Topological relaxation of shear-induced L_α to L_3 equilibrium & the energetics of membrane fusion

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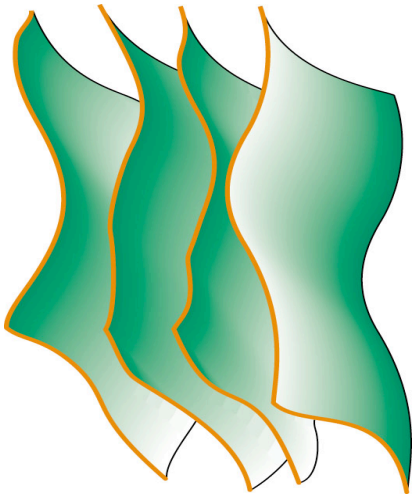

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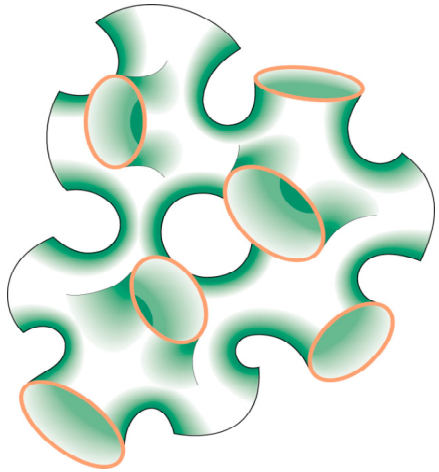
University of Sydney, Australia



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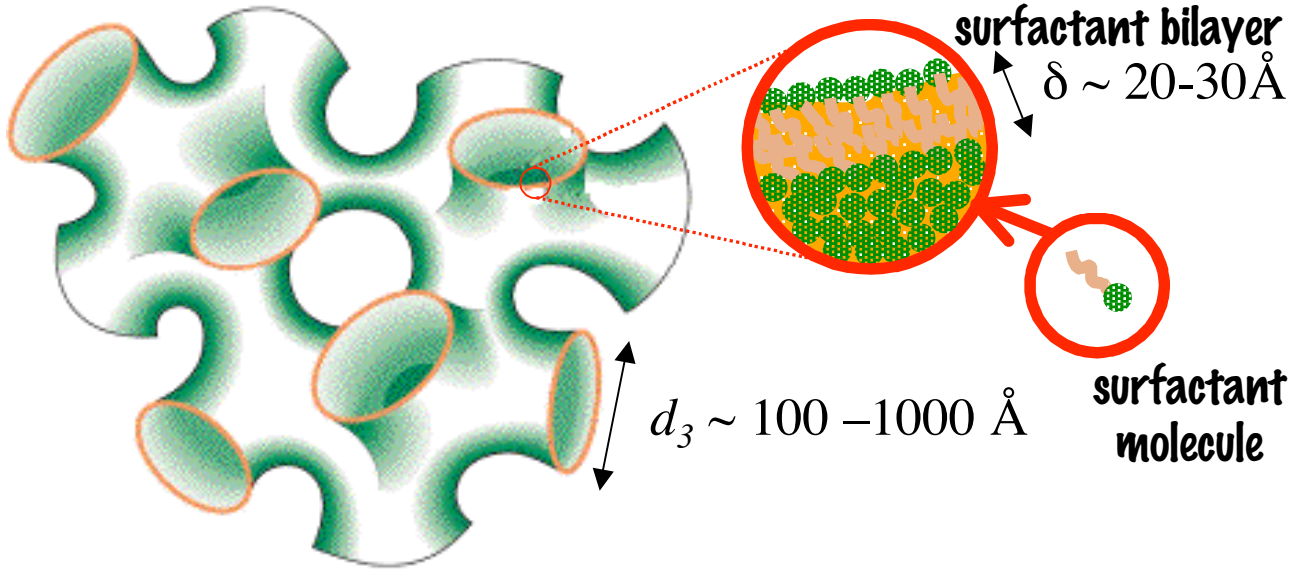
APS March Meeting, Montreal, 2004

L_3 "sponge" phases

Self assembled <u>isotropic</u> membrane phases



A convoluted solution spanning labyrinth of membrane passages (topologically "handles")

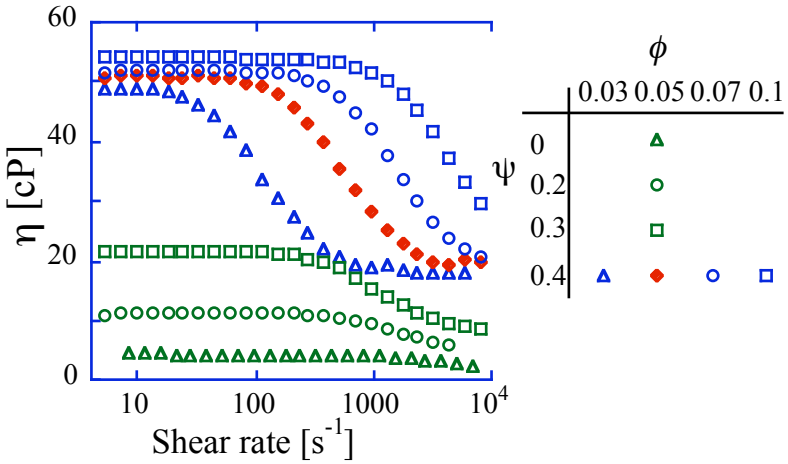
Typically <u>very</u> fluid
Not much of a response to applied shear ~ Newtonian
rapid realignment of passages relieves stress

