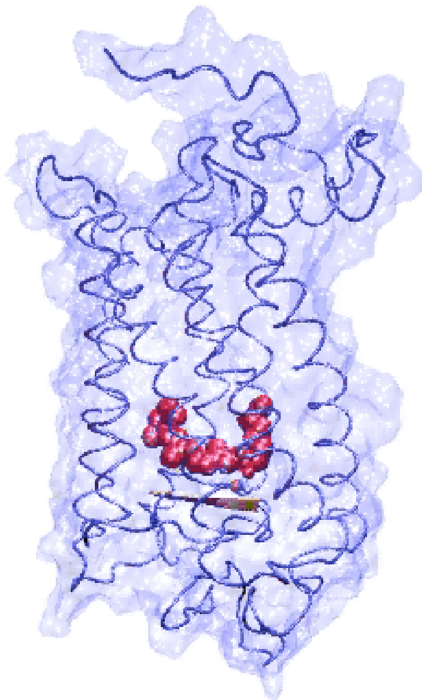
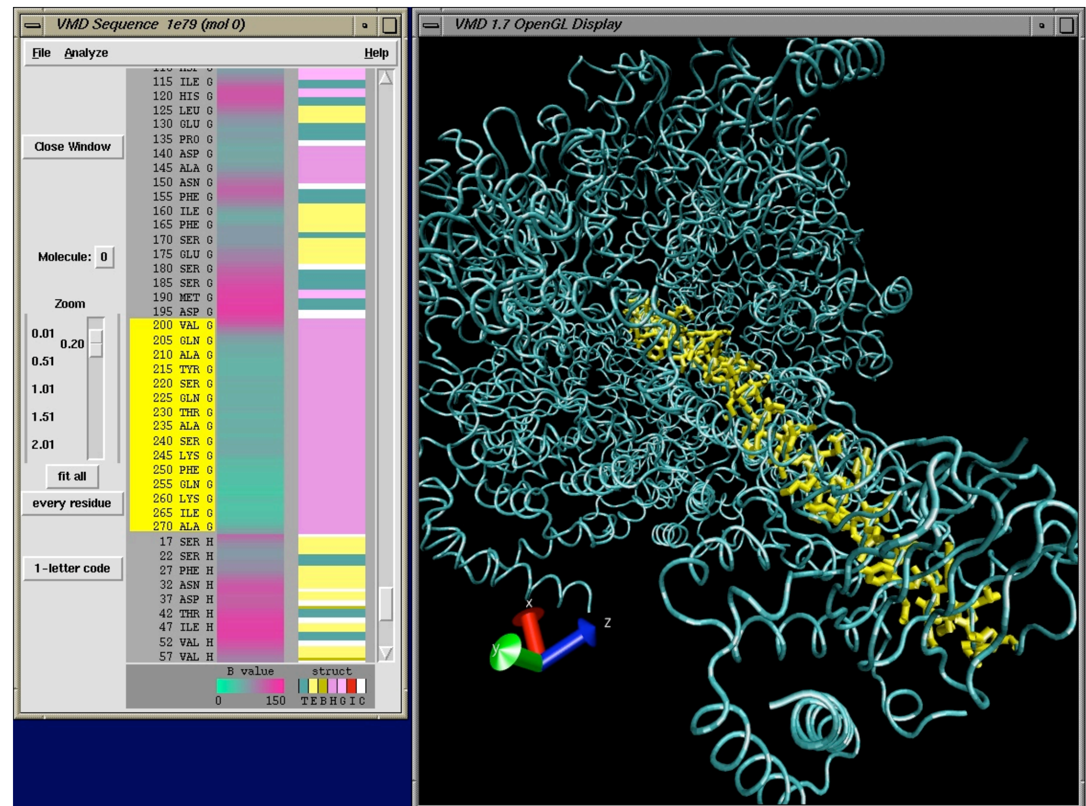
Molecular Graphics Perspective of Protein Structure and Function



