

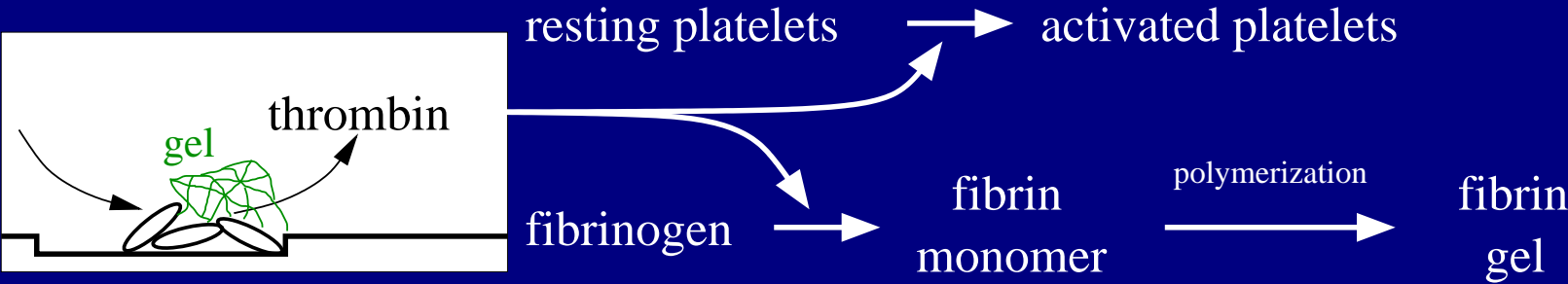
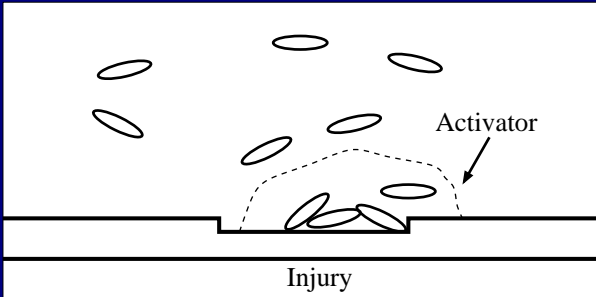
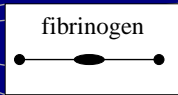
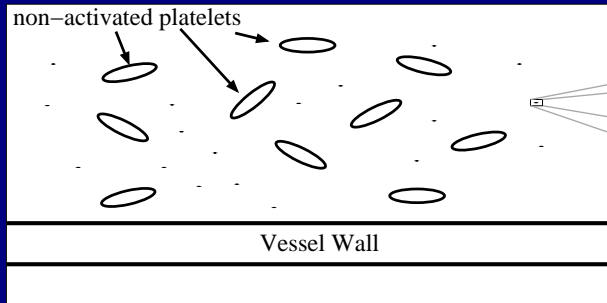
# **Modeling Fibrin Gel Formation: Continuous to Discrete**

Bob Guy

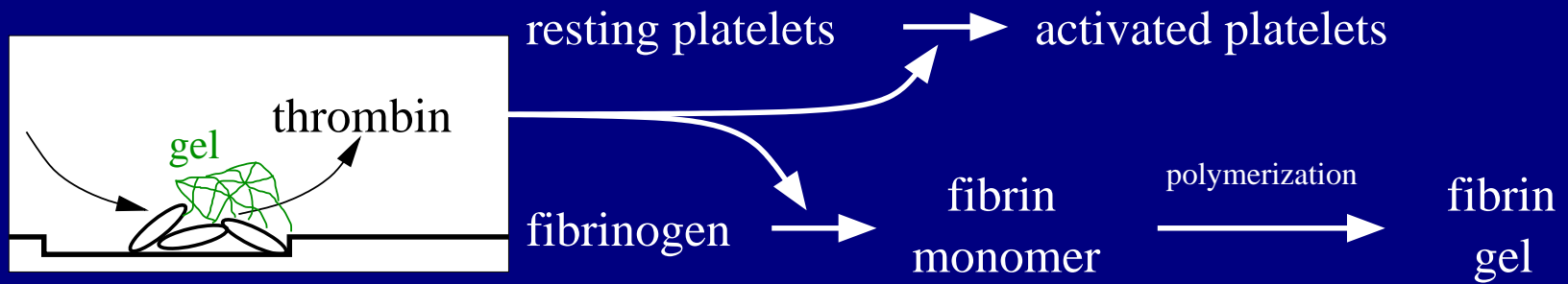
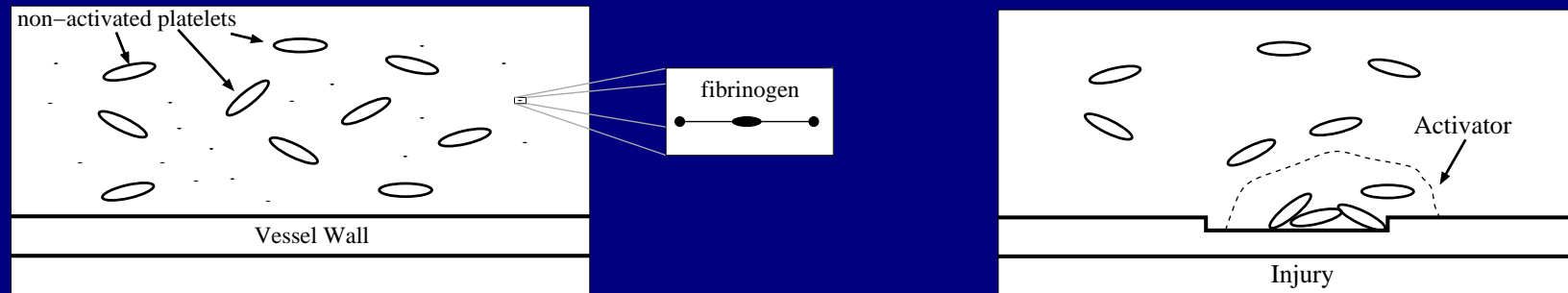
Department of Mathematics  
University of California Davis

