Robust and efficient likelihood-based docking of models into cryo-EM reconstructions

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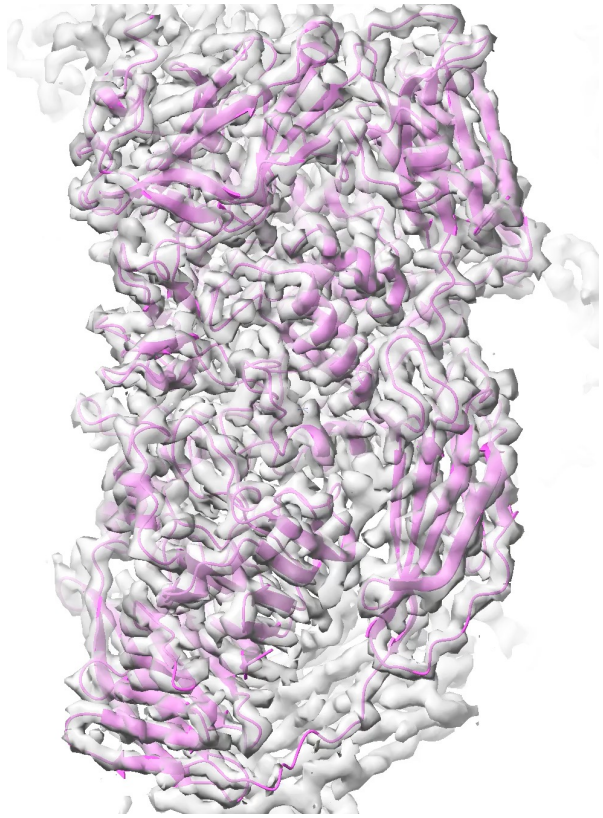
CIMR
Molecules
Mechanisms
Medicine

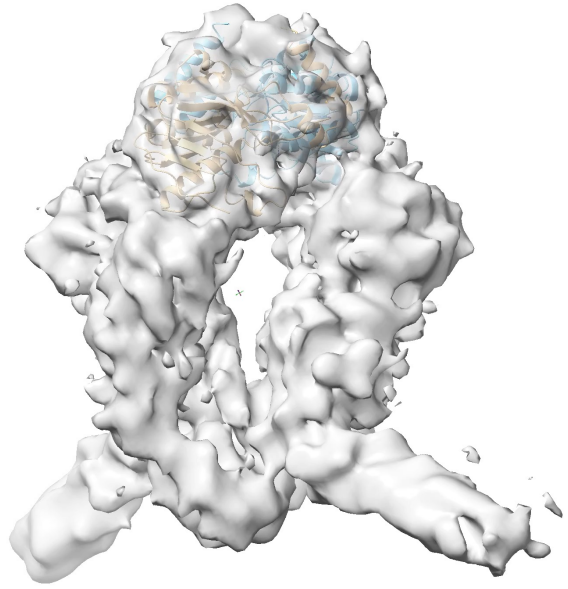
The docking problem in cryo-EM

- We have a map: how can we place an atomic model of a component in that map?
  - scoring problem
    - map correlations?
    - likelihood?
  - search problem: exploring rotations and translations
    - brute-force 6D search?
    - separate rotation and translation search?
  - decision problem
    - how confident can we be in the solution?

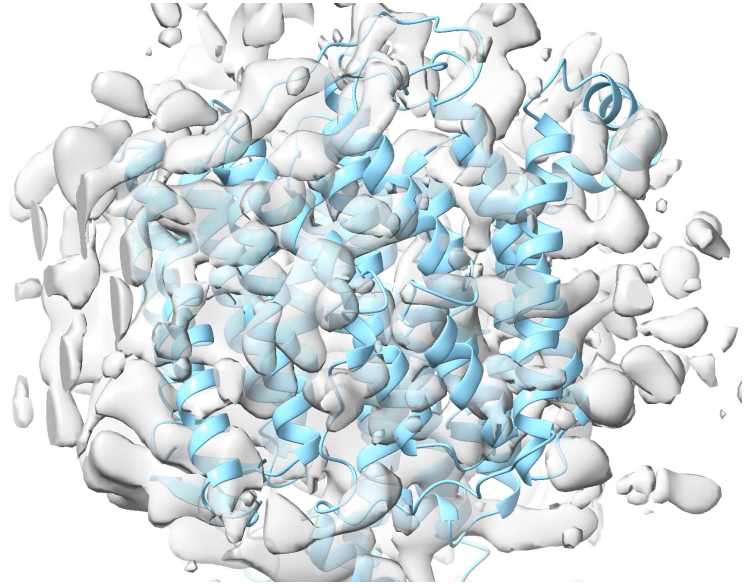
Which docking cases are important?



