United Nations Educational, Scientific and Cultural Organization

International Atomic Energy Agency

SMR 1746 - 6

WORKSHOP ON DRIVEN STATES IN SOFT AND BIOLOGICAL MATTER
18 - 28 April 2006

New Methods to Study Cell Migration

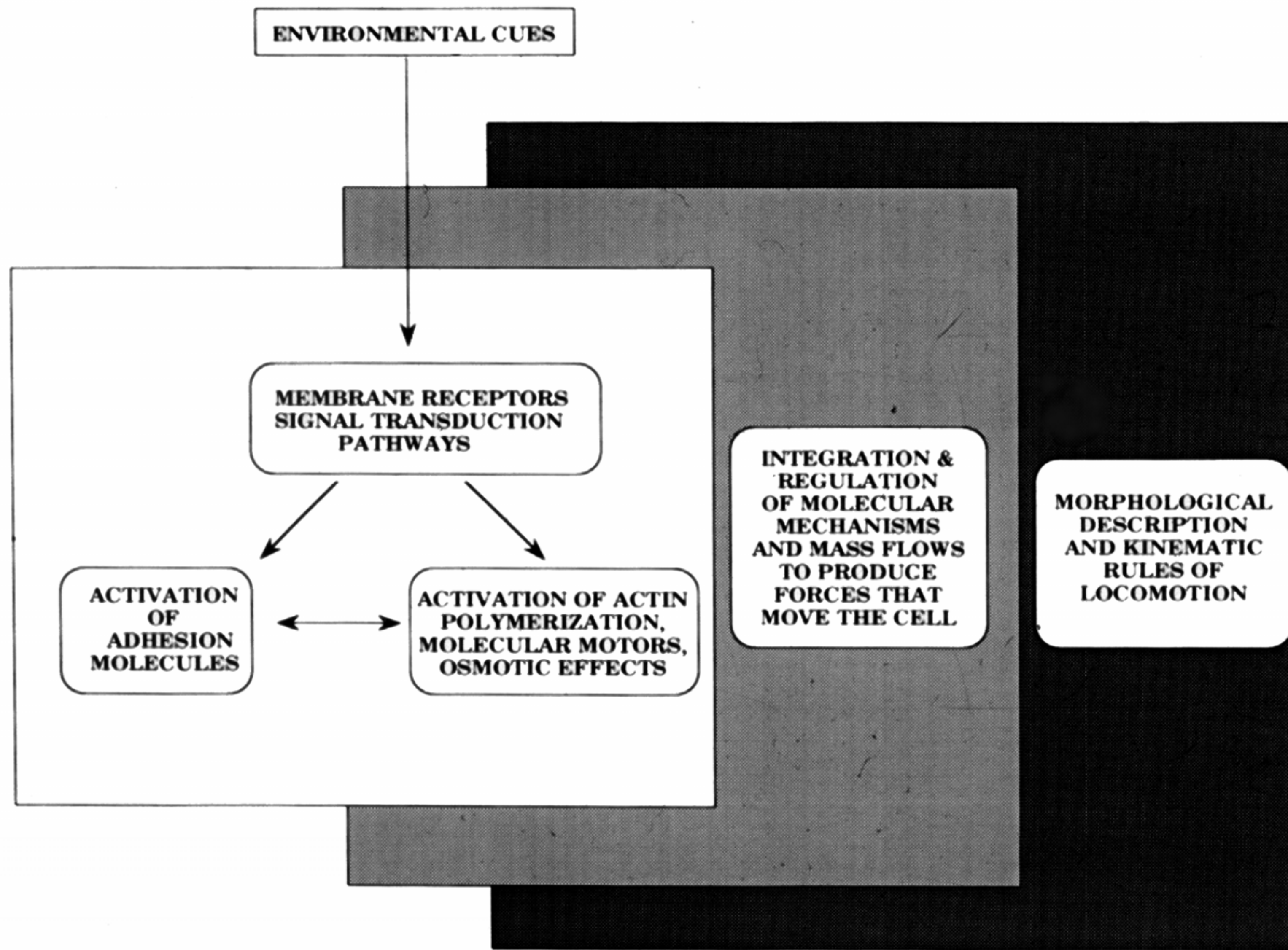
Part I

Kenneth JACOBSON
University of North Carolina at Chapel Hill,
School of Medicine, Dept. Cell & Development Biology
27599 Chapel Hill, U.S.A.

New methods to study cell migration

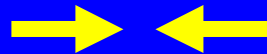
Topics

- The challenge of understanding cell migration
- Use of photoactivation & CALI to perturb cell migration-- a progress report
- Measurement of lamellipodial protrusive force
- An intermediate between the cell biologist's model and theory-causal mapping (CMAP)


ENVIRONMENTAL CUES
MEMBRANE RECEPTORS SIGNAL TRANSDUCTION PATHWAYS
ACTIVATION OF ADHESION MOLECULES
ACTIVATION OF ACTIN POLYMERIZATION, MOLECULAR MOTORS, OSMOTIC EFFECTS
INTEGRATION & REGULATION OF MOLECULAR MECHANISMS AND MASS FLOWS TO PRODUCE FORCES THAT MOVE THE CELL
MORPHOLOGICAL DESCRIPTION AND KINEMATIC RULES OF LOCOMOTION

How do we approach a quantitative understanding of cell movement?

Build up from molecular mechanisms



Top down modeling of integration of collective molecular mechanisms e.g. protrusion, contraction etc.

“Up close the paintings of Renoir & Monet look like ‘daubs of paint’, nothing more. Yet when we step back from the canvases, we see fields of flowers”

From Davidson’s review of A Different Universe by Robert Laughlin-NYTimes 6/19/05

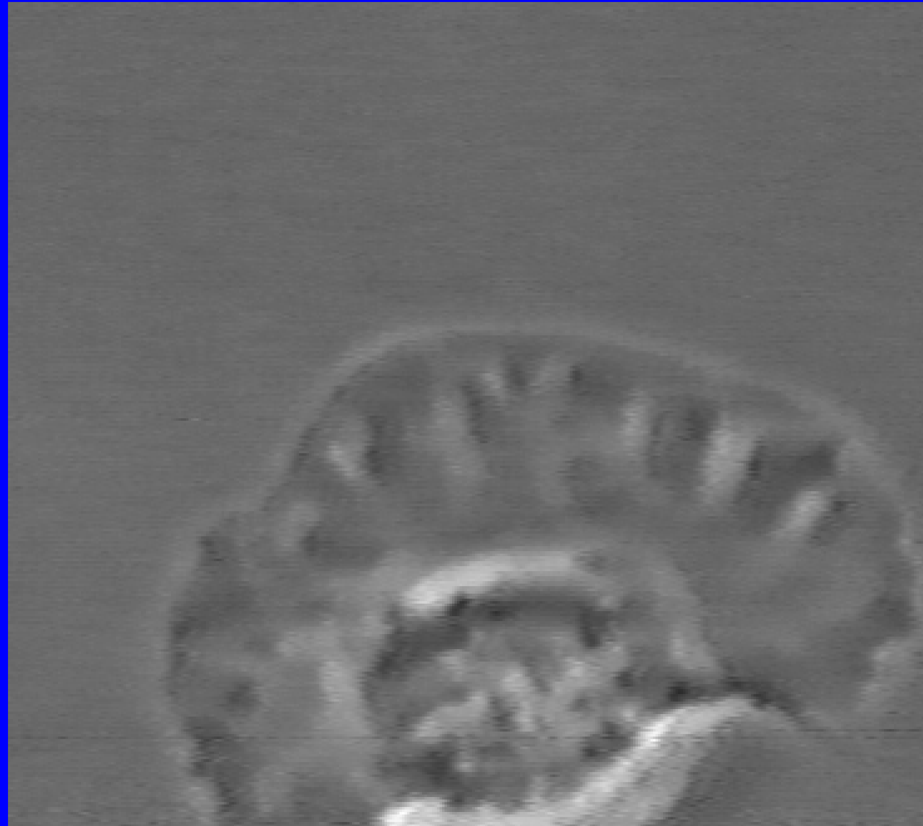
Philosophy of quantitative modeling

- Use model to simulate behavior & compare to experiment
- Revise model until concrete insight gained into key factors determining migration
- Test alternate models
- Overarching goal: Quantitatively organize information & ideas on migration mechanisms

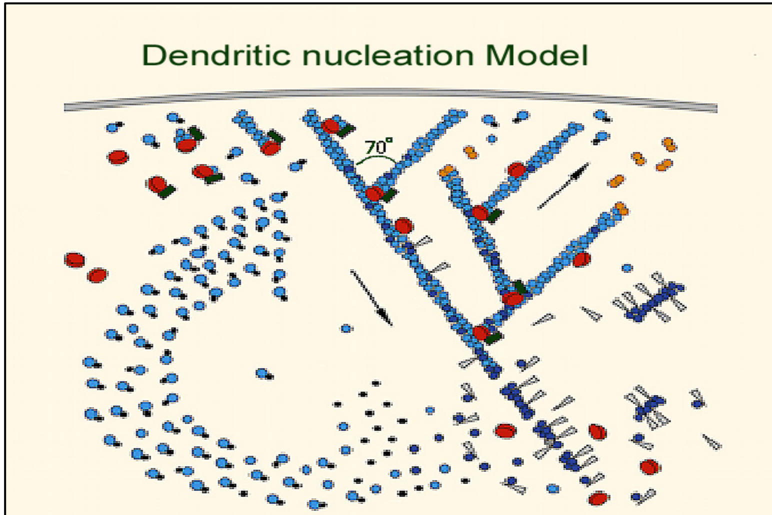
Advantages of Simple-shaped Cells for Biophysical Studies

