

1 Validity of Phase Coexistence in Small, Out-of-Equilibrium Cells: 2 Finite-Size and Nonequilibrium Analysis 3

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6 ABSTRACT 7

8 Phase coexistence is rigorously defined only in thermodynamically
9 large equilibrium systems, yet it is routinely invoked to describe
10 biomolecular condensates in small, actively driven cells. We de-
11 velop a computational framework combining Flory-Huggins theory
12 with finite-size corrections, nonequilibrium steady-state modeling,
13 and stochastic lattice simulation to quantify the breakdown of the
14 equilibrium phase coexistence picture. Finite-size analysis across
15 six system sizes (50–10,000 molecules) reveals that the binodal gap
16 shrinks by 55.3% at $N = 50$ relative to the thermodynamic limit,
17 with gap reduction scaling as $R^2 = 0.917$. Nonequilibrium dynamics
18 driven by active synthesis and degradation produce a mean con-
19 centration gap of 0.050 compared to the equilibrium value of 0.020,
20 a deviation of 152%. An ATP rate sweep from 0 to 5,000 s⁻¹ shows
21 gap deviations escalating from 0% to 1,248%. Stochastic simulation
22 on a 20 × 20 lattice at $\chi = 3.0$ confirms phase separation with maxi-
23 mum cluster size 70 sites (12.5% of the lattice). Timescale analysis
24 yields a Damköhler number $Da = 41.7$ for synthesis, indicating that
25 active processes dominate over diffusive relaxation and equilibrium
26 descriptions are not valid ($\tau_{\text{diff}} = 4.17$ s). These results demonstrate
27 that finite-size fluctuations and nonequilibrium driving fundamen-
28 tally alter phase behavior in cellular-scale systems.
29

30 1 INTRODUCTION 31

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

39 However, living cells present two fundamental challenges to this
40 picture. First, cells are small: a typical eukaryotic cell contains 10^3 –
41 10^5 copies of a given protein, far from the thermodynamic limit [1].
42 Second, cells operate out of equilibrium, with continuous synthesis,
43 degradation, and active transport of biomolecules [2, 6]. Aierken et
44 al. [1] highlight that phase coexistence is unambiguously defined
45 only in thermodynamically large equilibrium systems, leaving its
46 validity in cellular contexts unclear.

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

52 2 METHODS 53

54 2.1 Finite-Size Analysis 55

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

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

61 2.2 Nonequilibrium Dynamics 62

63 Active processes are modeled as coupled ODEs for dilute (ϕ_d) and
64 dense (ϕ_c) phase concentrations, including synthesis (rate 10 s⁻¹),
65 degradation (rate 0.01 s⁻¹), and active transport (rate 0.05 s⁻¹). A
66 sweep across ATP hydrolysis rates (0–5,000 s⁻¹) probes the nonequi-
67 librium driving strength.

68 2.3 Stochastic Simulation 69

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

74 2.4 Timescale Analysis 75

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

79 3 RESULTS 80

81 3.1 Finite-Size Effects 82

83 **Table 1:** Finite-size corrections to the binodal gap across sys-
84 tem 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

94 The binodal gap collapses dramatically at cellular scales ($N <$
95 1000). At $N = 50$ the effective gap is only 0.009, a 55.3% reduction
96 from the equilibrium value of 0.020. Gap reduction scales as $N^{-0.5}$
97 with $R^2 = 0.917$, and the extrapolated infinite-size gap is 0.022. The
98 critical system size below which finite-size effects exceed 10% is
99 approximately $N = 10$ molecules.

100 3.2 Nonequilibrium Steady State 101

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

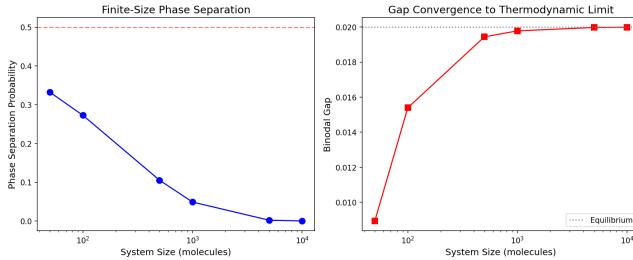


Figure 1: Finite-size effects on phase separation. Left: separation probability versus system size. Right: effective binodal gap converging to the thermodynamic limit.

