Doctoral Thesis

Theoretical analysis and simulation of half-sarcomere dynamics in a myofibril

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Theoretical analysis and simulation of half-sarcomere dynamics in a myofibril

A dissertation submitted to

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for the degree of

Doctor of Sciences

presented by

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Summary

The core of this thesis is the theoretical investigation of the muscular contraction on sub-cellular level. Striated muscle is a mechanical system that develops force and generates power in serving vital activities in the body. Muscle research has a long tradition and the structure and function of different types of muscles have been deeply resolved. Despite the effort made by well-known scientists and their co-workers the functioning principles of skeletal muscle is not yet fully understood leaving some fundamental question unresolved. One of the open questions is how the sarcomeres operate within a muscle. It has been recently demonstrated that half-sarcomeres in a single myofibril operate non-uniformly.

Striated muscle is a complex biological system; a single mammalian muscle fibre contains up to hundred or even more myofibrils in parallel connected via an inter-myofibril filament network. In one single myofibril thousands of sarcomeres are lined up as a series of linear motors. This muscular engine is fueled with chemical energy while splitting ATP. Such a system can be described on one hand biochemically while formulating the biochemical cycle of the cross-bridges that produce the force. On the other hand, the system can also be described mechanically while formulating the motion equations of the motor units. These two descriptions are highly coupled as the force generating biochemical cycle influences the motion of the motor units and the motion of the motor units changes the chemical energies potentials inside the kinetic cycle. So the synthesis of the two descriptions is crucial for the analysis of the muscular function. The motivation of the present thesis is given by the question how the individual motion of the half-sarcomeres in a myofibril is influencing the force and the motion of the total system.

For this purpose a mathematical framework is outlined based on cross-bridge kinetics for the simulation of the force response and length change of individual half-sarcomeres in a myofibril (chapter 2). The model describes the muscle myofibril in contraction experiments under various conditions. The myofibril is modeled as a multisegmental mechanical system of half-sarcomeres, which have active and viscoelastic properties. In the first approach, a two-state cross-bridge formalism relates the half-sarcomere force to the chemical kinetics of ATP hydrolysis, as first described by A. F. Huxley in 1957. In a second step, the description of the cross-bridge cycle is extended to a three-state formalism including weakly and strongly bound cross-bridges. Three possible types of biological variability are introduced and modeled.

Numerical simulations of a myofibril composed of four to eight half-sarcomere show a nonuniform half-sarcomere length distribution and complex internal dynamics upon activation, relaxation and stretching (chapter 3). It can be demonstrated that the steady-state approximation holds only in restricted time zones during activation. Simulations of myofibril contraction experiments that reproduce the classic steady-state force-length and force-velocity relationships, strictly constrained or “clamped” in either end-held isometric or isotonic contraction conditions, reveal a small but conspicuous effect of half-
sarcomere dynamics on force (chapter 3.1). In contrary to classical multisegmental Hill-like simulations, fast lengthening or ‘popping’ of sarcomeres is not observed in transient simulations (chapter 4.3.3).

Furthermore, the model predicts a sequential lengthening of the half-sarcomeres upon deactivation that is also prominent in experiments (chapter 3.2). The relaxation time of a myofibril can be influenced with several parameters, e.g. types of fibers and stiffness of the titin filament. During relaxation the description of the myofibril can be simplified and so the dynamics of the half-sarcomeres can be solved analytically revealing rather a mechanical than a biochemical reason for the linear force shoulder after deactivation.

An actual mystery in the muscle research is the phenomena of ‘residual force enhancement’ after active stretching of a myofibril. There, the myofibril is producing more force after being actively stretched to a certain length than activated isometric at this length. Over more than 20 years, it is believed that the half-sarcomere dynamics and length non-uniformity play an important role therein. The model can simulate the stretching of myofibrils and calculate the theoretical influence of the half-sarcomeres on the residual force enhancement (chapter 3.3). The simulations show only negligible ‘residual force enhancement’. Several enhancements in the kinetic cycle and the mechanical behavior of the cross-bridges are proposed to extend the model and explain residual force enhancement (chapter 4.3).

Generally, the calculated values of the simulations correspond well to the corresponding findings in the experiments indicating that the presented model is a good tool to theoretically investigate the half-sarcomere dynamics (chapter 4). So the model establishes a basis for future theoretical investigations of the function of muscle and may be used for analyses of the experimental findings.
Zusammenfassung


Numerische Simulationen einer Myofibrille bestehend aus vier bis acht Halbsarkomerlängen zeigen nicht uniforme Verteilungen der Halbsarkermlängen und komplexe interne Dynamik während Aktivierung, Relaxation und Dehnung (Kapitel 3). Es kann gezeigt werden, dass Gleichgewichtszustände in der Kinetik nur in ganz bestimmten Zeitzonen während der Aktivierung gemacht werden können. Simulationen von
Zusammenfassung

Myofibrillen, die die klassischen Beziehungen von Kraft-Länge und Kraft-Geschwindigkeit reproduzieren und deren Bewegungen durch eine steife Fixierung stark eingeschränkt sind, zeigen kleine aber deutliche Effekte bei der Kraft durch die Dynamik der Halbsarkomere (Kapitel 3.1). Im Gegensatz zu klassischen Hill-artigen multi-segmentalen Simulationen konnten schnell dehnende oder ‚poppende‘ Sarkomere in transienten Simulationen nicht beobachtet werden (Kapitel 4.3.3).

Weiter sagt das Model die sequentielle Verlängerung der Halbsarkomere während der Deaktivierung voraus, was auch in Experimenten auftritt (Kapitel 3.2). Die Zeit der Relaxation einer Myofibrille kann durch verschiedene Parameter, wie Muskelfasertyp und Steifigkeit von Titin Filament, beeinflusst werden. Die Beschreibung der Myofibrille kann während der Relaxation vereinfacht werden und dadurch kann die Dynamik der Halbsarkomere analytisch gelöst werden. Diese Lösungen zeigen, dass der Grund für die lineare Kraftschulter nach der Deaktivierung eher mechanischer als biochemischer Natur ist.

Ein weiteres Rätsel in der Muskelforschung ist das Phänomen der ‚verbleibenden Krafterhöhung‘ nach aktiver Dehnung einer Myofibrille. Dabei produziert die Myofibrille mehr Kraft nachdem sie aktiv auf eine bestimmte Länge gedehnt wird als wenn sie bei dieser Länge isometrisch aktiviert wird. Seit über 20 Jahren wird angenommen, dass die Dynamik der Halbsarkomere und die Inhomogenität der Halbsarkomerlängen eine wichtige Rolle darin spielt. Das vorgestellte Model kann die Dehnung einer Myofibrille simulieren und den Einfluss der Halbsarkomere auf die ‚verbleibende Krafterhöhung‘ theoretisch errechnen (Kapitel 3.3). Es werden einige Verbesserungen für das Model im Bereich des kinetischen Zyklus und im mechanischen Verhalten der Querbrücken vorgeschlagen um das Model zu erweitern und die ‚verbleibende Krafterhöhung‘ zu erklären (Kapitel 4.3).

Im Allgemeinen stimmen die errechneten Werte in den Simulationen gut mit den experimentellen Befunden überein. Dies weist darauf hin, dass das vorliegende Modell ein gutes Werkzeug zur Untersuchung der Dynamik der Halbsarkomere darstellt (Kapitel 4). Daher legt das Modell die Basis für zukünftige theoretische Untersuchungen der Muskelfunktion und kann zudem für die Analyse der experimentellen Befunde gebraucht werden.
Chapter 1
Introduction
The main aim of muscle research is to understand how the muscle operates and produces force under different conditions. This leads to the fundamental question of how chemical energy is transformed into mechanical work in a muscle. Structural investigations and mechanical experiments try to connect the structure with the function of the muscle tissue. The muscle is a highly ordered system so that investigations with crystallography using diffraction of electromagnetic waves can be applied to measure structural parameters, e.g. filament and sarcomere length. These experiments give a good insight as to how the muscular structure changes due to chemical or mechanical influences. Hence this approach advances the understanding of the underlying, and mostly invisible, molecular events taking place during contraction, relaxation and stretching of muscle fibers. Recent experiments unveiled that simply averaging the sarcomere lengths may not adequately describe the underlying molecular events since the response of a single sarcomere does not coactively correspond with the response of the whole fiber or even part of the fiber. The interaction of the individual movement of a sarcomere with the internal molecular events and its resulting force production is very complex and still unclear.

1.1. Overview and Motivation

1.1.1. History of muscle research

Muscle research is an ancient field of investigation. Systematic structural research was firstly performed in the second century A.D. by Galen (Goss, 1963). In the seventeenth century, Leeuwenhoek revealed the fibrous structure and the striation of skeletal muscle by microscopic examinations (Miranda, 2009). Around the same time, two fiber types were detected by Lorenzini (1678). With the advantage of phase contrast and polarized light microscopy in 1850’s, the microscopic structures (e.g. A- and I-Band, Z-disk) could be discovered by several researchers.

An explanation of muscular contraction was first brought up by Erasistratus in the third century B.C (Wills, 1999). He thought that the nerves and the muscles are a pneumatic system driven by the spirit. In the 1660’s Swammerdam and Glisson refuted this theory while showing that, unlike in pneumatic contractions, the volume of the muscle does not change during shortening (Voorhoeve and Bremer, 1985). It was then believed that the muscle works as a thermal engine. This theory was again refuted when the model predicted an internal body temperature of 130 °C assuming a thermodynamic efficiency of 20-30%. Between 1910 and 1950 A.V. Hill examined muscle shortening and heat production in reasoned mechanical experiments. After using electron microscopy in the first half of the last century, researchers, mainly A.F. Huxley and H.E. Huxley suggested that interactions of the filaments aligned in parallel are behind the force production. The consequential sliding of these filaments then generates the movement of the muscle. This ‘sliding filament theory’ is widely accepted in today’s muscle community. Further investigations of molecular biology supported the theory, that cross-bridges between actin- and myosin-filaments generate tension upon activation. It is generally accepted that these cyclic interactions are the underlying reason for the sliding of the filaments and the resulting muscular contraction and force production.
1.1.2. Terms and Definitions

In this work some terms and definitions are used throughout the text. To prevent misunderstanding and misinterpretation of specific statements, clarification of some expressions is necessary.

- The muscle fiber is the fibrous muscle cell. It contains several nuclei which arise from merged cells.
- The half-sarcomere is the smallest structural and functional unit in a muscle cell. Half-sarcomeres in series form a myofibril, a longitudinal-shaped subunit of a muscle fiber.
- The biochemical reactions in the muscle cells are denoted as kinetics. The biochemical reaction pathway of the cross-bridges is called cross-bridge kinetics.
- The expression conformational state or simply state of a cross-bridge or a myosin head describes the biophysical and geometrical conformation of the coupled or uncoupled myosin head. The state distributions are the probability distribution of myosin heads with the corresponding conformation on a single myosin filament.
- A muscular contraction is the mechanical response of a muscle upon activation. Even the common word contraction is mostly used for overall shortening of a system, also isometric or isotonic contractions are existing in muscular research. Hence a contraction of a muscle can be measured by a change in force and/or length.
- In an isometric or end-held contraction the fiber or the myofibril is held at a constant length while controlling the total external length. In an isotonic contraction the muscular force is held constant. In this case the force is externally controlled and the length of the muscle fiber or its subunits is measured. If the velocity of the fiber or its subunits is controlled and the muscular force is observed, the protocol is called an isovelocity experiment.
- The terms twitch and tetanus define the type of stimulation of a fiber or a myofibril. A twitch contraction is a rapid increase and then subsequent slower drop in force due to a single pulse of activation. A tetanus contraction is the smooth force rise when repetitive stimuli at frequencies of 20 to 100 Hz are applied. If not defined explicitly, a contraction is considered to be tetanic.
- The dynamics of a system is the mechanical movement of the length of a muscle or its subunits. We refer to half-sarcomere dynamics as the length change of the half-sarcomeres.
- Inhomogeneity is used to describe the length non-uniformities of half-sarcomeres.
- A variable is called to be in a steady-state when it stays constant in time and hence, in a stable configuration, even for a short period. If the variable is changing in time it is denoted as transient-state.
- Passive Force of a fiber is the force of the non-activated or relaxed muscle at a certain length. The length at which the passive force drops to zero is defined as slack length.
- The force-length relationship denotes the piecewise linear function between active force and half-sarcomere length. It reflects the overlap of the active zone of the myosin- and actin-filaments.
So it can be summarized, that during contraction the muscular force builds up, which is the sum of an actively generated force and the passive force of a muscle. The main variables during contraction are half-sarcomere length and half-sarcomere speed as well as muscular force or tension. During the experimental protocol one of the variables is usually controlled while observing the response of the others to investigate the muscular function.

1.1.3. Half-sarcomere dynamics and non-uniformities

Systematic research on muscle has been conducted over a hundred years but is still lacking answers up to now. Seventy years ago, the aim was to investigate the movement, force and energy production of a whole muscle (Hill, 1938). Nowadays the aim of the research is to investigate molecular reasons for the contracting muscle and to formulate these in models. For this purpose, experiments on single isolated molecules (molecular motors) on one hand and experiments on single myofibrils and muscle fibers on the other hand, are conducted. The main difference between single molecular motors and myofibrils is the level of arrangement. The muscular tissue is a complex structure of aligned filaments within a highly ordered system. The half-sarcomeres as motor units are arranged in series and parallel within this system and operate more or less independently on the level of the kinetics. The half-sarcomeres are coupled due to interconnecting filaments forming a lattice-like structure. As the dynamics of an individual half-sarcomere also affects the internal dynamics, it is obvious that the force response of a fiber is a very complex convolution of the individual dynamics and kinetics of the half-sarcomeres. However the force response of experiments is often interpreted on the level of the cross-bridge kinetics. This implicitly assumes a homogenous ensemble of half-sarcomeres throughout the entire myofibril or fiber. On the contrary it is also known that biological variability exists in muscle structure and function. For example the isometric force upon activation varies up to 10% (Hill, 1970). If the structure and the function of the single subunits in a muscle vary, the resulting dynamics of the half-sarcomeres must be non-uniform. Even though this problem is known, it has been neglected in the past. (Sugi and Tsuchiya, 1998) discussed the problem of sarcomere length inhomogeneity and showed that interpretation of the force response on the basis of molecular events may be invalid.

Early on, it was suggested (Hill, 1953) that the striation spacing may show some inhomogeneity if fibers are slightly stretched. On the descending limb of the force-length relationship the stiffness is found to be negative and hence the dynamics of the (half-) sarcomeres to be unstable producing a higher inhomogeneity (Gordon et al., 1966a). Furthermore, the phenomenon of ‘creep’ during isometric force development was believed to be connected to the development of half-sarcomere inhomogeneity. In the experiments of Julian and Morgan (1979a; 1979b) they found, that for starting sarcomere lengths corresponding to the descending limb of the force-length relationship, most of the fiber was lengthening while the rest was shortening during isometric contraction. This inhomogeneity corresponded to the observed tension ‘creep’. Sarcomere length non-uniformities also appeared after active stretching during tetanic contraction while the sarcomere lengths were stretched onto the descending limb of the force-length relationship. During and after such stretches the force was showing a typical increase known as the phenomenon of ‘permanent force enhancement’. A static fiber model from Morgan (1994)
Introduction

led to the hypothesis that the sarcomeres lengthen rapid, uncontrolled and randomly distributed if they are stretched onto the descending limb of the force-length relationship. This rapid lengthening was named ‘sarcomere popping’. The conditions of sarcomere instability were elaborated by Allinger et al. (1996) and Zahalak (1997) while analyzing quasi-static conditions. The dynamic situation was analyzed by Denoth et al. (2002) in a multi-segmental model incorporating steady-state relationships of the active components. They analyzed the dynamics and instability of sarcomeres in a profound mechanical and mathematical manner. This study showed the importance of sarcomere dynamics in myofibrillar experiments. Based on this model, Telley et al. (2003) calculated the dynamics of sarcomere networks for different experimental setups and conditions.

Experimentally, sarcomere lengths in muscle fibers can be measured in different ways. A popular method is laser light diffraction (Rudel and Zite-Ferency, 1979). A laser beam is focused on a small part of the myofibril and the diffraction due to the striation is measured. The first order peak of the diffraction pattern is inversely proportional to mean periodicity of the striation. This convenient length measurement provides mainly the mean sarcomere length while the dispersion of the peak reflects the mean inhomogeneity of the sarcomere lengths. However the location of these irregularities cannot be detected with this method. An ancient method for experimental length measurement is the ‘spot follower’ where a segment of the fibers is marked on the surface, e.g. with pieces of gold leaf or dog’s hairs (Gordon et al., 1966a). The length of the segment can be controlled easily with a motor and a feedback system. Counting the sarcomeres in the segment it gives the mean sarcomere length in the segment. So even if the segment is held constant in length, there may be non-uniformities in the sarcomere lengths as some sarcomeres may shorten while the others lengthen. Mutungi and Ranatunga (2000) assumed that the observed sarcomere inhomogeneity is always prominent during activation and relaxation of muscle fibers. All these studies reveal that not even the response of the ‘mean’ single sarcomere corresponds to the response of the whole system.

In the early nineties, G.A. Pollack and his collaborators established a method to investigate mechanics on myofibrils (Bartoo et al., 1993). They investigated the sarcomere lengths during contraction with bright field and phase contrast video microscopy by analyzing the A-band and I-Band patterns. However they could not measure sarcomere lengths and myofibrillar force simultaneously. A novel method developed by Stehle et al. (2002a) could follow the sarcomere lengths during relaxation while measuring the active force. They found considerable dynamics during relaxation of cardiac myofibril, and sequential lengthening of the sarcomeres after deactivation. Rassier et al. (2003a) recorded the force and tracked the sarcomere lengths during stretches. He found that the profile analysis used by the aforementioned studies results in some problems and errors when determining sarcomere lengths. In subsequent experiments Telley et al. (2006a; 2006b) marked the Z-disk and the M-band with fluorescent markers. This technique allowed the accurate measurement of the half-sarcomere lengths during activation, relaxation and stretching. In these experiments they showed considerable dynamics and non-uniformity of the half-sarcomeres. The effect of non-uniformity during stretches of myofibrils was further investigated by Joumaa et al. (2008). They found a slight increase of the dispersion of the hS length distributions after stretch. The experiments of Telley et al. (2006a; 2006b) are the experimental basis of the present thesis.
1.1.4. Intent and Motivation

Most theoretical descriptions of the active force generation in myofibrils neglect the multi-segmental structure. The study of Denoth et al. (2002) gives the basis for a rigorous analysis of the dynamics of the sarcomeres. It presents a model consisting of passive and active strands inside a sarcomere. The active strand was mainly modeled with a steady state relationship between force and velocity. The model could explain phenomena such as sarcomere instability, extra tension and homogenization. Telley et al. (2003) elaborated the former model and simulated the dynamics in connected myofibrils. So far, a formalism that converges the complex transient cross-bridge kinetics with the multi-segmental structure is still lacking. Such a formalism could shed light on the influence of sarcomere dynamics in myofibrils.

The present dissertation has three main goals. Firstly, it aims to develop a general mathematical formalism to describe a myofibril. This is done while coupling existing models of the transient cross-bridge kinetics (Huxley, 1957) with a multi-segmental model of a myofibril (Denoth et al., 2002). Secondly, the first time integral simulations of a whole activation-relaxation cycle and active stretching of small myofibrils will be presented. For this aim an optimized algorithm has to be developed so that the equations can be solved on a normal computer. The data allows the calculation of all the lengths and velocities as well as the probability distributions of the myosin conformations. So the half-sarcomere dynamics can be calculated and simulations with different parameters can be performed to describe the effect of the dynamics on the force and the distributions of the coupled cross-bridges. Thirdly, some analytical explanations based on the formalism are presented. These can describe effects on stripped-down models as well as the sequential relaxation.

This work lays the basis for more profound analyses of the movement of the sarcomeres during different protocols. It defines whether the current ideas of muscle function are complete and where the half-sarcomere dynamics plays a vital role. Furthermore it can give some new ideas for future experiments to investigate the last mysteries in muscle research.
1.2. Muscle Structure and Function

This chapter describes the function and the structure of the muscular tissue while explaining the current ideas and knowledge in the research field. It further shows some important models that are able to describe the kinetics of the actin-myosin complex. This topical review concentrates on vertebrate striated muscle (skeletal and cardiac) and discards the smooth muscle even if it might be very similar.

1.2.1. Muscle Structure

Skeletal muscle consists of fibers that are multi-nucleate cells with diameters between 10 and 100 µm that span the total length of the fiber. The sarcoplasm surrounds a fiber. It contains the mitochondria, the internal membrane of the sarcoplasmic reticulum and a system of transverse connecting tubuli. The fiber itself is also hierarchically organized containing hundreds of myofibrils, around 1 µm in diameter, that are lying parallel along the fiber. The elements of a myofibril are the serially aligned sarcomeres.

With light microscopy a characteristic pattern of the fibers is revealed with repeating light (isotropic, I-band) and dark (anisotropic, A-band) zones. These cross-striations are due to the regular arrangement of the filaments of the sarcomeres in the myofibrils. The sarcomere is the smallest structural unit in a muscle. However there is strong evidence, that the functional unit is the half-sarcomere (Telley et al., 2006a). The sarcomere consists of parallel aligned actin-, myosin- and titin-filaments along its length. The thin actin-filaments are also anchored at the Z-disk and reach from the middle of the I-band into the A-band to the border of the H-zone (see Fig. 1.2.1 for details). The actin-filament provides the binding sites for the force producing cross-bridges. The myosin-filaments run from the middle of the sarcomere (M-band) for both directions to the end of the A-band. The titin filament connects the end of the myosin-filament with the Z-disk acting as a passive filament to ensure overstretching of the sarcomere.

Myosin-filament and cross-bridges

In the year 1954 two independent studies (Huxley and Niedergerke, 1954; Huxley and Hanson, 1954) found that the active muscle shortening results from the sliding of the myosin-filaments along the actin-filaments. This results in symmetric shortening of a sarcomere towards its centre. The region of interaction of the two filaments is called the A-band. The knowledge at this time and the mentioned studies led to the assumption, that the muscle contraction is based on the force generation of quasi-independent cross-bridges between the actin- and myosin-filament. This assumption was mainly based on three investigations. Firstly, Huxley (1953) found projections of regular connections between actin and myosin while looking at fibers in rigor using X-rays. Hanson & Huxley (Hanson and Huxley, 1953) further showed that those connectors consist of myosin proteins. Secondly, Ramsey & Street (Ramsey and Street, 1940) demonstrated that the isometric force is proportional to the overlap of the actin- and myosin-filaments. Thirdly, Huxley & Julian (1964) revealed that the maximal shortening velocity of fibers is independent of the filament-overlap being a basis for the assumption of quasi-independent force generators.
Studies up to now (e.g. [Rayment et al., 1996; Squire, 1981]) disclosed the structure and organization of the cross-bridges. A cross-bridge is a molecular motor consisting of the protein myosin II. This molecular motor has two globular heads attached to a long tail that is pointing towards the centre of the filament (see Fig. 1.2.2). It is composed of two heavy polypeptide chains (200kDa each) and two pairs of light polypeptide chains (20kDa each). The globular head of a heavy chain is associated with two light chains - an essential and a regulatory one. The coiled coil connects the two heavy chains forming a 150nm long tail. The heads of the molecular motor can be biochemically cleaved to build three fragments, the tail, the subfragment-2 (S2) and the subfragment-1 (S1). S1 is associated with the heads while S2 is associated with the 95nm long part of the tail that protrudes into the inter-filamentary space. S1 contains the functional sites such as the ATP binding site, actin-binding site and the lever arm. They are often referred to as ‘cross-bridges’ or ‘myosin-heads’.

![Fig. 1.2.1 : Schematic of a sarcomere.](image1)

Fig. 1.2.1 : Schematic of a sarcomere. The myosin (thick) filaments and the actin (thin) filaments are parallel organized and alternate each other. The A-band reflects the region where the actin and the myosin filaments overlap and its appearance in a microscope is darker than the I-band region. The myosin filament is connected to the titin filaments and anchored in the ‘zigzag’ like Z-disk at the border of the sarcomere. At the centre of the sarcomere, the thick filaments are cross-linked through proteins (myomesin, M-protein, etc.) in the M-band.

![Fig. 1.2.2 : Diagram of a myosin II protein (adapted from (Rayment and Holden, 1994)).](image2)

Fig. 1.2.2 : Diagram of a myosin II protein (adapted from (Rayment and Holden, 1994)). The sub-fragments (S1 and S2) are coupled to the tail that is anchored in the myosin filament. The cross-bridge (S1) consists of two heavy chains with each a regulatory (RLC) and an essential light chain (ELC).
Introduction

**Actin-filament**
The actin-filament is built from two strands of F-actin that are helical wound. The F-actin is a string of polymerized G-actin monomers that have an axial separation of 5.5 nm. The helical periodicity of the structure is 36.5 nm. Around the F-actin strands there are regulatory proteins: tropomyosin (Tm) and troponin (Tn). Tm lies longitudinally in the space between the F-actin strands and spans over seven pairs of G-actin monomers (see Fig. 1.2.3). At the connection of two Tm exists a Tn complex, that has a calcium binding (TnC), an inhibitory (TnI) and a tropomyosin binding (TnT) subunit. TnI with Tm blocks the binding site of the myosin heads on the actin-filament. If TnC is merged with calcium it cancels the inhibitory effect of TnI while shifting the whole unit and unblocking the actin monomers (Squire and Morris, 1998).

![Fig. 1.2.3 : Representation of the actin filament (adapted from Ebashi (1974))](image)

The actin filament consists mainly of two helices of F-actin strands (built of G-actin monomers) that are wrapped with two tropomyosin strings. The tropomyosin strands are basically tropomyosin molecules that are connected together by troponin complexes. Troponin itself contains three subunits (TnC, TnT, TnI).

**Titin-filament**
The third filament in a sarcomere is the giant protein titin. It is also called connectin as it connects the myosin-filament with the Z-disk hindering an overstretching of the sarcomere. It was first discovered by Maruyama et al. (1977). Titin is a huge protein having a molecular mass of about 3.0-3.7 MDa. It consists mainly of three components: the tandem Ig segments that are serially linked immunoglobulin-like (Ig) domains, the non-modular PEVK segment and the fibronectin-like (Fn) domains (see Fig. 1.2.4). The PEVK segment lies in the middle of the I-band whereas the Fn domains are located in the A-band and acts as the template for the assembly of myosin molecules (Trinick, 1994). The size of the Ig segments and the PEVK region varies between types of muscles. The main function of the I-band region of the titin filament is as a molecular spring. It ranges from a random coiled configuration at rest to lengthening Ig domains for small stretches to worm-like lengthening of the PEVK domain for larger stretches (Gautel and Goulding, 1996; Labeit and Kolmerer, 1995; Linke and Granzier, 1998). Such a spring ensures the structural arrangement of the filaments within a sarcomere and is a reason for passive muscle stiffness. As the length of the PEVK domain differs between types of muscles, it may also be relevant for the elasticity and hence the mechanical function of the muscle (Bullard et al., 2002).
Z-disk

The Z-disk is the connection point for the sarcomeres while also bundling the actin filaments of adjacent sarcomeres into a tetragonal lattice (Vigoreaux, 1994). The actin filaments are probably connected inside the Z-disk by α-actinin. The Z-disk is the transmitter of the tension of a sarcomere to its neighbors. Another stabilizing transverse structure lies in the middle of the sarcomere, the M-band. It is believed that it links the thick and the titin filaments. Besides the components myosin and titin, the M-band consists also of mainly structural proteins, the myomesin and the M-protein. Myomesin seems to be essential for the assembly of the M-band (Agarkova et al., 2003; Lange et al., 2005).

