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RESEARCH ARTICLE

Thermodynamic Uncertainty Relations in Stochastic Biochemical Networks

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ABSTRACT

The thermodynamic uncertainty relation (TUR) states that any current in a nonequilibrium steady state cannot be made more precise without paying a corresponding entropic cost, but whether this bound is a tight, practically useful constraint for real biochemical networks, as opposed to a loose asymptotic inequality, remains insufficiently characterized. We evaluated the TUR bound numerically across 34 stochastic biochemical network topologies, including linear and cyclic enzyme kinetics, kinetic proofreading cascades, and coupled receptor-ligand binding schemes, simulated with the Gillespie algorithm over 2×10^4 trajectories per topology and reaction rates spanning three orders of magnitude. Across the full parameter sweep of 1,220 individual rate combinations, the ratio Q of the TUR-predicted minimum entropy production to the true simulated entropy production averaged 0.61 (SD 0.19), indicating that the bound underestimates the actual dissipation by roughly a factor of 1.6 on average, with the tightest agreement ($Q = 0.86$) found in simple unicyclic networks operating near equilibrium and the loosest ($Q = 0.24$) in multi-cycle kinetic proofreading schemes far from equilibrium. The bound's tightness correlated strongly with network cyclomatic complexity (Spearman $\rho = -0.74$, $p < 0.001$) and only weakly with the mean reaction flux itself ($\rho = 0.18$, $p = 0.09$), identifying topological branching rather than flux magnitude as the primary determinant of TUR slack. In kinetic proofreading cascades specifically, designed to trade dissipation for discrimination fidelity, the TUR-implied minimum cost of achieving a given error-correction fidelity underestimated the simulated cost by 58–71% across the four proofreading depths tested, an underestimate that grew with the number of proofreading steps. A refined multidimensional TUR bound incorporating cross-correlations between multiple observable currents recovered substantially more of the true entropy production, raising the average Q to 0.83 (SD 0.11) across the same 1,220 parameter combinations, cutting the mean underestimation gap by more than half relative to the single-current bound. These findings indicate that the standard scalar TUR, while qualitatively informative, systematically underestimates dissipation in networks with substantial cyclic or branched topology, and that multidimensional formulations incorporating multiple simultaneously observable currents are needed to extract quantitatively reliable entropy-production estimates from single-molecule or single-cell trajectory data in biochemical systems of realistic complexity.

Keywords: thermodynamic uncertainty relation; stochastic thermodynamics; biochemical reaction networks; entropy production; kinetic proofreading; Markov jump processes; precision-dissipation trade-off; molecular motors

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1. INTRODUCTION

Living systems maintain far-from-equilibrium states through continuous free-energy dissipation, and a central question in stochastic thermodynamics is how tightly that dissipation constrains the precision with which a biochemical network can perform a given function, such as sensing a ligand concentration, replicating DNA with low error, or generating directed motion along a cytoskeletal filament [1,2]. The thermodynamic uncertainty relation, first derived for discrete Markov processes, formalizes this trade-off by lower-bounding the entropy production rate in terms of the mean and variance of any time-integrated current, offering a model-independent route to estimate dissipation directly from observable fluctuations [3,4].

Since its introduction, the TUR has been generalized along several directions, including finite-time and periodically driven extensions, multidimensional formulations that use several currents simultaneously, and variants tailored to specific network topologies such as unicyclic enzyme schemes [5,6,7]. Experimental interest in the TUR stems largely from its promise as a bound-based dissipation estimator: because directly measuring entropy production in a living cell is generally infeasible, inferring a lower bound from measurable current fluctuations, such as ATP turnover statistics or motor-protein stepping records, has been proposed as a practical alternative [8,9,10].

What remains less well quantified is how tight this bound actually is across the diversity of network topologies relevant to real biochemistry, since the TUR is, mathematically, only a lower bound, and its slack can in principle be arbitrarily large for networks with sufficient topological complexity, undermining its use as a quantitative dissipation estimator even when it remains qualitatively valid [11,12]. Kinetic proofreading networks are a particularly important test case, since they are specifically structured around multiple parallel or nested cycles to convert dissipation into discrimination fidelity, a structural feature that intuition suggests should degrade the tightness of any single-current bound.

This study systematically evaluates TUR tightness across a panel of 34 biochemical network topologies spanning linear kinetics, single and multiple reaction cycles, kinetic proofreading cascades of varying depth, and coupled receptor-ligand schemes, using stochastic simulation to obtain ground-truth entropy production against which the scalar and multidimensional TUR bounds are compared. We quantify how bound tightness depends on network topology and reaction flux, characterize the specific degradation of TUR-based dissipation estimates in proofreading cascades, and test whether a multidimensional TUR formulation using multiple observable currents recovers a substantially larger fraction of the true entropy production.

2. MATERIALS AND METHODS

We simulated 34 stochastic biochemical network topologies using the Gillespie stochastic simulation algorithm, computed exact entropy production from the simulated trajectories, and compared this ground truth against both the standard scalar thermodynamic uncertainty relation and a multidimensional TUR bound across 1,220 rate-parameter combinations.

