TEMPy-ReFF + ChemEM

CCP-EM Icknield Model Building Workshop

05.11.2024

Maya Topf & Aaron Sweeney (CSSB Hamburg)

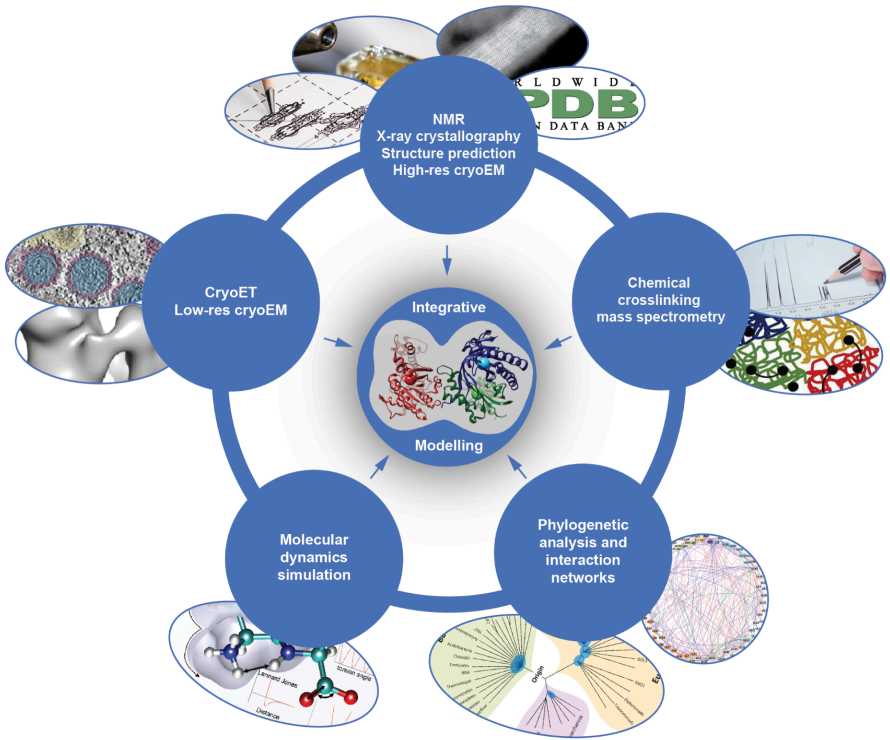
UKE HAMBURG

LIV

CSSB
Centre for Structural
Systems Biology

INTEGRATIVE MODELLING OF MACROMOLECULAR ASSEMBLIES

CSSB
Centre for Structural
Systems Biology

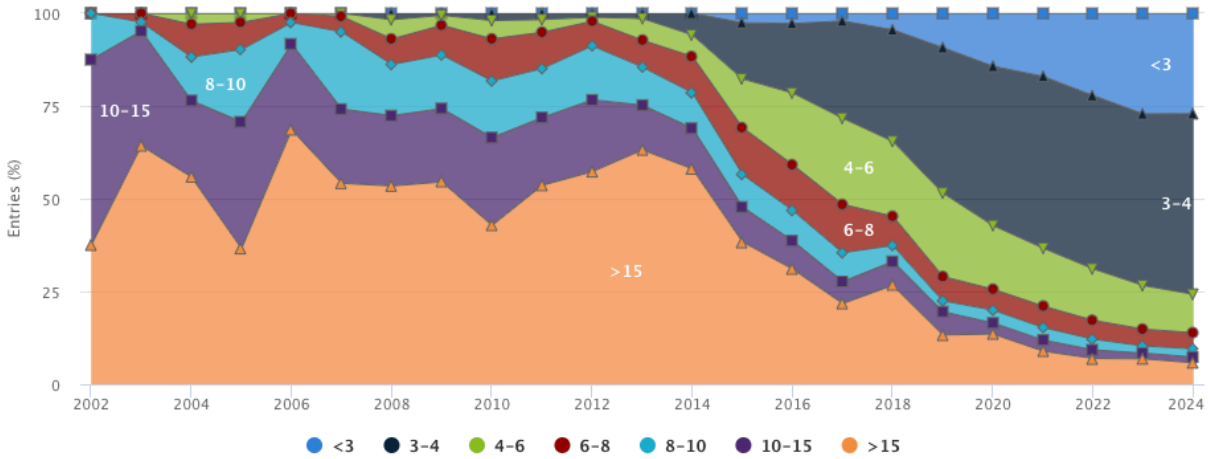


- **Develop** methods for integrative modelling of macromolecular assemblies
- **Apply** methods to model structures of viral assemblies (e.g. herpesviruses, arenaviruses)
- **Analyse** structural models to further understand their function
- **Distribute** and support new software for the structural biology community

UKE HAMBURG
LIV Leibniz Institute of Virology

Joesph et al. *Curr Opin Struct Biol*, 2017

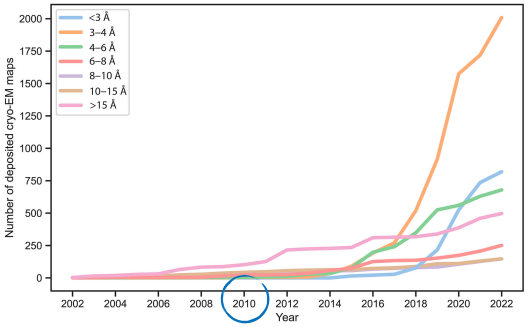
EMDB ENTRY RESOLUTION IN SHELLS PER YEAR

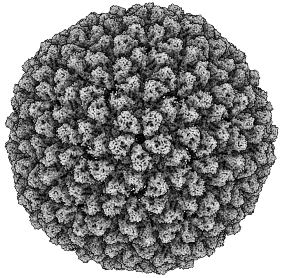


"The Resolution Revolution"
W Kühlbrandt, *Science* 2014

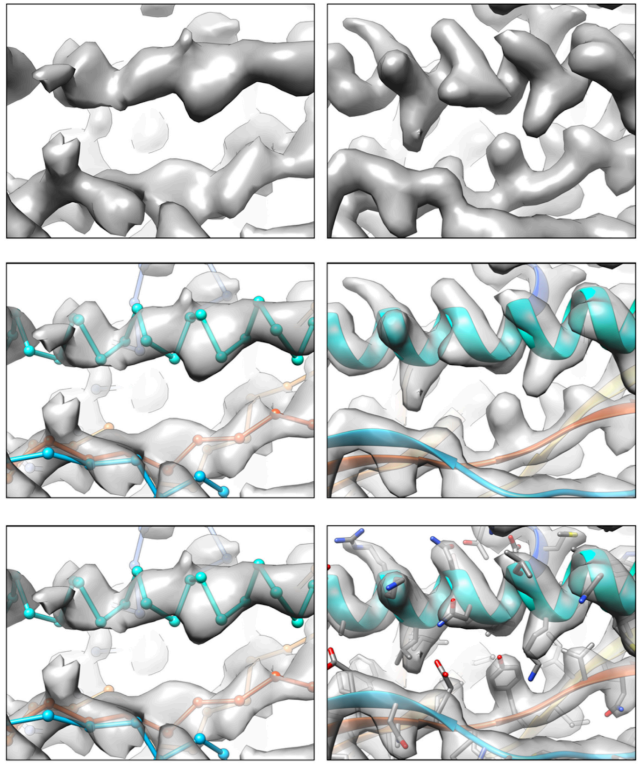
https://www.ebi.ac.uk/emdb/statistics/

THE "RESOLUTION REVOLUTION"





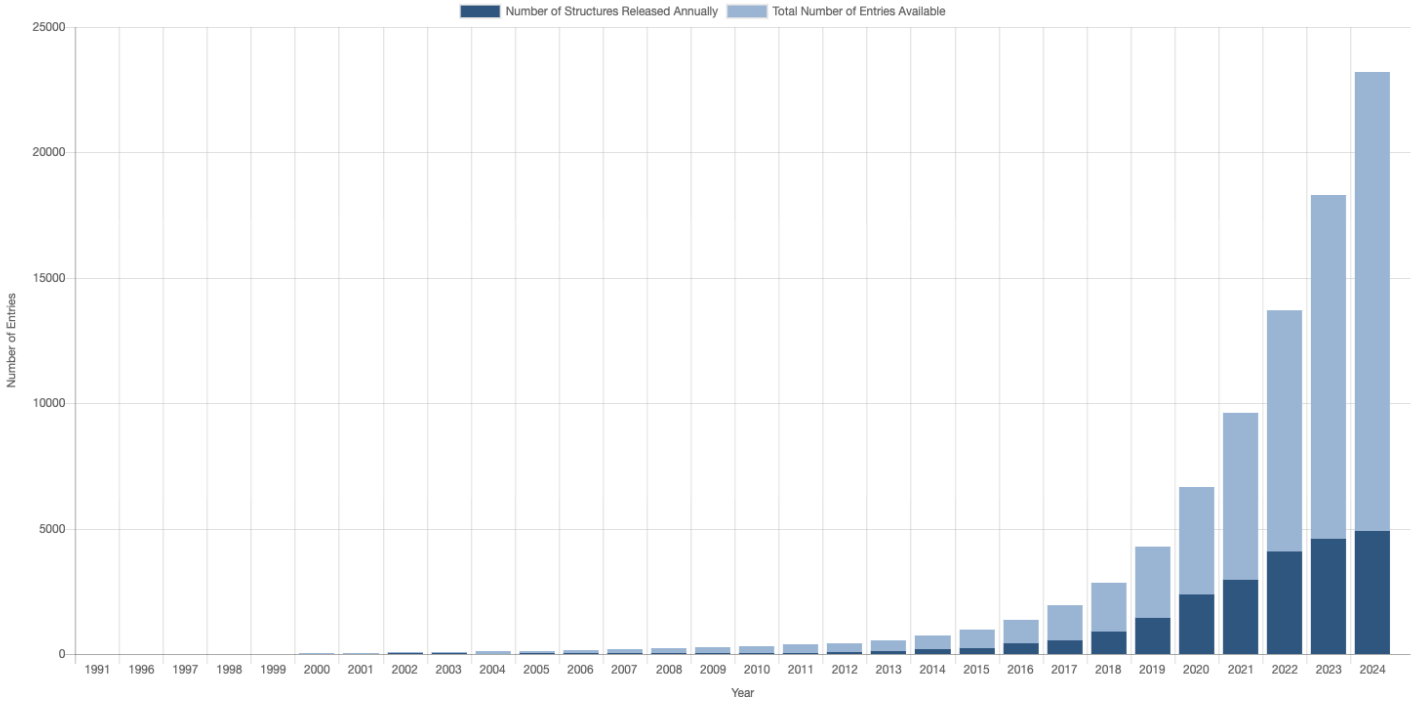
Bacteriophage P22
4Å (actually ~5Å)
EMDB 5137
Chen et al., 2011



Chen, 2011. *PNAS*
Non-validated C-alpha trace

Hryc, 2017. *PNAS*
***de novo* all-atom model**

NUMBER OF CRYOEM ENTRIES IN PDB

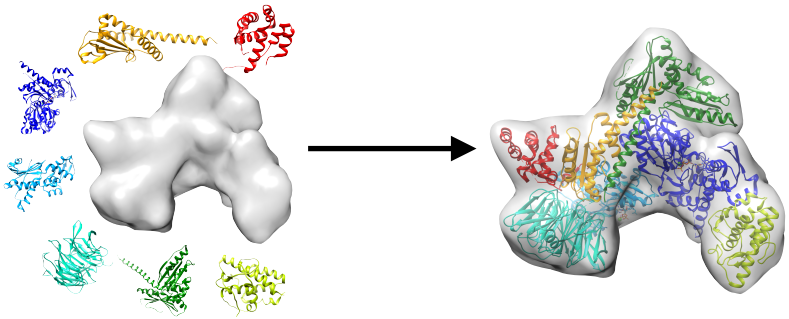


- ~10% of the entries in PDB are associated with maps in EMDB
- These days most entries are associated with either a single model or multiple models

https://www.rcsb.org/stats

What is it?

