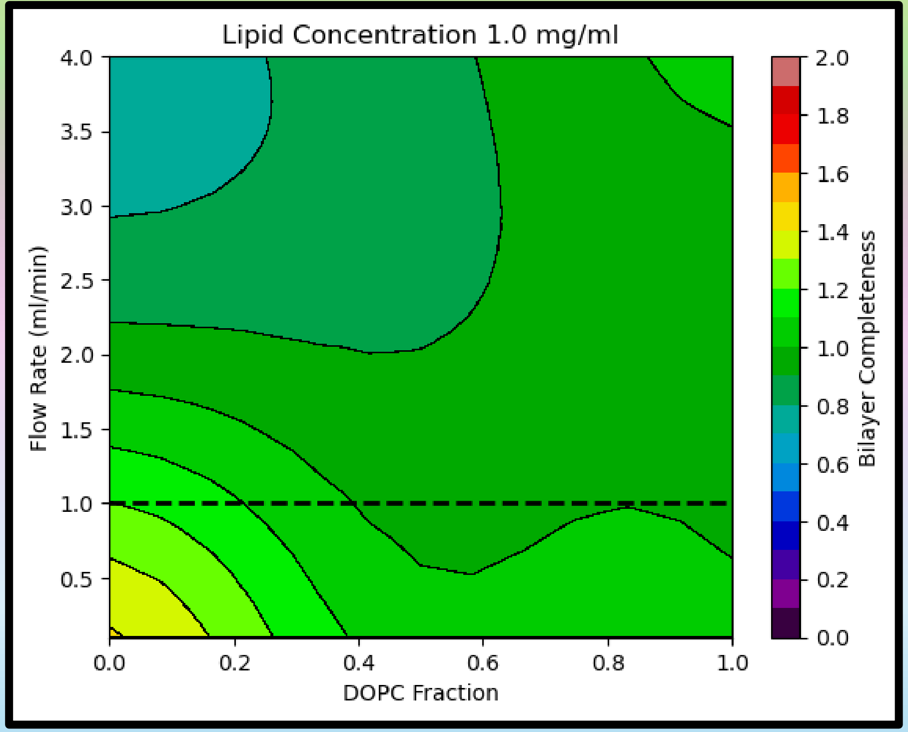
0 = pure DOPE, 1 = pure DOPC

Y-axis

Flow Rate (ml/min)

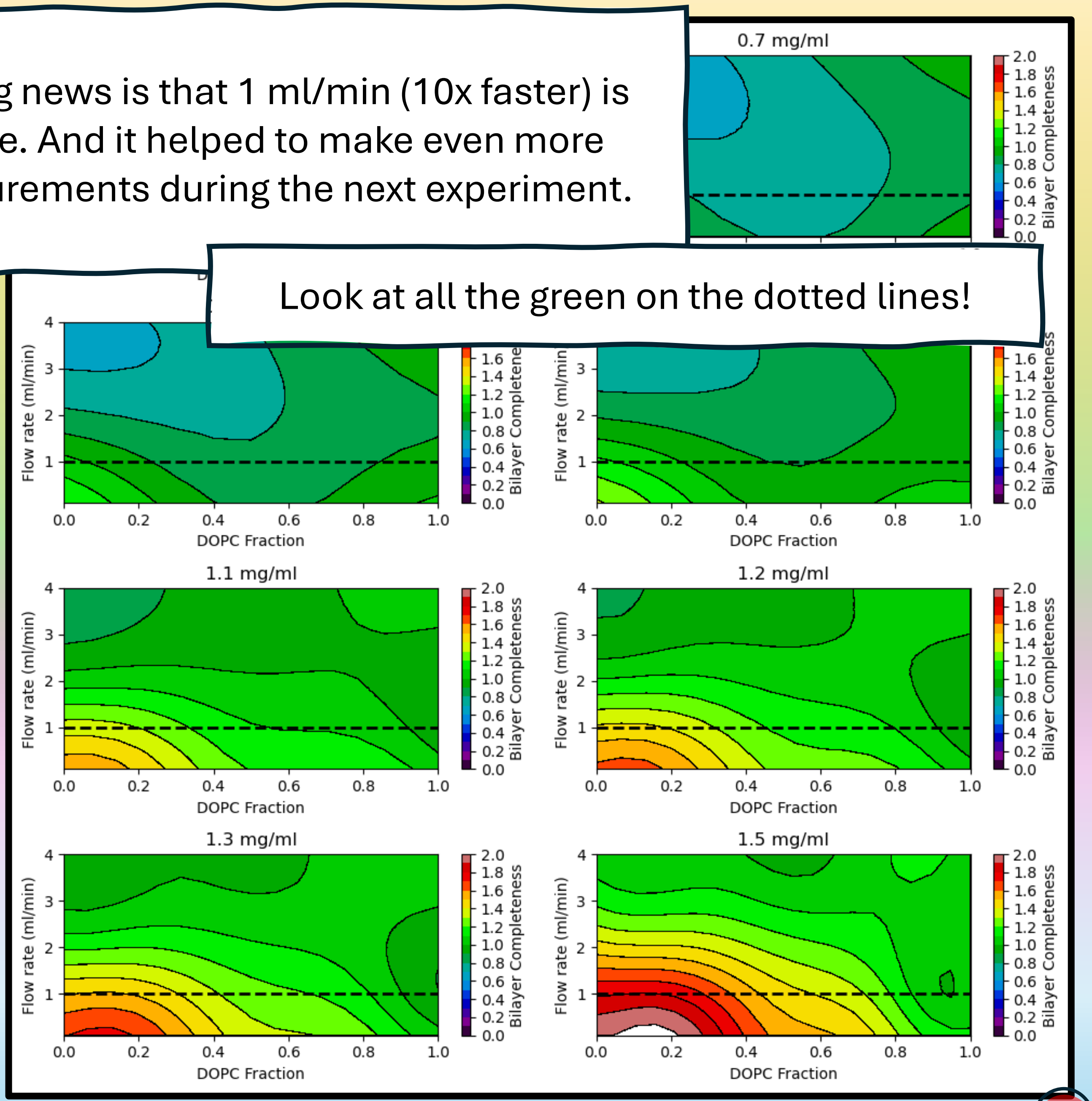
Color scale

Value of 1 signifies a complete bilayer (green or 🖤)



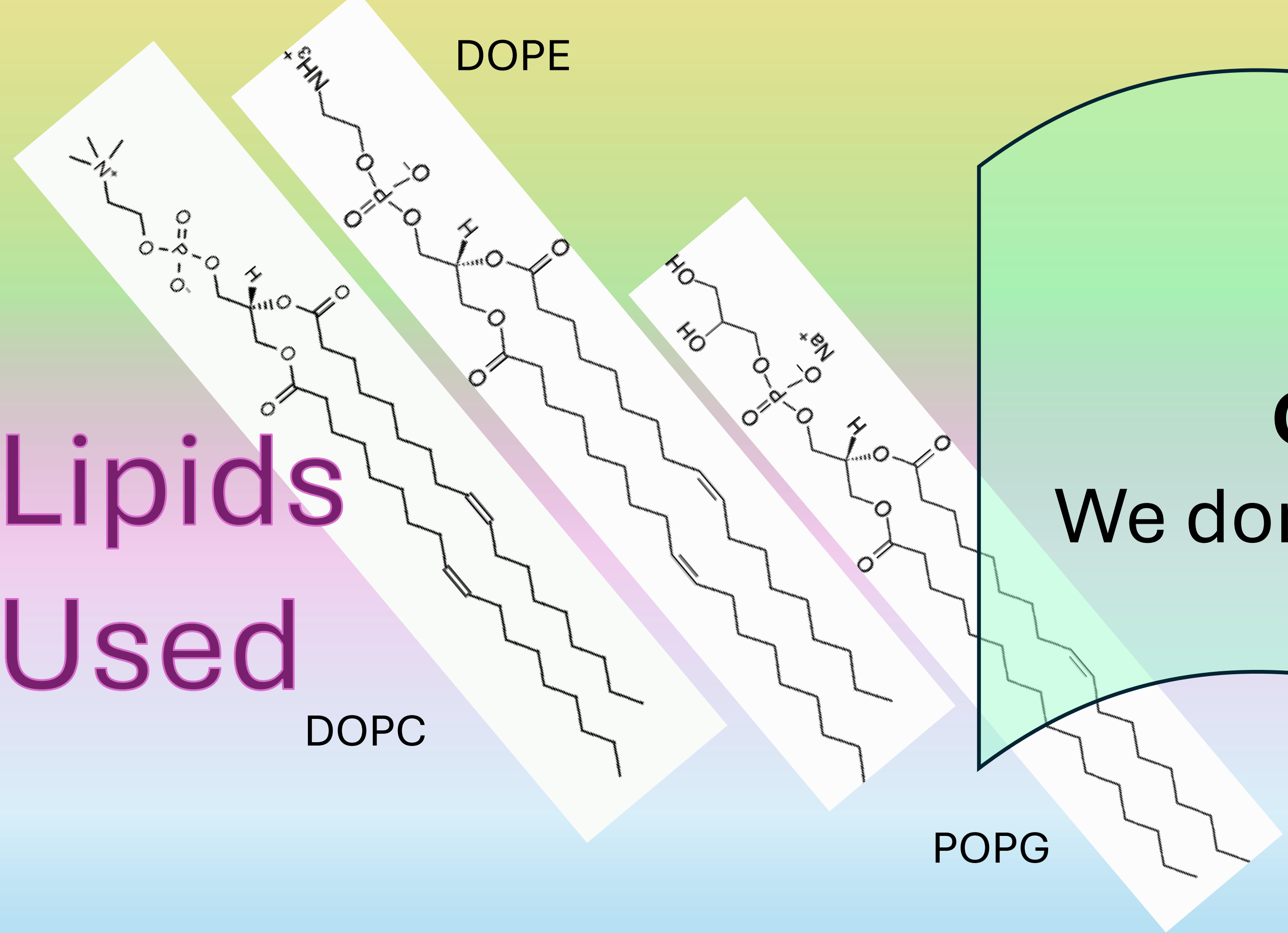
The big news is that 1 ml/min (10x faster) is viable. And it helped to make even more measurements during the next experiment.

