Cell motility and the mechanics of the extra-cellular matrix

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Collaborators & References

- Xiaoming Mao, University of Michigan
- Jingchen Feng, Rice University
- Herbert Levine, Rice University
- Refs:
  - A. M. Stein, D. A. Vader, D. A. Weitz, and L. Sander, *The micromechanics of three-dimensional collagen-I gels*, Complexity, 16, 22 (2011).
  - L. M. Sander, *Alignment Localization in Non-linear Biological Media,* J. Biomech. Eng. **135**, 071006 (2013).
  - L. M. Sander, *Modeling Contact Guidance and Invasion by Cancer Cells,* Cancer Research, in press (2014).
  - J-C Feng, X. Mao, L. M. Sander, arXiv.1402.2998

Outline

- Cells move through tissue in development, wound healing, spread of cancer.
- When cells move they deform tissue; often into the non-linear regime. Consider only the ECM.
  - Contracting cells *produce alignment*.
  - Motile cells *follow alignment* (contact guidance).
- First step to study this: a continuum, Landau-de Gennes theory for the non-linear elasticity of biopolymer gels.
  - Part of the order parameter: induced *nematic* order.
  - Our idea: non-linear elastic behavior arises from the onset of fiber alignment with induced strain.
- Compare to simulations of a disordered lattice model for biopolymers: do homogeneous deformations such as shear and extension.
- For cells, consider localized perturbations –a simple model for a contracting cell in ECM.

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Motivation: ECM *alignment* guides cancer cell invasion *in vitro*

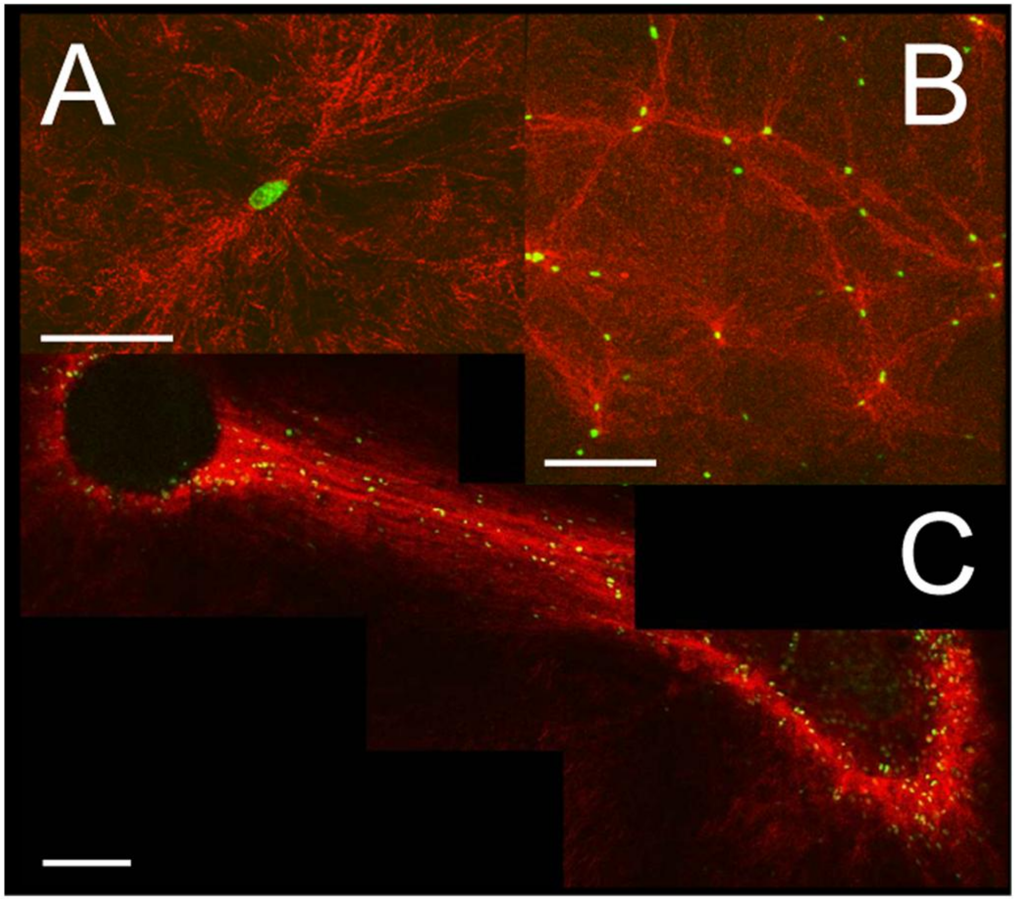

Figure 1. Collagen gel morphological changes induced by presence of cells. (A) Single U87 glioblastoma cell in a collagen network 10 hours after gel polymerization. bar = 50 mm. (B) Several U87 cells on the surface of a collagen gel 10 hours after gel polymerization. bar = 200 mm. (C) Two cell colonies embedded in a collagen matrix 48 hours after gel polymerization. bar = 200 mm. Fibers (artificial red color) are imaged through confocal reflectance; cell nuclei (green) are labeled with a GFP-histone heterodimer.
doi:10.1371/journal.pone.0005902.g001

D. Vader, A. Kabla, D. Weitz, and L. Mahadevan, *Strain-induced alignment in collagen gels, PloS one,* ***4 (2009).***

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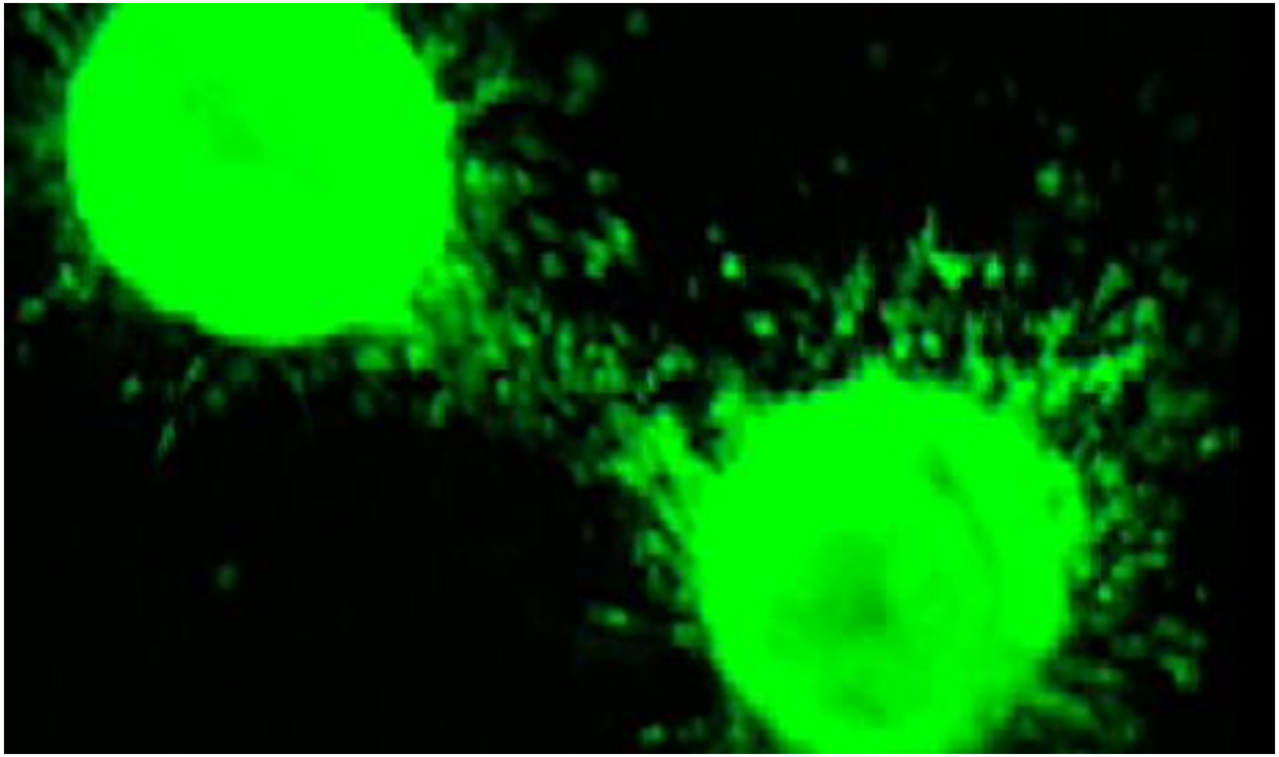
Cells following alignment

T. Demuth & M. Berens, unpublished



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Cells following alignment, cont.

