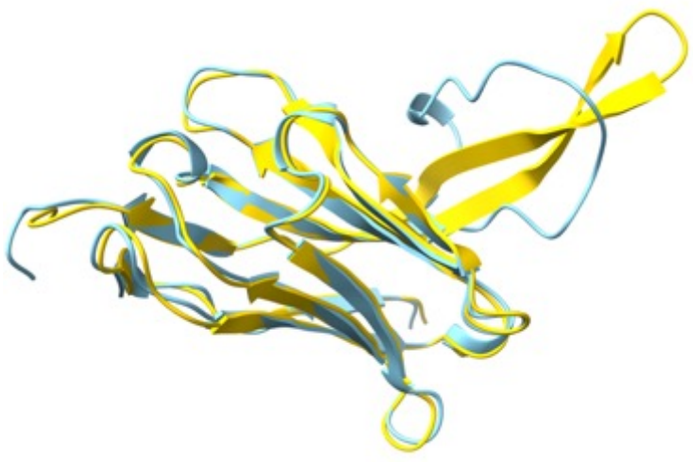
AlphaFold changes everything:

Incorporating predicted models in X-ray and Cryo-EM structure determination

Presentation available at:
phenix-online.org/presentations



How you can benefit from AlphaFold …

… by determining structures more easily

… and getting better models

January 18, 2022
RéNaFoBiS Webinar

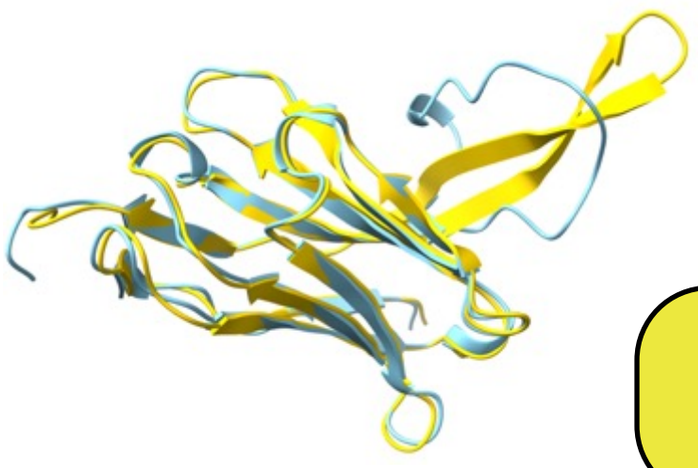
Tom Terwilliger

The New Mexico Consortium
Los Alamos National Laboratory

Randy Read, Tristan Croll, Claudia Millán, (Cambridge, University), Paul Adams, Billy Poon, Pavel Afonine, Christopher J. Schlicksup (Lawrence Berkeley National Laboratory); Jane Richardson (Duke University)

AlphaFold models …

*are great **hypotheses** for protein structures*



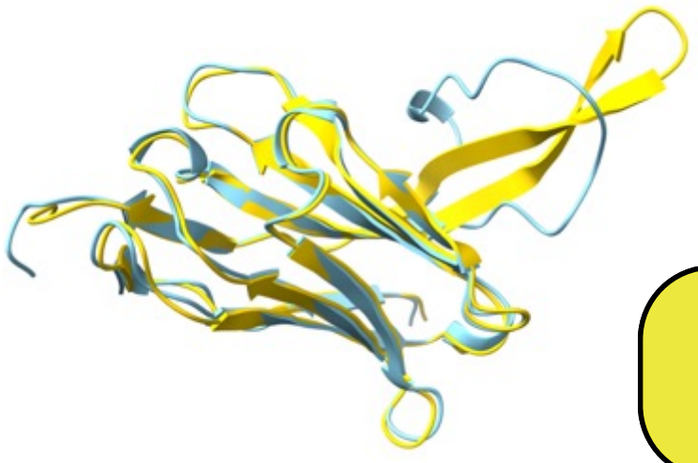
jump-start structure determination by X-ray and CryoEM

can be iteratively improved with a density map

allow a new work-flow for structure determination

AlphaFold models …

*are great **hypotheses** for protein structures*



jump-start structure determination by X-ray and CryoEM

can be iteratively improved with a density map

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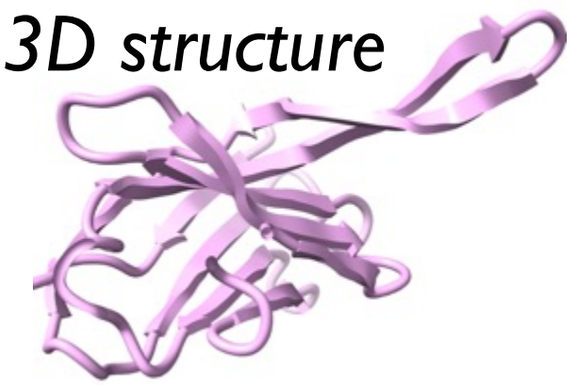
Machine-learning prediction of protein structure

Training

- *Sequence*
- *Multiple sequence alignment*
- *3D structure*

} *180,000 protein structures in the PDB*

```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M.........Q........
..................K.........Y....L........A.........
..............A.................................V......
....................A.............................
............................L....V....E.............
.........A.....................................Q.....
```



→ ***21 million parameters***

Prediction

- *Sequence*
- *Multiple sequence alignment*

```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M.........Q........
..................K.........Y....L........A.........
..............A.................................V......
....................A.............................
............................L....V....E.............
.........A.....................................Q.....
```

21 million parameters

Focus attention on important relationships

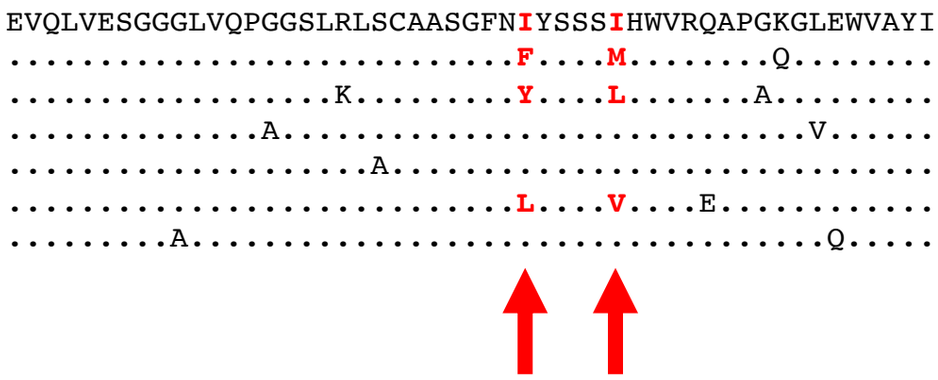
→ *3D prediction*

Confidence estimates (plDDT)

Multiple sequence alignment is key information for AlphaFold

Multiple sequence alignment

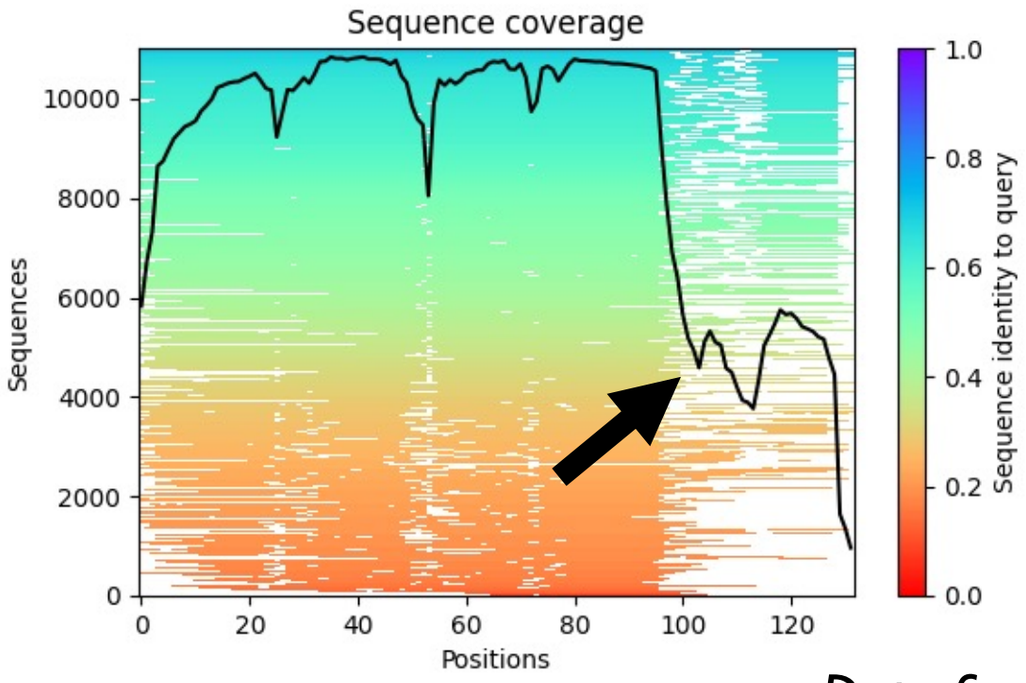
```
EVQLVESGGGLVQPGGSLRLSCAASGFNIYSSSIHWVRQAPGKGLEWVAYI
.............................F....M.........Q........
..................K.........Y....L........A.........
..............A..............................V.......
....................A.............................
.............................L....V....E............
.........A.....................................Q.....
```

