Sarah L. Keller

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Observations of lipid membranes in (roughly) 2D influenced by bulk water in 3D

Project 1: Dynamic critical phenomena

Project 2: Domain coarsening

Postdoctoral Fellowships in Biophysics
University of Washington, Seattle

http://depts.washington.edu/pbiopage/sackler

The Raymond and Beverly Sackler Scholars Program in Integrative Biophysics

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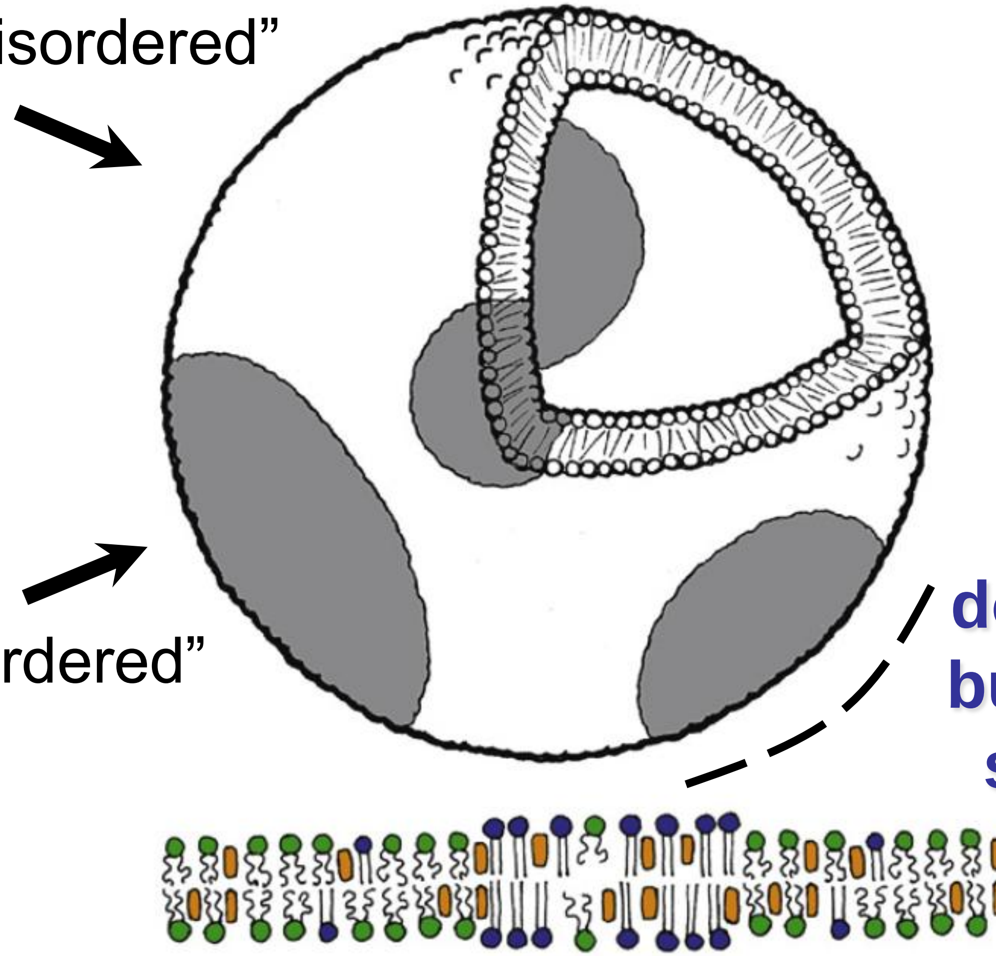
Giant unilamellar vesicles (GUVs)



In our expts, domains do not bulge out of the spherical shell

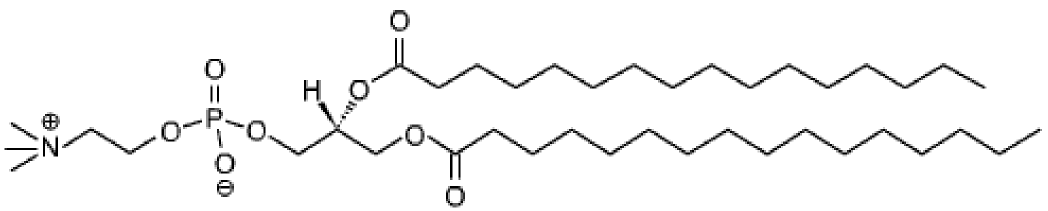
Drawing by Aurelia Honerkamp-Smith

Our vesicles (GUVs) contain 3 components (+ dye)

High Melting Temp

e.g. Saturated Lipids

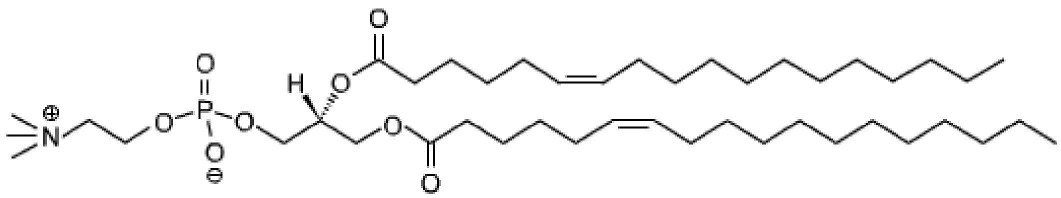
di(16:0)PC; DPPC



Low Melting Temp

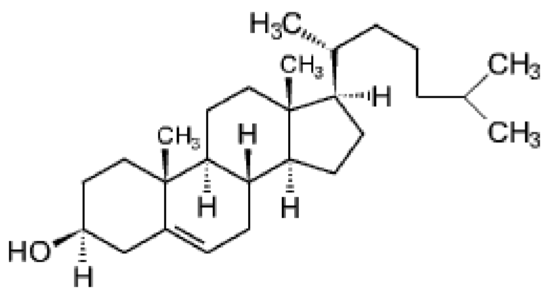
e.g. Unsaturated Lipids

or DiphytanoylPC

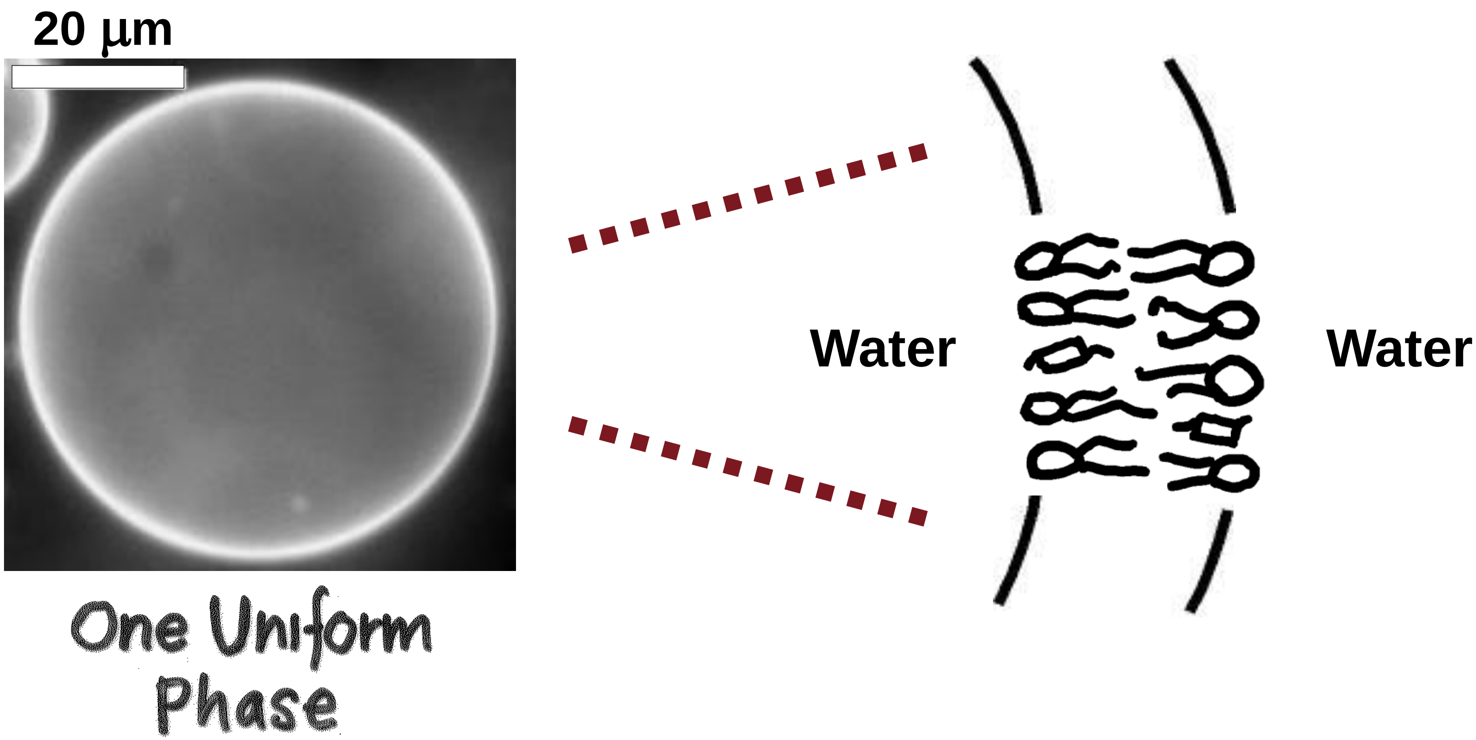


Cholesterol

or similar sterols

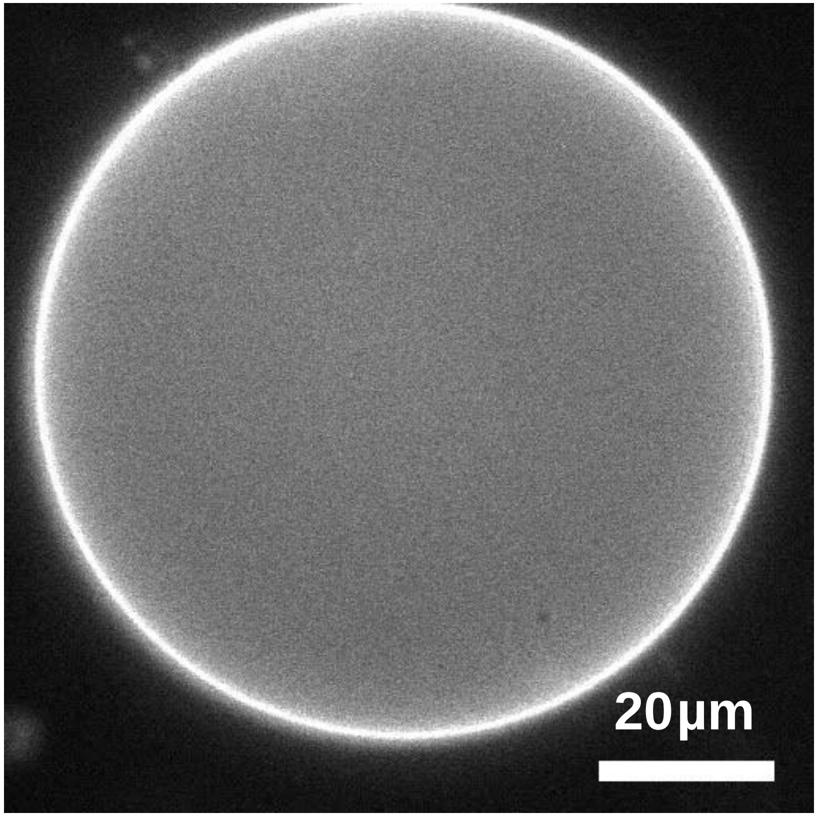


High temperature: uniform vesicle



Vesicles are free-floating in water,
not attached to or sitting on any substrate.

As temperature is lowered, micron-scale domains appear and coarsen.