Look at all the green on the dotted lines!



How does NaCl concentration affect lipid self-assembly?

Lipids Used



DOPE

DOPC

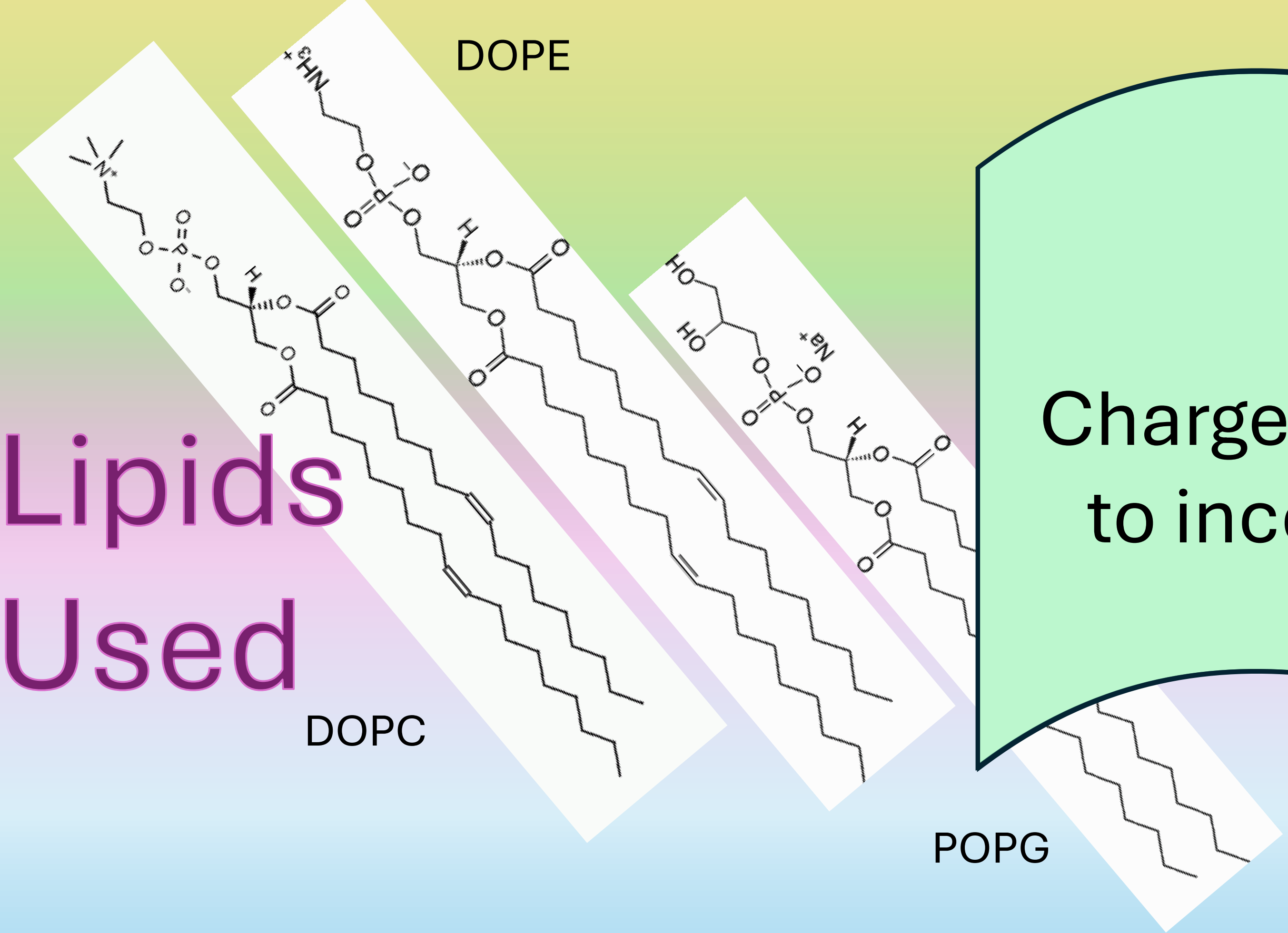
POPG

Current Knowledge:

We don’t know. Yay Pure Science!

How does NaCl concentration affect lipid self-assembly?

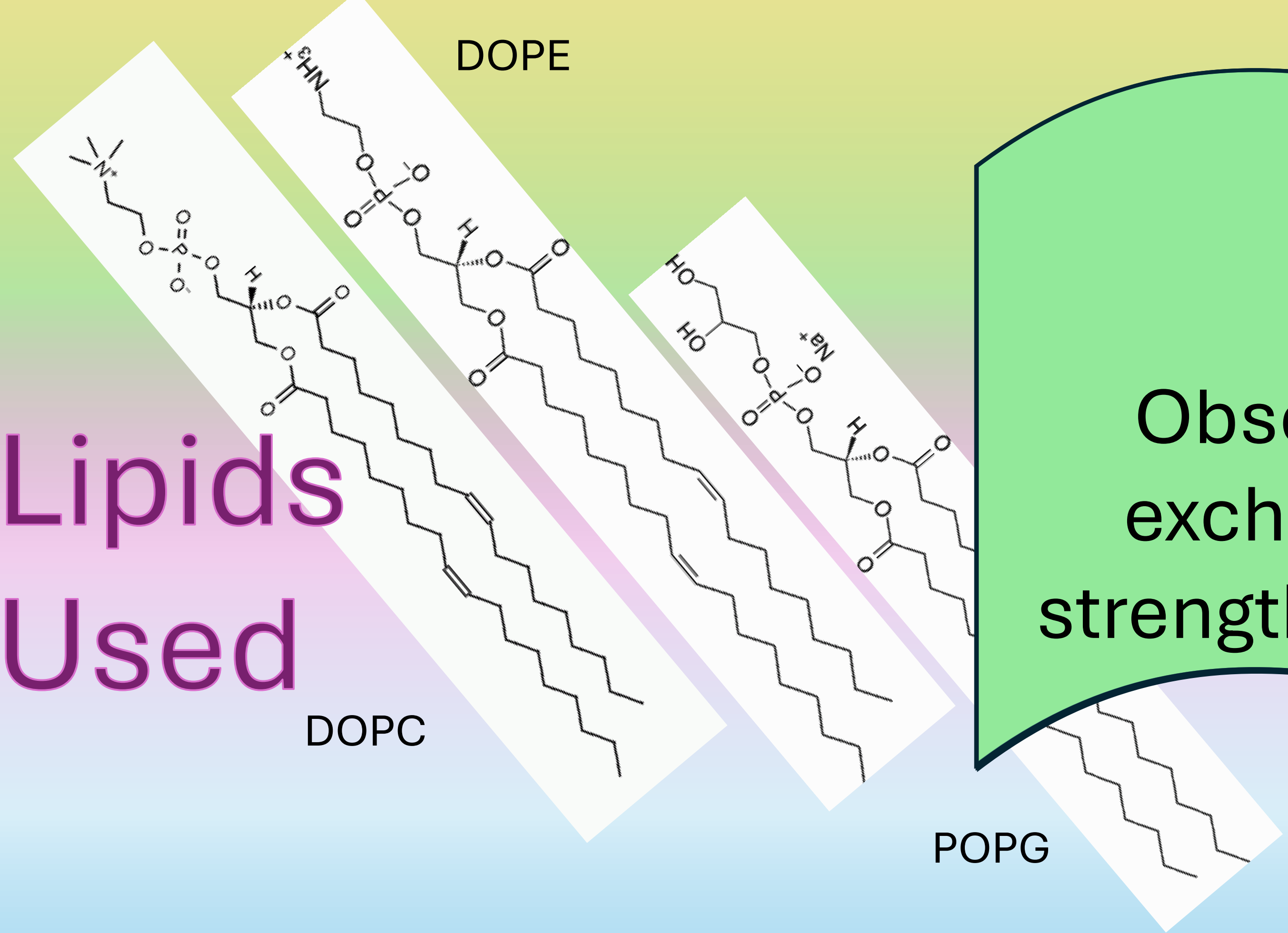
Lipids Used



Problem:
Charged lipids (POPG) are difficult to incorporate into good, planar bilayers.

How does NaCl concentration affect lipid self-assembly?