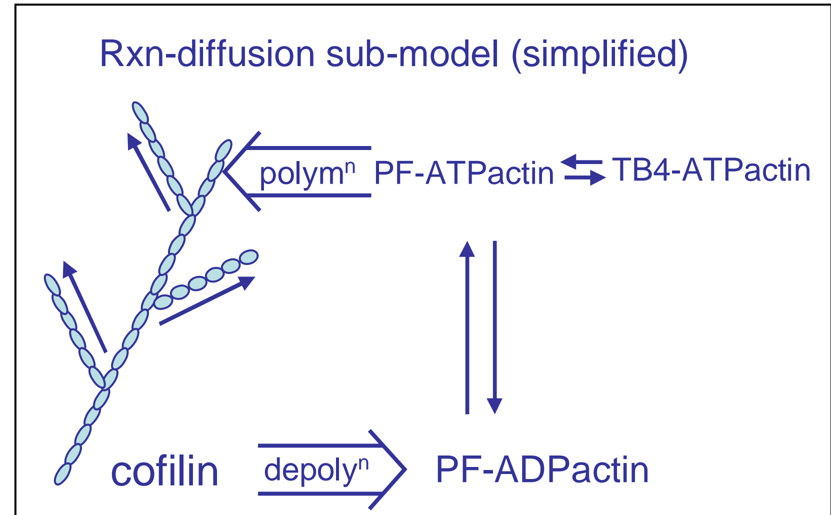
- Amenable to modeling
- Simple shape & migratory pattern: easy to see results of perturbation
- Simple, symmetric net traction stress pattern

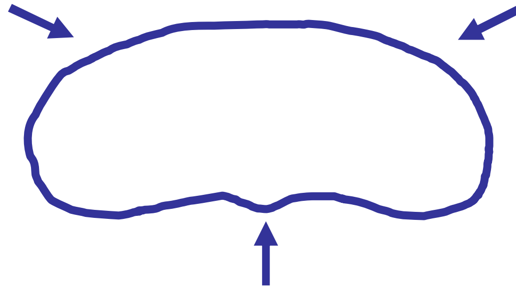
Gliding Fish Keratocyte

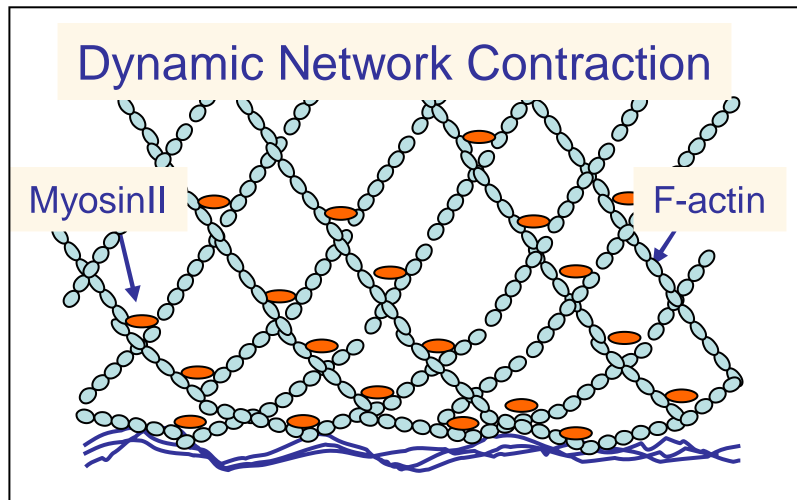


In the keratocyte, protrusion & retraction smoothly coordinated

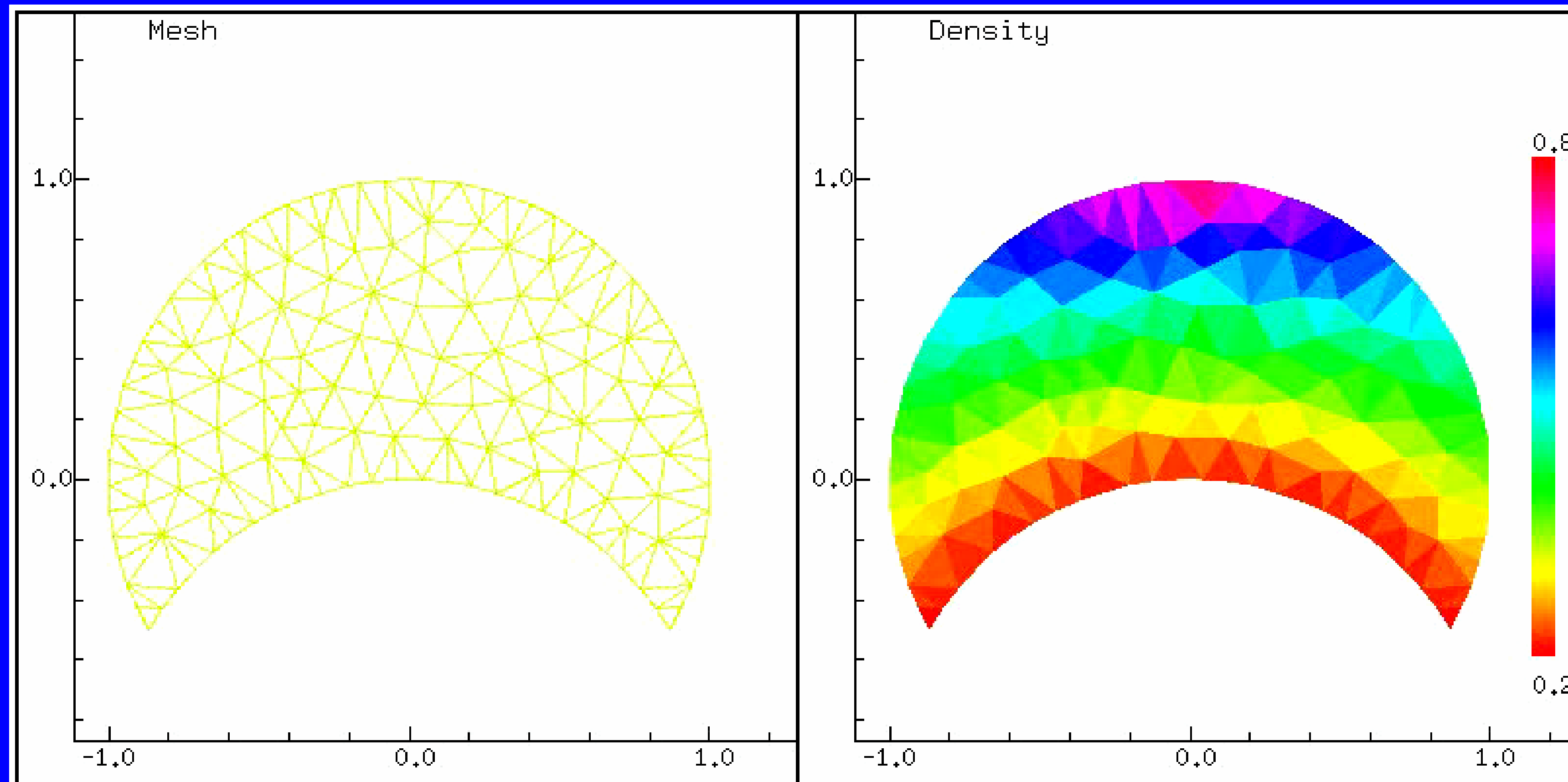

Dendritic nucleation Model
70°


Rxn-diffusion sub-model (simplified)
polym^n
PF-ATPactin
TB4-ATPactin
cofilin
depoly^n
PF-ADPactin