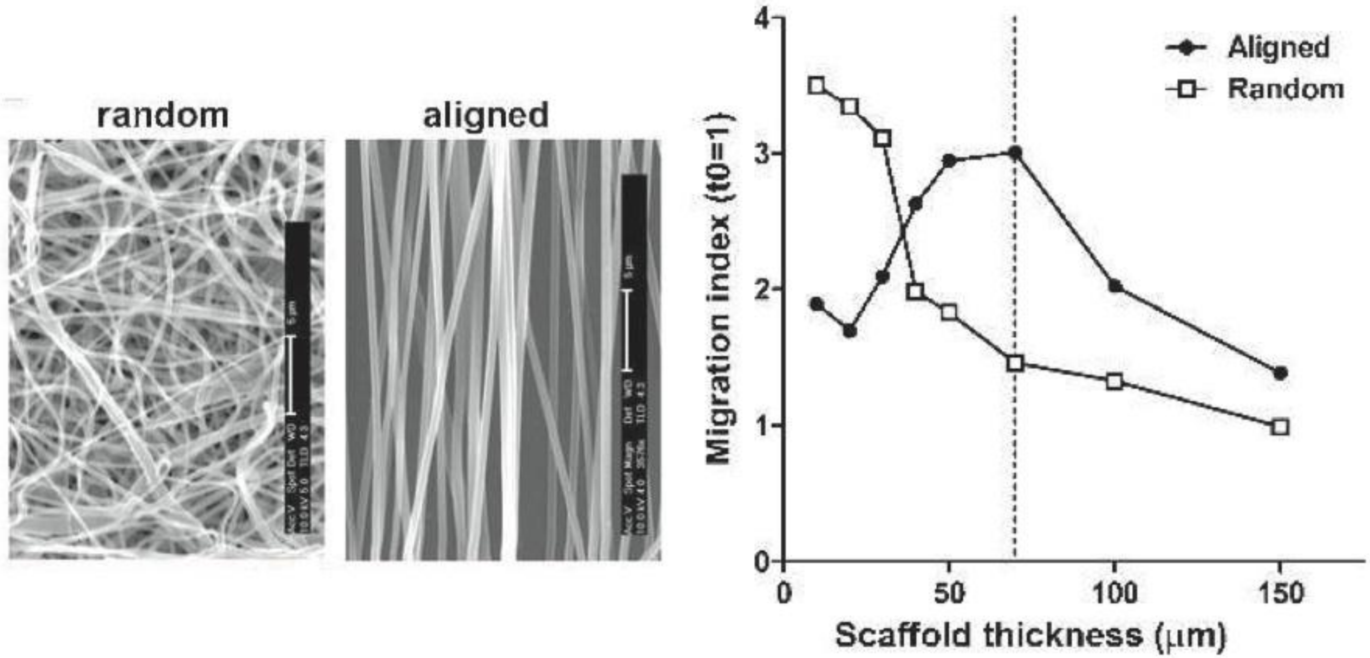

Figure 5: Left: Nanofibers are grown either amorphous or aligned. Scale bar is 5 μm. Right: Motility is much higher for the aligned case. From [32]. Courtesy of E. A. Chiocca.

Paula A Agudelo-Garcia, Jessica K De Jesus, et al. *Glioma cell migration on three- dimensional nanofiber scaffolds is regulated by substrate topography and abolished by inhibition of STAT3 signaling.* Neoplasia (New York, NY), 13(9):831, 2011.

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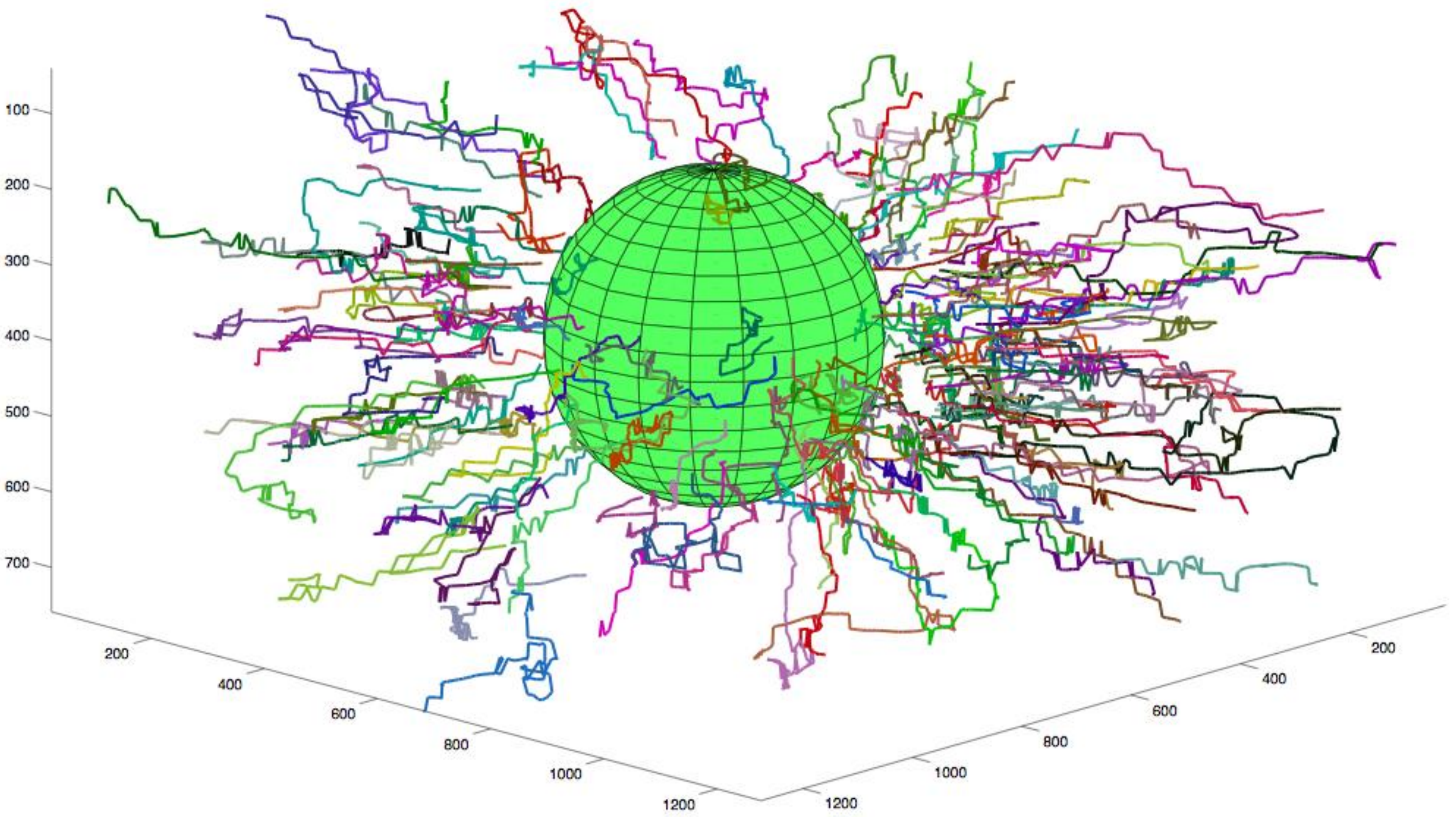
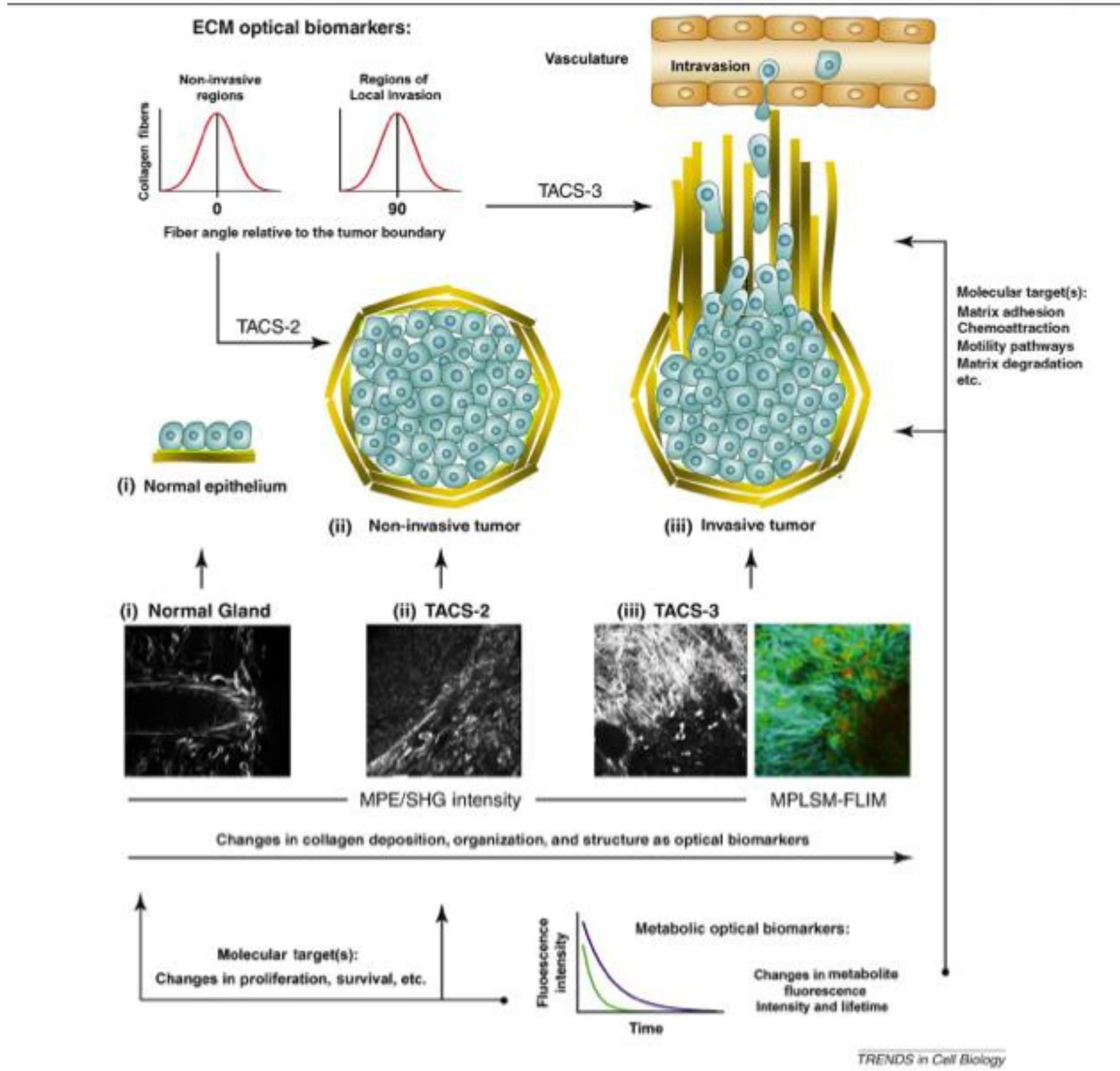
Cell tracks from confocal microscopy

