

<http://people.virginia.edu/~dta4n/biochem503/503.html>

## kinetics review!



for initial time, when  $[B] = 0$ :

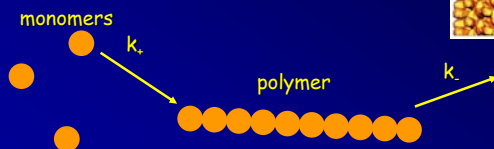
$$\frac{d[A]}{dt} = -k_{+}[A(t)] \quad \text{where } [A(t)] \text{ is concentration of } A \text{ at time } t$$

when  $[B] \neq 0$ :

$$\frac{d[A]}{dt} = -k_{+}[A(t)] + k_{-}[B(t)]$$

## kinetics of polymerization

consider non-covalent self-assembly of globular protein  
(e.g., G- to F-Actin; flagellin to flagella; pilin to pilus; tubulin to microtubule)



$$\frac{dn}{dt} = k_{+}c_1 - k_{-} \quad \text{for } n = \text{number of protomers in polymer, } c_1 = [\text{monomer}]$$

## kinetics of polymerization

rewriting,

$$\frac{dn}{dt} = k_{+}c_1 - k_{-} = k_{+}(c_1 - 1/K), \quad \text{where } K = k_{-}/k_{+}$$

$K$  is an equilibrium constant, while  $k_{+}, k_{-}$  are rate constants

what are the dimensions?

$k_{+}$  has dimensions of  $\text{mol}^{-1} \text{sec}^{-1}$

$k_{-}$  has dimensions of  $\text{sec}^{-1}$

$K$  has dimensions of  $\text{mol}^{-1}$

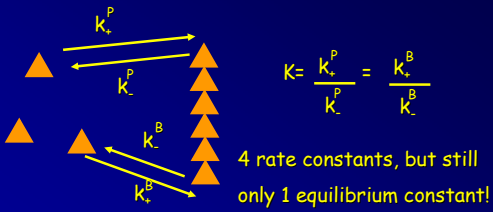
What are the regimes?

for  $c_1 > 1/K$ , growth

$c_1 < 1/K$ , depolymerization

$c_1^* = 1/K \equiv \text{critical monomer conc.}$

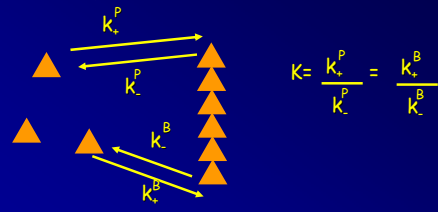
### Model too naïve, proteins not spheres



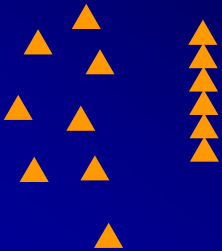
$$\frac{dn_p}{dt} = k_+^P c_1 - k_-^P = k_+^P (c_1 - 1/K)$$

$$\frac{dn_B}{dt} = k_+^B c_1 - k_-^B = k_+^B (c_1 - 1/K)$$

### Equilibrium vs. steady state



### "treadmilling" at steady state



generates a flux of subunits through a filament, can generate motion - requires an energy source (ATP, GTP)

### How can we measure these kinetics?

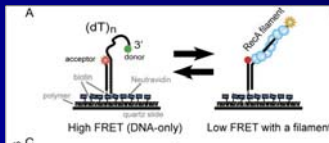
- bulk biochemical assays
- single molecule techniques

## How can we measure these kinetics?

### Real-Time Observation of RecA Filament Dynamics with Single Monomer Resolution

Cell, August, 2006

Chikina, Joo,<sup>1</sup> Sean A. McKinney,<sup>1</sup> Munehiko Nakamura,<sup>2</sup> Ivan Ruzick,<sup>1,3</sup> Sue Myung,<sup>1</sup> and Tsaijoo Hai<sup>1,4</sup>  
<sup>1</sup>Howard Hughes Medical Institute and Department of Physics  
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<sup>3</sup>University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA  
<sup>4</sup>Department of Physics, Emory University, Atlanta, GA 30302, USA  
 Contact: shai@uic.edu  
 DOI: 10.1016/j.cell.2006.06.042

