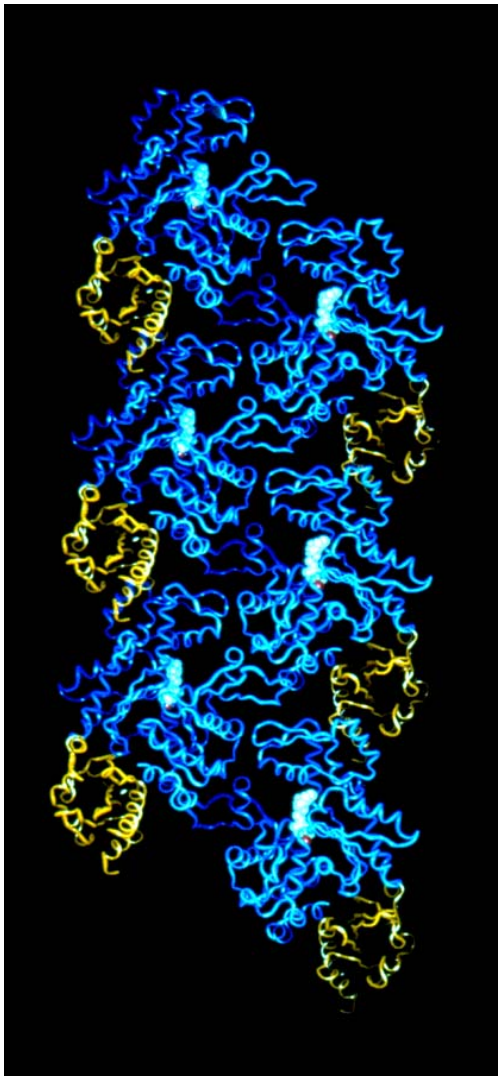
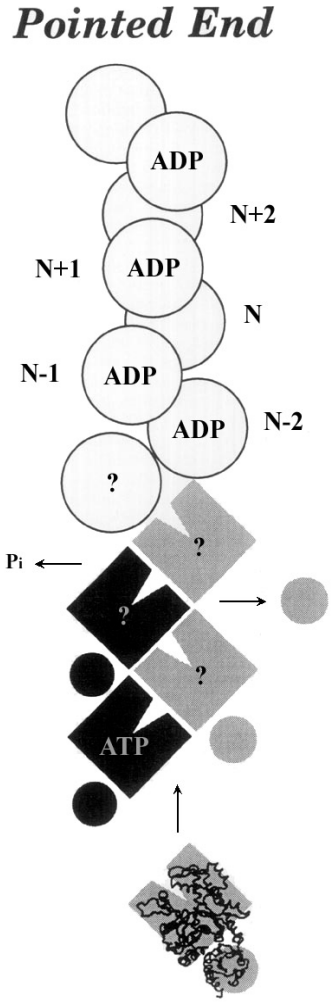
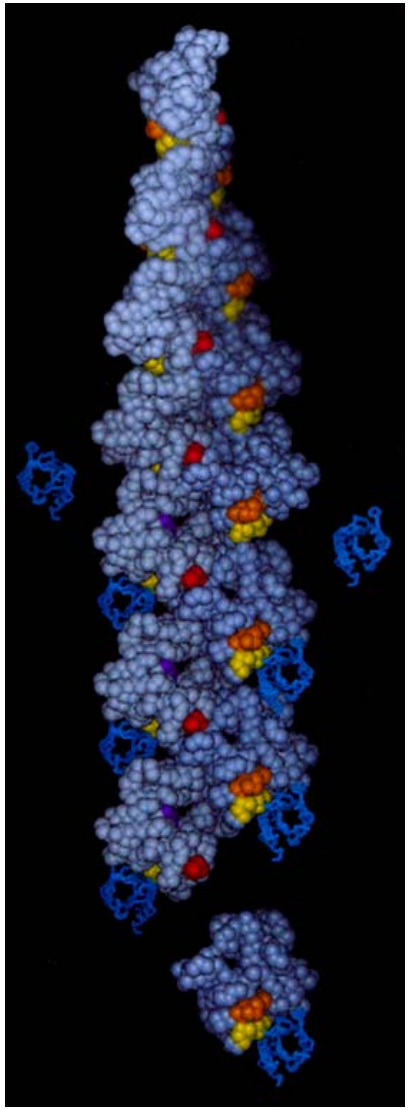
Structures of Actin-Binding Proteins

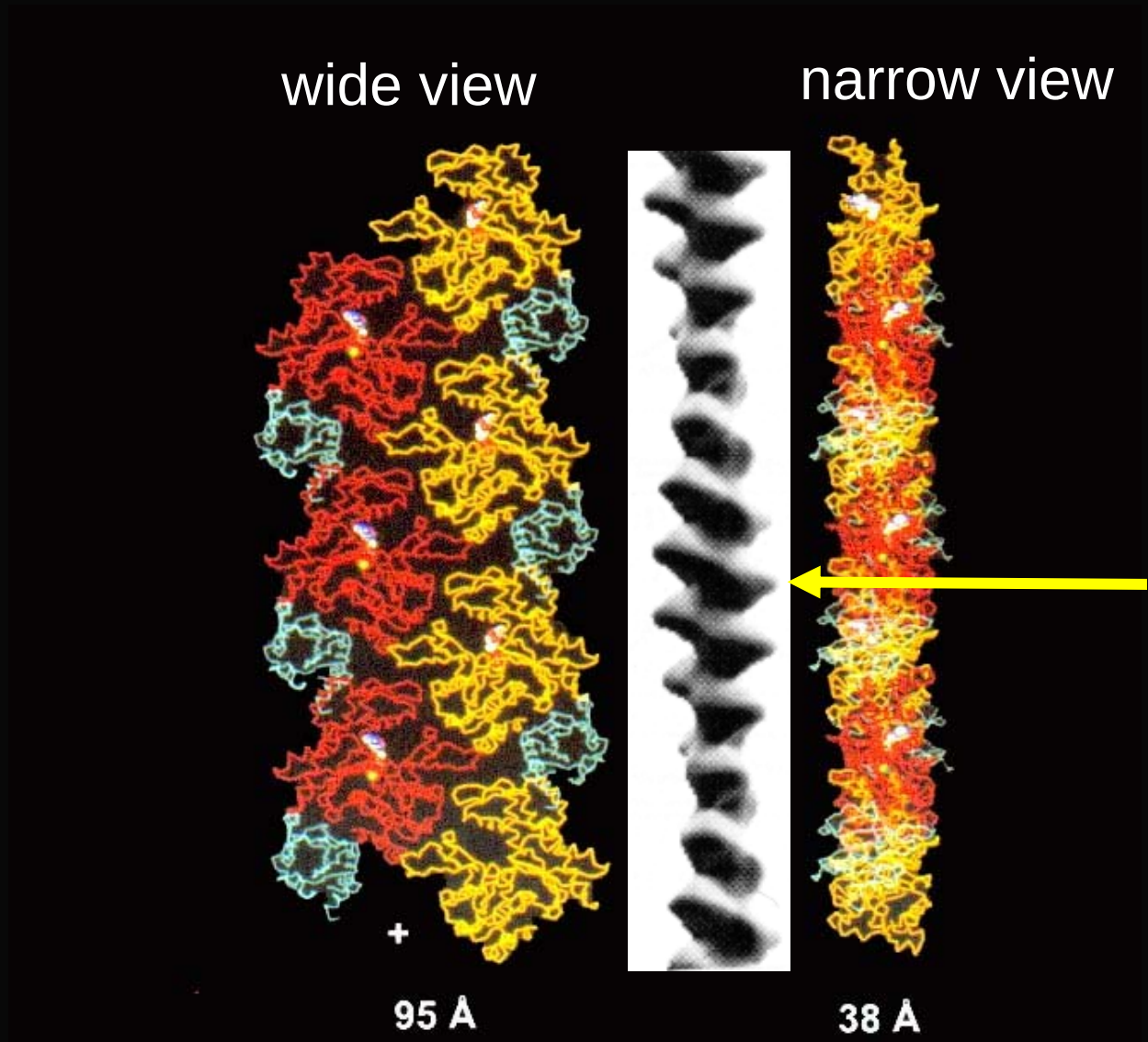
A new model for filament severing

Schutt, C., Lindberg, U., Karlsson, R., Weeds A., Pope, B., Plakantonakis, A.




Pointed End
ADP
N+2
N+1
ADP
N
N-1
ADP
ADP
N-2
?
?
Pi
?
?
ATP
Barbed End

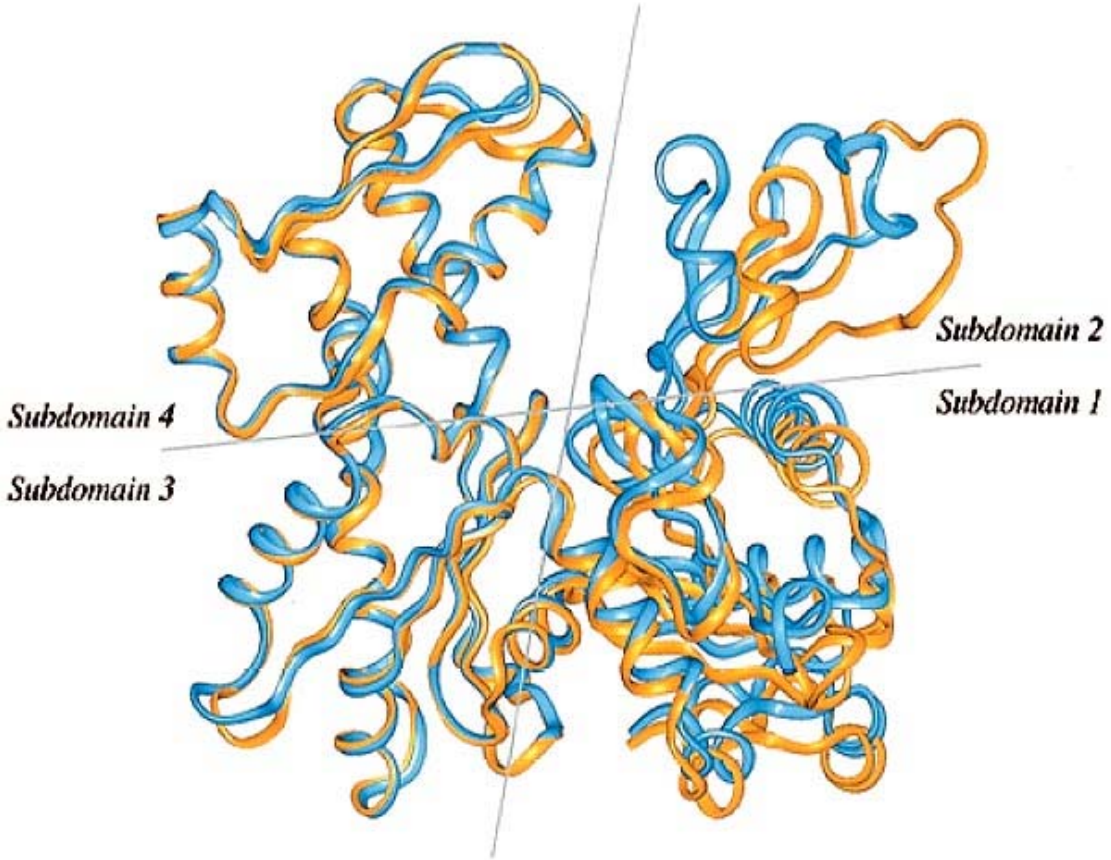




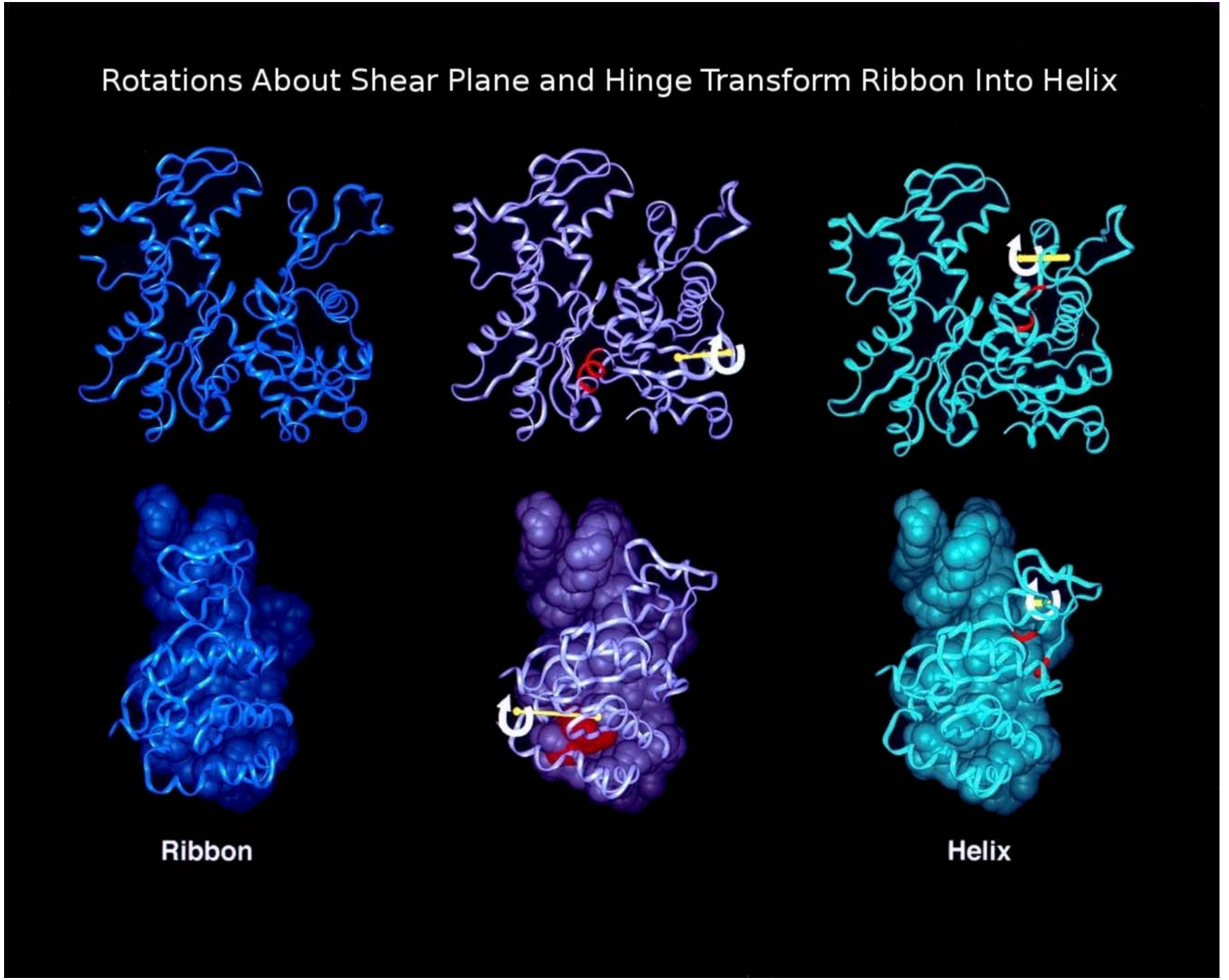
Schutt *et al*.
Nature
365, 810 (1994).

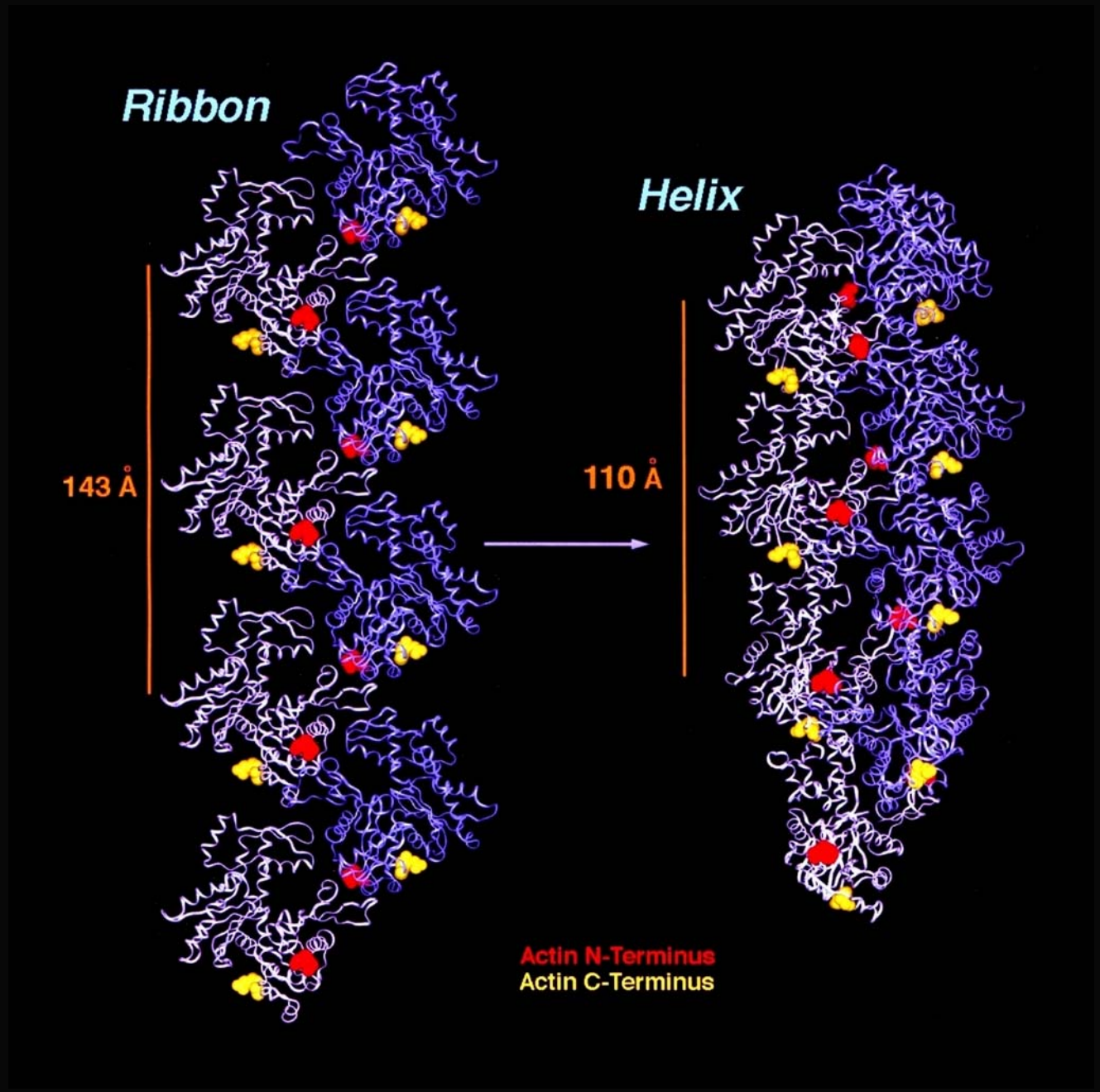
Lepault *et al*.
J. Struct. Biol.
112, 79-91 (1994).