C. Schneider-Mizell & LMS



Cancer invasion *in vivo*

- PP Provenzano, KW Eliceiri, JM Campbell, DR Inman, JG White, and PJ Keely. *Collagen reorganization at the tumor-stromal interface facilitates local invasion*. BMC medicine, 4(1):38, 2006.
- Paolo P Provenzano, Kevin W Eliceiri, and Patricia J Keely. *Shining new light on 3D cell motility and the metastatic process*. Trends in Cell Biology, 19(11):638–648, November 2009.



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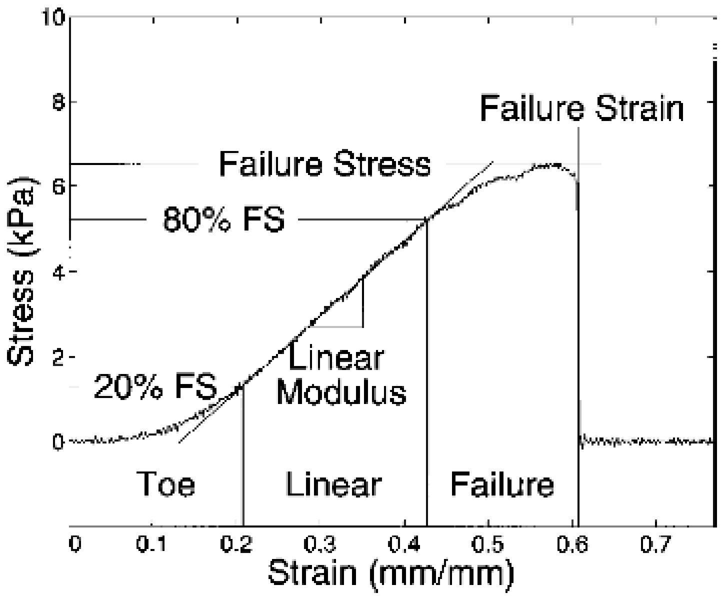
Cells & alignment

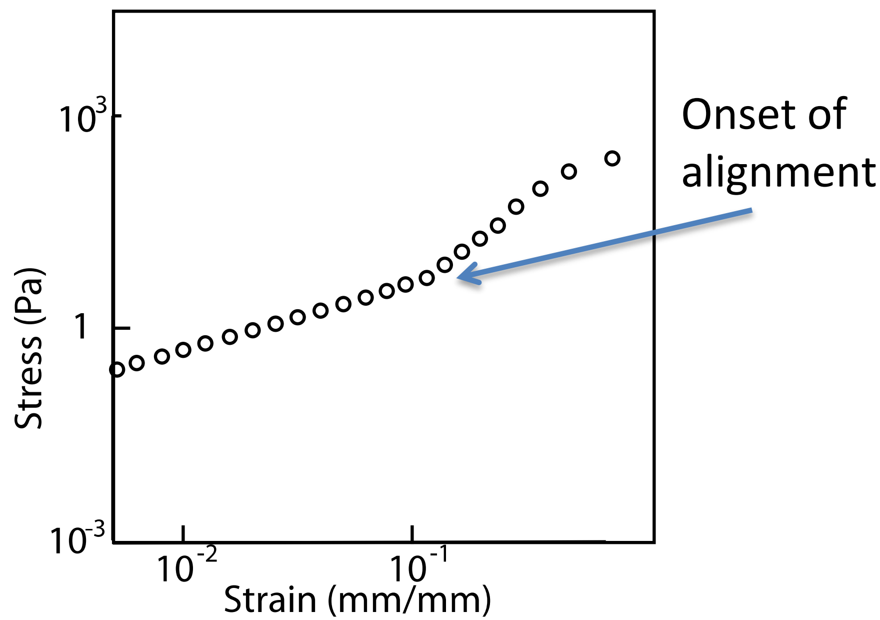
- Cells on the surface of a tumor, or isolated, pull on ECM and cause alignment.
- Cells are guided by alignment due to, *inter alia,* other cells.
- We first try to give a tractable theory of aligned athermal gels (like collagen-I), then put cells in.

ECM is non-linear

- Collagen and most biopolymers, have non-linear elasticity, and fibers *align at large strains*; this leads to *strain-stiffening*.
- Collagen-I is non-linear above a stress of ~1 Pa.



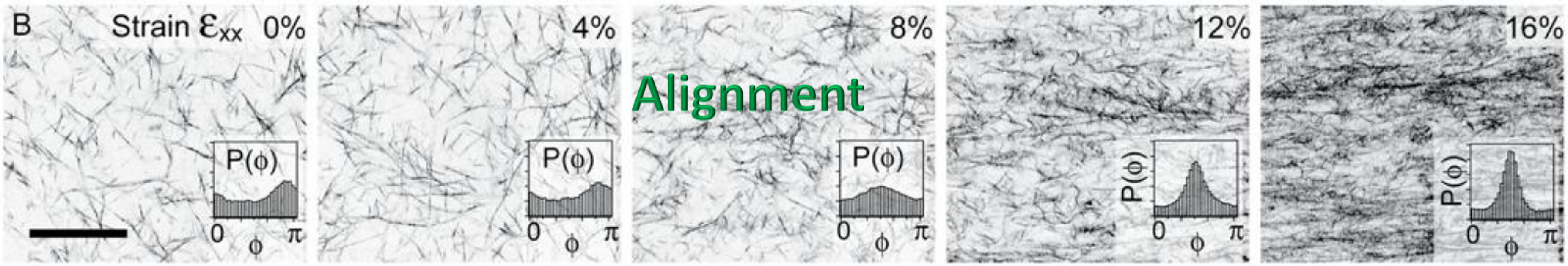
Roeder et. al., 2002

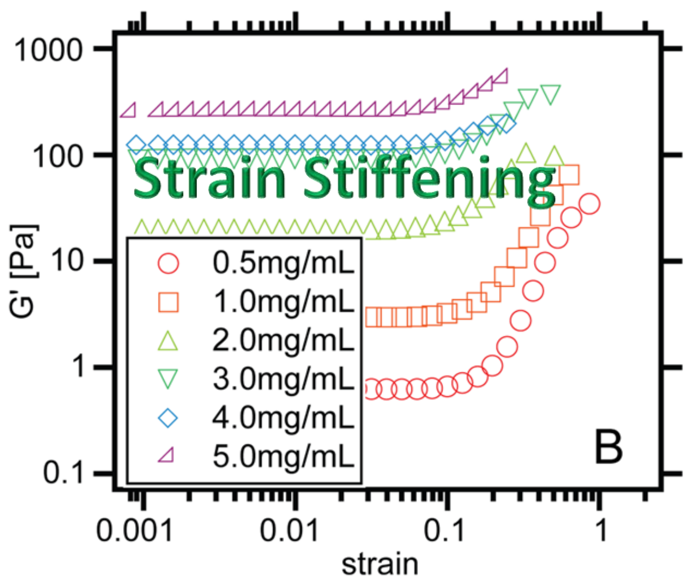


Stein, et al., 2011

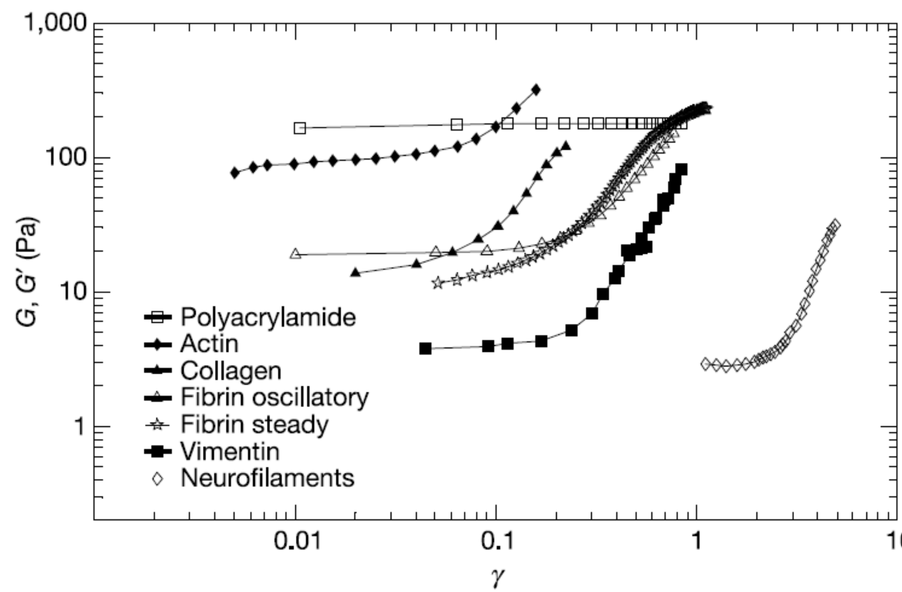
Non-linearity (cont.)

