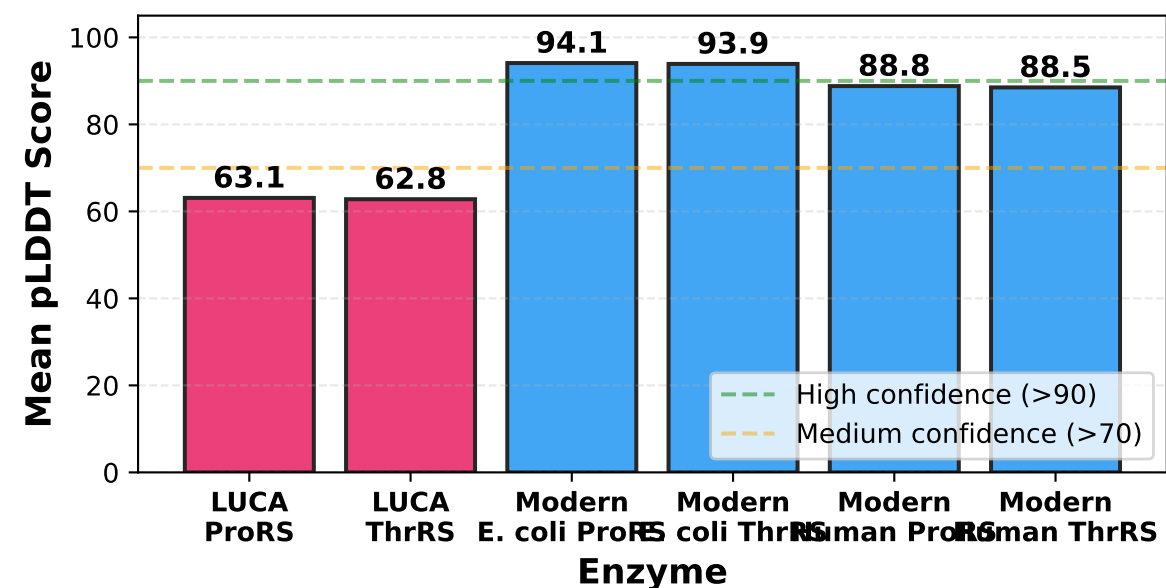
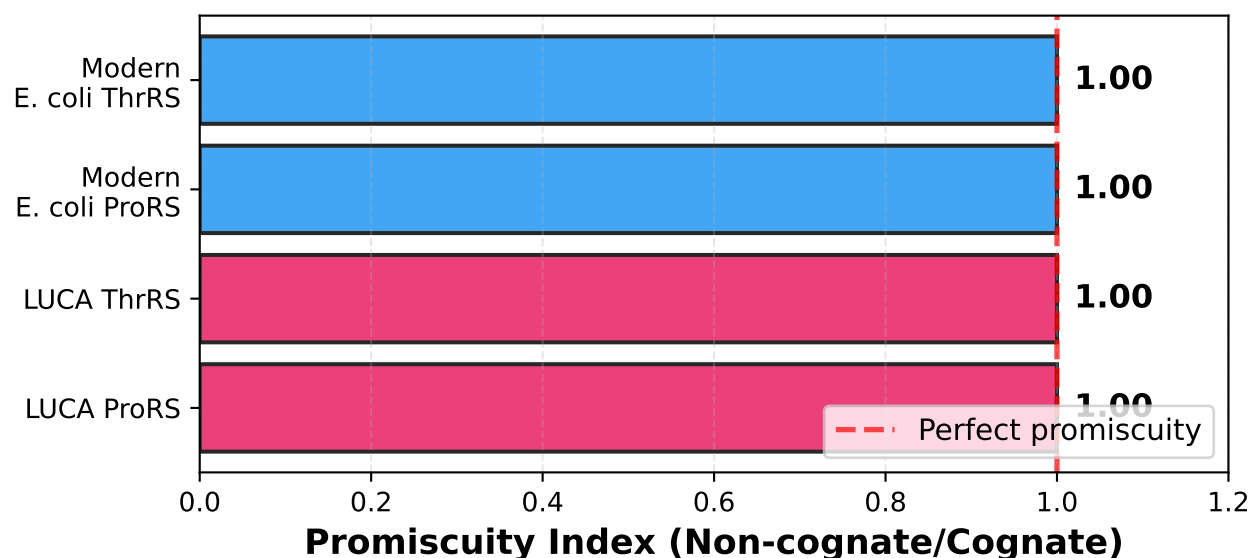
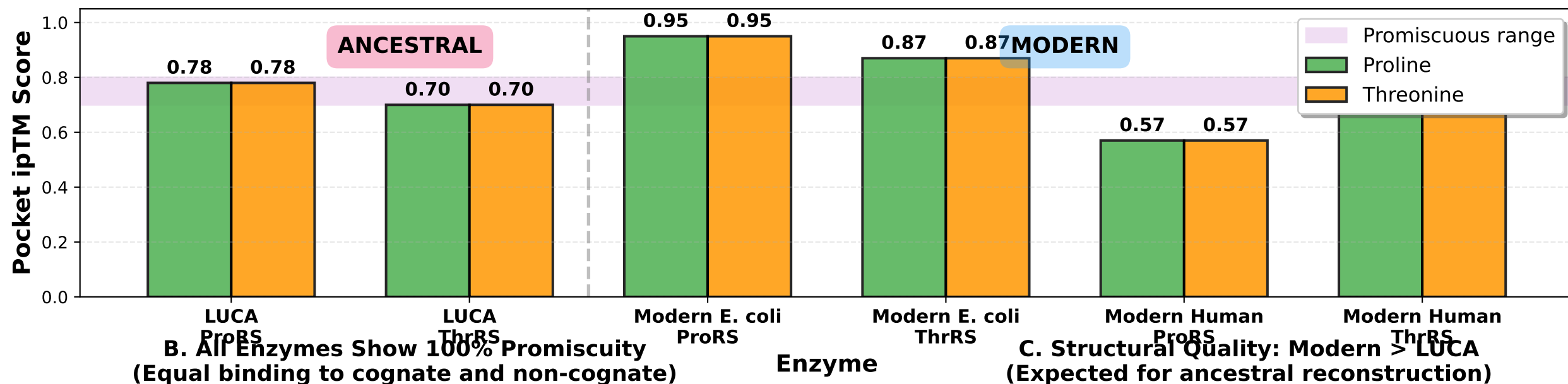


