# Investigations into Phase Separated Lipid Bilayer Dynamics and Transitions Using STORM

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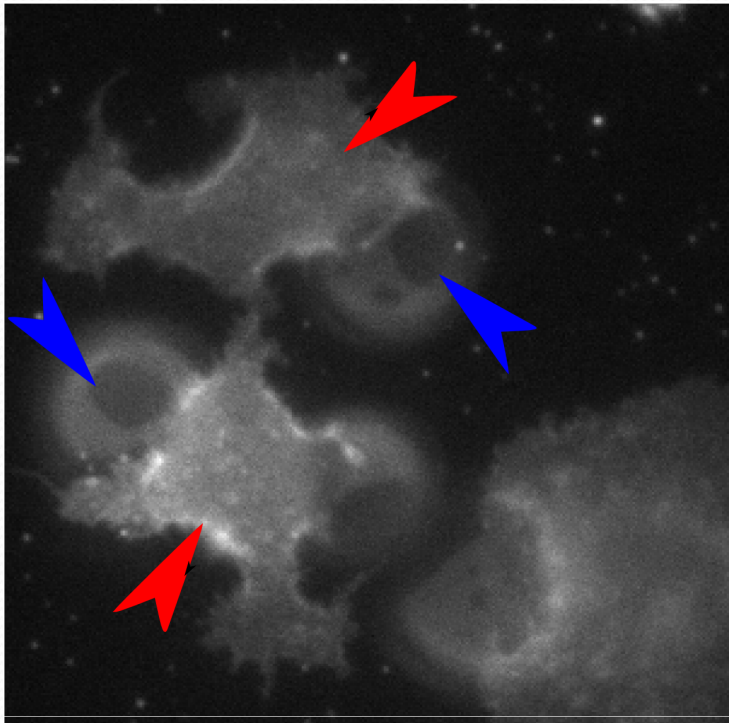
# Overview

- Cells are thought to have domains- areas of similar lipid composition and that these are important to membrane function
- This model, however, is very complicated and hard to study
- Model membrane systems are easier to understand, and we use them to connect back up to the complex cellular systems

# Phase Separated Blebs

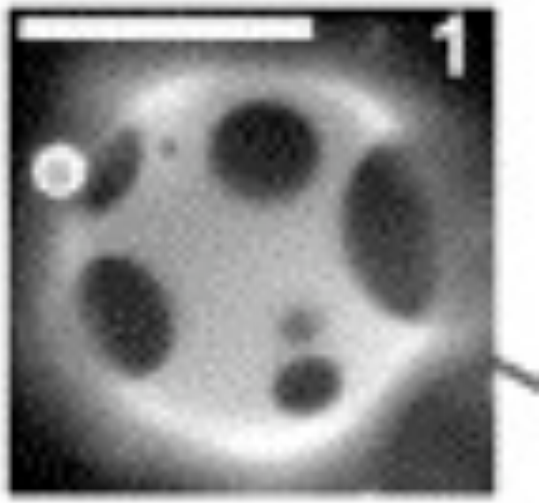
- Spherical membrane fragments harvested from cells, called blebs, are used as model systems for cellular membranes, since they contain all the components of real cellular membranes

Below: Red arrows point to cell, Blue point blebs budding from them. From Sarah Veatch



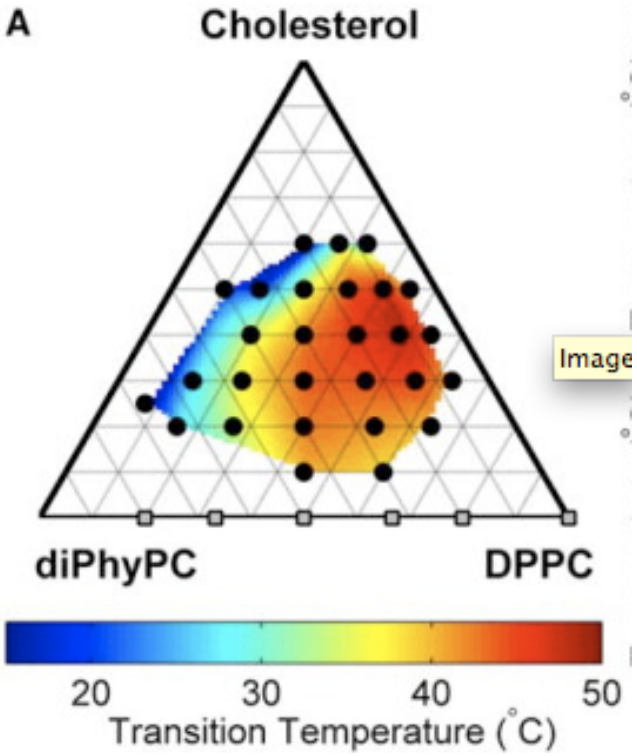
# A Simpler Model

- Instead of using blebs, whose composition cant really be controlled, I use artificial vesicles of predetermined lipid mixtures
- We use a low chain melting temperature lipid Di-Phytanol-PC, a high temp one DPPC, and cholesterol, which is an approximation of the many different lipids in the membrane
- Vesicles share many of the characteristics of blebs, such as separated domains and transitions



# Domains in Vesicles

- Vesicles of certain mixtures show large bright and dark domains that are two phases- ordered and disordered
- Different mixtures give different temperatures for transitions from one phase to two



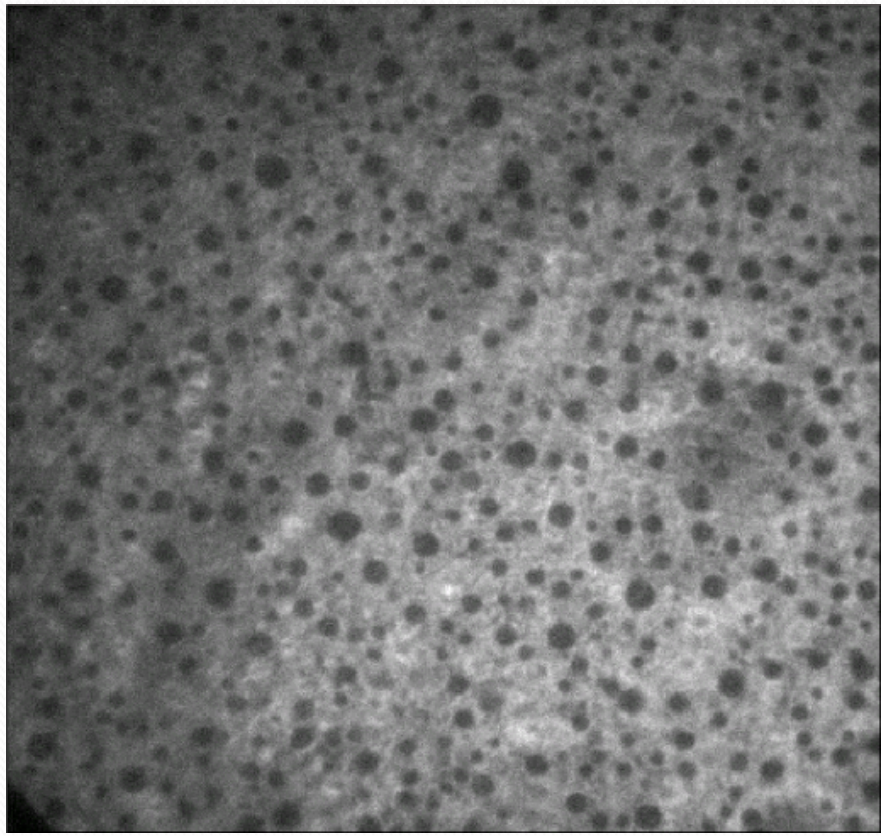

Sarah Veatch

# Issues with Vesicles

- The  dynamics of the components of vesicles and blebs are difficult to study because they are spherical
- We want to know how things behave in these model membranes so that we can try to correlate them with things we see in cellular membranes, so we would like to be able to image them with single particle tracking
- To do this, I developed a method for creating supported planar bilayers with large phase separated domains

