Expanding mutational screens from single proteins to entire proteomes

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How can we accelerate variant interpretation across the proteome?

Deep mutational scanning

Many mutations in a single protein

Miro

Mutations across the proteome

Miro: a method that measures the impact of mutations across the proteome

1. Non-canonical amino acids
2. tRNA charging
3. Proteome-wide mistranslation
4. Collection of protein variants
5. Biochemical selections with mass spec readout

- Thermal stability
- Solubility
- Turnover
- Enzymatic activity
- Small molecule binding
- Protein interactions

Analogs of interest

E1
F2
P1
W1

Measuring variant stability

Mistranslated proteome

30°C control treatment + Temperature gradient (46 to 58)

Quantify stability ratios ($R_s$)

$R_s = \log_{10} \frac{[\text{Variant}]_{\text{temp. grad.}}}{[\text{Variant}]_{30^\circ C}}$

Global impact of mistranslation

Destabilizing
No variants
Stabilizing

Yeast actin

(pdb: 1YAG)

E334 mediates actin's interaction with coflin

P332 is conserved in humans. P332A causes deafness, suggesting a critical role in actin stability