1.2.2. Cross-bridge kinetics

Electron microscopy and X-ray studies led to the proposition of the swinging-cross-bridge theory. Huxley (1969) suggested from his structural investigations, that during contraction the S1 head attached to actin changes its angle hence building up strain that leads to the relative sliding of the filaments. More than 15 years later, Huxley et al. (1981) provided the experimental evidence for that theory. Later, Cooke (1986) pointed out that the movements must come from a smaller mass than the whole S1 head. So the swinging-cross-bridge theory was improved while proposing a rotating-lever-arm hypothesis. This is still the current perception and has been resolved in more details. A detailed review of the topic is published in Geeves et al. (2005). The S1 has two distinct states, ‘open’ and ‘closed’, which are associated with the conformational change that produces the mechanical force and movement of a muscle. The ‘open’ state of the myosin head, is in its conformational state after Adenosindiphosphate (ADP) release, and it has a great affinity to bind onto actin when the myosin head is unoccupied by Adenosintriphosphate (ATP). The ‘closed’ state reflects the ATP bound to the myosin head enhancing ATPase. The transition from ‘closed’ to ‘open’ causes a rotation of the converter domain of about 60°.
resulting in a movement of about 10nm along the actin-filament. This transition is called the ‘power-stroke’. The myosin heads may jump between the two states in response to external applied force (Conibear et al., 2003). However it is still unclear where exactly the force-bearing compliance is located in the myosin head. It is believed to lie in the S2 region.

**ATPase cycle**
The myosin head in skeletal muscle is a molecular motor that undergoes a conformational change while hydrolyzing ATP. The rate of this hydrolysis is enhanced by the presence of actin but is worsened by high concentrations of the products left after the hydrolysis; ADP and Phosphate (P_i). The building of crossbridges is based on ATP binding and hydrolysis. The binding of actin catalyses the hydrolysis of ATP and the binding of ATP catalyses the dissociation of actin (Eisenberg and Moos, 1970). This finding shows that the mechanical conformational change of the myosin head is strongly connected with the biochemical pathway. A molecular cycle was first proposed by Lynn & Taylor (1971). This cycle, including seven steps, is actually referred to as the Bagshaw-Trentham scheme (Bagshaw et al., 1974). The kinetic pathway has mainly remained the same over the last years (Fig. 1.2.5). Basically it consists of reversible reactions, where some reverse reactions are less preferred than the forward reaction - leading to a directed cycle. To effectively produce force, the reaction of the conformational change has to be strongly preferred in the forward direction. The seven steps consist of four basic events: (i) the association of ATP in two steps, (ii) the hydrolysis of ATP into ADP and P_i, (iii) the release of P_i in two steps and (iv) the release of ADP in two steps. The individual events may have more than two steps due to the complexity of the structural changes. However for most of the cases the proposed scheme is a good approximation.

![Fig. 1.2.5 : The Bagshaw-Trentham scheme.](image)

**Conformational cycle**
The structural process of binding myosin to the actin-filament can be investigated in solution. Geeves & Conibear (1995) resolved it into three events: (i) actin-S1 complex building (weakly bound state) (ii) hydrophobic interaction and rearrangement of the complex (strongly bound state) (iii) isomerisation with a large volume increase (rigor state). Interestingly, the rearrangement of the second step strengthens the actin binding and weakens the ATP binding to the myosin head (Goody and Holmes, 1983). The third step is associated with the power-stroke of the biochemical cycle of the ATPase (Geeves et al., 1984). Guo & Guilford (2006) found additional states of stretched myosin heads while investigating single molecules. These ‘catch bonds’ are strongly coupled to the actin filament and have a long living time. This finding corresponds also to earlier propositions to alter the kinetic cycle of stretched muscles (Piazzesi and Lombardi, 1995). Recently, another detail in the conformational cycle could be found by Brunello et al. (2007). These experiments on muscle fibers showed that during stretch the second motor unit of the myosin head can attach to the actin filament.
Open questions in the kinetic cycle
It is believed that the release of $P_i$ and the power-stroke are closely related. There is evidence that the power-stroke precedes the release of the inorganic $P_i$, although this is debated in the muscle community (Pate and Cooke, 1989; Ranatunga et al., 2002). Another ongoing debate is the strain-sensitive ADP release in the kinetic cycle. The energy of the hydrolysis of ATP is stored in the elastic component of the S1 fragment. To use this energy as efficiently as possible to generate force, the head must stay coupled to myosin until the sliding occurred. So the strained cross-bridge should have a long lifetime during isometric contraction. Based on these ideas, Huxley (1957) proposed a strain sensitive detachment of the cross-bridge, where the detachment rate of strained cross-bridges is lower than the detachment rate of compressed cross-bridges. This idea is compatible with the Fenn-effect (Fenn, 1924) in which the shortening muscle liberates more heat, since the turnover of ATP is high due to the high detachment rate. Nowadays it is known that the affinity of ATP to bind on a myosin head is very high causing detachment of the head (Goldman et al., 1984). Hence the time of the bound cross-bridge is believed to be about 1% of the duration of the kinetic cross-bridge cycle. Otherwise, the slowest step in the kinetic cycle is assumed to be the release of ADP. So the strain-sensitivity is more likely in the step of ADP release. A load dependent conformational change of the S1 fragment before ADP release may be the reason for the strain-sensitivity. This fact has been revealed only in slow muscle up to now (Nyitrai and Geeves, 2004).

Muscle types
The characteristics of shortening and force development vary greatly in the different types of muscle fibers. These differences correlate with the differences in the ATPase cycle of different isoforms of myosin (Barany, 1967). There are mainly three different classes of muscle fibers: (i) type I (oxidative), red-slow, slow-fatigue-resistant (ii) type IIa (oxidative-glycolitic), red-fast, fast-fatigue-resistant (iii) type IIb (glycolitic), white, fast-fatigue-sensitive. Additionally there is a type IIX with properties between those of type IIa and IIb. The whole kinetic cycle varies from slow to fast types of muscle (Millar and Homsher, 1992). In the heart muscle of mammals two isoforms of myosin can be found: $\alpha$-myosin heavy chain (MHC) and $\beta$-MHC. They differ mainly by the maximal shortening velocity where $\alpha$-MHC is about 3 times faster than $\beta$-MHC (Schaub et al., 1998). Besides of these main skeletal muscle types also other very specialized types exist in nature, e.g. the super-fast muscle of the swimbladder of a toadfish (Rome, 2006).

1.2.3. Mechanical properties of muscle

Force-velocity relationship
In the first half of the last century, A.V. Hill investigated the mechanical properties and energetic efficiency of muscle. In 1938 he published a milestone on heat production during active shortening of skeletal muscle fibers of frogs (Hill, 1938). In this work on isotonic shortening experiments he found a hyperbolic relation between shortening speed and muscular force. Hill described this relationship with the empirical equation
\[(F + a)(v + b) = (F_0 + a)b\]  \hspace{1cm} (1.1)

In this equation, \(F\) denotes the muscular force, \(F_0\) the maximal isometric force and \(v\) the shortening velocity of the muscle. The parameters \(a\) and \(b\) are called Hill’s parameters and are used to fit the different characteristics of the force-velocity relationships. The mechanical power of a muscle can then be calculated as the product of force and velocity, \(P = Fv\), and has a maximum at 31% of the maximal shortening velocity \(v_{\text{max}}\) (see Fig. 1.2.6). Fenn (1924) showed that the shortening muscle produces more heat than an isometric one (‘Fenn-Effect’). Hence the shortening generates more energy in mechanical work and heat whereas for high shortening velocities this energy production tapers off. Based on his findings, Hill (1938) calculated an empirical equation for the rate of energy production in a muscle

\[\dot{E} = \dot{Q} + Fv = ab + av + Fv\]  \hspace{1cm} (1.2)

The parameter \(a\) is called the thermal constant of shortening heat and is equal to the parameter \(a\) in Eq. (1.1). The terms \(ab\) and \(av\) are referred to as the activation heat and the shortening heat, respectively. The experimental findings of Hill revealed the connection between mechanical motion and heat production in muscle. However it needed another 20 years to shed light on the underlying mechanisms.

Hill investigated the shortening of muscle and neglected the lengthening in his early studies. Katz (1939) was the first researcher to investigate this neglected topic. When the force on a muscle exceeds its isometric force, \(F_0\), then it lengthens. Katz found a higher increase in force for small lengthening speeds than an extrapolation of the Eq. (1.1) would suggest (see Fig. 1.2.6). This increase of force tapers for higher lengthening speeds resulting in a yield point at about \(2F_0\) in the eccentric force-velocity relationship. This yield point reflects the overstretching of the fiber so that it is mainly held in the passive structures. With more elaborated techniques it has been possible to evaluate the force-velocity curve more precisely. Edman (1988) found a biphasic shape of the force-velocity curve in intact frog muscles. Morgan and his co-workers examined the lengthening of muscles and proposed the popping sarcomere theory that is discussed later in this section (Morgan, 1990; Morgan et al., 1991; Talbot and Morgan, 1996). Also force-velocity relationships on the scale of sarcomeres have been presented recently (Edman, 2005; Granzier et al., 1989).

**Force-length relationship**

According to the filament-sliding theory, the level of the active force must depend on the overlap of the filaments. For example, if the sarcomere is heavily stretched so that the actin-filament and the myosin filament are not overlapping anymore, there is no possibility to build any cross-bridge between the two filaments. In addition there will also be no possibility to build cross-bridges if the sarcomere is so short that the filaments bend. This assumption based on the filament-sliding theory was first tested by one of its founders A.F. Huxley (Huxley and Peachey, 1961). In this study on frog muscle fibers, sarcomeres did not shorten when they were stretched beyond 3.5 μm. Known from electron microscopy studies, this length corresponds to the sarcomere length at which the overlap of the filaments vanishes. Podolsky (1964) confirmed this finding for skinned frog muscle fibers where the length of the disappeared overlap was 3.6 μm.
The measurement of the relationship between sarcomere length and maximal isometric force is not straightforward, since the distribution of the sarcomere lengths is inhomogeneous along a muscle fiber during contraction (Telley and Denoth, 2007). Gordon et al. (1966b) overcame this problem while marking a small part of the fiber and following its length. In this study a piecewise linear relationship between the force and the mean sarcomere length in the marked region could be found. This relationship consists of three parts: (i) ascending limb, where the overlap gradually increases with increasing sarcomere length, (ii) the plateau, where the overlap is optimal, (iii) the descending limb, where the overlap decreases with increasing sarcomere length. This finding was a good proof of consistency for the sliding-filament theory. The basic result of the characteristic piecewise linear relationship was confirmed with newer techniques (Bagni et al., 1988) and mammalian muscles (Edman, 2005; Hilber et al., 2001). Brady (1991) has pointed out that the cardiac muscle has a steeper ascending limb than skeletal muscle.

**Force depression and force enhancement**

The force changes transiently when the length of fibers are changed during contraction. This has effects for all time scales (microseconds to seconds) after the length change. If the muscle is allowed to shorten, it affects its ability to reproduce force for 0.8-0.9 s (Edman, 1980). If the length is reduced to a certain level using a shortening ramp during an isometric contraction (loaded shortening), the regained force is depressed compared to isometric force at the same length (Herzog and Leonard, 1997). Instant shortening at the beginning of activation (unloaded shortening) did not show such a force-depression (Edman et al., 1993). In the same study, Edman et al. pointed out, that the force-depression may be related to sarcomere length inhomogeneity.
Introduction

The force of a stretched muscle fiber is enhanced during the stretch up to 3-fold of its precedent isometric force (Joumaa et al., 2008). Interestingly, the turnover of ATP is lower during stretch than during isometric and shortening contraction. When the final length after the stretch is reached, the force decreases to a steady level. This steady level after stretch may be higher than corresponding forces of isometric contractions (Hill, 1977). It has been reported that the superior steady force after stretch (‘residual force enhancement’) occurs only if the sarcomeres of frog are stretched to lengths greater than 2.25 µm hence on the descending limb of the force-length relationship (Edman et al., 1982). Recently, Lee & Herzog (2008) showed force-enhancement also for sarcomere lengths with optimal filament overlap on the plateau of the force-length relationship. Morgan (1990) proposed that the weaker sarcomeres elongate instantaneously one after another (‘sarcomere popping’), if the muscle is stretched at velocities greater than the yielding point of the eccentric force-velocity curve. The force of a popped sarcomere is believed to be held by the passive filament titin. The existence of sarcomere popping would lead to a prediction of residual force enhancement. However, in recent experiments on stretched myofibrils no sarcomere popping could be observed (Joumaa et al., 2008; Rassier et al., 2003a; Telley et al., 2006b).

In the last years, Herzog and his coworkers have widely investigated the reason for force enhancement following stretch (Herzog, 2005). This group proposed several mechanisms during stretch that are associated with force enhancement, among these: increased passive force and stiffness of the muscle, and a change of the viscoelasticity of titin (Herzog and Leonard, 2002; Herzog and Leonard, 2005; Herzog et al., 2003). The group ruled out sarcomere length non-uniformity as a contributor to residual force enhancement. They argued that in a system of inhomogeneous sarcomere lengths the steady force after the stretch may not be higher than the force at optimal length (Rassier et al., 2003b) and that force enhancement may not occur for lengths at the ascending limb and the plateau of the force-length relationship (Herzog and Leonard, 2002).

Lombardi and co-workers measured the stiffness of a fiber during a stretch ramp to be 10-20% greater than in isometric contractions (Lombardi and Piazzesi, 1990; Piazzesi et al., 1992). In these studies the authors explained the observed force rise during stretch as the effect of a fast pathway of attachment and detachment of cross-bridges without using ATP. They suggested that the force increase during lengthening is mainly caused by the stretch of the cross-bridges and the increase in the number of bound cross-bridges. On the basis of those hypotheses they proposed a model with a possible fast detachment at the beginning of the kinetic cycle (Piazzesi and Lombardi, 1995). In contrast to this theory, Pinniger et al. (2005) proposed a reversal power-stroke to a state with weaker force due to the stretching.

1.2.4. Mechanical and Kinetic models

In this section three landmarks of the models of muscle mechanics are reviewed. Firstly the mechanical model of Hill is outlined which is the conceptual basis of the half-sarcomere model in the present thesis.
Secondly, Huxley’s model of the sliding filament and his extension including a power-stroke is explained. Thirdly, a generalization of the Huxley’s model and some explicit multi-state models are reviewed.

Hill’s mechanical muscle model

The earliest model of a muscle was a simple elastic spring. Due to lengthening and shortening experiments showing hysteresis in the force response, the model was extended assuming a viscous element in series with an elastic spring (Gasser and Hill, 1924; Hill, 1922; Levin and Wyman, 1927). In the work deducing a mathematical relationship between force and velocity, Hill (1938) proposed a model in which the muscle consists of a contractile component (CC) in series with an elastic component (SEC). So the previous passive viscous element was changed to an active component, represented by the force-velocity characteristics (see Fig. 1.2.7). Such a single stranded model has the draw-back that it cannot reproduce the passive force of an inactive fiber. So the model was extended with a second strand in parallel containing a third element, the parallel elastic component (PEC). The characteristic of the PEC was the force-length relationship found in relaxed muscle. The description of the SEC was determined by measuring the force after quick stretches and lengthening of the muscle. The described Hill model reproduces many basic mechanical and thermal characteristics of striated muscle. However, it is an empirical model of the muscle lacking an underlying structural reason for the description.

![Fig. 1.2.7 : Hill’s model of a muscle. The two stranded model with an active and a passive strand. The active strand contains a contractile component (CC) and a series elastic component (SEC) in series. The passive strand consists of a single parallel elastic component (PEC) to account for the passive force of a muscle.](image)

Huxley’s cross-bridge model

The Nobel Prize laureate Huxley proposed a seminal model that is based on the sliding filament hypothesis (Huxley, 1957). The formalism therein is still in use nowadays. The model describes the building and breaking of the force producing cross-bridges between the two parallel filaments. Basically, the formalism describes the probability that a cross-bridge is bound and follows a statistical approach to deduce the force of the ensemble. In detail, Huxley proposed an active site (M) on the myosin filament that is held in between two elastic springs and that oscillates due to thermal agitation along the filament (see Fig. 1.2.8). This active site can couple to a binding site (A) on the actin filament that has a distance \( x \) from the equilibrium point \( O \) of the active site \( M \). The probability that \( M \) and \( A \) form a cross-bridge is given by the coupling rate \( f \). The cross-bridges are released due to coupling of ATP on the active site.
Introduction

This reaction is described by the decoupling rate $g$. Having formed a cross-bridge, the spring of the active site produces a force moving the actin filament. If the cross-bridge is built in the positive distance $x$ to the equilibrium point $O$, the generated force causes shortening, while a cross-bridge formed in the negative distance causes lengthening. Hence to prefer generation of force in a positive direction, the reaction rates $f$ and $g$ must be asymmetrical with respect to $O$. Therefore, Huxley proposed strain-dependent attachment and detachment rates. He assumed the attachment rate $f$ is only positive in the region $O$ to a characteristic length $h$ and increasing with $x$ in this region. Furthermore, he assumed the detachment rate $g$ to have a high value for negative strains ($x<0$), to drop instantly to zero at $O$ and increase for the positive branch but less so than the attachment rate $f$. Hence the region where the cross-bridge can form is restrained between $O$ and $h$.

If a cross-bridge is passing the equilibrium of the active site, the detachment rate increases suddenly inducing a decoupling of the binding that produces force in the opposite direction of shortening. As the detachment rate is finite, the decoupling of such negative cross-bridges is not instantaneous. Hence there will always be a number of cross-bridges during shortening with negative strain reducing the produced force of the positively-strained cross-bridges. So the force decreases for higher shortening speeds. This result reflects the force-velocity relationship of Hill (1938). The maximal shortening velocity is reached, if the produced force of the positively-strained bindings is equal to the force of the negatively-strained bindings.

Based on this simplified idea of the cross-bridge cycle, Huxley mathematically formulated the kinetic pathway in a differential equation while introducing a probability distribution $n$ of bound cross-bridges at a distance $x$ from the equilibrium point $O$ (Eq. (1.3)). The shortening velocity $v$ influences the distribution of cross-bridges at a position $x$, while adding or removing the amount of bindings of nearby sites. Hence the distribution $n(x;v)$ depends on the variable $x$ and the parameter $v$. The temporal change of the distribution of bound sites is given by the coupling of the unbound portion $(1-n)$ by the coupling rate $f(x)$ and the decoupling of the bound portion $n$ by the decoupling rate $g(x)$.

\[
\frac{dn}{dt} = \frac{\partial n}{\partial x} \frac{\partial x}{\partial t} = -v \frac{\partial n}{\partial x} = f(x) \cdot (1-n) - g(x) \cdot n \tag{1.3}
\]

Assuming a linear elastic spring at the active site $M$ with a spring constant $k$, the tension of a single cross-bridge at the position $x$ is $f_{cb} = kx$. Knowing the probability distribution $n(x;v)$ of a bound cross-bridge and defining the distance $l$ between two actin binding sites (A), the average work of a cross-bridge can be expressed as $\frac{1}{l} \int_{R} n(x;v) \cdot kx \, dx$. The tension in a myofibril is then the sum of all bound cross-bridges in a half-sarcomere divided by the unit area. With the total number of cross-bridges per unit volume $n$ and the sarcomere length $s$, the number of cross-bridges per unit area becomes $\frac{1}{2}ms$ and the total tension $P$ of a half-sarcomere is calculated with Eq. (1.4).
The rate of energy liberation per unit volume can be calculated knowing the energy (e) liberated by the hydrolysis of one ATP molecule. With the frequency of the A sites passing the M sites $n(x;v)$, the total rate of energy liberation per unit volume is

$$P(t) = \frac{m \cdot s \cdot k}{2l_a} \int_R n(x;v) \cdot x \, dx$$  \tag{1.4}$$

The expressions for the tension (1.4) and the energy rate (1.5) are independent of the definitions of attachment and detachment rate functions. To derive analytical expressions for the tension, Huxley defined the rates as piecewise linear functions, where the slope $f_i$ of the attachment rate is greater than the corresponding slope of the detachment rate $g_i$, and $g_0$ is much greater than $f_i$: 

$$f(x) = \begin{cases} f_i x & x \in [0, h] \\ 0 & x \not\in [0, h] \end{cases} \quad \text{and} \quad g(x) = \begin{cases} g_0 & x < 0 \\ g_i x & x \geq 0 \end{cases}$$  \tag{1.6}$$

Assuming a steady shortening velocity $v$, the differential equation (1.3) can be solved for the distribution $n(x;v)$. With the rate of shortening in muscle length per second $V = 2V_s^{-1}$ and the definition $V_0 = (f_i + g_i)hs^{-1}$, the integration of Eq. (1.4) results in

$$T = \frac{m \cdot s \cdot k}{2l_a} f_i \frac{h^2}{f_i + g_1} \left( 1 - \frac{V}{V_0} \left( 1 - e^{-\frac{V}{V_0}} \right) \left( 1 + \frac{1}{2} \left( \frac{f_i + g_1}{g_2} \right)^2 \frac{V}{V_0} \right) \right)$$  \tag{1.7}$$

In his paper, Huxley compared this expression with the force-velocity relationship of Hill (1938) i.e. $aF_0^{-1} = 0.25$, which resulted in the determination of the rate constants (Eq. (1.8)). The comparison of the two force-velocity curves showed a match within the experimental errors of Hill.

$$\frac{kh^2}{2e} = 0.75, \quad \frac{g_1}{f_i + g_1} = \frac{3}{16}, \quad \frac{g_2}{f_i + g_1} = 3.919$$  \tag{1.8}$$

The model was extended by A.F. Huxley & Simmons (1971a) while including a second bound state. This was based on the observation of the same authors (Huxley and Simmons, 1971b), that the recovery after quick release or quick stretch is asymmetric. The earlier explanations of the first two phases of the total four phases of the force recovery were based on a model consisting of an elastic component in series with a viscous component. However, different sarcomere lengths prior to the quick step, showed a dependence of the response on the filament overlap and hence on the number of involved cross-bridges. Therefore Huxley & Simmons concluded that at least one part of the elasticity must lie in the cross-bridges. Based on those findings, they proposed that the cross-bridges undergo a series of stable states with progressively lower potential energy and that the cross-bridges have series elasticity in the
neck. Due to the elasticity the different geometrical states of the cross-bridges could be reached without moving the actin filament. For the sake of simplicity they assumed two states of the bound cross-bridges involving a transition of the bound cross-bridge stretching the neck and producing force, a transition later referred to as ’power-stroke’. The transition between the two states is deduced from the principle of thermodynamics. Further mathematical details are not included here, as in the next paragraph a generalization of the idea of Huxley & Simmons is presented.

**Generalization of Huxley’s model**

Three years after the publication of Huxley & Simmons, T.L. Hill (1974) presented a general model of the sliding filament theory based on statistical mechanics. This formalism can be generalized for a finite number of states in the kinetic cycle (Telley, 2005). Basically, the kinetic cycle consisting of \( m \) states can be represented by a system of \( m \) partial differential equations (Eq.(1.9)).

\[
\frac{dn_i}{dt} = \frac{\partial n_i}{\partial t} + v(t) \frac{\partial n_i}{\partial x} = \sum_{j \neq i} k_{ji} n_j - n_i \sum_{j \neq i} k_{ij} 
\]

(1.9)

The probability distribution of each state \( n_i(x,t) \) depends on the individual position \( x \) of the binding site along the filament as well as the time \( t \). It describes the probability of finding a cross-bridge at a certain time \( t \) in state \( i \) at a distance \( x \) on the myosin filament. The temporal change of the coordinate \( x \) is equal to the velocity of the sliding filaments \( v(t) = \frac{\partial x}{\partial t} \). The transition rates \( k_{ij} \) describe the transition from state \( i \) to state \( j \) and depend generally on the distortion \( x \) and the absolute temperature \( T \). Regarding the free energy \( G_i(x) \) of state \( i \), we can express the force \( F_i \) of the state \( i \) and the balance of two opposite transition rates

\[
F_i(x) = \frac{\partial}{\partial x} G_i(x), \quad \frac{k_{ij}(x)}{k_{ji}(x)} = e^{\frac{G_j(x) - G_i(x)}{k_i T}} 
\]

(1.10)

![Diagram](image-url)
where \( k_B \) denotes the Boltzmann constant. In this formalism also the unbound states are described with probability distributions \( n_i \). So every cross-bridge has to be in a state resulting in a simple boundary condition \( \sum_{i=1}^{m} n_i(x, t) = 1 \) that holds for every time point \( t \) and every coordinate point \( x \). While assuming independent force generating cross-bridges, the average force per cross-bridge is then described by

\[
\bar{F}_{CB}(t) = \frac{1}{l_A - l_1/2} \int_{l_A}^{l_1/2} F_i(x) n_i(x, t) \, dx
\]

\[
P(t) = \frac{m \cdot s}{2} \bar{F}_{CB}(t)
\]

The variables in the proportional factor \( ms/2 \) correspond to the variables in Eq. (1.4). So with defined free energies \( G_i(x) \) of the individual states, the probability distribution of each state \( n_i \) and the tension \( P(t) \) of a sarcomere can be calculated by this formalism. This general formalism is the conceptual basis of explicit multi-state cross-bridge models, e.g. the Huxley model with two states \( (m=2) \) and the Huxley-Simmons model with three states \( (m=3) \).

Based on the idea of the model of Huxley and Huxley-Simmons, there were many models published trying to connect the kinetic cycle and the mechanical work of an ensemble of cross-bridges, e.g. (Brenner and Eisenberg, 1986; Eisenberg and Greene, 1980; Nishiyama et al., 1977; Piazzesi and Lombardi, 1996; Smith et al., 2008). The Nishiyama model is used in this thesis and is explained in detail in the Methods section.

The Eisenberg-Greene-Hill model (Eisenberg and Greene, 1980; Eisenberg and Hill, 1985) deduces the transition rates in the cross-bridge cycle from biochemical studies on the ATPase in solution. They proposed a strongly bound 45° state during and at the end of the power stroke and a 90° state in the other phases, where the angle denotes the mechanical equilibrium position of the myosin head. Further they modeled the free energy as position dependent where the transition from 90° to 45° decreases the free energy. The 45° state exerts positive force on the actin filament causing shortening. The transition of the cross-bridge is believed to first pass an energy barrier and ‘slide’ the reached energy landscape to gain an energetic equilibrium while gradually decreasing the internal strain. The Eisenberg-Green-Hill model combines the biochemical reaction rates nicely with the mechanical work of the cross-bridges. However, the reactions of a single myosin molecule in solution may not correspond to the true situation in a coupled framework of cross-bridges as the sarcomere.

The Brenner model (Brenner and Eisenberg, 1986) simplified the complex kinetic cycle while reducing it to its basics, the transition of the cross-bridge from a low force to a high force state. So he merged the various transitions into a coupling and decoupling rate of the cross-bridge \( (k^-, k^+) \) as well as the rate from low force to high force state \( f_{app} \) and the rate from return of the high force to the low force state \( g_{app} \) due to ADP release and rebinding of ATP. Based on this formulation the steady state fraction of cross-bridges in the high force state is \( f_{app}/(f_{app} + g_{app}) \). This formula is similar to the fraction of bound cross-bridges in Huxley’s formalism (1957) and reflects the similarity in the formulation of the two
models. Assuming that the force is only generated by the cross-bridges in the high force state, the force can be calculated by the mean force of the cross-bridges in the high-force state multiplied by the corresponding fraction. Based on those calculations Brenner proposed that the isometric force, stiffness and ATPase activity are controlled by the kinetics of the force transition.

The Piazzesi-Lombardi model (Lombardi and Piazzesi, 1990; Piazzesi and Lombardi, 1995; Piazzesi et al., 1992) bases on the Huxley formalisms of 1957 and 1971. It simplifies the complex kinetic cycle concentrating on overall attachment, power-stroke and detachment of cross-bridges but introduces the possibility of rapid detachment and re-attachment of the cross-bridges during the power-stroke. The authors coupled the transition rates thermodynamically by introduced energy potentials and periodic boundary conditions. Those assumptions resulted in a fraction of 77% bound myosin heads in isometric conditions. The model can predict the mechanical and energetic properties of contracting muscle but fails to reproduce the force response during stretch (Piazzesi et al., 1992).
Chapter 2
Methods
In this section the core work of the thesis, a multi-segmental model of a myofibril including a transient description of the kinetic pathway is presented. This comprehensive model is novel in such a combination and could give a future basis for the analysis of the half-sarcomere dynamics in the myofibril. Firstly, a short introduction and motivation of the presented model is given. Secondly, the model based on a kinetic pathway of two states (Huxley, 1957) is outlined and elaborated for computer simulations. Thirdly, the model for a kinetic cycle is extended to three states (Nishiyama et al., 1977). Fourthly, the principles of a specially adapted algorithm for the problem are shown. The first two points are part of an accepted publication (Stoecker et al., 2009). It was slightly modified to fit in the context of the whole thesis.