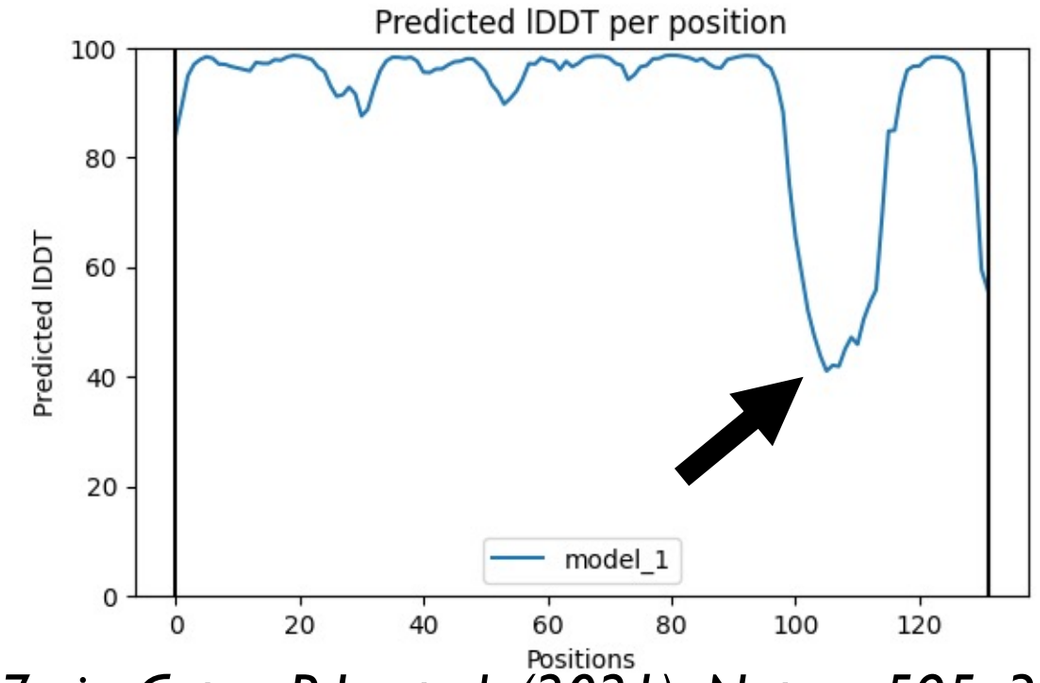


*Residues that **co-vary** are probably close in 3D structure*

All sequences in alignment should be compatible with the right structure

Sequence coverage → Confidence

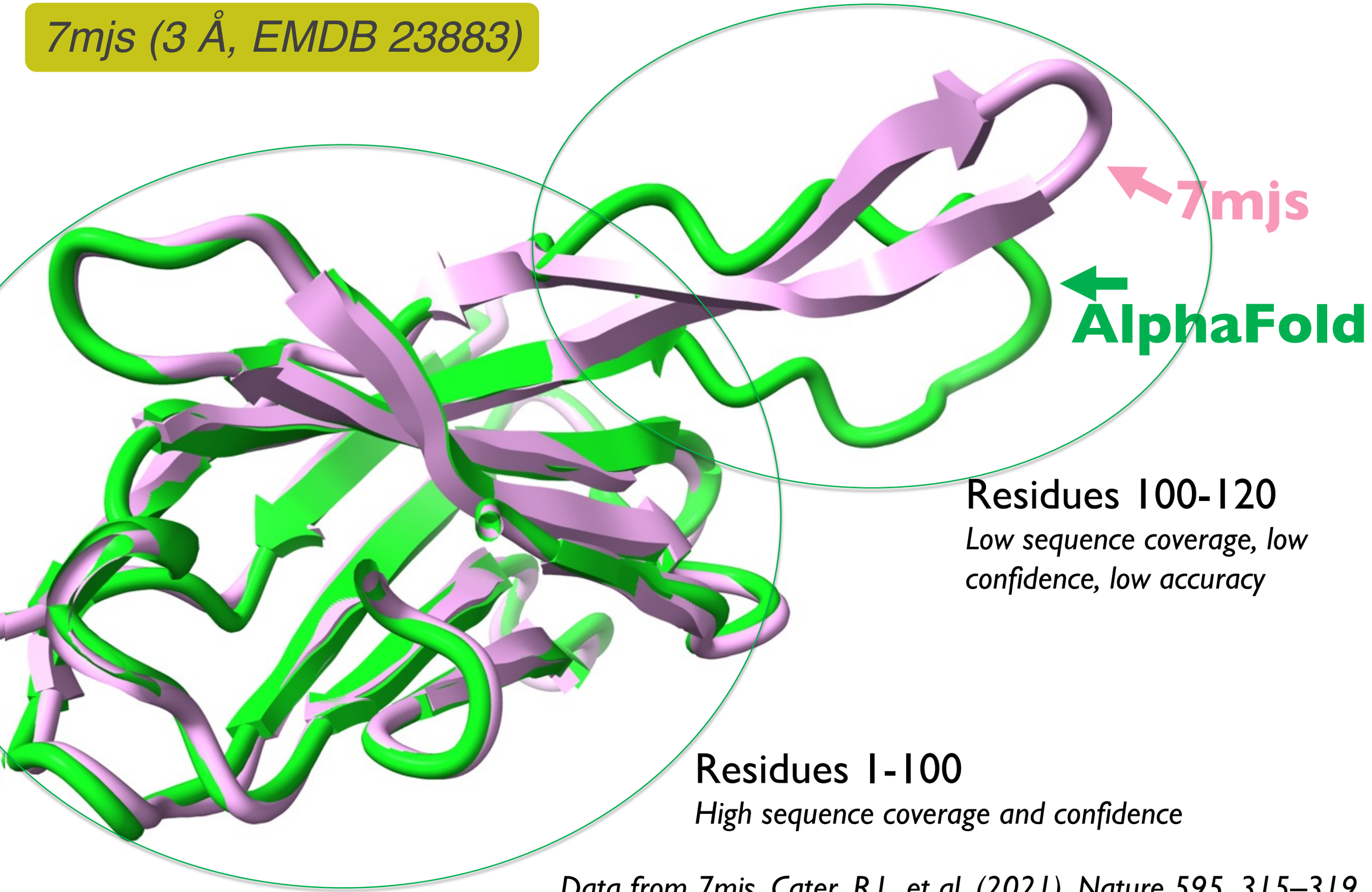




Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Models are accurate where sequence coverage is high

7mjs (3 Å, EMDB 23883)



Residues 100-120

Low sequence coverage, low confidence, low accuracy

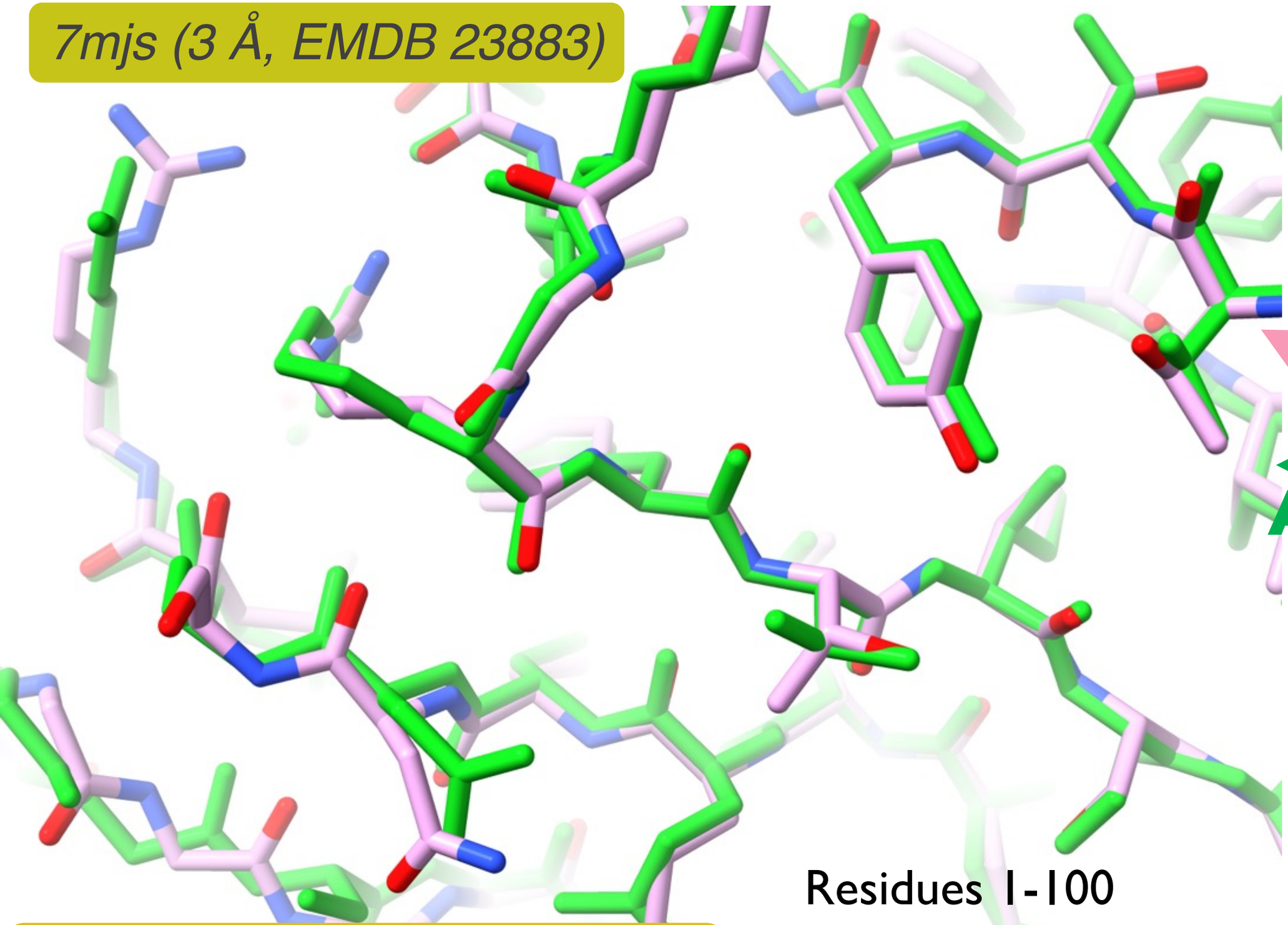
Residues 1-100

High sequence coverage and confidence

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Models are accurate where sequence coverage is high

7mjs (3 Å, EMDB 23883)



Residues 1-100

High sequence coverage and confidence

→High confidence residues can be accurate

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Limitations

- **Only protein** → ***No water, ions, covalent modifications, carbohydrates, ligands, DNA, RNA***

- **Trained on good and poor structures** → ***Parameters may systematically include poor geometry***