March 26, 2008

# What is Fibrin?



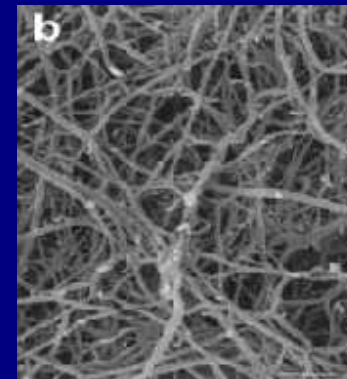
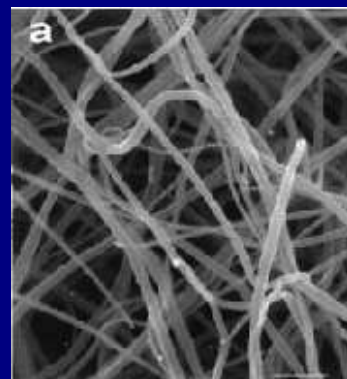
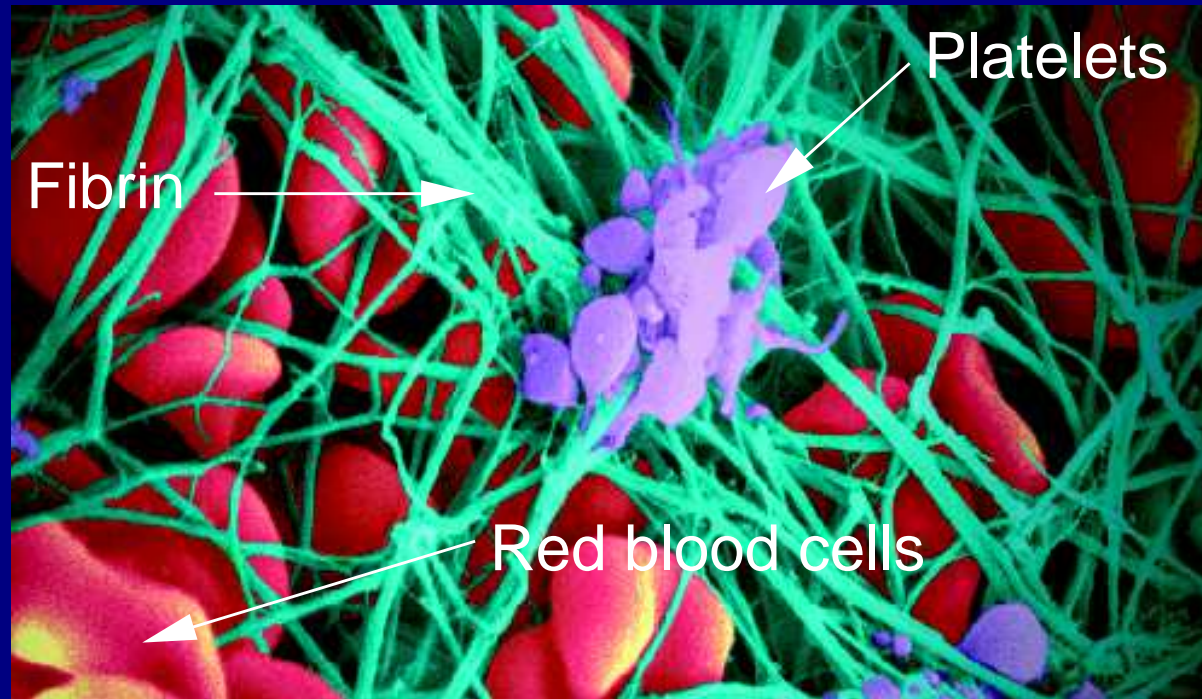
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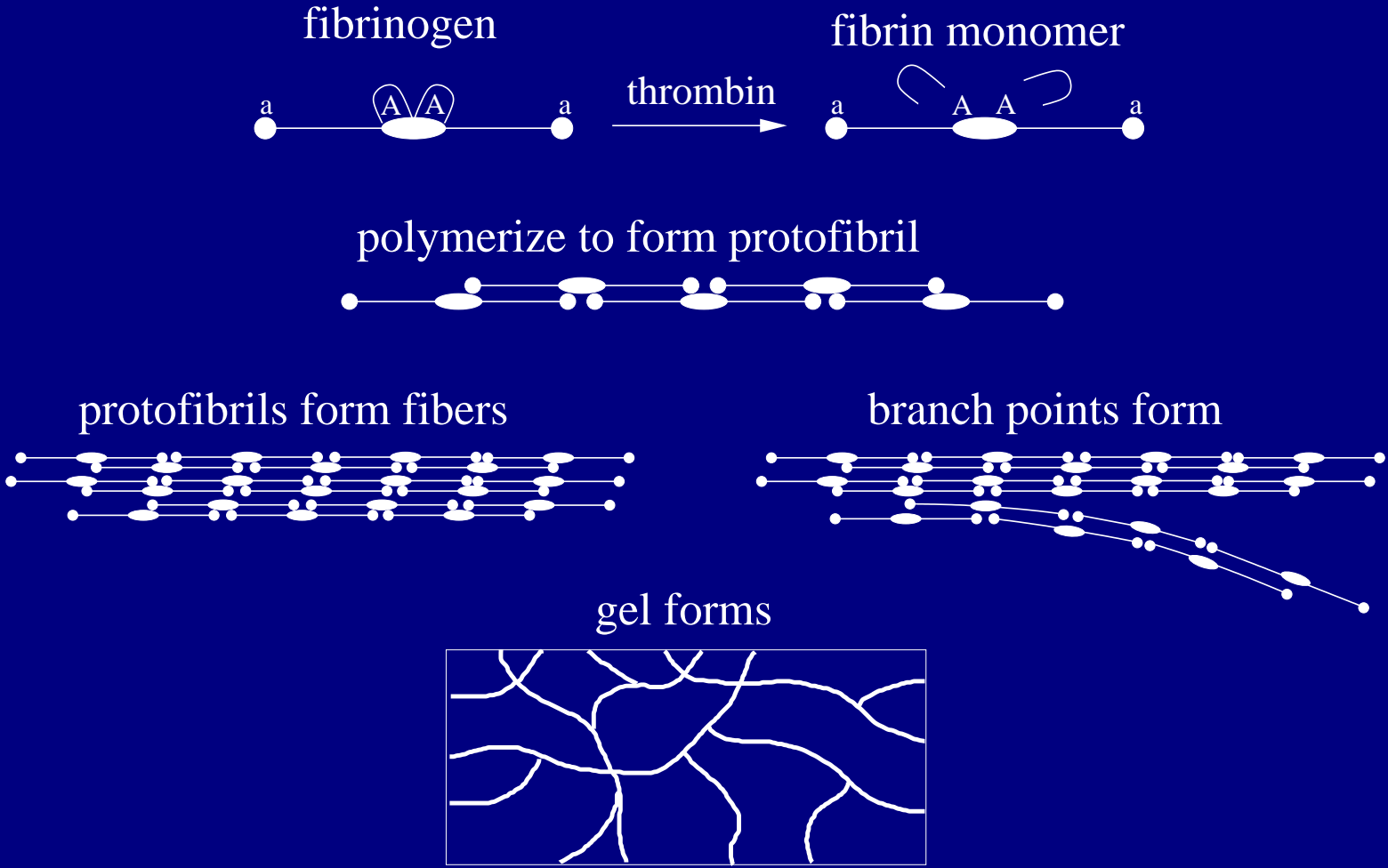
- ♦ High shear rates ( $\approx 1000 \text{ s}^{-1}$ ) clots mostly platelets
- ♦ Low shear rates ( $\approx 100 \text{ s}^{-1}$ ) clots mostly fibrin

Why?

