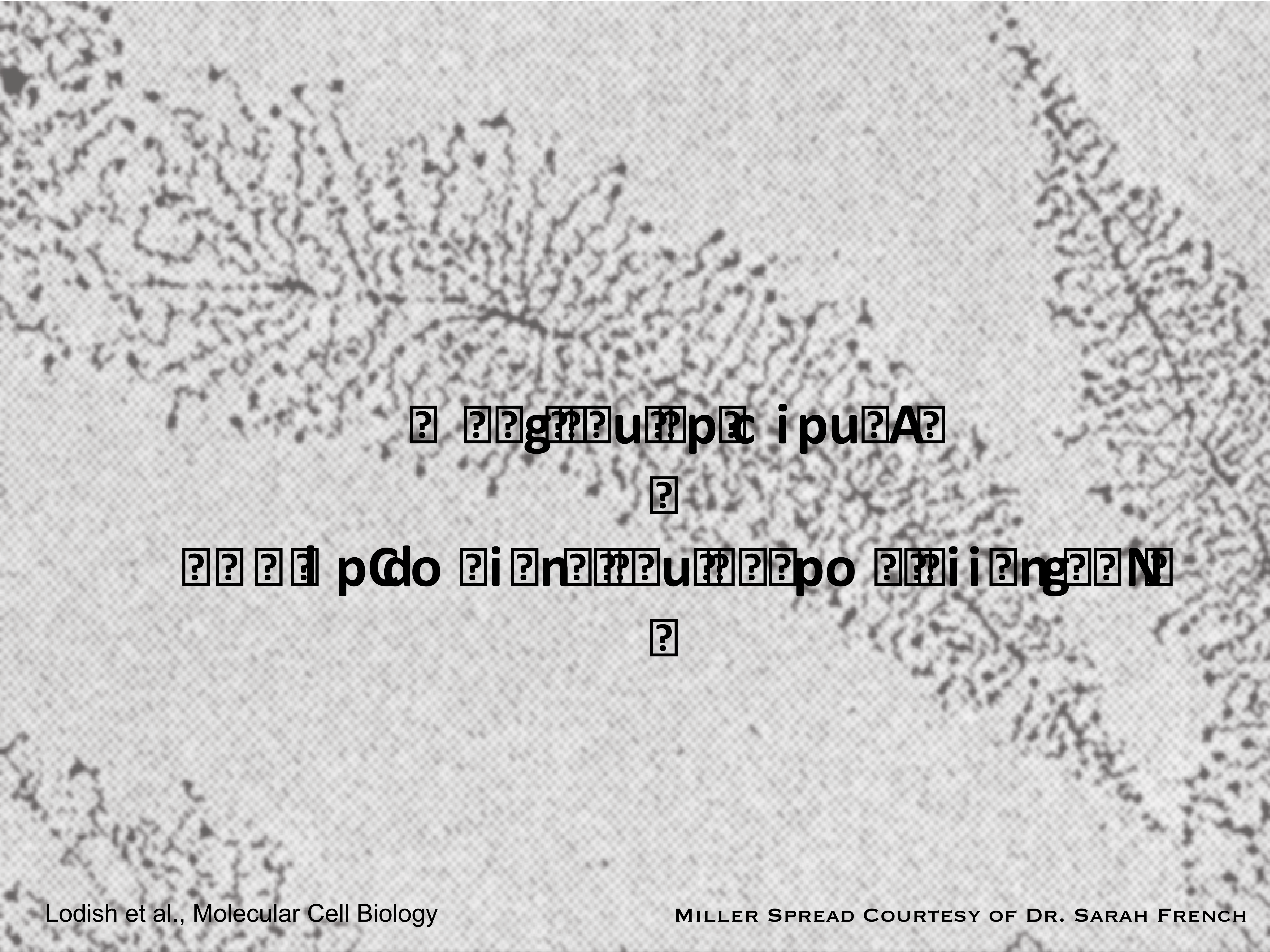
1. Coupling Transcription and DNA Repair with a dsDNA-Tracking Motor

2. Post-Translational Modification of Tubulin by Tubulin Tyrosine Ligase (TTL)

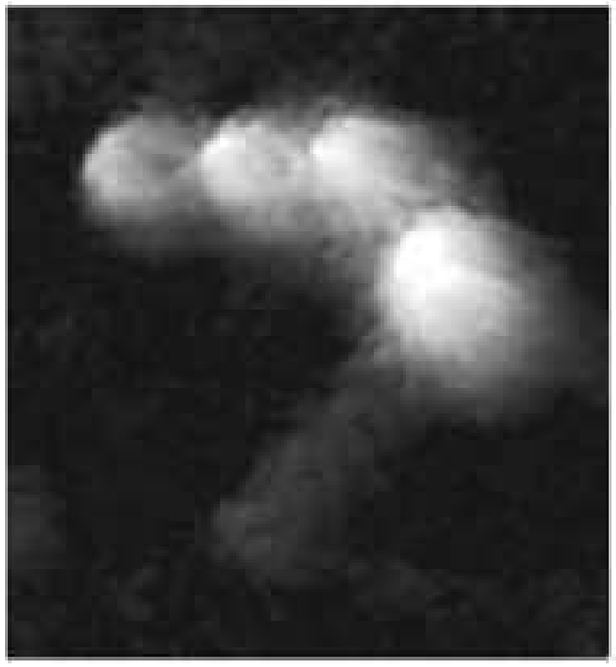
Miller Spread Courtesy of Dr. Sarah French



Lodish et al., Molecular Cell Biology

Miller Spread Courtesy of Dr. Sarah French

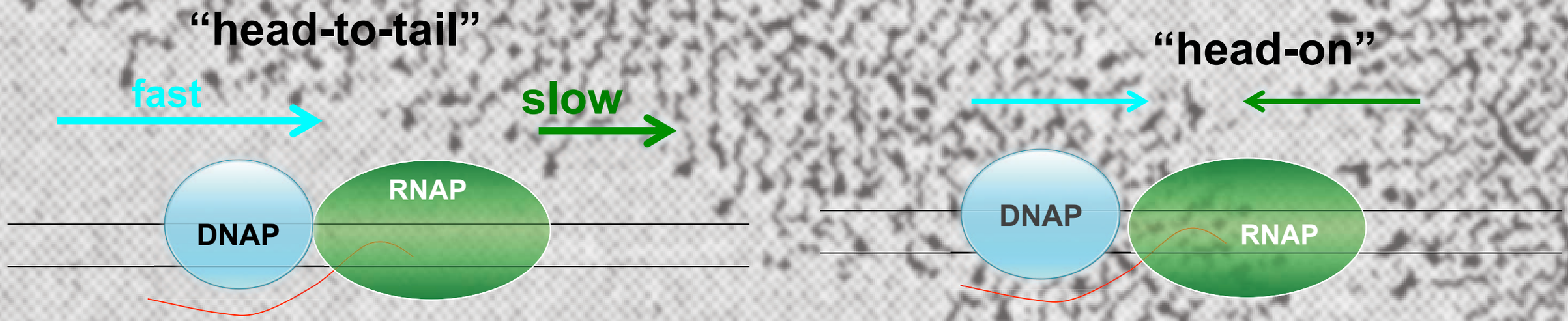
A Stalled RNA Polymerase Can Cause a Molecular Pile-up



AFM of transcription complexes on irradiated template

Trautinger et al., (2005) *Molecular Cell* 19, 247-254
Adapted from: http://www.cawlocal584.com/humour.html

- 3000 RNA Polymerase molecules/cell
- 10-20 DNA Polymerase III molecules/cell



Double strand breaks

Lodish et al., Molecular Cell Biology

Miller Spread Courtesy of Dr. Sarah French

[illegible]

- [illegible]
- [illegible]

Miller Spread Courtesy of Dr. Sarah French

RNAP Stalling Leads to Genomic Instability

- RNAP/DNAP collisions result in double-strand breaks
- DNA repair shapes the mutational landscape of cancer cells
- DNA repair is the **main** mechanism underlying the development of cancer in all living organisms
- Also essential for the mechanism of chemotherapeutic agents (irofulven)
- Essential for the etiology of accelerated-aging diseases (Cockayne Syndrome)

Deaconescu *et al.,* (2012) *Trends Biochem, Sci.37(12)* 543-552
Nudler, E. (2012) *Cell* 149(7) 1438-1445
Nik-Zainal *et al.,* (2012) *Cell* 149(5) 994-1007

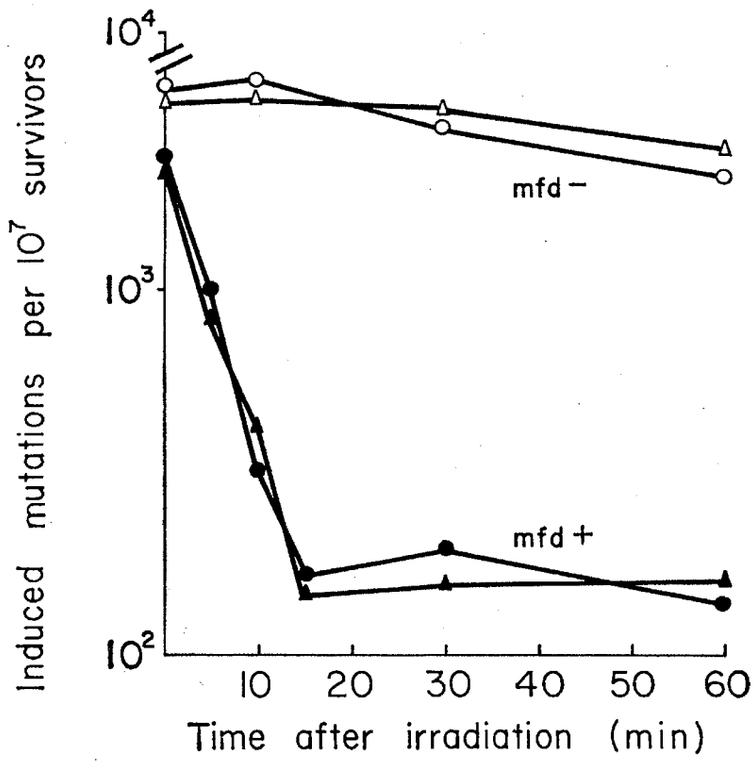
Mfd (mutation frequency decline)

Radiation-Induced Mutations and Their Repair

Evelyn M. Witkin

Science, New Series, Vol. 152, No. 3727 (Jun. 3, 1966), 1345-1353.

- mutant is UV sensitive
- 3-fold lower rate of excision of pyrimidine dimers



Witkin EM (1966) *Science 152, 1345-1353*

Mfd is the TRCF (transcription-repair coupling factor)

Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 11574–11578, December 1991
Biochemistry

Escherichia coli mfd mutant deficient in "mutation frequency decline" lacks strand-specific repair: *In vitro* complementation with purified coupling factor

(transcription-repair coupling/UV mutagenesis/SOS response/nonsense suppressors)

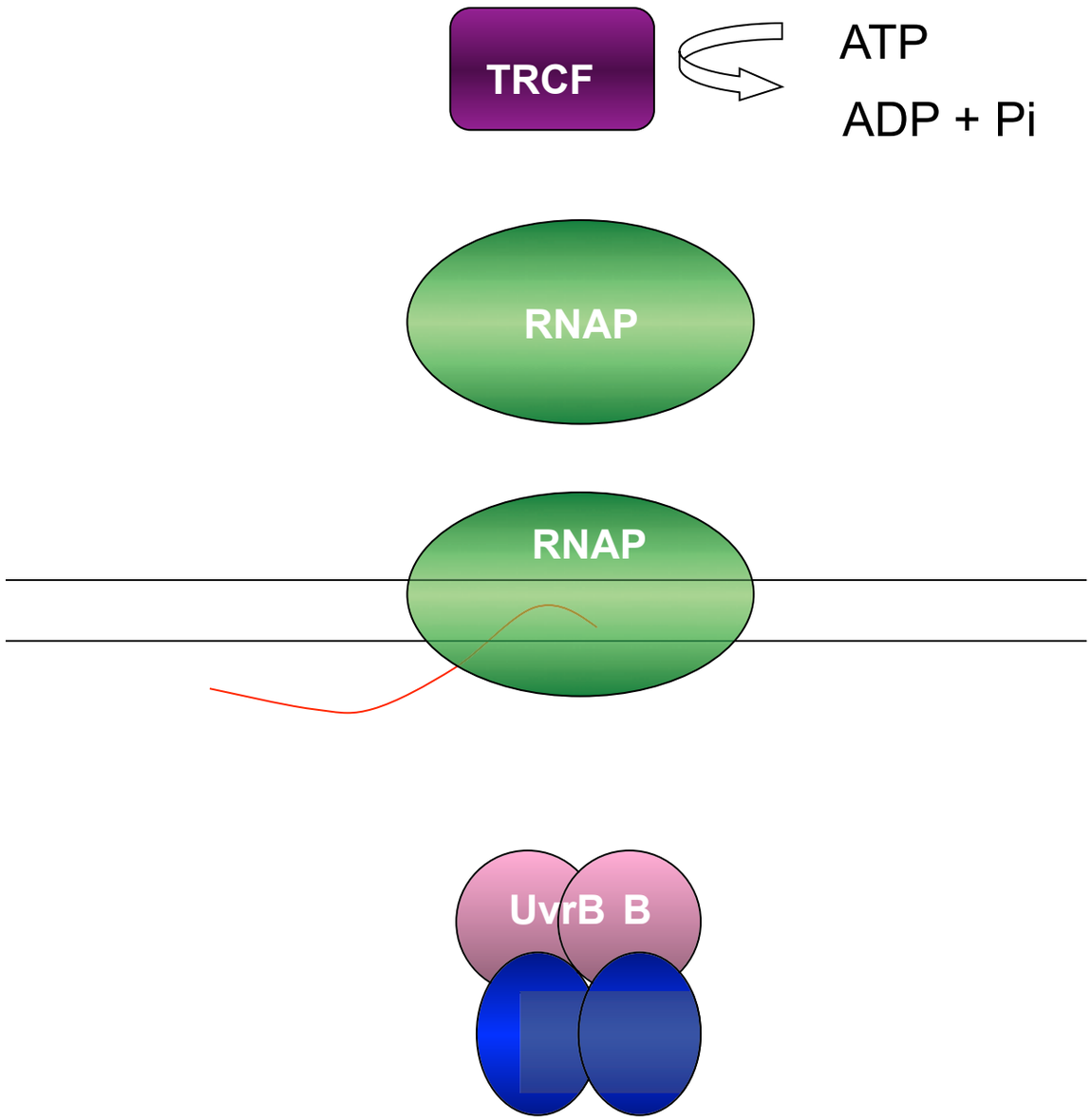
CHRISTOPHER P. SELBY*, EVELYN M. WITKIN†, AND AZIZ SANCAR*

*Department of Biochemistry and Biophysics, University of North Carolina School of Medicine, Chapel Hill, NC 27599; and †Waksman Institute of Microbiology, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, NJ 08854

