



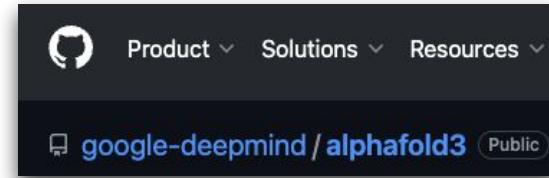
Accelerating biological research using AlphaFold 3

Oleg Kovalevskiy
Google DeepMind

DLS-CCP4 Workshop
2025

This talk

- AlphaFold 3 publication and AlphaFold Server released in May 2024
- Source code released in November 2024



- What do you **need to know** about AlphaFold 3?
- What are the **interesting features** of the AlphaFold 3 technology?
- What practical **applications** have we seen?
- What are the **keys for successful** AlphaFold 3 use?

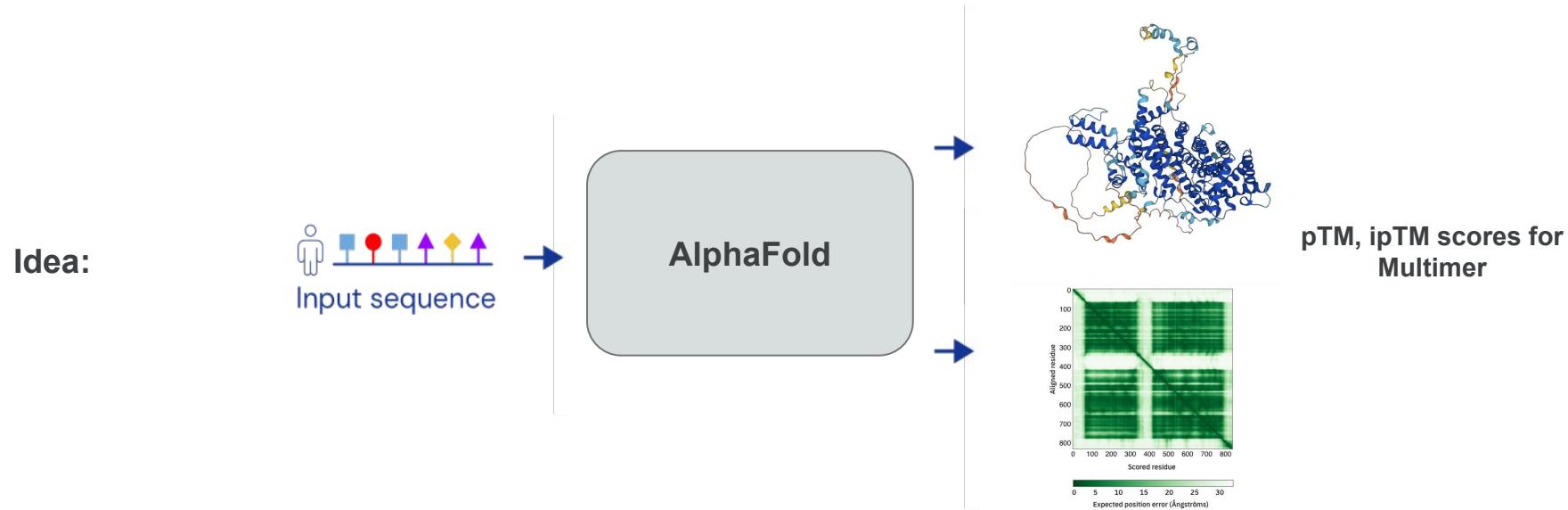
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AlphaFold 3: Basics

What is AlphaFold?

AlphaFold is a Deep Learning system trained to compute protein structures

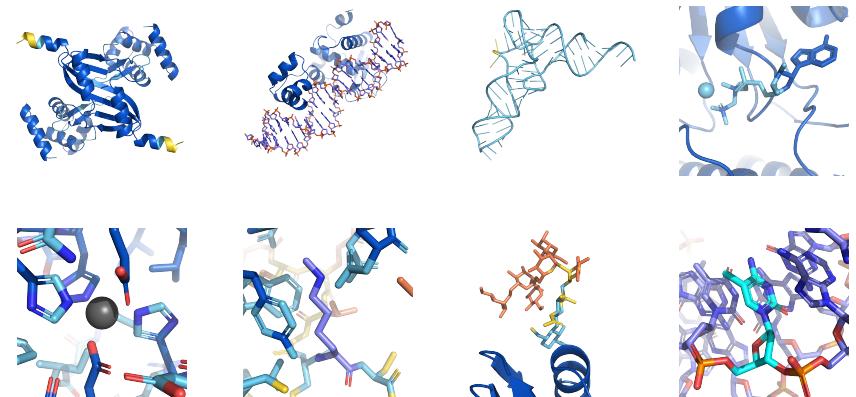
Enormous end-to-end differentiable function with lots of parameters, optimised to return the structure of a protein given its sequence (actually, multiple sequence alignment)



AlphaFold 3 (AF3), Google DeepMind and Isomorphic Labs

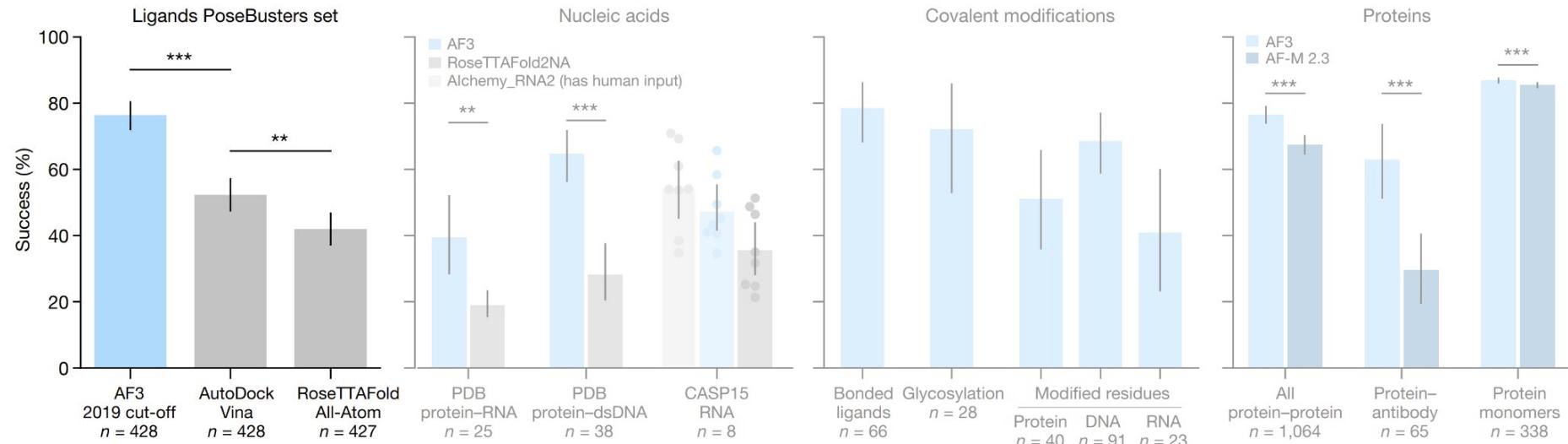
- ✓ Proteins
- ✓ RNA
- ✓ DNA
- ✓ Small molecules
- ✓ Ions
- ✓ Modified residues
- ✓ Glycosylation
- ✓ ...
- ✗ Water
- ✗ Hydrogen

Whole PDB as the goal



Accuracy across molecular types - Ligands

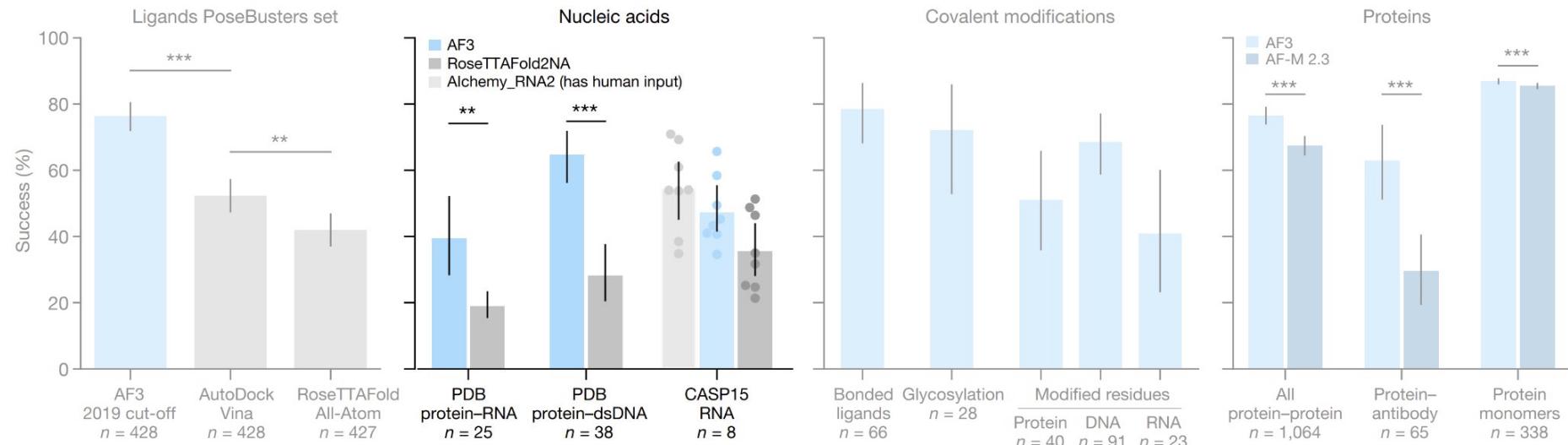
AF3 is light blue throughout



Classical docking tools and older ML models struggle on
Posebusters benchmark for ligands
AF3 excels, with implications for drug discovery