# Pictures



# Fibrin Polymerization



# Gelation – Classical Theory

What distinguishes a gel from polymers in solution?

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- ♦ Intuitively, gel is an “infinitely large molecule”

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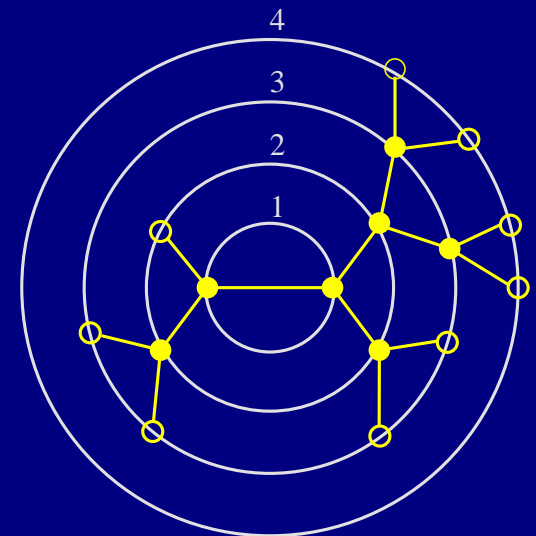
- ♦ Intuitively, gel is an “infinitely large molecule”
- ♦ Mathematical conditions derived by Flory (1941) and Stockmayer (1943)
- ♦ At gel point, average molecular weight becomes unbounded

$\alpha$  = Probability of branching

$Y_i$  = Expected number of branches

$Y_{i+1}/Y_i \approx 2\alpha$

$\alpha_c = 1/2$



# Gelation – Classical Theory

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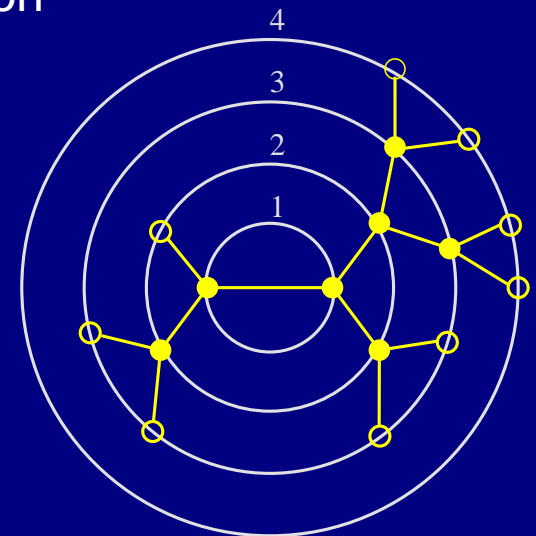
- ♦ Intuitively, gel is an “infinitely large molecule”
- ♦ Mathematical conditions derived by Flory (1941) and Stockmayer (1943)
- ♦ At gel point, average molecular weight becomes unbounded
- ♦ Early gelation theories foundations of percolation theory
- ♦ Statistical theories not well suited for kinetic gelation

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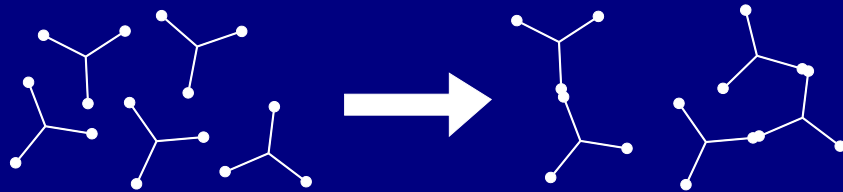
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$$\alpha_c = 1/2$$



# Simple Kinetic Gelation Model



monomers with  $n$  binding sites  
 $c_k$  concentration of  $k$ -mers

$$\frac{dc_k}{dt} = \underbrace{\sum_{i+j=k} a_i a_j c_i c_j}_{\text{formation of k-mer from smaller polymers}} - \underbrace{a_k c_k R}_{\text{k-mer reacts with other polymer}}$$

formation of k-mer from smaller polymers

k-mer reacts with other polymer

$$R = \sum_j a_j c_j + R_g$$

total binding sites

$$a_k = (n - 2)k + 2$$

Number of binding sites on k-mer  
 (assuming no cycles)

# Generating Function

Infinite set of ODEs for concentrations

$$\frac{dc_k}{dt} = \sum_{i+j=k} a_i a_j c_i c_j - a_k c_k R$$

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$$g(z, t) = \sum_k z^{a_k} c_k$$

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Introduce generating function

$$g(z, t) = \sum_k z^{a_k} c_k$$

Generating function satisfies the PDE

$$\frac{\partial g}{\partial t} = \frac{1}{2} \left( \frac{\partial g}{\partial z} \right)^2 - z \left( \frac{\partial g}{\partial z} \right) R$$

Moments obtained from derivatives at  $z = 1$

## More on Moments

$$M_0 = \sum c_k \quad \text{total amount of clusters}$$

$$M_1 = \sum k c_k \quad \text{total mass (total monomers)}$$

$$M_2 = \sum k^2 c_k \quad \text{????????}$$

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### Understanding $M_2$ :

$$k c_k \quad \text{number of monomers that are in } k\text{-mers}$$
$$p_k = \frac{k c_k}{M_1} \quad \text{probability a monomer is part of a } k\text{-mer}$$
$$M_w = \sum k p_k = \frac{M_2}{M_1} \quad \text{weight-average molecular weight}$$

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$M_2 \rightarrow \infty$  indicates gelation

