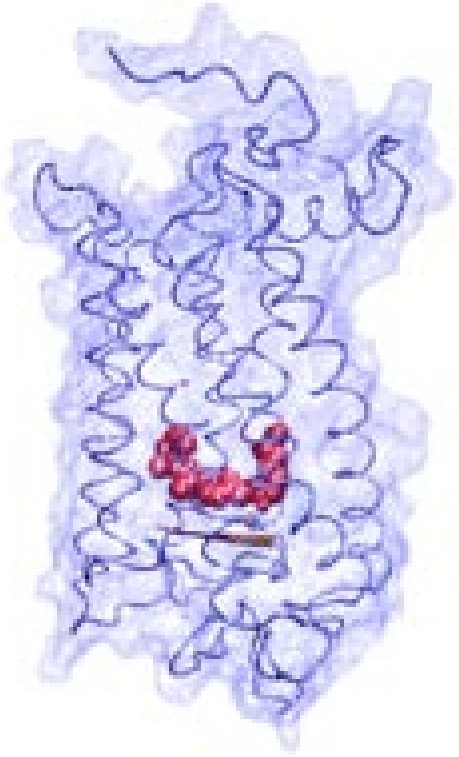
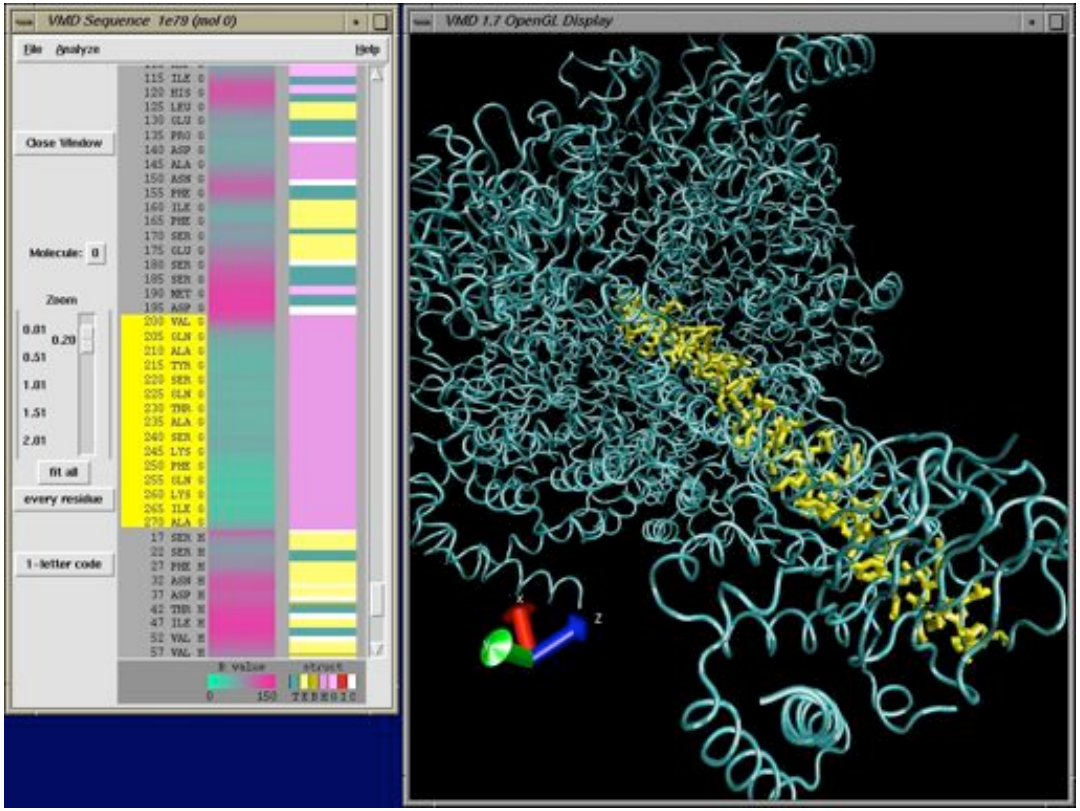
# Molecular Graphics Perspective of Protein Structure and Function

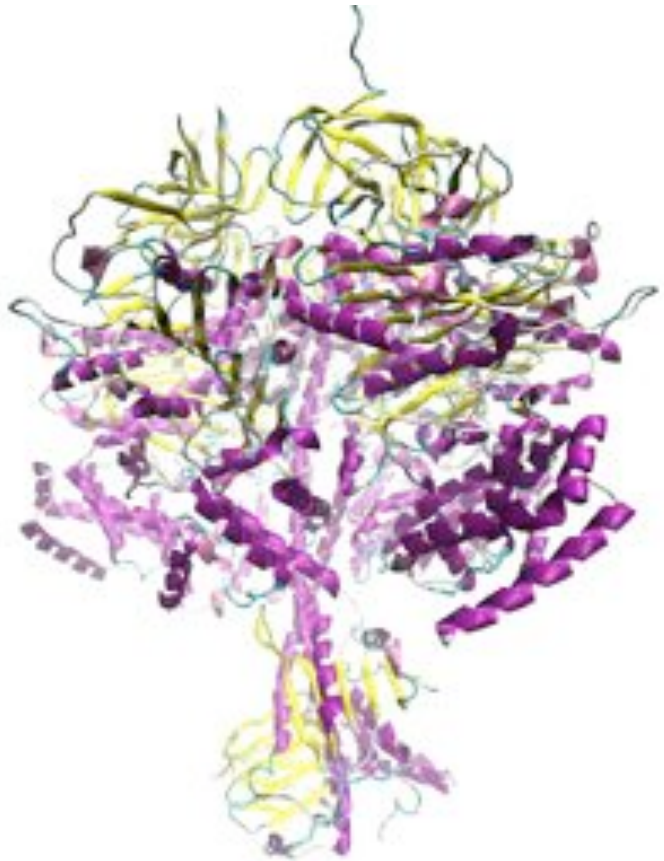





animation sequence structure

# VMD Highlights

- > 40,000 registered users
- Platforms:
  - Unix (16 builds)
  - Windows
  - MacOS X
- Display of large biomolecules and simulation trajectories
- Sequence browsing and structure highlighting
- Multiple sequence - structure analysis
- User-extensible scripting interfaces for analysis and customization

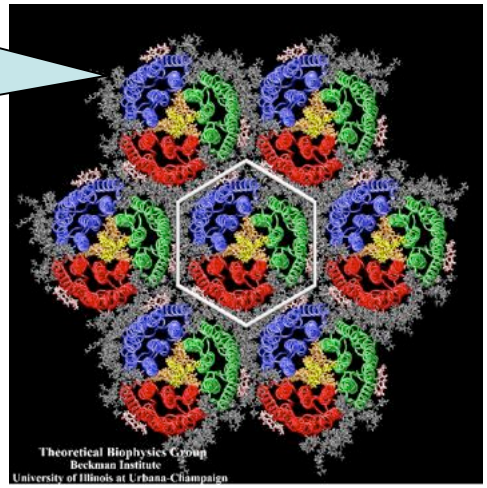


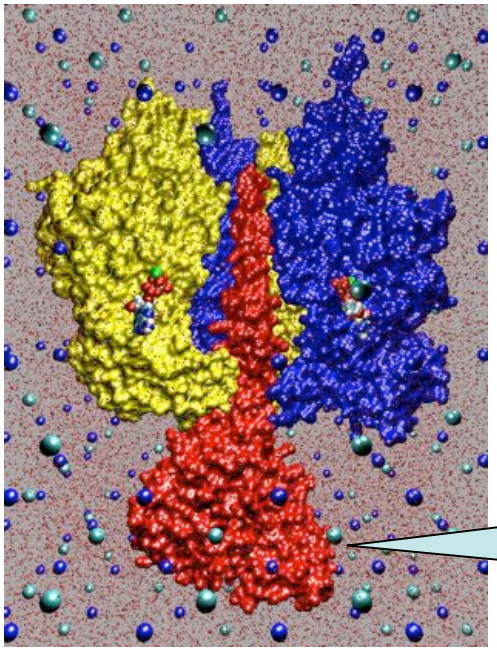
The program is used today more for preparation and analysis of modeling than for graphics

# VMD Permits Large Scale Visualization

- Large structures: 300,000 atoms and up
- Complex representations
- Long trajectories: thousands of timesteps
- Volumetric data
- Multi-gigabyte data sets break 32-bit barriers
- Handles large data sets, e.g., GlpF: each 5 ns simulation of 100K atoms produces a 12GB trajectory

