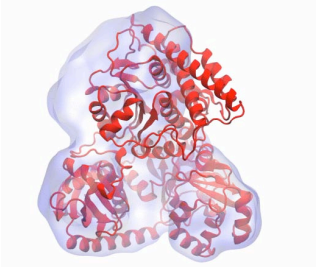
Modeling of Cryo-EM Maps

Workshop

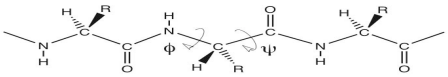
Baylor College of Medicine

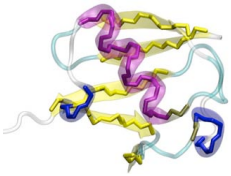
Klaus Schulten, U. Illinois at Urbana-Champaign

Molecular Modeling Flexible Fitting 2: Introduction to Method

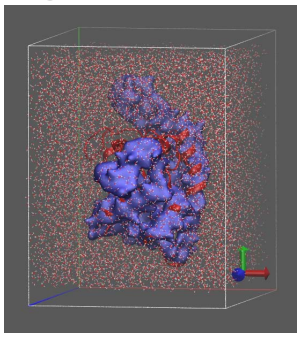


Fitting Structure to Map





Constraints against overfitting



Examples: here adenylate kinase

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Simulations for Hybrid Microscopy

X-ray crystallography

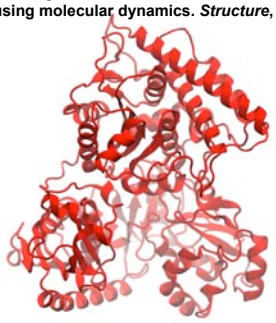
APS at Argonne

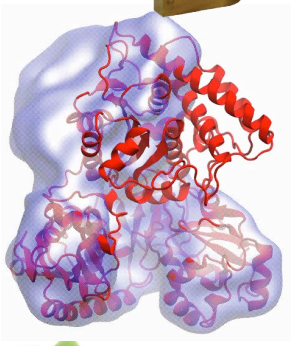
NCSA supercomputer

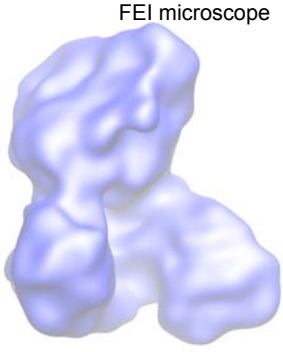
Electron microscopy

FEI microscope

L. Trabuco, E. Villa, K. Mitra, J. Frank, and K. Schulten. Flexible fitting of atomic structures into electron microscopy maps using molecular dynamics. *Structure*, 16:673-683, 2008.







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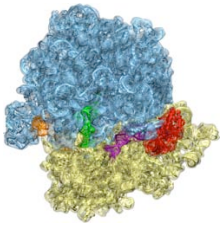
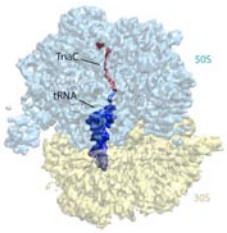
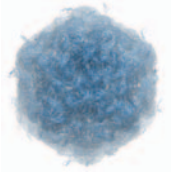
Current MDFF Applications



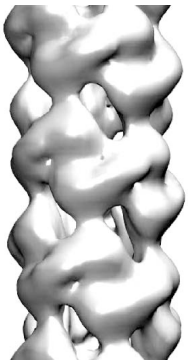
Genetic decoding [1]
J. Frank (Columbia U.)



Regulatory nascent chain [2]
R. Beckmann (U. Munich)

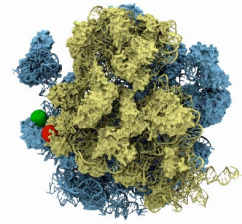


Poliovirus
J. Hogle (Harvard U.)