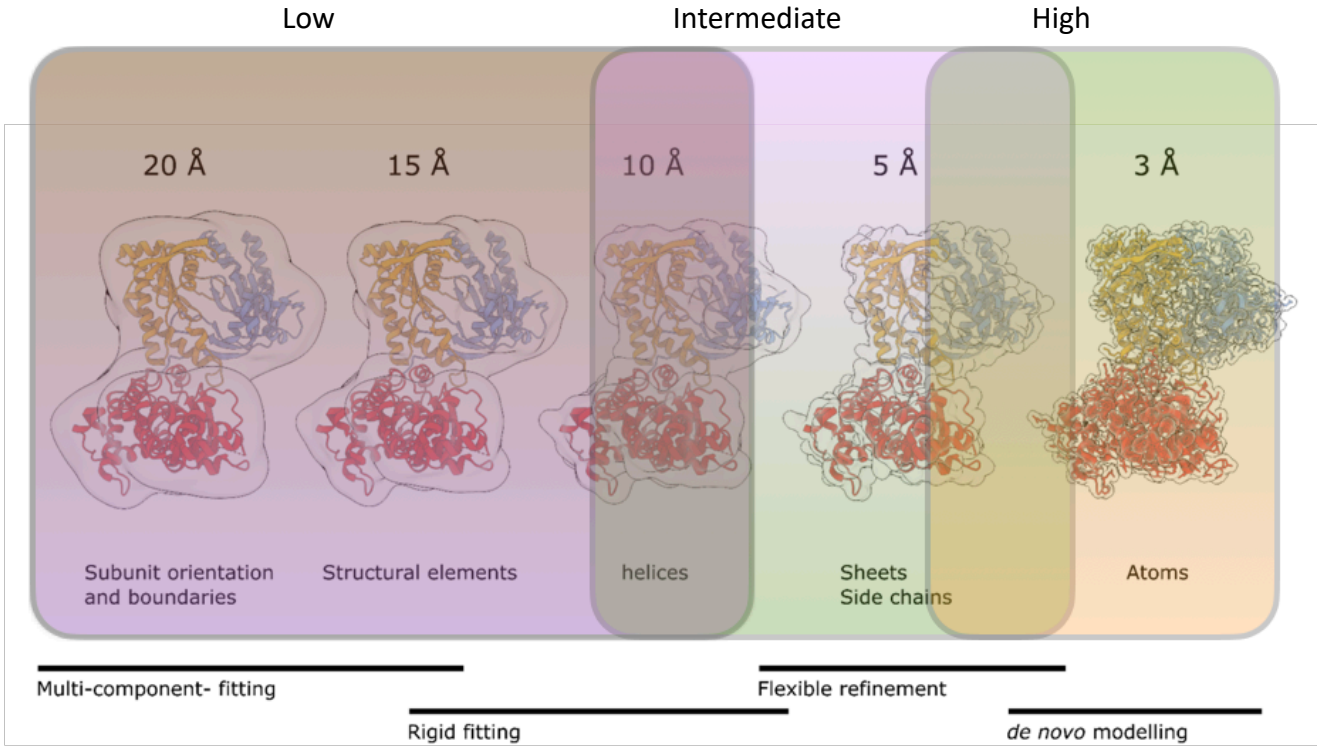
* Generation, representation, “manipulation” of the 3D structure of biological molecules
* A molecular model in cryoEM is a compact interpretation of a density map in light of everything known *a priori* about the structure-composition of the macromolecule of interest



Why we need it?

1. To get the atomic 3D structure of the molecules;
2. To know physicochemical characteristics of the molecules;
3. To compare the structure of a molecule with different molecules;
4. To visualize complexes formed between different small molecules and other macromolecules;
5. To help predict how new related molecules might look.
6. To simulated the dynamics of the model to better understand its function

STRUCTURAL FEATURES AT DIFFERENT RESOLUTION LEVELS



- If a complete experimental structure is available (and/or a reliable predicted model) 10 Å data may be sufficient.

- If no sequence/composition data is available even 3 Å may be challenging

Beton et al. WIREs Comput Mol Sci. 2023

Beton et al. WIREs Comput Mol Sci. 2023

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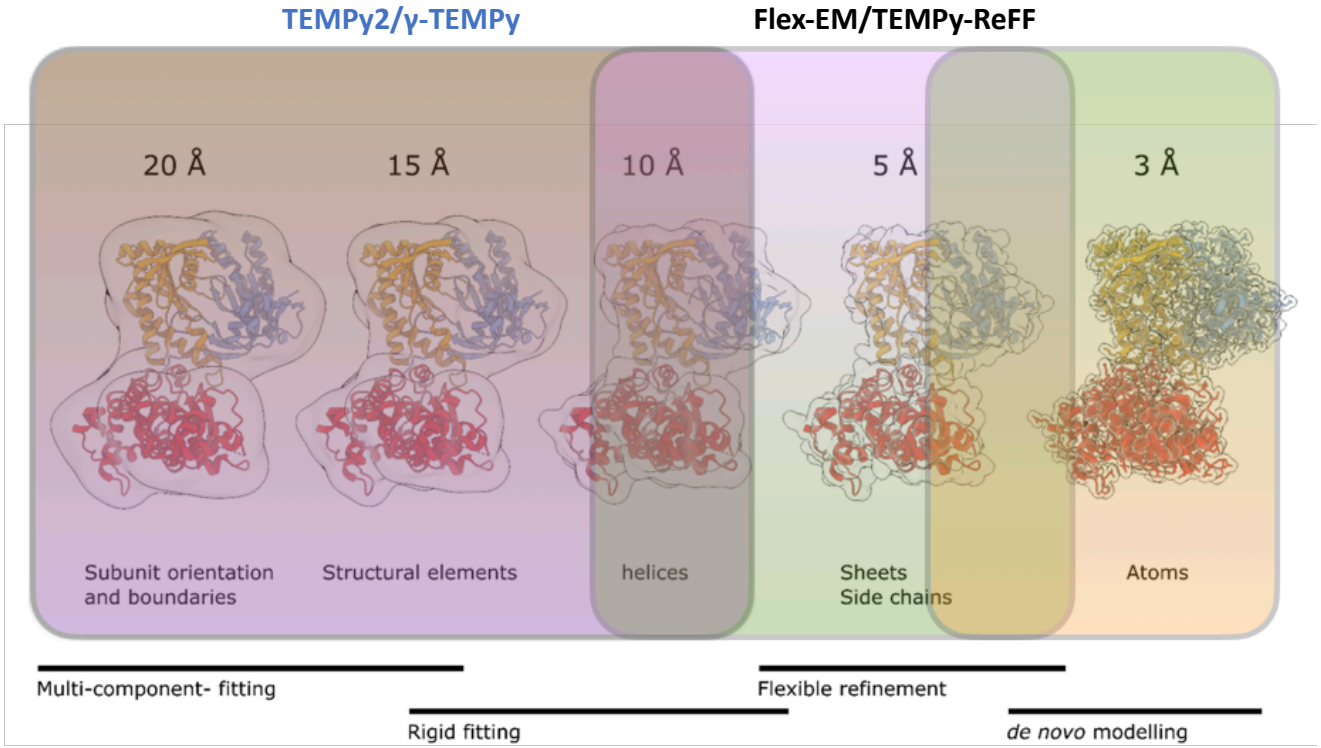

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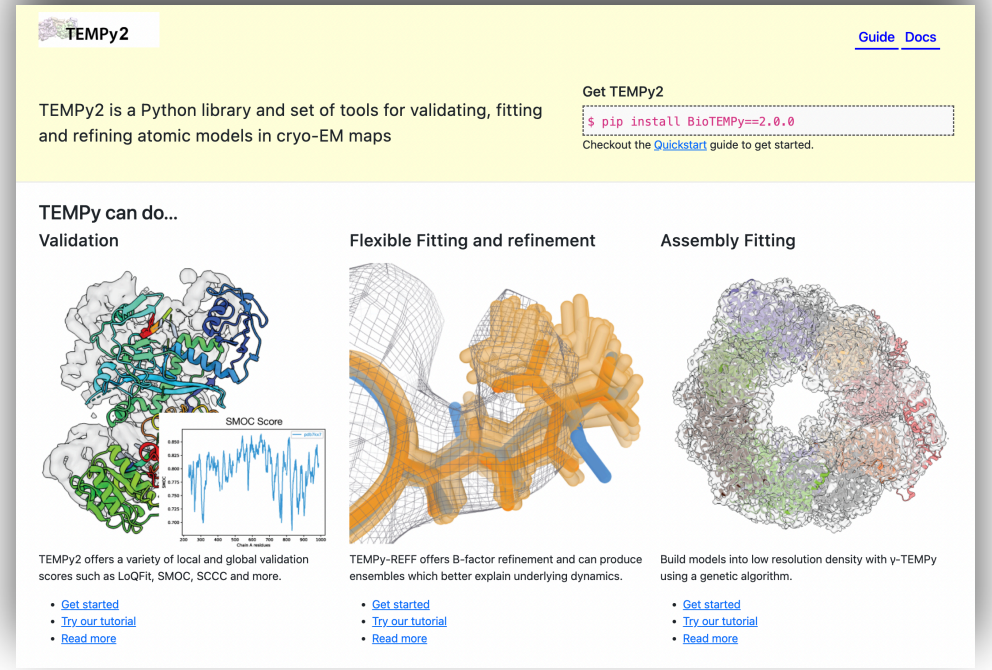
Beton et al. WIREs Comput Mol Sci. 2023

TEMPY2 SOFTWARE



Map processing
- Transformation (rotation/translation)
- Filters

Model processing
- Transformation (rotation/translation)
- Model-to-map
- Ensemble generation
- Clustering

Fitting
- Local random and exhaustive search
- Multicomponent fitting (γ-TEMPy)
- Refinement (TEMPy-REFF)

Global Scoring
- Density based
- Surface based

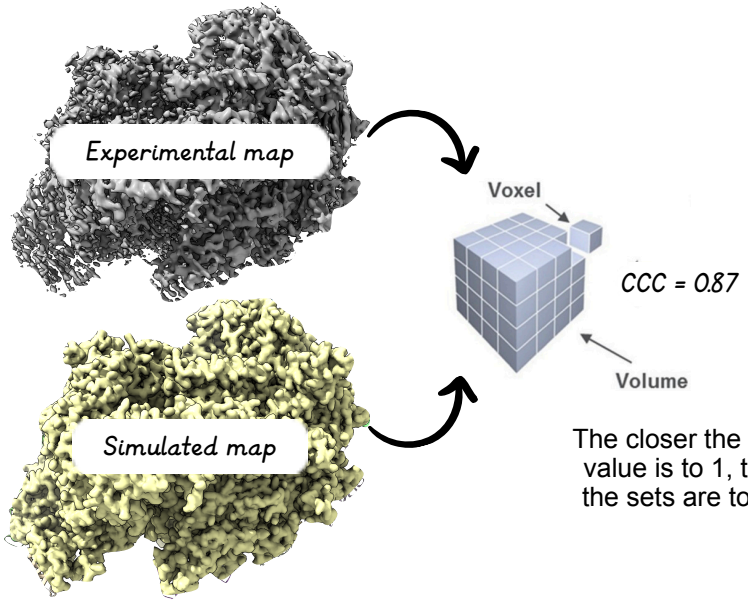
Local scoring
- SCCC
- SMOC
- LoQfit

Map comparison
- Difference maps
- FSC

***Chimera* scripts**
Plots
Attribute files
PDB files

Cragnolini et al. *Acta Crystallogr* D (2021)

https://topf-lab.gitlab.io/tempy



The closer the cross-correlation value is to 1, the more closely the sets are to being identical.

