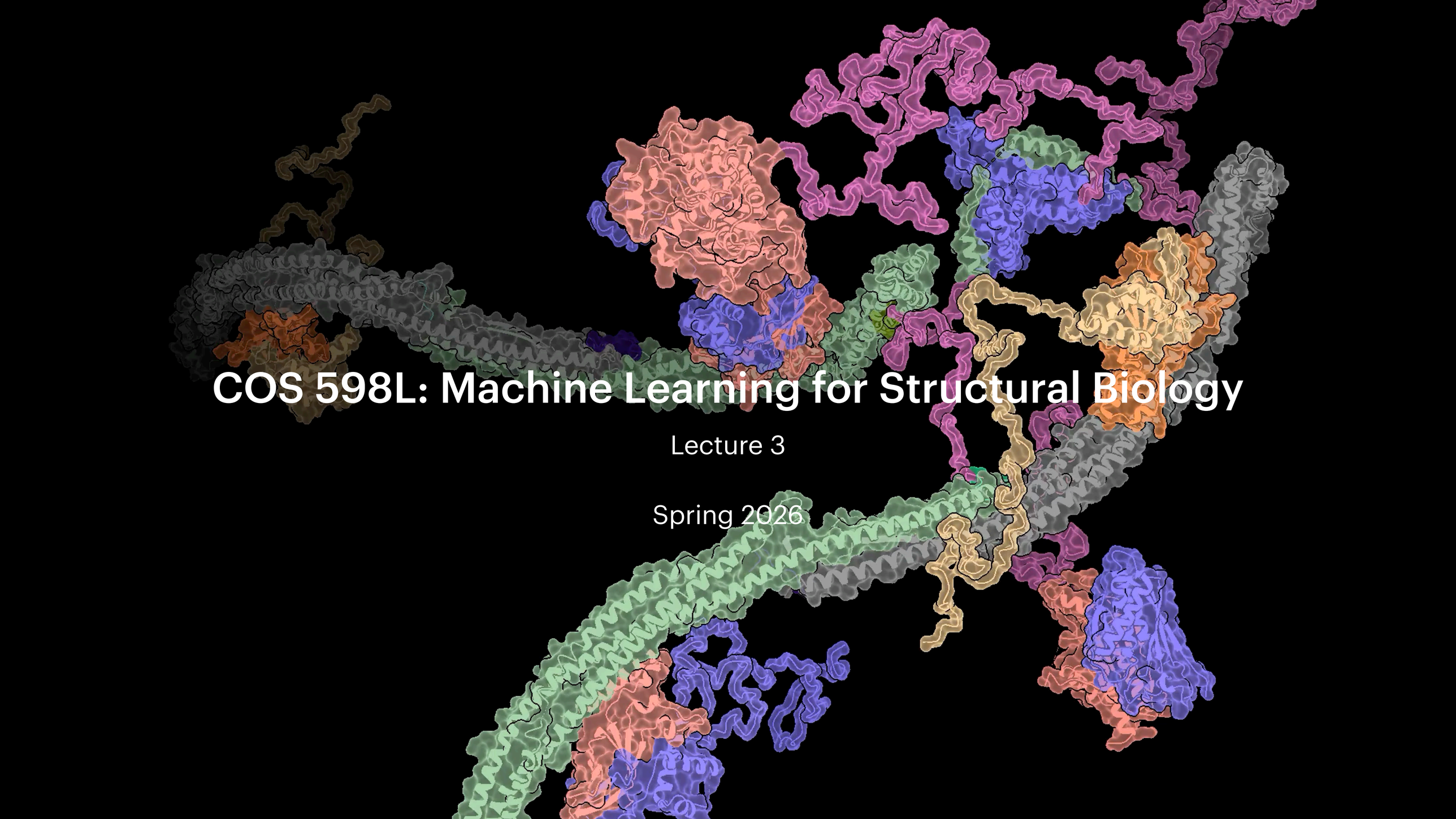


COS 598L: Machine Learning for Structural Biology

Lecture 3

Spring 2026

Course Logistics

- Today:
  - Jack Shaw, Maxwell Soh will present the AlphaFold2 and AlphaFold3 papers
  - Feedback: Robert Heeter, Yagiz Devre
- Next week: Protein Design! Please read one of:
  - **Generative models for graph-based protein design.** Ingraham et al. NeurIPS 2019.
  - **ESM-IF1: Learning inverse folding from millions of predicted structures.** Hsu et al. ICML 2022.