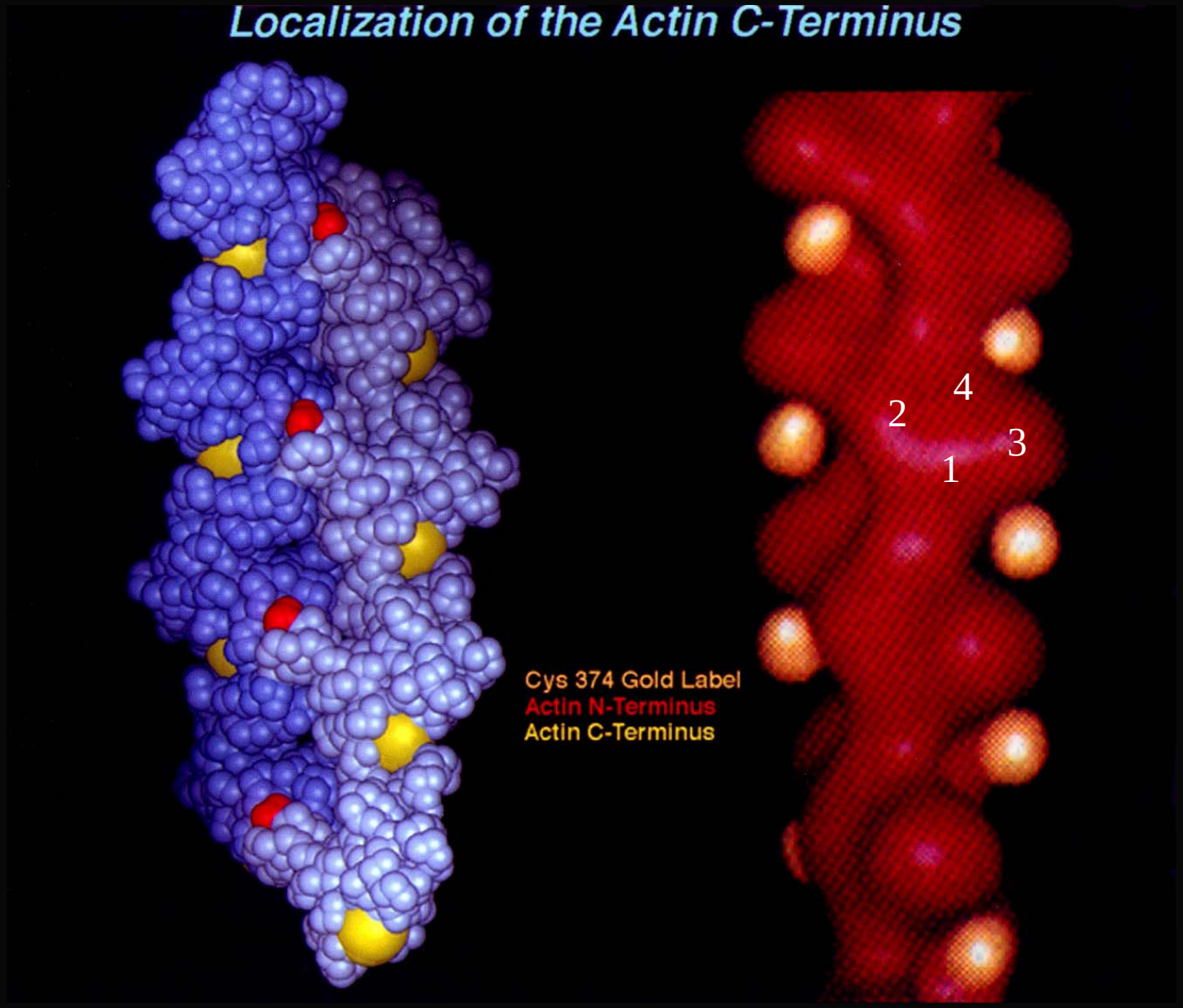
The actin filament is a twisted, flattened ribbon.



Chik, Lindberg & Schutt, *J. Mol. Biol.* **263**, 607-623 (1996)
Page, Lindberg & Schutt, *J. Mol. Biol.* **280**, 463-474 (1998)

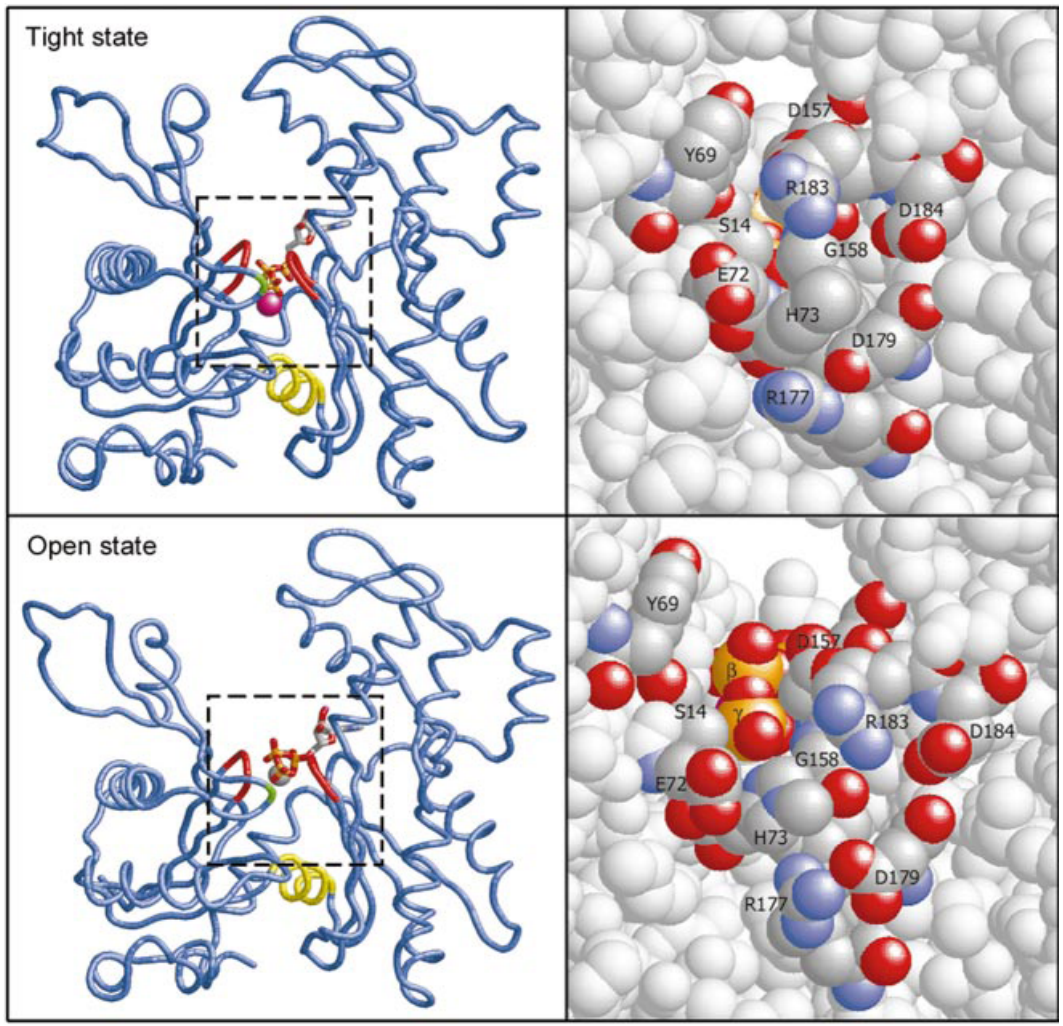

Rotations About Shear Plane and Hinge Transform Ribbon Into Helix
Ribbon
Helix


Ribbon
Helix
143 Å
110 Å
Actin N-Terminus
Actin C-Terminus



Milligan, Whittaker & Safer, *Nature* **348**, 217-221 (1990)

Mutational Analysis of Interdomain Connectivity in Actin



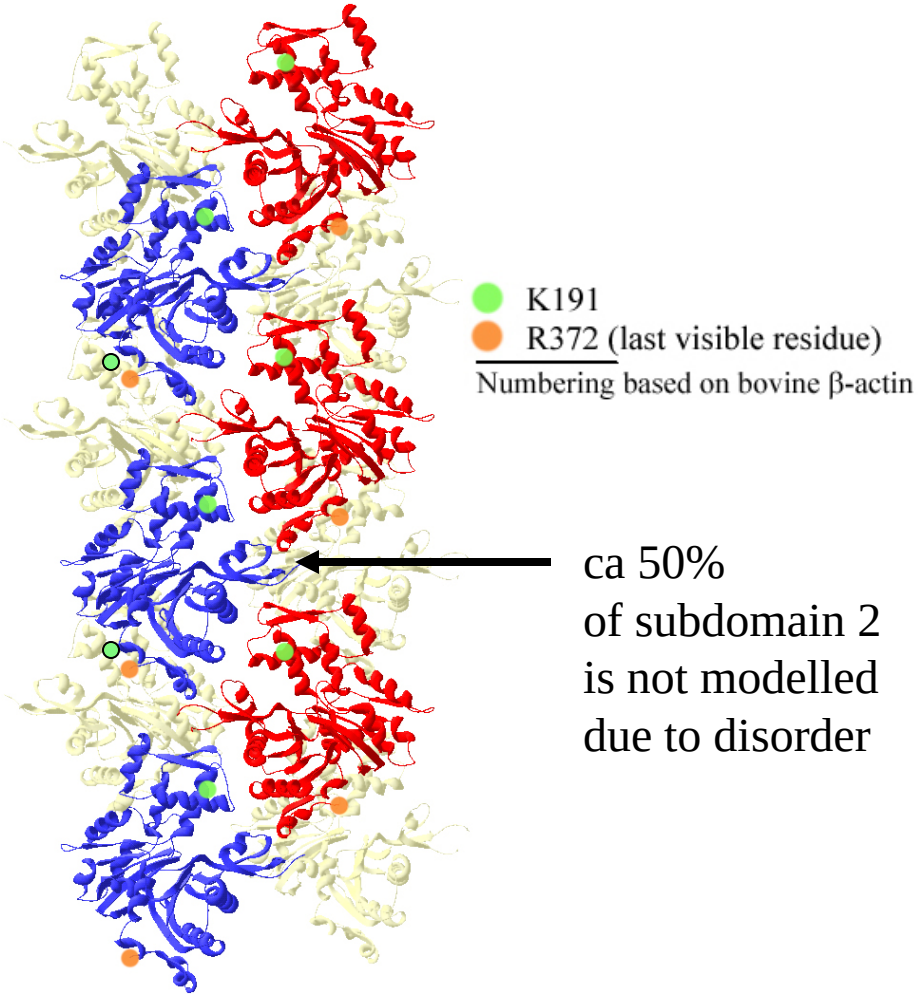
The “polar hub” of actin; its beating heart.

Nyman *et al.*, *J. Mol. Biol.* **317**, 577-589 (2002)

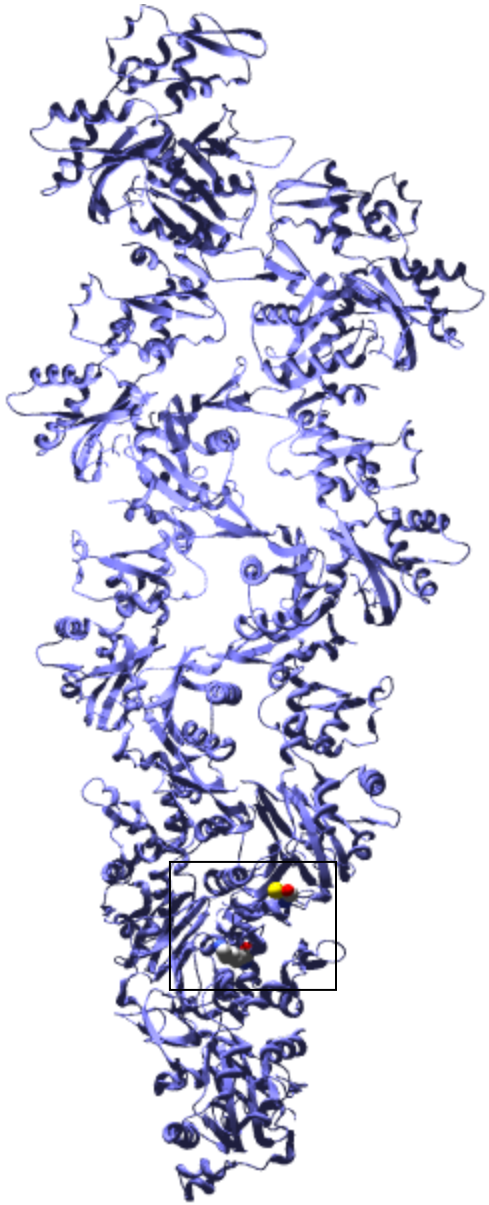
The Ribbon Appears in Crystals of X-linked Actin Trimers!

"Like other experimental data, the actin assemblies found in the **GS-1**-bound actin trimer crystals rule out the models both for F-actin and muscle contraction based on a single F-actin filament transitioning between the helical and ribbon forms described by Schutt *et al*."

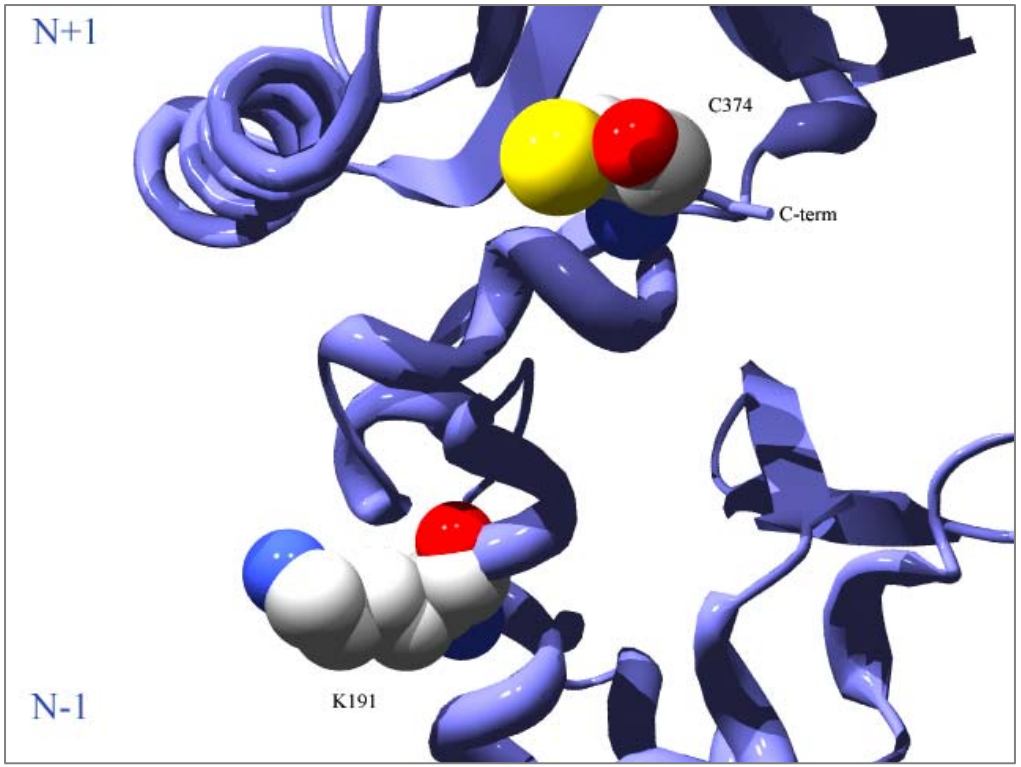
Dawson, Sablin, Spudich, & Fletterick
J. Biol. Chem. **278,** 1229-1238 (2003)



Note: GS-1 not shown.



