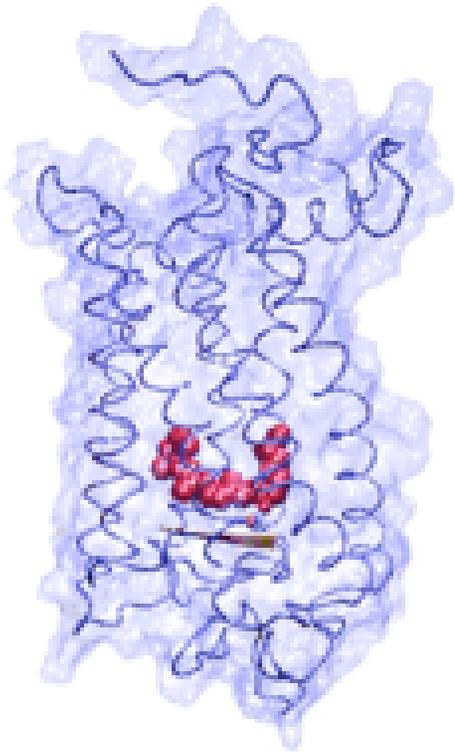
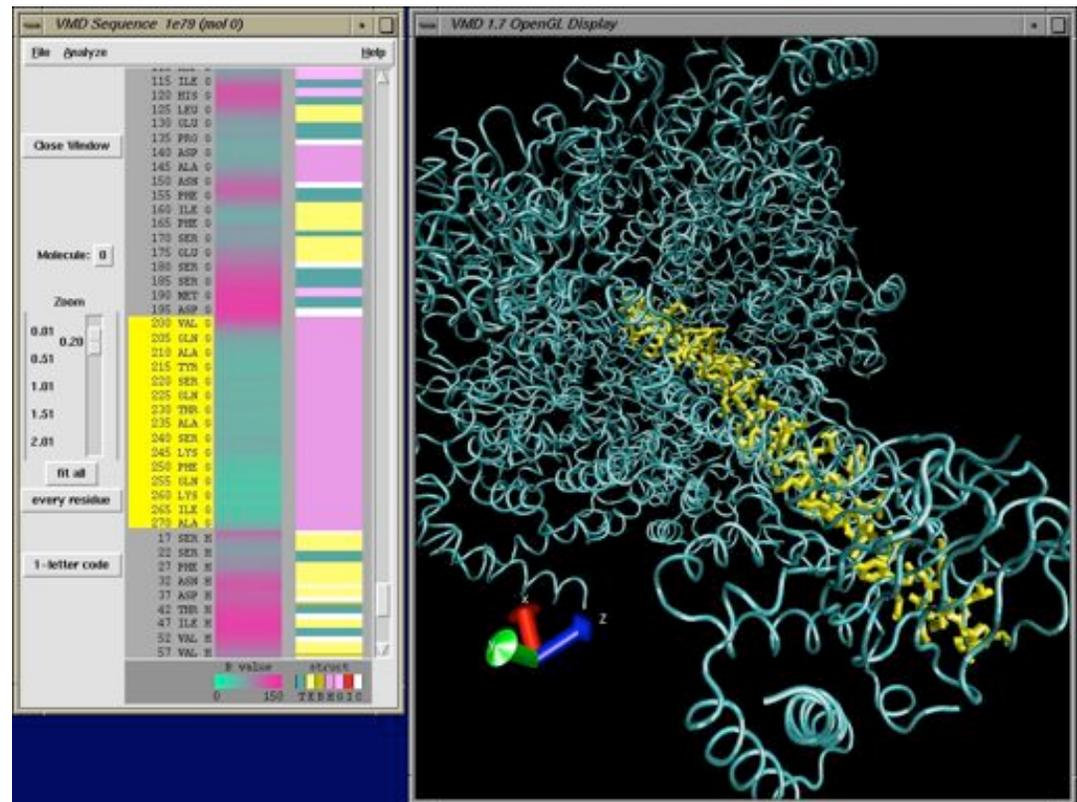


Molecular Graphics Perspective of Protein Structure and Function



animation

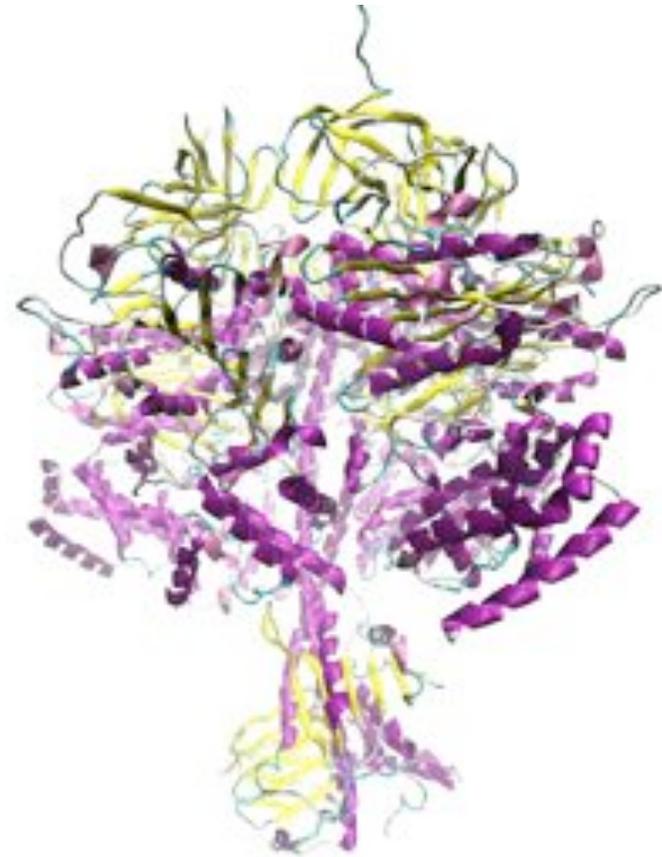


sequence

structure

VMD Highlights

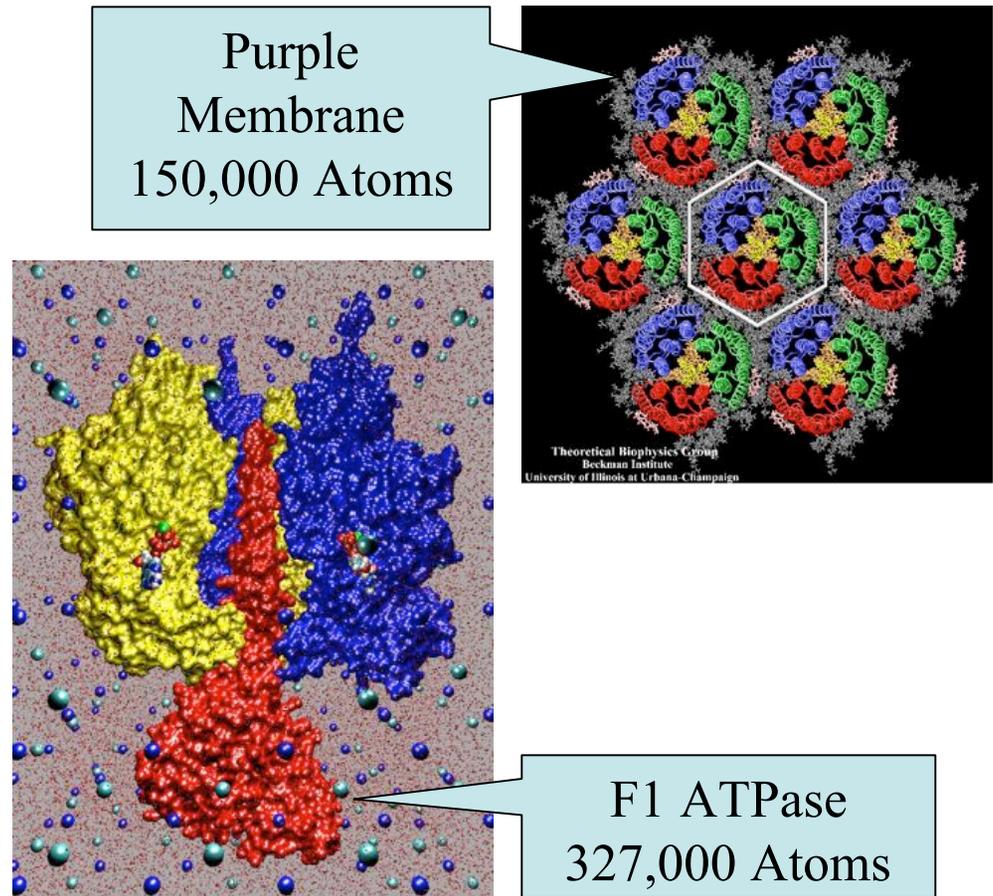
- > 40,000 registered users
- Platforms:
 - Unix (16 builds)
 - Windows
 - MacOS X
- Display of large biomolecules and simulation trajectories
- Sequence browsing and structure highlighting
- Multiple sequence - structure analysis
- User-extensible scripting interfaces for analysis and customization