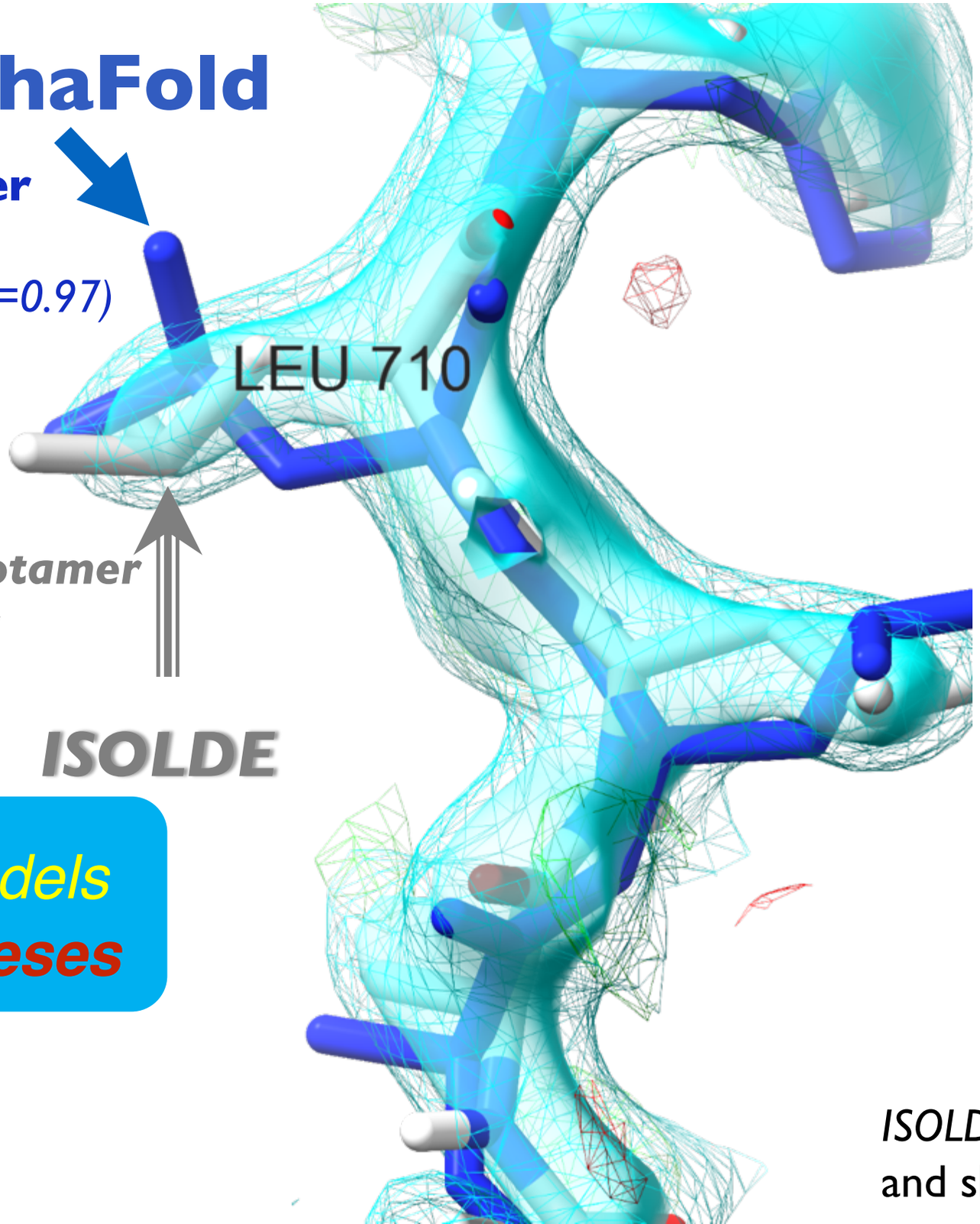
- **Little information about residues that are far apart** → ***Models may have distortions and incorrect domain relationships***

Limitations

Two interpretations of PDB entry 6te3 … which is right?

AlphaFold

Uncommon rotamer
Poor fit to density
High-confidence (plDDT =0.97)



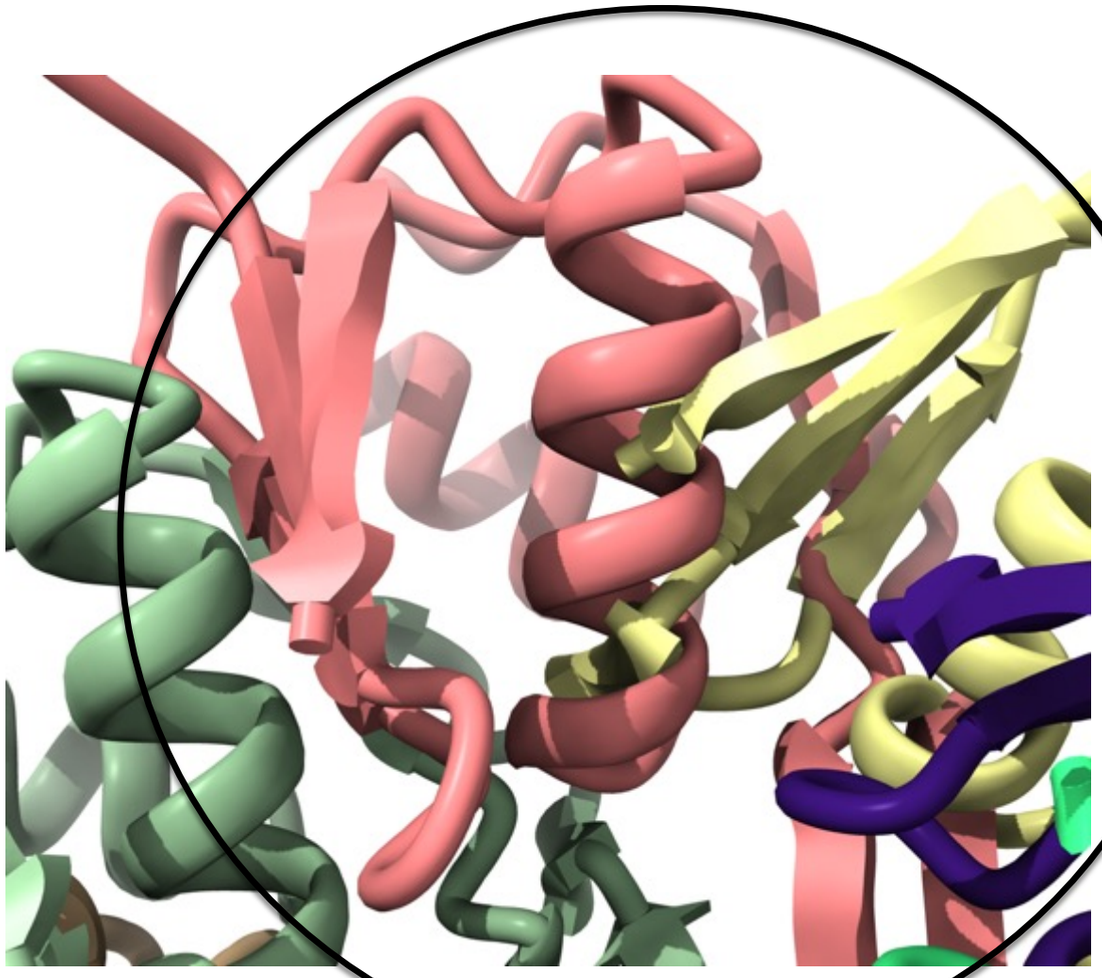
Common rotamer
Fits density

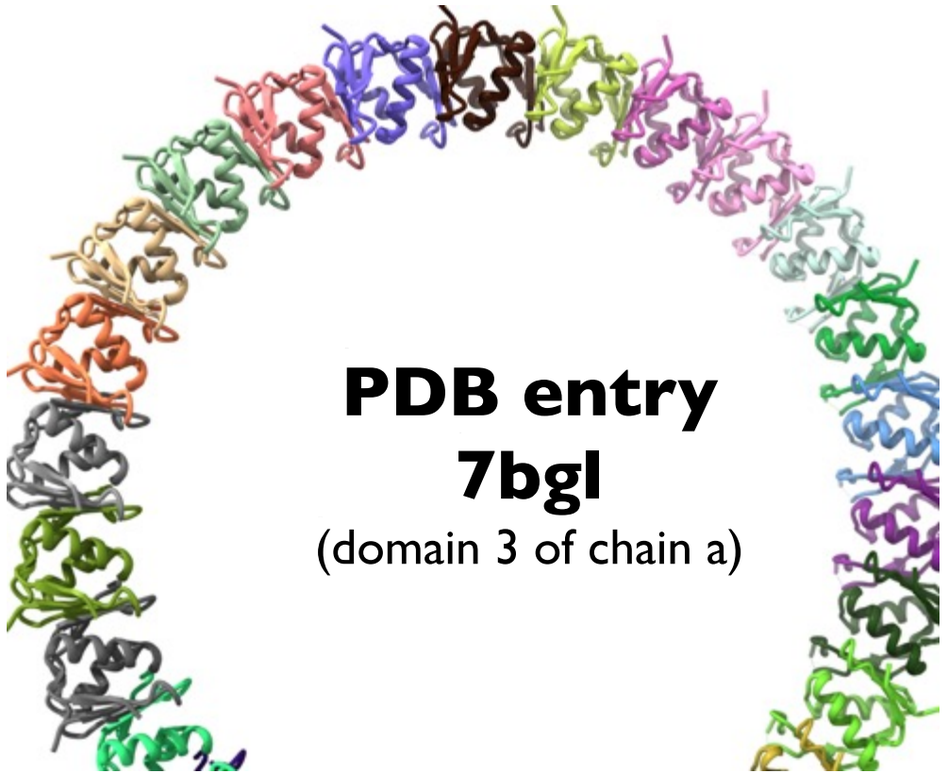
ISOLDE

→ *AlphaFold models are great* ***hypotheses***

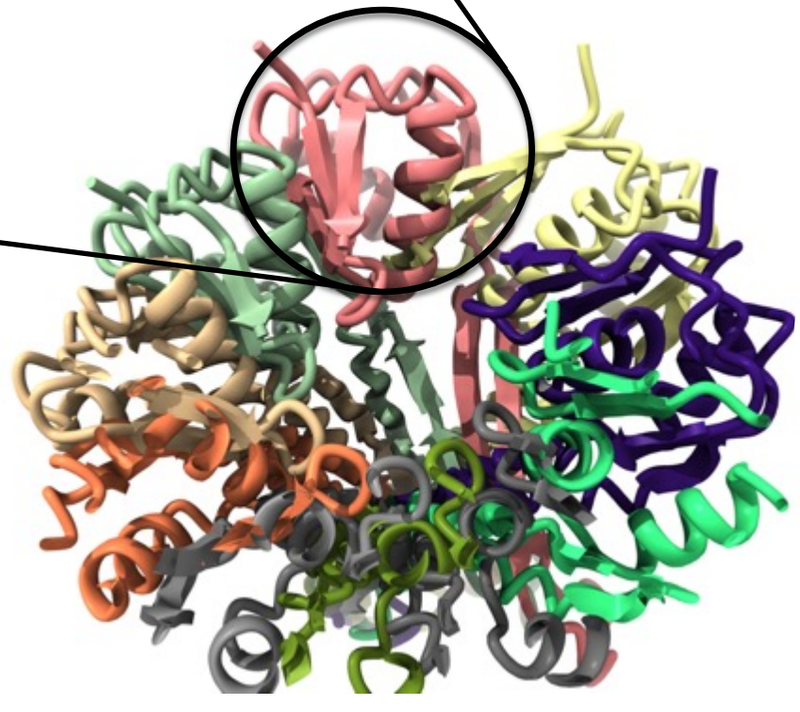
ISOLDE rebuilding of 6te3 and slide by Tristan Croll