β-galactosidase
2.2 Å



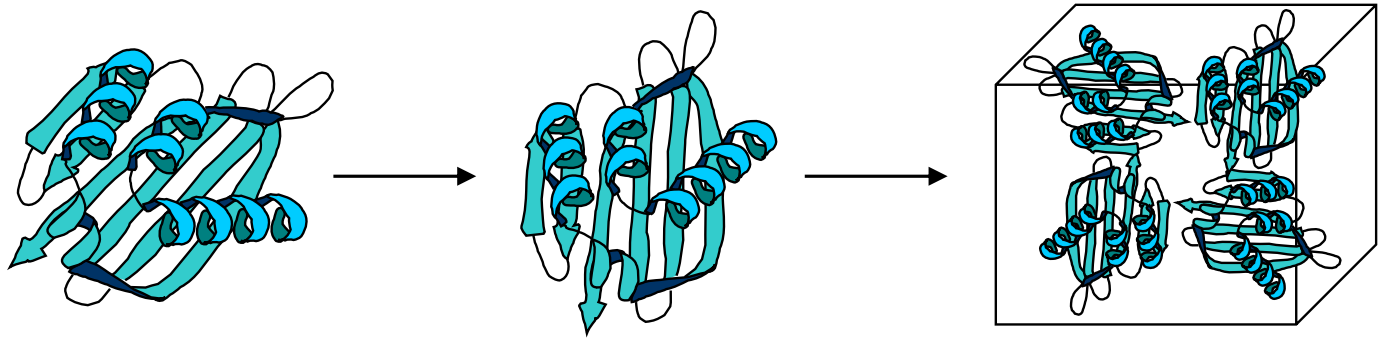
C-terminal domain of MutS
6.9 Å



Chain L of *E. coli* complex I
3.8 - 11 Å

Solving crystal structures by molecular replacement

- Rotate and translate atomic model
- Score the rotations and translations using likelihood in *Phaser*
  - accounts for errors in the data and in the model



- Some lessons can be applied to docking in cryo-EM
  - reconstructions are carried out in Fourier space *but they include phase information*

Advantages of likelihood for MR and docking

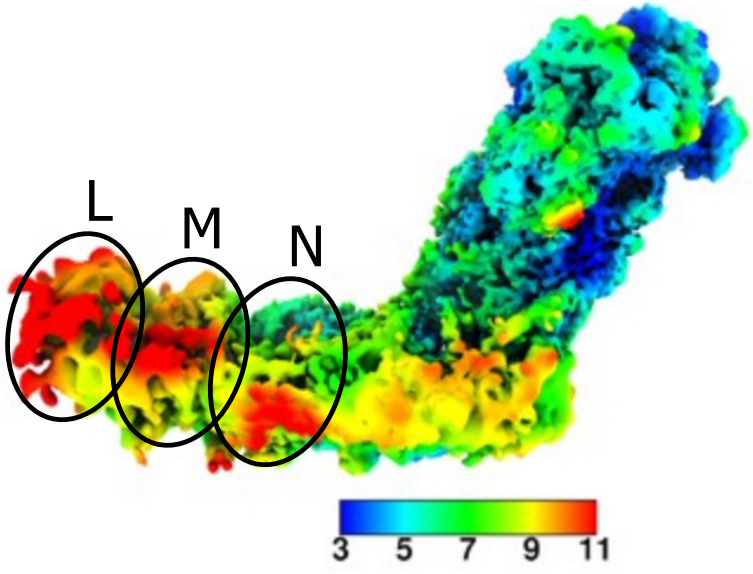
- Measures the consistency of the model (atomic model plus rigid-body rotation/translation parameters) with the data
  - probabilistic: accounts for errors in both model and data
- Likelihood is an absolute score
  - compare alternative hypotheses
  - judge confidence in solution
- Achievable score can be predicted from quality of model and data
  - optimise choices of strategy