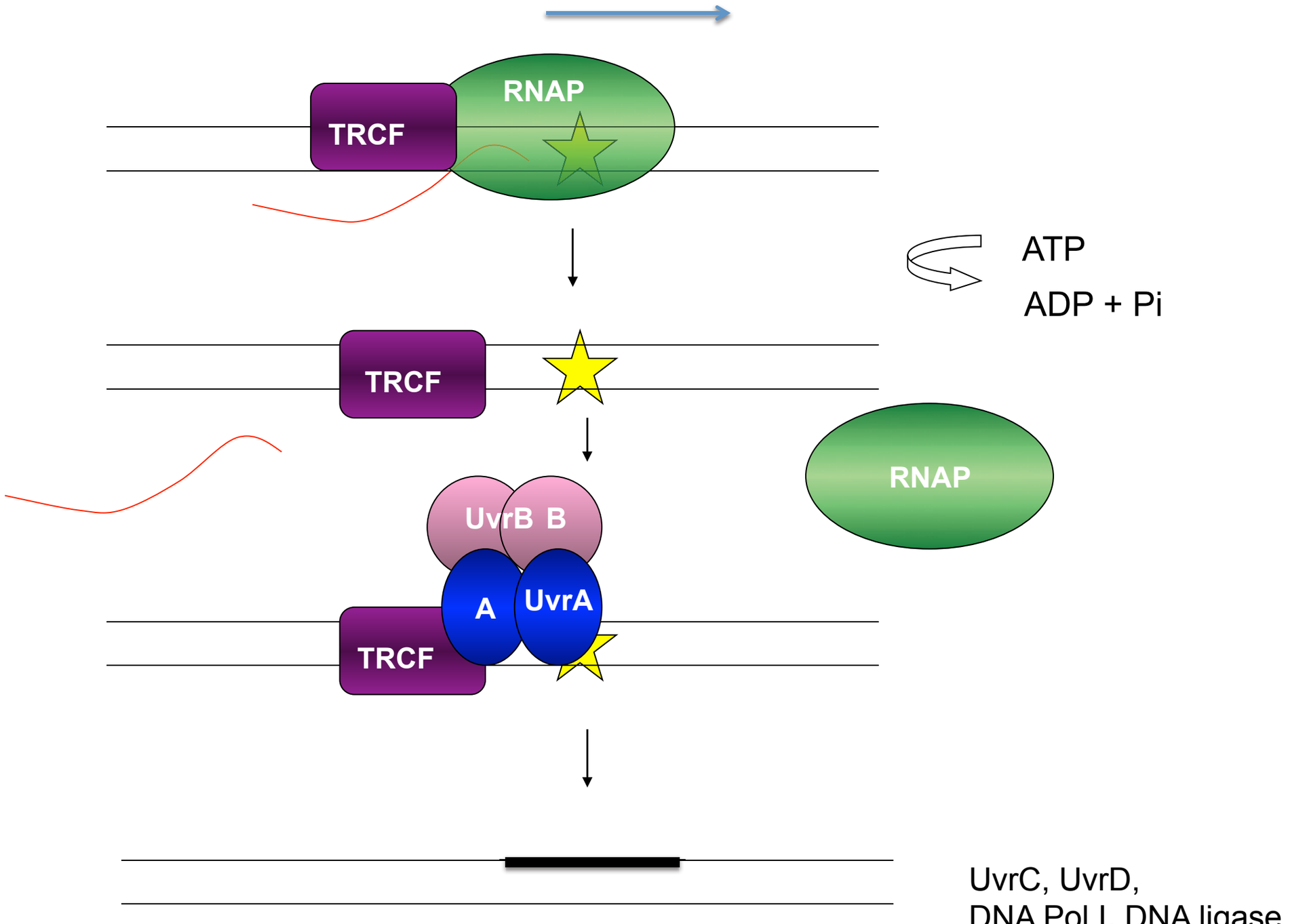
Contributed by Evelyn M. Witkin, September 26, 1991

Selby *et al.*, (1991) *PNAS* (88) 11574-11578

Main Players



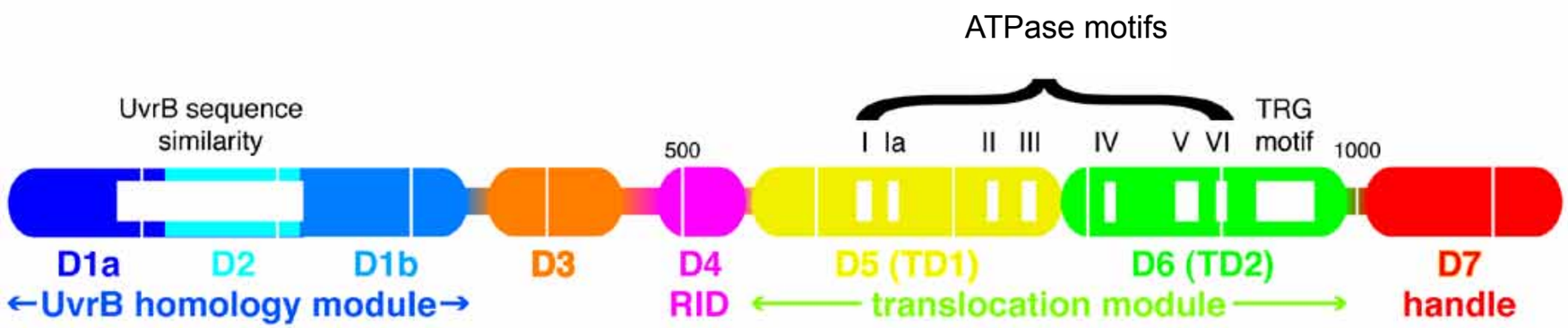
TRCF Mediates Transcription-Coupled Repair (TCR)

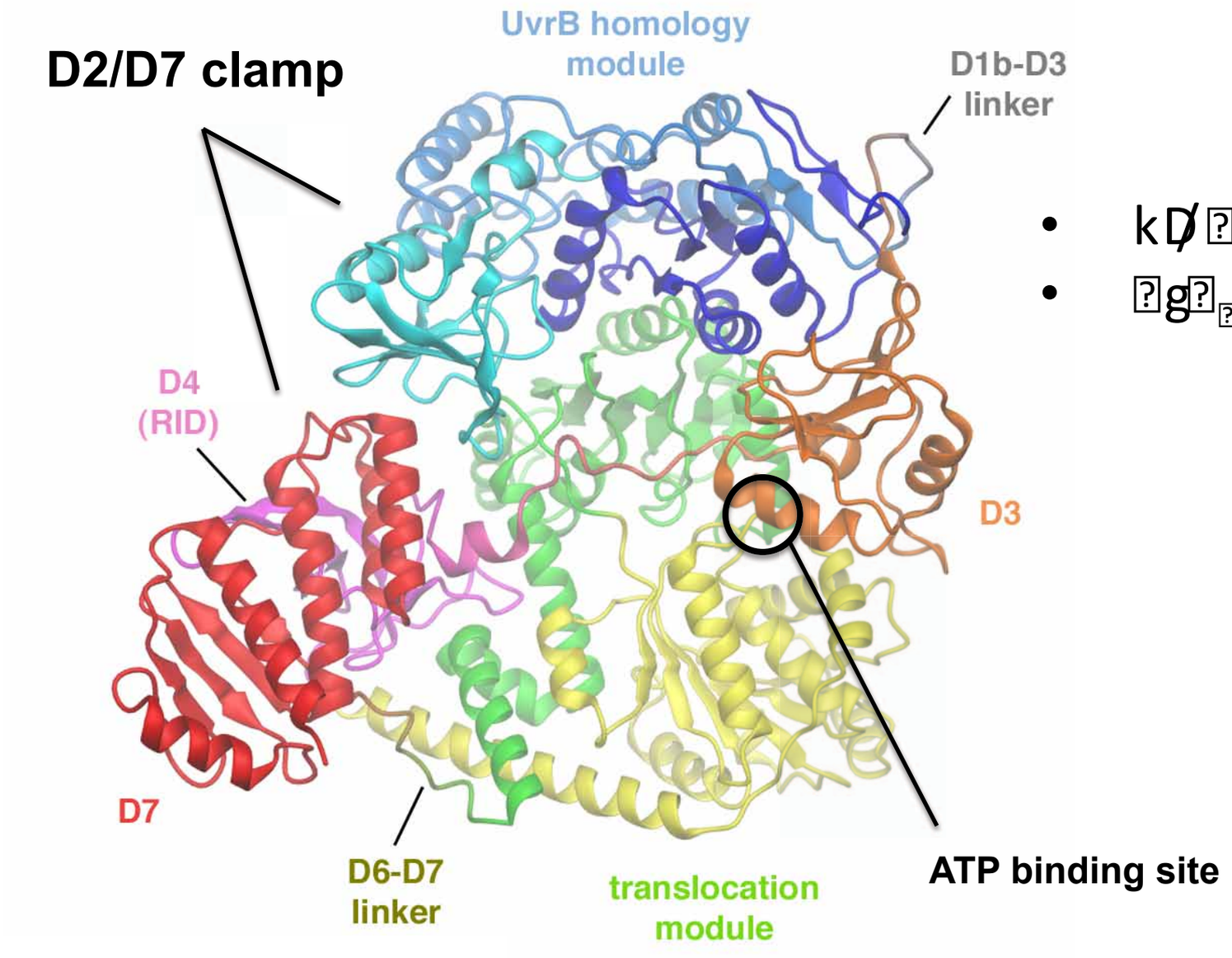


Question

How are the different TRCF activities integrated?

Multiple Activities Are Integrated in a Multi-Domain Protein

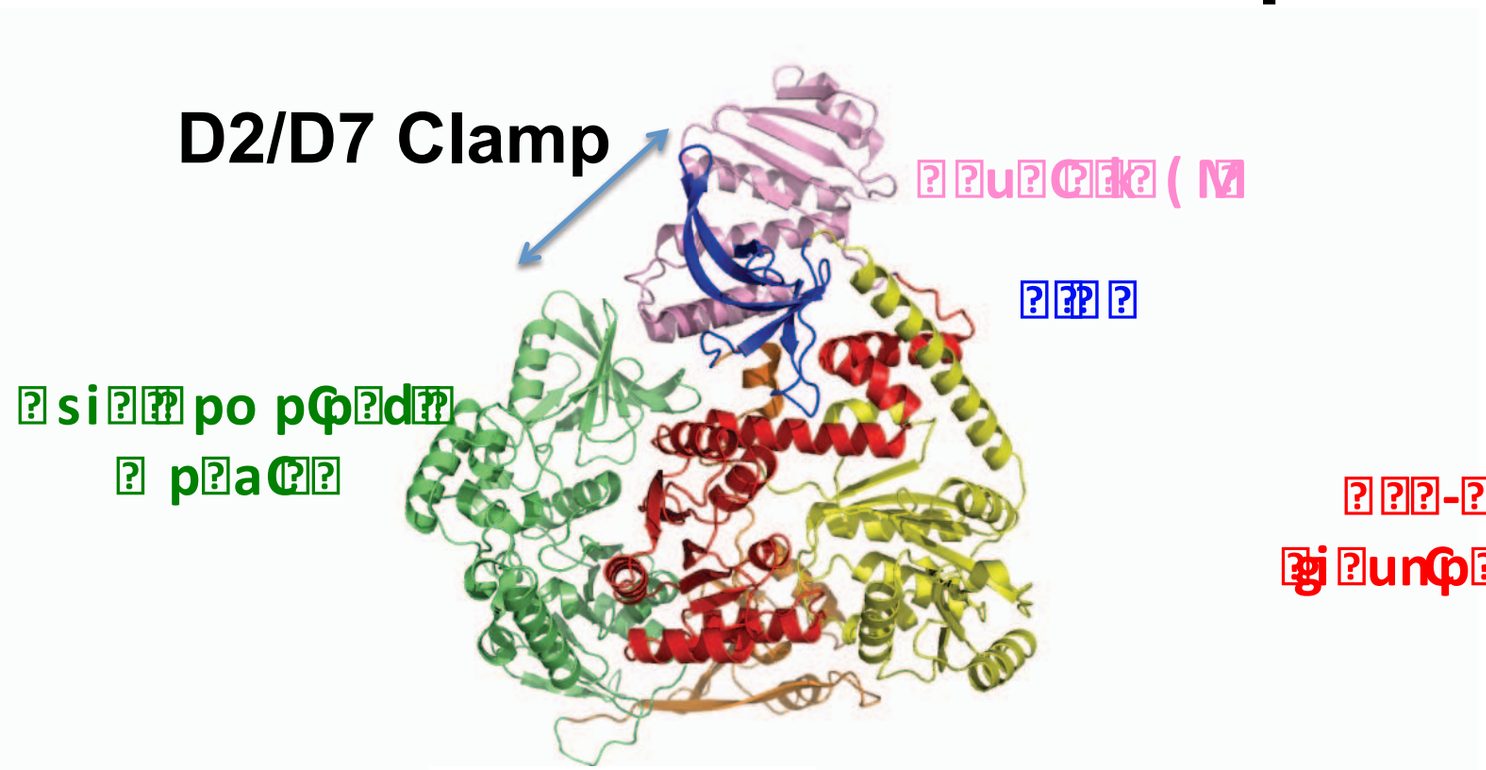


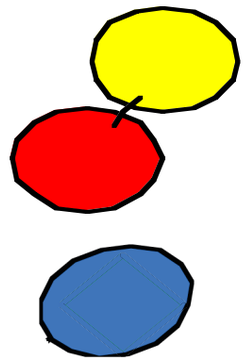


- kD/ mel halo
- g 3/kD 1 g/0D 4

[illegible]

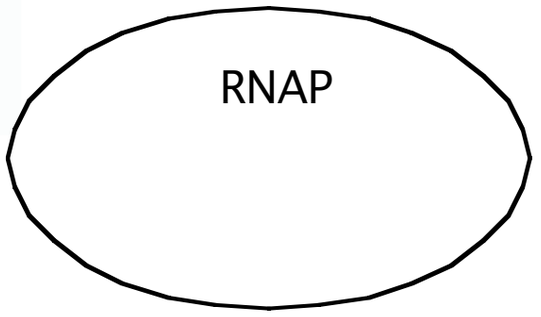
TRCF Induces Transcription Bubble Collapse

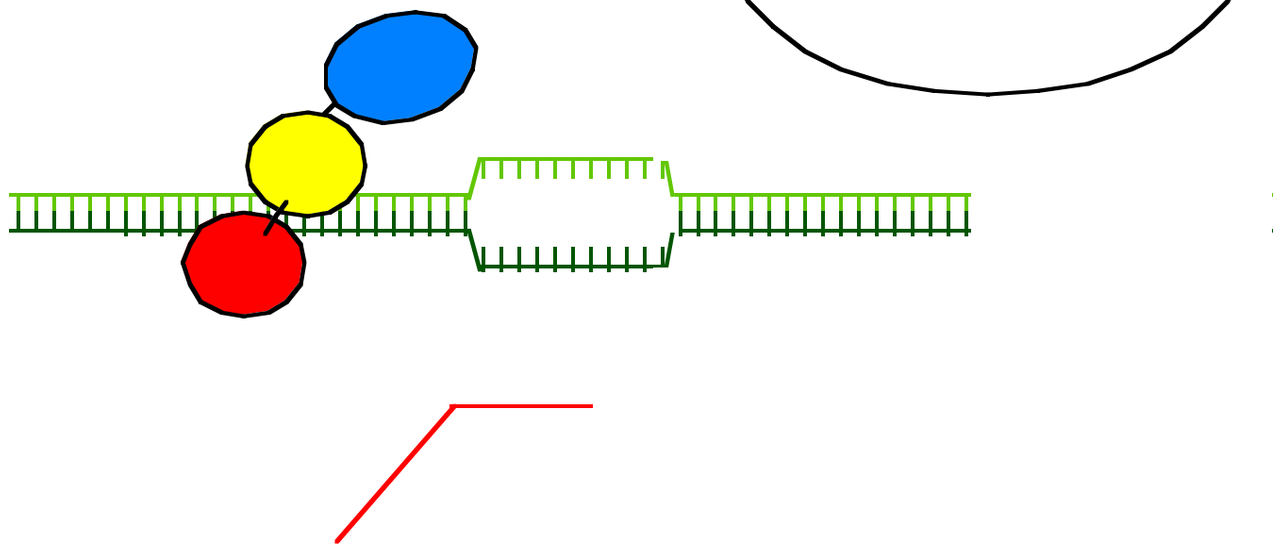




Translocation Domains
D5/D6

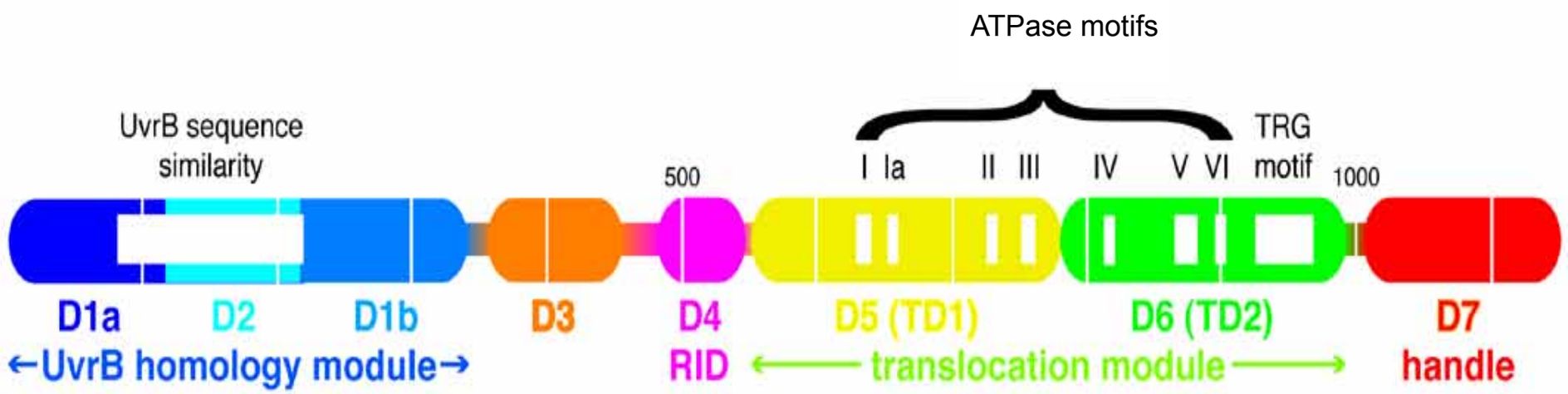
RID (RNAP Interacting Domain)

