A glimpse of computational methods in biological physics:

Case study on a ubiquitous protein

Jen Hsin
Theoretical and Computational Biophysics Group
April 14, 2010

- Introduction
  - Why am I here? And other administrative issues.
- What is ubiquitin?
  - Functions of ubiquitin
  - History of ubiquitin discoveries
  - More details on the ubiquitylation/protein degradation cycle
- Computational investigations of ubiquitin
  - Visualization of ubiquitin using VMD
  - What to look for in a protein?
  - Making proteins move: the molecular dynamics method
  - Simulations of ubiquitin
- Bibliography/Further readings

Administrative issues

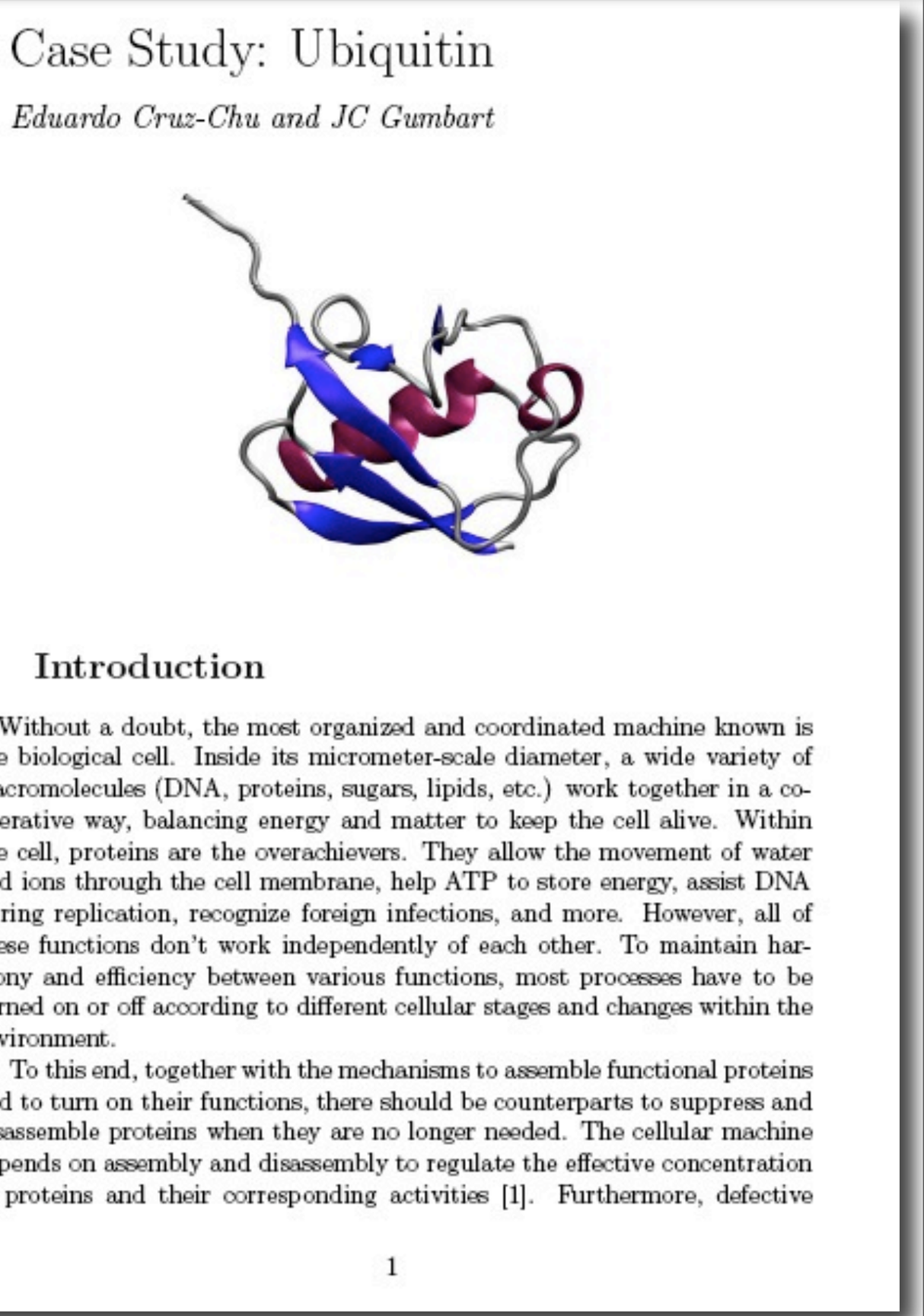
- The .pdf document of this lecture is available at http://www.ks.uiuc.edu/~jhsin/Lecture-04142010.pdf

- Assignment from this lecture: read the case study on ubiquitin and choose **FOUR** out of the six exercises to complete: http://www.ks.uiuc.edu/Training/CaseStudies/index.html#ubqcs

- You will have to download and install the software VMD: http://www.ks.uiuc.edu/Research/vmd/

- For any questions and assistance on the assignment, email jhsin@ks.uiuc.edu

- Assignments should be placed in the mailbox of **Yanxin Liu** on 2nd floor in Loomis by noon on April 19, 2010 (next Monday).



Case Study: Ubiquitin

Eduardo Cruz-Chu and JC Gumbart

1 Introduction

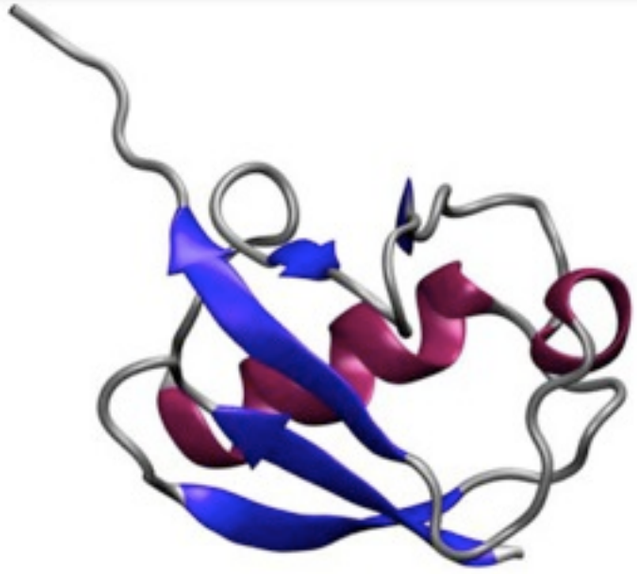
Without a doubt, the most organized and coordinated machine known is the biological cell. Inside its micrometer-scale diameter, a wide variety of macromolecules (DNA, proteins, sugars, lipids, etc.) work together in a cooperative way, balancing energy and matter to keep the cell alive. Within the cell, proteins are the overachievers. They allow the movement of water and ions through the cell membrane, help ATP to store energy, assist DNA during replication, recognize foreign infections, and more. However, all of these functions don't work independently of each other. To maintain harmony and efficiency between various functions, most processes have to be turned on or off according to different cellular stages and changes within the environment.

To this end, together with the mechanisms to assemble functional proteins and to turn on their functions, there should be counterparts to suppress and disassemble proteins when they are no longer needed. The cellular machine depends on assembly and disassembly to regulate the effective concentration of proteins and their corresponding activities [1]. Furthermore, defective

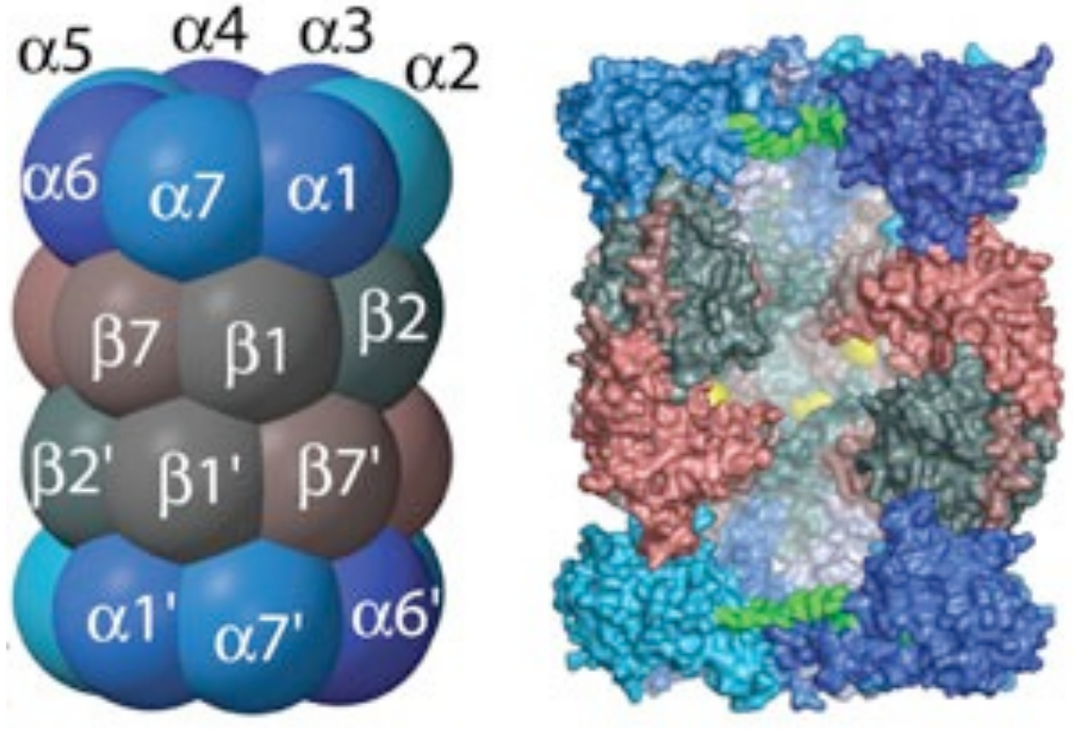
1

What is ubiquitin?

- A small protein expressed in all eukaryotes
- Small = 76 amino acids
- Ubiquitin functions as a “molecular flag”: it tags proteins that are to be destroyed
- Proteins tagged by ubiquitin are sent to proteasome, where the proteins are chopped up
- Later on, it was discovered that ubiquitin does more than protein degradation, a lot more!



A representation of ubiquitin colored by secondary structure element



A representation of proteasome, not drawn to scale compared to the ubiquitin above, as a proteasome is ~250 times bigger than a ubiquitin!

What is ubiquitin?

ubiquitin =
molecular “kiss of death”?

- A small protein expressed in all eukaryotes
- Small = 76 amino acids
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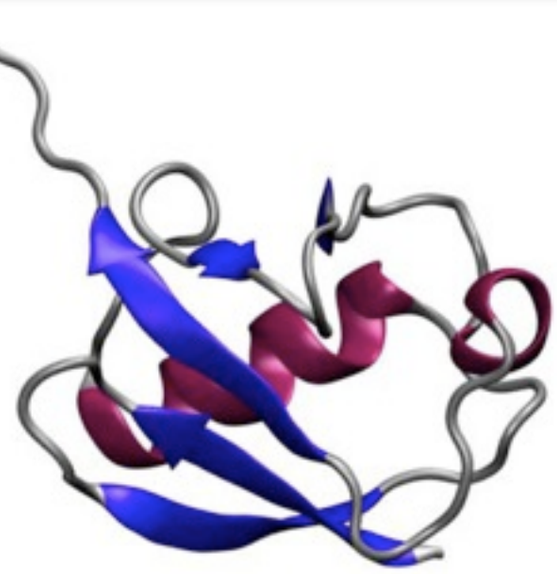
proteasome =
cellular garbage bin?



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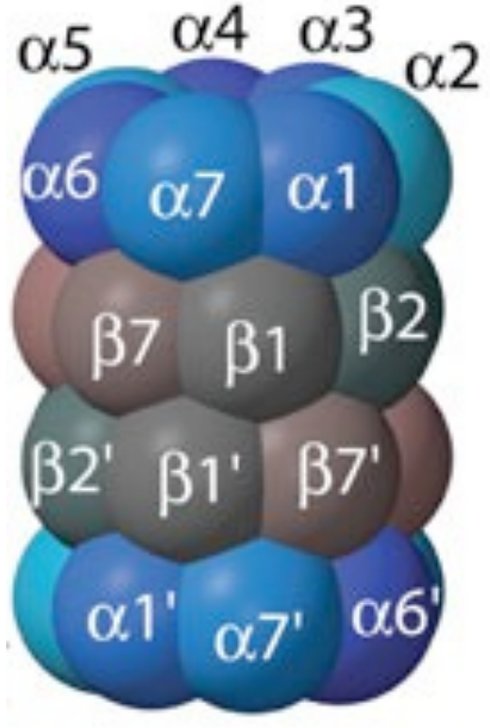
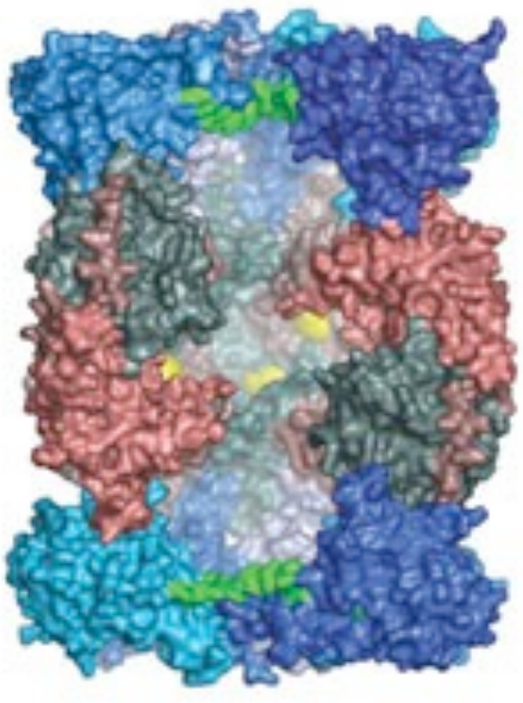
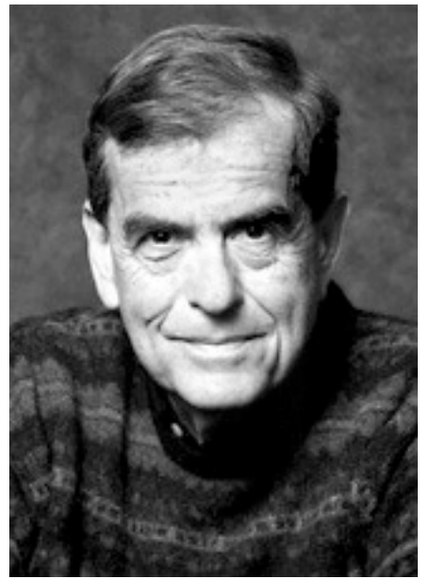


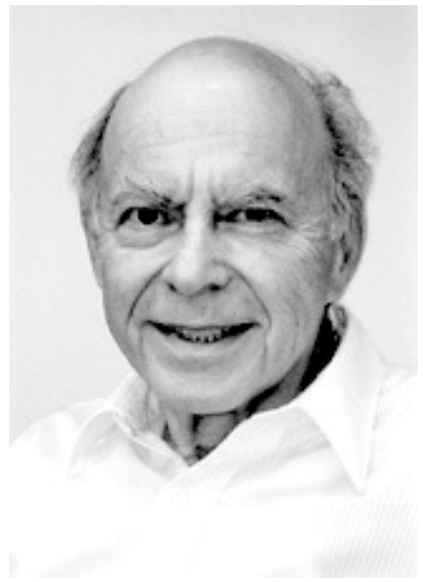


A representation of proteasome, not drawn to scale compared to the ubiquitin above, as a proteasome is ~250 times bigger than an ubiquitin!

