Protein structure prediction with in-cell photo-crosslinking mass spectrometry and deep learning

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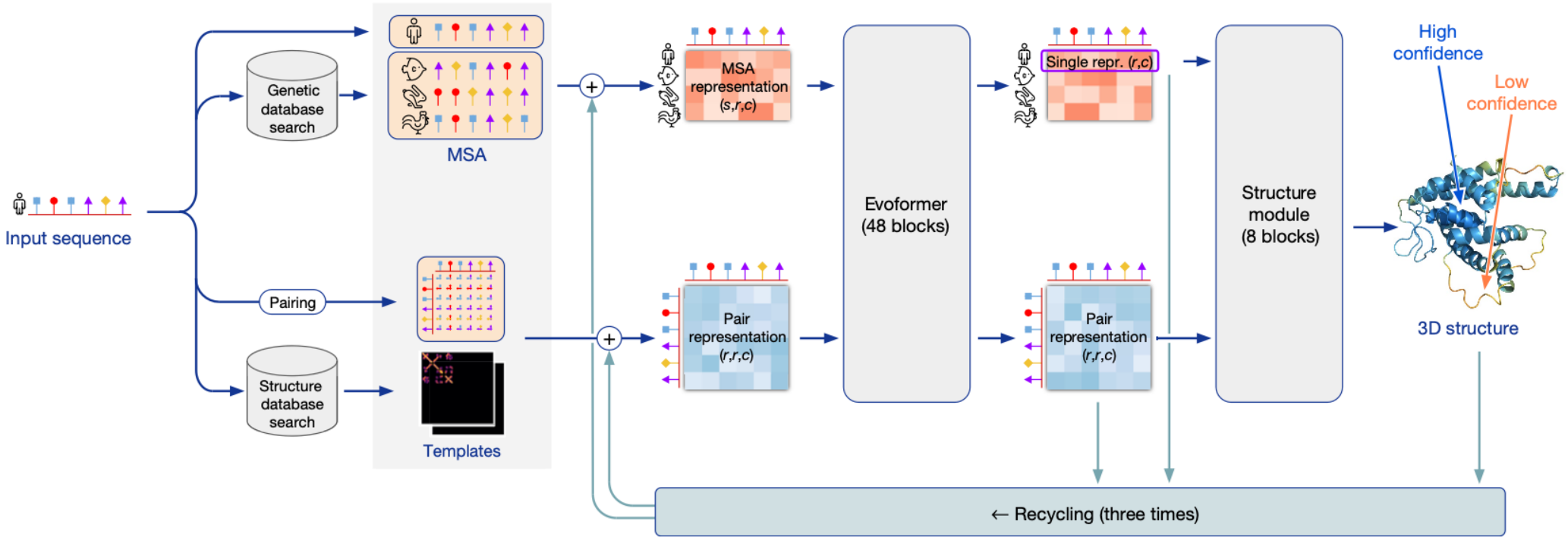
Jun 29, 2023

Presentation Line

- Background & Motivation
- AlphaLink architecture
- AlphaLink methods
- AlphaLink training data
- Main experimental results
- Discussions

Recap AlphaFold2



- Multiple Sequence Alignment (MSA) + Templates of Similar Protein Structures
- Evoformer (distance space)
- Structure Module (3D space)

Background

- AlphaFold2 predicts static models based on static input data, which is trained on two information sources, the protein structures in the Protein Data Bank (PDB) and multiple sequence alignments (MSAs).
- Challenged by targets with **insufficient evolutionary information**
  - viral proteins, proteins from understudied organisms, antibodies, and synthetic proteins have misleading information
  - structures underlying the model poorly reflect structural flexibility, multiple conformations, and dynamic interactions

Motivation

- **Structural restraints** observed on proteins in solution steer the prediction towards structural states occurring in situ under specific conditions.
- **Crosslinking mass spectrometry (MS): distance restraints**
  - photo amino acids (photo-AA) in prokaryotic and eukaryotic cells
  - photo-AA crosslinks: tight distance restraints that align well with co-evolutionary contacts.
  - Photo-leucine (photo-L): mapping conformations & binders.

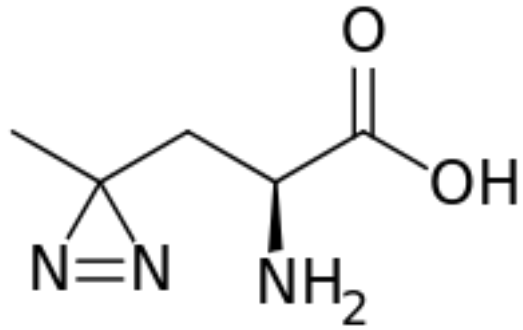
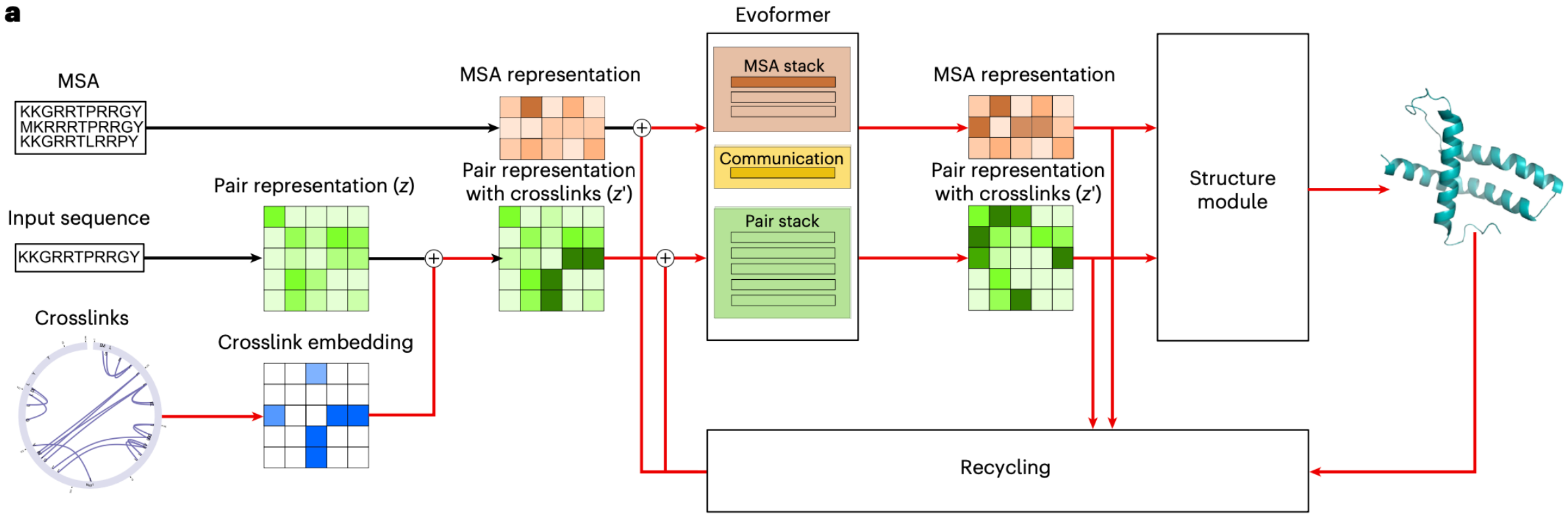