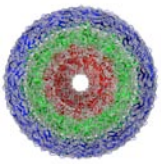


B. pumilus cyanide dihydratase
T. Sewell (U. Cape Town)

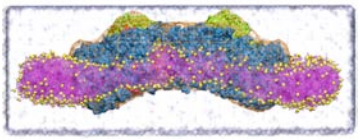
Protein translocation [3,4]
C. Akey (Boston U.)
R. Beckmann (U. Munich)



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



Flagellar hook
K. Namba (Osaka U.)



Membrane curvature [5,6]
N. Hunter (Sheffield U.)

[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Villa et al. *PNAS* (2009) 106:1063-1068.
[3] Sener et al. *Chem Phys* (2009) 357:188-197.
[4] Trabuco et al. *Methods* (2009) 49:174-180.
[5] Hsin et al. *Biophys J* (2009) 97:321-329.
[6] Gumbart et al. *Structure* (2009) In press.
[7] Seidelt et al. *Science* (2009) 326: 1412-1415.
[8] Becker et al. *Science* (2009) 326: 1369-1373.

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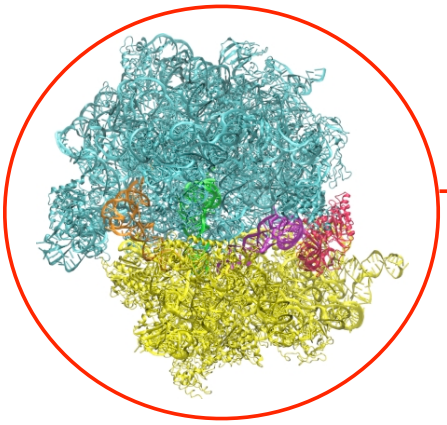
Application to Ribosome

X-ray crystallography

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

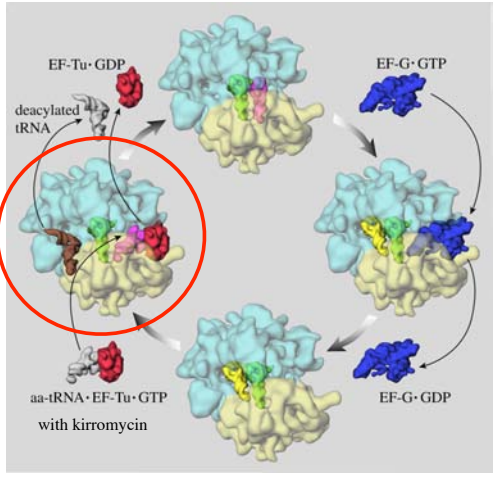


Crystal structures of ribosome and ligands

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)

Cryo-EM

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



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

(J. Frank. The dynamics of the Ribosome inferred from Cryo-EM, in Conformational Proteomics of Macromolecular Architectures, 2004)

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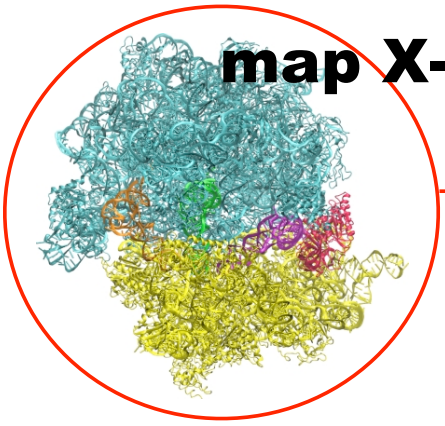
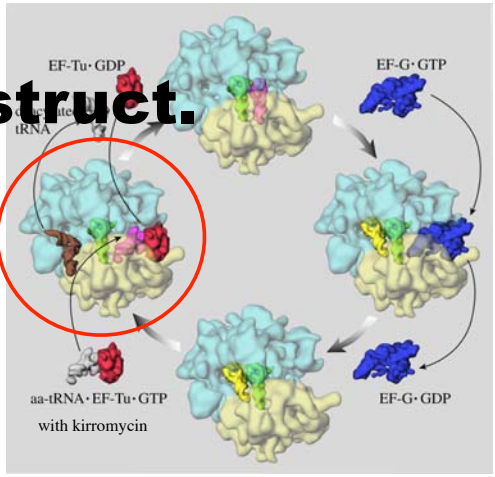
map X-ray struct.





