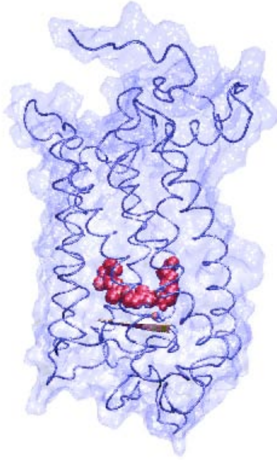
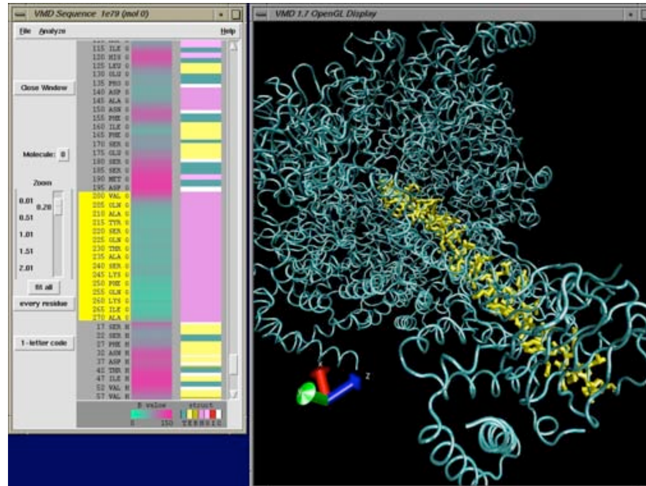
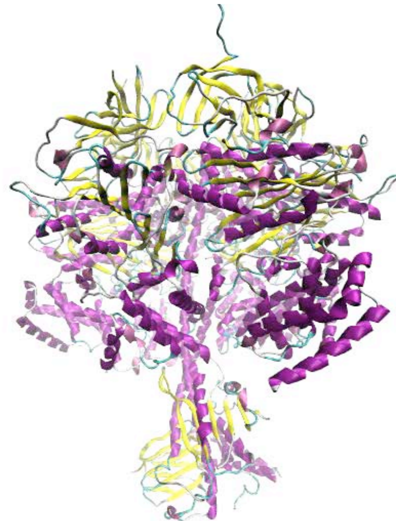
## Molecular Graphics Perspective of Protein Structure and Function





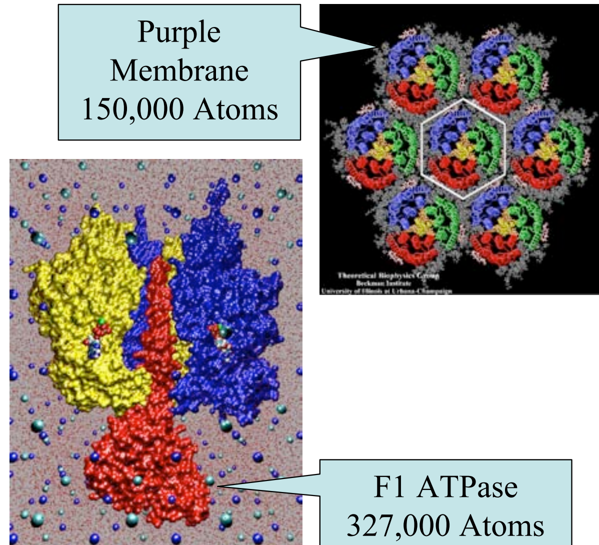
# VMD Highlights

- > 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

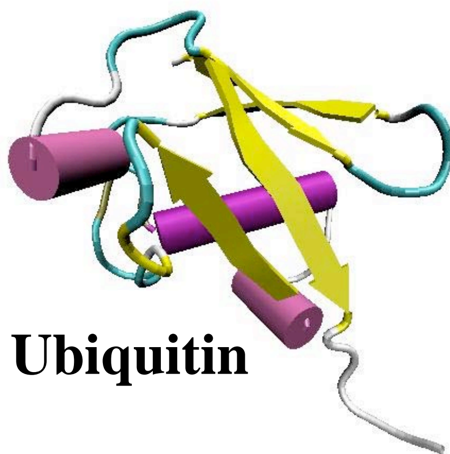
- Large structures: 300,000 atoms and up
- Complex representations
- Long trajectories: thousands of timesteps
- Volumetric data
- Multi-gigabyte data sets break 32-bit barriers
- Handles large data sets, e.g., GlpF: each 5 ns simulation of 100K atoms produces a 12GB trajectory