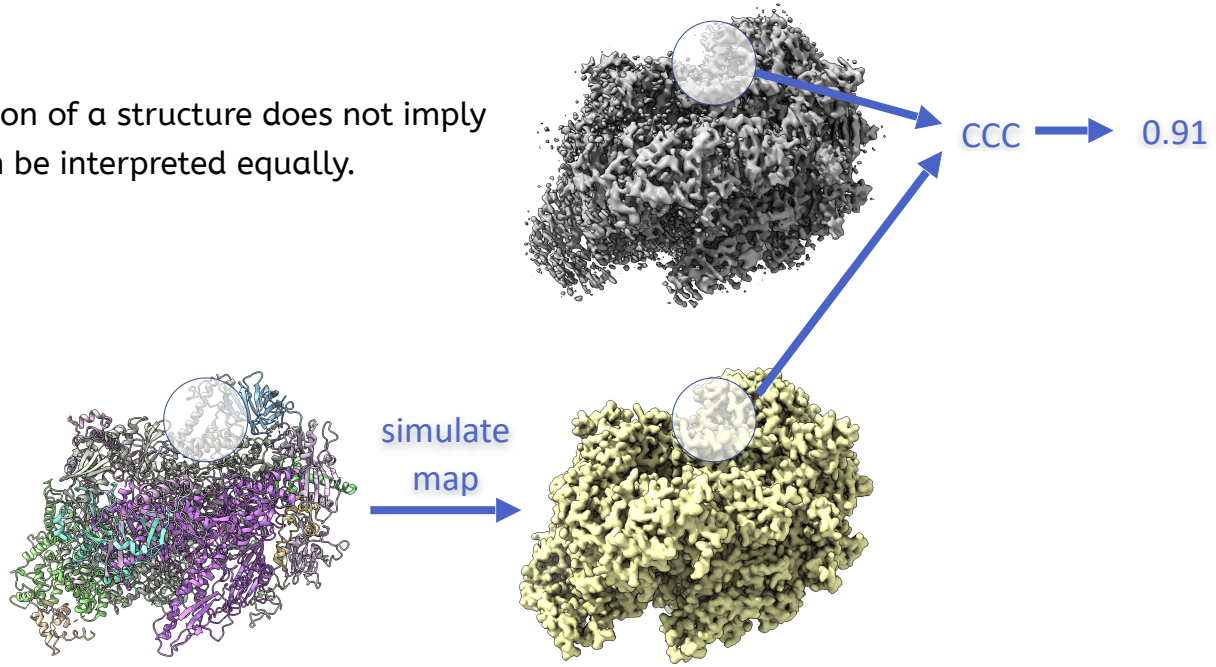
* When possible, a known or pre-calculated model is placed and fitted in the cryo-EM map as a rigid body.
* Often an exhaustive six dimensional (6D) grid search of the 3 translational and 3 rotational degrees of freedom is performed to locate the highest global cross-correlation.

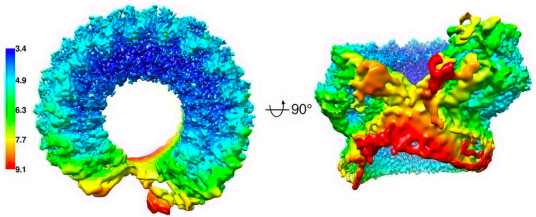
Cross Correlation Coefficient $$\mathbf{CCC}_{\rho,\mathrm{lin}} = \int_{\mathbf{x}} (\rho_{\mathbf{obs}}(\mathbf{x})\rho_{\mathbf{calc}}(\mathbf{x},\mathbf{m}))^2 d^3\mathbf{x}$$

Mutual information score $$I(X;Y) = \sum_{x\in X}\sum_{y\in Y} p(x,y)\log\frac{p(x,y)}{p(x)p(y)}$$

- Useful at intermediate resolutions; noisy maps;
- Less sensitive to relative intensity levels;

* The overall resolution of a structure does not imply that all regions can be interpreted equally.





Yu et al., *EMBO J* 2022

* Local resolution maps are useful for estimating resolution variability and map quality in general.

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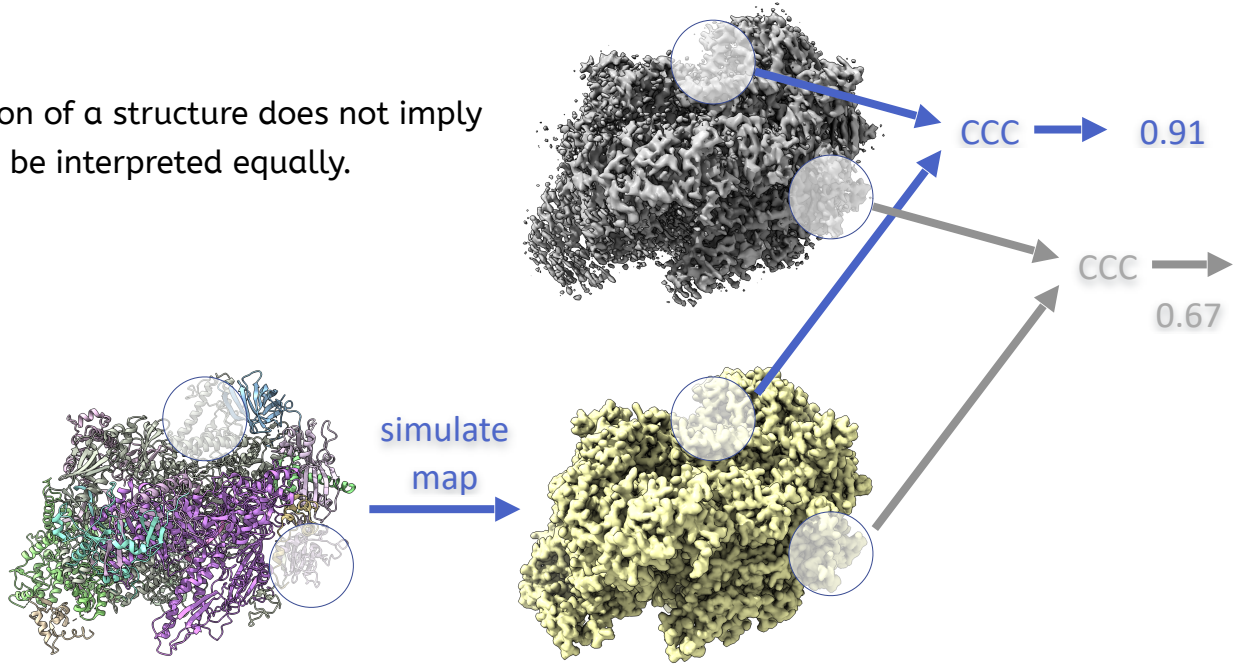
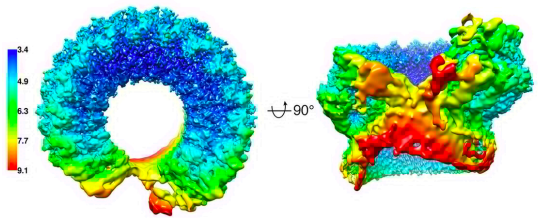




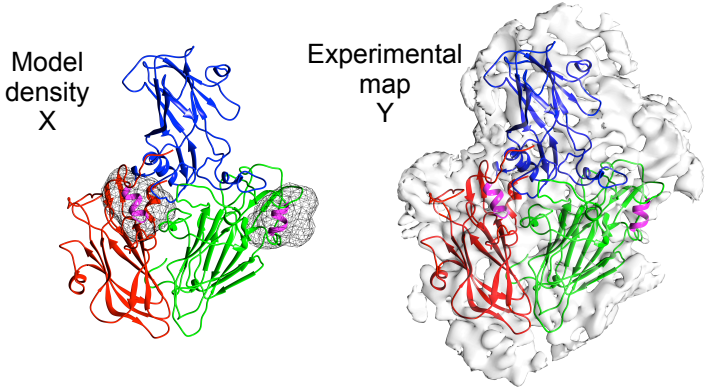
Yu et al., *EMBO J* 2022

* Local resolution maps are useful for estimating resolution variability and map quality in general.

LOCAL SCORING FOR MEDIUM-TO-HIGH RESOLUTION MAPS

SCCC: Segment-based cross-correlation coefficient:

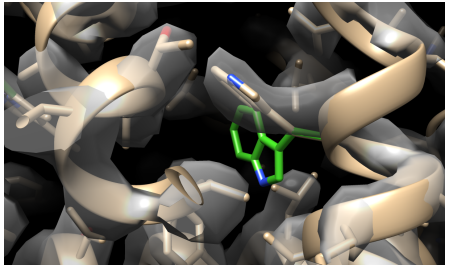
$$\text{SCCC} = \frac{\sum_{i=1}^{n}(X_i - \bar{X})(Y_i - \bar{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \bar{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i - \bar{Y})^2}}$$



Agnel Joseph

SMOC: An overlap coefficient is calculated over voxels covered by each **Residue** (and the local neighbourhood):

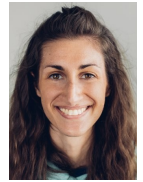
$$SMOC = \frac{\sum_{i=1}^{n} X_i Y_i}{\sqrt{\sum_{i=1}^{n} X_i^{\,2}}\sqrt{\sum_{i=1}^{n} Y_i^{\,2}}}$$



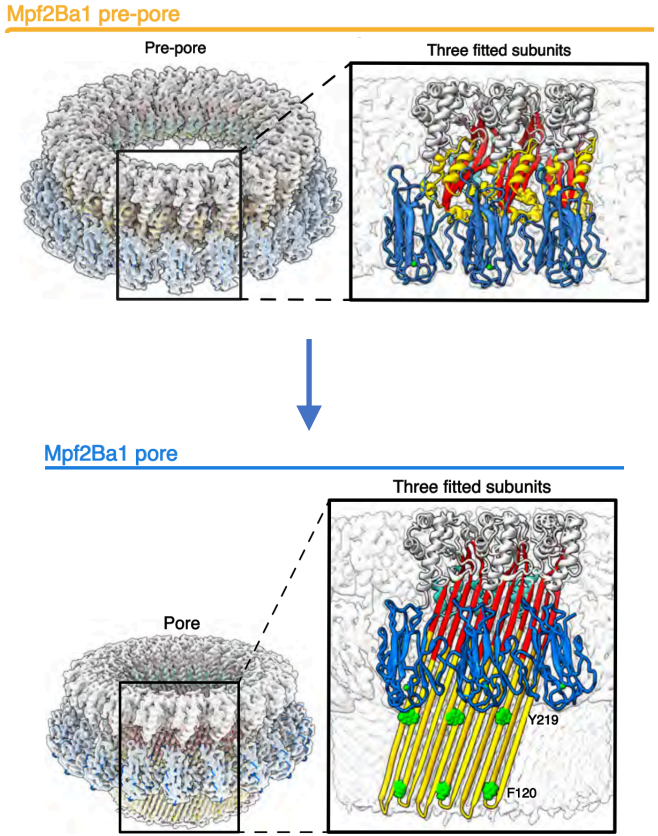
EMD-3488 (3.2Å)
Deposited model PDB: 5NI1

