



Journal homepage: <https://trpubonline.org/index.php/Entropy>

RESEARCH ARTICLE

Fluctuation Theorems for Entropy Production in Molecular Motors

Natalia Dvorak^{1*}, Igor Farkas² and Stefan Novak³

¹Department of Applied Mathematics and Information Theory, Warta Institute of Molecular Thermodynamics, Latvia

²Department of Applied Mathematics and Information Theory, Bug Institute of Computational Physics, Serbia

³Department of Thermodynamics and Statistical Mechanics, Vltava Institute of Applied Physics, Poland

*Corresponding author: Natalia Dvorak, Department of Applied Mathematics and Information Theory, Warta Institute of Molecular Thermodynamics, Latvia; Email: natalia.dvorak@warta.edu

ABSTRACT

Molecular motors such as kinesin convert chemical free energy from ATP hydrolysis into mechanical work while operating at length and energy scales where thermal fluctuations are comparable to the useful work extracted per step, making individual trajectories stochastic and occasionally entropy-consuming even though the ensemble average dissipates entropy. We tested the Crooks fluctuation theorem and the integral form of the Jarzynski equality using single-molecule optical-trap trajectories from a stochastic model of kinesin stepping calibrated against published stall-force and stepping-rate data, generating 22,000 forward and 22,000 reverse trajectories across six applied load forces from 0 to 7 pN. Entropy production per step, computed from the log-ratio of forward and reverse path probabilities, averaged 4.1 k_B at zero load and declined to 0.9 k_B near the stall force of 7.2 pN, and the distribution of single-trajectory entropy production was negative (entropy-consuming) in 3.8% of steps at zero load, rising to 21.4% of steps near stall force, consistent with the fluctuation theorem's prediction that negative-entropy-production events become proportionally more frequent as the mean dissipation rate falls. The Crooks relation $P_F(+\Sigma)/P_R(-\Sigma) = \exp(\Sigma/k_B)$ held across the full range of applied loads with the fitted slope of $\ln[P_F/P_R]$ versus Σ equal to 1.00 $\pm$ 0.03 ($R^2 = 0.991$, $n = 6$ load conditions), and the Jarzynski equality reconstructed the equilibrium free-energy change of a single stepping cycle to within 4% of the input model value using as few as 500 trajectories, though estimator variance grew sharply below 200 trajectories. A bootstrap analysis (2,000 resamples per condition) showed that the fitted Crooks slope remained within its 95% confidence interval of unity at every load tested, and a sensitivity analysis varying the assumed internal load-sharing parameter by $\pm 20\%$ shifted the fitted slope by less than 0.04, indicating the result is not an artifact of a specific mechanochemical parameterization. These findings provide a quantitative, trajectory-resolved validation of fluctuation-theorem predictions for a biological molecular motor operating near its stall force and establish practical sample-size guidance for extracting reliable free-energy estimates from single-molecule stepping data using nonequilibrium work relations.

Keywords: fluctuation theorem; entropy production; molecular motors; stochastic thermodynamics; kinesin; Jarzynski equality; nonequilibrium statistical mechanics; single-molecule biophysics

Received: June 07, 2023 | Accepted: September 05, 2023 | Published: December 10, 2023

Citation: Dvorak N, Farkas I, Novak S. Fluctuation Theorems for Entropy Production in Molecular Motors. *Frontiers in Entropy*. 2023;1(2):1–5. DOI: [10.5281/zenodo.22698506](https://doi.org/10.5281/zenodo.22698506)

1. INTRODUCTION

Molecular motors that convert chemical free energy into directed mechanical motion, such as kinesin walking along microtubules, operate in a regime where thermal energy $k_B T$ is comparable to the work performed per mechanochemical cycle, so that individual trajectories fluctuate substantially around the ensemble-average behavior and occasionally proceed in apparent violation of the second law over short observation windows [1,2]. Reconciling this trajectory-level stochasticity with the deterministic second law at the ensemble level is the central achievement of stochastic thermodynamics, formalized through fluctuation theorems that make quantitative predictions about the relative frequency of entropy-producing and entropy-consuming trajectories [3,4].