  - **One-shot design of functional protein binders with BindCraft.** Pacesa et al. Nature 2025.
  - Presenters: Jack McMahon, Joseph Clark, Md Toki Tahmid
- Week 5: Protein structure determination
  - Guest lecture on cryo-EM + cryoDRGN

This lecture

AlphaFold2

- AlphaFold2 presentation by Jack Shaw
- AlphaFoldDB figures
- AlphaFold3 presentation by Maxwell Soh

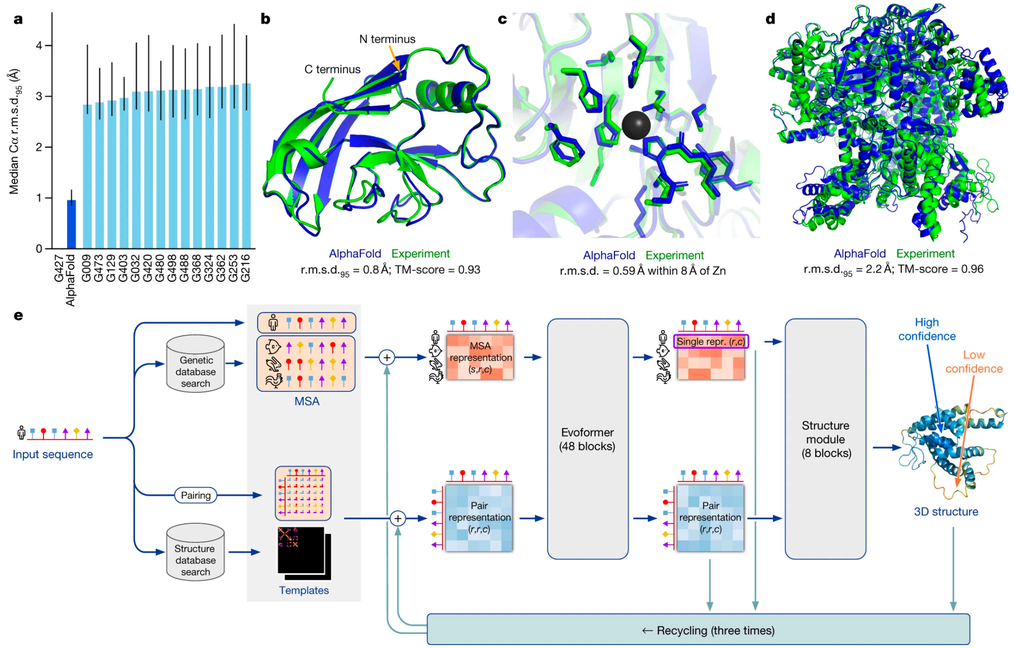
- Question:
  - What did you think of the papers?
  - Blog post?
  - AlphaFold2 vs. AFDB?
  - Any other thoughts/reflections?