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

VMD Permits Large Scale Visualization

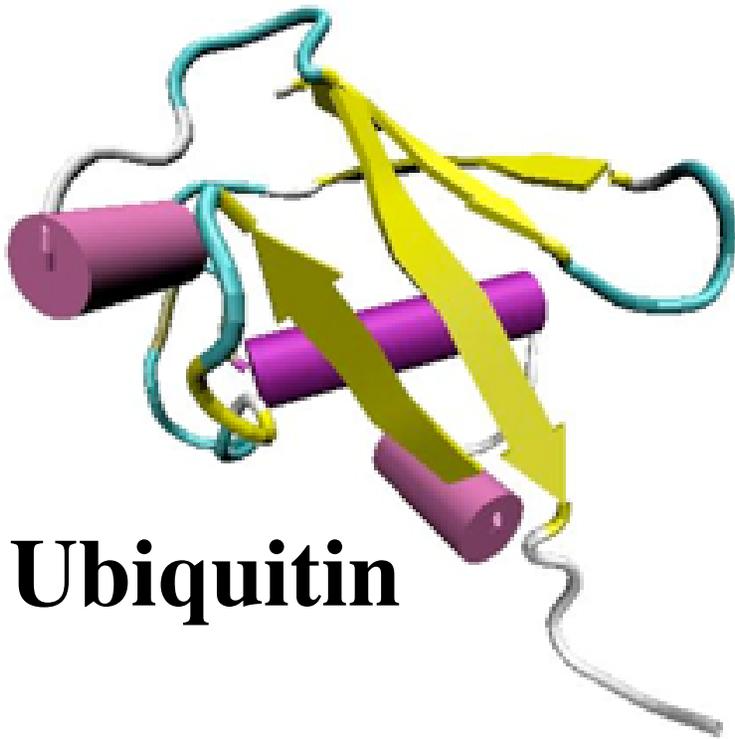
- Large structures: 300,000 atoms and up
- Complex representations
- Long trajectories: thousands of timesteps
- Volumetric data
- Multi-gigabyte data sets break 32-bit barriers
- Handles large data sets, e.g., GlpF: each 5 ns simulation of 100K atoms produces a 12GB trajectory
- Can load entire pdb (17 Gbytes) for data base wide analysis



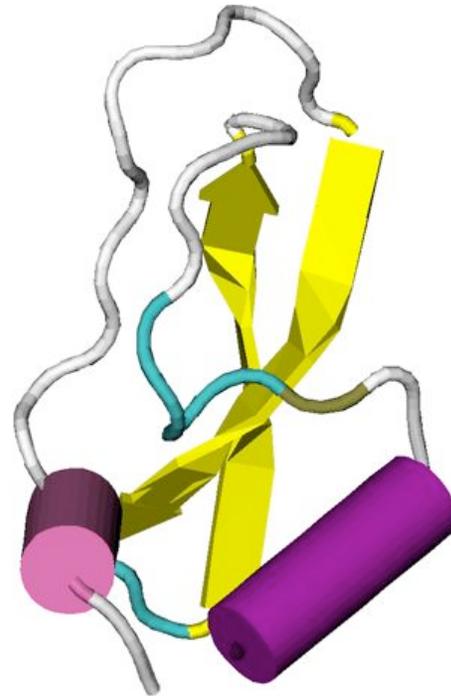
Focus on two proteins

Ubiquitin

Bovine Pancreatic Trypsin Inhibitor (BPTI)



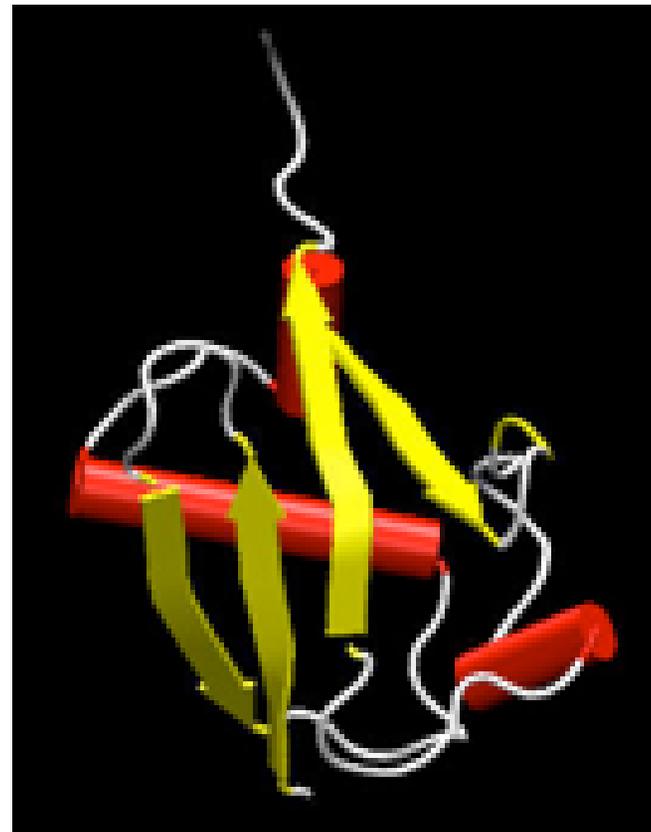
Ubiquitin



BPTI

Ubiquitin

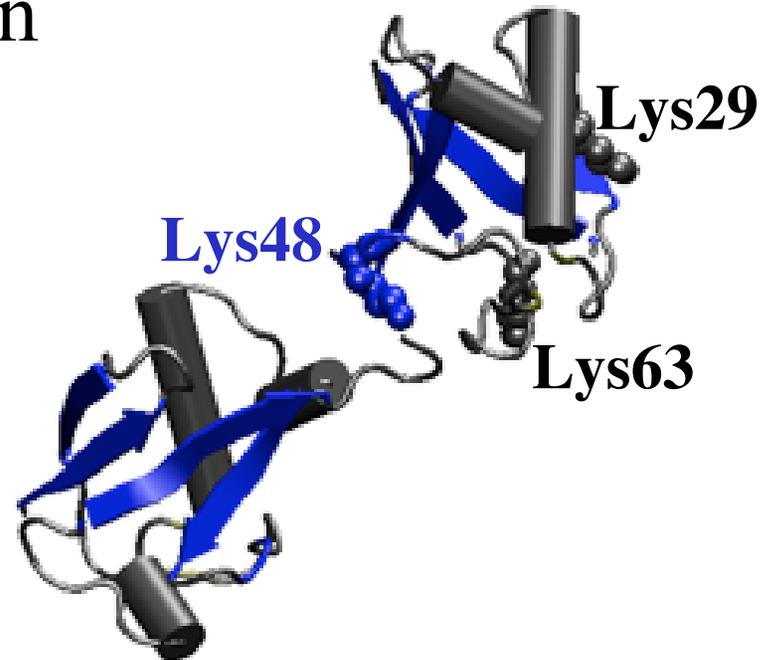
- 76 amino acids
- highly conserved
- covalently attaches to proteins and tags them for degradation
- other cell trafficking