# Gelation

Second moment related to  $g_{zz}(1, t)$

$$Y = g_{zz}(1, t) - g_z(1, t) = (n - 2)^2 M_2 + 2(n - 2) M_1$$

# Gelation

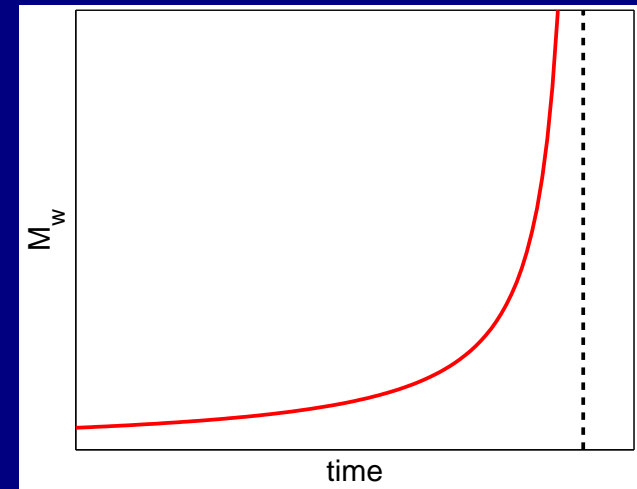
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$$\frac{dY}{dt} = Y^2$$

$$Y(t) = \frac{Y(0)}{1 - Y(0)t}$$

$$M_2 \rightarrow \infty \text{ as } t \rightarrow t_g = \frac{1}{Y(0)}$$



# Gelation

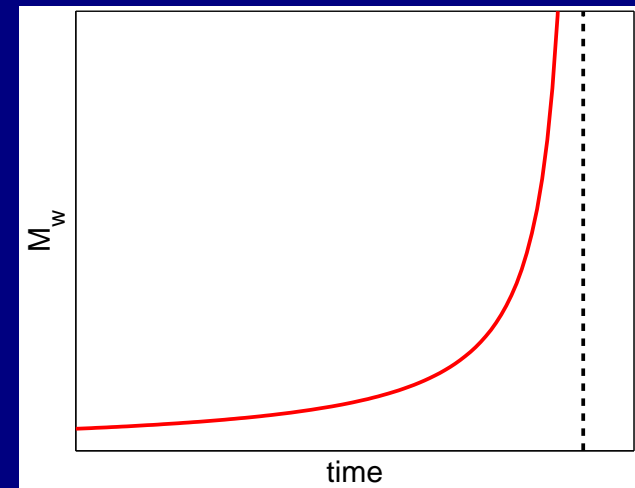
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$t_g$  is the gel time

## Gelation with Source and Sink

Given reaction rate  $\alpha$ , source rate  $S_k$ , removal rate  $\gamma$ , can gel form?

$$\frac{dc_k}{dt} = \alpha \left( \sum_{i+j=k} a_i a_j c_i c_j - a_k c_k R \right) + S_k - \gamma c_k$$

$$\frac{\partial g}{\partial t} = \alpha \frac{1}{2} \left( \frac{\partial g}{\partial z} \right)^2 - \alpha z \left( \frac{\partial g}{\partial z} \right) R + P(z) - \gamma g$$

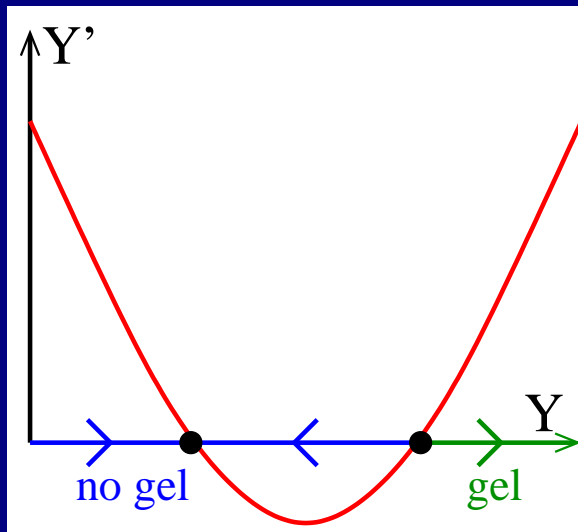
$$\frac{dY}{dt} = \alpha Y^2 - \gamma Y + \Pi$$

Behavior depends on roots of  $\alpha Y^2 - \gamma Y + \Pi = 0$

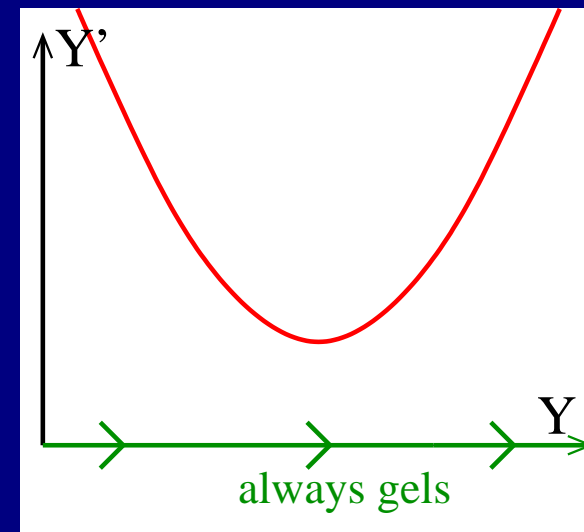
# Gelation Condition

$$\frac{dY}{dt} = \alpha Y^2 - \gamma Y + \Pi$$

$$\gamma > \sqrt{4\alpha\Pi}$$

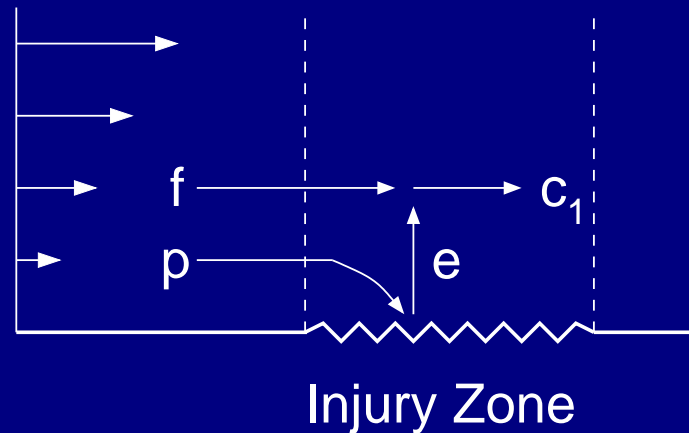


$$\gamma < \sqrt{4\alpha\Pi}$$



**Result:** For initial conditions of no polymer, gel forms if and only if  $\gamma < \sqrt{4\alpha\Pi}$ .

# Chemically Induced Gelation in a Shear Flow



- ✦ Prothrombin ( $p$ ) delivered from upstream
- ✦ Converted to thrombin ( $e$ ) near injury
- ✦ Thrombin converts fibrinogen ( $f$ ) to fibrin monomer ( $c_1$ )
- ✦ Monomers polymerize and are removed by the flow
- ✦ Average in  $x$ -direction over injury to give 1D model

