Hands-on Workshop on Computational Biophysics
May 30 - June 2, 2017
Pittsburgh Supercomputing Center

Emad Tajkhorshid
NIH Center for Macromolecular Modeling and Bioinformatics
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign
Serving the large and fast growing community of biomedical researchers employing molecular modeling and simulation technologies

- 103,000 VMD users
- 19,000 NAMD users
- 17,000 NIH funded
- 1.4 million web visitors
- 228,000 tutorial views
Serving a Large and Fast Growing Community

- Deploying Center’s flagship programs NAMD and VMD on all major computational platforms from commodity computers to supercomputers

- Consistently adding user-requested features
  - simulation, visualization, and analysis

- Covering broad range of scales (orbitals to cells) and data types

- Enhanced software accessibility
  - QwikMD, interactive MDFF, ffTk, simulation in the Cloud, remote visualization
Exploiting State of the Art Hardware Technology

• Software available and optimized on all national supercomputing platforms (even before they come online)

• Decade-long, highly productive relationship with NVIDIA

• The first CUDA Center of Excellence funded by NVIDIA

• Consistently exploring opportunities for new hardware technology
  • Remote visualization
  • Virtual Reality
  • Handheld devices
Computational Structural Biology
Describing Biomolecules at Nanoscale

Structure / Dynamics
@ nanoscale
Why Structural Biology at **Nanoscale**?

- Mechanisms in Molecular Biology
- Molecular Basis of Disease
- Drug Design
- Nano-biotechnology

Why Structural Biology at Nanoscale?

✦ Mechanisms in Molecular Biology
✦ Molecular Basis of Disease
✦ Drug Design
✦ Nano-biotechnology
Why Structural Biology at Nanoscale?

Drug binding to a GPCR
Dror, ..., Shaw, PNAS, 108:13118–13123 (2011)
Why Structural Biology at Nanoscale?

✧ Mechanisms in Molecular Biology
✧ Molecular Basis of Disease
✧ Drug Design
✧ Nano-biotechnology

Structural changes underlying function
M. Moradi & E. T. PNAS 2013
Why Structural Biology at Nanoscale?

- Mechanisms in Molecular Biology
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Structural changes underlying function
Nano-biotechnology
Microfluidic Sensing Devices

Functionalized nanosurface with antibodies

HIV subtype identification

Lab Chip 2012

Created by nanoBIO Node tools
Nano-biotechnology
Gold Nanoparticles as Delivery Vehicles

Schematic model with no prediction power

Cartoon representation of lipid Au NPs

Experiment:
Murphy Lab

Modeling/Simulation:
Tajkhorshid Lab

Yang, J. A.; Murphy, C. J. Langmuir 2012, 28, 5404–5416
Applications of Computational Methodologies to Structural Biology

Simulation of the dynamics of the molecular system (MD)
• Calculating ensemble-averaged properties of microscopic systems to compare to macroscopic measurements
• Providing a molecular basis for function
• Describing the molecular/structural changes underlying function
• …

- Thermal fluctuations of a phospholipid bilayer
- Hydration at the interface of viral shell proteins
- Membrane binding of a coagulation protein
Lipid Protein Interaction

Molecular Dynamics Simulations

Solving the Newtonian equations of motion for all particles at every time step

Major limitations:
- Time scale / sampling
- Force field approximations

Major advantage:
- Unparalleled spatial and temporal resolutions, simultaneously

SPEED LIMIT
1 fs
Steps in a Typical MD Simulation

1. Prepare molecule
   - Read in pdb and psf file
2. Minimization
   - Reconcile observed structure with force field used (T = 0)
3. Heating
   - Raise temperature of the system
4. Equilibration
   - Ensure system is stable
5. Dynamics
   - Simulate under desired conditions (NVE, NpT, etc)
   - Collect your data
6. Analysis
   - Evaluate observables (macroscopic level properties)
   - Or relate to single molecule experiments
QwikMD—Gateway to Easy Simulation

Applications of Computational Methodologies to Cell-Scale Structural Biology

Using computational methods as “structure-building” tools

All experimental Structural biological approaches heavily rely on computational methods to analyze their data

- NMR
- X-ray
- Electron Microscopy
- …

Structural model of HIV virus
Molecular Dynamics Flexible Fitting (MDFF)

Electron Microscope

(Ribosome-bound YidC)

APS Synchrotron

cryo-EM density map

Match through MD

Supercomputer

crystallographic structure

Applications of Computational Methodologies to Cell-Scale Structural Biology

Using simulations as a “structure-building” tool

The most detailed model of a chromatophore

Computational model of a minimal cell envelope
Automated Protein Embedding into Complex Membrane Structures