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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, identify 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

$$U_{total} = U_{MD} + U_{EM} + U_{SS}$$

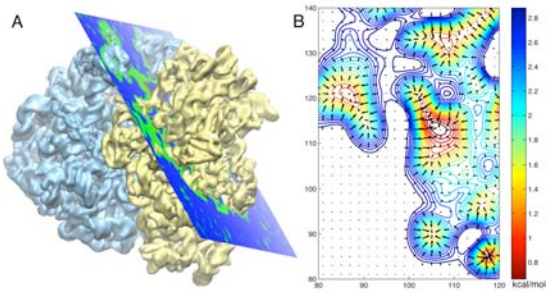
An external potential derived from the EM map is defined on a grid as

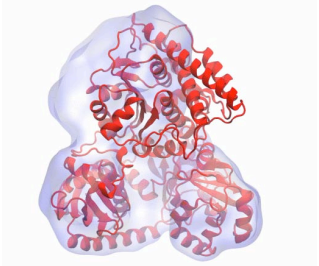
$$U_{EM}(\mathbf{R}) = \sum_j w_j V_{EM}(\mathbf{r}_j)$$

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

A mass-weighted force is then applied to each atom

$$\mathbf{f}_i^{EM} = -\nabla U_{EM}(\mathbf{R}) = -w_i \partial V_{EM}(\mathbf{r}_i)/\partial r_i$$





[1] Trabuco et al. *Structure* (2008) 16:673-683.
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[5] Hsin et al. *Biophys J* (2009) 97:321-329.
[6] Gumbart et al. *Structure* (2009) In press.
[7] Seidelt et al. *Science* (2009) 326: 1412-1415.
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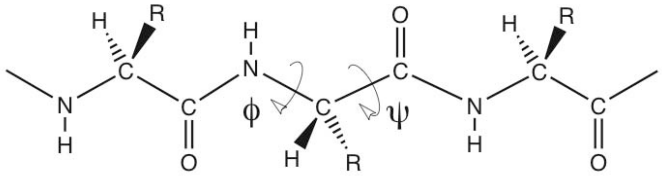
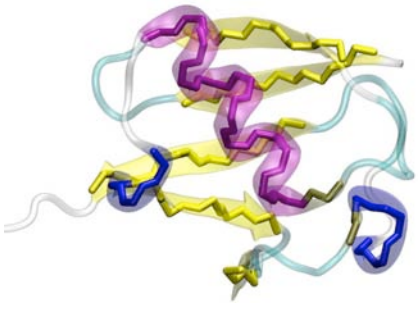
Protein Restraints

Harmonic restraints are applied to ϕ and ψ dihedral angles of amino acid residues in helices or β strands:

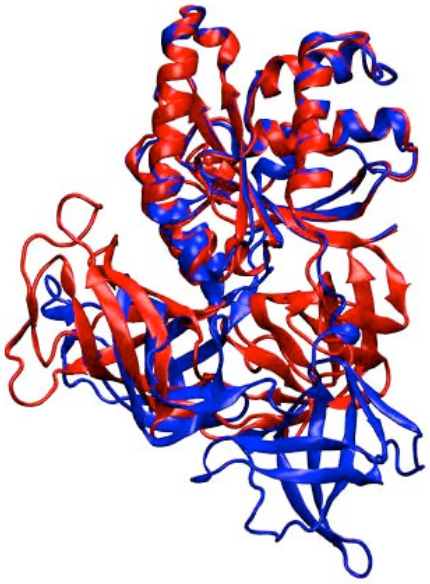
$$U_{restrain} = \frac{k}{2} \sum_i [(\phi_i - \phi_i^0)^2 + (\psi_i - \psi_i^0)^2]$$





[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Trabuco et al. *Methods* (2009) 49:174-180.

Validation Using EF-Tu
(test case for proteins)



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

- GTP-bound

  PDB 1EFT, Kjeldgaard et al., Structure 1: 35-50 (1993).