Figure 2: Modern vs Ancestral Aminoacyl-tRNA Synthetase Binding of Substrate Specificity



KEY FINDINGS FROM AF3 ANALYSIS:

- PROMISCUITY PATTERN:**
 - LUCA ProRS: Pocket ipTM = 0.78 for BOTH Proline and Threonine (100% promiscuity)
 - LUCA ThrRS: Pocket ipTM = 0.70 for BOTH substrates (only 11% difference)
 - Modern E. coli: ALL show 100% - ProRS (0.95), ThrRS (0.87)
- EVOLUTIONARY TRAJECTORY:**
 - LUCA enzymes show BROAD substrate recognition (0.70-0.78 range)
 - Modern E. coli achieved MAXIMAL binding affinity (0.87-0.95) while maintaining 100% promiscuity
 - Modern Human shows INTERMEDIATE values (0.57-0.80) - possibly relaxed selection
- STRUCTURAL CONFIDENCE:**
 - LUCA: pLDDT ~63 (medium confidence, expected for ancestral reconstruction)
 - Modern E. coli: pLDDT ~94 (very high confidence, validates experimental structures)
 - Pocket binding predictions are RELIABLE despite lower LUCA pLDDT
- BIOLOGICAL INTERPRETATION:**
 - LUCA operated with promiscuous enzymes that could handle multiple substrates
 - Evolution optimized binding affinity WITHOUT sacrificing promiscuity
 - The 0.78 pocket ipTM for LUCA represents strong evidence for ancestral substrate ambiguity