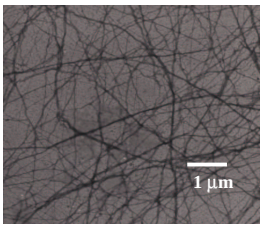


Mechanical Properties & Microstructure

Margaret Gardel, Maria Kilfoil, Jennifer Shin*, Alois Popp, Megan Valentine, John C. Crocker, Andreas R. Bausch and David A. Weitz**

Department of Physics, Harvard University
*Whitehead Institute, M.I.T.
**Chemical Engineering Dept., Univ. of Pennsylvania

Motivation

- Develop microrheology as a reliable tool to measure bulk rheology of μL volume samples
- Understand meaning of 1-particle microrheology in F-Actin networks
- Explore microscopic basis of bulk rheological properties
- Understand physics of entangled semiflexible polymer networks!

Sample Preparation

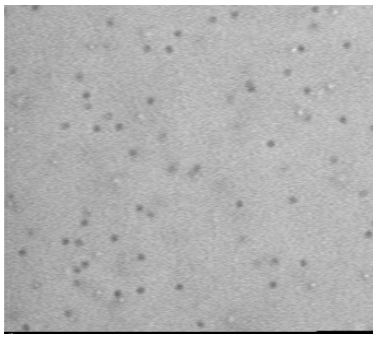
•Use lyophilized G-Actin
(a gift from Sackmann lab in Munich, Germ.)
•Dialyze against fresh G-Buffer for 2 days at 4 C
•Pre-incubate beads with G-Actin
•Incubate at 25 C for 1 hour

GOOD REPRODUCIBILITY

•Days after G-Actin prep
•Different Sample Preparations
•Fields of view within sample
•Polymerization temperatures
•Wait time before taking data

Multiparticle Tracking

(Crocker and Grier, 1996)



spatial resolution: 10 nm

temporal resolution: 1/30 sec

~ 100 particles in field of view (F.O.V.)

~ 3-15 minutes of video/ F.O.V.

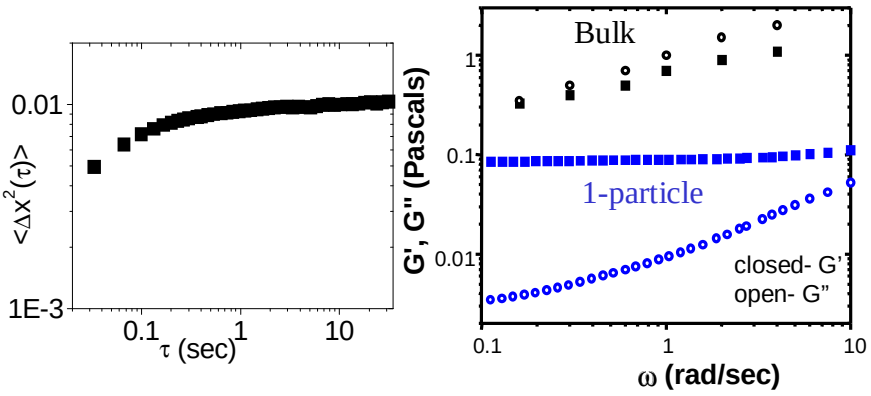
1.0 mg/mL F-Actin
1.0 CML beads
40x objective, bright field

→ individual tracks are minutes long!

1-Particle Microrheology

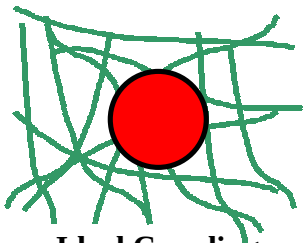
Ensemble Averaged m.s.d. → **GSER** → **Elastic, Loss Moduli**

1 μm COO^- beads; 1 mg/mL F-Actin

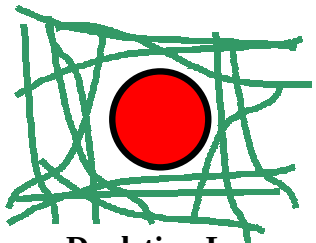


Why Discrepancy?!!

