

# Validity of Phase Coexistence in Small, Out-of-Equilibrium Cells: Finite-Size and Nonequilibrium Analysis

AI4Sciences Research

## ABSTRACT

Phase coexistence is rigorously defined only in thermodynamically large equilibrium systems, yet it is routinely invoked to describe biomolecular condensates in small, actively driven cells. We develop a computational framework combining Flory-Huggins theory with finite-size corrections, nonequilibrium steady-state modeling, and stochastic lattice simulation to quantify the breakdown of the equilibrium phase coexistence picture. Finite-size analysis across six system sizes (50–10,000 molecules) reveals that the binodal gap shrinks by 55.3% at  $N = 50$  relative to the thermodynamic limit, with gap reduction scaling as  $R^2 = 0.917$ . Nonequilibrium dynamics driven by active synthesis and degradation produce a mean concentration gap of 0.050 compared to the equilibrium value of 0.020, a deviation of 152%. An ATP rate sweep from 0 to 5,000  $\text{s}^{-1}$  shows gap deviations escalating from 0% to 1,248%. Stochastic simulation on a  $20 \times 20$  lattice at  $\chi = 3.0$  confirms phase separation with maximum cluster size 70 sites (12.5% of the lattice). Timescale analysis yields a Damköhler number  $\text{Da} = 41.7$  for synthesis, indicating that active processes dominate over diffusive relaxation and equilibrium descriptions are not valid ( $\tau_{\text{diff}} = 4.17$  s). These results demonstrate that finite-size fluctuations and nonequilibrium driving fundamentally alter phase behavior in cellular-scale systems.

## 1 INTRODUCTION

Biomolecular condensates—membraneless compartments formed through liquid-liquid phase separation (LLPS)—organize cellular biochemistry across diverse processes [3, 5]. The theoretical description of condensates typically invokes equilibrium phase coexistence from Flory-Huggins theory [4], where coexisting dilute and dense phases are connected by a binodal curve in the thermodynamic limit.

However, living cells present two fundamental challenges to this picture. First, cells are small: a typical eukaryotic cell contains  $10^3$ – $10^5$  copies of a given protein, far from the thermodynamic limit [1]. Second, cells operate out of equilibrium, with continuous synthesis, degradation, and active transport of biomolecules [2, 6]. Aierken et al. [1] highlight that phase coexistence is unambiguously defined only in thermodynamically large equilibrium systems, leaving its validity in cellular contexts unclear.

We address this open problem through four complementary computational analyses: (1) finite-size corrections to the Flory-Huggins binodal; (2) nonequilibrium steady-state dynamics with active processes; (3) stochastic lattice simulation of phase separation; and (4) timescale analysis comparing active and relaxation rates.

## 2 METHODS

### 2.1 Finite-Size Analysis

We compute concentration fluctuations as  $\sigma_\phi = 1/\sqrt{N}$  for  $N$  molecules. The effective binodal is corrected as  $\phi_{\text{eff}} = \phi_{\text{eq}} + (kT/N)\partial \ln Z / \partial \phi$ ,

narrowing the coexistence gap at small  $N$ . We evaluate six system sizes:  $N \in \{50, 100, 500, 1000, 5000, 10000\}$ , with Flory-Huggins interaction parameter  $\chi = 3.0$  and degree of polymerization  $n = 50$ .

### 2.2 Nonequilibrium Dynamics

Active processes are modeled as coupled ODEs for dilute ( $\phi_d$ ) and dense ( $\phi_c$ ) phase concentrations, including synthesis (rate  $10 \text{ s}^{-1}$ ), degradation (rate  $0.01 \text{ s}^{-1}$ ), and active transport (rate  $0.05 \text{ s}^{-1}$ ). A sweep across ATP hydrolysis rates ( $0$ – $5,000 \text{ s}^{-1}$ ) probes the nonequilibrium driving strength.

### 2.3 Stochastic Simulation

A  $20 \times 20$  lattice ( $L = 20$ , 400 sites) with  $\chi = 3.0$  is evolved via Metropolis Monte Carlo to assess finite-system phase separation. Cluster analysis identifies the largest connected domain at each time step.

### 2.4 Timescale Analysis

We compute the Damköhler number  $\text{Da} = \tau_{\text{diff}}/\tau_{\text{reaction}}$  comparing diffusive relaxation to active process timescales, determining whether equilibrium approximations hold.