History of ubiquitin discoveries

- Before 1980s, proteins were thought to be long-lived
- 1978-1983, it was discovered that some proteins can be conjugated to ubiquitin, and then destroyed later
- 1983, three types of enzymes (E1, E2, E3) were found to aid the process of ubiquitin-protein conjugation
- 1984-1990, the function of ubiquitin was established as a molecular flag that signals degradation
- 1989, other ubiquitin functions were identified that have nothing to do with protein degradation
- 1989, ubiquitin chain linked through Lysine 48 was discovered to be essential (polyubiquitin chain)
- 2004, three scientists were awarded the Nobel Prize in Chemistry for ubiquitin-related discoveries.
- 2006, various bacterial proteins were found to be similar to ubiquitin

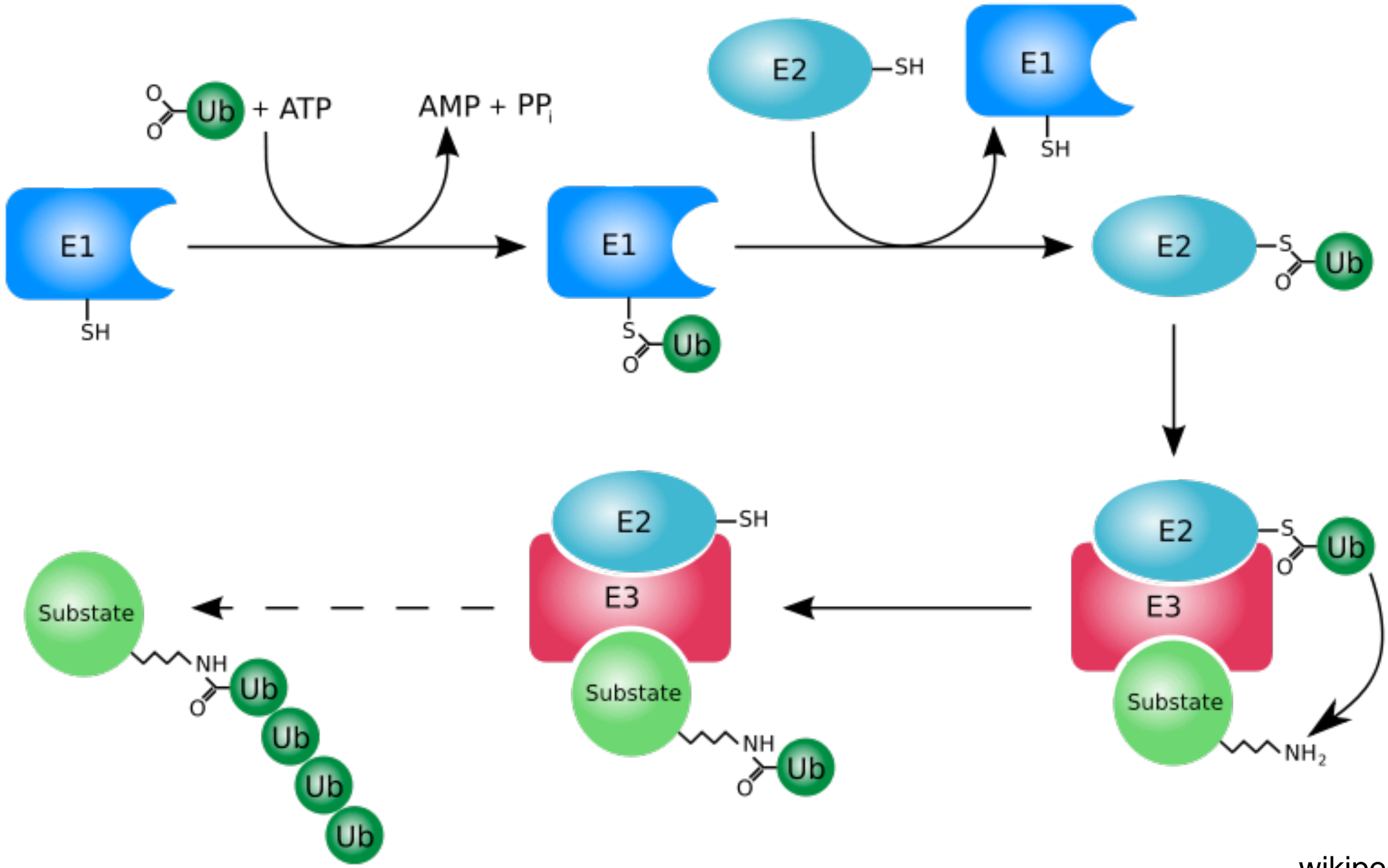




The Nobel Prize in Chemistry was awarded to A. Ciechanover, A. Hershko, and I. Rose in 2004 for their “discovery of ubiquitin-mediated protein degradation”

Details on ubiquitin's role in protein degradation

- The protein degradation cycle has the following key players: **ubiquitin**, enzymes **E1**, **E2**, **E3**, the protein to be destroyed (**substrate**), **proteasome**, and some **energy** input



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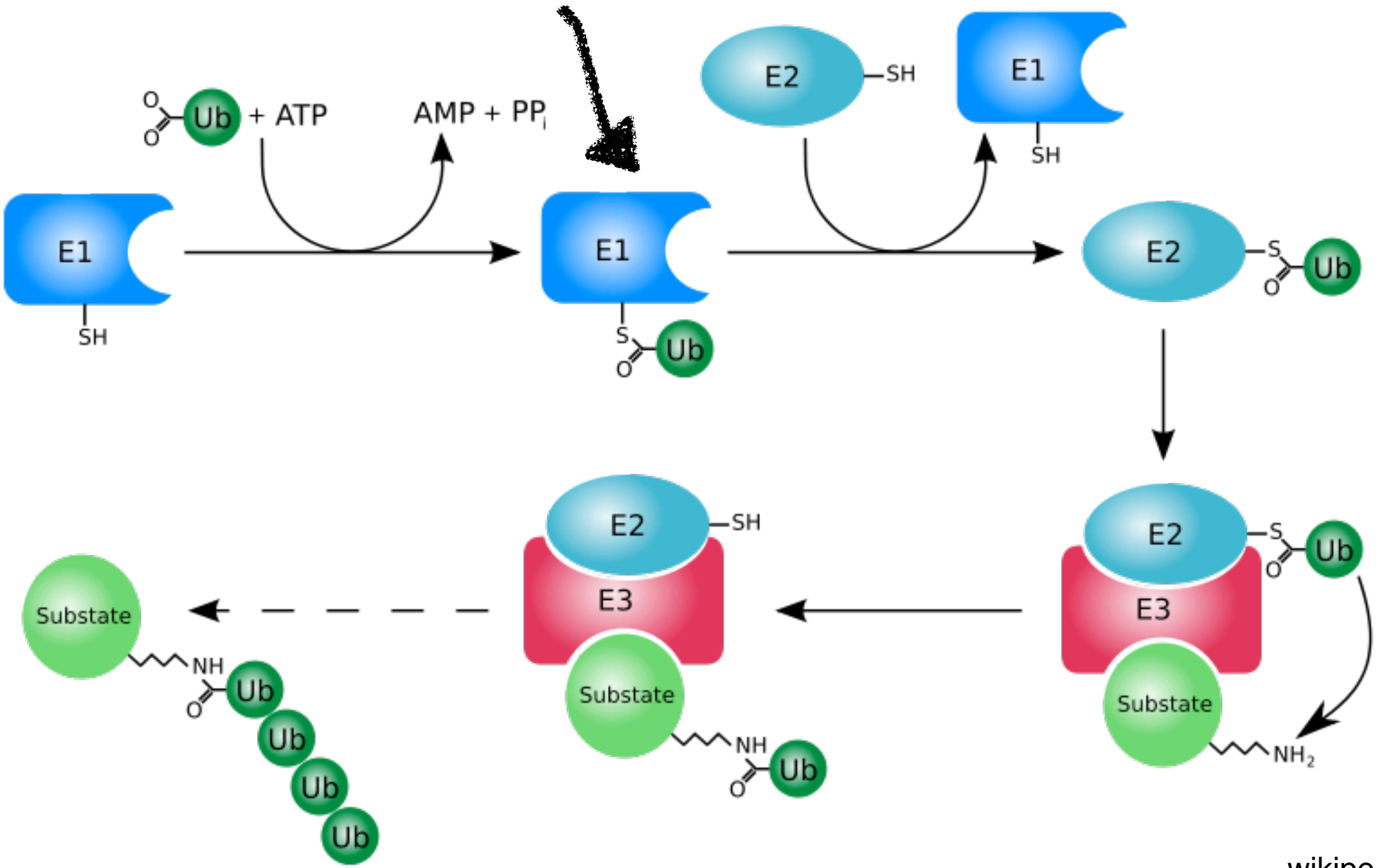
Details on ubiquitin's role in protein degradation

Step 1: E1 attaches to ubiquitin following energy input



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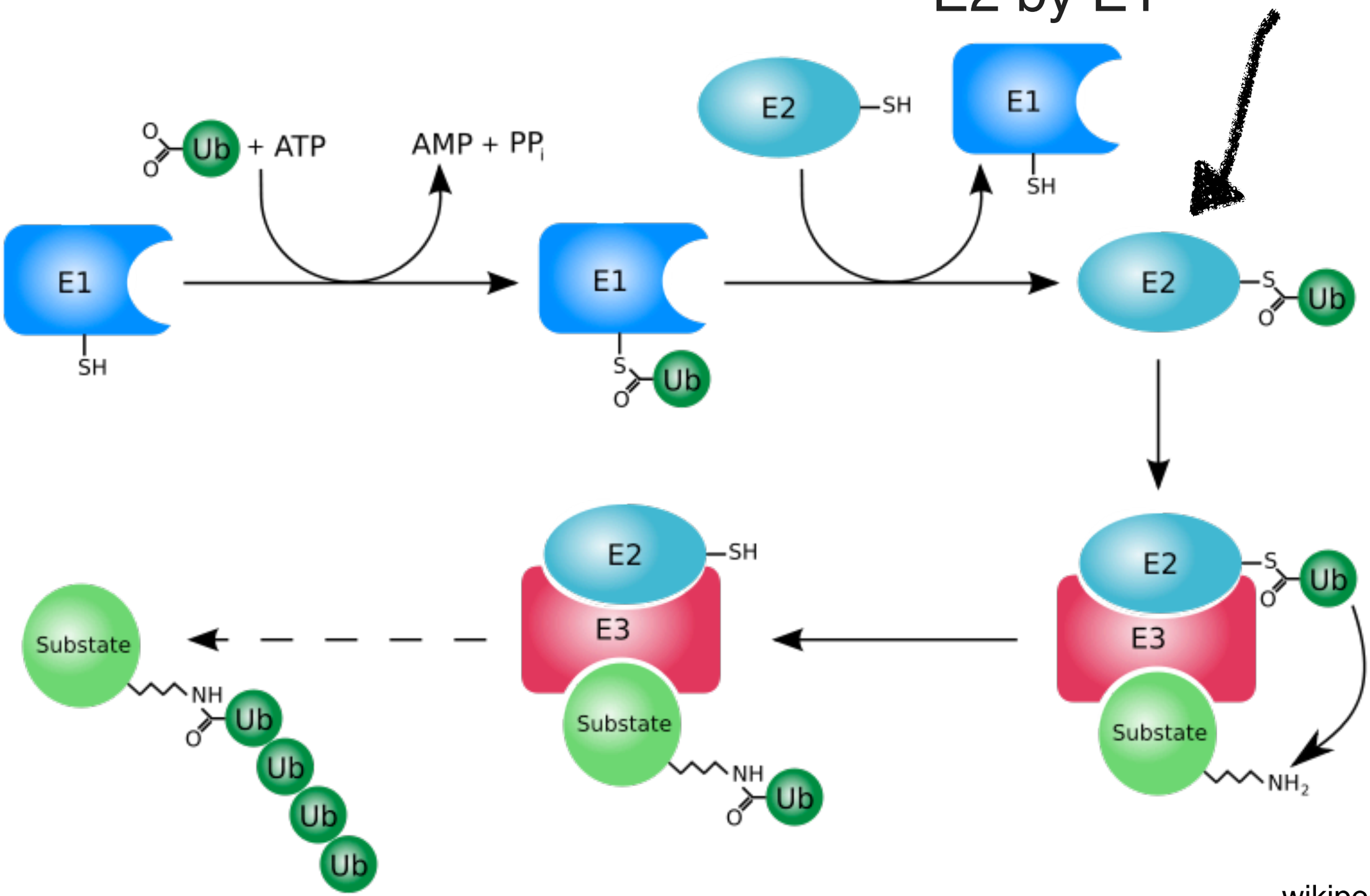
Details on ubiquitin's role in protein degradation

Step 2: ubiquitin is transferred to E2 by E1



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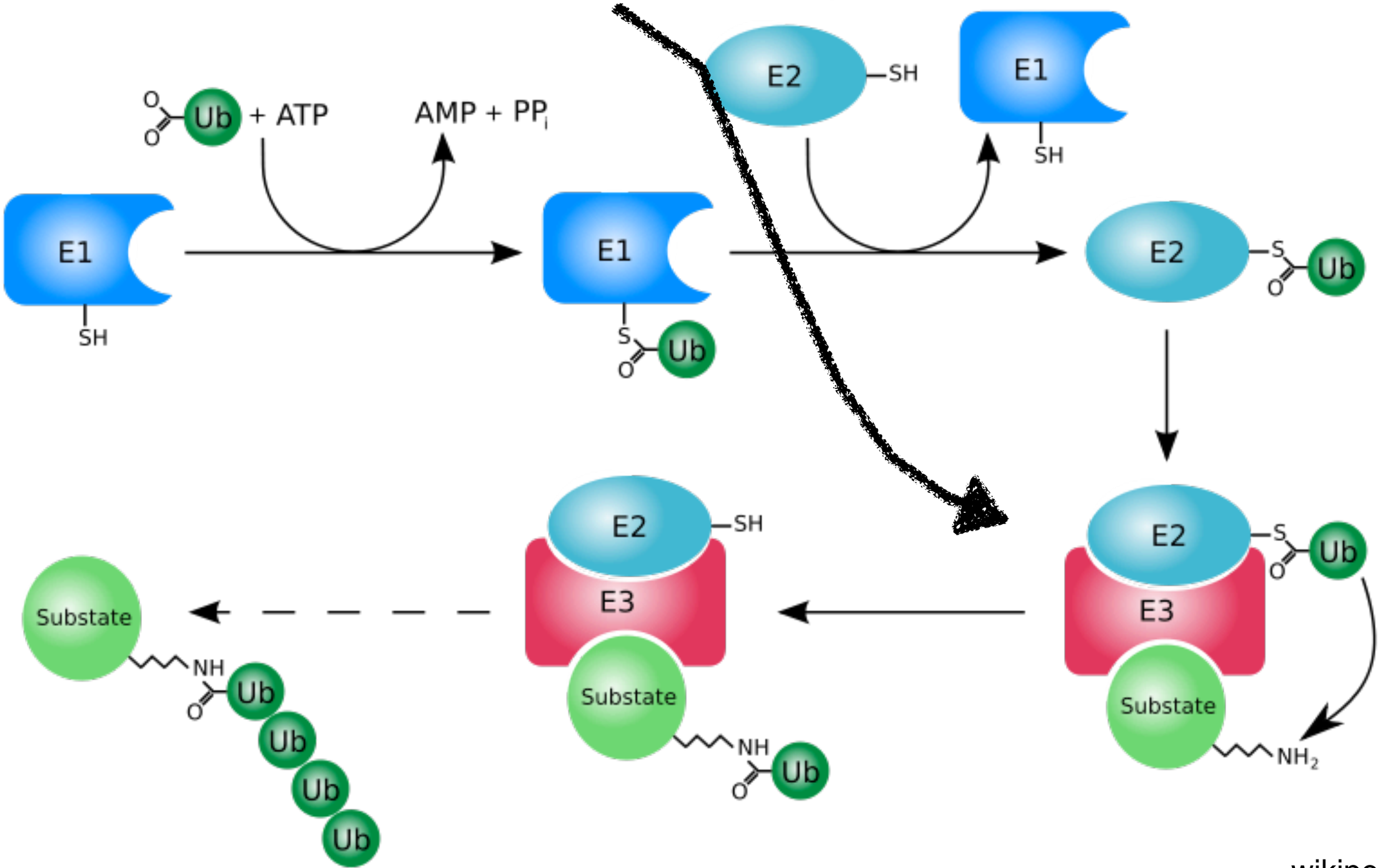
Details on ubiquitin's role in protein degradation