Lipids Used


DOPE

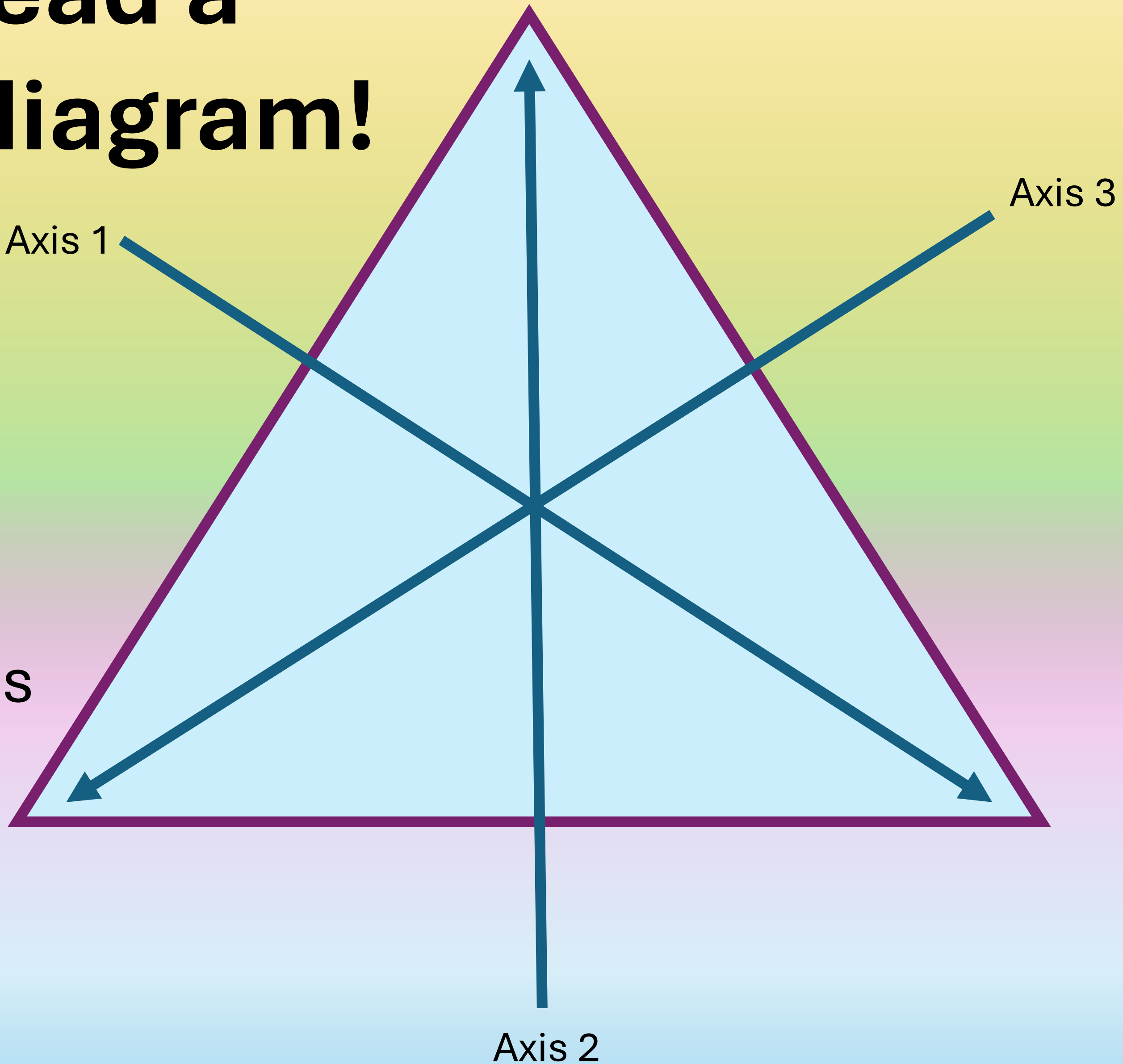
DOPC

POPG

Experiment:
Observe the effect of solvent exchanges of increasing ionic strength on lipid bilayer formation.

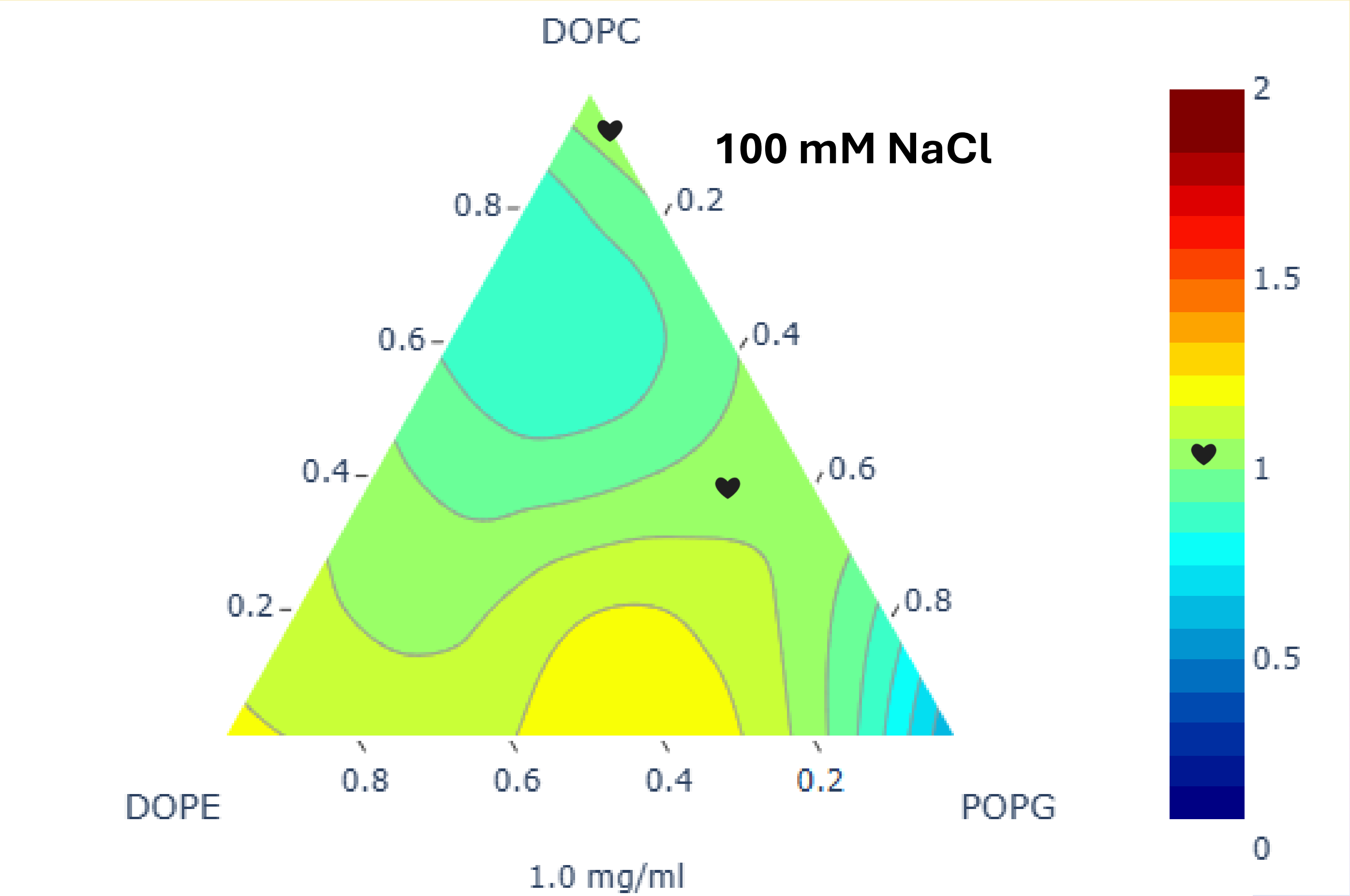
Good Info

How to read a ternary diagram!



At the arrowhead is 100 % of that axis
i.e. a pure solution

The centroid (where all axis' meet)
each axis is in equal fraction.