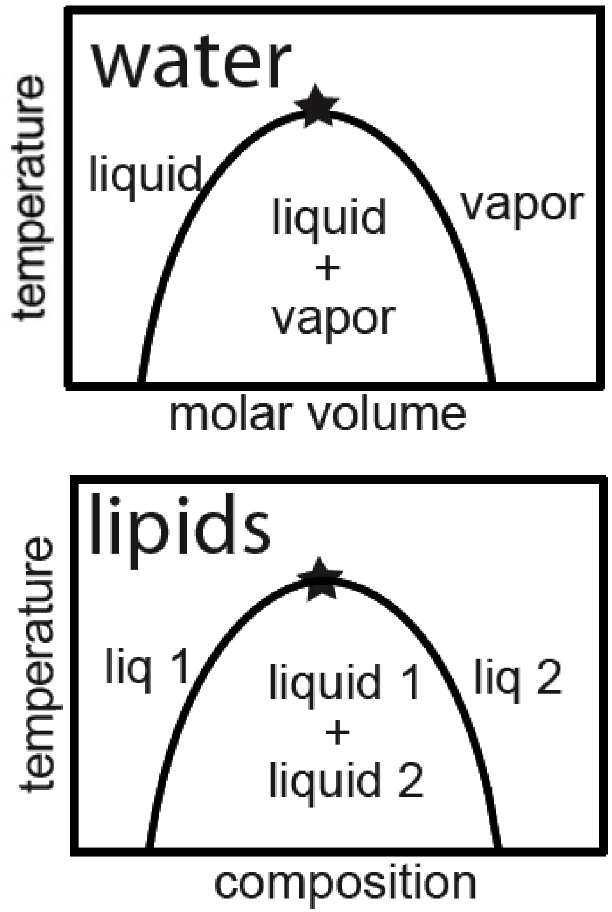
The image is 92μm on this edge.

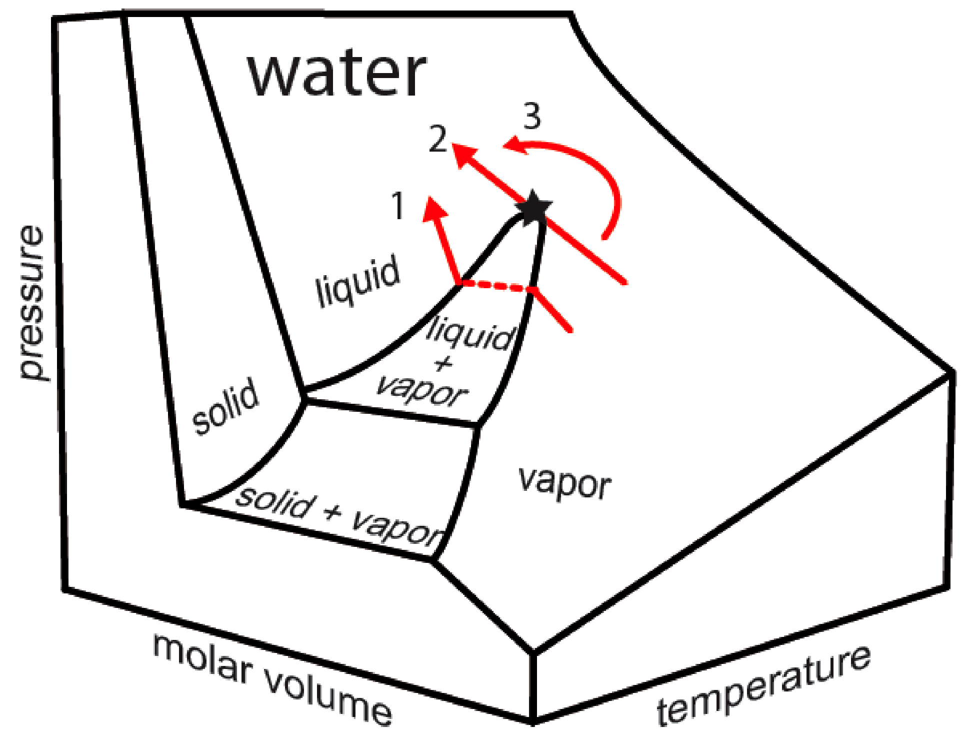
High temp:
Uniform membrane

Low temp:
Liquid domains / liquid background

Project 1: Dynamic critical phenomena

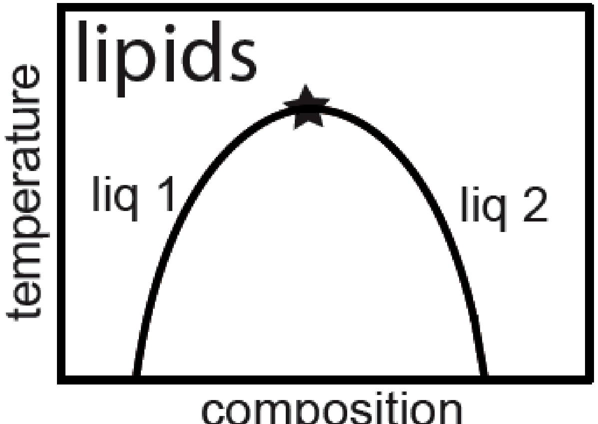
Critical points





A.R. Honerkamp-Smith et al.
BBA (2009)

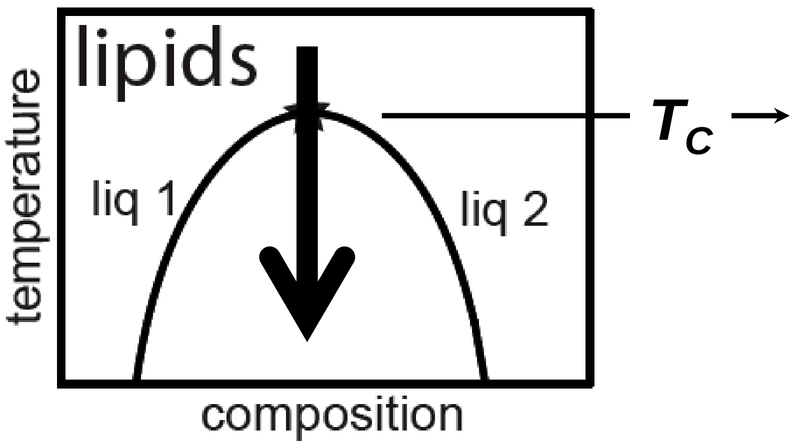
Fluctuations
in lipid composition
are associated
with critical points
(or plait points).



What does this
transition look like
in the microscope?

It's beautiful.

Fluctuations in lipid composition are associated with critical points (or plait points).



Way above Tc: Uniform, grey

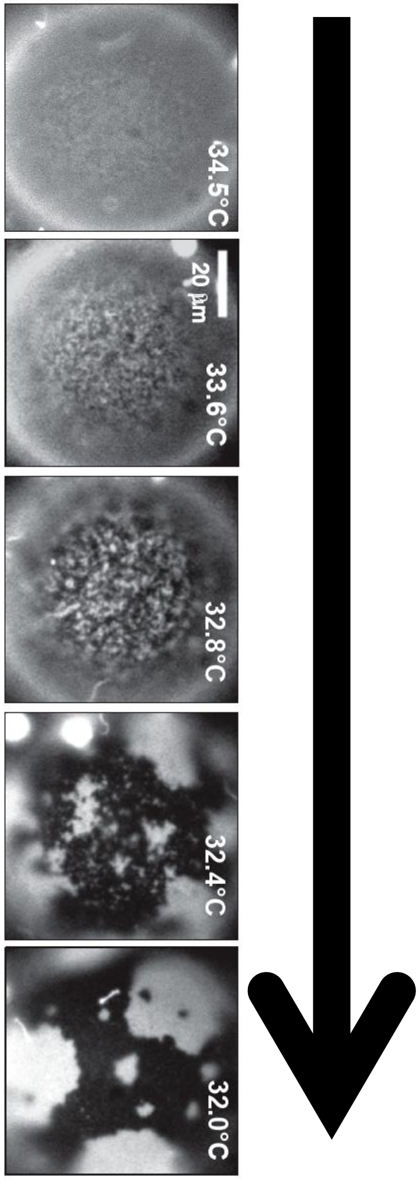
Above Tc: Tiny fluctuations

Which grow bigger…

And bigger…

Just below Tc: domains

Which become more circular…



Aurelia measured two time-independent quantities to find that membranes behave as 2D Ising models.

	Aurelia's Data	2-D Ising	3-D Ising
$\xi \sim \lvert T-T_c \rvert^{-\nu}$ correlation length	$\nu = 1.2 \pm 0.2$	$= 1$	~~$= 0.630$~~
$\Delta m \sim \lvert T-T_c \rvert^{\beta}$ order parameter	$\beta = 0.124 \pm 0.03$	$= 0.125$	~~$= 0.325$~~

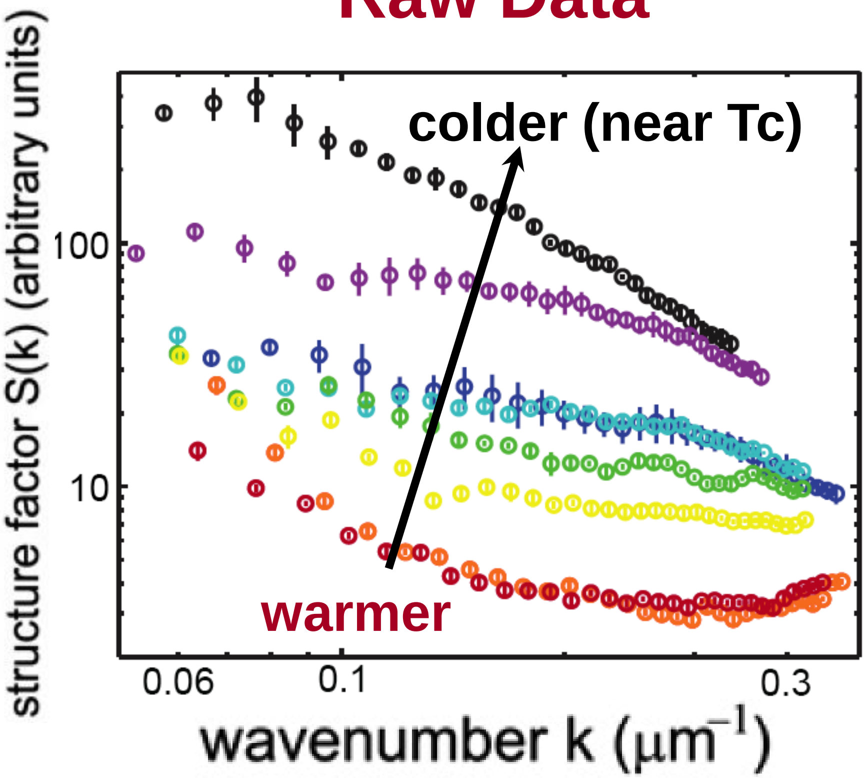
characterizes the biggest fluctuations we observe (correlation length)

characterizes the difference in composition of the two phases as *T* approaches *Tc* from below. (order parameter)

A.R. Honerkamp-Smith et al.
Biophys.J. (2008)

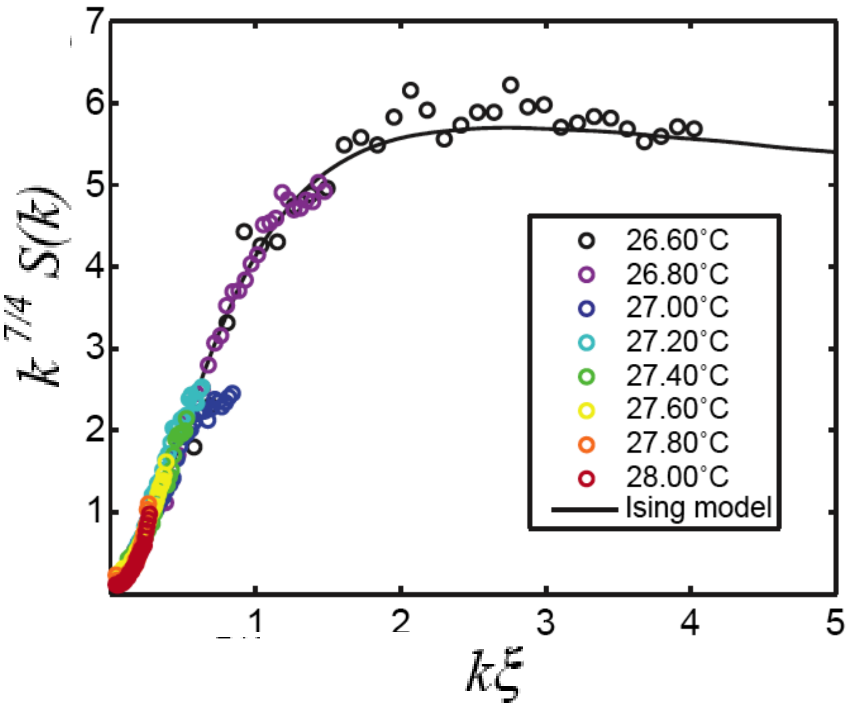
Example from one set of experiments: Applying scaling to find critical exponents