# Phase Separated Bi-layers

- Lipids deposited onto a glass slide coated in a very thin layer of agarose (to decouple the lipids from the glass) form bilayers that show the characteristics of phase separated blebs and vesicles
- They have large bright and dark domains that respond to temperature

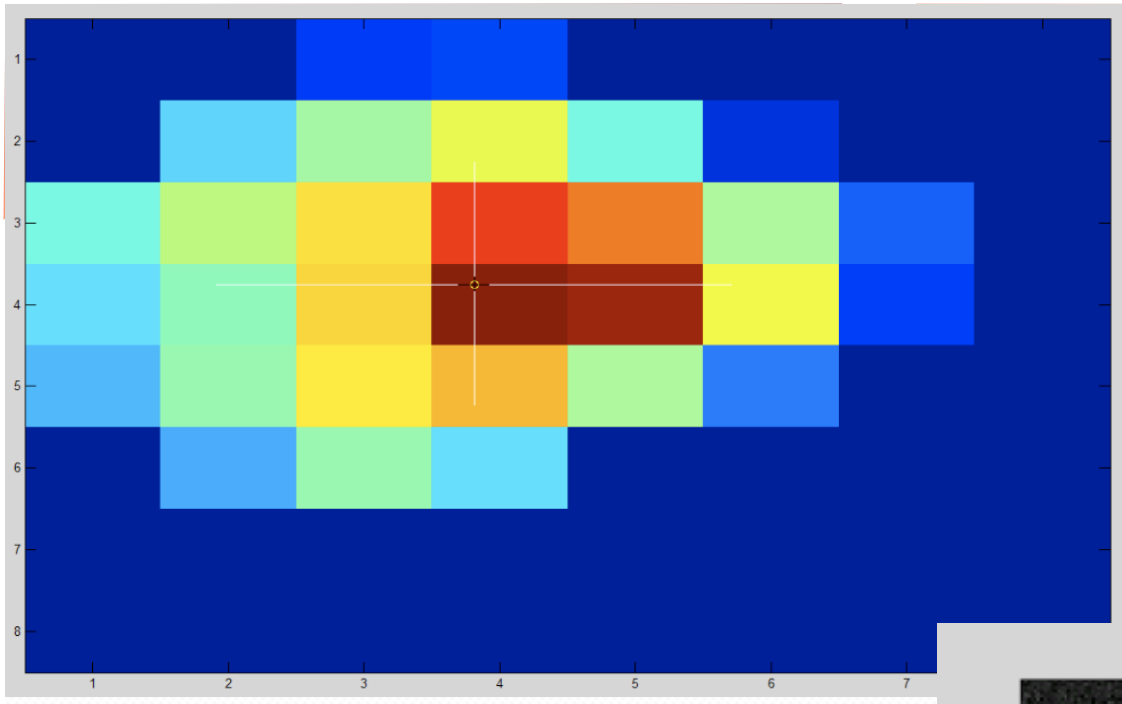


# What's So Great About This?

- We can use this system to investigate physical features such as diffusion of particles in various domains, molecule confinement to domains, and how temperature varies these things using single particle tracking techniques

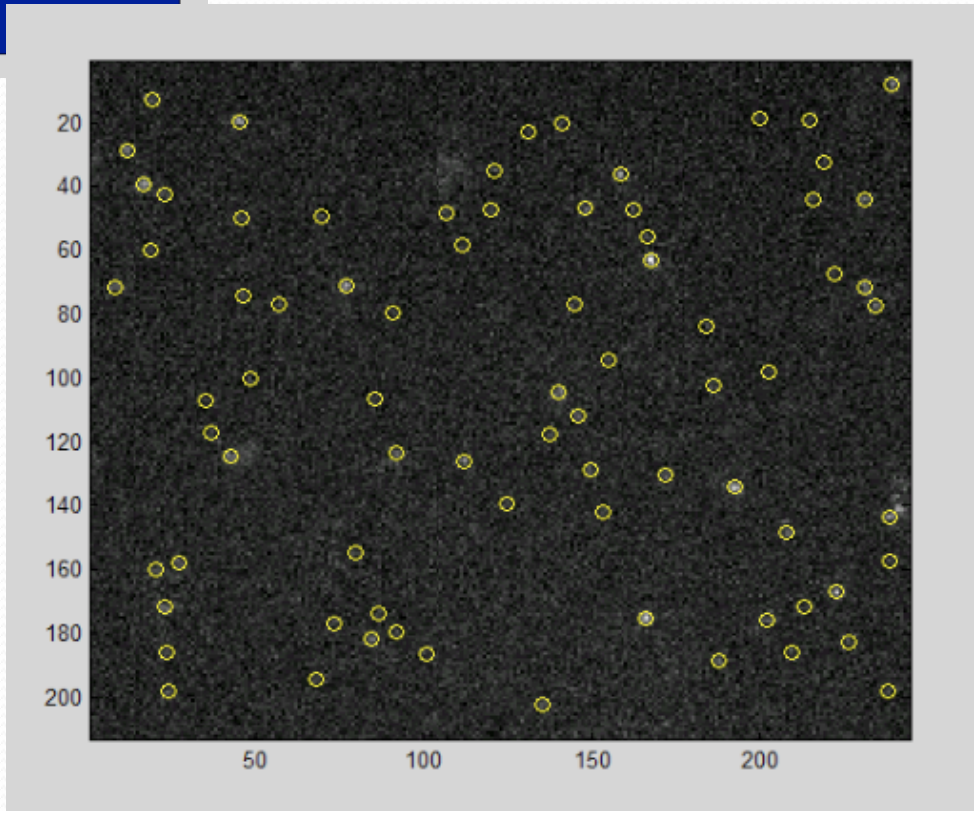
# Single Particle Tracking- Intro to STORM