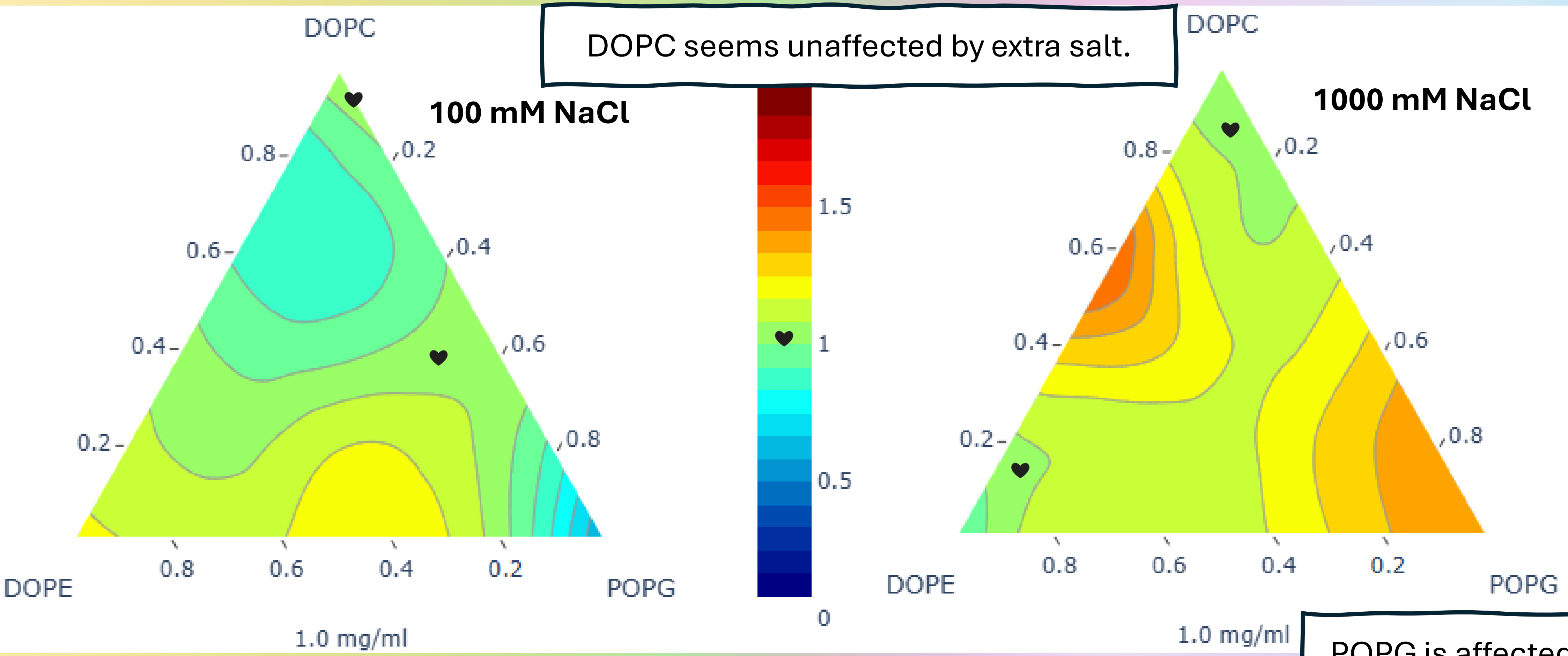


A Value of 1 represents a complete bilayer

Each triangle represents a TOTAL lipid concentration

Prediction based 162 measurements (8 removed)

Each point is 100% of that lipid

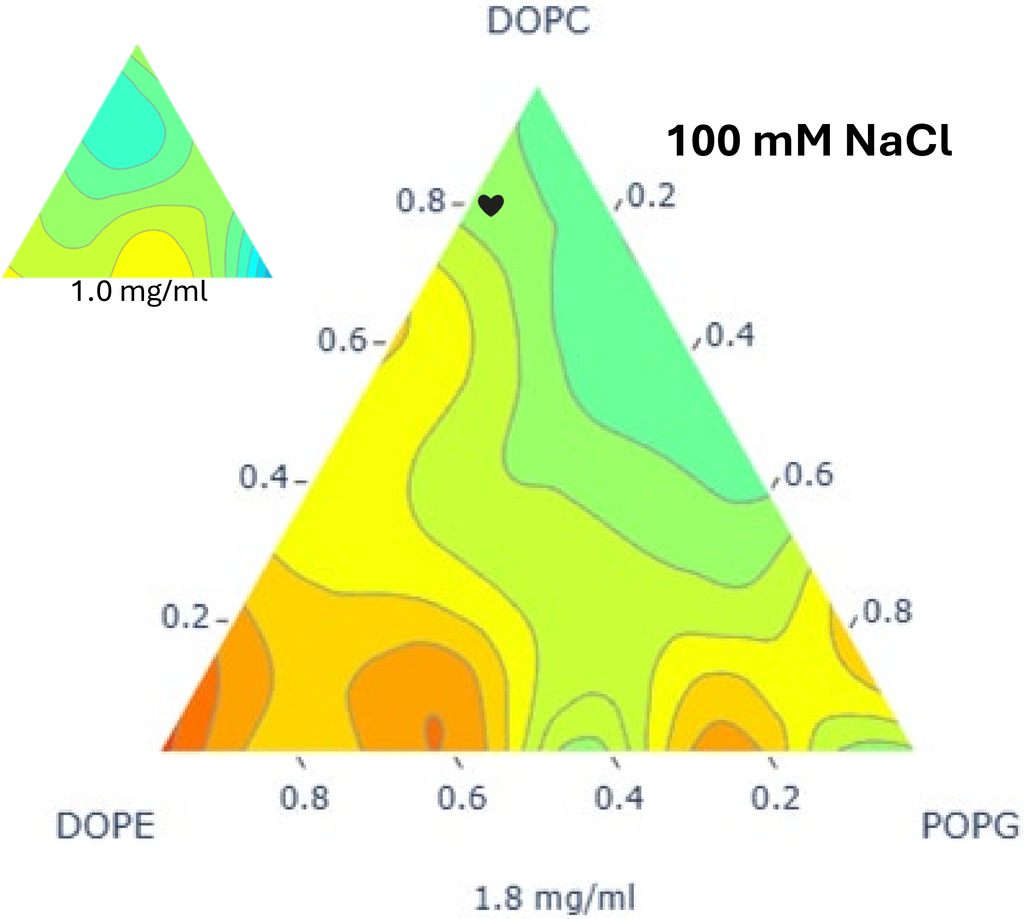


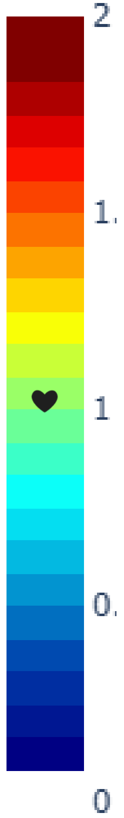
DOPC seems unaffected by extra salt.

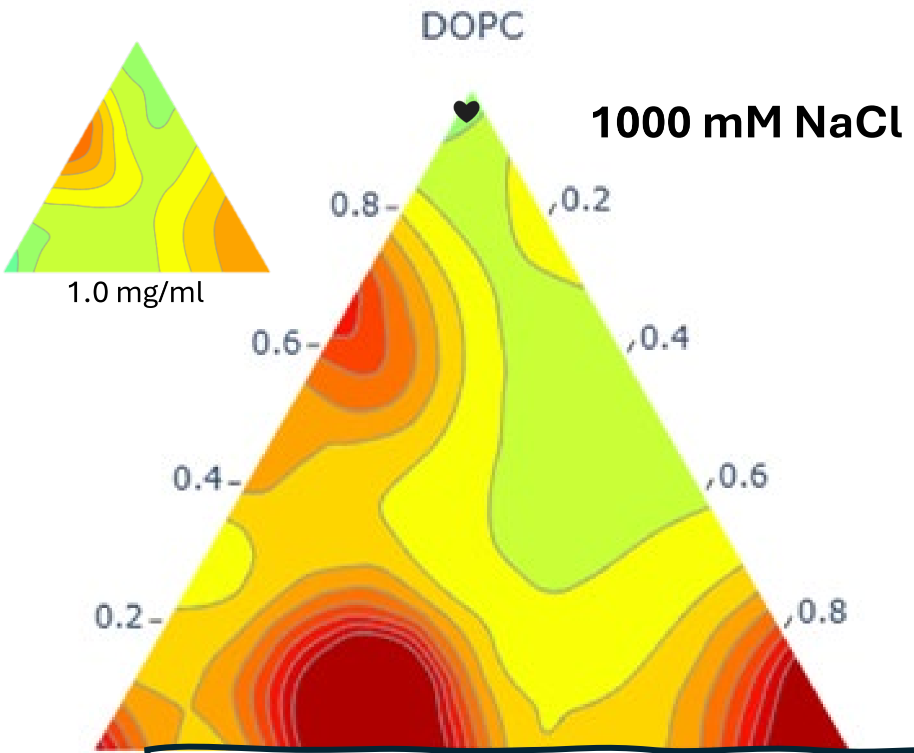
POPG is affected A LOT.

A Value of 1 represents a complete bilayer (look for the hearts!)

POPG is even worse at 1.8 mg/ml total lipid concentration!







What about those other big red dots? What is DOPE making over-complete bilayers?

A Value of 1 represents a complete bilayer (look for the hearts!)

How sure are we of predicted data?