Distribution of proteins across the membrane surface (dense environment)
- Ability to handle a variety of protein geometries
- Proper orientation of proteins in relation to the membrane surface
- Generalizable and automated method for membranes of arbitrary shape

Embedding proteins into the membrane
- Account for surface area occupied by proteins in inner and outer leaflets
- Proper lipid packing around embedded proteins
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- Proper lipid packing around embedded proteins
113 million Martini particles representing 1 billion atoms

Protein Components

- Aquaporin Z: 97
- Copper Transporter (CopA): 166
- F1 ATPase: 63
- Lipid Flipase (MsbA): 29
- Molybdenum transporter (ModBC): 130
- Translocon (SecY): 103
- Methionine transporter (MetNI): 136
- Membrane chaperon (YidC): 126
- Energy coupling factor (ECF): 117
- Potassium transporter (KtrAB): 148
- Glutamate transporter (GltTk): 41
- Cytidine-Diphosphate diacylglycerol (Cds): 50
- Membrane-bound protease (PCAT): 57
- Folate transporter (FolT): 134

Total: 1,397

3.7 M lipids (DPPC), 2.4 M Na\(^{+}\) & Cl\(^{-}\) ions, 104 M water particles (4 H\(_2\)O / particle)
Applications of Computational Methodologies to Cell-Scale Structural Biology

Guided Construction of Membranes from Experimental Data

Experimentally-Derived Membrane of Arbitrary Shape Builder

Terasaki Ramp
~4 Billion Atoms

- Outer Leaflet
- Inner Leaflet
- Cholesterol
- POPC
- POPE
- POPI
- POPS
- Sphingomyelin
- Cardiolipin

Terasaki et al., Cell, 2013.

Applications of Computational Methodologies to Cell-Scale Structural Biology

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Experimentally-Derived Membrane of Arbitrary Shape Builder

xMAS Builder

Obtain 3D mesh from an experimental technique
Molecular Dynamics Simulation

- Generating a thermodynamic ensemble (Sampling / Statistic)
- Taking into account fluctuations/dynamics in interpretation of experimental observables
- Describing molecular processes + free energy
- Help with molecular modeling
Classical Molecular Dynamics

\[ r(t + \delta t) = r(t) + \mathbf{v}(t) \delta t \]

\[ \mathbf{v}(t + \delta t) = \mathbf{v}(t) + \mathbf{a}(t) \delta t \]

\[ \mathbf{a}(t) = \frac{\mathbf{F}(t)}{m} \]

\[ \mathbf{F} = -\frac{d}{dr} U(r) \]
Potential Energy (hyper)Surface

What is Force?

\[ F = -\frac{d}{dx} U(x) \]
Classical Molecular Dynamics

\[
U(r) = \frac{1}{4\pi\varepsilon_0} \sum \frac{q_i q_j}{r_{ij}} + \varepsilon_{ij} \left[ \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - 2 \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^6 \right]
\]

\[
F(r) = \left( -\frac{1}{4\pi\varepsilon_0} \sum \frac{q_i q_j}{r_{ij}^2} - 12 \frac{\varepsilon_{ij}}{|r_{ij}|} \left[ \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^{12} - \left( \frac{R_{\text{min},ij}}{r_{ij}} \right)^6 \right] \right) \hat{r}_{ij}
\]
Classical Molecular Dynamics
Classical Molecular Dynamics

Bond definitions, atom types, atom names, parameters, ....
What is a Force Field?

In molecular dynamics a molecule is described as a series of charged points (atoms) linked by springs (bonds).

To describe the time evolution of bond lengths, bond angles and torsions, also the non-bonding van der Waals and electrostatic interactions between atoms, one uses a force field. The force field is a collection of equations and associated constants designed to reproduce molecular geometry and selected properties of tested structures.
Energy Functions

\[ U(\vec{R}) = \sum_{\text{bonds}} k_i^{\text{bond}} (r_i - r_0)^2 + \sum_{\text{angles}} k_i^{\text{angle}} (\theta_i - \theta_0)^2 + \]

\[ U_{\text{bond}} \]

\[ U_{\text{angle}} \]

\[ \sum_{\text{dihedrals}} k_i^{\text{dihe}} [1 + \cos (n_i \phi_i + \delta_i)] + \]

\[ U_{\text{dihedral}} \]

\[ \sum_{i} \sum_{j \neq i} 4\varepsilon_{ij} \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^6 + \sum_{i} \sum_{j \neq i} \frac{q_i q_j}{\varepsilon r_{ij}} \]

\[ U_{\text{nonbond}} \]

\[ U_{\text{bond}} = \text{oscillations about the equilibrium bond length} \]

\[ U_{\text{angle}} = \text{oscillations of 3 atoms about an equilibrium bond angle} \]

\[ U_{\text{dihedral}} = \text{torsional rotation of 4 atoms about a central bond} \]

\[ U_{\text{nonbond}} = \text{non-bonded energy terms (electrostatics and Lenard-Jones)} \]
Energy Terms Described in the CHARMM Force Field