Collagen gels





Not limited to Collagen:



- C. Storm et. al., Nature 435, 191 (2005).

- D. Vader et. al., *PloS one, **4*** e5902 (2009).

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Elasticity and alignment

- Formulate elasticity of collagen (or other ECM components) focusing on *alignment.*
- Introduce a nematic-like *tensor order parameter* ***Q(r)*** that describes orientations of the filaments in the network.

$$\mathbf{Q}(\mathbf{r}) \equiv \langle \hat{\nu}\hat{\nu} - \frac{1}{d} I \rangle.$$

-The director $\hat{\nu}$,gives the direction of the local fibers.

-Define *q*=Strength of alignment ~ eigenvalue of ***Q.***

In 2d we use 2 x eigenvalue = $\langle \cos(2\theta) \rangle$

- ECM is made of biopolymers with a long persistence length, intrinsically anisotropic at small scales. Simple elastic theory cannot be applied.

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Non-linearity from linear elements

- Our explanation for strain-stiffening in collagen-I:
  - Small strains the elasticity dominated by the (small) bending modulus
    - Different parts of the disordered material turn with respect to one another so that the deformation is non-affine.
  - Large strains, the material is aligned, and must stretch.
    - The large stretching modulus determines the elastic response; the deformation is affine as in ordinary elasticity theory.
  - Plausible guess: transition occurs when q achieves an appreciable value.
- Biopolymers composed of linear elements whose macroscopic response is non-linear: the non-linearity arises from a "hidden" variable, the alignment.

Landau-deGennes theory

- Two coupled order parameters:
  - ***Q(r)***, local alignment,
  - ***v***, non-linear strain tensor (left Cauchy-Green):

$$v_{ij} = \frac{1}{2}(\partial_i u_j + \partial_j u_i + \partial_l u_i \partial_l u_j)$$

- ***v*** and ***Q*** transform as tensors in deformed space.
- ***v•Q*** is a scalar.
- We assume isotropy on the average.

A few notions from finite strain theory

- Reference space, **r**, and target space, **R=r+u**.
- Basic object, Cauchy deformation tensor:

$$\Lambda_{ij} = \frac{\partial R_i}{\partial r_j} = \delta_{ij} + \partial_j u_i$$

- Left strain tensor $\mathbf{v} = \frac{1}{2}(\Lambda\Lambda^T - \mathbb{I})$
  - Tensor in target space
- Change in volume $\delta V = \det(\Lambda)\delta v$
  - In linear regime $\delta V/\delta v = 1 + \mathrm{Tr}(\mathbf{v})$
- Ref: T. Lubensky, R. Mukhopadhyay, L. Radzihovsky and X. Xing, *Symmetries and elasticity of nematic gels, Physical Review E* ***66*** *(1), 011702 (2002)*

Landau-deGennes free energy

- Our idea: attribute non-linearity to ***Q***, so use a linear theory (almost) for ***v***:

$$F = \int \Big[\frac{\lambda}{2}(\mathrm{Tr}\,\mathbf{v})^2 + \tilde{\mu}\mathrm{Tr}\,\mathbf{v}^2 + g(\mathrm{Tr}\,\mathbf{v})^3 - t\mathrm{Tr}(\mathbf{v}\cdot\mathbf{Q}) + V(\mathbf{Q})\Big] d^d r,$$

  - λ, μ Lame parameters. Tilde on μ means 'bare' value, not renormalized by ***Q***.
  - *t* coupling parameter; couple to traceless part of ***v***.
  - *V(**Q**)* potential for ***Q***.
  - $g(Tr\,\mathbf{v})^3$, non-linear kludge, explain later.

Shear-stiffening, linear

- Small shear strain, small ***Q***.
- Leading term in V: $V_l(Q) = \frac{A}{2}\mathrm{Tr}\mathbf{Q}^2.$
- Minimizing *F* for fixed *v* gives: $\mathbf{Q} = (t/A)\tilde{\mathbf{v}},$
  - Here the tilde indicates the traceless part of ***v***.
  - Strain induced alignment is a *linear response* for small deformations.
- Eliminate ***Q****:* $F_l = \int \left[\frac{\lambda}{2}(\mathrm{Tr}\mathbf{v})^2 + \mu\mathrm{Tr}\mathbf{v}^2\right] d^d r$
  - where $\mu = \tilde{\mu} - \frac{t^2}{2A},$ shear modulus *reduced by Q.*

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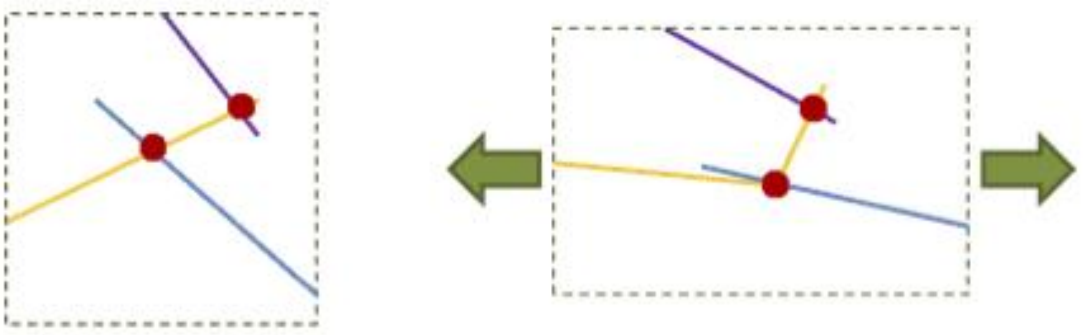
Shear-stiffening, non-linear

- Non-linear regime: *V(Q)* is non-linear (for 2d):

$$V(\mathbf{Q}) = \frac{A}{2}\mathrm{Tr}\ \mathbf{Q}^2 + \frac{C}{4!}\mathrm{Tr}\ \mathbf{Q}^4$$

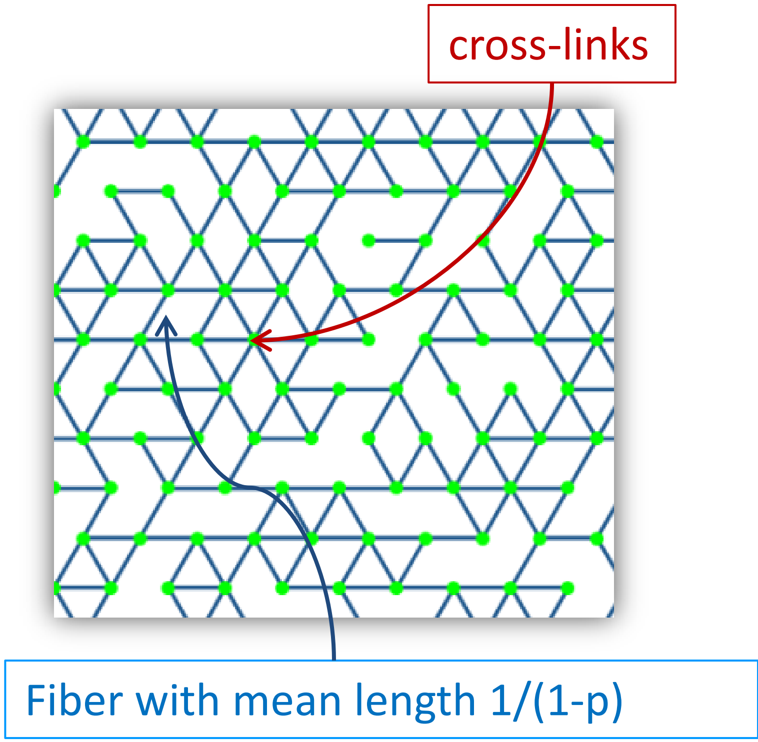
- Now $A\mathbf{Q} + (C/6)\mathbf{Q}^3 + \ldots = t\ \tilde{\mathbf{v}}$
- Now the renormalization is smaller, μ larger.
- Reason: 'exhaust' bending modes.



Test theory on lattice model

Diluted filamentous triangular lattice:

- Each bond is present with probability p
- Stretching stiffness k
- Bending stiffness (along straight lines) κ



$$E = \frac{k}{2a} \sum_{\langle ij \rangle} g_{ij} (|\mathbf{R}_{ij}| - a)^2 + \frac{\kappa}{2a} \sum_{\langle ijk \rangle} g_{ij} g_{jk} \Delta\theta_{ijk}^2$$

g_{ij} =0,1 for bond occupancy, θ_{ijk} =angle of 3 succesive nodes.