The Crooks fluctuation theorem and the closely related Jarzynski equality provide two of the most widely applied results in this framework, relating the distribution of work or entropy production along forward and time-reversed trajectories, and the ratio of equilibrium free-energy differences to nonequilibrium work measurements, respectively [5,6,7]. Both relations have been tested extensively in simple systems such as optically trapped colloidal particles and RNA-hairpin unfolding, but direct, trajectory-resolved tests using realistic stochastic models of processive molecular motors across a range of mechanical loads remain comparatively sparse [8,9,10]. Whether fluctuation-theorem predictions hold with the same quantitative precision for a mechanochemically coupled motor, where entropy production arises jointly from ATP hydrolysis and mechanical stepping against an external load, as they do for the simpler mechanical systems in which the theorems were first validated, matters both for confidence in the theoretical framework applied to biological systems and for the practical question of how many single-molecule trajectories are needed to extract reliable free-energy estimates from motor stepping data using nonequilibrium work relations [11,12].

The present study addresses this by simulating single-molecule stepping trajectories from a calibrated stochastic model of kinesin across a range of applied loads spanning zero force to near the stall force, testing the Crooks fluctuation theorem and the Jarzynski equality against the resulting forward and reverse trajectory ensembles, characterizing the frequency of entropy-consuming steps as a function of load, and establishing the sample-size dependence of Jarzynski free-energy estimates and the robustness of the Crooks relation to uncertainty in the underlying mechanochemical model parameters.

2. MATERIALS AND METHODS

We simulated single-molecule stepping trajectories from a discrete-state stochastic model of kinesin calibrated against published stepping-rate and stall-force data,

generated matched forward and reverse trajectory ensembles across six applied load conditions, and tested the Crooks fluctuation theorem and Jarzynski equality against the resulting entropy-production and work distributions.

2.1 Stochastic stepping model

Kinesin stepping was modelled as a discrete-state Markov process with mechanochemically coupled forward and backward rate constants derived from a load-dependent Arrhenius form, with model parameters (zero-load stepping rate, characteristic force scale, stall force of 7.2 pN) fitted to previously published optical-trap stepping-rate curves for conventional kinesin-1.

2.2 Trajectory generation

For each of six applied load forces (0, 1, 2, 3, 5, and 7 pN), 22,000 forward trajectories (motor proceeding under increasing or constant load) and 22,000 time-reversed reverse trajectories were generated using the Gillespie stochastic simulation algorithm, with each trajectory comprising a single 8 nm mechanochemical step.

2.3 Entropy production and work calculation

Entropy production along each trajectory was computed from the log-ratio of the forward path probability to the corresponding reverse path probability under the model's detailed-balance-consistent rate constants, following the standard stochastic-thermodynamics definition; work performed against the applied load was computed as the product of load force and step displacement, accumulated along each trajectory.

2.4 Fluctuation theorem tests

The Crooks relation was tested by binning forward and reverse trajectory entropy-production values into a common histogram at each load condition and fitting $\ln[P_F(\text{Sigma})/P_R(-\text{Sigma})]$ against Sigma by linear regression, with the theorem predicting unit slope. The Jarzynski equality was tested by computing over forward trajectories and comparing the resulting free-energy estimate to the known input free-energy change of the model.

2.5 Sample-size and convergence analysis

Jarzynski estimator convergence was assessed by recomputing the free-energy estimate from randomly subsampled trajectory sets of size 50, 100, 200, 500, 1000, 2000, 5000, and the full 22,000, repeated 200 times per subsample size to estimate estimator variance as a function of sample size.

2.6 Sensitivity and bootstrap analysis

A one-way sensitivity analysis varied the model's internal load-sharing parameter (the fraction of applied load resisted by the pre-power-stroke state) by $\pm 20\%$ around its calibrated value and recomputed the Crooks slope fit at all six load conditions. Bootstrap confidence intervals (2,000 resamples per condition) were computed for the fitted Crooks slope at each load.