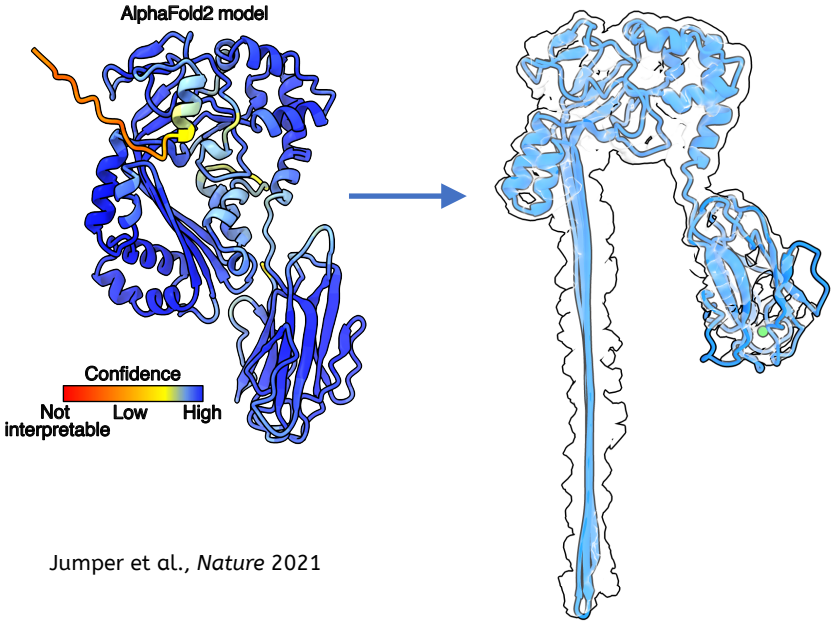
Beton et al. *WIREs Comput Mol Sci.* 2023;
Joseph et al. *Methods* 2016;
Farabella et al. *J Appl. Cryst.* 2015

ENTER THE "AI" ERA: GOOD INITIAL PREDICTIONS BUT…



Gwen Marini



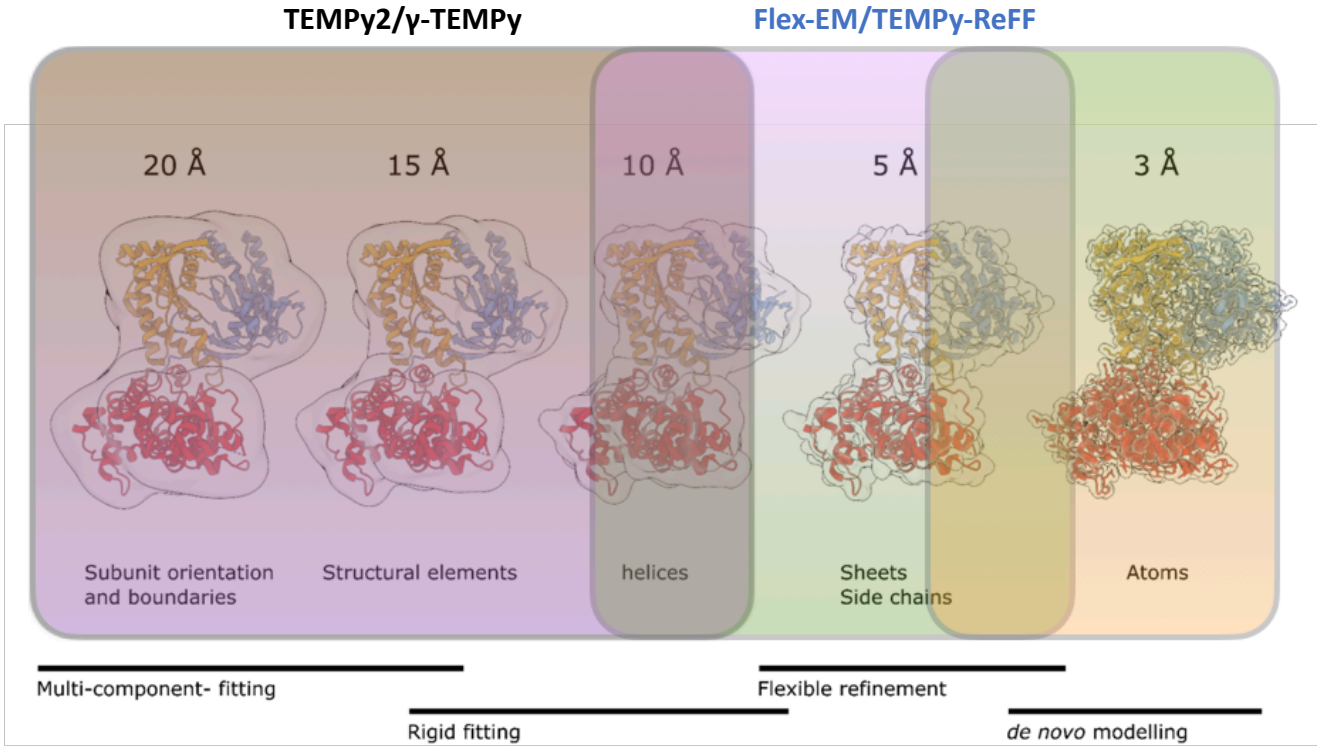


Jumper et al., *Nature* 2021

2-3 Å resolution

Marini et al., *Nat Comms* 2023

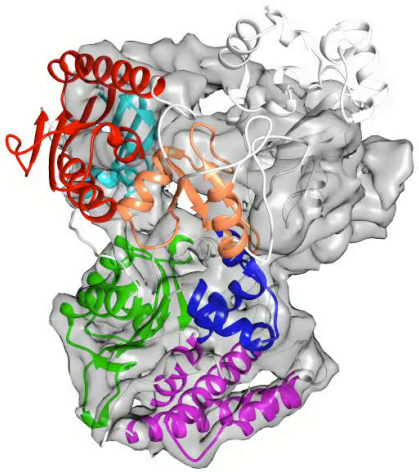
STRUCTURAL FEATURES AT DIFFERENT RESOLUTION LEVELS



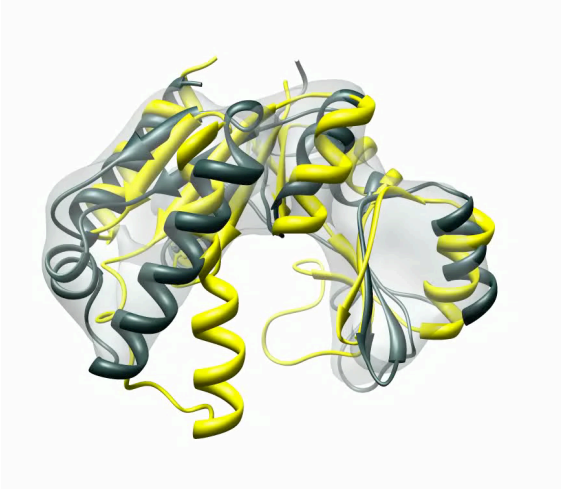
- What about higher resolutions?

Beton et al. WIREs Comput Mol Sci. 2023

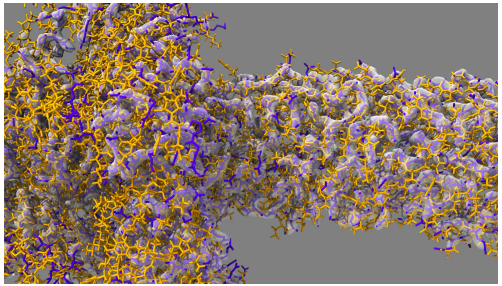
FLEXIBLE FITTING AND REFINEMENT AT LOW TO HIGH RESOLUTIONS



sub-domains

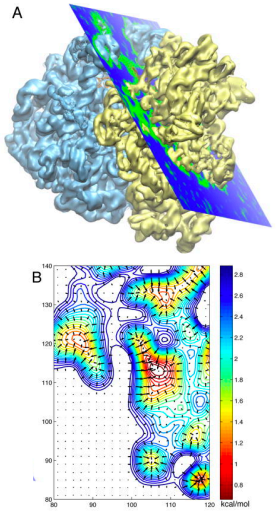


secondary structure elements



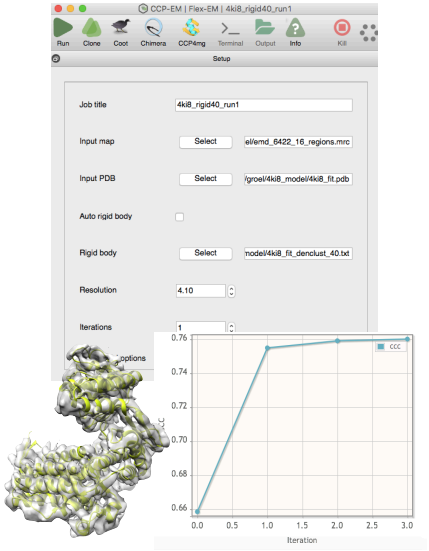
residue / all-atom

FLEXIBLE FITTING AND REFINEMENT



Molecule Dynamics Flexible Fitting (MDFF) uses a standard force field with a potential based on the density

Trabuco et al., *Methods* 2008

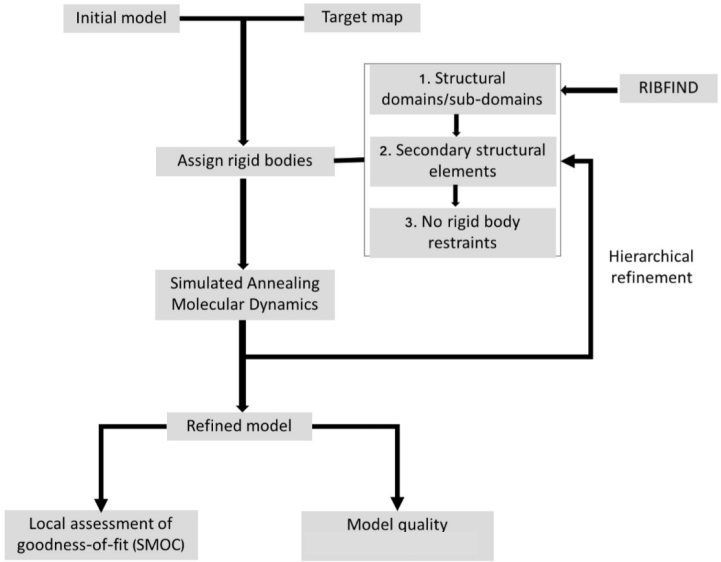


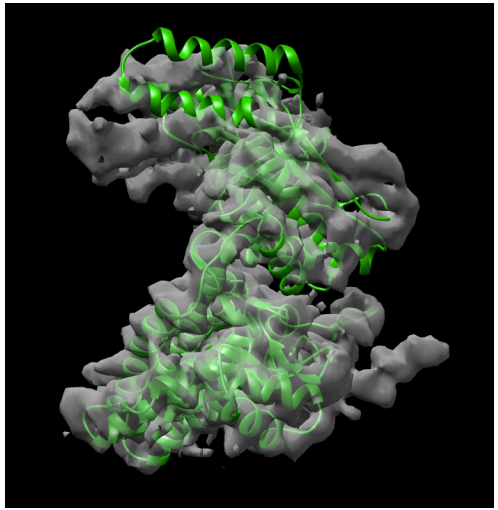
Flex-EM improves the correlation between the model sand the cryo-EM map