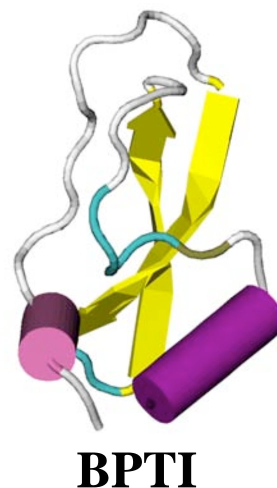



# Focus on two proteins

## Ubiquitin

## Bovine Pancreatic Trypsin Inhibitor (BPTI)

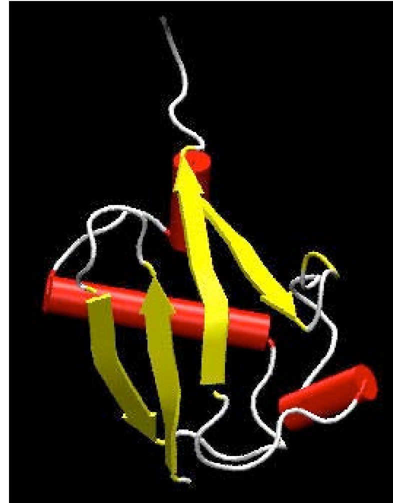


**BPTI**

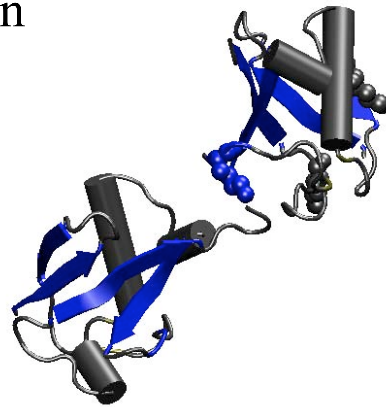
# Ubiquitin

- 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.

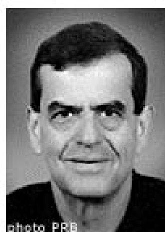


Two ubiquitins attached together through LYS 48. LYS 63 and LYS 29 are also shown there.

# Ubiquitination Pathway

The Nobel Prize in Chemistry 2004

"for the discovery of ubiquitin-mediated protein degradation"



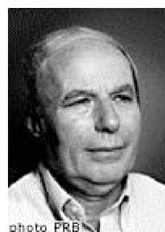

**Aaron Ciechanover**

1/3 of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1947



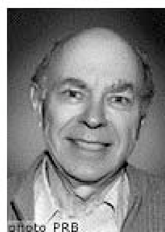

**Avram Hershko**

1/3 of the prize

Israel

Technion – Israel Institute of Technology
Haifa, Israel

b. 1937
(in Karcag, Hungary)