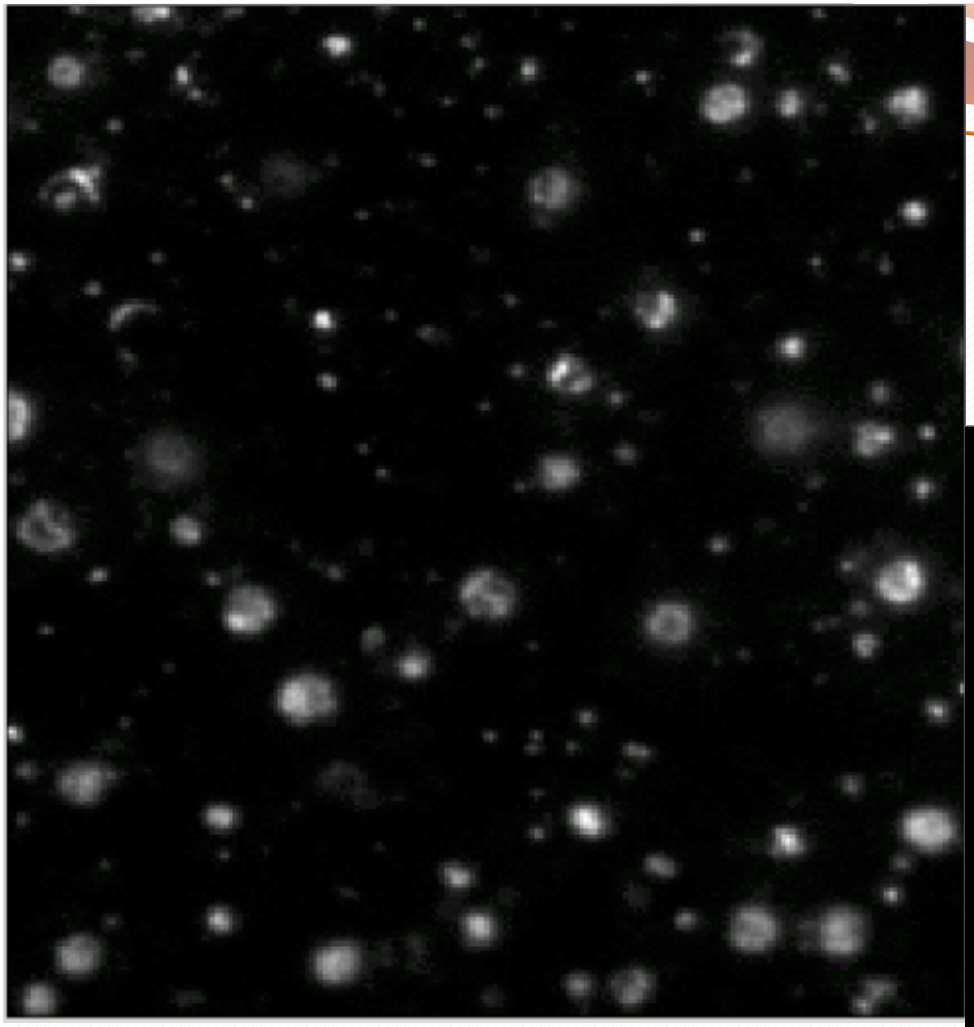
- The diffraction limit of light restricts what we can see to about 200nm at most, making it difficult to observe how labeled molecules or proteins diffuse around
- We get around this issue by having only a few probes on at any one time out of the thousands present. Using specific organic probes and buffers we can turn on only a small portion for a small amount of time
- A computer program then is able to pinpoint the probe and follow it as it goes around




Above: The program finds the center of a pixel intensity distribution to locate the probe

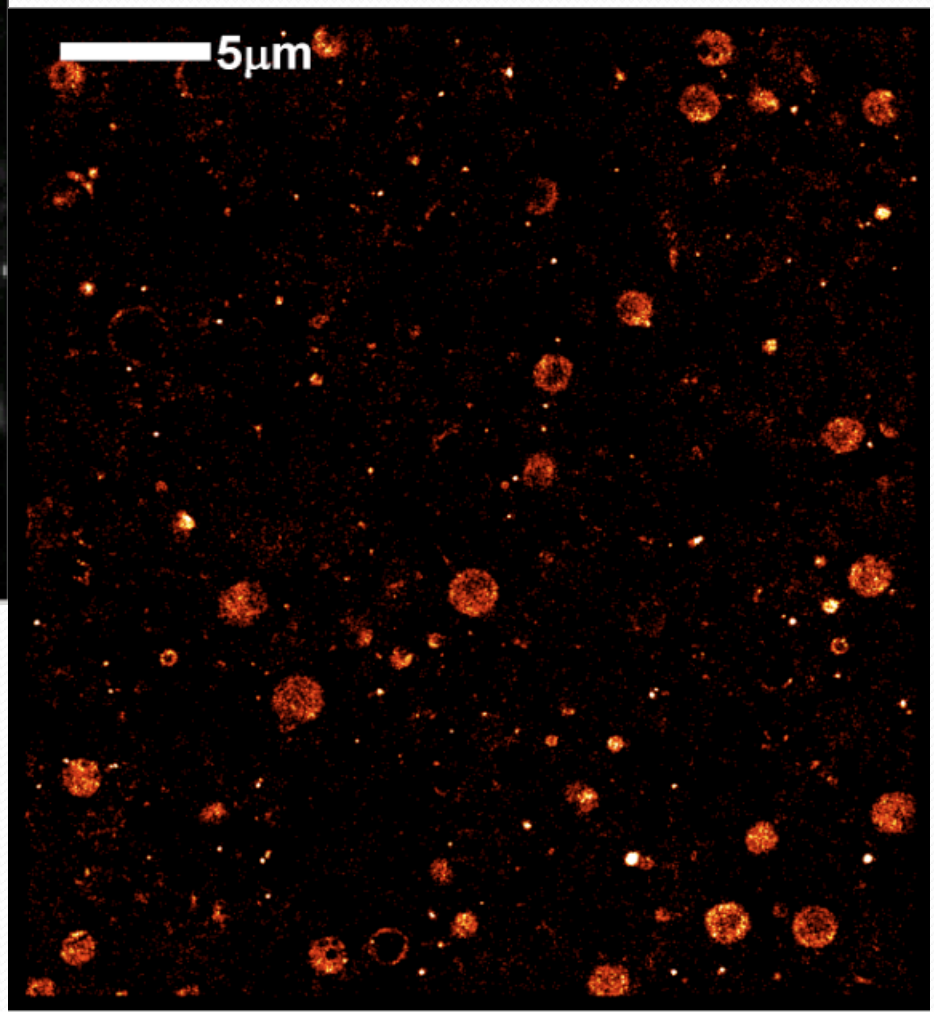
Right: It then does so for all the probes on in every frame