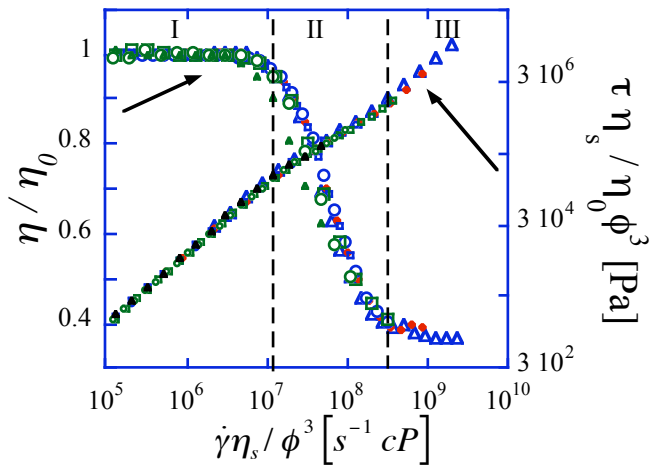
Scaling of dilution $d_3\phi$~const, volume fraction ϕ ~ 40% ... <1%
Transient birefringence for dilute samples d_3>500Å

The "sweetened" sponges

Strategy: add inert thickener to membrane solvent - viscosity η_s
Slows membrane dynamics - strengthens response to applied shear

*Rheology of Cetylpyridinium(CPCl)-Hexanol L_3 in **dextrose**-brine solvent*





Up to 40vol% dextrose in brine solvent η_s from 1 to 16.3cP

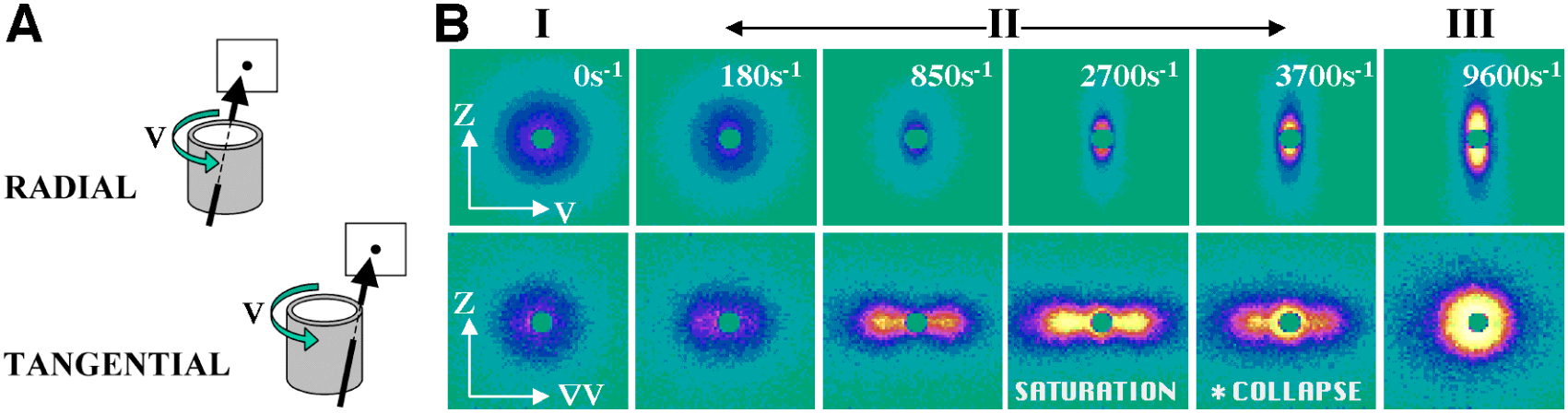
Rescaled shear rate parameter $\dot{\gamma}\eta_s/\phi^3$

Shear thins at high values

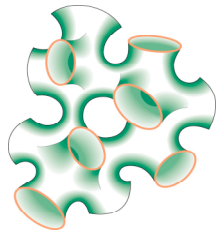
Structural response of the "sweetened" sponges

Small Angle Neutron Scattering from
ϕ=5vol% CPCl-hexanol in 40vol% dextrose-brine (η_s=16.3cP)

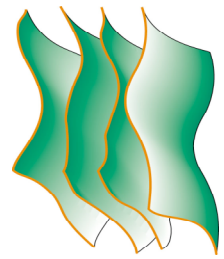
Equilibrium L_3 to Couette Shear-induced L_α transformation