Article | Open Access | Published: 15 July 2021

Highly accurate protein structure prediction with AlphaFold

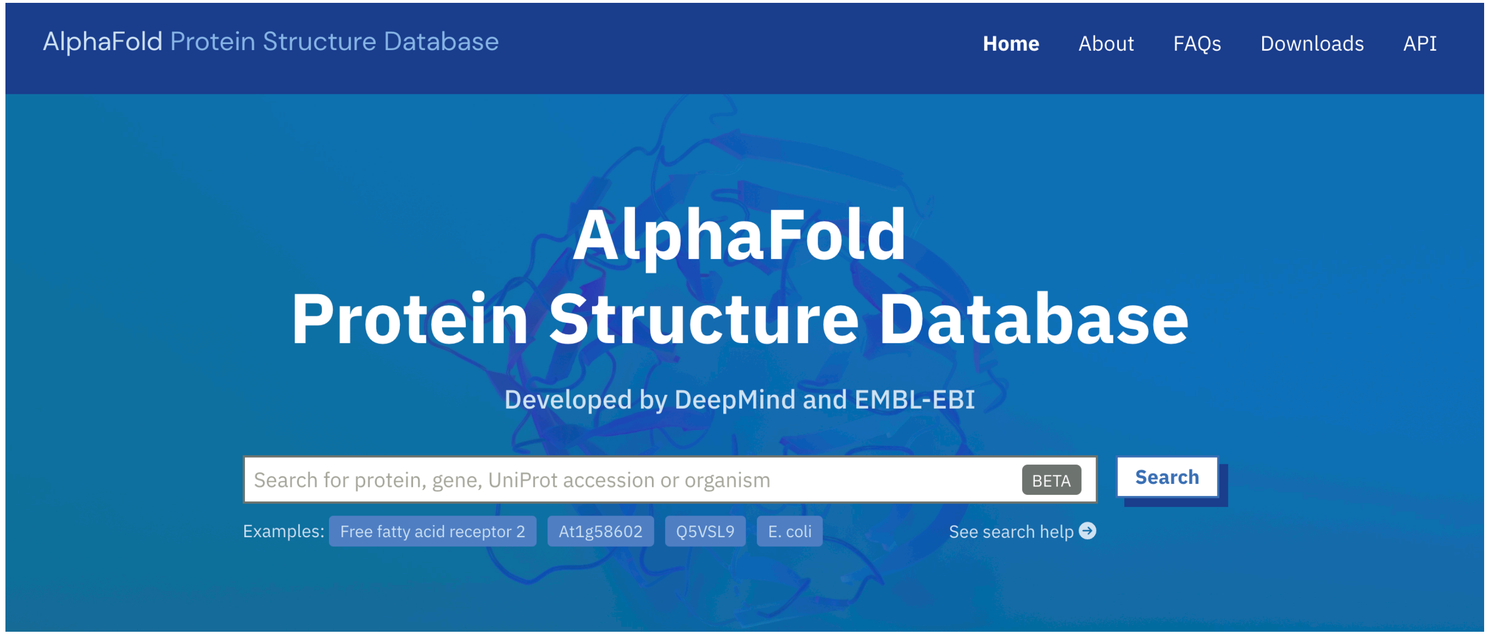
John Jumper, Richard Evans, Alexander Pritzel, Tim Green, Michael Figurnov, Olaf Ronneberger, Kathryn Tunyasuvunakool, Russ Bates, Augustin Žídek, Anna Potapenko, Alex Bridgland, Clemens Meyer, Simon A. A. Kohl, Andrew J. Ballard, Andrew Cowie, Bernardino Romera-Paredes, Stanislav Nikolov, Rishub Jain, Jonas Adler, Trevor Back, Stig Petersen, David Reiman, Ellen Clancy, Michal Zielinski, ... Demis Hassabis + Show authors

