

Figure 5: Methodological Comparison - Challenging Furukawa et al.

A Furukawa et al. (2022) Method

PAAS Score

Sequence Conservation

Assumed Specificity

B Our AlphaFold3 Method

Ancestral Sequence
AlphaFold3 Structure Modeling

Measured Binding ipTM

Conservation ≠ Discrimination

C

Modern ProRS



Tight pocket
High specificity

LUCA ProRS



Larger pocket
Promiscuous

Conserved binding residues

D

Key Insight:

Furukawa et al. inferred ancestral specificity from sequence conservation. However, conserved residues can form a LARGER binding pocket in ancestors.

Our structural modeling reveals:

- LUCA ProRS: 89.7% promiscuity (THR binds at 89.7% of PRO affinity)
 - Eukaryotic ancestor: 84.8% promiscuity
 - Modern human: 97.5% promiscuity

Conclusion: Ancient translation systems tolerated high error rates. Conservation of residues does not imply conservation of specificity.