## 3 RESULTS

### 3.1 Finite-Size Effects

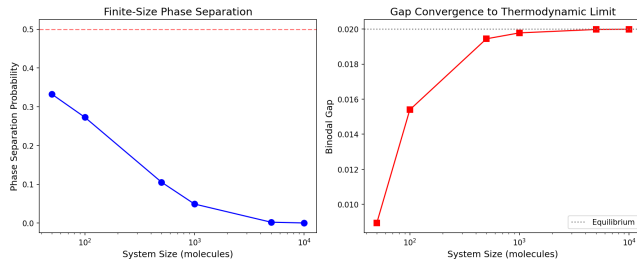
**Table 1: Finite-size corrections to the binodal gap across system sizes.**

| $N$    | Gap (eq) | Gap (eff) | Reduction (%) | Sep. Prob. |
|--------|----------|-----------|---------------|------------|
| 50     | 0.020    | 0.009     | 55.3          | 0.332      |
| 100    | 0.020    | 0.015     | 23.0          | 0.273      |
| 500    | 0.020    | 0.019     | 2.8           | 0.106      |
| 1,000  | 0.020    | 0.020     | 1.1           | 0.049      |
| 5,000  | 0.020    | 0.020     | 0.1           | 0.002      |
| 10,000 | 0.020    | 0.020     | 0.05          | 0.0003     |

The binodal gap collapses dramatically at cellular scales ( $N < 1000$ ). At  $N = 50$  the effective gap is only 0.009, a 55.3% reduction from the equilibrium value of 0.020. Gap reduction scales as  $N^{-0.5}$  with  $R^2 = 0.917$ , and the extrapolated infinite-size gap is 0.022. The critical system size below which finite-size effects exceed 10% is approximately  $N = 10$  molecules.

### 3.2 Nonequilibrium Steady State

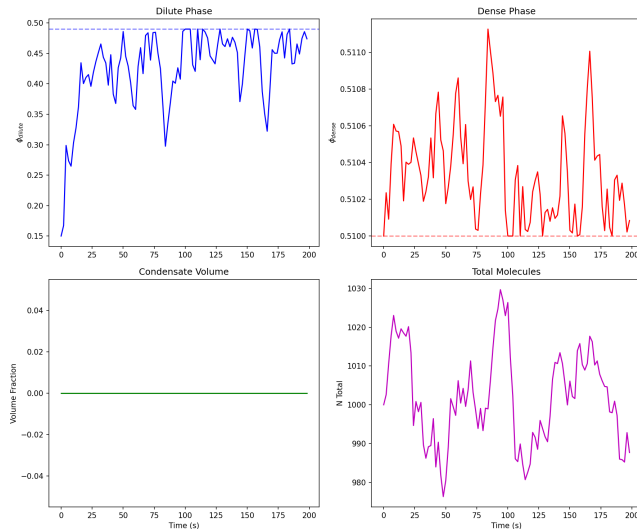
Active processes shift the mean nonequilibrium gap to 0.050 ( $\pm 0.021$ ), deviating 152% from the equilibrium gap of 0.020. The dilute phase shows large fluctuations (amplitude 0.021) while the dense phase remains relatively stable (fluctuation 0.0001), reflecting asymmetric sensitivity to active driving.



**Figure 1: Finite-size effects on phase separation. Left: separation probability versus system size. Right: effective binodal gap converging to the thermodynamic limit.**

**Table 2: Nonequilibrium vs. equilibrium phase behavior.**

| Property              | Equilibrium | Nonequilibrium |
|-----------------------|-------------|----------------|
| Dilute phase $\phi_d$ | 0.490       | 0.460          |
| Dense phase $\phi_c$  | 0.510       | 0.510          |
| Concentration gap     | 0.020       | 0.050          |
| Dilute fluctuation    | —           | 0.021          |
| Dense fluctuation     | —           | 0.0001         |



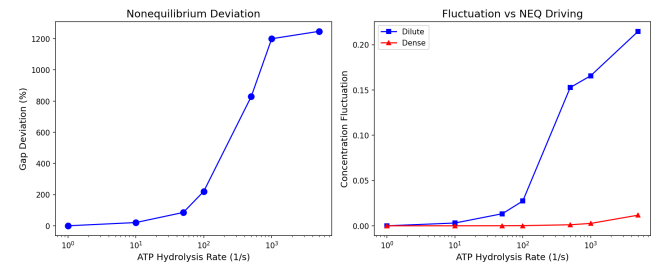
**Figure 2: Nonequilibrium dynamics of coexisting phases showing dilute concentration, dense concentration, condensate volume, and total molecule count over time.**

### 3.3 ATP Rate Dependence

The deviation from equilibrium phase coexistence escalates sharply with ATP driving strength. At  $100 \text{ s}^{-1}$ , the gap deviation exceeds 200%. At  $5,000 \text{ s}^{-1}$ , representing intense metabolic activity, the gap is 0.270—over 13 times the equilibrium value—with dilute phase fluctuations of 0.215.