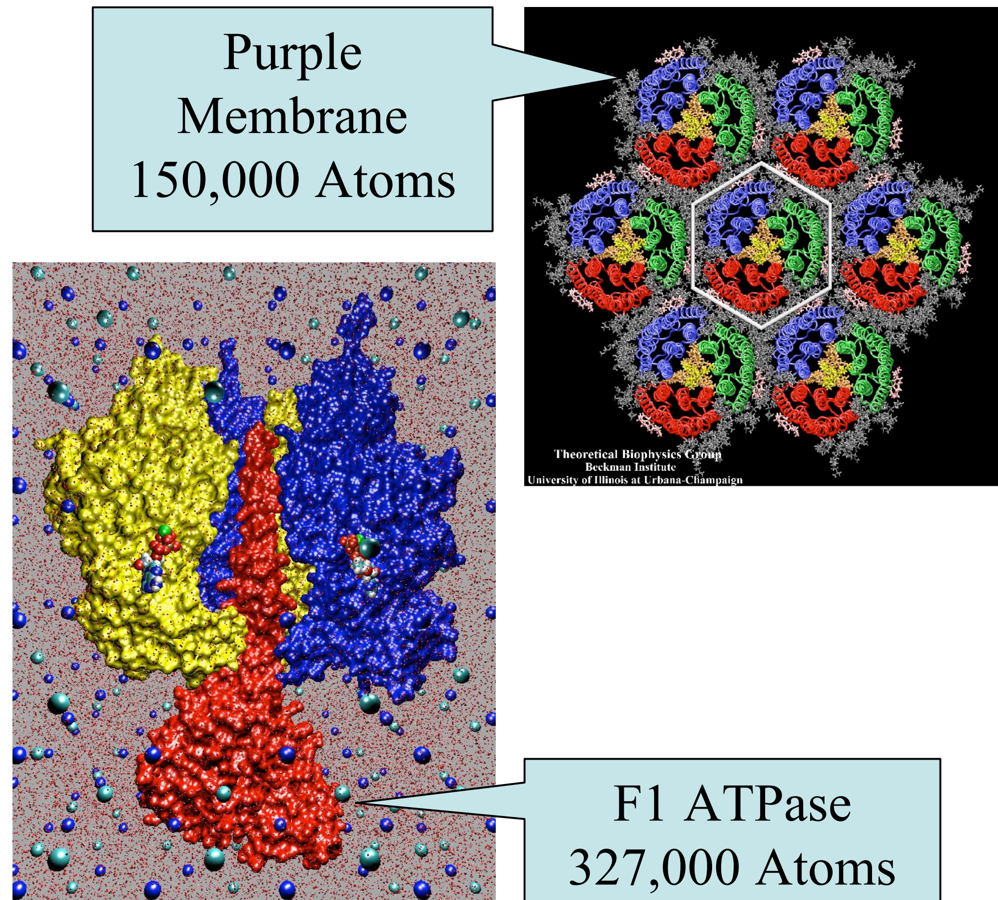


VMD Highlights

- > 20,000 registered Users
- Platforms:
  - Unix (16 builds)
  - Windows
  - MacOS X
- Display of large biomolecules and simulation trajectories
- Sequence browsing and structure highlighting
- User-extensible scripting interfaces for analysis and customization

VMD Permits Large Scale Visualization

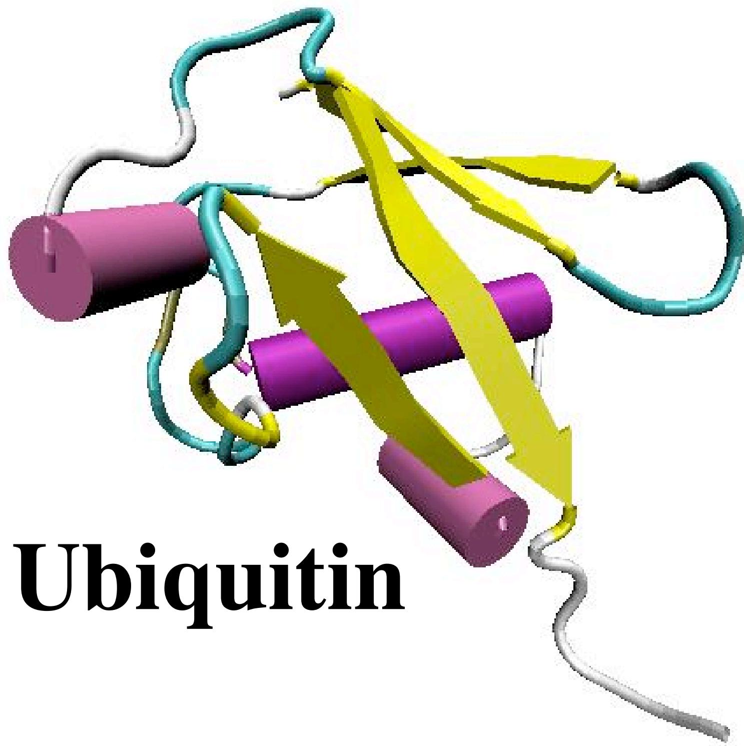
- Large structures: 300,000 atoms and up
- Complex representations
- Long trajectories: thousands of timesteps
- Volumetric data
- Multi-gigabyte data sets break 32-bit barriers
- GlpF: each 5 ns simulation of 100K atoms produces a 12GB trajectory