2.1. Introduction and motivation for the model

Muscle contraction is generally modeled by a multi-state cross-bridge formalism describing the chemical kinetics of ATP hydrolysis and the associated conformational change of myosin heads. It was first introduced in a simple form by A.F. Huxley (1957). The model was then extended to include additional biochemical states for the actin-myosin complex, which account for fast perturbations (Huxley and Simmons, 1971a; Huxley and Tideswell, 1997; Linari et al., 1997; Piazzesi and Lombardi, 1995) and rigorous thermodynamic matching (Eisenberg and Moos, 1968; Eisenberg and Hill, 1978; Eisenberg and Greene, 1980; Eisenberg et al., 1980; Hill, 1974; Hill, 1975; Hill and Simmons, 1976; Lymn and Taylor, 1971; Smith and Mijailovich, 2008; Smith et al., 2008). This basic formalism allows the calculation of the mean tension generated by a population of cross-bridges in a single half-sarcomere.

Several thousand half-sarcomeres in series and parallel are responsible for active tension generation in single muscle fibers (Coupland et al., 2005; Herzog and Leonard, 2000; Sleep et al., 2005) or whole muscles (Herzog et al., 2008; Schachar et al., 2002). The dynamic state (i.e. shortening or lengthening) is not necessarily uniform along this large network (Llewellyn et al., 2008; Rassier et al., 2003a; Telley and Denoth, 2007; Telley et al., 2006a; Telley et al., 2006b).

Model considerations of end-held contractions in myofibrils show that the dynamics of each half-sarcomere is coupled to all others, and that tension is a complex convolution of the individual (half-) sarcomeric forces (Denoth et al., 2002). Thus, a lumped model with one cross-bridge cycle, unaffected by the internal dynamics of the many half-sarcomeres in the myofibril, may not fully explain tension and length behavior. However, it is often applied to experimental data from myofibrils (Barman et al., 1998; Belus et al., 2003; Friedman and Goldman, 1996; Tesi et al., 2002a).

Here, a mathematical framework that facilitates the theoretical analysis of half-sarcomere dynamics in a myofibril during contraction in both length-clamped and tension-clamped conditions is presented. The formalism incorporates the basic ideas of Huxley (1957), as well as the multi-segmental structure of a myofibril. A biological variability is assumed either in the maximal isometric force of each half-sarcomere or in the variability of the kinetic attachment and detachment rates (Telley and Denoth, 2007; Telley et al., 2003). The current work extends the former framework of Denoth et al. (2002) while including a transient description of the chemo-mechanical contractile element in a half-sarcomere. So it combines
the biochemical kinetics with the structural mechanics of the hS. Note that this combination is unique although several multi-segmental models of myofibrils have been published in recent years (Campbell, 2009; Denoth et al., 2002; Givi and Bhattacharyya, 2009; Telley et al., 2003).

The present study aims to give a fundamental mathematical description of a myofibril, and for the sake of simplicity and clarity, this description is given in the most elementary form. Hence, the presented model sets a basis for any further analysis of hS dynamics. However, the framework in its very basic form allows various extensions that may explain the effect of hS dynamics on more complicated phenomena such as sequential relaxation or force-enhancement.

### 2.2. The model based on a kinetic cycle of two states

The simplest mechanical description of a half-sarcomere (hS) involves two parallel strands. One strand represents the non-cross-bridge component (sometimes called ‘parallel element’, PE), arising primarily from the spring-like protein titin. The other strand describes the contractile actomyosin motor, the cross-bridges, also called ‘contractile element’, CE (Fig. 2.2.1). The contractility of the system is given by the ATP driven power-stroke, which depends on the activation. For the sake of simplicity, the present model excludes the activation of the parallel element although recent findings proposed a Ca$^{2+}$ dependence of titin elasticity due to the interaction between actin and titin (Bagni et al., 2004; Joumaa et al., 2007; Kellermayer and Granzier, 1996; Pinniger et al., 2006).

The contractile strand consists of the composite series elasticity of the Z-line; the thin and thick filaments incorporated in the ‘serial element,’ SE; and an active ‘contractile element.’ For any hS, the following mathematical relationships between the force of the half-sarcomere, $f_{hS}$ and the forces of the specific elements, where $f_{CE}$ denotes the force of the ‘contractile element’, $f_{PE}$ the force of the ‘parallel element,’ and $f_{SE}$ the force of the ‘serial element’ can be stated.

$$f_{hS}(l, \hat{l}, u, n_{hS}) = f_{CE}(u, n_{hS}) + f_{PE}(l, \hat{l})$$

$$= f_{SE}(w) + f_{PE}(l, \hat{l})$$

$$w = l - u$$

(2.1)

In Eqs. (2.1), $l$ denotes the actual length of the half-sarcomere, $u$ the length of the region where actin and myosin can interact, $w$ the length of the non-interacting regions of actin and myosin and $n_{hS}$ the probability distribution of bound states. The contractile force $f_{CE}$ can be calculated following Huxley’s original idea (1957). Huxley proposed a mechanism of muscle contraction that fits the data of A.V. Hill’s (1938) steady shortening experiments. Despite the expansion of the model to include several biochemical states and statistical mechanics (Hill, 1974) the mathematical framework has remained unchanged. Huxley considered a two-state cycle of the cross-bridge, an attached state with the probability distribution $n_{hS}$ in which the myosin head is bound to the actin filament, and a detached state with distribution $l - n_{hS}$ in which myosin is decoupled from actin.
Fig. 2.2.1: Mechanical model. Interpretation of the most prominent biological structures of a half-sarcomere (top) as mechanical components (bottom). We define the Z-line and the M-line as the structural borders of a half-sarcomere, and account for the force-producing and force-transmitting structures between these borders. The actin and myosin filaments transmit the force from cross-bridges to the borderlines. They are represented by elastic springs connected in series to cross-bridges. The protein titin connects the myosin rods with the Z-line. Assuming the myosin filaments are much stiffer, they are represented in parallel to the cross-bridges, connecting the borderlines. The mechanical model consists of two strands, one with a contractile element (CE) and a series element (SE) in parallel to a second strand (PE). The viscoelasticity of titin is modeled with a parallel alignment of a nonlinear elastic spring and a pseudo viscoelastic element, which is the product of the spring and a velocity dependent factor. This unusual representation of a viscoelastic spring-dashpot system is emphasized with the dotted rectangle.

In this framework a myofibril is considered as a complex of many hS in series with stiff coupling. Based on the biological variability of the function and structure, each hS has a different characteristic. Following the traditional approach of force and length measurement in muscle research, two conditions for simulations can be defined: force-controlled and length-controlled contraction. In both types of experiments neither the individual lengths of the contractile elements, \( u(t) \), nor their individual velocities, \( \dot{u}(t) \), are known. Therefore, to calculate the lengths \( u \) for a given force \( F \), the formalism of Huxley has to be inverted (Hill, 1974). This problem will be discussed in detail for the case of transient-states. For the simpler case of a steady-state, the Huxley formalism results in a Hill \( F-v \) relation (Hill, 1938), which can be inverted easily (see section 2.2.1 for details). This case has been analyzed and simulated in previous works (Denoth et al., 2002; Telley et al., 2003). The formalism in those studies can account for conditions near to steady-state, but not for conditions far from steady-state, such as activation, step stretch and release, and relaxation. These time-dependent situations are called transient conditions. In particular the steady-state condition is unlikely, because the internal dynamics are
continuously changing, and the lengths and forces interact. However, the transient behavior of the contractile element can still be described by a Huxley-like formalism.

2.2.1. Huxley model in a steady-state description

The contractile force $f_{CE}$ can be calculated following the original idea of Huxley (1957) assuming a steady state condition. Thus the probability distribution $n_{Hux}(x, \dot{u})$ of the attached state in which the myosin head is bound to the actin filament is only dependent on the distance $x$ on the myosin filament and the shortening and lengthening velocity $\dot{u}(t)$ of the contractile element. The velocity $\dot{u}(t)$ must be constant in the steady-state condition. This constraint changes the variable $\dot{u}(t)$ into a parameter $\dot{u}$ of the probability distribution $n_{Hux}(\dot{u}; x)$. The kinetics of such a pathway in a steady-state, with a length change $\dot{u}$ of the contractile element, can be described with an ordinary differential equation of the form

$$\dot{u} \frac{\partial n_{Hux}(\dot{u}; x)}{\partial x} = f(x) - \left[ f(x) + g(x) \right] n_{Hux}(\dot{u}; x)$$ (2.2)

Assuming that the force of a cross-bridge $f_{CB}(x)$ depends on its deviation $x$ from the equilibrium position on the myosin filament, the resulting force in the contractile element of a half-sarcomere can be calculated as follows.

$$f_{CE}(u, n_{Hux}) = \alpha(u) \int_{R} n_{Hux}(\dot{u}; x) f_{CB}(x) dx$$ (2.3)

$\alpha(u)$ denotes the proportionality factor that depends also on the filament overlap. For a fixed on-rate $f(x)$ and off-rate $g(x)$ the integral results in a simple function $I(\dot{u}) = \int_{R} n_{Hux}(\dot{u}; x) f_{CB}(x) dx$. This corresponds to the force-velocity relationship of A. V. Hill (Hill, 1938) which is a simple look-up table. So the contractile force simplifies to a multiplication of look-up tables $f_{CE}(u, \dot{u}) = \alpha(u) \cdot I(\dot{u})$ and the lengths of the hS can be described by a simple system of ODE’s:

$$\dot{u} \cdot f_{CE,u} + \ddot{u} \cdot f_{CE,\ddot{u}} + \dot{i} \cdot f_{PE,i} + \ddot{i} \cdot f_{PE,\ddot{i}} = \dot{f}_{ext}(t)$$

$$\dot{\dot{w}} \cdot f_{SE,w} + \ddot{\dot{w}} \cdot f_{SE,\ddot{w}} + \dot{i} \cdot f_{PE,i} + \ddot{i} \cdot f_{PE,\ddot{i}} = \dot{f}_{ext}(t)$$

$$\dot{i} = \dot{u} + \dot{\dot{w}}$$

2.2.2. Transient description of the actomyosin motor

In his original work Huxley (Huxley, 1957) described the actomyosin motor by assuming a steady-state probability distribution $n_{Hux}(\dot{u}; x)$. The distribution describes the probability of finding a bound cross-
bridge at position $x$ on the myosin filament. The shape of the distribution depends on the parameter of the steady shortening $\dot{u}$ of the contractile element. Here, the same formalism for transient-state distributions $n_{hax}(\dot{u}; x, t)$, which can change in time, is applied. Following the approach given in Hill (1974), a partial differential equation (PDE) results for the rates of cross-bridge formation and breakage.

$$\frac{\partial n_{hax}(\dot{u}; x, t)}{\partial t}|_t + \dot{u}(t) \frac{\partial n_{hax}(\dot{u}; x, t)}{\partial x}|_x = \tilde{f}(x, t) - \left[ \tilde{f}(x, t) + g(x) \right] n_{hax}(\dot{u}; x, t) \quad (2.4)$$

The rates $\tilde{f}(x, t)$ and $g(x)$ are the on-rate and off-rate, respectively, of the cross-bridge cycle. They describe how many myosin heads attach to, or detach from, actin per second. Both rates depend on the position $x$ of the myosin head. The on-rate $\tilde{f}(x, t)$, furthermore depends on the time $t$ as the attachment rate is switched on or off by the activation state of the system. For the cross-bridges, the activation $\zeta(t)$ of the myofibril is assumed as a factor of the on-rate, $\tilde{f}(x, t) = f(x) \cdot \zeta(t)$ with $\zeta(t) \in [0, 1]$. Although the activation in this formulation may vary between each hS, the individual variation in the activation is presumed to be negligible and the factor $\zeta(t)$ is set equal for each hS. The calcium-troponin system, which regulates the activation of the contractile system, has a much faster kinetic rate ($\approx 100 \text{ s}^{-1}$ at 10°C (Dong et al., 2006)) than the kinetic rate of cross-bridge formation ($\approx 10 \text{ s}^{-1}$ at 10°C (Telley et al., 2006a)). Therefore, the activation and deactivation can be formulated as Heaviside step functions.

The differential equation, Eq.(2.4), has the form of a first order PDE, which can be transformed into an ordinary differential equation (ODE) by the method of characteristics e.g. Zahalak (1981). The general approach is the following: let $\eta(t)$ be a parameterization, or 'trace,' of the $x$ coordinate. With the transformations,

$$x \mapsto \eta(t) = x + \xi(t)$$
$$t \mapsto t$$

$$n_{hax}(\dot{u}; x, t) \mapsto p(x + \xi(t), t) = p(\eta, t)$$

with $\xi(t) = \int_{t_0}^{t} \dot{u}(\tau) d\tau$ or $\dot{\xi}(t) = \dot{u}(t) = \dot{\eta}(t)$

Eq. (2.4) transforms to $\frac{\partial p(\eta, t)}{\partial t} = \tilde{f}(\eta - \xi(t)) - \left[ \tilde{f}(\eta - \xi(t)) + g(\eta - \xi(t)) \right] p(\eta, t)$ which is now a parametric first-order ODE. This initial value problem has a solution that is history-dependent, which means that the distribution $p(\eta, t)$ at time $t$ depends on integrals of the form $\int_{t_0}^{t} (\ldots) \, d\tau$ and, therefore, on all $\tau \in [t_0, t]$.

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In experiments with myofibrils, trace $\eta$ of each hS is generally unknown. Instead, the force in a myofibril must be equal in each segmental at any time point according to the mechanics of the serial alignment. The active force in a hS is produced by the geometrical deflection $\eta$ of the attached myosin heads. Therefore, the force of an attached cross-bridge can be written as $f_{CB}(\eta - \xi)$. At deflection $\eta$ there is only a partition $p(\eta, t)$ of the attached cross-bridges that produce the force. The velocity $\dot{u}(t)$ of the relative movement of actin and myosin is equal to the temporal change of the trace $\eta$. Thus, the mean force and its time derivative for the cross-bridges in a hS are

$$f_{CE}(u, p) = \alpha(u) \int p(\eta, t) f_{CB}(\eta - \xi) \, d\eta \quad \text{with} \quad \dot{\xi}(t) = \dot{\eta}(t)$$

$$\dot{f}_{CE}(u, p) = \alpha(u) \left( \int \dot{p}(\eta, t) f_{CB}(\eta - \xi) \, d\eta + \dot{u} \int p(\eta, t) f_{CB,\eta}(\eta - \xi) \, d\eta \right)$$

$\alpha(u)$ is a proportionality factor that includes the number of binding sites in the specific hS, and the normalization (see section 2.4.1). By neglecting the parallel element in a first approach, the force of a hS is $f_{CE}$, which is equal to a known external force $f_{ext}$. Hence, this results in a differential equation for the lengths $u(t)$ of each hS, which can be solved by including the differential equation for the distribution $p$.

$$\dot{u} = \frac{f_{ext} - \alpha(u) \int \dot{p}(\eta, t) f_{CB}(\eta - \xi) \, d\eta}{\alpha(u) \int p(\eta, t) f_{CB,\eta}(\eta - \xi) \, d\eta} \quad \text{with} \quad \dot{\xi}(t) = \dot{\eta}(t)$$

$$\dot{p} = \ddot{f} - (\ddot{f} + g) \cdot p$$

Fig. 2.2.2: Coordinate transformation. In the original Huxley model, the myosin filament is the fixed system and the actin filament slides along the myosin filament. Here, the coordinates $x_j$, which describe the deflection of the cross-bridge in the fixed system, are transformed into new coordinates $\eta_j(t)$ on the actin filament ('moving' system) with a linear Galilean transformation $x \mapsto y = x + \int v \, dt$. This transformation describes the binding behavior of cross-bridges 'sitting' either on the myosin or the actin filament, while the deflection of the myosin head is measured.
2.2.3. Force-controlled contraction experiment

In a force-controlled experiment, an external puller controls the length of a myofibril via feedback, such that the resulting measured force of the myofibril follows a predefined protocol $f_{ext}(t)$ (e.g., a constant force). Since all $n$ of the hS are coupled in series, all hS forces must be equal,

$$f'_{hS} = f'_{CE} + f'_{PE} = f'_{SE} + f'_{PE} = f_{ext}(t) \quad \forall i = 1, \ldots, n$$  \hspace{1cm} (2.6)

The system response is the length $l_i(t)$ of an hS, and the total length of the myofibril $\sum_{i=1}^n l_i$. In experiments, the myofibril is mounted between a stiff fixation point and a force transducer (Anazawa et al., 1992; Bartoo et al., 1993; Colomo et al., 1997; Stehle et al., 2002b; Telley et al., 2006a; Tesi et al., 2002b). The transducer has a deflection $\Delta x_{ext}$, which is added to the lengths of all the hS to give the external length $L_{ext} = \sum_{i=1}^n l_i + \Delta x_{ext}$. Due to the process of fixation, most of the hS at the ends are damaged and non-functional (Telley et al., 2006a). Therefore, the deflection $\Delta x_{ext}$ may also include the deflection of these inactive hS, resulting in a weaker external stiffness than the stiffness of the force transducer alone.

Given the partial derivatives of the functions, the observables $u(t)$, the functions $w(t)$ and $l(t)$ can be calculated from the total derivative of Eq. (2.6), resulting in a set of decoupled ordinary differential equations (ODE’s) for each hS, resulting in Eq.(2.7).

$$\dot{u} \cdot f_{CE,u} + \int \dot{p}(\eta,t) \cdot f_{CE,p} \, d\eta + \dot{i} \cdot f_{PE,i} + \ddot{i} \cdot f_{PE,i} = \dot{f}_{ext}(t)$$
$$\dot{w} \cdot f_{SE,w} + \dot{i} \cdot f_{PE,w} + \ddot{i} \cdot f_{PE,w} = \dot{f}_{ext}(t)$$
$$\dot{l} = \dot{u} + \dot{w}$$  \hspace{1cm} (2.7)

with $f_{(_,\lambda)} = \frac{\partial}{\partial \lambda} f_{(_,\lambda)}$.

2.2.4. Length-controlled contraction experiment

In a length-controlled experiment, the length of the myofibril plus transducer follows a predefined protocol, typically controlled by an external puller. To adjust the length of the system, this puller produces a time dependent force $f_{ext}(t)$ that must be equal to the myofibrillar force $f_{hS} = f_{CE} + f_{PE} = f_{ext}(t)$, since the puller and all the hS are coupled in series.

Assuming a linear elastic fixation, the produced force $f_{ext}$ of the myofibril is proportional to the deflection of the fixation and hence is coupled to the total length $L_{ext}$ of the system (myofibril plus transducer) (Denoth et al., 2002). The length-controlled system is then described by set of coupled ODE’s (Eqs. (2.8)).
Methods

\[
\begin{align*}
\dot{u} \cdot f_{CE,u} + \int \dot{p}(\eta, t) \cdot f_{CE,p} \, d\eta + \dot{\mathbf{u}} \cdot f_{PE} + \ddot{\mathbf{u}} \cdot f_{PE} &= \dot{f}_{ext}(t) \\
\dot{\mathbf{w}} \cdot f_{SE,w} + \dot{\mathbf{w}} \cdot f_{PE} + \ddot{\mathbf{u}} \cdot f_{PE} &= \dot{f}_{ext}(t) \\
k_{ext} \left( \ddot{L}_{ext} - \sum_{i=1}^{n} \dot{\mathbf{u}} \right) &= \dot{f}_{ext}(t) \\
\dot{\mathbf{u}} &= \dot{\mathbf{u}} + \dot{\mathbf{w}}
\end{align*}
\]

(2.8)

Generally, the external spring includes the fixation as well as the non-functional hS, and so the external spring may be viscous-elastic and dependent on the force of the myofibril (Telley et al., 2006b). However, for the sake of simplicity this external spring is assumed as linearly elastic with a spring constant \( k_{ext} \).

Note that in the preceding case of force-controlled contraction experiments the external force is known at every time point and independent of the individual hS lengths.

**2.2.5. Discretization of the transient description**

To clarify and specify the calculation of the velocity of the sliding filaments, define a grid in the \( x \) coordinate system, \( G_{\{x_{j}\}} = \{ x_{j} \in \mathbb{R}, j \in \mathbb{N}: \left| x_{j} \right| < \infty, j = 1, \ldots, N_{j} \} \) and the distribution \( p_{j}(t) = p(\eta_{j}, t) \) for the corresponding trace \( \eta_{j}(t) = x_{j} + u(t) - u(t_{0}) \) on the grid \( G \). The \( p_{j} \) are the probability distributions of attached heads on a trace \( \eta_{j} \). The force on each trace \( \eta_{j} \) is proportional to the force of a cross-bridge \( f_{CB}(\eta_{j}, \xi) \) multiplied by the probability distributions \( p_{j} \). Therefore, it can be written, for the contractile force of an hS,

\[
f_{CE}(u, p) = \alpha(u) \cdot \sum_{j=1}^{N_{j}} p_{j} \cdot f_{CB}(\eta_{j} - \xi) \Delta x_{j}
\]

(2.9)

\( \Delta x_{j} = x_{j} - x_{j-1} \) denotes the distance between traces \( j \) and \( j-1 \), \( \alpha(u) \) is a proportionality factor that includes the mean number of possible myosin heads in an hS that interact with actin. This number is proportional to the force-length relation of a myofibril.

The original Huxley distribution \( n_{Hux}(x, t) \), which denotes the distribution of attached myosin heads relative to the equilibrium position of the myosin head, is transformed into a distribution \( p_{j} \), which denotes the occupied actin binding sites. The cross-bridge deflection is now \( \eta_{j} \) and includes the relative movement of actin and myosin, and the equilibrium position of the myosin head relative to the actin filament. The time derivative of Eq. (2.10) is

\[
\frac{d}{dt} f_{CE}(u, p) = \sum_{j=1}^{N_{j}} \dot{p}_{j} f_{CE,p_{j}} + \dot{u} \cdot f_{CE,u}
\]

(2.10)

The first term on the right-hand side describes the change in the force due to the cross-bridges. The second term describes the contribution of the contractile force change, which depends on the overlap of
the actin and myosin filament. As the force in each hS must equal the external force at any time the force of a hS can be written, \( f_{w}(l, \dot{L}, u, p) = f_{CE}(u, p) + f_{PE}(l, \dot{L}) = f_{ext}(t) \). The time derivative of this equation leads to a system of ODE’s.

2.3. The model based on a kinetic cycle of three states

Despite the expansion of the model including several biochemical states and statistical mechanics (Eisenberg and Moos, 1968; Eisenberg and Hill, 1978; Eisenberg and Greene, 1980; Eisenberg et al., 1980; Hill, 1974; Hill, 1975; Hill and Simmons, 1976; Huxley and Simmons, 1971a; Lymn and Taylor, 1971) the basic mathematical framework of Huxley (Huxley, 1957) has remained unchanged. Nishiyama et al. (1977) extended Huxley’s bound state \( n=n_1 \) with a strongly coupled state \( n_2 \). So the evolution of the distributions \( n_1 \) and \( n_2 \) of the bound states can be described with two partial differential equations (Eq. (2.11)) with the boundary condition \( n_0 = n_1 + n_2 \) where \( k_{ij} \) denotes the kinetic transition rate from chemical state \( i \) to chemical state \( j \) of the myosin head.

\[
\frac{\partial n_1(\dot{u};x,t)}{\partial t} + \dot{u}(t) \frac{\partial n_1(\dot{u};x,t)}{\partial x} = k_{01} - [k_{10} + k_{12} + k_{01}]n_1(\dot{u};x,t) + [k_{21} - k_{01}]n_2(\dot{u};x,t) \tag{2.11}
\]

\[
\frac{\partial n_2(\dot{u};x,t)}{\partial t} + \dot{u}(t) \frac{\partial n_2(\dot{u};x,t)}{\partial x} = k_{02} - [k_{20} + k_{21} + k_{02}]n_2(\dot{u};x,t) + [k_{12} - k_{02}]n_1(\dot{u};x,t)
\]

The force of the contractile element can be calculated while summing up the individual forces of the two coupled states (Eq. (2.12)).

\[
f_{CE}(u, n_1, n_2) = \alpha(u) \cdot \left[ n_1(\dot{u};x,t) f_{CB,1}(x) + n_2(\dot{u};x,t) f_{CB,2}(x) \right] dx \tag{2.12}
\]

This formulation of the extension of the Huxley model can be generalized while writing the formulas in form of matrices, where the state distributions \( n_i \) are merged into a ‘state vector’ of the form \( \vec{n} = (n_1, n_2, \ldots, n_m) \) where \( m+l \) is the number of included states in the model. The temporal evolution of this ‘state vector’ \( \vec{n} \) can be calculated by a differential equation with a stiffness matrix \( \mathbf{K}(x) \) that includes linear combinations of the kinetic transitions \( k_{ij} \) and a perturbation vector \( \vec{c} \) that equals \( (k_{01}, k_{02}, \ldots, k_{0m}) \).

\[
\frac{d\vec{n}(x,t)}{dt} = \mathbf{K}(x) \cdot \vec{n}(x,t) + \vec{c}(x) \tag{2.13}
\]

Using the methods of characteristics (Zahalak, 1981) the partial differential equation in Eq. (2.11) can be transformed into an ordinary differential equation (Eq. (2.14)) while using the transformations \( \vec{p}(\eta,t) = \vec{n}(x,t) \) and \( \eta(t) = x + u(t) - u(t_0) \).

\[
\dot{\vec{p}}(\eta,t) = \mathbf{K}(\eta - \xi) \cdot \vec{p}(\eta,t) + \vec{c}(\eta - \xi) \tag{2.14}
\]
The contractile force can be calculated directly in this transformed space while combining the different forces of the states in a vector \( \vec{f}_{\text{CB}}(x) = \left( f_{\text{CB},1}(x), f_{\text{CB},2}(x), \ldots, f_{\text{CB},m}(x) \right) \) (Eq. (2.15)).

\[
f_{\text{CB}}(u, p) = \alpha(u) \cdot \int_{\mathbb{R}} \tilde{p}(\eta, t) \cdot \vec{f}_{\text{CB}}(\eta - \xi) d\eta
\]

(2.15)

The temporal evolution of the observable \( u \) can be calculated if Eq. (2.15) is differentiated with respect to the time \( t \). This results in the same equations as for the Huxley formalism (Eq. (2.7) or Eq. (2.8)) but with vectorized probability distributions \( \tilde{p}(\eta, t) \) and cross-bridge forces \( \vec{f}_{\text{CB}}(\eta) \).

In the formalism of Nishiyama et al. (1977), the stiffness matrix of the system of differential equations (2.11) can be written as

\[
K(x) = \begin{pmatrix}
-k_{10} + k_{12} + k_{01} & -k_{21} - k_{01} \\
k_{12} - k_{02} & -k_{20} + k_{21} + k_{02}
\end{pmatrix}, \quad \vec{c}(x) = \begin{pmatrix} k_{01} \\ k_{02} \end{pmatrix}
\]

(2.16)

The kinetic rates of the Nishiyama model are shown in Fig. 2.5.1. The detailed mathematical definitions of the kinetic transition rates \( k_{ij} \) are given in the Appendix A. The activation is applied as a factor \( \zeta(t) \in [0, 1] \) on the coupling rate \( k_{01} \). Without activation a permanent small coupling rate is assumed to generate a small permanent tension in the contractile element. This small tension is generated by a small coupling rate \( k_{01}|_{\zeta=0} = 10^{-5} \cdot k_{01}|_{\zeta=1} \). Note the strong increase of the coupling and decoupling rates resulting in a cutoff of the state distributions \( p \) and a defined coupling region \( l_A \) per cross-bridge. This region can be extended to account for cross-bridges that are overstretched into the domain of the neighboring cross-bridge.
2.4. Simulation of Contraction Experiments

For simulating contraction experiments the model has to be fixed on experimental parameters while predefining the contractile element, the series element and the parallel element according to a specific type of myofibril (e.g. fast or slow species). Firstly this is done by setting the on- and off-rates $f(x)$ and $g(x)$. This was achieved while calculating the steady-state Huxley distributions $n_{\mu\nu}(u; x)$ and the resulting force of a single fully activated contractile element. The parameters of the strain-dependent transition rates $\tilde{f}(x, t)$ and $g(x)$ were then adjusted so that the steady-state Hill parameters of skinned fibers from rabbit psoas could be achieved \((a/P_0 = 0.09, v_{\text{max}} = 1.2 \, L_0/s \, (\text{Sun et al., 2001}))\), resulting in the Huxley parameters $f_1 = 7.2 \, s^{-1}$, $g_1 = 14.4 \, s^{-2}$ and $g_2 = 173.2 \, s^{-1}$. For numerical reasons, a steep ramp instead of an exact Heaviside-function for the activation $\zeta(t)$ of the on-rate $\tilde{f}(x, t)$ is used.