Dynamic Network Contraction
MyosinII
F-actin

A virtual keratocyte--A. Mogilner et al, UC-Davis

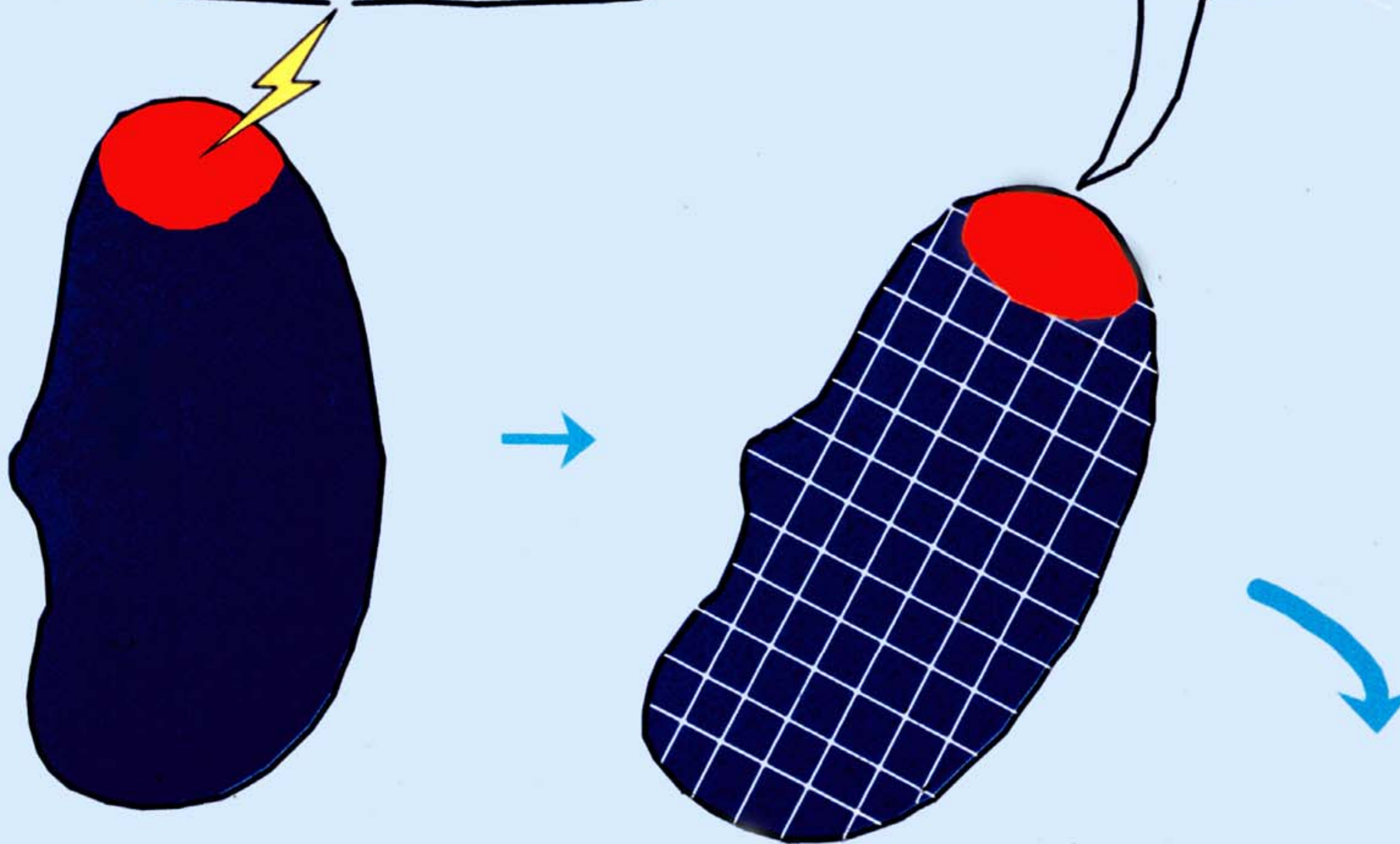


Density of f-actin plotted

Rubenstein et al SIAM J. 3:413 (2005)

Test robustness of in silico models of migration i.e. do we have the rules of integration of protrusion, retraction and adhesion correct?

Use light-directed methods to perturb molecular activities in single migrating cells in a spatially & temporally defined way--complement to genetic perturbations

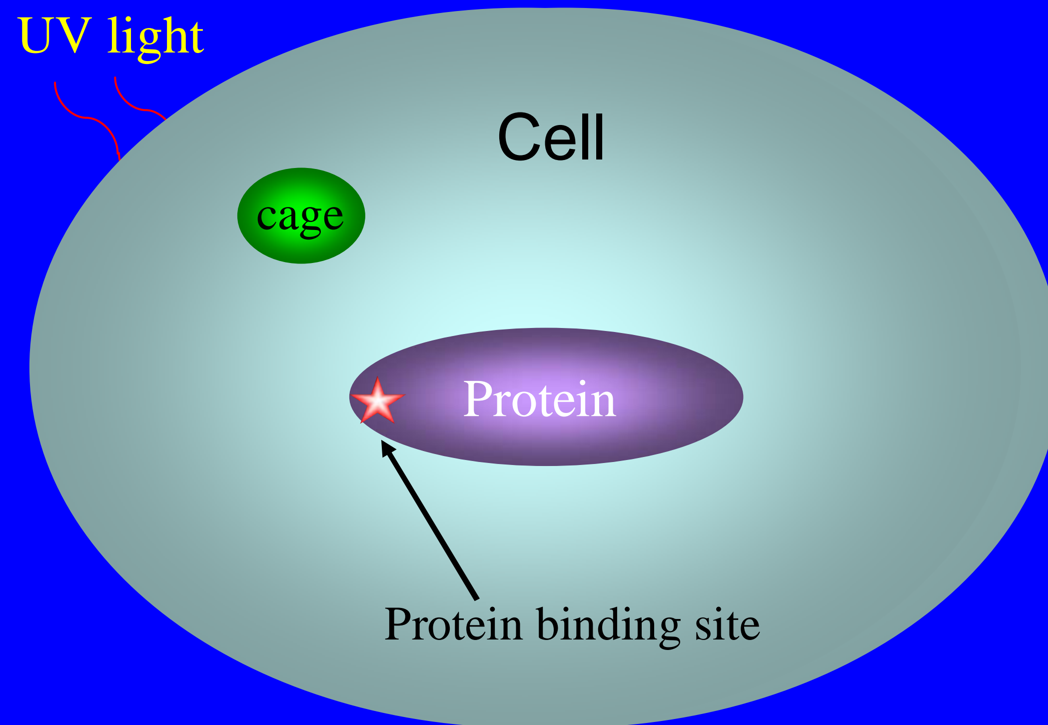

LOCAL, LIGHT DIRECTED PERTURBATION OF PROTRUSION, CONTRACTILITY OR ADHESION
MODEL PREDICTS EFFECT OF LOCAL PERTURBATION ON LOCOMOTION

Experimental Perturbation

Local ACTIVATION of molecule: photoactivation

Local INACTIVATION of molecule: CALI, photoactivation

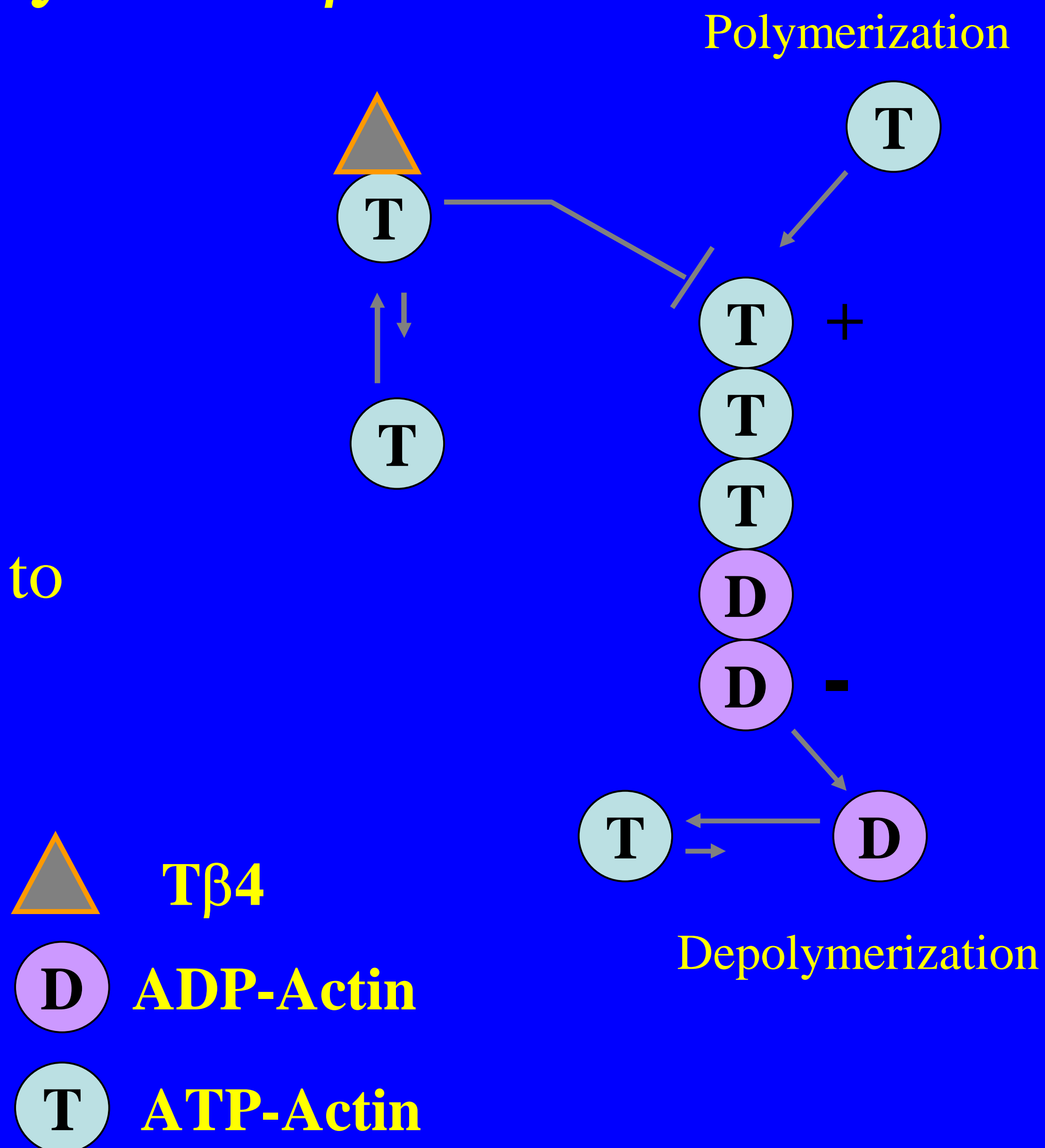
Photoactivation



Introduce a biologically inactive protein/peptide into the cell

Thymosin β4

- 5 kD protein
- Major G-actin sequestering protein
- Binds preferentially to ATP-G-actin



Caging of Thymosin β4

Caged Tβ4

Caging group — O — C(=O)

H — N

THYMOSIN β4
(inactive)

365 nm
UV
laser

Caging group

H — N — H

uncaged
THYMOSIN β4
(active)

