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An optical trap experiment to demonstrate Fluctuation Theorems in viscoelastic media

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Abstract

Conventional 19th century thermodynamics has limited our understanding of statistical physics to systems in the thermodynamic limit, and at or near-equilibrium. However, in the last decade two new theorems, collectively referred to as Fluctuation Theorems or FTs, were introduced that quantify the energy distributions of *small* systems that are driven out of equilibrium, possibly far-from-equilibrium, by an external field. As such the FTs represent a much needed extension of nonequilibrium thermodynamics that can potentially address systems of interest in the 21st century, including nano/micro-machines and single biomolecular function. Optical trapping has served as an ideal experimental technique for demonstrating these theorems. Measurement of picoNewton range forces over nanometre-sized displacements of a trapped micron-sized particle allows us to measure the energies to a fraction of thermal energy along the particle's trajectory - precisely what is needed to demonstrate the predictions of the FTs. Here we review the Fluctuation Theorems, as originally cast by Evans et al. [1, 2] and Crooks [3], and provide a discussion of their importance and a comparison of their arguments. We further demonstrate an optical trap experiment that confirms the FTs. We've chosen to review an optical trapping experiment that is identical to a previously published experiment [10], but where the solvent is viscoelastic rather than purely viscous. This represents the first experimental demonstration where dynamics of the colloidal particle are complex and not known apriori.

INTRODUCTION

Thermodynamics is the study of the flow of heat and the transformation of work into heat. Our understanding of thermodynamics is largely confined to equilibrium states. The field of "nonequilibrium thermodynamics" represents a simple-minded extension of the 19th century concepts of equilibrium thermodynamics to systems that are *close to*, or *near* equilibrium. Moreover these traditional concepts are limited in application to large systems, referred to as the "thermodynamic limit". However, in the last decade new theorems, referred to collectively as Fluctuation Theorems (FTs), lift the requirement of the thermodynamic limit, describing small systems that evolve far from equilibrium. In particular, the FT of Evans et al. [1, 2] can be thought of as a generalisation of the Second Law of Thermodynamics that applies to small systems over short timescales. The FT of Crooks[3] similarly describes the evolution of systems between equilibrium states. It implies that the change in free energy is related to the work done by an external field to drive a system between two states, at arbitrary rates, and not simply quasi-static rates as understood by conventional thermodynamics. Both of these theorems are at odds with a traditional understanding of 19th century thermodynamics where equilibrium is central and the Second law inviolate. However, these theorems should be critical to the application of thermodynamic concepts to systems of interest to scientists and engineers in the 21st century.

Much of the work done in developing and extending the theorem was accomplished by theoreticians and mathematicians interested in non-equilibiurm statistical mechanics. Until 2002, demonstrations of the theorems were limited to computer simulations and there were no practical experimental demonstrations of the theorems [5]. Consequently, these theorems have only recently received attention, spurred by the current interest in nano / micromachines, or devices that impose nanometre scale displacements $(1 \text{ nm} = 10^{-9} \text{ m})$ with picoNewton scale forces $(1 \text{ pN} = 10^{-12} \text{ N})$, or equivalently, have a work cycle comparable to k_BT or thermal energy $(1 k_BT = 4.1 \text{ pN} \cdot \text{nm})$ per degree of freedom. Such small machines will, according to the FT, sometimes operate in "reverse" and in contradiction to the Second Law of Thermodynamics over small timescales. In 2002 the FT of Evans *et al.* was demonstrated experimentally for the first time, using a single colloidal particle, localised in translating optical trap [5]. Since then, several other experiments have also confirmed the FT; however, all of these experiments, including the first 2002 experiment, were accomplished using systems whose dynamics are accurately described with a simple Langevin equation with uncorrelated, Gaussian noise. However, the deterministic proofs of Evans *et al.* and Crooks imply that the FTs apply generally to systems more complex than those described with white noise. In this paper we describe the first experiments confirming the applicability of these FTs to more complex systems, *i.e.*, an optically trapped colloidal particle suspended in a viscoelastic solvent.

The paper is organised in the following manner. In the following section we review the FTs, focussing upon their potential importance outside of the field of statistical mechanics and providing precise definitions of the arguments of the FTs. These FTs are usually studied as separate theorems; however, both describe the distribution of energies associated with the trajectories of systems that are driven out of equilibrium by an external field. Next, we introduce an optical trap experiment that we use to demonstrate these theorems. This experiment, referred to as the "capture" experiment, follows the dynamics of an individual micron-sized colloidal particle within an optical trap, whose strength is changed discontinuously. The colloidal particle corresponds to the system, whose trajectory we can easily record and the optical trap serves as the external potential. Furthermore, we can characterise the viscoelasticity of the suspending solvent using passive microrheology of the optically trapped particle to determine the frequency dependence of the local solvent's storage and loss modulii. The original capture experiment was accomplished in a purely viscous solution where the colloidal trajectories are well described using the stochastic equation of motion with white noise, here we describe a series of optical trapping experiments in a viscoelastic solvent and demonstrate the FTs simultaneously. Crooks' FT is satisfied in this system rather trivially while the Evans' FT is demonstrated with several thousand experimental trajectories of the optically trapped particle.

THE FLUCTUATION THEOREMS

Evans-Searles Fluctuation Theorem

In many areas of physical chemistry, researchers strive to understand new systems through deterministic equations of motion. They seek to quantify microscopic forces and understand how a system responds to external perturbations, using techniques such as Molecular Dynamics simulation. At the heart of this endeavour is the notion that if the equations of motion or trajectories of the system are known, then any question about that system may be answered. However, such deterministic equations (such as Newton's equations) are timereversible, so that for every trajectory there exists a time-reversed trajectory or "anti-" trajectory which is also a solution to the equations. The relative probabilities of observing bundles of conjugate trajectories quantifies the "reversibility" of the system: if the probability of observing all trajectories and their respective anti-trajectories are equal, the system is said to be perfectly reversible; on the other hand, if the probability of observing antitrajectories is vanishingly small, we say that the system is irreversible. The Second Law of Thermodynamics states that a system evolves irreversibly in one "time-forward" direction, *i.e.*, the probability of all anti-trajectories is zero. However, the Second Law strictly applies only to large systems over long time scales and does not describe the reversibility of small systems that are of current scientific interest, such as protein motors and nano-machines. This long-standing question of how irreversible macroscopic equations, as summarised by the Second Law of Thermodynamics, can be derived from reversible microscopic equations of motion was first noted by Loschmidt^[6] in 1876 and has been a paradox since the days of Boltzmann. Boltzmann's successors have simply side-stepped this issue by stating "as soon as one looks at bodies of such small dimension that they contain only very few molecules, the validity of this theorem [the Second Law of Thermodynamics] must cease" [7].