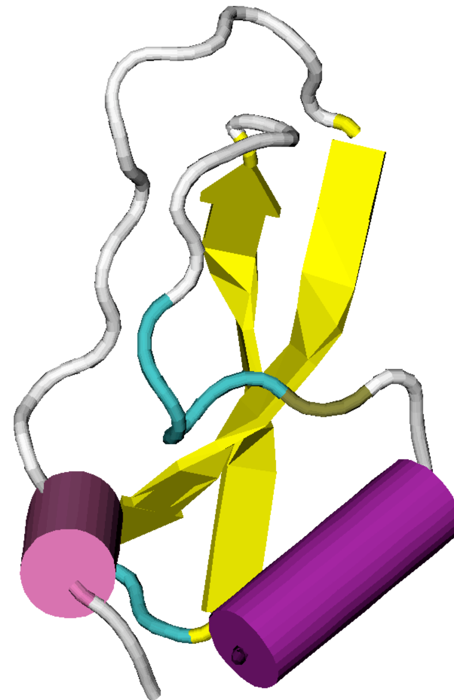
Focus on two proteins

Ubiquitin

Bovine Pancreatic Trypsin Inhibitor (BPTI)



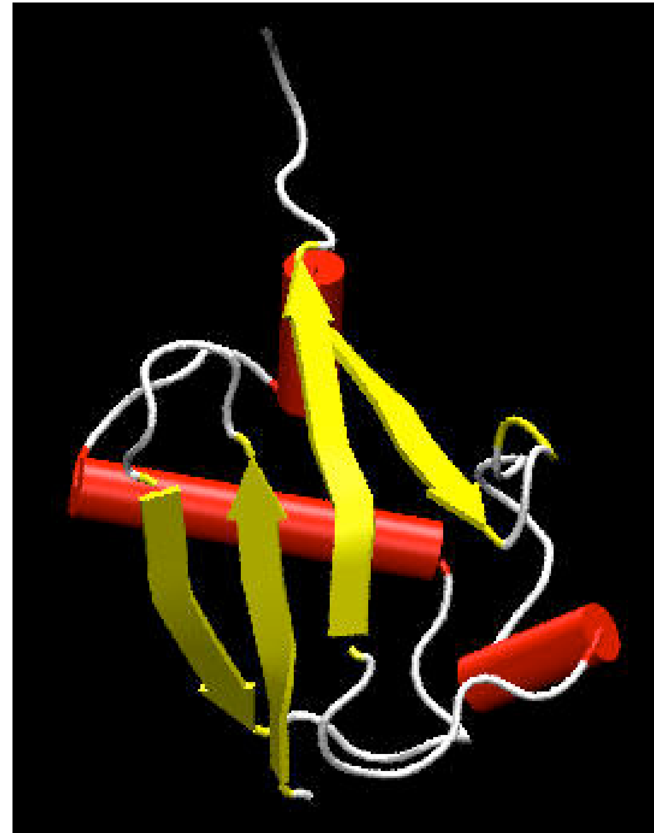
Ubiquitin



BPTI

Ubiquitin

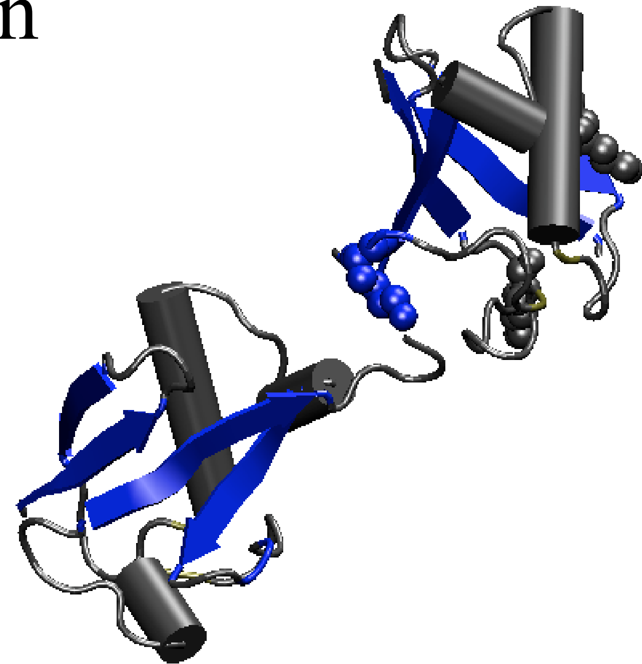
- 76 amino acids
- highly conserved
- Covalently attaches to proteins and tags them for degradation



• 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 on the ubiquitin.



2 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)

- Transfers to a cysteine residue on E2

  (ubiquitin conjugation enzyme)

- E3 transfers the ubiquitin to the substrate lysine residue.

- E3 recognizes the ubiquitination signal of the protein.

Ubiquitin Functions:

Tagging proteins to be degraded in proteasome.

- degrading misfolded proteins
- regulates key cellular processes such as cell division, gene expression, ...