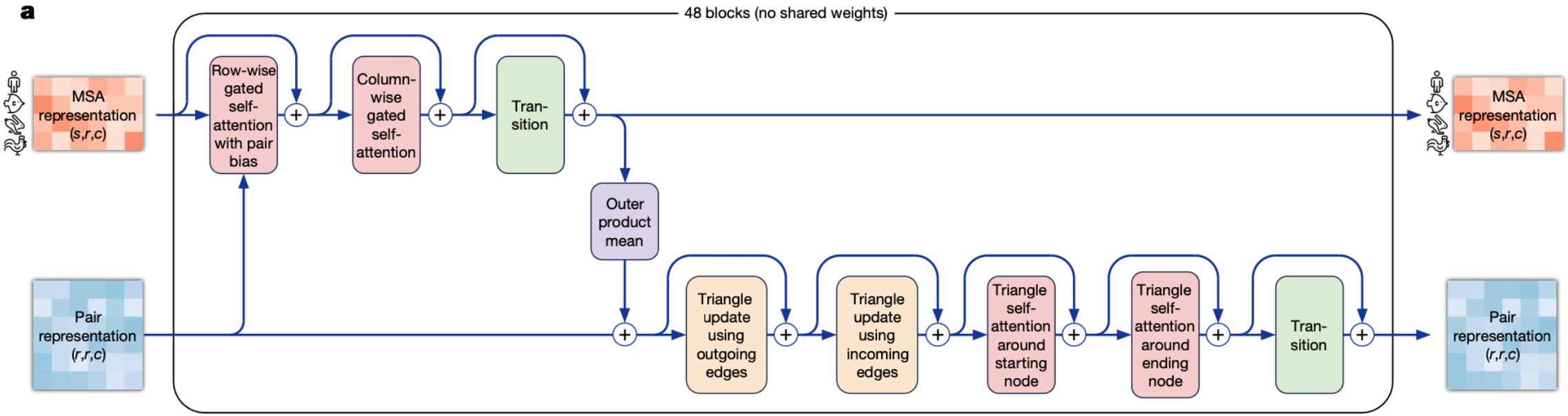
Photo-leucine, source from wikipedia

AlphaLink: integrating crosslinks into AlphaFold2 via OpenFold



- Crosslinks (blue) are embedded and added onto the pair representation (green)

