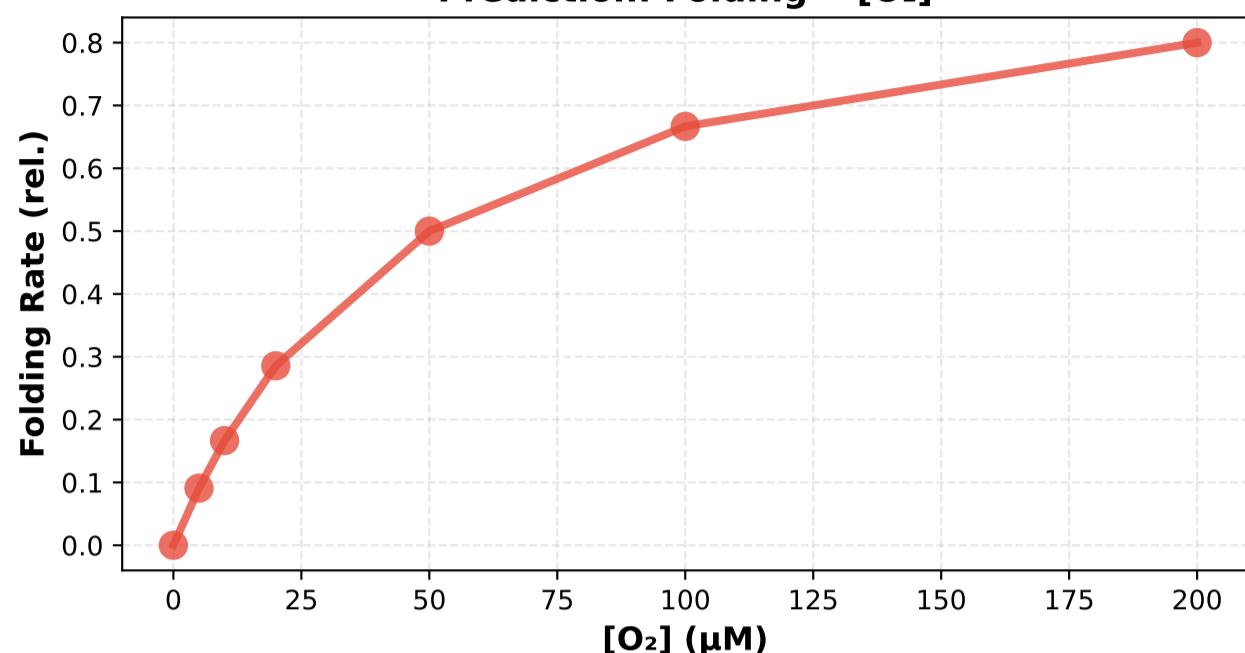


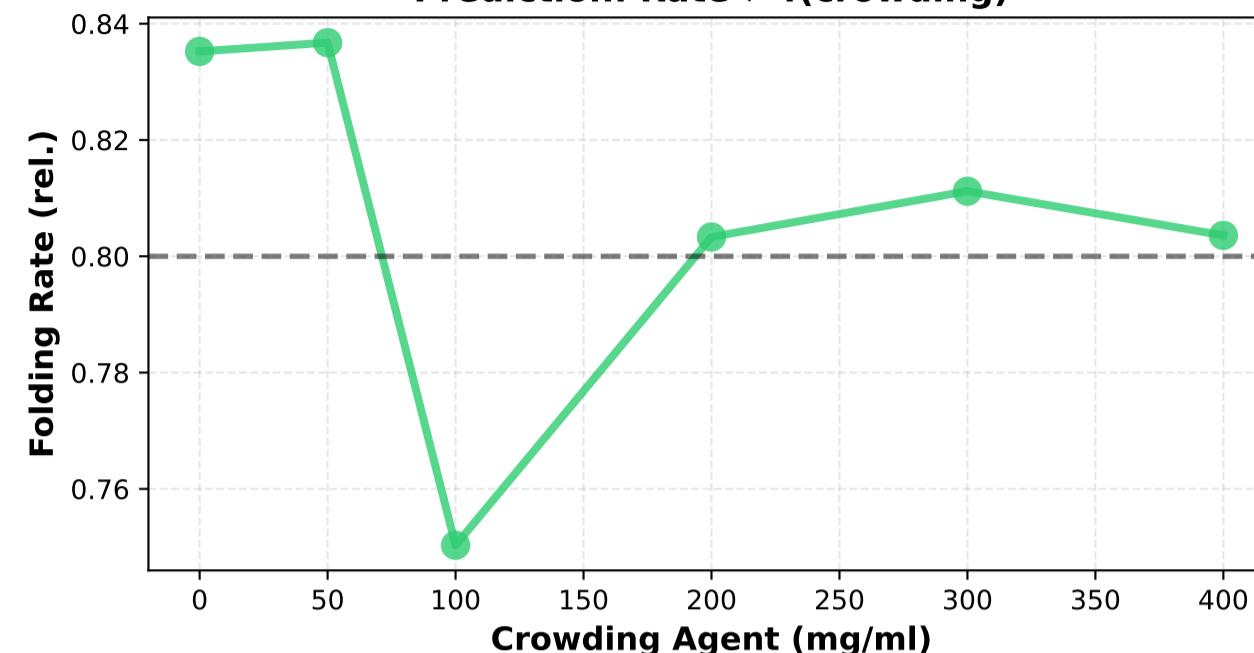
Experimental Predictions & Validation Protocols

