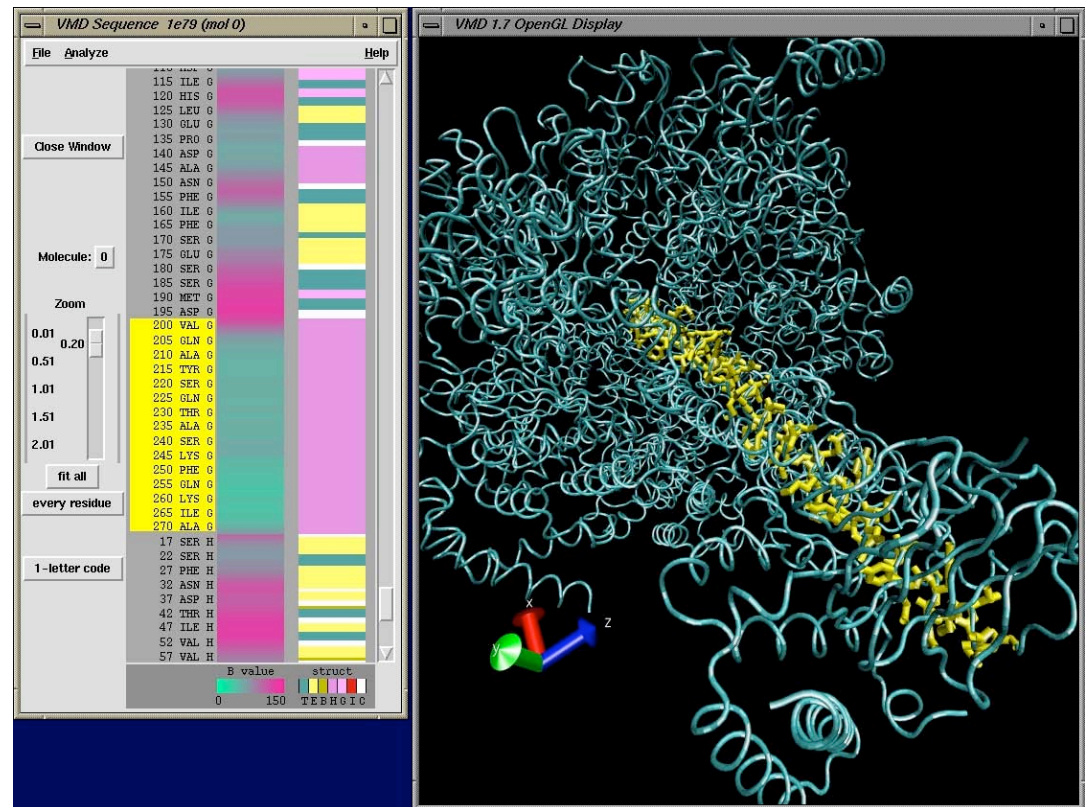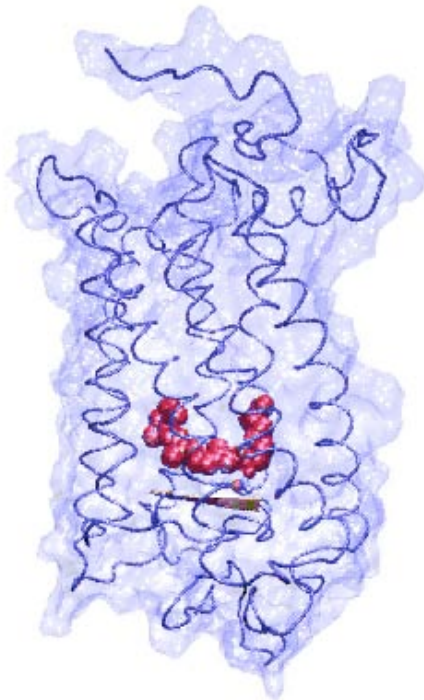# Molecular Graphics Perspective of Protein Structure and Function

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

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
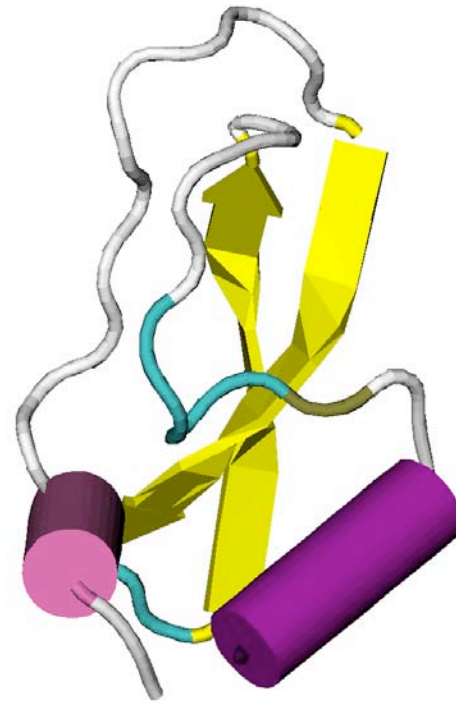