Recall Evoformer

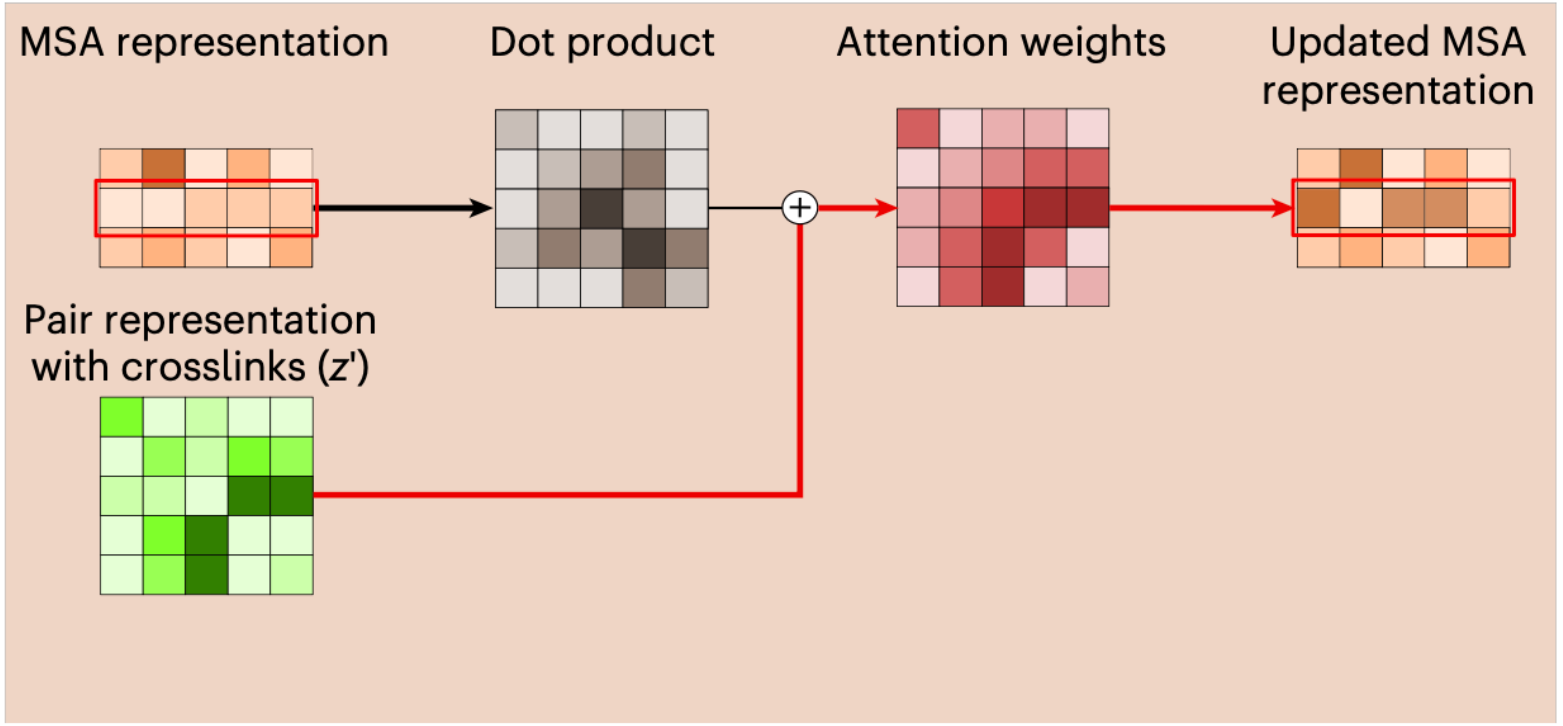


How to encode crosslink

- Two representations to encode crosslinking information
  - **soft labels**: each contact is weighted by the link-level false discovery rate (FDR) of the dataset (1-FDR)
  - **distance distributions** (distograms): uniformly distributed distograms for the given cutoff
- Use the same binning for the first 64 bins in Evoformer and extend the distogram further to 128 bins, spanning from 2.3125 Å to 42 Å.

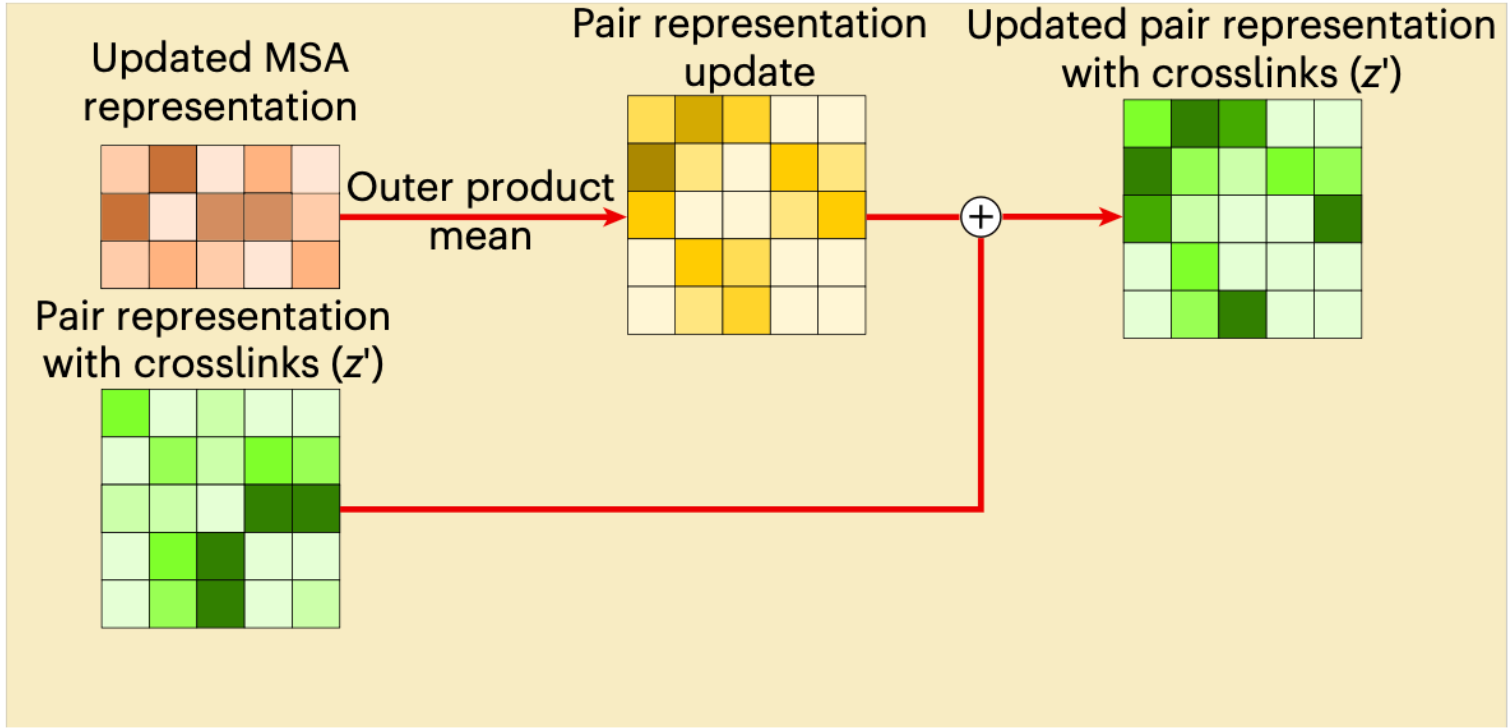
Crosslink as a bias in MSA transformer

- Crosslinks influence the retrieval of co-evolutionary information.

