

Modelling the effect of Brownian motion on the amount of Backwards steps in the Classical Three-beads Laser Trap Setup for Actin-Myosin Interaction

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INTRODUCTION

The properties of force and displacement of a single myosin molecule interacting with an actin filament attached between two optically trapped beads have been studied for more than a decade (Finer et al, 1994). According to the cross-bridge theory, the interaction between myosin and actin is governed by a deterministic process and the myosin molecule pulls the actin filament in one specific direction only (Huxley, 1957, Huxley and Simmons, 1971)

However, studies of single myosin-actin interactions have shown that cross-bridges pull actin filaments not only in a preferred but also in the opposite direction, thereby performing what is typically referred to as backward steps (e.g. Molloy et al, 1995). In a recent study, Jinha et al. (unpublished observations) found up to 30% backward steps, which is much greater than the average of about 10% of backward steps previously reported in these types of experiments. One possible explanation for this phenomenon could be, that due to the relatively soft laser trap setup used in Jinha's study ($k_t=0.005$ pN/nm) the Brownian motion acting on the actin filament has huge effects.

The aim of this study is to investigate, whether backwards steps could be explained by a cross-bridge model strictly based on Huxley's assumptions (Huxley, 1957), but incorporating large perturbations that are caused by Brownian motion, as observed in single cross-bridge-actin interactions.

METHODS AND PROCEDURES

We developed a mathematical model of the classical three-bead laser trap setup introduced by Finer et al. (1994, see Fig. 1). Briefly, this setup consists of two micron-sized beads attached to the ends of an actin filament. The beads are controlled by lasers for manipulation as well as for force and step size measurements. A heavy meromyosin (working part of a cross-bridge) is placed on a third bead fixed on a surface.

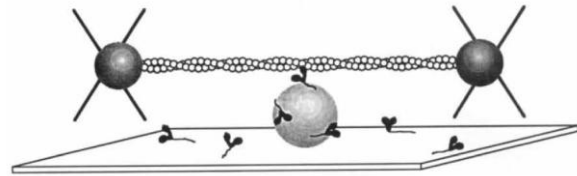


Figure 1. (Finer et al., 1994) Schematic diagram of two optical traps focused on beads which are attached to a single actin filament. The filament is held near a single heavy meromyosin molecule attached to a third bead.

The mathematical model is based on stochastic differential equations, the attachment and detachment rates of a single myosin are retrieved from the classical Huxley model (Huxley, 1957). This structure leads to coupled Fokker-Planck equations. If no analytical solution of the model can be found, a stable numerical algorithm will be used for analysing the model.

Different parameters for the laser trap stiffness are taken into account, since they influence Brownian motion which is a powerful force on the molecular level. The behaviour of a Brownian particle in a potential can be approximated by the

Boltzmann distribution, which describes the particle's steady state. The density function in Fig. 2 describes the influence of Brownian motion on the movement of the actin filament.

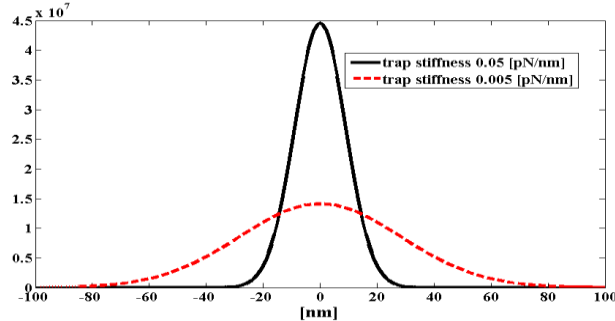


Figure 2. The density functions of Boltzmann distributions with different parameters for the trap stiffness. Further relevant parameters are: Boltzmann's constant ($k_B = 1.38065 \cdot 10^{-23}$ J/K), viscous damping coefficient on both beads attached to the actin filament ($b = 2 \cdot 10^{-8}$ N·s·m⁻¹) as well as the temperature ($T = 290.15$ °K).

The probability of a movement of the actin filament exceeding 10 nm is around 26 % for a trap stiffness of 0.05 pN/nm, whereas the same probability for a trap stiffness lowered by a factor of ten is approximately 72 %. We hypothesize that this dramatic change has an important and non-negligible effect on the attachment and detachment behaviour of cross-bridges.

DISCUSSION

Many approaches aimed at modelling the displacements of an optically trapped actin filament and its interaction with a myosin molecule have been published. Some of these models are based on the classical cross-bridge theory (Huxley, 1957). Backward steps in these models are accomplished with attachment rate functions that allow for such events a priori (e.g. Smith, 1998). Other approaches are based on stochastic models in which actin-myosin interactions are defined by Brownian ratchets in which directed

motion is achieved by exposing cross-bridges to switching potentials (e.g. Shimakawa et al., 2003).

In contrast to these approaches we suggest that backward steps can be explained by Brownian motion as a perturbation on the actin filament in a three bead laser trap setup (Figure 1) while maintaining rate functions for single myosin interactions converging to the classical, deterministic cross-bridge rate functions (Huxley, 1957) for large numbers of actin-myosin interactions.

SUMMARY

Studies on single cross-bridge interaction with an optically trapped actin filament report a significant number of “backward” steps. This result appears in contradiction with the classic cross-bridge theory. However, Brownian motion is a powerful force on the molecular level and is directly related to the laser trap stiffness. Therefore, we suggest, that the number of backward steps is directly related to the stiffness of the laser trap: the greater the stiffness, the smaller the Brownian motion and the less the percentage of backward steps. In order to demonstrate this relationship, we developed a model derived from the attachment and detachment rate functions of the classical cross-bridge model that incorporates Brownian noise as a function of trap stiffness.

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