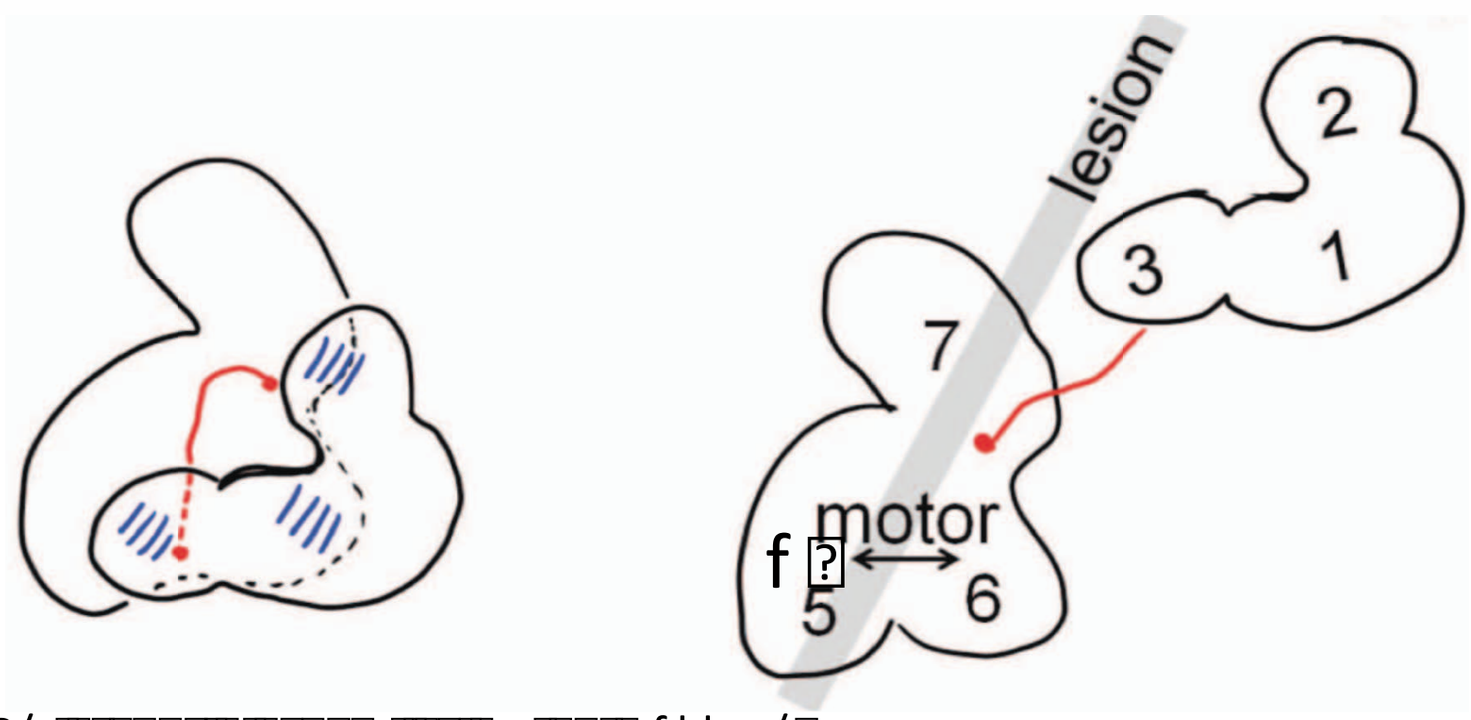




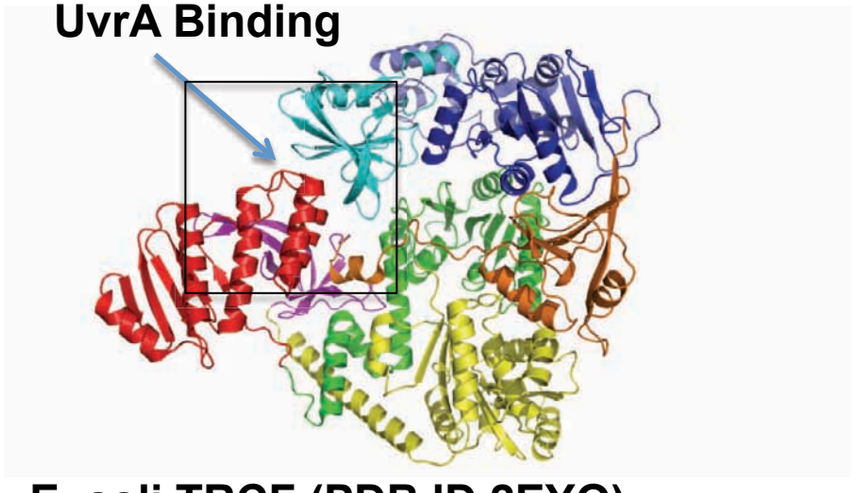
Park *et al*. (2002) *Cell* 109, 757

The D2-D7 Clamp Is Inhibitory to Translocation on Naked DNA

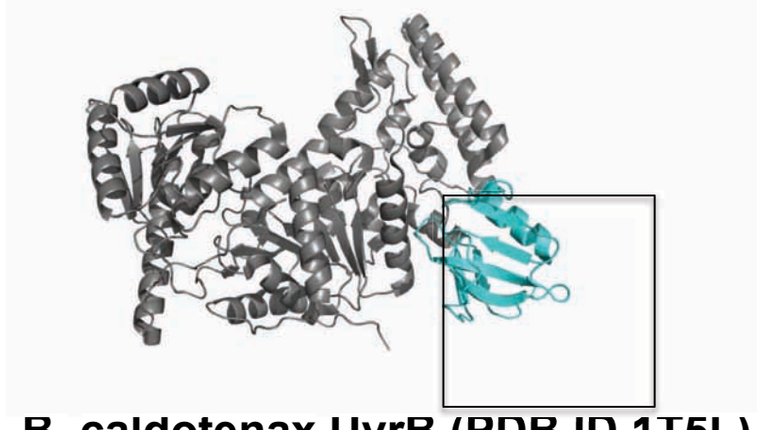




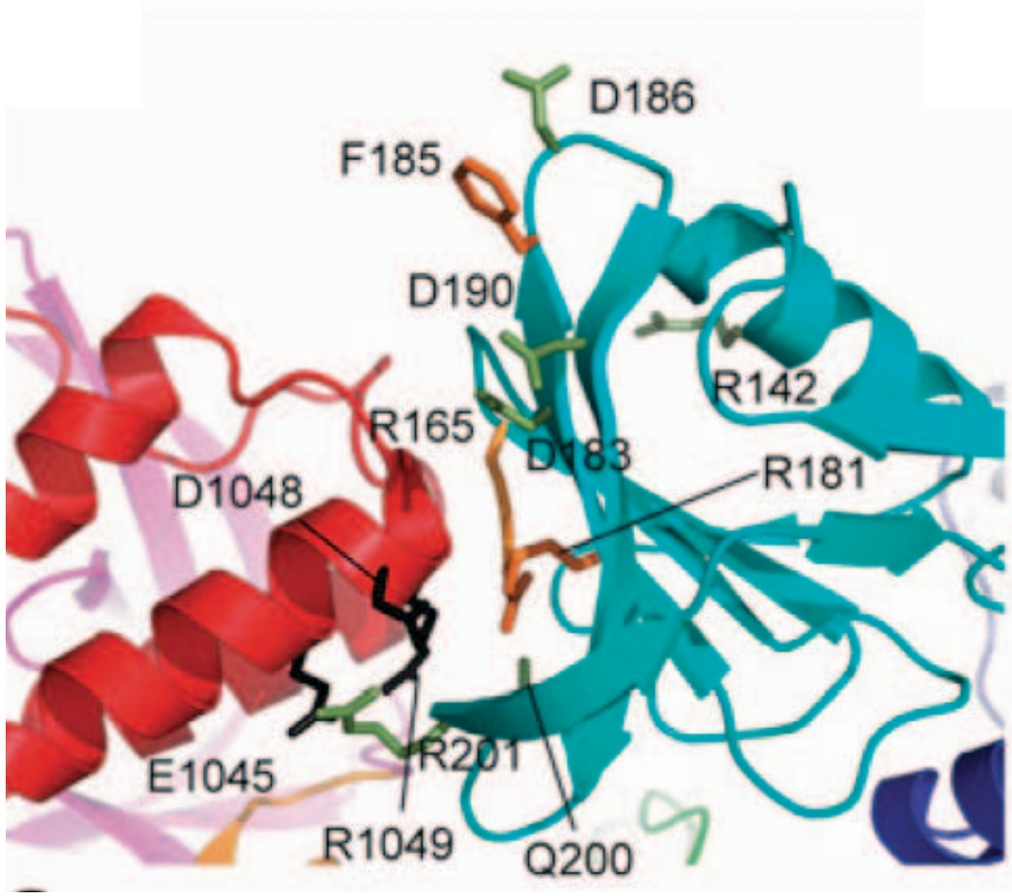
UvrA Recruitment Requires Opening of the D2/D7 Clamp



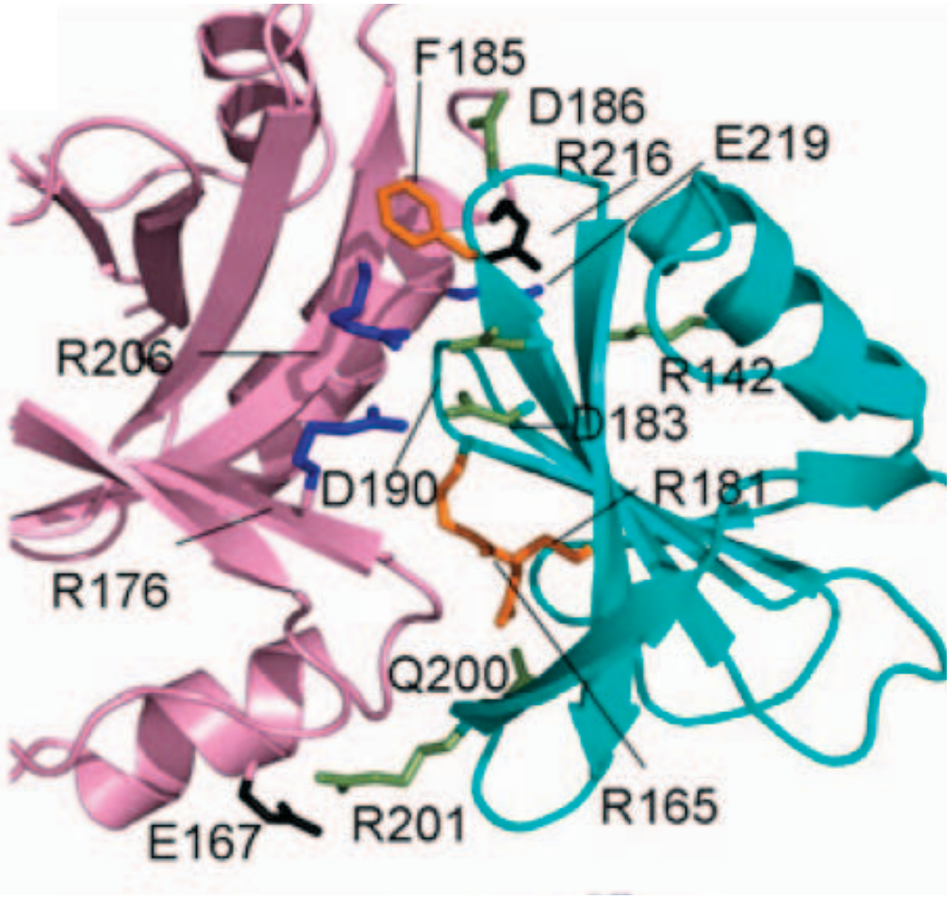
E. coli TRCF (PDB ID 2EYQ)