The Fluctuation Theorem (FT) of Evans & Searles [2] describes how a system's irreversibility develops in time from a completely time-reversible system at short observation times, to a thermodynamically irreversible one at infinitely long times. That is, it bridges the microscopic and macroscopic descriptions, relating a system's time-reversible equations of motion to the Second Law, and provides a resolution to the long-standing irreversibility paradox. Specifically, the FT relates the relative probabilities of observing trajectories of duration t characterised by the dissipation function, Ω_t , taking on arbitrary values a and -a, respectively:

$$\frac{P(\Omega_t = a)}{P(\Omega_t = -a)} = \exp\left[a\right].$$
(1)

The dissipation function, Ω_t , is, in general, a dimensionless dissipated energy, accumulated along the system's trajectory; expressions for Ω_t differ from system to system. We express Ω_t , and other energies introduced below, in units of $k_B T$ where k_B is Boltzmann's constant and T is the temperature of the surroundings with which the system is in contact and is initially at equilibrium. Any trajectory of the system that is characterised by a particular $\Omega_t = a$ has, under time-reversible deterministic mechanics, a conjugate or time-reversed trajectory with $\Omega_t = -a$. In this way, the LHS of the FT has also been interpreted as a ratio of the probabilities of observing trajectories to their respective time-reverse or anti-trajectories. The dissipation function, Ω_t , is an extensive property, *i.e.*, its magnitude scales with system size and observation time, t. Thus, eqn 1 also shows that as the system size gets larger or the observation time gets longer, anti-trajectories become rare and it becomes overwhelmingly likely that the system appears time-irreversible, in accord with the Second Law. That is, the evolution of a large macroscopic system proceeds preferentially in one direction. This concept is embodied in the Second Law of thermodynamics. The Second Law states that a macroscopic system evolves overwhelmingly in one, time-forward direction or is "irreversible". The Evans-Searles Fluctuation Theorem states that microscopic, systems evolve in both time-forward and time-reverse directions, and quantifies the system's "reversibility".

Crooks Fluctuation Theorem

From Classical thermodynamics, the work done by an external field to drive a system from one equilibrium state to another equilibrium state is equivalent to the change of free energy, ΔF , between the states, only in the special case where the path is traversed quasi-statically. That is the path between the two states must be traversed so slowly that intermediate, as well as the initial and final states of the system, are all in thermodynamic equilibrium.

Crook's Fluctuation Theorem [3] states something quite remarkable. In the case of paths that are traversed at arbitrary rate, ranging from quasi-static to "far-from-equilibrium", the distribution of trajectories, characterised by the work done by the external field over the lifetime of the trajectory, follows

$$\frac{P_f(W=a)}{P_r(W=-a)} = \exp\left[a - \Delta F\right].$$
(2)

This expression is similar to Evans' FT in that it relates distributions of trajectories, characterised by an energy, specifically the work, W, expressed in units of k_BT . Here T is the initial temperature of the system on which the external field does work, or equivalently the temperature of the surroundings with which the system is initially under equilibrium. While eqn 1 describes the asymmetry in the distribution of trajectories about a zero-energy $\Omega_t = 0$, Crooks FT, eqn 2, relates two different distributions: (i) a distribution of *forward* trajectories that connect states $A \to B$, or P_f where the free energy change between equilibrium states A and B is $\Delta F = F_B - F_A$, and the distribution of *reverse* trajectories, $B \to A$ or P_r , where the respective equilibrium free energy change is $-\Delta F$. Like the FT, Crook's FT also quantifies how irreversibility evolves out of reversible equations of motion. A perfectly reversible system is one where the work required to traverse $B \to A$ is equal but opposite in sign to the work required in the time-reversed trajectory, $A \to B$; under time-reversible mechanics, the RHS of eqn 2 is unity for these reversible paths and W = F, in agreement with classical thermodynamics.

From Crook's FT and the definition of ensemble averages,

$$\exp\left(-\Delta F\right) = \langle \exp\left(-W\right) \rangle_f. \tag{3}$$

This expression was first posed by Jarzynski in 1997 [8] and states that the free energy can be determined by measuring the work, W, done by an external field along dynamical paths that connect the two states. These paths may be traversed at arbitrary rates, so that the intervening states may not be in true thermodynamic equilibrium. This is a complete anathema to our understanding of classical thermodynamics. If instead of averaging the work, you average the exponential of the work, then you can calculate the equilibrium free energy difference from information obtained along *nonequilibrium* paths. The potential importance of eqn 3 has both theoretical and practical aspects. One could theoretically calculate ΔF using fictitious paths that "morph" between 2 states on a computationally convenient (not necessarily realistic) energy surface. (This differs from classical thermodynamics where considerable care must be made to measure the work along a path that is both quasi-static and accurate or realistic.) On the practical side, eqn 3 suggests that measuring work on small microscopic processes could yield thermodynamic quantities ΔF that are traditionally inferred by calorimetric measurements. The importance here is that in order to understand molecular-scale processes, it is necessary to probe them using molecular time/length scales.

Arguments of the FTs

To understand the FTs and their relationship, we need to define the energies dissipated along the trajectories, W and Ω_t . In the original derivations of the FTs, these energies were defined over trajectories within deterministic phase space, *i.e.*, where the system's degrees of freedom are given by the vectors of time-dependent positions, **q** and momenta, **p**, representing all components of the system. The internal energy, \mathcal{U} , of such a system is the sum of the kinetic energy, $K(\mathbf{p})$, the potential interactions amongst constituents, $\phi(\mathbf{q})$, and the external potential interacting with the degrees of freedom of the system, $\phi_{ext}(\mathbf{q}, \lambda)$:

$$\mathcal{U}(\mathbf{q}, \mathbf{p}) = K(\mathbf{p}) + \phi(\mathbf{q}) + \phi_{ext}(\mathbf{q}, \lambda).$$
(4)

Here λ parameterises the value of the external field strength as it changes from an initial value at s = 0 to a final value at time s = t. The work done by an external field on the degrees of freedom of the system is governed by the First Law

$$W = \int_0^t ds \left[\dot{\mathcal{U}}(s) - \dot{Q}(s) \right],\tag{5}$$

where Q(s) is the instantaneous rate of heat exchange with the system's surroundings. This expression is valid for any system. However, in the original derivation of Crooks FT and Jarzinksi's relation, the authors considered a system governed by a Hamiltonian, $\mathcal{H}(\mathbf{q}, \mathbf{p})$, where by definition, $\mathcal{H} \equiv \mathcal{U}$ and the system is isolated (there is no heat exchange with the surroundings, or $\dot{Q}(s) = 0$) and obeys Hamilton's equations,

$$\dot{\mathbf{q}} = \frac{\partial \mathcal{H}(\mathbf{q}, \mathbf{p})}{\partial \mathbf{p}} \tag{6}$$

$$\dot{\mathbf{p}} = -\frac{\partial \mathcal{H}(\mathbf{q}, \mathbf{p})}{\partial \mathbf{q}}.$$
(7)