Local accuracy better than global accuracy





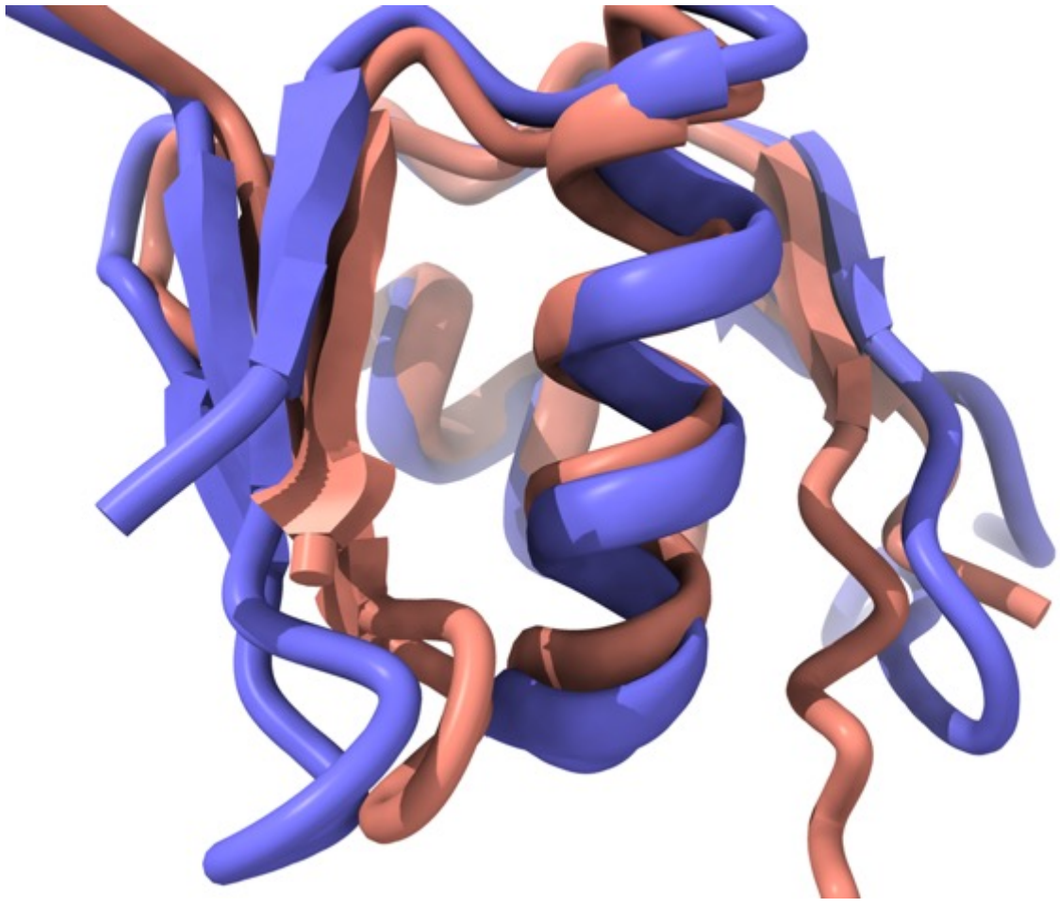
PDB entry
7bgl
(domain 3 of chain a)

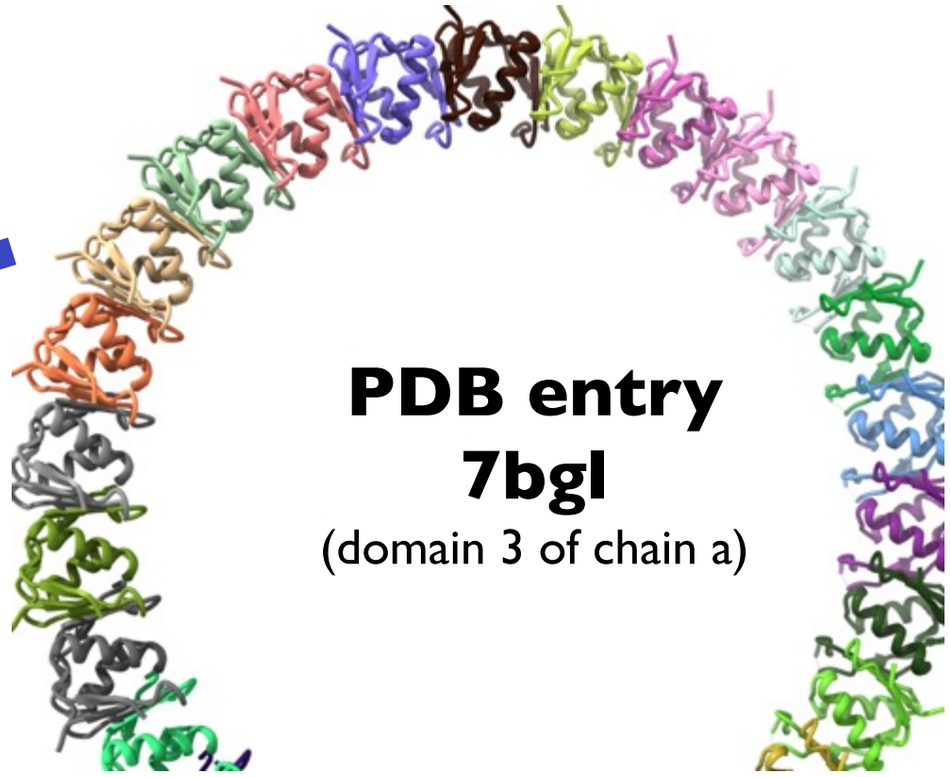


AlphaFold
(multimer prediction)

Data from 7bgl, Johnson, S. et al. (2021).
Nat Microbiol 6, 712–721

Local accuracy better than global accuracy





PDB entry
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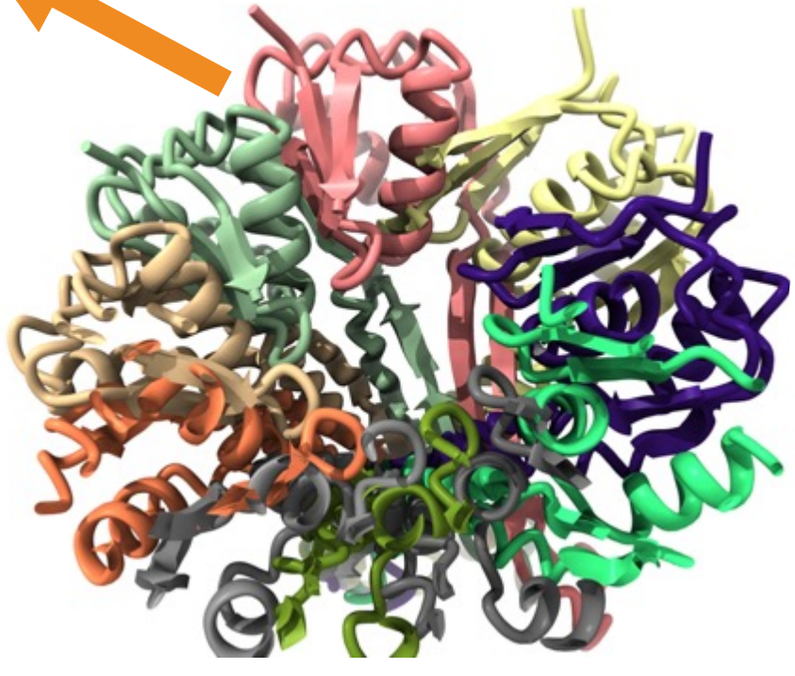
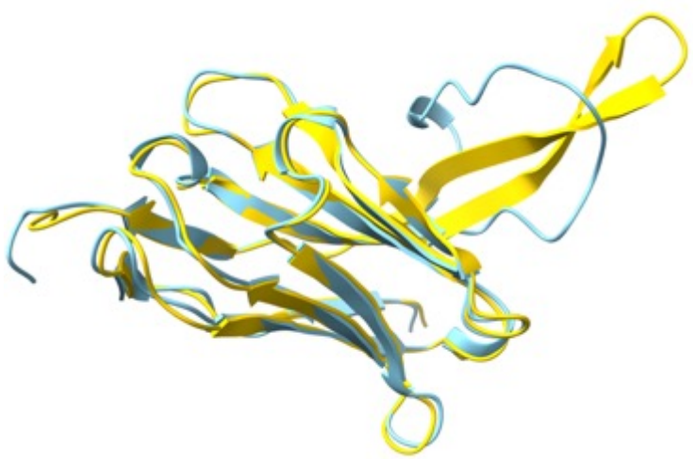


AlphaFold
(multimer prediction)

⟶ AlphaFold models are great ***hypotheses***

What can we expect from AlphaFold models?

