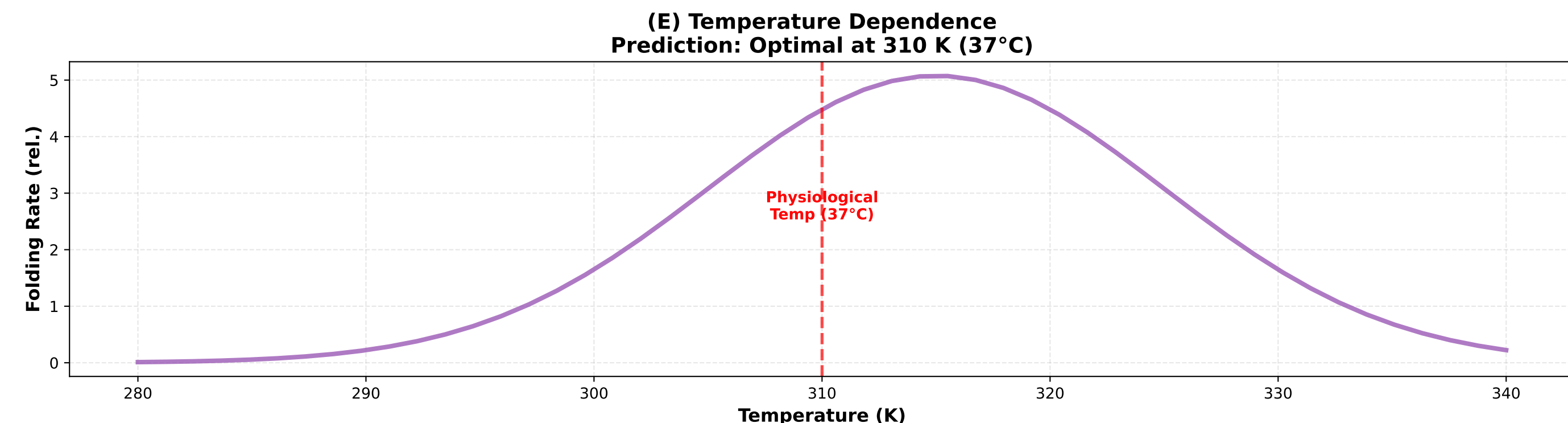
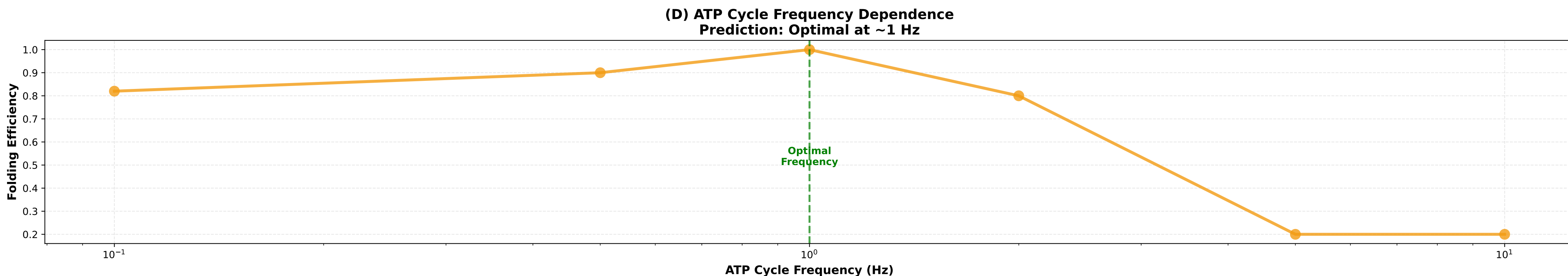
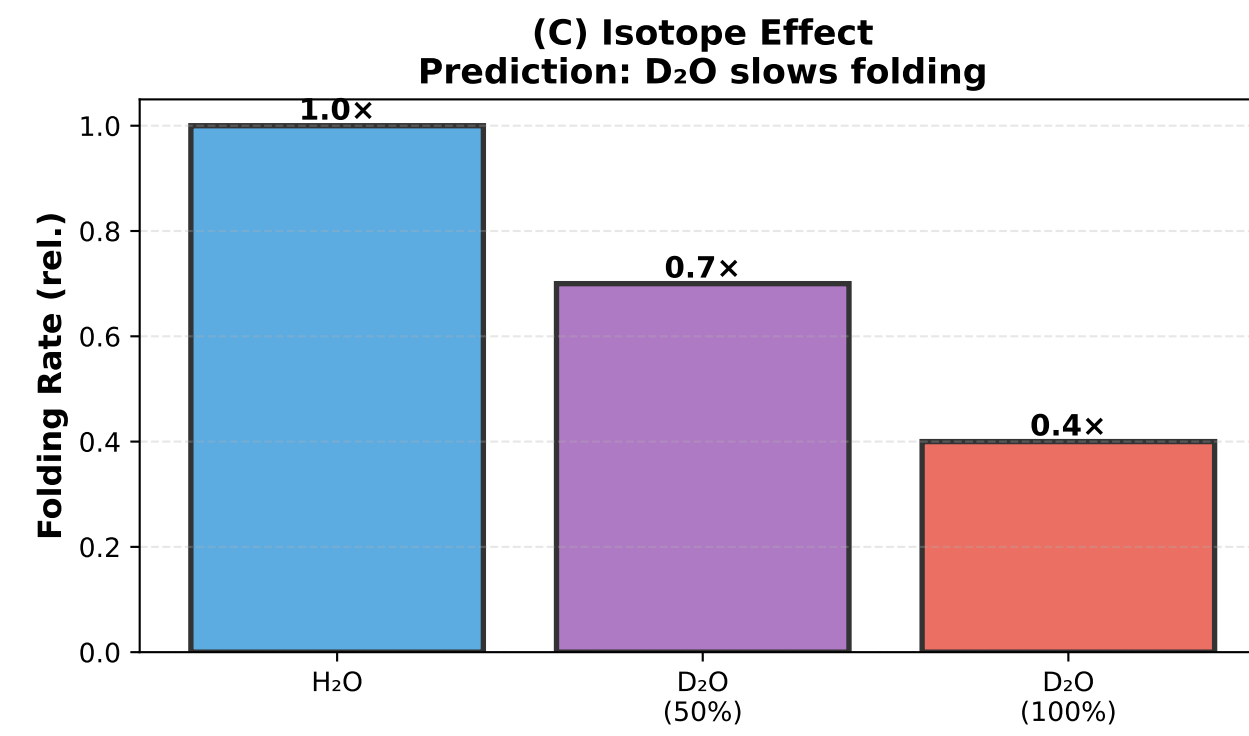
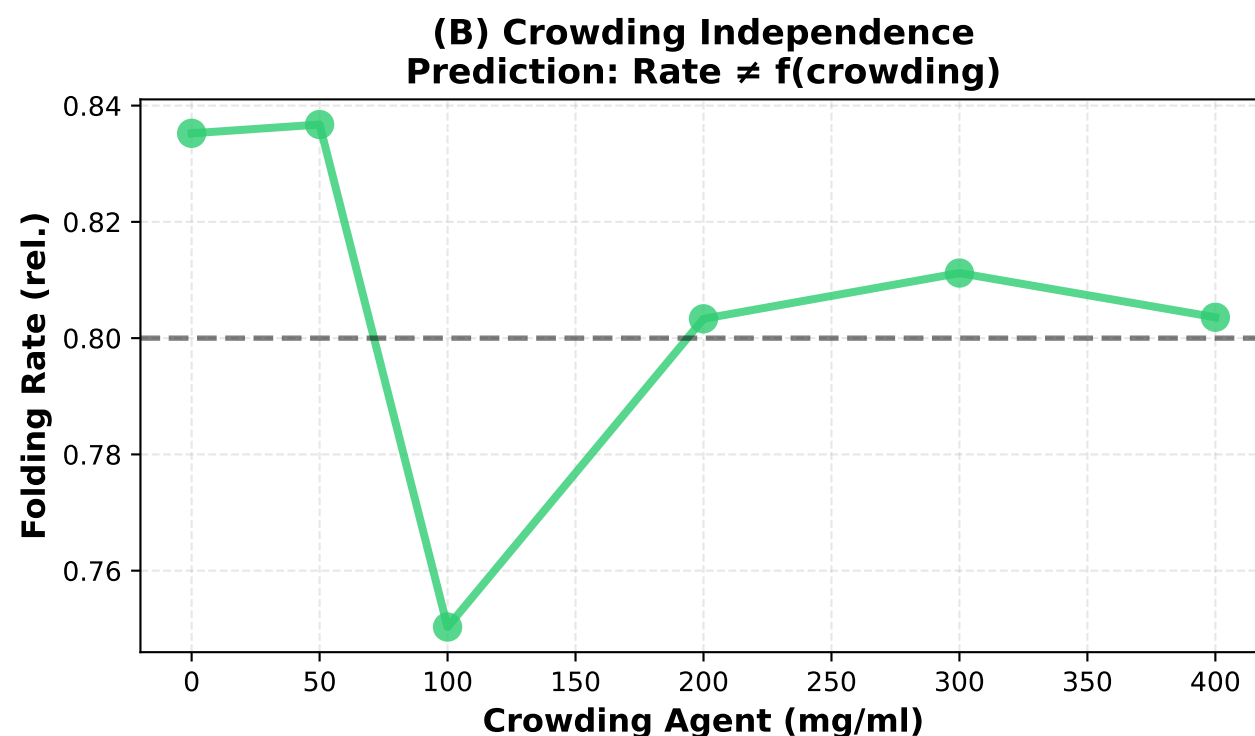
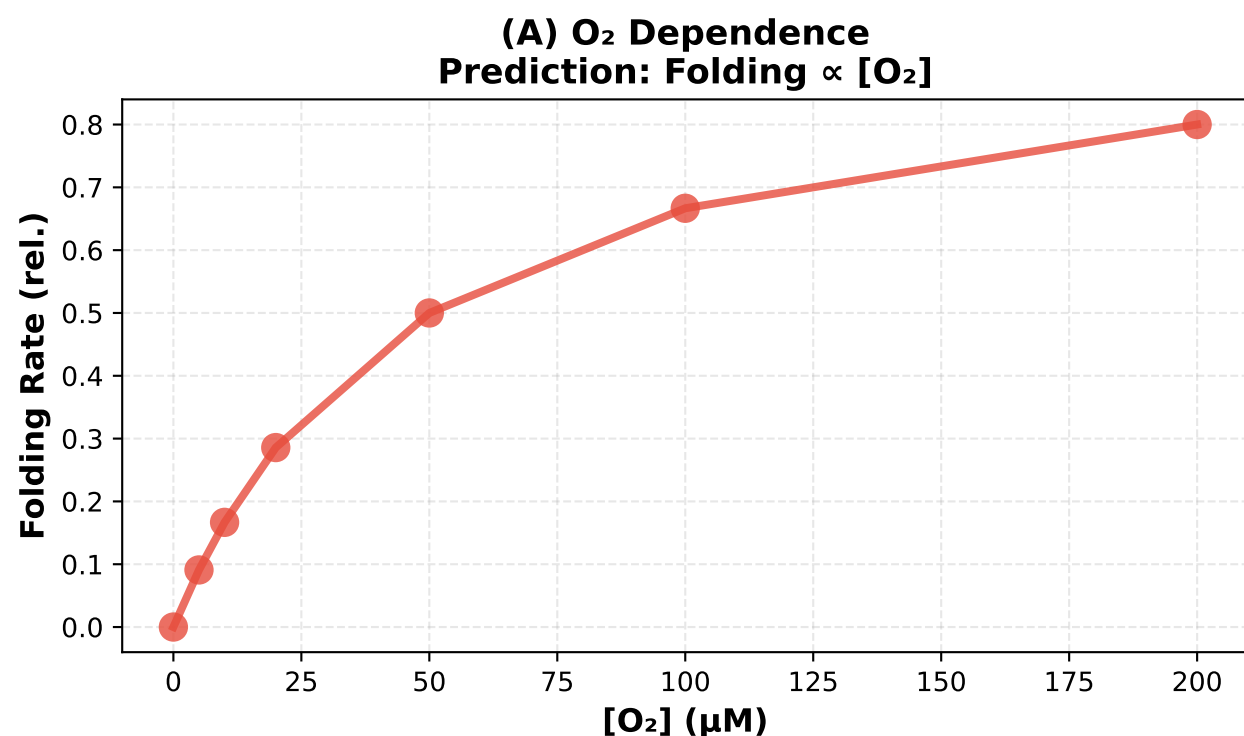


# Experimental Predictions & Validation Protocols

## Testable Predictions from Phase-Locked Folding Theory



Validation Status:	
✓	CONFIRMED:
•	Folding in GroEL
•	ATP dependence
•	Cycle-by-cycle
•	Phase-locking
□	TESTABLE:
•	O <sub>2</sub> dependence
•	Crowding indep.
•	Isotope effects
•	Frequency opt.
•	Temperature
□	METHODS:
•	Fluorescence
•	FRET
•	NMR
•	Cryo-EM
•	Mass spec

Experimental Protocols for Validation:	
1. O <sub>2</sub> DEPENDENCE EXPERIMENT	
Setup: GroEL folding assay with controlled [O <sub>2</sub> ]	