Step 3: E2 takes ubiquitin to E3, which is bound to the substrate already



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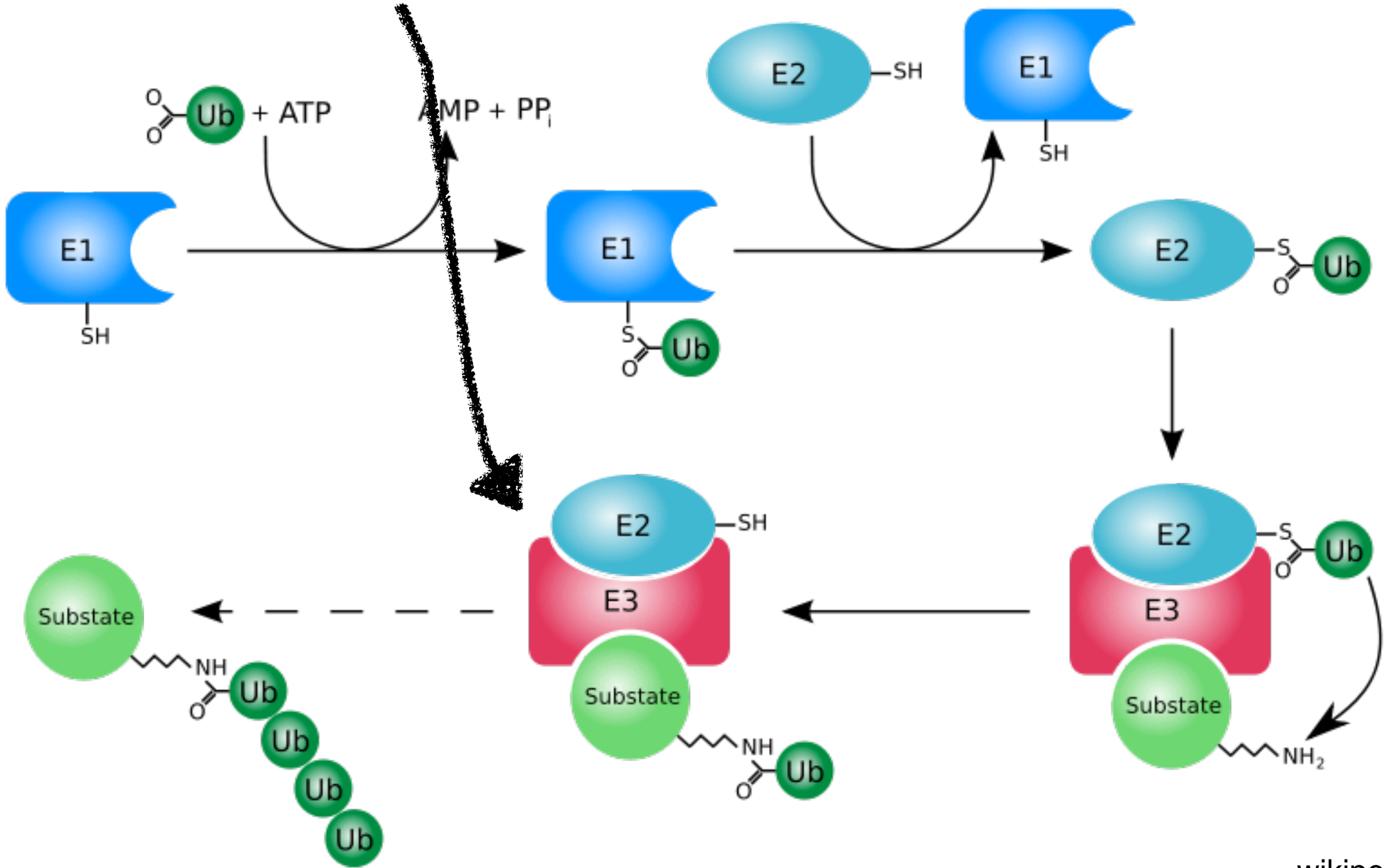
Details on ubiquitin's role in protein degradation

Step 4: ubiquitin is transferred to the substrate



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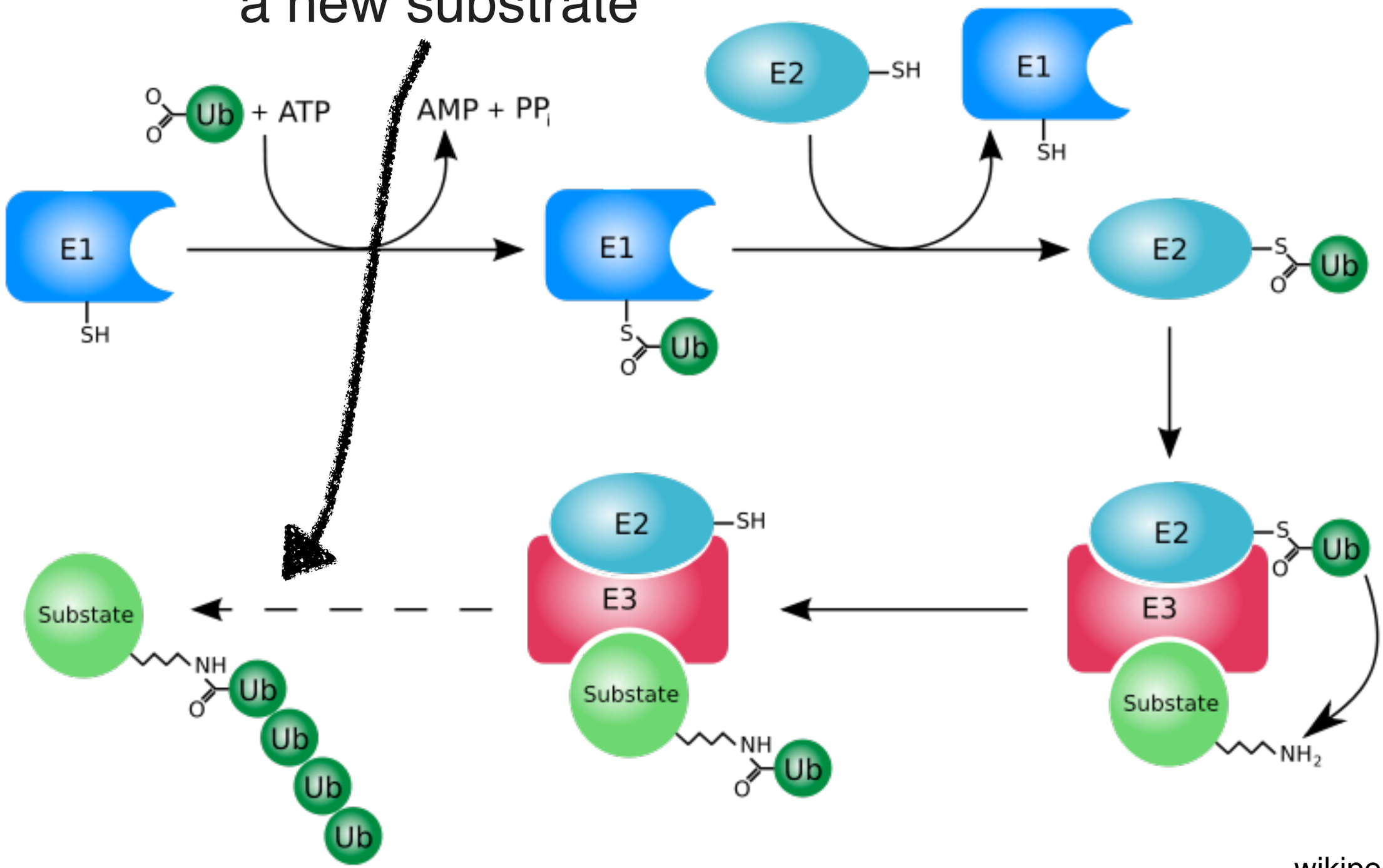
Details on ubiquitin's role in protein degradation

Step 5: repeat the previous steps, but treat the ubiquitin-substrate complex as a new substrate



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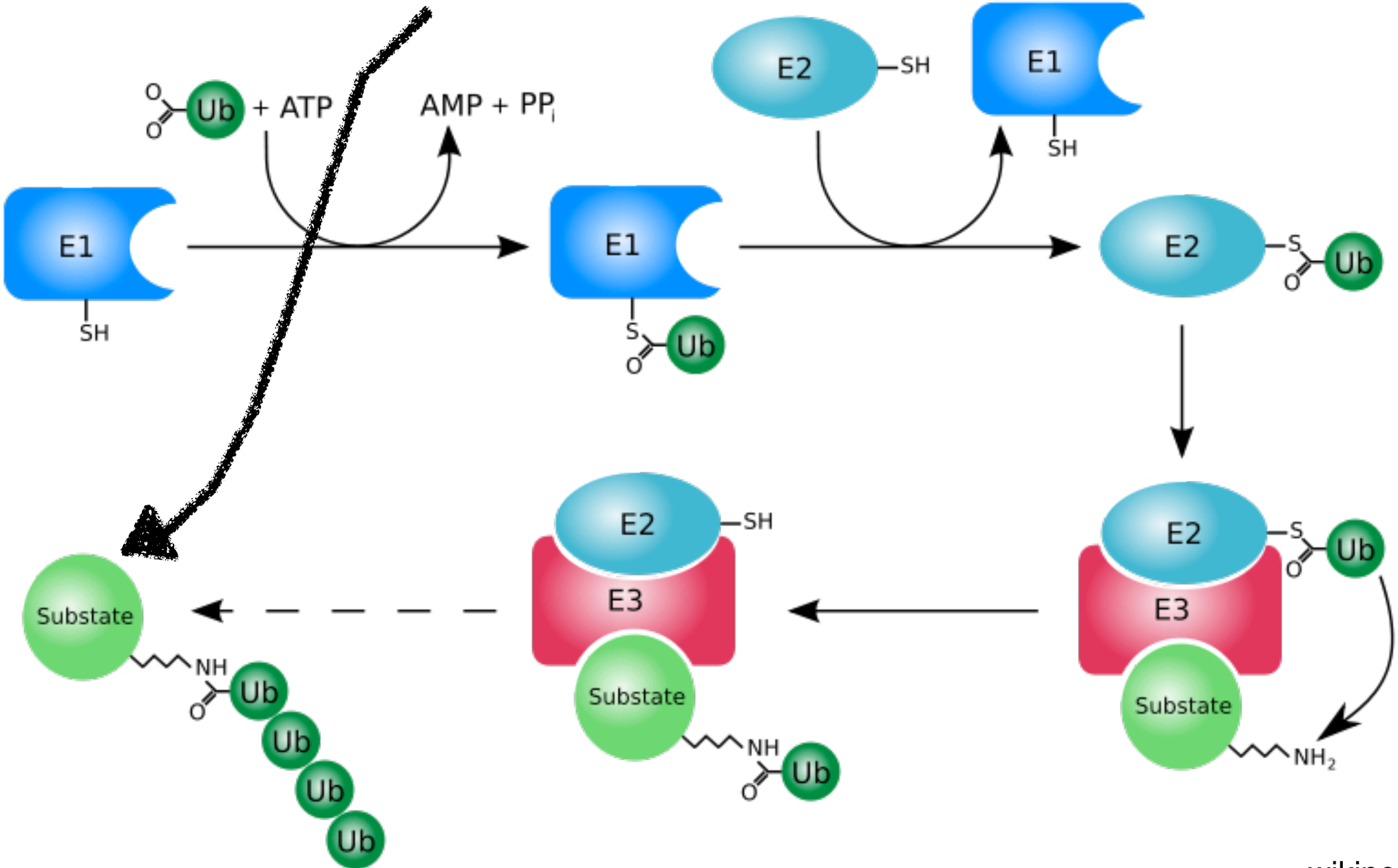
Details on ubiquitin's role in protein degradation

Get substrate tagged by chained ubiquitin



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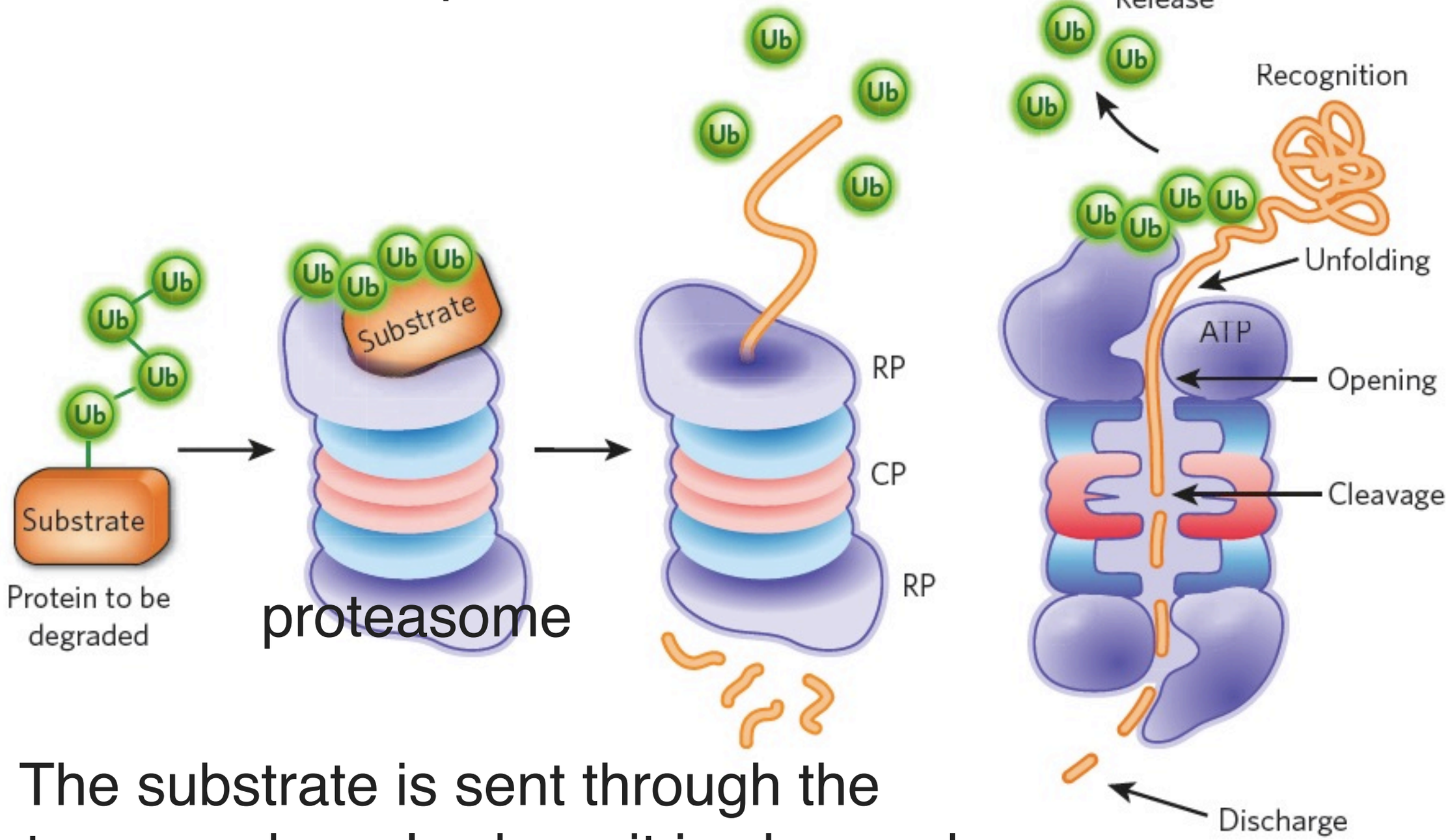
Details on ubiquitin's role in protein degradation