Raw Data



More structure near Tc

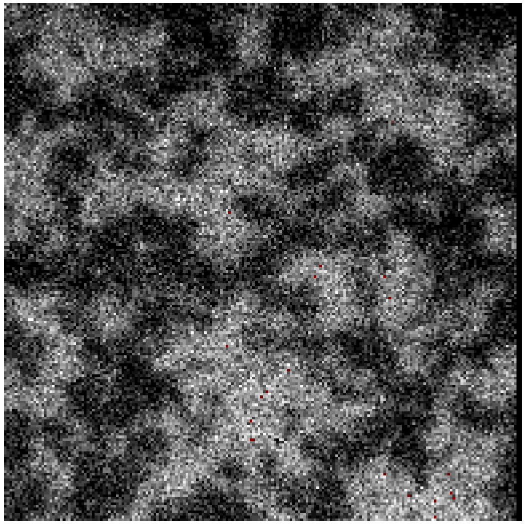
$k^{7/4}$ $S(k)$ vs. $k\xi$



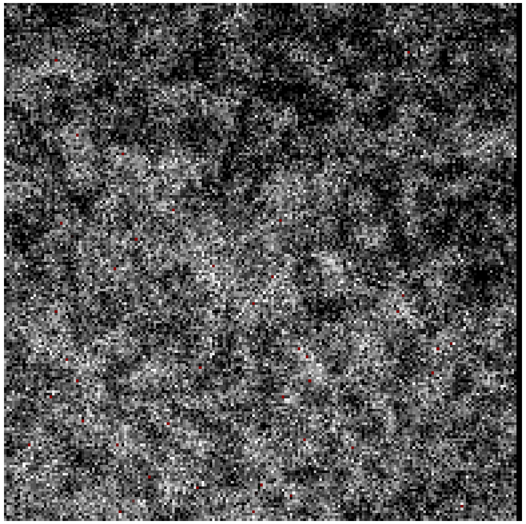
Aurelia extracted the value of ξ at each temp

$$\xi \sim |T-T_c|^{-\nu}$$

Now, what about any time <u>dependent</u> quantities?
Above T_c, no domains persist - only fluctuations.



Colder, 33˚C
(closer to T_c)

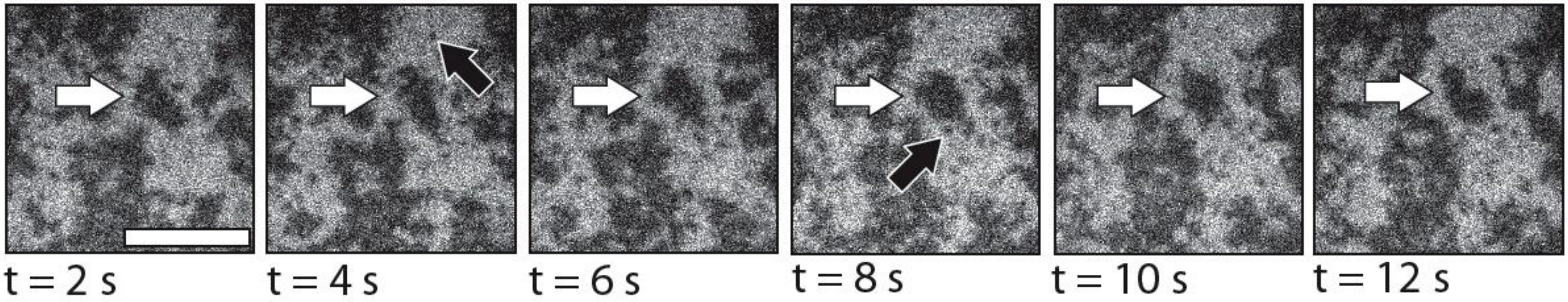


Warmer, 34˚C
(further from T_c)

Approach this problem by using scaling and by thinking about how size relates to time

Images ~30μm x 30μm

How does size relate to time?



How does size relate to time?

Hydrodynamic regime (where we usually live)	Critical regime (near a critical point)
$$\Gamma = k^2 D(k,\xi)$$	$$\Gamma = k^z \overline{\Omega}(k\xi)$$

Hydrodynamic regime:

- Γ: rate ($time^{-1}$)
- k: wavenumber ($length^{-1}$)
- D: diffusion coefficient ($\mu m^2/s$)

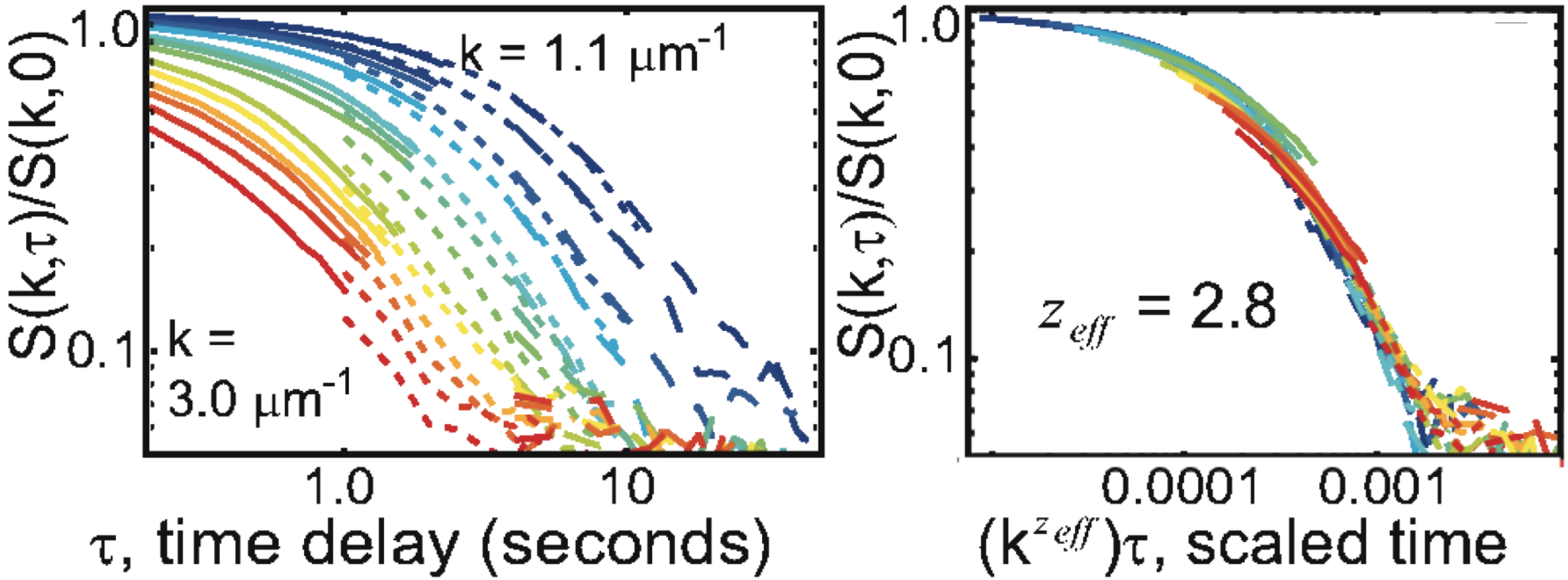
Critical regime:

- z: dynamic critical exponent
- $\overline{\Omega}$: dynamic scaling function

What should "z" be?

Model	Order parameter conserved?	Fluctuations dissipate via …	Value of z
2-D Model A (magnets)	No.		N/A
2-D Model B	Yes.	2D lipid diffusion	$z = 3.75$
2-D Model H (binary fluid)	Yes.	Collective 2D hydrodynamics	$z = 2$
Model HC (hydrodynamic coupling) (Inaura&Fujitani 2008; Haataja 2009)	Yes.	Same as above, but with coupling to bulk fluid ($L_{hydrodynamic} \neq 0$ $= \eta_{2D} / \eta_{3D}$)	$z = 3$ (z_{eff} crosses over from 2 to 3 as $T \longrightarrow T_c$)

Experimental data at the temperature closest to Tc that can be analyzed.



Aurelia.R. Honerkamp-Smith,
Ben Machta, SLK
In press *PRL* (2011)
ArXiv:1104.2613

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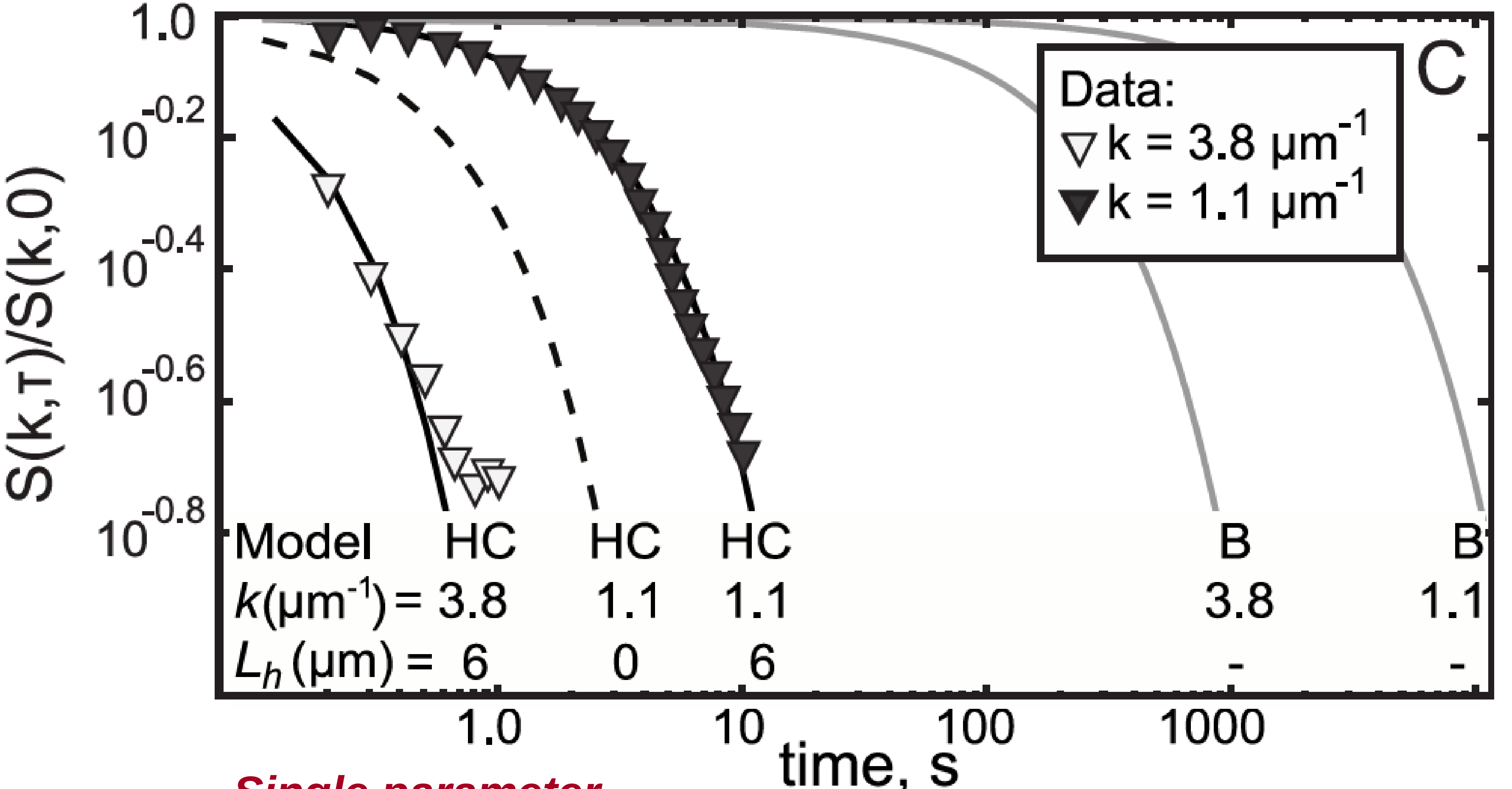
Excellent fit to structure factor predicted by “Model HC” (hydrodynamic coupling)