Picture of bi-layer with minority phase labeled

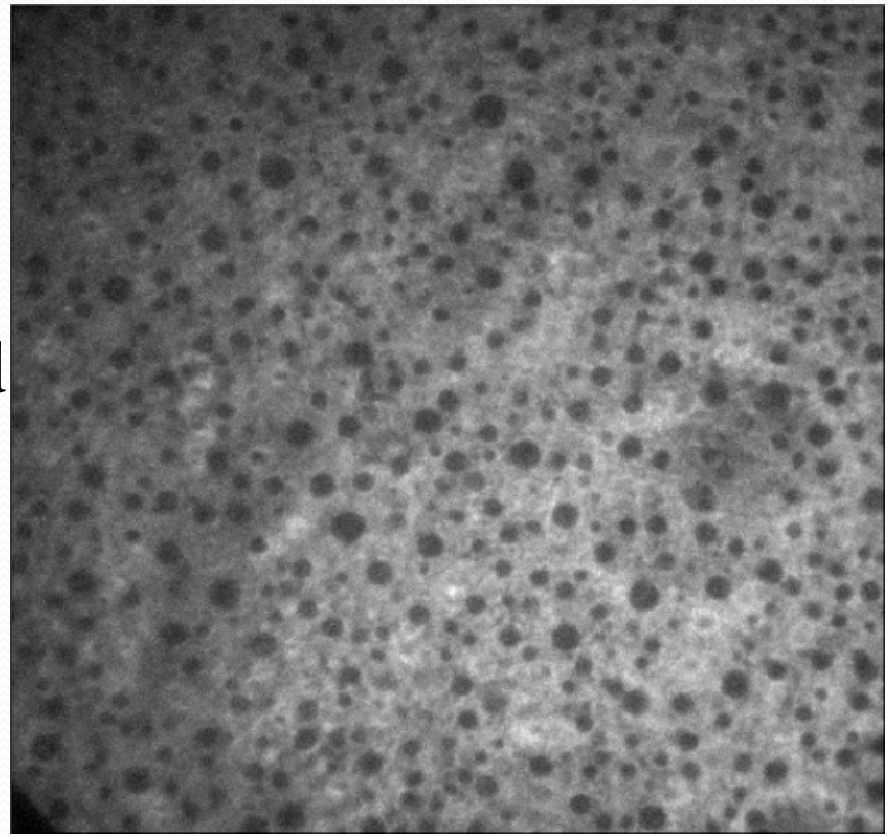
Single particle reconstruction of image using probe tracks

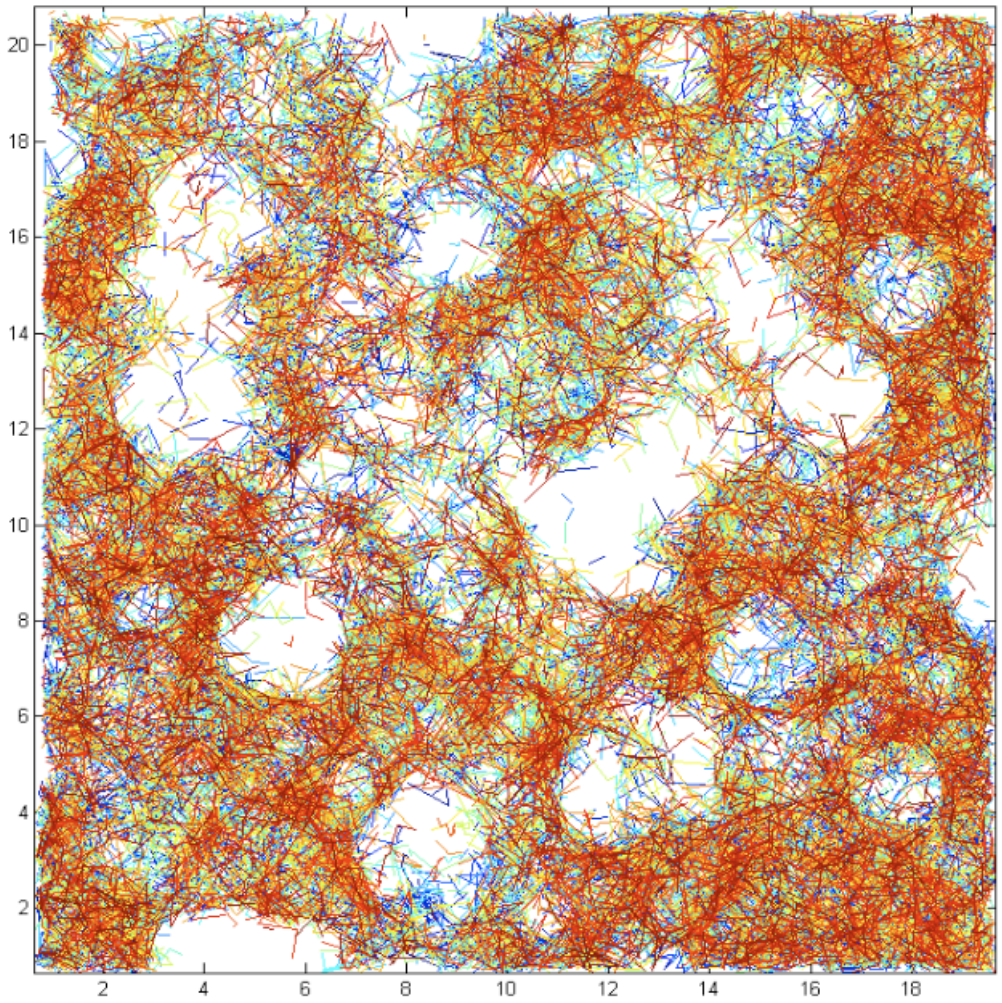


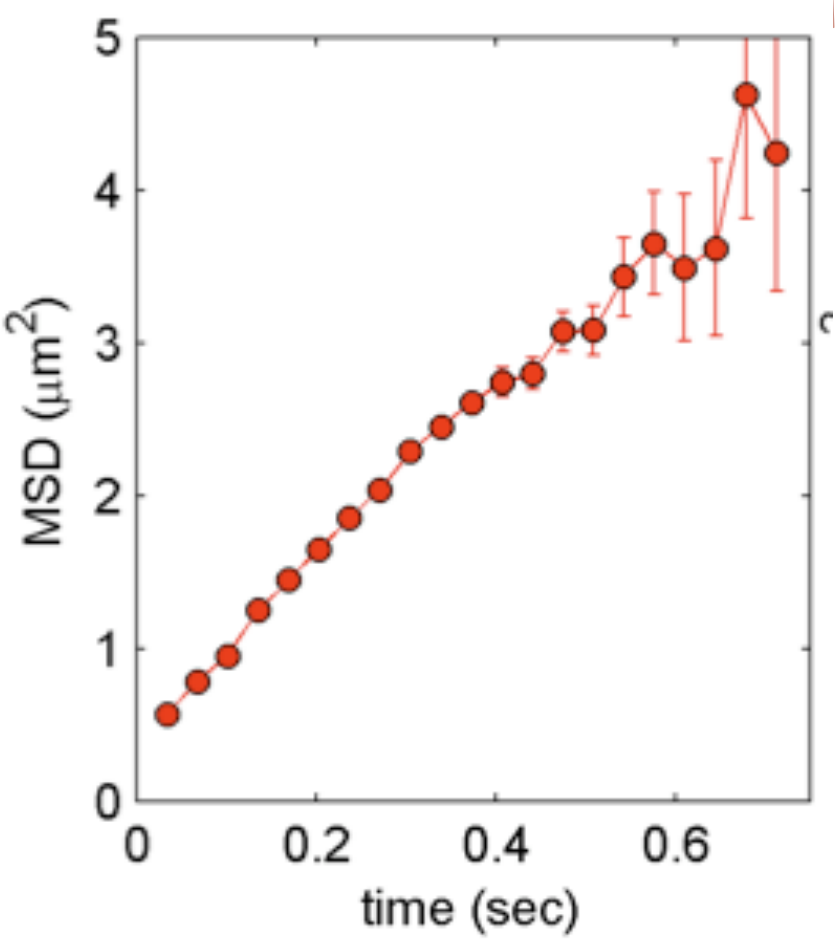
# Liquid Disordered

- The lipid mixture used was 2:1 Di-Phytanol-PC:DPPC plus 10% Cholesterol
- The disordered phase is the majority phase, here imaged with probes DiI or DiD, which preferentially partition into the disordered phase