The substrate-polyubiquitin complex is then sent to proteasome

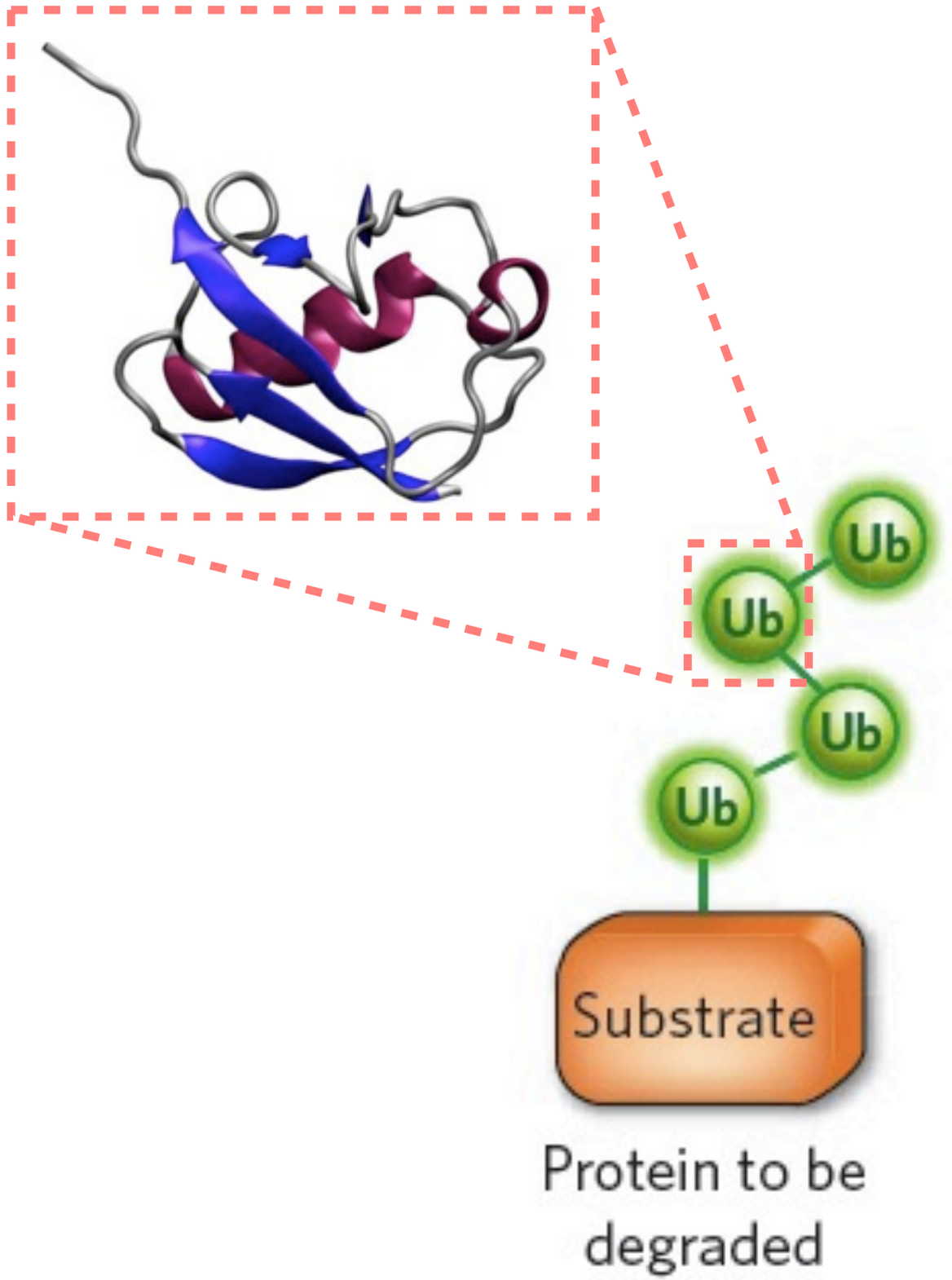


The substrate is sent through the proteasome barrel, where it is chopped up and recycled

M. Hochstrasser, *Nature*, 458. (2009)

Formation of polyubiquitin chain

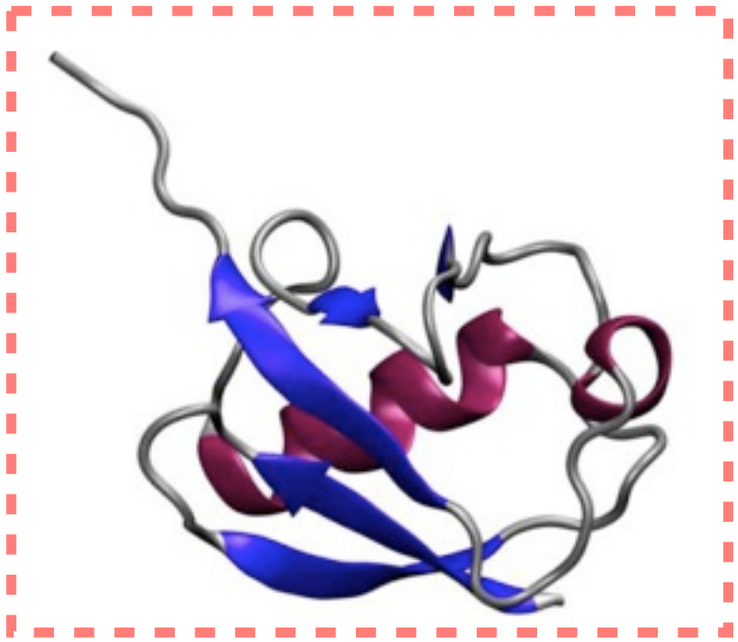
- You might have a few questions at this point
- How are the ubiquitins linked in a polyubiquitin chain?
- How does ubiquitin perform functions different than protein degradation?
- Maybe other questions?

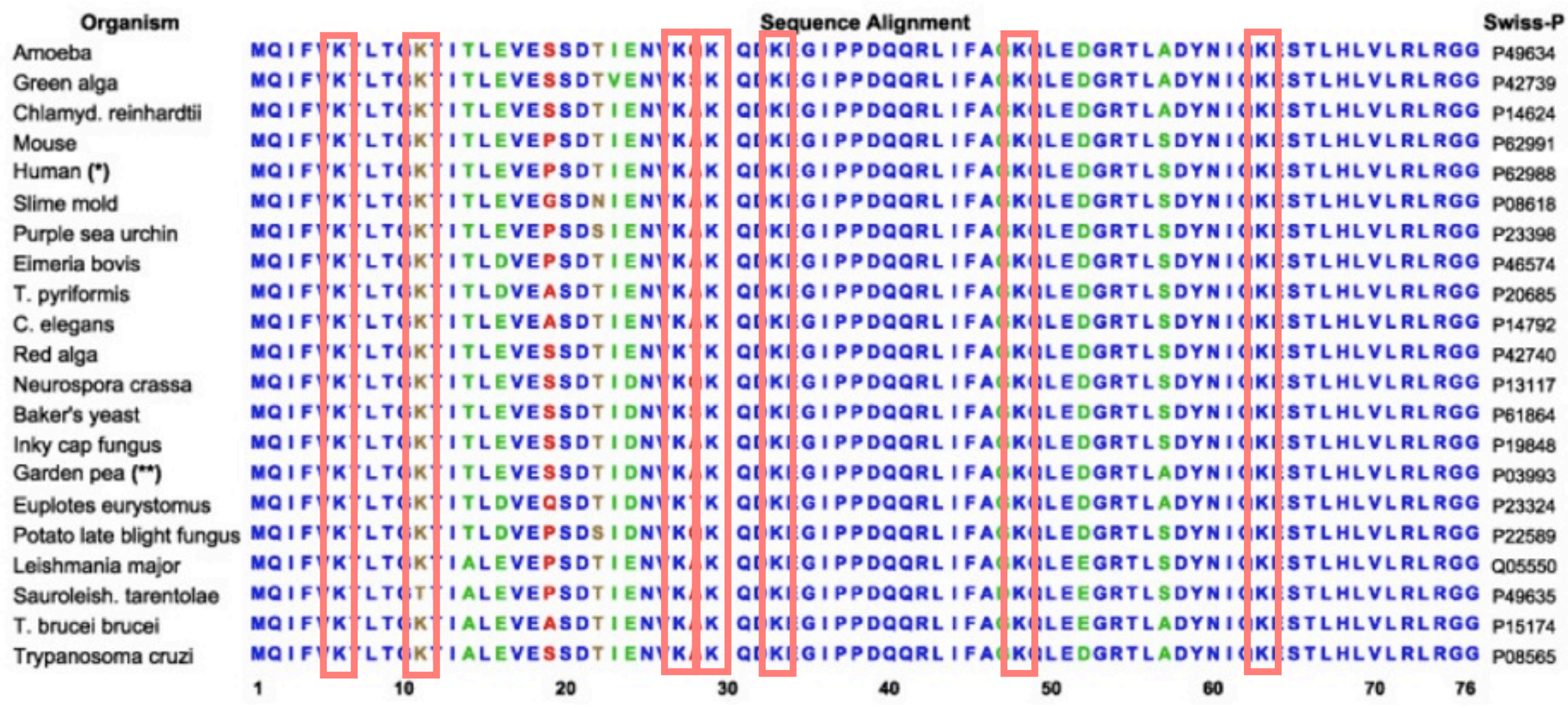


M. Hochstrasser, *Nature*, 458. (2009)

Formation of polyubiquitin chain

- The sequence of ubiquitin is highly conserved, in particular the seven lysine residues
- A lysine residue in a ubiquitin can be linked to the C-terminus of another ubiquitin
- By using different lysine for such linkage, ubiquitin is used for different cellular purposes





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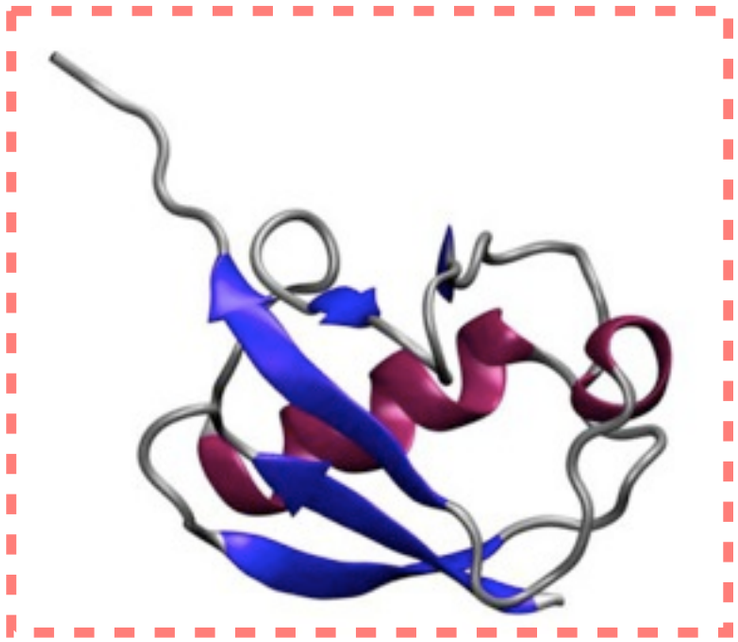
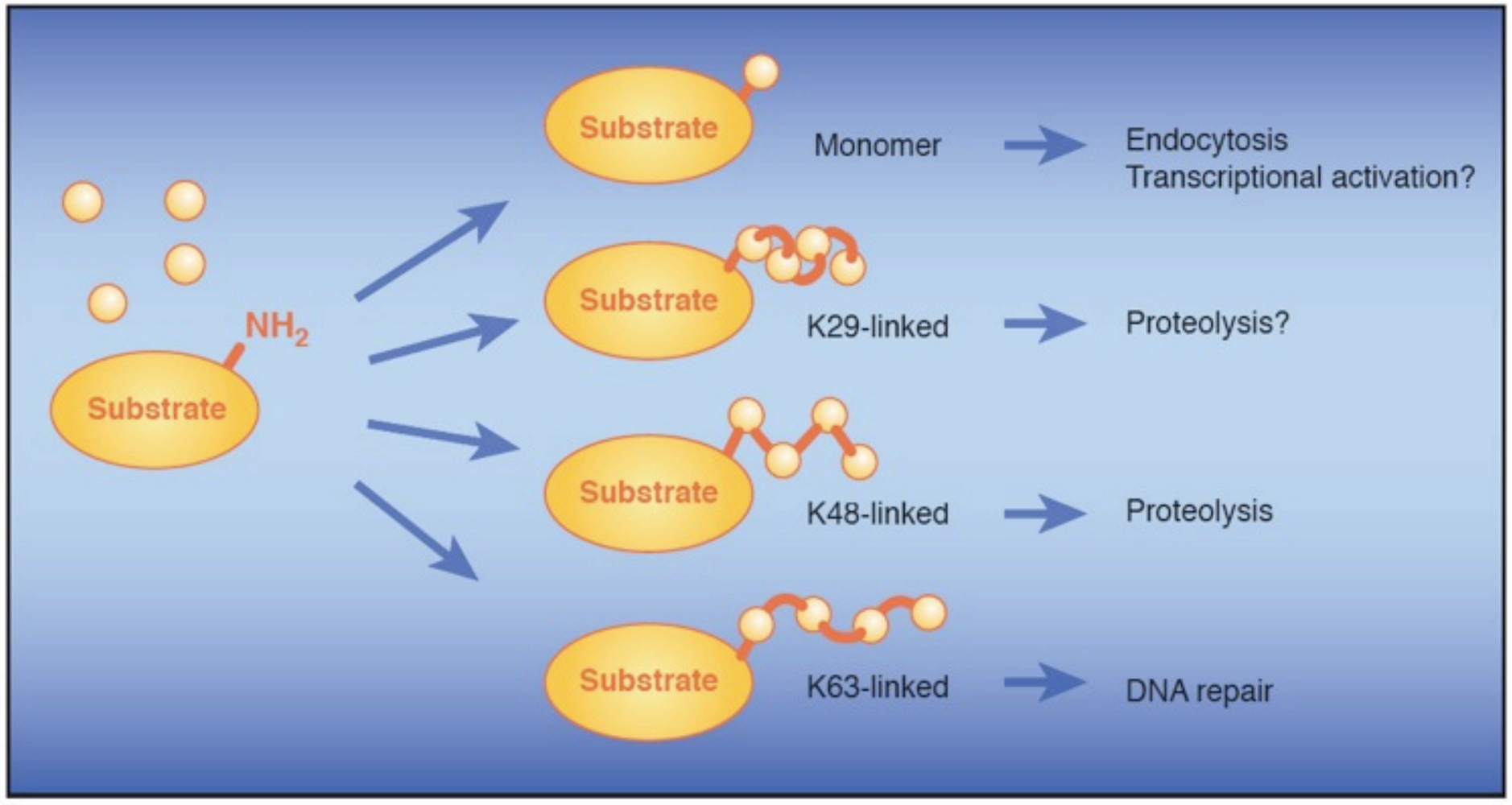
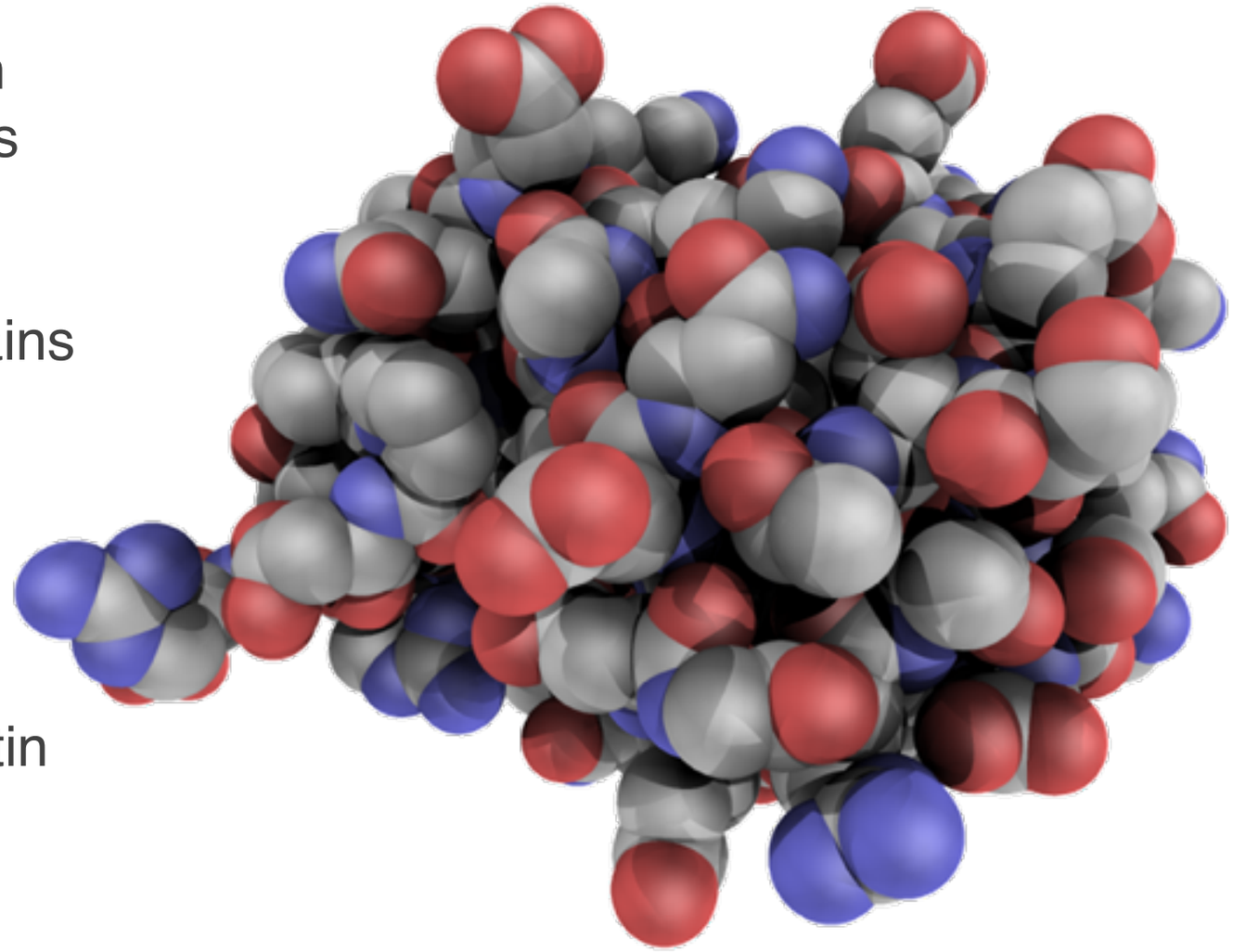




