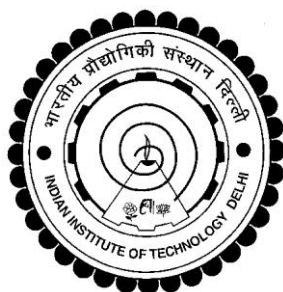


MOLECULAR MECHANISMS GOVERNING ENSEMBLE BEHAVIOUR OF MYOSIN II MOTORS UNDER UNLOADED CONDITIONS

KHUSHBOO RASTOGI



DEPARTMENT OF BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

October 2017

© Indian Institute of Technology Delhi (IITD), New Delhi, 2017

MOLECULAR MECHANISMS GOVERNING ENSEMBLE BEHAVIOUR OF MYOSIN II MOTORS UNDER UNLOADED CONDITIONS

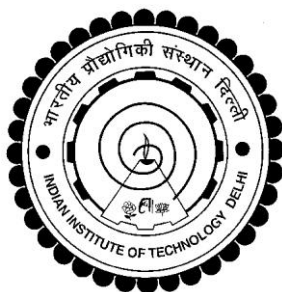
by

KHUSHBOO RASTOGI

Submitted

in fulfilment of the requirement of the degree of Doctor Of Philosophy

to the



Indian Institute of Technology Delhi

OCTOBER 2017

*Dedicated to
My Loved Ones*

CERTIFICATE

This is to certify that the thesis entitled “**Molecular Mechanisms Governing Ensemble Behaviour of Myosin II Motors Under Unloaded Conditions**”, being submitted by **Ms. Khushboo Rastogi** to the Indian Institute of Technology Delhi for the award of degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by her, which has been prepared under our supervision and guidance of conformity with the rules and regulations of “Indian Institute of Technology Delhi”. The research reports and the results presented in this thesis have not been submitted in part or full to any other University/ Institute for the award of any degree or diploma.

Dr. Ravikrishnan Elangovan

Assistant Professor

Dept. of Biochemical Engg & Biotechnology

Indian Institute of Technology Delhi

New Delhi- 110016.

Prof Sunil Nath

Professor

Dept. of Biochemical Engg. & Biotechnology

Indian Institute of Technology Delhi

New Delhi- 110016.

Date:

Place:

ACKNOWLEDGEMENTS

The whole journey of my Ph.D. has been a set combination of intricate as well as discernible events that played a crucial role in transforming and shaping my inner being. It was a great learning experience for me and completion of my thesis wouldn't have been possible without the encouragement and support of various individuals that I would like to acknowledge.

Firstly, I intend to express my deep sense of gratitude and indebtedness to Prof. Ravikrishnan Elangovan who gave me the opportunity to lead the study on myosin II motors in the Molecular Machines laboratory. His faith in me and constant motivation to overpower my fear in taking interdisciplinary research has finally brought me to successful submission of my thesis. He, as my mentor helped me develop ideas and nurtured my investigative abilities that serve an important part in a research career. His enthusiasm to deal with problems in science instilled a great amount of confidence in me to troubleshoot issues encountered during my study, without panicking. I immensely owe him for the time and efforts he invested in my research training.

I would like to thank Prof. Sunil Nath, for his invaluable assistance and suggestions during the course of my experimental work. He played a key role in boosting my confidence level during hard times. Throughout the course of this work, I learned immensely from his encyclopaedic knowledge in the field of molecular motors, patience and great scientific advice that I will forever be indebted to. Long, never ending hours of discussions to dissolve the knots in the experimental findings is something that can never be forgotten. It's been real pleasure to know him as a person and being mentored by him.

I am grateful to my SRC committee members: Prof. Prashant Mishra, Prof. Chinmoy S. Dey and Prof. Preeti Srivastava in taking out time from their busy schedule to review my work annually and provide with their valuable suggestions and feedback.