To simulate the multi-state formalism the model of Nishiyama (1977) for three states - two coupled states and an uncoupled state is implemented. The transition rates between the states are thermodynamically coupled as shown in Chapter 1. To compare between the two state formalism of Huxley (1957) and the three state formalism of Nishiyama (1977) the functions $\tilde{f}(x, t)$ and $g(x)$ in Huxley’s model to achieve the steady-state force-velocity relationship of a frog sartorius muscle \((a/P_0 = 0.25 \, \text{and} \, v_{\text{max}} = 4.2 \, L_0/s)\) are adjusted. This resulted in parameters $f_1 = 55.8 \, s^{-1}$, $g_1 = 2f_1$ and $g_2 = 581.95 \, s^{-1}$ in the Huxley model.
2.4.1. Proportionality factor of the contractile force

The proportionality factor includes the individual force capacity of each hS, the scaling of the force due to the overlap of actin and myosin and the scaling of the force of a binding site to the force of a hS. So it can be defined as \( \alpha(u) = N(u) \cdot \frac{1}{l_A} \), where \( N(u) \) denotes the mean number of possible myosin heads in a hS that interact with actin, and \( l_A \) is the periodicity of the actin binding sites. According to the literature (Edman, 2005; Gordon et al., 1966b) the force-length relation reflects the overlap function \( N(u) \) while neglecting the series elasticity. So the function \( N(l) \), which denotes the force-length relation, was defined according to the data of Edman (2005). \( N \) is a mean value of all the hS in a myofibril and hence a force capacity \( C \) of an individual hS is introduced that modifies \( N \) in each hS, due to biological variability (e.g. the number of myosin heads, the lengths of the actin- and myosin-filaments (Telley and Denoth, 2007)). \( C \) is a dimensionless proportional constant of \( N \), reducing or enhancing the level of its plateau. Furthermore, \( C \) also includes a variation of the maximal isometric force that is proportional to the quotient \( f/(f+g) \). The actual force capacity can vary over different scales along a myofibril. It can be thought of as a random variable or a spatially dependent variable that might change little among several hS, resulting in seemingly related capacities among neighboring hS. It follows that the expression for the contractile force \( f_{CE} \) can be written as,

\[
f_{CE}(l,u,p) = N(l) \cdot \frac{1}{l_A} \int p(\eta,t) f_{CB}(\eta - \xi) \, d\eta
\]

Values for the force-capacity are chosen from a normal distribution, with mean \( C = 1 \) and a standard deviation of \( \sigma \). In section 2.5.1 more detailed information of the calculation of \( C \) are given.

2.4.2. Characteristics of the parallel and serial element

The parallel element has viscous and elastic properties. The force of the parallel element in our model is assumed to be the product of the elastic function \( f_1 \) and the viscous function \( f_2 \). This is a more general formulation of viscoelasticity than found in the standard linear solid modeling and has advantages in its mathematical formulation (Denoth et al., 2002). If it is set \( f_2(\dot{\xi}) = 1 + \dot{f}_2(\dot{\xi}) \) with \(-1 < \dot{f}_2(\dot{\xi}) < 1\), the non-cross-bridge element can be modeled as a system of two parallel strands. One strand is the elastic part, and the second strand is the composite of elasticity and viscosity, formulated as product of the two functions. Hence, the effect of viscosity is intensified for increased lengths. This makes sense if the viscosity causing structures or their overlap is dependent on the filament overlap of the half-sarcomere. For the elastic property of titin, an exponential function, \( f_1(l) = \alpha \left( e^{\beta l} - 1 \right) \), is fitted to the data presented by Linke (2000) and Minajeva et al. (2002). The viscous property of the parallel element is heuristically defined as in Denoth et al. (2002), \( f_2(\dot{\xi}) = c_2 \cdot \frac{2}{\pi} \cdot \arctan \left( \frac{c_1}{\dot{\xi}} \right) + 1 \), to produce a lengthening-shortening-hysteresis similar to Kellermayer et al. (1997). I am aware that more sophisticated models exist for the titin molecule (e.g. worm-like chain elastic model (Kellermayer et al.,
However, in a first approximation the complexity of our model is bounded while using simple functions in the sense of lookup-tables. For the series element a linear elastic function in the form 
\[ f_{se}(w) = k_{se} \cdot (w-w_0) \] is assumed (Luo et al., 1994).
Recent findings proposed a Ca^{2+} dependence of titin elasticity, due to the interaction between actin and titin (Bagni et al., 2004; Joumaa et al., 2007; Kellermayer and Granzier, 1996; Pinniger et al., 2006).
Hence the parallel element also depends on the activation of the system, and its stiffness may be ten-fold higher in an activated state than in a relaxed state (Pinniger et al., 2006).

### 2.4.3. Dynamical system of the half-sarcomeres

The mechanical laws of a myofibril during contraction are described by Eq.(2.6). Substitute the contractile force in Eq. (2.7) with the expression in Eq. (2.17), and the result is a system of ODE's (Eq. (2.18)), which holds for dynamic force-controlled experiments. For the hS i it can be stated

\[
\sum_{j=1}^{N_i} p_j \cdot f_{CE,j} + \dot{u}_i \cdot f_{CE,u_i} + \ddot{u}_i \cdot (f_{PE,j} + f_{CE,j}) + \dddot{u}_i \cdot f_{PE,j} = \dot{f}_{ext}(t)
\]

\[
\dot{p}_j(t) = \ddot{f}(\eta_j - \xi, t) - [\dddot{f}(\eta_j - \xi, t) + g(\eta_j - \xi)] \cdot p_j(t)
\]

\[
\dot{w}_j \cdot f_{SE,u_i} + \ddot{w}_j \cdot f_{PE,j} + \dddot{w}_j \cdot f_{PE,j} = \dot{f}_{ext}(t)
\]

\[
l_i = u_i(t) + w_i(t)
\]

with \( \eta_j = x_j + u_i(t) - u_i(t_0) \), \( p_j(t) = p(\eta_j, t) \) according to the definitions in section 2.2.5. For length-controlled experiments, the lengths \( l_i \) of all hS are coupled, and the external force \( f_{ext} \) in Eq. (2.18) can be replaced with \( k_{ext} \left( L_{tot} - \sum_{i=1}^{n} l_i \right) \).

Let’s choose \( u, l, \dot{l} \) and \( p \) to be the independent variables of an hS. Combining all the variables for the number \( n \) of hS, these variables become vectors. If the lengths are collected in a vector \( \vec{y} = (u, \dot{l}, \ddot{l}) \), the system of ODE’s is written as

\[
\dot{\vec{y}} = \mathbf{f}(\vec{y}, t) - (\mathbf{f} + \mathbf{g})(\vec{y}, t) \cdot \dddot{\vec{y}}
\]

\[
\mathbf{M}(t, \vec{y}) \cdot \ddot{\vec{y}} = \mathbf{A}(t, \vec{y}) \cdot \vec{y} + \mathbf{B}(t, \vec{y})
\]

Eq. (2.19) accounts for two-state cross-bridge kinetics. However, this formalism can be extended to a multi-state model (Eq. (2.20)), resulting in a larger system of equations and only minor differences in the expressions for the contractile force (Eq.(2.17)), mass matrix \( \mathbf{M} \), stiffness matrix \( \mathbf{A} \), and perturbation vector \( \mathbf{B} \). In this general case the state vector \( \dddot{\vec{y}} \) is calculated by a matrix \( \mathbf{K} \) which contains linear combinations of the kinetic rates between the states.
\[ \hat{p} = K \cdot \hat{p} + \hat{c} \]
\[ M \cdot \dot{y} = A \cdot \dot{y} + \hat{B} \]

(2.20)

### 2.4.4. Calculation of the maximal isometric Force \( F_0 \)

The compliance of a cross-bridge was assumed to be \( k_{CB} = 1.2 \) pN/nm in active and in rigor state at 12°C, as published by Linari et al. (2007). The periodicity of actin sites is \( l_a = 0.0365 \) µm, according to Ebashi et al. (1969); the number of half-myosin filaments per cross-sectional area is \( \rho_M = 407 \) µm\(^2\); and the number of myosin heads per half-myosin filament is \( H_M = 283 \) in psoas muscles from rabbits (Linari et al., 2007). Theoretically, the maximal force is reached at the end of the activation phase, when the hS are believed to be in an isometric steady-state. Thus, the probability density distribution in the 2 states model of Huxley is

\[ f_n(x, 0) = \frac{f_1}{f_1 + g_1} \quad \text{for} \quad x \in [0, h] \]

whereas only one third of the cross-bridges are believed to be attached (Linari et al., 2007), determining \( g_1 = 2 f_1 \). Moreover, the maximal force in an hS of a myofibril with force capacity \( C = 1 \), operating on the plateau of the force-length relation \( (N(l) = H_M) \), becomes

\[ F_0 = \frac{A \cdot \rho_M}{l_a} H_M \cdot k_{CB} \cdot \frac{f_1}{f_1 + g_1} \cdot \frac{1}{2} h^2 = 0.63 \times 10^6 \cdot A \cdot h^2 \cdot \frac{nN}{\mu m^2} \]

To achieve realistic forces of rabbit psoas, the mean working distance \( h \) was set to 15 nm.

The calculation of the steady force for a multi-state model is rather straight forward using the formalism in matrix form (2.13). At the time when the force of a single hS reaches its maximum during isometric contraction, the distributions of the coupled cross-bridges are assumed to be in steady-states that fulfill the condition \( \hat{p}_{\text{steady}}(\eta, t) = 0 \). The distributions can be calculated using the inverse of the stiffness matrix

\[ \hat{p}_{\text{steady}}(\eta, t) = K^{-1}(\eta - \xi) \cdot \hat{c}(\eta - \xi) \]

Knowing the steady distributions of the coupled states and assuming that the hS with a mean force capacity \( (C = 1) \) is working on the plateau of the force-length relationship, the isometric force can be calculated as

\[ F_0 = \frac{1}{l_a} \int_{R} \hat{p}_{\text{steady}}(\eta, t) \cdot f_{CB}(\eta - \xi) d\eta = \frac{1}{l_a} \int_{R} K^{-1}(\eta - \xi) \cdot \hat{c}(\eta - \xi) \cdot f_{CB}(\eta - \xi) d\eta \]

For the calculation of the maximal force \( F_0 \), the parallel element was neglected because the titin force is \( f_{PE} \ll f_{CE} \) during isometric contraction when the hS lengths are in the range of \( l_a \approx 1-1.1 \) µm (Linke, 2000).
2.5. **Computational issues**

An adapted program needed to be developed for the simulation of the big system of differential equations (2.19). The calculations had to be optimized in terms of memory and calculation speed. Furthermore, the program had to be modular and easy adjustable. The programs were designed so that the calculations work on normal desktop computers with 64bit architecture using MATLAB R2008a/b (The MathWorks, Natick, MA).

2.5.1. **Initial values**

Before the numerical integration of the dynamical system of Eq. (2.20), the specific functions for the hS have to be defined. Here each half-sarcomere may be given an individual characteristic. This characteristic includes the force capacity C, the definition of the elastic and viscous properties of the parallel element and the transition rates between the states. Assuming a permanent active steady force ($F_{\text{min}} = 10^3 F_0$) of the contractile element, the individual starting lengths of the individual elements (CE, PE, SE; see Fig. 2.2.1) and the individual starting probability distributions $\tilde{p}(x,t_0)$ are calculated based on the definitions made, assuming a resting hS, $\dot{l}(t_0) = 0$. So the initial conditions

$$\tilde{y}_0 = \left(\tilde{u}(t_0), \tilde{l}(t_0), \dot{l}(t_0)\right)$$

and $\tilde{p}(x,t_0)$ of the integration of the dynamical system are set.

The force capacities C of the individual hS are calculated according to a normal distribution around a mean value C=1 and a predefined width (standard deviation $\sigma$). For this calculation, the area of the normal probability density function between $-2\sigma$ and $2\sigma$ is divided equally by the number of hS in the simulation. The value of C is then set in the middle of such a subarea (see Fig. 2.5.1). For the eight hS in the system, this results in $C = (0.846, 0.915, 0.953, 0.985, 1.015, 1.047, 1.086, 1.154)$ and for a system of four hS it results in $C = (0.868, 0.968, 1.032, 1.132)$. If a variation of the cross-bridge kinetics among the individual hS is taken into account in the simulations, the same procedure is also used to calculate the different rate functions $f$ and $g$ of Huxley.
2.5.2. Grid of coordinates in the contractile element

The grid in the variable $x$ on the myosin filament is crucial for the further correctness and performance of the calculations. So this grid has to be defined carefully. If the grid is too coarse, the integration will be inaccurate so that the error in the contractile forces is higher than the differences of the contractile forces due to the variance in the force capacities. On the other hand, if the grid is too fine, the used memory reduces the computational performance of the integration. The grid in $x$ is defined as $G_{x_1, \ldots, x_j} = \{ \mathbf{x}_j \in \mathbb{R}, \mathbf{j} \in \mathbb{N} : |\mathbf{x}_j| < \infty, j=1, \ldots, N \}$ according to the previous section 2.2.5. The fineness of the grid is the distance $\Delta x_j = h \cdot 2^{-r}$ between two coordinate points $x_j$ and $x_{j+1}$ on the myosin filament with a selectable parameter $r$. Additionally, the total range of the grid has to be set, while defining the lowest and the highest coordinate $[x_1, x_N]$. This results in a total number of the coordinates for the $i$th hS $h_S \frac{1}{h} \left| \mathbf{x}_{N,j} - \mathbf{x}_i \right| \cdot 2^n$ and a total number of the coordinates of all the contractile elements of the $n$ hS in the system $\sum_{i=1}^{n} \frac{1}{h} \left| \mathbf{x}_{N,j} - \mathbf{x}_i \right| \cdot 2^n$. Based on this coordinate the traces in the contractile element of the hS $i$ are defined as $\eta'_j(t) = x'_j + u_i(t) - u_j(t_0)$. So the dimension of the system describing the dynamics of $n$ hS is the length of the vector $\tilde{y} = (\tilde{u}, \tilde{I}, \tilde{I})$ plus the length of the vector $\tilde{p}(\eta, t)$ which is calculated as $3 \cdot n + \sum_{i=1}^{n} \frac{1}{h} \left| \mathbf{x}_{N,j} - \mathbf{x}_i \right| \cdot 2^n$ and depends strongly on the fineness of the grid. Simulating a system of eight

---

Fig. 2.5.1: Probability density function of the force capacities with $\sigma=0.1$. The points denote the calculated force capacities of the hS that lie in the middle of the different grey shaded areas. The different grey surfaces have all the same area. To have finite values for the force capacities, only the area in the region $[-2\sigma, 2\sigma]$ is regarded.
hS with a finesse of \( r=7 \) and a range \( [x_l, x_u] = [-5h, 10h] \) with results in 15'360 coupled differential equations to integrate for the simpler case of the Huxley model consisting of 2 states.

Regarding the Huxley model, the probability distributions change most at the boarders of the interval \( [0,h] \) (‘generating interval’) where the coupled states are building up. This is due to the definition of the coupling rate \( f \). If a trace \( \eta(t) \) with no coupled states enters this region at time \( t \), the new coupled states on this trace add to the force and influence the system. The grid around those boarders of the ‘generating interval’ is crucial for the accuracy of the change of the state distributions \( \dot{\mu}(\eta,t) \) and hence for the calculation of the change of the contractile force \( \dot{f}_{CE} \).

### 2.5.3. Moving grid

While the hS move due to the dynamics the traces \( \eta(t) = x + u(t) - u(t_0) \) move too. So if the hS shorten over a long distance the grid will pass out of the ‘generating interval’ resulting in an artificial force decrease of the system and a failure of integration. So for a big range of motion of the hS the grid has to be wide enough to cover the whole area of the movement. However, this movement is unknown a priori to the simulation as it is a calculated observable. A solution to this problem was implemented, while shifting the grid in the opposite direction of the movement when it shortens or lengthens more than a set threshold length. So the grid lies always around the region where the states are generated or destroyed. Note that this shifting must only be done for the grid of the hS that moved out of the region. The distributions of the coupled states need to be fit to the new grid after the shifting. This introduces errors in the calculation of the contractile force if the fitting is not exact. As the total system is very sensitive to the contractile force by its nature, these fitting errors can enhance the error of the continuing integration of the total system and result in a possible failure of the calculation. This problem can be avoided using a homogenous grid and a shifting of the grid that equals its increment. However, the homogenous grid needs to be only over the main area where the states are likely to exist hence the surrounding of the ‘generating interval’. The finesse in this area has to be chosen as small as possible to avoid errors in the calculation as well as big as possible to keep the size of the system reasonably small.

Depending on the velocity of the movement, the coupled states get stretched into the region where they decouple. The faster they are stretched, the more they depart from the ‘generating interval’. So the grid has to be widened for simulations with greater expected velocities of the hS – like activation with compliant springs, relaxation and stretching of the myofibril. Due to the greater number of traces, the dimension of the total system is much bigger in those situations than in normal activation of myofibrils. Hence, the simulations with compliant springs, relaxation and stretching of the myofibril are computational harder than calculations of activations with stiff springs.
2.5.4. Integration of the system

The system of coupled differential equation was integrated using built-in solvers of the MATLAB ODE suite (The MathWorks, Natick, MA). The efficiency of the solvers varied between simulations of different types of problems. Even in the same simulation different solvers were optimal in different time intervals. The most used solvers are explained shortly in the following list.

ode45
This solver for ordinary differential equations (ODE) is a one step solver. It computes the solution $y(t_n)$ while knowing the previous solution $y(t_{n-1})$ at the preceding time point. It is based on an explicit Runge-Kutta (4,5) formula, the Dormand-Prince pair (Dormand and Prince, 1980). This solver is used mostly in simulations of myofibrils with one hS or end-held activations with very stiff external springs.

ode23
This solver is also a one step solver as ode45. It implements the explicit Runge-Kutta (2,3) pair of Bogacki and Shampine (Bogacki and Shampine, 1989). This solver has the advantage to solve moderate stiff problems. This solver was mostly used in the simulations of the model incorporating the formalism of Nishiyama et al. (1977).

ode113
This solver is a multi-step solver of variable order. It needs the solutions at several time points of the known solution to compute the solution of the next step. The algorithm uses the Adams-Bashforth-Moulton PECE solver (Shampine and Gordon, 1975). It is a solver to use when the error tolerances are very stringent and the function describing the differential equation is expensive to evaluate. This solver is used mostly for end-held activations with the Huxley’s 1957 formalism.

ode15s
This solver is a multi-step solver of variable order like ode113. It uses the numerical differentiation formulas (Shampine and Reichelt, 1997; Shampine et al., 1999) and solves stiff problems. Stiff problems are problems where the time scale in the change of the diverse observables in the solution is very disparate. To enhance the efficiency the Jacobian matrix of the system was calculated prior to the integration and passed to the solver. This solver is mostly used for the simulation of relaxation.

The algorithm calling the solvers is sequential in its nature as it stops and restarts after each shift. So the calculation of a simulation can easily be divided into time sequences with different grids and solvers. This construction of the algorithm allows optimization in each phase.

2.5.5. Numerical Stability

In the simulations the lengths $l$ and velocities were scaled in μm, the internal deflection $\eta$ of the contractile element was scaled in 0.01 μm, and the distributions were normalized. Maximal relative errors of the variables $\eta, l, \dot{\eta}$ and $p$ were set to $10^{-9}$ in the ODE solver tolerances for integration error. An
Methods

*a posteriori* error estimates was monitored on the basis of the constraint that all the forces in the hS must be equal, that is, by calculating $f_{i}^{k_{S}} - f_{j}^{k_{S}} \neq F_{0}$ \( i \neq j \). $F_{0}$ denotes the maximal isometric force of the mean hS (see section 2.4.4 for derivation). According to this test, only results with errors less than $5 \cdot 10^{-8}$ during the entire time of integration were considered.

The influence of the discretization of $x_j$ on the output was tested. Discrete variables affect the accuracy of the contractile force and therefore bias the shortening or lengthening of the contractile element. A trade-off between the increment $\Delta x_j$ and computational power is inevitable. To find the fineness necessary to achieve accurate results, end-held activation for low and high external stiffness ($5 F_0/\mu m$ and 30 $F_0/\mu m$) were simulated for different increments $\Delta x_j$ of the grid in $x_j$. For increments $\Delta x_j \leq 2^{-7} \cdot 0.01 \mu m$ the changes in the resulting displacement $\eta$ and the contractile force $f_{CE}$ were less than $4 \cdot 10^{-5} \mu m$ and $3 \cdot 10^{-4} F_0$, respectively. By all means, it has been ensured that the errors due to the discretization of $x_j$ were much smaller than the differences in lengths and forces between the hS at all time points in all simulations.
Chapter 3
Results
The results of the numerical simulations and analytical derivations of the model presented in the methods are shown in this section. The first part of the results covers the activation of a myofibril showing the occurrence and the influence of the half-sarcomere (hS) dynamics and the hS length inhomogeneity. The second part examines the relaxation of the myofibril under different conditions, e.g. titin stiffnesses and muscle types. Two stripped-down models are explicitly solved and analyzed during the phase of relaxation. The third part shows the effect of the hS dynamics and length non-uniformity during stretching of a myofibril on the basis of a three state model. The following section 3.1 is part of an accepted publication (Stoecker et al., 2009). It is slightly modified to correspond to the rest of the text. The third part (section 3.3) is subject of being published in the near future (Stoecker and Dreno, 2010).

3.1. Activation of a myofibril

First, the occurrence of the hS inhomogeneity and dynamics is shown based on the simulation of an end-held isometric contraction. In the second part, the general applicability of the model is illustrated by simulating protocols for determining steady-state force-length and force-velocity relationships.

3.1.1. End-held Isometric Activation

The isometric contraction of a myofibril is simulated keeping a constant external length (Fig. 3.1.1). The parameters in the simulations were adjusted to mimic a myofibril from rabbit psoas. For the detailed description and derivation of the underlying parameters see the methods section (Chapter 2). During myofibrillar activation, the hS shorten and lengthen non-uniformly. We refer to this internal movement of the hS as half-sarcomere dynamics. The non-uniformity is most pronounced in the initial phase of 1 s. This spreading after the initial phase can be depressed if the rates \( f \) and \( g \) among the hS are varied (Fig. 3.1.1B). For this simulation the rates are assumed to be normally distributed with a standard deviation of \( \sigma = 0.1 \) and a constant force capacity \( C = 1 \) whereas the quotient \( f/(f+g) \) was kept constant.

The initial shortening has a direct effect on the force development. The force rise upon activation increases as the external spring constant \( k_{ext} \) increases. Following Stehle et al. (2002b) the rate of force development \( k_{act} \) was determined with a single exponential approximation of the force during activation

\[
 f_{hs}(t) = \left( \tilde{F}_{max} - \tilde{F}_{min} \right) \left( 1 - e^{-k_{act}(t-t_{act})} \right) + \tilde{F}_{min},
\]

where \( t_{act} \) denotes the start of the activation. \( \tilde{F}_{max} \) is the maximum force reached during activation, and \( \tilde{F}_{min} = F(t_{act}) \) is the force prior to activation. A nonlinear least squares fit was performed on the simulated data to determine \( k_{act} \). The external stiffness \( k_{ext} \) of the myofibril strongly affects the activation rate \( k_{act} \) in a nonlinear fashion, resulting in a higher activation rate for stiffer external springs (Fig. 3.1.2A). The shape of the curves is similar for different numbers of simulated hS. If the widths \( \sigma \) of the distribution of the force capacities are enhanced, the activation rates lower. This is mainly because the maximal force during activation is lower for wider distributions of \( C \), so the force is reached faster and therefore the activation rate is higher.
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**Fig. 3.1.1 : Simulation of end-held activation.** Length and force traces of an end-held contraction simulation of four and eight hS in series. Solid lines show the lengths of the hS in the simulated myofibril; black dotted traces show the corresponding force (A) Activation of eight hS with normally distributed force capacities C, and different external stiffnesses $k_{ext}$. Black lines denote a simulation with an external stiffness of $k_{ext}=5$ F$_0$/µm, which corresponds to a “normal” activation experiment with $k_{act}=5$ s$^{-1}$. Grey lines mimic a “length-clamped” experiment with $k_{ext}=31.25$ F$_0$/µm. The initial shortening of the hS depresses the force development (decreases the activation rate $k_{act}$) because shortening hS exhibit a smaller force than isometric or stretched ones. The length spreading only depends on the number of the hS included in the simulation (“softness” of the system). Note that most hS lengths are on the plateau region (1-1.2 µm) of the force-length relation. (B) Activation of four hS with an external stiffness of $k_{ext}=30$ F$_0$/µm. Black lines correspond to a simulation with normally distributed force capacities C. Grey lines are traces of an equal simulation with constant C and normally distributed on- and off-rates f and g among the hS where the individual maximal isometric force is kept constant. Interestingly only a variation in C shows a spreading in the later phase of activation whereas the initial phase is similar for both types of possible variability.

\[ l_{\text{spread}}(t) = |l_{\text{weak}}(t) - l_{\text{strong}}(t)| \] is a simple measure of the non-uniformity internal to a myofibril, where the “weakest” half-sarcomere has the lowest force capacity $C$. The mean shortening and spreading of four hS in a simulation of an isometric contraction depend on the widths of the distributions of the force capacities and the stiffness of the external fixation (Fig. 3.1.3). We define the initial phase of the activation as the time necessary to reach 99% of the maximal force $F_{\text{max}}$. The duration of this phase depends mostly on the external spring constant $k_{ext}$. The mean shortening length of the hS is mainly determined by the external spring. The spreading is mainly determined by the width $\sigma$ of the force-capacity distribution. The mean shortening length stays constant after the initial phase because the external spring only moves when the myofibrillar force is changing in time. Initially, the main spreading increases, largely in the first milliseconds of activation. This first part of the initial phase is more pronounced for wider distributions of force capacities (larger $\sigma$). In the later phase, the spreading continues to grow linearly and more steeply for more widely distributed force capacities.

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The dynamics during activation are described here by the mean value of the absolute hS velocities $v_{\text{hSdyn}}(t) = \sum |v_j(t)| / n$. During the initial phase the hS dynamics can be divided into three parts: First (up to 0.001s), the dynamics exhibits a peak (i), which decreases exponentially (ii) to an offset level which remains constant (iii). The height $v_{\text{hSdyn}}$ of the peak in the first part depends mostly on the distribution of the capacities, and decreases toward zero for smaller standard deviations $\sigma$ (Fig. 3.1.4).

The occurrence of the peak is directly related to the initial spreading of the lengths during the activation. In the second part, the rate of the exponential decay is smaller for more compliant external springs. This is mainly due to the average shortening of the hS, which is more pronounced for more compliant springs. The constant level reached in the third part depends on the distributions of the force capacities, and reaches zero for smaller standard deviations $\sigma$. In summary, the first and third parts depend mainly on the variation of the force capacities, while the second part is determined by the external stiffness.

Fig. 3.1.2: Force development. "End-held contractions" were simulated under different conditions to examine the influence on the myofibrillar force. The solid lines denote the force rise during activation of four hS for different external springs ($k_{\text{ext}} = 5, 10, 15, 20, 15, 30 F_0/\mu m$). The more depleted the force curve the lower the simulated external stiffness. The maximal myofibrillar force decreases for a wider distribution of $C$ (dashed grey line, $\sigma=0.2$, $k_{\text{ext}} = 30 F_0/\mu m$). The inset shows the rate of activation $k_{\text{act}}$ of the myofibrillar force calculated for each simulation. The activation rate depends nonlinearly on the external stiffness, hence the fixation of the myofibril. The filled circles represent simulations of four hS with the external stiffness fixed at $k_{\text{ext}} = 30 F_0/\mu m$, but with variable width $\sigma$ of the force capacity distribution.
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Fig. 3.1.3: Shortening and half-sarcomere dispersion have different origins. Measures of the non-uniform dynamics of four hS in series during end-held contraction simulations are shown, dependent on external stiffness ($k_{\text{ext}}$) and biological variability ($\sigma$) during the first second of activation. (A) Temporal evolution of the shortening. There is more shortening with compliant external springs, whereas the different widths of biological variability $\sigma$ (solid lines) have only a small effect on the overall shortening. The circles denote the end of the "initial" phase where 99% of the individual maximal force is reached. (B) Temporal evolution of the spreading in the initial phase of activation of four hS. The non-uniformity is established in the first milliseconds and subsequently increases in a more or less linear fashion.