B. caldotenax UvrB (PDB ID 1T5L)

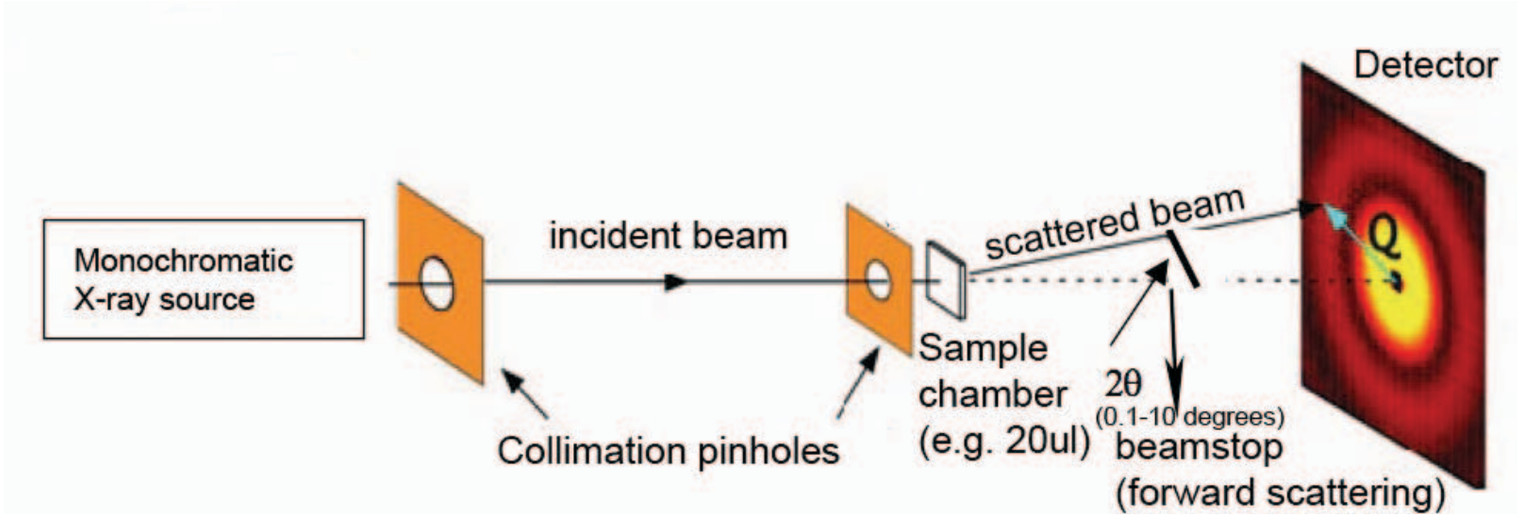


TRCF-D2: TRCF-D7



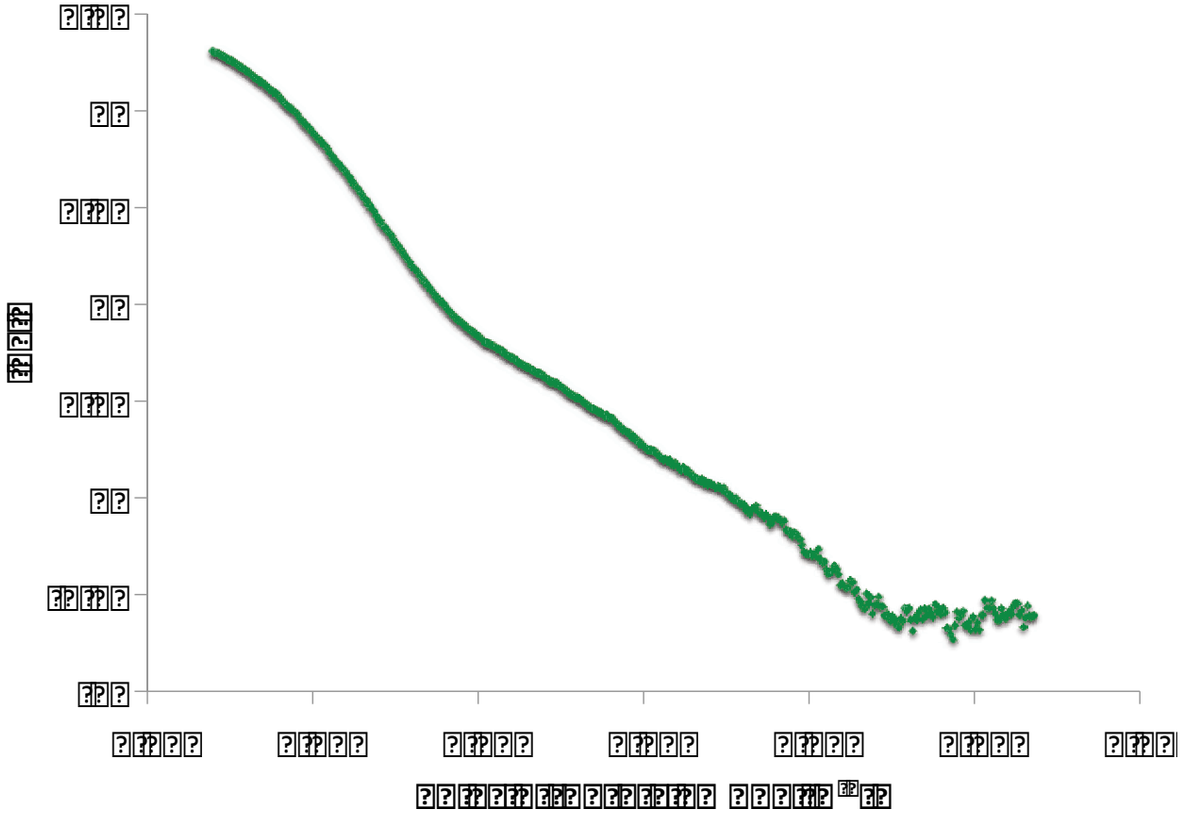
TRCF-D2: Truncated UvrA

SAXS Probes Structure in Solution

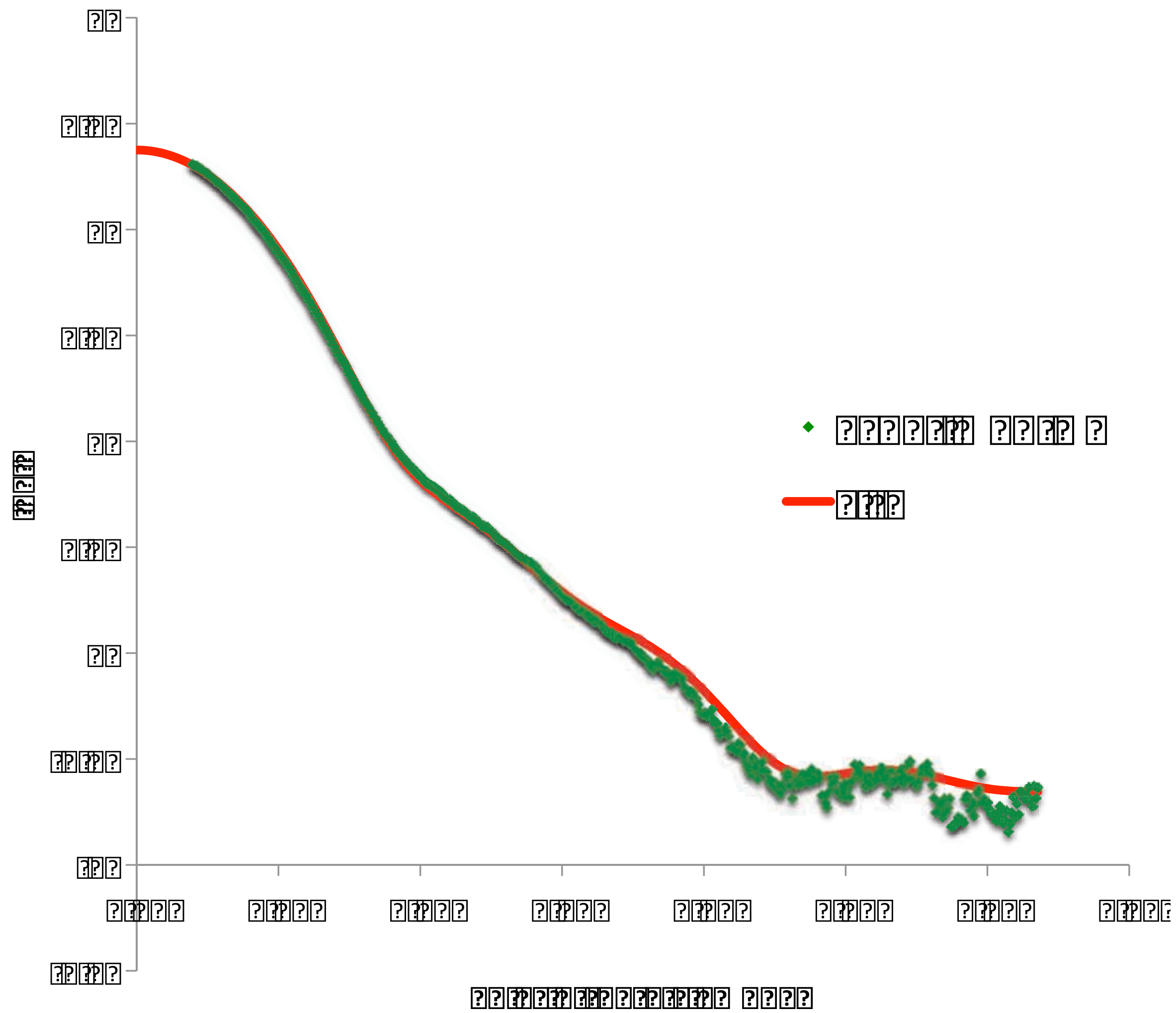


Adapted from Petoukhov, M., EMBL lecture

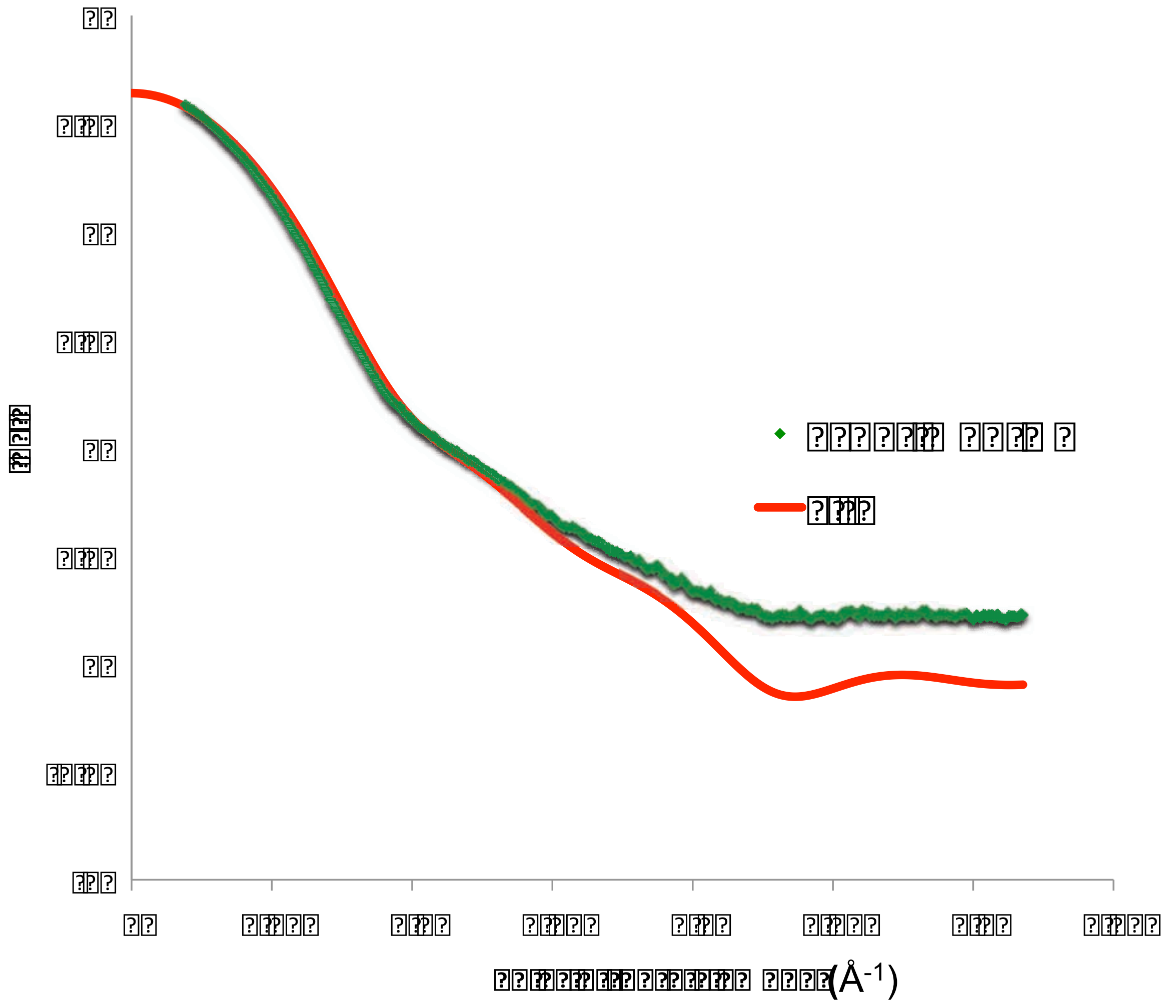
- Measure isotropic intensity distribution and radially average I(q)

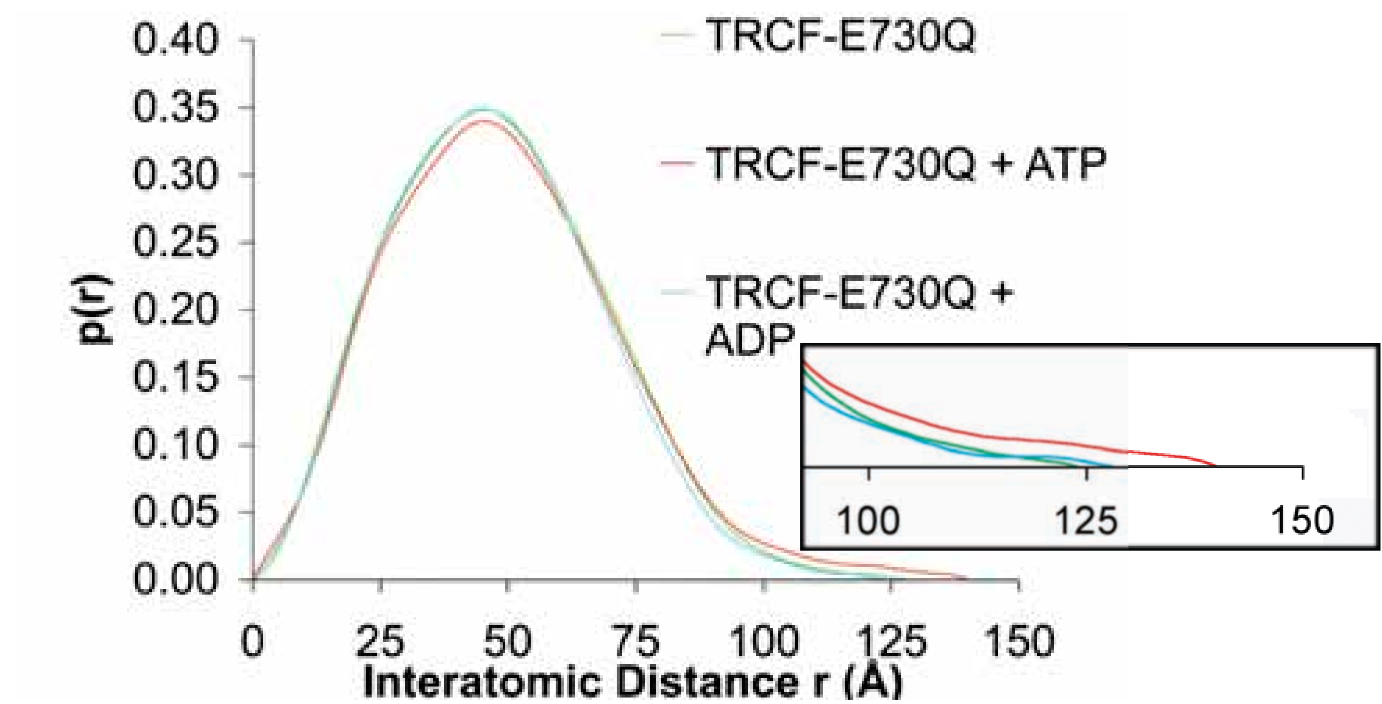


- Extract shape parameters:
  R_g (low q data)
  D_{max} (maximum intramolecular distance)



Deaconescu *et al.*, *PNAS* (2012) 109 (9): 3353-3358

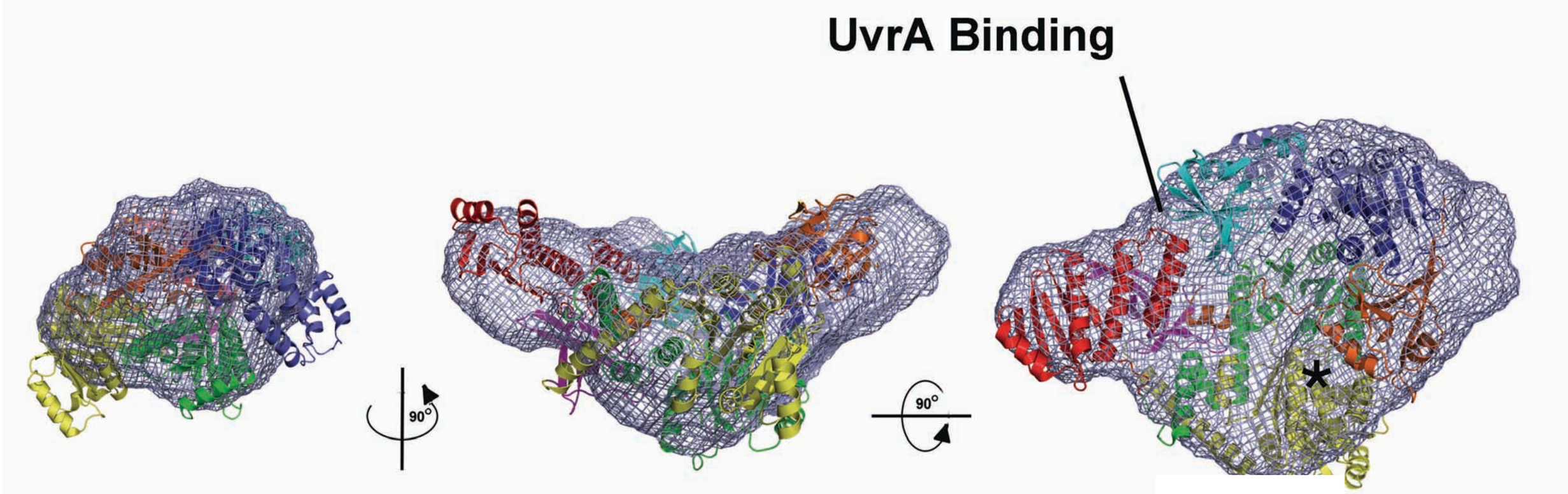




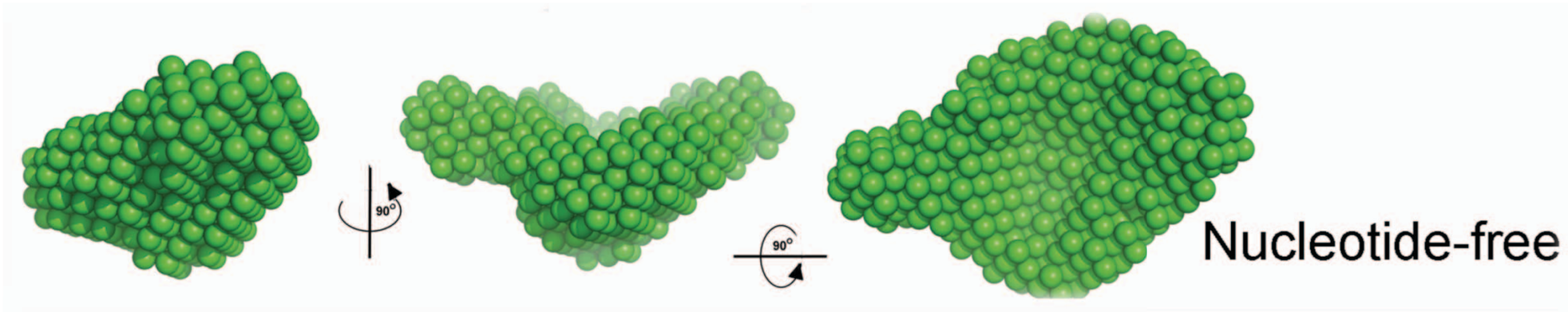
- Reconstruct 3D-envelope using *ab initio* algorithms starting with gas phase of "dummy atoms/residues" in a spherical search volume followed by minimization and fit against the scattering curve