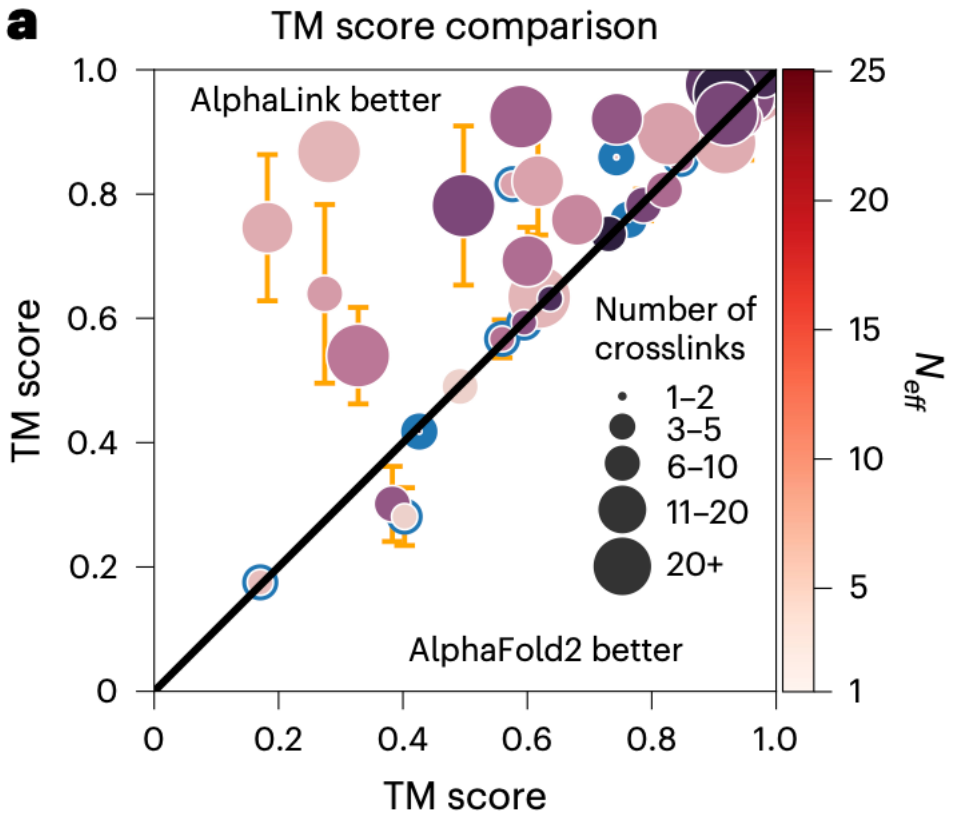


Crosslink as a bias in pair representations

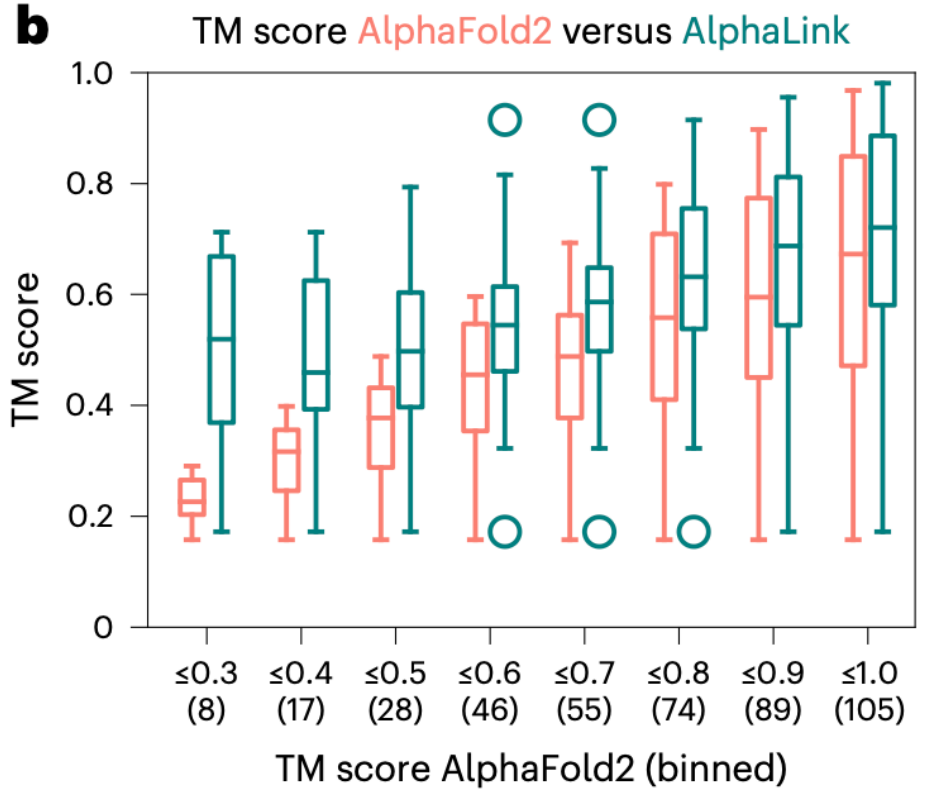
- The pair representation is updated with information from the MSAs that have been biased with the crosslinks.



Integrating photo-AA crosslinks enables noise-tolerant prediction of challenging targets



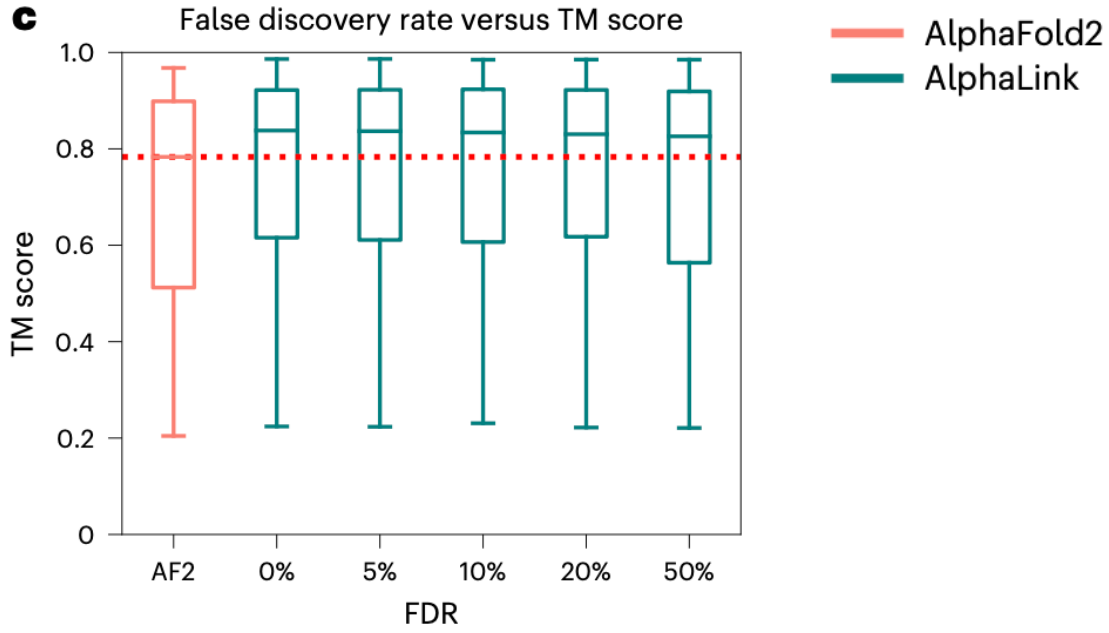
- TM score comparison on 49 CAMEO targets. TM score improves on average by 19.2%.