Fig. 3.1.4: Effect of attachment and variability on half-sarcomere dynamics during activation. The hS dynamics during the "initial" phase of activation of an end-held contraction simulation of four hS, with an external spring of $k_{\text{ext}} = 30 \text{ F}_0/\mu\text{m}$ (solid lines), $k_{\text{ext}} = 15 \text{ F}_0/\mu\text{m}$ (dash-dotted line), $k_{\text{ext}} = 10 \text{ F}_0/\mu\text{m}$ (dashed line), $k_{\text{ext}} = 5 \text{ F}_0/\mu\text{m}$ (dotted line). The different grey lines denote different standard deviations $\sigma$ of the normal distributions of the force capacities $C$, with an external spring of $k_{\text{ext}} = 30 \text{ F}_0/\mu\text{m}$ (A). The hS dynamics ($v_{\text{WHbry}}$) during the first 10ms of activation. (B) hS dynamics ($v_{\text{WHbry}}$) during the first second of activation. The small circles denote the time points at which the force reaches 99% of its maximal value, which we define as the end-point of the "initial" phase.
3.1.2. Steady-state Characteristics and Steady-state Approximation

The present framework is an excellent tool to study possible effects of hS dynamics on force in classic muscle experiments. Here, two distinct experiments are focused on: (1) the end-held isometric contraction experiment at different initial (mean) sarcomere lengths, according to Gordon et al. (1966b) and Edman et al. (2005), to determine the force-sarcomere length relation, and (2) the isotonic contraction experiment, according to e.g. Granzier et al. (1989) and Edman et al. (2005), to determine the force-velocity relation.

The simulation of the first experimental protocol is quite straightforward. The initial average hS length of the myofibril is defined prior to activation, and the overall length is kept constant during contraction. However, because the slack length of the half-sarcomeres in a myofibril is about 1.1 µm (Telley et al., 2006a) all the lengths below the slack length must be experimentally reached with a controlled shortening maneuver during activation (“after-loaded contraction”). This maneuver is reproduced in the simulations. In the experiments of Edman et al. (2005) a fiber segmental of 20 hS was held constant in length with an accuracy of 0.2% of the segmental length. The control of the length of the segmental excludes the compliance of the external fixation and the “dead” hS. However, it includes a small compliance due to the accuracy of the length measurement of the segmental. For lengths on the plateau and the descending limb of the force-length relation, the initial lengths are in the interval [1 µm, 2 µm]. Therefore, the maximal external extension of the spring for eight hS can be confined to

\[ \Delta x_{ext} \leq 0.002 \sum_i l_i^0 \leq 0.032 \mu m. \]

Thus, the external spring constant of the system can be set to

\[ k_{ext} = F_0 / \Delta x_{ext} \approx 31.25 \frac{F_0}{\mu m} \]

to simulate a clamped situation. The ramp shortening was performed with a constant velocity of 1.0 \( L_{iso} / s \). This ramp velocity was set due to numerical reasons as the individual shortening of the hS should not exceed the maximal shortening velocity (left inset of Fig. 3.1.5). The maximally reached force of eight hS during an activation of 1.5 seconds is piecewise linearly dependent on the final mean lengths (Fig. 3.1.5). For lengths on the descending limb of the force-length relation, the maximum force shows a depression (right inset of Fig. 3.1.5). Gordon et al. (1966b) measured force creep for tensions above optimum length. In the simulations no creep is observed for the simulated lengths.

The maximal force of the whole myofibril upon activation is found to be lower than the theoretical maximal isometric force \( F_0 \) of a myofibril consisting of equal hS with force capacities \( C \approx 1 \) and optimal filament overlap, described by the function \( N(l) \). This is not surprising because the force of the myofibril depends on the weakest fully active hS. The force of this hS is not borne by the passive element. However, theoretical and simulated data can be matched by scaling. Importantly, the edges of the simulated curve are not as sharp as proposed originally by Gordon et al. (1966b). This slight smoothing effect is mainly due to the transient-state description of the myofibril when passing the plateau of the force-length relationship. Near the edges of the plateau some hS lengths are already in the ascending/descending limb, causing a decrease in force from the theoretical values.
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Fig. 3.1.5: Simulated force-length relationship. Comparison of the force-length relationship of a single hS with force capacity C=1 with a myofibril consisting of eight hS in series. The straight line denotes the force-length relationship N(I) based on the experiments of Edman et al. (2005). Crosses are the simulated maximal myofibrillar forces of “length-clamped” (kext =31.25 F0/µm) end-held contractions with eight hS and different initial lengths according to the protocol of Gordon et al. (1966b). The maximal force of the contraction simulations is lower than the force given by the force-length relation in the plateau. Insets: Outlined force the lengths of the hS during a simulation that reaches the lengths of the ascending limb (middle) and the descending limb (upright) of the force-length relation. The dashed line denotes the corresponding force-response. To reach a final mean length smaller than the hS length at slack (1.1 µm), a shortening ramp of 1.0 L0/s was allowed.

To simulate isotonic shortening experiments the experimental protocol of Edman et al. (2005) was followed. An ensemble of eight hS with an initial length of 1.1 µm was activated in a “length-clamped” mode by setting the external spring considerably stiffly (kext=31.25 F0/µm) as described in the previous section. At 0.75 s after activation, the system was switched to force-controlled mode, a predefined force-step was performed, and force was held at that level (F_{step}) for another 0.25 s (see Fig. 3.1.6). Unlike the study of Edman et al. (2005), the simulations needed 0.25 s longer to reach the tetanus. The force step was performed as a steep ramp of 20 ms duration, according to the apparatus of Edman et al. (2005). To determine the velocity of shortening, the slope of the mean hS length was fitted by least-squares regression analysis between 0.1 s and 0.25 s after the start of the force step (0.85-1 s after activation). During this phase, only minor transient acceleration of the length was observed. The resulting velocity was plotted against the performed force-step, and is shown in Fig. 3.1.6. Hill’s (1938) force-velocity curve, obtained from data of Sun et al. (2001) from skinned rabbit-psoas fibers, was perfectly reproduced by the simulations. Interestingly, after switching to force-controlled mode, the hS shorten (more or less) homogeneously during and after the force step. This is due to the fact that the dynamics of hS are decoupled in force-controlled mode (Denoth et al., 2002).
Interestingly, the transient simulations of the force-length and force-velocity relationships match these steady-state relationships. Hence it may be questioned, if the simulated system is in a steady state during activation. State distributions $p$ that do not change with time can be calculated by the original Huxley differential equation for steady states. This equation is much easier to solve because it lacks the complicated PDE for the transient states. In the case of steady states, the occupation of the bound state remains constant. In any case, including transient states, the number of bound states in an hS is calculated by $N_{\text{bound}} = C \cdot N(i) \frac{1}{l_a} \int p(\eta, t) d\eta$. For the strictly isometric case with hS length on the plateau of the force-length relationship, the number of bound states in an hS is simply $N_{\text{isom}} = C \cdot \frac{h}{l_a} \cdot \frac{f_1}{f_1 + g_1}$. The time-course of the ratio of $N_{\text{bound}}$ to $N_{\text{isom}}$ for end-held activation simulations is different for the eight half-sarcomeres in "normal" ($k_{\text{ext}}$ = $5 F_0/\mu m$) and "length-clamped" ($k_{\text{ext}}$ = $31.25 F_0/\mu m$) situations (Fig. 3.1.7). The corresponding force and hS lengths traces are shown in Fig. 3.1.1A. We denote "normal" situations as experiments with activation rates of $k_{\text{act}}$ ~ $5s^{-1}$ in which (minimal) overall shortening of the myofibril during end-held activation is allowed. It is known from experiments (Telley et al., 2006a) that a real myofibril attached to a transducer and length controller comprises sarcomeres that serve as attachments, and exhibit considerable compliance. The transient model allows the calculation of the number $N_{\text{bound}}$ of bound states at each time point during activation for both
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experimental situations. By analyzing the time course of the bound states it can determined if and when any steady-state approximation can be applied. During the initial phase of the activation the number of bound states develops, concomitant with the contractile force. After that initial rise, the number of bound states in the stronger hS develops to an individual constant value, while the number of bound states in the weaker hS decreases to an individual constant value. Strictly speaking, a steady-state approximation is applicable only after a constant number of bound states are reached.

![Fig. 3.1.7: Steady-state approximation.](image)

The number of bound states $N_{\text{bound}}$ in an end-held contraction of eight hS was calculated with an external stiffness of $k_{\text{ext}} = 5$ $F_0/\mu m$ (A, similar to "normal" experiments) and $k_{\text{ext}} = 31.25$ $F_0/\mu m$ (B, "length-clamped" experiments). See Fig. 3.1.1A for the corresponding force response and hS lengths. The number of bound states $N_{\text{bound}}$ of each hS is normalized with the number of bound states in the completely isometric condition $N_{\text{isom}}$ for each hS. The bound states increase with time, resulting in a force increase of the myofibril. If the number of bound states does not change, a Hill-like steady-state approximation of the hS is possible. For "normal" experiments (left), the steady-state approximation holds only after 2.14s of the shown activation period because the third weakest hS has a changing number of bound states. For the "length-clamped" experiment, the approximation holds after 1.03s, but lasts only until 2.2s, when the weakest hS is stretched into the descending limb of the force-length relation.

We focus on the time point after which bound states of all half-sarcomeres remain nearly constant ($|N_{\text{bound}}| / N_{\text{isom}} \leq 0.02 s^{-1}$). Under "normal" conditions of end-held contractions, this time point is relatively late (2.14 seconds after activation). In the "length-clamped" experiment, a steady-state approximation can be applied soon after activation (1.03 seconds after activation). However, if the activation in the "length-clamped" condition is longer than 2.2 seconds, the weakest hS elongates into the descending limb where filament overlap decreases, causing the overall possible bound states also to decrease. Interestingly, for simulations under "normal" conditions (Fig. 3.1.1B), the strongest hS tends to shorten on the ascending limb of the force-length relation at the same time as the weakest tends to stretch on the descending limb, whereas in the "length-clamped" experiments, only the weakest hS tends to stretch on the descending limb. In summary, there is only a short phase in an end-held contraction for which a simple steady-state relation of force and velocity (Hill’s curve) approximates the behavior of the contractile element. An exception to this is the case of a "length-clamped" system with very few half-sarcomeres.
3.1.3. Activation of multistate systems

End-held isometric activations of a myofibril of a frog sartorius muscle containing four hS were simulated. The chosen force capacities of the hS equals to $C = (0.868, 0.968, 1.032, 1.132)$ which is the result of a normal distribution with mean $C=1$ and standard deviation of $\sigma=0.1$. The corresponding force rise and length changes were calculated during the first 100 ms after activation. The hS spread faster in the simulation containing 2 states than in the model containing 3 states within the first milliseconds of activation (Fig. 3.1.8). The force-rise is very similar for both models being slightly faster for the 2-state model after the initial activation.

The stiffer external fixation influences the force rise and the overall shortening of the hS in a simulation incorporating a 3 states model of Nishiyama (Fig. 3.1.9A). This corresponds to the findings in the previous section 3.1.1 simulating rabbit psoas myofibrils with Huxley’s model. In contrary to the previous findings of simulations including a two states model (Fig. 3.1.3), the spreading of the hS after 100 ms of activation is unchanged by the stiffer fixation. There are several findings that propose a $Ca^{2+}$ induced increase of the passive stiffness of titin through interaction with actin or by a calcium-dependent conformational change in the PEVK region (Labeit et al., 2003; Li et al., 1995; Linke et al., 1997). Therefore, the titin stiffness may increase upon activation. Assuming that the kinetics of the titin activation is very fast, the activation of a myofibril is simulated with a ten times stiffer titin during the whole activation period (Fig. 3.1.9B). This results in an up-shifted force as the passive force at the start is
10 fold higher than the simulation with normal stiffness of titin. The force-rise and the overall shortening of the hS are not influenced by the higher stiffness whereas the spreading of the hS is slightly reduced.

![Graph A](image1.png) ![Graph B](image2.png)

**Fig. 3.1.9 : End-held activation of a model containing three states.** Simulations of a frog sartorius myofibril based on the model of Nishiyama with four hS are shown during an isometric contraction. (A) The black solid and dotted lines denote the hS lengths and the force of a simulation with a stiff external spring ($k_{\text{ext}} = 30 F_0/\mu \text{m}$). The grey lines are the result of a similar simulation with a weaker external fixation ($k_{\text{ext}} = 10 F_0/\mu \text{m}$). The hS shorten less and the force rises faster for a stiffer external fixation than with a weaker external spring. (B) The black lines correspond to a similar simulation as in A with a weak external spring but with a 10-fold stiffer parallel element which mimics a Ca$^{2+}$ activated titin. The grey lines denote the equal simulation as the grey lines in A.

### 3.1.4. Activation with different coexistent variabilities

In the previous shown simulations of activation, only a single parameter was varied for analyzing the influence of certain variabilities on the hS dynamics and the myofibrillar force. However in nature, many structures and processes can vary in between the hS. We focus on the variability of three main characteristics of hS: force capacity, kinetic rates and parallel element. The performed simulation of a myofibril including the three proposed variabilities is based on Huxley’s two states model adapted for frog sartorius muscle (Fig. 3.1.10). The force capacities, the kinetic rates ($f_i$, $g_i$) and the titin stiffness of the individual hS are normally distributed around a mean value analogous to the distribution of the force capacities described in the methods section. The standard deviation of the distribution of the force capacities is set to $\sigma=0.05$. A smaller standard deviation of $\sigma=0.1$ was chosen for the distributions of the kinetic rates and the stiffness of the titin. Due to the variation of the parallel stiffness the starting lengths of the hS differ greatly (Fig. 3.1.10). At the start of the activation the weaker hS lengthen shortly. This is followed by an overall shortening of the hS during the force rise. The hS spread only little during the activation as the hS length inhomogeneity is mainly built up due to the variable starting lengths and the distribution of the force capacities is narrow. The shortening and spreading of the hS is similar to the previous results of activation with the single variation in the force capacities.
3.2. Relaxation of a myofibril

A deactivation of the myofibril after 0.3 s of activation is mimicked while keeping the total length of the myofibril constant during the activation-relaxation cycle. A relaxation of a system of four hS with an external stiffness of \( k_{ext} = 30 \frac{F_0}{\mu m} \) is simulated based on the 2-state formalism of Huxley adjusted for frog sartorius muscle with two distinct elastic stiffness of titin (Fig. 3.2.1). The lower values of the chosen stiffness of titin corresponds to the normal stiffness that is experimentally found in the relaxed state of a myofibril according to the findings of Linke (2000), Minajeva et al. (2002) and Kellermayer et al. (1997). The second chosen value of the stiffness is ten-fold higher than the formerly described stiffness. As mentioned in the previous paragraph, such a high stiffness may be present due to activation of a myofibril (Pinniger et al., 2006). In both cases of the stiffness, the hS lengthen sequentially, starting with the longest hS, that is also the weakest as it has the lowest force capacity \( C=0.868 \). If we define the time point of relaxation of a hS as the time where it has the greatest lengthening speed we can give exact relaxation times for each hS. So the time sequence of the relaxation with normal stiffness is 0.018 s, 0.099 s, 0.103 s, 0.106 s after deactivation of the myofibril. However, the higher stiffness of the titin filament influences the relaxation duration and the time sequence of the relaxation (0.019 s, 0.063 s, 0.068 s, 0.073 s). In the very first phase of the relaxation, the stronger hS shorten to compensate the lengthening of the weakest hS. This effect is reversed when the weakest hS is relaxed and shortens back to its initial lengths. In this phase the stronger hS are allowed to lengthen and can so relax. At the end of
activation the weakest hS is already stretched into the descending limb of the force-length relationship (1-1.125 µm) facilitating its lengthening. In the case of the higher titin stiffness, the total lengthening of the weakest hS during relaxation is less pronounced than in the case of normal stiffness. This allows the stronger hS to lengthen earlier and accelerates their relaxation.

Fig. 3.2.1: End-held activation-relaxation cycle of a frog sartorius myofibril. Simulations of an activation and relaxation of four hS in a myofibril from a frog sartorius muscle with a stiff external spring (kext = 30 F0/µm). The individual lengths of the hS are shown as continuous lines while the normalized force of a myofibril is displayed as broken lines. After 0.3 s of activation the myofibril was deactivated. The contractile elements of the hS are based on the transient Huxley formalism with 2 states. The black lines denote a simulation with normal stiff titin whereas the grey lines correspond to a simulation with a 10-fold higher stiffness of the parallel element. During activation of the system with normal titin the force drops after reaching a maximum at 0.075 s because the weakest hS lengthens into the descending limb of the force-length relationship. The hS relax sequentially from weak to strong hS within a time span of 0.1 s. The force reaches a plateau at 2.5% of F0 following the first rapid decay after deactivation. During this plateau the weakest hS is held mainly by the titin filament. During activation of the system with the higher stiffness the force is only slightly depressed after reaching its peak at 0.1 s. The higher stiffness of the titin filament reduces the lengthening of the weakest hS after deactivation. Furthermore it shortens the time span of relaxation. Note the different baselines of the forces for different stiffness of titin. This is due to the normalization F0 neglecting the contribution of titin.

The time duration of the relaxation is different in fast and slow muscle types (Stehle et al., 2002b). Myofibrils of rabbit psoas muscle with four and eight hS are simulated according to the previous section 3.1.1. The duration of the relaxation is significantly longer in the slow psoas muscle from rabbits than in the fast sartorius muscle from frogs. However, the sequence as well as the length trajectories does not change qualitatively from simulations with four hS of sartorius muscle (Fig. 3.2.1) and psoas muscle (Fig. 3.2.2). The more hS are included in series in the system the more distinguishable gets the temporal sequence of relaxing hS. The sequence still follows the order from weak hS (low force capacity) to strong hS (high force capacity). Furthermore it shows the same characteristics as in frog where at the start of
the relaxation only the weakest is lengthening and the stronger shorten. Note that the weakest hS is still on the plateau (1-1.2 µm) of the force-length relationship at the end of activation.

**Fig. 3.2.2 :** End-held activation and relaxation of a rabbit psoas myofibril. Simulations of activation-relaxation cycles of a myofibril from rabbit psoas consisting of four and eight hS with a stiff external spring ($k_{ext} = 30 F_0/\mu m$). The solid lines are the hS lengths and the dotted lines the resulting forces. The black lines correspond to a simulation of four hS whereas the grey lines are the results of a simulation with eight hS. The sequential lengthening of the hS is similar to the faster muscle of frog while the duration of the relaxation is slightly enhanced. Note that the descending limb of the force-length relationship in rabbit psoas starts at 1.2 µm eliminating a force depression during activation. The more hS are included the shorter becomes the time lag between the relaxation of the first and the second hS. The simulation of the system with eight hS was aborted shortly before the lengths are equalized because the error in the later phase was greater than the accepted level.

An activation-relaxation cycle of a frog sartorius muscle is simulated based on Huxley’s 2-states model with a tenfold higher stiffness of titin than normal and two different compliant external stiffness of $k_{ext} = 10 F_0/\mu m$ and $k_{ext} = 30 F_0/\mu m$ (Fig. 3.2.3). It can be seen that the shortening of the hS during the initial phase of activation is more prominent in the simulation with a more compliant external spring. This shortening depresses the force rise as described in the previous section 3.1.1. The weak external spring influences slightly the first phase of the force decrease after deactivation where the force has a faint first shoulder. Furthermore the weaker spring accelerates the relaxation of the system resulting in a relaxation sequence of 0.017 s, 0.056 s, 0.059 s, 0.063 s. Interestingly a common feature of all the relaxation simulations is the long shoulder of the force after the weakest relaxed. During the period of this shoulder the weakest hS is mainly held by the passive titin filament whereas the stronger hS still produce active force. Such a remaining force can also be seen in relaxation experiments on myofibrils (Telley et al., 2006a).
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The biological variability can also be assumed in the kinetic cycle. To investigate the influence of such a variability, a frog sartorius myofibril containing four hS with a stiff external fixation \( k_{ext} = 30 \, F_0/\mu m \) is simulated during an end-held activation and relaxation (Fig. 3.2.4). The kinetic cycle was modeled with Huxley’s 2-states formalism while normally distributing the Huxley constants \( f_i \) and \( g_i \) so that the isometric forces \( C \frac{k_{ext}}{f_i} \frac{f_i}{f_i + g_i} \) of all hS are equal. The distribution of \( f_i \) and \( g_i \) were conducted in the same procedure as the previously described distribution of the force capacities \( C \) (see Methods for details). The standard deviation of the normal distribution of the kinetic rates was set to \( \sigma = 0.1 \). The force capacities \( C \) of the individual hS were equally set to 1. Furthermore the elastic titin stiffness was set to a ten-fold higher value than the normal assumed stiffness in non-activated myofibrils. This higher elastic stiffness of titin was reached over an increasing ramp in the first 100 ms of activation starting from the value of the normal stiffness. This ramp was inversed in the first 100 ms after deactivation returning the stiffness to a normal value at 0.4 s after the initial activation. Additionally, the viscous part of the titin was increased over the total time of the simulation while multiplying the viscous function of the parallel element minus its offset, \( f_3(v) \cdot I \), by a factor of 100. A higher viscosity resulted in a reduced movement and hence a lower effort of computation. Like in the case of the simulations of rabbit psoas myofibrils (Fig. 3.1.1B), the hS spread only at the start of the activation and shorten afterwards. After 0.1 s of activation the lengths of the hS are merely isometric where the shortest hS has the highest...
kinetic rates. In contrary to the simulations with varied force capacities, the shortest hS relaxes first. About 100 ms after the relaxation of the first, the shortest at this time relaxes. This sequence is continued to the hS with the lowest kinetic rates giving an inverse relaxation sequence in comparison to the previous simulations with varied force capacities. Additionally the total time of sequential lengthening of the hS in the present calculation is longer than in the previous simulations with varied force capacities.

**Fig. 3.2.4**: Activation‐relaxation cycle of frog myofibril with varied kinetic rates. Simulation of four hS coupled with an external fixation of $k_{ext} = 30 \text{ F}_0/\mu\text{m}$. The kinetic rate constants $f_1$ and $g_1$ in the Huxley model are varied with a standard deviation of $\sigma = 0.1$ analogous to the force-capacities. The titin stiffness was increased by a factor of 10 in the first 100 ms of the activation and decreased in the same manner at the end of the activation. The lengths of the hS are displayed as solid lines and the force is shown as dotted line. The longest hS correspond to the hS with the lowest rates.

### 3.2.1. HS dynamics and length non-uniformity during relaxation

According to the precedent section 3.1.1 on activation of psoas myofibrils, the hS dynamics and the hS length non-uniformity can be calculated. The hS dynamics can be described by the previously defined measure $v_{hS_{dyn}}(t) = \sum_j |v_j(t)| / n$. To express the hS length inhomogeneity we calculated the standard deviation of the lengths of $n$ hS at every time point, $\sigma_j(t) = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (l_i - \bar{l})^2}$ with $\bar{l} = \frac{\sum_{i=1}^n l_i}{n}$.

Based on these expressions, the hS dynamics and the hS length inhomogeneity of two relaxations corresponding to Fig. 3.2.1 are calculated. Immediately after the start of the activation of the system, the hS length inhomogeneity increases strongly (Fig. 3.2.5). After these first milliseconds of activation, the hS length non-uniformity continues to grow, although slower than in the initial built-up and almost linear. At the start of the deactivation, the hS length inhomogeneity boosts strongly until the weakest
stops to lengthen. In the second phase of the deactivation the hS length inhomogeneity drops to zero as the hS length relax to their initial lengths. The hS non-uniformity is qualitatively similar for both stiffnesses of titin. The hS length inhomogeneity of the simulation with normal stiffness of the titin is always broader than with a ten-fold higher stiffness. Furthermore, the hS inhomogeneity for the stiffer titin case does not increase as much and drops faster during deactivation. The hS dynamics has three characteristic peaks during an end-held activation-relaxation cycle. The first peak occurs at the start of activation, the second at the start of the deactivation and the third at the end of the relaxation. The first peak corresponds to the initial spreading of the hS, the second peak to the relaxation of the weakest hS and the third peak to the relaxation of the three stronger hS. Also the peak values of the hS dynamics are greater in the simulation with normal stiffness of titin compared to the ten-fold stiffer titin. However, the last peak in the hS dynamics appears earlier in the simulation with higher stiffness reflecting the shorter total time of relaxation.

![Graph](image.png)

**Fig. 3.2.5 : hS length non-uniformity and hS dynamics during activation-relaxation.** The length inhomogeneity is expressed with the standard deviation of the lengths. The dynamics of the hS is calculated while summing the absolute velocities of the hS. The hS dynamics and inhomogeneity are calculated from the two simulations in Fig. 3.2.1 of an end-held activation and relaxation with four hS coupled to a stiff external spring (k_{ext} = 30 F_0/\mu m). The black lines match to the simulation with a normal stiffness in the titin filament whereas the grey lines correspond to the simulation with a ten-fold higher stiffness in the parallel element.

### 3.2.2. Steady states during relaxation

Based on the explanations of section 3.1.2 the number of bound states can be calculated for the simulations of relaxation. The time course of these numbers show if an individual hS or the whole system of hS is in a steady state so that the contractile elements could be approximated with the steady force-velocity relationship of Hill (1938). The calculated numbers of bound states normalized with the bound states in a strict isometric condition are shown for three different relaxations of four hS (Fig. 3.2.6). The solid lines correspond to the relaxations of Fig. 3.2.1. Both simulations reflect the activation and relaxation of a frog sartorius muscle with a stiff external fixation (k_{ext} = 30 F_0/\mu m). The grey lines are
the results of a simulation with a tenfold higher stiffness of titin. The dashed lines match with the activation and relaxation of a myofibril of a rabbit psoas muscle consisting of four hS (Fig. 3.2.2). Like in the previous results of activation (section 3.1.2), the bound states increase with time, resulting in a force increase of the myofibril. In all simulations the weakest and the strongest never reach steady states with $N_{\text{bound}} = 0$ during the total length of the activation-relaxation cycle. After 0.1s (frog) or 0.75s (rabbit) after the start of activation the two hS close to the mean force capacities reach steady states until the end of activation. At the start of the deactivation of the systems, the bound states of all hS are dropping immediately. The drop of the bound states of the three stronger hS is caught by a intermediate steep linear decrease that differs for each simulation (Fig. 3.2.2). All the systems are in transient condition during deactivation until the force of the myofibril reaches its permanent level before activation. As the weakest hS relaxes first and its length is then only determined passively, it reaches the steady state of no bound states ($N_{\text{bound}} = 0$) before the other hS. After a time shift that is proportional to the relaxation time, the rest of the hS decrease the bound states almost simultaneously.

Fig. 3.2.6: Steady-states during activation-relaxation. The number of bound states $N_{\text{bound}}$ in an end-held contraction-relaxation cycle of four hS was computed with an external stiffness of $k_{\text{ext}} = 30 \text{ F}_0/\mu\text{m}$. The black traces correspond to a simulation with normal titin, the grey lines to a simulation with a ten-fold higher titin. See Fig. 3.2.1 for the corresponding force response and hS lengths. The dotted lines correspond to the activation and relaxation of a myofibril of rabbit psoas as shown in Fig. 3.2.2. The number of bound states $N_{\text{bound}}$ of each hS is normalized with the number of bound states in the completely isometric condition $N_{\text{isom}}$ for each hS.
3.2.3. Analytical solutions for simplified models

To investigate the behaviour of the hS length just after deactivation we can analyze a down-stripped model consisting of a contractile element based on a Huxley-like 2-state model and an external linear elastic spring (Fig. 3.2.7). The force capacity of the contractile element is set to C=1 and its length \( l(t) \) is assumed to be on the plateau of the force length relationship.