Purple Membrane 150,000 Atoms



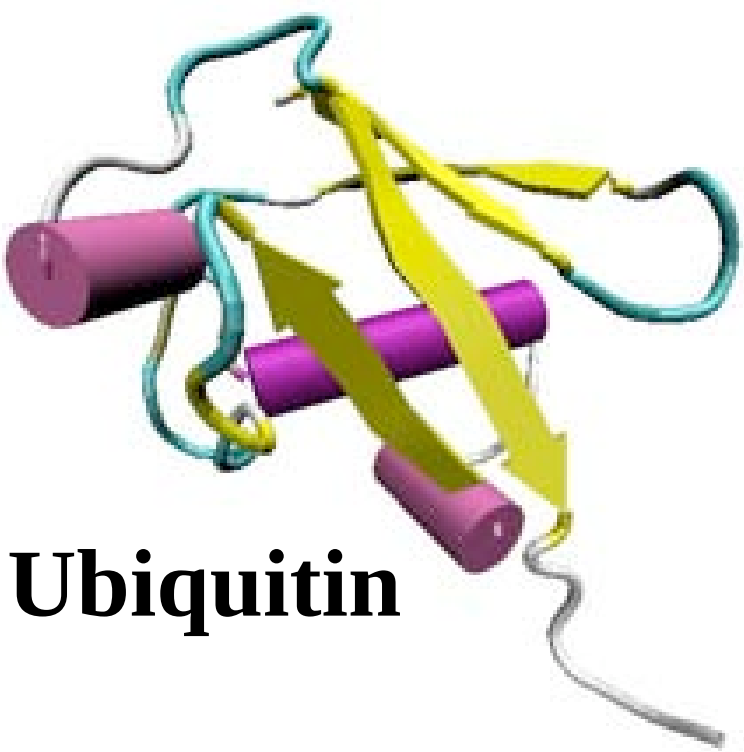



F1 ATPase 327,000 Atoms

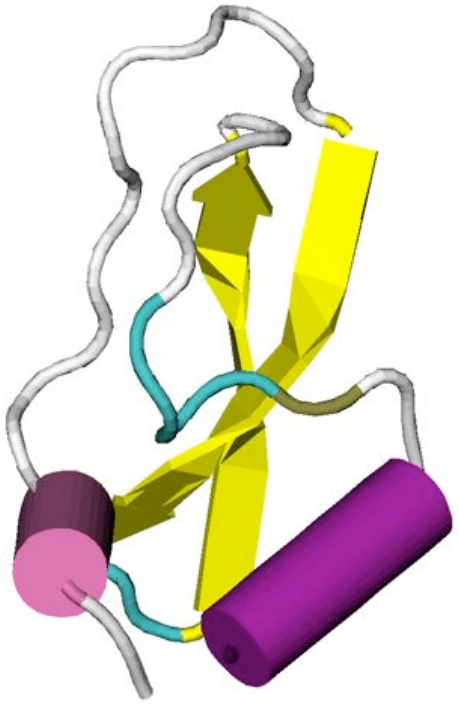
# Focus on two proteins

## Ubiquitin

## Bovine Pancreatic Trypsin Inhibitor (BPTI)



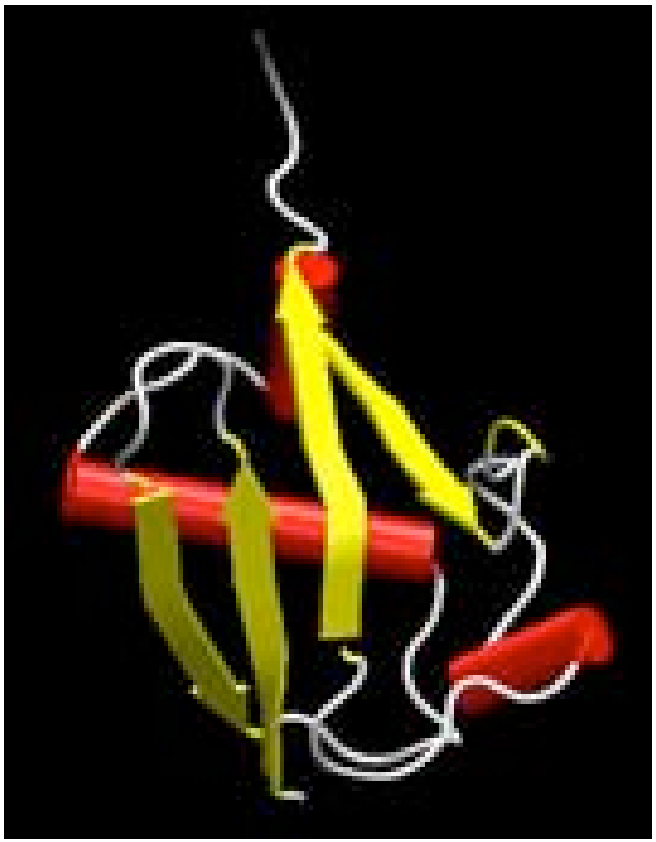
Ubiquitin



BPTI

# Ubiquitin

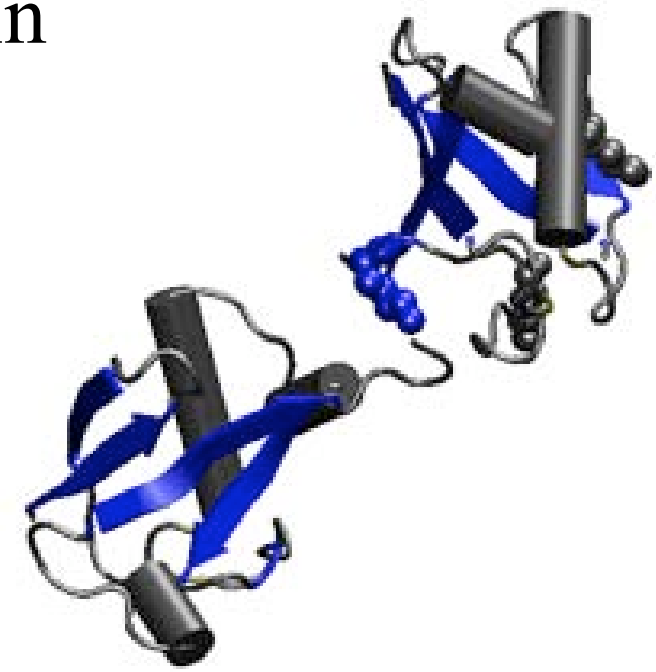
- 76 amino acids
- highly conserved
- covalently attaches to proteins and tags them for degradation
- other cell traficking



• Glycine at C-terminal attaches to the Lysine on the protein by an isopeptide bond.

• it can attach to other ubiquitin molecules and make a polyubiquitin chain.

There are 7 conserved lysine residues in ubiquitin.

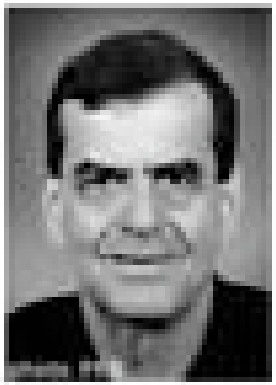


Two ubiquitins attached together through LYS 48. LYS 63 and LYS 29 are also shown there.

# Ubiquitination Pathway

The Nobel Prize in Chemistry 2004

"for the discovery of ubiquitin-mediated protein degradation"



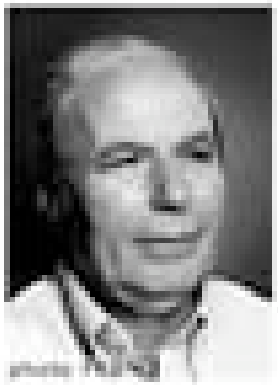
**Aaron Ciechanover**

½ of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1947



**Avram Hershko**

½ of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1937
(in Karcag, Hungary)