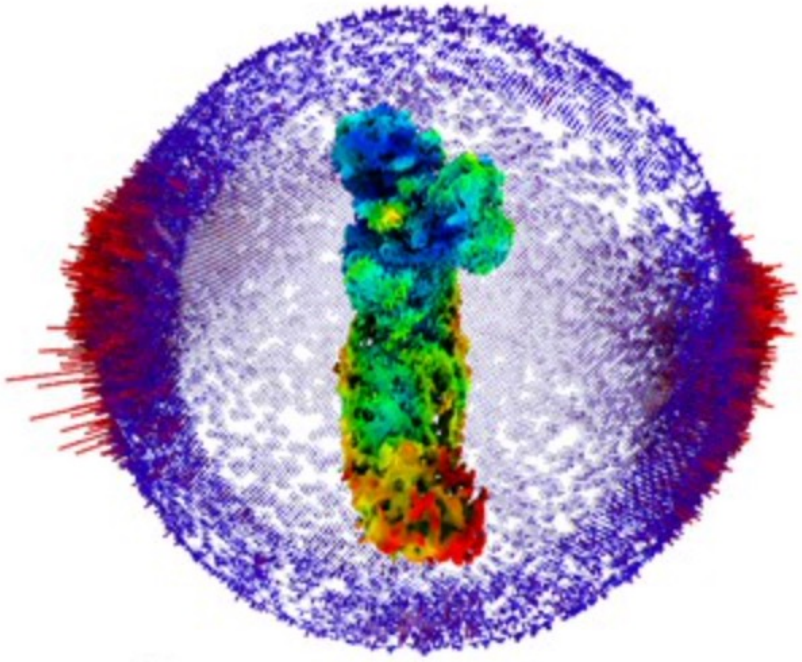
Likelihood: signal and noise in cryo-EM data

- Individual particle images are very noisy
  - average data from many particles to reduce noise
- Signal reduced by lack of reproducibility of the sample
  - different conformations, radiation damage
- Signal and noise strength are analysed by comparing half-maps
  - differs from one part of the map to another
  - described in Read, Millán, McCoy & Terwilliger *Structural Biology (Acta Cryst D)*, 2023

Example: EMDB 12654: PDB 7nyu

- *E. coli* respiratory complex 1 in lipid nanodisc
  - Kolata & Efremov, eLife, 2021
  - resolution ranges from 3.8 to 11 Å





orientation preference

Docking a model to a cryo-EM map

- Break 6D search into two 3D searches for efficiency, as in MR
  - rotation search: equivalent to the crystallographic rotation function
  - translation search: the phased cryo-EM likelihood function can be evaluated exactly with a single FFT
- Details of strategy adapt to the quality of the data and the model, through the expected log-likelihood-gain (eLLG)

The log-likelihood gain (LLG)

- Likelihood is probability of data set given model
- Log-likelihood gain: difference between logarithm of likelihood for tested model and an uninformative model
  - score of 60 or more: usually correct

- Related to how much information in the data is explained by the model

The expected log-likelihood-gain (eLLG)

- Inspired by MR: eLLG is used to devise optimal strategies
  - predict LLG that will be obtained given the quality and resolution of the data
- Rotation eLLG: much lower than for translation search
  - rotation is the hard step!
  - rotation LLG and eLLG can be increased by putting the relevant density in a smaller box: inversely proportional to box volume
    - this does require phase information!

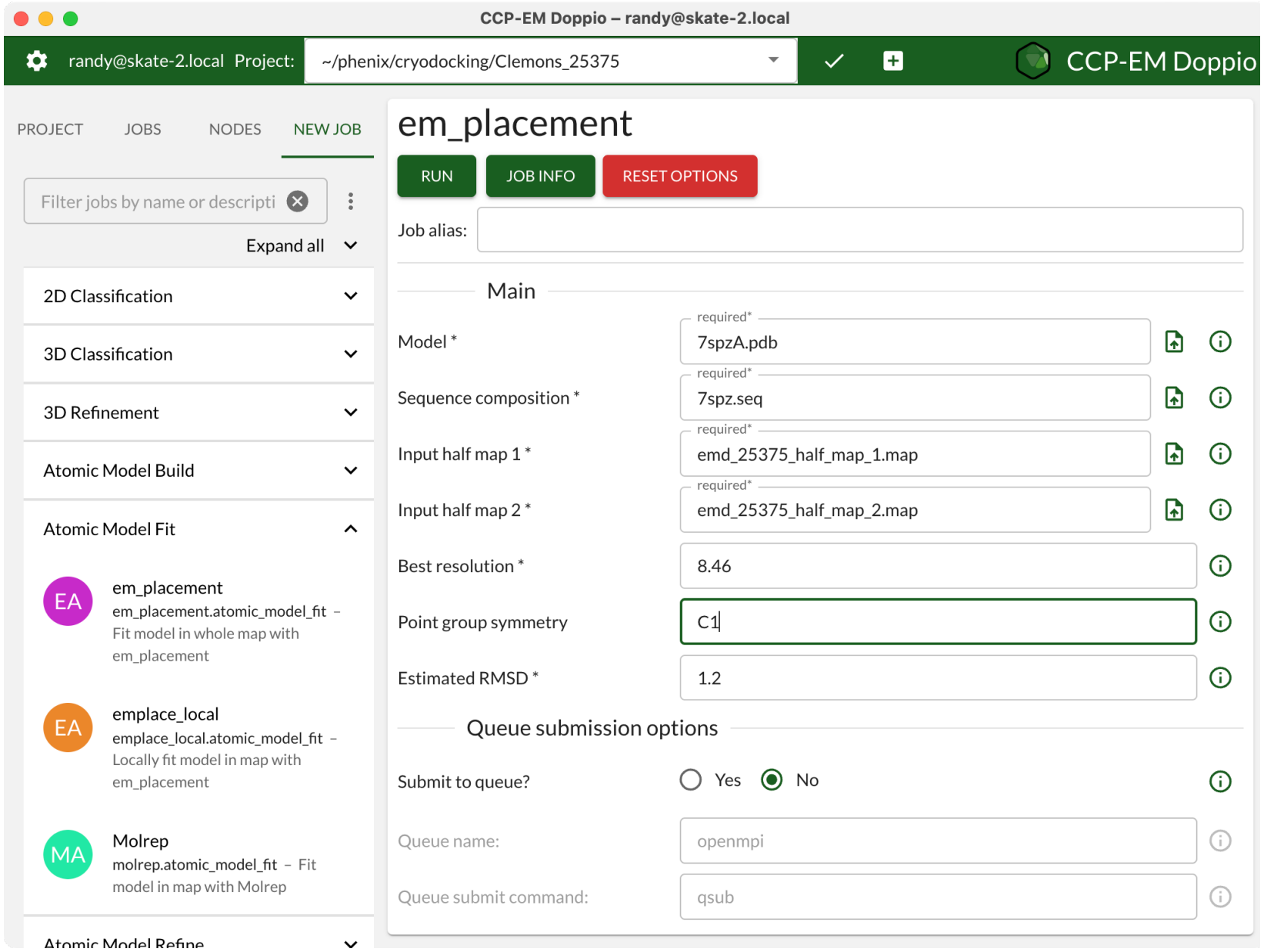
Overall docking strategy in *EM_placement*