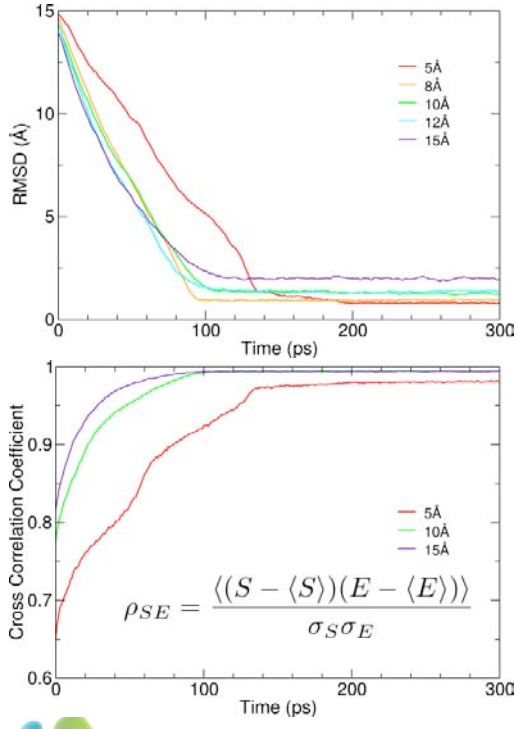
- GDP-bound

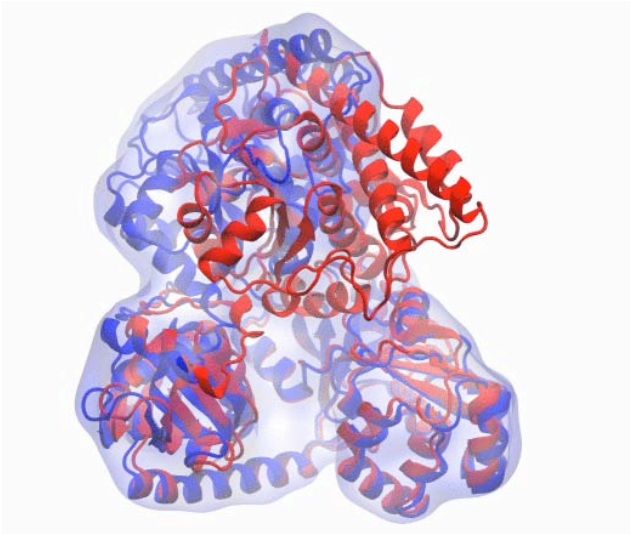
  PDB 1TUI; Polekhina et al., Structure 4: 1141-1451 (1996).

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

[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Trabuco et al. *Methods* (2009) 49:174-180.

Validation of the MDFF method





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[1] Trabuco et al. *Structure* (2008) 16:673-683.
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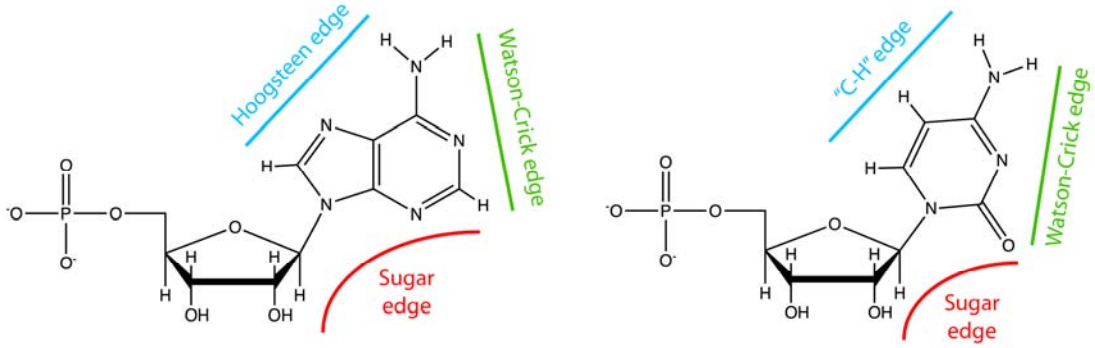
RNA restraints

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.



