Assessment of High Resolution CryoEM Features

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Assessment of High Resolution Features

• Background: map-model scores
  • Average map value at atom positions
  • Cross-correlation
  • Map-model FSC
  • EMRinger

• Q-score
  • Resolvability of atoms, side chains, bases
  • A measure of local resolution based on a fitted/derived model

• Examples
  • Protein, RNA
    • How resolvable are atoms, side chains, bases?
  • Water and Metal Ions
    • Can we really resolve waters and metal atoms in cryoEM maps?
    • At what resolutions can we see water and metal atoms?
    • How to validate water atoms and metal/salt ions?
• **Average map value** at atom positions

\[
\text{avg} = \frac{v_1 + v_2 + v_3 + v_4 \ldots v_7}{7}
\]
Cross-correlation

- Calculate a map from model (what resolution?)
  - \( r \) – vector of values at grid points in model map

- Interpolate map values
  - \( b \) - interpolated values at same points in CryoEM

\[
CC = \frac{\langle r, b \rangle}{|r||b|} = \frac{r_1 * b_1 + r_2 * b_2 + \cdots + r_5 * b_5}{\sqrt{r_1^2 + r_2^2 + \cdots + r_5^2} \cdot \sqrt{b_1^2 + b_2^2 + \cdots + b_5^2}}
\]
Map-model FSC

- Calculate a map from model
- Correlation between cryoEM map and model-map
- In Fourier Space

FSC

Resolution (Å)

CryoEM Map

Map From Model

Map from Model With B-Factors

CryoEM Map

Map From Model

Map from Model With B-Factors

Resolution (Å)

FSC

1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1

1000.00
19.61
9.90
6.62
4.98
3.98
3.32
2.85
2.49
2.22
2.00
1.81
1.66
1.54
1.43
1.33
**EMRinger**

- **EMRinger**
  - Barad et. al., 2015
  - [https://fraserlab.com/2015/02/18/EMringer/](https://fraserlab.com/2015/02/18/EMringer/)
  - Rotate Cβ-atom
  - Do peaks in map correspond to rotameric positions?
  - Average EMRinger score correlates with resolution

![Graph showing EMRinger score vs. resolution](image1.png)

![Graph showing map value vs. Chi1 angle](image2.png)
Q-Score

1.75Å
EMD:20026
PDB:3ajo, Leu26

Q=0.91

Radial Distance (Å)

Average Density

<table>
<thead>
<tr>
<th>Radial Distance (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
</tr>
<tr>
<td>0.5</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1.5</td>
</tr>
<tr>
<td>2</td>
</tr>
</tbody>
</table>

\[ Q = CC(u, v) = \frac{\langle u - u_{ave}, v - v_{ave} \rangle}{\|u - u_{ave}\|\|v - v_{ave}\|} \]

- \( u \) – avg. densities from map
- \( v \) – reference Gaussian

https://www.biorxiv.org/content/10.1101/722991v1
\[ Q = CC(u, v) = \frac{\langle u - u_{ave}, v - v_{ave} \rangle}{\| u - u_{ave} \| \| v - v_{ave} \|} \]

- \( u \) – avg. densities from map
- \( v \) – reference Gaussian

https://www.biorxiv.org/content/10.1101/722991v1
\[ Q = \text{CC}(\mathbf{u}, \mathbf{v}) = \frac{\langle \mathbf{u} - \mathbf{u}_{\text{ave}}, \mathbf{v} - \mathbf{v}_{\text{ave}} \rangle}{||\mathbf{u} - \mathbf{u}_{\text{ave}}||||\mathbf{v} - \mathbf{v}_{\text{ave}}||} \]

\( \mathbf{u} \) – avg. densities from map

\( \mathbf{v} \) – reference Gaussian


https://www.biorxiv.org/content/10.1101/722991v1
Q-score Example: Asparagine

$Q_{av_1}=0.94$

$Q_{av_2}=0.92$

$Q_{av_3}=0.89$

$Q_{av_4}=0.82$

$Q_{av_5}=0.62$

$Q_{av_6}=0.54$


https://www.biorxiv.org/content/10.1101/722991v1
Q-score Example: Glutamic Acid

<table>
<thead>
<tr>
<th>1.52 Å</th>
<th>1.54 Å</th>
<th>1.75 Å</th>
<th>2.3 Å</th>
<th>3.1 Å</th>
<th>3.9 Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>X-ray map</td>
<td>CryoEM</td>
<td>CryoEM</td>
<td>CryoEM</td>
<td>CryoEM</td>
<td>CryoEM</td>
</tr>
<tr>
<td>PDB: 3ajo 2fo-fc</td>
<td>EMD: 9865</td>
<td>EMD: 20026</td>
<td>EMD: 20027</td>
<td>EMD: 20028</td>
<td>EMD: 0140</td>
</tr>
<tr>
<td>Q_{avg} = 0.95</td>
<td>Q_{avg} = 0.91</td>
<td>Q_{avg} = 0.86</td>
<td>Q_{avg} = 0.78</td>
<td>Q_{avg} = 0.56</td>
<td>Q_{avg} = 0.47</td>
</tr>
</tbody>
</table>

https://www.biorxiv.org/content/10.1101/722991v1
Q-score Example: Tyrosine

1.52Å
X-ray map
PDB:3ajo 2fo-fc
Q\textsubscript{avg}=0.95

1.54Å
CryoEM
EMD:9865
Q\textsubscript{avg}=0.92

1.75Å
CryoEM
EMD:20026
Q\textsubscript{avg}=0.88

2.3Å
CryoEM
EMD:20027
Q\textsubscript{avg}=0.79

3.1Å
CryoEM
EMD:20028
Q\textsubscript{avg}=0.63

3.9Å
CryoEM
EMD:0140
Q\textsubscript{avg}=0.59

https://www.biorxiv.org/content/10.1101/722991v1
Maps and Models (Protein) from EMDB

\[ y = -0.1794x + 1.1244 \]
\[ r^2 = 0.91 \]


https://www.biorxiv.org/content/10.1101/722991v1
TMV with RNA Fragment (1.9Å)