- 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

1/3 of the prize
Israel

Technion - Israel
Institute of
Technology
Haifa, Israel

b. 1947



Avram Hershko

1/3 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

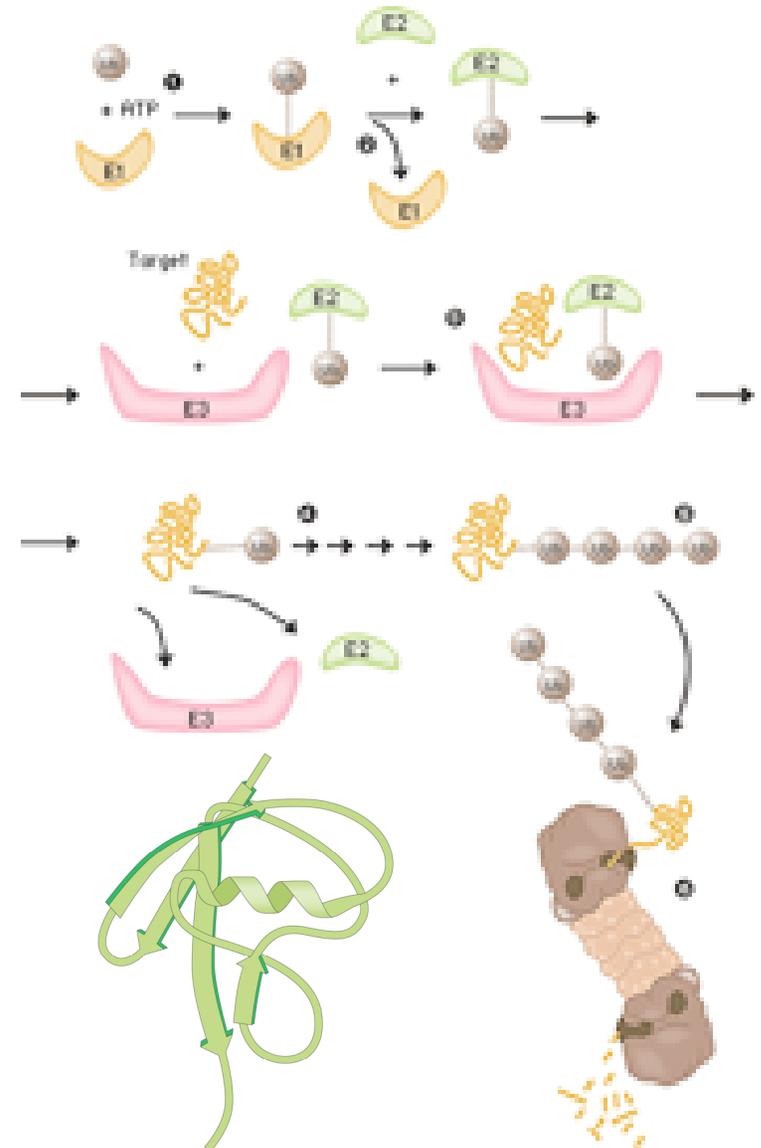
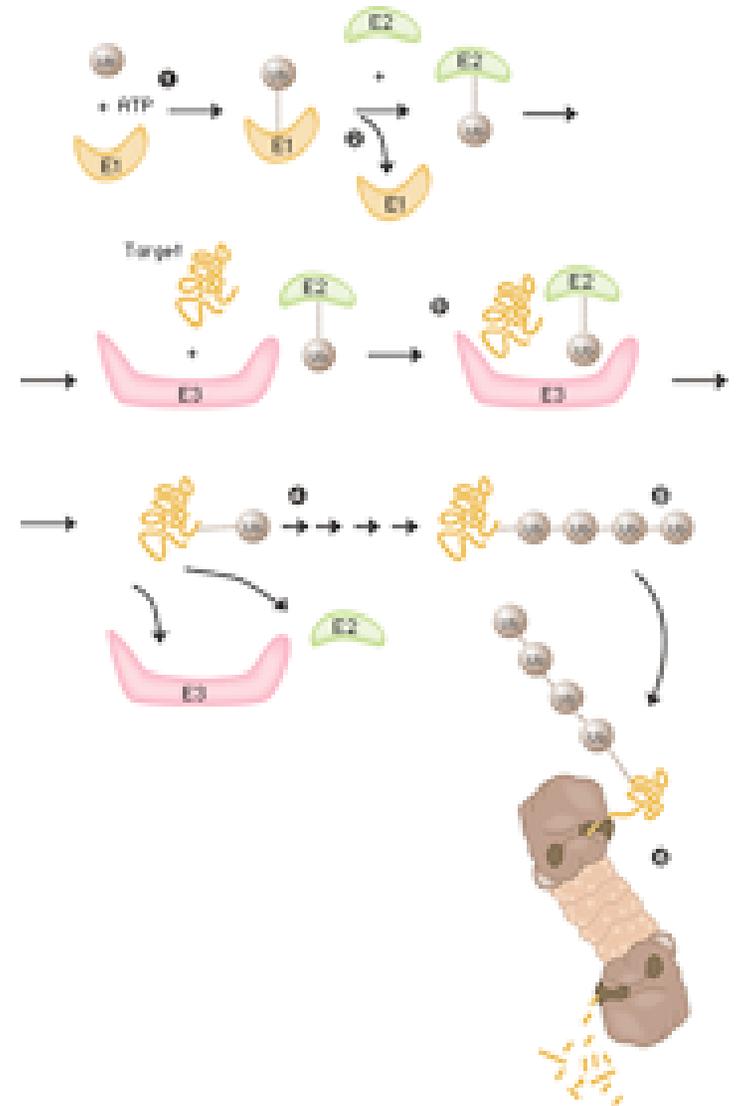


Illustration: Tgptorm

Ubiquitination Pathway

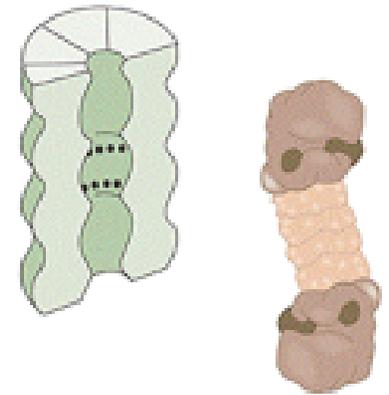
- Activation by E1 (ATP dependent process)
(thiol-ester linkage between a specific cysteine residue of E1 and Glycine on ubiquitin)
- Transfer to a cysteine residue on E2
(ubiquitin conjugation enzyme)
- Transfer of ubiquitin by E3 to the substrate lysine residue.
- E3 recognizes the ubiquitination signal 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, ...