# Model Equations

## Enzymes

$$p_t = Dp_{yy} - \frac{v}{L} (p - p_{up})$$

$$e_t = De_{yy} - \frac{v}{L} e - k_e e$$

## Reaction at $y = 0$

$$-Dp_y = -\frac{k_p p}{k_{sp} + p}$$

$$-De_y = \frac{k_p p}{k_{sp} + p}$$

## Fibrinogen & Fibrin

$$f_t = Df_{yy} - \frac{v}{L} (f - f_{up}) - \frac{k_f e f}{k_{sf} + f}$$

$$c_k t = \sum_{i+j=k} a_i a_j c_i c_j - a_k c_k R - \frac{v}{L} c_k + \delta_{1k} \left( \frac{k_f e f}{k_{sf} + f} \right)$$

## Velocity

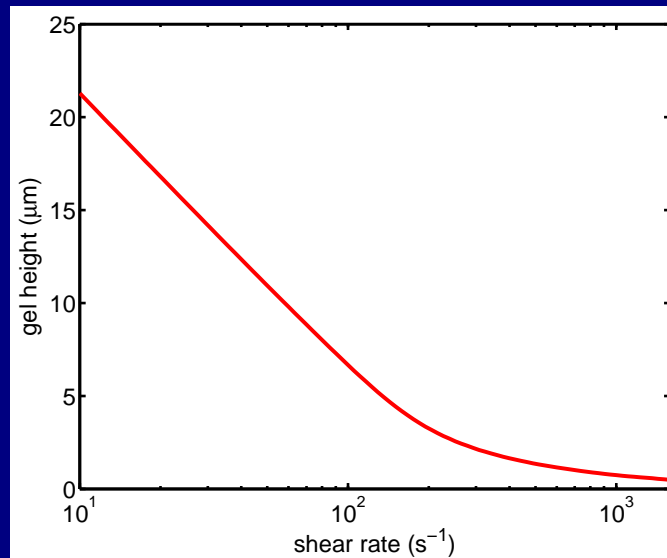
$$\mu v_{yy} - \xi(g)v = 0$$

## Finding the Maximum Gel Height

- ♦ Suppose gel is a given height
- ♦ Prothrombin, thrombin, fibrinogen in steady-state
- ♦ Check gelation condition based on source and sink strengths
- ♦ Increment gel height until no gelation

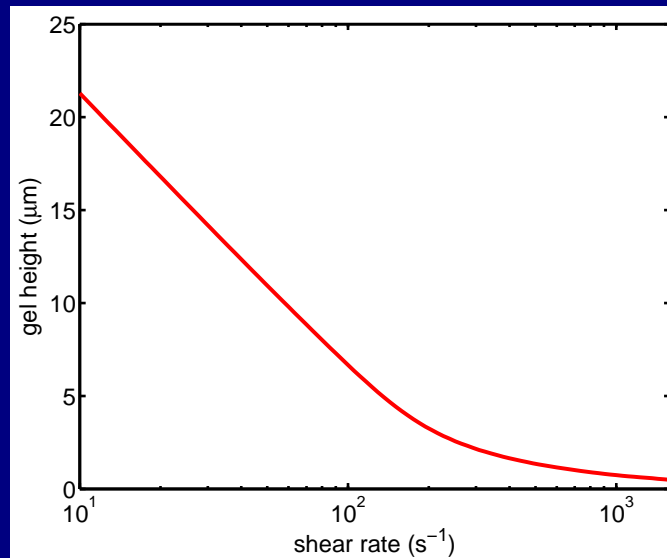
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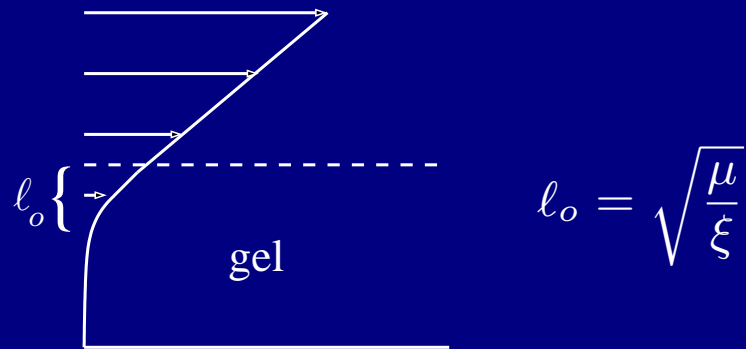
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- ♦ Possible mechanism for limiting growth
- ♦ Different behavior at low and high shear
- ♦ Explore what determines this behavior

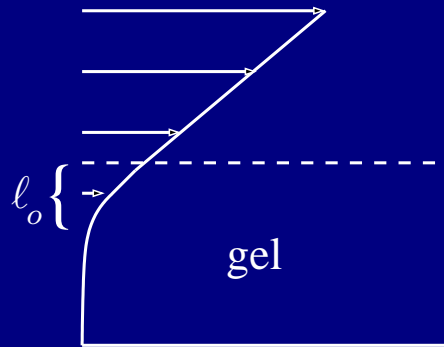
# Velocity Profile & Gel Permeability



for  $h \gg l_o$

$$v = \begin{cases} \gamma l_o \exp\left(\frac{y-h}{l_o}\right) & y < h \\ \gamma(y-h+l_o) & y \geq h \end{cases}$$

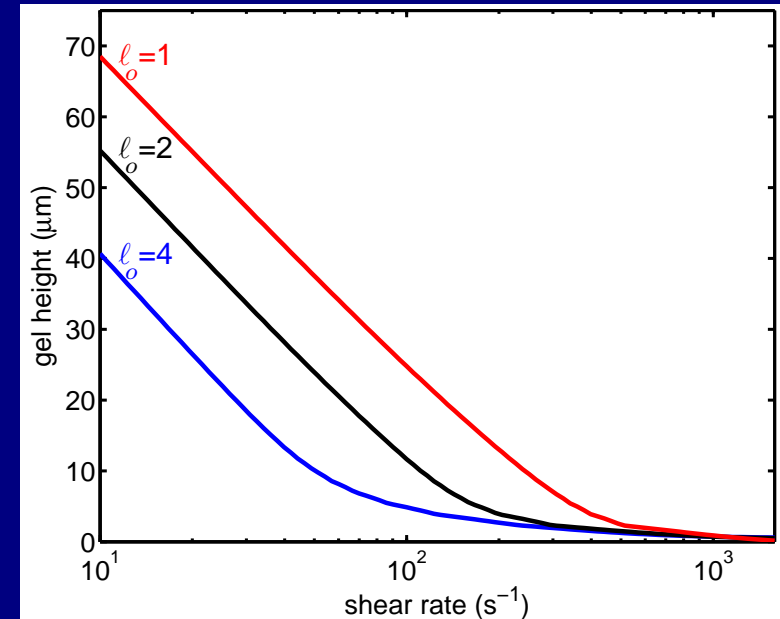
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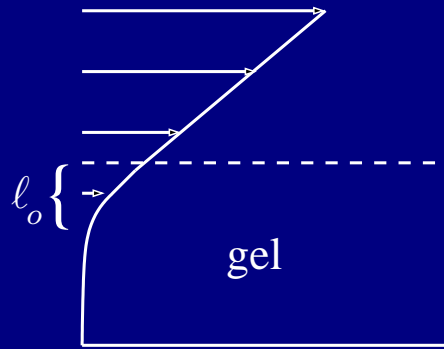
$$l_o = \sqrt{\frac{\mu}{\xi}}$$

