MapQ & Q-scores Tutorial

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MapQ v1.9.5 Chimera
Version 1.13+

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Overview

1. In this tutorial, we will use the MapQ Chimera plugin to first have a look through a map and model, to visually see how backbone and side chain residues fit the density.
2. Then, we will calculate Q-scores and visualize them on the ribbon, to spot areas that may not be resolved or fitted as well as we might expect at the given resolution.
3. Where 'expected-Q' score comes from
4. Example of areas where the map is not resolved
5. Example of areas where the model is not optimally fitted to the density

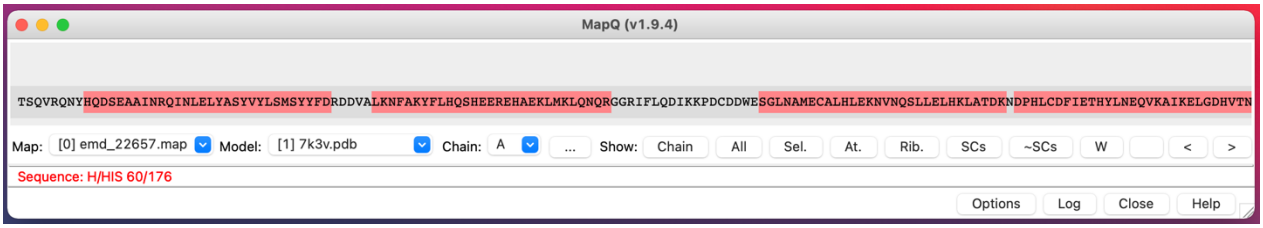
1. Install plugin, visualize map and model

- Download and install the latest version of the plugin
  - www.github.com/gregdp/mapq
  - Please not that on Mac, Chimera versions newer than 1.13 may appear to lag. In this tutorial, we use Chimera v1.13.

- Download and open the map EMD:22657, and the model, PDB:7k3v.
  - https://www.emdataresource.org/EMD-22657
  - https://www.rcsb.org/structure/7k3v

- Open the MapQ dialog:
  - From the Chimera window, Tools -> Volume Data -> MapQ.

- In the MapQ dialog, select the map and model in the Map: and Model: fields:
  - They may already be selected by default if they were already open.



- With Chain A selected, its sequence will be shown in the dialog.
  - The sequence is made up of amino acid residues (e.g. Tyrosine, T, Serine, S, Glutamine, Q, etc.)
  - Residues that are in 'loops' are shown with gray background.
  - Residues that are in 'alpha-helices' are shown with reddish background.
  - Residues that are in 'beta-sheets' are shown with blue background

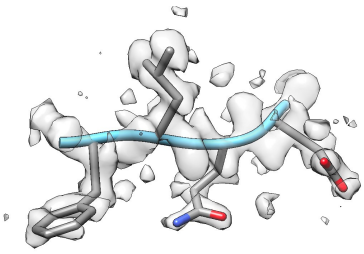
- The "…" button can be used to open new maps and models using the usual Chimera file selection dialog.
  - With this option, .cif files are opened using a custom parser, which should open faster than with the usual Chimera dialog.

- The buttons to the right of 'Show:' are:
  - '**Chain**' shows only the selected chain
  - '**All**' shows all chains
  - '**Sel**.' makes what is selected visible

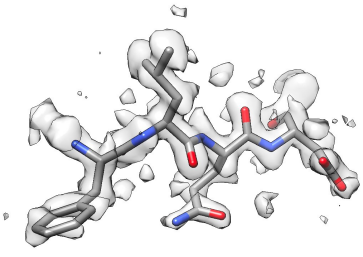
- Select an entire loop/helix (Ctrl+Click+Drag on the sequence).
  - This selects just that part of the protein (you should see a green outline around it)
  - The map around the selection will be extracted and shown.

- With some part of the protein selected, click '**At.**', '**Rib.**', '**SCs**', '**~SCs**', '**W**' to show/switch between seeing Atoms, Ribbon, Side Chains, No Side Chains, Wire-bonds, respectively. This will show the protein in different ways, as illustrated below.

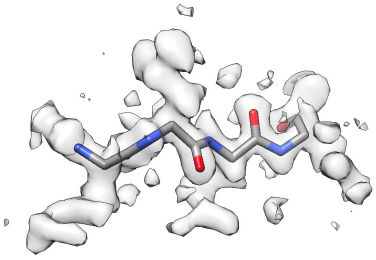
- The blank " " button can be used to hide anything that's currently selected.