Below: Image of 2:1 10% bi-layer with disordered phase labeled





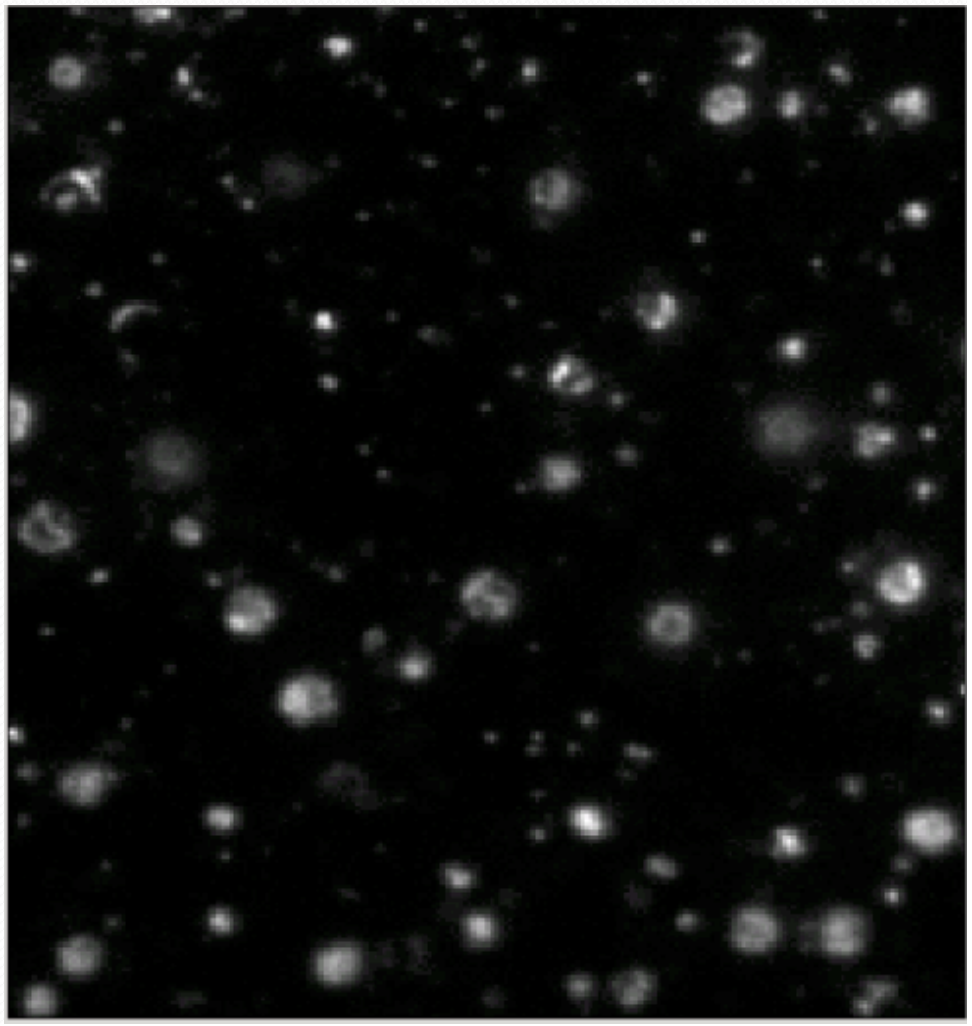



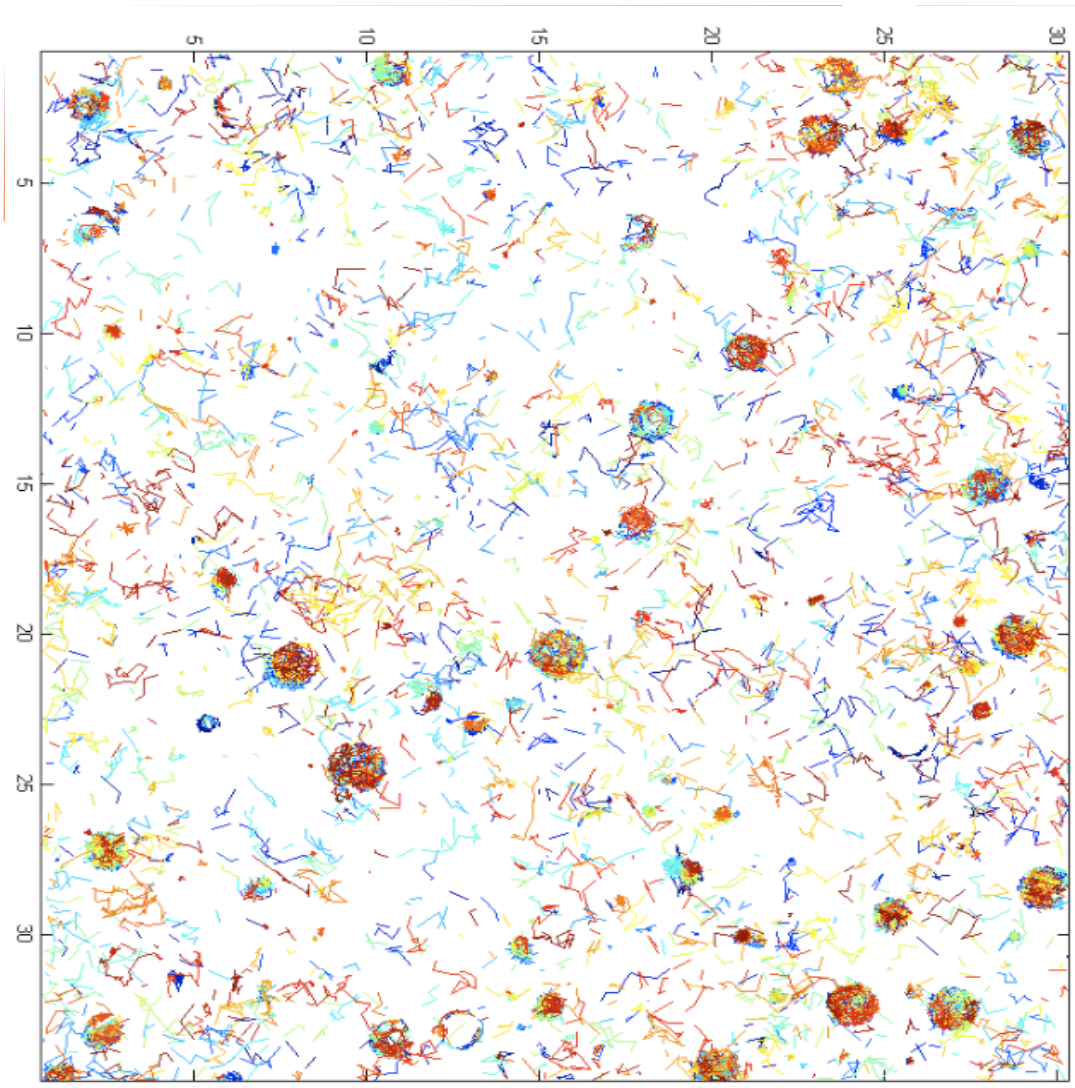

- Through STORM, we obtained thousands of single molecule tracks
- Tracked probes are mostly excluded from circular domains

