A126 · Deconvolving mutation effects on protein stability and function with disentangled protein language models

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Understanding how evolutionary constraints shape protein sequences is fundamental to deciphering the molecular mechanisms underlying protein stability and function, with broad implications for protein engineering and therapeutic development. Recent advances in protein language models (pLMs) have enabled accurate prediction of mutation effects, capturing the evolutionary pressure that governs protein sequence variation. A critical challenge, however, remains in disentangling the intertwined mutation effects on protein stability and function, as evolutionary signals conflate stability-driven and function-driven pressures, obscuring the mechanistic basis of mutation effects. In this work, we introduce DETANGO, a deep learning framework that explicitly deconvolves the mutation effects on protein functions by removing components attributable to stability perturbations from pLM-predicted mutation effects. DETANGO estimates a functional plausibility score for each single-point mutation that is the component of the mutation effect not accounted for by changes in stability. Single-point mutations with low functional plausibilities are predicted to be stable-but-inactive (SBI) variants, whose compromised activities are caused by direct perturbations on functional mechanisms rather than stability. Residues enriched for such variants are inferred to be functionally critical, as indicated by the strong pressures to maintain protein function. Through extensive benchmarking experiments, we show that DETANGO accurately identifies SBI variants and pinpoints functional sites across contexts, including ligand binding, catalysis, and allostery. Moreover, extending DETANGO from individual proteins to homologous protein families reveals shared and distinctive functional patterns across protein families. Collectively, these results establish DETANGO as a biologically grounded framework for disentangling evolutionary constraints and advancing mechanistic understanding of protein function.

Deconvolving mutation effects on protein stability and function with disentangled protein language models

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Introduction. Proteins evolve under coupled constraints to maintain structural stability while enabling diverse molecular functions such as catalysis, binding, and allosteric signaling. Mutation effects on stability and function can be concordant, where destabilization reduces abundance and thereby disrupts activity, or be tradeoffs, where activity-enhancing or specificity-shifting substitutions incur modest stability penalties, particularly in locally frustrated neighborhoods near active or binding sites[1]. Disentangling the intertwined effects of amino acid substitution on stability and function is central to understanding the protein sequence-structure-function relationship. Residues at which most substitutions affect function but not stability, termed 'stable but inactive' (SBI) variants[2], are often critical for mediating molecular interactions and other functional processes. While recent advances in protein language models (pLMs) have enabled accurate prediction of mutation effects through evolutionary information, most existing computational approaches collapse the distinct effects into a single deleteriousness score, obscuring mechanisms by which mutations impair function.

Methods. Here, we introduce DETANGO, a disentangled pLM that explicitly deconvolves the selective pressures shaping protein residues into function- and stability-driven constraints. We formalize mutation effect disentanglement by positing a multiplicative factorization of the evolutionary probability of a protein sequence $\boldsymbol{x}$ into structural and functional components:

$$p_e(\boldsymbol{x}) = p_s(\boldsymbol{x})p_f(\boldsymbol{x}), \tag{1}$$

where $p_e(\boldsymbol{x})$ denotes the evolutionary probability captured by pLMs as pseudo-likelihood, $p_s(\boldsymbol{x})$ the structural probability, and $p_f(\boldsymbol{x})$ the functional probability. For each single-mutation mutant sequence $\boldsymbol{x}^{\mathrm{MT}}$ of the target wild-type sequence $\boldsymbol{x}^{\mathrm{WT}}$, we define its *functional plausibility*, the component of the mutation effect specifically attributable to protein function, as the log functional probability difference between the wild-type protein sequence $\boldsymbol{x}^{\mathrm{WT}}$ and the mutant sequence $\boldsymbol{x}^{\mathrm{MT}}$:

$$f(\boldsymbol{x}^{\mathrm{MT}}) \triangleq \log \frac{p_f(\boldsymbol{x}^{\mathrm{MT}})}{p_f(\boldsymbol{x}^{\mathrm{WT}})} \triangleq \log \frac{p_e(\boldsymbol{x}^{\mathrm{MT}})}{p_e(\boldsymbol{x}^{\mathrm{WT}})} - \log \frac{p_s(\boldsymbol{x}^{\mathrm{MT}})}{p_s(\boldsymbol{x}^{\mathrm{WT}})} = e(\boldsymbol{x}^{\mathrm{MT}}) - s(\boldsymbol{x}^{\mathrm{MT}}), \tag{2}$$

where evolutionary plausibility $e(\boldsymbol{x}^{\mathrm{MT}}) = \log p_e(\boldsymbol{x}^{\mathrm{MT}}) - \log p_e(\boldsymbol{x}^{\mathrm{WT}})$ refers to the overall pLM-predicted mutation effect and structural plausibility $s(\boldsymbol{x}^{\mathrm{MT}}) = \log p_s(\boldsymbol{x}^{\mathrm{MT}}) - \log p_s(\boldsymbol{x}^{\mathrm{WT}})$ denotes the component attributable to changes in structural stability. Single-point mutations with low functional plausibility scores tend to directly perturb functional mechanisms and thus correspond to SBI variants. Residues enriched with such variants are likely to be functionally important residues. To derive functional and structural plausibilities, DETANGO decomposes the pLM representation into stability and function components, where the stability component is constrained to predict stability measurements (e.g., $\Delta\Delta G$ or cellular abundance), and the function component captures the residual signal beyond stability. Functional plausibility is inferred from this function representation, while structural plausibility is modeled from stability measurements. The training objective enforces that their combination reconstructs the original pLM likelihood, ensuring faithful decomposition of evolutionary signals.

Results. In evaluations, DETANGO accurately identifies SBI variants and outperforms post hoc heuristics that combine conservation with stability predictions, highlighting its effectiveness to mechanistically disentangle mutation effects. Across all proteins in Human Domainome 1[3], DETANGO assigned significantly higher function scores to functional sites compared to unannotated sites. The resulting functional-site maps of DETANGO generalize across contexts, including ligand binding (DNA, RNA, peptide, and small molecules), catalysis, and allostery. DETANGO substantially outperforms existing methods that do not disentangle stability from function in functional site identification and, despite being trained without residue-level functional annotations, achieves performance comparable to or even exceeding supervised baselines trained on annotated functional sites. Extending from individual proteins to homologous families, DETANGO reveals both shared and distinctive functional site patterns across families, highlighting differentially conserved residues that modulate functional specificity within each family and offering mechanistic insights into functional adaptation of protein families during evolution.

Availability: This work has been accepted to RECOMB 2026 and is available as a preprint at `https://doi.org/10.64898/2026.02.03.703560`. Code is available at `https://github.com/luo-group/DETANGO`.

References

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