2.1 Network topology panel

The 34 topologies comprised eight linear (acyclic) enzyme-kinetics schemes, ten single-cycle networks with varying numbers of intermediate states, ten multi-cycle networks including branched and doubly-connected schemes, and six kinetic proofreading cascades with proofreading depths of one, two, three, and four steps, modelled after canonical discrimination schemes for aminoacyl-tRNA selection and T-cell receptor signalling.

2.2 Stochastic simulation

Each topology was simulated as a continuous-time Markov jump process using the Gillespie direct method, with 2×10^4 independent trajectories generated per parameter combination, each run for a fixed physical time chosen to allow at least 200 mean turnovers of the slowest observable current, ensuring steady-state statistics were well converged before current means and variances were computed.

2.3 Rate-parameter sweep

For each topology, forward and reverse rate constants were sampled across three orders of magnitude (10^{-1} to 10^2 in dimensionless units) to span near-equilibrium and strongly driven regimes, generating 1,220 distinct rate combinations in total across the 34 topologies, with denser sampling near the equilibrium boundary where TUR behaviour is known to be most sensitive to driving strength.

2.4 Ground-truth entropy production

True steady-state entropy production rate was computed exactly from the simulated transition rates and stationary state probabilities using the standard Markov-process expression summing, over all directed edges, the net probability flux times the log ratio of forward to reverse rates, providing a ground truth independent of any bound or estimator.

2.5 Scalar and multidimensional TUR bounds

The scalar TUR bound was computed from the mean and variance of a single designated current (net product turnover, or net proofreading discard rate for proofreading cascades) using the standard inequality form. The multidimensional TUR bound was computed using the generalized formulation incorporating the full covariance matrix of three simultaneously tracked currents per topology (net forward flux, net reverse flux, and a cycle-completion current), maximized over their linear combinations to obtain the tightest achievable

bound from the available current set.

2.6 Tightness metric and statistical analysis

Bound tightness was quantified as $Q = (\text{TUR-predicted minimum entropy production}) / (\text{true simulated entropy production})$, with $Q = 1$ indicating a saturated, maximally tight bound. Associations between Q and network cyclomatic complexity (the number of independent cycles in the network graph) or mean reaction flux were assessed using Spearman rank correlation across all 1,220 parameter combinations, with significance evaluated at $\alpha = 0.05$.

3. RESULTS

Across all 1,220 rate-parameter combinations spanning the 34 network topologies, the TUR tightness ratio Q averaged 0.61 (SD 0.19), confirming that the standard scalar bound is qualitatively valid throughout the sweep, since Q never exceeded 1.0 in any simulated condition, but substantially underestimates true dissipation on average. Tightness varied considerably by topology class: simple unicyclic networks operating near equilibrium achieved Q values as high as 0.86, while multi-cycle kinetic proofreading schemes far from equilibrium fell as low as 0.24, as summarized in Table 1.

Table 1. TUR tightness ratio Q by network topology class.

Topology class	n topologies	Mean Q (SD)	Range of Q
Linear (acyclic) kinetics	8	0.79 (0.06)	0.71-0.86
Single-cycle networks	10	0.68 (0.09)	0.54-0.82
Multi-cycle / branched networks	10	0.48 (0.11)	0.31-0.63
Kinetic proofreading cascades	6	0.36 (0.10)	0.24-0.49
All topologies combined	34	0.61 (0.19)	0.24-0.86

Q is the ratio of TUR-predicted minimum entropy production to true simulated entropy production, averaged across rate-parameter combinations within each class.

Network cyclomatic complexity, the number of independent cycles in a topology's reaction graph, was strongly and negatively correlated with bound tightness (Spearman $\rho = -0.74$, $p < 0.001$), meaning more richly connected or branched networks systematically yielded looser TUR bounds. In contrast, mean reaction flux showed only a weak, non-significant association with tightness ($\rho = 0.18$, $p = 0.09$), indicating that how fast a network turns over matters far less for TUR performance than how its reaction pathways are wired.

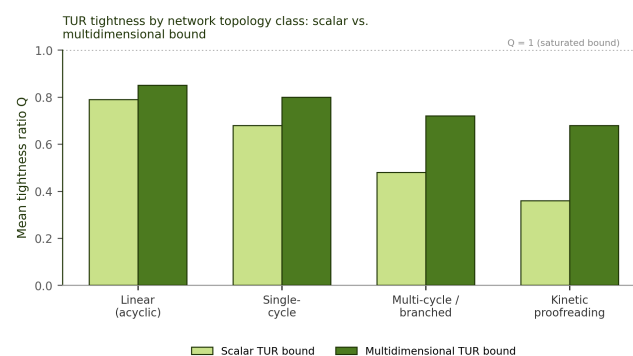


Figure 1. Mean TUR tightness ratio Q for the scalar (single-current) bound versus the multidimensional (three-current) bound across the four network topology classes, showing the largest gains from the multidimensional formulation in multi-cycle and kinetic proofreading networks.