**Irwin Rose**

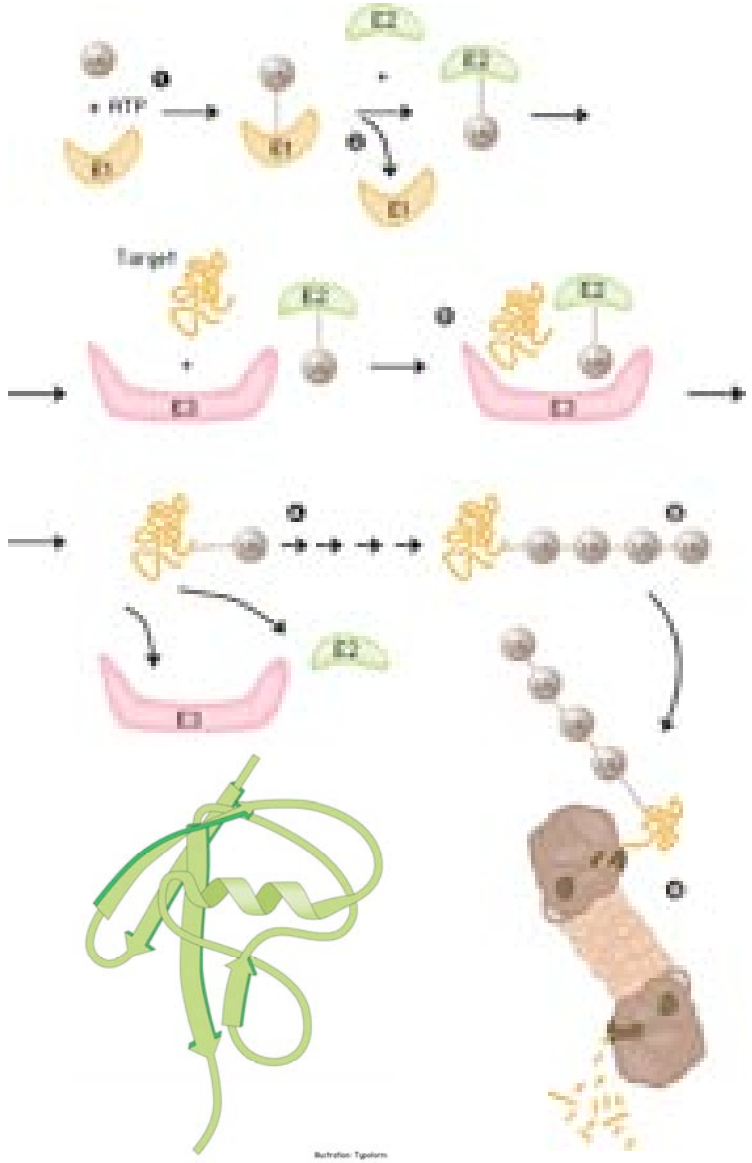
1/3 of the prize

USA

University of California
Irvine, CA, USA

b. 1926

Ubiquitin-mediated protein degradation



# Ubiquitination Pathway

- Activation by E1 (ATP dependent process)

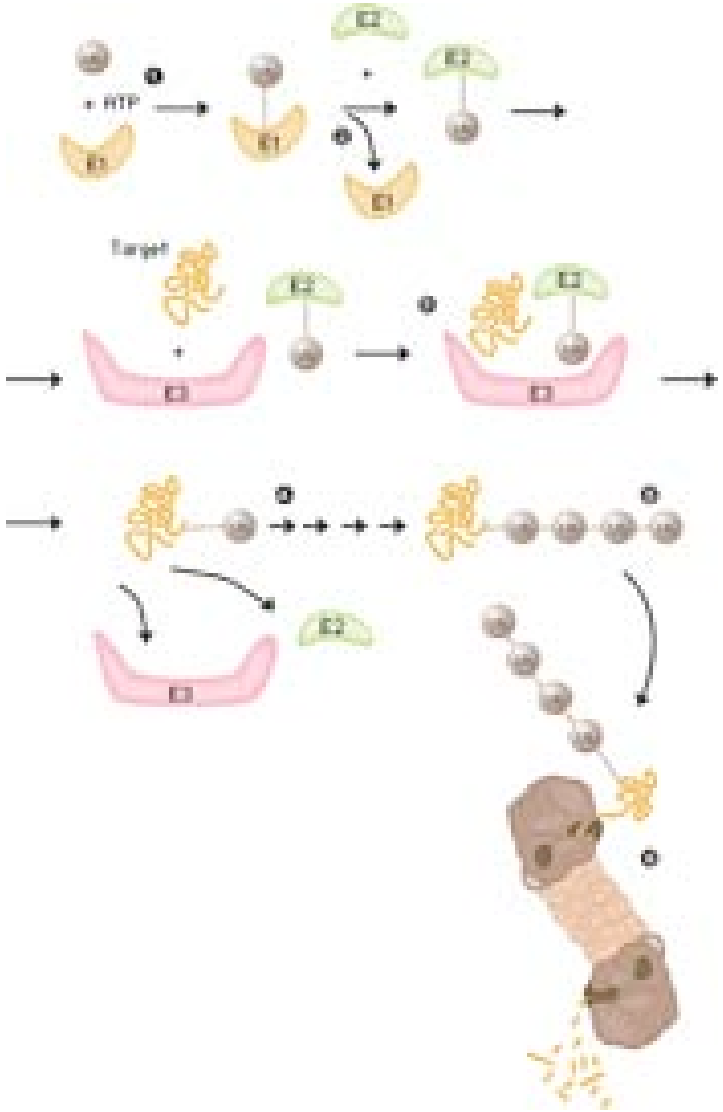
  (thiol-ester linkage between a specific cysteine residue of E1 and Glycine on ubiquitin)

- Transfer to a cysteine residue on E2

  (ubiquitin conjugation enzyme)

- Transfer of ubiquitin by E3 to the substrate lysine residue.

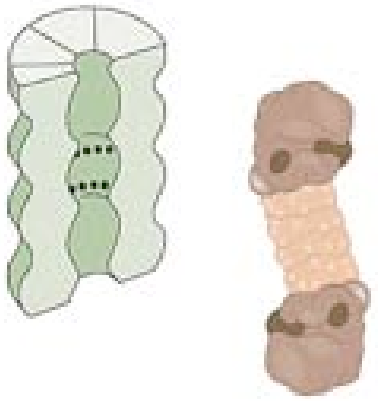
- E3 recognizes the ubiquitination signal of the protein.



# Ubiquitin Functions

• tagging misfolded proteins to be degraded in the proteasome (kiss of death).

• regulates key cellular processes such as cell division, gene expression, ...



The cell's waste disposer, the proteasome. The black spots indicate active, protein-degrading surfaces.

A chain of at least four ubiquitins is needed to be recognized by the proteasome.

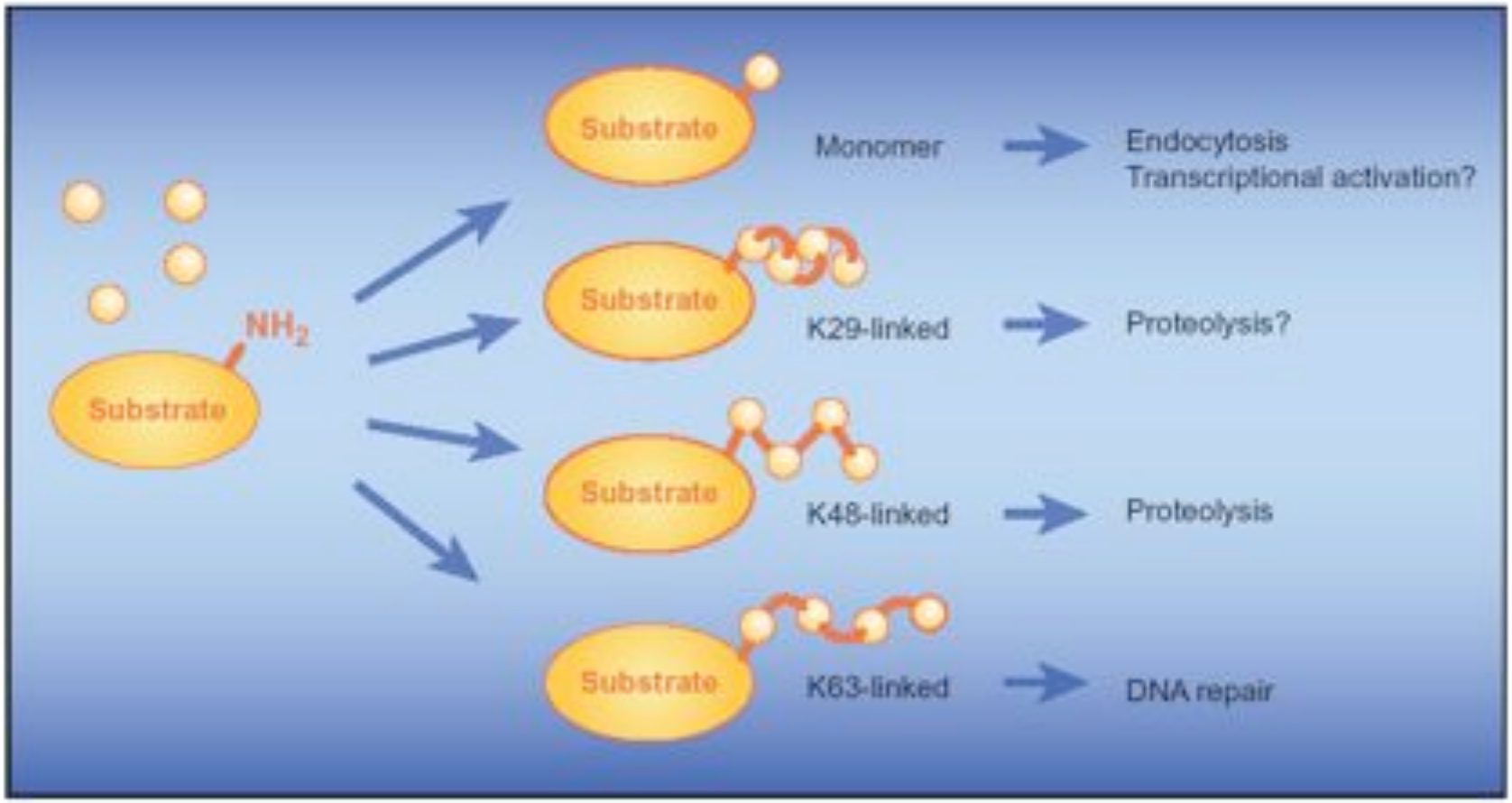
# Different types of ubiquitin signals arise from