- Evaluate signal and noise in entire reconstruction
  - will the rotation search probably succeed?
    - YES: run rotation search followed by translation search ← rotation eLLG
    - NO: will rotation search for minimal sub-volume succeed?
      - YES: divide map into sub-volumes, carry on as before
      - NO: do brute-force rotation and translation search ← translation eLLG
- Implementation and test cases (1.7-8.5Å resolution, 5-50% complete model) described in Millán, McCoy, Terwilliger & Read *Structural Biology (Acta Cryst D)*, 2023

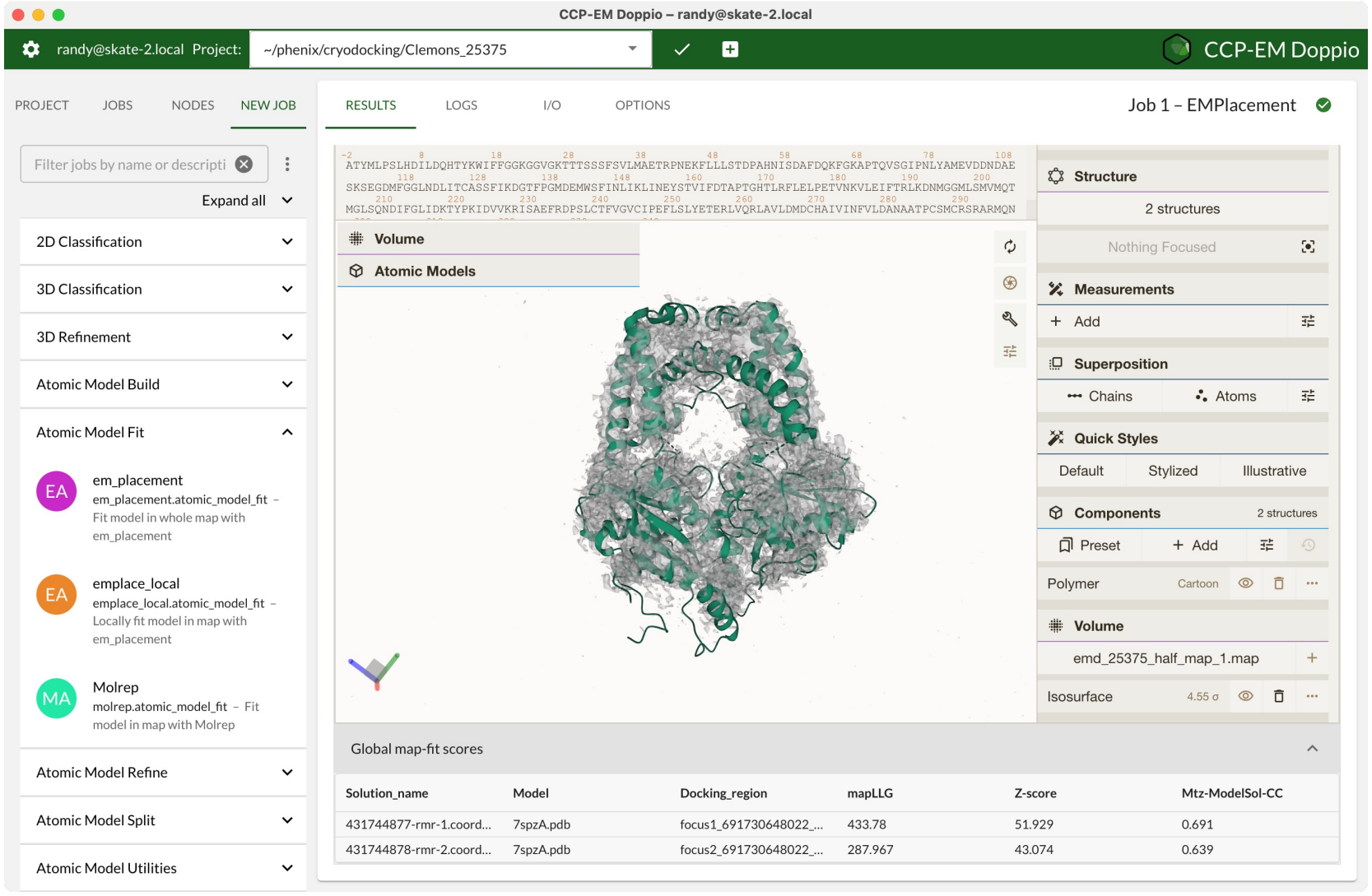
Practical aspects of running em_placement