Yang et al. (2003). Nucleic Acids Research **31**: 3450-3460.

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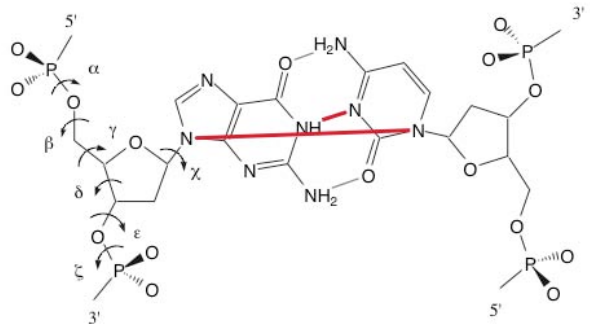
RNA restraints

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 (α, β, γ, δ, ε, and χ) and to two inter-atomic distances.



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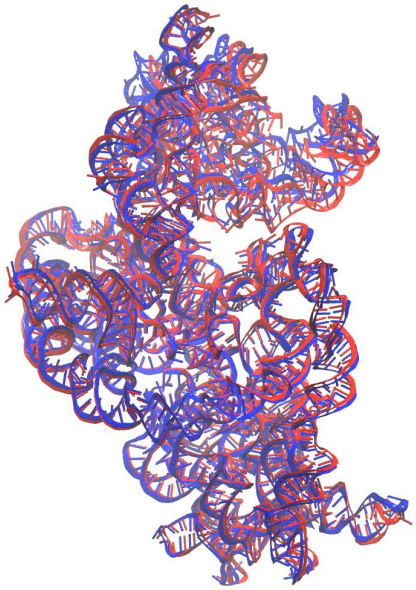
[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Trabuco et al. *Methods* (2009) 49:174-180.

Validation Using 16S rRNA
(test case for RNA)



X-ray structures of 16S rRNA in two states captured by the same crystal:

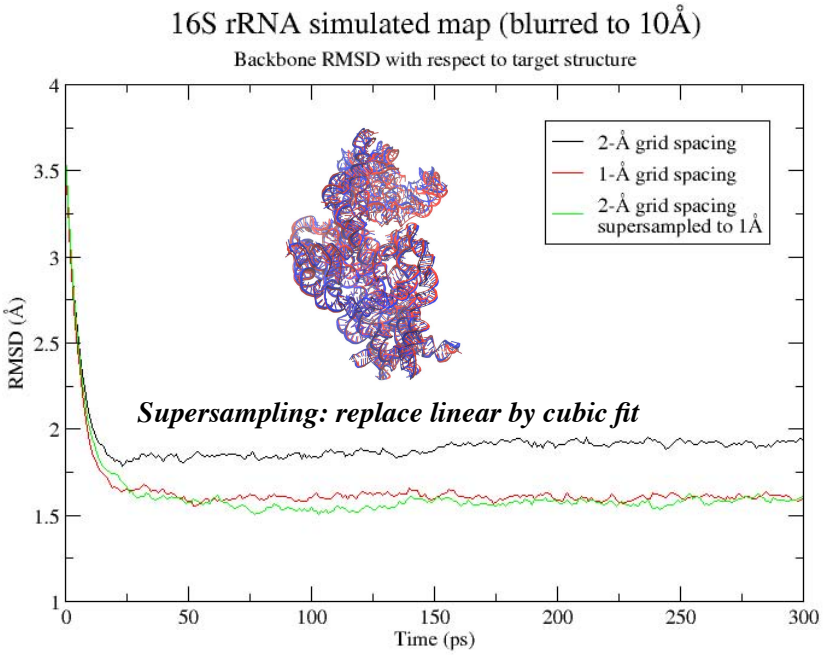
- 16S (2AVY)
- 16S (2AW7)

Schuwirth, et al., Science 310: 827-834 (2005).