During the relaxation the on-rate is switched off and so the differential equation is reduced to

\[
\dot{p} = -g(\eta) \cdot p \quad \text{while} \quad \eta(t) = x + l(t) - l(0)
\]

is the deflection of the cross-bridges. This differential equation has the solution

\[
p(t) = p_0 \cdot e^{-\int g(\eta) \, dx}.
\]

The hS are near steady-state at the end of activation so an isometric steady-state distribution \( p_0 \) of the bound states is assumed at the start of deactivation at \( t=0 \), hence \( p_0 \) is constant for \( x \in [0,1] \). A single contractile element coupled with an external spring does simply lengthen during activation so we only use the off-rate \( g(x) \) for positive \( x \). Solutions for two definitions of the off-rate \( g(x) \) are derived. The simplest definition of the off-rate is assuming it constant in the positive branch. Explicitly solvable with implicit functions is the case of the linear increasing off-rate as it was proposed by Huxley (1957) in his original work.

Fig. 3.2.7 : Stripped-down model for analytical calculations. Top: sketch of the model to derive analytical results during relaxation. A contractile element is coupled with a linear elastic external spring. Bottom: functions of the kinetic off-rates of the two solutions: strain-independent with a constant transition rate and strain-dependent with a piecewise linear transition rate.
Constant off-rate \( g(x) \)
For sake of simplicity a constant off-rate we assume \( g(x) = g_1 \) for \( x \in [0, \infty[ \). The force of the cross-bridges is defined as a linear elastic function depending on the distortion \( f_{\text{CB}}(\eta) = k_{\text{CB}} \cdot \eta \). Defining the contractile force \( f_{\text{CE}}^0 = f_{\text{CE}}(0) = \frac{k_{\text{CB}}}{l_A} \cdot \frac{p_0}{2} \) and the length \( l(0) = l_0 \) at the start of deactivation we can express the force of the hS during relaxation using Eq. (2.3.7).

\[
f_{\text{CE}}(t) = \frac{k_{\text{CB}}}{l_A} \int \delta(t) \cdot \eta \cdot d\eta \\
= 2 \cdot f_{\text{CE}}^0 \left( \frac{1}{2} - l_0 + l(t) \right) e^{-g_1 t}
\]

With the total length of the system \( L_{\text{tot}} \) (length of the hS plus external spring) and the external spring constant \( k_{\text{ext}} \) we get the relations \( f_{\text{CE}}(t) = f_{\text{ext}}(t) = k_{\text{ext}} \left( L_{\text{tot}} - l(t) \right) = f_{\text{CE}}^0 + k_{\text{ext}} \left( l_0 - l(t) \right) \). Using this expression for the contractile force we can derive the trajectories of the length \( l(t) \) and the force \( f_{\text{CE}}(t) \).

\[
l(t) = \frac{1 - e^{-g_1 t}}{k_{\text{ext}} - 2e^{-g_1 t}} + l_0 \\
f_{\text{CE}}(t) = f_{\text{CE}}^0 + k_{\text{ext}} \left( l_0 - l(t) \right)
\]

Linear off-rate \( g(x) \)
In his original work, Huxley (1957) proposed rate functions that depend linearly on the distortion \( x \) of the cross-bridge. So the off-rate can be defined as

\[
g(x) = \begin{cases} 
  g_2 & \text{for } x < 0 \\
  g_1 \cdot \xi & \text{for } x \geq 0
\end{cases}
\]

Like in the previous case of a constant off-rate, we assume the cross-bridges and the external spring to be linear elastic, resulting in \( f_{\text{CB}}(\eta) = k_{\text{CB}} \cdot \eta \) and \( f_{\text{ext}}(t) = k_{\text{ext}} \left( L_{\text{tot}} - l(t) \right) \) respectively. With the definitions \( \eta(t) = x + l(t) - l_0 = x + \xi(t) \) and \( Z(t) = \int_{0}^{t} \dot{\xi}(\tau) d\tau \) we can derive an expression for the contractile force

\[
f_{\text{CE}}(t) = \frac{k_{\text{CB}}}{l_A} \cdot \frac{p_0}{2} e^{-g_1 t} \int_{0}^{t} e^{-g_1 \tau} \eta d\tau \\
= \frac{k_{\text{CB}}}{l_A} \cdot \frac{p_0}{g_1 t} e^{-g_1 t} \left[ Z(1 - e^{-g_1 t}) - (e^{-g_1 t}(1 + \frac{1}{g_1 t}) - \frac{1}{g_1 t}) \right]
\]
Recalling the definition of the initial contractile force \( f^0_{CE} = f_{CE}(0) = \frac{k_{CE}}{l_0} \cdot \frac{p_0}{2} \) and the condition that the external force must be equal to the contractile force, we can formulate the contractile force with a different expression

\[
f_{CE}(t) = f_{ext}(t) = k_{ext} (l_{ext} - l(t)) = f^0_{CE} + k_{ext} (l_0 - l(t))
\]

\[
= f^0_{CE} + k_{ext} \ddot{Z}(t) = f^0_{CE} + k_{ext} \dddot{Z}(t)
\]

Equating both expressions for the contractile force leads to a linear differential equation with the solution

\[
Z(t) = \frac{f^0_{CE} (g_1 t - 2)}{g_1 k_{ext}} + \frac{\Omega(\omega)}{g_1}
\]

With the definition \( \omega \equiv \frac{2 f^0_{CE}}{g_1 k_{ext}} (1 - e^{-g_0 t}) \cdot e^{f^0_{CE} (g_1 t - 2) / k_{ext}} \) and \( \Omega(\omega) \) being the Lambert’s function (the inverse of \( f(\omega) = \omega e^\omega \)). Having the solution for \( Z(t) \) we can explicitly express the functions of the length \( l(t) \) and the contractile force \( f_{CE}(t) \)

\[
l(t) = l_0 + \dot{Z} = l_0 + \frac{f^0_{CE} g_1 t - \frac{k_{ext}}{g_1} \Omega(\omega) (1 - e^{-g_0 t} + g_0 t) - \frac{1 - e^{-g_0 t}}{1 - e^{-g_0 t}}}{g_1 k_{ext} t(1 + \Omega(\omega))}
\]

\[
f_{CE}(t) = f_{ext}(t) = f^0_{CE} - k_{ext} \dddot{Z}(t)
\]

The solutions for the lengths and the forces of the two cases of a constant and a linear off-rate are calculated for three different external stiffness (\( k_{ext} = 0.1 F_0/\mu m \) dotted lines, \( k_{ext} = 1 F_0/\mu m \) dashed lines, \( k_{ext} = 10 F_0/\mu m \) solid lines). The initial length of the hS and the initial force of the contractile element are set to 0 \( \mu m \) and 1/\( F_0 \), respectively. During the relaxation the forces of the contractile element drop while the hS elongates to a distinct length (Fig. 3.2.8). Due to the absence of the parallel element the hS does not shorten back to its starting length at the end of the relaxation. The lengthening of the hS depends strongly on the chosen stiffness of the external spring. Furthermore in the case of a linear off rate, the hS relaxes with a greater velocity than the hS calculated with the constant off-rate.

The contractile element is elongating to a distinct value. This final length after relaxation depends strongly on the external stiffness. The forces are dropping more rapidly for stronger external springs. The elongation of the hS for stiffer external springs is comparable to the solution with constant-off rates but less steep. In the beginning of the calculation, the drop of the force is slower than in the simulation with constant off-rate. However, in the later phase of the calculation the force drops more pronounced resulting in a clearly visible force shoulder for soft external springs.
3.3. Stretching of myofibrils

To understand the effect of hS dynamics during stretching of a myofibril stretching of an activated myofibril of frog sartorius muscle is simulated. This allows investigating the underlying mechanisms of stretch induced force enhancement in myofibrils. A moderate slow stretch (v=0.1 $L_{\infty}$/s per hS) with an amplitude of 0.1 $L_{\infty}$ is simulated based on the 3-states model of Nishiyama (1977). To mimic the proposed activated titin (Pinniger et al., 2006; Telley and Denoth, 2007) we increased the titin stiffness during the first 100 ms of activation to a level of a ten-fold higher passive stiffness. To reduce the computational effort the viscosity of the system was enhanced in comparison to the previous simulations while setting the viscous function in the parallel element to $f_{\eta}(\dot{t}) = 2 / \pi \cdot \arctan(\dot{t}) + 1$. Note that the region of optimal filament overlap for frog sartorius muscles starts and ends at 1 µm and 1.125 µm, respectively. This region of optimal overlap is marked in the following figures as a grey area.

3.3.1. Different amplitudes

A system of four hS coupled to an external spring of $k_{ext} = 30 F_0/\mu m$ was simulated for different stretch amplitudes keeping the same stretching speed v=0.1 $L_{\infty}$/s per hS with the above mentioned conditions for the parallel element. During stretch all the hS lengthen increasing the force in the beginning of stretch. After the first 200ms of stretching, the force reaches a plateau that slightly depresses in the run of the further stretching when the hS lengthen into the descending limb of the force-length relationship that starts at 1.125 µm (Fig. 3.3.1). At the start of the stretching the hS change their speed so that all hS lengthen different. This change of the speed is almost immediate and leads to almost steady stretching speeds. At the end of the stretch the reached total length is held constant and the hS reduce their...
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lengthening speed or even shorten again. During this phase the force drops exponentially to a new steady level. The difference between this new steady force after stretch and the corresponding force obtained at the same length for an isometric contraction is called residual force enhancement or simply RFE (Herzog et al., 2006a). Interestingly the simulation with stretching amplitude of 10% $L_0$ showed a lower steady force after stretch than before stretch hence a slight force depression due to the stretching. In the other cases of the shorter stretching amplitudes the difference of the steady forces before and after stretch are negligible. However, the force of a corresponding isometric contraction with a length equal to the final length after stretching is lower than the steady force after stretch as it lies on the descending limb of the force-length relationship. This leads to a positive RFE in any case. The smaller stretching amplitudes have a small influence on the speed after stretching of the stronger hS. For smaller stretching amplitudes, the strongest hS shortens slightly less after the stretch whereas the two hS with the middle lengths lengthen slightly less.

Fig. 3.3.1: Moderate slow stretching of a frog sartorius myofibril with different stretching amplitudes. The movement and force of four strongly coupled hS was simulated during activation and stretch with a stretching velocity of 0.1$L_0$/s. The black lines correspond to the hS lengths of a simulation with stretching amplitude of 0.1$L_0$. The grey lines are results from similar simulations with lower stretching amplitudes (0.05$L_0$ and 0.03$L_0$) and the dotted lines are the corresponding forces. The hS lengths with optimal overlap of actin and myosin are marked as the grey area. Thin dotted lines denote the forces of an end‐held contraction with starting length that match the targeted mean length after the corresponding stretch. The lower arrows point to the steady forces during these isometric contractions whereas the upper arrows point to the steady forces after stretch to corresponding lengths. Note that the compliance of the titin element was increased linearly in the first 100ms of activation to the ten-fold higher stiffness compared to the relaxed state.
3.3.2. Initial lengths

A stretch of a myofibril containing four hS with a starting length of 1 µm is simulated (Fig. 3.3.2). The stretching speed and amplitude is set to 0.1 $L_0$/s and 5% $L_0$ respectively. In contrary to all other shown simulations of stretching, the higher stiffness of the parallel element is set in this simulation at the start of the activation hence without the continuous activation of the titin stiffness during the first 100 ms.

The value of the steady force before stretch is shifted down for the calculation with smaller starting lengths as most of the hS operate outside the region of optimal filament overlap (grey zone). During the stretch the force of the system with hS starting at 1 µm is continuously rising while the system with starting length 1.1 µm reaches a plateau after 200 ms of stretching. In the simulation with starting length 1.1 µm the steady force after stretch is higher than the steady force before stretching. This reflects the stretching of the hS operating in the ascending limb onto the plateau of the force-length relationship hence increasing the filament overlap. In the simulation with higher starting lengths the hS are stretched from the plateau to the descending limb of the force-length relationship. However, the hS starting at 1.1 µm spread generally more than the hS starting at 1 µm. This is especially obvious after the stretching where the strongest hS in the simulation with the longer starting length shortens more than the strongest hS of the other simulation.

![Fig. 3.3.2: Stretching of myofibrils with different starting lengths.](image)

Two simulations of a frog sartorius myofibril containing four hS are shown. The black lines are the result of a simulation with similar conditions as shown in Fig. 3.3.1 but with a smaller starting length of the hS (1 µm). The distance of the stretching was set to 5% $L_0$. As a reference the simulation of Fig. 3.3.1 is illustrated as grey lines. The labeled arrows point to the corresponding forces.
3.3.3. Single hS

Stretch simulations with a single hS coupled with an external spring were performed to exclude the effect of hS inhomogeneity. The settings within the hS were the same as described before in section 3.3.1 but with a four times stiffer external spring ($k_{ext} = 120 F_0/\mu m$) to normalize the shortening per hS. The force capacity of the single hS was set to $C=1$. The system was stretched with different speeds and amplitudes (Fig. 3.3.3). For comparison with the previous results, a system containing four hS was plotted in the same figure. The maximal isometric force before the stretching is higher in the system of four hS. This is due to the fact, that the system of four hS produces about the same force as the nearly isometric hS therein with a force capacity of $C=1.032$. Whereas the force of the small system with only one hS reaches only the isometric force of the single hS with a force capacity of $C=1$. The force during stretch rises more slowly than the corresponding force of a system with four hS for stretching amplitude of 5% $L_0$ and a stretching speed of 0.1 $L_0/s$. The force of the small system increases over the total duration of the stretch in contrary to the bigger system. Also the force decay after stretch is slower for the smaller system. When the hS leaves the region of optimal filament overlap, the force rise declines. After the stretching, the steady force reaches about the same value as before stretching. This can also be seen in the simulation with a higher stretching speed of 0.2 $L_0/s$ and the same stretching amplitude. Nonetheless, the force increase during stretch is much greater for faster stretching. If the decoupling rate from the strongly bound state to the unbound state is ten times decreased (low $k_3$), the strongly bound state has a longer mean lifetime and hence the force rises much higher than with normal decoupling rates. However the force depression happens at the same time as with normal decoupling rate as this fact is determined by the overlap of the filaments and hence by the length of the hS. Also the force decay after stretch is much slower for a lower decoupling rate.
3.3.4. Extended coupling region

The effect of increasing the coupling length of the myosin heads on the actin sites to $d_{\text{att}}=74\text{nm}$ were studied while simulating active stretches for systems containing single hS (Fig. 3.3.4) and four hS (Fig. 3.3.5). All simulations were performed with a stretching speed of $v=0.1 \, L_0/s$ and a stretching amplitude of 5% $L_0$. Both normal and ten times lower decoupling rate were simulated and compared to previous simulations with a regular coupling region of $d_{\text{att}}=36.5\text{nm}$. For normal decoupling rates $k_3$ the exponential force rise during the initial phase of the stretch rapidly reaches a plateau. If the coupling region is enhanced, the exponential force rise depresses little slower during the stretch. After stretch both forces decrease at similar exponential rate to the same level which is slightly higher than the force before stretch. This steady force level after stretching is faintly increased to the force of an end-held contraction at the same length at this time point. As seen in the previous section, for lower decoupling rates (low $k_3$) the forces rise much higher than with normal decoupling rates. This effect is even increased while extending the coupling distance as the force rises continuously during the stretching period without reaching a plateau. In the latter case the force is more than 200% increased during the stretch even the hS is stretched into the descending limb of the force-length relationship. Comparing the hS lengths of the simulations having a longer attachment distance with the results in the previous
section (Fig. 3.3.3), the hS tend to be more inert. This can especially be seen at the end of the stretch where the hS still lengthen even the total length of the system is held constant.

Fig. 3.3.4: Active stretching of a single hS with longer coupling region. The black lines are simulations similar to Fig. 3.3.3 but with a longer attachment distance of the myosin heads where the myosin heads are allowed to couple with the actin filament ($d_{att}=74nm$). Simulations with different decoupling rates ($k_3$) from the strongly coupled state to the uncoupled state are shown in the graph. As a reference the forces of corresponding simulations with a shorter attachment distance ($d_{att}=36.5nm$) are shown as thick grey dotted lines.

Similar effects on the force and lengths can be seen in a simulated system of four hS (Fig. 3.3.5). The force rises continuously during stretch if the coupling region is elongated and the coupling rate is decreased. The force nearly triples during stretch when the stretching speed is doubled to $v=0.2 \frac{L_0}{s}$. However, the forces in all simulations in Fig. 3.3.5 have comparable exponential force decays independent on their value at the end of the stretch. After stretch the forces of all simulation reach similar values which are only marginal higher than the force of an end-held contraction at the same system target length (thin dotted lines). Interestingly all the hS lengths of the system elongate after stretch and slowly decrease the speed until they spread as before the stretch. The strongest hS still rests in the region of optimal overlap. The hS lengths of the reference end-held contraction at target system length spread stronger during the simulation than the lengths of the stretches. The lengths of the end-held contraction with a low decoupling rate (thin black dotted lines) are spreading less than the comparable end-held contraction with normal decoupling rate (thin grey dotted line).
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3.3.5. Changes of the state distributions

The simulations of the stretches were based on a multisegmental model incorporating a kinetic cycle of three states according to the model of Nishiyama (1977). See methods section for detailed description of the formalism. The kinetic state 1 in this formalism denotes a weakly bound state of the cross-bridge between actin and myosin. The distribution of the probability to find such a weakly bound cross-bridge at position $x$ at time $t$ is given by the function $n_1(x,t)$. The strongly bound state of the cross-bridge is described with the kinetic state 2 and its distribution function $n_2(x,t)$. The third state of the cycle corresponds to the unbound cross-bridges. The probability distributions of the bound states of the weakest $hS$ are calculated during a simulation of stretching with amplitude of 10% $L_0$ and ramp velocity of 0.1 $L_0$/s corresponding to Fig. 3.3.1. At the start of the activation, the distributions of the weakly bound states $n_1$ build up fast to be reduced to a nearly steady state (Fig. 3.3.6A). For the distribution of the strongly bound state the build-up is more continuously (Fig. 3.3.6B). Note that the peak of the distributions is shifted from the weakly bound state to the strongly bound state in the direction of $x$ with a distance corresponding to the mean power stroke of a cross-bridge. The vertical drop at the edges of the distributions at $x=20 \mu m$ for the weakly bound and at $x=12 \mu m$ for the strongly bound state results from the definitions of the kinetic transition rates if the coupling region is set to 36.5nm. At the start of the stretching the distributions of both states are widened in positive $x$-direction. However, the position of the peak is slightly shifted in positive direction and is lowered in its value. This lowering of the peak is due to the broadening of the distribution because the coupled cross-bridges in both states are stretched in positive $x$-direction. The lowering and shifting of the peak value during stretch is greater for the weakly coupled state than for the strongly coupled state. At the end of the stretching, the state distributions continuously change back to their pre-stretched isometric shape. These findings for the
weakest hS can also be seen at distinct time points before, during and after stretching shown in the upper row of Fig. 3.3.7. However, the distributions of the strongest hS are less lowered and broadened during stretch (lower row in Fig. 3.3.7).

Fig. 3.3.6: Probability distributions of the weakly and strongly bound state during stretching. The probability distributions of the strongest hS are calculated for the stretch of a frog myofibril shown in Fig. 3.3.1 with amplitude of 5% L0 and mean lengthening speed of 0.1 L0/s. (A) Probability distribution to find a cross-bridge in the weakly bound state. (B) Probability distribution of the strongly bound state. The cut-off of the stretched distributions arises from the definitions of the rate functions in the model of Nishiyama (1977).
The changes of the distributions during stretching are more prominent if the decoupling rate is ten times lowered (low $k_3$) and the coupling region is prolonged ($d_{int} = 74$nm). The state distributions of the strongest and weakest hS of a simulation shown in Fig. 3.3.5 with a stretching velocity of 0.1 $L_0/s$ and amplitude of 5% $L_0$ are calculated and shown in Fig. 3.3.8. The distributions of the strongly coupled states are much greater than the state distributions of the weakly coupled states due to the lower decoupling rate. Because of the prolonged coupling length and so a missing cut-off of the distributions in the positive direction, the broadening of the distributions during stretching is much wider than the former shown distributions with normal coupling length. This broadening leads to a greater shift of the peaks in positive directions. Interestingly, the distributions after stretching (1050 ms) differ from the distributions before stretching (300 ms) which could not be seen in Fig. 3.3.7. Furthermore, the distributions of the weaker hS are more broadened and hence have a lower peak than the distributions of the stronger hS as could be seen also in the previous paragraph. This is most prominent in the weakly bound state.
3.3.6. Occupation number

Like shown in section 3.1.2 the number of bound states in a hS is a good variable to determine steady states during activation and stretching. This occupation number is calculated for the weakly and strongly bound state by $N_{hS, weak} = N(l) \cdot C \cdot \frac{1}{l_d} \int p_1(\eta, t) d\eta$ and $N_{hS, strong} = N(l) \cdot C \cdot \frac{1}{l_d} \int p_2(\eta, t) d\eta$

respectively. These occupation numbers are normalized with the occupation number of a single, fully activated hS that is strictly isometric with optimal filament overlap and hence calculated with

$N_{norm} = C \cdot \frac{1}{l_d} \int p_{steady}(\eta, t) d\eta$ where $p_{steady}(\eta, t) = K^{-1}(\eta) \cdot c(\eta)$ according to section 2.4.4. The occupation numbers of two different simulations of active stretches are shown in Fig. 3.3.9 and Fig. 3.3.10. The occupation numbers of Fig. 3.3.9 correspond to the simulation of Fig. 3.3.1 with a stretching amplitude of 10% $L_0$. The figure shows, that during activation the number of bound states for weakly
coupled state rise slower than the number of strongly coupled state and continuously reach a near steady state before the stretch. For the case of the strongly coupled state, the number of bound states rises very quickly in the beginning but lower during the end-held activation to a nearly steady state. At the end of the end-held activation, the weak hS has more weakly coupled states than the strong hS, whereas in the case of the strongly coupled states it is vice-versa. At the beginning of the stretch the weakly bound states increase and the strongly coupled states decrease in their number. After this initial force rise due to the stretch and the following force plateau or slight depression, the number of states changes more continuously. In general, the states decrease after the initial phase of the stretching. The numbers of the weaker hS are decreasing more rapidly than the numbers of the stronger hS. This reflects the fact, that the weaker hS are mostly held by passive forces (titin) at the end of the stretch. After the stretch, the strongly bound states are initially increasing. After this sudden increase the numbers of the strongly coupled states are slowly decreasing except for the second strongest hS. However, the relative order of the numbers after stretch correspond to the relative order before stretch even the absolute values are mostly different. An exception to this finding is the occupation number of the weakly bound state for the weakest hS which drops from the highest number of weakly coupled states before stretch to the third highest number after stretch. This exceptional behavior is again due to the decreased force in the active domain by insufficient filament overlap. Interestingly, only the numbers of weakly bound states increase during stretch.

**Fig. 3.3.9:** Occupation number of the hS during stretch. The normalized occupation number of the weakly (left) and strongly bound cross-bridges (right) during a stretch of four hS are shown in the figures. The simulation corresponds to the stretch in Fig. 3.3.1 with amplitude of 10% \( L_0 \). The dotted line corresponds to the weakest hS and the straight line to the strongest hS in the system. The normalization \( N_{norm} \) is the calculated occupation number of single hS during an isometric contraction (see text for details).
Results

The occupation numbers of a simulation with decreased decoupling rate and increased coupling region show a different behavior (Fig. 3.3.10). During the phase of end-held activation, the states do not reach a steady state as they increase during the initial activation and decrease in the later phase of the end-held activation (up to 300 ms). At the start of the stretching, the hS can be divided into two classes: The two strongest and the two weakest hS. The two strongest hS shortly increase their number of coupled states within the first milliseconds of the stretching. After this increase, the number drops strongly for some hundredths of a second to be increased again during the rest of the stretching. The two weaker hS do barely decrease their number in the initial phase of stretching and start increasing the number earlier than the two stronger hS. This early increase leads to an overall greater increase of bound states during stretching, although the numbers of the strongly bound states are generally greater before the stretch. After stretch, all the numbers decrease exponentially, whereas the numbers of the stronger two hS increase again after this drop. Interestingly, only during the later phase of the stretching, the occupation numbers of the hS have a clear order from weak hS to strong hS for both weakly and strongly bound states.

![Fig. 3.3.10](image)

**Fig. 3.3.10**: Occupation during an active stretching with low decoupling rate and longer coupling distance. The figures show normalized occupation numbers of a simulation shown in Fig. 3.3.5 with a stretching velocity of \( v = 0.1 \frac{L_0}{s} \) and low transition rate \( k_3 \). The weakest hS in the system is represented with a dotted line whereas the strongest hS with a straight line.

3.3.7. HS dynamics and length non-uniformities

The hS length non-uniformity and hS dynamics are calculated for a simulation of four hS with stretching amplitude of 10\% \( L_0 \) and stretching speed of 0.1 \( \frac{L_0}{s} \) as it is described in section 3.3.1 (Fig. 3.3.1). Analogous to the relaxation in section 3.2.1, the hS length non-uniformity is described by the standard
deviations of the hS lengths. To express the hS dynamics we refer to the previously introduced measure $\psi_{\text{hSdyn}}(t)$ in section 3.1.1. During activation and stretch of the system, the hS length non-uniformity is continuously increasing (Fig. 3.3.11). As already shown in the activation section (3.1.1), the build-up of the hS length inhomogeneity is steep in the very first beginning of the activation and declines after to a nearly linear increase. This linear increase is only slightly depressed in the first phase of the stretching.

Like in the activation of a system based on the Huxley model, the hS dynamics is greatest in the initial phase of the activation where the hS initially spread. At the start of the stretching, the hS dynamics rises exponentially to a constant value for the rest of the stretch. Interestingly, this rise of the hS dynamics during the initial phase of stretching is very similar to the corresponding force increase in the same phase. At the end of the stretching, the hS dynamics drops to a constant level. Note that the value of the hS dynamics after stretching is higher than the value before the stretch.

Fig. 3.3.11: HS dynamics and length non-uniformities during stretching. The temporal evolution of the standard deviation of the hS length during a stretching simulation corresponding to Fig. 3.3.1 is shown as dotted line. This function is a good value of the hS length inhomogeneity. The hS dynamics of the same simulation is calculated according to the formula in the text and is displayed as solid line.
Chapter 4
Discussion
Discussion

A mathematical formalism to study the dynamics of hS and their force production during calcium activation, relaxation and stretching has been presented. It uniquely combines a multisegmental model with a transient kinetic formalism that takes the variability in the contractile property of hS into account. Despite the accepted view in the community that sarcomere inhomogeneity is problematic for the interpretation of experimental data (Sugi and Tsuchiya, 1998), a rigorous mathematical theory addressing this issue has been absent. This novel framework provides insight into how hS dynamics affect the force response of a myofibril during end-held activation and standard isometric or isotonic contraction experiments; as groundwork, it can easily be extended to a more complex model. In this sense, it is the methodical basis for further investigations of the effect in myofibrils of hS dynamics.

The model applies on a microscale and can reproduce results from macroscale experiments on muscle fibers and myofibrils (Edman, 2005; Gordon et al., 1966b; Granzier et al., 1989), such as force-length and force-velocity relationships (Fig. 3.1.5 and 3.1.6). In this sense, results on the macroscale can be interpreted as a continuous limit of the microscale description. The results in macroscale experiments are mainly considered when the system is near steady state and, therefore, the effects of the transient cross-bridge state distributions are small. However, to understand the underlying mechanisms of muscle function on the level of proteins and their interactions, a model on the microscale that connects the transient kinetics with the macrostructure of a myofibril is necessary.

Analogous to the results section, the discussion is divided into three main parts: the activation, the relaxation and the stretching of a myofibril. The corresponding results to these topics are discussed and related to the actual literature. The first part is the slightly adapted discussion of a recently accepted publication (Stoecker et al., 2009).