Accuracy across molecular types - Nucleic Acids

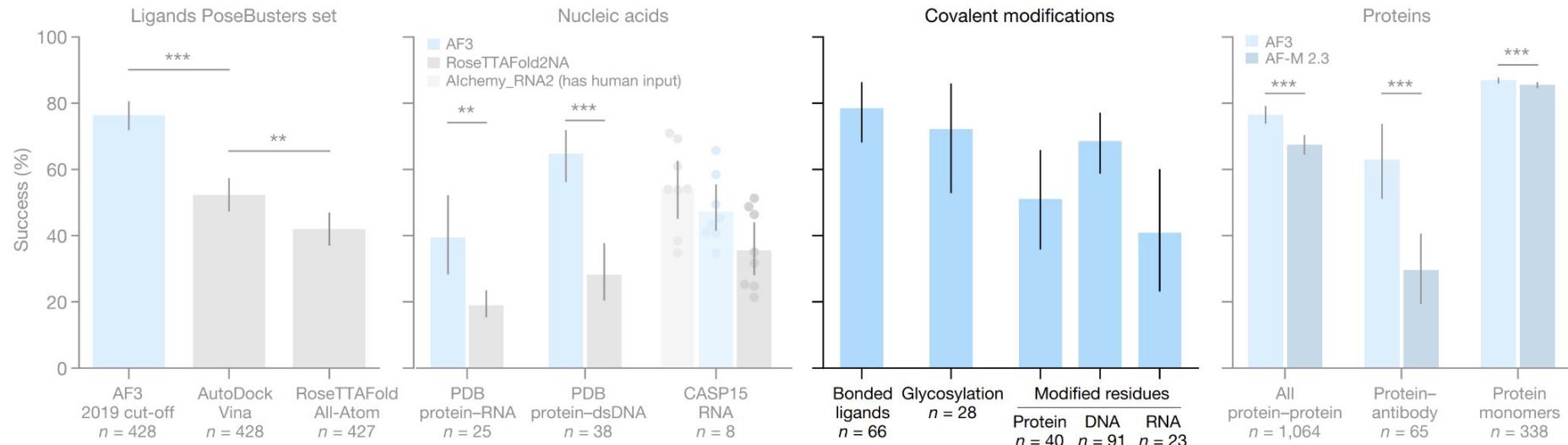
AF3 is light blue throughout



For protein-DNA and protein-RNA interactions, AF3 does well
For RNA alone human intervention is still better

Accuracy across molecular types - Covalent modifications

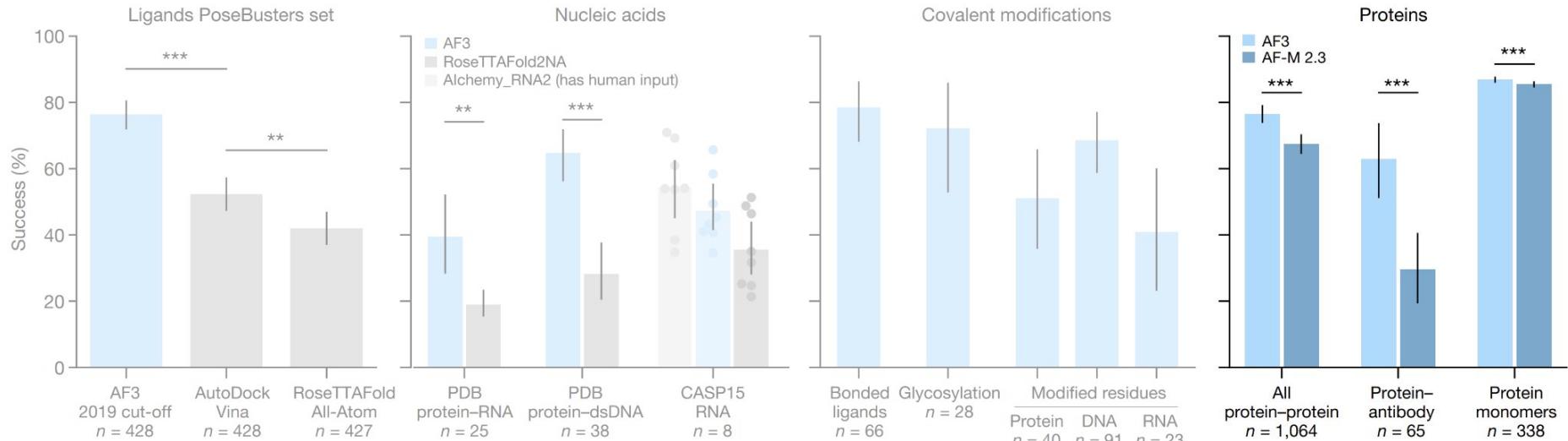
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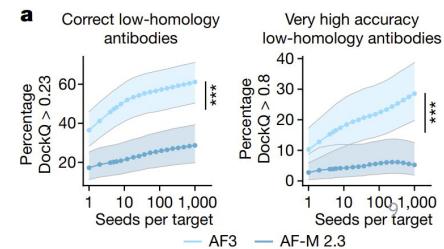
No model comparison possible at time of writing, but accuracy is enough to be useful

Accuracy across molecular types - Proteins

AF3 is light blue throughout

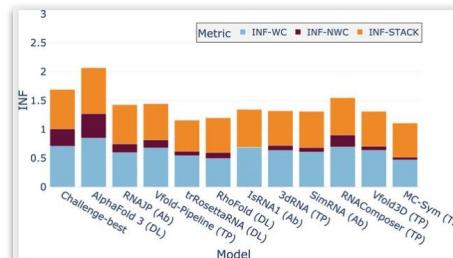


AlphaFold 3 outperforms AlphaFold-Multimer 2.3
(the previous SotA) in all categories



Is there any independent validation?

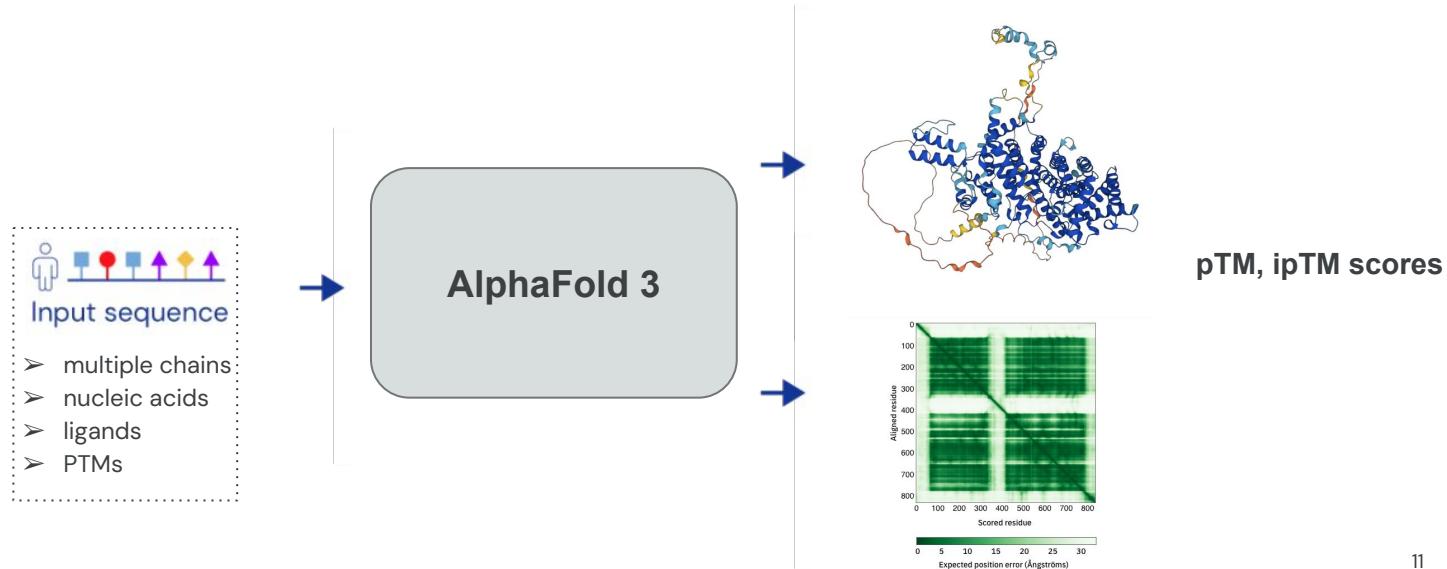
- Several successful attempts to reproduce AF3 results validate overall approach
- We are still waiting for comprehensive independent validation/benchmarking studies
- So far, we saw several preprints on checking particular aspects of AF3 performance:
 - Metal-protein interactions – "AF3 provides realistic predictions for metal ions" (Dürr and Rothlisberger, 2024).
 - Adversarial changes in the protein sequence – AF3 predicts ligand binding even if binding site is destroyed by mutations (Masters et al, 2024)
 - RNA structures – "*Through our benchmark, we showed that AlphaFold3 is a competitive method that outperforms most of the existing solutions. It yields better results for RNA-Puzzles and RNASolo, but remains outperformed by the best solutions from the CASP-RNA challenge.*" (Bernard et al., 2025)



Bernard, C., Postic, G., Ghannay, S. & Tahí, F. Has AlphaFold3 achieved success for RNA? (2025). Acta Cryst. D81, 49–62.

What do you need to know about AlphaFold 3

- Many practical details remain the same between AF2 and AF3
 - Just new types of input become available (nucleic acids, ligands, etc.)

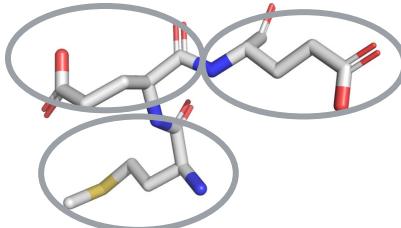


What do you need to know about AlphaFold 3

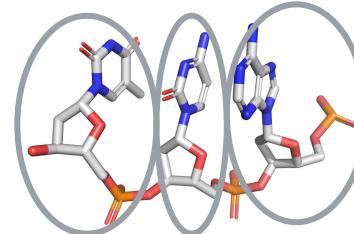
- Many practical details remain the same between AF2 and AF3, notably the use of **multiple sequence alignments** (MSAs) as the main input of the neural network
 - Although AF3 processes MSAs differently to AF2 and therefore it is less dependent on coevolutionary signal , **MSA quality** is still important!

What do you need to know about AlphaFold 3

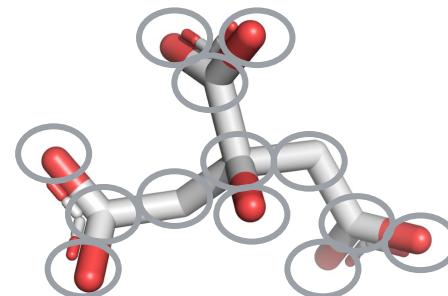
- Using transformer architecture, both AF2 and AF3 divide the complex of interest into “**tokens**”. In AF3, a **token** may correspond to:
 - One standard amino acid in the protein chain
 - One standard nucleotide in the nucleic acid chain
 - One atom of a ligand
 - One atom of an ion
 - One atom of a chemically-modified amino acid residue or nucleotide (applied to the whole residue/nucleotide).