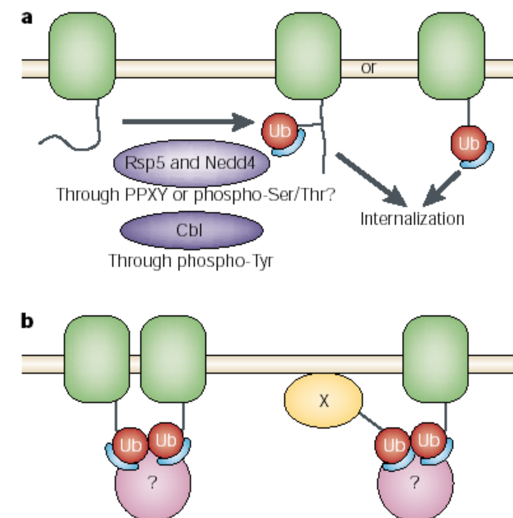
A chain of at least 4 ubiquitins is needed to be recognized by the proteasome.

Independent of proteasome degradation

1. Traffic Controller

- Directing the traffic in the cell.
  determines where the newly synthesized proteins should go

- Tagging membrane proteins for internalization

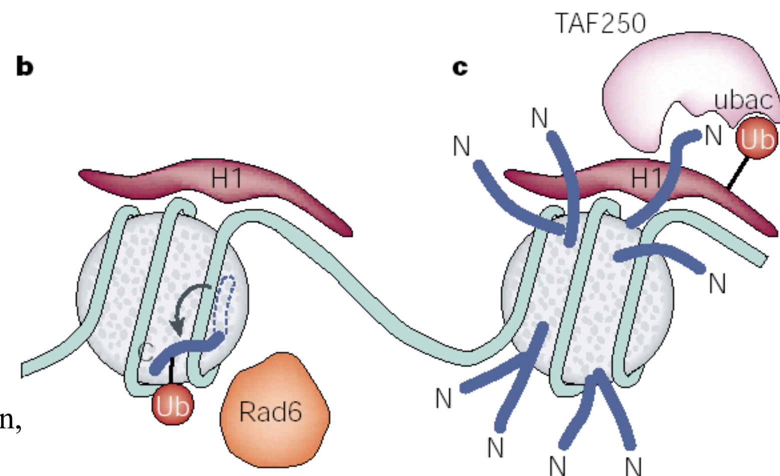


Hicke, L., Protein regulation by monoubiquitin, Nat. rev. mol cell biol., 2, 195-201 (2001)

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



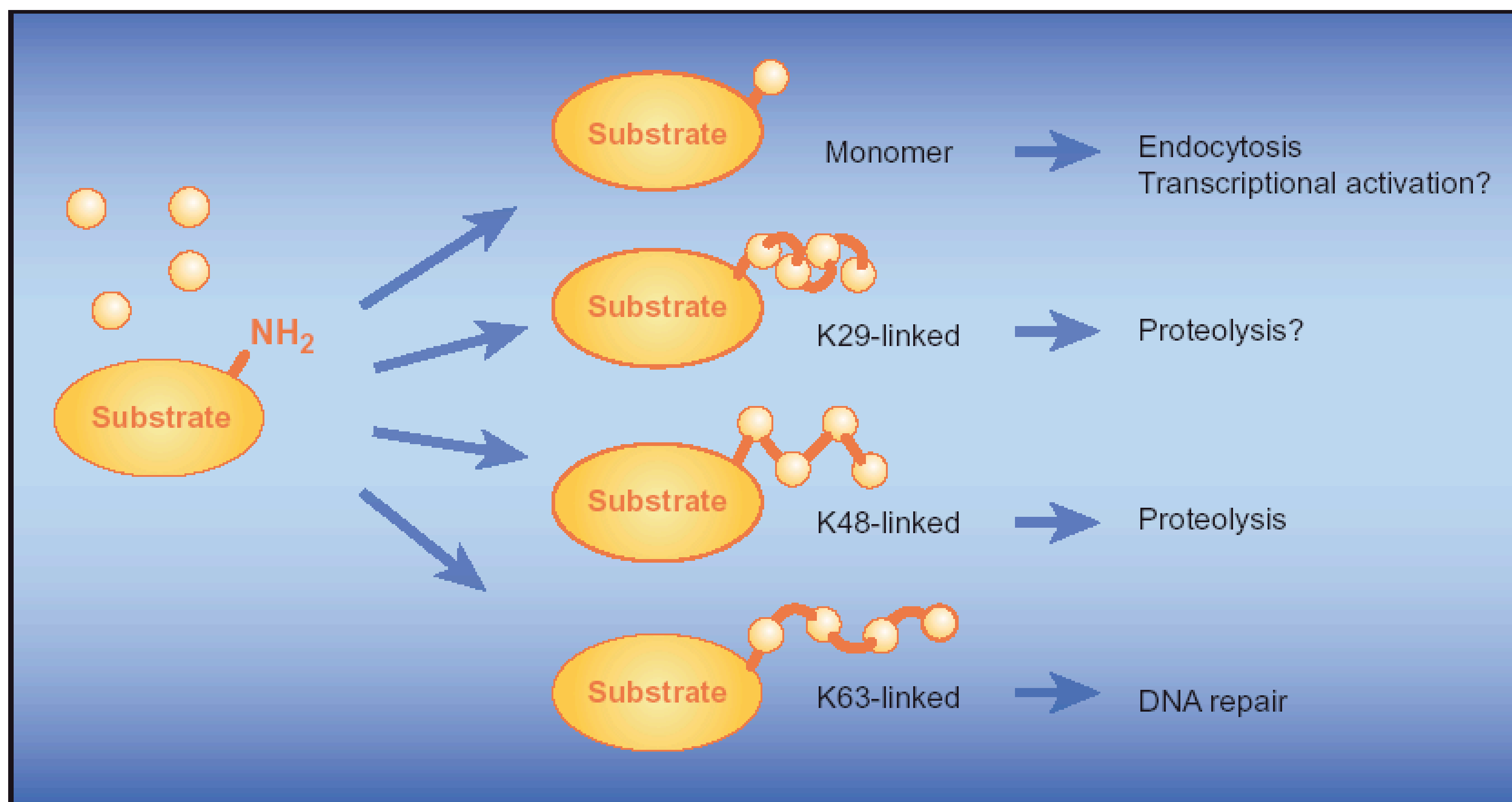
Hicke, L., Protein regulation by monoubiquitin, Nat. rev. mol cell biol., 2, 195-201 (2001)