**Irwin Rose**

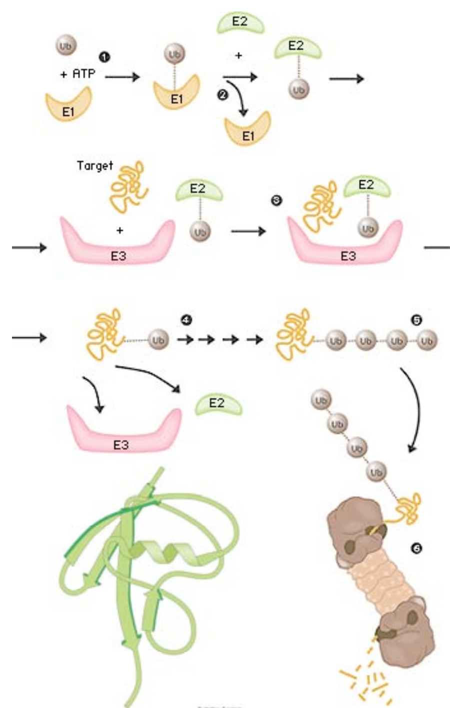
1/3 of the prize

USA

University of California
Irvine, CA, USA

b. 1926

Ubiquitin-mediated protein degradation




# Ubiquitination Pathway

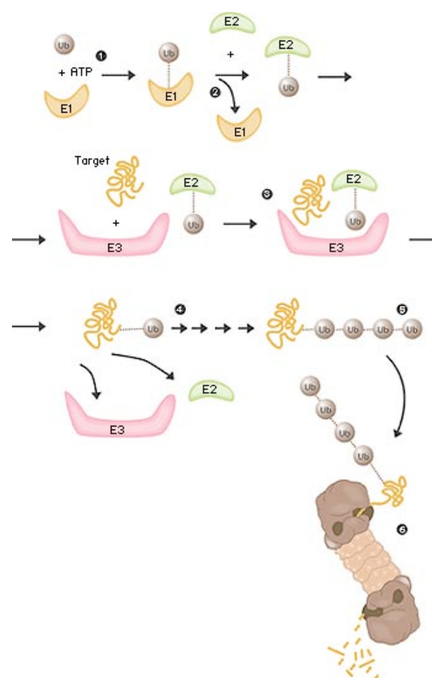
- Activation by E1 (ATP dependent process)

  (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.

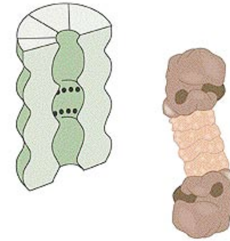


# Ubiquitin Functions

## Tagging proteins to be degraded in the proteasome.

• degrading misfolded proteins

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

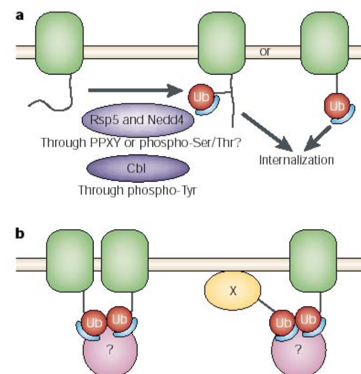


The cell's waste disposer, the proteasome. The black spots indicate active, protein-degrading surfaces.

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

# 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

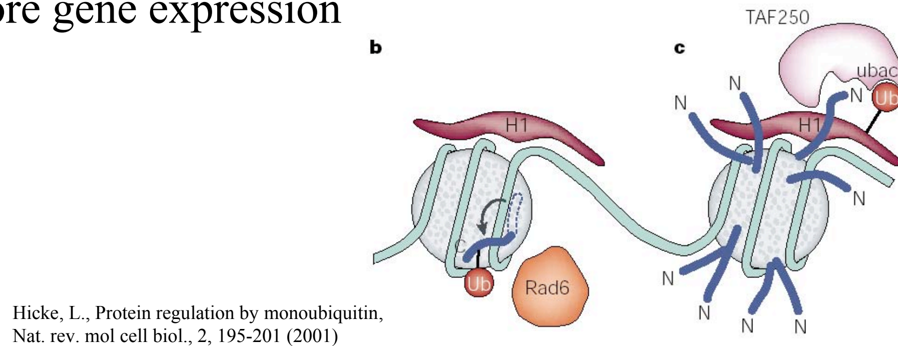



Hicke, L., Protein regulation by monoubiquitin, Nat. rev. mol cell biol., 2, 195-201 (2001)

## 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)

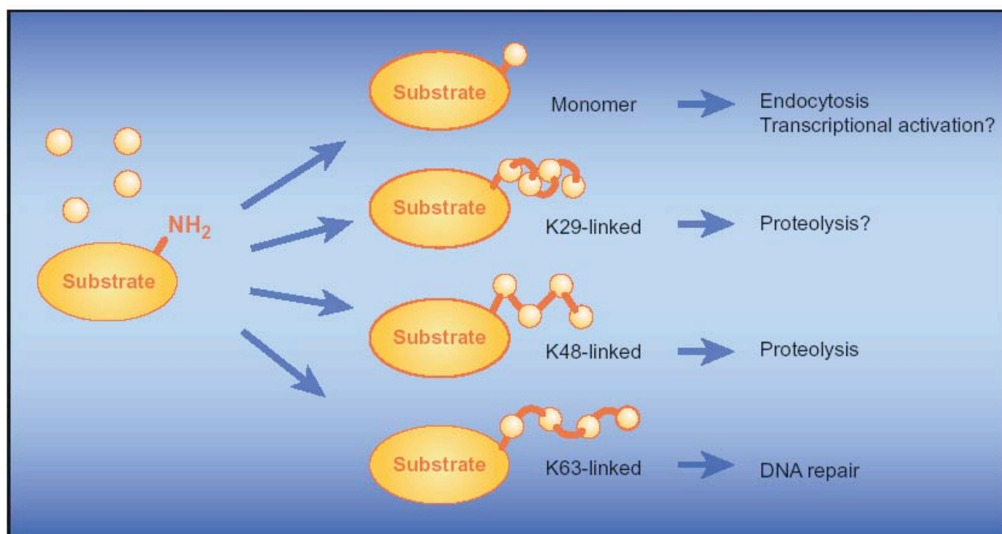
## Different types of ubiquitin signals arise from