My work dealt with the slaughtering of chicken to extract muscle proteins, and being a vegetarian, it was hard for me to attain a piece of live muscle tissue directly from the poultry shop. I cannot be more thankful to my lab colleagues Augustine and Vidhu, for their untimely help to procure fresh muscle tissue for my experimental proceedings. I am also grateful to Vidhu for being a great counsellor and instilling in me huge amount of positivity during the last days of my Ph.D.

I wish to express my thanks to my lab colleague and friend Jyoti Sharma for providing me with thousands of those wonderful moments of love, laughter, fun, support and encouragement. I couldn't have gone through this process, without the support of Vikas Pandey who acted as an influencer and co-operated in critical evaluation of my experimental findings with his expert knowledge of logistics and mathematical modeling. Also, I owe my lab member Saurabh for being a source of inspiration, dedication and hard work; and Abhishek for creating a light environment amidst work load with his funny commentaries.

I owe special thanks to my friend Rahul, who made this journey simple by constantly motivating me, providing me moral support and guiding me during tough times. I would like to thank my peers Neeti, Sanjay, Nudrat, Swati Jaiswal and Surabhi for always being available whenever I needed their help. I would also like to thank Mohita, Shilpi, and Pooja for their guidance during the initial years of my Ph.D.

I would love to thank The Almighty for always making me feel his presence around me and blessing me with world's most beautiful creation, my family, my world. I am highly grateful to my parents and siblings Umang, Kriti and Shubhangi; and can't thank them enough for their unparalleled support and unconditional love that has made me capable of what I am today.

I acknowledge Council of Scientific Research, New Delhi for providing me all the financial support in the form of scholarship to live my Ph.D. dream. In the end, I would also like to thank Indian Institute of Technology Delhi for providing a platform to enhance and polish my skills and knowledge to serve the mankind in a better way.

Khushboo Rastogi

ABSTRACT

Myosin II is a low duty ensemble motor that carries out vital physiological functions such as muscle contraction. *In vivo*, an ensemble of myosin molecules functions asynchronously to generate muscle contraction or tension development. Understanding the molecular/allosteric rules that govern the co-ordination and performance of an ensemble of myosin II molecules has been a longstanding puzzle for the research community. Though unloaded shortening and isometric tension have been well studied *in vivo*, it has been challenging to quantify the ensemble properties of Myosin II motor.

In this work, we present the first detailed analysis of processive sliding and breakage of actin filaments at various heavy meromyosin (HMM) densities, ATP concentrations and inorganic phosphate in IVMA. We observed that with addition of ATP solution, the actin filaments fragmented stochastically; we then determined mean length and velocity of surviving actin filaments post breakage. Using density of HMM molecules and length of actin, we estimated the number of HMM molecules per actin filament (N) that participate in processive sliding of actin.

Only those filaments that were able to interact with a minimum number of HMM molecules could sustain processive sliding. N was observed to be a function of ATP concentration, added inorganic phosphate and active binding sites on actin. Only those filaments that were able to interact with a minimum number of HMM molecules could sustain processive sliding. At 2 mM ATP, 88 ± 24 molecules and at 0.1 mM ATP, 54 ± 22 molecules are required for continuous sliding of actin in IVMA. Similarly, for added Pi (10 mM), $N = 72 \pm 30$ at 2 mM ATP and did not show any variation with change in HMM density. Processive sliding of actin filament was

observed only when N lay within the above range at each ATP concentration. These estimates helped arrive at a minimum lower limit (N_{min}) and a maximum upper limit (N_{max}) to the number of HMM molecules that can interact with a smooth sliding actin filament in IVMA under our experimental conditions. When $N < N_{min}$, the actin filament diffused away from the surface and processivity was lost, probably because at least one tightly bound myosin molecule is required to anchor the actin filament to the surface. When $N > N_{max}$, the filament underwent breakage eventually and could not sustain processive sliding. We postulate this maximum upper limit arises due to increased number of strongly bound myosin heads.

We also widened our perspectives to the understanding of sliding of regulated native thin filaments (NTF) and established a similar limit on N . Unlike unregulated actin, we observed no actin breakage of NTFs irrespective of any experimental condition.