Different types of ubiquitin signals

- Length of the ubiquitin chain.
- How they are attached together.
- Where it happens.

- multi-ubiquitin chains, linked through Lysine 48, target protein for proteasome degradation.
- K63 linkages are important for DNA repair and other functions.

Monoubiquitylation versus multi-ubiquitylation



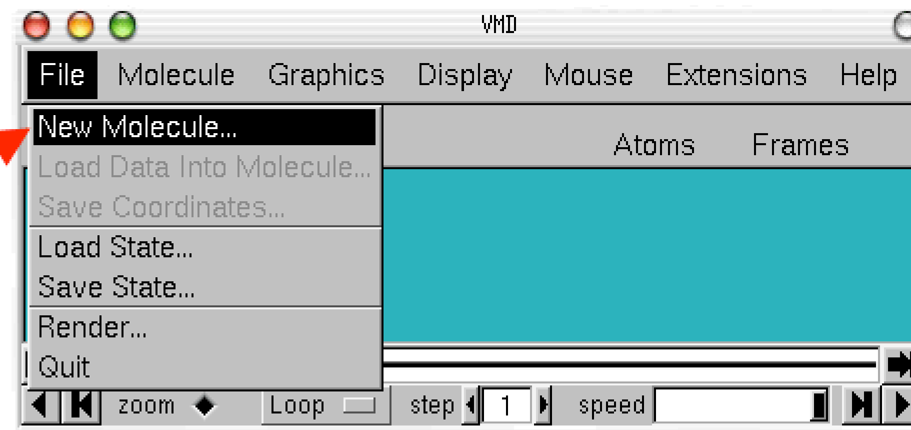
Multifaceted. Ubiquitin can attach to its various substrate proteins, either singly or in chains, and that in turn might determine what effect the ubiquitination has. (K29, K48, and K63 refer to the particular lysine amino acid used to link the ubiquitins to each other.)

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

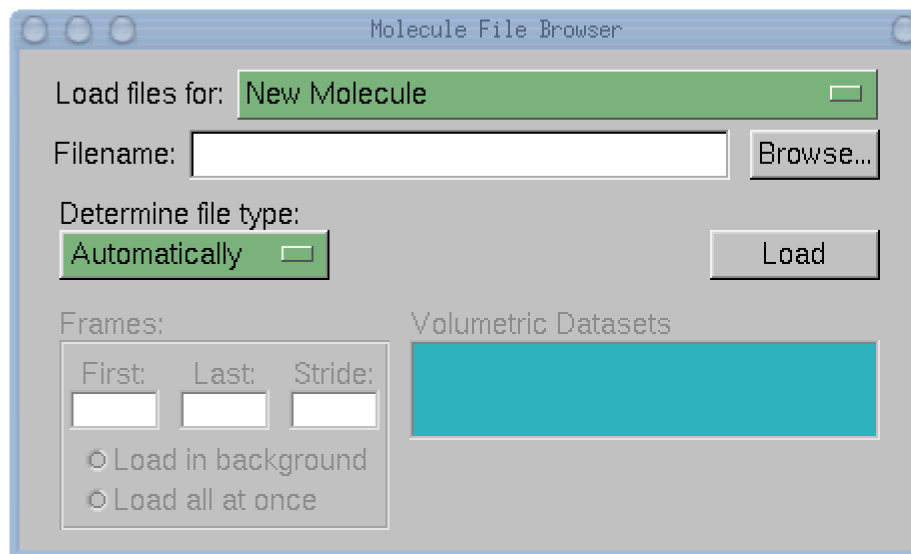
Basics of VMD

Loading a Molecule

New Molecule (a)



