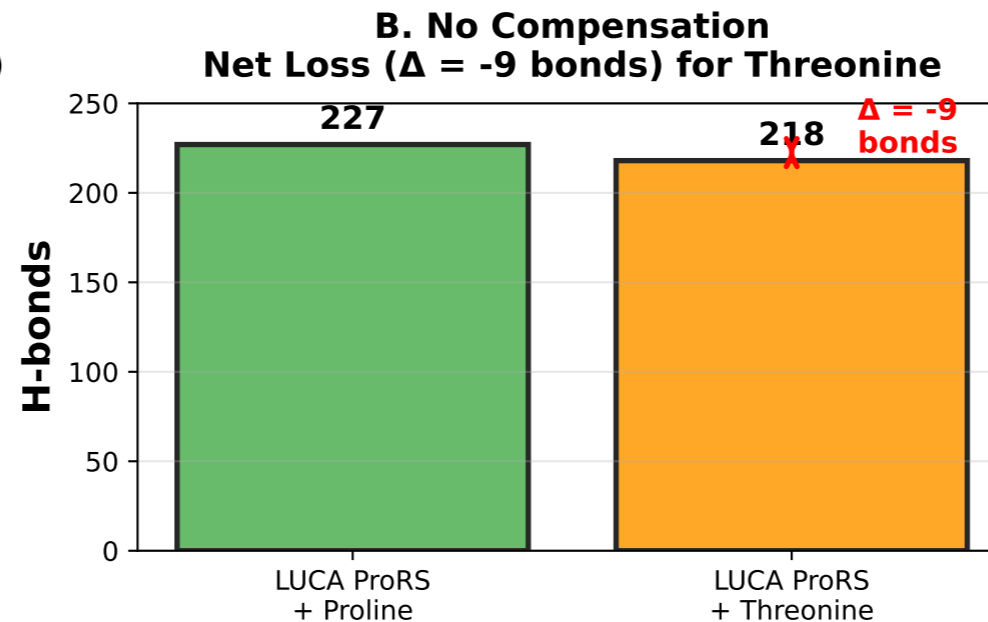
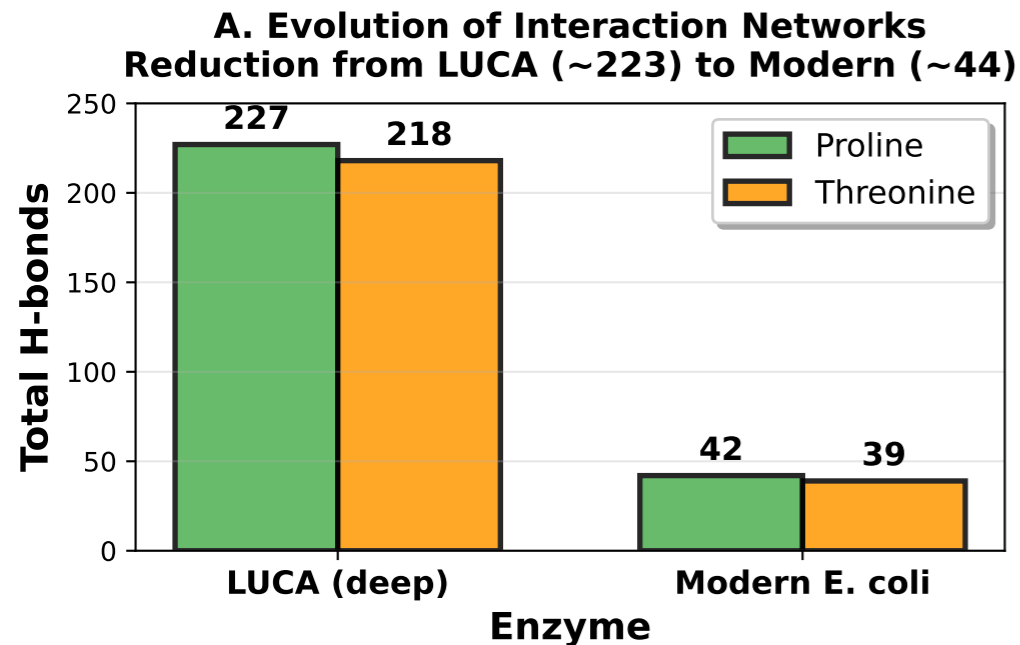


**Figure 4: Steric Permissiveness and Evolutionary Pruning**



**C. Structural Basis of Permissiveness**

STERIC PERMISSIVENESS:

LUCA ProRS (Ancestral):

- Proline: 227 H-bonds
- Threonine: 218 H-bonds (-9)
- Total ~223 bonds average

Modern E. coli ProRS:

- Proline: 42 H-bonds
- Threonine: 39 H-bonds (-3)
- Total ~41 bonds average

EVOLUTIONARY PRUNING:

- 81% reduction (223 → 41)
- Despite fewer contacts, promiscuity MAINTAINED

INTERPRETATION:

LUCA had promiscuous binding through STERIC PERMISSIVENESS (large pocket fits both Pro/Thr) rather than specific chemical engagement.

Modern enzymes PRUNED contacts while maintaining promiscuity via POCKET EXPANSION (2.5×).

Thr  $\beta$ -hydroxyl forms NO specific compensating interactions (0 bonds).