- Das, MacKintosh, Levine, *Physical Review Letters* **99**, 038101 (2007).

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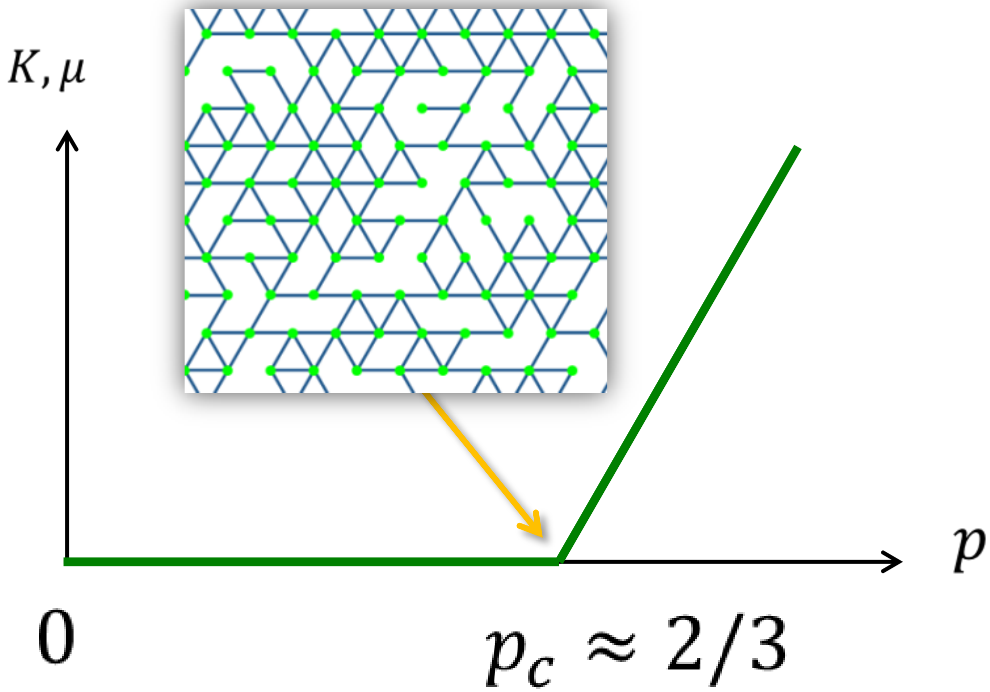
Bending and stretching

Bending stiffness (along straight lines) $\kappa=0$



- Thorpe, *J. Non-Cryst. Solids* **57**, 355 (1983).
- Feng and Sen, *PRL* **52**, 216 (1984).
- Feng and Thorpe, *PRB* **31**, 276 (1985).
- Feng, Sen, Halperin, and Lobb, *PRB* **30**, 5386 (1984).

Rigidity percolation:
(numerical simulation)
- Critical fluctuations
- Rigid percolating cluster is fractal

$\langle z \rangle = 6 p_c \approx 4 = 2d$ **CF isostatic point**

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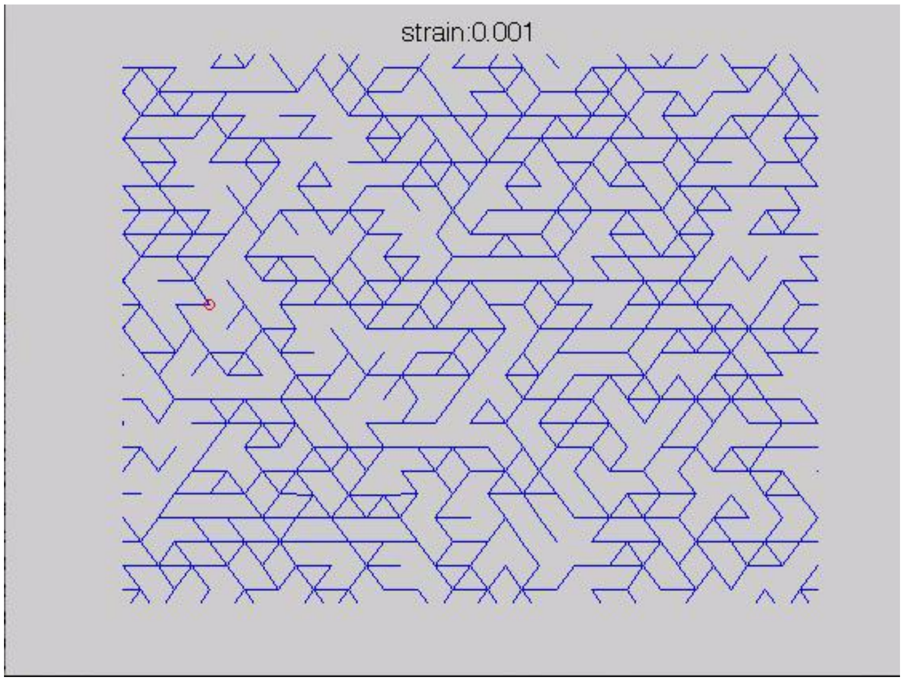
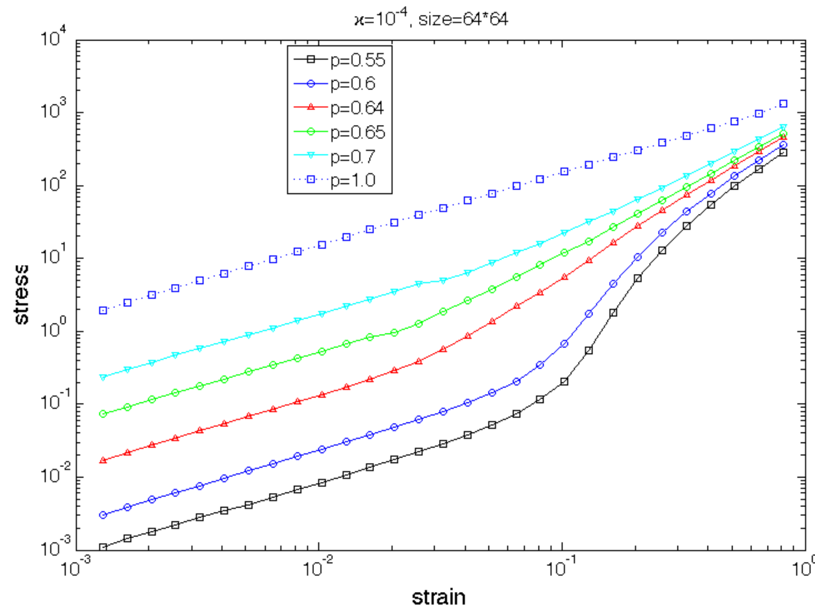
Simulations





- Strain-stiffening is stronger at small p
- "Turning point" $\gamma_c \to 0$ at p_c

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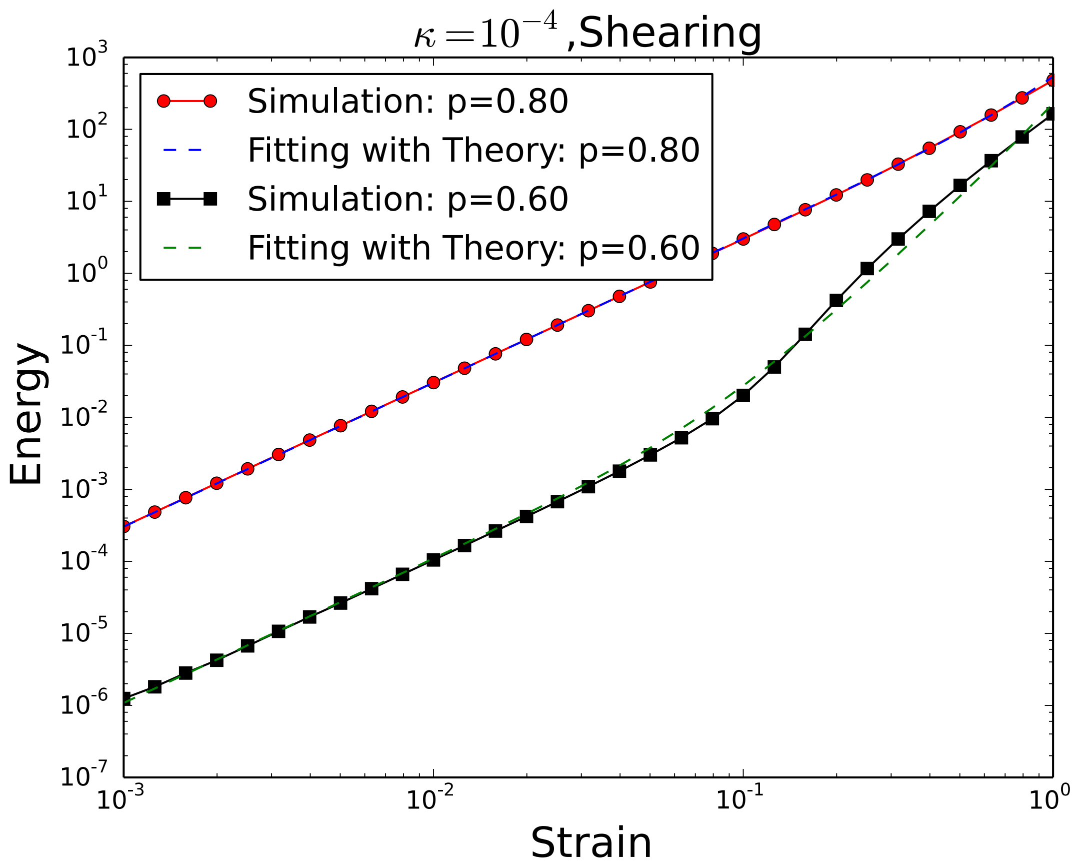
Fit Landau model to lattice model

- Find parameters for the Landau theory by fitting to stress-strain and q-strain curves from simulations: parameters are $\{\lambda, \tilde{\mu}, g, t, A, C, D\}$
- Shear modulus, μ, bulk modulus, K=λ+μ, t/A from linear slopes. Rest from non-linear curves.