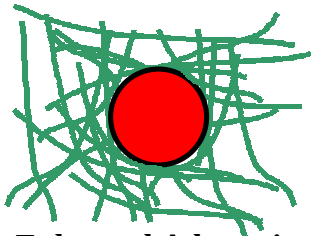
Microenvironments



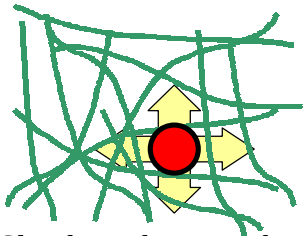
Ideal Coupling



Depletion Layer



Enhanced Adsorption



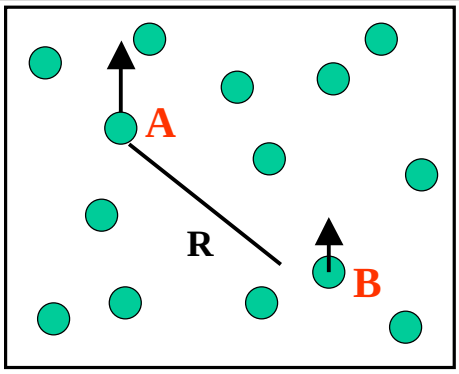
Slip through Network

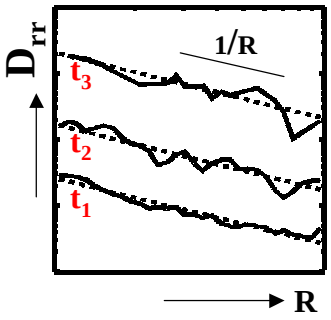
Two-point Microrheology

(J.C. Crocker et. al., 2000 and A.J. Levine and T. C. Lubensky, 2000)

Examine pairwise correlated motion of tracers

$$D_{\alpha\beta}(r,\tau)=\langle \Delta r_\alpha^i(t,\tau)\, \Delta r_\beta^j(t,\tau)\, \delta(r-R^{ij}(t))\rangle_{i=j,t}$$

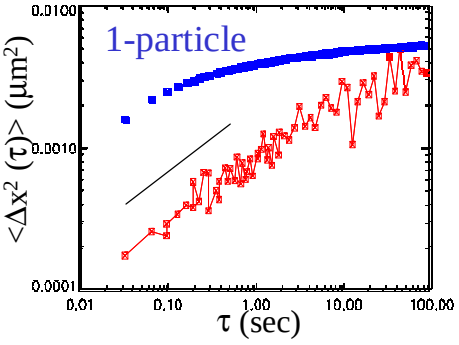


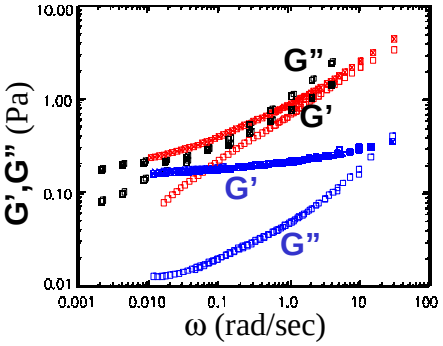


Define a 'distinct' m.s.d $\langle \Delta r^2(t,\tau)\rangle_D=(2r/a)D_{rr}(r,\tau)$

Two-Particle Results

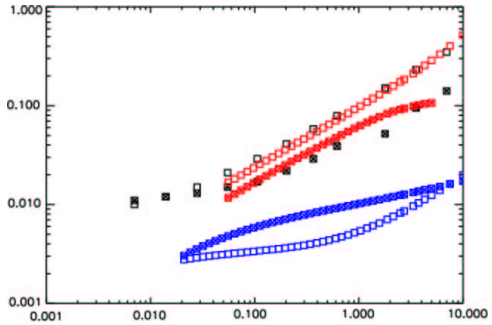
1.0 mg/mL F-Actin; 1.0 μm COO^- beads





2-Particle Results: 0.3 mg/mL

1.0 mg/mL F-Actin; 1.0 μm COO^- beads



BULK
1-Particle
2-Particle

■ G′

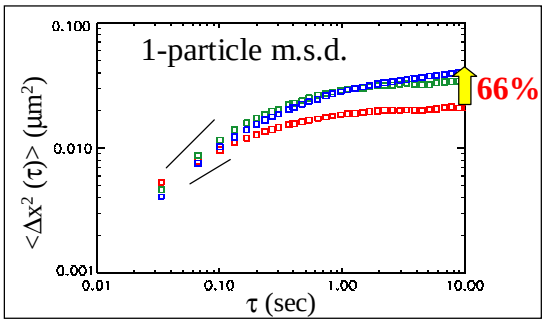
□ G″

Outline

How do 1- and 2-particle motions vary with:

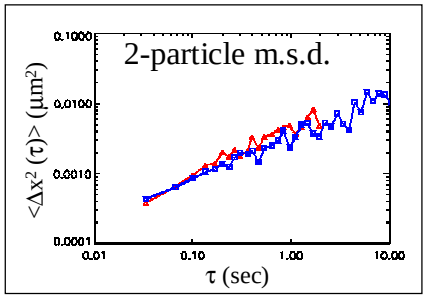
Bead Surface Chemistry?
Bead Size?
Actin Concentration?