As Ribbon, with Side Chains

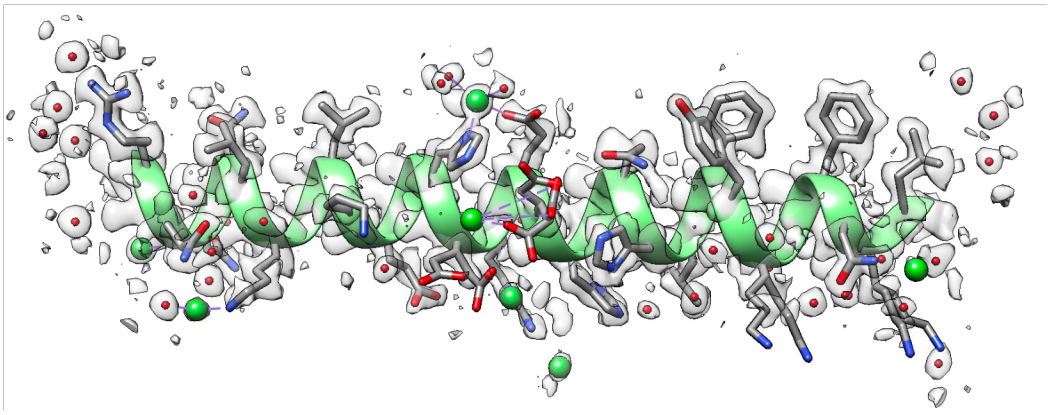


As 'Atoms', with Side Chains.



As 'Atoms', without Side Chains, i.e. just the Backbone

• Selecting the entire second helix:



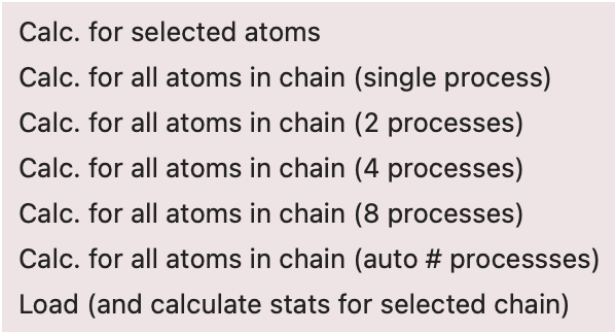
• **Options**

- The “Options” button at the bottom of the dialog will show another line in the dialog:

- Resolution – this value is used to compare Q-scores to expected Q-scores at a given resolution. ○ Sigma – by default, 0.6 is used in general, and works well for maps with resolutions of 1.5Å and lower. For higher-resolutions up to 1Å, 0.4 is used.
- Gaps – gaps are shown in the sequence with .
- Extract – when selecting part of the sequence, it will be shown by itself along with masked density
- Mesh – also adds a mesh when creating a masked map ○ Ligands – show ligands near selected sequence
- Keep – keeps previously shown selection, otherwise it is hidden
- +Map – also keeps previous masked map
- H – show hydrogen atoms (if SC are shown), otherwise they are hidden
- < - when using Keep, this reverts the last selection ○ Redo – Redo the last selection ○ Near – show residues near selection
- Zoom: ‘**-**‘ and ‘+’ decrease the sequence text size, ‘<’ and ‘>’ go to the beginning and end of sequence, respectively.

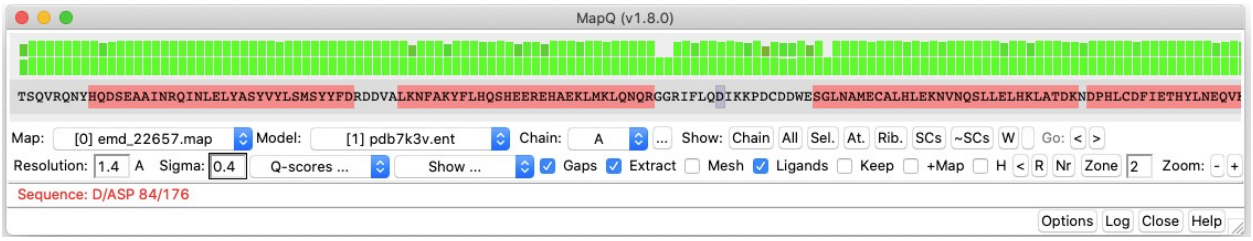
2. Calculating and visualizing Q-scores

- To calculate Q-scores, press the Q-scores … button, and select one of the options:

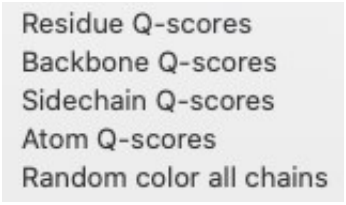


  - Press the second option for single processor (will take about 1min), or one of the multi-processing options which will be faster.
  - When using a single process, you will see the progress and ETA at the bottom of the main Chimera window. You can cancel the process by pressing the X.
  - When using multiple processes, you can keep track of the progress and ETA by navigating to the folder where the map and model are, going into the temp folder (has __Q-scores__mp__calculation__files__ in the name), and looking at the __stat.txt files (there is one for each process).
  - It can help to see status messages by opening the IDLE window (Tools -> General Controls -> IDLE), or press Log at the bottom of the MapQ window.

- After calculating Q-scores using the **Calc** button (or loading them with the **Load** option), you will see the area above the sequence show a bar graph of Q-scores for each residue.
  - On the bottom are backbone Q-scores, on the top are side chain Q-scores:

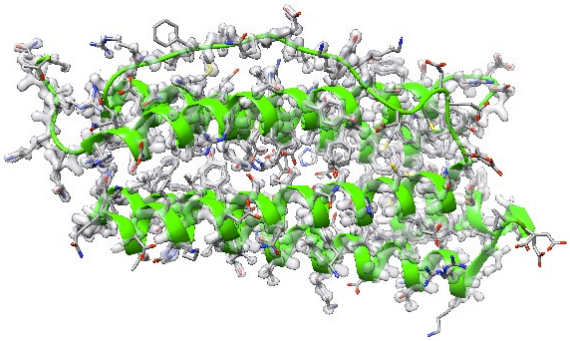


  - The resolution entered in the “Resolution:” field is used to calculate what the height of the bars should be, i.e. the expected Q-scores for a residue should be.
  - To visualize Q-scores on the model, press “Visualize…” button and select one of the options:

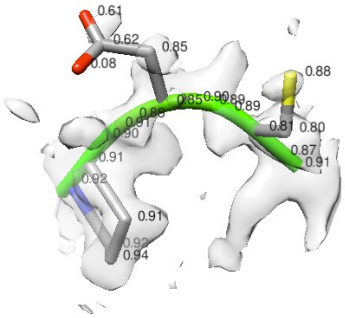


- **Residue** – colors ribbon by entire residue Q-score
- **Backbone** – colors the model ribbon by backbone score (green is high, red is lower Q-score). ○ **Sidechain** – colors ribbon by side chain score ○ **Atom** – shows a label with the Q-score next to each atom currently selected.
  When no atoms are selected, all previously shown labels are hidden.

- **Random color all chains** – colors each chain a random color



Colored backbone using Residue Q-scores (mostly green because all residues are on average well resolved)



Showing atom Q-scores, displayed as labels next to a few atoms. (Select the atoms, then the Show… Atom Q-scores option.)

