Molecular Graphics Perspective of Protein Structure and Function
VMD Molecular Graphics

- > 50,000 registered users
- Platforms:
  - Unix (16 builds)
  - Windows
  - MacOS X
- Display of large biomolecules and simulation trajectories
- Multiple sequence - structure analysis

VMD view of F1-ATPase

Electrostatic potential for an ATPase obtained with VMD’s PME plugin
VMD Permits Large Scale Visualization

- Large structures: million atoms and up (ribosome in solvent: 2.6 million)
- Complex representations (many types for pdb structures)
- Long trajectories: thousands of time steps (terabytes; on parallel processors)
- Volumetric data (many types of EM, electrostatics, quantum chemistry data)
- 64 bit, i.e., multi-gigabyte data don’t 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 pdb wide analysis

Ribosome  GroEL  STMV virus
Q: “How much does the length of arginine vary?”

Answer:

- A VMD scan of all structures in the PDB revealed only small fluctuations (less than 10%) in the length of arginine residues
- Scan measured 591,338 arginine residues
- The “length” was defined by the CA-CZ distance
- Measured length was 5.5 +/- 0.49Å
- Dominant factor in one-time scan is disk I/O
- Doing scan in memory runs 100x to 1000x faster
- Multiple instances of VMD can scan in parallel
Focus on two proteins

Ubiquitin
Bovine Pancreatic Trypsin Inhibitor (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
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.
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

Molecule file browser

Browse

Load
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

Create Representation (a)  Delete Representation (b)

Current Representation (d)

Multiple representations

Material (c)
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 appears in digestive system of mammalians.)
- blocks its active site.
Mechanism of cleavage of peptides with serine proteases.

**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 $\rightarrow$ 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.
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: Conservation is indicated by color using rainbow scale coloring (Blue to red = low to high)
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
VMD supports multiple sequence analysis

Version 1.8.3
(Zaida Luthey-Schulten + coworkers)
Features of VMD’s MultiSeq Tool

Analyze the Evolution of Sequence and Structure

Eliminate Redundancy

Plus More Functions