Isotropic L_3



Aligned L_α

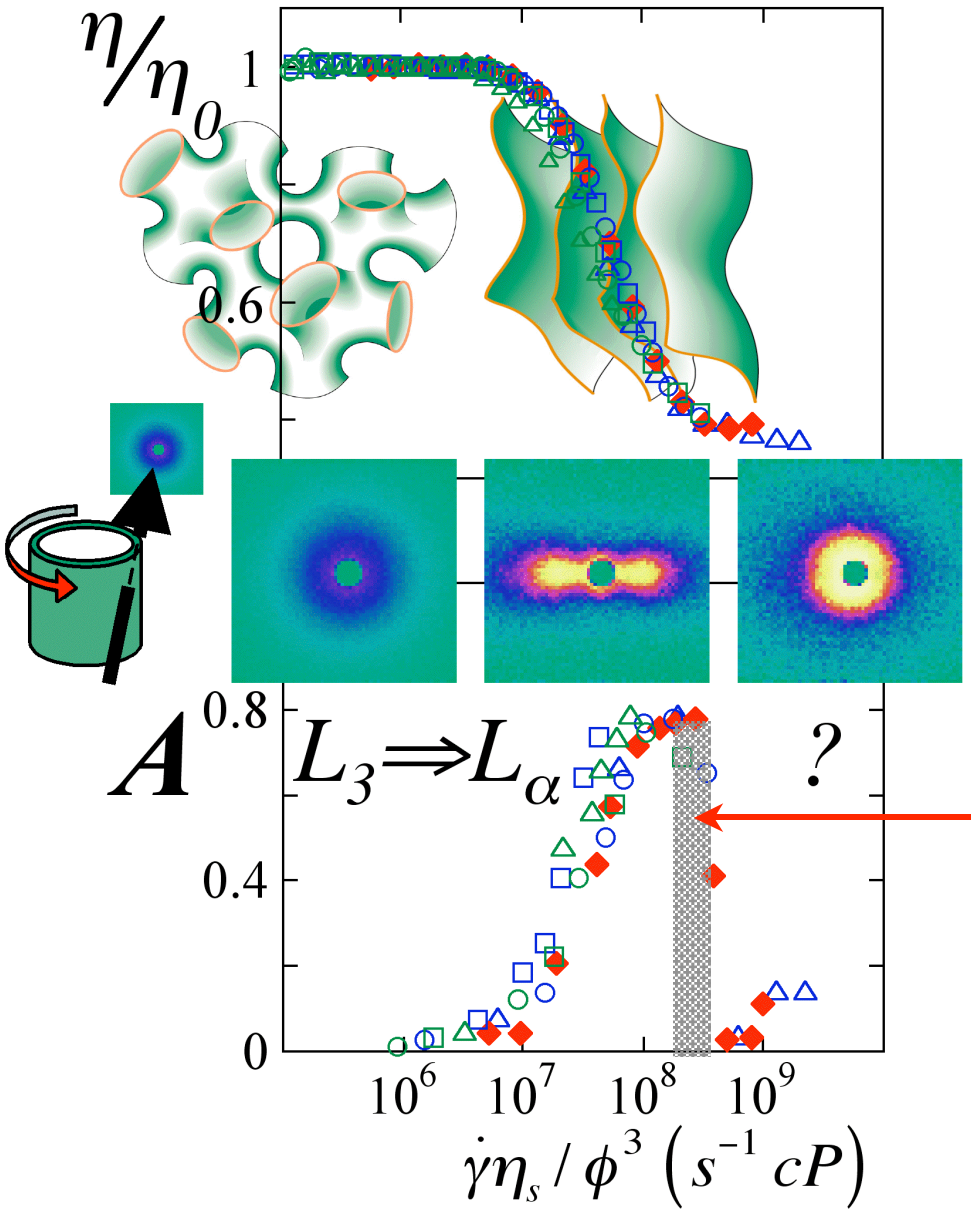


"c" alignment
membranes$\perp\nabla V$

Correlation peak Q_3 = 0.008 Å^{-1} *Bragg peak Q_α= 0.010 Å^{-1}*
passage size d_3 = 780 Å^{-1} *separation d_α = 630 Å^{-1}*
d_α / d_3~80% - same ratio as for equilibrium L_α & L_3 at same ϕ

1st shear $L_3 \rightarrow L_\alpha$ J. Yamamoto and H. Tanaka, PRL **77**, 4390(1996) *$C_{12}E_5$ ϕ<2%, our system ϕ<3-7%*

What we have so far ...



Tunable shear-induced L_3 to L_α transformation

A well characterized system

L. Porcar, W.A. Hamilton, P.D. Butler and G.G. Warr,
Physical Review Letters **89**,168301 (2002)
Langmuir **19**, 10779 (2003)

L_α signal saturates for

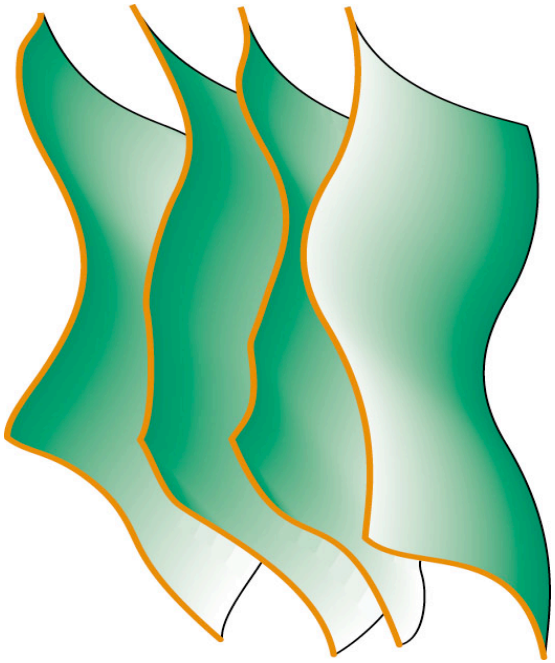
$$\dot{\gamma}\eta_s/\phi^3 \sim 2-4\times10^8\,cP/s$$

Easily accessible for high η_s

So what can we do with it?