Purple Membrane
150,000 Atoms

F1 ATPase
327,000 Atoms
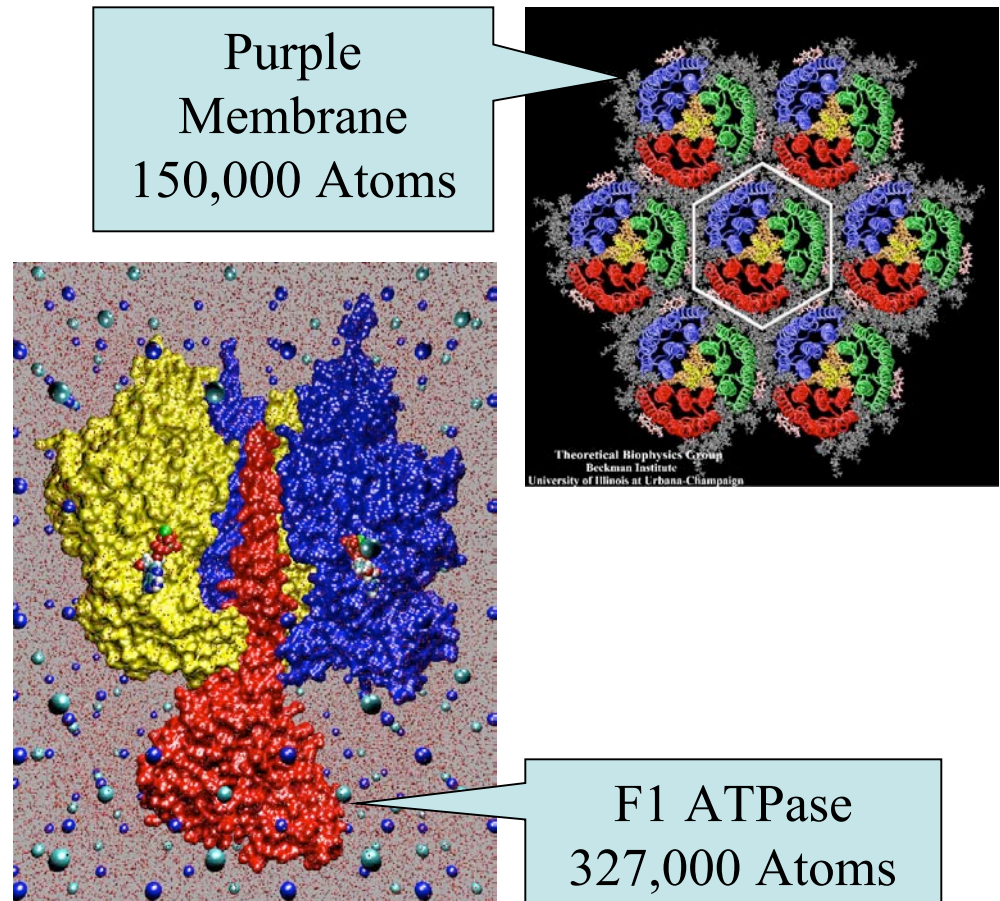
Focus on two proteins
Ubiquitin
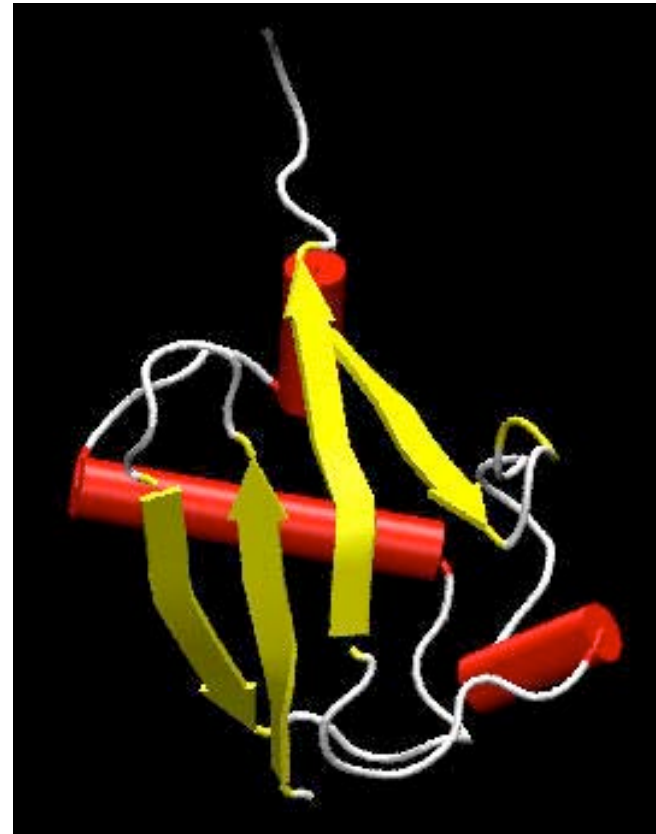## Bovine Pancreatic Trypsin Inhibitor (BPTI)
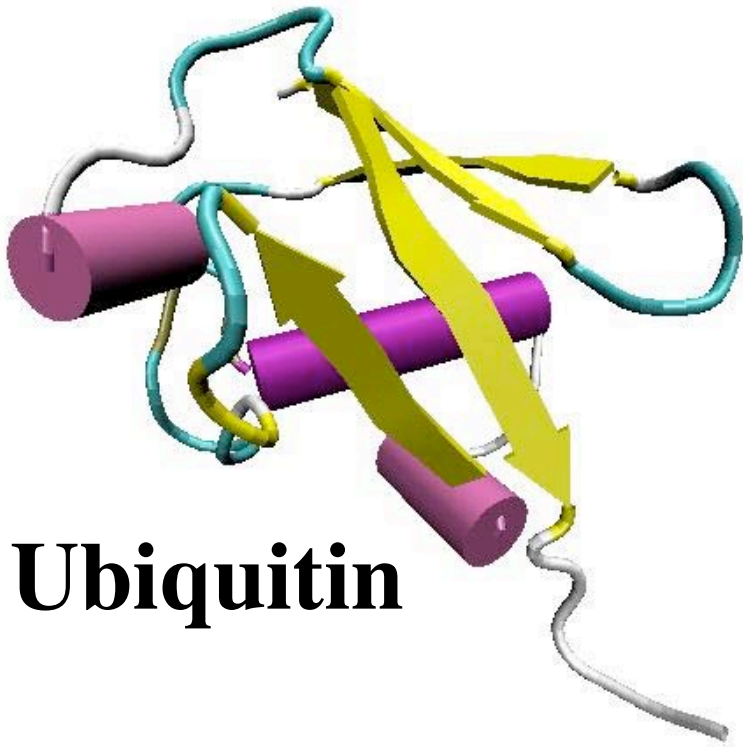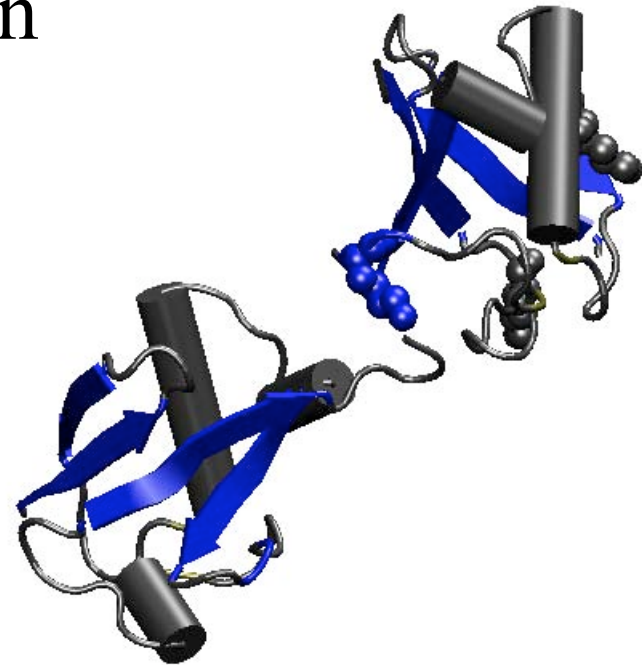