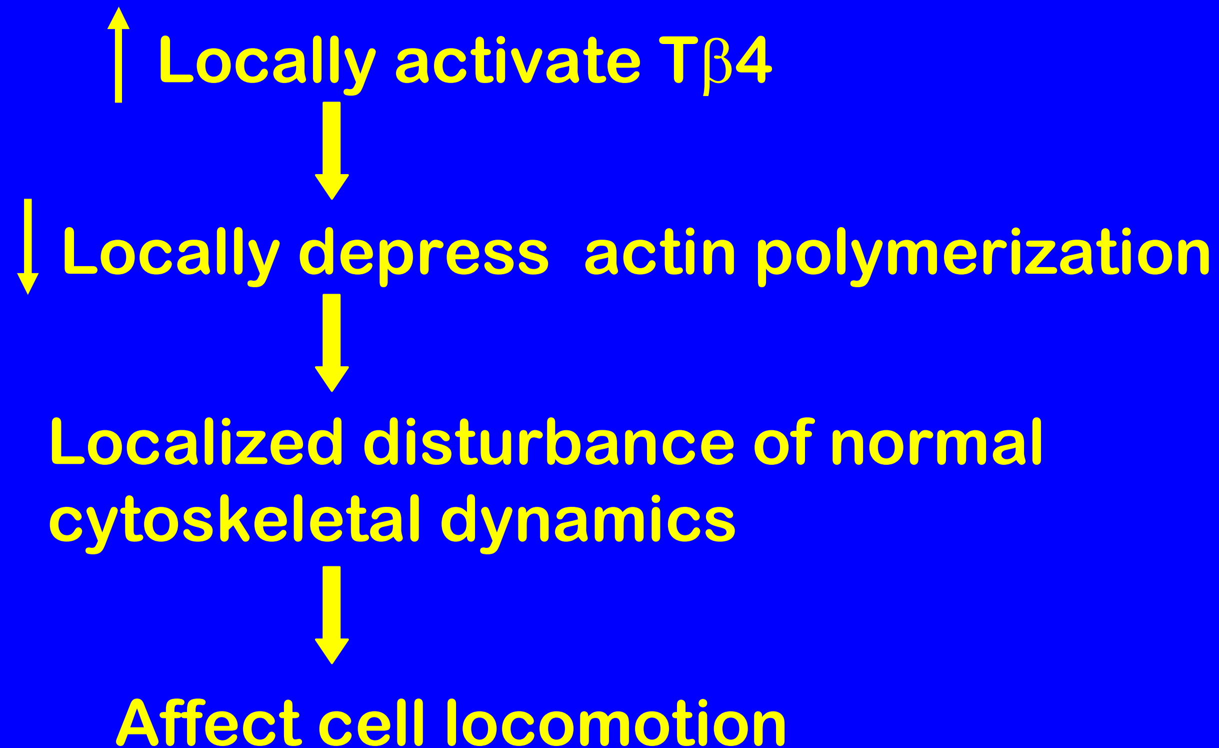
1 5 10 15 20 25 30 35 40

SD**K**PDMAEIE**K**FD**K**S**K**L**KK**TETQE**K**NPLPS**K**ETIEQE**K**QAGES

(N) (C)

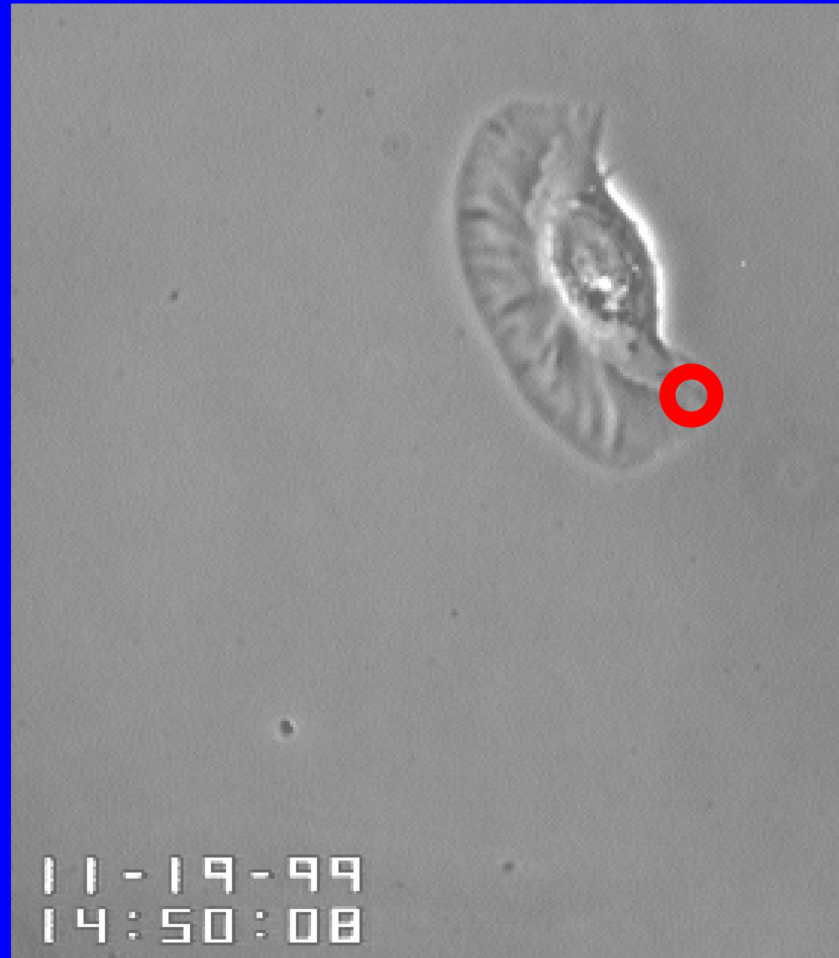
Actin binding

HYPOTHESIS



Turning of a keratocyte in response to photoreleased Tb4

Controls: no
effect of light alone
or of uncaging
cg’d Fl-dextran



10µW of 354nm
into~3 µm zone
for 100 ms

Two models for cell turning

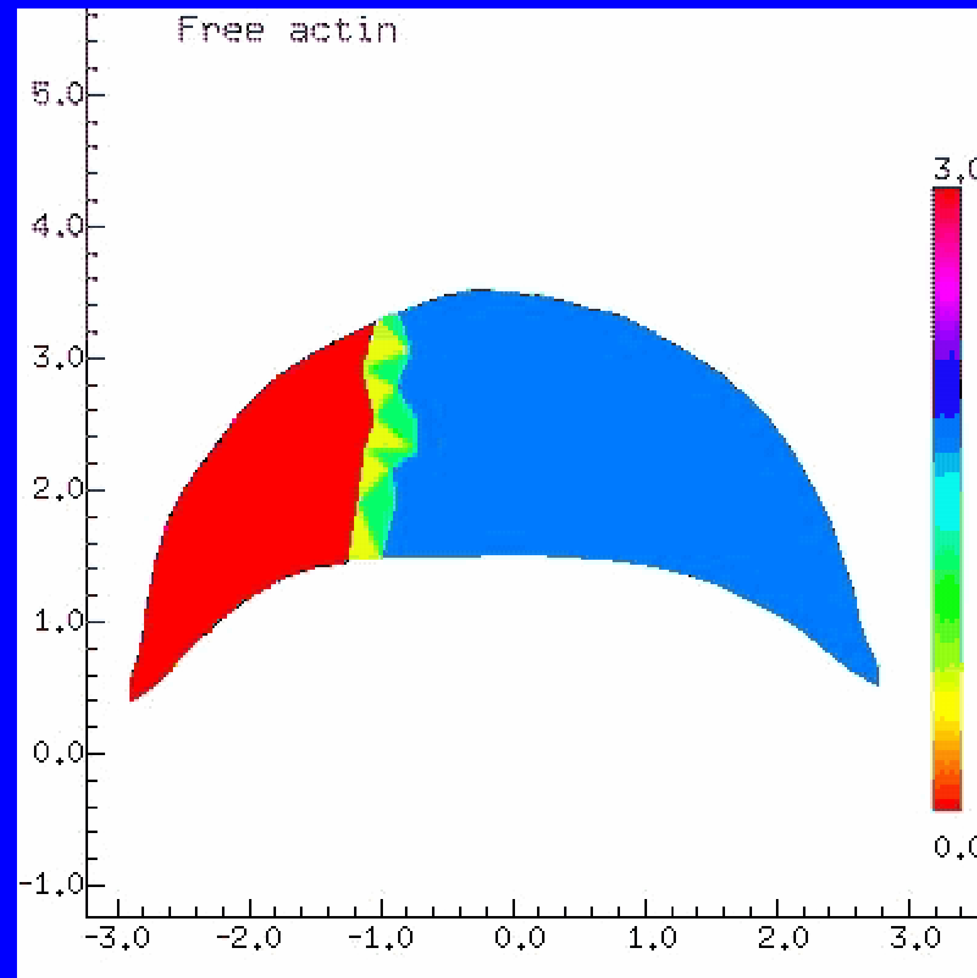
- Qualitative, cell biological model
  - more features but qualitatively interelated (Roy et al, JCB, 2001)
- Quantitative, computer generated model
  - fewer features but quantitatively interelated

Virtual keratocyte turning induced by photorelease of caged thymosin β4

- Tβ4 locally depresses free G-actin.
- Local lamellipod growth and contractility decrease as [G-actin] decreases.
- Adhesion is assumed to be unaffected.
- Because protrusion & retraction on opposite side are not changed, cell begins to pivot
- Diffusion & convection distributes the free G-actin

Alex Mogilner

Effect of locally depressing free G-actin w/pulse of TB4



Red = lower [G-actin]

Rubenstein et al SIAM J. 3:413 (2005)

CONCLUSION

- **Feasibility of photoactivation technique as a perturbation approach for studying cell locomotion.**
- **Specific effect of photoreleased Tβ4 in modulating cell locomotion was observed.**
- **Turning consistent with an *in vivo* role of Tβ4 in actin dynamics.**

Summary: photoactivation & migration

PROTEINS

- ***Thymosin β4***--local effect.