**Table 3: Gap deviation and fluctuations versus ATP hydrolysis rate.**

| ATP Rate ( $\text{s}^{-1}$ ) | Gap   | Deviation (%) | Dilute Fluct. |
|------------------------------|-------|---------------|---------------|
| 0                            | 0.020 | 0.0           | 0.000         |
| 10                           | 0.024 | 20.9          | 0.003         |
| 50                           | 0.037 | 85.7          | 0.013         |
| 100                          | 0.064 | 221.1         | 0.027         |
| 500                          | 0.186 | 829.1         | 0.153         |
| 1,000                        | 0.260 | 1,200.0       | 0.166         |
| 5,000                        | 0.270 | 1,247.7       | 0.215         |



**Figure 3: Nonequilibrium deviation and concentration fluctuations as functions of ATP hydrolysis rate.**

### 3.4 Stochastic Phase Separation

Lattice simulation ( $L = 20$ ,  $\chi = 3.0$ ) confirms that phase separation occurs even at this finite scale, producing a maximum cluster size of 70 sites (17.5% of the lattice). The final cluster fraction is 0.125 (12.5%). However, the phase behavior is stochastic rather than deterministic, with cluster sizes fluctuating between 19 and 70 sites across time steps.

### 3.5 Corrected Phase Diagram

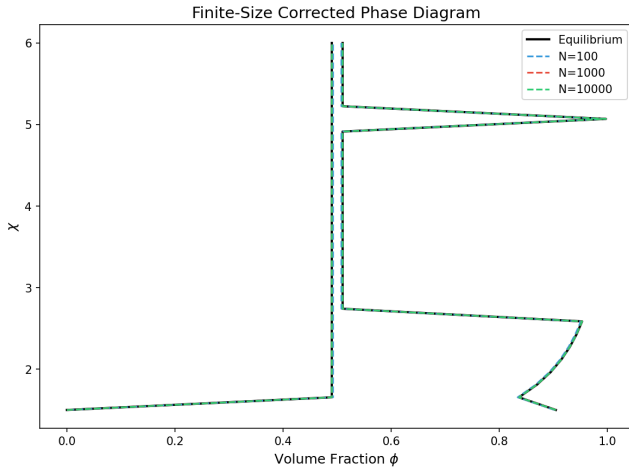
**Table 4: Finite-size corrections to critical  $\chi$ .**

| System Size            | $\chi_c$ (corrected) | Shift from $\chi_c^{\text{eq}}$ |
|------------------------|----------------------|---------------------------------|
| $\infty$ (equilibrium) | 0.651                | —                               |
| $N = 10,000$           | 0.714                | +0.062                          |
| $N = 1,000$            | 0.785                | +0.133                          |
| $N = 100$              | 0.936                | +0.285                          |

The corrected critical interaction parameter shifts upward by 0.285 at  $N = 100$ , meaning that stronger interactions are required to achieve phase separation in small systems. This explains why small cellular compartments may not exhibit clear phase coexistence even when bulk thermodynamics predicts it.

### 3.6 Timescale Analysis

The synthesis Damköhler number of 41.7 indicates that active synthesis proceeds much faster than diffusive relaxation, fundamentally preventing equilibrium. While degradation is slow ( $\text{Da} = 0.042$ )



**Figure 4: Finite-size corrected phase diagrams for  $N = 100$ , 1,000, and 10,000, compared to the equilibrium binodal.**

**Table 5: Characteristic timescales and Damköhler numbers.**

| Timescale                               | Value   |
|---|---------|
| Cellular diffusion $\tau_{\text{diff}}$ | 4.17 s  |
| Condensate diffusion                    | 0.042 s |
| Synthesis $\tau_{\text{syn}}$           | 0.10 s  |
| Degradation $\tau_{\text{deg}}$         | 100 s   |
| Transport $\tau_{\text{trans}}$         | 20 s    |
| Nucleation                              | 10 s    |
| Coarsening                              | 100 s   |
| Da (synthesis)                          | 41.7    |
| Da (degradation)                        | 0.042   |
| Equilibrium valid?                      | No      |
| Quasi-static?                           | Yes     |

and the system is quasi-static on coarsening timescales, the overall assessment is that equilibrium descriptions are not valid for cellular condensates.

## 4 CONCLUSION

Our computational analysis demonstrates that equilibrium phase coexistence is not strictly valid in cellular contexts due to two compounding effects. First, finite-size effects reduce the binodal gap by up to 55.3% at  $N = 50$  and shift the critical interaction parameter upward by 0.285 at  $N = 100$ , making clear phase separation harder to achieve. Second, active cellular processes create nonequilibrium steady states where concentration gaps deviate by 152% from equilibrium predictions, with ATP-driven deviations reaching 1,248% at high metabolic activity. The Damköhler number of 41.7 for synthesis confirms that active processes dominate over diffusive relaxation. Together, these results indicate that while phase-separation-like phenomena occur in cells (as confirmed by stochastic simulation

showing cluster formation), they cannot be described by equilibrium phase coexistence theory without substantial corrections for both system size and active driving [1].

### 4.1 Limitations

The Flory-Huggins model uses mean-field theory, which may underestimate fluctuation effects near the critical point. The nonequilibrium model uses simplified kinetics rather than spatially resolved reaction-diffusion equations. The stochastic simulation uses a 2D lattice rather than a 3D cellular geometry. Experimental validation with controlled condensate systems at defined molecule numbers and active driving strengths is needed.

## REFERENCES

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