We have thus also determined for the first time the number of simultaneously attached myosin molecules (N_a) to be $\sim 1-2$ at a given time despite changes in the experimental environment. Based on our experimental findings, we concluded that processive sliding of the actin filament depends on the relative values of the detachment and attachment rates. We concluded that the unloaded shortening velocity of the actin filaments is a function of ATP only (or ATP and Pi in experiments with external addition of Pi) and is independent of pCa and myosin density.

We believe these inferences from our experimental data will greatly aid in reaching a better understanding of the mechanisms underlying myosin motility in an ensemble. The results may also prove useful in the design of novel molecular motility-based devices in nanotechnology.

सार

मायोसिन द्वितीय एक कम कर्तव्य वाला सामूहिक मोटर होता है जो मांसपेशियों के संकुचन जैसे महत्वपूर्ण शारीरिक कार्य करता है। शरीर में, मायोसिन अणुओं का एक टुकड़ा असामान्य रूप से मांसपेशियों के संकुचन या तनाव पैदा करने के लिए कार्य करता है। आणविक / सबोस्टोरिक नियमों को समझना जो मायोसिन द्वितीय अणुओं के सामूहिक के समन्वयन और प्रदर्शन को नियंत्रित करते हैं, यह अनुसंधान समुदाय के लिए एक पुरानी पहली है। यद्यपि अनलोडेड शॉर्टिंग और आइसोमेट्रिक तनाव का शरीर में अच्छी तरह से अध्ययन किया गया है, यह मायोसिन द्वितीय मोटर के सामूहिक गुणों को मापने के लिए चुनौतीपूर्ण रहा है।

इस काम में, हम विभिन्न भारी मेरोमोओसिन (एचएमएम) घनत्व, एटीपी सांद्रता और ईवीएमए में फॉस्फेट पर प्रोटीसिव फिसलने और एक्टिन फिलामेंट्स के टूटने का पहला विस्तृत विश्लेषण प्रस्तुत करते हैं। हमने पाया कि एटीपी समाधान के साथ, एक्टिन तंतु प्रसंभात्य खंडित होते हैं। हमने तो लंबा और जीवित एक्टिन तंतु टूटने के बाद, उसके वेग की गति और लंबाई निर्धारित करी है। एचएमएम अणुओं के घनत्व और एक्टिन की लंबाई का उपयोग करके, हमने अनुमान लगाया है कि प्रति एक्टिन फिलामेंट, एचएमएम अणुओं (एन) की संख्या एक्टिन की प्रोसेसली फिसलने में भाग लेती है।

केवल उन तंतुओं जो एचएमएम अणुओं की न्यूनतम संख्या के साथ बातचीत करने में सक्षम थे, प्रोसेसिव फिसलने को बचा सकते हैं। एन एटीपी एकाग्रता का एक समारोह माना गया था, अकार्बनिक फॉस्फेट जोड़ा गया और एक्टिन पर सक्रिय बाध्यकारी साइटें। केवल उन तंतुओं जो एचएमएम अणुओं की न्यूनतम संख्या के साथ बातचीत करने में सक्षम थे, प्रोसेसिव फिसलने को बचा सकते हैं। 2 एमएम एटीपी में, 88 ± 24 अणुओं और 0.1 एमएम एटीपी पर, IVMA में एक्टिन की निरंतर स्लाइडिंग के लिए 54 ± 22 अणु आवश्यक हैं। इसी तरह, पीई (10 मिमी) के लिए, एन = 72 ± 30 2 एमएम एटीपी में और एचएमएम घनत्व में परिवर्तन के साथ कोई भिन्नता नहीं दिखा। एटीन रेशा की प्रक्रियात्मक स्लाइडिंग केवल तब देखी गई थी जब एन प्रत्येक एटीपी एकाग्रता में ऊपर की सीमा के भीतर था। इन अनुमानों ने न्यूनतम प्रयोगशाला (एनएमएन) और अधिकतम ऊपरी सीमा (एनएमएक्स) में एचएमएम अणुओं की संख्या तक पहुंचने में मदद की जो कि हमारे प्रयोगात्मक स्थितियों में एक चिकनी फिसलने एक्टिन फिलामेंट के साथ IVMA में बातचीत कर सकते हैं। जब एन < N_{min}, एक्टिन रेशा सतह से दूर फैल गया था और प्रसंस्करण खो गया था, शायद क्योंकि कम से कम एक कसकर बंधन myosin अणु सतह के लिए एक्टिन फिलामेंट एंकर करने के लिए आवश्यक है। जब एन > एनएमएक्स, फिलामेंट अंततः टूटता हुआ था और प्रोसेसली फिसलने को नहीं बचा सकता था। हम मानते हैं कि इस अधिकतम ऊपरी सीमा को मजबूती से बाध्य मायोसिन सिर की बढ़ी हुई संख्या के कारण उठता है।