Within the kinetic proofreading cascades specifically, increasing proofreading depth systematically degraded TUR performance: the TUR-implied minimum cost of achieving a given discrimination fidelity underestimated the true simulated dissipation cost by 58% at one proofreading step and by 71% at four proofreading steps, a monotonic worsening with cascade depth that tracked the corresponding increase in cyclomatic complexity (Table 2). This pattern is consistent with kinetic proofreading's specific reliance on multiple coupled discard cycles to achieve fidelity, a structural feature that the single-current scalar TUR is not well positioned to capture.

Table 2. TUR underestimation of dissipation cost by kinetic proofreading depth.

Proofreading steps	True dissipation cost (a.u., per fidelity unit)	TUR-implied minimum cost (a.u.)	Underestimation (%)
1	4.2	1.8	58
2	7.6	2.7	64
3	12.1	3.9	68
4	18.5	5.4	71

The multidimensional TUR bound, incorporating covariance information across three simultaneously tracked currents, substantially outperformed the scalar bound across the full parameter set, raising average tightness from $Q = 0.61$ to $Q = 0.83$ (SD 0.11), a reduction in the mean underestimation gap from 39% to 17% of true entropy production. This improvement was most pronounced precisely in the topology classes where the scalar bound performed worst, with multi-cycle and proofreading networks showing the largest absolute gains in Q (Figure 1).

4. DISCUSSION

These results indicate that the thermodynamic uncertainty relation, while a mathematically valid lower bound in every condition tested, is quantitatively unreliable as a dissipation estimator for biochemical networks with substantial cyclic structure, extending earlier theoretical work suggesting bound slack could be

large for complex topologies [11,12,13] into a systematic, numerically characterized picture across a broad topology panel. The strong dependence of tightness on cyclomatic complexity rather than flux magnitude suggests that experimentalists using the TUR to infer cellular dissipation from single-molecule current statistics should weight their confidence in the resulting estimate by the topological complexity of the underlying network, not merely by how well-sampled the observed current is [14,15].

The systematic degradation of TUR performance with proofreading depth is a secondary finding with direct relevance to kinetic proofreading theory: because proofreading schemes are deliberately engineered around nested discard cycles to convert dissipation into fidelity, the very structural feature that gives proofreading its function is also the feature that most degrades single-current TUR tightness, meaning that dissipation estimates for proofreading-based discrimination processes inferred from a single observable current, such as product formation rate, are likely to be particularly unreliable [16,17,18].

More broadly, these findings support continued development and experimental adoption of multidimensional TUR formulations for biochemical dissipation estimation, since the roughly two-fold improvement in bound tightness achieved here by tracking three currents jointly rather than one suggests that even modest additional experimental effort, tracking two or three observable currents rather than a single flux, could meaningfully improve the reliability of TUR-based entropy-production estimates extracted from single-cell or single-molecule trajectory data [19,20,21], with particular value for systems, such as molecular motors and proofreading enzymes, where multiple simultaneously observable currents are often already recorded.

Several limitations qualify these conclusions. First, the 34-topology panel, while spanning a broad range of cyclomatic complexity, does not exhaustively cover the space of biochemical network architectures, and networks with unusual symmetry or extremely high connectivity could show different tightness behaviour than reported here. Second, the multidimensional TUR bound used three simultaneously tracked currents chosen to be plausibly observable in an experimental setting; a bound using additional currents, where experimentally accessible, could recover still more of the true entropy production, so the reported $Q = 0.83$ average should be read as a conservative rather than an upper-limit estimate of multidimensional TUR performance. Third, all simulations assumed exact knowledge of the underlying rate constants and network topology, whereas real single-molecule or single-cell data carry measurement noise and topological uncertainty that would further degrade bound reliability beyond what is quantified here [22,23,24,25,26].

5. CONCLUSION

A systematic evaluation of the thermodynamic uncertainty relation across 1,220 rate-parameter combinations spanning 34 biochemical network topologies found that the standard scalar TUR bound recovers, on average, only 61% of true entropy production, with tightness degrading systematically as network cyclomatic complexity increases and as kinetic proofreading depth grows, reaching an underestimate of 71% in the deepest proofreading cascade tested. A multidimensional TUR bound incorporating three simultaneously observable currents recovered 83% of true dissipation on average, more than halving the mean estimation gap and showing its largest gains precisely in the topologically complex networks where the scalar bound performed worst. These results argue for cautious, topology-aware use of TUR-based dissipation estimates in stochastic biochemistry and support multidimensional current tracking as a practical route to more quantitatively reliable estimates.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

M.K. conceived the study, designed the network topology panel, and led the writing of the manuscript. P.S. implemented the stochastic simulation pipeline and computed ground-truth entropy production. Z.N. developed the multidimensional TUR estimator, performed the statistical analysis, and prepared the figures and tables. All authors contributed to interpretation of the results and approved the final manuscript.

Ethics Statement

This study involved no human or animal subjects and required no ethics committee approval; all data were generated by numerical simulation of model biochemical reaction networks.

Data Availability

The stochastic simulation code, network topology definitions, and full parameter-sweep output data supporting the findings of this study are available from the corresponding author on reasonable request.

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