3 tokens



3 tokens



13 tokens

- All confidence scores (like pLDDT, PAE) are calculated for the tokens rather than residues
- Size of the structure is also calculated in tokens (up to ~7,000 tokens can be modelled with A100 80G)

AlphaFold Server is made for biologists

AlphaFold Server BETA

Server About FAQs

Remaining jobs: 20

AlphaFold Server allows you to model a structure consisting of many biological molecules [Learn more](#)

Upload JSON Clear

Molecule type: Protein Copies: 2
MEERIERIKK 10 QLHAASYKL 20 PQREATVRVL 30 LENEEDHLSA 40 EDVYLLVKEK 50 SPEIGLATVY 60
RTLELLS ELK 70 VVDKINFQDG 80 VSRYDLRQEG 90 AQRFH HHLIC 100 TQC GAVQEQI 110 K 120
VERDWSFKVK 130 DHRLTFHGIC 140 KNCQENETDE 150 K
Molecule type: Ion Copies: 2 Zn²⁺
Molecule type: Ion Copies: 2 Fe²⁺

+ Add entity Save job Continue and preview job

<https://alphafoldserver.com/>



AlphaFold Server provides a user-friendly and free platform for molecular and cell biologists, biochemists, and other life scientists to leverage AlphaFold 3 in their research

More than 3M structures modelled in 6 months

AlphaFold Server BETA

Server About FAQs

Metalloprotein with iron and zinc

Back Download Clone and reuse Feedback on structure

Very high (pIDDT > 90) Confident (90 > pIDDT > 70) Low (70 > pIDDT > 50) Very low (pIDDT < 50)
ipTM = 0.84 pTM = 0.85 learn more

1 51 102 153 204 255 306
Aligned Residue
1 51 102 153 204 255 306
Scored Residue

AlphaFold Server is fast

2-3 minutes* to predict a structure with ~500 tokens**

6-8 minutes* to predict a structure with ~1,000 tokens**

8-12 minutes* to predict a structure with ~3,000 tokens**



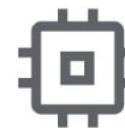
Powerful orchestrator

Ensures maximal parallelism within the pipeline; coordinates hardware requirements for each stage



MSA search parallelisation

Resolves the main bottleneck of the structure prediction by spreading MSA search over many CPUs



Inference on multiple accelerators

Speeds up inference and allows modelling of the large structures up to 5,000 tokens**

*depends on overall Server workload and may increase at peak hours due to queuing

**a token corresponds to one residue of a canonical amino acid, one nucleotide of unmodified RNA/DNA, or one atom otherwise

AlphaFold 3: Server vs GitHub

The screenshot shows the GitHub repository for AlphaFold 3. The repository has 572 forks and 51k stars. It contains 1 branch and 1 tag. The codebase includes files like Dockerfile, README, and various CMakeLists.txt and LICENSE files. The repository was created by Augustin-Zidek and updated by cdcf141. The repository is described as the "AlphaFold 3 inference pipeline".

	GitHub AlphaFold 3	AlphaFold Server
Protein, RNA, and DNA chains	✓	✓
Custom multiple sequence alignment (MSA) for protein and RNA chains	✓	Under development
Custom template structures for protein chains	✓	Under development
Custom ligands (via CCD codes, SMILES and CIFs)	✓	Selected ligands only
Covalent modifications (PTMs, glycosylation, modified nucleotides)	✓	Selected modifications only
Custom covalent bonds between entities	✓	✗
Multiple random seeds	✓	via multiple jobs
License	Non-commercial academic use ONLY	

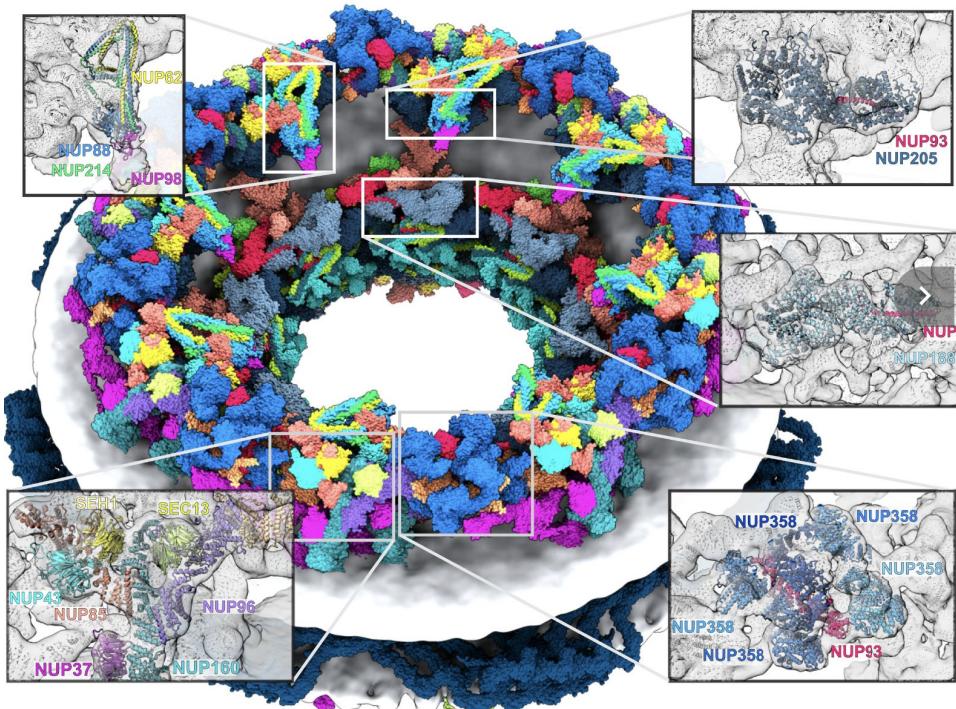
Should I use AlphaFold 2 or AlphaFold 3?

- In terms of functionality, AlphaFold 3 can do everything AlphaFold 2 can, and do it better. It also has additional capabilities, in particular predicting multiple types of molecules.
- **License terms:**
 - **AlphaFold 2** is open-source and is freely available for **both academic and commercial use** under permissive Apache 2 licence terms.
 - In contrast, **AlphaFold 3 is only currently available for non-commercial use, subject to Terms of Use**, which include a number of use restrictions.
- Importantly, you **must NOT**:
 - ✗ Use AF3 in connection with any **commercial activities**, including research on behalf of commercial organisations
 - ✗ Use AF3 or its outputs to **train machine learning models** or related technology for biomolecular structure prediction similar to AlphaFold
 - ✗ Circumvent access restrictions relating to the Model Parameters, including utilising, sharing or making available the Model Parameters (model weights)
- ✓ Please use **AlphaFold 3 for fundamental academic biological research**
- ✓ You are free to use **AlphaFold 2 for any activities**, including commercial ones.

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Variety of AlphaFold applications

Studying huge (and just large) complexes



Mosalaganti, S. et al. AI-based structure prediction empowers integrative structural analysis of human nuclear pores. *Science* (2022)

Ben Engel @bengelicious · Jul 23, 2021

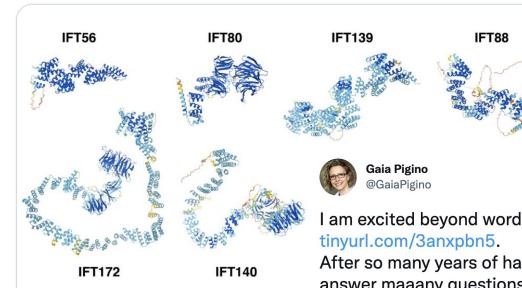
Well, this kept me up late! 😴 😴

...

#AlphaFold has very solid-looking predictions for many of the #IFT proteins! Tons of elaborate alpha solenoids and beta propellers! Hey @GaiaPigino (and twitterless Esben Lorentzen), is it time to build the train?



Team #Cilia is gonna crack it!



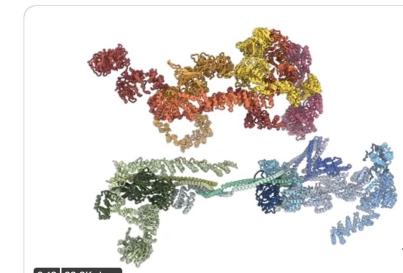
I am excited beyond words that this is now out:
tinyurl.com/3anxpbn5.

After so many years of hard work, we can finally answer maany questions about IFT... 🎉

Thanks Sam @stanuel02 Lacey, @Helen_Foster_, the cryo-EM facility @humantechopole, and notably also #AlphaFold2! ❤️

Lacey, S. E. et al. The molecular structure of IFT-A and IFT-B in anterograde intraflagellar transport trains.

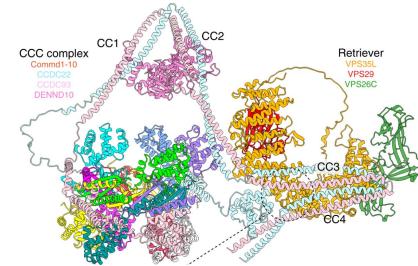
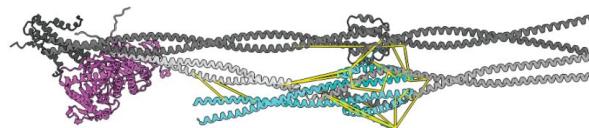
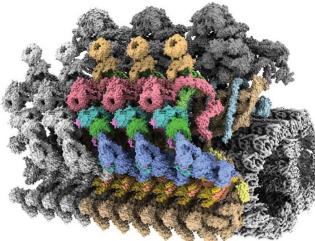
NSMB (2023)