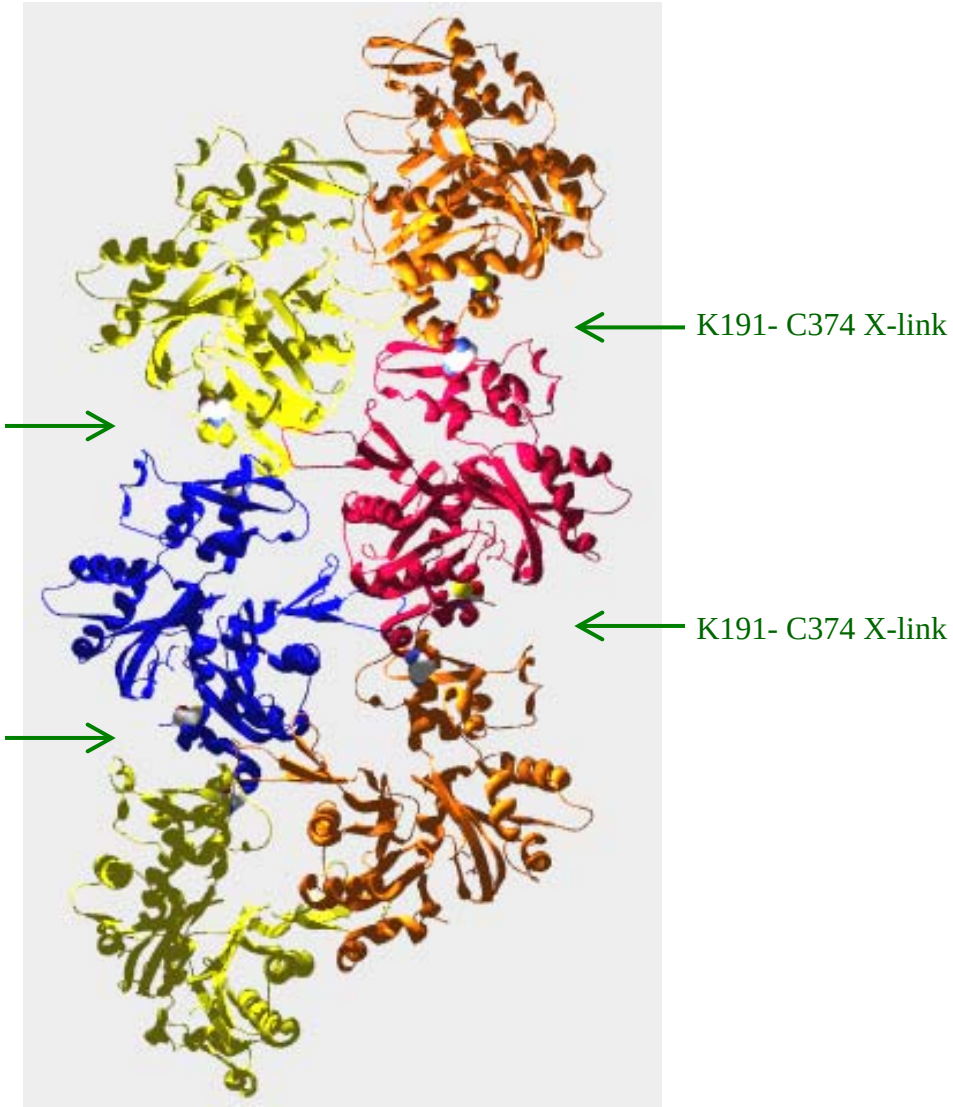
K191-C374 can be X-linked in the ribbon-derived helix by PDM (11.4Å) along the long pitch helix, as anticipated in 1978 by Knight & Offer.



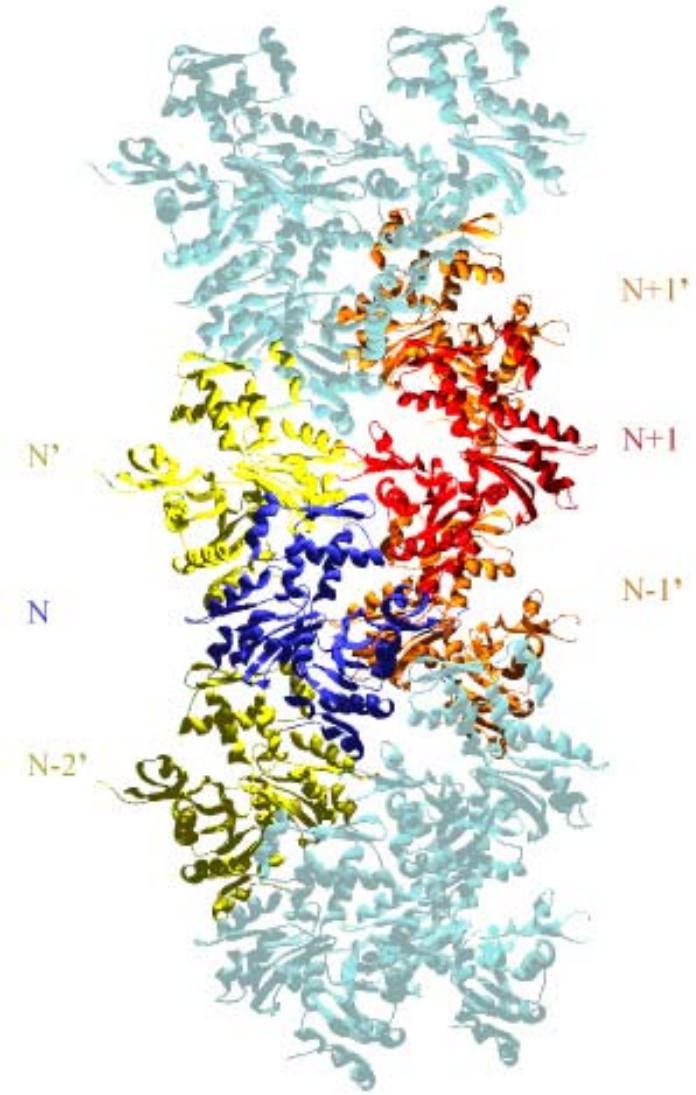
K191 εN – C374 S distance: 16.9 Å

NB: last residue seen in crystal is R372, indicating flexibility in the X-linked region.

Helix-Derived Trimers Are Consistent With X-links Deduced by Dawson *et al*.



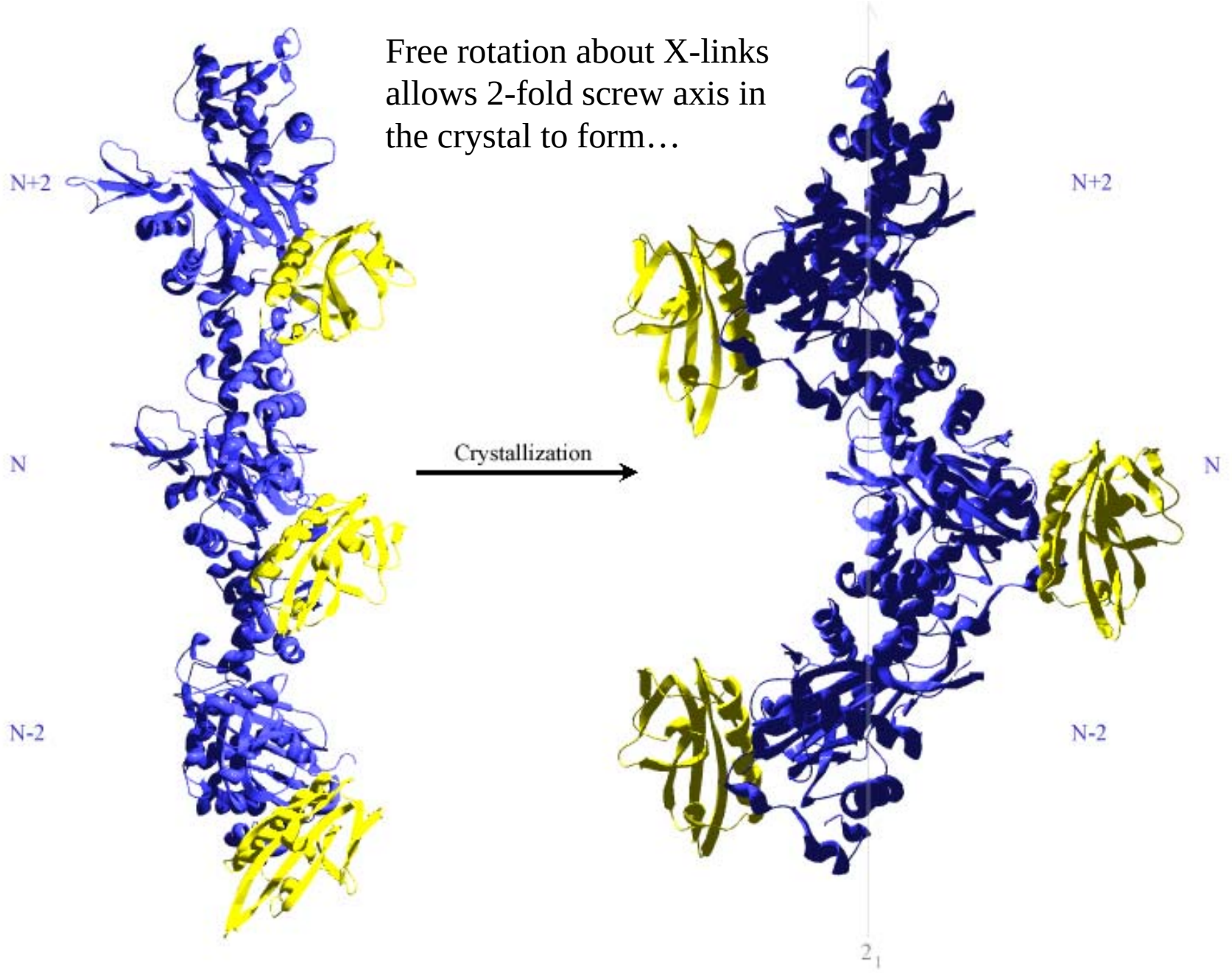
Ribbon-derived helix



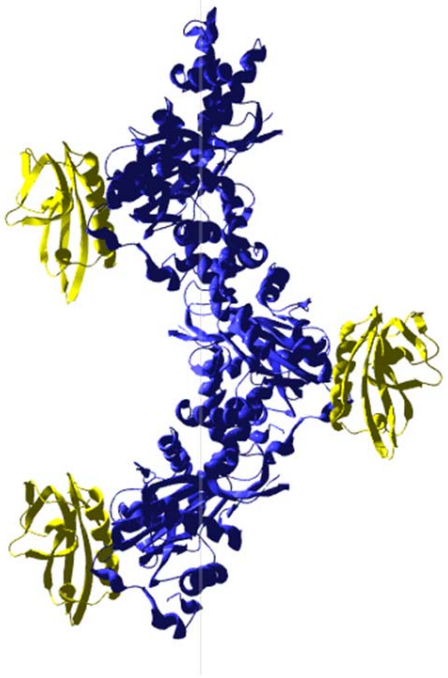
Dawson *et al*. crystal

Note: GS-1 not shown.

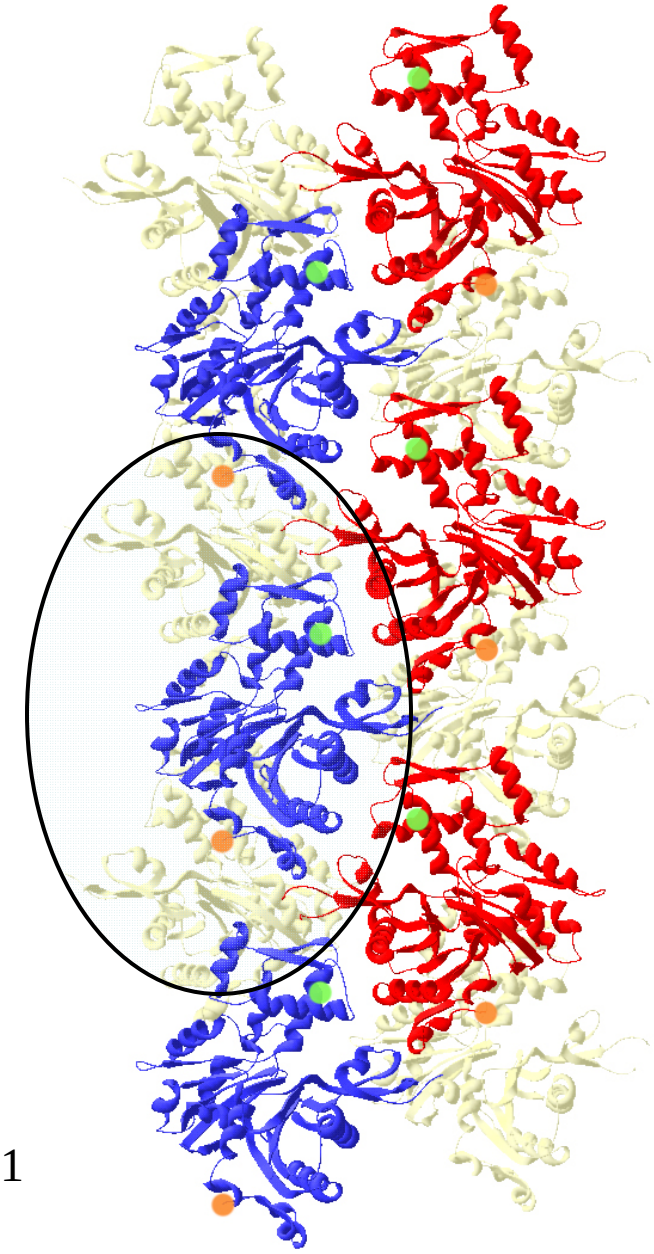
Free rotation about X-links allows 2-fold screw axis in the crystal to form…



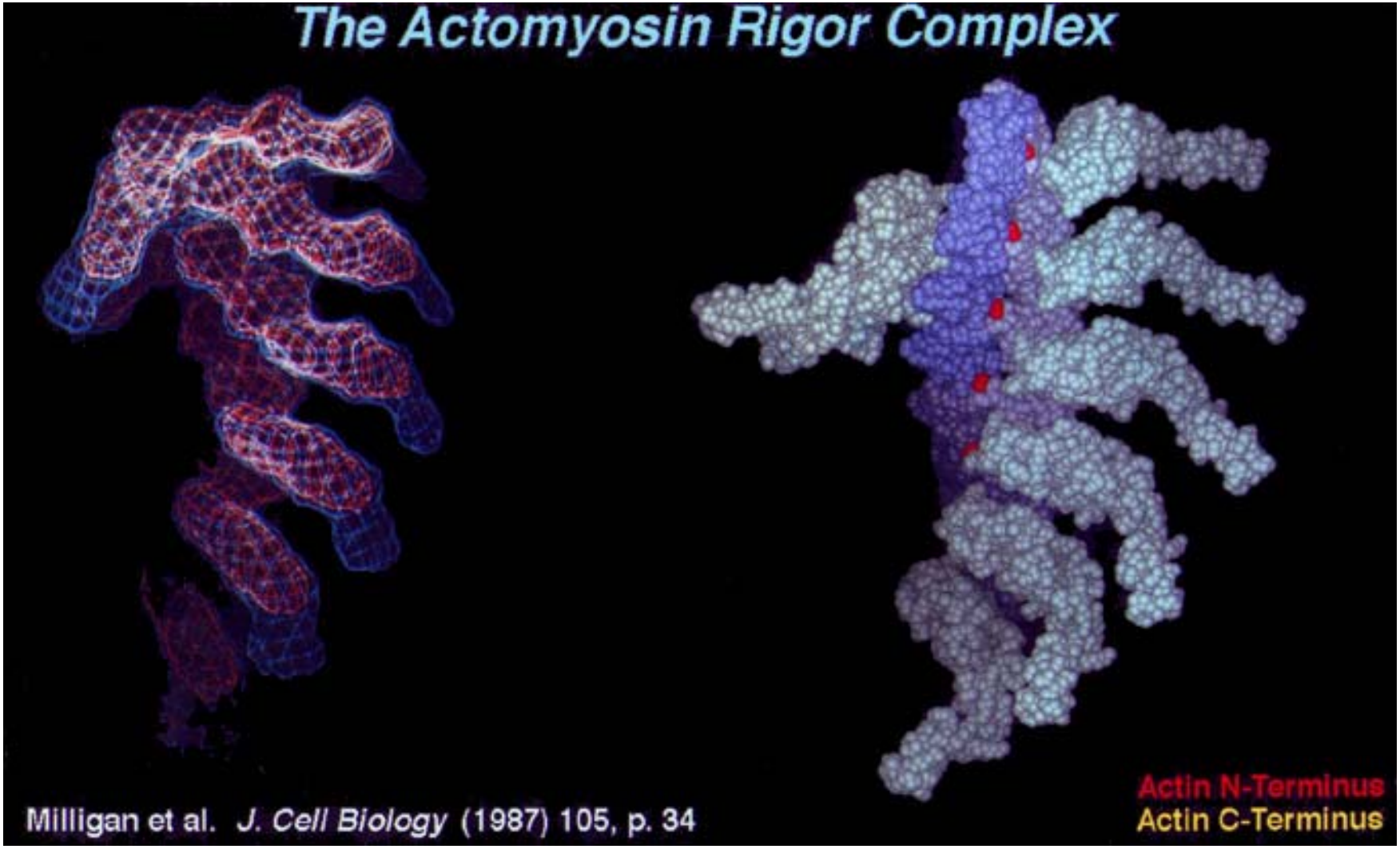
…therefore
ribbon organization
of actin ***not*** ruled out
by Dawson *et al*.