For such a Hamiltonian system, the rate of change of the internal energy is

$$\dot{\mathcal{U}}(s) = \dot{\mathcal{H}}(s) = \frac{\partial \mathcal{H}}{\partial \mathbf{p}} \dot{\mathbf{p}} + \frac{\partial \mathcal{H}}{\partial \mathbf{q}} \dot{\mathbf{q}} + \frac{\partial \mathcal{H}}{\partial \lambda} \dot{\lambda}$$
(8)

$$= \frac{\partial \mathcal{H}}{\partial \lambda} \dot{\lambda}, \tag{9}$$

and the work done by the external field is

$$W = \int_0^t ds \dot{\mathcal{U}} = \int_0^t ds \dot{\lambda} \frac{\partial \mathcal{H}}{\partial \lambda},\tag{11}$$

again, where the energies are expressed in units of $k_B T$ and where T is the temperature of the surroundings with which the system is initially at equilibrium. Although eqn 11 is the original definition used in the derivation of Crooks FT and the Jarzynski equality, it is not practical for small systems which are difficult to isolate from the surroundings. Most small systems are difficult to isolate from their surroundings, and even if possible, work performed on a small isolated system, as specified by eqn 11, will occur with a simultaneously increase in the temperature of the system. Whilst it can be problematic to attempt any description of the temperature of a small nonequilibrium system, the ΔF would then characterise the free energy change for that non-isothermal system. Indeed, for several decades, computer simulationists have studied necessarily small systems by imposing temperature constraints or thermostats in molecular dynamics simulations. Such temperature constraints are non-holonomic; *i.e.* we can append a system's equations of motion with a mathematical constraint to fix the temperature; however with such constraints it is no longer possible to express the dynamics using the Hamiltonian. For example, it is possible to append Hamilton's equations of motion with a mathematical constraint to fix the kinetic temperature [9], $f(\mathbf{q}, \mathbf{p})$:

$$\dot{\mathbf{q}} = \frac{\partial \mathcal{H}(\mathbf{q}, \mathbf{p})}{\partial \mathbf{p}} \tag{12}$$

$$\dot{\mathbf{p}} = -\frac{\partial \mathcal{H}(\mathbf{q}, \mathbf{p})}{\partial \mathbf{q}} - f(\mathbf{q}, \mathbf{p}).$$
(13)

Here \mathcal{H} corresponds to the Hamiltonian without the mathematical constraint, or the internal energy, \mathcal{U} of the system with the constraint. Several different mathematical constraints can be constructed, all of which may be argued to be "artificial", but which must satisfy the conditions that (i) additional thermostatting degrees of freedom introduced must be inert to an external field, ϕ_{ext} , (ii) that under a time-invariant ϕ_{ext} , the system relaxes to an equilibrium state, and (iii) that $\dot{\mathbf{q}}f(\mathbf{q},\mathbf{p}) = \dot{Q}(s)$. That the mathematical constraint is artificial is of no significance, is evident as one substitutes $\mathcal{U}(\mathbf{q},\mathbf{p}) = K(\mathbf{p}) + \phi(\mathbf{q}) + \phi_{ext}(\mathbf{q},\lambda)$, the kinetic energy $K(\mathbf{p}) = \mathbf{p} \cdot \mathbf{p}/m$, and the equations of motion into eqn 5 to show that the explicit value of $\dot{Q}(s)$ vanishes in the expression for W. That is not to say that W is independent of the heat exchange with the surroundings: the trajectory itself depends sensitively upon the mathematical constraint $f(\mathbf{q},\mathbf{p})$ or $\dot{Q}(s) = \dot{\mathbf{q}}f(\mathbf{q},\mathbf{p})$ through the system's equations of motion, eqns 13.

Having properly defined W for both an isolated Hamiltonian system, and a more general

deterministic system which allows heat exchange, we now turn our attention to the argument of the Evans-Searles FT, the dissipation function, Ω_t . The dissipation function is cast similarly to W as

$$\Omega_t = \int_0^t ds \left[\dot{\mathcal{U}}_0 - \dot{Q}(s) \right] \tag{14}$$

where \mathcal{U}_0 is the potential energy of the system where the contribution from the time-varying external field is neglected; although the trajectory over which \mathcal{U}_0 is evaluated is determined by the time-varying external field. That is.

$$\mathcal{U}_0 = \left[\mathcal{U} - \phi_{ext}(\mathbf{q}, \lambda) + \phi_{ext}(\mathbf{q}, \lambda = 0) \right].$$
(15)

For an isolated, Hamiltonian system, $\dot{Q}(s) = 0$ and

$$\dot{\mathcal{U}}_0 \equiv \dot{\mathcal{H}}_0 = \left[\dot{\mathcal{H}} - \dot{\phi}_{ext}(\mathbf{q}, \lambda) + \dot{\phi}_{ext}(\mathbf{q}, \lambda = 0)\right]$$
(16)

where

$$\frac{d\mathcal{H}}{ds} = \frac{\partial\mathcal{H}}{\partial\mathbf{p}}\dot{\mathbf{p}} + \frac{\partial\mathcal{H}}{\partial\mathbf{q}}\dot{\mathbf{q}} + \frac{\partial\mathcal{H}}{\partial\lambda}\dot{\lambda}$$
(17)

$$\frac{d\phi_{ext}(\mathbf{q},\lambda)}{ds} = \frac{\partial \mathcal{H}}{\partial \lambda} \dot{\lambda} + \frac{\partial \phi_{ext}(\mathbf{q},\lambda)}{\partial \mathbf{q}} \dot{\mathbf{q}}$$
(18)

$$\frac{d\phi_{ext}(\mathbf{q},\lambda=0)}{ds} = \frac{d\phi_{ext}(\mathbf{q},\lambda=0)}{d\mathbf{q}}\dot{\mathbf{q}},\tag{19}$$

leading to

$$\Omega_t = \int_0^t ds \left[\dot{\mathcal{U}}_0 - \Lambda(s) \right] = \int_0^t ds \dot{\mathbf{q}} \left[\frac{\partial \phi_{ext}(\lambda = 0)}{\partial \mathbf{q}} - \frac{\partial \phi_{ext}(\lambda)}{\partial \mathbf{q}} \right].$$
(20)