- 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.)

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

# Basics of VMD

## Loading a Molecule

New Molecule (a)

VMD

File Molecule Graphics Display Mouse Extensions Help

New Molecule...
Load Data Into Molecule...
Save Coordinates...
Load State...
Save State...
Render...
Quit

Atoms Frames

zoom Loop step 1 speed

(b) Molecule file browser

Molecule File Browser

Load files for: New Molecule

Filename: Browse...

(c) Browse

Determine file type:
Automatically

Load

(d) Load

Frames:
First: Last: Stride:
Load in background
Load all at once

Volumetric Datasets

# Basics of VMD

## Rendering a Molecule

Graphical Representations

Selected Molecule

0: 1UBQ

Create Rep | Delete Rep

| Style | Color | Selection |
|---|---|---|
| Lines | Name | all |

Current graphical representation (a)

Selected Atoms

all

(f) Selected Atoms

Draw style (b) — Draw style | Selections | Trajectory | Periodic

Coloring (c) — Coloring Method: Name — Material: Opaque

Drawing method (d) — Drawing Method: Lines — Default

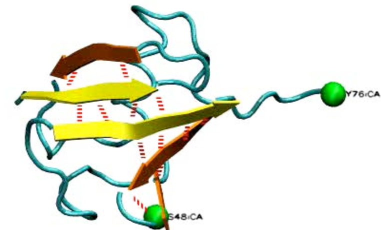
(e) Resolution, Thickness

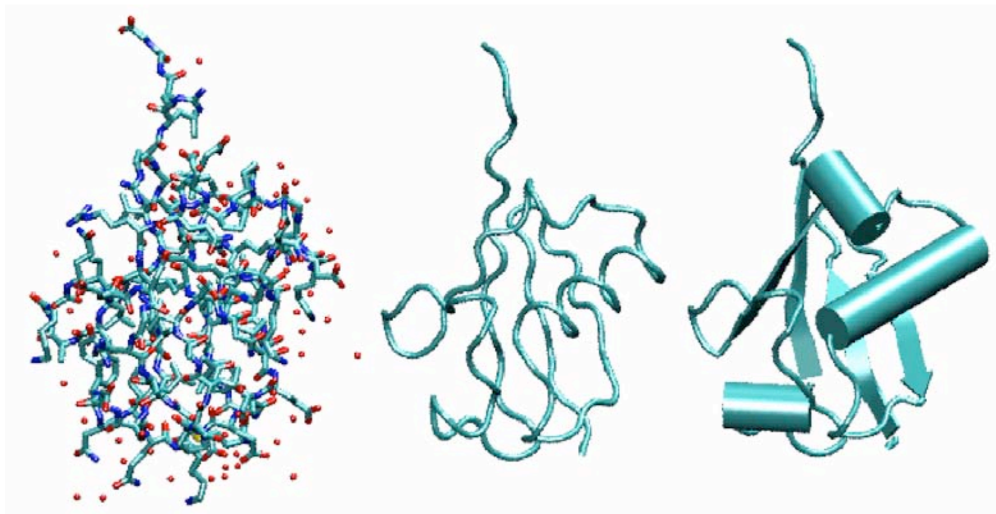
Thickness 1

Apply Changes Automatically — Apply

# Basics of VMD

## Change rendering style





**CPK** **tube** **cartoon**

# Basics of VMD

Graphical Representations

Selected Molecule

0: 1UBQ

Create Representation (a) → Create Rep

Delete Rep ← (b) Delete Representation

| Style | Color | Selection |
| --- | --- | --- |
| CPK | Name | protein |
| Ribbons | Structure | helix |
| Cartoon | Structure | betasheet |
| Cartoon | Molecule | (not helix)and |
| CPK | Name | (resid 1 76) a |
| Cartoon | Molecule | helix |

