Q&A September 8, 2021

Q Should we use relion postprocessed map as input for autosharpening?

A live answered

Q Are the sessions going to be uploaded to see on demand? (Because of time zones difference might be difficult to attend online)

A After formatting these lectures, we will have the recordings available on our website

Q is there any resolution limit for using density modification?

A live answered

Q what it the resolution limit here?

A live answered - Giving the approximate resolution limit is Ok here.

Q should the inout sequence only match the visible part of the map?

A You can give the entire sequence.

Q Since we are working on RNA, the sequence file input for nucleobases is same as the amino acids for protien structrues?

A live answered - You can give RNA sequence.

Q What resolution should we use?In CryoEM community, people were arguing wherther one could push up a resolution if you see higher resolution info during the process.

A For autosharpening and density modification, you can give resolution estimates from half-maps.

Q In case that my density map represent protein complex, should I create seq.dat file with sequences of all proteins in the complex?

A You should give the sequence of all proteins.

Q What is the resolution limit of input CryoEM map ?

A You could for example use the resolution limit from FSC curves using half maps.

Q Sould we go for autosharpen and density modification on the same map?

A live answered

Q What about heavily glycosylated protein?

A Are you asking about the sequence to input? I would give it a try with the protein sequence.

Q Can the speed of the computation for resolve cryo-EM be enhanced using multiple processors.

A Yes, there is an option for the number of processors in the GUI. However, on Windows, you are limited to one processor.

Q How do you quantify the "visual quality" for automatic density modification?

A You can look at the FSCref maps and compare the resolution limit of the maps before/after density modification.

Q After the density modification, do you usually perform the sharpening?

A live answered

Q Are these density modified maps preferably used for automated model building over the unmodified maps?

A If the modified map is better than the unmodified one, one use the better map for model building.

Q Thanks Dorothee, I think Chris answered inthe presentation.

A live answered

Q Since DM changes the actual map, how does one justify the validity of the new map, such as to a skeptical reviewer?

A The procedure is published, so you could for example cite the paper.

Q Do number of copies needs to be very precise? For example in helical reconstruction it may not be easy to get a map with integral copies without modifying/segmenting the (half)maps

A The best would be to try. Phenix has also a tool for map segmentation.

Q Could you explain why is it better not to sharpen at the same time as you do the density modification? Is the best strategy to use density modification followed by auto-sharpen?

A live answered

Q Can this density modification produce any artefacts in our EM map, which can be interepreted as some extra density which doesnt exist in reality?

A live answered

Q I was wondering of model-based sharpening could help furhter improvement after density modification and also wonder if there's any possible model biased sharpening could happen.

A Hi MinWoo, we try to minimize any model bias by not providing a model to the software during the sharpening or density modification stages. Usually I would suggest trying sharpening first, and then density modification as a second option, not do sharpening after density modification.

Q Can we do map sharpening and density modifcation for the low resolution maps like 7 or 8 A?

A You can always try and see if the map gets better. It might not work that well at these resolutions, though.

Q Do we have any thing like omit map here for cryo em too

A live answered

Q Does sequence file for density mod need to contain the entire sequence, or just the sequence that is covered by the map?

A The entire sequence.

Q Does density modification apply the same flattening to signal from detergent micelles in maps of membrane proteins? Does this play a role in the sharpening?

A If there is signal from the micelles, then the region should not be flatted.

Q What should be the resolution of the map to perform dock in map? does this work for discontinuous maps in certain regions with resolution around 5A

A You can try dock in map with ~5 Å resolution. Generally, the tasks get more and more challenging the lower the resolution is.

Q Is this example only for multiple same chains?

A live answered

Q Can we sharpening the composite map for a better electron density map?

A Autosharpen is taylored for cryo-EM.

Q Can combine_focused_map combine sub-particle volumes to generate a whole map?

A Yes, as long as the map can be aligned to a model. So if you have multiple maps that cover the whole particle, the program aligns the maps to the different parts of the model and then makes the full map based on the alignments.

Q Does combine_focused_maps work with half maps or post-processed maps?

A You can use any map, but I think most people use the post-processed map.

Q Say you refined in C1 but you want to check C2 symmetry. Would Phenix.symmetry pick this up?

A Possibly, if the higher symmetry is very well defined, e.g. the correlation of different parts of the map is high after applying a rotation operation, then the tool will suggest the higher symmetry.

Q Since I am beginer, Can we dock the cryoEM map from relion/cryoSPARC direclty to crystallographic model without/before the sharpning, density modification and other methods in the workflow? does it works?

A Yes, but depending on the resolution of the map, you may get better docking results after sharpening and/or density modification. For example, if side chains are not very visible, alignment/docking algorithms may have problems determining the sequence register.

Q The resulted Composite map can not be modified further?

A not the composite map from xtallography.