To develop an expression for Ω_t for small systems, it is important to account for heat exchange with the surroundings, or $\dot{Q}(s) \neq 0$. By appending Hamilton's equation with a thermostat constraint, eqns 17-19 become

$$\frac{d\mathcal{H}}{ds} = \left[\frac{\partial\mathcal{H}}{\partial\mathbf{p}}\dot{\mathbf{p}} + \frac{\partial\mathcal{H}}{\partial\mathbf{q}}\dot{\mathbf{q}}\right] + \frac{\partial\mathcal{H}}{\partial\lambda}\dot{\lambda} = \dot{Q}(s) + \frac{\partial\mathcal{H}}{\partial\lambda}\dot{\lambda}$$
(21)

$$\frac{d\phi_{ext}(\mathbf{q},\lambda)}{ds} = \frac{\partial \mathcal{H}}{\partial \lambda} \dot{\lambda} + \frac{\partial \phi_{ext}(\mathbf{q},\lambda)}{\partial \mathbf{q}} \dot{\mathbf{q}}$$
(22)

$$\frac{d\phi_{ext}(\mathbf{q},\lambda=0)}{ds} = \frac{d\phi_{ext}(\mathbf{q},\lambda=0)}{d\mathbf{q}}\dot{\mathbf{q}},\tag{23}$$

leading to eqn 20, *i.e.*, the same expression for the dissipation function of an isolated Hamiltonian system. This is *not* to say that the dissipation function is independent of heat exchange, as like W, exchange of heat will determine the system's trajectory through the deterministic equations of motion, eqn 13.

By analogy, it is now possible to express the dissipation function for any system's trajectory where the equations of motion and the heat exchange are not necessarily known, but where the external potential, $\phi_{ext}(\mathbf{q}, \lambda)$ is known. \mathcal{U}_0 can still be written in terms of the unknown quantity $\dot{Q}(s)$ as

$$\dot{\mathcal{U}}_0 = \dot{\mathcal{U}} - \dot{\phi}_{ext}(\mathbf{q}, \lambda) + \dot{\phi}_{ext}(\mathbf{q}, \lambda = 0)$$
(24)

$$= \dot{W} + \dot{Q}(s) - \dot{\phi}_{ext}(\mathbf{q}, \lambda) + \dot{\phi}_{ext}(\mathbf{q}, \lambda = 0)$$
(25)

$$= \left(\dot{\lambda}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\lambda} + \dot{Q}(s)\right) - \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}} + \dot{\lambda}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\lambda}\right) + \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda=0)}{\partial\mathbf{q}}\right) = \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}} + \dot{\lambda}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\lambda}\right) + \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda=0)}{\partial\mathbf{q}}\right) = \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}} + \dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}}\right) + \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda=0)}{\partial\mathbf{q}}\right) = \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}} + \dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}}\right) + \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda=0)}{\partial\mathbf{q}}\right) = \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}} + \dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}}\right) = \left(\dot{\mathbf{q}}\frac{\partial\phi_{ext}(\mathbf{q},\lambda)}{\partial\mathbf{q}}\right) = \left($$

$$= \dot{Q}(s) + \dot{\mathbf{q}} \frac{\partial(\phi_{ext}(\mathbf{q},\lambda=0) - \phi_{ext}(\mathbf{q},\lambda))}{\partial \mathbf{q}}.$$
(27)

So that $\int_0^t ds [\dot{\mathcal{U}}_0 - \dot{\mathcal{Q}}(s)]$ again yields equation eqn 20. Once again, it is important to note that Ω_t is independent of the explicit value of the heat exchanged at any point of the trajectory, although Ω_t does depend upon the exchange of heat through the time evolution of the coordinates, $\mathbf{q}(s)$. If the equations of motion are known, either as deterministic or stochastic, a value of Ω_t can either be solved for analytically or numerically; if the equations of motion are intractable, as in the case of an optically trapped particle in a viscoelastic solution, then Ω_t can be determined from experimentally measured trajectories, $\mathbf{q}(s)$, as $0 \leq s \leq t$.

Finally, it is important to maintain the convention adopted of expressing energies, including Ω_t and W, in units of $k_B T$ where T is the temperature of the surroundings, irrespective of whether the system is adiabatic or exchanges heat with the surroundings. All non-equilibrium trajectories in the FTs must be initiated under equilibrium conditions, by which we mean that the system and surroundings are in equilibrium and at the same temperature. Thus, the surrounding temperature is additionally, the most convenient reference temperature in FTs, particularly in closed systems where the external field is applied under adiabatic conditions, $\dot{Q} = 0$, and the system's temperature, if it were possible to define/measure in small, non-equilibrium systems, would increase as work is done on the system. T is not a scaling factor: normalising the energy terms Ω_t and W with any other temperature would render eqns 1 and 2 as nonequalities.

THE CAPTURE EXPERIMENT

In the capture experiment, the strength of the stationary optical trap is changed instantaneously, and the time-dependent relaxation of the particle's position from one equilibrium distribution to another distribution is recorded [10]. For this experiment, a particle is localised in a stationary trap of strength k_0 over a sufficiently long time s that its position is described by an equilbrium distribution. At time s = 0, the optical trap strength is increased discontinuously from k_0 to k_1 , $k_1 > k_0$, so that we more tightly confine or "capture" the particle. Alternatively we can decrease the trap strength from k_1 to k_0 to "release" or relax the particle. The particles position is recorded as it relaxes to its new equilibrium distribution and we evaluate the functions W and Ω over an ensemble of non-equilibrium trajectories. In the capture experiment, the external field parameter $\lambda(s)$ is the trap strength, k, which varies discontinuously

$$\lambda(s) \equiv k(s) = k_0 + (k_1 - k_0)H(s),$$
(28)

where H(s) is the heaviside function, $\dot{\lambda}(s) = (k_1 - k_0)\delta(s)$, so that

$$\phi_{ext}(s) = \frac{1}{2} \bigg[k_0 + (k_1 - k_0) H(s) \bigg] q^2(s).$$
(29)

Work, W, is simply the change in the internal energy that occurs upon the instantaneous change in the trapping constant. For the capture experiment it is

$$W = \int_0^t ds \dot{\lambda} \frac{\partial \phi_{eff}}{\partial \lambda} \tag{30}$$

$$= \int_{0}^{t} ds (k_1 - k_0) \delta(s) \frac{q^2(s)}{2}$$
(31)

$$= \frac{1}{2}(k_1 - k_0)q^2(0) \tag{32}$$

Note that W will always be positive if the trap strength is increased or $k_1 > k_0$ and consequently, distributions for W cannot be Gaussian. As all sampled trajectories initiate under equilibrium conditions, the probability distribution of $q(s = 0, k = k_0)$ is a Boltzmann distribution and the distribution of W is then simply

$$P_{k_0 \to k_1}(W) = \sqrt{\frac{k_0}{\pi (k_1 - k_0)W}} \exp\left[-\frac{k_0 W}{k_1 - k_0}\right].$$
(33)