Nature **596**, 583–589 (2021) | Cite this article



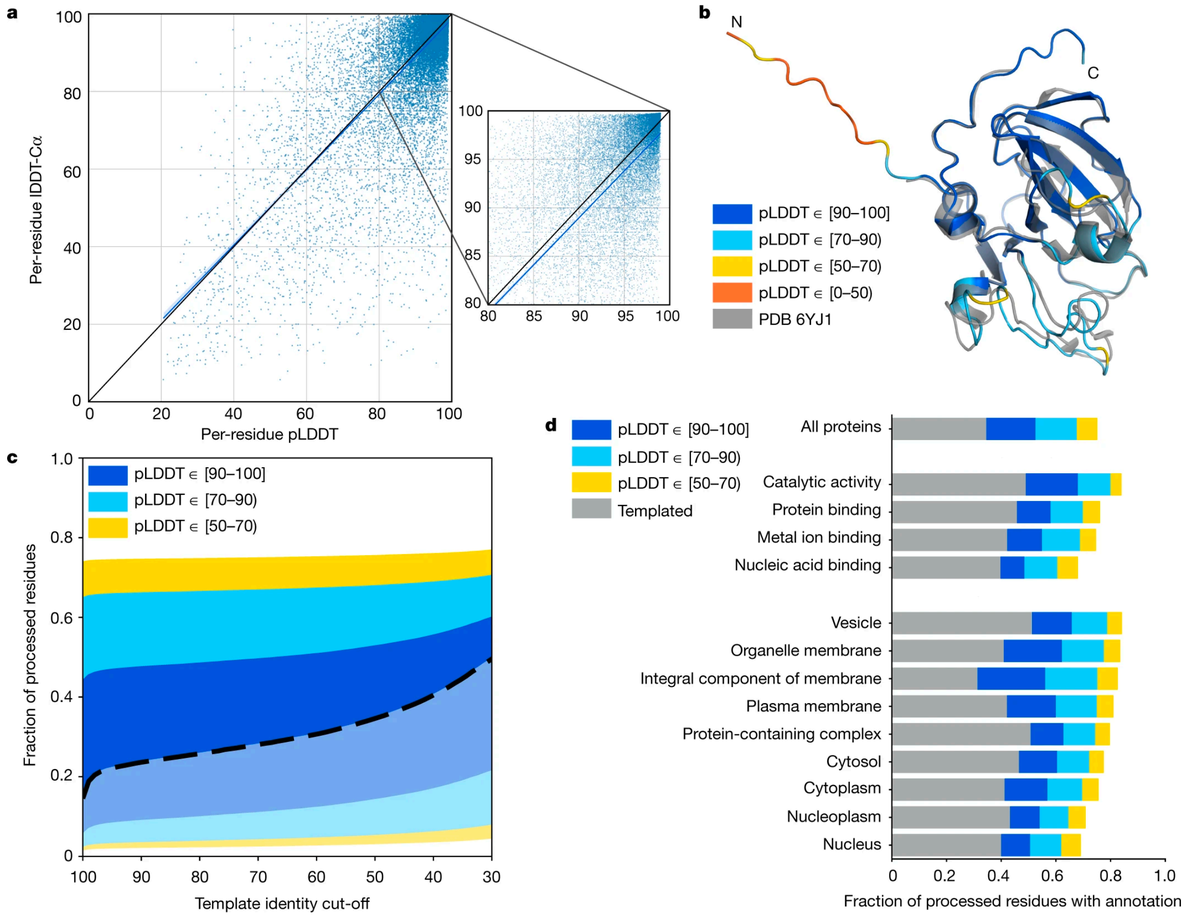
Highly accurate protein structure prediction for the human proteome

