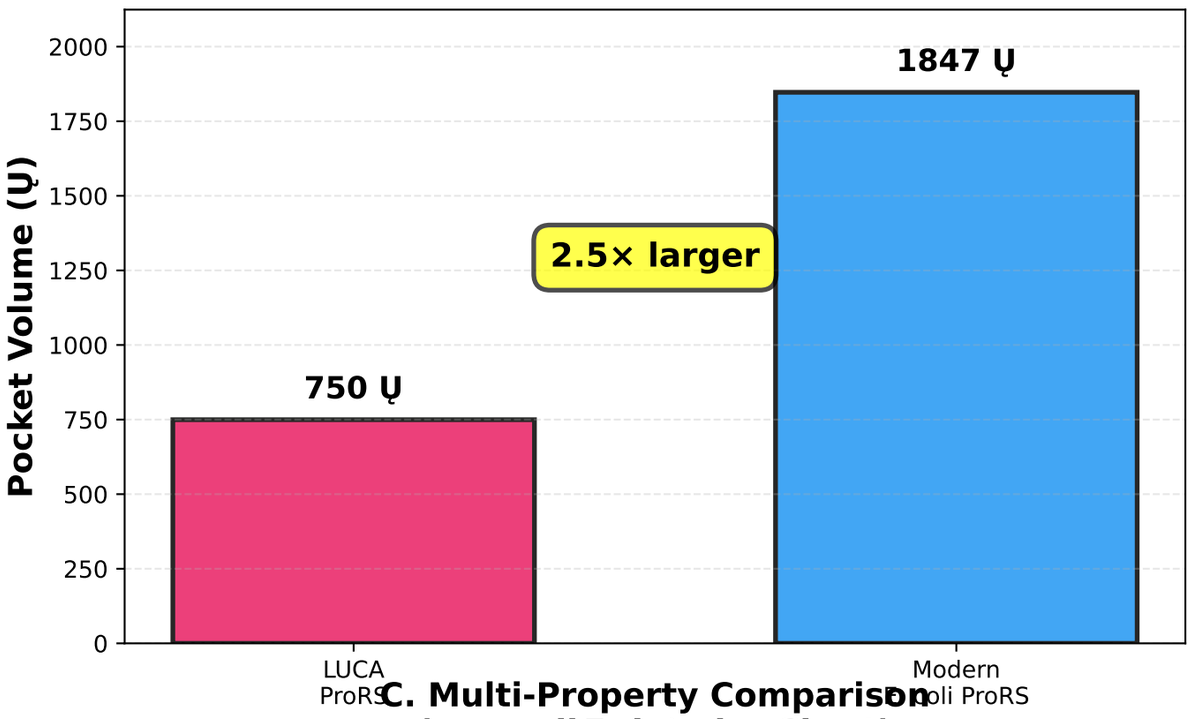
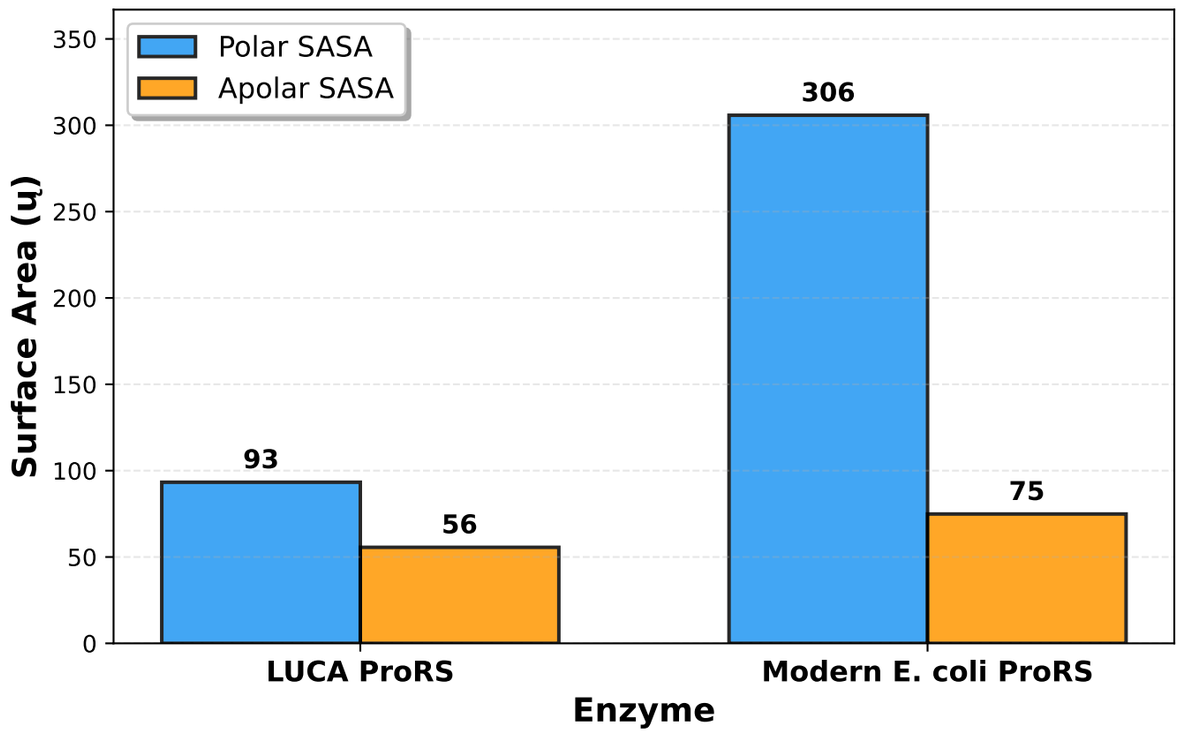
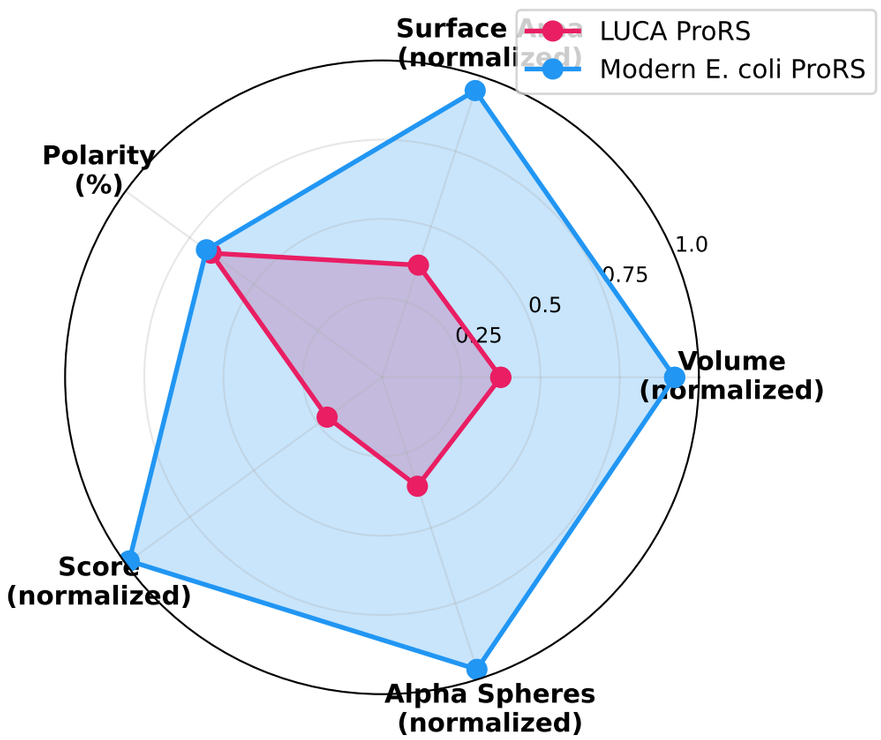
Figure 6: Pocket Binding Pocket Analysis - Evolution from LUCA to Modern