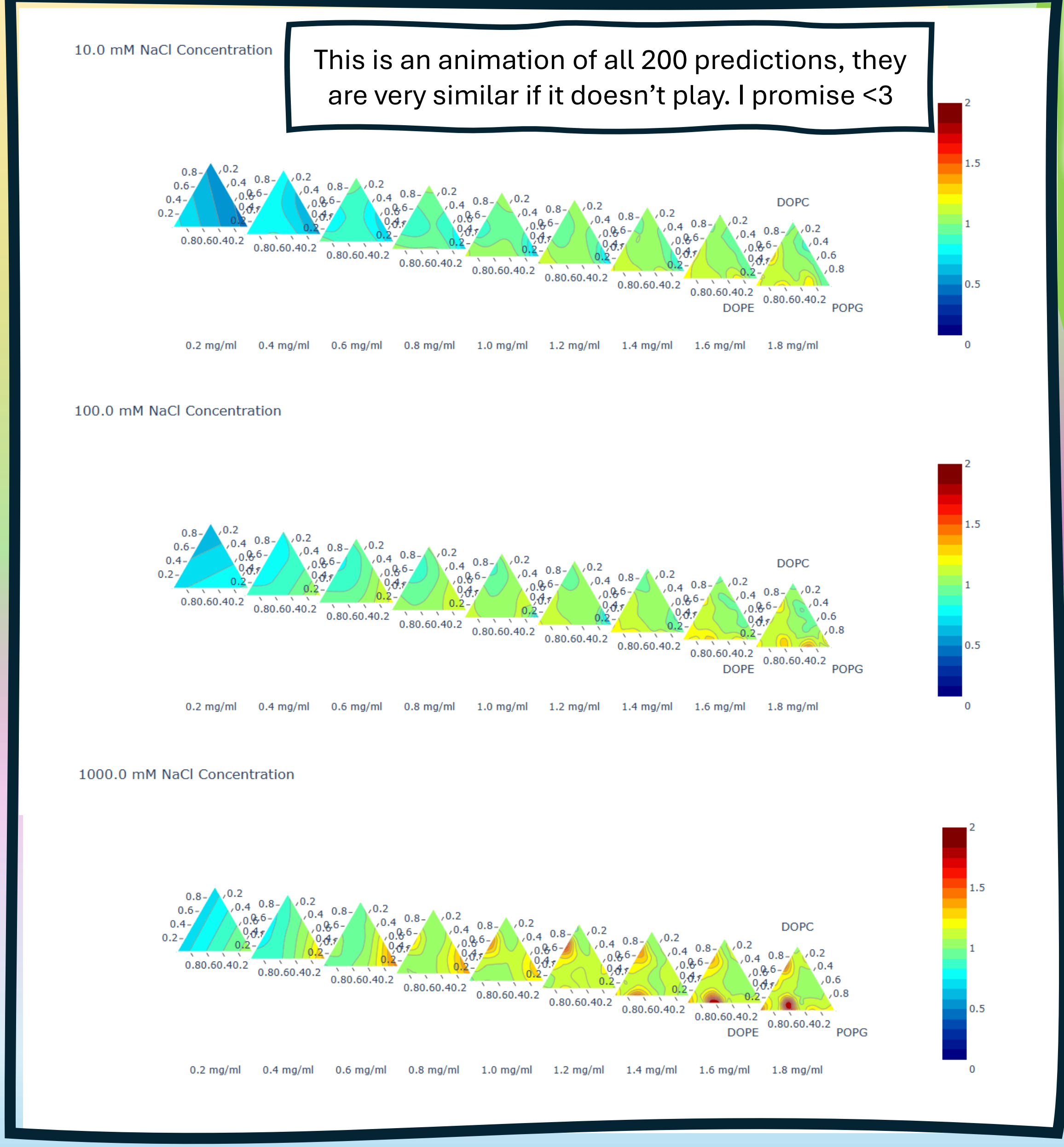
Key Questions

What does the prediction look like if some data points are missing?

How robust is the prediction against exclusion of different data points?

Bootstrapping

Sample with replacement
Ran prediction script 200 times



This is an animation of all 200 predictions, they are very similar if it doesn't play. I promise <3

We calculated a variance and standard deviation for each point in the predicted space.

On the left is:

10 mM NaCl solvent (top)

100 mM NaCl (middle)

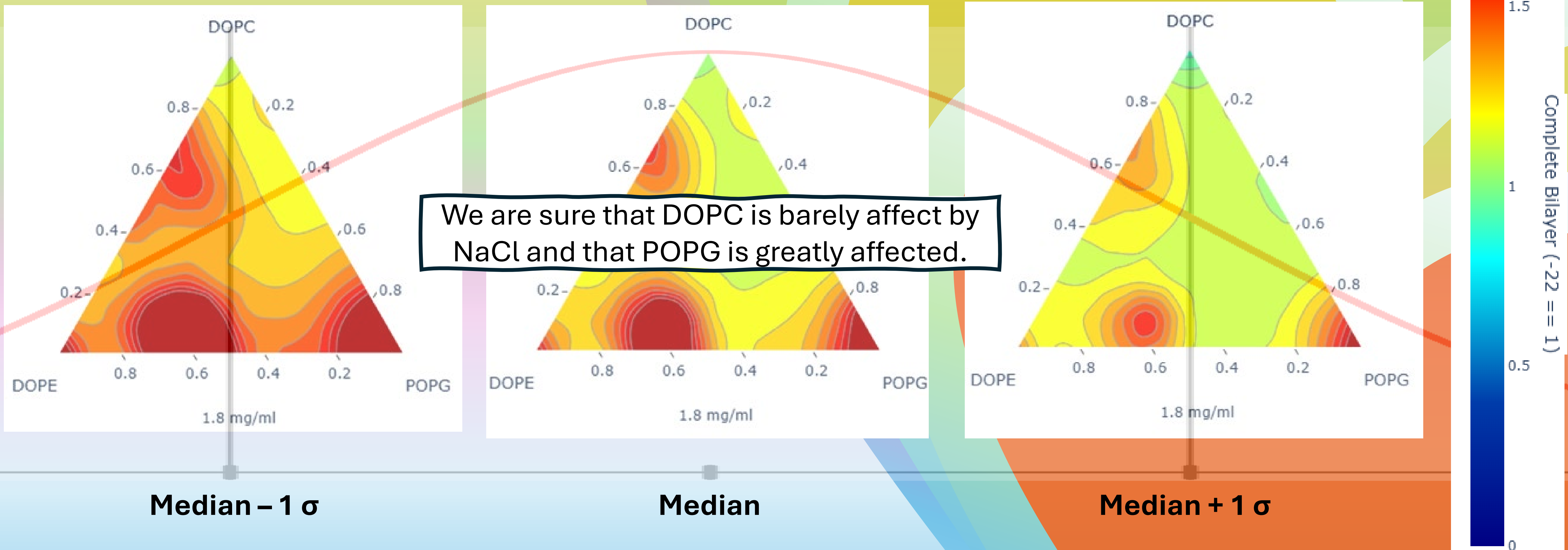
1000 mM NaCl (bottom)

How sure are we of predicted data?

Bootstrapping

Using the standard deviation of each point in space to show the 68% confidence interval.

1000 mM NaCl



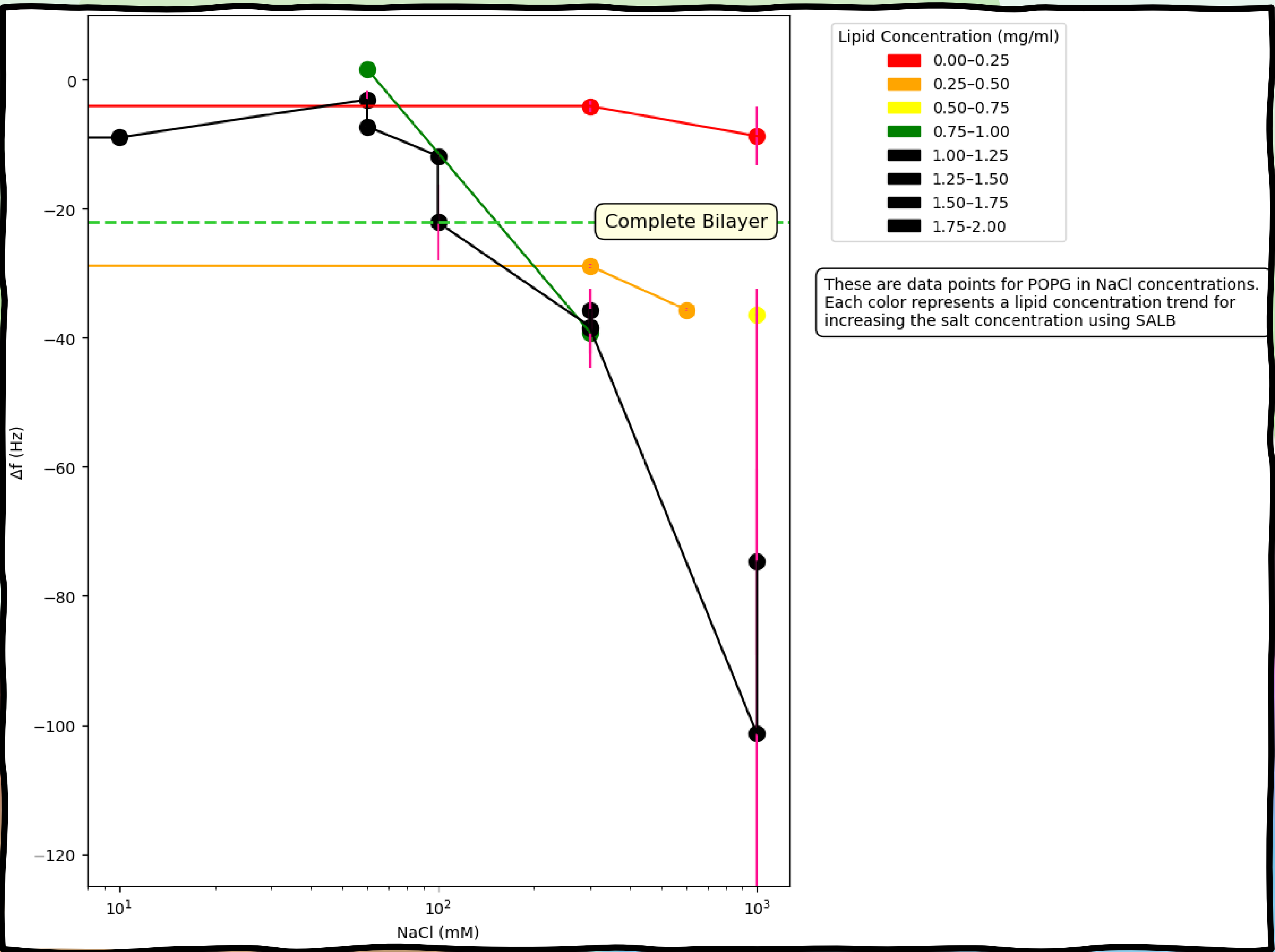
We are sure that DOPC is barely affect by NaCl and that POPG is greatly affected.