EMD:10129

Protein

RNA

PDB:4udv, High Threshold

0.81

0.95

0.86

0.94

0.82

0.86

0.81

GUA

ADE

ADE

Lower Threshold
Nucleic Acid (RNA) Q-score Examples

2.5Å (EMD:7025)  
G74.1

Q_{nt}=0.78
Q_{base}=0.84
Q_{bb}=0.73

3.0Å (EMD:4638)  
G194.A

Q_{nt}=0.73
Q_{base}=0.77
Q_{bb}=0.68

3.5Å (EMD:4125)  
G21.a

Q_{nt}=0.73
Q_{base}=0.58
Q_{bb}=0.62

4.0Å (EMD:2763)  
G95.2

Q_{nt}=0.51
Q_{base}=0.45
Q_{bb}=0.56

U73.1 G74.1

Q_{nt}=0.73
Q_{nt}=0.78

U193.A G194.A

Q_{nt}=0.69
Q_{nt}=0.73

U20.a G21.a

Q_{nt}=0.57
Q_{nt}=0.58

U94.2 G95.2

Q_{nt}=0.46
Q_{nt}=0.51
Maps and Models (Protein) from EMDB

Q-score vs. Estimated Resolution

Maps and Models (RNA) from EMDB

Avg. Q-score (Protein atoms)

Reported Resolution (Å)

Avg. Q-score (All atoms)

Reported Resolution (Å)

y = -0.18x + 1.12
r^2 = 0.91

y = -0.14x + 1.01
r^2 = 0.90
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    • At what resolutions can we see water and metal atoms?
    • How to validate water atoms and metal/salt ions?
Type III-A CRISPR-Csm (3.5Å)

M. Guo, K. Zhang, G. Pintilie, et al., Cell Research, 2019
Type III-A CRISPR-Csm (3.5Å)

M. Guo, K. Zhang, G. Pintilie, et al., Cell Research, 2019
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M. Guo, K. Zhang, G. Pintilie, et al., Cell Research, 2019
Apoferritin Example

Q-score can be low if:
• Map resolvability is low
• Model is not optimized

Per-residue Q-score – X-ray model

Automatically-built model

Q_residue

Q

Residue #
Assessment of High Resolution Features

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• Examples
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    • How resolvable are atoms, side chains, bases?
  • Water and Metal Ions
    • At what resolutions can we see water and metal atoms?
    • How to validate water atoms and metal/salt ions?
Water, Mg Atoms in Xray Map

PDB:3ajo model and 2fo-fc map (1.52Å)
Apo ferritin X-Ray Map 1.52 Å, PDB: 3ajo

**HOH – other atom distance**

- **HOH-O**: 2.8 Å
- **HOH-HOH**: 2.2 Å

**MG – other atom distance**

- **MG-HOH**: 2.2 Å

**Structures**

- **Trigonal planar**
- **Tetrahedral**
- **Octahedral**
Water, Mg from X-ray structure in 1.75Å CryoEM Map

PDB:3ajo model and 1.75Å Map
Water and Metal Atoms – Refinement

- X-ray model
- Refined Model

PDB:3ajo model and 1.75Å Map
Water and Metal Atoms – Refinement

**X-Ray Model**
- # HOH Atoms
- Q-Score

**Cryo-EM Map (Initial)**
- # MG Atoms
- Q-Score

**Cryo-EM Map (After Refinement)**
- Q-Score
Water and Metal Atoms in a Cryo-EM Map

Water atoms in CryoEM Map
1.54Å (EMD:9865)

Mg, Water atoms in cryoEM map
1.54Å (EMD:9865)
Validation of Water atoms using Half Maps
Apo ferritin – Water and Metal (sub-2.0Å)

1.52Å X-Ray Map (2Fo-Fc, PDB:3ajo, human)

1.54Å Cryo-EM Map (EMD:9865, mouse)

1.65Å Cryo-EM Map (EMD:0144, human)

1.75Å Cryo-EM Map (EMD:20026, human)
Apo ferritin – Water and Metal (2Å to 4Å)

1.75Å Cryo-EM Map (EMD:20026)

2.3Å Cryo-EM Map (EMD:20027)

3.1Å Cryo-EM Map (EMD:20028)

3.9Å Cryo-EM Map (EMD:0140)
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• Examples
  • Protein, RNA
  • Water and Metal Ions
    • Waters and Magnesium are reproducible in cryoEM (compared to X-ray)
    • At what resolutions can we see water and metal atoms?
      • Metal/salt at ~4Å and higher
      • Water atoms at ~3Å and higher
    • How to validate water atoms and metal/salt ions?
      • Distances to nearby polar atoms
      • Q-scores in map, half maps, and related maps