Single parameter fit: L_h = 6.0 ± 1.5 μm

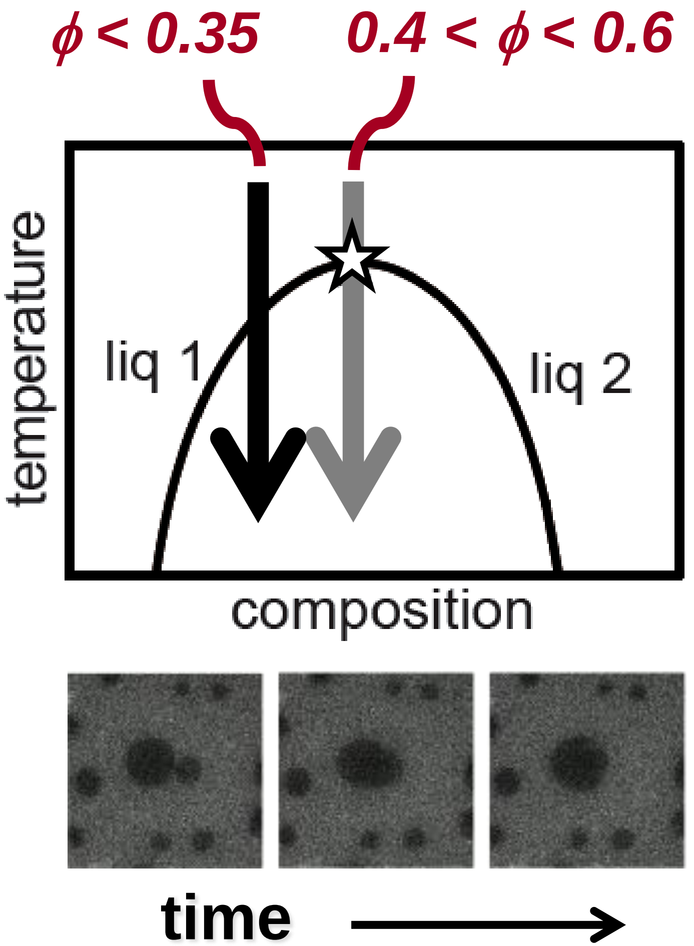
Aurelia.R. Honerkamp-Smith, Ben Machta, SLK
In press *PRL* (2011)
ArXiv:1104.2613

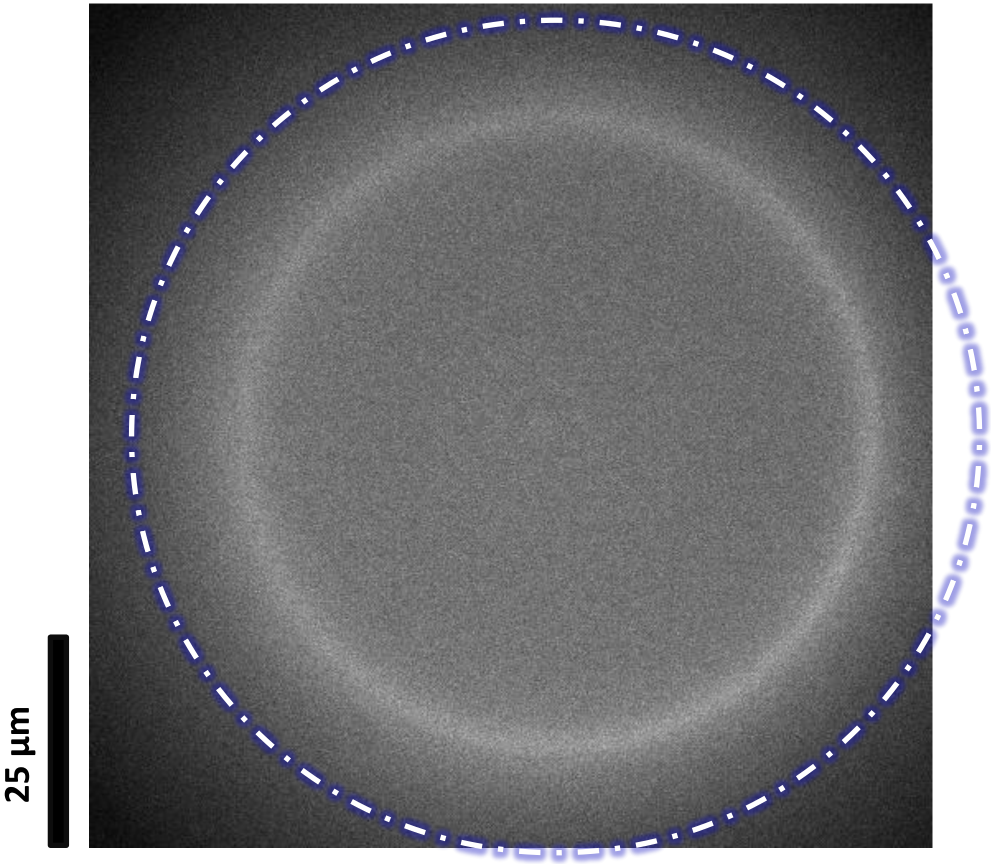
Conclusion from this section:

Dynamics of membrane fluctuations are consistent with a recent theory that invokes hydrodynamic coupling between the membrane and bulk fluid such that Ising degrees of freedom are coupled to momentum modes.

Project 2: Domain coarsening

Quenches that are not through the critical point produce circular domains that merge with time.





Cynthia Stanich, Aurelia Honerkamp-Smith, et al.

Which equations are relevant for diffusing domains?

Saffman-Delbrück (1975)

small domains and/or high membrane viscosity

$$D(r) = \frac{k_B T}{4\pi \eta_{2D}} \left[\ln \frac{\eta_{2D}}{(\eta_{3D})(r)} - \gamma + \frac{1}{2} \right]$$

η_{3D} = 3D bulk viscosity of solvent
η_{2D} = 2D viscosity of membrane
γ = 0.5572

Hughes-Pailthorpe-White (1981)

de Koker (1996), Seki-Ramachandran-Komura (2011)

large domains and/or low membrane viscosity

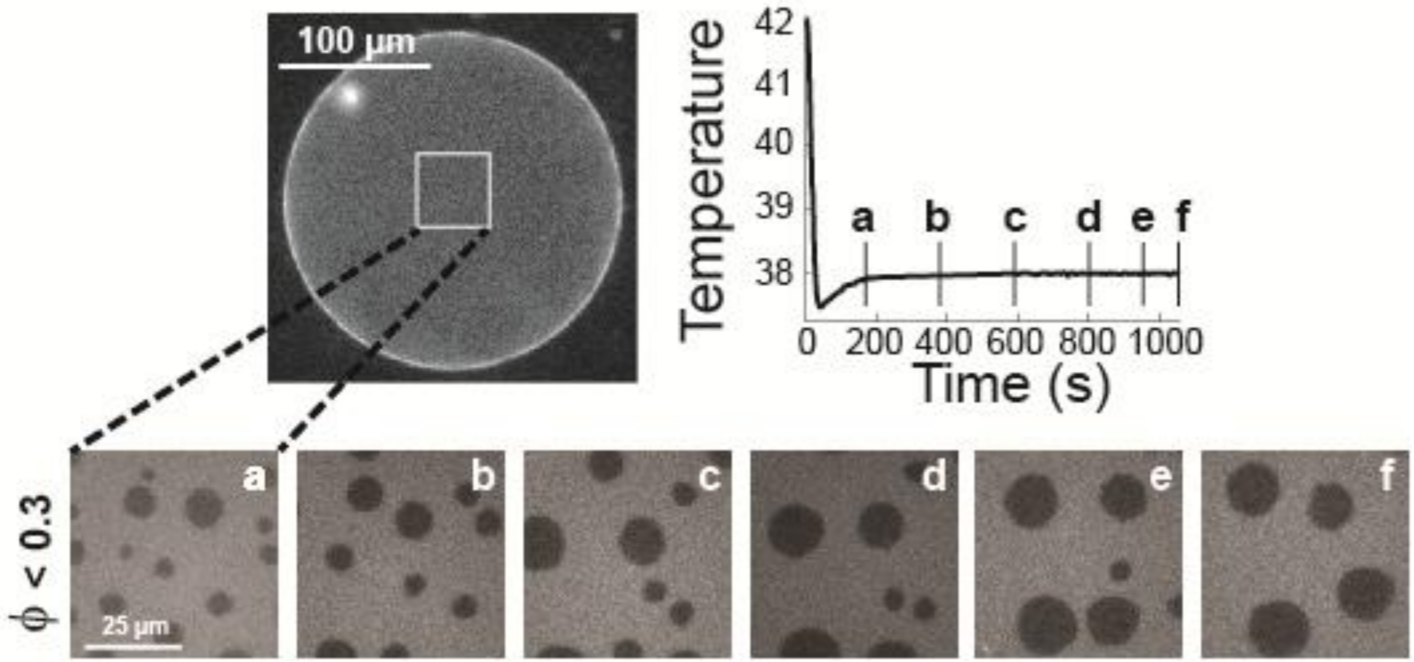
$$D(r) = \frac{k_B T}{16 \eta_{3D} r} \qquad D(r) = \frac{2 k_{\mathrm{B}} T}{3\pi^2 \eta_{3\mathrm{D}} r}$$

A log-log plot of *D* vs. *r* should be a straight line.

In between?

Numerical solution by HPW, nice approximation by Petrov & Schwille

Conceptually simple: Track domains of different size vs. time



time →

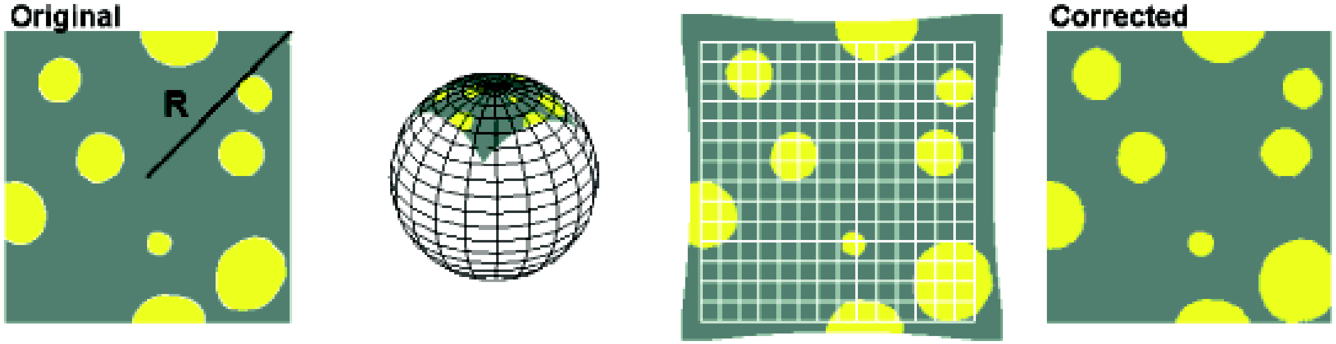
Cynthia Stanich, Aurelia Honerkamp-Smith, et al.

Experimental headaches:

1) Isothermal.
2) Vesicles are huge (80-250μm) to maximize flat area.
3) Vesicles are free-floating.
4) Rotating vesicles are deleted (17 retained out of >100).
5) Domains do not bulge out of the spherical surface.
6) Deletion of merging domains.
7) Parameter choice to avoid under-sampling and errors due to domains diffusing in and out of view.

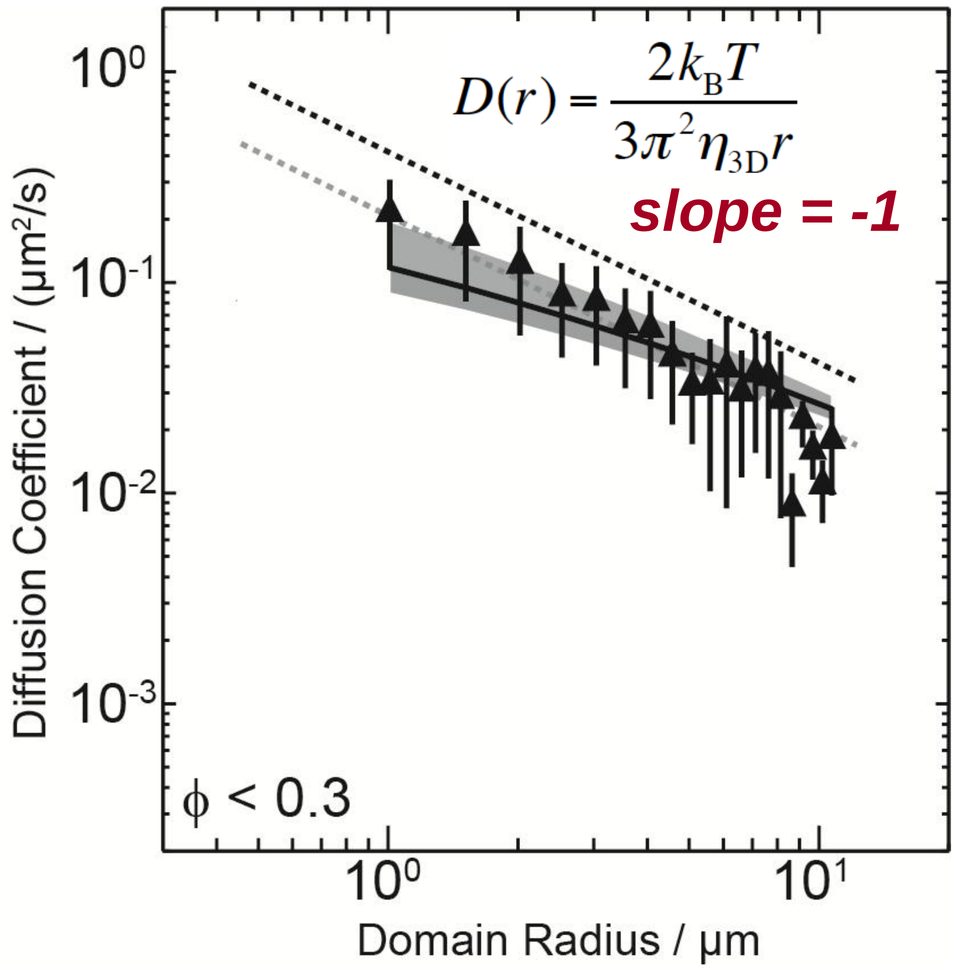
$$\text{Normalized length } R = \frac{\text{Area of minority phase in view}}{\text{Perimeter of all domains in view}}$$

8) Projection from 2D picture to 3D surface.