0:13 | 23.3K views

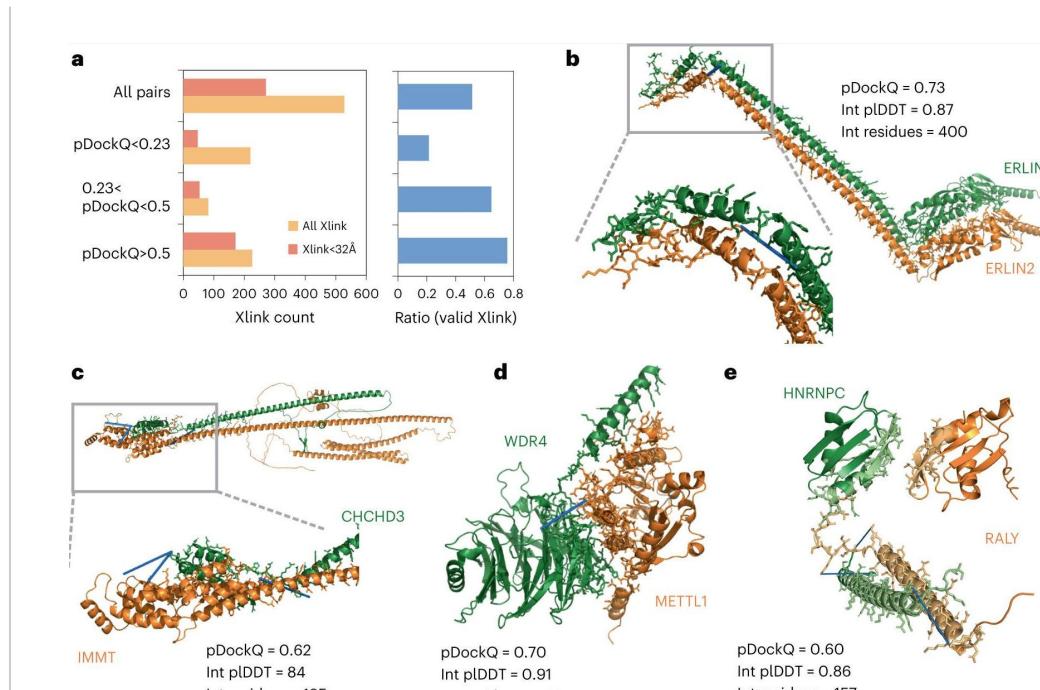
Studying huge (and just large) complexes

- Nuclear pore complex – **70 MDa** assembly (*Mosalaganti et al. 2022, Fontana et al. 2022*)
- Intraflagellar train IFT-A and IFT-B – **0.8 MDa** (*Ma et al. 2023, McCafferty et al. 2022, Hesketh et al. 2022*)
- Commander endosomal trafficking complex – **0.5 MDa** (*Healy et al. 2023*)
- Full-length kinesin-1 heavy chain and kinesin-1 heavy and light chains – **0.4 MDa** complex (*Tan et al. 2023*)
- Augmin complex (*Gabel et al. 2022, Zupa et al. 2022*)
- Components of the yeast small subunit processome (*Zhao et al. 2022*)
- Component of the eukaryotic lipid transport machinery (*Cai et al. 2022*)
- Community members conclude that “**better structure reconstruction can be obtained by combining AlphaFold predicted structure models and cryo-EM data**” (*Giri et al. 2023*)



Large scale predictions of protein interactions

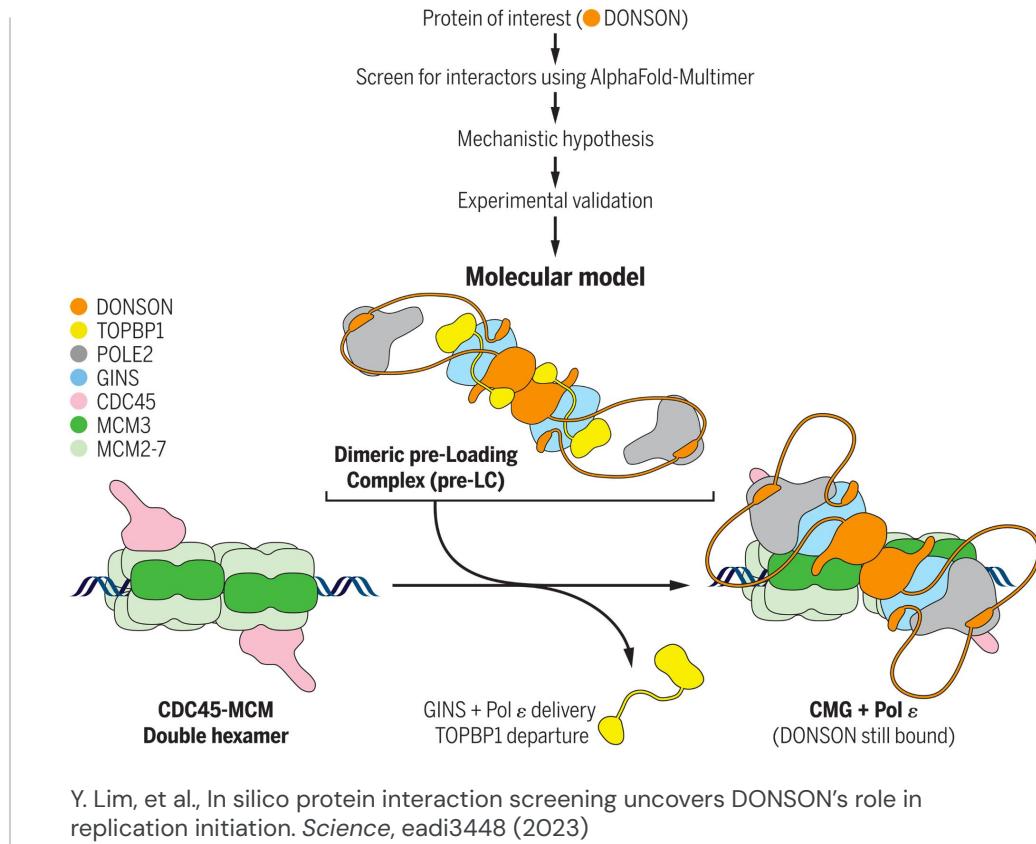
- Predicted the structures of **65,000** pairs of *human* proteins, obtained **3,000** high-confidence pairs
- 1,400** of the newly predicted high-confidence complexes lacked homology to existing structures
- Cross-linking validation:
 - Experimental evidence confirmed 171 out of 246 (**70%**) complexes predicted with high confidence



Burke, D. F. et al., Towards a structurally resolved human protein interaction network. Nat. Struct. Mol. Biol. (2023).

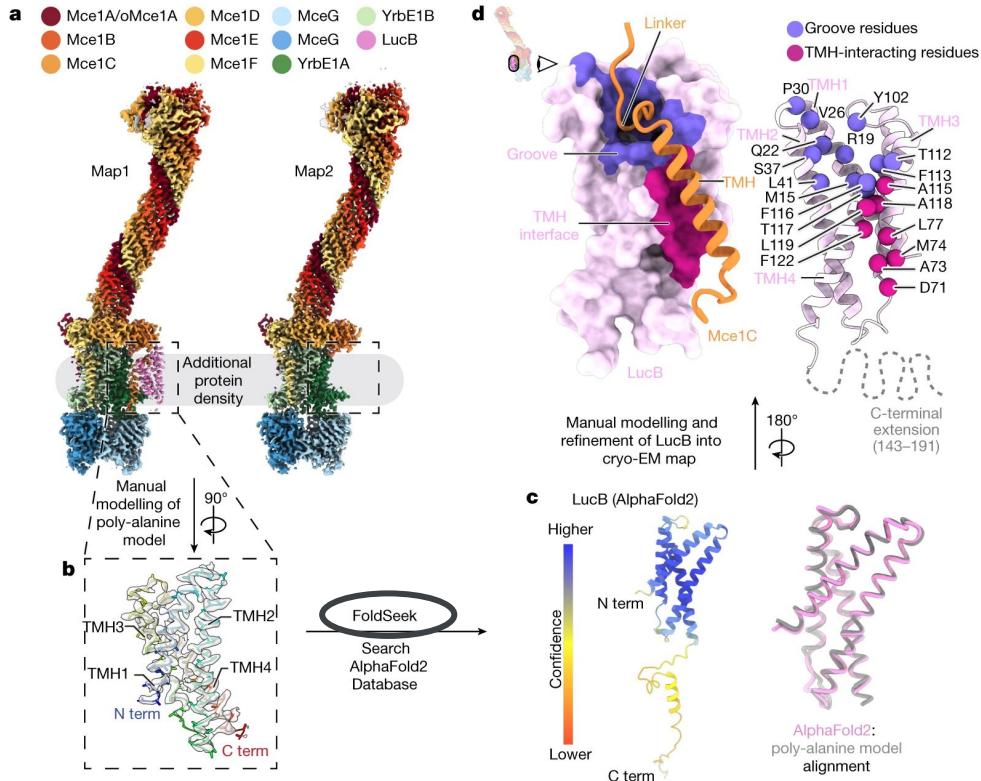
Focused prediction of protein interactions

- Screened DONSON protein against 70 DNA replication factors
- Built a structural model of a pre-Loading Complex in which DONSON interacts with GINS, TOPBP1, and Pol ε
- Used coimmunoprecipitation and site directed mutagenesis to confirm the model experimentally



Use of the AlphaFold Database

Identification of proteins in cryoEM density

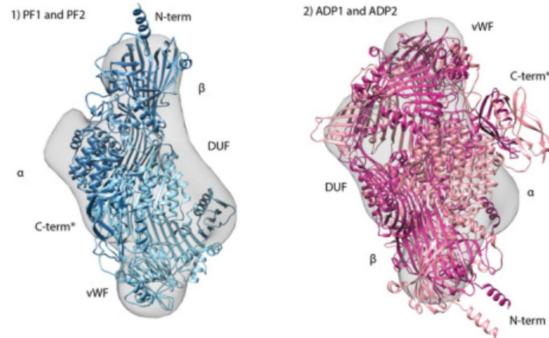




New possibilities unlocked by
AlphaFold 3

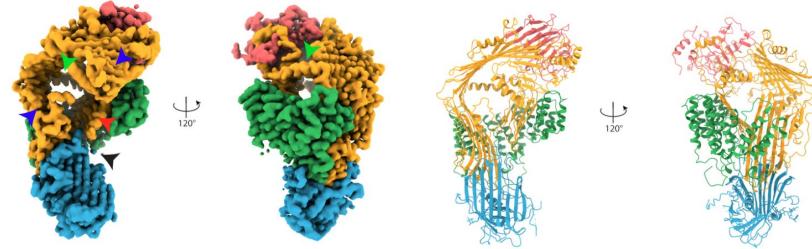
Honey bees story

Honey bee vitellogenin (Vg) – protein central for bee's immunity



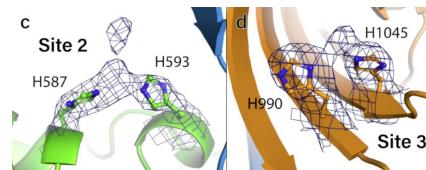
2021 vitellogenin (Vg) study:

- AlphaFold 2 modelling of Vg confirmed by low-resolution negative staining EM
- New structural features identified:
 - vWD domain position with respect to the lipid binding module
 - Identification of a putative Ca binding site
 - Mapping of functional polymorphisms



2024 cryo-EM reconstruction:

- Confirmed AF2 predictions (RMSD 2.3 Å)
- AF3 allowed even better prediction (**RMSD 1.7 Å**)
- AF3 modelled **Zn and Ca binding**; some metal sites confirmed experimentally

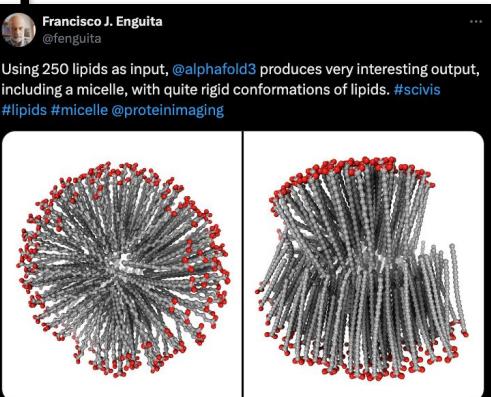
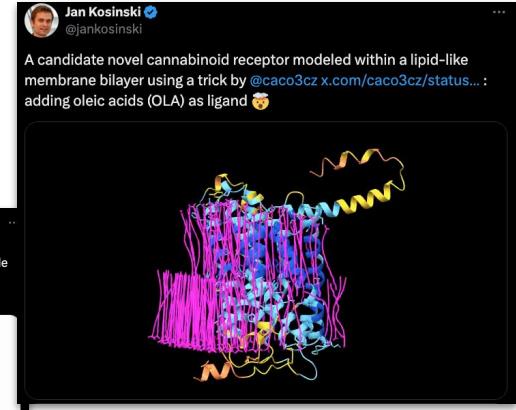
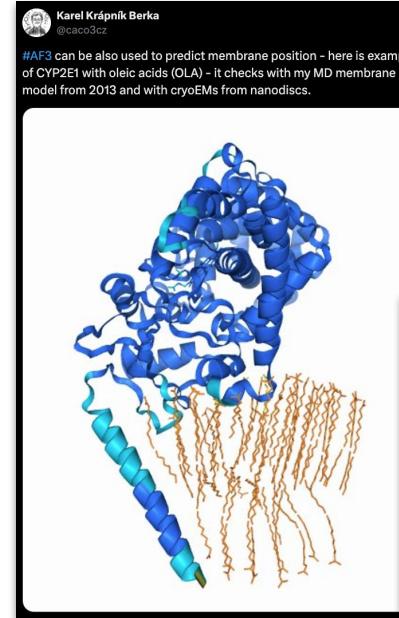


H. Luecke et al. Cryo-EM structure of native honey bee vitellogenin, preprint at Research Square (2024)

Modelling transmembrane domains with fatty acids

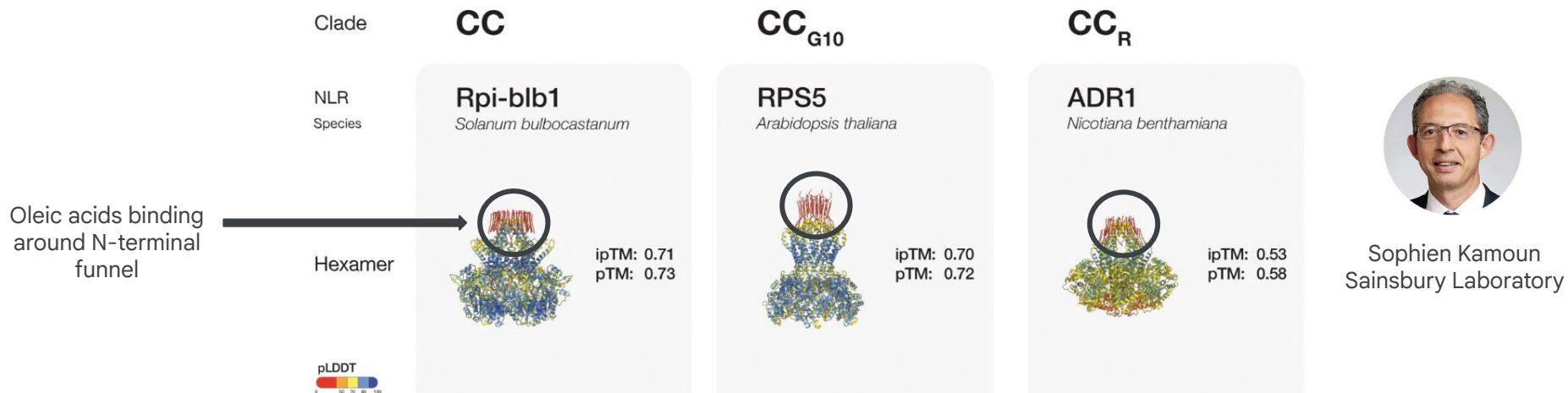
**AlphaFold 2
(AlphaFold Database)**

The screenshot shows the AlphaFold 2 interface. On the left, there is a sequence alignment for AF-P00533-F1, which includes a sequence logo and a sequence bar. The main area displays a 3D ribbon model of a protein structure, likely a transmembrane domain, colored by component. To the right of the structure is a heatmap plot showing residue-residue contacts or scores. The x-axis is labeled "Scored residue" and ranges from 0 to 1.2. The y-axis is labeled "Aligned residue" and ranges from 0 to 1k. Two specific regions of the heatmap are circled in black.



Modelling transmembrane domains with fatty acids

- NLR proteins form a key part of the innate immune response of plants:
 - "The N-terminal funnels of activated CC-NLR resistosomes are often not resolved in experimental structures... due to depletion of lipids during purification"*
 - "AlphaFold 3 can be used to fill out gaps in resistosome structures that have remained elusive due to technical limitations."*
 - "...we have been using AlphaFold predictions to complement phylogenetic grouping and other data to triage NLRs into functional categories and prioritize them for functional analyses"*



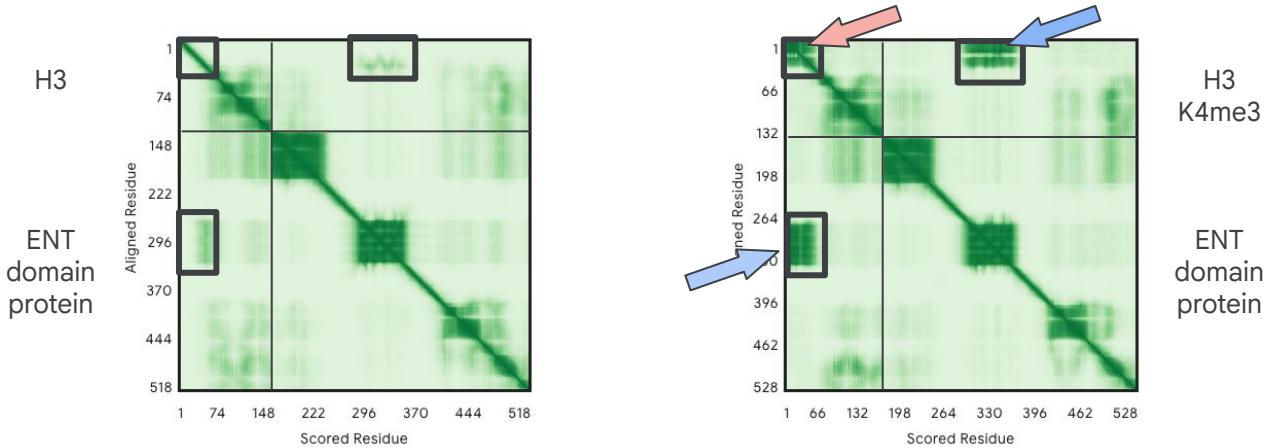
Madhuprakash et al., A disease resistance protein triggers oligomerization of its NLR helper into a hexameric resistosome to mediate innate immunity. Sci. Adv. 10, eadr2594 (2024)

Ibrahim et al., A helper NLR targets organelular membranes to trigger immunity. bioRxiv preprint (2024) <https://doi.org/10.1101/2024.09.19.613839>