Thus, if we consider the ensemble average, $\langle \exp[-W] \rangle$, then

$$\langle \exp\left[-W\right] \rangle = \int_{0}^{\infty} dW P(W) \exp\left[-W\right]$$
 (34)

$$=\sqrt{\frac{k_1}{k_0}}\tag{35}$$

$$= \exp\left[-\Delta F\right] \tag{36}$$

where the change in free energy associated with changing the trapping constant from k_0 to k_1 is $\Delta F = \ln \left[\sqrt{k_1/k_0}\right]$ from classical statistical thermodynamics. Furthermore, if we consider the probability distribution of W for both the *forward* or capture directions, *i.e.* $W_{k_0 \to k_1}$ where the trap strength increases from k_0 to k_1 , and the *reverse* or release direction, *i.e.* $W_{k_1 \to k_0}$,

$$P_{k_1 \to k_0}(W) = \sqrt{\frac{k_1}{\pi (k_1 - k_0)W}} \exp\left[\frac{k_1 W}{k_1 - k_0}\right],\tag{37}$$

one can see that these distributions trivially obey Crook's FT,

$$\frac{P_{k_0 \to k_1}(W=a)}{P_{k_1 \to k_0}(W=-a)} = \exp\left[a - \Delta F\right].$$
(38)

Notice that in the context of the capture experiment, Crook's FT depends only upon the equilibrium distribution of particle positions within the optical trap. This distribution is independent of the viscoelastic response of the surrounding fluid. Consequently, this experimental demonstration of Crook's FT depends solely upon the ability to sample an equilibrium or Boltzmann distribution of particle positions within the particle well. An alternative experiment, say where the trapping constant changes linearly over some time period is different: there W is accumulated over the time period over which k is changing and the distribution of W depends upon the response or microrheology of the fluid.

In contrast, the dissipation function, Ω_t depends sensitively upon the material properties of the surrounding fluid as this determines the time-response of the particle position to an instantaneous change in the trap constant. For any particle trajectory, irrespective of the solvent, we can express the dissipation function Ω_t as

$$\Omega_t = \int_0^t ds \dot{q} \left[\frac{\partial \phi_{ext}(\lambda = 0)}{\partial q} - \frac{\partial \phi_{ext}(\lambda)}{\partial q} \right]$$
(39)

$$= \int_{0}^{t} ds \left[k_{0} q \dot{q} - \left(k_{0} + (k_{1} - k_{0}) H(s) \right) q \dot{q} \right]$$
(40)

$$= (k_0 - k_1) \int_0^t ds q \dot{q}$$
 (41)

$$= \frac{k_0 - k_1}{2} \left(q^2(t) - q^2(0) \right). \tag{42}$$

For a particle embedded in a purely viscous solvent, the motion of the particle can be expressed by the stochastic Langevin equation

$$\xi \frac{dq}{ds} = -k_1 q + g(s) \tag{43}$$

where ξ is a friction coefficient and g(s) is uncorrelated Gaussian noise with zero mean and $\langle g(s)g(s')\rangle = 2\xi k_B T \delta(s - s')$. As demonstrated by Wang *et al.*[11], it is possible to construct an analytic expression for the probability distributions $P(\Omega_t = A)$ for the capture experiment in purely viscous solvents. From these analytic expressions for $P(\Omega_t)$, one can show that the FT, eqn 1, does indeed hold. However, we are unable to construct such distributions for a particle in a viscoelastic solvent, where g(s) is time-correlated. Under these conditions, it is necessary to construct distribution of $P(\Omega_t)$ from a large ensemble of *experimentally* recorded particle trajectories and inspect the development of asymmetry in the distributions to determine if the FT does indeed still hold. Despite our inability to describe generally the non-equilibrium trajectories of a particle in a viscoelastic solution, it is possible to state long time limiting values of Ω_t , where equilibrium distributions of particle positions are expected. First, as any trajectory evolves over infinite time,

$$\lim_{t \to \infty} \Omega_t = -\left(W_{k_0 \to k_1} + W_{k_1 \to k_0} \right). \tag{44}$$

That is, the dissipation function over the long time limit is equivalent to the work done by the external field to "capture" a particle equilibrated in a trap of strength k_0 , and to "release" a particle equilibrated in a trap of strength k_1 . In addition, the ensemble average of Ω_t in the long time limit is, by equipartition [4]:

$$\lim_{t \to \infty} \langle \Omega_t \rangle = \frac{(k_0 - k_1)^2}{2k_1 k_0} > 0.$$
(45)

Note that the long time limit is predicted to be independent of solvent condition and that as the difference in trapping constants vanish, the trajectory becomes more reversible, and Ω_t approaches 0.

In order to demonstrate the FT in a viscoleastic solution, it is imperative that we demonstrate that the fluid response is viscoelastic over the time and length scales explored in the capture experiment. In accord with the fluctuation-dissipation theorem, the thermal fluctuations of an optically trapped particle can be used to quantify the micro-rheological response of the surrounding fluid [12, 13]. Let q(t) represent the the temporal displacement of the particle of radius R due to a force, F(t). Then the response function or complex compliance of the single particle motion, expressed in the frequency domain, $\tilde{\alpha} = \tilde{\alpha}' + i\tilde{\alpha}''$, relates the Fourier transforms of the displacement and force, \tilde{q} and \tilde{F} respectively:

$$\tilde{q} = \tilde{\alpha}\tilde{F}.\tag{46}$$

The response function is inversely proportional to the complex shear modulus of the fluid, \tilde{G} , by a Generalised Stokes-Einstein Relation (GSER) or

$$\tilde{G} = \frac{1}{6\pi R\tilde{\alpha}}.$$
(47)

Here $\tilde{G} = \tilde{G}' + i\tilde{G}''$ where \tilde{G}' , the real component, is identified with the elastic or storage modulus and \tilde{G}'' , the imaginary component, is the loss modulus.

$$\tilde{G}' = \frac{1}{6\pi R} \frac{\tilde{\alpha}'}{{\tilde{\alpha}'}^2 + {\tilde{\alpha}''}^2},\tag{48}$$

$$\tilde{G}'' = \frac{1}{6\pi R} \frac{-\tilde{\alpha}''}{\tilde{\alpha}'^2 + \tilde{\alpha}''^2}.$$
(49)