(b) Molecule file browser



(c) Browse

(d) Load

Basics of VMD

Rendering a Molecule

Current graphical representation (a)

Draw style (b)

Coloring (c)

Drawing method (d)

(f) Selected Atoms

(e) Resolution, Thickness

Graphical Representations

Selected Molecule

0: 1UBQ

Create Rep

Delete Rep

Style	Color	Selection
Lines	Name	all

Selected Atoms

all

Draw style | Selections | Trajectory | Periodic

Coloring Method

Name

Material

Opaque

Drawing Method

Lines

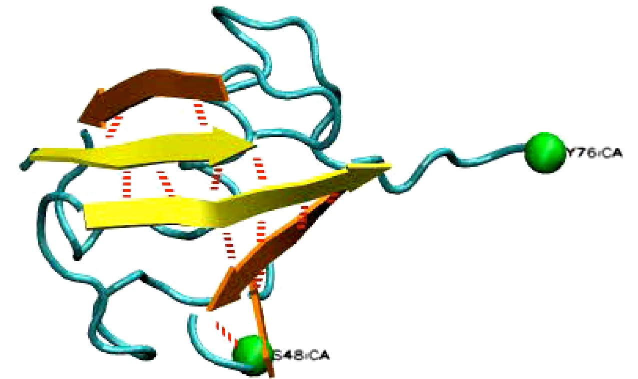
Default

Thickness 1

Apply Changes Automatically

Apply

Basics of VMD



Change rendering style

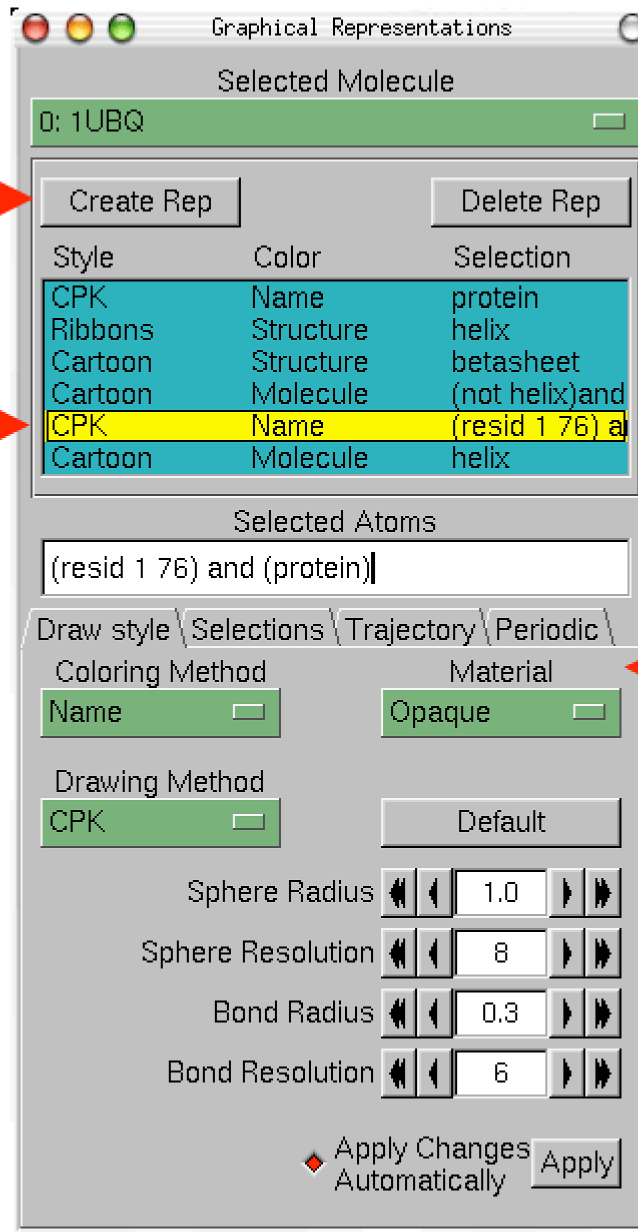
CPK | tube | cartoon

Basics of VMD

Create Representation (a)

Current Representation (d)