- TM score performance on 60 CASP14 and 45 CAMEO targets (15.2% improvement)

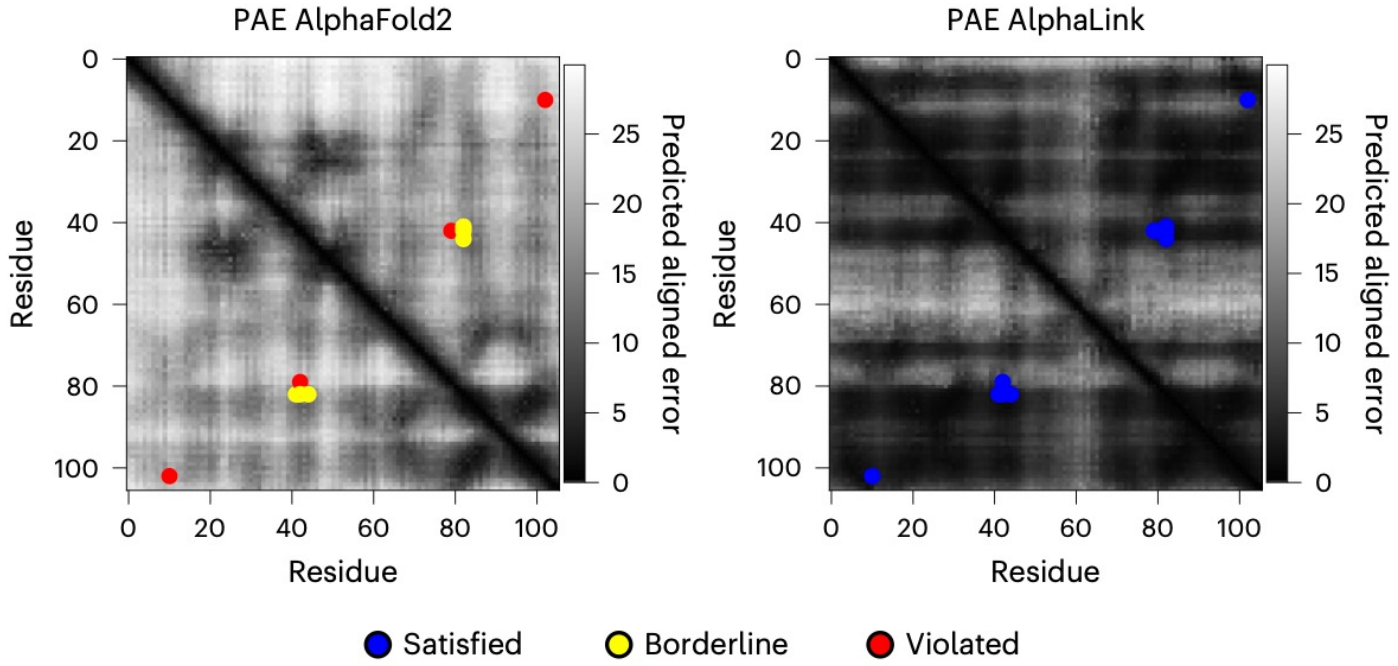
Performance on different noise levels

- Performance on 60 CASP14 targets with different noise levels (FDR 0%, 5%, 10%, 20% and 50%)
- AlphaLink improves in the median for all noise levels. Performance shows robust noise rejection.

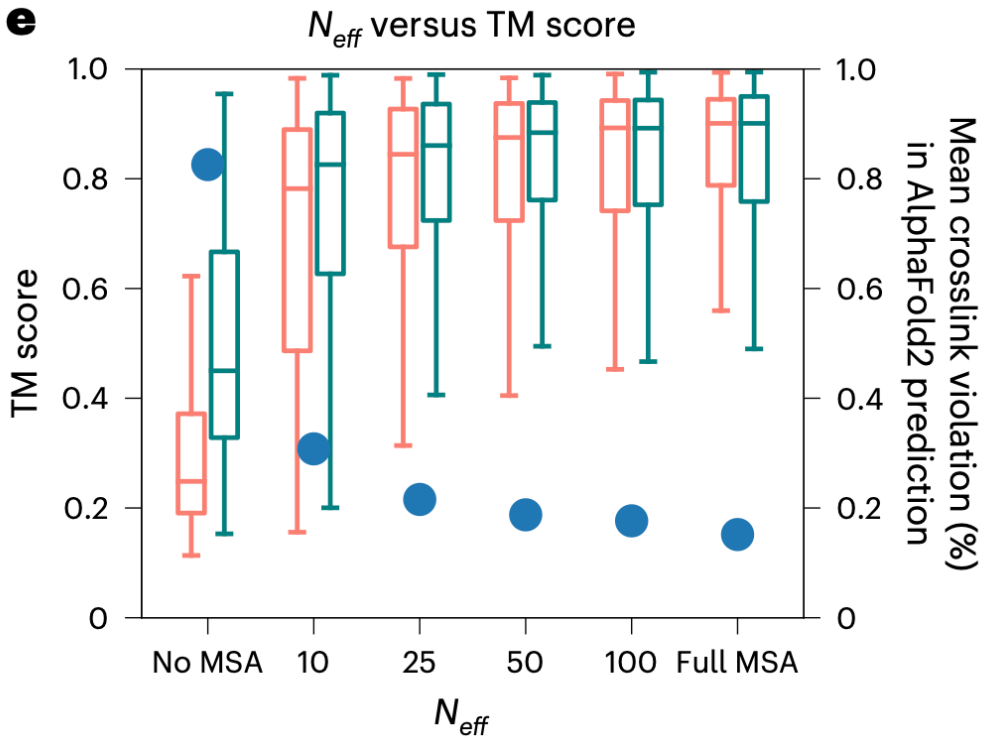


Sparse restraints decrease uncertainty across the whole protein

- Predicted aligned error of AlphaFold2 (left) and AlphaLink (right) on T1064.
- Satisfied crosslinks <10 Å Cα–Cα, borderline crosslinks (10–15 Å Cα–Cα), violated crosslinks >15 Å Cα–Cα



Crosslink diminishes with increasing MSA size



- Performance on 60 CASP14 targets as a function of MSA size (N = 100, 10 MSAs and 10 crosslink sets)
- Blue dots represent the mean percentage of nonsatisfied crosslinks (>10 Å Cα–Cα) in the AlphaFold2 prediction.