J. Marx, *Science*, 297. (2002)

Summary on ubiquitin

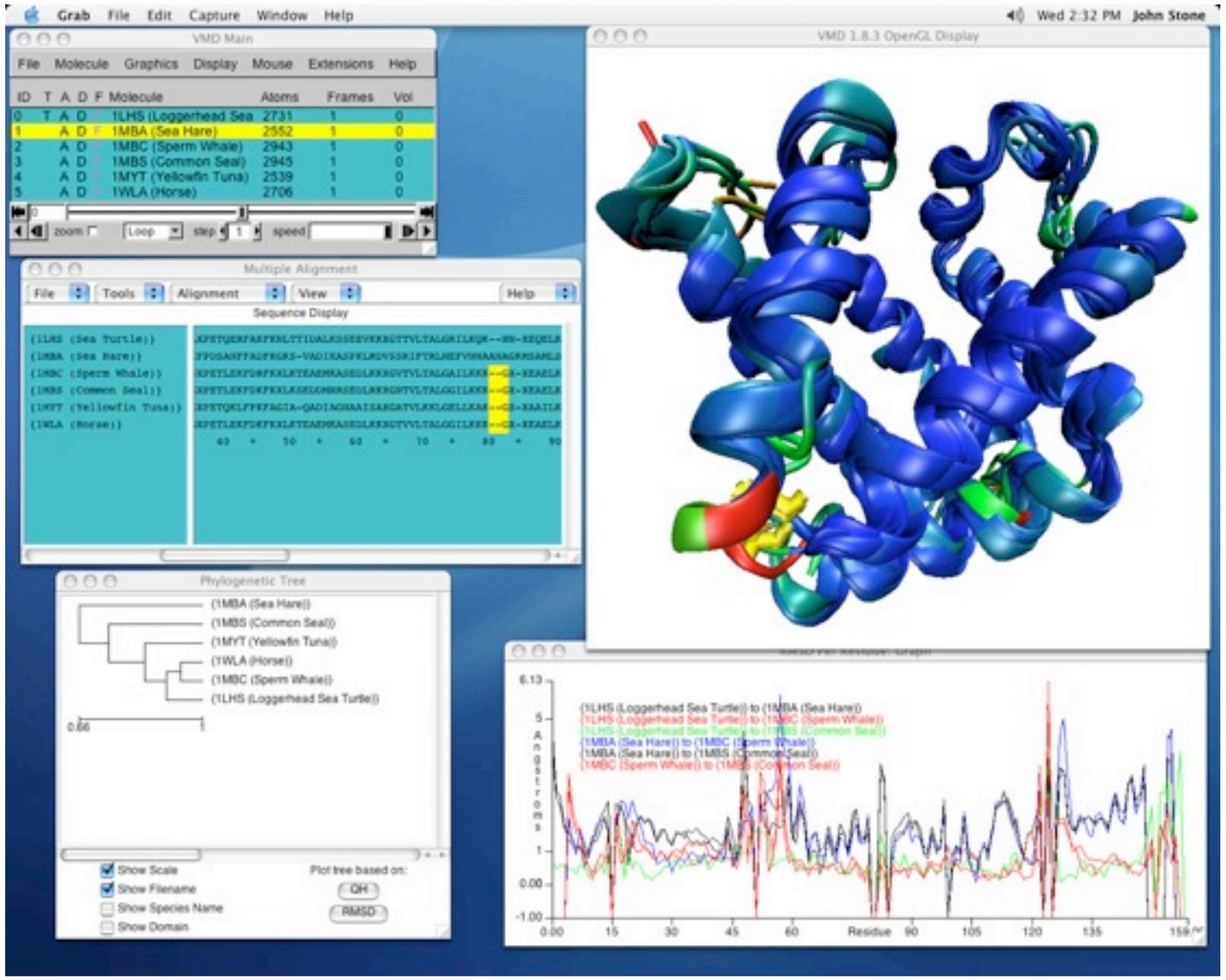
- Ubiquitin is found in all eukaryotes, with nearly identical sequences across species
- The seven lysine residues can bind to other ubiquitins, forming polyubiquitin chains
- By using a different lysine, the popyubiquitin chain performs different functions
- The most well-known function of ubiquitin is protein degradation
- Protein degradation takes place in proteasome, where the amino acids in the protein are separated and recycled



wikipedia.org

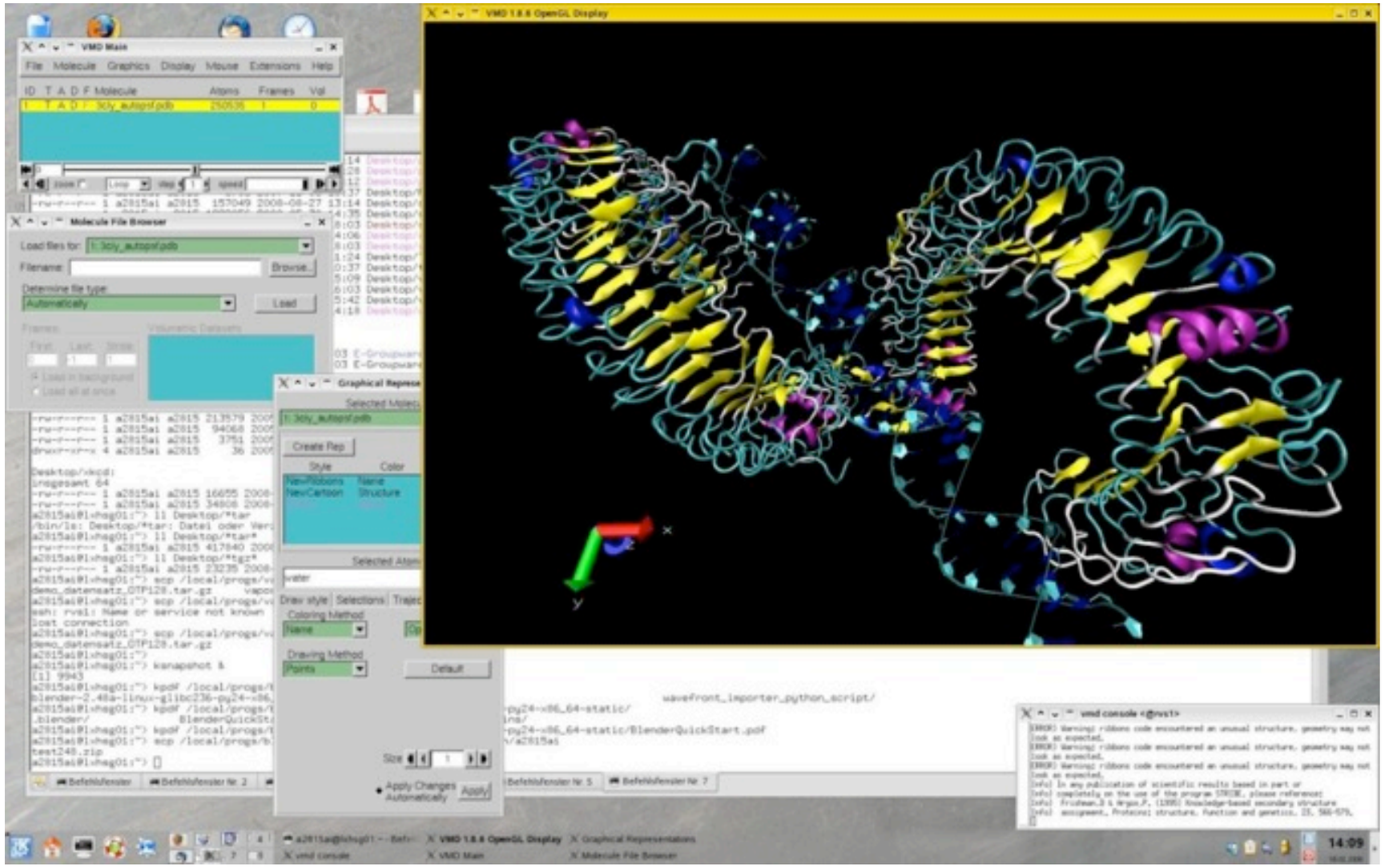
Visualization of ubiquitin with VMD

- Visualization can greatly speed up the learning/communication process
- For biomolecules, many softwares for visualization of proteins are available
- VMD is an example of such software, developed here at UIUC at Klaus Schulten's lab (http://ks.uiuc.edu)
- Here we will use VMD to review what we've learned about ubiquitin
- Feel free to repeat these procedures on your own after class, as a mean to learn VMD (and probably helps you with the assignment as well!)



Visualization of ubiquitin with VMD

- Visualization of a molecule generally follow the steps below (you can try this procedure with your favorite protein):
- Step 1: load you molecule
- Step 2: find a good representation for your molecule
- Step 3: find a good coloring method for your molecule
- Step 4: highlighting important aspects that you might want to explore



We will now give it a try!!

If after the class, you forget how to do something on VMD, here is a quick tutorial:
http://www.ks.uiuc.edu/~jhsin/papers/HSIN2008.pdf

The molecular dynamics method

- Why simulate proteins?
- Snapshots of proteins obtained from crystallography or microscopy reveal important information about the proteins, but cannot provide a full picture
- Molecular dynamics method allows one to explore the possible motions of proteins
- How a protein moves often tells one how the protein does its job

a snapshot of a soccer game does not reveal how the game is played

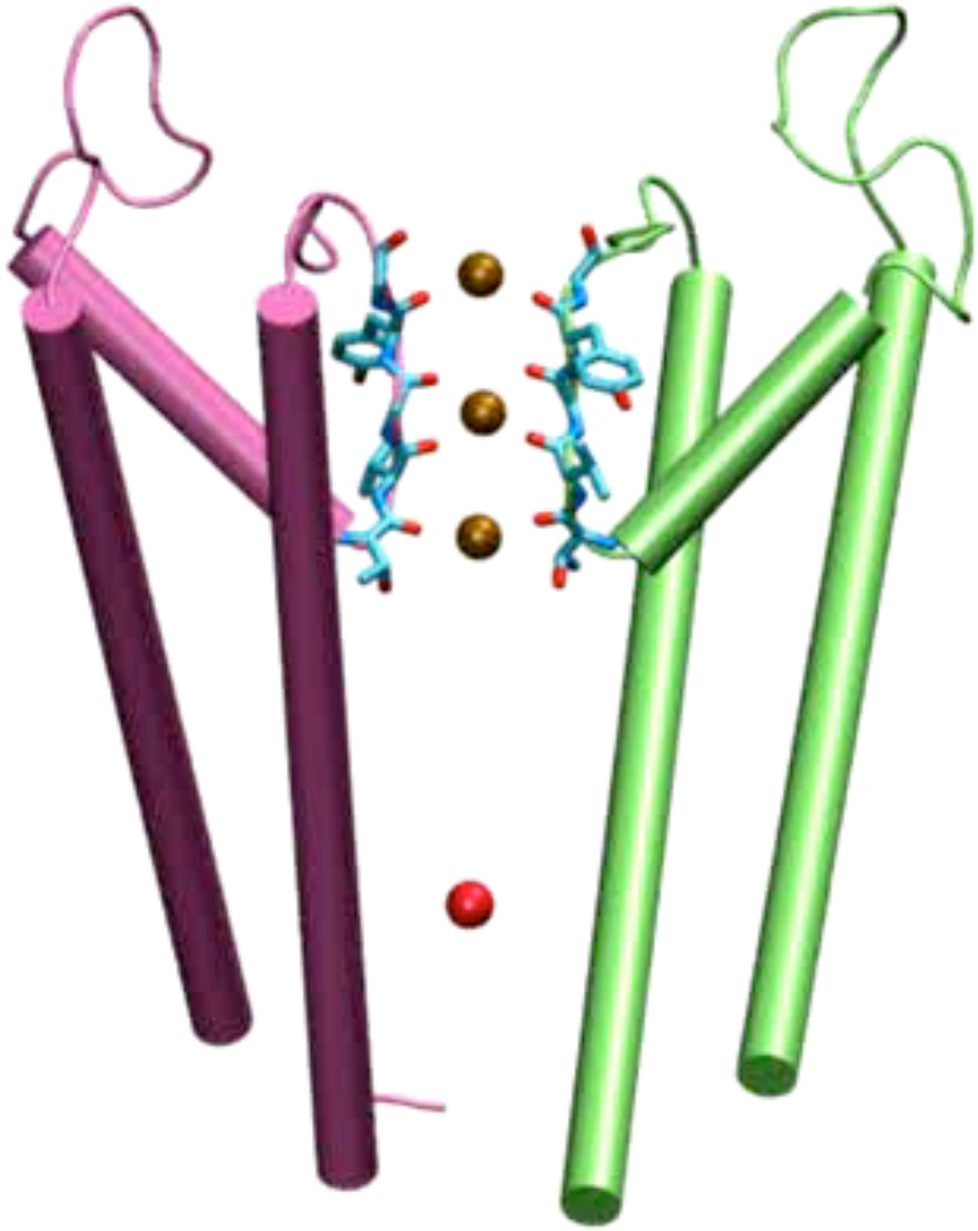
The molecular dynamics method: an example