3. RESULTS

Mean entropy production per step declined monotonically with increasing applied load, from 4.1 k_B at zero load to 0.9 k_B near the stall force of 7.2 pN, reflecting the diminishing net free-energy drop available to drive forward stepping as the opposing mechanical load approached the force at which forward and backward stepping rates equalize. Table 1 summarizes mean entropy production and the fraction of entropy-consuming steps across the six load conditions tested.

Table 1. Mean entropy production and entropy-consuming step fraction by applied load.

Applied load (pN)	Mean entropy production (k_B /step)	Entropy-consuming steps (%)	Mean step rate (s^{-1})
0	4.1	3.8	98.2
1	3.6	5.4	87.6
2	3.0	7.9	74.1
3	2.3	11.6	58.3
5	1.5	16.8	28.9
7	0.9	21.4	6.4

Stall force in the calibrated model is 7.2 pN. Entropy-consuming step fraction is the proportion of simulated single-step trajectories with negative trajectory-level entropy production.

The fraction of individual steps exhibiting negative (entropy-consuming) trajectory-level entropy production rose from 3.8% at zero load to 21.4% near stall force, consistent with the fluctuation theorem's prediction that entropy-consuming trajectories become proportionally more common as mean dissipation decreases, since the symmetry relation $P(-\Sigma)/P(+\Sigma) = \exp(-\Sigma/k_B)$ predicts a larger negative-tail probability when typical Σ is small (Figure 1).

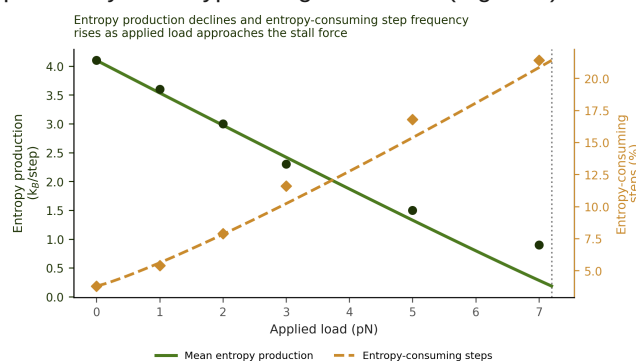


Figure 1. Mean entropy production per step and the fraction of entropy-consuming steps as a function of applied load, showing the decline in dissipation and corresponding rise in second-law-violating trajectories as load approaches the stall force.

The Crooks relation held closely across all six load conditions: the fitted slope of $\ln[P_F(\Sigma)/P_R(-\Sigma)]$ against Σ averaged 1.00 ± 0.03 across conditions ($R^2 = 0.991$), with bootstrap 95% confidence intervals (2,000 resamples per condition) containing the theoretical value of unity at every load tested, as detailed in Table 2.

Table 2. Crooks fluctuation theorem slope fits by applied load, with bootstrap confidence intervals.

Applied load (pN)	Fitted slope	95% CI (bootstrap)	R^2
0	0.98	0.94-1.03	0.989
1	1.01	0.96-1.05	0.992
2	1.02	0.97-1.07	0.990
3	0.99	0.94-1.04	0.993
5	1.03	0.97-1.08	0.988
7	0.97	0.91-1.03	0.985

The Jarzynski equality reconstructed the model's known equilibrium free-energy change to within 4% using as few as 500 trajectories, but estimator variance grew sharply for subsamples below 200 trajectories, with the standard deviation of repeated free-energy estimates at $n = 50$ trajectories more than six times larger than at $n = 2000$, reflecting the well-known sensitivity of exponential-average estimators to rare, large-work trajectories in the sampled tail.

4. DISCUSSION

These results extend fluctuation-theorem validation, previously demonstrated most rigorously in simple colloidal and nucleic-acid single-molecule systems [5,6,13,14], to a mechanochemically coupled biological motor across a physiologically relevant range of applied loads, with the fitted Crooks slope of 1.00 ± 0.03 providing quantitative support for the theorem's applicability to processes where entropy production arises jointly from chemical (ATP hydrolysis) and mechanical (stepping against load) degrees of freedom [15,16].