Performance without MSAs

- Performance without MSAs on 60 CASP14 and 45 CAMEO targets.
- AlphaLink predicts the correct fold (TM score >0.5) for 43/105 (13/105 for AlphaFold2).
- Error bars represent the 95% confidence interval (N = 10).

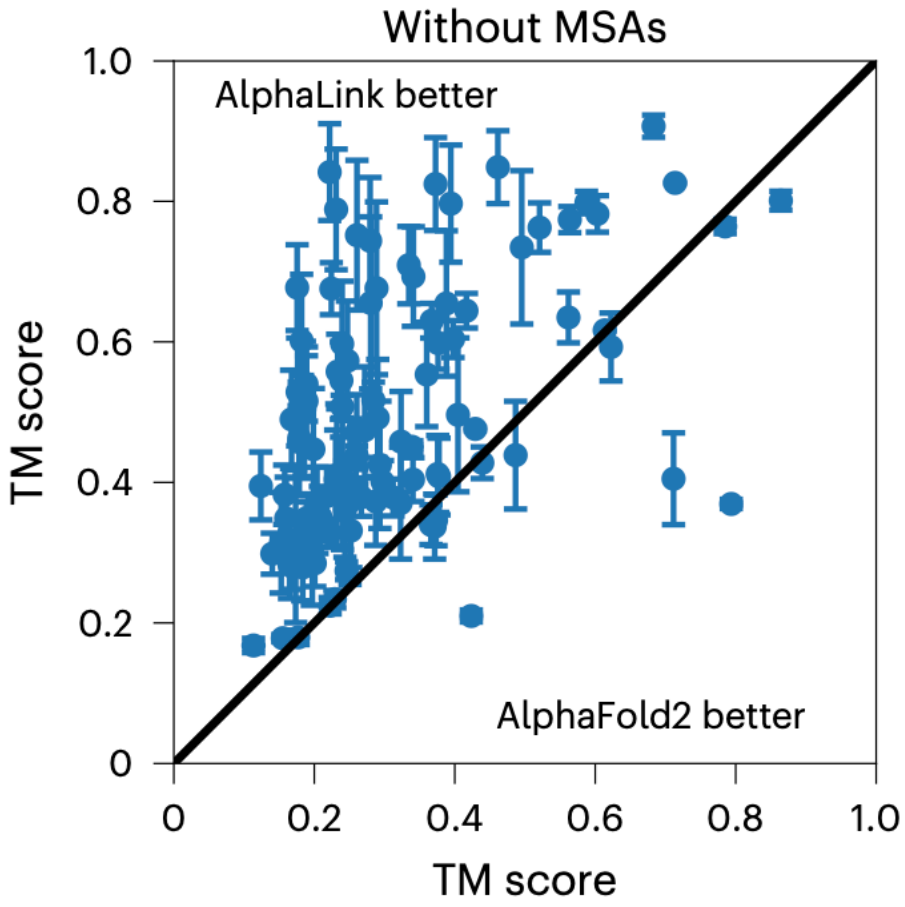
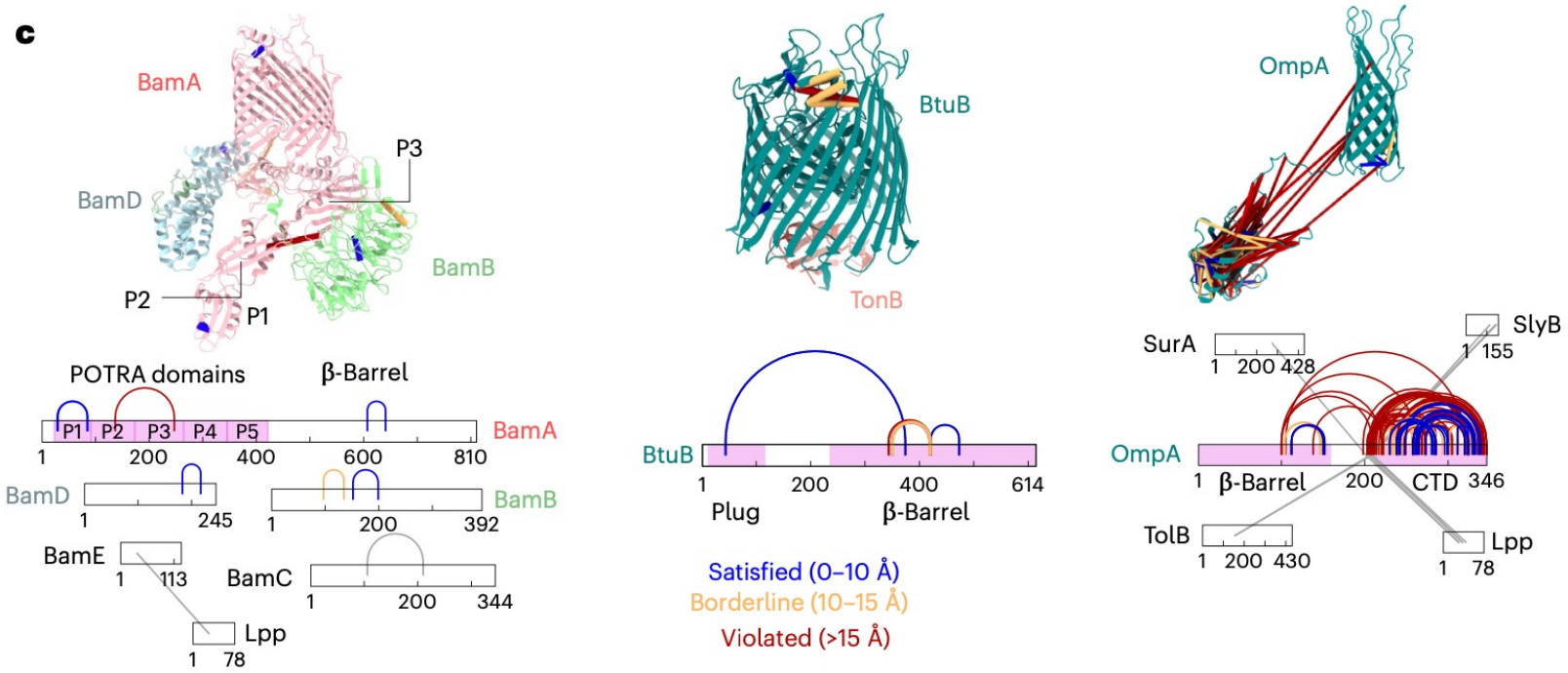