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



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

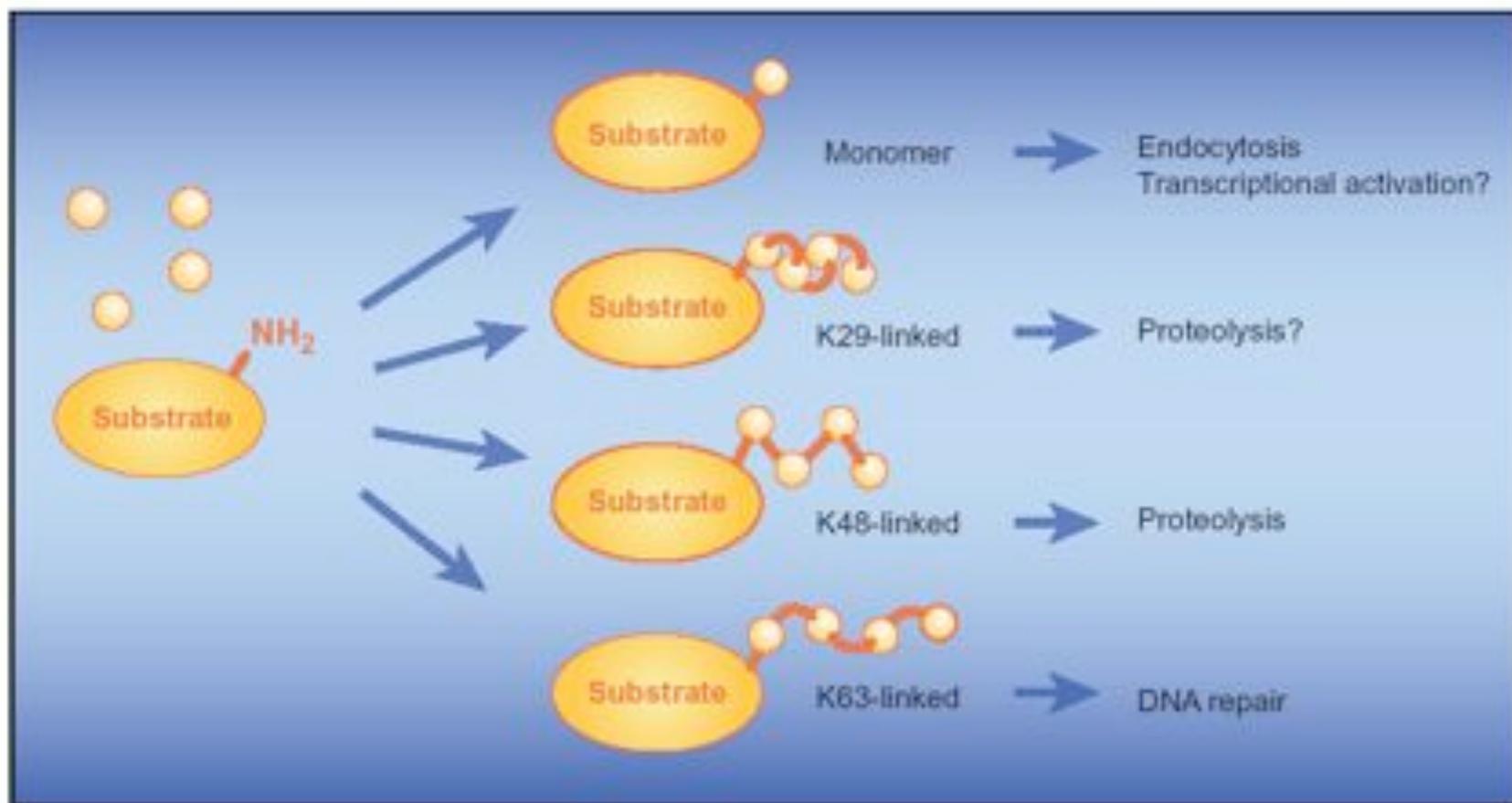
Different types of ubiquitin signals arise from

- 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.)

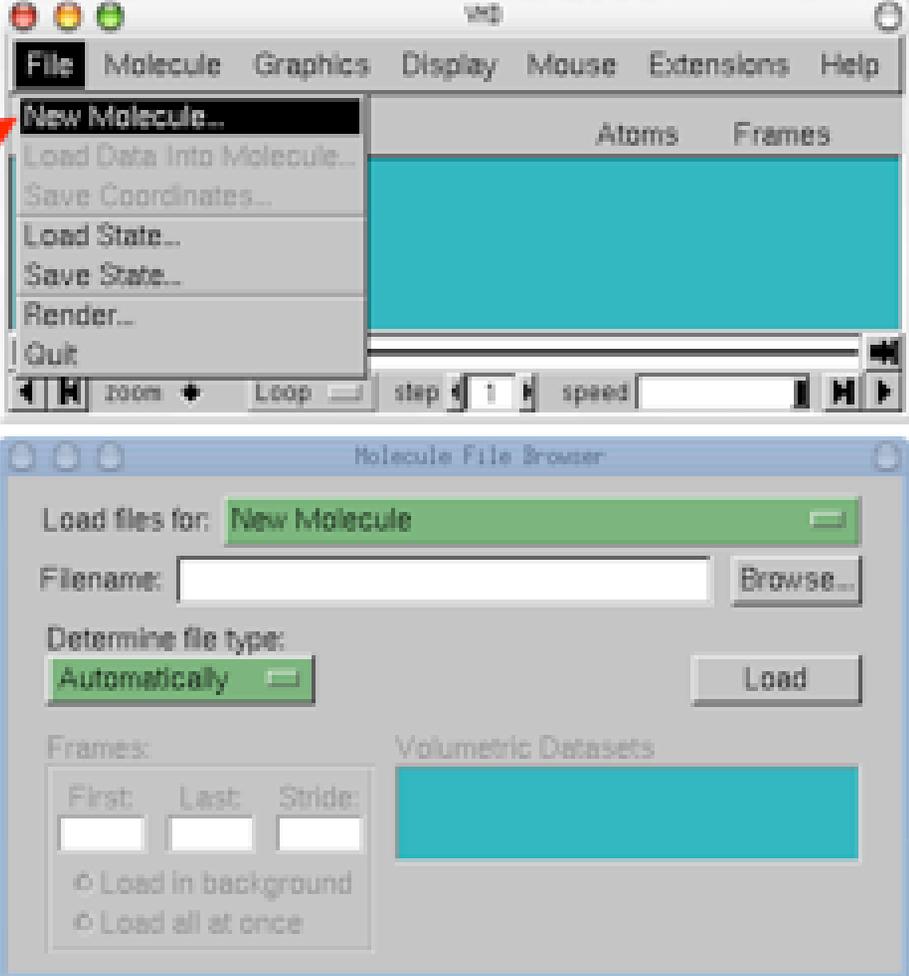
Marx, J., Ubiquitin lives up its name, *Science* 297, 1792-1794 (2002)

Inspect ubiquitin with VMD

Basics of VMD

Loading a Molecule

New Molecule (a)



The image shows two windows from the VMD software. The top window is the main VMD interface with the 'File' menu open. The 'New Molecule...' option is highlighted. A red arrow points from the text 'New Molecule (a)' to this option. The bottom window is the 'Molecule File Browser' dialog. A red arrow points from the text '(b) Molecule file browser' to the dialog's title bar. Another red arrow points from the text '(c) Browse' to the 'Browse...' button. A third red arrow points from the text '(d) Load' to the 'Load' button. The dialog also shows a 'Filename:' field, a 'Determine file type:' dropdown set to 'Automatically', and 'Frames:' and 'Volumetric Datasets' sections.

(b) Molecule file browser

(c) Browse

(d) Load

Basics of VMD

Rendering a Molecule

The screenshot shows the 'Graphical Representations' window in VMD. The 'Selected Molecule' is '0: 1UBQ'. The 'Style' is 'Lines', 'Color' is 'Name', and 'Selection' is 'all'. The 'Selected Atoms' field contains 'all'. The 'Draw style' is 'Lines', 'Coloring Method' is 'Name', and 'Material' is 'Opaque'. The 'Drawing Method' is 'Lines'. The 'Thickness' is set to 1. The 'Apply Changes Automatically' checkbox is checked.

Current graphical representation (a) →

Draw style (b) →

Coloring (c) →

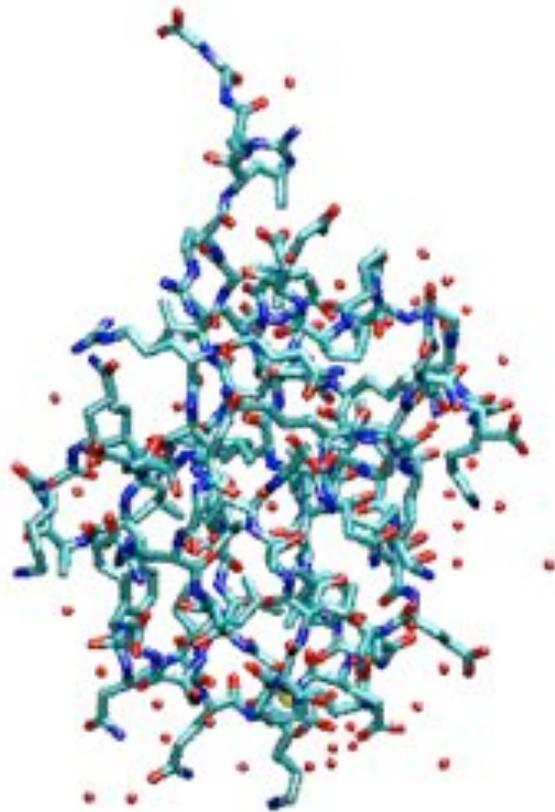
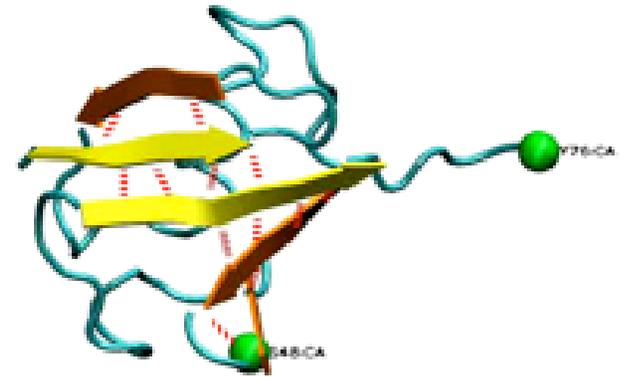
Drawing method (d) →

