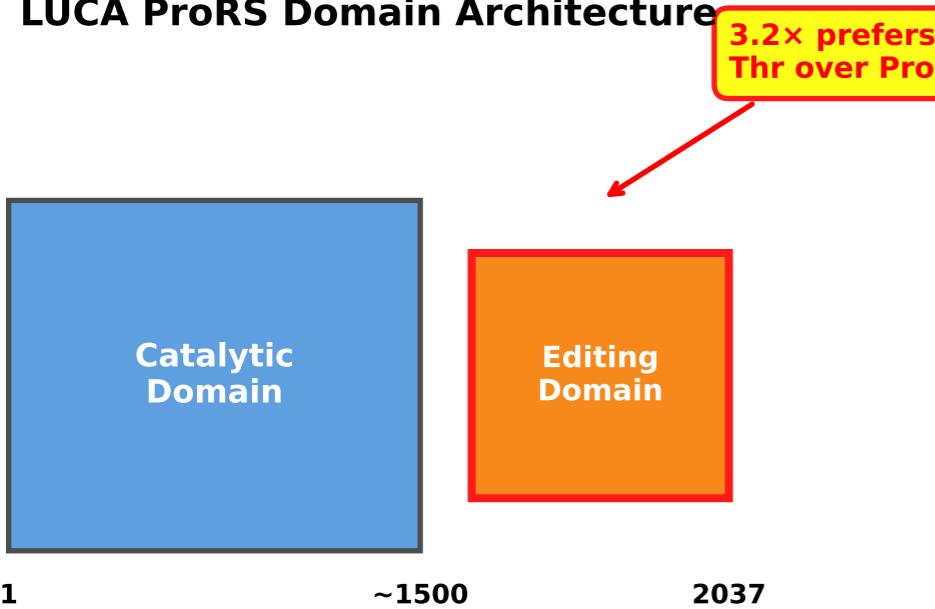


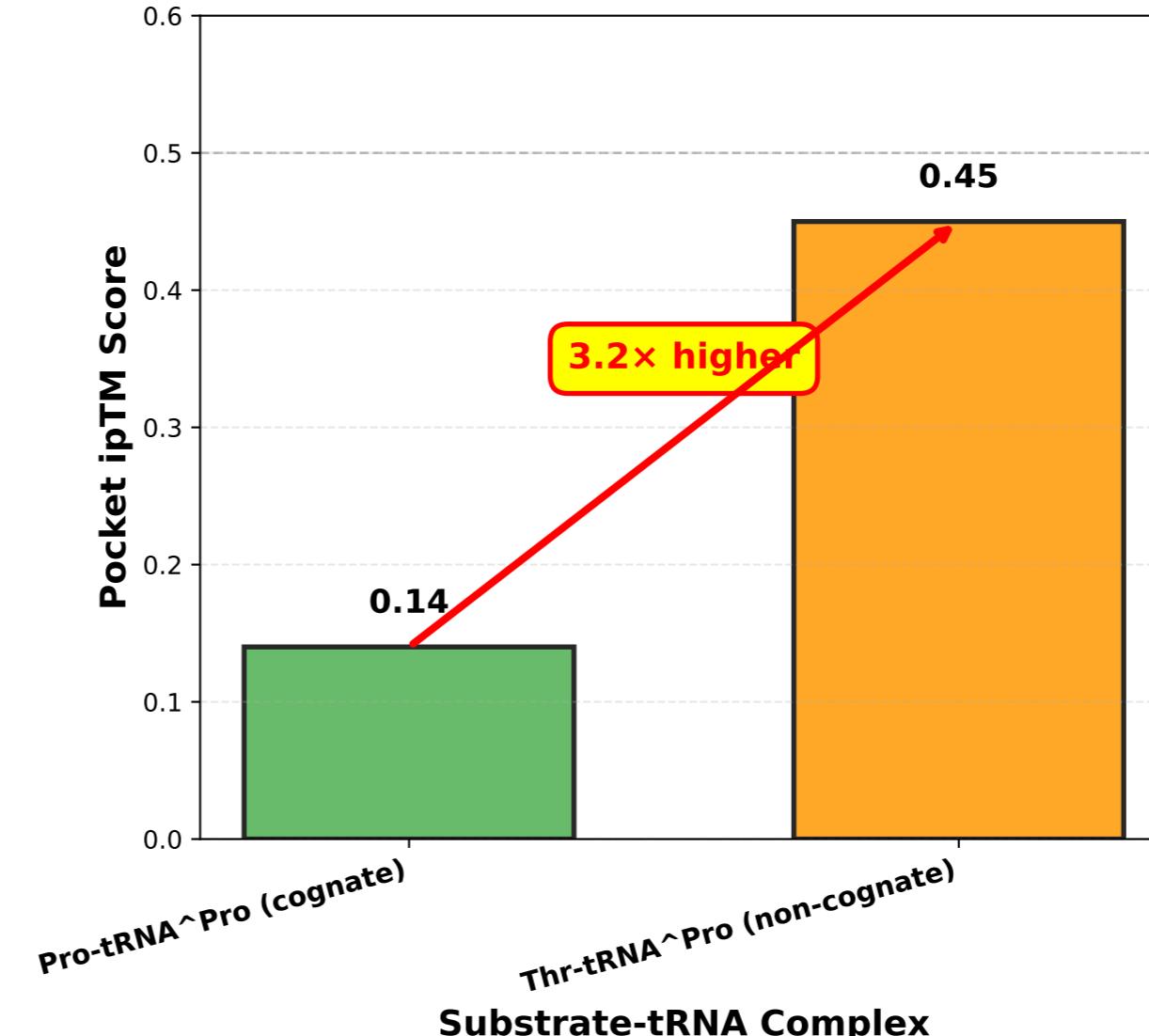
Figure 3: Ancestral Editing Domain Displays Inverted Substrate Preference

A. Domain Architecture

LUCA ProRS Domain Architecture



B. Editing Domain Shows 3.2x Preference for Non-Cognate Substrate



INVERTED PREFERENCE:

Editing domain **PREFERS** the **WRONG** substrate ($\text{Thr-tRNA}^{\text{Pro}}$) over the correct one ($\text{Pro-tRNA}^{\text{Pro}}$).

This is **OPPOSITE** to the catalytic domain, which binds both equally (promiscuity).

BIOLOGICAL FUNCTION:
The editing domain's job is to **HYDROLYZE** mis-acetylated tRNA ($\text{Thr-tRNA}^{\text{Pro}}$), so it **SHOULD** preferentially bind the error product, not the correct product.

This inverted preference validates the editing domain's proofreading function in ancestral ProRS.