- Length of the ubiquitin chain
- How ubiquitins are attached together
- Where the signals are read

**Examples:**

- multi-ubiquitin chains, linked through Lysine 48, target protein for proteasome degradation
- K63 linkages direct DNA repair

# Mono-ubiquitylation versus multi-ubiquitylation



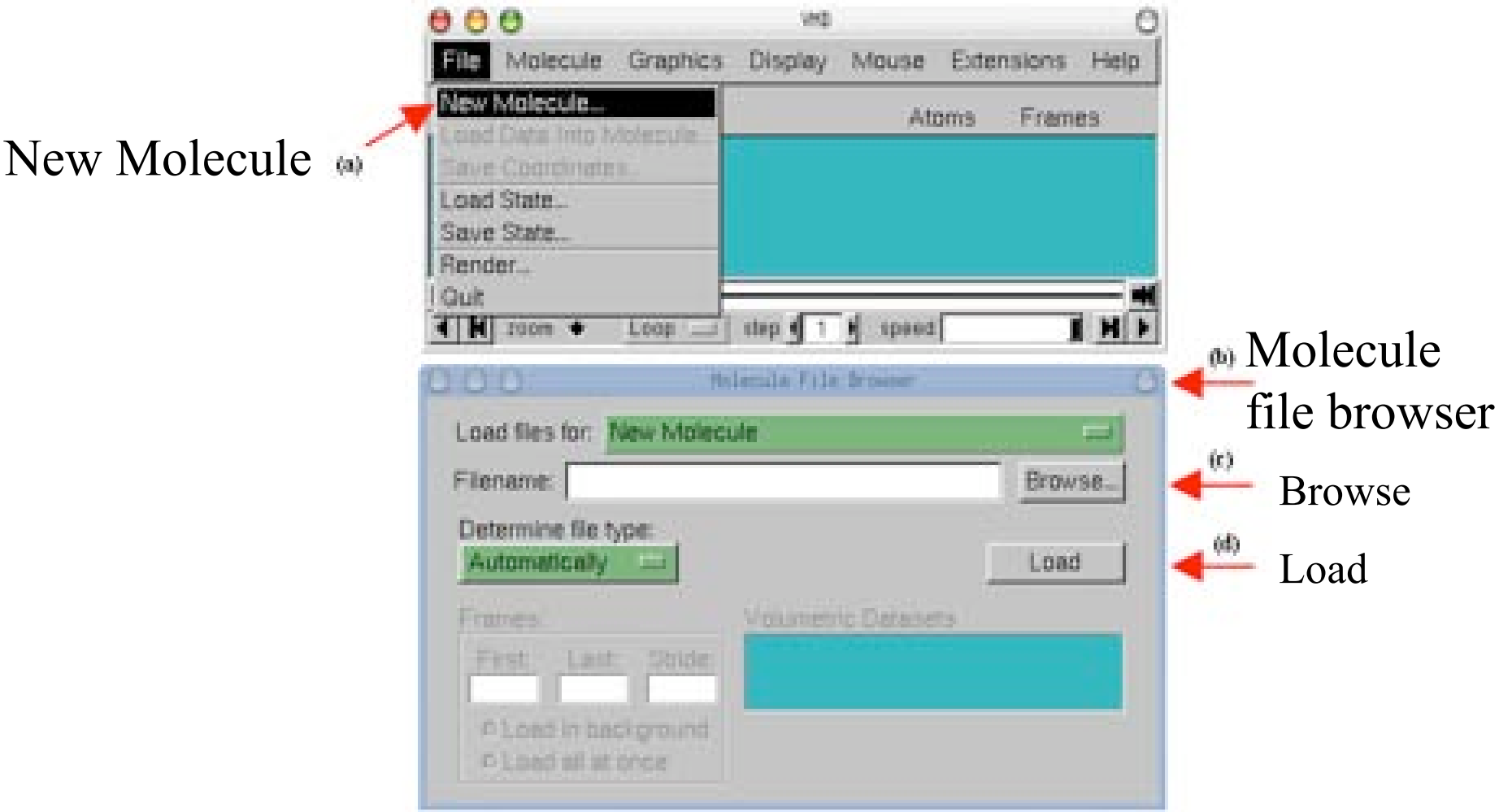

**Multifaceted.** Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

Marx, J., Ubiquitin lives up its name, Science 297, 1792-1794 (2002)