Testable Predictions from Phase-Locked Folding Theory

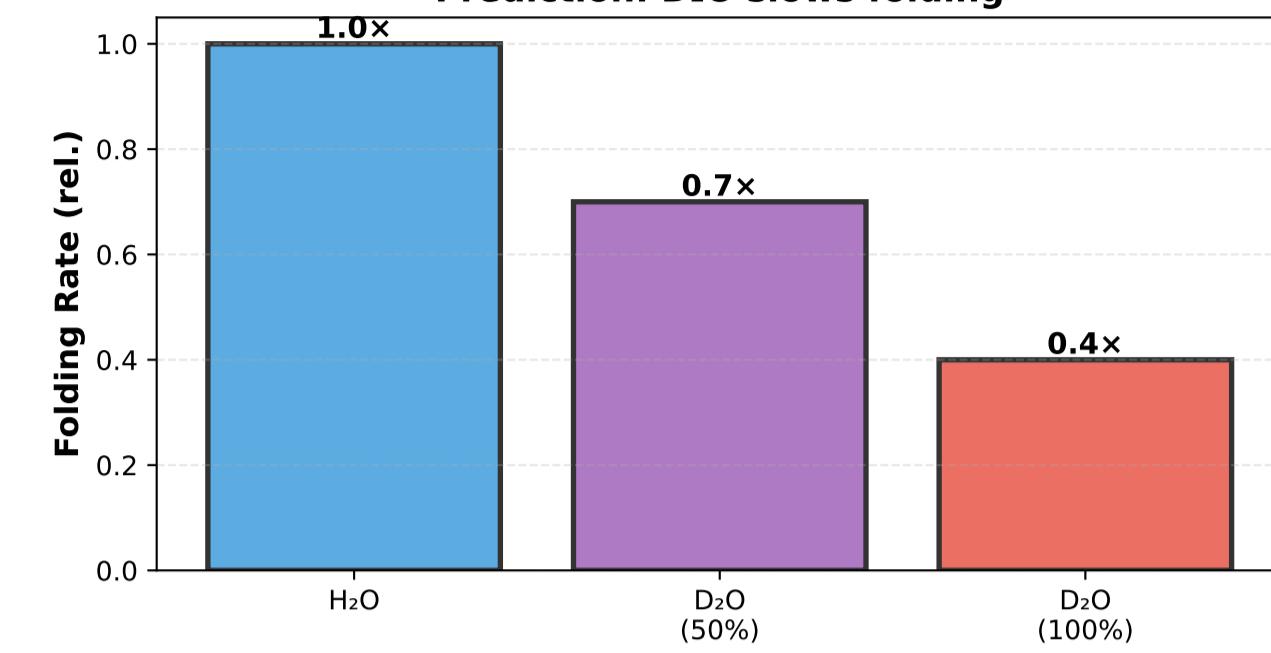
(A) O₂ Dependence
Prediction: Folding \propto [O₂]