Current Representation (d) → CPK / Name / (resid 1 76) a

Selected Atoms

(resid 1 76) and (protein)

Draw style | Selections | Trajectory | Periodic

Coloring Method: Name

Material: Opaque ← (c) Material

Drawing Method: CPK

Default

Sphere Radius 1.0

Sphere Resolution 8

Bond Radius 0.3

Bond Resolution 6

Apply Changes Automatically

Apply

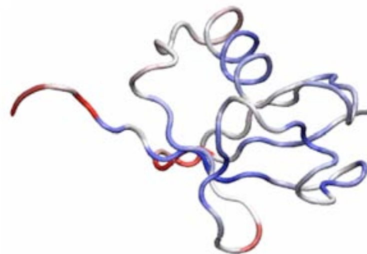
## Multiple representations

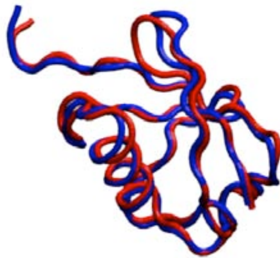
# VMD Scripting

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

Color Controls

Assign colors to categories:

| Categories | Names | Colors |
| --- | --- | --- |
| Display | | 0 blue |
| Axes | | 1 red |
| Name | | 2 gray |
| Type | | 3 orange |
| Resname | | 4 yellow |
| Restype | | 5 tan |

Color Definitions | Color Scale

Method: RGB

Offset 0.10

Midpoint 0.50

The Color Controls window showing the Color Scale tab.

# VMD Sequence Window

(a)

Beta Value (b)

List of the residues (e)

Structure (c)

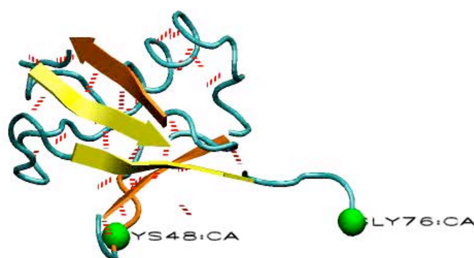
Zoom (f)

(d)

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

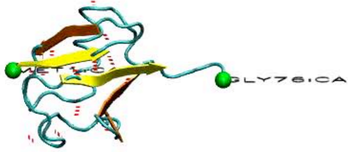
# VMD Macros to Color Beta Strands

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

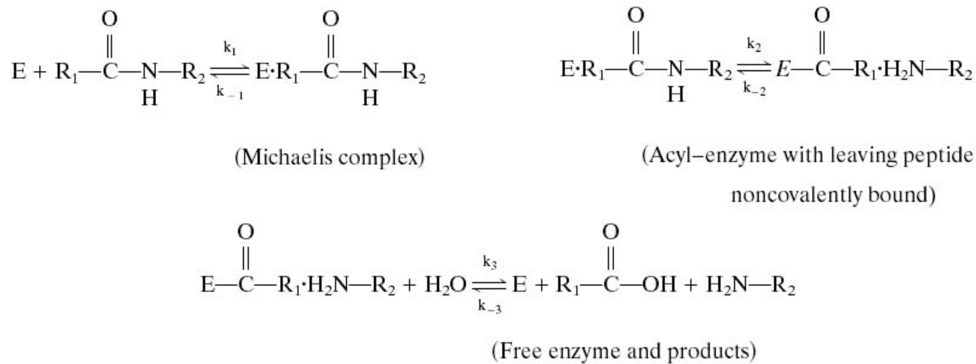



**Ubiquitin stretched between the C and the N termini extends fully!**

# 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.




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.

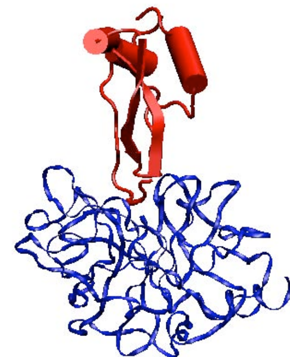
- Very little **structural changes** in Trypsin or BPTI

  several H-bonds between backbone of the two proteins chai

  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