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

[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Trabuco et al. *Methods* (2009) 49:174-180.

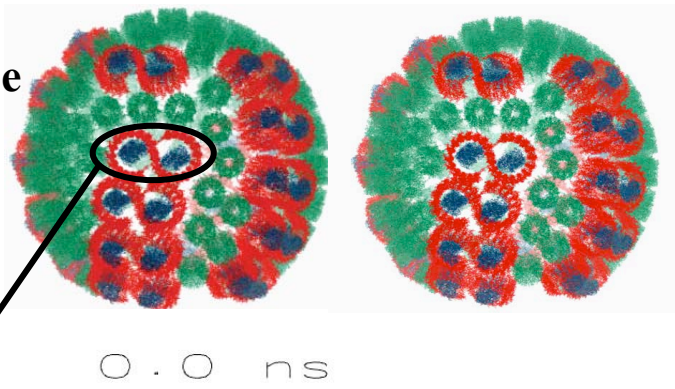
Effect of Supersampling the Map



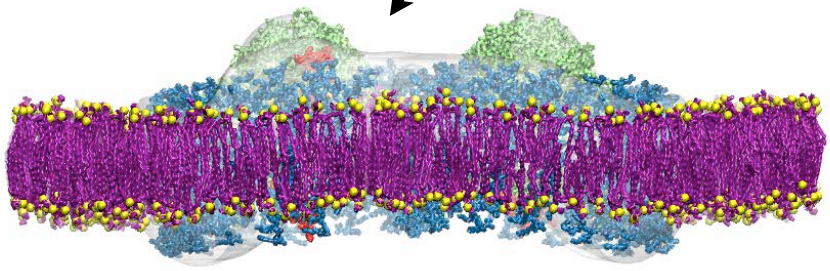
[1] Trabuco et al. *Structure* (2008) 16:673-683.
[2] Trabuco et al. *Methods* (2009) 49:174-180.

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



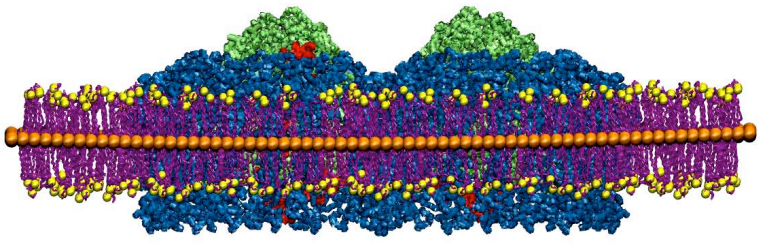
water not shown

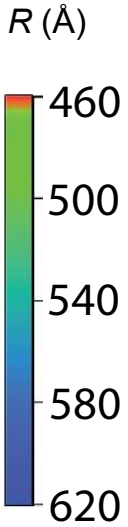


J. Hsin, J. Gumbart, L. T. Trabuco, E. Villa, P, Qian, C. N. Hunter, and K. Schulten. *Biophys. J.* 97: 321-329 (2009).

Membrane Curvature Analysis

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



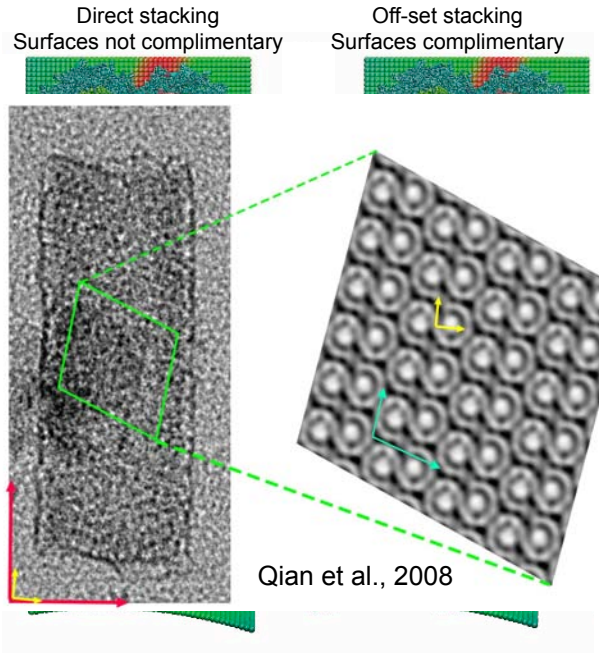


* Radius of curvature within range of experimental value
* "Twisting" of the membrane quantified -- axis of maximum curvature slanted