Median – 1 σ

Median

Median + 1 σ

Future Work



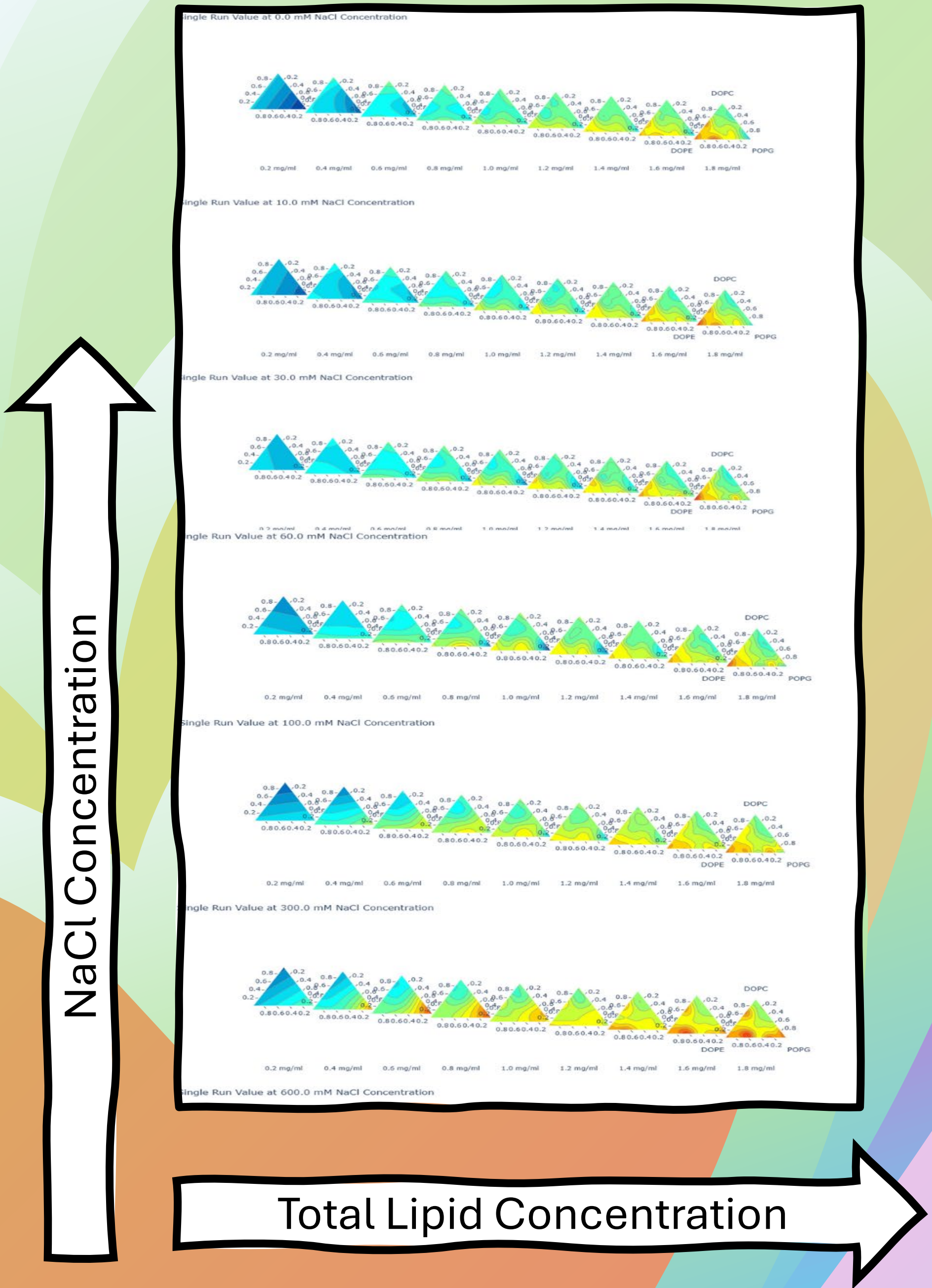
These are data points for POPG in NaCl concentrations. Each color represents a lipid concentration trend for increasing the salt concentration using SALB

This data is only POPG and NaCl

From possible DOPC/DOPE/POPG compositions

ACTUAL MEASUREMENTS

We see that when POPG > 1 mg/ml, bilayer aggregation increases



What if we predicted the space of multiple anionic lipids similar to POPG?

like POPS and POPA

Is there more change vertically (NaCl influence) or horizontally (lipid influence)

OR!

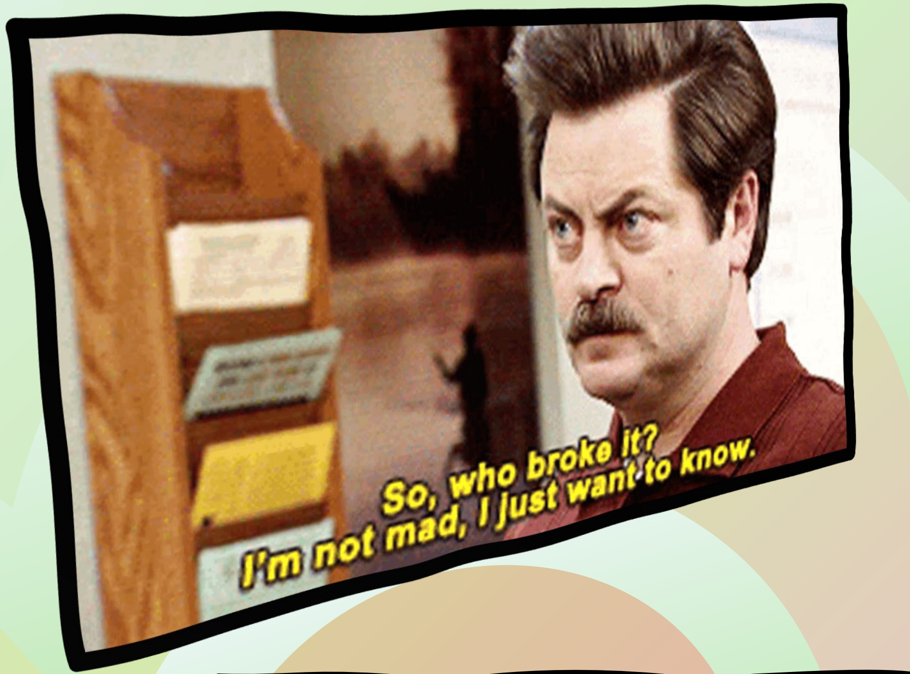
Is there a physics-based (vertical) or conformation-based (horizontal) influence

i.e. **a really quick and scalable way to explore molecular interactions**

:ERRO:

.rSr

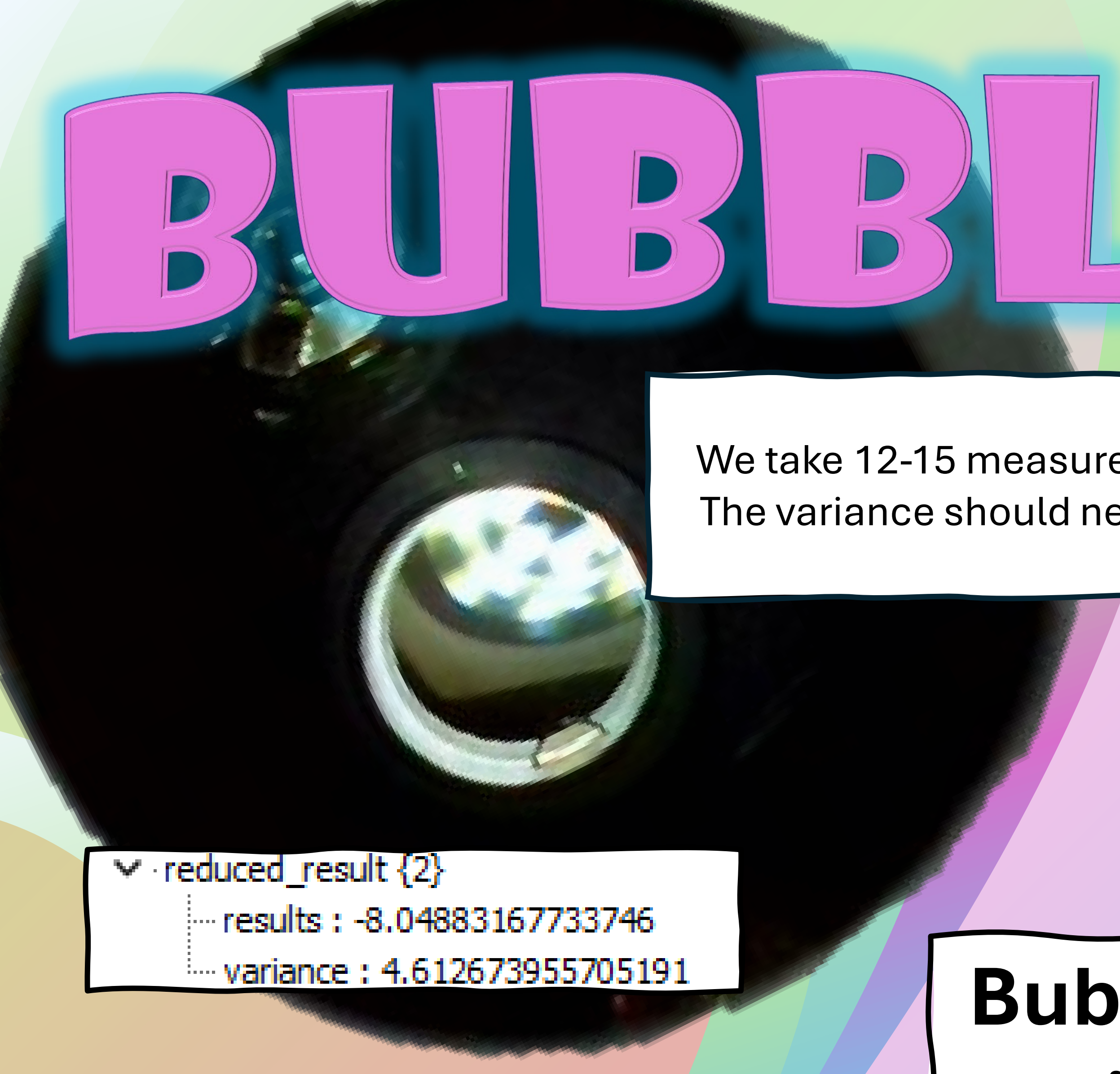
```
7092025-152754(DOPC lipid binding.v2.day2)', 'na
ate': '2025-07-09T19:32:06.546', 'resultGUID': '7a3
D_Dir  e': 'Run Mode', 'SOURCE_ZONE': 'Sample Zone', 'SO
: 'False', 'I  'SECOND INJECTION VOLUME': '0', 'AIR GAP': '0', 'INJ
UBBLE_STEP_  5', 'OUTSIDE_RINSE_VOLUME': '0.5'}], 'sampleTracking
e'}, 'sampleTra  ne': 'Air', 'well': '', 'trackEvent': 'Aspirate Air', 'content': 'A
'Aspirate Rese  servoir', 'volume': '0.502', 'currentUnitForVolume': 'mL', 's
servoir', 'volum  lume': 'mL', 'syringe': '642H1N079:A', 'orderID': 4}, {'iteratio
tUnitForVo  'orderID': 5}, {'iteration': '1', 'zone': 'Sample Zone', 'v
derID': 6}, {'iteration': '1', 'zone': 'Air', 'well': '', 'trac
9:A', 'orderID': 7}, {'iteration': '1', 'zone': 'Injection Z
'trackEvent': 'Aspirate Reservoir',
'trackEvent': 'Dispense',
'volume': '0.0375', 'curre
e': '642H1N079:A', 'order
on Zone', 'well': '1', 'track
```

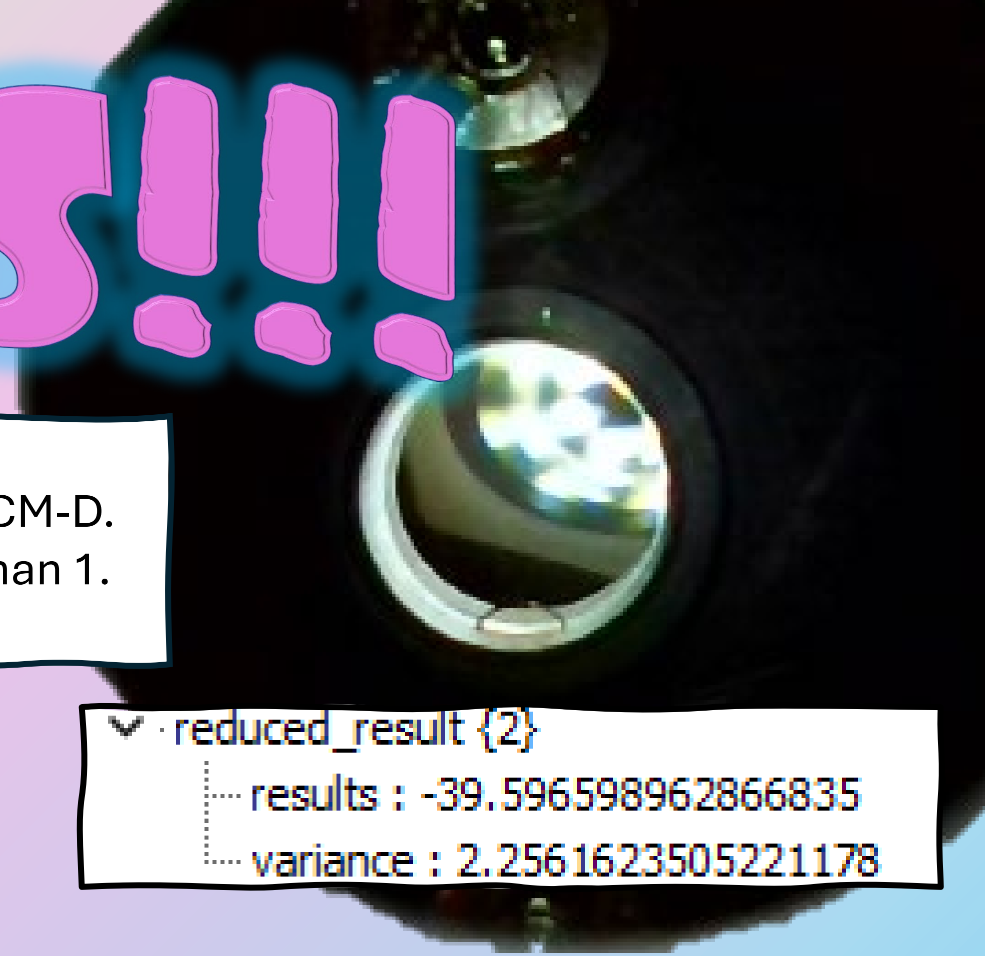


Not actually me, but....

The liquid handler had a recurring valve error that needs to be resolved before optimal use of ROADBot

BUBBLES!!!





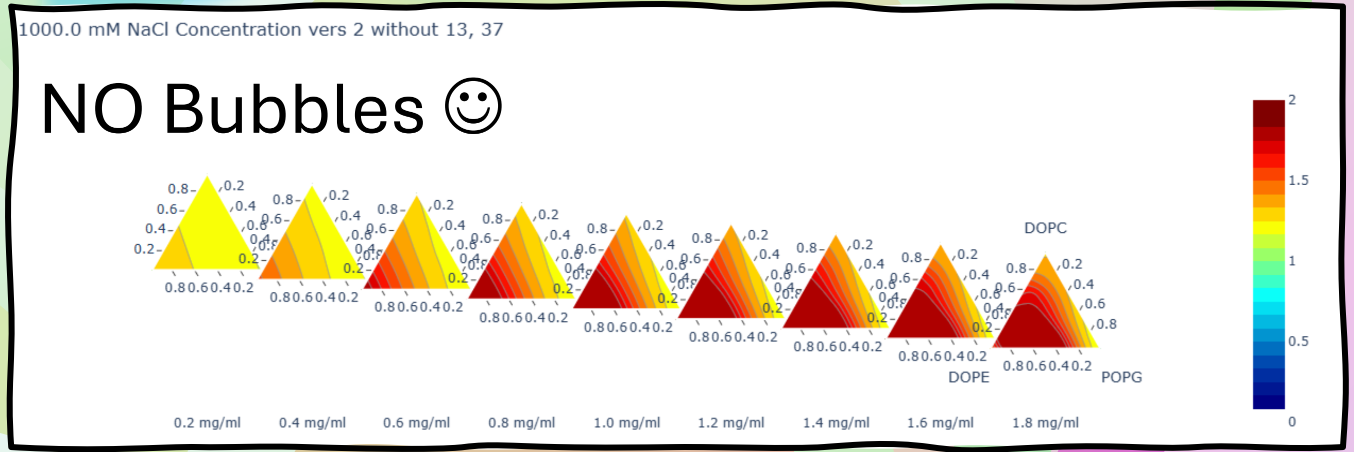
We take 12-15 measurements in the QCM-D.
The variance should never be greater than 1.

```
reduced_result {2}
    results : -39.596598962866835
    variance : 2.2561623505221178
```

```
reduced_result {2}
    results : -8.04883167733746
    variance : 4.612673955705191
```

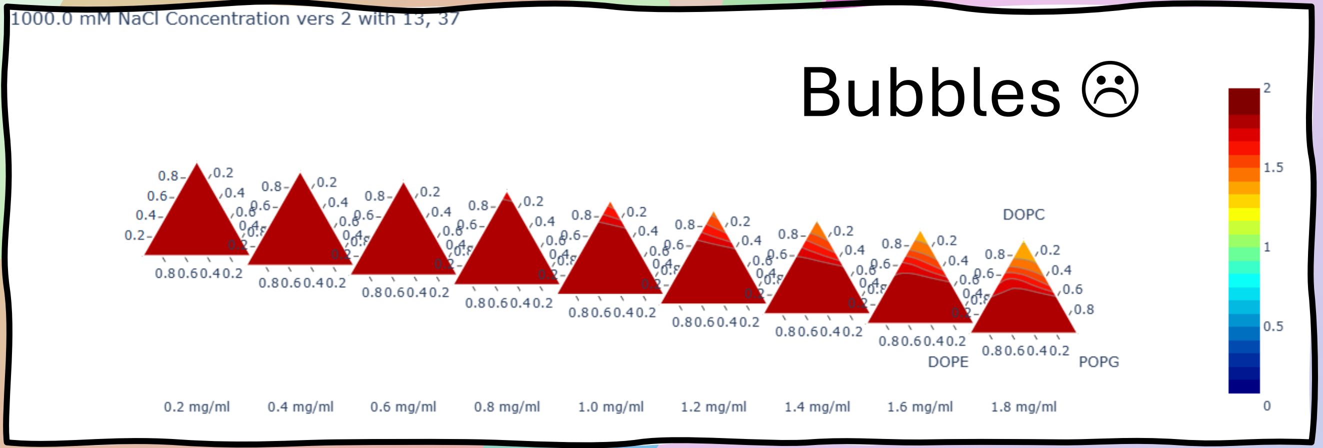
Bubbles in the QCM-D introduce error

BUBBLES!!!