हमने अपने परिप्रेक्ष्य को विनियामक देशी पतली तंतुओं (एनटीएफ) की फिसलने की समझ को चौड़ा कर दिया और एन पर एक समान सीमा की स्थापना की। अनियमित एक्टिन के विपरीत, हमने किसी भी प्रयोगात्मक हालत के बावजूद एनटीएफ की कोई क्रियात्मक क्षति नहीं देखी।

इस प्रकार हमने प्रायोगिक माहौल में परिवर्तन के बावजूद पहली बार एक साथ समय पर 1/2 होना करने के लिए एक साथ जुड़े मायोसिन अणुओं (एनए) की संख्या निर्धारित की है। हमारे प्रयोगात्मक निष्कर्षों के आधार पर, हमने निष्कर्ष निकाला कि एक्टिन फिलामेंट की प्रोसेसिव स्लिसिंग टुकड़ी और लगाव दर के सापेक्ष मूल्यों पर निर्भर करता है। हमने निष्कर्ष निकाला है कि एक्टिन फिलामेंट्स की उतार-चढ़ाव के वेग एटीपी (या एटीपी और पीआई के बाह्य जोड़ के प्रयोग के साथ प्रयोग में है) और पीसीए और मायोसिन घनत्व से स्वतंत्र है।

हम मानते हैं कि इन प्रयोगों से हमारे प्रयोगात्मक आंकड़ों से बहुत अधिक सहायता मिलेगी जो कि मेसिन गतिशीलता के आधार पर एक पहनावे में बेहतर ढंग से समझने में सहायता करेगी। परिणाम नैनो में उपन्यास आणविक गतिशीलता-आधारित उपकरणों के डिजाइन में भी उपयोगी साबित हो सकते हैं।

CONTENTS

Title	Page No.
CERTIFICATE.....	i
ACKNOWLEDGEMENTS.....	ii
ABSTRACT.....	v
CONTENTS.....	vii
LIST OF FIGURES.....	xi
LIST OF TABLES.....	xiv
ABBREVIATIONS.....	xv
Chapter 1 INTRODUCTION.....	1
Chapter 2 REVIEW OF LITERATURE.....	10
2.1 Skeletal Muscle Anatomy.....	11
2.1.1 Muscle Fiber and Myofibrils	11
2.1.2 Sarcomere.....	12
2.1.3 Thick Filament.....	15
2.1.3.1 X-Ray Crystallographic Sstructure of Myosin S1 Head.....	17
2.1.4 Thin Filament	19
2.1.4.1 X-Ray Crystallographic Structure of G-actin.....	22
2.2 Physiology of Skeletal Muscle Activation: E-C Coupling.....	23
2.3 Acto-Myosin ATPase Cycle (Cross-Bridge Cycle).....	25
2.4 Duty Ratio.....	27
2.5 Single Acto-Myosin Event.....	28
2.6 Reductionist Approach: From Muscle to Single Molecule.....	30
2.6.1 Whole Muscle and Muscle Fiber Studies.....	32