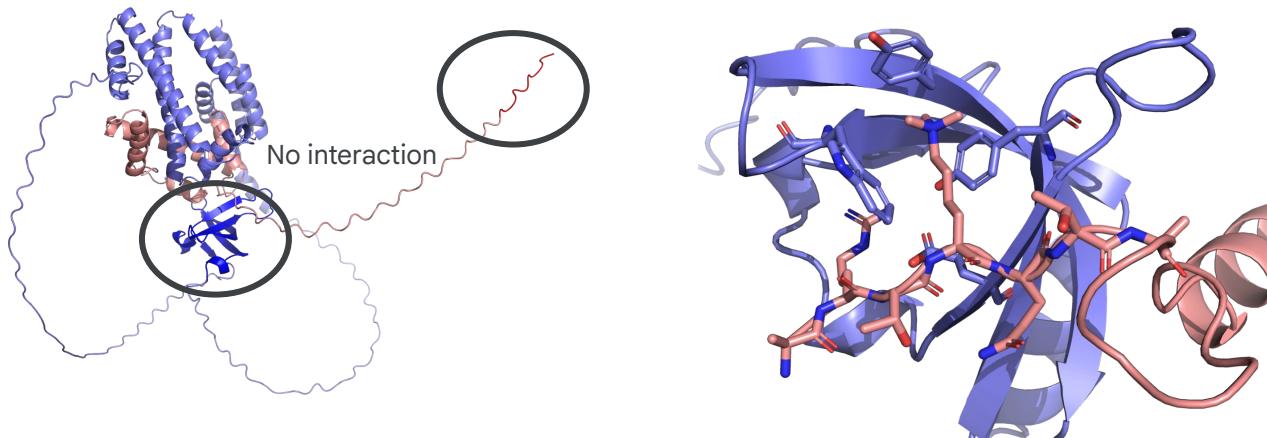


Sophien Kamoun
Sainsbury Laboratory

Protein-protein interactions induced by PTM

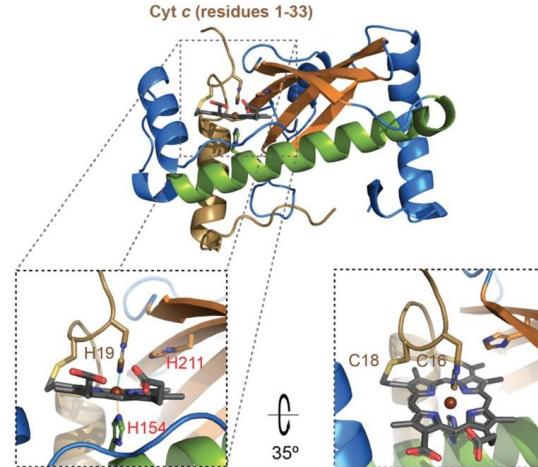


Jake Harris
University of Cambridge



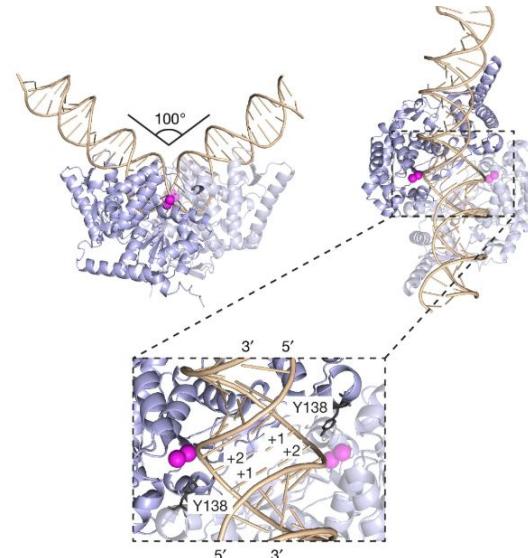
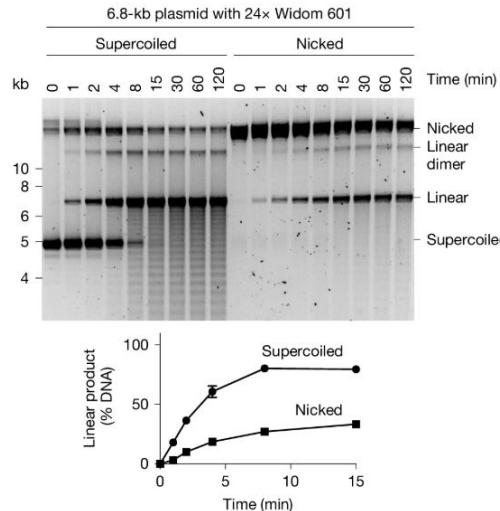
Modelling proteins with ligand

- Study of the essential mitochondrial electron transport chain in *Plasmodium* malaria parasites:
 - used AlphaFold 3 to model a complex between *P. falciparum* HCCS, heme, and the first 33 residues of the CytC N-terminus
 - Prediction suggests a key intermediate in CytC hemylation in which HCCS-bound heme is positioned by opposing His coordination to facilitate thioether bond formation between the Cys residues of CytC and heme vinyl groups
 - This model is consistent with prior mechanistic suggestions for human HCCS maturation of CytC based on biochemical experiments



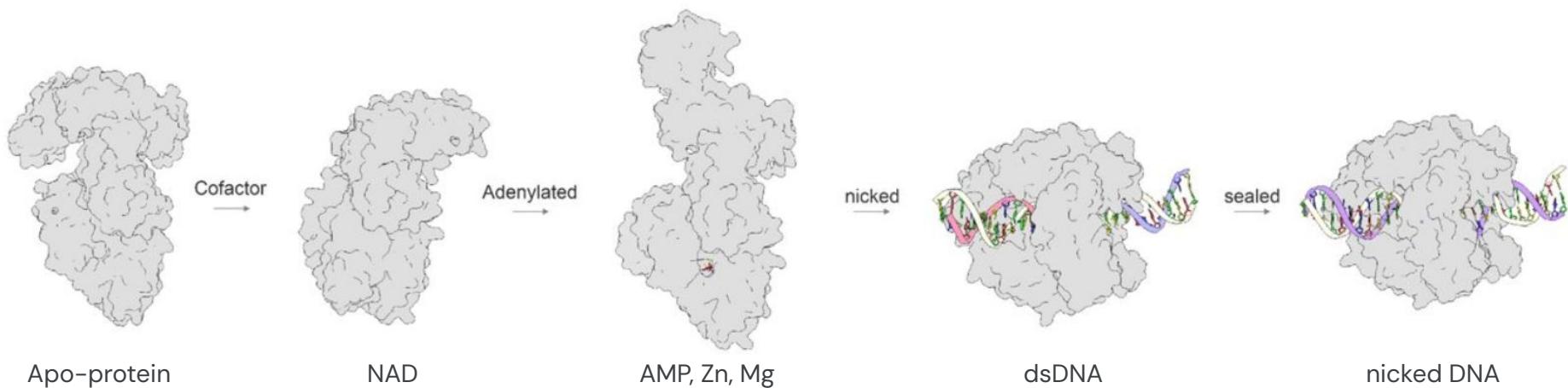
Protein-DNA complexes

- [In the AF3 prediction] “*The substrate is bent at an angle of 100° with underwound DNA strands at the centre of the complex, consistent with the observed preference of SPO11 for bendable and negatively supercoiled DNA.*”
- Metal coordination is confirmed: “*we found that mutation of E224 to alanine abolishes the DNA-cleavage activity*”
- “*AlphaFold modelling suggests that SPO11 complexes across eukaryotes bend the DNA substrate before cleavage. This could lead to preferential DSB induction at sites under topological stress, and potentially provide the energy required to drive the cleavage reaction forward*”



AlphaFold for modeling structural dynamics

- “We utilized AlphaFold3 (AF3) to investigate the structural dynamics and mechanisms of enzymes involved in DNA homeostasis, using NAD-dependent Taq ligases and human DNA Ligase 1 as a case example. Modifying the input parameters for AF3 yielded detailed conformational states of a DNA-binding enzyme, thereby offering enhanced mechanistic insights”.
- Authors obtained different structural states by adding different ligands and metal ions, and different DNA substrates; also they used multiple seeds to analyse potential dynamics of the complexes via PCA





How to use AlphaFold 3 correctly?

Confidence metrics

"Nothing in **biology** makes sense except in the light of **evolution** "

Theodosius Dobzhansky, 1973

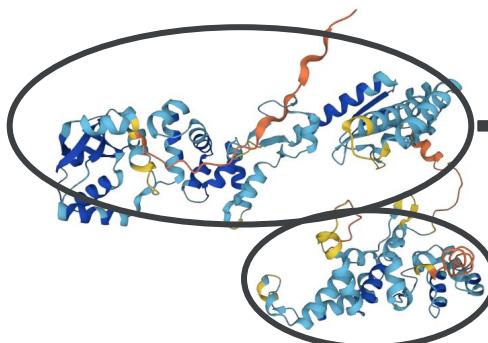
Confidence metrics

"Nothing in AlphaFold predictions makes sense except in the light of confidence scores"

Confidence metrics

"Nothing in AlphaFold predictions makes sense except in the light of confidence scores"

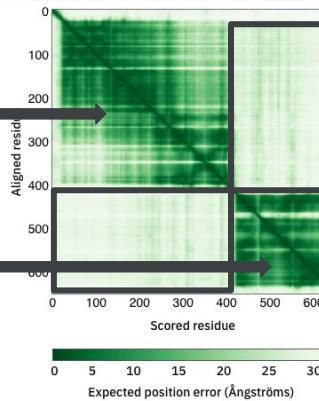
- Two major types of uncertainties:
 - of the position of an **amino acid** relative to its close neighbours (residue **pLDDT score**)
 - of the orientation of a **larger part** of the structure, e.g. a domain, relative to other domains (**PAE plot**)



Model Confidence

Very low (pLDDT < 50)
Low (70 > pLDDT > 50)
Confident (90 > pLDDT > 70)
Very high (pLDDT > 90)

pLDDT score
colouring



Predicted Aligned
Error (PAE) plot

Empty space here means no predicted connections between the domains, meaning they are positioned randomly

Confidence metrics

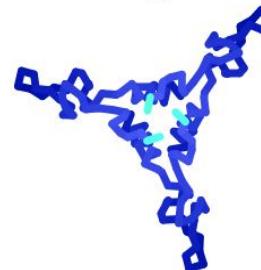
"Nothing in AlphaFold predictions makes sense except in the light of confidence scores"

2022-03-15 07:56:06,840 Running model_1
2022-03-15 08:02:00,946 model_1 took 354.1s (3 recycles) with pLDDT 95.9 and ptmscore 0.845

colored by chain



colored by pLDDT

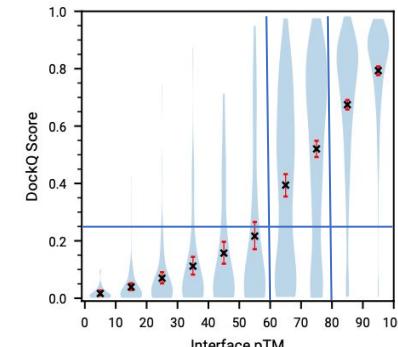
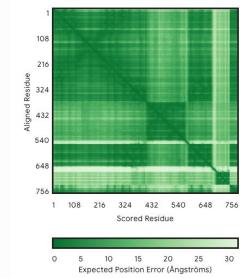
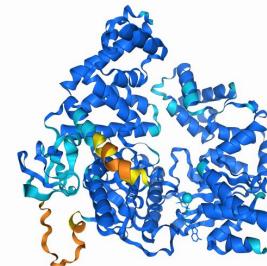
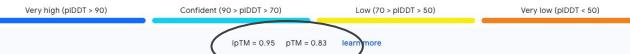