Edge-on view:
Schutt-Lindberg-derived trimer with GS-1



Plane view: X-linked actin trimers in crystal


The Actomyosin Rigor Complex
Milligan et al. J. Cell Biology (1987) 105, p. 34
Actin N-Terminus
Actin C-Terminus

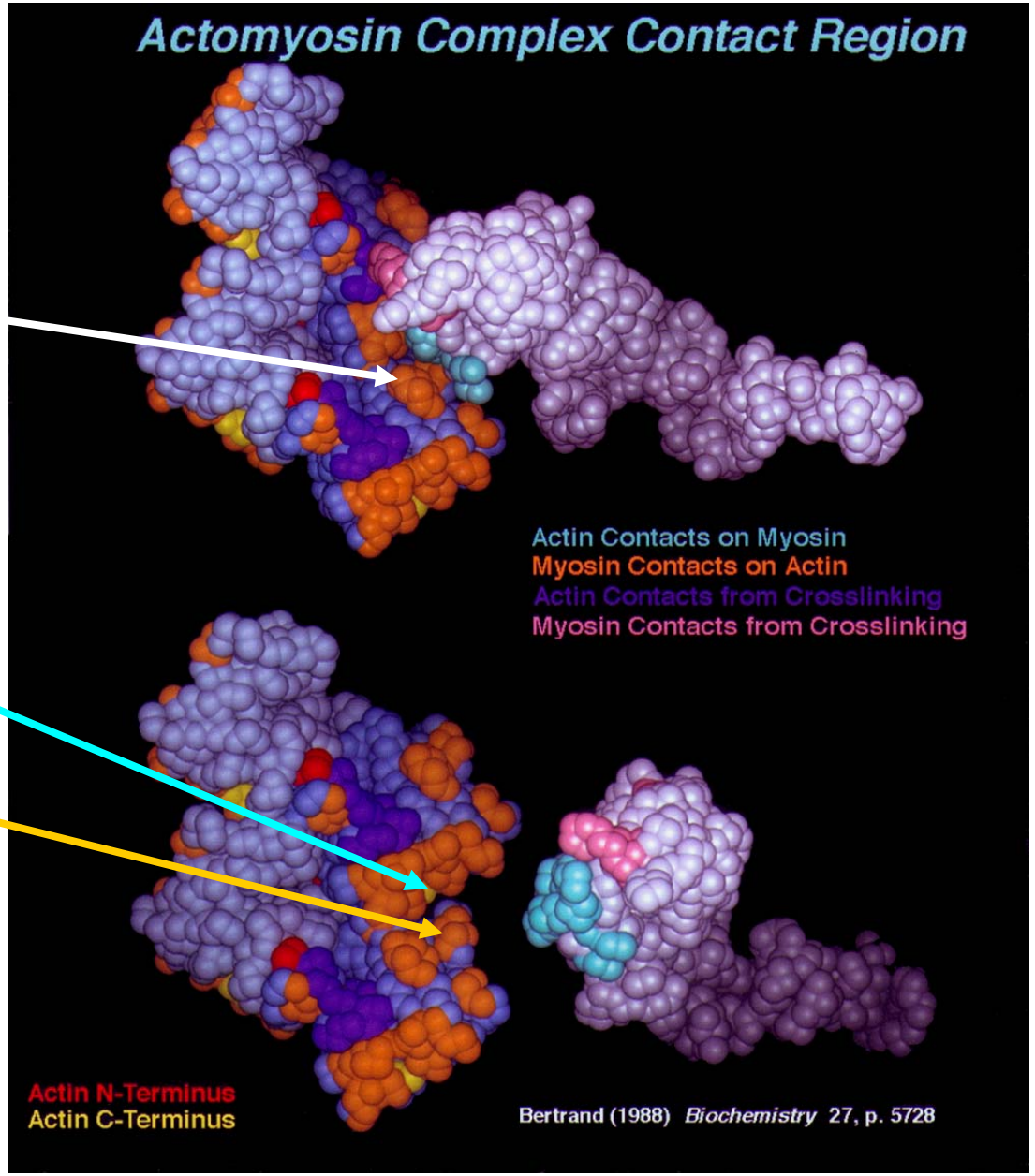
hydrophobic plug

(Rubenstein)

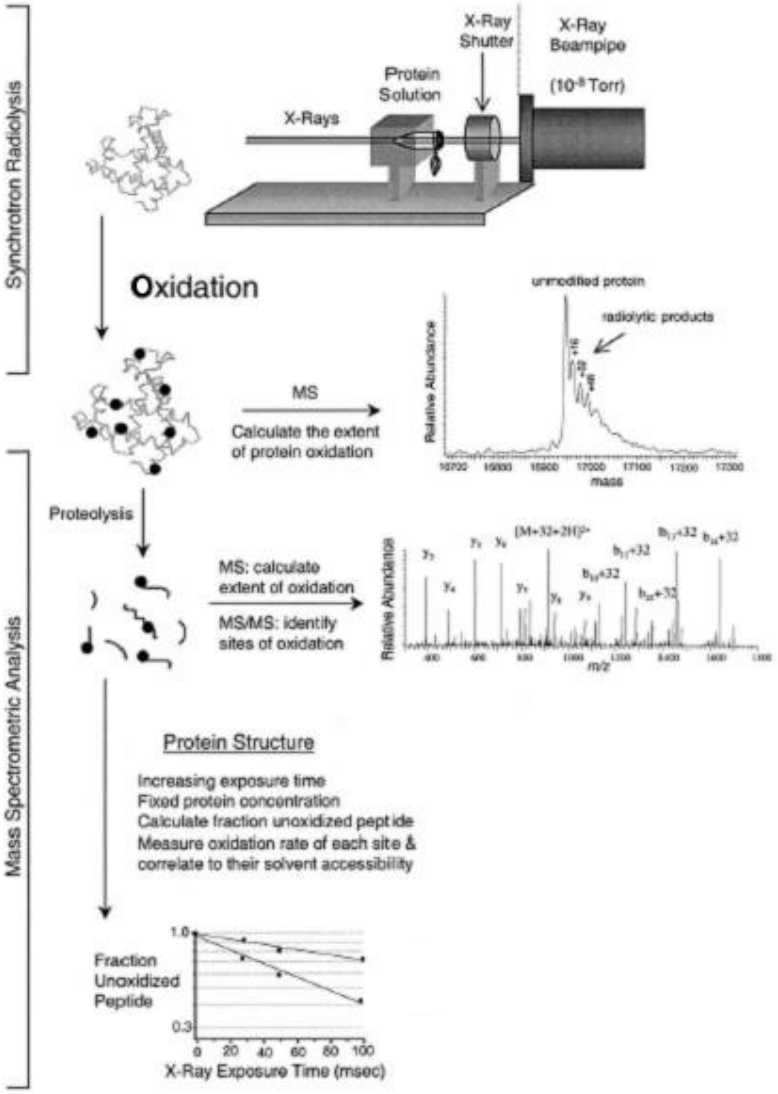
Cys 374

Lys 191

(Knight & Offer, 1978)

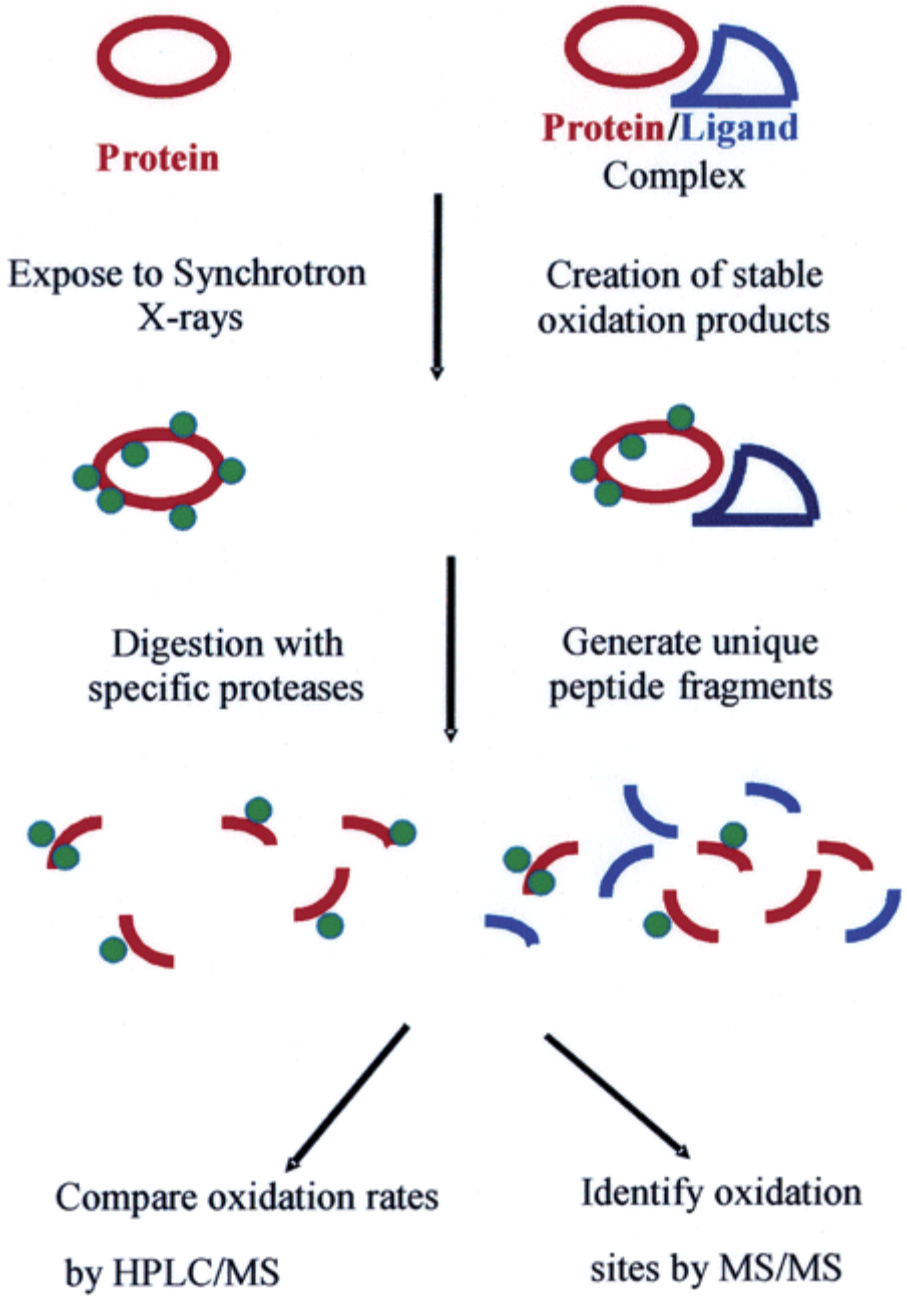


Synchrotron Protein Footprinting

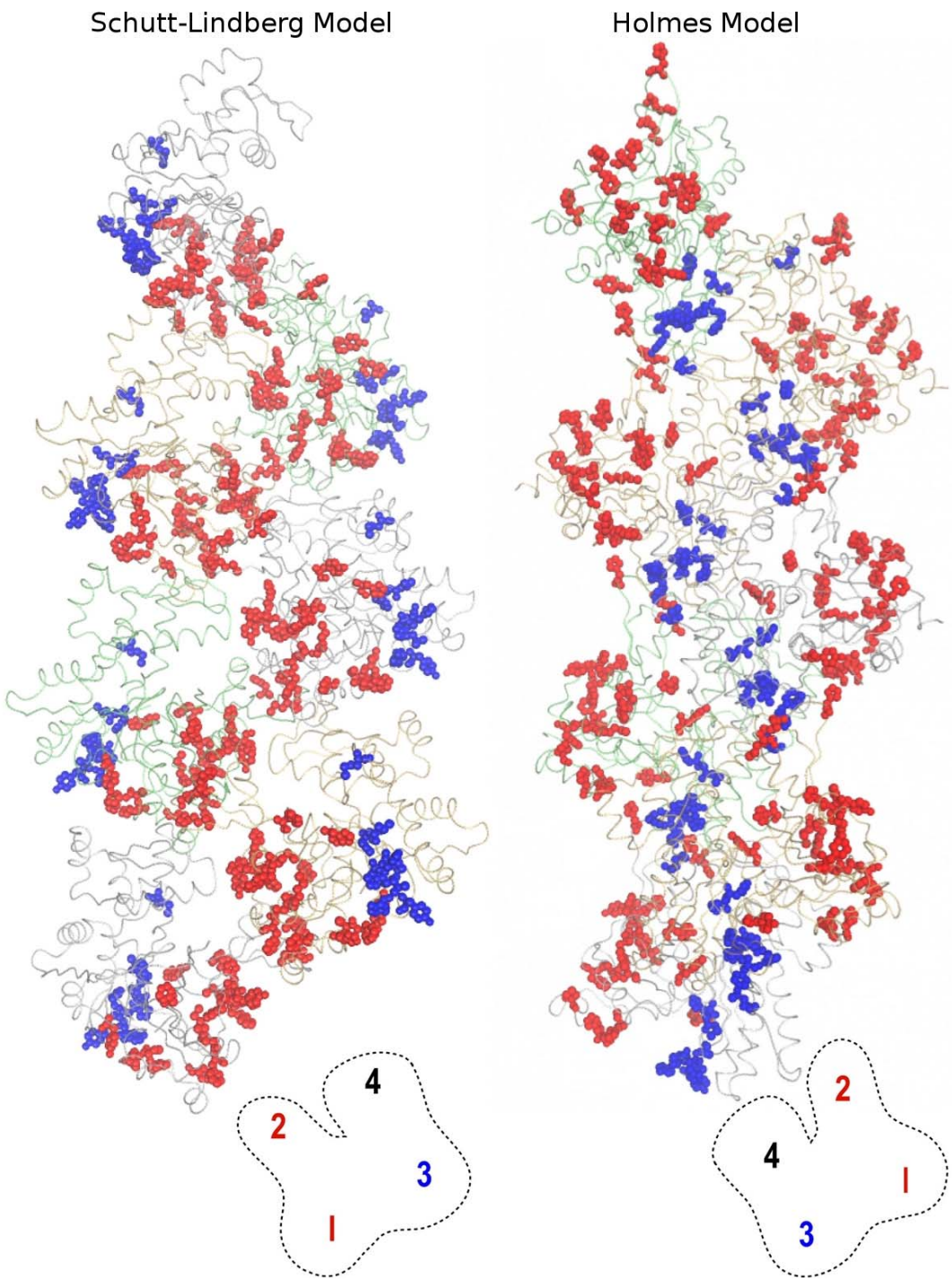


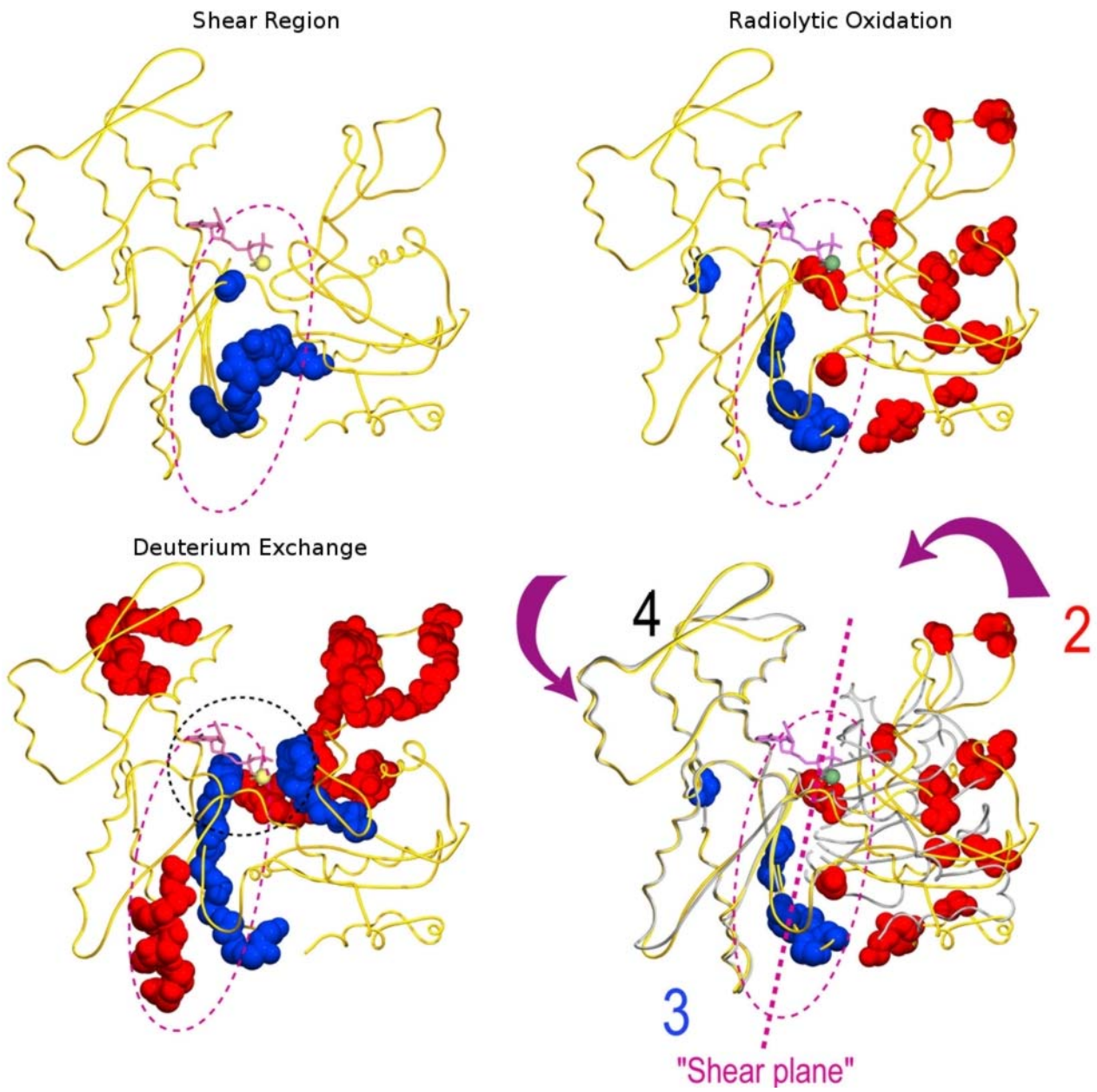
Adapted from Mileknia et al., *Anal. Biochem.*, **289,** 103–115 (2001)