2.6.2 Single Molecule Studies	35
2.7 Insights from the In Vivo Studies of Thin Filaments.....	37
2.7.1 Cooperativity in Thin Filament Activation.....	38
2.7.1.1 pCa-Force Relationship – Activation by Binding of Ca ²⁺	38
2.7.1.2 Activation by Strong Cross Bridge Attachment	40
2.8 Regulation of Reconstituted Thin Filaments in the Motility Assay.....	41
2.9 Models of Thin Filament Regulation.....	43
2.9.1 Two State Model	44
2.9.2 Three State Model	44
Chapter 3 MATERIALS AND METHODS.....	47
3.1 Extraction of Contractile Proteins.....	48
3.1.1 Myosin.....	48
3.1.2 HMM.....	49
3.1.3 Actin.....	50
3.2 Protein Purification.....	51
3.3 Construction of Flow Chamber.....	52
3.4 In Vitro Motility Assay.....	52
3.5 Measurement of HMM and Myosin Surface Density.....	55
3.6 Temperature Controller.....	56
3.7 Observation Setup and Data Acquisition.....	56
3.8 Pixel calibration of Camera.....	61
3.9 Length of Actin Filaments.....	62
3.10 Native Thin Filaments	63
3.10.1 Protein Extraction and Purification.....	63

3.10.2 In Vitro Motility Assay with Regulated Actin.....	64
3.10.3 Imaging and Data Acquisition.....	65
Chapter 4 RESULTS (UNREGULATED FILAMENTS).....	67
4.1 Protein Purification.....	68
4.2 IVMA Control Experiments.....	69
4.3 Actin Filament Length Determination.....	71
4.4 HMM Surface Density on Coverslip.....	74
4.5 Effect of HMM Density on Actin Filament Breakage.....	75
4.6 Evidence of Actin Breakage in the Presence of Full Length Myosin.....	79
4.7 Effect of [ATP] on Actin Filament Breakage.....	80
4.8 Sequence of Actin Fragmentation.....	83
4.9 Number of HMM Molecules Participating in Continuous Actin Sliding.....	84
4.10 Actin Sliding Velocity Dependence on [ATP].....	87
4.11 Effect of Inorganic Phosphate on Actin Sliding Velocity and Filament Breakage.....	89
4.12 Effect of Inorganic Phosphate on the Number of HMM Molecules (<i>N</i>) on Processive Sliding.....	92
Chapter 5 RESULTS (NATIVE THIN FILAMENTS).....	94
5.1 Protein Purification of Native Thin Filaments.....	95
5.2 Control IVMA Experiments with Myosin.....	96
5.3 Estimation of Myosin Surface Density.....	97
5.4 Effect of Different Myosin Concentration and pCa on Sliding Velocity of NTF.....	99
5.5 Analysis of Movement of Native Thin Filaments at Different Myosin Concentration and pCa.....	100
5.6 Role of the Number of Myosin Molecules in Activation of Thin Filaments.....	103
Chapter 6 DISCUSSION.....	106

6.1 Optimization of Experimental Conditions.....	107
6.2 Actin Breakage in IVMA.....	108
6.3 Number of Motors Participating During Processive Sliding in IVMA.....	110
6.4 Number of Attached Motors during Processive Sliding.....	110
6.5 Maximum and Minimum Limit to Processive Actin Sliding.....	111
6.6 Inorganic Phosphate Affecting Kinetics of Actomyosin Interaction.....	115
6.7 Effect on Sliding Velocities of Native Thin Filaments.....	117
6.8 Regulation of Native Thin Filaments by Calcium and Myosin Heads.....	120
Chapter 7 CONCLUDING REMARKS AND FUTURE DIRECTIONS.....	122
BIBLIOGRAPHY.....	125
APPENDIX.....	138
RESUME OF THE AUTHOR.....	157

