

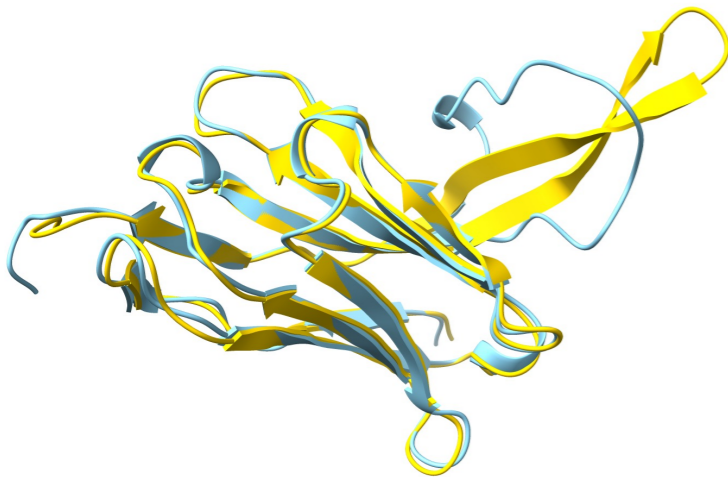
Using AlphaFold predictions for structure determination

Phenix Workshop

October 14, 2023, 80th Pittsburgh Diffraction Conference

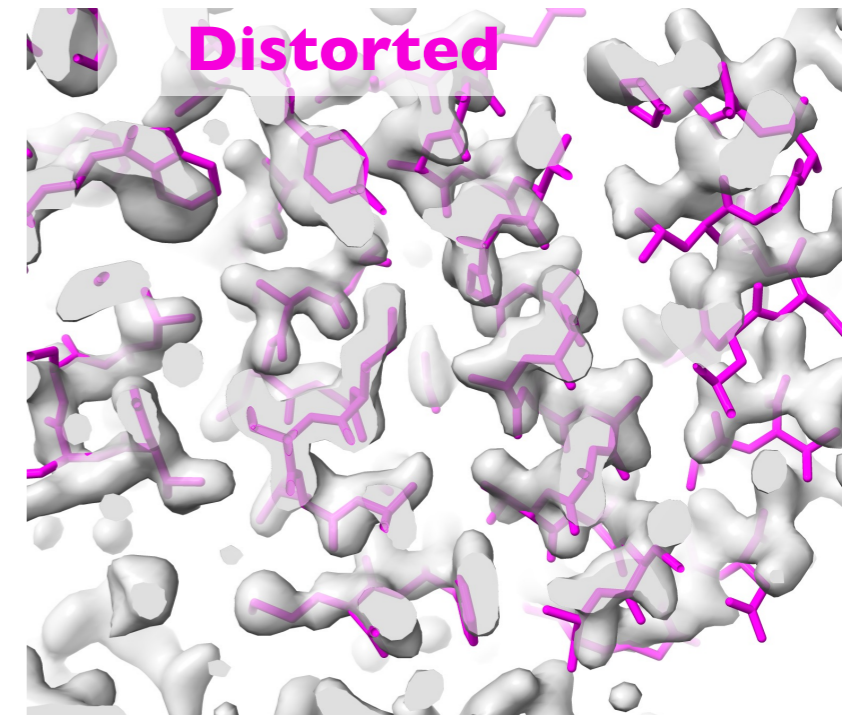
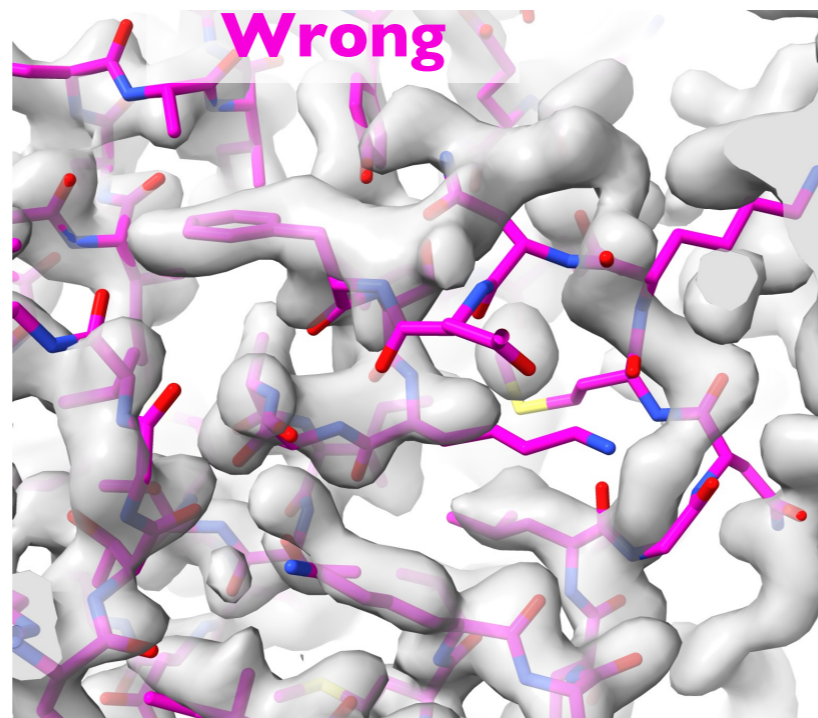
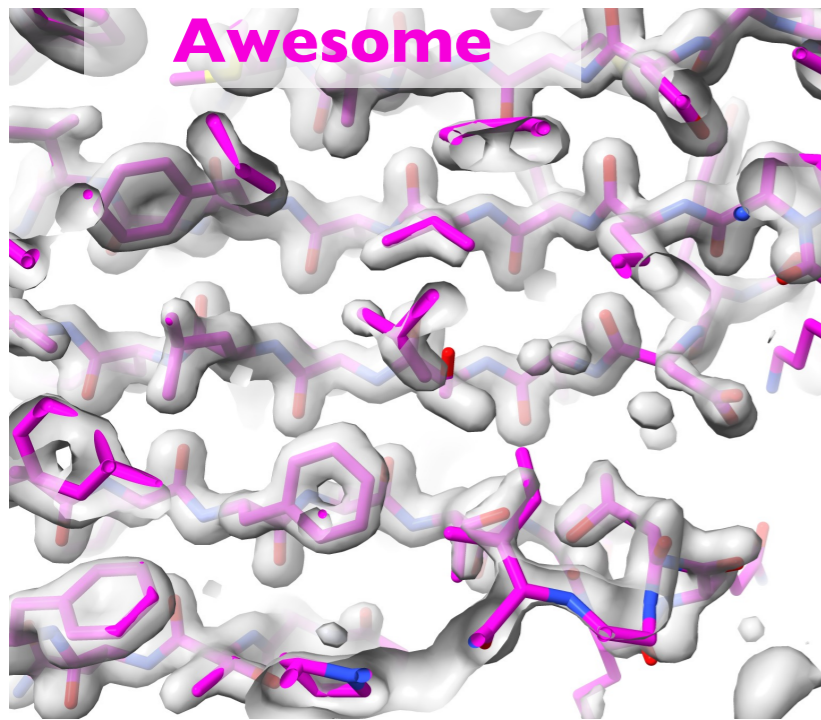
Tom Terwilliger