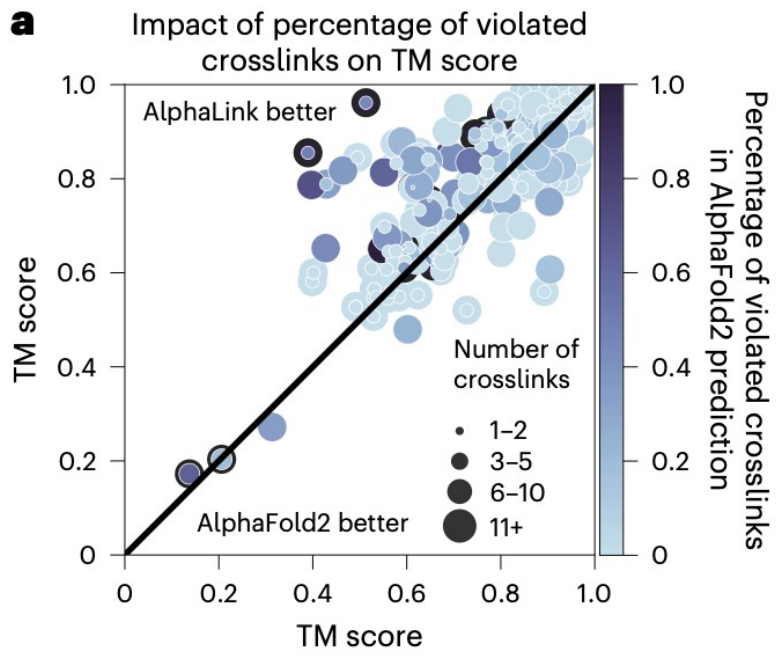
Photo-Leucine as an in situ structural probe

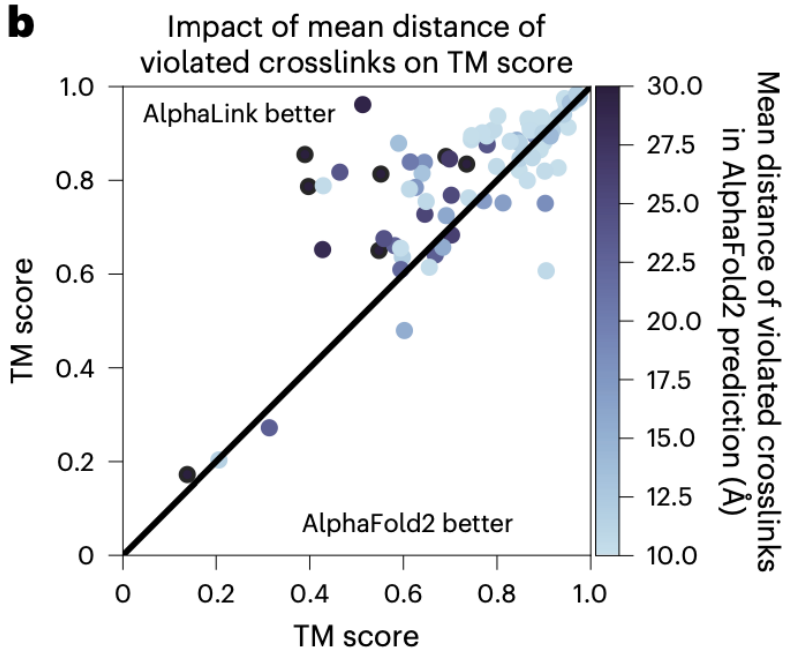
- large-scale experimental photo-AA dataset with in situ structural restraints on the *Escherichia coli* membrane fraction
- 615 residue pairs involving 112 proteins at 5% link-level FDR
- Photo-Leucine provides in situ conformation of multiprotein complexes



