

# Figure 5 - Detailed Interpretation

## FIGURE 5: THE "RECEIVER-FIRST" PATTERN

### KEY INSIGHT:

The substrate binding pocket (receiver) evolved HIGH specificity ( $\text{ipTM } 0.78$ ) BEFORE the global protein structure achieved full coordination ( $\text{ipTM } 0.28$ ).

### EVIDENCE FROM AF3 PREDICTIONS:

1. LUCA ProRS (Ancestral, 4 billion years ago):
  - Pocket  $\text{ipTM: } 0.78$  (RIGID binding site - high confidence)
  - Global  $\text{ipTM: } 0.28$  (FLEXIBLE structure - low confidence)
  - Interpretation: Binding pocket was already well-formed, but the rest of the protein structure was still evolving/flexible
2. Modern *E. coli* ProRS (Contemporary):
  - Pocket  $\text{ipTM: } 0.95$  (RIGID binding site - very high confidence)
  - Global  $\text{ipTM: } 0.95$  (RIGID structure - very high confidence)
  - Interpretation: Both pocket AND global structure are now fully optimized

### BIOLOGICAL SIGNIFICANCE:

1. MODULAR EVOLUTION:
  - Evolution proceeded in a modular fashion
  - Critical functional domains (binding pocket) evolved first
  - Structural scaffold (global fold) optimized later
2. FUNCTION-FIRST PRINCIPLE:
  - Enzymatic function (substrate binding) is prioritized
  - Structural elegance comes secondary to catalytic activity
  - LUCA could perform aminoacylation despite imperfect global fold
3. EVOLUTIONARY CONSTRAINTS:
  - Binding pocket under STRONG purifying selection (must bind substrate)
  - Global structure under WEAKER selection (just needs to be stable enough)
  - Modern enzymes optimize BOTH for maximum efficiency
4. IMPLICATIONS FOR PROTEIN ENGINEERING:
  - Focus first on active site architecture
  - Global structure can be more flexible/tolerant
  - Receiver-first approach may be useful for designing new enzymes

### COMPARISON TO OTHER EVOLUTIONARY MODELS:

#### Traditional Model (REJECTED):

Entire protein evolves as a unit → specificity emerges late

#### Receiver-First Model (SUPPORTED BY DATA):

Binding pocket evolves first → global structure coordinates later

#### Evidence:

LUCA pocket  $\text{ipTM (0.78)} >> \text{global ipTM (0.28)}$   
50 percentage point difference indicates UNCOUPLING

### FIGURE INTERPRETATION GUIDE:

#### Panel A (LUCA):

- Wavy outline = flexible global structure (low confidence)
- Solid pocket = rigid binding site (high confidence)
- Shows functional pocket in a still-evolving scaffold

#### Panel B (Modern):

- Smooth outline = rigid global structure (high confidence)
- Solid pocket = rigid binding site (very high confidence)
- Shows fully optimized enzyme with coordinated structure

#### Panel C (Timeline):

- Shows 4 billion year evolutionary trajectory
- Pocket specificity emerges early ( $0.78$ )
- Global coordination optimizes later ( $0.28 \rightarrow 0.95$ )

#### Panel D (Quantitative):

- Bar chart shows the uncoupling in LUCA
- Green shading: pocket already high confidence
- Orange shading: global still low confidence
- Modern shows convergence (both  $0.95$ )

### RELATED FIGURES:

- Figure 3: Shows promiscuity is maintained (separate phenomenon)
- Figure 5: Shows pocket evolved before global coordination
- Both are compatible: promiscuous but well-formed pocket came first