LIST OF FIGURES

Figure No.	Title	Page No.
1.1	Kinetics involved in Myosin II ATPase cycle.	6
1.2	Pictorial representation of ensemble of ensemble behaviour of Myosin II motor.	8
2.1	Hierarchy of skeletal muscle organization.	12
2.2	Electron micrograph and pictorial representation of a sarcomere.	14
2.3	Thick filaments structure and organization.	16
2.4	Myosin II and its proteolytic fragments.	17
2.5	Features of the tertiary crystal structure of chicken skeletal muscle myosin S1 in the near-rigor state compatible with nucleotide binding.	19
2.6	A schematic of the molecular arrangement of thin filament and its regulation.	21
2.7	Structure of F-actin.	22
2.8	A schematic showing a flow through diagram of different steps of regulation of muscle contraction via excitation-contraction coupling.	25
2.9	The acto-myosin ATPase kinetic pathway as described by Lynn and Taylor.	26
2.10	Relationship between myosin duty ratio and strong binding lifetimes.	28
2.11	Time course of a single acto-myosin event.	30
2.12	A schematic of reductionist approach to study skeletal muscle in great detail.	31
2.13	Muscle fiber studies on sarcomere length and shortening in response to force.	33
2.14	Single molecule studies of myosin II motor using laser trap.	37
2.15	pCa - Force relationship in regulation of thin filament in muscle fiber.	39

2.16	Calcium regulation of fully activated thin filaments in a motility assay.	42
2.17	A diagrammatic representation of the McKillop-Geeves three state model of thin filament activation.	46
3.1	Enzymatic cleavage of myosin II yielding active HMM and inactive LMM.	50
3.2	Construction of flow chamber for <i>in vitro</i> motility assay.	53
3.3	Fluorescence microscopic setup and custom built temperature controller for IVMA experiments.	57
3.4	Thresholding of fluorescently labelled actin filaments for analysis.	59
3.5	Analysis of tracks of sliding actin filaments using <i>Mtrack 2</i> .	59
3.6	Snapshot of custom built program in Labview version 10.01.1 for estimation of frame to frame velocity of single actin filament based on filament track.	60
3.7	Calibration of pixels for Andor Neo camera.	61
3.8	TIRF setup for Native Thin Filament data acquisition.	66
4.1	15% SDS-PAGE of myosin, HMM and G-actin from chicken pectoralis skeletal muscle.	68
4.2	Histogram of observed actin sliding velocities ($\mu\text{m/s}$) for myosin and HMM under controlled conditions i.e. 2mM ATP at 30°C.	71
4.3	Snapshot of actin filaments in the absence and presence of HMM.	72
4.4	Histogram plot of actin filament length in the presence and absence of 2 mM ATP.	73
4.5	Surface density estimation of HMM using SDS-PAGE analysis.	75
4.6	Effect of HMM density on actin filament breakage.	76
4.7	Histogram of actin length distribution at 2mM ATP and various HMM density conditions.	77
4.8	Actin sliding velocity (V_F) as a function of HMM density.	78
4.9	Histogram of actin length distribution for myosin molecules at 2 mM ATP and various motor densities.	80
4.10	Effect of ATP concentration on actin filament breakage.	82