Cynthia Stanich, Aurelia Honerkamp-Smith, et al.

When domains are circular, D ~ 1/radius



- Qualitatively, D ~ 1/r

- Quantitatively, must fit through approximation.

Membrane viscosity found by the fit consistent with previous measurements.

η_{2D} (Pa s m)	Reference
(3.3 ± 1.1) x 10^{-9}	Stanich et al. 2012
(6.0 ± 1.5) x 10^{-9}	Honerkamp-Smith et al. 2012
(4 ± 1) x 10^{-9}	Camley et al. 2010

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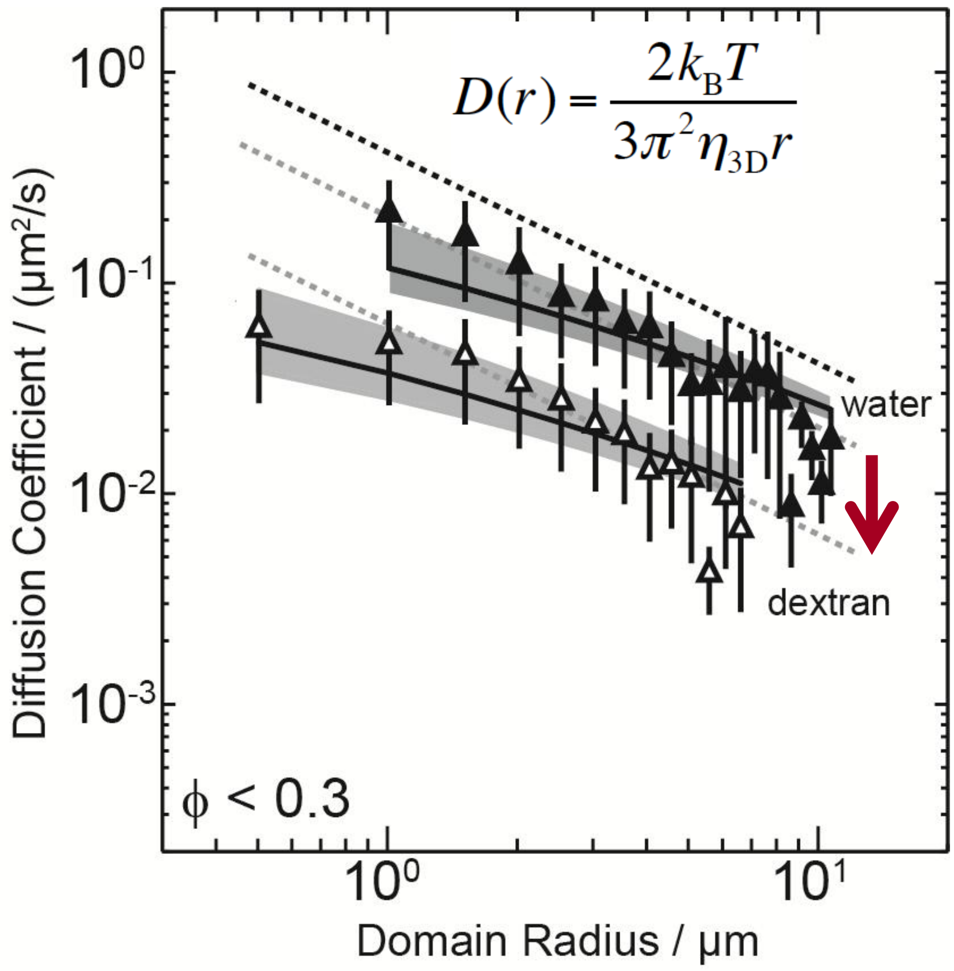
Prediction: Increasing bulk viscosity slows diffusion



Divide by 3.3

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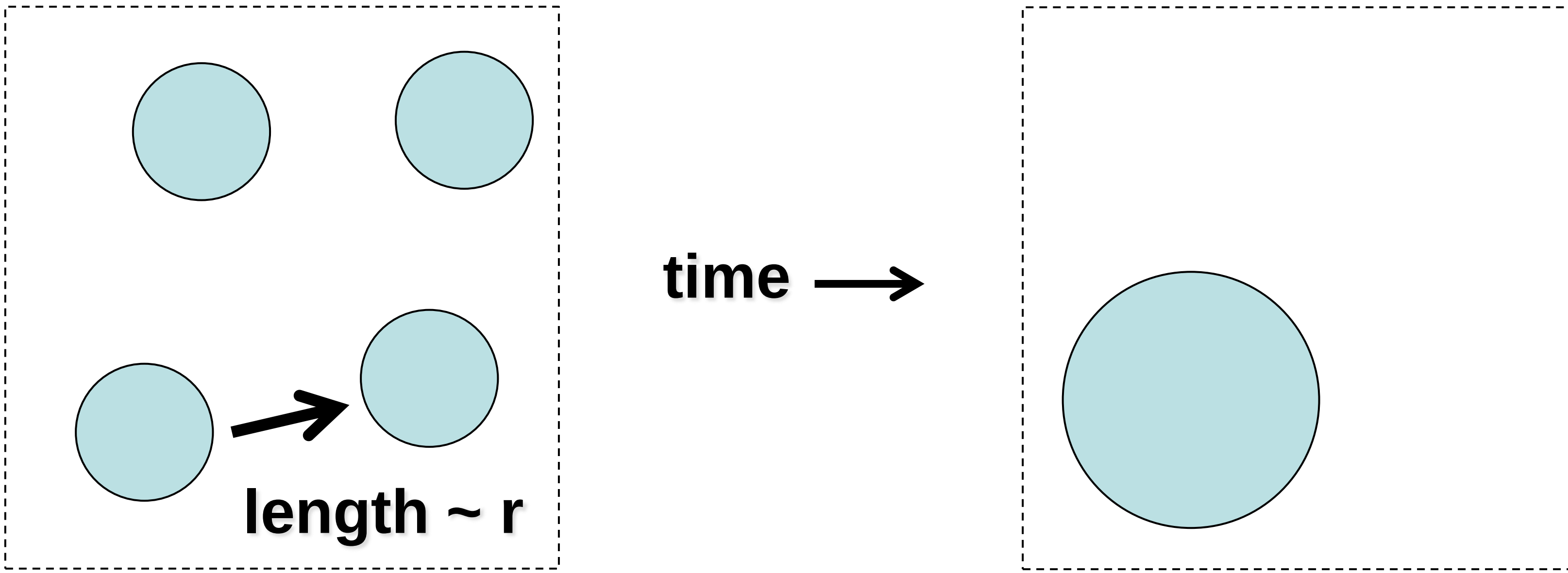
We've just learned that D ~ 1/radius.
Let's say that domain radius grows as $r \sim t^{\alpha}$. What is α?



Assume domains grow entirely by collision/coalescence.

Brownian motion: $\text{length}^2 \sim Dt$

So $\text{radius}^2 \sim Dt$.

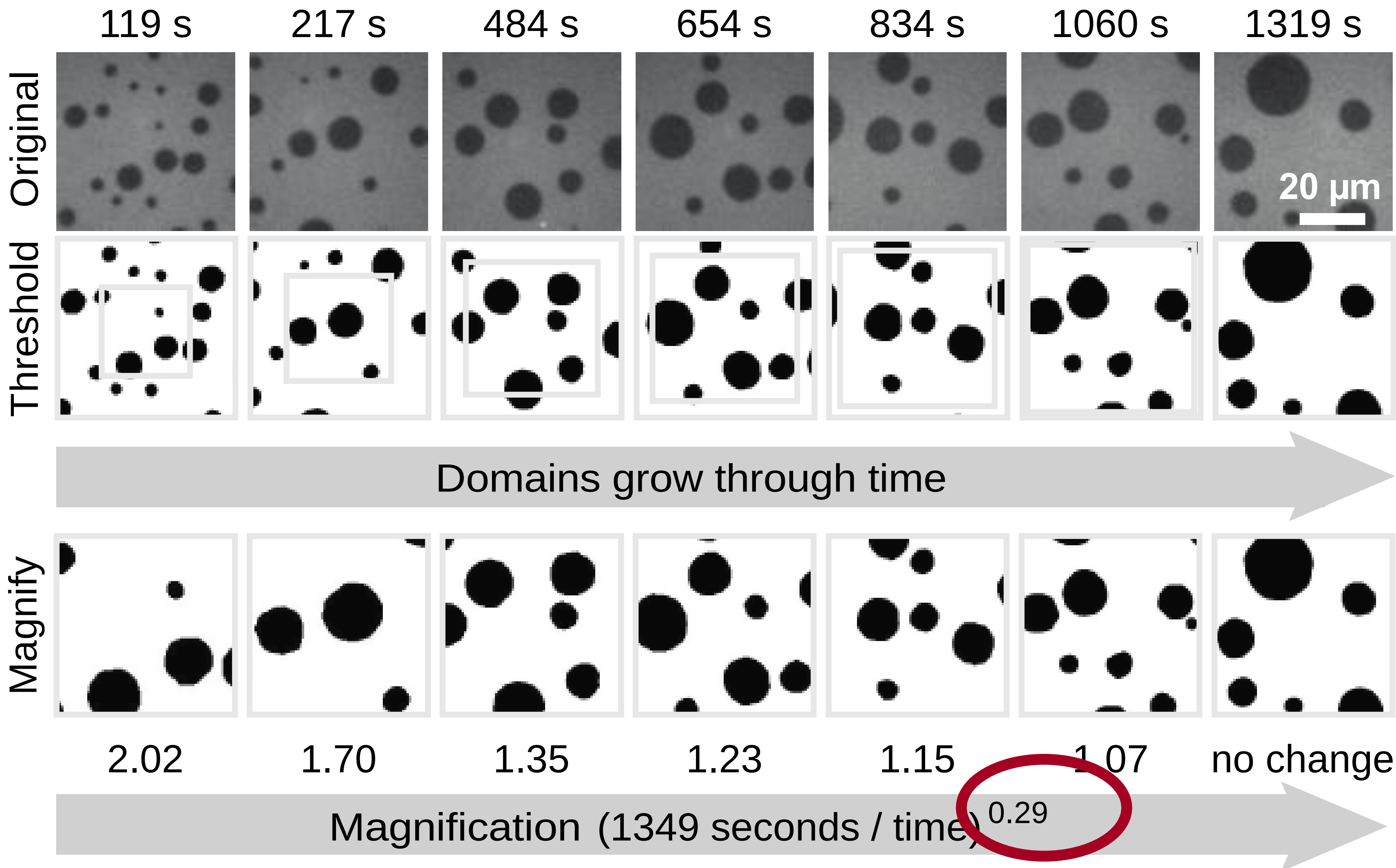
When D ~ 1/radius, $\alpha = 1/3$.

Simulations with $\phi \leq 0.3$

α	SIMULATION	REFERENCE
0.3	Dissipative particle dynamics. $\phi = 0.3$	Taniguchi 1996
1/3	Purely dissipative dynamics. $\phi = 0.3$	Laradji et al. 2004
0.31	200 spherical caps on a vesicle. $\phi = 0.09$	Putzel (Northwestern U.)
1/3	Continuum approach with hydrodynamics	Fan et al 2010
1/3	Stochastic phase field model + hydrodynamics	Camley et al. 2010

When D ~ 1/radius, $\alpha = 1/3$.