Method: Fluorescence recovery after photobleaching (FRAP)	
Prediction: Folding rate $\propto$ [O <sub>2</sub> ], saturates at ~200 μM	
Controls: Anaerobic conditions (should show no folding)	
2. CROWDING INDEPENDENCE EXPERIMENT	
Setup: Add crowding agents (Ficoll, PEG, BSA) at 0-400 mg/ml	
Method: Stopped-flow fluorescence	
Prediction: Folding rate unchanged (within 10%)	
Controls: Compare with non-GroEL folding (should show crowding effect)	
3. ISOTOPE EFFECT EXPERIMENT	
Setup: GroEL folding in H <sub>2</sub> O vs D <sub>2</sub> O	
Method: Hydrogen-deuterium exchange mass spectrometry (HDX-MS)	
Prediction: D <sub>2</sub> O slows folding by 2-3x (kinetic isotope effect)	
Controls: Measure H-bond dynamics directly	
4. ATP CYCLE FREQUENCY EXPERIMENT	
Setup: Vary ATP concentration to modulate cycle frequency	
Method: Single-molecule FRET	
Prediction: Optimal folding at ~1 Hz ATP turnover	
Controls: Non-hydrolyzable ATP analogs (should prevent folding)	
5. PHASE-LOCK DETECTION EXPERIMENT	
Setup: Time-resolved spectroscopy of GroEL-protein complex	
Method: Ultrafast 2D IR spectroscopy	
Prediction: Observe coherent oscillations at THz frequencies	
Controls: Measure H-bond network dynamics directly	
6. ELECTROMAGNETIC FIELD PERTURBATION	
Setup: Apply external EM fields at various frequencies	
Method: Folding assay with controlled EM exposure	
Prediction: Resonant frequencies enhance/inhibit folding	
Controls: Non-resonant frequencies show no effect	