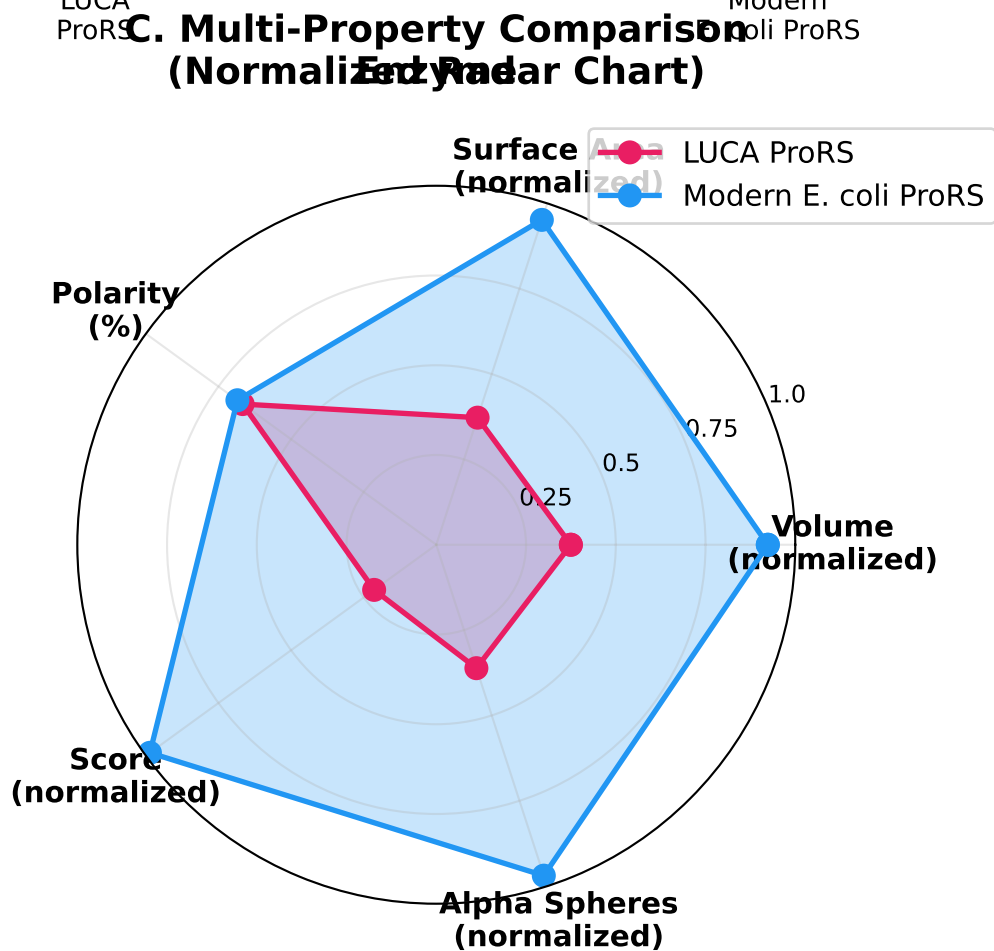
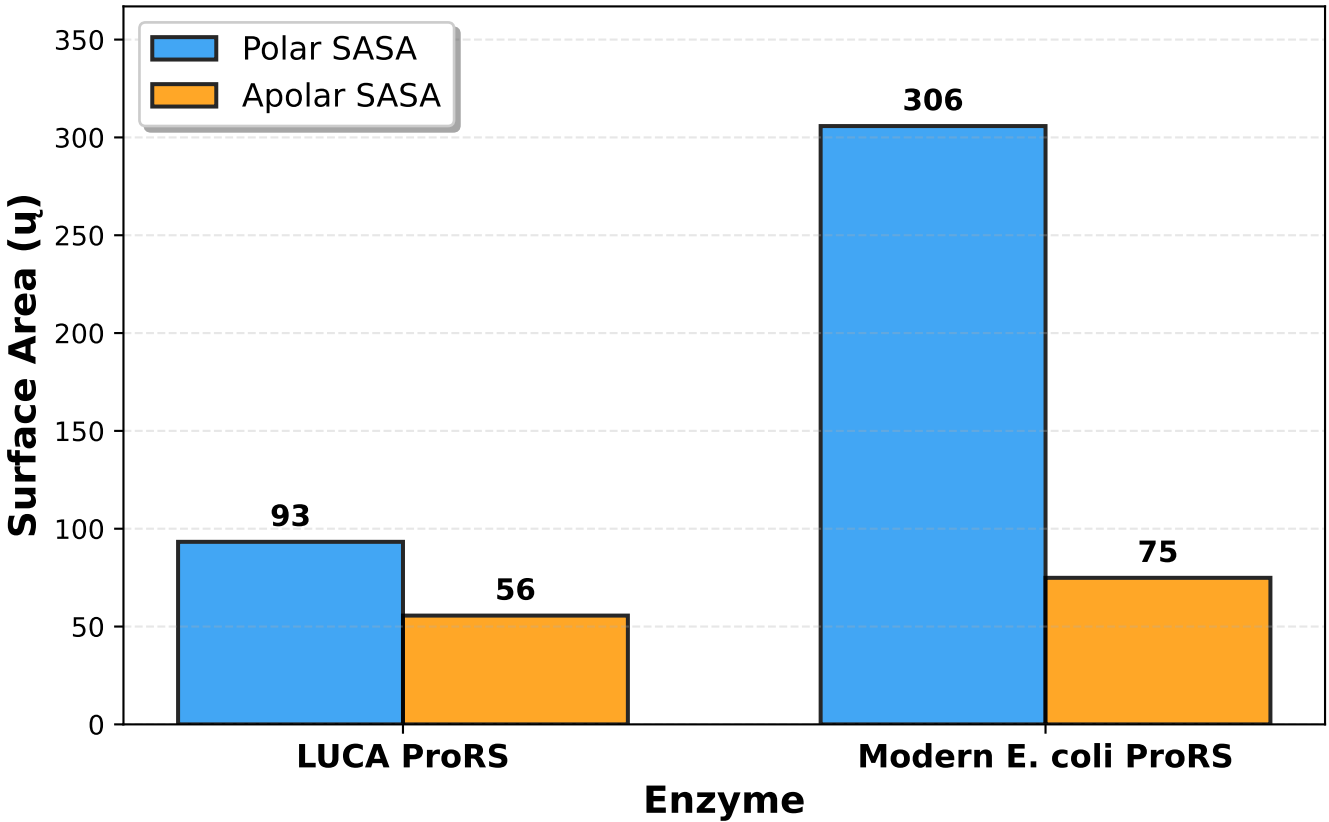
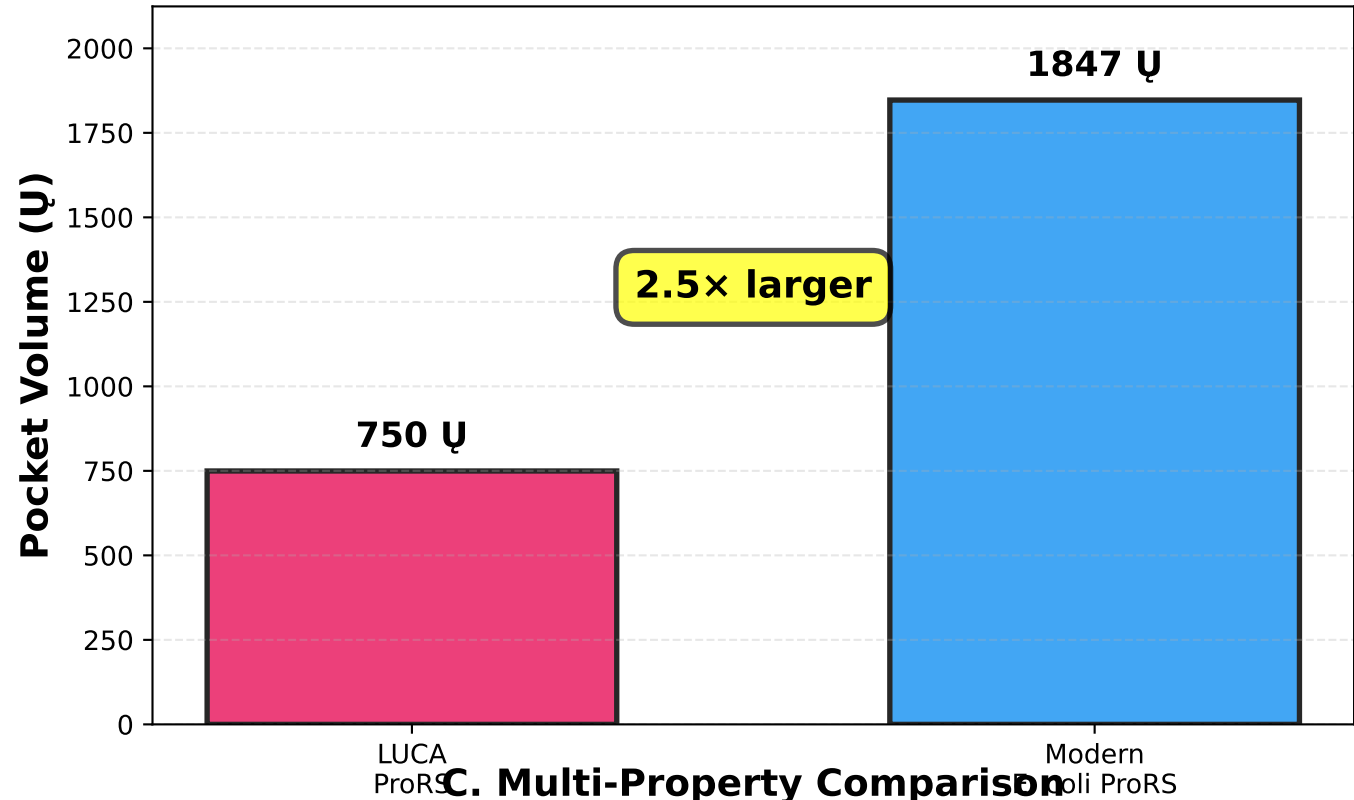


Figure 6: ProR Binding Pocket Analysis - Evolution from LUCA to Modern Enzymes
Modern is 2.5× Larger than LUCA
Both Primarily Polar (Hydrophilic)



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

Property	LUCA ProRS	Modern E. coli	Fold Change
Volume (Å³)	750	1847	2.5×
Total SASA (Å²)	149	381	2.6×
Polar SASA (Å²)	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:

- VOLUME EXPANSION:** Modern pocket is 2.5× larger (1847 vs 750 Å³)
 - More spacious substrate binding site
 - Potentially accommodates diverse conformations
- SURFACE AREA:** Modern has 2.6× more surface area (381 vs 149 Å²)
 - Increased protein-ligand interactions
 - More contact points for substrate recognition
- POLARITY MAINTAINED:** Both ~67-68% polar
 - Hydrophilic character conserved across evolution
 - Consistent with binding charged amino acids (Pro, Thr)
- QUALITY SCORE:** Modern scores 4.6× higher (1.48 vs 0.32)
 - Better-defined binding pocket geometry
 - More optimal for substrate binding
- 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).