Topf et al., *Structure* 2008;
Joseph et al., *Methods* 2016

FLEXIBLE FITTING AT MEDIUM RESOLUTION BY HIERARCHICAL REFINEMENT

Agnel Joseph



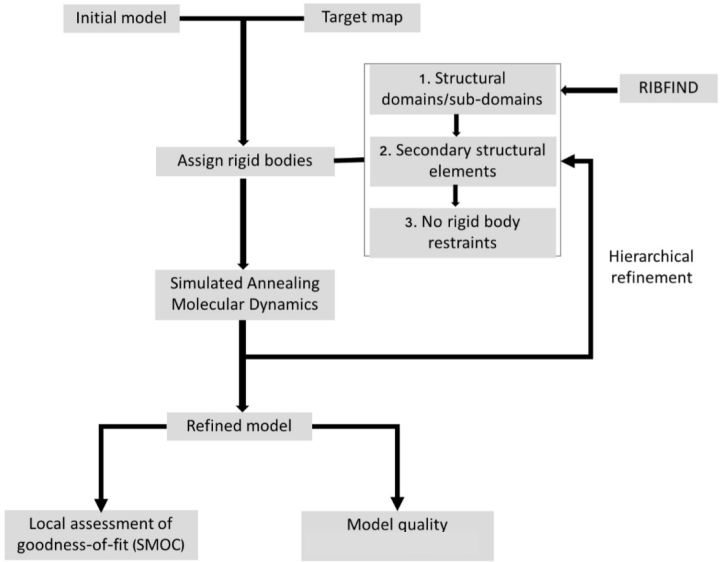


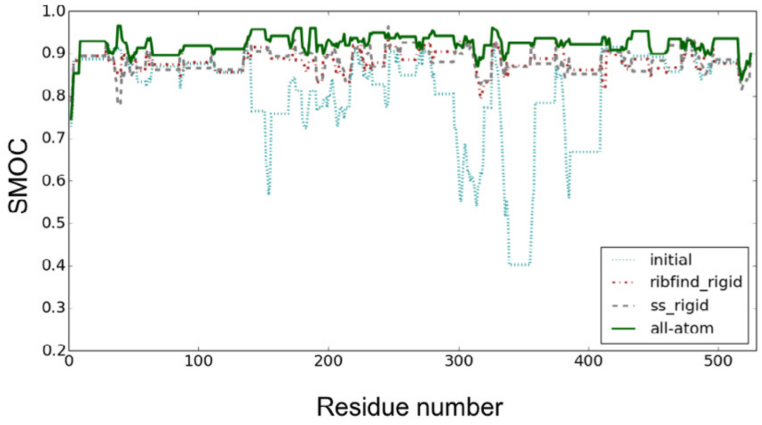
Unliganded GroEL at 4.2 Å resolution (EMD-5001)

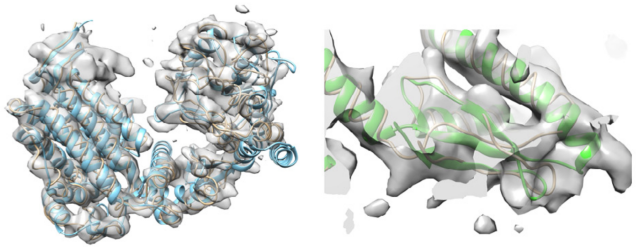
Using validation approaches, we can **iteratively** improve the fit to the density

Joseph et al. *Methods* 2016

FLEXIBLE FITTING AT MEDIUM RESOLUTION BY HIERARCHICAL REFINEMENT







Unliganded GroEL at 4.2 Å resolution (EMD-5001)
Initial model: ADP-bound GroEL (PDB: 4KI8)
Refined model
Deposited model (PDB: 3CAU)

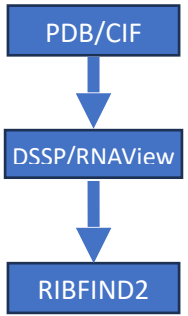
Joseph A.P. et al. *Methods* 2016

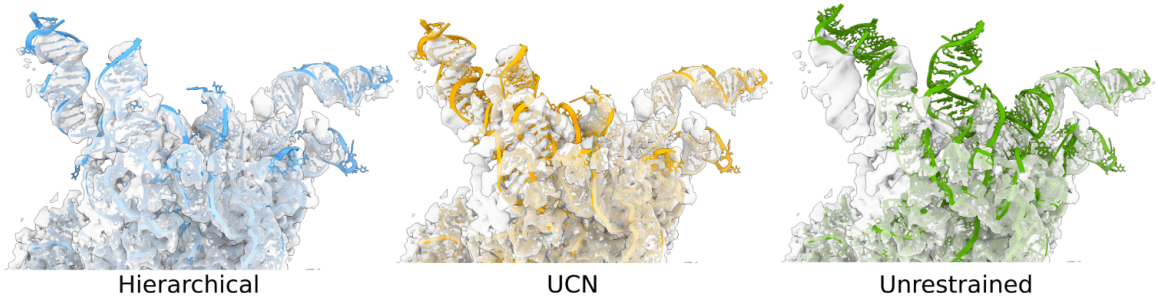
RIBFIND2 - HELPS TO AVOID GETTING "STUCK" IN LOCAL OPTIMA

Thomas Mulvaney

Sony Malhotra

- **human SSU processome**
- **pre-A1 into state post-A1**
- **EMD-23938, 2.7 Å resolution**

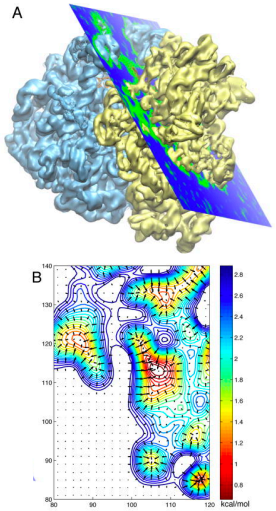




Malhotra, Mulvaney et al. *NAR* 2023;
Pandurangan *et al. J Struct Biol* 2012

https://ribfind.topf-group.com/

FLEXIBLE FITTING AND REFINEMENT



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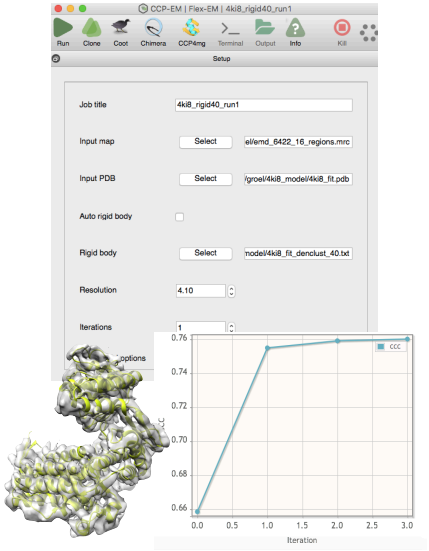

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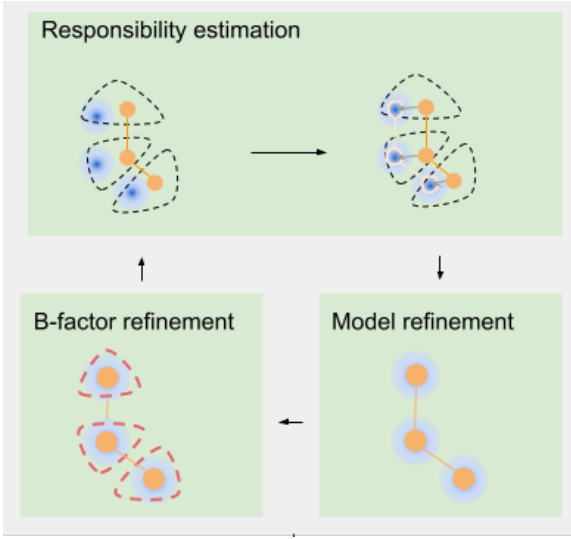


TEMPy-ReFF (Responsibility-based Flexible-Fitting)

Cragnolini et al., *Proteins* 2021;
Beton*, Mulvaney* et al, *Nat Comm* 2024

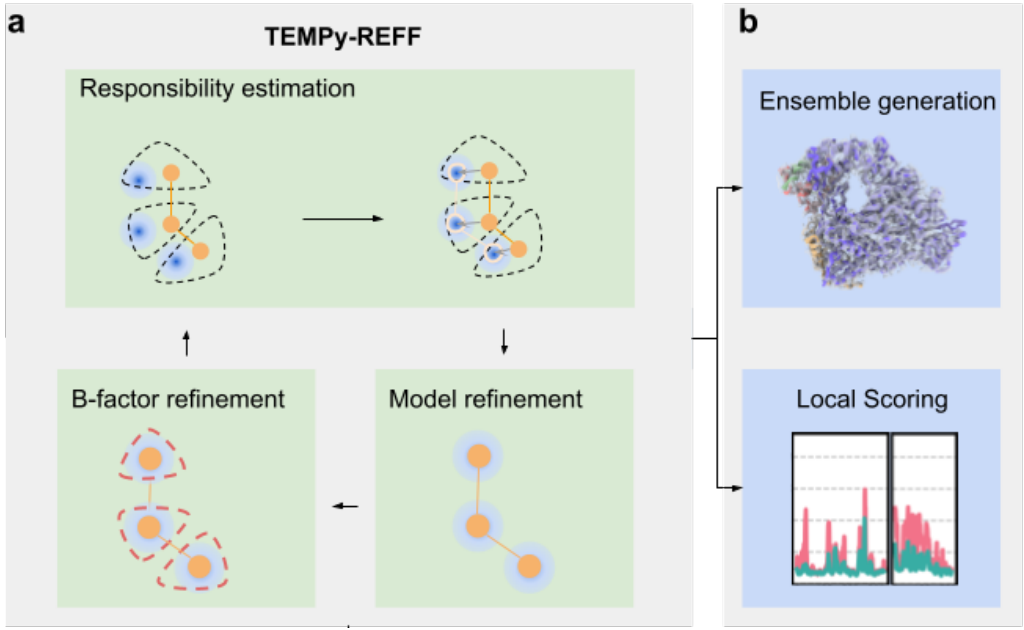
TEMPY-REFF: FLEXIBLE FITTING AND REFINEMENT

Thomas Mulvaney

Joseph Betoni

Tristan Cragnolini

- Use an expectation-maximisation approach
- Use one gaussian per atom, and a background noise term
- Improves atomic position and variance (B-factor)



TEMPy and OpenMM

Amber14 forcefield

Generalised Born implicit solvent model

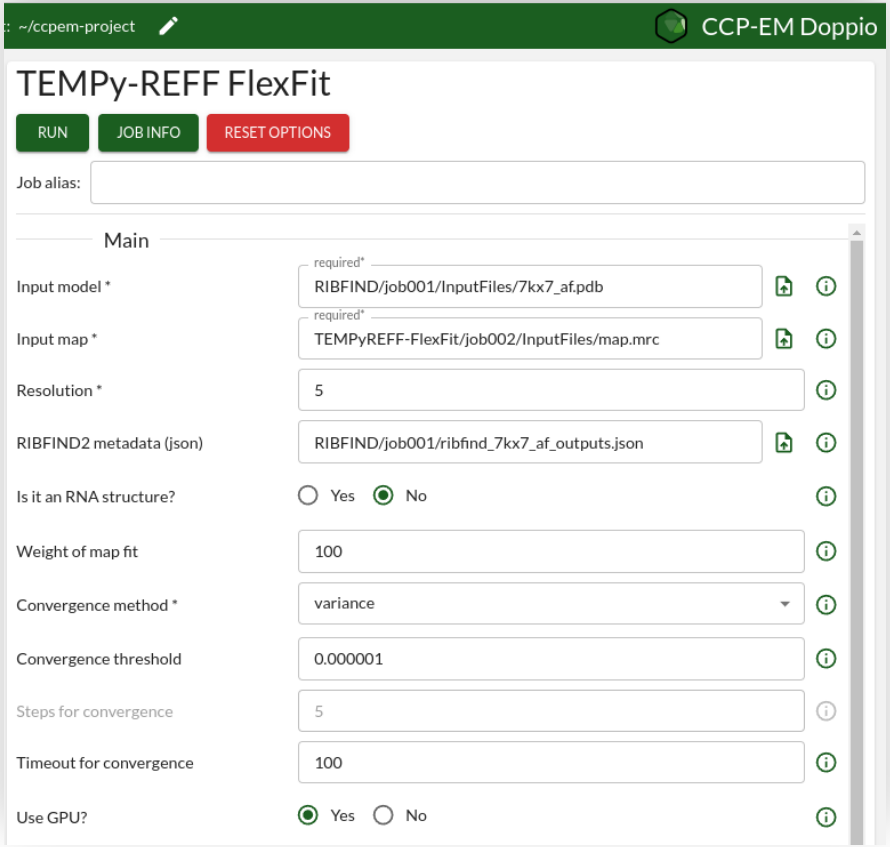
Integration with Langevin dynamics, at 100K