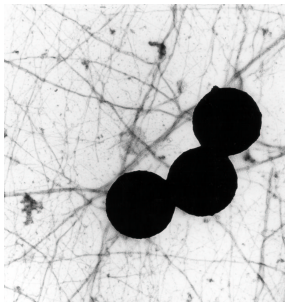
Effect of Surface Chemistry I: Inert Beads



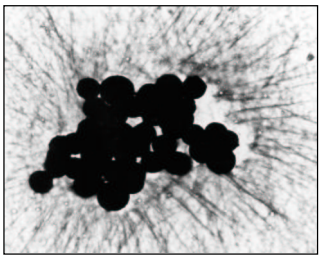
0.5 mg/mL F-Actin

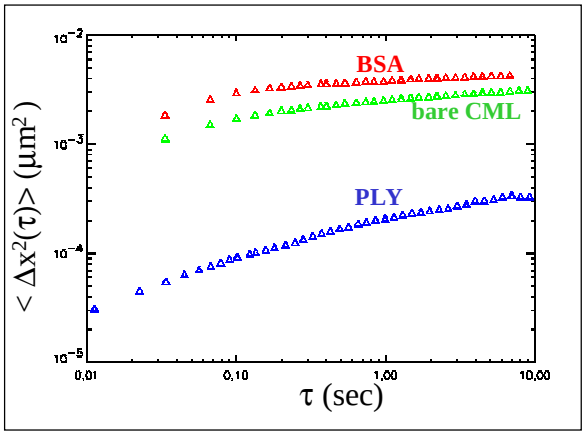
- 1 μm COO⁻
- 1 μm PEG coated COO⁻ (thanks Megan)
- 1 μm BSA coated COO⁻





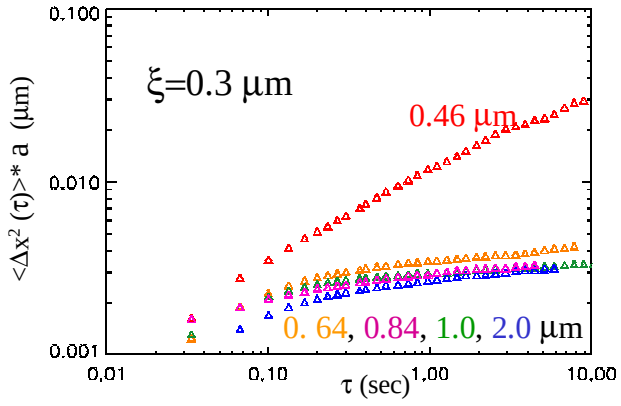
Effect of Surface Chemistry I: Sticky Beads

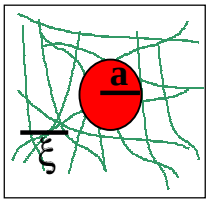




Effect of Bead Size: 1-particle microheology

1 mg/mL F-Actin: **m.s.d * a**



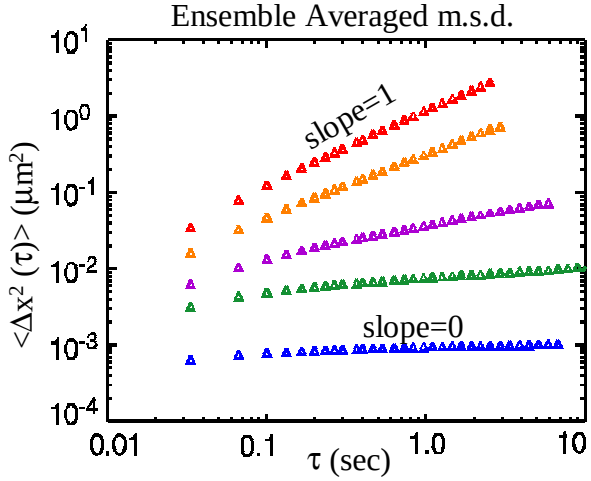


$$G_o \sim \frac{k_B T}{a * \Delta r_{max}^{\ 2}(\tau)}$$ → Go = 0.13 Pa

Effect of Mesh Size: 0.46 μm beads

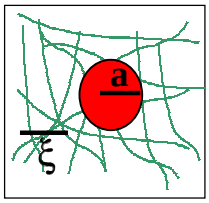
$$\xi = 0.3/\sqrt{[G - Actin]}$$

Ensemble Averaged m.s.d.

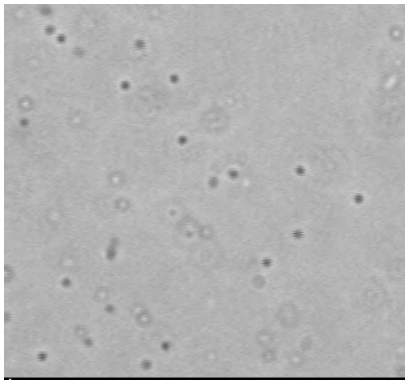


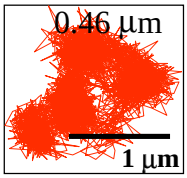
Mesh (μm)