1000.0 mM NaCl Concentration vers 2 without 13, 37

NO Bubbles ☺

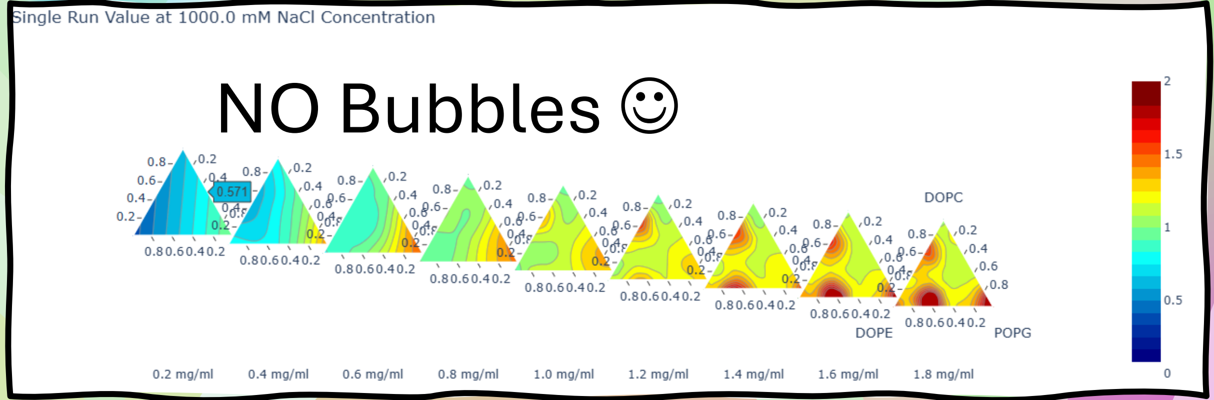


1000.0 mM NaCl Concentration vers 2 with 13, 37

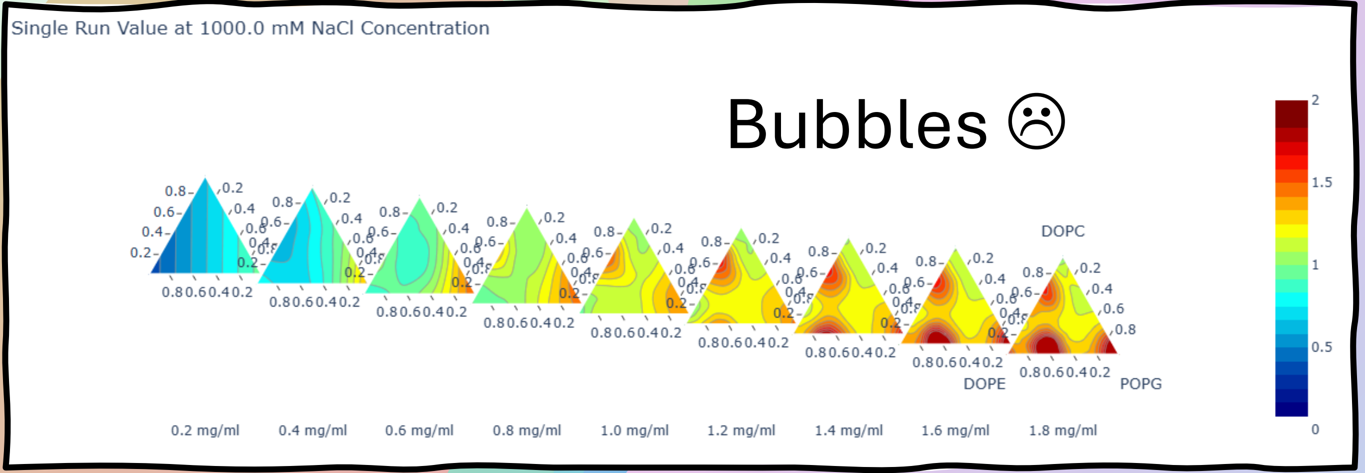
Bubbles ☹

On a smaller data set (37), bubbles can drastically change the prediction of the phase space

BUBBLES!!!



NO Bubbles ☺



Bubbles ☹

On a bigger data set (170), bubbles can change the prediction of the phase space, but less likely

As for what I learned at NIST

A lot about lipids

An appreciation for the mathematical rigor that foundations AI

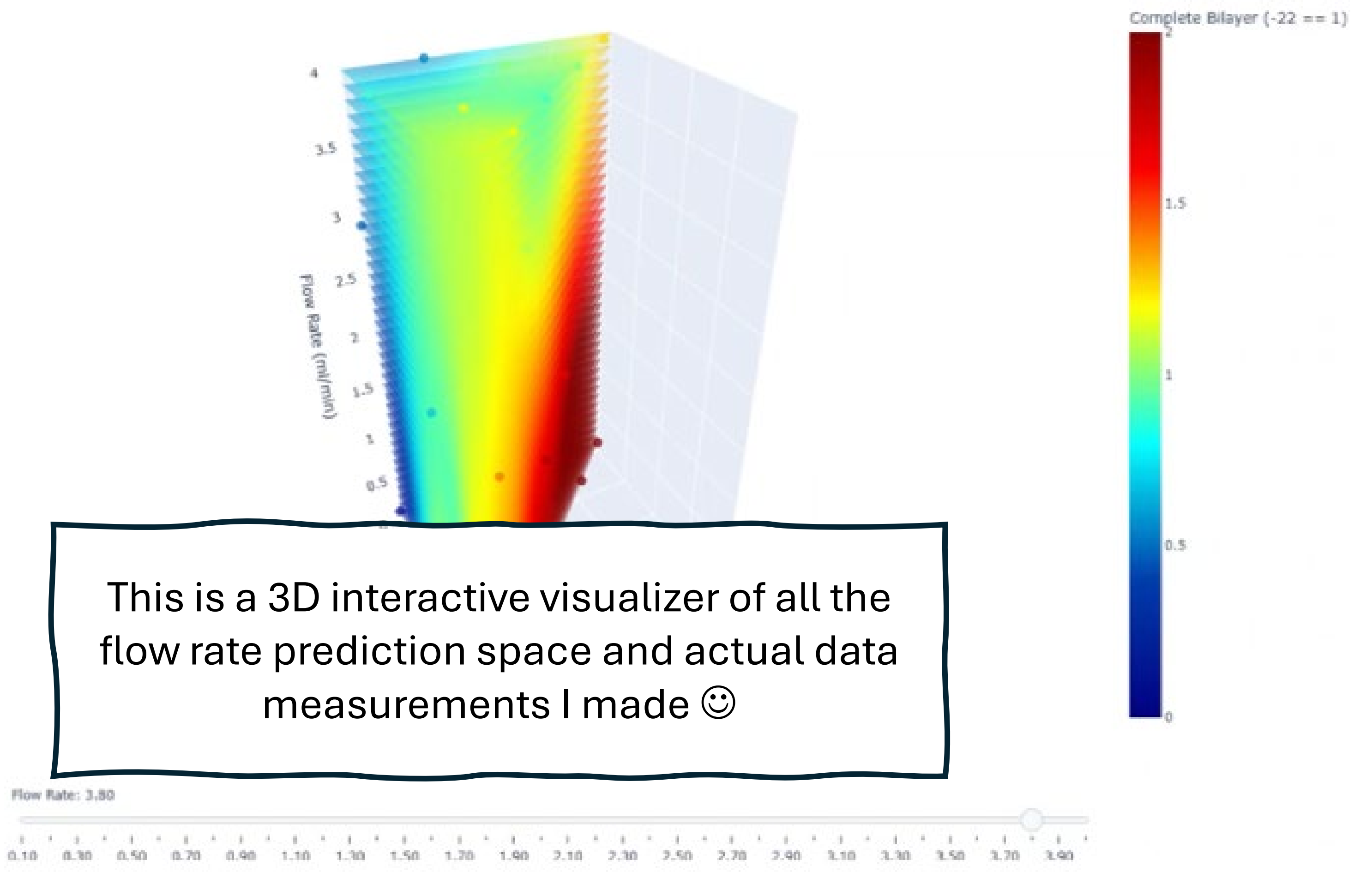
I don’t always use `if i == variable:`

Sometimes I use `i in [list]`

As for what I learned at NIST

But mostly

And finally learned to program



This is a 3D interactive visualizer of all the flow rate prediction space and actual data measurements I made ☺

I learned that neutrons are really cool...

...and I want to learn more

Thank you

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