(e) Selected Atoms

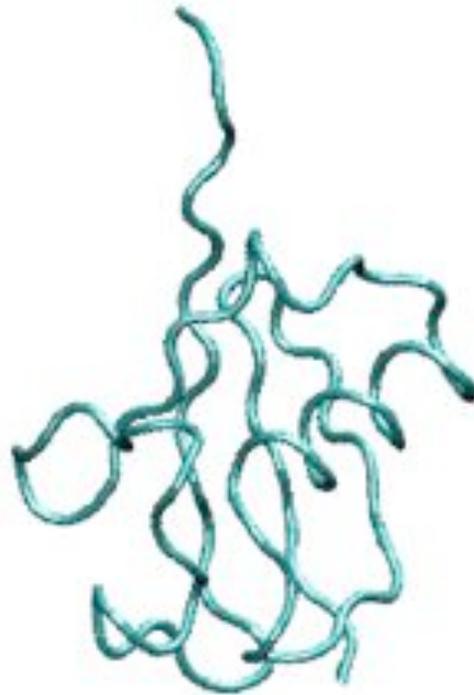
(e) Resolution, Thickness

Basics of VMD

Change rendering style



CPK



tube



cartoon

Basics of VMD

Create Representation (a)

(a)

Create Rep

Delete Representation (b)

(b)

Delete Rep

Current Representation (d)

(d)

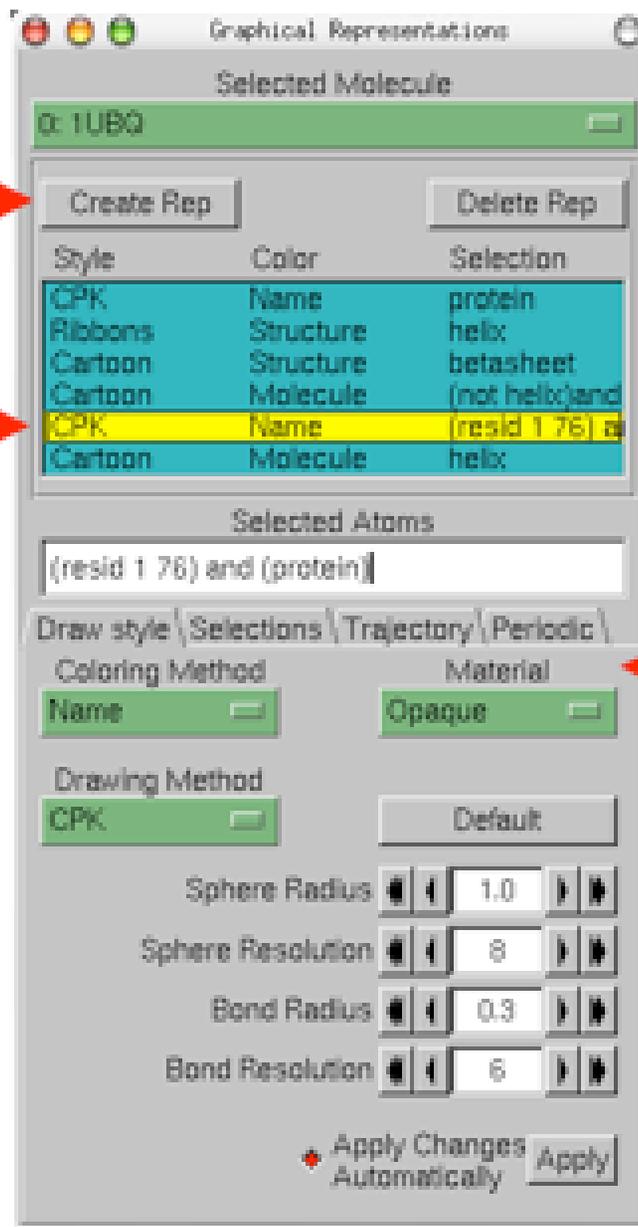
CPK

Material (c)

(c)

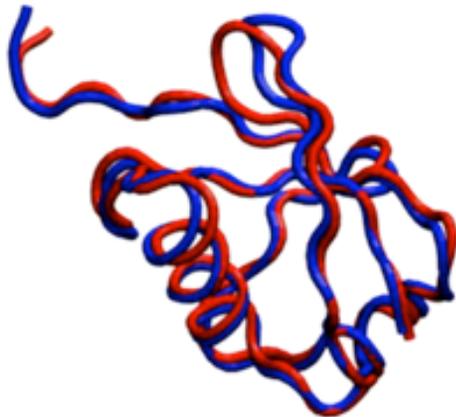
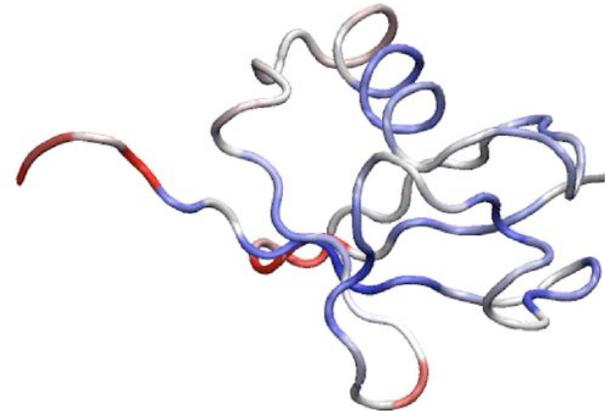
Material