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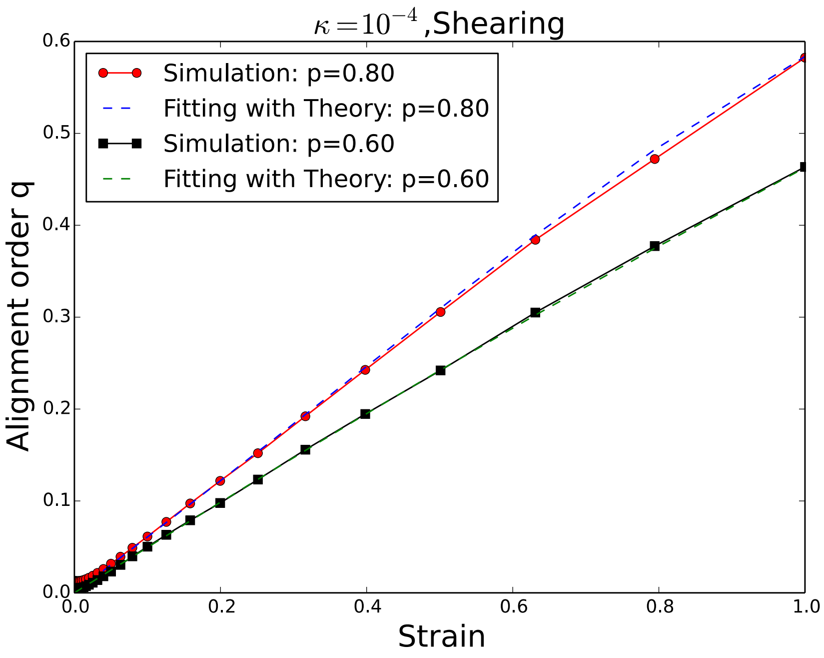
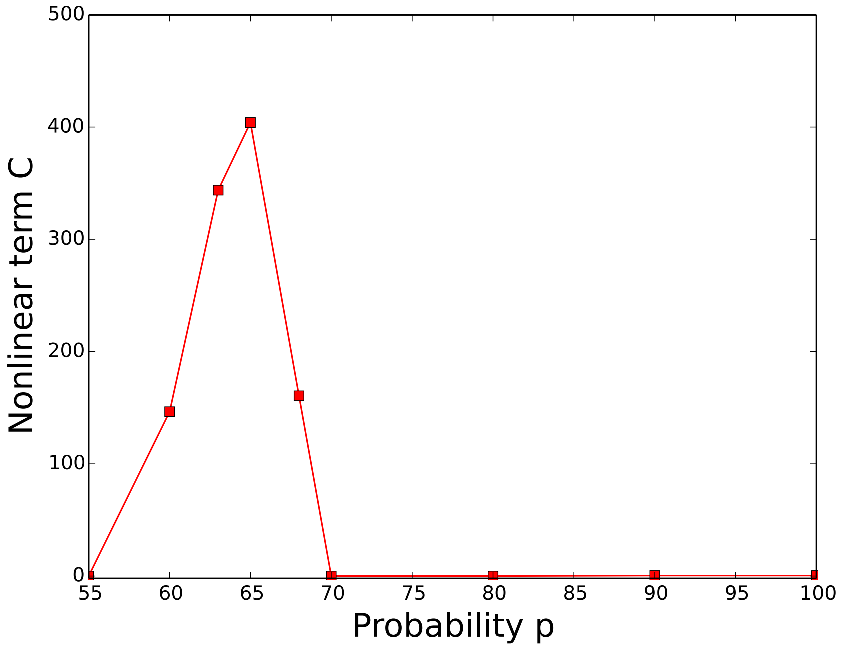
Shear, (cont.)

- Alignment works fine
- Non-linear parameter, C, peaks at p_c, as we expect.





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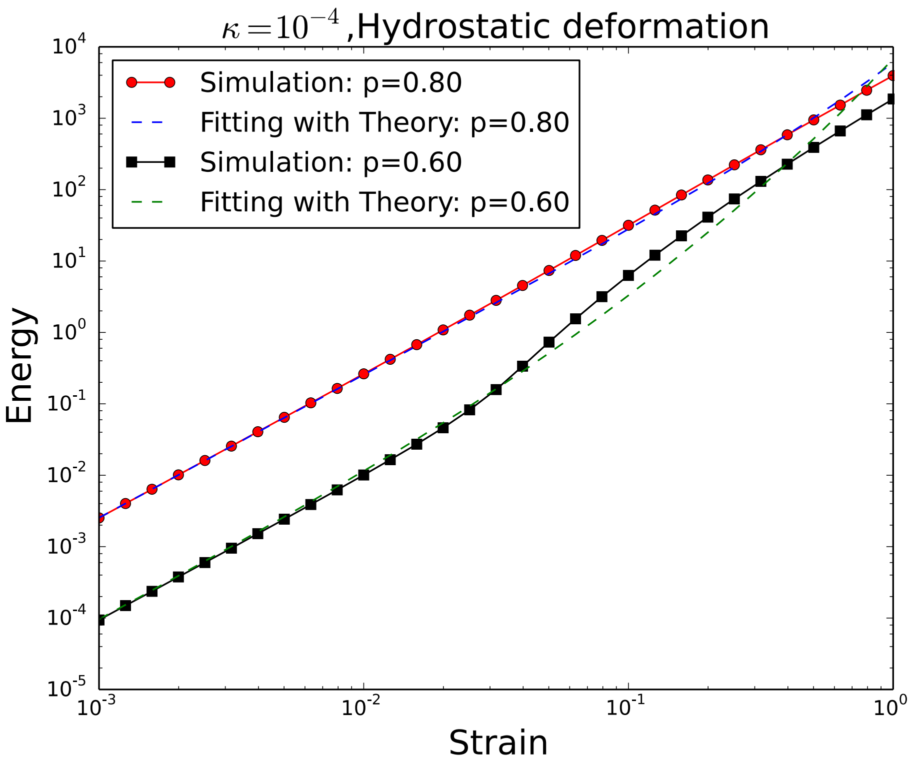
Hydrostatic &uniaxial strain

- Now Tr ***v*** ≠ 0. Uniaxial = Shear + Hydrostatic
- Hydrostatic: no alignment, on the average. So no non-linearity?
  - For lattice model for $p<p_c$ there is still non-linear behavior.



This is why we put in the term *g(Tr* **v***)*^{3}*.*
Fit for stress-strain is not bad.

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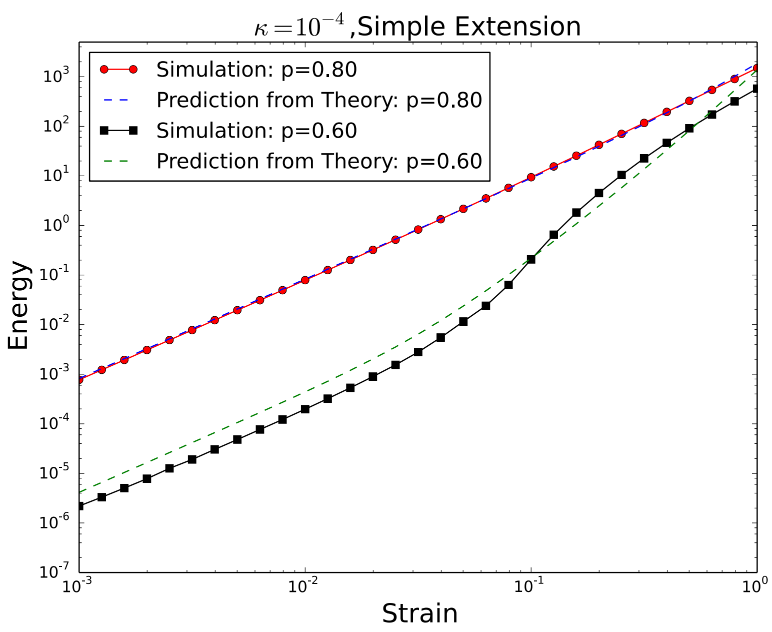
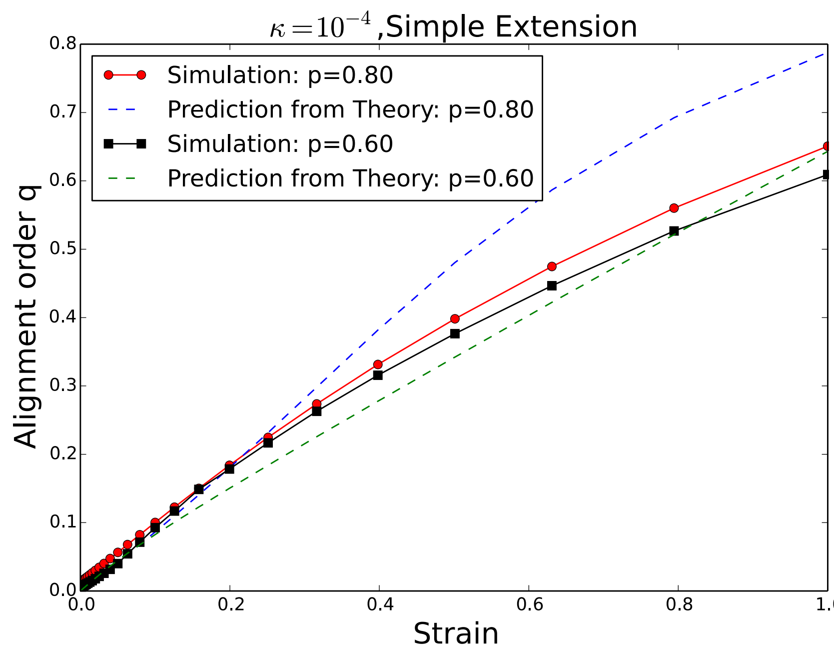
Uniaxial alignment