Q Does phenix.sequence_from_map take into consideration that certain residues (Asp/Glu) frequently have poor density due to damage from the electron beam?

A Sort of, the algorithm will try to fit different side chains into the density. If the density is poor, the fit will fail. But as long as other side chains can be confidently placed, the sequence should still be placed properly.

Q Does sequence-to-map make any assumptions about map (e.g. its power spectrum?). Have you seen sequence-to-map fail more often on maps from certain software packages - either after refinmenet or some post processing?

A It does not, the program works by trying different side chains and rotamers into the density. So it's more likely to work in higher resolution maps where the side chain density is better defined.

Q Basically, how robust is it to different modifications of maps, but that are interpretable to a human.

A Sorry, it is not so clear what your question is.

Q Can we use a sharpened map for using map_to_model tool? What are the inputs we need to use this tool

A You can use a sharpened map. Check out the documentation (+ video tutorial) for map_to_model.
https://phenix-online.org/documentation/reference/map_to_model.html
(basically you only need map, sequence, resolution limit)

Q with my experimental map at 3.5A resolution, after running map to model, I get just bunch of non-connected backbone. Any suggstions how to improve?

A Try to improve the map with autosharpen and/or density modification. You can also supply the partial model to map_to_model and run it again.

Q Can you determine the RNA sequence for a segement of map at 2.6 to 3 anstrogm from phenix.sequence_from_map without sequence file in hand?

A phenix.sequence_from_map works also with RNA

Q just to followup to my last question, I got a CryoEM map around 7 A resolution with RNA, does the docking my map with the model works?

A You should give it a try. It is difficult to say "tool X works at such-and-such resolution for sure". The best is simply to try and see if it gives a result.

Q Now you have a model in your map. What is the next step to build more of the model into weaker density?

A It depends on the map. You could try several rounds of map_to_model with the partial model as starting point. You might also use a graphics program to do some manual work.

Q does douse have any criterion for hydrogen bonding connectivity?

A I think same H-bond criteria as water building in xtallography.

Q From which resolution would you say you can use douse to asign reliably waters in your structure?

A It isn't clear yet up to what resolution it is possible to build water reliably into cryo-EM maps. If you can see water peaks, you can try douse.

Q What resolution would you suggest a map should have in order to run phenix.douse and know that it is not picking random noise as water molecules? Thank you! :)

A It is not clear yet up to what resolution it is possible to build water reliably into cryo-EM maps. I'd say if you can see water peaks in the map, then you can try douse.

Q When would you use map_to_model vs RealSpaceRefine?

A Map to model is for generating the initial model. Refinement is for fixing geometry issues and fine tuning the atomic positions to the map.

Q What would be best choice for restraints and weightings in phenix real space refinement for models refined in maps of 5-9A resolution?

A Phenix determines the weights automatically. You could enable Ramachandran restraints and secondary structure restraints.

Q Does using Ramachandran restraints invalidate using the Ramachandran plot for validation?

A You should still inspect the Ramachandran plot and especially the Rama-Z score.

Q How many macro cycles would you normally try? and also where do you indicate the geometric restraints you'd like to use?

A Default number of cycles is typically fine.

Q How concerned should we be about overfitting and are there any methods for gauging this? Are approaches like CNS's torsion angle dynamics available to reduce model freedom?

A Overfitting is less a concern, as you have experimental phases from the map.

Q What approach would you suggest to refine a model against maps with different pixel sizes? I have been resampling the maps to avoid issues with fitting. Resampling will compromise the resolution a little bit, but it works. Is there another or better way to do this?

a Hi Dario, I don't think you need to resample the maps, it would just be an issue of making sure both maps are aligned with the model before inputting them to real space refine. If you keep having issues, please send us an email and we can take a look at the maps. help@phenix-online.org

Q how does phenix_refine deal with map local resolution differences?

A The refinement currently does not take local resolution into account.

Q What is the minmum resolution required to start modeling from density map?

A live answered

Q How do you judge the quality of the refinement?

A The validation criteria are a good start. It provides geometric metrics of the model and map correlation values.

Q How much precise has to be the resolution for phenix real space refinement? What happens in case of over estimation?

A It does not need to be very precise. But don't use 1.0 if the map resolution is closer to 2.0.

Q Where should one start to optimize if the default setup of real space refine does not work well?

A It depends a lot on the resolution and content.
You could try secondary structure restraints and Ramachandran restraints.

Q My phenix real space refinement mostly work fine on Mac intel chip but when I run the refinements with the same map and model on M1 chip I keep getting some errors related to model (Ter presence/ map not overlapping properly etc and sometimes just runtime error) and only works fine when I am not doing morphing and simulated annealing. What could be the reason?

A Can you send a message to help@phenix-online.org? We'll need your model and map for debugging. For the same Phenix version, they should behave the same on both architectures.