Multiple representations

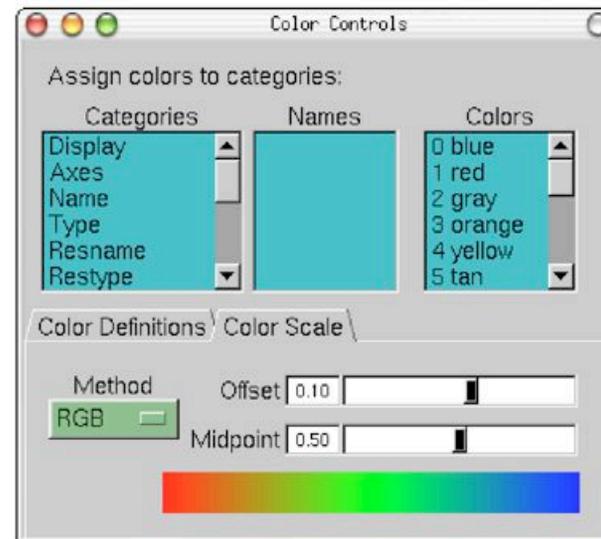


VMD Scripting

```
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)

(e) List of the residues

(f) Zoom

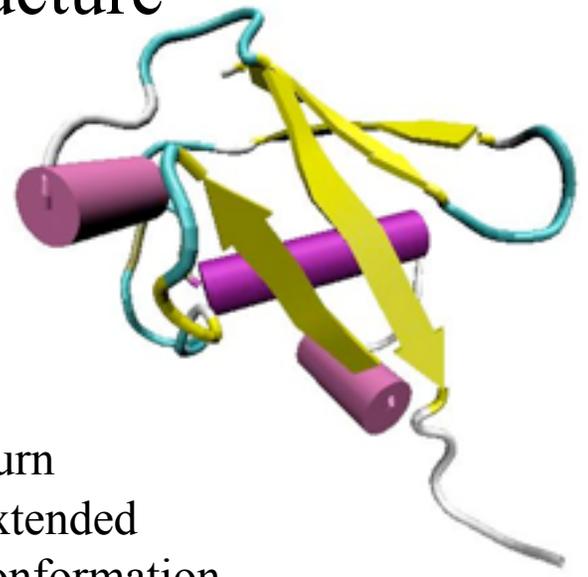
(d)

The screenshot shows the VMD Sequence window for a protein of 64 residues. The window title is 'VMD Sequence 1000 (mol 0)'. The main area contains a table with columns for residue number, 3-letter code, 1-letter code, B value, and structure. The B value column is color-coded from green (low) to red (high). The structure column is color-coded by secondary structure type. A legend at the bottom identifies the colors: B value (0 to 150) and structure (TEBHCIC). The zoom slider is set to 1.00.

Residue	3-letter code	1-letter code	B value	Structure
23	ILE	I	Low	Turn
24	GLU	E	Low	Turn
25	ASN	N	Low	Turn
26	VAL	V	Low	Turn
27	LYS	K	Low	Turn
28	ALA	A	Low	Turn
29	LYS	K	Low	Turn
30	ILE	I	Low	Turn
31	GLN	Q	Low	Turn
32	ASP	D	Low	Turn
33	LYS	K	Low	Turn
34	GLU	E	Low	Turn
35	GLY	G	Low	Turn
36	ILE	I	Low	Turn
37	PRO	P	Low	Turn
38	PRO	P	Low	Turn
39	ASP	D	Low	Turn
40	GLN	Q	Low	Turn
41	GLN	Q	Low	Turn
42	ARG	R	Low	Turn
43	LEU	L	Low	Turn
44	ILE	I	Low	Turn
45	PHE	F	Low	Turn
46	ALA	A	Low	Turn
47	GLY	G	Low	Turn
48	LYS	K	Low	Turn
49	GLN	Q	Low	Turn
50	LEU	L	Low	Turn
51	GLU	E	Low	Turn
52	ASP	D	Low	Turn
53	GLY	G	Low	Turn
54	ARG	R	Low	Turn
55	THR	T	Low	Turn