# Inspect ubiquitin with  VMD

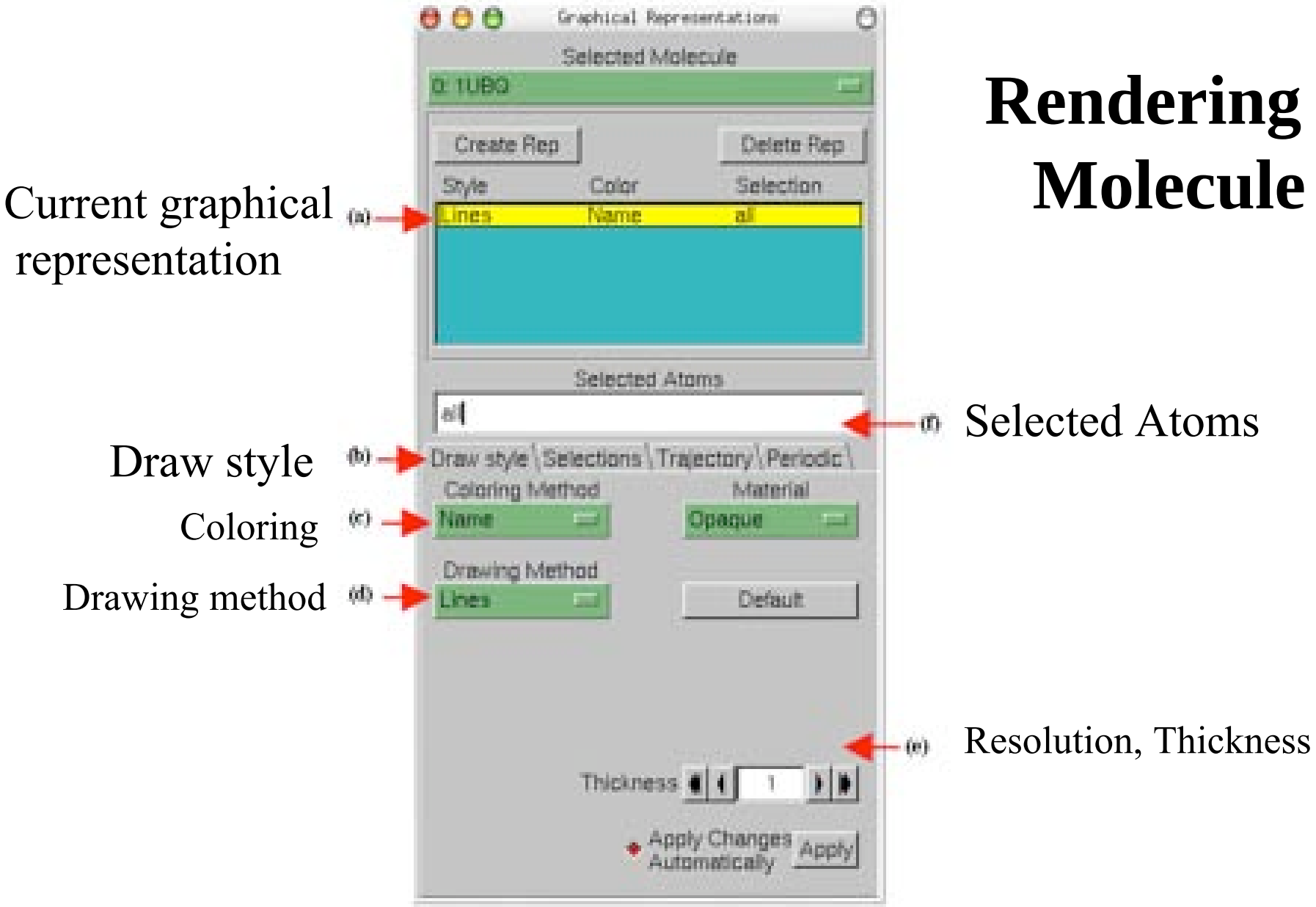
# Basics of VMD

## Loading a Molecule



# Basics of VMD

## Rendering a Molecule




Current graphical representation

Draw style

Coloring

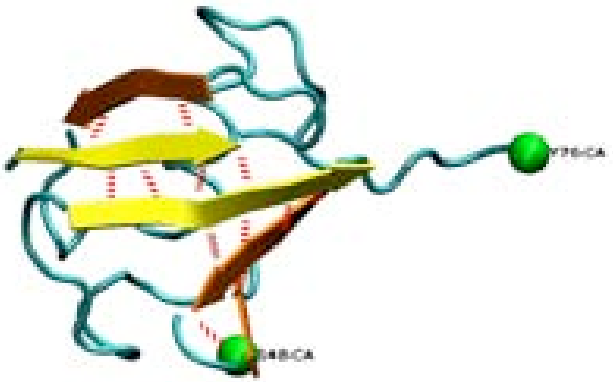
Drawing method

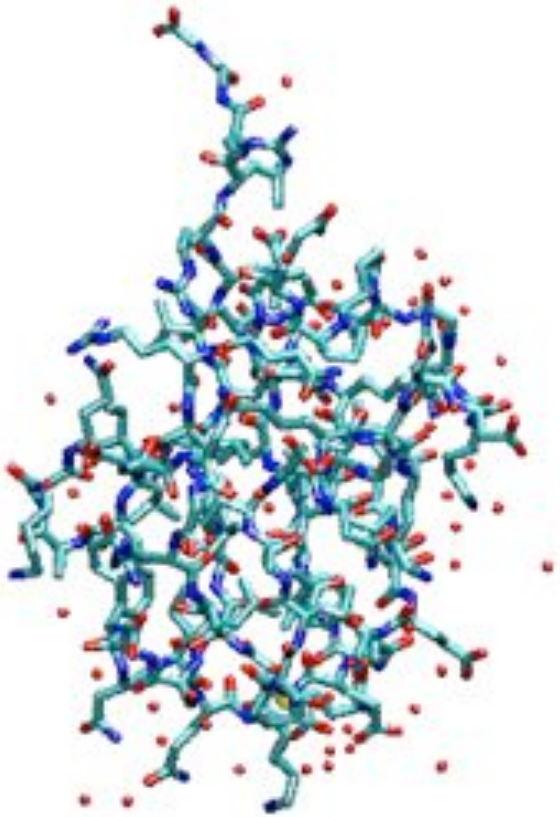
Selected Atoms

Resolution, Thickness

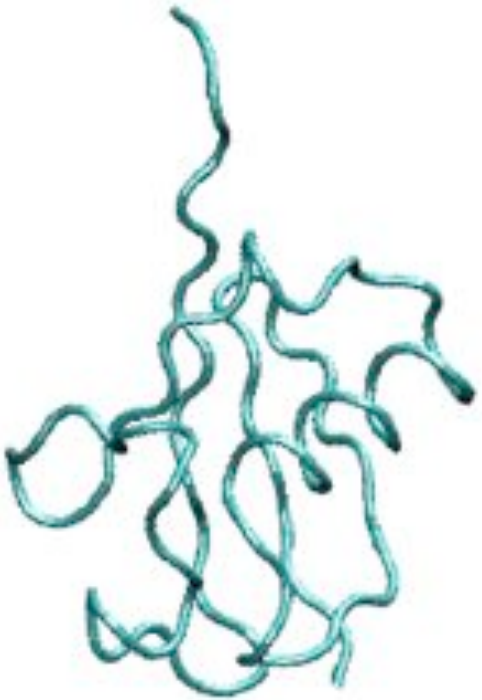
# Basics of VMD

## Change rendering style





CPK

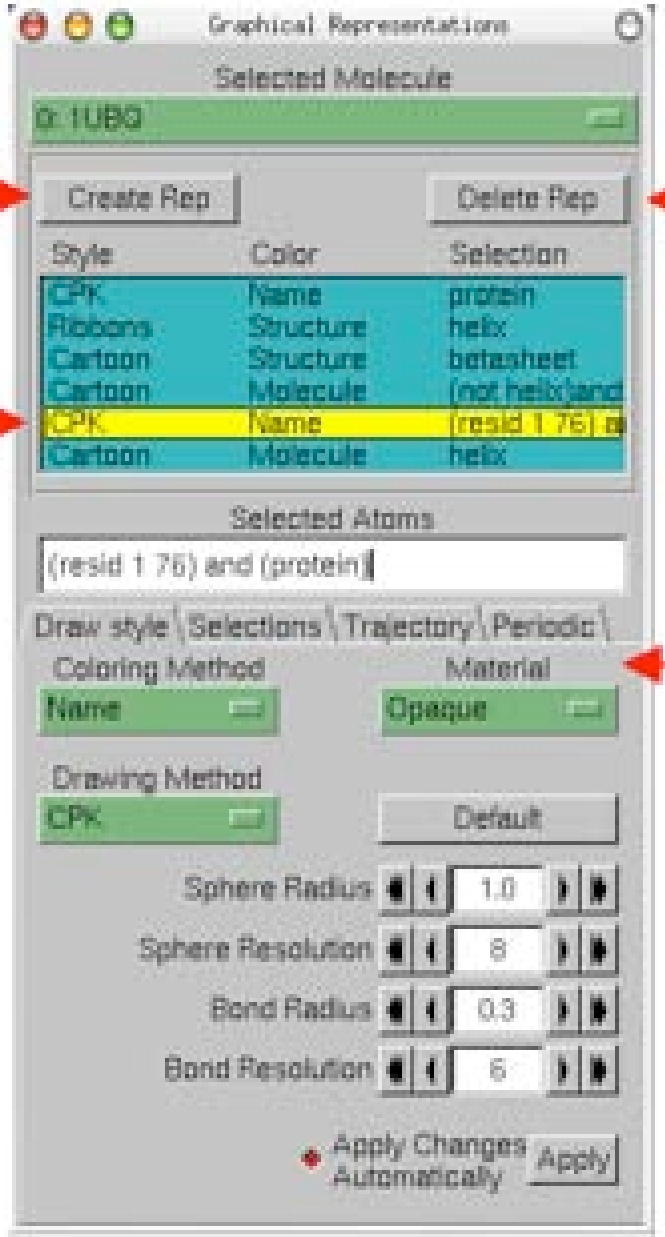


tube

cartoon

# Basics of VMD

**Multiple representations**



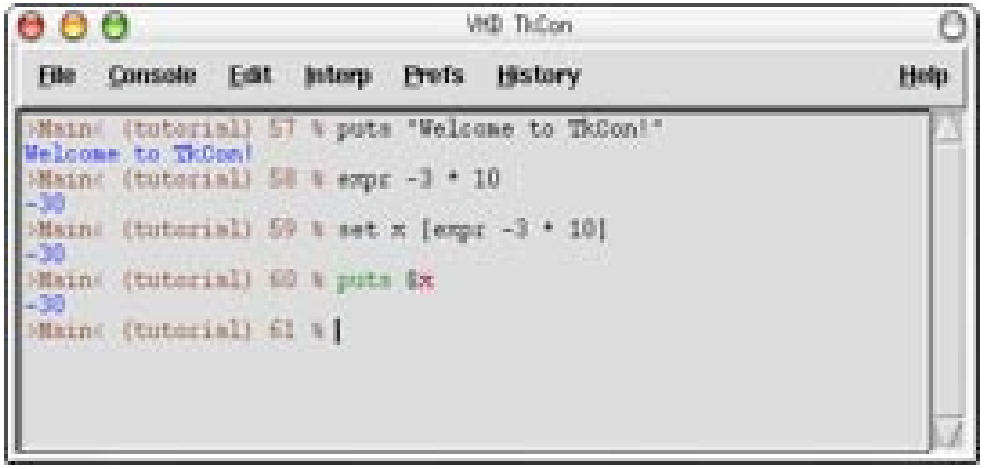

Create Representation (a)

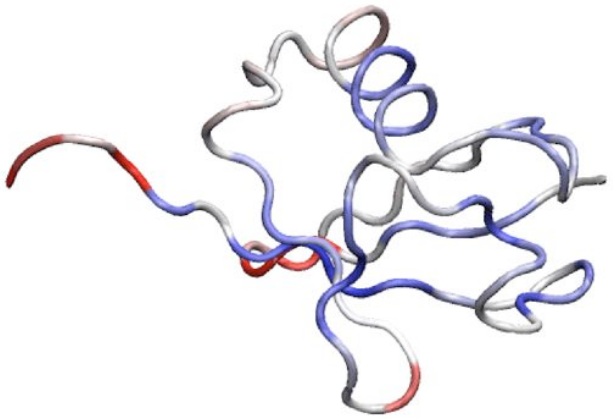
Delete Representation (b)