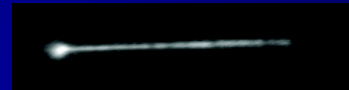


## How can we measure these kinetics?

### Direct observation of individual RecA filaments assembling on single DNA molecules

Nature, September, 2006

Roberts-Galloni<sup>1,2</sup>, John Aronson<sup>1,2</sup>, Ronald J. Bakker<sup>1</sup> & Stephen C. Kowalczykowski<sup>1,2</sup>



## We have ignored nucleation....

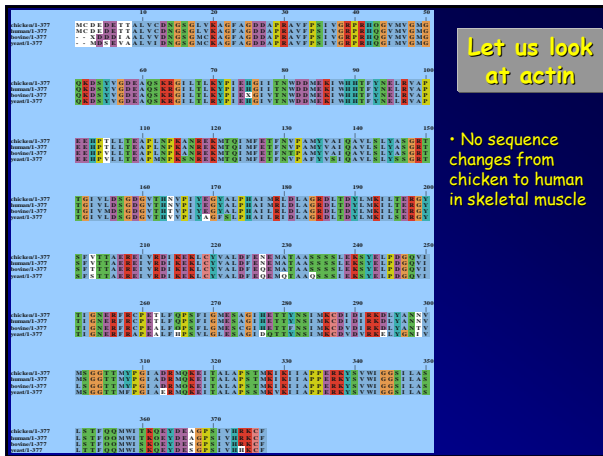
Early experiment with flagellin:  
 length increased linearly with number of added monomers  
 What does this imply?

Kinetics of nucleation different from kinetics of elongation:

$i_0$  = size of nucleus  
 $k_n$  = rate constant for nucleation  
 rate  $\sim k_n c_1^{i_0}$

## In summary

In absence of energy source, single equilibrium constant for monomer-polymer equilibrium  
 Polar polymer will have different ends, but same equilibrium constant at each end  
 With energy source (ATP, GTP) can have different equilibrium constants, subunit flux at *steady state*  
 In general, nucleation is greatly suppressed - kinetically unfavorable association of small oligomers



### Extensive evidence for active role of actin in acto-myosin motility

- Drummond D.R., Peckham M., Sparrow J.C. & White D.C. Alteration in crossbridge kinetics caused by mutations in actin. *Nature* 348, 440-442 (1990).
- Prochniewicz E. & Yanagida T. Inhibition of sliding movement of F-actin by crosslinking emphasizes the role of actin structure in the mechanism of motility. *J. Mol. Biol.* 216, 761-772 (1990).
- Prochniewicz E., Katayama E., Yanagida T. & Thomas D.D. Cooperativity in F-actin: chemical modifications of actin monomers affect the functional interactions of myosin with unmodified monomers in the same actin filament. *Biophys. J.* 65, 113-123 (1993).
- Schwyter D.H., Kron S.J., Toyoshima Y.Y., Spudich J.A. & Reisler E. Subtilisin cleavage of actin inhibits in vitro sliding movement of actin filaments over myosin. *J. Cell Biol.* 111, 465-470 (1990).
- Kim E. *et al.* Intrastrand cross-linked actin between Gln-41 and Cys-374, III. Inhibition of motion and force generation with myosin. *Biochemistry* 37, 17801-17809 (1998).