- An example of a molecular mechanism revealed by molecular dynamics simulations: the potassium channel

- Potassium channels reside in the cellular membrane, functioning as selective gates for the potassium ions

- The channels can open and close, generating electrical signals and impulses in nervous systems

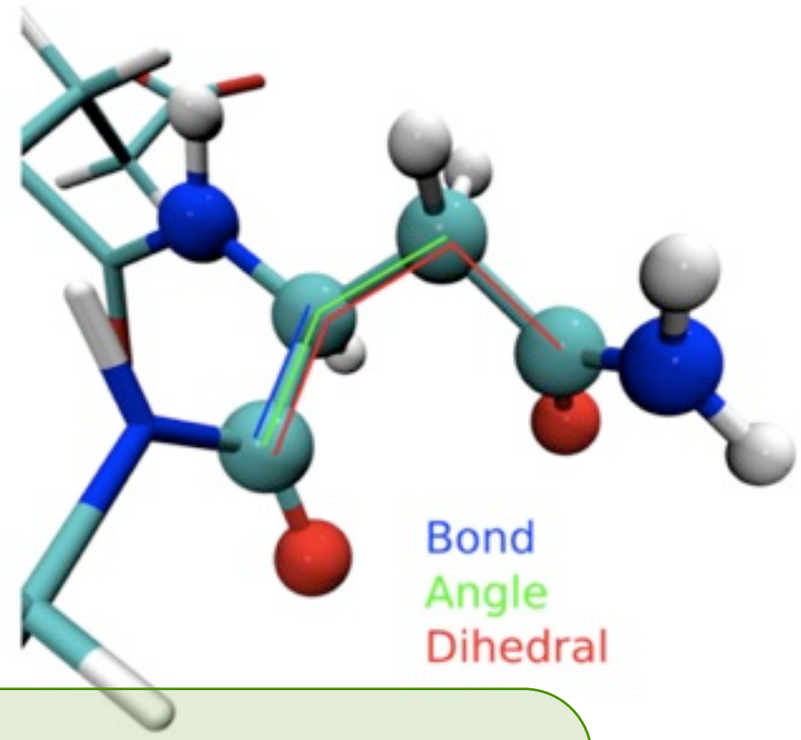
- Molecular dynamics simulations revealed how the potassium ions pass through a potassium channel: they wait in line



F. Khalili-Araghi et al., *Biophysical Journal*, 91. (2006)

Classical molecular dynamics

- Model biological systems with classical mechanics
- Simulate atoms as point masses by integrating Newton's equation of motion
- Assume a potential of form:



$$U_{MD}(\mathbf{R}) =$$

Bonded interactions

$$\underbrace{\sum_{bonds} k_\alpha (r_\alpha - r_\alpha^0)^2}_{U_{bond}} + \underbrace{\sum_{angles} k_\beta (\theta_\beta - \theta_\beta^0)^2}_{U_{angle}}$$

$$+ \underbrace{\sum_{dihedrals} k_\gamma([1 + cos(n_\gamma \psi_\gamma + \delta_\gamma)])}_{U_{dihedral}} + \underbrace{\sum_{impropers} k_\delta (\phi_\delta - \phi_\delta^0)^2}_{U_{improper}}$$

Nonbonded interactions

$$+ \underbrace{\sum_i \sum_{i \neq j} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right]}_{U_{vdW}} + \underbrace{\sum_i \sum_{i \neq j} \frac{q_i q_j}{4\pi\epsilon_0 \epsilon r_{ij}}}_{U_{elec}}$$

Classical molecular dynamics

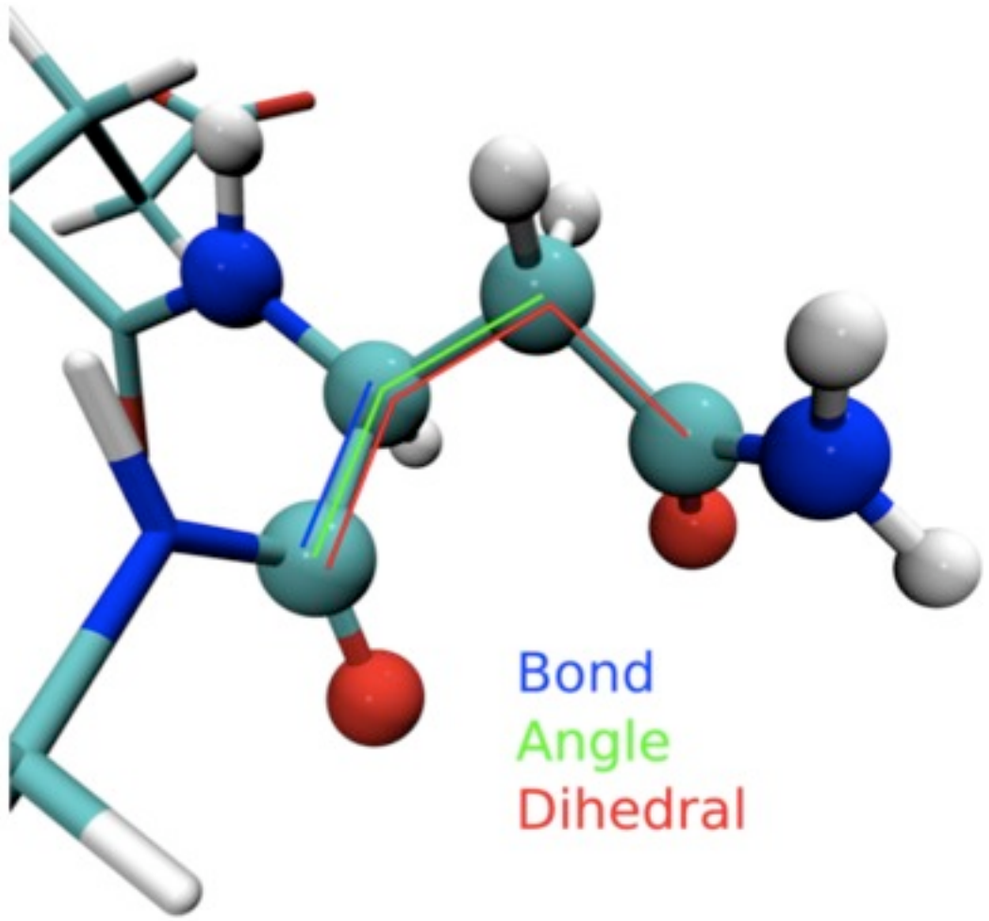
- Parameters of MD potential are derived from *ab initio* QM calculations

- Computational protocol is used to iteratively solve the Langevin equation

$$m\ddot{\mathbf{r}} = \mathbf{F} - \gamma\mathbf{v} + \psi(t)$$

- Dynamics is computed in discrete timestep typically 1 or 2 fs

- Appropriate for simulating mechanical motions of biomolecules, not electronic changes



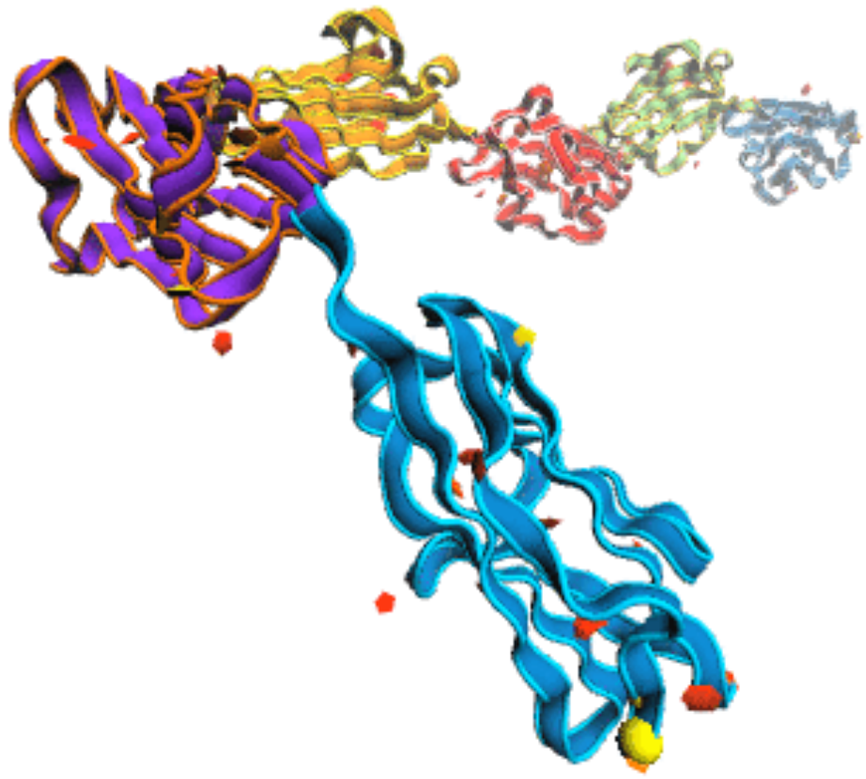
Common applications of molecular dynamics

Common uses for MD include:

- Identifying molecular interactions
- Understanding the structural effects of mutations or ligand binding
- Testing the structural transition of biomolecules in response to environmental factors (force, voltage, fluid flow, etc...)
- Calculating free energies of binding or conformational change

Limited mainly by:

- accessible timescales (ns-ms)
- system size (up to few million atoms)
- accuracy of the potential used



Lee et al., *Biophysical Journal*, 98. (2010)

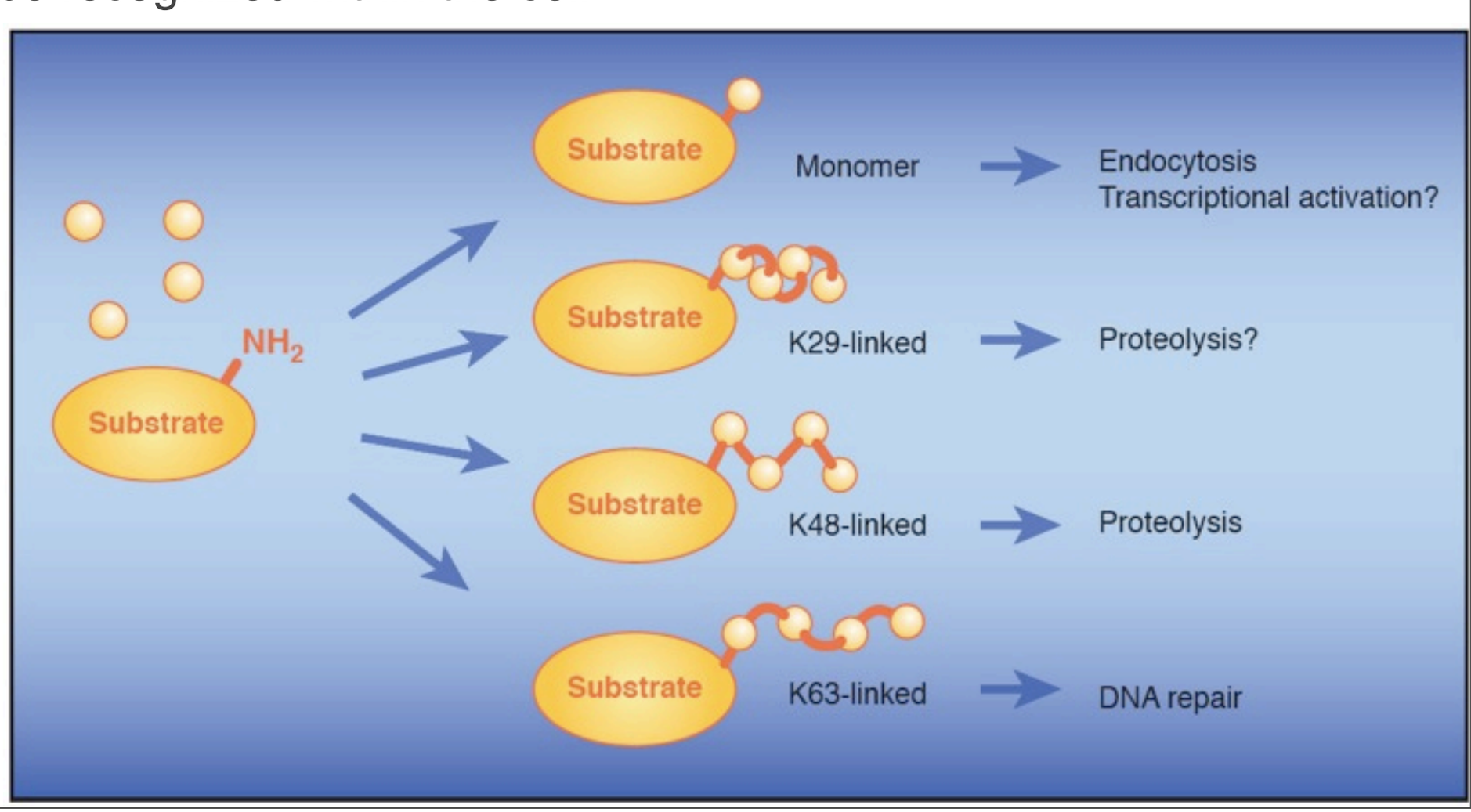
Molecular dynamics simulation of ubiquitin

- How can molecular dynamics contribute to the understanding of ubiquitin structure/ function?

- Thinking hat: how do different polyubiquitin chains linked through a different lysine residue lead the tagged protein to a different cellular pathway?