Yes, for circular domains, $\alpha \approx 1/3$ (only one experiment here)



α = 0.29, close to 1/3

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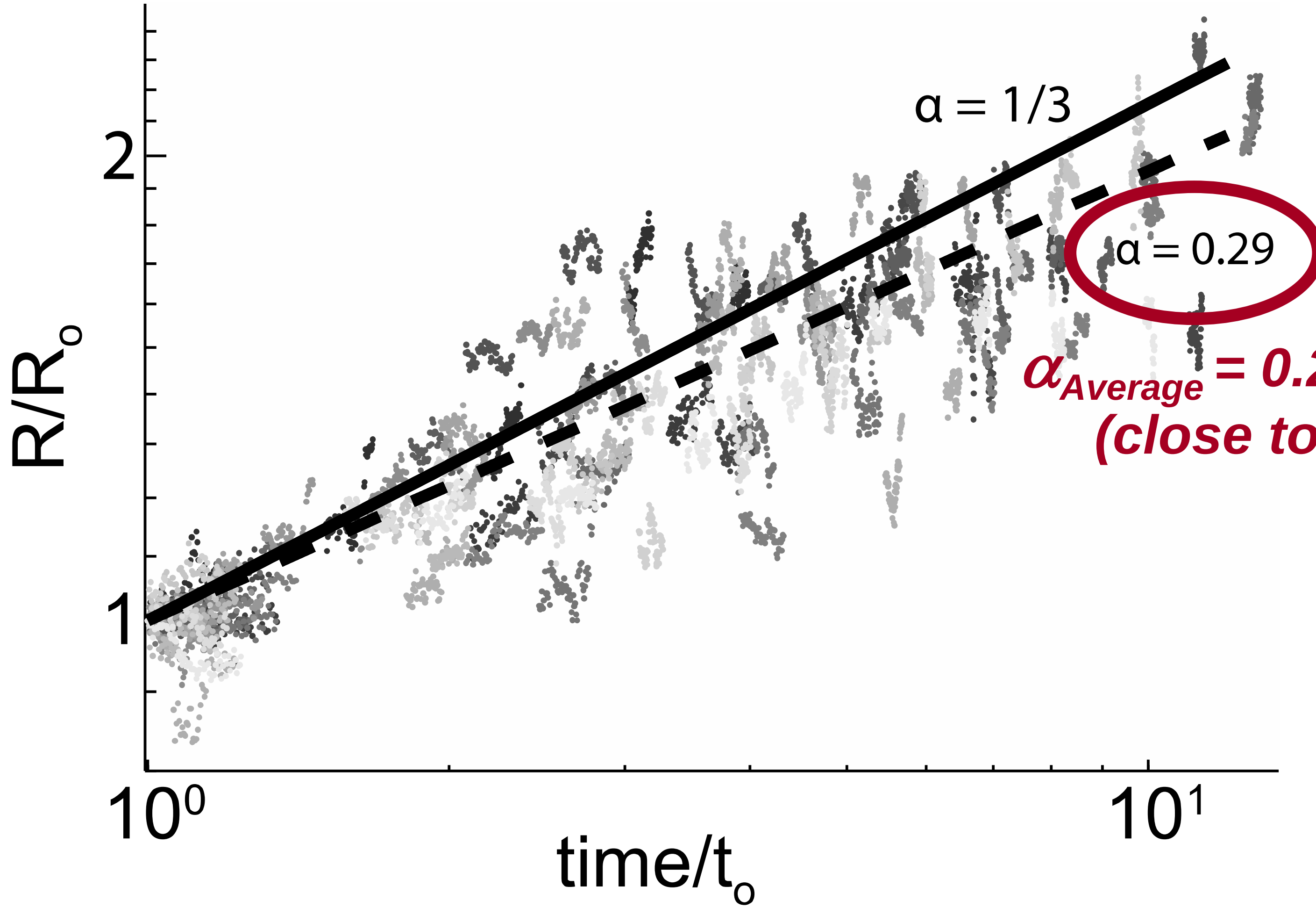
Yes, for circular domains, $\alpha \approx 1/3$ (all 17 experiments here)



$\alpha_{Average}$ = 0.29 ± 0.05
(close to 1/3)

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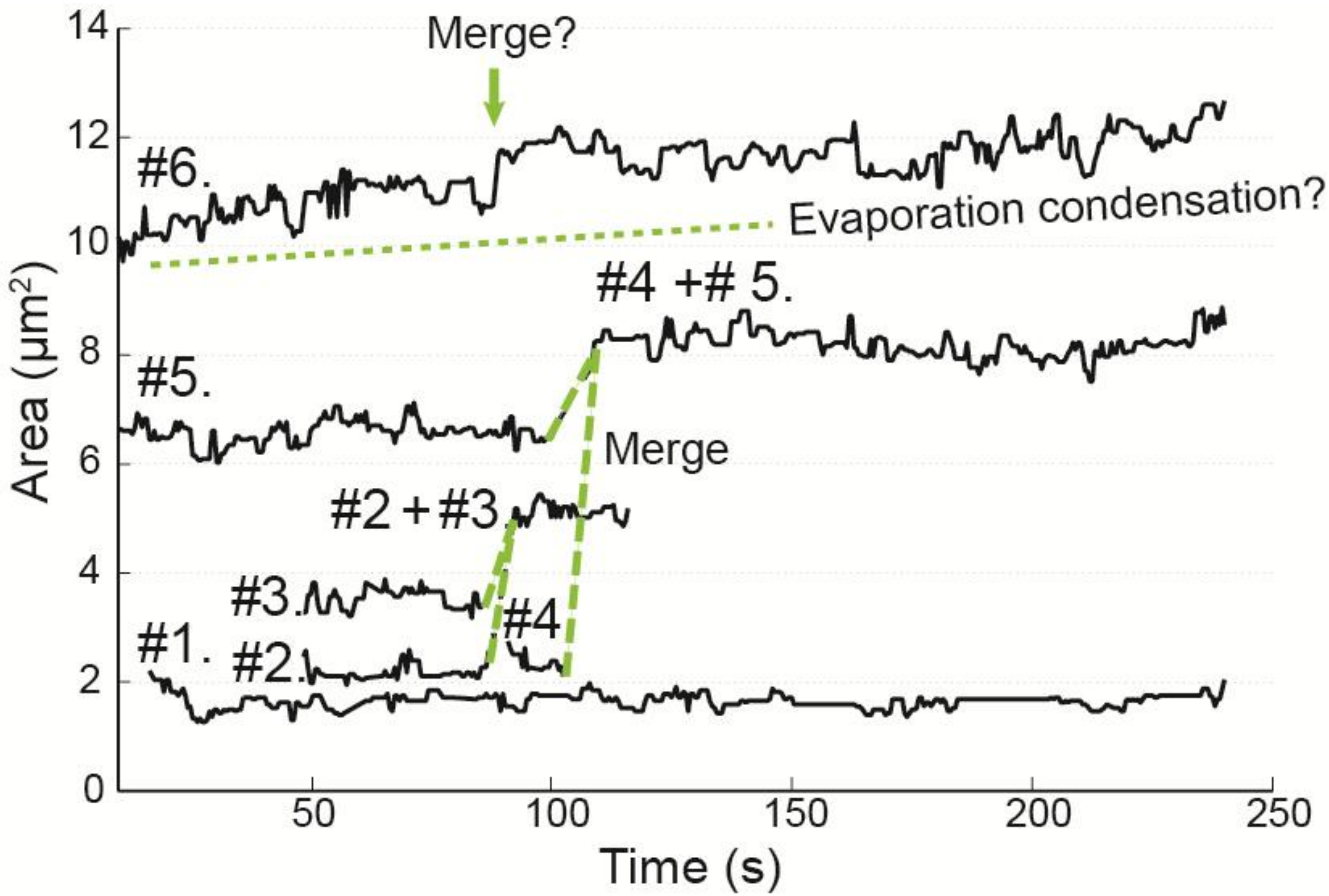
Coarsening of domains appears to be dominated by merges (not by evaporation-condensation / Ostwald ripening)



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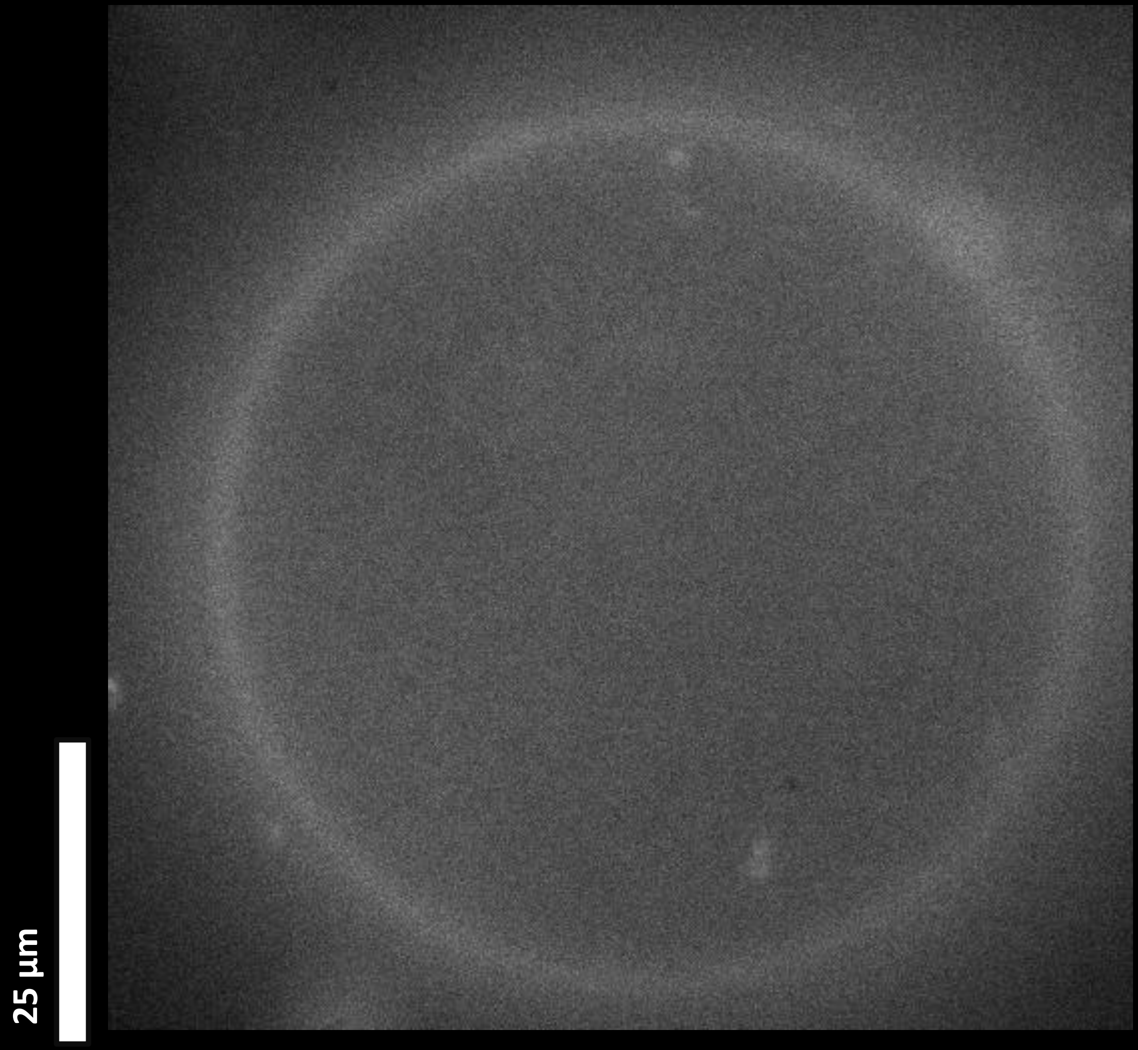
What happens when area fraction (ϕ) is close to ½?

In other words, what happens after a quench through a critical point?