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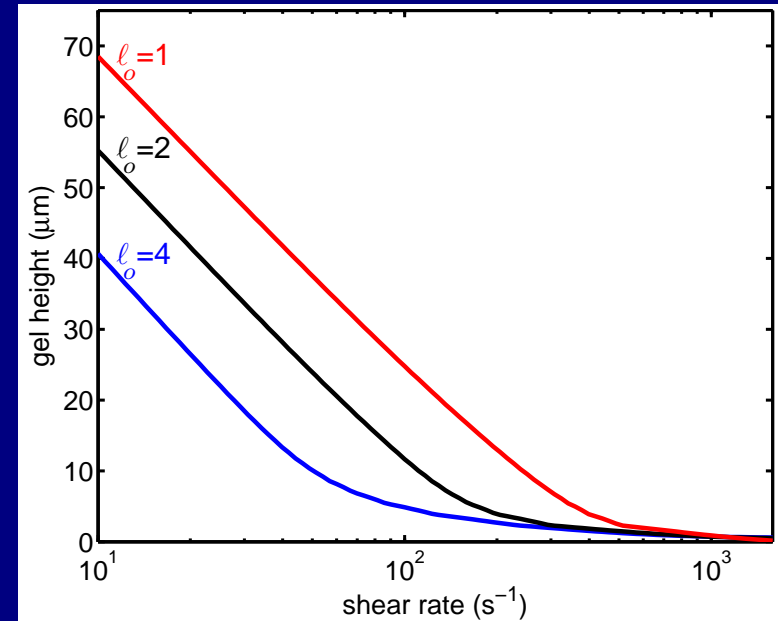
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Permeability determines transition between low and high shear

# Gel Permeability

$$v_h = \gamma \ell_o \tanh \left( \frac{h}{\ell_o} \right)$$

Low shear

$$h \gg \ell_o, \quad v_h \approx \gamma \ell_o$$

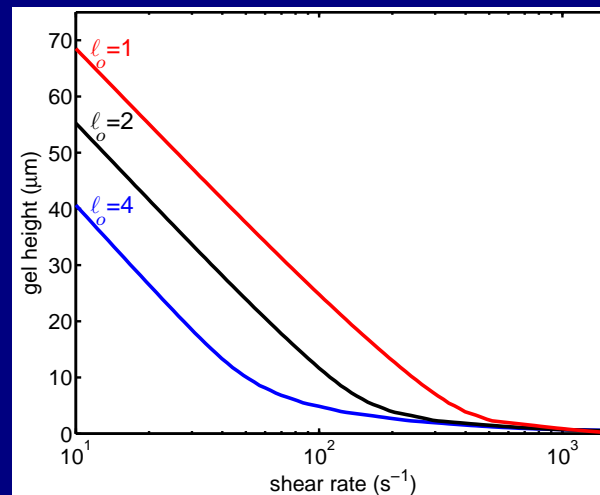
Velocity independent of  $h$

High shear

$$h < \ell_o, \quad v_h \approx \gamma h$$

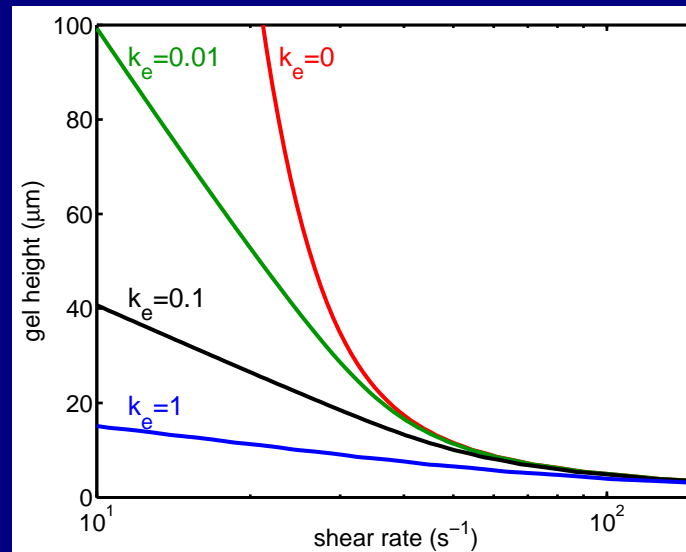
Velocity independent of  $\ell_o$

Transition occurs when  $h \approx \ell_o$



# Thrombin Inhibition

Thrombin inhibition rate ( $k_e$ ) controls growth at low shear



- ♦ At high shear, removal by flow faster than inhibition
- ♦ At low shear (thick gel), flow in gel is negligible  
Thrombin is degraded as it diffuses to the gel interface

# Steady State Model Summary/Questions

## Summary

- ♦ Thrombin inhibition limits gel height at low shear
- ♦ At high shear, removal by flow prevents significant gel growth
- ♦ Gel permeability determines transition shear rate

# Steady State Model Summary/Questions

## Summary

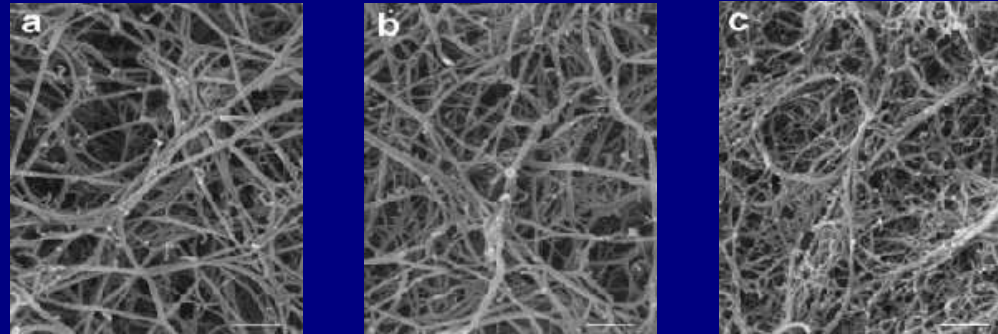
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## Questions