- *They are great **hypotheses** for protein structures*
- *Parts of AlphaFold models are accurate*
- *Parts are completely wrong*
- *The confidence measure is helpful but may not fully reflect accuracy*



AlphaFold models …

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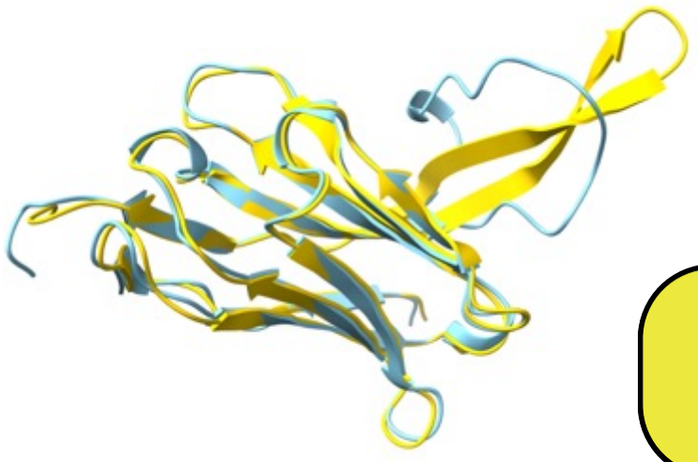


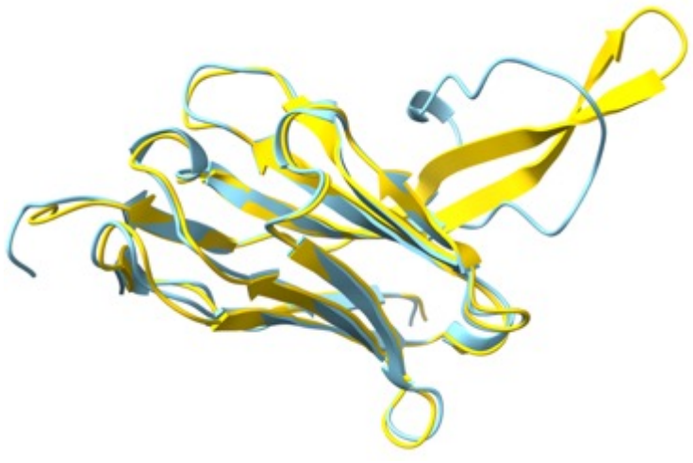
jump-start structure determination by X-ray and CryoEM (and NMR, cryo-ET, neutron…)

can be iteratively improved with a density map

allow a new work-flow for structure determination

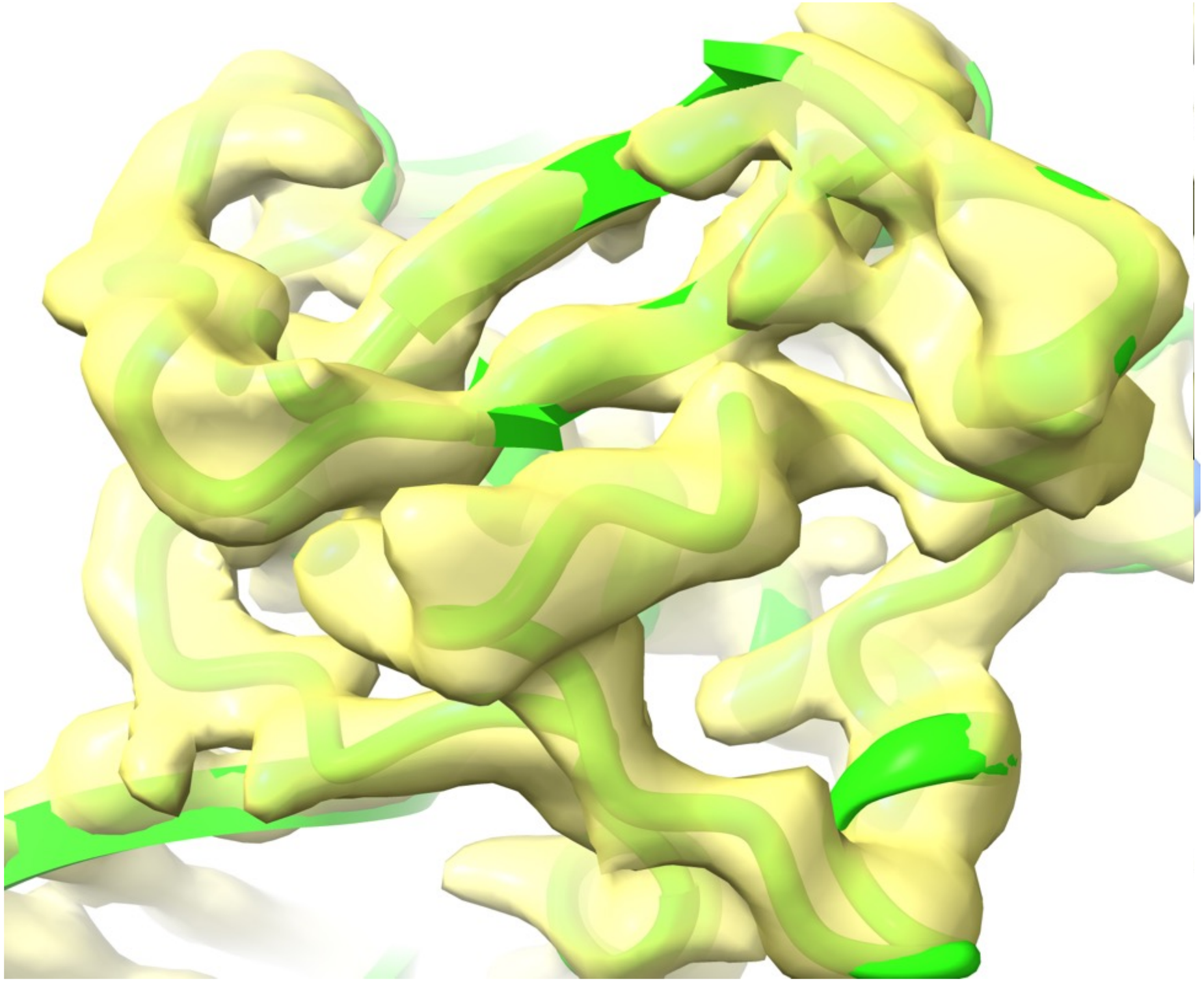
AlphaFold models are great for jump-starting structure determination

- *Confidence measure (plDDT) allows pruning of worst parts of models*
- *High-confidence parts are often accurate*
- *Better than a homology model: no insertions and deletions in the sequence*



Example: Finishing a difficult crystal structure

Repressor – DNA complex, solved with 2.6 Å SeMet SAD data and refined against 3.1 Å native data



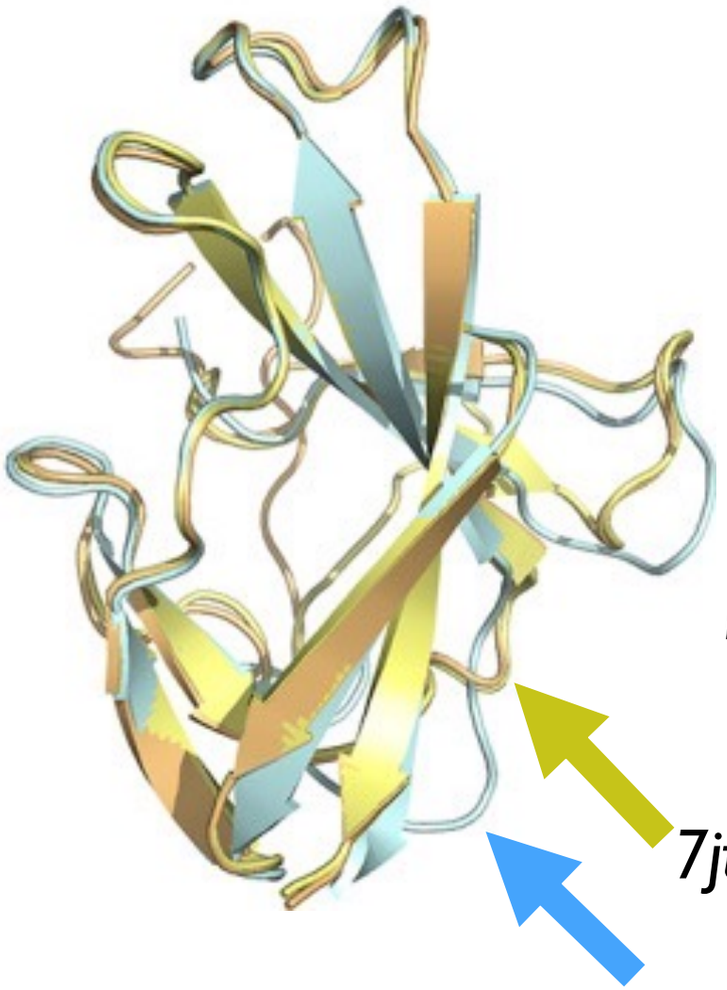
Before AlphaFold, R/Rfree = 0.27/0.29

AlphaFold model:
*A **hypothesis** about this structure*

After AlphaFold, R/Rfree = 0.21/0.24
(it was a good hypothesis)