Tunyasuvunakool et al. Nature 2021



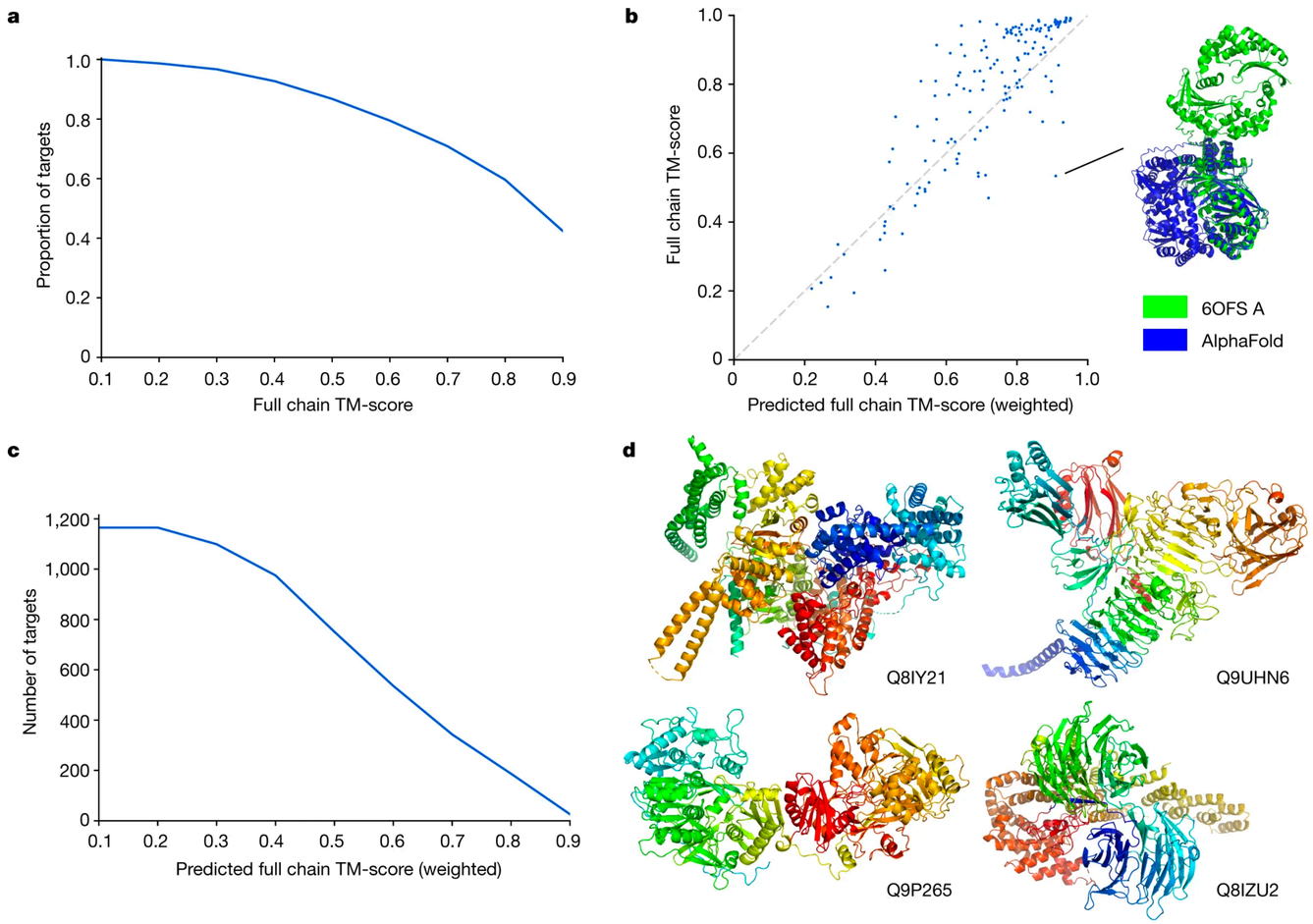
- The structures of around 100k unique proteins have been determined, but this represents a small fraction of the billions of known protein sequences
  - 17% of the total residues in human protein sequences are covered by an experimentally determined structure
- Here, they apply AlphaFold2 at proteome scales, covered 98.5% of the human proteins
  - 58% of residues have a confident prediction (pLDDT > 70)
  - 36% of residues have a highly confident prediction (pLDDT > 90)
- Identify strong multi-domain predictions, regions that are likely to be disordered
- Release of a database providing structural hypotheses