- Requires two half-maps
  - assess signal (correlation) and noise (difference)
  - also used to define ordered volume in the reconstruction
- Requires sequence information to define the total content of the reconstruction
  - set threshold for ordered volume determination
  - how much of the ordered volume is in a spherical subvolume?
    - is there enough to contain my search model?
- Resolution limit can be estimated, but useful to provide it
- Models should be edited appropriately

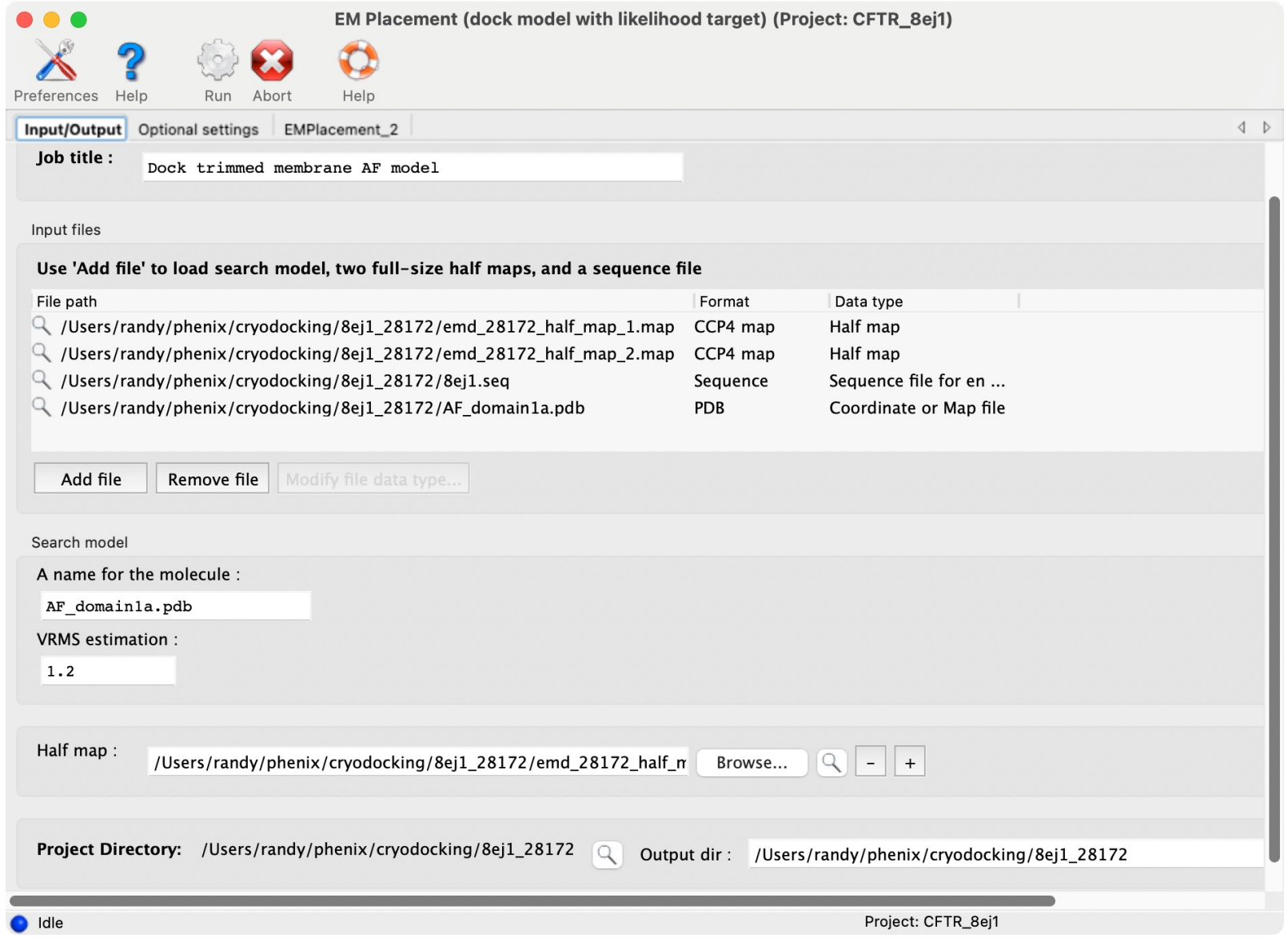