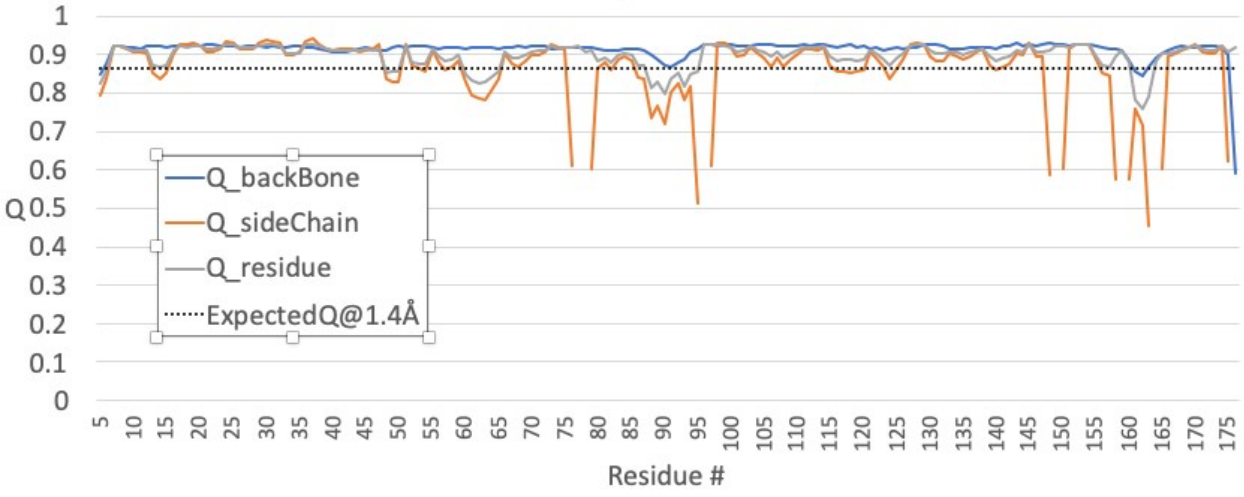
- After Q-scores are calculated you will see two new files in the folder where your model is saved:
  - [model_name]_Q_[map_name].pdb
    - This file stores Q-scores in the occupancy column
    - Q-scores are loaded from this file when you press the **Load** button, if it exists ○ [model_name]_Q_[map_name]_[chain].txt
    - This is a text file which saves per-residue Q-scores
    - You can load it in Excel or another graphing program to create per-residue Q-score plots as shown below.
    - At each residue, the average Q score over all atoms in the residue are in the Q_residue column. Average Q-scores for backbone and side chain atoms are in the Q_backBone and Q_sideChain columns.

- There are a few columns in which Q-scores from nearby residues are averaged (with 1,2,3 and 5 nearby residues). This makes the plot a bit nicer if the Q-scores fluctuate a lot.
- In the last column in each section, the expected Q-score at a resolution of xxÅ is shown, where xx is the value entered in the Resolution:[_] field in the MapQ dialog. Where this value comes from is explained below.

- Notes:
  - These files are created only if you loaded your model from a file on disk; if you loaded it with Fetch or copied it from another model somehow, you will not see these files
  - The files will only have Q-scores for the chain selected; if 'All' is selected, Qscores for all chains will be calculated and included in the files above
  - If you want Q-scores for all chains in the .txt file, make sure to select 'All' next to chains before hitting the **Calc** button. Otherwise, only the Q-scores for the selected chain will be calculated.

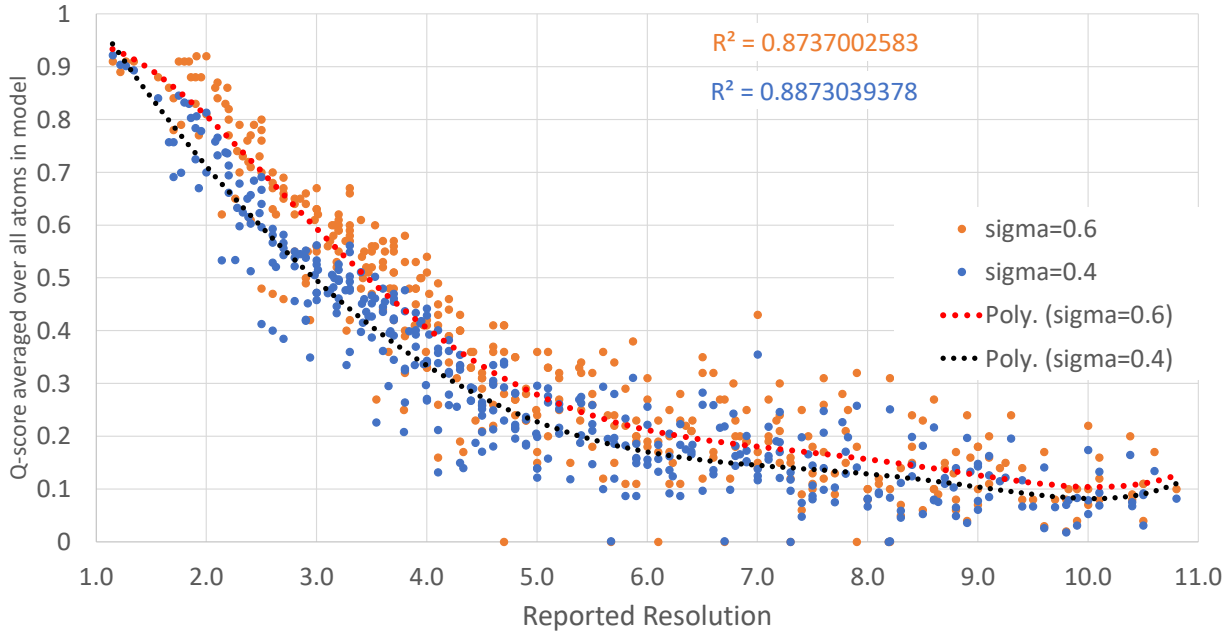
- Per-residue Q-scores can be plotted from the .txt file, e.g. using Excel as below.
- The Q-scores are mostly above the expected Q-score at the given resolution (1.4Å, here we used sigma 0.4 instead of the default 0.6 which should be used for 1.5Å and lower resolutions).
- Q-scores for some side chains dip below the expected Q-score line. Such residues should be inspected to see if a better fit to the map can be found, or if the map is simply not too well-resolved there (e.g. side chains may have alternate conformations, or are not resolved due to a more dynamical nature).
- Some per-residue side chain Q-scores are missing (breaks in the line) for residues like GLY which don't have side chain atoms. Note how side chain scores dip just before and after a GLY.