Fig. 1: Model confidence and added coverage



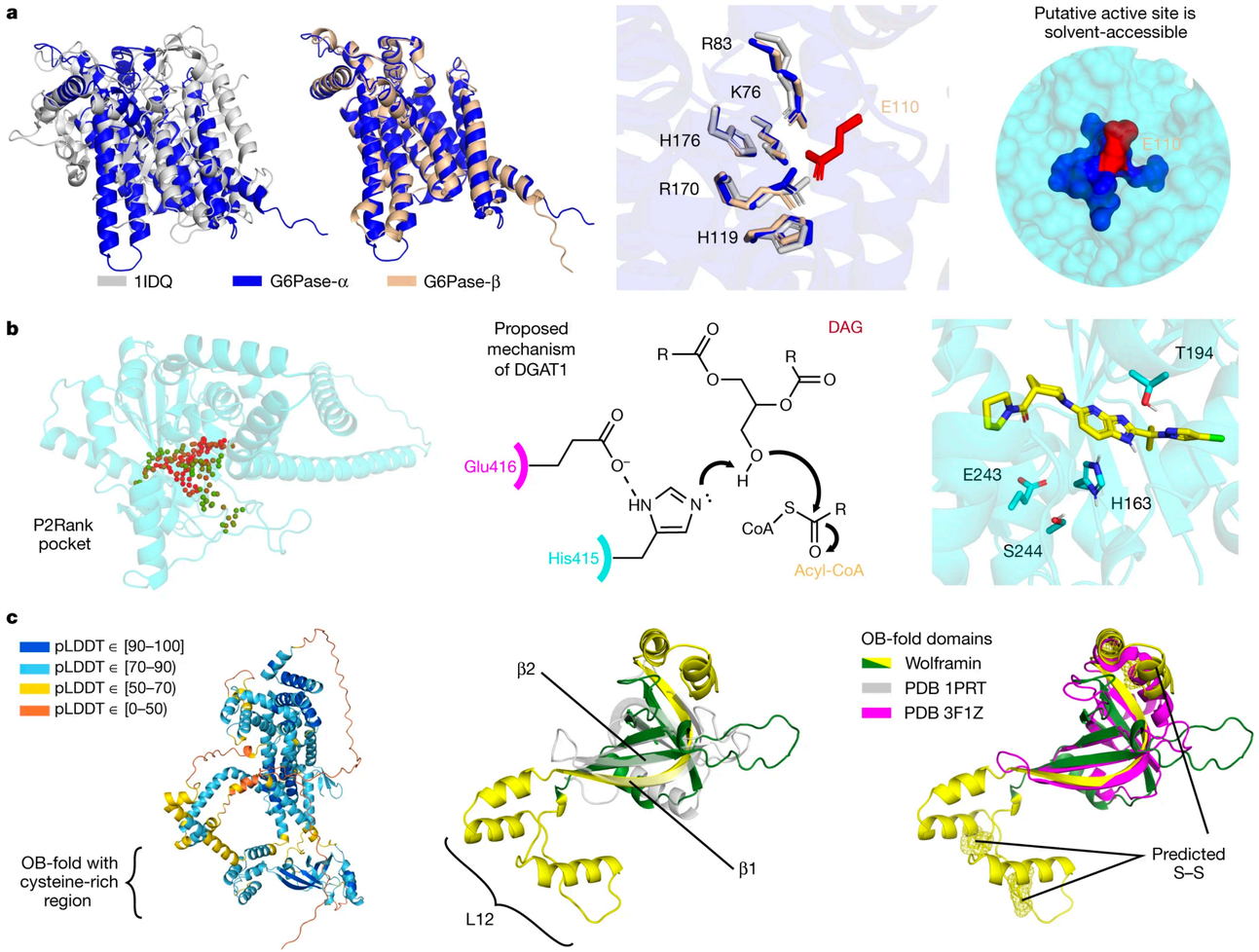
a, Correlation between per-residue pLDDT and lDDT-Cα. Data are based on a held-out set of recent PDB chains (Methods) filtered to those with a reported resolution of <3.5 Å (n = 10,215 chains and 2,756,569 residues). The scatterplot shows a subsample (1% of residues), with the blue line showing a least-squares linear fit and the shaded region a 95% confidence interval estimated with 1,000 bootstrap samples. The black line shows $x = y$, for comparison. The smaller plot is a magnified region of the larger one. On the full dataset, the Pearson's $r = 0.73$ and the least-squares linear fit is $y = (0.967 \pm 0.001) \times x + (1.9 \pm 0.1)$. **b**, AlphaFold prediction and experimental structure for a CASP14 target (PDB: 6YJ1)[64]. The prediction is coloured by model confidence band, and the N terminus is an expression tag included in CASP but unresolved in the PDB structure. **c**, AlphaFold model confidence on all residues for which a prediction was produced (n = 10,537,122 residues). Residues covered by a template at the specified identity level are shown in a lighter colour and a heavy dashed line separates these from residues without a template. **d**, Added residue-level coverage of the proteome for high-level GO terms, on top of residues covered by a template with sequence identity of more than 50%. Based on the same human proteome dataset as in **c** (n = 10,537,122 residues).

