A Report on "Highly Accurate Protein Structure Prediction with AlphaFold" by Jumper et al. (2021)

Reviewer 2

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I am wiser than this person; for it is likely that neither of us knows anything fine and good, but he thinks he knows something when he does not know it, whereas I, just as I do not know, do not think I know, either. I seem, then, to be wiser than him in this small way, at least: that what I do not know, I do not think I know, either.

Plato, *The Apology of Socrates*, 21d

To err is human. All human knowledge is fallible and therefore uncertain. It follows that we must distinguish sharply between truth and certainty. That to err is human means not only that we must constantly struggle against error, but also that, even when we have taken the greatest care, we cannot be completely certain that we have not made a mistake.

Karl Popper, 'Knowledge and the Shaping of Reality'

Overview

Citation: Jumper, J., Evans, R., Pritzel, A., Green, T., Figurnov, M., Ronneberger, O., Tunyasuvunakool, K., Bates, R., Žídek, A., Potapenko, A., Bridgland, A., Meyer, C., Kohl, S. A. A., Ballard, A. J., Cowie, A., Romera-Paredes, B., Nikolov, S., Jain, R., Adler, J., Back, T., Petersen, S., Reiman, D., Clancy, E., Zielinski, M., Steinegger, M., Pacholska, M., Berghammer, T., Bodenstein, S., Silver, D., Vinyals, O., Senior, A. W., Kavukcuoglu, K., Kohli, P., & Hassabis, D. (2021). Highly Accurate Protein Structure Prediction with AlphaFold. *Nature*, Vol 596, pp. 583–589.

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Abstract Summary: This paper introduces AlphaFold, a novel neural network-based computational method designed to solve the protein folding problem by predicting 3*D* protein structures from amino acid sequences with atomic accuracy. AlphaFold demonstrated accuracy competitive with experimental methods in the challenging CASP14 assessment.

Key Methodology: Deep learning neural network (AlphaFold) architecture featuring Evoformer blocks, a structure module for end-to-end 3*D* coordinate prediction, iterative refinement (recycling), and training using self-distillation.

Research Question: Can a computational method predict protein structures from sequence with atomic accuracy, even when no similar structure is known?

Summary

Is It Credible?

This article by Jumper et al. presents AlphaFold, a deep learning system designed to predict the three-dimensional structure of proteins from their amino acid sequences. The authors posit that this method provides the "first computational method that can regularly predict protein structures with atomic accuracy," effectively addressing the structure prediction component of the decades-old "protein folding problem" (p. 583). The central claim is that AlphaFold achieves accuracy competitive with experimental methods, even in cases where no homologous structure is available. The primary evidence supporting this is the system's performance in the 14th Critical Assessment of protein Structure Prediction (CASP14), a blind test where AlphaFold achieved a median backbone accuracy of 0.96 Å (r.m.s.d.95), vastly outperforming the next best method which scored 2.8 Å (p. 584).

The credibility of the article's headline claims is exceptionally high, primarily due to the nature of the validation. The reliance on CASP14, a blind, external assessment, provides a robust shield against the overfitting and selection biases that often plague computational biology papers. The reported gap in performance between AlphaFold and competitor methods is large enough that minor methodological quibbles are unlikely to alter the qualitative conclusion that a step-change in capability has occurred. Furthermore, the authors validate the model on a large set of PDB structures released after the training period, demonstrating that the CASP results generalize to a broader distribution of proteins (p. 584). The inclusion of confidence metrics (pLDDT) that correlate well with actual error adds a layer of reliability, allowing users to trust the predictions when the model itself indicates high confidence.

However, the claim of "regularly" predicting structures with atomic accuracy requires careful qualification regarding the boundary conditions of the system. The

authors are transparent about these limitations, noting that accuracy "decreases substantially when the median alignment depth is less than around 30 sequences" (p. 588). This indicates that the method is not a solution for all proteins *ab initio*, but rather relies heavily on evolutionary history encoded in multiple sequence alignments (MSAs). Consequently, the system is less effective for orphan proteins or those with shallow alignments. Additionally, the article admits that the model is "much weaker for proteins that have few intra-chain or homotypic contacts," such as bridging domains in larger complexes (p. 588). This suggests that while the "protein folding problem" for single, well-conserved chains may be effectively solved, the broader challenge of predicting complex biological assemblies and proteins with limited evolutionary data remains open.

From a methodological standpoint, the architectural contributions—such as the Evoformer and the recycling mechanism—are plausible, though the evidence attributing specific gains to specific components is slightly weaker than the overall performance claims. The ablation studies provided in the supplementary material were conducted without re-tuning hyperparameters for the ablated models (Suppl. p. 51). As the authors acknowledge, this could exaggerate the apparent importance of removed components, as the baseline hyperparameters might simply be ill-suited for the simplified architectures. Furthermore, the training of the recycling mechanism involves stopping gradients between iterations (Suppl. p. 42), a biased approximation that theoretically deviates from ideal recurrent optimization. While the empirical success of the final model renders these points moot regarding the *utility* of AlphaFold, they introduce some uncertainty regarding the theoretical optimality of the specific architecture described.

Ultimately, Jumper et al. present a credible and transformative advance in structural biology. The limitations regarding MSA depth and protein complexes are disclosed transparently and define the current operating envelope of the technology rather than undermining its core achievement. The use of blind testing via CASP provides

the strongest possible evidence for the system's efficacy. While the precise contribution of each architectural sub-component is difficult to disentangle due to the lack of hyperparameter re-tuning in ablations, the aggregate performance of the system is indisputable.