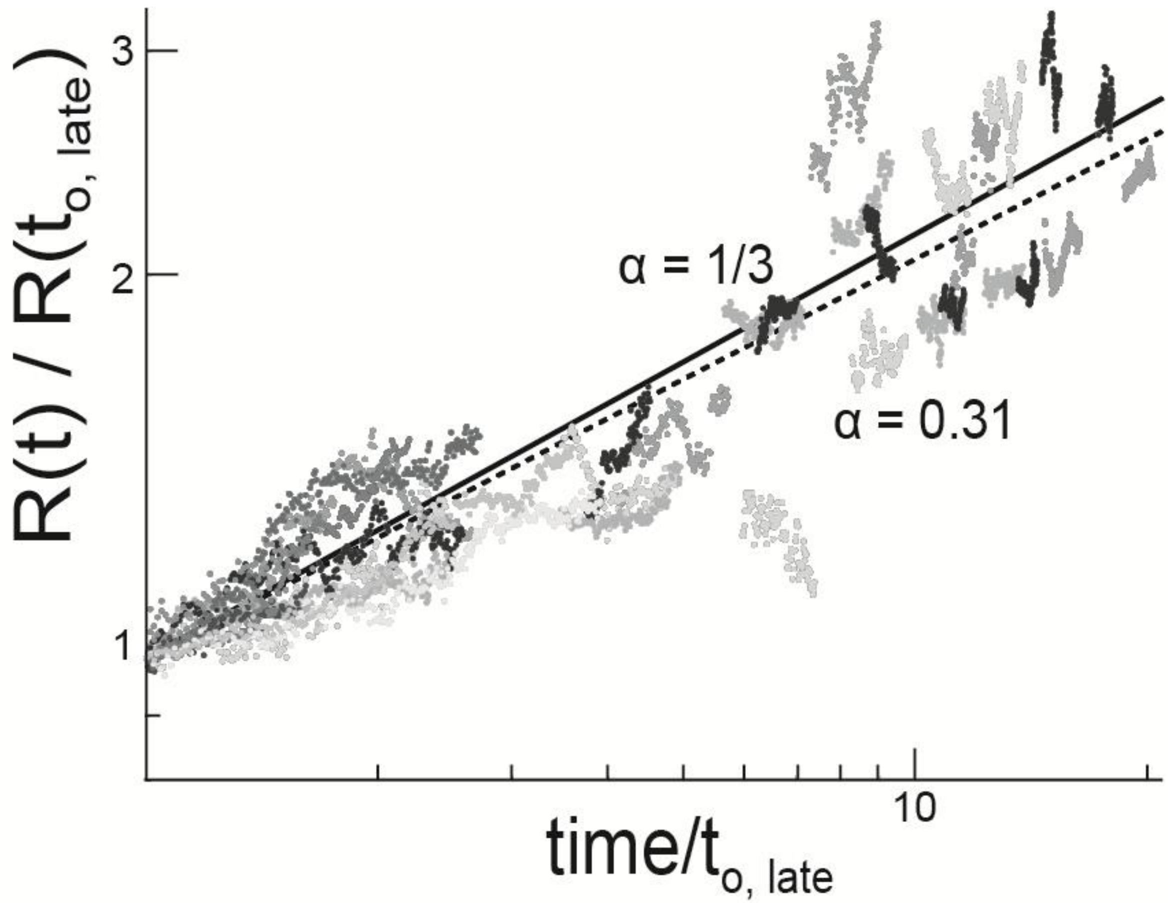
(Note: Isothermal critical point, or plait point)

0.4 ≤ Area Fraction ≤ 0.6

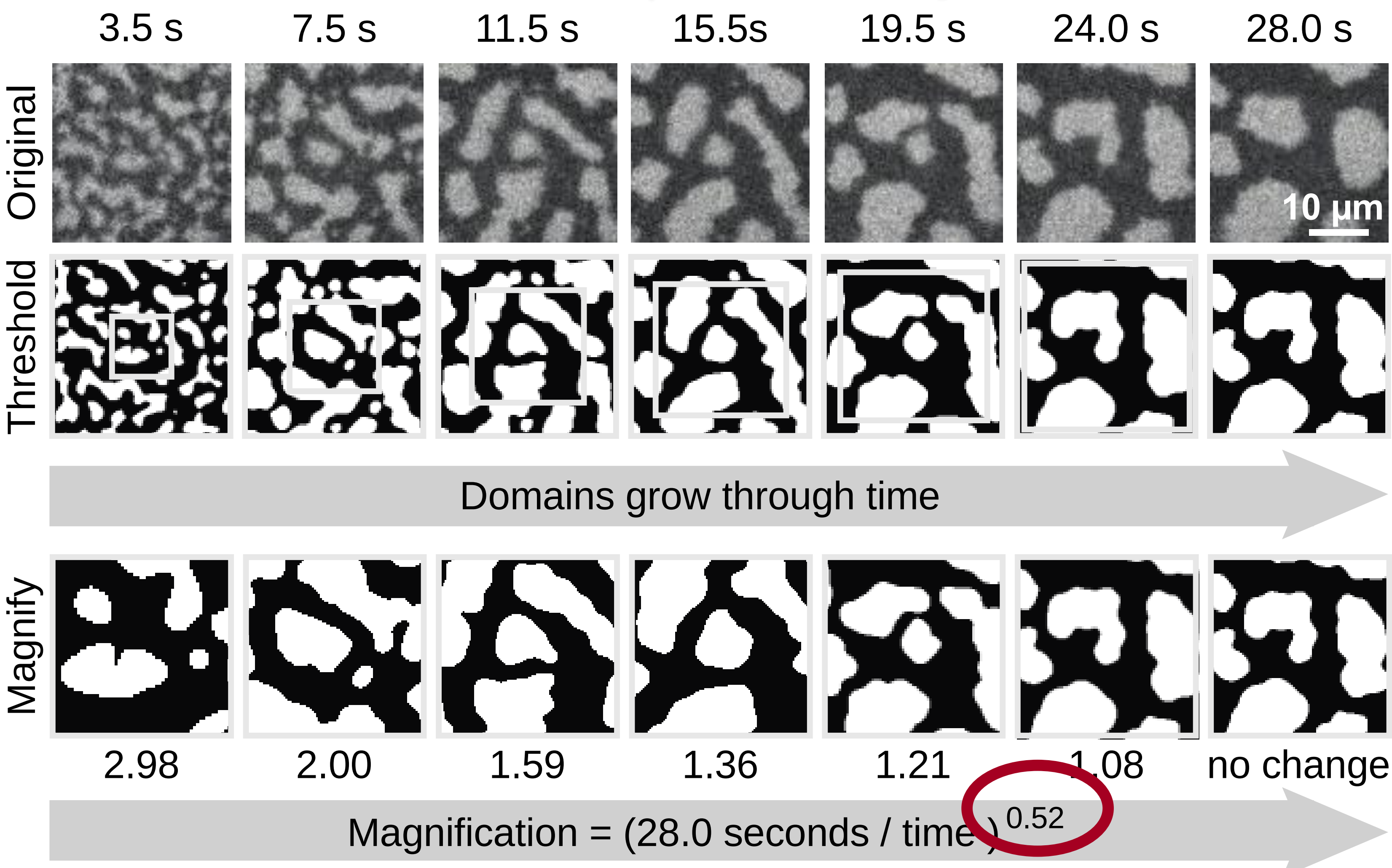
Early (temp stabilizing), Intermediate (noncircular), Late (circular)



Late times, $0.4 \le \phi \le 0.6$, 10 experiments, $\alpha = 0.31 \pm 0.05$



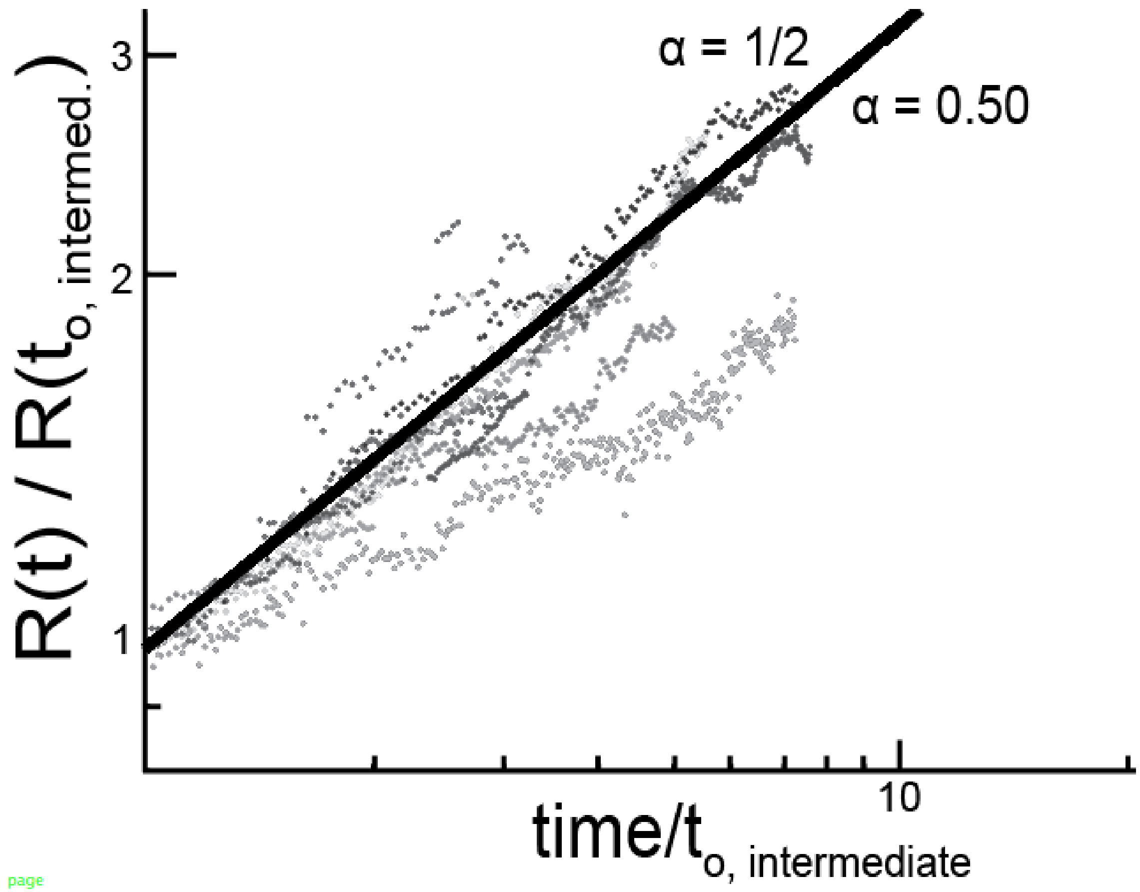
Intermediate times, $0.4 \leq \phi \leq 0.6$, 8 expts, $\alpha = 0.50 \pm 0.16$



α = 0.52, close to 1/2.

Consistent with simulations by Ramachandran et al. 2009, Laradji et al. 2005, Fan et al. 2010, and Camley et al. 2011.

Intermediate times, $0.4 \le \phi \le 0.6$, 8 expts, $\alpha = 0.50 \pm 0.16$



page

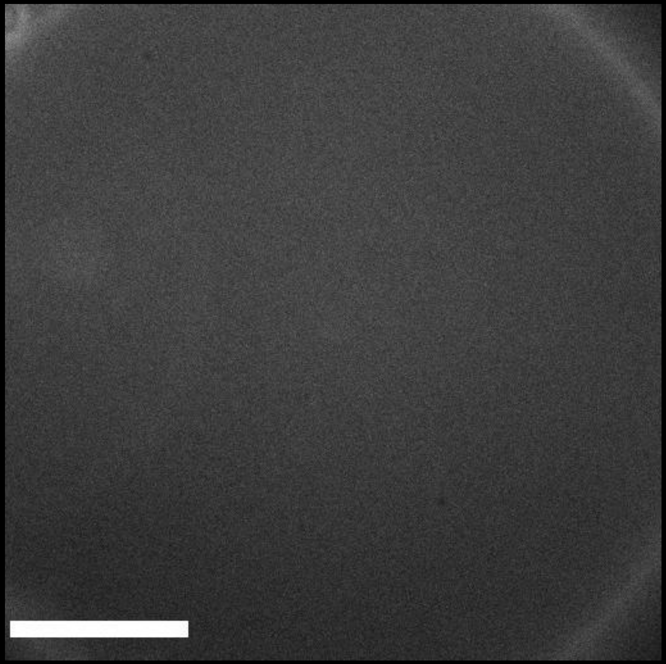
Worth noting…

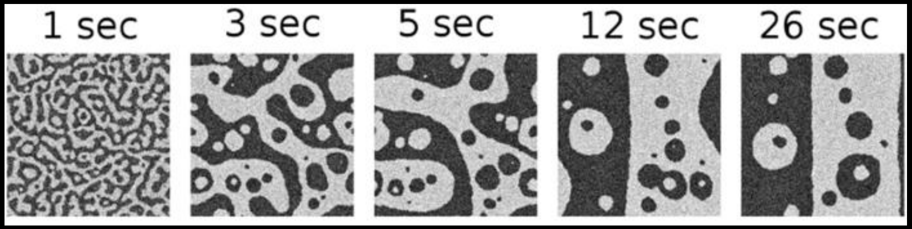
Observing different exponents at different times means scaling is only apparent, not universal.

This is seen in simulations by:
Fan, Han, and Haataja (2010)
Camley and Brown (2011)

Both groups say that if experiments see any apparent scaling in a membrane with noncircular domains near a critical point, then α should be ~ 1/2.