Fig. 2: Full chain structure prediction



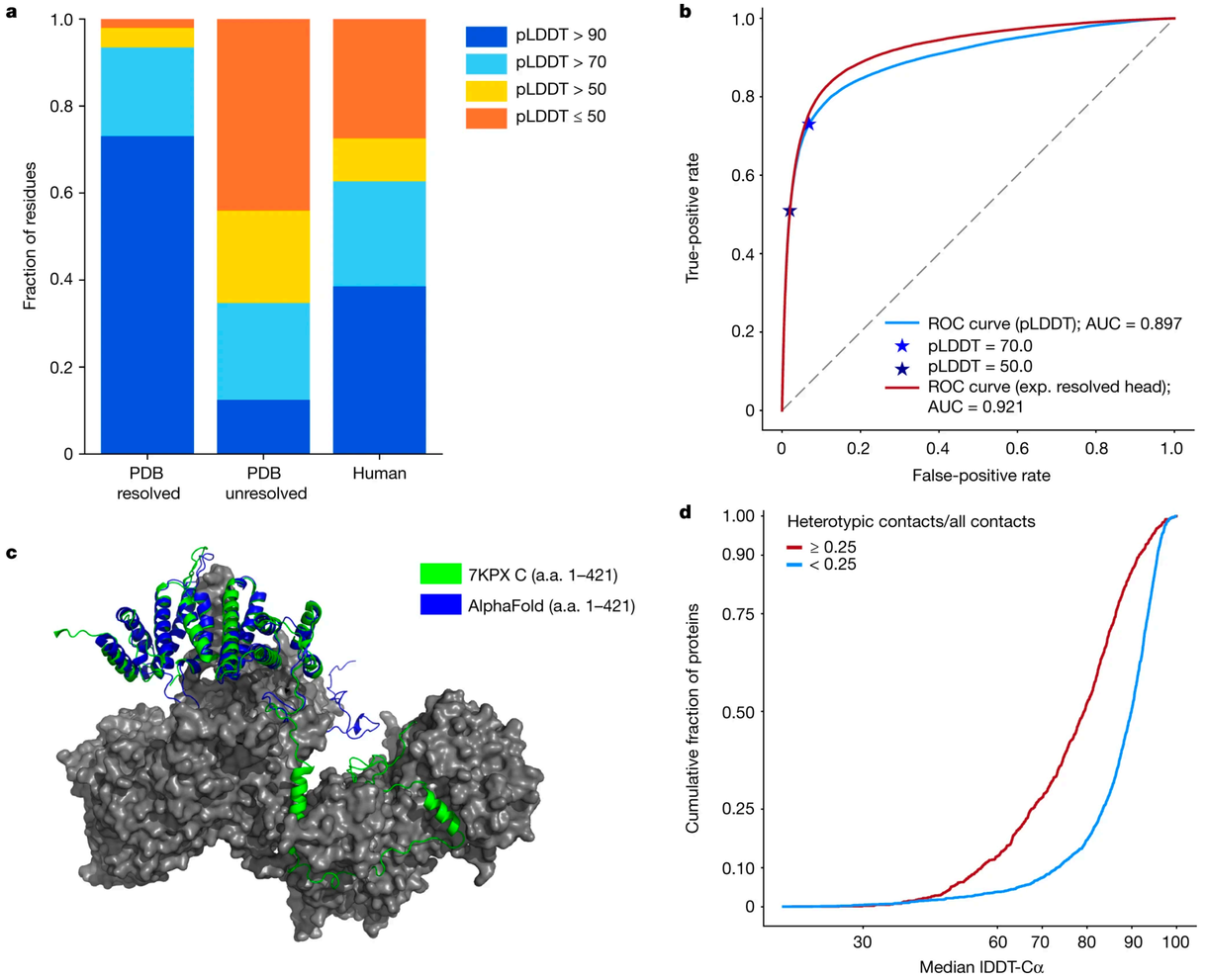
a, TM-score distribution for AlphaFold evaluated on a held-out set of template-filtered, long PDB chains ($n = 151$ chains). Includes recent PDB proteins with more than 800 resolved residues and best 50% coverage template below 30% identity. **b**, Correlation between full chain TM-score and pTM on the same set ($n = 151$ chains), Pearson's $r = 0.84$. The ground truth and predicted structure are shown for the most over-optimistic outlier (PDB: 6OFS, chain A). **c**, pTM distribution on a subset of the human proteome that we expect to be enriched for structurally novel multidomain proteins ($n = 1{,}165$ chains). Human proteome predictions comprise more than 600 confident residues (more than 50% coverage) and no proteins with 50% coverage templates. **d**, Four of the top hits from the set shown in **c**, filtering by pTM > 0.8 and sorting by number of confident residues. Proteins are labelled by their UniProt accession. For clarity, regions with pLDDT < 50 are hidden, as are isolated smaller regions that were left after this cropping.