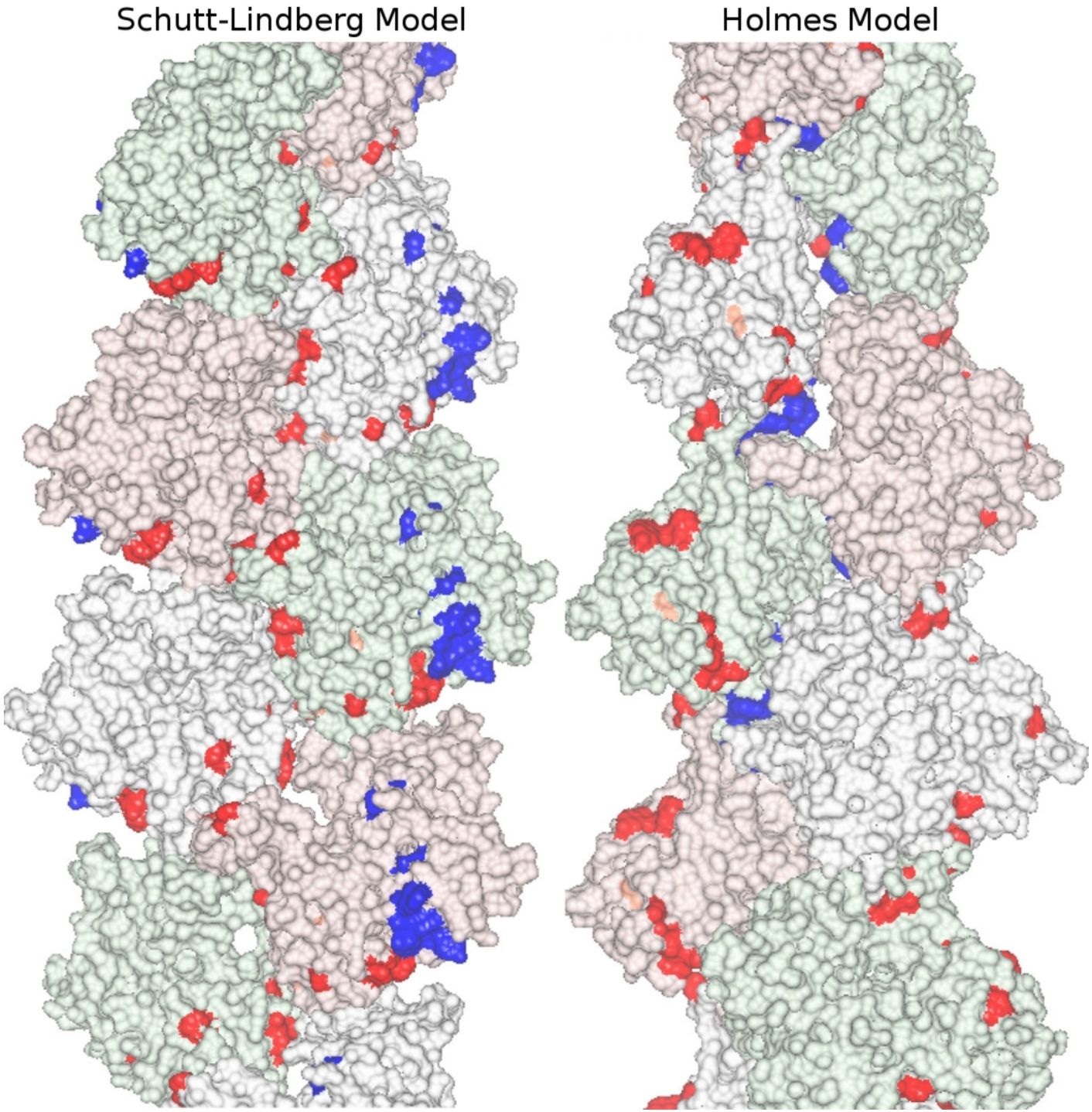
Synchrotron Protein Footprinting



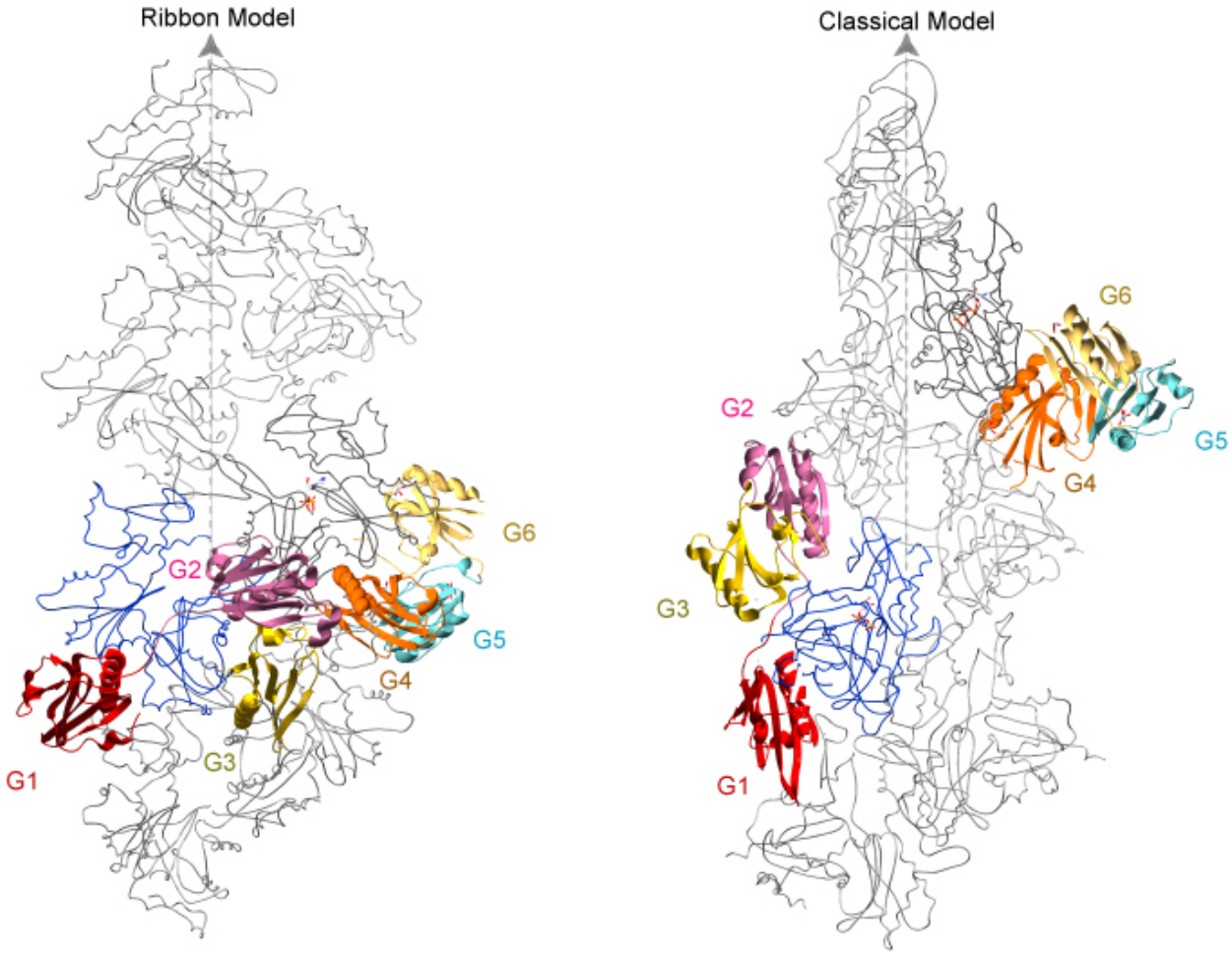
Adapted from Guan *et al.*, *Acc. Chem. Res.*, **37**, 221 -229 (2004)


Schutt-Lindberg Model
Holmes Model
4
2
3
I
2
4
I
3


Shear Region
Radiolytic Oxidation
Deuterium Exchange
4
2
3
"Shear plane"


Schutt-Lindberg Model
Holmes Model

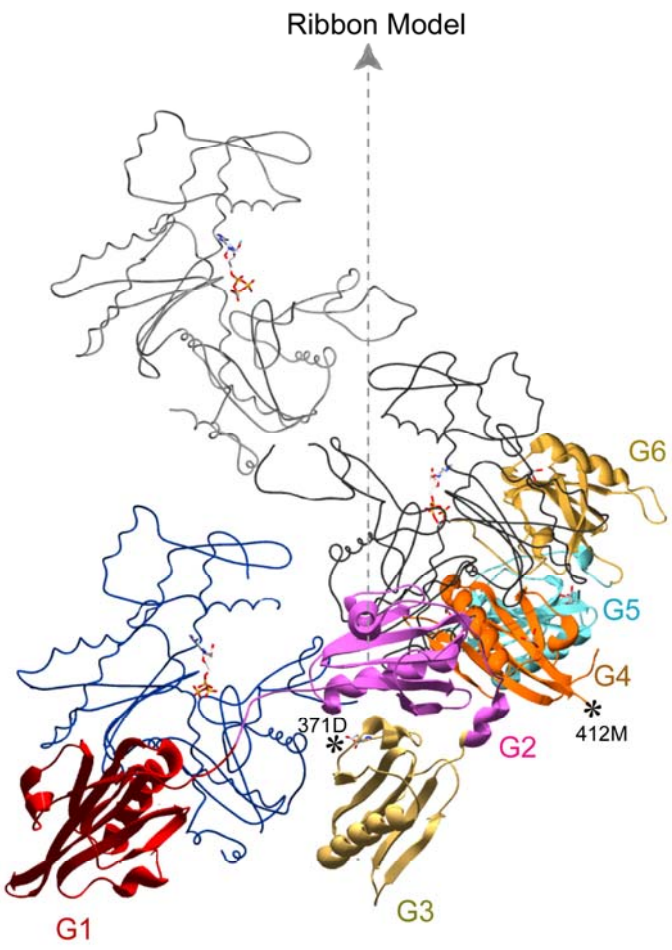
Binding of Gelsolin to F-Actin

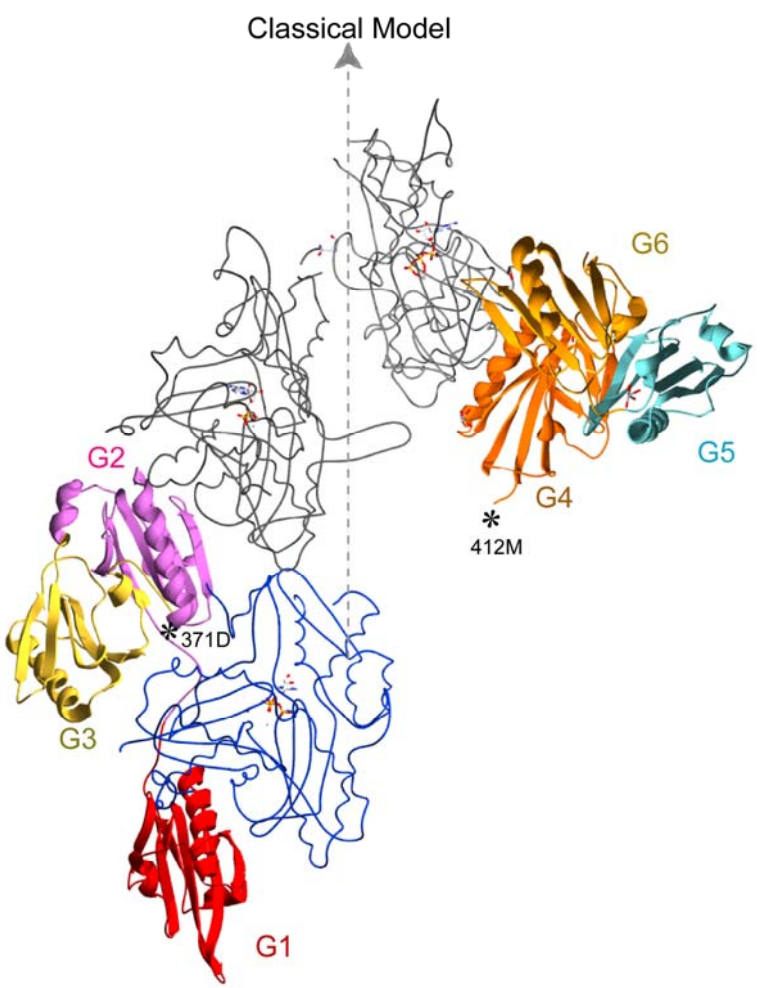


G1-3_actin: Burtnick *et al*. *EMBO J*. **23**, 2713-2722 (2004)

G4-6_actin: Choe *et al*., *J. Mol. Biol*. **324**, 691-702 (2002)

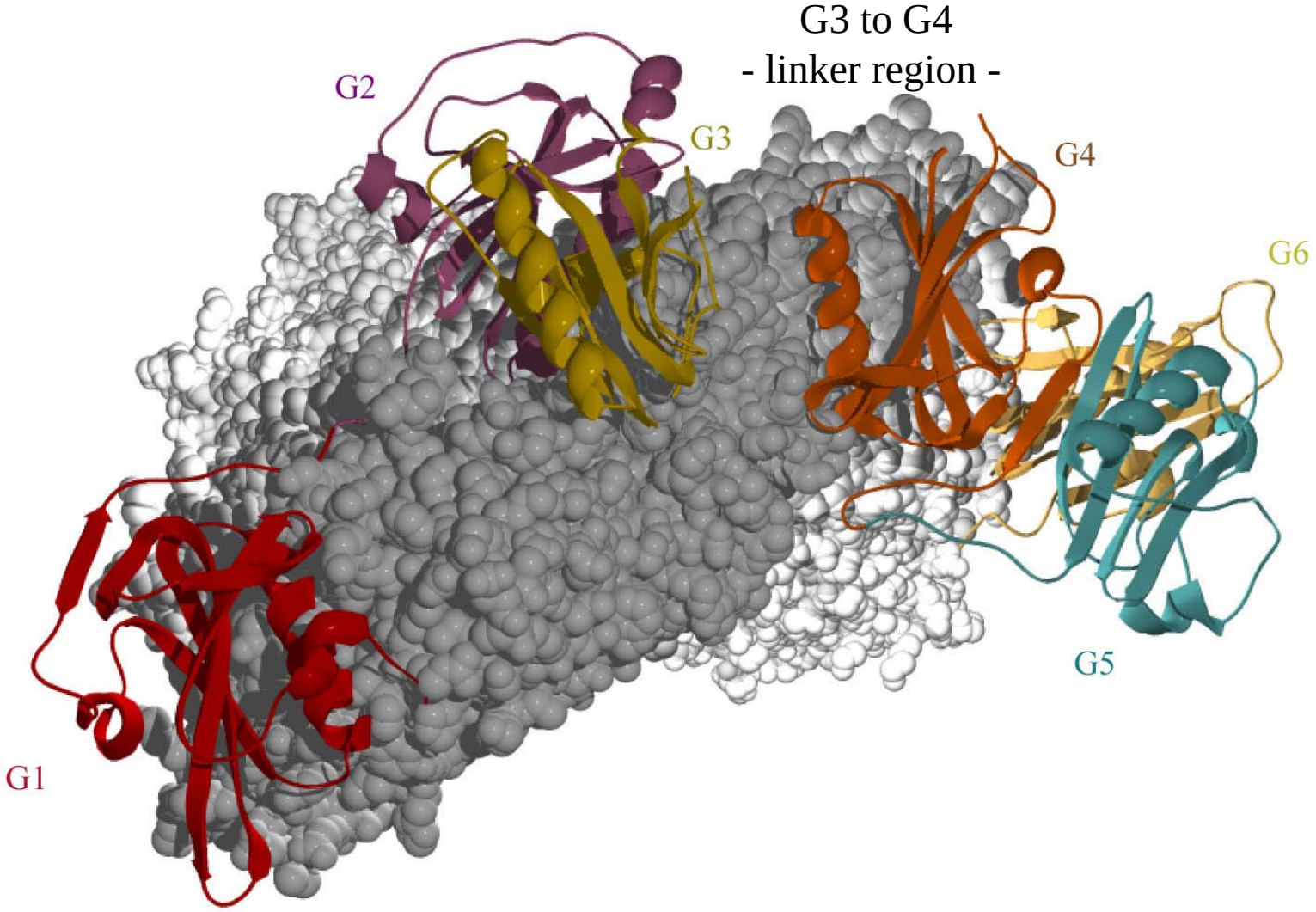
Capping of the Barbed End of F-Actin

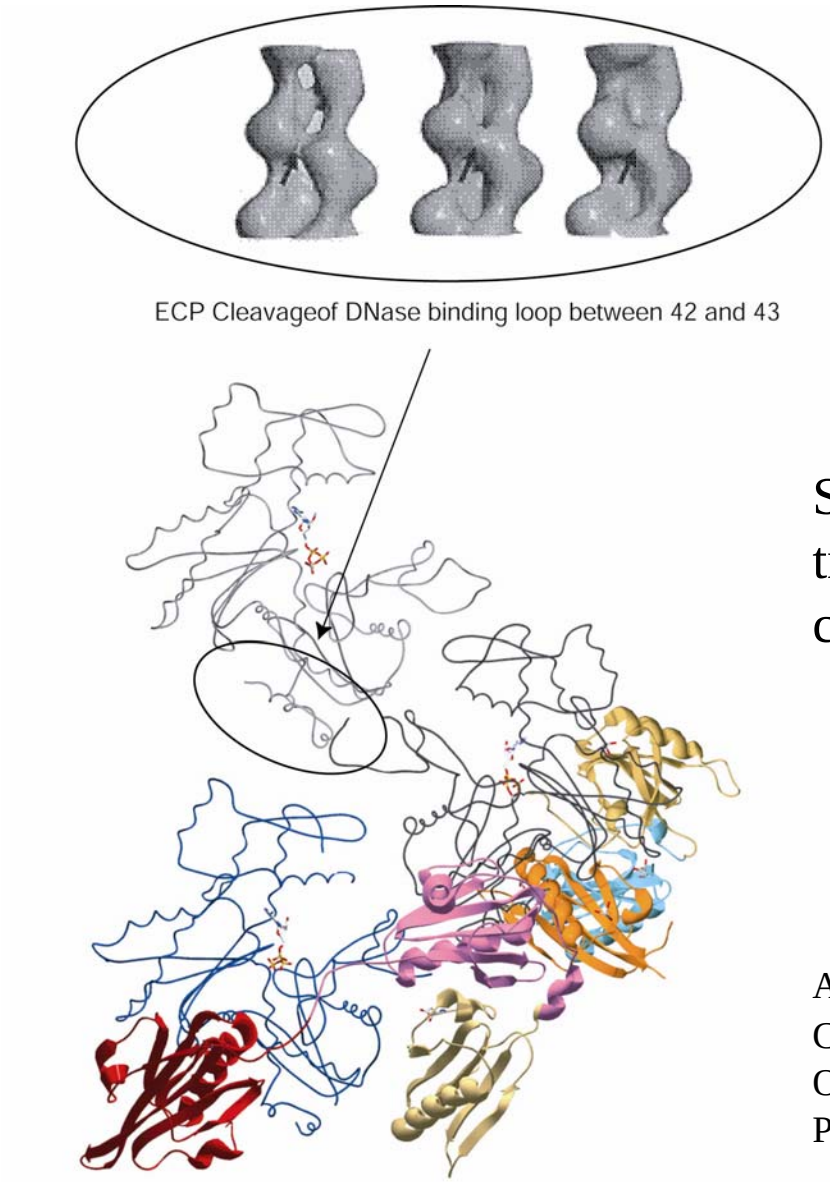




G1-3_actin: Burtnick *et al*. *EMBO J*. **23**, 2713-2722 (2004)
G4-6_actin: Choe *et al*., *J. Mol. Biol*. **324**, 691-702 (2002)