CCP-EM EM_placement GUI



CCP-EM EM_placement GUI



Phenix EM_placement GUI

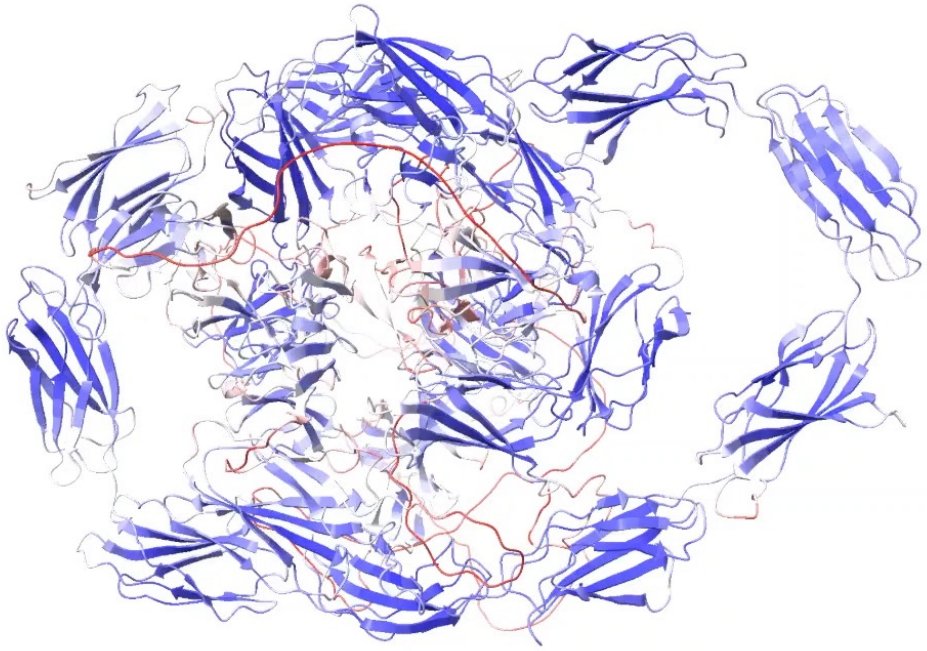


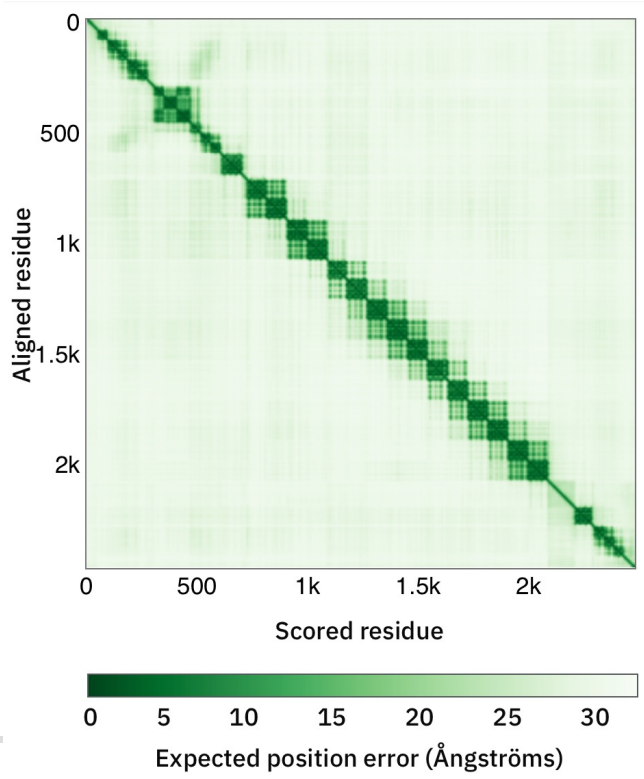
Process_predicted_model

- Docked models from AlphaFold (or other machine-learning methods) are an excellent starting point for model-building
- AlphaFold models have regions of high and low confidence
  - trim off low-confidence regions (pLDDT < 70)
  - turn pLDDT into sensible corresponding B-factors!
- Relative orientations of domains may be poorly predicted
  - PAE matrix is very useful