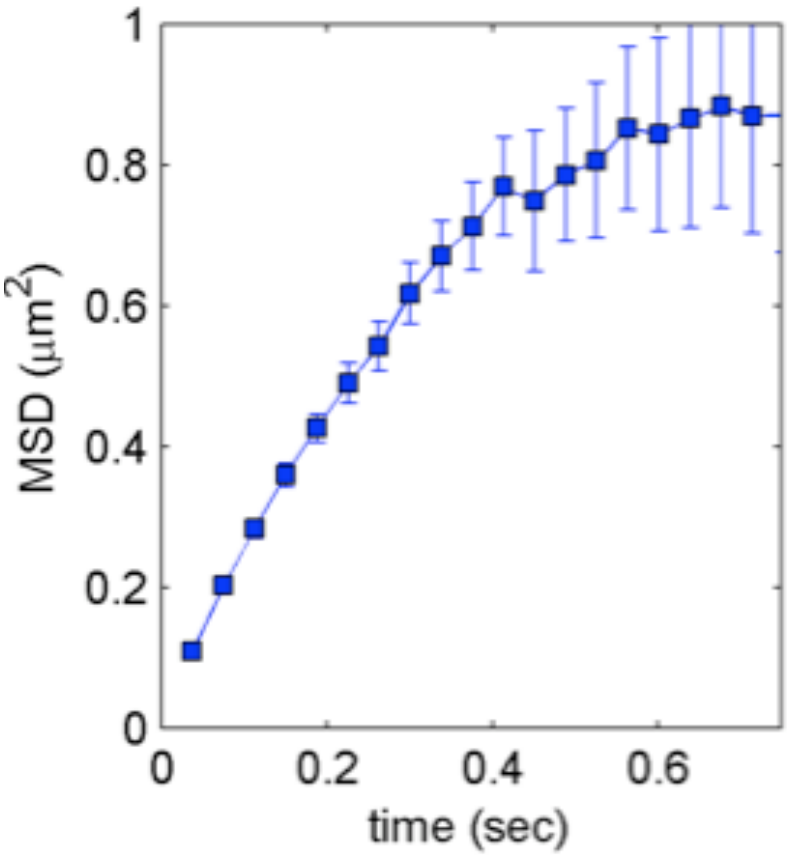
- Mean Squared Displacement is linear with time
- This means diffusion is not confined
- 1.5 $\mu^2$/sec diffusion constant

# Liquid Ordered and Phase Transition

- In our 2:1 10% mixture, the ordered phase is the minority circular phase, which makes it easier to see what happens when temperature is varied.