Ubiquitin

BPTI

Ubiquitin

- 76 amino acids

- highly conserved

- Covalently attaches to proteins and tags them for degradation

• 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

- 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 proteins to be degraded in the proteasome.

• degrading misfolded proteins

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

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

# Ubiquitin acts independent of proteasome degradation

- Controlling the traffic in the cell

- Directing the traffic in the cell, i.e., determining where the newly synthesized proteins should go

- Tagging membrane proteins for internalization

Hicke, L., Protein regulation by monoubiquitin, Nat. rev. mol cell biol., 2, 195-201 (2001)
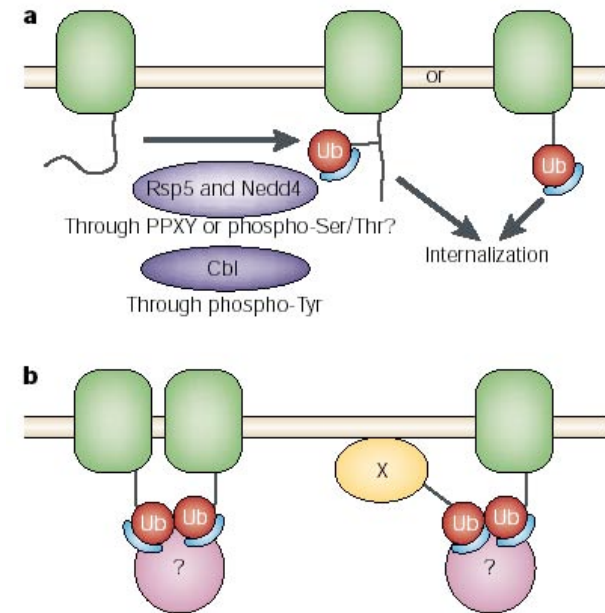
2. Regulating gene expression:

(indirectly, by destruction of some of the involved proteins)

• Recruiting Transcription Factors (proteins needed for gene expression)