Jamie Wallen, Western Carolina University

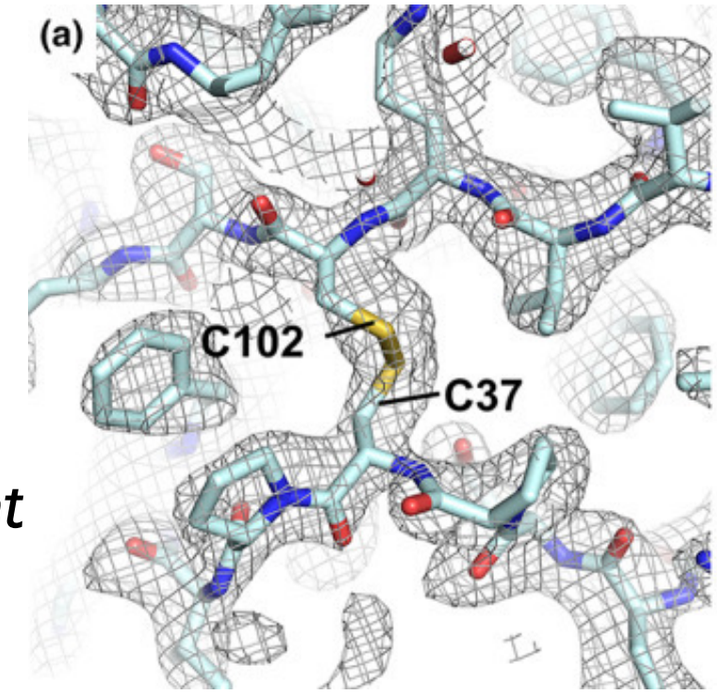
Molecular replacement



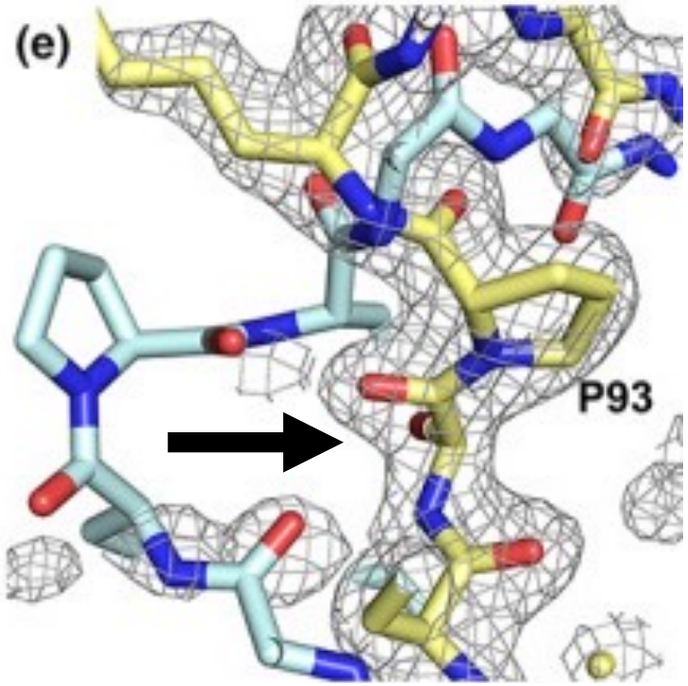
Molecular Replacement (2 Å)

7jtl (2 chains)

AlphaFold



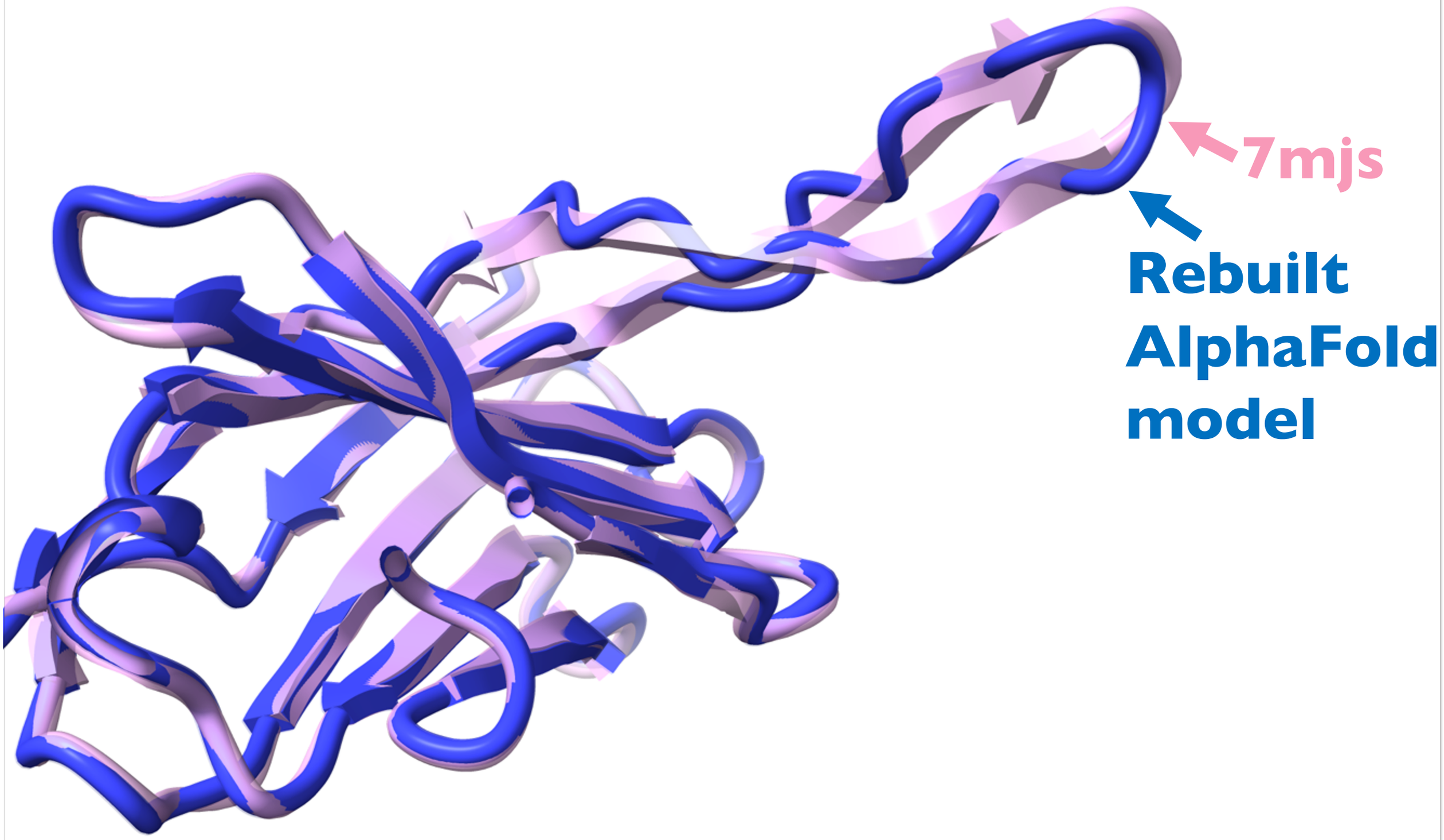
Accurate parts



Parts of main chain required rebuilding

Flower TG, Hurley JH. (2021) Crystallographic molecular replacement using an in silico-generated search model of SARS-CoV-2 ORF8. Protein Science 30:728–734

Cryo-EM (7mjs 3 Å, EMDB 23883)



AlphaFold models …

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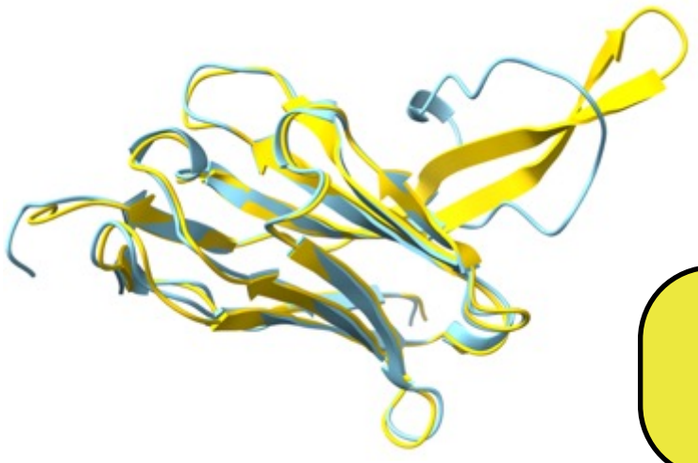

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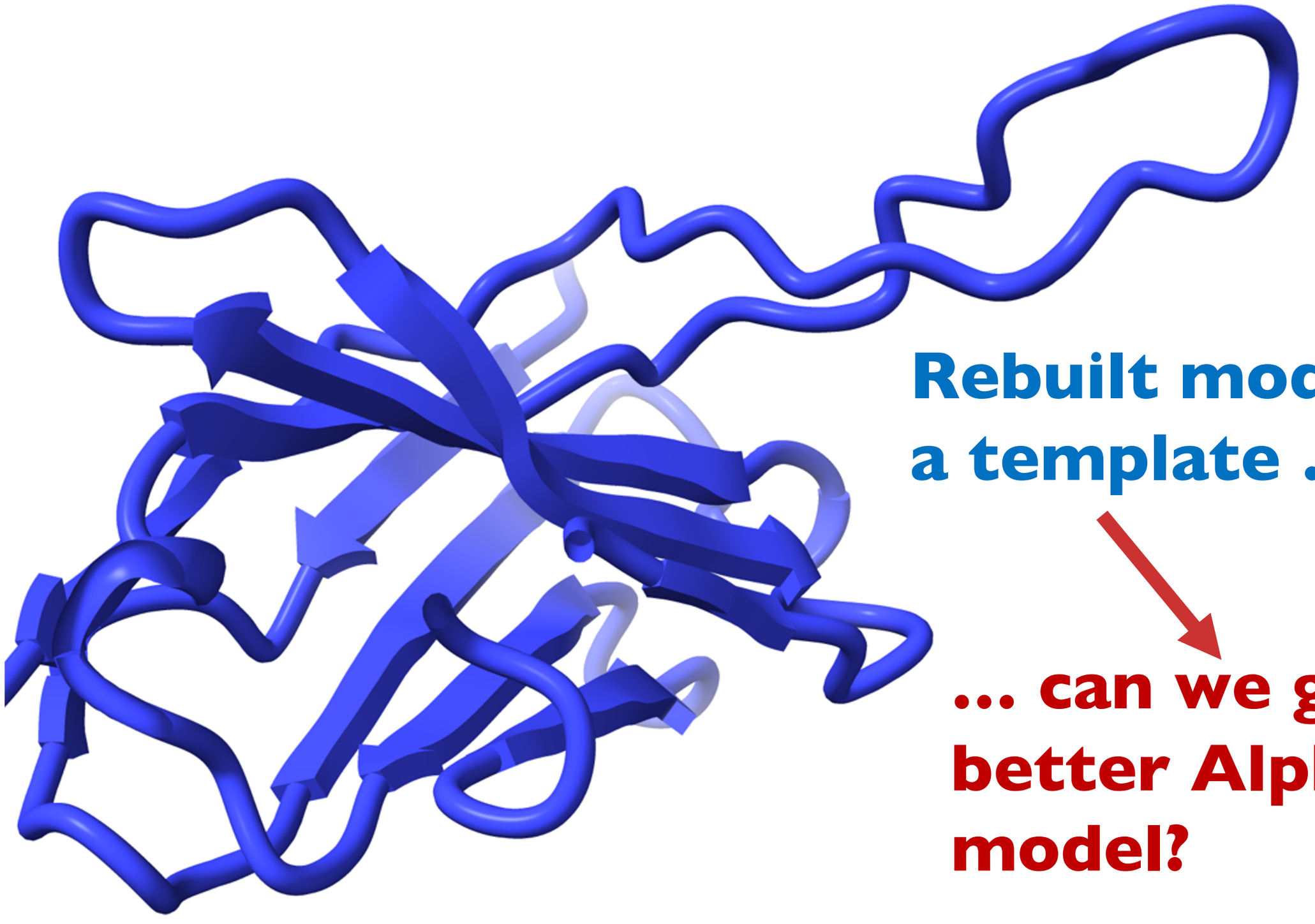
Iterative AlphaFold prediction and rebuilding



Rebuilt model as a template …

… can we get a better AlphaFold model?