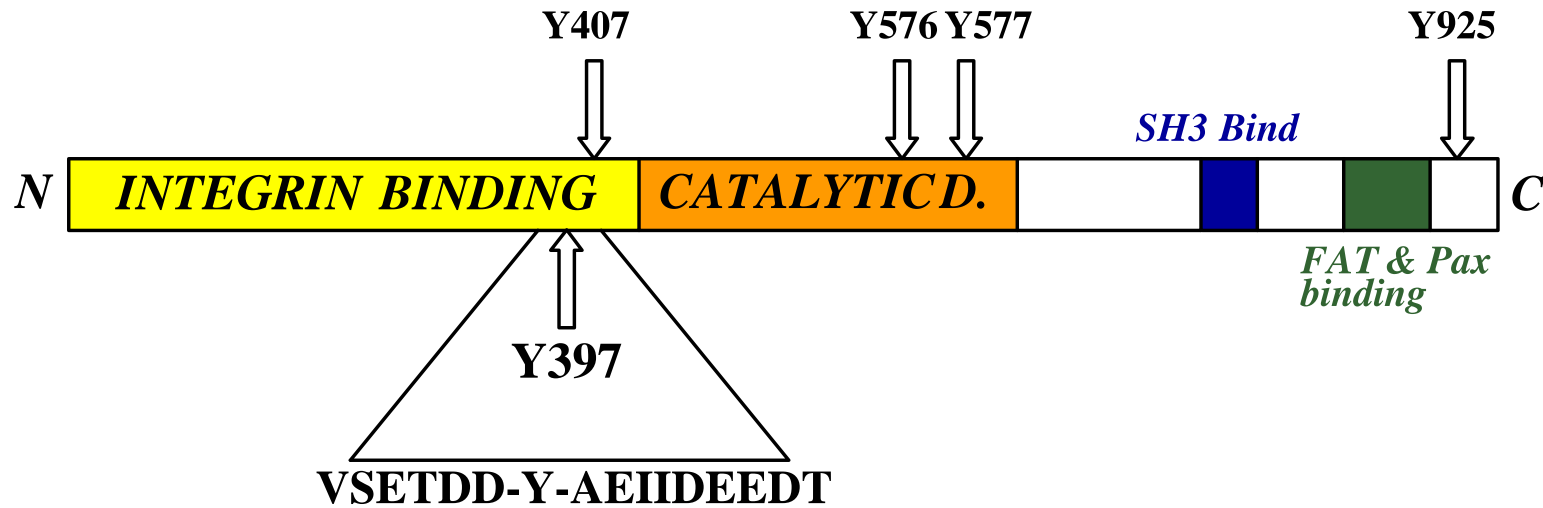
- ***Cofilin***: local uncaging induces local protrusion because of increase in free barbed ends caused by severing. [Ghosh et al, Science 304:743 (2004)].

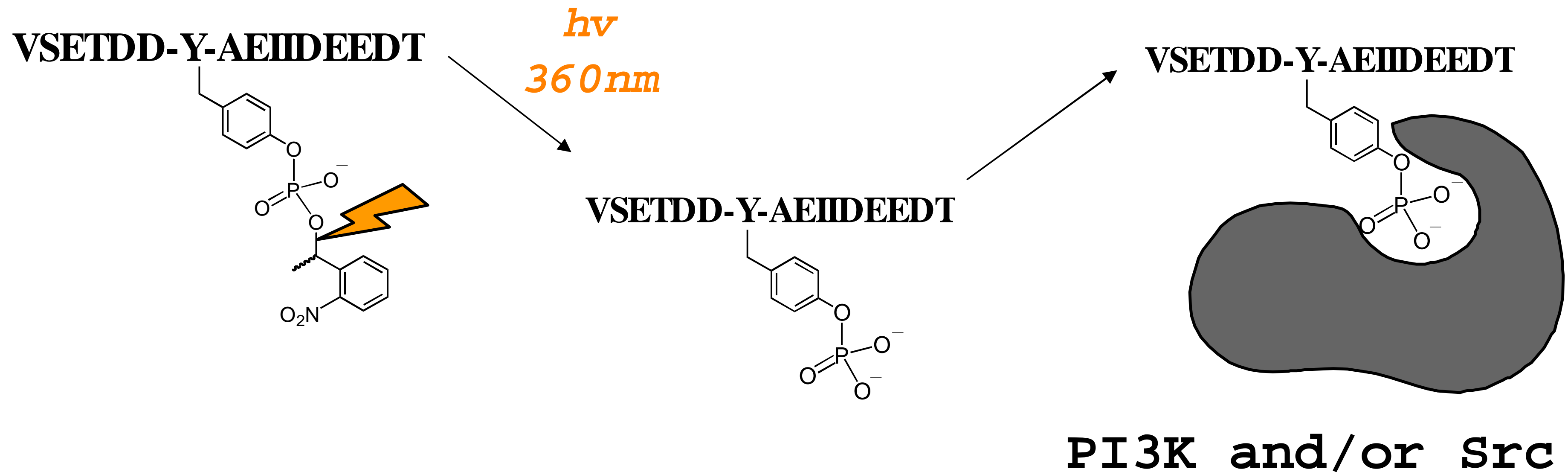
PEPTIDES

- ***Small inhibitor peptides for MLCK***:Local uncaging-no effect; global uncaging-cell stops [Walker et al PNAS 95:1568 (1998)].

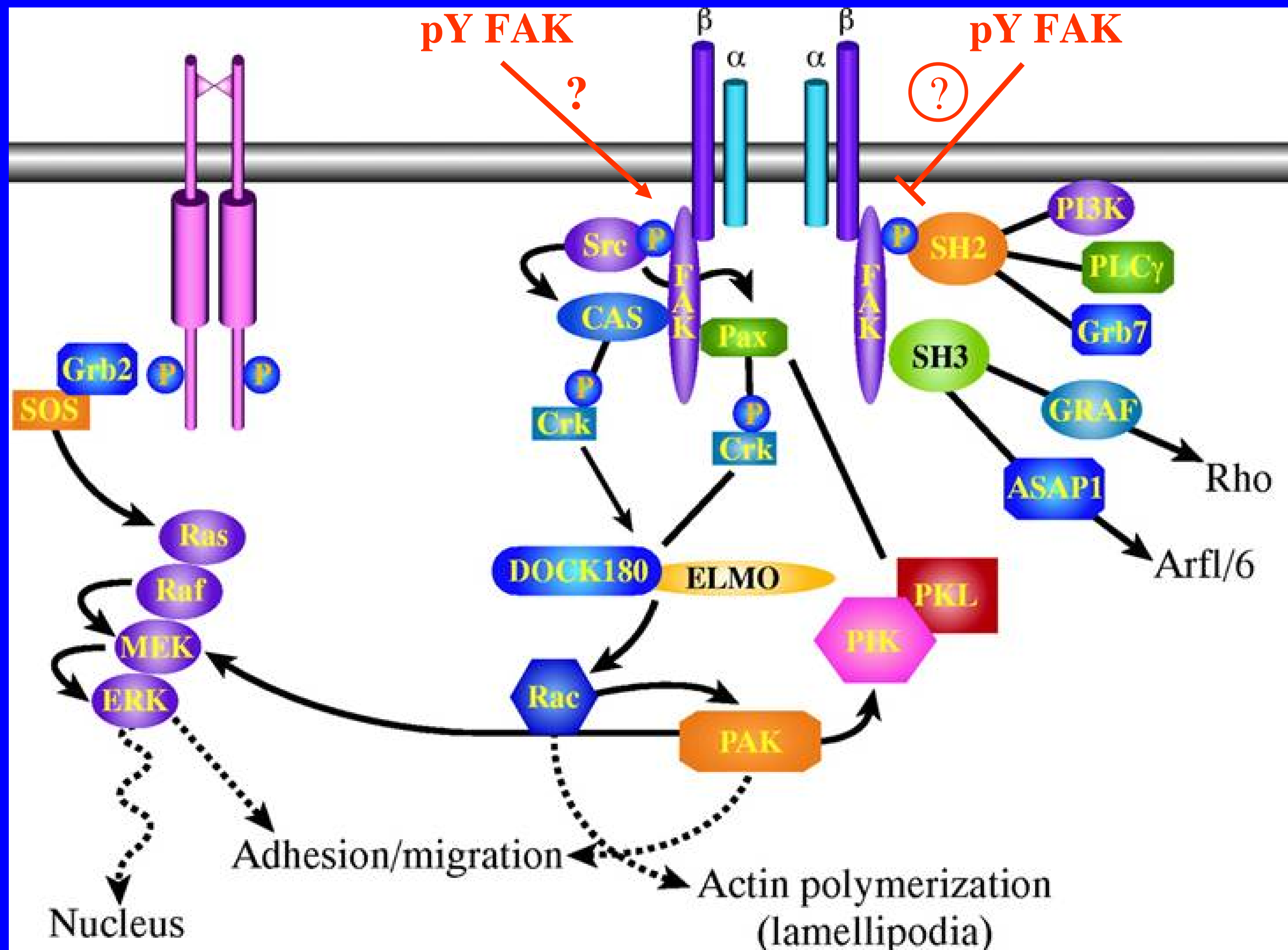
- ***Caged phosphotyrosine FAK peptides:*** global uncaging produces transient lamellar arrest [Humphrey et al J Biol Chem 280:22091(2005)]