- Recall: K48 linked polyubiquitin signals protein degradation

- This must mean that there are differences in the physical or chemical properties of different polyubiquitins that can be recognized within the cell

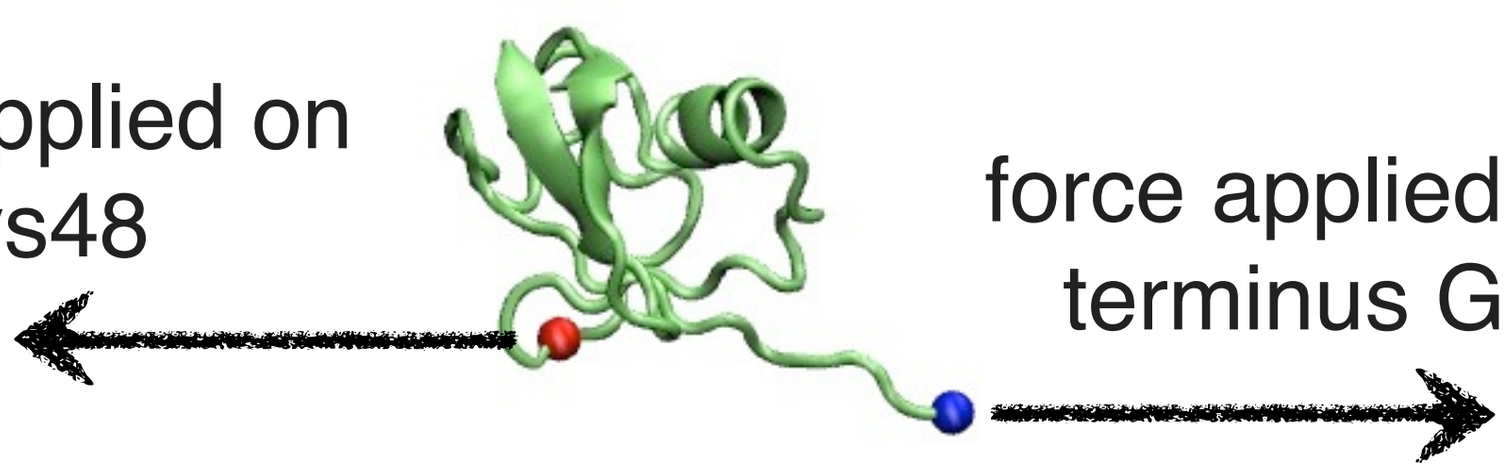


J. Marx, *Science*, 297. (2002)

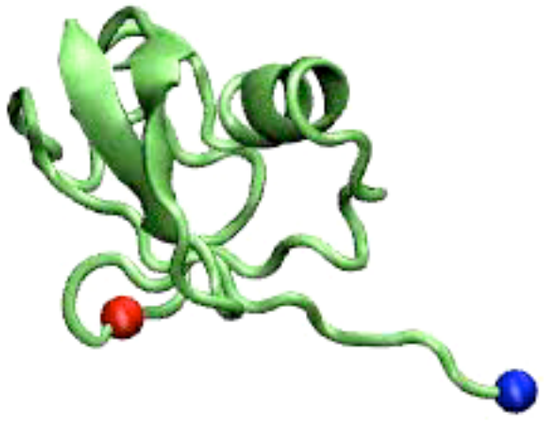
Molecular dynamics simulation of ubiquitin

- One can apply forces on the ends of ubiquitin, and observe the force required to disrupt the ubiquitin structure
- Forces can be applied on different lysine residues, i.e., Lys48, or Lys63, or on the termini
- We can see if different amount of force is required

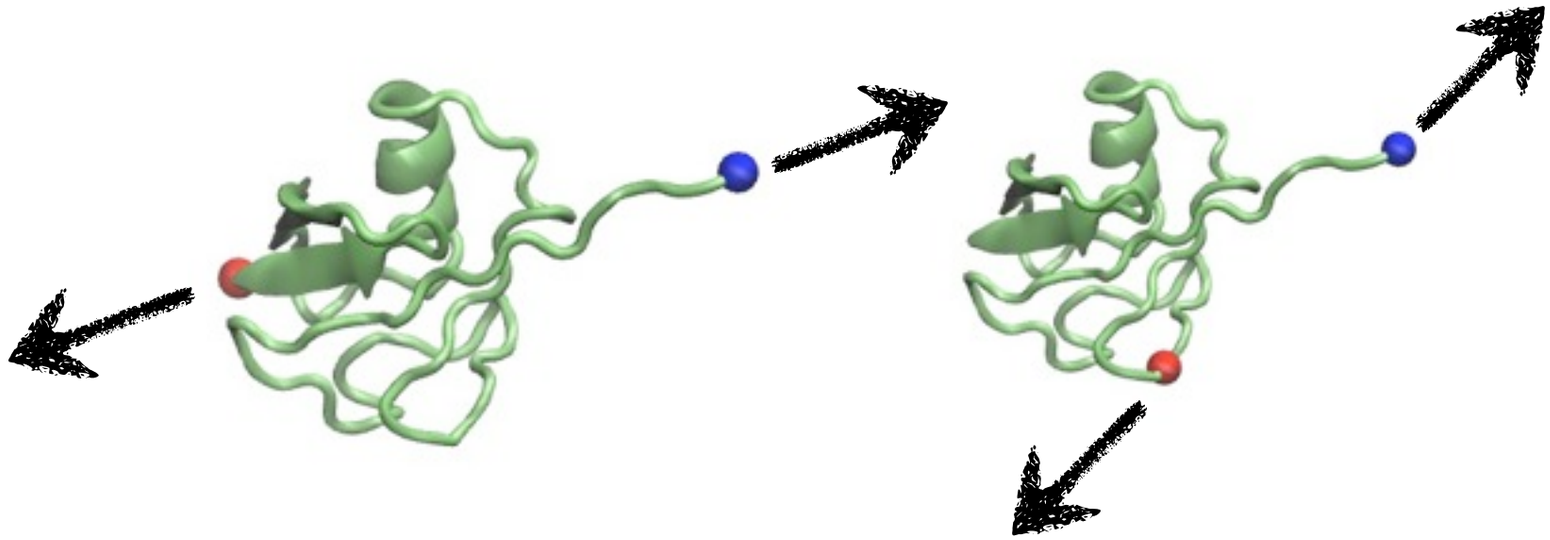
force applied on Lys48



force applied on C-terminus Gly76



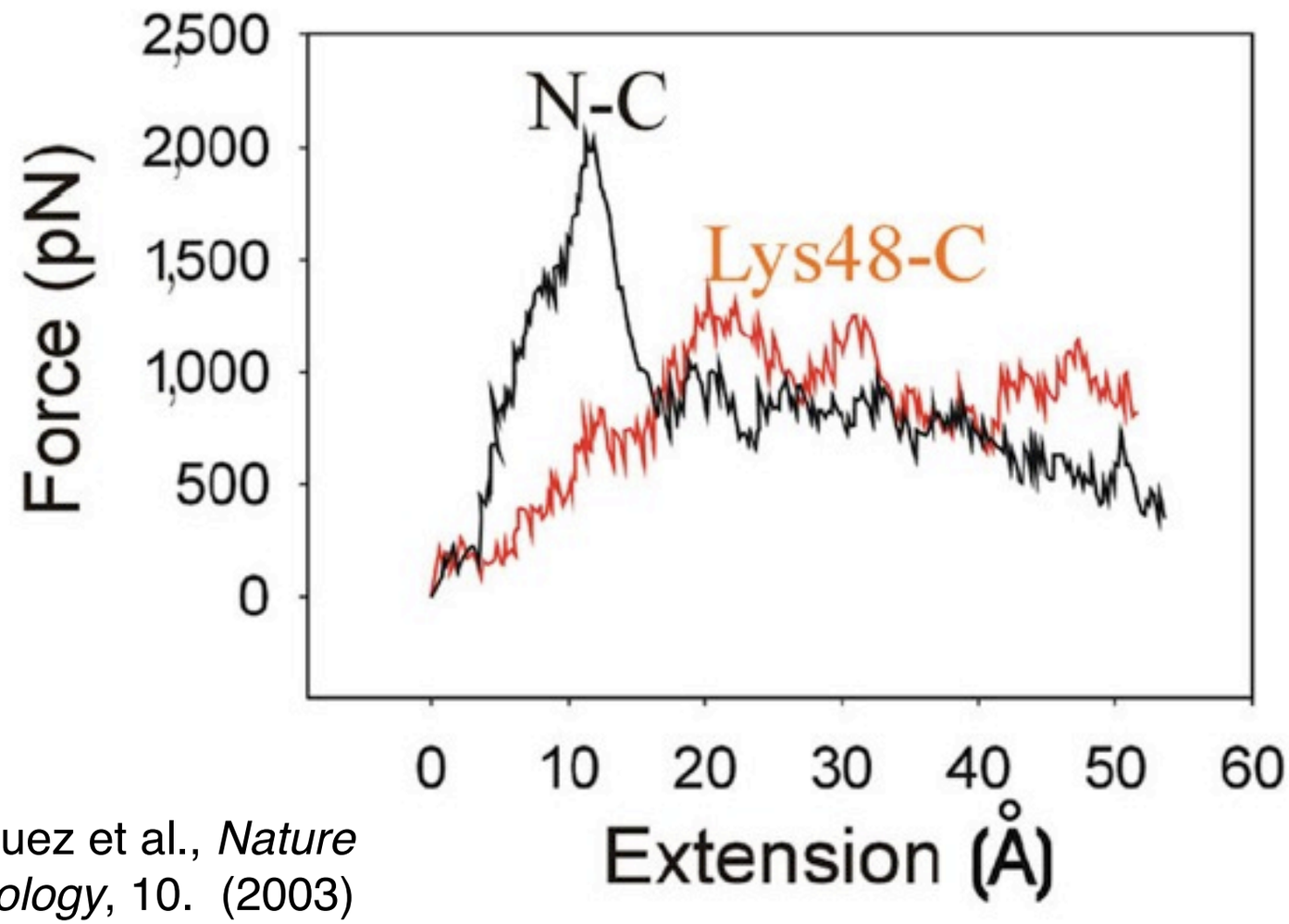
Molecular dynamics simulation of ubiquitin



- Indeed, forces required to rupture ubiquitin depends on where one applies the force

- Pulling between N- and C-terminus requires higher force than pulling between Lys48 and C-termini

- This is because the rupturing process involves dissociation of the hydrogen bonds that hold together the secondary structure of the protein

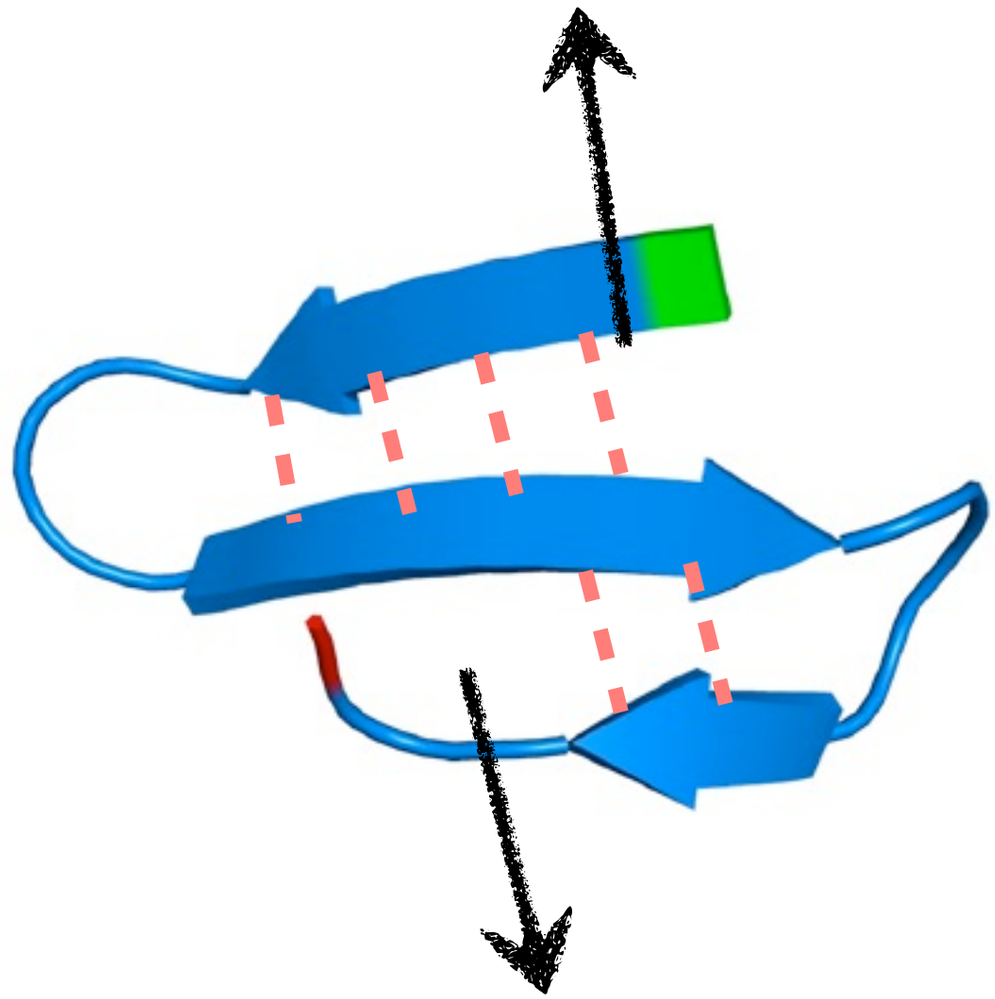


M. Carrion-Vazquez et al., *Nature Structural Biology*, 10. (2003)

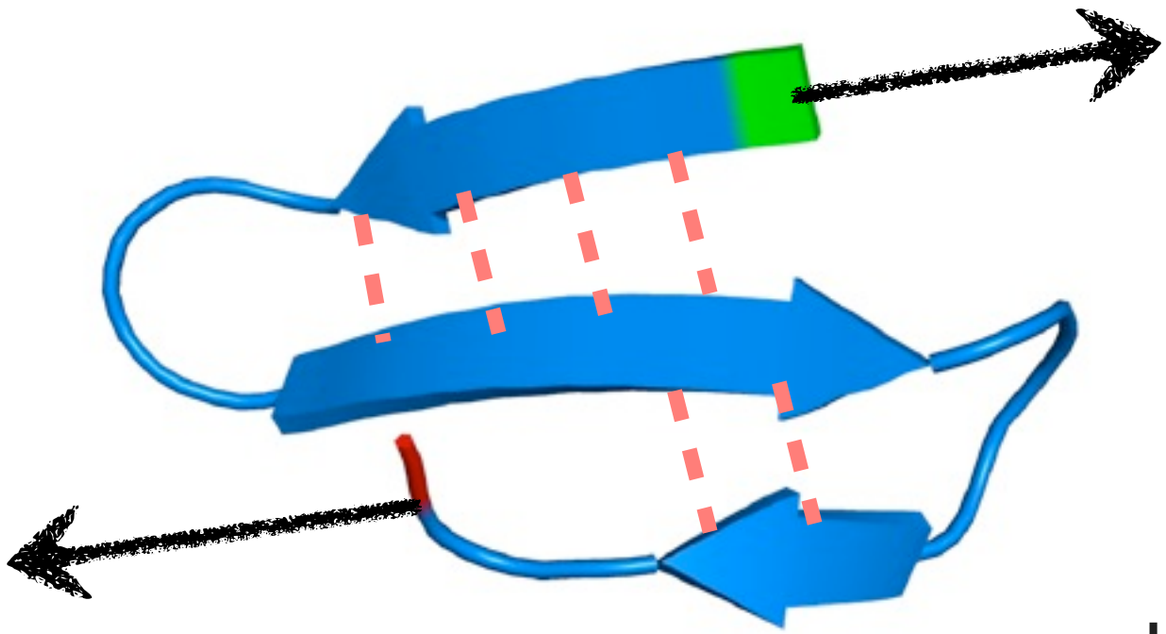
Molecular dynamics simulation of ubiquitin