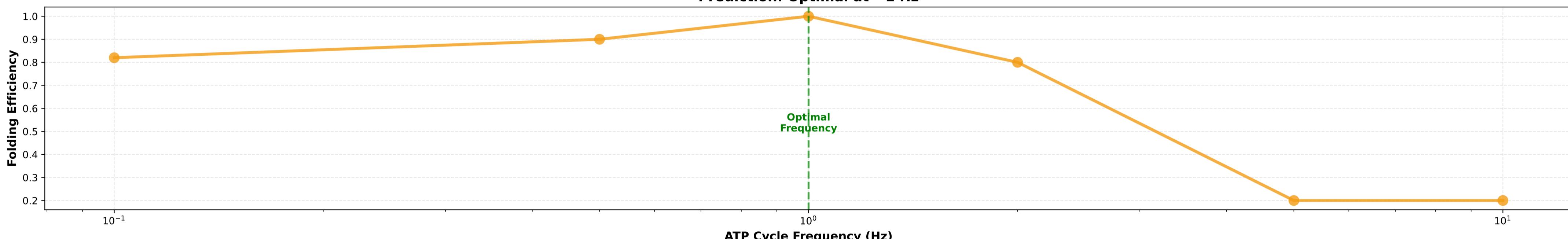
(B) Crowding Independence
Prediction: Rate \neq f(crowding)



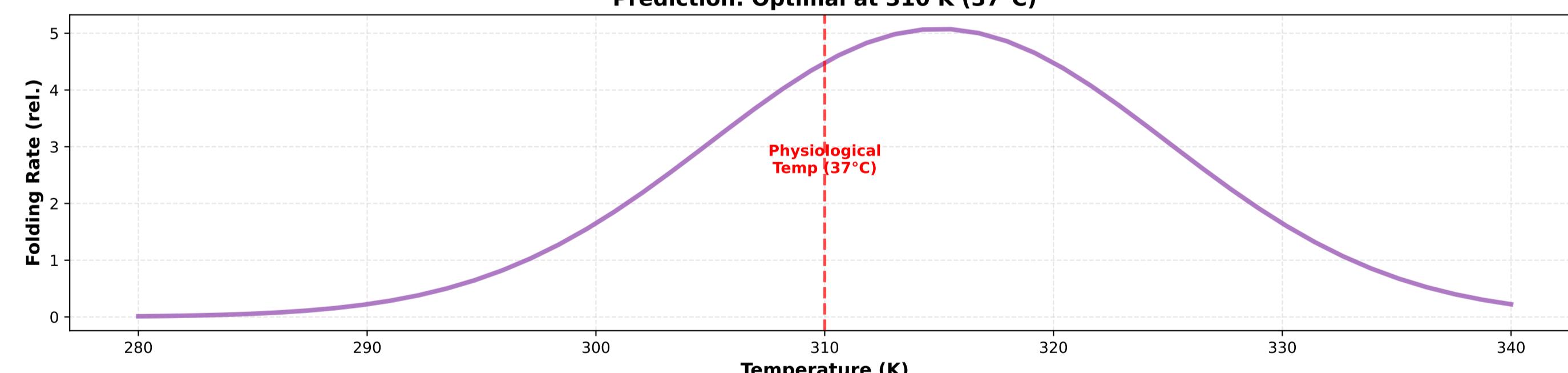
(C) Isotope Effect
Prediction: D₂O slows folding



(D) ATP Cycle Frequency Dependence
Prediction: Optimal at ~1 Hz



(E) Temperature Dependence
Prediction: Optimal at 310 K (37°C)



VALIDATION STATUS:

- ✓ CONFIRMED:
 - Folding in GroEL
 - ATP dependence
 - Cycle-by-cycle
 - Phase-locking

- TESTABLE:
 - O₂ dependence
 - Crowding indep.
 - Isotope effects
 - Frequency opt.
 - Temperature

- METHODS:
 - Fluorescence
 - FRET
 - NMR
 - Cryo-EM
 - Mass spec

EXPERIMENTAL PROTOCOLS FOR VALIDATION:

1. O₂ DEPENDENCE EXPERIMENT
 - Setup: GroEL folding assay with controlled [O₂]
 - Method: Fluorescence recovery after photobleaching (FRAP)
 - Prediction: Folding rate \propto [O₂], 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 H₂O vs D₂O
 - Method: Hydrogen-deuterium exchange mass spectrometry (HDX-MS)
 - Prediction: D₂O 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