### Two most likely possibilities:

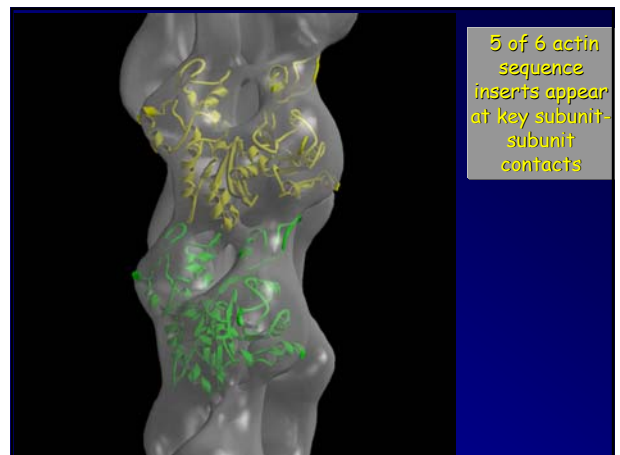
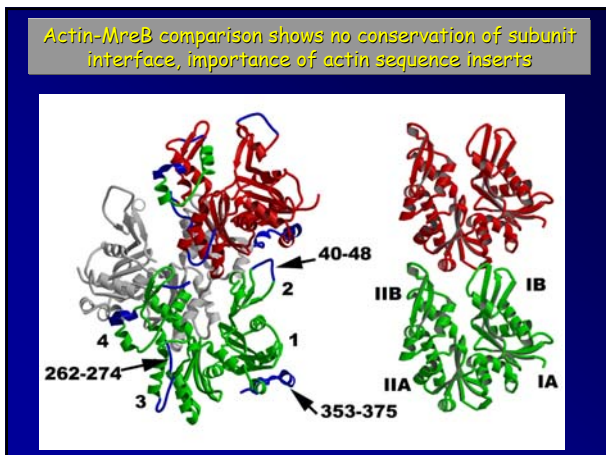
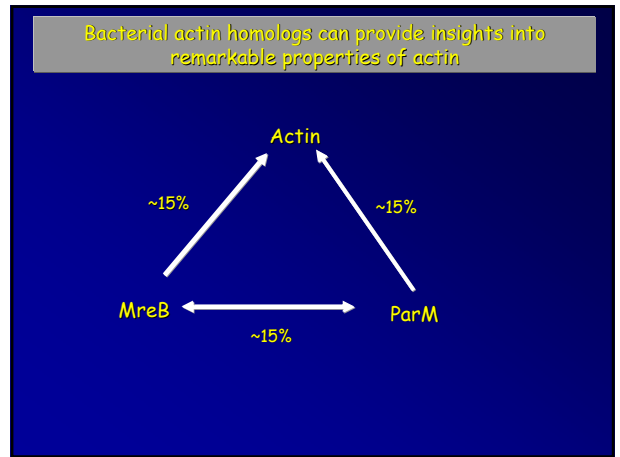
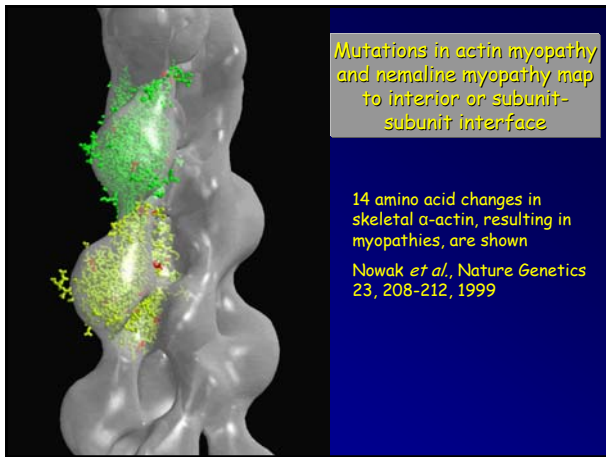
- Actin filament has dynamic changes, essential for force production in the actomyosin interaction, that are inhibited by these modifications
- Actin is static, but myosin binds to a second site on actin after ATP hydrolysis, and these modifications all affect that second site
- Will look at what is known about internal dynamics of F-actin
- We will ask how these properties of actin may explain the remarkable sequence conservation

### Structural studies can begin to make sense of the conservation

red=39 (out of 375) residues different between yeast and bovine b-cytoplasmic actin

EM reconstruction is yeast F-actin (Orlova *et al.*, 2001); G-actin crystal structure (Chik *et al.*, 1996)