• Conformational changes in Histone, necessary before gene expression
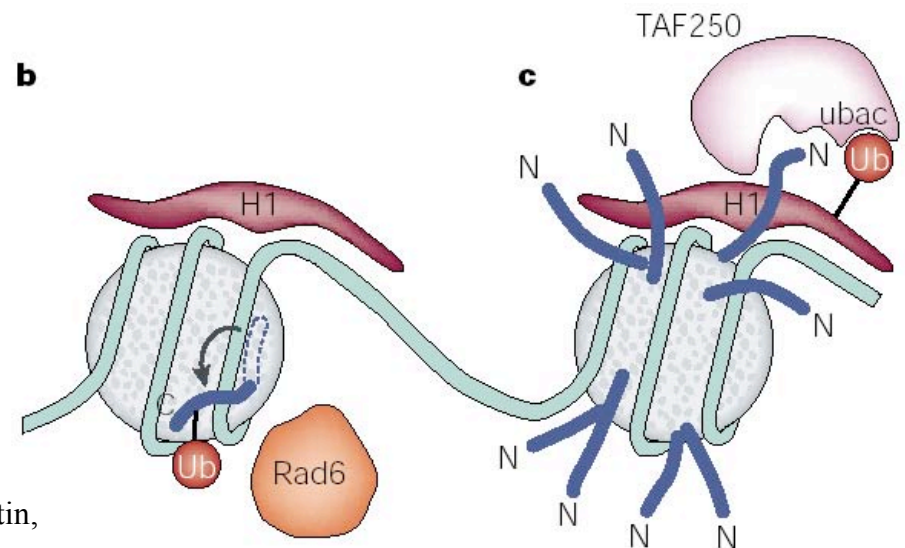
Hicke, L., Protein regulation by monoubiquitin, Nat. rev. mol cell biol., 2, 195-201 (2001)
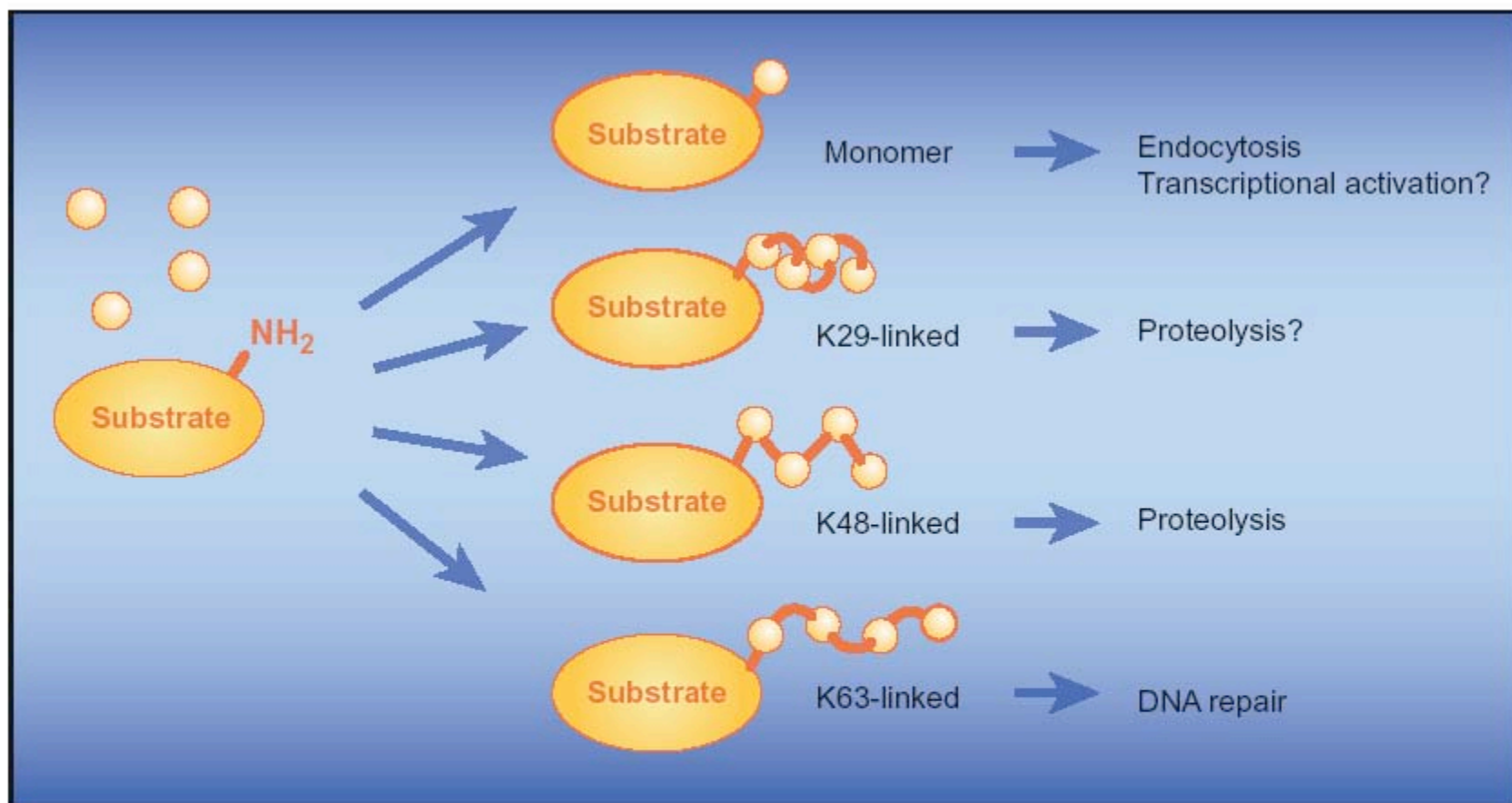
Different types of ubiquitin signals arise from