The Bottom Line

The claim that AlphaFold achieves atomic-level accuracy in protein structure prediction is highly credible and supported by rigorous blind testing. However, this accuracy is conditional on the availability of sufficient evolutionary data (multiple sequence alignments) and applies primarily to single protein chains rather than complex heteromeric interactions. While the theoretical attribution of performance to specific network components is slightly obscured by methodological shortcuts in the ablation studies, the practical utility of the final model is firmly established.

Potential Issues

Acknowledged boundary conditions for high accuracy: The article's headline claims of "highly accurate" and "atomic accuracy" prediction are subject to several important boundary conditions that are disclosed in the text. The model's performance is conditional and may be significantly lower for certain classes of proteins. First, accuracy is critically dependent on the availability of evolutionary information, and "decreases substantially when the median alignment depth is less than around 30 sequences" (p. 588, Fig. 5a). This suggests the method may not be effective for orphan proteins or those from sparsely sequenced families. Second, the model is "much weaker for proteins that have few intra-chain or homotypic contacts compared to the number of heterotypic contacts," meaning it struggles with proteins whose structures are primarily defined by interactions with other protein chains in a complex (p. 588). Finally, the model is trained on the Protein Data Bank (PDB) and learns to predict structures that conform to the biases of this database. The authors state, "AlphaFold is trained to produce the protein structure most likely to appear as part of a PDB structure" (p. 588). This implies that its high accuracy may not generalize to proteins that are underrepresented in the PDB, such as intrinsically disordered proteins or those with significant conformational dynamics, which are often excluded from the validation sets by design (p. 589).

Methodological limitations of the ablation studies: The article's claims about the importance of specific architectural innovations are supported by a series of ablation studies, but the methodology used may exaggerate the impact of removing each component. The authors acknowledge in the supplementary information that when a component was removed, the model's hyperparameters were not re-tuned (Suppl. p. 51). Since the optimal hyperparameters for the full model are unlikely to be optimal for an architecturally different, ablated version, the performance of these ablated models may be artificially low. This makes it difficult to distinguish the true contri-

bution of a given component from the artifact of using suboptimal hyperparameters for the reduced model. The authors are transparent about this choice, stating that it "could make ablations appear more significant than in a properly tuned model," but this acknowledged limitation means the reported magnitudes of each component's contribution should be interpreted with caution (Suppl. p. 51).

Biased approximation in the training of the recycling feature: The "recycling" procedure, where the network's outputs are fed back as inputs for refinement over multiple iterations, is trained using a computationally efficient but biased approximation. To make the process tractable, gradients are prevented from flowing between recycling iterations during training (Suppl. pp. 41–42, Algorithm 31). This means the network is not optimized to produce outputs at one step that are ideal inputs for the next in a fully recurrent manner, but rather to produce a good final structure from a fixed input at each step. The authors acknowledge this methodological choice, stating that "stopping the gradients between iterations leads to a bias in the gradients," but assert based on their empirical results that this "does not hamper training" (Suppl. p. 42). While the model's overall success supports this empirical claim, the use of this biased training scheme is a notable deviation from a fully recurrent optimization.

Minor methodological and presentation issues: Several minor issues regarding the methodology and its presentation are noted. First, the loss function weights were determined through a "hand-selected and only lightly-tuned" process rather than a systematic optimization, a fact the authors disclose in the supplement (Suppl. p. 32). Second, the procedure for handling failures in the final Amber relaxation step for the CASP14 competition is ambiguously described; the supplement states that "targets with unresolved violations were re-run," without specifying what "re-run" entails (Suppl. p. 45). Finally, the article's highest-accuracy results for CASP14 were generated using a computationally intensive ensembled model, while the impressive speed claims are based on a faster, non-ensembled version with slightly lower accu-

racy. The article is transparent about this trade-off, but it creates a minor disconnect between the configurations used for the headline accuracy and speed claims (p. 589; Suppl. p. 60).

Future Research

Single-sequence prediction capabilities: Future work should focus on reducing the dependency on deep multiple sequence alignments (MSAs). Since the current model's accuracy degrades significantly with fewer than 30 sequences, research could investigate the integration of protein language models (PLMs) trained on massive sequence databases to replace or augment explicit MSAs. This would address the current limitation regarding orphan proteins and those from sparsely sequenced families.

End-to-end complex prediction: To address the identified weakness in predicting proteins dominated by heterotypic contacts, future research should extend the architecture to explicitly model protein-protein interactions within the end-to-end framework. Rather than predicting chains in isolation, training regimes that input multiple distinct sequences simultaneously could allow the network to learn interface geometry and folding constraints imposed by quaternary structure, moving beyond the single-chain paradigm.

Rigorous component analysis: To better understand the theoretical underpinnings of the architecture, future studies should perform rigorous ablation analyses where hyperparameters are re-tuned for each ablated variant. This would isolate the true contribution of mechanisms like the Invariant Point Attention (IPA) and recycling, distinguishing fundamental architectural necessities from artifacts of the specific hyperparameter configuration used in the final model.

Conformational ensemble prediction: Since the model is trained to predict the single structure most likely to appear in the PDB, it may be biased toward crystal packing artifacts or rigid states. Future research should investigate methods to modify the network to predict conformational ensembles or alternative states, perhaps by stochastically sampling the MSA or latent representations, to capture the dynamic

nature of proteins that is currently smoothed over by the training objective.

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