- **SYSTEM IS UNDERDETERMINED, MULTIPLE SOLUTIONS ARE POSSIBLE**

The D2/D7 Clamp is Maintained in Solution in the Nucleotide-Free State

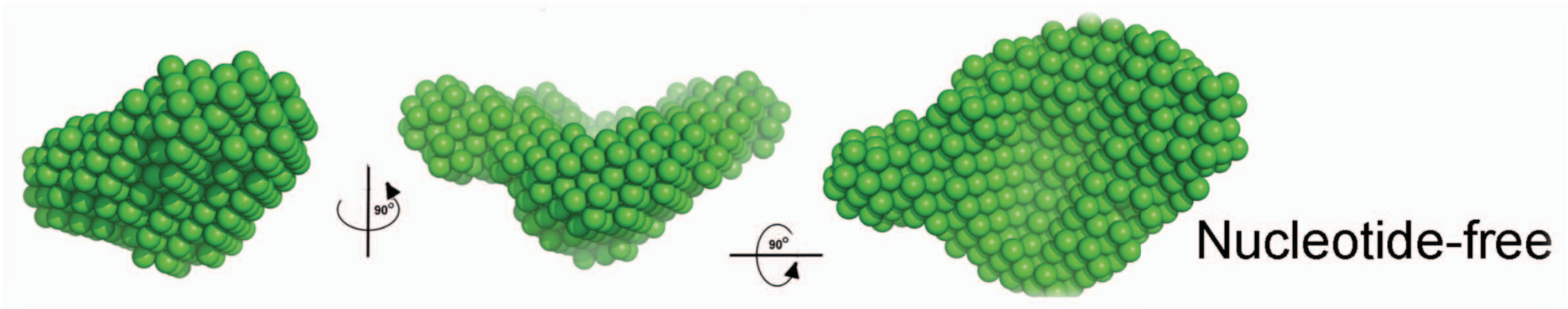


The Catalytic Cycle Reorganizes Interdomain Contacts

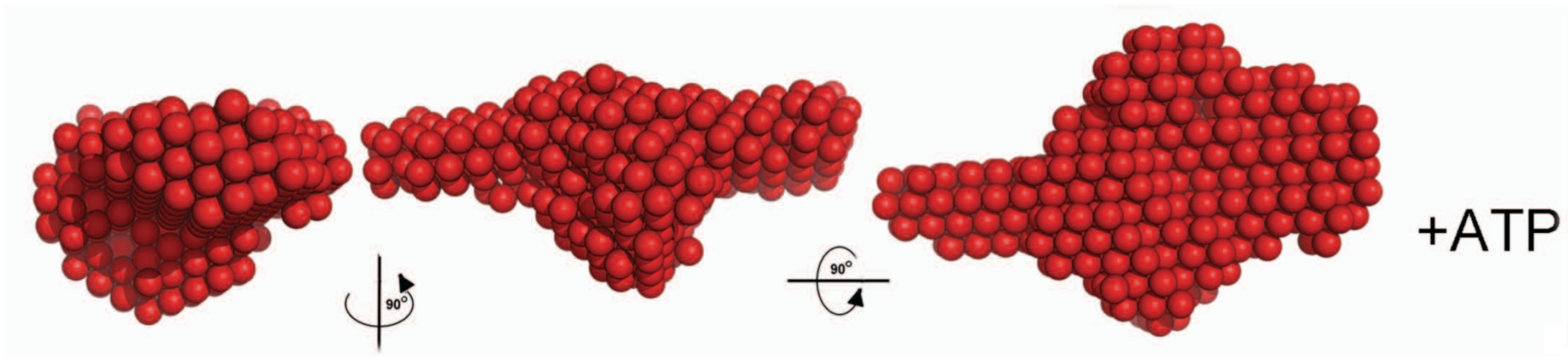


Deaconescu *et al.*, *PNAS* (2012) 109 (9): 3353-3358

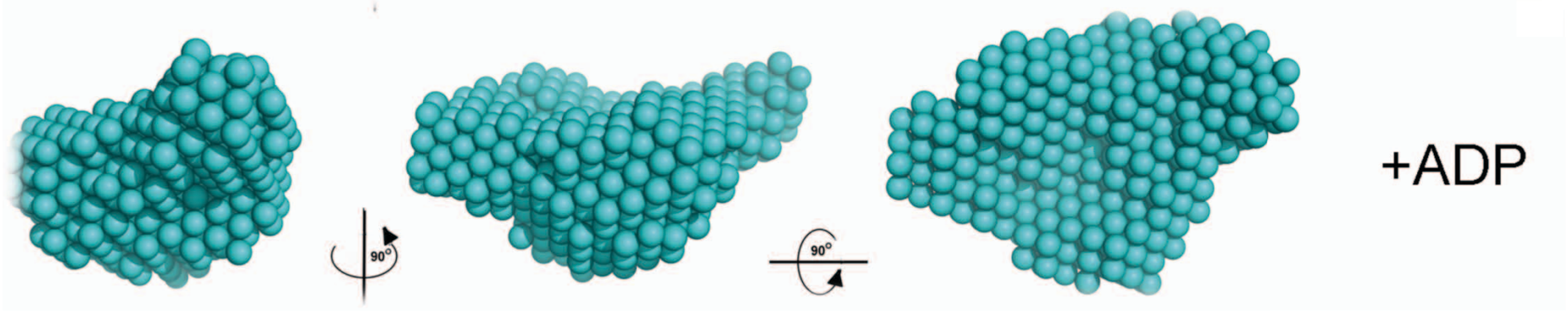
The Catalytic Cycle Reorganizes Interdomain Contacts



No DNA binding
Dmax = 124 ± 5Å
R_g =37.6 ± 0.2 Å



DNA binding
Dmax = 140 ± 5Å
R_g =38.2 ± 0.1 Å



No DNA binding
Dmax = 126 ± 5Å
R_g =36.8 ± 0.1 Å

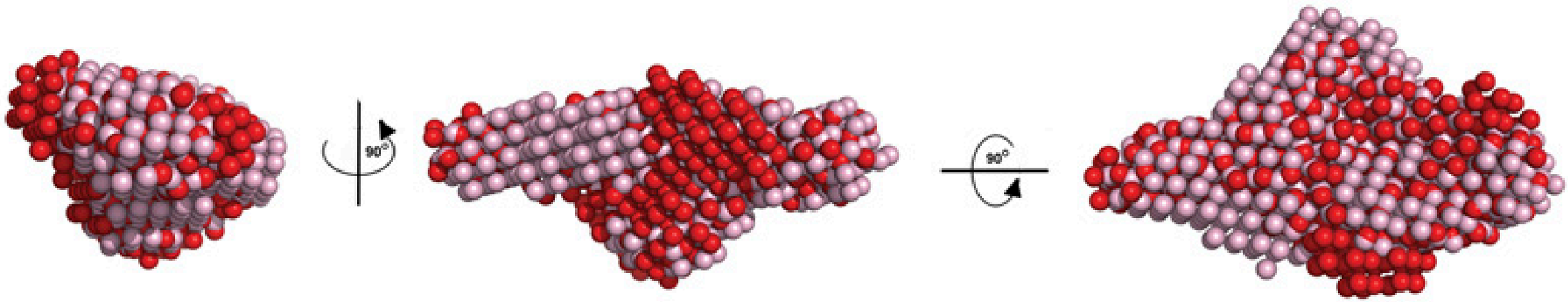
Deaconescu *et al.*, *PNAS* (2012) 109 (9): 3353-3358

ATP•TRCF Simulations are Robust and Reproducible

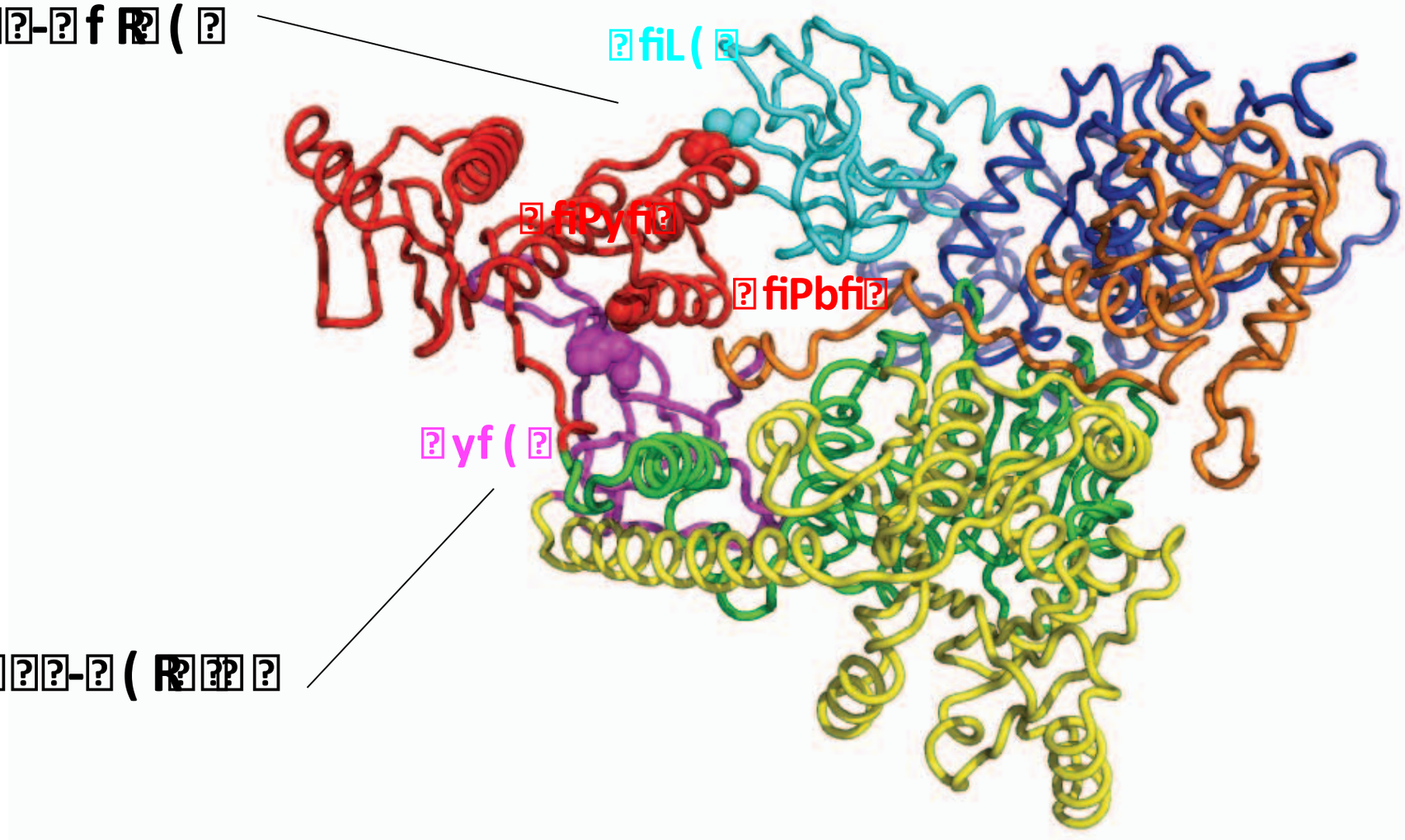
- Use the PDB of apo-TRCF as starting model (input) to seed the simulation process

Ab initio simulation

PDB-seeded simulation

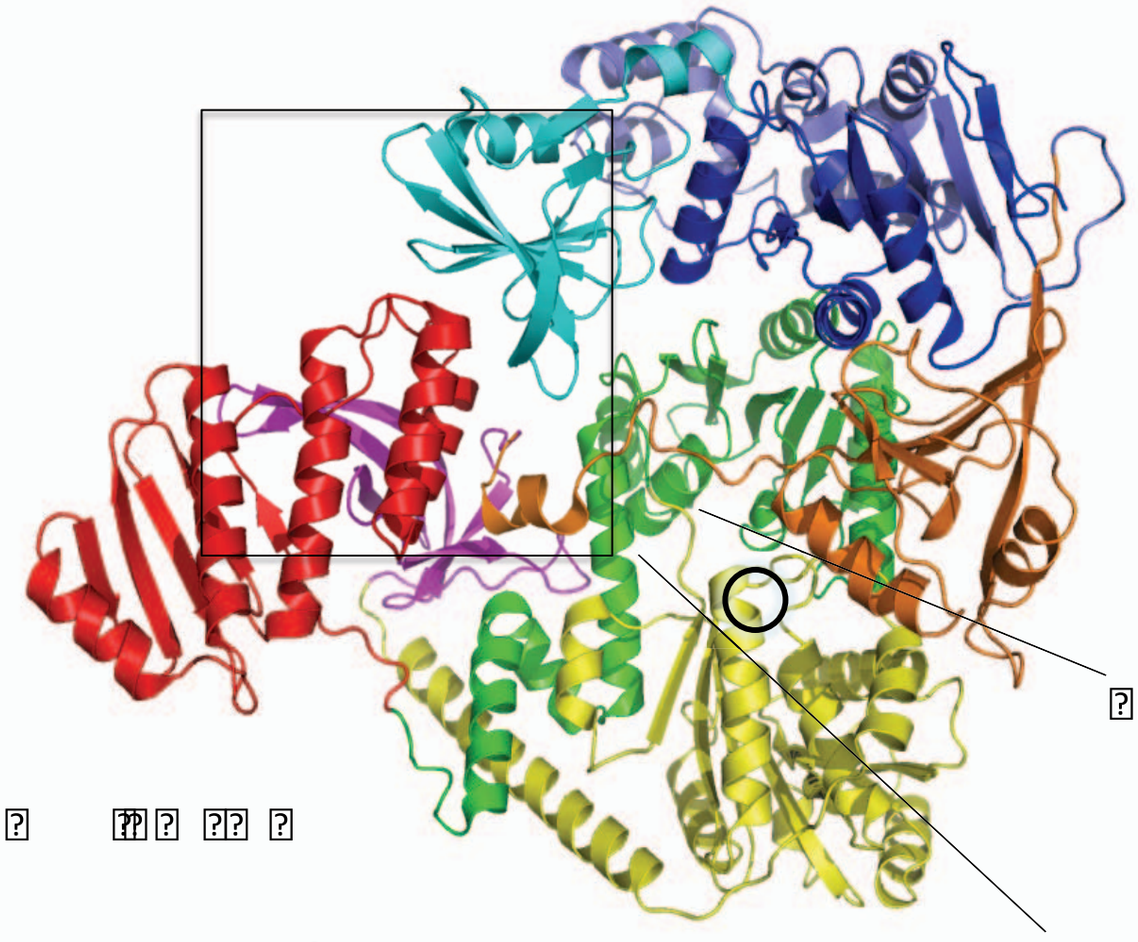


Probing Clamp Opening Using Interdomain Disulfide Engineering



TRCF Variant	Crosslinking w/ ATP	Crosslinking w/ UvrA
TRCF-D7:RID (open, more extended)	+++	+++
TRCF-D2:D7 (closed, more compact)	---	---