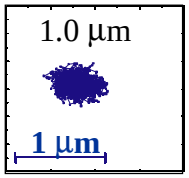
0.78
0.55
0.30
0.25
0.17

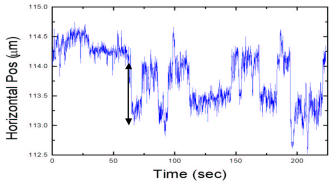


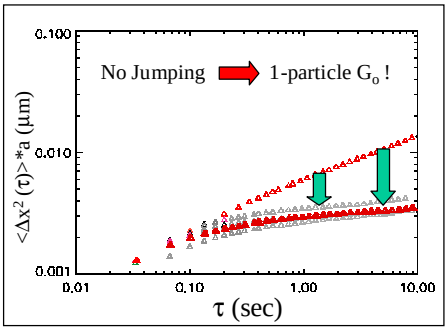
Jumps



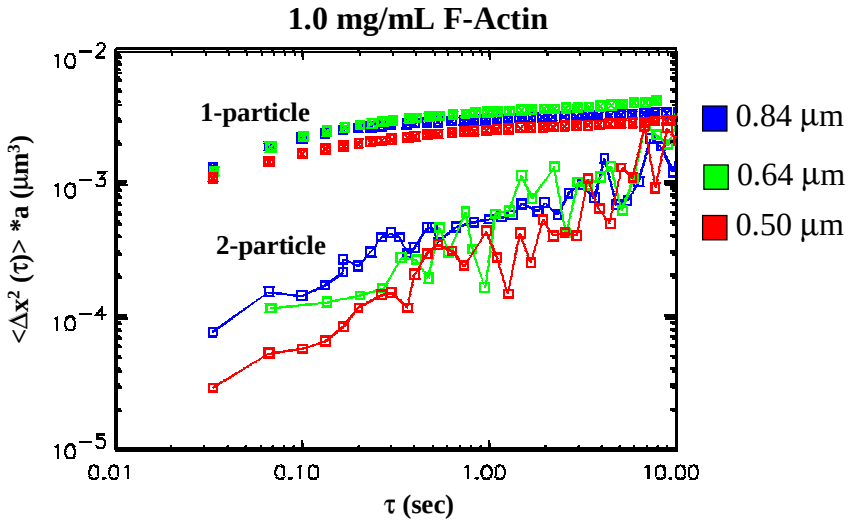




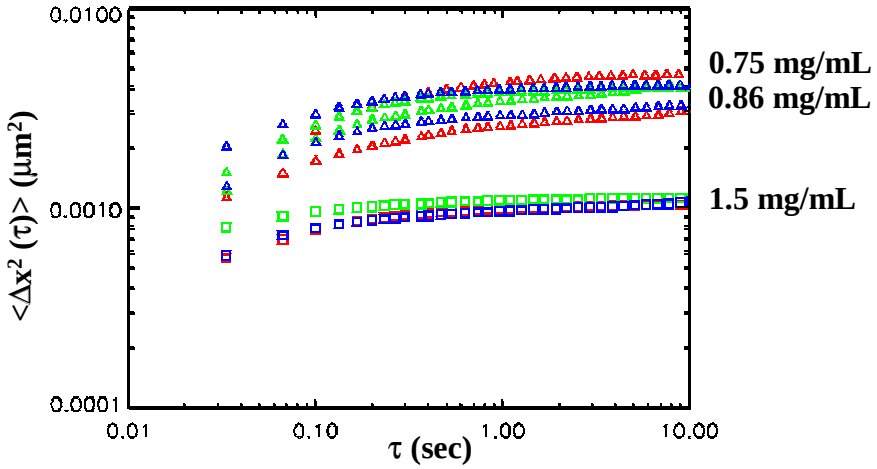




Effect of bead size: 2-particle microrheology

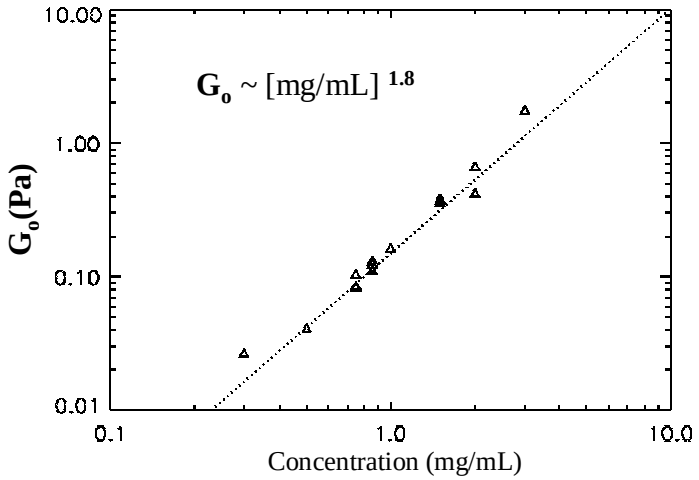


Concentration Dependence: 1-particle

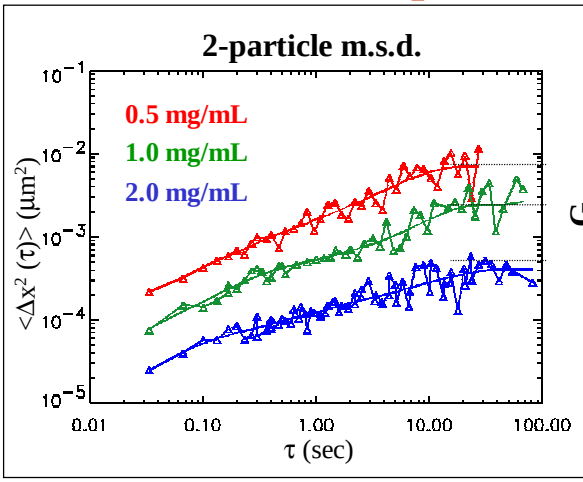


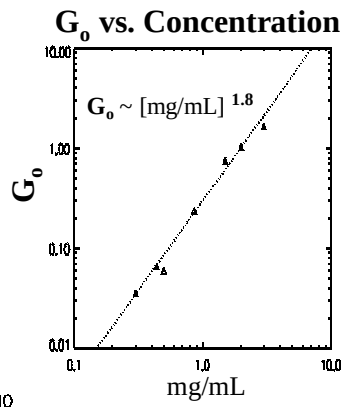
Bead Sizes: ▲ 0.46 μm ▲ 0.64 μm ▲ 1.0 μm

Scaling of 1-particle modulus



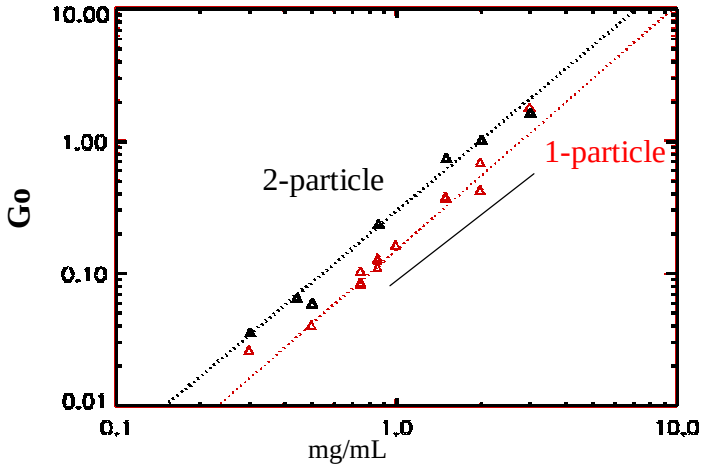
Concentration dependence: 2-particle



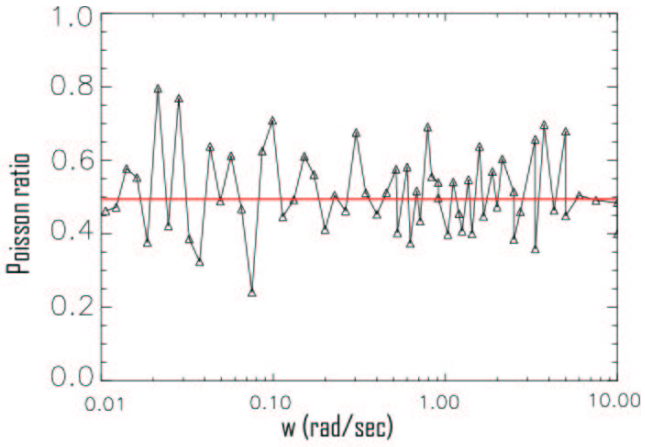


•Why do 1-and 2-particle have similar scaling, magnitude of G_o ???

Comparison: 1-particle vs. 2-particle



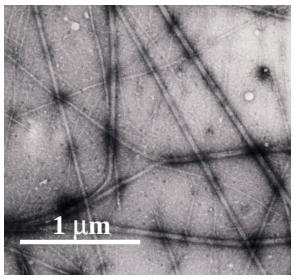
Poisson Ratio



Conclusions

- 2-particle microrheology reproduces bulk G(ω) !
- 2-particle eliminates local effects of bead surface chemistry, size
- When a ~ ξ, bead motions give additional info about microstructure
- 1-particle microrheology measures $G_o \sim [\text{G-Actin}]^{1.8}$
- 2-particle microrheology measures $G_o \sim [\text{G-Actin}]^{1.8}$

Crosslinked & Bundled Actin Networks



Bundling Protein: Scruin
(Jennifer Shin, P. Matsudaira MIT)

WHY?!
- many actin binding proteins found in nature
- new material!