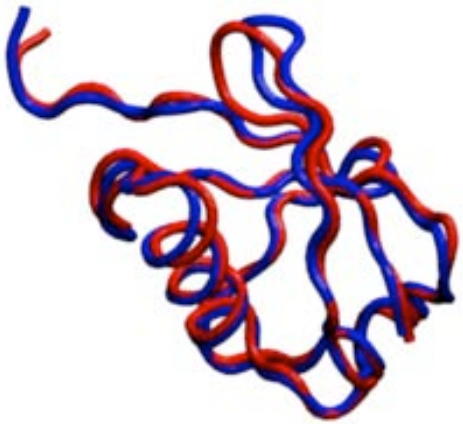
Current Representation (d)

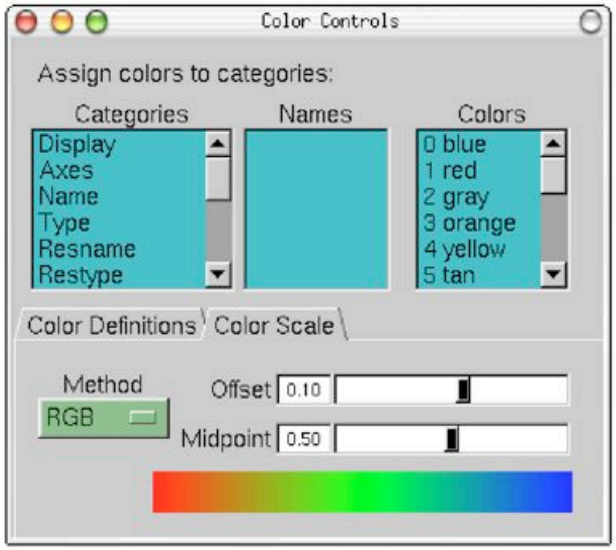
Material (c)

# VMD Scripting



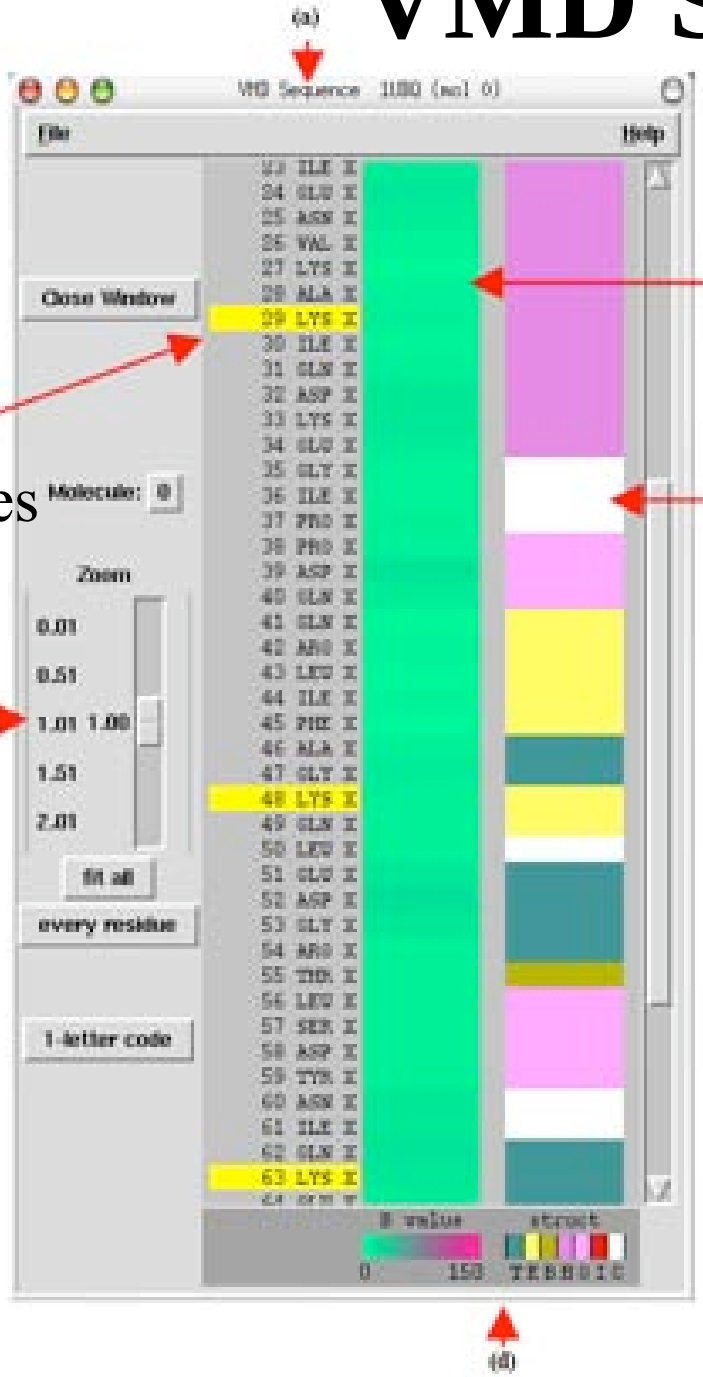





Left: Initial and final states of ubiquitin after spatial alignment
Right (top): Color coding of deviation between initial and final

The Color Controls window showing the Color Scale tab.

# VMD Sequence Window

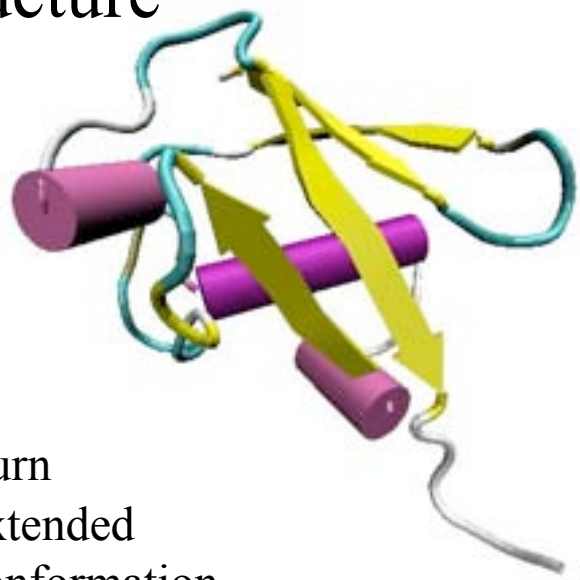



List of the residues

Zoom

Beta Value

Structure



T: Turn
E: Extended conformation
H: Helix
B: Isolated Bridge
G: 3-10 helix
I: Phi helix

# VMD Macros to Color Beta Strands

Use VMD scripting features to color beta strands separately; show hydrogen bonds to monitor the mechanical stability of ubiquitin

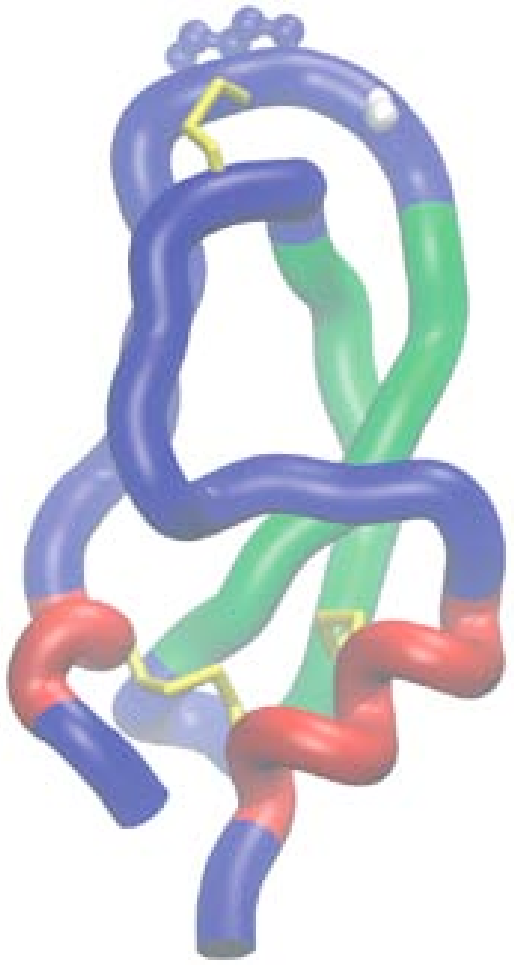

**Ubiquitin stretched between the C terminus and K48  does not fully extend!**

# Discovering the Mechanical Properties of Ubiquitin

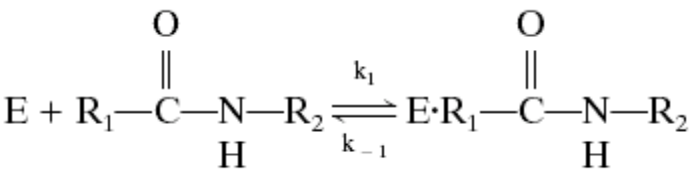
**Ubiquitin stretched between the C and the N termini extends fully!**

# Discover BPTI on your own!

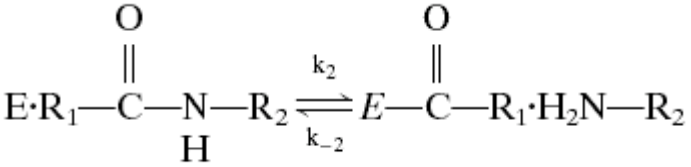
*bovine pancreatic trypsin inhibitor*



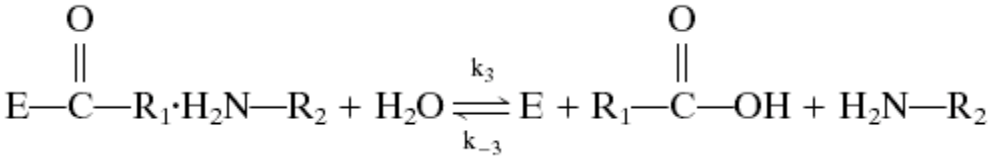
- small (58 amino acids)
- rigid
- binds as an inhibitor to Trypsin
  (a serine proteolytic enzyme, that appea
  system of mammalians.)
- blocks its active site.