AlphaFold model of human fibronectin

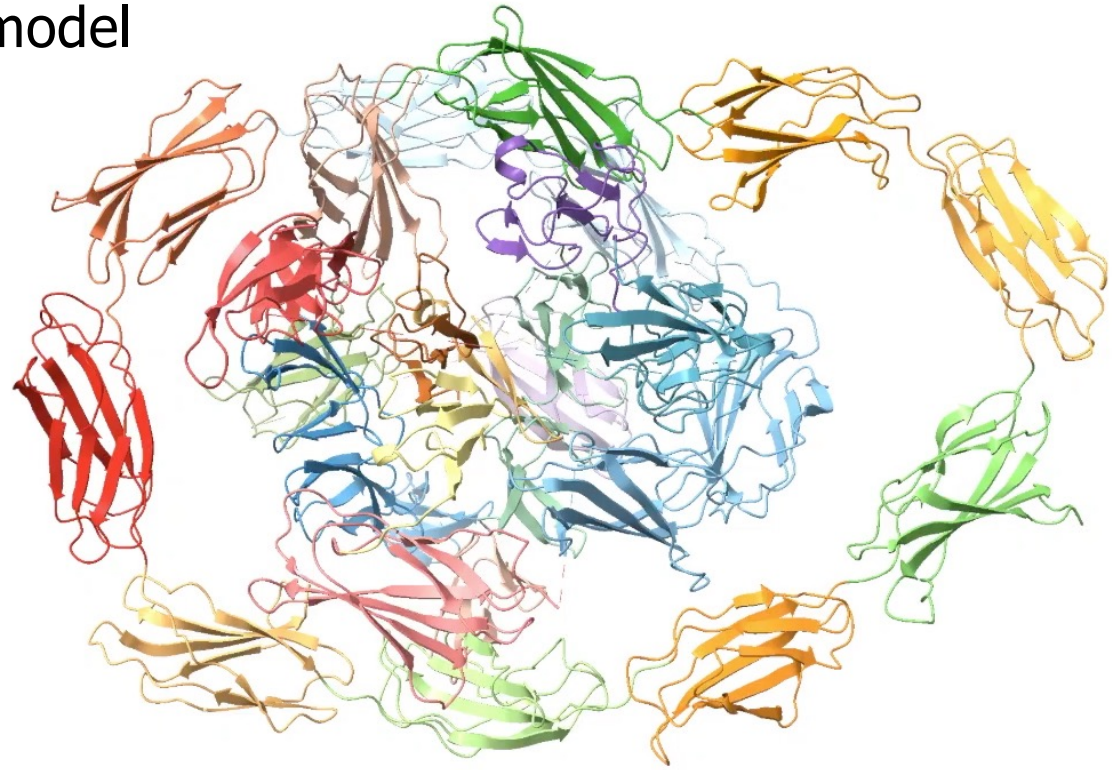
- Fibronectin repeats often have different relative orientations
- Large segments (in red) poorly predicted (or disordered)





Fibronectin parsed into domains

- Community clustering of PAE matrix (Tristan Croll)
  - phenix.process_predicted_model
    - cctbx library
  - CCP4
  - ChimeraX