- Change mechanical properties of actin network
- Form spatial (temporal?) heterogeneities

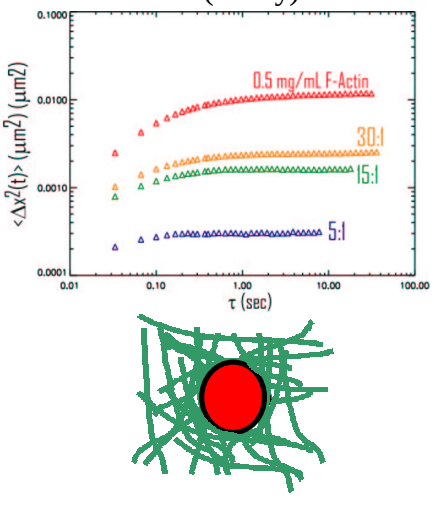
Imaging (Jennifer)

Multiparticle Tracking (Margaret)

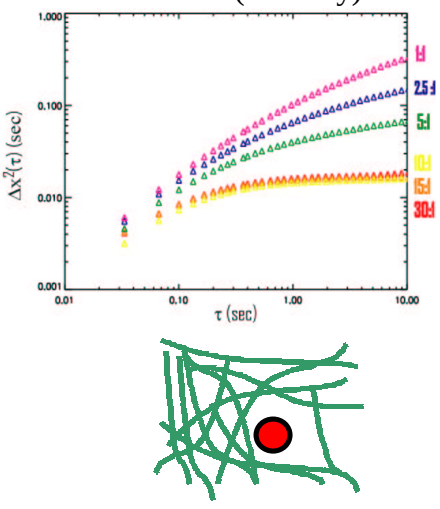
Bulk Rheology (Maria,Margaret, Alois)

1-Particle Microrheology

BSA (sticky)

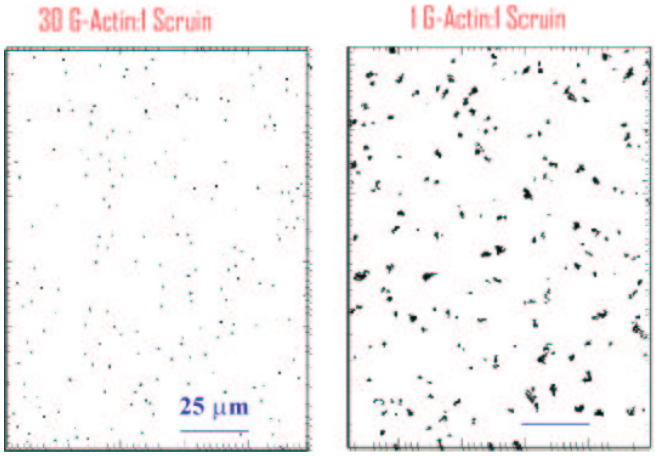


PEG (unsticky)



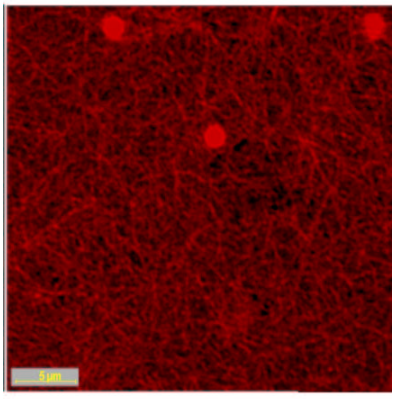
Spatial trajectories

1 μm PEG



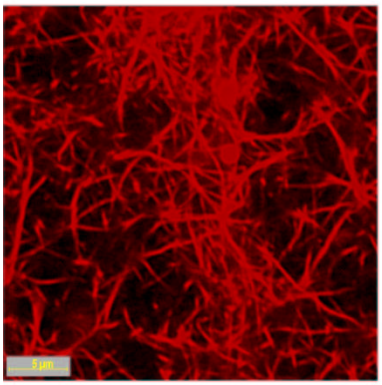
10 minutes long!!!!!