J. Hsin, J. Gumbart, L. T. Trabuco, E. Villa, P, Qian, C. N. Hunter, and K. Schulten. *Biophys. J.* 97: 321-329 (2009).

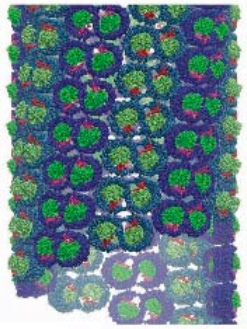
Local Curvature Properties and Long-Range Order

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



Tubular vesicle
w/ direct stacking
Not observed

Tubular vesicle
w/ off-set stacking
Helical arrangement
observed



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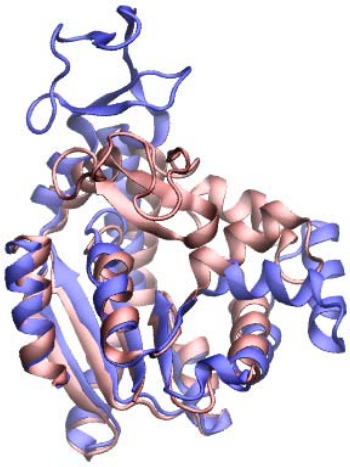
Molecular Dynamics Flexible Fitting Tutorial: A Simple Example



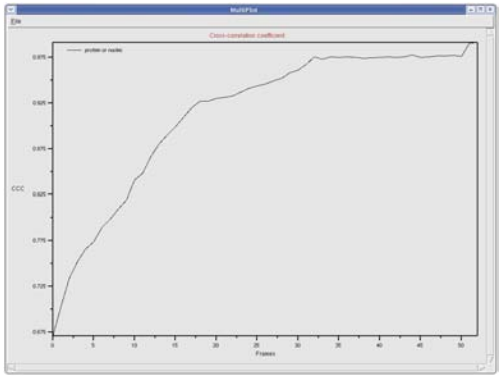
Initial (red) and target (blue) structures of adenylate kinase. This protein catalyzes the interconversion of adenine nucleotides, i.e., 2 ADP -> 1 ATP + 1 AMP

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.

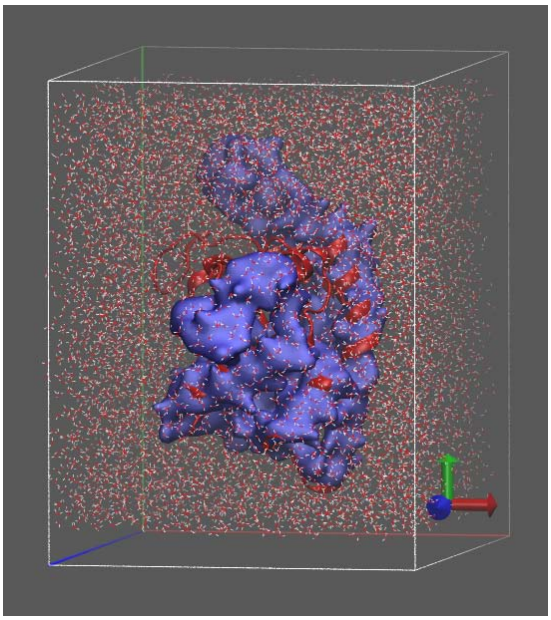
Molecular Dynamics Flexible Fitting Tutorial: A Simple Example

- 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

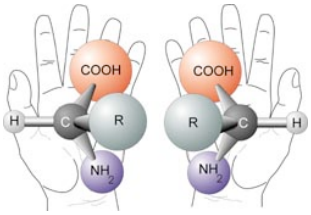
Repeating the MDFF calculation in an explicit solvent.