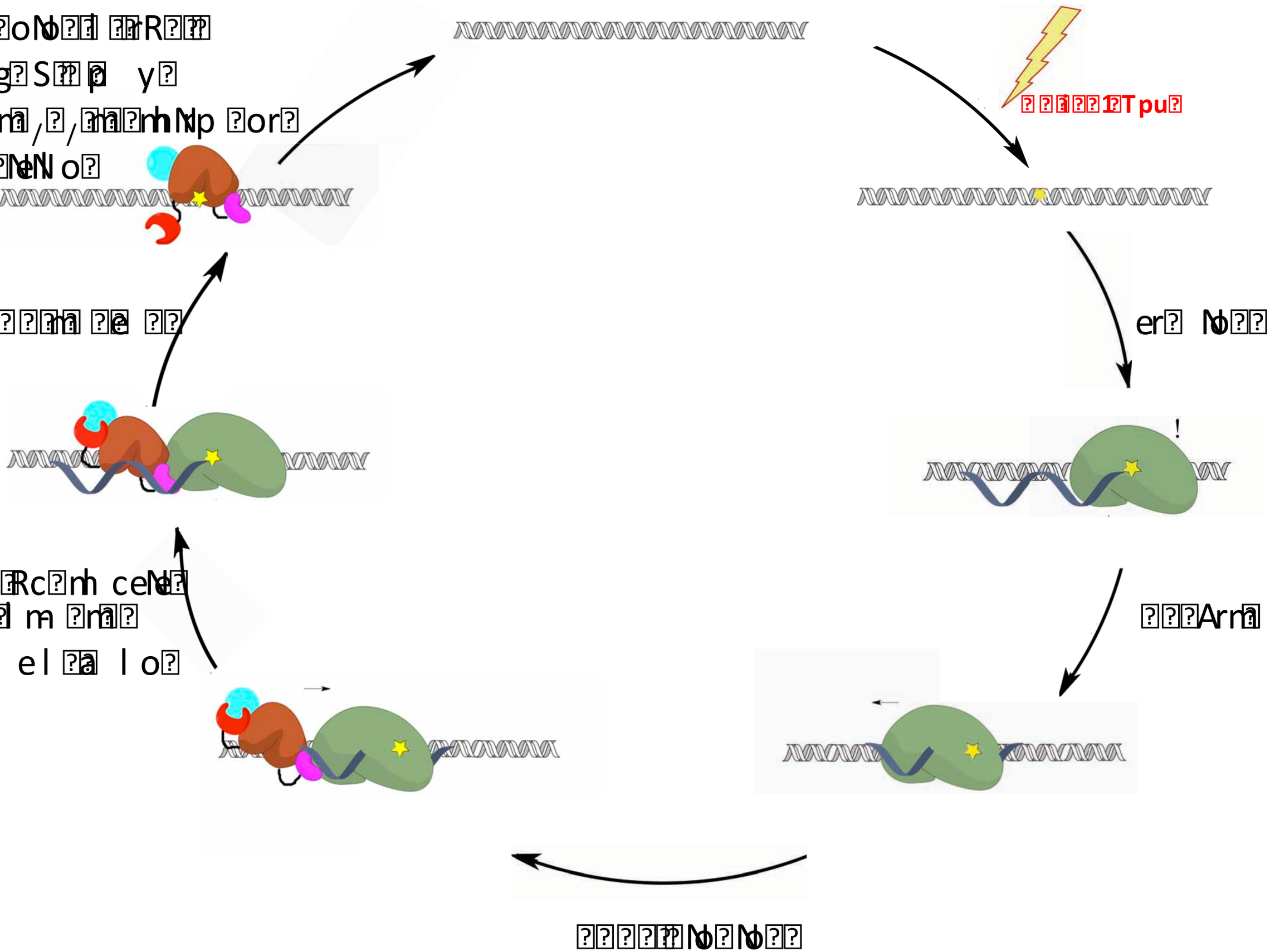
The D2/D7 Clamp Is Ideally Positioned to Restrain the TRCF Motor and Provide Spatiotemporal Control



E. coli TRCF

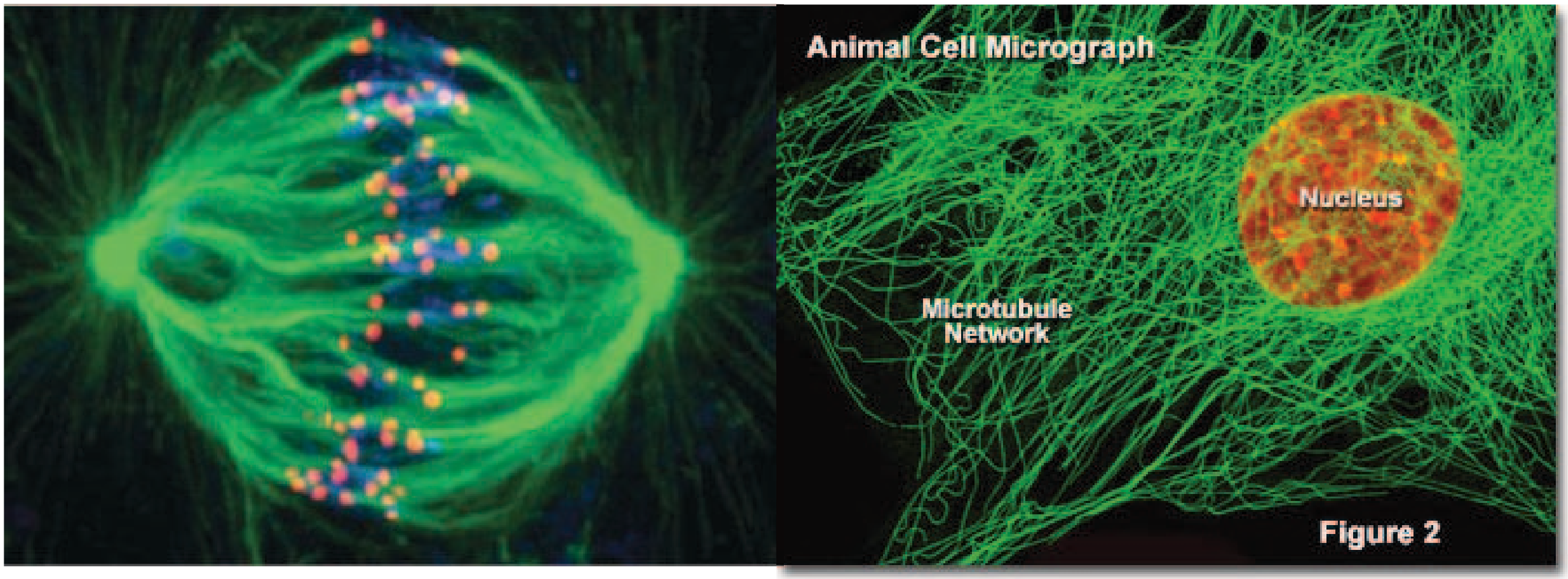
Prevents UvrA Binding
Compromises DNA binding
Impairs ATP turnover (in the absence of RNAP)
Still releases RNAP off templates due to stimulation by the RNAP elongation complex →

UvrA may be recruited after release of RNAP/transcript from the elongation complex



Post-translational modification of tubulin by a tubulin tyrosine ligase (TTL)

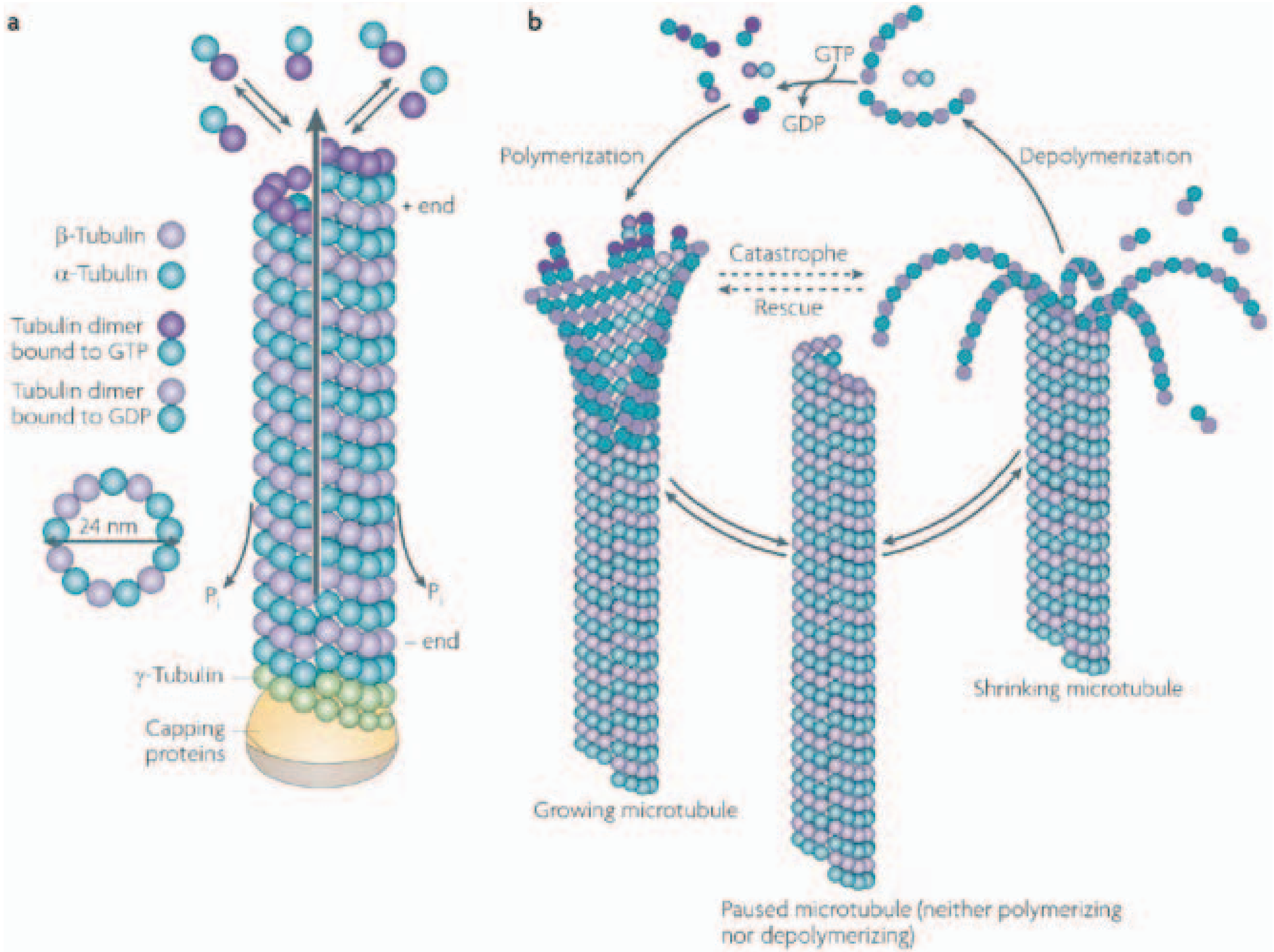
(a collaboration with the Roll-Mecak Lab, NIH)



http://schulmanlab.jhu.edu/research.html

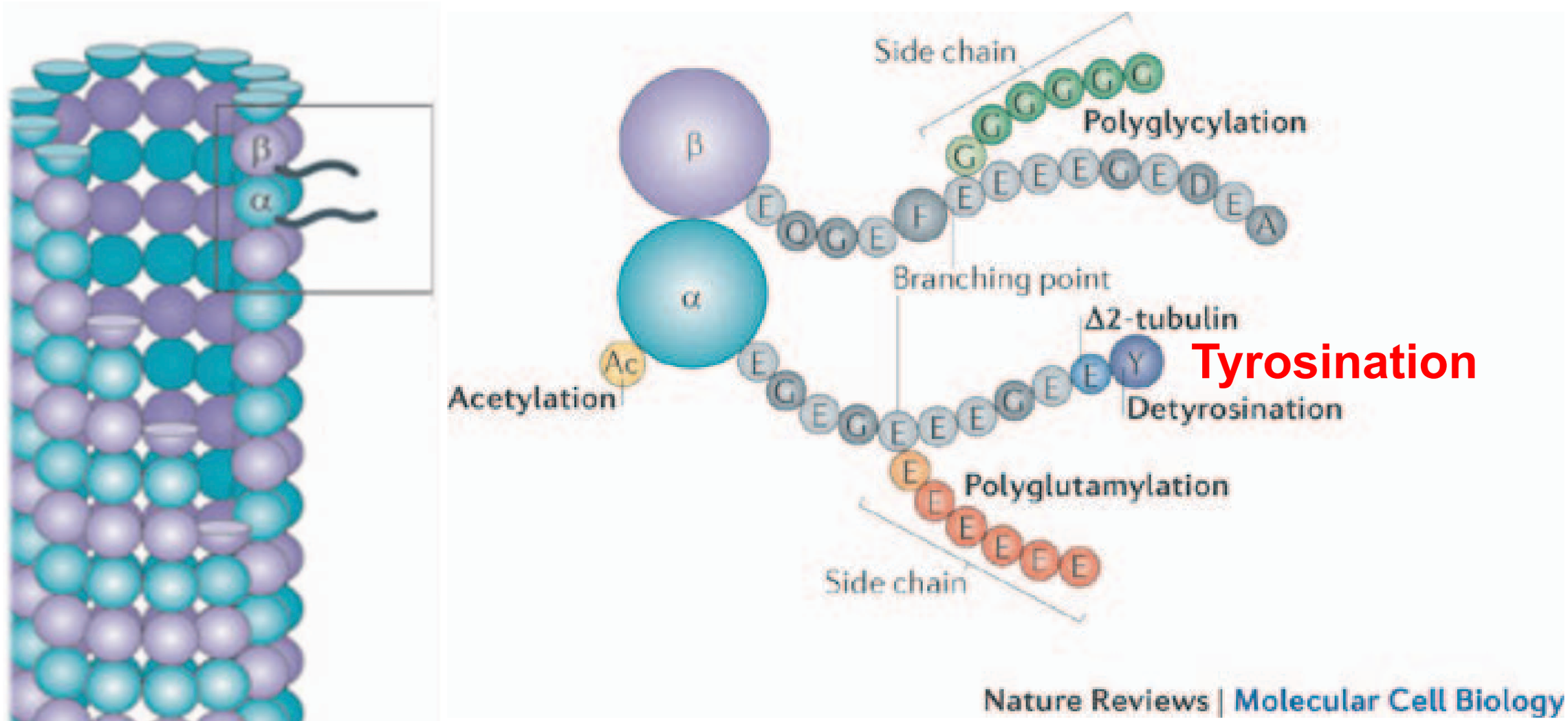
http://micro.magnet.fsu.edu/cells/microtubules/microtubules.html