2022-03-15 08:02:20,109 Running model_2
2022-03-15 08:03:21,560 model_2 took 61.4s (3 recycles) with pLDDT 95.5 and ptmscore 0.832

colored by chain

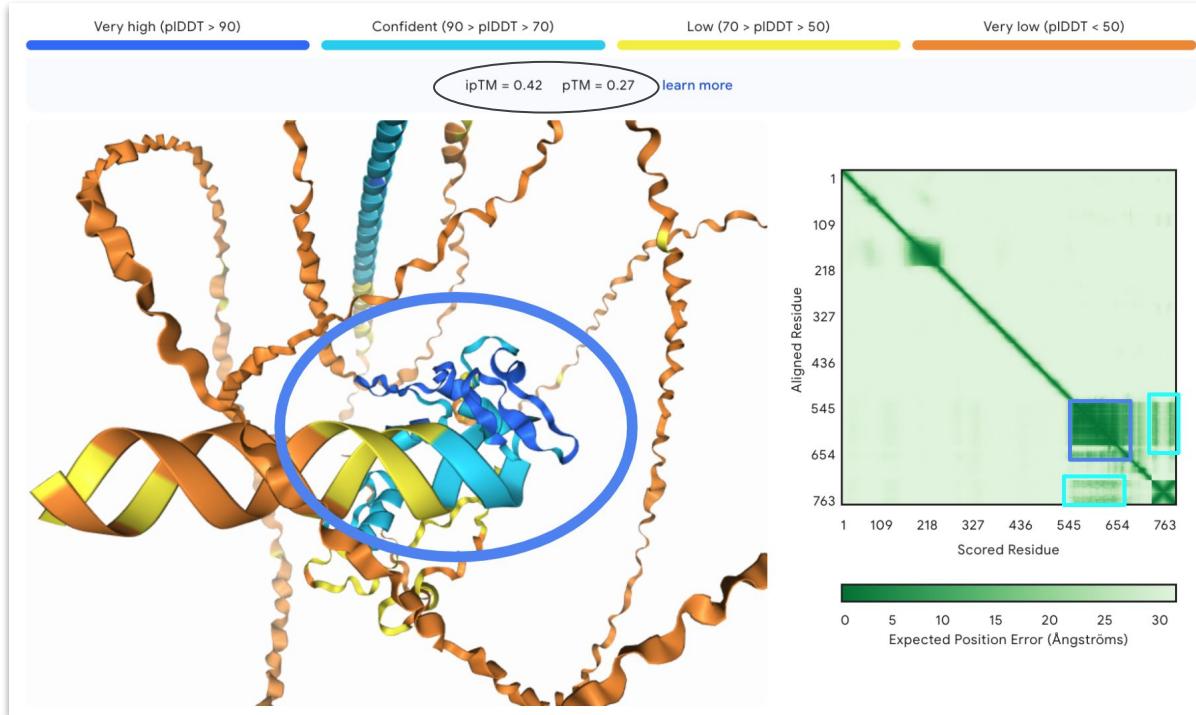


colored by pLDDT



Confidence metrics

"Nothing in AlphaFold predictions makes sense except in the light of confidence scores"

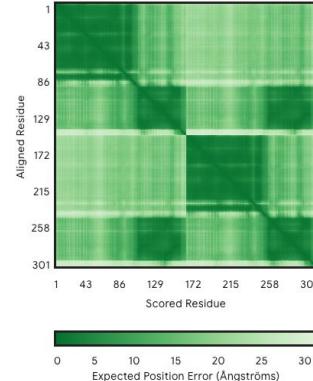
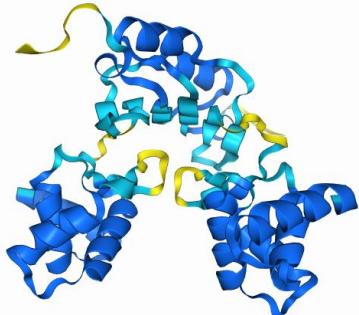


AlphaFold 3 looks for full structural context

- **Confidence metrics** of AlphaFold 3 (pLDDT, PAE, pTM, ipTM) may depend on having the **full structural context**:
 - For instance, if you model metal-binding protein **without metal ions**, it could be modelled with **lower confidence**, indicating that important parts of the structure are missing
 - Adding metal ions will result in the confident prediction as all parts of the structure are in place

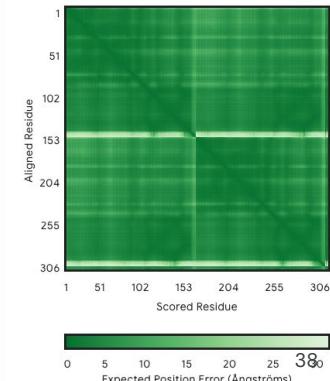
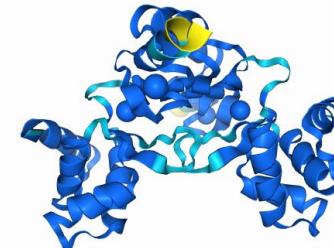
no Zn, no Fe

ipTM = 0.54 pTM = 0.54



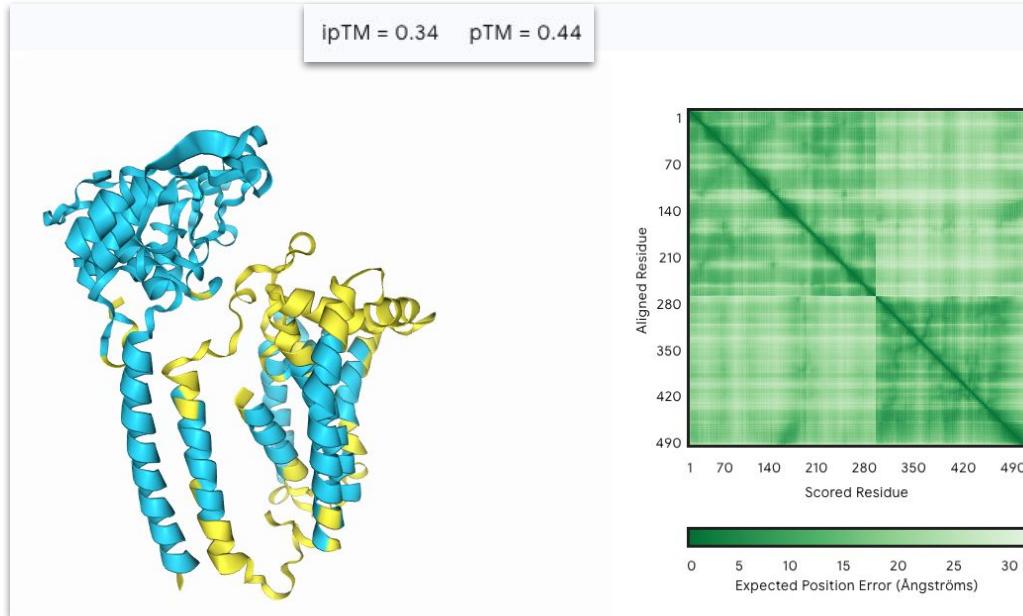
plus Zn, plus Fe

ipTM = 0.84 pTM = 0.85



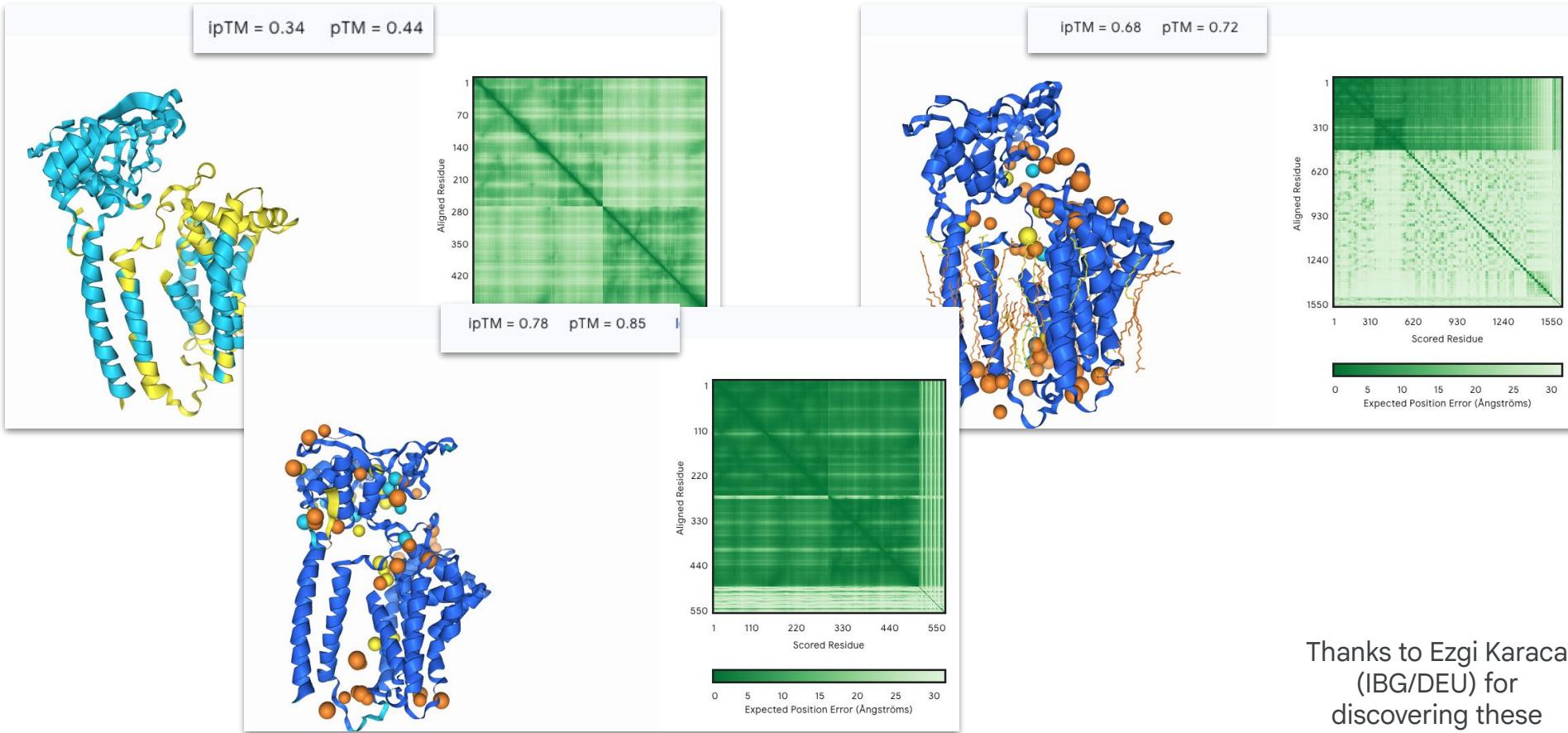
AlphaFold 3 looks for full structural context

- **Confidence metrics** of AlphaFold 3 (pLDDT, PAE, pTM, ipTM) may depend on having the **full structural context**:
 - For protein–protein interactions, presence of other molecules can greatly increase confidences without changing positions of the predicted atomic coordinates