FAK derived peptides (Y397)



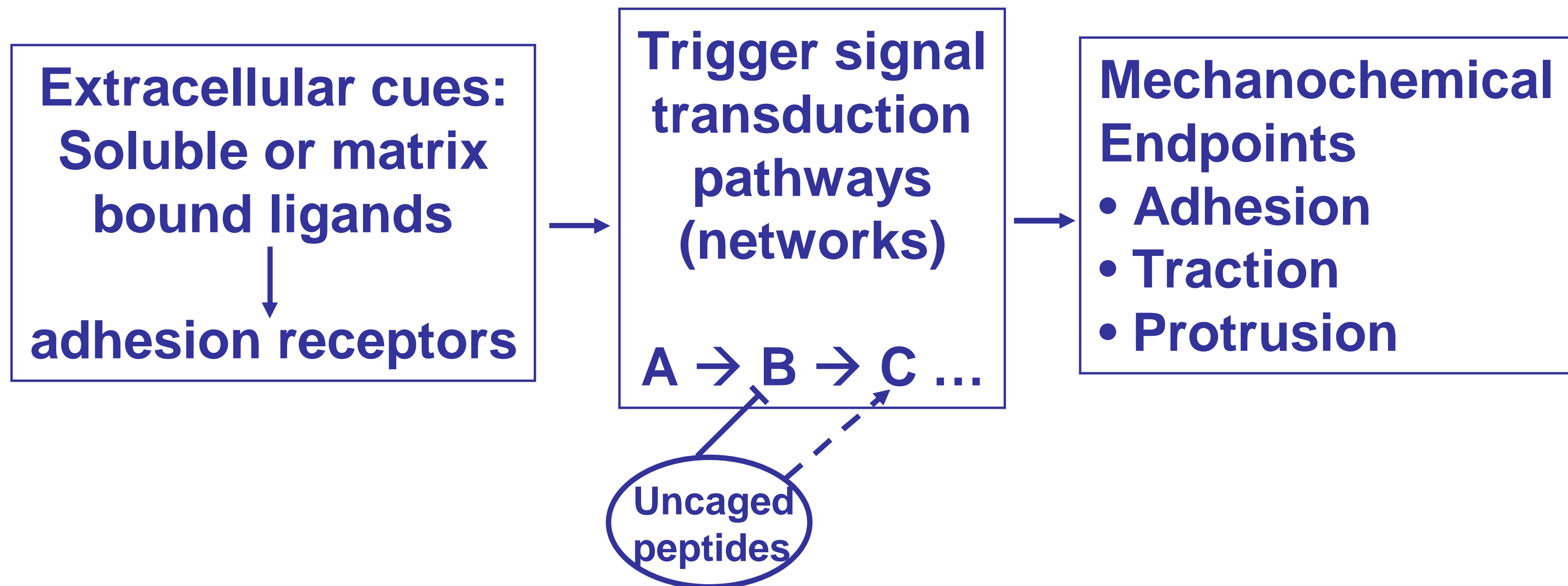


FAK is a multifacted regulator of cell migration, survival, cell cycle & cancer



Adapted from: JT Parsons, J.Cell Sci. 116, 1409-1416 (2003)

CENTRAL CHALLENGE: MECHANISM



CALI: Chromophore Assisted Laser Inactivation [Dan Jay]

Chromophore Assisted Laser Inactivation (CALI)

Light-mediated loss-of-function tool

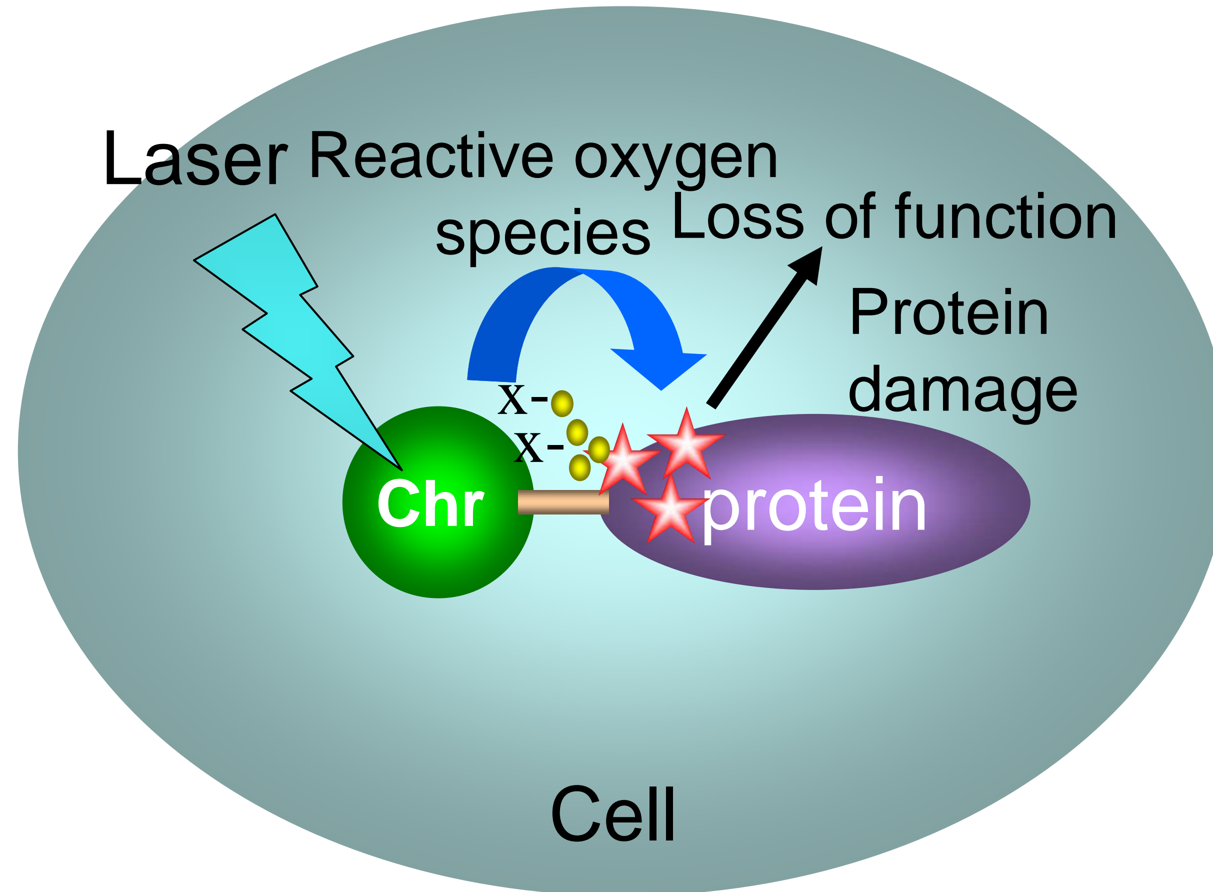
High spatial resolution

- Subcellular inactivation
- High selectivity

High temporal resolution

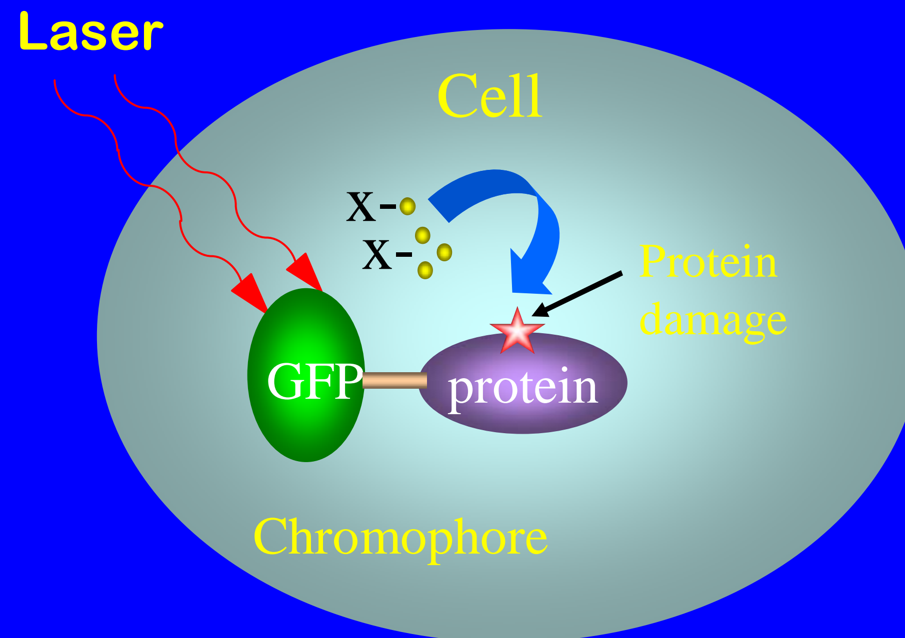
- Instantaneous inactivation
- Eliminates genetic/molecular compensation

CALI Mechanism



- Chromophore excitation leads to production of free radicals
- Free radicals are highly destructive, causing protein damage
  - short half-life (nm destruction radius)
- Potential for local, instantaneous inactivation of adjacent protein

