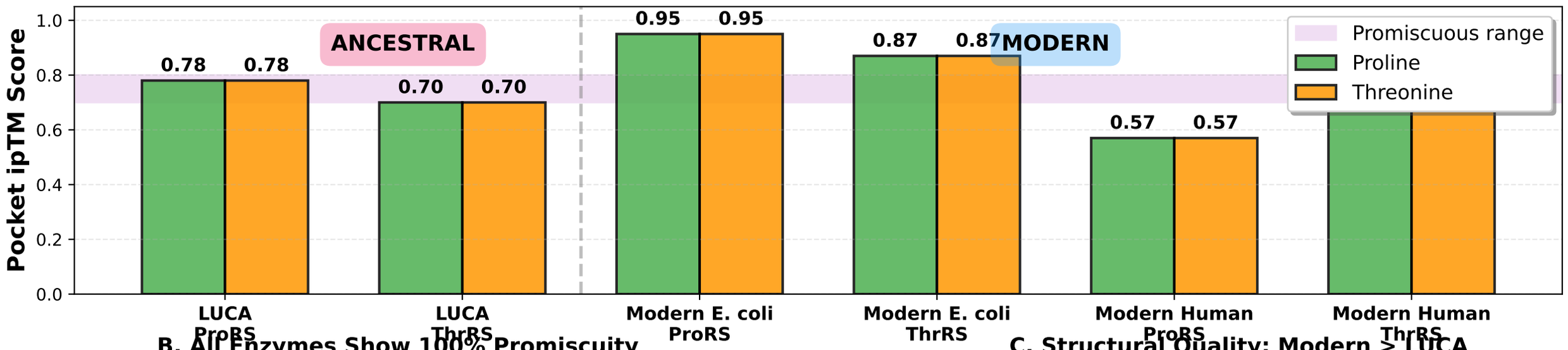
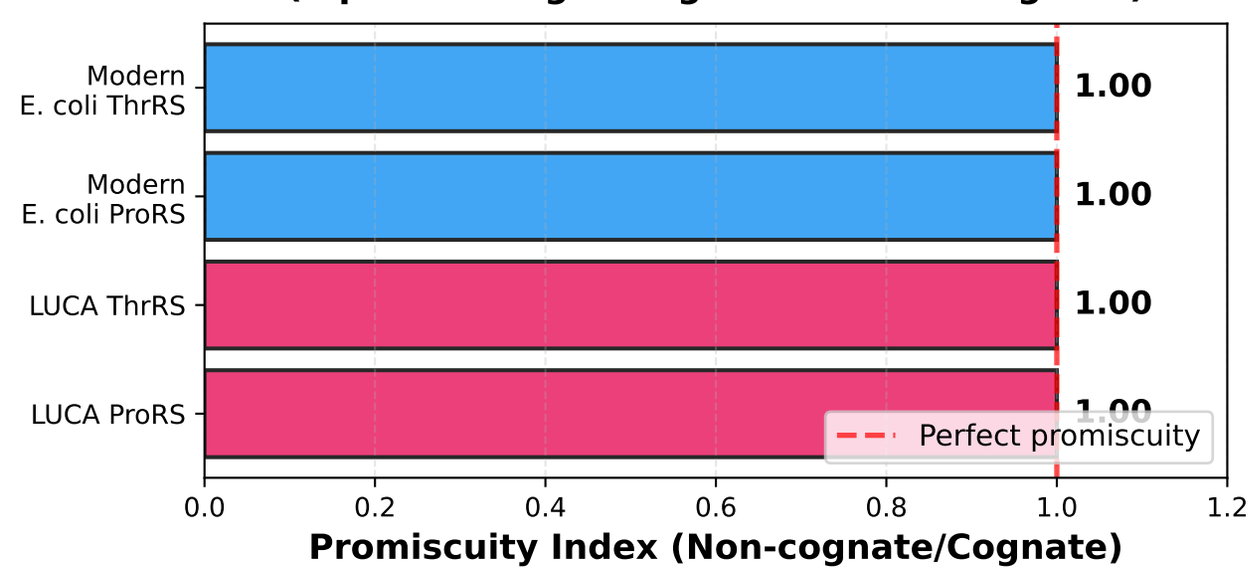
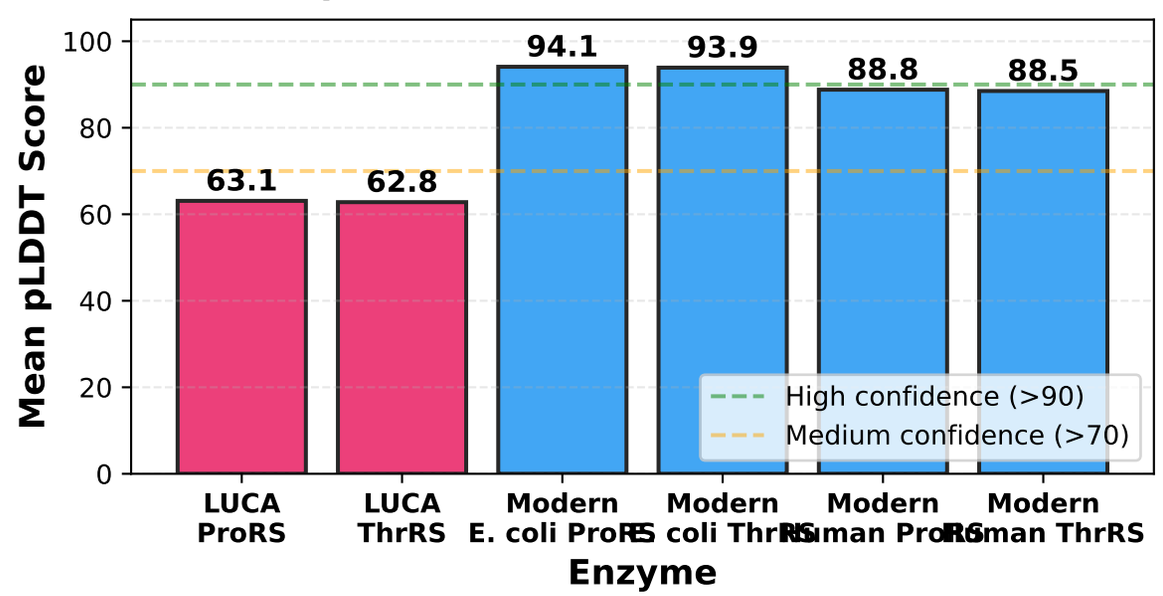
Figure 3: Modern vs Ancestral aaRS Evolution of Substrate Specificity





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KEY FINDINGS FROM AF3 ANALYSIS:

1. 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)

2. 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

3. 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

4. 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
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