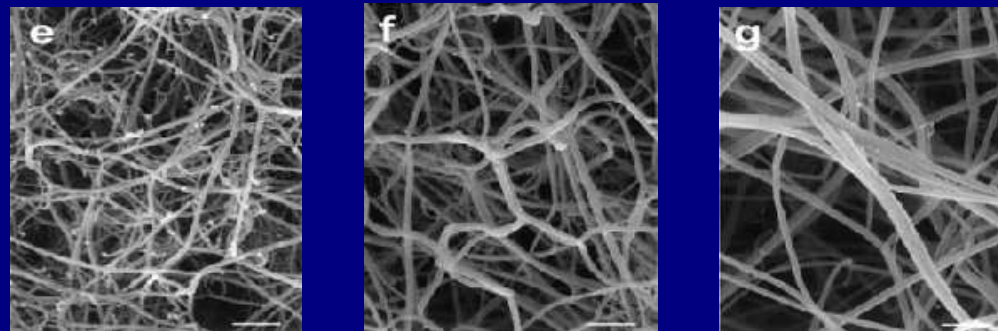
- ♦ Why steady state model? — dynamic gel density is difficult
- ♦ Would results be different if gel density is inhomogeneous?
- ♦ Permeability is important, but what does it depend on?

# Fibrin Structure

## Different thrombin concentrations



## Different CaCl<sub>2</sub> concentrations



Images from J. Weisel

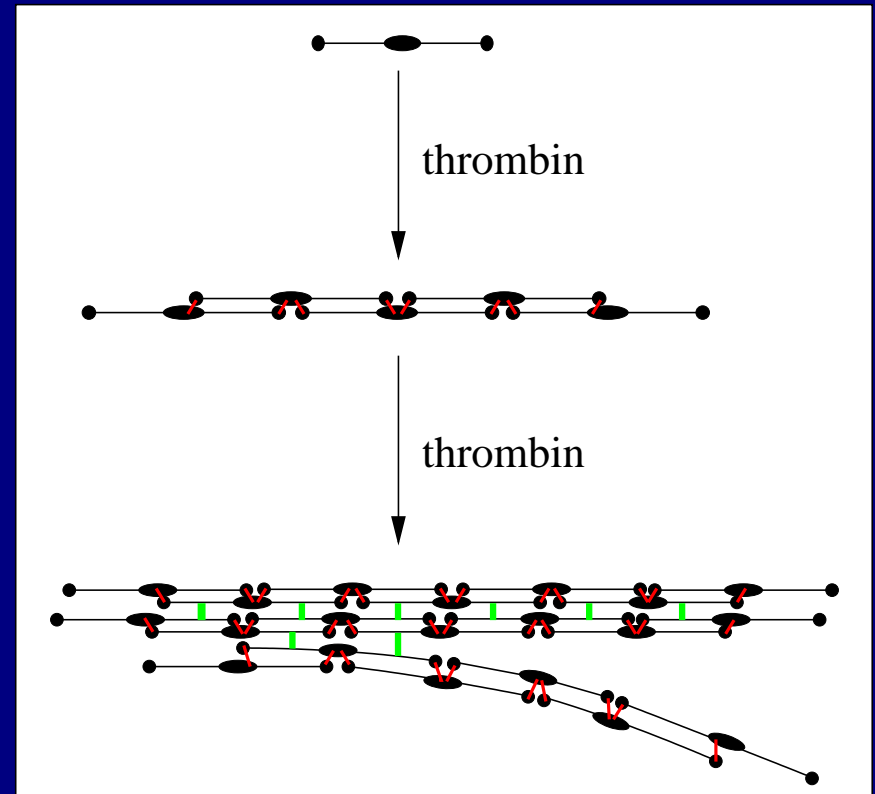
# Fibrin Polymerization Revisited

## Two kinds of bonds

1. Linear bonds
2. Lateral bonds

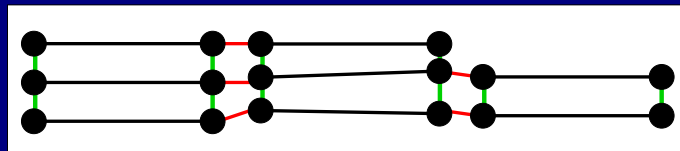
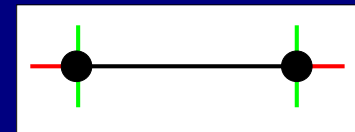
## Lateral bonds differ from linear

- ♦ Thrombin kinetics
- ♦ Formation/breaking rates
- ♦ Response to force loading



# Discrete MC Polymerization Simulations

- ◆ Represent fibrin monomer as dumbbell with a stiff spring
- ◆ Each monomer has two linear and four lateral binding sites
- ◆ Monomers experience random thermal forces
- ◆ Bonds form and break randomly
- ◆ Develops a network of springs and hinges



[Movie](#)

[B.A.M.](#)

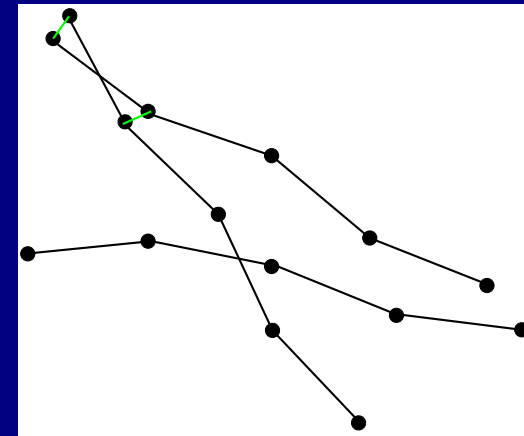
# Simplified Model

- Previous simulations require a very small time step – not possible to simulate on physical time scale without simplifying model
- Since little is known about lateral aggregation, we explore this by itself.