Beton*, Mulvaney* et al, *Nat Comm* 2024

https://tempy-reff.topf-group.com/

TEMPY-REFF: FLEXIBLE FITTING AND REFINEMENT

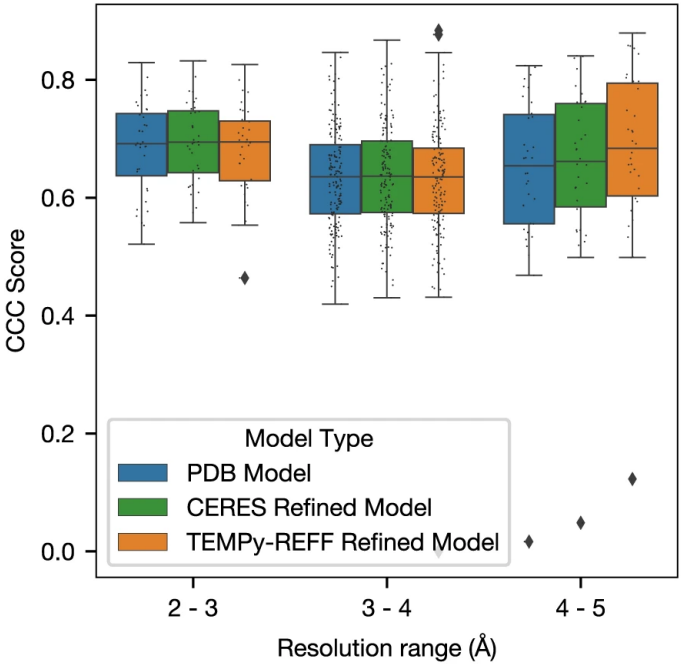
- **Flexible fitting**
  - Flexible fitting of proteins intermediate resolutions using density guided force + AMBER.
  - Faster – built on top of OpenMM, can handle much larger models
  - Hierarchical rigid body protocol using **RIBFIND2**
- **Refinement**
  - Optimises fit of GMM to data using molecular dynamics with OpenMM and AMBER.

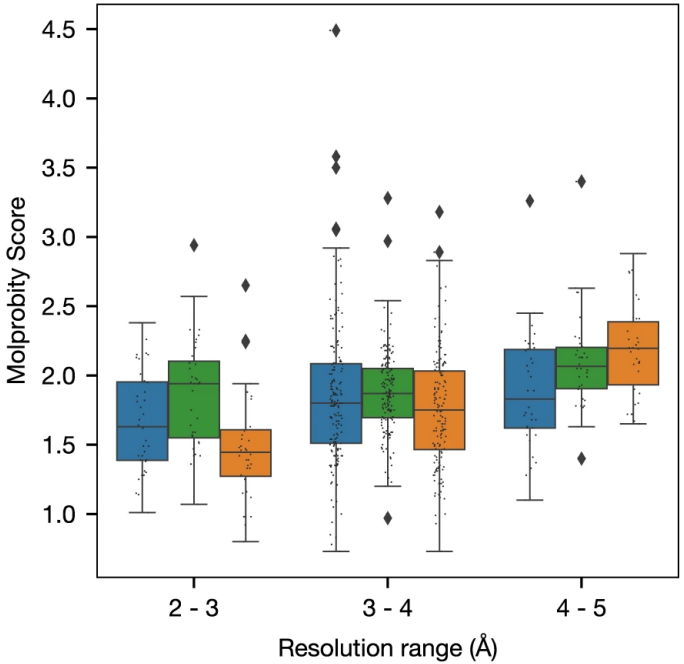


Fancy GUI in CCPEM Doppio

Beton*, Mulvaney* et al, *Nat Comm* 2024

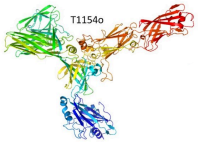
COMPARISON AGAINST 229 CERES MODELS

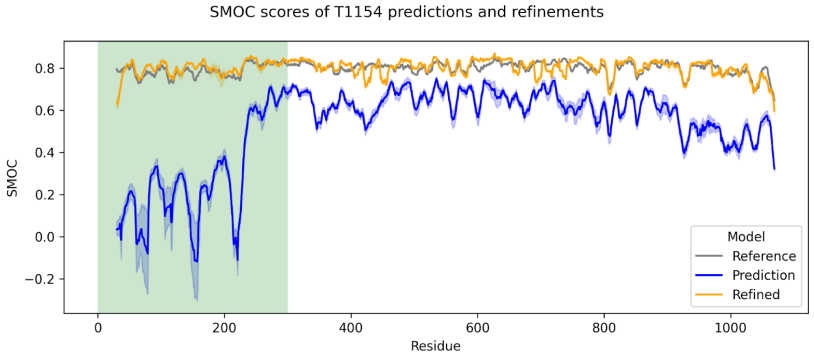


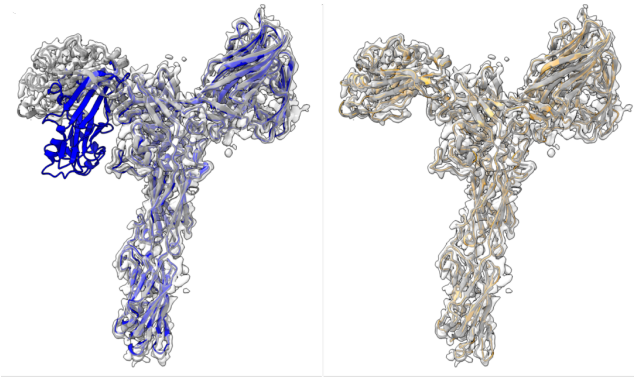


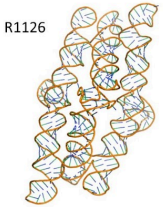
https://cci.lbl.gov/ceres

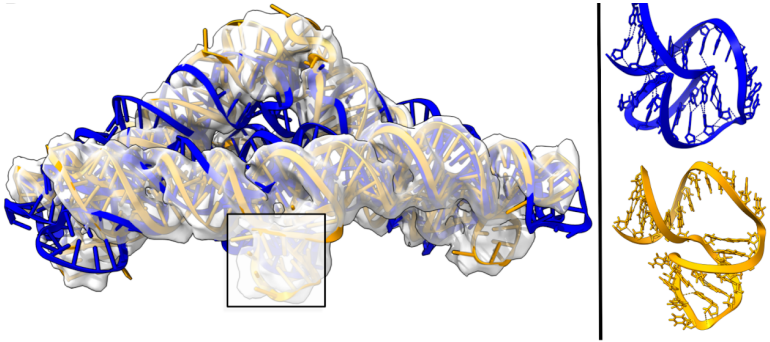
TEMPY-REFF REFINEMENT OF CASP15 MODELS (PROTEIN COMPLEXES AND RNA)







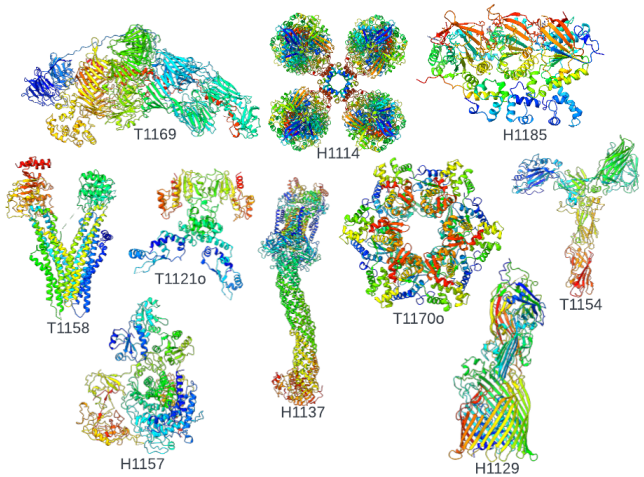


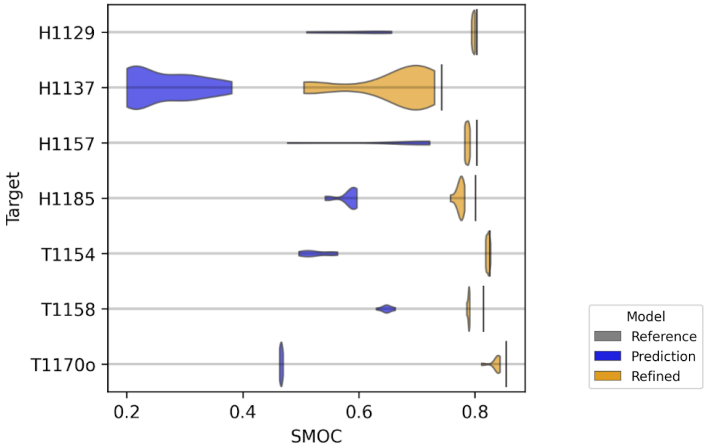


Mulvaney et al, *Proteins* 2023 (CASP15 specital edition)

TEMPy2

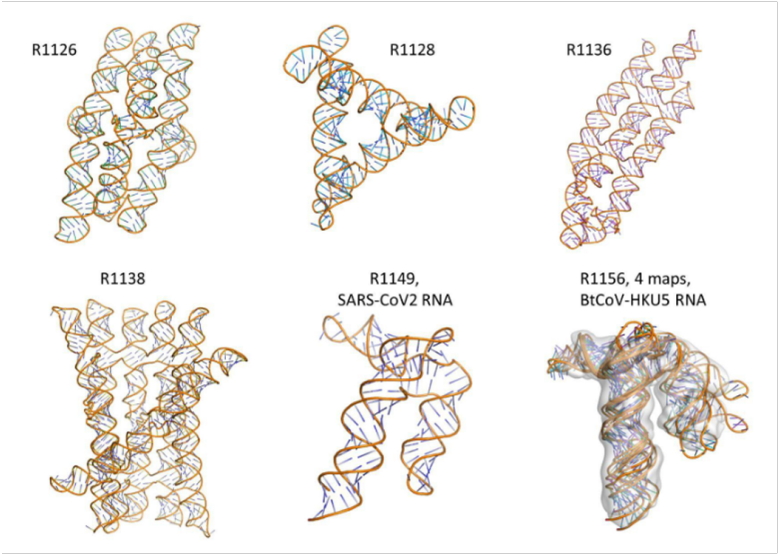
TEMPY-REFF REFINEMENT OF CASP15 MODELS (PROTEIN COMPLEXES AND RNA)

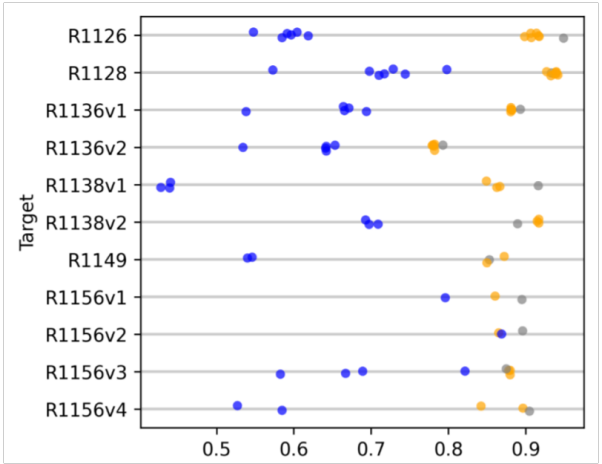




Mulvaney et al, *Proteins* 2023 (CASP15 specital edition)