Relaxation is now a topological transformation

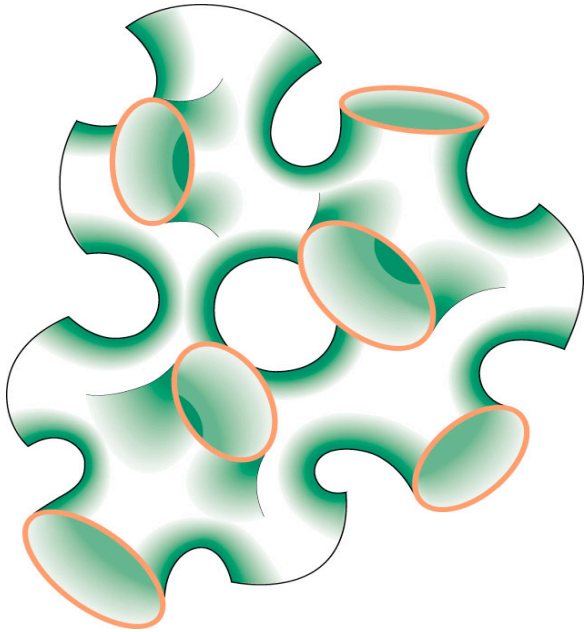
Shear-induced L_α



~ NO passages

stop shear

Equilibrium L_3

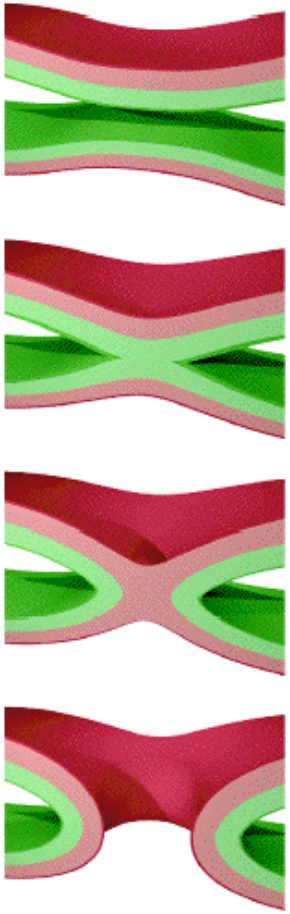


~ ALL passages
("handles" in membrane manifold)

Offers a strong clear signal
of membrane solution passage creation. So ... ?

Solution passage/pore formation ...

The "stalk" hypothesis of membrane fusion



(a) Approach to small distances

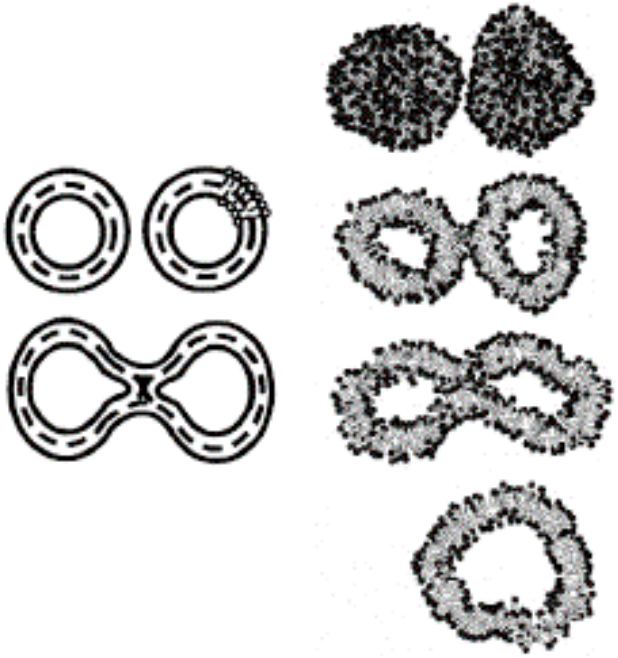
(b) Merger of proximal monolayers
"Stalk" formation

(c) Merger of distal monolayers
Stalk to TMC evolution

(d) Expansion to pore formation

from R. Jahn and H. Grubmüller,
Current Opinion in Cell Biology **14**, 488 (2002)