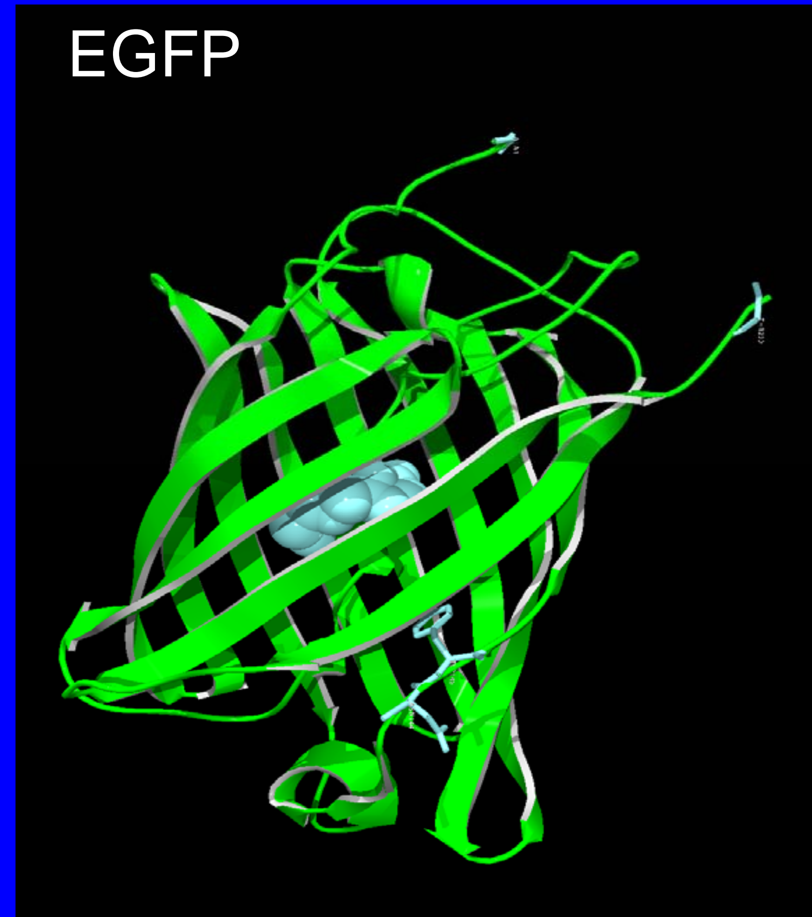
Can GFP be used as *in vivo* CALI chromophore??



Surrey et al., PNAS 95:4293 (1998)

EGFP as a CALI Chromophore

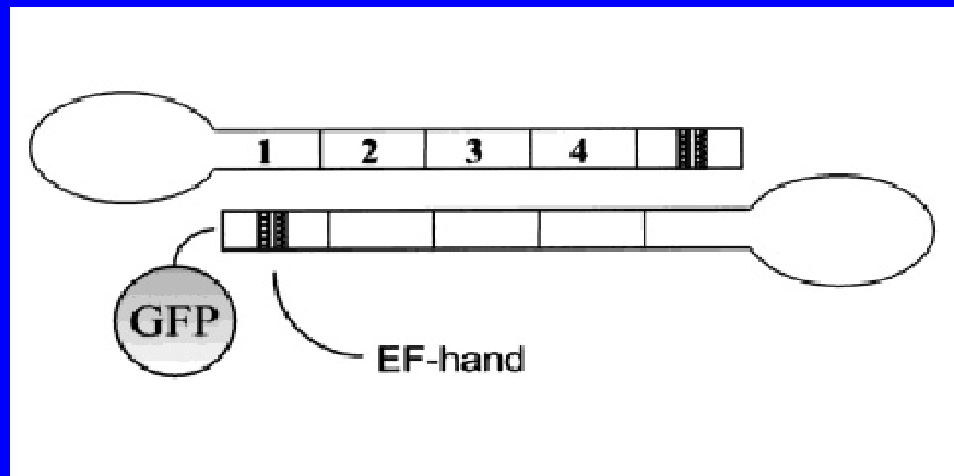
- **Advantages**
  - Genetically encoded
  - Covalent linkage to protein of interest insures specificity
  - Widely used
- **Disadvantages**
  - Photostable
    Ineffective ROS generator
  - 200-1000X less efficient than other dyes



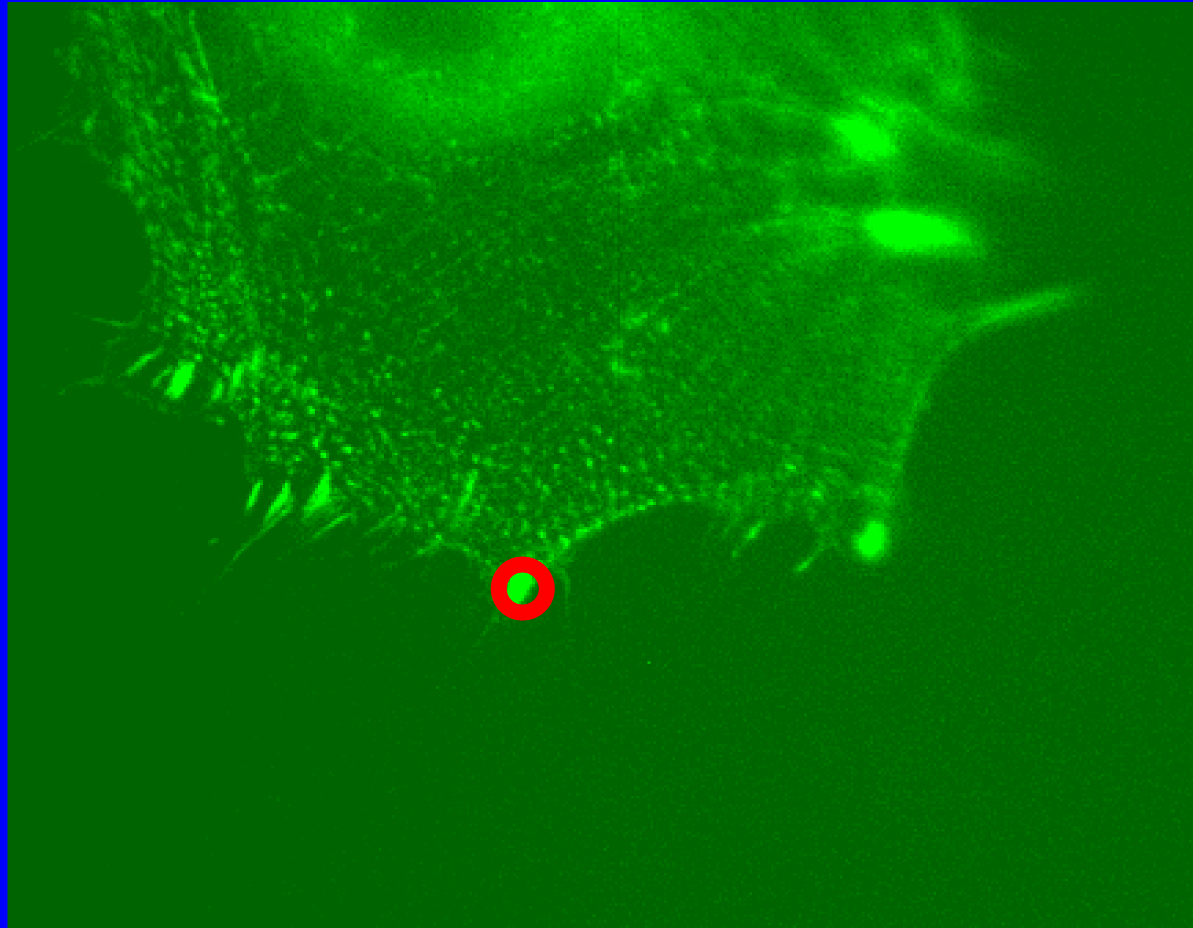
Photostability may also be an advantage in that there are separate regimes for imaging and inactivation

EGFP α-actinin

- permanently expressed in Swiss 3T3 at 1-5% endogenous levels
- dimerizes with native a-actinin
- EGFP fused to C terminus
- a-actinin key component of focal adhesions & stress fibers