To determine these moduli from the position of the optically trapped particle, we first construct a power spectral density, S(f), of particle fluctuations:

$$S(f) = \lim_{t \to \infty} \frac{2}{t} \tilde{q}_t(f) \tilde{q}_t^*(f)$$
(50)

where $\tilde{q}_t(f)$ and $\tilde{q}_t^*(f)$ represent the Fourier transform, $\tilde{q}_t(f) = \int_{-t/2}^{t/2} dsq(s) \exp[2\pi i f s]$, and its complex conjugate. The imaginary component of the complex response function $\tilde{\alpha}''$ is then

$$\tilde{\alpha}'' = \frac{\pi}{2k_B T} fS(f), \tag{51}$$

and the real component of the complex response function is given by a Kramers-Kronig relation, or

$$\tilde{\alpha}' = \frac{2}{\pi} \int_0^\infty d\omega \frac{f \tilde{\alpha}''(\omega)}{f^2 + \omega^2}.$$
(52)

For a purely viscous fluid, $\tilde{G}'' = 2\pi\eta f$, and there is no elastic component of the modulus. or $\tilde{G}' = 0$. However, confinement of the particle's motion to the harmonic optical trap gives rise to an elastic contribution to the measured storage modulus, $\tilde{G}'_{measured}$, so that

$$\tilde{G}'_{measured} = \tilde{G}' + \frac{k}{6\pi R}.$$
(53)

 $\tilde{G}'_{measured}$ is determined from the experimentally measured power spectral density, S(f) and eqns 51 and 52.

For a Brownian particle with uncorrelated Gaussian noise, one can show that the power spectral density is given by [14]

$$S(f) = \frac{k_B T}{\xi \pi^2 (f_c^2 + f^2)}$$
(54)

where f_c is the "corner frequency",

$$f_c = \frac{k}{2\pi\xi},\tag{55}$$

 $\xi = 6\pi\eta R$ is Stokes drag coefficient, and $\eta = \alpha(f = 0)$ is the solution viscosity. For frequencies smaller than the corner frequency, $f \ll f_c$, the power spectral density is nearly constant at a "plateau" value of

$$f \ll f_c \Rightarrow S(f) \approx S_0 = \frac{2k_B T}{\pi k f_c},$$
(56)

while at high frequencies, the power spectral density obeys a power law behaviour,

$$f >> f_c \Rightarrow S(f) \approx \frac{1}{f^2}.$$
 (57)

The corner frequency is determined by the intersection of these two regimes. Indeed, the low-frequency plateau value S_0 and the corner frequency, f_c , provide an alternative method to calibrating the strength, or trapping constant k, of the optical trap. This method is particularly advantageous over the equipartition method (where k is determined from the variance in the particle position, or $k \equiv k_B T/\langle q(t)^2 \rangle$) when the trap strength is large. Note that the variance in the particle displacement, $\langle q(t)^2 \rangle$, is independent of the microrheological properties of the suspending liquid. The integral over the power spectral density corresponds to the variance and depends only upon trap strength and temperature; however the power spectral density does indeed depend upon the microrheological properties [13]. The corner frequency and shape of S(f) depend upon these rheological properties of the suspending liquid. Indeed, the power law behaviour of S(f) at high frequencies is used to qualitatively describe the fluid: $S(f) \approx f^{-z}$ where z = 2 for purely viscous solutions and z < 2 for viscoelastic solutions.

EXPERIMENTAL

The optical trap setup consists of a Nikon DIAPHOT 300 inverted microscope equipped with a $100 \times (N.A.=1.3)$ oil-immersion objective lens and a 4 W Nd:YAG laser, $\lambda = 1064$ nm (Coherent, U.S.A.). The optical trap strength is controlled by adjusting the laser intensity using an LCD power modulator (LPC-NIR, Brockton Electrooptic Corporation, USA). Changes in the laser intensity are recorded using a photodiode (SDP86001, Honeywell, USA) located above the sample cell. The position of a trapped particle is detected by projecting its image onto a quadrant photodiode, which has a resolution of 15 nm (S4349 Hamamatsu, Japan). Labview and a PCI-6014 data acquisition card (National Instruments, USA) are used for automatic data collection.

The sample cell holds approximately 8 ml of liquid to minimise any localised heating from the optical trap. The cell consists of a glass coverslide, a teflon box, and is sealed using either mineral oil or a second coverslide. The sample cell is filled with either 8 ml of deionised water or an aqueous solution containing 0.1 wt% of 8×10^6 MW polyethyleneoxide (PEO). Approximately 3000 polystyrene particles, with a diameter of 6.3 μ m, are injected locally into the sample cell. One particle is optically trapped and moved at least 2 mm from the injection site to ensure its isolation from other particles and allowed to equilibrate within the optical trap for at least 1 hour before measurements taken for microrheology or FT. The position of the particle is then recorded at 20 kHz for 100 s.

Microrheology

First, power spectral densities are constructed from particle positions in optical traps of fixed strength, using water and PEO solutions to demonstrate microrheological differences in the two solutions. Figure 1 shows the power spectral density of a particle in PEO solution, $S_P(f)$, sampled at 20 kHz and the power spectral density of a similarly sized particle in water, $S_W(f)$. At the high frequencies sampled, $S_W(f)$ obeys the power law behaviour, $S_W(f) \sim f^{-2}$ that is expected for solvents where there is no elastic response. From the fit of $S_W(f)$ to eqn 54, we obtain the corner frequency, f_c and the plateau value S_0 , from which we determine the optical trapping constant to be $k = 2.3 \text{ pN}/\mu\text{m}$ and the solution's viscosity $\eta = 0.96 \times 10^{-3}$ Pa.s, in good agreement with the viscosity of water at room temperature, known to be 1.0×10^{-3} Pa.s. The power spectral density of the polymer solution, $S_P(f)$, exhibits a high frequency power law behaviour $S_W(f) \sim f^{-1.85}$, suggesting that an elastic response is present in the PEO solution. From the corner frequency, f_c , and plateau value, S_0 , the trap strength is calculated to be $k = 2.4 \text{ pN}/\mu\text{m}$, the Stokes drag coefficient is $\xi = 0.475 \text{ pN.s}/\mu\text{m}^2$ and the zero-frequency viscosity is therefore $\eta(0) = 7.0 \times 10^{-3}$ Pa.s.

The storage and loss moduli are calculated from the measured particle position, q(t), and eqns 48 - 52. Figure 2a shows the storage and loss moduli, \tilde{G}'_W and \tilde{G}''_W , calculated for the pure water solution using $S_W(f)$. For water, within the frequency range shown, the storage modulus, \tilde{G}'_W , is roughly constant at $\tilde{G}'_W = 4.0 \times 10^{-2}$. This value compares well with the contribution of the optical trap to the measured $\frac{k}{6\pi r} = 3.9 \times 10^{-2}$, indicating that the elastic component of the particle motion is entirely the result of the confining optical trap with no elastic contribution from the solvent at these frequencies [13]. The figure also shows that the loss modulus, \tilde{G}''_W , is linear in frequency, $G'' = 2\pi\eta f$, as expected for a purely viscous solution, where η is independent of frequency over the sampled frequency range. A least squares fit to \tilde{G}''_W provides a viscosity value of $\eta = 0.96 \times 10^{-3}$ Pa.s, agreeing with that obtained from the Lorentzian fitting. Figure 2b shows the complex moduli for the viscoelastic PEO solution. It differs considerably from that obtained in pure water: the storage modulus, \tilde{G}'_P , is no longer a constant and the loss modulus, \tilde{G}''_P , no longer has a simple linear relationship with frequency.