- Bond
- Angle
- Dihedral
- Improper
Classical Dynamics

\( F=ma \) at 300K

Energy function:

\[ U(\vec{r}_1, \vec{r}_2, \cdots \vec{r}_N) = U(\vec{R}) \]

used to determine the force on each atom:

\[ m_i \frac{d^2 \vec{r}_i}{dt^2} = \vec{F}_i = -\nabla U(\vec{R}) \]

yields a set of 3N coupled 2\(^{nd}\)-order differential equations that can be propagated forward (or backward) in time.

Initial coordinates obtained from crystal structure, velocities taken at random from Boltzmann distribution.

Langevin dynamics deals with each atom separately, balancing a small friction term with Gaussian noise to control temperature:

\[ m \ddot{\vec{r}} = \vec{F}(\vec{r}) - \gamma m \dot{\vec{r}} + \vec{R}(t) \]
The most serious bottleneck

- Rotation of buried sidechains
- Local denaturations
- Allosteric transitions
- Hinge bending
- Rotation of surface sidechains
- Elastic vibrations
- Bond stretching
- Molecular dynamics timestep

<table>
<thead>
<tr>
<th>Time Unit</th>
<th>Steps</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>$10^{15}$</td>
</tr>
<tr>
<td>ms</td>
<td>$10^{12}$</td>
</tr>
<tr>
<td>µs</td>
<td>$10^{9}$ (year)</td>
</tr>
<tr>
<td>ns</td>
<td>$10^{6}$ (day)</td>
</tr>
<tr>
<td>ps</td>
<td>$10^{3}$</td>
</tr>
<tr>
<td>fs</td>
<td>$10^{0}$</td>
</tr>
</tbody>
</table>

$\delta t = 1 \text{ fs}$

SPEED LIMIT
Initial coordinates have bad contacts, causing high energies and forces (due to averaging in observation, crystal packing, or due to difference between theoretical and actual forces).

Minimization finds a nearby local minimum.

Heating and cooling or equilibration at fixed temperature permits biopolymer to escape local minima with low energy barriers.

Initial dynamics samples thermally accessible states.
Molecular Dynamics to Sample Energy Landscape

Longer dynamics access other intermediate states; one may apply external forces to access other available states in a more timely manner.
Patience is required to observe Molecular Events

Stochastic behavior

dihedral angle $\phi$ (degree)

time (ps)
Steps in a Typical MD Simulation

• 1. Prepare molecule
  – Read in pdb and psf file
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  – Evaluate observables (macroscopic level properties)
  – Or relate to single molecule experiments
Preparing Your System for MD Solvation

Biological activity is the result of interactions between molecules and occurs at the interfaces between molecules (protein-protein, protein-DNA, protein-solvent, DNA-solvent, etc).

Why model solvation?
• many biological processes occur in aqueous solution
• solvation effects play a crucial role in determining molecular conformation, electronic properties, binding energies, etc

How to model solvation?
• explicit treatment: solvent molecules are added to the molecular system
• implicit treatment: solvent is modeled as a continuum dielectric
Classical Molecular Dynamics

\[ r(t + \delta t) = r(t) + v(t)\delta t \]

\[ v(t + \delta t) = v(t) + a(t)\delta t \]

\[ a(t) = \frac{F(t)}{m} \]

\[ F = -\frac{d}{dr}U(r) \]
Maxwell Distribution of Atomic Velocities

\[ p(v_\sigma) = \sqrt{\frac{m}{2\pi k_B T}} \exp \left[-\frac{mv^2_\sigma}{2k_B T}\right] \]

\[ \sigma = x, y, z \]
Equilibrium Properties of Proteins

Ubiquitin

Root Mean Squared Deviation: measure for equilibration and protein flexibility

\[ \text{RMSD}(t) = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (R_i(t) - R_i(0))^2} \]

Protein sequence exhibits characteristic permanent flexibility!