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

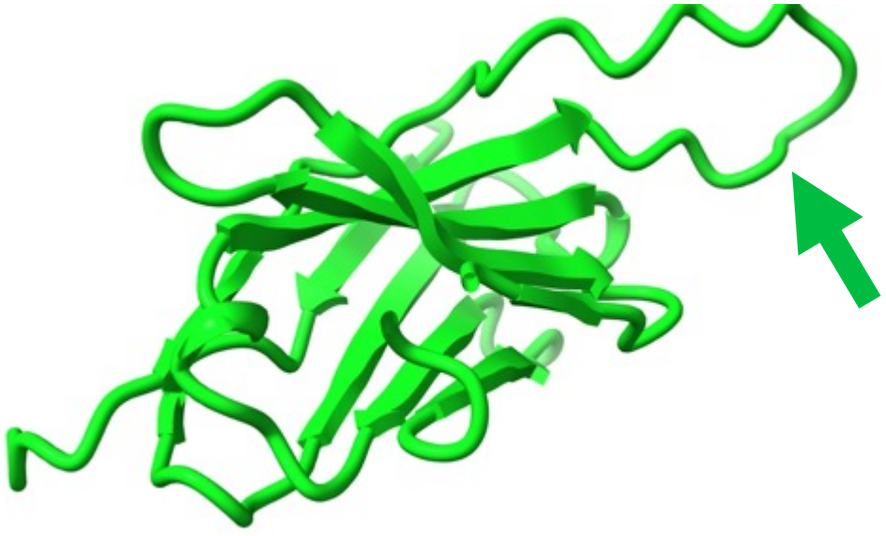
Initial AlphaFold prediction …

- *Sequence*
- *Multiple sequence alignment*

```
EVQLVESGGGLVQPGGSLRLSCAAASGFNIYSSSIHWVRQAPGKGLEWVAYI
.............................F....M.........Q........
..................K.........Y....L........A.........
..............A................................V......
....................A.............................
.............................L....V....E............
.........A.....................................Q.....
```

21 million parameters

3D prediction



→ *The prediction is poor in the loop region*

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

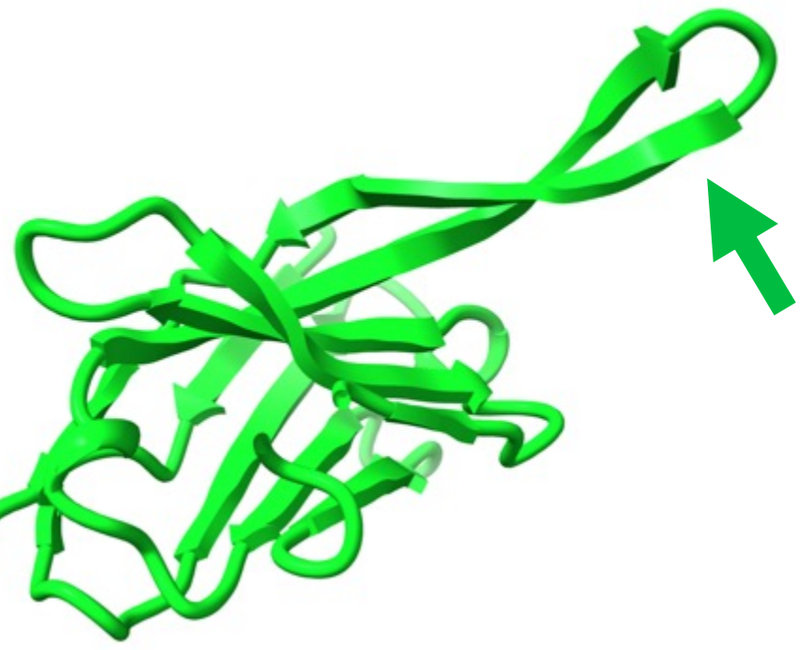
AlphaFold prediction with a template

- *Sequence*
- *Multiple sequence alignment*

```
EVQLVESGGGLVQPGGSLRLSCAAASGFNIYSSSIHWVRQAPGKGLEWVAYI
............................F....M.........Q........
..................K.........Y....L........A.........
..............A................................V......
....................A.............................
............................L....V....E.............
.........A.....................................Q.....
```

21 million parameters

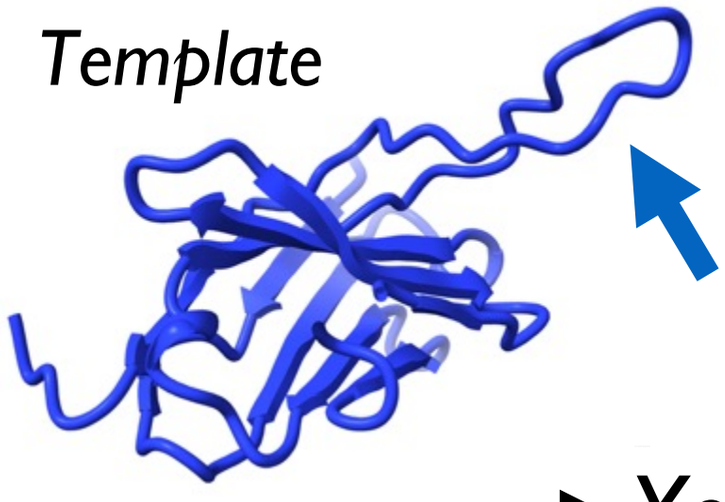
3D prediction



Backbone angles

Focus attention on residues that are close

Template

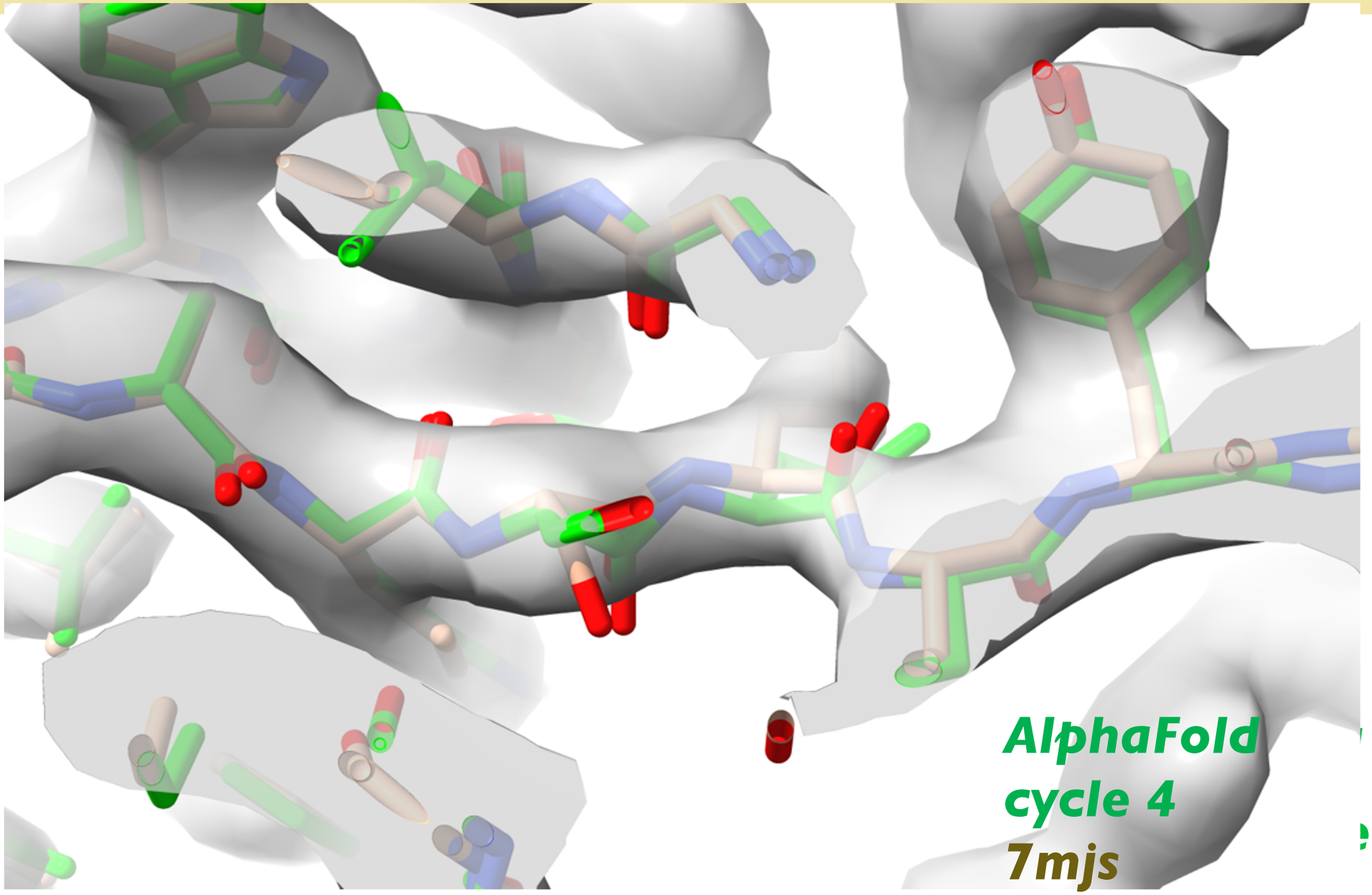


→ *Yes, the template improves prediction*

→ *The new prediction is even better than the template*

Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Iterative AlphaFold prediction and rebuilding