Example in which no scaling is observed:





Compare to simulation
by Camley and Brown 2011

Conclusion from this section:

- Qualitatively, micron-scale domains in membranes of our composition diffuse with $D \sim 1/r$.

- Quantitatively, we find membrane viscosities that agree with values found by other methods.

- Circular domains coarsen with a growth exponent of $\alpha \approx 1/3$.

- Noncircular domains coarsen with an apparent growth exponent of $\alpha \approx 1/2$.

- The hydrodynamic length scale, L_h, holds the key to understanding a range of membrane phenomena.

Thank You

Data from current group members

Cynthia Stanich (coarsening), Matt Blosser,

Ben Horst, Joan Bleecker, Thomas Portet

Data/Code from group alumnae

Aurelia Honerkamp-Smith (U. Cambridge), Sarah Veatch (U Michigan)

Collaborations

Ben Machta (Cornell - this project), Pietro Cicuta (U. Cambridge),

Michael Schick (U. Washington), Marcel den Nijs (U. Washington),

Ray Goldstein (U. Cambridge), Sharona Gordon (U. Washington)

Funding for this project

NSF MCB-0744852

Partner

Rob Carlson

If you are interested in pursuing graduate or postdoctoral research in my lab, please contact me and let's scheme about funding.

Postdoctoral Fellowships in Biophysics
University of Washington, Seattle

http://depts.washington.edu/pbiopage/sackler

The Raymond and Beverly Sackler Scholars Program in Integrative Biophysics

- Up to two years of funding ($50,000 yearly for stipend / benefits, $3000 yearly for professional development / conference travel)
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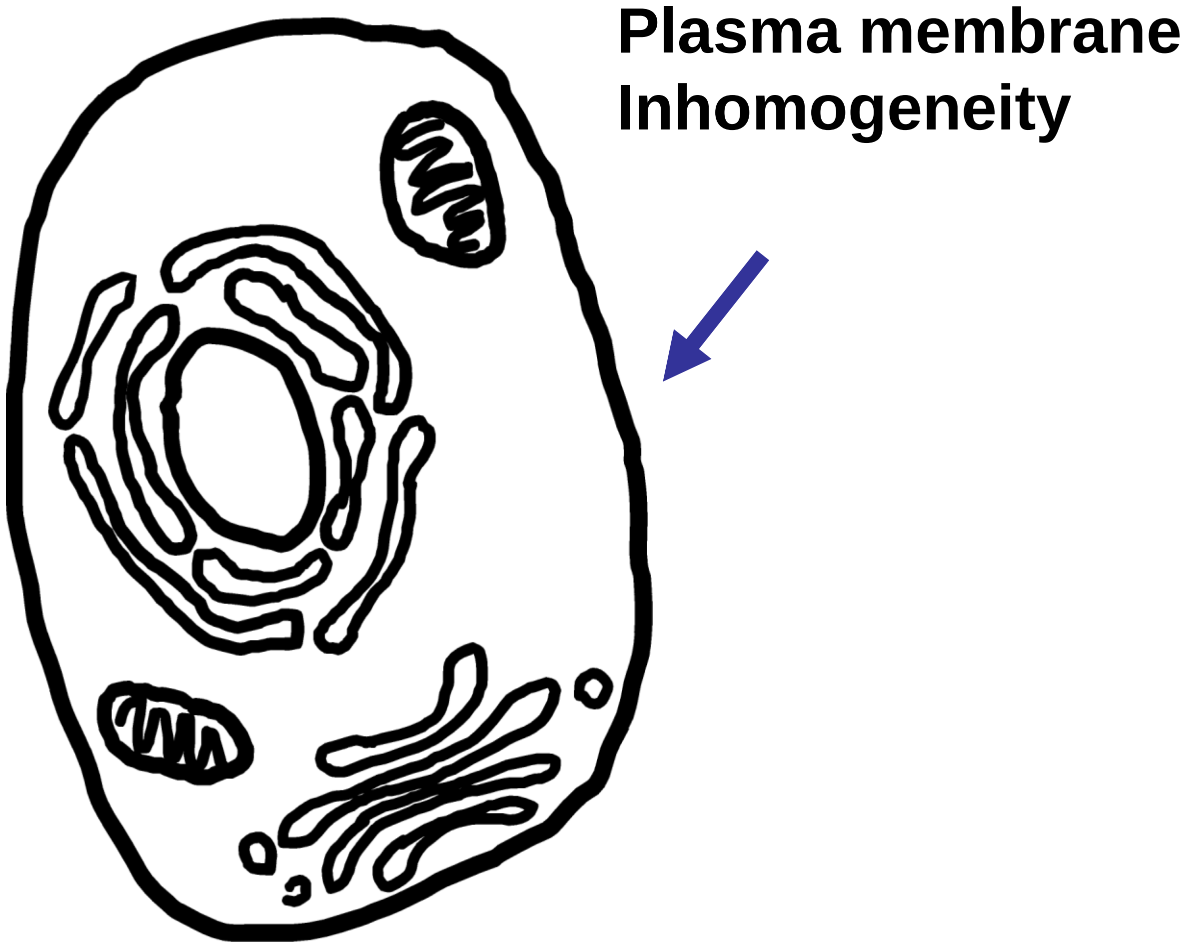
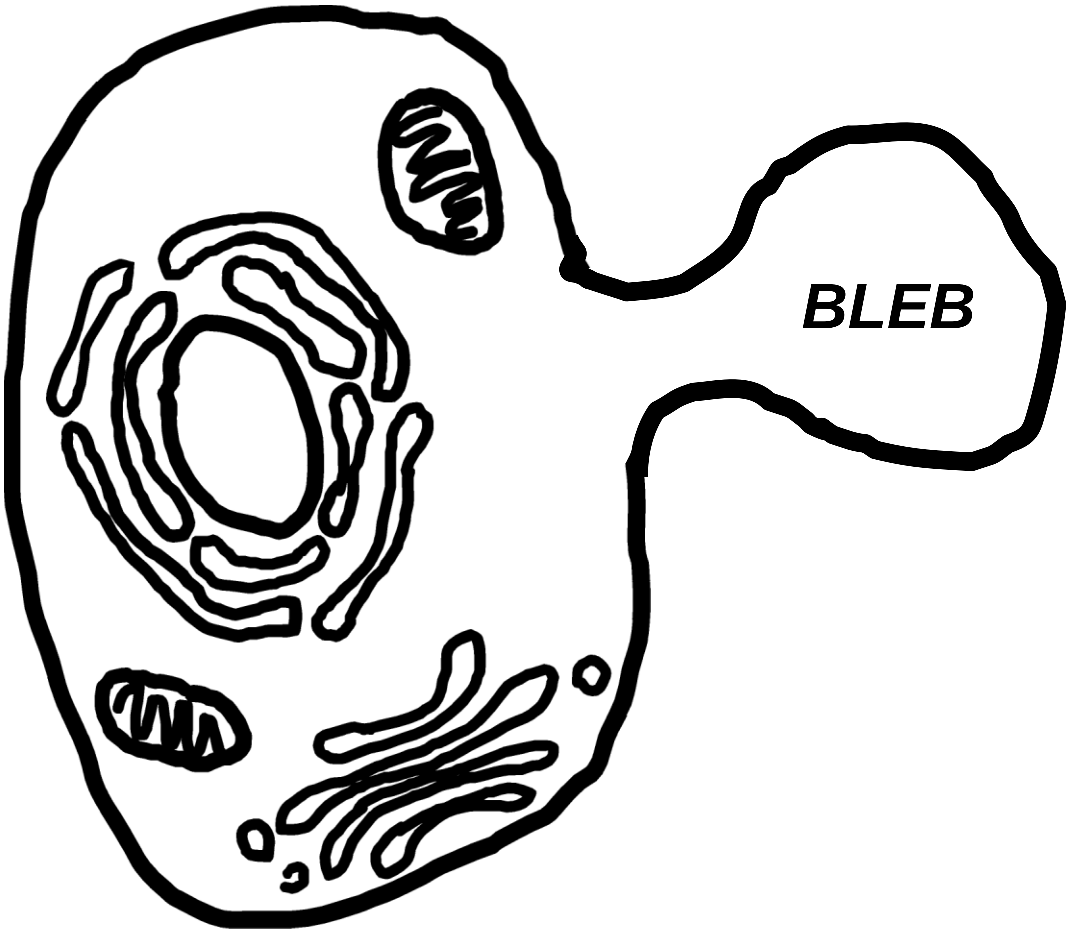
UW seeks the most outstanding early career scientists, regardless of nationality. Applications accepted on a rolling basis. Apply at *http://depts.washington.edu/pbiopage/sackler/app_process.php*

Inspiration:



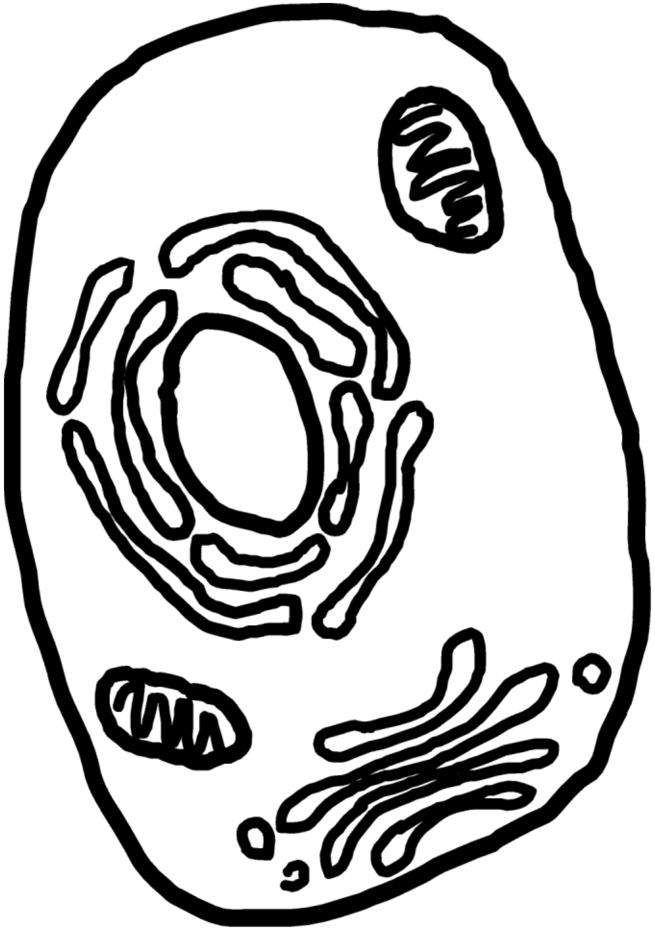
Sarah Veatch's Results:
In collaboration with Baird / Holowka Lab at Cornell



RBL cells

Sarah Veatch
Assistant Professor
University of Michigan

Sarah Veatch's Results:
In collaboration with Baird / Holowka Lab at Cornell



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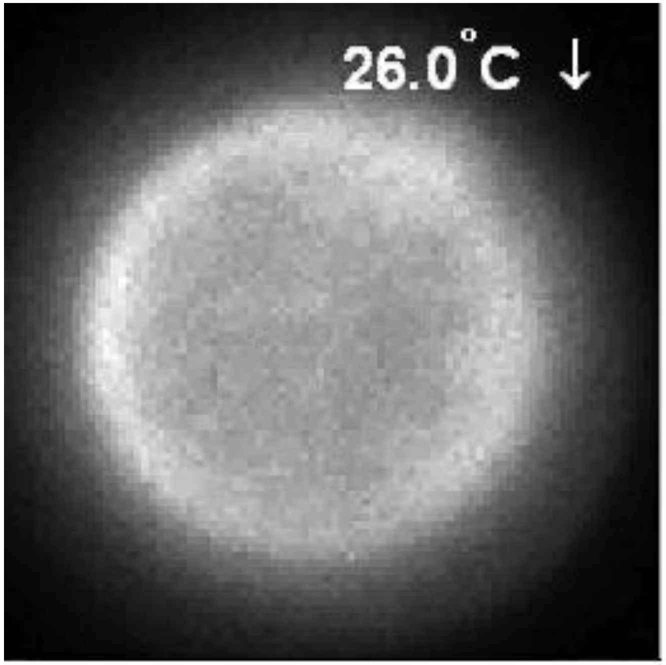

S.L. Veatch et al.
ACS Chem Biol (2008) 3:287

In all cases,
T_{Growth} >
$T_{Critical}$

So, presumably, at the growth temperature, sub-micron fluctuations are indeed present in the membrane.

Pretty cool.