Fig. 3: Highlighted structure predictions



a, Left, comparison of the active sites of two G6Pases (G6Pase-α and G6Pase-β) and a chloroperoxidase (PDB 1IDQ). The G6Pases are glucose-forming enzymes that contain a conserved, solvent-accessible glutamate (red; right) opposite the shared active-site residues (middle). **b**, Left, pocket prediction (P2Rank[65]) identifies a putative binding pocket for DGAT2, which is involved in body-fat synthesis. Red and green spheres represent the ligandability scores by P2Rank of 1 and 0, respectively. Middle, a proposed mechanism for DGAT1[51] activates the substrate with Glu416 and His415, which have analogous residues in the DGAT2 pocket. The docked inhibitor is well placed for polar interactions with His163 and Thr194 (right). The chemical structure (middle) is adapted from ref. 51. **c**, Predicted structure of wolframin, mutations in which cause Wolfram syndrome. Although there are regions in wolframin with low pLDDT (left), we could identify an OB-fold region (green/yellow), with a comparable core to a prototypical OB-fold (grey; middle). However, the most similar PDB chain (magenta; right) lacks the conserved cysteine-rich region (yellow) of our prediction. This region forms the characteristic β1 strand and an extended L12 loop, and is predicted to contain three disulfide bridges (yellow mesh).

Fig. 4: Low-confidence regions



a, pLDDT distribution of the resolved parts of PDB sequences (n = 3,440,359 residues), the unresolved parts of PDB sequences (n = 589,079 residues) and the human proteome (n = 10,537,122 residues). **b**, Performance of pLDDT and the experimentally resolved head of AlphaFold as disorder predictors on the CAID Disprot-PDB benchmark dataset (n = 178,124 residues). **c**, An example low-confidence prediction aligned to the corresponding PDB submission (7KPX chain C)[66]. The globular domain is well-predicted but the extended interface exhibits low pLDDT and is incorrect apart from some of the secondary structure. a.a., amino acid. **d**, A high ratio of heterotypic contacts is associated with a lower AlphaFold accuracy on the recent PDB dataset, restricted to proteins with fewer than 40% of residues with template identity above 30% (n = 3,007 chains) (Methods). The ratio of heterotypic contacts is defined as: heterotypic/(intra-chain + homomeric + heterotypic).