Microtubules are Dynamic



Conde & Caceres (2009) Nat Rev Neuroscience

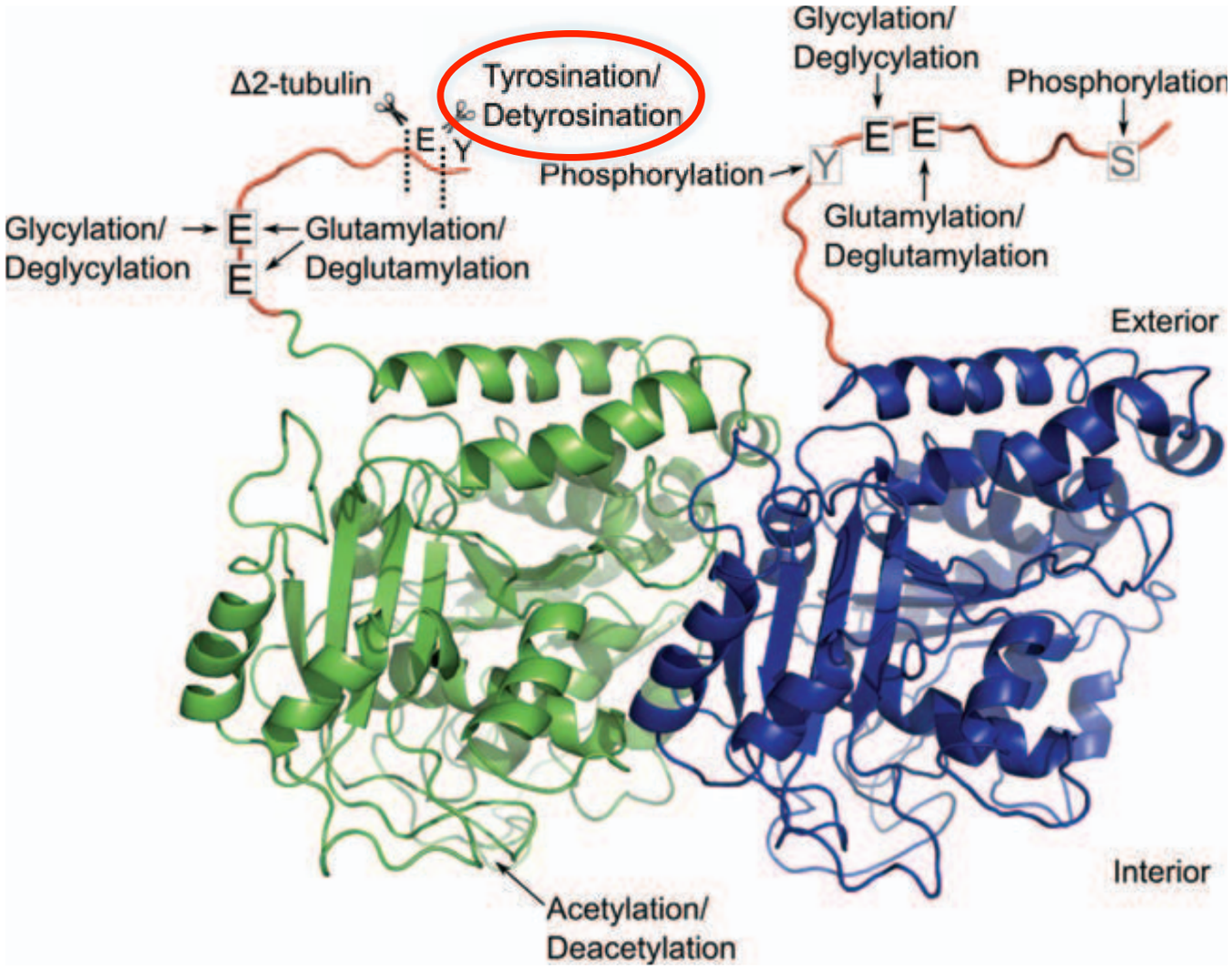
Microtubules are Heterogeneous



Carsten Janke & Jeannette Chloë Bulinski

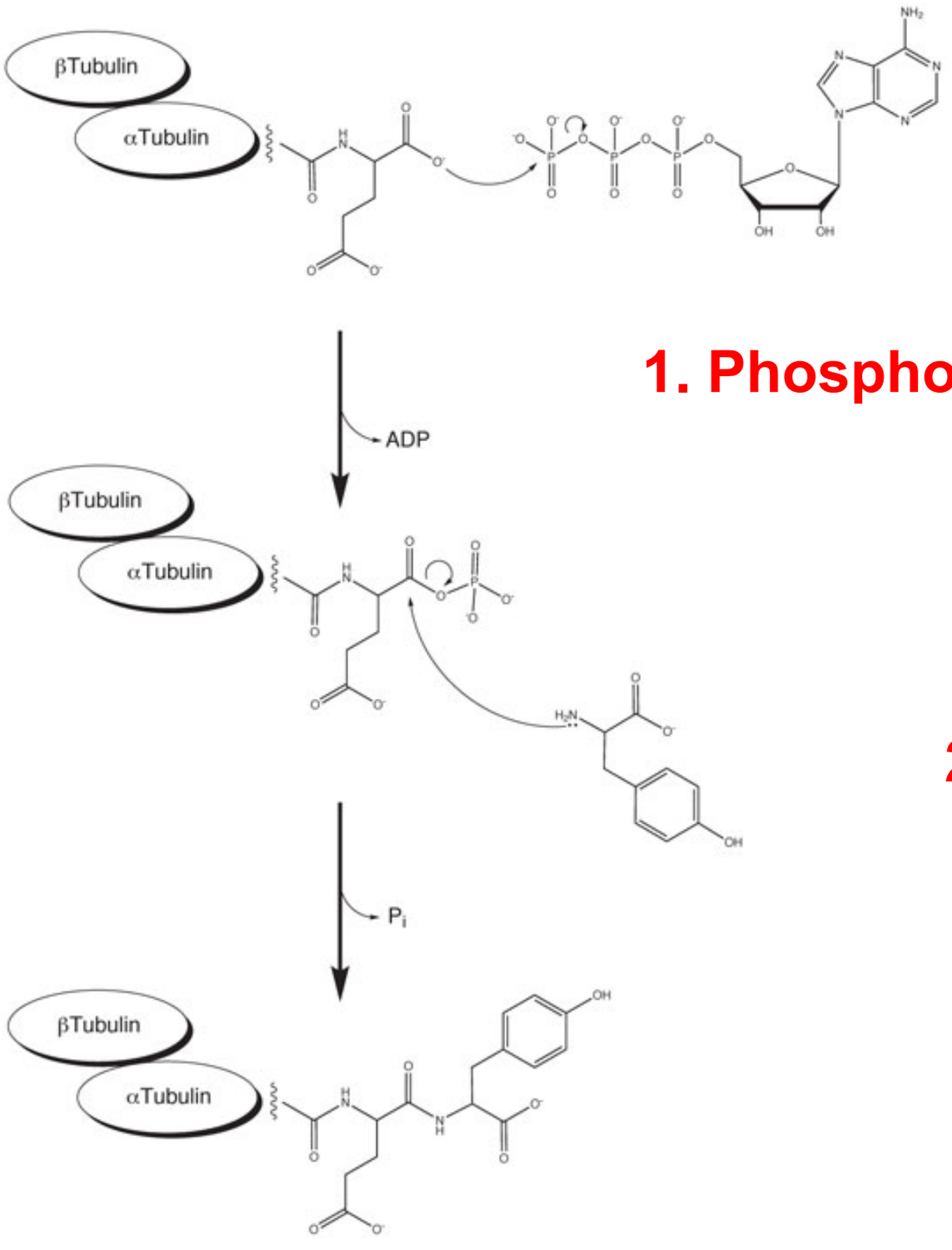
Nature Reviews Molecular Cell Biology (2010) 12, 773-786

Tubulin Tyrosine Ligase (TTL) Modifies the α-Tubulin Tail



Garnham & Roll-Mecak. Cytoskeleton (2012) Volume 69, Issue 7, pages 442-463

A Putative Mechanism for Tyrosination

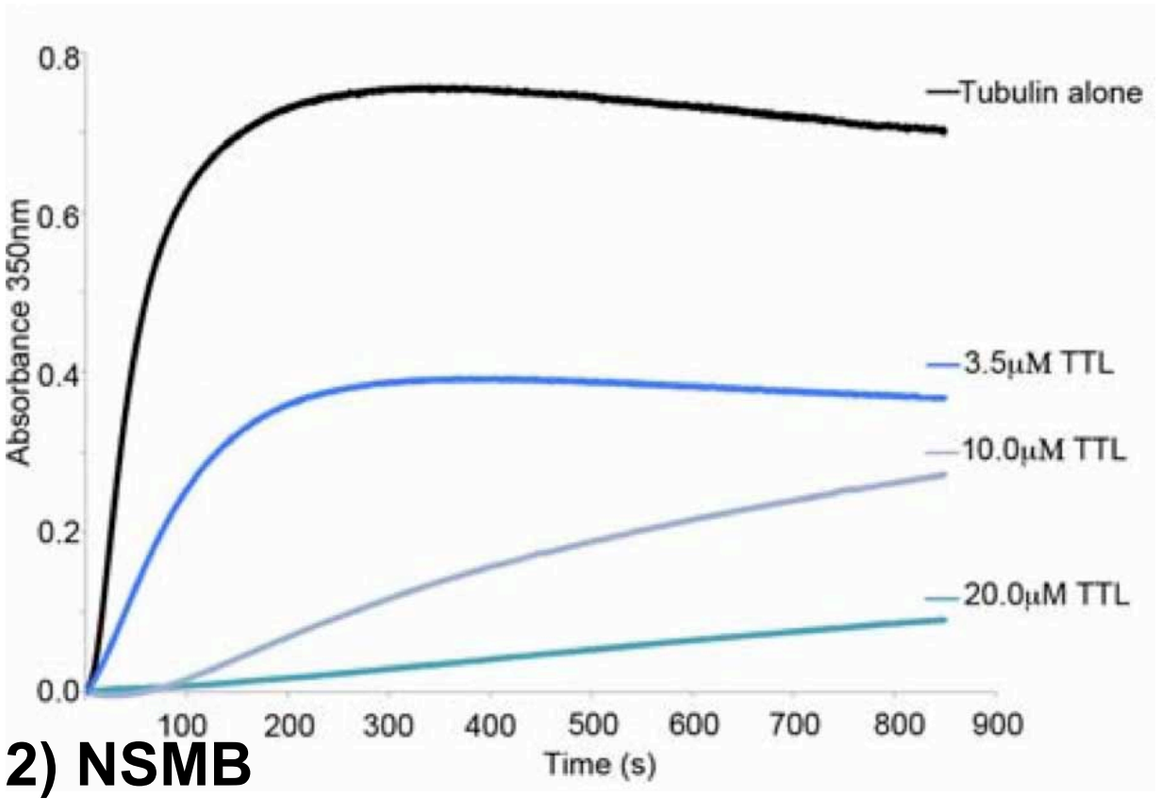


1. Phosphorylation of the α-carboxylate

2. Nucleophilic attack on the phosphorylated carboxylate

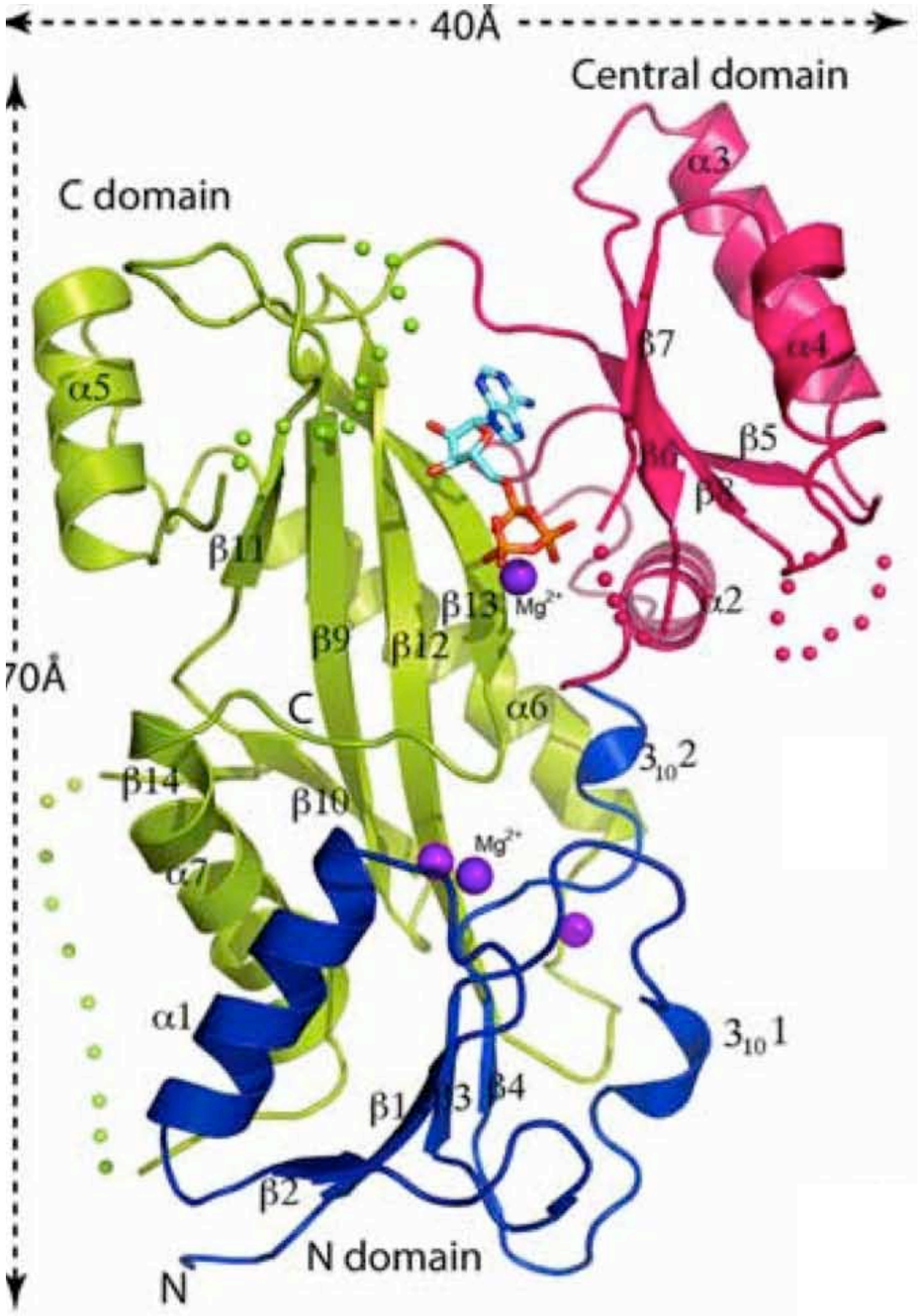
TTL-How Does It Work, and Why Is It Important?

- Important for organization of neural networks
- Microtubules in dendrites are enriched in Tyr tubulin
- TTL suppression leads to cancer
- TTL prefers to tyrosinate free tubulin heterodimer over tubulin that has been incorporated into a lattice (e.g. microtubules)
- TTL inhibits tubulin polymerization

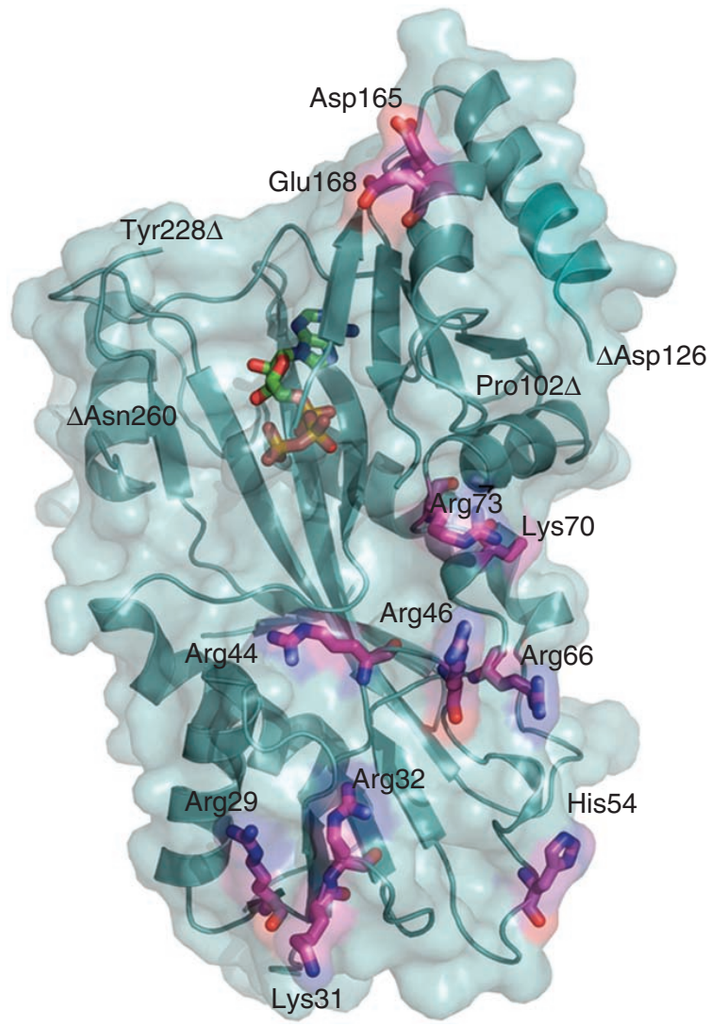


Szyk et al., (2012) NSMB

TTL Binds Tubulin Not only Through Its Tail



K_d (tubulin) ~ 1 μM
K_d (peptide) ~ 144 μM

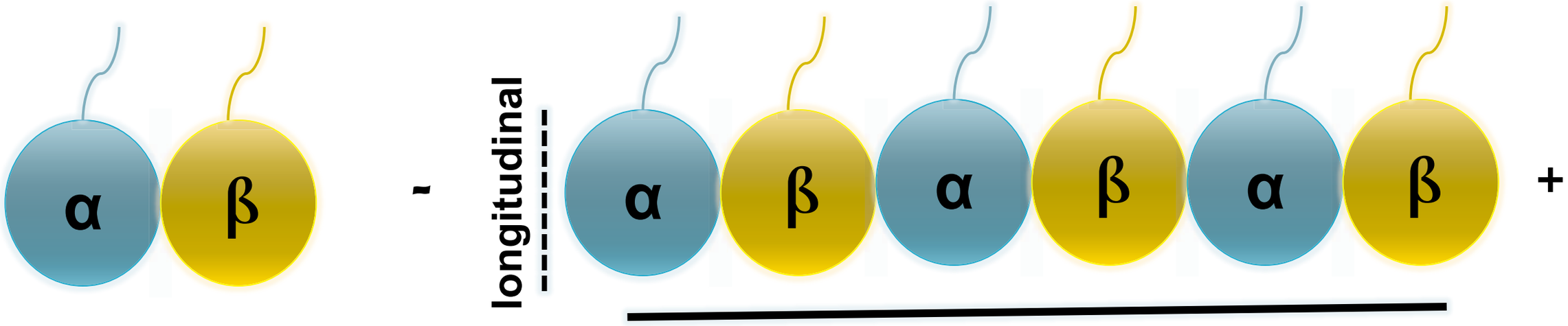


X. tropicalis TTL (Monomer)

Szyk et al., (2012) NSMB

What is the basis for this substrate specificity?