CALI of GFP-α-actinin in Swiss 3T3

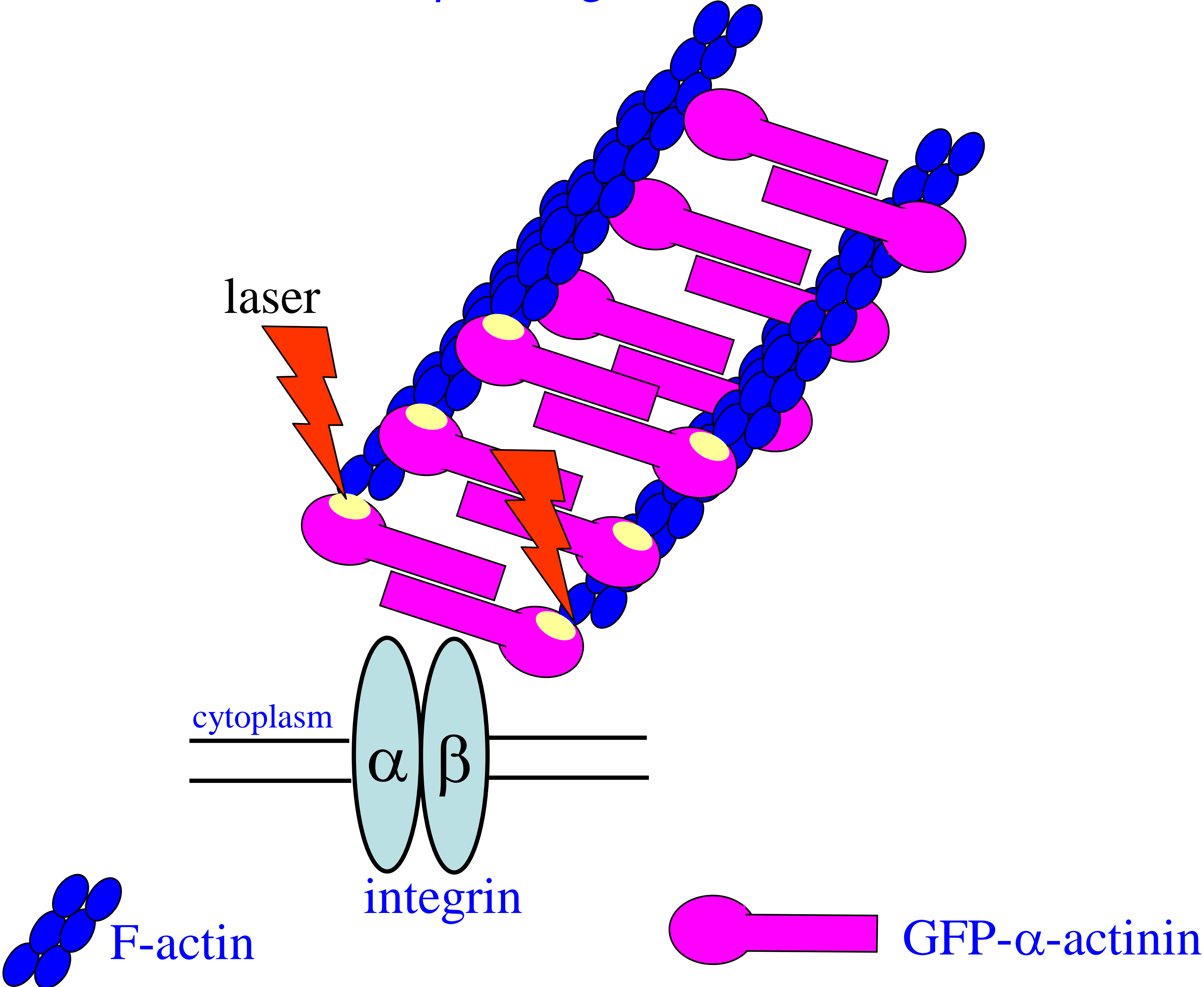


40 mW of
488nm into
2.2μm spot
for 100ms

No SF detachment with EGFP-FAK or
Paxillin

CALI-induced detachment of GFP-α-actinin from the focal adhesion

β1-integrin

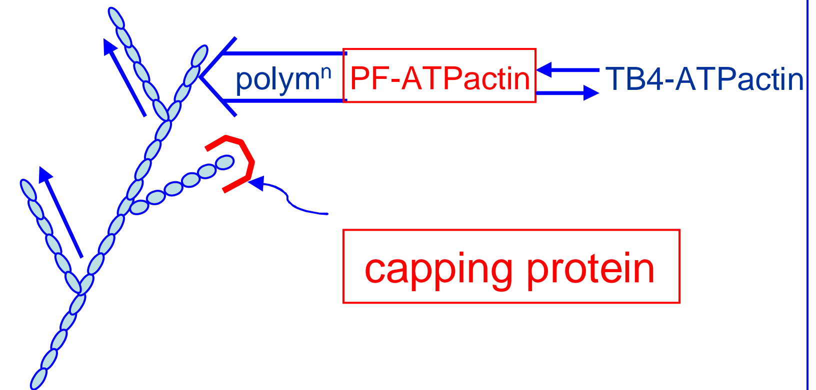


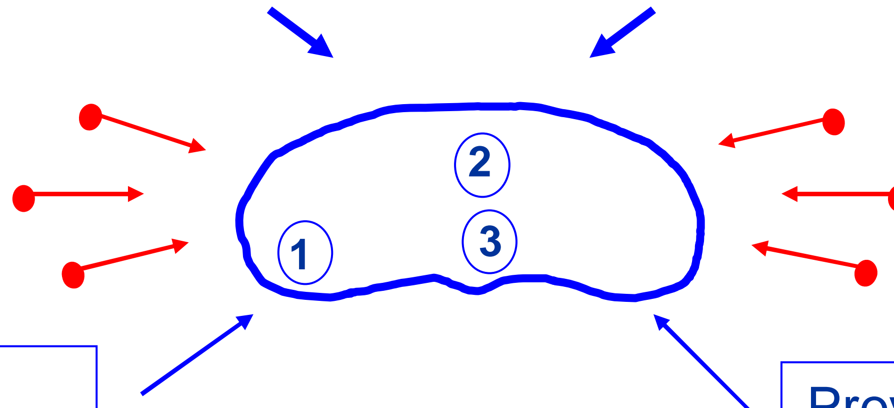
Current CALI/FALI reagents

- Introduced, labeled non-function-blocking abs
  - Malachite green, fluorescein
- Expressed reagents-GFPs
  - -actinin, Mena;Cx43 & Aurora B kinase using MPM (Tanabe et al, Nat. Methods2: 503-5 (2005)
  - Killer red (585nm)-PH domains (Bulina et al Nat. Biotech, 2005)
- Hybrid expressed/added reagents
  - FlAsH [synaptogamin I; Marek&Davis, Neuron 36:805 (2002)]
  - ReAsH--[connexin43 & L-type Ca channels; Tour et al Nature Biotech. 21: 1505(2003)].
  - FK-FALI--Marks et al PNAS 101:9982 (2004).

Concentration of players as numerical input to Mogilner model

G, F-actin, Tβ4, profilin etc.





Release or inactivate at different points in cell

Provide simultaneous traction & network dynamics maps with photoactivation/CALI operation

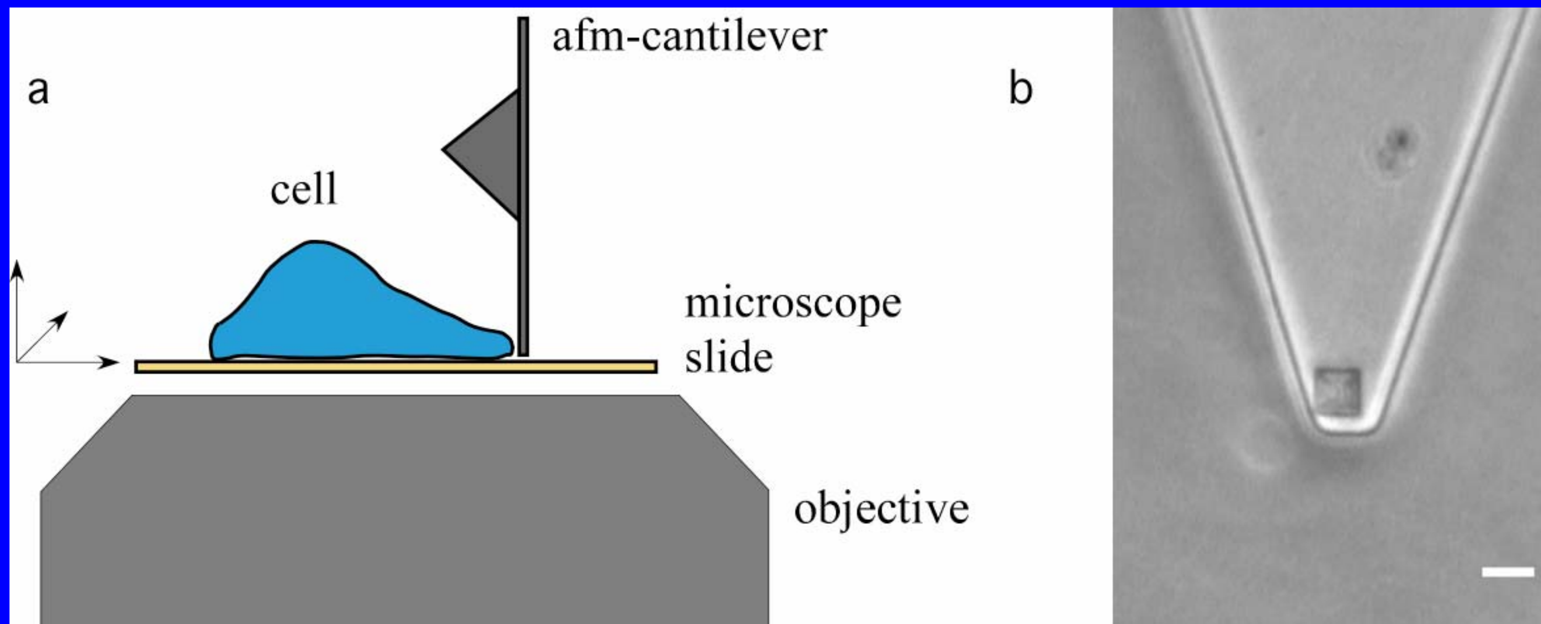
Direct measurement of the lamellar protrusive force in migrating cells

Marcus Prass, Ken Jacobson, Alex Mogilner & Manfred Radmacher

Objectives

- **Measure force-velocity relation for lamellar protrusion *in vivo [ie how motor system responds to load force]***
- **Measure stall force for protruding lamella**
- **Mechanical benchmark for theory**

Measurement Concept



Trick: get tip close enough to substrate so that cell doesn't slip under but not so close that tip touches substrate

Time record of cantilever interacting with cell