NMR structures aligned together to see flexibility

MD simulation

The color represents mobility of the protein through simulation (red = more flexible)
Thermal Motion of Ubiquitin from MD

RMSD values per residue

![RMSD graph showing thermal motion of ubiquitin residues with corresponding structures](image-url)
RMS deviations for the KcsA protein and its selectivity filter indicate that the protein is stable during the simulation with the selectivity filter the most stable part of the system.

Temperature factors for individual residues in the four monomers of the KcsA channel protein indicate that the most flexible parts of the protein are the N and C terminal ends, residues 52-60 and residues 84-90. Residues 74-80 in the selectivity filter have low temperature factors and are very stable during the simulation.
Battling the Timescale

non-Equilibrium MD simulations

Reduced Representations
Battling the Timescale - Case I

Steered Molecular Dynamics is a non-equilibrium method by nature

- A wide variety of events that are inaccessible to conventional molecular dynamics simulations can be probed.

- The system will be driven, however, away from equilibrium, resulting in problems in describing the energy landscape associated with the event of interest.

Second law of thermodynamics \[ W \geq \Delta G \]
In principle, it is possible to obtain free energy surfaces from repeated non-equilibrium experiments.
AqpZ vs. GlpF

- Both from *E. coli*
- AqpZ is a pure water channel
- GlpF is a glycerol channel
- We have high resolution structures for both channels
Steered Molecular Dynamics

constant force
(250 pN)

constant velocity
(30 Å/ns)
SMD Simulation of Glycerol Passage

Trajectory of glycerol pulled by constant force
Constructing the Potential of Mean Force

4 trajectories

\( v = 0.03, \ 0.015 \ \text{Å/ps} \)

\( k = 150 \ \text{pN/Å} \)

\[
f(t) = -k[z(t) - z_0 - vt]
\]

\[
W(t) = \int_0^t dt' \ \nu f(t')
\]
- Captures major features of the channel
- The largest barrier $\approx 7.3$ kcal/mol; exp.: $9.6 \pm 1.5$ kcal/mol

Features of the Potential of Mean Force

Asymmetric Profile in the Vestibules

Artificial induction of glycerol conduction through AqpZ

Three fold higher barriers

AqpZ 22.8 kcal/mol
GlpF 7.3 kcal/mol

Could it be simply the size?

Battling the Timescale - Case II
Biased (nonequilibrium) simulations


- **Neurotransmitter Uptake**
  - Norepinephrine, serotonin, dopamine, glutamate,…

- **Gastrointestinal Tract**
  - Active absorption of nutrients
  - Secretion of ions

- **Kidneys**
  - Reabsorption
  - Secretion

- **Pharmacokinetics of all drugs**
  - Absorption, distribution, elimination
  - Multi-drug resistance in cancer cells
Alternating Access Mechanism

Outward-facing


Inward-facing

Diverse Structural Transitions Involved

Non-equilibrium methods are required.
Complex Processes Require Complex Treatments

I.1 Defining Practical Collective Variables
Empirical search for practical collective variables for inducing the conformational changes involved in the transition.

I.2 Optimizing the Biasing Protocols
Systematic search for a practical biasing protocol by using different combinations of collective variables.

II. Optimizing the Transition Pathway
Use all of the conformations available to generate the most reliable transition pathway:
1. Bayesian approach for combining the data
2. Post-hoc string method (analysis tool)
3. String method with swarms of trajectories

III.1 Free Energy Calculations
Using the most relevant collective variables (from I.1), biasing protocol (from I.2), and initial conformations (from I.2).

III.2 Assessing the Sampling Efficiency
Detecting the poorly sampled, but potentially important regions, e.g., by using PCA.

Empirical search for reaction coordinates and biasing protocols

Aggressive Search of the Space
Non-equilibrium Driven Molecular Dynamics: Applying a time-dependent external force to induce the transition along various pathways/mechanisms (collective variables)

Harmonic constant: $U_{dr}(x, t) = \frac{1}{2} k \left( \xi(x) - \xi_A + (\xi_B - \xi_A) \frac{t}{T} \right)^2$

Biasing potential

Initial state

Final state

Collective variables: RMSD, distance, $R_g$, angle, ...