- Now apply *same parameters* to uniaxial strain.
- Stress-strain is not bad.
- Alignment, *q,* doesn't work very well for strains > 0.3.





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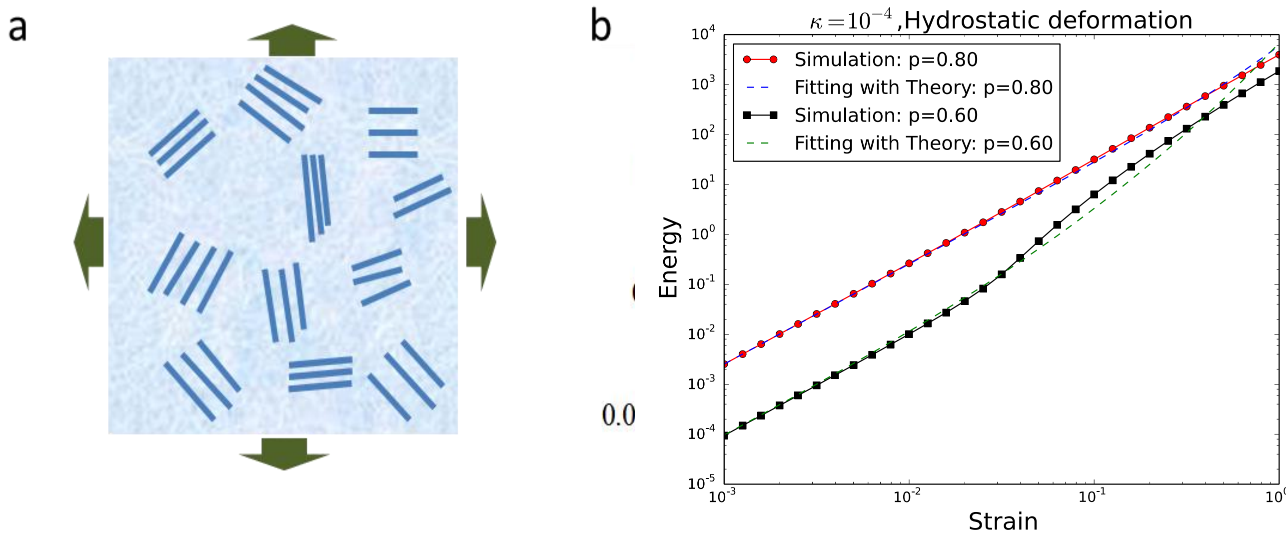
Hydrostatic deformation revisited

- In fact, for p<1, we expect *local* alignment even for hydrostatic deformation, even though <***Q***>=0.



For the ordered lattice (p=1) stress-strain is linear for all strain.

For p<1, especially $p<p_c$, local regions align under stress.

For large strain, affine.

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Quenched random field

- Represent the effect of missing bonds by introducing a random tensor field, ***h***, which couples to ***Q.***
- *<**h**>=0,* ***<h(r)h(s)> =*** *g**I** δ(r-s)* . One new parameter, *g.*
- Couple to non-linear expression for the volume change:

$$F = \tilde{\mu}\mathrm{Tr}(\tilde{v}^2) - t\mathrm{Tr}(\tilde{v}\cdot Q)$$

$$+(\tilde{K}/2)Y^2 - Y\mathrm{Tr}(h\cdot Q)$$

$$+(A/2)Q^2 + (C/4!)Q^4$$

$$Y = 2(\sqrt{\mathrm{det}\Lambda} - 1) \to \mathrm{Tr}v$$

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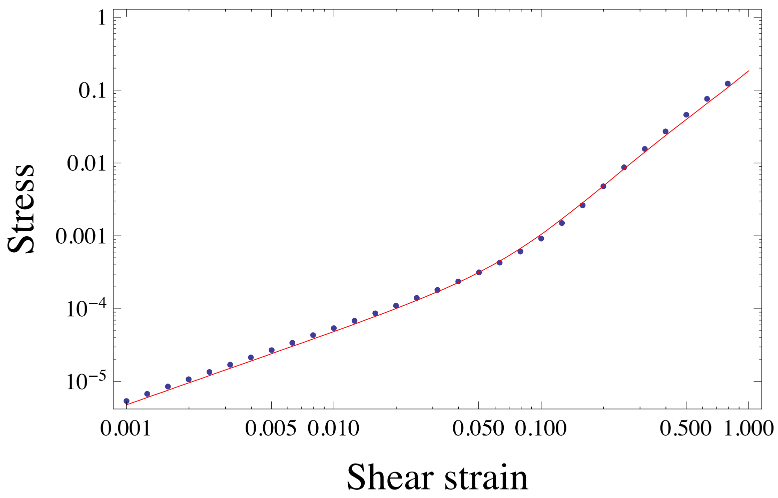
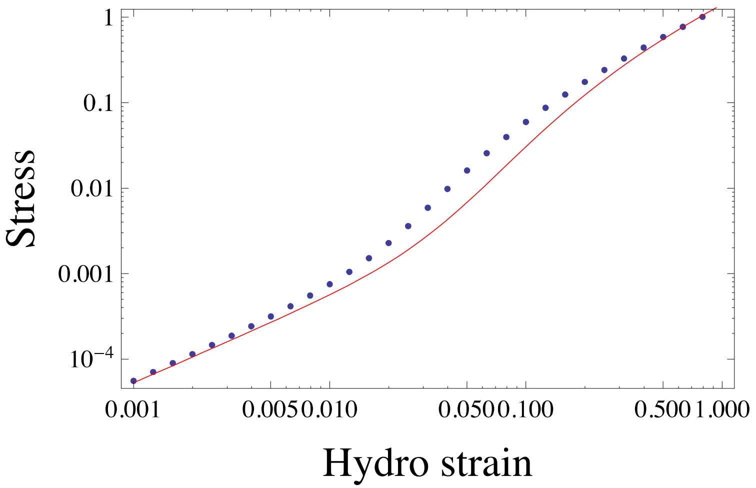
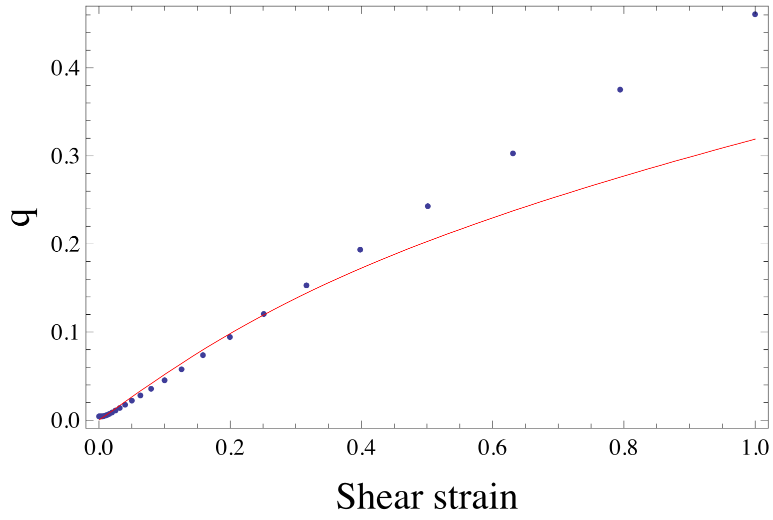
Random field coupling

- Now we have the same mechanism as before:
  - Quenched disorder induces quenched, random ***Q***:
    - ***Q(r) = [****Y/A****] h(r) -> [Tr(v)/A] h(r).***
    - K → K – gd/2A in the linear regime.
      (Reduced bulk modulus.)
  - Non-linear regime, ***Q*** is smaller, so the renormalization of the Lame parameter is less, and we have strain stiffening, even for hydrostatic deformations.
- Outside the linear regime we can minimize *F* take the disorder average, and find the best fit, all without measuring the random ***Q***.