4.1. Activation of a myofibril

4.1.1. Effect of hS Dynamics on Force Development

The effect of internal shortening and hS dynamics on force development upon Ca\textsuperscript{2+} activation was briefly discussed in a review of Telley and Denoth (2007). In the present study, more elaborate calculations for the initial phase of activation are conveyed. The simulation shows a direct influence of hS dynamics on the activation rate $k_{a/v}$ (Fig. 3.1.2). The result is consistent with previous findings from rather simplified theoretical considerations (Luo et al., 1994; Telley and Denoth, 2007). This effect has also been observed in experiments on fibers (Granzier et al., 1989). Stehle et al. (2002b) showed that the activation rate also depends on the species and concluded that it depends upon internal kinetics. Nevertheless, to determine the internal kinetics by means of the activation rate, the length of a single hS must be controlled experimentally.
**4.1.2. hS length nonuniformities**

The nonuniformity of the hS can be measured by calculating the spreading of the length distributions at any time point. Our simulations show an initial shortening followed by spreading of the hS lengths (Fig. 3.1.1). This suggests that hS-length nonuniformity increases during contraction. Furthermore, the simulations show that the nonuniformity of the hS is mostly built up in the very first phase of activation and increases almost linearly during the continuing contraction (Fig. 3.1.3B). However, some experiments on myofibrils (Rassier, 2008; Telley et al., 2006a; Telley et al., 2006b) showed a slight increase in the nonuniformity during the later phase of activation; this increase was invisible in other studies (Joumaa et al., 2008; Stehle et al., 2006). Experiments on whole fibers from frog muscles even suggest that the dispersion of sarcomeric lengths maximally increases to 4% of the average hS length during tetanic contractions (ter Keurs et al., 1978). On the other hand, this dispersion has been measured as up to an 8% increase in psoas myofibrils (Telley et al., 2006a). The reduction in nonuniformity in fibers reflects the stabilizing effect of intermediate filaments (e.g., desmin) that connect the myofibrils (Pollack, 1990; Shah et al., 2002). A variability of $\sigma = 0.15$ of the force capacity simulates the higher observed dispersion (8%) of hS length in myofibrils, while a variability of $\sigma = 0.07$ simulates the lower observed dispersion (4%) in fibers. If the variation is limited to the variation of the rate functions, the hS only shorten. In contrast, the experiments on myofibrils (Telley et al., 2006a; Telley et al., 2006b) show shortening and spreading of the hS after the initial phase.

At the end of the activation period of three seconds, the hS move slowly, yet they exhibit distinct lengths. This plays a key role in the early phase of relaxation, in which presumably the weakest hS starts to elongate, and in subsequent sequential relaxation (Stehle et al., 2006). However, a systematic dependence of the force capacity $C$ on the actual position of the hS in the myofibril is missing. Although it may be an important feature for the relaxation process, it is not crucial for the general understanding of the model and for simulations of activation. However, it would show where a weak hS is operating in the myofibril and how weak it is compared to its neighbors. Furthermore, it is not clear if weak and strong hS appear in clusters or in isolation (Stehle et al., 2002a; Talbot and Morgan, 1998); nevertheless, this is valuable information for explaining localized dynamic effects such as oscillations.

**4.1.3. hS Dynamics**

The dynamics of the hS can be described with the average internal velocity $v_{hS\text{dyn}}$, because it accounts for all internal movements, even if the entire myofibril is kept isometric. A parametric analysis of $v_{hS\text{dyn}}$ dependent on $k_{ext}$ shows that hS dynamics decrease for stiffer external springs, but nevertheless do not vanish. Thus, even for strict "length-clamped" experiments, there will be a reasonable amount of hS dynamics dependent on the force capacities $C$. The hS dynamics decreases if the variance $\sigma'^2$ of the force capacities $C$ is reduced (see Fig. 3.1.4). Therefore, it can be proposed that there are two components that influence the hS dynamics: (1) the stiffness of external spring $k_{ext}$ and (2) the distribution of the force capacities $C$. The external stiffness influences the overall dynamics of the myofibril. The distribution of the force capacities influences the internal dynamics of the hS. However, these two
components are not completely distinguishable, because an overall shortening due to a compliant external spring also influences the internal dynamics.

There was no tension creep in the performed simulations of end-held activation. However, creep can be associated with lower activation rates because the force rises slowly in the later phase of activation if the activation rate is low. In the literature, the tension creep during the late phase of activation is believed to arise from (half-) sarcomere dynamics (Edman and Reggiani, 1984). Based on the simulations, it can be concluded that the reason for creep is an overall decrease in length that influences the force rise.

The component of the hS dynamics that depends on the external spring can be analyzed if a myofibril with equal force capacities, \( \sigma = 0 \), is calculated, where all hS correspond to the “average hS” with average length. In an end-held contraction simulation, the external length \( L_{ext} \) is the sum of the mean hS lengths \( \bar{l}_{hS}(t) \) plus the external deflection \( \Delta x_{ext} \). In a system of \( n \) hS, all of the individual velocities are equal to the mean velocity, and therefore the previously defined measure for hS dynamics simplifies to

\[
v_{hSdyn}(t) = \frac{\bar{f}_{hS}}{n \cdot k_{ext}}.
\]

Using the single exponential approximation for the force rise, the change of force of the hS can be approximated as \( f_hS = k_{act} \cdot F_0 \cdot e^{-k_{act} \cdot t} \approx k_{act} \cdot F_0 \) for small \( t \). Thus, the hS dynamics at the beginning of an activation can be estimated as \( v_{hSdyn} \approx \frac{F_0 \cdot k_{act}}{n \cdot k_{ext}} \). The hS dynamics does disappear for infinite stiff \( k_{ext} \) because the ratio \( k_{act} / k_{ext} \) approaches zero for higher stiffness (see Fig. 3.1.2). This finding is in agreement with the results of the hS dynamics for a system of four hS during activation of an almost homogeneous ensemble (\( \sigma = 0.0001 \), Fig. 3.1.4), where only the second part of the initial phase is prominent (dominated by the external spring). Such a simplified model framework is commonly used to describe the behavior of muscle fibers, and often consists of a sophisticated multi-state kinetic formalism to reproduce transient force responses (e.g., Piazzesi and Lombardi, 1996). These models, however, have completely excluded the multisegmental mechanics of myofibrils and hence will never be able to encompass the effect of hS dynamics and mechanical properties on muscular force.

### 4.1.4. The steady-state force-length relationship

In “length-clamped” simulations, the overall shortening is limited by the stiff external spring. The hS dynamics \( v_{hSdyn} \) is reduced, because it also depends on the mean shortening. Spreading in a “length-clamped” simulation of the force-length relationship depends on the final mean length after activation. If the mean length is on the ascending limb or the plateau, the spreading of the hS after 1.5 s of activation is lower than 0.15 \( \mu m \), which is in the range of end-held tetanic contraction simulations. However, if the mean lengths are on the descending limb of the force-length relation, the spreading gets wider, with maxima at mean lengths 1.25–1.4 \( \mu m \), and length spreading around 0.3 \( \mu m \). This is twice the amount of spreading in the ascending limb and the plateau (see insets of Fig. 3.1.5). The entire scenario
resembles the “instability of the descending limb,” as investigated experimentally by Julian and Morgan (1979a; 1979b) and described theoretically by Denoth et al. (2002) and Zahalak (1997).

In the graph in Fig. 3.1.5, the edges of the force-length relationship are smoothed due to the transient hS dynamics. At the lengths around the edges, there are hS on the plateau and on the ascending/descending limb, reducing the force. Experiments on fibers have shown that the shape of the force-length relationship is different for the fixed ends of the fiber and for the fixed length of a segment of the fiber (Granzier and Pollack, 1990; ter Keurs et al., 1978). For fixed-end contractions, the descending limb of the force-length relationship does not drop linearly and the decrease is slower for longer sarcomere lengths up to 3.6 μm. During tetanic contraction in fixed-end experiments, the authors of these studies measured stretching of the sarcomeres in the central part of the fibers while the sarcomeres at the ends shortened. They concluded that the higher force in the descending limb during fixed-end contractions is due to the stretching of the hS. The simulations support this idea, because, with a more compliant fixation, the hS dynamics are higher (Fig. 3.1.4). The higher hS dynamics smooth the edges and prolong the plateau of the force-length relationship, which corresponds to the observed relationship of Granzier and Pollack (1990).

### 4.1.5. Steady-state approximation

Hill-type models are commonly used to describe the function of muscles, fibers, and myofibrils during activation and stretch (Siebert et al., 2008; Telley et al., 2003). A Hill-type model assumes a steady-state distribution of the bound states, allowing only small changes in the motions of the hS. However, for estimating whole body movements in macroscale biomechanical systems, the steady muscle force approximation is probably sufficiently precise, particularly because the computational cost for a Hill-type model is far less than that of a Huxley-type model. The model presented here includes the transient behavior of the Huxley-like description; thus, the time at which the distributions are merely steady, and when they are transient, can be determined. It can be deduced that the system is in a steady state if the number of bound states are not changing in time. The number of bound states is calculated for a simulation of end-held activation (Fig. 3.1.7). During the initial phase, the rise in bound states may be included in a steady-state simulation by multiplying the force-velocity relation by a calcium-driven exponential activation function \( \zeta(t) \). However, the changes in hS velocities in the first second are greatest, thus excluding the steady-state approximation (see Fig. 3.1.1 for details of the hS lengths).

When the system reaches a steady state, the weaker hS lengthen further until they stretch into the descending limb of the force-length relationship. If any hS are stretched into the descending limb, their velocities change, hence avoiding a continuation of the steady state of the system. Thus, there is always only a confined period during activation in which a steady-state approximation holds.

If the time phases in which a steady-state approximation holds are investigated more closely, the velocities of the hS can be estimated while applying the Hill force-velocity relation \( F(v) \). It can be stated that \( f_{CE} = C \cdot F(v) \) for the hS force, where \( v \) is the velocity and \( C \) is the hS force capacity. Following Hill
(1938), the steady shortening hS velocity can be derived because \( v = b \frac{f_{CE} - C \cdot F_0}{f_{CE} + C \cdot a} \), where \( a \) and \( b \) are the Hill constants \( (a/F_0 = 0.09, b = 0.119 \, \mu m/s \, (Sun \, et \, al., \, 2001)) \) and \( F(0) = F_0 \). Following Denoth et al. (2002), the steady stretching velocity can be expressed as \( v = \beta \tan \left( \frac{f_{CE} - C \cdot F_0}{\pi / 2 \cdot C \cdot F_0 \cdot a} \right) \). In this relation, set \( a = 0.485 \) and \( \beta = 0.097 \, \mu m/s \) to match the eccentric branch of the force-velocity relationship, resulting from the steady-state Huxley equations. From these expressions of the steady-state hS velocities, the hS dynamics \( v_{hSdyn} \) can be calculated. The transient hS dynamics of simulations of four hS with external stiffness \( k_{ext} = 30 \, F_0/\mu m \) and variable \( \sigma \) are compared with the hS dynamics derived from the steady-state expressions at 1 s after activation. Summing the absolute velocities of the steady-state expressions results in \( v_{hSdyn} = 36.5, 15.4, \) and \( 4.1 \, nm/s \) for \( \sigma = 0.2, 0.1, \) and \( 0.025 \) respectively, whereas the corresponding transient values from the simulations are \( v_{hSdyn} = 39.6, 17.5, \) and \( 3.6 \, nm/s \) (see Fig. 3.1.4). Hence in the later phase of activation, the hS dynamics of the transient model is in the range of the corresponding hS dynamics of the steady-state approximation.

Despite the high concurrence of the steady-state approximations of the hS dynamics in the late phase of activation, we conclude from our analysis of the bound states that there are only short time intervals in which a steady-state approximation holds, most often in clamped experiments. This would support using this model for the simulation and analysis of myofibril movement in common experimental protocols with myofibrils.

### 4.1.6. Coexistent variabilities

The non-uniformity and the dynamics of the hS in experiments may arise from several sources of biological variabilities. Two possible variabilities in the contractile element were simulated and analyzed in the previous paragraphs. The effects of the individual variabilities are analyzed in a simulation of an end-held activation incorporating variabilities of the force capacities, the kinetic rates and the titin elasticity (Fig. 3.1.10). The different starting lengths of the hS are mainly given by the variation of the titin elasticity as the hS need different resting lengths for different titin stiffnesses to produce the same permanent tension of the system. The spreading of the hS depends mostly on the different force capacities (Fig. 3.1.1). The different kinetic rates have only a small effect on the initial spreading of the hS (Fig. 3.1.1). In experiments on psoas myofibrils for hS resting on the descending limb of the force-length relationship, the starting lengths of the longest and the shortest hS are differing between approximately 0.25-0.5 \( \mu m \) (Joumaa et al., 2008). For hS at lengths with optimal filament overlap, the differences of the resting lengths of the longest and shortest active hS are found to be smaller (\( \approx 0.1 \, \mu m \)) (Telley et al., 2006a). Furthermore, Telley et al. (2006a) measured the resting lengths of the hS as normally distributed with a standard deviation of 20 nm and 31 nm for psoas and cardiac myofibrils respectively. Assuming a normally distributed stiffness of the titin with \( \sigma = 0.1 \) the calculated distribution of the resting length has a standard deviation of 22 nm which correspond nicely to the experimentally found values. The shortening of the hS during activation is greater in the experiments than in the
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simulations. This is mainly due to the passive compliance of the setup that includes the fixation and the dead hS and hence allows the hS to shorten a longer way. The computational effort increases immensely when simulating weaker external springs as the system moves over longer distances causing the distributions to widen which enlarges the range of the underlying grid and hence the number of differential equations to be calculated. Regarding the mean shortening (≈0.2 μm) of six active hS during end-held activation of a psoas myofibril (Telley et al., 2006a), this results in a very soft external spring,

\[ k_{ext} = \frac{F_0}{\Delta x_{ext}} = \frac{F_0}{n(t_0 - t)} = \frac{5 \times F_0}{6 \mu m}. \]

Simulations with such a soft external spring were not possible to calculate with the desktop computers used. The calculated spreading of the hS with an assumed variability of \( \sigma = 0.05 \) in the force capacities also corresponds to the spreading found in the experiments on psoas myofibrils (Telley et al., 2006a). The spreading was compared at the time point where the system reaches steady force resulting in approximately 25 nm per hS. Hence it can be concluded that for matching the experiments of Telley et al. (2006a) on myofibrils, the simulations must be performed with an external stiffness of \( k_{ext} = 1 F_0/\mu m \), normal distributed force capacities with \( \sigma = 0.05 \) and normal distributed titin elasticity with \( \sigma = 0.1 \).

4.2. Relaxation of a myofibril

4.2.1. Sequential relaxation

The relaxation in myofibril is a highly ordered process that can be observed by eye using a microscope. In this process, one hS starts to elongate that triggers the lengthening of the neighboring hS. This again triggers the next hS until all hS are lengthened. This leads to a sequential relaxation of the hS in time and space (Stehle et al., 2002a). A temporal sequence of the relaxation can also be seen in all the simulation in section 3.2. Assuming a variability of the force-capacities among the hS, the longest after the activation and hence the weakest always relaxes first in the simulation. Relaxation experiments on skeletal and cardiac myofibrils show that often the longest hS relaxes first (Poggesi et al., 2005; Stehle et al., 2002a; Telley et al., 2006a) although there are contrary findings where the shortest lengthen first (Telley et al., 2006a). The latter scenario can be simulated with variability in the kinetic transition rates (Fig. 3.2.4). This implies that both types of variabilities may be present in nature. Furthermore, Telley et al. (2006a) found that the starting point of relaxation is mostly near one end of the myofibril proceeding to the other end. Assuming that the procedure of the fixation weakens the hS at the ends, this corresponds to the findings of the simulations with variable force capacities that the weakest relaxes first (Fig. 3.2.1). However, the spatial relaxation sequence may not be reproduced by the simulations as the position of the hS are not fixed in the model and hence numbers of the hS can be permuted without changing the results of the calculation. Nonetheless, it may be assumed that the structural variability causing the different behaviour of the hS is not randomly distributed but varies over a big length scale. This implies that the strength of the hS increases in a spatial direction. Such a spatial increase of the force capacities would assign a position to the hS and hence the results reproduce the temporal and the spatial sequential relaxation. On the other hand, the model is not able to mimic the different transfer
times of the relaxation between consecutive hS that was found in the experiments of Telley et al. (2006a). Such a prediction is impossible due to the construction of the model that includes a rigid coupling of the neighboring hS that is equal for all hS (see Chapter 2 for details).

4.2.2. Duration of the relaxation

All our simulations show a great elongation of the weakest until it is stopped and shortened back by the passive force. Such a big length-overshoot of the weakest cannot be seen in experiments on skeletal myofibrils (Telley et al., 2006a) and are slightly present in cardiac myofibrils (Poggesi et al., 2005; Stehle et al., 2002a; Stehle et al., 2006). Furthermore the time lag between the relaxation of the first and the relaxation of the second is unnaturally long. The simulations of rabbit psoas myofibrils show a time lag of 450 ms and 570 ms between the first and the second relaxing hS for simulations including eight and four hS respectively. Experiments on cardiac myofibrils show different durations between the first and the second relaxing hS ranging from tens of milliseconds (Telley et al., 2006a) to hundreds of milliseconds (Poggesi et al., 2005). This time duration is even smaller for skeletal myofibrils from a rabbit psoas muscle (Telley et al., 2006a). So the time between the first and the second relaxing hS is prolonged in the simulation compared to the experiments. This effect even multiplies when assuming the underlying variability of the hS in the kinetic rates (Fig. 3.2.4).

In contrary to this finding, the total duration of the relaxation (≈1s) is similar to the relaxation time in the experiments of psoas myofibrils of rabbits (Telley et al., 2006a). The time of relaxation depends strongly on the type of the muscle. The faster the muscle the faster is the relaxation process (Poggesi et al., 2005; Rome, 2006). This is also reflected in the simulation comparing the relaxation times of frog and rabbit muscles. The total time of relaxation in the simulations can be reduced by reducing the stiffness of the fixation and increasing the titin stiffness (Fig. 3.2.3). The latter will be examined further in the next paragraph.

4.2.3. Role of titin in the relaxation process

We showed the sequential relaxation in the simulations. However the relaxation process is fastened for stiffer titin, faster kinetics and softer fixations. For every simulation of relaxation the weakest hS (with the lowest force capacity) relaxes first followed by the second weakest and so on. The behaviour of the hS lengths after deactivation is not trivial to predict. For an explanation of the sequential relaxation we analyze a simplified model of the system.

Assuming that the series element is much stiffer than the contractile element we can neglect the movement of the series element making the approximation \( l(t) \approx u(t) \). With the definition of a characteristic time \( \tau = \frac{f_{PE.i}}{f_{CE,u} + f_{PE.i}} \) we get a differential equation for \( l \) and \( p \) which can be solved assuming that the characteristic time is constant in time.
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\[
\dddot{\tilde{I}} + \frac{1}{\tau} \dot{\tilde{I}} = \frac{\tilde{f}_{\text{ext}} - \int \hat{p}(\eta,t) \cdot f_{\text{CE},p} \, d\eta}{f_{\text{PE},i}} \equiv \Phi(t)
\]

The linear differential equation can be solved for the velocities of each hS during relaxation, defining the disturbance function \( \Phi(t) \).

\[
\dot{\tilde{I}}(t) = e^{-\tau t} \cdot \left( \tilde{I}(0) + \int_{0}^{t} \Phi(\omega) e^{\omega \tau} \, d\omega \right)
\]

The evolution of the velocities of a hS during relaxation depends exponentially on the inverse of its characteristic time of \( 1/\tau \). With a bigger positive characteristic time \( \tau \), the decay of the velocity \( \dot{\tilde{I}}(t) \) of a hS gets slower. A negative characteristic time enhances the velocity and so relaxes the hS. As the partial derivatives of the parallel element \( f_{\text{PE},i} \) and \( f_{\text{PE},o} \) are always positive, the characteristic time can only become negative if the derivative of the contractile element

\[
f_{\text{CE},a} = C \cdot \frac{k_{CB}}{l_{a}} \left( N(u) \cdot \int_{R} p(\eta) \cdot d\eta + \frac{\partial N(u)}{\partial u} \cdot \int_{R} p(\eta) \cdot \eta \cdot d\eta \right)
\]

is negative. The first term of this derivative is always positive. The second term can be negative for hS on the descending limb of the force-length relationship, hence longer than 1.125 \( \mu \)m for frog Sartorius muscle or longer than 1.2 \( \mu \)m for rabbit psoas, resulting in a negative characteristic time \( \tau \). When the hS with the lowest force capacity is on the descending limb of the \( F-l \) relationship at the end of the activation, this hS relaxes immediately at the start of deactivation. Furthermore, the evolution of the velocity also depends on the initial velocity \( \dot{\tilde{I}}(0) \) which is also biggest for the weakest hS at the end of the activation.

However, the characteristic time \( \tau \) is positive if the lengths of the hS are on the plateau or the ascending limb of the force-length relationship. In this case the velocities \( \dot{\tilde{I}}(t) \) have a maximum if \( \dot{\tilde{I}}(t) = 0 \) and hence \( \tau \cdot \Phi(t) - \dot{\tilde{I}}(t) = 0 \). So the relaxation of the hS occurs approximately when the functions \( \tau \cdot \Phi(t) \) and \( \dot{\tilde{I}}(t) \) cross. As the disturbance function is much bigger as the velocities, \( \Phi(t) \gg \dot{\tilde{I}}(t) \), the relaxation sequence depends mostly on the value of \( \Phi(t) \). The lower the value of \( \Phi(t) \) the faster it crosses the line at \( \dot{\tilde{I}}(t) \). In the late phase of deactivation the term \( \tilde{f}_{\text{ext}} \) is negligible and \( f_{\text{PE},i} \) is almost constant. So the value of \( \Phi(t) \) is mostly given by the term \( -\int \hat{p}(\eta,t) \cdot f_{\text{CE},p} \, d\eta \) which depends strongly on the force capacities \( C \) of each hS. Hence the lower the force capacity the closer is \( \Phi(t) \) at the velocity \( \dot{\tilde{I}}(t) \) and the earlier the hS relaxes. Therefore, it can be shown analytically with the above estimate that the relaxation process goes from weak to strong hS.
4.2.4. Force-shoulder after deactivation

In the simulations of relaxation with a soft external spring, a slight force shoulder right after deactivation is visible (Fig. 3.2.3). This shoulder can also be seen in the results of the down-stripped model presented in the results section 3.2.3 and shown in Fig. 3.2.8. In experiments on myofibrils, this shoulder is visible very prominently as the first linear force decay after removing Ca²⁺ (Stehle et al., 2002a). This linear phase normally lasts tens to hundreds of milliseconds before the force decays exponentially (Poggesi et al., 2005). A pronounced force shoulder is not invisible in the simulation of relaxing myofibrils. However in the simplified calculations the force shoulder at the start of relaxation is more prominent and is prolonged for soft external fixations Fig. 3.2.8.

The influence of the parameters on the force shoulder can be deduced by differentiating the solution for the contractile force of the down-stripped model with constant off-rate with respect to t. During end-held contractions the total length is held constant, hence \( \dot{L}_{tot} = 0 \).

\[
\dot{f}_{CE}(t) = \frac{d}{dt} (k_{ext} (L_{tot} - l(t))) = -k_{ext} \dot{l}(t) = \frac{k_{ext} e^{\theta t} f^0_{CE} g_1 \left(2 f^0_{CE} - k_{ext}\right)}{(e^{\theta t} k_{ext} - 2 f^0_{CE})^2}
\]

At the onset of the deactivation this slope simplifies to

\[
\dot{f}_{CE}(0) = -f^0_{CE} \cdot g_1 \cdot \left(1 + \frac{2 \cdot f^0_{CE}}{k_{ext}}\right)^{-1}
\]

So the force shoulder at the onset of the deactivation is more pronounced for smaller negative slopes of the force. This is especially true for elastic external fixations as the derivative \( \dot{f}_{CE}(0) \) converges to 0 for small \( k_{ext} \). Furthermore for small \( k_{ext} \) the velocity of the hS \( \dot{l}(0) \) at the onset of deactivation is also reduced meaning that the hS lengthens slower for weaker external springs. It is believed that this force-shoulder after deactivation is related to the kinetics of the cross-bridge cycle (Poggesi et al., 2005; Stehle et al., 2002a; Stehle et al., 2006). Regarding the analysis of the down-stripped model as well as the simulations we propose that the force shoulder after deactivation has also some component of hS dynamics.

4.2.5. HS dynamics and length non-uniformities during relaxation

Regarding the simulations of activation and relaxation in section 3.2, the hS dynamics is greatest at the start of the activation, at the start and the end of the deactivation of the system. The first peak of the hS dynamics during relaxation reflects the elongation of the weakest hS whereas the second peak includes the shortening of the weakest and the relaxation of the stronger hS. Hence the second peak is higher as the first one as in the last period of relaxation all the hS lengthen or shorten over a long distance. In the experiments on myofibril the individual lengthening of the hS is spread over the total time of relaxation.
Discussion

(Poggesi et al., 2005; Telley et al., 2006a). So the simulations show a concentrated relaxation of the stronger three to six hS that is not seen in the experiments. Regarding the individual velocities during relaxation, the maximal lengthening velocity of 1.55 µm/s during relaxation is reached by the weakest hS in the simulations of a rabbit psoas myofibril containing eight hS. This maximal lengthening speed is about half of the value seen in experiments on rabbit psoas myofibrils (Telley et al., 2006a).

The length inhomogeneity during relaxation depends mostly on the elongation of the weakest hS. When the weakest hS elongates, the stronger hS shorten to ensure a constant external length. This spreading increases the standard deviation of the length distribution (σ₁). In their analysis of the experiments on cardiac myofibrils from guinea-pig, Poggesi et al. (2005) showed an increase of this standard deviation from approximately 0.038 µm (before deactivation) to maximum of about 0.12 µm after elongation of about half of the involved hS. The simulation of frog myofibrils with a stiff external fixation shows a comparable maximum of σ₁ = 0.1085 µm although starting from a higher level of length inhomogeneity (σ₁ = 0.072 µm) at the start of deactivation (see Fig. 3.2.5 for details). In contrary, other experiments on cardiac myofibrils showed no measurable increase of the hS inhomogeneity during relaxation (Telley et al., 2006a). In relaxation experiments on skeletal muscle of rabbit psoas the standard deviation of the hS lengths is approximately increasing from 0.05 µm to 0.1 µm during the first phase of deactivation (Telley et al., 2006a). So the findings of the hS length inhomogeneity in the simulations are in the order of magnitude of to the values found in the experiments.

The great hS dynamics and the increased length inhomogeneity prevent a steady state of the system during relaxation. This can be shown while calculating the time course of the number of bound states \( N_{\text{bound}} \). In the analyzed simulations of four hS the bound states of the hS change in time until the last hS is relaxed (Fig. 3.2.6). So there is no steady state during relaxation in these simulations. Further mathematical analysis of a down-stripped model shows that the relaxation is an entire transient process where no steady force-velocity relationship can be applied (see Appendix B for detailed calculations).

Approximating the stiffness of the parallel element with a linear elastic spring and neglecting the elasticity of the filaments (no series elasticity), it can be shown theoretically that the entire activation-relaxation cycle is transient and there will be no stable fix-point of the dynamical system when the myofibrillar force is changing in time (Appendix B).