TEMPY-REFF REFINEMENT OF CASP15 MODELS (PROTEIN COMPLEXES AND RNA)

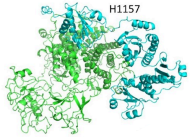


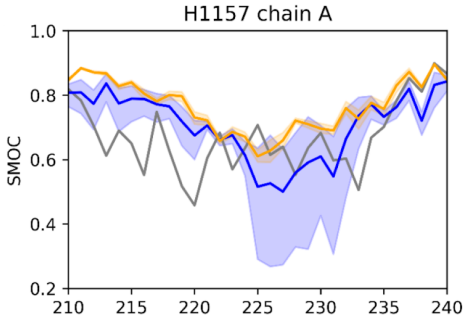


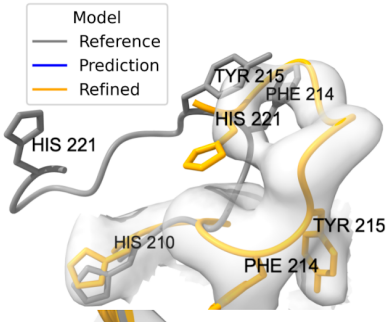
Rachael Kretsch,
Das Lab, Stanford

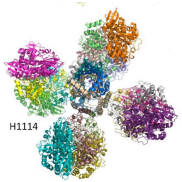
Mulvaney et al, *Proteins* 2023 (CASP15 specital edition)

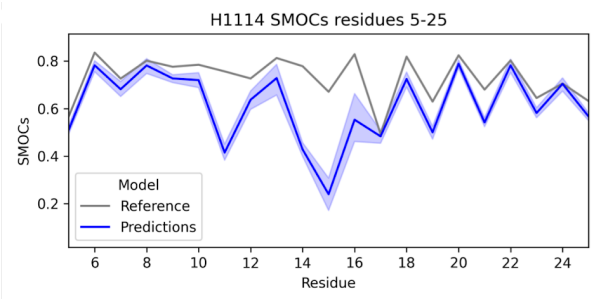
EXPERIMENTAL VALIDATION AND REFINEMENT BY CRYO-EM (CASP15)

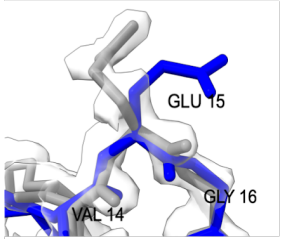


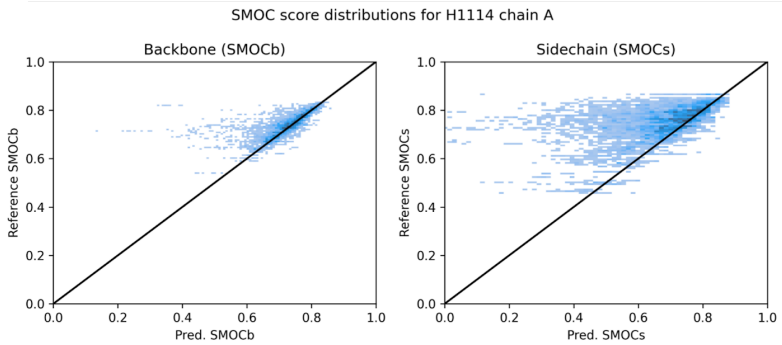




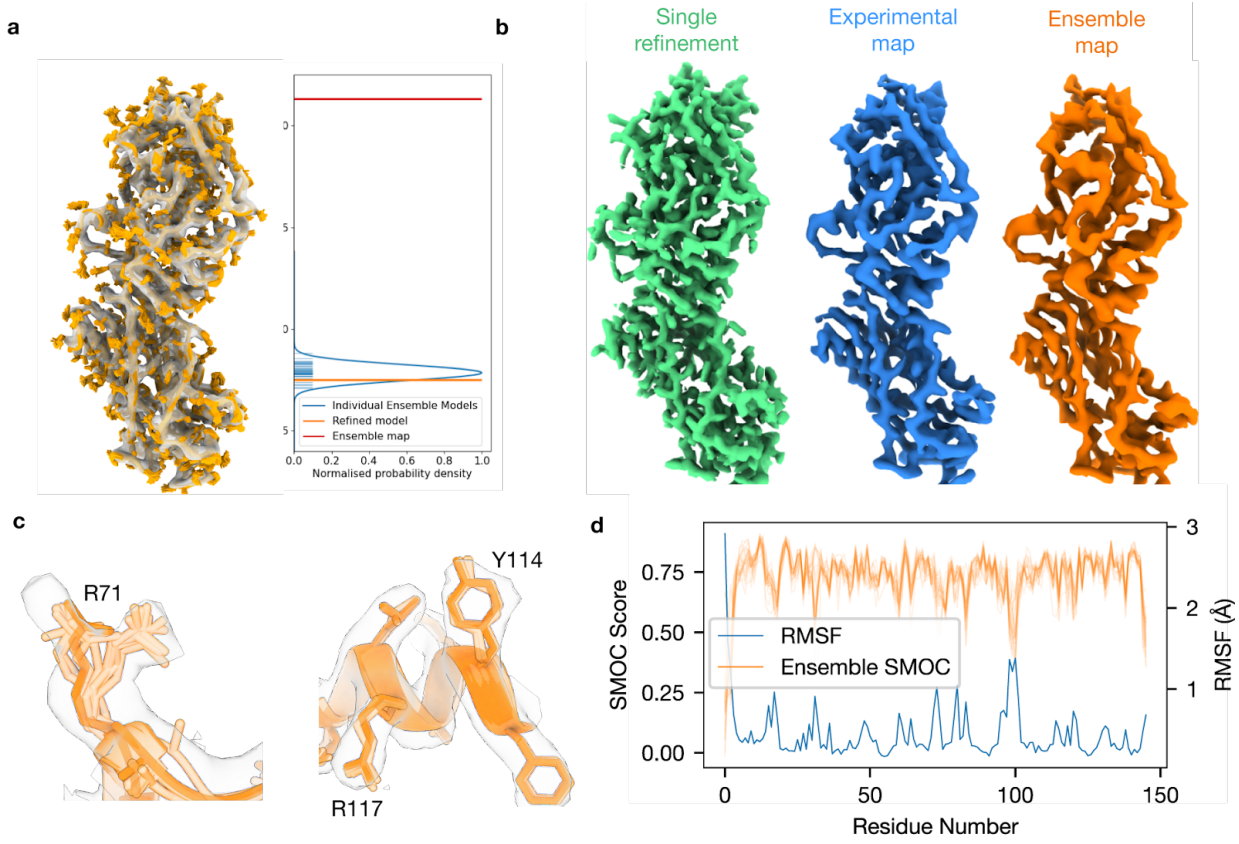




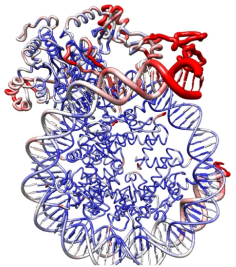




TEMPY-REFF CAN GENERATE ENSEMBLES

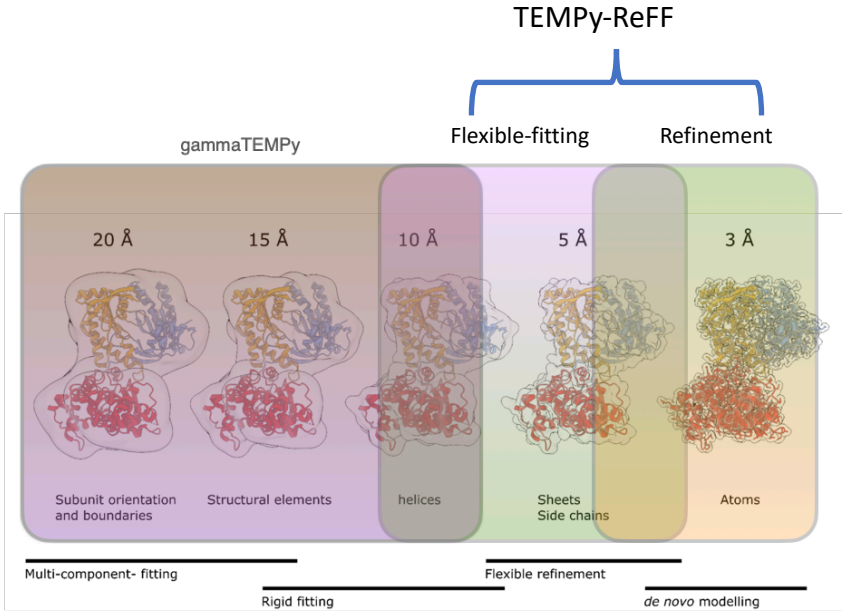


Color: TEMPy-ReFF Bfactor
Width: ResMap



Beton, Mulvaney et al. *Nat. Comm* 2024

- The quality of the fit to the density often varies in different parts of the cryoEM map
- Proteins are dynamic
- Some of these parts may be better represented by multiple models
- **TEMPy-ReFF** is useful across a range of resolutions.
- **RIBFIND2** can generate rigid bodies for Protein and RNA.
- **TEMPy-ReFF** ensembles can model uncertainty/heterogeneity.
- **CASP15** predictions were good starting points for models for flexible fitting and refinement.



- **More attention is needed for small molecules…**

ChemEM: flexible docking of small molecules in Cryo-EM structures

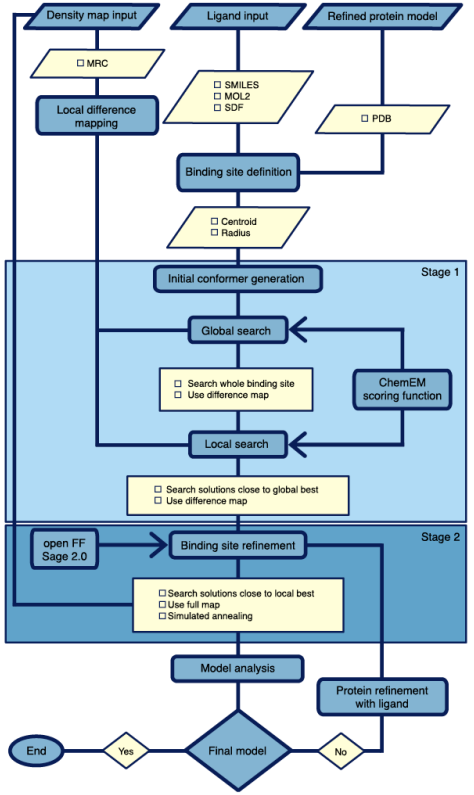
ChemEM

Uses Molecular docking, Molecular Dynamics and Density Difference mapping.

Fits ligands and refines binding site residues.

Acurate ligand fitting at 2.2-5.6 Å resolution.

Can fit multiple ligands in parralel to a single site.



Using ChemEM : Stage 1

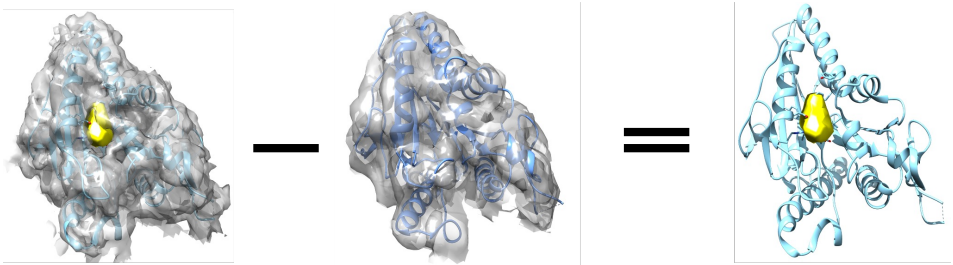
ChemEM: Ligand Placement and Refinement Workflow

- **Stage 1: Initial Ligand Docking**
  - Segment ligand density from the electron density map.
  - Perform molecular docking using ligand density as a ‘restraint’.
  - Score solutions by combining:
    - **Docking Score** for physicochemical reasonableness.
    - **Mutual Information Score** for alignment with density.