Q any program can be used to generate map from crystal model ?

A Phenix.f_model can calculate a model based map for xtallography.

Q What if the map has large variation in local resolution, will real space refine determine weight locally automatically?

A Hi yen-li, this is an idea we are interested in. However it is not currently implemented.

Q If your complex contains only beta sheets. do we need to use any specific strategy to solve the structure?

A The procedure should be the same.

Q Just a follow up to Dorothee, I used them all the time, and my resolution is typically better than 3.5. My protein is somewhat unusual (amyloid), would this affect the refinement?

A You should give it a try. Maybe secondary structure restraints may help.

Q what value is good for average map vaule? it matehcs well
but only 0.577.

A as Tom said, exact value is not that meaningful, just use relative values... i.e. if it gets higher the fit may be better

Q General ChimeraX question: is there any way to update an installed version directy from the UI or do I need to download and install every new release from the website?

A bundles can be updated from within the UI but yes, for getting the whole ChimeraX itself (with the built-in functionality) you would go to the website download page

Q Does Alphafold work for protein-DNA complexes too?

A AlphaFold Database (and I think the AlphaFold method) is currently for single chain only, and also protein only.

Q David Baker's latest method, also based on a machine learning algorithm, seems to deal with both individual protein structure prediction and protein:protein interface structure prediction. Is that preferable over alphafold?

A We also plan to take a look at RosettaFold to see if that can be connected to ChimeraX but have not done so yet. At least according to articles I've read, it is pretty good for complexes.

Q why no report on correlation after FIT?

A It depends on what is used for fitting. The atom-in-map fitting method uses average map value. The map-in-map fitting uses correlation. In the Options of the Fit in Map tool, you can specify to calculate a map from the atoms and use the map-in-map method if you want. See https://rbvi.ucsf.edu/chimerax/docs/user/tools/fitmap.html

Q why ideas how to handle low confidence (red/orange) parts of the sequence?

A That is an awfully broad question that would appy to any modeling. Some general thoughts are to incorporate any biochemical knowledge you might have about the specific protein and try other modeling approaches (e.g. Modeller refine). However, the short answer is to simply keep in mind that those areas are lower confidence, and perhaps disregard or discount them in subsequent modeling work on the structure.

Q Since we are working on RNA, the sequence file input for nucleobases is same as the amino acids for protien structrues in alphafold data base? does it works?

A AlphaFold is for protein chains only, here is the paper https://www.nature.com/articles/s41586-021-03819-2

Q Does AlphaFold have an option to build a model that is missed or omitted in other homologue structures? For example, I wated to build a model for a long loop region at low resomution map so I can try MD simulation.

A Tom is showing now how to run a new calculation It's also described in the ChimeraX User Guide, see https://rbvi.ucsf.edu/chimerax/docs/user/tools/alphafold.html
AlphaFold just builds the whole chain, however. As far as I know there isn't any option to just do one area or loop.
ChimeraX also has a "modeller loops" command in the daily build (no GUI yet) that will fill in the missing parts. but that uses Modeller, not AlphaFold.
https://rbvi.ucsf.edu/chimerax/docs/user/commands/modeller.html

Q Does alphafold work with modified proteins like stapled peptides, cyclic peptide or un-natural amino acids?

A My guess would be no, but we are not the alphafold developers so I haven't looked at anything like that... you can take a look at the alphafold paper https://www.nature.com/articles/s41586-021-03819-2

Q show some side chains

A live answered

Q I mean, what types of things can you do in Chimera, to handly low confidence regions?

A ChimeraX daily build has "modeller loops" command that you can build alternative conformations for loops or missing segments. No GUI yet, uses Modeller.
https://rbvi.ucsf.edu/chimerax/docs/user/commands/modeller.html

You can change or manually rotate torsion angles if you have some biochemical evidence of a specific conformation.

You can analyze sidechain rotamers with the Rotamers tool, look for clashes with the Find Clashes tool, etc.Actually one of the best options in the context of modeling for cryoEM is probably to use the ISOLDE plugin to ChimeraX that Tristan will be presenting later in this workshop, see also
https://cxtoolshed.rbvi.ucsf.edu/apps/chimeraxisolde
https://isolde.cimr.cam.ac.uk/

Q How do I access the alphaphold window in chimeraX daily build?

A menu: Tools.. Structure Prediction... AlphaFold.

Q Is there another option to run alpha fold in chimeraX rather than google colab?

A No. You can fetch precalculated from AlphaFold Database, but for running a new calculation from ChimeraX, google colab is the only option.

Q Does Alpha Fold predict the structure of a higher molecular weight oligomers for which no model is available?

A I believe that it predicts single chain only, i.e. monomers. Then you can put them together into multimers yoursself, but the multimerization is not predicted by AlphaFold.

