Modeling of Cryo-EM Maps
Workshop
Baylor College of Medicine
Klaus Schulten, U. Illinois at Urbana-Champaign

Molecular Modeling Flexible Fitting 2: Introduction to Method

Fitting Structure to Map
Constraints against overfitting
Examples: here adenylate kinase

Simulations for Hybrid Microscopy

X-ray crystallography
Electron microscopy

APS at Argonne
NCSA supercomputer
FEI microscope

Current MDFF Applications

- Genetic decoding [1]
  J. Frank (Columbia U.)
- Regulatory nascent chain [2]
  R. Beckmann (U. Munich)
- Protein translocation [3,4]
  C. Akey (Boston U.)
  R. Beckmann (U. Munich)
- Poliovirus
  J. Hogle (Harvard U.)
- Flagellar hook
  K. Namba (Osaka U.)
- B. pumilus cyanide dihydratase
  T. Sewell (U. Cape Town)
- Membrane curvature [5,6]
  N. Hunter (Sheffield U.)


Ribosome ratcheting
J. Frank (Columbia U.)
T. Ha (UIUC)

Structures of the ribosome at different stages obtained by Cryo-EM
Application to Ribosome

**X-ray crystallography**
- High resolution (3-5Å)
- Crystal packing makes it difficult to determine functional state

**Cryo-EM**
- Lower resolution (typically 8-12Å)
- Many functional states can be obtained

Crystal structures of ribosome and ligands:
- 30S and 50S from 2I2U/2I2V (Berk et al., 2006);
- L1 protuberance based on 1MZP (Nikulin et al., 2003);
- L1 protein using MODELLER (Sali and Blundell, 1993) with 1ZHO as template (Nevskaya et al., 2006);
- A-site finger using 1TWB (Tung and Sanbonmatsu, 2004) as template;
- tRNAs from Selmer et al., 2006;
- ternary complex from 1OB2 (P:Nissen, unpublished)

Structures of the ribosome at different stages of the elongation cycle obtained by Cryo-EM

Obtaining High Resolution Images of Representative Functional States in Soccer

**Team photo**
- High resolution in close packing

**Match photo**
- Lower resolution during free action

Map players from team photo to match photo, bodies being flexible, obeying proper body mechanics, and being “drawn” into players identified in match photo; “proper” implies restraints to avoid overfitting.

EM: body mechanics = molecular dynamics; restraints = secondary structure conserving; “draw” through artificial forces that only weight density, as architectural are maintained through molecular dynamics.
Molecular dynamics flexible fitting (MDFF)

Two terms are added to the MD potential

\[ U_{total} = U_{MD} + U_{EM} + U_{SS} \]

An external potential derived from the EM map is defined on a grid as

\[ U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j) \]

\[ V_{EM}(\mathbf{r}) = \begin{cases} \xi & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr}, \\ \xi \left(1 - \frac{\Phi(\mathbf{r}) - \Phi_{thr}}{\Phi_{max} - \Phi_{thr}}\right) & \text{if } \Phi(\mathbf{r}) < \Phi_{thr}. \end{cases} \]

A mass-weighted force is then applied to each atom

\[ f_i^{EM} = -\nabla U_{EM}(\mathbf{R}) = -w_i \frac{\partial V_{EM}(\mathbf{r}_i)}{\partial r_i} \]


Protein Restraints

Harmonic restraints are applied to \( \phi \) and \( \psi \) dihedral angles of amino acid residues in helices or \( \beta \) strands:

\[ U_{restrain} = \frac{k}{2} \sum_i [(\phi_i - \phi_i^0)^2 + (\psi_i - \psi_i^0)^2] \]

Validation Using EF-Tu
(test case for proteins)

X–ray structures of EF–Tu in two states:

- **GTP–bound**

- **GDP–bound**

Red structure was fitted into simulated map from blue one (resolution of 10 Å).