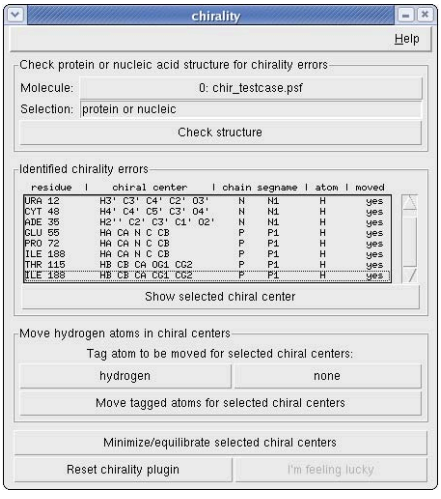
- Preparing the initial structure.
- Preparing the density map.
- Running the MDFF simulation using NAMD.
- Visualizing the MDFF trajectory.
- Analyzing the results.

Structure Check Tutorial

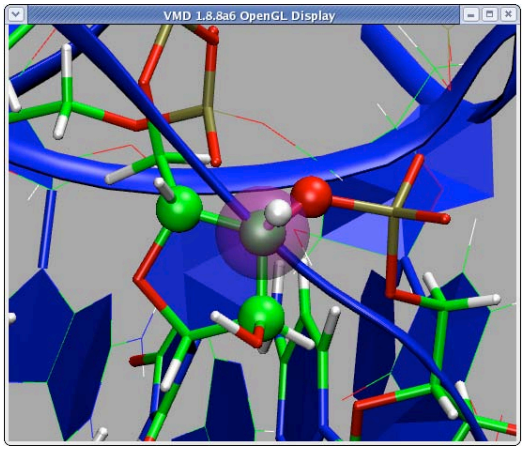
Chirality in proteins and nucleic acids



- Checking stereochemistry of structure
- Correcting chirality



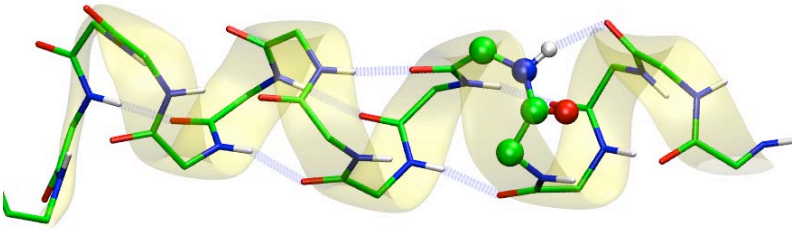
Identified unusual chiral center configurations in the chirality GUI window.



Representation of the chiral error in U12 of the tRNA.

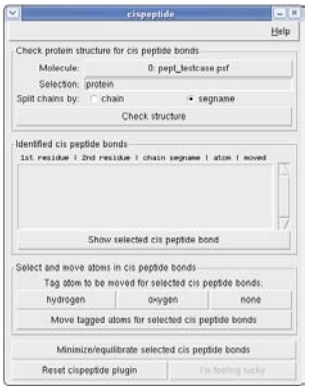
Structure Check Tutorial

cis peptide bonds in proteins

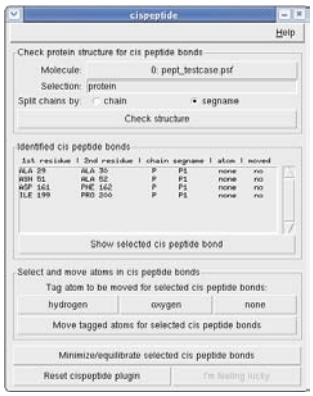


Structurally optimized α-helix containing a *cis*peptide bond. The *cis* peptide bond is shown in CPK, while hydrogen bonds within the helix are shown as thick dashed blue lines. The *cis* configuration of the peptide bond disrupts the hydrogen bond network stabilizing the helix. Note, the hydrogen bond 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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