The New Mexico Consortium
Los Alamos National Laboratory



AlphaFold predictions are great hypotheses

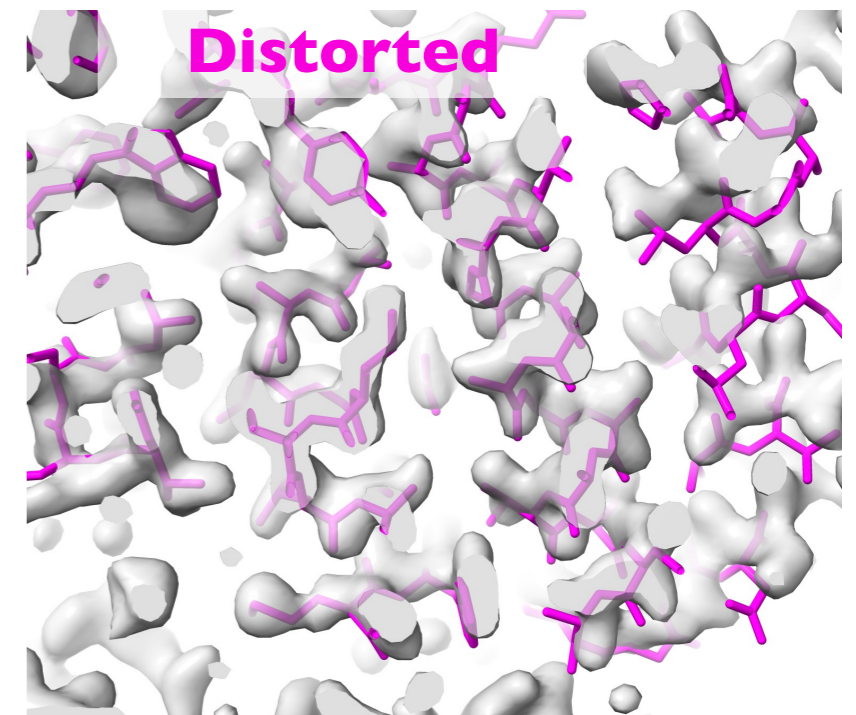
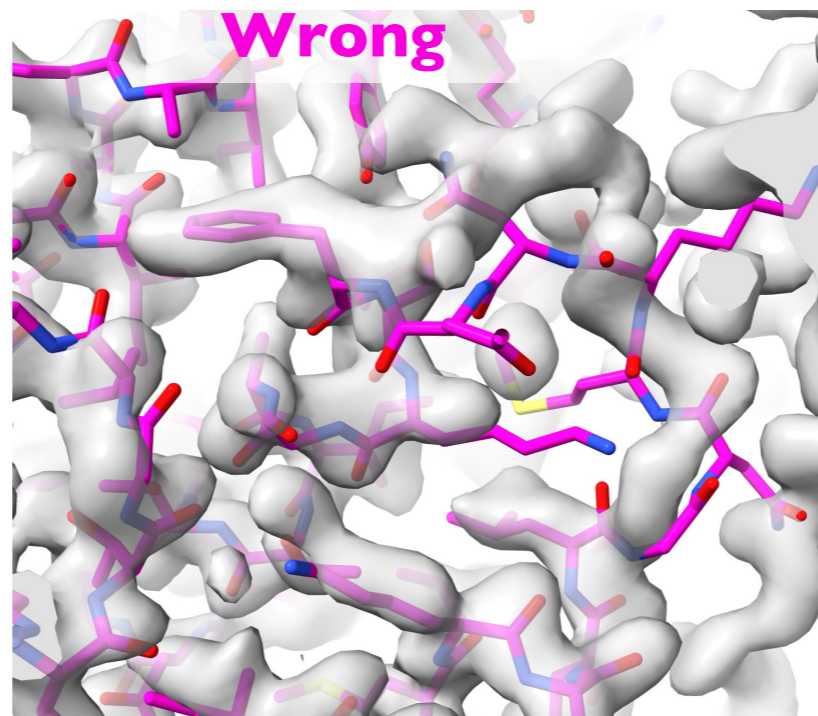
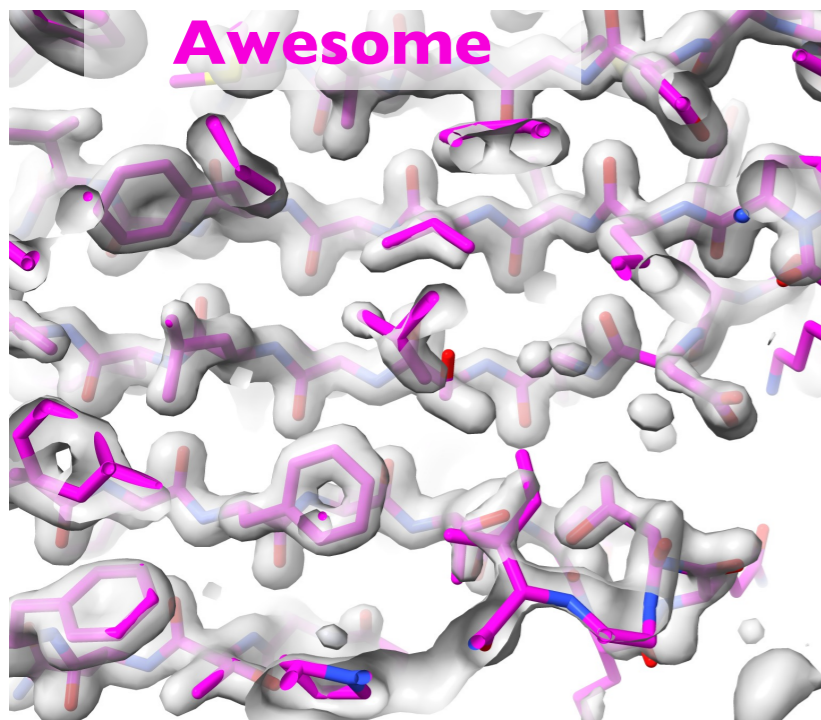
*AlphaFold models
can be....*