Orientation quaternion

Total simulation time

Progressively Optimizing the Biasing Protocol/Collective Variable using non-Equilibrium Work as a Measure of the Path Quality

Example set taken from a subset of 20 ns biased simulations
Mechanistic Insight From Transition Pathways in ABC exporters from Non-Equilibrium Simulations

NBD Doorknob Mechanism

Describing a Complete Cycle (Adding Substrate) Requiring a Combination of Multiple Collective Variables

12 replicas x 40 ns (H1/H7)
50 replicas x 20 ns (10 Hs)

12 replicas x 40 ns (H1/H7)
24 replicas x 20 ns (H1/H7)
200 replicas (2D) x 5 ns
50 replicas x 20 ns

150 replicas

30 r x 20 ns
30 r x 20 ns

30 r x 20 ns
30 r x 20 ns
30 r x 20 ns
## Simulation protocols

<table>
<thead>
<tr>
<th>Transition</th>
<th>Technique</th>
<th>Collective Variables</th>
<th># of Replicas × Runtime</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IF\textsubscript{a} ↔ OF\textsubscript{a}</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Q\textsubscript{7})</td>
</tr>
<tr>
<td>2</td>
<td>BEUS</td>
<td>(Q)</td>
<td>50 × 20 ns = 1 \mu s</td>
</tr>
<tr>
<td>3</td>
<td>BEUS</td>
<td>(Q)</td>
<td>1000 × 1 ns = 1 \mu s</td>
</tr>
<tr>
<td>4</td>
<td>IF\textsubscript{a} ↔ IF\textsubscript{b}</td>
<td>BEUS</td>
<td>Z\textsubscript{Pi}</td>
</tr>
<tr>
<td>5</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Z\textsubscript{Pi})</td>
<td>30 × 40 ns = 1.2 \mu s</td>
</tr>
<tr>
<td>6</td>
<td>OF\textsubscript{a} ↔ OF\textsubscript{b}</td>
<td>BEUS</td>
<td>Z\textsubscript{Pi}</td>
</tr>
<tr>
<td>7</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Z\textsubscript{Pi})</td>
<td>30 × 40 ns = 1.2 \mu s</td>
</tr>
<tr>
<td>8</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Q\textsubscript{7})</td>
<td>24 × 20 ns = 0.5 \mu s</td>
</tr>
<tr>
<td>9</td>
<td>BEUS</td>
<td>Z\textsubscript{Pi}</td>
<td>15 × 30 ns = 0.5 \mu s</td>
</tr>
<tr>
<td>10</td>
<td>IF\textsubscript{b} ↔ OF\textsubscript{b}</td>
<td>2D BEUS</td>
<td>(\Delta\text{RMSD}, Z\textsubscript{Pi})</td>
</tr>
<tr>
<td>11</td>
<td>SMwST</td>
<td>(Q\textsubscript{1}, Z\textsubscript{Pi})</td>
<td>1000 × 1 ns = 1 \mu s</td>
</tr>
<tr>
<td>12</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Z\textsubscript{Pi})</td>
<td>50 × 20 ns = 1 \mu s</td>
</tr>
<tr>
<td>13</td>
<td>Full Cycle</td>
<td>BEUS</td>
<td>(Q\textsubscript{1}, Z\textsubscript{Pi})</td>
</tr>
</tbody>
</table>

**Total Simulation Time**: 18.7 \mu s

### GlpT

Crystal Structure

1. **BEUS**
2. **SMwST**
3. **BEUS**
4. **SMwST**
5. **BEUS**
6. **BEUS**
7. **SMwST**
8. **SMwST**
9. **SMwST**
10. **SMwST**
11. **SMwST**
12. **SMwST**
13. **Full Cycle**

---

Combining multiple replica simulations and coarse-grained models to describe membrane fusion
Workflow for Multi-Scale Modeling

Parametrically Defined Sine Function

Simulation box: periodic image

Initial Frame → Final Frame

Christopher Mayne, Tajkhoshid Lab
Workflow for Multi-Scale Modeling

Christopher Mayne, Tajkhorshid Lab
Highly Mobile Membrane Mimetic model

Advantages
- Increased mobility of lipids
- Retain explicit headgroups allowing for atomic details

Battling the Timescale - Case IV
Reduced Representations

Full model

HMMM model


Mark Arcario
Zenmei Ohkubo
Taras Pogorelov
Josh Vermaas
Javier Baylon
Membrane Binding of a Coagulation Factor

Z. Ohkubo and E. Tajkhorshid, Structure 2008
Enhanced Lipid Lateral Diffusion

Without Compromising Atomic Details of the Headgroups

Conventional membrane (10 ns)  

HMMM membrane (1 ns)
PS-Dependent Spontaneous Insertion of FVII-GLA
HMMM - More Efficient Computational Model for Membrane Proteins
R E M E M B E R:

One of the most useful advantages of simulations over experiments is that you can modify the system as you wish: You can do modifications that are not even possible at all in reality!

This is a powerful technique to test hypotheses developed during your simulations. Use it!

Animation available at the Nobel web site
Electrostatic Stabilization of Water Bipolar Arrangement