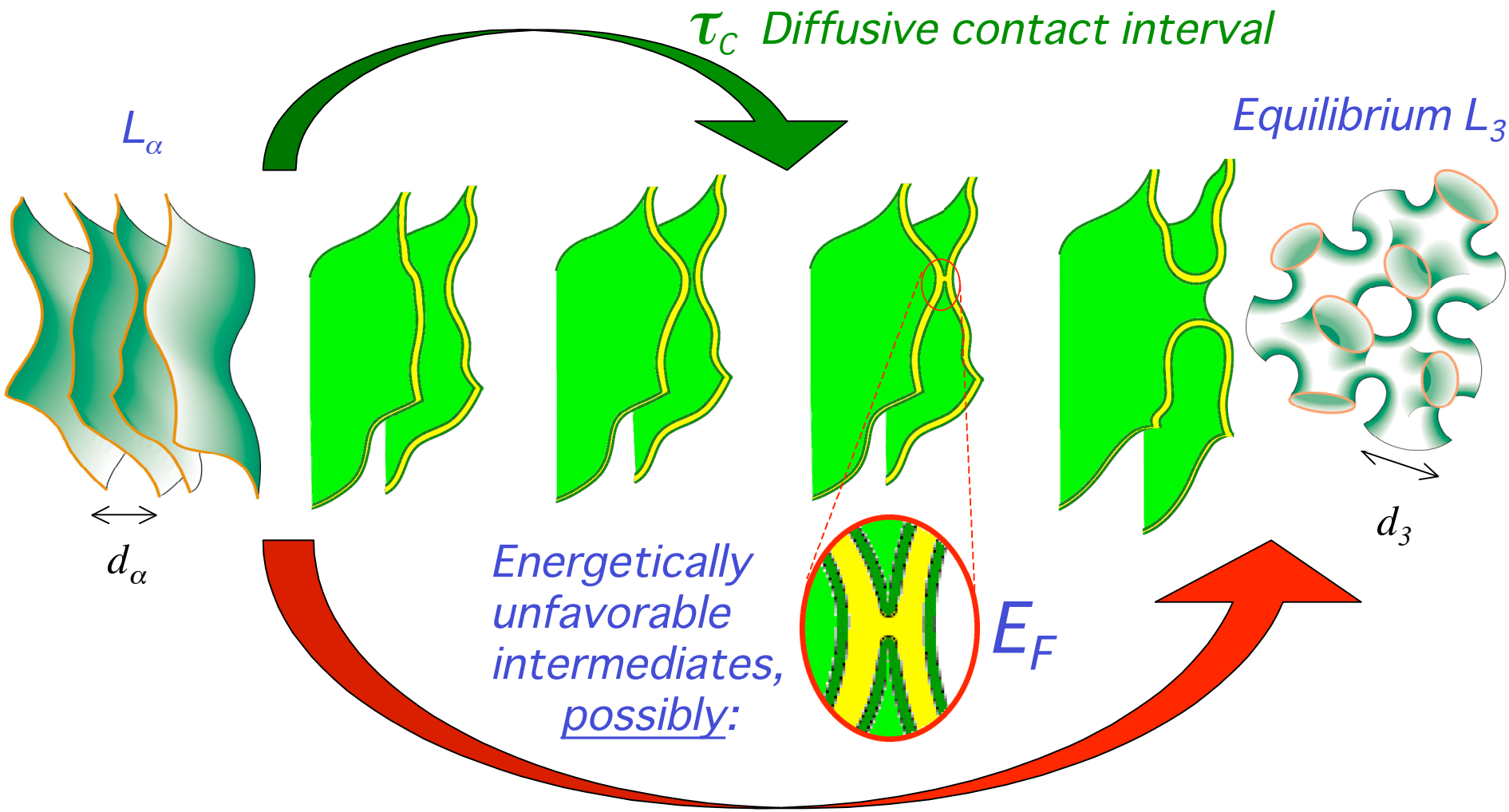
MD simultations



from H. Noguchi and M. Takasu,
J. Chem. Phys. **115**, 9547 (2001)

Stalk, TMC, or some ***other*** *intermediate structure*
⇒ energy barrier to solution passage formation

Energetics of passage formation



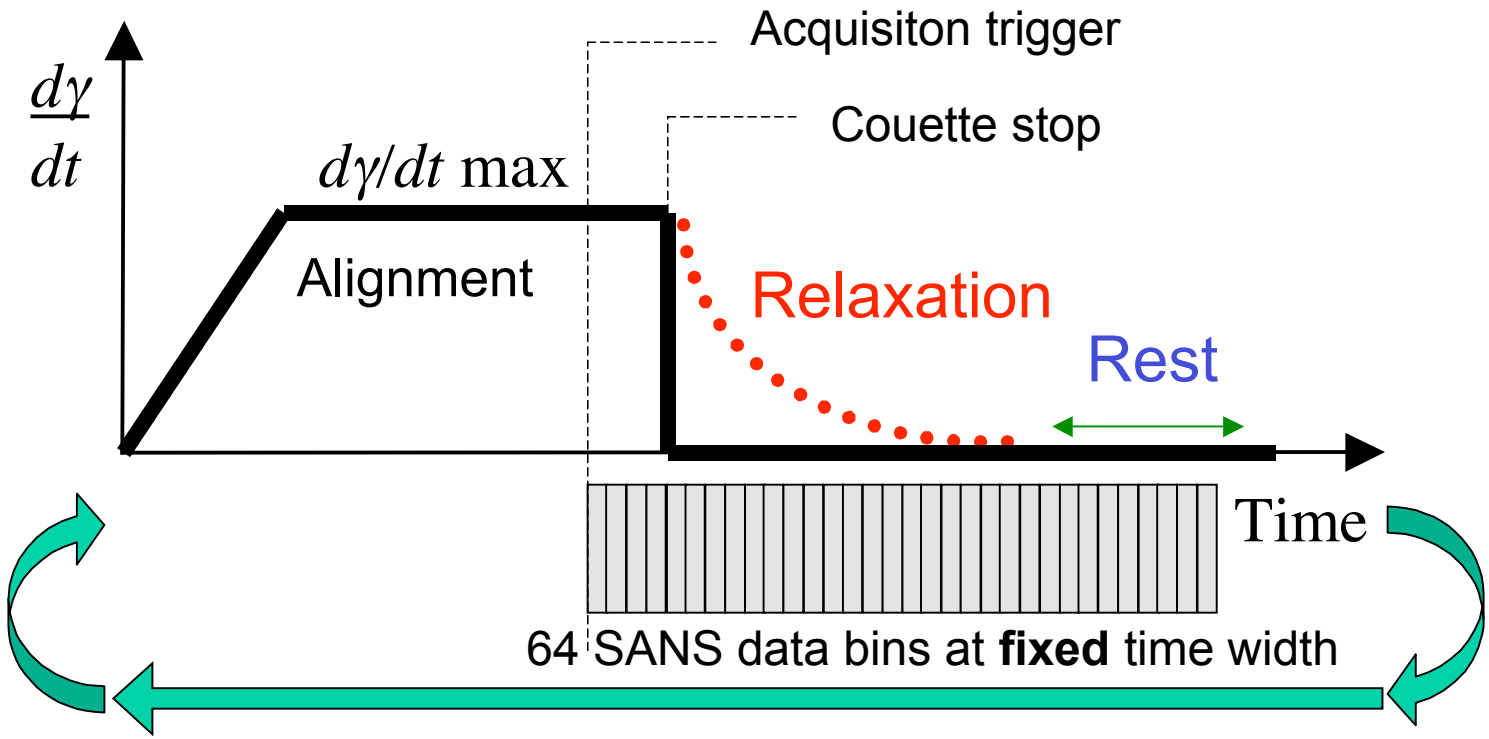
Topological relaxation time $\tau_R = \tau_C \exp[-E_F/k_BT]$

S. T. Milner, M.E. Cates and D. Roux, *J. Phys.* (Paris) 51, 2629 (1990)

When things are (a little) too fast for SANS - "t-SANS"

Even at highest viscosity full topological relaxation τ_R *~seconds*

Very fast (for SANS) ⇒ "time sliced" cycled SANS (NIST-NG7)