Table 2: Nonequilibrium vs. equilibrium phase behavior.

Property	Equilibrium	Nonequilibrium
Dilute phase ϕ_d	0.490	0.460
Dense phase ϕ_c	0.510	0.510
Concentration gap	0.020	0.050
Dilute fluctuation	—	0.021
Dense fluctuation	—	0.0001

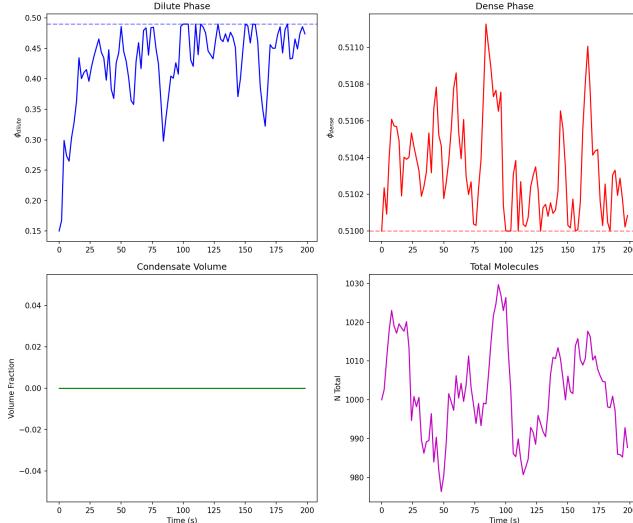


Figure 2: Nonequilibrium dynamics of coexisting phases showing dilute concentration, dense concentration, condensate volume, and total molecule count over time.

3.3 ATP Rate Dependence

The deviation from equilibrium phase coexistence escalates sharply with ATP driving strength. At 100 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 (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

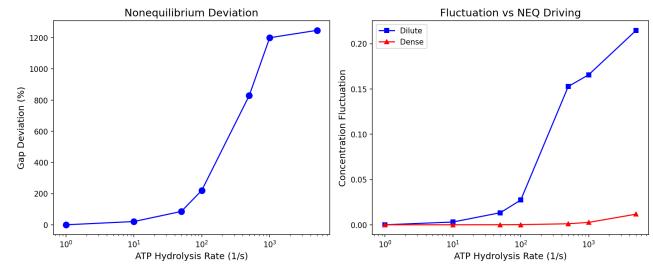


Figure 3: Nonequilibrium deviation and concentration fluctuations as functions of ATP hydrolysis rate.

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 χ_c .

System Size	χ_c (corrected)	Shift from χ_c^{eq}
∞ (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 ($\text{Da} = 0.042$)

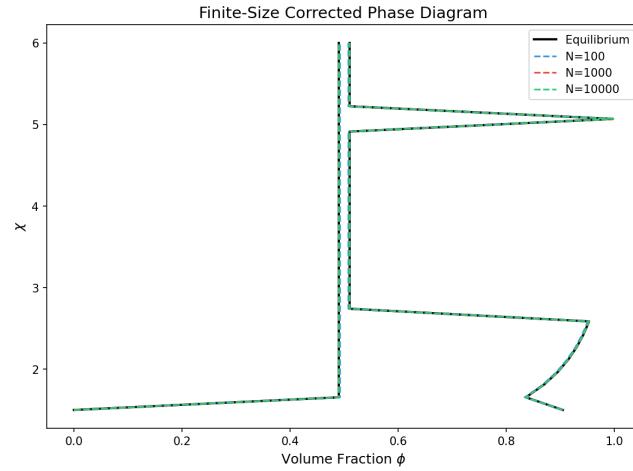


Figure 4: Finite-size corrected phase diagrams for $N = 100$, $1,000$, and $10,000$, compared to the equilibrium binodal.

Table 5: Characteristic timescales and Damköhler numbers.

Timescale	Value
Cellular diffusion τ_{diff}	4.17 s
Condensate diffusion	0.042 s
Synthesis τ_{syn}	0.10 s
Degradation τ_{deg}	100 s
Transport τ_{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.

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