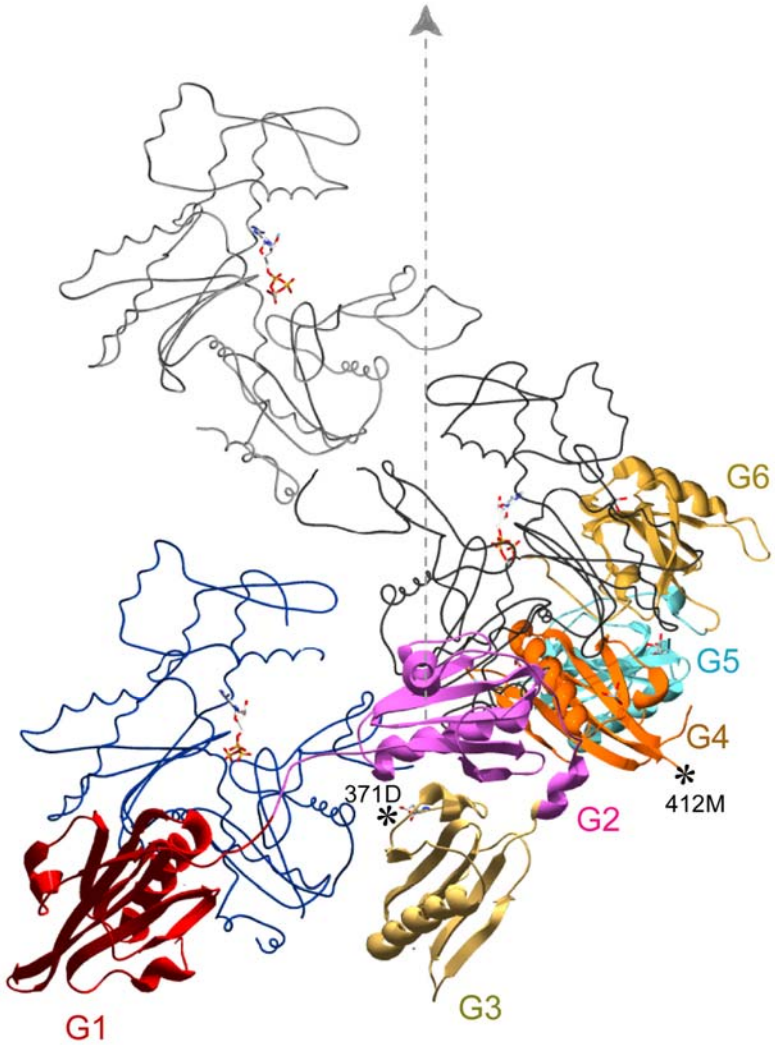
Gelsolin Capping the Barbed End – End View



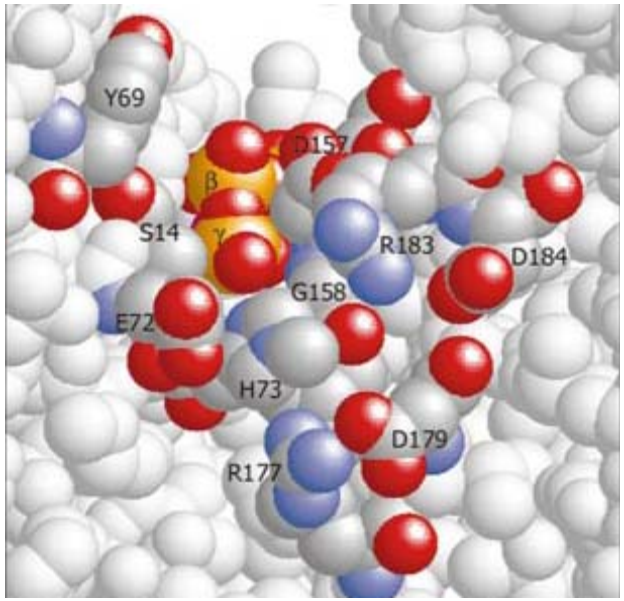


Severing by gelsolin is based on transmission of conformational changes in actin.

Aspenström et al. *FEBS Lett*. **329**, 163-70, (1993)
Crosbie *et al*., *Biophys. J*. **67**, 1957-1964 (1994)
Orlova & Egelman, *J. Mol. Biol*. **245**, 582-597 (1995)
Prochniewicz *et al*., *J. Mol. Biol*. **260**, 756-766 (1996)

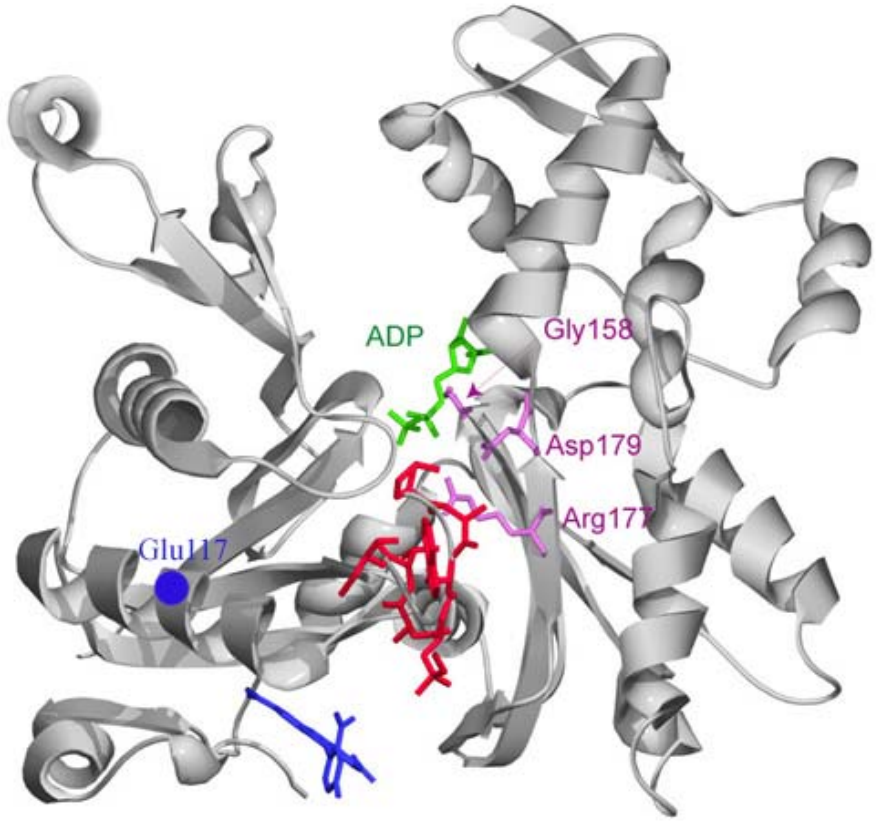


Gelsolin-capped barbed end



The polar hub of actin

Phalloidin Binding Site on Actin



Rhodamine binds in the "G1 cleft".

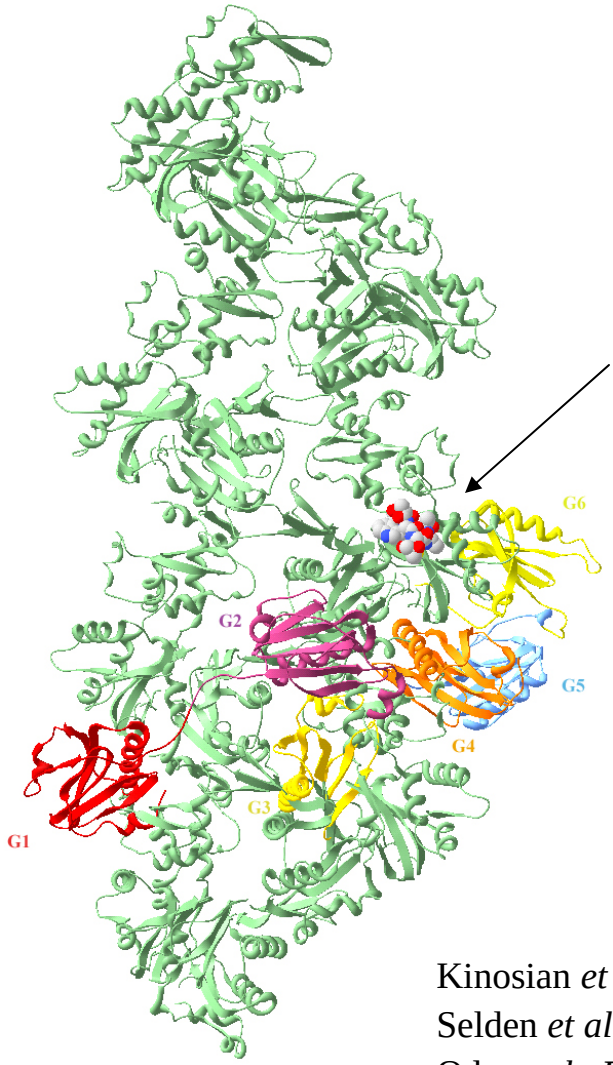
Phalloidin binds to G158, D179, R177.

Phalloidin (red) (Ala-Trp-Ala-Thr-Cys) could bridge the two sites.

Note also cross-linking data by Vandekerckhove *et al.*, *EMBO J.* **4**, 2815-2828 (1985): E117, M119, M355.

Rhodamine from Otterbein *et al.*, Science **293**, 708-711 (2001).

Phalloidin Inhibits Gelsolin Severing Activity



Phalloidin binding on the polar hub.

Kinosian *et al*., *Biochemistry* **35**, 16550-16556 (1996)
Selden *et al*., *Biophys. J*. **75**, 3092-3100 (1998)
Oda *et al*., *Biophys J*. **88**, 2727-2736 (2005)

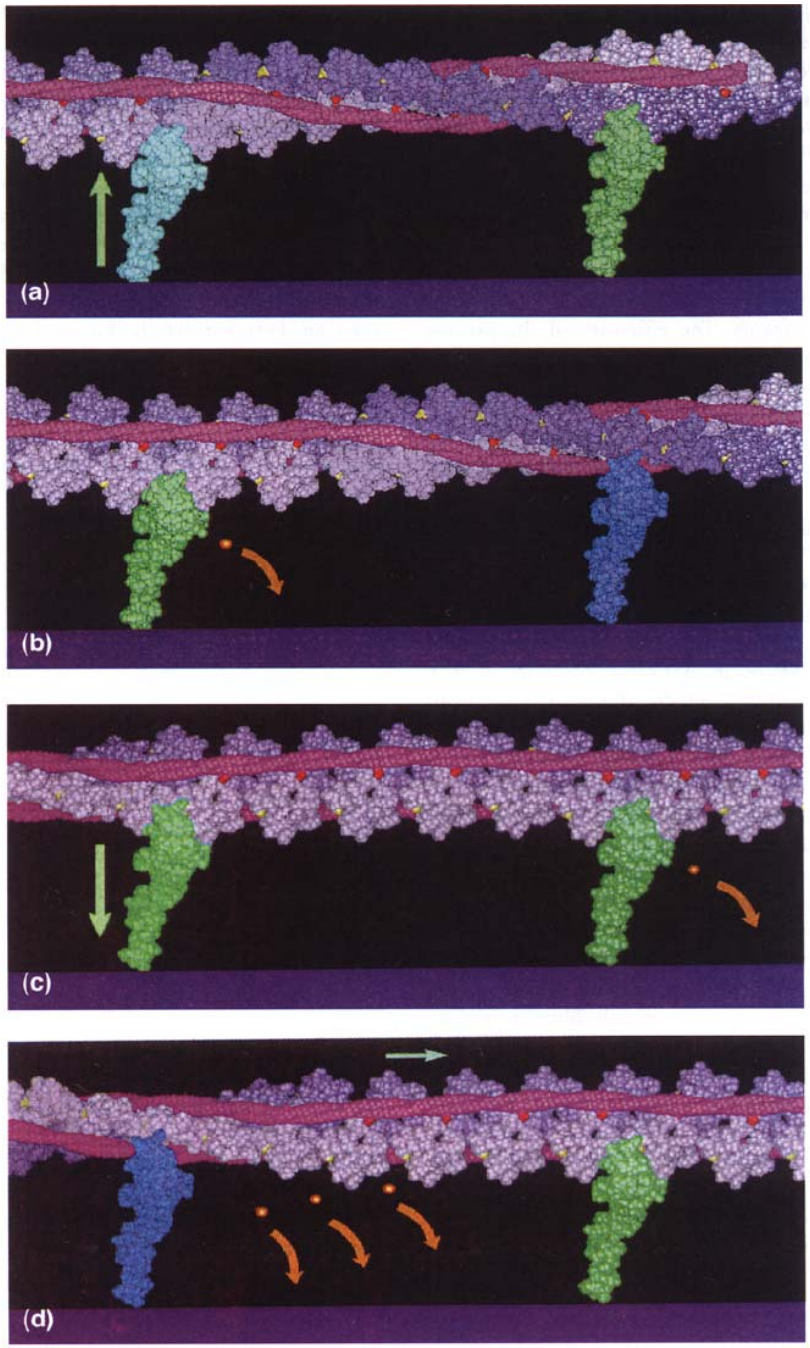
Actin Motor Model

Myosin induces a change in actin filaments.

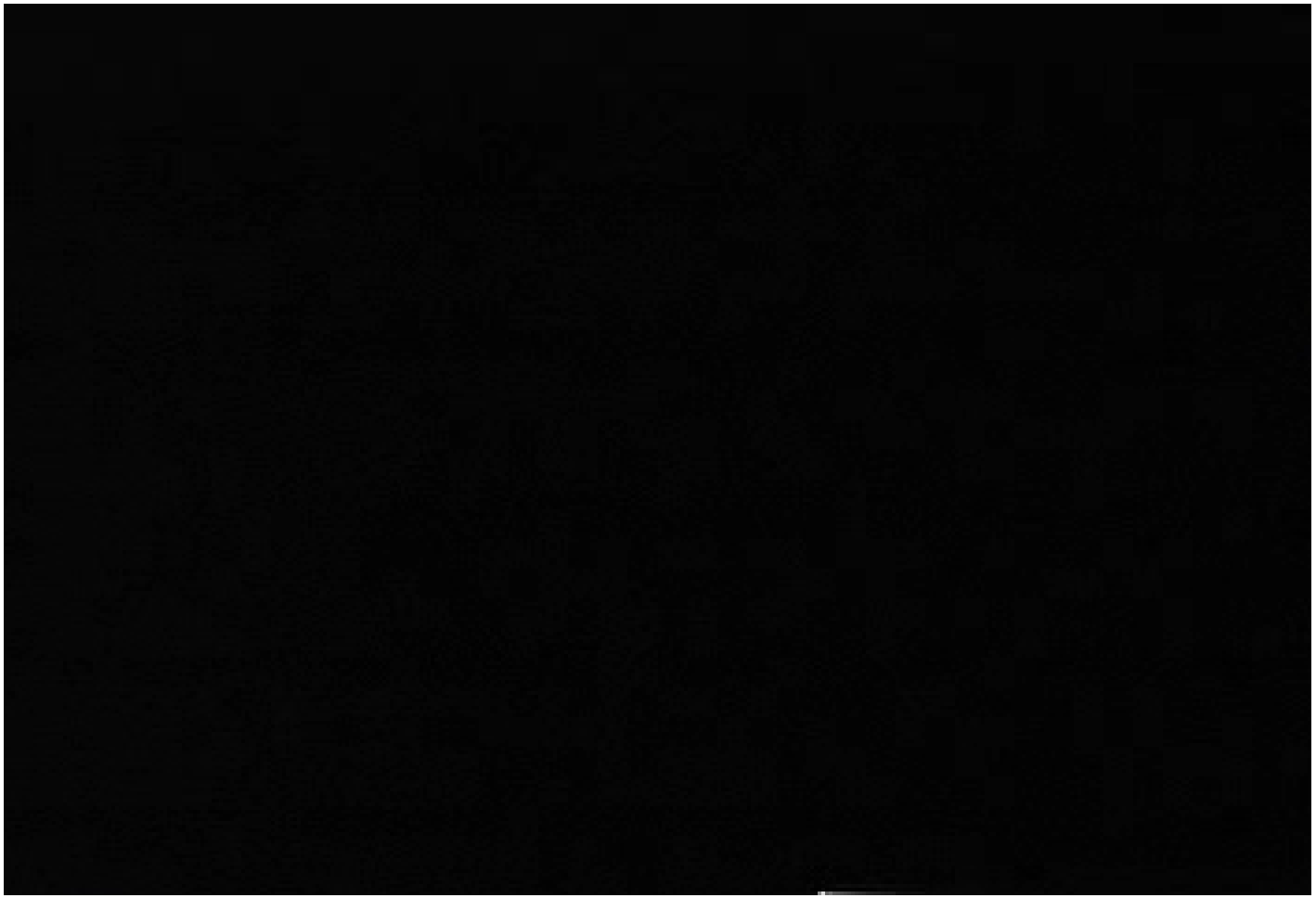
The change is propagated along actin filaments.