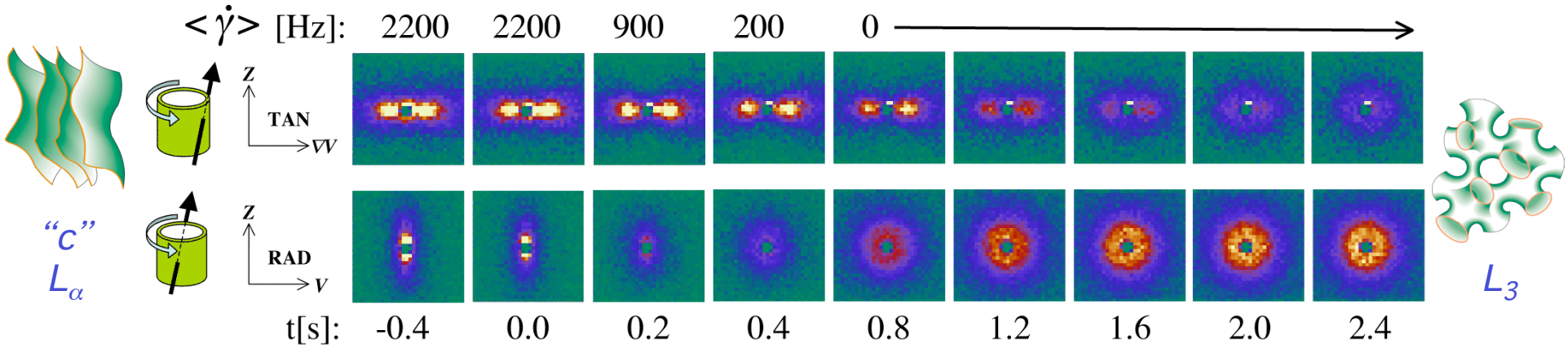
Cycle ***alignment-relaxation-rest*** *to build up statistics in SANS time bins*

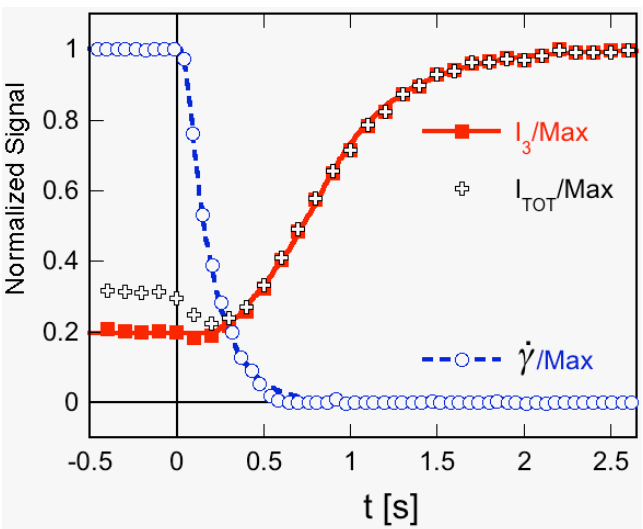
"Relaxation of a shear-induced lamellar phase measured with time resolved small angle neutron scattering",
L. Porcar, W.A. Hamilton, P.D. Butler and G.G. Warr, *Physica B* (2004) in press
Once upon a time: "Fast Relaxation of a Hexagonal Poiseuille Shear-induced Near-Surface Phase in a Threadlike Micellar Solution",
W.A. Hamilton, P.D. Butler, L.J. Magid, Z. Han and T.M. Slawecki, *Physical Review E (Rapid Communications)* **60**, 1146 (1999)

Determination of τ_R - t-SANS

t-SANS Shear-induced L_α to equilibrium L_3 relaxation

ϕ=5vol% CPCl-hexanol in 40vol% dextrose-brine (η_s=16.3cP)





Shear aligned at

$$\dot{\gamma}\eta_s/\phi^3 \sim 3\times10^8 cP/s$$

~center L_α signal plateau

When Couette cell is stopped
L_3 signal (passages) re-established

τ_R=0.40±0.08 s

Determination of τ_c

Shear-induced L_α signal saturates for

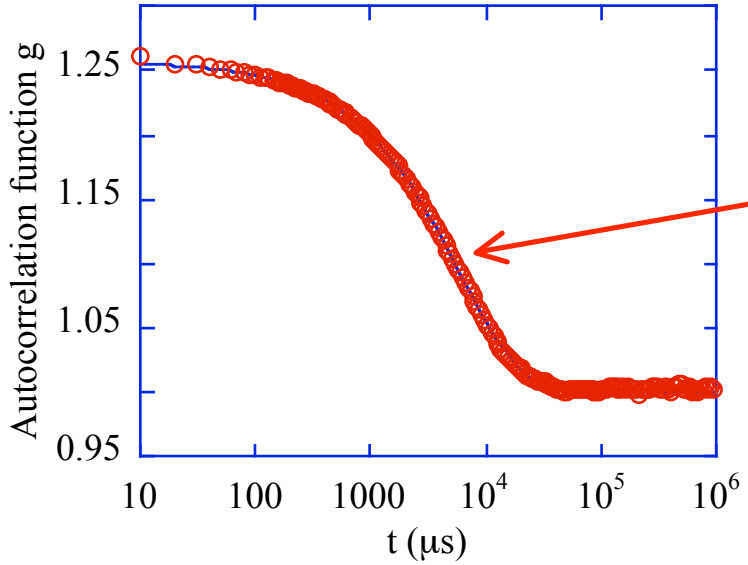
$$\dot{\gamma}\eta_s/\phi^3 \sim 2-4\times10^8 cP/s$$

Applied shear rate s^{-1}
Represents 1/time which totally frustrates
(re)formation of disrupted membrane passages

so $$\tau_c \sim \frac{1}{\dot{\gamma}_{Saturation}} \sim \frac{\eta_s/\phi^3}{2-4\times10^8 cP/s}$$