(Michaelis complex)



(Acyl–enzyme with leaving peptide noncovalently bound)



(Free enzyme and products)

Mechanism of cleavage of peptides with serine proteases.
Radisky E. and Koshland D. Jr., Proc. Natl. Acad. Sci., USA, 99, 10316-10321

Trypsin: A proteolytic enzyme that hydrolyzes peptide bonds on the carboxyl side of Arg or Lys.

# BPTI: A "standard mechanism" inhibitor

- Binds to Trypsin as a substrate.

  forms an acyl-enzyme intermediate rapidly.

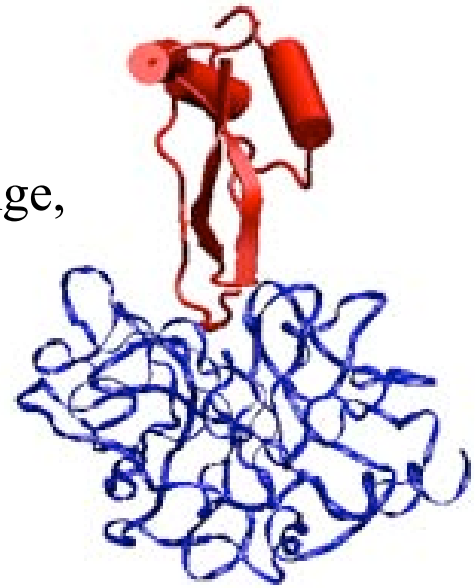
- Very little **structural changes** in trypsin or BPTI.

  several H-bonds between backbone of the two proteins change,

  little reduction in conformational entropy → binds tightly

- Remains uncleaved.

  hydrolysis is $10^{11}$ times slower than for other substrates



Structures of the protease binding region, in the proteins of all 18 families of standard mechanism inhibitors are similar.

# Why does Trypsin cleave BPTI so slowly?

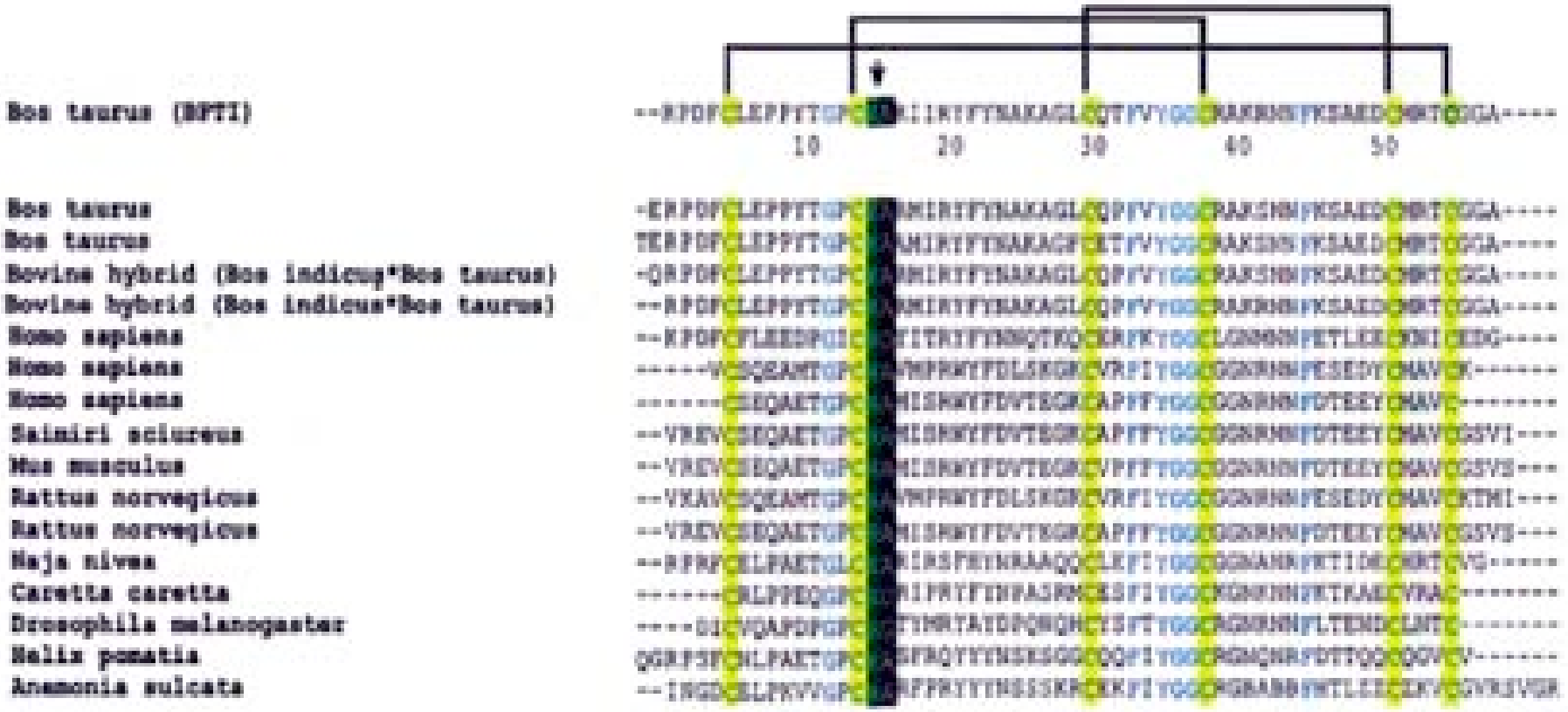
• Disruption of the non-covalent bonds in the tightly bonded enzyme-inhibitor complex increases the energy of transition states for bond cleavage.

• Water molecules do not have access to the active site, because of the tight binding of Trypsin and BPTI.

• After the cleavage of the active-site peptide bond, the newly formed termini are held in close proximity, favoring reformation of the peptide bond.

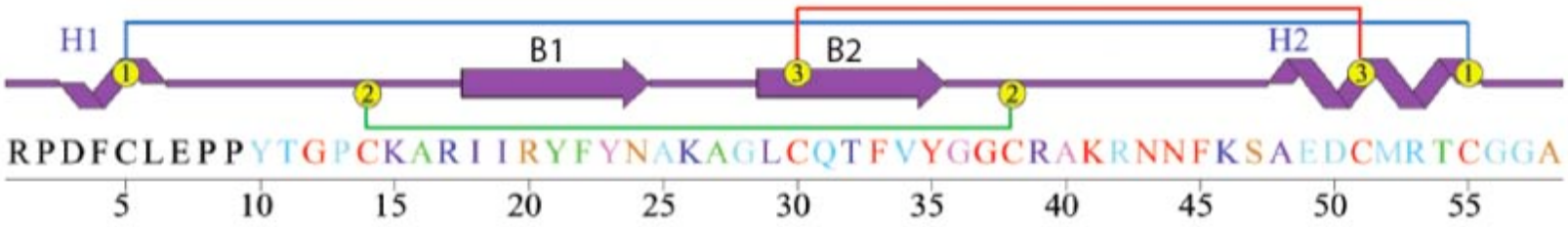
• The rigidity of BPTI may also contribute by not allowing necessary atomic motions.