ChemDock Score: Includes terms for Bonding, Pi-Pi stacking and hydrophobic interactions

$$\begin{aligned} Docking\ Score = & Bias + w1 * \mathrm{HB}_{dist} + w2 * \mathrm{HB}_{angle} + w3 * \mathrm{Vina}_{g1} + w4 * \mathrm{Vina}_{g2} + w5 * \mathrm{Vina}_{steric} + \\ & w6 * \mathrm{LogP}_{HPI} + w7 * \mathrm{Aromatic}_{dist} + w8 * \mathrm{Aromatic}_{angle} + w9 * \mathrm{Intra}_{vg1} + w10 * \mathrm{Intra}_{vg2} + w11 * \mathrm{Intra}_{v\,steric} \end{aligned}$$

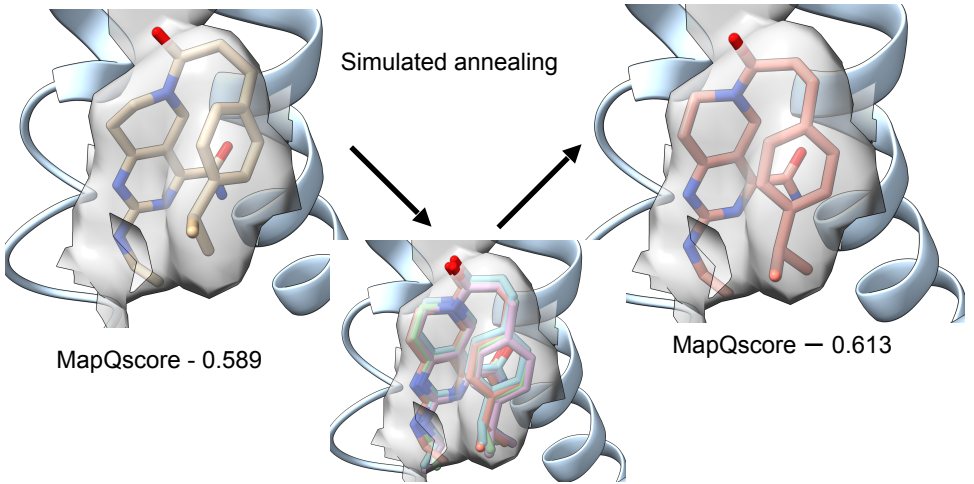
Segmenting Ligand density: Currently using a density difference map method.



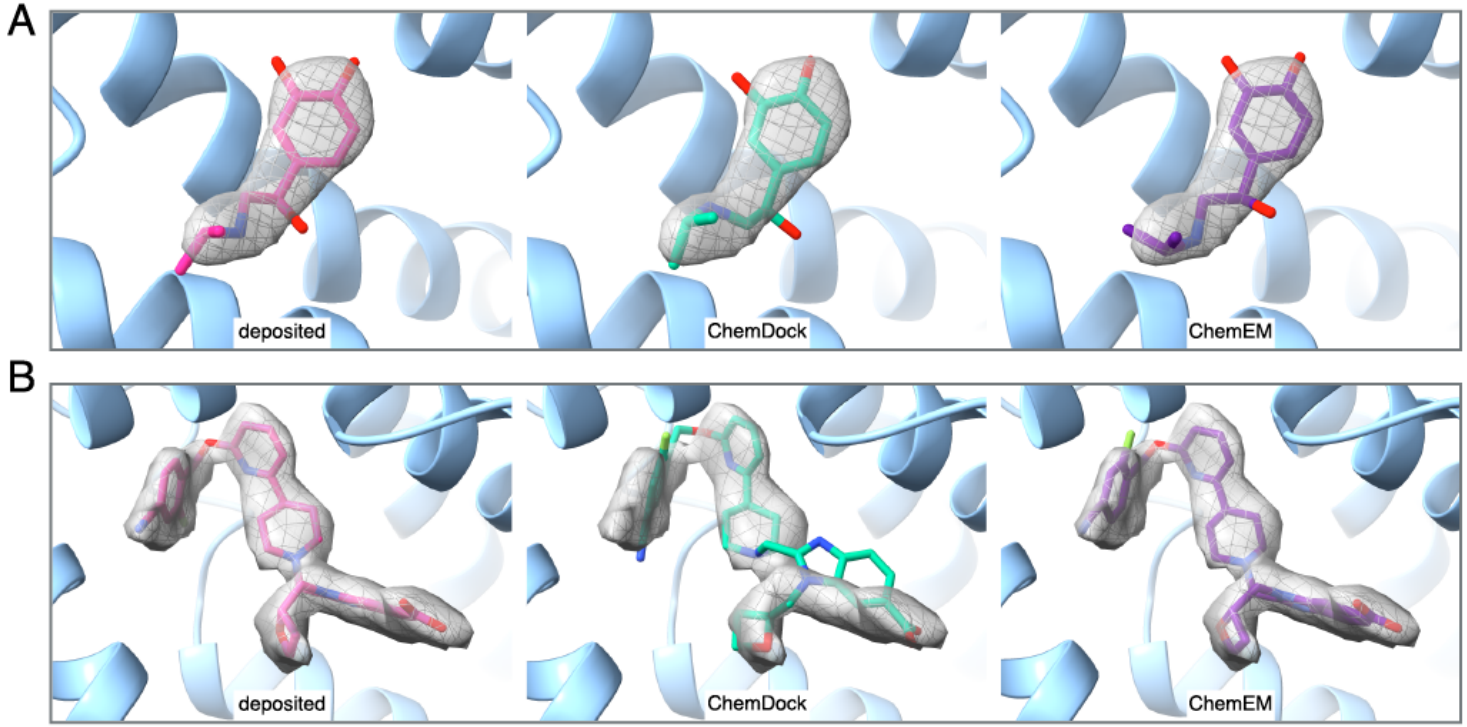
Using ChemEM : Method Overview

Stage 2: Molecular Dynamics Refinement

- Select top solutions from Stage 1.
- Refine ligand placement using molecular dynamics:
  - **Amber Parameters** for protein atoms
  - **OpenFF Parameters** for ligand molecules.



Using ChemEM : Examples



Using ChemEM : Examples

CSSB Centre for Structural Systems Biology

- ChemEM can be downloaded from
- It runs in terminal and all data is passed in from a configuration file.
- The enable stages block defines what protocols to run
- The rest defines the data and output locations
- ChemEM is also capable of fitting multiple ligand to the same site.

```
#ChemEM config file

#enable stages
pre_process = 1
pre_process_split_density = 1
auto_split_point = 1
auto_split_zone = 0
fitting = 1
dock_only = 0
post_process = 0
rescore = 0

#data and working directory
protein = ~/test_data/6tti_protein.pdb
ligand = CC(=O)Nc1cc(no1)C(F)(F)F
ligand = CC(=O)Nc1cc(no1)C(F)(F)F
ligand = CS(=O)C
ligand = CS(=O)C
densmap = ~/maps/6tti.mrc
resolution = 2.5
map_contour = 0.07
centroid = ( 43.908,  54.415,  49.755)
output = ~/multi_fit
```

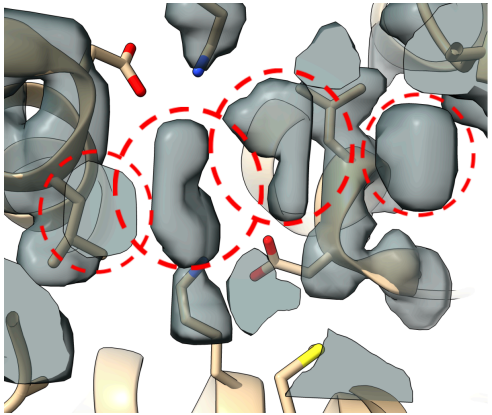
HLIV Leibniz Institute of Virology

UKE HAMBURG

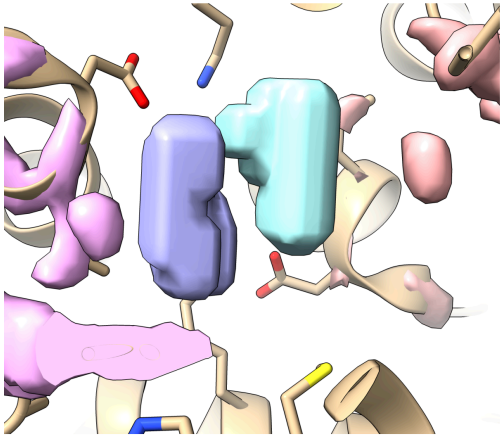
Using ChemEM : Examples

- The density is segmented by the difference mapping technique. This yields a single density map (That has a lot of extra density)

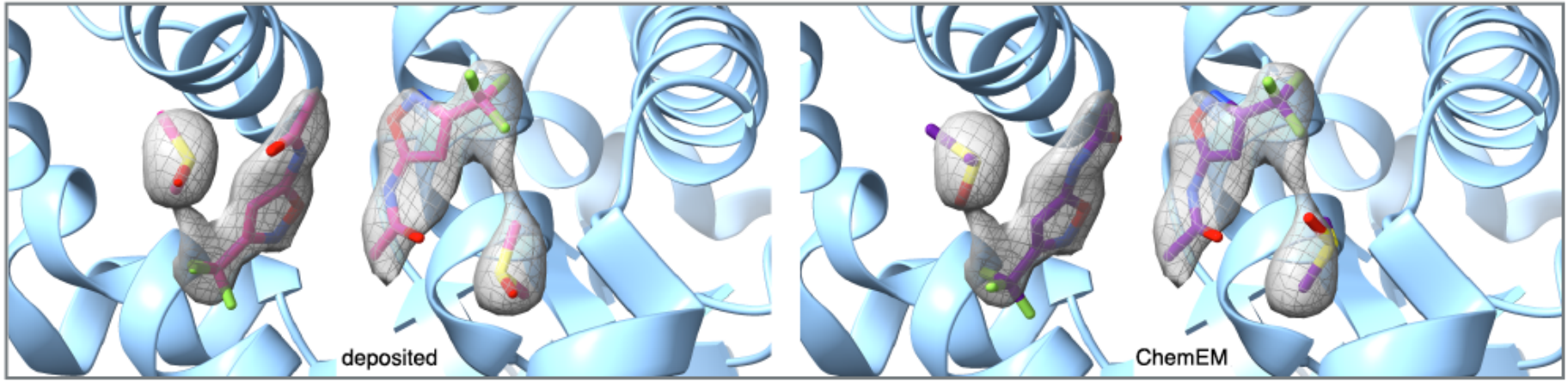
- The results can be improved and calculations sped up by specifying multiple centroid using this initial map. And assigning a ligand to each ‘density’.



```
...
centroid = ( 43.908,   54.415,   49.755)
centroid = ( 39.049,   58.586,   49.760)
centroid = ( 40.625,   62.434,   52.641)
centroid = ( 42.391,   50.558,   52.642)
...
```



Using ChemEM : Multi-ligand fitting



ChemEM:

- **Recent improvement to ChemEM include:**
  - Ions can be included to the docking calculation
  - Previously known ligand positions can be included
  - Protonation state assignment
  - Improved post-processing algorithms enable selective refinement of specific ligand atoms.
- **Coming soon:**
  - Better handling of water molecules and binding site solvation.
  - Lead optimization tools to predict the impact of R-group modifications on ligand affinity.

THANKS…

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Bundesministerium für Bildung und Forschung
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DFG
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