ATP is hydrolyzed on actin.

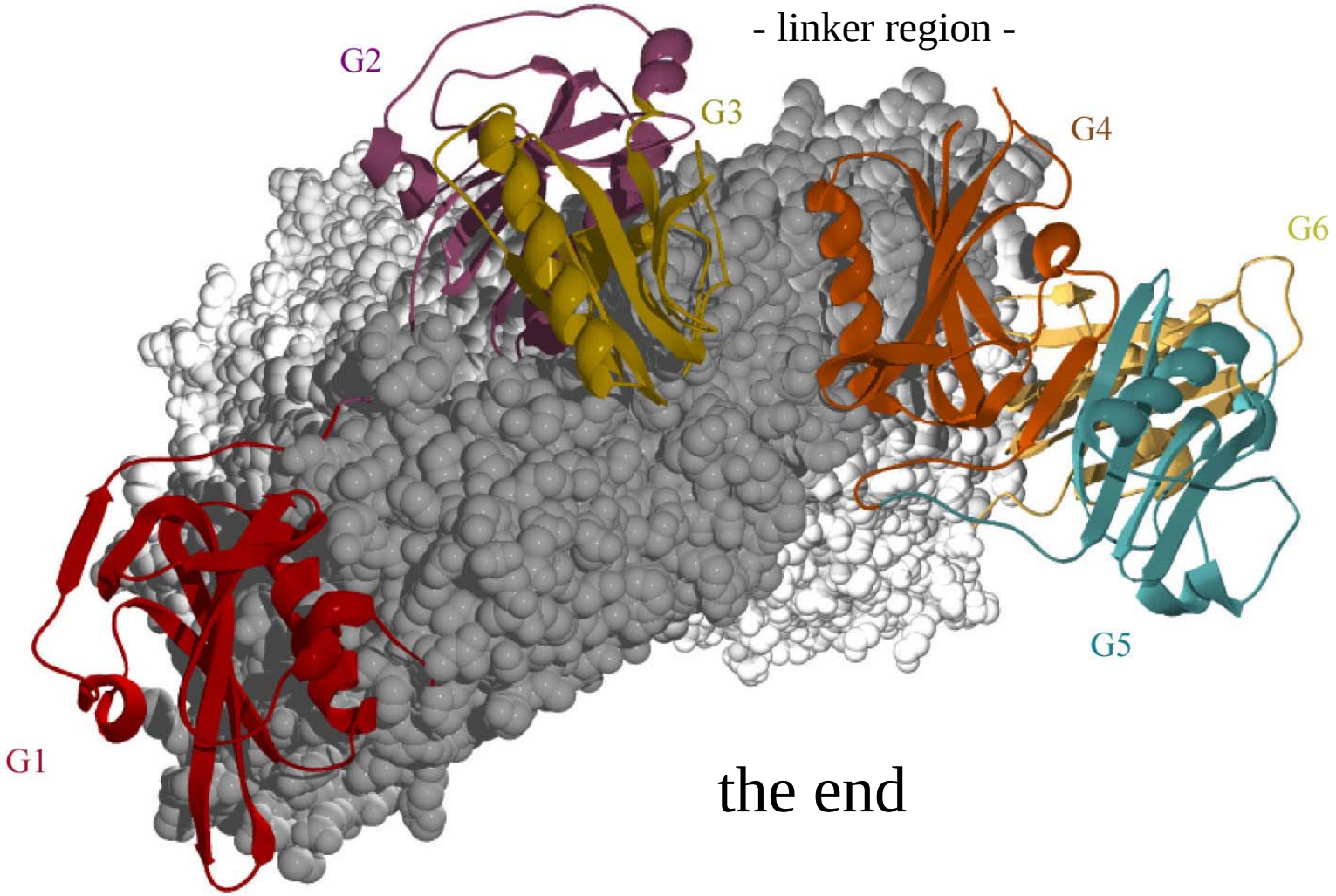
Force is generated as actin expels phosphate.



Actin as the Generator of Tension



G3 to G4
- linker region -



the end

Acknowledgements

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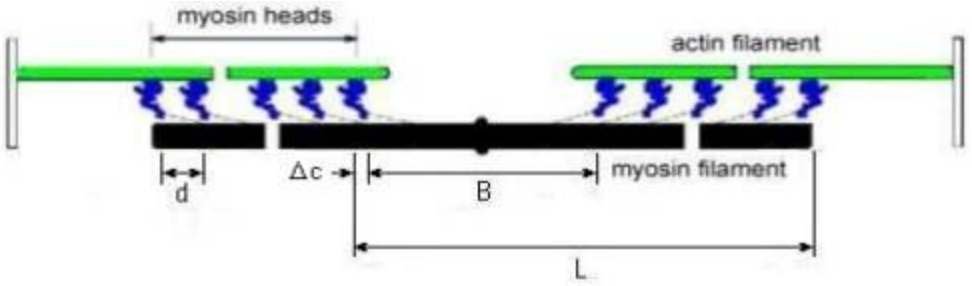
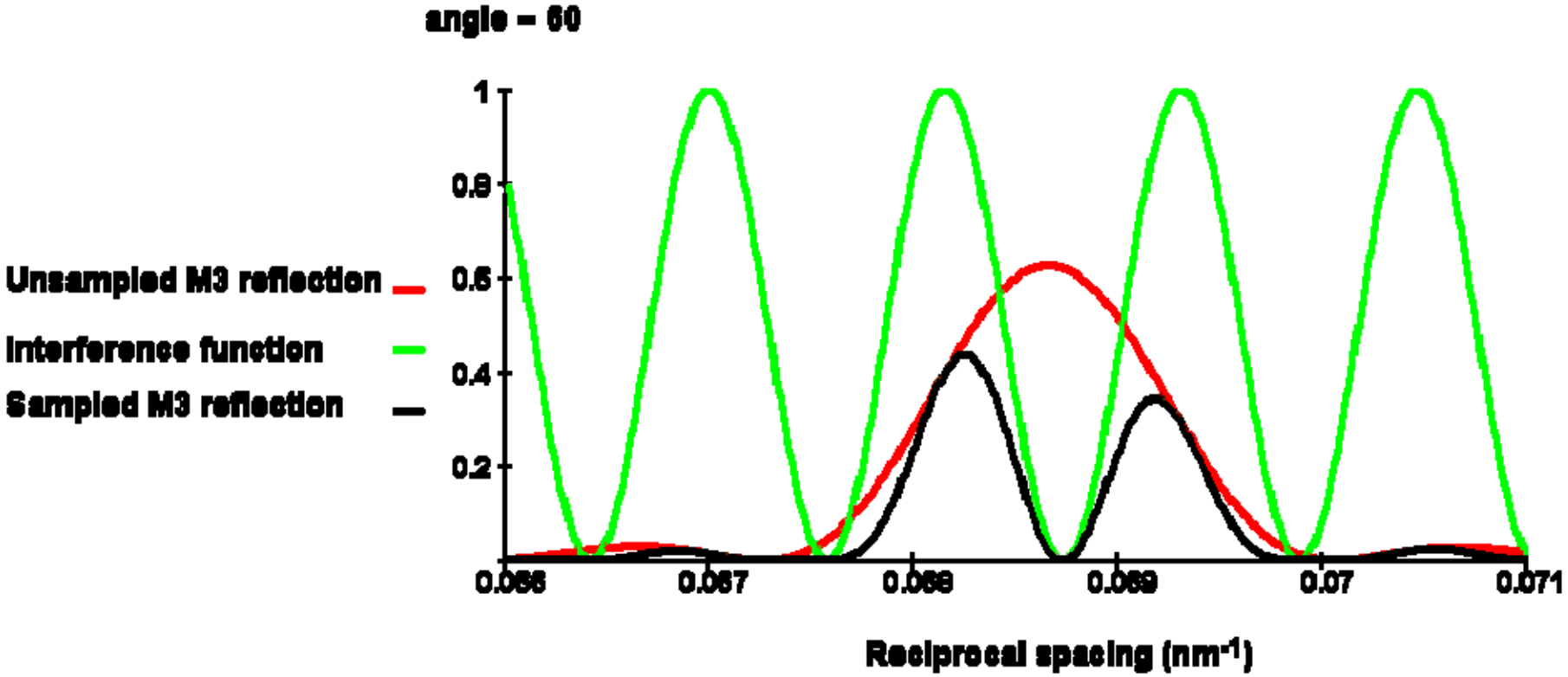
Volodymyr Gelfand
Alex Plakantonakis

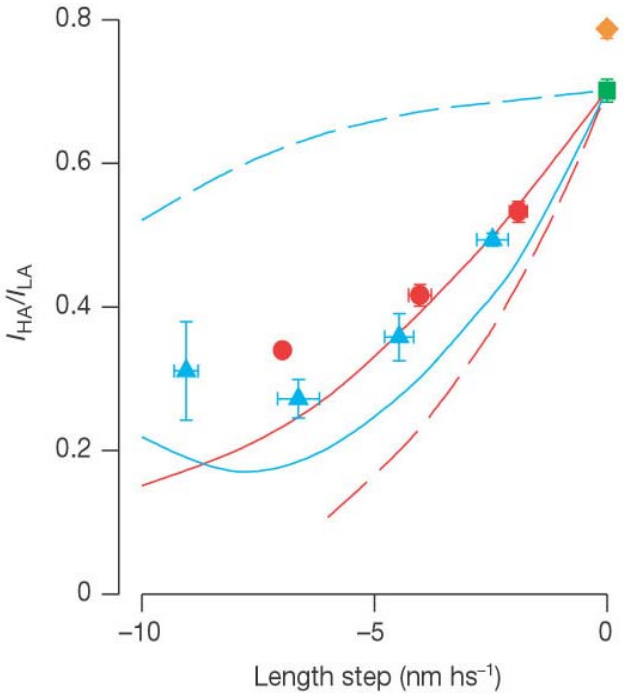
Lindberg Lab
Stockholm University

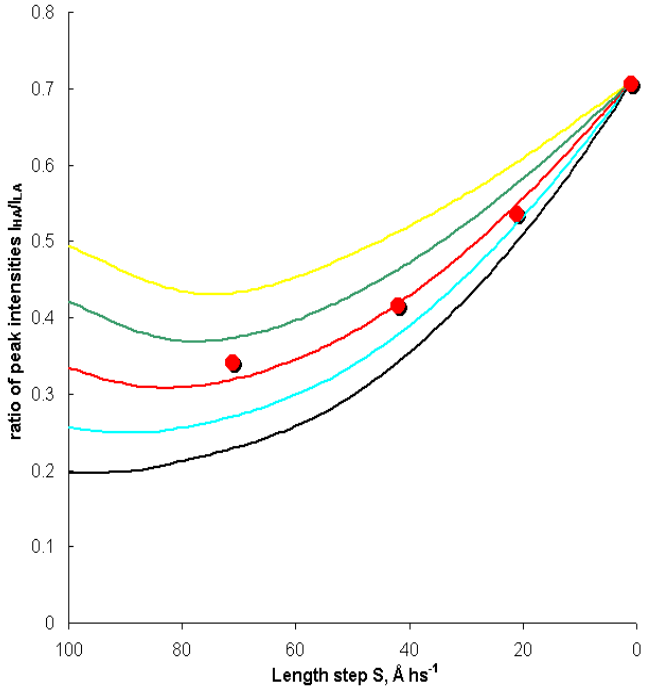
Roger Karlsson
Tomas Nyman
Herwig Schuler
Maria Nyakern-Meazza
Elena Korenbaum



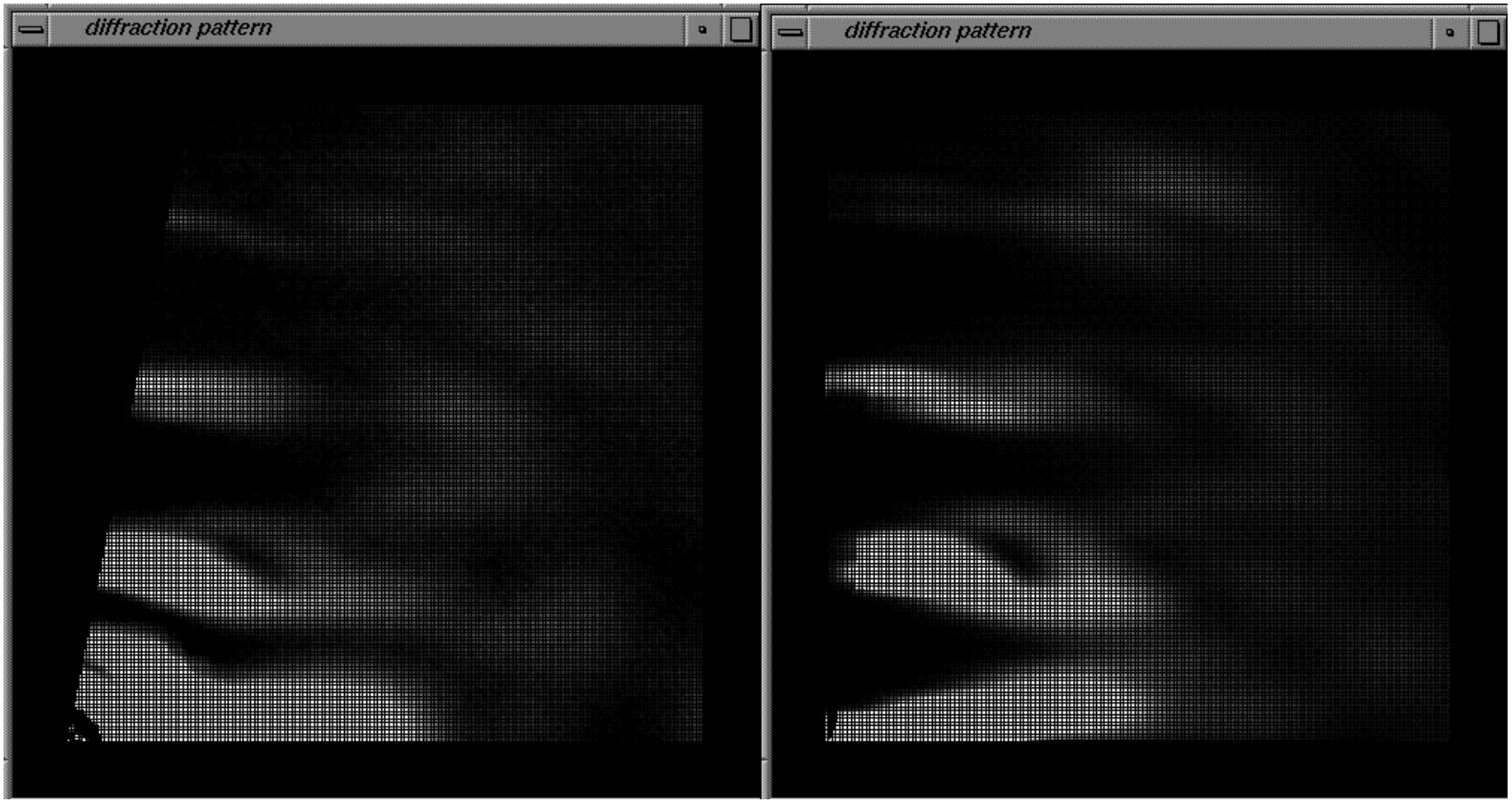


http://www.unifi.it/dfisio/web%20page%20v2.3/

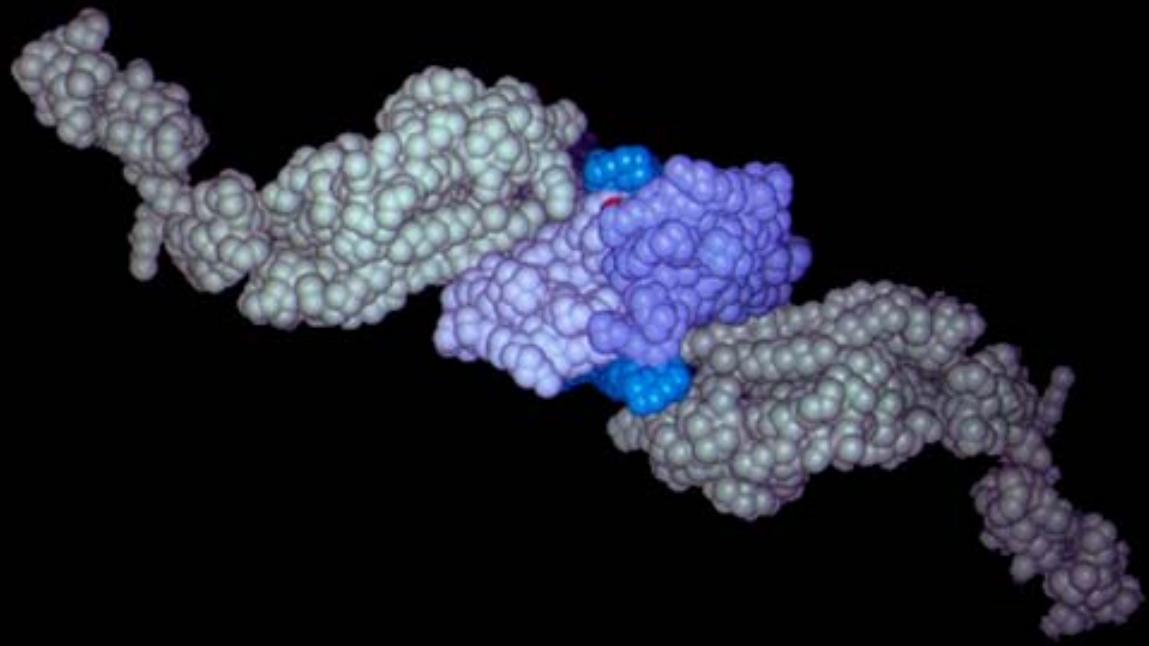




Piazzesi et al., *Nature* **415**, 659-662 (2002)