56	LEU	L	Low	Turn
57	SER	S	Low	Turn
58	ASP	D	Low	Turn
59	TYR	Y	Low	Turn
60	ASN	N	Low	Turn
61	ILE	I	Low	Turn
62	GLN	Q	Low	Turn
63	LYS	K	Low	Turn
64	GLY	G	Low	Turn

(b) Beta Value

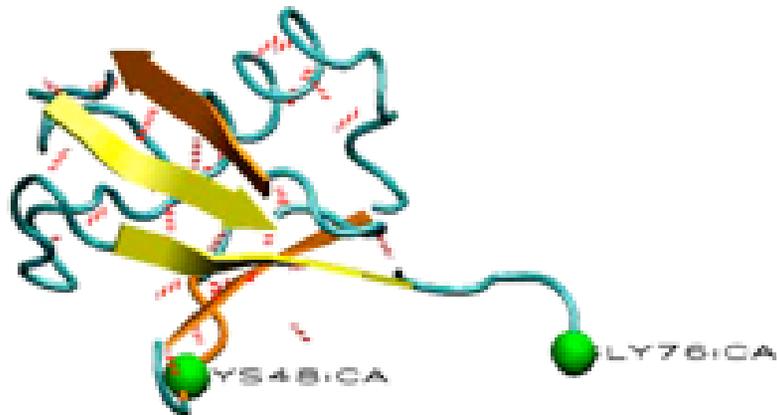
(c) Structure



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

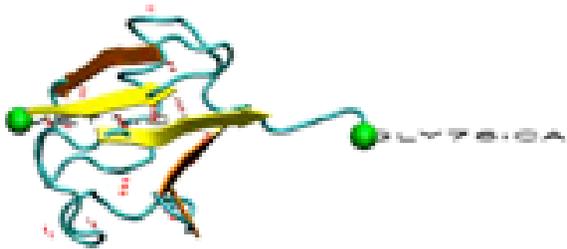
VMD Macros to Color Beta Strands

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

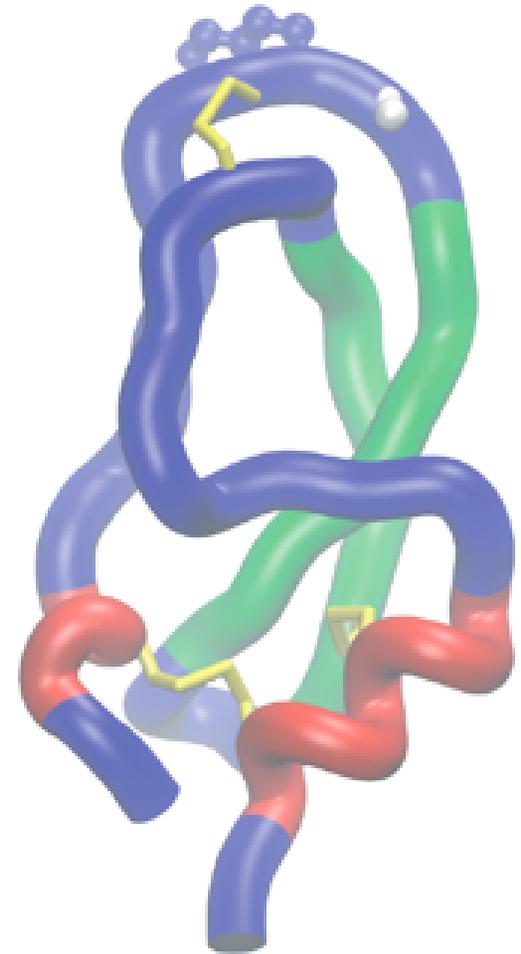


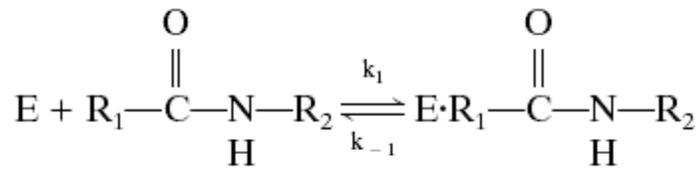
Ubiquitin stretched between the C and the N termini extends fully!

Discover BPTI on your own!

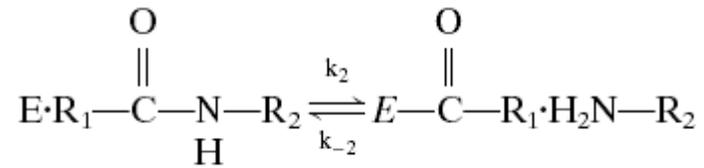
bovine pancreatic trypsin inhibitor

- small (58 amino acids)
- rigid
- binds as an **inhibitor** to Trypsin
(a serine proteolytic enzyme, that appears in the digestive system of mammals.)
- blocks its active site.

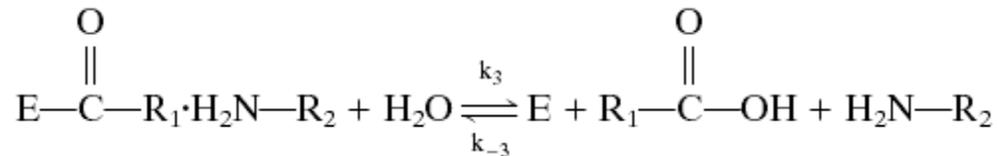




(Michaelis complex)



(Acyl-enzyme with leaving peptide
noncovalently bound)



(Free enzyme and products)

Mechanism of cleavage of peptides with serine proteases.

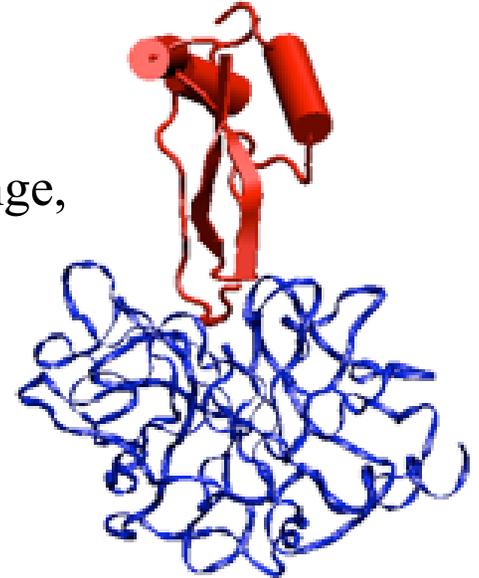
Radisky E. and Koshland D. Jr., Proc. Natl. Acad. Sci., USA, 99, 10316-10321

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

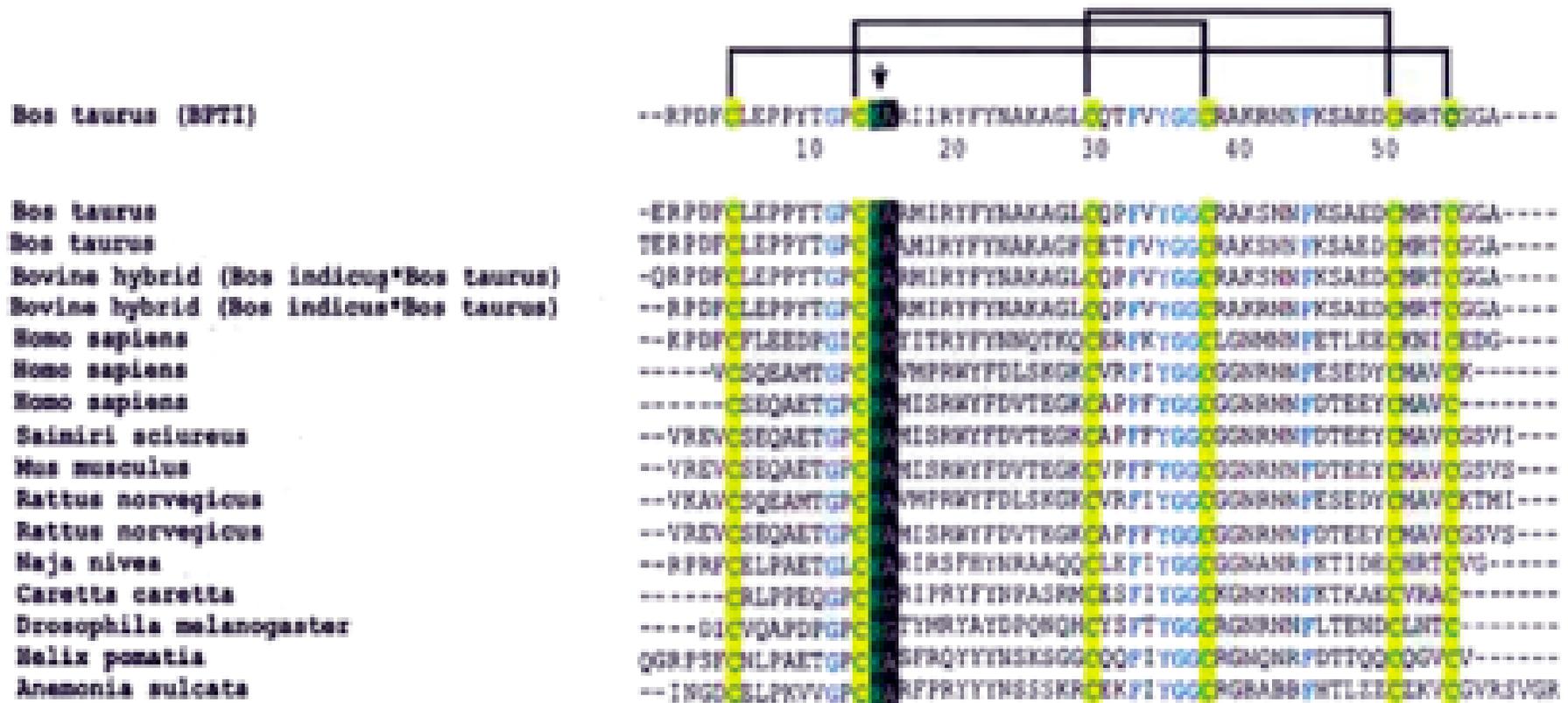
Structures of the **protease binding region**, in the proteins of all 18 families of standard mechanism inhibitors are similar.