AlphaFold predictions and confidence estimates

Residue-specific confidence (pLDDT) identifies where errors are more likely

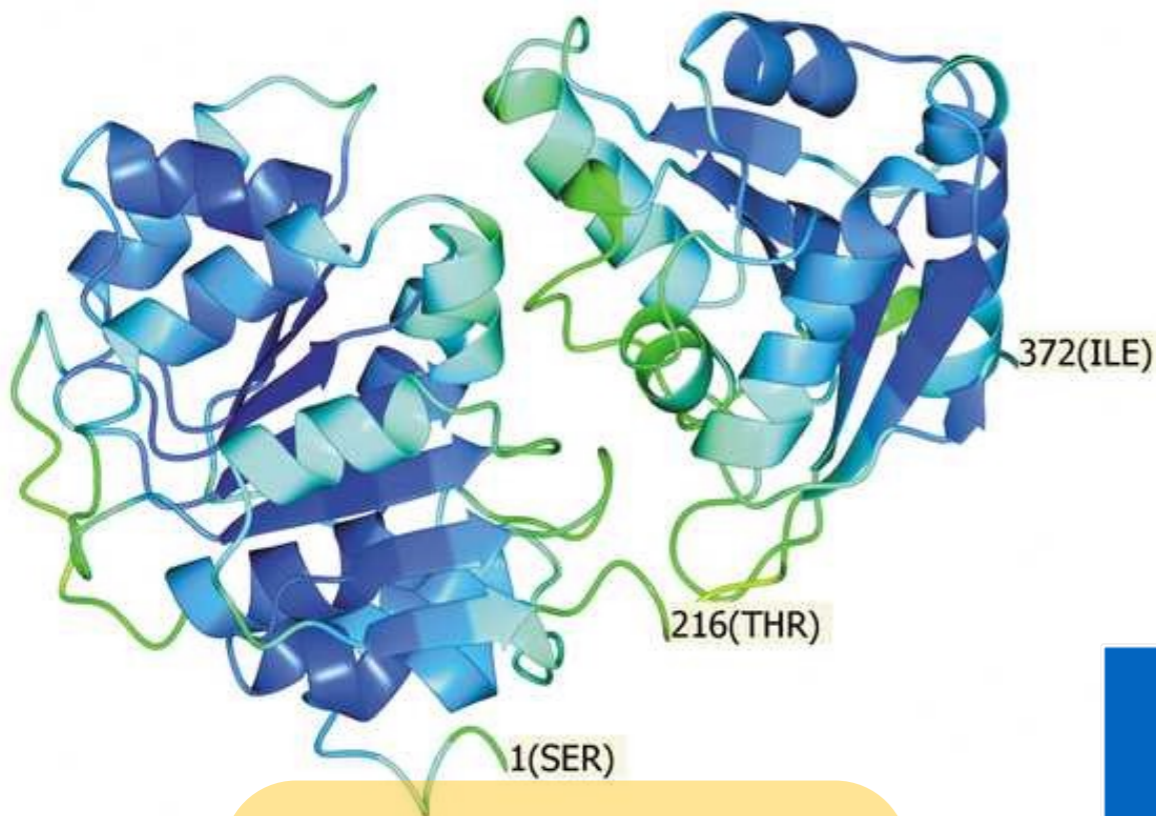
AlphaFold confidence (pLDDT)	Median prediction error (Å)	Percentage with error over 2 Å
>90	0.6	10
80 - 90	1.1	22
70 - 80	1.5	33
<70	3.5	77



Terwilliger et al. (2023), AlphaFold predictions are valuable hypotheses, and accelerate but do not replace experimental structure determination. BioRxiv 2022.11.21.517405

AlphaFold confidence measure

(pLDDT, Predicted difference distance test)



Confidence:

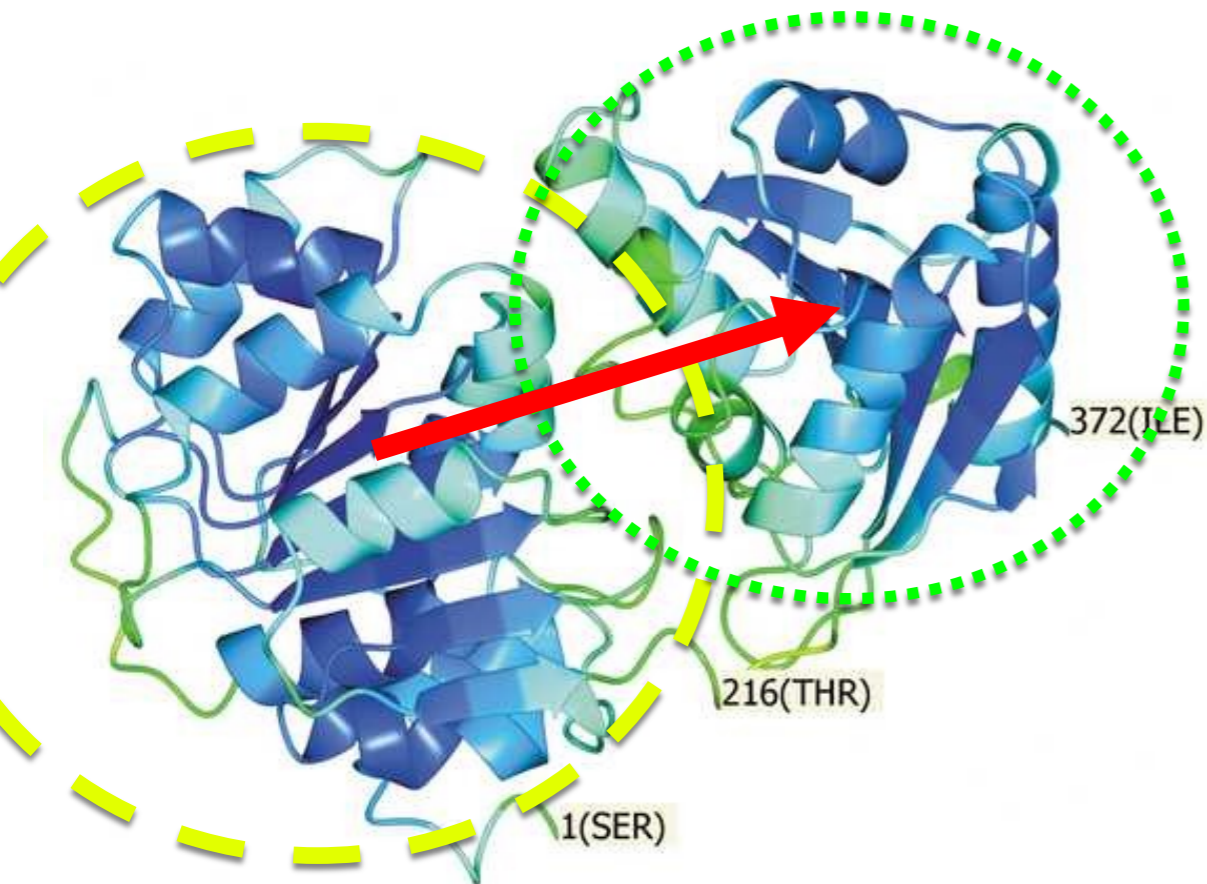
Blue: > 90

Green: 80 - 90

AlphaFold prediction for
RNA helicase
(PDB entry 6i5i)

AlphaFold confidence (pLDDT)	Median prediction error (Å)	Percentage with error over 2 Å