Direct Imaging



15:1(A:S) side ~32μm

15 Actin:1 Scruin



2:1 (A:S) side: ~ 32μm

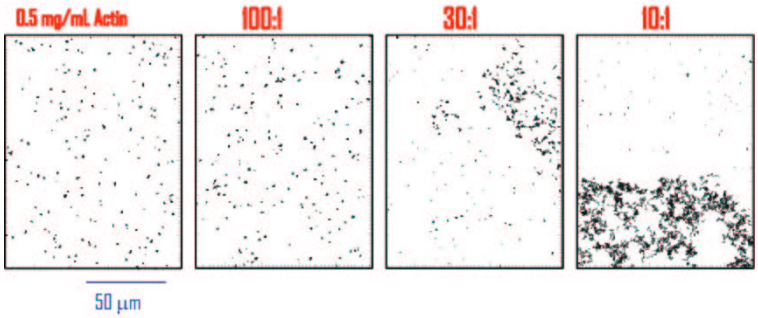
2 Actin:1 Scruin

Outlook: Imaging and Structure

Directly map local mechanical properties to structure

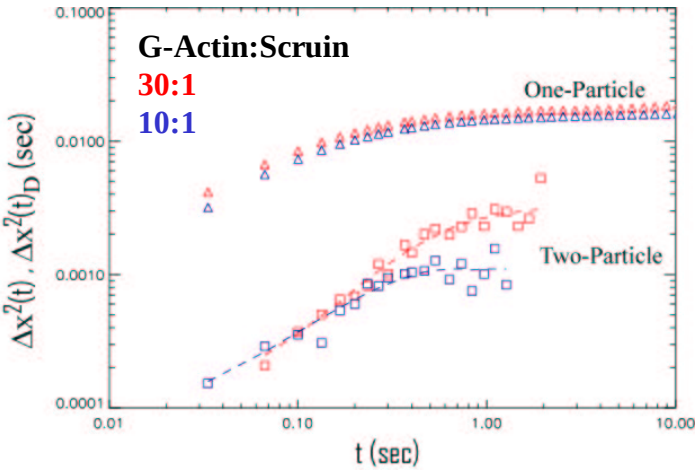
Other x-linkers/bundlers

Actin/α-Actinin Networks



2-Particle Microrheology: Actin/Scruin

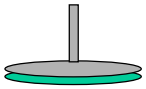
1 μm PEG (non stick) beads; 0.5 mg/mL



0.3 Pa
0.85 Pa

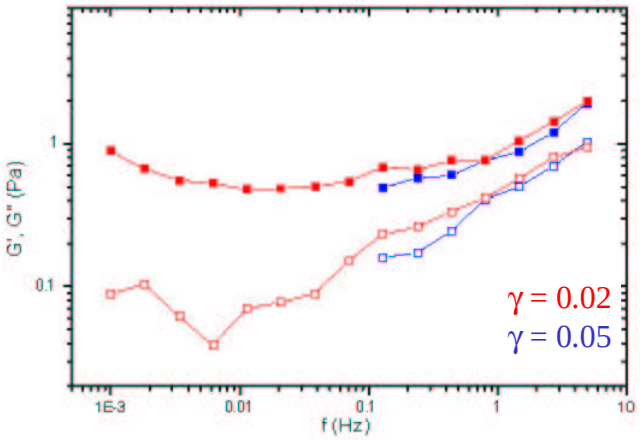
Bulk Rheology: Actin/Scruin

- 40 mm parallel plate, 100 μm gap
- Bohlin stress-control rheometer
- 10 °C

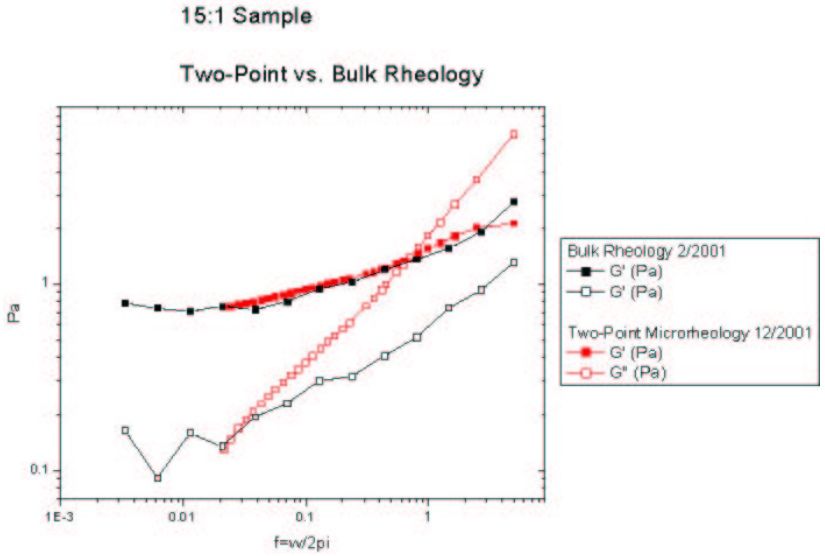


Bulk Rheology: Actin/Scruin

10:1 G-actin:scruin

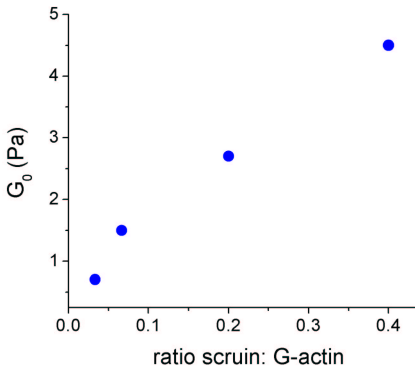


Bulk Rheology and Two-Point



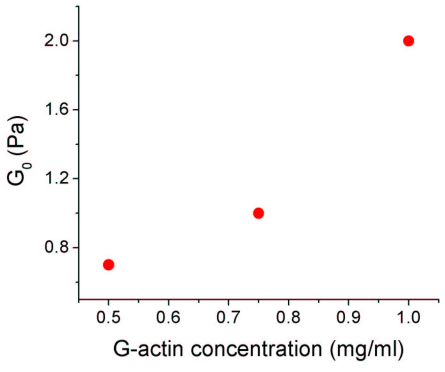
Plateau Modulus: actin/scruin

with scruin concentration:



0.5 mg/mL G-actin

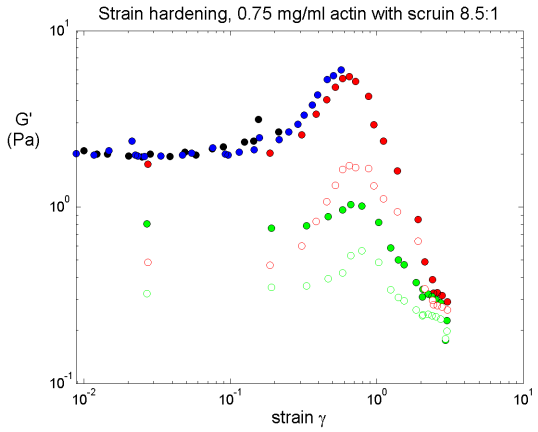
with concentration of G-actin:



30:1 G-actin: scruin

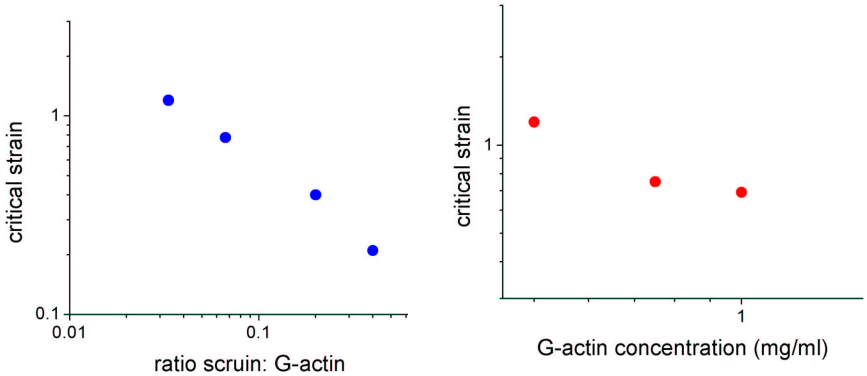
Strain Hardening: Actin/Scruin

0.75 mg/ml G-Actin, 30 G-actin:1 scruin



Critical strain

with scruin concentration: *with concentration of G-actin:*

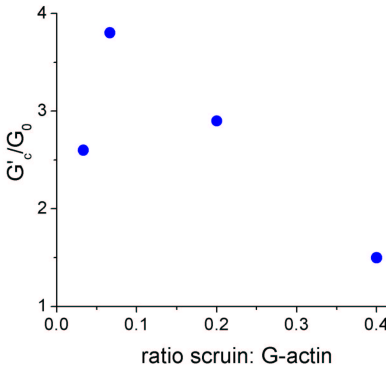


0.5 mg/mL G-actin

30:1 G-actin: scruin

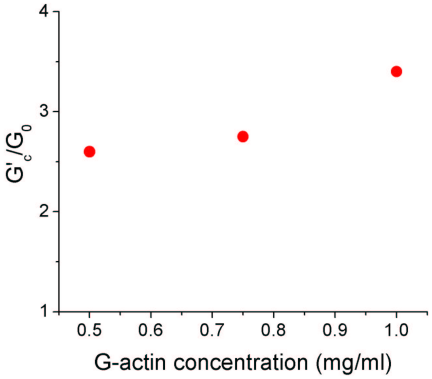
Degree of Strain Hardening

with scruin concentration:



0.5 mg/mL G-actin

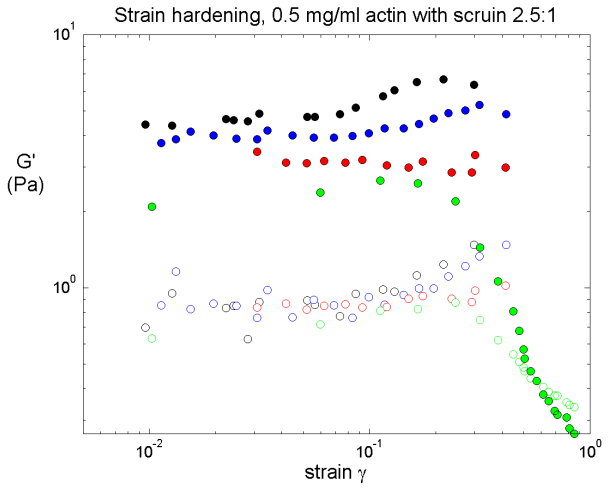
with concentration of G-actin:



30:1 G-actin: scruin

Strain Hardening: Actin/Scruin

0.75 mg/ml G-Actin, 2.5 G-actin:1 scruin



Conclusions

- Plateau modulus clearly increases with scruin concentration
- G_0 clearly increases with G-actin concentration
- Critical strain clearly occurs earlier with increasing scruin concentration
- Critical strain clearly occurs earlier with increasing actin concentration
- Theory ???