Why does Trypsin cleave BPTI so slowly?

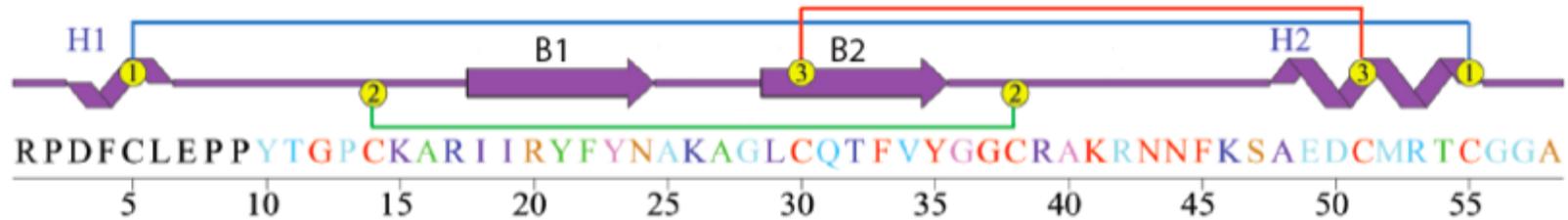
- Disruption of the non-covalent bonds in the **tightly bonded** enzyme-inhibitor complex increases the energy of transition states for bond cleavage.
- Water molecules do not have access to the active site, because of the **tight binding** of Trypsin and BPTI.
- After the cleavage of the active-site 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



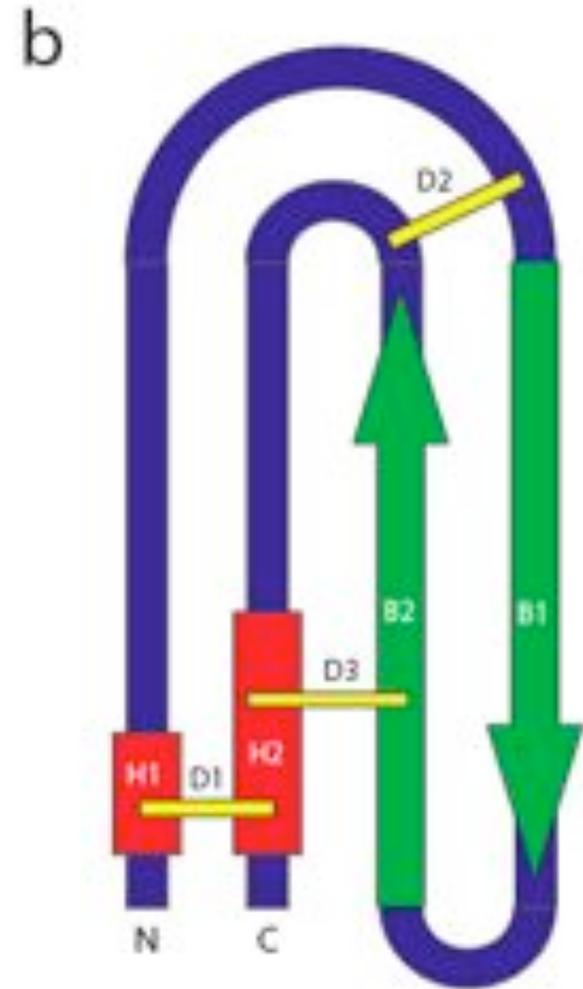
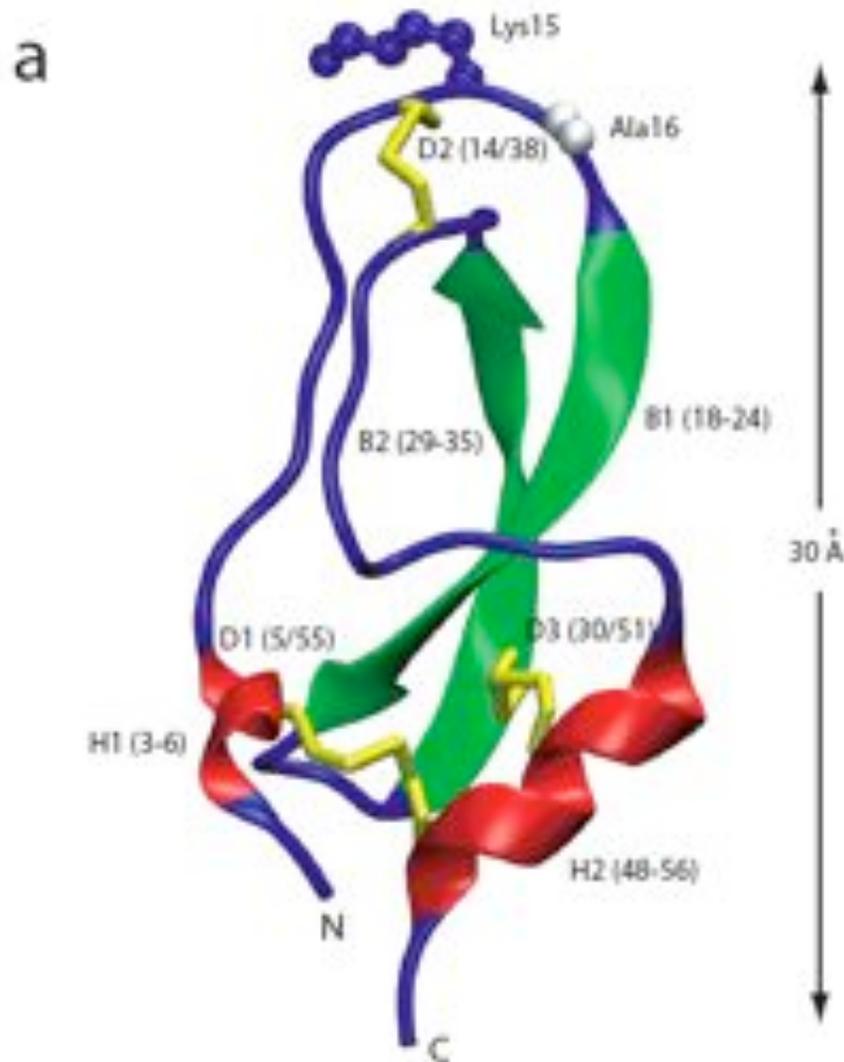
Reactive conserved P and P' residues are highlighted in black and indicated by the arrow. Six conserved cysteine residues are highlighted in yellow. Three disulfide bonds formed by the cysteines are indicated by black lines. Other residues that are conserved in all proteins are labeled in blue.

BPTI secondary structure

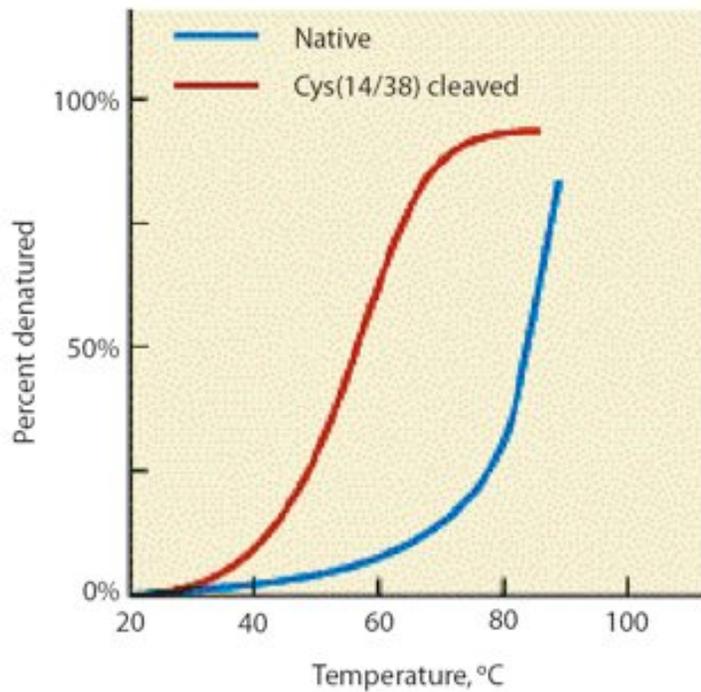


BPTI secondary structure: Conservation is indicated by color using rainbow scale coloring (Blue to red= low to high)

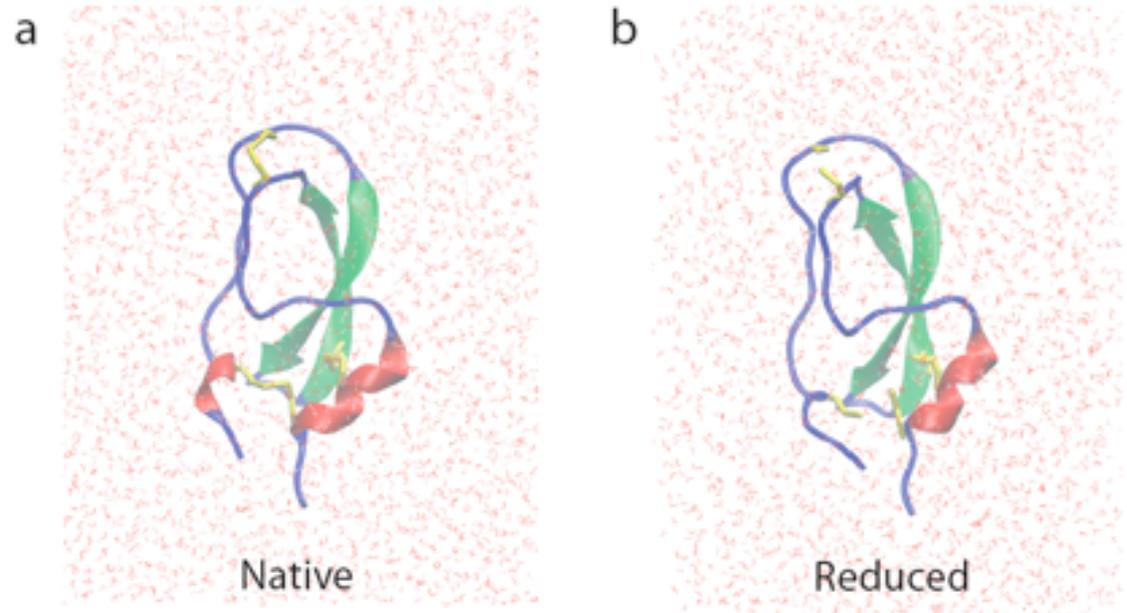
BPTI Tertiary Structure



Stability of native and reduced BPTI



Experiment



Can be tested through simulation

BPTI case study

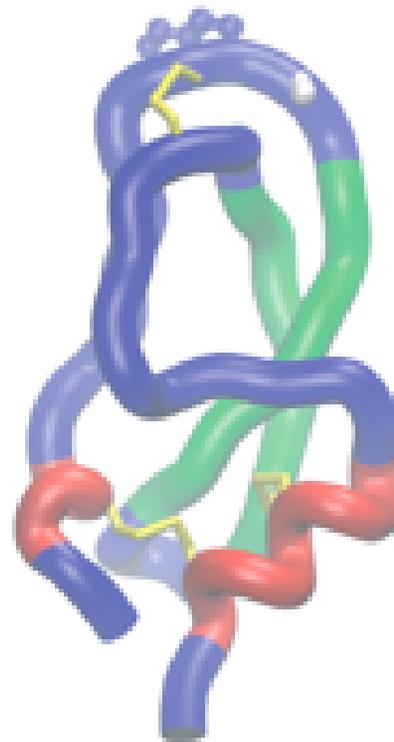
Chalermpol Kanchanawarin

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