• 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

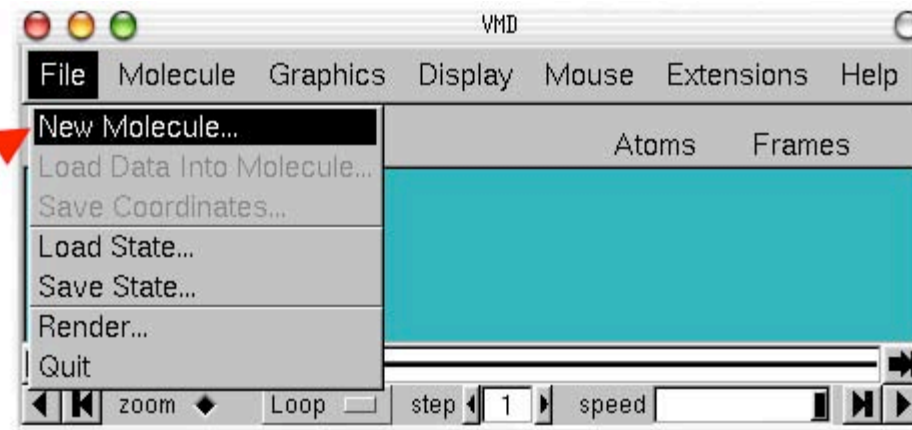# Mono-ubiquitylation versus multi-ubiquitylation

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.)