Fluctuation Theorem

To demonstrate the FT, eqn 1, in a viscoelastic solvent, we record and analyse the trajectory of a particle in the PEO solution as the trap strength is changed discontinuously from k_0 to k_1 . The value of the trap strength is determined by recording the position of



FIG. 1: The power spectral density constructed from particle fluctuations measured in water, $S_W(f)$, and in the PEO solvent $S_P(f)$. Both power spectral densities display a high frequency power law behaviour: $S_W(f) \sim f^{-2}$, indicative of particle motion in a purely viscous solvent, and $S_P(f) \sim f^{-1.85}$, indicative of particle motion in a viscoelastic solvent.

the optically trapped particle at 500 Hz for 600 s and applying the equipartition theorem to determine the trapping constant k. Due to the possibility of an asymetric optical trap, the equipartition theorem was calculated along each orthogonal axis within the focal plane separately, resulting in $(k_{0,x}, k_{0,y}) = (2.24, 2.22) \text{ pN}/\mu\text{m}$ and $(k_{1,x}, k_{1,y}) = (4.54, 4.37) \text{ pN}/\mu\text{m}$. These values compare favourably with the fitting parameters of the power spectral density, sampled at 20 kHZ for 100 seconds. We generated several thousand trajectories of an individual particle by cycling the trap strength discontinuosly between k_0 and k_1 with a period of 8 seconds: *i.e.*, the optical trap strength is k_0 for $0 \le t < 4$ s and k_1 for $4 \le t < 8$ s. The characteristic relaxation times of particle motion in optical traps of strength k_0 and k_1 is $(2\pi f_c)^{-1} \equiv \xi/k_0 = 0.212$ s and $(2\pi f_c)^{-1} \equiv \xi/k_1 = 0.104$ s; consequently, the par-



FIG. 2: The storage modulus, \tilde{G}' and real component of the loss modulus, \tilde{G}'' versus frequency, f for (a) water, and (b) PEO solution. In the sampled frequency range in (a), the storage modulus for water is independent of frequency and $\tilde{G}'_W \approx 4.0 \times 10^{-2}$. This value is roughly equal to the expected elastic contribution from the optical trap, $\frac{k}{6\pi r} = 3.9 \times 10^{-2}$, indicating that the storage modulus of the solution is zero, as expected for a purely viscous solution. The loss modulus, \tilde{G}''_W , is linear in frequency. A least squares fit yields, $\eta = 0.96 \times 10^{-3}$ Pa.s, agreeing with the values obtained from analysis of the Lorentzian, eqn 54, fitted to the power spectral density, $S_W(f)$. For 19 the shear moduli measured by particle fluctuations in the PEO solution, (b) shows that \tilde{G}'_P differs from that of water as it is no longer constant, indicating that the solution is now viscoelastic. Furthermore, \tilde{G}''_P no longer has a simple linear relationship with frequency, f.

ticle position obeys a Boltzmann distribution at the instances when the trap constant is discontinuously changed.

Histograms of the dissipation function, *i.e.* the number of trajectories, N_a having a value of the dissipation function, Ω_t at time t within the range $a \pm da$, are constructed from over 25000 particle trajectories and shown in Figure 3. Figures 3(a-c) show the dissipation function for "capture" where the trapping constant changes from k_0 to k_1 and the dissipation function for each trajectory at time t is evaluated from $\Omega_t = \frac{1}{2}(k_0 - k_1)[q^2(t) - q^2(0)]$. Figures 3(d-f) show the dissipation function for "release" where the trapping constant changes from a high value of k_1 to a lower value k_0 and $\Omega_t = \frac{1}{2}(k_1 - k_0)[q^2(t) - q^2(0)]$ for each trajectory at time t. In Figures 3a and 3d, corresponding to data recorded only 2 ms after the change in the optical trap constant, the data is approximately symmetric about zero with only a slightly larger number of trajectories having a dissipation function with positive values. The range of values of Ω_t in Figure 3a is wider than that shown in Figure 3d: this reflects that under an initially weaker trap strength, there is a wider distribution of values of q(0) than when the trap strength is initially high and is lowered as in the case of Figure 3d. The number of trajectories with a positive dissipation function increases with time for both k_0 to k_1 and k_1 to k_0 . Figures 3b and 3e show the histograms after 20 ms, *i.e.*, only a fraction of the characteristic relaxation time, has elapsed. Here you can see that the number of trajectories with $\Omega_t > 0$ have increased and that the distribution of Ω_t tends to be more asymmetric. Figures 3c and 3f, show distributions of Ω_t evaluated 200 ms after the discontinuous change in k, corresponding to trajectories of duration comparable to the characteristic relaxation time of the new trap strength: 200 ms is on the order of the characteristic relaxation time for trajectories in Figures 3c, and twice that for trajectories in Figures 3f. These distributions remain unchanged for Ω_t evaluated over trajectories of duration t > 200ms, indicating that the system (particle + trap) has relaxed to a new equilibrium distribution.

The FT plots in Figure 4 show $\ln\left(\frac{N_a}{N_{-a}}\right)$ plotted vs $\Omega_t = a$, where N_a is the number of trajectories where $\Omega_t = a \pm da$ and N_{-a} is the number of trajectories where $\Omega_t = -a \mp da$, as calculated from the respective histograms in Figure 3. Figures 4(a-c) are constructed from Figures 3(a-c), *i.e.* from trajectories of a "captured" individual particle in viscolealstic solvent as the trap strength is changed discontinuously from k_0 to k_1 . Figures 4(d-f) are constructed from Figure 3(d-f), *i.e.* from trajectories of a "released" individual particle in



FIG. 3: Histograms of Ω_t from 3500 trajectories of an optically trapped particle in a viscoelastic solvent. In (a) - (c) the optical trapping constant is increased from k_0 to k_1 , to more tightly confine or capture the particle. The histograms are recorded at (a) 2 ms, corresponding to 2% of the characteristic relaxation time of the particle in the trap of strength k_1 , (b) 20 ms or 20% of the relaxation time, and (c) 200 ms or twice the relaxation time of the particle. In (d) - (f) the optical trapping constant is decreased from k_1 to k_0 so as to partially release the confinement of the particle. The histograms are also shown at (d) 2 ms, corresponding to 1% of the characteristic relaxation time of the particle in the trap of strength k_0 , (e) 20 ms or 10% of the relaxation time, and (f) 200 ms, or roughly the relaxation time of the particle motion.