•Tracked probes are mostly included in circular domains

•Mean Squared Displacement is not linear with time

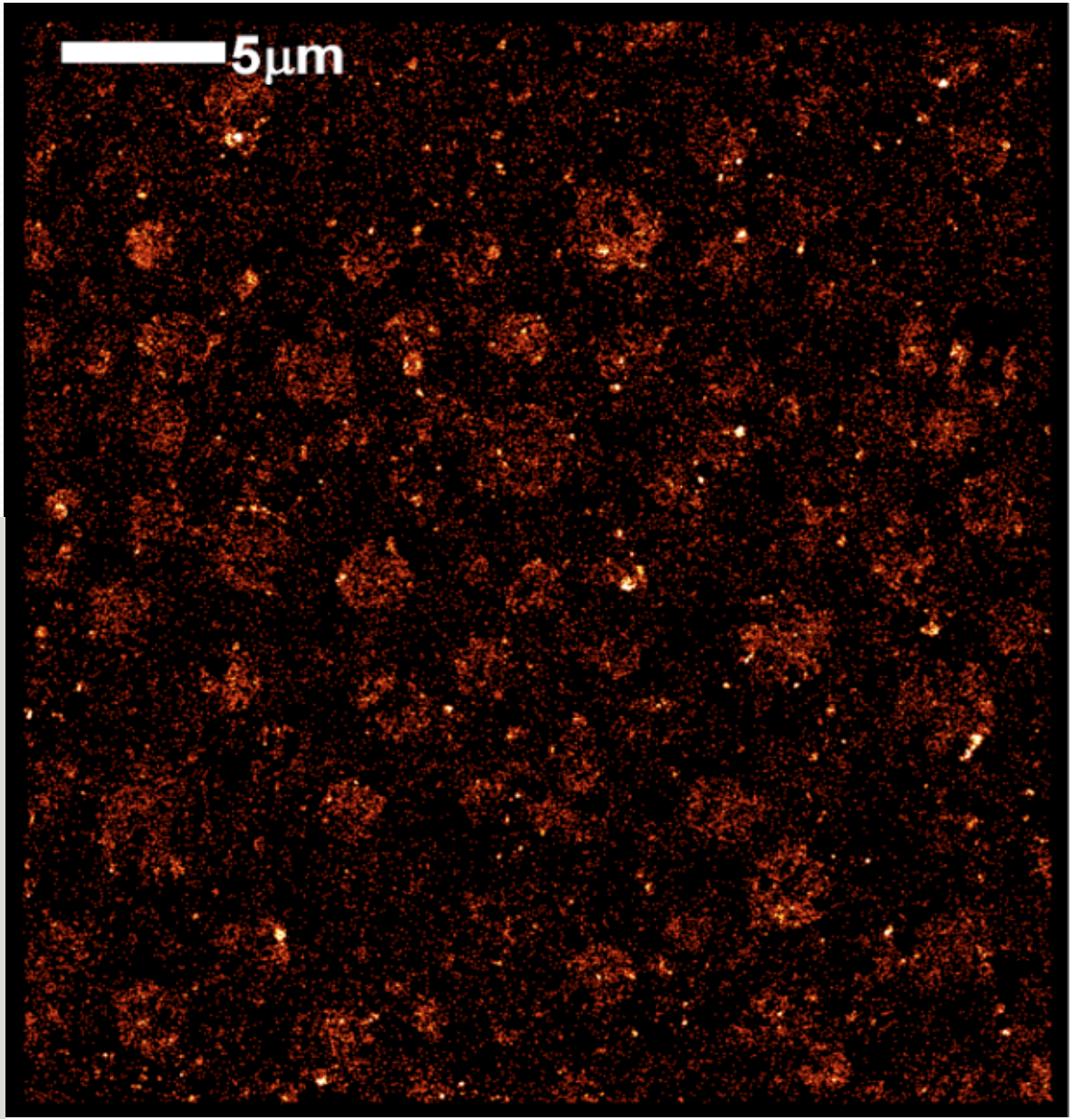
•This means diffusion is confined

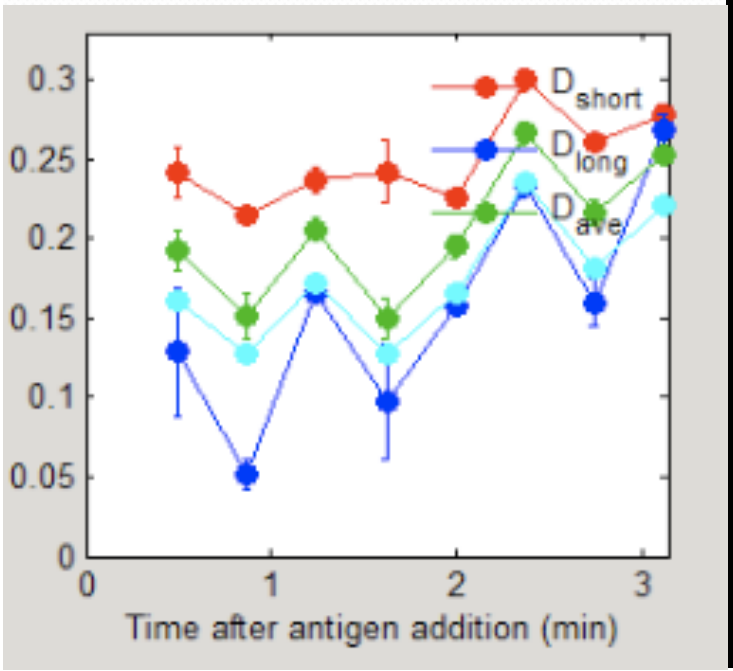
•.4 μ²/sec diffusion constant

# Temperature Series

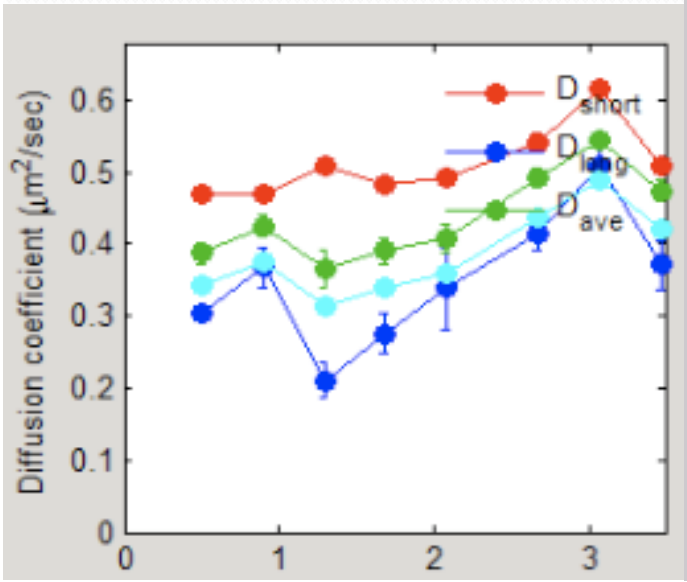
- The transition from a two phase system into a one phase at high enough temperatures is a defining characteristic of phase separated blebs and vesicles
- The bilayers also have this feature, which I looked at using STORM
- Would expect that as temperature increases towards the transition, domains would get smaller and diffusion constants would increase

