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Evolutionary constraints guide AlphaFold2 in predicting alternative conformations and inform rational mutation design

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Investigating structural variability is essential to understand protein biological functions. Although AlphaFold2 accurately predicts static structures, it fails to capture the full spectrum of functional states. Recent methods have used AlphaFold2 to generate diverse structural ensembles, but they offer limited interpretability and overlook the evolutionary signals underlying predictions. In this work, we enhance the generation of conformational ensembles and identify sequence patterns that influence alternative fold predictions for several protein families. Building on prior research that clustered Multiple Sequence Alignments to predict fold-switching states, we introduce a refined clustering strategy that integrates protein language model representations with hierarchical clustering, overcoming limitations of density-based methods. Our strategy effectively identifies high-confidence alternative conformations and generates abundant sequence ensembles, providing a robust framework for applying Direct Coupling Analysis (DCA). Through DCA, we uncover key coevolutionary signals within the clustered alignments, leveraging them to design mutations that stabilize specific conformations, which we validate using alchemical free energy calculations from molecular dynamics. Notably, our method extends beyond fold-switching, effectively capturing a variety of conformational changes.

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