Note this AlphaFold model is superimposed, not refined

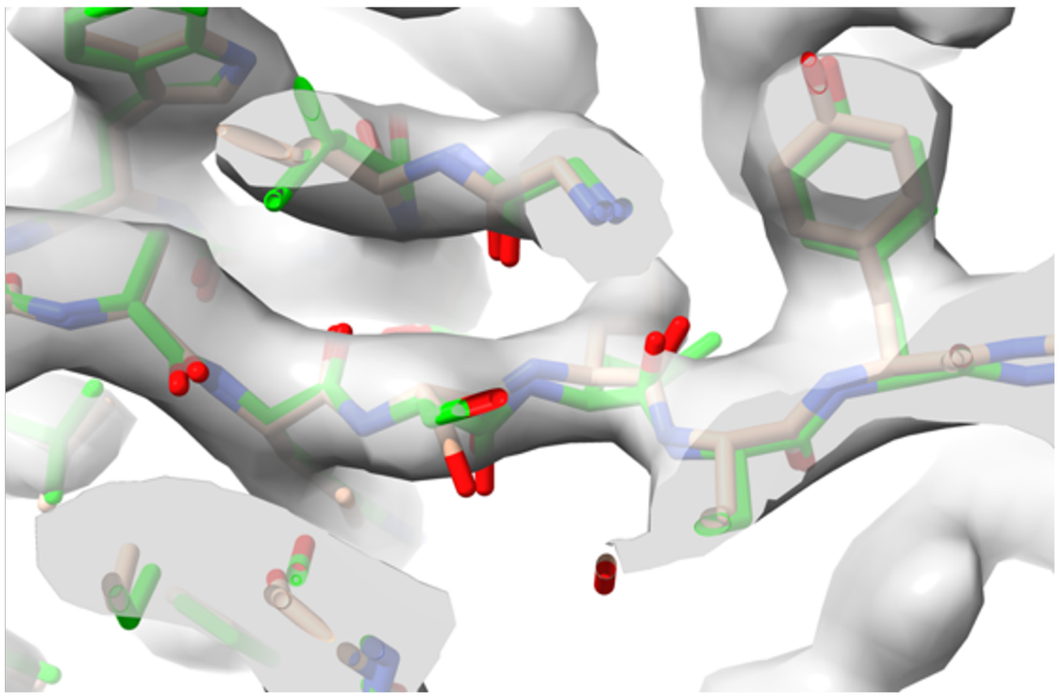
Data from 7mjs, Cater, R.J., et al. (2021). Nature 595, 315–319

Iterative AlphaFold prediction and rebuilding

→ *Rebuild AlphaFold model with density map*

→ *Rebuilt model improves next AlphaFold prediction*

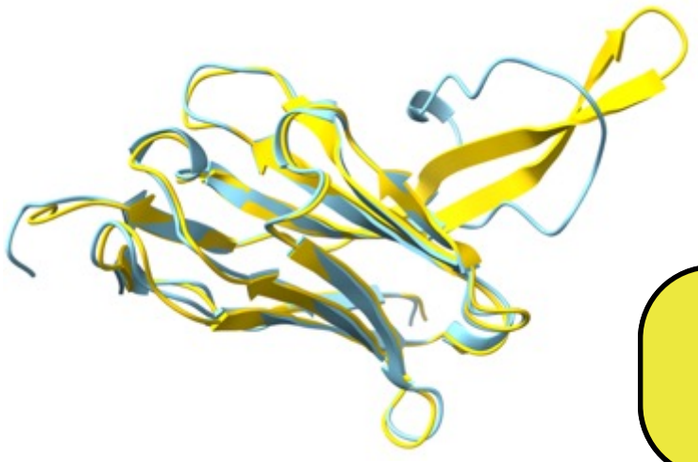
→ *Iterate to improve model*



Terwilliger et al. (2022). Improving AlphaFold modeling using implicit information from experimental density maps. BioRxiv 2022.01.07.475350

AlphaFold models …

*are great **hypotheses** for protein structures*



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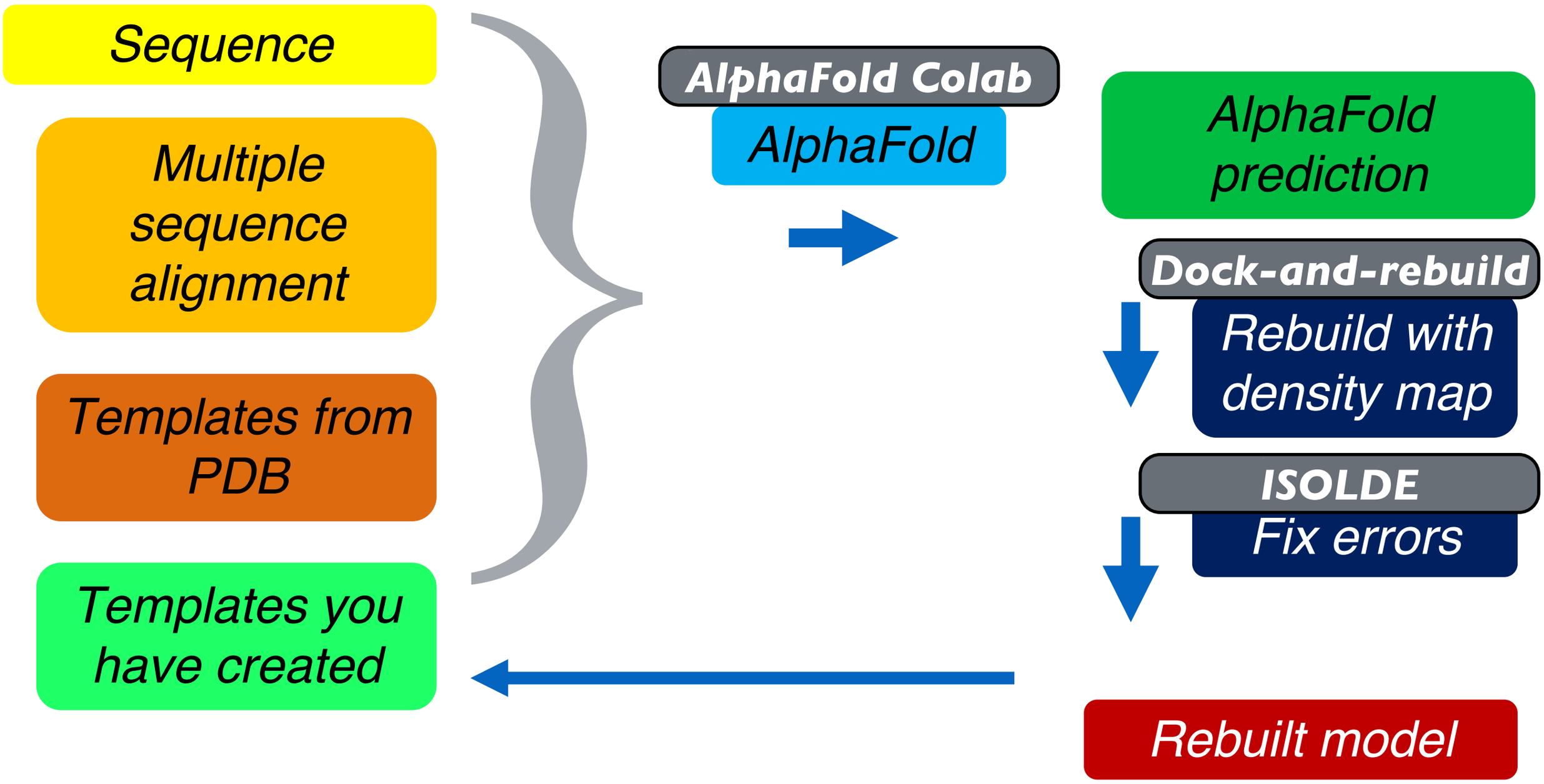
Workflow for getting the best AlphaFold model



Workflow for cryo-EM

Density map

AlphaFold prediction for each chain

Dock-and-rebuild

Rebuild AlphaFold predictions

Model for each chain with full exact sequence

ISOLDE, real-space refine

Fix errors, refine

Refined model for each chain

AlphaFold Colab

AlphaFold with refined model for each chain

Workflow for crystallography

X-ray data

AlphaFold prediction for each chain

Phaser

Molecular replacement

MR model

AutoBuild

Automated rebuilding

Autobuilt model

Density map

AlphaFold prediction

Dock-and-rebuild

Rebuild AlphaFold predictions

Rebuilt model for each chain with full exact sequence

ISOLDE, Real-space refine

Fix errors, refine

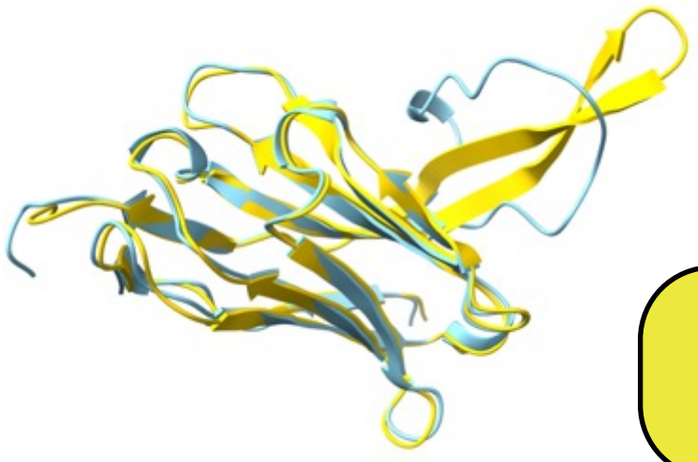
Refined model for each chain

AlphaFold Colab

AlphaFold with supplied template on each chain

AlphaFold models …

*are great **hypotheses** for protein structures*



jump-start structure determination by X-ray and CryoEM

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The Phenix Project

Lawrence Berkeley Laboratory

Paul Adams, Pavel Afonine, Dorothee Liebschner, Nigel Moriarty, Billy Poon, Christopher Schlicksup, Oleg Sobolev

New Mexico Consortium
Los Alamos National Laboratory

Tom Terwilliger, Li-Wei Hung

University of Cambridge

Randy Read, Airlie McCoy, Tristan Croll, Claudia Millán Nebot, Rob Oeffner, Duncan Stockwell

Baylor College of Medicine

Matt Baker, Corey Hyrc

Duke University

Jane & David Richardson, Chris Williams, Vincent Chen

An NIH/NIGMS funded Program Project

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