- For example, consider a small beta sheet maintained by hydrogen bonds

- It's easier to pull this beta sheet apart in the direction perpendicular to the beta sheet (or, parallel to the hydrogen bonds)



easier to rupture; lower force required

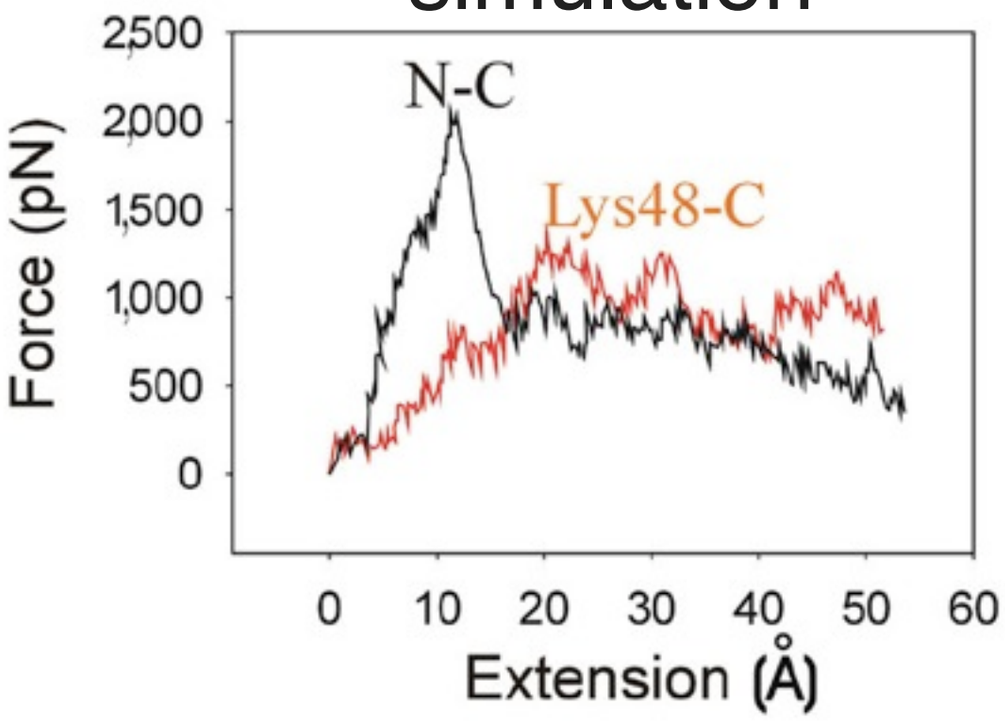


more difficult to rupture; higher force required

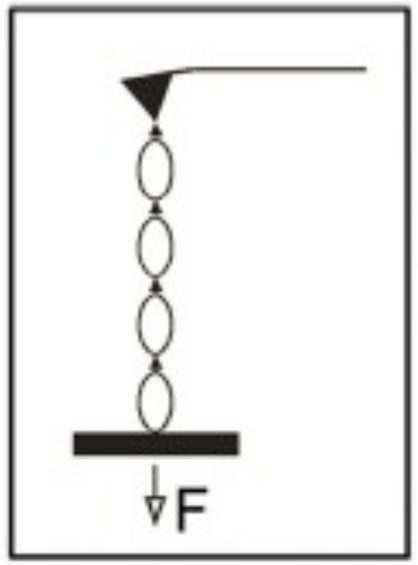
Experimental rupturing of polyubiquitin chains

- Results from simulation can be verified by experiment!
- The method like atomic force microscopy (AFM) can pull apart polyubiquitin chains and measure the required force

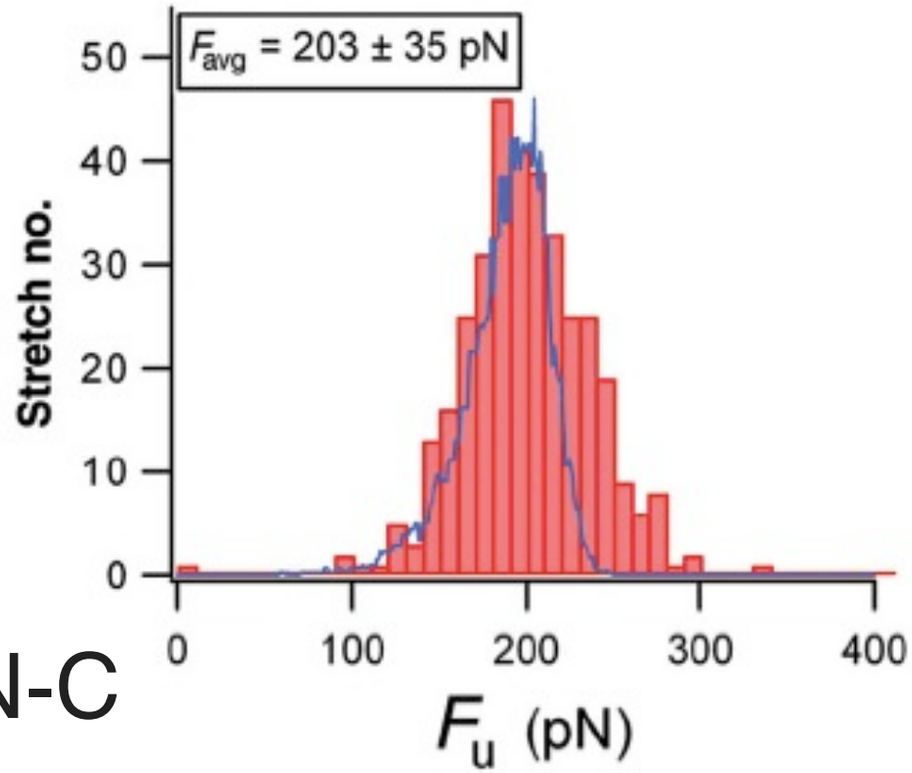
simulation

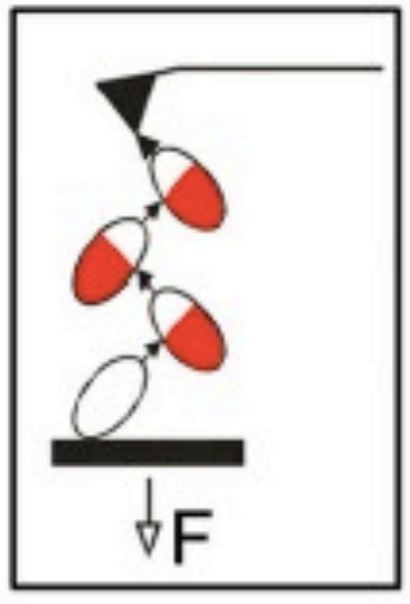


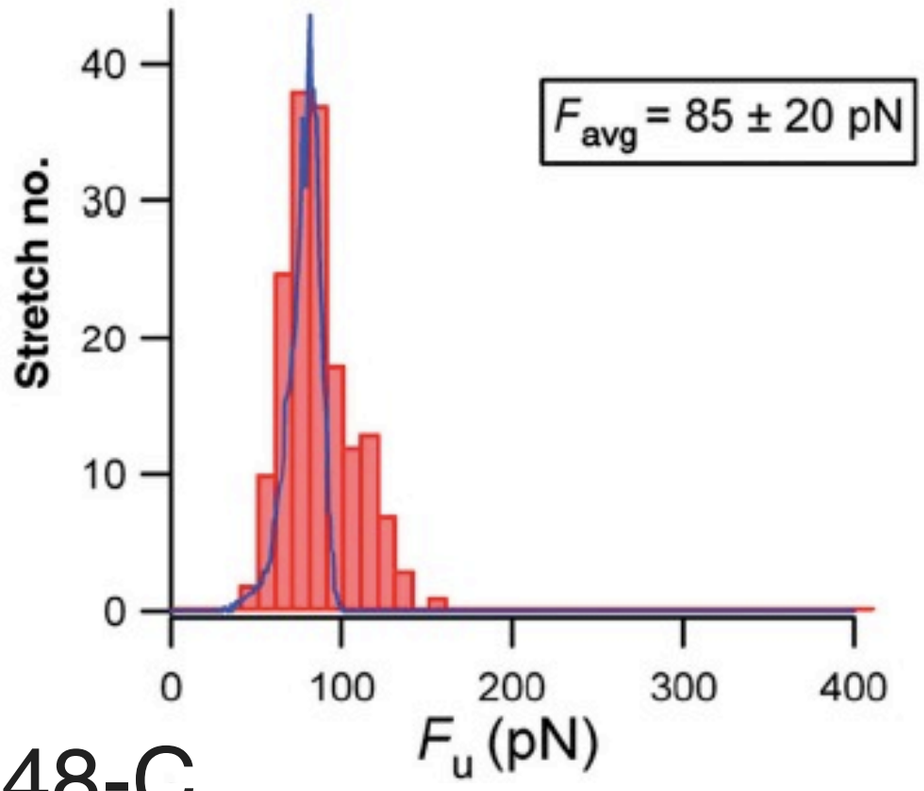
linked through N-C terminus



experiment







M. Carrion-Vazquez et al., *Nature Structural Biology*, 10. (2003)

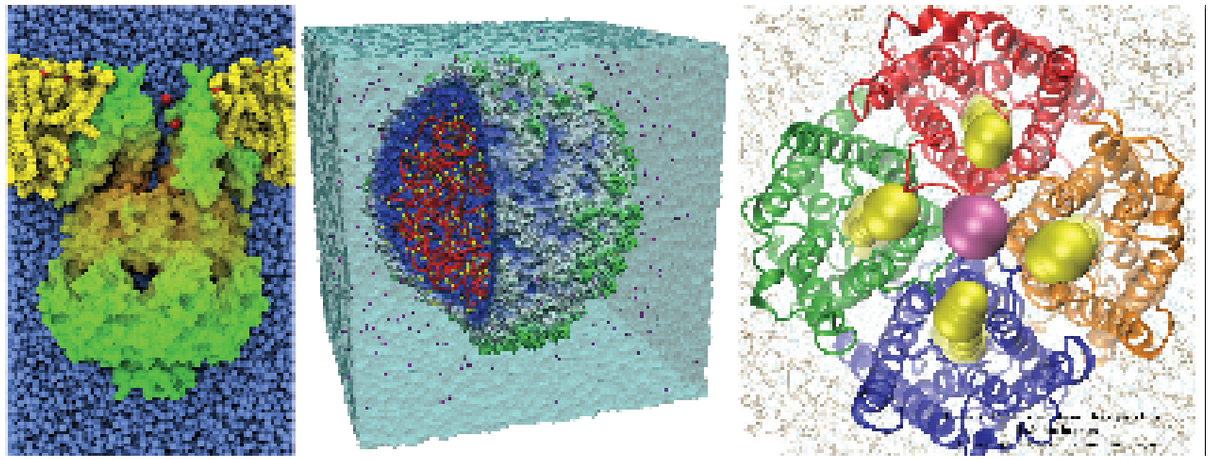
linked through Lys48-C

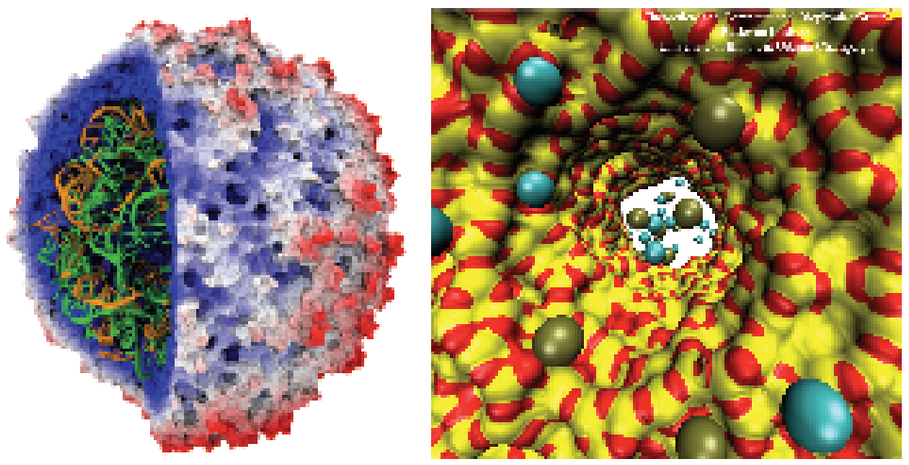
Summary on molecular dynamics

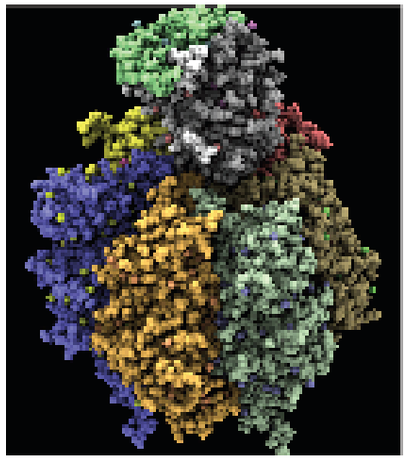
- Visualization of biomolecules can help us understand the relationship between protein structure and function

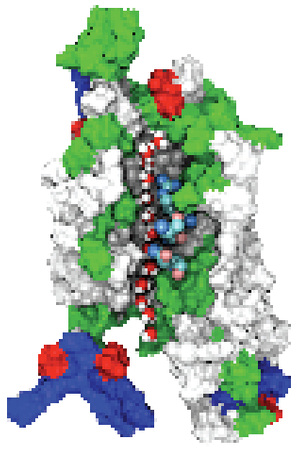
- Molecular dynamics simulation is a useful tool for answering some important biological inquires

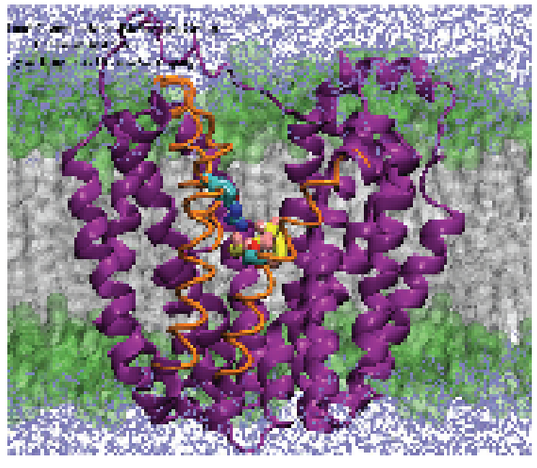
- Direct comparison between experimental and computational results is already feasible, making the two methods complimentary and hand-in-hand











Most molecular rendering in this class is done with VMD, and all simulations were performed with NAMD.
More information can be found on http://www.ks.uiuc.edu

Bibliography/Further readings

As a non-expert of ubiquitin, during the past few days I found the following sources extremely helpful in preparing for this class.

http://en.wikipedia.org/wiki/Ubiquitin

J. Marx, “Ubiquitin lives up to its name.” *Science*, 297. (2002)

M. Hochstrasser, “Origin and function of ubiquitin-like proteins.” *Nature*, 458. (2009)

A. Varshavsky, “The early history of the ubiquitin field.” *Protein Science*, 15. (2006)

C. M. Pickart, “Back to the future with ubiquitin.” *Cell*, 116. (2004)

M. Carrion-Vazquez et al., “The mechanical stability of ubiquitin is linkage dependent.” *Nature Structure Biology*, 10. (2003)

http://nobelprize.org/nobel_prizes/chemistry/laureates/2004/

If you need to get a quick lesson on VMD, here is a short tutorial:

http://www.ks.uiuc.edu/~jhsin/papers/HSIN2008.pdf