>90	0.6	10
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PAE matrix (Predicted aligned error)



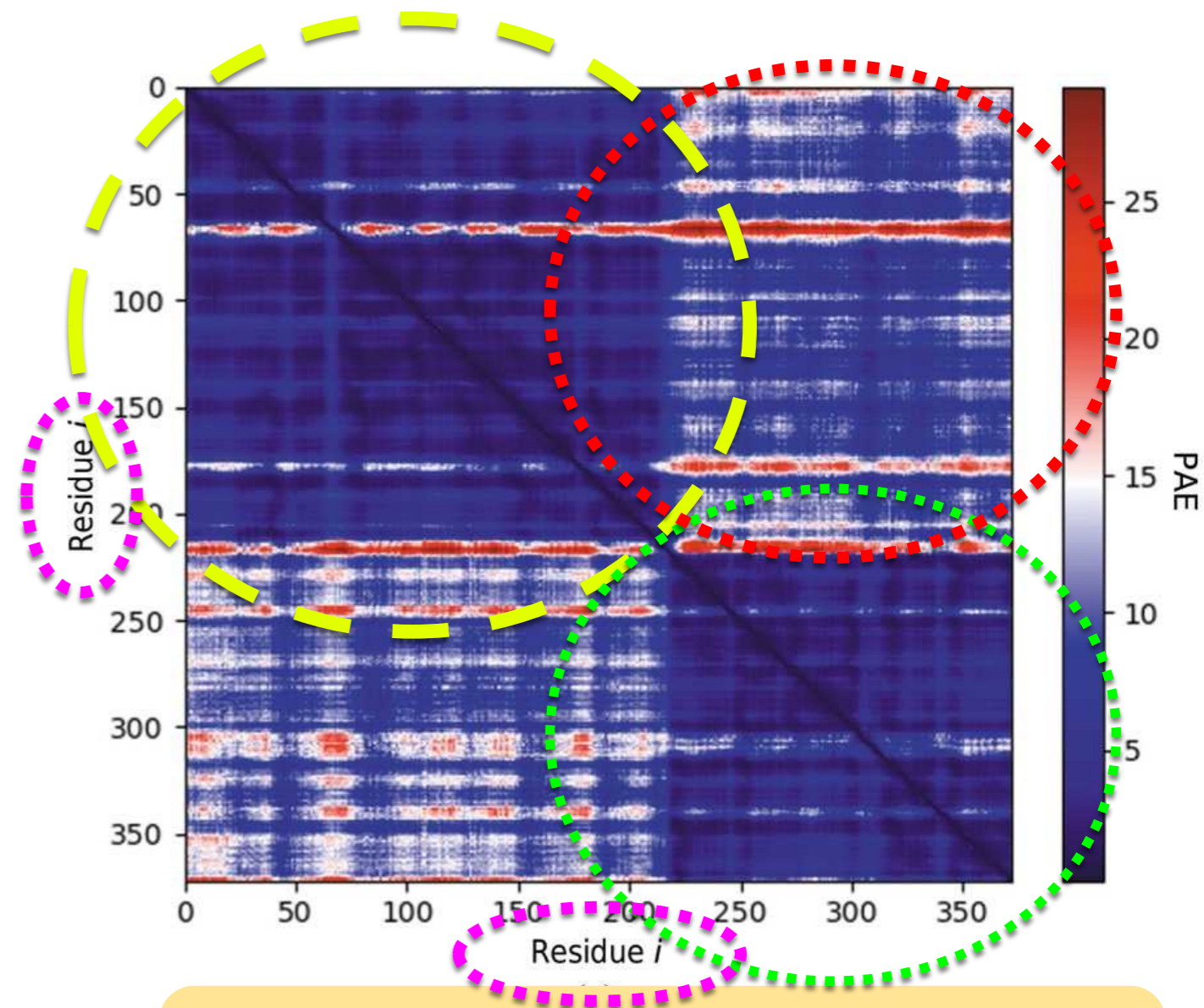
Confidence:

Blue: > 90

Green: 80 - 90

AlphaFold prediction for
RNA helicase
(PDB entry 6i5i)

PAE matrix identifies
accurately-predicted domains



Dark blue: uncertainty in
relative positions $< 5 \text{ \AA}$

Strategy for structure determination in the AlphaFold era

1. Predict your structure

Design your experiment based on predicted models
(choose experimental approach, consider trimming at domain boundaries)

2. Solve your structure

Cryo-EM or X-ray MR with trimmed predicted model, SAD

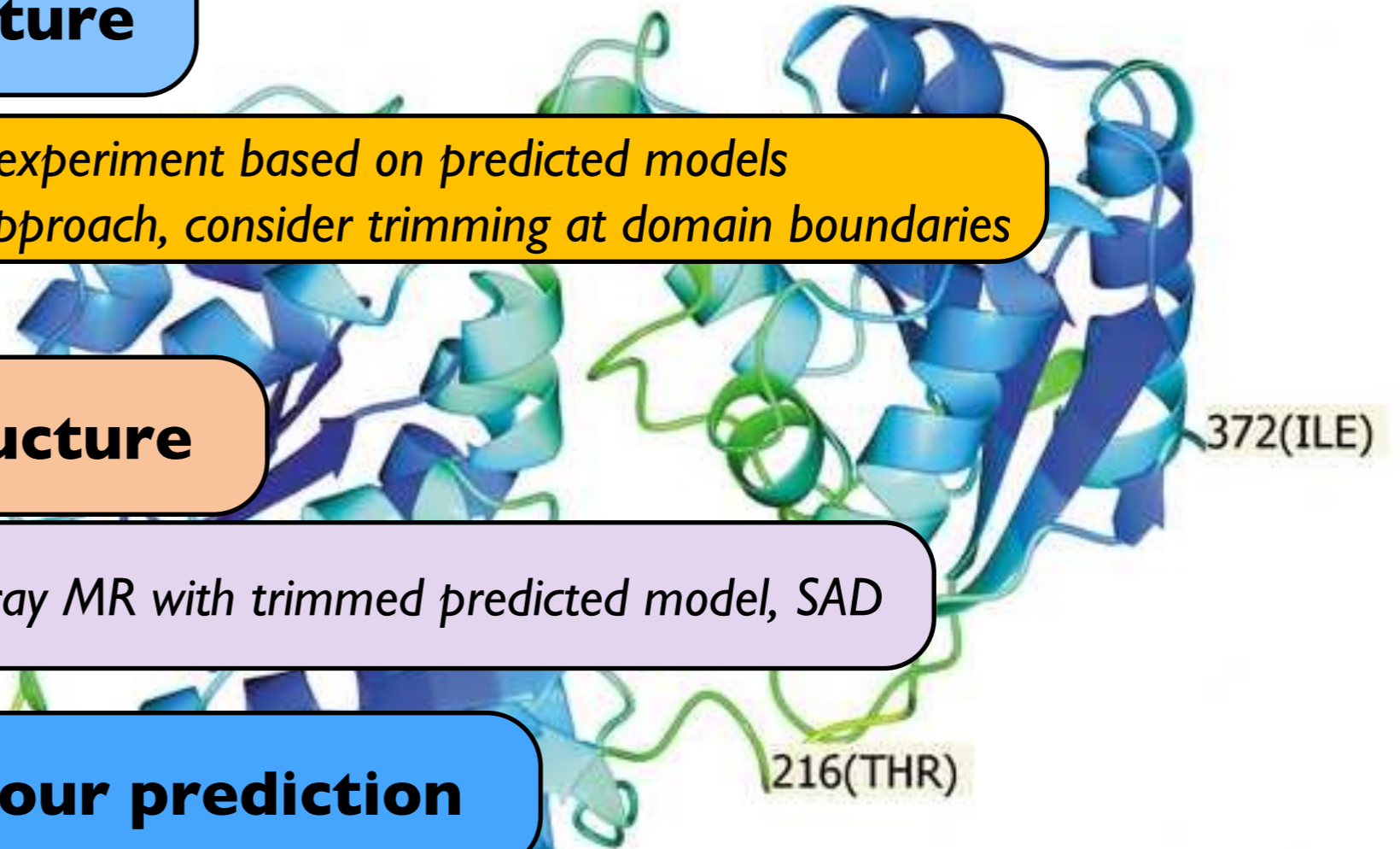
3. Update your prediction

Run AlphaFold with your best model as a template

4. Improve your structure

Use your new predictions as hypotheses

Iterate



Phenix AlphaFold prediction server

Available from the Phenix GUI

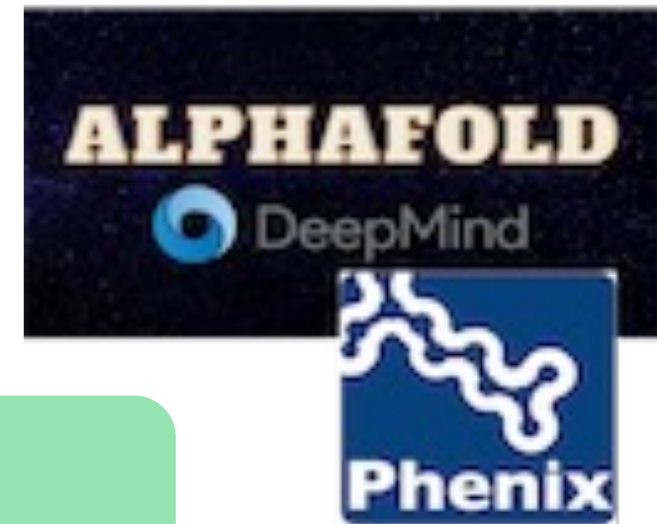
*Predicts structures of protein chains
(one at a time)*