Plot of per-residue Q-scores



3. Where 'expected Q-score' comes from

- The expected Q-score is calculated for a given resolution (entered in the Resolution field in the MapQ dialog) based on the relationship seen between Q-scores and reported resolution for a number of maps and models in the EMDB, as shown below.
- Two sigmas are possible, and each one gives a slightly different expected Q-score; sigma=0.4 is used for maps up to resolution of 1Å, and sigma=0.6 is used for maps up to resolution 1.5Å. Either sigma is fine to use, but if your map is higher than 1.5Å, sigma=0.4 should be used to maintain the nearly-linear relationship up to 1.0Å resolution.



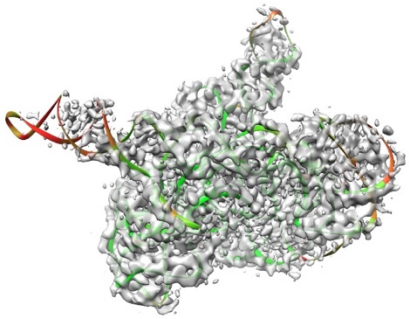
Plot of model Q-score vs reported resolution of map, for ~400 maps and models in the EMDB with resolutions evenly spread between ~1 and 11Å. From the shown fitted curves relating y and x, i.e. average Q-score and reported resolution, we can calculate what is the expected Q-score at a given resolution. Two sigmas are used, 0.4, and 0.6. For 0.4, the expected Q-score increases up to ~1.Å resolution, whereas for 0.6, it starts to taper of beyond 1.5Å as we approach 1.0Å. Hence sigma=0.4 is more appropriate to use for resolutions up to ~1Å.

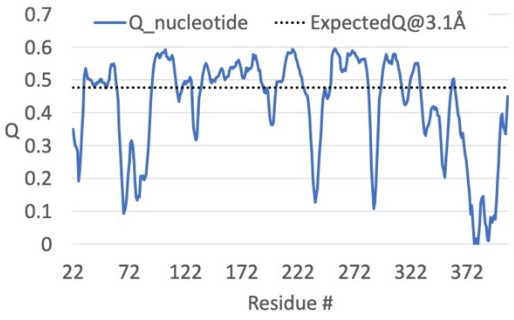
- Equation for sigma=0.4
  - $y = 0.0000121733x^6 - 0.0003668610x^5 + 0.0040966965x^4 - 0.0225427321x^3 + 0.0930645229x^2 - 0.4500934403x + 1.3652087689$
- Equation for sigma=0.6
  - $y = -0.0000027257x^6 + 0.0002420020x^5 - 0.0058960622x^4 + 0.0611398758x^3 - 0.2790872603x^2 + 0.3548162472x + 0.8108389026$

4. Example of areas where the map is not resolved

- Download the following map and model from the EMDB:
  - https://www.emdataresource.org/EMD-31385
  - https://www.rcsb.org/structure/7ez0

- Calculating and visualizing Q-scores as above, we can see in some parts of the model that are likely very flexible, are not resolved in the map, and Q-scores are accordingly low.





5. Example of areas where the model is not fitted properly to the map

- Download the following map and model from the EMDB:
  - https://www.emdataresource.org/EMD-22657
  - https://www.rcsb.org/structure/7k3v

- Calculating and visualizing Q-scores as above, we can see a potential problem in the region colored reddish. Upon closer, look, we can see the model may not be fitted optimally to the map.