Or ... we can use Dynamic Light Scattering (DLS)
to measure membrane diffusion rates
$\Rightarrow$ time to bring membranes separated by
an average separation d_α into contact

Determination of τ_c - DLS



Homodyne autocorrelation function:

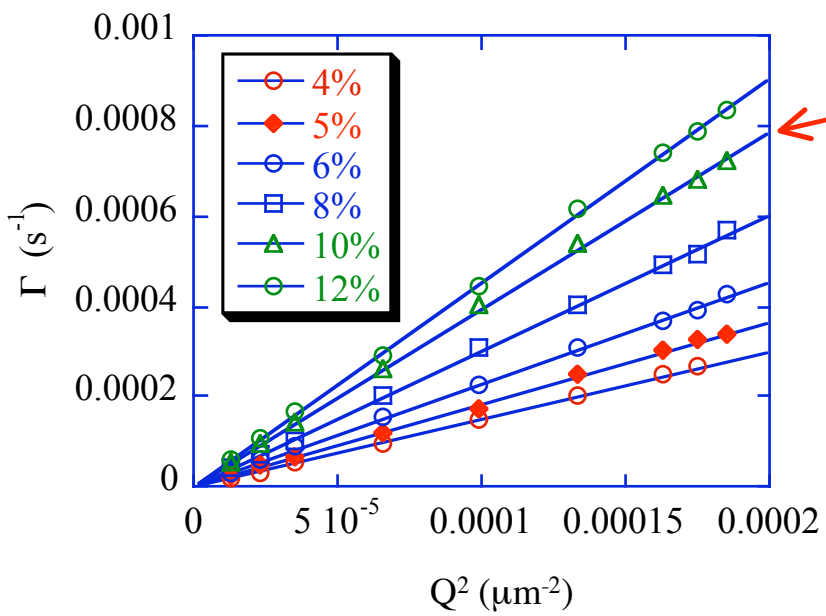
$$1 + A \exp[-2(\Gamma t)^{\beta}]$$

Stretched exponential β=0.7-0.8

$\Gamma[Q]=DQ^2$

$\Rightarrow$ simple cooperative diffusion process

$D \propto \phi$



Shear-induced L_α separation $d_\alpha \propto 1/\phi$

$\Rightarrow \tau_c \approx 1/\Gamma[Q_\alpha]=1/DQ_\alpha^2$

where $Q_\alpha=2\pi/d_\alpha \propto \phi$

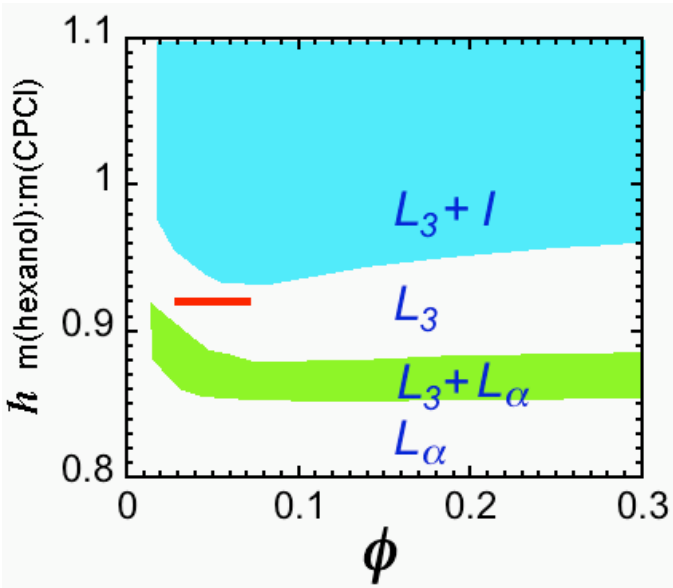
$\Rightarrow \tau_c \propto 1/\phi^3$

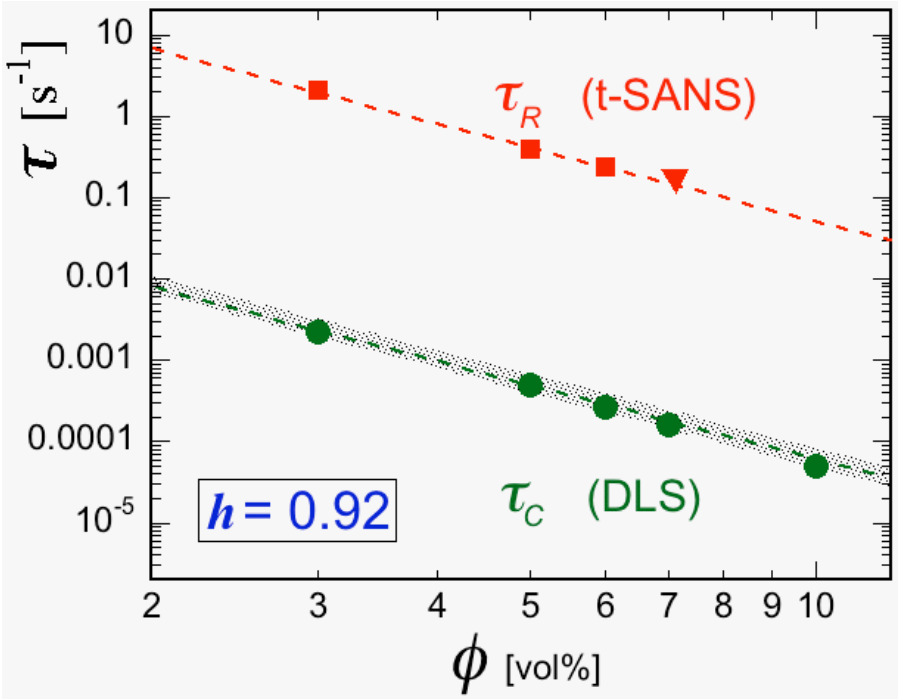
agrees with Shear-induced L_α plateau

τ_R and τ_C versus membrane volume fraction ϕ

Constant membrane composition, i.e. properties

Dilution series: $d_3\phi \approx$ const, $d_\alpha\phi \approx$ const





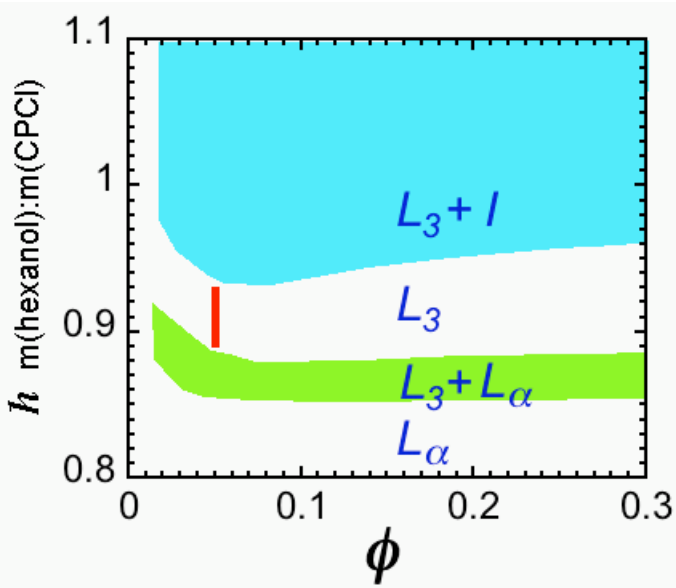
τ_R and τ_C scale as ϕ^{-3} (as you might expect per shear response)

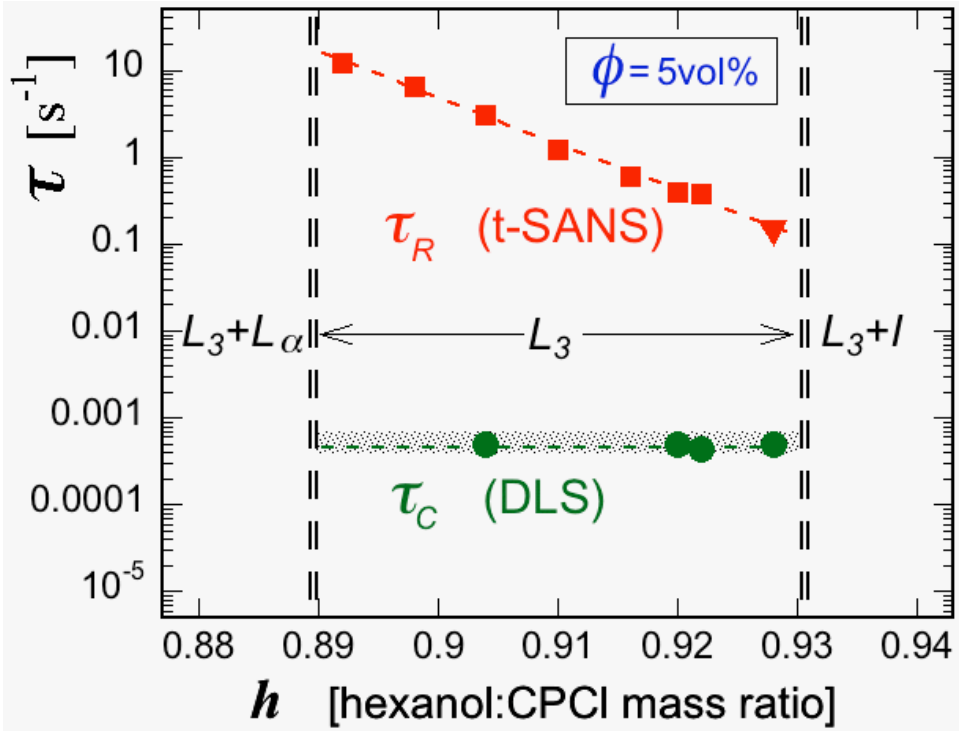
Constant Arrhenius relationship (despite >2X scaling)

$$\tau_R = \tau_C \exp[-E_F/k_BT] \Rightarrow E_F = 6.7k_BT \quad (170\ \text{meV})$$