Can use a template to guide the prediction

You do not need an MSA (multiple sequence alignment) if you supply a template

The template should not be an AlphaFold model

Many thanks for AlphaFold, ColabFold scripts, and the MMseqs2 server for MSAs



Process predicted model

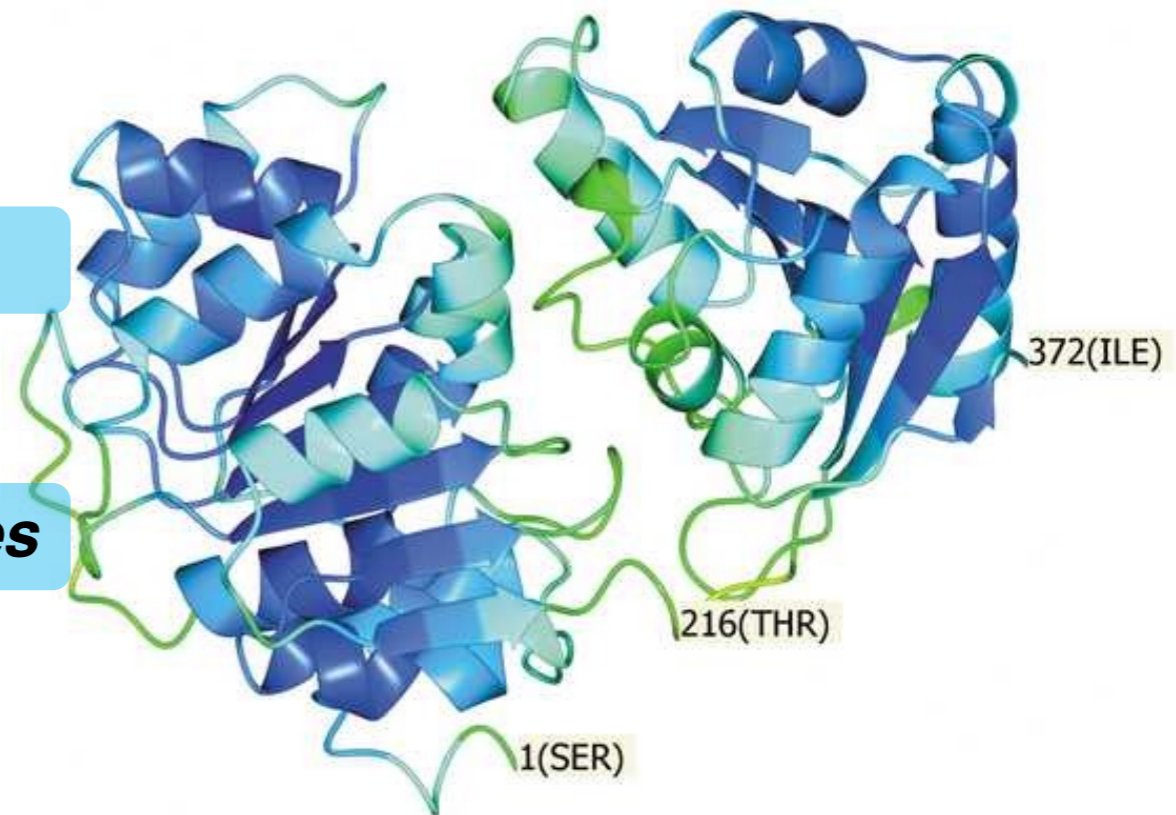
Convert pLDDT to B-value

Trim low-confidence parts of model

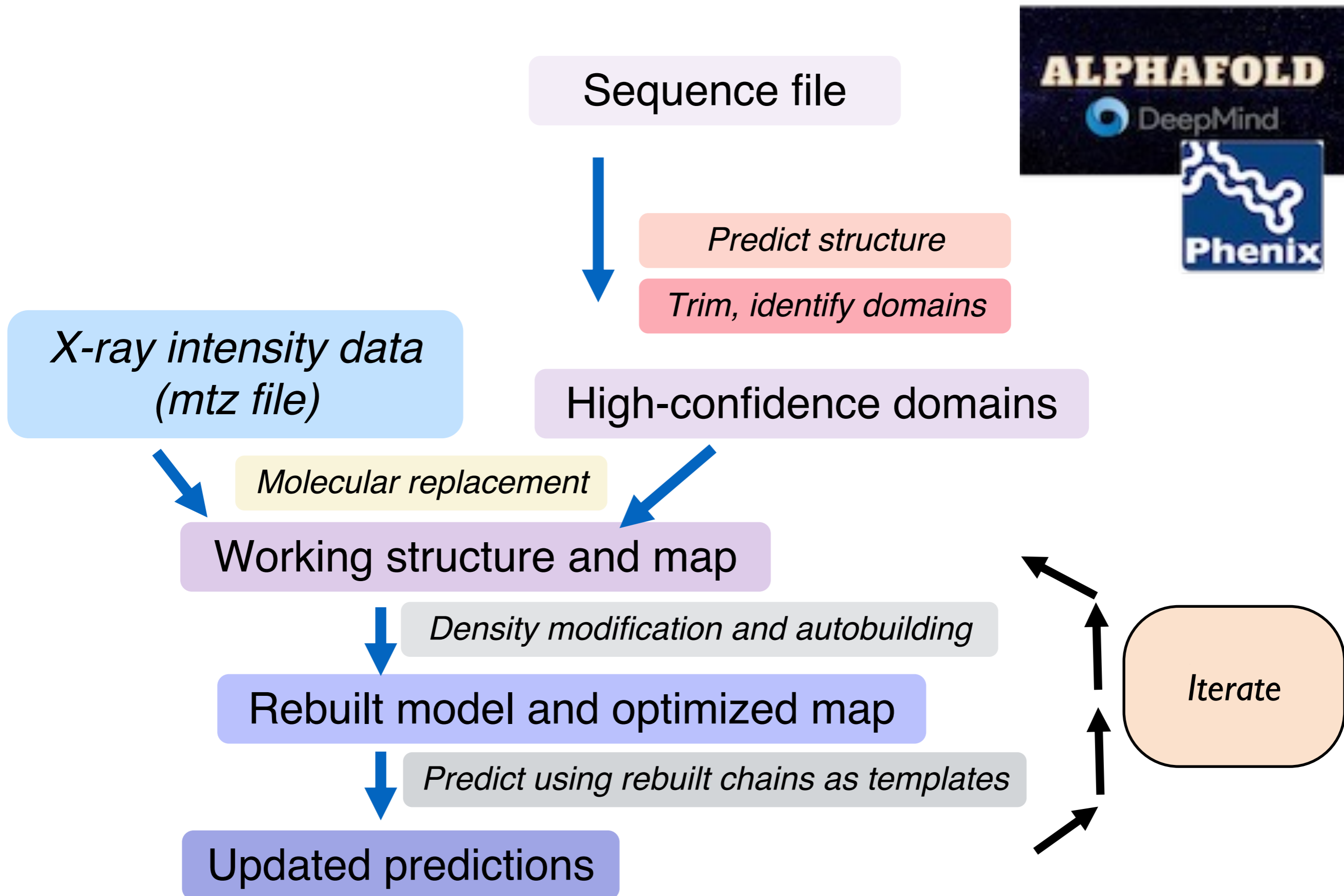
Identify high-confidence domains

Compact high-confidence regions

Groupings of residues with low PAE values



X-ray structure determination with AlphaFold



Input and output from structure determination with AlphaFold

Input

Experimental data (maps or X-ray data)

Contents of asymmetric unit (sequence file)

Output

*Rebuilt model
Optimized map*

*Map and model ready
for next steps*

Docked predicted models

*Useful as high-quality
reference models*

Phenix tools for structure determination with AlphaFold

PredictModel (Predict with AlphaFold)

AlphaFold
models

ProcessPredictedModel (Trim and identify domains)

ResolveCryoEM, LocalAnisoSharpen (map improvement)

EMPlacement, DockInMap (Docking of single, multiple chains)

Cryo-EM

DockAndRebuild (Morphing and rebuilding)

RealSpaceRefine (Refinement)

Phaser-MR (Molecular replacement)

AutoBuild (Density modification and rebuilding)

X-ray

Phenix.refine (Refinement)

PredictAndBuild (Prediction and structure determination)

Full
automation



The Project



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Oleg Sobolev,
Christopher Schlicksup



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UTHealth

Matt Baker



Duke University

Jane Richardson, Vincent
Chen, Michael Prisant,
Christopher Williams,



An NIH/NIGMS funded
Program Project

Liebschner D, *et al.*, Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in *Phenix*. Acta Cryst. 2019 **D75**:861–877