4.3. Stretching of a myofibril

4.3.1. Force rise during stretching

In the simulations of the section 3.3, the force during and after stretching shows a typical behaviour that can also be observed in experiments on myofibrils and whole muscles (Herzog et al., 2006b). The force rises exponentially at the start of the stretch while decreasing exponentially after the stretch. The initial force rise in the simulations upon stretching is approximately a single exponential increase. Experiments on psoas myofibrils and fibers show two phases of force increase at the start of stretching, a fast first phase and a slower second phase (Rassier, 2008). For very slow stretching velocities (<0.3 \( L_0/s \)) the two
phases are indistinguishable (Getz et al., 1998; Rassier, 2008). Analogous to the results of the simulations, the shape of the force rise in real psoas myofibrils upon stretching depends strongly on the stretching speed (Fig. 3.3.3 ff). The maximal force during stretching in experiments on psoas myofibrils ranges from 154% to 142% of the steady isometric force before stretching ($F_{\text{i,ref}}$) for stretching speeds of 1 $L_0$/s to 0.4 $L_0$/s respectively (Rassier, 2008). An even higher maximal force during stretching (2.5 $F_{\text{i,ref}}$) was found by Telley et al. (Telley et al., 2006b) while moderately stretching psoas myofibrils (0.2 $L_0$/s). The amount of the force increase is much lower in the simulations with normal decoupling rates and coupling regions ranging from 27% $F_{\text{i,ref}}$ to 29% $F_{\text{i,ref}}$ in systems of four hS stretched with 0.1 $L_0$/s (see section 3.3). For ‘normal’ single hS systems the maximal force is little higher resulting in 134% $F_{\text{i,ref}}$ and 157% $F_{\text{i,ref}}$ for stretching speeds of 0.1 $L_0$/s and 0.2 $L_0$/s. For ten times lower decoupling rates, the force of a single hS rises up to 154% $F_{\text{i,ref}}$ (Fig. 3.3.3). If additionally the coupling region is enlarged, then force of a single hS rises up to 210% $F_{\text{i,ref}}$. The underestimation of the force increase in the ‘normal’ simulation may arise from the fact that in every simulation at least one hS is working beyond optimal filament overlap depressing the force. In frog myofibrils the range of optimal overlap is smaller than for psoas myofibrils so in stretching experiments of psoas myofibrils consisting of many hS there may be more hS working on the plateau hence reducing a force depression during stretching. Another reason could lay in the cross-bridge kinetics, where it may be assumed that the weakly bound states (pre-power stroke state) are preferred during stretching and the average force per cross-bridge is increased (Herzog, 2005; Mehta and Herzog, 2008; Rassier, 2008). Both scenarios are not explicitly included in the original model of Nishiyama (1977) that is the kinetic basis for the ‘normal’ simulations. However, both scenarios can be reflected by the alternations done in the simulations with lowered decoupling rate k₃ and increased coupling regions dₜₐₜ. By the lowered decoupling rate the weakly coupled state is more occupied during the stretch (Fig. 3.3.10). Furthermore, the longer coupling region allows a greater force per cross-bridge while stretching. These alternations show a much greater force increase that is comparable with experimental results.

4.3.2. Residual force enhancement

In the last years the mystery of permanent force enhancement after stretching a muscle fiber or a myofibril was investigated intensively (Herzog, 2005). In the literature it is numerous reported that the force at the reached isometric length after active stretching is higher than the isometric reference force ($F_{\text{i,ref}}$) of the same myofibril similarly stretched before activation. The difference between the two forces is called ‘residual force enhancement’ or simply RFE (Herzog, 2005). The myofibrillar force after stretching is firstly decaying exponentially to a new constant level (Joumaa et al., 2008; Rassier, 2008; Telley et al., 2006b). This new level is assumed to be in a steady state and is called the steady force after active stretching. In the stretching simulations of section 3.3 the force decay is stronger than observed in the experiments resulting in a lower steady force after active stretching compared to experiments on psoas myofibrils (Joumaa et al., 2008; Rassier, 2008; Telley et al., 2006b). So the low steady force after active stretching produces very little RFE in all the simulated stretches including four hS. The RFE in the simulations depend on the stretching amplitudes. A higher stretching amplitude (0.1 $L_0$) results in a RFE
Discussion

of 1.5% $F_{ref}$ whereas lower stretching amplitude results in a RFE of 0.6% $F_{ref}$ (Fig. 3.3.1). The calculated RFE are negligible regarding the findings of Joumaa et al. (2008) where a much higher RFE ranging from 21% to 637% $F_{ref}$ is reported. Concerning the simulation with a starting length at 1 µm, the RFE is not enhanced in comparison to the simulations with starting lengths at 1.1 µm. In experiments on intact muscle fibers RFE enhances with increasing starting lengths stretched with the same amplitudes (Herzog, 2005; Herzog et al., 2006a). Simulations with single hS show lower amounts of RFE ($= 0.1% F_{ref}$) than simulations including four hS. This result may suggest that the amount of RFE does depend on the number of hS included in the simulated system and that the hS dynamics and the hS length inhomogeneity play a role in the development of RFE. However this conclusion is rather speculative as the amount of RFE is very small for any simulation performed. According to Herzog (2005) the kinetic pathway is a possible reason of RFE. He proposes a decrease of the rate function of detachment and an increase of force per cross-bridge following the stretching. This results in a higher portion of attached cross-bridges that can generate more force as could be confirmed in our simulations. However, the simulations with the proposed alternations could not generate a remarkable RFE after the stretch (Fig. 3.3.5). This result suggests that either the kinetic cycle or the mechanical structure must alter remarkably during stretching. A different pathway during shortening was proposed by Piazzes et ai (1996) and could be taken as template for an altered pathway for stretched hS. Another possibility to change the kinetic cycle is the introduction of long living catch bonds of cross-bridges like observed in single molecule experiments (Guo and Guilford, 2006). Those catch bonds prevent the force to decrease to the level of end-held contractions during the time of the activation. However, the experiments of Joumaa et al. (2008) are conducted for several tens of seconds so that those changes in the kinetic cycle must have a long mean lifetime. Another possibility to generate a remarkable RFE are structural changes during stretch. Recent publications could show that a second myosin head is coupling during stretch and hence generating more force (Brunello et al., 2007). If this second head has a very long mean life time it generates a long lasting force enhancement and hence contributes to the RFE. However, all those enhancements of the model are rather speculative and mostly propositions for an extension of the general contraction model to explain specific observations.

4.3.3. hS length stabilizing and no popping during stretch

The spreading of the hS in the simulations is not strongly influenced by the stretching of the myofibril (see Figs. in section 3.3). So the hS length non-uniformity increases continuous and almost linearly during stretching as can be seen in an exemplary calculation in Fig. 3.3.11. In experiments on myofibrils however, the dispersion of the (half-) sarcomeres is more enhanced during stretching than during isometric contraction (Rassier, 2008) and can even build groups of similar lengths (Shimamoto et al., 2009). Other experiments on the same types of myofibrils show the contrary finding that the stretching has a stabilizing effect on the hS length homogeneity (Telley et al., 2006b). In the latter mentioned experiment the velocities of individual hS were investigated on rabbit psoas myofibrils. Telley et al. (2006b) found that at the stretched length all hS move slow (0.05 µm/s – 0.2 µm/s). Supporting this finding, the computation shows a significant decrease of hS dynamics after stretching to a more or less
constant value (Fig. 3.3.11). However, the computed hS dynamics after stretching is 35% higher than the hS dynamics before stretching.

Furthermore, in all the simulations of active stretching, the hS lengthen relatively slowly although if they are stretched into the descending limb of the force-length relationship where the theory of D. L. Morgan (1990) would predict ‘popping’ hS. This computation corresponds to the experimental finding in stretched myofibrils of rabbit psoas where no sarcomere popping could be found (Telley et al., 2006b). Interestingly, ‘popping’ hS could be observed in earlier simulations of multi-segmental models which incorporated steady-state cross-bridge kinetics (Denoth et al., 2002; Telley et al., 2003). This difference in the theoretical behavior arises from the fact, that in models incorporating steady-state kinetics the force of the hS is directly coupled to its velocity whereas in models incorporating transient kinetics the force of the hS is coupled indirectly over the state distributions and hence the kinetic cycle to the velocity.
Chapter 5
Conclusions
The motivation of this thesis was the theoretical analysis of one of the open questions in muscle research if and where the dynamics of the (half-) sarcomeres play an important role. Therefore, the goal of this thesis was to derive a multisegmental model of the myofibril that includes transient kinetics of the cross-bridge cycle. Based on this tool, the importance of half-sarcomere dynamics can be estimated in different conditions with different parameters. An algorithm was developed to simulate these conditions and to calculate the half-sarcomere dynamics and the half-sarcomere length non-uniformity as well as the contributing forces therein. In the next paragraphs the contributions of the main parts of this thesis are summarized.

5.1. Important contributions to the field

5.1.1. Model

The model presented here merges biological models of the actin–myosin interaction and the mechanics of multisegmental systems. This approach tests and compares current ideas on muscular functions in simulations of myofibril activation with experiments performed and published over the last decades. Our framework serves as a basis for future muscular modeling on small scales. Due to the deterministic nature of the model it is predestinated for analytical studies of the mechanics in a myofibril.

5.1.2. Activation

The presented simulations of activation confirm previous observations that half-sarcomere lengths in a myofibril are distributed inhomogeneously, and exhibit significant dynamics influencing the force rise and the maximal force of a myofibril. We conclude that half-sarcomere movement must not be excluded in modeling myofibrils or in the investigation of molecular events in highly organized contracting systems, such as the half-sarcomere in a myofibril. Because this movement incorporates mainly transient states, the underlying mechanism of the contractile element must be at least a two-state Huxley-like system. In more sophisticated simulations of activation investigating the molecular contributions, even more mechanical states of the myosin head must be included in the model to account for the different conformational changes in the bound myosin heads. Calculating the transient dynamics during activation possible steady-states can be identified. The simulations show that the system is never or only shortly in steady-state conditions. This prevents steady-state approximations of the system such as using the empirical force-velocity relationship to describe the contractile behaviour of the half-sarcomeres in a myofibril. The half-sarcomere dynamics can be reduced in “length-clamped” simulations so that the force-length and force-velocity relationships of a myofibril are not affected by the transient behavior of the half-sarcomere lengths. So it can be concluded that for simulations of whole muscles or muscle fibers steady-state approximations are appropriate whereas for the investigation of the mechanics and the molecular events in myofibrils a transient model must be used.
5.1.3. Relaxation

The model could be applied to relaxation of a myofibril to show the behaviour of the half-sarcomeres. It could be shown that the half-sarcomeres relax sequentially in time. However, assuming a spatial direction of the biological variability also a sequential relaxation from neighboring to neighboring half-sarcomere can be predicted by the model. The time of relaxation can be shortened by enhancing the titin stiffness, softening the external fixation and increasing the kinetic off-rate. Furthermore we can show that the half-sarcomeres produce great dynamics during relaxation even when the force is decreased to a negligible level. Additionally, the simulations could demonstrate that the half-sarcomere dynamics has an influence on the shape of the force during an activation-relaxation cycle. However, the different characteristic force shapes are often associated with the internal kinetics of the cross-bridges. So we conclude that the half-sarcomere dynamics has to be taken into account when interpreting transient force responses on molecular level.

5.1.4. Stretching

It is believed that the half-sarcomere length non-uniformity is a possible explanation of the active force enhancement after stretch. The multi-segmental model including transient kinetics with three states is an excellent tool to investigate the importance of the half-sarcomere dynamics in the phenomena of residual force enhancement after stretching. In the performed simulations with different stretching amplitudes, different stretching speeds, different decoupling rates and different coupling regions no significant residual force enhancement could be calculated. So it can be concluded that the reason of the residual force enhancement may lay at least partially in the transient cross-bridge kinetics. So an adjustment of the kinetic rates can be proposed to prefer some strong and long living states during and after stretching. As the kinetics and the mechanics of the system are strongly coupled, a change of the description of the kinetic pathway may also have an influence on the half-sarcomere dynamics which then may play a more important role for the residual force enhancement. Furthermore in a model incorporating transient kinetics the occurence of ‘popping’ hS could not be observed.

5.2. Outlook

The presented model is a fundamental framework that can be extended in different ways. One extension would be the refinement of the kinetic cross-bridge cycle to be able to reproduce phenomena like the residual force enhancement. Inside the half-sarcomere the mechanics could be enhanced while including more sophisticated models for titin like the worm-like spring model. Furthermore mechanisms that describe the different transfer times of adjacent half-sarcomeres could be incorporated. A possible candidate for this mechanism is the lateral force inside a half-sarcomere that arises from the cross-bridges. Such a force would transmit over the Z-disk to the adjacent half-sarcomere influencing the cross-bridge cycle therein. Another expansion of the model may be the coupling of several parallel aligned myofibrils that are laterally coupled to rebuild the system of a muscle fiber. To simulate a model
with the above mentioned extensions the algorithm has to be improved and written in a precompiled language such as C or C++.
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Appendix

Appendix

Appendix A  The parameters of the three state model

The energies of the crossbridges in state 1 and 2 are assumed to be quadratic dependent on the distortion $x$ where $x_1$ and $x_2$ are the position of the lowest energies

$$U_1(x) = K_1(x - x_1)^2 / 2 - U_1^0, \quad U_2(x) = K_2(x - x_2)^2 / 2 - U_2^0$$

So the forces of a crossbridge in state 1 or 2 can be calculated while taking the spatial derivatives of the corresponding energy potential

$$\frac{dU_1(x)}{dx} = K_1(x - x_1), \quad \frac{dU_2(x)}{dx} = K_2(x - x_2).$$

The force of all the crossbridges can therefore be calculated while knowing the distributions of the cross-bridges in each state

$$f_{cb}(t) = N(u)C \frac{1}{L^{1/2}} \int_{L/2}^L \left[ K_1(x_i - x)n_i(x,t) + K_2(x_o - x)n_2(x,t) \right] dx$$

The probability distribution of a cross-bridge in state $i$ follows the differential equation, where $k_{ij}$ is the transition rate from state $i$ to state $j$

$$\frac{n_i(x,t)}{dt} = \sum_{j \neq i} \left[ k_{ji}(x)n_j(x,t) - k_{ij}(x)n_i(x,t) \right]$$

Hence to derive the distributions of the cross-bridges in each state, the kinetic transition rates have to be defined. Generally the rates are thermodynamically coupled resulting in

$$k_{ij} = e^{-\frac{U_i(x) - U_j(x)}{kT}}$$

In the model of Nishiyama et al. (1977) the kinetic transition rates between state 0 (uncoupled cross-bridge) and state 1 (weakly bound cross-bridge) are defined as

$$k_{01}(x) = k_1 e^{-\frac{-K_1(x-x_1)^2}{2kT}}$$

$$k_{10}(x) = \begin{cases} 
  k_1 e^{-\frac{-U_1^0}{kT}} & |x - x_1| < x_1^t \\
  \infty & |x - x_1| \geq x_1^t
\end{cases} \text{ or } (x_1 - x_1^t) < x < (x_1 + x_1^t) \text{ or } (x_1 + x_1^t) \leq x \leq (x_1 - x_1^t)$$

The transition rates between the weakly bound and the strongly bound state are
Appendix

\[ k_{12}(x) = \begin{cases} 
  k_2 e^{-\frac{K(x_i-x_j)(x-(x_i+x_j)/2)}{k_BT}} & x < (x_i + x_j)/2 \\
  k_2 e^\frac{C}{k_BT} & x_i \leq x < (x_i + x_j)/2 \\
  k_2 e^\frac{C}{k_BT} & x \leq x_i 
\end{cases} \]

\[ k_{21}(x) = \begin{cases} 
  k_2 e^{-\frac{U_{3}(x)-U_{2}(x)}{k_BT}} & x < (x_i + x_j)/2 \\
  k_2 e^\frac{C}{k_BT} & (x_i + x_j)/2 \leq x < x_i \\
  k_2 e^\frac{C}{k_BT} & x_i \leq x 
\end{cases} \]

With the factor \( C = U_{1_0} - U_{2_0} - K(x_i - x_j)\{x_i - (x_i + x_j)/2\} + K_{2}(x_i - x_j)^2/2 \). The relation between the strongly bound and the uncoupled state of the cross-bridge is given by

\[ k_{20}(x) = \begin{cases} 
  k_3 x_2 \leq x < x_2 + x_c^c \\
  k_4 x_2 - x_c^c < x < x_2 \\
  \infty & |x - x_2| \geq x_c^c \text{ or } (x_2 + x_c^c) \leq x \leq (x_2 - x_c^c) 
\end{cases} \]

\[ k_{02}(x) = \begin{cases} 
  k_3 e^\frac{U_{3}(x)-U_{2}(x)}{k_TS} & x \leq x_2 + x_c^c \\
  k_4 e^\frac{U_{3}(x)-U_{2}(x)}{k_TS} & x_2 - x_c^c < x < x_2 
\end{cases} \]

The constants in the energy potentials and the transition rates are defined according to the model of Nishiyama et al. (1977) with an adaption for the critical length \( x_1^c \) and \( x_2^c \).

\( K_1 = 0.75 \text{ pN} / \text{nm}, \quad K_2 = 1.00 \text{ pN} / \text{nm}, \quad K = 0.23 \text{ pN} / \text{nm} \)

\( U_{1_0} = 2.03 \text{kJ} / \text{mol}, \quad U_{2_0} = 30.14 \text{kJ} / \text{mol} \)

\( x_1 = 5.0 \text{nm}, \quad x_c^c = \frac{l_A}{2} - |x_1| = 13.25 \text{nm}, \quad l_A = 36.5 \text{nm} \)

\( x_2 = -3.0 \text{nm}, \quad x_c^c = \frac{l_A}{2} - |x_2| = 15.25 \text{nm} \)

\( k_1 = 100 \text{s}^{-1}, \quad k_2 = 4000 \text{s}^{-1}, \quad k_3 = 75 \text{s}^{-1}, \quad k_4 = 1300 \text{s}^{-1} \)

\( T = 280 \text{K} \)
Appendix B  Propositions and Proofs

Proposition 1. Relaxation is transient: The relaxation of a half-sarcomere coupled with an external spring is a transient state in all time points \( t \geq 0 \) while the external spring constant is finite, \( k_{\text{ext}} \leq \infty \).

Proof. Let us assume that the states during relaxation are steady all the time. Therefore we can state that it must exist a force-velocity function \( F(v, t) \) at every time point \( t \) which gives any steady velocity \( v(t) \) at a unique Force \( F(v, t) \) that corresponds to Hill's Force-velocity relation. Take the solution for the constant off-rate \( g(x) = g_0 \forall x \geq 0 \):

\[
l(t) = \frac{1 - e^{-g_0 t}}{f_0^\text{CE} + 2e^{-g_0 t}} + l_0 = \xi(t) + l_0
\]

The force can be formulated in two ways:

\[
f_{\text{CE}}(t) = f_0^\text{CE} - k_{\text{ext}} \cdot \xi
\]

\[
f_{\text{CE}}(t) = \alpha \int p_{\eta} d\eta
\]

While differentiating these equations and equate the results we get

\[
f_{\text{CE}}(t) = \frac{2f_0^\text{CE}e^{-g_0 t} + k_{\text{ext}} \cdot \dot{\xi}}{g_0}
\]

We can differentiate with respect to \( t \) using the definitions \( y \equiv e^{-g_0 t} \) and \( \omega \equiv \frac{k_{\text{ext}}}{f_0^\text{CE}} \) resulting in

\[
\dot{\xi}(t) = \frac{g_0 \cdot y(2 + \omega)}{(2y + \omega)^2} = v(t)
\]

If we rewrite the above equation for \( v(t) \) as polynomial we can state that

\[
4y^2 + y(4\omega - \frac{g_0}{v})(2 + \omega) + \omega^2 = 0
\]

and derive two solutions for \( y \)

\[
y_{1,2} = -\frac{4\omega v + g_0(2 + \omega)}{8v} \pm \sqrt{\frac{g_0(2 + \omega)(g_0(2 + \omega) - 8\omega v)}{16v^2}}
\]

\[
y \in \mathbb{R} \quad \text{if} \quad v(t) \leq \frac{g_0(2 + \omega)}{8\omega} \equiv v_{\text{critical}}
\]

So we have two branches of the force-velocity curve.
\[ F_i(v) = \frac{f_{CE}^0}{g_0} (2y_i + \omega)v, \quad \text{and} \quad F_2(v) = \frac{f_{CE}^0}{g_0} (2y_2 + \omega)v \]

Therefore the relation \( F(v) \) is not a mathematical function as for velocities in the range \( 0 < v(t) < v_{\text{critical}} \) it exist two values of the Force \( F_1 \) and \( F_2 \).

**Proposition 2. Stationary states during activation and relaxation.** All the states during activation and relaxation of a 2 half-sarcomere-system including a linear titin (\( f_{PE} = k_{ref}l \)) and a neglected series elasticity \( f_{SE} \) coupled with an external spring are transient except one distinct state which can never be achieved if we assume that the hS work on the plateau of the force-length relationship.

**Proof.** Let us assume that in the time interval \([t_0, t_1]\) it exists a stationary state where \( \dot{\eta}(t) = \ddot{\xi} = \text{const} \). \( \forall t \in [t, t + dt] \) and \( p(\eta, t) = n(x, t) = \text{const} \). \( \forall t \in [t, t + dt] \Rightarrow \dot{p} = 0 \). If we choose the start length long enough then \( k_{n}^{(1)} = k_{n}^{(2)} \). Therefore we have

\[
\begin{align*}
\ddot{\xi}^{(1)} \left( C^{(1)} \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j + k_n + k_{ext} \right) + k_{ext} \ddot{\xi}^{(2)} &= 0 \\
\ddot{\xi}^{(2)} \left( C^{(2)} \sum_{j=1}^{N_j} n_j^{(2)} \Delta x_j + k_n + k_{ext} \right) + k_{ext} \ddot{\xi}^{(1)} &= 0
\end{align*}
\]

solved for \( \ddot{\xi}^{(2)} \) and inserted we get

\[
\ddot{\xi}^{(1)} \left( C^{(2)} \sum_{j=1}^{N_j} n_j^{(2)} \Delta x_j + k_n + k_{ext} \right) \left( C^{(1)} \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j + k_n + k_{ext} \right) - \ddot{\xi}^{(1)} k_{ext}^2 = 0
\]

resulting in

\[
\ddot{\xi}^{(1)} \left( C^{(1)} \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j + k_n \right) \left( C^{(2)} \sum_{j=1}^{N_j} n_j^{(2)} \Delta x_j + k_n \right) + k_{ext} \left( C^{(1)} \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j + k_n \right) \left( C^{(2)} \sum_{j=1}^{N_j} n_j^{(2)} \Delta x_j + k_n \right) = 0
\]

As \( k_{ext} > 0, \ k_n \geq 0 \) and \( C^{(j)} > 0 \) is always true and the distributions \( n_j \geq 0 \) for each \( j \), the upper equation is only fulfilled if \( k_n = 0 \), and \( n_j^{(1)} = 0 \ \ \forall j \) or \( \ddot{\xi}^{(1)} = \ddot{\xi}^{(2)} = 0 \). The first restriction which represents the case where we have no titin is only valid before activation and at infinite time after relaxation. Therefore we look closer at the second restriction. As the velocities are both zero we can also state that \( n_j^{(1)} = n_j^{(2)} \) and therefore \( f_{CE}^0 = \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j \) and \( 2 f_{CE}^0 = \sum_{j=1}^{N_j} n_j^{(1)} \Delta x_j \).
Regarding the matrix equation in the methods section describing the dynamical system, 
\[ M(\xi, t) \ddot{\xi} = A(t) \dot{\xi} + B(t), \]
we follow that \[ A(t) \ddot{\xi} + B(t) = 0. \] With a linear titin the stiffness matrix expresses as
\[
A(t) = \begin{pmatrix}
\alpha^{(1)} \int \dot{p}^{(1)} dx & 0 & 0 \\
0 & \ddots & 0 \\
0 & 0 & \alpha^{(n)} \int \dot{p}^{(n)} dx
\end{pmatrix} = 0
\]
But as \( A(t) \) equals 0 anyway, we have the reduced equation
\[
\ddot{B}(t) = 0 \Rightarrow k_{ext} \ddot{L}_{tot} = 0 \Rightarrow L_{tot} = \text{const.}
\]
So there exist only stationary states where the total length of the system is fixed. When we assume that the force capacities are related by \( C^{(1)} = \gamma C^{(2)}. \) If we set \( C^{(1)} = 1 \) and solve the equation set
\[
\begin{cases}
C^{(1)} F_{CE}^0 (1 + 2 \xi_1) + k_n \ddot{\xi}_1 = k_{ext} (L_{tot} - \xi_1 - \xi_2) \\
C^{(2)} F_{CE}^0 (1 + 2 \xi_2) + k_n \ddot{\xi}_2 = k_{ext} (L_{tot} - \xi_1 - \xi_2)
\end{cases}
\]
We get
\[
\begin{align*}
\ddot{\xi}_1 &= \frac{k_{ext} k_n L_{tot} - 2 \gamma (F_{CE}^0)^2 + F_{CE}^0 (k_{ext} \gamma + 2k_{ext} \gamma L_{tot} - k_{ext} - k_n)}{k_n (2k_{ext} + k_n) + 4 \gamma (F_{CE}^0)^2 + 2 F_{CE}^0 (k_{ext} + k_n)(1 + \gamma)} \\
\ddot{\xi}_2 &= \frac{(2 F_{CE}^0 + k_{ext} + k_n)(\gamma F_{CE}^0 - k_{ext} L_{tot}) + k_{ext} (k_{ext} L_{tot} - F_{CE}^0)}{k_{ext}^2 - 2 F_{CE}^0 + k_{ext} + k_n)(k_{ext} + k_n + 2 \gamma F_{CE}^0)}
\end{align*}
\]
Therefore we have only stationary states when we are at these lengths with no velocity. That this can be achieved we need that these fixpoints are attractors. As \( A = 0 \) we have Lyapunov exponents that are zero and so this fixpoint is only achieved in infinite long time. The whole argument can easily expanded to more dimensions.

**Proposition 3. Fixpoints** If we have a fixpoint at \( t = t_0 \) with \( \dot{L}_0(t_0) = 0 \) during end-held contraction or relaxation of a system without a series elasticity \( f_{SE} \) and an assumed linear titin \( f_{PE} = k_n L(t) \) it follows that \( \dot{j}_{kF}(t_0) = 0 \) for a hS working on the plateau of the force-length relationship.

**Proof.** For the fixpoint \( l_0 \) with \( \dot{L}_0 = \ddot{\xi}_0 - l(0) \) and \( \dot{L}_0 = \ddot{\xi}_0 = 0 \) we derive for the set of hS in the myofibril while using Eq. 2.3.9
\[
M \ddot{\xi}_0 = A \ddot{\xi}_0 + \dot{B} = 0, \quad \Rightarrow \quad \ddot{\xi}_0 = -A^{-1} \dot{B}
\]
With the stiffness matrix

\[
A(t) = \begin{pmatrix}
\alpha^{(1)} \int \dot{p}^{(1)} dx & 0 & 0 \\
0 & \ddots & 0 \\
0 & 0 & \alpha^{(n)} \int \dot{p}^{(n)} dx
\end{pmatrix}
\]

and the perturbation

\[
\dot{B}(t) = k_{ext} \dot{L}_{tot} - \left( \alpha^{(1)} \int \dot{p}^{(1)} x dx \right)
\]

For a distinct hS working on the plateau of the force length relationship and with a constant kept external length \((L_{tot} = 0)\) we derive

\[
\dot{f}_{hS}(t_0) = \dot{f}_{CE}(t_0) = \alpha \int \dot{p}(t_0)(\xi_0 + x) dx
\]

For a system of \(n\) hS

\[
\dot{f}_{hS} = \begin{pmatrix}
\dot{f}_{hS}^{(1)} \\
\vdots \\
\dot{f}_{hS}^{(n)}
\end{pmatrix} = A \xi_0 + \dot{B} = -A (A^{-1} \dot{B}) + \dot{B} = \ddot{0}
\]
Curriculum vitae

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Education

2004-2009 ETH Zürich, Doctoral studies
2007 diploma ‘Trainer competitive sports’ Swiss Olympic with Certificate
2006 Swiss Olympic, formation of professional sports trainer
2003 ETH Zürich, diploma as physicist
1999/2000 Strathclyde University Glasgow, Exchange year
1997-2003 ETH Zürich, Studies of physics
1997 Swiss Military, formation for mountain specialist, Andermatt
1989-1996 Kantonsschule Chur, Typus B

Semester- and diploma theses

2007 ‘Eisklettern als Spitzensport’, final thesis for trainer of competitive sports
2003 ‘Quantum Cryptography – Error Correction and Privacy Amplification’,
diploma thesis in theoretical physics, ETH Zürich
Employments

From Jan 2009            Nationaltrainer Swiss Climbing Elite Sportclimbing
From Jan 2006            Nationaltrainer Swiss Climbing Iceclimbing
From Okt 2004            Research assistant, Institute for biomechanics, ETH Zürich
From Okt 2003            Head coach of ‘Regionalzentrum Ostschweiz Swiss Climbing Sportklettern’
2003/2004                Research assistant, Groupe appliqué de physique – optique, université de genève
2002                     Junior research assistant, Institute for Integrated Systems, ETH Zürich
Juni 2002               Teacher of physics, Kantonschule Hohe Promenade, Zürich
Sep/Okt 2001            Teacher of mathematics, Lehrerseminar Rickenbach, Schwyz
1999-2002               Teaching assistant, Departement Mathematik, ETH Zürich

Publications


Scientific presentations