MD-Based Refinement and Validation at Sub-5 Å Resolution

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Cryo-EM from Cells to Molecules: Multi-Scale Visualization of Biological Systems
Lake Tahoe, CA

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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

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

Molecular Dynamics Flexible Fitting at 3-5 Angstrom Resolution
Combination of “Enhanced-sampling” with Flexible Fitting

Multiple stereoisomers in the same 1.8 Angstrom data

J Am. Chem. Soc. 2015, 137, 8810-8818
Results of the Cryo-EM Structure Challenge (EM193)

emb_5778

emb_5995
Model-uncertainty: RMSF (ensemble measure)
RMSF and inherent flexibility

- (a) β-gal (2.2 Å), $R^2 = 0.97$, RMSF = 0.37
- (b) TRPV1 (3.4 Å), $R^2 = 0.96$, RMSF = 0.64
- (c) γ-sec (3.4 Å), $R^2 = 0.83$, RMSF = 0.54
RMSF and Quality of Map

![Graph showing RMSF and quality of map as a function of B-factor. The graph includes data points for RMSF and EMRinger data, with a curve fit for RMSF, and an experimental RMSF at a B-factor of -75.](Image)
What should we report?

- Local per-residue cross correlation
- Integrated FSC
- RMSF or similar uncertainty measures
- EM-ringer
- Molprobity statistics
- Source of initial model
New Force Fields and CaBLAM Analysis

β-galactosidase

<table>
<thead>
<tr>
<th>Force Field</th>
<th>Rama-favored</th>
<th>Outliers</th>
<th>Rot-favored</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARMM</td>
<td>89.90%</td>
<td>2.70%</td>
<td>89.2%</td>
<td>4.40%</td>
</tr>
<tr>
<td>CHARMM-36m</td>
<td>93.01%</td>
<td>1.45%</td>
<td>90.3%</td>
<td>2.69%</td>
</tr>
<tr>
<td>36m + CaBLAM</td>
<td>94.04%</td>
<td>1.08%</td>
<td>92.3%</td>
<td>2.12%</td>
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Looking Forward: **Pushing the Resolution limit of de novo Modeling**

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- Richardson (Duke)
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**eposters**

- aMDFF, MELD-MDFF, SegMDFF, *Model Maker*

In-house MDFF applications:  
- *Cell*, 170, 1234 (2017)  
- *PNAS*, 113, 7816 (2016)