Structure prediction with Photo-Leucine data

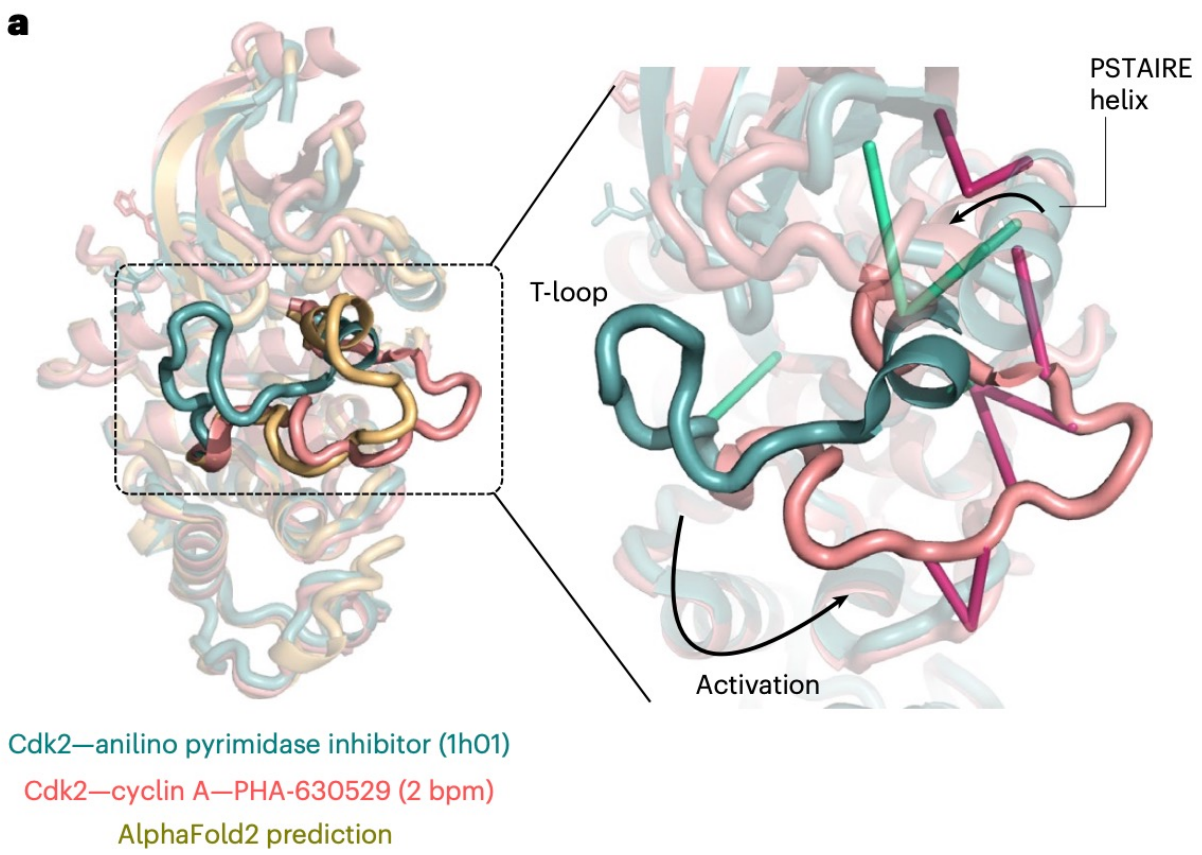
- 31 targets with high-resolution structures
- **a**: performance improvement is bigger for targets with a higher percentage of nonsatisfied crosslinks in the base prediction
- **b**: predictions that improved the most have unsatisfied crosslinks with large distances





Probing conformational dynamics in situ

- a proof-of-concept experiment on the human cyclin-dependent protein kinase Cdk2, a drug target in cancer therapy.
- Activation of Cdk2 in the S phase proceeds via a conformational change in the T-loop (residues 145–165) and the PSTAIRE helix (residues 45–55) triggered by binding of cyclin A.

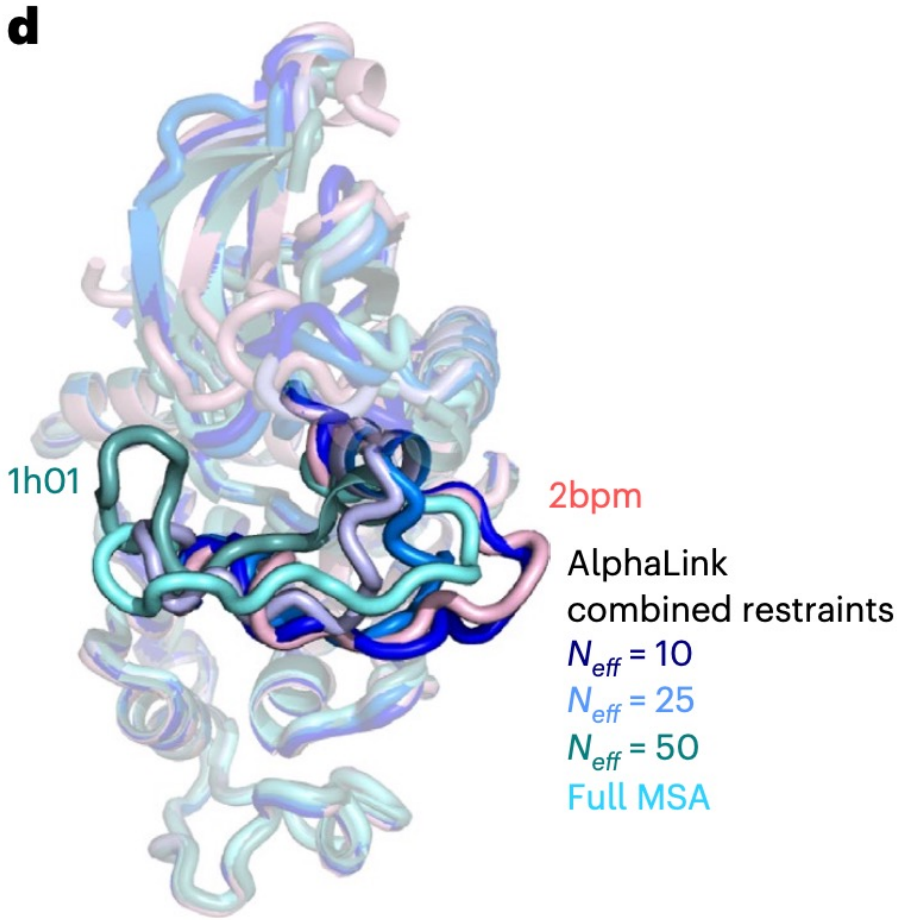


Comparison on active/inactive conformation

- photo-crosslinking: protein was acquired in either its inhibited or in its cyclin A-bound states.
- Cdk2 structure using AlphaLink with these restraints, showing that the loop structure is driven towards the appropriate conformation.
- all AlphaFold2 predictions converge to the cyclin A-bound state, failing to predict the inactive conformation.

Outcome with a combined set of restraints

- At low N_{eff} (*number of effective sequences*) values, the crosslinks drive the prediction towards the cyclin E-bound state.
- As the MSA information increases, the prediction is steered more towards the inhibited state and closer to the AlphaFold2 prediction.
- Crosslinking is weighted against the MSA depending on the information content and size of both strands of information.



Conclusion

- AlphaLink integrates experimental data from photo-AA crosslinking directly into the AlphaFold2 architecture to merge co-evolutionary relationships and crosslinking data in distance space, exploiting the complementary nature of the data.
- AlphaLink can leverage noisy experimental contacts to improve predictions.
- AlphaLink performs a large-scale crosslinking MS study with photo-Leucine, identifying 615 in situ residue-residue contacts in *Escherichia coli* membrane fractions.
- Even sparse crosslinking MS data can anchor predictions to particular conformational states, opening up the possibility of hybrid experimental/deep learning approaches.

Questions