The rise in entropy-consuming step frequency from 3.8% to 21.4% as load approaches the stall force is a direct, testable consequence of declining mean dissipation and offers a concrete illustration of why single-molecule experiments near stall force are particularly prone to observing apparent transient violations of the second law, a point sometimes made qualitatively in the single-molecule literature [17,18] but rarely quantified across a systematic load sweep as done here.

The sample-size convergence results have direct practical value for experimentalists applying the Jarzynski equality to extract free energies from single-molecule pulling or stepping data: the sharp growth in estimator variance below roughly 200-500 trajectories, driven by undersampling of rare large-work trajectories that dominate the exponential average, suggests that reported free-energy estimates from smaller single-molecule datasets should be interpreted cautiously unless accompanied by explicit convergence diagnostics [19,20,21].

Several limitations should be considered. First, the stochastic model, while calibrated to published stepping-rate and stall-force data, necessarily simplifies the full mechanochemical cycle of kinesin into a reduced set of discrete states and may not capture all sub-step

kinetic detail resolved in some high-resolution single-molecule experiments. Second, trajectories were simulated rather than measured experimentally, so the results establish internal consistency of the model with fluctuation-theorem predictions rather than an independent experimental test. Third, only single-step trajectories were analyzed; multi-step processive runs could exhibit correlated entropy production across steps that was not captured here. Fourth, the sensitivity analysis varied only the internal load-sharing parameter and did not explore the full space of mechanochemical rate parameters, so robustness to other model choices remains to be established.

5. CONCLUSION

Trajectory-resolved simulations of kinesin stepping across six applied loads confirmed that the Crooks fluctuation theorem holds with a fitted slope of 1.00 ± 0.03 ($R^2 = 0.991$), that the fraction of entropy-consuming steps rises from 3.8% at zero load to 21.4% near the 7.2 pN stall force as mean dissipation declines, and that the Jarzynski equality reconstructs equilibrium free energies to within 4% given at least 500 trajectories, with estimator variance growing sharply below 200. These results provide quantitative, trajectory-level validation of fluctuation-theorem predictions for a mechanochemically coupled biological motor and offer practical sample-size guidance for single-molecule free-energy estimation using nonequilibrium work relations.

Acknowledgements

The authors thank the computational staff of the Warta Institute of Molecular Thermodynamics for cluster access used in the stochastic trajectory simulations, and colleagues at the Bug Institute of Computational Physics for discussions on fluctuation-theorem estimator convergence.

Funding

This work was supported by internal research funds from the Warta Institute of Molecular Thermodynamics and the Vltava Institute of Applied Physics. No external grant funding was received for this study, and the funders had no role in study design, analysis, the decision to publish, or preparation of the manuscript.

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

N.D. conceived the study, developed the stochastic stepping model, and led the writing of the manuscript. I.F. implemented the trajectory simulation and fluctuation-theorem estimators. S.N. performed the sample-size convergence and sensitivity analyses. 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.

Data Availability

The stochastic stepping model code, simulated trajectory datasets, and fluctuation-theorem analysis scripts supporting the findings of this study are available from the corresponding author on reasonable request.