viscolealstic solvent as the trap strength is changed discontinuously from k_1 to k_0 . The line in each of these figures corresponds to the prediction of the Fluctuation Theorem, eqn 1. In all but Figure 4a, the experimental data agrees with the Fluctuation Theorem. The experimental data shown in Figure 4a does not clearly match the FT prediction; *i.e.* at 2ms after the trap strength is increased, the resulting distributions of Ω_t do not seem to obey the FT. Note that in comparison, the FT is obeyed 2 ms after trap strength is *decreased*, Figure 4d, albeit the range of Ω_t is much reduced, reflecting the initially more tightly confined particle. Thus, we might expectedly attribute this lack of agreement in Figure 4a to inadequate sampling of non-equilibrium trajectories. Those trajectories that initiate at particle positions far from the focal point are represented in the wings of the equilibrium Boltzmann distribution and are somewhat rare, but nevertheless more frequently sampled in weak traps than in strong ones. These rare, initial positions can evolve to more confined positions, as expected from the tightening trap, resulting in trajectories where Ω_t is large and negative. Insufficient sampling of these non-rare trajectories can lead to seemingly "disproportionate" number of large, negative or positive Ω_t .

Figure 5 shows $\langle \Omega_t \rangle$, *i.e.*, the average dissipation function, accumulated to time t after the instantaneous change in trap strength, versus time, t, for (a) capture $(k_0 \rightarrow k_1, k_1 > k_0)$ and (b) release $(k_1 \rightarrow k_0)$. The points correspond to averages accumulated over 3500 trajectories of the colloidal particle in the viscoelastic solution and the lines correspond to the predicted values of $\langle \Omega_t \rangle$ for a purely viscous solution, as determined from the Langevin equation with Gaussian noise and identical trapping constants. As demonstrated previously, the results of the capture experiment accomplished in pure water are nearly identical to the predictions of the Langevin equation. It is clear that the elastic component of the suspending polymer solution has a marked effect upon $\langle \Omega_t \rangle$ for both capture and release. In the long time limit, $\langle \Omega_t \rangle$, is independent of solvent (*i.e.*, whether viscoelastic or purely viscous), independent of path (*i.e.*, whether the particle is captured or released) and approaches the average long time limit value of $\lim_{t\to\infty} \langle \Omega_t \rangle = 0.26$ predicted by eqn 45.

CONCLUSIONS

In this paper we reviewed the Fluctuation Theorems of Evans *et al.* and Crooks and an optically trapping experiment that demonstrating these theorems. First, we showed that the arguments of the theorems, the dissipation function Ω_t and the work done by the external field, W, do not explicitly depend upon the exchange of heat between system and surroundings. This is *not* to say that Ω_t and W are independent of \dot{Q} as heat exchange will determine the system's trajectory, over which these energies are accumulated. This is very convenient



FIG. 4: Logarithm of the ratio of the numbers of experimental trajectories having dissipation function $\Omega_t = a \pm da$ and $\omega_t = -a \mp da$, $\ln [N_a/N_{-a}]$, versus the dissipation function, $\Omega_t = a$, constructed from the histograms of experimental trajectories in Figure 3. The points represent the experimental data and the line represents the FT prediction. In the top row, (a) - (c), the optical trapping constant is varied from k_0 to k_1 and the dissipation function is accumulated after (a) t = 2 ms or 2% of the relaxation time, (b) t = 20 ms, and (c) t = 200 ms or twice the relaxation time. A least squares fit to the data results in slopes of (a) 0.42 ± 0.47 , (b) 0.84 ± 0.37 and (c) 1.08 ± 0.36 , as compared with the FT prediction of a slope of unity. In the bottom row, (d) - (f), the optical trapping constant is varied from k_1 to k_0 and the dissipation function is accumulated after (a) t = 2 ms or 1% of the relaxation time, (b) t = 20 ms, and (c) t = 200 ms or roughly the relaxation time. A least squares fit to the data results in slopes of (d) 1.17 ± 0.65 , (e) 1.00 ± 0.36 , and (f) 1.21 ± 0.34 , as compared with the FT prediction of a slope of unity.



FIG. 5: The average dissipation function, $\langle \Omega_t \rangle$ versus time, t for (a) capture, $k_0 \to k_1$ and (b) for release, $k_1 \to k_0$. The data points are constructed from averages over 3500 trajectories of a colloidal particle in the viscoelastic solution where the trapping constants are cycled between $k_0 = 2.24$ and $k_1 = 4.54$ pN/ μ m. The lines are predictions from the Langevin equation, $\langle \Omega_t \rangle =$ $\lim_{t\to\infty} \langle \Omega_t \rangle (1 - \exp[-2tk_1/\xi]$ for capture and $\langle \Omega_t \rangle = \lim_{t\to\infty} \langle \Omega_t \rangle (1 - \exp[-2tk_0/\xi]]$ for release. The limiting value, $\lim_{t\to\infty} \langle \Omega_t \rangle = 0.26$, is independent of solvent and path, *i.e.* whether capture or release.

result and quite necessary for the application of the FTs to small experimental systems where it is difficult to isolate the system from surroundings, control the heat exchange between system and surrounding, or even define and measure the temperature of the small system. Experimentally, it is only necessary to know the temperature of the surroundings with which the system is in initial equilibrium and to record the trajectories of the system in response to the applied external field. Second, we showed that in addition to the similarities in form and arguments of the FTs, the Crooks' & Evans' FTs can be applied to the same set of non-equilibrium trajectories; specifically, the set of trajectories of a colloidal particle localised in an optical trap whose strength is changed discontinuously. The Crooks FT is shown to be trivially satisfied in this experiment. Our previous optical trap experiments used a colloidal particle suspended in a purely viscous solvent, *i.e.*, a system whose dynamics are accurately described by the Langevin equation with uncorrelated Gaussian noise[4, 10]. Recently a derivation of Evans' FT from the Langevin equation was given[11], confirming these experimental results. However, the deterministic derivation of the FT suggests that the theorem applies more generally, to systems whose dynamics are more complex than the Langevin equation with white noise. Consequently, we presented here an optical trapping experiment using a colloidal particle suspended in a viscoelastic solvent. The dynamics of the particle can be cast in stochastic form but with time-correlated noise and the FT has not been derived from such an equation of motion. Thus the experimental demonstration of the FTs in viscoelastic solvents, presented here, represents the first experimental confirmation with a system involving complex dynamics.

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