Multiple representations



(b) Delete Representation

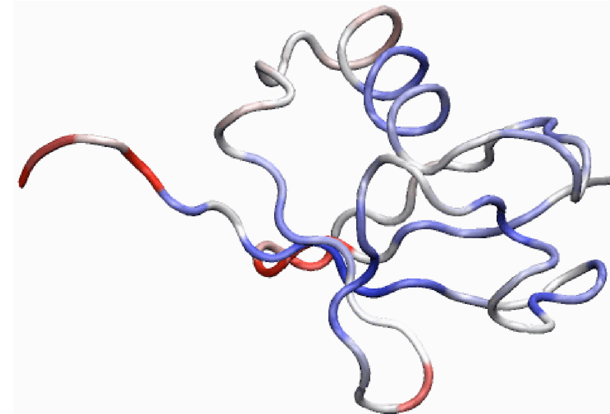
(c) Material

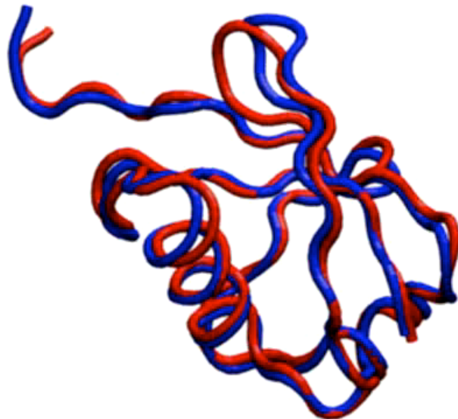
VMD Scripting

VMD TkCon

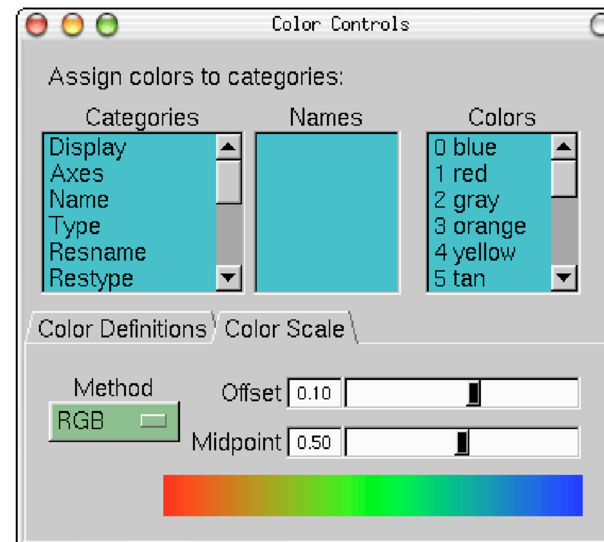
File Console Edit Interp Prefs History Help

```
>Main< (tutorial) 57 % puts "Welcome to TkCon!"
Welcome to TkCon!
>Main< (tutorial) 58 % expr -3 * 10
-30
>Main< (tutorial) 59 % set x [expr -3 * 10]
-30
>Main< (tutorial) 60 % puts $x
-30
>Main< (tutorial) 61 %
```





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

(a)

VMD Sequence 1UBQ (mol 0)

File Help

Close Window

Molecule: 0

Zoom

0.01
0.51
1.01 1.00
1.51
2.01

fit all

every residue

1-letter code

23 ILE X
24 GLU X
25 ASN X
26 VAL X
27 LYS X
28 ALA X
29 LYS X
30 ILE X
31 GLN X
32 ASP X
33 LYS X
34 GLU X
35 GLY X
36 ILE X
37 PRO X
38 PRO X
39 ASP X
40 GLN X
41 GLN X
42 ARG X
43 LEU X
44 ILE X
45 PHE X
46 ALA X
47 GLY X
48 LYS X
49 GLN X
50 LEU X
51 GLU X
52 ASP X
53 GLY X
54 ARG X
55 THR X
56 LEU X
57 SER X
58 ASP X
59 TYR X
60 ASN X
61 ILE X
62 GLN X
63 LYS X

B value 0 150

struct T E B H G I C

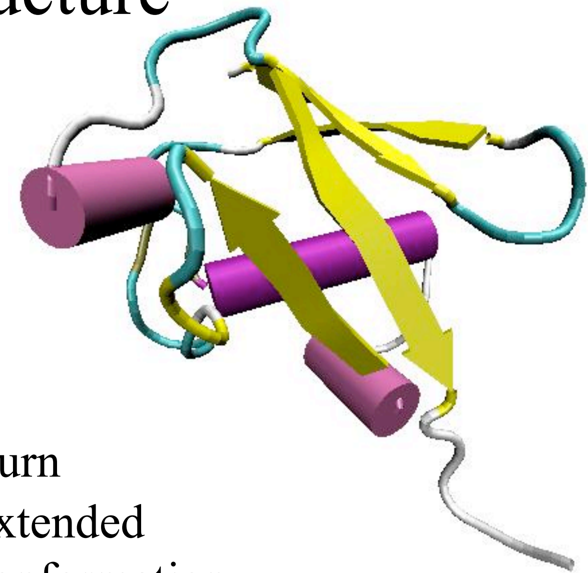
(d)

(b) Beta Value

(c) Structure

List of the residues (e)

Zoom (f)