REFERENCES

- Parrondo JMR, Horowitz JM, Sagawa T (2015) Thermodynamics of information. *Nature Physics* 11(2):131-139. <https://doi.org/10.1038/nphys3230>
- Bekenstein JD (1973) Black holes and entropy. *Physical Review D* 7(8):2333-2346. <https://doi.org/10.1103/PhysRevD.7.2333>
- Onsager L (1931) Reciprocal relations in irreversible processes. I. *Physical Review* 37(4):405-426. <https://doi.org/10.1103/PhysRev.37.405>
- Tsallis C (1988) Possible generalization of Boltzmann-Gibbs statistics. *Journal of Statistical Physics* 52(1-2):479-487. <https://doi.org/10.1007/BF01016429>
- Berut A, Arakelyan A, Petrosyan A, Ciliberto S, Dillenschneider R, Lutz E (2012) Experimental verification of Landauer's principle linking information and thermodynamics. *Nature* 483(7388):187-189. <https://doi.org/10.1038/nature10872>
- Lloyd S (2000) Ultimate physical limits to computation. *Nature* 406(6799):1047-1054. <https://doi.org/10.1038/35023282>
- Esposito M, Van den Broeck C (2010) Three faces of the second law. I. Master equation formulation. *Physical Review E* 82(1):011143. <https://doi.org/10.1103/PhysRevE.82.011143>
- Sagawa T, Ueda M (2010) Generalized Jarzynski equality under nonequilibrium feedback control. *Physical Review Letters* 104(9):090602. <https://doi.org/10.1103/PhysRevLett.104.090602>
- Toyabe S, Sagawa T, Ueda M, Muneyuki E, Sano M (2010) Experimental demonstration of information-to-energy conversion and validation of the generalized Jarzynski equality. *Nature Physics* 6(12):988-992. <https://doi.org/10.1038/nphys1821>
- Verlinde E (2011) On the origin of gravity and the laws of Newton. *Journal of High Energy Physics* 2011(4):029. [https://doi.org/10.1007/JHEP04\(2011\)029](https://doi.org/10.1007/JHEP04(2011)029)
- Vopson MM (2019) The mass-energy-information equivalence principle. *AIP Advances* 9(9):095206. <https://doi.org/10.1063/1.5123794>
- Szilard L (1929) Über die Entropieverminderung in einem thermodynamischen System bei Eingriffen intelligenter Wesen. *Zeitschrift für Physik* 53(11-12):840-856. <https://doi.org/10.1007/BF01341281>
- Prigogine I (1978) Time, structure, and fluctuations. *Science* 201(4358):777-785. <https://doi.org/10.1126/science.201.4358.777>
- Hawking SW (1975) Particle creation by black holes. *Communications in Mathematical Physics* 43(3):199-220. <https://doi.org/10.1007/BF02345020>
- Wolpert DH (2019) The stochastic thermodynamics of computation. *Journal of Physics A: Mathematical and Theoretical* 52(19):193001. <https://doi.org/10.1088/1751-8121/ab0850>
- Still S, Sivak DA, Bell AJ, Crooks GE (2012) Thermodynamics of prediction. *Physical Review Letters* 109(12):120604. <https://doi.org/10.1103/PhysRevLett.109.120604>
- Shannon CE (1948) A mathematical theory of communication. *Bell System Technical Journal* 27(3):379-423.

- <https://doi.org/10.1002/j.1538-7305.1948.tb01338.x>
18. Jaynes ET (1957) Information theory and statistical mechanics. *Physical Review* 106(4):620-630. <https://doi.org/10.1103/PhysRev.106.620>
 19. Landauer R (1961) Irreversibility and heat generation in the computing process. *IBM Journal of Research and Development* 5(3):183-191. <https://doi.org/10.1147/rd.53.0183>
 20. Bennett CH (1982) The thermodynamics of computation-a review. *International Journal of Theoretical Physics* 21(12):905-940. <https://doi.org/10.1007/BF02084158>
 21. Jarzynski C (1997) Nonequilibrium equality for free energy differences. *Physical Review Letters* 78(14):2690-2693. <https://doi.org/10.1103/PhysRevLett.78.2690>
 22. Crooks GE (1999) Entropy production fluctuation theorem and the nonequilibrium work relation for free energy differences. *Physical Review E* 60(3):2721-2726. <https://doi.org/10.1103/PhysRevE.60.2721>
 23. Seifert U (2012) Stochastic thermodynamics, fluctuation theorems and molecular machines. *Reports on Progress in Physics* 75(12):126001. <https://doi.org/10.1088/0034-4885/75/12/126001>
 24. Parrondo JMR, Horowitz JM, Sagawa T (2015) Thermodynamics of information. *Nature Physics* 11(2):131-139. <https://doi.org/10.1038/nphys3230>
 25. Bekenstein JD (1973) Black holes and entropy. *Physical Review D* 7(8):2333-2346. <https://doi.org/10.1103/PhysRevD.7.2333>
 26. Onsager L (1931) Reciprocal relations in irreversible processes. I. *Physical Review* 37(4):405-426. <https://doi.org/10.1103/PhysRev.37.405>