τ_R and τ_C versus membrane composition h

Change membrane composition, i.e. properties across L_3 phase region for constant ϕ





Increasing hexanol to CPCl mass ratio h

⇒ Increasing Gaussian curvature modulus of membranes

⇒ <u>Decreasing</u> energy cost of passages (and stalk structures)

4% increase in h $E_F = 10.3 k_B T$ (260 meV) down to $5.8 k_B T$ (150 meV)

Summary/Conclusions/Future

"Sweetened" Cetylpyridinium-Hexanol/dextrose-brine L_3 sponges
Viscosity tuning $\Rightarrow$ accessible shear-induced L_3 to L_α transition

Topological relaxation of shear-induced to L_α to equilibrium L_3

t-SANS measurement of membrane passage formation time τ_R

DLS determination and alignment shear rates agreement

on interval between diffusion driven membrane contacts τ_C

Activation energy for membrane fusion (handle creation)

$$\tau_R = \tau_C \exp[-E_F/k_BT] \Rightarrow E_F \sim 5 - 10\ k_BT$$

E_F constant wrt ϕ ($\Rightarrow$ constant barrier state - stalk/TMC ?)

E_F linear decrease wrt h across L_3 phase region
($\Rightarrow$ Gaussian curvature modulus of membranes)

Future: Application of technique to biological system?
Identify (or engineer) lipid system with suitable relaxation mode