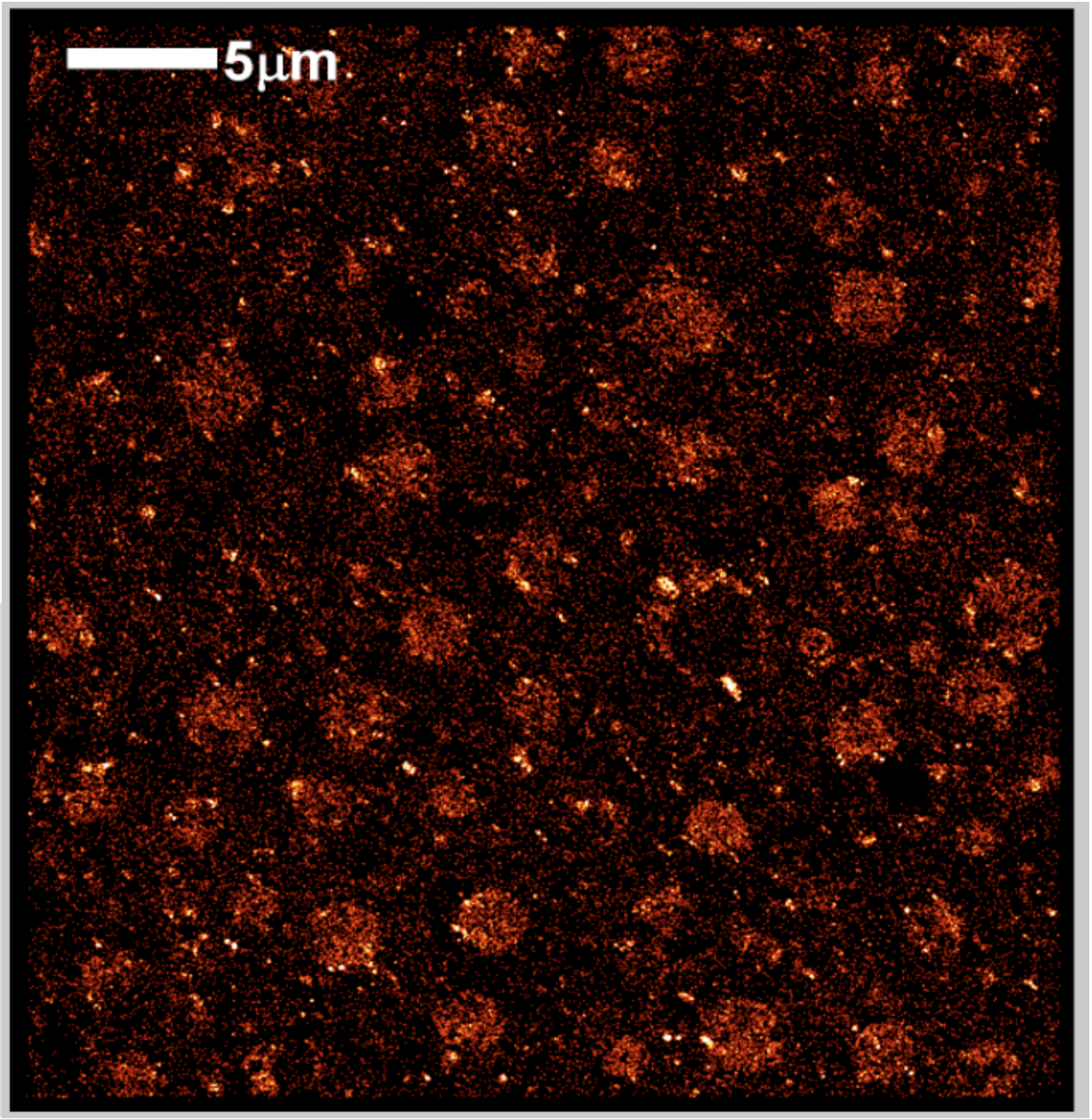
# 27° C


5μm

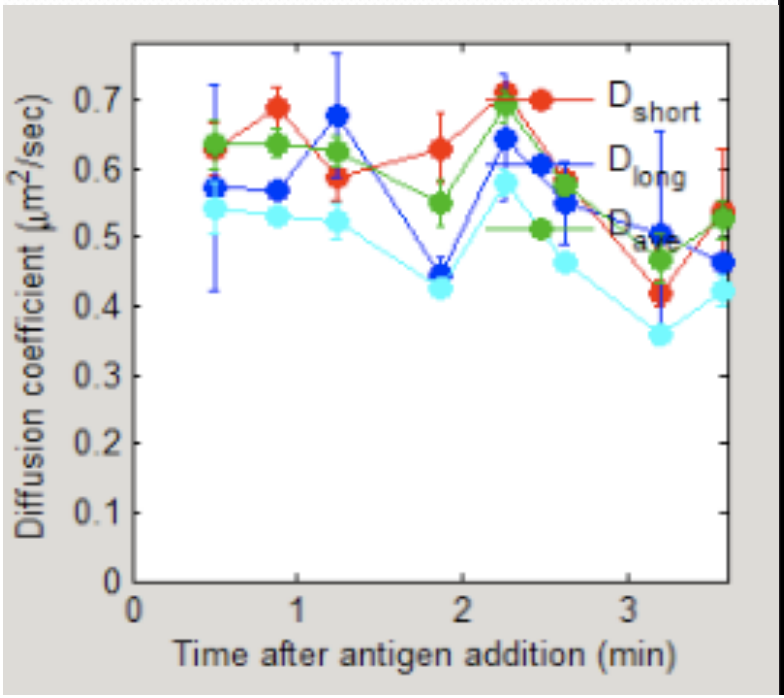

0.3
0.25
0.2
0.15
0.1
0.05
0
0
1
2
3
Time after antigen addition (min)
D short
D long
D ave

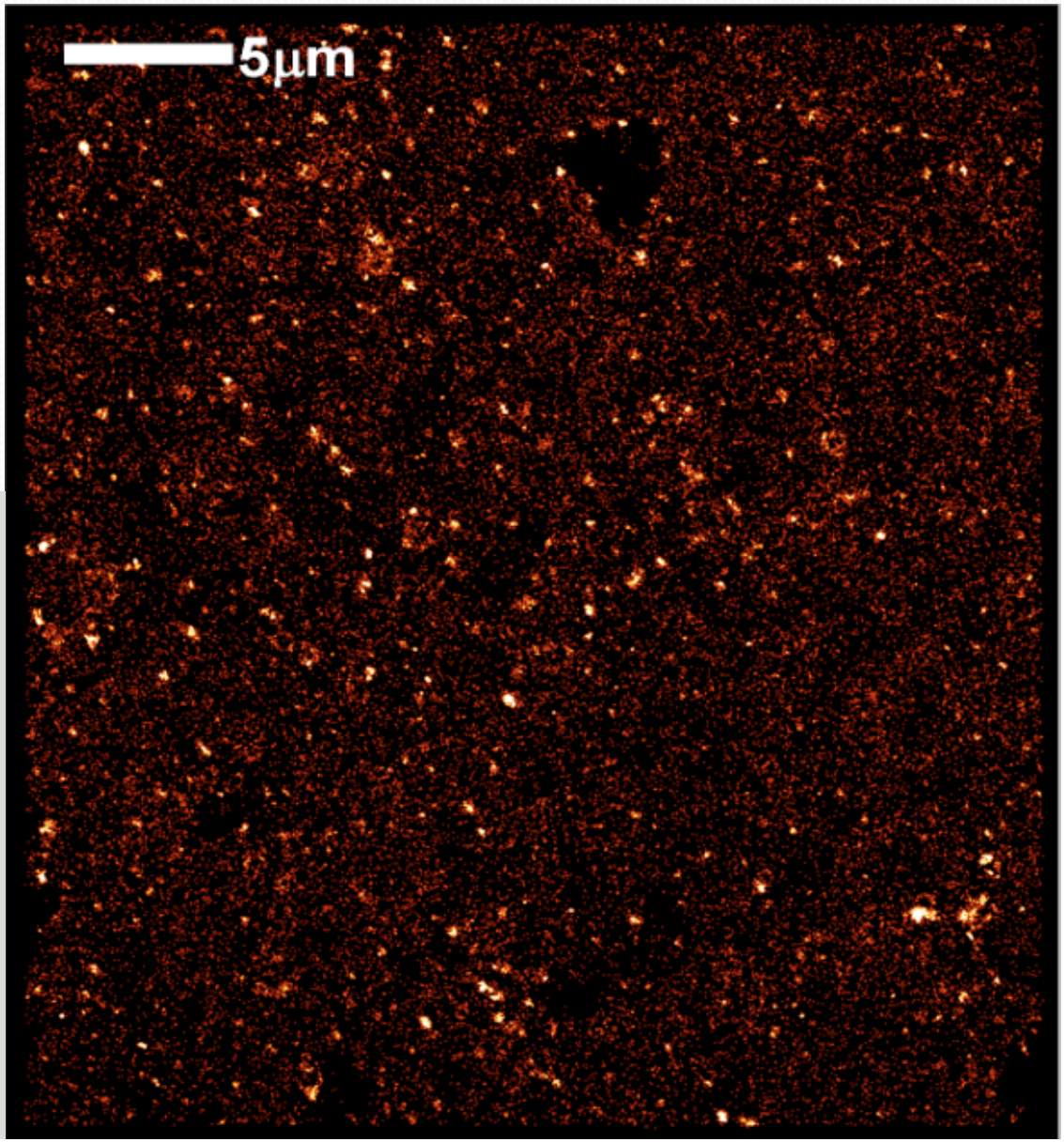
# 32° C


Diffusion coefficient (μm²/sec)
0
0.1
0.2
0.3
0.4
0.5
0.6
0
1
2
3
D short
D long
D ave


5μm

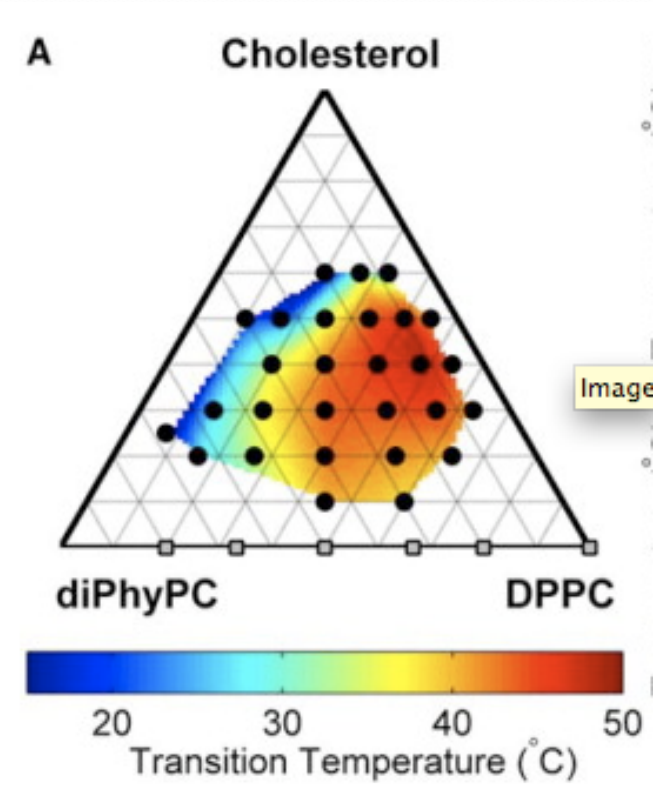
# 37° C


Diffusion coefficient (μm²/sec)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
Time after antigen addition (min)
D short
D long
D ave


5μm

# Onwards!

- In the future I'll go through the previous series with smaller temperature step sizes to get closer to imagining the transition as it happens
- I will also do all this with different ratio mixtures and probes
- We will one day use this information and apply it to the biological systems data we have and will collect

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