D. Summary Table: Modern Pocket is Larger & Higher Quality

Property	LUCA ProRS	Modern E. coli	Fold Change
———	———	———	———
Volume (Ų)	750	1847	2.5×
Total SASA (ų)	149	381	2.6×
Polar SASA (ų)	93	306	3.3×
Polarity (%)	66.7	68.5	~Equal
fpocket Score	0.32	1.48	4.6×
Alpha Spheres	47	126	2.7×

KEY FINDINGS:

1. VOLUME EXPANSION: Modern pocket is 2.5× larger (1847 vs 750 Ų)
→ More spacious substrate binding site
→ Potentially accommodates diverse conformations

2. SURFACE AREA: Modern has 2.6× more surface area (381 vs 149 ų)
→ Increased protein-ligand interactions
→ More contact points for substrate recognition

3. POLARITY MAINTAINED: Both ~67-68% polar
→ Hydrophilic character conserved across evolution
→ Consistent with binding charged amino acids (Pro, Thr)

4. QUALITY SCORE: Modern scores 4.6× higher (1.48 vs 0.32)
→ Better-defined binding pocket geometry
→ More optimal for substrate binding

5. COMPLEXITY: Modern has 2.7× more alpha spheres (126 vs 47)
→ More sophisticated pocket architecture
→ Refined geometric organization

BIOLOGICAL INTERPRETATION:
Evolution EXPANDED the binding pocket while maintaining its chemical properties (polarity). Larger volume may allow promiscuous binding of both Pro and Thr, consistent with ipTM data (Figure 3).