Validation of the MDFF method

\[ \rho SE = \frac{\langle (S - \langle S \rangle)(E - \langle E \rangle) \rangle}{\sigma S \sigma E} \]

RNA restraints

1. RNAView is used to identify and classify base pairs; the following base pair types are selected: W/W, W/H, W/S, H/H, H/S, and stacked.

2. Harmonic restraints are applied to 7 dihedrals ($\alpha$, $\beta$, $\gamma$, $\delta$, $\epsilon$, and $\chi$) and to two inter-atomic distances.
Validation Using 16S rRNA
(test case for RNA)

X-ray structures of 16S rRNA in two states captured by the same crystal:
- 16S (2AVY)
- 16S (2AW7)
Red structure was fitted into simulated map from blue one (resolution of 10 Å).

Effect of Supersampling the Map

16S rRNA simulated map (blurred to 10 Å)
Backbone RMSD with respect to target structure

Supersampling: replace linear by cubic fit

**MDFF Simulation of Photosynthetic Membrane**

- In an MDFF simulation, RC-LH1-PufX dimer atoms are steered into high-density regions of the EM map
- 5 ns of MDFF, followed by a 29 ns of equilibration was performed

*water not shown*

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**Membrane Curvature Analysis**

Evolution of membrane geometry during the 34 ns MDFF+equilibration trajectory:

* Radius of curvature within range of experimental value
* “Twisting” of the membrane quantified -- axis of maximum curvature slanted
Local Curvature Properties and Long-Range Order

* Helical stacking of RC-LH1-PufX explained through local curvature properties

Direct stacking
Surfaces not complimentary

Off-set stacking
Surfaces complimentary

Tubular vesicle w/ direct stacking
Not observed

Tubular vesicle w/ off-set stacking
Helical arrangement observed


Molecular Dynamics Flexible Fitting Tutorial: A Simple Example

Initial (red) and target (blue) structures of adenylate kinase. This protein catalyzes the interconversion of adenine nucleotides, i.e., 2 ADP -> 1 ATP + 1 AMP
Molecular Dynamics Flexible Fitting Tutorial: A Simple Example

Generate an ideal electron density map of the target structure. Target structure shown in blue cartoon, density map shown as blue mesh.

Molecular Dynamics Flexible Fitting Tutorial: A Simple Example

• Prepare the initial structure.
• Defining secondary structure constraints.
• Rigid-body docking of the initial structure into density map.
• Running the MDFF simulation using NAMD.
• Visualizing the MDFF trajectory.
• Calculating the root mean square deviation.
• Calculating the cross-correlation coefficient.
Molecular Dynamics Flexible Fitting Tutorial: A Simple Example

Repeating the MDFF calculation in an explicit solvent.

- Preparing the initial structure.
- Preparing the density map.
- Running the MDFF simulation using NAMD.
- Visualizing the MDFF trajectory.
- Analyzing the results.

Structure Check Tutorial

Chirality in proteins and nucleic acids

- Checking stereochemistry of structure
- Correcting chirality

Identified unusual chiral center configurations in the chirality GUI window.

Representation of the chiral error in U12 of the tRNA.
Structure Check Tutorial

cis peptide bonds in proteins

Structurally optimized α-helix containing a cis peptide bond. The cis peptide bond is shown in CPK, while hydrogen bonds within the helix are shown as thick dashed blue lines. The cis configuration of the peptide bond disrupts the hydrogen bond network stabilizing the helix. Note, the hydrogen bond network is broken not only locally.

- Checking configuration of peptide bonds
- Converting cis peptide into trans configurations

The cis peptide GUI window. Identified cis peptide bonds in the cis peptide GUI window.

MDFF software

- MDFF plugin for setup and analysis available in VMD 1.8.8
- MDFF support available in NAMD version 2.7
- Tutorial for performing flexible fitting with MDFF released
- For more information visit http://www.ks.uiuc.edu/Research/mdff
- Planned development:
  - GPU-accelerated volumetric map manipulation
  - Interactive MDFF
  - Support for implicit solvent model
  - Helical symmetry restraints
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