diffraction pattern
diffraction pattern

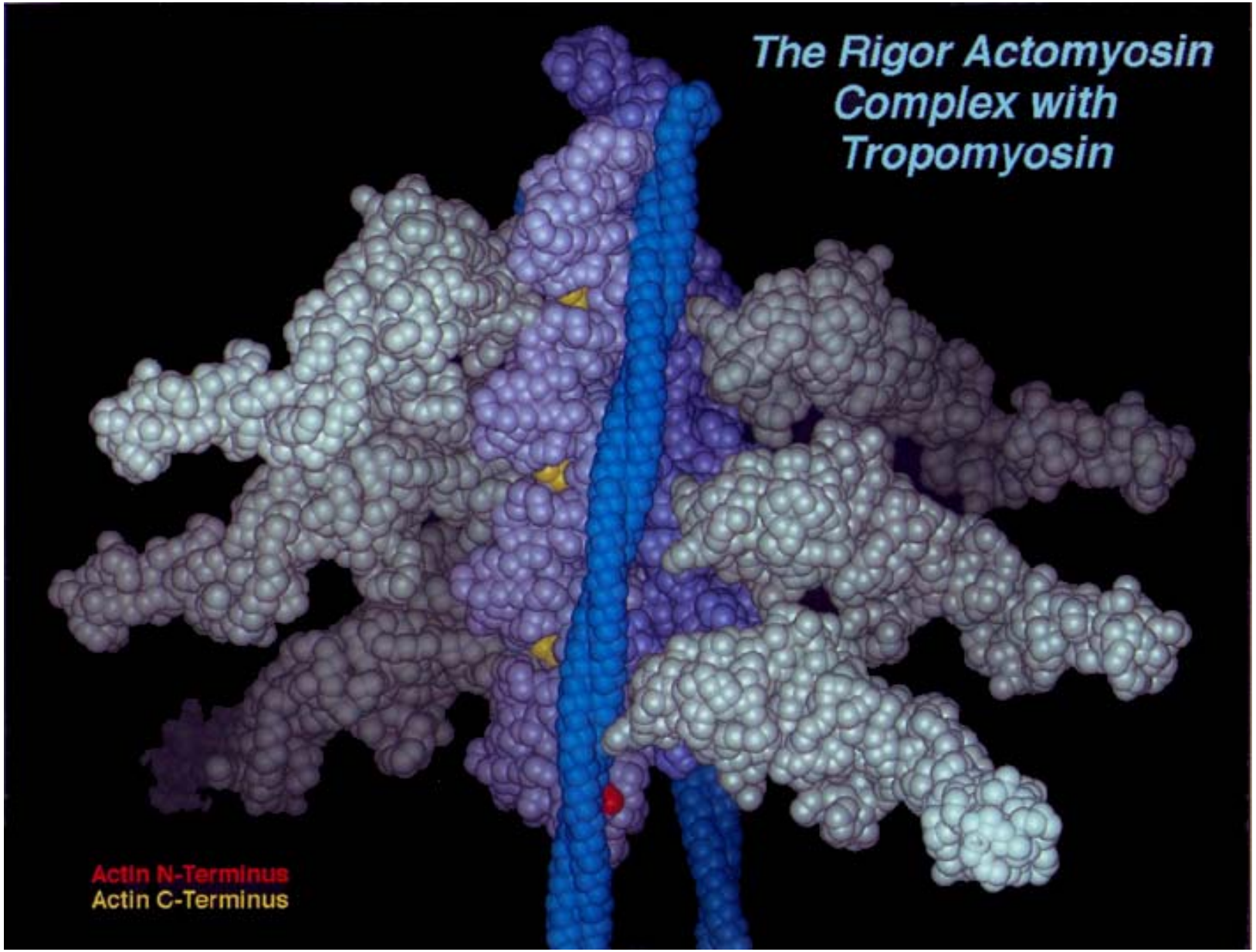
Localization of Tropomyosin



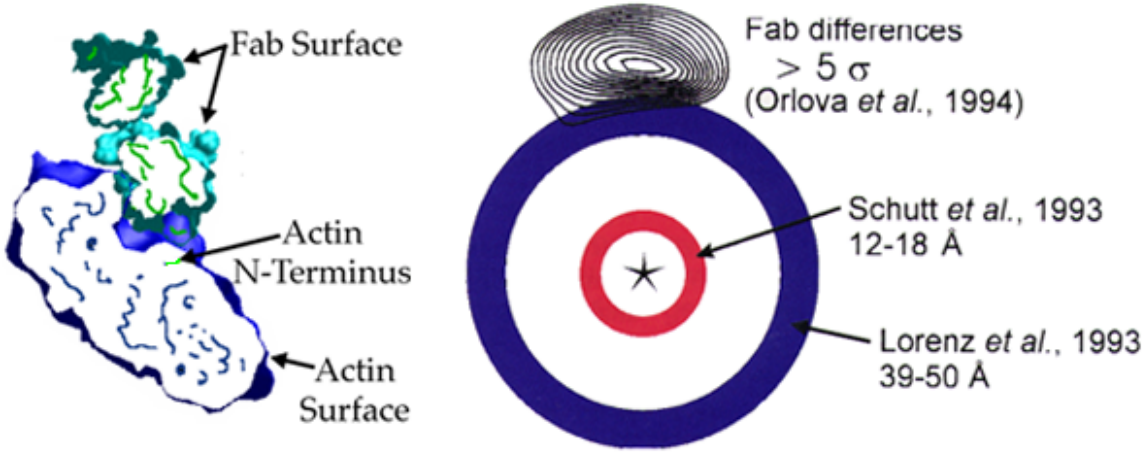
Actin-Tropomyosin-S1

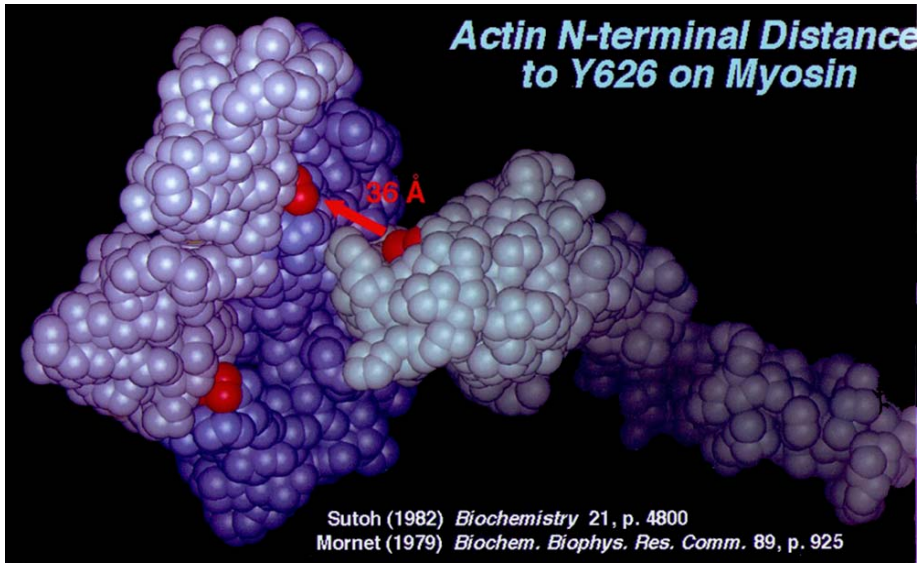
Actin-S1

Milligan et al. *J. Cell Biology* (1987) 105, p.34

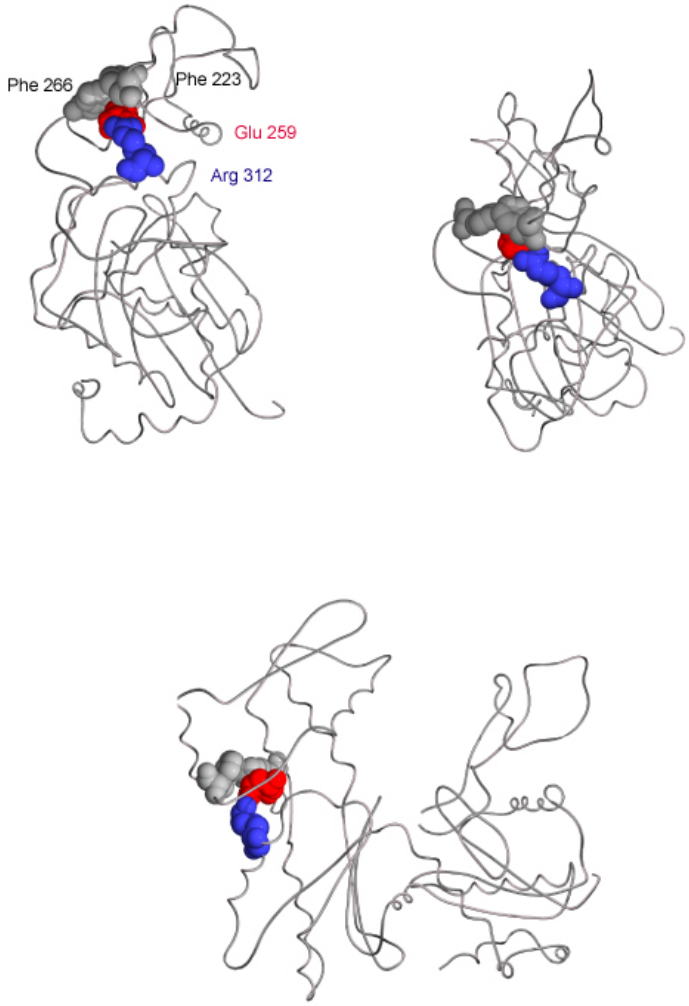

The Rigor Actomyosin Complex with Tropomyosin
Actin N-Terminus
Actin C-Terminus

Location of the actin N-terminus in the ribbon-derived filament



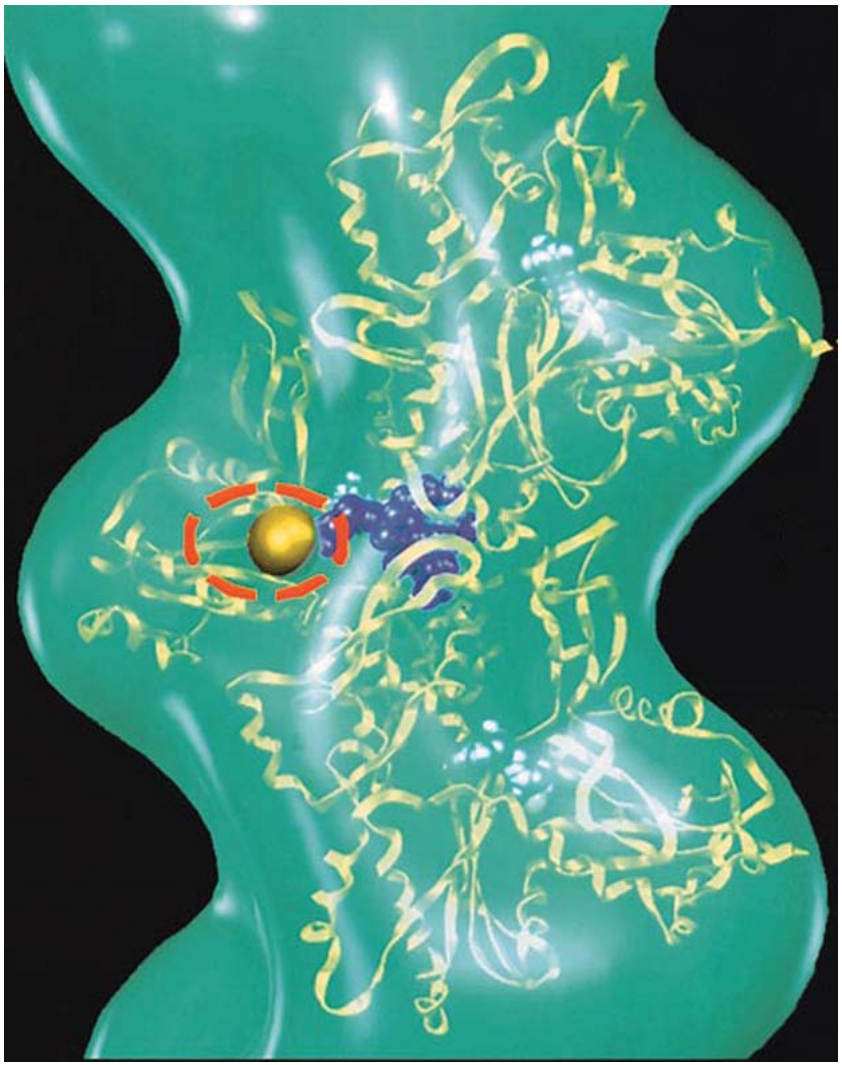


The “hydrophobic plug”

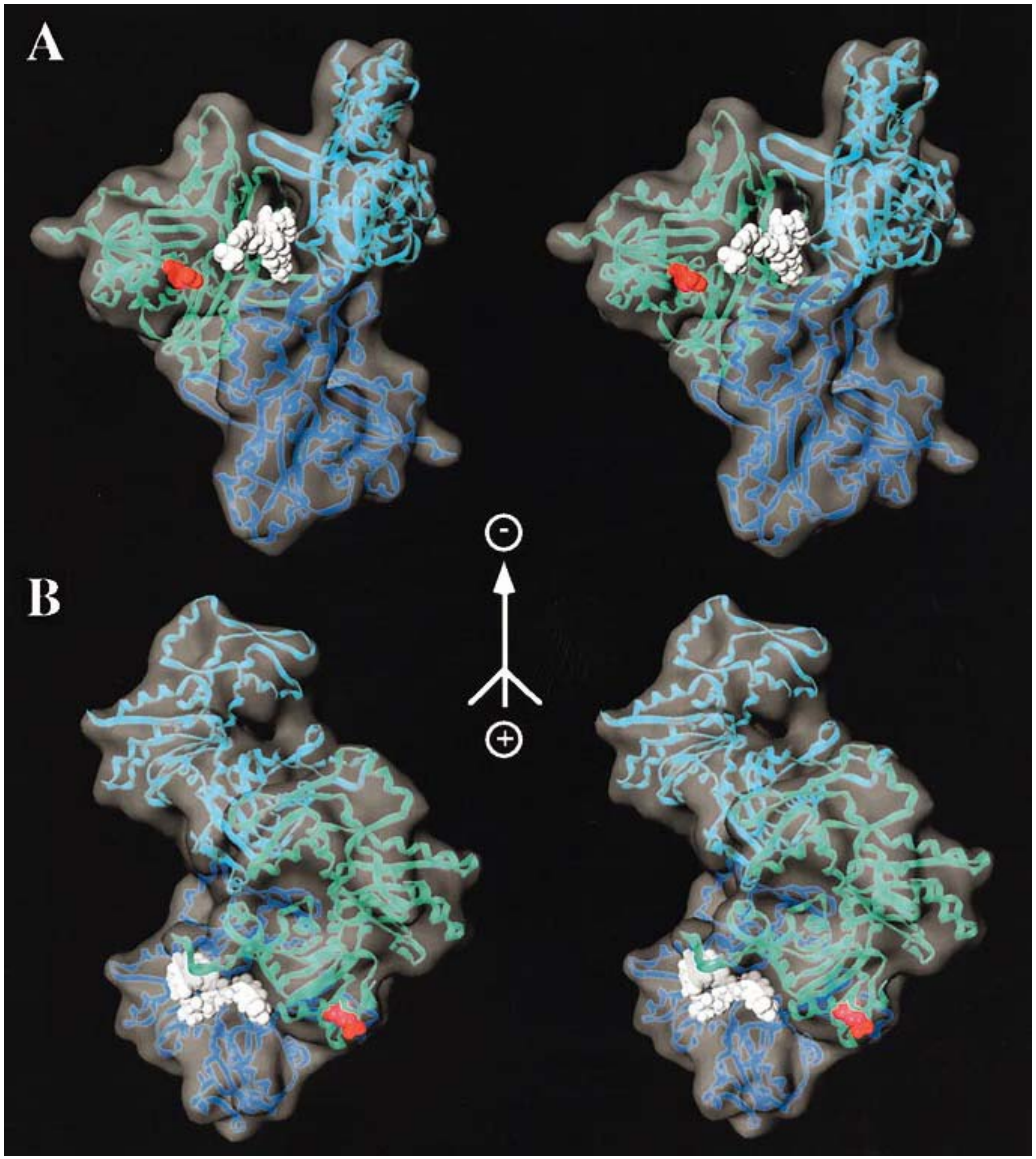


STEM of Undecagold-tagged Phalloidin on F-actin

Au_{11} mapped



Steinmetz *et al*., *J. Mol. Biol.* **276**, 1-6 (1998)



Phalloidin is thought to bind at a three-way contact inside the filament…

Lorenz, Popp and Holmes
J. Mol. Biol. **234**, 826-836 (1993)

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…but this new model for gelsolin severing strongly suggests that phalloidin is on the surface of F-actin.