4.11	Histogram plot of actin length distribution for 100 $\mu\text{g/ml}$ HMM density and various ATP concentrations.	83
4.12	Snapshots of actin breakage event imaged at 100 frames per second rate.	84
4.13	Estimation of number of HMM molecules as a function of HMM and ATP concentration.	86
4.14	Velocity of actin filament as a function of ATP at two different HMM densities; 100 $\mu\text{g/ml}$ and 600 $\mu\text{g/ml}$.	88
4.15	Actin sliding velocity (V_F) as a function of HMM density in the absence and presence of 10 mM Pi, 2 mM ATP concentration at 30°C.	90
4.16	Effect of inorganic phosphate on actin filament length as a function of HMM density.	91
4.17	Number of interacting myosin molecules (N) as a function of 10 mM Pi.	92
5.1	15% gel image of native thin filament protein composition.	95
5.2	Histogram of observed native thin filament sliding velocities ($\mu\text{m/s}$) under controlled conditions i.e. 2 mM ATP at 30°C, pCa 5.	97
5.3	Estimation of myosin surface density using SDS-PAGE analysis.	98
5.4	V_F of native thin filaments is plotted against myosin density ($\mu\text{g/ml}$) at 2mM ATP at three different pCa (5, 6.5, and 7).	100
5.5	Fraction of filaments undergoing different types of movements at varied pCa (4-9) at 500 $\mu\text{g/ml}$ myosin concentration.	101
5.6	Fraction of actin filaments exhibiting different kinds of movements as a function of myosin density at different pCa.	102
5.7	Fraction of sliding and fixed thin filaments as function of number of myosin molecules.	105
6.1	Schematic displaying the three major kinetic events that comprise the ATPase cycle, where $T_c = T_{on} + T_h (1/k_h) + T_{att} (1/k_{att})$.	112
6.2	Dependence of velocity (V_F) and cycle time of a single HMM molecule (T_c) on ATP concentration.	114
6.3	Estimation of attachment rate (k_{att}) in the absence and presence of 10 mM Pi.	115

LIST OF TABLES

Table No.	Title	Page No.
3.1	Buffer solutions (2X) for IVMA experiments with unregulated filaments.	55
3.2	Buffer solutions (1X) for IVMA experiments with Native Thin Filaments.	65
6.1	Parameter values for IVMA experimental conditions.	113

ABBREVIATIONS

ADP	-	Adenosine Diphosphate
ATP	-	Adenosine Triphosphate
β ME	-	2- β Mercapto Ethanol
BSA	-	Bovine serum albumin
EMCCD	-	Electron Multiplying Charge Coupled Device
ELC	-	Essential Light Chain
Ca^{2+}	-	Calcium (divalent ions)
CrP	-	Creatine Phosphate
CPK	-	Creatine Phosphokinase
d	-	Working stroke of myosin II motor
DTT	-	Dithiothreitol
EDTA	-	Ethylene Diamine Tetraacetic Acid
EGTA	-	Ethylene Glycol Tetraacetic Acid
HMM	-	Heavy Meromyosin
IVMA	-	<i>In Vitro</i> Motility Assay
KCl	-	Potassium chloride
k_{-ADP}	-	Rate constant for ADP release
k_{+ATP}	-	Second order rate constant of ATP binding
kDa	-	Kilo Dalton
K_m	-	Apparent binding constant for [MgATP]
k_{-Pi}	-	Rate constant for Pi release

KPi	-	Potassium phosphate buffer
LMM	-	Light Meromyosin
MgCl ₂	-	Magnesium chloride
mM	-	Millimolar
MOPS-3	-	(N-mopholino)propanesulfonic acid
n	-	Hill Coefficient
NaN ₃	-	Sodium Azide
N.A	-	Numerical aperture
NTF	-	Native thin filaments
pCa	-	Negative logarithm (base 10) of Ca ²⁺ ion concentration
PMSF	-	Phenylmethylsulfonyl fluoride
Pi	-	Inorganic Phosphate
RLC	-	Regulatory Light Chain
S1	-	Sub-fragment 1
S2	-	Sub-fragment 2
sCMOS	-	scientific Complementary Metal Oxide Semiconductor
S.D	-	Standard Deviation
S.E	-	Standard Error
SDS PAGE	-	Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis
TAME	-	N-alpha-p-tosyl-L-arginine methyl ester hydrochloride
TPCK	-	N-alpha-tosyl-L-phenylalanine chloromethane ketone
Tm	-	Tropomyosin
Tn C	-	Troponin C

Tn I	-	Troponin I
Tn T	-	Troponin T
T_{on}	-	Time of attachment
Tris-Cl	-	Tris hydrochloride
TRITC	-	Tetramethyl Rhodamine Isothiocyanate
V_F	-	Velocity of thin filament sliding in the IVMA
V_{max}	-	Velocity of thin filament sliding at saturating [MgATP]