## Simplified model

- Represent fixed length protofibrils using bead-spring model
- Lateral bonds can form at any angle

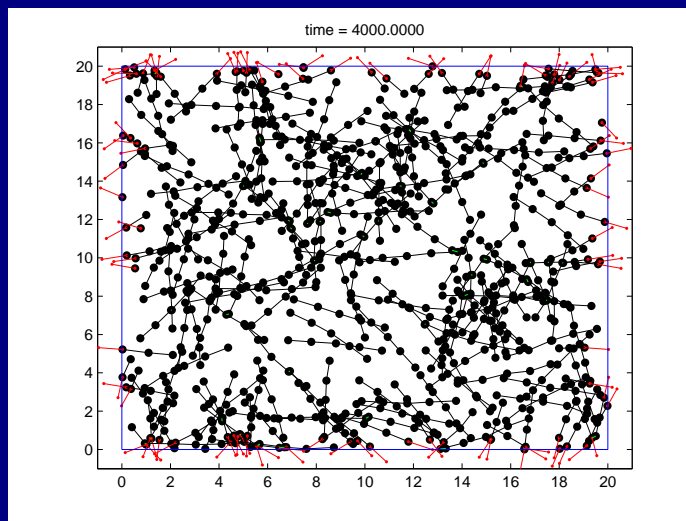
[Movie](#)



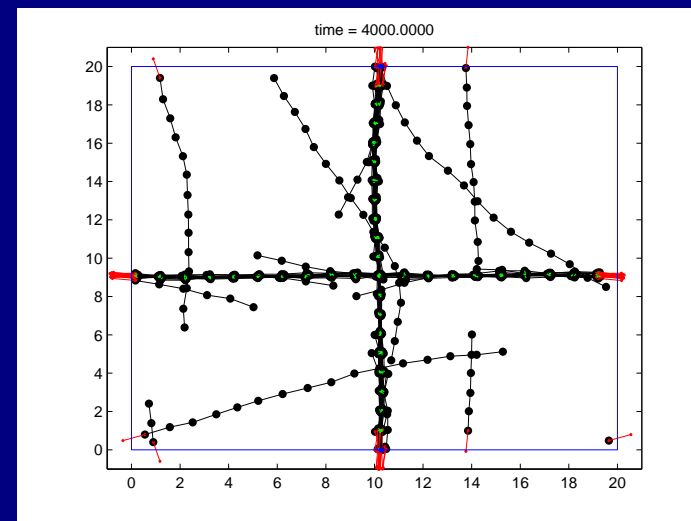
# Exploring Length

Simulate different length protofibrils for fixed monomer concentration

Short protofibrils

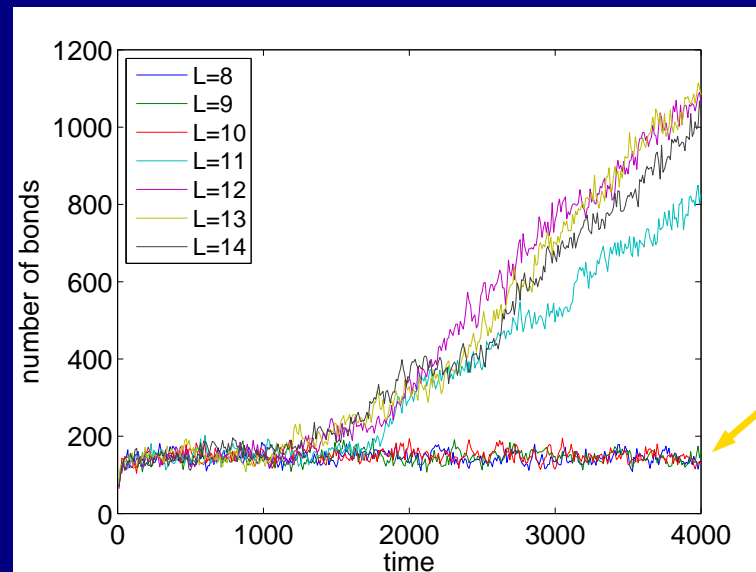


Long protofibrils



# Phase Transition?

Plot of number of bonds for different length filaments

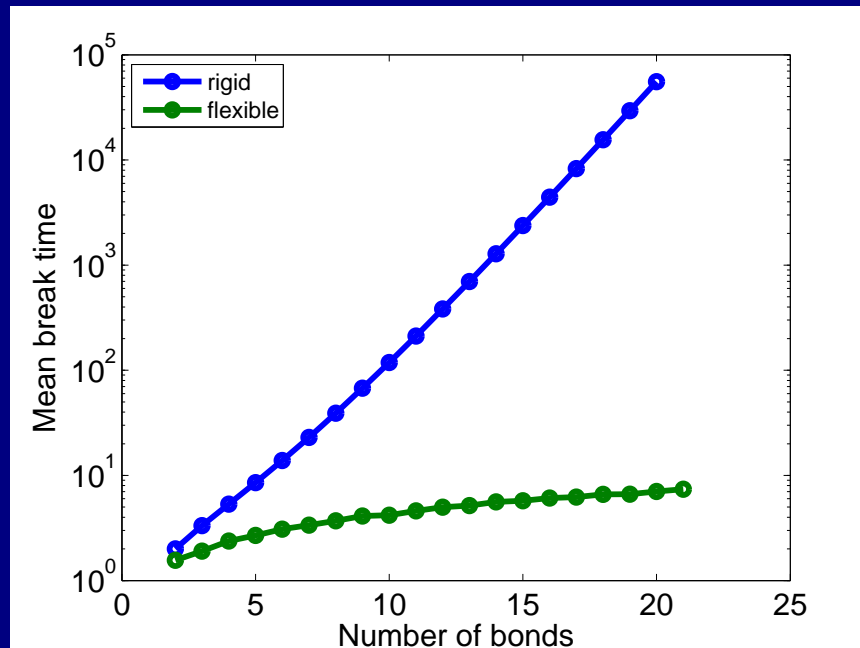
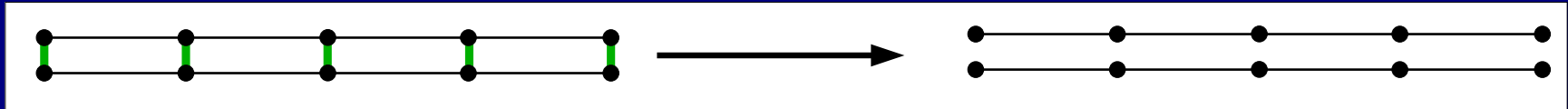


short polymers  
do not bundle  
for  $L < 11$

- ♦ There appears to be a critical length for lateral aggregation
- ♦ Observed experimentally, but it is not understood

# Mean Rupture Time

Measure the mean time of fully zippered pair to separate



- ♦ Very slow increase in breaking time for flexible filaments
- ♦ No obvious transition in length
- ♦ Probably not a two filament phenomenon

bundle

no bundle

# Summary

## Continuous Model

- ♦ Model of flow, chemistry, and gelation used to explore fibrin clot growth
  - ♦ Key parameters: shear rate, thrombin inhibition rate, and gel permeability
  - ♦ Working on time dependent version

## Discrete Model

- ♦ Using discrete models to explore development of microstructure
  - ♦ How do branch points form?
  - ♦ Little is known about lateral aggregation
- ♦ Working to understand length dependent bundle formation

## Acknowledgments

Joint work with John Weisel (Penn) and Aaron Fogelson (Utah).