Basics of VMD

Loading a Molecule
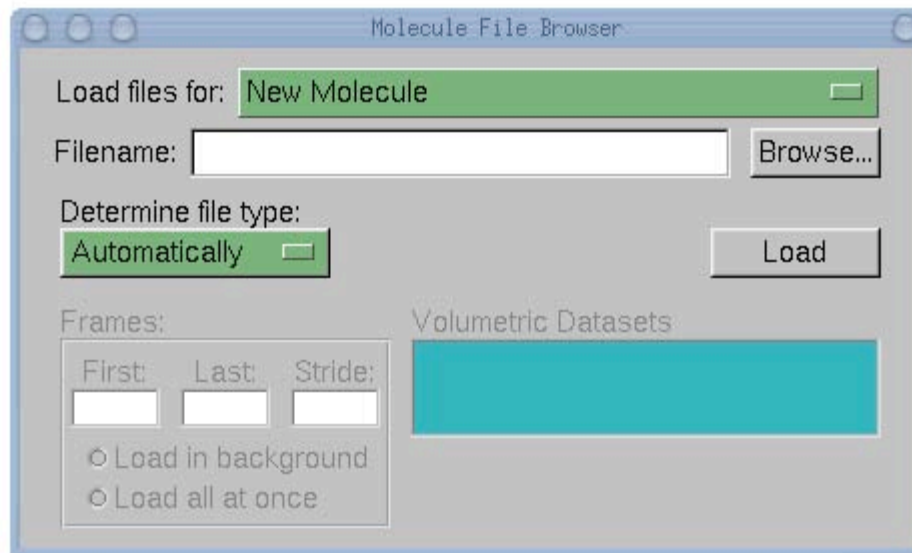
Basics of VMD

Rendering a Molecule
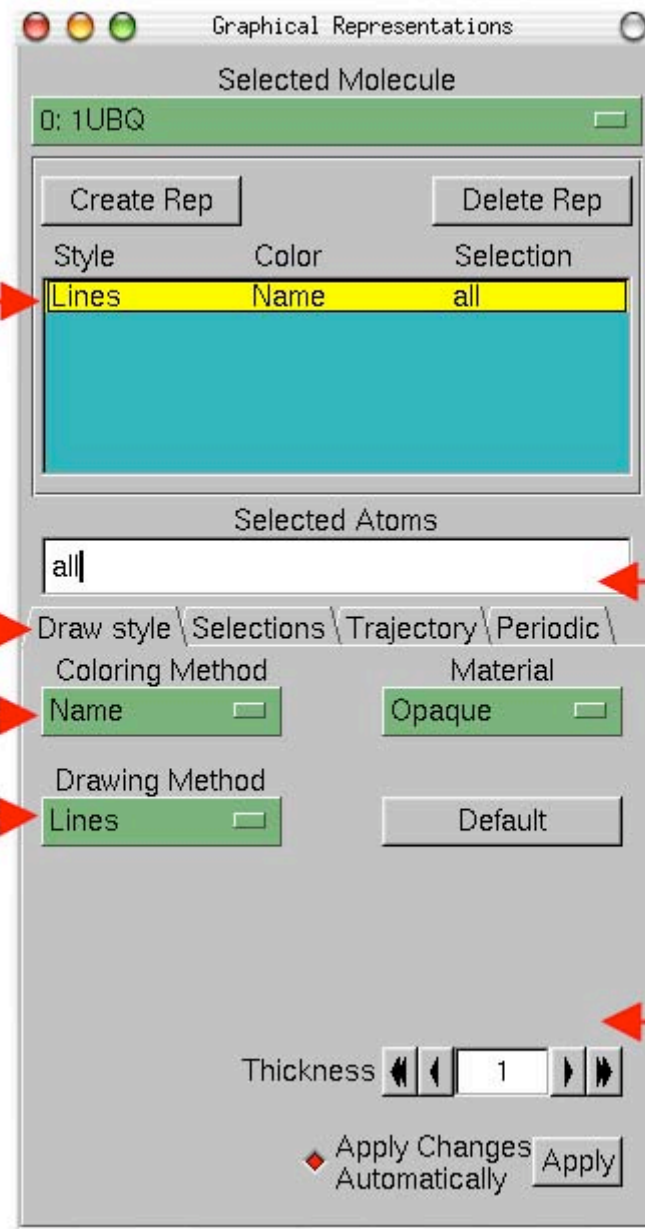
Basics of VMD

Change rendering style
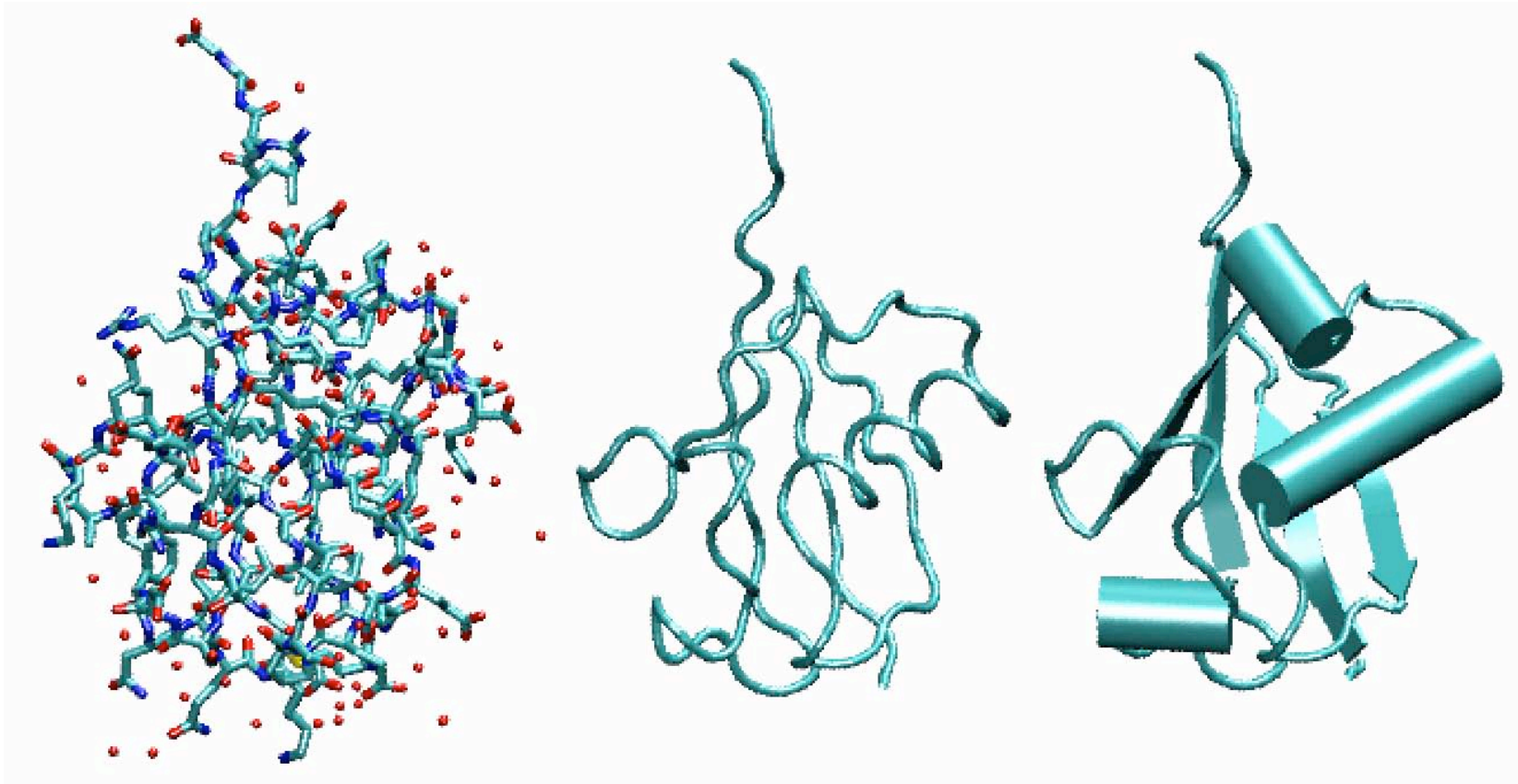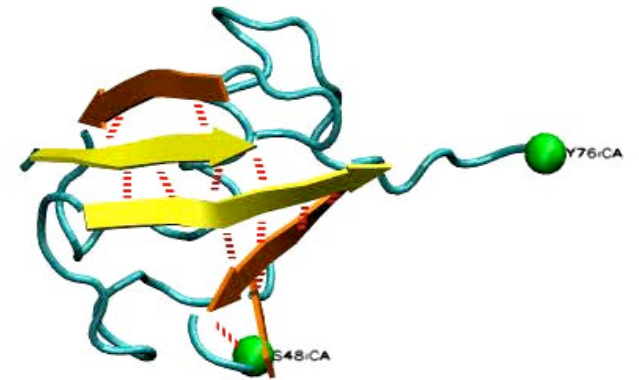
CPK   tube   cartoon

