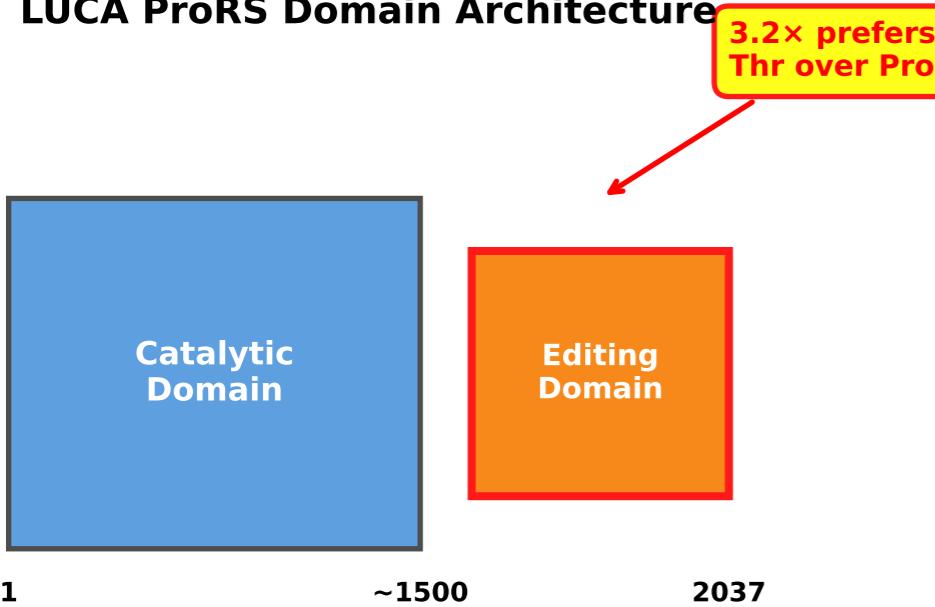


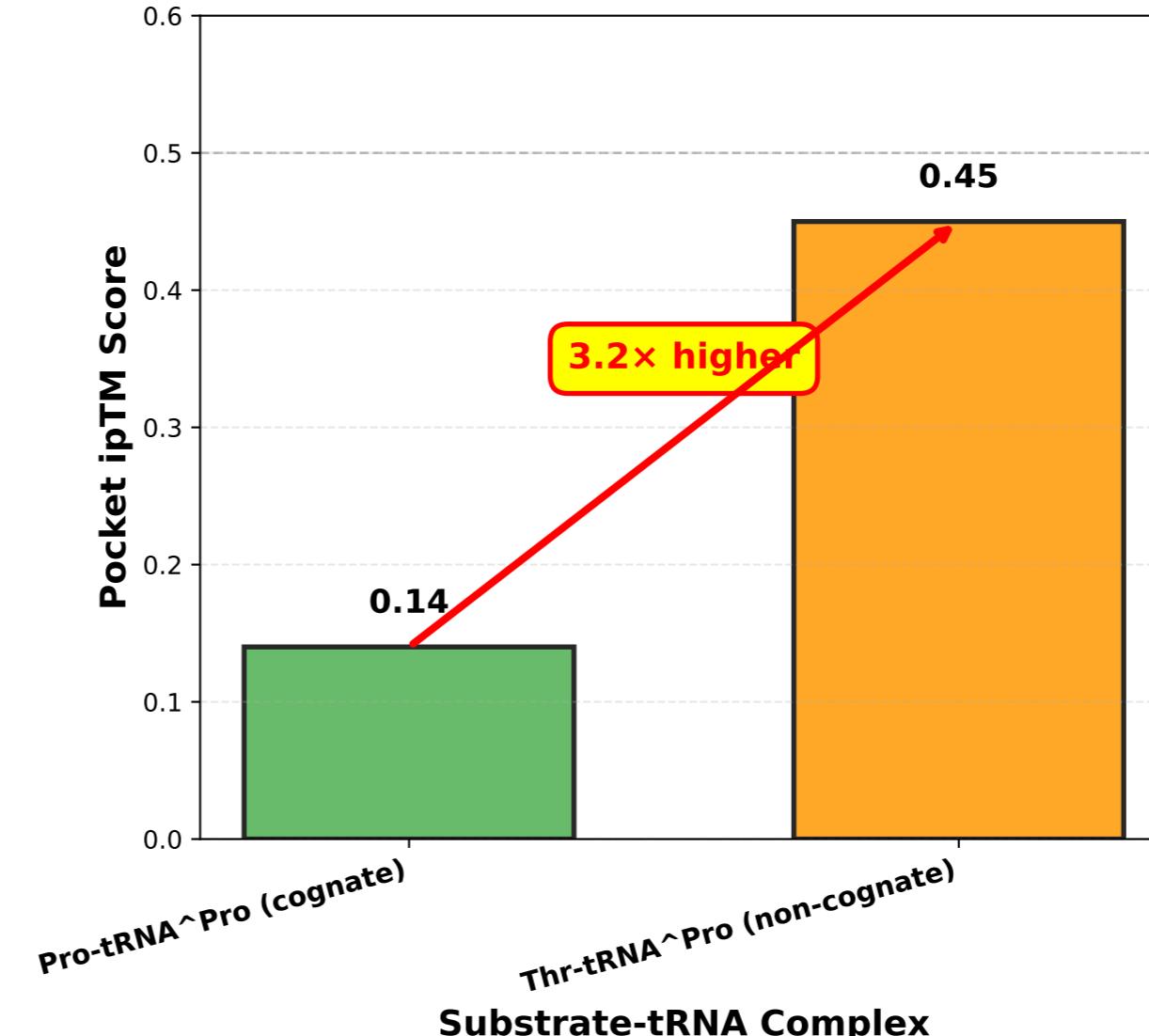
**Figure 3: Ancestral Editing Domain Displays Inverted Substrate Preference**

**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.