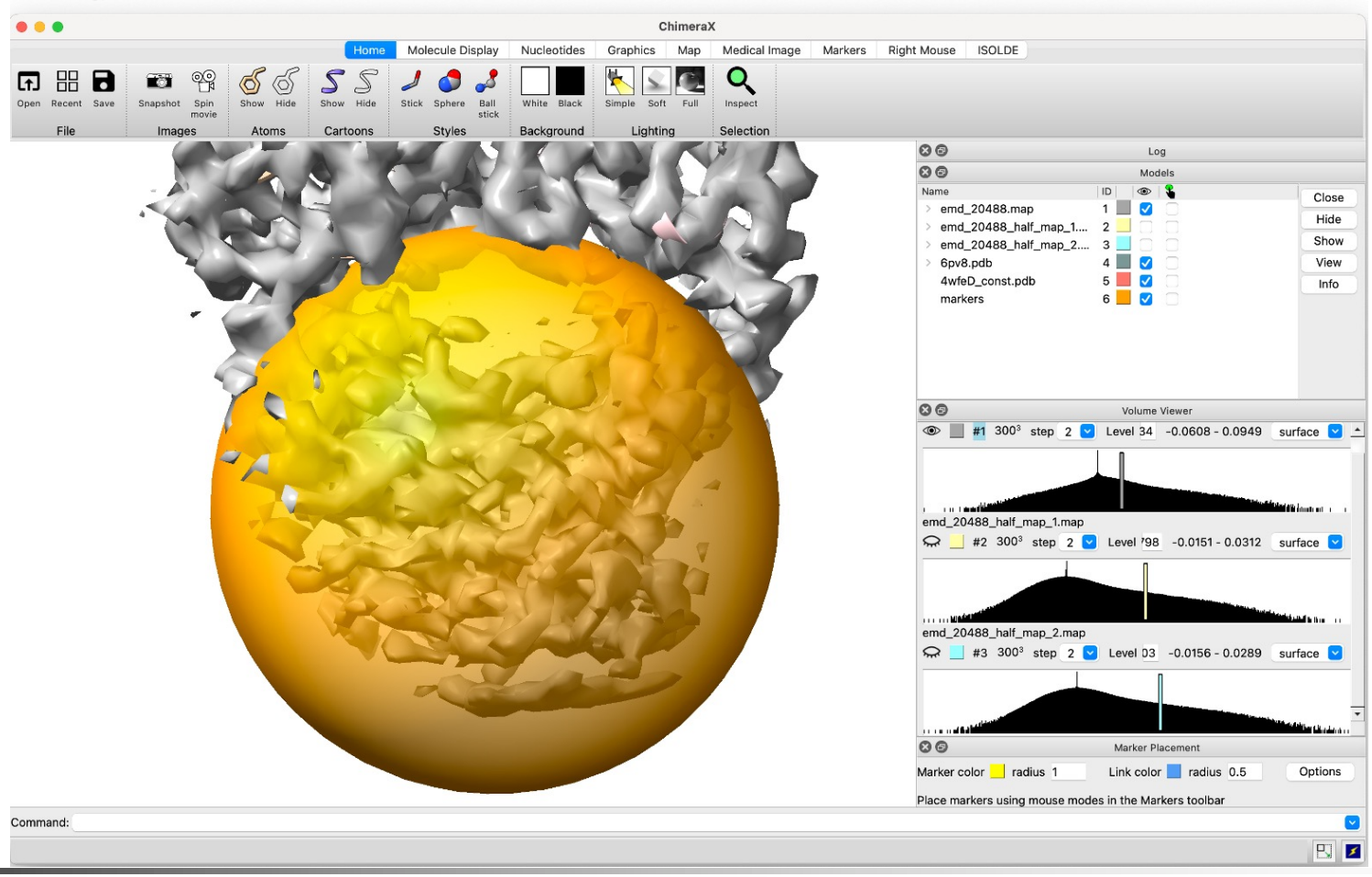


Searching in a defined sphere: *emplace_local*

- More sensitive (and much faster) if you know approximately where a molecule should go
  - half-maps are recommended but not essential
- Easiest to run from ChimeraX plugin
  - see YouTube tutorials by Dorothee Liebschner
    - https://www.youtube.com/c/phenixtutorials
    - Phenix/ChimeraX playlist

- Read, Pettersen, McCoy, Croll, Terwilliger, Poon, Meng, Liebschner & Adams. *Structural Biology (Acta Cryst D)*, 2024

ChimeraX emplace_local GUI



New feature: accounting for placed components

- Most difficult cases: poorly-ordered component next to a well-ordered component in the map
  - docking tends to be confused by a large part of the signal being unexplained by the search model
- Address this by masking out the explained part of the map?
  - no, this violates an assumption that noise is distributed pretty evenly over the map
- Subtract contribution of placed components from map?
  - this works and is easier to implement
- Add contribution of placed component to moving component
  - possibly better in theory, implementation in progress

Demos

- EM-placement in Doppio
  - Get3 targeting factor in closed conformation at 8.5 Å resolution
    - asymmetric dimer
    - EMD-25375
  - Use monomer from closed form as a model
- emplace_local in ChimeraX
  - place 60 copies of monomer in icosahedral virus capsid
    - EMD-28022
    - 2.4 Å resolution

Future plans

- Account explicitly for point-group or even helical symmetry
- Search automatically for multiple components
- Adapt search strategy to local map quality
- Move likelihood function closer to raw data?

Software availability

- Underlying algorithms in open-source CCTBX library

- *em_placement:* Phenix, CCP-EM Doppio
- *emplace_local:* Phenix, ChimeraX, CCP-EM Doppio

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Phenix

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