Basics of VMD

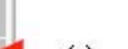Create Representation (a) → ← (b) Delete Representation

Current (d) → Representation

Multiple representations ← (c) Material

VMD Scripting
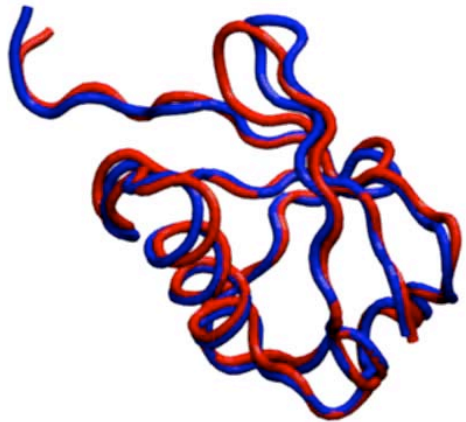
Left: Initial and final states of
ubiquitin after spatial alignment
Right (top): Color coding of
deviation between initial and final
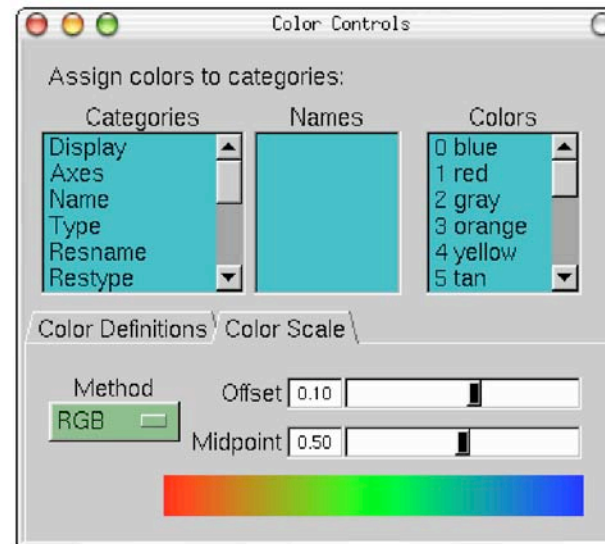
The Color Controls window showing the Color Scale tab.

VMD Sequence Window

List of the residues (e)

Molecule: 0

Zoom

0.01

0.51

Zoom (f) 1.01 1.00

1.51

2.01

fit all

every residue

1-letter code

(b) Beta Value

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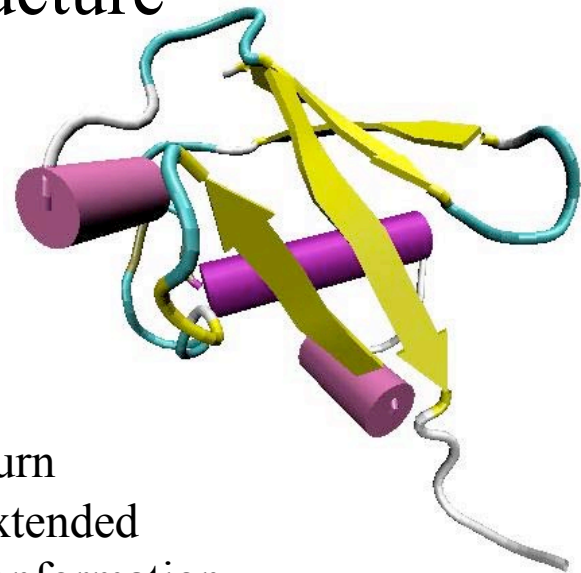
T: Turn
E: Extended
   conformation
H: Helix
B: Isolated Bridge
G: 3-10 helix
 I: Phi helix

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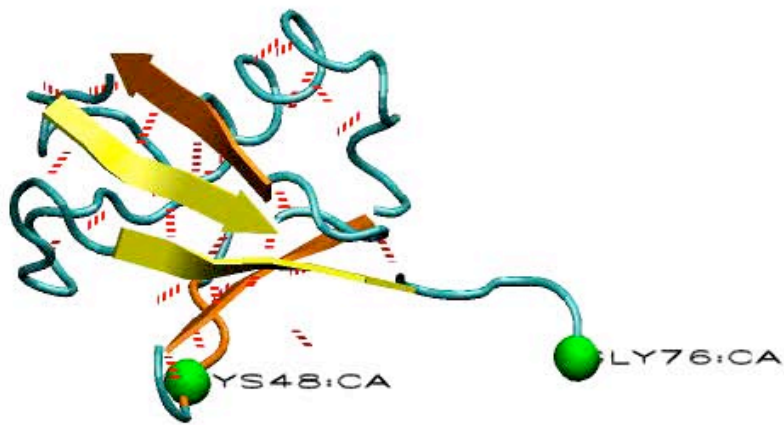
Ubiquitin stretched between the C terminus and K48 does not fully extend!