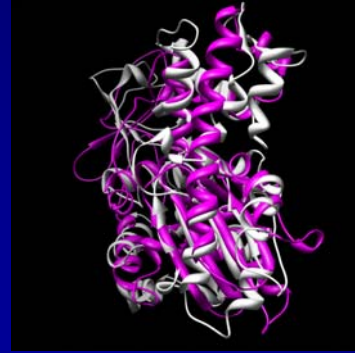
- Multitude of actin-binding proteins has been used to explain sequence conservation...
  - but actin binding proteins are not highly conserved (e.g., yeast & human cofilin share 37% identity)
  - divergence of actin-binding proteins can explain actin divergence, not conservation!
- actin-actin interactions in filament dynamics - e.g., subdomain 2
- allosteric interactions within actin
- multiple binding sites for some actin-binding proteins



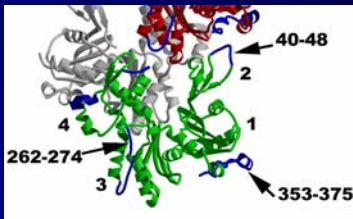
ParM and Actin Monomers Differ at Regions of Subunit-Subunit Contacts for Actin



ParM and Actin Monomers Differ at Regions of Subunit-Subunit Contacts for Actin



Actin insertions are allosterically coupled



- \* C-terminus/DNase I loop coupling exists in G- and F-actin
- \* Hydrophobic plug/DNase I loop interaction
- \* C-terminus/Hydrophobic plug coupling in F-actin

Allostery arises from internal networks of coupled residues

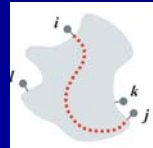
Evolutionarily conserved networks of residues mediate allosteric communication in proteins

Görel M. Süel<sup>1,2</sup>, Steve W. Lockless<sup>1,2</sup>, Mark A. Wall<sup>1</sup> and Rama Ranganathan<sup>1</sup>

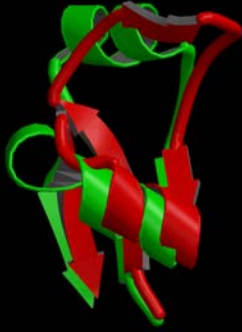
<sup>1</sup>Published online 16 December 2002; doi:10.1038/08881

A fundamental goal in cellular signaling is to understand allosteric communication, the process by which signals originating at one site in a protein propagate reliably to affect distant functional sites. The general principles of protein structure that underlie this process remain unknown. Here, we describe a sequence-based statistical method for quantitatively mapping the global network of amino acid interactions in a protein. Application of this method for three structurally and functionally distinct protein families (G-protein-coupled receptors, the chymotrypsin class of serine proteases and hemoglobin) reveals a surprisingly simple architecture for amino acid interactions in each protein family: a small subset of residues forms physically connected networks that link distant functional sites in the tertiary structure. Although small in number, residues comprising the network show excellent correlation with the large body of mechanistic data available for each family. The data suggest that evolutionarily conserved sparse networks of amino acid interactions represent structural motifs for allosteric communication in proteins.

Nature Structural Biology 10(1), 59, 2003



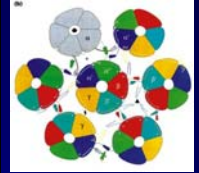
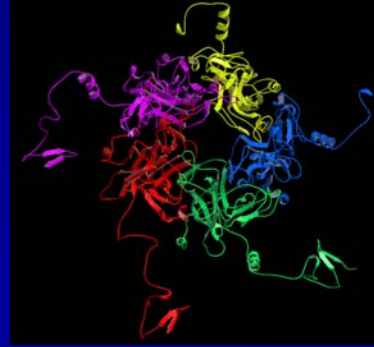
Different G-actin structure reinforces concept of subdomain 2 being a switch



Kabsch *et al.*  
(1990), actin-  
DNase I complex

Otterbein *et al.*  
(2001), modified  
G-actin

Subunit switches have been observed in viral capsids



SV40 all pentamer capsid  
from Stehle *et al.*, 1996

"flexible yet specific"