Q Can you please remind me where I find the AlphaFold tool?

A menu: Tools.. Structure Prediction... AlphaFold

Q Yeah, our company licensed it

A That's interesting. I wonder if they are continuously training it and updating the models, or whether you just get it once and that's it.

Q How can we run AlphaFold for commercial use?

A Hmmm may need to contact google.. I only see this page that says it's for noncommercial use only https://github.com/deepmind/alphafold/#model-parameters-license. AlphaFold code itself is open source but then maybe to get these parameters you would have to train the neural net yourself? I don't know details, our group does not develop AlphaFold. (We develop ChimeraX and just added this alphaFold tool recently l to connect to the database and Google colab calculations.)

Q Can we use the average FSC resolution for model-map to calculate the map-model score?

A live answered

Q Does Q square avaiable on overall structure or locall region? Should we use SCIPION? Also, what throushold is suggested to use for map-model FSC? 0.5? 0.37?

A not answered

Q why you did not consider the higer point on the same line although it has a higher Q value?

A not answered

Q Do you ever see a scaling factor in MDFF where you see a difference in half map correlations (implying overfitting) without notable red flags in the geometry statistics? In orther words, is there a scaling factor between 0.3 and 5000 where the structural statistics would look good, and you'd only realize overfitting by checking half maps?

A not answered

Q Is the Q-score valid if a large region of the map has not been modelled by the an atomic model?

A live answered

Q How do you choose the best parameters for smoothing and grouping?

A not answered

Q Could lower threshold (or sigma) after 'proper' sharpening, although the map might have more noises around, could give a better Q core?

A not answered

Q At high resolutions do you observe anisotropic Q-scores?

A live answered

Q Or really anisotropic sigma values?

A live answered

Q Got that! Thanks!

A live answered

Q possibly scaling factor (b=-100/-300) applied the same amount on variable local resolution affect Q-score?

A live answered

Q how to differentiate between different ion modelling in cryo EM map?

A not answered

Q How confident are we for the ion densities at different resolutions? Any possibilities to assign the densities to certain ion (or ion group) based on the different apperance or surrounding environment?

A not answered

Q How does the Q-score get affected by atom-types? Does a well-resolved carbon atom has the same Q-score of a well-resolved Nitrogen atom?

A not answered

Q Sorry if I missed it; is Segger also available in ChimeraX?

A not answered

Q Greg, great talk. You mentioned Q-score for ligands. Is it possible to calculate this already?

A Thanks! Yes, you can do it with the MapQ plugin, select the atoms and then the 'Calculate for selected atoms'. This plugin is only for Chimera so far, I haven't had a chance to get it into ChimeraX yet…

Q Thanks Greg, I should use Q this for the next paper. Would you recommend to use the most recent version of Chimera for MapQ?

A Great! Yes you can use the MapQ plugin in the most recent version of Chimera. You could also install the latest version of the plugin from the github page (https://github.com/gregdp/mapq). The Q-scores will be the same, but the UI has been updated a bit, it may be easier to use in the more recent version.

Q Greg, great talk! Do you need to extract the segmented map to calculate the Q-score?

A Thanks…. no, actually you should use the entire map for the Q-scores

Q Hi Jasmin, I might have missed it but can we deposit updated map and models using the same PDB IDs?

A Currently you can update models without change in maps using the same PDB IDs.

Q Oh, if we have improved maps (meaning improved resolution and including more map regions from the same cryo-EM data sets) and updated models, then should we get new deposit?

A If you have new maps along with new models to be replaced, they will need to be deposited under new EMDB IDs and PDB IDs. However, PDB can link previous entry with new entry together if needed.

Q Is there a point at which we force people to deposit half-maps?

A We have discussed about this at the PDB, but we do not have a plan yet.

Q What if I have multiple maps (i.e. focused refinement) and refine into a single model? Should I upload all maps? But the validation report is one map per model. What should I do?

A live answered

Q Any suggestions for checking ligand geometry prior to submitting the PDB validation for ligands that are not currently in the PDB?

A not answered

Q What if the resolution determined from the EMDB validation report using the half maps is different from the resolution reported from Relion/CryoSparc? I have seen this in my own data so I have been unsure what to report.

A not answered

Q What do you suggest, if we have clear density but no sequence annotation in the uniprot or any other database, do we still leave it as poly-A

A live answered

Q Just a small opinion. I guess scary part of depositing half-maps and masks are the anyone could find do variable opmtions using those information such as map-modification using phase & amplitude and come up with low-Q scores or somehting from the models. It seems it depends this also follows other general scientific policy: honesity and validation.

A not answered

Q how do we zflip the map in chimeraX? in chimera is vop zflip #1

A not answered

AlphaFold source on github https://github.com/deepmind/alphafold,