Discovering the Mechanical Properties of Ubiquitin
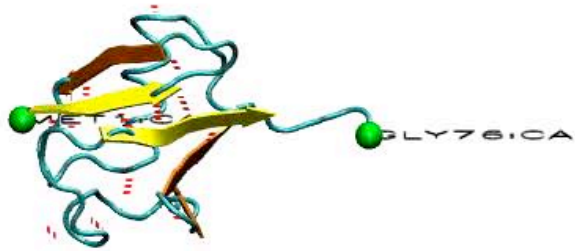
Ubiquitin stretched between the C and the N termini extends fully!

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 appears in digestive
system of mammalians.)

• Blocks its active site.

$$E + R_1-\overset{\overset{\textstyle O}{\|}}{C}-\underset{\underset{\textstyle H}{}}{N}-R_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\cdot}R_1-\overset{\overset{\textstyle O}{\|}}{C}-\underset{\underset{\textstyle H}{}}{N}-R_2$$

(Michaelis complex)
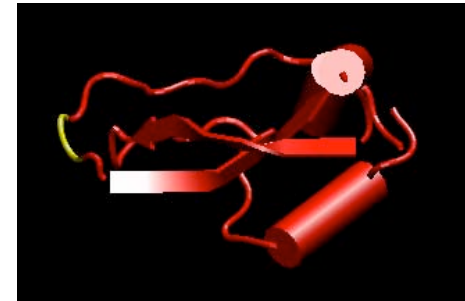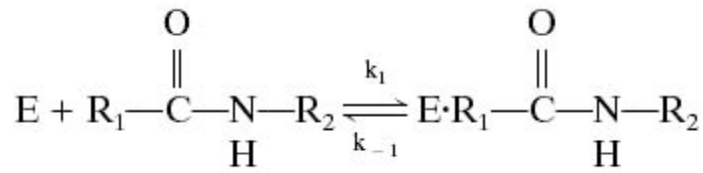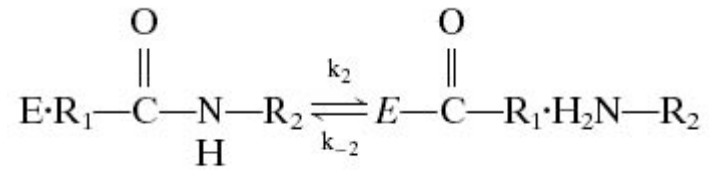
$$E{\cdot}R_1-\overset{\overset{\textstyle O}{\|}}{C}-\underset{\underset{\textstyle H}{}}{N}-R_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E-\overset{\overset{\textstyle O}{\|}}{C}-R_1{\cdot}H_2N-R_2$$

(Acyl–enzyme with leaving peptide noncovalently bound)

$$E-\overset{\overset{\textstyle O}{\|}}{C}-R_1{\cdot}H_2N-R_2 + H_2O \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + R_1-\overset{\overset{\textstyle O}{\|}}{C}-OH + H_2N-R_2$$
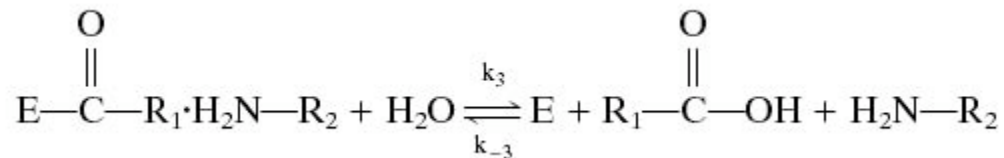
(Free enzyme and products)

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 char

  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.
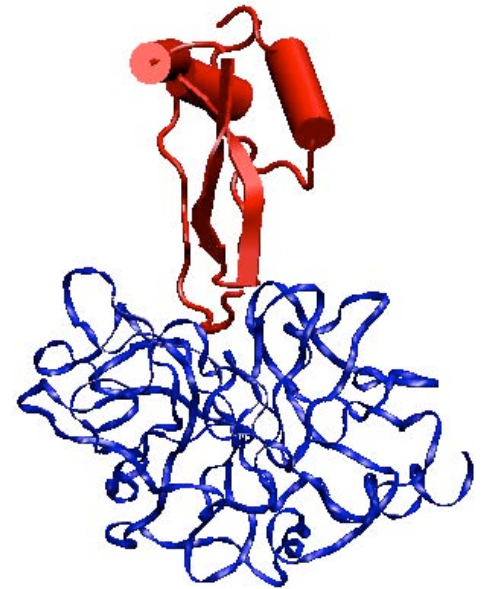
Why does Trypsin cleave BPTI so slowly?

• 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.

Dance of Ubiquitin