Results of fitting parameters







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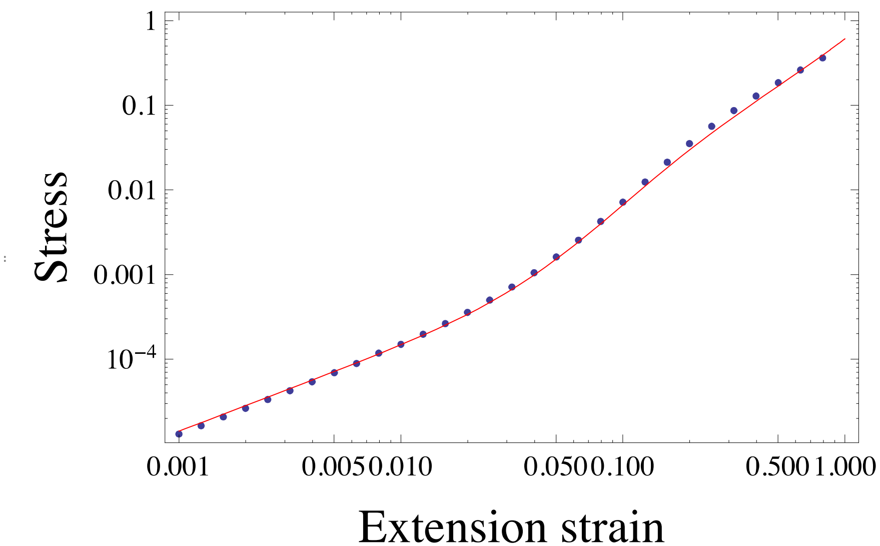
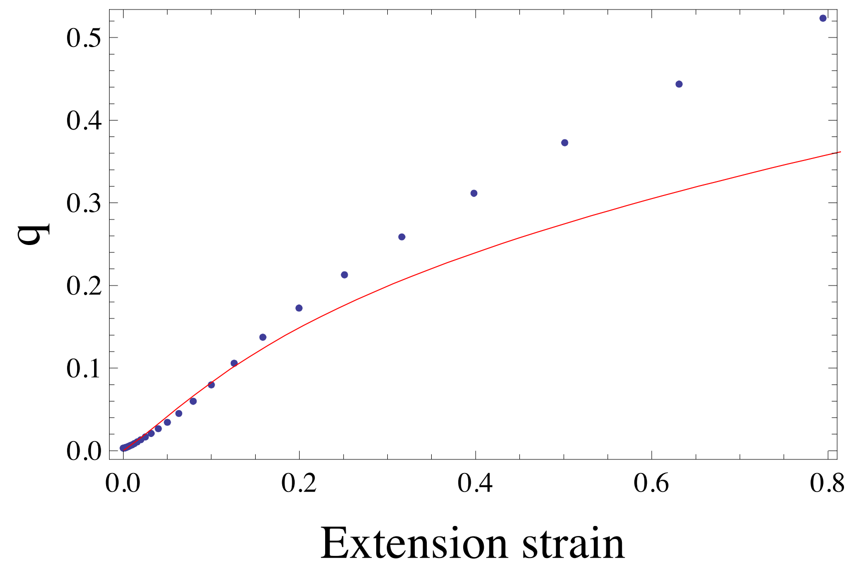
Same parameters, extension

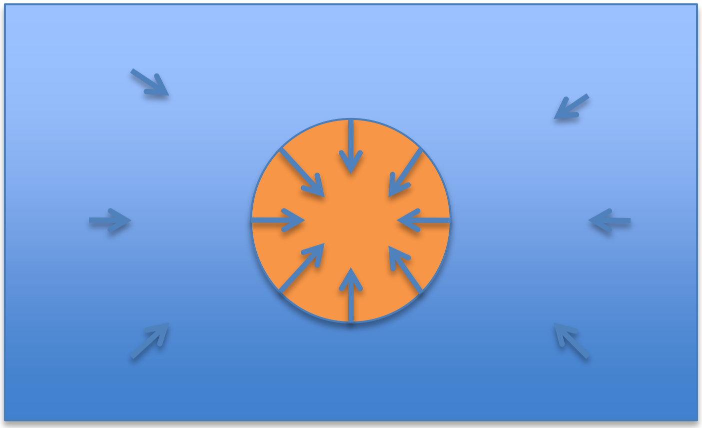




Reasonable account for bulk strains with a few Landau parameters!

Back to cells

- Think of a cell as a localized source of strain, e.g. a cavity in the material with a contractile force.
  - The simplest way to think of a contractile force is a negative pressure.
  - Now the problem has spherical symmetry: spherical deformation, u(r), and spherical alignment, q(r).
- Minimize *F* w.r.t. two functions, u, q.



Coupled ODE's for localized strain

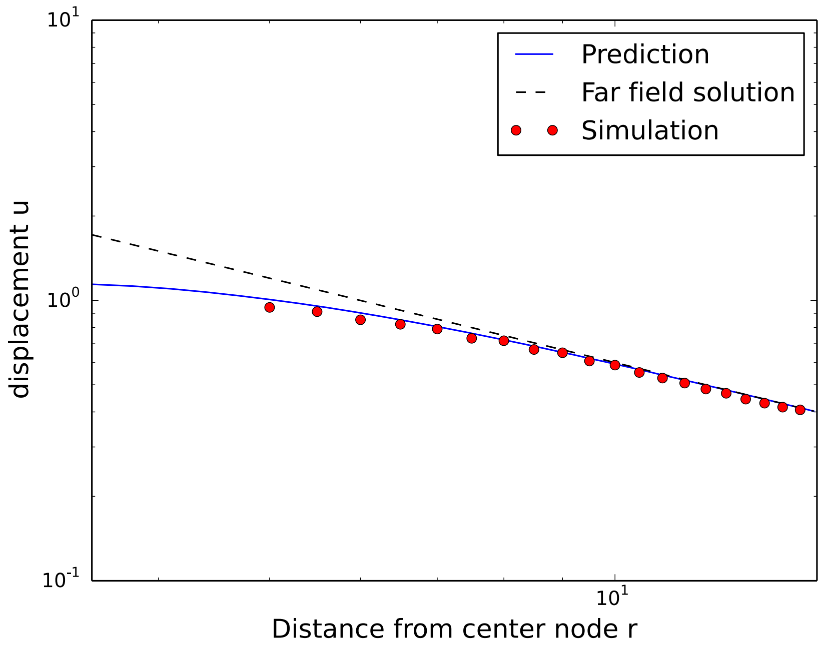
- Result of minimization:

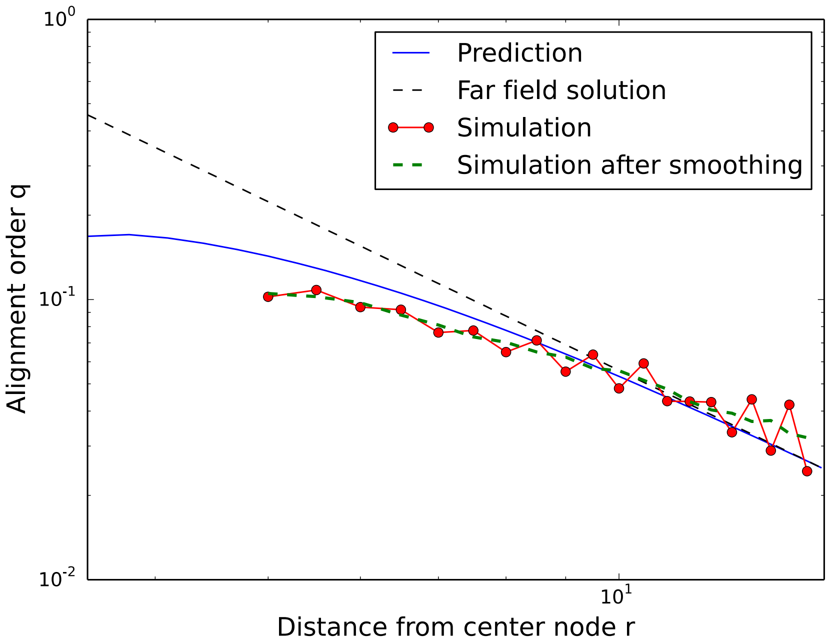
$$u'' + \frac{2u'}{r} - \frac{2u}{r^2} = \frac{2t}{\lambda + 4\mu} \left(\frac{q'}{3} + \frac{q}{r} \right))$$

$$\frac{2t}{3} \left(u' + \frac{u}{r} \right) = V'(q).$$

- Linear regime, far away, easy to show:
  u ~ 1/r (classical result); q~ $1/r^2$
- Non-linear regime: numerically solve the equations.

Results for localized strain





Summary

- We have a theory which accounts for non-linear elasticity as a result of alignment.
- Good results for shear.
- Extension and hydrostatic deformations require a random field, ***h***, and a random **Q**.
- Cell deformation under control.
- Possible equation of motion for cells based on symmetry: D_o= effective diffusion coefficient:

$$\mathbf{D} = D_\circ(1 + \alpha\mathbf{Q})$$

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