Thanks to Ezgi Karaca
(IBG/DEU) for
discovering these
examples

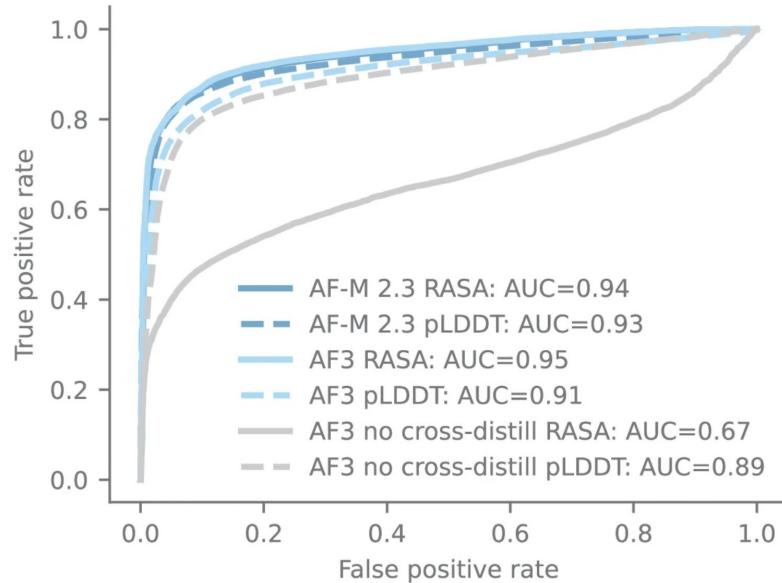
AlphaFold 3 looks for full structural context



Thanks to Ezgi Karaca
(IBG/DEU) for
discovering these
examples

Peculiarity of the AF3 - hallucinations

- **Hallucinations** were not fully removed – the further from the training set, the more hallucinations. Hallucinations nearly always have very low pLDDT, but may still disrupt structure prediction.
 - One workaround is to rerun the model with low confidence regions removed.



Hallucinations

First, we modeled two ChREBP-MLX complexes with the ChoRE sequence from the promoter region of the target gene encoding pyruvate kinase (PK) by AlphaFold 3. The model showed that the MLX phosphorylation motif lies in a flexible region close to the alpha-helix which binds to the E-box (Fig. 3a). Interestingly, a model with **phosphorylated** MLX showed a **drastic conformational change** in ChREBP-MLX dimers, **stabilizing the flexible region as alpha-helices** (Fig. 3b).

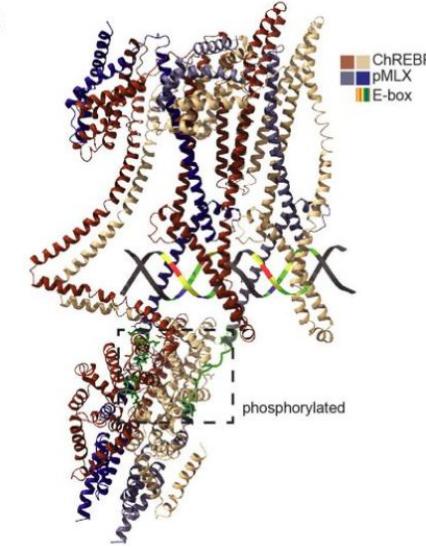
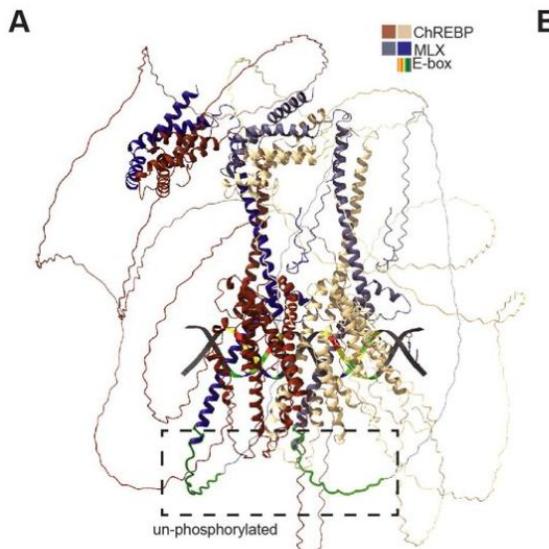
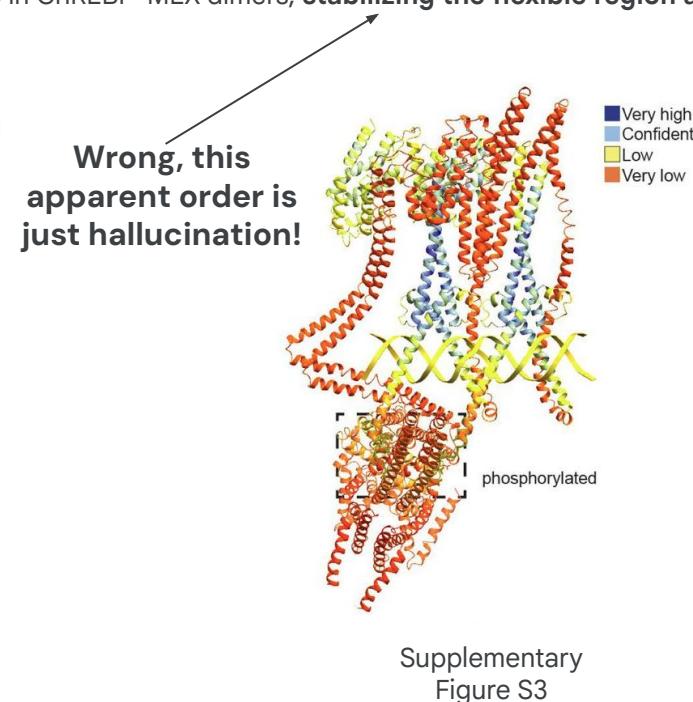


Fig. 3



Supplementary Figure S3

Check the documentation!

AlphaFold Server BETA

Server About FAQ & Guides ▾

Overview:



Section 1: Introducing AlphaFold 3



Section 2: AlphaFold Server: Your gateway to AlphaFold 3



Section 3: Interpreting results from AlphaFold Server



Section 4: Conclusions



The screenshot shows the AlphaFold online tutorial course page. At the top, there's a navigation bar with links to EMBL-EBI Home, Services, Research, Training, About us, and a search bar. Below the navigation is a banner with the text "ONLINE TUTORIAL AlphaFold A practical guide". The main content area has a heading "Proteins are essential components of life, predicting their 3D structure enables researchers to get an insight into its function and role. AlphaFold is an artificial intelligence (AI) system, developed by Google DeepMind, that predicts a protein's 3D structure based on its primary amino acid sequence. It regularly achieves accuracy competitive with experiment." There's also a "Course overview", "Course contents", "Getting started", and "Competencies" section. On the left, there's a sidebar with sections like "Time to complete: 3 hours", "This course includes: Activities, Quizzes, Videos", and "Written by: Paulyna Gabriela Magana Gomez, Oleg Kovallevskiy". The bottom right corner features the Google DeepMind logo.

The screenshot shows a GitHub repository for "google-deepmind/alphafold". The "input.md" file is open, showing documentation for "AlphaFold 3 Input". It includes sections for "Specifying Input Files" and "Input Format". The "Input Format" section notes that AlphaFold 3 uses a custom JSON input format different from the AlphaFold Server JSON input format. It lists several ways to specify inputs: single input file, multiple input files, specifying protein, RNA, and DNA chains, specifying custom MSA, specifying structural templates, and specifying ligands using Chemical Component Dictionary (CCD) codes or SMILES.

<https://www.ebi.ac.uk/training/online/courses/alphafold/>
<https://alphafoldserver.com/guides>
<https://github.com/google-deepmind/alphafold3/>

Correct and incorrect uses of AlphaFold

- **Correct uses of AlphaFold:**

- Predicting structures of macromolecules and complexes
 - to accelerate experimental structural biology
 - to make testable hypotheses about the function and check them biochemically
 - to map mutations and hypothesise about mechanism of their action
 - to analyse positions of the structured domains and disordered regions
- Assessing (and screening) interactions based on confidence scores
- Checking folding of the designed proteins
- Structural search using predicted structure to find distant homologues

- **Dubious uses of AlphaFold:**

- Modelling of the point mutants – likely not see any difference
- Attempts to interpret low-confidence regions or interactions
- Ignoring PAE plot and making interpretations of the randomly-positioned domains
- Docking of the ligands directly into the predicted structures – MD may help though
 - Docking vs cofolding (AF3) – cofolding often works better

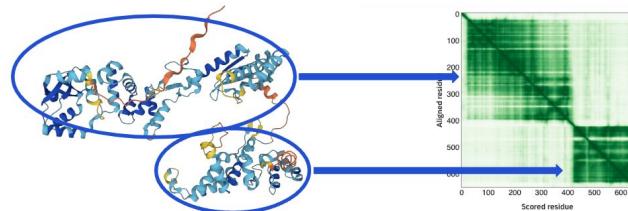
AlphaFold is a scientific method

- **Experiment** with it
 - ...but do not forget to **validate** the results!
- Try it, play with it
- Invent new uses
- Think how you can use predictions in your research
- Get inspired by others working in the field
 - ...but beware of limitations



Not a rival to the experimental methods

- In no way do we see AlphaFold as a rival to the experimental methods of structure determination
- We believe that initial structural hypotheses provided by AlphaFold may facilitate and guide experimental structure determination, complement low-resolution structural data and assist their interpretation, help solving phase problem in crystallography and overall accelerate structural studies
- Also, hypotheses based on the AlphaFold predictions (e.g. proteome-wide screenings of protein–protein interactions) may help to advance research, including biochemical or cell biology experiments.



Thanks to everyone who made AlphaFold 3 possible!

AlphaFold 3

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The wider team at
Google DeepMind



AlphaFold Server

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Our wonderful collaborators at
EMBL-EBI



The CASP
community



AlphaFold 3 on GitHub

Augustin Žídek, Andrew Cowie, Bella Hansen, Charlie Beattie, Chris Jones, Grace Margand, Jacob Kelly, James Spencer, Josh Abramson, Kathryn Tunyasuvunakool, Kuba Perlin, Lindsay Willmore, Max Bileschi, Molly Beck, Oleg Kovalevskiy, Sebastian Bodenstein, Sukhdeep Singh, Tim Green, Toby Sargeant, Uchechi Okereke, Yotam Doron, John Jumper

PDB & the experimental
structural biology community

