PROTEUS-REPAIR

Compensatory mutations in protein stability

James Hong & Adam Shen
Independent student research | Exploratory analysis | Not peer reviewed

RESEARCH QUESTION

When a second mutation improves the stability of a damaged protein variant, how much reflects the partner's own effect, and how much reflects a specific interaction? We re-examine a candidate screen derived from published folding-stability measurements.

AVAILABLE EVIDENCE

26	16	4
Selected candidates	Domain identifiers	Negative standalone effects

Counts were recomputed from the existing selected-candidate summary. They describe a selected subset, not the rescue prevalence of the full experimental library.

ILLUSTRATIVE CANDIDATES FROM THE SUMMARY

Domain / partner	Both methods / tested	Standalone effect (kcal/mol)
3DKM / N56G	14 / 14	+0.438
4G3O / D28C	14 / 15	+0.030
2K28 / E16F	15 / 17	-0.132
2K5N / R27Y	12 / 27	-0.323

Positive effects indicate stabilization under the project convention. Standalone effects are medians; passing fractions are descriptive, not general repair probabilities.

INTERPRETATION AND LIMITS

The supplied 2K28 matrix reports E16F/W/Y passing on the same 15 of 17 R9 substitutions. All 17 backgrounds occur at **one damaged position**. The methods share underlying measurements, so their agreement is not independent replication. Matched alternative-partner base rates, per-combination interaction effects, and dependence-calibrated significance remain unresolved.

The original same-site comparison controls protein, damaged substitution, and partner position. Its small conditional p-values still rely on exchangeability and independence assumptions. No new experimental validation or general-purpose repair mechanism is claimed.

NEXT VALIDATION STEPS

1. Reconstruct all tested combinations and complete event definitions. **2.** Estimate continuous interaction effects with shared-measurement uncertainty. **3.** Calibrate a structured null and evaluate held-out positions or domains where feasible. **4.** Plan independent wild-type, single-mutant, and double-mutant stability measurements with a laboratory partner.

MATERIALS AND CORRESPONDENCE

proteusrepair.pages.dev - candidate data, interactive explanations, audit code, and provenance.
Project contact: jameshongbc@gmail.com

Experimental source: Tsuboyama, K. et al. *Mega-scale experimental analysis of protein folding stability in biology and design.* Nature **620**, 434-444 (2023). doi:10.1038/s41586-023-06328-6. Experimental credit belongs to the original investigators; this brief describes a student secondary analysis.