# Amino acid sequence alignment of BPTI-like proteins



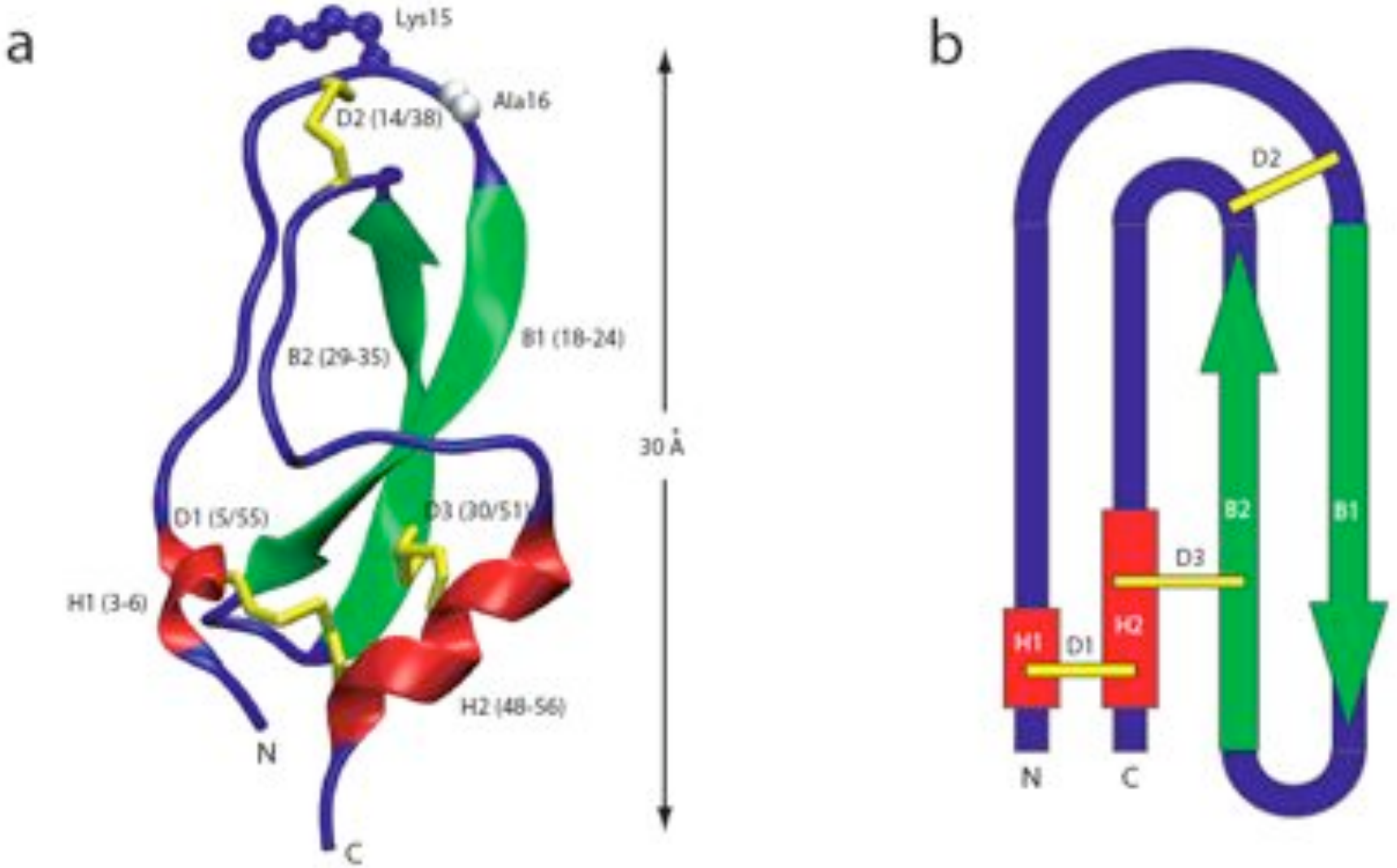

Reactive conserved P and P’ residues are highlighted in black and indicated by the arrow. Six conserved cysteine residues are highlighted in yellow. Three disulfide bonds formed by the cysteines are indicated by black lines. Other residues that are conserved in all proteins are labeled in blue.

# BPTI secondary structure

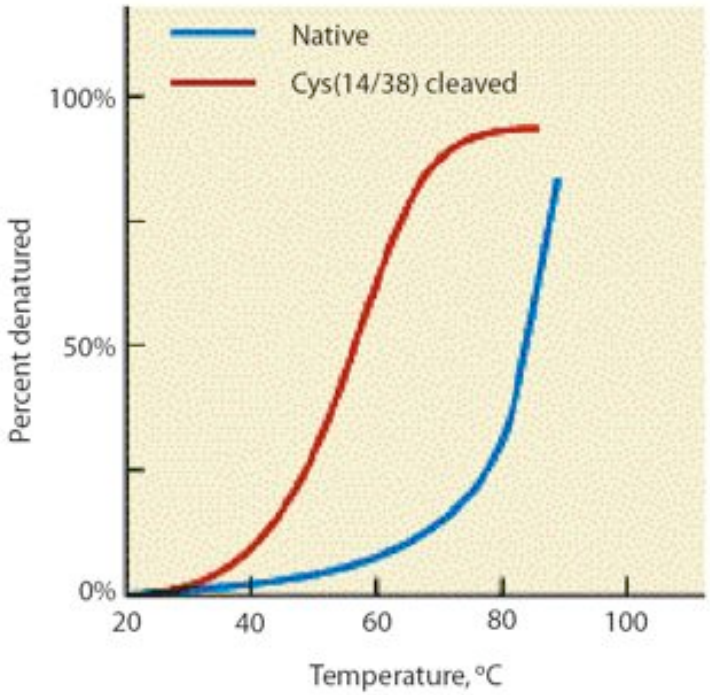


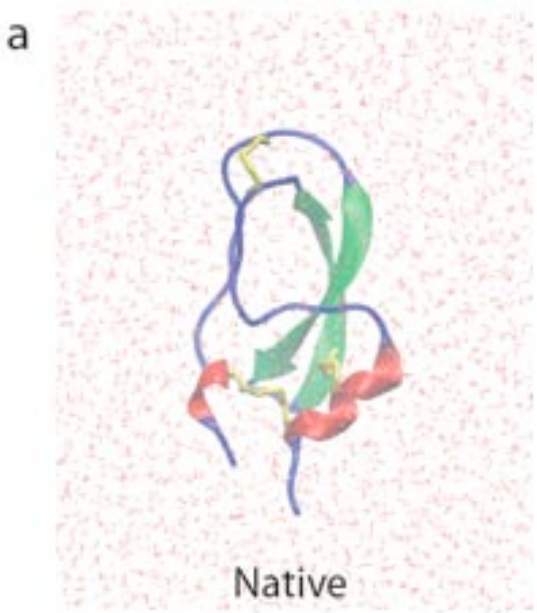

BPTI secondary structure: Consevation is indicated by color using rainbow scale coloring (Blue to red= low to high)

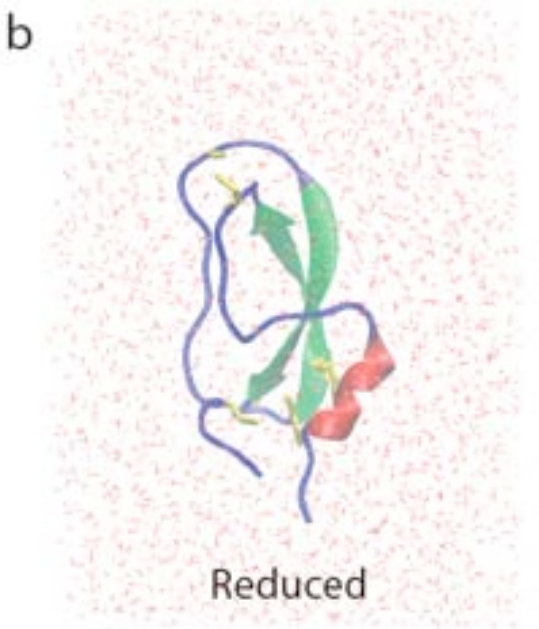
# BPTI Tertiary Structure



# Stability of native and reduced BPTI



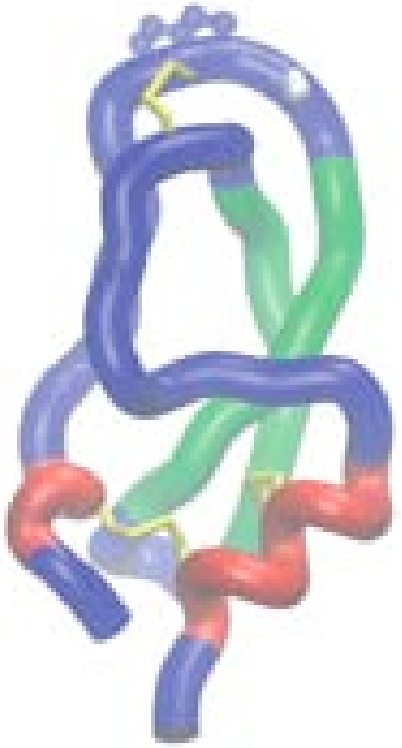

Experiment

Can be tested through simulation

# BPTI case study

Chalermpol Kanchanawarin
Department of Physics and Beckman Institute,
University of Illinois at Urbana-Champaign
Urbana, IL 61801, USA

Date: Tuesday 11th January 2005



# Inspect BPTI with  VMD