T: Turn

E: Extended conformation

H: Helix

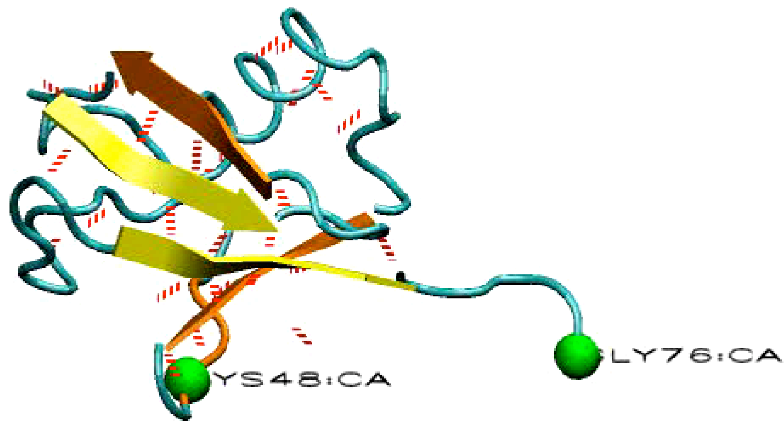
B: Isolated Bridge

G: 3-10 helix

I: Phi helix

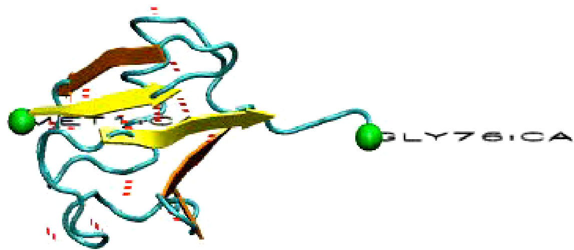
VMD Macros to Color Beta Strands

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!

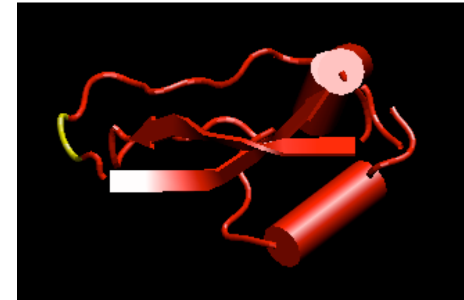
Discovering the Mechanical Properties of Ubiquitin



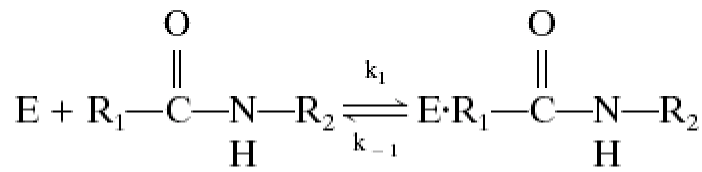
Ubiquitin stretched between the C and the N termini extends fully!

Discover BPTI on your own!

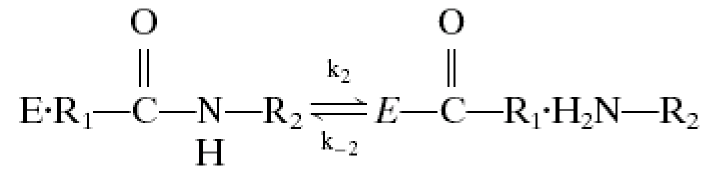
bovine pancreatic trypsin inhibitor



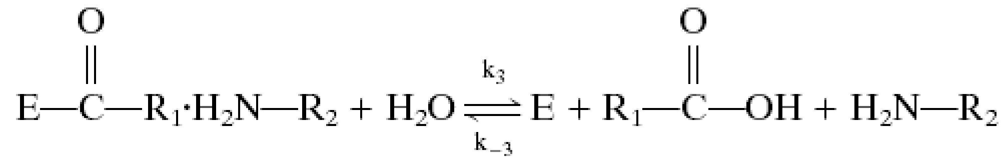
- Small (58 amino acids)
- rigid
- Binds as an inhibitor to Trypsin
  (a serine proteolytic enzyme, that appears in digestive system of mammalians.)
- Blocks its active site.



(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. (has a reactive site)

  forms an acyl-enzyme intermediate rapidly.

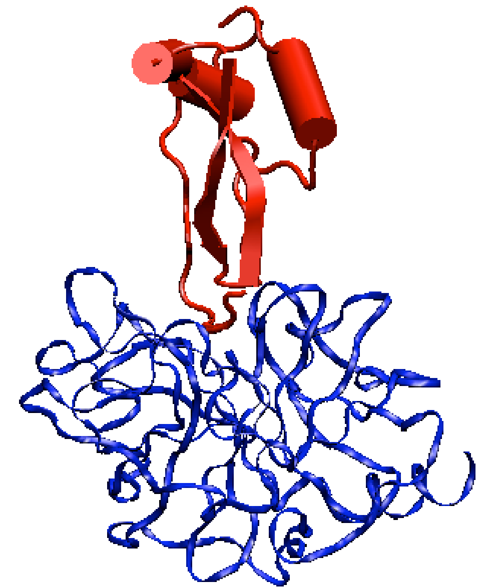
- Very little **structural changes** in Trypsin or BPTI

  several H-bonds between backbone of the two proteins

  little reduction in conformational entropy → binds tightly

- Remains uncleaved.

  (hydrolysis is 10^{11} times slower than 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.