Heterodimer versus Polymer

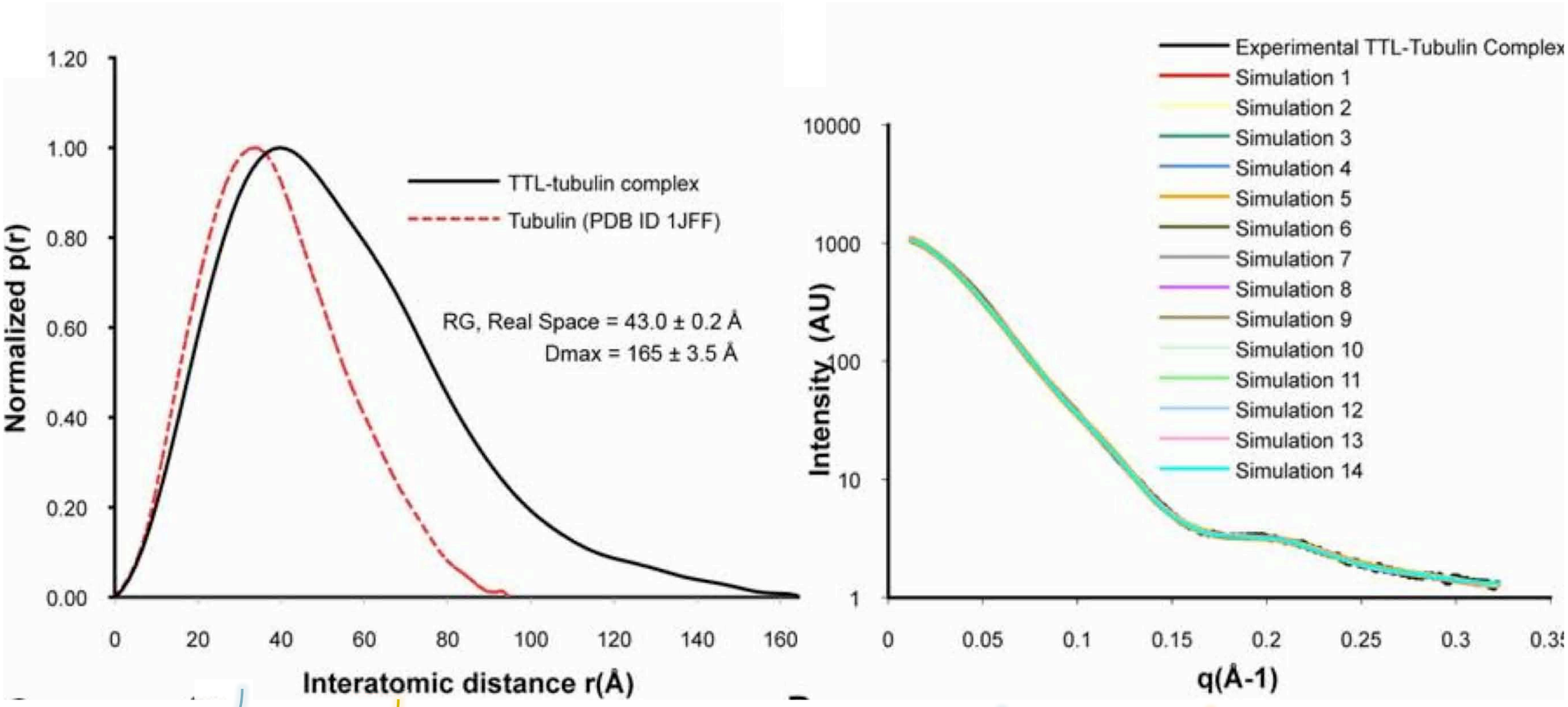


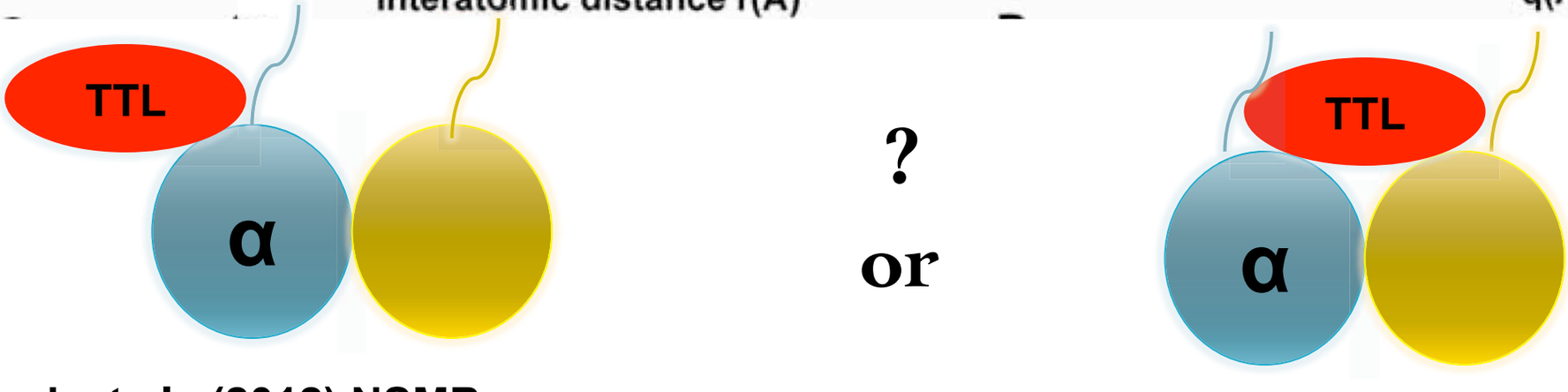
(lateral, between protofilaments)

Hypothesis

TTL binds to a surface that is buried in the polymeric tubulin form

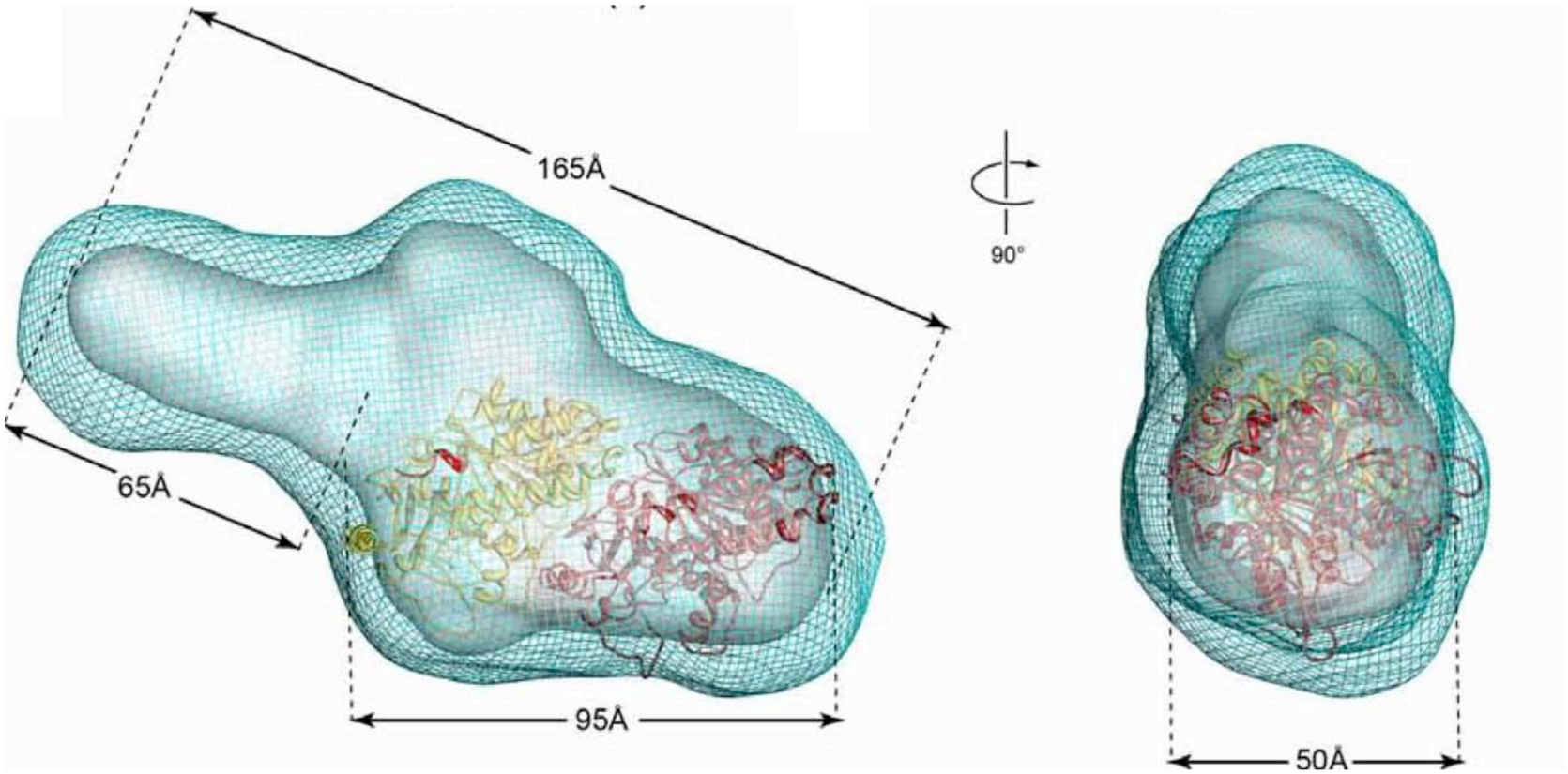
The TTL-Tubulin Complex is Elongated





Szyk et al., (2012) NSMB

The TTL Contacts Primarily α-Tubulin

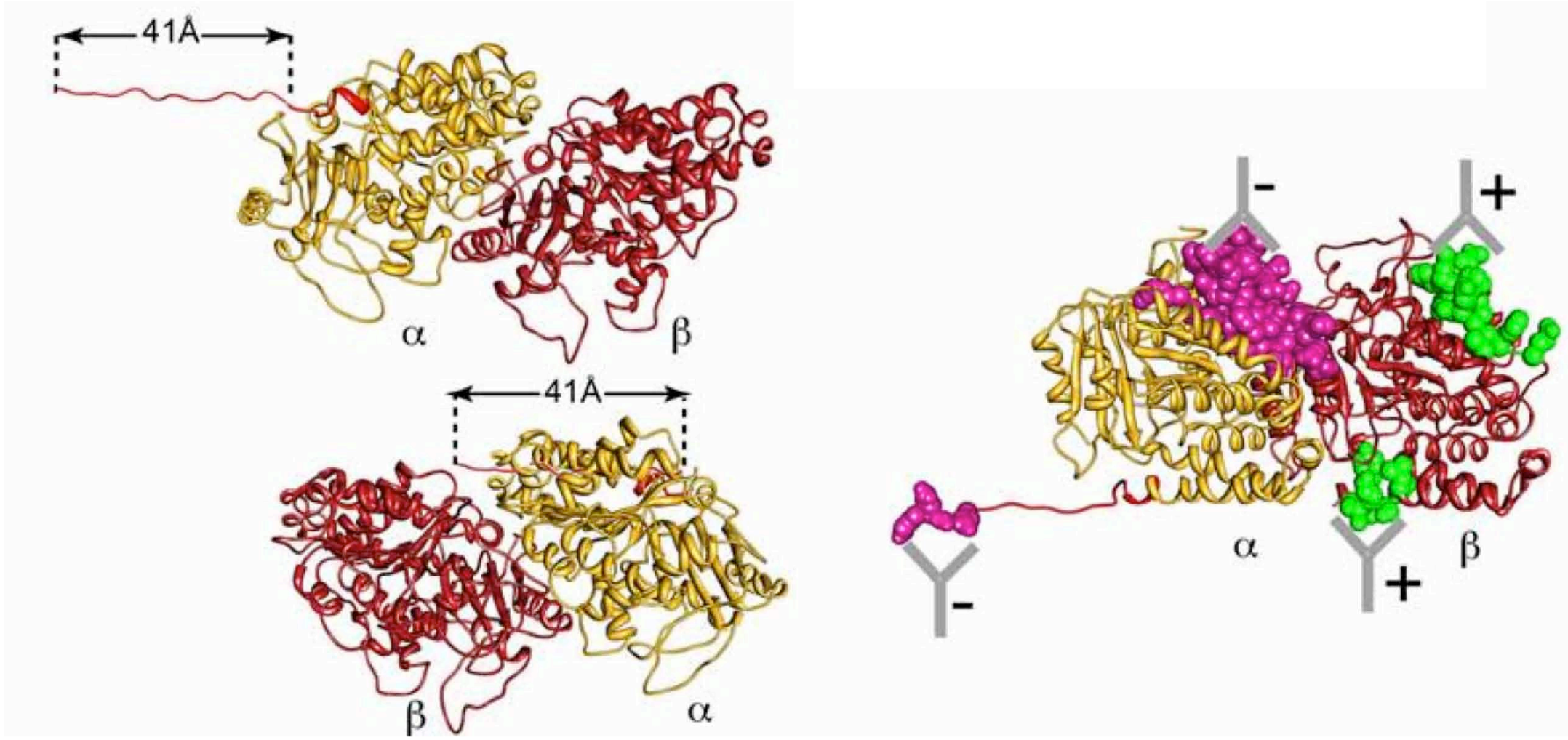


Is the tubulin heterodimer flipped?

- AUC confirmed complex formation and 1:1 stoichiometry
- From frictional ratio→ aspect ratio is 3.8 (versus 2.2 for tubulin) denoting an elongated structure

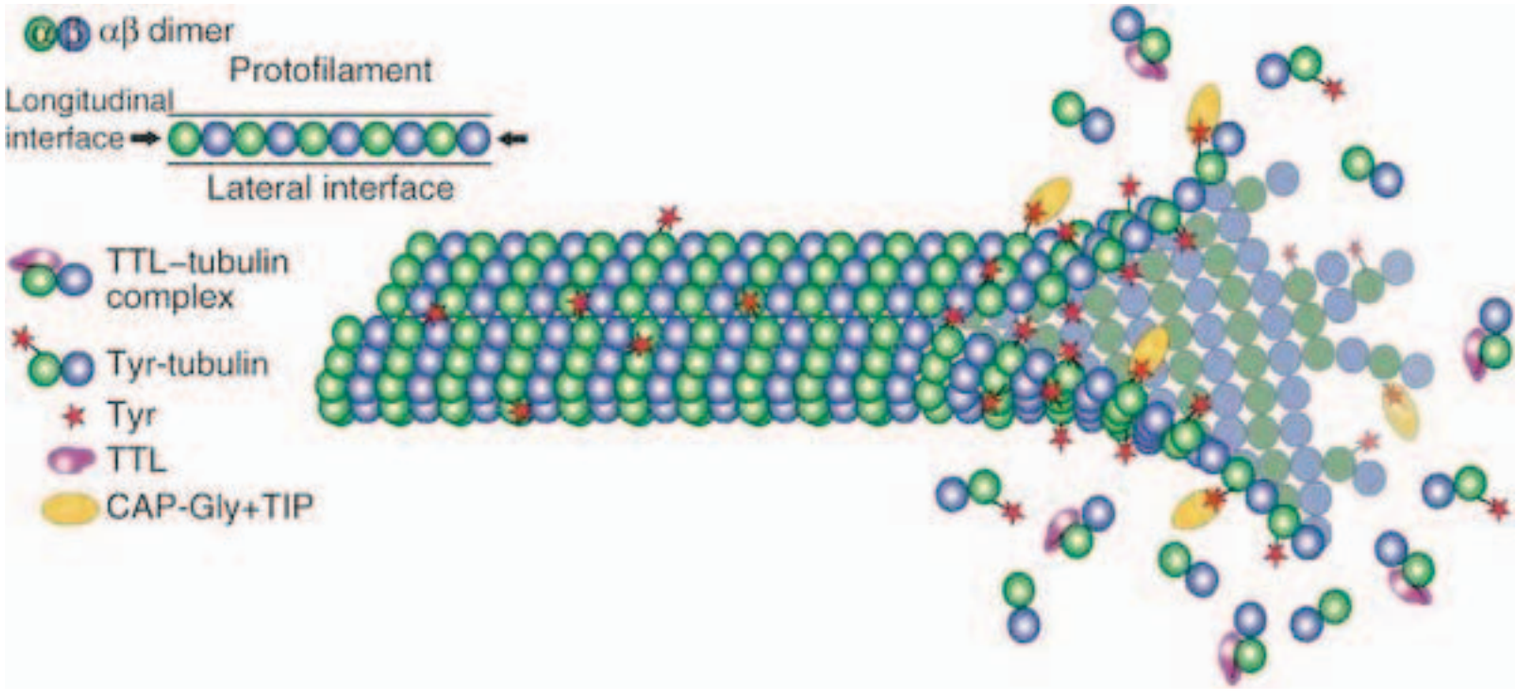
Szyk et al., (2012) NSMB

Antibodies against α-Tubulin Inhibit Tyrosination



Szyk et al., (2012) NSMB

TTL May Modulate the Partitioning of Heterdimeric versus Polymeric Tubulin and Tune Motors and End-Binding Proteins



Szyk et al., (2012) NSMB

Acknowledgements

The Grigorieff Lab (Brandeis University)

Dr. Niko Grigorieff

Drs. J. Gelles and J. Kondev

Collaborators

Artsimovitch Lab (Ohio State University)

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